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Patent

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Patent Number	12,013,326
Issue Date	June 18, 2024
Application Number	18/506,853
Filing Date	November 10, 2023
First Named Inventor	Catherine R. O'RIORDAN
Attorney Docket Number	15979-20103.03

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☐ If the Patentee was not the applicant for patent (37 CFR 1.42), then a Statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is enclosed or was filed on _____. See 37 CFR 3.71.

☑ Attorney or agent of record. Registration Number 60243.

☐ Patent practitioner acting in a representative capacity whose correspondence address is the correspondence address of record. Notice has been given to the patentee or owner. Registration Number _____.

Signature /Seth Snyder/

Typed or Printed Name Seth Snyder

Date December 13, 2024	Telephone +1 (415) 268-7046

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Mail Stop Post Issue, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	**12/13/2024 03:29:50 PM Z ET**	**15979-20103.03**

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	12013326
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	68364855	FILING DATE	11/10/2023
CUSTOMER #	89300	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Seth Snyder

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Change_of_Corr.pdf	2	Change of Address	203 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Change_of_Corr.pdf	6E311682EF02C254AF80793C1C445A8339723C88B48770D4F2F8F9D5EB1FD7678EB0F2A50560C69F6B266E4F201D10810BF9EEF04F0D887BE2249F3075CF526D

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

89300 7590 06/18/2024

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EXAMINER	
GILL, RACHEL B	

ART UNIT	PAPER NUMBER
1648	

NOTIFICATION DATE	DELIVERY MODE
06/18/2024	ELECTRONIC

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pair_mofo@firsttofile.com

APPLICATION NO.	ISSUE DATE	PATENT NO.
18/506,853	18-Jun-2024	12013326

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EGRANT NOTIFICATION

Your electronic patent grant (eGrant) is now available, which can be accessed via Patent Center at https://patentcenter.uspto.gov

The electronic patent grant is the official patent grant under 35 U.S.C. 153. For more information, please visit https://www.uspto.gov/electronicgrants

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	06/18/2024	12013326	15979-20103.03	3457

89300 7590 05/29/2024

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at https://patentcenter.uspto.gov/. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO's Certified Copy Center at https://certifiedcopycenter.uspto.gov/index.html. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Center (https://patentcenter.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

INVENTOR(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional inventors):

Catherine R. O'RIORDAN, Cambridge, MA;
Brenda Burnham, Hopkinton, MA;

APPLICANT(s) (Please see PATENT CENTER site https://patentcenter.uspto.gov for additional applicants):

Genzyme Corporation, Cambridge, MA;

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

89300 7590 05/23/2024

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EXAMINER	
GILL, RACHEL B	

ART UNIT	PAPER NUMBER
1648	

NOTIFICATION DATE	DELIVERY MODE
05/23/2024	ELECTRONIC

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pair_mofo@firsttofile.com

PTOL-90A (Rev. 04/07)

Response to Rule 312 Communication	Application No. 18/506,853	Applicant(s)
	Examiner	Art Unit

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address –

1. ☒ The amendment filed on *08 May 2024* under 37 CFR 1.312 has been considered, and has been:

a) ☒ entered.

b) ☐ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	**05/08/2024 06:50:03 PM Z ET**	**15979-20103.03**

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	65433316	FILING DATE	11/10/2023
CUSTOMER #	89300	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Seth Snyder

Documents

TOTAL DOCUMENTS: 4

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
Copy_Notice.pdf		3	Applicant Response to Pre-Exam Formalities Notice	102 KB
After_Allowance_amendment.pdf		3	-	85 KB
After_Allowance_amendment-A.NA.pdf	(1-1)	1	Amendment after Notice of Allowance (Rule 312)	77 KB
After_Allowance_amendment-SPEC.pdf	(2-2)	1	Specification	78 KB
After_Allowance_amendment-REM.pdf	(3-3)	1	Applicant Arguments/Remarks Made in an Amendment	78 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Copy_Notice.pdf	87E72D2641DCC1998487D44D707C0937AA891E9B9F2406C11F577D1646E731E65C9A4463A443C1C4E4238E38F9D1E94F9A8992A2F4EE9462FB4C3A9FFB8C09A6
After_Allowance_amendment.pdf	2F1390FBF4D3C5F2445EE2CC421EDF15B3F4F88B129DDD75662485F1479A3259DDD349B0E3C3C46D9ED6B1D2F16A29497CB7804A3E1B28361F8F211C20AF4DA5
After_Allowance_amendment-A.NA.pdf	87CCF2B82709C05FF2EE778E04005B7482BB89A36253956A4BC1689FC793BD83E4B2218B75263ECB5B1D94804D73D7A4F3EDCC16B907A43148B0D8C0D6EE0AD8
After_Allowance_amendment-SPEC.pdf	0C6E92413CD5CDCF0F28B1E92A0CFC0295F53FE99A42059AC58297B6DB3C80005F381C49A5F36496454B80658C757BB4D0B2F420B5E7C02D4A40E09B08F5AC45
After_Allowance_amendment-REM.pdf	B28E1163F87B9D693DE0C052037E6BCABDAAAE5B01E3EF0DCFA0CB3B45DBA47566520DD14032507269C4D9C006019DC9609F9ED37882E34970A0FDA2C8028D5E

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Sarepta Exhibit 1002, Page 12

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

89300 7590 05/01/2024

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EXAMINER	
GILL, RACHEL B	

ART UNIT	PAPER NUMBER
1648	

NOTIFICATION DATE	DELIVERY MODE
05/01/2024	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

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Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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UNITED STATES PATENT AND TRADEMARK OFFICE

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Application No. : 18506853
Applicant : O'Riordan
Filing Date : 11/10/2023
Date Mailed : 05/01/2024

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given two (2) months from the mail date of this Notice within which to respond. This time period for reply is extendable under 37 CFR 1.136(a) for only TWO additional MONTHS.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED.

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Naran Hem/
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Application No. 18506853

SPECIFICATION REFERS TO ITS OWN PAGE/PARAGRAPH NUMBER(S) AND/OR SPECIFICATION REFERS TO NONEXISTENT CLAIM(S)

☐ The patent will be printed in numbered columns with intercolumnar line numbers and without paragraph numbers. Any reference(s) within the applicant-filed specification to its own page number(s) or paragraph number(s) will have no meaning in the printed patent.

In response to this notice, the applicant either should file an amendment to the specification that removes the reference(s) to the page number(s) or paragraph number(s) or should submit a letter stating that the patent specification should be printed with the reference(s) to the page number(s) or paragraph number(s).

See Page(s)/Paragraph(s) .

☒ The specification refers by number to one or more claims that are not present in the allowed application – that is, the claim number(s) mentioned in the specification are higher than the total number of allowed claims.

In response to this notice, the applicant either should file an amendment to the specification that removes the reference(s) to the nonexistent claim(s) or should submit a letter stating that the patent specification should be printed with the reference(s) to the nonexistent claim(s).

See Page(s)/Paragraph(s) page 9, paragraph [0027].

Docket No.: 15979-20103.03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Catherine R. O'RIORDAN et al.

Application No.: 18/506,853

Filed: November 10, 2023

For: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Confirmation No.: 3457

Art Unit: 1648

Examiner: R. Gill

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

INTRODUCTORY COMMENTS

This communication is in response to the Notice to File Corrected Application Papers ("Notice") dated May 1, 2024, for which a response is due on July 1, 2024. Accordingly, this response is timely filed. A copy of the Notice is enclosed with this reply.

Amendments to the Specification begin on page 2 of this paper.

Remarks/Arguments begin on page 3 of this paper.

sf-5945990

AMENDMENTS TO THE SPECIFICATION

Please amend paragraph **[0027]** as follows:

[0027] In some aspects, the invention provides methods for evaluating a process for the production of recombinant viral particles comprising the method of any one of claims 1 to 30 [[67]], wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes compared to the relative amount of empty capsid particles and/or recombinant viral capsid particles with variant recombinant viral genomes compared to a reference preparation of recombinant viral particles indicates an improvement in the production of recombinant viral particles. In some embodiments, the recombinant viral particle is a recombinant adeno-associated viral (AAV) particle, a recombinant adenovirus particle, a recombinant lentivirus particle or a recombinant herpes simplex viral (HSV) particle. In some embodiments, the rAAV particles are produced from a producer cell line. In other embodiments, the rAAV particles are produced by triple transfection of i) nucleic acid encoding AAV rep and cap, ii) rAAV vector sequences, and iii) nucleic acid encoding adenovirus helper functions. In other embodiments, the recombinant viral particles are produced by an AAV/HSV hybrid. In other embodiments, the recombinant viral particles are produced from a baculovirus cell. In some embodiments, the recombinant viral particles are produced by transient transfection of nucleic acid encoding AAV vector sequences, AAV rep and cap coding regions, and AAV helper virus functions to a suitable host cell. In some embodiments, the recombinant viral particles are produced by introduction of one or more nucleic acids encoding AAV vector sequences, AAV rep and cap coding regions, and AAV helper virus functions to a suitable host cell, wherein the one or more nucleic acids are introduced to the cell using a recombinant helper virus. In some embodiments, the recombinant helper virus is an adenovirus or a herpes simplex virus. In some embodiments, the recombinant viral particles comprise a self-complementary AAV (scAAV) genome. In some embodiments, the method is used to detect the presence of recombinant viral particles comprising the monomeric form of a scAAV genome or the dimeric form of a scAAV genome.

REMARKS

The Notice indicates that the specification's has been amended to amend paragraph **[0027]** that starts on page 9. The claims as filed on November 10, 2023 in the present application canceled claims 1-95 and added new claims 96-125. Accordingly, they will be renumbered as claims 1-30. No new matter has been added and entry of the amendment is respectfully requested.

Applicant believes this communication is fully responsive to the Notice to File Corrected Application Papers. However, should the Office believe that further response on the part of the Applicant is required, the Office is requested to contact the undersigned at the earliest convenience.

In the event the U.S. Patent and Trademark Office determines that an extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. **03-1952** referencing docket no. **15979-20103.03**.

Dated: May 8, 2024

Respectfully submitted,

Electronic signature: /Seth Snyder/
Seth Snyder
Registration No.: 60,243
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, CA 94304-1018
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

EOfficePA@mofo.com
PatentDocket@mofo.com
pair_mofo@firsttofile.com

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Application No. : 18506853
Applicant : O'Riordan
Filing Date : 11/10/2023
Date Mailed : 05/01/2024

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given two (2) months from the mail date of this Notice within which to respond. This time period for reply is extendable under 37 CFR 1.136(a) for only TWO additional MONTHS.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED.

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Naran Hem/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 18506853

SPECIFICATION REFERS TO ITS OWN PAGE/PARAGRAPH NUMBER(S) AND/OR SPECIFICATION REFERS TO NONEXISTENT CLAIM(S)

☐ The patent will be printed in numbered columns with intercolumnar line numbers and without paragraph numbers. Any reference(s) within the applicant-filed specification to its own page number(s) or paragraph number(s) will have no meaning in the printed patent.

In response to this notice, the applicant either should file an amendment to the specification that removes the reference(s) to the page number(s) or paragraph number(s) or should submit a letter stating that the patent specification should be printed with the reference(s) to the page number(s) or paragraph number(s).

See Page(s)/Paragraph(s) .

☒ The specification refers by number to one or more claims that are not present in the allowed application – that is, the claim number(s) mentioned in the specification are higher than the total number of allowed claims.

In response to this notice, the applicant either should file an amendment to the specification that removes the reference(s) to the nonexistent claim(s) or should submit a letter stating that the patent specification should be printed with the reference(s) to the nonexistent claim(s).

See Page(s)/Paragraph(s) page 9, paragraph [0027].

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

89300 7590 04/23/2024
Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

Aurelia Caparas	(Typed or printed name)
/Aurelia Caparas/	(Signature)
April 25, 2024	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

TITLE OF INVENTION: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	07/23/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
GILL, RACHEL B	1648	435-005000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Morrison & Foerster LLP
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: GENZYME CORPORATION

(B) RESIDENCE: (CITY and STATE OR COUNTRY): CAMBRIDGE, MASSACHUSETTS

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☒ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

☒ Electronic Payment via the USPTO patent electronic filing system ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 03-1952

5. **Change in Entity Status** (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

☐ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

☐ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Seth Snyder/ Date April 25, 2024

Typed or printed name Seth Snyder Registration No. 60243

PTOL-85 Part B (11/23) Approved for use through 03/31/2026 OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	04/25/2024 07:29:09 PM Z ET	15979-20103.03

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	65251335	AUTHORIZED BY	Seth Snyder
CUSTOMER #	89300	FILING DATE	11/10/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 031952	E20244OJ30357510	Aurelia Caparas

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1501	UTILITY ISSUE FEE	1200.00	1	1200.00
			TOTAL AMOUNT:	$1,200.00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C.

371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	**04/25/2024 07:29:09 PM Z ET**	**15979-20103.03**

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	65251335	FILING DATE	11/10/2023
CUSTOMER #	89300	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Seth Snyder

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
Issue_Fee.pdf	1	Issue Fee Payment (PTO-85B)	167 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
Issue_Fee.pdf	04706892C7708F52A01A190567D43B300625B8832A171B8B3D BE55990D5C7A8E7E2869E38952D383F705A9958AD609F5826E 275DFAF301294779B6D65A34DD72

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

89300 7590 04/23/2024

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EXAMINER
GILL, RACHEL B

ART UNIT	PAPER NUMBER
1648	

DATE MAILED: 04/23/2024

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

TITLE OF INVENTION: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	07/23/2024

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via the USPTO patent electronic filing system.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

89300 7590 04/23/2024
Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via the USPTO patent electronic filing system or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

TITLE OF INVENTION: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1200	$0.00	$0.00	$1200	07/23/2024

EXAMINER	ART UNIT	CLASS-SUBCLASS
GILL, RACHEL B	1648	435-005000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1_____
2_____
3_____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required)

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via the USPTO patent electronic filing system ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29
NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

89300 7590 04/23/2024

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EXAMINER	
GILL, RACHEL B	

ART UNIT	PAPER NUMBER
1648	

DATE MAILED: 04/23/2024

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. The United States Patent and Trademark Office (USPTO) collects the information in this record under authority of 35 U.S.C. 2. The USPTO's system of records is used to manage all applicant and owner information including name, citizenship, residence, post office address, and other information with respect to inventors and their legal representatives pertaining to the applicant's/ owner's activities in connection with the invention for which a patent is sought or has been granted. The applicable Privacy Act System of Records Notice for the information collected in this form is COMMERCE/PAT-TM-7 Patent Application Files, available in the Federal Register at 78 FR 19243 (March 29, 2013).
https://www.govinfo.gov/content/pkg/FR-2013-03-29/pdf/2013-07341.pdf

Routine uses of the information in this record may include disclosure to:

1) law enforcement, in the event that the system of records indicates a violation or potential violation of law;
2) a federal, state, local, or international agency, in response to its request;
3) a contractor of the USPTO having need for the information in order to perform a contract;
4) the Department of Justice for determination of whether the Freedom of Information Act (FOIA) requires disclosure of the record;
5) a Member of Congress submitting a request involving an individual to whom the record pertains, when the individual has requested the Member's assistance with respect to the subject matter of the record;
6) a court, magistrate, or administrative tribunal, in the course of presenting evidence, including disclosures to opposing counsel in the course of settlement negotiations;
7) the Administrator, General Services Administration (GSA), or their designee, during an inspection of records conducted by GSA under authority of 44 U.S.C. 2904 and 2906, in accordance with the GSA regulations and any other relevant (i.e., GSA or Commerce) directive, where such disclosure shall not be used to make determinations about individuals;
8) another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c));
9) the Office of Personnel Management (OPM) for personnel research purposes; and
10) the Office of Management and Budget (OMB) for legislative coordination and clearance.

If you do not furnish the information requested on this form, the USPTO may not be able to process and/or examine your submission, which may result in termination of proceedings, abandonment of the application, and/or expiration of the patent.

Notice of Allowability	Application No. 18/506,853	Applicant(s) O'RIORDAN et al.	
	Examiner RACHEL B GILL	Art Unit 1648	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to response filed on 04/09/2024.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 96-124 and 126 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some* c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/RACHEL B GILL/
Primary Examiner, Art Unit 1648

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Disposition of Claims

Claims 96-125 were pending. Claims 1-95 and 125 are cancelled. Amendments to claims 96-97, 109-116, and 121-124 are acknowledged and entered. New claim 126 is acknowledged and entered. Claims 96-124 and 126 will be examined on their merits.

Examiner's Note

Applicant is encouraged to utilize the new web-based Automated Interview Request (AIR) tool for submitting interview requests; more information can be found at https://www.uspto.gov/patent/laws-and-regulations/interview-practice.

This application is being examined under the accelerated "Track One" Program. Applicant is reminded of the provisions of this prioritized examination program as per MPEP §708.02(b). Specifically noted is the application must contain, or be amended to contain, no more than four independent claims and no more than thirty total claims. In addition, the application must not contain any multiple dependent claims. After prioritized examination has been granted in an application, an amendment that results in more than four independent claims or thirty total claims, or a multiple dependent claim, will result in termination of prioritized examination.

Response to Arguments

Applicant's arguments filed 04/09/2024 regarding the previous Office action dated 01/09/2024 have been fully considered. In light of applicant's arguments, applicant's amendments, and the filing and acceptance of terminal disclaimers, all outstanding objections and/or rejections have been **withdrawn** as noted herein.

Terminal Disclaimer

The terminal disclaimers filed on 04/09/2024 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of **US Pat. Nos. 11,639,887 and 10,429,288 and any patents maturing from US Apps. 18/188,176 and 18/513,970** have been reviewed and are accepted. The terminal disclaimers have been recorded.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 04/09/2024 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Specification

(**Objection withdrawn**.) The objection to the abstract is **withdrawn** in light of the amendments to the abstract.

Claim Rejections - 35 USC § 112(b); Second Paragraph

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

(**Rejection withdrawn**.) The rejection of Claim 115 and dependent claims 116-125 thereof under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, is **withdrawn** in light of the amendments to the claims.

Claim Rejections - 35 USC § 112(a); First Paragraph

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

(**Rejection withdrawn**.) The rejection of Claims 96-125 under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, is **withdrawn** in light of the amendments to the claims and applicant arguments.

Claim Rejections - 35 USC § 102

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

(**Rejection withdrawn**.) The rejection of **Claims 96, 98-99, 108, 112-115, 117-118, and 122-125** under 35 U.S.C. 102(a)(1) as being anticipated by Le Bec et. al. (WO2014125101A1, Priority 02/15/2013; CITED ART OF RECORD; hereafter "Le Bec"; NB: the English translation of Le Bec utilized for this rejection was provided in parent application 16/547,144; the original French WIPO publication and English translation utilized for this rejection has been included for reference in this Office action), is **withdrawn** in light of the amendments to the claims and applicant arguments.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

(**Rejection withdrawn**.) The rejection of **Claims 97, 100-107, 109-111, 116, and 119-121** under 35 U.S.C. 103 as being unpatentable over Le Bec as applied to **claims 96, 98-99, 108, 112-115, 117-118, and 122-125** above, and further in view of McLaughlin et. al. (US20170362608A1, Pub. 12/21/2017, Priority 12/12/2014; hereafter "McLaughlin"), Berkowitz et. al. (Berkowitz SA, et. al. *Anal Biochem*. 2007 Mar 1;362(1):16-37. Epub 2006 Dec 20.; CITED ART OF RECORD; hereafter "Berkowitz"); and Cole et. al. (Cole JL, et. al *Methods Cell Biol*. 2008;84:143-79.; CITED ART OF RECORD; hereafter "Cole"), is **withdrawn** in light of the amendments to the claims and applicant arguments.

Double Patenting

The text regarding nonstatutory double patenting was presented in a previous Office action.

(**Rejection withdrawn**.) The rejection of Claims 96-125 on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of U.S. Patent No. 11,639,887 in view of Le Bec (supra) is **withdrawn** in light of the terminal disclaimer filed and accepted over the '887 patent.

(**Rejection withdrawn**.) The rejection of Claims 96-125 on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 5-9, and 15-34 of U.S. Patent No. 10,429,288 B2. NB: Claims 4 and 10-14 were recently disclaimed by Patent Owner in the '288 patent) is **withdrawn** in light of the terminal disclaimer filed and accepted over the '288 patent.

(**Rejection withdrawn**.) The provisional rejection of Claims 96-125 on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 5, 10, 12, 18, 30-37, 42-44, 48, 58, and 61 of copending Application No. 18/188,176 in view of Le Bec (*supra*) and Berkowitz (*supra*) is **withdrawn** in light of the terminal disclaimer filed and accepted over the '176 application.

(**Rejection withdrawn**.) The provisional rejection of Claims 96-125 on the ground of nonstatutory double patenting as being unpatentable over claims 96-125 of copending Application No. 18/513,970 in view of Le Bec (*supra*) and Berkowitz (*supra*) is **withdrawn** in light of the terminal disclaimer filed and accepted over the '970 application.

Conclusion

Claims 96-124 and 126 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to RACHEL B GILL whose telephone number is (571)272-3129. The examiner can normally be reached on M to F 8:00 AM to 5:00 PM Eastern.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JANET ANDRES can be reached on 571-272-0867. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/RACHEL B GILL/
Primary Examiner, Art Unit 1648

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/506,853	O'RIORDAN et al.
	Examiner	Art Unit
	RACHEL B GILL	1648

CPC - Searched*		
Symbol	**Date**	**Examiner**
(G01N15/042 or C12N7/00 or G01N1/405 or G01N1/4077 or C12N2710/10351 or C12N2750/14151 or G01N2001/4083 or G01N2015/045).cpc.	04/15/2024	RG

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

/RACHEL B GILL/ Primary Examiner, Art Unit 1648	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/506,853	O'RIORDAN et al.
	Examiner	Art Unit
	RACHEL B GILL	1648

Search Notes		
Search Notes	**Date**	**Examiner**
IPR2023-01044 Reviewed Petition for IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01045 Reviewed Petition for IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01044 Reviewed Patent Owner's Preliminary Response in IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01045 Reviewed Patent Owner's Preliminary Response in IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01044 Reviewed Third Party Observation Ex.1012 in IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01045 Reviewed Third Party Observation Ex.1012 in IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01044 Reviewed Dec. Jeffrey Hansen Ex.2001 in IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01045 Reviewed Dec. Jeffrey Hansen Ex.2001 in IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01044 Reviewed Dec. Steven Berkowitz Ex.1020 in IPR of US Pat. No. 10,429,288	01/03/2024	RG
IPR2023-01045 Reviewed Dec. Steven Berkowitz Ex.1020 in IPR of US Pat. No. 10,429,288	01/03/2024	RG
PubMed inventor, assignee, and keyword search (see attached)	01/04/2024	"
PALM inventor search (see attached)	01/04/2024	"
PE2E inventor, assignee, and keyword search (see attached)	01/04/2024	"
Google inventor, assignee, and keyword search (see attached)	01/04/2024	"
Updated PubMed, PE2E, and Google searches (see attached)	04/15/2024	RG
PDP Inventor search (see attached)	04/15/2024	"
Reviewed most recent filings in IPR2023-01044	04/15/2024	"
Reviewed most recent filings in IPR2023-01045	04/15/2024	"

/RACHEL B GILL/ Primary Examiner, Art Unit 1648	

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/506,853	O'RIORDAN et al.
	Examiner	Art Unit
	RACHEL B GILL	1648

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**
	PE2E inventor, assignee, CPC, and keyword interference search (see attached)	04/15/2024	RG

/RACHEL B GILL/ Primary Examiner, Art Unit 1648	

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/506,853	O'RIORDAN et al.
	Examiner	**Art Unit**
	RACHEL B GILL	1648

CPC						
Symbol					**Type**	**Version**
G01N	/	15	/	042	F	2013-01-01
C12N	/	7	/	00	I	2013-01-01
G01N	/	1	/	405	I	2013-01-01
G01N	/	1	/	4077	I	2013-01-01
G01N	/	2001	/	4083	A	2013-01-01
C12N	/	2710	/	10351	A	2013-01-01
C12N	/	2750	/	14151	A	2013-01-01
G01N	/	2015	/	045	A	2013-01-01

CPC Combination Sets							
Symbol					**Type**	**Set**	**Ranking**	**Version**
	/		/					

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	30	
/RACHEL B GILL/ Primary Examiner, Art Unit 1648	15 April 2024	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	none

U.S. Patent and Trademark Office — Part of Paper No.: 20240415

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/506,853	O'RIORDAN et al.
	Examiner	**Art Unit**
	RACHEL B GILL	1648

INTERNATIONAL CLASSIFICATION				
CLAIMED				
G01N15/04	/	15	/	04
C12N7/00	/	7	/	00
G01N1/40	/	1	/	40

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 30	
/RACHEL B GILL/ Primary Examiner, Art Unit 1648 (Primary Examiner)	15 April 2024 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office

Part of Paper No.: 20240415

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	18/506,853	O'RIORDAN et al.
	Examiner	**Art Unit**
	RACHEL B GILL	1648

☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☑ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner)	(Date)	**Total Claims Allowed:** 30	
/RACHEL B GILL/ Primary Examiner, Art Unit 1648 (Primary Examiner)	15 April 2024 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure none

U.S. Patent and Trademark Office Part of Paper No.: 20240415

Doc code: IDS
Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (01-22)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	18506853
Filing Date	2023-11-10
First Named Inventor	Catherine R. O'RIORDAN
Art Unit	1648
Examiner Name	R. Gill
Attorney Docket Number	15979-20103.03

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

If you wish to add additional U.S. Patent citation information please click the Add button.

U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20240085301	A1	2024-03-14	O'riordan, Catherine R.	

If you wish to add additional U.S. Published Application citation information please click the Add button.

FOREIGN PATENT DOCUMENTS

Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

If you wish to add additional Foreign Patent Document citation information please click the Add button

NON-PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /R.B.G/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	18506853
	Filing Date	2023-11-10
	First Named Inventor	Catherine R. O'RIORDAN
	Art Unit	1648
	Examiner Name	R. Gill
	Attorney Docket Number	15979-20103.03

	1		☐

If you wish to add additional non-patent literature document citation information please click the Add button

EXAMINER SIGNATURE

Examiner Signature	/RACHEL B GILL/	Date Considered	04/15/2024

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /R.B.G/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	18506853
	Filing Date	2023-11-10
	First Named Inventor	Catherine R. O'RIORDAN
	Art Unit	1648
	Examiner Name	R. Gill
	Attorney Docket Number	15979-20103.03

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☒ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Seth Snyder/	Date (YYYY-MM-DD)	2024-04-09
Name/Print	Seth Snyder	Registration Number	60,243

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /R.B.G/

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /R.B.G/

Inventor Information

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
O'RIORDAN, CATHERINE	08020483	Not Issued		157	VIRAL VECTOR CONSTRUCTS	KAUSHAL,SUMESH	02/08/1998
O'RIORDAN, CATHERINE	11934148	10744210	20080069261	150	GENE THERAPY FOR SPINAL CORD DISORDERS	WEHBE,ANNE MARIE	11/02/2007
O'RIORDAN, CATHERINE	12328267	9034836	20100287612	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	12/04/2008
O'RIORDAN, CATHERINE	12417910	9890394	20080286857	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	04/03/2009
O'RIORDAN, CATHERINE	14692469	Not Issued	20160067312	161	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	04/21/2015
O'RIORDAN, CATHERINE	15127757	10383963	20170173183	150	GENE THERAPY FOR RETINITIS PIGMENTOSA	BURKHART,MICHAEL	09/20/2016
O'RIORDAN, CATHERINE	15549885	10450563	20180023002	150	VARIANT RNAI	CHONG,KIMBERLY	08/08/2017
O'RIORDAN, CATHERINE	15858303	10820245	20180230490	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	12/29/2017
O'RIORDAN, CATHERINE	16335653	11608377	20210041451	150	METHODS FOR DETECTING AAV	XU,XIAOYUN	02/14/2019
O'RIORDAN, CATHERINE	16503081	11103588	20200046851	150	GENE THERAPY FOR RETINITIS PIGMENTOSA	BURKHART,MICHAEL	07/03/2019
O'RIORDAN, CATHERINE	16546070	11554161	20200101138	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	08/20/2019
O'RIORDAN, CATHERINE	16586585	10780079	20200109401	150	VARIANT RNAI	CHONG,KIMBERLY	09/19/2019
O'RIORDAN, CATHERINE	16910834	Not Issued	20210008227	41	GENE THERAPY FOR SPINAL CORD DISORDERS	WEHBE,ANNE MARIE	06/24/2020
O'RIORDAN, CATHERINE	16941303	11781137	20210047841	150	VARIANT RNAI	CHONG,KIMBERLY	07/28/2020
O'RIORDAN, CATHERINE	17162356	Not Issued	20210261625	41	MODIFIED ADENO-ASSOCIATED VIRAL CAPSID PROTEINS FOR OCULAR GENE THERAPY AND METHODS OF USE THEREOF	PENNINGTON,KATIE	01/29/2021
O'RIORDAN, CATHERINE	17383062	Not Issued	20220054657	30	GENE THERAPY FOR RETINITIS PIGMENTOSA	BURKHART,MICHAEL	07/23/2021
O'RIORDAN, CATHERINE	18068149	Not Issued	20230384200	30	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CENTRAL,DOCKET	12/14/2022
O'RIORDAN, CATHERINE	18296283	Not Issued	20230365868	30	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY	CENTRAL,DOCKET	04/05/2023
O'RIORDAN, CATHERINE	18321542	Not Issued	20240044810	30	METHODS FOR DETECTING AAV	CENTRAL,DOCKET	05/22/2023
O'RIORDAN, CATHERINE	18483474	Not Issued	0	30	VARIANT RNAI	CENTRAL,DOCKET	10/08/2023
O'RIORDAN, CATHERINE	18628784	Not Issued		19	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY		01/01/0001
O'RIORDAN, CATHERINE	60135082	Not Issued		158	VIRAL VECTOR CONSTRUCTS	NOT,DEFINED	02/08/1998
O'RIORDAN, CATHERINE	60827977	Not Issued		158	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS		10/03/2006

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
O'RIORDAN, CATHERINE	61045077	Not Issued		159	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RdCVF)		04/15/2008
O'RIORDAN, CATHERINE	61869027	Not Issued		159	GENE THERAPY FOR RETINITIS PIGMENTOSA		03/21/2014
O'RIORDAN, CATHERINE	62105714	Not Issued		159	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES		01/20/2015
O'RIORDAN, CATHERINE	62114575	Not Issued		159	NOVEL NUCLEIC ACIDS FOR IMPROVED GENE DELIVERY		02/10/2015
O'RIORDAN, CATHERINE	62114576	Not Issued		159	VARIANT RNAi		02/10/2015
O'RIORDAN, CATHERINE	62375314	Not Issued		159	METHODS FOR DETECTING AAV		08/15/2016
O'RIORDAN, CATHERINE	62581934	Not Issued		159	COMPOSITIONS AND METHODS OF TREATING HUNTINGTON'S DISEASE		09/22/2017
O'RIORDAN, CATHERINE	62987416	Not Issued		159	MODIFIED ADENO-ASSOCIATED VIRAL CAPSID PROTEINS FOR OCULAR GENE THERAPY AND METHODS OF USE THEREOF		01/29/2020
O'RIORDAN, CATHERINE	63480375	Not Issued		159	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY		02/03/2023
O'RIORDAN, CATHERINE	63490736	Not Issued		159	TREATMENT OF DRY AGE-RELATED MACULAR DEGENERATION		03/16/2023
O'RIORDAN, CATHERINE	63494453	Not Issued		159	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY		04/05/2023
O'RIORDAN, CATHERINE	63589417	Not Issued		20	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY		10/11/2023
O'RIORDAN, CATHERINE	63607419	Not Issued		20	TREATMENT OF DRY AGE-RELATED MACULAR DEGENERATION		12/07/2023
O'RIORDAN, CATHERINE E.	09604349	Not Issued		161	CHROMATOGRAPHIC PURIFICATION OF ADENOVIRUS	SALIMI,ALI	06/27/2000
O'RIORDAN, CATHERINE E.	10470604	7015026	20040152183	250	PURIFICATION OF ADENOVIRUS AND AAV	SALIMI,ALI	03/02/2004
O'RIORDAN, CATHERINE E.	11178885	7579181	20050244942	250	PURIFICATION OF ADENOVIRUS AND AAV	MOSHER,MARY	07/12/2005
O'RIORDAN, CATHERINE E.	12504073	Not Issued	20100279385	161	PURIFICATION OF ADENOVIRUS AND AAV	MOSHER,MARY	07/16/2009
O'RIORDAN, CATHERINE E.	08011826	6143548		150	CHROMATOGRAPHIC PURIFICATION OF ADENOVIRUS AND AAV	SALIMI,ALI	06/29/1998
O'RIORDAN, CATHERINE E.	60071674	Not Issued		159	ADENOVIRAL VECTORS WITH MODIFIED CAPSID PROTEINS		01/16/1998
O'RIORDAN, CATHERINE E.	60087853	Not Issued		159	CATIONIC COMPLEXES OF POLYMER-MODIFIED ADENOVIRUS		08/24/1998

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
O'RIORDAN, CATHERINE R	60183296	Not issued		159	Adenoviral system		02/17/2000
O'RIORDAN, CATHERINE R.	09582509	Not issued		161	ADENOVIRAL VECTORS WITH MODIFIED CAPSID PROTEINS	GUZO,DAVID	06/26/2000
O'RIORDAN, CATHERINE R.	09801022	Not issued		161	NUCLEIC ACID DELIVERY VEHICLES	GUZO,DAVID	07/24/2000
O'RIORDAN, CATHERINE R.	10349630	Not issued	20030180261	161	Polymer-modified viruses	NAFF,DAVID	01/22/2003
O'RIORDAN, CATHERINE R.	12936659	9567382	20110034546	150	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	BURKHART,MICHAEL	10/06/2010
O'RIORDAN, CATHERINE R.	14888385	10821154	20160074474	150	COMPOSITIONS AND METHODS FOR TREATING SPINAL MUSCULAR ATROPHY	SCHULTZ,JAMES	02/08/2015
O'RIORDAN, CATHERINE R.	15397455	10035829	20170369541	150	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	BURKHART,MICHAEL	01/03/2017
O'RIORDAN, CATHERINE R.	15544496	10429288	20180180525	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	07/18/2017
O'RIORDAN, CATHERINE R.	16022410	Not issued	20190055293	161	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	BURKHART,MICHAEL	06/28/2018
O'RIORDAN, CATHERINE R.	16547144	11639887	20200225139	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	08/21/2019
O'RIORDAN, CATHERINE R.	16649042	11603529	20200216848	150	VARIANT RNAI	ZARA,JANE	03/19/2020
O'RIORDAN, CATHERINE R.	17033443	Not issued	20210121523	161	COMPOSITIONS AND METHODS FOR TREATING SPINAL MUSCULAR ATROPHY	SCHULTZ,JAMES	09/25/2020
O'RIORDAN, CATHERINE R.	18066175	Not issued	20230322873	30	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	CENTRAL,DOCKET	12/14/2022
O'RIORDAN, CATHERINE R.	18169779	Not issued	20230392149	30	VARIANT RNAI	CENTRAL,DOCKET	02/15/2023
O'RIORDAN, CATHERINE R.	18188178	Not issued	20230288308	41	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	03/22/2023
O'RIORDAN, CATHERINE	18304519	Not issued	20240000886	30	Compositions and Methods for Treating Spinal Muscular Atrophy	CENTRAL,DOCKET	04/21/2023

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
R.							
O'RIORDAN, CATHERINE R.	18506853	Not Issued	20240077402	71	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	11/10/2023
O'RIORDAN, CATHERINE R.	18513870	Not Issued	20240086301	41	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	11/20/2023
O'RIORDAN, CATHEIRINE R.	61818448	Not Issued		159	COMPOSITIONS AND METHODS FOR TREATING SPINAL MUSCULAR ATROPHY		06/01/2013
O'RIORDAN, CATHERINE R.	62561843	Not Issued		159	VARIANT RNAI		09/23/2017
O'RIORDAN, CATHERINE R.	08118054	Not Issued		162	METHODS FOR PURIFYING CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	WAX,ROBERT	09/08/1993
O'RIORDAN, CATHEIRINE R.	08785107	5899538		250	METHODS FOR PURIFYING CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATION	CARLSON,KAREN	01/21/1997
O'RIORDAN, CATHERINE R.	09409803	6566428	20020034488	250	TRESYL-MONOMETHOXYPOLYETHYLENE GLYCOL-MODIFIED VIRUSES HAVING VIRAL INFECTIVITY	NAFF,DAVID	09/30/1999
O'RIORDAN, CATHERINE R.	09439680	6287857	0	250	NUCLEIC ACID DELIVERY VEHICLES	SANDALS,WILLIAM	10/25/1999
O'RIORDAN, CATHEIRINE R.	60002987	Not Issued		159	PURIFICATION OF ADENOVIRUS AND AAV		08/30/1995
O'RIORDAN, CATHERINE R.	60107471	Not Issued		159	NUCLEIC ACID DELIVERY VEHICLES		11/06/1998
O'RIORDAN, CATHERINE R.	60110899	Not Issued		159	DRY POWDER COMPLEXES FOR GENE DELIVERY		12/04/1998

Bibliographic Data

Application No: 18/506,853

Foreign Priority claimed: ○ Yes ◉ No

35 USC 119 (a-d) conditions met: ☐ Yes ☑ No ☐ Met After Allowance

Verified and Acknowledged: /RACHEL B GILL/ (Examiner's Signature) | (Initials)

Title: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/10/2023	435	1648	
RULE			

APPLICANTS

Genzyme Corporation, Cambridge, MA, UNITED STATES

INVENTORS

Catherine R. O'RIORDAN,

Brenda Burnham,

CONTINUING DATA

This application is a CON of 18188176 03/22/2023

18188176 is a CON of 16547144 08/21/2019 PAT 11639887

16547144 is a CON of 15544498 07/18/2017 PAT 10429288

15544498 is a 371 of PCT/US2016/013947 01/19/2016

PCT/US2016/013947 has PRO of 62105714 01/20/2015 *

(*) Data provided by applicant is not consistent with PTO record

FOREIGN APPLICATIONS

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#19	•••	›	Search: **#12 and #3** Sort by: **First Author**	2	15:36:01
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#14	•••	›	Search: **#13 and #12** Sort by: **First Author**	89	15:35:07
#13	•••	›	Search: **"AAV" or "adeno-associated virus" or parvovirus or dependovirus** Sort by: **First Author**	31,394	15:34:57
#12	•••	›	Search: **"analytical ultracentrifugation" or "AUC"** Sort by: **First Author**	137,589	15:34:41
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#10	•••	›	Search: **#4 and #3** Sort by: **First Author**	2	15:34:05
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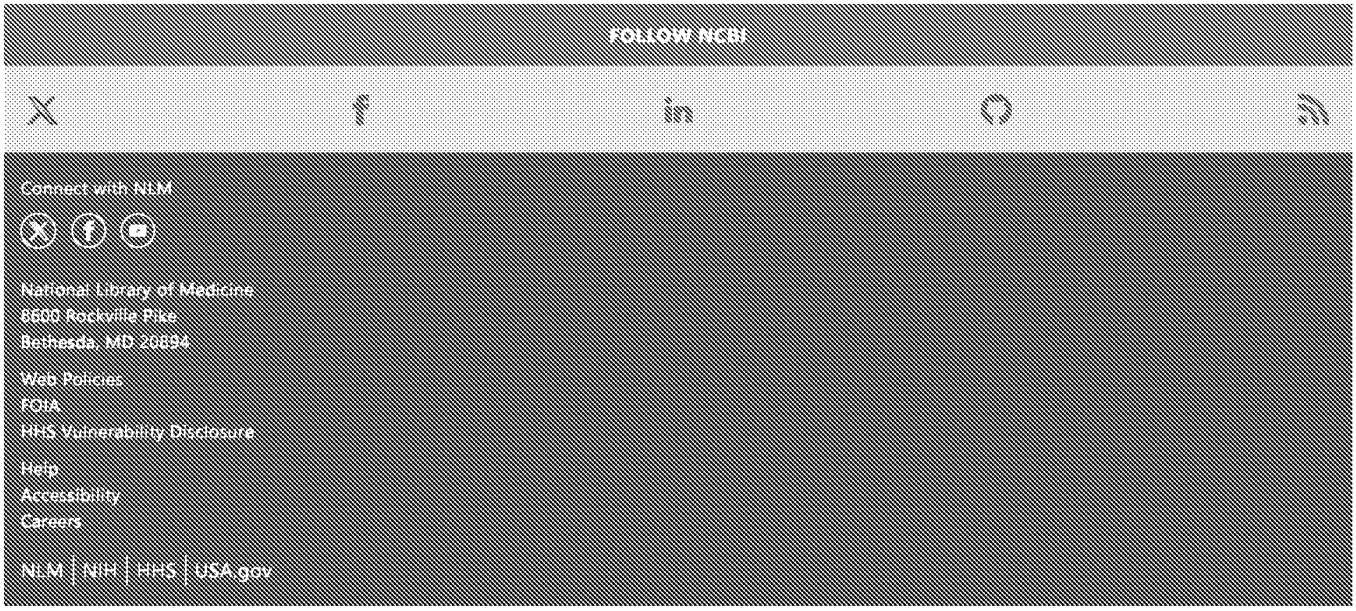
#8	•••	›	Search: **#5 and #2** Sort by: **First Author**	14	15:33:59
#7	•••	›	Search: **#5 and #3** Sort by: **First Author**	4	15:33:57
#6	•••	›	Search: **#5 and #4** Sort by: **First Author**	9,456	15:33:55
#5	•••	›	Search: **sanofi** Sort by: **First Author**	59,261	15:33:46
#4	•••	›	Search: **Genzyme** Sort by: **First Author**	12,068	15:33:33
#3	•••	›	Search: **Burnham, B** Sort by: **First Author**	92	15:33:13
#2	•••	›	Search: **O'RIORDAN, C** Sort by: **First Author**	89	15:32:31
#1	•••	›	Search: **O'RIORDAN, Catherine R.; Burnham, Brenda** Sort by: **First Author**	4	15:32:10

Showing 1 to 20 of 20 entries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Inventor Information

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
BURNHAM, BRENDA	15544498	10428288	20180180625	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	07/19/2017
BURNHAM, BRENDA	16547144	11639987	20200225139	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	08/21/2019
BURNHAM, BRENDA	17222659	Not Issued	20210361778	41	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF	HILL,KEVIN	04/05/2021
BURNHAM, BRENDA	18188178	Not Issued	20230288308	41	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	03/22/2023
BURNHAM, BRENDA	18508853	Not Issued	20240077402	71	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	11/10/2023
BURNHAM, BRENDA	18513970	Not Issued	20240085301	41	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	11/20/2023
BURNHAM, BRENDA	62105714	Not Issued		159	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES		01/20/2015
BURNHAM, BRENDA	63005833	Not Issued		159	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF		04/08/2020
BURNHAM, BRENDA	63084800	Not Issued		159	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF		10/21/2020
BURNHAM, BRENDA	63145258	Not Issued		159	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF		02/03/2021

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	455	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:47 AM
L2	89174	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:47 AM
L3	130	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:47 AM
L4	46	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:48 AM
L5	4797	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:48 AM
L6	6	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:48 AM
L7	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:49 AM
L29	480	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L30	93749	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L31	137	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L32	48	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L33	5018	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L34	8	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2019/05/16 08:32 AM

			JPO; DERWENT; IBM_TDB)				
L35	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
L36	63192	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L37	2374	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L38	68558	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L39	4138	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L40	1	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L41	195	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L42	2314	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
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L44	416	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:35 AM
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L51	176	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L52	55	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L53	69299	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L54	12	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L55	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
L56	705	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2020/11/14 03:15 PM

			IBM_TDB)				
L57	138043	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L58	176	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L59	55	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L60	69299	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L61	12	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L62	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
L63	76966	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L64	3965	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L65	78532	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L66	6213	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L67	3	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L68	257	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L69	2663	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L70	32	L42 and L39	(US-PGPUB; USPAT;	OR	ON	ON	2020/11/14

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				03:15 PM
L71	519	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L72	87036	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L73	87036	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L74	223	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L75	279513	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L76	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2021/06/23 11:05 AM
L77	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L78	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2021/06/23 11:06 AM

			JPO; DERWENT; IBM_TDB)				
L79	185	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L80	58	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L81	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L82	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L83	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L84	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L85	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L86	185	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L87	58	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L88	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L89	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L90	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L91	81986	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L92	4212	"analytical ultracentrifugation".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2021/06/23 11:06 AM

		or "AUC".clm.	JPO; DERWENT; IBM_TDB)				
L93	83582	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L94	6895	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L95	3	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L96	294	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L97	2826	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L98	44	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L99	569	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L100	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L101	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L102	249	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM

		C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)					
L103	296377	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L104	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L105	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L106	185	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L107	58	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L108	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L109	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L110	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L111	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L112	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L113	185	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L114	58	L31 and	(US-PGPUB; USPAT;	OR	ON	ON	2021/06/23

		(sedimentation)	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:06 AM
L115	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L116	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L117	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L118	81986	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L119	4212	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L120	83582	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L121	6895	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L122	3	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L123	294	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L124	2826	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L125	44	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L126	569	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L127	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2021/06/23 11:06 AM

		C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	IBM_TDB)				
L128	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L129	249	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L130	296377	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L131	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2021/06/23 11:06 AM
L132	1271	("lentivirus" OR "retrovirus" OR "HIV" OR "SIV") AND ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 08:15 PM
L133	2	("lentivirus".clm. OR "retrovirus".clm. OR "HIV".clm. OR "SIV".clm.) AND ("analytical ultracentrifugation".clm.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 08:16 PM
L134	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L135	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT;	OR	ON	ON	2022/03/07

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				12:20 PM
L136	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L137	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L138	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L139	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L140	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L141	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L142	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L143	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L144	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L145	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L146	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L147	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L148	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L149	4479	"analytical	(US-PGPUB; USPAT;	OR	ON	ON	2022/03/07

		ultracentrifugation".clm. or "AUC".clm.	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				12:20 PM
L150	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L151	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L152	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L153	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L154	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L155	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L156	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L157	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L158	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L159	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

		C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)					
L160	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L161	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L162	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L163	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L164	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L165	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L166	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L167	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L168	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L169	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L170	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

L171	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L172	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L173	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L174	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L175	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L176	4479	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L177	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L178	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L179	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L180	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L181	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L182	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L183	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L184	96056	(G01N15/042 or G01N1/4077 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2022/03/07 12:20 PM

		G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	JPO; DERWENT; IBM_TDB)				
L185	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L186	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L187	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L188	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/03/07 12:20 PM
L189	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L190	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L191	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L192	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/03/07 12:20 PM

			IBM_TDB)				
L193	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L194	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L195	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L196	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L197	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L198	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L199	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L200	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L201	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L202	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L203	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L204	4479	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L205	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L206	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/03/07 12:20 PM

			IBM_TDB)				
L207	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L208	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L209	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L210	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L211	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L212	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L213	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L214	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L215	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

		C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.					
L216	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L217	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L218	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L219	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L220	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L221	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L222	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L223	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L224	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L225	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L226	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L227	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L228	13	L32 and L33	(US-PGPUB; USPAT;	OR	ON	ON	2022/03/07

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				12:20 PM
L229	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L230	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L231	4479	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L232	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L233	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L234	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L235	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L236	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L237	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L238	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L239	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L240	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

		G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.					
L241	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L242	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L243	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/03/07 12:20 PM
L244	1371	("lentivirus" OR "retrovirus" OR "HIV" OR "SIV") AND ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L245	3	("lentivirus".clm. OR "retrovirus".clm. OR "HIV".clm. OR "SIV".clm.) AND ("analytical ultracentrifugation".clm.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L246	5	("5622856" OR "6001650" OR "20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/03/07 12:24 PM
L247	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L248	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L249	203	L1 and L2	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L250	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L251	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L252	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L253	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L254	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L255	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L256	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L257	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L258	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L259	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L260	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L261	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L262	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L263	98099	"adeno-associated	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

		virus" or "AAV"	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L264	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L265	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L266	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L267	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L268	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L269	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L270	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L271	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L272	324	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

		C12Q1/70).CPC.)					
L273	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L274	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L275	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L276	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L277	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L278	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L279	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L280	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L281	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L282	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L283	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L284	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

L285	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L286	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L287	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L288	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L289	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L290	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L291	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L292	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L293	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L294	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L295	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L296	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L297	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

		G01N2001/4083).cpc.					
L298	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L299	324	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L300	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L301	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 11:54 AM
L302	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L303	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L304	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L305	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L306	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

L307	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L308	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L309	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L310	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L311	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L312	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L313	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L314	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L315	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L316	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L317	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L318	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L319	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L320	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

L321	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L322	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L323	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L324	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L325	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L326	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L327	324	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L328	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L329	826	ultracentrifugation.clm.	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L330	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L331	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L332	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L333	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L334	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L335	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L336	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L337	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L338	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L339	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L340	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L341	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L342	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L343	94076	"analytical	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

		ultracentrifugation" or "AUC"	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L344	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L345	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L346	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L347	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L348	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L349	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L350	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L351	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L352	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L353	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L354	324	L44 AND (	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

		(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L355	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L356	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 11:54 AM
L357	1483	("lentivirus" OR "retrovirus" OR "HIV" OR "SIV") AND ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L358	4	("lentivirus".clm. OR "retrovirus".clm. OR "HIV".clm. OR "SIV".clm.) AND ("analytical ultracentrifugation".clm.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L359	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L360	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L361	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L362	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L363	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

L364	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L365	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L366	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L367	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L368	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L369	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L370	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L371	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L372	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L373	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L374	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L375	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L376	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L377	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

L378	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L379	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L380	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L381	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L382	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L383	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L472	5	("5622856" OR "6001650" OR "20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 04:08 PM
L473	888	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L474	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L475	218	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L476	71	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM

L477	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L478	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L479	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L480	888	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L481	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L482	218	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L483	71	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L484	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L485	15	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L486	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L487	101233	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L488	5164	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L489	105412	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L490	10245	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM

L491	14	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L492	489	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L493	3477	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L494	117	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L495	812	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L496	108501	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L497	108501	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L498	384	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L499	370050	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM

		C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.					
L500	888	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L501	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L502	218	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L503	71	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L504	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L505	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L506	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L507	888	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L508	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L509	218	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L510	71	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L511	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L512	15	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2023/10/21 01:43 PM

			JPO; DERWENT; IBM_TDB)				
L513	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L514	101233	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L515	5164	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L516	105412	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L517	10245	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L518	14	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L519	489	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L520	915	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L521	182500	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L522	223	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L523	72	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L524	112860	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L525	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM

L526	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
L527	915	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L528	182500	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L529	223	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L530	72	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L531	112860	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L532	15	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L533	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
L534	102946	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L535	5287	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L536	107275	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L537	10605	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L538	15	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L539	505	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM

L540	3549	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L541	124	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L542	848	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L543	109132	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L544	109132	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L545	410	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L546	373753	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L547	915	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L548	182500	viral.clm. or virus.clm.	(US-PGPUB; USPAT;	OR	ON	ON	2024/01/03

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				01:44 PM
L549	223	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L550	72	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L551	112860	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L552	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L930	954	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L931	186328	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L932	227	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L933	74	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L934	115479	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L935	16	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L936	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/04/15 03:32 PM
L937	954	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L938	186328	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2024/04/15 03:32 PM

			IBM_TDB)				
L939	227	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L940	74	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L941	115479	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L942	16	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L943	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/04/15 03:32 PM
L944	105279	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L945	5424	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L946	109745	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L947	11070	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L948	15	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L949	539	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L950	3655	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L951	150	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L952	914	("adeno-associated	(US-PGPUB; USPAT;	OR	ON	ON	2024/04/15

		virus" or "AAV") and ("analytical ultracentrifugation")	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				03:32 PM
L953	109806	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L954	109806	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L955	452	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L956	376959	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L957	954	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L958	186328	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L959	227	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L960	74	L3 and (sedimentation)	(US-PGPUB; USPAT;	OR	ON	ON	2024/04/15

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				03:32 PM
L961	115479	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L962	16	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L963	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/04/15 03:32 PM
L964	954	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L965	186328	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L966	227	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L967	74	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L968	115479	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L969	16	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L970	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/04/15 03:32 PM
L971	105279	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L972	5424	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L973	109745	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L974	11070	"adeno-associated	(US-PGPUB; USPAT;	OR	ON	ON	2024/04/15

		virus".clm. or "AAV".clm.	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				03:32 PM
L975	15	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L976	539	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L977	3655	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L978	150	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L979	914	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L980	109806	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L981	109806	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L982	452	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L983	376959	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2024/04/15 03:32 PM

		C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	IBM_TDB)				
L984	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2024/04/15 03:32 PM
L985	954	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L986	186328	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L987	227	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L988	74	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L989	115479	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L990	16	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L991	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/04/15 03:32 PM
L992	954	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L993	186328	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L994	227	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM
L995	74	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/04/15 03:32 PM

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N8	953	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:44 AM
N9	25	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N10	25	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N11	1	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N12	1	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N13	1750	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N14	18	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N15	1	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N16	1	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N17	1	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N18	18	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N19	18	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N20	455	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N21	89174	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N22	130	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N23	2	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N24	1	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N25	1	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N26	1	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N27	1	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N28	1	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10

							08:46 AM
N49	983	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N50	27	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N51	27	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N52	1	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N53	1	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N54	1781	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N55	18	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N56	1	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N57	1	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N58	1	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N59	18	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N60	18	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N61	480	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N62	93749	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N63	137	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N64	2	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N65	1	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N66	1	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N67	1	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N68	1	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N69	1	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N70	2374	"analytical ultracentrifugation".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:34 AM

		or "AUC".clm.					
N71	4138	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:34 AM
N72	2	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N73	1	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N74	1	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N75	1	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N76	1	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N77	1	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N78	16590	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:40 AM
N79	16590	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:43 AM
N80	46560	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N81	10453	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N82	7	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N83	40	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N84	10359	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N85	7	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16

							08:44 AM
N86	1	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N87	1	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N88	6	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N89	37	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N90	1	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N91	1	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N92	1037	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N93	38	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N94	38	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N95	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N96	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N97	1881	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N98	24	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N99	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N100	2	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N101	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N102	24	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N103	24	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N104	556	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N105	103267	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N106	152	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N107	3	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM

N108	3	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N109	3	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N110	3	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N111	3	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N112	3	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N113	1037	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N114	38	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N115	38	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N116	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N117	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N118	1881	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N119	24	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N120	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N121	2	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N122	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N123	24	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N124	24	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N125	556	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N126	103267	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N127	152	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N128	3	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N129	3	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N130	3	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14

							03:15 PM
N131	3	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N132	3	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N133	3	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N134	2758	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N135	5192	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N136	3	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N137	3	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N138	3	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N139	3	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N140	3	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N141	3	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N142	19091	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N143	19091	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N144	53858	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM

N145	12033	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N146	10	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N147	46	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N148	11966	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N149	10	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N150	3	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N151	3	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N152	8	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N153	42	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N154	2	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N155	2	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N156	22364	(G01N15/042 OR C12N7/00 OR G01N1/405 OR G01N1/4077 OR C12N2710/10351 OR C12N2750/14151 OR G01N2001/4083 OR G01N2015/045).cpc.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 11:55 AM
N157	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N158	46	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N159	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N160	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N161	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N162	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N163	26	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N164	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM

N165	2	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N166	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N167	26	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N168	26	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N169	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N170	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N171	172	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N172	3	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N173	3	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N174	3	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N175	3	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N176	3	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N177	3	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N178	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N179	46	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N180	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N181	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N182	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N183	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N184	26	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N185	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N186	2	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N187	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

							11:55 AM
N188	26	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N189	26	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N190	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N191	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N192	172	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N193	3	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N194	3	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N195	3	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N196	3	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N197	3	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N198	3	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N199	3305	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N200	7320	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N201	3	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N202	3	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N203	3	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N204	3	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N205	3	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N206	8	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N207	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM

N208	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N209	65719	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N210	14097	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N211	10	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N212	49	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N213	14028	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N214	10	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N215	3	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N216	3	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N217	8	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N218	45	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N219	2	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N220	2	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N221	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N222	46	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N223	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N224	3	(oriordan with catherine).in. and	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM

		(burnham with brenda).in.					
N225	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N226	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N227	26	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N228	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N229	2	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N230	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N231	26	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N232	26	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N233	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N234	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N235	172	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N236	3	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N237	3	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N238	3	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N239	3	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N240	3	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N241	3	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N242	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N243	46	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N244	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N245	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N246	3	(oriordan).in. and	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

		(burnham).in.					11:55 AM
N247	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N248	26	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N249	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N250	2	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N251	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N252	26	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N253	26	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N254	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N255	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N256	172	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N257	3	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N258	3	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N259	3	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N260	3	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N261	3	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N262	3	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N263	3305	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N264	7320	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N265	3	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N266	3	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N267	3	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N268	3	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N269	3	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM

N270	8	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N271	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N272	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N273	65719	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N274	14097	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N275	10	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N276	49	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N277	14028	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N278	10	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N279	3	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N280	3	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N281	8	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N282	45	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N283	2	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N284	2	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N285	22364	(G01N15/042 OR	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07

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Gill, Rachel

Sent: Thursday, January 4, 2024 8:56 AM
Subject: 18506853 search notes

Google:
"analytical ultracentrifugation" "adenovirus"
"analytical ultracentrifugation" "herpes"
boundary sedimentation ultracentrifugation adenovirus
boundary sedimentation ultracentrifugation HSV
boundary sedimentation ultracentrifugation HIV
boundary sedimentation ultracentrifugation HIV "genzyme"
boundary sedimentation ultracentrifugation HSV "genzyme"
analytical ultracentrifugation adenovirus "genzyme"
Genzyme R&D Center, Framingham, MA. Appropriately engineered recombinant adeno-associated viral. (rAAV) vectors represent a ... ultracentrifugation (AUC) to characterize the homogeneity of preparations of rAAV ... by B Burnham · 2015
analytical ultracentrifugation HSV
analytical ultracentrifugation adenovirus
adenovirus lentivirus analytical ultracentrifugation
HIV lentivirus analytical ultracentrifugation
HIV boundary sedimentation ultracentrifugation
homogeneity HIV preparation ultracentrifugation
boundary sedimentation ultracentrifugation

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“analytical ultracentrifugation” lentivirus empty particle
“analytical ultracentrifugation” empty virus particle
Buoyancy lentivirus particle empty centrifugation

Segura MM, Garnier A, Kamen A. Purification and characterization of retrovirus vector particles by rate zonal ultracentrifugation. J Virol Methods. 2006 Apr;133(1):82-91. doi: 10.1016/j.jviromet.2005.10.030. Epub 2005 Dec 1. PMID: 16325928.
"analytical ultracentrifugation" genome resolution
AAV nucleic acid analytical ultracentrifugation
nucleic acid analytical ultracentrifugation
DNA analytical ultracentrifugation

Google Patents:

Lingappa HIV ultracentrifugation
US7348134B2
Lingappa empty HIV ultracentrifugation
empty HIV ultracentrifugation
empty HIV analytical ultracentrifugation
empty virion analytical ultracentrifugation
("analytical ultracentrifugation" "AAV") before:priority:20150119
US20170362608A1
("analytical ultracentrifugation" "DNA") before:priority:20150119
("analytical ultracentrifugation" "nucleic acid") before:priority:20150119

Notes:
Lingappa JR, Thielen BK. Assembly of immature HIV-1 capsids using a cell-free system. Methods Mol Biol. 2009;485:185-95. doi: 10.1007/978-1-59745-170-3_13. PMID: 19020826; PMCID: PMC2678559.
Lingappa JR, Hill RL, Wong ML, Hegde RS. A multistep, ATP-dependent pathway for assembly of human immunodeficiency virus capsids in a cell-free system. J Cell Biol. 1997 Feb 10;136(3):567-81. doi: 10.1083/jcb.136.3.567. PMID: 9024688; PMCID: PMC2134302.
Le Bec from IPR WO2014125101A1

Application Number	Application/Control No.	Applicant(s)/Patent under Reexamination	
* 18/506,853 *	18/506,853	O'RIORDAN et al.	
	Examiner	Art Unit	
	GILL, RACHEL B	1648	

Document Code - DISQ	Internal Document - DO NOT MAIL

TERMINAL DISCLAIMER	☑ APPROVED	☐ DISAPPROVED
Date Filed: 09 April 2024	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:

2/Tds both approved.
/LAWANA R HIXON/

Technology Center: OPLC

Telephone: (571)272-6074

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PTO/SB/08a (01-22)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Not for submission under 37 CFR 1.99)

Application Number	18506853
Filing Date	2023-11-10
First Named Inventor	Catherine R. O'RIORDAN
Art Unit	1648
Examiner Name	R. Gill
Attorney Docket Number	15979-20103.03

U.S.PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1					

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U.S.PATENT APPLICATION PUBLICATIONS

Examiner Initial*	Cite No	Publication Number	Kind Code[1]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear
	1	20240085301	A1	2024-03-14	O'riordan, Catherine R.	

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Examiner Initial*	Cite No	Foreign Document Number[3]	Country Code[2] i	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages,Columns,Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1							☐

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.	T[5]

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	18506853
	Filing Date	2023-11-10
	First Named Inventor	Catherine R. O'RIORDAN
	Art Unit	1648
	Examiner Name	R. Gill
	Attorney Docket Number	15979-20103.03

	1		☐

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Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	18506853
	Filing Date	2023-11-10
	First Named Inventor	Catherine R. O'RIORDAN
	Art Unit	1648
	Examiner Name	R. Gill
	Attorney Docket Number	15979-20103.03

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☒ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☒ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☐ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Seth Snyder/	Date (YYYY-MM-DD)	2024-04-09
Name/Print	Seth Snyder	Registration Number	60,243

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these record s.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	04/09/2024 05:02:47 PM Z ET	15979-20103.03

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	65024196	AUTHORIZED BY	Seth Snyder
CUSTOMER #	89300	FILING DATE	11/10/2023
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 031952	E202449H04015947	Aurelia Caparas

PRE-AUTHORIZED ACCOUNT	PRE-AUTHORIZED CATEGORY
031952	37 CFR 1.19 (Document supply fees); 37 CFR 1.21 (Miscellaneous fees and charges)

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1814	STATUTORY DISCLAIMER, INCLUDING TERMINAL DISCLAIMER	170.00	2	340.00
			TOTAL AMOUNT:	$340.00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d)

and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/AIA/26 (04-14)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) 15979-20103.03

In re Application of: Catherine R. O'RIORDAN et al.

Application No.: 18/506,853

Filed: November 10, 2023

For: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

The applicant, Genzyme Corporation, owner of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of **prior patent** No. **11,639,887 and 10,429,288** as the term of said **prior patent** is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.

I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 60,243

/Seth Snyder/ — Signature
April 9, 2024 — Date

Seth Snyder — Typed or printed name

Agent of Record — Title
+1 (650) 813-4046 — Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) included.

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This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Doc Code: PTA.IDS
Document Description: Patent Term Adjustment Stmt Under 37 CFR 1.704(d)

PTO/SB/133 (02-23)

PATENT TERM ADJUSTMENT STATEMENT UNDER 37 CFR 1.704(d)		
	Application Number	18506853
	Filing Date	2023-11-10
	First Named Inventor	Catherine R. O'RIORDAN
	Art Unit	1648
	Examiner Name	R. Gill
	Practitioner Docket No.	15979-20103.03

APPLICANT HEREBY STATES THE FOLLOWING (please review 37 CFR 1.704(d) before filing this form):

☑ Each item of information contained in the information disclosure statement was first cited in any communication from a patent office in a counterpart foreign or international application or from the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

AND/OR

☐ Each item of information contained in the information disclosure statement is a communication that was issued by a patent office in a counterpart foreign or international application or by the Office, and this communication was not received by any individual designated in 37 CFR 1.56(c) more than thirty days prior to the filing of the information disclosure statement.

INSTRUCTIONS:

- **This form will not satisfy the requirement of 37 CFR 1.97(e).** The present statement is filed under 37 CFR 1.704(d) and will not substitute for compliance with any of the requirements of 37 CFR 1.97 and 1.98. For an information disclosure statement to comply with 37 CFR 1.97(c) or (d), the information disclosure statement must be accompanied by a statement under 37 CFR 1.97(e) notwithstanding any statement filed under 37 CFR 1.704(d).
- The present form (PTO/SB/133) should be filed concurrently with the information disclosure statement to derive benefit under 37 CFR 1.704(d).

Signature	/Seth Snyder/	Date	April 9, 2024
Typed or Printed Name	Seth Snyder	Practitioner Registration Number	60,243

Note: *Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.*

☐ *Total of _____ forms are submitted.

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION	Docket Number (Optional) 15979-20103.03

In re Application of: Catherine R. O'RIORDAN et al.

Application No.: 18/506,853

Filed: November 10, 2023

For: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

The owner*, Genzyme Corporation, of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending **reference** Application Number **18/188,176**, filed **March 22, 2023** and Application Number **18/513,970**, filed **November 20, 2023**, as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the **reference** application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said **reference** application, "as the term of any patent granted on said **reference** application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending **reference** application," in the event that: any such patent: granted on the pending **reference** application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Check either box 1 or 2 below, if appropriate.

1. ☐ For submissions on behalf of a business/organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the business/organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 60,243

/Seth Snyder/ — Signature

April 9, 2024 — Date

Seth Snyder — Typed or printed name

+1 (650) 813-4046 — Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) is included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner). Form PTO/SB/96 may be used for making this statement. See MPEP § 324.

This collection of information is required by 37 CFR 1.321. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Docket No.: 15979-20103.03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Catherine R. O'RIORDAN et al.

Application No.: 18/506,853

Filed: November 10, 2023

For: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Confirmation No.: 3457

Art Unit: 1648

Examiner: R. Gill

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. 1.111

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

INTRODUCTORY COMMENTS

This is in response to the non-final Office Action dated January 9, 2024, for which a response is due on April 9, 2024. Accordingly, this response is timely filed. Reconsideration and allowance of the pending claims, as amended, in light of the remarks presented herein are respectfully requested.

Amendments to the Abstract begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks/Arguments begin on page 8 of this paper.

AMENDMENTS TO THE ABSTRACT

Please substitute the following paragraph(s) for the abstract now appearing in the currently filed specification:

Provided herein are methods to characterize preparations of recombinant viral particles using analytical ultracentrifugation. Recombinant viral particles include recombinant adeno-associated viral particles, recombinant adenoviral particles, recombinant lentiviral particles and recombinant herpes simplex virus particles. Variant species of recombinant viral particles including empty capsids and recombinant viral particles with variant genomes ([[*e.g.*,]] truncated genomes, aggregates, recombinants) can be identified and quantitated. The methods can be used to characterize preparations of recombinant viral particles regardless of the sequence of the recombinant viral genome or the serotype of the recombinant viral capsid.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

In the claims:

Claims 1-95 (Canceled).

Claim 96 (Currently amended): A method of determining the size of one or more fragmented genomes in a ~~an AAV vector~~ preparation of viral particles comprising recombinant adeno-associated viral (rAAV) vectors encapsidated into viral capsids, said method comprising:

(i) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate one or more sedimenting boundaries, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm;

(ii) measuring the rate of movement or migration of the one or more sedimenting boundaries~~boundary~~, wherein movement or migration of the viral particles ~~fragmented genomes~~ results in distinct ~~different~~ sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable viral particle ~~vector species~~, and wherein one or more of the viral particles comprise a fragmented genome, ~~thereby~~ and determining the sedimentation coefficients of the viral particles comprising one or more of the fragmented genomes in the ~~AAV vector~~ preparation; and

(iii) determining the size of the one or more fragmented genomes as a function of the sedimentation coefficients of the viral particles comprising the one or more fragmented genomes.

Claim 97 (Currently amended): The method of claim 96, wherein step (iii) comprises comparing the sedimentation coefficients of the one or more viral particles comprising the fragmented genomes with a standard curve generated [[by]]from sedimentation coefficients of viral particles comprising recombinant AAV genomes of known nucleotide sizes, thereby determining the size of the one or more fragmented genomes.

Claim 98 (Previously presented): The method of claim 96, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm.

Claim 99 (Previously presented): The method of claim 96, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm.

Claim 100 (Previously presented): The method claim 99, wherein the boundary sedimentation is performed at a temperature of from about 4°C to about 20°C.

Claim 101 (Previously presented): The method of claim 100, wherein the boundary sedimentation is performed at a temperature of about 4°C.

Claim 102 (Previously presented): The method of claim 96, wherein the fragmented genomes are sub-genomic DNA molecules.

Claim 103 (Previously presented): The method of claim 96, wherein the centrifugation is run from about 0.5 hours to about 2 hours.

Claim 104 (Previously presented): The method of claim 103, wherein the centrifugation is run from about 1 hours to about 2 hours.

Claim 105 (Previously presented): The method of claim 103, wherein the centrifugation is run for about 1 hour.

Claim 106 (Previously presented): The method of claim 103, wherein the centrifugation is run for about 1.2 hours.

Claim 107 (Previously presented): The method of claim 96, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is greater than $5x10^{11}$ vg/mL.

Claim 108 (Previously presented): The method of claim 96, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is from about $1x10^{11}$ vg/mL to about $1x10^{13}$ vg/mL.

Claim 109 (Currently amended): The method of claim 96, further comprising quantifying ~~one or more of~~ the viral particles comprising the one or more fragmented genomes in the ~~AAV vector~~ preparation.

Claim 110 (Currently amended): The method of claim 96, comprising determining the size of all of the fragmented genomes in the ~~rAAV~~ preparation.

Claim 111 (Currently amended): The method of claim 110, further comprising quantifying all of the fragmented genomes in the ~~AAV vector~~ preparation.

Claim 112 (Currently amended): The method of claim 96, wherein the ~~AAV vector~~ preparation comprises AAV9 capsid proteins.

Claim 113 (Currently amended): The method of claim 112, wherein the ~~AAV vector~~ preparation further comprises at least one AAV2 ITR.

Claim 114 (Currently amended): The method of claim 111, wherein the ~~AAV vector~~ preparation comprises self-complementary AAV viral genomes.

Claim 115 (Currently amended): A method of determining the ~~purity of~~ molar concentrations of each species of individual viral particles in a heterogeneous mixture of viral particles comprising recombinant adeno-associated viral (rAAV) vectors encapsidated into viral capsids, ~~an AAV vector preparation,~~ said method comprising:

(i) subjecting the ~~preparation~~ heterogeneous mixture of viral particles to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate sedimenting

boundaries, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm;

(ii) measuring the rate of movement or migration of the sedimenting ~~boundary~~boundaries, wherein movement or migration of each species of the individual viral particles in the heterogeneous mixture of viral particles ~~AAV vector preparation~~ results in distinct~~different~~ sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable ~~vector~~ species of viral particle, and wherein the heterogeneous mixture of viral particles ~~vector preparation~~ comprises full genomes, fragmented genomes and empty capsids without genome;

(iii) determining the sedimentation coefficients of each species of the individual viral particles in the heterogeneous mixture of viral particles ~~AAV vector preparation~~; and

(iv) quantifying the molar concentration ~~amount~~ of each species of the individual viral particles ~~resolvable vector species~~ in the heterogeneous mixture of viral particles ~~AAV vector preparation,~~

~~thereby determining the purity of the~~ AAV vector preparation~~~~.

Claim 116 (Currently amended): The method of claim 115, further comprising determining the size of each species of the individual viral particles after step (iii) and prior to step (iv) by comparing the sedimentation coefficients of each species of the individual viral particles with a standard curve generated [[by]]from sedimentation coefficients of viral particles comprising recombinant AAV genomes of known nucleotide sizes.

Claim 117 (Previously presented): The method of claim 115, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm.

Claim 118 (Previously presented): The method of claim 115, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm.

Claim 119 (Previously presented): The method of claim 115, wherein the boundary sedimentation is performed at a temperature of about 4°C.

Claim 120 (Previously presented): The method of claim 115, wherein the centrifugation is run from about 0.5 hours to about 2 hours.

Claim 121 (Currently amended): The method of claim 115, wherein the total concentration of viral particles in the ~~AAV vector preparation~~heterogeneous mixture prior to step (i) is greater than $5x10^{11}$ vg/mL.

Claim 122 (Currently amended): The method of claim 115, wherein the total concentration of viral particles in the ~~AAV vector preparation~~heterogeneous mixture prior to step (i) is from about $1x10^{11}$ vg/mL to about $1x10^{13}$ vg/mL.

Claim 123 (Currently amended): The method of claim 115, wherein the viral particles comprise ~~AAV vector preparation comprises~~ AAV9 capsid proteins.

Claim 124 (Currently amended): The method of claim 123, wherein the viral particles comprise ~~AAV vector preparation further comprises~~ at least one AAV2 ITR.

Claim 125 (Cancelled)

Claim 126: (New) The method of claim 115, further comprising determining the relative percentage of each species of the individual viral particles in the heterogeneous mixture of viral particles.

REMARKS

Claims 96-125 were pending in the present application. By virtue of this response, claim 125 has been cancelled, claims 96, 97, 109-116, and 121-124 have been amended, and new claim 126 has been added. Accordingly, following entry of this amendment, claims 96-124 and 126 will be pending and under consideration.

Support for the amended claims and new claim 126 can be found, for instance, at paragraphs [006], [007], [0014], [0047], [0057]-[0059], [0087], [0100], [0102], [0110]-[0111], [0119, [0147], and in Examples 1 and 5 of the application as filed. No new matter has been added by virtue of the claim amendments.

Amendments to certain claims or cancellation of claims is not to be construed as a dedication to the public of any of the subject matter of the claims as previously presented.

Abstract

The abstract of the disclosure stands objected to because of the use of legal phraseology (e.g. "e.g."). To comply with MPEP § 608.01 (b), the abstract has been amended to remove reference to “e.g.”. As such, withdrawal of this objection is respectfully requested.

Rejections under 35 U.S.C. § 112(b); Second Paragraph

Claims 115-125 stand rejected under 35 U.S.C. 112(b), second paragraph, as allegedly being incomplete for omitting essential structural cooperative relationships of elements, such omission amounting to a gap between the necessary structural connections. The Examiner asserts the omitted structural cooperative relationships are: what is considered a "purified" AAV vector preparation. The Examiner alleges it is unclear if the purity is meant to be compared to cell culture contaminants (e.g. presence/absence of cell lysate, cell growth media, nutrients, salts, etc.), presence of helper virus that is necessary to generate AAV in cell culture (e.g. presence of Adenovirus (AdV) in the sample, or if the "purified" AAV vector

preparation is meant to be one that is substantially free of empty AAV capsids or immature AAV capsids.

Applicant respectfully disagrees that the term "purity" is unclear. Nonetheless, to expedite prosecution, Applicant has deleted the term "purity" from claim 115. Claim 115 now recites a method of determining the relative amounts of individual species of viral particles in a heterogeneous mixture of viral particles comprising recombinant adeno-associated viral vectors (rAAVs) encapsidated into viral capsids.

The Examiner further asserts that applicant defines "vector" as a recombinant plasmid or virus that comprises a nucleic acid to be delivered into a host cell, either in vitro or in vivo ([0054]), the interpretation of an adeno-associated virus (AAV) "vector" may be the entire AAV virion (e.g., mature virus with genome) or may be the nucleic acid generated by the virus separate from the virion (e.g., the single-stranded linear DNA genome of approximately 4.8 kb or any intermediates thereof.) The Examiner alleges that as this definition provides further ambiguity (e.g., the "vector" desired may only be the genome separated from the virion or the "vector" desired may be mature AAV virions which encapsulate full-sized genomes), and that it makes the metes and bounds of what is being claimed within the method unclear.

Applicant disagrees with the Examiner's assessment that the term "vector," as used in the pending claims, is unclear. Nonetheless, to advance prosecution, claim 115 has been recast to specify that the preparation is composed of viral particles comprising recombinant adeno-associated viral (rAAV) vectors encapsidated into viral capsids. Paragraph [0058] of the specification as provided a clear definition of a recombinant AAV (rAAV). Specifically, an rAAV vector is defined as comprising a heterologous nucleic acid sequence flanked by at least one inverted terminal repeat (ITR). Moreover , paragraph [0058] of the specification further specifies that an rAAV vector can be packaged into an AAV virus capsid to generate an rAAV particle. Accordingly, the term rAAV vector, as used in the specification, does not refer to the full rAAV particle (virion) but rather to the nucleic acid component of the rAAV particle. Therefore, claims 96 and 115, as amended, and their various dependencies, are not ambiguous.

Accordingly, Applicant requests that the rejections under 35 U.S.C. 112(b), second paragraph, be reconsidered and withdrawn.

Rejections under 35 U.S.C. § 112(a), First Paragraph

Claims 96-125 stand rejected under 35 U.S.C. 112(a), first paragraph. The Examiner asserts that the specification, while being enabling for methods using analytical ultracentrifugation (AUC) to determine the difference between adeno-associated virus (AAV) genome-containing virions/capsids by size (e.g., dimeric genomes, monomeric genomes, partial genome fragments, empty capsids) and for methods of determining the percentage of other immature virions or other contaminants in a heterogeneous AAV virion preparation, does not reasonably provide enablement for utilizing AUC in the methods as claimed to resolve the difference in AAV capsid-free genomes or other AAV nucleic acids. The Examiner further asserts that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Without agreeing with the propriety of the rejection and merely to expedite prosecution, Applicant has amended independent claims 96 and 115 to specify viral particles comprising recombinant adeno-associated viral vectors (rAAVs) encapsidated into viral capsids. Amendments have also been made to dependent claims, as necessary. Therefore, the claims do not relate to utilizing AUC to resolve differences in AAV capsid-free genomes.

Accordingly, Applicant requests that the rejections under 35 U.S.C. 112(a), first paragraph, be reconsidered and withdrawn.

Prior Art Rejections

The Examiner has rejected all of the pending claims under 35 U.S.C. § 102, and/or 35 U.S.C. § 103 and/or for double patenting. Prior to addressing these rejections, Applicant provides the following comments regarding the claimed invention.

The inventors of the present application discovered that analytical centrifugation (AUC) can be used to fully resolve AAV viral particles in a population of AAV viral particles and quantify the amount of each of the individual viral particles based on sedimentation coefficients. Importantly, as set forth in the application as filed, preparations of AAV viral particles include particles with fragmented genomes, in addition to full genomes and empty species. *See* paragraph [0042] and FIG. 12A of the application as filed. The presence of capsids with fragmented genomes may result in aberrant or absent expression of transgenes of interest. Indeed, heterogeneity associated with AAV preparations have been reported to result from packaging of fragmented genomes or AAV-encapsidated DNA impurities. (Kapranov et al. (2012) Hum. Gene Ther., 23:46-55). Therefore, as of the filing date of the present application, the characterizing and quantifying of AAV particles with fragmented genomes was an important but unmet goal, and hence was a focus of the disclosure as filed.

Methods described in the present application for determining the molar concentrations of individual viral particles in an AAV preparation require knowing the extinction coefficients of the particles. *See* paragraphs [0100], [0101], [0147], [0149], [0153], [0154] of the application as filed. The extinction coefficients are a function of molecular weight of the genome in a viral particle in accordance with Beer's Law. *See* paragraphs [0089], [0100], [0147], [0154] of the application as filed. Therefore, if the molecular weight of the genome in a viral particle is known, the molar concentration of the viral particle in the preparation can be determined. Thus, determining the molar concentration of a viral particle with a full genome with a known molecular weight is possible by applying Beer's Law. However, molar concentrations of a viral particle can only be determined if the molecular weights of the genomes within the viral particle are known. The molecular weights of fragmented genomes produced by standard methods (e.g., triple transfection) are unknown. *See* paragraph [0101] of the application

as filed. Therefore, extinction coefficients cannot readily be determined for these fragmented genomes and hence, determining the molar concentrations of viral particles having fragmented genomes was not possible prior to the present invention. *See* paragraphs [001]-[0102] of application as filed.

