Extraction-free ddPCR quantification of host-cell derived E1A impurities in rAAV samples

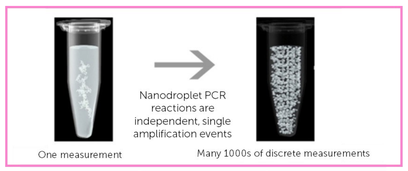
S Beer, P Zanotti, T Klötzler, F Thoennissen, S Geiger, S M Schermann, R Kober

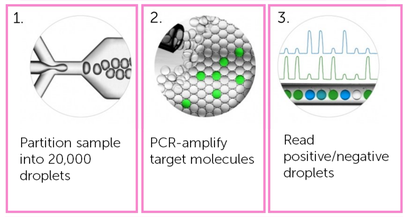
Poster 1842

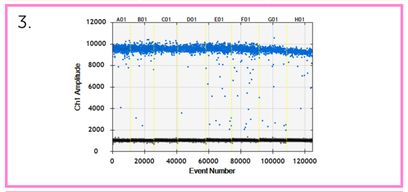
Abstract

Packaged residual DNA impurities in recombinant adeno-associated virus (rAAV) products arise during manufacturing and pose a risk to the patient safety, product efficacy and persistence of gene therapy. In production processes using HEK293 cells, the integrated adenoviral E1A gene is a potential contaminant from host cell DNA with cell-transforming potential. Therefore, the level of contaminating E1A is a critical quality attribute for AAV produced in HEK293.
Ascend has developed and qualified a ddPCR for the absolute quantification of E1A copy numbers in rAAV batches using sequence-specific primers and probe. While most currently used release methods require DNA extraction to achieve the required sensitivity, the ddPCR method developed and presented here enables analysis without any sample pre-treatment or DNA extraction. Thus, our E1A ddPCR method avoids experimental errors due to insufficient or poor DNA extraction and saves sample volume and time while maintaining high sensitivity. It was developed for purified rAAV samples such as drug substance and drug products, allowing very low sample dilutions without matrix interference.

Droplet digital PCR





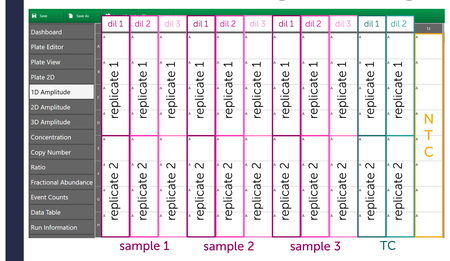


→ Absolute quantification using Poisson statistics

- End point assay
  - Digital (yes/no) read out
  - No requirement for standard curves, simplifying the assay, reducing costs & eliminating the need for bridging
- Less dependent on amplification efficiency – superior resistance to matrix interference/inhibitors

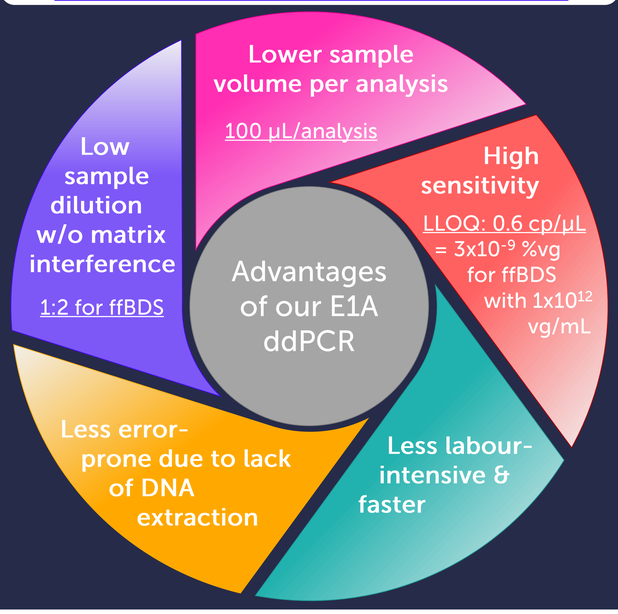
Adapted from: Bulletin_6407.pdf (bio-rad.com)

Rare event detection with the QX Manager "Merge" function



1. Positive and negative droplets from four wells are added by the software
2. Poisson statistic on more droplets for the absolute quantification

→ Increased sensitivity and robustness for low concentration samples



Qualification parameters & results for the E1A ddPCR based on ICH Q2(R2) (rAAV9 ffBDS, 200L scale EpyQ® platform)

Range

Linearity & LLOQ
- At least 5 consecutive dilutions
- $r^2 \geq 0.98$
- Dilution recovery to lowest dilution 80-120%
- CVs ≤ 20% of dilutions

Results - Sample Linearity
- rAAV ffBDS
- 8 valid dilutions
- $r^2 = 1.00$
- CVs ≤ 20%
- 91 – 108% recovery
- LLOQ: 0.6 copies/µL

Results - Plasmid Linearity
- Linearized plasmid
- 5 valid dilutions
- $r^2 = 1.00$
- CVs ≤ 14%
- 92 - 102% recovery
- LLOQ: 2.0 copies/µL

Repeatability

- 9 determinations (3 samplings, 3 concentrations each)
- Data from 1 experiment
- CV ≤ 20% across 9 determinations

Result
- CV = 3%

Intermediate precision

- 4 samplings
- 3 concentrations each
- Data from 4 experiments
- CV ≤ 20% of mean values across all sample measurements

Result
- CV = 3%

Robustness

General
- 2 Operators
- Equipment
  - ➢ 2 droplet generators
  - ➢ 3 cyclers
  - ➢ 3 readers
- Days of assay performance
- Supermix lots
- CV ≤ 20% of TC mean values across experiments

Result
- CV (6 experiments) = 3%

Assay specific
- Storage of droplets at RT for 90 min before cycling
- Recovery to ref. 80-120%
- Sample CV ≤ 20%

Result
- 94% recovery to ref.
- Sample CV = 8%

Specificity

- Spiking of rAAV sample with production plasmid to mimic DNA impurities
- Recovery to non-spiked control 80 - 120%
- CVs ≤ 20% (dilutions & overall)

Result
- 97-101% recovery
- CVs ≤ 8%

Accuracy

- Recovery of plasmid copies 80-120% compared to orthogonal spectrophotometric measurement
- CVs ≤ 20% (dilutions & overall)

Result
- 92 – 102% recovery
- CVs ≤ 14%

LLOQ – lower limit of quantification; CV – coefficient of variation; ffBDS – filtered, formulated bulk drug substance; TC – trending control; RT – room temperature; min – minutes; ref. - reference

Summary

Based on ICH Q14, we have developed an E1A ddPCR for rAAV drug substance (DS) and drug product (DP) and qualified the method according to ICH Q2(R2). Our E1A ddPCR assay exhibits high precision, accuracy and sensitivity across 5 AAV serotypes analyzed. It enables product-specific validation with very limited effort and can be applied as a robust and accurate drug substance and drug product release assay in line with regulatory expectations. Analysis of an ffBDS produced in 200L scale revealed an E1A impurity of 5.6 x 10^{-7} % vg and confirms the high quality of AAV produced with our inhouse EpyQ production platform.

Aim higher

Ascend Advanced Therapies GmbH | Fraunhoferstraße 9b | 82152 Planegg | Germany

www.ascend-adv.com
sabine.geiger@ascend-adv.com