Two for one: A single QC assay to quantify two plasmid impurities (cap/kanR) across a number of serotypes reduces time & costs for rAAV batch release

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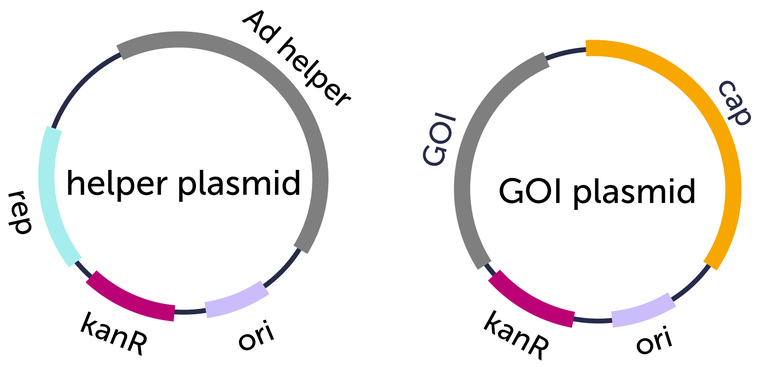
P0197

Abstract

Plasmid derived DNA impurities present in recombinant adeno-associated virus (rAAV) products arise during the manufacturing process and are undesirable byproducts. More importantly, they pose a risk to patient safety and could trigger immune responses impacting product efficacy and long-term expression. As there are different sources of DNA impurities, multiplex ddPCR assays are an excellent way to monitor more than one impurity in a single assay reducing the cost and time of rAAV batch release. Ascend has developed a duplex ddPCR assay with specific primer/probe sets to simultaneously quantify cap and kanamycin resistance gene (nptII; kanR) sequences in one reaction. Our universal cap primer and probe design detects common serotypes including AAV1, 2, 3B, 5, 6, 8, 9, hu37, rh10 and any engineered capsids carrying the respective target sequences such as LK03.

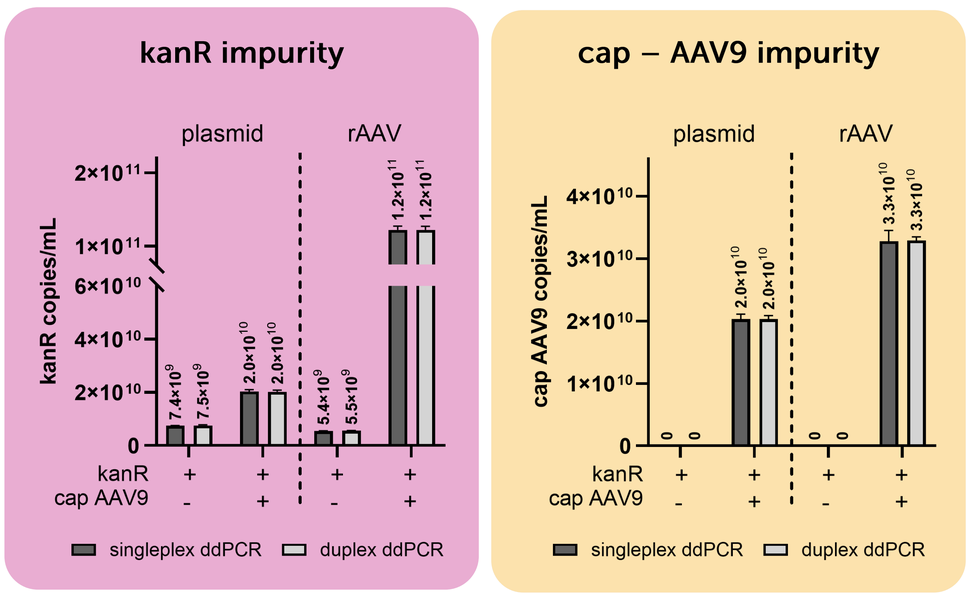
Ascend's EpyQ™ split 2 plasmid system



- kanR (nptII) is present on the helper plasmid and the gene of interest (GOI) plasmid.
- cap is present on the GOI plasmid.
- Ascend has plasmids for AAV1, 2, 3B, 4, 5, 6, 8, 9 that can accommodate clients GOI.
- Universal cap primers and a serotype specific probe enable duplex ddPCR for various AAV serotypes.