As described in the present application, AUC is used to separate all of the viral particles, including viral particles with fragmented genomes. *See* paragraph [0042] and FIG. 12A of the application as filed. The sedimentation coefficients of the fragmented genomes are used to determine the molecular weight of the fragmented genomes. *See* paragraphs [0047] and [0101] of the application as filed. The inventors of the present application discovered that sedimentation coefficients can be used to accurately calculate the molecular weight of the genomes in every viral particle of the preparation, including the molecular weights of the fragmented genomes. The linear relationship between sedimentation coefficients and molecular weights of genomes was not appreciated in the art prior to the present invention, and it was not disclosed or suggested in the art applied in the Office Action, as discussed below. Therefore, the present application provides for the first time a means of calculating the size of each of the individual genomes in the population of viral particles. *See* paragraphs [0007], [0038], and [0167] of the application as filed. Moreover, based on the aforementioned discovery, the molar concentrations, and hence the molar concentrations and relative amounts of species of the individual viral particles in a heterogeneous population (see claim 115 and new claim 126) can be determined by application of Beer's Law. Again, nothing in the prior art disclosed or suggested the ability to determine the molar concentrations of genomes of unknown molecular weights (e.g., fragmented genomes) in a population of viral particles.

Rejections under 35 U.S.C. § 102

Claims 96, 98-99, 108, 112-115, 117-118, and 122-125 are rejected under 35 U.S.C. 102(a)(1) as allegedly being anticipated by Le Bec et. al. (WO2014125101A1, Priority 02/15/2013; CITED ART OF RECORD; hereafter "Le Bec". (NB: the English translation of Le Bec utilized for this rejection was provided in parent application 16/547,144; the original French

WIPO publication and English translation utilized for this rejection has been included for reference in this Office action.). Applicant respectfully disagrees.

Present independent claim 96 relates to a method of determining the size of one or more fragmented genomes in a preparation of viral particles comprising recombinant adeno-associated viral (rAAV) vectors encapsidated into viral capsids, said method comprising: (i) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate one or more sedimenting boundaries, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm; (ii) measuring the rate of movement or migration of the one or more sedimenting boundaries, wherein movement or migration of the viral particles results in distinct sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable viral particle, and wherein one or more of the viral particles comprise a fragmented genome, thereby and determining the sedimentation coefficients of the viral particles comprising one or more of the fragmented genomes in the preparation; and(iii) determining the size of the one or more fragmented genomes as a function of the sedimentation coefficients of the viral particles comprising the one or more fragmented genomes.

Present independent claim 115 relates to a method of determining molar concentrations of each species of individual viral particles in a heterogeneous mixture of viral particles comprising recombinant adeno-associated viral (rAAV) vectors encapsidated into viral capsids, said method comprising: (i) subjecting the heterogeneous mixture of viral particles to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate sedimenting boundaries, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm; (ii) measuring the rate of movement or migration of the sedimenting boundaries, wherein movement or migration of each species of individual viral particles in the heterogeneous mixture of viral particles results in distinct sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable species of viral particle, and wherein the heterogeneous mixture of viral particles comprises full genomes, fragmented genomes and empty capsids without genome; (iii) determining the sedimentation coefficients of each species of the individual viral particles in the heterogeneous mixture of viral particles; and (iv) quantifying the

molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles.

In support of the rejection, on page 12 of the Office Action, the Examiner alleges that, "Le Bec teaches the AUC allowed for analysis of empty and mature AAV particles, wherein the sedimentation coefficient of the various AAV viral particles (empty, mature, aggregate) and other present populations (subparticles, contaminating proteins, aggregate) …" In that regard, Le Bec only teaches analyzing capsids that are empty (i.e., contain no genome) and capsids that contain full genomes. Le Bec does not teach determining the size of one or more fragmented genomes comprised within the capsids. In fact, Figure 2 and Figure 3 of Le Bec and the accompanying text on page 18 only show that Le Bec analyzed empty (65S), full single stranded (80S) and full double stranded (110S) genomes.

On page 13 of the Office Action, the Examiner refers to Le Bec mentioning "quantifying" the different species present in the AAV9 viral preparation. However, Le Bec does not mention or suggest quantifying fragmented genomes. Moreover, with respect to pending claim 96, Le Bec is silent in regards to "determining the size of the one or more fragmented genomes as a function of sedimentation coefficients of the one or more fragmented genomes." The mere noting of "…the density of mature AAV particles (11 OS; 1.45 g/cm"3 for a genome size of 5 4.8kB) and empty AAV particles (60S, 1.32 g/cm"3), and that the difference is sufficiently significant to distinguish them through AUC in a heterogeneous preparation of AAV virions (Results, item 2, p. 4)…," as quoted on page 13 of the Office Action, does not anticipate the claimed subject matter of "…(iii) determining the size of the one or more fragmented genomes as a function of sedimentation coefficients of the one or more fragmented genomes." Le Bec only shows recognition of empty capsids versus capsids containing full genomes—this is especially evident in FIGS. 2 and 3 of Le Bec, where all sedimentation peaks are classified as either empty or full. As set forth above, determining the size of fragmented genomes based on sedimentation coefficients was discovered by the inventors. This aspect was not part of the disclosure of Le Bec, nor was there any indication in Le Bec as to how this can be achieved.

In contrast, the determination of the size of one or more fragmented genomes as a function of sedimentation coefficients of one or more fragmented genomes is clearly demonstrated as depicted in FIGS. 6B, 6C and 8 and as described throughout the present application. For example, sedimentation peaks 63S, 75S, and 92S of FIG. 6B represent an empty capsid, a capsid containing a fragmented genome, and a capsid containing a full genome, respectively. Likewise in FIG. 6C peaks 64S, 76S, 84S and 94S, show an empty capsid, capsids containing fragmented genome species (both 76S and 84S), and capsids having a full genome, respectively.

Further, with respect to claim 115, Le Bec does not teach determining the relative amounts of individual species amongst empty capsid, capsids containing fragmented genome species, and capsids having a full genome. Specifically, Le Bec does not teach measuring the relative amounts of individual species amongst various fragmented genome species such as those represented by peaks 76S and 84S of FIG. 6C. Moreover, FIG. 8 of the present application clearly shows experimental results that correlate the S coefficient of a peak to the packaged nucleotides (i.e., the size of each genome species and each fragmented genome species).

Therefore, Applicant contends that the Le Bec disclosure does not anticipate the claims of the present application under 35 U.S.C. 102. In view of the foregoing, Applicant respectfully requests withdrawal of this rejection of the claims.

Rejections under 35 U.S.C. § 103

Claims 97, 100-107, 109-111, 116, and 119-121 stand rejected under 35 U.S.C. 103 as allegedly being unpatentable over Le Bec as applied to claims 96, 98-99, 108, 112-115, 117-118, and 122-125 above, and further in view of McLaughlin et. al. (US20170362608A1, Pub. 12/21/2017, Priority 12/12/2014; hereafter "McLaughlin"), Berkowitz et. al. (Berkowitz SA, et. al. *Anal Biochem*. 2007 Mar 1;362(1):16-37. Epub 2006 Dec 20.; CITED ART OF RECORD; hereafter "Berkowitz"); and Cole et. al. (Cole JL, et. al *Methods Cell Biol*. 2008;84:143-79.; CITED ART OF RECORD; hereafter "Cole".). Applicant respectfully disagrees.

The Examiner alleges Le Bec teaches the methods of AUC in order to separate AAV virion species from one another and from impurities, and the Examiner states Le Bec fails to detail specifics about the method, such as time and temperature of centrifugation. The Examiner alleges Le Bec teaches the AAV virions should be concentrated to greater than about 1x10^11, and Examiner states Le Bec fails to specifically state that it must be greater than 5x10^11 vg/ml. The Examiner alleges Le Bec teaches that the sedimentation velocities correspond to the viral particle and genome comprised within, and states Le Bec fails to teach generation of a standard curve to determine the size of AAV particles with genomes in unknown AAV samples. Nonetheless, the Examiner alleges all of these steps would be routine optimization steps to one of skill in the art, as evidenced by the teachings of McLaughlin, Cole, and Berkowitz. Applicant respectfully disagrees.

The Examiner applies Le Bec as set forth in the rejection under 35 U.S.C. 102 and further alleges that Le Bec teaches that sedimentation velocities correspond to viral particles and genomes comprised within. For reasons discussed with respect to the rejection under 35 U.S.C. 102, Applicant disagrees with the Examiner's characterization of the teachings of Le Bec. Moreover, the Examiner has not established why it would be routine optimization to determine the size of the one or more fragmented genomes as a function of sedimentation coefficients of the one or more fragmented genomes, as recited in independent claim 96. Furthermore, the Examiner has not established why it would be routine optimization to quantify the amount of each of the resolvable vector species in the AAV vector preparation, as recited in independent claim 115. In fact, in alleging routine optimization, the Examiner is relying on improper hindsight based on Applicant's disclosure.

Initially, one of skill in the art would have no motivation to modify the teachings of Le Bec so as to arrive at the present claims. Le Bec discloses using absorbance at 276 nm to analyze its rAAV preparation by AUC ("sedimentation was followed by absorbance at the wavelength of 276 nm.", Le Bec, page 15, lines 23-28). The present application details the challenge the use of extinction coefficients had posed for determining the size and amounts of fragmented species at similar wavelengths and identified an innovative way to overcome this challenge. Le Bec, however, is silent regarding extinction coefficients of fragmented species at

276 nm. Thus, one of skill in the art would not have motivation to modify Le Bec, nor would one of skill in the art have sufficient guidance to modify Le Bec so as to arrive at the methods of the present claims with any reasonable expectation of success. Moreover, as discussed below, McLaughlin, Cole, and Berkowitz fail to cure the deficiencies of Le Bec.

The Examiner alleges that McLaughlin teaches that AAV viral particles can comprise both single-stranded and self-complementary genomes, and the analysis of these particles via analytical ultracentrifugation (AUC) can resolve the difference between these AAV genomic particle subtypes. The Examiner alleges that McLaughlin teaches intermediate replicative species are also packaged into viral particles, and AAV particles produced in vitro may comprise scAAV, scAAV intermediate species, ssAAV monomers, and ssAAV repaired from scAAV, and the Examiner alleges McLaughlin teaches these scAAV species may be generated by deleting portions of the AAV genome known as the Rep nicking sequence, which results in a truncated genome. However, none of these features, when combined with the disclosure of Le Bec, teach all of the limitations of independent claims 96 and 115. Specifically, the combination of Le Bec and McLaughlin does not disclose "determining the size of the one or more fragmented genomes as a function of the sedimentation coefficients of the viral particles comprising the one or more fragmented genomes" or "quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles." For at least this reason, the combination of Le Bec and McLaughlin cannot render the pending claims obvious.

The Examiner alleges that Cole teaches that Analytical ultracentrifugation (AUC) is a versatile and powerful method for the quantitative analysis of macromolecules in solution (abstract) and can be utilized to determine the size and shape of macromolecules in solution, such as viruses. The Examiner alleges that Cole teaches subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S) and integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral particle. The Examiner further provides a detailed summary of the methodology described

in Cole to optimize the AUC procedure. To the extent the Examiner's characterization of Cole is correct, the reference still fails to cure the deficiencies of Le Bec and McLaughlin. Cole does not disclose "determining the size of the one or more fragmented genomes as a function of the sedimentation coefficients of the viral particles comprising the one or more fragmented genomes" or "quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles."

The Examiner alleges Berkowitz teaches the use of a type of AUC in a solution containing a high concentration of CsCL in order to obtain a high resolution of different viral particle subtypes. The Examiner states Berkowitz is teaching the AUC methods with respect to adenovirus particles, said methods would be applicable to the analogous art of Le Bec and would be obvious to a skilled artisan to use in order to obtain greater resolution of viral subspecies. The Examiner alleges Berkowitz teaches the revised AUC method can be completed in a shorter time. The Examiner alleges Berkowitz teaches the AdV compositions may comprise empty capsids, incomplete particles, aberrant particles, sub 10 particles, and/or aggregates. The Examiner alleges Berkowitz teaches the AUC was run at 15,000 rpm at a temperature of 20C (instant claims 100, 119).

As an initial matter, one of skill in the art would not have predicted with a reasonable expectation of success that the methods described in Berkowitz for adenovirus could be applied to rAAV. Adenoviruses are far larger than AAVs in terms of both genome size and actual particle size. Specifically, adenovirus particles are about 150 MDa in mass, while AAV particles are only about 3.9 MDa. Importantly, adenoviral genomes are made up of double-stranded DNA and are between 26 and 48 kb in length, while AAV genomes are made up of single-stranded DNA and are typically 4.7 kb in length. Therefore, the absolute difference in molecular weight between a particle with an intact genome and a particle without an intact genome would be far greater for an adenovirus than for an AAV.

A person of skill in the art would expect adenoviruses and AAVs to behave differently in AUC based on the difference in DNA content and the consequent difference in molecular weight of the viral particles. As noted in the specification, "the sedimentation

coefficient depends on the molecular weight (larger particles sediment faster) and also on molecular shape" (see paragraph [0076]). Due to the smaller difference in molecular weight between an AAV particle with an intact genome and a particle without an intact genome, a person of skill in the art would not assume that a method with a step of comparing the amount of recombinant AAV particles having an S values that do not correspond to recombinant AAV particles comprising intact viral genomes to the amount of recombinant AAV particles having an S value that corresponds to recombinant viral particles comprising intact AAV genomes or to the total amount of recombinant AAV particles in the preparation would work with a reasonable expectation of success. Therefore, based on Berkowitz, a person of skill in the art would not have had a reasonable expectation that using AUC as claimed to measure the relative amount of viral particles comprising variant recombinant viral genomes or empty viral particles in a preparation of recombinant adeno-associated virus (AAV) particles would work.

Moreover, Berkowitz does not teach "determining the size of the one or more fragmented genomes as a function of the sedimentation coefficients of the viral particles comprising the one or more fragmented genomes" or "quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles," as recited in pending independent claims 96 and 115, respectively. Even if Berkowitz teaches an improved method of resolving vector species in a heterogeneous preparation, this observation does not translate to determining sizes of any genome, let alone an rAAV genome, as a function of sedimentation coefficient nor does it suggest determining the molar concentrations of fragmented genomes within a heterogeneous rAAV particle preparation. Accordingly. Berkowitz does not cure the deficiencies of Le Bec, McLaughlin and Cole. The references, taken alone or together, fail to teach or suggest all of the limitations recited in the independent claims. The Examiner's reliance on routine optimization does not resolve this deficiency. Once again, the Examiner is applying improper hindsight based on the presently disclosed invention.

Further, Le Bec does not teach measuring a resolvable species amongst various fragmented genome species such as those represented by peaks 76S and 84S of FIG. 6C. Le Bec does not teach determining genome size of unknown fragmented genome species via a standardization curve comprising S values for rAAV viral particles with known genome size. As

discussed above, the inventors of the present application discovered that sedimentation coefficients can be used to accurately calculate the molecular weight of the genomes in every viral particle of the preparation, including the molecular weights of the fragmented genomes. The linear relationship between sedimentation coefficients and molecular weights of genomes was not disclosed in Le Bec. For at least this additional reason, Le Bec and the cited prior art does not teach or suggest the present claims.

For at least these reasons, Applicant requests that the rejections under 35 U.S.C. 103 be reconsidered and withdrawn.

Double Patenting

Claims 96-125 stand rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 1-22 of U.S. Patent No. 11,639,887 in view of Le Bec (supra).

Without agreeing with the propriety of the rejection and merely to expedite prosecution, Applicant is filing a terminal disclaimer over U.S. Patent No. 11,639,887 concomitant with this response. It is noted that the filing of a terminal disclaimer to obviate a rejection based on non-statutory double patenting is not an admission of the propriety of the rejection. *See, e.g., Quad Environmental Technologies Corp. v. Union Sanitary Distric*t, 946 F.2d 870, 20 USPQ2d 1392 (Fed. Cir. 1991) ("filing of a terminal disclaimer simply serves the statutory function of removing the rejection of double patenting, and raises neither a presumption nor estoppel on the merits of the rejection."). Applicant thereby requests that the double patenting rejection be withdrawn.

Claims 96-125 stand rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 1-3, 5-9, and 15-34 of U.S. Patent No. 10,429,288.

Without agreeing with the propriety of the rejection and merely to expedite prosecution, Applicant is filing a terminal disclaimer over U.S. Patent No. 10,429,288 concomitant with this response. It is noted that the filing of a terminal disclaimer to obviate a

rejection based on non-statutory double patenting is not an admission of the propriety of the rejection. *See, e.g., Quad Environmental Technologies Corp. v. Union Sanitary Distric*t, 946 F.2d 870, 20 USPQ2d 1392 (Fed. Cir. 1991) ("filing of a terminal disclaimer simply serves the statutory function of removing the rejection of double patenting, and raises neither a presumption nor estoppel on the merits of the rejection."). Applicant thereby requests that the double patenting rejection be withdrawn.

Claims 96-125 stand provisionally rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 1-3, 5, 10, 12, 18, 30-37, 42-44, 48, 58, and 61 of copending Application No. 18/188,176 in view of Le Bec (*supra*) and Berkowitz (*supra*).

Without agreeing with the propriety of the rejection and merely to expedite prosecution, Applicant is filing a terminal disclaimer over copending Application No. 18/188,176 concomitant with this response. It is noted that the filing of a terminal disclaimer to obviate a rejection based on non-statutory double patenting is not an admission of the propriety of the rejection. *See, e.g., Quad Environmental Technologies Corp. v. Union Sanitary Distric*t, 946 F.2d 870, 20 USPQ2d 1392 (Fed. Cir. 1991) ("filing of a terminal disclaimer simply serves the statutory function of removing the rejection of double patenting, and raises neither a presumption nor estoppel on the merits of the rejection."). Applicant thereby requests that the double patenting rejection be withdrawn.

Claims 96-125 stand provisionally rejected on the ground of nonstatutory double patenting as allegedly being unpatentable over claims 96-125 of copending Application No. 18/513,970 in view of Le Bec (*supra*) and Berkowitz (*supra*).

Without agreeing with the propriety of the rejection and merely to expedite prosecution, Applicant is filing a terminal disclaimer over copending Application No. 18/513,970 concomitant with this response. It is noted that the filing of a terminal disclaimer to obviate a rejection based on non-statutory double patenting is not an admission of the propriety of the rejection. *See, e.g., Quad Environmental Technologies Corp. v. Union Sanitary Distric*t, 946 F.2d 870, 20 USPQ2d 1392 (Fed. Cir. 1991) ("filing of a terminal disclaimer simply serves

the statutory function of removing the rejection of double patenting, and raises neither a presumption nor estoppel on the merits of the rejection."). Applicant thereby requests that the double patenting rejection be withdrawn.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark Office determines that an extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. **03-1952** referencing docket no. **15979-20103.03**. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: April 9, 2024

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APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	**04/09/2024 05:02:47 PM Z ET**	**15979-20103.03**

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	65024196	FILING DATE	11/10/2023
CUSTOMER #	89300	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Seth Snyder

Documents

TOTAL DOCUMENTS: 8

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
SB133_SIDS.pdf	2	Patent Term Adjustment Statement under 37 CFR 1.704(d) filed with an IDS	230 KB
TD_over_11_639_887_and_10_429_288.pdf	1	Terminal Disclaimer Filed	208 KB
SB08_SIDS.pdf	4	Information Disclosure Statement (IDS) Form (SB08)	80 KB
Warning: This is not a USPTO supplied IDS fillable form. Data in the form cannot be automatically loaded to other USPTO systems.			
TD_over_18_188_176_and_1	1	Terminal Disclaimer Filed	267 KB

8_513_970_.pdf				
ROA.pdf		23	-	176 KB
ROA-A...pdf	(1-1)	1	Amendment/Request for Reconsideration-After Non-Final Rejection	83 KB
ROA-ABST.pdf	(2-2)	1	Abstract	106 KB
ROA-CLM.pdf	(3-7)	5	Claims	91 KB
ROA-REM.pdf	(8-23)	16	Applicant Arguments/Remarks Made in an Amendment	160 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
SB133_SIDS.pdf	451462D87615DC1B787AE91DA7D2A4A52916AF7B7A091CF7EC4404C38DD2C88079509BD0C8576A0B480328680EA636B4BD054FDF0F65906E75892641C449A8DA
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ROA-A....pdf	741126AF5F7BEA59C9513E10B68B8B8C373C3F77FD83A1609E23DBE8AD02F3E3CEEA68DED071DEC9293201D97B69241568C6C11AA9739A1595E533AB5704309A
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ROA-REM.pdf	33BCE6BF6F47FF69774D4C486E6D8989723D38B8ADBE16D8DF765989DAEE0CB6DDA5736070D6A7A7930964802DE75E268D79F3C0698BDF8F339AABA732D15332

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 18/506,853	Filing Date 11/10/2023	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $480 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT	04/09/2024	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	*** 3	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

		(Column 1)		(Column 2)	(Column 3)			
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	/ZETA T ADAMS/
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03

CONFIRMATION NO. 3457

89300
Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

PUBLICATION NOTICE

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Date Mailed: 03/07/2024

Title:ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Publication No.US-2024-0077402-A1
Publication Date:03/07/2024

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently https://portal.uspto.gov/pair/PublicPair. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

89300 7590 01/09/2024

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EXAMINER	
GILL, RACHEL B	

ART UNIT	PAPER NUMBER
1648	

NOTIFICATION DATE	DELIVERY MODE
01/09/2024	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

EOfficePA@mofo.com
PatentDocket@mofo.com
pair_mofo@firsttofile.com

Office Action Summary	Application No. 18/506,853	Applicant(s) O'RIORDAN et al.	
	Examiner RACHEL B GILL	Art Unit 1648	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 10 November 2023.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 96-125 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 96-125 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☑ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 10 November 2023 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Disposition of Claims

Claims 96-125 are pending.

Examiner's Note

Applicant is encouraged to utilize the new web-based Automated Interview Request (AIR) tool for submitting interview requests; more information can be found at https://www.uspto.gov/patent/laws-and-regulations/interview-practice.

This application is being examined under the accelerated "Track One" Program. Applicant is reminded of the provisions of this prioritized examination program as per MPEP §708.02(b). Specifically noted is the application must contain, or be amended to contain, no more than four independent claims and no more than thirty total claims. In addition, the application must not contain any multiple dependent claims. After prioritized examination has been granted in an application, an amendment that results in more than four independent claims or thirty total claims, or a multiple dependent claim, will result in termination of prioritized examination.

Optional Authorization to Initiate Electronic Communications

The Applicant's representative may wish to consider supplying a written authorization in response to this Office action to correspond with the Examiner via electronic mail (e-mail). This authorization is **optional** on the part of the Applicant's representative, but it should be noted that the Examiner may not initiate nor respond to communications via electronic mail unless and until Applicant's representative authorizes such communications in writing within the official record of the patent application. A sample authorization is available at MPEP § 502.03, part II. If Applicant's representative chooses to provide this authorization, please ensure to include a valid e-mail address along with said authorization.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on 11/10/2023 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Notably, the disclosure statement filed lists a Search Report. The listing of the references cited in a Search Report itself is not considered to be an information disclosure statement (IDS) complying with 37 CFR 1.98. 37 CFR 1.98(a)(2) requires a legible copy of: (1) each foreign patent; (2) each publication or that portion which caused it to be listed; (3) for each cited pending U.S. application, the application specification including claims, and any drawing of the application, or that portion of the application which caused it to be listed including any claims directed to that portion, unless the cited pending U.S. application is stored in the Image File Wrapper (IFW) system; and (4) all other information, or that portion which caused it to be listed. In addition, each IDS must include a list of all patents, publications, applications, or other information submitted for consideration by the Office (see 37 CFR 1.98(a)(1) and (b)), and MPEP § 609.04(a), subsection I. states, "the list ... must be submitted on a separate paper." Therefore, the references cited in the Search Report have not been considered. Applicant is advised that the date of submission of any item of information or any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing the IDS, including all "statement" requirements of 37 CFR 1.97(e). See MPEP § 609.05(a).

Note: If copies of the individual references cited on the Search Report are also cited separately on the IDS (and these references have not been lined-through) they have been considered.

Specification

Applicant is reminded of the proper language and format for an abstract of the disclosure.

The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words in length. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details.

The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc. In addition, the form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided.

The abstract of the disclosure is objected to because of the use of legal phraseology (e.g. "e.g." stands for "*exempli gratia*", which is Latin for "for example"). Correction is required. See MPEP § 608.01(b).

Claim Rejections - 35 USC § 112(b); Second Paragraph

> The following is a quotation of 35 U.S.C. 112(b):
> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.
>
> The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 115 and dependent claims 116-125 thereof are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

Claims 115-125 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being incomplete for omitting essential structural cooperative relationships of elements, such omission amounting to a gap between the necessary structural connections. See MPEP § 2172.01. The omitted structural cooperative relationships are: what is considered a "purified" AAV vector preparation. It is unclear if the purity is meant to be compared to cell culture contaminants (e.g. presence/absence of cell lysate, cell growth media, nutrients, salts, etc.), presence of helper virus that is necessary to generate AAV in cell culture (e.g. presence of Adenovirus (AdV) in the sample, or if the "purified" AAV vector preparation is meant to be one that is substantially free of empty AAV capsids or immature AAV capsids. The claimed methods fail to provide a clear benchmark, so it is unclear to a

skilled artisan if the determination of purity is meant to be compared to non-AAV components, other non-mature AAV components, or a combination of both (e.g. a "purified" AAV vector composition is substantially free of cell culture contaminants and has less than 1% of non-mature AAV virions.) Further, as applicant defines "vector" as a recombinant plasmid or virus that comprises a nucleic acid to be delivered into a host cell, either in vitro or in vivo (¶[0054]), the interpretation of an adeno-associated virus (AAV) "vector" may be the entire AAV virion (e.g. mature virus with genome) or may be the nucleic acid generated by the virus separate from the virion (e.g. the single-stranded linear DNA genome of approximately 4.8 kb or any intermediates thereof.) As this definition provides further ambiguity (e.g. the "vector" desired may only be the genome separated from the virion or the "vector" desired may be mature AAV virions which encapsulate full-sized genomes), it makes the metes and bounds of what is being claimed within the method unclear.

Since a skilled artisan would not be reasonably apprised as to the metes and bounds of the claimed invention, instant Claim 115 is rejected on the grounds of being indefinite. Claims 116-125 are also rejected since they depend from claim 115, but do not remedy these deficiencies of claim 115.

Claim Rejections - 35 USC § 112(a); First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

> (a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 96-125 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, because the specification, while being enabling for methods using analytical ultracentrifugation (AUC) to determine the difference between adeno-associated virus (AAV) genome-containing virions/capsids by size (e.g. dimeric genomes, monomeric genomes, partial genome fragments, empty capsids) and for methods of determining the percentage of other immature virions or other contaminants in a heterogeneous AAV virion preparation, does not reasonably provide enablement for utilizing AUC in the methods as claimed to resolve the difference in AAV capsid-free genomes or other AAV nucleic acids. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The legal considerations that govern enablement determinations pertaining to undue experimentation have been clearly set forth. Enzo Biochem, Inc., 52 U.S.P.Q.2d 1129 (C.A.F.C. 1999). In re Wands, 8 U.S.P.Q.2d 1400 (C.A.F.C. 1988). See also MPEP § 2164.01(a) and § 2164.04. Ex parte Forman 230 U.S.P.Q. 546 (PTO Bd. Pat. App. Int., 1986). The courts concluded that several factual inquiries should be considered when making such assessments including: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in that art, the predictability or unpredictability of the art and the breadth of the claims. In re Rainer, 52 C.C.P.A. 1593, 347 F.2d 574, 146 U.S.P.Q. 218 (1965). The disclosure fails to provide adequate guidance pertaining to a number of these considerations as follows:

Nature of the invention/Breadth of the claims. The claims are drawn to methods of determining the size of one or more fragmented genomes in an AAV vector preparation, said method comprising:(i) subjecting the preparation to analytical centrifugation under boundary sedimentation velocity conditions, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm; (ii) measuring the rate of movement or migration of the sedimenting boundary, wherein movement or migration of the fragmented genomes results in different sedimenting boundaries, each sedimenting boundary corresponding to a resolvable vector species, thereby determining the sedimentation coefficients of the one or more of the fragmented genomes in the AAV vector preparation; and (iii) determining the size of the one or more fragmented genomes as a function of sedimentation coefficients

of the one or more fragmented genomes. The claims are also drawn to methods of determining the purity of an AAV vector preparation, said method comprising:(i) subjecting the preparation to analytical centrifugation under boundary sedimentation velocity conditions, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm; (ii) measuring the rate of movement or migration of the sedimenting boundary, wherein movement or migration of individual viral particles in the AAV vector preparation results in different sedimenting boundaries, each sedimenting boundary corresponding to a resolvable vector species, and wherein the vector preparation comprises full genomes, fragmented genomes and empty capsids without genome; (iii) determining sedimentation coefficients of the individual viral particles in the AAV vector preparation; and (iv) quantifying the amount of each of the resolvable vector species in the AAV vector preparation, thereby determining the purity of the AAV vector preparation.

As per the specification, "vector" is defined as a recombinant plasmid or virus that comprises a nucleic acid to be delivered into a host cell, either in vitro or in vivo (¶[0054]). Therefore, under the definition provided by applicant, the broadest reasonable interpretation of an adeno-associated virus (AAV) "vector" may be describing either the entire AAV virion (e.g. mature virus with genome/nucleic acid) or may be the nucleic acid generated by the virus separate from the virion/capsid (e.g. the single-stranded linear DNA genome of approximately 4.8 kb or any fragments or intermediates thereof.)

State of the prior art/Predictability of the art. The art appears to teach the use of AUC to separate AAV mature or full capsids from empty capsids, and unassembled viral proteins and other contaminants from a heterogeneous mixture (See e.g. Figs. 2-3 of WO2014125101A1, CITED ART OF RECORD; hereafter "Le Bec".) It is not clear that AUC was used to resolve the differences between AAV nucleic acids not associated with an AAV virion, as other methods or techniques appear in the art to have been preferable to analyze AAV nucleic acids or genomes from virions or other contaminants, such as isolation of the nucleic acid using phenol/chloroform extraction, Cesium chloride (CsCl) gradient centrifugation, solid phase extraction, magnetic bead isolation, coupled with analysis of the isolated nucleic acid using PCR (such as RT-PCR), Southern and northern blotting, 260/280 nm absorbance, and other biosensor methods (See e.g. Rose JA, et. al. *Proc Natl Acad Sci U S A*. 1966 Jul;56(1):86-92.; Cervelli T, et. al. *PLoS One*. 2011;6(8):e23474. Epub 2011 Aug 10.; Hauck B, et. al. *J Virol*. 2004

Dec;78(24):13678-86.) However, AUC has been used to resolve nucleic acids within solution, but require different rotor speeds and different density gradients than what is required to resolve whole virions (ROSENBLOOM J, et. al. Biochemistry. 1963 Nov-Dec;2:1206-11.; Hatters DM, et. al. *Biophys J.* 2001 Jul;81(1):371-81.)

Working examples. The working examples disclosed in the specification note that the capsids contain both "mature capsids", wherein the virion comprises a full length genome, and immature or empty capsids, wherein the virion contains only partial genomes or are completely empty (p. 62, ¶1). The homogeneity of AAV capsids is necessary for their use in gene therapy and other biopharmaceutical methods, so the Applicants developed a method to determine a way to monitor product quality with regards to homogeneity and purity (¶2, p. 62). The AUC data analysis appeared to monitor percent full virions (i.e. capsids with full length genomes) (pp. 63-64, ¶ bridging pages, to p. 64, ¶2). Applicant appears to use the term "vector" in the methods to mean "virions" or "capsids" and not the definition of "plasmids" (see ¶[0147]). In order to determine the difference between capsids which were empty, partially full, or full, Applicants developed a method that developed a relationship between S value and genome size (¶[0148]). Applicants developed AAV genomes which varied in size, such as genomes which had heterologous transgenes (¶[0151]). Applicants took purified mixtures of AAV empty capsids and full capsids at known concentrations, and subjected them to the AUC methods (¶[0152]), plotted the differential sedimentation coefficient distribution value (C(S)) versus the sedimentation coefficient (in Svedberg units, S) to yield peaks for the empty and genome-containing capsids (Fig. 1B), and their analysis as outlined (¶[0152-0154]) allowed them to show that AUC may be used to accurately distinguish ***and*** quantify empty capsids and genome-containing capsids from a heterogeneous capsid preparation.

Applicants then compared different detection methods for AUC to determine if the detection method altered the ability to quantitate and quantify AAV mature and immature virions (Example 2 starting at ¶[0157]). Interference detection was compared to the absorbance method utilized in Example 1, but the peak sizes generated by both methods showed a disconnect between peak height and concentration (e.g. comparison of "empty capsid" peaks of Figs. 3A and 3B).

Applicants then compared methods of production for AAV virion resolution, namely comparing the "triple transfection" and "producer cell line" methods of AAV virion production (Example 3 starting at

¶[0160]). This analysis showed the presence of an additional peak, the 75S species, which they labeled as capsids with fragmented genomes (¶[0162]). Applicants also compared the use of anion exchange chromatography (AEX) to remove empty capsids (Example 4 starting at ¶[0164]), and showed the AEX methods could also purify mixtures so that the final product comprised full AAV virions (Fig. 7). At Example 5, the analysis of fragmented genomes of unknown size and sequence was performed (starting at ¶[0166]). A standard curve was generated of known AAV2 capsids to correlate genome size and S value (Fig. 8; Table 5). Example 6 (starting at ¶[0170]) analyzed different factors that influenced the packaging of AAV genomes into the capsid, such as methods of production, cap serotypes, and overall genome size. Example 7 focused on AdV, not AAV, and the use of AUC to determine AdV mature and empty virions.

Nowhere did it appear as though the alternate use of the term "vector" was applied to the AUC method, in that they did not appear to use AUC to analyze for the presence of AAV genomes or nucleic acid which were not capsid-associated.

Guidance in the specification. The specification provides guidance towards the use of AUC to resolve differences in intact capsids or intact virions, insomuch that AUC can differentiate between empty capsids, full capsids, and capsids with fragmented, larger, or duplicated genomes. Nowhere does it appear as though AUC was used to determine or quantitate AAV nucleic acid, such as AAV genomes, which were isolated and/or not associated with AAV capsids.

Amount of experimentation necessary. Additional research is required in order to determine how effective the method of AUC would be in resolving AAV vectors of both types according to Applicant's definition; it appears as though AUC can distinguish between AAV virions/capsids which comprise different amounts of nucleic acid, but plasmids or AAV nucleic acid free from a virion/capsid was not resolved using AUC.

For the reasons discussed above, it would require undue experimentation for one skilled in the art to make and/or use the claimed methods.

Claim Interpretation

The claims in this application are given their broadest reasonable interpretation using the plain meaning of the claim language in light of the specification as it would be understood by one of ordinary skill in the art.

Claim 96 is drawn to a method of determining the size of one or more fragmented genomes in an AAV vector preparation, said method comprising:(i) subjecting the preparation to analytical centrifugation under boundary sedimentation velocity conditions, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm; (ii) measuring the rate of movement or migration of the sedimenting boundary, wherein movement or migration of the fragmented genomes results in different sedimenting boundaries, each sedimenting boundary corresponding to a resolvable vector species, thereby determining the sedimentation coefficients of the one or more of the fragmented genomes in the AAV vector preparation; and (iii) determining the size of the one or more fragmented genomes as a function of sedimentation coefficients of the one or more fragmented genomes.

Further limitations on the method of claim 96 are wherein step (iii) comprises comparing the sedimentation coefficients of the one or more fragmented genomes with a standard curve generated by sedimentation coefficients of recombinant AAV genomes of known nucleotide sizes, thereby determining the size of the one or more fragmented genomes (claim 97); wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm (claim 98); wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm (claim 99); wherein the boundary sedimentation is performed at a temperature of from about 4°C to about 20°C (claim 100), wherein the boundary sedimentation is performed at a temperature of about 4°C (claim 101); wherein the fragmented genomes are sub-genomic DNA molecules (claim 102); wherein the centrifugation is run from about 0.5 hours to about 2 hours (claim 103), wherein the centrifugation is run from about 1 hours to about 2 hours (claim 104), wherein the centrifugation is run for about 1 hour (claim 105), wherein the centrifugation is run for about 1.2 hours (claim 106); wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is greater than 5x10^11 vg/mL (claim 107); wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is from about 1x10^11 vg/mL to about 1x10^13vg/mL (claim 108); further comprising quantifying one or more of the fragmented genomes in the AAV vector preparation

(claim 109); comprising determining the size of all of the fragmented genomes in the rAAV preparation (claim 110), further comprising quantifying all of the fragmented genomes in the AAV vector preparation (claim 111), wherein the AAV vector preparation comprises self- complementary AAV viral genomes (claim 114); and wherein the AAV vector preparation comprises AAV9 capsid proteins (claim 112), wherein the AAV vector preparation further comprises at least one AAV2 ITR (claim 113).

Claim 115 is drawn to a method of determining the purity of an AAV vector preparation, said method comprising:(i) subjecting the preparation to analytical centrifugation under boundary sedimentation velocity conditions, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm; (ii) measuring the rate of movement or migration of the sedimenting boundary, wherein movement or migration of individual viral particles in the AAV vector preparation results in different sedimenting boundaries, each sedimenting boundary corresponding to a resolvable vector species, and wherein the vector preparation comprises full genomes, fragmented genomes and empty capsids without genome; (iii) determining sedimentation coefficients of the individual viral particles in the AAV vector preparation; and (iv) quantifying the amount of each of the resolvable vector species in the AAV vector preparation, thereby determining the purity of the AAV vector preparation.

Further limitations on the method of claim 115 are wherein the method is further comprising determining the size of the individual viral particles after step (iii) and prior to step (iv) by comparing the sedimentation coefficients of the individual viral particles with a standard curve generated by sedimentation coefficients of recombinant AAV genomes of known nucleotide sizes (claim 116); wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm (claim 117); wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm (claim 118); wherein the boundary sedimentation is performed at a temperature of about 4°C (claim 119); wherein the centrifugation is run from about 0.5 hours to about 2 hours (claim 120); wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is greater than 5x10^11 vg/mL (claim 121); wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is from about 1x10^11 vg/mL to about 1x10^13 vg/mL (claim 122); and wherein the AAV vector preparation comprises AAV9 capsid proteins (claim 123), wherein the AAV vector preparation further comprises at

least one AAV2 ITR (claim 124), wherein the AAV vector preparation comprises self- complementary AAV viral genomes (claim 125).

Claim Rejections - 35 USC § 102

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis (i.e., changing from AIA to pre-AIA) for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale, or otherwise available to the public before the effective filing date of the claimed invention.
>
> (a)(2) the claimed invention was described in a patent issued under section 151, or in an application for patent published or deemed published under section 122(b), in which the patent or application, as the case may be, names another inventor and was effectively filed before the effective filing date of the claimed invention.

Claims 96, 98-99, 108, 112-115, 117-118, and 122-125 are rejected under 35 U.S.C. 102(a)(1) as being anticipated by Le Bec et. al. (WO2014125101A1, Priority 02/15/2013; CITED ART OF RECORD; hereafter "Le Bec"; NB: the English translation of Le Bec utilized for this rejection was provided in parent application 16/547,144; the original French WIPO publication and English translation utilized for this rejection has been included for reference in this Office action.)

The Prior Art

Le Bec teaches analytic ultracentrifugation (AUC) analysis of a heterogeneous preparation of AAV9 viral particles (Figs. 2-3). Le Bec teaches the AUC allowed for analysis of empty and mature AAV particles, wherein the sedimentation coefficient of the various AAV viral particles (empty, mature, aggregate) and other present populations (subparticles, contaminating proteins, aggregate) in the purified products was determined by centrifugation in real time. The centrifugation of the samples was carried out

at a speed of 16000 rpm using 100µl or 400 µl of undiluted pure vectors, the sedimentation was followed by absorbance at the wavelength of 276 nm, the sedimentation coefficient of the different populations was obtained using the SEDFIT software (p. 4, 3.d.; **instant claims 96, 98-99, 112, 117-118, 123**.) Le Bec goes on to further note the density of mature AAV particles (110S; 1.45 g/cm^3 for a genome size of 4.8kB) and empty AAV particles (60S, 1.32 g/cm^3), and that the difference is sufficiently significant to distinguish them through AUC in a heterogeneous preparation of AAV virions (Results, item 2, p. 4). Le Bec notes they are able to perform the AUC on the AAV particles to quantify the different species present in a viral preparation of AAV, such as sub-particles, contaminating proteins, aggregates, empty particles, and full particles (Results, item 2, p. 4; Figs. 2-3; **instant claim 115**.) Le Bec notes that peaks smaller than 60S correspond to unassembled viral proteins and/or other contaminants, while those particles at 65S are empty AAV particles, those at 90S represent AAV with a single copy of the genome (ssDNA) and those at 105S have two copies (dsDNA or scDNA) of the genome (AAV is a single-stranded DNA (ssDNA) genome and is packaged as such, but the ssDNA genomes of (+) and (-) polarity are encapsidated into mature virions with equal frequency, and self-complementary AAV (scAAV) genomes have been developed as noted by Le Bec and can be packaged, wherein the scAAV are double-stranded and allow for immediate transcription of the genome in both dividing and non-dividing cells; **instant claims 114, 125**.) Le Bec notes the viral genome prior to the AUC step is concentrated to at least 1x10^11 vg/mL (2. Purification; pp.3-4; **instant claims 108, 122**). Le Bec teaches the AAV genome may be derived from any known AAV serotype, such as AAV1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, namely AAV2 ("Detailed Description of Invention", p.2; **instant claims 113, 124**).

For at least these reasons, Le Bec teaches the limitations of **instant claims 96, 98-99, 108, 112-115, 117-118, and 122-125**, and anticipates the invention encompassed by said claims.

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis

(i.e., changing from AIA to pre-AIA) for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims the examiner presumes that the subject matter of the various claims was commonly owned as of the effective filing date of the claimed invention(s) absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and effective filing dates of each claim that was not commonly owned as of the effective filing date of the later invention in order for the examiner to consider the applicability of 35 U.S.C. 102(b)(2)(C) for any potential 35 U.S.C. 102(a)(2) prior art against the later invention.

Claims 97, 100-107, 109-111, 116, and 119-121 are rejected under 35 U.S.C. 103 as being unpatentable over Le Bec as applied to **claims 96, 98-99, 108, 112-115, 117-118, and 122-125** above, and further in view of McLaughlin et. al. (US20170362608A1, Pub. 12/21/2017, Priority 12/12/2014; hereafter "McLaughlin"), Berkowitz et. al. (Berkowitz SA, et. al. *Anal Biochem*. 2007 Mar 1;362(1):16-37. Epub 2006 Dec 20.; CITED ART OF RECORD; hereafter "Berkowitz"); and Cole et. al. (Cole JL, et. al *Methods Cell Biol*. 2008;84:143-79.; CITED ART OF RECORD; hereafter "Cole".)

The Prior Art

The teachings of Le Bec have been set forth *supra*. While Le Bec teaches the methods of AUC in order to separate AAV virion species from one another and from impurities, Le Bec fails to detail specifics about the method, such as time and temperature of centrifugation. While Le Bec teaches the AAV virions should be concentrated to greater than about 1x10^11, Le Bec fails to specifically state that it must be greater than 5x10^11 vg/mL. While Le Bec teaches that the sedimentation velocities correspond to the viral particle and genome comprised within, Le Bec fails to teach generation of a standard curve to determine the size of AAV particles with genomes in unknown AAV samples. However, all of these steps would be routine optimization steps to one of skill in the art, as evidenced by the teachings of McLaughlin, Cole, and Berkowitz.

McLaughlin teaches AAV viral particles can comprise both single-stranded and self-complementary genomes, and the analysis of these particles via analytical ultracentrifugation (AUC) can resolve the difference between these AAV genomic particle subtypes (¶[0073]). McLaughlin teaches intermediate replicative species are also packaged into viral particles, and AAV particles produced in vitro may comprise scAAV, scAAV intermediate species, ssAAV monomers, and ssAAV repaired from scAAV (¶[0074), and McLaughlin teaches these scAAV species may be generated by deleting portions of the AAV genome known as the Rep nicking sequence, which results in a truncated genome (¶[0132]; **instant claims 102, 109-110**).

Cole teaches that Analytical ultracentrifugation (AUC) is a versatile and powerful method for the quantitative analysis of macromolecules in solution (abstract) and can be utilized to determine the size and shape of macromolecules in solution, such as viruses (p. 2, ¶2). Cole teaches subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (p. 3, ¶1), plotting the differential sedimentation coefficient distribution value (C(s)) (p. 5, ¶3) versus the sedimentation coefficient (s) (p. 3, ¶1) in Svedberg units (S) (p. 3, ¶3-4; p. 4, ¶3; p. 15, ¶2; See "B. Data Analysis" starting on p. 15, Table 4) and integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral particle (abstract; p. 4, ¶1; p. 5, ¶1; pp. 15-16, ¶ bridging pages; See "B. Data Analysis" starting on p. 15, esp. ¶ bridging pp. 15-16.)

Cole teaches the sedimentation from aqueous solutions is monitored by absorbance, interference, or fluorescence (abstract). Cole teaches adjusting the optics to account for the solvent component interference in the sample solution (p. 7, ¶2) and the use of reference solutions in absorbance optics for AUC (p. 7, ¶5). Cole teaches that the C(s) values are determined by an algorithm that comprises Lamm equation solutions (p. 3, ¶2-4; p. 16, ¶3); wherein the algorithm is the SEDFIT algorithm (p. 12, ¶3; p. 16, ¶ 3; p. 17, ¶2). Cole teaches that sedimentation is monitored according to the sedimentation coefficient of the sample, and should take a boundary at least 2 hours to sediment the full length of a cell to the bottom of a sector (p. 15; ¶2). Cole teaches optimizing solvents according to their compatibility with the sample cell and with optical systems (p. 2, ¶2). Cole describes the instrumentation, and details the rotors, sample holders, and optical systems, including:

"To view the sample, the analytical rotor has holes through it to hold sample containers commonly called cells. Each cell contains a centerpiece, with chambers (called channels) to hold the liquid samples. The centerpiece, in turn, is sealed between windows to permit the passage of light through the channels, thus allowing the cell contents to be viewed."

The instrumentation described would allow monitoring of the sedimentation of particles within the AUC through an optical window (p. 6, ¶2). Cole describes the "scans" performed to view the sedimentation (p. 6, ¶3) and states when observing the system using absorbance readings it is best to scan at least 30-40 times (p. 15, ¶2) and depending on the scanning method used, the scans can be performed once every second up to once every 300 seconds (Table 2.) In analyzing the data using SEDFIT, regularization procedures can be performed to produce a smoother distribution function (p, 16, ¶3). Cole describes that depending on what you want to know about your sample and how you are scanning the sample will alter the velocity of the rotor, and that depending on the S (Svedberg units) of the sample, you will use a velocity of anywhere from 10,000-55,000 RPM (Table 3), and with absorbance scanning (especially involving multi-wavelength scanning), one may wish to use a lower speed (Table 3; p. 19, ¶3-4, 6,000-12,000 RPM, p. 7, ¶4) and that larger biomolecules may aggregate or degrade, so one can detect the loss of material by measuring the OD at low speed (3,000 RPM; pp. 12-13, ¶ bridging pages). Cole describes that with some AUCs it is difficult to use the machine with a temperature below 4

°C and that most experiments are performed at about 20 °C (pp. 14-15, ¶ bridging pages; **instant claims 100-101; 119**).

Berkowitz teaches the use of a type of AUC in a solution containing a high concentration of CsCL in order to obtain a high resolution of different viral particle subtypes (p. 19, "Analytical density gradient sedimentation equilibrium in CsCl"). While Berkowitz is teaching the AUC methods with respect to Adenovirus (AdV) particles, said methods would be applicable to the analogous art of Le Bec and would be obvious to a skilled artisan to use in order to obtain greater resolution of viral subspecies. Berkowitz teaches the revised AUC method can be completed in a shorter time (p. 19, rt. Col., ¶) Berkowitz teaches the AdV compositions may comprise empty capsids, incomplete particles, aberrant particles, sub particles, and/or aggregates (pp. 16-17, ¶ bridging pages; p. 26, rt. Col., ¶2). Berkowitz teaches the AUC was run at 15,000 rpm at a temperature of 20C (Fig. 1; **instant claims 100, 119**).

As shown by the teachings of Le Bec and McLaughlin, the use of AUC to separate AAV virion species was known in the art. As evidenced by the further teachings of Berkowitz and Cole, optimizations for AUC, especially with respect to separation of viral particles, was well-known in the art, and parameters to optimize AUC routinely included temperature and time of running the sample, rendering the limitations of **instant claims 103-106 and 120** obvious to a skilled artisan with a reasonable expectation of success, especially given the motivation provided by Berkowitz. Further, the operating temperatures of the AUC would be obvious to the methods of Le Bec in view of Cole and Berkowitz, rendering the limitations of **instant claims 100-101 and 119** obvious to a skilled artisan with a reasonable expectation of success. Further optimization, such as the input of a larger concentration of isolated viral particles, would be obvious given the teachings of Le Bec and further supported by the prior art methods such as Cole and Berkowitz which provide motivation to optimize parameters, thus rendering the limitations of **instant claims 107 and 121** obvious to a skilled artisan. Further, Le Bec and McLaughlin both teach plotting the data as C(s) vs. S graphs with respect to AAV particle separation, as does Berkowitz, and Le Bec and McLaughlin both teach expected sedimentation coefficients for empty and full AAV were known, thus giving a reasonable expectation of success to a skilled artisan in generating a standard curve of other viral particles based on sedimentation coefficients, thus rendering the limitations of **instant claims 97, 109-111, and 116** obvious.

It would have been obvious to one of ordinary skill in the art to modify the methods taught by Le Bec in order to optimize specific parameters for AUC, such as time of run, temperature of run, and concentration of viral input prior to the run. One would have been motivated to do so, given the suggestion by Cole and Berkowitz that AUC methods can and should be optimized according to input, temperature, and time. There would have been a reasonable expectation of success, given the knowledge that AUC was applied to separation of empty viral particles from mature, full viral particles, as taught by Le Bec, Berkowitz, and McLaughlin, especially with respect to separating AAV viral particles as taught by Le Bec and McLaughlin, and also given the knowledge that AAV viral particles existed in a variety of species, not just "empty" and "full", as taught by Le Bec and McLaughlin. Thus the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or

claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The filing of a terminal disclaimer by itself is not a complete reply to a nonstatutory double patenting (NSDP) rejection. A complete reply requires that the terminal disclaimer be accompanied by a reply requesting reconsideration of the prior Office action. Even where the NSDP rejection is provisional the reply must be complete. See MPEP § 804, subsection I.B.1. For a reply to a non-final Office action, see 37 CFR 1.111(a). For a reply to final Office action, see 37 CFR 1.113(c). A request for reconsideration while not provided for in 37 CFR 1.113(c) may be filed after final for consideration. See MPEP §§ 706.07(e) and 714.13.

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The actual filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/apply/applying-online/eterminal-disclaimer.

Claims 96-125 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-22 of U.S. Patent No. 11,639,887 in view of Le Bec (supra).

Both sets of claims are drawn to a method of characterizing a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), and

c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral particle. Both sets of claims are drawn to methods to assess vector genome integrity of recombinant viral particles in a preparation of recombinant viral particles through the same method noted above. Both sets of claims are drawn to additional steps further comprising integrating the area under each peak in the C(s) distribution to determine the relative concentration of each species of recombinant viral particles. Both sets of claims are drawn to the method outlined above also measuring the relative amount of empty capsids in a preparation of recombinant viral particles, wherein the presence of a peak that corresponds to the S value of empty capsid particles indicates the presence of empty capsid particles. Both sets of claims are drawn to multiple peaks indicating the presence of full viral particles versus aggregates, truncated genomes, or other DNA impurities. Both sets of claims are drawn to the use of reference samples, the determination of C(s) values using Lamm equation solutions, namely the SEDFIT algorithm, in order to generate standard curves. Both sets of claims are drawn to wherein the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000 rpm.

The main difference between the two sets of claims is that the ‘887 claims are drawn to the recombinant viral particles being lentivirus particles, while the instant claims are drawn to the recombinant viral particles being AAV particles. However, such a difference would be an obvious change to the ‘887 claims, given what was known in the art as evidenced by Le Bec. Through simple substitution of one known element (e.g. recombinant lentivirus particles) with another known element (e.g. AAV particles), one of skill in the art would find it obvious to use this known technique of the ‘887 claims and apply it to separation of other recombinant viral particles. Le Bec provides motivation in that he teaches monitoring the homogeneity of AAV preparations using analytical ultracentrifugation (AUC). Le Bec teaches through AUC one can separate and determine the presence of different viral particles in a preparation, including intact, mature particles, aggregates, empty capsids, and smaller incomplete particles or subparticles (see detailed analysis *supra*.) Applying the known technique of the ‘887 claims to the previously performed methods of Le Bec would yield predictable results, in that a skilled artisan would reasonably conclude that one could separate different viral particle formulations for AAV given that it worked with the lentiviruses of the ‘887 claims.

Therefore, the Office maintains that the instant claims are an obvious variant of the '887 claims, especially in light of the teachings of Le Bec.

Claims 96-125 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 5-9, and 15-34 of U.S. Patent No. 10,429,288 B2. NB: Claims 4 and 10-14 were recently disclaimed by Patent Owner in the '288 patent.)

Both sets of claims are drawn to a method of characterizing a preparation of recombinant AAV viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), and

c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral particle. Both sets of claims are drawn to methods to assess AAV genome size/integrity of recombinant viral particles in a preparation of recombinant AAV particles through the same method noted above. Both sets of claims are drawn to additional steps further comprising integrating the area under each peak in the C(s) distribution to determine the relative concentration of each species of recombinant viral particles. Both sets of claims are drawn to the method outlined above also measuring the relative amount of empty capsids in a preparation of recombinant viral particles, wherein the presence of a peak that corresponds to the S value of empty capsid particles indicates the presence of empty capsid particles. Both sets of claims are drawn to multiple peaks indicating the presence of full viral particles versus aggregates, truncated genomes, or other DNA impurities. Both sets of claims are drawn to the use of reference samples, the determination of C(s) values using Lamm equation solutions, namely the SEDFIT algorithm, especially in order to generate standard curves to assess genome size within viral particles. Both sets of claims are drawn to wherein the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000 rpm.

Therefore, the Office maintains that the instant claims are an obvious variant of the '288 claims, and are not patentably distinct.

Claims 96-125 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-3, 5, 10, 12, 18, 30-37, 42-44, 48, 58, and 61 of copending Application No. 18/188,176 in view of Le Bec (*supra*) and Berkowitz (*supra*). Both sets of claims are drawn to methods of characterization of recombinant viral particles comprising the use of AUC, plotting the C(s) vs. S, and determining the relative concentration of each viral species. Both sets of claims assess genomic integrity or genomic size within the recombinant viral particles using said AUC method, including assessment of the relative amount of empty capsids in the viral preparation. Both claim sets are drawn to assessing the presence of other non-mature viral impurities, including empty virions, aggregates, virions comprising truncated genomes, and other impurities. Both claim the use of reference samples. Both claim the AUC is performed at about 3000 rpm to about 20,000 rpm. The main difference is the '176 claims are drawn to the viral particles being from AdV or Herpes simplex virus (HSV), while the instant claims are drawn to the viral particles being from AAV. However, such a difference would be a simple substitution of one known element with another, given the teachings of Le Bec and Berkowitz (outlined in detail *supra*). Le Bec teaches how the methods instantly claimed and claimed in the '176 application can be used to separate AAV viral species, and Berkowitz teaches how the methods instantly claimed and claimed in the '176 application can be applied to AdV. A skilled artisan would be able to substitute one well-characterized element with another well-characterized element, especially given these prior art teachings which show AUC using AdV and AAV. Therefore, the difference between these two claim sets would be obvious to a skilled artisan, and the claimed inventions are not patentably distinct.

This is a provisional nonstatutory double patenting rejection.

Claims 96-125 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 96-125 of copending Application No. 18/513,970 in view of Le Bec (*supra*) and Berkowitz (*supra*). Both sets of claims are drawn to methods of characterization of recombinant AAV particles comprising the use of AUC, plotting the C(s) vs. S, and determining the relative concentration of

each viral species. Both sets of claims assess genomic integrity or genomic size within the recombinant viral particles using said AUC method, including assessment of the relative amount of empty capsids in the viral preparation. Both claim sets are drawn to assessing the presence of other non-mature viral impurities, including empty virions, aggregates, virions comprising truncated genomes, and other impurities. Both claim the use of reference samples and the generation of standard curves. Both claim the AUC is performed at about 3000 rpm to about 20,000 rpm. Both claim the method is performed at about 4 deg C, and wherein the fragmented genomes are subgenomic DNA molecules. Both claim the same run times of about 0.5 to about 2 hours. Both claim the same concentration of AAV prior to input in the AUC method. Both claim the presence of AAV2 ITR, AAV9 capsid proteins, and self-complementary AAV viral genomes.

The main difference is the '970 claims comprise more specific conditions for the AUC method, such as the cells used in AUC, and that the method is run until the lightest sediment clears the optical window. However, such a difference would be routine optimizations made in viral vector separation and analysis using AUC, as taught by Cole (outlined in detail *supra*). Cole teaches how the methods instantly claimed and claimed in the '970 application can be used to separate viral species, and the routine optimizations one must make in order to perform AUC, such as the cell usage and optical windows. A skilled artisan would find it routine optimization to arrive at the further limitations claimed by the '970 claims, and would find it an indispensable part of performing the instantly claimed method. Therefore, the difference between these two claim sets would be obvious to a skilled artisan, and the claimed inventions are not patentably distinct, especially in light of the teachings of Cole.

This is a provisional nonstatutory double patenting rejection.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to RACHEL B GILL whose telephone number is (571)272-3129. The examiner can normally be reached on M to F 8:00 AM to 5:00 PM Eastern.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, JANET ANDRES can be reached on 571-272-0867. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/RACHEL B GILL/
Primary Examiner, Art Unit 1648

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	Examiner RACHEL B GILL	Art Unit 1648	Page 1 of 2

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	Examiner	Art Unit
	RACHEL B GILL	1648

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

/RACHEL B GILL/ Primary Examiner, Art Unit 1648	

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	RACHEL B GILL	1648

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PubMed inventor, assignee, and keyword search (see attached)	01/04/2024	"
PALM inventor search (see attached)	01/04/2024	"
PE2E inventor, assignee, and keyword search (see attached)	01/04/2024	"
Google inventor, assignee, and keyword search (see attached)	01/04/2024	"

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**

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First Named Inventor	Catherine R. O'Riordan
Art Unit	Not Yet Assigned
Examiner Name	Not Yet Assigned
Attorney Docket Number	15979-20103.03

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	Examiner Name	Not Yet Assigned
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	40.	Notice of Filing Date Accorded to Petition and Time for Filing Patent Owner Preliminary Response for U.S. Patent No. 10,429,288, mailed July 19, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 6 pages.	
	41.	Notice of Filing Date Accorded to Petition and Time for Filing Patent Owner Preliminary Response for U.S. Patent No. 10,429,288, mailed July 11, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 6 pages.	
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	43.	Patent Owner's Mandatory Notices Pursuant to 37 C.F.R. Section 42.8 for U.S. Patent No. 10,429,288, dated July 11, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 5 pages.	
	44.	Patent Owner's Mandatory Notices Pursuant to 37 C.F.R. Section 42.8 for U.S. Patent No. 10,429,288, dated July 11, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 5 pages.	
	45.	Patent Owner's Power of Attorney for U.S. Patent No. 10,429,288, dated July 11, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		
	Application Number	Not Yet Assigned
	Filing Date	Concurrently Herewith
	First Named Inventor	Catherine R. O'Riordan
	Art Unit	Not Yet Assigned
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	15979-20103.03

NON-PATENT LITERATURE DOCUMENTS			
	46.	Patent Owner's Power of Attorney for U.S. Patent No. 10,429,288, dated July 10, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	47.	Patent Owner's Preliminary Response for U.S. Patent No. 10,429,288, dated October 19, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 71 pages.	
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	49.	Petition for Inter Partes Review for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 80 pages.	
	50.	Petition for Inter Partes Review for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 80 pages.	
	51.	Petitioner Novartis Gene Therapies, Inc.'s Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	52.	Petitioner Novartis Gene Therapies, Inc.'s Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	53.	Petitioner Novartis Pharmaceuticals Corporation's Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	54.	Petitioner Novartis Pharmaceuticals Corporation's Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	55.	Petitioner's Exhibit List for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 4 pages.	

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	Art Unit	Not Yet Assigned
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	Attorney Docket Number	15979-20103.03

NON-PATENT LITERATURE DOCUMENTS			
	56.	Petitioner's Exhibit List for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 4 pages.	
	57.	Petitioner's Supplemental Paper and Notice Ranking Petitions for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 5 pages.	
	58.	Petitioner's Supplemental Paper and Notice Ranking Petitions for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 5 pages.	
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	Examiner Name	Not Yet Assigned
	Attorney Docket Number	15979-20103.03

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EXAMINER SIGNATURE			
Examiner Signature	/RACHEL B GILL/	Date Considered	01/03/2024

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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	Examiner Name	Not Yet Assigned
	Attorney Docket Number	15979-20103.03

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	Examiner Name	Not Yet Assigned
	Attorney Docket Number	15979-20103.03

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e) (1).

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☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e) (2).

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☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

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SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Seth Snyder/	Date (YYYY-MM-DD)	2023-11-10
Name/Print	Seth Snyder	Registration Number	60,243

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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /R.B.G/

Inventor Information

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
O'RIORDAN, CATHERINE	11934148	10744210	20090069261	150	GENE THERAPY FOR SPINAL CORD DISORDERS	WEHBE,ANNE MARIE	11/02/2007
O'RIORDAN, CATHERINE	12328267	9034836	20100287612	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	12/04/2008
O'RIORDAN, CATHERINE	12417910	9890394	20090286857	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	04/03/2009
O'RIORDAN, CATHERINE	14692468	Not Issued	20160087312	161	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	04/21/2015
O'RIORDAN, CATHERINE	15127757	10383963	20170173183	150	GENE THERAPY FOR RETINITIS PIGMENTOSA	BURKHART,MICHAEL	09/20/2016
O'RIORDAN, CATHERINE	15549885	10450563	20180023082	150	VARIANT RNAI	CHONG,KIMBERLY	08/09/2017
O'RIORDAN, CATHERINE	15858303	10820245	20180030490	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	12/29/2017
O'RIORDAN, CATHERINE	16335653	11698377	20210041451	150	METHODS FOR DETECTING AAV	XU,XIAOYUN	02/14/2019
O'RIORDAN, CATHERINE	16503081	11103588	20200046851	150	GENE THERAPY FOR RETINITIS PIGMENTOSA	BURKHART,MICHAEL	07/03/2019
O'RIORDAN, CATHERINE	16546070	11554161	20200101138	150	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CHEN,SHIN LIN	08/20/2019
O'RIORDAN, CATHERINE	16586565	10780079	20200109401	150	VARIANT RNAI	CHONG,KIMBERLY	09/10/2019
O'RIORDAN, CATHERINE	16910834	Not Issued	20210008227	41	GENE THERAPY FOR SPINAL CORD DISORDERS	WEHBE,ANNE MARIE	06/24/2020
O'RIORDAN, CATHERINE	16941303	11781137	20210047841	150	VARIANT RNAI	CHONG,KIMBERLY	07/28/2020
O'RIORDAN, CATHERINE	17162358	Not Issued	20210261825	41	MODIFIED ADENO-ASSOCIATED VIRAL CAPSID PROTEINS FOR OCULAR GENE THERAPY AND METHODS OF USE THEREOF	PARK,ILKYOO	01/29/2021
O'RIORDAN, CATHERINE	17383062	Not Issued	20220054657	30	GENE THERAPY FOR RETINITIS PIGMENTOSA	BURKHART,MICHAEL	07/23/2021
O'RIORDAN, CATHERINE	18068149	Not Issued	20230384200	30	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS	CENTRAL,DOCKET	12/14/2022
O'RIORDAN, CATHERINE	18298293	Not Issued	20230385868	30	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY	CENTRAL,DOCKET	04/05/2023
O'RIORDAN, CATHERINE	18321542	Not Issued	0	30	METHODS FOR DETECTING AAV	CENTRAL,DOCKET	05/22/2023
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O'RIORDAN, CATHERINE	60827977	Not Issued		158	GENE THERAPY FOR AMYOTROPHIC LATERAL SCLEROSIS AND OTHER SPINAL CORD DISORDERS		10/03/2006
O'RIORDAN, CATHERINE	61045077	Not Issued		158	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RdCVF)		04/15/2008
O'RIORDAN, CATHERINE	61969027	Not Issued		158	GENE THERAPY FOR RETINITIS PIGMENTOSA		09/21/2014
O'RIORDAN, CATHERINE	62105714	Not Issued		158	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES		01/20/2015

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
O'RIORDAN, CATHERINE	62114575	Not Issued		159	NOVEL NUCLEIC ACIDS FOR IMPROVED GENE DELIVERY		02/10/2015
O'RIORDAN, CATHERINE	62114578	Not Issued		159	VARIANT RNAI		02/10/2015
O'RIORDAN, CATHERINE	62375314	Not Issued		159	METHODS FOR DETECTING AAV		08/15/2016
O'RIORDAN, CATHERINE	62561934	Not Issued		159	COMPOSITIONS AND METHODS OF TREATING HUNTINGTON'S DISEASE		09/22/2017
O'RIORDAN, CATHERINE	62967416	Not Issued		159	MODIFIED ADENO-ASSOCIATED VIRAL CAPSID PROTEINS FOR OCULAR GENE THERAPY AND METHODS OF USE THEREOF		01/28/2020
O'RIORDAN, CATHERINE	63483075	Not Issued		20	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY		02/03/2023
O'RIORDAN, CATHERINE	63494483	Not Issued		20	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY		04/05/2023
O'RIORDAN, CATHERINE	63588417	Not Issued		20	TARGETED GENE THERAPY FOR DM-1 MYOTONIC DYSTROPHY		10/11/2023
O'RIORDAN, CATHERINE E.	09604349	Not Issued		161	CHROMATOGRAPHIC PURIFICATION OF ADENOVIRUS	SALIMI,ALI	06/27/2000
O'RIORDAN, CATHERINE E.	10470804	7015028	20040152183	250	PURIFICATION OF ADENOVIRUS AND AAV	SALIMI,ALI	03/03/2004
O'RIORDAN, CATHERINE E.	11179965	7579181	20050244942	250	PURIFICATION OF ADENOVIRUS AND AAV	MOSHER,MARY	07/12/2005
O'RIORDAN, CATHERINE E.	12504073	Not Issued	20100279386	161	PURIFICATION OF ADENOVIRUS AND AAV	MOSHER,MARY	07/16/2009
O'RIORDAN, CATHERINE R	80183396	Not Issued		159	Adenoviral system		02/17/2000
O'RIORDAN, CATHERINE R.	09582509	Not Issued		161	ADENOVIRAL VECTORS WITH MODIFIED CAPSID PROTEINS	GUZO,DAVID	06/26/2000
O'RIORDAN, CATHERINE R.	09601022	Not Issued		161	NUCLEIC ACID DELIVERY VEHICLES	GUZO,DAVID	07/24/2000
O'RIORDAN, CATHERINE R.	10349830	Not Issued	20030180261	161	Polymer-modified viruses	NAFF,DAVID	01/23/2003
O'RIORDAN, CATHERINE R.	12936659	9567382	20110034548	150	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	BURKHART,MICHAEL	10/06/2010
O'RIORDAN, CATHERINE R.	14886385	10821154	20160074474	150	COMPOSITIONS AND METHODS FOR TREATING SPINAL MUSCULAR ATROPHY	SCHULTZ,JAMES	02/08/2015

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
O'RIORDAN, CATHERINE R.	15387455	10035829	20170369541	150	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	BURKHART,MICHAEL	01/03/2017
O'RIORDAN, CATHERINE R.	15544496	10429288	20180180525	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	07/18/2017
O'RIORDAN, CATHERINE R.	16022410	Not Issued	20190056293	161	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	BURKHART,MICHAEL	06/28/2018
O'RIORDAN, CATHERINE R.	16547144	11639887	20200225139	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	08/21/2019
O'RIORDAN, CATHERINE R.	16849042	11603529	20200216848	150	VARIANT RNAi	ZARA,JANE	03/18/2020
O'RIORDAN, CATHERINE R.	17033443	Not Issued	20210121523	161	COMPOSITIONS AND METHODS FOR TREATING SPINAL MUSCULAR ATROPHY	SCHULTZ,JAMES	09/25/2020
O'RIORDAN, CATHERINE R.	18068175	Not Issued	20230322873	30	METHODS TO PRODUCE ROD-DERIVED CONE VIABILITY FACTOR (RDCVF)	CENTRAL,DOCKET	12/14/2022
O'RIORDAN, CATHERINE R.	18189779	Not Issued	20230392149	30	VARIANT RNAi	CENTRAL,DOCKET	03/16/2023
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O'RIORDAN, CATHERINE R.	18304518	Not Issued	20240000886	30	Compositions and Methods for Treating Spinal Muscular Atrophy	CENTRAL,DOCKET	04/21/2023
O'RIORDAN, CATHERINE R.	18506853	Not Issued	0	37	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	11/10/2023
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O'RIORDAN, CATHERINE R.	61618448	Not Issued		159	COMPOSITIONS AND METHODS FOR TREATING SPINAL MUSCULAR ATROPHY		06/01/2013
O'RIORDAN, CATHERINE R.	62581843	Not Issued		159	VARIANT RNAi		09/22/2017

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#10	•••	>	Search: Rose JA Proc Natl Acad Sci U S A. 1966 Jul;56(1):86-92.;	0	18:24:06
#9	•••	>	Search: Rose JA Proc Natl Acad Sci U S A. 1966 Jul;56(1):86-92.; - Articles found by alternative search	1	18:24:06
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“analytical ultracentrifugation” lentivirus empty particle
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"analytical ultracentrifugation" genome resolution
AAV nucleic acid analytical ultracentrifugation
nucleic acid analytical ultracentrifugation
DNA analytical ultracentrifugation

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empty virion analytical ultracentrifugation
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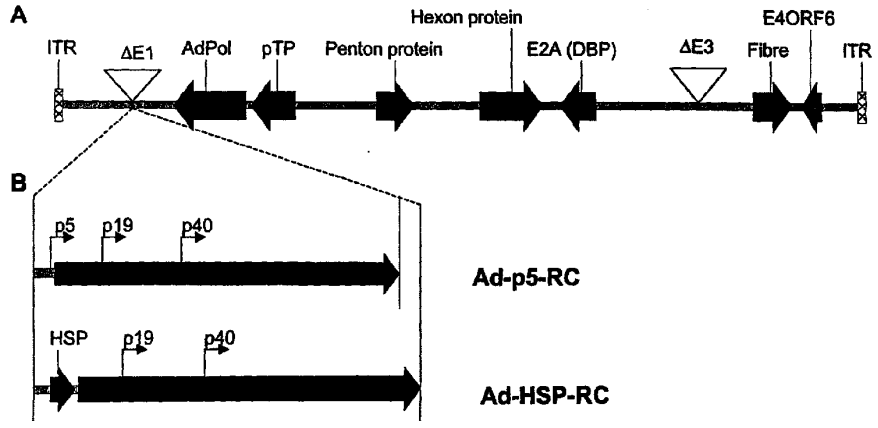
(54) Title: PRODUCTION OF RECOMBINANT AAV USING ADENOVIRUS COMPRISING AAV REP/CAP GENES



(57) Abstract: This invention relates to novel adenoviruses useful in the production of high titers of recombinant adeno-associated virus (rAAV) comprising a foreign DNA insert and methods of making these adenoviruses. The adenovirus comprises the AAV *rep* gene in which the p5 promoter of *rep* is replaced by a minimal promoter or by no promoter. The invention also provides methods of producing high levels of rAAV as a substantially homogeneous preparation and compositions of rAAV.

WO 01/25462 A1

PRODUCTION OF RECOMBINANT AAV USING ADENOVIRUS COMPRISING AAV REP/CAP GENES

TECHNICAL FIELD OF THE INVENTION

This invention relates to novel adenoviruses useful in the production of high titers of recombinant adeno-associated virus (rAAV) comprising a foreign DNA insert and methods of making these adenoviruses. The adenovirus comprises the AAV *rep* gene in which the p5 promoter of *rep* is replaced by a minimal promoter or by no promoter. The invention also provides methods of producing high levels of rAAV as a substantially homogeneous preparation and compositions of rAAV.

BACKGROUND OF THE INVENTION

A recombinant virus carrying a foreign DNA insert may be used to deliver genes to cells, where the gene may be expressed, if desired, to permit production of recombinant proteins *in vitro* or *in vivo*, vaccination of human and non-human mammals, or treatment or amelioration of diseases or genetic defects in humans or in non-human mammals. One may treat or ameliorate diseases or genetic defects by providing some effective level of normal gene products, increased levels

of gene products or by blocking endogenous production of a gene, whose expression would be deleterious to the cell or organism.

Methods for delivering an exogenous gene to a mammalian cell include the use of mammalian viral vectors, such as those that are derived from retroviruses, adenoviruses, herpes viruses, vaccinia viruses, polio viruses, adeno-associated viruses, hybrid viruses (e.g., hybrid adenovirus-AAV, see U.S. Pat. No. 5,856,152) and the like. Other methods include direct injection of DNA, biolistic administration of DNA, electroporation, calcium phosphate precipitation, as well as methods of administration which utilize ligand-DNA conjugates, liposome conjugates of DNA, polycation-DNA complexes or adenovirus-ligand-DNA conjugates.

Adeno-associated virus (AAV) systems have many advantages that can be exploited for delivery of transgenes. AAV is a helper-dependent DNA parvovirus which belongs to the genus *Dependovirus*. AAV requires helper function in order for a productive infection to occur. Helper functions may be provided by a number of agents, but generally co-infection with an unrelated helper virus, either adenovirus, herpesvirus or vaccinia, is used. In the absence of such co-infection, AAV establishes a latent state by insertion of its genome into a host cell chromosome. Subsequent infection by a helper virus rescues the integrated copy which can then replicate to produce infectious viral progeny. AAV has a wide host range and is able to replicate in cells from any species so long as there is also a successful co-infection of such cells with a suitable helper virus. AAV has not been associated with any human or animal disease and does not appear to alter the

biological properties of the host cell upon integration. For a review of AAV, see, e.g., Berns and Bohenzky (1987) Advances in Virus Research (Academic Press, Inc.) 32:243-307.

AAV has a genome of about 4.7 kb in length, including inverted terminal repeats (ITRs) that often, but not necessarily are 145 nucleotides in length. The AAV genome encodes two genes, *rep* and *cap*, each of which expresses a family of related proteins from separate open reading frames and which are produced by alternative mRNA splicing and different transcriptional and translational start sites. Rep polypeptides (Rep78, Rep68, Rep52, and Rep40) are involved in replication, rescue and integration of the AAV genome. Rep78 and Rep68 have the same amino-terminal sequence and share the same promoter, p5, but Rep78 contains an exon that is alternatively spliced out in *rep*68. Similarly, Rep52 and Rep40 have the same amino-terminal sequence and share the p19 promoter, which is downstream from the p5 promoter, but *rep*52 contains an exon that is alternatively spliced out in *rep*68. Cap proteins (VP1, VP2, and VP3) form the virion capsid. *Cap* gene transcription is driven by the p40 promoter. See Fig. 2B for a schematic diagram of the *rep* and *cap* genes and promoters p5, p19 and p40. Flanking the *rep* and *cap* open reading frames at the 5' and 3' ends of the AAV genome are the ITRs. In certain AAV genomes, the ITRs are 145 nucleotides in length, the first 125 bp of which are capable of forming Y- or T- shaped duplex structures. The entire nucleic acid encoding *rep* and *cap* can be excised and replaced with a transgene [B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp.155-168 (1990)]. The ITRs represent the minimal

sequence required for replication, rescue, packaging, and integration of the AAV genome if other sources of *rep* and *cap* are provided.

When AAV infects a human cell, the viral genome integrates into chromosome 19 resulting in latent infection of the cell. Upon introduction of helper functions into the cell, such as by infection with a helper virus, the AAV provirus is rescued and amplified. The rescued AAV genomes are packaged into preformed protein capsids (icosahedral symmetry approximately 20 nm in diameter) and released as infectious virions that have packaged either + or - single stranded DNA genomes following cell lysis.

Replacing the *rep* and *cap* sequences with a desired transgene yields a rAAV capable of delivering the transgene to target host cells. In current methods, the deleted *rep* and *cap* sequences are supplied to the host cells by other viruses or plasmids where they are transiently or stably expressed. There are also a number of cell lines that stably express *rep* and *cap*. The host cells also require helper functions in order for the rAAV to replicate and excise from the host cell genome. The helper functions usually are provided by helper viruses (either wildtype or crippled viruses), plasmids containing the helper virus functions or physical methods.

Although it is known that *rep* is required for replication and excision of AAV, the amount of Rep proteins required for effective rAAV production is, as yet, unclear. U.S. Pat. No. 5,354,678 states that Rep proteins may be toxic to certain cell lines, and WO 97/06272, WO 98/46728 and Li et al. suggest that attenuation of Rep78/68 production results in higher levels of production of rAAV.

In contrast, other art, such as U.S. Pat. No. 5,658,776, explicitly states that high expression of Rep proteins -- a result of replacing the native p5 promoter with a strong promoter, such as the human immunodeficiency virus long terminal repeat (HIV LTR) -- results in high level expression of rAAV. Similarly, U.S. Pat. No. 5,837,484 states that the p5 promoter should be replaced by a strong constitutive promoter or inducible promoter, such as the metallothionein promoter, in order to overcome the strong feedback inhibition by Rep of its own transcription. Thus, U.S. Pat. Nos. 5,658,776 and 5,837,484 suggest that high expression of Rep78/68 is required for efficient rAAV production.

One method that has been used to produce recombinant AAV (rAAV) vectors comprises co-transfecting eukaryotic cells with a plasmid containing rAAV sequences (the *cis* plasmid) and a plasmid containing *rep* and *cap* (the *trans* plasmid), and infecting the cells with a helper virus (e.g., adenovirus or herpes virus). See U.S. Pat. No. 5,753,500. Li et al. (J. Virol. 71:5236-5243, 1997) have modified this method by altering the translation initiation codon of the Rep78/68 proteins in the *trans* plasmid to decrease the translation of the Rep protein and increase production of rAAV. However, the disadvantage of the methods taught by U.S. Pat. No. 5,753,500 and Li et al. is that co-transfection of two plasmids along with infection by a helper virus is inefficient, may exhibit poor reproducibility, may result in generation of pseudo-wildtype replication-competent AAV (rcAAV), and cannot be easily scaled up for industrial production of rAAV. rcAAV, comprising *rep* and *cap* flanked by ITRs, is produced when the *rep* and *cap*

genes recombine with the ITRs flanking the transgene which results in deletion of the transgene.

A second method that has been used to produce rAAV involves co-transfection of three plasmids into eukaryotic cells. In this method, one plasmid carries the transgene and ITRs (the *cis* plasmid), a second plasmid encodes the *rep* and *cap* genes (the *trans* plasmid), and the third plasmid encodes the helper virus functions, i.e. adenoviral genes such as E1a, E1b, E2a and E4 (the *helper* plasmid). The disadvantages of the first method are shared with this method.

