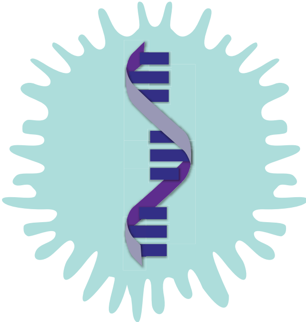
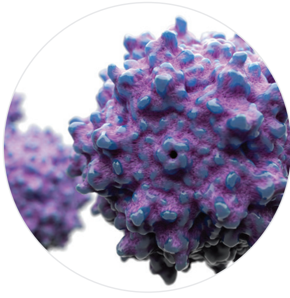
Catalent CELL & GENE THERAPY | LumaCyte



TECHNICAL NOTE
Radiance® Label-Free Monitoring of AAV Transfection in HEK293 Cells Using Laser Force Cytology™

Executive Summary

Gene therapy, which delivers DNA into a patient's cells to replace or add genes that may be abnormal or missing, is delivering results in the clinic after 30 years of development. With the FDA approval of the second adeno-associated virus (AAV) based gene therapy treatment in May of 2019[1], it is truly an exciting time for the advanced therapeutics. AAV has proven to be a powerful tool for gene therapy purposes for its broad tropism, inability to replicate on its own *in vivo*, minimal immunogenicity, and ability to deliver effective and long-lasting results[2]. However, there are several challenges related to the development and manufacturing of AAV-based processes, including characterization, quantification, and downstream purification[3, 4]. Manufacturing challenges in particular include the need for consistently high purity, potency, and safety for AAV products, all while maintaining acceptable large-scale manufacturing costs[5]. While there are several methods for AAV production, including transient transfection in human (HEK293) cells[6], baculovirus driven production in insect (Sf9) cells[7], or recombinant helper viruses such as HSV in mammalian cells[8, 9], the importance of precise and predictive analytics remains regardless of the production system. LumaCyte's Radiance® instrument, which uses Laser Force Cytology™ (LFC) to measure the intrinsic biophysical and biochemical properties of single cells[10, 11], has the potential to improve the characterization of cell-based AAV transfection and production, improving the efficiency and accuracy of both processes and shortening development time. As a leader in the gene therapy field, Catalent Cell and Gene Therapy strives to incorporate the most advanced analytics in its processes in order to provide high quality, innovative solutions for its customers. In this technical note, collaborative efforts are described between Catalent and LumaCyte to compare AAV production with three different transfection reagents using both LFC and a digital droplet PCR (ddPCR) based viral genome titer assay. A strong correlation was generated between the LFC based measurements, which are available in near real-time (5 minutes analysis time per sample) and the ddPCR results which take significant time and labor, demonstrating the utility of LFC for rapid process monitoring. Additional applications of LFC throughout the AAV production process include adventitious agent monitoring to rapidly detect potential contamination as well as cell line characterization during process development and scale-up.

