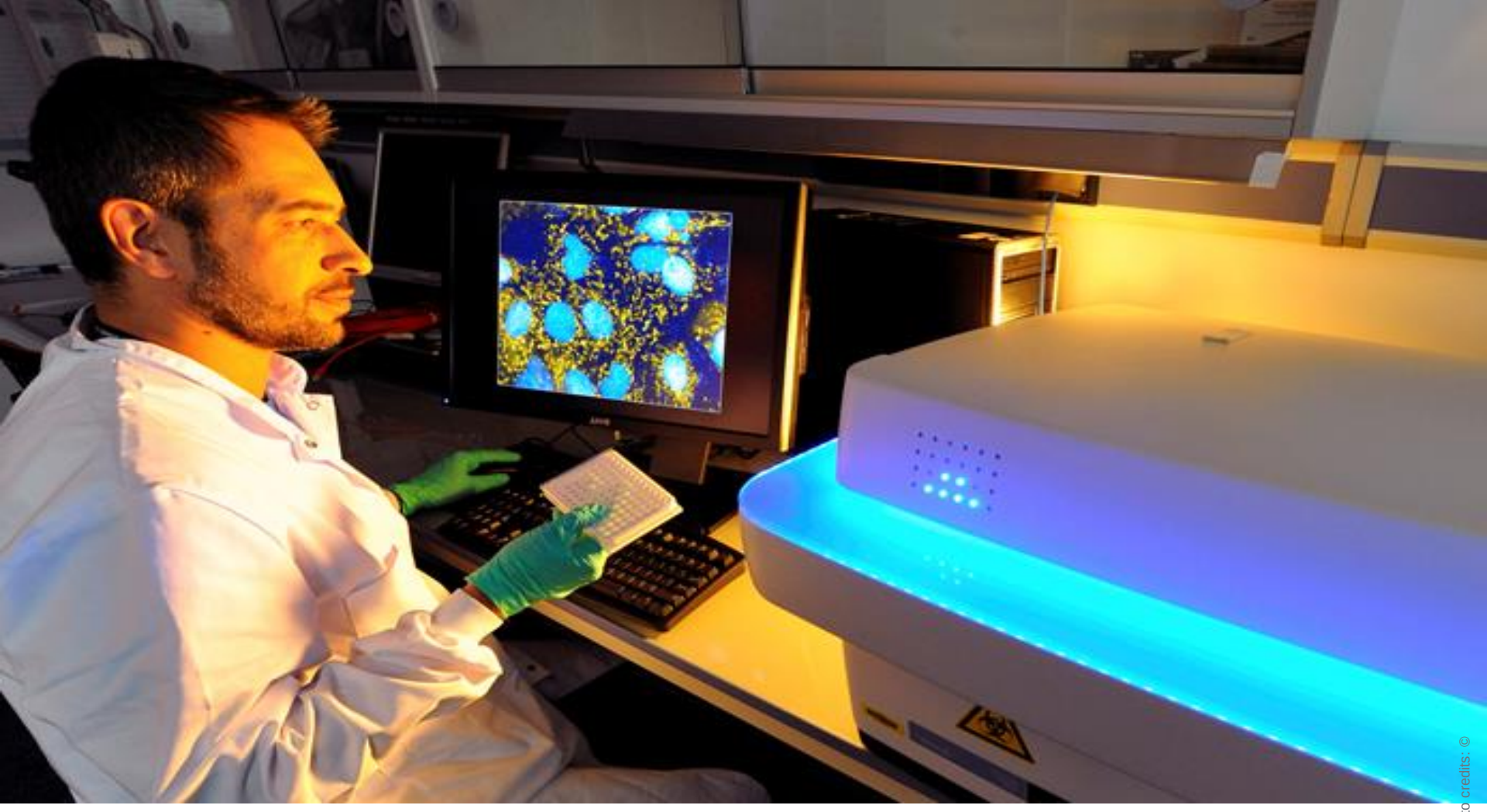


Photo credits: ©

SANOFI

Development and qualification of a potency assay to support gene therapy

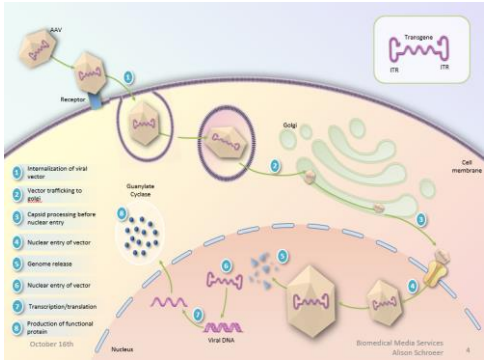
Hannah Maheno, CASSS Bioassay 2020

Outline

- Potency assays for gene therapy 3
- Development Objectives 7
- Cell line engineering 10
- Developing the reporter gene potency assay 13
- Method Crossover strategy 24
- Final Conclusion 26

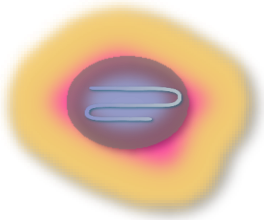
Sanofi Method vs. Industry Requirements

✓ 1) Demonstrates MoA



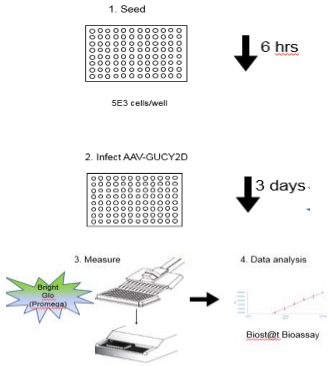
- Mirrors infectivity, gene expression, and generation of functional protein
- Preferably a single assay
- Reflects pre-clinical & clinical response

