

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SAREPTA THERAPEUTICS, INC.,

Petitioner

v.

GENZYME CORPORATION,

Patent Owner

U.S. Patent No. 12,013,326

“Analytical Ultracentrifugation for
Characterization of Recombinant Viral Particles”

IPR2026-00149

PETITION FOR *INTER PARTES* REVIEW

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EX1006	WO 2014/125101 A1 (PCT/EP2014/052978) and certified English translation, “Methods for the Production of Double-Stranded AAV Viral Particles,” published on August 21, 2014 (“Le Bec”)
EX1007	de la Maza and Carter, “DNA Structure of Incomplete Adeno-Associated-Virus Particles” in REPLICATION OF MAMMALIAN PARVOVIRUSES (Ward, D., and Tattersall, P., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 193-204 (1978) (“de la Maza”)
EX1008	de la Maza and Carter, “Molecular Structure of Adeno-Associated Virus Variant DNA” <i>J. Biol. Chem.</i> 255:3194-3203 (1980) (“de la Maza (April 1980)”)
EX1009	Cole <i>et al.</i> , “Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium” in METHODS IN CELL BIOLOGY, VOL. 84, 143-179 (2008) (“Cole”)
EX1010	Sommer <i>et al.</i> , “Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement” <i>Molecular Therapy</i> 7:122-28 (2003) (“Sommer”)

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EX1025	Cole and Hansen, “Analytical Ultracentrifugation as a Contemporary Biomolecular Research Tool” <i>J. Biomolecular Techniques</i> 10:163-176 (1999) (“Cole (1999)”)
EX1026	Gao <i>et al.</i> , “Empty virions in AAV8 vector preparations reduce transduction efficiency and may cause total viral particle dose-limiting side effects” <i>Molecular Therapy – Methods & Clinical Development</i> 9:1-8 (2014) (“Gao”)
EX1027	Gombold <i>et al.</i> , “Lot Release and Characterization Testing of Live-Virus-Based Vaccines and Gene Therapy Products, Part 2” <i>BioProcess International</i> 4:56-65 (2006) (“Gombold”)
EX1028	Hoque <i>et al.</i> , “Chimeric Virus-like Particle Formation of Adeno-Associated Virus” <i>Biochemical and Biophysical Research Communications</i> 266:371–376 (1999) (“Hoque”)
EX1029	European Medicines Agency, Committee for Medicinal Products for Human Use, Glybera Assessment Report (July 19, 2012)
EX1030	Ward and Tattersall, Preface, in REPLICATION OF MAMMALIAN PARVOVIRUSES (Ward, D., and Tattersall, P., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ix-x (1978) (“Ward”)
EX1031	Schuck, “Size-Distribution Analysis of Macromolecules by Sedimentation Velocity Ultracentrifugation and Lamm Equation Modeling” <i>Biophysical Journal</i> 78:1606-1619 (2000) (Schuck”)

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EX1033	Carter, “Adeno-associated virus and the development of adeno-associated virus vectors: a historical perspective” <i>Molecular Therapy</i> 10:981-89 (2004) (“Carter (2004)”).
EX1034	Carter, “My Pathway to Adeno-Associated Virus and Adeno-Associated Virus Gene Therapy: A Personal Perspective” <i>Human Gene Therapy</i> 31:494-98 (2020) (“Carter (2020)”)
EX1035	Tratschin <i>et al.</i> , “A human parvovirus, adeno-associated virus as a eukaryotic vector: transient expression and encapsidation of the prokaryotic gene for chloramphenicol acetyl transferase” <i>Molecular Cell Biology</i> 4:2072–81 (1984) (“Tratschin”)
EX1036	Hermonat and Muzyczka, “Use of adeno-associated virus as a mammalian DNA cloning vector: transduction of neomycin resistance into mammalian tissue culture cells” <i>Proc. Natl. Acad. Sci. USA</i> 81:6466-70 (1984) (“Hermonat”)
EX1037	Carter <i>et al.</i> , “Specific Cleavage of Adenovirus-Associated Virus DNA by Restriction Endonuclease R·EcoRI – Characterization of Cleavage Products” <i>Virology</i> 63:523-528 (1975) (“Carter (1975)”)
EX1038	Carter <i>et al.</i> , “Genome Localization of Adeno-Associated Virus RNA” <i>J. Virology</i> 19:1044-1053 (1976) (“Carter (1976)”)
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EX1041	Lock <i>et al.</i> , “Analysis of particle content of recombinant adeno-associated virus serotype 8 vectors by ion-exchange chromatography” <i>Human Gene Therapy Methods</i> 23(1):56-64 (2012) (“Lock”)

LIST OF CHALLENGED CLAIMS

Claim	Element
1 [pre]	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:
1[a]	(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;
1[b]	(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, and determining the sedimentation coefficients of the viral particles comprising one or more of the fragmented genomes in the preparation; and
1[c]	(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.
2	The method of claim 1, wherein step (iii) comprises comparing the sedimentation coefficients of the one or more viral particles comprising the fragmented genomes with a standard curve generated 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	Element
3	The method of claim 1, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm.
4	The method of claim 1, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm.
5	The method claim 4, wherein the boundary sedimentation is performed at a temperature of from about 4°C to about 20°C.
7	The method of claim 1, wherein the fragmented genomes are sub-genomic DNA molecules.
8	The method of claim 1, wherein the centrifugation is run from about 0.5 hours to about 2 hours.
9	The method of claim 8, wherein the centrifugation is run from about 1 hours to about 2 hours.
12	The method of claim 1, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is greater than 5×10^{11} vg/mL.
13	The method of claim 1, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is from about 1×10^{11} vg/mL to about 1×10^{13} vg/mL.
14	The method of claim 1, further comprising quantifying of the viral particles comprising the one or more fragmented genomes in the preparation.
15	The method of claim 1, comprising determining the size of all of the fragmented genomes in the preparation.
16	The method of claim 15, further comprising quantifying all of the fragmented genomes in the preparation.
17	The method of claim 1, wherein the preparation comprises AAV9 capsid proteins.

Claim	Element
18	The method of claim 17, wherein the preparation further comprises at least one AAV2 ITR.
19	The method of claim 16, wherein the preparation comprises self-complementary AAV viral genomes.
20 [pre]	A method of determining the 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:
20[a]	(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;
20[b]	(ii) measuring the rate of movement or migration of the sedimenting boundaries, wherein movement or migration of each species of the 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;
20[c]	(iii) determining the sedimentation coefficients of each species of the individual viral particles in the heterogeneous mixture of viral particles; and
20[d]	(iv) quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles.

Claim	Element
21	The method of claim 20, 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 from sedimentation coefficients of viral particles comprising recombinant AAV genomes of known nucleotide sizes.
22	The method of claim 20, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm.
23	The method of claim 20, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm.
25	The method of claim 20, wherein the centrifugation is run from about 0.5 hours to about 2 hours.
26	The method of claim 20, wherein the total concentration of viral particles in the heterogeneous mixture prior to step (i) is greater than 5×10^{11} vg/mL.
27	The method of claim 20, wherein the total concentration of viral particles in the heterogeneous mixture prior to step (i) is from about 1×10^{11} vg/mL to about 1×10^{13} vg/mL.
28	The method of claim 20, wherein the viral particles comprise AAV9 capsid proteins.
29	The method of claim 28, wherein the viral particles comprise at least one AAV2 ITR.
30	The method of claim 20, further comprising determining the relative percentage of each species of the individual viral particles in the heterogeneous mixture of viral particles.

Sarepta Therapeutics, Inc. (“Sarepta” or “Petitioner”) respectfully requests *inter partes* review of claims 1-5, 7-9, 12-23 and 25-30 (the “challenged claims”) of U.S. Patent No. 12,013,326 (“the ’326 patent”). EX1001. The ’326 patent is assigned to Genzyme Corporation (“Genzyme” or “Patent Owner”). EX1013, ¶31.

I. INTRODUCTION

The ’326 patent relates to analyzing recombinant adeno-associated virus (“rAAV”) preparations using analytical ultracentrifugation (“AUC”). AUC can be used to separate different viral particle species in an AAV preparation, including AAV particles containing fragmented genomes.

During prosecution, Patent Owner obtained allowance of the challenged claims by arguing that “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.” EX1002 (04/09/24 Amendment and Response), 143. According to Patent Owner, the named inventors “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.” *Id.* In particular, Patent Owner argued that “[t]he ***linear relationship*** between sedimentation coefficients and molecular weights of genomes was not appreciated in the art prior to the present invention.” *Id.* (emphasis added). However, as shown below, Patent Owner’s representations during prosecution were incorrect.

A book chapter published in 1978 (referred to herein as “de la Maza”) describes methods using ultracentrifugation to characterize and quantify AAV particles with fragmented genomes. EX1007. De la Maza was not cited or considered during prosecution. However, contrary to Patent Owner’s arguments, this reference expressly discloses the correlation between “[t]he density of the incomplete virus particles” and “the size of the incomplete DNA” within these particles. EX1007, 203. As de la Maza explains in a subsequent article discussing the earlier experiments, “the particle density has an approximately *direct linear relationship* to the strand length.” EX1008, 3202 (emphasis added). Thus, the allegedly distinguishing characteristic that Patent Owner relied on during prosecution to obtain allowance of the challenged claims was well understood and disclosed in the prior art more than 35 years before the earliest priority application for the ’326 patent.

Moreover, Patent Owner was aware of the groundbreaking work in the research group of de la Maza’s advisor and co-author, Barrie Carter, involving the characterization of AAV. Indeed, Patent Owner cites a different article from the Carter lab twice in the specification of the ’326 patent. That article, published in 1980, discloses the mechanism by which the viral genome is inserted into a fully formed capsid to form a mature AAV particle. EX1001, 43:26-29 (citing EX1012), 45:17-20 (same). Notably, however, Patent Owner never cited either of the

contemporaneous de la Maza publications from the Carter lab characterizing AAV particles with fragmented genomes. This was a material omission that allowed Patent Owner to overcome the Examiner's rejections during prosecution and obtain allowance of the challenged claims of the '326 patent.

The Examiner's allowance was in error because the Examiner (i) was not aware of relevant prior art references (*i.e.*, de la Maza (1978) (EX1007) and de la Maza (April 1980) (EX1008)) that were highly material to the patentability of the challenged claims, and (ii) was led astray by Patent Owner's argument that the named inventors were the first to recognize the linear relationship between sedimentation coefficients and the molecular weights of fragmented genomes in AAV particles.

Petitioner respectfully submits that the challenged claims are unpatentable. This conclusion is supported by the prior art of record, Patent Owner's arguments and admissions, and the declaration of Dr. Steven Berkowitz (EX1004), an expert in AUC, including its application to viral preparations (EX1005).

II. MANDATORY NOTICES

A. Real Parties-in-Interest (37 C.F.R. §42.8(b)(1))

Petitioner identifies Sarepta Therapeutics, Inc. and Sarepta Therapeutics Three, LLC as real parties-in-interest.

B. Related Matters (37 C.F.R. §42.8(b)(2))

Petitioner identifies the following related matters. The '326 patent is being asserted in currently-pending litigation: *Genzyme Corp. v. Sarepta Therapeutics, Inc., et al.*, C.A. No. 24-cv-00882-RGA (D. Del.). EX1014.

Petitioner is unaware of any other lawsuits involving the '326 patent.

C. Related Patent Office Proceedings

This is the first petition challenging a claim of the '326 patent.

Petitioner is also concurrently filing a petition requesting *inter partes* review of claims 1-7 and 10-30 of a related patent, U.S. Patent No. 12,031,894 ("the '894 patent). The '894 patent and the '326 patent both claim priority to U.S. Patent Application No. 15/544,498, filed on January 19, 2016, and U.S. Provisional Application No. 62/105,714, filed on January 20, 2015.

D. Lead and Back-up Counsel and Service Information

Petitioner provides the following counsel and service information. Pursuant to 37 C.F.R. §42.10(b), a Power of Attorney accompanies this Petition.

Lead Counsel	Robert Wilson (Reg. No. 45,227) Quinn Emanuel Urquhart & Sullivan, LLP 295 Fifth Avenue, 9th Floor New York, New York 10016 robertwilson@quinnemanuel.com (212) 849-7000
Back-Up Counsel	Anne Toker (Reg. No. 53,692) Quinn Emanuel Urquhart & Sullivan, LLP 295 Fifth Avenue, 9th Floor New York, New York 10016 annetoker@quinnemanuel.com (212) 849-7000
	James Glass (Reg. No. 46,729) Quinn Emanuel Urquhart & Sullivan, LLP 295 Fifth Avenue, 9th Floor New York, New York 10016 jimglass@quinnemanuel.com (212) 849-7000

III. REQUIREMENTS FOR IPR

A. Payment of Fees

The undersigned authorizes the Office to charge the fee required for this Petition for *inter partes* review to Deposit Account No. 50-5708.

B. Grounds for Standing

Petitioner certifies that the '326 patent is available for IPR and Petitioner is not barred or estopped from requesting IPR on the grounds identified herein. Petitioner further certifies that the prohibitions of 35 U.S.C. §§315(a)-(b) are inapplicable.

