Development of a Reporter Cell Line and Assay for Merck's Ebola Vaccine (rVSV-ΔG-ZEBOV-GP)

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Overview

- Recombinant Vesicular Stomatitis Virus Ebola Vaccine (rVSV-ΔG-ZEBOV-GP)
- Background and rationale for developing a cell-based reporter assay vs. plaque potency
- Reporter cell line development: JM-1
- Assay development with JM-1 and rVSV-ΔG-ZEBOV-GP
- Conclusions
- Acknowledgements
- References

Recombinant Vesiculostomatitis Virus (rVSV)-Based Ebola Vaccine

- rVSV was generated in a cell line using reverse genetics, which included the glycoprotein gene from the Ebolavirus-Zaire, Kitwit strain
- rVSV-ΔG-ZEBOV-GP is grown in cell culture followed by purification, manufacture of drug substance, and drug product
- Infectivity by plaque titration was utilized to determine final titers of the drug substance, drug product, and stability samples
- A cell-based reporter assay was investigated as an alternative to infectivity by plaque titration for rVSV-ΔG-ZEBOV-GP

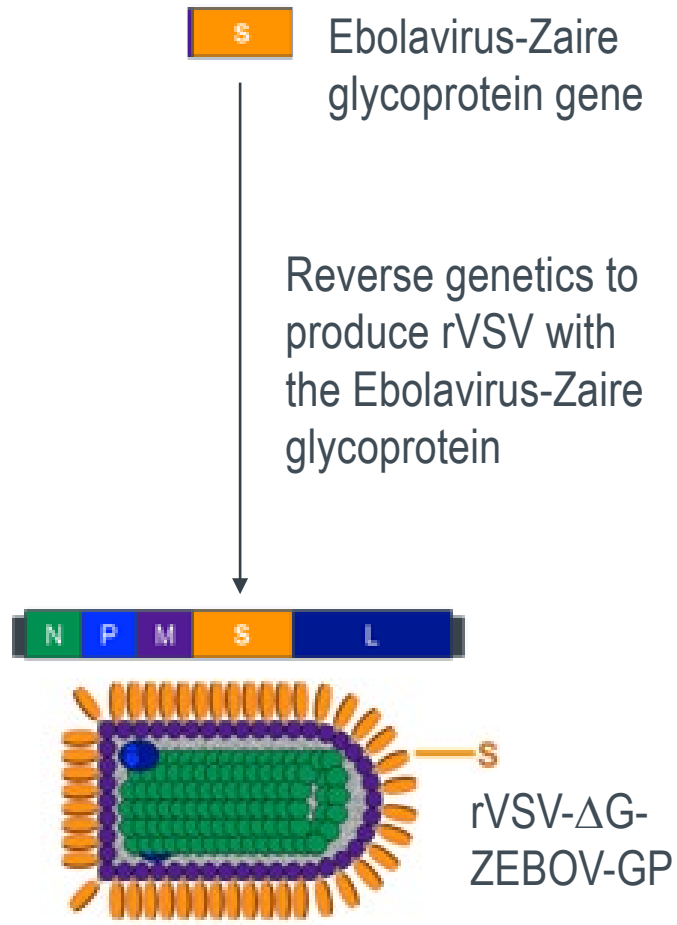

Figure 1. Generation of rVSV-ΔG-ZEBOV-GP

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Background and Rationale: Cell-based Reporter Assays vs. Plaque Potency

Goal: Determine if a cell-based reporter assay could be developed that would be more efficient than the plaque potency assay for a Merck rVSV-based vaccine (Ebola, rVSV-ΔG-ZEBOV-GP)

- Plaque assay
  - The industry standard for determining potency of live virus vaccines
    - Assay infectivity via plaque titration
  - Low throughput, requires significant resources, and has high variability
  - Long turnaround times can slow down vaccine development
- Cell-based reporter assays
  - Designed to be fast and with low variability
  - Acceleration of viral vaccine development is critical in the event of an outbreak

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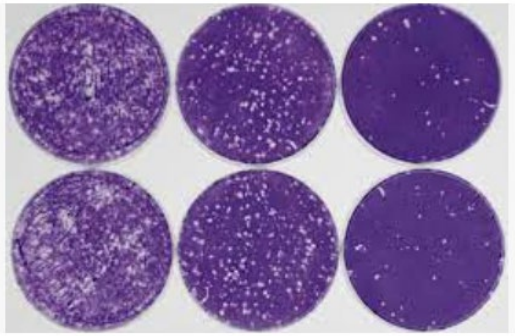

