Host-cell DNA impurity sizing in rAAV by an 18S rRNA gene-based ddPCR approach

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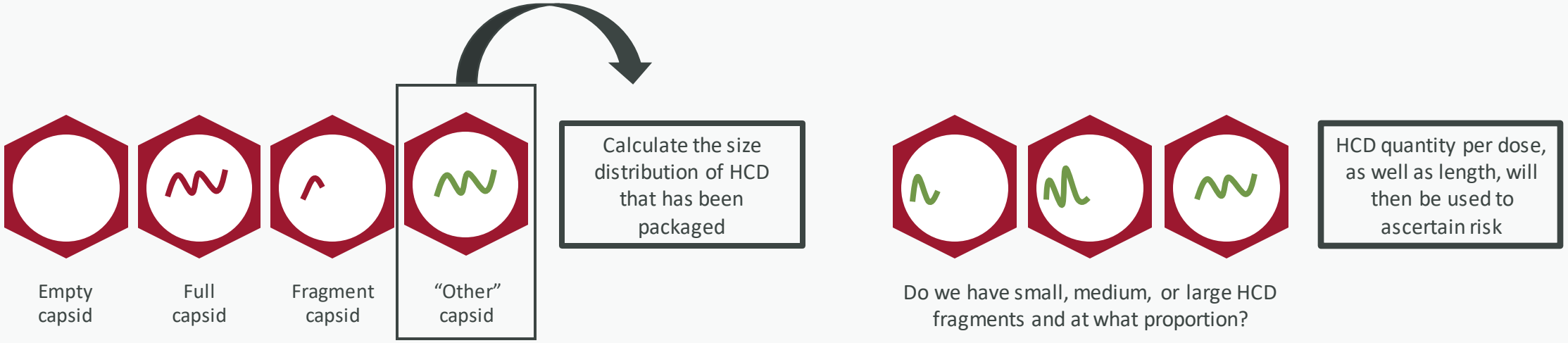
Host-cell-derived DNA (HCD) impurities are an undesirable byproduct of vector manufacturing

- HCD is comprised of heterogeneous fragments of DNA derived from the producer cell line and is partially packaged into AAV capsids
- The potential expression of oncogenes, immunogenic polypeptides, or other potentially harmful DNA sequences could be a risk to patients who receive rAAV gene therapy
- Regulatory authorities require characterization of the quantity and size of HCD impurities to support an assessment of the risk to patient safety



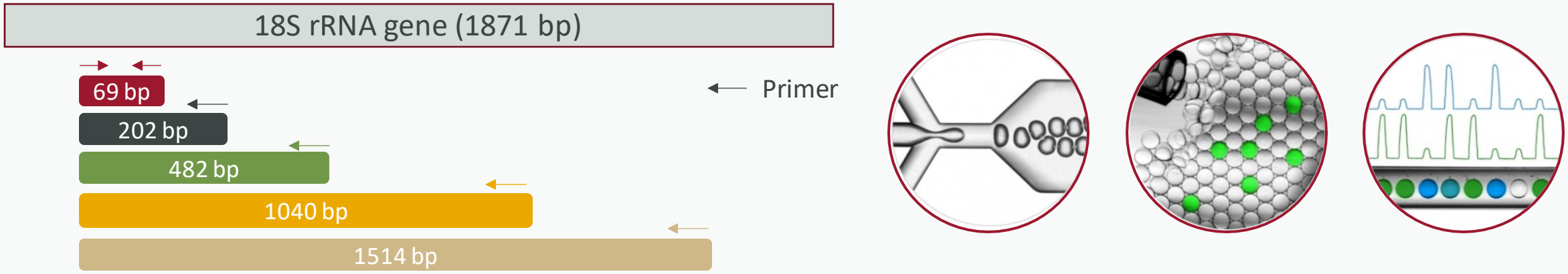
AAV, adeno-associated virus; HCD, host-cell-derived DNA; rAAV, recombinant adeno-associated virus

The 18S rRNA gene locus as surrogate gene for HCD sizing

- Since heterogeneity of HCD makes testing of all potential nucleic acid sequences a challenge, high-copy-number genes or repeat elements are commonly used as surrogates for analysis
- The 18S rRNA gene (1871 bp) integrated in the 45S ORF is present 50 to >800 times in diploid cells