C. Statement of Relief Requested

Petitioner respectfully requests review and cancellation of claims 1-5, 7-9, 12-23, and 25-30 of the '326 patent. The challenged claims should be found unpatentable on the following grounds:

Prior Art References	
Le Bec (EX1006); published in 2014; prior art under AIA §102(a)(1).	
de la Maza (EX1007); published in 1978; prior art under AIA §102(a)(1).	
Cole (EX1009); published in 2008; prior art under AIA §102(a)(1).	
Sommer (EX1010); published in 2003; prior art under AIA §102(a)(1).	

Ground	Claims	Description
1	1-4, 7, 12-13, 15, 17-18	Obvious over Le Bec and de la Maza
2	5, 8-9	Obvious over Le Bec and de la Maza, in view of Cole
3	14, 16, 19, 20-23, 26-30	Obvious over Le Bec and de la Maza, in view of Sommer
4	25	Obvious over Le Bec and de la Maza, in view of Sommer and Cole

De la Maza (EX1007) was not cited to or considered by the Examiner during prosecution. EX1001 ("References Cited"); EX1002.

Cole (EX1009) is listed on the '326 patent and is cited in the specification. EX1001, 20:9-12. Cole was the subject of a §103 rejection during prosecution. EX1002 (01/09/24 Office Action), 175-78. In response, Patent Owner did not attempt to distinguish Cole, but instead, argued that the prior art did not disclose methods for determining the size of the DNA in AAV particles with fragmented genomes. EX1002 (04/09/24 Amendment and Response), 148-49.

Sommer (EX1010) is listed on the '326 patent and is cited in the specification. EX1001, 24:38-41, 44:51-55, 46:42-45. Sommer was not the subject of a rejection during prosecution. EX1002.

Le Bec (EX1006) was the subject of §102 and §103 rejections during prosecution. EX1002 (01/09/24 Office Action), 172-78. In response, Patent Owner argued that Le Bec was distinguishable because the prior art did not disclose the linear relationship between sedimentation coefficients and the size of the DNA in AAV particles with fragmented genomes. EX1002 (04/09/24 Amendment and Response), 142-43, 150-51. However, Patent Owner's arguments were incorrect in light of the disclosures in de la Maza. EX1007, 195 (Table 1), 196 (Figure 1).

De la Maza reports the linear relationship between the density of an AAV particle and the size of the nucleic acid inside the capsid. EX1007, 203; EX1008, 3194, 3202; EX1011, 449. The allegedly distinguishing characteristic of the

challenged claims was disclosed in the prior art, contrary to Patent Owner's arguments during prosecution. Thus, the challenged claims are unpatentable.

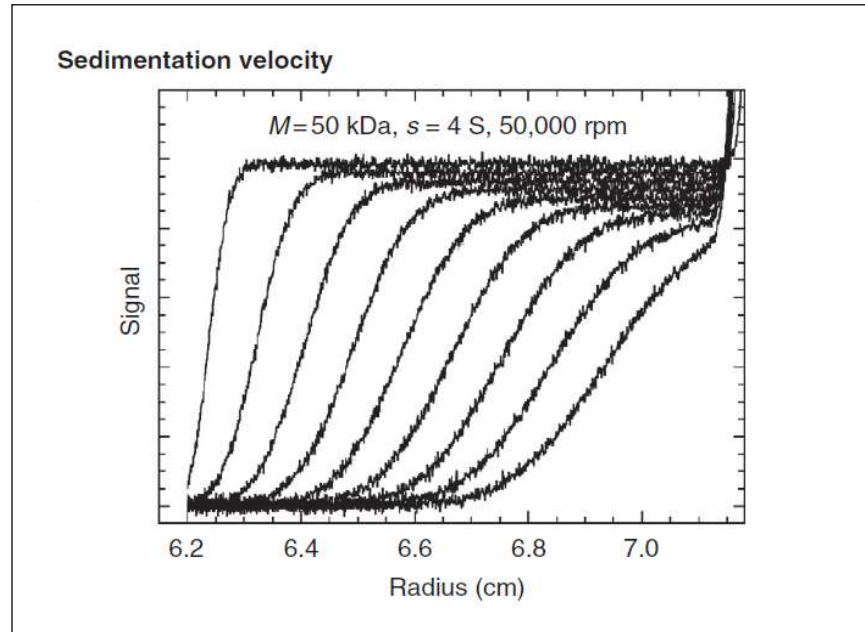
IV. BACKGROUND OF THE TECHNOLOGY

A. Analytical Ultracentrifugation (AUC)

Theodore Svedberg invented the analytical ultracentrifuge and was awarded the Nobel Prize in Chemistry in 1926. EX1004, ¶23. The fundamental principles of analytical ultracentrifugation have changed little since that time. EX1004, ¶¶23-29; EX1023, 144; EX1025, 163; EX1001, 19:37-43.

The claims of the '326 patent involve a type of AUC called sedimentation velocity analytical ultracentrifugation ("SV-AUC"). EX1001, 55:13-36 (claim 1), 56:17-42 (claim 20). SV-AUC is a technique used to characterize macromolecules and nanoparticles in solution. EX1004, ¶¶23, 25-31. SV-AUC involves spinning a sample fast enough to force suspended particles or dissolved molecular species to migrate in solution while collecting data on their differing sedimentation velocities (*i.e.*, rates of movement). *Id.*, ¶¶30-31, 43. Particles moving at the same rate form a boundary in response to the applied centrifugal force, and a series of optical scans are taken to measure the rate of movement (sedimentation velocity) of the boundaries over time. *Id.*, ¶¶31-33, 43; EX1025, 165. The figure below illustrates a representative scanning profile of boundary sedimentation velocity depicting the

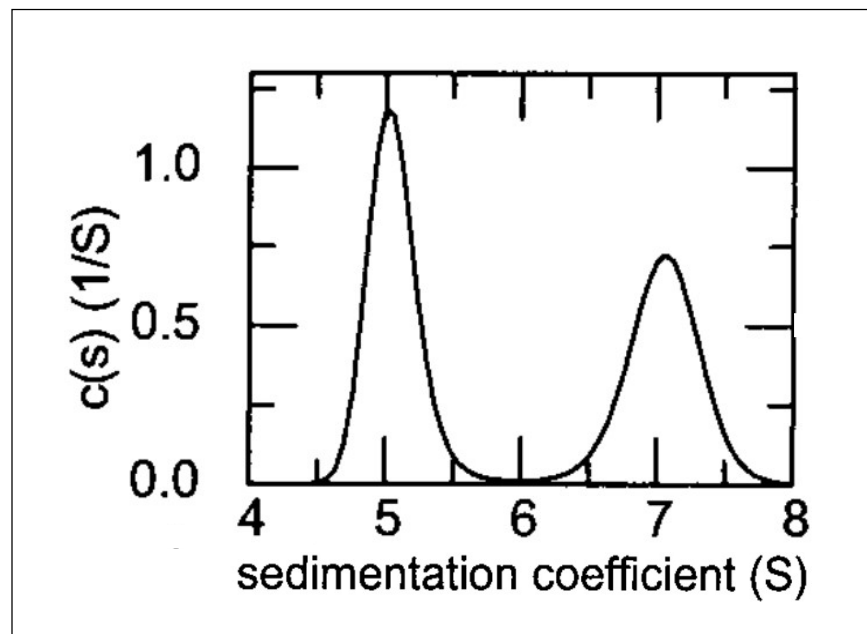
absorbance (signal) versus the radius (cm) for a single macromolecule species (a 50 kDa protein) measured at 20-minute intervals.



EX1009, 162 (Figure 1A).

Sedimentation velocity is a function of the mass, shape, and density of a particle, and is reported as a sedimentation coefficient (s) measured in Svedberg units, S (10^{-13} s). EX1009, 146; EX1004, ¶¶33-39. SV-AUC can separate, identify, and quantify different species of particles in a heterogenous mixture by their sedimentation coefficients. EX1004, ¶¶65-66. A higher S-value indicates a molecule that sediments more quickly, suggesting a higher mass or a more compact shape, while a lower S-value indicates a molecule that sediments more slowly, suggesting a lower mass or a less compact shape. *Id.*, ¶¶39-42.

Prior to 2015, a variety of computer software programs were publicly available to determine sedimentation coefficients from SV-AUC data. *Id.*, ¶¶44-49; EX1009, 146-47; EX1024, 228. For example, in 2000, Peter Schuck created a freely downloadable program called SEDFIT, which models the sedimentation boundaries measured in an SV-AUC experiment and calculates the sedimentation coefficient for each of the observed species in a sample. EX1004, ¶45; EX1031, 1610. Programs such as SEDFIT can also graphically display sedimentation coefficient distribution profiles and determine the relative concentrations of the observed species. EX1004, ¶¶45-47. The figure below illustrates the distribution profile generated by the SEDFIT program from the SV-AUC data for a sample with two species:



EX1032, 1109 (Figure 10).

In the figure, the sedimentation coefficient, measured in Svedberg units (S), is plotted along the x-axis. EX1004, ¶49. A peak at a specific S-value corresponds to a species with that sedimentation rate. *Id.* The concentration of species with a given sedimentation coefficient, expressed as $c(s)$, is plotted along the y-axis. *Id.* The area under each peak can be integrated to determine the relative concentration of each species in the sample. *Id.* In this example, the distribution profile shows two species with sedimentation coefficients 5S and 7S. *Id.*; EX1032, 1109 (Figure 10).

The relationship between sedimentation coefficient and molar mass can be expressed mathematically according to the following equation, which was published by Svedberg in 1926 and is now widely known as the “Svedberg equation:”

$$s = M (1 - \bar{v}\rho) / (N_a f)$$

where s is the sedimentation coefficient of the particle, M is the molar mass of the particle, $\bar{v}$ is the partial specific volume of the particle (the inverse of the density of the particle), ρ is the density of the solvent, N_a is Avogadro’s number, and f is the frictional coefficient of the particle. *See, e.g.*, EX1025, 165-66; EX1009, 146; EX1024, 216.

For particles having about the same size and shape, such as rAAV particles, the frictional coefficient (f) is approximately constant. Thus, for mixtures of rAAV particles, the Svedberg equation provides that the sedimentation coefficient (s) is

directly proportional to the molar mass (M) and density ($1/\bar{v}$) of the particle. EX1004, ¶¶41-42.

B. Adeno-Associated Virus (AAV)

Adeno-associated viruses (AAV) were discovered over 60 years ago. *See* EX1033, 982; EX1034, 494. In the decades that followed, AAV was studied and characterized extensively. *See* EX1004, ¶¶50-61. In the 1970s and early 1980s, this research focused on the molecular biology of AAV. EX1033, 982-83; EX1034, 495.

For example, working in Barrie Carter's lab at the NIH, Luis de La Maza analyzed the variant genomes that accumulate with undiluted passage of wild-type AAV. EX1034, 495; *see* EX1007, 196-97, 200-201; EX1008, 3202. These experiments confirmed that there is a linear relationship between the density of an AAV particle and the length of the genome packaged inside the capsid. EX1007, 203; EX1008, 3194, 3202; EX1011, 449. Also in the Carter lab, Maureen Myers studied the formation of AAV particles. EX1034, 495. Myers showed that single-stranded AAV genomes are packaged into preformed capsids to form viral particles of approximately the same size, regardless of the length of the DNA. EX1033, 983-84, EX1034, 495; EX1012, 71-72, 79-82.

This early research laid the foundation for the development of AAV vectors for gene therapy applications. EX1034, 495. In 1984, the Carter lab and Nicholas Muzyczka's lab at the University of Florida separately published papers disclosing

the first rAAV vectors that could express foreign genes. EX1033, 985-86; EX1034, 496; EX1035; EX1036; *see also* EX1040, 491 (describing Carter and Muzyczka as the “fathers of rAAV vectors”). By the mid-1980s, there was definitive proof-of-concept that rAAVs could transduce mammalian cells to express foreign transgenes. EX1033, 986. By the end of the 1980s, work had begun in the Carter lab on a specific AAV vector for the treatment of cystic fibrosis (CF). EX1034, 496; *see also* EX1004 ¶¶62-64.

C. Characterization of AAV Preparations Using AUC

By 2015, SV-AUC had been identified as a useful tool for characterizing rAAV particles developed for gene therapy applications. EX1004, ¶¶65-66; EX1027, 4-5; EX1025.

For example, a 2012 Assessment Report from the European Medicines Agency identified analytical ultracentrifugation as a method used to “determine mass, density and [SV-AUC] distribution profiles” for Glybera, a gene therapy product comprising a replication deficient AAV vector. EX1029, 15; *see also* EX1028, 373-374, Fig. 3; EX1026, 6.

Similarly, Le Bec used SV-AUC to characterize preparations of rAAV particles and reported the results in a patent application published in 2014. *See* EX1006. Thus, before the earliest priority date for the ’326 patent, the field was

aware of SV-AUC, its usefulness in characterizing biomolecules of different types, and its actual application to the development of gene therapy products.

V. THE '326 PATENT

The '326 patent is generally directed to “methods to characterize preparations of recombinant viral particles using analytical ultracentrifugation.” EX1001, Abstract. The patent acknowledges that AUC is a tool that “may be applied to determine the biophysical properties of many types of particles across a wide range of particle concentrations and sizes” and that AUC had been available for decades. EX1001, 19:37-43. The challenged claims recite methods for using AUC to characterize preparations of rAAV viral vectors, including AAV particles with fragmented genomes. EX1001, 55:13-57:6.