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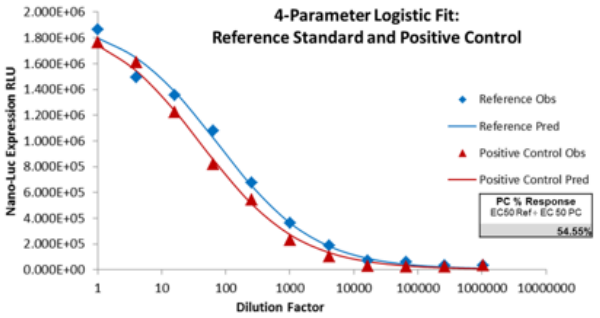


Figure 2. A) Plaque assay B) Cell-based reporter assays

Reporter Cell Line Development: JM-1

Goal: Develop a reporter cell line that could be used with rVSV-ΔG-ZEBOV-GP

- Decision was made to generate a cell line that would constitutively express a reporter protein in the cell, selected NanoLuc (Promega, engineered from the deep sea shrimp *Oplophorus gracilirostris*[2])
- Generated a vector construct consisting of the CMV promoter driving NanoLuc. Isolated stable transfectants using G418 selection and cloned JM-1
- Hypothesis was NanoLuc would be released from the cell following infection by rVSV-ΔG-ZEBOV-GP
- Demonstrated proof-of-concept with rVSV-ΔG-ZEBOV-GP (Ebola vaccine, ERVEBO®)[3]

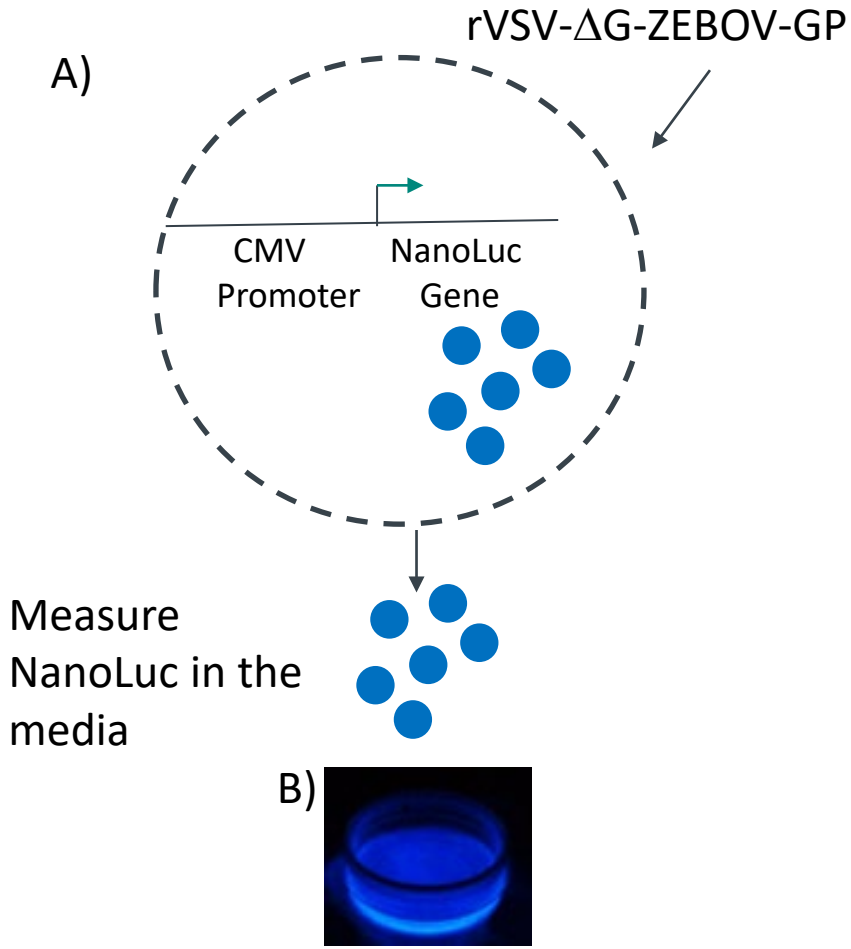


Figure 3. A) Illustration of JM-1 and release of NanoLuc following infection with rVSV-ΔG-ZEBOV-GP, B) Cell culture plate of JM-1 infected with rVSV-ΔG-ZEBOV-GP and substrate for NanoLuc

JM-1 Cell Line: Test Proof-of-Concept

Goal: Evaluate proof-of-concept that an increase in rVSV-ΔG-ZEBOV-GP would result in a concomitant increase in NanoLuc expression in the media

- Selected a condition to obtain proof-of-concept
- A range of virus was tested in a 96-well plate
- Substrate added and luminescence measured