Method

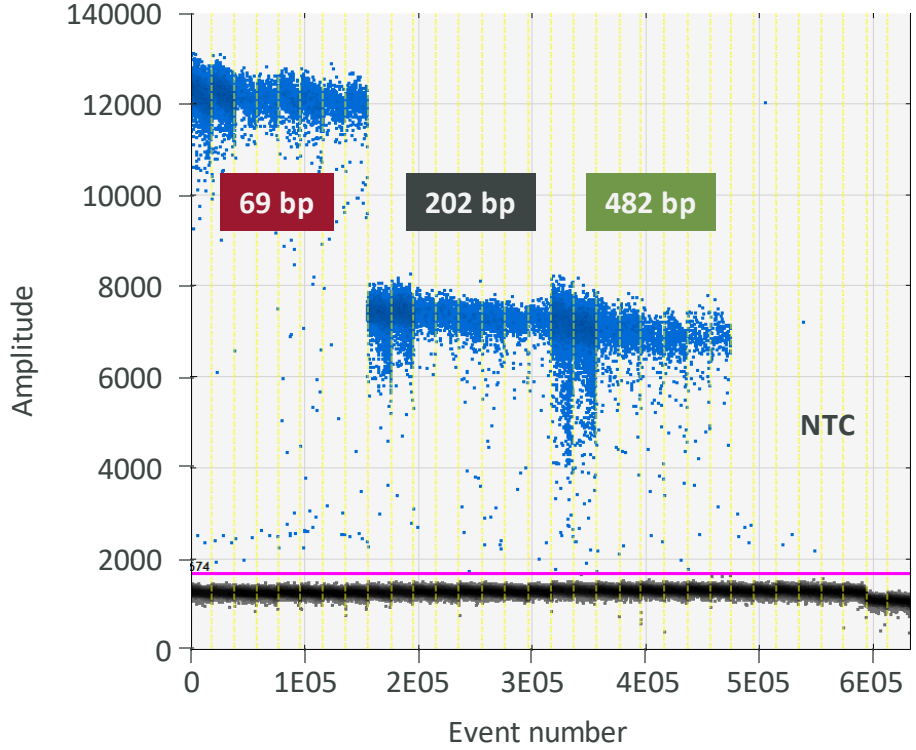
- In contrast to qPCR, ddPCR is less dependent on amplification efficiency
- This makes it suitable to assess larger fragments by a singleplex or duplex approach

