Development of a ddPCR protocol for the quantification of AAV viral genomes in purified samples

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AAV (adeno-associated virus) is a non-pathogenic virus that is used as a delivery vehicle to transfer therapeutic genes into a patient's cells. Accurate quantification of AAV genome copies is essential for the optimization of the production and purification processes, preclinical studies, and clinical dosage of AAV-based gene therapy products. Several methods exist for the determination of the viral genome (vg) titer with quantitative PCR (qPCR) and droplet digital (dd)PCR being the two most widely used and accepted ones. ddPCR is preferred over qPCR because of the independence of the amplification efficacy, higher precision, and robustness.

The aim of this study was to develop a ddPCR protocol for the quantification of AAV viral genomes in purified samples. Unpackaged contaminant DNA, amongst others DNA from the production plasmids and/or cell line, needs to be eliminated before viral genomes can accurately be quantified. We tested several protocols for their ability to remove unpackaged DNA while maintaining accurate packaged viral genome titers. To do so, we spiked plasmid DNA into purified AAV2 vector and evaluated the following sample treatment conditions: (A) incubation with DNAseI only; (B) DNAseI incubation followed by EDTA inactivation, 72°C heat inactivation, proteinase K treatment, and finally 95°C heat inactivation; (C) the same as condition B but with a final heat inactivation at 72°C instead of 95°C (D) no sample pretreatment. Plasmid and AAV genome copies were quantified through a duplex ddPCR (QX200 droplet digital PCR system, Bio-Rad). In contrast to condition D, where no sample treatment was performed, no plasmid DNA was detected in conditions A, B and C. A trend was observed towards lower AAV2 vg titers in condition B compared to conditions A and C (Figure 1). Interestingly, comparable vg titers were noted between conditions A and C. A similar observation was made when repeating the experiment with purified AAV9.

Our findings indicate that heat inactivation at 95°C is detrimental to AAV vg titration and DNAseI treatment only is sufficient for the removal of unpackaged DNA without affecting vg titers. In a next step, we aim to validate this ddPCR protocol and evaluate robustness, specificity, linearity, range, accuracy, precision, and quantification limits.

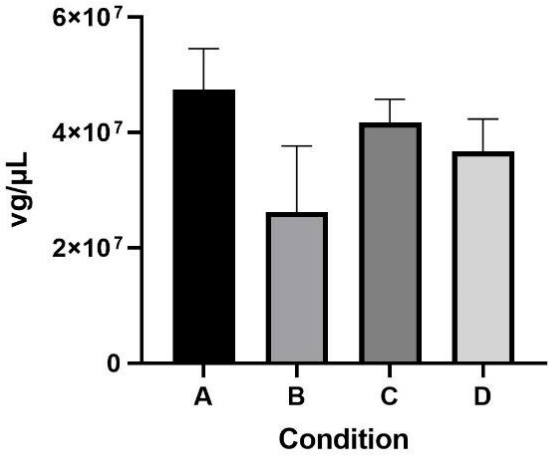

Figure 1. AAV2 viral genome titers after the following sample treatment conditions: (A) incubation with DNAseI only; (B) DNAseI incubation followed by EDTA inactivation, 72°C heat inactivation, proteinase K treatment, and finally 95°C heat inactivation; (C) the same as condition B but with a final heat inactivation at 72°C instead of 95°C (D) no sample pretreatment. N = 8