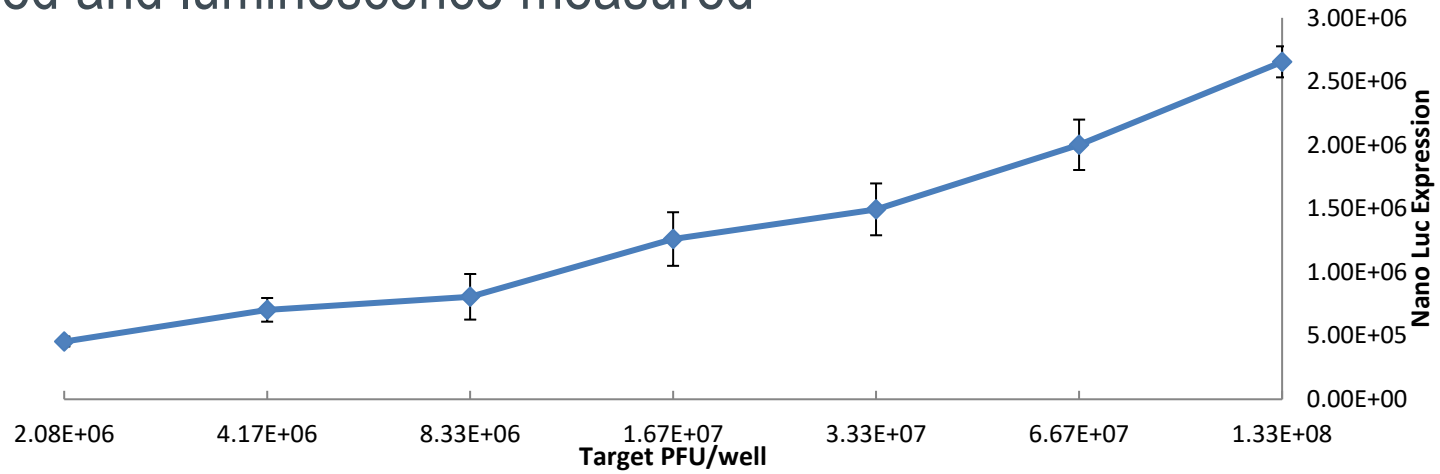


Figure 4. JM-1 infected with an increasing titer of rVSV-ΔG-ZEBOV-GP

Conclusion: Proof-of-Concept completed, an increasing titer of rVSV-ΔG-ZEBOV-GP resulted in an increase in NanoLuc

JM-1 Cell Line: Stability-indicating

Goal: Determine if JM-1 was stability-indicating for rVSV-ΔG-ZEBOV-GP by measuring NanoLuc activity in the media

- Samples of rVSV-ΔG-ZEBOV-GP were incubated at accelerated temperatures for 0, 3, and 7 days
  - Accelerated temperatures will result in lower potency of rVSV-ΔG-ZEBOV-GP
- Samples were assayed and media measured for NanoLuc activity