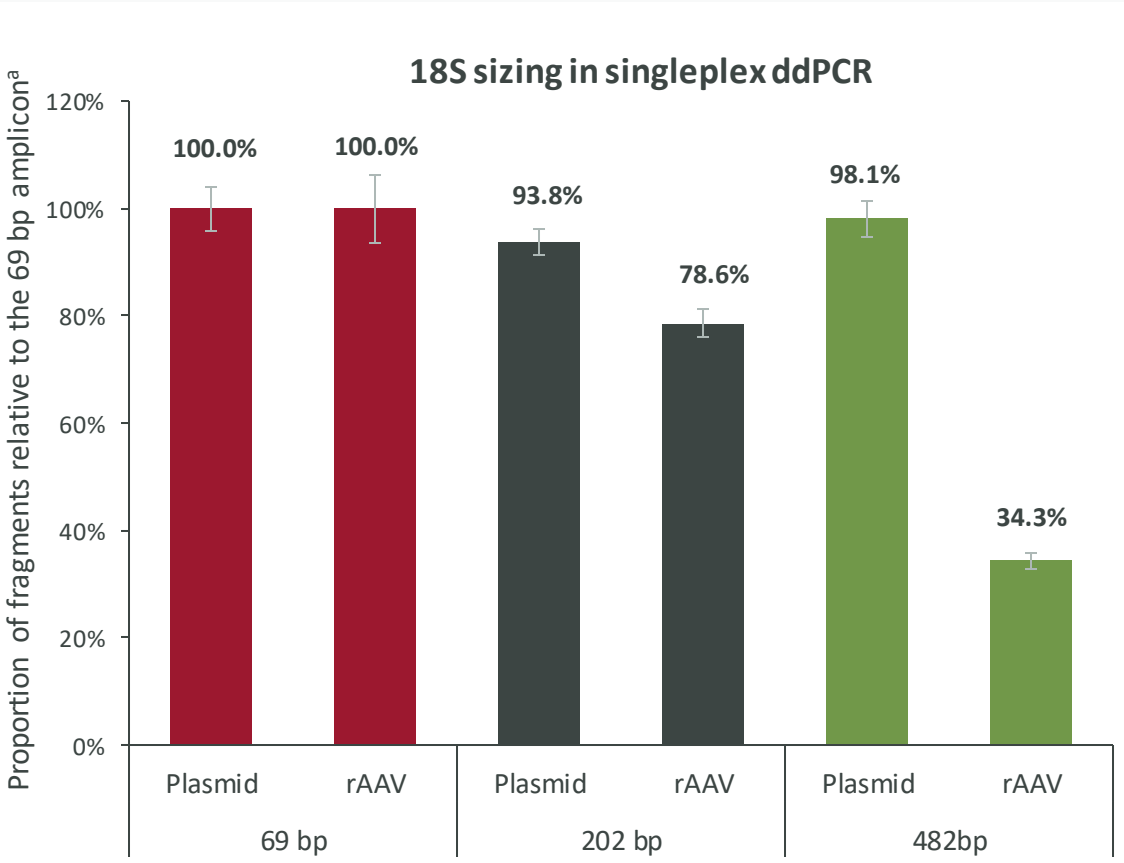


Images taken from: https://link.springer.com/article/10.1208/s12248-021-00560-6/

bp, base pairs; ddPCR, droplet digital polymerase chain reaction; HCD, host-cell-derived DNA; ORF, open reading frame; qPCR, quantitative PCR; rRNA, ribosomal RNA

Good recovery for full-length 18S rRNA plasmid confirms suitability of singleplex ddPCR to assess fragments up to 500-600 bp





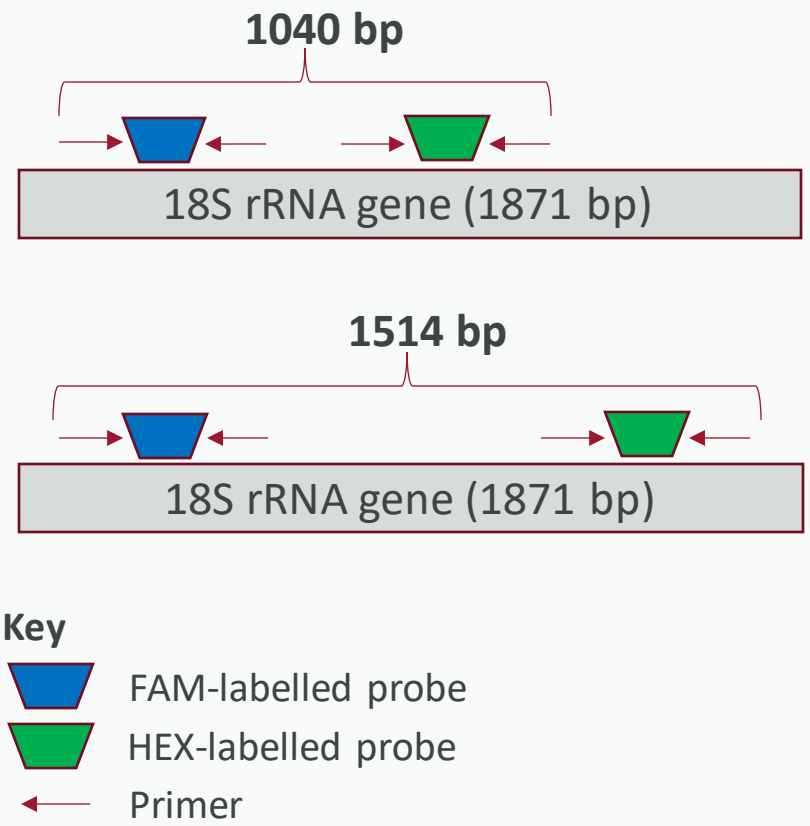
[a]Data for the 202 and 482 bp amplicons are normalized to the concentrations determined for the 69 bp amplicon of the plasmid or the rAAV respectively
bp, base pairs; ddPCR, droplet digital polymerase chain reaction; NTC, no template control; rAAV, recombinant adeno-associated virus; rRNA, ribosomal RNA