During prosecution, Patent Owner argued that the prior art did not disclose methods for determining the length of DNA in AAV particles with fragmented genomes as a function of the sedimentation coefficient. EX1002 (04/09/24 Amendment and Response), 142-43. However, contrary to Patent Owner’s argument, the sedimentation characteristics of AAV particles with fragmented genomes were well understood and the relationship between the density of an AAV particle and the size of the genome had been disclosed decades before the earliest priority date for the '326 patent. EX1006; EX1007; EX1008; EX1011.

A. Priority Date

The '326 patent claims priority to U.S. Application No. 15/544,498, filed on January 19, 2016, and U.S. Provisional Application No. 62/105,714, filed on January 20, 2015. EX1001, (60), (63); EX1003. Thus, the earliest priority date for the challenged claims is January 20, 2015.

B. The Claims

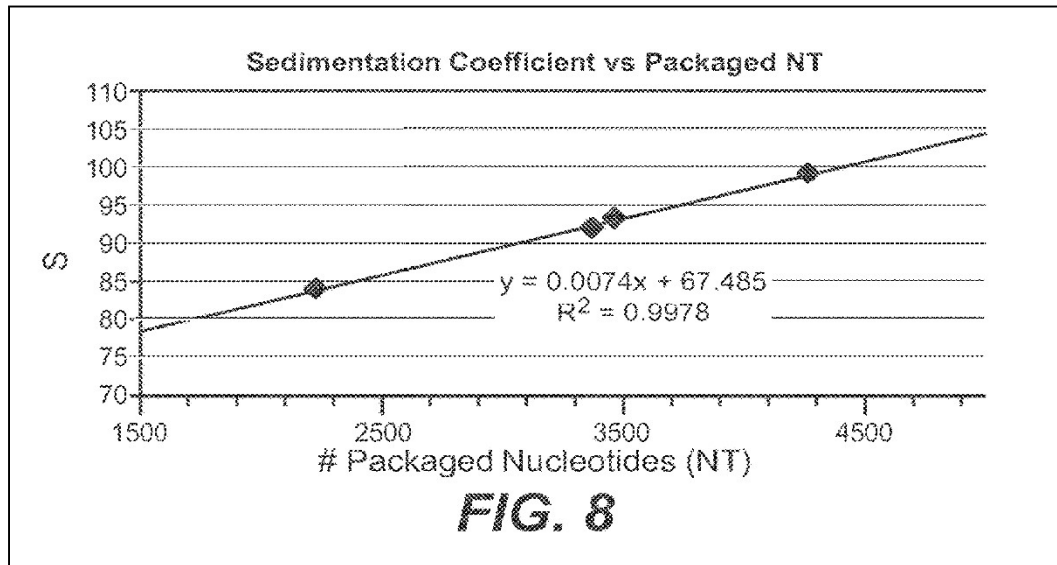
Claims 1-19 of the '326 patent are directed to methods for determining the size of one or more fragmented genomes in a preparation of rAAV particles. EX1001, 55:13-56:16. Claim 1 is an independent claim. Claims 2-19 are dependent claims.

Claims 20-30 are directed to methods for determining the molar concentration of each of the viral particles in a heterogeneous mixture of rAAV particles. EX1001, 56:17-57:6. Claim 20 is an independent claim. Claims 21-30 are dependent claims.

C. The Specification

The '326 patent acknowledges that a POSA would know how to optimize AUC parameters (*e.g.*, time, temperature, rotor speed) for the analysis of rAAV particles. EX1001, 26:19-21 (“It is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles.”), 30:1-3 (“Suitable ultracentrifugation conditions, analysis algorithms, and other parameters may be determined empirically through methods known in the art.”).

To characterize AAV with fragmented genomes, applicants measured the S values of rAAV vectors with genomes of known nucleotide length. *Id.*, 44:64-45:5, 49:58-67. Applicants plotted the S values as a function of genome length in nucleotides (“NT”). This data is shown in Figure 8, which is reproduced below:



Id., Figure 8. Figure 8 is a plot of four data points showing the measured S values for rAAVs with packaged genomes of approximately 2200, 3400, 3450, and 4300 nucleotides in length. *Id.*, 9:36-40, Figure 8. A best fit line for these data is also shown, along with the formula for the best fit line and the calculated R^2 value for the measured data. *Id.*

Applicants used the best fit line in Figure 8 as a “standard curve” to calculate the predicted genome length for rAAVs that sediment at selected S-values. *Id.*, 49:64-67. Applicants also calculated the extinction coefficient at 260 nm for rAAVs with a predicted genome length according to the formulas published in Sommer. *See*

id., 24:38-41, 44:51-55, 46:42-45 (citing EX1010). These calculated values are reported in Table 5. *Id.*, 50:1-19 (Table 5).

D. The Prosecution History

The '326 patent issued from U.S. Application No. 18/506,853 (“the '853 application”), filed on November 10, 2023. The '853 application is a continuation of U.S. Application No. 15/544,498 (“the '498 application”), filed on January 19, 2016. EX1001 (21), (22), (63).

1. U.S. Application No. 15/544,498

The '498 application (the parent application of the '326 patent) issued as U.S. Patent No. 10,429,288 (“the '288 patent”) on October 1, 2019. EX1015, (10) (21), (45). The claims of the '288 patent are generally directed to SV-AUC methods for characterizing full and empty capsids in preparations of rAAV particles. *See* EX1015, 54:20-58:47.

On May 19, 2023, Genzyme sued Novartis Gene Therapies, Inc. and Novartis Pharmaceuticals Corporation (“Novartis”) for alleged infringement of the '288 patent. EX1017.

On June 30, 2023, Novartis filed two IPR petitions, challenging the claims of the '288 patent as anticipated and/or obvious in light of Le Bec. EX1018, 2-3; EX1019, 2. Le Bec was not cited during prosecution of the '288 patent. EX1016. In its preliminary response in IPR2023-01045, Genzyme disclaimed claims 4 and

10-14. *See* EX1021, 2. In January 2024, the Board granted institution of both IPRs on all remaining claims. EX1020, 41; EX1021, 28. On March 4, 2024, the Board granted the parties' motion to terminate the IPRs due to settlement of the district court litigation. EX1022, 3.

2. U.S. Application No. 18/506,853

During prosecution of the '326 patent, the Examiner rejected the pending claims as anticipated and/or obvious over Le Bec in combination with McLaughlin, Berkowitz, or Cole. EX1002 (01/09/24 Office Action), 172-78.

In response, Patent Owner argued that “[t]he 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,” including AAV “particles with fragmented genomes.” EX1002 (04/09/24 Amendment and Response), 142.

Patent Owner argued that “determining the molar concentrations of viral particles having fragmented genomes was not possible prior to the present invention.” *Id.*, 143. According to Patent Owner, “molar concentrations of a viral particle can only be determined if the molecular weights of the genomes within the viral particle are known,” but “[t]he molecular weights of fragmented genomes produced by standard methods (*e.g.*, triple transfection) are unknown.” *Id.*, 142.

Patent Owner argued that “[t]he 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.” *Id.*, 143. “As described in the present application, AUC is used to separate all of the viral particles, including viral particles with fragmented genomes,” and “[t]he sedimentation coefficients of the fragmented genomes are used to determine the molecular weight of the fragmented genomes.” *Id.* In particular, Patent Owner argued:

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.

Id. (emphasis added). As Patent Owner emphasized, “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.” *Id.*

Patent Owner distinguished the cited prior art references on this basis. *Id.*, 145-49. Patent Owner argued that “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.” *Id.*, 145. Patent Owner concluded:

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.

Id., 150-51 (emphasis added).

The Examiner accepted Patent Owner’s arguments and allowed the claims to issue. EX1002 (04/23/24 Notice of Allowance), 34.

VI. OVERVIEW OF THE PRIOR ART

A. de la Maza

De la Maza is a book chapter titled “DNA Structure of Incomplete Adeno-Associated-Virus Particles.” EX1007. De la Maza was published in 1978 and is prior art under AIA §102(a)(1). *Id.*

De la Maza characterizes the physical properties of “incomplete” genomes isolated from a preparation of AAV particles. EX1007, 193 (“We summarize here the physical properties of the incomplete AAV genomes.”); EX1004, ¶¶87-103.

Linear Relationship Between Density and DNA. De la Maza discloses that there is a linear relationship between the density of AAV particles with “incomplete”

genomes and the length of the DNA packaged inside the capsid. EX1007, 203 (“The density of the incomplete virus particles appears to be *correlated* with the size of the incomplete DNA.”) (emphasis added); *see also* EX1008, 3194 (“The density of the virus particle is generally *correlated* with the size of the variant DNA.”) (emphasis added); EX1011, 449 (“Elsewhere we have reported an analysis of the physical structure of DNA in each class of AAV particle (de la Maza and Carter, 1978). Briefly, the densities of the AAV particles which are lighter than 1.41 g/cm³ are *proportional* to the size of the encapsidated DNA molecule.”) (emphasis added).

As de la Maza explains in a follow-on article describing the earlier results, “[t]hese molecules appear to be encapsidated in AAV virions with usually only one strand per particle; thus, the particle density has an approximately *direct linear relationship* to the strand length.” EX1008, 3202 (emphasis added).

Fractionation of Virus Particles in CsCl Gradients. In de la Maza, virus particles in lysates of KB cells infected with AAV2 and Ad2 were fractionated by equilibrium density centrifugation in CsCl. EX1007, 194. The regions containing visible bands at densities of 1.45, 1.41, 1.35, and 1.32 g/cm³ were pooled and centrifuged to equilibrium in CsCl two or three more times. *Id.*

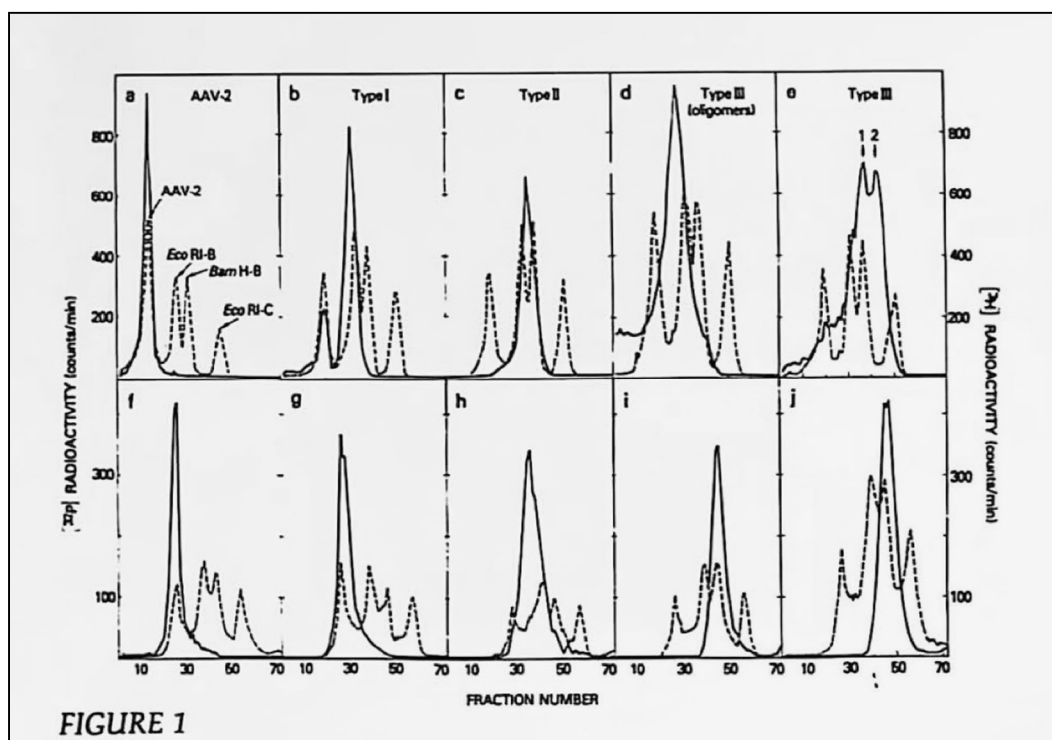
Fractionation of Incomplete DNA from Purified Virions. DNA was released from virions by sedimentation in alkaline sucrose gradients, then reannealed in formamide and fractionated in neutral sucrose gradients. *Id.* When DNA was

prepared from AAV virions of different densities, several classes of incomplete molecules were obtained – designated Type I, II, III, and IV. *Id.*, 194-96. The sedimentation coefficients and molecular weights of the different classes of incomplete DNA molecules are summarized in Table 1, which is reproduced below.

DNA	Density of virion ^a (g/cm ³)	Sedimentation coefficient and molecular weight of isolated DNA ^b			
		alkaline sucrose		neutral sucrose	
		S	m.w. × 10 ⁻⁶	S	m.w. × 10 ⁻⁶
Intact	1.41	15.5	1.4	14.5	2.8
Type I	1.41	15.5	1.4	11.5	1.4
Type II	1.41	12.0	0.8	10.0	0.8
Type III (oligomer)	1.35	8.5	0.3	8–15	~1.4
Type III	1.35	8.5	0.3	(1) 8.2 (2) 7.0	0.6 0.3
Type IV	1.32	5.5	~0.13	5.0	~0.13

Id., 195 (Table 1).