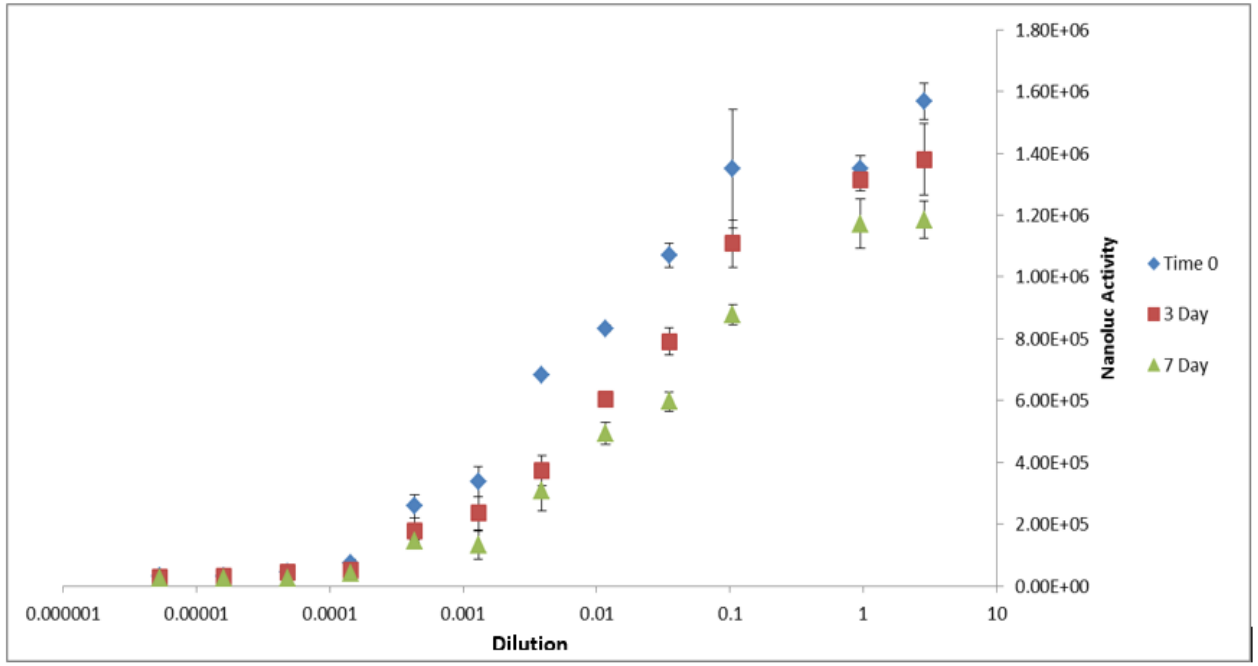


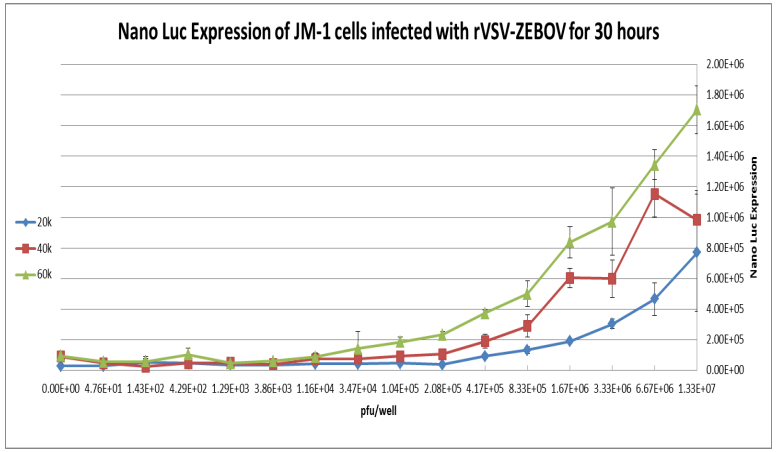
Figure 5. JM-1 is stability-indicating for rVSV-ΔG-ZEBOV-GP

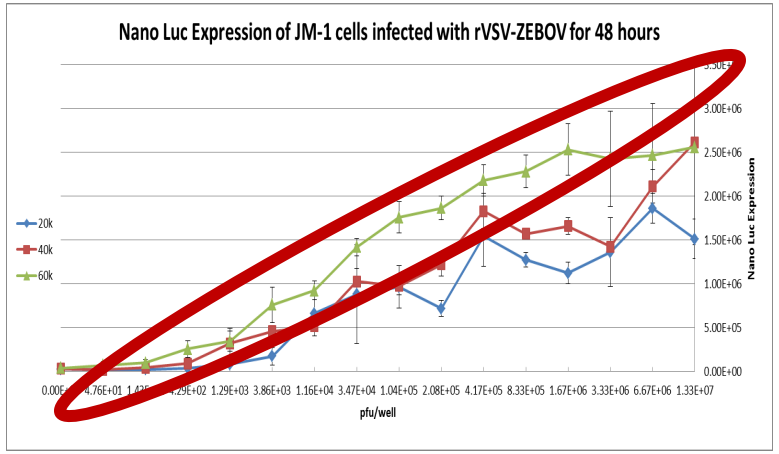
Conclusion: JM-1 was stability-indicating

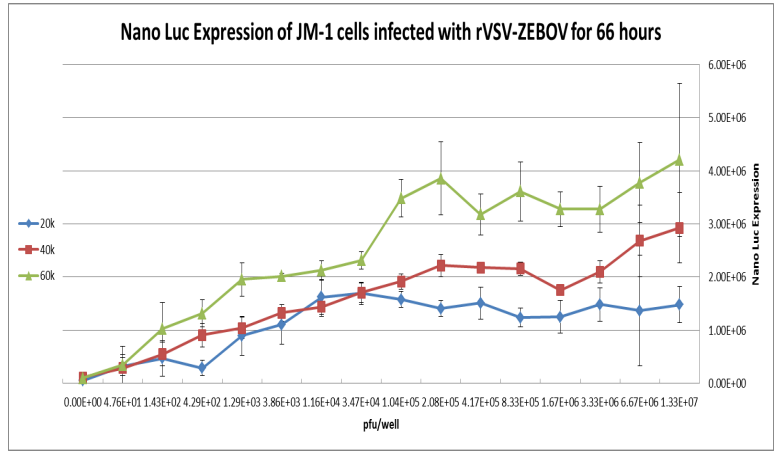
JM-1 Cell Line: Optimize conditions for infection

Goal: Optimize cells/well and incubation time for assay development

- Evaluated 2E4, 4E4, and 6E4 JM-1 cells/well
- Infection time of 30, 48, and 66 hours, followed by luminescence measurement







30 Hours	R^2
20k cells/well	0.5518
40k cells/well	0.6573
60k cells/well	0.7232

48 Hours	R^2
20k cells/well	0.8844
40k cells/well	0.9236
60k cells/well	0.9511

66 Hours	R^2
20k cells/well	0.5820
40k cells/well	0.9092
60k cells/well	0.8766

Figure 6. Experimental results of cell number and infection time with rVSV-ΔG-ZEBOV-GP

