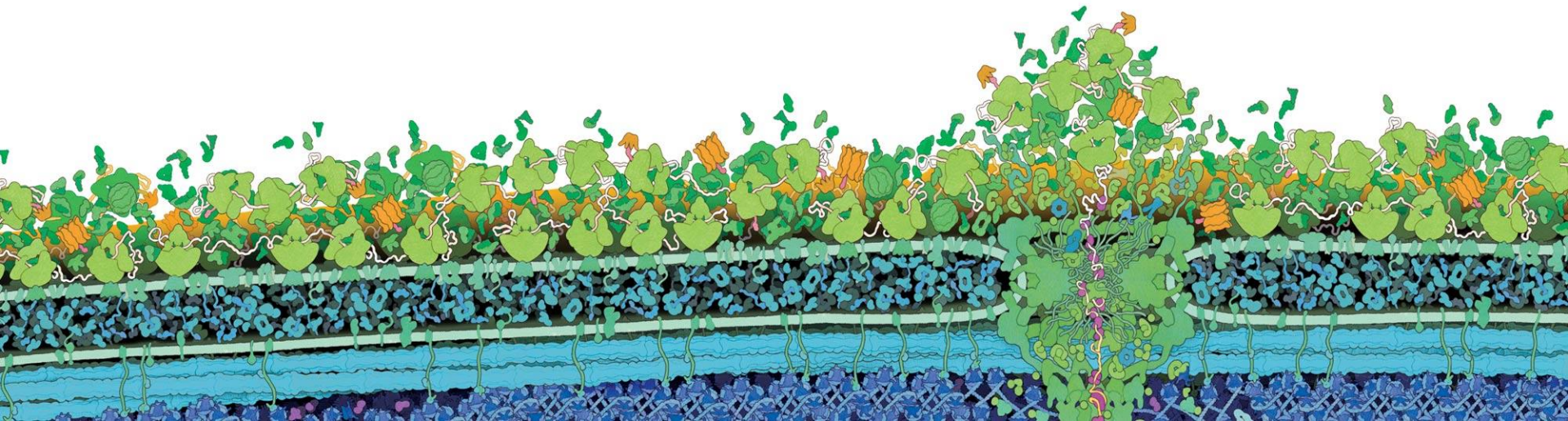


A New Application of Bioluminescence for In-process Potency Testing of T Cell Redirecting Viral Vectors

Julia Gilden

Senior Scientist, Promega Corp.

