Qualification of an mRNA expression assay for GOI, stability insights, and process evolution detection

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Poster 0097

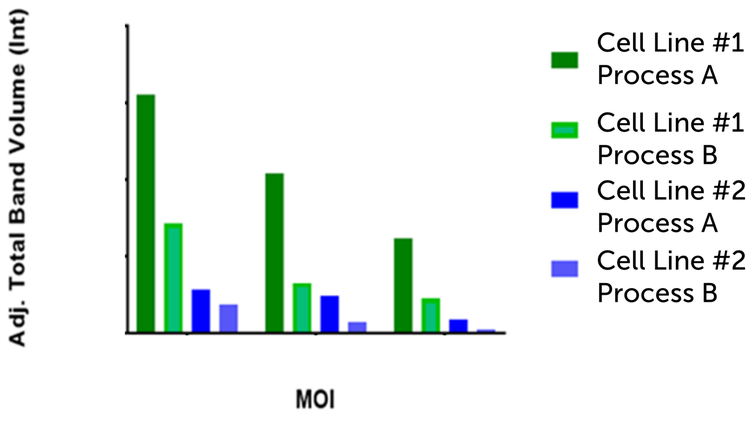
Abstract

Potency assays for clinical gene therapy products are multifaceted, requiring continuous efforts throughout preclinical and clinical phases. Gene therapy potency measurements face many unique challenges including transduction, transcription, translation, protein modification, cellular localization, and ultimately, protein function. A potency test, along with several other tests, assesses product conformance: release testing, stability programs and comparability studies when manufacturing changes are made. Potency tests are critical to measure rAAV product attributes such as quality, identity, purity, strength, and stability. Here, we report the establishment of a sensitive and specific in vitro cell-based assay to measure mRNA expression from our client's ophthalmic gene therapy vector, using multiplexed qPCR. Measurement of mRNA expression after transduction with our AAV vector was achieved in three phases: cell transduction, cell harvesting/RNA isolation, and detection via gene-specific RT-qPCR. We successfully optimized this gene expression assay and qualified the assay for use as a functional potency assay. Qualification evaluated the assay's sensitivity, specificity, accuracy, precision, RT-qPCR linearity, and total assay linearity. The abstract presents an overview of a study conducted over a period of time, focusing on the analysis of mRNA expression levels across various projects. Data collected encompassed assessments of stability, process comparability, and device compatibility. Through trending analysis, this research aimed to discern any alterations in mRNA expression patterns and derive meaningful conclusions.

mRNA Expression Assay Overview

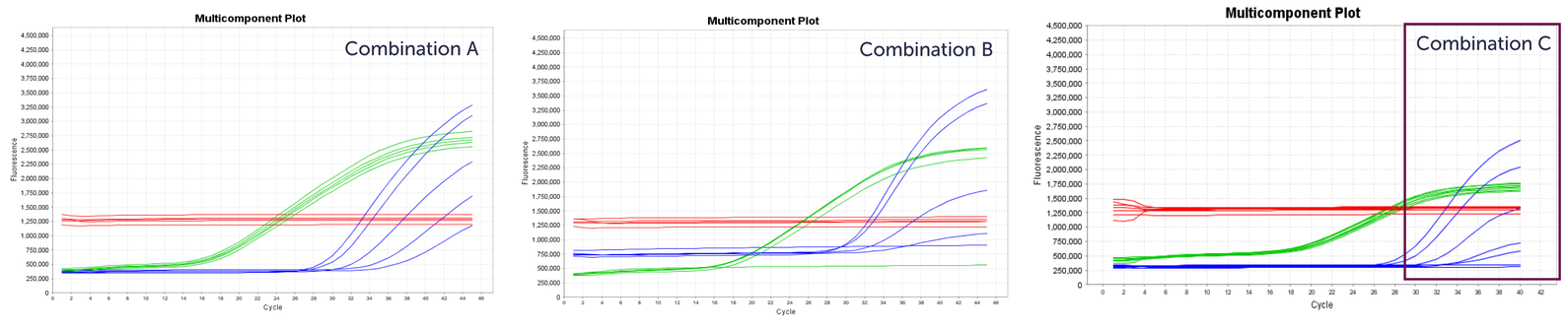
Cell seeding	Cell transduction	RNA isolation	1 Step RT-qPCR
Seed 20,000 cells/well in 96 well plates	Co-transduction of human Ad5 + GOI Vector at 5 MOI's	Extraction with RNeasy Mini Plus Kit.	Multiplex qPCR of GOI and GAPDH cDNA

Comparison of Protein Expression



- A western blot was used to semi-quantitatively assess *in-vitro* protein expression. Cell Line #1, a transgenic cell line and an improvement in manufacturing process (Process A), provided greater protein expression.