✓ 2) Relevant cell line



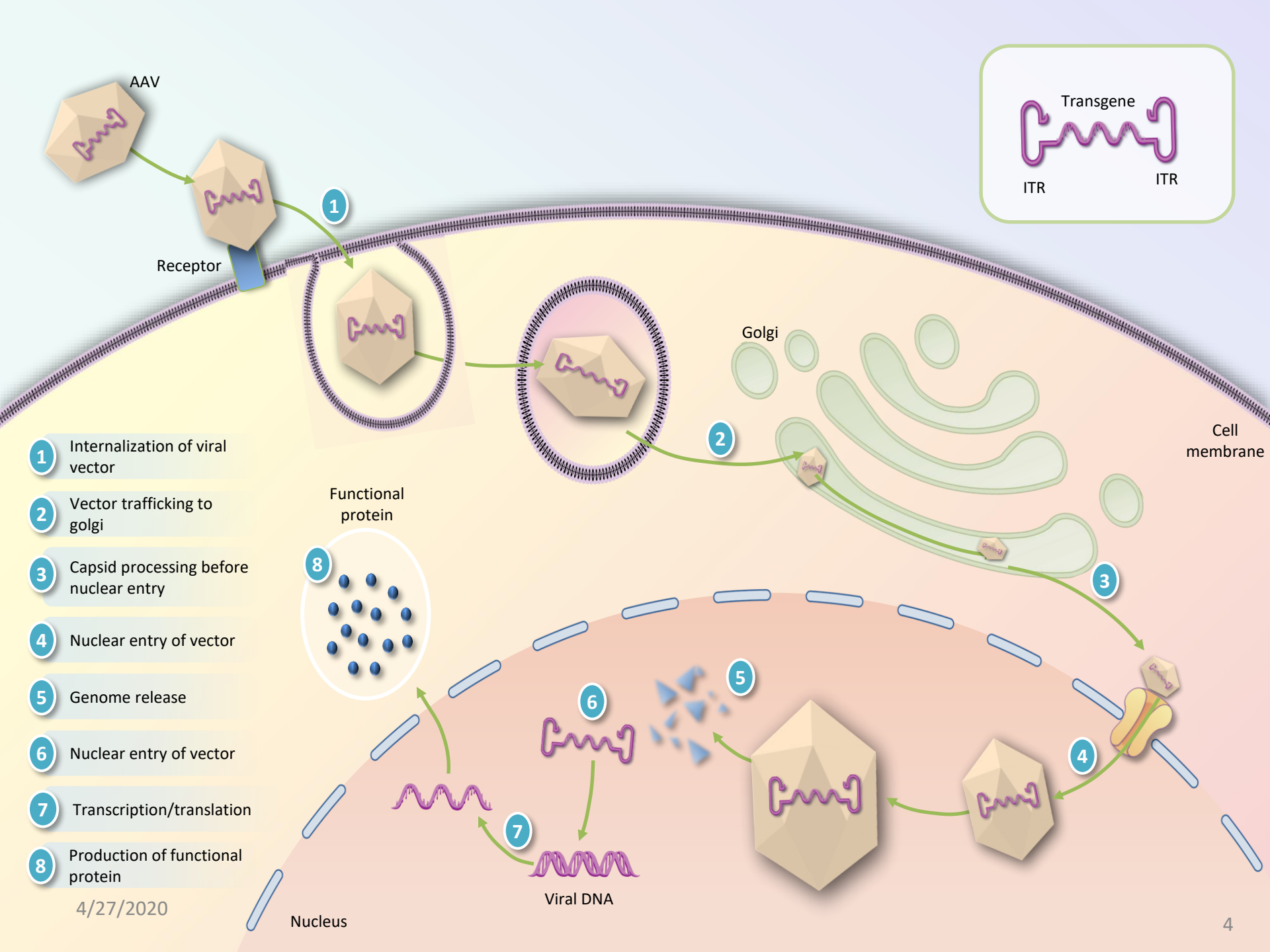
- Needs to be relevant to the biological activity of the product (or bridged)
- Permissive

✓ 3) Quantitative Read-out



- Simple
- Platformable (across multiple serotypes)
- Robust
- Automatable
- Sensitive and quantitative analysis

Industry Average: Complex, semi-quantitative assay lacking robustness & precision


AAV
Receptor
Transgene
ITR
ITR
Golgi
Cell membrane
Functional protein
Nucleus
Viral DNA
1 Internalization of viral vector
2 Vector trafficking to golgi
3 Capsid processing before nuclear entry
4 Nuclear entry of vector
5 Genome release
6 Nuclear entry of vector
7 Transcription/translation
8 Production of functional protein

Development of a cell-based potency assay for an early phase gene therapy product

SANOFI

Objective

- Transition gene therapy potency method during GMP sample release and stability
- Evolve assay platform from a ELISA endpoint to a reporter gene endpoint via luciferase detection