A third method involves the use of a packaging cell line such as one including AAV functions *rep* and *cap*. See U.S. Pat. Nos. 5,658,785 and 5,837,484 and PCT US98/19463. The packaging cell line may be transfected with a *cis* plasmid comprising the transgene and ITRs, and infected by wild-type adenovirus (Ad) helper. See U.S. Pat. No. 5,658,785. Alternatively, the packaging cell line may be co-infected by a hybrid Ad/AAV, in which a hybrid Ad vector carries the *cis* plasmid in the E1 locus (see U.S. Pat. No. 5,856,152), and by a wild-type or mutant Ad that supplies E1. See, e.g., Reference 7. The disadvantage of this method is that it requires making a cell line that expresses sufficient levels of *rep* and *cap*, and requires multiple components -- including the cell line, the rAAV genome, and an adenovirus -- to produce rAAV, which do not lend themselves to easy and convenient downstream manufacturing processes. In addition, some of these packaging cell lines do not produce high levels of rAAV.

A fourth method is provided by a prophetic example in U.S. Pat. No. 5,354,678. The method involves using a recombinant adenovirus in which the *rep*

and *cap* genes of AAV replace a part of the adenovirus genome not essential for helper virus functions. In this method, an AAV/EBV plasmid vector comprising an rAAV genome is introduced into a cell to produce an rAAV producer cell. It is presumed that the *rep* gene is driven by its native p5 promoter or by a strong inducible promoter. The recombinant adenovirus comprising *rep* and *cap* is then introduced into the cell and their production is induced such that rAAV is produced by the cells. U.S. Pat. No. 5,354,678 does not disclose the levels of rAAV, if any, produced by this method.

As described above, current rAAV production methods are not amenable for production of sufficient rAAV for pharmaceutical applications in a convenient manner. However, the problem of reproducibly generating high levels of substantially homogeneous replication-deficient rAAV by an efficient method that is applicable to large-scale industrial production is solved by the present invention.

SUMMARY OF THE INVENTION

The instant invention provides an alternative production method that results in high yields of rAAV vector and is amenable to large-scale industrial applications. The invention provides a novel adenovirus vector comprising *rep* and *cap* genes, thus providing AAV *rep* and *cap* and adenovirus helper functions in one component. In the adenovirus vector of the instant invention, the native AAV p5 promoter upstream of *rep* is removed and replaced with a minimal promoter or with no promoter. This novel vector, when infected into cells containing a nucleic acid

sequence comprising a transgene flanked by AAV ITRs, results in the production of high levels of rAAV. The nucleic acid sequence comprising the transgene flanked by AAV ITRs may be established in the host cell by stable integration into the host cell chromosome, secondary infection with an adenovirus or other viral vector carrying the transgene flanked by ITRs (see, e.g, U.S. Pat. No. 5,856,152), infection with an rAAV comprising the transgene, or any other method known in the art, such as transfection, lipofection or microinjection, of plasmid DNA comprising the transgene flanked by ITRs.

In one embodiment of the invention, *rep*, operably linked to a minimal promoter or to no promoter, is inserted into either the E1 or E3 regions of an adenovirus. The adenovirus is deleted in E1 or E3 alone, or a combination of both. In another embodiment, the adenovirus vector is further deleted in E4. In this embodiment, *rep* sequences may be inserted in E4, while upstream of these *rep* sequences there may be no promoter or a minimal promoter. In a preferred embodiment, *cap* is inserted along with the *rep* gene into the adenoviral vector. In another aspect of the invention, the adenoviral vector comprising the minimal promoter or promoterless *rep* is used in a method to produce rAAV. The advantage of this method is that it is easily scaled for industrial production of rAAV.

In the method of the invention, the host cell is supplied with an rAAV genome, and the adenovirus comprising the minimal promoter or promoterless *rep* is infected into the cell. In one embodiment of the invention, the host cell is either simultaneously or sequentially co-infected with two adenoviruses,

wherein one adenovirus comprises *cap* and *rep* driven from a minimal promoter or no promoter, as described above, and the other adenovirus comprises an rAAV genome. In another preferred embodiment, an adenovirus comprising *cap* and *rep* driven from a minimal promoter or no promoter is used to infect a host cell comprising a stably expressed rAAV genome.

In a preferred embodiment of the invention, the method is one in which a high titer of substantially homogeneous rAAV lysates and stocks is achieved.

In any of these embodiments, the host cell may stably express those adenoviral sequences that are deleted from the adenovirus comprising *rep* and *cap*. For instance, a cell line such as 293 cells, which express E1, 84-31 cells, which express E1 and E4 (Ref. 1), or 10-3 cells, which express E1 and E4ORF6 (Ref. 11), may be used. Alternatively, a second helper virus is co-infected into the host cell and expresses those adenoviral sequences deleted from the adenovirus comprising *rep* and *cap*. For instance, if a second adenovirus comprising a transgene cassette is used to infect the host cell, this adenovirus could supply the deleted adenoviral sequences.

In another embodiment of this invention, the recombinant virus carrying the *rep* gene may be any virus in which *rep* interferes with its replication. In this embodiment, the recombinant viral vector comprises a *rep* gene in which the native p5 promoter of *rep* is removed and replaced with a minimal promoter or with no promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Construction of recombinant shuttle plasmids pAV2cisEGFP, pAd-p5-RC and pAd-HSP-RC.

Fig. 2A. Genome of the parental E1/E3 deleted adenovirus.

Fig. 2B. Schematic diagrams of the recombinant adenoviruses Ad-p5-RC and Ad-HSP-RC showing insertion of p5-rep-cap or HSP-rep-cap DNA sequences into the E1 locus of the parental viral genome to generate Ad-p5-RC or Ad-HSP-RC, respectively.

Fig. 3A. Schematic diagram of the *rep-cap* insert in the E1 locus of Ad-p5-RC or Ad-HSP-RC showing the location of PCR primers relative to the viral genome and expected PCR DNA products I, II, III, and IV. PCR analysis is used to determine the integrity of AAV *rep-cap* DNA sequences inserted into the adenovirus genome.

Fig. 3B. Ethidium bromide stained agarose gel of PCR products using primers whose locations are shown in Fig. 3A. Lanes 1, 3, 5, 7 are PCR products from viral DNA of Ad-p5-RC. Lanes 2, 4, 6, 8 are PCR products from viral DNA of Ad-HSP-RC. M, 1 kb DNA ladder size marker (Gibco BRL).

Fig. 4. Production of rAAV after infection of 293-CG3 cells with Ad-HSP-RC.

Fig. 5. Production of rAAV after co-infection of 293 cells with Ad-HSP-RC and Ad-AAV-LacZ.

Fig. 6. Time-course study of rAAV production after co-infection of 293 cells with Ad-HSP-RC and Ad-AAV-LacZ.

Fig. 7. Multiplicity of infection study of rAAV production after co-infection of 293 cells with Ad-HSP-RC and Ad-AAV-LacZ.

Fig. 8. Ethidium bromide stained agarose gel of Hirt DNA fractionated to detect replicating rAAV DNA in 293 cells (lanes 1-5) or in control B50 cells (lane 6). Lane 1, Ad-p5-RC infection of 293 cells; lane 2, Ad-HSP-RC infection of 293 cells; lane 3, Ad-AAVLacZ infection of 293 cells; lane 4, Ad-HSP-RC and Ad-AAV-LacZ co-infection of 293 cells; lane 5, Ad-p5-RC and Ad-AAV-LacZ co-infection of 293 cells; lane 6, sub100r and Ad-AAV-LacZ stepwise infection of B50 cells. M, 1 kb DNA Ladder size marker (Gibco BRL).

Fig. 9A. Ethidium bromide stained agarose gel of Hirt DNA samples from 293 cells (lanes 2-6) or from control B-50 cells (lane 7) DNA samples.

Fig. 9B. Southern blot analysis of the gel shown in Fig. 9A hybridized to a *lac*Z DNA probe. Lane 1, *lac*Z DNA fragment as a positive control; lane 2, Ad-HSP-RC infection of 293 cells; lane 3, Ad-p5-RC infection of 293 cells; lane 4, Ad-AAVLacZ infection of 293 cells; lane 5, Ad-HSP-RC and Ad-AAV-LacZ co-infection of 293 cells; lane 6, Ad-p5-RC and Ad-AAV-LacZ co-infection of 293 cells; lane 7, sub100r and Ad-AAV-LacZ stepwise infection of B50 cells. M, 1 kb DNA Ladder size marker (Gibco BRL)

Fig. 10. Western blot analysis of Rep and Cap protein expression in 293 cells infected with or Ad-p5-RC or Ad-HSP-RC viruses. Lane 1, Ad-p5-RC alone; lane 2, Ad-p5-RC + Ad-AAV-LacZ; lane 3, Ad-HSP-RC alone; lane 4, Ad-AAV-LacZ alone; lane 5, Ad-HSP-RC + Ad/AAV-LacZ.

DETAILED DESCRIPTION OF THE INVENTION

The instant invention relates to a novel adenoviral vector and a method for producing high titer stocks of rAAV using this vector. The adenoviral vector comprises a *rep* gene in which the native AAV p5 promoter upstream of the *rep* coding sequences has been deleted or effectively rendered inactive by mutation or partial deletion and replaced by a minimal promoter or no promoter.

Although decreasing maximal production of Rep78 and Rep68 may increase rAAV production (see WO 97/06272 and WO 98/46728), there has been no evidence that one could obtain rAAV production if one replaced the p5 promoter with either a minimal promoter that promotes only basal expression of Rep78/68 or with no promoter, i.e., removing the promoter altogether and incorporating *rep* into an adenovirus.

In a preferred embodiment, Rep78 and Rep68 are produced at much lower levels than Rep52 and Rep40 in 293 cells or other E1-complementing cell lines infected with an adenovirus containing a minimal promoter driving expression of rep78 and rep68. See, e.g., Example 9 and Fig. 10. In another preferred embodiment, host cells are infected with an adenovirus vector comprising a *rep* gene that lacks any promoter. Although the exact amounts of Rep78 and Rep68 protein expressed in host cells infected by an adenovirus lacking any promoter upstream of *rep* coding sequences are unknown, it is expected that Rep78 and Rep68 protein levels would be expressed from this recombinant adenovirus at much lower levels than Rep52 and Rep40 or at levels much lower than that expressed by wildtype AAV during infection.

In one embodiment of the invention, the total amount of Rep78 and Rep68 protein is less than 80%, more preferably less than 50%, of the total amount of Rep52 and Rep40 produced in the infected cells. In a more preferred embodiment, the total amount of Rep78 and Rep68 is less than 25% of the total amount of Rep52 and Rep40 produced in the infected cells. In an even more preferred embodiment, the total amount of Rep78 and Rep68 is less than 15%, more preferably 10%, and more preferably is less than 5% of the total amount of Rep52 and Rep40 produced in the infected cells. One may measure the amount of Rep proteins by any method known in the art. These methods include, without limitation, immunoprecipitation of metabolically labeled Rep proteins followed by separation on SDS polyacrylamide gel electrophoresis and quantitation of the labeled protein, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunofluorescence of infected cells, and quantitative Western blot analysis using radioactive or enzymatic labeling of an anti-Rep antibodies.

Experiments performed using an adenovirus vector comprising a promoterless *rep* gene demonstrate that the infected host cells also produce rAAV. Without wishing to be bound by any theory, it is possible that in the absence of a promoter upstream of *rep*, low-level transcription of Rep78 and Rep68 may occur from the upstream ITR of adenovirus or from sequences downstream of the ITR that are upstream of the *rep* gene.

The instant invention demonstrates that adenoviral vectors comprising a *rep* gene with its native p5 promoter are unstable when infected into host cells while adenoviral vectors comprising a *rep* gene with a minimal promoter

or no promoter are stably propagated in host cells. See Example 4 and Figs. 3A and 3B. Example 4 demonstrates that Ad-p5-RC, which is an adenovirus containing p5, *rep* and *cap* in the E1 site of the adenovirus vector, undergoes a rearrangement or deletion event in the *rep-cap* DNA sequences of the adenovirus vector when passaged in 293 cells. In contrast, Ad-HSP-RC, which contains a minimal heat shock protein promoter (HSP), *rep* and *cap*, is stable after insertion into the E1 locus of the adenoviral genome and does not appear to undergo any rearrangement when passaged in the same cells. In a preferred embodiment, a minimal promoter or promoterless *rep*-containing adenovirus of the instant invention is one which is stable upon propagation in a defined host cell system, such as 293 cells.

The deletion or rearrangement of *rep* and *cap* is further borne out by Examples 8-9 and Figs. 8-10. Southern blot analysis demonstrates that Hirt DNA from 293 cells co-infected with Ad-HSP-RC and Ad-AAV-LacZ (an adenoviral vector in which a transgene cassette comprising lacZ flanked by AAV ITRs is inserted in E1 of adenovirus) contains LacZ DNA sequences in AAV replicating form (RF) DNA, while Hirt DNA from 293 cells co-infected with Ad-p5-RC and Ad-AAV-LacZ does not contain LacZ DNA sequences in AAV RF DNA. In addition, 293 cells express Rep and Cap proteins when co-infected with Ad-HSP-RC and Ad-AAV-LacZ (see lane 5 of Fig. 10), but do not express these proteins when co-infected with Ad-p5-RC and Ad-AAV-LacZ (see lane 4 of Fig. 10).

The effect of the deletion or rearrangement in Ad-p5-RC is shown by the levels of rAAV produced using this adenovirus vector. Little or no

replicating rAAV is produced in cells co-infected with Ad-p5-RC and Ad-AAV-LacZ, while replicating rAAV is observed in 293 cells co-infected with Ad-HSP-RC and Ad-AAV-LacZ. See Example 8 and Figs. 8, 9A and 9B. Similarly, sufficient amounts of replicating rAAV is produced in cells that have been infected with an adenovirus vector comprising *rep* sequences downstream of no promoter.

Definitions and General Techniques

Unless otherwise defined, all technical and scientific terms used herein have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, virology and immunology. See, e.g., Sambrook et al., 1989, Ausubel et al., 1992, Harlow et al. 1989 (which are incorporated herein by reference).

A "recombinant adeno-associated virus (rAAV) genome" comprises all or a part of an AAV genome, wherein the viral genome may be wild type or may contain point mutations or deletions, and optionally comprises a transgene operably linked to expression control sequences. The transgene may be regulated in *cis* or in *trans*. In a preferred embodiment, the rAAV genome comprises a transgene flanked by AAV inverted terminal repeats (ITRs). The rAAV genome of the invention may be embedded in the genome of an adenovirus vector to form a hybrid Ad/AAV. See U.S. Pat. No. 5,586,152, herein incorporated by reference. Alternatively, the rAAV genome may be introduced into a host cell by any route known in the art.

The rAAV genome can be expressed transiently or stably in the host cell.

A "recombinant adeno-associated virus" or "rAAV" is the AAV derived from the rAAV genome described above. The rAAV preferably comprises a transgene. The rAAV comprising a transgene is capable of transducing mammalian cells and delivering the transgene thereto.

A "flanking element" or "flanking nucleic acid" is a nucleic acid sequence which, when located in positions flanking a transgene, permits the packaging of the transgene into an rAAV. The flanking elements of AAV are inverted terminal repeats (ITRs). Flanking elements may be the naturally-occurring ITRs from any one of AAV serotypes 1-6 or may be artificial nucleic acid elements, e.g. mutated sequences of ITRs, that have the same or equivalent packaging function.

A "transgene" is a nucleic acid sequence that is to be delivered or transferred to a mammalian cell. A transgene may encode a protein, peptide or polypeptide that is useful as a marker, reporter or therapeutic molecule. The transgene also may be a selection gene, such as one for antibiotic resistance. A transgene may also encode a protein, polypeptide or peptide that is useful for protein production, diagnostic assays or for any transient or stable gene transfer *in vitro* or *in vivo*. Alternatively, a transgene may not encode a protein but rather be used as a sense or antisense molecule, ribozyme or other regulatory nucleic acid to modulate replication, transcription or translation of a nucleic acid to which it is complementary or to target a complementary mRNA for degradation.

"Expression control sequences" are nucleic acid sequences that

regulate the expression of a gene by being operably linked to the gene of interest.

"Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion.

A "transgene cassette" is a nucleic acid sequence comprising a transgene operably linked to expression control sequences in which the transgene and expression control sequences are flanked by AAV flanking sequences. In a preferred embodiment, the flanking sequences are AAV ITRs.

An "adenovirus genome" is the nucleic acid molecule backbone of an adenovirus particle. The adenovirus genome may contain point mutations, deletions or insertions of nucleotides. The adenovirus genome may further comprise a foreign gene.

An "adenovirus" is an encapsidated adenovirus genome capable of binding to a mammalian cell and delivering the adenovirus genome to the cell's nucleus. The term "adenovirus" encompasses both recombinant and non-recombinant adenoviruses. The term "adenovirus" also encompasses both wildtype and mutant adenoviruses.

A "recombinant adenovirus" is an adenovirus which contains one or more genes that are foreign to a wildtype adenovirus. Recombinant adenoviruses include, without limitation, those that include foreign genes such as *rep* and/or *cap*, as well as adenoviruses that comprise an rAAV genome.

An "adenovirus vector" is a recombinant adenovirus comprising one or more foreign genes, wherein the adenovirus vector is capable of binding to a mammalian cell and delivering the foreign gene to the cell's nucleus. The foreign genes include, without limitation, genes such as *rep* and *cap*, rAAV genomes, such as transgenes and expression control sequences, or any foreign gene that is useful in increasing production of rAAV.

A "locus" is a site within a virus wherein a particular gene normally resides. For instance, the "adenovirus E1 locus" is the site at which E1 resides in adenovirus. If a foreign gene or nucleic acid is inserted into a locus, it may either replace the gene that resides there or it may be inserted at the site in addition to the gene that resides there.

An "AAV p5 promoter" or "p5 promoter" is one that is derived from any AAV serotype, including AAV Serotypes 1 to 6, as well as any AAV that infects non-human species, such as avian and bovine AAV. The p5 promoter of AAV-2 directs the expression of rep78 and rep68, and is downregulated by the Rep protein, and is upregulated by certain adenoviral proteins, including E1.

As used herein, the term "deleted p5 promoter" refers to a p5 promoter that has been completely deleted from the AAV genome or to a p5 promoter that has been effectively deleted or attenuated such that the p5 promoter

is less active when compared to the wildtype p5 promoter in promoting transcription in a cell into which it has been introduced. The p5 promoter may be effectively deleted or attenuated by any method, including, without limitation, removing or mutating a sufficient number of nucleotides to render the p5 promoter less active or inactive or moving the promoter relative to the coding sequences of *rep* such that the promoter is less active. In an alternative embodiment, one may increase the distance between the p5 promoter and the start codon of the *rep* gene to decrease promoter activity. For instance, one may move the p5 promoter downstream of the *rep* gene or insert nucleotide sequences between the p5 promoter and the downstream ATG start codon. One may measure whether a p5 promoter is effectively deleted by measuring the transcription of a gene operably linked to the mutated or partially deleted p5 promoter and comparing the gene's transcription to the transcription of a gene in a promoterless construct.

In a preferred embodiment, a p5 promoter is effectively deleted when it promotes less than 80% of wildtype p5 promoter activity, more preferably less than 50% of wildtype p5 promoter activity. In a more preferred embodiment, a p5 promoter is effectively deleted when it promotes less than 25% of wildtype p5 promoter activity. In an even more preferred embodiment, a p5 promoter is effectively deleted when it promotes less than 15%, more preferably promotes less than 10%, and more preferably less than 5% of wildtype p5 promoter activity. In another preferred embodiment, a p5 promoter is effectively deleted when the *rep* gene to which it is operably linked is not rearranged or deleted when an adenovirus comprising the effectively deleted p5 promoter and *rep* gene is infected into a host

cell, such as 293 cells. In another preferred embodiment, a p5 promoter is effectively deleted when a host cell infected with an adenovirus comprising *rep* and the deleted p5 promoter produces rAAV at a high titer. In a preferred embodiment, the titer is at least 10^2 particles per cell; preferably at least 10^3 particles per cell; more preferably at least 10^4 particles per cell; and, even more preferably, at least 10^5 or 10^6 particles per cell. In general, there are approximately 1 x 10^3 to 3 x 10^3 particles per transducing units (TU). The number of particles required to produce one TU varies based upon the transgene, purification method and assay method.

A "minimal promoter" is one that essentially comprises only a TATA box and promotes only very low or basal levels of transcription of *rep*78 and *rep*68. A promoter is a nucleotide sequence that promotes the initiation of transcription at a particular site by the cell's transcriptional machinery.

In a preferred embodiment, a minimal promoter promotes transcription that is less than 80% of the wildtype p5 promoter, more preferably less than 50% of the wildtype p5 promoter, even more preferably less than 25% of the wildtype p5 promoter. In a more preferred embodiment, a minimal promoter is one that promotes transcription that is less than 20% of the wildtype p5 promoter, even more preferably less than 15% of the wildtype p5 promoter. In an even more preferred embodiment, a minimal promoter is one that promotes transcription that is less than 10% of the wildtype p5 promoter, even more preferably less than 5%, even more preferably less than 1% of the wildtype p5 promoter.

A minimal promoter also may be defined by functional measures. A minimal promoter is one in which the *rep* gene to which it is operably linked is not

rearranged or deleted when an adenovirus comprising the minimal promoter and *rep* gene is infected into a host cell, such as 293 cells. A minimal promoter is one in which a host cell infected with an adenovirus comprising a minimal promoter that regulates transcription of *rep*78 and *rep*68 produces rAAV at a high titer. In a preferred embodiment, the titer is at least 10^2 particles per cell; preferably at least 10^3 particles per cell; more preferably at least 10^4 particles per cell; and, even more preferably, at least 10^5 or 10^6 particles per cell.

Many minimal promoters are known in the art. Alternatively, an artificial minimal promoter may be constructed by using a sequence or a consensus sequence of a TATA box and adding nucleotide sequences to the 5' and 3' ends of the TATA box. The activity of the minimal promoter may be measured by measuring the transcription of the artificial minimal promoter and comparing it to an natural minimal promoter, such as the Drosophila heat shock protein promoter.

*Rep*78/68 is "promoterless" or has "no promoter" when the p5 promoter has been deleted or effectively deleted, as defined *supra*, and no promoter has been inserted in its place. Alternatively, *rep*78/68 is promoterless when the p5 promoter has been deleted and is replaced by a heterologous promoter that does not promote transcription in the host cell in which the adenovirus has been infected. For instance, *rep*78/68 would be considered promoterless if p5 were substituted by a promoter that was active in bacterial or insect cells, for example, but that was inactive in a mammalian host cell. In another embodiment, *rep*78/68 is promoterless when the p5 promoter has been deleted and replaced by an inducible promoter that permits low-level expression of *rep*78/68.

The Adenoviral Vector

A large number of adenoviruses and adenoviral vectors are known, including human adenoviruses types 1-46, chimpanzee adenoviruses, canine adenoviruses, bovine adenoviruses [all available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209], and ovine adenoviruses (Both et al., WO 97/06826 A1). Any of these adenoviruses may be used in this invention, provided that the adenovirus is able to infect the target host cell. For instance, a human adenovirus would generally be used to infect a human cell, while a bovine adenovirus would be used to infect a bovine cell.

In one embodiment, the adenoviral vector comprises the AAV *rep* gene downstream of a minimal promoter or no promoter (this is alternatively referred to as a minimal promoter or promoterless AAV *rep* gene), and sufficient helper virus functions for rAAV production in a host cell. In a preferred embodiment, the adenoviral vector further comprises the AAV *cap* gene. The type of adenoviral sequences required for replication and encapsidation of the rAAV genome depends upon whether the host cell expresses any helper functions or whether other vectors or viruses are introduced into the host cell which express helper functions. For instance, if the adenovirus is to be used to infect a cell line that expresses E1, e.g., the 293 cell line, then the adenoviral vector could comprise *rep* and *cap*, and could also comprise those helper virus functions required in addition to E1 (e.g., E2a, E4ORF6 and VAI RNA). If the adenovirus is used to infect a cell line such as 84-31, which expresses E1 and E4, then the adenoviral vector could express *rep*, *cap*, E2a and VAI RNA. If the adenovirus is used to

infect a cell line that does not express any helper functions, then the adenovirus vector could comprise, at least, E1 (both E1a and E1b) and E2a, and, optionally, may comprise E4ORF6 and VAI RNA. In an alternative embodiment, helper functions may be supplied by chemical or physical methods or by other helper viruses.

The recombinant adenovirus comprising the *rep* gene downstream of a minimal promoter or no promoter may be produced by any method known in the art. In one preferred method, the recombinant adenovirus of the instant invention is produced using homologous recombination. In another preferred method, the recombinant adenovirus is produced using Cre-*lox* recombination (12).

In an alternative embodiment, some or all of the helper virus functions may be provided by nucleic acid sequences that are introduced into the host cell. For instance, the host cell may be co-infected with a second virus, such as an adenovirus, that expresses some or all of the required helper functions. In a preferred embodiment, a second adenoviral vector comprises a transgene cassette and further comprises helper functions that are not expressed by either the host cell or the adenovirus comprising the *rep* gene. See, e.g., Examples 6-8. Alternatively, some or all of the helper virus functions may be provided by any method known in the art, such as by transfection or direct injection, as discussed above.

Based on this description, other embodiments of the adenoviral vector will be readily apparent to those of ordinary skill in the art. Other viral vectors in which Rep interferes with viral replication also may be used.

Rep and *Cap* Nucleic Acids

In AAV's life cycle, both *rep* and *cap* are required for excision, replication and encapsidation of the recombinant viral genome into an infectious recombinant vector or virus. The Rep and Cap proteins may have a naturally occurring sequence derived from any serotype of AAV, including serotypes 1 to 6. In one preferred embodiment, the *rep* and *cap* genes are derived from the same AAV serotype. In another preferred embodiment, the *rep* and *cap* genes are derived from different AAV serotypes to permit the production a pseudotyped rAAV. Pseudotyped rAAV is desirable in cases in which the rAAV is to be administered to a patient as a gene therapy vector and there are existing neutralizing antibodies in the patient's serum to the capsid proteins of one AAV serotype and not to another AAV serotype. The *cap* gene from the serotype to which there is an antibody response may be exchanged by the *cap* gene from a different serotype of AAV to which there is no antibody response. For example, the *rep* gene from AAV-2 may be used with the *cap* gene from AAV-1 to produce a pseudotyped rAAV-2, or *vice-versa*.

In an alternative embodiment, the Rep and/or Cap proteins may have a mutated sequence, including insertions, deletions, fragments or point mutations of particular amino acid residues, so long as the mutated Rep and/or Cap proteins retain their respective excision, replication and encapsidation functions. In a preferred embodiment, the naturally-occurring Rep and Cap amino acid sequences from AAV- 2 are used. In one embodiment, a single adenovirus comprises the nucleic acid sequences encoding the Rep and Cap proteins. In another embodiment,

the nucleic acid sequences encoding the Rep and Cap proteins are inserted at a single site within one adenovirus, e.g., both *rep* and *cap* are inserted at E1, E3 or E4 of adenovirus. Alternatively, the nucleic acid sequences encoding Rep and Cap, respectively, are each inserted at different loci in the adenovirus genome, e.g., the nucleic acid sequence encoding Rep may be in E1, and the nucleic acid sequence encoding Cap may be in E4, and other combinations thereof. Alternatively, a cell line comprising the *cap* gene may be used, in which case only the *rep* gene would be required on the adenoviral vector. See, e.g., WO 98/27204.

The minimal promoter that regulates expression of Rep78 or Rep68 may be any promoter that promotes only basal expression of the *rep* gene in a host cell. In general, the promoter is one that essentially contains a TATA box as its only regulatory element. In a preferred embodiment, the minimal promoter is the Drosophila heat shock promoter (HSP). In another preferred embodiment, the minimal promoter is the minimal promoter derived from the adenovirus E1b gene that provides only basal promoter activity. In another preferred embodiment, the minimal promoter is a 70 nucleotide DNA element derived from the promoter region upstream of the adenovirus pIX gene. The minimal pIX promoter comprises a TATA box and an Sp1 box, and, in Ad5, corresponds to nucleotides 3511 to 3580. Many other adenovirus serotypes contain the pIX gene and its upstream promoter as well, and the minimal promoters derived from these pIX promoters are encompassed by this invention as well. Other minimal promoters are well known in the art and may be used in the practice of this invention. In another preferred embodiment, the p5 promoter is deleted altogether and replaced by no promoter at

all.

In another embodiment of the invention, the activity of the Rep78/68 proteins are attenuated by mutating the *rep*78/68 genes to produce Rep78/68 proteins that are less active than wildtype Rep78/68. This may be done by altering the coding sequence of the Rep78/68 proteins to make them less active. Alternatively, one may alter the DNA sequence of *rep*78/68 to destabilize the RNA encoded by the gene and thus decrease the amount of Rep78/68 proteins produced.

One may determine whether a DNA sequence is appropriate for use as a minimal promoter by inserting the DNA sequence upstream of the *rep*78/68 ATG codon in a plasmid construct or an adenoviral vector and transfecting or infecting, respectively, a host cell that comprises an rAAV genome, incubating the host cell under conditions in which rAAV is produced, measuring the titer of rAAV produced, and comparing the titer to that produced using a control plasmid or adenovirus comprising a *rep* gene whose expression is regulated by a minimal promoter. The host cell may comprise the rAAV genome stably or transiently. Alternatively, one may measure the level of Rep78 and Rep68 produced in the host cell after infection to determine if sufficiently low levels of Rep78 and Rep68 are produced.

Helper Functions

As discussed above, AAV requires helper functions for excision, replication and encapsidation of AAV. AAV helper functions can be provided by adenovirus, herpesvirus [including herpes simplex virus type 1 (HSV-1) or type 2

(HSV-2), cytomegalovirus (CMV) and pseudorabies virus (PRV)] or by exposure of the cells to different chemical or physical agents. Alternatively, one of skill in the art may determine which helper functions are required by producing rAAV using the compositions and methods disclosed in the instant specification.

To identify which helper functions are required for high levels of rAAV production, one may transfect a host cell containing an rAAV genome with a plasmid comprising *rep* and *cap* and then transfect with one or nucleic acids encoding various potential helper functions to determine which potential helper functions are required for rAAV production. The rAAV genome may be stably integrated into the host cell or may be transfected or infected into the host cell by methods known in the art. After transfecting the host cell with the nucleic acid encoding the potential helper function, one may then measure the titer of the rAAV that is produced to determine if the nucleic acid encodes a helper function.

In a preferred embodiment, the helper functions are nucleic acids derived from a virus. In a more preferred embodiment, the helper functions are derived from adenovirus types 2 or 5, HSV-1, HSV-2, CMV or PRV. In an even more preferred embodiment, the helper functions are E1a, E1b, E2a, E4ORF6 proteins and VAI RNA from adenovirus. In another preferred embodiment, the nucleic acid encodes the helper functions from the helicase-primase complex of HSV (UL5, UL8 and UL52) and the major single-stranded DNA binding protein of HSV (UL29). Alternatively, helper functions for recombinant AAV may be provided by chemical or physical agents, including ultraviolet light, cycloheximide, hydroxyurea and various carcinogens.

The required helper functions for production of a rAAV may be delivered to the host cell by any method known in art. The helper functions may be delivered by transfection with a vector, such as a plasmid, by infection with a viral vector comprising the helper functions, or by any other method known in the art, including those discussed above (e.g., biolistic injection of DNA, use of DNA conjugates, etc.). The transfection or infection may be stable or transient. Alternatively, the cell line may stably express (either on an extrachromosomal episome or through integration in the cell's genome) the helper functions. In addition, some of the helper functions may be expressed by the mammalian cell line while other helper functions are introduced by a vector. Thus, for production of rAAV in 293 cells (ATCC CRL-1573), which constitutively produce adenoviral E1a and E1b proteins, only E2a, E4ORF6 and VAI must be introduced into the host cell by transfection or infection of a vector.

In a preferred embodiment, the helper functions are transduced into the host cells by an adenovirus. In a more preferred embodiment, some or all of the helper functions are transduced into the host cell by the adenovirus that comprises the *rep* and/or *cap* genes. In a preferred embodiment, the native helper function sequences are used. However, mutated helper function sequences may be used so long as they retain their helper function activity. The helper function nucleic acids may be supplied with its native promoter or may be under the regulatory control of a variety of promoters, constitutive or inducible, such as the CMV immediate-early promoter/enhancer or the zinc-inducible metallothionein promoter, respectively, as known in the art or as described above.

The rAAV Transgene Cassette

In order to manufacture a rAAV containing a transgene, the method of the present invention begins with a desired transgene, then associates the transgene with appropriate expression regulatory sequences (ERS), e.g., promoter, enhancer, polyadenylation site, then inserts this ERS-transgene construct between AAV flanking sequences, e.g., the ITRs, in place of *rep* and *cap* genes normally found therein. Where the length of the ERS-transgene cassette is shorter than the AAV *rep* and *cap* sequences, and that shorter length would pose an obstacle to proper packaging, an optional spacer or "stuffer" sequence may be inserted in order to maintain the proper length for packaging. The transgene cassette comprised of the ERS-transgene bordered by the AAV flanking sequences may then be embedded in an adenovirus vector separate from that which carries the *rep* gene. Alternatively, the transgene cassette may be inserted into a plasmid vector and transfected into a host cell. The transgene cassette may be maintained in the host cell stably, either by integration into the host cell genome or as an episome, or may be introduced transiently, such as by infection with a hybrid Ad/AAV virus. See, e.g., Examples 3 and 6-8. Each element of the transgene cassette is further described below:

The Transgene

A transgene is a nucleic acid encoding a protein of interest; it may be a gene to allow for genetic or drug selection, e.g., a gene conferring resistance to antibiotics, or a reporter gene allowing detection, e.g., by color in the case of the

use of green fluorescent protein. Alternatively, the transgene may be one that is useful for corrective applications. For instance, a transgene may be a normal gene that replaces or augments the function of a patient's defective gene. The transgene may be one that counteracts the effects of a disease, such as introduction and expression of a gene that is distinct from the one that it replaces or augments, but which has the same function or compensates for the defective gene's function. The transgene may be a gene which blocks or represses the expression of a malfunctioning, mutated, or viral gene in the patient, thereby giving rise to a corrective effect. A transgene may also be a protective gene, such as one that prevents cellular apoptosis, injury, toxicity or death. A transgene may also be used for immunization against various agents, by provoking an immunogenic response in an animal. Delivery of therapeutic transgenes to a patient thus effects some level of correction of a defect or is beneficial for prevention of disease. The transgene also may be a gene which would confer sensitivity to a reagent that results in cellular toxicity, e.g., introduction of HSV thymidine kinase, which confers sensitivity to gancyclovir. The transgene also may be one which is useful for production of proteins *in vitro*, such as for large-scale production of therapeutic proteins.

Many gene therapy methods involve supplying an exogenous gene to overcome a deficiency in the expression of a gene in a patient. Some of these deficiencies are congenital and are due to a mutation in a particular gene in all the cells of the patient. For instance, in cystic fibrosis, there are one or more mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) which prevents the CFTR protein from functioning properly. In other

cases, a deficiency in gene expression is due to an accident or disease that occurs during the patient's life. For instance, in Type I diabetes mellitus, the β pancreatic islet cells, which produce insulin, are destroyed, such that patients with this disease can no longer synthesize insulin. In other cases, the endogenous gene may be structurally normal but is not transcribed and/or translated in high enough quantities due to disease, medical treatment or other environmental conditions, or mutations in the regulatory elements of the endogenous gene. For example, there are a number of blood disorders, such as anemia, in which there is insufficient production of red blood cells, which may be treated with erythropoietin (EPO) or with a transgene encoding EPO. Conversely, gene therapy methods may be used where overexpression of a particular gene results in a disease state. For instance, overexpression of c-*myc* by the immunoglobulin heavy chain promoter results in leukemia. Transgenes may also be used for genetic immunization, i.e., to elicit an immune response to a pathogen in an animal, including humans. For instance, a transgene may include a sequence from a viral, bacterial or fungal pathogen, such as influenza virus, human immunodeficiency virus (HIV), or mycobacterium tuberculosis.

Appropriate genes for expression in the cell include, without limitation, those genes which are normally expressed in cells but whose products are produced in abnormal amounts due to over- or under-expression. Alternatively, the appropriate gene for expression is one which expresses a normal gene product which replaces a defective gene product, encodes ribozymes or antisense molecules which repair or destroy mutant cellular RNAs expressed from mutated genes, or

modifies or destroys viral RNAs. Transgenes used for production of proteins *in vitro* include proteins such as secreted factors, including hormones, growth factors and enzymes.

The composition of the transgene sequence depends upon the intended use for the resulting rAAV. For example, one type of transgene sequence comprises a reporter or marker sequence, which upon expression produces a detectable signal. Such reporter or marker sequences include, without limitation, DNA sequences encoding *E. coli* β-lactamase, β-galactosidase (*LacZ*), alkaline phosphatase, HSV thymidine kinase, green fluorescent protein (GFP), bacterial chloramphenicol acetyltransferase (CAT), firefly luciferase, eukaryotic membrane bound proteins including, for example, CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art, to which high affinity antibodies directed to them exist or can be made routinely, and fusion proteins comprising a membrane bound protein appropriately fused to an antigen tag domain from, among others, hemagglutinin or *myc*.

These sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, including enzymatic, radiographic, colorimetric, fluorescence or other spectroscopic assays, fluorescent activated cell sorting assay and immunological assays, including ELISA, RIA and immunohistochemistry. For example, where the transgene is the *LacZ* gene, the presence of a rAAV is detected by assays for β-galactosidase activity. Similarly, where the transgene is luciferase, the rAAV gene expression may be measured by light production in a luminometer.

However, desirably, the transgene is a non-marker gene which can be delivered to a cell or an animal via the rAAV produced by this method. The transgene may be selected from a wide variety of gene products useful in biology and medicine, such as proteins, sense or antisense nucleic acids (e.g., RNAs), or catalytic RNAs. The invention may be used to correct or ameliorate gene deficiencies, wherein normal genes are expressed but at greater than normal or less than normal levels, and may also be used to correct or ameliorate genetic defects wherein a functional gene product is not expressed. A preferred type of transgene sequence is a therapeutic gene which expresses a desired corrective gene product in a host cell at a level sufficient to ameliorate the disease, including partial amelioration. These therapeutic nucleic acid sequences typically encode products which, upon expression, are able to correct, complement or compensate an inherited or non-inherited genetic defect, or treat an epigenetic disorder or disease. However, the selected transgene may encode any product desirable for study. The selection of the transgene sequence is not a limitation of this invention. Choice of a transgene sequence is within the skill of the artisan in accordance with the teachings of this application.

The invention also includes methods of producing rAAV and compositions thereof which can be used to correct or ameliorate a gene defect caused by a multi-subunit protein. In certain situations, a different transgene may be used to encode each subunit of the protein. This may be desirable when the size of the DNA encoding the protein subunit is large, e.g., for an immunoglobulin or the platelet-derived growth factor receptor. In order for the cell to produce the

multi-subunit protein, a cell would be infected with rAAV expressing each of the different subunits.

Alternatively and more preferably, different subunits of a protein may be encoded by the same transgene. In this case, a single transgene would include the DNA encoding each of the subunits, with the DNA for each subunit separated by an internal ribosome entry site (IRES). The use of IRES permits the creation of multigene or polycistronic mRNAs. IRES elements are able to bypass the ribosome scanning model of 5' methylated cap-dependent translation and begin translation at internal sites (Pelletier and Sonenberg, 1988). For example, IRES elements from hepatitis C and members of the picornavirus family (e.g., polio and encephalomyocarditis) have been described, as well an IRES from a mammalian mRNA (Macejak and Sarnow, 1991). IRES elements can be linked to heterologous open reading frames. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Thus, multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message. This is preferred when the size of the DNA encoding each of the subunits is sufficiently small that the total of the DNA encoding the subunits and the IRES is no greater than the maximum size of the DNA insert that the virus can encompass. For instance, for rAAV, the insert size can be no greater than approximately 4.8 kilobases; however, for an adenovirus which lacks all of its helper functions, the insert size is approximately 28 kilobases.

Useful gene products include hormones and growth and differentiation factors including, without limitation, insulin, glucagon, growth

hormone (GH), parathyroid hormone (PTH), calcitonin, growth hormone releasing factor (GRF), thyroid stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), prolactin, melatonin, vasopressin, β-endorphin, met-enkephalin, leu-enkephalin, prolactin-releasing factor, prolactin-inhibiting factor, corticotropin-releasing hormone, thyrotropin-releasing hormone (TRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), chorionic gonadotropin (CG), vascular endothelial growth factor (VEGF), angiopoietins, angiostatin, endostatin, granulocyte colony stimulating factor (GCSF), erythropoietin (EPO), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), bFGF2, acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), transforming growth factor α (TGFα), platelet-derived growth factor (PDGF), insulin-like growth factors I and II (IGF-I and IGF-II), any one of the transforming growth factor β (TGFβ) superfamily comprising TGFβ, activins, inhibins, or any of the bone morphogenic proteins (BMP) BMPs 1-15, any one of the heregulin/neuregulin/ARIA/neu differentiation factor (NDF) family of growth factors, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins NT-3, NT-4/5 and NT-6, ciliary neurotrophic factor (CNTF), glial cell line derived neurotrophic factor (GDNF), neurtuin, persephin, agrin, any one of the family of semaphorins/collapsins, netrin-1 and netrin-2, hepatocyte growth factor (HGF), ephrins, noggin, sonic hedgehog and tyrosine hydroxylase.

Other useful gene products include proteins that regulate the immune system including, without limitation, cytokines and lymphokines such as thrombopoietin (TPO), interleukins (IL) IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6,

IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, and IL-17, monocyte chemoattractant protein (MCP-1), leukemia inhibitory factor (LIF), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), monocyte colony stimulating factor (M-CSF), Fas ligand, tumor necrosis factors α and β (TNFα and TNFβ), interferons (IFN) IFN-α, IFN-β and IFN-γ, stem cell factor, flk-2/flt3 ligand. Gene products produced by the immune system are also encompassed by this invention. These include, without limitations, immunglobulins IgG, IgM, IgA, IgD and IgE, chimeric immunoglobulins, humanized antibodies, single chain antibodies, T cell receptors, chimeric T cell receptors, single chain T cell receptors, class I and class II MHC molecules, as well as engineered MHC molecules including single chain MHC molecules. Useful gene products also include complement regulatory proteins such as membrane cofactor protein (MCP), decay accelerating factor (DAF), CR1, CR2 and CD59.

Still other useful gene products include any one of the receptors for the hormones, growth factors, cytokines, lymphokines, regulatory proteins and immune system proteins. Examples of such receptors include *flt-1*, *flk-1*, TIE-2; the *trk* family of receptors such as *TrkA*, MuSK, Eph, PDGF receptor, EGF receptor, HER2, insulin receptor, IGF-1 receptor, the FGF family of receptors, the TGFβ receptors, the interleukin receptors, the interferon receptors, serotonin receptors, α-adrenergic receptors, β-adrenergic receptors, the GDNF receptor, p75 neurotrophin receptor, among others. The invention encompasses receptors for extracellular matrix proteins, such as integrins, counter-receptors for

transmembrane-bound proteins, such as intercellular adhesion molecules (ICAM-1, ICAM-2, ICAM-3 and ICAM-4), vascular cell adhesion molecules (VCAM), and selectins E-selectin, P-selectin and L-selectin. The invention encompasses receptors for cholesterol regulation, including the LDL receptor, HDL receptor, VLDL receptor, and the scavenger receptor. The inventions encompasses the apolipoprotein ligands for these receptors, including ApoAI, ApoAIV and ApoE. The invention also encompasses gene products such as steroid hormone receptor superfamily including glucocorticoid receptors and estrogen receptors, Vitamin D receptors and other nuclear receptors. In addition, useful gene products include antimicrobial peptides such as defensins and maginins, transcription factors such as *jun*, *fos*, max, mad, serum response factor (SRF), AP-1, AP-2, *myb*, MRG1, CREM, Alx4, FREAC1, NF-κB, members of the leucine zipper family, C2H4 zinc finger proteins, including Zif268, EGR1, EGR2, C6 zinc finger proteins, including the glucocorticoid and estrogen receptors, POU domain proteins, exemplified by Pit1, homeodomain proteins, including HOX-1, basic helix-loop-helix proteins, including *myc*, MyoD and myogenin, ETS-box containing proteins, TFE3, E2F, ATF1, ATF2, ATF3, ATF4, ZF5, NFAT, CREB, HNF-4, C/EBP, SP1, CCAAT-box binding proteins, interferon regulation factor 1 (IRF-1), Wilms tumor protein, ETS-binding protein, STAT, GATA-box binding proteins, e.g., GATA-3, and the forkhead family of winged helix proteins.

Other useful gene products include carbamoyl synthetase I, ornithine transcarbamylase, arginosuccinate synthetase, arginosuccinate lyase, arginase, fumarylacetoacetate hydrolase, phenylalanine hydroxylase, alpha-1 antitrypsin,

glucose-6-phosphatase, porphobilinogen deaminase, factor VII, factor VIII, factor IX, factor II, factor V, factor X, factor XII, factor XI, von Willebrand factor, superoxide dismutase, glutathione peroxidase and reductase, heme oxygenase, angiotensin converting enzyme, endothelin-1, atrial natriuetic peptide, pro-urokinase, urokinase, plasminogen activator, heparin cofactor II, activated protein C (Factor V Leiden), Protein C, antithrombin, cystathione beta-synthase, branched chain ketoacid decarboxylase, albumin, isovaleryl-CoA dehydrogenase, propionyl CoA carboxylase, methyl malonyl CoA mutase, glutaryl CoA dehydrogenase, insulin, beta-glucosidase, pyruvate carboxylase, hepatic phosphorylase, phosphorylase kinase, glycine decarboxylase (also referred to as P-protein), H-protein, T-protein, Menkes disease protein, tumor suppressors (e.g., p53), cystic fibrosis transmembrane regulator (CFTR), the product of Wilson's disease gene PWD, Cu/Zn superoxide dismutase, aromatic aminoacid decarboxylase, tyrosine hydroxylase, acetylcholine synthetase, prohormone convertases, protease inhibitors, lactase, lipase, trypsin, gastrointestinal enzymes including chyromotrypsin, and pepsin, adenosine deaminase, α1 anti-trypsin, tissue inhibitor of metalloproteinases (TIMP), GLUT-1, GLUT-2, trehalose phosphate synthase, hexokinases I, II and III, glucokinase, any one or more of the individual chains or types of collagen, elastin, fibronectin, thrombospondin, vitronectin and tenascin, and suicide genes such as thymidine kinase and cytosine deaminase. Other useful proteins include those involved in lysosomal storage disorders, including acid β-glucosidase, α-galactosidase a, α-l-iduronidase, iduroate sulfatase, lysosomal acid α-glucosidase, sphingomyelinase, hexosamina\idase A, hexominidases A and B, arylsulfatase A,

acid lipase, acid ceramidase, galactosylceramidase, α-fucosidase, α-, β-mannosidosis, aspartylglucosaminidase, neuramidase, galactosylceramidase, heparan-N-sulfatase, N-acetyl-α-glucosaminidase, Acetyl-CoA: α-glucosaminide N-acetyltransferase, N-acetylglucosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfate sulfatase, arylsulfatase B, β-glucuoronidase and hexosaminidases A and B.

Other useful transgenes include non-naturally occurring polypeptides, such as chimeric or hybrid polypeptides or polypeptides having a non-naturally occurring amino acid sequence containing insertions, deletions or amino acid substitutions. For example, single-chain engineered immunoglobulins could be useful in certain immunocompromised patients. Other useful proteins include truncated receptors which lack their transmembrane and cytoplasmic domain. These truncated receptors can be used to antagonize the function of their respective ligands by binding to them without concomitant signaling by the receptor. Other types of non-naturally occurring gene sequences include sense and antisense molecules and catalytic nucleic acids, such as ribozymes, which could be used to modulate expression of a gene.

Other useful transgenes include those that encode antigenic peptides capable of generating an immune response. Recombinant vectors comprising these transgenes can be used for genetic immunization. Useful transgenes include those that encode peptides specific for Epstein Barr virus; HIV; simian immunodeficiency virus (SIV); human T-cell leukemia viruses I and II (HTLV-I and HTLV-II); hepatitis A, B, C, D, E and SEN; pseudorabies virus; rabies virus; cytomegalovirus; respiratory syncytial virus; parainfluenza virus types 1-4; mumps virus; rubella virus;

polio virus; measles virus; influenza virus types A, B and C; rotavirus; herpes simplex viruses types 1 and 2; varicella-zoster virus; human herpes virus type 6, 7 and 8; hantavirus; denguevirus, sindbisvirus, adenoviruses; chlamydia pneumoniae; chlamydia trachomatis; mycoplasma pneumoniae; mycobacterium tuberculosis; atypical mycobacteria; feline leukemia virus; feline immunodeficiency virus; bovine immunodeficiency virus; equine infectious anemia virus; caprine arthritis encephalitis virus; visna virus; *Staphlococcus* species and *Streptococcus* species. The transgenes may also be directed against peptides from tumor antigens to provide immunization for tumors and cancers.

Expression Control Sequences

A great number of expression control sequences -- native, constitutive, inducible and/or tissue-specific -- are known in the art and may be utilized to drive expression of the transgene and the nucleic acid sequences encoding the replication and encapsidation functions of the rAAV, the helper functions and the ligand. The choice of expression control sequence depends upon the type of expression desired. For eukaryotic cells, expression control sequences typically include a promoter, an enhancer, such as one derived from an immunoglobulin gene, SV40, cytomegalovirus, etc., and a polyadenylation sequence. The polyadenylation sequence generally is inserted following the transgene sequences and before the 3' flanking sequence of the transgene. A transgene-carrying molecule useful in the present invention may also contain an intron, desirably located between the promoter/enhancer sequence and the

transgene. One possible intron sequence is also derived from SV40 and is referred to as the late16S/19S intron. Another vector element that may be used is an internal ribosome entry site (IRES), as described above. An IRES element is used to produce more than one polypeptide from a single transcript. An IRES element can be used for the transgene or for any of the other nucleic acid sequences encoding the replication and encapsidation polypeptides, the helper functions or the ligand. Selection of these and other common vector elements are conventional and many such sequences are available [see, e.g., Sambrook et al, and references cited therein at, for example, pages 3.18-3.26 and 16.17-16.27 and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989].

In one embodiment, high-level constitutive expression will be desired. Examples of such promoters include, without limitation, the retroviral Rous sarcoma virus (RSV) LTR promoter/enhancer, the cytomegalovirus (CMV) immediate early promoter/enhancer [see, e.g., Boshart et al, Cell, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the cytoplasmic β-actin promoter and the phosphoglycerol kinase (PGK) promoter.

In another embodiment, inducible promoters may be desired. Inducible promoters are those which are regulated by exogenously supplied compounds, either in *cis* or in *trans*, including without limitation, the zinc-inducible metallothionine (MT) promoter; the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter; the T7 polymerase promoter system [WO 98/10088]; the ecdysone insect promoter [No et al, Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)]; the tetracycline-repressible system [Gossen et al,

Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)]; the tetracycline-inducible system [Gossen et al., Science, 268:1766-1769 (1995); see also Harvey et al., Curr. Opin. Chem. Biol., 2:512-518 (1998)]; the RU486-inducible system [Wang et al., Nat. Biotech., 15:239-243 (1997) and Wang et al., Gene Ther., 4:432-441 (1997)]; and the rapamycin-inducible system [Magari et al., J. Clin. Invest., 100:2865-2872 (1997); Rivera et al., Nat. Medicine, 2:1028-1032 (1996)]. Other types of inducible promoters which may be useful in this context are those which are regulated by a specific physiological state, e.g., temperature, acute phase, or in replicating cells only.

In another embodiment, the native promoter for the transgene or nucleic acid sequence of interest will be used. The native promoter may be preferred when it is desired that expression of the transgene or the nucleic acid sequence should mimic the native expression. The native promoter may be used when expression of the transgene or other nucleic acid sequence must be regulated temporally or developmentally, or in a tissue-specific manner, or in response to specific transcriptional stimuli. In a further embodiment, other native expression control elements, such as enhancer elements, polyadenylation sites or Kozak consensus sequences may also be used to mimic the native expression.

In one embodiment, the recombinant viral genome comprises a transgene operably linked to a tissue-specific promoter. For instance, if expression in skeletal muscle is desired, a promoter active in muscle may be used. These include the promoters from genes encoding skeletal α-actin, myosin light chain 2A, dystrophin, muscle creatine kinase, as well as synthetic muscle promoters with

activities higher than naturally-occurring promoters [see Li et al., Nat. Biotech., 17:241-245 (1999)]. Examples of promoters that are tissue-specific are known for liver [albumin, Miyatake et al. J. Virol., 71:5124-32 (1997); hepatitis B virus core promoter, Sandig et al., Gene Ther., 3:1002-9 (1996); alpha-fetoprotein (AFP), Arbuthnot et al., Hum. Gene Ther., 7:1503-14 (1996)], bone [osteocalcin, Stein et al., Mol. Biol. Rep., 24:185-96 (1997); bone sialoprotein, Chen et al., J. Bone Miner. Res., 11:654-64 (1996)], lymphocytes [CD2, Hansal et al., J. Immunol., 161:1063-8 (1998); immunoglobulin heavy chain; T cell receptor α chain], neuronal [neuron-specific enolase (NSE) promoter, Andersen et al. Cell. Mol. Neurobiol., 13:503-15 (1993); neurofilament light-chain gene, Piccioli et al., Proc. Natl. Acad. Sci. USA, 88:5611-5 (1991); the neuron-specific vgf gene, Piccioli et al., Neuron, 15:373-84 (1995)]; among others.

Of course, not all vectors and expression control sequences will function equally well to express all of the transgenes or other nucleic acid sequences of this invention. However, one of skill in the art may make a selection among these expression control sequences without departing from the scope of this invention. Suitable promoter/enhancer sequences which function in the appropriate host cell of choice may be selected by one of skill in the art using the guidance provided by this application. Such selection is a routine matter and is not a limitation of the molecule or construct.

One may identify a suitable expression control sequence for a desired transgene by selecting one or more expression control sequences and operably linking the expression control sequence to the nucleic acid sequence to be

regulated. Then, one may insert these operably linked sequences comprising the expression control sequence and regulated sequence into the genome of the adenovirus vector. In one embodiment, one may insert a recombinant viral genome comprising the expression control sequence and the transgene into a vector of the instant invention. After following one of the methods for producing and packaging the rAAV as taught in this specification one may infect suitable cells *in vitro* or *in vivo*. The number of copies of the transgene in the cell may be monitored by Southern blotting or quantitative PCR; the level of RNA expression may be monitored by Northern blotting or quantitative RT-PCR; and the level of protein expression may be monitored by Western blotting, immunohistochemistry, ELISA, RIA, tests of the transgene's gene product's biological activity, either *in vitro* or *in vivo*, or tests for correction or amelioration of a genetic defect.

Flanking Elements

The naturally-occurring AAV ITRs consist of repeated sequences, usually but not necessarily approximately 145 nucleotides in length, at the 5' and 3' ends of the AAV genome. The AAV ITRs are required for replication, excision and encapsidation of both wild type and recombinant AAV virions. The ITRs flank the transgene when the AAV DNA integrates into a host cell chromosome. When rAAV is rescued from the host chromosome, the ITRs excise along with the transgene and remain in flanking positions surrounding the rescued DNA, in a form suitable for packaging into virions. The ITRs may be derived from any one of the adeno-associated viruses known, including AAV serotypes 1 to 6. In a preferred

embodiment of the invention, the rAAV comprises a selected transgene operably linked to expression regulatory sequences and AAV flanking elements.

Host Cells

Any type of mammalian cell that can be adapted to cell culture may be used as a host cell to produce the recombinant viral genome. In general, a host cell used in this invention is one that may be infected by the adenovirus vector of the instant invention. Another preferred characteristic of the host cell is that it is able to replicate the rAAV at high levels.

Appropriate host cells include, without limitation, CHO, BHK, MDCK and various murine cells, e.g., 10T1/2 and WEHI cells, African green monkey cells such as VERO, COS 1, COS 7, BSC 1, BSC 40, and BMT 10, and human cells such as WI38, MRC5, A549, human embryonic retinoblast (HER), human embryonic kidney (HEK), human embryonic lung (HEL) and HT1080 cells. In a preferred embodiment, appropriate cells include 293 cells (human embryonic kidney cells that express adenoviral E1a and E1b proteins), 911 or PER.C6 cells (human embryonic retinoblast cells that express adenoviral E1; see WO 97/00326), B50 cells (HeLa cells that express AAV *rep* and *cap,* see PCT US98/19463), 84-31 cells (293-based cells that express adenovirus E1a, E1b and E4, Ref. 4), 10-3 cells (293-based cells that express adenovirus E1a, E1b and E4ORF6, Ref. 11), 3T3 cells (mouse embryonic fibroblast cell line), NIH3T3 cells (subline of 3T3 cells), HepG2 cells (human liver carcinoma cell line), Saos-2 cells (human osteogenic sarcoma cell line), HuH7 cells or HeLa cells (human carcinoma cell line).

In addition to the host cells listed above, other host cells may be used. One may determine whether a cell line would be suited for use as a mammalian host cell by infecting the cell line with an adenovirus of the instant invention in the presence of *rep*, *cap* and all required helper functions, culturing the cells under conditions in which rAAV is produced, and then measuring the titer of infectious rAAV. One may then compare the titer of infectious rAAV produced in the potential host cell with the titers produced by other host cells to determine whether the cell line is good for rAAV production.

Methods of Producing Recombinant Adeno-Associated Virus from Adenovirus

Another aspect of the instant invention is a method of producing rAAV from the adenovirus of the instant invention. The method comprises the steps of:

1. Infecting host cells comprising an rAAV genome with an adenovirus comprising a *rep* gene under the regulatory control of a minimal promoter or no promoter;

2. growing the infected host cells under conditions in which the rAAV genome is excised, replicated and encapsidated; and

3. collecting the rAAV from the mammalian host cells.

The host cells may be any mammalian cell known in the art or as described herein. The host cell, prior to infection by the adenovirus comprising the *rep* gene, may be one that expresses one or more of the following genetic elements: 1) the *cap* gene, 2) some or all necessary helper functions (e.g., 293 cells), and/or 3)

an rAAV genome. Alternatively, the host cell may comprise none of these genetic elements prior to infection by the adenovirus. In this case, the genetic elements are supplied by the adenovirus comprising the *rep* gene, other viral vectors, and/or plasmids.

The host cells may be infected by the adenovirus by any method known in the art or as described herein. Methods for infecting host cells with adenoviruses are well known in the art and are also described herein. Once the host cell has been infected, the helper functions are activated and *rep* and *cap* are produced. Low levels of Rep78 and Rep68 are produced because the p5 promoter has been replaced by a minimal promoter or by no promoter. This, combined with levels of Rep52 and Rep40, both expressed from the adenoviral p19 promoter, and the capsid proteins, expressed from the AAV p40 promoter, are sufficient to produce high titers of rAAV.

Methods of producing rAAV using other viral vectors in which Rep interferes with viral replication also may be performed following the teachings of the instant specification.

The rAAV may be purified from the supernatant produced by the host cells or from cell lysates by any method known in the art or as described herein. A method of collecting and purifying rAAV is described in Examples 5 and 6.

The method is easily scaled to industrial production because it does not require transfection of a large number of host cells to produce rAAV. In a preferred embodiment, only a single infection of host cells by an adenovirus is

required to produce rAAV in large amounts at high titers. See, e.g., Example 5. In another preferred embodiment, host cells are co-infected with two different adenoviruses, one comprising *rep* downstream of a minimal promoter or no promoter and *cap*, and the other adenovirus comprising the rAAV genome. Infection of host cells by adenovirus is highly efficient and may be easily scaled to a large number of cells.

The instantly described method produces rAAV at a high titer. In a preferred embodiment, the titer is at least 10^2 particles per cell; preferably at least 10^3 particles per cell; more preferably at least 10^4 particles per cell; and, even more preferably, at least 10^5 or 10^6 particles per cell. The instant invention also encompasses lysates and supernatants of host cells comprising rAAV. These lysates and supernatants differ from those produced by prior art methods because of the higher level of rAAV contained therein without concentration.

rAAV Compositions

The rAAV produced by the method of this invention may be formulated as a pharmaceutical or pharmacological composition for use for any form of transient and stable gene transfer *in vivo* and *in vitro*. The composition comprises at least the rAAV and a pharmaceutically acceptable carrier. The rAAV may be used for *in vivo* and *ex vivo* gene therapy, genetic immunization, *in vitro* protein production and diagnostic assays.

For gene therapy, the rAAV may be introduced into cells *ex vivo* or *in vivo*. Where the virus is introduced into a cell *ex vivo*, the rAAV may be used to

infect a cell *in vitro*, and then the cell may subsequently be introduced into a mammal (e.g., into the portal vein or into the spleen), if desired. Alternatively, the rAAV may be administered to a mammal directly, e.g., intravenously or intraperitoneally. A slow-release device, such as an implantable pump, may be used to facilitate delivery of the virus to a cell. Where the virus is administered to a mammal, the specific cells to be infected may be targeted by controlling the method of delivery. For example, intravascular administration of rAAV to the portal vein or to the hepatic artery may be used to facilitate targeting rAAV to a liver cell.

The rAAV produced by the above-described method may be administered to a patient, preferably suspended in a biologically compatible solution or pharmaceutically acceptable delivery vehicle. A suitable vehicle includes sterile saline. Other aqueous and non-aqueous sterile suspensions known to be pharmaceutically acceptable carrier and well known to those of skill in the art may be employed for this purpose.

The rAAV is administered in sufficient amounts to infect the desired cells and provide sufficient levels of transduction and expression of the selected transgene (or viral gene products in the case of a vaccine) to provide some level of a corrective effect without undue adverse or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include direct administration to the target organ, tissue or site; intranasal; intravenous; intramuscular; subcutaneous; intradermal; oral and other parenteral routes of administration. Routes of administration may be combined, if desired.

Dosages of rAAV will depend primarily on factors such as the condition being treated and the selected gene. The dosage may also vary depending upon the age, weight and health of the patient. For example, an effective human dosage of rAAV is generally in the range of from about 0.5 ml to 50 ml of saline solution containing rAAV at concentrations of 1×10^7 or 1×10^8 or 1×10^9 or 1×10^{10} or 1×10^{11} or 1×10^{12} or 1×10^{13} or 1×10^{14} or 1×10^{15} or 1×10^{16} particles per dose administered. The dosage will be adjusted to balance the corrective benefits against any adverse side effects. The levels of expression of the selected gene may be monitored to determine the type and frequency of dosage administration.

The following examples of the present inventions are illustrative only, and are not intended to limit the scope of the invention.

EXAMPLE 1
Cell Lines and Viruses and Maintenance and Propagation Thereof

All cell lines are maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco BRL) supplemented with 10% FBS (Hyclone) and 50 μg/ml of penicillin, 50 μg/ml of streptomycin, and 10 μg/ml of neomycin (Gibco BRL). Human embryonic kidney cell line 293 is obtained from ATCC (CRL 1573). 293-derived 84-31 cells (1) which express adenovirus E1/E4orf6 proteins, and HeLa-derived B50 cells (7) which express AAV-2 Rep and Cap proteins from the native p5 promoter, are obtained from Dr. Guangping Gao, Institute for Human Gene Therapy, University of Pennsylvania. 293-CG3 is a 293-derived cell line carrying

stably integrated copies of AAV ITRs flanking GFP as marker gene (Chen et al., unpublished data). Human adenovirus type 5 (ATCC VR-5) and derived recombinant adenoviruses are propagated on 293 cells and purified through CsCl gradient centrifugation according to the method of Jones and Shenk with modification (2).

EXAMPLE 2
Construction of Plasmids and Generation of Recombinant Adenoviruses

Standard recombinant DNA techniques are employed to create recombinant plasmids (3). DNA containing the *rep* and *cap* sequences of pAV2 (ATCC 37216) between DraIII site (nucleotide 241, upstream of the AAV-2 p5 promoter) and NcoI site (nucleotide 4489, downstream of the polyA signal) is removed and replaced through multiple cloning steps with a DNA cassette containing GFP under the transcriptional control of elongation factor 1 alpha (EF1α) promoter and upstream of the SV40 polyA signal to create pAV2cisEFGFP (Fig.1). The AAV-2 *rep* and *cap* genes located between a Dra III site (nucleotide 241, upstream of the p5 promoter) and a BsaI site (nucleotide 4464, downstream of the polyA signal) are further subcloned to obtain pAd-p5-RC (Fig.1). A small DNA fragment between nucleotides 241 and 287 of pAd-p5-RC containing the p5 promoter is removed and replaced with a *Drosophila melanogaster* minimal heat shock protein (HSP) promoter from pIND (Invitrogen) to create pAd-HSP-RC (Fig.1). Recombinant adenoviruses Ad-p5-RC and Ad-HSP-RC (Fig. 2) are generated according to standard protocols known in the art (see, e.g., Refs. 4 and

12). The recombinant adenoviruses are passaged five to six times on appropriate mammalian cells to generate a stock of recombinant adenovirus that is used for production of rAAV.

EXAMPLE 3

Transfection of 293 Cells and Selection of the 293-CG3 Stable Cell Line

293 cells are grown to ~70% confluency in 6-cm tissue culture dishes and co-transfected overnight with 1 μg pIRES1neo and 10 μg pAV2cisEFGFP by the calcium phosphate transfection method. The monolayer is replenished with fresh medium containing 10% FBS and cultured for 24 hours. Following trypsinization, cells are seeded at a 1:20 dilution in fresh medium containing 10% FBS. After incubation for another 24 hours, fresh medium containing 1,250 μg/ml of G418 (Gibco BRL) is added to the cell monolayer for genetic selection of G418-resistant cells. The medium containing G418 is replaced every 3-4 days to allow formation of G418-resistant cell colonies. A total of fifty colonies are picked, six of which demonstrate constitutive GFP expression. These six clones are expanded and tested for their ability to rescue functional rAAV by transfection with pBV-EiOV-RC, a plasmid that carries adenovirus E2A, E4ORF6, and VAI genes as well as AAV *rep-cap* genes. One cell clone, 293-CG3, shows high efficiency of rAAV rescue and is expanded and used for further experiments.

EXAMPLE 4

PCR Analysis of AAV-2 *rep* and *cap* Genes Inserted into the Adenovirus Genome

In order to determine the integrity of AAV-2 *rep* and *cap* genes recombined into the adenovirus genome, a polymerase chain reaction (PCR) assay is employed. Four sets of primer pairs overlapping the entire DNA sequence encoding AAV-2 *rep* and *cap* genes, as well as the junctions between adenoviral and *rep-cap* sequences, are synthesized. These primers are:

HC#30 (5'-CGTAACCGAGTAAGATTTGG-3'; SEQ ID NO: 1),

HC#31 (5'-ATGTTGGTGTTGGAGGTGAC-3'; SEQ ID NO: 2),

HC#32 (5'-TGGACCAGAAATGCAAGTCC-3'; SEQ ID NO: 3),

HC#33 (5'-AGCCTTGACTGCGTGGTGGT-3'; SEQ ID NO: 4),

HC#34 (5'-GTACCTGTATTACTTGAGCA-3'; SEQ ID NO: 5),

HC#35 (5'-ACGAGTCAG GTATCTGGTGC-3'; SEQ ID NO: 6),

HC#36 (5'-GGACTTTACTGTGGACACTA-3'; SEQ ID NO: 7), and

HC#37 (5'-GACCCAGACTACGCTGACGA-3'; SEQ ID NO: 8).

To obtain adenoviral DNA for the PCR assay, a miniprep method is employed. Briefly, 293 cells are grown in 10-cm dishes until nearly confluent and then infected with either Ad-HSP-RC or Ad-p5-RC for 3 days. Infected cells are harvested, pelleted and lysed in DOC lysis buffer (100 mM Tris-HCl, pH9.0, 20% ethanol, 0.4% sodium deoxycholate). Cellular nucleic acids are precipitated by spermine-HCl and supernatant is collected. The supernatant is then treated with RNase A and pronase, followed by extraction with phenol-chloroform. Adenoviral DNA in the supernatant is precipitated with isopropanol and dissolved in TE/RNase

buffer (10 mM Tris-HCl, pH8.0, 1 mM EDTA, 20 μg/ml RNase).

The PCR assay is performed using the Robocycler Gradient 96 thermal cycler (Stratagene), PCR products are separated on a 1% agarose gel and DNA is stained with ethidium bromide. As shown in Fig. 3, all four expected DNA PCR products are obtained when using Ad-HSP-RC DNA as template, indicating that the full-length AAV-2 *rep-cap* DNA is present in the genome of this recombinant. However, the 880 bp PCR product III is not amplified from Ad-p5-RC DNA, suggesting a rearrangement or deletion event in *rep-cap* DNA sequences of this recombinant. These results indicate the integrity and stability of HSP-rep-cap DNA after insertion into the E1 locus of the adenoviral genome, and point out that p5-rep-cap DNA sequences inserted into the same locus are unstable.

EXAMPLE 5
Production of rAAV through Infection of 293-CG3 Cells with Ad-HSP-RC

Since Ad-HSP-RC is shown to contain full length AAV-2 *rep-cap* DNA sequences, its utility to produce rAAV is analyzed. 293-CG3 cells are seeded in 6-well plates at a density of $1.0x10^6$ cells/well. Twelve to fifteen hours later, the culture media is removed from the cell monolayer and dilutions of Ad-HSP-RC virus in 0.5 ml of serum-free DMEM are added to the cells. Following a 30 min. incubation, an additional 2.5 ml of DMEM containing 10% FBS is added and the infection is allowed to proceed for a total of 3 days. Infected cells are harvested and pelleted. Cell pellets are lysed in 1 ml of lysis buffer (50 mM Tris-HCl, pH7.4, 1.0 mM $MgCl_2$, 0.5% DOC) with sonication for 3x1 min. Cell debris is removed by

centrifugation at 3,000 rpm using a Beckman GS-6R centrifuge for 10 min. The lysate supernatant is collected for titration of rAAV.

To titrate the rAAV in the lysate, 84-31 cells (1) are plated 3-4 hours before use on 24-well plates at a density of $2x10^5$ cells/well. The rAAV lysate is diluted in DMEM containing 10% FBS and heated at 56°C for 60 min to inactivate contaminating Ad-HSP-RC. The rAAV lysate is added to the monolayer, cells are incubated for approximately 24 hours, and GFP-expressing cells are scored as transducing units (TU).

As shown in Fig.4, the results demonstrate that rAAV is successfully produced through infection of 293-CG3 cells with the recombinant Ad-HSP-RC. The rAAV titer increases by increasing the multiplicity of infection (MOI) of Ad-HSP-RC. Using an MOI of 250 particles/cell, as much as 70 TU/cell of rAAV is produced. However, further increasing the MOI of the Ad-HSP-RC virus does not increase the yield of rAAV. Instead, a slight decrease in rAAV yield is observed, probably due to the increased cytotoxicity associated with higher MOI of Ad-HSP-RC virus.

EXAMPLE 6
Production of rAAV through Co-Infection of 293 Cells with Ad-HSP-RC and Ad-AAV-LacZ

In the previous example, AAV vector sequences are stably integrated into the host cell chromosome while *rep-cap* functions necessary for their rescue and packaging into rAAV are provided by Ad-HSP-RC. As a separate measure to determine the functionality of the Ad-HSP-RC recombinant, AAV

vector sequences are delivered exogenously into 293 cells to determine whether they could be rescued and packaged into rAAV particles following infection with Ad-HSP-RC. To perform this experiment, 293 cells are seeded on 6-well plates at a density of $1x10^6$ cells/well. 12-15 hours later, the cells are co-infected with Ad-HSP-RC and Ad-AAV-LacZ, an E1-deleted adenovirus containing the *E. coli lacZ* gene flanked by AAV-2 ITR's inserted into the E1 locus (4).

To further investigate the effects of varying MOI's of either virus on rAAV yield, the following experiment is performed. Different quantities of the two viruses (50 to 450 particles/cell) are mixed together, keeping the total inoculum at a constant 500 particles/cell (Fig. 5). The virus mixture is diluted in 0.5 ml of serum-free DMEM at various particle/cell ratios (50:450, 100:400, 200:300, 300:200, 400:100, and 450:50) and is added to the cell monolayer. Thirty minutes later, an additional 2.5 ml of DMEM containing 10% FBS is added and the cells are incubated in the presence of virus inoculum for 3 additional days.

Infected cells are harvested, lysed, and rAAV titrated as described in Example 5 except that rAAV transduction is scored cells by X-gal staining according to standard protocols (5). Cell stain is possible because the rAAV in this experiment carries *lacZ* as a transgene. Briefly, the rAAV transduced cells are first fixed with 0.5 ml of 0.05% glutaraldehyde for 10 min and then rinsed with 3x0.5 ml of PBS. Fixed cells are stained with 0.5 ml of X-gal solution at room temperature overnight. The X-gal solution is removed, 0.5 ml of 70% ethanol is added to terminate the reaction, and blue-staining cells are scored as transducing units.

As shown in Fig. 5, the results clearly demonstrate that co-infection

of 293 cells by the two recombinant adenoviruses, Ad-HSP-RC and Ad-AAV-LacZ, can produce high titers of rAAV in 293 cells. The data indicate that with decreasing MOI of Ad-HSP-RC and increasing MOI of Ad-AAV-LacZ, the yield of rAAV produced remains relatively steady until the Ad-HSP-RC MOI reaches 100 particles/cell or less. Further decrease of the MOI of Ad-HSP-RC dramatically decreases the rAAV yield, presumably due low levels of Rep and Cap proteins that are produced at the lower MOI's. On the other hand, increasing the MOI of Ad-AAV-LacZ does not increase the rAAV yield. While the conventional method for rAAV production using plasmid co-transfection is limited in its yield of rAAV, the current invention provides the means to easily and efficiently produce high yields of rAAV.

EXAMPLE 7
Time-Course and Particle Ratio Studies of rAAV Production through Co-Infection of 293 Cells with Ad-HSP-RC and Ad-AAV-LacZ

To further study the conditions required for optimal production of rAAV through co- infection of 293 cells with Ad-HSP-RC and Ad-AAV-LacZ, separate time-course and MOI studies are performed. 293 cells are seeded in 6-well plates as described in Example 6 and are infected with both viruses for different time intervals or at different particles/cell ratios. Infected cells are harvested and lysed, and rAAV is titrated as described in Example 6. For the time-course study, 100 particles/cell each of Ad-HSP-RC and Ad-AAV-LacZ are used to infect 293 cells for various times.

As shown in Fig.6, the results demonstrate that rAAV is detected as

early as 24 hours post-infection by the two viruses, but its levels peaks at 72 hours after infection. More than 200 TU/cell of rAAV is produced between 48 and 96 hours after infection by the two viruses. To determine the optimal MOI of the two viruses required for high level rAAV production, 293 cells are infected by the two viruses at equal MOI's for 72 hours and harvested and rAAV titers are determined. As shown in Fig. 7, it is apparent that with increasing input of Ad-HSP-RC and Ad-AAV-LacZ viruses, the yield of rAAV increases up to an MOI of 125 particles/cell of each virus. Further increasing the input adenovirus MOI does not increase the rAAV yield and instead results in a slight decrease of rAAV yield. This may be due to higher cytopathic effects resulting from higher MOI's of input adenovirus which in turn may affect rAAV yields from the infected cell.

EXAMPLE 8
Analysis of rAAV DNA Replication Following Co-Infection of 293 Cells with Ad-HSP-RC and Ad-AAV-LacZ

To analyze the excision and replication of rAAV DNA from the Ad-AAV-LacZ hybrid genome, extrachromosomal DNA is analyzed in 293 cells co-infected by Ad-HSP-RC and Ad-AAV-LacZ using the method of Hirt (6). As a positive control for rAAV DNA rescue and replication, a separate system previously shown to produce rAAV at high titers is similarly assayed in parallel. The control system is based on the use of the B50 cell line, that was previously created by stably transfecting into HeLa cells a *rep/cap*-containing plasmid utilizing endogenous AAV-2 promoters (7). rAAV production in this cell line occurs in a two-step process: B50 cells are initially infected with sub100r, an adenovirus

temperature-sensitive mutant in the E2b gene, to induce *rep* and *cap* expression and provide helper functions. 24 h later, cell are infected by a hybrid E1-deleted adenovirus in which the AAV vector sequence is cloned in the E1 region of a replication-defective adenovirus. In the presence of Rep and Cap proteins expressed by the cell, as well as adenoviral helper functions expressed from sub100r, the rAAV genome delivered by the hybrid vector is rescued, replicated and encapsidated into rAAV particles (7).

293 cells are grown in 10-cm dishes to subconfluency and are either co-infected with Ad-HSP-RC plus Ad-AAV-LacZ or with Ad-p5-RC plus Ad-AAV-LacZ at 200 particles/cell of each virus. Positive control B50/sub100r experiments are carried out in a similar fashion except that Ad-AAV-LacZ is added to B50 cells 24 hours after addition of sub100r, each at 1,000 particles/cell. Negative controls include single virus infections of 293 cells with either Ad-p5-RC, Ad-HSP-RC or Ad-AAV-LacZ alone. Infected cells are harvested 72 hours post-infection and lysed in 0.85 ml of Hirt solution (10 mM Tris-HCl, pH 7.4, 100 mM EDTA, 0.6% SDS). The lysate is mixed with 0.25 ml of 5 M NaCl and incubated at 4°C overnight. After centrifugation at 14,000 RPM for 40 min. in a Sorvall centrifuge, supernatant is collected and extracted three times with phenol-chloroform. The low molecular weight DNA is precipitated by isopropanol, dissolved in TE/RNase buffer, fractionated by electrophoresis on a 0.8% agarose gel and stained with ethidium bromide.

The results of this experiment are presented in Fig. 8. Co-infection of 293 cells by Ad-HSP-RC and Ad-AAV-LacZ results in the generation of DNA

bands of 4.8 and 9.6 kb which correspond to monomeric and dimeric forms of replicating, double-stranded rAAV DNA (Fig. 8, lane 4). The positive control also shows the same two DNA species following infection of B-50 cells with sub100r and Ad-AAV-LacZ (Fig. 8, lane 6). Neither DNA band is observed in negative controls in which 293 cells are infected singly with either Ad-p5-RC (Fig. 8, lane 1), Ad-HSP-RC (Fig. 8, lane 2), or Ad-AAV-LacZ (Fig. 8, lane 3). Furthermore, co-infection of 293 cells by Ad-p5-RC and Ad-AAV-LacZ also does not result in the formation of either rAAV DNA species (Fig. 8, lane 5), suggesting that Ad-p5-RC is defective in its *rep/cap* functions for rescue or replication of rAAV DNA.

To confirm that the 4.8 and 9.6 kb extrachromosomal DNA species detected in Fig. 8 does indeed contain the *lac*Z transgene, and to confirm that low quantities of such DNA species are indeed absent in negative control lanes, a Southern blot analysis is performed. Hirt-extracted DNA, isolated from either 293 cells co-infected by Ad-HSP-RC and Ad-AAV-LacZ or B50 cells infected by sub100r and Ad-AAV-LacZ, is separated on a 0.8% agarose gel (Fig. 9A). The gel is transferred to a nitrocellulose membrane and hybridized with a digoxigenin labeled, *lac*Z DNA probe using the DIG High Prime DNA Labeling Detection Starter Kit II from Boehringer Mannheim (Fig. 9B). A *lac*Z DNA fragment, isolated from a bacterial plasmid, is used as positive control for the Southern blot (Fig. 9A and 9B, lane 1).

Both monomeric and dimeric forms of replicating rAAV DNA, isolated from either 293 cells co-infected by Ad-HSP-RC and Ad-AAV-LacZ (Fig. 9A and 9B, lane 5) or B50 cells infected by sub100r and Ad-AAV-LacZ (Fig. 9A

and 9B, lane 7), hybridize to the *lac*Z probe. Southern blotting does not detect any replicated rAAV DNA species following co-infection of 293 cells by Ad-p5-RC and Ad-AAV-LacZ (Fig. 9A and 9B, lane 6), or from the negative controls which include Hirt DNA from 293 cells infected singly by either Ad-HSP-RC (Fig. 9A and 9B, lane 2), Ad-p5-RC (Fig. 9A and 9B, lane 3), or Ad-AAV LacZ (Fig. 9A and 9B, lane 4). Taken together, these results indicate that co-infection of 293 cells by Ad-HSP-RC and Ad-AAV-LacZ results in rescue and replication of rAAV DNA from the Ad-AAV-LacZ genome, and that replicating rAAV DNA indeed contains the *lac*Z transgene. Moreover, the lack of replicating rAAV DNA after co-infection of 293 cells by Ad-p5-RC and Ad-AAV-LacZ supports earlier observations that Ad-p5-RC is indeed defective in its *rep/cap* functions for rescue or replication of rAAV DNA.