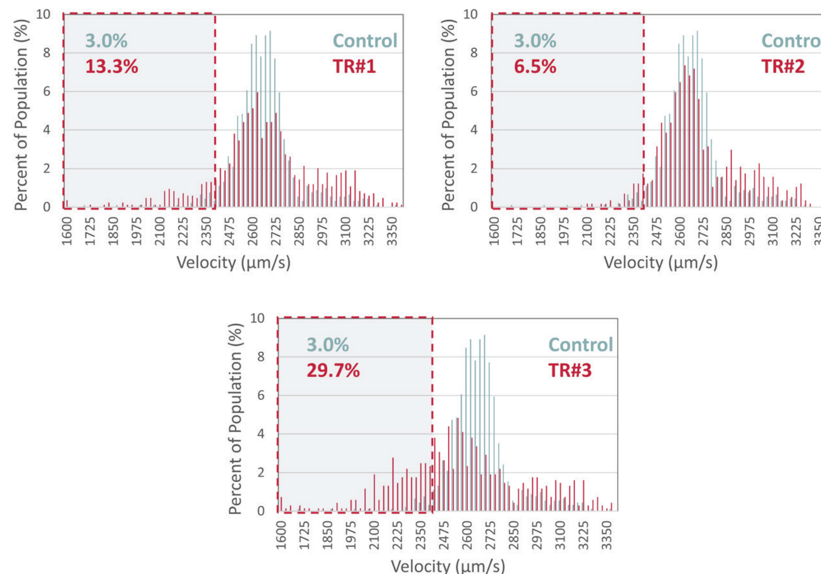


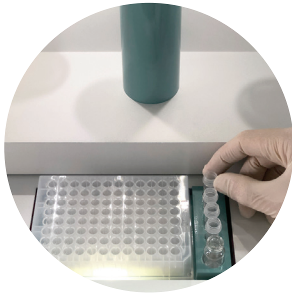
Experimental Results

One important aspect of optimizing AAV production is the selection of an appropriate transfection reagent. In this study, Radiance® demonstrated LFC's ability to monitor AAV transfection in a label-free manner in order to select the optimal transfection reagent (TR). HEK293 cells grown in suspension were transfected with the plasmids to produce AAV using one of three different reagents and then incubated for 72 hours. An untreated control population was also seeded and incubated for 72 hours for comparison purposes. At the time of harvest, an aliquot of cells was removed from culture for each condition and resuspended in LumaCyte Stabilization Fluid prior to analysis with Radiance®. A powerful laser within Radiance® illuminates each cell as it flows through the microfluidic channel, thereby inducing a momentum transfer to the cell as a result of the optical force. This optical force changes the travel velocity of each cell as a function of its biochemical and biophysical properties, which means that as the cell's properties change due to transfection, photons generate forces differently, causing a greater change in the velocity with transfection. Thus, a cell's velocity is proportional to its optical force.

FIGURE 1 shows velocity histograms for each of the transfection reagents compared to the control. For all three treatments, the transfection resulted in a broadening of the histogram distribution, indicating that there is a greater amount of heterogeneity in the transfected populations. This can be seen by comparing the standard deviation of the velocity for each condition (162.4±8.8 μm/s for the control versus 284.6±28.5, 220.7±5.6, and 347.1±20.1 μm/s for reagents 1, 2, and 3, respectively). Notably, all three reagents resulted in a population of lower velocity cells that comprised a higher percentage of the population than the untreated sample (3.0±1.8 % for the control versus 13.3±2.1, 6.5±0.3, and 29.7±2.7 % of the population for reagents 1, 2, and 3, respectively). As shown in **FIGURE 1**, the percentage of cells below the threshold velocity of 2400 μm/s can be used to differentiate each of the transfection reagents from both untreated controls and each other.

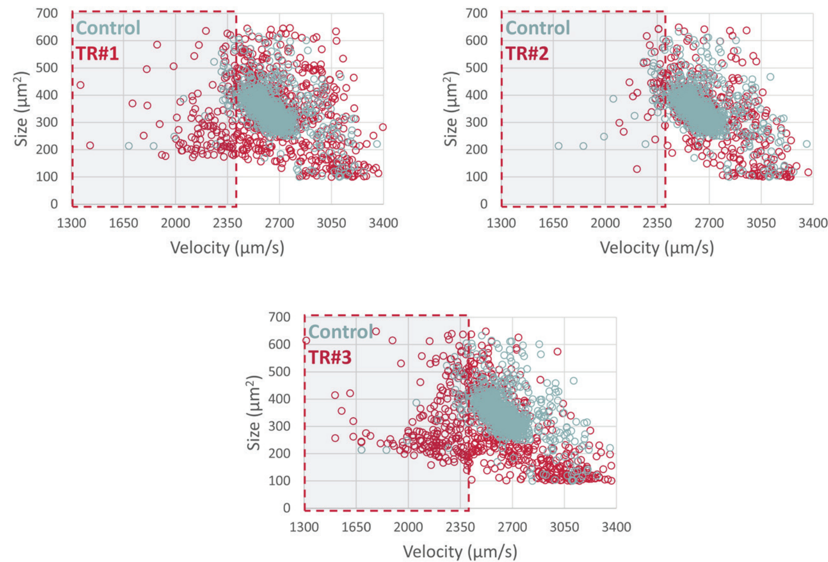
FIGURE 1 Velocity histograms comparing control HEK293 cells to cell populations transfected with AAV production plasmids using three different transfection reagents. Transfection resulted in a clear difference between each population and the control, although the shift in velocity was different in the different reagents. The percentage of cells in the population with a velocity below 2400 μm/s is shown numerically and graphically for each of the three transfection reagents.





