Evaluation of a Novel Strand-Specific Approach for AAV Genome Titer and Genome Integrity Quantification Using DNA Hybridization Biolayer Interferometry

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Introduction

High specificity AAV genome titer is one of the most important assays, traditionally done using qPCR and digital PCR. Although both approaches have specific advantages and limitations, the availability of other target specific quantitative assays, which could fill some gaps, is limited. One of the promising developments in this field is a novel method using DNA hybridization biolayer interferometry (BLI) that shows promise of high accuracy, fast run time and high repeatability and precision. Biolayer interferometry (BLI) is a label free biomolecular detection method where biomolecular interactions are detected by measuring the interference pattern of white light reflected from the surface of a biosensor. As BLI compares the interference pattern of white light reflected from an internal reference layer within a layer of immobilized biomolecules on the surface chemistry of the biosensor, it can be used for various applications. We have evaluated BLI as a novel approach for vector genome titer quantification (Figure 1). Moreover, as evaluation of vector genome integrity is becoming more and more important, we have adapted the assay in a way to provide the strand specific results on quantity of full-length and fragmented genomes.

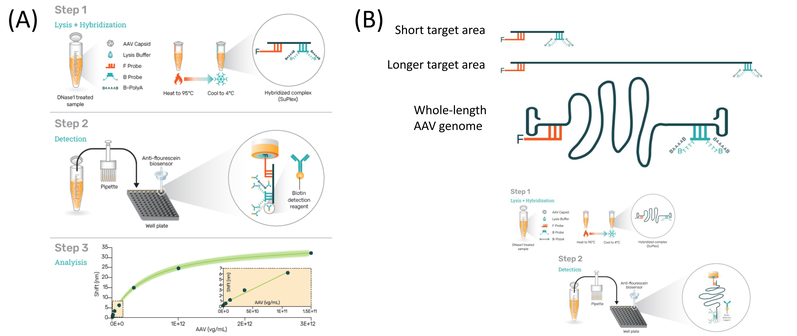

Figure 1: (A) Schematic representation of Gator Bio BLI approach to vector genome titer quantification. First step includes single tube lysis plus hybridization of the two oligos, one labelled with fluorescein and other with biotin. In second step detection of hybridized targets is performed by Gator BLI system (in our case Gator Plus). After detection, analysis is performed in third step, where the wavelength shift can be analysed, and standard curve can later be used for unknown sample quantification. (B) Schematic representation of novel BLI approach to vector genome integrity quantification. The principle of the technology is the same, but different oligos need to be used.

Methods

We've used the Gator® Plus instrument to measure the AAV vector genome titer, using heat lysis plus oligo probe hybridization and proprietary biotin detection. Strand specific oligos targeted different parts of the AAV vector genome construct.

Repeatability of the measured responses for positive and negative strands was evaluated on AAV8 serotype, using the standard curve prepared from ddPCR characterized AAV8 sample. Three test samples with different expected concentrations were prepared and analyzed on the BLI system. The 30-minute assay involves single tube AAV capsid lysis plus a single hybridization step with DNA probes, followed by interferometry detection. Our focus was on repeatability, accuracy and dynamic range.

Furthermore, we have evaluated the possibility for quantification of full-length viral vectors using the same approach as for the genome titer but with different oligo combination.

Key benefits of novel BLI AAV genome titer and genome integrity assay

Strand specific quantitative determination of genome titer (specific target or full-length genome)

Short time to the result

Robust and repeatable results

Results - Vector genome titer assay – CMV enhancer (positive strand)

The responses (binding rates) of the standard curve samples were repeatable over the whole tested range, however, variability was higher at high concentration (1E+12 vg/mL) (Figure 2 and Table 1).

In terms of quantification of AAV8 vector genomes, we have shown that variability between runs of the same day (n=3) and variability between days (n=9) is comparable and is below 20%. This variability is not dependent on sample concentration.

Accurate range of quantification is narrower than the standard curve range, especially the high concentrations are less accurately quantified (33.6 % difference to expected) (Table 2), which is due to higher variability in the responses of the standard curve sample at 1E+12 vg/mL. For intermediate sample concentrations the determined titer was close to the expected value (~7% difference) (Table 2).