EXAMPLE 9
Analysis of Rep/Cap Protein Expression in 293 Cells Infected with Ad-HSP-RC Virus

To analyze the expression of Rep and Cap proteins in cells infected by Ad-HSP-RC virus, Western blotting is performed. 293 cells are grown in 10-cm plates to subconfluency and are either infected with Ad-HSP-RC, Ad-AAV-LacZ, or Ad-p5-RC using 200 particles/cell of each virus. 72 hours post-infection, cells are harvested, pelleted, and processed as described by Xiao et al. (8). Briefly, the infected cell pellet from one 10-cm dish is lysed in 0.5 ml lysis buffer containing 10 mM Tris-HCl, pH 8.2, 1% Triton X-100, 1% SDS and 150 mM NaCl by sonication. Samples are separated by SDS-polyacrylamide gel electrophoresis

(PAGE) on a 4%~20% gradient polyacrylamide gel and transferred to a nitrocellulose membrane. The Rep proteins are detected using monoclonal antibody 303.9 and Cap proteins are detected using monoclonal antibody B1 (American Research Products, Inc.), both at a 1:20 dilution. Protein-antibody complexes are visualized using the ECL™ Western Blotting Analysis System (Amersham).

As shown in Fig.10, Rep and Cap proteins are expressed in 293 cells infected with Ad-HSP-RC alone (Lane 3), or co-infected with Ad-HSP-RC and Ad-AAV-LacZ (Lane 5), but not in 293 cells infected with Ad-p5-RC alone (Lane 1), Ad-p5-RC plus Ad-AAV-LacZ (Lane 2), or Ad-AAV-LacZ (Lane 4). Levels of Rep52 and Rep40 proteins expressed following Ad-HSP-RC infection are much higher than those of Rep78 and Rep68, a phenomenon that has been previously reported to contribute to higher levels of rAAV produced (9). The low basal transcriptional activity of the HSP promoter may indeed play an important role in the success of creating the Ad-HSP-RC recombinant adenovirus since it is known that expression of AAV Rep proteins may interfere with adenovirus replication (10). Low level expression of these proteins may minimize the interference but provide enough Rep function for excision and replication of the rAAV genome.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be

readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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CLAIMS

1. An adenovirus vector for the manufacture of a rAAV, wherein the adenovirus vector comprises an AAV *rep* gene, and wherein the AAV p5 promoter is deleted upstream of the AAV *rep* gene.

2. The adenovirus vector of claim 1, wherein the AAV *rep* gene comprises a minimal promoter in place of the p5 promoter.

3. The adenovirus vector of claim 1, wherein the AAV *rep* gene contains no promoter in place of the p5 promoter.

4. The adenovirus vector of any one of claims 1 to 3, wherein the adenovirus vector further comprises an AAV *cap* gene.

5. The adenovirus vector of claim 2, wherein the minimal promoter contains a TATA box.

6. The adenovirus vector of claim 2, wherein the minimal promoter is a minimal Drosophila heat shock promoter.

7. The adenovirus vector of claim 2, wherein the minimal promoter is a minimal adenoviral E1b promoter.

8. The adenovirus vector of claim 4, wherein the *rep* gene and the *cap* gene are inserted in place of at least a portion of one or more of the E1, E3 or E4 genes of adenovirus in a locus of the adenovirus vector.

9. The adenovirus vector of claim 8, wherein both the *rep* gene and the *cap* gene are inserted within the same locus of the adenovirus vector.

10. The adenovirus vector of claim 8, wherein the *rep* gene and the *cap* gene are inserted within different loci of the adenovirus vector.

11. The adenovirus vector of claim 4, wherein the *rep* and *cap* genes are derived from different AAV serotypes from each other.

12. A method for producing recombinant rAAV, comprising the steps of:

a) infecting the adenovirus vector according to any one of claims 1 to 11 into a host cell containing an rAAV genome;

b) growing the host cells under conditions in which rAAV is produced; and

c) collecting the rAAV from the host cells.

13. The method according to claim 12, wherein the rAAV genome is stably integrated in a chromosome of the host cells.

14. The method according to claim 12, wherein an adenovirus vector comprises the rAAV genome and is co-infected with the adenovirus vector comprising the *rep* gene into the host cell.

15. The method according to claim 12, wherein the adenovirus vector provides necessary helper functions for rAAV production.

16. The method according to claim 12, wherein the host cell is 293 cells.

17. The method according to any one of claims 12 to 16, further comprising the step of purifying the rAAV.

18. The method according to claim 12, wherein the host cells are selected from CHO, BHK, MDCK, 10T1/2, WEHI cells, COS, BSC 1, BSC 40, BMT 10, VERO, WI38, MRC5, A549, HT1080, 293, B-50, 3T3, NIH3T3, HepG2, Saos-2, Huh7, HER, HEK, HEL, or HeLa cells.

19. A lysate or supernatant comprising the rAAV produced by the method according to any one of claims 12 to 16 or 18.

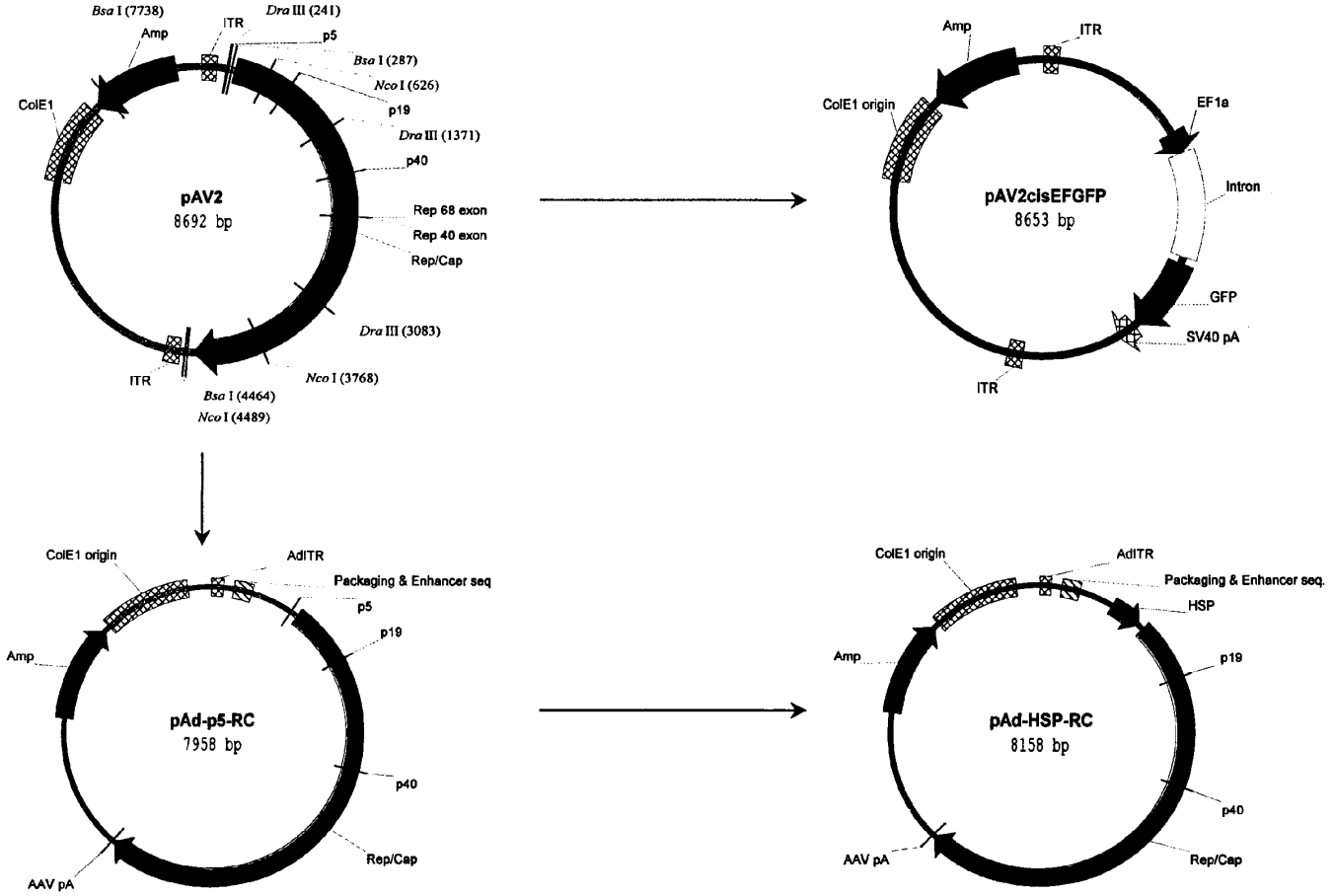
20. A purified rAAV produced by the method according to claim 19.

21. A pharmaceutical composition comprising the rAAV according to claim 20 and further comprising a pharmaceutically acceptable carrier.

22. A method for transient or stable gene transfer of a desired transgene to a mammalian cell, comprising the step of infecting the mammalian cell with the rAAV according to claim 20.

23. The method according to claim 22, wherein said transient or stable gene transfer is for genetic immunization, correction of genetic defects or production of proteins *in vitro, in vivo,* or *ex vivo.*

24. The vector according to claim 15, wherein said helper functions are encoded by at least one gene product selected from the group consisting of adenoviral genes E1A, E1B, E2A, E4orf6 and VAI, or at least one gene product selected from the group consisting of HSV type 1 genes UL5, UL8, UL52, and UL29.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Amp
ITR
Dra III (241)
p5
Bsa I (287)
Nco I (626)
p19
Dra III (1371)
p40
ColE1
pAV2
8692 bp
Rep 68 exon
Rep 40 exon
Rep/Cap
Dra III (3083)
ITR
Nco I (3768)
Bsa I (4464)
Nco I (4489)
Amp
ITR
ColE1 origin
EF1a
Intron
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GFP
SV40 pA
ITR
ColE1 origin
AdITR
Packaging & Enhancer seq
p5
p19
Amp
pAd-p5-RC
7958 bp
p40
Rep/Cap
AAV pA
ColE1 origin
AdITR
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p19
pAd-HSP-RC
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AAV pA
Rep/Cap

Figure 1

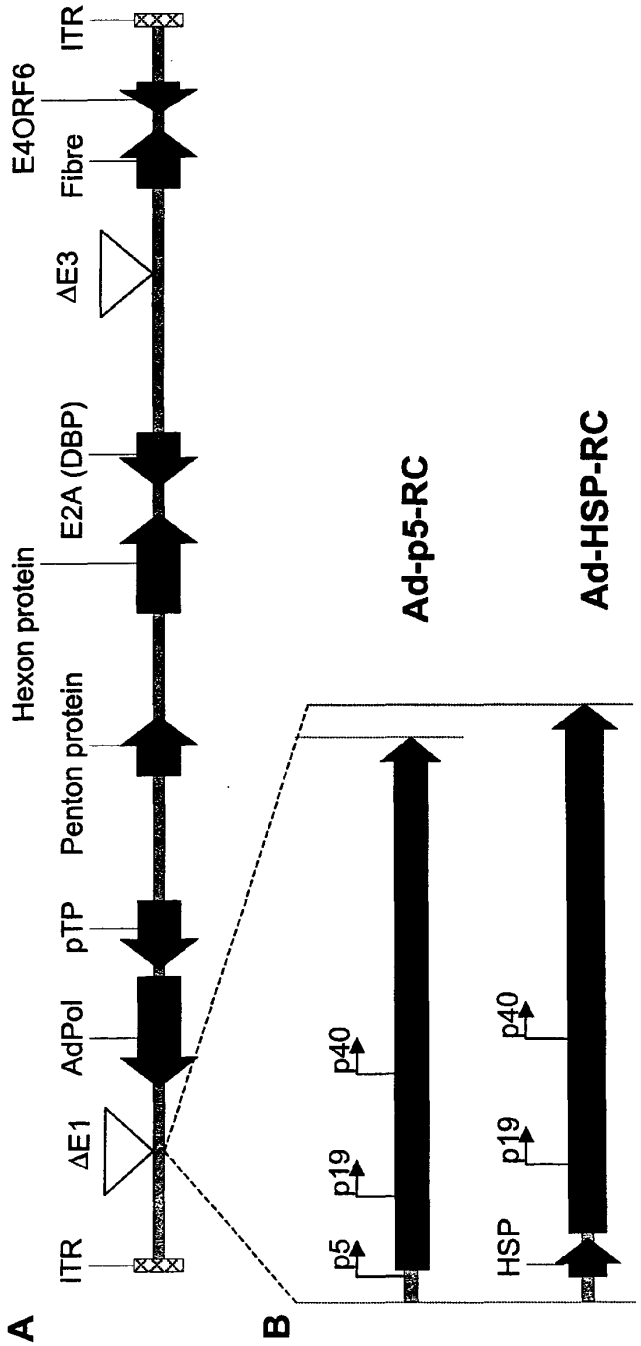

Figure 2

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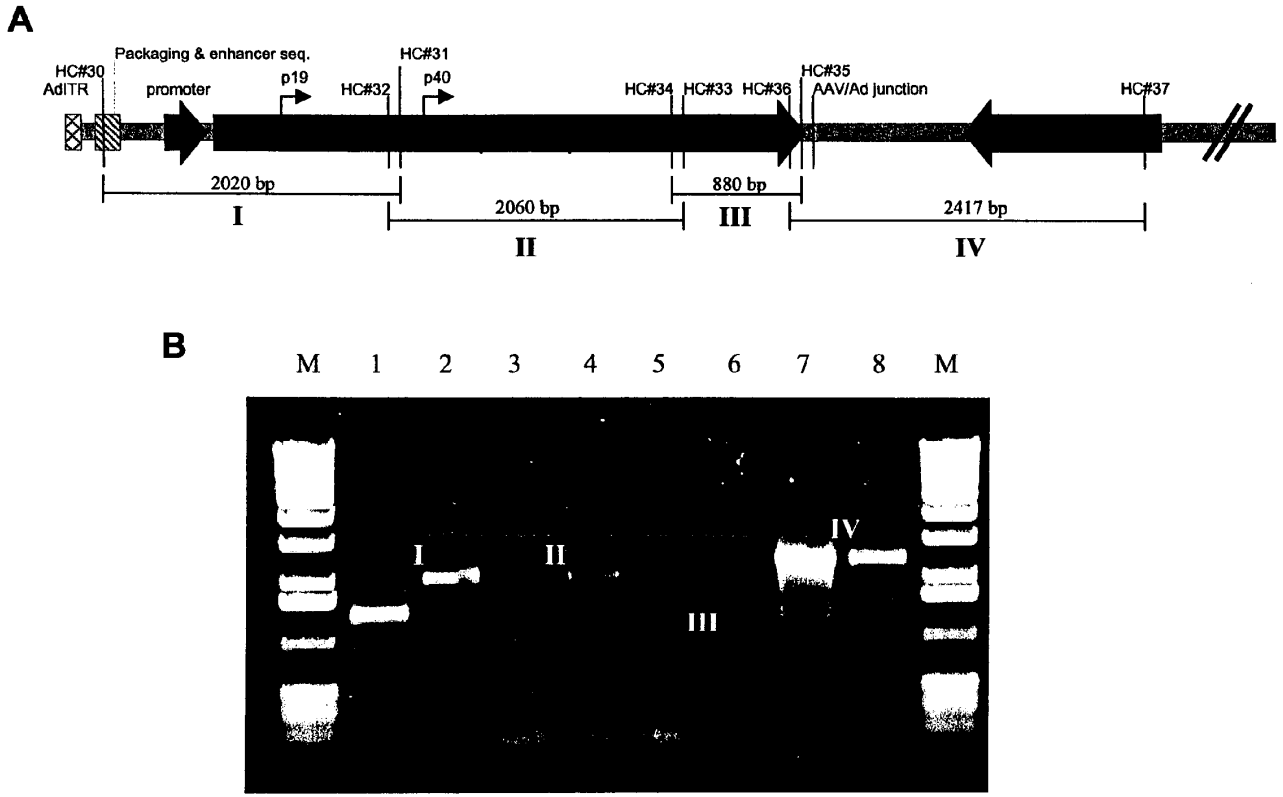
A
Packaging & enhancer seq.
HC#31
HC#30
AdITR
promoter
p19
HC#32
p40
HC#34
HC#33
HC#36
HC#35
AAV/Ad junction
HC#37
2020 bp
880 bp
2060 bp
2417 bp
I
II
III
IV
B
M 1 2 3 4 5 6 7 8 M
I
II
III
IV

Figure 3

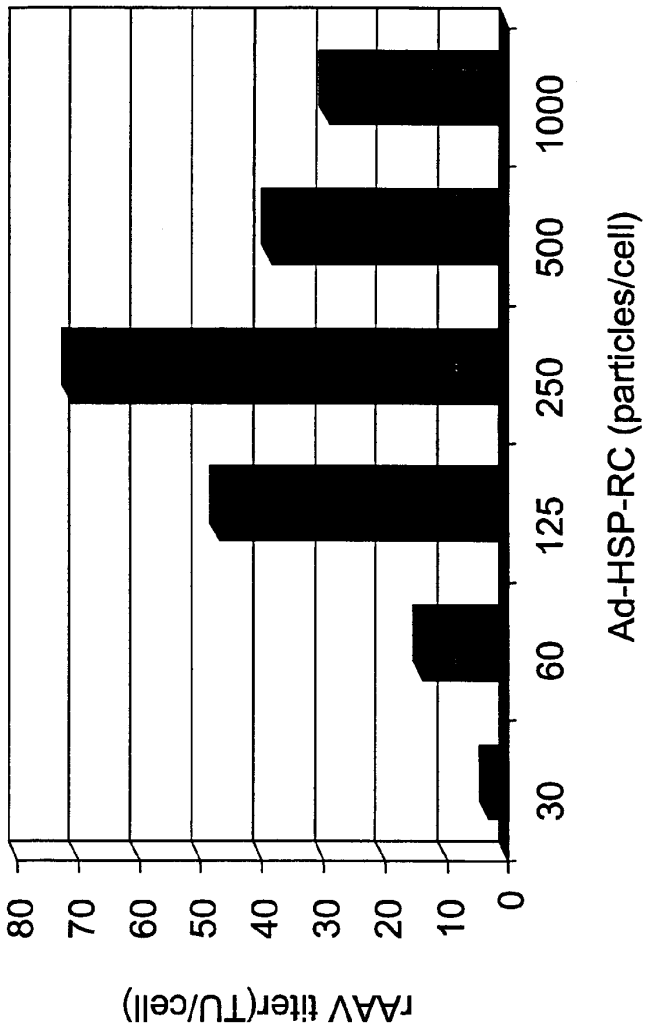
rAAV titer(TU/cell)
0
10
20
30
40
50
60
70
80
30
60
125
250
500
1000
Ad-HSP-RC (particles/cell)

Figure 4

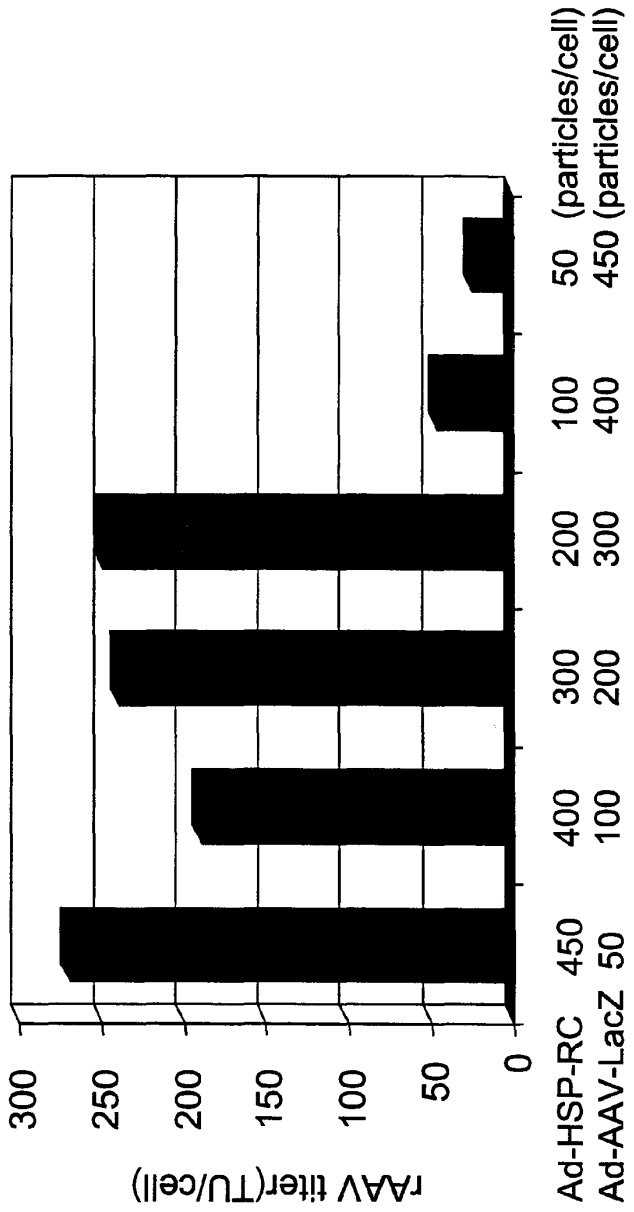

Figure 5

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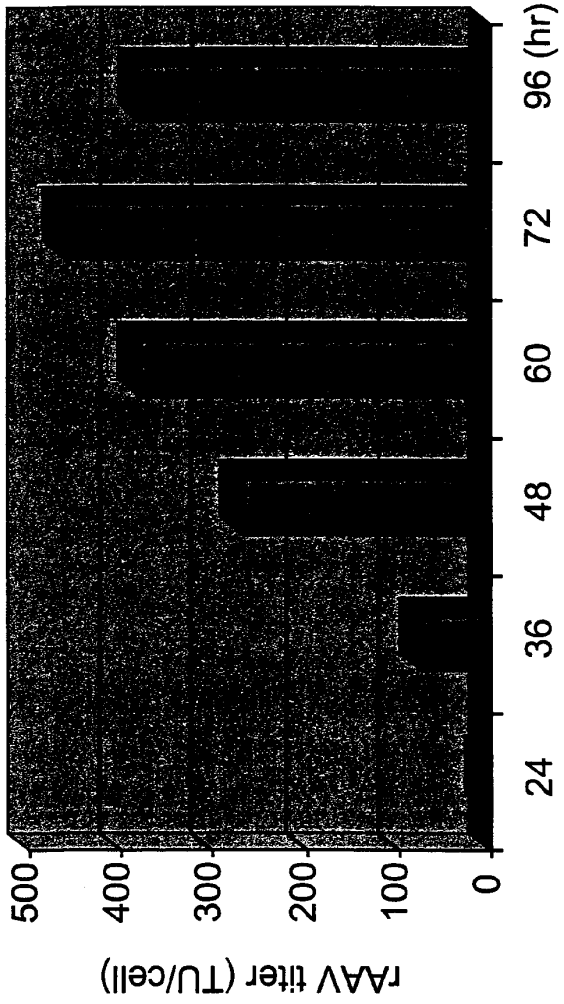
rAAV titer (TU/cell)
500
400
300
200
100
0
24
36
48
60
72
96 (hr)
Time after virus infection

Figure 6

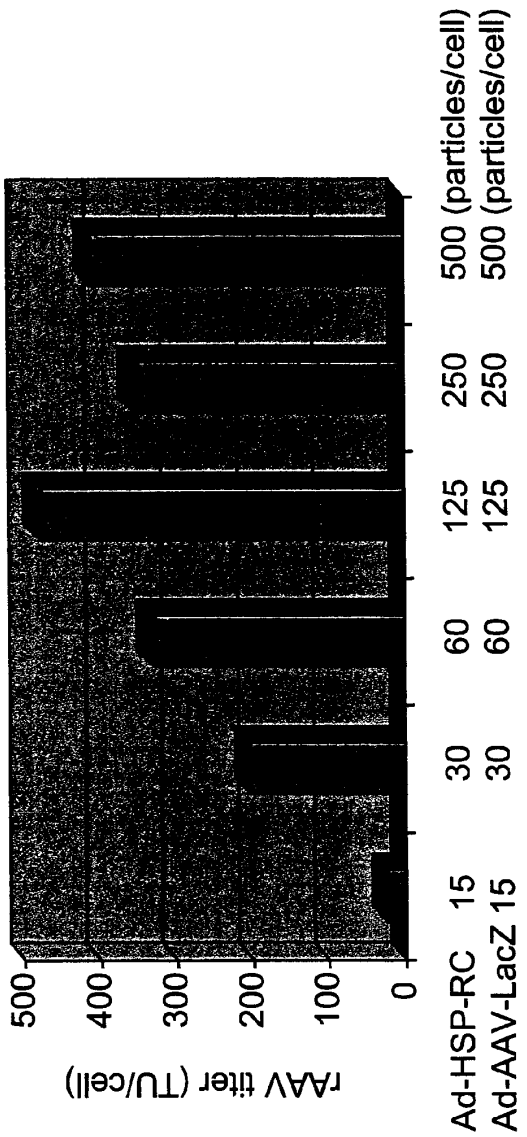

Figure 7

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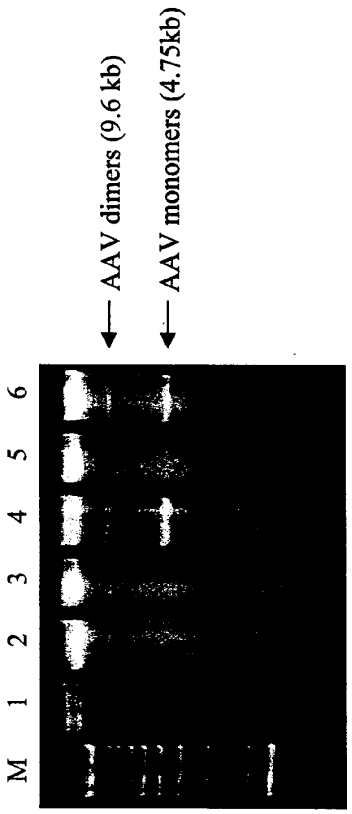

Figure 8

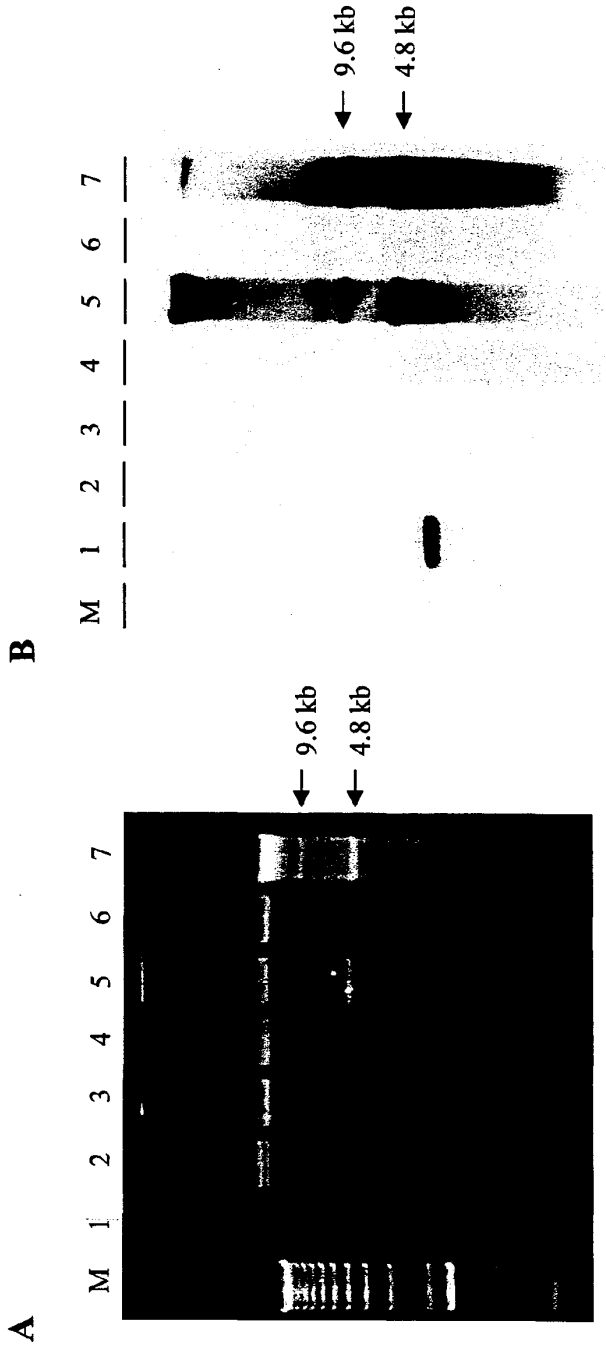

Figure 9

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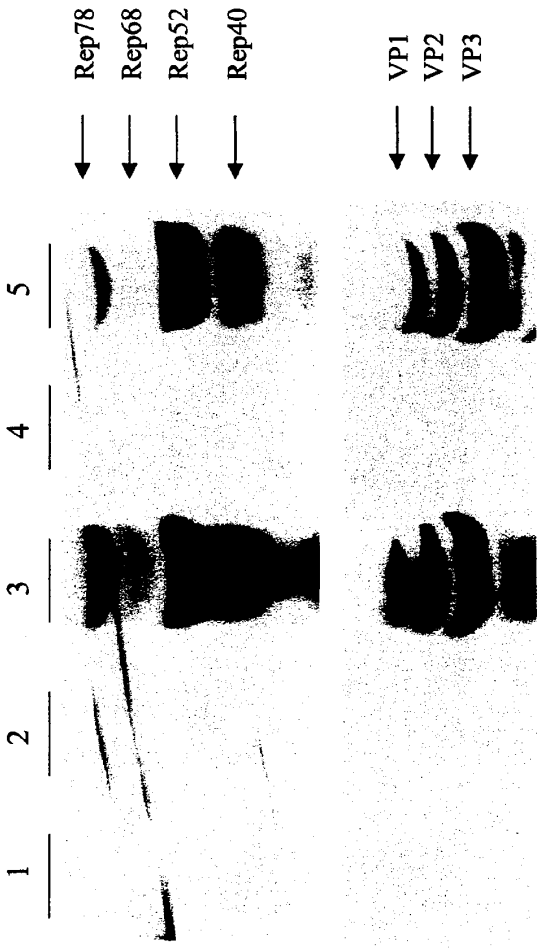

Figure 10

INTERNATIONAL SEARCH REPORT

Intern nal Application No
PCT/US 00/26948

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 A61K48/00 A61K39/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 780 280 A (MCNALLY MAUREEN A ET AL) 14 July 1998 (1998-07-14) the whole document ---	1-24
Y	WO 98 46728 A (UNIV NORTH CAROLINA ;CELL GENESYS INC (US)) 22 October 1998 (1998-10-22) cited in the application the whole document ---	1-24
A	WO 98 06864 A (US HEALTH ;MOONEN CHRIT (US)) 19 February 1998 (1998-02-19) abstract ---	6
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 January 2001	02/02/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

Form PCT/ISA/210 (second sheet) (July 1992)

Sarepta Exhibit 1002, Page 286

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 00/26948

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 13598 A (UNIV PENNSYLVANIA ;WILSON JAMES M (US); FISHER KRISHNA J (US); KEL) 9 May 1996 (1996-05-09) examples 1,2 claims 1-263 ---	14
A	KYÖSTIÖ S.R.M. ET AL.: "Negative regulation of the Adeno-Associated Virus (AAV) P5 promoter involves both the P5 Rep binding site and the consensus ATP-binding motif of the AAV Rep68 protein." J. VIROL., vol. 69, no. 11, November 1995 (1995-11), pages 6787-6796, XP002158144 the whole document ---	1-24
A	WANG X.S. & SRIVASTAVA A.: "Rescue and autonomous replication of Adeno-Associated Virus Type 2 genomes containing Rep-binding site mutations in the viral P5 promoter." J. VIROL., vol. 72, no. 6, June 1998 (1998-06), pages 4811-4818, XP002158145 the whole document -----	1-24

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Sarepta Exhibit 1002, Page 287

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-18, 24

An adenovirus vector for the manufacture of a rAAV, wherein the adenovirus vector comprises an AAV rep gene, and wherein the AAV p5 promoter is deleted upstream of the AAV rep gene. AM method for producing rAAV using said adenovirus vector.

2. Claims: 19-23

A lysate or supernatant comprising rAAV or a purified rAAV, a corresponding pharmaceutical composition and therapeutical applications thereof.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr. ıal Application No
PCT/US 00/26948

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5780280 A	14-07-1998	US 5354678 A	11-10-1994
		US 5173414 A	22-12-1992
		US 5681731 A	28-10-1997
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		JP 10507928 T	04-08-1998
		US 5871982 A	16-02-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

Inventor Information

Inventor Name	Application Number #	Patent Number	PG Pub Number	Status	Title	Examiner Name	Date Filed
BURNHAM, BRENDA	15644498	10429288	20180180525	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	07/18/2017
BURNHAM, BRENDA	16547144	11639887	20200225139	150	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	08/21/2019
BURNHAM, BRENDA	17223658	Not Issued	20210381778	41	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF	HILL,KEVIN	04/06/2021
BURNHAM, BRENDA	18188175	Not Issued	20230288308	41	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	03/22/2023
BURNHAM, BRENDA	18508853	Not Issued	0	37	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	GILL,RACHEL	11/10/2023
BURNHAM, BRENDA	18513970	Not Issued	0	37	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	CENTRAL,DOCKET	11/20/2023
BURNHAM, BRENDA	62105714	Not Issued		159	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES		01/20/2015
BURNHAM, BRENDA	63005833	Not Issued		159	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF		04/06/2020
BURNHAM, BRENDA	63094800	Not Issued		159	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF		10/21/2020
BURNHAM, BRENDA	63145258	Not Issued		159	ADENO-ASSOCIATED VIRUS COMPOSITIONS FOR IDS GENE TRANSFER AND METHODS OF USE THEREOF		02/03/2021

PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	455	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:47 AM
L2	89174	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:47 AM
L3	130	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:47 AM
L4	46	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:48 AM
L5	4797	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:48 AM
L6	6	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2018/08/10 08:48 AM
L7	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:49 AM
L29	480	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L30	93749	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L31	137	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L32	48	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L33	5018	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L34	8	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2019/05/16 08:32 AM

			JPO; DERWENT; IBM_TDB)				
L35	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
L36	63192	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L37	2374	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:32 AM
L38	68558	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L39	4138	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L40	1	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L41	195	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L42	2314	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L43	12	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:33 AM
L44	416	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:35 AM
L45	73405	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:40 AM
L46	73405	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:43 AM

		C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.					
L47	165	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:44 AM
L48	232575	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2019/05/16 08:44 AM
L49	705	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L50	138043	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L51	176	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L52	55	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L53	69299	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L54	12	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L55	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
L56	705	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2020/11/14 03:15 PM

			IBM_TDB)				
L57	138043	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L58	176	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L59	55	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L60	69299	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L61	12	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L62	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
L63	76966	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L64	3965	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L65	78532	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L66	6213	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L67	3	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L68	257	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L69	2663	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L70	32	L42 and L39	(US-PGPUB; USPAT;	OR	ON	ON	2020/11/14

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				03:15 PM
L71	519	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L72	87036	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L73	87036	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L74	223	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L75	279513	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2020/11/14 03:15 PM
L76	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2021/06/23 11:05 AM
L77	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L78	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2021/06/23 11:06 AM

			IBM_TDB)				
L79	185	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L80	58	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L81	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L82	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L83	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L84	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L85	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L86	185	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L87	58	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L88	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L89	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L90	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L91	81986	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L92	4212	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2021/06/23 11:06 AM

			IBM_TDB)				
L93	83582	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L94	6895	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L95	3	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L96	294	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L97	2826	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L98	44	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L99	569	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L100	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L101	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L102	249	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM

		G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)					
L103	296377	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L104	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L105	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L106	185	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L107	58	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L108	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L109	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L110	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L111	736	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L112	145607	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L113	185	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L114	58	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2021/06/23 11:06 AM

			JPO; DERWENT; IBM_TDB)				
L115	78391	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L116	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L117	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2021/06/23 11:06 AM
L118	81986	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L119	4212	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L120	83582	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L121	6895	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L122	3	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L123	294	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L124	2826	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L125	44	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L126	569	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L127	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM

		C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.					
L128	91390	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L129	249	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L130	296377	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 11:06 AM
L131	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2021/06/23 11:06 AM
L132	1271	("lentivirus" OR "retrovirus" OR "HIV" OR "SIV") AND ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 08:15 PM
L133	2	("lentivirus".clm. OR "retrovirus".clm. OR "HIV".clm. OR "SIV".clm.) AND ("analytical ultracentrifugation".clm.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2021/06/23 08:16 PM
L134	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L135	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

L136	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L137	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L138	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L139	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L140	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L141	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L142	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L143	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L144	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L145	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L146	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L147	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L148	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L149	4479	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

L150	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L151	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L152	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L153	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L154	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L155	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L156	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L157	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L158	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L159	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

		G01N2001/4083 OR C12Q1/70).CPC.)					
L160	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L161	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L162	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L163	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L164	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L165	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L166	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L167	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L168	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L169	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L170	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L171	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/03/07 12:20 PM

			IBM_TDB)				
L172	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L173	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L174	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L175	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L176	4479	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L177	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L178	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L179	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L180	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L181	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L182	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L183	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L184	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

		C12N2710/10351 or G01N2001/4083).cpc.					
L185	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L186	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L187	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L188	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/03/07 12:20 PM
L189	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L190	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L191	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L192	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L193	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/03/07 12:20 PM

			IBM_TDB)				
L194	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L195	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L196	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L197	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L198	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L199	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L200	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L201	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L202	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L203	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L204	4479	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L205	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L206	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L207	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2022/03/07 12:20 PM

			IBM_TDB)				
L208	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L209	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L210	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L211	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L212	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L213	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L214	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L215	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

L216	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L217	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L218	191	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L219	61	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L220	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L221	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L222	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM
L223	766	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L224	155552	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L225	191	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L226	61	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L227	89087	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L228	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L229	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/03/07 12:20 PM

L230	87492	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L231	4479	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L232	89658	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L233	7789	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L234	6	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L235	353	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L236	3013	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L237	64	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L238	640	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L239	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L240	96056	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM

L241	287	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L242	313955	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L243	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/03/07 12:20 PM
L244	1371	("lentivirus" OR "retrovirus" OR "HIV" OR "SIV") AND ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L245	3	("lentivirus".clm. OR "retrovirus".clm. OR "HIV".clm. OR "SIV".clm.) AND ("analytical ultracentrifugation".clm.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/03/07 12:20 PM
L246	5	("5622856" OR "6001650" OR "20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/03/07 12:24 PM
L247	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L248	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L249	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L250	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2022/12/07 11:54 AM

			JPO; DERWENT; IBM_TDB)				
L251	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L252	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L253	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L254	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L255	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L256	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L257	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L258	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L259	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L260	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L261	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L262	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L263	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L264	8896	"adeno-associated virus".clm. or	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2022/12/07 11:54 AM

		"AAV".clm.	JPO; DERWENT; IBM_TDB)				
L265	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L266	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L267	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L268	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L269	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L270	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L271	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L272	324	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L273	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

		C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.					
L274	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L275	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L276	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L277	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L278	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L279	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L280	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L281	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L282	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L283	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L284	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L285	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L286	13	L32 and L33	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L287	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L288	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L289	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L290	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L291	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L292	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L293	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L294	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L295	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L296	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L297	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L298	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

		G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.					
L299	324	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L300	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L301	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 11:54 AM
L302	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L303	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L304	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L305	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L306	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L307	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L308	2	("6989264"|"8137948").	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

		PN.					11:54 AM
L309	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L310	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L311	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L312	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L313	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L314	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L315	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L316	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L317	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L318	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L319	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L320	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L321	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L322	3233	"analytical	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

		ultracentrifugation"	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L323	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L324	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L325	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L326	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L327	324	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L328	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L329	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L330	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2022/12/07 11:54 AM

			JPO; DERWENT; IBM_TDB)				
L331	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L332	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L333	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L334	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L335	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L336	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L337	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L338	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L339	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L340	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L341	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L342	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L343	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L344	4810	"analytical ultracentrifugation".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO;	OR	ON	ON	2022/12/07 11:54 AM

		or "AUC".clm.	JPO; DERWENT; IBM_TDB)				
L345	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L346	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L347	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L348	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L349	3233	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L350	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L351	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L352	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L353	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L354	324	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM

		C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)					
L355	353368	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L356	3	("20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 11:54 AM
L357	1483	("lentivirus" OR "retrovirus" OR "HIV" OR "SIV") AND ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L358	4	("lentivirus".clm. OR "retrovirus".clm. OR "HIV".clm. OR "SIV".clm.) AND ("analytical ultracentrifugation".clm.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L359	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L360	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L361	203	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L362	65	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L363	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L364	13	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L365	2	("6989264"|"8137948").	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

		PN.					11:54 AM
L366	826	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L367	166820	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L368	203	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L369	65	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L370	100871	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L371	13	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L372	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:54 AM
L373	94076	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L374	4810	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L375	98099	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L376	8896	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L377	8	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L378	415	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L379	3233	"analytical	(US-PGPUB; USPAT;	OR	ON	ON	2022/12/07

		ultracentrifugation"	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				11:54 AM
L380	82	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L381	702	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L382	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L383	104984	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2022/12/07 11:54 AM
L472	5	("5622856" OR "6001650" OR "20090017542" OR "6989264" OR "8137948").pn.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 04:08 PM
L473	888	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L474	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L475	218	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L476	71	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L477	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM

L478	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L479	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L480	888	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L481	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L482	218	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L483	71	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L484	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L485	15	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L486	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L487	101233	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L488	5164	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L489	105412	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L490	10245	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L491	14	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM

L492	489	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L493	3477	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L494	117	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L495	812	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L496	108501	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L497	108501	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L498	384	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L499	370050	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L500	888	ultracentrifugation.clm.	(US-PGPUB; USPAT;	OR	ON	ON	2023/10/21

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				01:43 PM
L501	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L502	218	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L503	71	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L504	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L505	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L506	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L507	888	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L508	179386	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L509	218	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L510	71	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L511	110761	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L512	15	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L513	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2023/10/21 01:43 PM
L514	101233	"analytical	(US-PGPUB; USPAT;	OR	ON	ON	2023/10/21

		ultracentrifugation" or "AUC"	USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)				01:43 PM
L515	5164	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L516	105412	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L517	10245	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L518	14	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L519	489	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2023/10/21 01:43 PM
L520	915	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L521	182500	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L522	223	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L523	72	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L524	112860	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L525	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L526	2	("6989264"|"8137948").PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
L527	915	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM

L528	182500	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L529	223	L29 and L30	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L530	72	L31 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L531	112860	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L532	15	L32 and L33	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L533	2	("6989264"|"8137948"). PN.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
L534	102946	"analytical ultracentrifugation" or "AUC"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L535	5287	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L536	107275	"adeno-associated virus" or "AAV"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L537	10605	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L538	15	L39 and L37	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L539	505	L39 and L36	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L540	3549	"analytical ultracentrifugation"	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L541	124	L42 and L39	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	OR	ON	ON	2024/01/03 01:44 PM

			IBM_TDB)				
L542	848	("adeno-associated virus" or "AAV") and ("analytical ultracentrifugation")	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L543	109132	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L544	109132	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L545	410	L44 AND ((C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L546	373753	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L547	915	ultracentrifugation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L548	182500	viral.clm. or virus.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L549	223	L1 and L2	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM

L550	72	L3 and (sedimentation)	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L551	112860	sedimentation.clm.	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM
L552	15	L4 and L5	(US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB)	OR	ON	ON	2024/01/03 01:44 PM

PE2E SEARCH - Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
N8	953	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:44 AM
N9	25	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N10	25	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N11	1	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N12	1	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N13	1750	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N14	18	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N15	1	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N16	1	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N17	1	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N18	18	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:45 AM
N19	18	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N20	455	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N21	89174	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N22	130	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM

N23	2	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N24	1	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N25	1	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N26	1	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N27	1	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N28	1	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2018/08/10 08:46 AM
N49	983	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N50	27	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N51	27	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N52	1	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N53	1	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N54	1781	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N55	18	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N56	1	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N57	1	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N58	1	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N59	18	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N60	18	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N61	480	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N62	93749	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N63	137	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N64	2	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N65	1	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16

							08:32 AM
N66	1	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N67	1	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N68	1	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N69	1	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:32 AM
N70	2374	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:34 AM
N71	4138	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:34 AM
N72	2	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N73	1	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N74	1	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N75	1	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N76	1	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N77	1	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:35 AM
N78	16590	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:40 AM
N79	16590	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:43 AM
N80	46560	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM

		G01N2001/4083 OR C12Q1/70).CPC.					
N81	10453	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N82	7	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N83	40	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N84	10359	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N85	7	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N86	1	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N87	1	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:44 AM
N88	6	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N89	37	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N90	1	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N91	1	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2019/05/16 08:45 AM
N92	1037	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N93	38	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N94	38	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N95	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N96	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N97	1881	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N98	24	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N99	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N100	2	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N101	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N102	24	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14

							03:15 PM
N103	24	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N104	556	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N105	103267	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N106	152	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N107	3	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N108	3	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N109	3	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N110	3	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N111	3	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N112	3	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N113	1037	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N114	38	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N115	38	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N116	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N117	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N118	1881	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N119	24	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N120	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N121	2	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N122	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N123	24	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N124	24	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM

N125	556	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N126	103267	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N127	152	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N128	3	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N129	3	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N130	3	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N131	3	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N132	3	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N133	3	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N134	2758	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N135	5192	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N136	3	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N137	3	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N138	3	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N139	3	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N140	3	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N141	3	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N142	19091	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N143	19091	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM

		G01N2001/4083 or C12N2750/14151).cpc.					
N144	53858	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N145	12033	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N146	10	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N147	46	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N148	11966	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N149	10	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N150	3	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N151	3	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N152	8	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N153	42	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N154	2	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N155	2	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2020/11/14 03:15 PM
N156	22364	(G01N15/042 OR C12N7/00 OR G01N1/405 OR G01N1/4077 OR C12N2710/10351 OR C12N2750/14151 OR G01N2001/4083 OR G01N2015/045).cpc.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 11:55 AM
N157	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N158	46	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N159	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N160	3	(oriordan with	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

		catherine).in. and (burnham with brenda).in.					11:55 AM
N161	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N162	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N163	26	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N164	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N165	2	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N166	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N167	26	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N168	26	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N169	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N170	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N171	172	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N172	3	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N173	3	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N174	3	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N175	3	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N176	3	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N177	3	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N178	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N179	46	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N180	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N181	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM

N182	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N183	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N184	26	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N185	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N186	2	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N187	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N188	26	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N189	26	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N190	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N191	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N192	172	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N193	3	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N194	3	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N195	3	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N196	3	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N197	3	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N198	3	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N199	3305	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N200	7320	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N201	3	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N202	3	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N203	3	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N204	3	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N205	3	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

							11:55 AM
N206	8	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N207	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N208	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N209	65719	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N210	14097	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N211	10	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N212	49	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N213	14028	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N214	10	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N215	3	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N216	3	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N217	8	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N218	45	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N219	2	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N220	2	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM

N221	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N222	46	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N223	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N224	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N225	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N226	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N227	26	N13 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N228	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N229	2	N11 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N230	2	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N231	26	N10 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N232	26	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N233	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N234	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N235	172	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N236	3	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N237	3	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N238	3	N22 and N10	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N239	3	N22 and N11	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N240	3	N22 and N12	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N241	3	N22 and N13	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N242	1138	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N243	46	(oriordan with	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

		catherine).in.					11:55 AM
N244	47	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N245	3	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N246	3	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N247	2006	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N248	26	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N249	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N250	2	N52 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N251	2	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N252	26	N51 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N253	26	N50 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N254	660	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N255	118713	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N256	172	N61 and N62	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N257	3	N63 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N258	3	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N259	3	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N260	3	N63 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N261	3	N63 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N262	3	N63 and N54	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N263	3305	"analytical ultracentrifugation".clm. or "AUC".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N264	7320	"adeno-associated virus".clm. or "AAV".clm.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM

N265	3	N70 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N266	3	N70 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N267	3	N70 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N268	3	N70 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N269	3	N70 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N270	8	N70 and N71	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N271	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N272	22364	(G01N15/042 or G01N1/4077 or G01N1/405 or C12N7/00 or G01N2015/045 or C12N2750/14151 or C12N2710/10351 or G01N2001/4083 or C12N2750/14151).cpc.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N273	65719	(C07K2317/92 OR C07K14/005 OR A61K39/12 OR C12N15/86 OR C12N2750/14143 OR C12N15/864 OR C12N2750/14152 OR G01N33/68 OR G01N2001/4083 OR C12Q1/70).CPC.	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N274	14097	N79 and N80	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N275	10	N81 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N276	49	N81 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N277	14028	N81 and "7"	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N278	10	N81 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N279	3	N81 and N52	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N280	3	N81 and N53	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07

							11:55 AM
N281	8	N71 and N82	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N282	45	N71 and N83	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N283	2	N70 and N88	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N284	2	N70 and N89	(US-PGPUB; USPAT)	OR	ON	ON	2022/12/07 11:55 AM
N285	22364	(G01N15/042 OR C12N7/00 OR G01N1/405 OR G01N1/4077 OR C12N2710/10351 OR C12N2750/14151 OR G01N2001/4083 OR G01N2015/045).cpc.	(US-PGPUB; USPAT)	AND	ON	ON	2022/12/07 11:55 AM
N286	8	("11639887"|"20090017542"|"20200225139"|"5622856"|"6001650"|"6989264"|"7348134"|"8137948").PN.	(US-PGPUB; USPAT)	AND	ON	ON	2023/10/21 01:48 PM
N287	10	("10429288"|"11639887"|"20090017542"|"20200225139"|"20230288308"|"5622856"|"6001650"|"6989264"|"7348134"|"8137948").PN.	(US-PGPUB; USPAT)	AND	ON	ON	2024/01/03 01:44 PM
N288	1171	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N289	54	(oriordan with catherine).in.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N290	55	(oriordan with catherine).in. or (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N291	5	(oriordan with catherine).in. and (burnham with brenda).in.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N292	5	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N295	3	N12 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N299	31	N9 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N301	127468	viral.clm. or virus.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N302	190	N20 and N21	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N303	4	N22 and N8	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N304	5	N22 and N9	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N309	1171	genzyme.as.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N313	5	(oriordan).in. and (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N314	2108	(oriordan).in. or (burnham).in.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N315	31	N54 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N318	3	N53 and N49	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N321	724	ultracentrifugation.clm.	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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N325	5	N63 and N50	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
N326	5	N63 and N51	(US-PGPUB; USPAT)	OR	ON	ON	2024/01/03 01:44 PM
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(54) **Title** : METHODS FOR THE PRODUCTION OF DOUBLE-STRANDED AAV VIRAL PARTICLES

(54) **Titre** : METHODES POUR LA PRODUCTION DE PARTICULES VIRALES AAV DOUBLE BRIN

(57) **Abstract** : The invention relates to methods for producing double-stranded AAV viral particles using the insect/baculovirus cell system to produce sufficient amounts of product for therapeutic applications such as gene therapy and/or DNA vaccination, in uses during clinical or commercial trials.

(57) **Abrégé** : L'invention concerne des méthodes pour la production de particules virales AAV double brin utilisant le système de cellules d'insecte / baculovirus pour la fabrication de quantités suffisantes de produit pour des applications thérapeutiques telles que la thérapie génique et/ou la vaccination à ADN, dans des utilisations lors d'essais cliniques ou commerciales.

METHODES POUR LA PRODUCTION DE PARTICULES VIRALES AAV DOUBLE BRIN

DOMAINE DE L'INVENTION

La présente invention concerne des méthodes pour la production de particules virales AAV double brin utilisant le système de cellules d'insecte / baculovirus pour la fabrication de quantités suffisantes de produit pour des applications thérapeutiques telles que la thérapie génique et/ou la vaccination à ADN, dans des utilisations lors d'essais cliniques ou commerciales.

ARRIERE PLAN TECHNOLOGIQUE

Les principaux types de vecteurs utilisés aujourd'hui en thérapie génique sont les vecteurs viraux, dont les vecteurs recombinants de virus adéno associé ("recombinant adeno-associated virus" ou "rAAV"). De nombreux essais chez l'animal ont démontré que ces vecteurs peuvent transduire efficacement des tissus tels que le système nerveux central, le muscle, le foie, la rétine et que l'expression du transgène persiste à long terme[1,2,3]. De plus, les vecteurs rAAV présentent l'avantage d'être non pathogènes pour l'homme donc peu dangereux.

Dans les vecteurs rAAV, les gènes rep et cap encadrés par les séquences ITR (inverted terminal repeat) codant respectivement pour les protéines régulatrices de la réplication et les protéines structurales de la capside sont excisés et remplacés par le transgène (gène thérapeutique). Les séquences ITR sont conservées et sont les éléments nécessaires pour la réplication et l'encapsidation du génome viral. Pour produire des vecteurs rAAV, la procédure classique consiste à co-transfecter des cellules humaines 293 adhérentes avec un plasmide vecteur rAAV, le plasmide rep-cap sans les ITR et le plasmide auxiliaire contenant les gènes adénoviraux nécessaires à la réplication du virus AAV. Les vecteurs sont généralement purifiés et concentrés par centrifugation ou par chromatographie[4,5,6]. Cette procédure est lourde à mettre en œuvre et relativement inefficace pour générer des lots d'AAV à haut titre, sans contamination et en grande quantité nécessaires pour des essais précliniques chez des modèles animaux de grande taille ou pour des essais cliniques.

Les vecteurs rAAV sont actuellement utilisés dans de nombreux essais cliniques de thérapie génique en phase I/II pour le traitement de l'hémophilie B, le déficit en alpha-1 antitrypsine, la maladie de Parkinson, les maladies de dystrophie musculaire comme l'alpha-sarcoglycanopathie et l'amaurose congénitale de Leber[1,7,8,9]. De nouveaux modes de production et de purification ont été développés pour augmenter à la fois le titre, c'est-à-dire la concentration relative en vecteurs et la qualité des vecteurs tout en assurant une adaptation à grande échelle pour un procédé GMP. A ce jour, les systèmes les plus utilisés reposent sur 1) l'utilisation de lignées stables d'encapsidation, 2) l'infection en suspension des cellules d'insectes sf9 par des vecteurs baculovirus d'expression, ou 3) l'infection en suspension des cellules d'hamster BHK par des vecteurs HSV (herpès simplex)[10,11]. Les cellules sf9 sont notamment utilisées aujourd'hui pour produire des vecteurs AAV simple brin à l'échelle industrielle.

Le génome de l'AAV est composé d'une molécule d'ADN simple brin de 4,7 kb (inactif) dont la conversion en conformation double brin (forme active) est une étape limitante qu'il est possible de contourner en produisant des vecteurs AAV « double brin » notés scAAV (self complementary)[12,13]. Bien que ce nouveau type de vecteur soit très efficace et expérimenté en phase I/II pour l'essai clinique dans l'hémophilie B, la production des vecteurs scAAV n'est encore à ce jour réalisée qu'en utilisant le système traditionnel de transfection transitoire des cellules HEK293 en adhérence, un système de production inefficace pour le traitement par thérapie génique d'un grand nombre de patients atteints d'hémophilie[6]. De plus, dus à des problèmes inhérents au vecteur double brin : i) la proportion de particules virales AAV vides, c'est-à-dire de particules dépourvues d'ADN viral est largement prédominante et représente plus de 90%, un produit inactif contribuant à l'immunogénicité du vecteur ; ii) le génome viral n'est pas exclusivement sous forme d'ADN double brin.

La présente invention vise à pallier ces problèmes et à fournir un procédé pour la production de particules rAAV double brin avec une amélioration du ratio de particules contenant de l'ADN viral par rapport aux particules vides avec une proportion augmentée de particules contenant un génome double brin.

RESUME DE L'INVENTION

L'invention concerne un procédé pour la production d'un vecteur AAV double brin (scAAV) à haut titre et à grande échelle, comprenant la mise en culture en suspension de cellules d'insectes infectées par un ou plusieurs baculovirus recombinants codant les protéines Rep et Cap d'AAV et codant pour un génome viral d'AAV contenant un transgène d'intérêt, ledit génome viral d'AAV étant susceptible de former un génome double brin dans la particule virale. L'invention permet la production à grande échelle de vecteurs de thérapie génique de grade humain dans le respect des bonnes pratiques de fabrication. Ce procédé permet d'envisager une production industrielle de vecteurs scAAV de thérapie génique.

L'invention concerne plus particulièrement un procédé pour la production d'un vecteur scAAV, comprenant:

a) l'introduction, dans une cellule d'insecte, des séquences nucléotidiques suivantes au moyen d'au moins un vecteur baculoviral:

- une séquence codant un transgène d'intérêt compris entre des ITR d'AAV, la séquence étant choisie de manière à produire un génome AAV double brin;
- au moins une séquence codant des protéines de capside d'AAV; et
- au moins une séquence codant des protéines Rep d'AAV;

b) la culture de ladite cellule d'insecte dans des conditions permettant la production d'un vecteur rAAV et la récolte dudit vecteur rAAV.

Selon un mode particulier de réalisation de l'invention, la cellule d'insecte est une cellule sf9. Selon un autre mode particulier de réalisation, les séquences nucléotidiques sont introduites dans la cellule d'insecte au moyen d'au moins deux vecteurs baculoviraux. Selon un autre mode de réalisation, les protéines de capside produites sont des protéines de capside d'un AAV de sérotype 9. Selon un mode de réalisation supplémentaire, des protéines de Rep de sérotype 2 sont mises en oeuvre. Selon un autre mode de réalisation, le vecteur rAAV est un vecteur pseudotypé (par exemple un vecteur scAAV2/9, c'est-à-dire un vecteur AAV comprenant un génome viral double brin de sérotype 2 encapsidé dans une particule virale AAV de sérotype 9.

Selon une caractéristique avantageuse, le procédé selon l'invention permet de générer plus de 20 % de particules virales rAAV pleines, en particulier plus de 30 %, plus de 40 %, plus de 50

%, plus de 60, 70 %, voire plus de 80 % de particules virales rAAV pleines, c'est-à-dire comprenant un génome AAV encapsidé à l'intérieur desdites particules. Selon une autre caractéristique avantageuse, le procédé de l'invention permet la production de particules rAAV pleines essentiellement composées de génome viral sous forme double brin (par exemple au moins 20, 30, 40 ou au moins 50 % des particules contiennent un génome double brin). La production d'une telle proportion de particules pleines, qui plus est contenant de l'ADN double brin, n'a jamais été montrée au suggérée dans l'état de la technique.

LEGENDE DES FIGURES

La **figure 1** présente (A) une photographie d'un gel SDS-Page coloré au nitrate d'argent et (B) une photographie d'un western blot réalisé sur une membrane de nitrocellulose avec un anticorps monoclonal B1 permettant l'identification des protéines virales Vp. Les échantillons sont déposés en duplicat à 5.10^9 vg et 10^{10} vg, le puits 1 et 2 correspondent à un lot contrôle interne (ssAAV8-GFP, produit avec le système sf9), les puits 3 et 4 correspondent au lot de scAAV 9 n°A.11003.DEV produit dans les cellules sf9, les puits 5 et 6 correspondent à un autre lot (n°12D0106) de scAAV9 produit dans les cellules sf9 et les puits 7 et 8 correspondent au lot de scAAV9 produit dans des cellules HEK293F (lot n°12D0055).

La **figure 2** est un graphique montrant le profil de distribution en ultracentrifugation analytique du lot de scAAV9 n°A.12026.DEV#4 produit avec le système sf9 (trait pointillé) et du lot 12D0055 produit avec le système HEK293 (en trait plein)

La **figure 3** est un graphique montrant le profil de distribution en ultracentrifugation analytique de 3 lots scAAV9-SMN produits avec le système sf9.

DESCRIPTION DETAILLEE DE L'INVENTION

La présente invention concerne des procédés pour la production d'un vecteur rAAV recombinant double brin (ou self-complementary AAV – scAAV – en anglais). Le procédé repose sur l'utilisation du système cellule d'insecte / baculovirus qui est utilisé aujourd'hui pour la production de vecteurs AAV simple brin à l'échelle industrielle mais qui n'avait jamais été utilisé pour la production de vecteurs scAAV. Les inventeurs ont pu montrer que ce système permet la production de particules virales scAAV de meilleure qualité lorsque qu'elles sont comparées à celles produites par le système classique utilisant des cellules HEK293 transfectées au moyen de plasmides codant tous les éléments nécessaires pour la

production d'un rAAV. Les inventeurs ont notamment pu montrer que le procédé selon l'invention permet de générer une proportion élevée de particules virales rAAV pleines et que les particules produites sont essentiellement composées de génome viral sous forme double brin. Une telle qualité dans la production de vecteurs scAAV était totalement inattendue et présente des avantages pour la production à grande échelle de virus scAAV destinés à la thérapie génique.

Les éléments du vecteur produit selon l'invention peuvent être dérivés de tout sérotype d'AAV. En particulier, chaque élément peut être dérivé d'un AAV de sérotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ou 11. Par ailleurs, les éléments constitutifs du vecteurs rAAV ne sont pas nécessairement tous dérivés du même sérotype. Ainsi, un vecteur rAAV peut être composé d'un génome dérivé d'un AAV de sérotype 2, par exemple, encapsidé par des protéines de capside dérivées d'un AAV de sérotype 9. On parle alors d'un vecteur pseudotypé. Les séquences codant pour les protéines Rep peuvent également être dérivées de tout sérotype d'AAV.

Selon un mode particulier de réalisation, la séquence codant les protéines de capside est dérivée d'un AAV de sérotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ou 11. Selon une variante spécifique, la séquence code des protéines de capside d'AAV de sérotype 9 (AAV9).
Selon un mode particulier de réalisation, la séquence codant les protéines Rep est dérivée d'un AAV de sérotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ou 11. Selon une variante spécifique, la séquence code des protéines de capside d'AAV de sérotype 2 (AAV2).
Selon un mode particulier de réalisation, la séquence codant le génome viral d'AAV contenant le transgène d'intérêt est dérivée d'un AAV de sérotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ou 11. Selon une variante spécifique, le génome viral est dérivé d'un génome d'AAV2.

Le génome viral d'AAV est codé par une séquence comprenant le transgène d'intérêt flanqué de séquences ITR d'AAV. Les moyens d'obtenir un génome AAV double brin sont bien connus de l'homme du métier. Il pourra notamment se référer aux informations des références 12 et 13 ci-dessous, de McCarty et al. et de Wang et al. En résumé, la suppression du Site Terminal de Résolution (trs) au niveau d'un des deux ITR a pour autre caractéristique de ne pas séparer le brin formé du brin néonatal et ainsi de permettre la production d'un génome viral AAV sous forme double brin. Néanmoins, ce système n'est pas efficace et la cellule humaine, qui est la cellule hôte naturelle du système de régulation de l'AAV, est capable de

séparer et de former un génome viral AAV simple brin. Par ailleurs, il a été décrit que dans ce système, la production de particules AAV contenant un génome AAV est faible et ne représente que 20% de la totalité des particules virales[6].

Le transgène d'intérêt peut correspondre à une protéine ou à un ARN dont l'expression est souhaitée dans une cellule de mammifère. Le transgène d'intérêt peut en particulier coder une protéine ou un ARN ayant un intérêt thérapeutique. L'homme du métier a à sa disposition une grande quantité d'information sur les protéines ou ARN dont l'expression peut avoir un intérêt pour compenser l'absence d'expression ou l'expression anormale de ladite protéine, ou pour réguler l'expression d'une telle protéine ou d'un ARN. A titre illustratif, les exemples montrent la production d'une particule rAAV double brin dont le transgène d'intérêt est la protéine SMN. Cette protéine est mutée dans l'amyotrophie spinale. Un scAAV produit selon l'invention permettrait l'expression d'une protéine sauvage restaurant la fonction absente due à la mutation chez un patient.

Les différentes séquences codant les éléments constitutifs d'une particule virale AAV sont introduites dans un ou plusieurs baculovirus recombinants différents. Ainsi, selon une alternative, les différentes séquences listées ci-dessus peuvent être introduites dans un seul et même baculovirus recombinant. Selon un mode de réalisation alternatif, deux baculovirus recombinants sont utilisés, l'un codant les protéines Rep et Cap d'AAV (de sérotype(s) identique ou différents) et l'autre portant le génome viral de l'AAV. Selon un autre mode de réalisation, trois baculovirus sont utilisés, chacun portant l'une des trois séquences mentionnées ci-avant. Les gènes codant les protéines de l'AAV peuvent être placés sous le contrôle des promoteurs du baculovirus, régulés par les cellules d'insectes[16, 17].

La cellule d'insecte utilisée peut être toute cellule permettant la production d'un rAAV. On peut citer à titre d'exemple les cellules sf9 et sf21. Selon un mode particulier de réalisation, la cellule utilisée est une cellule sf9.

La production des vecteurs AAV est basée sur l'infection des cellules avec un ou plusieurs des baculovirus décrits ci-dessus. La culture peut être réalisée en suspension, dans un milieu approprié. A titre de milieu approprié pour la culture de cellules sf9, on peut citer le milieu Sf900III (Life technologies/Invitrogen). Les cellules sont cultivées dans un bioréacteur de volume approprié, par exemple supérieur à 100 mL, notamment supérieur à 500 mL, à 1 L, à

2 L, à 5 L, à 10 L, voire supérieur à 50 L. Selon un mode particulier de réalisation, les cellules sont infectées par le ou les baculovirus recombinant à une densité comprise entre $0,01.10^6$ et 5.10^6 cellules/mL, en particulier à une densité cellulaire comprise entre $0,8.10^6$ et 2.10^6 cellules/mL, et à une multiplicité d'infection (MOI) comprise entre 0,01 et 0,1 en particulier à une MOI de 0,05. La culture cellulaire est ensuite maintenue en suspension dans des conditions de culture permettant la production d'un AAV recombinant. Si la cellule d'insecte utilisée est une cellule sf9, elles seront maintenues en suspension à environ 27°C pendant 96 à 144 heures.

Les vecteurs sont ensuite purifiés. La purification peut comprendre une première étape de lyse des cellules. La lyse peut comprendre des étapes de lyse chimique, mécanique ou enzymatique, notamment chimique. Selon une variante de réalisation, une lyse chimique est mise en œuvre, par addition d'une solution de détergent aux cellules, notamment de Triton-X100. L'ADN est ensuite dégradé par digestion enzymatique, notamment par addition de benzonase. L'étape de purification peut notamment également comprendre une étape de clarification (e.g. sur filtre 0,45 µm). Les particules virales peuvent ensuite être davantage purifiées sur colonne(s) de chromatographie ou par ultracentrifugeuse ou par une combinaison des deux approches. Selon un mode particulier de réalisation, cette étape de purification sur colonne(s) comprend une ou plusieurs chromatographies échangeuses d'ions. Selon un mode spécifique de réalisation, les chromatographies échangeuses d'ions emploient successivement:
1) une résine cationique, par exemple SP sepharose (GE Healthcare);
2) une résine anionique en mode négatif, par exemple Fractogel® EMDTMAE, Merck-Millipore), puis
3) une résine anionique telle que la résine POROS 50HQ (Life Technologies Invitrogen). Les particules virales rAAV peuvent ensuite être concentrées et formulées dans un tampon, notamment tampon PBS, par exemple par filtration tangentielle (TFF) pour être à une concentration finale d'environ $\geq 10^{11}$ vg/ml. Ces particules concentrées peuvent être filtrées, notamment sur un filtre de 0,2 µm.

Selon un aspect particulier, l'invention se rapporte donc également à un procédé de purification de particules rAAV comprenant la soumission d'un lysat cellulaire contenant des particules rAAV aux étapes successives suivantes:
- une étape de chromatographie sur résine cationique en mode capture;
- une étape de chromatographie sur résine anionique en mode négatif; puis

- une étape de chromatographie sur résine anionique en mode capture.

Le lysat cellulaire soumis à ces étapes de chromatographie peut être issus des étapes de production développées ci-dessus.

Selon un autre aspect, l'invention concerne également un ensemble de particules scAAV, en particulier scAAV9, produit et éventuellement purifiées selon les modalités décrites ci-dessus. Plus particulièrement, l'invention concerne une composition comprenant des particules rAAV double brin, au moins 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, voire au moins 80 % des particules étant pleines, c'est-à-dire comprenant un génome AAV encapsidé à l'intérieur desdites particules et ces particules étant essentiellement composées de génome viral sous forme double brin, i.e. au moins 30 %, en particulier au moins 40 %, voire au moins 50% des particules contiennent un génome viral sous forme double brin.

L'invention sera davantage détaillée dans les exemples ci-dessous qui décrivent un mode de réalisation particulier, non limitatif, de l'invention.

EXEMPLES

Matériels et Méthodes

1. Culture cellulaire
   a. *Système de cellules d'insecte (sf9)*

La production des vecteurs scAAV9-SMN avec le système sf9/baculovirus est basée sur l'infection des cellules d'insectes sf9 dont la culture est réalisée en suspension dans le milieu Sf900III. Brièvement, dans un bioréacteur de 10L, lorsque la concentration cellulaire est comprise entre $0,8.10^6$ à 2.10^6 cellules/ml, les cellules sont infectées à une MOI de 0,05 par deux baculovirus, l'un codant pour les protéines Rep de sérotype 2 et Cap de sérotype 9 du vecteur AAV et l'autre pour le génome viral de l'AAV (transgène scSMN). La culture cellulaire est maintenue en suspension à 27°C entre 96h et 144h.

   b. *Système de cellules humaines (HEK293F)*

La production des vecteurs scAAV9-SMN avec le système HEK293F/plasmide est basée sur la transfection de ces cellules dont la culture est réalisée en suspension dans un milieu

dépourvu en protéine animale (F17). Brièvement, dans un bioréacteur de 10L, les cellules sont transfectées à la concentration cellulaire de 10^6 cellules/ml par 3 plasmides:

- le plasmide auxiliaire pXX6 contenant les gènes de l'adenovirus essentiels pour la réplication du vecteur AAV
- le plasmide codant pour les protéines Rep de sérotype 2 et Cap de sérotype 9 du vecteur AAV
- le plasmide codant pour le génome viral du vecteur AAV (transgène scSMN)

avec 20 mg total de plasmide en quantité équimolaire et 25 mL de PEIpro™ à 1 mg/ml (Polyplus). La culture cellulaire est maintenue en suspension à 37°C pendant 72h.

2. Purification

La purification des vecteurs AAV est réalisée à partir de 2 L de culture et se déroulent en 2 grandes étapes dont la première est légèrement différente pour les 2 types de culture.

a) Etape 1

Système cellules d'insecte (sf9) :

Les cellules et les baculovirus sont lysés chimiquement par addition d'une solution de Triton-X100 à 0,5% puis l'ADN cellulaire est dégradé par addition de benzonase (25U/ml de culture). La solution est ensuite clarifiée sur filtre 0,45µm. A cette étape, l'échantillon est dénommé « vrac » et titré par qPCR en utilisant une sonde et deux amorces spécifiques du génome viral AAV. La séquence nucléotidique de la sonde est 5'-TAGTTAATGATTAACCC-3' (SEQ ID NO:1), celle de l'amorce 1 (sens) est 5'-CTCCATCACTAGGGGTTCCTTG-3' (SEQ ID NO:2) et de l'amorce 2 (antisens) est 5'-GTAGATAAGTAGCATGGC-3' (SEQ ID NO:3).

Système cellules humaines (HEK293F) :

La culture est concentrée, formulée dans 500 ml par filtration tangentielle (TFF), les cellules sont ensuite lysées mécaniquement et l'ADN cellulaire est dégradé par addition de benzonase (50U/ml de culture). La solution est ensuite clarifiée sur filtre 0,45µm. A cette étape, l'échantillon est dénommé « vrac » et titré par qPCR en utilisant la sonde et les amorces dont les séquences sont fournies ci-dessus.

b) Etape 2

Les étapes de chromatographies échangeuses se décompose en trois parties :

Le lysat filtré sur membrane 0,45 µm est dilué dans un tampon MES 50 mM pH 6.0 puis chargé sur une résine cationique (SP sepharose, GE healthcare) ; l'élution est effectuée, avec variante de réalisation, à forte concentration en sel jusqu'à 200 mM. L'éluât de chromatographie est dilué dans un tampon Trizma base 50 mM afin d'avoir une concentration en sel d'environ 25 mM et un pH 8.0 avant d'être chargé en mode négatif sur une résine anionique (Fractogel® EMDTMAE, Merck-Millipore). La fraction non retenue est collectée et le pH est ajusté à pH 9.0 puis la solution est chargée sur une résine anionique (POROS 50HQ, Life tehnologies Invitrogen) utilisée comme une étape de capture. L'élution est réalisée à forte concentration en sel.

Les particules virales AAV sont concentrées et formulées dans un tampon PBS par filtration tangentielle (TFF) utilisant un seuil de coupure de 100KDa pour être à une concentration finale d'environ $\geq 10^{11}$ vg/ml et filtrées sur 0,2µm.

3. Analyses et caractérisation du vecteur scAAV9

a) Titration en génome viral (vg/ml) - qPCR

Le titre en génome viral (vg/mL) des échantillons représentant des intermédiaires de purification et des produits purifiés a été déterminé par PCR quantitative en utilisant le couple sonde/amorce1/amorce2 correspondant à un amplicon situé dans la région ITR du plasmide transgène dont les séquences sont fournies ci-dessus.

b) Pureté : SDS-PAGE, coloration au nitrate d'argent

Le degré de pureté des vecteurs AAV a été évalué en réalisant un gel d'électrophorèse des protéines en conditions dénaturantes (SDS-PAGE) suivie d'une coloration au nitrate d'argent. Tous les échantillons ont été déposés à vg équivalent (5.10^9 vg et 10^{10} vg), la masse molaire des protéines a été déterminée à l'aide d'un marqueur de protéines standards de masses molaires connues.

c) Identité des protéines virales Vp de l'AAV: Western blot

Les protéines virales de la capside de l'AAV (Vp1, Vp2 et Vp3) ont été identifiées en réalisant un gel SDS-PAGE suivie d'un transfert sur membrane de nitrocellulose et d'une détection par immunofluorescence avec l'anticorps monoclonal B1. Tous les échantillons ont

été déposés à vg équivalent (5.10^9 vg et 10^{10} vg), la masse molaire des protéines a été déterminée à l'aide d'un marqueur de protéines standards de masses molaires connues.

d) Analyse des particules virales AAV vides et pleines : Ultracentrifugation analytique

Le coefficient de sédimentation des différentes particules virales d'AAV (vide, pleine, agrégat) et autres populations présentes (subparticules, protéines contaminantes, agrégat) dans les produits purifiés a été déterminé par centrifugation en temps réel. La centrifugation des échantillons a été réalisée à une vitesse de 16000 rpm en utilisant 100µl ou 400 µl de vecteurs purs non dilués, la sédimentation a été suivie par absorbance à la longueur d'onde de 276 nm, le coefficient de sédimentation des différentes populations a été obtenu en utilisant le logiciel SEDFIT.

Résultats

1. Culture cellulaire et titre en génome viral à l'étape 1 de purification.

Dans un volume de bioréacteur de 10L, deux lots de cellules humaines HEK293F (n° 12D0004 et 12D0082) a été transfecté et deux lots de cellules d'insectes sf9 (n° A.12026.DEV et 12D0066) ont été infectés, puis 2L de culture ont été purifiés par filtration. A cette étape, la production des particules virales d'AAV a été quantifiée par PCR quantitative (tableau 1).

Le vrac 12D0055 et 12D0082 ont été obtenus respectivement à partir de la culture 12D0004 et 12D0082 (HEK293F), le vrac A.12026.DEV-4 à partir de la culture A.12026.DEV (sf9) et les 3 vracs 12D0066, 12D0070 et 12D0068 à partir de la culture 12D0066 (sf9). A cette étape de purification, les 2 systèmes de culture montrent une productivité similaire de particules virales d'AAV en termes de génomes viraux produits par cellule (de l'ordre de 10^4 vg/cellule) et de quantité totale de génomes viraux (environ 10^{13} vg).

2. Caractérisation du vecteur scAAV9-SMN final (après étape 2 de purification)

La purification des vracs a été poursuivie par 3 chromatographies successives à échange d'ions (étape 2) en appliquant un procédé décrit dans la littérature pour l'obtention de vecteurs AAV de grade précliniques et cliniques[14]. Cependant, la résine anionique POROS 50HQ, décrite comme non appropriée pour purifier les vecteurs AAV9 produits avec la méthode classique (cellules humaines HEK293 adhérentes), s'est révélée efficace pour

capturer les vecteurs AAV9 produits en suspension. Nous avons ainsi obtenu des préparations virales scAAV9-SMN dont le titre en génome viral, la pureté, l'identité et la quantification des particules virales vides et pleines ont été déterminés. A partir des 6 vracs récoltés à l'étape 1, deux lots avec le système HEK293F et quatre lots avec le système sf9 ont été générés, le lot 12D0106 ayant été obtenu en réunissant les vracs 12D0066 et 12D0070 (tableau 2).

a) Titration en génome viral (vg/ml)

Les 2 systèmes conduisent à une productivité similaire en ce qui concerne le nombre de génome viral (de l'ordre de 10^{11} à 10^{12} vg) et donc de particules virales d'AAV9 contenant le transgène d'intérêt SMN (dénommées particules d'AAV pleines).

b) Pureté et identité

Le profil en SDS-Page et Western blot de 2 lots 12D0106 et A.12026.DEV-4 (système sf9) sont identiques avec la présence des 3 protéines virales Vp1, Vp2 et Vp3 de masses molaires respectivement de 87 kDa, 72kDa et 62 kDa et les quantités relatives de 1 :1 :10, correspondant à celles attendues et au contrôle interne (fig1A et 1B). Ceci montre que les vecteurs scAAV9-produits avec le système sf9 sont purs et que la particule virale est conforme.

Le profil en SDS-Page et en Western blot du lot 12D0055 (système HEK293F) montre la présence de très nombreuses protéines dont certaines sont reconnues par l'anticorps B1 et qui correspondent à protéines virales dégradées (fig1A et 1B). Ceci indique que le vecteur scAAV9 produit avec le système HEK293F n'est pas pur.

Le procédé de chromatographie mis en place aujourd'hui et identique aux 2 systèmes de culture, ne permet pas d'obtenir des lots purs de vecteurs scAAV9 produits avec le système HEK293F.

c) Analyse des particules d'AAV vides et pleines

Il a été décrit dans la littérature que la densité est approximativement de 1,32 g/cm3 (équivalent à 60S) pour les particules virales AAV vides, c'est-à-dire les vecteurs dépourvus de génome viral et de 1,45 g/cm3 (équivalent à 110S) pour les particules virales AAV pleines, c'est-à-dire ceux contenant un génome viral de taille maximale de 4800 bases[15]. Sachant que la différence de densité est suffisamment significative pour les distinguer par centrifugation, nous avons aujourd'hui mis en place une méthode pour séparer analytiquement par

ultracentrifugation et quantifier les différentes espèces présentes dans une préparation virale d'AAV.