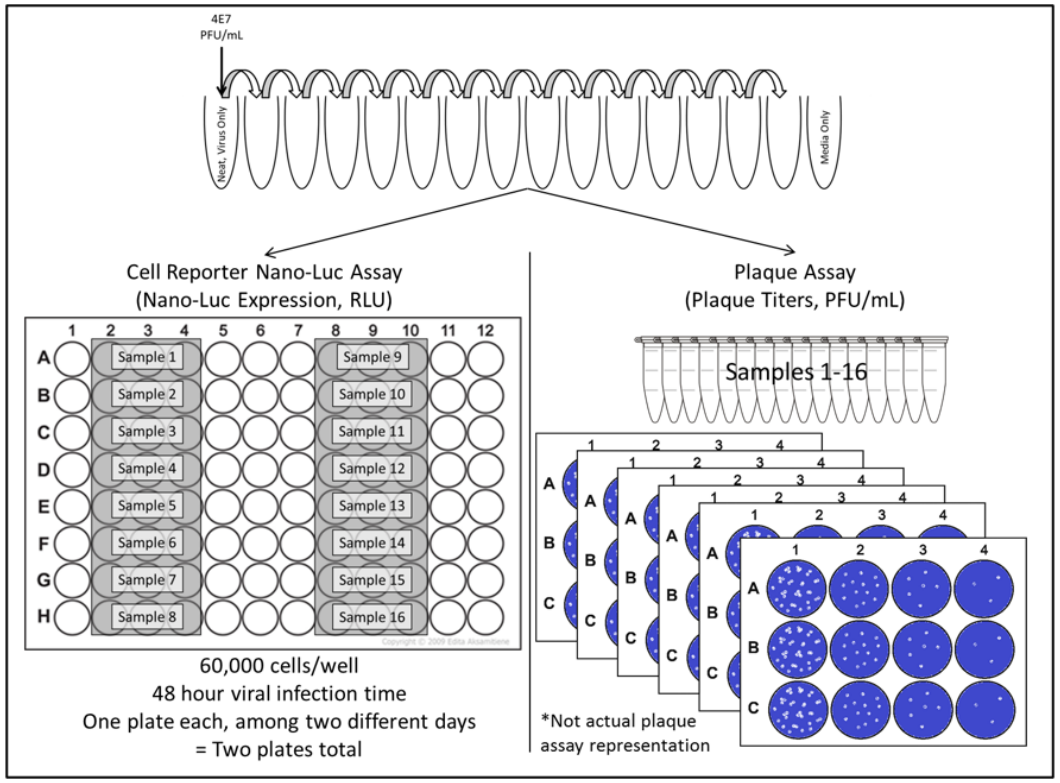
Conclusion: 6E4 cells/well infected for 48 hours had the highest R^2 under these experimental conditions

JM-1 Cell Line: Evaluate correlation to plaque potency assay

Goal: Determine if there was a correlation between the JM-1 and plaque potency assays

- Prepared a sample set of rVSV-ΔG-ZEBOV-GP, submitted to the plaque potency lab, and tested with JM-1

A)



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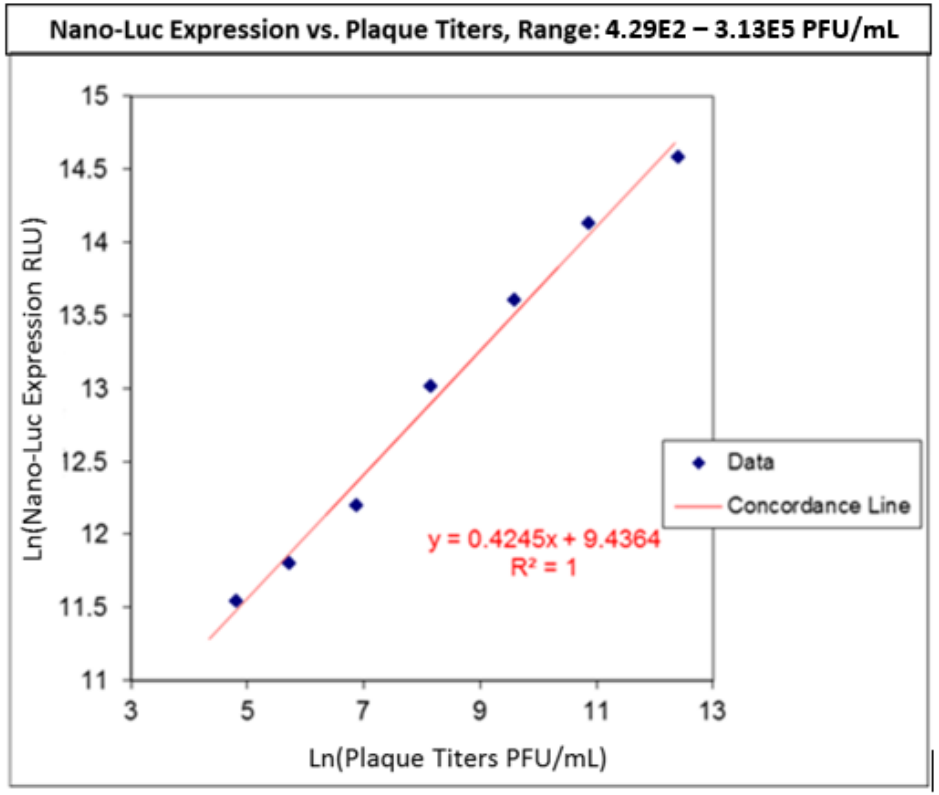


