

Development and qualification of a robust *in vitro* relative potency assay using thaw-and-use cells for AVB-101: A recombinant AAV9 investigational gene therapy for frontotemporal dementia with progranulin mutations

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OBJECTIVE

To develop and qualify an *in vitro* relative potency assay for AVB-101, using the previously generated engineered HEK293 cells with enhanced AAV9 permissibility and CRISPRa-mediated neuronal promoter activation¹.

INTRODUCTION

- AVB-101 is a recombinant AAV9-based investigational gene therapy for frontotemporal dementia with progranulin mutations (FTD-GRN), requiring a qualified relative potency assay for GMP batch release and regulatory compliance.
- Engineered HEK293 cells with enhanced AAV9 permissibility and CRISPRa-mediated neuronal promoter activation¹ showed high variability between a 10-passage range (up to 78% CV), preventing development of a reliable relative potency assay.
- Here we describe the implementation of a thaw-and-use cell banking strategy with the engineered cells to enable the development of a robust relative potency assay.
- In addition, we also present the relative potency assay's pre-qualification results, establishing critical parameters including specificity, precision, accuracy, linearity, and range, while also defining system and sample suitability criteria.

RESULTS

Implementation of thaw and use cells

- When cells were transduced across a range of passages, PGRN expression declined due to heterogeneous dCas9-VPR cell population with variable genomic integration sites (Figure 2) resulting in poor assay robustness (CV: 58-78%) unsuitable for a quality control assay.
- Multiple factors could have contributed to this instability, such as epigenetic silencing, selection pressure favoring less active cells, and cellular defense mechanisms against foreign DNA.
- A "thaw and use" approach was developed as an alternative to a lengthy single-cell cloning procedure, which dramatically improved precision with CV <15% across all conditions (Figure 3).
- Transduction controls (AAV9-CMV-PGRN) showed good reproducibility in both systems, confirming variability stems from dCas9-VPR activation, not the transduction process.
- The "thaw and use" approach then allowed further development of a relative potency assay suitable for QC use (Figure 4).

METHOD

An overview of the method is shown in Figure 1.