L'analyse en ultracentrifugation analytique des vecteurs scAAV9-SMN a été réalisée dans un premier temps en comparant un lot produit en système sf9 (A.12026.DEV-4) avec le lot 12D0055 produit en système HEK293 (figure 2). Le profil de distribution des espèces permet d'identifier 2 catégories de populations:

a) < 60S correspondant à des protéines virales non assemblées et/ou contaminants. Cette population est quasiment absente dans le lot A.12026.DEV-4 alors qu'elles sont présentes en quantité non négligeable dans le lot 12D0055. Ces impuretés ont également été détectées en SDS-Page (figure 1).
b) 60S - 110S correspondant à des particules virales AAV intactes

La population « 60S-110S » se décline en 2 sous-populations avec les particules virales d'AAV vides à 65S et les particules virales d'AAV pleines entre 80S et 110S avec le génome viral sous la forme simple brin à 90S et sous la forme double brin à 105S. Ces valeurs sont spécifiques aux vecteurs scAAV9-SMN et seront redéfinies en fonction du type du génome. De façon très inattendue, nous avons détecté très peu de particules virales d'AAV vides avec le système sf9 comparée au système HEK293. De plus, les particules virales AAV pleines sont nettement plus enrichies en génome sous forme double brin (105S) avec le système sf9 comparée au système HEK293.
Le profil de distribution du lot 12D0082 est identique à celui du lot 12D0055, ce qui confirme que le système HEK293 produit essentiellement très peu de particules AAV pleines (< 25%) avec seulement 10% de particules virales contenant le génome sous forme double brin.

Une seconde expérience avec les 3 lots de vecteurs scAAV9-SMN produits en système sf9 (A.12026.DEV-4, 12D0106, 12D0068) a été réalisée de la même façon par ultracentrifugation analytique (figure 3). Ces 3 lots montrent tous un profil de distribution similaire et confirme que le système sf9 permet de produire des particules virales d'AAV essentiellement composées de particules d'AAV pleines (>80%) avec une très forte proportion de génome viral sous forme double brin (>50%).

Culture cellulaire			Purification: Etape 1				
Type	Lot N°	Cellule/ml	Vrac N°	Volume (L)	Titre (vg/ml)	Titre (vg/cellule)	Total Vg
HEK293F	12D0004	1,0E+06	12D0055	0,5	4,0E+10	4,0E+04	2,0E+13
	12D0082	1,0E+06	12D0082	2	1,2E+10	1,2E+04	2,4E+13
sf9	A.12026.DEV	1,0E+06	A.12026.DEV-4	2	2,2E+10	1,3E+04	4,5E+13
	12D0066	1,9E+06	12D0066	2	1,9E+10	1,0E+04	3,8E+13
			12D0070	2	2,0E+10	1,0E+04	4,0E+13
			12D0068	2	6,4E+09	3,4E+03	1,3E+13

Tableau 1 : Description de la concentration cellulaire dans le bioréacteur de 10L avant transfection ou infection, des volumes (L) et des titres en génomes viraux (vg/ml) obtenus après la purification (étape 1) de 2L culture cellulaire.

Culture cellulaire		Purification				
Type	Lot N°	Etape 1	Etape 2			
		Vrac N°	Lot N°	Vol (ml)	Titre (vg/mL)	Total Vg
HEK293F	12D0004	12D055	12D0055	0,6	1,6E+12	9,5E+11
	12D0082	12D0082	12D0082	0,8	5,1E+11	4,1E+11
sf9	A.12026.DEV	A.12026.DEV-4	A.12026.DEV-4	1,2	1,4E+12	1,7E+12
	12D0066	12D0066	12D0106	0,8	9,4E+11	7,5E+11
		12D0070				
		12D0068	12D0068	0,5	1,6E+12	8,0E+11

Tableau 2 : Description du volume, du titre en génomes viraux (vg/ml), quantité totale de génomes viraux de particules d'AAV9 (vg) obtenus après l'étape 2 de purification

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REVENDICATIONS

1. Procédé pour la production d'un vecteur AAV double brin (scAAV) à haut titre et à grande échelle, comprenant la mise en culture en suspension de cellules d'insectes infectées par un ou plusieurs vecteurs baculoviraux recombinants codant les protéines Rep et Cap d'AAV et pour un génome viral d'AAV contenant un transgène d'intérêt, ledit génome viral d'AAV étant susceptible de former un génome double brin dans la particule virale.

2. Procédé pour la production de particules virales scAAV, le procédé permettant la production de particules scAAV dont plus de 20 % ont encapsidé de l'ADN viral et sont fonctionnelles, comprenant la mise en culture en suspension de cellules d'insectes infectées par un ou plusieurs vecteurs baculoviraux recombinants codant les protéines Rep et Cap d'AAV et codant pour un génome viral d'AAV contenant un transgène d'intérêt, ledit génome viral d'AAV étant susceptible de former un génome double brin dans la particule virale.

3. Procédé selon la revendication 1 ou 2, la cellule d'insecte étant une cellule sf9.

4. Procédé selon l'une quelconque des revendications 1 à 3, les cellules d'insecte étant infectées au moyen de deux baculovirus, l'un des deux vecteurs baculoviraux codant les protéines Rep et Cap, et l'autre vecteur baculoviral codant le génome viral d'AAV.

5. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel le vecteur scAAV produit est de sérotype 9.

6. Procédé selon l'une quelconque des revendications 1 à 5, comprenant en outre une étape de purification du vecteur scAAV.

7. Procédé selon la revendication 6, la purification comprenant les deux étapes suivantes:
a) lyse des cellules d'insecte; et
b) passage du lysat ainsi obtenu sur des colonnes de chromatographie échangeuse d'ions.

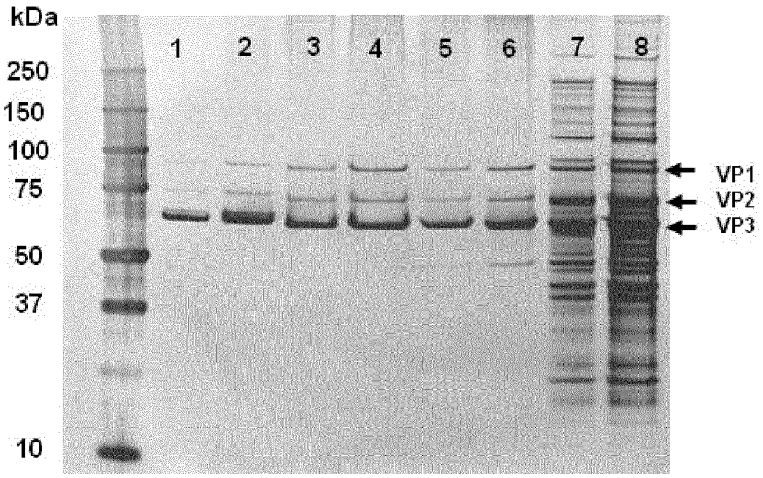
8. Procédé selon la revendication 7, dans lequel l'étape b) de chromatographie échangeuse d'ions comprend successivement un passage de l'échantillon sur une résine cationique en

mode capture, un passage sur une résine anionique en mode négatif et un passage sur une autre résine anionique en mode capture.

9. Procédé de purification d'un scAAV comprenant la soumission d'un lysat de cellule produisant des scAAV sur des colonnes de chromatographie échangeuse d'ions, comprenant successivement un passage de l'échantillon sur une résine cationique en mode capture, un passage sur une résine anionique en mode négatif et un passage sur une autre résine anionique en mode capture.

10. Composition comprenant des particules scAAV, au moins 20 % des particules comprenant un génome AAV encapsidé à l'intérieur desdites particules et ces particules étant essentiellement composées de génome viral sous forme double brin.

A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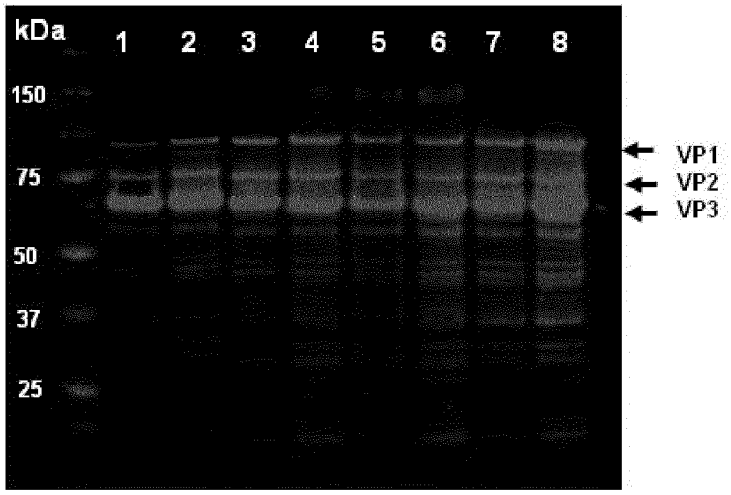
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Figure 1

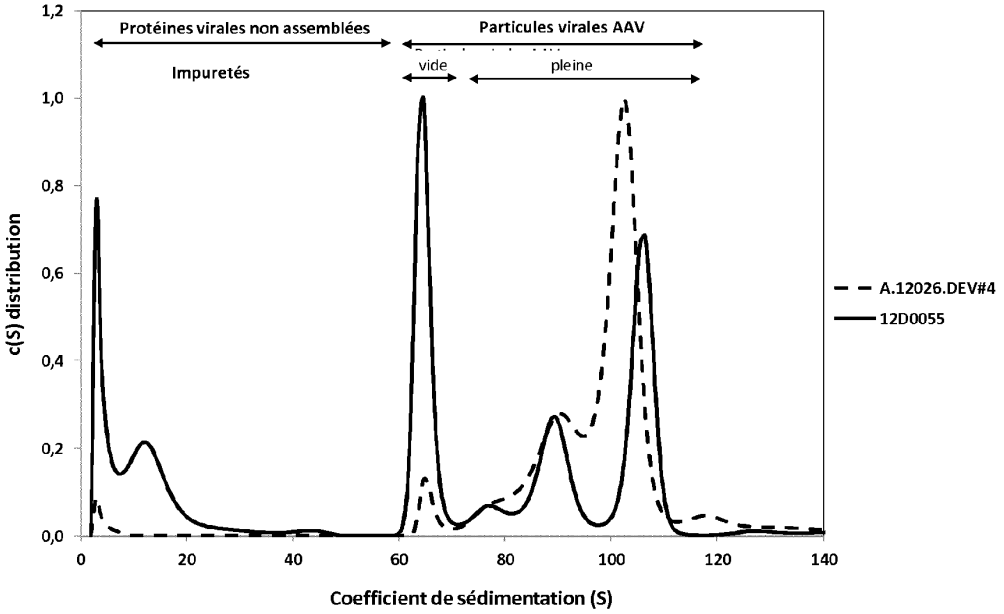

Figure 2

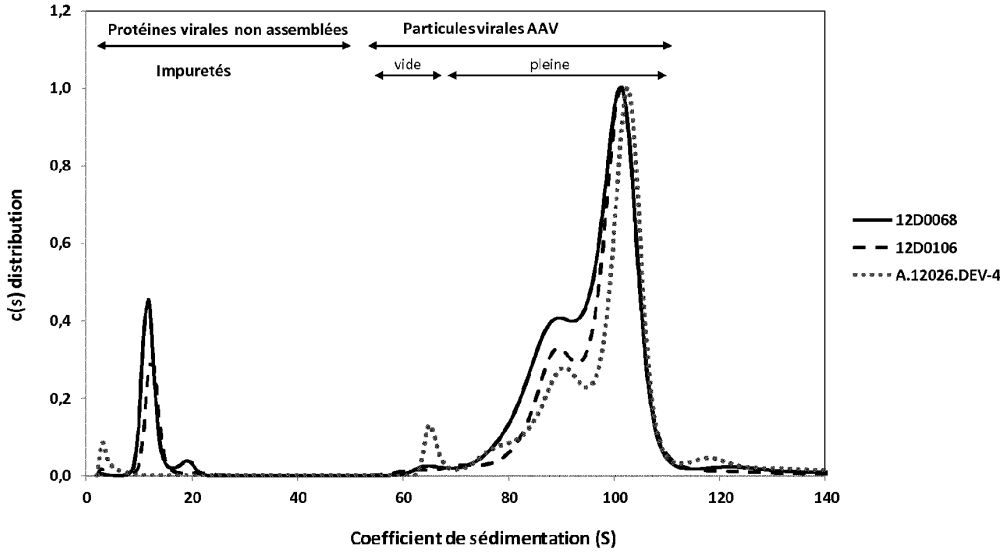

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/052978

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N7/02 C12N15/86 A61K48/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/122950 A1 (AMSTERDAM MOLECULAR THERAPEUTICS AMT IP B V [NL]; LUBELSKI JACEK [NL];) 6 October 2011 (2011-10-06)	1-6,10
Y	page 6 - page 10; claims 23-29, 42-44	7-9

Y	WO 2011/094198 A1 (PHILADELPHIA CHILDREN HOSPITAL [US]; WRIGHT JOHN FRASER [US]; QU GUANG) 4 August 2011 (2011-08-04) page 14 - page 17	7-9

A	WO 2011/012724 A1 (ASS INST DE MYOLOGIE [FR]; GENETHON [FR]; CENTRE NAT RECH SCIENT [FR];) 3 February 2011 (2011-02-03) page 11	1-5

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2014	03/04/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Deleu, Laurent

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/052978

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/084773 A2 (ASKLEPIO BIOPHARMACEUTICAL INC [US]; SAMULSKI RICHARD J [US]; CHEN HAI) 26 July 2007 (2007-07-26) claim 1	1-4
A	TAMAS VIRAG ET AL: "Producing Recombinant Adeno-Associated Virus in Foster Cells: Overcoming Production Limitations Using a Baculovirus-Insect Cell Expression Strategy", HUMAN GENE THERAPY, vol. 20, no. 8, August 2009 (2009-08), pages 807-817, XP055052744, ISSN: 1043-0342, DOI: 10.1089/hum.2009.092 figures 1,4	1-4
A	WO 2008/016391 A2 (UNIV LELAND STANFORD JUNIOR [US]; KAY MARK A [US]; GRIMM DIRK [US]) 7 February 2008 (2008-02-07) page 26 - page 37	1-4

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/052978

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011122950 A1	06-10-2011	NONE	
WO 2011094198 A1	04-08-2011	AU 2011209743 A1	23-08-2012
		CA 2787827 A1	04-08-2011
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		WO 2008016391 A2	07-02-2008

RAPPORT DE RECHERCHE INTERNATIONALE

Demande internationale n°
PCT/EP2014/052978

Cadre N° I Séquence(s) de nucléotides ou d'acides aminés (suite du point 1.c de la première feuille)

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A. CLASSEMENT DE L'OBJET DE LA DEMANDE
INV. C12N7/02 C12N15/86 A61K48/00
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Selon la classification internationale des brevets (CIB) ou à la fois selon la classification nationale et la CIB

B. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultée (système de classification suivi des symboles de classement)
C12N A61K

Documentation consultée autre que la documentation minimale dans la mesure où ces documents relèvent des domaines sur lesquels a porté la recherche

Base de données électronique consultée au cours de la recherche internationale (nom de la base de données, et si cela est réalisable, termes de recherche utilisés)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie*	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
X	WO 2011/122950 A1 (AMSTERDAM MOLECULAR THERAPEUTICS AMT IP B V [NL]; LUBELSKI JACEK [NL];) 6 octobre 2011 (2011-10-06)	1-6,10
Y	page 6 - page 10; revendications 23-29, 42-44	7-9

Y	WO 2011/094198 A1 (PHILADELPHIA CHILDREN HOSPITAL [US]; WRIGHT JOHN FRASER [US]; QU GUANG) 4 août 2011 (2011-08-04) page 14 - page 17	7-9

A	WO 2011/012724 A1 (ASS INST DE MYOLOGIE [FR]; GENETHON [FR]; CENTRE NAT RECH SCIENT [FR];) 3 février 2011 (2011-02-03) page 11	1-5

	-/--	

[X] Voir la suite du cadre C pour la fin de la liste des documents

[X] Les documents de familles de brevets sont indiqués en annexe

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Date à laquelle la recherche internationale a été effectivement achevée	Date d'expédition du présent rapport de recherche internationale
24 mars 2014	03/04/2014

Nom et adresse postale de l'administration chargée de la recherche internationale	Fonctionnaire autorisé
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RAPPORT DE RECHERCHE INTERNATIONALE

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PCT/EP2014/052978

C(suite). DOCUMENTS CONSIDERES COMME PERTINENTS

Catégorie*	Identification des documents cités, avec, le cas échéant, l'indication des passages pertinents	no. des revendications visées
A	WO 2007/084773 A2 (ASKLEPIO BIOPHARMACEUTICAL INC [US]; SAMULSKI RICHARD J [US]; CHEN HAI) 26 juillet 2007 (2007-07-26) revendication 1	1-4
A	TAMAS VIRAG ET AL: "Producing Recombinant Adeno-Associated Virus in Foster Cells: Overcoming Production Limitations Using a Baculovirus-Insect Cell Expression Strategy", HUMAN GENE THERAPY, vol. 20, no. 8, août 2009 (2009-08), pages 807-817, XP055052744, ISSN: 1043-0342, DOI: 10.1089/hum.2009.092 figures 1,4	1-4
A	WO 2008/016391 A2 (UNIV LELAND STANFORD JUNIOR [US]; KAY MARK A [US]; GRIMM DIRK [US]) 7 février 2008 (2008-02-07) page 26 - page 37	1-4

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RAPPORT DE RECHERCHE INTERNATIONALE

Renseignements relatifs aux membres de familles de brevets

Demande internationale n°
PCT/EP2014/052978

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)	Date de publication
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		US 2007253936 A1	01-11-2007
		WO 2008016391 A2	07-02-2008

Patents PCT/EP2014/052978,

Methods for the production of double-stranded aav viral particles

Abstract

The invention relates to methods for producing double-stranded AAV viral particles using the insect/baculovirus cell system to produce sufficient amounts of product for therapeutic applications such as gene therapy and/or DNA vaccination, in uses during clinical or commercial trials.

Classifications

A61K48/0091 Purification or manufacturing processes for gene therapy compositions

View 6 more classifications

WO2014125101A1

WIPO (PCT)

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Other languages: French

Inventor: Christine Le Bec, Mohammed RIFKI

Worldwide applications

2013 - FR 2014 - WO

Application PCT/EP2014/052978 events

2014-02-15 • Application filed by Genethon

2014-08-21 • Publication of WO2014125101A1

Info: Patent citations (6), Non-patent citations (1), Cited by (7), Legal events, Similar documents, Priority and Related Applications

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Claims

Hide Dependent

A method for producing a large-scale, high-titre, double-stranded AAV vector (scAAV) comprising culturing in suspension of insect cells infected with one or more recombinant baculovirus vectors encoding Rep and Cap proteins of AAV and for an AAV viral genome containing a transgene of interest, said AAV viral genome being capable of forming a double-stranded genome in the viral particle.

2. Process for the production of scAAV viral particles, the process for the production of scAAV particles of which more than 20% have encapsidated viral DNA and are functional, comprising culturing in suspension of infected insect cells by or a plurality of recombinant baculovirus vectors encoding the AAV Rep and Cap proteins and encoding an AAV viral genome containing a transgene of interest, said AAV viral genome being capable of forming a double-stranded genome in the viral particle.

3. Method according to claim 1 or 2, the insect cell being a sf9 cell.

4. Method according to any one of claims 1 to 3, the insect cells being infected with two baculoviruses, one of two baculovirus vectors encoding the Rep and Cap proteins, and the other baculovirus vector encoding the genome. viral AAV.

The method of any one of claims 1 to 4, wherein the scAAV vector produced is serotype 9.

The method of any one of claims 1 to 5, further comprising a step of purifying the scAAV vector.

7. The method of claim 6, the purification comprising the two following steps: a) lysis of the insect cells; and

b) passage of the lysate thus obtained on ion exchange chromatography columns.

The process according to claim 7, wherein the ion exchange chromatography step b) comprises successively a passage of the sample on a cationic resin in capture mode, a passage on an anionic resin in negative mode and a passage on another anionic resin in capture mode.

9. A process for purifying a scAAV comprising subjecting a cell lysate producing scAAVs on ion exchange chromatography columns, successively comprising a passage of the sample on a cationic resin in capture mode, a passage on an anionic resin in negative mode and a passage on another anionic resin in capture mode.

10. A composition comprising scAAV particles, at least 20% of the particles comprising an AAV genome packaged inside said particles and these particles being essentially composed of viral genome in double-stranded form.

Description

METHODS FOR THE PRODUCTION OF DOUBLE AAV VIRAL PARTICLES

STRAND

FIELD OF THE INVENTION

The present invention relates to methods for producing double-stranded AAV viral particles using the insect cell / baculovirus system for the manufacture of sufficient quantities of product for therapeutic applications such as gene therapy and / or DNA vaccination, in uses in clinical or commercial trials.

BACKGROUND TECHNOLOGY

The main types of vectors used today in gene therapy are the viral vectors, including the recombinant vectors of adeno-associated virus ("recombinant adeno-associated virus" or "rAAV"). Numerous animal tests have shown that these vectors can efficiently transduce tissues such as the central nervous system, muscle, liver, retina and that transgene expression persists in the long term [1, 2, 3]. In addition, the rAAV vectors have the advantage of being non-pathogenic for humans and therefore not very dangerous.

In the rAAV vectors, the rep and cap genes flanked by the inverted terminal repeat (ITR) sequences coding respectively for the replication regulatory proteins and the capsid structural proteins are excised and replaced by the transgene (therapeutic gene). The ITR sequences are conserved and are the necessary elements for the replication and encapsidation of the viral genome. In order to produce rAAV vectors, the conventional procedure is to co-transfect human 293 cells adherent with an rAAV vector plasmid, the rep-cap plasmid without the ITRs and the helper plasmid containing the adenoviral genes necessary for replication of the AAV virus. The vectors are generally purified and concentrated by centrifugation or by [4, 5, 6] chromatography. This procedure is cumbersome and relatively inefficient to generate high-titre, contamination-free, high-volume AAV batches required for preclinical testing in large animal models or for clinical trials. RAAV vectors are currently used in numerous Phase I / II gene therapy clinical trials for the treatment of hemophilia B, alpha-1 antitrypsin deficiency, Parkinson's disease, muscular dystrophy diseases such as alpha - sarcoglycanopathy and congenital amaurosis of Leber [1, 7, 8, 9]. Novel modes of production and purification have been developed to increase both the titre, i.e. the relative vector concentration and the vector quality, while ensuring large scale adaptation for a GMP process. To date, the most used systems rely on 1) the use of stable encapsidation lines, 2) the suspension infection of sf9 insect cells by baculovirus expression vectors, or 3) the infection. suspension of hamster BHK cells by HSV vectors (herpes simplex) [10, 11]. The sf9 cells are especially used today to produce single-stranded AAV vectors on an industrial scale.

The genome of AAV is composed of a 4.7 kb single-stranded DNA molecule (inactive) whose conversion into double-stranded conformation (active form) is a limiting step that can be circumvented by producing AAV "double-stranded" vectors scAAV (self complementary) [12, 13]. Although this new type of vector is very effective and tested in phase I / II for the clinical trial in hemophilia B, the production of scAAV vectors is still only achieved using the traditional transfection system. transient adherence of HEK293 cells, an inefficient production system for gene therapy treatment of large numbers of patients with hemophilia [5]. In addition, due to the problems inherent in the double-stranded vector: i) the proportion of empty AAV viral particles, that is particles devoid of viral DNA is largely predominant and represents more than 90%, an inactive product contributing to the immunogenicity of the vector; ii) the viral genome is not exclusively in the form of double-stranded DNA.

The present invention aims to overcome these problems and to provide a method for the production of double-stranded rAAV particles with an improvement in the ratio of particles containing viral DNA to empty particles with an increased proportion of particles containing a double-stranded genome . SUMMARY OF THE INVENTION

The invention relates to a method for the production of a large-scale, high-titre double-stranded AAV vector (scAAV), comprising culturing in suspension of insect cells infected with one or more recombinant baculoviruses encoding the Rep proteins. and AAV cap and encoding an AAV viral genome containing a transgene of interest, said AAV viral genome being capable of forming a double-stranded genome in the viral particle. The invention enables the large-scale production of human-grade gene therapy vectors in accordance with good manufacturing practices. This method makes it possible to envisage an industrial production of scAAV gene therapy vectors.

The invention more particularly relates to a method for producing a scAAV vector, comprising:

a) introducing, into an insect cell, the following nucleotide sequences using at least one baculovirus vector:

a sequence encoding a transgene of interest comprised between AAV ITRs, the sequence being chosen so as to produce a double-stranded AAV genome,

at least one sequence encoding AAV capsid proteins; and

at least one sequence encoding AAV Rep proteins,

b) culturing said insect cell under conditions permitting the production of an rAAV vector and harvesting said rAAV vector.

According to a particular embodiment of the invention, the insect cell is a sf9 cell. According to another particular embodiment, the nucleotide sequences are introduced into the insect cell by means of at least two baculovirus vectors. According to another embodiment, the capsid proteins produced are capsid proteins of a serotype 9 AAV. According to a further embodiment, serotype 2 Rep proteins are used. According to another embodiment, the rAAV vector is a pseudotyped vector (for example a scAAV2 / 9 vector, that is to say an AAV vector comprising a double-stranded viral genome of serotype 2 packaged in a serotype AAV viral particle. 9.

According to an advantageous characteristic, the method according to the invention makes it possible to generate more than 20% of solid rAAV viral particles, in particular more than 30%, more than 40%, more than 50%. %, more than 60, 70%, or even more than 80% of full rAAV viral particles, that is to say comprising an AAV genome packaged inside said particles. According to another advantageous characteristic, the method of the invention allows the production of solid rAAV particles essentially composed of viral genome in double-stranded form (for example at least 20, 30, 40 or at least 50% of the particles contain a double-stranded genome). The production of such a proportion of solid particles, moreover containing double-stranded DNA, has never been shown as suggested in the state of the art.

LEGEND OF FIGURES

FIG. 1 shows (A) a photograph of a silver nitrate stained SDS-Page gel and (B) a photograph of a western blot made on a nitrocellulose membrane with a monoclonal antibody B1 allowing the identification of proteins. viral Vp. The samples are loaded in duplicate at 5.10^9 vg and 10^{10} vg, the wells 1 and 2 correspond to an internal control lot (ssAAV8-GFP product with Sf9 system), the wells 3 and 4 correspond to the batch of scAAV 9 n ° A.11003.DEV produced in sf9 cells, wells 5 and 6 correspond to another lot (# 12D0106) of scAAV9 produced in sf9 cells and wells 7 and 8 correspond to the lot of scAAV9 produced in HEK293F cells (Lot No 12D0055).

Figure 2 is a graph showing the **analy**tic ultracentrifugation distribution profile of the batch of scAAV9 # A.12026. DEV # 4 produced with sf9 system (dashed line) and lot 12D0055 produced with HEK293 system (solid line)

Figure 3 is a graph showing the **analy**tic ultracentrifugation distribution profile of 3 scAAV9-SMN lots produced with the sf9 system.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for producing a double-strand recombinant rAAV vector (or self-complementary AAV - scAAV). The method relies on the use of the insect cell / baculovirus system which is used today for the production of single-strand AAV vectors on an industrial scale but which has never been used for the production of scAAV vectors. The inventors have been able to show that this system allows the production of better quality scAAV viral particles when compared to those produced by the conventional system using HEK293 cells transfected with plasmids encoding all the elements necessary for the production of an rAAV. The inventors have in particular been able to show that the method according to the invention makes it possible to generate a high proportion of solid rAAV viral particles and that the particles produced are essentially composed of viral genome in double-stranded form. Such quality in the production of scAAV vectors was totally unexpected and has advantages for the large scale production of scAAV viruses for gene therapy.

The elements of the vector produced according to the invention may be derived from any serotype of AAV. In particular, each element can be derived from an AAV of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11. Furthermore, the constituent elements of the rAAV vector are not necessarily all derived from the same serotype. Thus, an rAAV vector may be composed of a genome derived from a serotype 2 AAV, for example, packaged with capsid proteins derived from a serotype 9 AAV. This is called a pseudotyped vector. The coding sequences for the Rep proteins can also be derived from any AAV serotype.

According to a particular embodiment, the sequence encoding the capsid proteins is derived from an AAV serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11. According to a specific variant, the Serotype 9 AAV Capsid Protein (AAV9) coding sequence.

According to one particular embodiment, the sequence coding for the Rep proteins is derived from an AAV of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11. According to a specific variant, the sequence serotype 2 AAV capsid protein code (AAV2).

According to a particular embodiment, the sequence encoding the AAV viral genome containing the transgene of interest is derived from an AAV of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or According to a specific variant, the viral genome is derived from an AAV2 genome.

The viral AAV genome is encoded by a sequence comprising the transgene of interest flanked by AAV ITR sequences. The means for obtaining a double-stranded AAV genome are well known to those skilled in the art. in particular, he may refer to the information in references 12 and 13 below. by McCarty et al. and Wang et al. In

summary, the deletion of the Resolution Terminal Site (trs) at one of the two ITRs has another characteristic of not separating the strand formed from the neonatal strand and thus allowing the production of an AAV viral genome in double form. strand. Nevertheless, this system is not efficient and the human cell, which is the natural host cell of the AAV regulation system, is capable of separate and form a single-stranded AAV viral genome. Furthermore, it has been described that in this system, the production of AAV particles containing an AAV genome is low and represents only 20% of the totality of the viral particles [5] .

The transgene of interest may be a protein or RNA whose expression is desired in a mammalian cell. The transgene of interest can in particular encode a protein or an RNA having a therapeutic interest. Those skilled in the art have at their disposal a large amount of information on the proteins or RNA whose expression may be of interest to compensate for the lack of expression or the abnormal expression of said protein, or for regulating the expression of such a protein or RNA. By way of illustration, the examples show the production of a double-stranded rAAV particle whose transgene of interest is the SMN protein. This protein is mutated in spinal muscular atrophy. A scAAV produced according to the invention would allow the expression of a wild-type protein restoring the function absent due to mutation in a patient.

The different sequences encoding the constitutive elements of an AAV viral particle are introduced into one or more different recombinant baculoviruses. Thus, according to one alternative, the various sequences listed above can be introduced into a single recombinant baculovirus. According to an alternative embodiment, two recombinant baculoviruses are used, one encoding the AAV Rep and Cap proteins (of the same or different serotype (s)) and the other carrying the viral AAV genome. According to another embodiment, three baculoviruses are used. each carrying one of the three sequences mentioned above. The genes encoding the AAV proteins may be placed under the control of the promoters of the baculovirus, regulated by insect cells [10 11].

The insect cell used may be any cell allowing the production of an rAAV. By way of example, mention may be made of the sf9 and sf21 cells. According to a particular embodiment, the cell used is a sf9 cell.

The production of AAV vectors is based on the infection of cells with one or more of the baculoviruses described above. The culture can be carried out in suspension, in a suitable medium. As a suitable medium for the culture of sf9 cells, mention may be made of Sf900II medium (Life Technologies / Invitrogen). The cells are cultured in a bioreactor of appropriate volume, for example greater than 100 mL, especially greater than 500 mL, 1 L, 2 L, 5 L, 10 L, or greater than 50 L. According to a particular embodiment, cells are infected with recombinant baculo virus or to a density of between 5.10 and 0.01.10 * [6] cells / mL , in particular at a cell density of between 0.8 x 10 [6] and 2.10 [6] cells / mL, and at a multiplicity of infection (MOI) of between 0.01 and 0.1, in particular at an MOI of 0.05. The cell culture is then kept in suspension under culture conditions allowing the production of a recombinant AAV. If the insect cell used is a sf9 cell, they will be suspended at about 27 ° C for 96 to 144 hours.

The vectors are then purified. The purification may comprise a first step of lysing the cells. The lysis may comprise chemical, mechanical or enzymatic lysis, in particular chemical lysis steps. According to one embodiment variant, chemical lysis is carried out by adding a detergent solution to the cells, in particular Triton-X100. The DNA is then degraded by enzymatic digestion, in particular by the addition of benzonase. The purification step may in particular also comprise a clarification step (e.g., on a 0.45 µm filter). The viral particles can then be further purified on a chromatography column (s) or ultracentrifuge or a combination of both approaches. According to a particular embodiment, this column purification step (s) comprises one or more ion exchange chromatograms. According to a specific embodiment, the ion exchange chromatographs successively use:

1) a cationic resin, for example SP sepharose (GE Healthcare);

2) a negative mode anionic resin, for example Fractogel® EMDTMAE, Merck-Millipore), then

3) an anionic resin such as POROS 50HQ resin (Life Technologies Invitrogen). RAAV viral particles can then be concentrated and formulated in a buffer, including PBS buffer, for example by tangential filtration (TFF) to be at a final concentration of about> 10 [13] vg / ml. These concentrated particles can be filtered, in particular on a 0.2 µm filter.

According to a particular aspect, the invention therefore also relates to a process for purifying rAAV particles comprising subjecting a cell lysate containing rAAV particles to the following successive stages:

a step of cationic resin chromatography in capture mode;

an anionic resin chromatography step in negative mode, then an anionic resin chromatography step in capture mode.

The cell lysate subjected to these chromatography steps may be derived from the production steps developed above.

According to another aspect, the invention also relates to a set of scAAV particles, in particular scAAV9, produced and optionally purified according to the modalities described above. More particularly, the invention relates to a composition comprising double-stranded rAAV particles, at least 20%, 30%, 40%, 50%, 60%, 70%, or even at least 80% of the particles being solid, that is, ie comprising an AAV genome packaged inside said particles and these particles being essentially composed of viral genome in double-stranded form, ie at least 30%, in particular at least 40%, or even at least 50% of the particles contain a viral genome in double-stranded form.

The invention will be further detailed in the examples below which describe a particular, non-limiting embodiment of the invention.

EXAMPLES

Materials and methods

1. Cell culture

at Insect cell system (sf9)

The production of the scAAV9-SMN vectors with the sf baculovirus system is based on the infection of the sf9 insect cells whose culture is carried out in suspension in Sf900II medium. Briefly, in a 10L bioreactor, when the cell concentration is between 0.8 x 10 [6] to 2 x 10 [6] cells / ml, the cells are infected at an MOI of 0.05 with two baculo viruses, one coding for the Rep proteins. serotype 2 and Cap serotype 9 of the AAV vector and the other for the viral genome of AAV (transgene scSMN). The cell culture is kept in suspension at 27 ° C between 96h and 144h. b. Human cell system (HEK293F)

The production of the scAAV9-SMN vectors with the HEK293F / plasmid system is based on the transfection of these cells, the culture of which is carried out in suspension in a medium lacking in animal protein (F 17). Briefly, in a 10L bioreactor, the cells are transfected at a cell concentration of 10 [6] cells / ml with 3 plasmids:

the auxiliary plasmid pXX6 containing the adeno virus genes essential for the replication of the AAV vector

the plasmid encoding serotype 2 serotype 2 and serotype 9 cape proteins of the AAV vector

the plasmid encoding the viral genome of the AAV vector (transgene scSMN) with 20 mg total plasmid in equimolar amount and 25 mL of PEIpro ™ 1 mg / ml (Polyplus). The cell culture is kept in suspension at 37 ° C for 72h.

2. Purification

The purification of the AAV vectors is carried out from 2 L of culture and take place in two large stages, the first of which is slightly different for the two types of culture. a) Step 1

Insect Cells System (sf9):

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The cells and the baculo viruses are chemically lysed by addition of a 0.5% Triton-X100 solution and then the cellular DNA is degraded by addition of benzonase (25U / ml of culture). The solution is then clarified on a 0.45 µm filter. At this stage, the sample is called "bulk" and titrated with qPCR using a probe and two specific primers of the AAV viral genome. The nucleotide sequence of the probe is 5'-TAGTTAATGATTAACCC-3 '(SEQ ID NO: 1), that of primer 1 (sense) is 5'-CTCCATCACTAGGGGTTCCTTG-3' (SEQ ID NO: 2) and the Primer 2 (antisense) is 5'-GTAGATAAGTAGCATGGC-3 '(SEQ ID NO: 3).

Human cell system (HEK293F):

The culture is concentrated, formulated in 500 ml by tangential filtration (TFF), the cells are then mechanically lysed and the cellular DNA is degraded by addition of benzonase (50 U / ml of culture). The solution is then clarified on a 0.45 µm filter. At this stage, the sample is called "bulk" and titrated with qPCR using the probe and primers whose sequences are provided above. b) Step 2

The stages of exchange chromatography is divided into three parts: The lysate filtered on a 0.45 µm membrane is diluted in a 50 mM MES buffer pH 6.0 and then loaded onto a cationic resin (SP sepharose, GE Healthcare); the elution is carried out, with variant embodiment, with a high salt concentration of up to 200 mM. The chromatography eluate is diluted in a 50 mM Trizma base buffer in order to have a salt concentration of about 25 mM and pH 8.0 before being loaded in negative mode on an anionic resin (Fractogel® EMDTMAE, Merck -Millipore). The non-retained fraction is collected and the pH is adjusted to pH 9.0 and the solution is loaded onto an anionic resin (POROS 50HQ, Life tehnologies invitrogen) used as a capture step. Elution is carried out with high salt concentration.

The AAV viral particles are concentrated and formulated in a PBS buffer by tangential filtration (TFF) using a cut-off threshold of 10KDa to be at a final concentration of approximately- $10^{\,n}$ vg / ml and filtered through 0.2µm.

3. **Analy**sis and characterization of the scAAV9 vector

a) Titration in viral genome (vg / ml) - qPCR

The viral genome titre (vg / mL) of the samples representing purification intermediates and purified products was determined by quantitative PCR using the pair probe / amorcel / primer2 corresponding to an amplicon located in the ITR region of the transgene plasmid whose sequences are provided above.

b) Purity: SDS-PAGE, silver nitrate stain

The degree of purity of the AAV vectors was evaluated by performing an electrophoresis gel of the proteins under denaturing conditions (SDS-PAGE) followed by staining with silver nitrate. All samples were plated at 5% equivalent ($5.10^{\,9}$ and $10^{\,5}$), and the protein molecular weight was determined using a standard protein marker of known molecular weights. c) Identity of viral proteins Vp of the V: Western blot

The viral proteins of the AAV capsid (Vp1, Vp2 and Vp3) were identified by performing an SDS-PAGE gel followed by nitrocellulose membrane transfer and immuno fluorescence detection with the monoclonal antibody B1. . All samples have The molar mass of the proteins was determined using a standard protein marker of known molar masses, measured at 5% equivalent ($5.10^{\,9}$ and $10^{\,10}$). d) **Analy**sis of empty and solid AA V viral particles: **Analy**tical ultracentrifugation The sedimentation coefficient of the various AAV viral particles (empty, solid, aggregate) and other present populations (subparticles, contaminating proteins, aggregate) in the purified products was determined by centrifugation in real time. The centrifugation of the samples was carried out at a speed of 16000 rpm using 100µl or 400 µl of undiluted pure vectors, the sedimentation was followed by absorbance at the wavelength of 276 nm, the sedimentation coefficient of the different populations was obtained using the SEDFIT software.

Results

1. Cell Culture and Title in Viral Genome at Purification Step 1

In a 10L bioreactor volume, two batches of human HEK293F cells (# 12D0004 and 12D0082) were transfected and two batches of sf9 insect cells (# A.12026.DEV and # 12D0066) were infected, followed by 2L of culture were purified by filtration. At this stage, the production of the AAV viral particles was quantified by quantitative PCR (Table 1).

Bulk 12D0055 and 12D0082 were obtained respectively from crop 12D0004 and 12D0082 (HEK293F), bulk A.12026.DEV-4 from crop A.12026.DEV (sf9) and 3 bulks 12D0066, 12D0070 and 12D0068 from culture 12D0066 (sf9). At this purification step, the 2 culture systems show a similar productivity of AAV viral particles in terms of viral genomes produced per cell (of the order of $10^{\,4}$ vg / cell) and of total amount of viral genomes (approximately $10^{\,13}$ (vg).

2. Characterization of the final scAAV9-SMN vector (after purification step 2)

Bulk purification was continued by 3 successive ion exchange chromatographies (step 2) using a method described in the literature for obtaining preclinical and clinical grade AAV vectors [14]. However, the POROS 50HQ anionic resin, described as not suitable for purifying the AAV9 vectors produced with the conventional method (adherent human HEK293 cells), has proved effective for capture the AAV9 vectors produced in suspension. We thus obtained scAAV9-SMN viral preparations whose title in viral genome, the purity, the identity and the quantification of empty and full viral particles were determined. From the 6 bulks collected in step 1, two batches with the HEK293F system and four batches with the sf9 system were generated, the batch 12D0106 having been obtained by combining the bulks 12D0066 and 12D0070 (Table 2). a) Titration in viral genome (vg / ml)

The two systems lead to a similar productivity with regard to the number of viral genomes (of the order of $10^{\,11}$ to $10^{\,12}$ vg) and therefore of viral particles of AAV9 containing the transgene of interest SMN (called particles of AAV full). b) Purity and identity

The SDS-Page and Western blot profile of 2 lots 12D0106 and A.12026.DEV-4 (system sf9) are identical with the presence of the 3 viral proteins Vp1, Vp2 and Vp3 of molar masses of 87 kDa, 72 kDa and 62 kDa, respectively. kDa and the relative amounts of 1: 1: 10, corresponding to those expected and internal control (figA and 1B). This shows that the scAAV9-vectors produced with the sf9 system are pure and that the virus particle is compliant.

The SDS-Page and Western blot profile of lot 12D0055 (HEK293F system) shows the presence of a large number of proteins, some of which are recognized by the B1 antibody and which correspond to degraded viral proteins (figA and 1B). This indicates that the scAAV9 vector produced with the HEK293F system is not pure.

The chromatography method set up today and identical to the 2 culture systems, does not allow to obtain pure batches of scAAV9 vectors produced with the HEK293F system. c) **Analy**sis of empty and solid particules V particles

It has been reported in the literature that the density is approximately 1.32 g / cm3 (equivalent to 60S) for empty AAV viral particles, i.e. those lacking a viral genome and 1.45 g / cm3 (equivalent to 110S) for solid AAV viral particles, that is to say those containing a viral genome with a maximum size of 4800 bases [15]. Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have today set up a method to separate **analy**tically by ultracentrifugation and quantify the different species present in a viral preparation of AAV.

Analytical ultracentrifugation **analy**sis of the scAAV9-SMN vectors was performed initially by comparing a batch produced in system sf9 (A.12026.DEV-4) with lot 12D0055 produced in HEK293 system (FIG. 2). The distribution profile of the species makes it possible to identify 2 categories of populations:

a) <60S corresponding to unassembled viral proteins and / or contaminants.

This population is almost absent in lot A.12026.DEV-4 while they are present in a significant amount in lot 12D0055. These impurities were also detected in SDS-Page (FIG. 1).

b) 60S - 110S corresponding to intact AAV viral particles

The "60S-110S" population consists of 2 subpopulations with empty AAV viral particles at 65S and full AAV viral particles between 80S and 110S with the viral genome in the single stranded form at 90S and under the double-stranded at 105 S. These values are specific to the scAAV9-SMN vectors and will be redefined according to the genome type. Very unexpectedly, we detected very few empty AAV virus particles with the sf9 system compared to the HEK293 system. In addition, the solid AAV viral particles are significantly more enriched in the double-stranded (105 S) genome with the sf9 system compared to the HEK293 system.

The distribution profile of lot 12D0082 is identical to lot 12D0055, which confirms that the HEK293 system produces essentially very few solid AAV particles (<25%) with only 10% of viral particles containing the double-stranded genome.

A second experiment with the 3 batches of scAAV9-SMN vectors produced in system sf9 (A.12026.DEV-4, 12D0106, 12D0068) was carried out in the same way by analytical ultracentrifugation (FIG. 3). These 3 lots all show a similar distribution profile and confirms that the sf9 system produces AAV viral particles essentially composed of solid AAV particles (>80%>) with a very high proportion of viral genome in double form. strand (>50%>). Cell culture Purification: Step 1

Volume Title Title

Type Lot No. Cell / ml Bulk No. Total Vg

(L) (vg / ml) (vg / cell)

12D0004 L 0E + 06 12D0055 0.5 4.0E + 10 4.0E + 04 2.0E + 13

HEK293F

12D0082 L 0E + 06 12D0082 2 1,2E + 10 L 2E + 04 2,4E + 13

A.12026.DEV L 0E + 06 A.12026.DEV-4 2 2.2E + 10 L 3E + 04 4.5E + 13

12D0066 2 1,9E + 10 L 0E + 04 3,8E + 13 sf9

12D0066 L 9E + 06 12D0070 2 2.0E + 10 L 0E + 04 4.0E + 13

12D0068 2 6.4E + 09 3.4E + 03 1.3E + 13

Table 1: Description of the cell concentration in the 10L bioreactor before transfection or infection, volumes (L) and titers of viral genomes (vg / ml) obtained after the purification (step 1) of 2L cell culture.

Culture cellulaire		Purification				
		Etape 1	Etape 2			
Type	Lot N°	Vrac N°	Lot N°	Vol (ml)	Titre (vg/mL)	Total Vg
HEK293F	12D0004	12D055	12D0055	0,6	1,6E+12	9,5E+11
	12D0082	12D0082	12D0082	0,8	5,1E+11	4,1E+11
sf9	A.12026.DEV	A.12026.DEV-4	A.12026.DEV-4	1,2	1,4E+12	1,7E+12
	12D0066	12D0066	12D0106	0,8	9,4E+11	7,5E+11
		12D0070				
		12D0068	12D0068	0,5	1,6E+12	8,0E+11

Table 2: Description of volume, titre in viral genomes (vg / ml), total amount of viral genomes of AAV9 (vg) particles obtained after purification step 2

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Patent Citations (5)

Publication number	Priority date	Publication date	Assignee	Title
WO2007084773A2 *	2006-01-20	2007-07-26	University Of North Carolina At Chapel Hill	Enhanced production of infectious parvovirus vectors in insect cells
WO2008016891A2 *	2006-01-31	2008-02-07	The Board Of Trustees Of The Leland Stanford Junior University	Self-complementary parvoviral vectors, and methods for making and using the same
WO2011012774A1 *	2009-07-31	2011-02-03	Association Institut De Myologie	Widespread gene delivery to the retina using systemic administration of aav vectors
WO2011094198A1 *	2010-01-28	2011-08-04	The Children's Hospital Of Philadelphia Research Institute, Abramson Research Center	A scalable manufacturing platform for viral vector purification and viral vectors so purified for use in gene therapy
WO2011122950A1 *	2010-04-01	2011-10-06	Amsterdam Molecular Therapeutics (Amt) ip B.V.	Monomeric duplex aav vectors
Family To Family Citations				

* Cited by examiner, † Cited by third party

Non-Patent Citations (1)

Title
TAMAS VIRAG ET AL: "Producing Recombinant Adeno-Associated Virus in Foster Cells: Overcoming Production Limitations Using a Baculovirus-Insect Cell Expression Strategy", HUMAN GENE THERAPY, vol. 20, no. 8, August 2009 (2009-08-01), pages 807 - 817, KP055052744, ISSN: 1043-0342, DOI: 10.1089/hum.2009.092 *

* Cited by examiner, † Cited by third party

Cited By (7)

Publication number	Priority date	Publication date	Assignee	Title
WO2016118520A1	2015-01-20	2016-07-28	Genzyme Corporation	Analytical ultracentrifugation for characterization of recombinant viral particles
WO2020214526A1 *	2019-04-13	2020-10-22	University Of Florida Research Foundation, Incorporated	Enhanced transduction of aav vectors encoding micromas
CN113862267A *	2021-08-30	2021-12-31	南京贝恩奥生物科技有限公司	Method and kit for rapidly detecting size of viral genome
EP3387138B1	2015-12-11	2022-01-26	The Trustees Of The University Of Pennsylvania	Scalable purification method for aav9
US11713449B2	2015-12-11	2023-08-01	The Trustees of the University of Pennsylvania	Scalable purification method for AAVRH10
US11713450B2	2015-12-11	2023-08-01	The Trustees of the University of Pennsylvania	Scalable purification method for AAV1
US11718885B2	2015-12-11	2023-08-08	The Trustees of the University of Pennsylvania	Scalable purification method for AAV8
Family To Family Citations				

* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

Publication	Publication Date	Title
WO2014125101A1	2014-08-21	Methods for the production of double-stranded aav viral particles
US11732245B2	2023-08-22	Scalable purification method for AAV9
US20210277864A1	2021-09-09	Scalable purification method for aav8
US11015173B2	2021-05-25	Scalable purification method for AAV1
EP1155120B1	2006-07-05	Method for obtaining a purified viral preparation
JP6522506B2	2018-05-09	Method for generating a high titer helperless preparation of recombinant AAV vectors
EP3535388B1	2023-05-31	Adeno-associated virus purification methods
JP6588008B2	2019-10-09	Promoters, expression cassettes, vectors, kits, and methods for the treatment of single color vision and other diseases
KR102528156B1	2023-05-03	Dna impurities in a composition comprising a parvoviral virion
JP2017060487A	2017-03-30	Promoter, expression cassette, vector, kit, and method for treatment of achromatopsia and other diseases
JP2022501087A	2022-01-06	Compositions and Methods for Producing Gene Therapy Vectors
EP2737072B1	2018-02-14	Baculovirus system for the expression of a gene therapy vector

JP2022505106A	2022-01-14	Expression vector for large-scale production of rAAV in the baculovirus / Sf9 system
Negrete et al.	2008	Strategies for manufacturing recombinant adeno-associated virus vectors for gene therapy applications exploiting baculovirus technology
KR20230134682A	2023-08-25	In vitro assembly of anellovirus capsid encapsulating RNA
CN112980884A	2021-06-18	Method for improving infection efficiency of adeno-associated virus infected cells
EP3484917B1	2022-09-28	Baculovirus expression system
US20230212538A1	2023-07-06	Materials and methods for viral purification
US20220251538A1	2022-08-11	Method for removal of nucleic acids impurities from liquid composition comprising genetically engineered particles or proteins
US20230416695A1	2023-12-28	Scalable purification method for aav9
TW202246516A	2022-12-01	Controlled expression of viral proteins
CN117083070A	2023-11-17	Proparvovirus and tetrapparvovirus compositions and methods for gene therapy
CN116887863A	2023-10-13	In vitro assembly of RNA-encapsulating finger ring viral capsids

Priority And Related Applications

Applications Claiming Priority (2)

Application	Filing date	Title
FR1351295A	2013-02-15	METHODS FOR THE PRODUCTION OF DOUBLE-STRANDED AAV VIRAL PARTICLES
FR1351295	2013-02-15	

Legal Events

Date	Code	Title	Description
2014-10-08	121	Ep: the epo has been informed by wipo that ep was designated in this application	**Ref document number:** 14705744 **Country of ref document:** EP **Kind code of ref document:** A1
2015-08-17	NENP	Non-entry into the national phase	**Ref country code:** DE
2016-03-09	122	Ep: pct application non-entry in european phase	**Ref document number:** 14705744 **Country of ref document:** EP **Kind code of ref document:** A1

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
18/506,853	11/10/2023	Catherine R. O'RIORDAN	15979-20103.03	3457

89300 7590 12/15/2023

Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

EXAMINER
CENTRAL, DOCKET

ART UNIT	PAPER NUMBER
OPAP	

NOTIFICATION DATE	DELIVERY MODE
12/15/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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PatentDocket@mofo.com
pair_mofo@firsttofile.com

Decision Granting Request for Prioritized Examination (Track I)	**Application No.** 18/506,853	**Applicant(s)** O'RIORDAN et al.	
	Examiner FIKIRTE A GEREMEW	**Art Unit** OMBL	**AIA (FITF) Status** Yes

1. THE REQUEST FILED 10 November 2023 IS **GRANTED** .

   The above-identified application has met the requirements for prioritized examination
   - A. ☑ for an original nonprovisional application (Track I).
   - B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination**. The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:
   - A. filing a **petition for extension of time** to extend the time period for filing a reply;
   - B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims** , or a multiple dependent claim;
   - C. filing a **request for continued examination** ;
   - D. filing a notice of appeal;
   - E. filing a request for suspension of action;
   - F. mailing of a notice of allowance;
   - G. mailing of a final Office action;
   - H. completion of examination as defined in 37 CFR 41.102; or
   - I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to FIKIRTE GEREMEW at (703) 756-1930. In his/her absence, calls may be directed to Petition Help Desk at (571) 272-3282.

/FIKIRTE A GEREMEW/ PROGRAM SUPPORT ASSISTANT, OMBL	

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
18/506,853	11/10/2023		2820	15979-20103.03	30	2

CONFIRMATION NO. 3457

89300
Sanofi/Genzyme c/o Morrison & Foerster LLP
755 Page Mill Road
Palo Alto, CA 94304

FILING RECEIPT

OC00000006503162 1

Date Mailed: 11/29/2023

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

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Inventor(s)

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Applicant(s)

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Power of Attorney: The patent practitioners associated with Customer Number 89300

Domestic Priority data as claimed by applicant

This application is a CON of 18/188,176 03/22/2023
which is a CON of 16/547,144 08/21/2019 PAT 11,639,887
which is a CON of 15/544,498 07/18/2017 PAT 10,429,288 *
which is a 371 of PCT/US2016/013947 01/19/2016
which claims benefit of 62/105,714 01/20/2015

(*) Data provided by applicant is not consistent with PTO records.

Foreign Applications for which priority is claimed (You may be eligible to benefit from the Patent Prosecution Highway program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 11/27/2023
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 18/506,853**
Projected Publication Date: 03/07/2024
Non-Publication Request: No
Early Publication Request: No
Title

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Preliminary Class

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 18/506,853

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	320
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	700
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	800
TOTAL CLAIMS (37 CFR 1.16(i))	30 minus 20 =	* 10			OR	x 100 =	1000
INDEPENDENT CLAIMS (37 CFR 1.16(h))	2 minus 3 =	*				x =	0
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							0
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL			TOTAL	2820

APPLICATION AS AMENDED - PART II

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
AMENDMENT A	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
AMENDMENT B	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

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The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	**11/10/2023 06:08:29 PM Z ET**	**15979-20103.03**

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	63219170	AUTHORIZED BY	Seth Snyder
CUSTOMER #	89300	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
DA / 031952	**E2023A0I18418512**	**Aurelia Caparas**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
1830	PROCESSING FEE, EXCEPT IN PROVISIONAL APPLICATIONS	140.00	1	140.00
1817	REQUEST FOR PRIORITIZED EXAMINATION	4200.00	1	4200.00
1111	UTILITY PATENT APPL. SEARCH FEE	700.00	1	700.00
1011	BASIC FILING FEE - UTILITY (PAPER FILING ALSO REQUIRES NON-ELECTRONIC FILING FEE UNDER 1.16(T))	320.00	1	320.00

1311	PATENT APPL. EXAMINATION FEE	800.00	1	800.00
1202	EACH CLAIM IN EXCESS OF 20	100.00	10	1000.00
			TOTAL AMOUNT:	**$7,160.00**

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If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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Docket No.: 15979-20103.03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Catherine R. O'RIORDAN et al.

Application No.: Not Yet Assigned

Confirmation No.: Not yet Assigned

Filed: Concurrently Herewith

Art Unit: Not Yet Assigned

For: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Examiner: Not Yet Assigned

PRELIMINARY AMENDMENT UNDER 37 C.F.R. 1.115

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

INTRODUCTORY COMMENTS

Prior to examination on the merits, Applicant respectfully requests entry of this Preliminary Amendment for the above-captioned patent application.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 7 of this paper.

sf-5668369

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-95 (Canceled).

96. (New) A method of determining the size of one or more fragmented genomes in an AAV vector preparation, said method comprising:

(i) subjecting the preparation to analytical centrifugation under boundary sedimentation velocity conditions, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm;

(ii) measuring the rate of movement or migration of the sedimenting boundary, wherein movement or migration of the fragmented genomes results in different sedimenting boundaries, each sedimenting boundary corresponding to a resolvable vector species, thereby determining the sedimentation coefficients of the one or more of the fragmented genomes in the AAV vector preparation; and

(iii) determining the size of the one or more fragmented genomes as a function of sedimentation coefficients of the one or more fragmented genomes.

97. (New) The method of claim 96, wherein step (iii) comprises comparing the sedimentation coefficients of the one or more fragmented genomes with a standard curve generated by sedimentation coefficients of recombinant AAV genomes of known nucleotide sizes, thereby determining the size of the one or more fragmented genomes.

98. (New) The method of claim 96, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm.

99. (New) The method of claim 96, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm.

100. (New) The method claim 99, wherein the boundary sedimentation is performed at a temperature of from about 4°C to about 20°C.

101. (New) The method of claim 100, wherein the boundary sedimentation is performed at a temperature of about 4°C.

102. (New) The method of claim 96, wherein the fragmented genomes are sub-genomic DNA molecules.

103. (New) The method of claim 96, wherein the centrifugation is run from about 0.5 hours to about 2 hours.

104. (New) The method of claim 103, wherein the centrifugation is run from about 1 hours to about 2 hours.

105. (New) The method of claim 103, wherein the centrifugation is run for about 1 hour.

106. (New) The method of claim 103, wherein the centrifugation is run for about 1.2 hours.

107. (New) The method of claim 96, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is greater than $5x10^{11}$ vg/mL.

108. (New) The method of claim 96, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is from about $1x10^{11}$ vg/mL to about $1x10^{13}$ vg/mL.

109. (New) The method of claim 96, further comprising quantifying one or more of the fragmented genomes in the AAV vector preparation.

110. (New) The method of claim 96, comprising determining the size of all of the fragmented genomes in the rAAV preparation.

111. (New) The method of claim 110, further comprising quantifying all of the fragmented genomes in the AAV vector preparation.

112. (New) The method of claim 96, wherein the AAV vector preparation comprises AAV9 capsid proteins.

113. (New) The method of claim 112, wherein the AAV vector preparation further comprises at least one AAV2 ITR.

114. (New) The method of claim 111, wherein the AAV vector preparation comprises self-complementary AAV viral genomes.

115. (New) A method of determining the purity of an AAV vector preparation, said method comprising:

(i) subjecting the preparation to analytical centrifugation under boundary sedimentation velocity conditions, wherein the boundary sedimentation velocity is from about 3,000 rpm to about 20,000 rpm;

(ii) measuring the rate of movement or migration of the sedimenting boundary, wherein movement or migration of individual viral particles in the AAV vector preparation results in different sedimenting boundaries, each sedimenting boundary corresponding to a resolvable vector species, and wherein the vector preparation comprises full genomes, fragmented genomes and empty capsids without genome;

(iii) determining sedimentation coefficients of the individual viral particles in the AAV vector preparation; and

(iv) quantifying the amount of each of the resolvable vector species in the AAV vector preparation,

thereby determining the purity of the AAV vector preparation.

116. (New) The method of claim 115, further comprising determining the size of the individual viral particles after step (iii) and prior to step (iv) by comparing the sedimentation coefficients of the individual viral particles with a standard curve generated by sedimentation coefficients of recombinant AAV genomes of known nucleotide sizes.

117. (New) The method of claim 115, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm.

118. (New) The method of claim 115, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm.

119. (New) The method of claim 115, wherein the boundary sedimentation is performed at a temperature of about 4°C.

120. (New) The method of claim 115, wherein the centrifugation is run from about 0.5 hours to about 2 hours.

121. (New) The method of claim 115, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is greater than $5x10^{11}$ vg/mL.

122. (New) The method of claim 115, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is from about $1x10^{11}$ vg/mL to about $1x10^{13}$ vg/mL.

123. (New) The method of claim 115, wherein the AAV vector preparation comprises AAV9 capsid proteins.

124. (New) The method of claim 123, wherein the AAV vector preparation further comprises at least one AAV2 ITR.

125. (New) The method of claim 123, wherein the AAV vector preparation comprises self-complementary AAV viral genomes.

REMARKS

Claims 1-95 are pending. By virtue of this amendment, claims 1-95 have been cancelled and claims 96-125 have been newly added. Accordingly, upon entry of this amendment, claims 96-125 will be pending and under consideration.

Support for new claims 96-125 is found throughout the specification and the originally filed claims. Exemplary support for the new claims is found in the specification, for example, as set forth in the table below.

Claim(s)	Exemplary Support
96, 97, 102, 109-111, 115, 116	[0007]; [0038]; [0048]; [0088]-[0089]; [0101]-[0103]; [0179]; Example 1; Example 5; originally filed claims 2 and 3
98, 99, 117, 118	[0024]; [0107]; [0109]; originally filed claims 62 and 63
100, 101, 119	[0076]; [109]; originally filed claims 65 and 66
103-106, 120	[0019]; [0098]
107, 108, 121, 122	[0095]
112-114, 123-125	[0017]-[0121]

No new matter is believed to have been added by way of the introduction of the new claims. Entry of the claim amendments is respectfully requested.

Cancelation of certain claims is not to be construed as a dedication to the public of any of the subject matter of the claims as previously presented. Applicants expressly reserve the right to file one or more continuation and/or divisional applications claiming benefit of the present application during the pendency of this application.

CONCLUSION

If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark Office determines that an extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. **03-1952** referencing docket no. **15979-20103.03**. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: November 10, 2023

Respectfully submitted,

Electronic signature: /Seth Snyder/
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+1 (650) 813-4046

Docket No.: 15979-20103.03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Catherine R. O'RIORDAN et al.

Application No.: Not Yet Assigned

Filed: Concurrently Herewith

For: ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Confirmation No.: Not Yet Assigned

Art Unit: Not Yet Assigned

Examiner: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97 & § 1.98

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Pursuant to 37 C.F.R. § 1.97 and § 1.98, Applicant submits for consideration in the above-identified application the documents listed on the attached Form PTO/SB/08a/b. Copies of U.S. Patent Publications and Patents are not submitted herewith pursuant to 37 C.F.R. § 1.98(a)(2)(ii). A copy of foreign document (document numbered 7) and copies of non-patent literature (documents numbered 1, 2, 12-22, 26, 28, 31, 37, 38, 40, 41, 43-58, 60, 67, 68, 74, and 75) are submitted herewith. However, copies of the remaining documents cited in the attached Form PTO/SB/08a/b were previously submitted in an Information Disclosure Statement and/or Office Action, directed to the related U.S. Patent Application Number 16/547,144, filed August 21, 2019, now issued as U.S. Patent Number 11,639,887 and U.S. Patent Application Number 15/544,498, filed July 18, 2017, now issued as U.S. Patent Number 10,429,288, and, accordingly, copies are not included herewith. This protocol conforms with 37 C.F.R. § 1.98(d) and M.P.E.P. 609.02(A)(2). The Examiner is requested to make these documents of record in the application.

sf-5652982

This Information Disclosure Statement is submitted:

☒ With the application; accordingly, no fee or separate requirements are required.

☐ With the filing of a Request for Continued Examination under 37 C.F.R. § 1.114; accordingly, no fee or separate requirements are required.

☐ Before the mailing of a first Office Action after the filing of a Request for Continued Examination under 37 C.F.R. § 1.114; accordingly, no fee or separate requirements are required. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.

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Applicant would appreciate the Examiner initialing and returning the Form PTO/SB/08a/b, indicating that the information has been considered and made of record herein.

The information contained in this Information Disclosure Statement under 37 C.F.R. § 1.97 and § 1.98 is not to be construed as a representation that: (i) a complete search has been made; (ii) additional information material to the examination of this application does not exist; (iii) the information, protocols, results and the like reported by third parties are accurate or enabling; or (iv) the above information constitutes prior art to the subject invention.

Dated: November 10, 2023

Respectfully submitted,

Electronic signature: /Seth Snyder/
Seth Snyder

MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, CA 94304-1018
+1 (650) 813-4046

CLAIMS

What is claimed is:

1. A method of characterizing a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), and

c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral recombinant viral particle.

2. A method to assess vector genome integrity of recombinant viral particles in a preparation of recombinant viral particles comprising

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value C(s) versus the sedimentation coefficient in Svedberg units (S), and

c) identifying species of recombinant viral particles in the preparation by presence of peaks on the plot corresponding to an S value, wherein the genome size of a particular species of recombinant viral recombinant viral particles is calculated by comparing the S value of the species to a standard curve generated by S values of recombinant viral particles comprising encapsidated viral genomes of known nucleotide sizes.

3. The method of claim 2 further comprising integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral recombinant viral particles.

4. A method to determine the presence of empty capsids or capsid particles comprising variant sized recombinant viral genomes in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, and

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant viral genomes indicates that presence of capsid particles comprising variant sized genomes and/or empty capsids.

5. A method of measuring the relative amount empty capsids in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, and

d) comparing the amount of recombinant viral particles having an S value corresponding to empty capsid particles to the amount of recombinant viral particles having an S value corresponding to recombinant viral particles comprising intact viral genomes or the total amount of recombinant viral particles in the preparation.

6. A method of measuring the relative amount of capsid particles comprising variant recombinant viral genomes or empty viral capsid particles in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles,

d) comparing the amount of recombinant viral particles having an S values that do not correspond to recombinant viral particles comprising intact viral genomes to the amount of recombinant viral particles having an S value that corresponds to recombinant viral particles comprising intact viral genomes or to the total amount of recombinant viral particles in the preparation.

7. A method of measuring the relative amount of capsid particles comprising variant recombinant viral genomes in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles,

d) comparing the amount of recombinant viral particles having an S values that do not correspond to recombinant viral particles comprising intact viral genomes or empty capsid particles to the total amount of recombinant viral particles in the preparation.

8. A method of measuring the relative amount of recombinant viral particles comprising intact viral genomes in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles,

d) comparing the amount of recombinant viral particles having an S values corresponding to recombinant viral particles comprising intact viral genomes to the amount of recombinant viral particles having an S value corresponding to empty capsid particles, to capsid particles comprising variant recombinant viral genomes, and/or to the total amount of recombinant viral particles in the preparation.

9. A method of monitoring the removal of empty capsids and/or capsid particles comprising variant recombinant viral genomes during the purification of a preparation of recombinant viral particles, the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and analyzing the sample for the relative amount of empty capsids and/or capsid particles comprising variant recombinant viral genomes according to the method of any one of claims 5-8, wherein a decrease in the relative amount of empty capsids and/or capsids comprising variant genomes to full capsids indicates removal of empty capsids from the preparation of recombinant viral particles.

10. The method of any one of claims 4-9, wherein the presence of a peak that corresponds to the S value of empty capsid particles indicates the presence of empty capsid particles.

11. The method of any one of claims 4-9, wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant viral genomes or empty capsid particles indicates that presence of capsid particles comprising variant sized genomes.

12. The method of claim 11, wherein the capsid particles comprising variant sized genomes comprises truncated genomes, aggregates, recombinants and/or DNA impurities.

13. A method of determining the heterogeneity of recombinant viral particles in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of peaks in addition to the peak representing capsids comprising an intact viral genome indicates heterogeneity of recombinant particles in the preparation.

14. The method of claim 13, wherein the presence of additional peaks indicates the presence of empty capsid particles and/or recombinant viral particles comprising variant genomes.

15. The method of claim 14, wherein the variant genomes are truncated viral genomes, aggregates, recombinants and/or DNA impurities.

16. The method of any one of claims 13-15 further comprising integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles.

17. A method of monitoring the homogeneity of recombinant viral particles during the purification of a preparation of recombinant viral particles, the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and determining the heterogeneity of recombinant viral particles according to the method of claim 16, wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes indicates an increase in the homogeneity of full viral particles in the preparation of recombinant viral particles.

18. The method of any one of claims 1-17, wherein the sedimentation of recombinant viral particles is monitored by absorbance.

19. The method of claim 18, wherein the absorbance is at about 230 nm, 260 nm or 280 nm.

20. The method of claim 18 or 19, wherein the absorbance is at about 260 nm.

21. The method of any one of claims 1-20, wherein the sedimentation of recombinant viral particles is monitored by interference.

22. The method of claim 21, wherein the interference is Rayleigh interference.

23. The method of any one of claims 1-22, wherein the preparation is an aqueous solution.

24. The method of claim 23, wherein the aqueous solution comprises a pharmaceutical formulation.

25. The method of claim 23 or 24, wherein the aqueous solution comprises a buffer.

26. The method of claim 25, wherein the buffer is at physiological pH.

27. The method of claim 25 or 26, wherein the buffer is at physiological osmolality.

28. The method of any one of claims 24-27, wherein the pharmaceutical formulation comprises phosphate buffered saline (PBS).

29. The method of claim 28, wherein the PBS has pH of about 7.2 and an osmolality of about 300 mOsm/L.

30. The method of any one of claims 24-29, wherein the monitoring further comprises comparison to a reference sample, wherein the reference sample comprises the aqueous solution without recombinant viral particles.

31. The method of any one of claims 1-30, wherein the C(S) values are determined by an algorithm that comprises Lamm equation solutions.

32. The method of claim 31, wherein the algorithm is the SEDFIT algorithm.

33. The method of any one of claims 1-32, wherein sedimentation is monitored until the recombinant viral particles with the lowest density sediments to the bottom of a sector of an ultracentrifuge.

34. The method of any one of claims 1-32, wherein the ultracentrifugation utilizes an ultracentrifuge comprising an ultracentrifuge velocity cell.

35. The method of any one of claims 1-33, wherein sedimentation is monitored until recombinant viral particles sediment to the bottom of an ultracentrifuge velocity cell.

36. The method of claim 34, wherein sedimentation is monitored until the recombinant viral particles with the lowest density sediments and clears an optical window.

37. The method of any one of claims 33-36, wherein at least 30 scans are used to monitor sedimentation of recombinant viral particles.

38. The method of claim 37, wherein about 30 scans are used to monitor sedimentation of recombinant viral particles.

39. The method of claim 37, wherein about 30 to about 75 scans are used to monitor sedimentation of recombinant viral particles.

40. The method of claim 37, wherein about 30 to about 50 scans are used to monitor sedimentation of recombinant viral particles.

41. The method of claim 37, wherein about 50 to about 75 scans are used to monitor sedimentation of recombinant viral particles.

42. The method of claim any one of claims 31-41, wherein a regularization is applied to a fitting level with a confidence level of F statistic of at least about 0.68.

43. The method of claim 42, wherein the regularization is a second derivative regularization.

44. The method of claim 42, wherein the regularization is Max entropy regularization.

45. The method of any one of claims 42-44, wherein the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68 to about 0.90.

46. The method of any one of claims 42-44, wherein the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68 to about 0.99.

47. The method of any one of claim 42-44, wherein the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68.

48. The method of any one of claims 31-47, wherein the following C(S) parameters are held constant: resolution of about 200S to about 5000S, S min is about 1S to about 100S, S max is about 100S to about 5000S, and frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software.

49. The method of claim 48, wherein resolution is about 200S to about 1000S.

50. The method of claim 48 or 49, wherein resolution is about 200S.

51. The method of any one of claims 48-50, wherein S min is about 1.

52. The method of any one of claims 48-51, wherein Smax is about 100S to about 1000S.

53. The method of any one of claims 48-51, wherein Smax is about 200S to about 5000S.

54. The method of any one of claims 48-51, wherein Smax is about 200S.

55. The method of any one of claims 48-54, wherein the frictional ratio is left to float to a value determined by centrifugation software.

56. The method of claim any one of 48-54, wherein the frictional ratio is about 1.0.

57. The method of any one of claims 31-56, wherein radial invariant (RI) and time invariant (TI) noise subtractions are applied.

58. The method of any one of claims 1-57, wherein the sedimentation of recombinant viral particles is monitored about every 10-60 seconds.

59. The method of claim 58, wherein the sedimentation of recombinant viral particles is monitored about every 10 seconds.

60. The method of claim 58, wherein the sedimentation of recombinant viral particles is monitored about every 60 seconds

61. The method of any one of claims 1-60, wherein the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000 rpm.

62. The method of claim 61, wherein the boundary sedimentation velocity is performed at about 3,000 rpm to about 10,000 rpm.

63. The method of claim 61, wherein the boundary sedimentation velocity is performed at about 10,000 rpm to about 20,000 rpm.

64. The method of claim 61, wherein the boundary sedimentation velocity is performed at about 15,000 rpm to about 20,000 rpm.

65. The method of any one of claims 1-64, wherein the boundary sedimentation velocity is performed at about 4°C to about 20 °C.

66. The method of claim 65, wherein the boundary sedimentation velocity is performed at about 4°C.

67. The method of any one of claims 1-66, wherein the recombinant viral particle is a recombinant adeno-associated viral (AAV) particle, a recombinant adenovirus particle, a recombinant lentivirus particle or a recombinant herpes simplex viral (HSV) particle.

68. A method of evaluating a process for the production of recombinant viral particles comprising the method of any one of claims 1 to 67, wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes compared to the relative amount of empty capsid particles and/or recombinant viral capsid particles with variant recombinant viral genomes compared to a reference preparation of recombinant viral particles indicates an improvement in the production of recombinant viral particles.

69. The method of claim 68, wherein the viral particles are rAAV particles.

70. The method of claim 69, wherein the rAAV particles are produced from a producer cell line.

71. The method of claim 69, wherein the rAAV particles are produced by triple transfection of i) nucleic acid encoding AAV rep and cap, ii) rAAV vector sequences, and iii) nucleic acid encoding adenovirus helper functions.

72. The method of claim 69, wherein the recombinant viral particles are produced by an AAV/HSV hybrid

73. The method of claim 69, wherein the recombinant viral particles are produced from a insect cell.

74. The method of claim 69, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding AAV vector sequences, AAV rep and cap coding regions, and AAV helper virus functions to a suitable host cell.

75. The method of claim 69, wherein the recombinant viral particles are produced by introduction of one or more nucleic acids encoding AAV vector sequences, AAV rep and cap coding regions, and AAV helper virus functions to a suitable host cell, wherein the one or more nucleic acids are introduced to the cell using a recombinant helper virus.

76. The method of claim 75, wherein the recombinant helper virus is an adenovirus, a herpes simplex virus or a baculovirus.

77. The method of claim 68, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding adenovirus vector sequences and adenovirus replication and packaging sequences to a suitable host cell.

78. The method of claim 68, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding lentivirus vector sequences and/or lentivirus replication and packaging sequences to a suitable host cell.

79. The method of claim 68, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding HSV vector sequences and/or HSV replication and packaging sequences to a suitable host cell.

80. A method for preparing recombinant viral particles with reduced empty capsids and/or recombinant viral particles comprising variant genomes, the method comprising

a) culturing host cells under conditions suitable for recombinant viral production, wherein the cells comprise

i) nucleic acid encoding a heterologous transgene flanked by at least one AAV ITR,

ii) nucleic acid comprising AAV rep and cap coding regions, wherein the nucleic acid comprises a mutated p5 promoter wherein rep expression from the p5 promoter is reduced compared to a wild-type p5 promoter, and

iii) nucleic acid encoding AAV helper virus functions;

b) lysing the host cells to release recombinant viral particles;

c) isolating the recombinant viral particles produced by the host cell; and

d) analyzing the recombinant viral particles for the presence of empty capsids and/or recombinant viral particles with variant genomes by analytical ultracentrifugation by the methods of any one of claims 1 to 79.

81. The method of claim 80, wherein the p5 promoter is located 3' to the rep and/or cap coding region.

82. The method of claim 80 or 81, wherein the AAV helper virus functions comprise adenovirus E1A function, adenovirus E1B function, adenovirus E2A function, adenovirus VA function and adenovirus E4 orf6 function.

83. The method of any one of the preceding claims wherein the recombinant viral particles have been purified using one or more purification steps.

84. The method of claim 67, 69-76, 80-83 wherein the recombinant viral particles comprise a self-complementary AAV (scAAV) genome.

85. The method of claim 84, wherein the method is used to detect the presence of recombinant viral particles comprising the monomeric form of a scAAV genome or the dimeric form of a scAAV genome.

86. The method of any one of claims 67, 69-76, 80-85,wherein the recombinant viral particle comprises an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an

AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, or a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV / human bocavirus virus 1).

87. The method of any one of claims 67, 69-76, 80-86, wherein the recombinant viral particle comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, an AAV12 ITR, an AAV DJ ITR, a goat AAV ITR, a bovine AAV ITR, or a mouse AAV ITR.

88. The method of claim 86 or 87, wherein the AAV capsid comprises a tyrosine mutation or a heparin binding mutation.

89. The method of claim 67 or 68, where the recombinant viral particle is a recombinant adenoviral particle.

90. The method of claim 89, wherein the recombinant adenoviral particle comprises an capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, , AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3.

91. The method of claim 90, wherein the recombinant adenoviral particle comprises a variant of an adenovirus serotype 2 capsid or a variant of an adenoviral serotype 5 capsid.

92. The method of claim 67 or 68, where the recombinant viral particle is a recombinant lentiviral particle.

93. The method of claim 92, wherein the recombinant lentiviral particle is pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein.

94. The method of claim 67 or 68, where the recombinant viral particle is a rHSV particle.

95. The method of claim 94, wherein the HSV particle is an HSV-1 particle or an HSV-2 particle.

ABSTRACT

Provided herein are methods to characterize preparations of recombinant viral particles using analytical ultracentrifugation. Recombinant viral particles include recombinant adeno-associated viral particles, recombinant adenoviral particles, recombinant lentiviral particles and recombinant herpes simplex virus particles. Variant species of recombinant viral particles including empty capsids and recombinant viral particles with variant genomes (*e.g.*, truncated genomes, aggregates, recombinants) can be identified and quantitated. The methods can be used to characterize preparations of recombinant viral particles regardless of the sequence of the recombinant viral genome or the serotype of the recombinant viral capsid.

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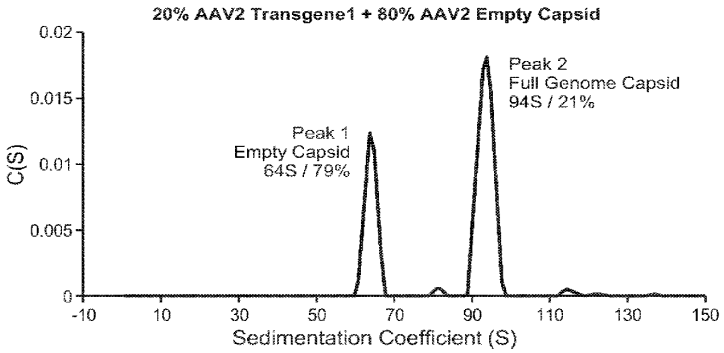

FIG. 1B

(57) Abstract: Provided herein are methods to characterize preparations of recombinant viral particles using analytical ultracentrifugation. Recombinant viral particles include recombinant adeno- associated viral particles, recombinant adenoviral particles, recombinant lentiviral particles and recombinant herpes simplex virus particles. Variant species of recombinant viral particles including empty capsids and recombinant viral particles with variant genomes (e.g., truncated genomes, aggregates, recombinants) can be identified and quantitated. The methods can be used to characterize preparations of recombinant viral particles regardless of the sequence of the recombinant viral genome or the serotype of the recombinant viral capsid.

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ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/105,714, filed on January 20, 2015, the content of which is hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to methods to characterize recombinant viral vectors; *e.g.*, recombinant adeno-associated viral (AAV) particles, recombinant adenoviral (rAd) particles, recombinant lentiviral particles and recombinant Herpes simplex viral (rHSV) particles using analytical ultracentrifugation.

BACKGROUND OF THE INVENTION

[0003] Recombinant viruses show great promise and utility as a vehicle to deliver therapeutic nucleic acids for gene therapy applications. A number of different recombinant viruses are used in these gene therapy applications based on a number of factors including the size of the nucleic acid to be delivered, the target cell or tissue to deliver the nucleic acid, the need for short or long term expression of the therapeutic nucleic acid, and integration of the therapeutic nucleic acid into the recipient's genome. Examples of viruses used in gene therapy applications include adeno-associated virus (AAV), adenovirus, lentivirus and herpes simplex virus (HSV).

[0004] The generation of recombinant viral vectors for the clinic requires an analytical method that monitors drug product quality with regard to homogeneity, purity and consistency of manufacturing, yet to date no method to support such a characterization has been established. Typically, the DNA content of recombinant viral DNA viral vectors is measured by Southern blot analysis using a sequence specific probe. Viral capsids or envelopes may be characterized by immunoassay using an antibody that binds specifically to a capsid or envelope protein of a particular recombinant virus. For example, Steinbach, S *et al.*, (1997) *J. Gen. Virol.*, 78:1453-

1462 provides an immunoassay for rAAV serotypes. What is needed is a generic assay to characterize recombinant viral preparations regardless of the nucleic acid sequence of the recombinant viral genome or the serotype of the capsid.