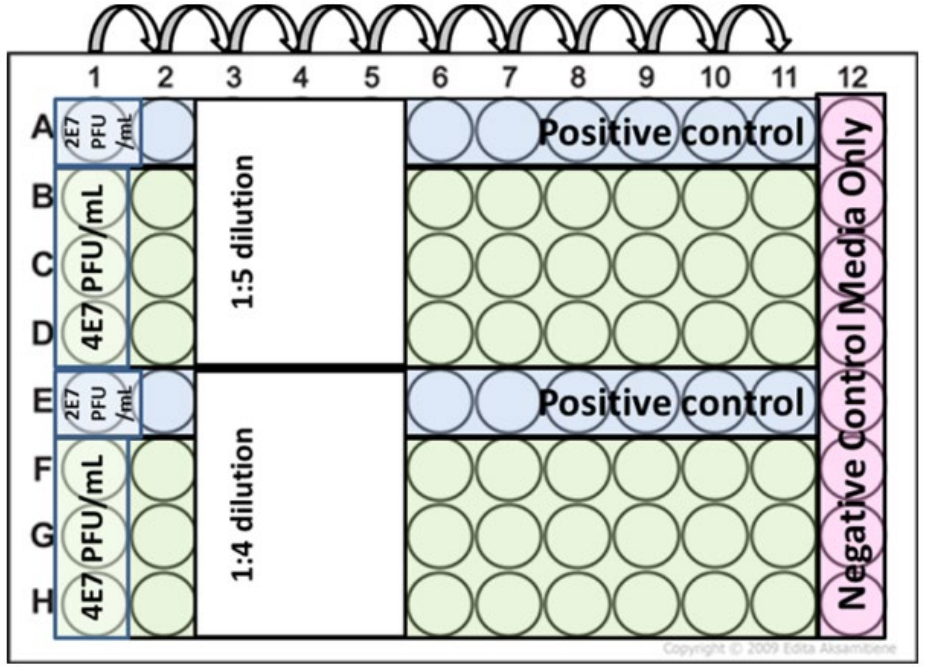
Figure 7. A) Experimental design, B) Concordance analysis of the NanoLuc vs. plaque potency

Conclusions: Determined there was a correlation between the NanoLuc and plaque potency assays

Evaluate optimum dilution for curve generation

Goal: Determine the optimized dilution to obtain a standard curve and evaluate parallelism

A)



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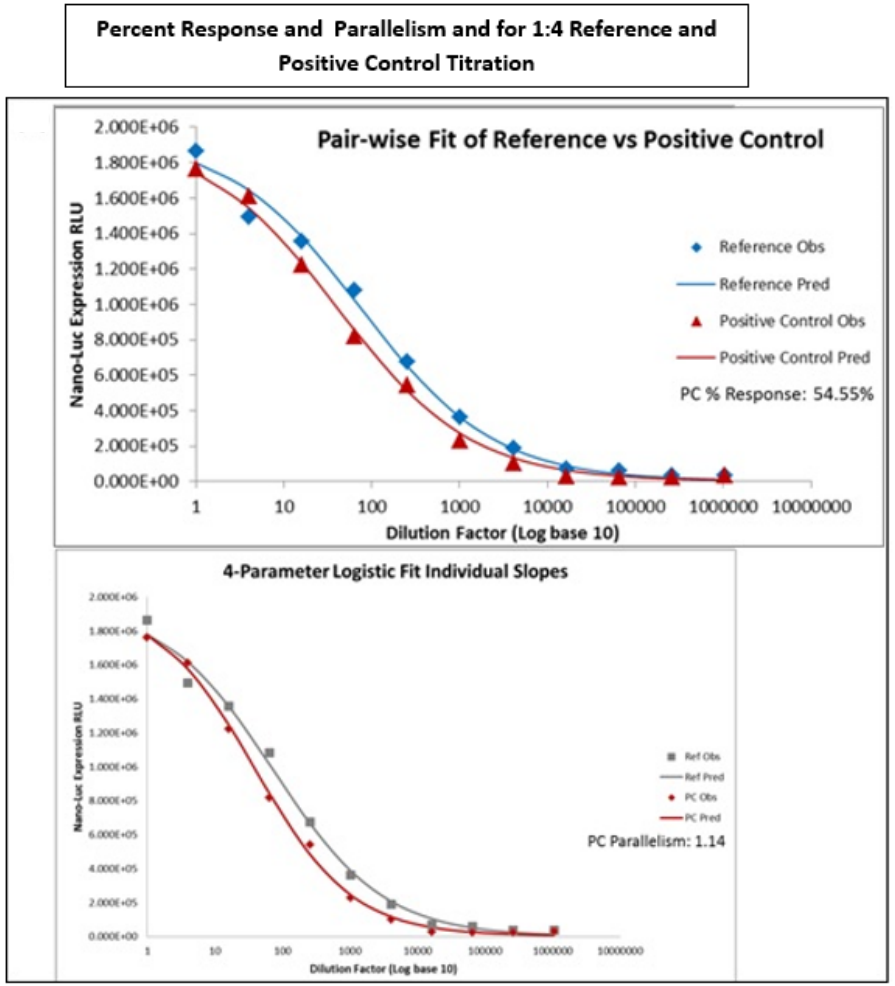