Deliverables

1) Deliver a robust potency assay to support GMP release and stability

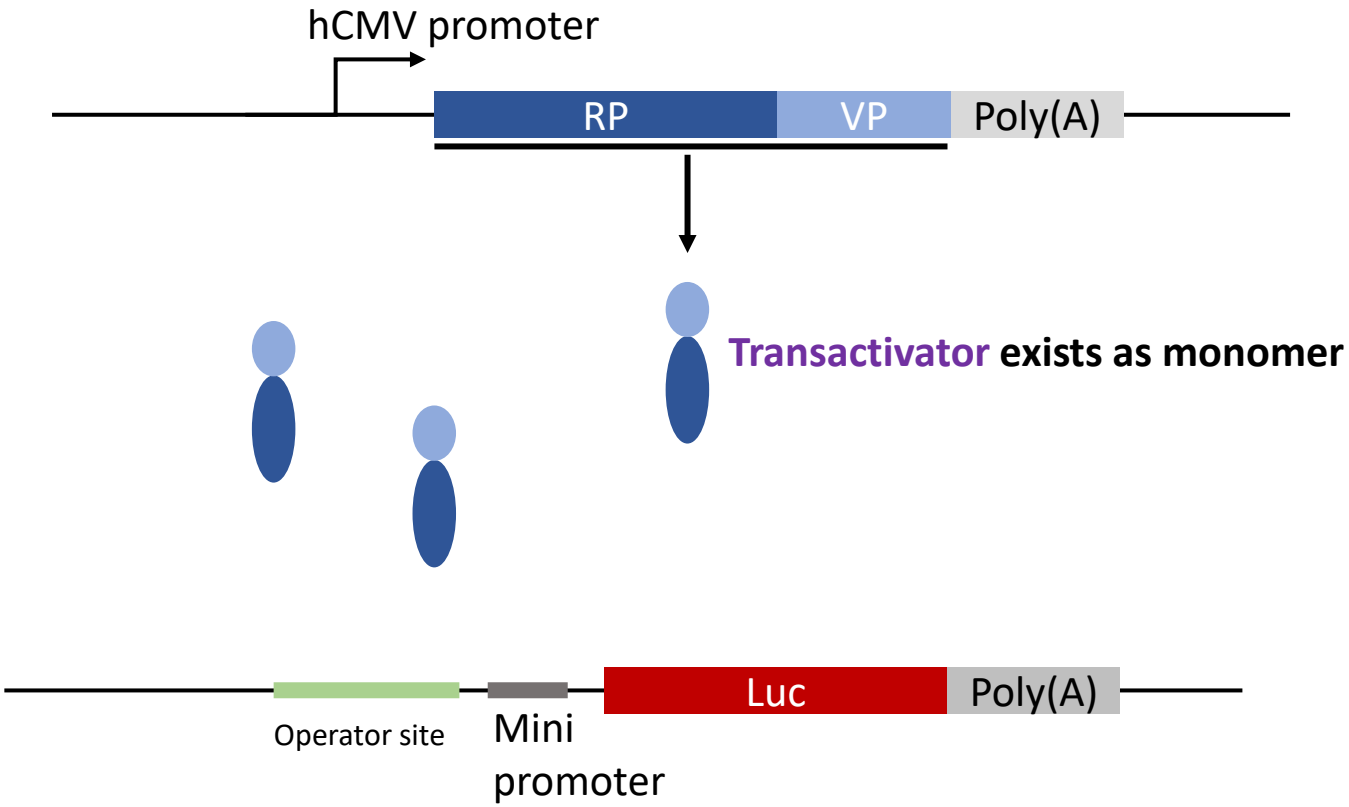
2) Cross-over data between the different platforms to bridge the on-going stability study

Both platforms target the same second messenger molecule response as a measure of potency

Reporter Gene cell line engineering

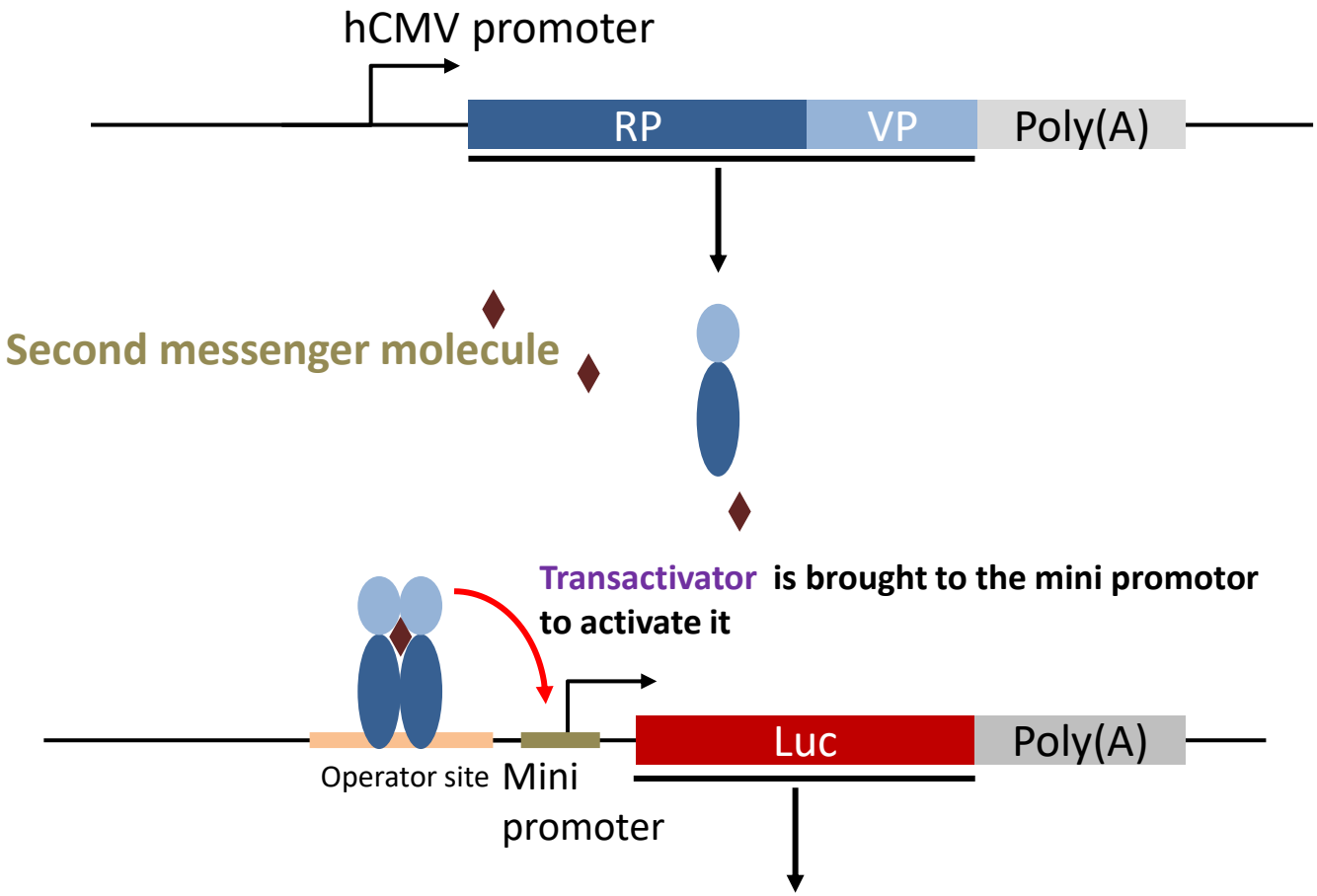
- Cell line A engineered by Svar Life Science
  - Stably transfected with activation protein (Sanofi plasmid with Gene Y)
  - Luciferase reporter system (proprietary) responsive to second messenger molecule
  - Characterization and manufacture
    - Clone stability, cell viability, and second messenger response was evaluated during manufacture
    - Assay Ready Cells delivered