[0005] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0006] In some aspects, the invention provides methods of characterizing a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), and c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral recombinant viral particle.

[0007] In some aspects, the invention provides methods to assess vector genome integrity of recombinant viral particles in a preparation of recombinant viral particles comprising a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, b) plotting the differential sedimentation coefficient distribution value C(s) versus the sedimentation coefficient in Svedberg units (S), and c) identifying species of recombinant viral particles in the preparation by presence of peaks on the plot corresponding to an S value, wherein the genome size of a particular species of recombinant viral recombinant viral particles is calculated by comparing the S value of the species to a standard curve generated by S values of recombinant viral particles comprising encapsidated viral genomes of known nucleotide sizes. In some embodiments, the methods further comprise integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral recombinant viral particles.

[0008] In some aspects, the invention provides methods to determine the presence of empty capsids or capsid particles comprising variant sized recombinant viral genomes in a preparation of

recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, and b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant viral genomes indicates that presence of capsid particles comprising variant sized genomes and/or empty capsids.

[0009] In some aspects, the invention provides, methods of measuring the relative amount empty capsids in a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, and d) comparing the amount of recombinant viral particles having an S value corresponding to empty capsid particles to the amount of recombinant viral particles having an S value corresponding to recombinant viral particles comprising intact viral genomes or the total amount of recombinant viral particles in the preparation.

[0010] In some aspects, the invention provides methods of measuring the relative amount of capsid particles comprising variant recombinant viral genomes or empty viral capsid particles in a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, d) comparing the amount of recombinant viral particles having an S values that do not correspond to recombinant viral particles comprising intact viral genomes to the amount of recombinant viral particles having an S value that corresponds to recombinant viral particles comprising intact viral genomes or to the total amount of recombinant viral particles in the preparation.

[0011] In some aspects, the invention provides methods of measuring the relative amount of capsid particles comprising variant recombinant viral genomes in a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, d) comparing the amount of recombinant viral particles having an S values that do not correspond to recombinant viral particles comprising intact viral genomes or empty capsid particles to the total amount of recombinant viral particles in the preparation.

[0012] In some aspects, the invention provides methods of measuring the relative amount of recombinant viral particles comprising intact viral genomes in a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, d) comparing the amount of recombinant viral particles having an S values corresponding to recombinant viral particles comprising intact viral genomes to the amount of recombinant viral particles having an S value corresponding to empty capsid particles, to capsid particles comprising variant recombinant viral genomes, and/or to the total amount of recombinant viral particles in the preparation.

[0013] In some aspects, the invention provides, methods of monitoring the removal of empty capsids and/or capsid particles comprising variant recombinant viral genomes during the purification of a preparation of recombinant viral particles, the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and analyzing the sample for the relative amount of empty capsids and/or capsid particles comprising variant recombinant viral genomes according to the method of any one of claims 5-8, wherein a decrease in the relative amount of empty capsids and/or capsids comprising variant genomes to full capsids indicates removal of empty capsids from the

preparation of recombinant viral particles. In some embodiments, the presence of a peak that corresponds to the S value of empty capsid particles indicates the presence of empty capsid particles. In some embodiments, the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant viral genomes or empty capsid particles indicates that presence of capsid particles comprising variant sized genomes. In some embodiments, the capsid particles comprising variant sized genomes comprise truncated genomes, aggregates, recombinants and/or DNA impurities.

[0014] In some aspects, the invention provides methods of determining the heterogeneity of recombinant viral particles in a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of peaks in addition to the peak representing capsids comprising an intact viral genome indicates heterogeneity of recombinant particles in the preparation. In some embodiments, the presence of additional peaks indicates the presence of empty capsid particles and/or recombinant viral particles comprising variant genomes. In some embodiments, the variant genomes are truncated viral genomes, aggregates, recombinants and/or DNA impurities. In some embodiments, the methods further comprise integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles.

[0015] In some aspects, the invention provides methods of monitoring the homogeneity of recombinant viral particles during the purification of a preparation of recombinant viral particles, the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and determining the heterogeneity of recombinant viral particles according to the above method, wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes indicates an increase in the homogeneity of full viral particles in the preparation of recombinant viral particles.

[0016] In some embodiments of the above aspects, sedimentation of recombinant viral particles is monitored by absorbance. In some embodiments, the absorbance is at about 230 nm, 260 nm or 280 nm. In some embodiments, the absorbance is at about 260 nm. In some

embodiments, sedimentation of recombinant viral particles is monitored by interference. In some embodiments, the interference is Rayleigh interference.

[0017] In some embodiments of the above aspects, the preparation is an aqueous solution. In further embodiments, the aqueous solution comprises a pharmaceutical formulation. In some embodiments, the aqueous solution comprises a buffer. In some embodiments, the buffer is at physiological pH. In some embodiments, the buffer is at physiological osmolality. In some embodiments, the pharmaceutical formulation comprises phosphate buffered saline (PBS). In some embodiments, the PBS has pH of about 7.2 and an osmolality of about 300 mOsm/L. In some embodiments, the monitoring further comprises comparison to a reference sample, wherein the reference sample comprises the aqueous solution without recombinant viral particles.

[0018] In some embodiments of the above aspects, the C(S) values are determined by an algorithm that comprises Lamm equation solutions. In some embodiments, the algorithm is the SEDFIT algorithm. In some embodiments, sedimentation is monitored until the recombinant viral particles with the lowest density sediments to the bottom of a sector of an ultracentrifuge; for example, the sector may be a portion of the ultracentrifuge comprising a detection system. In some embodiments, the ultracentrifugation utilizes an ultracentrifuge comprising an ultracentrifuge velocity cell. In some embodiments, sedimentation is monitored until recombinant viral particles sediment to the bottom of ultracentrifuge velocity cell. In some embodiments, sedimentation is monitored until the recombinant viral particles with the lowest density sediments and clears the optical window.

[0019] In some embodiments, the radial concentration is recorded for at least about any of 0.5 hours, 0.75 hours, 1.0 hours, 1.5 hours, 2.0 hours, 3.0 hours, 4.0 hours, or 5.0 hours. In some embodiments, the radial concentration is recorded for about 1.0 hour. In some embodiments, the radial concentration is recorded for about 1.2 hours. In some embodiments, the radial concentration is recorded from about 0.5 hours to about 2.0 hours. In some embodiments, the radial concentration is recorded from about 1.0 hours to about 2.0 hours.

[0020] In some embodiments of the above aspects, at least 30 scans are used to monitor sedimentation of recombinant viral particles. In some aspects, about 30 scans are used to monitor sedimentation of recombinant viral particles. In other embodiments, about 30 to about 75 scans

are used to monitor sedimentation of recombinant viral particles. In other embodiments, about 30 to about 50 scans are used to monitor sedimentation of recombinant viral particles. In other embodiments, about 50 to about 75 scans are used to monitor sedimentation of recombinant viral particles.

[0021] In some embodiments of the above aspects, a regularization is applied to a fitting level with a confidence level of F statistic of at least about 0.68. In some embodiments, the regularization is a second derivative regularization. In some embodiments, the regularization is Max entropy regularization. In some embodiments, the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68 to about 0.90. In some embodiments, the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68 to about 0.99. In some embodiments, the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68.

[0022] In some embodiments of the above aspects, the following C(S) parameters are held constant: resolution of about 200S to about 5000S, S min is about 1S to about 100S, S max is about 100S to about 5000S, and frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software. In some embodiments, resolution is about 200S to about 1000S. In some embodiments, resolution is about 200S. In some embodiments, S min is about 1. In some embodiments, Smax is about 100S to about 1000S. In other embodiments, Smax is about 200S to about 5000S. In other embodiments, Smax is about 200S. In some embodiments, the frictional ratio is left to float to a value determined by centrifugation software. In some embodiments, the frictional ratio is about 1.0. In some embodiments, radial invariant (RI) and time invariant (TI) noise subtractions are applied.

[0023] In some embodiments of the above aspects, the sedimentation of recombinant viral particles is monitored about every 10-60 seconds. In some embodiments, sedimentation of recombinant viral particles is monitored (*e.g.*, scanned) about every 10 seconds. In other embodiments, the sedimentation of recombinant viral particles is monitored about every 60 seconds. In some embodiments, the sedimentation velocity of recombinant viral during ultracentrifugation is determined by monitoring the sedimentation of recombinant viral particles once in more than about every 15 seconds, 30 seconds, 45 seconds, 1 minute (60 seconds), 2

minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes.

[0024] In some embodiments of the above aspects, the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000 rpm. In some embodiments, the boundary sedimentation velocity is performed at about 3,000 rpm to about 10,000 rpm. In other embodiments, the boundary sedimentation velocity is performed at about 10,000 rpm to about 20,000 rpm. In other embodiments, the boundary sedimentation velocity is performed at about 15,000 rpm to about 20,000 rpm.

[0025] In some embodiments of the above aspects, the boundary sedimentation velocity is performed at about 4°C to about 20 °C. In some embodiments, the boundary sedimentation velocity is performed at about 4°C.

[0026] In some embodiments of the above aspects, the recombinant viral particle is a recombinant adeno-associated viral (AAV) particle, a recombinant adenovirus particle, a recombinant lentivirus particle or a recombinant herpes simplex viral (HSV) particle. In some embodiments, the recombinant viral particle comprises an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, or a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV / human bocavirus virus 1). In some embodiments, the recombinant viral particle comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, or an AAV12 ITR. In some embodiments, the AAV capsid comprises a tyrosine mutation or a heparin binding mutation. In other embodiments, the recombinant viral particle is a recombinant adenoviral particle. In some embodiments, the recombinant adenoviral particle comprises an capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, , AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine

Ad, or porcine Ad type 3. In some embodiments, the recombinant adenoviral particle comprises a variant of an adenovirus serotype 2 capsid or a variant of an adenoviral serotype 5 capsid. In other embodiments, the recombinant viral particle is a recombinant lentiviral particle. In some embodiments, the recombinant lentiviral particle is pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein. In other embodiments, the recombinant viral particle is a rHSV particle. In some embodiments, the HSV particle is an HSV-1 particle or an HSV-2 particle.

[0027] In some aspects, the invention provides methods for evaluating a process for the production of recombinant viral particles comprising the method of any one of claims 1 to 67, wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes compared to the relative amount of empty capsid particles and/or recombinant viral capsid particles with variant recombinant viral genomes compared to a reference preparation of recombinant viral particles indicates an improvement in the production of recombinant viral particles. In some embodiments, the recombinant viral particle is a recombinant adeno-associated viral (AAV) particle, a recombinant adenovirus particle, a recombinant lentivirus particle or a recombinant herpes simplex viral (HSV) particle. In some embodiments, the rAAV particles are produced from a producer cell line. In other embodiments, the rAAV particles are produced by triple transfection of i) nucleic acid encoding AAV rep and cap, ii) rAAV vector sequences, and iii) nucleic acid encoding adenovirus helper functions. In other embodiments, the recombinant viral particles are produced by an AAV/HSV hybrid. In other embodiments, the recombinant viral particles are produced from a baculovirus cell. In some embodiments, the recombinant viral particles are produced by transient transfection of nucleic acid encoding AAV vector sequences, AAV rep and cap coding regions, and AAV helper virus functions to a suitable host cell. In some embodiments, the recombinant viral particles are produced by introduction of one or more nucleic acids encoding AAV vector sequences, AAV rep and cap coding regions, and AAV helper virus functions to a suitable host cell, wherein the one or more nucleic acids are introduced to the cell using a recombinant helper virus. In some embodiments, the recombinant helper virus is an adenovirus or a herpes simplex virus. In some embodiments, the recombinant viral particles comprise a self-complementary AAV (scAAV) genome. In some embodiments, the method is

used to detect the presence of recombinant viral particles comprising the monomeric form of a scAAV genome or the dimeric form of a scAAV genome.

[0028] In some embodiments of the above aspect, the recombinant viral particles are produced by transient transfection of nucleic acid encoding adenovirus vector sequences and adenovirus replication and packaging sequences to a suitable host cell. In other embodiments, the recombinant viral particles are produced by transient transfection of nucleic acid encoding lentivirus vector sequences and/or lentivirus replication and packaging sequences to a suitable host cell. In other embodiments, the recombinant viral particles are produced by transient transfection of nucleic acid encoding HSV vector sequences and/or HSV replication and packaging sequences to a suitable host cell.

[0029] In some aspects the invention provides methods for preparing recombinant viral particles with reduced empty capsids and/or recombinant viral particles comprising variant genomes, the method comprising a) culturing host cells under conditions suitable for recombinant viral production, wherein the cells comprise i) nucleic acid encoding a heterologous transgene flanked by at least one AAV ITR, ii) nucleic acid comprising AAV rep and cap coding regions, wherein the nucleic acid comprises a p5 promoter, and iii) nucleic acid encoding AAV helper virus functions; b) lysing the host cells to release recombinant viral particles; c) isolating the recombinant viral particles produced by the host cell; and d) analyzing the recombinant viral particles for the presence of empty capsids and/or recombinant viral particles with variant genomes by analytical ultracentrifugation by the above methods. In some aspects the invention provides methods for preparing recombinant viral particles with reduced empty capsids and/or recombinant viral particles comprising variant genomes, the method comprising a) culturing host cells under conditions suitable for recombinant viral production, wherein the cells comprise i) nucleic acid encoding a heterologous transgene flanked by at least one AAV ITR, ii) nucleic acid comprising AAV rep and cap coding regions, wherein the nucleic acid comprises a mutated p5 promoter wherein rep expression from the p5 promoter is reduced compared to a wild-type p5 promoter, and iii) nucleic acid encoding AAV helper virus functions; b) lysing the host cells to release recombinant viral particles; c) isolating the recombinant viral particles produced by the host cell; and d) analyzing the recombinant viral particles for the presence of empty capsids and/or recombinant viral particles with variant genomes by analytical ultracentrifugation by the above methods. In some embodiments, the p5 promoter is located 3' to the rep and/or cap coding region.

In some embodiments, the AAV helper virus functions comprise adenovirus E1A function, adenovirus E1B function, adenovirus E2A function, adenovirus VA function and adenovirus E4 orf6 function.

[0030] In some embodiments, of any of the preceding embodiments, the recombinant viral particles have been purified using one or more purification steps.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] **FIGS. 1A and 1B** show that analytical ultracentrifugation (AUC) can be used to characterize recombinant viral vector particles. (**FIG. 1A**) A representative scanning profile of boundary sedimentation velocity depicting the absorbance (260 nm) versus the radius (cm) of an AAV2 mixture over a time interval (T) of 1.2 hours. The AAV2 mixture contained empty capsids ("Empty Cap") and full genome capsids ("Intact Vector"). (**FIG. 1B**) A plot of concentration in units of detection, C(S), versus sedimentation coefficient (Svedberg units, S) showing that AUC can be used to measure the concentration of empty capsids and full genome capsids from an 80%/20% mixture. Each peak is labeled with the particle species and its corresponding sedimentation coefficient (S) and relative abundance (%).

[0032] **FIGS. 2A and 2B** show the AUC profiles of pure populations of empty AAV2 capsids (**FIG. 2A**) and genome-containing AAV2-transgene 1 capsids (**FIG. 2B**). Each peak is labeled with the capsid species and its sedimentation coefficient (S).

[0033] **FIGS. 3A and 3B** show a comparison between interference and absorbance detection methods by AUC. (**FIG. 3A**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a 1:1 mixture of empty and genome-containing capsids generated using interference optical detection. The sedimentation coefficient and relative abundance (%) for each species are labeled. (**FIG. 3B**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a 1:1 mixture of empty and genome-containing capsids generated using absorbance optical detection (260 nm). The sedimentation coefficient and relative abundance (%) for each species are labeled.

[0034] **FIG. 4** illustrates the triple transfection method for AAV vector production. The three vectors, containing the gene of interest ("pVector"), AAV Rep and Cap genes ("pHLP"), and adenoviral components ("pIAdeno") are labeled. Note that both genome-containing (labeled with the "ITR-Transgene-ITR" graphic) and empty capsids (blank) are produced.

[0035] **FIG. 5** illustrates the producer cell line method for AAV vector production. As labeled, the HeLa S3 cell line contains integrated Rep, Cap, and Puromycin resistance genes, along with an ITR-flanked transgene of interest. This cell line is infected with adenovirus ("*Ad5*") to stimulate recombinant viral production. Note that both genome-containing (labeled "recombinant viral Vector") and empty capsids are produced, in addition to adenovirus particles.

[0036] **FIGS. 6A, 6B and 6C** shows that vector production by the producer cell line and triple transfection methods yields different vector preparations, as revealed by AUC analysis. (**FIG. 6A**) A schematic of the AAV2-transgene 2 vector and its 3.4 kb genome. (**FIG. 6B**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a vector preparation produced by the producer cell line method. The sedimentation coefficient and relative abundance (%) for each species are labeled. (**FIG. 6C**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a vector preparation produced by the triple transfection method. The sedimentation coefficient and relative abundance (%) for each species are labeled.

[0037] **FIGS. 7A, 7B and 7C** show that the AUC method may be used to monitor the quality and efficacy of vector purification. (**FIG. 7A**) A plot showing the purification of full-genome AAV2-transgene 1 capsids from empty capsids using anion exchange chromatography. Peak fractions corresponding to each species are labeled. (**FIG. 7B**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a vector preparation after elution from the anion exchange column. The sedimentation coefficient and relative abundance (%) for each species are labeled. (**FIG. 7C**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a vector preparation before chromatography. The sedimentation coefficient and relative abundance (%) for each species are labeled.

[0038] **FIG. 8** shows the linear relationship between sedimentation coefficient and vector genome size. A standard curve plotting sedimentation coefficient (S) versus genome size is depicted, along with a line of best fit, its formula, and its associated R^2 value.

[0039] **FIGS. 9A, 9B and 9C** show that assessment of capsid genome size using AUC data correlates with assessment of genome size by Southern blot. (**FIG. 9A**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a scAAV9 EGFP vector preparation. Single stranded monomeric (82S) and double stranded dimeric (101S) species are labeled with the corresponding sedimentation coefficients and relative abundance values (%). A schematic of the vector is also provided. (**FIG. 9B**) Alkaline Southern blot analysis of the DNA from scAAV9 EGFP (lane 1) and single stranded AAV9 EGFP (lane 2) vector capsids. Corresponding bands are labeled as described in the blot legend. 4.2 and 2.4 kb size standards are provided as labeled. (**FIG. 9C**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a single stranded AAV9 EGFP vector preparation. 82S and 99S (full genome) peaks are labeled with the corresponding sedimentation coefficient and relative abundance values (%).

[0040] **FIGS. 10A, 10B and 10C** show that the Rep/Cap promoter position affects genome packaging in recombinant viral vectors produced by the triple transfection method. (**FIG. 10A**) A schematic of the self-complementary scAAV2 EGFP vector, with estimated sedimentation coefficients for the dimeric and monomeric genome species. (**FIG. 10B**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a scAAV2 EGFP vector preparation produced using a "wild-type" helper plasmid with the endogenous p5 promoter driving Rep 78/68 expression ("WT Rep"). Peaks for single stranded monomeric (80S) and double stranded dimeric (100S) species are labeled with the corresponding relative abundance values (%). (**FIG. 10C**) A plot of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yields the distribution of sedimentation coefficients for a scAAV2 EGFP vector preparation produced using a "wild-type" helper plasmid with the p5 promoter driving Rep 78/68 expression moved downstream of the cap2 sequence ("pHLP Rep"). Peaks for single

stranded monomeric (82S) and double stranded dimeric (100S) species are labeled with the corresponding relative abundance values (%).

[0041] **FIGS. 11A, 11B, 11C and 11D** show that the Rep/Cap promoter position affects genome packaging in two additional AAV vectors. (**FIGS. 11A and 11B**) Plots of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yield the distribution of sedimentation coefficients for the single stranded AAV5 Factor IX vector (AAV5 hFIX16) containing a cap5 sequence produced with a helper plasmid having an endogenous p5 promoter ("WT Rep," **FIG. 11B**) or a p5 promoter downstream of the cap5 sequence ("pHLP Rep," **FIG. 11A**). (**FIGS. 11C-11D**) Plots of differential sedimentation coefficient distribution value, c(s), vs sedimentation coefficient in Svedberg units (S), yield the distribution of sedimentation coefficients for the single stranded AAV5hSMN vector (AAV5SMN) containing a cap5 sequence produced with a helper plasmid having an endogenous p5 promoter ("WT Rep," **FIG. 11D**) or a p5 promoter downstream of the cap5 sequence ("pHLP Rep," **FIG. 11C**).

[0042] **FIGS. 12A and 12B** reveal that Southern blot analysis correlates with AUC analysis but misses some fragmented genomes detectable by AUC. (**FIG. 12A**) Southern blot analysis of vector DNA from AAV5SMN preparations made with the pHLP helper plasmid (lane 2) or the WT Rep plasmid (lane 1). 4.6 and 2.4 kb size standards are provided as labeled. (**FIG. 12B**) Southern blot analysis of vector DNA from AAV5FIX preparations made with the pHLP helper plasmid (lane 1) or the WT Rep plasmid (lane 2). 4.3, 3.0, and 1.9 kb size standards are provided as labeled.

[0043] **FIG. 13** provides a map of the AAV5 Factor IX vector indicating the positions of the hFIX transgene, ITR, Rep origin, and *AmpR* marker gene, among other features. Note that the *AmpR* marker is upstream of the ITR, enhancer, and promoter region.

[0044] **FIGS. 14A and 14B** show that WT Rep vector genomes, unlike pHLP Rep vector genomes, package sequences upstream of the 5' ITR in the AAV5 Factor IX vector. (**FIG. 14A**) Southern blot analysis using an hFIX transgene-specific probe comparing pHLP Rep (lane 1) and WT Rep (lane 2) vector genomes. (**FIG. 14B**) Southern blot analysis using a Rep ori/*AmpR*-specific probe comparing pHLP Rep (lane 1) and WT Rep (lane 2) vector genomes.

[0045] **FIGS. 15A and 15B** show the fragmentation of oversized AAV vector genomes, as demonstrated by AUC analysis. (**FIG. 15A**) Plot of concentration, C(S), versus sedimentation coefficient (S) generated by AUC for an AAV vector with an oversized genome. This genome contains a full-length chicken β-actin (CBA) promoter driving expression of β-phosphodiesterase (ssAAV2/5CBA-βPDE). Peaks for detected species are labeled by observed sedimentation coefficient (S) and relative abundance values (%). (**FIG. 15B**) Plot of concentration, C(S), versus sedimentation coefficient (S) generated by AUC for an AAV vector with a truncated genome. This genome contains a CBA promoter with a reduced-size intron driving expression of β-phosphodiesterase (AAV5 minCBAPDE6B). Peaks for detected species are labeled by observed sedimentation coefficient (S) and relative abundance values (%).

[0046] **FIG. 16** shows the AUC profiles of pure populations of adenovirus capsids. The sedimentation coefficient (S) and interference values are given for each peak.

DETAILED DESCRIPTION

[0047] The present invention provides methods of characterizing preparations of viral particles using analytical ultracentrifugation. By subjecting preparations to analytical ultracentrifugation (AUC) under boundary sedimentation velocity conditions, the sedimentation of viral particles can be monitored at time intervals (*e.g.*, one or more times). The differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S) is then plotted and the area under each peak in the C(s) distribution is integrated to determine the relative concentration of each peak. Each peak represents a species of viral particle reflective of its molecular weight. The species that can be detected by these methods include, but are not limited to, recombinant adeno-associated viral (rAAV) particles, recombinant adenoviral (rAd) particles, recombinant lentiviral particles, and recombinant herpes simplex viral (rHSV) particles. To use rAAV particles as an illustrative example, these methods allow the detection of rAAV species including rAAV capsid particles comprising intact rAAV genomes (*e.g.*, full capsids), empty viral capsids wherein no rAAV genomes have been encapsidated into viral capsids, and rAAV particle variants in which variant rAAV genomes are encapsidated in viral capsids (*e.g.*, particles containing AAV-encapsidated DNA impurities, truncated viral genomes, aggregates, and the like). These methods can be applied to preparations of viral particles regardless of nucleotide sequence of the viral genome or, in the case of recombinant viral

particles, the serotype of the recombinant viral capsid. These methods can be applied to rAAV, rAd, recombinant lentivirus and rHSV viral particles.

[0048] In some aspects, the invention provides methods to assess vector genome integrity of recombinant viral particles in a preparation of recombinant viral particles by subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times). By plotting the differential sedimentation coefficient distribution value C(S) versus the sedimentation coefficient in Svedberg units (S), species of recombinant viral particles in the preparation can be identified by presence of peaks on the plot corresponding to an S value. The genome size of a particular species of recombinant viral particles can be calculated, for example, by comparing the S value of the species to a standard curve generated by S values of recombinant viral particles comprising encapsidated viral genomes of different known size. The vector genomes that can be assessed by these methods include, but are not limited to, recombinant viral capsid particles comprising intact recombinant viral genomes (*e.g.*, full capsids), empty viral capsids wherein no recombinant viral genomes have been encapsidated into viral capsids, and recombinant viral particle variants in which variant recombinant viral genomes (*e.g.*, particles containing AAV-encapsidated DNA impurities, truncated viral genomes, aggregates and the like) are encapsidated in viral capsids. In some embodiments, the viral particles are rAAV, rAd, recombinant lentivirus or rHSV viral particles.

[0049] In some embodiments the invention provides methods of determining the heterogeneity of recombinant viral particles (*e.g.*, rAAV, rAd, lentivirus or rHSV particles) in a preparation of recombinant viral particles by AUC under boundary sedimentation velocity conditions wherein the presence of peaks in a plot of C(S) v. S, in addition to the peak representing capsids comprising an intact viral genome, indicates heterogeneity of recombinant viral particles in the preparation. In some embodiments, the relative amounts of each recombinant viral species in the preparation are calculated by integrating the area for each peak in the plot.

[0050] In some embodiments of the invention, AUC is used to determine the presence of empty capsids and/or recombinant viral particle variants in a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentivirus or rHSV particles), wherein the presence of peak that corresponds to the S value of empty capsid particles and/or recombinant viral particle variants in a

plot of C(S) vs. S indicates the presence of empty capsid particles and/or recombinant viral particle variants. In some embodiments, the relative amount of empty capsids and/or recombinant viral particle variants in a preparation of recombinant viral particles is determined by integrating the area under each peak in a plot of C(S) versus S and comparing the amount of recombinant viral particles having an S value corresponding to empty capsid particles and/or recombinant viral particle variants to the amount of recombinant viral particles having an S value corresponding to recombinant viral particles comprising intact viral genomes. In some embodiments, the amount of recombinant viral particles having an S value corresponding to empty capsid particles and/or recombinant viral particle variants is compared to the total amount of all recombinant viral particles in the preparation by integrating and summing and the area under all the peaks of the plot.

[0051] In some embodiments, the invention provides methods of monitoring the removal of empty capsids and/or recombinant viral particle variants during the purification of a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentivirus or rHSV particles) by using AUC. Samples of the recombinant viral particles from the preparation following one or more steps in the purification process are analyzed for the relative amount of empty capsids and/or recombinant viral particle variants wherein a decrease in the relative amount of empty capsids and/or recombinant viral particle variants to full capsid particles indicates removal of empty capsids and/or recombinant viral particle variants from the preparation of recombinant viral particles.

[0052] In some embodiments, the invention provides methods of evaluating processes for the production of recombinant viral particles (*e.g.*, rAAV, rAd, lentivirus or rHSV particles) by AUC. The preparation of recombinant viral particles is analyzed for the presence of intact full viral capsid particles, empty particles and/or recombinant viral particle variants. An increase in the relative amount of recombinant viral particles comprising intact viral genomes compared to the relative amount of empty capsid particles and/or recombinant viral particle variants (*e.g.*, particles containing AAV-encapsidated DNA impurities, truncated viral genomes, aggregates, and the like) compared to a reference preparation of recombinant viral particles (*e.g.*, a standard recombinant viral preparation process) indicates an improvement in the production of recombinant viral particles.

I. General Techniques

[0053] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2012); *Current Protocols in Molecular Biology* (F.M. Ausubel, *et al.* eds., 2003); the series *Methods in Enzymology* (Academic Press, Inc.); *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds., 1995); *Antibodies, A Laboratory Manual* (Harlow and Lane, eds., 1988); *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications* (R.I. Freshney, 6th ed., J. Wiley and Sons, 2010); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., Academic Press, 1998); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, Plenum Press, 1998); *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., J. Wiley and Sons, 1993-8); *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds., 1996); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis *et al.*, eds., 1994); *Current Protocols in Immunology* (J.E. Coligan *et al.*, eds., 1991); *Short Protocols in Molecular Biology* (Ausubel *et al.*, eds., J. Wiley and Sons, 2002); *Immunobiology* (C.A. Janeway *et al.*, 2004); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita *et al.*, eds., J.B. Lippincott Company, 2011).

II. Definitions

[0054] A "vector," as used herein, refers to a recombinant plasmid or virus that comprises a nucleic acid to be delivered into a host cell, either in vitro or in vivo.

[0055] The term "polynucleotide" or "nucleic acid" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term

includes, but is not limited to, single-, double- or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases, or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively, the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidates and thus can be an oligodeoxynucleoside phosphoramidate ($P\text{-}NH_2$) or a mixed phosphoramidate- phosphodiester oligomer. In addition, a double-stranded polynucleotide can be obtained from the single stranded polynucleotide product of chemical synthesis either by synthesizing the complementary strand and annealing the strands under appropriate conditions, or by synthesizing the complementary strand de novo using a DNA polymerase with an appropriate primer.

[0056] The terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[0057] A "recombinant viral vector" refers to a recombinant polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of viral origin). In the case of recombinant AAV vectors, the recombinant nucleic acid is flanked by at least one inverted terminal repeat sequence (ITR). In some embodiments, the recombinant nucleic acid is flanked by two inverted terminal repeat sequences (ITRs).

[0058] A "recombinant AAV vector (recombinant adeno-associated viral vector)" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of AAV origin) that are flanked by at least one AAV inverted terminal repeat sequences (ITR). In some embodiments, the recombinant nucleic acid is flanked by two inverted terminal repeat sequences (ITRs). Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper virus (or that is expressing suitable helper functions) and that is expressing AAV rep and cap gene products (*i.e.* AAV Rep and Cap proteins). When a recombinant viral vector is incorporated into a larger polynucleotide (*e.g.,* in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the recombinant viral vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of AAV packaging functions and suitable helper functions. A recombinant viral vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, for example, an AAV particle. A recombinant viral vector can be packaged into an AAV virus capsid to generate a "recombinant adeno-associated viral particle (recombinant viral particle)".

[0059] An "rAAV virus" or "rAAV viral particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated rAAV vector genome.

[0060] A "recombinant adenoviral vector" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of adenovirus origin) that are flanked by at least one adenovirus inverted terminal repeat sequence (ITR). In some embodiments, the recombinant nucleic acid is flanked by two inverted terminal repeat sequences (ITRs). Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that is expressing essential adenovirus genes deleted from the recombinant viral genome (*e.g.*, E1 genes, E2 genes, E4 genes, etc.). When a recombinant viral vector is incorporated into a larger polynucleotide (*e.g.,* in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the recombinant viral vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of adenovirus packaging functions. A recombinant viral vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, for example, an adenovirus

particle. A recombinant viral vector can be packaged into an adenovirus virus capsid to generate a "recombinant adenoviral particle."

[0061] A "recombinant lentivirus vector" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of lentivirus origin) that are flanked by at least one lentivirus terminal repeat sequences (LTRs). In some embodiments, the recombinant nucleic acid is flanked by two lentiviral terminal repeat sequences (LTRs). Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper functions. A recombinant lentiviral vector can be packaged into a lentivirus capsid to generate a "recombinant lentiviral particle."

[0062] A "recombinant herpes simplex vector (recombinant HSV vector)" refers to a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of HSV origin) that are flanked by HSV terminal repeat sequences. Such recombinant viral vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been infected with a suitable helper functions. When a recombinant viral vector is incorporated into a larger polynucleotide (*e.g.,* in a chromosome or in another vector such as a plasmid used for cloning or transfection), then the recombinant viral vector may be referred to as a "pro-vector" which can be "rescued" by replication and encapsidation in the presence of HSV packaging functions. A recombinant viral vector can be in any of a number of forms, including, but not limited to, plasmids, linear artificial chromosomes, complexed with lipids, encapsulated within liposomes, and encapsidated in a viral particle, for example, an HSV particle. A recombinant viral vector can be packaged into an HSV capsid to generate a "recombinant herpes simplex viral particle."

[0063] "Heterologous" means derived from a genotypically distinct entity from that of the rest of the entity to which it is compared or into which it is introduced or incorporated. For example, a polynucleotide introduced by genetic engineering techniques into a different cell type is a heterologous polynucleotide (and, when expressed, can encode a heterologous polypeptide). Similarly, a cellular sequence (*e.g.*, a gene or portion thereof) that is incorporated into a viral vector is a heterologous nucleotide sequence with respect to the vector.

[0064] The term "transgene" refers to a polynucleotide that is introduced into a cell and is capable of being transcribed into RNA and optionally, translated and/or expressed under appropriate conditions. In aspects, it confers a desired property to a cell into which it was introduced, or otherwise leads to a desired therapeutic or diagnostic outcome. In another aspect, it may be transcribed into a molecule that mediates RNA interference, such as siRNA.

[0065] The terms "genome particles (gp)," "genome equivalents," or "genome copies" as used in reference to a viral titer, refer to the number of virions containing the recombinant viral DNA genome or RNA genome, regardless of infectivity or functionality. The number of genome particles in a particular vector preparation can be measured by procedures such as described in the Examples herein, or for example, in Clark *et al.* (1999) *Hum. Gene Ther.*, 10:1031-1039; Veldwijk *et al.* (2002) *Mol. Ther.*, 6:272-278.

[0066] The terms "infection unit (iu)," "infectious particle," or "replication unit," as used in reference to a viral titer, refer to the number of infectious and replication-competent recombinant viral vector particles as measured by the infectious center assay, also known as replication center assay, as described, for example with AAV, in McLaughlin *et al.* (1988) *J. Virol.*, 62:1963-1973.

[0067] The term "transducing unit (tu)" as used in reference to a viral titer, refers to the number of infectious recombinant viral vector particles that result in the production of a functional transgene product as measured in functional assays such as described in Examples herein, or for example regarding AAV, in Xiao *et al.* (1997) *Exp. Neurobiol.*, 144:113-124; or in Fisher *et al.* (1996) *J. Virol.*, 70:520-532 (LFU assay).

[0068] An "inverted terminal repeat" or "ITR" sequence is a term well understood in the art and refers to relatively short sequences found at the termini of viral genomes which are in opposite orientation.

[0069] An "AAV inverted terminal repeat (ITR)" sequence, a term well-understood in the art, is an approximately 145-nucleotide sequence that is present at both termini of the native single-stranded AAV genome. The outermost 125 nucleotides of the ITR can be present in either of two alternative orientations, leading to heterogeneity between different AAV genomes and between the two ends of a single AAV genome. The outermost 125 nucleotides also contains several

shorter regions of self-complementarity (designated A, A', B, B', C, C' and D regions), allowing intrastrand base-pairing to occur within this portion of the ITR.

[0070] A "terminal resolution sequence" or "trs" is a sequence in the D region of the AAV ITR that is cleaved by AAV rep proteins during viral DNA replication. A mutant terminal resolution sequence is refractory to cleavage by AAV rep proteins.

[0071] "AAV helper functions" refer to functions that allow AAV to be replicated and packaged by a host cell. AAV helper functions can be provided in any of a number of forms, including, but not limited to, helper virus or helper virus genes which aid in AAV replication and packaging. Other AAV helper functions are known in the art such as genotoxic agents.

[0072] A "helper virus" for AAV refers to a virus that allows AAV (which is a defective parvovirus) to be replicated and packaged by a host cell. A helper virus provides "helper functions" which allow for the replication of AAV. A number of such helper viruses have been identified, including adenoviruses, herpesviruses, poxviruses such as vaccinia and baculovirus. The adenoviruses encompass a number of different subgroups, although Adenovirus type 5 of subgroup C (Ad5) is most commonly used. Numerous adenoviruses of human, non-human mammalian and avian origin are known and are available from depositories such as the ATCC. Viruses of the herpes family, which are also available from depositories such as ATCC, include, for example, herpes simplex viruses (HSV), Epstein-Barr viruses (EBV), cytomegaloviruses (CMV) and pseudorabies viruses (PRV). Examples of adenovirus helper functions for the replication of AAV include E1A functions, E1B functions, E2A functions, VA functions and E4orf6 functions. Baculoviruses available from depositories include *Autographa californica* nuclear polyhedrosis virus.

[0073] A preparation of rAAV is said to be "substantially free" of helper virus if the ratio of infectious AAV particles to infectious helper virus particles is at least about 10^2:1; at least about 10^4:1, at least about 10^6:1; or at least about 10^8:1 or more. In some embodiments, preparations are also free of equivalent amounts of helper virus proteins (*i.e.*, proteins as would be present as a result of such a level of helper virus if the helper virus particle impurities noted above were present in disrupted form). Viral and/or cellular protein contamination can generally be observed

as the presence of Coomassie staining bands on SDS gels (*e.g.*, the appearance of bands other than those corresponding to the AAV capsid proteins VPl, VP2 and VP3).

[0074] As used herein, "differential coefficient distribution value" or "C(S)" is a variant of the distribution of Lamm equation solutions to describe distributions of sedimenting particles; for example during ultracentrifugation.

[0075] As used herein, "Svedberg units" refers to a unit for sedimentation rate. The sedimentation rate for a particle of a given size and shape measures how fast the particle sediments. One Svedberg unit is equivalent to 10^{-13} seconds. For example, Svedberg units are often used to reflect the rate at which a molecule travels under the centrifugal force of a centrifuge.

[0076] As used herein, "sedimentation velocity conditions" or "boundary sedimentation velocity conditions" may refer to any experimental conditions under which a sample solution is subjected to sedimentation velocity analysis. Sedimentation velocity allows the study of particles over a wide range of pH and ionic strength conditions and at temperatures 4 to 40 °C. The rate at which the sedimentation boundary moves is a measure of the sedimentation coefficient of the sedimenting species. The sedimentation coefficient depends on the molecular weight (larger particles sediment faster) and also on molecular shape. The minimum width of the sedimentation boundary is related to the diffusion coefficient of the molecule; the presence of multiple species with similar sedimentation coefficients will cause the boundary to be broader than expected on the basis of diffusion alone. Sedimentation velocity conditions may include without limitation any conditions related to the rotor speed, distance between sample and rotor center, temperature, solvent, sample, buffer, ultracentrifugation time, time interval for detection, sector and optical window characteristics, AUC instrumentation (including ultracentrifuge and detection apparatus), equilibrium dialysis of reference solvent, and data analysis algorithms.

[0077] As used herein, the term "analytical density gradient sedimentation equilibrium" relates to methods for measuring the buoyant density of a particle, or using differences in buoyant density to separate different species of particles. These methods may use, for example, AUC sedimentation equilibrium techniques. In these methods, a particle solution (*e.g.,* without limitation, a solution of a polypeptide, polynucleotide, or viral capsids) may be subjected to

ultracentrifugation in a gradient solvate, such as a cesium chloride or cesium sulfate gradient, until equilibrium with the solvate is attained. At equilibrium, the particle solution will concentrate, or band, at the position in the gradient where the density of the particle is equal to that of the solvate. The position of bands may be used to calculate particle density, or a band may be extracted to isolate a single species of particle.

[0078] As used herein, the "SEDFIT algorithm" is an algorithm that allows one to analyze hydrodynamic data such as sedimentation velocity (Schuck (2000) *Biophys. J.*, 78:1606-19). In the SEDFIT algorithm, a grid of sedimentation coefficients across an expected range is created. Sedimentation boundaries are simulated using solutions to the Lamm equation for each sedimentation coefficient, assuming constant particle shape and solvent frictional ratio.

[0079] As used herein, the term "F statistic" or "F ratio" refers to the confidence level. This parameter controls the amount of regularization used. It has a different meaning for different ranges: From 0 to 0.5, no regularization is used. Values from 0.5 to 0.999 correspond to probabilities P (confidence levels). From these P-values, the desired chi-square increase allowed for the parsimony constraint of the regularization is calculated with F-statistics. A value of 0.51 will cause very little regularization; values of 0.68 to 0.90 would correspond to commonly used confidence levels (usually, with 50 scans or more the chi-square increase corresponding to a probability of 0.7 is of the order of 0.1%), while values close to 0.99 would cause very high regularization. The relationship of these values with probabilities can be examined using the F-statistics calculator. If numbers > 1 are entered, they are taken directly as chi-square ratios (as there are no probabilities > 1). For example, a value of 1.1 will result in regularization with 10% chi-square increase.

[0080] To "reduce" is to decrease, reduce or arrest an activity, function, and/or amount as compared to a reference. In certain embodiments, by "reduce" is meant the ability to cause an overall decrease of 20% or greater. In another embodiment, by "reduce" is meant the ability to cause an overall decrease of 50% or greater. In yet another embodiment, by "reduce" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater.

[0081] A "reference" as used herein, refers to any sample, standard, or level that is used for comparison purposes. For example, when measuring absorbance or refraction of AAV in an

aqueous solution, the absorbance or refraction of the solution is compared to the absorbance or refraction of the aqueous solution without AAV (*i.e.* a reference solution). In other examples, a reference may refer to a standard procedure known in the art. For example, when analyzing a procedure for improved quality of AAV production (*e.g.*, homogeneity), the AAV produced by the candidate procedure is compared to procedures known in the art (*i.e.* reference procedures).

[0082] An "isolated" molecule (*e.g.*, nucleic acid or protein) or cell means it has been identified and separated and/or recovered from a component of its natural environment. Thus, for example, isolated rAAV particles may be prepared using a purification technique to enrich it from a source mixture, such as a culture lysate or production culture supernatant. Enrichment can be measured in a variety of ways, such as, for example, by the proportion of DNase-resistant particles (DRPs) present in a solution, or by infectivity, or it can be measured in relation to a second, potentially interfering substance present in the source mixture, such as contaminants, including production culture contaminants or in-process contaminants, including helper virus, media components, and the like, as defined below.

[0083] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X."

[0084] As used herein, the singular form of the articles "a," "an," and "the" includes plural references unless indicated otherwise. For example, the phrase "a rAAV particle" includes one or more rAAV particles.

[0085] It is understood that aspects and embodiments of the invention described herein include "comprising," "consisting," and/or "consisting essentially of" aspects and embodiments.

III. Analytical ultracentrifugation

[0086] Analytical ultracentrifugation is a means to evaluate the molecular weight and the hydrodynamic and thermodynamic properties of a protein or other macromolecule. Heterogeneity of a protein or macromolecule by sedimentation velocity over a range of conditions including concentration, temperature, ionic strength, and pH. For example, a protein may be analyzed in a

clinically relevant formulation. Use of analytical ultracentrifugation to characterize adenovirus preparations is provided by Berkowitz, SA & Philo JS, (2007) *Anal. Biochem.*, 362:16-37.

[0087] In certain aspects, the present invention provides methods of characterizing preparations of viral particles using analytical ultracentrifugation (AUC). For example, in some embodiments, the invention provides methods to assess vector genome integrity of recombinant adeno-associated viral (rAAV) particles in preparations of rAAV particles using AUC to distinguish viral particles with full, intact genomes, empty viral capsids and viral particles with variant (*e.g.,* truncated, aggregates, impurities and the like) viral genomes. In others embodiments, these methods may be applied in a similar way to analyze adenovirus, lentivirus, and herpes simplex virus (HSV) particles. AUC analysis refers to quantitative methods for characterizing the biophysical properties of particles (*e.g.,* polypeptides, polynucleotides, and viral capsids) by measuring their migration through a solvent in a centrifugal field. AUC analysis has been well characterized over many decades and is highly versatile. Because AUC analysis relies upon first-principle hydrodynamic and thermodynamic information, AUC may be applied to determine the biophysical properties of many types of particles across a wide range of particle concentrations and sizes. AUC analysis typically encompasses two basic types of experiment: sedimentation velocity and sedimentation equilibrium. Sedimentation equilibrium analysis yields thermodynamic properties of particles that may be used to measure characteristics such as stoichiometry and association constants. Sedimentation velocity yields hydrodynamic properties of particles that may be used to measure characteristics such as size, shape, and concentration. A feature of AUC analysis of viral preparations is that the same assay conditions may be used to analyze different preparations of viral particles regardless of nucleotide sequence of the viral genome or serotype of the capsid.

[0088] Certain aspects of the present disclosure relate to the use of sedimentation velocity analysis to characterize viral capsid properties. In some embodiments, sedimentation velocity analysis uses an ultracentrifuge velocity cell with two sectors in dialysis equilibrium (one for an experimental sample and one for a solvent-only reference sample), each containing two optical windows that allow light to pass through the compartment. Ultracentrifugation applies an angular velocity to the cell and leads to rapid sedimentation of the solute particles towards the bottom of the sector. As sedimentation occurs, solute is depleted near the meniscus at the top of the cell, creating a sedimenting boundary between the depleted region and the sedimenting solute. The

rate of movement or migration of the sedimenting boundary is measured by taking measurements that compare the properties of the sample and reference sectors at specific time intervals (for sedimentation velocity, these intervals are typically on the order of minutes). If multiple species of solute are present, this may lead to the formation of multiple sedimenting boundaries, each corresponding to a resolvable species.

[0089] Several methods for optically detecting a sedimenting boundary and measuring its rate of movement or migration are known in the art (for reference, see Cole *et al.* (2008) *Methods Cell Biol.,* 84:143-79). In some embodiments, the reference and sample sectors may be assayed using absorbance detection. In this detection method, the absorbance at a particular wavelength may be measured for the sample and reference sectors at different radial positions within each sector. Alternatively, the time course of absorbance at a single radial position may be measured. Beer's Law provides a mathematical relationship between absorbance and a solute's extinction coefficient.

[0090] In some embodiments, the reference and sample sectors may be assayed using interference detection (*e.g.*, Rayleigh interference detection). In the Rayleigh interference detection method, the interference optical system contains two parallel slits. A single, coherent beam of light is split such that it passes through both windows, and then the two beams are re-merged. When these two light waves are merged, they form an interference pattern of alternating light and dark fringes. If the sample and reference samples were to have an identical refractive index, the resulting interference fringes would be perfectly straight. Increasing the concentration of solute increases the solution's refractive index, thereby retarding the sample light beam and causing a vertical fringe shift. By measuring this fringe shift, one may measure the concentration of solute in the sample. Unlike absorbance detection, which measures absolute values for the sample and reference, interference detection measures a relative difference between the sample and reference. However, interference detection yields integrated peaks that are directly proportional to concentration, and it may be used for types of samples that do not absorb significantly. For a reference on using Rayleigh interference optics with AUC, see Furst (1997) *Eur. Biophys. J.* 35:307-10.

[0091] Measurement of the rate at which the sedimentation boundary moves may be used to derive many physical properties of solute particles. The rate of the boundary movement

determines the sedimentation coefficient, which is based on the mass and shape (frictional coefficient) of the particle. The sedimentation coefficient of a particle, *s*, refers to the ratio of its velocity to the acceleration applied to it by a centrifugal field. Sedimentation coefficients are expressed in Svedberg units, S (one Svedberg unit is equivalent to 10^{-13} seconds). The sedimentation coefficient of a particle or solution of particles depends upon its properties, for example molecular weight (corrected for buoyancy), and the properties of the solvent.

[0092] The change in the concentration boundary of a solute over time during ultracentrifugation may be determined using the Lamm equation (Schuck (2000) *Biophys. J.*, 78:1606-19). Briefly, the Lamm equation calculates the change in the concentration boundary of a solute over time in response to the competing forces of sedimentation (which concentrates the solute) and diffusion (which disperses the solute), taking into account the sector-shaped cell and the centrifugal field generated by the rotor. The Lamm equation may be expressed as:

Equation 1: $\partial c/\partial t = D\,[(\partial^2 c/\partial r^2) + 1/r(\partial c/\partial r)] - s\omega^2\,[r(\partial c/\partial r) + 2c]$

where c is the solute concentration, D represents the solute diffusion constant, s represents the sedimentation coefficient, ω represents the angular velocity of the rotor, r is the radius, and t is time.

[0093] By fitting raw AUC data to solutions of the Lamm equation, it is possible to determine solute characteristics such as the sedimentation coefficient and the change in concentration distribution. For example, experimentally determined values for the rate of change of a sedimenting boundary may be modeled using the Lamm equation to derive the sedimentation coefficient, molecular mass, or concentration of the solute forming the boundary. Several programs known in the art, such as SEDFIT (Schuck (2000) *Biophys. J.*, 78:1606-19), may be used to model the Lamm equation to AUC data. These programs are also able to apply the Lamm equation to solutions containing multiple solutes or multiple sedimenting boundaries.

[0094] One example of a suitable program for the determination of solute characteristics is the SEDFIT algorithm. In some embodiments, the SEDFIT algorithm may be used to calculate a differential coefficient distribution value, or C(S), using AUC data from a solution containing a mixture of particle species (for reference, see Schuck (2000) *Biophys. J.*, 78:1606-19). In the SEDFIT algorithm, a grid of sedimentation coefficients across an expected range is created.

Sedimentation boundaries are simulated using solutions to the Lamm equation for each sedimentation coefficient, assuming constant particle shape and solvent frictional ratio. Actual AUC data are then fit to these Lamm solutions to derive the differential coefficient distribution value, or C(S). Many other programs useful for analyzing AUC data may be found in Cole and Hansen (1999) *J. Biomol. Tech.* 10:163-76.

[0095] In some embodiments of the invention, recombinant viral particles are highly purified, suitably buffered, and concentrated. In some embodiments, the viral particles are concentrated to at least about any of 1 x 10^7 vg/mL, 2 x 10^7 vg/mL, 3 x 10^7 vg/mL, 4 x 10^7 vg/mL, 5 x 10^7 vg/mL, 6 x 10^7 vg/mL, 7 x 10^7 vg/mL, 8 x 10^7 vg/mL, 9 x 10^7 vg/mL, 1 x 10^8 vg/mL, 2 x 10^8 vg/mL, 3 x 10^8 vg/mL, 4 x 10^8 vg/mL, 5 x 10^8 vg/mL, 6 x 10^8 vg/mL, 7 x 10^8 vg/mL, 8 x 10^8 vg/mL, 9 x 10^8 vg/mL, 1 x 10^9 vg/mL, 2 x 10^9 vg/mL, 3 x 10^9 vg/mL, 4 x 10^9 vg/mL, 5 x 10^9 vg/mL, 6 x 10^9 vg/mL, 7 x 10^9 vg/mL, 8 x 10^9 vg/mL, 9 x 10^9 vg/mL, 1 x 10^{10} vg/mL, 2 x 10^{10} vg/mL, 3 x 10^{10} vg/mL, 4 x 10^{10} vg/mL, 5 x 10^{10} vg/mL, 6 x 10^{10} vg/mL, 7 x 10^{10} vg/mL, 8 x 10^{10} vg/mL, 9 x 10^{10} vg/mL, 1 x 10^{11} vg/mL, 2 x 10^{11} vg/mL, 3 x 10^{11} vg/mL, 4 x 10^{11} vg/mL, 5 x 10^{11} vg/mL, 6 x 10^{11} vg/mL, 7 x 10^{11} vg/mL, 8 x 10^{11} vg/mL, 9 x 10^{11} vg/mL, 1 x 10^{12} vg/mL, 2 x 10^{12} vg/mL, 3 x 10^{12} vg/mL, 4 x 10^{12} vg/mL, 5 x 10^{12} vg/mL, 6 x 10^{12} vg/mL, 7 x 10^{12} vg/mL, 8 x 10^{12} vg/mL, 9 x 10^{12} vg/mL, 1 x 10^{13} vg/mL, 2 x 10^{13} vg/mL, 3 x 10^{13} vg/mL, 4 x 10^{13} vg/mL, 5 x 10^{13} vg/mL, 6 x 10^{13} vg/mL, 7 x 10^{13} vg/mL, 8 x 10^{13} vg/mL, 9 x 10^{13} vg/mL. In some embodiments, the viral particles are concentrated to of about 1 x 10^7 vg/mL to about 1 x 10^{13} vg/mL, about 1 x 10^8 vg/mL to about 1 x 10^{13} vg/mL, about 1 x 10^9 vg/mL to about 1 x 10^{13} vg/mL, about 1 x 10^{10} vg/mL to about 1 x 10^{13} vg/mL, about 1 x 10^{11} vg/mL to about 1 x 10^{13} vg/mL, about 1 x 10^{12} vg/mL to about 1 x 10^{13} vg/mL, about 1 x 10^7 vg/mL to about 1 x 10^{12} vg/mL, about 1 x 10^8 vg/mL to about 1 x 10^{12} vg/mL, about 1 x 10^9 vg/mL to about 1 x 10^{12} vg/mL, about 1 x 10^{10} vg/mL to about 1 x 10^{12} vg/mL, about 1 x 10^{11} vg/mL to about 1 x 10^{12} vg/mL, about 1 x 10^7 vg/mL to about 1 x 10^{11} vg/mL, about 1 x 10^8 vg/mL to about 1 x 10^{11} vg/mL, about 1 x 10^9 vg/mL to about 1 x 10^{11} vg/mL, about 1 x 10^{10} vg/mL to about 1 x 10^{11} vg/mL, about 1 x 10^7 vg/mL to about 1 x 10^{10} vg/mL, about 1 x 10^8 vg/mL to about 1 x 10^{10} vg/mL, about 1 x 10^9 vg/mL to about 1 x 10^{10} vg/mL, about 1 x 10^7 vg/mL to about 1 x 10^9 vg/mL, about 1 x 10^8 vg/mL to about 1 x 10^9 vg/mL, or about 1 x 10^7 vg/mL to about 1 x 10^8 vg/mL.

[0096] In some embodiments, viral particles are generated in a suitable host cells and purified. In some embodiments, the viral particles are purified by affinity chromatography. Methods to

purify viral particles (*e.g.*, AAV particles, adenovirus particles, lentivirus particles, HSV particles) are known in the art. For example, by use of an antibody of a viral capsid protein or binding ligand of a viral capsid protein immobilized on a chromatography media. Examples of viral capsid affinity chromatographies include but are not limited to AVB affinity chromatography for AAV (GE Healthcare), metal affinity chromatography for adenovirus and HSV, and heparin affinity chromatography for AAV and lentivirus, and the like. Methods to purify adenovirus particles are found, for example, in Bo, H *et al.*, (2014) *Eur. J. Pharm. Sci.* 67C:119-125. Methods to purify lentivirus particles are found, for example, in Segura MM, *et al.*, (2013) *Expert Opin Biol Ther.* 13(7):987-1011. Methods to purify HSV particles are found, for example, in Goins, WF et al., (2014) Herpes Simplex Virus Methods in Molecular Biology 1144:63-79.

[0097] In some embodiments, the recombinant viral particles are formulated in a pharmaceutical composition. In related embodiments, the pharmaceutical composition contains a buffer having physiological pH and/or physiological osmolality. A nonlimiting example of a pharmaceutical formulation is phosphate buffered saline (PBS) and in some embodiments, the PBS can be at physiological osmolality (*e.g.*, about pH 7.2 and about 300 mOsm/L). In some embodiments, sample adjustments are made to target concentration by optical density measurement at 260nm from 0.1 to 1.0. In some examples, this concentration results in reproducible and consistent AUC data. In some examples, concentration of viral particles is adjusted either by direct dilution with PBS or further concentration; for example, by using a centrifugal filter device.

[0098] In some embodiments of the invention, sedimentation velocity analytical ultracentrifugation (SV-AUC) analysis is performed using an analytical ultracentrifuge that is capable of characterizing a sample in its native state under biologically relevant solution conditions (*e.g.*, ProteomeLab™ XL-I (Beckman Coulter)). When using the ProteomeLab™ XL-1, sample is loaded into the sample sector of a two sector velocity cell, a vehicle control (*e.g.*, PBS without recombinant viral) is loaded into the corresponding reference sector. The sample is placed in the four-hole rotor and allowed to equilibrate in the instrument until a temperature of about 20 ^{o}C and full vacuum are maintained for about one hour. In an exemplary embodiment, sedimentation velocity centrifugation is performed at about 20,000 RPM, about 20 °C, and about 0.003 cm radial step setting, with no delay and with no replicates. As noted below, different parameters may be used for centrifugation. In some embodiments, absorbance (260 nm) and/or

interference optics (*e.g.*, Rayleigh interference optics) are used to simultaneously record radial concentration as a function of time until the smallest sedimenting component clears the optical window. In some embodiments, the radial concentration is recorded until the sedimenting species with the lowest density clears the sector. In some embodiments, sedimentation is monitored until the recombinant viral particles with the lowest density sediments to the bottom of a sector of an ultracentrifuge. A sector may be a portion of an ultracentrifuge; for example an ultracentrifuge velocity cell. In some embodiments, a sector may be a portion of an ultracentrifuge where samples are detected. In some embodiments, the ultracentrifugation utilizes an ultracentrifuge comprising an ultracentrifuge velocity cell. In some embodiments, is monitored until recombinant viral particles sediment to the bottom of an ultracentrifuge velocity cell. In some embodiments, sedimentation is monitored until the recombinant viral particles with the lowest density sediments and clears the optical window. In some embodiments, the radial concentration is recorded for at least about any of 0.5 hours, 0.75 hours, 1.0 hours, 1.5 hours, 2.0 hours, 3.0 hours, 4.0 hours, or 5.0 hours. In some embodiments, the radial concentration is recorded for between any of about 0.5 hours to about 0.75 hours, about 0.75 hours to about 1.0 hours, about 1.0 hours to about 1.5 hours, about 1.5 hours to about 2.0 hours, about 2 hours to about 3 hours, about 3 hours to about 4 hours, about 4 hours to about 5 hours. In some embodiments, the radial concentration is recorded for about 1.2 hours. Optimizing runs conditions may include, for example, continuing the run until all of the sedimenting species are fully sedimented to the bottom of the sector, with the temperature held constant at 20 °C and a speed between 18,000 rpm and 20,000 rpm. As noted below, other temperatures and speeds may be used.

[0099] The percent full capsid is determined by analyzing a multiple of scans (*e.g.*, 75) from each detection method using the SEDFIT continuous size C(S) distribution model. Second (2nd) derivative regularization is applied to the fitting. In some embodiments, the confidence level of F statistic is about 0.68. In some embodiments, the confidence level of F statistic is more than about any of 0.68, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95 or 0.99. In some embodiments, the confidence level of F statistic is about 0.68 to about 0.90. In some embodiments, the confidence level of F statistic is about 0.68 to about 0.99. In some embodiments, the following C(S) parameters are held constant: resolution of about 200S to about 5000S, S min is about 1S to about 100S, S max is about 100S to about 5000S, and frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software. In some embodiments, the resolution is about any of 200S, 300S,

400S, 500S, 600S, 700S, 800S, 900S, or 1000S. In some embodiments, the resolution is between any of about 200S to about 1000S, 200S to about 900S, 200S to about 800S, 200S to about 700S, 200S to about 600S, 200S to about 500S, 200S to about 400S, 200S to about 300S, 300S to about 1000S, 300S to about 900S, 300S to about 800S, 300S to about 700S, 300S to about 600S, 300S to about 500S, 300S to about 400S, 400S to about 1000S, 400S to about 900S, 400S to about 800S, 400S to about 700S, 400S to about 600S, 400S to about 500S, 500S to about 1000S, 500S to about 900S, 500S to about 800S, 500S to about 700S, 500S to about 600S, 600S to about 1000S, 600S to about 900S, 600S to about 800S, 600S to about 700S, 700S to about 1000S, 700S to about 900S, 700S to about 800S, 800S to about 1000S, 800S to about 900S, or 900S to about 1000S. In some embodiments, the resolution is about 200S. In some embodiments, the Smax is about any of 100S, 200S, 300S, 400S, 500S, 600S, 700S, 800S, 900S, or 1000S. In some embodiments, the Smax is between any of about 100S to about 1000S, 100S to about 900S, 100S to about 800S, 100S to about 700S, 100S to about 600S, 100S to about 500S, 100S to about 400S, 100S to about 300S, 100S to about 200S, 200S to about 1000S, 200S to about 900S, 200S to about 800S, 200S to about 700S, 200S to about 600S, 200S to about 500S, 200S to about 400S, 200S to about 300S, 300S to about 1000S, 300S to about 900S, 300S to about 800S, 300S to about 700S, 300S to about 600S, 300S to about 500S, 300S to about 400S, 400S to about 1000S, 400S to about 900S, 400S to about 800S, 400S to about 700S, 400S to about 600S, 400S to about 500S, 500S to about 1000S, 500S to about 900S, 500S to about 800S, 500S to about 700S, 500S to about 600S, 600S to about 1000S, 600S to about 900S, 600S to about 800S, 600S to about 700S, 700S to about 1000S, 700S to about 900S, 700S to about 800S, 800S to about 1000S, 800S to about 900S, or 900S to about 1000S. In some embodiments, Smax is about 200S to about 5000S. In some embodiments, wherein Smax is about 200S. In some embodiments, the frictional ratio is left to float to a value determined by centrifugation software. In some embodiments, the frictional ratio is about 1.0. In some embodiments, radial invariant (RI) and time invariant (TI) noise subtractions are applied. In some embodiments, the meniscus position is allowed to float, letting the software choose the optimal position. In some embodiments, the frictional ratio is allowed to float, letting the software choose the optimal position. The model fits the data to the Lamm equation, and the resulting size distribution is a "distribution of sedimentation coefficients" that looks like a chromatogram with the area under each peak proportional to concentration in units of Fringes or OD_{260} units. The sedimentation coefficient (in Svedberg units) and the relative concentration (in OD units) are determined for each component in the distribution. In some

embodiments, multiple AUC runs are independent assays, and each analysis the following attributes are monitored to ensure quality of results: goodness of fit (rmsd), the ratio of $OD_{260\ nm}$/ interference signal in fringes (A260/IF ratio) for each peak, consistency of sedimentation coefficients for each species between runs, and overall quality of the scans.

[0100] In some embodiments of the invention, extinction coefficients are used to calculate molar concentration and the actual percent value of the intact vector peak from absorbance data. Molar absorbance extinction coefficients for both empty capsids ($\epsilon_{260}/_{capsid}$=3.72e6) and intact vector ($\epsilon_{260}/_{vector}$=3.00e7) can be calculated based on published formulae (Sommer *et al.* (2003) *Mol Ther.*, 7:122-8). Extinction coefficients are available for empty capsid and intact vector peaks. The C(S) values can be determined using the SEDFIT algorithm described by Schuck (2000) *Biophys. J.,* 78:1606-19. Molar concentration of both intact vector and empty capsid can be calculated using Beer's Law and the percentage of full capsid are calculated from these values. In some embodiments, values are reported in terms of the percentage of full capsid.

[0101] In some embodiments, it is not possible to determine empirically the extinction coefficient of particular species of recombinant viral particles (*e.g.*, viral particles with fragmented genomes of unknown size and sequence). A relationship between S value and genome size may be established by analyzing recombinant viral vector preps with encapsidated viral genomes of known nucleotide size and a corresponding S value are determined as described herein. The calculated S values can be plotted to generate a standard curve to which recombinant viral species of unknown molecular weight or genome size can be compared to determine the molecular weight of the unknown species.

[0102] In some aspects, the invention provides methods of characterizing a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times), b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral particle. In some embodiments, the species of recombinant viral particle identified by the methods of the invention include, but are

not limited to; full recombinant viral particles comprising intact recombinant viral genomes, empty recombinant viral capsid particles, and recombinant viral particles comprising variant recombinant viral genomes. In some embodiments the variant genomes are smaller than the intact recombinant viral genome (*e.g.*, truncated genomes). In some embodiments, the variant genomes are larger than the intact recombinant viral genome (*e.g.*, aggregates, recombinants, *etc.*). In some embodiments, the invention provides methods to assess vector genome integrity of recombinant viral particles in a preparation of recombinant viral particles comprising a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times), b) plotting the differential sedimentation coefficient distribution value C(s) versus the sedimentation coefficient in Svedberg units (S), c) identifying species of recombinant viral particles in the preparation by presence of peaks on the plot corresponding to an S value, wherein the genome size of a particular species of recombinant viral particles is calculated by comparing the S value of the species to a standard curve generated by S values of recombinant viral particles comprising encapsidated viral genomes of different known size. In some embodiments, the methods further comprise integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles. In some embodiments, the sedimentation of recombinant viral particles is monitored at one time interval. In some embodiments, the sedimentation of recombinant viral particles is monitored at more than one time interval.

[0103] In some embodiments of the invention, the sedimentation of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) is monitored by measuring optical density or absorbance at about 260 nm. Means of measuring absorbance are known in the art. In some embodiments, an ultracentrifuge used for AUC is equipped with means for measuring absorbance. In other embodiments, the sedimentation of recombinant viral particles is monitored by interference. In some embodiments, the sedimentation of recombinant viral particles is monitored by Rayleigh interference. Means of measuring interference are known in the art (Furst (1997) *Eur. Biophys. J.* 35:307-10). In some embodiments, an ultracentrifuge used for AUC is equipped with means for measuring interference. In some embodiments, the sedimentation of recombinant viral particles is monitored by both absorbance and interference. In some embodiments, the absorbance and/or interference are measured using a reference standard. In some embodiments,

the reference standard matches the solution of the recombinant viral preparation with the exception that the recombinant viral is not present. For example, the recombinant viral preparation may comprise recombinant viral in a buffer such as phosphate buffered saline. In this example, the reference standard may be phosphate buffered saline without recombinant viral particles.

[0104] In some embodiments of the invention, the preparation of viral particles is in a pharmaceutical formulation. Such formulations are well known in the art (see, *e.g.*, Remington's Pharmaceutical Sciences, 15th Edition, pp. 1035-1038 and 1570-1580). Such pharmaceutical formulations can be sterile liquids, such as water and oil, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and the like. Saline solutions and aqueous dextrose, polyethylene glycol (PEG) and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. The pharmaceutical formulation may further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like. In some embodiments of the invention the pharmaceutical formulation comprises phosphate buffered saline.

[0105] In some embodiments of the invention, the sedimentation velocity of viral particles during ultracentrifugation is determined by monitoring the sedimentation of viral particles continuously during ultracentrifugation. It is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles. Without wishing to be bound to theory, a range of AUC settings that allows the analysis of both AAV and adenovirus particles should enable the analysis of other viral particles including lentivirus and HSV since the size of HSV and lentiviral particles is between that of AAV and adenovirus particles. In some embodiments, data acquisition for rAAV, rHSV, lentiviral, and/or rAd particles is performed with an AUC speed of between about 3,000 and about 20,000 rpm. In some embodiments, data analysis for rAAV, HSV, lentiviral, and/or adenoviral particles is performed with an S_{min} of about 1S and an S_{max} of about 1000S. In some embodiments, data analysis for rAAV, rHSV, lentiviral, and/or rAd particles is performed with a resolution of about 200S to about 1,000S. In some embodiments, the resolution is about any of 200S, 300S, 400S, 500S, 600S, 700S, 800S, 900S, or 1000S. In some embodiments, the resolution is between any of about 200S to about 1000S, 200S to about 900S, 200S to about 800S, 200S to about 700S, 200S to about 600S, 200S to about 500S,

200S to about 400S, 200S to about 300S, 300S to about 1000S, 300S to about 900S, 300S to about 800S, 300S to about 700S, 300S to about 600S, 300S to about 500S, 300S to about 400S, 400S to about 1000S, 400S to about 900S, 400S to about 800S, 400S to about 700S, 400S to about 600S, 400S to about 500S, 500S to about 1000S, 500S to about 900S, 500S to about 800S, 500S to about 700S, 500S to about 600S, 600S to about 1000S, 600S to about 900S, 600S to about 800S, 600S to about 700S, 700S to about 1000S, 700S to about 900S, 700S to about 800S, 800S to about 1000S, 800S to about 900S, or 900S to about 1000S. In some embodiments, the resolution is about 200S. data analysis for rAAV, rHSV, lentiviral, and/or rAd particles is performed with an Smax of about any of 100S, 200S, 300S, 400S, 500S, 600S, 700S, 800S, 900S, or 1000S. In some embodiments, the Smax is between any of about 100S to about 1000S, 100S to about 900S, 100S to about 800S, 100S to about 700S, 100S to about 600S, 100S to about 500S, 100S to about 400S, 100S to about 300S, 100S to about 200S, 200S to about 1000S, 200S to about 900S, 200S to about 800S, 200S to about 700S, 200S to about 600S, 200S to about 500S, 200S to about 400S, 200S to about 300S, 300S to about 1000S, 300S to about 900S, 300S to about 800S, 300S to about 700S, 300S to about 600S, 300S to about 500S, 300S to about 400S, 400S to about 1000S, 400S to about 900S, 400S to about 800S, 400S to about 700S, 400S to about 600S, 400S to about 500S, 500S to about 1000S, 500S to about 900S, 500S to about 800S, 500S to about 700S, 500S to about 600S, 600S to about 1000S, 600S to about 900S, 600S to about 800S, 600S to about 700S, 700S to about 1000S, 700S to about 900S, 700S to about 800S, 800S to about 1000S, 800S to about 900S, or 900S to about 1000S. In some embodiments, Smax is about 200S to about 5000S. In some embodiments, wherein Smax is about 200S. In some embodiments, radial invariant (RI) and time invariant (TI) noise subtractions are applied. In some embodiments, the meniscus position is allowed to float, letting the software choose the optimal position. In some embodiments, the frictional ratio is allowed to float, letting the software choose the optimal position. In some embodiments, data analysis for rAAV and/or adenoviral particles is held constant at 1. In some embodiments, data analysis for rAAV, HSV, lentiviral, and/or adenoviral particles is allowed to float by using the FIT command with a value optimized using non-linear regression.

[0106] With respect to recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles), in some embodiments, the sedimentation velocity of recombinant viral during ultracentrifugation is determined by monitoring (*e.g.*, scanning) the sedimentation of recombinant viral particles once in more than about every 15 seconds, 30 seconds, 45 seconds, 1 minute (60

seconds), 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes. Scans may be continuously acquired without delay as quickly as the optical systems allow. Interference scans are rapid, and a single scan is complete in ~10-15 seconds, while absorbance scans require ~ 60 seconds. When dual detection is used the speed of scan acquisition for both are determined by the absorbance system. In some embodiments of the invention, more than about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 scans are used to monitor sedimentation of recombinant viral particles during ultracentrifugation. In some embodiments, a minimum of 30 scans is required for analysis, and scans are collected until the sedimentation process is complete. In some embodiments, the sedimentation process may typically be described by between 40 and 75 scans. In some embodiments, the sedimentation velocity of recombinant viral particles is determined based on about 75 scans. In some embodiments, the sedimentation velocity of recombinant viral particles is determined based on about 55 scans to about 75 scans. In some embodiments, the sedimentation velocity of recombinant viral particles is determined based on about 55 scans to about 60 scans. In some embodiments, the sedimentation velocity of recombinant viral particles is determined based on about 60 scans to about 75 scans. In some embodiments, the sedimentation velocity of recombinant viral particles is determined based on about 60 scans to about 70 scans. In some embodiments, the sedimentation velocity of recombinant viral particles is determined based on multiple ultracentrifugations (runs). In some embodiments, the sedimentation velocity of recombinant viral particles is determined based on any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more ultracentrifugation runs. In some embodiments, the sedimentation velocities are used to determine C(S) values using the SEDFIT algorithm. In some embodiments, a second derivative regularization is applied to a fitting level with a confidence level of F statistic of about 0.68. In some embodiments, the following C(S) parameters are held constant: resolution 100S to about 200S, S min is about 1, S max is about 200S to 300S, and frictional ratio is about 1.0 to 1.2S. In some embodiments, radial invariant (RI) and time invariant (TI) noise subtractions are applied.

[0107] In some embodiments of the invention, the boundary sedimentation velocity of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) in a preparation of recombinant viral particles by ultracentrifuging the preparation of recombinant viral particles at more than about any of 5,000 rpm; 10,000 rpm; 15,000 rpm; 20,000 rpm; 25,000 rpm; 30,000 rpm; 35,000 rpm; 40,000 rpm; 45,000 rpm; or 50,000 rpm. In some embodiments, the

ultracentrifugation is run at between any of about 5,000 rpm and about 50,000 rpm; about 10,000 rpm and about 50,000 rpm; about 15,000 rpm and about 50,000 rpm; about 20,000 rpm and about 50,000 rpm; about 25,000 rpm and about 50,000 rpm; about 30,000 rpm and about 50,000 rpm; about 35,000 rpm and about 50,000 rpm; about 40,000 rpm and about 50,000 rpm; about 45,000 rpm and about 50,000 rpm; about 5,000 rpm and about 45,000 rpm; about 10,000 rpm and about 45,000 rpm; about 15,000 rpm and about 45,000 rpm; about 20,000 rpm and about 45,000 rpm; about 25,000 rpm and about 45,000 rpm; about 30,000 rpm and about 45,000 rpm; about 40,000 rpm and about 45,000 rpm; about 5,000 rpm and about 40,000 rpm; about 10,000 rpm and about 40,000 rpm; about 15,000 rpm and about 40,000 rpm; about 20,000 rpm and about 40,000 rpm; about 25,000 rpm and about 40,000 rpm; about 30,000 rpm and about 40,000 rpm; about 35,000 rpm and about 40,000 rpm; about 5,000 rpm and about 35,000 rpm; about 10,000 rpm and about 35,000 rpm; about 15,000 rpm and about 35,000 rpm; about 20,000 rpm and about 35,000 rpm; about 25,000 rpm and about 35,000 rpm; about 30,000 rpm and about 35,000 rpm; about 5,000 rpm and about 30,000 rpm; about 10,000 rpm and about 30,000 rpm; about 15,000 rpm and about 30,000 rpm; about 20,000 rpm and about 30,000 rpm; about 25,000 rpm and about 30,000 rpm; about 5,000 rpm and about 25,000 rpm; about 10,000 rpm and about 25,000 rpm; about 20,000 rpm and about 25,000 rpm; about 5,000 rpm and about 20,000 rpm; about 10,000 rpm and about 20,000 rpm; about 15,000 rpm and about 20,000 rpm; about 5,000 rpm and about 15,000 rpm; about 10,000 rpm and about 15,000 rpm; or about 5,000 rpm and about 10,000 rpm. In some embodiments of the invention, the boundary sedimentation velocity of recombinant viral particles in a preparation of recombinant viral particles by ultracentrifuging the preparation of recombinant viral particles at about 20,000 rpm. In some embodiments of the invention, the boundary sedimentation velocity of recombinant viral particles in a preparation of recombinant viral particles by ultracentrifuging the preparation of recombinant viral particles at about 15,000 rpm to about 20,000 rpm.

[0108] In some embodiments of the invention, the boundary sedimentation velocity of recombinant viral particles in a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) by ultracentrifuging the preparation of recombinant viral particles at about or more than 4 °C, 10 °C, 15 °C, 20 °C, 25 °C, or 30 °C. In some embodiments, the ultracentrifugation is run as between any of about 4 °C and about 30 °C, about 4 °C and about 25 °C, about 4 °C and about 20 °C, about 4 °C and about 15 °C, about 4 °C and about 10 °C, about

10 °C and about 30 °C, about 10 °C and about 25 °C, about 10 °C and about 20 °C, about 10 °C and about 15 °C, about 15 °C and about 30 °C, about 15 °C and about 25 °C, about 15 °C and about 20 °C, about 20 °C and about 30 °C, or about 20 °C and about 25 °C. In some embodiments, the boundary sedimentation velocity of recombinant viral particles in a preparation of recombinant viral particles by ultracentrifuging the preparation of recombinant viral particles at about 20 °C. In some embodiments, the boundary sedimentation velocity of recombinant viral particles in a preparation of recombinant viral particles by ultracentrifuging the preparation of recombinant viral particles at about 15 °C to about 20 °C.

[0109] As disclosed herein, numerous types of recombinant viral particles may be analyzed by the methods of the present disclosure (*e.g.,* AAV, adenoviral, lentiviral, and/or HSV particles). Suitable ultracentrifugation conditions, analysis algorithms, and other parameters may be determined empirically through methods known in the art. Exemplary parameters for AAV, adenoviral, lentiviral, and HSV particles, along with guidance for the selection of specific parameter options, are provided without limitation in Table 1 below.

Table 1. Exemplary parameters for AAV, adenoviral, lentiviral, and HSV particles.

	AAV	**Ad**	**Lentivirus**	**HSV**
Exemplary buffers	Phosphate based buffer at physiologic pH, and physiologic osmolality ~ 300 mOsM/L	Phosphate based buffer at physiologic pH, and physiologic osmolality ~ 300 mOsM/L L	Phosphate based buffer at physiologic pH, and physiologic osmolality ~ 300 mOsM/L	Phosphate based buffer at physiologic pH, and physiologic osmolality ~ 300 mOsM/L
Exemplary algorithms for determining C(S)	Any algorithm using Lamm equation solutions; e.g., SEDFIT C(S)	Any algorithm using Lamm equation solutions; e.g., SEDFIT C(S)	Any algorithm using Lamm equation solutions; e.g., SEDFIT C(S)	Any algorithm using Lamm equation solutions; e.g., SEDFIT C(S)
Exemplary number of scans (minimum, maximum, ranges)	30-999 *excess scans can always be collected and then excluded from analysis (such as skip every other scan) -scans that occur after complete sedimentation of virus can be	30-999	30-999	30-999

	excluded			
Exemplary confidence level of the F statistic	F=0.68	F=0.68	F=0.68	F=0.68
Exemplary ranges for Smin	1-100S	1-100S	1-100S	1-100S
Exemplary ranges for Smax	100-1000S	100-5000S	100-5000S	100-5000S
Exemplary ranges for resolution	*Resolution depends on S Max 200S-1000S	200-5000S	200-5000S	200-5000S
Exemplary frictional ratio	Use the FIT command to determine frictional ratio. Since AAV is ~spherical, in embodiments, 1 may be used as the frictional ratio.	Use FIT command to determine frictional ratio or set at 1.	Use FIT command to determine frictional ratio	Use FIT command to determine frictional ratio
Exemplary ranges for AUC speed	10,000-20,000 rpm	3,000-10,000 rpm	3,000-10,000 rpm	3,000-10,000 rpm
Exemplary absorbances for monitoring sedimentation of viral particles	260nm 280 nm 230 nm	260nm 280 nm 230 nm	260nm 280 nm 230 nm	260nm 280 nm 230 nm
Exemplary methods for monitoring sedimentation of viral particles	IF Absorbance	IF Absorbance	IF Absorbance	IF Absorbance
Radial invariant (RI) and time invariant (TI) noise subtractions, alternative subtractions/calculations	Ti and RI noise correction required for interference detection. May or may not be used with absorbance detection	Ti and RI noise correction required for interference detection. May or may not be used with absorbance detection	Ti and RI noise correction required for interference detection. May or may not be used with absorbance detection	Ti and RI noise correction required for interference detection. May or may not be used with absorbance detection
Scanning frequency	*when using absorbance detection system, limited by speed of absorbance scan (~60 Seconds)-scan as fast as system	Scan with no delay through scan with 60 second delay	Scan with no delay through scan with 60 second delay	Scan with no delay through scan with 60 second delay

	allows with no delay IF only: collect every 10 -60 seconds (10-60 second delay)			
Temperature ranges	4°C -20°C	4°C -20°C	4°C -20°C	4°C -20°C

[0110] In some aspects, the invention provides methods to determine the presence of empty capsids in a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times), b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of peak that corresponds to the S value of empty capsid particles indicates that presence of empty capsid particles. In some embodiments, the invention provides methods of measuring the relative amount empty capsids in a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times), b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, d) comparing the amount of recombinant viral particles having an S value corresponding to empty capsid particles to the amount of recombinant viral particles having an S value corresponding to recombinant viral particles comprising intact viral genomes. In some embodiments, the amount of recombinant viral particles having an S value corresponding to empty capsid particles is compared to the total amount of all recombinant viral particles in the preparation by integrating all peaks on the plot of C(S) vs. S.

