

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

**DECLARATION OF STEVEN BERKOWITZ, PH.D.
IN SUPPORT OF PETITION FOR *INTER PARTES* REVIEW OF
U.S. PATENT NO. 12,013,326**

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3.	“(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;”	114
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I. INTRODUCTION

1. I have been retained by Quinn Emanuel Urquhart & Sullivan, LLP on behalf of Petitioner Sarepta Therapeutics, Inc. (“Sarepta”) as an independent expert consultant in the above referenced *inter partes* review (“IPR”) proceeding. I am being compensated for my time in connection with this IPR at my standard consulting rate, which is \$100 per hour.

2. I understand Sarepta is petitioning for *inter partes* review of U.S. Patent No. 12,013,326 (EX1001, “the ’326 patent”). I have been retained to provide information and opinions on the teachings of the prior art and the state of the art, as relevant to the issued claims of the ’326 patent.

II. BACKGROUND AND QUALIFICATIONS

3. In 1968, I graduated from Fairleigh Dickinson University and received a Bachelor of Science degree in biology. In 1975, I received a Ph.D. in Biochemistry from New York University, School of Medicine, under the supervision of Dr. Loren Day. My dissertation work focused on applying biophysical tools (predominately analytical ultracentrifugation and light scattering techniques) to characterize the structure and physicochemical properties of a group of filamentous bacteriophages and its DNA.

4. I then spent 1975-1980 as a post-doctoral fellow at Yale University in the biology department under Dr. Robley Williams, Jr., the chemistry department

under Dr. Julian Sturtevant, and at the NIH under Dr. Jan Wolff. During my post-doctoral work, I was involved in research focused on studying the biochemical and biophysical structure and properties of microtubules.

5. After my post-doctoral work, I held various positions at Celanese Research Company (conducting analytical work on characterizing synthetic polymers), J.T. Baker (developing chromatography materials and automating analytical instruments and pilot plant equipment), and Lederle Laboratories (developing analytical and purification methods relating to monitoring and making of polysaccharide vaccines and pursuing efforts to automate these areas) before taking a position at Biogen. I spent more than twenty years working at Biogen in the areas of analytical and biophysical characterization, purification and formulation development on a wide range of biopharmaceutical products (involving proteins and complex drugs such as virus like particles and viral vectors for potential gene therapy applications). I retired from my position as a senior principal investigator at the end of 2013.

6. Since retiring, I have continued to participate in science by working as a consultant on a part time basis. My consulting work has generally focused on the biophysical characterization of biopharmaceuticals where I have continued to be active in publishing novel and original work and have occasionally served as an expert scientific reviewer for various scientific journals. Much of the scientific work

I have been involved with in my retirement has focused on the application of analytical ultracentrifugation (“AUC”) to gene therapy products, specifically the characterization of adeno-associated virus (“AAV”). This work started as early as 2014 when I did consulting work regarding how to do boundary sedimentation velocity AUC analysis on AAV using the software program SEDFIT and CsCl density gradient sedimentation equilibrium (a very common technique used for purifying viruses), but in an analytical mode, as described in my 2007 paper (with John Philo) for Adenovirus. EX1023 (Berkowitz).

7. Overall, my technical areas of expertise are concentrated in the separation and physical sciences associated with the characterization of a wide range of biopolymers and synthetic polymers. Much of my work has centered on assessing the physicochemical properties, micro-heterogeneity, aggregation properties, and higher-order structure of biopharmaceuticals using a range of biochemical and biophysical techniques. This work has predominately involved the use of dynamic and static (or intensity) light scattering, AUC, chromatography, electrophoresis, microcalorimetry and various forms of spectroscopy.

8. In applying these techniques, I have published approximately 35 peer reviewed papers and book chapters, have co-edited a book titled “Biophysical Characterization of Proteins in Developing Biopharmaceuticals,” and have given a

number of invited scientific talks that demonstrate novel extension of the techniques mentioned in the paragraphs above.

9. EX1005 is a copy of my *curriculum vitae* setting forth additional information concerning my background, credentials, and publications. Based on my education, training, and over 25 years of biopharmaceutical industry experience (including the January 2015 time frame) in pharmaceutical product development, which involved drug purification, formulation and analytical characterization and release testing, I am qualified to provide useful technical information regarding the subject matter of this case.

III. MATERIALS CONSIDERED

10. In formulating my opinions and preparing this declaration, I considered all of the references cited herein, including the references listed in the table below. I also relied on my experience, education, and knowledge of the art.

Exhibit Number	Description
EX1001	U.S. Patent No. 12,013,326 (“326 patent”)
EX1002	Prosecution history of U.S. Patent No. 12,013,326 (“326 patent prosecution history”)
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”)

Exhibit Number	Description
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”)
EX1011	Carter <i>et al.</i> , “Adeno-Associated Virus Autointerference” <i>Virology</i> 92:449-462 (1979) (“Carter”)
EX1012	Myers and Carter, “Assembly of Adeno-Associated Virus” <i>Virology</i> 102:71-82 (1980) (“Myers”)
EX1013	de la Maza and Carter, “Heavy and Light Particles of Adeno-Associated Virus” <i>J. Virol.</i> 33:1129-1137 (1980) (“de la Maza (March 1980)”)
EX1023	Berkowitz and Philo, “Monitoring the homogeneity of adenovirus preparations (a gene therapy delivery system) using analytical ultracentrifugation” <i>Anal. Biochem.</i> 362:16-37 (2007) (“Berkowitz”)

Exhibit Number	Description
EX1024	Berkowitz and Philo, “Characterizing Biopharmaceuticals using Analytical Ultracentrifugation” in BIOPHYSICAL CHARACTERIZATION OF PROTEINS IN DEVELOPING BIOPHARMACEUTICALS (Houde, D., and Berkowitz, S., eds.), Elsevier, 211-260 (2015), available online September 12, 2014.(“Berkowitz (2015)”)
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”)
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”)
EX1032	Schuck <i>et al.</i> , “Size-Distribution Analysis of Proteins by Analytical Ultracentrifugation: Strategies and Application to Model Systems” <i>Biophysical Journal</i> 82:1096-1111 (2002) (Schuck (2002)”)
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)”).

Exhibit Number	Description
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)”)
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)”)
EX1040	Srivastava <i>et al.</i> , “A Tribute to Barrie J. Carter” <i>Human Gene Therapy</i> 31:491-493 (2020) (“Srivastava”)
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”)

IV. LEGAL STANDARDS

11. In this section, I describe my understanding of certain legal standards relating to the issue of obviousness that I have been asked to consider for claims 1-5, 7-9, 12-23, and 25-30 of the '326 patent. These legal standards have been explained to me in connection with the preparation of this declaration. I have applied these standards in my analysis, as described in the sections below.

12. I have been informed by counsel and I understand that an obviousness analysis concerns whether the differences between the prior art and the subject matter claimed in the patent are such that, at the time the invention was made, the

subject matter as a whole would have been obvious to a “person of ordinary skill in the art” (“POSA”) to which the subject matter pertains.

13. I have been informed by counsel and I understand that obviousness is a factual inquiry, and that the factors guiding the analysis include: (1) the scope and content of the prior art; (2) the differences between the prior art and the claims at issue; (3) the level of ordinary skill in the art; and (4) any objective evidence of obviousness or non-obviousness, also referred to as “secondary considerations,” if present. I understand that secondary considerations may include, by way of example, commercial success, long-felt but unmet need, unexpected results, and copying of the invention.

14. I have been informed by counsel and I understand that obviousness may be shown by a single prior art reference or by a combination of prior art references. For obviousness based on a combination of prior art references, I understand that a POSA must have a motivation to combine the references. I understand that the prior art references themselves may provide a suggestion, motivation, or reason to combine. I further understand that a motivation to combine two or more prior art references need not be express, but may be based upon common sense or the knowledge available to a POSA.

15. I have been informed by counsel and I understand that, to evaluate the obviousness of the ’326 patent claims over a given combination of prior art

references, I should consider whether the prior art references disclose every limitation of the challenged claims either explicitly or inherently, as those references are read by the POSA at the time of the invention.

16. In addition, I understand that a POSA must have a reasonable expectation of success in modifying or combining the prior art to arrive at the claimed invention. However, I understand that obviousness cannot be avoided merely because there is some degree of unpredictability in the art.

17. Finally, I understand that it is impermissible to evaluate obviousness from a hindsight perspective, using the teachings of the patent as a guide. However, I understand that a POSA is not an automaton, and is assumed to have a reasonable degree of creativity. As such, I understand that an analysis of obviousness may take account of the inferences and creative steps that a POSA would employ.

V. SUMMARY OF INVALIDITY GROUNDS

18. The table below summarizes the invalidity grounds for claims 1-5, 7-9, 12-23, and 25-30 of the '326 patent that I address in this declaration.

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, 19-23, 26-30	Obvious over Le Bec and de la Maza, in view of Sommer

Ground	Claims	Description
4	25	Obvious over Le Bec and de la Maza, in view of Sommer and Cole

19. Le Bec (EX1006) was published in August 2014, before the earliest priority date listed on the face of the '326 patent, January 10, 2015. EX1001 ('326 patent), (60); EX1006 (Le Bec). I understand that Le Bec was the subject of anticipation and obviousness rejections during prosecution. EX1002 ('326 patent prosecution history), 172-178. I understand that to overcome these rejections, Patent Owner did not dispute that Le Bec is prior art to the '326 patent, but rather argued that Le Bec was distinguishable because the prior art does not disclose the linear relationship between sedimentation coefficients and molecular weights of genomes in AAV particles. *Id.*, 142-43, 150-51.

20. De la Maza (EX1007) was published in 1978, before the earliest priority date listed on the face of the '326 patent, January 10, 2015. EX1001 ('326 patent), (60); EX1007 (de la Maza). I understand that de la Maza was not cited or considered by the Patent Office during prosecution. EX1001 (the '326 patent) ("References Cited"); EX1002 ('326 patent prosecution history).

21. Sommer (EX1010) was published in 2003. Sommer is listed on the face of the '326 patent and is cited in the specification. EX1001 ('326 patent), 24:38-

41, 44:51-55. I understand that Sommer was not the subject of any rejection during prosecution. EX1002 ('326 patent prosecution history).

22. Cole (EX1009) was published in 2008. Cole is listed on the face of the '326 patent and is cited in the specification. EX1001 ('326 patent), 20:9-12; EX1009 (Cole). I understand that Cole was the subject of an obviousness rejection during prosecution. EX1002 ('326 patent prosecution history), 173-178. I understand that during prosecution, Patent Owner did not dispute that Cole is prior art to the '326 patent. *Id.*, 148-49.

VI. TECHNICAL BACKGROUND AND STATE OF THE PRIOR ART

A. Analytical Ultracentrifugation (“AUC”)

23. Analytical ultracentrifugation (“AUC”) is a versatile and powerful method for the quantitative analysis of macromolecules in solution. EX1009 (Cole), 174. The development of AUC-based techniques dates to the early twentieth century. EX1024 (Berkowitz (2015)), 211. It initially played a key role in demonstrating the existence of macromolecules via the pioneering studies carried out by Theodor Svedberg and his colleagues. *Id.* Svedberg was awarded the Nobel Prize in Chemistry for his work in 1926. *See* <https://www.nobelprize.org/prizes/chemistry/1926/svedberg/facts/>. In the years following this ground-breaking work, the analytical ultracentrifuge was widely used to characterize the macromolecular properties of biological and synthetic polymers, with a focus in characterizing

homogeneity, paucidispersity, or polydispersity of these macromolecular materials via size-distribution analysis (by assessing sedimentation coefficient distributions, $c(s)$ vs S). *See, e.g.*, EX1024 (Berkowitz (2015)), 211, EX1031 (Schuck), 1606-07; EX1032 (Schuck (2002)), 1096. Overall, the analytical ultracentrifuge has found its most effective application in the biological sciences where it has been employed to study the physical and chemical properties of the enormous range of macromolecules and other materials present in the biological world, *e.g.*, proteins, nucleic acids, polysaccharides, and supramolecular structures (such as ribosomes, multi-subunit enzymes, and viruses) by assessing the presence/absence and the amounts or distribution of the different components present in a biological sample and studying their sedimentation behavior. EX1024 (Berkowitz (2015)), 211.

24. In the 1980s timeframe, the advent of simple, inexpensive and high throughput techniques such as chromatography/electrophoresis, along with the eventual developments in mass spectroscopy, led to the temporary phasing out of interest in AUC. EX1024 (Berkowitz (2015)), 211-212; EX1023 (Berkowitz), 17.

25. However, development of Beckman's second generation automated analytical ultracentrifuge, the ready availability of computational power via inexpensive personal computers, the associated development of powerful computer software, and the renewed interest and need in applying biophysics to understand biological and biochemical processes all converged in the early to mid-1990s to

drive AUC's reemergence during the first decade of the twenty-first century. EX1024 (Berkowitz (2015)), 212; EX1025 (Cole (1999)), 164.

26. A particularly important attribute of AUC that made it highly valued in the biotechnology area was the ability to place virtually any biological sample in the ultracentrifuge, with little to no sample preparation, to acquire characterization information about its structure, physicochemical properties and assess its purity in its native environment. EX1025 (Cole (1999)), 164 (“biomolecules are characterized during analytical ultracentrifugation in their native state under biologically relevant solution conditions” and “[s]ample requirements are fairly modest, and preparation is straightforward.”). Avoiding sample processing steps, commonly needed when employing other techniques, greatly reduces the exposure of these delicate biological materials to hostile environments that can lead to altered data.

27. Analytical ultracentrifuges differ from other centrifuges that might be found in a laboratory setting by the ability to allow the operator to watch and monitor quantitatively the migration of particles or molecules of interest during the centrifugation process. EX1024 (Berkowitz (2015)), 212; EX1025 (Cole (1999)), 164. Depending on the nature of the sample and data sought, this monitoring can be performed using a range of detectors that include UV absorbance, refractometric (Raleigh interference, or refractometry), or fluorescence detection. At present,

Beckman Coulter is the only commercial supplier of AUC instruments on the market. EX1024 (Berkowitz (2015)), 212. Beckman has been supplying commercial centrifuges since the 1940s.

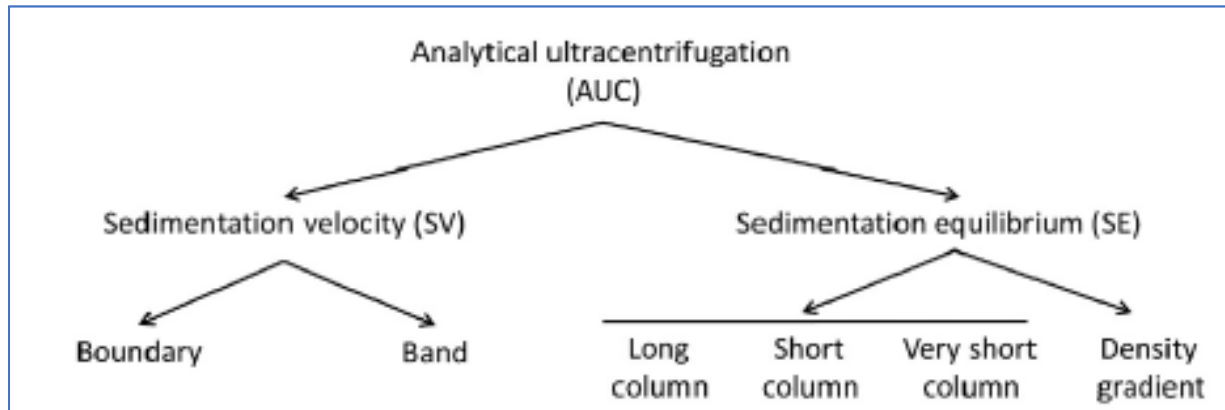
28. For a typical AUC experiment, a sample and reference solution (reference buffer or formulation buffer) are loaded into separate chambers or channels within a cell's centerpiece that is sandwiched between two window assemblies. *Id.* Inclusion of the reference buffer solution in one chamber allows the operator to account for any optical properties of the buffer solution. The cells are then sealed and precisely aligned into a titanium rotor so that the optical detection systems scan both sample and reference buffer solutions while rotating at speeds of up to 60,000 rpm and under very high vacuum at a defined and controlled temperature. EX1009 (Cole), 163-164.

29. The basic design and operation of analytical ultracentrifuge cells has changed remarkably little since the initial development of the analytical ultracentrifuge by Svedberg and colleagues in the 1920s. EX1024 (Berkowitz (2015)), 213.

1. Basic Principles

30. Despite the technological and analytical advances that have occurred over the many years, the core physics underlying AUC have remained constant. Simply put, a sample is loaded into an optically accessible cell in a centrifuge and

spun to induce a centrifugal (or gravitational) field and the movement of any solute particles (or molecular species) is monitored as a function of time. AUC experiments can be broadly classified as either sedimentation equilibrium (“SE-AUC”) or sedimentation velocity (“SV-AUC”):



EX1024 (Berkowitz (2015)), 214; Figure 9.2; *see also* EX1025 (Cole (1999)), 164-65.

31. The key difference between SV-AUC and SE-AUC experiments is rotor speed, which controls the strength of the centrifugal field applied to the sample. EX1024 (Berkowitz (2015)), 214. In the case of SV-AUC, the experimenter spins the rotor at a fast enough speed to force suspended particles or dissolved molecular species present in a sample to migrate (due to the net interaction of three forces, *see* ¶36 below) to the bottom of the cell while collecting data on their differing rates of movement (velocities) as they transit the cell. EX1025 (Cole (1999)), 165; EX1024 (Berkowitz (2015)), 214-215. In the case of SE-AUC, the experimenter spins the rotor at a much slower speed, which allows each suspended particle or dissolved

molecular species present to establish its own unique static exponential concentration distribution, which is a function of its buoyant mass. EX1024 (Berkowitz (2015)), 241.

32. The strength of a centrifugal field generated in any AUC experiment (the centrifugal acceleration) is equal to the product of the angular velocity, ω (expressed in radians/s), squared times the radial distance from the center of rotation, r (expressed in cm). As a result, the strength of the centrifugal field in an AUC experiment is not a constant, but rather increases linearly with radial distance:

$$\text{Centrifugal field} = \omega^2 r = [(\text{rpm})(2\pi)/60]^2 r \text{ (expressed as cm/s}^2\text{)}$$

33. For SV-AUC experiments, this field is usually strong enough that all particle or molecular species of interest will transit the length of the cell over a period of time (typically up to a few hours) depending on the buoyant mass (see ¶¶35-42 below) and size and shape of the particle and the viscosity and density of the solution through which it moves. EX1024 (Berkowitz (2015)), 214-15. This migration rate as a function of time is of key importance in these experiments and is characterized by an experimental molecular parameter called the sedimentation coefficient, s . The value of s for a given particle, macromolecule or molecule is a unique parameter that is specific to each species in a particular solvent run under specific experimental conditions (*e.g.*, temperature). Experimentally, s is simply defined as the ratio of the

velocity, v , with which a molecular entity moves with respect to the strength of the centrifugal field:

Sedimentation coefficient (s) = velocity/centrifugal field = $v/[\omega^2 r]$

See, e.g., EX1009 (Cole), 146; EX1025 (Cole (1999)), 165; EX1024 (Berkowitz (2015)), 215.

34. In practice, sedimentation coefficients of many important biological molecules range from roughly 10^{-13} to 10^{-11} sec. EX1024 (Berkowitz (2015)), 215. Therefore, s -values are usually reported in Svedberg units (using the symbol S), where $1 S = 10^{-13}$ sec. *Id.* Hence a sedimentation coefficient value of 5.3 S is equal to 5.3×10^{-13} sec. *Id.*

35. A molecule's velocity, and therefore its s value in an SV-AUC experiment, is determined by the summation of three forces (F), that are influenced by the physical and chemical properties of a particle or molecular entity in a specific formulation buffer in combination with the centrifugal field and other defined experimental conditions, *e.g.,* solvent composition and temperature. EX1009 (Cole), 146; EX1024 (Berkowitz (2015)), 215.

36. These three forces include:

- The centrifugal force (F_c) that drives molecules to the base of the AUC cell, which is equal to the product of the centrifugal acceleration ($\omega^2 r$) times the unique mass of the particle or

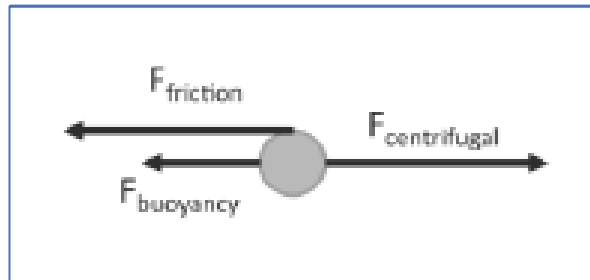
molecular entity. If M is the molar mass of the particle or molecule, dividing it by Avogadro's number (N_a) yields the mass of one particle or molecule, and hence $F_c = (M/N_a)\omega^2 r$.

- The buoyant force (F_b), which opposes F_c , is the force which the solvent molecules put on the solute molecules placed into the solvent solution which is equal to the mass of solvent displaced by the particle or molecular entity when it is placed into the solvent (M_s) multiplied by the strength of the centrifugal field, $F_b = -M_s(\omega^2 r)$, where the negative sign indicates that this force is in the opposite direction of F_c . M_s is equal to the product of the mass of the particle or molecular entity (M/N_a) times its partial specific volume, $\bar{v}$ (cm^3/g) times the solvent density, ρ (g/cm^3):

$$M_s = (M/N_a)(\bar{v}\rho).$$
- The frictional force (F_f) expressed as $-f v$, also opposes F_c (hence the negative sign), where f is the frictional coefficient of the particle or molecular entity which uniquely characterizes its hydrodynamic frictional properties as it moves through a solution under a net driving force, and v is the velocity. The frictional coefficient depends on the overall size and shape of the

particle. EX1025 (Cole (1999)), 166; EX1024 (Berkowitz (2015), 215.

