GeneSwift: A PCR-Free Genome Titer Method for Rapid Bioprocess Monitoring

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Introduction

Standard methods for determining adenovirus associated virus (AAV) titers, such as qPCR and dPCR/ddPCR, are time-consuming, labor-intensive, and require multiple serial dilutions. These methods are also prone to interference from PCR inhibitors. Additionally, mRNA-based gene therapy presents challenges that PCR cannot adequately address, particularly in distinguishing between single-stranded (ss) and double-stranded (ds) RNA, the latter of which can trigger cytotoxic responses. To overcome these limitations, we developed GeneSwift, a PCR-free assay that integrates DNA hybridization, immunochemistry, and biolayer interferometry (BLI) for rapid and accurate gene titer quantification.

Methods

AAV capsids are mixed with gene-specific fluorescein-labeled (F*) and biotin-labeled (B*) oligonucleotides, designed using the online oligo tool (primerdigital.com/tools/gator.html). The capsids are lysed by heating in a hybridization buffer, and upon cooling, the labeled oligos hybridize to the gene of interest (GoI), forming stable hybridized complexes. These complexes are then captured using anti-fluorescein-coated biosensors, followed by the addition of a Biotin Detection Solution to generate BLI signals. By comparing the results to a calibration curve constructed with known GoI concentrations, accurate titer quantification is achieved. The entire assay is rapid and efficient, with a total run time of just 35 minutes

Genome Titer

Fig. 1A. GeneSwift Workflow

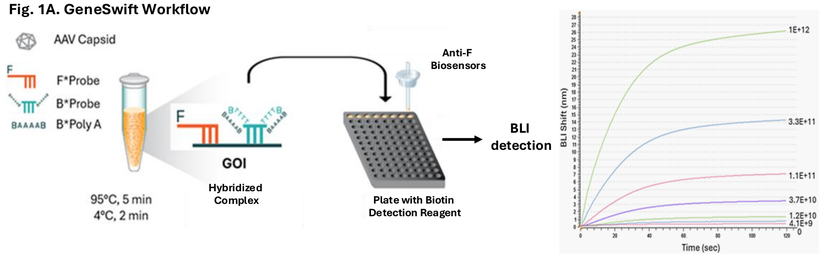

Fig. 1B. GeneSwift Dose-Response Curves in Different Challenging Matrices

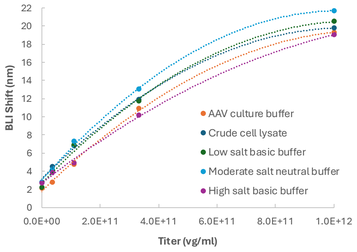

Table 1. Titer Comparison by GeneSwift and ddPCR

	Determined AAV Titer (vg/ml)	
Bioprocess Sample	**ddPCR**	**GeneSwift**
Clarified Harvest	**8.10E+10**	**6.64E+10**
TFF1 Retentate	**4.87E+11**	**6.04E+11**
AFF Neutralized Eluate	**8.45E+11**	**8.66E+11**
TFF2 Retentate	**3.00E+11**	**3.33E+11**
AEX Full	**2.93E+12**	**2.21E+12**
Purified AAV	**1.59E+13**	**1.99E+13**

Fig. 1A. GeneSwift workflow and data showing the detection range of AAV from 4E+9 to 1E+12 vg/ml. **Fig. 1B.** GeneSwift dose-response curves of AAV-GFP spiked into various upstream and downstream manufacturing matrices. The results show robust signals and dose-response curves in all tested bioprocess media, demonstrating a broad compatibility of the assay with various challenging matrices. **Table 1**. Titer comparison of the customer's AAV engineered bioprocess samples by GeneSwift and ddPCR showing very similar titer values.

Highlights

Extremely suitable for AAV bioprocess monitoring and rapid titer justification

- Very fast assay compared to ddPCR (35 min vs. 5 hrs) and no tedious serial sample dilutions required.
- Very accurate assay and data highly correlated to ddPCR.
- Compatible with complex bioprocess matrices and broad applications for gene therapy.

Linearity and Precision Studies

Fig. 2. Linearity Curve in Crude Lysate

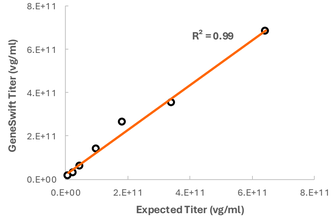

Table 2. Precision Comparison Between GeneSwift and ddPCR

	N = 16 Individual AAV Samples (2E+10 vg/ml) in Crude Cell Lysate	
	GeneSwift assay	**ddPCR assay**
CV (%)	**10.1%**	**9.1%**

Fig. 2. Linearity curve of AAV in the insect crude cell lysate. The sample titers determined by GeneSwift are compared with the expected titer values. **Table 2.** Precision comparison between GeneSwift and ddPCR for 16 individual AAV samples shows very similar performance.