[0111] In some aspects, the invention provides methods to determine the presence of recombinant viral particle variants in a preparation of recombinant viral particles (*e.g.*, rAAV,

rAd, lentiviral, or rHSV particles) comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times), b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of peak that corresponds to the S value that differs from the S value of recombinant viral capsid particles comprising a full intact recombinant viral genome indicates that presence of recombinant viral particle variants. In some embodiments, the invention provides methods of measuring the relative amount recombinant viral particle variants in a preparation of recombinant viral particles comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times), b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, d) comparing the amount of recombinant viral particles having an S value corresponding to empty capsid particles to the amount of recombinant viral particles having an S value corresponding to recombinant viral particles comprising intact viral genomes. In some embodiments, the amount of recombinant viral particles having an S value that differs from the S value of recombinant viral capsid particles comprising a full intact recombinant viral genome is compared to the total amount of all recombinant viral particles in the preparation by integrating all peaks on the plot of C(S) vs. S. In some embodiments, the recombinant viral particle variants comprise recombinant viral genomes that are smaller (*e.g.*, truncated) or larger than the full length intact viral genome. Other viral-encapsidated DNA impurities can also be detected.

[0112] In some embodiments, the invention provides methods of monitoring the removal of empty capsids and/or recombinant viral particles with variant genomes during the purification of a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and analyzing the sample for the relative amount of empty capsids using AUC as described herein. A decrease in the relative amount of

empty capsids and/or recombinant viral particles comprising variant genomes to full capsids indicates removal of empty capsids from the preparation of recombinant viral particles.

[0113] In some embodiments, the invention provides methods of determining the heterogeneity of recombinant viral particles in a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) comprising the steps of a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals (*e.g.*, one or more times), b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of peaks in addition to the peak representing capsids comprising an intact viral genome indicates heterogeneity of recombinant viral particles in the preparation. In some embodiments, the species of recombinant viral particle identified by the methods of the invention include, but are not limited to full recombinant viral particles comprising intact recombinant viral genomes, empty recombinant viral capsid particles, and recombinant viral particles comprising variant recombinant viral genomes. In some embodiments the variant genomes are smaller than the intact recombinant viral genome (*e.g.*, truncated genomes). In some embodiments, the variant genomes are larger than the intact recombinant viral genome (*e.g.*, aggregates, recombinants, *etc.*). In some embodiments the variant genomes include genomes that are smaller and larger than the intact recombinant viral genome.

[0114] In some embodiments, the invention provides methods of monitoring the heterogeneity of recombinant viral particles during the purification of a preparation of recombinant viral particles (*e.g.*, rAAV, rAd, lentiviral, or rHSV particles) the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and determining the relative amount of full capsids comprising an intact recombinant viral genome, empty capsids and/or recombinant viral particles with variant genomes using AUC as described herein, wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes indicates an increase in the homogeneity of full viral particles in the preparation of recombinant viral particles.

[0115] In embodiments of the embodiments described above, the recombinant viral particles have been purified using one or more purification steps. Examples of purification steps include but are not limited to equilibrium centrifugation, anion exchange filtration, tangential flow

filtration (TFF), apatite chromatography, heat inactivation of helper virus, hydrophobic interaction chromatography, immunoaffinity chromatography, size exclusion chromatography (SEC), nanofiltration, cation exchange chromatography, and anion exchange chromatography.

[0116] In embodiments of the embodiments described above, the recombinant viral particles comprise a self-complementary AAV (scAAV) genome. In some embodiments, the recombinant AAV genome comprises a first heterologous polynucleotide sequence (*e.g.*, a therapeutic transgene coding strand) and a second heterologous polynucleotide sequence (*e.g.*, the noncoding or antisense strand of the therapeutic transgene) wherein the first heterologous polynucleotide sequence can form intrastrand base pairs with the second polynucleotide sequence along most or all of its length. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a sequence that facilitates intrastrand basepairing; *e.g.*, a hairpin DNA structure. Hairpin structures are known in the art, for example in siRNA molecules. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a mutated ITR. In some embodiments, the scAAV viral particles comprise a monomeric form of an scAAV genome. In some embodiments, the scAAV viral particles comprise the dimeric form of and scAAV genome. In some embodiments, AUC as described herein is used to detect the presence of rAAV particles comprising the monomeric form of an scAAV genome. In some embodiments, AUC as described herein is used to detect the presence of rAAV particles comprising the dimeric form of an scAAV genome. In some embodiments, the packaging of scAAV genomes into capsid is monitored by AUC described herein.

[0117] In embodiments of the embodiments described above, the rAAV particles comprise an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid (*e.g.*, a wild-type AAV6 capsid, or a variant AAV6 capsid such as ShH10, as described in U.S. PG Pub. 2012/0164106), an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAVrh8R, an AAV9 capsid (*e.g.*, a wild-type AAV9 capsid, or a modified AAV9 capsid as described in U.S. PG Pub. 2013/0323226), an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, a tyrosine capsid mutant, a heparin binding capsid mutant, an AAV2R471A capsid, an AAVAAV2/2-7m8 capsid, an AAV DJ capsid (*e.g.*, an AAV-DJ/8 capsid, an AAV-DJ/9 capsid, or any other of the capsids described in U.S. PG Pub. 2012/0066783), an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an

AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, a mouse AAV capsid, or an AAV capsid described in U.S. Pat. No. 8,283,151 or International Publication No. WO/2003/042397. In embodiments of the above embodiments described above, the rAAV particles comprise at least one AAV1 ITR, AAV2 ITR, AAV3 ITR, AAV4 ITR, AAV5 ITR, AAV6 ITR, AAV7 ITR, AAV8 ITR, AAVrh8 ITR, AAV9 ITR, AAV10 ITR, AAVrh10 ITR, AAV11 ITR, AAV12 ITR, AAV DJ ITR, goat AAV ITR, bovine AAV ITR, or mouse AAV ITR. In some embodiments, the rAAV particles comprise ITRs from one AAV serotype and AAV capsid from another serotype. For example, the rAAV particles may comprise a therapeutic transgene flanked by at least one AAV2 ITR encapsidated into an AAV9 capsid. Such combinations may be referred to as pseudotyped rAAV particles.

IV. Viral particles

[0118] The methods disclosed herein may find use, *inter alia,* in characterizing species of interest in a variety of viral particles (*e.g.,* viral particles with a full genome, as compared to viral particles with truncated genomes and/or viral particles comprising DNA impurities).

[0119] In some embodiments, the viral particle is a recombinant AAV particle comprising a nucleic acid comprising a transgene flanked by one or two ITRs. The nucleic acid is encapsidated in the AAV particle. The AAV particle also comprises capsid proteins. In some embodiments, the nucleic acid comprises the protein coding sequence(s) of interest (*e.g.*, a therapeutic transgene) operatively linked components in the direction of transcription, control sequences including transcription initiation and termination sequences, thereby forming an expression cassette. The expression cassette is flanked on the 5' and 3' end by at least one functional AAV ITR sequences. By "functional AAV ITR sequences" it is meant that the ITR sequences function as intended for the rescue, replication and packaging of the AAV virion. See Davidson *et al.*, *PNAS*, 2000, 97(7)3428-32; Passini *et al.*, *J. Virol.*, 2003, 77(12):7034-40; and Pechan *et al.*, *Gene Ther.*, 2009, 16:10-16, all of which are incorporated herein in their entirety by reference. For practicing some aspects of the invention, the recombinant vectors comprise at least all of the sequences of AAV essential for encapsidation and the physical structures for infection by the rAAV. AAV ITRs for use in the vectors of the invention need not have a wild-type nucleotide sequence (*e.g.*, as described in Kotin, *Hum. Gene Ther.*, 1994, 5:793-801), and may be altered by the insertion, deletion or substitution of nucleotides or the AAV ITRs may be derived from any of several AAV

serotypes. More than 40 serotypes of AAV are currently known, and new serotypes and variants of existing serotypes continue to be identified. See Gao *et al.*, *PNAS*, 2002, 99(18): 11854-6; Gao *et al.*, *PNAS*, 2003, 100(10):6081-6; and Bossis *et al.*, *J. Virol.*, 2003, 77(12):6799-810. Use of any AAV serotype is considered within the scope of the present invention. In some embodiments, a rAAV vector is a vector derived from an AAV serotype, including without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh.10, AAV11, AAV12, a tyrosine capsid mutant, a heparin binding capsid mutant, an AAV2R471A capsid, an AAVAAV2/2-7m8 capsid, an AAV DJ capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, or a mouse AAV capsid, or the like. In some embodiments, the nucleic acid in the AAV comprises an ITR of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, AAV12 or the like. In further embodiments, the rAAV particle comprises capsid proteins of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh.10, AAV11, AAV12 or the like. In further embodiments, the rAAV particle comprises capsid proteins of an AAV serotype from Clades A-F (Gao, *et al. J. Virol.* 2004, 78(12):6381).

[0120] Different AAV serotypes are used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). A rAAV particle can comprise viral proteins and viral nucleic acids of the same serotype or a mixed serotype. For example, a rAAV particle can comprise AAV9 capsid proteins and at least one AAV2 ITR or it can comprise AAV2 capsid proteins and at least one AAV9 ITR. In yet another example, a rAAV particle can comprise capsid proteins from both AAV9 and AAV2, and further comprise at least one AAV2 ITR. Any combination of AAV serotypes for production of a rAAV particle is provided herein as if each combination had been expressly stated herein.

[0121] In some embodiments, the AAV comprises at least one AAV1 ITR and capsid protein from any of AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV2 ITR and capsid protein from any of AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV3 ITR and capsid protein from any of AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV

comprises at least one AAV4 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV5 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV6, AAV7, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV6 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV7, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV7 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV8, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV8 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV9, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV9 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh.8, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAVrh8 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV8, AAV9, AAVrh10, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAVrh10 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV11, and/or AAV12. In some embodiments, the AAV comprises at least one AAV11 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAVrh10, and/or AAV12. In some embodiments, the AAV comprises at least one AAV12 ITR and capsid protein from any of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV rh8, AAV9, AAVrh10, and/or AAV11.

Self-complementary AAV viral genomes

[0122] In some aspects, the invention provides viral particles comprising a recombinant self-complementing genome. AAV viral particles with self-complementing genomes and methods of use of self-complementing AAV genomes are described in US Patent Nos. 6,596,535; 7,125,717; 7,765,583; 7,785,888; 7,790,154; 7,846,729; 8,093,054; and 8,361,457; and Wang Z., *et al.*, (2003) *Gene Ther* 10:2105-2111, each of which are incorporated herein by reference in its entirety. A rAAV comprising a self-complementing genome will quickly form a double stranded DNA molecule by virtue of its partially complementing sequences (*e.g.*, complementing coding and non-coding strands of a transgene). In some embodiments, the invention provides an AAV

viral particle comprising an AAV genome, wherein the rAAV genome comprises a first heterologous polynucleotide sequence (*e.g.*, a therapeutic transgene coding strand) and a second heterologous polynucleotide sequence (*e.g.*, the noncoding or antisense strand of the therapeutic transgene) wherein the first heterologous polynucleotide sequence can form intrastrand base pairs with the second polynucleotide sequence along most or all of its length. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a sequence that facilitates intrastrand basepairing; *e.g.*, a hairpin DNA structure. Hairpin structures are known in the art, for example in siRNA molecules. In some embodiments, the first heterologous polynucleotide sequence and a second heterologous polynucleotide sequence are linked by a mutated ITR (*e.g.*, the right ITR). The mutated ITR comprises a deletion of the D region comprising the terminal resolution sequence. As a result, on replicating an AAV viral genome, the rep proteins will not cleave the viral genome at the mutated ITR and as such, a recombinant viral genome comprising the following in 5' to 3' order will be packaged in a viral capsid: an AAV ITR, the first heterologous polynucleotide sequence including regulatory sequences, the mutated AAV ITR, the second heterologous polynucleotide in reverse orientation to the first heterologous polynucleotide and a third AAV ITR.

[0123] In some embodiments, the viral particle is an adenoviral particle. In some embodiments, the adenoviral particle is a recombinant adenoviral particle, *e.g.,* a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of adenoviral origin) between two ITRs. In some embodiments, the adenoviral particle lacks or contains a defective copy of one or more E1 genes, which renders the adenovirus replication-defective. Adenoviruses include a linear, double-stranded DNA genome within a large (~950Å), non-enveloped icosahedral capsid. Adenoviruses have a large genome that can incorporate more than 30kb of heterologous sequence (*e.g.,* in place of the E1 and/or E3 region), making them uniquely suited for use with larger heterologous genes. They are also known to infect dividing and non-dividing cells and do not naturally integrate into the host genome (although hybrid variants may possess this ability). In some embodiments, the adenoviral vector may be a first generation adenoviral vector with a heterologous sequence in place of E1. In some embodiments, the adenoviral vector may be a second generation adenoviral vector with additional mutations or deletions in E2A, E2B, and/or E4. In some embodiments, the adenoviral vector may be a third generation or gutted adenoviral vector that lacks all viral coding genes, retaining only the ITRs

and packaging signal and requiring a helper adenovirus *in trans* for replication, and packaging. Adenoviral particles have been investigated for use as vectors for transient transfection of mammalian cells as well as gene therapy vectors. For further description, see, *e.g.,* Danthinne, X. and Imperiale, M.J. (2000) *Gene Ther.* 7:1707-14 and Tatsis, N. and Ertl, H.C. (2004) *Mol. Ther.* 10:616-29.

[0124] In some embodiments, the viral particle is a recombinant adenoviral particle comprising a nucleic acid comprising a transgene. Use of any adenovirus serotype is considered within the scope of the present invention. In some embodiments, the recombinant adenoviral vector is a vector derived from an adenovirus serotype, including without limitation, AdHu2, AdHu 3, AdHu4, AdHu5, AdHu7, AdHu11, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, and porcine Ad type 3. The adenoviral particle also comprises capsid proteins. In some embodiments, the recombinant viral particles comprise an adenoviral particle in combination with one or more foreign viral capsid proteins. Such combinations may be referred to as pseudotyped recombinant adenoviral particles. In some embodiments, foreign viral capsid proteins used in pseudotyped recombinant adenoviral particles are derived from a foreign virus or from another adenovirus serotype. In some embodiments, the foreign viral capsid proteins are derived from, including without limitation, reovirus type 3. Examples of vector and capsid protein combinations used in pseudotyped adenovirus particles can be found in the following references (Tatsis, N. *et al.* (2004) *Mol. Ther.* 10(4):616-629 and Ahi, Y. *et al.* (2011) *Curr. Gene Ther.* 11(4):307-320). Different adenovirus serotypes can be used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). Tissues or cells targeted by specific adenovirus serotypes, include without limitation, lung (*e.g.* HuAd3), spleen and liver (*e.g.* HuAd37), smooth muscle, synoviocytes, dendritic cells, cardiovascular cells, tumor cell lines (*e.g.* HuAd11), and dendritic cells (*e.g.* HuAd5 pseudotyped with *reovirus type 3, HuAd30, or HuAd35*). For further description, see Ahi, Y. *et al.* (2011) *Curr. Gene Ther.* 11(4):307-320, Kay, M. *et al.* (2001) *Nat. Med.* 7(1):33-40, and Tatsis, N. *et al.* (2004) *Mol. Ther.* 10(4):616-629.

[0125] In some embodiments, the viral particle is a lentiviral particle. In some embodiments, the lentiviral particle is a recombinant lentiviral particle, *e.g.,* a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of lentiviral origin) between

two LTRs. Lentiviruses are positive-sense, ssRNA retroviruses with a genome of approximately 10 kb. Lentiviruses are known to integrate into the genome of dividing and non-dividing cells. Lentiviral particles may be produced, for example, by transfecting multiple plasmids (typically the lentiviral genome and the genes required for replication and/or packaging are separated to prevent viral replication) into a packaging cell line, which packages the modified lentiviral genome into lentiviral particles. In some embodiments, a lentiviral particle may refer to a first generation vector that lacks the envelope protein. In some embodiments, a lentiviral particle may refer to a second generation vector that lacks all genes except the gag/pol and tat/rev regions. In some embodiments, a lentiviral particle may refer to a third generation vector that only contains the endogenous rev, gag, and pol genes and has a chimeric LTR for transduction without the tat gene (see Dull, T. *et al.* (1998) *J. Virol.* 72:8463-71). For further description, see Durand, S. and Cimarelli, A. (2011) *Viruses* 3:132-59.

[0126] In some embodiments, the viral particle is a recombinant lentiviral particle comprising a nucleic acid comprising a transgene. Use of any lentiviral vector is considered within the scope of the present invention. In some embodiments, the lentiviral vector is derived from a lentivirus including, without limitation, human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), equine infectious anemia virus (EIAV), bovine immunodeficiency virus (BIV), Jembrana disease virus (JDV), visna virus (VV), and caprine arthritis encephalitis virus (CAEV). The lentiviral particle also comprises capsid proteins. In some embodiments, the recombinant viral particles comprise a lentivirus vector in combination with one or more foreign viral capsid proteins. Such combinations may be referred to as pseudotyped recombinant lentiviral particles. In some embodiments, foreign viral capsid proteins used in pseudotyped recombinant lentiviral particles are derived from a foreign virus. In some embodiments, the foreign viral capsid protein used in pseudotyped recombinant lentiviral particles is Vesicular stomatitis virus glycoprotein (VSV-GP). VSV-GP interacts with a ubiquitous cell receptor, providing broad tissue tropism to pseudotyped recombinant lentiviral particles. In addition, VSV-GP is thought to provide higher stability to pseudotyped recombinant lentiviral particles. In other embodiments, the foreign viral capsid proteins are derived from, including without limitation, Chandipura virus, Rabies virus, Mokola virus, Lymphocytic choriomeningitis virus (LCMV), Ross River virus (RRV), Sindbis virus, Semliki Forest virus (SFV), Venezuelan equine

encephalitis virus, Ebola virus Reston, Ebola virus Zaire, Marburg virus, Lassa virus, Avian leukosis virus (ALV), Jaagsiekte sheep retrovirus (JSRV), Moloney Murine leukemia virus (MLV), Gibbon ape leukemia virus (GALV), Feline endogenous retrovirus (RD114), Human T-lymphotropic virus 1 (HTLV-1), Human foamy virus, Maedi-visna virus (MVV), SARS-CoV, Sendai virus, Respiratory syncytia virus (RSV), Human parainfluenza virus type 3, Hepatitis C virus (HCV), Influenza virus, Fowl plague virus (FPV), or Autographa californica multiple nucleopolyhedro virus (AcMNPV). Examples of vector and capsid protein combinations used in pseudotyped Lentivirus particles can be found, for example, in Cronin, J. *et al.* (2005). *Curr. Gene Ther.* 5(4):387-398. Different pseudotyped recombinant lentiviral particles can be used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). For example, tissues targeted by specific pseudotyped recombinant lentiviral particles, include without limitation, liver (*e.g.* pseudotyped with a VSV-G, LCMV, RRV, or SeV F protein), lung (*e.g.* pseudotyped with an Ebola, Marburg, SeV F and HN, or JSRV protein), pancreatic islet cells (*e.g.* pseudotyped with an LCMV protein), central nervous system (*e.g.* pseudotyped with a VSV-G, LCMV, Rabies, or Mokola protein), retina (*e.g.* pseudotyped with a VSV-G or Mokola protein), monocytes or muscle (*e.g.* pseudotyped with a Mokola or Ebola protein), hematopoietic system (*e.g.* pseudotyped with an RD114 or GALV protein), or cancer cells (*e.g.* pseudotyped with a GALV or LCMV protein). For further description, see Cronin, J. *et al.* (2005). *Curr. Gene Ther.* 5(4):387-398 and Kay, M. *et al.* (2001) *Nat. Med.* 7(1):33-40.

[0127] In some embodiments, the viral particle is a herpes simplex virus (HSV) particle. In some embodiments, the HSV particle is a rHSV particle, *e.g.,* a polynucleotide vector comprising one or more heterologous sequences (*i.e.*, nucleic acid sequence not of lentiviral origin) between two TRs. HSV is an enveloped, double-stranded DNA virus with a genome of approximately 152 kb. Advantageously, approximately half of its genes are nonessential and may be deleted to accommodate heterologous sequence. HSV particles infect non-dividing cells. In addition, they naturally establish latency in neurons, travel by retrograde transport, and can be transferred across synapses, making them advantageous for transfection of neurons and/or gene therapy approaches involving the nervous system. In some embodiments, the HSV particle may be replication-defective or replication-competent (*e.g.,* competent for a single replication cycle through

inactivation of one or more late genes). For further description, see Manservigi, R. *et al.* (2010) *Open Virol. J.* 4:123-56.

[0128] In some embodiments, the viral particle is a rHSV particle comprising a nucleic acid comprising a transgene. Use of any HSV vector is considered within the scope of the present invention. In some embodiments, the HSV vector is derived from a HSV serotype, including without limitation, HSV-1 and HSV-2. The HSV particle also comprises capsid proteins. In some embodiments, the recombinant viral particles comprise a HSV vector in combination with one or more foreign viral capsid proteins. Such combinations may be referred to as pseudotyped rHSV particles. In some embodiments, foreign viral capsid proteins used in pseudotyped rHSV particles are derived from a foreign virus or from another HSV serotype. In some embodiments, the foreign viral capsid protein used in a pseudotyped rHSV particle is a Vesicular stomatitis virus glycoprotein (VSV-GP). VSV-GP interacts with a ubiquitous cell receptor, providing broad tissue tropism to pseudotyped rHSV particles. In addition, VSV-GP is thought to provide higher stability to pseudotyped rHSV particles. In other embodiments, the foreign viral capsid protein may be from a different HSV serotype. For example, an HSV-1 vector may contain one or more HSV-2 capsid proteins. Different HSV serotypes can be used to optimize transduction of particular target cells or to target specific cell types within a particular target tissue (*e.g.*, a diseased tissue). Tissues or cells targeted by specific adenovirus serotypes include without limitation, central nervous system and neurons (*e.g.* HSV-1). For further description, see Manservigi, R. *et al.* (2010) *Open Virol J* 4:123-156, Kay, M. *et al.* (2001) *Nat. Med.* 7(1):33-40, and Meignier, B. *et al.* (1987) *J. Infect. Dis.* 155(5):921-930.

V. Production of Viral Vectors

[0129] Numerous methods are known in the art for production of rAAV vectors, including transfection, stable cell line production, and infectious hybrid virus production systems which include adenovirus-AAV hybrids, herpesvirus-AAV hybrids (Conway, JE et al., (1997) *J. Virology* 71(11):8780-8789) and baculovirus-AAV hybrids. rAAV production cultures for the production of rAAV virus particles all require; 1) suitable host cells, including, for example, human-derived cell lines such as HeLa, A549, or 293 cells, or insect-derived cell lines such as SF-9, in the case of baculovirus production systems; 2) suitable helper virus function, provided by wild-type or mutant adenovirus (such as temperature sensitive adenovirus), herpes virus,

baculovirus, or a plasmid construct providing helper functions; 3) AAV rep and cap genes and gene products; 4) a transgene (such as a therapeutic transgene) flanked by at least one AAV ITR sequences ; and 5) suitable media and media components to support rAAV production. In some embodiments, the AAV rep and cap gene products may be from any AAV serotype. In general, but not obligatory, the AAV rep gene product is of the same serotype as the ITRs of the rAAV vector genome as long as the rep gene products may function to replicated and package the rAAV genome. Suitable media known in the art may be used for the production of rAAV vectors. These media include, without limitation, media produced by Hyclone Laboratories and JRH including Modified Eagle Medium (MEM), Dulbecco's Modified Eagle Medium (DMEM), custom formulations such as those described in U.S. Patent No. 6,566,118, and Sf-900 II SFM media as described in U.S. Patent No. 6,723,551, each of which is incorporated herein by reference in its entirety, particularly with respect to custom media formulations for use in production of recombinant AAV vectors. In some embodiments, the AAV helper functions are provided by adenovirus or HSV. In some embodiments, the AAV helper functions are provided by baculovirus and the host cell is an insect cell (e.g., Spodoptera frugiperda (Sf9) cells).

[0130] Suitable rAAV production culture media of the present invention may be supplemented with serum or serum-derived recombinant proteins at a level of 0.5%-20% (v/v or w/v). Alternatively, as is known in the art, rAAV vectors may be produced in serum-free conditions which may also be referred to as media with no animal-derived products. One of ordinary skill in the art may appreciate that commercial or custom media designed to support production of rAAV vectors may also be supplemented with one or more cell culture components know in the art, including without limitation glucose, vitamins, amino acids, and or growth factors, in order to increase the titer of rAAV in production cultures.

[0131] In some aspects, the invention provides methods for preparing rAAV particles with reduced empty capsids comprising a) culturing host cells under conditions suitable for rAAV production, wherein the cells comprise i) nucleic acid encoding a heterologous transgene flanked by at least one AAV ITR, ii) nucleic acid comprising AAV rep and cap coding regions, wherein the nucleic acid comprises a mutated p5 promoter wherein expression from the p5 promoter is reduced compared to a wild-type p5 promoter, and iii) nucleic acid encoding AAV helper virus functions; b) lysing the host cells to release rAAV particles; c) isolating the rAAV particles produced by the host cell; and d) analyzing the rAAV particles for the presence of empty capsids

and/or rAAV particles with variant genomes by analytical ultracentrifugation as described above. In some embodiments, the p5 promoter of the nucleic acid encoding AAV rep and cap regions is located 3' to the rep and/or cap coding region. In some embodiments, the nucleic acid encoding AAV rep and cap coding regions is plasmid pHLP, pHLP19, or pHLP09 (see US Patent Nos. 5,622,856; 6,001,650; 6,027,931; 6,365,403; 6,376,237; and 7,037,713; the content of each is incorporated herein in its entirety). In some embodiments, the AAV helper virus functions comprise adenovirus E1A function, adenovirus E1B function, adenovirus E2A function, adenovirus VA function and adenovirus E4 orf6 function.

[0132] rAAV production cultures can be grown under a variety of conditions (over a wide temperature range, for varying lengths of time, and the like) suitable to the particular host cell being utilized. As is known in the art, rAAV production cultures include attachment- dependent cultures which can be cultured in suitable attachment-dependent vessels such as, for example, roller bottles, hollow fiber filters, microcarriers, and packed-bed or fluidized-bed bioreactors. rAAV vector production cultures may also include suspension-adapted host cells such as HeLa, 293, and SF-9 cells which can be cultured in a variety of ways including, for example, spinner flasks, stirred tank bioreactors, and disposable systems such as the Wave bag system.

[0133] rAAV vector particles of the invention may be harvested from rAAV production cultures by lysis of the host cells of the production culture or by harvest of the spent media from the production culture, provided the cells are cultured under conditions known in the art to cause release of rAAV particles into the media from intact cells, as described more fully in U.S. Patent No. 6,566,118). Suitable methods of lysing cells are also known in the art and include for example multiple freeze/thaw cycles, sonication, microfluidization, and treatment with chemicals, such as detergents and/or proteases.

[0134] Numerous methods are known in the art for production of adenoviral vector particles. For example, for a gutted adenoviral vector, the adenoviral vector genome and a helper adenovirus genome may be transfected into a packaging cell line (*e.g.,* a 293 cell line). In some embodiments, the helper adenovirus genome may contain recombination sites flanking its packaging signal, and both genomes may be transfected into a packaging cell line that expresses a recombinase (*e.g.,* the Cre/loxP system may be used), such that the adenoviral vector of interest is packaged more efficiently than the helper adenovirus (see, *e.g.,* Alba, R. *et al.* (2005) *Gene Ther.*

12 Suppl 1:S18-27). Adenoviral vectors may be harvested and purified using standard methods, such as those described herein.

[0135] Numerous methods are known in the art for production of lentiviral vector particles. For example, for a third-generation lentiviral vector, a vector containing the lentiviral genome of interest with gag and pol genes may be co-transfected into a packaging cell line (*e.g.,* a 293 cell line) along with a vector containing a rev gene. The lentiviral genome of interest also contains a chimeric LTR that promotes transcription in the absence of Tat (see Dull, T. *et al.* (1998) *J. Virol.* 72:8463-71). Lentiviral vectors may be harvested and purified using methods (*e.g.*, Segura MM, *et al.*, (2013) *Expert Opin Biol Ther.* 13(7):987-1011) described herein.

[0136] Numerous methods are known in the art for production of HSV particles. HSV vectors may be harvested and purified using standard methods, such as those described herein. For example, for a replication-defective HSV vector, an HSV genome of interest that lacks all of the immediate early (IE) genes may be transfected into a complementing cell line that provides genes required for virus production, such as ICP4, ICP27, and ICP0 (see, *e.g.,* Samaniego, L.A. *et al.* (1998) *J. Virol.* 72:3307-20). HSV vectors may be harvested and purified using methods described (*e.g.*, Goins, WF et al., (2014) Herpes Simplex Virus Methods in Molecular Biology 1144:63-79).

VI. Purification of rAAV Vectors

[0137] At harvest, rAAV production cultures of the present invention may contain one or more of the following: (1) host cell proteins; (2) host cell DNA; (3) plasmid DNA; (4) helper virus; (5) helper virus proteins; (6) helper virus DNA; and (7) media components including, for example, serum proteins, amino acids, transferrins and other low molecular weight proteins. In addition, rAAV production cultures further include rAAV particles having an AAV capsid serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAV9, AAV10, AAVrh10, AAV11, or AAV12. In some embodiments, the rAAV production cultures further comprise empty AAV capsids (*e.g.,* a rAAV particle comprising capsid proteins but no rAAV genome). In some embodiments, the rAAV production cultures further comprise rAAV particles comprising variant rAAV genomes (*e.g.,* a rAAV particle comprising a rAAV genome that differs from an intact full-length rAAV genome). In

some embodiments, the rAAV production cultures further comprise rAAV particles comprising truncated rAAV genomes. In some embodiments, the rAAV production cultures further comprise rAAV particles comprising AAV-encapsidated DNA impurities.

[0138] In some embodiments, the rAAV production culture harvest is clarified to remove host cell debris. In some embodiments, the production culture harvest is clarified by filtration through a series of depth filters including, for example, a grade DOHC Millipore Millistak+ HC Pod Filter, a grade A1HC Millipore Millistak+ HC Pod Filter, and a 0.2 μm Filter Opticap XL1O Millipore Express SHC Hydrophilic Membrane filter. Clarification can also be achieved by a variety of other standard techniques known in the art, such as, centrifugation or filtration through any cellulose acetate filter of 0.2 μm or greater pore size known in the art.

[0139] In some embodiments, the rAAV production culture harvest is further treated with Benzonase® to digest any high molecular weight DNA present in the production culture. In some embodiments, the Benzonase® digestion is performed under standard conditions known in the art including, for example, a final concentration of 1-2.5 units/ml of Benzonase® at a temperature ranging from ambient to 37°C for a period of 30 minutes to several hours.

[0140] rAAV particles may be isolated or purified using one or more of the following purification steps: equilibrium centrifugation; flow-through anionic exchange filtration; tangential flow filtration (TFF) for concentrating the rAAV particles; rAAV capture by apatite chromatography; heat inactivation of helper virus; rAAV capture by hydrophobic interaction chromatography; buffer exchange by size exclusion chromatography (SEC); nanofiltration; and rAAV capture by anionic exchange chromatography, cationic exchange chromatography, or affinity chromatography. These steps may be used alone, in various combinations, or in different orders. In some embodiments, the method comprises all the steps in the order as described below. Methods to purify rAAV particles are found, for example, in Xiao *et al.*, (1998) *Journal of Virology* 72:2224-2232; US Patent Numbers 6,989,264 and 8,137,948 and WO 2010/148143. Methods to purify adenovirus particles are found, for example, in Bo, H *et al.*, (2014) *Eur. J. Pharm. Sci.* 67C:119-125. Methods to purify lentivirus particles are found, for example, in Segura MM, *et al.*, (2013) *Expert Opin Biol Ther.* 13(7):987-1011. Methods to purify HSV particles are found, for example, in Goins, WF et al., (2014) Herpes Simplex Virus Methods in Molecular Biology 1144:63-79.

EXAMPLES

[0141] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Example 1: Characterization of recombinant adeno-associated viral vector preparations by analytical ultracentrifugation

[0142] Adeno-associated viruses (AAV) have features that make them attractive as vectors for gene therapy. Wild-type AAV consists of two open reading frames (rep and cap) which code for all structural and regulatory elements required for assembly, replication and infection. The rep ORF codes for Rep 78 and 68 proteins which have genome replication functions as well as Rep 52 and 40 proteins which are involved in single strand replication and packaging. The cap ORF codes for the three structural capsid proteins: VP1, VP2 and VP3. Recombinant AAV vector is typically produced by the triple transfection method using the "gutless" vector approach (Xiao, X, *et al.*, 1998, *J. Virol.* 3:2224-2232). The rep and cap genes are replaced with the therapeutic gene and its regulatory elements sandwiched between a 5' and 3' inverted terminal repeat (ITR), the rep and cap genes are provided in trans on a separate plasmid and a third plasmid contributes required adenoviral helper genes. It is postulated that the viral capsids are fully assembled and the ITR flanked vector genome is then inserted into the capsid via a capsid pore (Myers, MW & Carter, BJ, 1980, *Virology*, 102:71-82). The resulting population of capsids contains both non-genome containing capsids (empty capsids) as well as genome containing capsids. In addition, capsids may contain incomplete portions of the recombinant viral genome. The vector prep may then purified by affinity chromatography to isolate the capsids from the cellular debris and can be further processed to enrich for intact vector by anion exchange chromatography.

[0143] Based on their recent approval for use in gene therapy, adeno-associated viral (AAV) vectors have emerged as an important class of novel biopharmaceutical drug products. The generation of AAV vector products requires an analytical method that monitors product quality

with regard to homogeneity, purity, and consistency of manufacturing, yet to date no method to support AAV vector characterization has been established. To meet this demand, the potential use of analytical ultracentrifugation (AUC) as a technique to characterize the homogeneity of AAV vectors was investigated.

Methods

Sample preparation

[0144] In order to support accurate AUC assessment, vector product (AAV2-transgene 2) was highly purified, suitably buffered, and concentrated to greater than 5 x 10^{11} vg/mL. To achieve this, cell supernatants runs were purified using AVB affinity chromatography (GE Healthcare) and buffer-exchanged into PBS, pH 7.2 using a 10K MWCO Slide-a-Lyzer (Thermo Scientific). Product concentration was determined by optical density measurement at 260nm (OD_{260}) by spectrophotometric methods. To generate reproducible and consistent AUC data, sample adjustments were made to target concentration by optical density measurement at 260nm from 0.1 to 1.0, either by direct dilution with PBS or further concentration using Amicon Ultra-0.5 /30K MWCO Centrifugal Filter Device.

Sedimentation velocity AUC data acquisition

[0145] Sedimentation velocity analytical ultracentrifugation (SV-AUC) analysis was performed using a ProteomeLab™ XL-I (Beckman Coulter). 400 µL sample was loaded into the sample sector of a two sector velocity cell, and 400 µL PBS was loaded into the corresponding reference sector. The sample was placed in the four-hole rotor and allowed to equilibrate in the instrument until a temperature of 20°C and full vacuum were maintained for one hour. Sedimentation velocity centrifugation was performed at 20,000 RPM, 20°C, 0.003 cm radial step setting, with no delay and with no replicates. Absorbance (260 nm) and Raleigh interference optics were used to simultaneously record radial concentration as a function of time until the smallest sedimenting component cleared the optical window (1.2 hour). Assay throughput was limited to a single sample per run based on absorbance scan collection times of greater than one minute, as well as the large size and rapid sedimentation of AAV.

AUC data analysis

[0146] The percent full capsid was determined by analyzing approximately 75 scans from each detection method using the SEDFIT (NIH/see worldwide web at analyticalultracentrifugation.com) continuous size C(S) distribution model. Second (2nd) derivative regularization was applied to the fitting with a confidence level of F statistic=0.68. The following C(S) parameters were held constant: resolution=200S, S min=1, S max=200 and frictional ratio=1.0. RI and TI noise subtractions were applied, and the meniscus position was allowed to float, letting the software choose the optimal position. This model fit the data to the Lamm equation, and the resulting size distribution was a "distribution of sedimentation coefficients" that looked like a chromatogram with the area under each peak proportional to concentration in units of Fringes or OD_{260} units. The sedimentation coefficient (in Svedberg units) and the relative concentration (in OD units) were determined for each component in the distribution. Each AUC run was an independent assay, and each analysis was monitored for the following attributes to ensure quality of results: goodness of fit (rmsd), the ratio of $OD_{260\ nm}$/ interference signal in fringes (A260/IF ratio) for each peak, consistency of sedimentation coefficients for each species between runs, and overall quality of the scans.

Absorbance optics (260 nm)

[0147] Extinction coefficients were used to calculate molar concentration and the actual percent value of the intact vector peak from absorbance data. Molar absorbance extinction coefficients for both empty capsids ($\epsilon_{260}/_{capsid}$=3.72e6) and intact vector ($\epsilon_{260}/_{vector}$=3.00e7) were calculated based on published formulae (Sommer *et al.* (2003) *Mol Ther.*, 7:122-8). Extinction coefficients were available for empty capsid and intact vector peaks. The C(S) values were determined using the SEDFIT algorithm described by Schuck (2000) *Biophys. J.*, 78:1606-19. Molar concentration of both intact vector and empty capsid were calculated using Beer's Law, and the percentage of full capsid was calculated from these values. Values were reported in terms of the percentage of full capsid.

Generation of an AUC standard curve

[0148] Because it is not possible to determine empirically the extinction coefficient of fragmented genomes of unknown size and sequence, a relationship between S value and genome

size was established. To achieve this, rAAV vector preps with encapsidated viral genomes of known nucleotide size were analyzed by AUC, and a corresponding S value was determined as described above.

Production of rAAV by transient transfection

[0149] Recombinant AAV vector was produced by the triple transfection method using the "gutless" vector approach (Xiao *et al.* (1998) *J. Virol.*, 3:2224-32). In this approach, the rep and cap genes were replaced with the therapeutic gene and its regulatory elements, both sandwiched between a 5' and 3' inverted terminal repeat (ITR). The rep and cap genes were provided in trans on a separate plasmid, and a third plasmid contributed the required adenoviral helper genes. Without wishing to be bound to theory, it is postulated that the viral capsids are fully assembled, and the ITR flanked vector genome is then inserted into the capsid via a capsid pore (Myers & Carter (1980) *Virology*, 102:71-82). The resulting population of capsids contained both non-genome-containing capsids (empty capsids) and genome-containing capsids.

Production of rAAV by producer cell platform

[0150] The AAV producer cell line is an alternative production platform used to generate clinical rAAV vectors. With this method, a HeLa S3 cell, adapted to growth in suspension, was engineered to have integrated copies of the AAV rep and cap genes required for vector replication and packaging, in addition to vector sequences and the selectable marker (*see, e.g.*, Puro: Thorne *et al.* (2009) *Hum. Gene Ther.*, 20:707-14). Once infected with WT Adenovirus, which provides the helper functions required for replication, the cell produced recombinant AAV vector as well as adenovirus, which was subsequently removed during the purification process using ion-exchange chromatography.

Other methods

[0151] Synthetic transgenes were cloned into a plasmid that contained a promoter of choice and bovine growth hormone polyadenylation signal sequence (polyA). The entire transgene expression cassette was then cloned into previral plasmid vector pAAVDC64 containing AAV2 inverted terminal repeats. The total size of the resulting AAV genomes in the respective expression plasmids (including the region flanked by ITRs) was 4 - 4.6 kb. The recombinant

vectors were produced by triple transfection of 293 cells using helper plasmids expressing rep2/cap sequences and Adenovirus helper functions, pAd Helper (Stratagene, La Jolla, CA USA) The rep/cap helper expressed rep from AAV serotype 2, while the cap sequence encoded one of the following sequences: AAV cap 1, 2, 5, 9, or rh8R. Vectors were purified by affinity chromatography and in some cases were further purified to remove empty particles (see, *e.g.*, Qu *et al.* (2007) J. *Virol. Methods.* 140:183-92).

Results

[0152] Analytical ultracentrifugation (AUC) using classical boundary sedimentation velocity was used to reveal the particle heterogeneities of recombinant adeno-associated virus (rAAV) vector preps. A mixture containing 20% rAAV2 particles with the full genome and 80% empty capsids was created by mixing together purified empty capsids and purified genome-containing capsids at defined ratios. The empty and full capsids were generated by $CsCl_2$ gradient purification of a mixture of empty and full capsids following triple transfection production. To monitor the movement of rAAV2 particles in response to a centrifugal force, this mixture of rAAV2 capsids was scanned at an absorbance of 260nm along a centrifugal field at defined time intervals. **FIG. 1A** shows a representative scanning profile following centrifugation of the AAV2 mixture at 20,000 rpm for 1.2 hrs (until the smallest sedimenting species cleared the optical window). Scans represented the acquisition of concentration data as a function of radius *r*, at times *t*, to yield a series of concentration scans that revealed the complete migration pattern of constituent vector particles in the rAAV2 vector prep. In these sigmoidal curves or boundaries, the leading edge of the curve represented the faster sedimenting species (*i.e.*, the genome-containing rAAV2 capsid), and the trailing edge of the curve represented the slower sedimenting species (*i.e.*, the "empty" rAAV2 capsids) (**FIG. 1A**).

[0153] Plotting the differential sedimentation coefficient distribution value, C(S), versus the sedimentation coefficient (in Svedberg units, S) yielded distinct peaks with unique sedimentation coefficients for both the empty and genome-containing capsid species (**FIG. 1B**). The C(S) values were determined using the SEDFIT algorithm described by Schuck (2000) *Biophys. J.*, 78:1606-19. In order to calculate molar concentrations and percent value for each capsid species from the absorbance data, extinction coefficients were used according to Table 2.

Table 2. Extinction coefficients and molar concentrations for capsid species

Species	Signal (Abs $_{260nm}$)	ε_{260nm}	Molar concentration (M)	Relative abundance (%)
Peak 1	0.0479	3.73E+06	1.28E-08	79
Peak 2	0.0909	2.59E+07	3.51E-09	21
Sum			1.63E-08	

[0154] Molar absorbance extinction coefficients for both the empty capsid (ε_{260} /$_{capsid}$ = 3.72e6) and genome containing capsid (ε_{260} /$_{capsid}$ = 3e7) were calculated using genome size and published formulae (Sommer *et al.*, 2003). Molar concentrations of both genome-containing and empty capsids were then calculated using Beer's Law. The molar concentration of each species was used to calculate its relative abundance, expressed as a percentage of total capsids (**FIG. 1**). These results demonstrated that AUC may be used to accurately distinguish and quantify empty capsids and genome-containing capsids from a heterogeneous vector preparation.

[0155] For the rAAV2 vector prep shown in **FIG. 1**, capsids containing a full genome were represented by the peak sedimenting at 94S and accounted for 21% of the vector prep. Empty capsids sedimented with an S value of 64S and accounted for 79% of the vector prep. These sedimentation coefficient values were confirmed by AUC analysis of pure populations of empty (**FIG. 2A**) or genome containing particles (**FIG. 2B**). The AUC profile of a pure population of rAAV2 empty capsids revealed a single peak with a sedimentation coefficient of 64S, whereas the AUC profile of a pure population of rAAV2AUC genome-containing capsids revealed a single peak with a higher sedimentation coefficient of 94S. These results agreed with the values generated from a heterogeneous preparation and further confirmed that AUC methods may be used to quantify genome-containing and empty AAV capsids from a heterogeneous preparation containing both species.

[0156] The AUC method was further assessed for reproducibility by performing five independent AUC runs of the same vector sample (scAAV2/9 LP2), as shown in Table 3. The sedimentation coefficients for both genome-containing and empty AAV2 capsids were highly reproducible, yielding coefficients of variation from 0.5-0.6%. Similarly, the relative abundance (expressed as a percentage of the total) of genome-containing capsids was determined with a coefficient of variation of approximately 2%. These results indicated that the AUC method for

quantifying genome-containing and empty AAV2 capsids yields highly reproducible and consistent values.

Table 3. Five independent assays on scAAV2/9 LP2 sample.

AUC Run	Empty Capsid, Peak 1 (S)	Full Capsid, Peak 3	
		% Full Capsids	S
20110927A	64.3	34.1	84.0
20111003A	64.6	34.2	84.6
20111005A	65.1	35.0	84.5
20111005B	64.2	35.2	83.8
20111011A	64.1	33.5	84.5
Mean	64.5	34.4	84.3
Standard Deviation	0.4	0.7	0.4
% CV	0.6	2.1	0.5

Example 2: Comparison of interference and absorbance detection methods for AUC

[0157] An alternative optical detection method for AUC, Rayleigh Interference Optics, was also evaluated. This detection method measures the sample concentration based on refractive index differences between a reference solution and the AAV containing sample. Like absorbance detection, interference detection can be applied to any rAAV regardless of the sequence of the genome. Unlike absorbance detection, which requires an extinction coefficient, interference detection yields integrated peaks that are directly proportional to concentration.

[0158] Pure populations of empty and genome-containing AAV2 capsids were mixed at a 1:1 ratio and analyzed by AUC using both interference (**FIG. 3A**) and absorbance detection methods (**FIG. 3B**). Interference detection revealed two populations of AAV capsids at the approximate expected ratios of 43% empty and 57% genome-containing (**FIG. 3A**). Both detection methods yielded similar abundance ratios. However, comparing the peak sizes generated by both methods illustrates the disconnect between peak height and concentration with absorbance detection (compare size of "empty capsid" peaks in **FIG. 3A** and **3B**). The data generated by both methods are compared in Table 4. The ratio of absorbance signal to interference signal (A_{260nm}/IF) can be used in a fashion analogous to the 260/280 ratio of absorbance data, and this assisted in identifying peaks in the C(S) distribution.

Table 4. S values and relative abundance generated by absorbance and interference detection.

Peak	Absorbance (260 nm)		Interference			A_{260nm}/IF
	Sedimentation coefficient (S)	Relative abundance (%)	Sedimentation coefficient (S)	Signal (fringes)	Relative abundance (%)	
Peak 1	63	47	62	0.080	43	0.41
Peak 2	93	53	92	0.104	57	2.38
Sum				0.184		

[0159] Although interference optics offers precision and resolution, it may require a high concentration of sample. Moreover, interference optics may be affected by a mismatch between the reference and AAV sample buffer. AAV samples, however, typically contain a low protein concentration, and it may be necessary to completely match the AAV sample and reference buffers.

Example 3: Influence of production method on AAV vector heterogeneity

[0160] The previous examples demonstrated that the AUC method is a highly accurate and reproducible way to resolve and quantify empty and genome-containing AAV capsids from a heterogeneous mixture. This capability could be advantageous for a variety of applications to evaluate the quality of AAV vector preparations. For example, a major problem in producing pure AAV vector preparations is the presence of capsids with partial or fragmented genomes. To illustrate the utility of the AUC method for resolving these species, AAV vectors generated by two different methods, termed the "triple transfection" and "producer cell line" methods, were analyzed by AUC.

[0161] An AAV2 vector harboring the transgene 2 was produced using either the triple transfection method (**FIG. 4**) or the producer cell line method (**FIG. 5**). For a description of these methods, see Example 1. Following chromatographic purification, both vector preps were analyzed by AUC. **FIG. 6A** shows a schematic of this AAV2 vector genome.

[0162] The AUC profiles of vector preps produced by these methods were remarkably different. Using the producer cell line method, 74% of capsids contained a full genome, represented by the 92S species (**FIG. 6B**). 19% were empty capsids, with the remainder containing a fragmented genome (75S species, 7%). In contrast, 82% of the capsids produced by

the triple transfection method were empty, 64S species (**FIG. 6C**), with 11% of capsids containing a fragmented genome (76S and 84S species) and only 8% of capsids having a full genome (94S).

[0163] These results demonstrate that vector preparations generated using the producer cell line technology may have high quality, containing predominantly capsids with a full genome. The vast majority of capsids produced by the triple transfection method are empty, with a greater proportion of capsids having a fragmented genome. These results also highlight the ability of the AUC method to resolve capsids with fragmented genomes, in addition to full genome-containing and empty capsids. Moreover, they illustrate the power of the AUC method in evaluating the quality and homogeneity of vector preparations.

Example 4: Use of AUC to assess removal of empty capsids from vector preps

[0164] The AUC method was evaluated as a tool to monitor removal of empty capsids using chromatographic methods (for methods, see Qu *et al.* (2007) *J. Virol. Methods*, 140:183-92). Separation of empty and genome-containing capsids was performed using anion exchange chromatography (**FIG. 7A**). AUC was performed on the resolved peaks to demonstrate that the genome-containing rAAV2 particles were enriched in the later fractions eluted from the resin ("Full Genome Capsid" in **FIG. 7A**).

[0165] As shown in **FIG. 7B**, AUC analysis revealed that genome-containing capsids represented 94% of the vector prep upon elution from the column. This later fraction yielded a single peak with a sedimentation coefficient of 92S. In contrast, the rAAV2 vector prep prior to the chromatographic step (**FIG. 7C**) had a substantial level of empty capsids. AUC analysis revealed two peaks with S values of 63 and 93, with the 63S peak (empty capsid) representing 52% of the total capsid population. These results show that chromatographic methods are highly effective in removing empty capsids from AAV vector preparations. Importantly, they demonstrate the utility of applying the AUC method to evaluate vector quality upon purification. The AUC method is a useful tool for evaluating different vector purification protocols or techniques.

Example 5: Assessment of viral genome integrity by the AUC method

[0166] As illustrated in Example 3, AAV vector preparations may contain capsids packaged with fragmented genomes, in addition to full genome and empty species. A major problem in generating homogeneous AAV preparations for therapeutic or research applications is the presence of capsids with fragmented genomes, which may result in aberrant or absent expression of transgenes of interest. Indeed, heterogeneity associated with AAV vector preps has been reported to result from packaging of fragmented genomes or AAV-encapsidated DNA impurities. (Kapranov *et al.* (2012) *Hum. Gene Ther.*, 23:46-55). Therefore, AUC was evaluated as a tool to quantify the aberrant packaging of fragmented genomes in rAAV vector preps.

[0167] Because it is not possible to determine empirically the extinction coefficient of fragmented genomes of unknown size and sequence, a relationship between S value and genome size was established. To achieve this, rAAV vector preps with encapsidated viral genomes of known size were analyzed by AUC, and their corresponding S values were determined, as shown in Table 5. A standard curve was then generated to correlate genome size and S value (**FIG. 8**). This demonstrated a highly linear relationship (R^2=0.9978) between sedimentation coefficient and genome size.

Table 5. S values for rAAV vectors with known genome size.

Predicted Trend Line		**Calculated values**	
Sedimentation coefficient (S)	**Genome size (# NT)**	**MW**	**Extinction coefficient (260 nm)**
(y)	**(x)**		
Empty capsid	N/A		3.72E+06
74	880	2.7E+05	9.17E+06
78	1421	4.4E+05	1.25E+07
82	1961	6.1E+05	1.59E+07
84	2232	6.9E+05	1.75E+07
88	2772	8.6E+05	2.09E+07
92	3313	1.0E+06	2.42E+07
96	3853	1.2E+06	2.76E+07
100	4394	1.4E+06	3.09E+07
104	4934	1.5E+06	3.43E+07
108	5475	1.7E+06	3.76E+07

[0168] To demonstrate the utility of AUC to detect genome fragments, a self-complementary vector comprising AAV2 ITRS, a minimal CBA promoter, and an EGFP transgene was packaged

into an AAV9 capsid (AAV2/9minCBAEGFP; see schematic in **FIG. 9A**). The vector particles were purified to eliminate empty capsids and analyzed by AUC. The standard curve was then used to assign genome size to each of the resolved genome containing capsids. Approximately 25% of the vector prep sedimented as a 101S species, representing an encapsidated genome of ~ 4.3kb (**FIG. 9A**). This 101S peak represented the double stranded dimeric vector genome, which has a predicted size of ~4.3kb. However, the majority of the vector prep (75%), sedimented with an S value of 82, which corresponds to a vector genome size of ~ 2kb (**FIG. 9A**), consistent with packaging of the single stranded monomer. The packaging of monomeric genomes with self-complementary vectors is well documented and is often a result of inadvertent terminal resolution at spurious "trs like" sequences despite the presence of an ITR with a mutated D sequence (McCarty *et al.* (2001) *Gene Ther.,* 8:1248-54).

[0169] **FIG. 9B** shows an alkaline Southern blot of the same vector, scAAV9 EGFP, which revealed two vector populations with genomes ~ 4.3kb and ~2kb in size, corroborating the AUC data in **FIG. 9A**. The Southern blot also confirmed that the monomeric viral genome was preferentially packaged over the dimeric genome. Interestingly, AUC analysis of a single stranded AAV9 EGFP vector (~4kb) revealed a single predominant peak with a measured S value of 99S, corresponding to approximately 4.1kb by the standard curve and 84% of capsid abundance (**FIG. 9C**). These results suggest that single stranded AAV vectors may be packaged in a more homogeneous manner than double stranded vectors. Again, in agreement with the AUC method, Southern blot analysis of this vector prep revealed homogeneous encapsidation of a viral genome of the predicted size of ~4kb (lane 2, **FIG. 9B**). These results demonstrate that the AUC method may be used to measure the size of AAV vector genomes, yielding genome size data in agreement with the standard Southern blotting technique. Using the AUC method, single stranded AAV vectors were found to produce more homogeneous vector preparations than double stranded ones. These results show that the AUC method is a powerful tool to identify and quantify capsid species with incomplete genomes from vector preparations.

Example 6: Use of AUC to assess factors that influence packaging of vector genomes

[0170] The AUC method was next used as a tool to identify factors that influence the packaging of intact AAV vector genomes.

[0171] As discussed in Example 3, the production of rAAV vectors by transient transfection methods requires the use of three plasmids including a rep/cap helper, an ITR vector plasmid, and a pAd helper (see **FIG. 4**). AUC was used to assess the effect of the rep/cap helper on vector genome packaging for both single stranded and self-complementary AAV vectors. First, a self-complementary AAV vector harboring an EGFP transgene (**FIG. 10A**) was produced using one of two methods. In the first method (**FIG. 10B**), a helper plasmid was used in which rep 78/68 expression was driven by the endogenous p5 promoter ("WT Rep" construct). In the second method (**FIG. 10C**), the helper was modified such that 78/68 expression was reduced by moving the p5 promoter downstream of the cap2 sequence as well as mutating the TATA box ("pHLP Rep" construct). The full scAAV2 EFGP capsid was predicted to have a sedimentation coefficient of 100S in dimeric genome form and 80S in monomeric genome form (**FIG. 10A**).

[0172] AUC analysis of these scAAV2EGFP vector preps revealed a significant difference in vector genome packaging. In the presence of reduced rep78/68 (pHLP), more than half (55%) of the vector prep contained dimeric genomes, represented by the 100S species (**FIG. 10C**). This was the expected sedimentation coefficient for a capsid containing a dimeric genome of 4.4kb. In contrast, the scAAV2EGFP prep generated with the full complement of rep78/68 had significantly less packaged dimeric genomes (26%), with the majority of the capsids containing monomeric genomes and sedimenting at 80S (**FIG. 10B**). These results uncovered a significant difference in genome packaging induced by shifting the P5 promoter of the helper plasmid, leading to reduced rep 78/68 protein levels.

[0173] A single stranded AAV5 Factor IX vector, AAV5FIX, (**FIG. 11A-B**) and a single stranded AAV5hSMN vector (**FIG. 11C-D**) were generated using rep/cap helpers that differed in rep expression as described above, but cap sequences of AAV2 were replaced by cap sequences of AAV5. Based on the nucleotide size of the FIX expression cassette (4.3kb), the predicted sedimentation coefficient for the AAV5 FIX vector capsid was approximately 101S. AUC analysis of AAV2/5FIX made in the presence of reduced rep78/68 ("pHLP19 Rep") revealed a homogenous profile with the majority of the vector (90%) sedimenting with an S value of the expected size, ~101S (**FIG. 11A**). In contrast, AAV5 FIX vector generated using a rep/cap5 helper expressing wild-type levels of rep 78/68 proteins ("WT Rep") generated a strikingly different AUC profile (**FIG. 11B**). Instead of a predominant peak at 101S, this profile revealed more capsid heterogeneity, with the majority of the AAV5 FIX (80%) sedimenting at a lower S

value of 86S, likely representing packaging of a fragmented genome. Moreover, in this vector sample only 15% of the AAV5 FIX vector capsids sedimented at the correct S value of ~ 104S (**FIG. 11B**).

[0174] AAV5SMN vectors made using these same wild-type and mutated p5 rep/cap helpers also had strikingly different AUC profiles. As seen with the single stranded AAV5FIX vectors, AAV5 SMN vectors generated in the presence of reduced rep78/68 showed less heterogeneity by AUC analysis, with a single capsid species sedimenting at the S value of 101S, consistent with packaging of a genome of the predicted size of ~ 4.4kb (**FIG. 11C**). In contrast, the AUC profile for the same vector genome packaged using "wild-type" levels of rep78/68 protein revealed three distinct AAV vector species, with sedimentation coefficients of 100S (predicted S value for the full vector genome of 4400nt), 92S (representing a fragmented genome of approximately 3300nt) and 80S (representing a fragmented genome of 2000 nucleotides) (**FIG. 11D**). These results confirm a significant difference in genome packaging induced by shifting the P5 promoter of the helper plasmid using two additional AAV vectors.

[0175] Further analysis of the AAV5SMN and AAV5FIX vector preps was performed by Southern blot analysis of vector DNA. In agreement with the AUC method, Southern blot analysis of AAV5SMN generated with wild-type rep78/68 protein levels revealed packaging of full length (4.4kb) and fragmented (less than 4.4 kb) SMN genomes (**FIG. 12A**, lane 1). In contrast, the AAV5SMN vector generated in the presence of reduced rep78/68 protein contained largely capsids with a full length SMN genome (**FIG. 12A**, lane 2). Interestingly, a comparison of the two AAV5FIX vector preps by Southern analysis revealed the presence of a FIX full length genome even when vector was produced in the presence of wild-type levels of rep 78/68 (**FIG. 12B**, lane 2). However, AUC analysis of this AAV5FIX vector (**FIG. 11B**) showed that 80% of the capsids contained a fragmented genome (~ 3000 nucleotides), which were undetected by the FIX probe.

[0176] Further analysis of the FIX vector preps generated under the two experimental conditions was performed by generating probes to discrete regions of the vector plasmid, including regions of the backbone. A map of this vector is provided in **FIG. 13**. **FIG. 14** shows Southern blotting analyses using these probes to compare these FIX vector preps generated under different conditions. As shown in **FIG. 14A**, both vector preparations (pHLP rep, lane 1; WT rep,

lane 2) contained the hFIX transgene. However, **FIG. 14B** lane 2confirms that the vector genome species sedimenting at 86S observed in the WT Rep preparation (**FIG. 11B**) was a ~ 3kb fragment. Moreover, this species reacted with an Amp^R specific probe (**FIG. 14B**, lane 2), suggesting that packaging upstream of the 5'ITR had occurred in a rep dependent manner. In contrast, there was no evidence of an Amp^R containing fragment in the rAAV5 FIX vector preps that were generated in the presence of reduced levels of rep 68 / 78 (**FIG. 14B**, lane 1).

[0177] DNA impurities in the AAV FIX preps were also assessed by Q-PCR using primers and probes specific for Amp^R. By Q-PCR, approximately 35% Amp^R titer was detected in AAV5FIX vector preps generated in the presence of "wt" rep, in contrast to less than 1% when the same vector plasmid was used to generate AAV5FIX vector in the presence of reduced rep68/78 (data not shown). These results underscore the utility of AUC analysis for revealing the presence of packaged genomes that would otherwise go undetected by gene specific Southern blot analysis.

[0178] The packaging capacity of AAV vectors has been studied extensively, and although numerous reports have demonstrated successful transduction with vectors packaging oversized AAV genomes, the latter have been shown to be fragmented into subgenomic – length DNA. To further explore the applicability of the AUC method, the heterogeneity of AAV vectors produced using oversized genomes was evaluated. An expression cassette harboring the full length CBA promoter driving expression of the β-phosphodiesterase transgene was packaged as an oversized genome of 5.4kb (**FIG. 15A**) or as a wild type size genome of 4.6kb (**FIG. 15B**). To generate the 4.6kb genome, the CBA promoter was truncated by reducing the size of the intron as previously reported (Gray, SJ *et al.*, (2011) *Hum. Gene Ther.* 22(9):1143-1153).

[0179] As shown in **FIG. 15A**, the AUC profile of the AAV vector prep generated using the oversized vector genome demonstrated that nearly half of the vector prep sedimented as a 93S species, consistent with packaging a fragmented vector genome of approximately 3.5kb. 30% of the preparation was represented by another sub-genomic vector species of approximately 4.9kb sedimenting at 105S. There was no evidence of packaging of a full-length 5.4kb genome, which was predicted to sediment at 108-109S. In contrast, AUC analysis revealed that the same transgene under control of the abbreviated CBA promoter sedimented predominantly as a vector species of 102S, consistent with packaging of the predicted, full-length vector genome of 4.6 kb (**FIG. 15B**). These results demonstrate the utility of AUC analysis in profiling AAV vectors with

oversized genomes, and this profiling is critical, given the observed incidence of genome fragmentation.

[0180] This example demonstrated that the AUC method is highly effective in analyzing the genome size of AAV vector capsids in a heterogeneous preparation. By resolving genome-containing capsids by size (*e.g.*, dimeric and monomeric genomes, or partial fragments thereof), the AUC method represents a powerful tool for assaying the quality of AAV vector preps produced under different conditions. Moreover, the results from three distinct vector systems demonstrated that the AUC method is widely useful for quality control and optimization of conditions to yield improved AAV vector preparations. Importantly, the AUC method is able to detect fragmented genomes that are not detectable by Southern blot analysis. Whereas Southern blotting relies on the presence of DNA probe sequence for detection, the AUC method is sequence-independent. The AUC method has also been demonstrated to be an effective tool in analyzing oversized AAV genomes. In total, these results demonstrate the highly advantageous and effective implementation of AUC methods to analyze multiple types of AAV vector preparations, which have been found to display dramatically variable effects on genome packaging.

Example 7: Characterization of recombinant adenoviral vector preparations by analytical ultracentrifugation

[0181] Adenovirus (Ad) vectors have features that make them attractive as vectors for gene therapy. The generation of Ad vector products requires an analytical method that monitors product quality with regard to homogeneity, purity, and consistency of manufacturing. To meet this demand, the potential use of analytical ultracentrifugation (AUC) as a technique to characterize the homogeneity of Ad vectors was investigated.

Methods

Sample preparation

[0182] In order to support accurate AUC assessment, a recombinant adenovirus serotype 2 vector (Ad2) was prepared and highly purified ty CsCl gradient ultrafiltration to enrich for genome containing particles. Product concentration was determined by optical density

measurement at 260nm (OD_{260}) by spectrophotometric methods. To generate reproducible and consistent AUC data, sample adjustments were made to target concentration by optical density measurement at 260nm from 0.1 to 1.0, either by direct dilution with PBS or further concentration using Amicon Ultra-0.5 /30K MWCO Centrifugal Filter Device.

Sedimentation velocity AUC data acquisition

Sedimentation velocity analytical ultracentrifugation (SV-AUC) analysis was performed using a ProteomeLab™ XL-I (Beckman Coulter). 400 µL sample was loaded into the sample sector of a two sector velocity cell, and 400 µL PBS was loaded into the corresponding reference sector. The sample was placed in the four-hole rotor and allowed to equilibrate in the instrument until a temperature of 20°C and full vacuum were maintained for one hour. Sedimentation velocity centrifugation was performed at 6,000 RPM, 20°C, 0.003 cm radial step setting, with no delay and with no replicates. Raleigh interference optics were used to simultaneously record radial concentration as a function of time until the smallest sedimenting component cleared the optical window (1.2 hour). Assay throughput was limited to a single sample per run based on absorbance scan collection times of greater than one minute, as well as the large size and rapid sedimentation of Ad2.

AUC data analysis

[0183] The percent full capsid was determined by analyzing approximately 75 scans from interference detection method using the SEDFIT (NIH/see worldwide web at analyticalultracentrifugation.com) continuous size C(S) distribution model. Second (2nd) derivative regularization was applied to the fitting with a confidence level of F statistic/ratio=0.68. The following C(S) parameters were held constant: resolution=250S, S min=10, S max=1500 and frictional ratio=1.86935. RI and TI noise subtractions were applied, and the meniscus position was allowed to float, letting the software choose the optimal position. This model fit the data to the Lamm equation, and the resulting size distribution was a "distribution of sedimentation coefficients" that looked like a chromatogram with the area under each peak proportional to concentration in units of Fringes or OD_{260} units. The sedimentation coefficient (in Svedberg units) and the relative concentration (in OD units) were determined for each component in the distribution. Each AUC run was an independent assay, and each analysis was monitored for the

following attributes to ensure quality of results: goodness of fit (rmsd), the ratio of $OD_{260\ nm}$/ interference signal in fringes (A260/IF ratio) for each peak, consistency of sedimentation coefficients for each species between runs, and overall quality of the scans. The rmsd of this representative example was 0.006584.

Results

[0184] Analytical ultracentrifugation (AUC) using classical boundary sedimentation velocity was used to reveal the particle heterogeneities of recombinant adenovirus serotype 2 vectdor (rAd2) vector preps. To monitor the movement of rAd2 particles in response to a centrifugal force, this mixture of rAd2 capsids was scanned using interference optics along a centrifugal field at defined time intervals. Scans represented the acquisition of concentration data as a function of radius *r*, at times *t*, to yield a series of concentration scans that revealed the complete migration pattern of constituent vector particles in the rAd2 vector prep. Plotting the differential sedimentation coefficient distribution value, C(S), versus the sedimentation coefficient (in Svedberg units, S) yielded distinct peaks with unique sedimentation coefficients rAd2 species (**FIG. 16**). The C(S) values were determined using the SEDFIT algorithm described by Schuck (2000) *Biophys. J.*, 78:1606-19.

[0185] For the rAd2 vector prep shown in **FIG. 16**, 87.8% of the rAd2 vector preparation sedimented with an S value of 731, consistent with a vector preparation consisting predominantly of genome containing capsids. These data confirm that adenoviral particles can be resolved by AUC.

CLAIMS

What is claimed is:

1. A method of characterizing a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), and

c) integrating the area under each peak in the C(s) distribution to determine the relative concentration of each peak, wherein each peak represents a species of recombinant viral recombinant viral particle.

2. A method to assess vector genome integrity of recombinant viral particles in a preparation of recombinant viral particles comprising

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value C(s) versus the sedimentation coefficient in Svedberg units (S), and

c) identifying species of recombinant viral particles in the preparation by presence of peaks on the plot corresponding to an S value, wherein the genome size of a particular species of recombinant viral recombinant viral particles is calculated by comparing the S value of the species to a standard curve generated by S values of recombinant viral particles comprising encapsidated viral genomes of known nucleotide sizes.

3. The method of claim 2 further comprising integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral recombinant viral particles.

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4. A method to determine the presence of empty capsids or capsid particles comprising variant sized recombinant viral genomes in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals, and

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant viral genomes indicates that presence of capsid particles comprising variant sized genomes and/or empty capsids.

5. A method of measuring the relative amount empty capsids in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles, and

d) comparing the amount of recombinant viral particles having an S value corresponding to empty capsid particles to the amount of recombinant viral particles having an S value corresponding to recombinant viral particles comprising intact viral genomes or the total amount of recombinant viral particles in the preparation.

6. A method of measuring the relative amount of capsid particles comprising variant recombinant viral genomes or empty viral capsid particles in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles,

d) comparing the amount of recombinant viral particles having an S values that do not correspond to recombinant viral particles comprising intact viral genomes to the amount of recombinant viral particles having an S value that corresponds to recombinant viral particles comprising intact viral genomes or to the total amount of recombinant viral particles in the preparation.

7. A method of measuring the relative amount of capsid particles comprising variant recombinant viral genomes in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles,

d) comparing the amount of recombinant viral particles having an S values that do not correspond to recombinant viral particles comprising intact viral genomes or empty capsid particles to the total amount of recombinant viral particles in the preparation.

8. A method of measuring the relative amount of recombinant viral particles comprising intact viral genomes in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S),

c) integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles,

d) comparing the amount of recombinant viral particles having an S values corresponding to recombinant viral particles comprising intact viral genomes to the amount of recombinant viral particles having an S value corresponding to empty capsid particles, to capsid particles comprising variant recombinant viral genomes, and/or to the total amount of recombinant viral particles in the preparation.

9. A method of monitoring the removal of empty capsids and/or capsid particles comprising variant recombinant viral genomes during the purification of a preparation of recombinant viral particles, the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and analyzing the sample for the relative amount of empty capsids and/or capsid particles comprising variant recombinant viral genomes according to the method of any one of claims 5-8, wherein a decrease in the relative amount of empty capsids and/or capsids comprising variant genomes to full capsids indicates removal of empty capsids from the preparation of recombinant viral particles.

10. The method of any one of claims 4-9, wherein the presence of a peak that corresponds to the S value of empty capsid particles indicates the presence of empty capsid particles.

11. The method of any one of claims 4-9, wherein the presence of one or more peaks other than the peak for full capsid particles comprising intact recombinant viral genomes or empty capsid particles indicates that presence of capsid particles comprising variant sized genomes.

12. The method of claim 11, wherein the capsid particles comprising variant sized genomes comprises truncated genomes, aggregates, recombinants and/or DNA impurities.

13. A method of determining the heterogeneity of recombinant viral particles in a preparation of recombinant viral particles comprising the steps of

a) subjecting the preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions wherein the sedimentation of recombinant viral particles is monitored at time intervals,

b) plotting the differential sedimentation coefficient distribution value (C(s)) versus the sedimentation coefficient in Svedberg units (S), wherein the presence of peaks in addition to the

peak representing capsids comprising an intact viral genome indicates heterogeneity of recombinant particles in the preparation.

14. The method of claim 13, wherein the presence of additional peaks indicates the presence of empty capsid particles and/or recombinant viral particles comprising variant genomes.

15. The method of claim 14, wherein the variant genomes are truncated viral genomes, aggregates, recombinants and/or DNA impurities.

16. The method of any one of claims 13-15 further comprising integrating the area under each peak in the C(S) distribution to determine the relative concentration of each species of recombinant viral particles.

17. A method of monitoring the homogeneity of recombinant viral particles during the purification of a preparation of recombinant viral particles, the method comprising removing a sample of the recombinant viral particles from the preparation following one or more steps in the purification process and determining the heterogeneity of recombinant viral particles according to the method of claim 16, wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes indicates an increase in the homogeneity of full viral particles in the preparation of recombinant viral particles.

18. The method of any one of claims 1-17, wherein the sedimentation of recombinant viral particles is monitored by absorbance.

19. The method of claim 18, wherein the absorbance is at about 230 nm, 260 nm or 280 nm.

20. The method of claim 18 or 19, wherein the absorbance is at about 260 nm.

21. The method of any one of claims 1-20, wherein the sedimentation of recombinant viral particles is monitored by interference.

22. The method of claim 21, wherein the interference is Rayleigh interference.

23. The method of any one of claims 1-22, wherein the preparation is an aqueous solution.

24. The method of claim 23, wherein the aqueous solution comprises a pharmaceutical formulation.

25. The method of claim 23 or 24, wherein the aqueous solution comprises a buffer.

26. The method of claim 25, wherein the buffer is at physiological pH.

27. The method of claim 25 or 26, wherein the buffer is at physiological osmolality.

28. The method of any one of claims 24-27, wherein the pharmaceutical formulation comprises phosphate buffered saline (PBS).

29. The method of claim 28, wherein the PBS has pH of about 7.2 and an osmolality of about 300 mOsm/L.

30. The method of any one of claims 24-29, wherein the monitoring further comprises comparison to a reference sample, wherein the reference sample comprises the aqueous solution without recombinant viral particles.

31. The method of any one of claims 1-30, wherein the C(S) values are determined by an algorithm that comprises Lamm equation solutions.

32. The method of claim 31, wherein the algorithm is the SEDFIT algorithm.

33. The method of any one of claims 1-32, wherein sedimentation is monitored until the recombinant viral particles with the lowest density sediments to the bottom of a sector of an ultracentrifuge.

34. The method of any one of claims 1-32, wherein the ultracentrifugation utilizes an ultracentrifuge comprising an ultracentrifuge velocity cell.

35. The method of any one of claims 1-33, wherein sedimentation is monitored until recombinant viral particles sediment to the bottom of an ultracentrifuge velocity cell.

36. The method of claim 34, wherein sedimentation is monitored until the recombinant viral particles with the lowest density sediments and clears an optical window.

37. The method of any one of claims 33-36, wherein at least 30 scans are used to monitor sedimentation of recombinant viral particles.

38. The method of claim 37, wherein about 30 scans are used to monitor sedimentation of recombinant viral particles.

39. The method of claim 37, wherein about 30 to about 75 scans are used to monitor sedimentation of recombinant viral particles.

40. The method of claim 37, wherein about 30 to about 50 scans are used to monitor sedimentation of recombinant viral particles.

41. The method of claim 37, wherein about 50 to about 75 scans are used to monitor sedimentation of recombinant viral particles.

42. The method of claim any one of claims 31-41, wherein a regularization is applied to a fitting level with a confidence level of F statistic of at least about 0.68.

43. The method of claim 42, wherein the regularization is a second derivative regularization.

44. The method of claim 42, wherein the regularization is Max entropy regularization.

45. The method of any one of claims 42-44, wherein the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68 to about 0.90.

46. The method of any one of claims 42-44, wherein the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68 to about 0.99.

47. The method of any one of claim 42-44, wherein the regularization is applied to a fitting level with a confidence level of F statistic of about 0.68.

48. The method of any one of claims 31-47, wherein the following C(S) parameters are held constant: resolution of about 200S to about 5000S, S min is about 1S to about 100S, S max is about 100S to about 5000S, and frictional ratio is about 1.0 or is left to float to a value determined by centrifugation software.

49. The method of claim 48, wherein resolution is about 200S to about 1000S.

50. The method of claim 48 or 49, wherein resolution is about 200S.

51. The method of any one of claims 48-50, wherein S min is about 1.

52. The method of any one of claims 48-51, wherein Smax is about 100S to about 1000S.

53. The method of any one of claims 48-51, wherein Smax is about 200S to about 5000S.

54. The method of any one of claims 48-51, wherein Smax is about 200S.

55. The method of any one of claims 48-54, wherein the frictional ratio is left to float to a value determined by centrifugation software.

56. The method of claim any one of 48-54, wherein the frictional ratio is about 1.0.

57. The method of any one of claims 31-56, wherein radial invariant (RI) and time invariant (TI) noise subtractions are applied.

58. The method of any one of claims 1-57, wherein the sedimentation of recombinant viral particles is monitored about every 10-60 seconds.

59. The method of claim 58, wherein the sedimentation of recombinant viral particles is monitored about every 10 seconds.

60. The method of claim 58, wherein the sedimentation of recombinant viral particles is monitored about every 60 seconds

61. The method of any one of claims 1-60, wherein the boundary sedimentation velocity is performed at about 3,000 rpm to about 20,000 rpm.

62. The method of claim 61, wherein the boundary sedimentation velocity is performed at about 3,000 rpm to about 10,000 rpm.

63. The method of claim 61, wherein the boundary sedimentation velocity is performed at about 10,000 rpm to about 20,000 rpm.

64. The method of claim 61, wherein the boundary sedimentation velocity is performed at about 15,000 rpm to about 20,000 rpm.

65. The method of any one of claims 1-64, wherein the boundary sedimentation velocity is performed at about 4°C to about 20 °C.

66. The method of claim 65, wherein the boundary sedimentation velocity is performed at about 4°C.

67. The method of any one of claims 1-66, wherein the recombinant viral particle is a recombinant adeno-associated viral (AAV) particle, a recombinant adenovirus particle, a recombinant lentivirus particle or a recombinant herpes simplex viral (HSV) particle.

68. A method of evaluating a process for the production of recombinant viral particles comprising the method of any one of claims 1 to 67, wherein an increase in the relative amount of recombinant viral particles comprising intact viral genomes compared to the relative amount of empty capsid particles and/or recombinant viral capsid particles with variant recombinant viral genomes compared to a reference preparation of recombinant viral particles indicates an improvement in the production of recombinant viral particles.

69. The method of claim 68, wherein the viral particles are rAAV particles.

70. The method of claim 69, wherein the rAAV particles are produced from a producer cell line.

71. The method of claim 69, wherein the rAAV particles are produced by triple transfection of i) nucleic acid encoding AAV rep and cap, ii) rAAV vector sequences, and iii) nucleic acid encoding adenovirus helper functions.

72. The method of claim 69, wherein the recombinant viral particles are produced by an AAV/HSV hybrid

73. The method of claim 69, wherein the recombinant viral particles are produced from a insect cell.

74. The method of claim 69, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding AAV vector sequences, AAV rep and cap coding regions, and AAV helper virus functions to a suitable host cell.

75. The method of claim 69, wherein the recombinant viral particles are produced by introduction of one or more nucleic acids encoding AAV vector sequences, AAV rep and cap

coding regions, and AAV helper virus functions to a suitable host cell, wherein the one or more nucleic acids are introduced to the cell using a recombinant helper virus.

76. The method of claim 75, wherein the recombinant helper virus is an adenovirus, a herpes simplex virus or a baculovirus.

77. The method of claim 68, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding adenovirus vector sequences and adenovirus replication and packaging sequences to a suitable host cell.

78. The method of claim 68, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding lentivirus vector sequences and/or lentivirus replication and packaging sequences to a suitable host cell.

79. The method of claim 68, wherein the recombinant viral particles are produced by transient transfection of nucleic acid encoding HSV vector sequences and/or HSV replication and packaging sequences to a suitable host cell.

80. A method for preparing recombinant viral particles with reduced empty capsids and/or recombinant viral particles comprising variant genomes, the method comprising

a) culturing host cells under conditions suitable for recombinant viral production, wherein the cells comprise

i) nucleic acid encoding a heterologous transgene flanked by at least one AAV ITR,

ii) nucleic acid comprising AAV rep and cap coding regions, wherein the nucleic acid comprises a mutated p5 promoter wherein rep expression from the p5 promoter is reduced compared to a wild-type p5 promoter, and

iii) nucleic acid encoding AAV helper virus functions;

b) lysing the host cells to release recombinant viral particles;

c) isolating the recombinant viral particles produced by the host cell; and

d) analyzing the recombinant viral particles for the presence of empty capsids and/or recombinant viral particles with variant genomes by analytical ultracentrifugation by the methods of any one of claims 1 to 79.

81. The method of claim 80, wherein the p5 promoter is located 3' to the rep and/or cap coding region.

82. The method of claim 80 or 81, wherein the AAV helper virus functions comprise adenovirus E1A function, adenovirus E1B function, adenovirus E2A function, adenovirus VA function and adenovirus E4 orf6 function.

83. The method of any one of the preceding claims wherein the recombinant viral particles have been purified using one or more purification steps.

84. The method of claim 67, 69-76, 80-83 wherein the recombinant viral particles comprise a self-complementary AAV (scAAV) genome.

85. The method of claim 84, wherein the method is used to detect the presence of recombinant viral particles comprising the monomeric form of a scAAV genome or the dimeric form of a scAAV genome.