Figure 1: Schematic representation of AVB-101 relative potency method

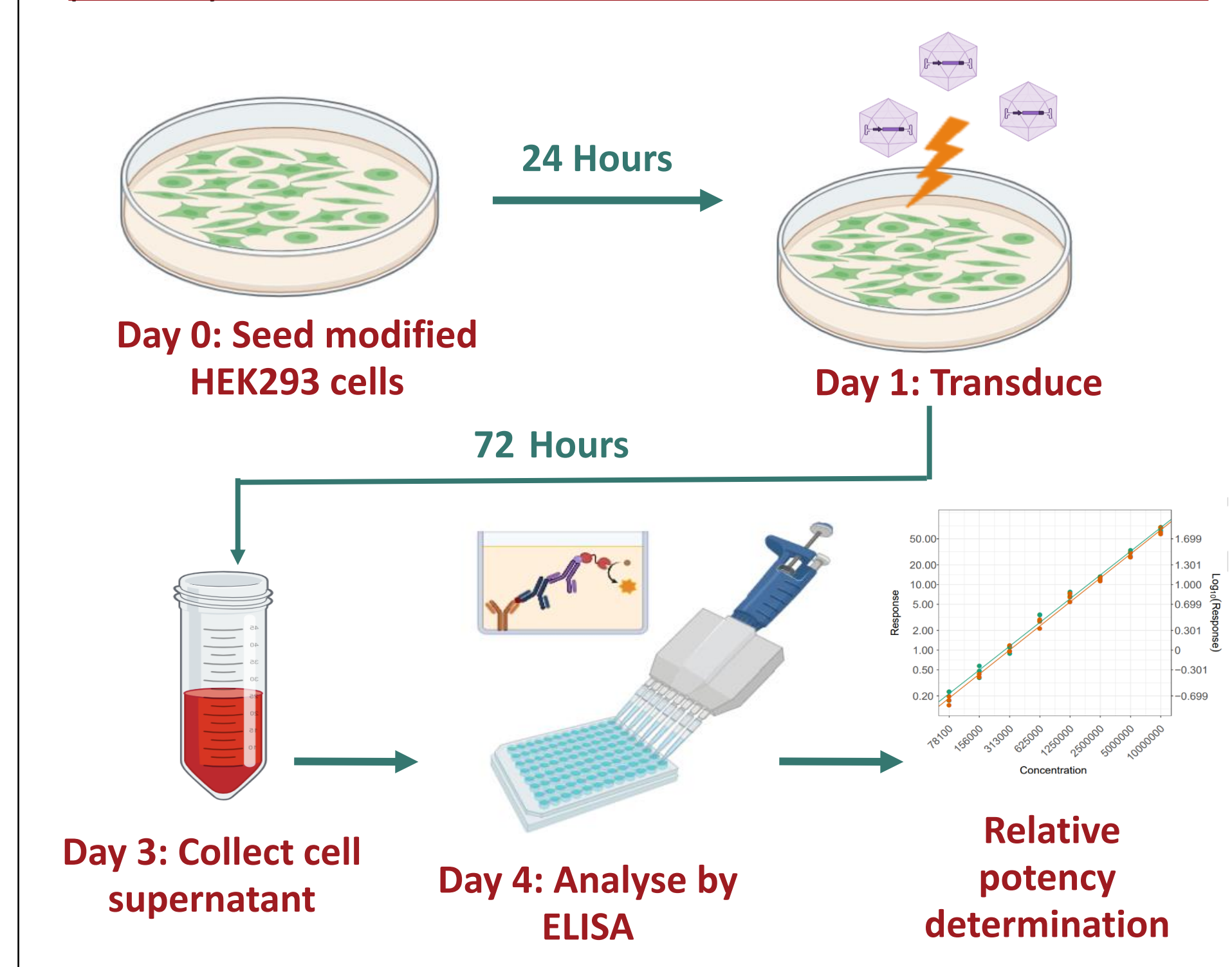


Figure 2: PGRN expression levels from AVB-101 transduced onto modified HEK293 cells at 3 MOIs.