Physical Properties of Incomplete AAV DNA. The physical properties and molecular weights of Type I, II, and III DNA molecules were examined by sedimentation in alkaline and neutral sucrose gradients. *Id.*, 96. These results are shown in Figure 1, which is reproduced below.



Id., 196 (Figure 1).

The molecular weights and sedimentation coefficients listed in Table 1 were determined from sucrose gradient profiles using molecular-weight markers for AAV DNA, as shown in Figure 1: duplex monomer AAV2 DNA and restriction fragments *EcoRI* B (38% of AAV2 genome), *Bam* B (22%), and *EcoRI* C (4.5%). *Id.*, 195 (Table 1, fn.b), 196 (Figure 1, legend). These molecular-weight markers were developed in the Carter lab and published in 1975 and 1976. EX1037; EX1038.

De la Maza summarized the results as follows. EX1007, 201-03. The largest molecules analyzed (Type I) have a molecular weight of 1.4×10^6 in both alkaline and neutral sucrose gradients and appear to be hairpin duplexes. *Id.*, 201. Restriction-endonuclease cleavage indicated that both the right and left ends of the

molecule are present and that only 10-20% of the internal portion of the genome is missing from this population of molecules. *Id.* Type II molecules have a similar structure but contain only 20-30% of the left or the right terminal region. *Id.* Type III molecules have a more heterogeneous structure. *Id.* These molecules contain only the left or right 10-20% of the genome and form either hairpin molecules or non-cross-linked duplexes. *Id.* Some of these molecules may form oligomeric species held by the “cohesive ends” of the AAV DNA termini. *Id.* Finally, Type IV DNA molecules contain little other than the inverted terminal repetitions which comprise 147 nucleotides at each terminus. *Id.*, 200.

De la Maza concluded: “*The density of the incomplete virus particles appears to be correlated with the size of the incomplete DNA*, which suggests that each particle contains only one DNA molecule.” *Id.*, 203 (emphasis added). This implies that “virus assembly occurs by encapsidation of DNA into preformed capsids, since any model for crystallization of viral capsids around the DNA might be expected to yield particles with aberrant morphology with smaller DNA.” *Id.*

De la Maza was published as a collection of papers presented during a meeting at Cold Spring Harbor in 1977. EX1030, ix. In April 1980, de la Maza published an updated report of these experiments characterizing the physical properties of AAV variant DNA in the Journal of Biological Chemistry. *See* EX1008, 3194 (“A preliminary account of some of the properties of AAV variant DNA has been

presented before.”) (citing EX1007). In de la Maza (April 1980), the authors again note that “[t]he density of the virus particle is generally correlated with the size of the variant DNA.” EX1008, 3194 (emphasis added).

De la Maza (April 1980) further highlights the “direct linear relationship” between the density of an AAV particle and the length of the encapsidated DNA:

The variant DNA molecules extracted from AAV particles are a heterogenous population of strands ranging in size from nearly full genome length (4700 nucleotides) to only about 3% of genome length (150 nucleotides). These molecules appear to be encapsidated in AAV virions with usually only one strand per particle; *thus, the particle density has an approximately direct linear relationship to the strand length.*”

Id., 3202 (emphasis added).

The teachings in de la Maza confirm that the observed sedimentation characteristics of AAV particles are consistent with the Svedberg equation, whereby the sedimentation coefficient is directly proportional to mass and density for particles having approximately the same size and shape. EX1004, ¶¶100-103.

B. Le Bec

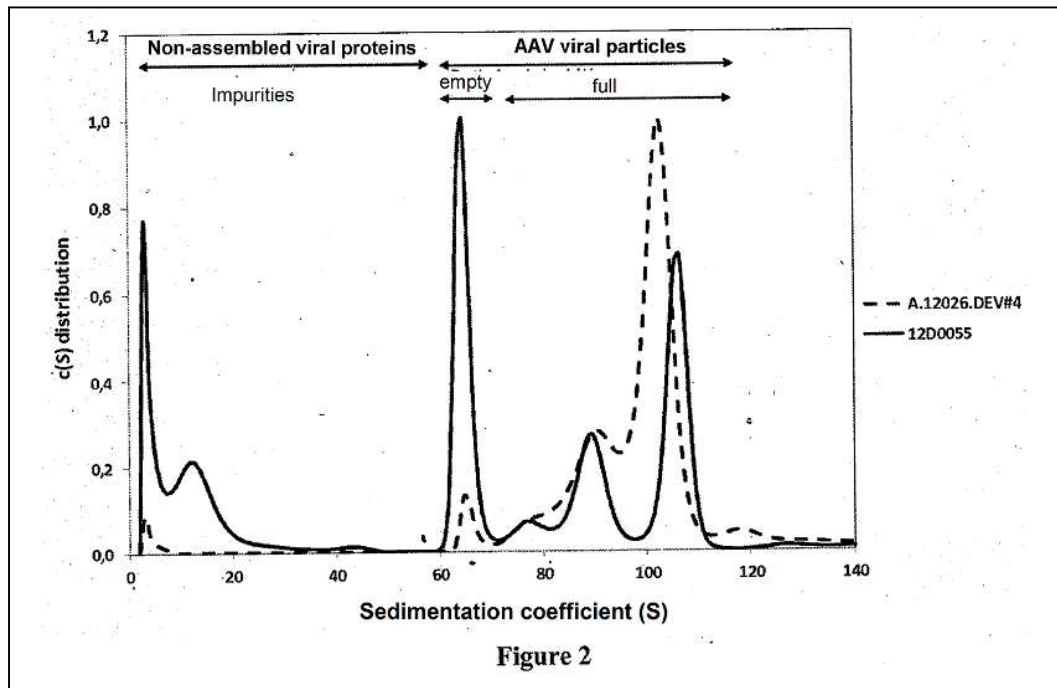
Le Bec is a PCT Publication of International Application Number PCT/EP2014/052978, titled “Methods for the Production of Double-Stranded AAV Viral Particles.” *Id.*, (21), (54). Le Bec was published on August 21, 2014 and

qualifies as prior art under AIA §102(a)(1). *Id.*, (43). Le Bec was published in French, and a certified translation has been submitted with this petition. *Id.*

Le Bec discloses methods to produce AAV particles with double-stranded, “self-complementary” genomes (“scAAV”). EX1006, 3:4-4:10; EX1004, ¶¶104-113. Le Bec reports characterization data for scAAV prepared using two different cell lines – insect derived sf9 cells and human embryonic kidney (HEK293) cells. *Id.*, 6:14-34, 14:21-15:28. This characterization includes “[a]nalysis of empty and full AAV viral particles” using “analytical ultracentrifugation.” *Id.*, 15:17-18.

Le Bec notes that the densities of empty and full AAV viral particles had been reported in the literature: “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.* vectors lacking a viral genome, and 1.45 g/cm³ (equivalent to 110S) for full AAV viral particles, *i.e.* those containing a viral genome with a maximum size of 4800 bases.” *Id.*, 17:29-18:1. Le Bec cites a March 1980 article by Carter and de la Maza, which reports these densities and the corresponding S-value for full AAV particles. *Id.*, 18:1, n.15 (citing EX1013). As Le Bec explains, “[k]nowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.” *Id.*, 18:1-6.

Figure 2 is a $c(s)$ v. S plot from the AUC analysis comparing batches of scAAV produced in sf9 cells (A.12026.DEV-4, dotted line) and HEK293 cells (12D0055, solid line). *Id.*, 6:3-7, 18:8-11. Figure 2 is reproduced below:



Id., Figure 2.

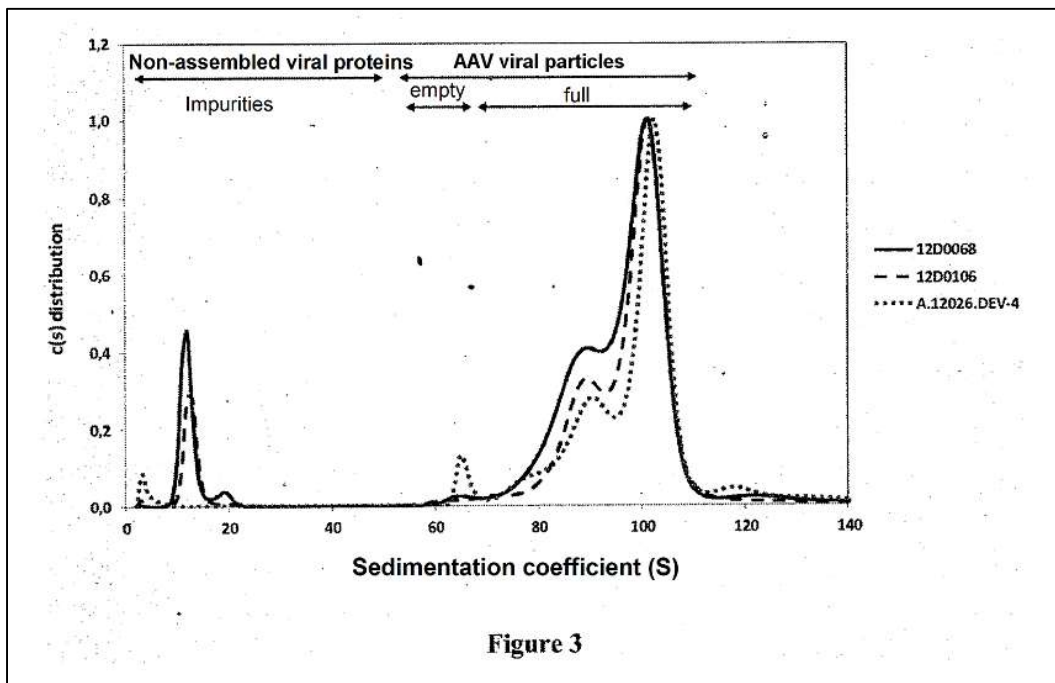
Le Bec identifies “two categories of populations” in Figure 2: (a) <60S population corresponding to non-assembled viral proteins and/or contaminants, and (b) 60S-110S population corresponding to intact AAV viral particles. *Id.*, 18:11-20. Le Bec notes that the <60S population is “virtually absent in batch A.12026.DEV-4, while it is present in a non-negligible amount in batch 12D0055.” *Id.*, 18:15-17. For the 60S-110S population, Le Bec identifies “two subpopulations:” empty AAV viral particles at 65S and “full” AAV viral particles

between 80S and 110S. *Id.*, 18:21-23. Included in the population of “full” particles, Le Bec identifies AAVs “with the viral genome in single-stranded form at 90S and double-stranded form at 105S.” *Id.*, 18:21-25.

Le Bec concludes that “[h]ighly unexpectedly, we detected very few empty AAV viral particles with the sf9 system compared to the HEK293 system.” *Id.*, 18:27-29. Moreover, the “full” AAV viral particles were “significantly more enriched in the genome in double-stranded form (105S) with the sf9 system compared to the HEK293 system.” *Id.*, 18:29-32.

Le Bec reports that the distribution profile for a second batch of scAAV produced in HEK293 cells (12D0082) was “identical” to the profile for batch 12D0055 in Figure 2. *Id.*, 19:1-2. This result “confirms that the HEK293 system essentially produced very few full AAV particles (< 25%), with only 10% of viral particles containing the genome in double-stranded form.” *Id.*, 19:2-5.

A second experiment with three batches of scAAV vectors produced in the sf9 system (A.12026.DEV-4, 12D0106, 12D0068) was carried out in the same way by analytical ultracentrifugation. *Id.*, 6:8-10, 19:7-10. The distribution profiles for these three batches are shown in Figure 3, which is reproduced below:



Id., Figure 3.

Le Bec concludes that “these three batches all show a similar distribution and confirm that the sf9 system makes it possible to produce AAV viral particles essentially composed of full AAV particles (> 80%), with a very high percentage of viral genome in double-stranded form (> 50%).” *Id.*, 19:11-15.

C. Cole

Cole is a chapter in *Methods in Cell Biology*, titled “Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium.” EX1009, 143. Cole was published in 2008 and qualifies as prior art under AIA §102(a)(1). *Id.*, 143.

Cole provides an overview of analytical ultracentrifugation techniques – including SV-AUC – and explains the parameters to be adjusted depending on the

experimental system and samples being tested. Cole notes that AUC is considered “a versatile and powerful method,” with “broad applications for the study of biomacromolecules in a wide range of solvents and over a wide range of solvent concentrations.” EX1009, 144; EX1004, ¶¶114-118.

Cole teaches that “[s]edimentation coefficient distributions are used widely in the pharmaceutical industry to assess the stability of protein formulations and to characterize preparations of inherently heterogeneous samples (e.g., vaccines based on bacterial cell wall preparations).” *Id.*, 149.

Cole describes the instrumentation routinely used in SV-AUC analysis. Cole further instructs how to optimize parameters for an SV-AUC experiment and how to perform the analytical steps necessary to interpret the data. *Id.*, 161-168.