CASSS Bioassays 2023





Lentiviral Vectors in CAR-T Manufacturing

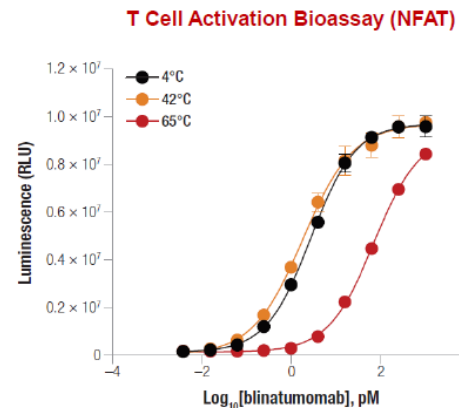
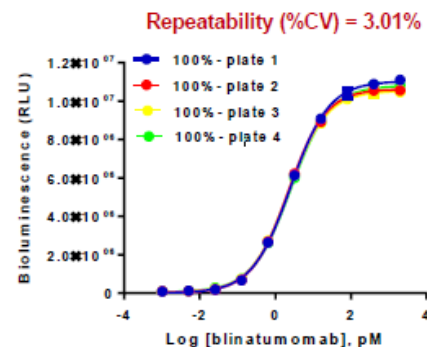
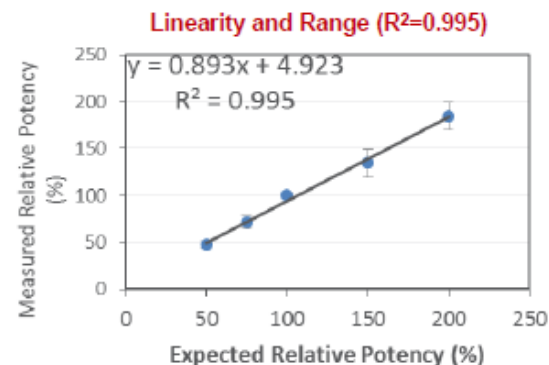
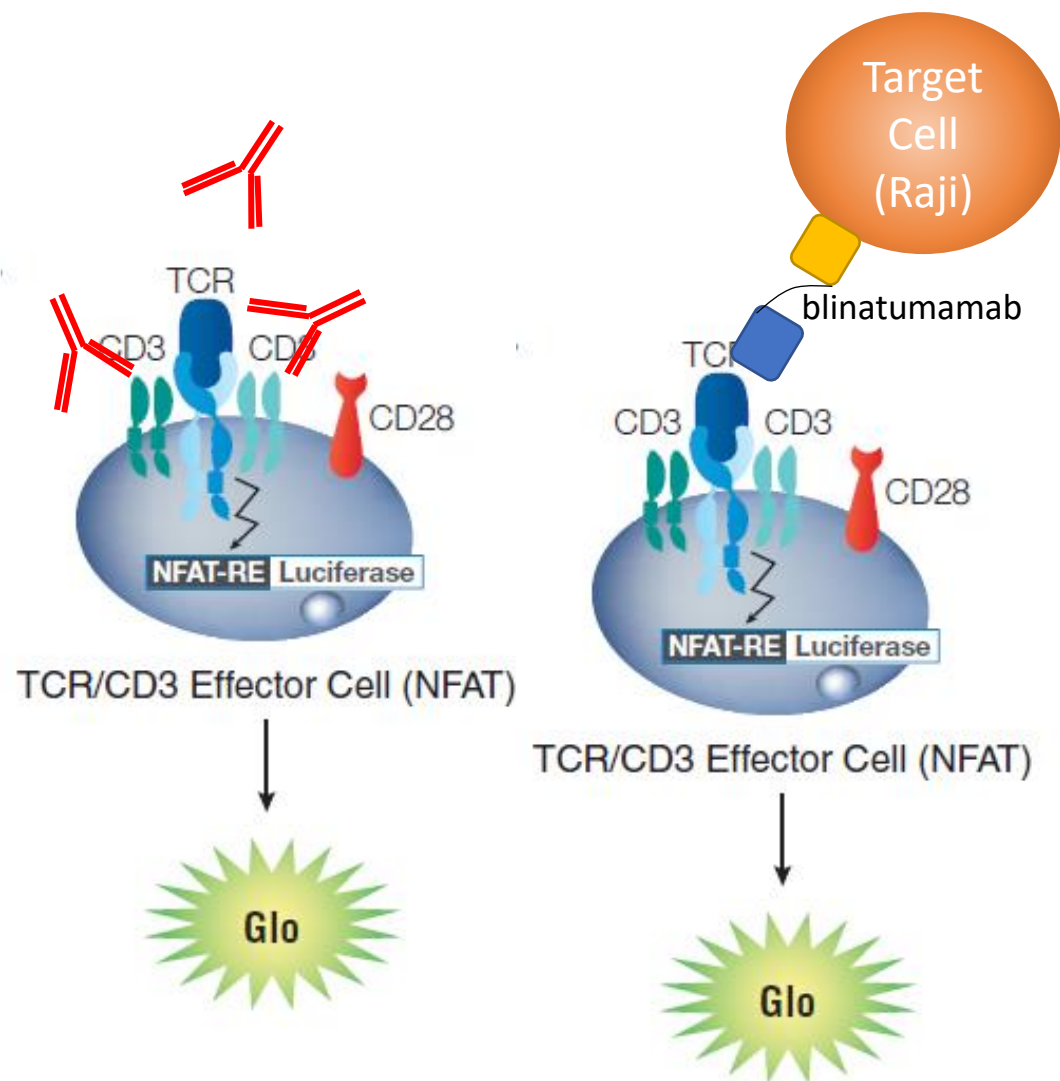
FDA Draft Guidance (2022):

- Recommends a complete Drug Substance section for viral vector.
- “Additional measures of potency” to support marketing application [beyond transgene expression]

Analytical	Functional
Particle counting • DLS • Nanoparticle tracking	HEK293 transduction → Flow Cytometry for transgene or reporter
p24 ELISA	HEK293 transduction → qPCR of genomic DNA
Nucleic acid quantification in particles • qPCR • Fluorescent probe	
PERT	