- intracellular reporter system



No expression of firefly luciferase activity

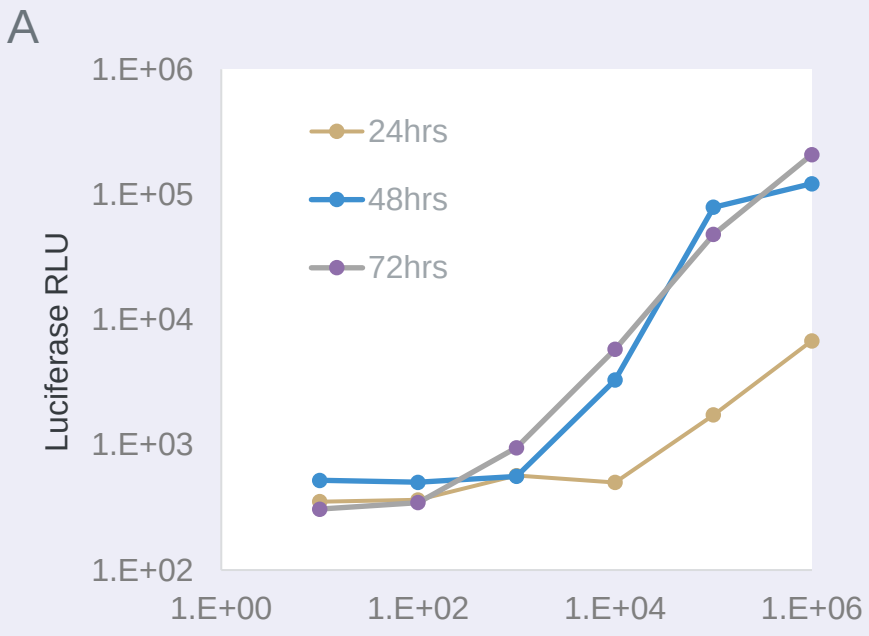
+ intracellular reporter system

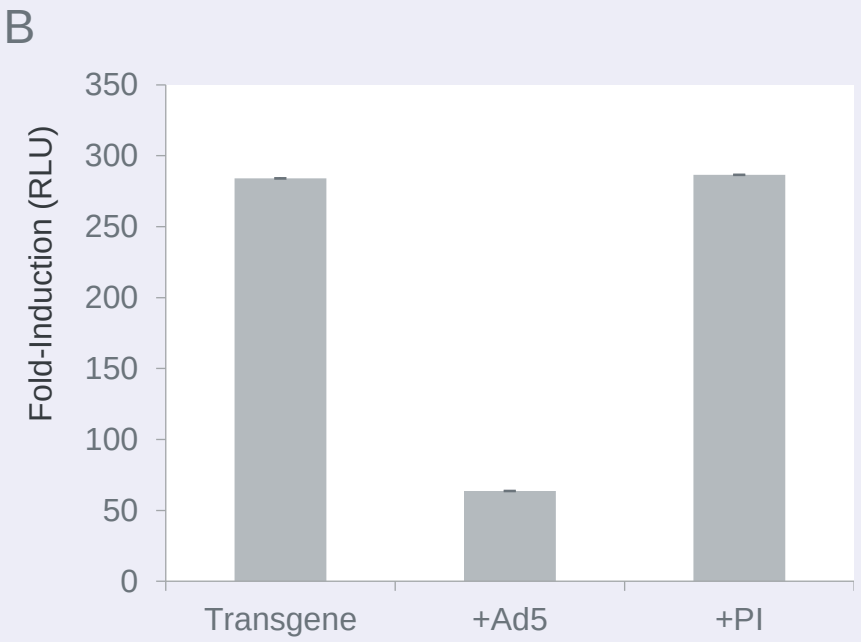


binds to transactivator, forming a homodimer that activates transcription of promoter

Expression of luciferase activity is directly proportional to the intracellular level of second messenger

Confirming reporter gene cell line demonstrates MoA

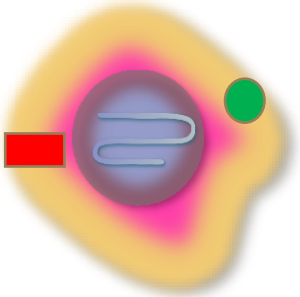




A) AAV produced the highest luciferase signal T=72 hours post-infection.

B) Ad5 or proteasome inhibitors are not required to generate transduction-induced luciferase activity.