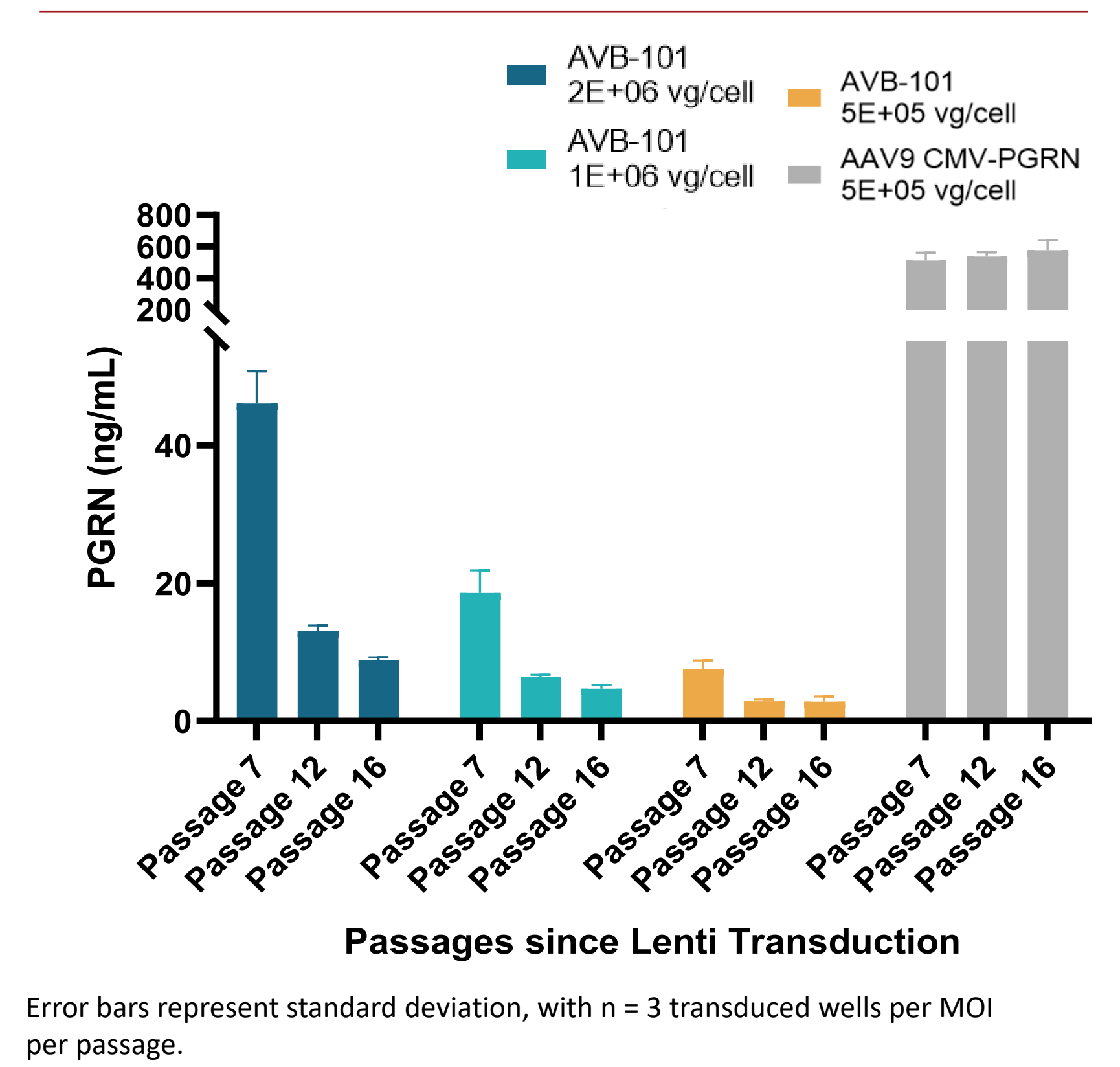


Figure 3: "Thaw-and-use" approach eliminates variability

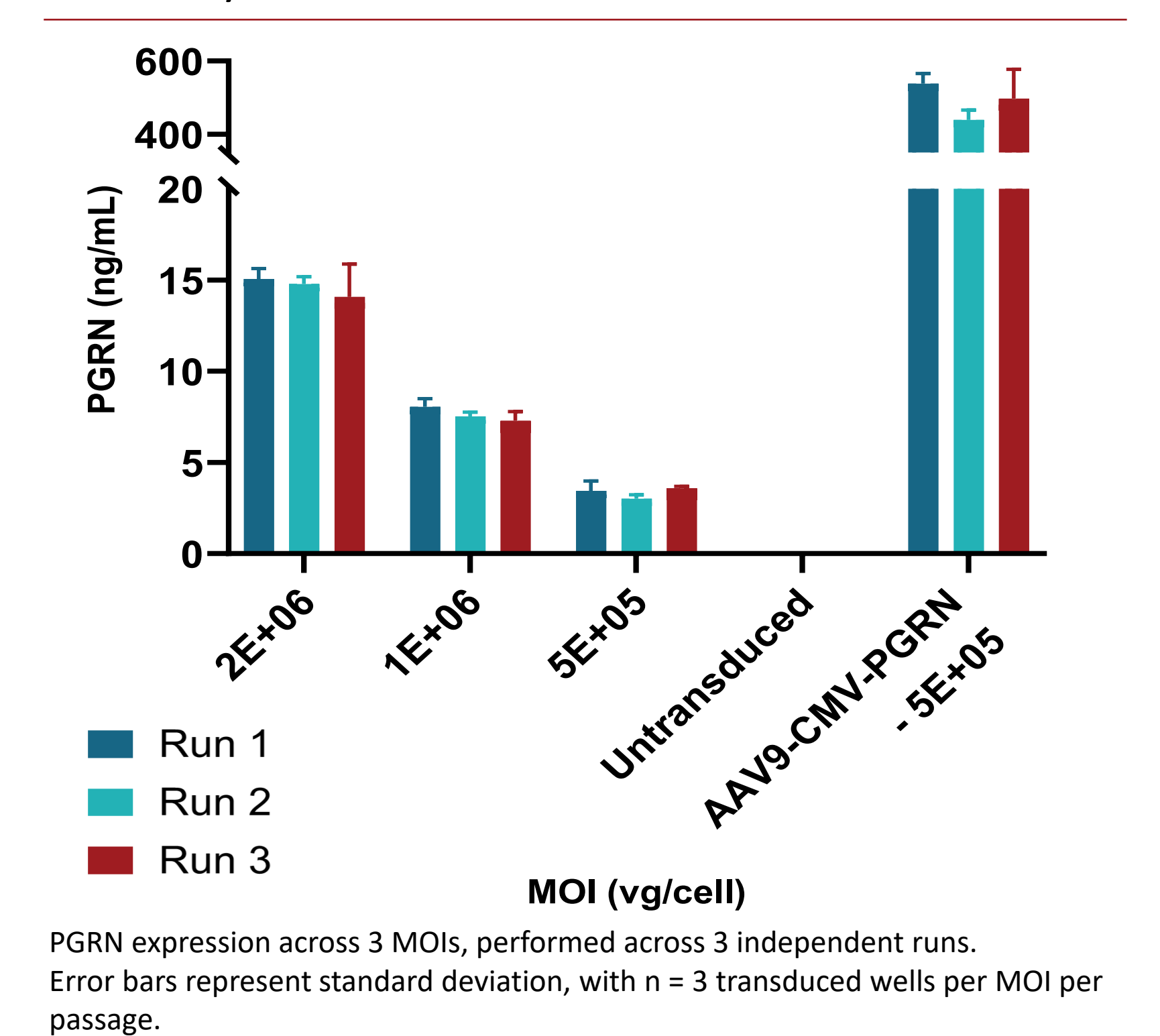