37. The figure below summarizes the interplay between these forces:



38. Upon reaching the desired rotor speed in an SV-AUC experiment, these three forces will very quickly equilibrate ($F_{\text{friction}} + F_{\text{buoyancy}} = F_{\text{centrifugal}}$), and the particle will travel at a unique constant velocity defined by its sedimentation coefficient. By substituting the relationship defined in paragraph 33 for each force and rearranging the resulting equation, one obtains the well-known 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. EX1025 (Cole (1999)), 165-166; EX1024 (Berkowitz (2015)), 215-16; EX1009 (Cole), 146.

39. Thus, as set forth in the Svedberg equation, sedimentation velocity is a function of the mass, shape, and density of a particle. As a particle's molar mass (M) increases, the sedimentation coefficient (s) increases. Similarly, as a particle's density increases, the partial specific volume ($\bar{v}$) of the particle decreases because partial specific volume is the inverse of density of the particle. As $\bar{v}$ decreases, the term $(1-\bar{v}\rho)$ will increase, and thus the sedimentation coefficient (s) will increase. *See generally* EX1009 (Cole), 146; EX1024 (Berkowitz (2015)), 215. And as a particle's frictional coefficient decreases, the sedimentation coefficient (s) increases.

40. A higher s value therefore corresponds with a particle that sediments more quickly, suggesting a higher mass, higher density, and/or a more compact shape. A lower s value signals a particle that sediments more slowly, suggesting a lower mass, lower density, and/or a less compact shape.

41. As will be described in more detail below, a POSA in 2015 would have known that intact, non-aggregated AAV particles have approximately uniform size and shape, because AAV virus assembly occurs via insertion of DNA into preformed spherical capsids composed of the same number of protein subunits. EX1007 (de la Maza), 203; EX1012 (Myers), 71-72; *see also* EX1026 (Gao), 2, Figure 1 (showing transmission electron microscopy images of full and empty AAV particles with the same size and shape); EX1041 (Lock), 59, Figure 2 (same). As such, for AAV particles, the size and shape of a particle is about the same, and thus the frictional

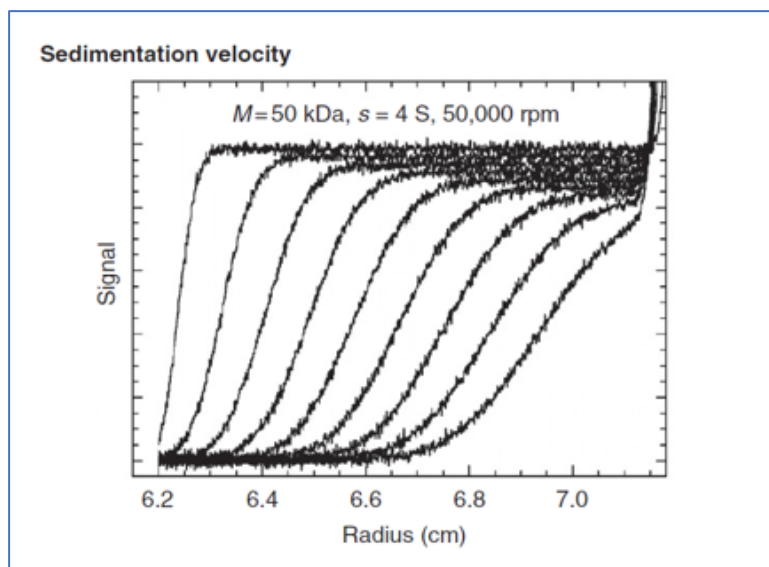
coefficient for AAV particles is approximately constant for empty particles, particles containing full genomes, and particles containing fragmented genomes.

42. Furthermore, solvent density ρ will be constant for a given solution at a given temperature. In applying the Svedberg equation, $s = M(1 - \bar{v}\rho)/(N_A f)$, to a solution of AAV particles, the only remaining variables are molar mass (M) and partial specific volume ($\bar{v}$) of the particle (*i.e.*, the inverse of the particle's density). For intact, non-aggregated AAV particles in the same solvent and at the same temperature, every parameter in the Svedberg equation is constant except for particle molar mass and partial specific volume (the inverse of density). Thus, a POSA in 2015 would understand from the Svedberg equation that the sedimentation coefficient (s) for an AAV particle is directly proportional to its molar mass and partial specific volume.

2. Boundary Sedimentation Velocity Analytical Ultracentrifugation

43. For nearly the past twenty years in the biopharmaceutical industry, the most common form of AUC conducted is boundary SV-AUC. EX1024 (Berkowitz (2015)), 219. The primary reasons for this are the high information content of this method, as well as the typically much shorter time required to conduct these experiments. *Id.* In conducting a boundary SV-AUC experiment on a homogeneous biopharmaceutical sample the resulting data output consists of a series of data scans, which typically are separated by a constant time interval over the course of the SV-

AUC run. *Id.*, 219-20. Particles or molecular entities 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.* The figure below illustrates a representative sedimentation velocity AUC experiment plotting absorbance (signal) against the radius (cm) for a single macromolecule species (a 50 kDa protein) at 50,000 rpm, measured at 20-minute intervals:



EX1009 (Cole), 162 (Figure 1A).

44. The Lamm equation, derived by Ole Lamm during his Ph.D. studies in the late 1920s, has been long known as the equation that describes the sedimentation of a component or molecular species under ultracentrifugation in traditional sector-shaped cells. EX1024 (Berkowitz (2015)), 228. The Lamm equation can be used by computer software programs to generate a sedimentation coefficient distribution

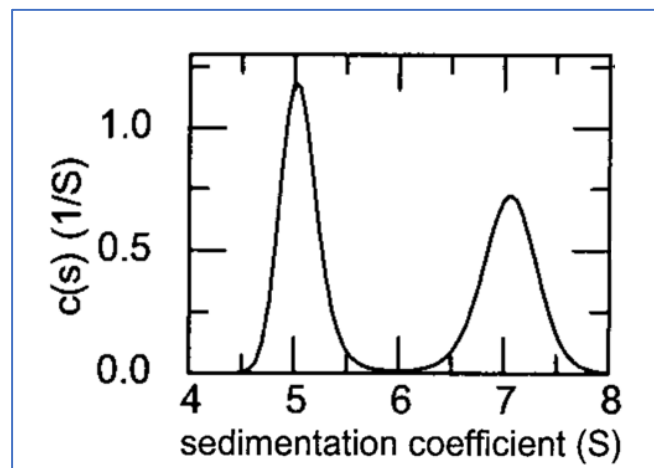
profile from SV-AUC data. EX1009 (Cole), 146-147, 166-167; EX1024 (Berkowitz (2015)), 228-233.

45. By way of example, in 2000, Peter Schuck created and freely distributed to the public a computer software program called SEDFIT. EX1031 (Schuck). Schuck explains that the Lamm equation can be used to model boundaries of sedimenting particles using a number of different models, the most common being that the sample consists of one or more independent ideal behaving discrete species (commonly referred to as the continuous $c(s)$ model). *Id.*, 1607. Schuck “describes an extension of the Lamm equation analysis for the characterization of continuous size distributions of macromolecules.” *Id.* The computational methods taught in Schuck were implemented into a Windows-based ultracentrifugal analysis program called SEDFIT. *Id.*, 1610.

46. SEDFIT is a versatile software tool for the analysis of SV-AUC data. A POSA in 2015 would have been well aware of the existence of the SEDFIT software and similar tools used for analyzing SV-AUC data. *See, e.g.*, EX1009 (Cole), 159, 166; EX1024 (Berkowitz (2015)), 228, 230-32. Programs such as SEDFIT may be used to graphically display sedimentation coefficient distribution profiles and allow for the determination of relative concentrations of the species in the sample at each peak. EX1023 (Berkowitz), 25.

47. In a typical SV-AUC experiment using SEDFIT, data is obtained on an analytical ultracentrifuge for one or more samples, and the resulting scan data is exported to the SEDFIT software. For a $c(s)$ analysis, the user will specify certain parameters depending on what is already known about the sample, and SEDFIT will attempt to find the $c(s)$ distribution that best fits the experimental data. EX1024 (Berkowitz (2015)), 231-32.

48. The following figure shows a sedimentation coefficient distribution profile generated by the SEDFIT program from SV-AUC data for a sample with two species (EX1032 (Schuck (2002)), 1109 (Figure 10)):



49. The sedimentation coefficient, measured in Svedberg units (S), is plotted along the x-axis (from 4 to 8 Svedberg units). A peak at a specific s value corresponds to a species with that sedimentation rate. $C(s)$, plotted on the y-axis, is the differential sedimentation coefficient distribution, which is to say it is a function that quantitates the amount of particles that are present at a certain s value. When

c(s) is plotted versus S the resulting plot yields the sedimentation coefficient distribution or c(s) distribution that characterizes the sample. *See* EX1023 (Berkowitz), 25 (“This c(s) distribution is similar to a chromatogram: each species produces a peak at its characteristic sedimentation coefficient and the area under each peak gives the concentration of that species (in signal units of OD or fringes).”). In this example, the distribution profile shows two species with sedimentation coefficients of approximately 5S and 7S, respectively.

B. Adeno-Associated Virus (“AAV”)

50. Adeno-associated virus (“AAV”) is a replication-defective human parvovirus that has been studied for decades as a possible gene therapy vector. *See, e.g.,* EX1008 (de la Maza (April 1980)), 3194; EX1033 (Carter (2004)), 981; EX1006 (Le Bec), 1:23-29. Recombinant AAVs (“rAAVs”) are AAVs in which the wild-type AAV genes have been replaced by other DNA sequences. EX1006 (Le Bec), 1:33-2:3 (“In rAAV vectors, the rep and cap genes flanked by the ITR (inverted terminal repeat) sequences respectively coding for the replication-regulating proteins and the structural proteins of the capsid are excised and replaced by the transgene (therapeutic gene).”). AAVs occur naturally in a variety of different serotypes, each with its own specific genome sequence and physical properties. *See, e.g.,* EX1006 (Le Bec), 7:1-9 (“The elements of the vector produced according to the invention may be derived from any serotype of AAV. In particular, each element

may be derived from an AAV of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11.”); EX1033 (Carter (2004)), 984 (“We now know that expanding the AAV work to a variety of serotypes offers a greater range of biology and sophistication in development of vectors.”); *see also* EX1001 (’326 patent), 34:56-58 (“More than 40 serotypes of AAV are currently known, and new serotypes and variants of existing serotypes continue to be identified.”).

51. AAVs were discovered in the 1960s in the laboratories of Robert Atchison at the University of Pittsburgh and Wallace Rowe at the National Institutes of Health (“NIH”). EX1033 (Carter (2004)), 982. The first AAV vectors were described in the 1980s, and the first human clinical trial of an AAV vector was initiated in the mid-1990s. *Id.*, 981. Following its discovery, AAV was studied extensively. In the 1970s and into the 1980s, the research largely focused on the molecular biology of AAV. *Id.*, 982-83; EX1034 (Carter (2020)), 495. For example, James Rose and Ken Berns at the NIH reported that AAV packages plus and minus single-strand DNA genomes into separate particles, and demonstrated how to separate these complementary strands. EX1033 (Carter (2004)), 982-83; EX1034 (Carter (2020)), 494.

52. Researchers working in the laboratory of Barrie Carter at the NIH were heavily involved in work to identify the basic elements of the AAV genome. EX1033 (Carter (2004)), 982-983; EX1034 (Carter (2020)), 495. Carter and his

colleagues published several articles between 1975 and 1980 describing, amongst other things, the structure of AAV DNA, the palindromic arrangement of the AAV inverted terminal repeats (“ITRs”), the mechanism of AAV DNA replication, and the packaging of progeny AAV genomes into preformed capsids during replication. *See generally* EX1033 (Carter (2004)).

53. Carter has been described as one of the “fathers of rAAV vectors.” EX1040 (Srivastava), 491. An overview of some of the AAV characterization work conducted in the Carter laboratory is described in the following section.

C. Characterization of AAVs

54. In the early 1970s, Carter and George Khoury conducted basic studies on the AAV genome and adenovirus helper functions, reporting the unusual properties of the AAV DNA terminal sequences, which ultimately led to their characterization as ITRs. EX1033 (Carter (2004)), 983; EX1034 (Carter (2020)), 495. Carter and Khoury later analyzed specific cleavage of AAV2 DNA by the restriction endonuclease *EcoRI*. EX1037 (Carter (1975)), 523. Carter and Khoury reported that AAV2 DNA duplexes were cleaved at two specific sites to provide three unique fragments A, B, and C, which are equivalent to approximately 57.2, 38.2, and 4.6% of the AAV genome, respectively. *Id.* The authors also were able to deduce the likely physical order of the three fragments within the duplex genome. *Id.*, 524, 536-37. Molecular weights of the three AAV DNA fragments were

determined via sedimentation in neutral and alkaline sucrose, gel electrophoresis, and distribution of ^{32}P isotope in each fragment. *Id.*, 532-33, Table 1.

55. Additional restriction endonuclease work by the Carter group and others characterized AAV DNA restriction fragments resulting from cleavage with *HindII*, *HindIII*, and *BamHI*. EX1038 (Carter (1976)), Abstract. Carter reported the physical order of the specific AAV DNA fragments obtained from cleavage with *EcoRI*, *HindII*, *HindIII*, and *BamHI*, and determined that stable AAV RNA was transcribed from 70% of the minus DNA strand. *Id.*, 1044. RNA-DNA hybridization experiments conducted using the cleavage fragments were used to obtain a more accurate map of the AAV genome represented in stable RNA. *Id.*

56. Cleavage of AAV DNA with *BamHI* was determined to produce two fragments, labeled *Bam A* and *Bam B* by the authors, as shown by sedimentation in neutral sucrose. *Id.*, 1048. Isotope distribution experiments determined that the *Bam A* and *Bam B* fragments contained 78% and 22% of the AAV2 genome, corresponding to molecular weights of 2.2×10^6 and 6.2×10^5 , respectively. *Id.*, 1049.

57. Carter and colleagues would later use selected *EcoRI* and *BamHI* AAV fragments as markers to determine the sedimentation coefficients and molecular weights of incomplete genomes of unknown length released from AAV particles. In 1978, de la Maza and Carter published a book chapter describing experiments to

characterize the physical properties of “incomplete” AAV genomes isolated from a preparation of AAV particles. EX1007 (de la Maza), 193.

58. Sedimentation coefficients and molecular weights of the incomplete AAV DNA molecules were computed from alkaline and neutral sucrose gradient profiles using the above-referenced *EcoRI* and *BamHI* fragments as molecular-weight markers. EX1007 (de la Maza), 195, Table 1. The DNA preparations of incomplete genomes used in the sedimentation analyses were then cleaved with restriction endonuclease. *Id.*, 197. Cleavage showed that as sizes of incomplete genomes decreased, the terminal regions of the AAV genome were progressively enriched and the internal regions progressively lost, with inverted repeated sequences present in all virions. *Id.*, 199-200. De la Maza concluded that the “density of the incomplete virus particles appears to be correlated with the size of the incomplete DNA.” *Id.*, 203. In other words, higher density AAV particles contain larger DNA genomes. This correlation “implies that virus assembly occurs by encapsidation of DNA into preformed capsids.” *Id.*

59. In 1980, de la Maza and Carter published an updated report of these experiments characterizing the physical properties of variant AAV DNA. *See* EX1008 (de la Maza (April 1980)), 3194-95. De la Maza (April 1980) again highlighted the “direct linear relationship” between the density of an AAV particle and the length of the encapsidated DNA. *Id.*, 3202. De la Maza (April 1980) further

reiterated that incomplete AAV DNA “appear to be encapsidated in AAV virions with usually only one strand per particle.” *Id.*

60. This work by de la Maza and Carter led to several important insights, including the observation that incomplete AAV DNA molecules are internally deleted and enriched for the ITRs, which helped to establish the importance of the ITR as the replication origin. EX1034 (Carter (2020)), 495. Furthermore, because these incomplete genomes were less than half-size, they could be encapsidated as dimeric molecules that snapped back to form duplex molecules, which was the basis for the idea of self-complementary AAV vectors. *Id.*, 495; *see* EX1007 (de la Maza), 196-97, 200-201; EX1008 (de la Maza (April 1980), 3202. De la Maza and Carter’s work also taught that solutions of AAV DNA particles contain a heterogeneous mixture of particles, including empty particles, particles containing full genomes, and particles containing fragmented genomes. De la Maza and Carter further established the “direct linear relationship” between the density of an AAV particle and the length of the encapsidated DNA, and described the encapsidation of one DNA strand per AAV particle. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980), 3202.

61. Finally, a 1980 publication by Maureen Myers and Carter reported additional experiments regarding the assembly of AAV particles. Amongst other analyses, Myers addressed the question of whether, during AAV assembly, the viral

genome is condensed into a preformed capsid, or whether the capsid assembles around the genome. EX1012 (Myers), 71-72. Myers taught that assembly of AAVs occurs via condensation (insertion) of DNA into preformed capsids. *Id.* During AAV assembly, DNA progeny strands are supplied by strand displacement replication from duplex replicating intermediates. *Id.*, 79. AAV protein assembles into the empty capsids which then associate with the progeny DNA strands to form intermediate structures. *Id.*, 80-81. The intermediate structures are converted to mature particles containing either standard (full) or fragmented genomes. *Id.* Myers thus confirmed that virus assembly occurs by insertion of DNA into preformed capsids.¹

D. AAV Use in Gene Therapy Applications

62. The above-referenced AAV research laid the foundation for the development of AAV as a vector for gene therapy. EX1034 (Carter (2020)), 495. Gene therapy refers to the concept of delivering corrective genetic material into an organism to combat a disease. *See, e.g.*, EX1023 (Berkowitz), 16. Several different

¹ Myers is cited in the '326 patent specification. EX1001 ('326 patent), 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, B J, 1980, *Virology*, 102:71-82).”); *see also id.*, 45:17-20.

viruses, including rAAVs, have been studied as potential gene therapy vectors to deliver therapeutic DNA to target cells. EX1006 (Le Bec), 1:23-29 (“The main types of vectors used today in gene therapy are viral vectors, including recombinant adeno-associated virus or rAAV vectors.”); *see also* EX1001 (’326 patent), 1:32-43 (“Examples of viruses used in gene therapy applications include adeno-associated virus (AAV), adenovirus, lentivirus and herpes simplex virus (HSV).”).

63. By the mid-1980s, there was definitive proof-of-concept that rAAV particles could be generated and could transduce mammalian cells to express foreign transgenes from either AAV or non-AAV transcription promoters. EX1033 (Carter (2004)), 986. By the end of the decade, the Carter lab had started to work on a specific AAV vector for treatment of cystic fibrosis. EX1034 (Carter (2020)), 496.

64. By the early 2000s, companies were developing gene therapy products comprising viral-based vectors. *See, e.g.*, EX1033 (Carter (2004)), 981. By 2015, the earliest priority date for the ’326 patent, it had been known for some time that the “main types of vectors used today in gene therapy are viral vectors, including recombinant adeno-associated virus or rAAV vectors.” EX1006 (Le Bec), 1:23-29; *see also* EX1023 (Berkowitz), 16 (describing “the use of specifically engineered viruses that can carry the desired genetic material into the target cell”); *see also* EX1001 (’326 patent), 1:32-34 (“Recombinant viruses show great promise and utility as a vehicle to deliver therapeutic nucleic acids for gene therapy

applications.”) Multiple viral platforms had been developed, which included “adeno-associated virus (AAV), adenovirus, lentivirus and herpes simplex virus (HSV).” EX1001 (’326 patent), 1:40-43.

65. As noted in the prior art, SV-AUC methods had been used in quality control applications for gene therapy products, including rAAV preparations, long before the ’326 patent priority date. *See, e.g.*, EX1026 (Gao), 6 (“[A]nalytic ultracentrifugation technology is a powerful tool for quantitative characterization of structural heterogeneity of rAAV preparations, allowing precise and selective observation of viral capsid sedimentation in real time.”); EX2029 (Glybera[®] EMA Assessment Report), 15 (AUC identified as a method used to “determine mass, density and distribution profiles” for AAV-based gene therapy Glybera[®]); *see also* EX1023 (Berkowitz), Abstract (“We demonstrate that a single sedimentation velocity run on an adenovirus sample can detect and accurately quantify a number of different forms of virus particles and subvirus particles” including “(a) intact virus monomer particles, (b) virus aggregates, (c) empty capsids (ECs), and (d) smaller assembly intermediates.”); EX1009 (Cole), 144-145 (“For over 75 years, analytical ultracentrifugation (AUC) has proven to be a powerful method for characterizing solutions of macromolecules and an indispensable tool for the quantitative analysis of macromolecular interactions. [...] The range of molecular weights suitable for AUC exceeds that of any other solution technique from a few hundred Daltons (e.g.,

peptides, dyes, oligosaccharides) to several hundred-million Daltons (e.g., viruses, organelles).”). Thus, by 2015 SV-AUC was recognized as a suitable technique for analyzing purity and homogeneity of viral-based products, including rAAV particles developed for gene therapy.

66. Indeed, a patent application published in August 2014 by Le Bec and colleagues detailed experiments using SV-AUC to characterize preparations of rAAV particles for gene therapy applications. *See* EX1006 (Le Bec). Before the earliest possible priority date for the ’326 patent in 2015, those working in the field were aware of the use of SV-AUC to characterize different types of biomolecules, as well as the application of SV-AUC to the development and manufacture of gene therapy products by identifying and quantifying the different species in preparations of rAAV particles.