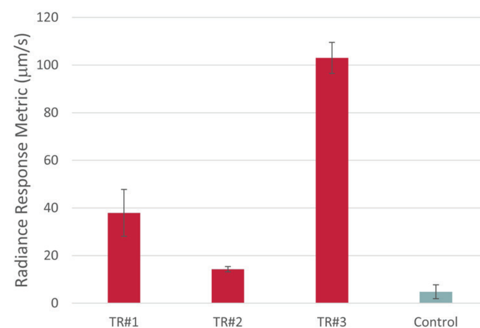
To further characterize the results of each transfection reagent, numerous other Radiance® metrics such as size, shape factors, and deformability can be used. As one example, **FIGURE 2** shows the size versus velocity scatter plots for each treatment. In TR#1 and TR#3 there is clear decrease in both velocity and size as a result of the transfection when compared to the control cells. Cells that decreased in size and velocity experienced a much greater optical force than larger cells at a similar or higher velocity, demonstrating the significant biochemical and biophysical differences between these populations. There is also a population of high velocity and small size cells in both the TR#1 and TR#3 populations. These cells experienced a much lower optical force.

FIGURE 2 Scatter plots of size versus velocity for each transfection treatment compared to the control. TR#1 and TR#3 have populations of cells with a smaller size and lower velocity, demonstrating that they experienced greater optical force during LFC analysis. The population of cells with a velocity below 2400 μm/s is highlighted in the box.



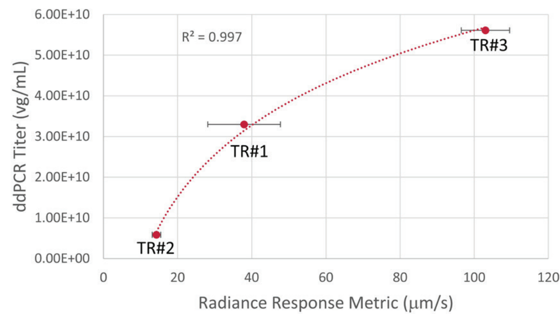
In order to quantify the success of transfection, a Radiance® Response Metric (RRM) can be calculated by multiplying the percentage of cells below the threshold velocity of 2400 by the population's velocity standard deviation. **FIGURE 3** shows the average value of the metric for each treatment. Using this bivariate RRM, all of the reagents could be readily distinguished from one another and the untreated control. Because TR#3 has the highest percentage of cells compared to the untreated control, it was predicted that the greatest success of transfection occurred in TR#3, followed by TR#1 and TR#2.

FIGURE 3 Bar plot of the Radiance® Response Metric (% Low Velocity Cells Velocity<2400 x Standard Deviation Velocity). This Radiance® bivariate response metric allowed for the clear distinction in the transfection results in the three different reagents with TR#3 having the highest success and TR#2 having the lowest success of transfection as measured by ddPCR. Error bars show the standard deviation of the mean (N=3).



In order to confirm the prediction that TR#3 had the highest success of transfection, supernatant samples were collected for later AAV titration using a ddPCR based TCID50 assay, which was completed at a later point in time. **FIGURE 4** shows the correlation between the RRM and the ddPCR titer for all treatments with a coefficient of determination (R^2) of 0.997, indicating a strong correlation between the RRM and AAV titer. This plot is significant in that it not only demonstrates the ability of Radiance® to monitor AAV production in order to optimize transfection conditions, but also shows the ability to establish a quantitative relationship between Radiance® data and titer across different transfection reagent types, making this metric an excellent and robust predictor of titer. By establishing a strong correlation between the Radiance® metrics and titer, LFC is able to give titer results in 5 minutes post cell harvest, as opposed to ddPCR, which takes significant time and labor for amplification. It is also important to note that by looking at the single-cell data, cell groups can be identified that are not readily apparent by looking only at the population wide averages. For example, while there are some differences in the average velocity and almost no differences in the average size between treatments, using a combination of metrics at the single-cell level allows for the identification of a population of high optical force cells that correlates with the AAV production. This demonstrates the utility of LFC for in-process measurements to provide quantitative information that can be used to improve efficiency, reduce labor, and increase the speed of process development.