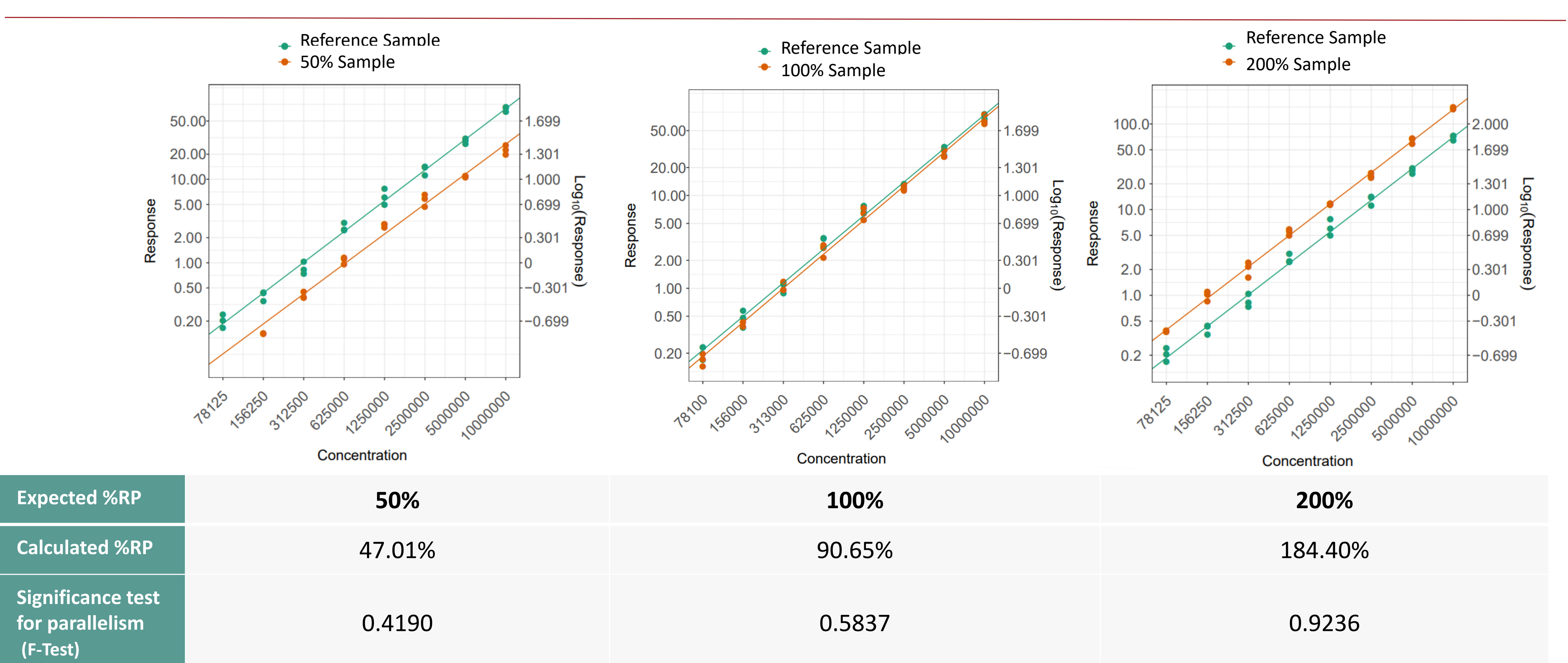