Correlation Studies

Fig. 3A. Clarified Harvest AAV

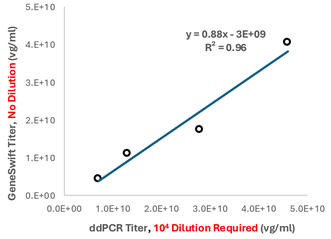

Fig. 3B. AEX Purified AAV

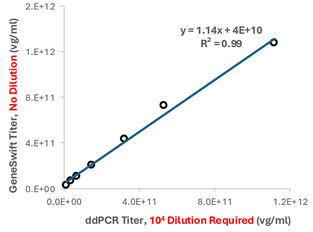

Table 3. Summary of Correlation Coefficient and Slope

Bioprocess Sample	R^2	Correlation Slope
Clarified Harvest	**0.96**	**0.88**
TFF1 Retentate	**0.97**	**0.77**
AFF Neutralized Eluate	**0.99**	**1.09**
TFF2 Retentate	**0.97**	**0.91**
AEX Full	**0.99**	**1.14**
Purified AAV	**0.98**	**0.89**

Fig. 3A and **3B** show the correlation curves of the engineered bioprocess samples Clarified Harvest AAV and AEX Purified AAV respectively. **Table 3** summaries the correlation coefficient and slope of different upstream and downstream bioprocess samples. All tested samples show very good correlation with ddPCR and coefficient $R^2 > 0.95$, indicating the superior correlation between GeneSwift and ddPCR.

Genome Integrity Assessment

Fig. 4A. GFP Oligos

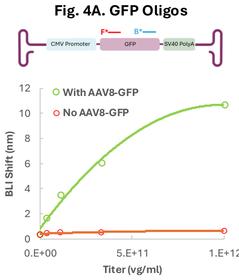

Fig. 4B. CMV Oligos

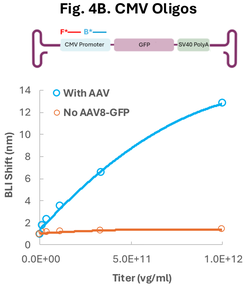

Fig. 4C. CMV and SV40 Oligos

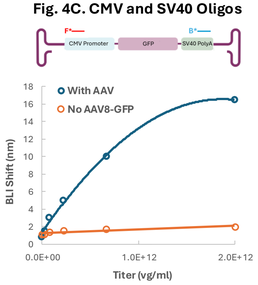

Fig. 4A-C show the dose-response curves done by using AAV8-GFP and oligo pairs hybridizing to different specific genome components. The results show that different oligo pairs, including the CMV and SV40 oligo pair which covers almost the whole genome, are able to generate robust signals and dose-response curves, confirming the assay's potential to evaluate genome integrity.

Detection of dsDNA, ssRNA and dsRNA

Fig. 5A. dsDNA

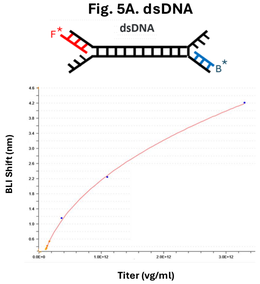

Fig. 5B. ssRNA

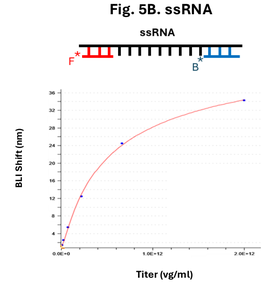

Fig. 5C. dsRNA

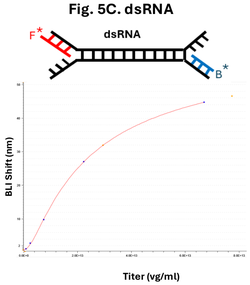

Fig. 5. Broad applications of GeneSwift to detect dsDNA, ssRNA and dsRNA. Detection of dsDNA/RNA requires selection of the oligo pair to hybridize to the opposite strands. All results still show very good signals and dose-response curves, demonstrating the assay's versatility in measuring dsDNA, ssRNA and dsRNA titers.

Conclusion

1. GeneSwift delivers titer results in just 35 minutes with most undiluted bioprocess samples, while ddPCR typically requires ≥10^4-fold dilutions and 5 hours of processing time, making GeneSwift a significantly more efficient titer assay.
2. Compatible with various AAV manufacturing matrices and its accuracy and precision performance are very comparable to ddPCR.
3. Enables genome integrity and mRNA detection, making it a versatile tool for gene therapy research and bioprocessing.

Acknowledgements

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