D. Sommer

Sommer is an article titled “Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement.” EX1010, 122. Sommer was published in 2003 and is prior art under AIA §102(a)(1). *Id.*

Sommer discloses “a simple, rapid, and direct method for quantifying vector genomes and capsid proteins in solution.” EX1010, Abstract; EX1004, ¶¶119-127. Sommer notes that “[m]ethods for largescale production of highly purified AAV for clinical use have improved considerably in recent years.” *Id.*, 122. However, as Sommer explains, “[e]mpty capsids that are typically formed during AAV vector

production copurify with genome-containing vector particles during chromatographic purification, and the excess of empty capsids confounds simple determinations of vector genome concentration by absorbance.” *Id.*, 122.

The method disclosed in Sommer “takes into account the capsid ratio, deduced from the A260/A280 ratio, to determine vector genome (vg) concentrations.” *Id.*, 122. This approach results in “a simple, rapid, and accurate quantification method for vector genomes at concentrations above 5×10^{11} vg/ml in the presence of up to 40-fold excess empty capsids.” *Id.*, 122-23.

For the calculation of capsid ratio and vector genome concentration, Sommer discloses a formula for determining the extinction coefficient for an rAAV vector. *Id.*, 127. The extinction coefficient for rAAV can be calculated by adding the extinction coefficient of the vector DNA to the extinction coefficient for the AAV capsid. *Id.*, Abstract, 127.

$$\epsilon_{(\text{rAAV vector})} = \epsilon_{(\text{DNA})} + \epsilon_{(\text{capsid})}$$

Sommer discloses formulas for calculating the molar extinction coefficient for AAV2-based vectors at 260 nm and 280 nm, as set forth below. *Id.*, 127.

$$\epsilon_{260(\text{rAAV vector})} = 20.0 \times \text{MW}_{\text{DNA}} + 3.72 \times 10^6$$

$$\epsilon_{280(\text{rAAV vector})} = 11.1 \times \text{MW}_{\text{DNA}} + 6.31 \times 10^6$$

Sommer notes that related AAV serotypes differ slightly in their capsid composition, which may result in slightly different extinction coefficients. *Id.*, 126.

For example, Sommer reports that calculations based on the capsid sequences of other AAV serotypes suggest that the predicted extinction coefficients at 280 nm would be 1 to 2% lower for AAV3 and AAV6 and 4.5% lower for AAV5 compared to AAV2. *Id.*

Using the formulas disclosed in Sommer, the extinction coefficient for each rAAV particle species in a sample may be calculated if the molecular weight of the encapsidated DNA is known. *Id.*, 127. Applying Beer's Law, the extinction coefficient and absorbance at a given wavelength may be used to calculate the molar concentration of each rAAV species. *See* EX1001, 24:35-47, 44:49-61, 46:42-50; EX1009, 152; EX1004, ¶127. The molar concentrations may then be used to calculate the percentage of each rAAV species in the preparation. *See* EX1001, 24:45-47, 44:58-61, 46:47-50; EX1004, ¶¶127, 148, 180.

VII. LEVEL OF ORDINARY SKILL IN THE ART

A POSA working in the field of the '326 patent in January 2015 would have had at least a Ph.D. in biology, chemistry, biochemistry, biophysics, pharmaceutical sciences, or a related discipline, with two or more years of industry, laboratory, and/or clinical experience in analyzing or characterizing biomolecules, including viruses or viral vectors. Alternatively, a POSA would have had at least a Master's or Bachelor's Degree in biology, chemistry, biochemistry, biophysics, pharmaceutical sciences, or a related discipline, with a corresponding number of

additional years of experience analyzing or characterizing biomolecules, including viruses or viral vectors.

VIII. CLAIM CONSTRUCTION

The Board construes claims per *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005). 37 C.F.R. §42.100(b). Claims should only be construed to the extent necessary to resolve a controversy. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017). For this proceeding, no terms require construction because the disclosures in the prior art are commensurate with the disclosures in the '326 patent and Patent Owner's arguments regarding the scope of the challenged claims during prosecution. The prior art reads on the claims under any construction consistent with *Phillips*. For purposes of this proceeding, the petition analyzes the claim terms under their plain and ordinary meaning.

IX. DETAILED EXPLANATION OF GROUNDS

A. Ground 1: Claims 1-4, 7, 12-13, 15 and 17-18 Are Obvious Over Le Bec and de la Maza

A POSA would have been motivated to combine Le Bec and de la Maza because both references relate to the identification and characterization of viral particles in AAV preparations. Le Bec discloses SV-AUC methods for evaluating preparations of rAAV particles for use in therapeutic applications, such as gene therapy. EX1006, 1:5-19, 3:24-4:10. De la Maza teaches that there is a linear relationship between the density of an AAV particle and the length of the DNA

inside the capsid. EX1007, 203; EX1008, 3194, 3202; EX1011, 449. A POSA would have been motivated to apply the teachings in de la Maza to the analysis of SV-AUC distribution profiles, obtained using the methods in Le Bec, to identify and characterize the various species present in a preparation of rAAV particles, including AAV particles with fragmented genomes.

Le Bec teaches that SV-AUC is a powerful tool for assessing the composition of rAAV vectors for use in gene therapy applications. EX1006, 15:17-28, 17:29-19:15. Le Bec discloses SV-AUC methods to evaluate the quality of high-titer batches of rAAV during “the large-scale production of human-grade gene therapy vectors in compliance with good manufacturing practice.” EX1006, 3:32-4:9. As Le Bec shows, SV-AUC is a useful method for quality control testing that may be applied to the industrial-scale production of rAAV vectors for gene therapy. EX1006, 4:9-10, 15:17-28, 17:29-19:15.

In view of (i) the importance of drug product quality to achieve FDA approval (EX1023, 16; EX1026, 1, 6; EX1027, 4-5), and (ii) the recognized benefits of using SV-AUC methods for analyzing such aspects of biological samples (EX1006, 15:17-28, 17:29-19:15; EX1009, 145, 149, 161-168), a POSA would have been motivated to choose SV-AUC when seeking to characterize preparations of rAAV particles, including AAV particles with fragmented genomes, and to quantify the presence of

different rAAV species in a consistent and reliable way during the manufacture of rAAV vectors. EX1004, ¶¶132-134.

A POSA would have been further motivated to combine the SV-AUC methods in Le Bec with teachings from the Carter lab regarding the physical properties of AAV. The Carter lab is a recognized leader in the development of AAV as a vector for gene therapy applications. EX1040, 491-93. In light of this established reputation, a POSA would have been motivated to combine the teachings in de la Maza regarding the physical properties of AAV with fragmented genomes to analyze the SV-AUC results for preparations of rAAV obtained according to the methods of Le Bec. EX1004, ¶¶135-140.

Indeed, Le Bec includes an express motivation to combine the disclosed SV-AUC methods with the teachings in de la Maza. Le Bec cites an article published by de la Maza and Carter in March 1980 reporting the separation and characterization of empty and full AAV particles using centrifugation in a CsCl gradient. EX1006, 17:30-18:1 (citing EX1013). As Le Bec notes, “[i]t 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. vectors lacking a viral genome, and 1.45 g/cm³ (equivalent to 110S) for full AAV viral particles, i.e. those containing a viral genome with a maximum size of 4800 bases.” *Id.*

Le Bec applies the teachings of de la Maza and Carter to the analysis of SV-AUC profiles for rAAVs containing self-complementary genomes. EX1006, 18:8-32. Specifically, Le Bec uses the density measurements (and equivalent S-values) reported by de la Maza and Carter as benchmarks for analyzing the SV-AUC results for scAAVs in Figures 2 and 3. Le Bec identifies the peak at 65S as empty AAV capsids – corresponding to the empty AAV capsids identified by de la Maza and Carter with a density of approximately 1.32 g/cm^3 (equivalent to 60S). EX1006, 17:30-33, 18:21-22. Likewise, Le Bec identifies the peak at 105S as full AAV particles with a complete, double-stranded, self-complementary genome – corresponding to the full AAV particles with a complete, wild-type genome identified by de la Maza and Carter at a density of approximately 1.45 g/cm^3 (equivalent to 110S). EX1006, 17:33-18:1, 18:21-25.

In light of these express disclosures, a POSA would have been motivated to combine Le Bec with de la Maza (first published in 1978 and reported again in April 1980) disclosing the linear relationship between AAV particle density and the size of the encapsidated DNA. EX1007, 203; EX1008, 3194, 3202; EX1011, 449. A POSA would have been motivated to apply the teachings in de la Maza to the analysis of rAAV preparations containing particles with fragmented genomes – particularly given the reliance in Le Bec on the disclosures in the contemporaneous

de la Maza and Carter article published in March 1980 as a framework for the analysis of the SV-AUC profiles in Figures 2 and 3.

1. Claim 1

- a. “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”**

Le Bec, in combination with de la Maza, discloses a method for determining the size of one or more fragmented genomes in a preparation of rAAV vectors. EX1004, ¶¶142-147. Le Bec teaches an SV-AUC method for analytically separating, detecting, and quantifying the different species (empty AAV particles, complete AAV particles, and AAV particles with fragmented genomes) in an rAAV preparation. EX1006, 17:29-19:15; EX1004, ¶142.

Le Bec identifies the size of the genomes in the AAV particles corresponding to the peaks in Figures 2 and 3 between 60S and 110S. EX1006, 18:19-20, Figures 2 and 3. Specifically, Le Bec identifies (i) empty particles at 65S, (ii) particles with a complete double-stranded, self-complementary genome at 105S, and (iii) particles with a single-stranded genome at 90S. EX1006, 18:21-25. Additionally, Figure 2 shows a distinct peak at about 75S, which a POSA would understand reflects an AAV particle with a fragmented genome that is shorter than the single-stranded genome. EX1006, Figure 2 (12D0055); EX1004, ¶¶143-145.

De la Maza characterizes the fragmented genomes in a preparation of AAV particles. EX1007, 193 (“We summarize here the physical properties of the incomplete AAV genomes.”); EX1008, 3194 (“In this report, we have analyzed the DNA structure of each density class of AAV particles. We show that all classes contain AAV DNA molecules. The size of these DNA molecules ranges from full length to about 3% of genome length.”); EX1004, ¶146. De la Maza teaches that there is a linear relationship between AAV particle density and the size of the DNA packaged inside the capsid. EX1007, 203; EX1008, 3194, 3202; EX1011, 449.

Le Bec, in combination with de la Maza, therefore meets this limitation of claim 1. EX1004, ¶148.

- b. “(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”**

Le Bec discloses subjecting an rAAV preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate one or more sedimenting boundaries. EX1006, 15:17-28; EX1004, ¶149. Le Bec further discloses that “centrifugation of the samples was carried out at a speed of 16,000 rpm,” which is within the claimed range. EX1006, 15:23-25; EX1004, ¶155.

Le Bec describes subjecting an rAAV preparation to SV-AUC conditions to determine the sedimentation coefficients of rAAV particles. EX1006, 15:17-28. SV-AUC methods determine sedimentation coefficients by looking at boundary sedimentation velocity data. EX1009, 146-47; EX1004, ¶¶43-47, 150. The determination in Le Bec of the “sedimentation coefficient[s] of the various AAV viral particles,” and plotting those coefficients in a distribution profile, confirms that Le Bec was measuring the velocities at which different sedimentation boundaries were moving and, thereby, performing SV-AUC. EX1004, ¶¶150-154; *see also* EX1009, 146-47.

The AUC parameters described in Le Bec result in boundary sedimentation conditions for rAAV-sized particles. EX1004, ¶¶150-154; EX1001, 17:29-51. Le Bec reports that “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm using 100 µl or 400 µl of undiluted pure vectors, sedimentation was followed by the absorbance at the wavelength of 276 nm, and the sedimentation coefficient of the various populations was obtained using the software SEDFIT.” EX1006, 15:23-28. The rotor speed of 16,000 rpm in Le Bec falls within the range identified in the ’326 patent for use with AAV particles (between 10,000 rpm and 20,000 rpm). EX1001, 29-30 (Table 1). Similarly, the measurement of absorbance at 276 nm in Le Bec is consistent with the identification in the ’326 patent of “exemplary

absorbances” for viral particles at “about 230nm, 260nm or 280nm.” *See* EX1001, 4:42-43; *see also* EX1001, 29-30 (Table 1).

Le Bec further reports acquiring data in “real-time,” which means that the sedimentation of rAAV particles was monitored at time intervals. EX1006, 15:19-23; EX1004, ¶152. The distribution profiles reported in Figures 2 and 3 and the calculation of sedimentation coefficients of the various populations using the SEDFIT software confirms that sedimentation was monitored at time intervals. EX1006, 15:23-28, Figures 2 and 3; EX1004, ¶152. In light of these disclosures, a POSA would understand that Le Bec applied boundary sedimentation velocity conditions to measure the physical characteristics of preparations of rAAV particles. EX1004, ¶152.

Le Bec therefore meets this limitation of claim 1. EX1004, ¶156.

- c. **“(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, and determining the sedimentation coefficients of the viral particles comprising one or more of the fragmented genomes in the preparation”**

Le Bec discloses using SV-AUC to measure the rate of movement or migration of the distinct sedimenting boundaries in a preparation of rAAV particles,

where each distinct sedimenting boundary corresponds to a resolvable viral particle. Section IX.A.1.b.¹; *see also* EX1006, 15:17-28; EX1004, ¶¶157-159. Further, Le Bec determines the sedimentation coefficients of the rAAV particles in the preparation, including the sedimentation coefficients of particles with fragmented genomes. EX1006, 15:17-28, Figures 2 and 3; EX1004, ¶¶160-161.