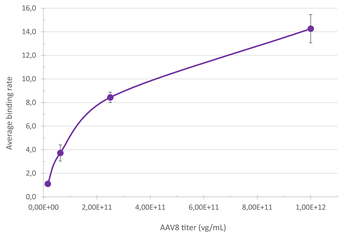

Figure 2: Repeatability of wavelength shift responses for CMV enhancer positive strand assay, tested on three different days, with three runs each day (total 9 data points per concentration).

Sample	Expected concentration (vg/mL)	Day	Average Binding Rate Per Day (n=3)	CV % (n=3)	Average Binding Rate Per Sample	SD (n=9)	CV % (n=9)
S1	1,00E+12	1	13,90	10,2	14,26	1,20	8,4
		2	15,40	4,6			
		3	13,49	3,5			
S2	2,50E+11	1	8,14	2,6	8,44	0,44	5,3
		2	8,79	7,3			
		3	8,38	1,7			
S3	6,25E+10	1	3,50	8,9	3,72	0,68	18,4
		2	3,89	22,1			
		3	3,78	25,3			
S4	1,56E+10	1	1,09	14,2	1,11	0,15	13,6
		2	1,15	19,5			
		3	1,08	10,4			

Table 1: Repeatability of the binding rates for standard curve samples at four different concentrations. Results are for runs completed within three different days, with three runs each day (total 9 data points per concentration).

Sample	Expected concentration (vg/mL)	Day	Average calculated concentration (vg/mL) (n=3)	CV % (n=3)	% difference (expected /calculated)	Average calculated concentration (vg/mL) (n=9)	CV %	% difference (expected /calculated)
U1	6,58E+11	1	8,64E+11	6,7	31,4	8,79E+11	14,0	33,6
		2	9,35E+11	16,0	42,1			
		3	8,37E+11	19,8	27,2			
U2	2,19E+11	1	2,69E+11	8,7	22,5	2,34E+11	15,2	6,6
		2	2,3E+11	6,0	4,9			
		3	2,02E+11	15,6	-7,7			
U3	7,38E+10	1	7,74E+10	10,2	4,8	7,91E+10	16,4	7,1
		2	8,94E+10	10,9	21,1			
		3	6,98E+10	18,6	-5,4			

Table 2: Repeatability results for quantification of test samples using standard curve at four concentration points. Samples were tested on three different days, with three runs each day (total 9 data points per concentration).

Conclusions

- The novel AAV vector genome tittering and genome integrity quantification approach based on BLI has shown promising results
- Quantification relies on the well characterized standard curve
- Strand specific quantification of defined vector genome length can be achieved without amplification
- Repeatable results in relatively short time (30 min)
- Currently testing options for a more standardized and simplified quantification by using more defined standard curve sample

Results – Vector genome integrity – CMVenhancer-SV40 polyA

The responses of the standard curve samples was similar as with genome titer assay, with higher variability at highest concentration point (Figure 3).

We have used two different approaches for quantification:

1) by quantifying the samples considering only one run (4 points of standard curve and single replicates of three samples),
2) by quantifying the samples against an average standard curve by considering all nine individual standard curves for average calculation.

Higher variability was observed between runs for standard curve (Figure 3 and Figure 4) and for quantification of samples (Table 3 and Table 4). Nevertheless, the average determined concentration of full-length genomes was in general relatively close to the expected ones when calculated as single runs or to average of standard curves for positive strand assay (Table 3). For negative strand assay the quantitative results were better when calculated as single runs.

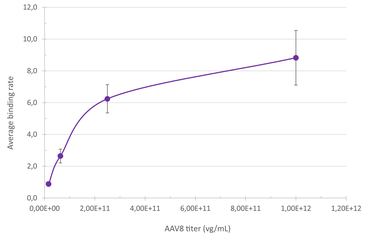

Figure 3: Repeatability of wavelength shift responses for genome integrity positive strand assay, tested on three different days, with three runs each day (total 9 data points per concentration).

