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Revamp, Replace, or Revolutionize? Strategic Decisions in Potency Assay Evolution through Research and CMC

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09Jun2026

Early stage mRNA expression assay for AAV gene therapy

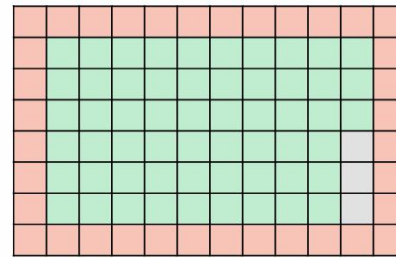
Obtained from research:

- ✓ Cell lines
- ✓ qPCR primer/probes optimized

Platformed from other programs:

- ✓ mRNA extraction kit and method
- ✓ RT kit

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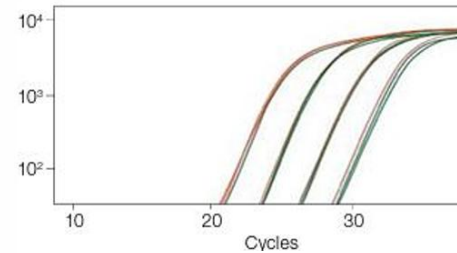


Seed cells overnight 96-well plate, infect in the A.M.

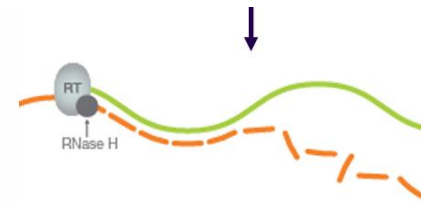
2-7 days
→



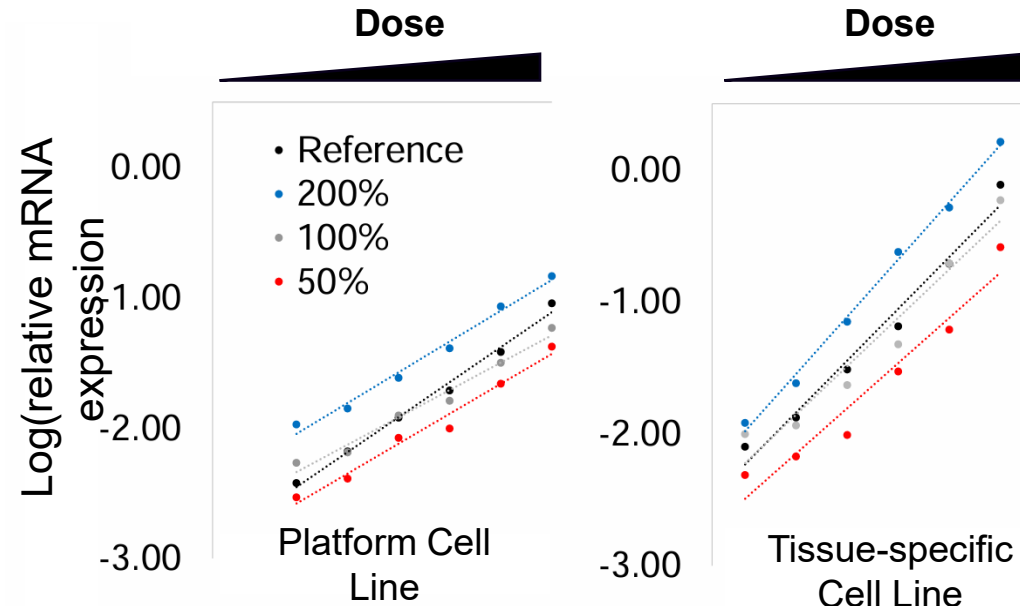
Semi-automated mRNA purification



qPCR amplification



Reverse transcription



AAV transduction; cell trafficking

AAV unpackaging, genome replication, **transcription**

AAV

gene product

Target Gene Expression

Downstream Biomarkers' Gene Expression

Early stage inhibition MOA assay

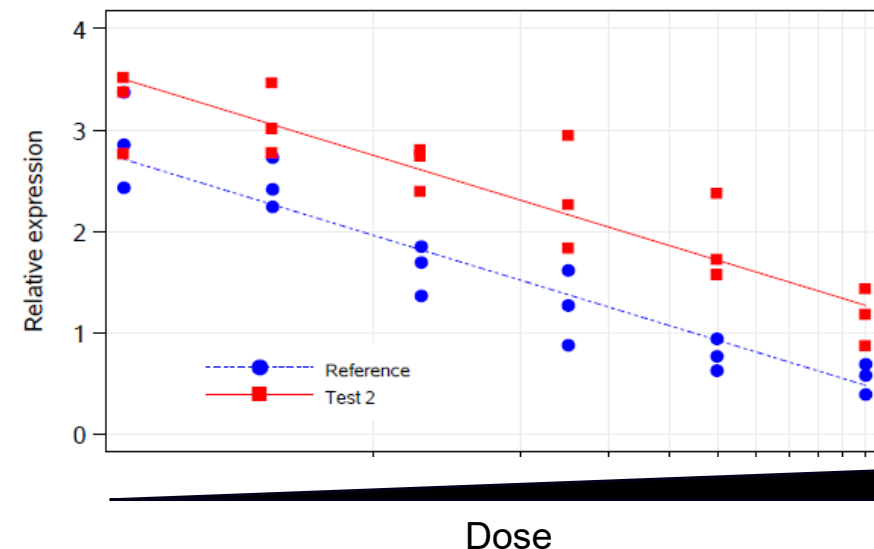
Obtained from research:

- ✓ Cell lines
- ✓ qPCR primer/probes optimized

Optimized:

- ✓ RT-ddPCR protocol and temperatures
- ✓ Intermediate precision

	Replicate %CV	
Dose	qPCR	ddPCR
1	30.4	9.09
2	14.0	12.7
3	16.0	7.05
4	30.9	10.0
5	23.4	8.21



ddPCR plate prepared on ice:

	2	3	4	5	6	7	8	9	10	11
B	1.62	1.78	1.94	2.01	2.02	2.06	2.06	2.17	2.15	2.12
C	1.65	1.77	1.94	2	2.11	2.06	2.11	2.09	2.13	2.19
D	1.56	1.84	1.99	1.98	2.06	2.19	2.07	2.07	2.17	2.14
E	1.64	1.85	1.96	2	2.03	2.09	2.13	2.09	2.16	
F	1.63	1.79	1.94	1.97	1.99	2.1	2.05	2.15	2.12	
G	1.59	1.79	1.95	1.97	1.98	2.09	2.08	2.19	2.13	

Prepared at RT and equilibrated after addition of RNA:

B	2.1	1.99	2.08	2.13	2.24	2.11	2.15	2.14	2.12	2.37
C	2.09	2.15	2.07	2.14	2.11	2.16	2.16	2.1	2.17	2.13
D	2.08	2.01	2.01	2.17	2.23	2.01	2.14	2.13	2.04	2.15
E	2.12	2.01	2.07	2.03	2.12	2.15	2.11	2.12	2.1	
F	2.1	2.04	2.02	2.03	2.17	2.09	2.04	2.08	2.1	
G	2.12	2.07	2.02	2.16	2.14	2.09	2.05	2.19	2.11	

Sample	mRNA Expression RP	MOA RP
1	106%	112%
2	94%	94%
3	102%	97%
4	82%	77%
5	113%	105%
6	120%	116%

Late-stage MOA biomarker gene regulation: leveraging research, building new models