Figure 8. A) Experimental design for rVSV-ΔG-ZEBOV-GP positive control and reference and four parameter fit with 1:4 and 1:5 dilutions; B) Common slope and Individual fits models

Conclusion: 1:4 dilution had better linearity and parallelism closer to 1

Plate format evaluation

Goal: Perform a statistical evaluation of the sample plate

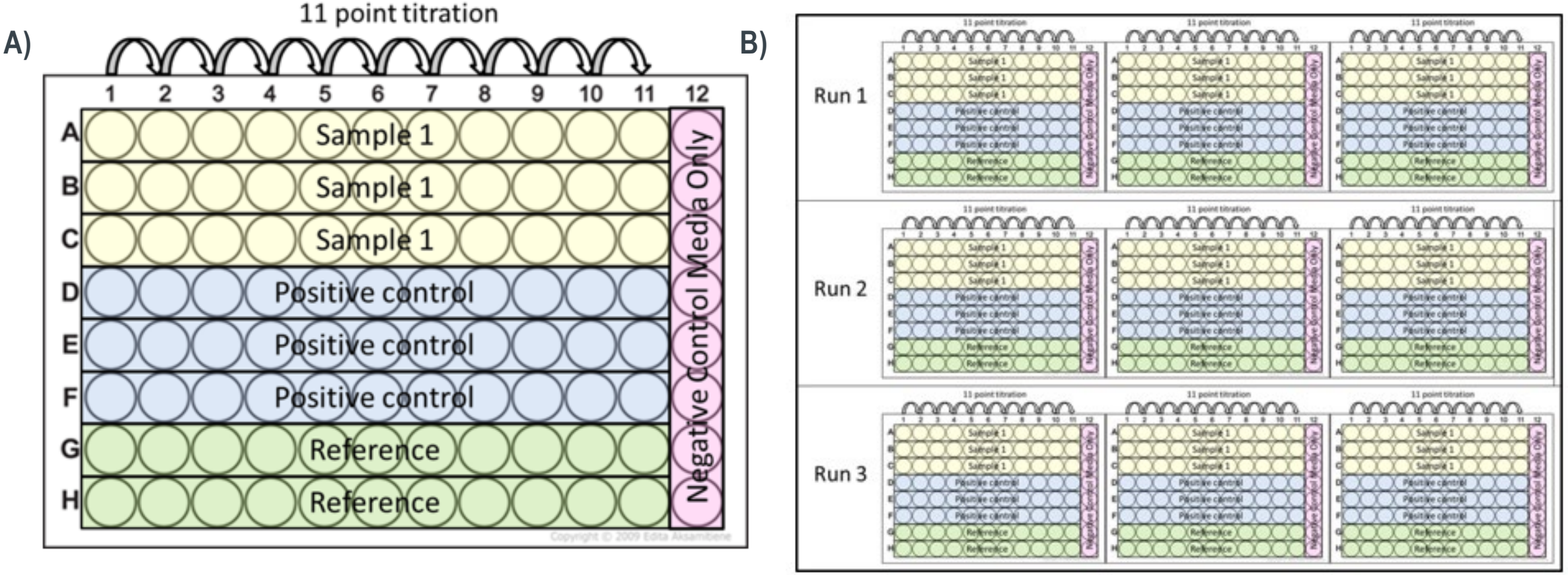

Figure 9. A) Plate layout, B) Design for replicate-to-replicate (Intra-plate), plate-to-plate (Inter-plate), and run-to-run