VII. THE ’326 PATENT

67. The ’326 patent is titled “Analytical ultracentrifugation for characterization of recombinant viral particles.” EX1001 (’326 patent), (54). The ’326 patent names Catherine R. O’Riordan and Brenda Burnham as inventors. *Id.*, (72). The ’326 patent issued on June 18, 2024. *Id.*, (45). The ’326 patent identifies Genzyme Corporation as the assignee. *Id.*, (73).

68. The ’326 patent issued from U.S. Application No. 18/506,853, filed on November 10, 2023. *Id.*, (21), (22). The ’326 patent claims priority to U.S.

Application No. 15/544,498, filed on January 19, 2016. *Id.*, (63). The '326 patent also claims priority to Provisional Application No. 62/105,714, filed on January 20, 2015. *Id.*, (60). I understand that January 20, 2015 is the earliest possible priority date for the '326 patent.

A. The Claims

69. The challenged claims of the '326 patent recite methods of determining the size of fragmented genomes of AAV particle species in a preparation of viral particles, and methods of determining the molar concentrations of each species of individual viral particles in a sample, including viral particles with full, fragmented, and no genomes (empty capsids).

70. I have reproduced the challenged claims in the table below.

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[i]	(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[ii]	(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

Claim	Element
	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[iii]	(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.
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.

Claim	Element
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.
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[i]	(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[ii]	(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

Claim	Element
	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[iii]	(iii) determining the sedimentation coefficients of each species of the individual viral particles in the heterogeneous mixture of viral particles; and
20[iv]	(iv) quantifying the molar concentration of each species of the individual viral particles in the heterogeneous mixture
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.

Claim	Element
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.

71. As indicated in the table, claims 1 and 20 are independent claims. EX1001 ('326 patent), 55:12-36, 56:17-42. The remaining claims are dependent claims, which recite additional elements. *Id.*, 55:37-56:16, 56:43-57:6.

B. The Specification

72. The '326 patent relates to methods of analyzing recombinant AAV preparations using AUC. Generally, the '326 patent discloses methods of analyzing viral preparations which involve (a) subjecting the viral preparations to SV-AUC conditions and monitoring them, (b) plotting the resulting data as c(s) v. S plots, (c) integrating the area under specific S-values and/or (d) making inferences about the preparation and the relative or absolute amounts of different species therein based on the data. EX1001 ('326 patent), 1:65-2:9.

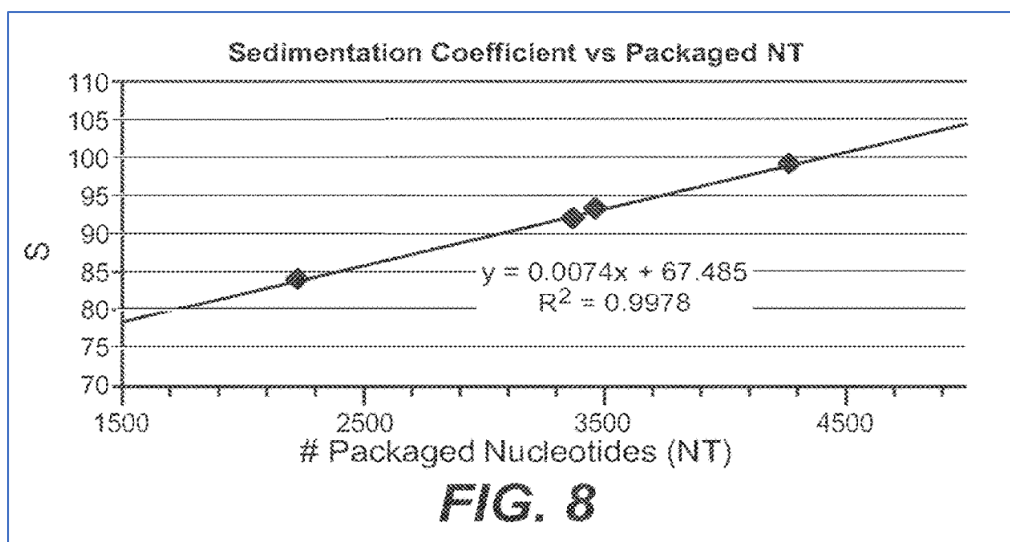
73. The '326 patent acknowledges that “[t]he techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art.” *Id.*, 13:6-9.

The '326 patent also acknowledges that “[i]t is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles.” *Id.*, 26:19-21. The '326 patent further states that “[s]uitable ultracentrifugation conditions, analysis algorithms, and other parameters may be determined empirically through methods known in the art.” *Id.*, 30:1-3. I agree that optimizing SV-AUC conditions for viruses, including rAAVs, would have been well known to a skilled practitioner as of the 2015 priority date, and that as of 2015 suitable ultracentrifugation conditions could be determined through methods known in the art.

74. The '326 patent also cites other references teaching the use of AUC to evaluate viral preparations for gene therapy applications. *Id.*, 19:19-21 (“Use of analytical ultracentrifugation to characterize adenovirus preparations is provided by Berkowitz, S A & Philo J S, (2007) *Anal. Biochem.*, 362:16-37.”). I also note that the '326 patent cites Cole as disclosing methods for optically detecting a sedimenting boundary and measuring its rate of movement or migration: “Several methods for optically detecting a sedimenting boundary and measuring its rate of movement or migration are known in the art (for reference, see Cole et al. (2008) *Methods Cell Biol.*, 84:143-79).” *Id.*, 20:9-12 (citing EX1009 (Cole)). I further agree that Cole teaches that multiple well-known methods of optically detecting a sedimenting boundary were known in the art as of the earliest priority date for the '326 patent.

75. In Example 1, the '326 patent discloses performance of an SV-AUC assay on an AAV preparation using a ProteomeLab™ XL-I analytical ultracentrifuge from Beckman Coulter at “20,000 RPM, 20°C, 0.003 radial step setting with no delay and with no replicates” and acquiring data using either optical absorbance or Rayleigh interference. *Id.*, 44:3-18. Data analysis was conducted using the SEDFIT software. *Id.*, 44:20-45.

76. Example 5 of the '326 patent is titled “Assessment of Viral Genome Integrity by the AUC Method.” *Id.*, 49:41-42. The '326 patent notes that “AAV vector preparations may contain capsids packaged with fragmented genomes, in addition to full genome and empty species.” *Id.*, 49:44-46. To characterize AAV particles with fragmented genomes, the '326 patent discloses a process involving measuring the S values of rAAV vectors with known genome sizes expressed in terms of number of nucleotides. *Id.*, 44:64-45:5, 49:58-67. The S values were plotted as a function of genome length, expressed in nucleotides (“NT”). This data is shown in Figure 8 of the '326 patent:



Id., Figure 8.

77. Figure 8 depicts 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. Figure 8 also shows a line of best fit for these data, including the formula and associated R^2 value for the measured data. *Id.*

C. The Prosecution History

78. During prosecution, the Examiner rejected the pending claims as anticipated by the prior art Le Bec reference, which discloses the SV-AUC analysis of heterogeneous preparations of AAV viral particles. EX1002 ('326 patent prosecution history), 172-178. The Examiner also rejected the claims as obvious over Le Bec in further view of additional references, including Cole. *Id.* In response,

Patent Owner amended the claims and made representations to the Patent Office regarding the scope of the claimed invention. *Id.*, 132-154.

79. Specifically, to overcome the Examiner's prior art rejections, Patent Owner represented to the patent office that:

[A]s of the filing date of the present application, the characterizing and quantifying of AAV particles with fragmented genomes was an important but unmet goal, and hence was a focus of the disclosure as filed.

...

The inventors of the present application discovered that sedimentation coefficients can be used to accurately calculate the molecular weight of the genomes in every viral particle of the preparation, including the molecular weights of the fragmented genomes. ***The linear relationship between sedimentation coefficients and molecular weights of genomes was not appreciated in the art prior to the present invention***, and it was not disclosed or suggested in the art applied in the Office Action, as discussed below. Therefore, the present application provides for the first time a means of calculating the size of each of the individual genomes in the population of viral particles.

Id., 142-43.

80. Further, I understand that Patent Owner argued that “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” and that “[t]he linear relationship between sedimentation coefficients and molecular weights of genomes was not disclosed in Le Bec.” *Id.*, 150-51. Patent Owner further

argued that the prior art “does not teach determining genome size of unknown fragmented genome species via a standardization curve comprising S values for rAAV viral particles with known genome size.” *Id.*

81. Furthermore, Patent Owner argued that “determining the molar concentrations of individual viral particles in an AAV preparation require[s] knowing the extinction coefficients of the particles.” *Id.*, 142 “The extinction coefficients are a function of molecular weight of the genome in a viral particle in accordance with Beer’s Law.” *Id.* Thus, Patent Owner argued that “molar concentrations of a viral particle can only be determined if the molecular weights of the genomes within the viral particle are known.” *Id.*

82. In response, the Examiner allowed the pending claims to issue “in light of the amendments to the claims and applicant arguments.” *Id.*, 34. During prosecution, I understand that Patent Owner did not cite de la Maza and the Examiner did not consider de la Maza in allowing the ’326 patent claims to issue.

VIII. THE PERSON OF ORDINARY SKILL IN THE ART

83. From counsel, I understand that obviousness must be analyzed from the perspective of a person of ordinary skill in the art (“POSA”) in the same field as the challenged patent as of the priority date of the claims. I understand that the earliest possible priority date for the ’326 patent is January 20, 2015. I further understand that a POSA is a hypothetical individual presumed to know the relevant art as of the

priority date and who has the same level of skill as the ordinary practitioner of the art at issue.

84. I have been asked to consider the level of education and experience of a POSA for the '326 patent. In evaluating the qualifications of a POSA, I have considered the following factors: (i) the types of problems encountered in the art, (ii) prior art solutions to those problems, (iii) the rapidity with which innovations are made, (iv) the sophistication of the technology, and (v) the educational level of active workers in the field. I have also relied on my experience working with and supervising others in the field of analytical methods relevant to gene therapies.

85. In my opinion, a POSA working in the field of the '326 patent on January 20, 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.

86. For this declaration, I have applied this definition of a POSA, and my opinions are offered from the perspective of a POSA as that hypothetical person would have understood matters on January 20, 2015.

IX. THE PRIOR ART

A. de la Maza

87. De la Maza is a chapter in the book “Replication of Mammalian Parvoviruses” entitled “DNA Structure of Incomplete Adeno-Associated-Virus Particles.” EX1007 (de la Maza). It was published in 1978 as part of a collection of papers that had been presented at a meeting at Cold Spring Harbor in 1977. EX1030 (Ward), ix. I understand that de la Maza qualifies as prior art to the '326 patent.

88. De la Maza describes experiments to characterize the physical properties of incomplete AAV genomes. EX1007 (de la Maza), 193 (“We have examined the AAV DNA present in both ‘full’ and ‘empty’ particles. These studies revealed that all density classes of AAV virions appear to contain varying proportions of incomplete genomes. [...] We summarize here the physical properties of the incomplete AAV genomes.”).

89. De la Maza and colleagues grew AAV2 in KB3 spinner cells with Ad2 as the helper virus. *Id.*, 194. Virus particles present in lysates of KB cells infected with AAV2 and Ad2 were fractionated via equilibrium density centrifugation in CsCl. *Id.*, 194. The authors identified and analyzed particle components which

banded in CsCl at densities of 1.45 g/cm³, 1.41 g/cm³, 1.35 g/cm³, and 1.32 g/cm³.

Id. In subsequent publications, Carter and colleagues referred to these classes of virus as AAV(1.45), AAV(1.41), AAV(1.35) and AAV(1.32) particles. *See, e.g.*, EX1011 (Carter).

90. The authors identified the vast majority of AAV particles in the bands at densities of 1.45 g/cm³ and 1.41 g/cm³ as “full” and a large proportion of those present in the 1.35 g/cm³ and 1.32 g/cm³ bands as “empty.” Pooling and concentrating the areas between the visible bands, following by rebanding in CsCl, indicated the presence of “incomplete” virus particles of different densities spread through the gradient. EX1007 (de la Maza), 194.

91. De la Maza further describes releasing the DNA from virions by sedimentation in alkaline sucrose gradients, reannealing in formamide, and further fractionation in neutral sucrose gradients. *Id.* The authors defined several classes of incomplete DNA molecules (Types I-IV) that were obtained from AAV virions of different densities, as detailed in Table 1:

TABLE 1
Sedimentation Properties of Incomplete AAV-DNA Molecules

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.

92. “Intact” DNA as defined in Table 1 is “the full-length infectious DNA of AAV2.” *Id.* Intact, or full-length, DNA made up the majority (90%) of the virions banding at 1.4 g/cm³. *Id.* Incomplete DNA Types I-IV, as designated by the authors, were obtained as follows: virions from the 1.41 or 1.45 g/cm³ band of a CsCl gradient were sedimented in alkaline sucrose, resulting in about 90% of DNA forming a single peak at 15.5S, and the remainder sedimented at about 12S. *Id.*, 195. The 15.5S DNA component from the alkaline sucrose gradient was reassociated and sedimented in neutral sucrose to yield a major 14.5S component (intact duplex AAV DNA) and a minor 11.5S component which was designated as “Type I” incomplete DNA. *Id.* The 12S DNA component was pooled, neutralized, and reassociated, and designated by the authors as “Type II” incomplete DNA. *Id.*, 194-95.

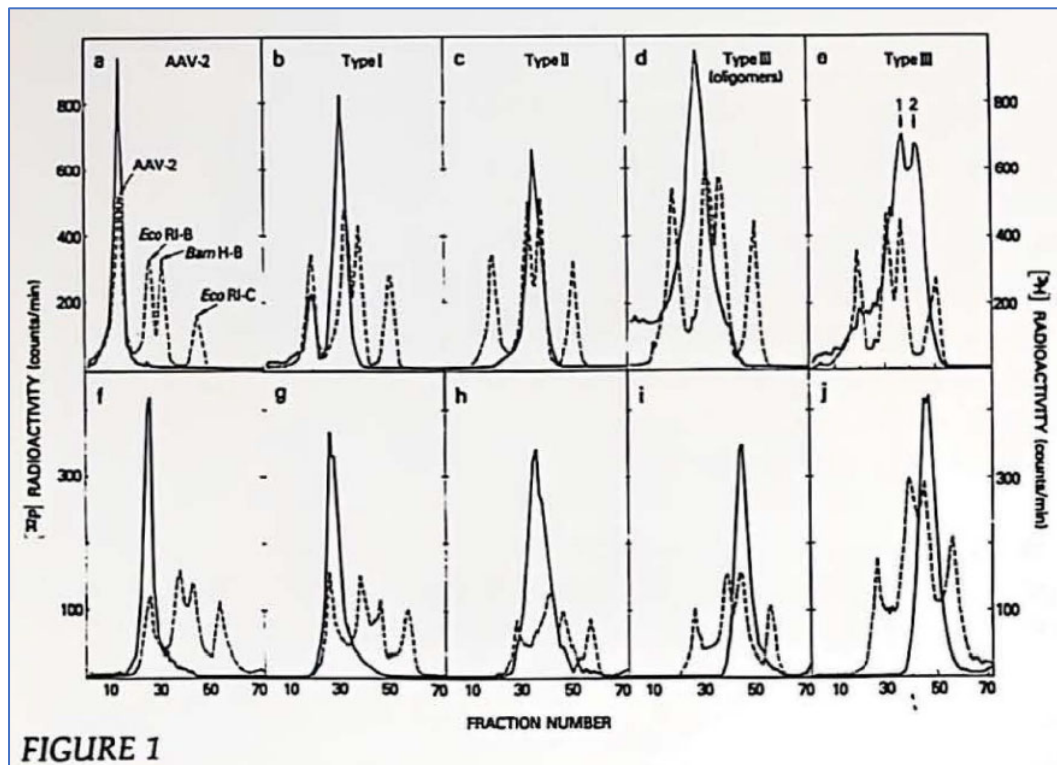
93. When virions that banded in CsCl at a density of 1.35 g/cm^3 were sedimented in alkaline sucrose, all AAV DNA sedimented at 8.5S. *Id.*, 195. The authors designated this DNA as “Type III” incomplete DNA. *Id.* Sedimentation of Type III incomplete DNA in neutral sucrose resulted in broad distribution of DNA from 8S to 15.5S, which the authors split into leading and trailing edges designated as “Type III oligomers” and “Type III” respectively. *Id.*, 195-96.

94. Sedimentation in alkaline sucrose of the virions that banded in CsCl at 1.32 g/cm^3 released a peak of DNA that sedimented at about 5S, which the authors designated as “Type IV” incomplete DNA. *Id.*, 196.

95. The molecular weights and sedimentation coefficients in Table 1 were calculated from sucrose gradient profiles using molecular-weight markers. *Id.*, 195, Table 1. All gradients contained ^3H -labeled duplex monomer AAV2 DNA or fragmented genomes representing 38% (*EcoRI* B), 22% (*Bam* B), and 4.5% (*EcoRI* C) of the AAV2 genome, respectively. *Id.*, 196, Figure 1. From these standards, de la Maza calculated the molecular weights and sedimentation coefficients of the incomplete AAV DNA strands, as detailed in Table 1 above.

96. Type I DNA sedimented in both neutral and alkaline sucrose gradients as a single component with molecular weight of 1.4×10^6 . *Id.*, 196-197. Type II DNA sedimented in both neutral and alkaline sucrose gradients as a single component with molecular weight of 0.8×10^6 . *Id.* Type III DNA sedimented in

alkaline sucrose with mean molecular weight of 0.3×10^6 . *Id.*, 197. Type III DNA sedimented in neutral sucrose as linear duplexes of molecular weight of 0.6×10^6 , “snapback” duplexes of molecular weight of 0.3×10^6 , and oligomeric structure of molecular weight of $\sim 1.4 \times 10^6$. *Id.*. The results of these sedimentation experiments are depicted in Figure 1:



Id., 196, Figure 1.

97. Figures 1a-e (top) show neutral sucrose gradients, and figures 1f-j (bottom) show alkaline sucrose gradients. *Id.* The dotted line represents ^3H -labeled DNA molecular weight markers (intact duplex monomer AAV DNA and fragments of 38% of the genome (*EcoRI* B), 22% of the genome (*Bam* B), and 4.5% of the

genome (*EcoRI* C)). *Id.* The solid line represents ³²P-labeled DNA preparations of intact AAV-2DNA and incomplete fragment Types I-III. *Id.*

98. The DNA preparations used in the alkaline and neutral sucrose sedimentation analyses were then cleaved with restriction endonuclease *HhaI* and electrophoresed in an acrylamide slab gel. *Id.*, 197. De la Maza teaches that the sizes of the uncleaved incomplete DNA fragments estimated by gel electrophoresis agree with those derived from neutral sucrose gradients. *Id.* Cleavage showed that as sizes of incomplete genomes decreased, the terminal regions of the AAV genome were progressively enriched and the internal regions progressively lost. *Id.*, 197, 199. Inverted repeated sequences of AAV DNA were present in all virions. *Id.*, 199-200.

99. Based on these sedimentation and cleavage experiments, de la Maza teaches that Type I incomplete DNA molecules appear to be hairpin duplexes containing either the left or the right terminal 30-50% of the genome, and Type II incomplete molecules have a similar structure but contain only 20-30% of the left or the right terminal region. *Id.*, 201. The authors reported that Type III molecules have a more heterogeneous structure configuration, containing only the left or right 10-20% of the genome and forming hairpin molecules or non-crosslinked duplexes. *Id.* Some of the Type III molecules may form oligomeric species held by the “cohesive ends” of the AAV DNA termini. *Id.* Type IV DNA molecules reportedly

contained little other than the inverted terminal repetitions, made up of 147 nucleotides at each terminus. *Id.*, 199-200.

100. Finally, de la Maza concludes that the “density of the incomplete virus particles appears to be correlated with the size of the incomplete DNA.” *Id.*, 203. 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.” *Id.*

101. In 1980, de la Maza and Carter published an updated report of these experiments characterizing the physical properties of variant AAV DNA in the Journal of Biological Chemistry. *See* EX1008 (de la Maza (April 1980)), 3194 (noting that “[a] preliminary account of some of the properties of AAV variant DNA has been presented before” and citing EX1007 (de la Maza)). Consistent with de la Maza (the 1978 book chapter), de la Maza (April 1980) again notes that “[t]he *density of the virus particle is generally correlated with the size of the variant DNA.*” EX1008 (de la Maza (April 1980)), 3194 (emphasis added).

102. In the discussion, de la Maza (April 1980) highlights the “direct linear relationship” between the density of an AAV particle and the length of the encapsidated DNA, teaching that “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).

103. Based on the teachings of de la Maza, a POSA would understand that there is a linear relationship between the density of AAV particles and the size of the DNA fragment contained therein. 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, as discussed above.

B. Le Bec

104. Le Bec is a publication of International Application Number PCT/EP2014/052978, entitled “Methods for the Production of Double-Stranded AAV Viral Particles.” EX1006 (Le Bec), (21), (54). I understand that Le Bec was published on August 21, 2014 and that it qualifies as prior art to the ’326 patent.

105. Le Bec discloses methods to produce compositions of AAV particles with double-stranded, “self-complementary” genomes (“scAAV”) for gene therapy applications using two different methods, one involving insect-derived sf9 cells, and another employing a human embryonic kidney cell line called HEK293. *Id.*, 6:14-

16, 12:2-33. These scAAV particles are recombinant AAV particles where the AAV genome has been replaced with a DNA sequence encoding a therapeutic protein. Le Bec reports characterization data for its AAV preparations, including “[a]nalysis of empty and full AAV viral particles” using AUC. *Id.*, 15:17-18.