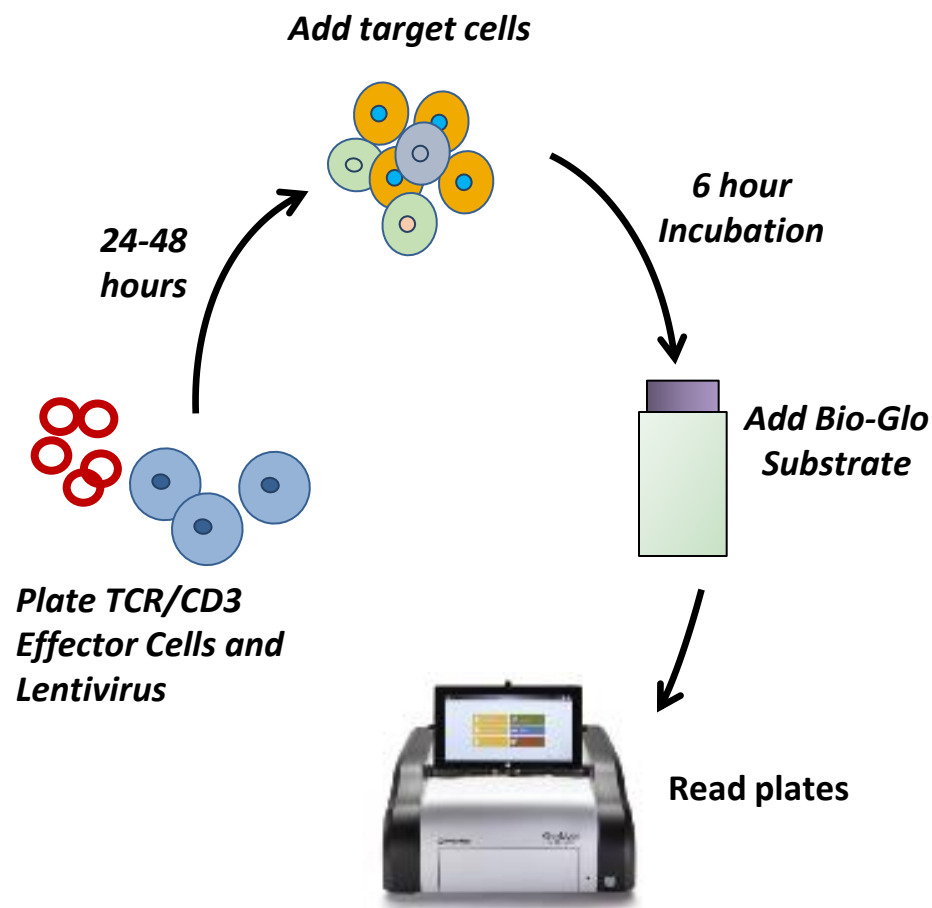
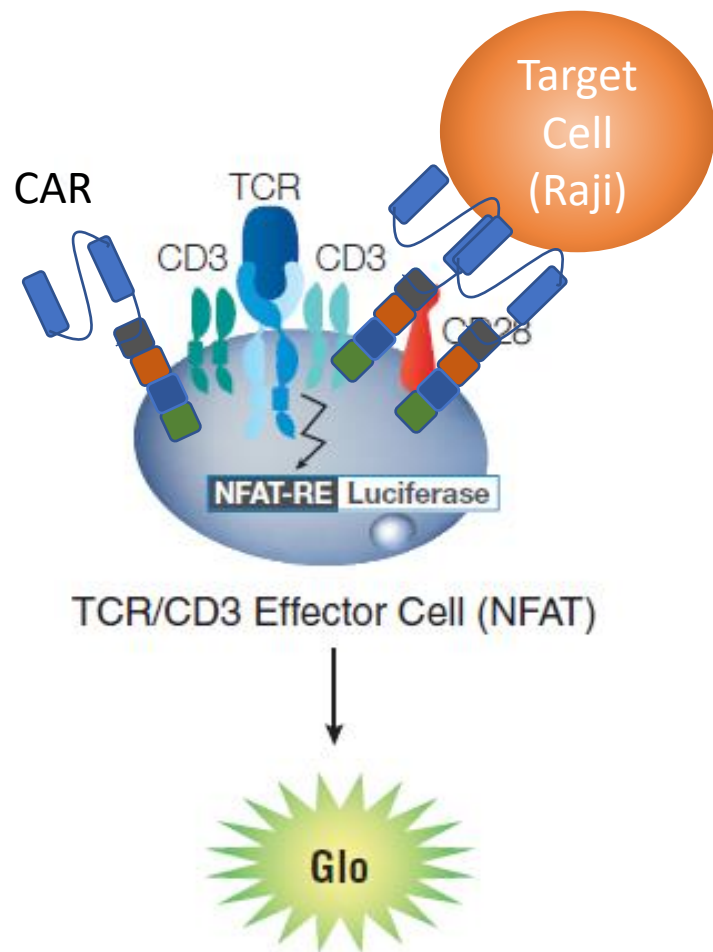
Need: An MOA-based potency assay for T cell redirecting lentiviral vectors