Le Bec discloses that “the sedimentation coefficient of the various populations was obtained using the software SEDFIT.” EX1006, 15:27-28. Le Bec identifies the S-values for rAAV particle species based on the peaks shown in the distribution profiles in Figures 2 and 3. EX1006, 18:8-32. Le Bec reports that the peak at 65S corresponds to empty rAAV particles and that the peak at 105S corresponds to an rAAV particle with a complete double-stranded, self-complementary genome. EX1006, 18:21-25. Figures 2 and 3 also show rAAV particles with a fragmented genome at 90S, which corresponds to particles with a single-stranded genome. EX1006, 18:23-25, Figures 2 and 3. Finally, Figure 2 shows a peak at about 75S, which corresponds to an rAAV particle with a fragmented genome that is shorter than the single-stranded genome. EX1006, Figure 2 (12D0055); EX1004, ¶161.

Le Bec therefore meets this limitation of claim 1. EX1004, ¶162.

¹ Citations to “Section ____” refer to the analysis and discussion in earlier sections in this Petition.

d. “(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”

Le Bec discloses SV-AUC methods for determining the sedimentation coefficients for rAAV particles, including rAAV particles with fragmented genomes. EX1006, 15:17-28, 18:8-32, Figures 2 and 3; EX1004, ¶163. Le Bec identifies the size of a fragmented genome in at least one species of rAAV particle (with a single-stranded, monomeric genome) as a function of the sedimentation coefficient (90S), compared to the sedimentation coefficients of empty rAAV particles (65S) and rAAV particles with a complete double-stranded, self-complementary genome (105S). EX1006, 18:19-25; EX1004, ¶163.

De la Maza teaches that there is a linear relationship between the density of an AAV particle and the size of the DNA packaged within the capsid. EX1007, 203; EX1008, 3194, 3202; EX1011, 449; EX1004, ¶164. As de la Maza explains, “[t]he density of the incomplete virus particles appears to be correlated with the size of the incomplete DNA, which suggests that each particle contains only one DNA molecule.” EX1007, 203; *see also* EX1008, 3202. This correlation “implies that virus assembly occurs by encapsidation of DNA into preformed capsids, since any model for crystallization of viral capsids around the DNA might be expected to yield particles with aberrant morphology with smaller DNA.” EX1007, 203.

The mechanism for AAV assembly was confirmed in a 1980 article, also published by the Carter lab. EX1012, 79-82; EX1004, ¶165. In that article, Myers and Carter report that “newly synthesized AAV proteins are rapidly assembled into empty capsids which in turn serve as precursors to stable, mature (full) particles that contain either a standard or deleted (defective-interfering) virus genome.” EX1012, Abstract. The results of these experiments are “consistent with a model for AAV assembly in which progeny DNA strands are packaged into preformed capsids.” EX1012, Abstract; *see also* EX1012, 72 (“Our studies suggest assembly of AAV by condensation of DNA into preformed capsids.”). Consistent with this mechanism, Myers and Carter report that individual AAV genomes “containing deletions of up to 90% of genome length are packaged into AAV particles having an apparently normal capsid.” EX1012, 82; *see also* EX1026, 2, Figure 1 (showing transmission electron microscopy images of full and empty AAV particles with the same size and shape); EX1041, 59, Figure 2 (same); EX1004, ¶¶165-167.

The specification of the '326 patent also refers to this mechanism for AAV particle formation, and cites Myers and Carter in support. EX1001, 43:26-29 (“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).”); 45:17-20 (“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).”).

A POSA would understand that the size and shape of intact, non-aggregated AAV particles are approximately the same, regardless of the size of the genome packaged in the capsid. EX1007, 203; EX1008, 3202; EX1004, ¶169. And because the size and shape of the capsid is relatively constant among AAV particles (regardless of whether they are empty, or contain a full or fragmented genome), the density (and sedimentation coefficient) of an AAV particle is directly proportional to the size of the genome, as taught in de la Maza and as predicted by the Svedberg equation. EX1007, 203; EX1008, 3194, 3202; EX1011, 449; EX1004, ¶168.

Applying the teachings of de la Maza to the SV-AUC methods in Le Bec, a POSA would have been able to determine the size of fragmented genomes as a function of the sedimentation coefficients of the AAV particles. A POSA would have understood that the sedimentation coefficient depends on the size, shape, and mass of a particle. EX1004, ¶168. For example, heavier particles typically sediment faster and have higher sedimentation coefficients. *Id.* Lighter particles typically sediment more slowly and have smaller sedimentation coefficients. *Id.*

For mixtures of rAAV particles, a POSA would understand that the size and shape of the particles are approximately the same. EX1007, 203, EX1008, 3202; EX1004, ¶169. Given the similarity in size and shape, a POSA would understand

that observed differences in the measured sedimentation coefficients would depend upon the difference in mass and density among different rAAV particles. EX1004, ¶¶169-170. In view of the teachings in de la Maza, a POSA would understand that there is a linear relationship between the density of an AAV particle (and the rate at which it will sediment – *i.e.*, the sedimentation coefficient) and the size of the genome packaged inside the capsid. *Id.* Given the linear relationship between the size of the genome in an rAAV particle and the measured sedimentation coefficient, a POSA would have been able to determine the size of the DNA in AAVs with fragmented genomes by comparison to the sedimentation coefficients for rAAV particles with genomes of known length. EX1004, ¶171.

For example, applying the teachings of de la Maza to the distribution profile in Figure 2 of Le Bec, a POSA would have understood that one way to estimate the size of the fragmented genome in rAAV particles with a sedimentation coefficient of about 75S is by comparison to the sedimentation coefficients for rAAV particles with a known genome size – *i.e.*, empty rAAV particles, particles with a complete double-stranded, self-complementary genome, and rAAV particles with a single-stranded, monomeric genome. EX1006, Figure 2 (12D0055); EX1004, ¶171. Assuming that the double-stranded genome for the rAAV particles in Le Bec is approximately the same size as a wild-type genome – about 4700 nucleotides, as reported in Le Bec (*see* EX1006, 3:4-9) and de la Maza (*see* EX1008, 3194, 3202)

– a POSA would have been able to estimate the size of the fragmented genome of the rAAV particles sedimenting at 75S as about 980 nucleotides long. EX1004, ¶172.

Le Bec, in combination with de la Maza, therefore meets this limitation of claim 1. EX1004, ¶173.

2. **Claim 2: “The method of claim 1, wherein step (iii) comprises comparing the sedimentation coefficients of the one or more viral particles comprising the fragmented genomes with a standard curve generated 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”**

Le Bec discloses SV-AUC methods for measuring the distribution profiles for rAAV particles with genomes of known length. EX1006, 18:19-25, Figures 2 and 3. EX1004, ¶174. Given the linear relationship between the size of the genome and the density of an AAV particle, as taught in de la Maza, a POSA would have been able to use the measured sedimentation coefficients for rAAVs with genomes of known size as a “standard curve” to determine the size of the fragmented genomes in rAAV particles containing DNA of an unknown length. Section IX.A.1.d.; EX1004, ¶¶174-175.

The use of molecular weight markers to determine the size of unknown samples was a well-established technique in the art. EX1004, ¶176. For example,

De la Maza discloses the use of DNA molecular weight markers to estimate the length of DNA in AAVs with fragmented genomes. EX1007, 196-97, Figure 1. De la Maza uses the AAV2 genome and restriction fragments comprising 38%, 22%, and 4.5% of the total AAV2 genome as size markers in alkaline and neutral sucrose gradients of fragmented genomes of unknown length. EX1007, Figure 1 (legend). De la Maza compares the sedimentation profiles of the unknown DNA (Types I-IV) to the known DNA markers to determine the molecular weight of the unknown, fragmented genomes. EX1007, 196-97, Table 1.

Applying the teaching in de la Maza regarding the linear relationship between sedimentation coefficient and genome size, a POSA would have been able to determine the size of an unknown, fragmented genome by comparing the sedimentation coefficients measured by SV-AUC for rAAVs with genomes of known length. EX1004, ¶177. For example, in Figure 2 of Le Bec, a POSA would have been able to estimate the size of the fragmented genome in rAAV particles with a sedimentation coefficient of about 75S by comparison to the sedimentation coefficients for rAAV particles with a known genome size – *i.e.*, empty rAAV particles (65S), particles with a complete, double-stranded, self-complementary genome (105S), and rAAV particles with a single-stranded, monomeric genome (90S). EX1006, Figure 2 (12D0055); EX1004, ¶178. Assuming that the complete, double-stranded genome for the rAAV particles in Le Bec is approximately the same

size as a wild-type genome – about 4700 nucleotides, as reported in Le Bec (*see* EX1006, 3:4-9) and de la Maza (*see* EX1008, 3194, 3202) – a POSA would have been able to estimate the size of the fragmented genome of the rAAV particles sedimenting at 75S as about 980 nucleotides long. EX1004, ¶179.

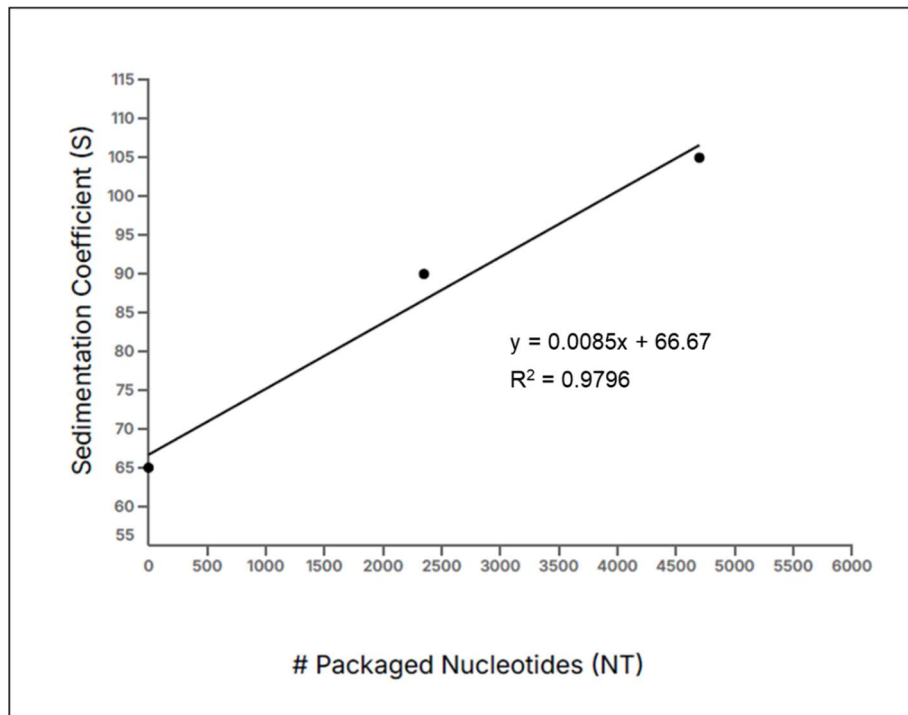
Likewise, using the data in Figure 2 of Le Bec, a POSA would have been able to generate a “standard curve” using the sedimentation coefficient values for the rAAV species with genomes of known size in Figure 2 of Le Bec. EX1004, ¶¶180-183. Specifically, applying a linear regression analysis to the data in Figure 2, a POSA would have obtained a standard curve with the following formula and R^2 value:

$$y = 0.0085x + 66.67$$

$$R^2 = 0.9796$$

where y is the sedimentation coefficient (S) and x is the size of the genome, expressed as the number of nucleotides. EX1004, ¶182.

The linear relationship between the sedimentation coefficient and the length of the packaged genome based on the data in Le Bec is illustrated in the figure below:



Le Bec, in combination with de la Maza, therefore meets the additional limitation of claim 2. EX1004, ¶184.

3. Claim 3: “The method of claim 1, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm”

Le Bec discloses that “centrifugation of the samples was carried out at a speed of 16,000 rpm.” EX1006, 15:23-25; EX1004, ¶185.

Le Bec therefore meets the additional limitation of claim 3. EX1004, ¶186.

4. Claim 4: “The method of claim 1, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm”

Le Bec discloses centrifugation at 16,000 rpm. Section IX.A.3.

Le Bec therefore meets the additional limitation of claim 4. EX1004, ¶188.

5. Claim 7: “The method of claim 1, wherein the fragmented genomes are sub-genomic DNA molecules”

Le Bec discloses SV-AUC methods for determining the sedimentation coefficients for rAAV particles containing sub-genomic DNA molecules. EX1006, 15:17-28, Figure 2; EX1004, ¶189. For example, Figure 2 in Le Bec shows a peak at about 75S, which corresponds to an rAAV particle with a fragmented genome that is shorter than the single-stranded, monomeric genome in AAV particles that sediment at about 90S. EX1006, Figure 2 (12D0055); EX1004, ¶190.

De la Maza measures the molecular weights of sub-genomic DNA molecules in a preparation of AAV particles. EX1007, 194-97, Table 1; EX1008, 3195-96, Table 1; EX1004, ¶191. De la Maza discloses that the fragmented DNAs designated Type I, II, III and IV are all missing a portion of the AAV genome. EX1007, 200-201; EX1008, 3202.

Le Bec and de la Maza therefore both meet the additional limitation of claim 7. EX1004, ¶192.