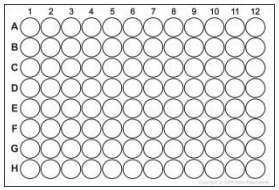
Reporter gene potency assay workflow



Cell Line A
+ Gene Y
+ Luciferase Reporter

1. Seed

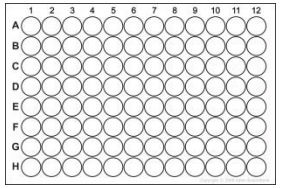
5E3 cells/well



Seeding density
Flask Confluency

37C 5% CO2
6 hrs

2. Infect

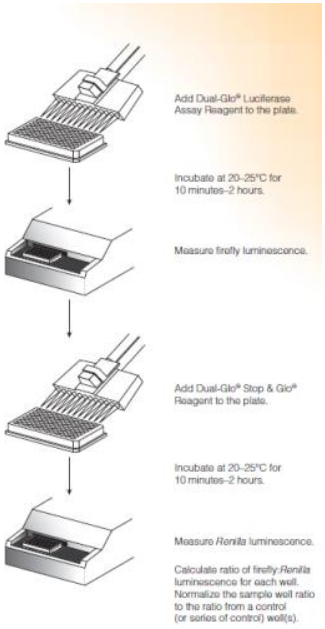


Dose
Optimization

+ AAV

37C 5% CO2
3 days

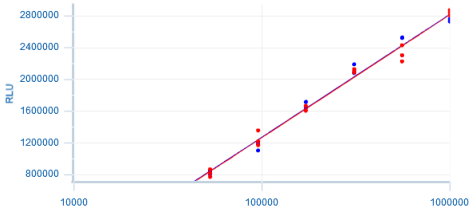
3. Measure



Bright
Glo
(Promega)

Plate Uniformity

4. Data Analysis



BioSt@t
Parallel Line Analysis

Pre-Assay Culture Conditions

Objective:

- Investigate the impact that flask confluency at time of seeding

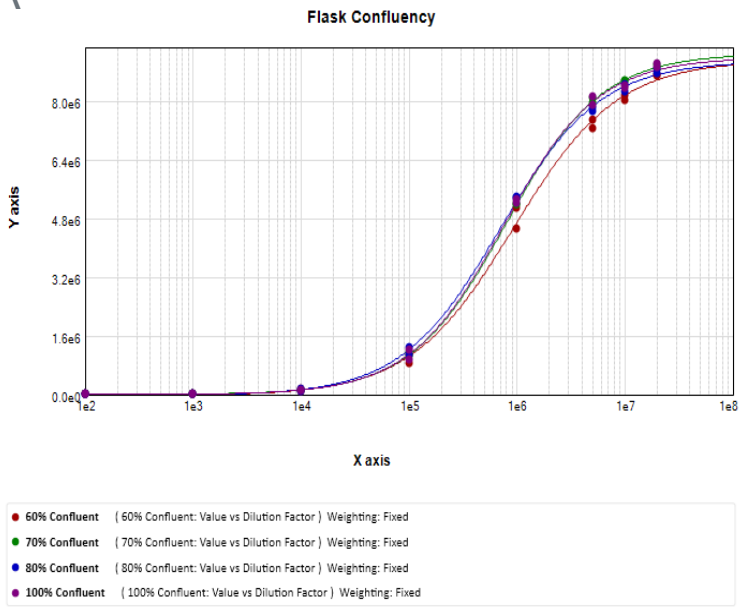
Procedure:

- Examine multiple flask densities targeting 60, 70, 80, 100% confluency
- Perform assay from each flask on a single assay plate using same seeding density and optimal drug exposure previously defined

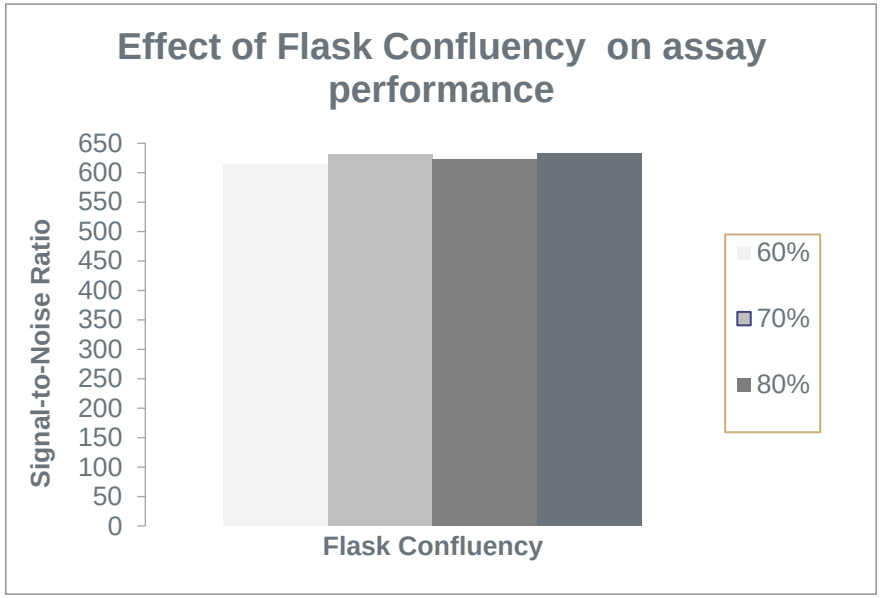
Seeding Density	Cells/Flask	Targeted Confluency at Seed	Confluency (%)	Viable Cell Concentration (E5/mL)	Viability (%)
5E3 cells/well	5.70E+05	60%	65	2.5	93.5
	7.10E+05	70%	75	3.2	95.2
	8.50E+05	80%	80	4.0	95.2
	1.00E+06	100%	95	4.7	95.9

Flask Confluency Results

A



B



C

Flask Confluency	R^2	EC50	S/N
60	0.998	9.26E5	614
70	1.00	7.89E5	630
80	1.00	7.00E5	623
100	0.999	7.47E5	632

A. An initial dose response curve was observed
B. Signal-to-Noise ratio was 600-fold for all conditions
C. Robustness with respect to flask confluency from 70-100%.