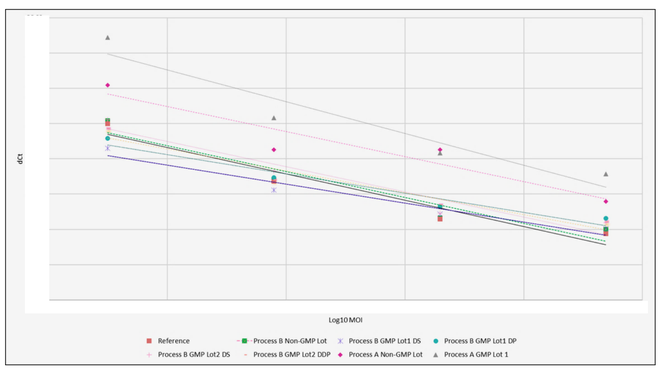
Screening Primer & Probe Designs



Eight novel assay designs were screened using 2 unique forward primers, 4 unique reverse primers, and 3 unique probes in various combinations using the gblock. In all assays, the forward primer spans the 5' SV40 splice site and GOI to confer specificity to the assay. Assay designs were evaluated based on the efficiency of amplification, robustness of amplification (observed on multicomponent plot), and specificity (lack of amplification in non-transduced cells). Based on the data from this experiment, designs Combination A, Combination B and Combination C were identified as the best performing primer probe combinations. The three combinations were further tested across MOI range and No RT (reverse transcriptase reactions) to confirm robust amplification of the analyte. Combination C had superior specificity, which was especially evident at the higher MOI's.

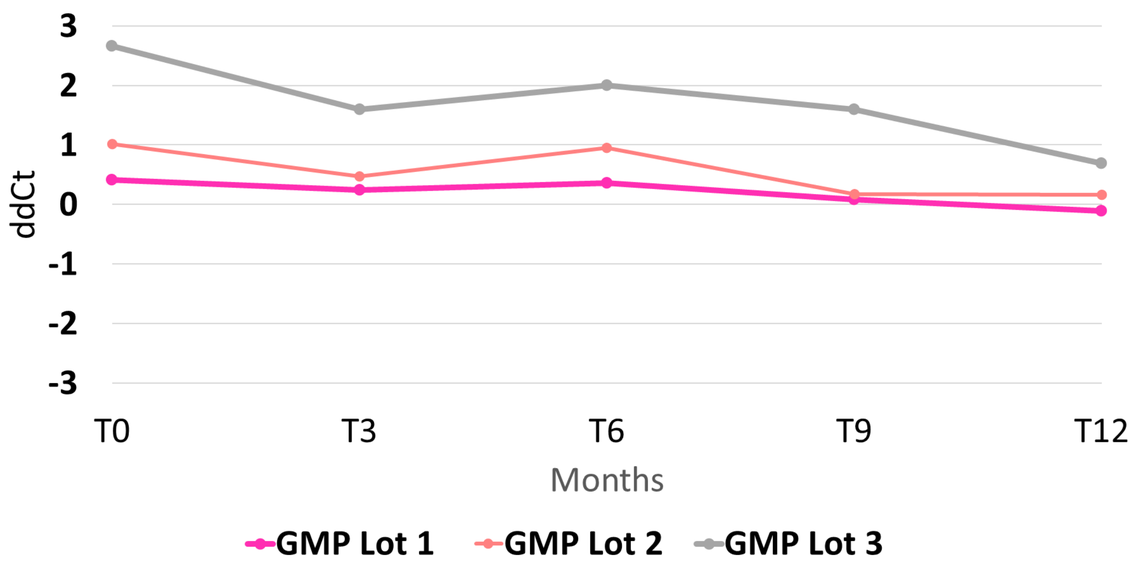
Process Comparability

Parameters	Results
Precision	Intra-assay: dCt %CVs ≤8 % Inter-assay: dCt %CVs ≤ 14%
Efficiency	%E = 98.03%
Linearity	R^2 = 0.9617
Specificity	NTC, and NRT control Cts all >39 (both targets); Non-transduced, Ad5 only Cts all >39
Sensitivity	Limit of Blank dCt = 15.83-19.88
Dynamic Range	1e5 to 1.6e2 MOIs



- The expression assay was successfully qualified testing different parameters per the ICH2 (R2) guidelines.
- All parameters passed the validity criteria and was deemed fit for purpose.
- The expression assay was used to compare mRNA expression levels of different lots made by Process A and Process B. A difference between the two processes was seen, however between different lots manufactured with the same process, results were consistent.

mRNA Expression of GOI with Different GMP lots over time



- Stability of mRNA expression of different GMP lots was assessed over a year.
- Variations <2 ddCT were seen amongst the different lots overtime and is regarded as insignificant, deeming the lots stable over the screened timepoints.

Summary

Potency assays play a crucial role in evaluating the effectiveness and reliability of pharmaceutical products. Among these assays, measuring expression levels is essential for assessing lot-to-lot comparability, stability, and overall efficacy. These assessments ensure that each batch of a product meets consistent quality standards, thereby reinforcing the product's safety and therapeutic effectiveness. One of the main requirements of assay development, is the selection of a cell line capable of providing enough mRNA and/or protein expression to be detected by the endpoint analytical method, which in this case was qPCR. Furthermore, once fully developed and qualified, the method was found to be sensitive in lot-lot comparability and assessment of stability of the drug product lot at the specified timepoints. This method was developed and qualified before the development of a potency assay for activity measurement. Please visit **Poster0068** for the development of a potency assay with an activity readout and **Poster 0067** for the usage of these assays to assess potency within a device compatibility study.

Aim higher

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