FIGURE 4 Correlation curve of ddPCR physical titer versus the Radiance® Response Metric (% Low Velocity Cells Velocity<2400 x Standard Deviation Velocity). The Radiance® metrics correlated very well with ddPCR titer indicating LFC's usefulness in monitoring AAV transfection and calculating titers with accuracy.



Cell Culture, Transfection & Harvest

All experimental procedures were conducted at Catalent Gene Therapy facilities in Baltimore, MD. HEK293 cells were grown in suspension in serum-free growth media in a 37°C incubator per Catalent protocols. Transfection complexes were prepared using each of the reagents and then added to the cultures in a controlled manner to ensure uniform conditions across the flasks. After a 72 hour incubation period post transfection, cells were harvested, centrifuged at 200 g for 5 minutes, and then resuspended at a concentration of approximately 600,000 cells/mL in LumaCyte's Stabilization Fluid (Part #SF 1800) before loading 200 mL of each sample into a LumaCyte 96-well plate (Part #WP3110) prior to Radiance® analysis. Samples were taken at the same time to analyze using the ddPCR assay.

REFERENCES

1. FDA Approves Zolgensma, Landmark AAV-Delivered Gene Therapy to Treat Spinal Muscular Atrophy. 2019 [cited 2020 06Jan2020]; Available from: https://www.asgct.org/research/news/may-2019/fda-approves-zolgensma-gene-therapy.
2. Naso, M.F., et al., Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. BioDrugs, 2017. 31(4): p. 317-334.
3. Clement, N. and J.C. Grieger, Manufacturing of Recombinant Adeno-Associated Viral Vectors for Clinical Trials. Mol Ther Methods Clin Dev, 2016. 3: p. 16002.
4. van der Loo, J.C. and J.F. Wright, Progress and Challenges in Viral Vector Manufacturing. Hum Mol Genet, 2016. 25(R1): p. R42-52.
5. Wright, J.F., Transient Transfection Methods for Clinical Adeno-Associated Viral Vector Production. Hum Gene Ther, 2009. 20(7): p. 698-706.
6. Matsushita, T., et al., Adeno-Associated Virus Vectors can be Efficiently Produced Without Helper Virus. Gene Ther, 1998. 5(7): p. 938-45.
7. Kotin, R.M. and R.O. Snyder, Manufacturing Clinical Grade Recombinant Adeno-Associated Virus Using Invertebrate Cell Lines. Hum Gene Ther, 2017. 28(4): p. 350-360.
8. Thomas, D.L., et al., Scalable Recombinant Adeno-Associated Virus Production Using Recombinant Herpes Simplex Virus Type 1 Coinfection of Suspension-Adapted Mammalian Cells. Hum Gene Ther, 2009. 20(8): p. 861-70.
9. Adamson-Small, L., et al., Sodium Chloride Enhances Recombinant Adeno-Associated Virus Production in a Serum-Free Suspension Manufacturing Platform Using the Herpes Simplex Virus System. Hum Gene Ther Methods, 2017. 28(1): p. 1-14.
10. Hebert, C.G., et al., Rapid Quantification of Vesicular Stomatitis Virus in Vero Cells Using Laser Force Cytology. Vaccine, 2018. 36(41): p. 6061-6069.
11. Bommareddy, P.K., et al., MEK Inhibition Enhances Oncolytic Virus Immunotherapy Through Increased Tumor Cell Killing and T Cell Activation. Sci Transl Med, 2018. 10(471).

Discover more solutions at biologics.catalent.com/gene
GLOBAL +1 888 SOLUTION (765-8846)
EU 00800 88 55 6178
solutions@catalent.com

Discover more at lumacyte.com
GLOBAL +1 888 472 9295
info@lumacyte.com

© 2020 LumaCyte, LLC and Catalent, Inc. All rights reserved.
Catalent is a registered trademark of Catalent, Inc. and its affiliates.
Laser Force Cytology is a trademark of LumaCyte.
LumaCyte & Radiance are registered trademarks of LumaCyte, LLC.