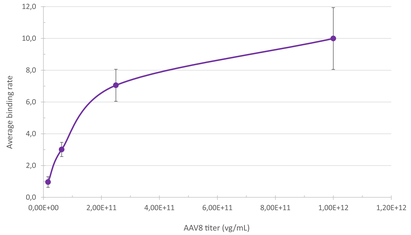

Figure 4: Repeatability of wavelength shift responses for genome integrity negative strand assay, tested on three different days, with three runs each day (total 9 data points per concentration).

Sample	Expected concentration (vg/ml)	Day	Quantification by using standard curve of each run: Average calculated concentration (vg/ml) (n = 2 to 3)	CV %	% difference (expected / calculated)	Average calculated concentration (vg/ml) (n = 7 or 9)	CV %	% difference (expected / calculated)	Quantification by using standard curve average (n=9): Average calculated concentration (vg/ml) (n = 2 or 3)	CV %	% difference (expected / calculated)	Average calculated concentration (vg/ml) (n = 8 or 9)	CV %	% difference (expected / calculated)
U1	6,60E+11	1	8,82E+11	9,5	33,6	1,18E+12	69,4	79,2	1,51E+12	104,6	129,1	1,01E+12	72,3	53,6
		2	1,47E+12	77,8	122,2				9,02E+11	56,0	36,7			
		3	1,06E+12	93,7	60,4				7,94E+11	24,6	20,3			
U2	2,20E+11	1	1,93E+11	11,5	-12,4	2,58E+11	43,7	17,2	2,66E+11	3,0	20,8	2,42E+11	29,5	10,0
		2	3,64E+11	39,2	65,6				2,66E+11	46,1	21,1			
		3	2,17E+11	29,0	-1,5				1,94E+11	5,0	-11,8			
U3	7,40E+10	1	7,37E+10	2,7	-0,4	6,92E+10	20,3	-6,6	9,41E+10	6,4	27,1	7,17E+10	26,3	-3,1
		2	5,49E+10	29,3	-25,9				5,16E+10	6,7	-30,3			
		3	7,89E+10	8,2	6,6				6,94E+10	2,9	-6,2			

Table 3: Repeatability results for genome integrity quantification by **positive strand assay**. Three different concentrations of test samples were quantified using standard curve at four concentration points. Samples were tested on three different days, with three runs each day (total 9 data points per concentration).

Sample	Expected concentration (vg/ml)	Day	Quantification by using standard curve of each run: Average calculated concentration (vg/ml) (n = 1 to 3)	CV %	% difference (expected / calculated)	Average calculated concentration (vg/ml) (n = 5 to 9)	CV %	% difference (expected / calculated)	Quantification by using standard curve average (n=9): Average calculated concentration (vg/ml) (n = 2 or 3)	CV %	% difference (expected / calculated)	Average calculated concentration (vg/ml) (n = 8 or 9)	CV %	% difference (expected / calculated)
U1	6,60E+11	1	5,24E+11	49,0	-20,6	5,99E+11	40,3	-9,2	8,00E+11	75,80%	21,2	1,18E+12	90,9	78,9
		2	5,39E+11	NA	-18,3				1,48E+12	105,99%	124,6			
		3	8,84E+11	NA	33,9				1,30E+12	99,09%	96,9			
U2	2,20E+11	1	1,73E+11	22,2	-21,2	2,28E+11	44,3	3,5	1,72E+11	20,35%	-21,8	1,95E+11	30,0	-11,4
		2	2,09E+11	26,3	-4,8				2,11E+11	36,54%	-4,1			
		3	3,37E+11	50,2	53,0				2,05E+11	40,70%	-6,8			
U3	7,40E+10	1	9,08E+10	6,8	22,6	8,99E+10	15,5	21,5	1,38E+11	62,36%	87,1	1,07E+11	51,7	45,1
		2	8,08E+10	6,4	9,2				7,57E+10	16,07%	2,3			
		3	9,85E+10	20,2	33,1				1,08E+11	38,82%	45,8			

Table 4: Repeatability results for genome integrity quantification by **negative strand assay**. Three different concentrations of test samples were quantified using standard curve at four concentration points. Samples were tested on three different days, with three runs each day (total 9 data points per concentration).

Acknowledgements

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