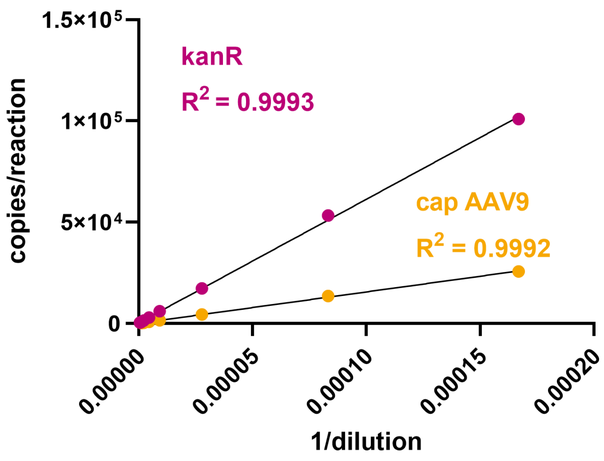
Method development

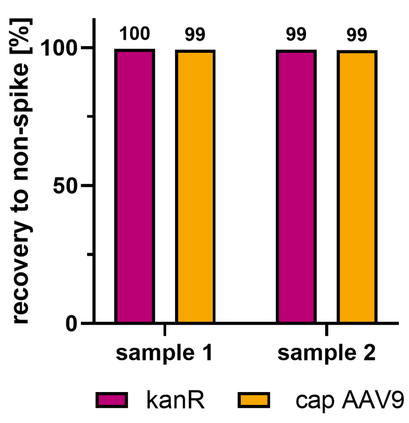
Comparing singleplex ddPCR with duplex ddPCR



- Method development was performed with an AAV9 specific duplex assay.
- kanR is detected in all plasmid and rAAV samples.
- Specificity for cap is demonstrated with AAV9 positive (AAV9+) and negative (AAV9-) plasmids and rAAV samples.
- Singleplex and duplex ddPCR generate comparable results.

Method qualification according to ICH Q2 (R2)





Linearity is demonstrated over a working range of up to four logs.

Specificity is demonstrated by rAAV samples spiked with 500 pg gDNA of the production host cell.

Parameter	kanR	cap
Repeatability	≤ 2% CV	≤ 2% CV
Intermediate precision	≤ 4% CV	≤ 5% CV
Accuracy	93% - 99 %	93% - 99%

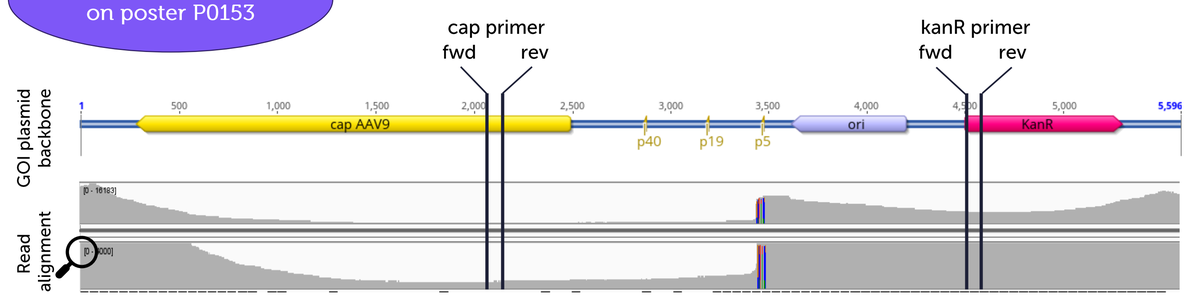
Precision is demonstrated by repeated measurements of the same sample, varying operators, days, devices, etc.

Accuracy is demonstrated by a plasmid sample of a known concentration.

The duplex ddPCR for the dectection of kanR and cap impurities in rAAV samples is a reliable method with high precision and specificity.

Nanopore sequencing complements primer design

Detailed NGS data on poster P0153



- Nanopore (Oxford Nanopore Technologies) sequencing alignment for the backbone of a GOI plasmid used for the rAAV9 production in a 50L bioreactor.
- Universal cap primers are designed to recognize the same position of mispackaged fragments in the 5' region of different serotypes.
- KanR (nptII) primers detect 5' region including the ATG start codon.
- In-depth characterization of rAAV samples verified ddPCR results.

AAV serotypes covered by the duplex assay

Serotype	cap/kanR duplex	Serotype	cap/kanR duplex
AAV1	X	AAV6	X
AAV2	X	AAV8	X
AAV3B	X	AAV9	X
LK03	X	hu37	X
AAV5	X	rh10	X

Summary

Here, we demonstrate the development, qualification and application of a ddPCR assay to analyse cap and kanR impurities for different rAAV serotypes in a single assay. Our universal cap primer and probe design detects common serotypes as well as any engineered capsids carrying the respective target sequences. Since the primer/probe sets are designed to recognize the same position of mispackaged cap fragments, our approach guarantees a fair comparison of impurity data between different serotypes and/or production platforms.
The generic background of the assay allows an accelerated product specific validation to apply the duplex ddPCR as a QC release assay quantifying the two plasmid derived impurities at once. This enables a faster turnover of results, and costs as well as sample volumes needed for rAAV batch release are reduced.

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