86. The method of any one of claims 67, 69-76, 80-85,wherein the recombinant viral particle comprises an AAV1 capsid, an AAV2 capsid, an AAV3 capsid, an AAV4 capsid, an AAV5 capsid, an AAV6 capsid, an AAV7 capsid, an AAV8 capsid, an AAVrh8 capsid, an AAV9 capsid, an AAV10 capsid, an AAVrh10 capsid, an AAV11 capsid, an AAV12 capsid, an AAV2R471A capsid, an AAV2/2-7m8 capsid, an AAV DJ capsid, an AAV2 N587A capsid, an AAV2 E548A capsid, an AAV2 N708A capsid, an AAV V708K capsid, a goat AAV capsid, an AAV1/AAV2 chimeric capsid, a bovine AAV capsid, or a mouse AAV capsid rAAV2/HBoV1 (chimeric AAV / human bocavirus virus 1).

87. The method of any one of claims 67, 69-76, 80-86, wherein the recombinant viral particle comprises an AAV1 ITR, an AAV2 ITR, an AAV3 ITR, an AAV4 ITR, an AAV5 ITR, an AAV6 ITR, an AAV7 ITR, an AAV8 ITR, an AAVrh8 ITR, an AAV9 ITR, an AAV10 ITR, an AAVrh10 ITR, an AAV11 ITR, an AAV12 ITR, an AAV DJ ITR, a goat AAV ITR, a bovine AAV ITR, or a mouse AAV ITR.

88. The method of claim 86 or 87, wherein the AAV capsid comprises a tyrosine mutation or a heparin binding mutation,

89. The method of claim 67 or 68, where the recombinant viral particle is a recombinant adenoviral particle.

90. The method of claim 89, wherein the recombinant adenoviral particle comprises an capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, , AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3.

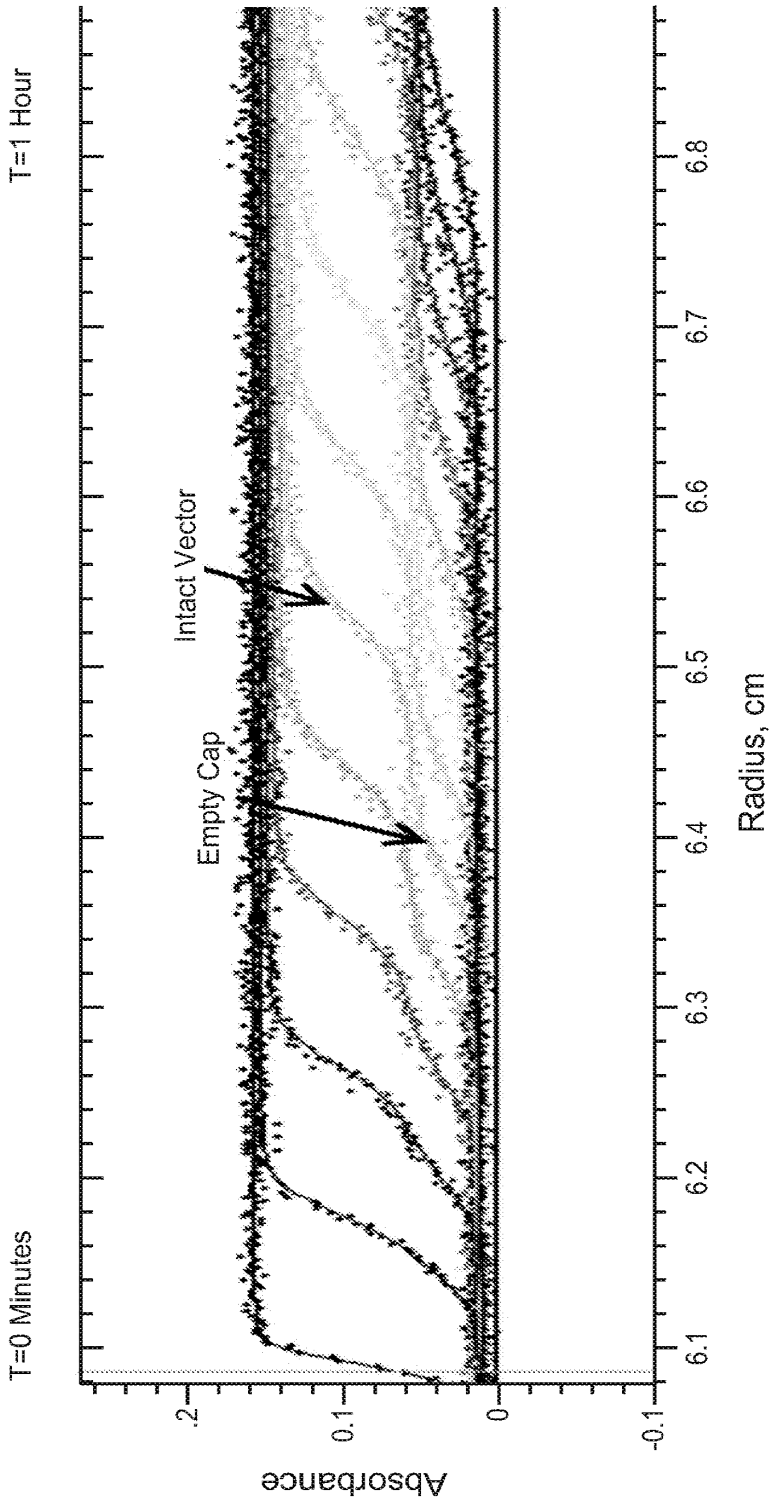
91. The method of claim 90, wherein the recombinant adenoviral particle comprises a variant of an adenovirus serotype 2 capsid or a variant of an adenoviral serotype 5 capsid.

92. The method of claim 67 or 68, where the recombinant viral particle is a recombinant lentiviral particle.

93. The method of claim 92, wherein the recombinant lentiviral particle is pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein.

94. The method of claim 67 or 68, where the recombinant viral particle is a rHSV particle.

95. The method of claim 94, wherein the HSV particle is an HSV-1 particle or an HSV-2 particle.


T=1 Hour
T=0 Minutes
Intact Vector
Empty Cap
Absorbance
.2
0.1
0
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6.1
6.2
6.3
6.4
6.5
6.6
6.7
6.8
Radius, cm

FIG. 1A

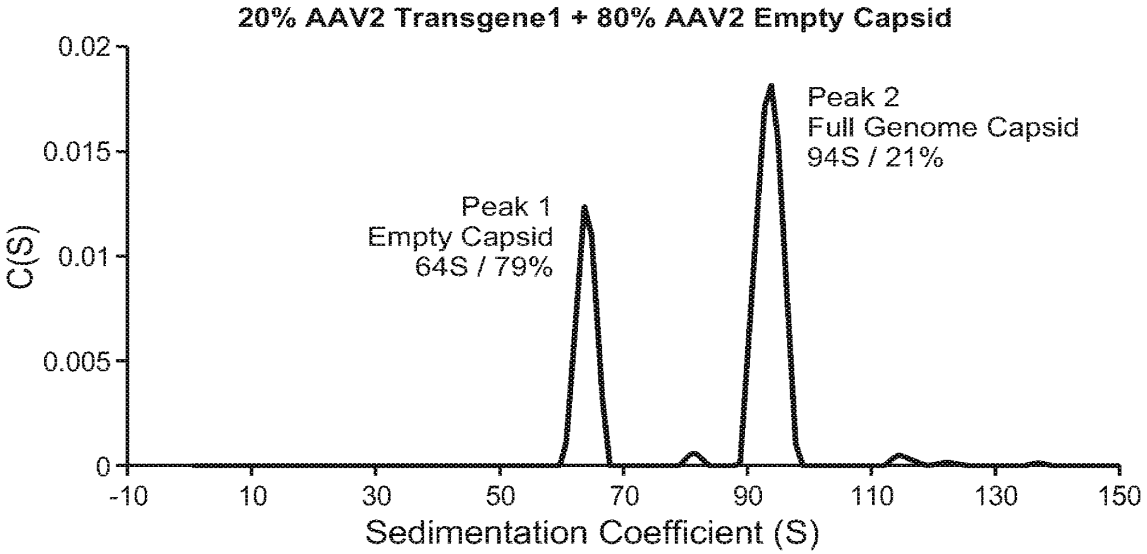
20% AAV2 Transgene1 + 80% AAV2 Empty Capsid
0.02
0.015
0.01
0.005
0
C(S)
Peak 1
Empty Capsid
64S / 79%
Peak 2
Full Genome Capsid
94S / 21%
-10
10
30
50
70
90
110
130
150
Sedimentation Coefficient (S)

FIG. 1B

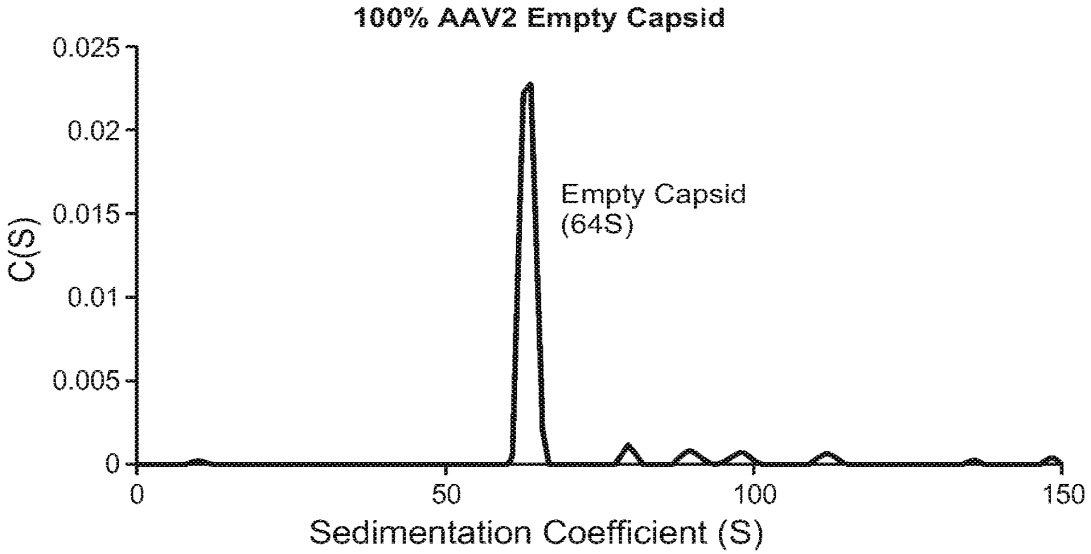
100% AAV2 Empty Capsid
0.025
0.02
0.015
0.01
0.005
0
C(S)
Empty Capsid
(64S)
0
50
100
150
Sedimentation Coefficient (S)

FIG. 2A

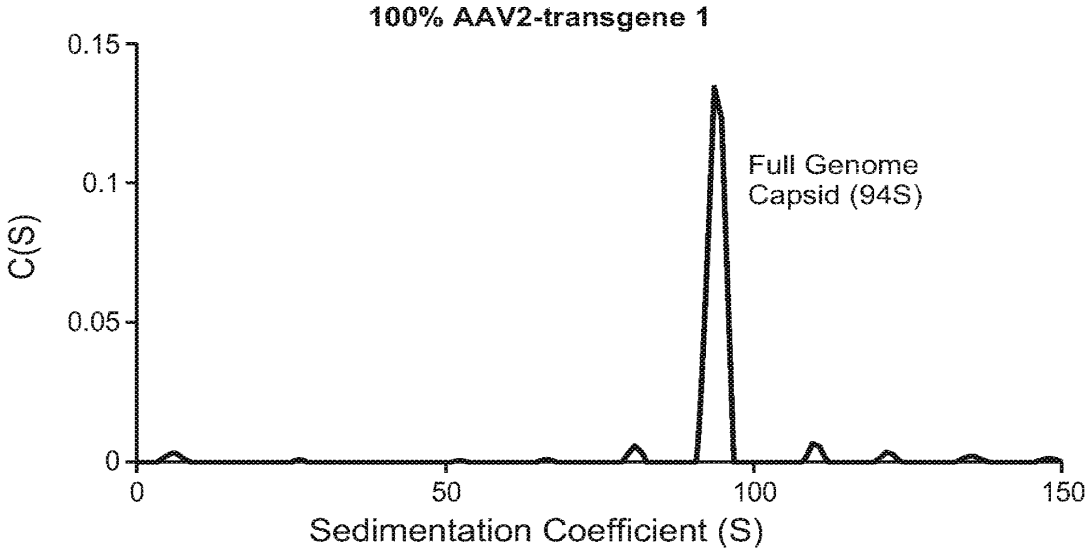
100% AAV2-transgene 1
0.15
0.1
0.05
0
C(S)
Full Genome
Capsid (94S)
0
50
100
150
Sedimentation Coefficient (S)

FIG. 2B

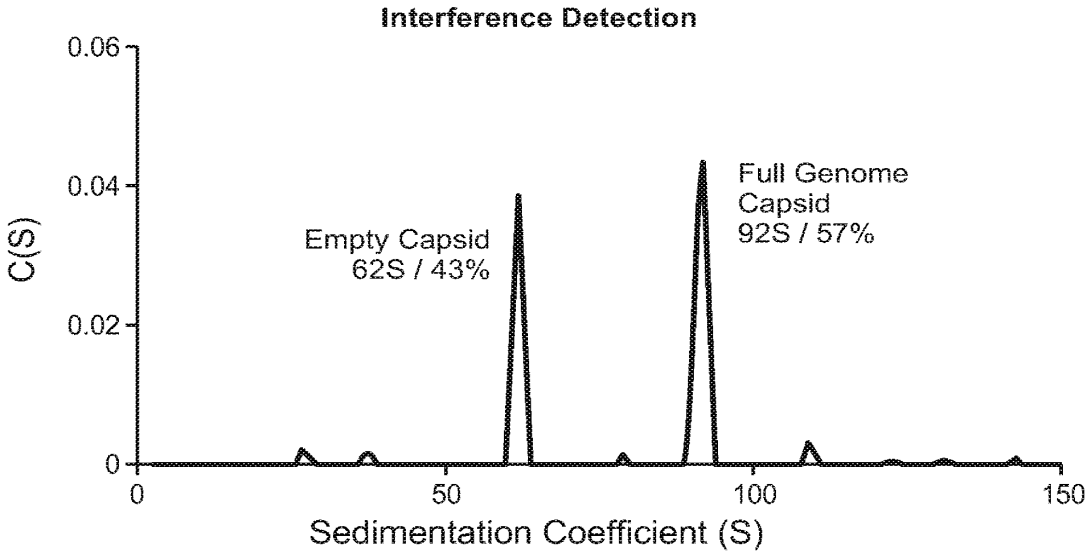
Interference Detection
0.06
0.04
0.02
0
C(S)
Empty Capsid
62S / 43%
Full Genome
Capsid
92S / 57%
0
50
100
150
Sedimentation Coefficient (S)

FIG. 3A

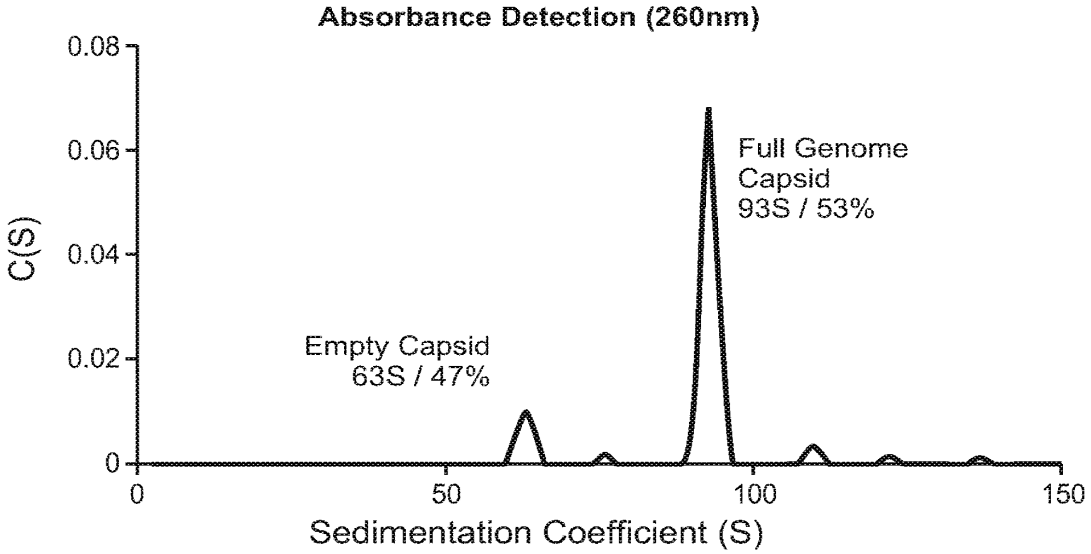
Absorbance Detection (260nm)
0.08
0.06
0.04
0.02
0
C(S)
Empty Capsid
63S / 47%
Full Genome
Capsid
93S / 53%
0
50
100
150
Sedimentation Coefficient (S)

FIG. 3B

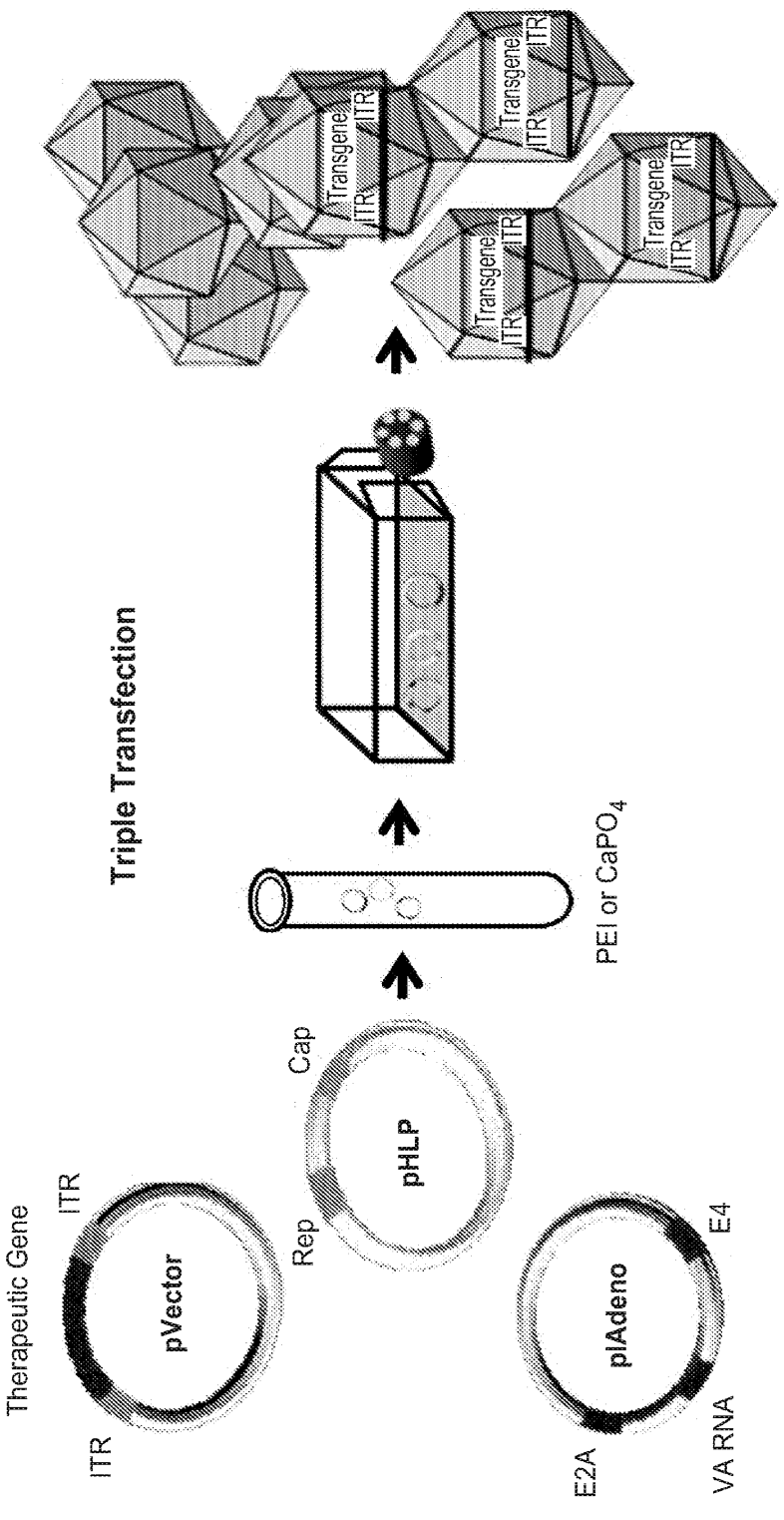

FIG. 4

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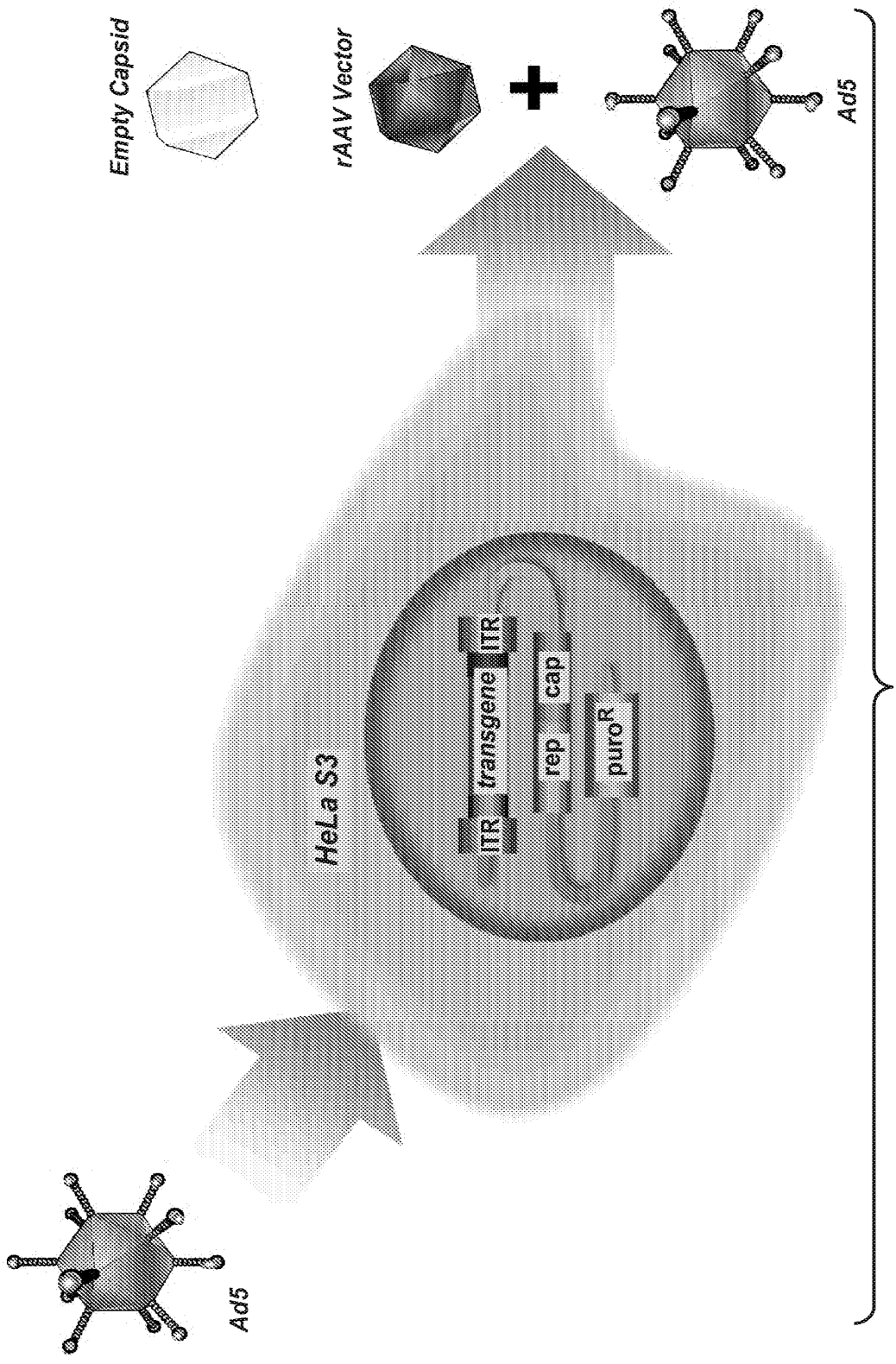
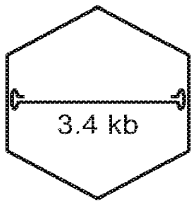

Empty Capsid
rAAV Vector
Ad5
HeLa S3
ITR
transgene
ITR
rep
cap
puroR
Ad5

FIG. 5

FIG. 6A



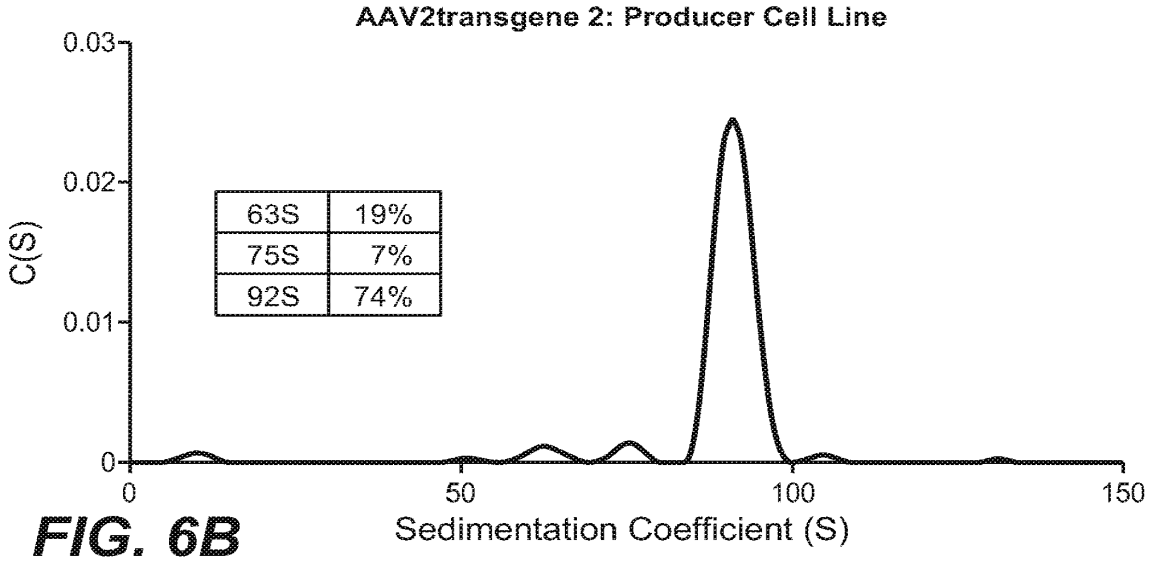


FIG. 6B

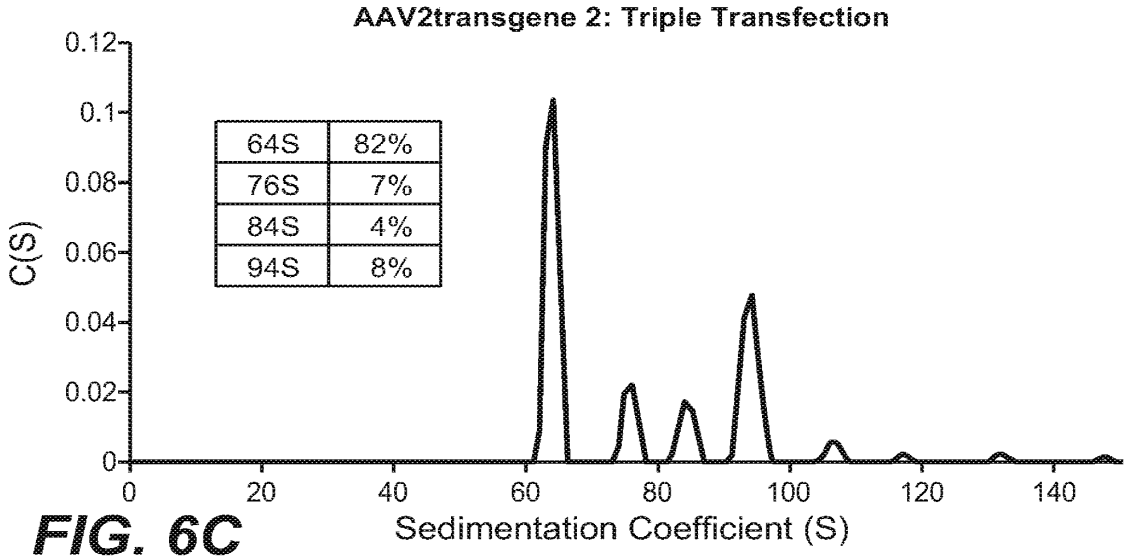


FIG. 6C

SUBSTITUTE SHEET (RULE 26)

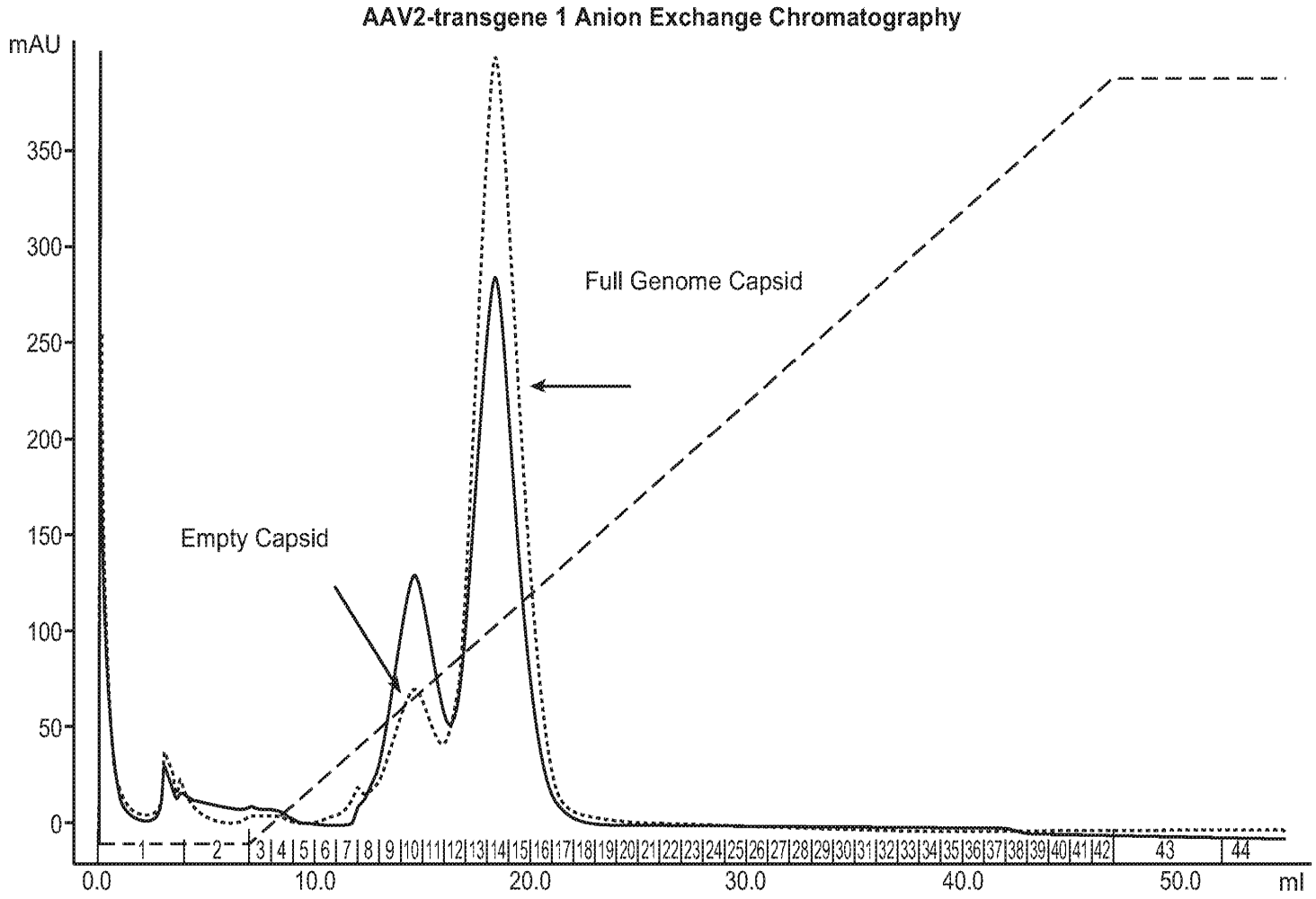


FIG. 7A

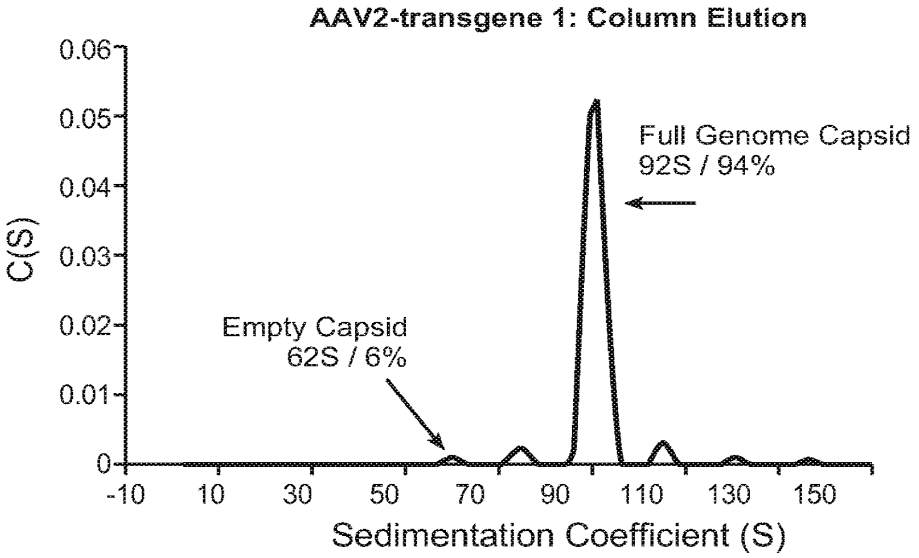

AAV2-transgene 1: Column Elution
0.06
0.05
0.04
0.03
0.02
0.01
0
C(S)
Full Genome Capsid
92S / 94%
Empty Capsid
62S / 6%
-10
10
30
50
70
90
110
130
150
Sedimentation Coefficient (S)

FIG. 7B

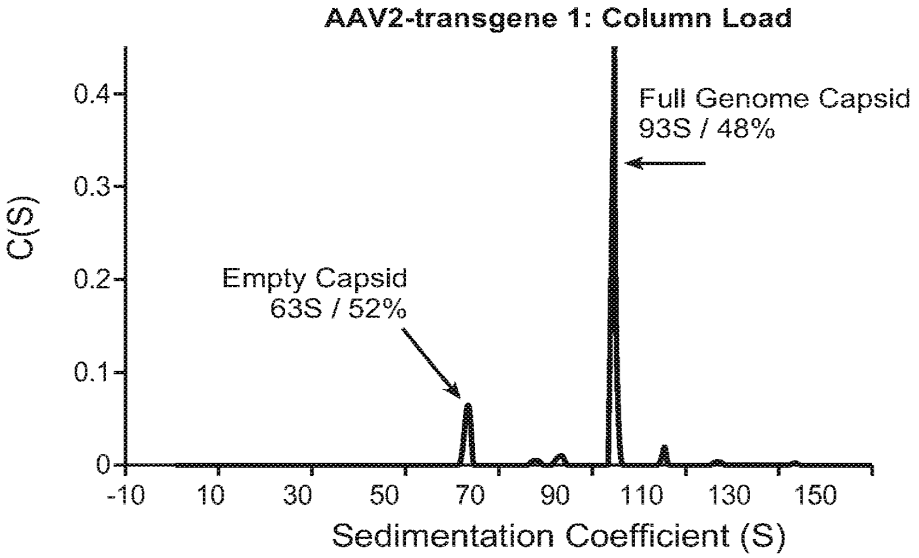

AAV2-transgene 1: Column Load
0.4
0.3
0.2
0.1
0
C(S)
Full Genome Capsid
93S / 48%
Empty Capsid
63S / 52%
-10
10
30
50
70
90
110
130
150
Sedimentation Coefficient (S)

FIG. 7C

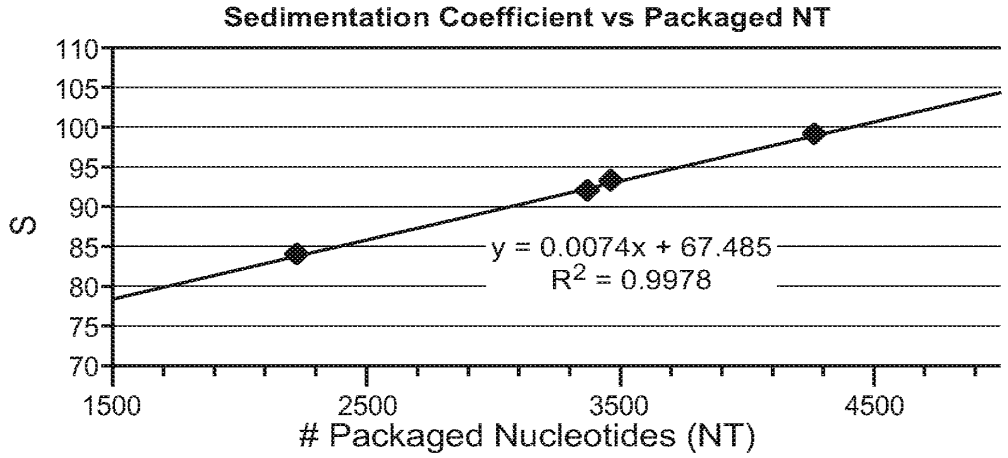

Sedimentation Coefficient vs Packaged NT
S
110
105
100
95
90
85
80
75
70
y = 0.0074x + 67.485
R2 = 0.9978
1500
2500
3500
4500
Packaged Nucleotides (NT)

FIG. 8

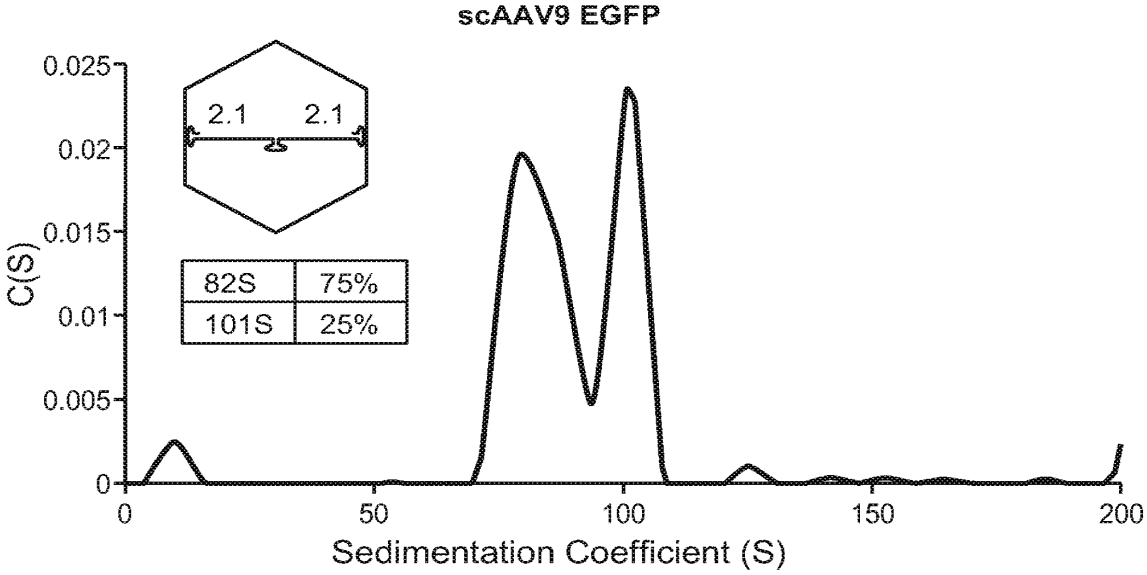

scAAV9 EGFP
C(S)
0.025
0.02
0.015
0.01
0.005
0
2.1
2.1
82S
75%
101S
25%
0
50
100
150
200
Sedimentation Coefficient (S)

FIG. 9A

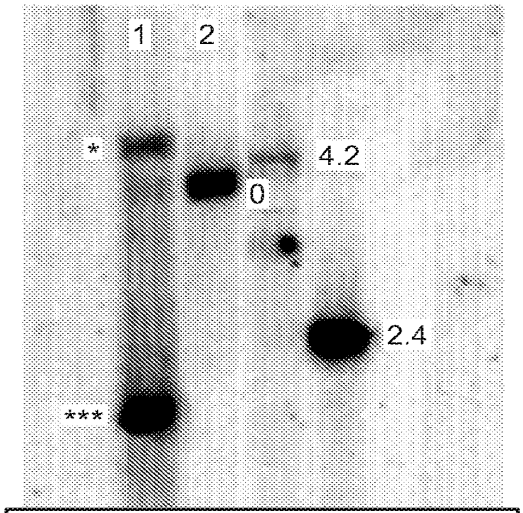

1
2
*
4.2
0
2.4
***
0 ss Monomer (4000b) ss Vector
* Dimer (4400b)
*** Monomer (2200b)
sc Vector

FIG. 9B

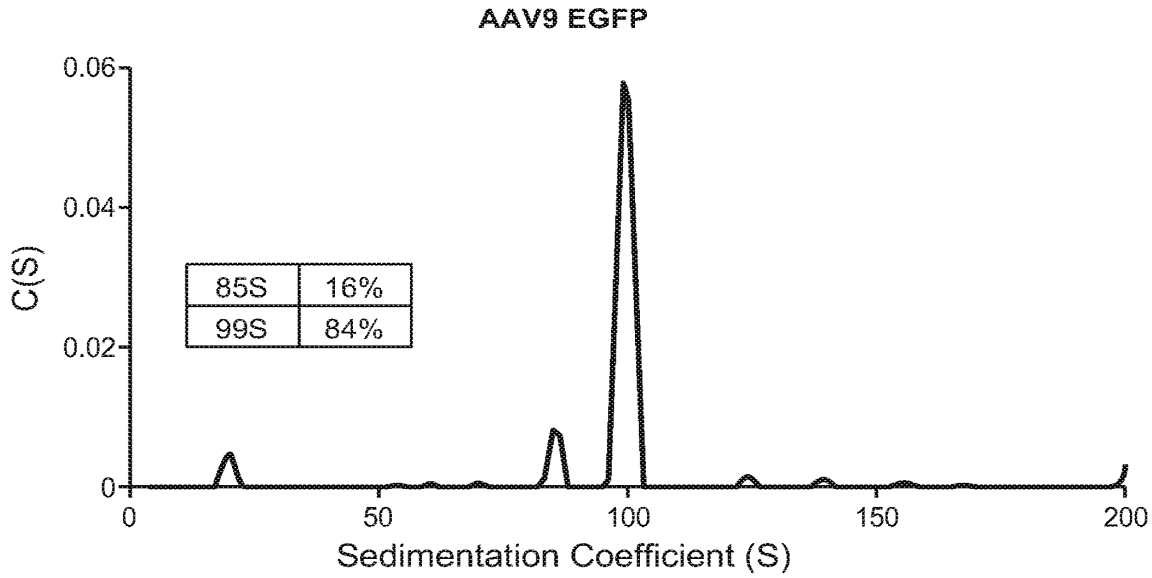
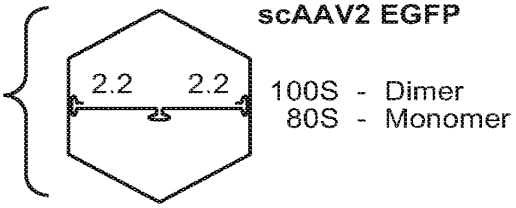

AAV9 EGFP
0.06
0.04
0.02
0
C(S)
85S 16%
99S 84%
0
50
100
150
200
Sedimentation Coefficient (S)

FIG. 9C

FIG. 10A



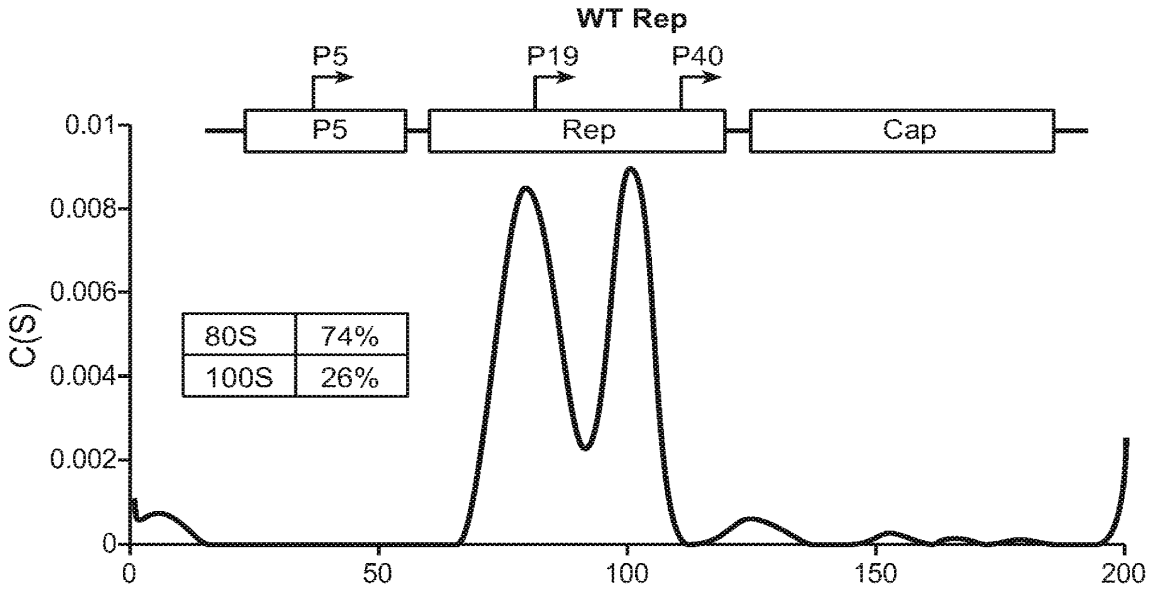


FIG. 10B

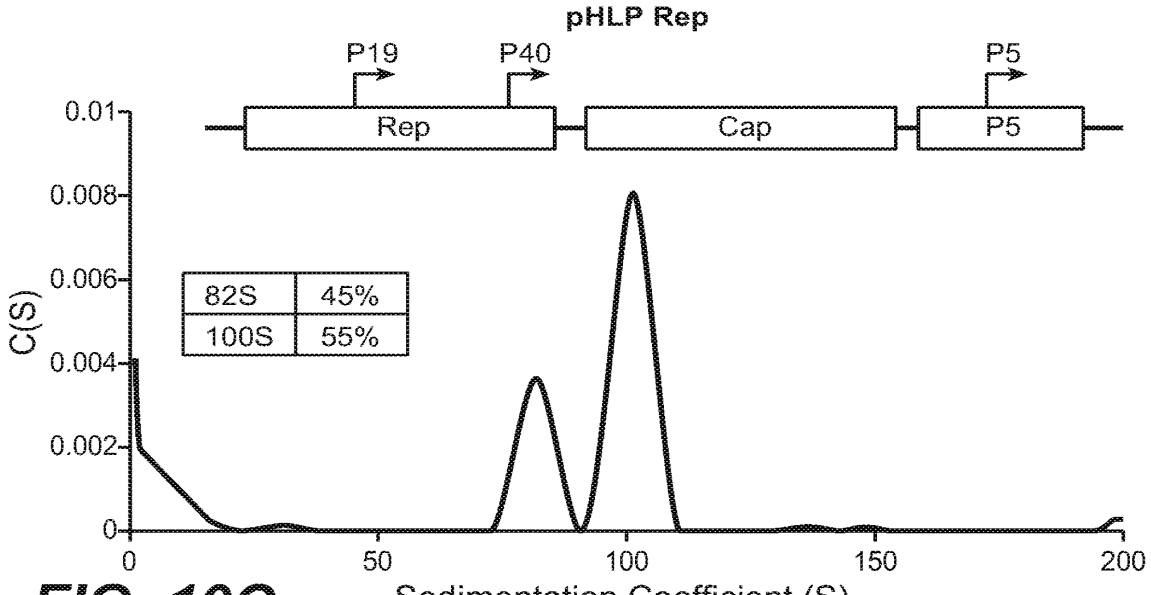


FIG. 10C

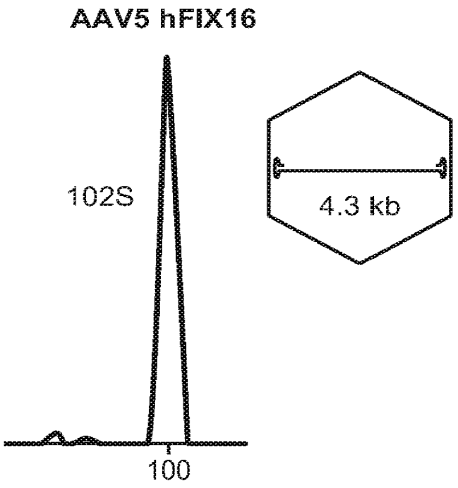


FIG. 11A

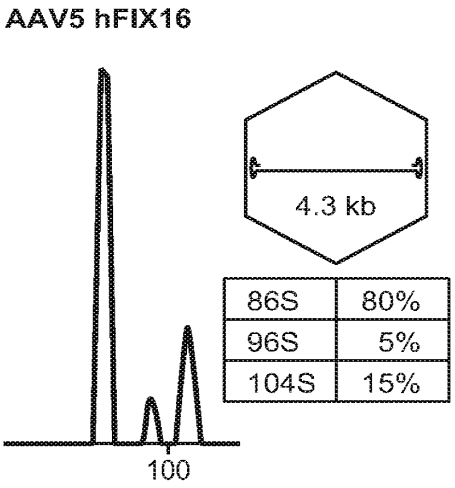


FIG. 11B

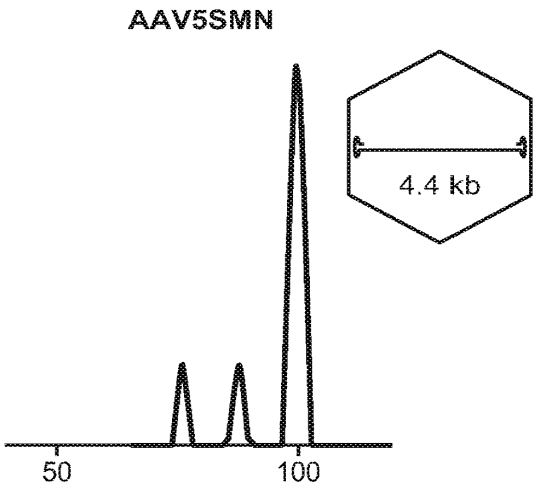


FIG. 11C

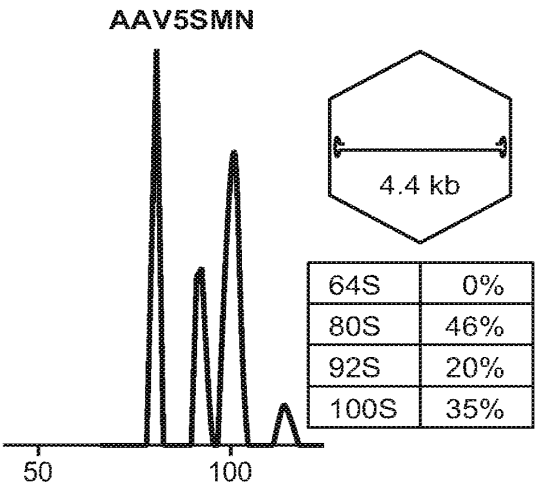
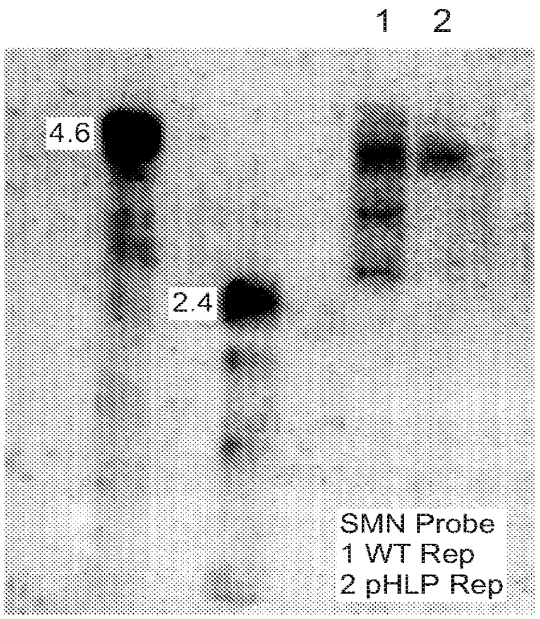
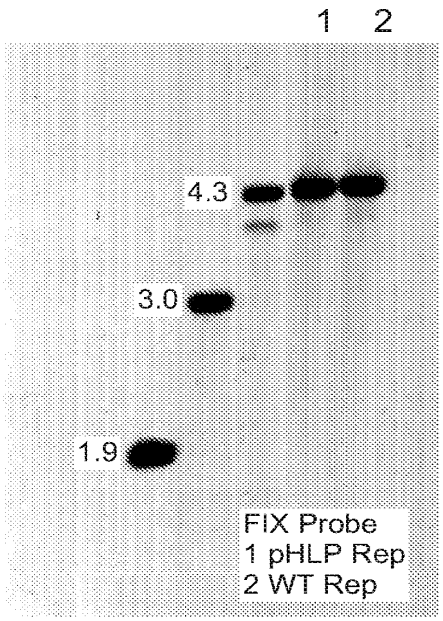


FIG. 11D



SMN Probe

FIG. 12A



FIX Probe

FIG. 12B

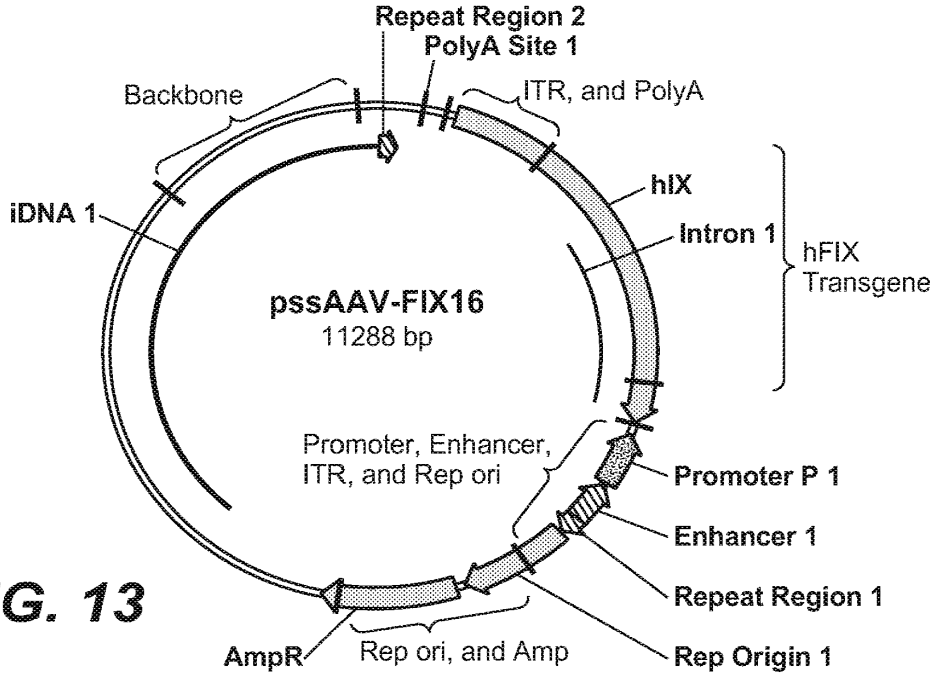
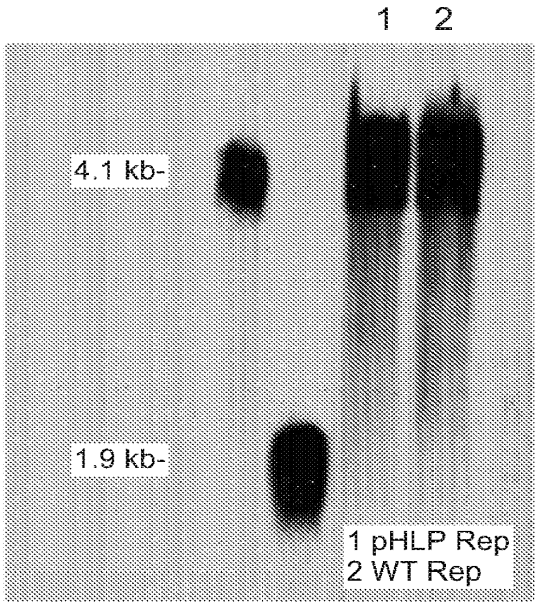
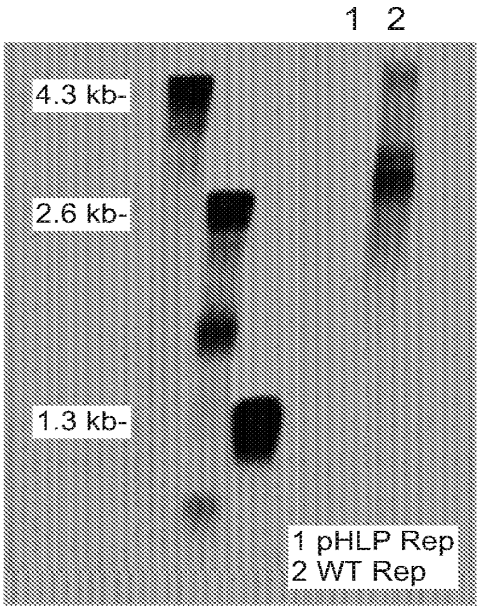


FIG. 13



Transgene

FIG. 14A



Rep ori & Amp

FIG. 14B

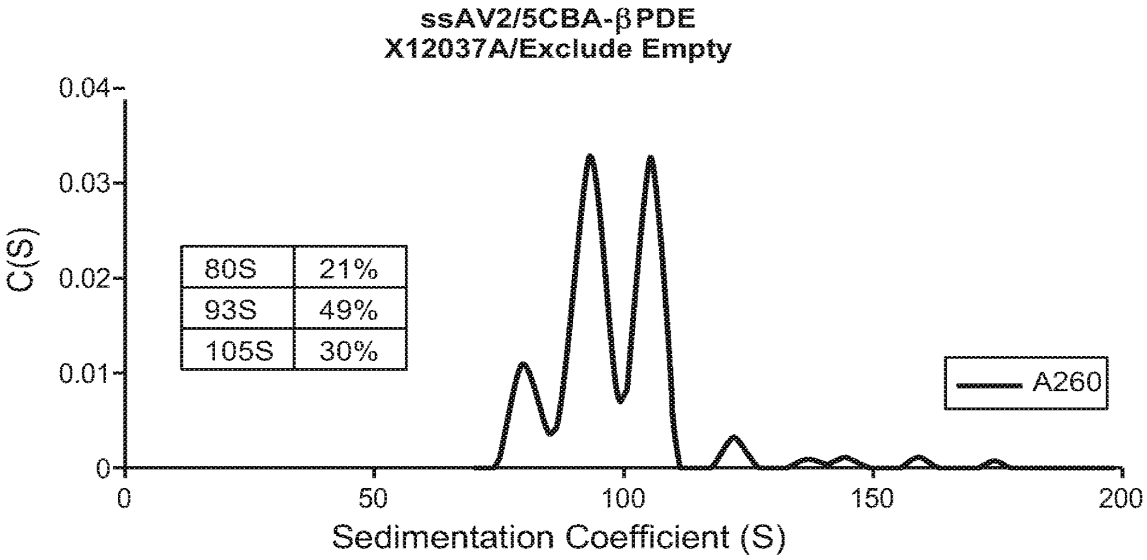


FIG. 15A

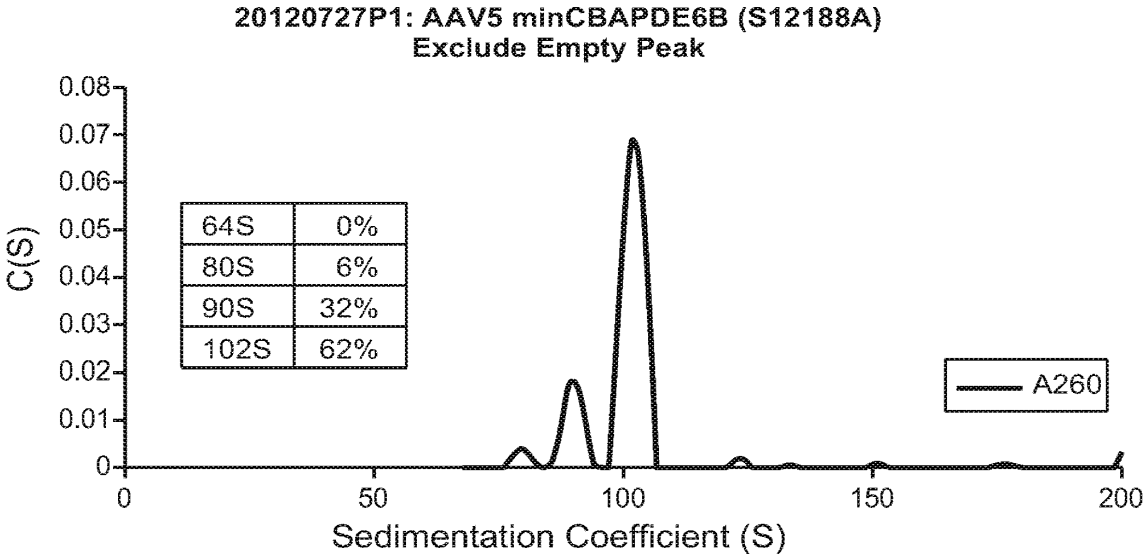


FIG. 15B

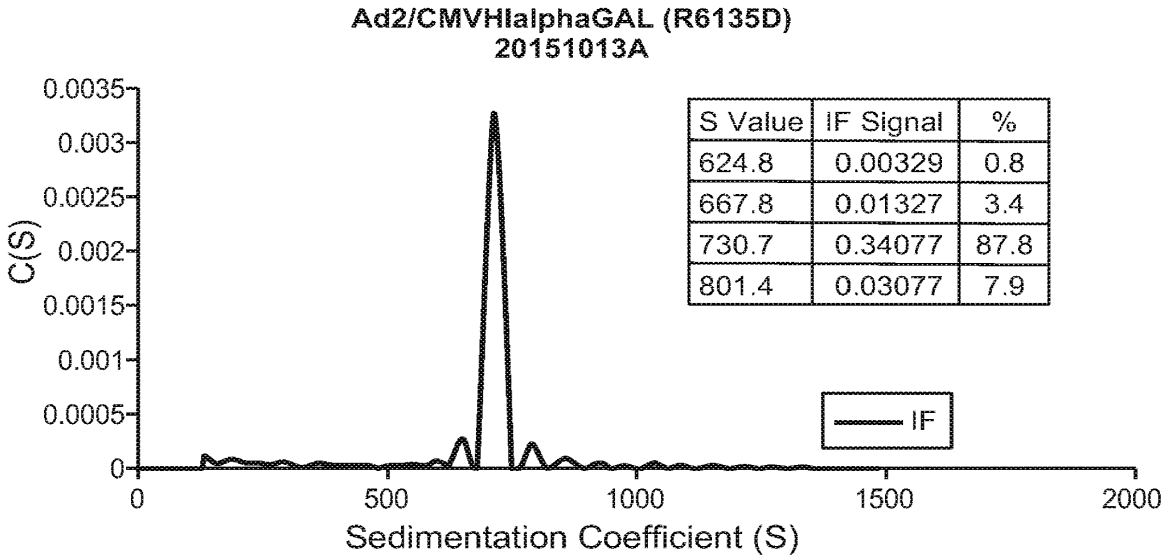

Ad2/CMVHIalphaGAL (R6135D)
20151013A
C(S)
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
0
500
1000
1500
2000
Sedimentation Coefficient (S)
IF
S Value | IF Signal | %
624.8 | 0.00329 | 0.8
667.8 | 0.01327 | 3.4
730.7 | 0.34077 | 87.8
801.4 | 0.03077 | 7.9

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/013947

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N7/02 G01N9/30
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ewoud Van Tricht ET AL: "MSc Chemistry Analytical Sciences Virus Particle Characterization Techniques to Quantify Virus Particle Aggregation and Integrity", , 1 July 2013 (2013-07-01), pages 1-51, XP055258342, Retrieved from the Internet: URL:https://esc.fnwi.uva.nl/thesis/centraal/files/f318319369.pdf [retrieved on 2016-03-15] Whole doc., in particular p.23-24 (esp. Fig.7); p.44 (bottom para.)-45; Table 2 (p.46); Table 3 (p.48). ----- -/--	1-95

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 April 2016	12/04/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Roscoe, Richard

2

Form PCT/ISA/210 (second sheet) (April 2005)

Sarepta Exhibit 1002, Page 520

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BERKOWITZ ET AL: "Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation", ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS INC, NEW YORK, vol. 362, no. 1, 1 March 2007 (2007-03-01) , pages 16-37, XP022056699, ISSN: 0003-2697, DOI: 10.1016/J.AB.2006.11.031 Whole doc., in partic. abstract; p.17; p.18(col.2, l. 19-21); Fig.8C; p.31, col.1	1-95
X	XIAOYU YANG ET AL: "Determination of Particle Heterogeneity and Stability of Recombinant Adenovirus by Analytical Ultracentrifugation in CsCl Gradients", JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 97, no. 2, 1 February 2008 (2008-02-01), pages 746-763, XP055258325, WASHINGTON, US ISSN: 0022-3549, DOI: 10.1002/jps.21008 Whole doc., in partic. abstract; p.749, col.2; p.751, col.2; p.753	1-95
A	J. FRASER WRIGHT: "Product-Related Impurities in Clinical-Grade Recombinant AAV Vectors: Characterization and Risk Assessment", BIOMEDICINES, vol. 2, no. 1, 3 March 2014 (2014-03-03), pages 80-97, XP009183697, Whole doc., in partic. abstract; table 2 (p.85)	1-95
X,P	BRENDA BURNHAM ET AL: "Analytical Ultracentrifugation as an Approach to Characterize Recombinant Adeno-Associated Viral Vectors", HUMAN GENE THERAPY METHODS, vol. 26, no. 6, 1 December 2015 (2015-12-01), pages 228-242, XP055258343, ISSN: 1946-6536, DOI: 10.1089/hgtb.2015.048 Whole doc. - applicants scientific disclosure of invention.	1-95

2

PTO/AIA/82B (07-13)
Approved for use through 11/30/2014. OMB 0651-0051
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number	Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)

[X] I hereby appoint Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above: 89300

OR

[ ] I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter to:

[X] The address associated with the above-mentioned Customer Number

OR

[ ] The address associated with Customer Number:

OR

Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

GENZYME CORPORATION

[ ] Inventor or Joint Inventor (title not required below)

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature	/Andrea Q. Ryan/	Date (Optional)	July 23, 2014
Name	Andrea Q. Ryan		
Title	Attorney-In-Fact		

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

[X] Total of 1 forms are submitted.

sf-3407816

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Catherine	R.	O'RIORDAN	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Cambridge	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Sanofi, Patent Department
Address 2	450 Water Street

City	Cambridge	State/Province	MA
Postal Code	02141	Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Brenda		BURNHAM	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Hopkinton	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	c/o Sanofi, Patent Department
Address 2	450 Water Street

City	Cambridge	State/Province	MA
Postal Code	02141	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

PTO/AIA/14 (02-18)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

☐ **An Address is being provided for the correspondence Information of this application.**			
Customer Number	89300		
Email Address	PatentDocket@mofo.com	Add Email	Remove Email

Application Information:

Title of the Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES		
Attorney Docket Number	15979-20103.03	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	17	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	89300		

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Continuation of	18/188,176	2023-03-22

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
18/188,176	Continuation of	16/547,144	2019-08-21	11639887	2023-05-02

Prior Application Status	Patented				Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16/547144	Continuation of	15/544,498	2016-01-19	10429288	2019-10-01

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
15/544,498	a 371 of international	PCT/US2016/013947	2016-01-19

Prior Application Status	Expired		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
PCT/US2016/013947	Claims benefit of provisional	62/105,714	2015-01-20

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (02-18)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Genzyme Corporation		
Mailing Address Information For Applicant:			
Address 1	450 Water Street		
Address 2			
City	Cambridge	**State/Province**	MA
Country	US	Postal Code	02141
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Seth Snyder/			Date (YYYY-MM-DD)	2023-11-10
First Name	Seth	Last Name	Snyder	Registration Number	60,243

Additional Signature may be generated within this form by selecting the Add button. Add

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Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	15979-20103.03
	Application Number	
Title of Invention	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES	

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Doc Code: TRACK1.REQ
Document Description: TrackOne Request

PTO/AIA/424 (04-14)

CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

First Named Inventor:	Catherine R. O'RIORDAN	Nonprovisional Application Number (if known):	Not Yet Assigned
Title of Invention:	ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES		

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.

1. The processing fee set forth in 37 CFR 1.17(i)(1) and the prioritized examination fee set forth in 37 CFR 1.17(c) have been filed with the request. The publication fee requirement is met because that fee, set forth in 37 CFR 1.18(d), is currently $0. The basic filing fee, search fee, and examination fee are filed with the request or have been already been paid. I understand that any required excess claims fees or application size fee must be paid for the application.

2. I understand that the application may not contain, or be amended to contain, more than four independent claims, more than thirty total claims, or any multiple dependent claims, and that any request for an extension of time will cause an outstanding Track I request to be dismissed.

3. The applicable box is checked below:

I. ☑ Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.

---OR---

(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed inventor's oath or declaration under 37 CFR 1.63 or 37 CFR 1.64 for each inventor, **or** the application data sheet meeting the conditions specified in 37 CFR 1.53(f)(3)(i) is filed with the application.

II. ☐ Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

Signature /Seth Snyder/	Date November 10, 2023
Name (Print/Typed) Seth Snyder	Practitioner Registration Number 60,243

Note: *This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.**

☐ *Total of _____ forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Box No. VIII (iv) DECLARATION: INVENTORSHIP (only for the purposes of the designation of the United States of America)

The declaration must conform to the following standardized wording provided for in Section 214; see Notes to Boxes Nos. VIII, VIII(i) to (v) (in general) and the specific Notes to Box No. VIII (iv). If this Box is not used, this sheet should not be included in the request.

Declaration of Inventorship (Rules 4.17(iv) and 51*bis*.1(a)(iv))
for the purposes of the designation of the United States of America:

I hereby declare that I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

This declaration is directed to the international application of which it forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/ US2016/013947 (if furnishing declaration pursuant to Rule 26*ter*.)

I hereby declare that the above identified international application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

Name: Catherine R. O'RIORDAN

Residence: Waban, Massachusetts
(city and either US state, if applicable, or country)

Mailing Address: c/o Sanofi
55 Corporate Drive
Mail Code: 55A-505A
Bridgewater, New Jersey, 08807: UNITED STATES OF AMERICA

Catherine O'Riordan 3/16/2016
Inventor's Signature Date
(The signature must be that of the inventor, not that of the agent)

Name: Brenda BURNHAM

Residence: Hopkinton, MA
(city and either US state, if applicable, or country)

Mailing Address: c/o Sanofi
55 Corporate Drive
Mail Code: 55A-505A
Bridgewater, New Jersey 08807: UNITED STATES OF AMERICA

Inventor's Signature Date
(The signature must be that of the inventor, not that of the agent)

Name:

Residence:
(city and either US state, if applicable, or country)

Mailing Address:

Inventor's Signature Date
(The signature must be that of the inventor, not that of the agent)

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)".

Box No. VIII (iv) DECLARATION: INVENTORSHIP (only for the purposes of the designation of the United States of America)

The declaration must conform to the following standardized wording provided for in Section 214; see Notes to Boxes Nos. VIII, VIII(i) to (v) (in general) and the specific Notes to Box No. VIII (iv). If this Box is not used, this sheet should not be included in the request.

Declaration of inventorship (Rules 4.17(iv) and 51*bis*.1(a)(iv)) for the purposes of the designation of the United States of America:

I hereby declare that I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

This declaration is directed to the international application of which it forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/ US2016/013947 (if furnishing declaration pursuant to Rule 26*ter*.)

I hereby declare that the above identified international application was made or authorized to be made by me.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

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Inventor's Signature _____ Date _____
(The signature must be that of the inventor, not that of the agent)

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Brenda Burnham 3/16/2016
Inventor's Signature Date
(The signature must be that of the inventor, not that of the agent)

Name:

Residence:
(city and either US state, if applicable, or country)

Mailing Address:

Inventor's Signature _____ Date _____
(The signature must be that of the inventor, not that of the agent)

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)".

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
18/506,853	**11/10/2023 06:08:29 PM Z ET**	**15979-20103.03**

Title of Invention

ANALYTICAL ULTRACENTRIFUGATION FOR CHARACTERIZATION OF RECOMBINANT VIRAL PARTICLES

Application Information

APPLICATION TYPE	Utility - Nonprovisional Application under 35 USC 111(a)	PATENT #	-
CONFIRMATION #	3457	FILED BY	Aurelia Caparas
PATENT CENTER #	63219170	FILING DATE	-
CUSTOMER #	89300	FIRST NAMED INVENTOR	Catherine R. O'RIORDAN
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Seth Snyder

Documents

TOTAL DOCUMENTS: 55

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
Track_1.pdf		2	Track One Request	139 KB
ADS.pdf		9	Application Data Sheet	1227 KB
SPEC.pdf		93	-	423 KB
SPEC-SPEC.pdf	(1-78)	78	Specification	396 KB
SPEC-CLM.pdf	(79-92)	14	Claims	112 KB
SPEC-ABST.pdf	(93-93)	1	Abstract	84 KB
Decs.pdf		2	Oath or Declaration filed	131 KB

Prelim_.pdf		8	-	111 KB
Prelim_-A.PE.pdf	(1-1)	1	Preliminary Amendment	76 KB
Prelim_-CLM.pdf	(2-6)	5	Claims	102 KB
Prelim_-REM.pdf	(7-8)	2	Applicant Arguments/Remarks Made in an Amendment	78 KB
SB08_IDS.pdf		10	Information Disclosure Statement (IDS) Form (SB08)	268 KB

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POA.pdf	1	Power of Attorney	826 KB
Figs.pdf	17	Drawings-other than black and white line drawings	1659 KB
FOR_7_WO2016118520A1.pdf	107	Foreign Reference	7674 KB
Arisaka_2011_375-377.pdf	8	Non Patent Literature	1222 KB
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Curriculum Vitae of Jeffrey C. Hansen_AUG222022_IPR2023-1044_8pgs.pdf	8	Non Patent Literature	203 KB
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Declaration of Steven A. Berkowitz_JUN302023_IPR2023-1045_EP_132pgs.pdf	132	Non Patent Literature	5978 KB
Defendants Initial Invalidity Contentions_NOV062023_34pgs.pdf	34	Non Patent Literature	609 KB
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Patent Owners Mandatory Notices_JUL112023_IPR2023-01044_5pgs.pdf	5	Non Patent Literature	110 KB
Patent Owners Mandatory Notices_JUL112023_IPR2023-01045_5pgs.pdf	5	Non Patent Literature	110 KB
Genzyme POA_JUL112023_IPR2023-01045_4pgs.pdf	4	Non Patent Literature	107 KB
Genzyme POA_JUL102023_IPR2023-01044_4pgs.pdf	4	Non Patent Literature	108 KB
Genzyme_Prelim_Response_OCT192023_IPR2023-01045_71pgs.pdf	71	Non Patent Literature	1396 KB
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IDS_CL.pdf	3	Transmittal Letter	79 KB
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Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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Declaration of Steven A. Berkowitz_JUN302023_IPR2023-1045_EP_132pgs.pdf	0D016714A83647DACC85AC94464AADECC41C2BC45F0718219588F54111954C7A097EFA3D8586904CA50DDF5B2F397F7C48A243B37E8D220D464A2F7F23C0DBF6
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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	Art Unit	Not Yet Assigned
	Examiner Name	Not Yet Assigned
	Attorney Docket Number	15979-20103.03

U.S. PATENTS

Examiner Initial*	Cite No	Patent Number	Kind Code[1]	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1.	US-5,622,856-A		04-22-1997	Natsoulis et al.	English Equivalent to JPH11-512926A
	2.	US-6,989,264-B2		01-24-2006	Atkinsin et al.	
	3.	US-6,001,650-A		12-14-1999	Colosi, P.	
	4.	US-7,348,134-B2		03-2008	Lingappa; Jaisri R.	
	5.	US-8,137,948-B2		05-21-2003	Qu et al.	
	6.	US-10,429,288-B2		10-01-2019	O'riordan et al.	
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	2.	US-2020/0225139-A1		07-16-2020	O'riordan et al.	
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Examiner Initial*	Cite No	Country Code[2]	Foreign Document Number[3]	Kind Code[4]	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear	T[5]
	1.	JPH11-512926A			11-09-1999	Avigen, Inc.	English equivalent WO-1997006272-A2,A3	X
	2.	JP2003-511037A			03-25-2003	Genovo, Inc.	English equivalent WO01/025462	X
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	6.	WO-2014125101-A1			08-21-2014	Genethon		
	7.	WO-2016118520-A1			07-28-2016	Genzyme Corporation		

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Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[5]
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	14.	Curriculum Vitae of Jeffrey C. Hansen for U.S. Patent No. 10,429,288, dated August 22, 2022, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 8 pages.	
	15.	Curriculum Vitae of Jeffrey C. Hansen for U.S. Patent No. 10,429,288, dated August 22, 2022, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 8 pages.	
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	41.	Notice of Filing Date Accorded to Petition and Time for Filing Patent Owner Preliminary Response for U.S. Patent No. 10,429,288, mailed July 11, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 6 pages.	
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	44.	Patent Owner's Mandatory Notices Pursuant to 37 C.F.R. Section 42.8 for U.S. Patent No. 10,429,288, dated July 11, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 5 pages.	
	45.	Patent Owner's Power of Attorney for U.S. Patent No. 10,429,288, dated July 11, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	

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	46.	Patent Owner's Power of Attorney for U.S. Patent No. 10,429,288, dated July 10, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	47.	Patent Owner's Preliminary Response for U.S. Patent No. 10,429,288, dated October 19, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 71 pages.	
	48.	Patent Owner's Preliminary Response for U.S. Patent No. 10,429,288, dated October 11, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 81 pages.	
	49.	Petition for Inter Partes Review for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 80 pages.	
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	51.	Petitioner Novartis Gene Therapies, Inc.'s Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	52.	Petitioner Novartis Gene Therapies, Inc.'s Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	53.	Petitioner Novartis Pharmaceuticals Corporation's Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	54.	Petitioner Novartis Pharmaceuticals Corporation's Power of Attorney and Designation of Lead and Backup Counsel for U.S. Patent No. 10,429,288, dated June 20, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 3 pages.	
	55.	Petitioner's Exhibit List for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1045, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 4 pages.	

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	56.	Petitioner's Exhibit List for U.S. Patent No. 10,429,288, dated June 30, 2023, Case No. IPR2023-1044, Petitioner Novartis Gene Therapies Inc. and Novartis Pharmaceuticals Corporation, Patent Owner Genzyme Corporation, 4 pages.	
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EXAMINER SIGNATURE			
Examiner Signature		Date Considered	

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

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[1] See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. [2] Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). [3] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document.
[4] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [5] Applicant is to place a check mark here if English language translation is attached.

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	Attorney Docket Number	15979-20103.03

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e) (1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e) (2).

☐ See attached certification statement.

☐ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Seth Snyder/	Date (YYYY-MM-DD)	2023-11-10
Name/Print	Seth Snyder	Registration Number	60,243

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