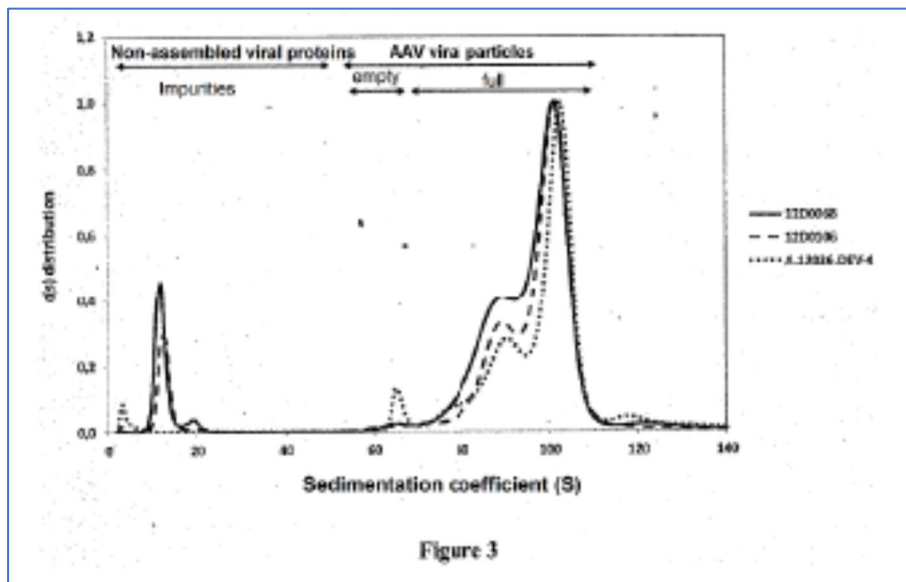
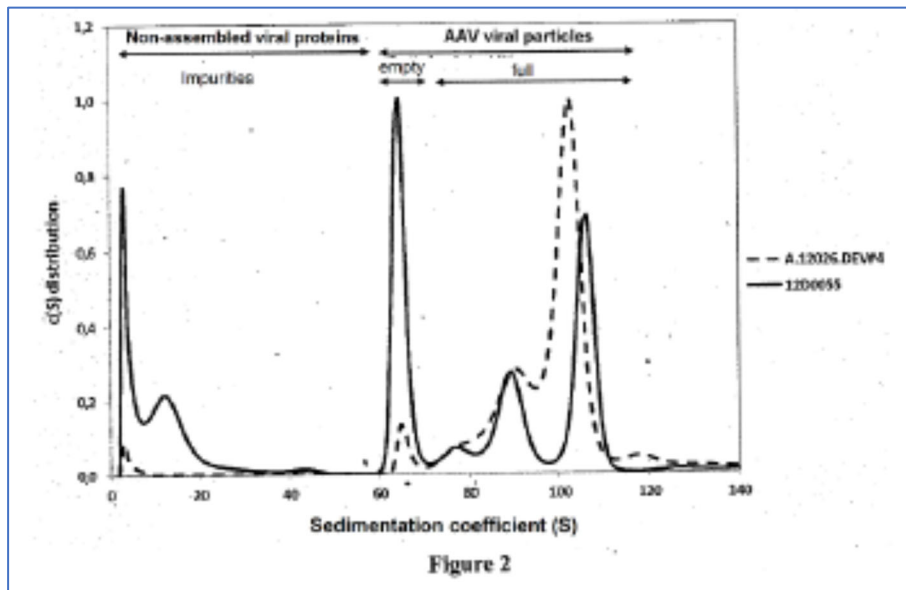
106. Le Bec discloses that “[t]he sedimentation coefficient of the various AAV viral particles (empty, full, aggregate) and other present populations (subparticles, contaminant proteins, aggregate) in the purified products was determined by real-time centrifugation.” *Id.*, 15:19-23. Further, “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm using 100 μ l or 400 μ l of undiluted pure vectors, sedimentation was followed by absorbance at the wavelength of 276 nm, and the sedimentation coefficient of the various populations was obtained using the software SEDFIT.” *Id.*, 15:23-28.

107. Finally, Le Bec explains that the densities of empty and full AAV viral particles had been reported in the literature, and that “[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.*, 17:30-18:6. Le Bec cites a de la Maza and Carter publication, “Heavy and Light Particles of Adeno-Associated Virus,” *J. Virol.*, 33 (1980): 1129-1137, as teaching that the density of empty AAV particles is approximately 1.32 g/cm³ (equivalent to 60S) and

the density of full AAV particles is approximately 1.45 g/cm³ (equivalent to 110S).

Id., 17:30-18:1 (citing EX1013 (de la Maza (March 1980))).

108. Figures 2 and 3 of Le Bec depict c(s) v. S plots:



Id., Figures 2, 3.

109. Figure 2 compares the AAV particles produced in the sf9 system (dashed line) with those prepared in the HEK293 system (solid line). Le Bec

explains that the distribution profile showed “two categories of populations”: (a) a population at <60S corresponding to non-assembled viral proteins and/or contaminants, and (b) a population at 60S-110S corresponding to intact AAV viral particles. *Id.*, 18:11-20. Within the 60S-110S population, Le Bec further delineates “two subpopulations:” “empty AAV particles at 65S and full AAV viral particles between 80S and 110S.” *Id.*, 18:21-25. Le Bec defines “full” particles as “comprising an AAV genome encapsidated within said particles.” *Id.*, 5:10-12, 11:21-22. Thus, a POSA would understand that Le Bec uses the term “full” to refer to any AAV particles containing a viral genome, including the complete double-stranded form of the viral genome, as well as the monomeric, single-stranded form and other fragmented genomes.

110. For example, the population of “full” particles in Figure 2 includes a peak at 90S corresponding to particles with the viral genome in single-stranded form, and a peak at 105S corresponding to particles with the viral genome in complete, double-stranded form. *Id.*, 18:14-25. Le Bec discloses that “[t]hese values are specific to the scAAV9-SMN vectors and will be redefined according to the type of genome.” *Id.*, 18:25-27. In addition to the single-stranded and double-stranded forms of full viral genomes at approximately 90S and 105S, a POSA viewing Figures 2 and 3 of Le Bec would also recognize an additional peak in the distribution profile

at approximately 75S. This peak corresponds to a fragmented genome that is smaller than the monomeric, single-stranded viral genome.

111. Le Bec indicates that the results in Figure 2 show the viral preparation made with the HEK293 system has less than 25% full particles (only 10% of which containing the double stranded genome). *Id.*, 19:1-5. Empty AAV particles make up a substantial portion of this composition, as shown by the large peak at 65S in Figure 2.

112. The data show that viral particles made with the sf9 system contained very few empty particles and were enriched in particles containing the complete, double-stranded genome relative to those produced using HEK293. *Id.*, 19:7-15. As shown in Figure 3 (a c(s) v. S plot of three lots of sf9-produced AAV particles), the AUC analysis showed consistent distribution profiles between lots, with few empty viral particles. *See id.*, Figure 3.

113. During prosecution of the '326 patent, Patent Owner admitted that Le Bec “teaches analyzing capsids that are empty (i.e., contain no genome) and capsids that contain full genomes” but nevertheless argued that “Le Bec does not teach determining the size of one or more *fragmented* genomes comprised within the capsids.” EX1002 ('326 patent prosecution history), 145.

C. Cole

114. Cole is a chapter in *Methods in Cell Biology*, Vol. 84 entitled “Analytical Ultracentrifugation: Sedimentation Velocity and Sedimentation Equilibrium.” Cole was published in 2008, and I understand that it qualifies as prior art to the ’326 patent. EX1009 (Cole). Cole was cited in the specification of the ’326 patent. EX1001 (’326 patent), 20:9-12.

115. Cole provides a general overview of various analytical ultracentrifugation techniques, including SV-AUC, and explains the various parameters and conditions that can be adjusted depending on the experimental system and samples being tested.

116. Cole explains that a common application of SV-AUC is to determine the distribution of sedimentation coefficients for different macromolecules, *e.g.*, by their $c(s)$ values, explaining that “often a simple relationship between s [sedimentation coefficient] and M [effective particle mass] may be used to identify particular peaks as belonging to certain oligomers (*e.g.*, dimer, trimer, etc.) or certain fragments of the monomer.” EX1009 (Cole), 149. In particular, “[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.” *Id.*

117. Cole also describes the instrumentation routinely used in SV-AUC analyses. Analytical ultracentrifuges are distinguished from other high-speed centrifuges by “the specialized rotors, sample holders and optical systems that permit the observation of samples during sedimentation.” *Id.*, 150. Samples are loaded into cells that permit the passage of light allowing for optical assessments during centrifugation. These “fundamental measurements” are known as “scans” and “are acquired at intervals ranging from minutes (for velocity sedimentation) to hours (for equilibrium sedimentation).” *Id.*, 151.

118. Cole provides detailed instructions on how to determine appropriate parameters to use for an SV-AUC experiment and how to perform the data analysis steps necessary to interpret the data. *Id.*, 161-168. For example, Cole teaches that the “useful temperature range” for SV-AUC experiments on the Beckman Coulter XLI is between 4 °C-35 °C and states that “most experiments are conducted at 20 °C.” *Id.*, 163. With respect to centrifugation time for SV-AUC experiments, Cole teaches 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. *Id.*, 164. Table III of Cole discloses “maximum recommended rotor speeds for various sedimentation coefficients” to enable “[a]bout 2 h of data acquisition” at the listed centrifuge rotor speed. *Id.*, 164, Table III, note c.

D. Sommer

119. Sommer is an article titled “Quantification of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement.” EX1010 (Sommer),

122. I understand that Sommer was published in 2003 and qualifies as prior art to the '326 patent.

120. Sommer is listed on the face of the '326 patent and is cited in the specification. EX1001 ('326 patent), 24:38-41, 44:51-55.

121. Sommer teaches “a simple, rapid, and direct method for quantifying [AAV] vector genomes and capsid proteins in solution.” EX1010 (Sommer), Abstract. Sommer notes that as of 2003, “[m]ethods for large scale production of highly purified AAV for clinical use have improved considerably in recent years.” *Id.*, 122. It was known in the art at that time that “[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.* To solve this issue, Sommer developed a method for quantifying vector genomes using UV absorbance at 260 and 280 nm to develop a ratio of A_{260}/A_{280} . *Id.*, Abstract.

122. The method taught in Sommer “takes into account the capsid ratio, deduced from the A_{260}/A_{280} ratio, to determine vector genome (vg) concentrations.”

Id., 122. This approach led to “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.

123. To calculate vector genome concentration, Sommer teaches a formula that can be used to determine the extinction coefficient for a recombinant AAV vector particle. *Id.*, 127. The basic calculation involves adding the extinction coefficient of the vector genome to the extinction coefficient for the AAV capsid to arrive at the extinction coefficient for the recombinant AAV vector particle. *Id.*

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

124. For AAV2 vectors, Sommer discloses equations for calculating extinction coefficients at 260 nm and 280 nm. *Id.* First, the molar extinction coefficients of AAV2 capsids at 260 and 280 nm were determined to be 3.72×10^6 and $6.31 \times 10^6 \text{ M}^{-1} \text{ cm}^{-1}$, respectively. *Id.* Sommer then determined the molar extinction coefficients for the vector genome at 260 and 280 nm to be $20.0 \text{ g}^{-1} \text{ cm}^{-1} \times \text{MW}_{\text{DNA}}$ and $11.1 \text{ g}^{-1} \text{ cm}^{-1} \times \text{MW}_{\text{DNA}}$, respectively. *Id.*

125. Sommer then teaches that “the individual absorbance of capsid protein and vector genome can be added to arrive at the extinction coefficient for a vector particle. *Id.* Combining the above molar extinction coefficients results in the following formulas for calculating the molar extinction coefficients for AAV2-based vector particles at 260 nm and 280 nm. *Id.*

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

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

126. As disclosed in Sommer, related AAV serotypes differ slightly in their capsid composition, which may result in slightly different extinction coefficients. *Id.*, 126. Sommer teaches 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 than for the AAV2 serotype reported in Sommer. *Id.*

127. Sommer thus teaches formulas that can be used to calculate the extinction coefficient for each recombinant AAV particle species if the molecular weight of the encapsidated DNA is known. *Id.*, 127. A POSA would then be able to calculate the concentration of each rAAV species in a preparation of AAV particles using Beer's Law, once the extinction coefficient and absorbance at a given wavelength are known. Indeed, the '326 patent specification states that molar extinction coefficients can be calculated based on the formulae in Sommer, and that once molar extinction coefficients are known, molar concentration can be calculated using Beer's Law. EX1001 ('326 patent), 44:49-62; 46:27-47.

X. CLAIM CONSTRUCTION

128. Challenged claims 1-5, 7-9, 12-23, and 25-30 of the '326 patent recite methods of determining the size of fragmented genomes of AAV particle species in

a preparation of viral particles, and methods of determining the molar concentrations of each species of individual viral particles in a sample, including viral particles with full, fragmented, and no genomes (empty capsids). EX1001 ('326 patent), 55:12-57:6.

129. Certain dependent claims recite additional limitations, including comparing the sedimentation coefficients of fragmented genomes with a standard curve generated from sedimentation coefficients of rAAVs of known nucleotide sizes, specific rpm and temperature ranges, specific centrifugation times, concentrations of viral particles, and specific AAV capsid proteins and ITRs. *Id.*, 55:12-57:6.

130. I have analyzed the terms recited in claims 1-5, 7-9, 12-23, and 25-30 according to their plain and ordinary meaning.

XI. GROUND 1: CLAIMS 1-4, 7, 12-13, 15, AND 17-18 ARE OBVIOUS OVER LE BEC AND DE LA MAZA

131. In my opinion, claims 1-4, 7, 12-13, 15, and 17-18 of the '326 patent are obvious over Le Bec and de la Maza.

132. A POSA would have been motivated to combine Le Bec and de la Maza because both are directed to separation, identification, and characterization of viral particles in AAV vector preparations, including analyzing AAV particles with fragmented genomes. Both Le Bec and de la Maza disclose the use of ultracentrifugation and sedimentation coefficient data to characterize AAVs. Le Bec

discloses the application of SV-AUC methods to preparations of rAAV particles for use in gene therapy applications. EX1006 (Le Bec), 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 size of the DNA in the capsid. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3194, 3202. A POSA would have been motivated to apply the linear relationship taught in de la Maza to the analysis of SV-AUC distribution profiles obtained using the methods taught in Le Bec, to identify and characterize the species in a preparation of rAAV particles, including AAV particles with fragmented genomes.

133. As stated in Berkowitz, “[t]o achieve drug licensure, drug product quality (with regard to homogeneity and purity) and consistency of manufacturing are of key importance.” EX1023 (Berkowitz), 16. By 2015, a POSA would have known that SV-AUC methods had been developed to analyze preparations of rAAV viral vectors for use in gene therapy applications and to quantify various AAV particle species contained therein, such as empty particles, particles containing complete genomes, and particles containing fragmented genomes. EX1006 (Le Bec), 15:17-28, 17:29-19:15. Specifically, Le Bec teaches that SV-AUC may be used to monitor product quality of batches of rAAV during “the large-scale production of human-grade gene therapy vectors in compliance with good manufacturing practice.” *Id.*, 3:32-4:9. Given the importance of drug product

quality (in particular homogeneity and purity), and the known benefit of utilizing SV-AUC methods for analyzing homogeneity and purity of drug products, including for preparations of rAAV used in gene therapy applications, a POSA would have been motivated to use SV-AUC to characterize preparations of rAAV vectors, and quantify different species of particles contained therein, including characterization of rAAV particles having fragmented genomes.

134. Le Bec teaches using SV-AUC to separate and quantify species in an AAV viral preparation for use in therapeutic applications such as gene therapy. *Id.*, 1:5-19, 3:24-4:10, 15:17-28, 17:29-19:15. Indeed, Patent Owner admitted during prosecution of the '326 patent that Le Bec “teaches analyzing capsids that are empty (i.e., contain no genome) and capsids that contain full genomes.” EX1002 ('326 patent prosecution history), 145. Furthermore, the '326 patent specification acknowledges that “AUC analysis has been well characterized over many decades and is highly versatile” and that it “may be applied to determine the biophysical properties of many types of particles across a wide range of particle concentrations and sizes.” EX1001 ('326 patent), 19:37-43.

135. To apply the SV-AUC methods of Le Bec to rAAV particles with fragmented genomes, a POSA would have been motivated to look to the characterization work on fragmented AAV genomes conducted by de la Maza and Carter in the 1970s and 1980s. Carter has been described as one of the “fathers of

rAAV vectors.” EX1040 (Srivastava), 491. A POSA would know that Carter and de la Maza conducted detailed characterization work on the properties of AAV particles with incomplete, or fragmented, genomes, and would have been motivated to combine the teachings in de la Maza about the physical properties of AAV with fragmented genomes with the methods taught in Le Bec.

136. Le Bec itself provides a POSA with explicit motivation to combine Le Bec’s SV-AUC methods with the teachings of de la Maza by citing a 1980 publication by de la Maza and Carter as teaching the density and corresponding sedimentation coefficients of empty and full AAV particles. EX1006 (Le Bec), 17:30-18:1 (citing EX1013 (de la Maza (March 1980))). As Le Bec states, “[i]t has been reported in the literature that the density is approximately 1.32 g/cm^3 (equivalent to 60S) for empty AAV viral particles, i.e. vectors lacking a viral genome, and 1.45 g/cm^3 (equivalent to 110S) for full AAV viral particles, i.e. those containing a viral genome with a maximum size of 4800 bases.” *Id.*, 17:30-18:1 (citing EX1013 (de la Maza (March 1980))).

137. Le Bec uses de la Maza and Carter’s sedimentation coefficient teachings as a benchmark for the analysis of the SV-AUC experiments carried out in Le Bec on batches of scAAV vectors. *Id.*, 18:8-32. Figures 2 and 3 of Le Bec disclose SV-AUC distribution profiles of four different batches of scAAV. *Id.*, 6:3-10; Figures 2 and 3. Based on the density of empty particles reported by de la Maza

and Carter of 1.32 g/cm^3 (corresponding to 60S), Le Bec identifies the peak at 65S in Figure 2 as empty AAV capsids. *Id.*, 17:30-33, 18:21-22, Figure 2. Based on the density of full AAV viral particles containing a complete wild type viral genome of 4800 bases, reported by de la Maza and Carter to be approximately 1.45 g/cm^3 (corresponding to 110S), Le Bec identifies the peak at 105S in Figure 2 as full AAV particles with complete, double-stranded self-complementary genome. *Id.*, 17:33-18:1, 18:21-25, Figure 2. Le Bec also identifies the peak at 90S in Figure 2 as AAV particles with the viral genome in single-stranded form. *Id.* A POSA would understand the single-stranded genome to be about half the size of the complete double-stranded genome.

138. Given the citation in Le Bec to work from de la Maza and Carter, a POSA would have been motivated to combine Le Bec with other teachings from the Carter lab regarding the physical properties of incomplete, or fragmented AAV. Specifically, a POSA would have been motivated to combine Le Bec with de la Maza, which teaches the linear relationship between AAV particle density and the size of the packaged DNA. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3194, 3202.

139. Indeed, a POSA viewing the SV-AUC profiles in Figures 2 and 3 of Le Bec would have recognized there is a direct linear relationship between the sedimentation coefficient and the size of the DNA packaged in an AAV particle, as

taught by de la Maza. The S values for empty AAV particles (65S), AAV particles with a monomeric, single-stranded genome (90S), and AAV particles with a complete, double-stranded, self-complementary genome (105S) are directly correlated to the size of the packaged DNA. EX1006 (Le Bec), 18:8-32, Figures 2 and 3. Assuming that the complete, double-stranded self-complementary genome is approximately the same size as a wild type AAV genome – about 4700 kb, as reported in Le Bec (*see* EX1006 (Le Bec), 3:4-9) and de la Maza (April 1980) (*see* EX1008 (de la Maza (April 1980)), 3194, 3202) – a POSA would understand that the monomeric, single-stranded genome would be approximately 2350 kb in size. Plotting sedimentation coefficient against genome size with these three data points provides a direct linear relationship, in agreement with the teachings of de la Maza.

140. In my opinion, a POSA would have been motivated to apply the direct linear relationship taught in de la Maza to the SV-AUC methods in Le Bec to analyze and quantify the different viral species in preparations of rAAV particles.

