Quality over quantity: where is the trade off?

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Introduction

One of the biggest challenges in rAAV manufacturing is tailoring the AAV production platform in such a manner to improve virus productivity, and simultaneously to uniformly improve a multitude of critical quality attributes, to meet both regulatory and commercial demands. The final design of the process should aim at high productivity with a high percentage of full-length genome packed viruses, high vector potency but also with lowest possible DNA impurity levels (mispackaged plasmid derived and host cell derived sequences).

Our platform is an end-to-end process solution that is differentiated by being developed with the primary aim of the best possible quality at high yield, to ensure patient safety throughout a product life cycle in a rapidly maturing field and to make AAV gene therapy accessible to large patient populations and for indications with more challenging risk benefit profiles. This platform is fully scalable and proven for a number of capsid serotypes, based on HEK293 transient transfection using our proprietary split 2-plasmid system.

Transient transfection is widely used to produce rAAV as it enables the agility and speed from gene to GMP typically being a major focus for product development companies. Besides cell line choice and media composition, plasmid design and the transfection procedure used are amongst the main critical process steps in upstream processing and are the major "driving force" for low product derived impurities and high potency product formation. The main goal of our study was to compare two transfection reagents currently available on the market and widely used for AAV manufacturing, to our standard process and to determine the impact on the final product quantity and quality, as part of our continuous platform innovation strategy.

Our current upstream process platform

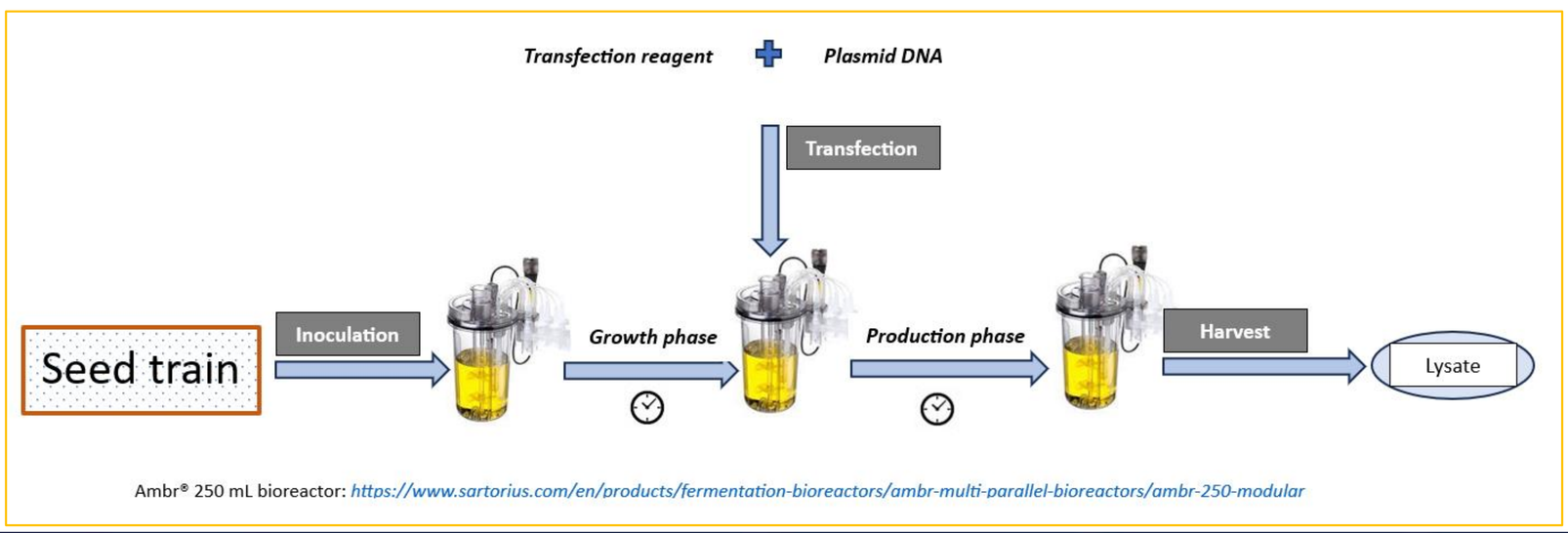
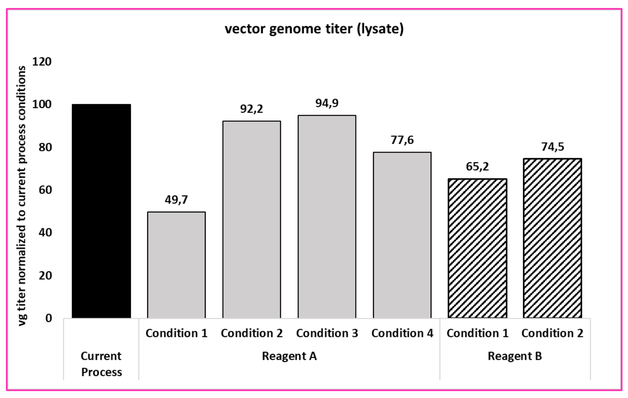