T Cell Activation Reporter Bioassay





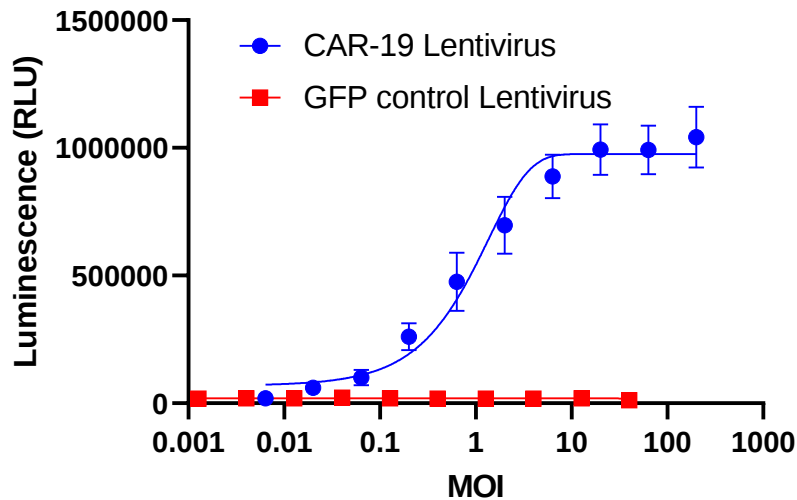
Lentiviral Bioassay Concept



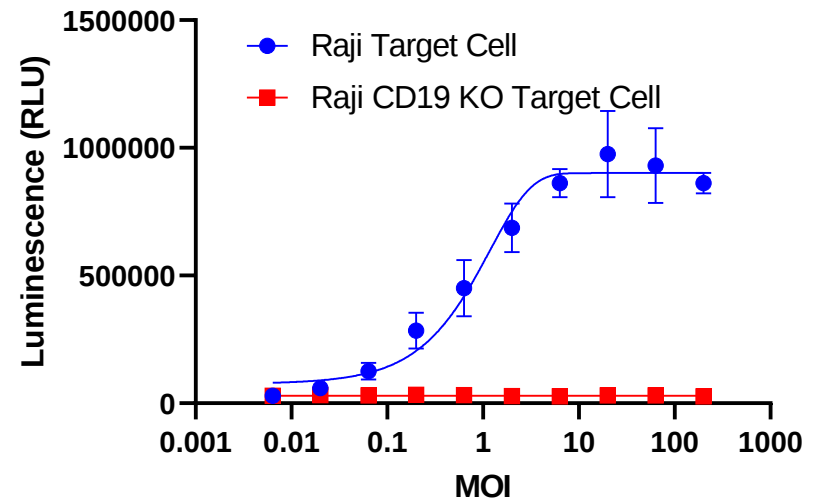


Lentivirus Bioassay Specificity

**T Cell Activation
Reporter Bioassay**



**T Cell Activation
Reporter Bioassay**

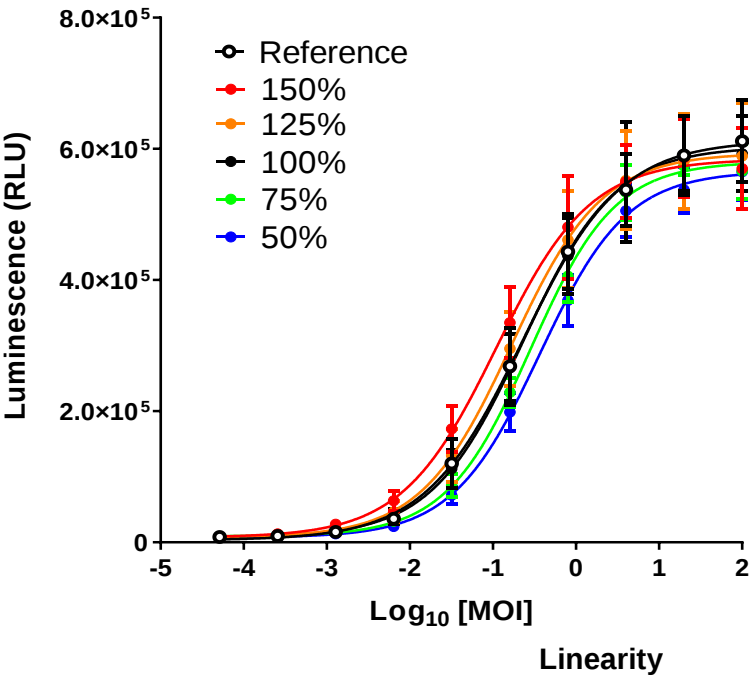


Key Factors:

- E:T Ratio
- Plate Type
- Mixing/Resuspension

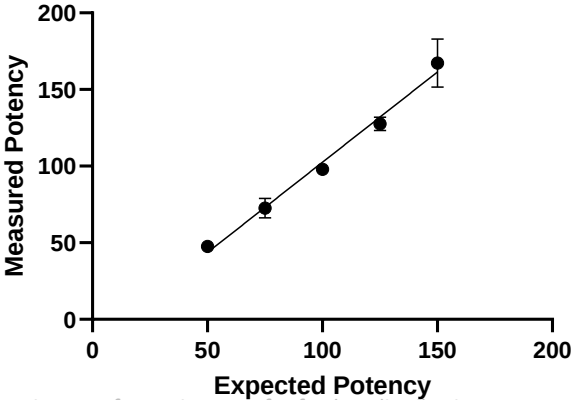


Assay Pre-qualification



Expected Relative Potency	Measured Relative Potency	Accuracy (% Recovery)	Precision (%CV)
50	46.6	93%	4%
75	73.1	97%	6%
100	97.8	98%	1%
125	128.1	102%	3%
150	160.3	107%	10%
Intermediate Precision			5%

n=3

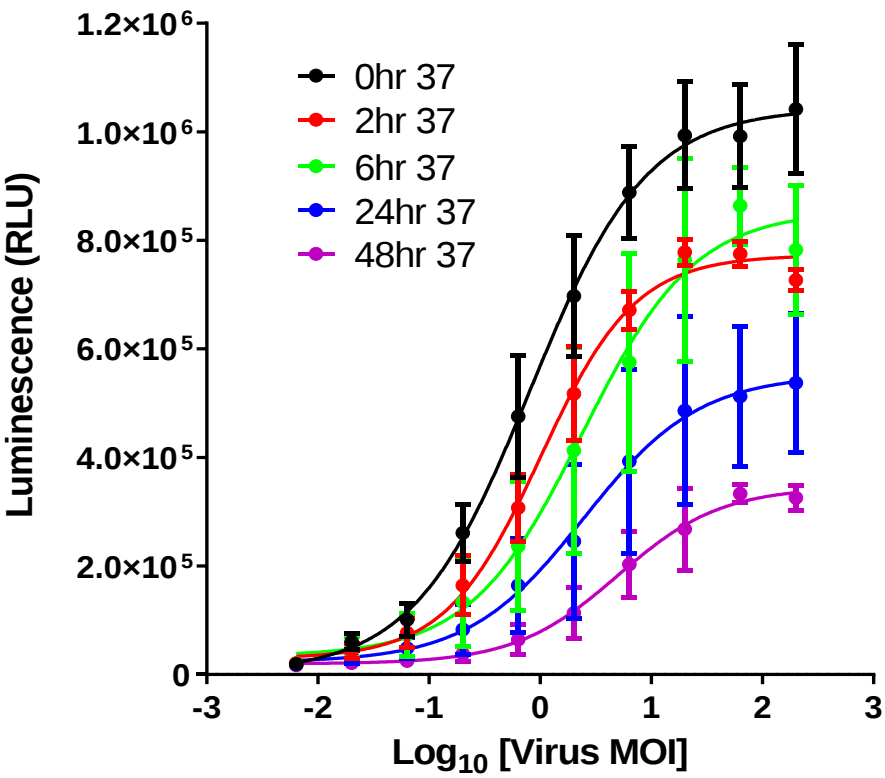


$r^2 = 0.97$
 $y = 1.178x - 15.24$



Lentiviral Bioassay is Stability Indicating

Heat Treatment of CAR-19 Lentivirus



Heat Treatment Time (hrs)	EC50	% Inhibition of Max
0	0.81	Reference
2	1.0	26%
6	2.4	18%
24	2.5	47%
48	5.0	67%



Summary

- The T Cell Activation Bioassay platform can be adapted for lentiviral potency testing
- The assay is specific, sensitive, and reproducible, with little hands-on time
- The assay is stability indicating
- The assay can be adapted to target cells expressing other tumor-associated antigens

Acknowledgments

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