A. Claim 1

- 1. “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:”**

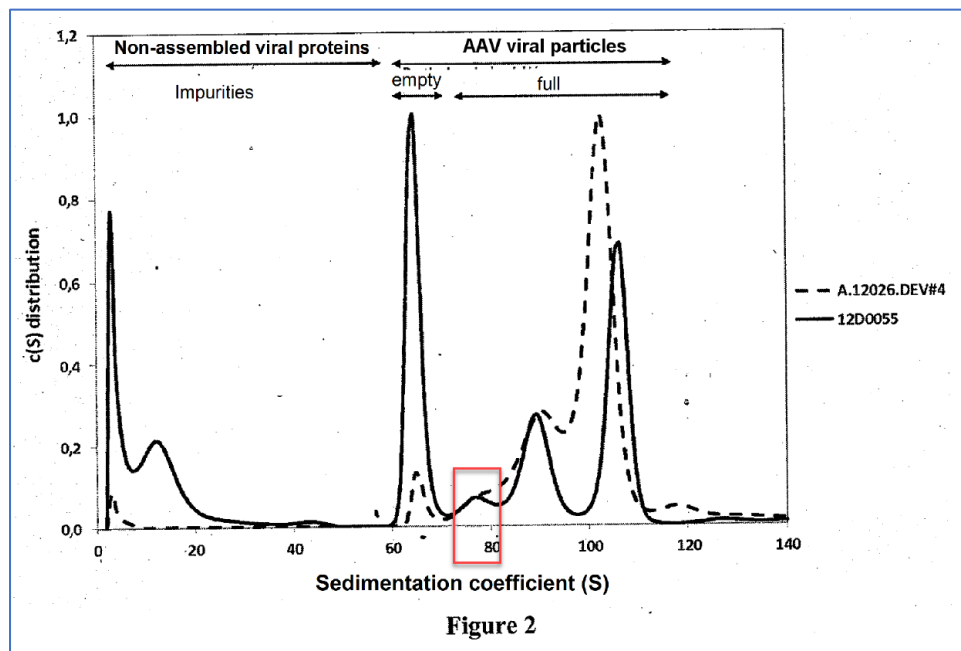
141. As an initial matter, I note that de la Maza uses the terms “incomplete” or “variant” to refer to fragmented genomes. *See* EX1007 (de la Maza), 193 (“We

summarize here the physical properties of the *incomplete AAV genomes*.”) (emphasis added); *see also* EX1008 (de la Maza (April 1980)), 3194 (“We have analyzed ... the molecular structure of the *variant AAV DNA molecules* obtained from these light density particles.”) (emphasis added). I will use the term “fragmented” in my analysis with this understanding.

142. Le Bec discloses a method of analyzing a preparation of rAAV vectors. Specifically, Le Bec “implemented a method ... for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.” EX1006 (Le Bec), 18:1-6. Le Bec discloses an SV-AUC method capable of analytically separating, detecting, and quantifying empty AAV particles, AAV particles with complete genomes, and AAV particles with fragmented genomes in an rAAV preparation. *Id.*, 15:17-28, 17:29-19:15.

143. Le Bec characterizes the SV-AUC distribution profiles in Figures 2 and 3 as containing “two subpopulations” of AAV viral particles, namely “empty AAV viral particles at 65S and full AAV viral particles between 80S and 110S.” *Id.*, 18:21-25. Le Bec further states that within the subpopulation of full AAV viral particles, there exists “viral genome in single-stranded form at 90S and double-stranded form at 105S.” *Id.*

144. In addition to the single-stranded and double-stranded viral genomes at approximately 90S and 105S, Figure 2 discloses an additional peak in the distribution profile at approximately 75S, highlighted in the figure below:



Id., Figure 2 (batch 12D0055).

145. As shown in Figure 2, Le Bec categorizes all AAV particles containing a DNA strand as “full,” regardless of whether the capsid contains a complete, double-stranded viral genome, or some smaller sized DNA. *Id.*, Figure 2; *see also id.*, 5:10-12 (defining “full viral rAAV particles” to mean “comprising an AAV genome encapsidated within said particles.”). Peaks in the distribution profile between the empty capsid population (at 65S) and the complete, double-stranded genome (at 105S) correspond to rAAVs with fragmented genomes – *i.e.*, rAAVs that contain less than a complete double-stranded, self-complementary genome. Thus,

for example, the peak at approximately 75S in Figure 2 corresponds to a capsid containing a fragmented genome that is smaller than the monomeric, single-stranded genome.

146. De la Maza discloses the size of fragmented (*i.e.*, incomplete) genomes in a preparation of wild type AAV particles. EX1007 (de la Maza), 193 (“We summarize here the physical properties of the incomplete AAV genomes.”); EX1008 (de la Maza (April 1980)), 3194 (“We have analyzed ... the molecular structure of the variant AAV DNA molecules obtained from these light density particles. The size of variant DNA molecules ranged from 100 to 3% of genome length.”).

147. 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 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3194, 3202, EX1011 (Carter), 449. Applying this teaching in de la Maza to the analysis of the sedimentation profiles obtained using the SV-AUC methods in Le Bec, a POSA would have been able to determine the size of one or more fragmented genomes in a preparation of recombinant AAV vectors.

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

2. “(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;”

149. Le Bec teaches a method of subjecting an rAAV preparation to AUC to generate sedimenting boundaries. EX1006 (Le Bec), 15:23-28 (“Centrifugation of the samples was carried out at a speed of 16,000 rpm using 100 μ l or 400 μ l of undiluted pure vectors, sedimentation was followed by absorbance at the wavelength of 276 nm, and the sedimentation coefficient of the various populations was obtained using the software SEDFIT.”). As discussed in detail below, the AUC methods disclosed in Le Bec reflect boundary sedimentation velocity conditions.

150. First, Le Bec teaches that sedimentation coefficients of the various populations of rAAV particles were determined, confirming that the method used in Le Bec required measurement of the velocity at which different boundaries sedimented. *Id.*, 18:8-32. A POSA would use SV-AUC if they sought to obtain sedimentation coefficients or sedimentation coefficient distribution profiles from a sample.

151. Le Bec further states that in the AUC experiments disclosed therein, “sedimentation was followed by absorbance at the wavelength of 276 nm.” *Id.*, 15:25-26. Measurement of absorbance at 276 nm is consistent with the identification in the ’326 patent of “exemplary absorbances” for sedimentation of viral particles at

“about 230 nm, 260 nm or 280 nm.” *See* EX1001 (’326 patent), 4:42-43; *see also id.*, 29-30 (Table 1). A POSA would understand absorbance at 276 nm to be a typical detection approach for monitoring migration of AAV particles during an SV-AUC experiment.

152. Additionally, Le Bec discloses two c(s) v. S plots generated from AUC data. EX1006 (Le Bec), Figures 2 and 3. Creating such plots requires data generated from samples sedimenting under boundary sedimentation velocity conditions. That is how a POSA would acquire data about the unique sedimentation velocities of species in a composition necessary to construct such a plot. Such plots are typically generated by a software program, like the SEDFIT software identified in Le Bec. *Id.*, 15:27-28 (“the sedimentation coefficient of the various populations was obtained using the software SEDFIT”). Use of the SEDFIT software to construct these plots and determine the unique sedimentation velocities of the particle species in the rAAV preparation confirms that sedimentation was monitored at time intervals, as is necessary for an SV-AUC experiment. Le Bec explicitly states that data was acquired in “real time,” which a POSA would understand to refer to monitoring sedimentation of distinct populations of rAAV particles at time intervals. *Id.*, 15:19-23. In view of these disclosures in Le Bec, a POSA would understand that Le Bec applies boundary sedimentation velocity AUC conditions to rAAV particles.

153. Moreover, Le Bec discloses that rAAV particles of interest will range from approximately 60S to 110S. *Id.*, 17:30-18:25. As described above, boundary sedimentation results when a sufficient centrifugal force is applied to a sample such that particles migrate at characteristic velocities. This force is achieved by setting an appropriate rotor speed based on the known or expected sedimentation coefficients of the particles of interest, generally using the fastest rotor speed available that will permit enough scans/cell to be acquired. 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 ('326 patent), 26:19-21. Knowing the range of expected S values, a POSA may determine an appropriate rotor speed for particles with those anticipated sedimentation coefficients based on the knowledge in the art. By way of example, Cole generally teaches SV-AUC and discloses maximum rotor speeds for analyzing species at 90S and 270S, respectively:

Table III Maximum Rotor Speeds for Sedimentation Velocity Experiments		
S^a	M_{app}^b	rpm ^c
10	200,000	55,000
15	400,000	50,000
30	1,000,000	30,000
90	5,000,000	20,000
270	25,000,000	10,000

EX1009 (Cole), 164 (Table 3).

154. Knowing that 20,000 rpm is an approximate maximum rotor speed for SV-AUC experiments of species at 90S, and 10,000 rpm is an approximate maximum rotor speed for SV-AUC experiments of species at 270S, a POSA would understand that the rotor speed of 16,000 rpm used in Le Bec is sufficient to create sedimentation boundary conditions for the characterization of rAAV particles with sedimentation coefficients of approximately 60S to 110S. The rotor speed of 16,000 rpm also falls within the range identified in the '326 patent for use with AAV particles (between 10,000 rpm and 20,000 rpm). EX1001 ('326 patent), 29-30 (Table 1).

155. Finally, Le Bec teaches centrifugation at 16,000 rpm. EX1006 (Le Bec), 15:23-25 ("centrifugation of the samples was carried out at a speed of 16,000 rpm."). 16,000 rpm falls within the claimed range of 3,000-20,000 rpm.

156. Thus, Le Bec meets this limitation of claim 1.

3. **"(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"**

157. Le Bec teaches a method of subjecting a preparation of rAAV particles to AUC to generate sedimenting boundaries, measuring the rate of migration of the

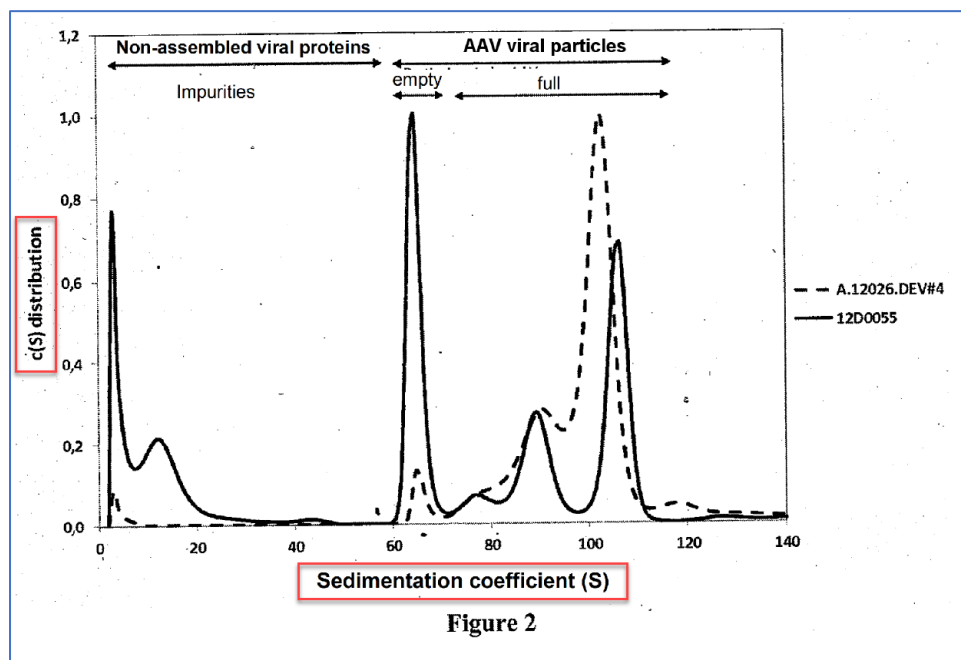
sedimenting boundaries, and determining sedimentation coefficients of the particles contained in the viral preparation. EX1006 (Le Bec), 15:23-28. As discussed above, a POSA would understand Le Bec to teach using SV-AUC to analyze preparations of rAAV particles. A POSA would further understand that the data output for an SV-AUC experiment consists of a series of data scans, which typically are separated by a constant time interval over the course of the SV-AUC run, wherein 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 of the boundaries over time.

158. Distinct populations of viral particles will sediment with distinct sedimenting boundaries. For example, a POSA would understand that viral particles of different densities (*e.g.*, empty rAAV particles, rAAV particles containing complete genomes, and rAAV particles containing fragmented genomes) will sediment as distinct boundaries given their different masses and densities. Thus, the migration of viral particles under SV-AUC conditions would result in distinct sedimenting boundaries, each distinct sedimenting boundary corresponding to a resolvable viral particle.

159. Le Bec discloses that “sedimentation was followed by absorbance at the wavelength of 276 nm.” EX1006 (Le Bec), 15:25-26. A POSA would understand that obtaining absorbance data refers to measuring the rate of migration

of the sedimenting boundaries. Furthermore, Le Bec teaches that the sedimentation coefficient of various rAAV particles was determined using the SEDFIT software. *Id.*, 15:27-28.

160. Figures 2 and 3 of Le Bec show “analytical ultracentrifugation distribution profile[s]” generated by the SEDFIT software. *Id.*, 6:3-10. In both figures, “c(s) distribution” is plotted on the y-axis and “Sedimentation coefficient (S)” is plotted on the x-axis. *Id.*, Figures 2 and 3:



161. Le Bec specifically identifies sedimentation coefficients for rAAV particle species based on the peaks in the distribution profiles in Figures 2 and 3. *Id.*, 18:8-32. Le Bec reports that the peak at 65S corresponds to empty rAAV particles, the peak at 105S corresponds to an rAAV particle with a complete, double-stranded, self-complementary genome, and that the peak at 90S corresponds to

particles with a monomeric, single stranded genome. *Id.*, 18:21-25. A POSA would understand the peak at 90S to correspond to an rAAV with a fragmented genome – *i.e.*, a genome that is less than the complete double-stranded, self-complementary genome. Figure 2 also shows a peak at about 75S, which corresponds to an rAAV particle with a fragmented genome that is smaller than the monomeric, single-stranded genome. *Id.*, Figure 2 (batch 12D0055).

162. Thus, Le Bec meets this limitation of claim 1.

4. “(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.”

163. Le Bec discloses methods of determining the sedimentation coefficients for species of rAAV particles, including those with fragmented genomes, by SV-AUC. EX1006 (Le Bec), 15:17-28, 18:8-32, Figures 2, 3. Le Bec identifies the size of a fragmented genome in at least one species of rAAV particle as a function of its sedimentation coefficient. Specifically, Le Bec identifies the size of the monomeric, single stranded genome as a function of the sedimentation coefficient (90S), as compared to the sedimentation coefficients of empty rAAV particles (65S) and rAAV particles with a complete, double-stranded, self-complementary genome (105S). *Id.*, 18:19-25. A POSA would understand that the peak at 90S corresponds to a particle with a fragmented genome because the particles contain less than the complete double-stranded, self-complementary genome.

164. De la Maza teaches that there is a linear relationship between the density of AAV virus particles and the size of the encapsidated DNA. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3194, 3202. De la Maza explicitly teaches that “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 (de la Maza), 203; *see also* EX1008 (de la Maza (April 1980), 3202 (“particle density has an approximately direct linear relationship to the [DNA] strand length”).

165. De la Maza teaches that the correlation between particle density and DNA size “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 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3202 (“variant DNA molecules extracted from AAV particles ... appear to be encapsidated in AAV virions with usually only one strand per particle”). The mechanism for virus assembly was confirmed by further work in the Carter laboratory published in 1980. EX1012 (Myers). Specifically, Myers and Carter confirmed 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.” *Id.*, Abstract.

Myers and Carter thus concluded that their experimental results were “consistent with a model for AAV assembly in which progeny DNA strands are packaged into preformed capsids.” *Id.*; *see also id.*, (Myers), 72 (“Our studies suggest assembly of AAV by condensation of DNA into preformed capsids.”).

166. As of 2015, a POSA would have been familiar with the mechanism for AAV assembly. Indeed, the specification of the ’326 patent refers to this known mechanism of AAV particle formation, citing Myers and Carter’s publication from 35 years earlier. EX1001 (’326 patent), 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).”); *see also id.*, 45:17-20.

167. Because AAV assembly occurs via packaging of DNA into preformed capsids, a POSA would understand that empty AAV particles, particles containing fragmented genomes, and particles containing complete genomes are all approximately the same size and shape. *See* EX1007 (de la Maza), 203; EX1012 (Myers); *see also* EX1026 (Gao), 2, Figure 1 (showing transmission electron microscopy images of full and empty AAV particles with the same size and shape); EX1041 (Lock), 59, Figure 2 (same). Because all particles are approximately the same size and shape, the density of an AAV particle—and thus its sedimentation coefficient, which is a function of particle density—is directly proportional to the

size of the encapsidated DNA, as explicitly taught by de la Maza. EX1007 (de la Maza), 203; *see also* EX1008 (de la Maza (April 1980), 3202 (“particle density has an approximately direct linear relationship to the [DNA] strand length”).

168. A POSA would have been able to apply the teachings of de la Maza to the SV-AUC methods taught by Le Bec to determine the size of fragmented genomes as a function of the sedimentation coefficients of viral particles. As discussed above, a POSA would have understood from the Svedberg equation that sedimentation coefficients are a function of the mass, shape, and density of a particle. *See* § VI.A.1; *see also* EX1009 (Cole), 146. A higher sedimentation coefficient (s) value corresponds to a particle that sediments more quickly, suggesting a higher mass, higher density, and/or a more compact shape. A lower sedimentation coefficient (s) value corresponds to a particle that sediments more slowly, suggesting a lower mass, lower density, and/or a less compact shape.

169. The size and shape of intact, non-aggregated rAAV particles are about the same, regardless of the size of the genome packaged in the capsid, such that the frictional coefficient for AAV particles is constant in a given solvent and at a given temperature. *See* § VI.A.1. Thus, for AAV particles, every component in the Svedberg equation is constant except for particle density (*i.e.*, partial specific volume) and particle molar mass. A POSA would understand that any observed differences in measured sedimentation coefficients would be the result of differences

in mass and density between different species of AAV particles. Given the constant contribution of mass and density from the empty AAV capsid, the resulting increase in sedimentation coefficient value for an empty AAV capsid filled with increasing amounts of DNA is directly (linearly) related to the length (*i.e.*, size or molecular weight) of the genome packaged in the capsid. The experimental results disclosed in de la Maza demonstrate empirically that there is a linear relationship between an intact, non-aggregated AAV particle's sedimentation coefficient and the length or size of the genome therein, as predicted by the Svedberg equation.

170. This relationship between sedimentation coefficient and the physical characteristics of a particle is also noted in the specification of the '326 patent. EX1001 ('326 patent), 17:35-39 ("The rate at which the sedimentation boundary moves is a measure of the sedimentation coefficient of the sedimenting species. The sedimentation coefficient depends on the molecular weight (larger particles sediment faster) and also on molecular shape."); 20:46-49 ("The rate of the boundary movement determines the sedimentation coefficient, which is based on the mass and shape (frictional coefficient) of the particle.").

171. With the knowledge that a linear relationship exists between the sedimentation coefficient and the size of the genome in an rAAV particle, 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. By way of example, a POSA would have been able to determine the approximate size of the fragmented genome in the rAAV particles with sedimentation coefficient of about 75S in Figure 2 of Le Bec by comparison to the sedimentation coefficients of the rAAV particles with genomes of known length in Figure 2 – that is, the empty rAAV particles at 65S, the particles with a complete, double-stranded, self-complementary genome at 105S, and the rAAV particles with a monomeric, single-stranded genome at 90S. EX1006 (Le Bec), Figure 2 (batch 12D0055).

172. Assuming that the complete, double-stranded genome for the rAAV particles in Le Bec is approximately the same size as a wild-type AAV genome (4700 nucleotides, *see* EX1006 (Le Bec), 3:5-6; EX1008 (de la Maza (April 1980), 3194, 3202), a POSA would be able to determine the approximate size of the fragmented genome in the rAAV particles with a sedimentation coefficient of about 75S. One exemplary approach to making this determination is by comparing the sedimentation coefficient of the 75S particles to a standard curve prepared from the sedimentation coefficients and genome sizes for the empty rAAV particles at 65S, the particles with a complete, double-stranded, self-complementary genome at 105S, and the rAAV particles with a monomeric, single-stranded genome at 90S. *See* § XI.B, below. Using this approach, a POSA would calculate the approximate size

of the fragmented genome of the rAAV particles at 75S to be about 980 nucleotides.

See § XI.B, below.

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

B. 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.”

174. Figures 2 and 3 of Le Bec disclose SV-AUC distribution profiles for rAAV particles of known genome size. EX1006 (Le Bec), 18:19-25, Figures 2, 3. A POSA would know, based on the teachings of de la Maza, that there is a linear relationship between genome size and the density of an AAV particle. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3202. In light of this known linear relationship, a POSA would have been able to use experimentally generated sedimentation coefficients for rAAV particles with genomes of known genome size to generate a standard curve.

175. A standard curve, sometimes also referred to as a calibration curve, is a general analytical approach used to plot measured values against a set of standards of a given parameter, such as size or concentration. The standard curve essentially shows the relationship between two parameters (*e.g.*, measured response and known

size/concentration). The standard curve may then be used to calculate the parameter of interest as a function of the measured value. Here, a POSA would have been able to use the standard curve generated from sedimentation coefficients for rAAV particles of *known* genome size to determine the genome size of fragmented genomes in particles containing DNA of *unknown* size.

176. A POSA would have known that molecular weight markers were a well-established, commonly used technique in the art for determining the molecular weight of unknown macromolecules. For example, de la Maza discloses the use of DNA molecular weight markers to determine molecular weight and genome length of DNA in fragmented AAV genomes. EX1007 (de la Maza), 196-197, Figure 1. De la Maza used molecular weight markers comprised of duplex monomer AAV2 DNA, as well as fragmented genomes representing 38%, 22%, and 4.5% of the AAV2 genome, in alkaline and neutral sucrose gradients to measure fragmented genomes of unknown length. *Id.*, 196, Figure 1. A comparison of the sedimentation of the unknown fragmented DNA (labeled Types I-IV by the authors) to the sedimentation of the known molecular weight markers was used to determine molecular weights of the unknown, fragmented genomes. *Id.*, 195, Table 1.

177. Applying the teachings of de la Maza regarding the direct linear relationship between particle density and DNA strand length to the SV-AUC methods in Le Bec, a POSA would have been able to determine the size of an

unknown, fragmented genome by comparison to the sedimentation coefficients (s) for rAAVs with genomes of known length.