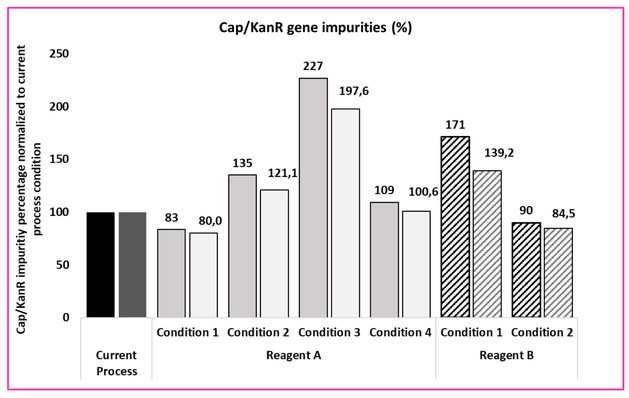
Diagram of our current upstream rAAV process platform (Ambr® 250 mL bioreactor scale).
After thaw and expansion, HEK293 cells were transfected using either transfection reagent A or B or transfection reagent used in our current process platform offering. In this study, different total amounts of plasmid DNA per batch and different transfection reagent : DNA amount ratios were also tested (shown as a different conditions for transfection reagent).
After the production phase, the transfected cells were harvested, and the bulk lysate was analyzed. Furthermore, the harvested product was affinity-column purified to be able to determine plasmid derived (cap and kanamycin resistance (kanR)) and host cell derived DNA (HCD) impurities.

Vector genome titers



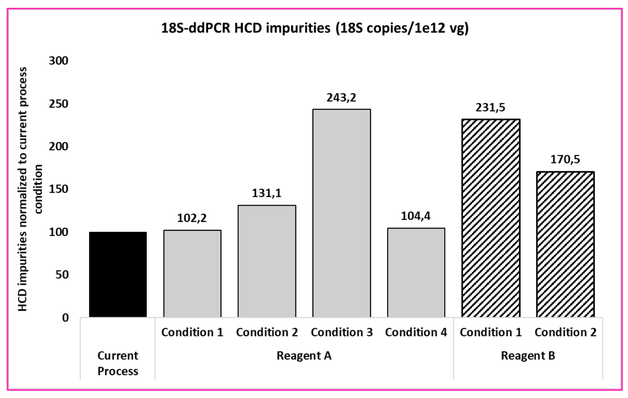
Relative vector genome titer of lysate (vg/mL) normalized to our control process run.
Our current process drives the highest vector genome titers amongst all transfection reagents and conditions tested.
Determination of vector genome titers was done by transgene-specific ddPCR.