Cell Seeding Density/Incubation Time DOE

Objective:

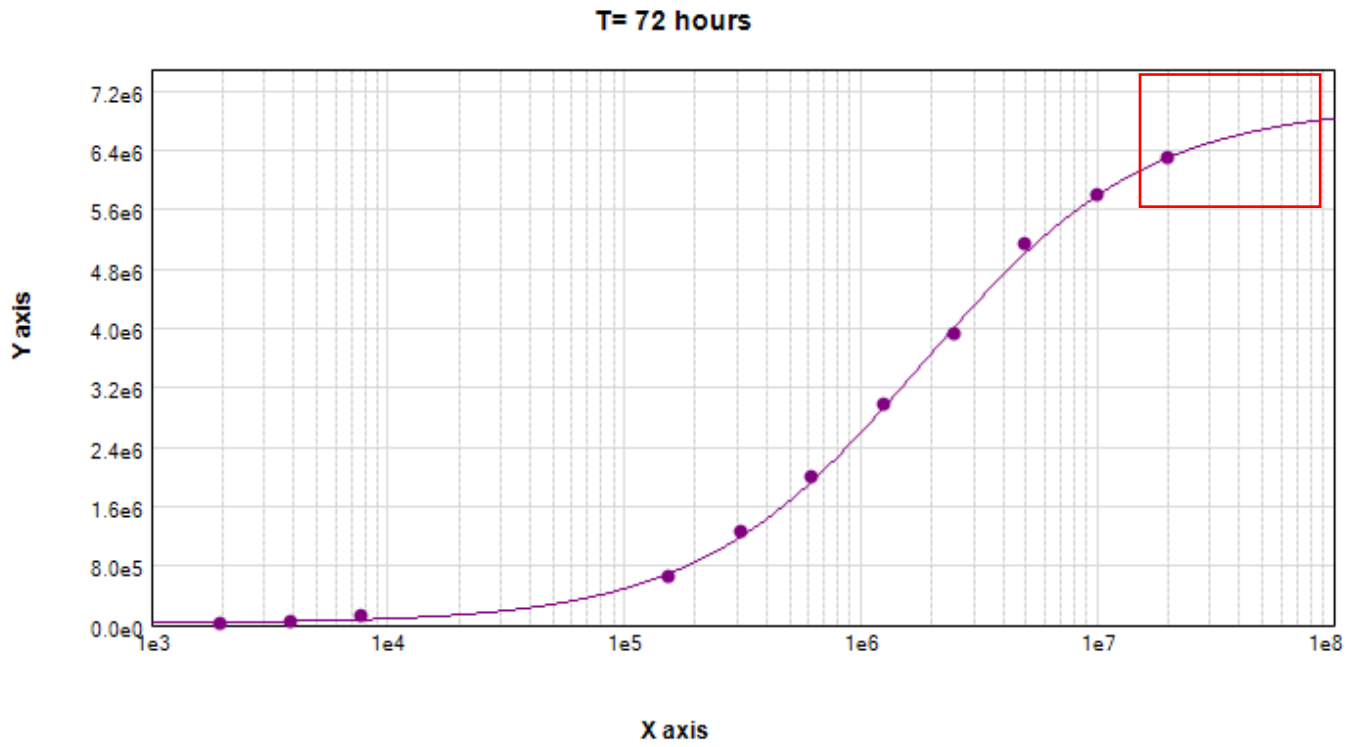
- Determine the optimal infection incubation time
- Determine the optimal seeding density

Procedure:

- Seeding Density: 5E3, 1.25E4, 2.5E4, 1E5 cells/well
- Infection Incubation Time: 2, 3, 4 days
- Evaluate across a wide MOI series

		1	2	3	4	5	6	7	8	9	10	11	12
SD1	A	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0
	B	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0
SD2	C	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0
	D	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0
SD3	E	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0
	F	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0
SD4	G	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0
	H	2.00E+07	1.00E+07	5.00E+06	2.50E+06	1.25E+06	6.25E+05	3.13E+05	1.56E+05	3.13E+04	6.25E+03	1.25E+03	0

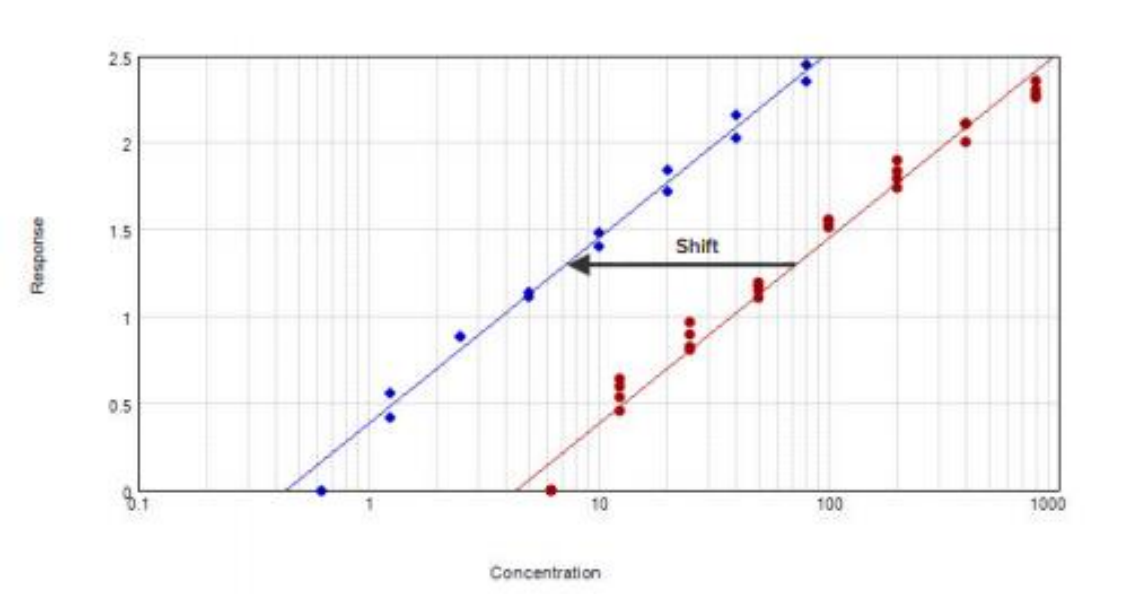
Optimal Infection Day/Seeding Density



4-Parameter Logistic Regression (4PL)
-One point in upper asymptote (3 is optimal)