178. As an example, the sedimentation profile in Figure 2 of Le Bec is illustrative. As discussed above, Figure 2 shows a sedimentation profile with sedimentation coefficients for three subpopulations of rAAV particles of known genome size: empty rAAV particles at 65S (with no DNA), rAAV particles with a monomeric, single-stranded genome at 90S, and rAAV particles with a complete double-stranded, self-complementary genome at 105S. EX1006 (Le Bec), Figure 2 (batch 12D0055). Figure 2 also depicts a population of rAAV particles with fragmented genomes of unknown size at approximately 75S. A POSA would have been able to determine the size of the fragmented genomes in particles of unknown size by comparison to the sedimentation coefficients for the rAAV particles of known genome size (*i.e.*, the empty, single-stranded, and double-stranded populations).

179. Le Bec does not report the number of nucleotides in the genome of the disclosed scAAV vectors. However, assuming that the complete, double-stranded genome for the scAAV vectors in Le Bec is approximately the same size as a wild-type AAV genome (4700 nucleotides (*see* EX1006 (Le Bec), 3:5-6; EX1008 (de la Maza (April 1980)), 3194, 3202)), a POSA would have been able to calculate the

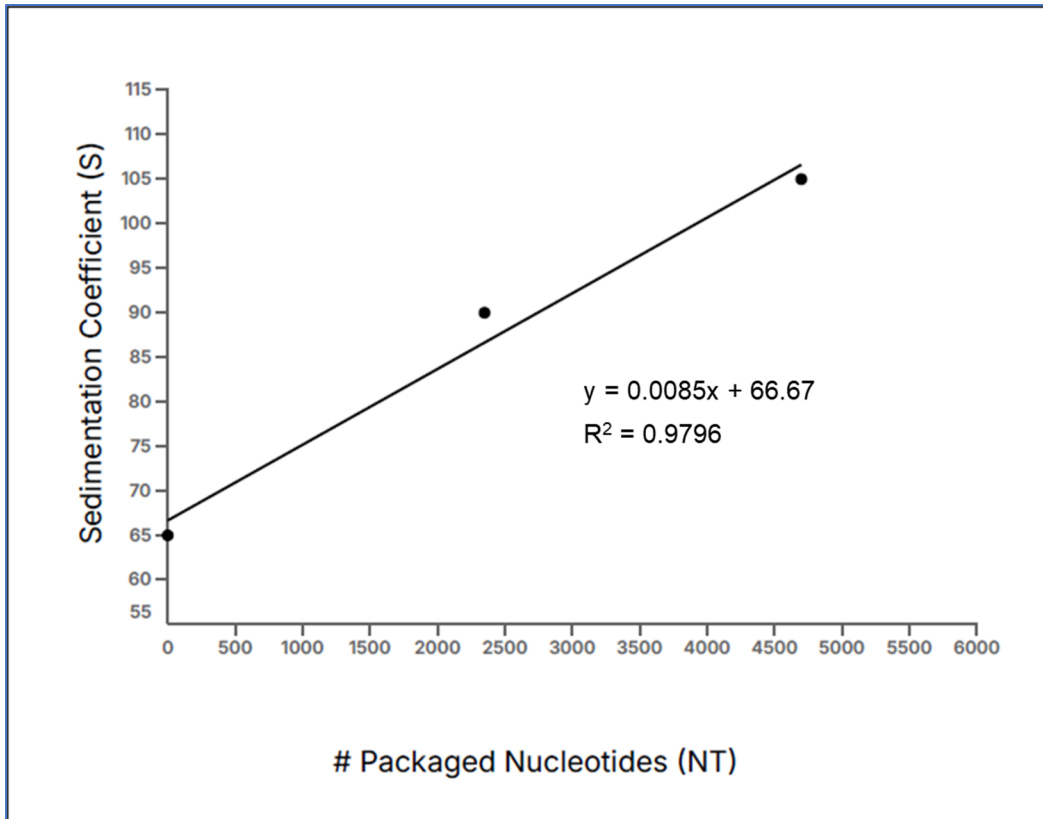
approximate size of the fragmented genome in the population of rAAV particles at 75S to be about 980 nucleotides.

180. The size of the fragmented genome may be calculated by comparing the sedimentation coefficient for rAAV particles with a fragmented genome of unknown length (75S) to a standard curve generated from the sedimentation coefficients and genome sizes disclosed in Figure 2 of Le Bec, as detailed in Table 1 below:

Population	Sedimentation coefficient	# nucleotides
Empty AAV particles	65	0
Single-stranded AAV viral genome	90	2350
Double-stranded AAV viral genome	105	4700

Table 1: Sedimentation coefficients and number of nucleotides for rAAV particles in Le Bec Figure 2

181. Applying a linear regression analysis to the data in Table 1 (derived from Le Bec Figure 2), a POSA would have obtained the following standard curve:



182. The equation of the best fit line is $y = 0.0085x + 66.67$, where y is the sedimentation coefficient and x is genome size, in nucleotides. The R^2 value is 0.9796. Using this equation, a POSA would be able to calculate the approximate genome size x (in nucleotides) for the unknown fragmented genome at 75S by plugging in $y = 75S$. Solving the equation for x results in a genome size of about 980 nucleotides.

183. Combining the SV-AUC methods in Le Bec with the teachings in de la Maza regarding the direct linear relationship between density and genome size, a POSA would have been able to determine the size of the genome in rAAV particles

with fragmented genomes by comparison to a standard curve generated from the sedimentation coefficients for rAAV particles with genomes of known length.

184. Thus, Le Bec, in combination with de la Maza, meets the additional limitation of dependent claim 2.

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

185. Le Bec discloses SV-AUC analyses wherein “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm.” EX1006 (Le Bec), 15:23-25. Centrifugation at 16,000 rpm as taught in Le Bec falls within the range of 10,000-20,000 rpm.

186. Thus, Le Bec meets the additional limitation of dependent claim 3.

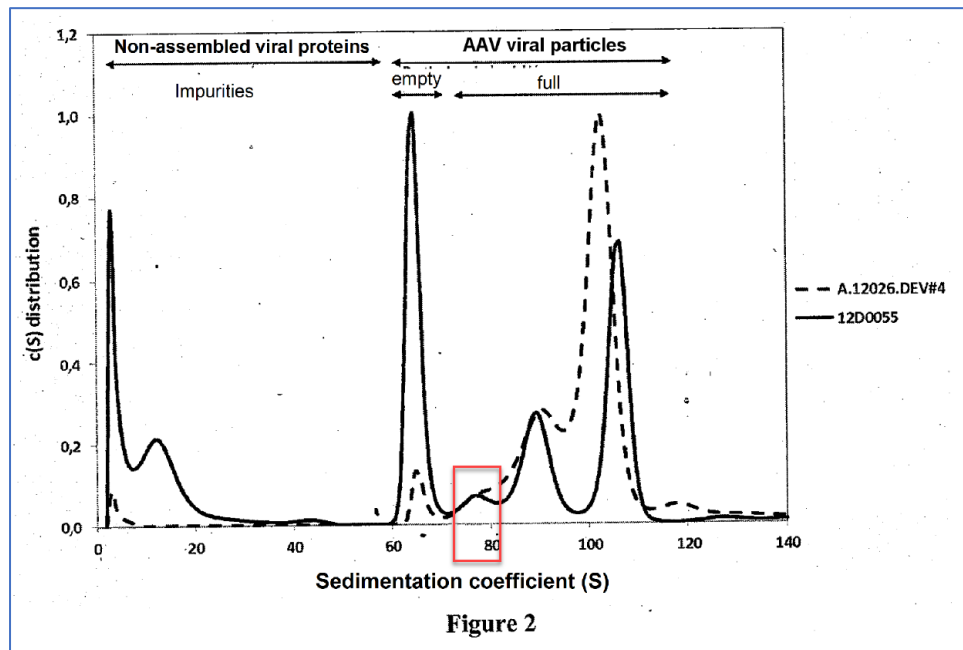
D. Claim 4: “The method of claim 1, wherein the boundary sedimentation velocity is from about 15,000 rpm to about 20,000 rpm.”

187. Le Bec discloses SV-AUC analyses wherein “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm.” EX1006 (Le Bec), 15:23-25. Centrifugation at 16,000 rpm as taught in Le Bec falls within the range of 15,000-20,000 rpm.

188. Thus, Le Bec meets the additional limitation of dependent claim 4.

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

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



Id., Figure 2 (batch 12D0055), 18:21-25.

190. A POSA would understand that the peak at 75S in Le Bec Figure 2 corresponds to a fragmented genome that is a sub-genomic DNA molecule.

191. De la Maza determines the molecular weights of sub-genomic DNA molecules in a preparation of AAV particles, by using molecular weight markers to

compute molecular weights and sedimentation coefficients for incomplete AAV DNA molecules. EX1007 (de la Maza), 194-97, Table 1; EX1008 (de la Maza (April 1980)), 3195-96, Table 1. De la Maza discloses that the incomplete (*i.e.*, fragmented) DNAs designated Type I, II, III and IV are each missing part of the AAV genome. EX1007 (de la Maza), 200-201; EX1008 (de la Maza (April 1980)), 3202 (“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).”). A POSA would understand that these fragmented genomes, which are missing part of the AAV genome, are sub-genomic DNA molecules.

192. Thus, Le Bec and de la Maza each meet the additional limitation of dependent claim 7.

F. 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.”

193. Le Bec discloses methods for producing high-titer, large-scale quantities of rAAV vectors for gene therapy applications. EX1006 (Le Bec), 1:5-10 (“The invention relates to methods for producing double-stranded AAV viral particles using the insect/baculovirus cell system to produce sufficient amounts of product for therapeutic applications such as gene therapy and/or DNA vaccination in uses during clinical or commercial trials.”); *id.*, 3:33-34 (“The invention relates

to a method for producing a high-titer and large-scale double-stranded AAV vector (scAAV)"); *see also id.*, 1:14-19, 2:19:3:2, 4:6-10, 6:14-33, 21:3-10 (claim 1).

194. Le Bec teaches preparing samples of rAAV viral particles to “a final concentration of approximately $\geq 10^{11}$ vg/mL.” *Id.*, 10:28-32; *id.*, 14:15-19 (“The AAV viral particles are concentrated and formulated in PBS buffer by tangential filtration (TFF) using a cutoff threshold of 100 KDa to a final concentration of approximately $\geq 10^{11}$ vg/mL and filtered through a 0.2 μ m filter.”).

195. Table 2 of Le Bec discloses the titer for different batches of rAAV vectors after purification. *Id.*, 19:23-20:3. As set forth in Table 2, each batch of rAAV vector has a concentration between 5.1×10^{11} and 1.6×10^{12} vg/mL, which is greater than the concentration of 5×10^{11} vg/mL recited in claim 12:

Cell culture		Purification				
Type	Batch no.	Step 1	Step 2			
		Bulk no.	Batch no.	Vol (ml)	Titer (vg/mL)	Total Vg
HEK293F	12D0004	12D055	12D0055	0.6	1.6E+12	9.5E+11
	12D0082	12D0082	12D0082	0.8	5.1E+11	4.1E+11
sf9	A.12026.DEV	A.12026.DEV-4	A.12026.DEV-4	1.2	1.4E+12	1.7E+12
	12D0066	12D0066	12D0106	0.8	9.4E+11	7.5E+11
		12D0070				
		12D0068				
			12D0068	0.5	1.6E+12	8.0E+11

Table 2: Description of the volume and titer of viral genomes (vg/mL), total amount of viral genomes of AAV9 particles (vg) obtained after purification step 2

Id.

196. Thus, Le Bec meets the additional limitation of dependent claim 12.

G. 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.”

197. Le Bec discloses methods for producing high-titer, large-scale quantities of rAAV vectors for gene therapy applications. EX1006 (Le Bec), 1:5-10 (“The invention relates to methods for producing double-stranded AAV viral particles using the insect/baculovirus cell system to produce sufficient amounts of product for therapeutic applications such as gene therapy and/or DNA vaccination in uses during clinical or commercial trials.”); *id.*, 3:33-34 (“The invention relates to a method for producing a high-titer and large-scale double-stranded AAV vector (scAAV)”); *see also id.*, 1:14-19, 2:19:3:2, 4:6-10, 6:14-33, 21:3-10 (claim 1).

198. Le Bec teaches preparing samples of AAV vectors to “a final concentration of approximately $\geq 10^{11}$ vg/mL.” *Id.*, 10:28-32; *see also id.*, 14:15-19 (“The AAV viral particles are concentrated and formulated in PBS buffer by tangential filtration (TFF) using a cutoff threshold of 100 KDa to a final concentration of approximately $\geq 10^{11}$ vg/mL and filtered through a 0.2 μ m filter.”).

199. Table 2 of Le Bec discloses the titer for rAAV vectors after purification. *Id.*, 19:23-20:3. As set forth in Table 2, each batch of rAAV vector has a concentration between 5.1×10^{11} and 1.6×10^{12} vg/mL, which is within the range of about 1×10^{11} vg/mL and about 1×10^{13} vg/mL recited in claim 13:

Cell culture		Purification				
Type	Batch no.	Step 1	Step 2			
		Bulk no.	Batch no.	Vol (ml)	Titer (vg/mL)	Total Vg
HEK293F	12D0004	12D055	12D0055	0.6	1.6E+12	9.5E+11
	12D0082	12D0082	12D0082	0.8	5.1E+11	4.1E+11
sf9	A.12026.DEV	A.12026.DEV-4	A.12026.DEV-4	1.2	1.4E+12	1.7E+12
	12D0066	12D0066	12D0106	0.8	9.4E+11	7.5E+11
		12D0070				
		12D0068				
			12D0068	0.5	1.6E+12	8.0E+11

Table 2: Description of the volume and titer of viral genomes (vg/mL), total amount of viral genomes of AAV9 particles (vg) obtained after purification step 2

Id., 19:23-20:3.

200. Thus, Le Bec meets the additional limitation of dependent claim 13.

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

201. Le Bec teaches methods for determining the sedimentation coefficients of rAAV particles using SV-AUC. Le Bec also determines the size of at least one fragmented genome from a species of rAAV particles, namely the size of the single-stranded scAAV genome, as a function of its sedimentation coefficient. § XI.A.4.

202. De la Maza determines the molecular weight of all of the fragmented genomes obtained from a preparation of AAV particles. EX1007 (de la Maza), 194-97, Table 1; EX1008 (de la Maza (April 1980)), 3195-96, Table 1. De la Maza further teaches that there is a direct linear relationship between density of an AAV particle and the size of the DNA packaged within the capsid. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3202.

203. Applying the teachings of Le Bec and de la Maza (§ XI.A.4), a POSA would also be able to determine the size of all of the fragmented genomes in a preparation of rAAV particles.

204. Thus, Le Bec, in combination with de la Maza, meets the additional limitation of dependent claim 15.

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

205. Le Bec teaches that elements of vectors produced according to the invention “may be derived from *any serotype of AAV*” and that the constituent elements of rAAV vectors need not be derived from the same serotype. EX1006, 7:1-9 (emphasis added). Le Bec provides the example of an rAAV vector made up of a genome derived from an AAV of serotype 2 encapsidated by capsid proteins derived from an AAV of serotype 9. *Id.* Le Bec also teaches that according to one embodiment, the preparation of viral particles comprises capsid proteins of AAV9 serotype. *Id.*, 7:14-19 (“According to a specific variant, the sequence codes for AAV capsid proteins of serotype 9 (AAV9).”); *see also id.*, 4:31-33 (“According to another embodiment, the capsid proteins produced are capsid proteins of an AAV of serotype 9.”); *id.*, 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.”).

206. Le Bec further discloses AUC analysis of a preparation of viral particles comprising AAV9 capsids. *Id.*, 15:17-28, 18:8-19:15, Figures 2-3. Le Bec describes Figure 2 as “a graph showing the analytical ultracentrifugation distribution profile” of two batches of “scAAV9.” *Id.*, 6:3-7. Similarly, Figure 3 is described as “a graph showing the analytical ultracentrifugation distribution profile of three scAAV9-SMN batches.” *Id.*, 6:8-10. Each of these batches was prepared with either baculoviruses or plasmids coding for the Cap protein of AAV serotype 9. *Id.*, 12:1-14, 12:16-33. A POSA would understand that the scAAV preparations analyzed by AUC in Le Bec were preparations comprising AAV9 capsid proteins.

207. Thus, Le Bec meets the additional limitation of dependent claim 17.

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

208. Le Bec discloses a preparation of rAAV vectors comprising “a sequence coding for a transgene of interest comprised [sic] between AAV ITRs.” EX1006 (Le Bec), 4:12-19; *see also* 1:33-2:6 (“In rAAV vectors, the rep and cap genes flanked by the ITR (inverted terminal repeat) sequences respectively coding for the replication-regulating proteins and the structural proteins of the capsid are excised and replaced by the transgene (therapeutic gene). The ITR sequences are preserved and are necessary elements for the replication and encapsidation of the viral genome.”); *id.* 7:30-31 (“The AAV viral genome is coded for by a sequence comprising the transgene of interest flanked by AAV ITR sequences.”).

209. Le Bec teaches that “each element” of the vector produced according to the invention “may be derived from any serotype of AAV” and that the constituent elements of rAAV vectors need not be derived from the same serotype. *Id.*, 7:1-9. Le Bec gives the example of a preparation of rAAV vectors made up of a genome derived from AAV2 encapsidated by capsid proteins derived from AAV9. *Id.*; see also *id.*, 4:34-5:4 (“According to another embodiment, the rAAV vector is a pseudotyped vector (for example an scAAV2/9 vector, i.e. an AAV vector comprising a double-stranded viral genome of serotype 2 encapsidated in an AAV viral particle of serotype 9).”); *id.*, 7:24-28 (“According to a specific variant, the viral genome is derived from an AAV2 genome.”).

210. Thus, Le Bec meets the additional limitation of dependent claim 18.

K. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

211. In my opinion, a POSA would have had a reasonable expectation of success in combining Le Bec with de la Maza to obtain the subject matter recited in claims 1-4, 7, and 12-19.

212. A POSA would have had a reasonable expectation of success in using SV-AUC to separate and analyze rAAV particles using the methods disclosed in Le Bec. SV-AUC was a well-known method in the art and within the skill of a POSA to optimize. EX1001 (’326 patent), 19:37-43; 26:19-21. Le Bec teaches that rAAV particles (including rAAV particles with fragmented genomes) could be effectively

separated, characterized, and quantified by AUC. EX1006 (Le Bec), 15:17-28, 18:8-32.

213. 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/cm^3 (equivalent to 60S) for empty AAV viral particles, i.e. vectors lacking a viral genome, and 1.45 g/cm^3 (equivalent to 110S) for full AAV viral particles, i.e. those containing a viral genome with a maximum size of 4800 bases.” *Id.*, 17:30-18:1 (citing EX1013 (de la Maza (March 1980))).

214. Likewise, Le Bec teaches that AAV particles that can be separated using a CsCl density gradient (as in de la Maza) can also be resolved by SV-AUC, citing de la Maza (March 1980). *Id.* Le Bec explains that “[k]nowing 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.” *Id.*, 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.

215. A POSA would have also had a reasonable expectation of success in applying the teachings of de la Maza to analyze the sedimentation coefficient values obtained using the SV-AUC methods of Le Bec to determine the size of the DNA in rAAVs with fragmented genomes. De la Maza teaches the linear relationship between AAV particle density and genome size. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980), 3194, 3202; EX1011 (Carter), 449. As discussed above, based on this known linear relationship, a POSA would have been able to determine the size of the DNA in rAAVs with fragmented genomes by comparison to the sedimentation coefficients for rAAVs with genomes of known size, for example by using a standard curve.

L. Secondary Considerations Do Not Change the Conclusion of Obviousness

216. For evidence of “secondary considerations” to be informative of obviousness, I understand that there must be a “nexus” or link between the alleged secondary consideration and the subject matter recited in the Asserted Claims. I am not aware of any secondary considerations of non-obviousness with the required nexus to the claims of the ’326 patent. For example, I am not aware of any commercial success attributable to an analytical method of determining the size of fragmented genomes in a preparation of viral particles using the well known

technique of AUC.² Similarly, I am not aware of any licenses directed specifically to the '326 patent or the subject matter recited in challenged the claims.

217. Finally, I am not aware of any unexpected results having a nexus to the claimed subject matter. The '326 patent does not disclose any unexpected properties of the claimed methods. The AUC methods perform as expected from the prior art, and the analysis of the sedimentation profiles obtained using the claimed AUC methods is merely a matter of applying well-known relationships between the sedimentation properties of rAAV particles and the size of the packaged genome.

218. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 1-4, 7, 12-13, 15, and 17-18 of the '326 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness, discussed above. Thus, secondary considerations do not alter my opinion that claims 1-4, 7, 12-13, 15, and 17-18 of the '326 patent are obvious over the combination of Le Bec and de la Maza.

² If Patent Owner attempts to rely on the commercial success of Sarepta's gene therapy treatment for Duchenne muscular dystrophy – Elevidys[®] – I am not aware of any nexus to the challenged claims of the '326 patent. Specifically, I am not aware of any nexus between the commercial success of Elevidys[®] and the AUC methods recited in the challenged claims.

XII. GROUND 2: CLAIMS 5, 8, AND 9 ARE OBVIOUS OVER LE BEC AND DE LA MAZA, IN VIEW OF COLE

219. In my opinion, claims 5, 8, and 9 of the '326 patent are obvious over Le Bec and de la Maza, in view of Cole.

220. As discussed above, a POSA would have been motivated to combine the SV-AUC methods disclosed in Le Bec with the teachings of de la Maza regarding the properties of AAV particles to characterize the species in a preparation of rAAV viral particles. *See* § XI.