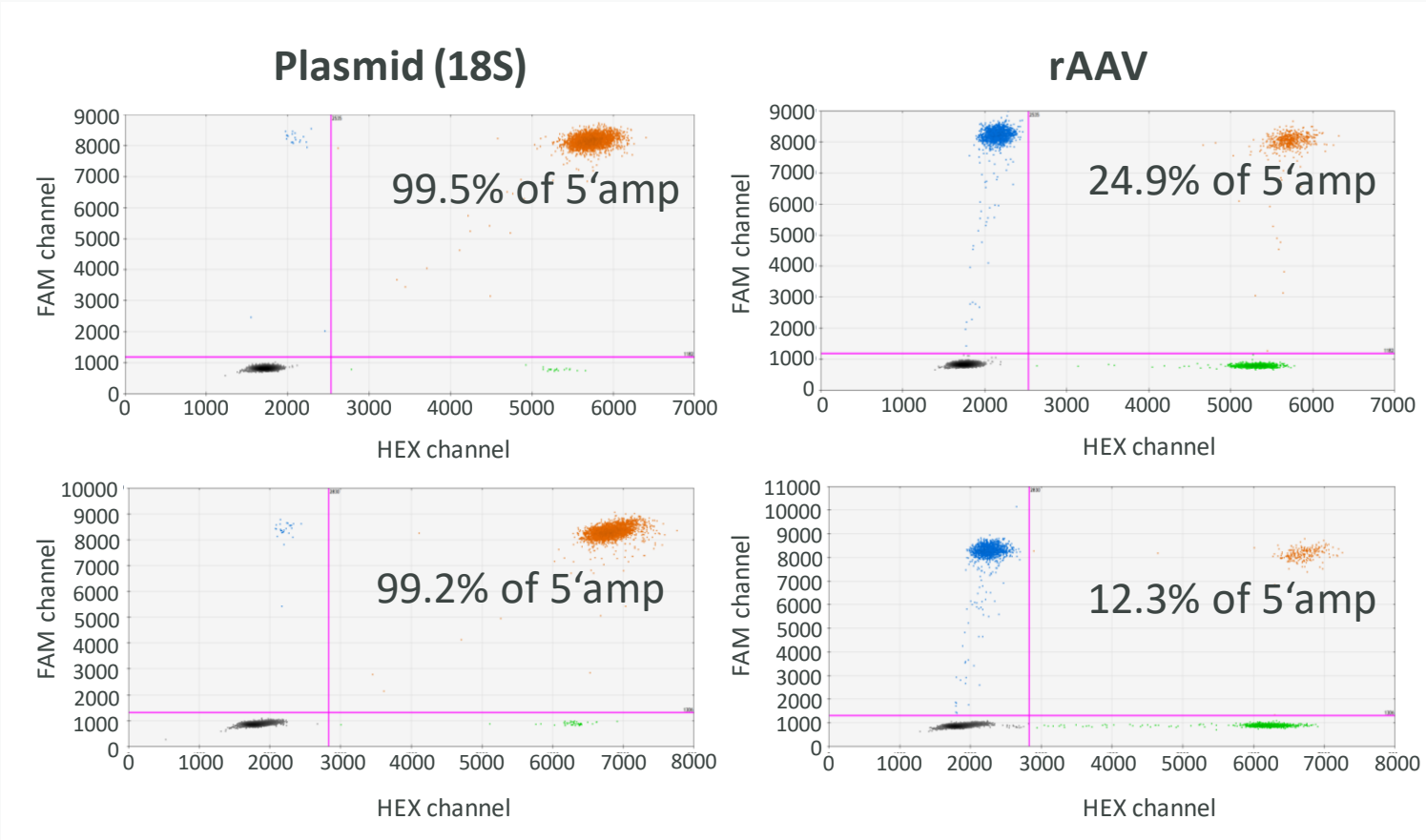
18S rRNA gene singleplex ddPCR meets acceptance criteria to assess fragments of 69 bp, 202 bp, and 482 bp