Figure 4: Relative potency assay development



- Assay specificity was confirmed by the absence of signal in untransduced cells and lack of PGRN expression when cells were transduced with an alternative product.
- The assay range demonstrating precision, accuracy, and linearity spans 50-200% relative potency.

Figure 5: Linearity assessment

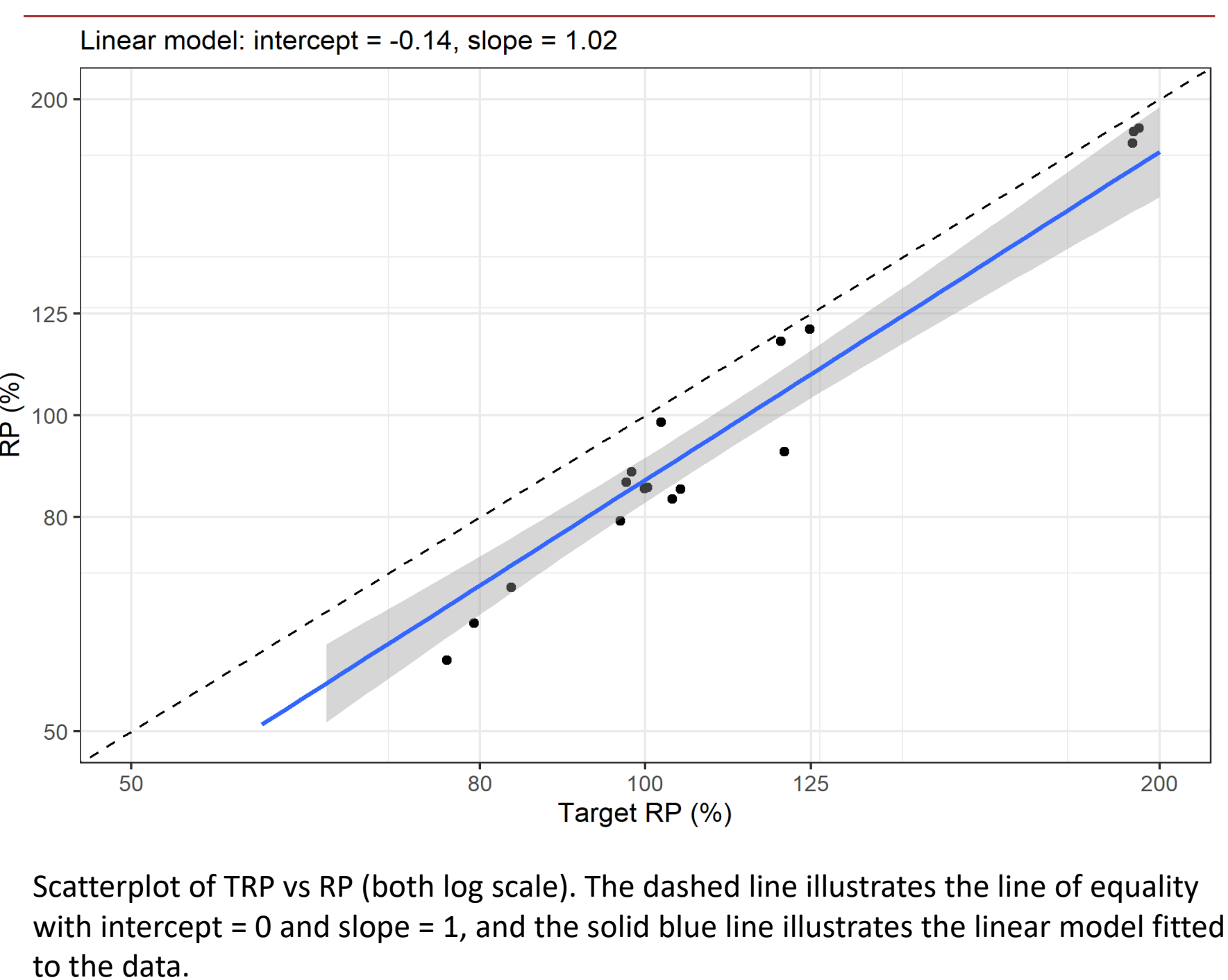


Table 1: Accuracy

Target Relative Potency	Relative Accuracy	
	%RB	90% CI on %RB
50%	-7.4%	(-20.57%, 7.99%)
80%	-20.9%	(-30.87%, -9.43%)
100%	-13.8%	(-17.31%, -10.10%)
125%	-12.5%	(-31.80%, 12.37%)
200%	-7.5%	(-10.16%, -4.79%)

Assessment of accuracy via the relative bias (%RB) at target relative potencies from 50% to 200%.

Table 2: Relative potency precision

Target Relative Potency	Intermediate Precision	Repeatability
	%GCV	%GCV
50%	9.5%	-
80%	8.3%	-
100%	8.4%	4.7%
125%	16.0%	-
200%	1.7%	-

Table 3: Sample batch precision

Test Sample Batch	Relative Potency	Intermediate Precision
	Geomean (95% CI)	%GCV
Batch 1	133.3% (98.8%, 179.9%)	12.8%
Batch 2	90.7% (69.3%, 118.7%)	11.4%
Batch 3	98.9% (91.8%, 106.6%)	3.0%

CONCLUSIONS

- Successfully developed a 6-day QC-compatible relative potency assay for neuronal-specific investigational gene therapy with robust reproducibility enabling GMP CRO tech transfer.
- Achieved an assay range of 50-200% relative potency with demonstrated precision, accuracy, and linearity meeting regulatory pre-qualification standards.
- Created transferable "thaw-and-use" cell platform and engineering workflow applicable to other advanced gene therapies targeting the CNS.
- Enables critical batch-to-batch potency comparisons essential for consistent AVB-101 manufacturing control, supporting progression toward ongoing clinical trials for FTD-GRN patients.

REFERENCES: ¹Lyth RL, et al. Poster presentation at ESGCT Annual Congress 2024.

ABBREVIATIONS: AAV9: adeno-associated virus serotype 9; CI: confidence interval; CV: coefficient of variation; dCas9-VPR: deactivated cas9 fused to VP64-p65-Rta; GCV: geometric coefficient of variation; MOI: multiplicity of infection; PGRN: progranulin; RB: relative bias; RP: relative potency; TRP: target relative potency.

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