Plasmid derived impurities



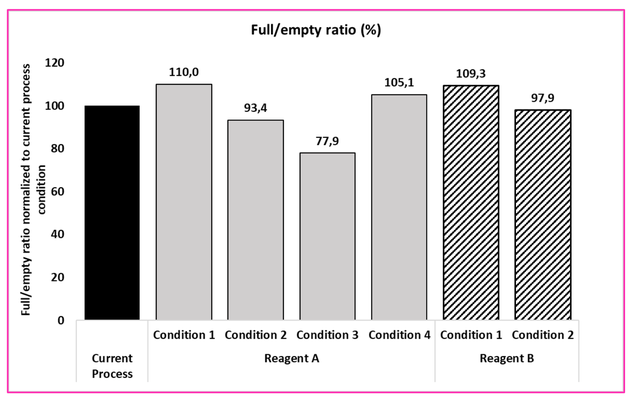
For each condition, relative capsid derived impurities (left bars) and kanR DNA impurity levels (right bars) normalized to standard conditions of our current process are indicated.
The data show an approximately doubling of plasmid-derived impurity levels for at least three conditions for reagent A and for one condition for reagent B.
cap and kanR gene sequences were quantified by duplex ddPCR.

Host cell-derived DNA (HCD) impurity



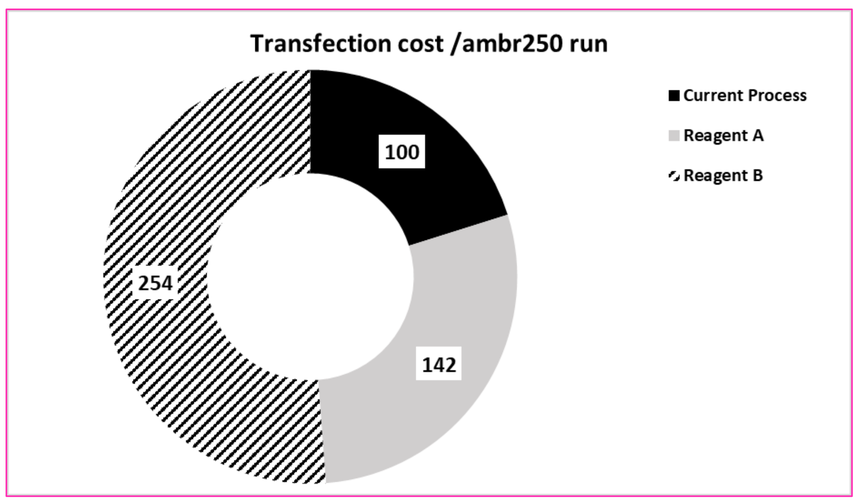
Relative HCD packaging levels normalized to our standard process.
Data shown demonstrate the lowest possible HCD levels with our current platform process.
Determination of host cell-derived DNA (HCD) impurity levels in rAAV productions was done by ddPCR using the 18S rDNA locus of the 45S open reading frame (ORF) in the human cellular genome as a surrogate for HCD.

Full/empty ratios



Relative F/E (full to empty) ratios determined by Refeyn2 Mass Photometry normalized to standard conditions.
Except for reagent A (condition 3), comparable F/E ratios were obtained across all conditions analysed.

Economic feasibility



Transfection process cost (including transfection reagent and plasmids costs) per Ambr® 250 mL bioreactor based on the current market price.
Our current process platform is the most economically feasibly process so far. Since the cost of the transfection could have a huge impact and thus dictate the final cost of the batch at a larger scale, our current process, using the current transfection reagent, minimizes batch costs from a transfection step point of view.

Summary

Two alternative transfection reagents from different suppliers were tested in our current platform process and compared to our standard transfection conditions. Higher vector genome yields and lower DNA impurity levels (both, plasmid-derived and HCD) when using our current manufacturing platform offering were observed.

From an economical point of view, our current process is also most economical when comparing total transfection costs at all conditions analysed.

We are continuously challenging and optimizing our platform process, and while doing so, we always keep a quality mindset. Our aim is the production of the highest quality AAVs, that are scalable and economically feasible for manufacturing at a commercial scale, to support our clients and patients with the safest possible products.

Poster downloads

Aim higher

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