221. A POSA would have been further motivated to combine Le Bec and de la Maza with Cole. Cole provides a general overview of AUC techniques – including SV-AUC – and explains the various parameters and analyses that may be adjusted depending on the experimental system and samples being tested. Cole provides detailed instructions on how to determine appropriate parameters to use for an SV-AUC experiment and how to perform the steps necessary to analyze and interpret the data. EX1009 (Cole), 161-168.

222. As stated in the specification of the '326 patent, “[i]t is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles.” EX1001 ('326 patent), 26:19:21. Such AUC parameters include “any conditions related to the rotor speed, distance between sample and rotor center, temperature, solvent, sample, buffer, ultracentrifugation time, time interval for detection, sector and optical window characteristics, AUC instrumentation

(including ultracentrifuge and detection apparatus), equilibrium dialysis of reference solvent, and data analysis algorithms.” *Id.*, 17:44-51.

223. A POSA would have been motivated to combine Cole with Le Bec and de la Maza to optimize the separation of rAAV particles using SV-AUC.

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

224. Cole teaches that the “useful temperature range” for SV-AUC experiments is between 4 °C-35 °C and that “[m]ost experiments are conducted at 20 °C.” EX1009 (Cole), 163.

225. Thus, Cole meets the additional limitation of claim 5.

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

226. As stated in Cole, “the correct rotor speed for a SV experiment depends what you want to know about your sample, what the expected component size distribution is, and which optical systems will be used.” EX1009 (Cole), 164.

227. Cole teaches 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. *Id.*, 164. Table III of Cole discloses “maximum recommended rotor speeds for various sedimentation coefficients” to enable “[a]bout 2 h of data acquisition” at the listed centrifuge rotor speed. *Id.*, 164, Table III, note c.

228. Thus, Cole meets the additional limitation of claim 8.

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

229. As stated in Cole, “the correct rotor speed for a SV experiment depends what you want to know about your sample, what the expected component size distribution is, and which optical systems will be used.” EX1009 (Cole), 164.

230. Cole teaches 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. *Id.*, 164. Table III of Cole discloses “maximum recommended rotor speeds for various sedimentation coefficients” to enable “[a]bout 2 h of data acquisition” at the listed centrifuge rotor speed. *Id.*, 164, Table III, note c.

231. Thus, Cole meets the additional limitation of claim 9.

D. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

232. As discussed above, it is my opinion that a POSA would have had a reasonable expectation of success in applying the teachings of de la Maza to the SV-AUC methods disclosed in Le Bec. *See* § XI.K. Additionally, in my opinion, a POSA would have had a reasonable expectation of success applying the teachings of Cole to optimize the separation of rAAV particles when using the SV-AUC methods disclosed in Le Bec. Consistent with the statements in the ’326 patent specification, optimizing AUC parameters for different types of viral particles would have been routine for a POSA. EX1001 (’326 patent), 26:19-21.

E. Secondary Considerations Do Not Change the Conclusion of Obviousness

233. As discussed above, I am not aware of any secondary considerations of non-obviousness with the required nexus to the challenged claims of the '326 patent. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 5, 8, and 9 of the '326 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness. Thus, secondary considerations do not alter my opinion that claims 5, 8, and 9 of the '326 patent are obvious over the combination of Le Bec and de la Maza, in view of Cole.

XIII. GROUND 3: CLAIMS 14, 16, 19-23, AND 26-30 ARE OBVIOUS OVER LE BEC AND DE LA MAZA, IN VIEW OF SOMMER

234. In my opinion, claims 14, 16, 19-23 and 26-30 are obvious over Le Bec and de la Maza, in view of Sommer.

235. As discussed above, a POSA would have been motivated to combine the SV-AUC methods disclosed in Le Bec with the teachings of de la Maza regarding the properties of AAV particles to characterize the species in preparations of rAAV viral particles. *See* § XI.

236. A POSA would have been further motivated to combine Le Bec and de la Maza with Sommer. Sommer relates to the identification and characterization of viral particles in preparations of AAV, such as rAAV vectors for use in gene therapy

applications, and teaches a direct method for quantifying AAV vector genomes and empty capsids. EX1010 (Sommer), 122. Sommer discusses the increasing promise of gene therapies, and the importance of accurately characterizing AAV vector products manufactured for clinical use. *Id.* A POSA would have been motivated to combine Le Bec and de la Maza with Sommer to obtain additional information regarding the composition of rAAV preparations for gene therapy applications.

237. As discussed above, Le Bec, in combination with de la Maza, teaches SV-AUC methods for determining the size of the DNA in rAAVs with fragmented genomes. Sommer teaches mathematical formulas used to calculate the extinction coefficients of rAAV particles based on the molecular weight of the encapsidated DNA. *Id.*, 127. A POSA would have been motivated to use the formulas in Sommer to calculate the molar concentrations of rAAV particles in a preparation, including rAAV particles with fragmented genomes.

238. Specifically, a POSA would have been motivated to calculate the extinction coefficients for rAAV particles with fragmented genomes, by applying the formulas in Sommer and using the DNA size (*i.e.*, molecular weight) measured for each species of AAV particle according to the methods taught by Le Bec, in combination with de la Maza. *See Id.* With extinction coefficients for each species of AAV particle in hand, a POSA would have readily calculated the molar

concentration of each species of AAV virion, according to Beer's Law. EX1001 ('326 patent), 24:41-47, 44:58-61, 46:42-50; EX1009 (Cole), 152.

239. In my opinion, a POSA would have been motivated to combine the teachings of Le Bec and de la Maza with the mathematical formulas of Sommer to calculate the molar concentration and percentage of each species in a heterogeneous mixture of rAAV particles, using calculated extinction coefficients and measured absorbance values.

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

240. Le Bec teaches a method “for analytically separating by ultracentrifugation and *quantifying* the different species present in an AAV viral preparation.” EX1006 (Le Bec), 18:1-6 (emphasis added).

241. Le Bec discloses the preparation of batches of scAAV particles made using two different systems, HEK293 and sf9, and compares the results obtained using each system. *Id.*, 16:29-33, 18:8-19:15. Le Bec reports that the distribution profiles for the two batches produced by the HEK293 system confirm 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.*, 19:1-5. For scAAV particles prepared using the sf9 system, Le Bec discloses that “we detected very few empty AAV viral particles with the sf9 system compared to

the HEK293 system” and 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.*, 18:27-32, 19:12-15, Figure 3.

242. A POSA would understand that the percentages reported in Le Bec are relative concentrations of various AAV particle species in the sample, and that these values were obtained by integrating the peaks of interest using the SEDFIT software. As discussed above, the c(s) v. S plots in Figures 2 and 3 of Le Bec were generated using the SEDFIT software program. *Id.*, 15:27-28 (“the sedimentation coefficient of the various populations was obtained using the software SEDFIT.”). SEDFIT is a versatile software tool for the analysis of SV-AUC data that has been publicly available since at least 2000. EX1031 (Schuck), 1610 (“All computational methods were implemented into the Windows-based ultracentrifugal analysis program SEDFIT, which is available on request, or can be downloaded from <http://www.biochem.uthscsa.edu/auc/software>, and from the RASMB network at ftp://rasmb.bbri.org/rasmb/spin/ms_dos/.”). Version 14.4d of SEDFIT, which was publicly available and freely downloadable prior to the publication date of Le Bec and the priority date of the ’326 patent, included the ability to determine relative concentrations by integrating the area under the curve of a specific region or peak selected by the user. See <https://sedfitsedphat.nibib.nih.gov/software> (SEDFIT

software version 14.4d (“sedfit144d.zip”) dated October 29, 2013); *see also* EX1001 (’326 patent), 4:24-27, 12:35-40, 25:33-36.

243. Furthermore, as discussed below, using absorbance values and the calculated extinction coefficients derived from the formulas in Sommer, a POSA would have been able to calculate molar concentration and percentage for each species of rAAV particle by simply applying Beer’s Law. § XIII.D.5.

244. Thus, Le Bec and Sommer each meet the additional limitation of dependent claim 14.

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

245. As discussed below, Le Bec teaches SV-AUC methods for quantifying the different species present in a preparation of rAAV viral particles. § XIII.D. Le Bec teaches using the publicly-available SEDFIT software to integrate the area under the curve for the peaks in a $c(s)$ v. S distribution profile to determine the relative concentrations of the rAAV particles in the sample, including rAAVs with fragmented genomes.

246. Furthermore, using absorbance values and the calculated extinction coefficients derived from the formulas in Sommer, a POSA would have been able to calculate molar concentration and percentage for all rAAV species in the preparation by simply applying Beer’s Law. § XIII.D.5.

247. Thus, Le Bec and Sommer each meet the additional limitation of dependent claim 16.

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

248. Le Bec teaches the preparation of rAAV vectors comprising self-complementary AAV (“scAAV”) viral genomes. *See generally* EX1006 (Le Bec), 3:4-9 (“The AAV genome is composed of a singled-stranded DNA molecule of 4.7 kb (inactive), the conversion of which to a double-stranded conformation (active form) is a limiting step that can be bypassed by producing ‘double-stranded’ AAV vectors referred to as scAAV (self complementary) vectors.”); *see also id.*, 3:33-34 (“The invention relates to a method for producing a high titer and large-scale double-stranded AAV vector (scAAV)”; *id.*, 4:12-13 (“The invention relates more specifically to a method for producing an scAAV vector”); *id.*, 6:14-16 (“The present invention relates to methods for producing a double-stranded recombinant rAAV vector, or self-complementary AAV (scAAV).”); *id.*, 18:8-32 (“Analytical ultracentrifugation analysis of the scAAV9-SMN vectors was [] carried out.”).

249. Thus, Le Bec meets the additional limitation of dependent claim 19.

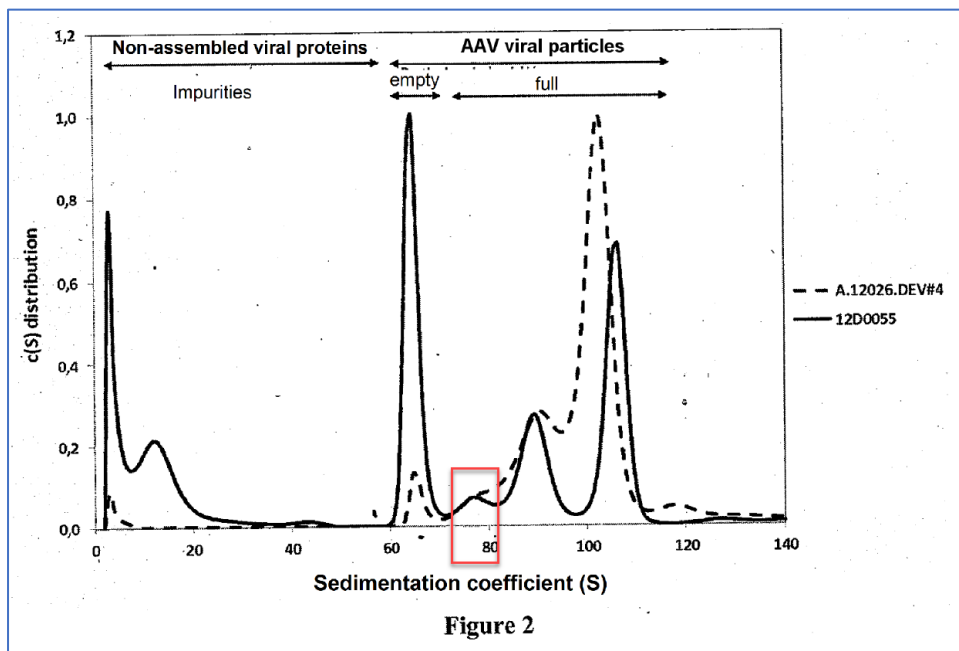
D. Claim 20

- 1. “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:”**

250. Le Bec discloses a method of analyzing a heterogeneous mixture of viral particles comprising rAAV vectors encapsidated into viral capsids. Specifically, Le Bec “implemented a method ... for analytically separating by ultracentrifugation and quantifying the different species present in an AAV viral preparation.” EX1006 (Le Bec), 18:1-6. Le Bec discloses an SV-AUC method capable of analytically separating, detecting, and quantifying empty AAV particles, AAV particles with complete genomes, and AAV particles with fragmented genomes in an rAAV preparation. *Id.*, 15:17-28, 17:29-19:15.

251. Le Bec characterizes the SV-AUC distribution profiles in Figures 2 and 3 as containing “two subpopulations” of AAV viral particles, namely “empty AAV viral particles at 65S and full AAV viral particles between 80S and 110S.” *Id.*, 18:21-25. Le Bec further states that within the subpopulation of full AAV viral particles, there exists “viral genome in single-stranded form at 90S and double-stranded form at 105S.” *Id.*

252. In addition to the single-stranded and double-stranded viral genomes at approximately 90S and 105S, Figure 2 discloses an additional peak in the distribution profile at approximately 75S, highlighted in the figure below:



Id., Figure 2 (batch 12D0055).

253. As shown in Figure 2, Le Bec categorizes all AAV particles containing a DNA strand as “full”, regardless of whether the capsid contains a complete, double-stranded viral genome, or some smaller sized DNA. *Id.*, Figure 2; *see also id.*, 5:10-12 (defining “full viral rAAV particles” to mean “comprising an AAV genome encapsidated within said particles.”). Peaks in the distribution profile between the empty capsid population (at 65S) and the complete, double-stranded genome (at 105S) correspond to rAAVs with fragmented genomes – *i.e.*, rAAVs that contain less than a complete double-stranded, self-complementary genome. Thus,

for example, the peak at approximately 75S in Figure 2 corresponds to a capsid with a fragmented genome that is smaller than the monomeric, single-stranded genome.

254. De la Maza determined the size of fragmented genomes in a preparation of wild type AAV particles. EX1007 (de la Maza), 193 (“We summarize here the physical properties of the incomplete AAV genomes.”); EX1008 (de la Maza (April 1980)), 3194 (“We have analyzed ... the molecular structure of the variant AAV DNA molecules obtained from these light density particles. The size of variant DNA molecules ranged from 100 to 3% of genome length.”).

255. De la Maza separated AAV particles via centrifugation in CsCl. EX1007 (de la Maza), 194; EX1008 (de la Maza (April 1980)), 3195. Following fractionation of the particles, DNA was released from each separated band of particles by sedimentation in alkaline sucrose gradients. EX1007 (de la Maza), 194-95; EX1008 (de la Maza (April 1980), 3195. De la Maza then measured the size of the fragmented DNA released from each band of particles, designated Types I, II, III, and IV. EX1007 (de la Maza), 194-97, 200-201; EX1008 (de la Maza (April 1980), 3195-96. To determine the size of the extracted DNA fragments, de la Maza then compared the fragmented DNA to markers of known molecular weight in alkaline and neutral sucrose gradients. EX1007 (de la Maza), Table 1, Figure 1. De la Maza reported that the molecular weights for fragmented genome Types I to IV

were 1.4, 0.8, 0.3, and about 0.13×10^6 Da, respectively. EX1007 (de la Maza), 194-97, Table 1.

256. De la Maza teaches that there is a linear relationship between the density of an AAV particle and the size of the DNA packaged inside the capsid. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3194, 3202, EX1011 (Carter), 449. Applying this teaching in de la Maza to the analysis of the sedimentation profiles obtained using the SV-AUC methods in Le Bec, a POSA would have been able to determine the size of the fragmented genomes in a heterogeneous mixture of recombinant AAV vectors.

257. Sommer discloses mathematical formulas for calculating the extinction coefficient of an rAAV particle based on the molecular weight of the DNA packaged inside the capsid. EX1010 (Sommer), 127. As discussed above, after determining the extinction coefficients, a POSA would have been able to calculate the molar concentration of each species of rAAV particle in in a mixture of viral particles using Beer's Law. *See* EX1001 ('326 patent), 24:41-47, 44:58-61, 46:42-50; EX1009 (Cole), 152.

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

2. **“(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;”**

259. As discussed above, Le Bec teaches subjecting a heterogenous mixture of rAAV particles to AUC under boundary sedimentation velocity conditions to generate one or more sedimenting boundaries, wherein the centrifugation was carried out at 16,000 rpm. *See* § XI.A.2.

260. For the same reasons discussed above in my analysis of claim 1(i), Le Bec meets this limitation of claim 20. *See* § XI.A.2.

3. **“(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;”**

261. As discussed above, Le Bec teaches a method of using SV-AUC to measure the rate of migration of the distinct sedimenting boundaries in a heterogeneous mixture of rAAV particles, where each distinct sedimenting boundary corresponds to a resolvable viral particle. *See* § XI.A.3.; *see also* EX1006 (Le Bec), 15:17-28.

262. Le Bec also discloses two distribution profiles plotted from SV-AUC data for heterogenous mixtures of scAAV particles, each of which includes full, double stranded genomes, fragmented genomes, and empty capsids. EX1006 (Le Bec), 15:17-28, 18:8-32, Figures 2 and 3.

263. Thus, Le Bec meets this limitation of claim 20.

4. “(iii) determining the sedimentation coefficients of each species of the individual viral particles in the heterogeneous mixture of viral particles; and”

264. Le Bec teaches using the SEDFIT software to determine the sedimentation coefficients of the various AAV viral particles in the heterogenous mixture. EX1006 (Le Bec), 15:17-28. Figures 2 and 3 of Le Bec show “analytical ultracentrifugation distribution profile[s]” generated by the SEDFIT software. *Id.*, 6:3-10. In both figures, “c(s) distribution” is plotted on the y-axis and “Sedimentation coefficient (S)” is plotted on the x-axis. *Id.*, Figures 2 and 3.

265. The distribution profiles in Figures 2 and 3 include multiple peaks. Le Bec determines the sedimentation coefficients for the various rAAV particle species in the mixture based on the peaks shown in these distribution profiles. *Id.*, 18:8-32. Le Bec reports that the peak at 65S corresponds to empty rAAV particles, the peak at 105S corresponds to an rAAV particle with a complete, double-stranded, self-complementary genome, and that the peak at 90S corresponds to particles with a monomeric, single stranded genome. *Id.*, 18:21-25. A POSA reading Le Bec would

understand that the peak at 90S corresponds to a fragmented genome – *i.e.*, a DNA that is less than the complete double-stranded, self-complementary genome. Figure 2 also shows a peak at about 75S, which corresponds to an rAAV particle with a fragmented genome that is smaller than the monomeric, single-stranded genome. *Id.*, Figure 2 (batch 12D0055).

266. Thus, Le Bec meets this limitation of claim 20.

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

267. As discussed above, Le Bec in combination with de la Maza teaches how to determine the size of the genome contained within each species in a mixture of rAAV particles as a function of the sedimentation coefficient, including rAAV particles with fragmented genomes. *See* § XI.A.4.

268. Using the molecular weight of DNA packaged within each species of rAAV particle, a POSA would have been able to calculate the extinction coefficient for each species using the mathematical formulas taught by Sommer. EX1010 (Sommer), 127.

269. With calculated extinction coefficients in hand for each species of rAAV particle, a POSA would then have been able to calculate the molar concentration of each species of rAAV particle in a preparation of AAV particles

using Beer's Law. Beer's Law provides that absorbance is directly proportional to solute concentration according to the following formula:

$$A = \epsilon cl$$

where A is the absorbance at a particular wavelength, ϵ is the extinction coefficient of the particle at that wavelength, c is the concentration, and l is the sample path length. EX1009 (Cole), 152.

270. Using the absorbance values for each rAAV particle in the heterogenous mixture, a POSA would have been able to calculate the molar concentration of each rAAV particle by simply applying Beer's Law to known absorbance, extinction coefficient (calculated using the formulas in Sommer), and sample path length values. *See, e.g.*, EX1009 (Cole), 152; *see also* EX1001 ('326 patent), 24:41-47, 44:58-61, 46:42-50.

271. Thus, Le Bec and de la Maza, in combination with Sommer, meet this limitation of claim 20.

E. 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.”

272. Figures 2 and 3 of Le Bec disclose SV-AUC distribution profiles for rAAV particles of known genome size. EX1006 (Le Bec), 18:19-25, Figures 2, 3.

A POSA would know, based on the teachings of de la Maza, that there is a linear relationship between genome size and the density of an AAV particle. EX1007 (de la Maza), 203; EX1008 (de la Maza (April 1980)), 3202. In light of this known linear relationship, a POSA would have been able to use experimentally generated sedimentation coefficients for rAAV particles with genomes of known genome size to generate a standard curve.

273. A standard curve, sometimes also referred to as a calibration curve, is a general analytical approach used to plot measured values against a set of standards of a given parameter, such as size or concentration. The standard curve essentially shows the relationship between two parameters (*e.g.*, measured response and known size/concentration). The standard curve may then be used to calculate the parameter of interest as a function of the measured value. Here, a POSA could use the standard curve generated from sedimentation coefficients for rAAV particles of *known* genome size to determine the genome size of fragmented genomes in particles containing DNA of *unknown* size.

274. A POSA would have known that molecular weight markers were a well-established, commonly used technique in the art for determining the molecular weight of unknown macromolecules. For example de la Maza discloses the use of DNA molecular weight markers to determine molecular weight and genome length of DNA in fragmented AAV genomes. EX1007 (de la Maza), 196-197, Figure 1.

De la Maza used molecular weight markers comprised of duplex monomer AAV2 DNA, as well as fragmented genomes representing 38%, 22%, and 4.5% of the AAV2 genome, in alkaline and neutral sucrose gradients to measure of fragmented genomes of unknown length. *Id.*, 196, Figure 1. A comparison of the sedimentation of the unknown fragmented DNA (labeled Types I-IV by the authors) to the sedimentation of the known molecular weight markers was used to determine molecular weights of the unknown, fragmented genomes. *Id.*, 195, Table 1.