6. Claim 12: “The method of claim 1, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is greater than 5×10^{11} vg/mL”

Le Bec is directed to methods for producing “high-titer and large-scale” quantities of rAAV vectors for clinical applications. EX1006, 3:33-34; *see also* EX1006, 1:5-10, 1:14-19, 2:19:3:2, 4:6-10, 6:14-33, 21:3-10 (claim 1).

Le Bec discloses that, following purification, “[t]he viral rAAV particles can then be concentrated and formulated in a buffer . . . in order to be at a final concentration of approximately $\geq 10^{11}$ vg/mL.” EX1006, 10:28-32; *see also* EX1006, 14:15-19; EX1004, ¶¶193-195.

Le Bec reports the vector genome concentration of batches of scAAV-SMN after purification – ranging from 5.1×10^{11} vg/mL to 1.6×10^{12} vg/mL. EX1006, 19-20 (Table 2).

Le Bec therefore meets the additional limitation of claim 12. EX1004, ¶196.

7. Claim 13: “The method of claim 1, wherein the total concentration of viral particles in the AAV vector preparation prior to step (i) is from about 1×10^{11} vg/mL to about 1×10^{13} vg/mL”

Le Bec discloses formulations of rAAV vector particles with a final concentration ranging from 5.1×10^{11} vg/mL to 1.6×10^{12} vg/mL. Section IX.A.6.

Le Bec therefore meets the additional limitation of claim 13. EX1004, ¶200.

8. Claim 15: “The method of claim 1, comprising determining the size of all of the fragmented genomes in the preparation”

Le Bec discloses SV-AUC methods for determining the sedimentation coefficients for rAAV particles, and identifies the size of a fragmented genome in at least one species of rAAV particle (with a single-stranded, monomeric genome) as a function of the sedimentation coefficient. Section IX.A.1.d.

De la Maza reports the molecular weights of fragmented genomes in a preparation of AAV particles, and teaches that there is a linear relationship between the density of an AAV particle and the size of the DNA packaged within the capsid. Section IX.A.1.d.

Applying the teachings of de la Maza to the SV-AUC methods in Le Bec, a POSA would have been able to determine the size of all of the fragmented genomes in a preparation of rAAV particles as a function of the corresponding sedimentation coefficients. Section IX.A.1.d.

Le Bec, in combination with de la Maza, therefore meets the additional limitation of claim 15. EX1004, ¶¶201-204.

9. Claim 17: “The method of claim 1, wherein the preparation comprises AAV9 capsid proteins”

Le Bec discloses preparations of recombinant AAV vectors having an AAV9 capsid. EX1006, 7:16-18 (“According to a specific variant, the sequence codes for AAV capsid proteins of serotype 9 (AAV9).”), 11:14-17 (“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.”); *see also* EX1006, 12:1-33, 14:21-15:28, 16:16-17:27, 18:8-19:15; EX1004, ¶¶206-206.

Le Bec therefore meets the additional limitation of claim 17. EX1004, ¶207.

10. Claim 18: “The method of claim 17, wherein the preparation further comprises at least one AAV2 ITR”

Le Bec discloses preparations of recombinant AAV vectors where the “AAV viral genome is coded for by a sequence comprising the transgene of interest flanked by AAV ITR sequences.” EX1006, 7:30-31; *see also* 1:33-2:6, 4:12-19; EX1004, ¶208.

Le Bec discloses that the ITRs may be from AAV serotype 2. EX1006, 7:27-28 (“According to a specific variant, the viral genome is derived from an AAV2 genome.”); EX1004, ¶209.

Le Bec further discloses preparations of rAAV vectors comprising an AAV2 viral genome in an AAV9 capsid. EX1006, 7:6-9 (“[A]n rAAV vector may be composed of a genome derived from an AAV of serotype 2, for example, encapsidated by capsid proteins derived from an AAV of serotype 9.”); *see also* EX1006, 4:34-5:4; EX1004, ¶209.

Le Bec therefore meets the additional limitation of claim 18. EX1004, ¶210.

11. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in applying the SV-AUC methods of Le Bec to determine the sedimentation coefficients of rAAVs with fragmented genomes. The SV-AUC method was well-known in the art and within the skill of a POSA to optimize. EX1001, 19:37-43; EX1004, ¶¶211-212.

The sedimentation coefficients of empty and full rAAV particles were known and reported in the literature, and Le Bec taught that rAAV particles (including rAAV particles with fragmented genomes) could be effectively separated, characterized, and quantified by analytical ultracentrifugation. EX1006, 15:17-28, 18:8-32; EX1004, ¶212.

Le Bec equates the peaks in Figures 2 and 3 to the bands observed by Carter and de la Maza for empty and full AAV particles using equilibrium centrifugation in a CsCl gradient: “[i]t 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. vectors lacking a viral genome, and 1.45 g/cm³ (equivalent to 110S) for full AAV viral particles, i.e. those containing a viral genome with a maximum size of 4800 bases.” EX1006, 17:30-18:1 (citing EX1013); EX1004, ¶213.

Le Bec teaches that AAV particles that can be separated using equilibrium centrifugation in a CsCl gradient, as in de la Maza, may also be separated and quantified using SV-AUC methods. EX1006, 18:1-6; EX1004, ¶214. As Le Bec explains: “Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation [using a CsCl gradient], we have implemented a method today for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.” EX1006, 18:1-6. Thus, a POSA would have had a reasonable expectation of success that AAV particles with

fragmented genomes that could be separated using a CsCl gradient may also be separated and quantified using the SV-AUC methods in Le Bec. EX1004, ¶214. As the European Patent Office explained in a communication during prosecution of the corresponding EU application, “[a]ny virus particles that can be separated [sic] by CsCl density gradient obviously can be resolved by AUC since the method effectively is a modern real-time form of readout of the movement of particles in a density gradient.” EX1039 (Annex), 4.

A POSA would have had a reasonable expectation of success in applying the teachings of de la Maza to analyze the sedimentation coefficients obtained using the SV-AUC methods of Le Bec and determine the size of the DNA in rAAVs with fragmented genomes. De la Maza teaches the linear relationship between AAV particle density and the length of the genome. EX1007, 203; EX1008, 3194, 3202; EX1011, 449. A POSA would have had a reasonable expectation of success in applying the teachings of de la Maza to determine the size of the DNA in rAAVs with fragmented genomes by comparison to the S-values for rAAVs with genomes of known length. EX1004, ¶215.

12. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner is unaware of any secondary considerations that would outweigh the compelling evidence of obviousness.

B. Ground 2: Claims 5 and 8-9 Are Obvious Over Le Bec and de la Maza, in view of Cole

A POSA would have been motivated to combine the SV-AUC methods in Le Bec with the teachings in de la Maza regarding the sedimentation properties of AAV particles – in order to characterize the different species in a preparation of rAAV particles, including rAAV particles with fragmented genomes. Section IX.A.

A POSA would have been further motivated to combine Le Bec and de la Maza with Cole. EX1004, ¶221. Cole discusses the instrumentation used for SV-AUC analysis, and provides guidance for preparing samples and selecting the optimum parameters to run SV-AUC experiments. EX1009.

As the '326 patent acknowledges, “[i]t is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles.” EX1001, 26:19:21. A POSA would have been motivated to apply the guidance in Cole in order to optimize the separation of rAAV particles using SV-AUC according to Le Bec. EX1004, ¶¶222-223.

1. Claim 5: “The method claim 4, wherein the boundary sedimentation is performed at a temperature of from about 4° C to about 20° C”

Cole teaches that SV-AUC is typically performed in the temperature range between 4° C and 35° C. EX1009, 163. Cole further teaches that “most experiments are conducted at 20° C.” EX1009, 163.

Cole therefore meets the additional limitation of claim 5. EX1004, ¶225.

2. Claim 8: “The method of claim 1, wherein the centrifugation is run from about 0.5 hours to about 2 hours”

Cole explains that “[i]t should take a boundary at least 2 h to sediment the full length of the cell” to ensure sufficient scans will be acquired. EX1009, 164. Using this criterion, Cole discloses “maximum recommended rotor speeds for various sedimentation coefficients” so that “[a]bout 2 h of data acquisition will be available.” EX1009, 164, Table III, note c.

Cole therefore meets the additional limitation of claim 8. EX1004, ¶228.

3. Claim 9: “The method of claim 8, wherein the centrifugation is run from about 1 hours to about 2 hours”

Cole teaches an AUC run time of about 2 hours. Section IX.B.2.

Cole therefore meets the additional limitation of claim 9. EX1004, ¶231.

4. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in applying the teachings of de la Maza to the SV-AUC methods in Le Bec. Section IX.A.11.

A POSA would also have had a reasonable expectation of success in applying the teachings of Cole to optimize the separation of rAAV particles using the SV-AUC methods of Le Bec. EX1004, ¶232. As the ’326 patent acknowledges, the

selection of optimal conditions for SV-AUC was a matter of routine and well within the level of ordinary skill. EX1001, 26:19-21; EX1004, ¶232.

5. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner is unaware of any secondary considerations that would outweigh the compelling evidence of obviousness.

C. Ground 3: Claims 14, 16, 19-23 and 26-30 Are Obvious over Le Bec and de la Maza, in view of Sommer

A POSA would have been motivated to combine the SV-AUC methods in Le Bec with the teachings in de la Maza regarding the sedimentation properties of AAV particles – in order to characterize the different species in a preparation of rAAV particles, including rAAV particles with fragmented genomes. Section IX.A.

A POSA would have been further motivated to combine Le Bec and de la Maza with Sommer. EX1004, ¶236. Like Le Bec and de la Maza, Sommer relates to the identification and characterization of viral particles in preparations of AAV, such as rAAV vectors for use in gene therapy applications. *See* EX1010, 122. A POSA would have been motivated to combine Le Bec and de la Maza with Sommer in order to obtain additional information regarding the composition of an rAAV preparation. EX1004, ¶236.

Sommer discloses mathematical formulas for calculating the extinction coefficient of an rAAV particle based on the size of the DNA packaged in the capsid.

EX1010, 127. Indeed, the '326 patent reports using the formulas in Sommer to calculate the concentration of empty and full rAAV particles, where the size of the DNA is known. EX1001, 24:38-41, 44:51-55, 46:42-45.

A POSA would have been motivated to use the formulas in Sommer to calculate the molar concentrations of rAAV particles with fragmented genomes. EX1004, ¶¶237-238. Specifically, a POSA would have been motivated to use the DNA size measured for each identified species, according to the methods of Le Bec and de la Maza, to calculate the extinction coefficients for rAAV particles with fragmented genomes, according to the formulas in Sommer. *See* EX1010, 127. Further, a POSA would have been motivated to use the calculated extinction coefficients and measured absorbance values to calculate the molar concentration and percentage of each species of rAAV particle in order to evaluate the composition of the heterogeneous mixture. EX1001, 24:35-47, 44:49-61, 46:42-50, 46:42-50; EX1009, 152; EX1004, ¶239.

1. Claim 14: “The method of claim 1, further comprising quantifying of the viral particles comprising the one or more fragmented genomes in the preparation”

Le Bec teaches methods for using SV-AUC to quantify the viral particles in an AAV preparation. EX1006, 16:26-29. As Le Bec discloses: “Knowing that the difference in density is sufficiently significant to distinguish them by centrifugation, we have implemented a method today for analytically separating by

ultracentrifugation and *quantifying the different species present in an AAV viral preparation.*” *Id.*, 18:1-6 (emphasis added).

Le Bec reports that “[t]he distribution profile . . . confirms that the HEK293 system essentially produced very few full AAV particles (< 25%).” *Id.*, 19:1-5. A POSA would understand from Figures 2 and 3 that Le Bec’s reference to “full AAV particles” includes scAAV particles with a double-stranded genome, particles with a fragmented, single-stranded genome, and particles with a fragmented genome that is shorter than the single-stranded genome. *Id.*, Figure 2 (identifying “AAV viral particles” that are designated “full”), Figure 3 (same). Le Bec further reports that “only 10%” of the AAV viral particles contain the genome in double-stranded form. *Id.*, 19:1-5.

By contrast, Le Bec reports: “Highly unexpectedly, we detected very few empty AAV viral particles with the sf9 system compared to the HEK293 system. Moreover, the full AAV viral particles are significantly more enriched in the genome in double-stranded form (105S) with the sf9 system compared to the HEK293 system.” *Id.*, 18:27-32. Le Bec explains that “the sf9 system makes it possible to produce AAV viral particles essentially composed of full AAV particles (> 80%), with a very high percentage of viral genome in double-stranded form (> 50%).” *Id.*, 19:12-15, Figures 2 and 3.

A POSA would understand that the disclosed percentages in Le Bec are relative concentrations of the rAAV particle species, obtained by integrating the area under the curve in the sedimentation coefficient distribution profile for the peaks of interest using the SEDFIT software. EX1006, 15:23-28, EX1004, ¶¶240-242; *see also* EX1001, 4:24-27, 12:35-40, 25:33-36.

