Streamlined process for automated plasmid purification reduces labour time and increases titres for AAV production

Sanne Rönning*, Hannes Thorell*, Magdalena Malm, Johan Rockberg

Dept. of Protein science; KTH - Royal Institute of Technology; Stockholm; SE-106 91; Sweden

*Contributed equally

Introduction

Adeno-associated virus (AAV)-based therapies can revolutionise the treatment of many genetic diseases. More and more of these therapies are receiving approval, and the number in development are steadily increasing. AAV manufacturing for drug candidate screening require a large amount of different high-quality plasmids. Each transfection requires three essential plasmids (Fig 1) carrying genes for replication and the capsid proteins (RepCap), the therapeutic gene to be delivered (Cargo) and viral helper genes (Helper). Purification of these plasmids can be both labour-intensive and time-consuming, leaving room for improvement of the process.

In this study, we have compared the automated plasmid purification system Biotage® PhyPrep (PhyPrep) with a commercially available manual plasmid purification kit. The plasmids required for production of AAV9, a serotype of interest in therapeutics and clinical trials, were purified in Giga scale on both systems, followed by a comparison of plasmid quality, plasmid preparation time and AAV titres when produced in the commercial mammalian production system AAV-MAX (Thermo Fisher Scientific).

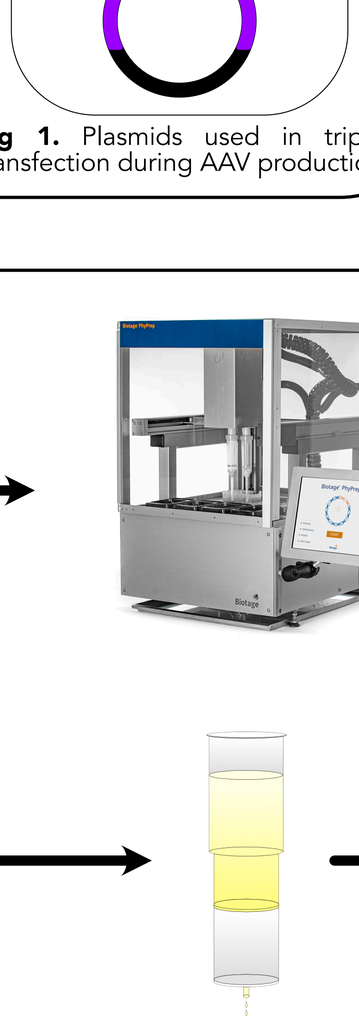


Fig 1. Plasmids used in triple transfection during AAV production

Results

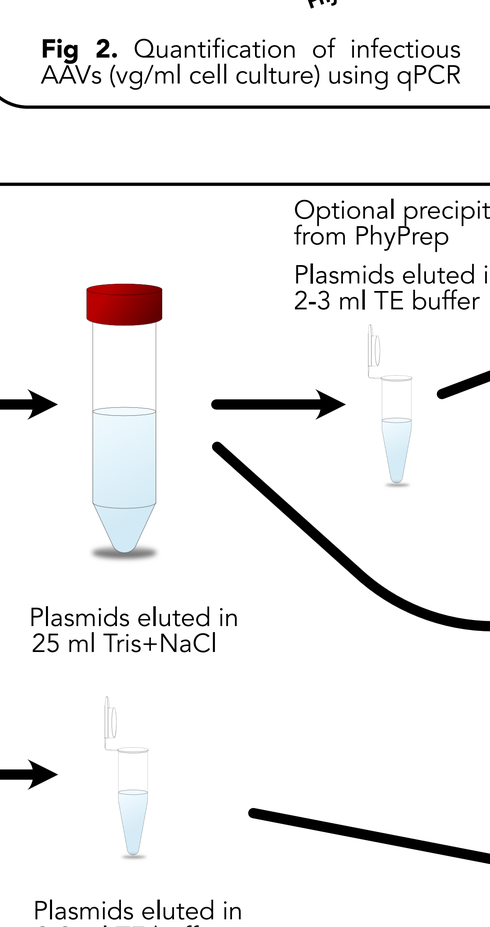


Fig 2. Quantification of infectious AAVs (vg/ml cell culture) using qPCR

Overnight bacterial cultures were pelleted and divided for purification according to each manufacturer's instructions. The preparation time when purifying plasmids from four different cultures resulted in a 90% cut of manual labour time when using the PhyPrep compared to the manual kit (Table 1). The hands-on labour time was 35 minutes for the PhyPrep system compared to close to 9 hours required for the manual kit. The total process time was reduced by almost half.

An optional precipitation step of the plasmids isolated from the PhyPrep added 70 minutes to the preparation time. This resulted in an 80% reduction of the active time compared to the 90% without precipitation, and a total process time at 70% of that of the manual kit.

Initial results from small-scale productions of AAV9 showed a 20-fold increase in infectious AAV9 titres using plasmids prepared with Phyprep (3.8E+11 infectious AAVs per ml) compared to plasmids isolated with the manual kit (1.4E+10).

Workflow

Preparation Time

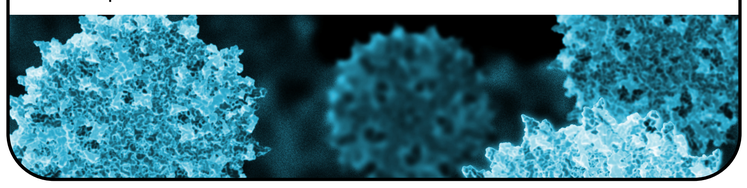
Table 1. Time required for lysis and purification of four plasmids according to manufacturer's instructions. Active time exclude process steps above 30 minutes (walk-away time). Total time is the process time from start to finish (active time + walk-away time).

	Active Time	Total Time
Manual Kit	8 h 45 min	9 h 45 min
PhyPrep	35 min	5 h
PhyPrep + precipitation	1 h 45 min	6 h 15 min

Conclusion

One limiting factor in AAV drug candidate screening is the large amount of high-quality plasmids needed for AAV production, as plasmid purification can be both labour-intensive and time-consuming.

The results from our comparison indicate that the automated PhyPrep purification system can vastly cut plasmid preparation time while also provide plasmids resulting in enhanced AAV yields compared to a manual kit.



This project is part of the GeneNova project with funding from Vinnova and Biotage AB

Contact:
Sanne Rönning
sronning@kth.se
Hannes Thorell
hannesth@kth.se