Reporter cell line seeded at 5E3 cells/well, and infected for 3 days with AAV

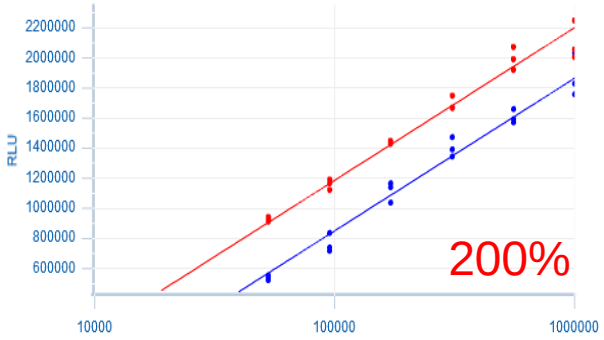
Parallel Line Analysis

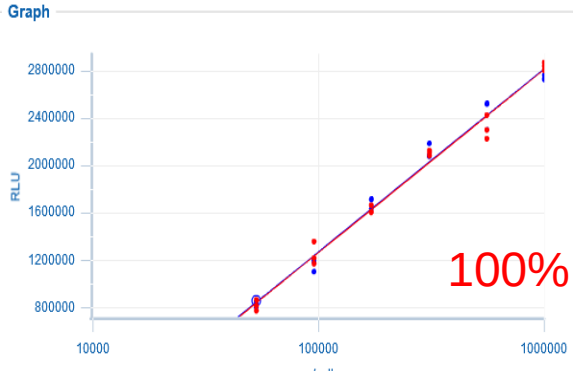


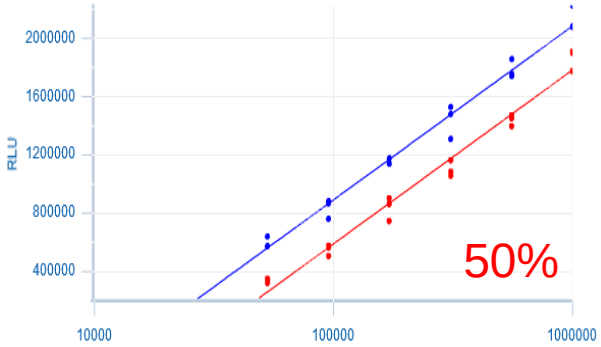
Molecular Devices
Relative Potency and Parallel Line analysis in Softmax Pro

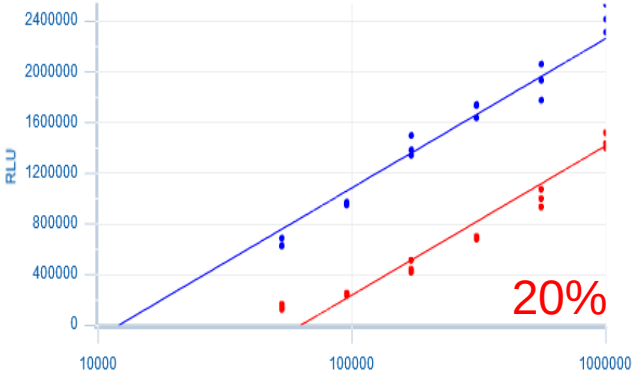
- Compare dose-response curves to reference in order to assess differences
- Shift relative to reference indicates change in potency that can be measured