Version 14.4d of SEDFIT – which was publicly available and freely downloadable before the priority date of the '326 patent and at the time of Le Bec – included the ability to determine relative concentrations by integrating the area under the curve of a specific region or peak selected by the user. EX1004, ¶242. A POSA would have been aware of this functionality of the SEDFIT software and how to implement it. *Id.*

Additionally, based on the size of the DNA packaged within the capsid, a POSA would have been able to use the mathematical formulas disclosed in Sommer to calculate the extinction coefficient for each type of rAAV particle. EX1010, 127. With these calculated extinction coefficients, a POSA would have been able to quantify the molar concentration of each species of rAAV particle in the sample. EX1004, ¶¶243, 268-270.

Beer's Law provides:

$$A = \epsilon cl$$

where A is the absorbance at a particular wave length (typically 260 nm or 280 nm), ϵ is the extinction coefficient of the particle, l is the path length through the sample, and c is the concentration. EX1009, 152; EX1004, ¶269. Using the absorbance values measured for each rAAV particle in the sample and the calculated extinction coefficients, a POSA would have been able to calculate the molar concentration for each species of rAAV particle according to Beer's Law. *See* EX1001, 24:41-47, 44:58-61, 46:42-50; EX1009, 152; EX1004, ¶¶268-270. A POSA would also have been able to use the calculated molar concentrations to calculate percentages of each species of rAAV particle in the heterogeneous mixture. *See* EX1001, 24:45-47, 44:58-61, 46:45-50; EX1004, ¶270.

Thus, Le Bec and Sommer both meet the additional limitation of claim 14. EX1004, ¶244.

2. Claim 16: “The method of claim 15, further comprising quantifying all of the fragmented genomes in the preparation”

Le Bec teaches SV-AUC methods for quantifying the different species present in an AAV viral preparation using the publicly-available SEDFIT software to integrate the area under the curve for the peaks in a c(s) v. S distribution profile. Section IX.C.1.

Sommer teaches formulas for calculating extinction coefficients for rAAV particles that can be used to calculate the molar concentrations and percentages of all of the rAAV species in the preparation. Section IX.C.1.

Thus, Le Bec and Sommer both meet the additional limitation of claim 16. EX1004, ¶247.

3. Claim 19: “The method of claim 16, wherein the preparation comprises self-complementary AAV viral genomes”

Le Bec discloses preparations of recombinant AAV vectors having self-complementary AAV genomes. EX1006, 3:33-34 (“The invention relates to a method for producing a high titer and large-scale double-stranded AAV vector (scAAV) . . .”); 6:14-16 (“The present invention relates to methods for producing a double-stranded recombinant rAAV vector, or self-complementary AAV (scAAV).”); *see also* EX1006, 3:4-9, 4:12-13, 6:25-33, 21:4-22:27 (claims 1-10); EX1004, ¶248.

Le Bec therefore meets the additional limitation of claim 19. EX1004, ¶249.

4. Claim 20

- a. “A method of determining the 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”**

Le Bec, in combination with de la Maza, discloses a method for determining the size of the genome in each species of rAAV vector in a heterogeneous mixture of viral particles. EX1004, ¶¶250-256; Section IX.A.1.a.

Sommer discloses mathematical formulas for calculating the extinction coefficient for each species of rAAV particle based on the size of the DNA packaged inside the capsid. EX1010, 127. With these calculated extinction coefficients, a POSA would have been able to calculate the molar concentration of each species of rAAV particle in the sample using Beer’s Law. *See* EX1001, 24:41-47, 44:58-61, 46:42-50; EX1009, 152; EX1004, ¶¶257, 268-270.

Le Bec and de la Maza, in combination with Sommer, therefore meets this limitation of claim 20. EX1004, ¶258.

- b. “(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”**

Le Bec discloses subjecting an rAAV preparation to analytical ultracentrifugation under boundary sedimentation velocity conditions to generate

sedimenting boundaries, where the centrifugation of the samples is carried out at 16,000 rpm. Section IX.A.1.b.

Le Bec therefore meets this limitation of claim 20. EX1004, ¶260.

- c. **“(ii) measuring the rate of movement or migration of the sedimenting boundaries, wherein movement or migration of each species of the 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”**

Le Bec discloses using SV-AUC to measure the rate of movement or migration of the distinct sedimenting boundaries in a heterogeneous mixture of rAAV particles comprising viral particles with full genomes, fragmented genomes, and empty capsids. Section IX.A.1.c.

Le Bec therefore meets this limitation of claim 20. EX1004, ¶263.

- d. **“(iii) determining the sedimentation coefficients of each species of the individual viral particles in the heterogeneous mixture of viral particles”**

Le Bec discloses determining the sedimentation coefficients of each species in a heterogeneous mixture of rAAV particles. Section IX.A.1.c.

Le Bec therefore meets this limitation of claim 20. EX1004, ¶266.

e. “(iv) quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture of viral particles”

Le Bec, in combination with de la Maza, discloses SV-AUC methods for determining the size of the genome of each species of rAAV particle in a heterogeneous mixture. Section IX.A.1.d.

Based on the size of the genome, a POSA would have been able to use the mathematical formulas disclosed in Sommer to calculate the extinction coefficient for each species of rAAV particle. EX1010, 127. Using the measured absorbance values for the rAAV particles and the calculated extinction coefficients, a POSA would have been able to calculate the molar concentration for each species of rAAV particle in the heterogeneous mixture according to Beer’s Law. Section IX.C.1.

Le Bec and de la Maza, in combination with Sommer, therefore meets this limitation of claim 20. EX1004, ¶¶268-271.

5. Claim 21: “The method of claim 20, 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 from sedimentation coefficients of viral particles comprising recombinant AAV genomes of known nucleotide sizes”

Le Bec, in combination with de la Maza, teaches using the measured sedimentation coefficients for rAAVs with genomes of known size as a “standard

curve” to determine the size of the genome in each species of rAAV particles containing DNA of an unknown length. Section IX.A.2.

Le Bec, in combination with de la Maza, therefore meets the additional limitation of claim 21. EX1004, ¶282.

6. Claim 22: “The method of claim 20, wherein the boundary sedimentation velocity is from about 10,000 rpm to about 20,000 rpm”

Le Bec discloses centrifugation at 16,000 rpm. Section IX.A.3.

Le Bec therefore meets the additional limitation of claim 22. EX1004, ¶284.

7. Claim 23: “The method of claim 20, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm”

Le Bec discloses centrifugation at 16,000 rpm. Section IX.A.3.

Le Bec therefore meets the additional limitation of claim 23. EX1004, ¶286.

8. Claim 26: “The method of claim 20, wherein the total concentration of viral particles in the heterogeneous mixture prior to step (i) is greater than 5×10^{11} vg/mL”

Le Bec discloses formulations of rAAV vector particles with a final concentration ranging from 5.1×10^{11} vg/mL to 1.6×10^{12} vg/mL. Section IX.A.6.

Le Bec therefore meets the additional limitation of claim 26. EX1004, ¶290.

9. Claim 27: “The method of claim 20, wherein the total concentration of viral particles in the heterogeneous mixture prior to step (i) is from about 1×10^{11} vg/mL to about 1×10^{13} vg/mL”

Le Bec discloses formulations of rAAV vector particles with a final concentration ranging from 5.1×10^{11} vg/mL to 1.6×10^{12} vg/mL. Section IX.A.6.

Le Bec therefore meets the additional limitation of claim 27. EX1004, ¶294.

10. Claim 28: “The method of claim 20, wherein the viral particles comprise AAV9 capsid proteins”

Le Bec discloses preparations of rAAV vectors having an AAV9 capsid. Section IX.A.9.

Le Bec therefore meets the additional limitation of claim 28. EX1004, ¶297.

11. Claim 29: “The method of claim 28, wherein the viral particles comprise at least one AAV2 ITR”

Le Bec discloses preparations of rAAV vectors comprising a genome that includes at least one AAV2 ITR. Section IX.A.10.

Le Bec therefore meets the additional limitation of claim 29. EX1004, ¶300.

12. Claim 30: “The method of claim 20, further comprising determining the relative percentage of each species of the individual viral particles in the heterogeneous mixture of viral particles”

Le Bec teaches methods for using SV-AUC to determine the relative concentration of each species of the rAAV particles in a heterogeneous mixture. Section IX.C.1.

Sommer teaches formulas for calculating extinction coefficients that can be used to determine the molar concentration and percentage of each species of rAAV particle in a heterogeneous mixture. Section IX.C.1.

Thus, Le Bec and Sommer both meet the additional limitation of claim 30. EX1004, ¶305.

13. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success combining the SV-AUC methods of Le Bec with the teachings in de la Maza to determine the size of the DNA in each species of rAAV in a heterogeneous mixture. Section IX.A.11.

A POSA would have had a reasonable expectation of success in using publicly available software, such as SEDFIT, to generate distribution profiles from the SV-AUC data and to integrate the area under the observed peaks to determine the relative concentrations of the rAAV species in the preparation, including rAAV particles with fragmented genomes. EX1004, ¶307.

A POSA would also have had a reasonable expectation of success in applying the mathematical formulas in Sommer to calculate the extinction coefficients for each species of rAAV particle in a heterogeneous mixture based on the size of the DNA packaged in the capsid. EX1009, 152; EX1004, ¶308. Finally, a POSA would have had a reasonable expectation of success in determining the molar concentration

and percentage of each species of rAAV particle. *Id.* A POSA would have had a reasonable expectation of success in applying known mathematical formulas, such as Beer's Law, as they involve straightforward calculations routinely performed by those skilled in the art. *Id.*

14. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner is unaware of any secondary considerations that would outweigh the compelling evidence of obviousness.

D. Ground 4: Claim 25 Is Obvious Over Le Bec and de la Maza, in view of Sommer and Cole

A POSA would have been motivated to combine the SV-AUC methods in Le Bec with de la Maza and Sommer in order to determine the molar concentration of each species of rAAV particle in a heterogeneous mixture. Section IX.A; IX.C.

A POSA would have been further motivated to combine Le Bec, de la Maza, and Summer with Cole. EX1004, ¶311. Cole discusses the instrumentation used for SV-AUC analysis, and provides guidance for preparing samples and selecting the optimum parameters to run SV-AUC experiments. EX1009. A POSA would have been motivated to apply the guidance in Cole in order to optimize the separation of rAAV particles using SV-AUC methods according to Le Bec. EX1004, ¶¶312-313.

1. Claim 25: “The method of claim 20, wherein the centrifugation is run from about 0.5 hours to about 2 hours”

Cole teaches an AUC run time of about 2 hours. Section IX.B.2.

Cole therefore meets the additional limitation of claim 25. EX1004, ¶316.

2. A POSA Would Have Had a Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining the SV-AUC methods in Le Bec with the teachings in de la Maza and Sommer. Section IX.C.13.

A POSA would also have had a reasonable expectation of success in applying the teachings of Cole to optimize the separation of rAAV particles using the SV-AUC methods of Le Bec. EX1004, ¶317.

3. Secondary Considerations Do Not Change the Conclusion of Obviousness

Petitioner is unaware of any secondary considerations that would outweigh the compelling evidence of obviousness.

X. CONCLUSION

Sarepta respectfully requests institution of IPR for claims 1-5, 7-9, 12-23, and 25-30 of the '326 patent.

November 25, 2025

Respectfully submitted,

By: /Robert B. Wilson/

Robert B. Wilson (Reg. No. 45,227)
Anne S. Toker (Reg. No. 53,692)
James M. Glass (Reg. No. 46,729)
Quinn Emanuel Urquhart & Sullivan,
LLP
295 Fifth Avenue, 9th Floor
New York, New York 10016
Tel.: 212-849-7000
Fax: 212-849-7100

*Attorneys for Petitioner Sarepta
Therapeutics, Inc.*

WORD COUNT CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), Petitioner hereby certifies, in accordance with and reliance on the word count provided by the word-processing system used to prepare this Petition, that the number of words in this paper is 13,974. Pursuant to 37 C.F.R. §42.24(d), this word count excludes the table of contents, table of authorities, mandatory notices under §42.8, certificate of service, certificate of word count, appendix of exhibits, and any claim listing.

November 25, 2025

Respectfully submitted,

By: /Robert B. Wilson/

Robert B. Wilson (Reg. No. 45,227)
Anne S. Toker (Reg. No. 53,692)
James M. Glass (Reg. No. 46,729)
Quinn Emanuel Urquhart & Sullivan,
LLP
295 Fifth Avenue, 9th Floor
New York, New York 10016
Tel.: 212-849-7000
Fax: 212-849-7100

*Attorneys for Petitioner Sarepta
Therapeutics, Inc.*

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §42.6 (e) and 37 C.F.R. §42.105, I hereby certify that on November 25, 2025, I caused the foregoing Petition for *Inter Partes* Review, Power of Attorney, and Exhibits 1001-1041 to be served on Patent Owner by depositing them for shipment with Federal Express to the correspondence address of record listed on the Patent Center:

5487 - LISA P. RASMUSSEN
SANOFI
450 WATER STREET
Cambridge, MA 02141
UNITED STATES

November 25, 2025

Respectfully submitted,

By: /Robert B. Wilson/

Robert B. Wilson (Reg. No. 45,227)
Anne S. Toker (Reg. No. 53,692)
James M. Glass (Reg. No. 46,729)
Quinn Emanuel Urquhart & Sullivan,
LLP
295 Fifth Avenue, 9th Floor
New York, New York 10016
Tel.: 212-849-7000
Fax: 212-849-7100

*Attorneys for Petitioner Sarepta
Therapeutics, Inc.*