Parameter	Acceptance criteria	Result	Status
Specificity	Criteria for each amplicon size (used spike P-150): • 80–20% recovery compared to non-spiked sample • %CV ≤15% (each dilution and overall CV) • No positive droplets (≤4 copies/reaction) in non-spiked/spiked NTC	 • 90.9–105.3% • 2.3–11.1% • ≤4 copies/reaction in all spiked and non-spiked NTCs	**Passed**
Intra-assay precision (repeatability)	• %CV of sample preparation mean values ≤15% for each amplicon size • %CV of sample preparation mean proportion normalized to the 69 bp amplicon ≤15% for each amplicon size	• 5.6–9.1% • ≤2.1%	**Passed**
Inter-assay precision (intermediate precision)	• %CV of assay mean values ≤30% for each amplicon size • %CV of assay mean proportion normalized to the 69 bp amplicon ≤30% for each amplicon size	• rAAV1: 6.4–8.6% • rAAV2: 2.2–4.1% • rAAV1: ≤3.3% • rAAV2: ≤5.5%	**Passed**
Linearity	• R^2 ≥0.98 for each amplicon size • %CVs of the mean ≤15% over all dilutions for each amplicon size	• Plasmid: 0.9998–1 • rAAV: 0.9997–0.9999 • Plasmid: 4.4–6.0% • rAAV: 1.9–3.1%	**Passed**
Limit of quantification	• Lowest concentration at which recovery of plasmid is 80–120% and CV is ≤15% for each amplicon size compared to NanoPhotometer measurement	• LOQ: 80 copies/20 µL reaction	**Passed**
Accuracy	• 80–120% of target concentration for each amplicon size • CV ≤15% for replicates at each amplicon size	• 85.9–96.1% • 1.1–7.7%	**Passed**

bp, base pairs; CV, coefficient of variation; ddPCR, droplet digital polymerase chain reaction; LOQ, limit of quantification; NTC, no template control; rAAV recombinant adeno-associated virus; rRNA, ribosomal RNA

Using duplex ddPCR, droplets that are positive for both fluorophores indicate the presence of 18S rRNA gene sequence fragments

