

Quantification of Adeno-Associated Viral Particles using CX300 and ParaySelect™

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INTRODUCTION

Adeno-associated virus (AAV) platforms have recently emerged as an important focus for translational biomedical research due to their exceptional attributes as gene delivery vehicles. AAVs exhibit a remarkably high safety profile, elicit minimal immune responses, and possess the ability to mediate long-term gene expression in both dividing and non-dividing cells, making them ideal candidates for gene therapy applications. Furthermore, their ability to transduce a wide range of cell types, including neurons, hepatocytes, and muscle cells, has unlocked a vast array of therapeutic opportunities, targeting a diverse range of genetic and acquired diseases.

AAVs are generated by infection of large-scale mammalian cell cultures. Production of virus vectors for gene therapy presents many challenges that must be overcome for successful translation from the laboratory to clinical application. Key challenges include a need for precise optimisation of steps in large-scale viral production, such as cell transfection, vector genome replication, and purification. High vector production efficiency (often $>10^{12}/\text{mL}$) remains a challenge, especially for large-scale manufacturing, which is necessary to meet the demands of clinical trials and potential commercialisation. Of critical importance is the need for rapid analysis of viral load during culture optimisation and subsequent virus purification. Current methods of viral particle enumeration and characterisation include ELISA, and size exclusion chromatography which are both time-consuming and labour intensive. Here we have used the Paraytec CX300 together with ParaySelect™ to investigate the utility of CX300 for rapid enumeration of AAV titres.

METHOD

AAV serotype 1 (Catalogue RS-AAV1-ET) was obtained from Charles Rivers Laboratories. Virus (2.26×10^{12} particles per mL) was serially diluted 1:3 in 10 mM histidine, pH 6.0 (Fig. 1), prior to tagging using 2.0 ng/mL AFDye™ 488 - labelled mouse monoclonal anti-AAV1 (Progen Ltd) for 1 h. Fluorescently-tagged material (100 μL) was applied to a ParaySelect™ cartridge equilibrated in histidine buffer as above and subsequently 7.5 μL of each sample was analysed using CX300 (Fig. 1) with data collection for 60 seconds.

RESULTS

Fig. 1 displays the real-time analyses output, showing the viral concentration-dependent increase in events captured by CX300. These data can be converted into a standard curve for each viral serotype, as shown in Fig. 2.

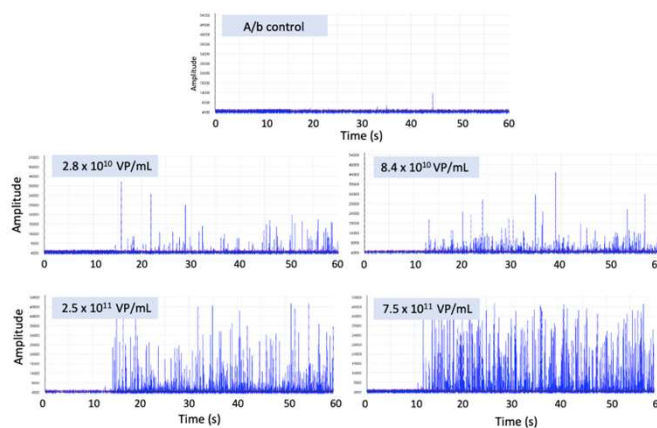


Fig. 1 CX300 response profiles of AAV1 species at the indicated concentrations of viral particles.

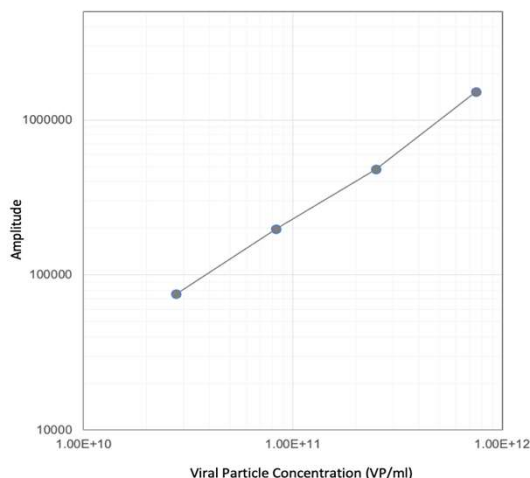


Fig. 2 Standard curve of CX300 response versus AAV concentrations analysed.

CONCLUSIONS

CX300 is a platform for the rapid and sensitive analysis of AAV concentration.

- Fast analysis - 5 min.
- Small sample requirement - 30 μL
- Broad application and range capability