275. 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 an unknown, fragmented genome by comparison to the sedimentation coefficients (S) for rAAVs with a genomes of known length.

276. As an example, the sedimentation profile in Figure 2 of Le Bec is illustrative. As discussed above, Figure 2 shows a sedimentation profile with sedimentation coefficients for three subpopulations of rAAV particles of known genome size: empty rAAV particles at 65S, rAAV particles with a monomeric, single-stranded genome at 90S, and rAAV particles with a complete double-stranded, self-complementary genome at 105S. EX1006 (Le Bec), Figure 2 (batch 12D0055). Figure 2 also depicts a population of rAAV particles with fragmented genomes of unknown size at approximately 75S. A POSA would have been able to determine the size of the fragmented genomes in the particles of unknown size by

comparison to the sedimentation coefficients for the rAAV particles of known genome size (*i.e.*, the empty, single-stranded, and double-stranded populations).

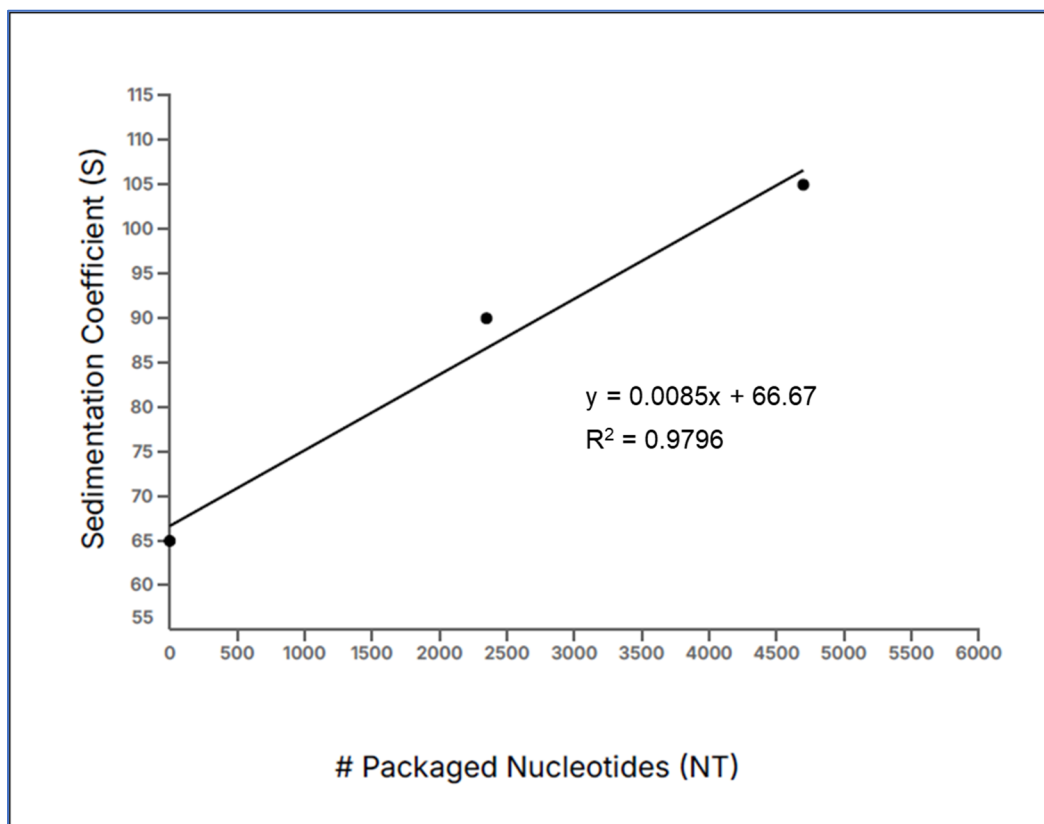
277. Le Bec does not report the number of nucleotides in the genome of the disclosed scAAV vectors. However, assuming that the complete, double-stranded genome for the scAAV vectors in Le Bec is approximately the same size as a wild-type AAV genome (4700 nucleotides (*see* EX1006 (Le Bec), 3:5-6; EX1008 (de la Maza (April 1980)), 3194, 3202)), a POSA would have been able to calculate the approximate the size of the fragmented genome in the population of rAAV particles at 75S to be about 980 nucleotides.

278. The size of the fragmented genome may be calculated by comparing the sedimentation coefficient for rAAV particles with a fragmented genome of unknown length (75S) to a standard curve generated from the sedimentation coefficients and genome sizes disclosed in Figure 2 of Le Bec, as detailed in Table 1 below:

Population	Sedimentation coefficient	# nucleotides
Empty AAV particles	65	0
Single-stranded AAV viral genome	90	2350
Double-stranded AAV viral genome	105	4700

Table 1: Sedimentation coefficients and number of nucleotides for rAAV particles in Le Bec Figure 2

279. Applying a linear regression analysis to the data in Figure 2, a POSA would have obtained the following standard curve:



280. The equation of the best fit line is $y = 0.0085x + 66.67$, where y is the sedimentation coefficient and x is genome size, in nucleotides. The R^2 value is 0.9796. Using this equation, a POSA would be able to calculate the approximate genome size x (in nucleotides) for the unknown fragmented genome at 75S by plugging in $y=75(S)$. Solving the equation for x results in a genome size of about 980 nucleotides.

281. Combining the SV-AUC methods in Le Bec, with the teachings in de la Maza regarding the direct linear relationship between density and AAV genome

size taught by de la Maza, a POSA would have been able to determine the size of the genome in rAAV particles with fragmented genomes by comparison to a standard curve generated from the sedimentation coefficients for rAAV particles with genomes of known length.

282. Thus, Le Bec, in combination with de la Maza, meets the additional limitation of dependent claim 21.

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

283. Le Bec discloses SV-AUC analyses wherein “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm.” EX1006 (Le Bec), 15:23-25. Centrifugation at 16,000 rpm as taught in Le Bec falls within the range of 10,000-20,000 rpm.

284. Thus, Le Bec meets the additional limitation of dependent claim 22.

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

285. Le Bec discloses SV-AUC analyses wherein “[c]entrifugation of the samples was carried out at a speed of 16,000 rpm.” EX1006 (Le Bec), 15:23-25. Centrifugation at 16,000 rpm as taught in Le Bec falls within the range of 15,000-20,000 rpm.

286. Thus, Le Bec meets the additional limitation of dependent claim 23.

H. 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.”

287. Le Bec discloses methods for producing high-titer, large-scale quantities of rAAV vectors for gene therapy applications. EX1006 (Le Bec), 1:5-10 (“The invention relates to methods for producing double-stranded AAV viral particles using the insect/baculovirus cell system to produce sufficient amounts of product for therapeutic applications such as gene therapy and/or DNA vaccination in uses during clinical or commercial trials.”); *id.*, 3:33-34 (“The invention relates to a method for producing a high-titer and large-scale double-stranded AAV vector (scAAV)”); *see also id.*, 1:14-19, 2:19:3:2, 4:6-10, 6:14-33, 21:3-10 (claim 1).

288. Le Bec teaches preparing samples of rAAV viral particles to “a final concentration of approximately $\geq 10^{11}$ vg/mL.” *Id.*, 10:28-32; *id.*, 14:15-19 (“The AAV viral particles are concentrated and formulated in PBS buffer by tangential filtration (TFF) using a cutoff threshold of 100 KDa to a final concentration of approximately $\geq 10^{11}$ vg/mL and filtered through a 0.2 μ m filter.”).

289. Table 2 of Le Bec discloses the titer for different batches of rAAV vectors after purification. *Id.*, 19:23-20:3. As set forth in Table 2, each batch of rAAV vector has a concentration between 5.1×10^{11} and 1.6×10^{12} vg/mL, which is greater than the concentration of 5×10^{11} vg/mL recited in claim 26:

Cell culture		Purification				
Type	Batch no.	Step 1	Step 2			
		Bulk no.	Batch no.	Vol (ml)	Titer (vg/mL)	Total Vg
HEK293F	12D0004	12D055	12D0055	0.6	1.6E+12	9.5E+11
	12D0082	12D0082	12D0082	0.8	5.1E+11	4.1E+11
sf9	A.12026.DEV	A.12026.DEV-4	A.12026.DE V-4	1.2	1.4E+12	1.7E+12
	12D0066	12D0066	12D0106	0.8	9.4E+11	7.5E+11
		12D0070				
		12D0068			1.6E+12	8.0E+11

Table 2: Description of the volume and titer of viral genomes (vg/mL), total amount of viral genomes of AAV9 particles (vg) obtained after purification step 2

Id., 19:23-20:3 (Table 2).

290. Thus, Le Bec meets the additional limitation of dependent claim 26.

I. 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.”

291. Le Bec discloses methods for producing high-titer, large-scale quantities of rAAV vectors for gene therapy applications. EX1006 (Le Bec), 1:5-10 (“The invention relates to methods for producing double-stranded AAV viral particles using the insect/baculovirus cell system to produce sufficient amounts of product for therapeutic applications such as gene therapy and/or DNA vaccination in uses during clinical or commercial trials.”); *id.*, 3:33-34 (“The invention relates to a method for producing a high-titer and large-scale double-stranded AAV vector (scAAV)”); *see also id.*, 1:14-19, 2:19:3:2, 4:6-10, 6:14-33, 21:3-10 (claim 1).

292. Le Bec teaches preparing samples of AAV vectors to “a final concentration of approximately $\geq 10^{11}$ vg/mL.” *Id.*, 10:28-32; *id.*, 14:15-19 (“The

AAV viral particles are concentrated and formulated in PBS buffer by tangential filtration (TFF) using a cutoff threshold of 100 KDa to a final concentration of approximately $\geq 10^{11}$ vg/mL and filtered through a 0.2 μ m filter.”).

293. Table 2 of Le Bec discloses the titer for batches of rAAV vectors after purification. *Id.*, 19:23-20:3. As set forth in Table 2, each batch of rAAV vector has a concentration between 5.1×10^{11} and 1.6×10^{12} vg/mL, which is within the range of about 1×10^{11} vg/mL and about 1×10^{13} vg/mL recited in claim 27:

Cell culture		Purification				
Type	Batch no.	Step 1	Step 2			
		Bulk no.	Batch no.	Vol (ml)	Titer (vg/mL)	Total Vg
HEK293F	12D0004	12D0055	12D0055	0.6	1.6E+12	9.5E+11
	12D0082	12D0082	12D0082	0.8	5.1E+11	4.1E+11
sf9	A.12026.DEV	A.12026.DEV-4	A.12026.DEV-4	1.2	1.4E+12	1.7E+12
	12D0066	12D0066	12D0106	0.8	9.4E+11	7.5E+11
		12D0070				
		12D0068	12D0068	0.5	1.6E+12	8.0E+11

Table 2: Description of the volume and titer of viral genomes (vg/mL), total amount of viral genomes of AAV9 particles (vg) obtained after purification step 2

Id., 19:23-20:3.

294. Thus, Le Bec meets the additional limitation of dependent claim 27.

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

295. Le Bec teaches that elements of vectors produced according to the invention “may be derived from *any serotype of AAV*” and that the constituent elements of rAAV vectors need not be derived from the same serotype. EX1006,

7:1-9 (emphasis added). Le Bec provides the example of an rAAV vector made up of a genome derived from an AAV of serotype 2 encapsidated by capsid proteins derived from an AAV of serotype 9. *Id.* Le Bec also teaches that according to one embodiment, the preparation of viral particles comprises capsid proteins of AAV9 serotype. *Id.*, 7:14-19 (“According to a specific variant, the sequence codes for AAV capsid proteins of serotype 9 (AAV9).”); *id.*, 4:31-33 (“According to another embodiment, the capsid proteins produced are capsid proteins of an AAV of serotype 9.”); *id.*, 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.”).

296. Le Bec further discloses AUC analysis of a preparation of viral particles comprising AAV9 capsids. *Id.*, 15:17-28, 18:8-19:15, Figures 2-3. Le Bec describes Figure 2 as “a graph showing the analytical ultracentrifugation distribution profile” of two batches of “scAAV9.” *Id.*, 6:3-7. Similarly, Figure 3 is described as “a graph showing the analytical ultracentrifugation distribution profile of three scAAV9-SMN batches.” *Id.*, 6:8-10. Each of these batches was prepared with either baculoviruses or plasmids coding for the Cap protein of AAV serotype 9. *Id.*, 12:1-14, 12:16-33. A POSA would understand that the scAAV preparations analyzed by AUC in Le Bec were preparations comprising AAV9 capsid proteins.

297. Thus, Le Bec meets the additional limitation of dependent claim 28.

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

298. Le Bec discloses a preparation of rAAV vectors comprising “a sequence coding for a transgene of interest comprised [sic] between AAV ITRs.” EX1006 (Le Bec), 4:12-19; *see also id.* 1:33-2:6 (“In rAAV vectors, the rep and cap genes flanked by the ITR (inverted terminal repeat) sequences respectively coding for the replication-regulating proteins and the structural proteins of the capsid are excised and replaced by the transgene (therapeutic gene). The ITR sequences are preserved and are the elements necessary for the replication and encapsidation of the viral genome.”); *id.* 7:30-31 (“The AAV viral genome is coded for by a sequence comprising the transgene of interest flanked by AAV ITR sequences.”).

299. Le Bec teaches that “each element” of the vector produced according to the invention “may be derived from any serotype of AAV” and that the constituent elements of rAAV vectors need not be derived from the same serotype. *Id.*, 7:1-9. Le Bec gives the example of a preparation of rAAV vectors made up of a genome derived from AAV2 encapsidated by capsid proteins derived from AAV9. *Id.*; *see also id.*, 4:34-5:4 (“According to another embodiment, the rAAV vector is a pseudotyped vector (for example an scAAV2/9 vector, i.e. an AAV vector comprising a double-stranded viral genome of serotype 2 encapsidated in an AAV viral particle of serotype 9).”); *id.*, 7:24-28 (“According to a specific variant, the viral genome is derived from an AAV2 genome.”).

300. Thus, Le Bec meets the additional limitation of dependent claim 29.

L. 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.”

301. Le Bec teaches the implementation of a method “for analytically separating by ultracentrifugation and *quantifying* the different species present in an AAV viral preparation.” EX1006 (Le Bec), 18:1-6 (emphasis added).

302. Le Bec discloses the preparation of batches of scAAV particles made using two different systems, HEK293 and sf9, and compares the results obtained using each system. *Id.*, 16:29-33, 18:8-19:15. Le Bec reports that the distribution profiles for the two batches produced by the HEK293 system confirm 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.*, 19:1-5. For scAAV particles prepared using the sf9 system, Le Bec discloses that “we detected very few empty AAV viral particles with the sf9 system compared to the HEK293 system” and 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.*, 18:27-32, 19:12-15, Figure 3.

303. A POSA would understand that the percentages reported in Le Bec are the relative concentrations of various AAV particle species in the sample, and that

these values were obtained by integrating the peaks of interest using the SEDFIT software. As discussed above, the $c(s)$ v. S plots in Figures 2 and 3 of Le Bec were generated using the SEDFIT software program. *Id.*, 15:27-28 (“the sedimentation coefficient of the various populations was obtained using the software SEDFIT”). SEDFIT is a versatile software tool for the analysis of SV-AUC data that has been publicly available since at least 2000. EX1031 (Schuck), 1610 (“All computational methods were implemented into the Windows-based ultracentrifugal analysis program SEDFIT, which is available on request, or can be downloaded from <http://www.biochem.uthscsa.edu/auc/software>, and from the RASMB network at ftp://rasmb.bbri.org/rasmb/spin/ms_dos/.”). Version 14.4d of SEDFIT, which was publicly available and freely downloadable prior to the publication date of Le Bec and the priority date of the ’326 patent, included the ability to determine relative concentrations by integrating the area under the curve of a specific region or peak selected by the user. *See* <https://sedfitsedphat.nibib.nih.gov/software> (SEDFIT software version 14.4d (“sedfit144d.zip”) dated October 29, 2013).

304. Furthermore, Sommer teaches formulas for calculating extinction coefficients for rAAV particles. Using measured absorbance values and the calculated extinction coefficients derived from the formulas in Sommer, a POSA would have been able to calculate molar concentration and percentage for each species of rAAV particle by simply applying Beer’s Law. § XIII.D.5.

305. Thus, Le Bec and Sommer each meet the additional limitation of dependent claim 30.

M. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

306. As discussed above, a POSA would have had a reasonable expectation of success in applying the teachings of de la Maza to the SV-AUC methods disclosed in Le Bec. *See* § XI.K.

307. In my opinion, a POSA would also have had a reasonable expectation of success using the publicly available SEDFIT software (or other similar, publicly available software programs for SV-AUC) to generate distribution profiles from the SV-AUC data. A POSA would have been able to use the SEDFIT program to integrate the area under the curve for peaks in the distribution profile to determine the relative concentrations of the rAAV species in the preparation, including rAAV particles with fragmented genomes.

308. Finally, a POSA would have had a reasonable expectation of success applying the mathematical formulas taught in Sommer to calculate the extinction coefficients, molar concentrations, and percentages for all of the rAAV species in a heterogeneous mixture, including rAAV particles with fragmented genomes. To do so would involve a simple application of known mathematical equations – namely the formulas disclosed in Sommer and Beer’s Law, which would have been well-known to a POSA. *See, e.g.*, EX1010 (Sommer), 127; EX1009 (Cole), 152. A

POSA would have a reasonable expectation of success in applying these mathematical formulas to calculate the molar concentration of each species in a heterogeneous mixture of rAAV particles.

N. Secondary Considerations Do Not Change the Conclusion of Obviousness

309. As discussed above, I am not aware of any secondary considerations of non-obviousness with the required nexus to the challenged claims of the '326 patent. To the extent Patent Owner attempts to raise secondary considerations that have only a marginal nexus, if any, to claims 14, 16, 19-23 and 26-30 of the '326 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness. Thus, secondary considerations do not alter my opinion that claims 14, 16, 19-23 and 26-30 of the '326 patent are obvious over the combination of Le Bec and de la Maza, in view of Sommer.

XIV. GROUND 4: CLAIM 25 IS OBVIOUS OVER LE BEC AND DE LA MAZA, IN VIEW OF SOMMER AND COLE

310. As discussed above, a POSA would have been motivated to combine the SV-AUC methods disclosed in Le Bec with de la Maza and Sommer to calculate the molar concentrations of different species in a heterogeneous mixture of rAAV particles. *See* § XIII.

311. A POSA would have been further motivated to combine Le Bec, de la Maza, and Sommer with Cole. Cole provides a general overview of AUC techniques

– including SV-AUC – and explains the various parameters and analyses that may be adjusted depending on the experimental system and samples being tested. Cole provides detailed instructions on how to determine appropriate parameters to use for an SV-AUC experiment and how to perform the steps necessary to analyze and interpret the data. EX1009 (Cole), 161-168.

312. As stated in the specification of the '326 patent, “[i]t is within the purview of the skilled artisan to optimize the parameters of AUC for different types of viral particles.” EX1001 ('326 patent), 26:19-21. Such AUC parameters include “any conditions related to the rotor speed, distance between sample and rotor center, temperature, solvent, sample, buffer, ultracentrifugation time, time interval for detection, sector and optical window characteristics, AUC instrumentation (including ultracentrifuge and detection apparatus), equilibrium dialysis of reference solvent, and data analysis algorithms.” *Id.*, 17:44-51.

313. A POSA would have been motivated to combine the teachings of Cole with Le Bec, de la Maza, and Sommer to optimize the separation of rAAV particles using SV-AUC.

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

314. As stated in Cole, “the correct rotor speed for a SV experiment depends what you want to know about your sample, what the expected component size distribution is, and which optical systems will be used.” EX1009 (Cole), 164.

315. Cole teaches 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. *Id.*, 164. Table III of Cole discloses “maximum recommended rotor speeds for various sedimentation coefficients” to enable “[a]bout 2 h of data acquisition” at the listed centrifuge rotor speed. *Id.*, 164, Table III, note c.

316. Thus, Cole meets the additional limitation of claim 25.

C. A POSA Would Have Had a Reasonable Expectation of Success in Making the Claimed Combination

317. As discussed above, it is my opinion that a POSA would have had a reasonable expectation of success in applying the teachings of de la Maza and Sommer to the SV-AUC methods disclosed in Le Bec. *See* § XIII.M. Additionally, in my opinion, a POSA would have had a reasonable expectation of success applying the teachings of Cole to optimize the separation of rAAV particles when using the SV-AUC methods disclosed in Le Bec. Consistent with the statements in the ’326 patent specification, optimizing AUC parameters for different types of viral particles would have been routine for a POSA. EX1001 (’326 patent), 26:19-21.

D. Secondary Considerations Do Not Change the Conclusion of Obviousness

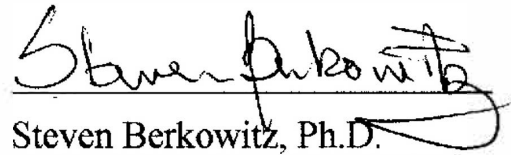
318. As discussed above, I am not aware of any secondary considerations of non-obviousness with the required nexus to the challenged claims of the ’326 patent. To the extent Patent Owner attempts to raise secondary considerations that have only

a marginal nexus, if any, to claim 25 of the '326 patent, such evidence of secondary considerations should not outweigh the compelling evidence of obviousness. Thus, secondary considerations do not alter my opinion that claim 25 is obvious over the combination of Le Bec and de la Maza, in view of Sommer and Cole.

XV. DECLARATION

319. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Date: 11-24-25


Steven Berkowitz, Ph.D.