Summary of Statistical Evaluation for the JM-1 Cell-Based Reporter Assay

Table 1. JM-1 Assay Evaluation

Variance Components Analysis, ANOVA	
Rep-to-Rep (Intra-Plate)	22%
Plate-to-Plate (Inter-Plate)	18%
Run-to-Run	5%
Assay Variability	29%

JM-1 Cell-Based Reporter Assay: Final Plate Format

Goal: Establish the 96-well plate format for the JM-1 NanoLuc assay

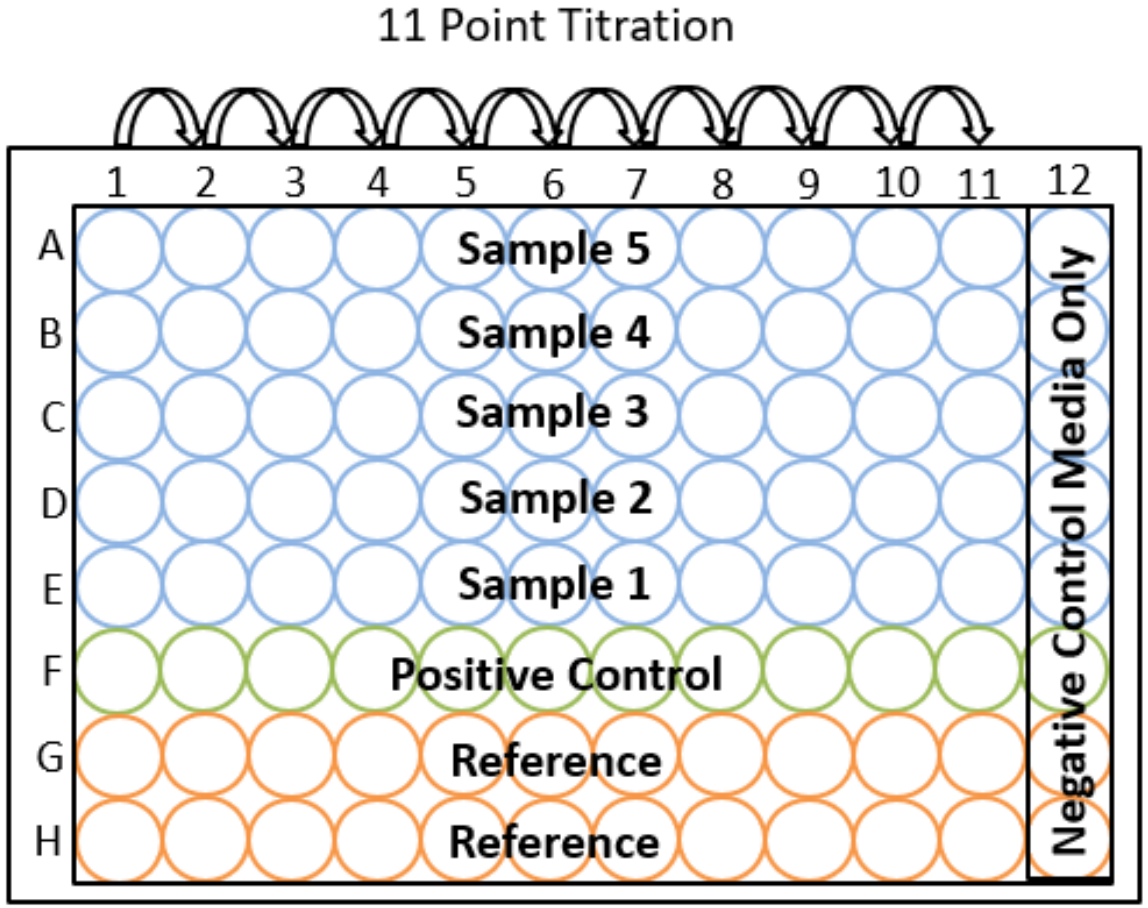

Figure 10. Plate format for the JM-1 NanoLuc assay

JM-1 Cell Line: Characteristics of the NanoLuc and plaque potency assays

Table 2. Comparison of the JM-1 and plaque potency assays

Reagent / Item	JM-1	Plaque Potency Assay
Tissue Culture Plate	3 (96-well)	~11 (12-well)
Detection Reagents, volume	Substrate, <2 mL	Coomassie blue, up to 48 mL/plate
Measurement	Automated/ Luminometer	Manual Counting with a light box
Total assay time and data reporting	1 week	1 week

Conclusions

- Developed a Nanoluc reporter cell line, JM-1 for rVSV-ΔG-ZEBOV-GP
- Demonstrated a correlation between the cell-based reporter and plaque potency assays
- Demonstrated JM-1 was stability-indicating for rVSV-ΔG-ZEBOV-GP
- Established an assay for the JM-1 cell line and completed a statistical evaluation

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