Qualification: Reporter gene relative potency assay

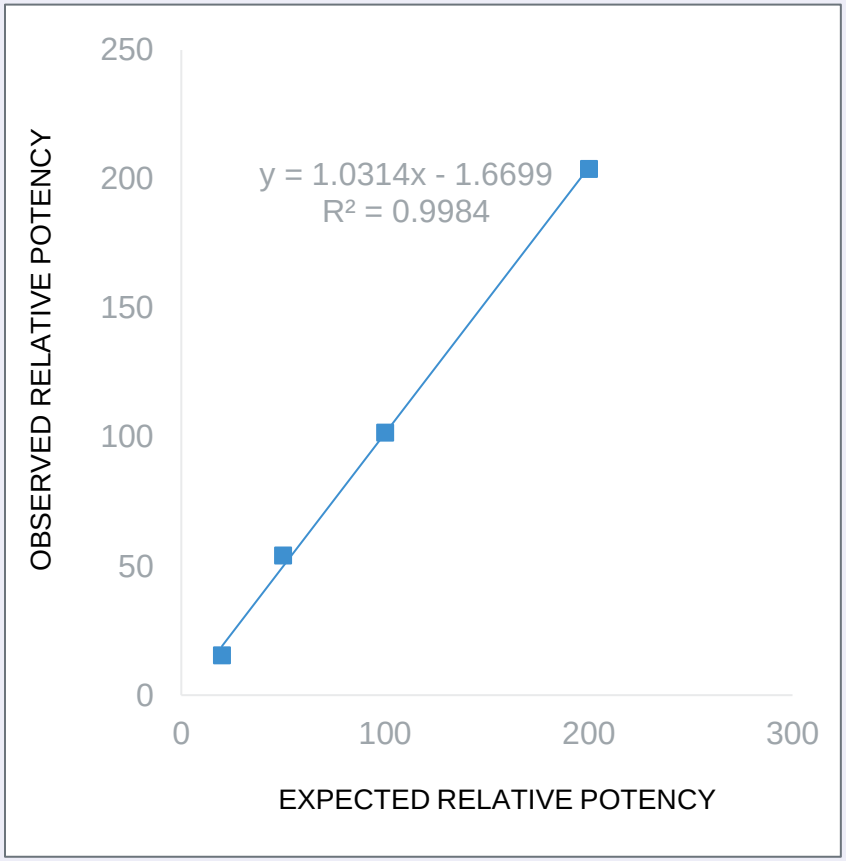








Preliminary Qualification Results



Qualification Parameter	Reporter Gene Potency Qualification Results
Accuracy	75 – 118%
Intermediate Precision	17%
Repeatability	7%
Range	20 – 200 %
Linearity	R^2 = 0.990
Specificity	Method is specific to DS and DP samples vs. buffer only

Comparing Workflows: ELISA vs. Reporter Gene endpoints

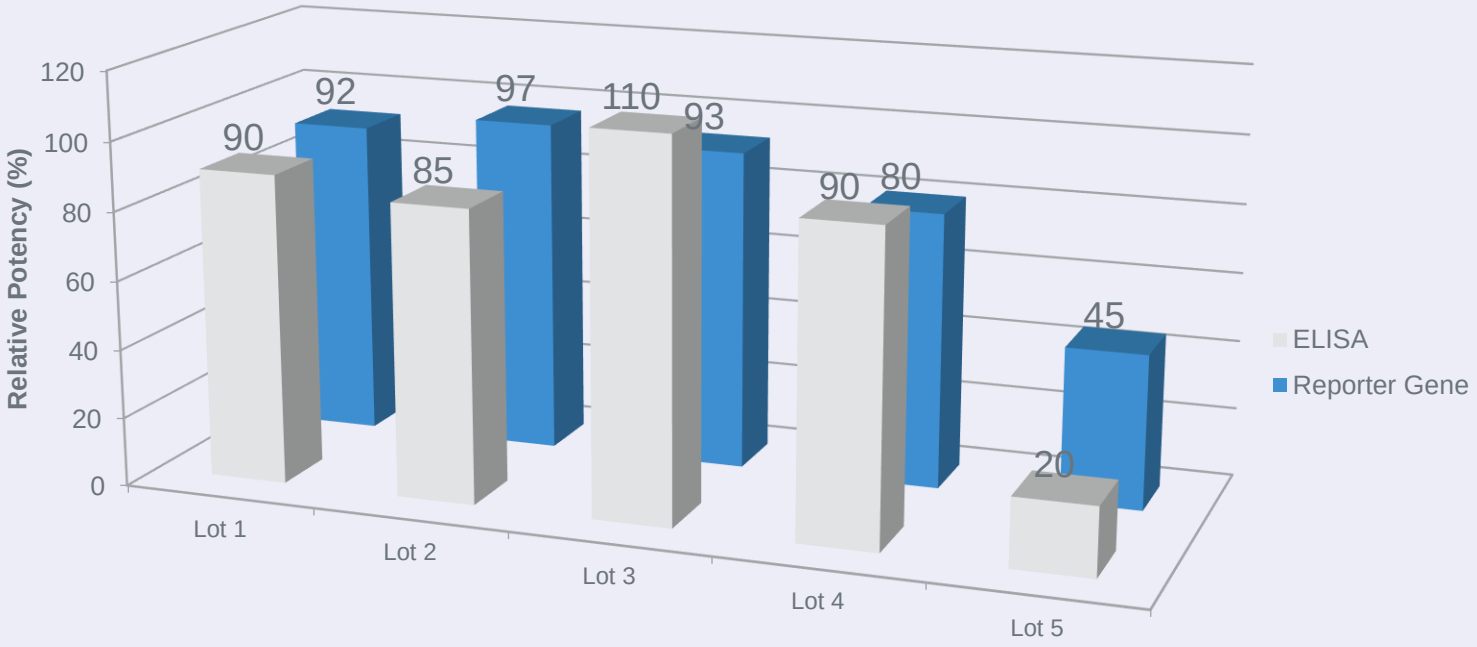
Assay Step	Current Method	Reporter Method
Plate format	24-well	96-well
Cell line	Parental	Reporter gene
Plasmid transfection	transient	Stable
Infection MOI	5 points in quadruplicate	6 points in triplicate
Ad5 required?	Yes	No
Sample Throughput	2 plates /1 sample	2 plates / 4 samples
Infection time	3 days	3 days
Harvest/Lysis	1 day	Not required
Detection	ELISA	Luciferase
Data Analysis	PLA	PLA
Total Assay Time	6 days	4 days
Controls	• WRS • ELISA standard • negative control	• WRS • negative control • Positive Control

Cross over Strategy

- Generating similar results for platform comparability was not attainable because ELISA method was not robust

- Instead, selective historical data was used as a comparison
  - Lot 5 obtained lower potency compared to reference using the ELISA platform.
    - *Can reporter gene method detect differences observed in potency?*

Cross over Results



Final Conclusion

- Reporter gene assay was implemented to test DS and DP samples on release/stability
  - Replaced variable ELISA method
  - Increased method accuracy, range, throughput, and sensitivity
- Reporter gene assay not intended for early phase release testing but transitioned early due to program need

Value to Gene Therapy programs

- Potency is only method relevant to product efficacy in gene therapy and therefore has significant value

  - Structure/Function studies
  - Process characterization
  - Batch-to-batch consistency
  - Comparability studies
  - interrogates the product

THANK YOU

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