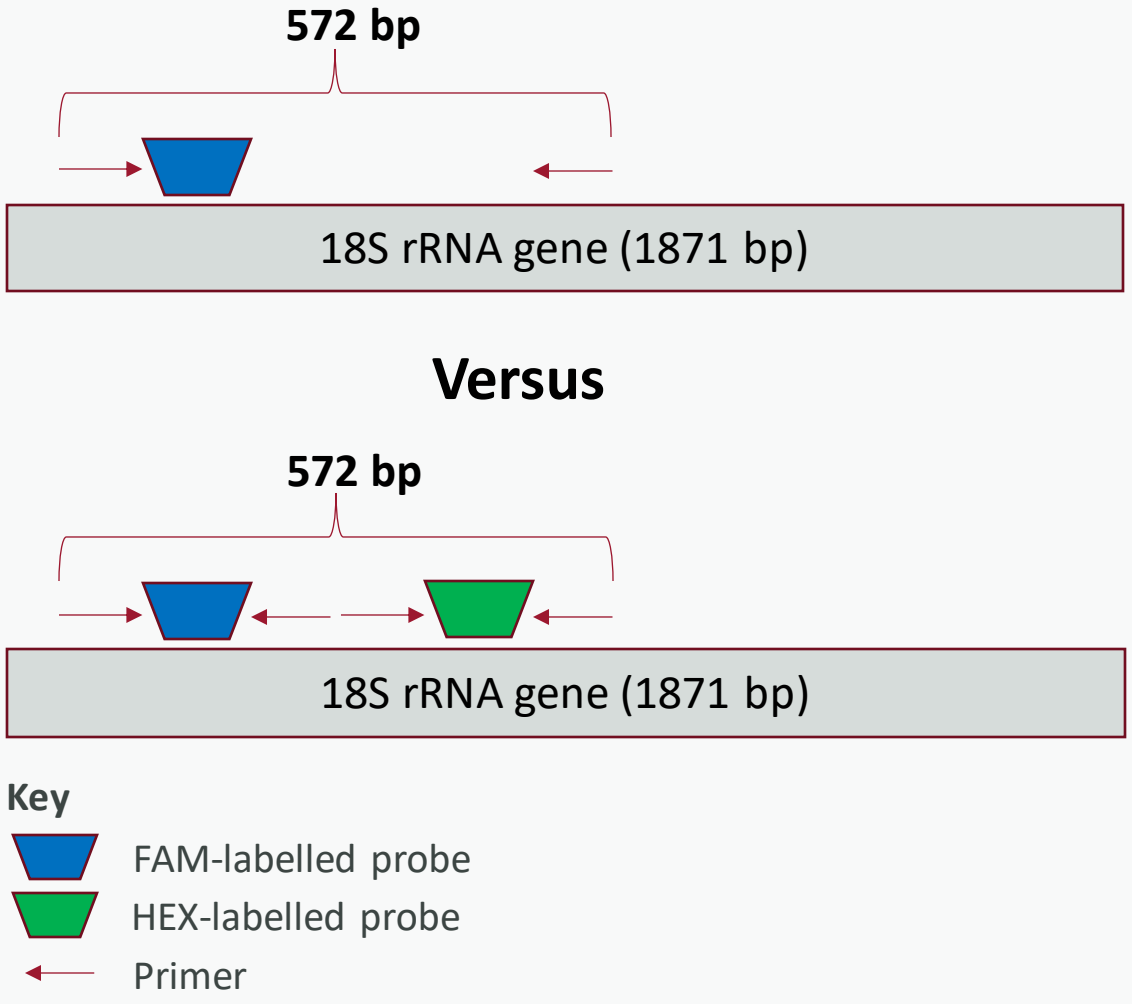


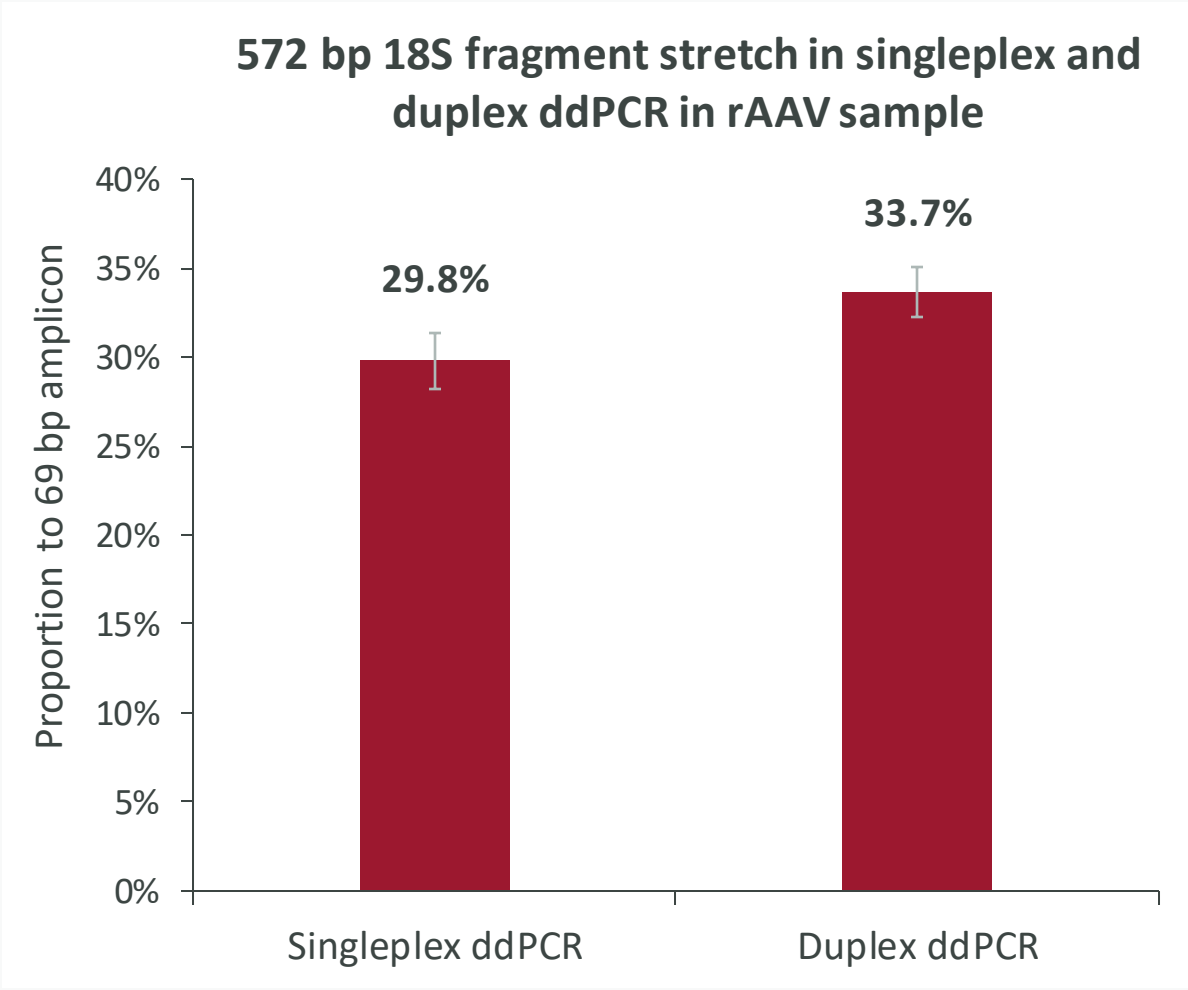


5’amp represents the 69 bp amplicon

bp, base pairs; ddPCR, digital droplet polymerase chain reaction; FAM, fluorescein; HCD, host-cell-derived DNA; HEX, hexachlorofluorescein; rAAV, recombinant adeno-associated virus; rRNA ribosomal RNA

572 bp fragment shows comparable results in singleplex and duplex ddPCR

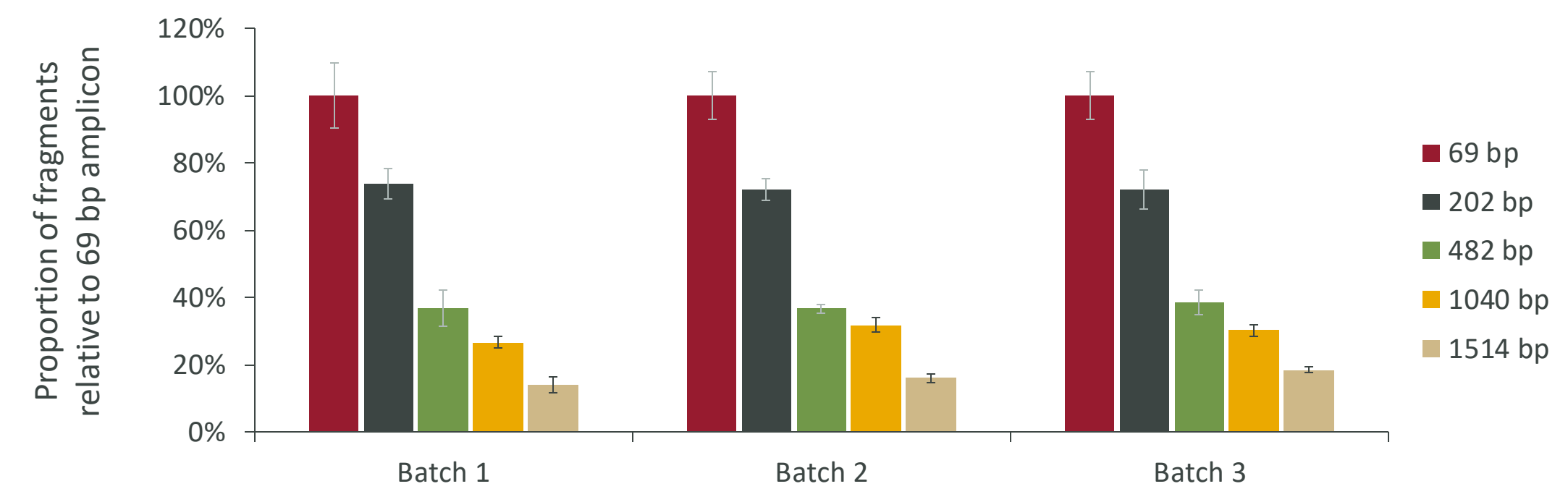




bp, base pairs; ddPCR, droplet digital polymerase chain reaction; FAM, fluorescein; HEX, hexachlorofluorescein; rAAV, recombinant adeno-associated virus

Three large-scale batches of one rAAV product manufactured using our iCELLis® production platform indicate consistent sizing profiles

- HCD packaging frequency decreases as the size of the fragment increases



- Observed sizing profile agrees with long-read next-generation sequencing data that have been generated for one rAAV batch

bp, base pairs; HCD, host-cell-derived DNA, rAAV, recombinant adeno-associated virus

Conclusions

- The method described here meets regulatory requirements for analysis, in a quality-controlled environment, of the sizing of packaged HCD impurities
- The packaging length of the 18S gene may not be completely representative for the whole genome. However, the assay enables the assessment of the effects of different process parameters on the HCD impurity sizing profile
- The data generated from this method can form part of a comprehensive assessment of the potential risk to patients from packaged HCD

HCD, host-cell-derived DNA