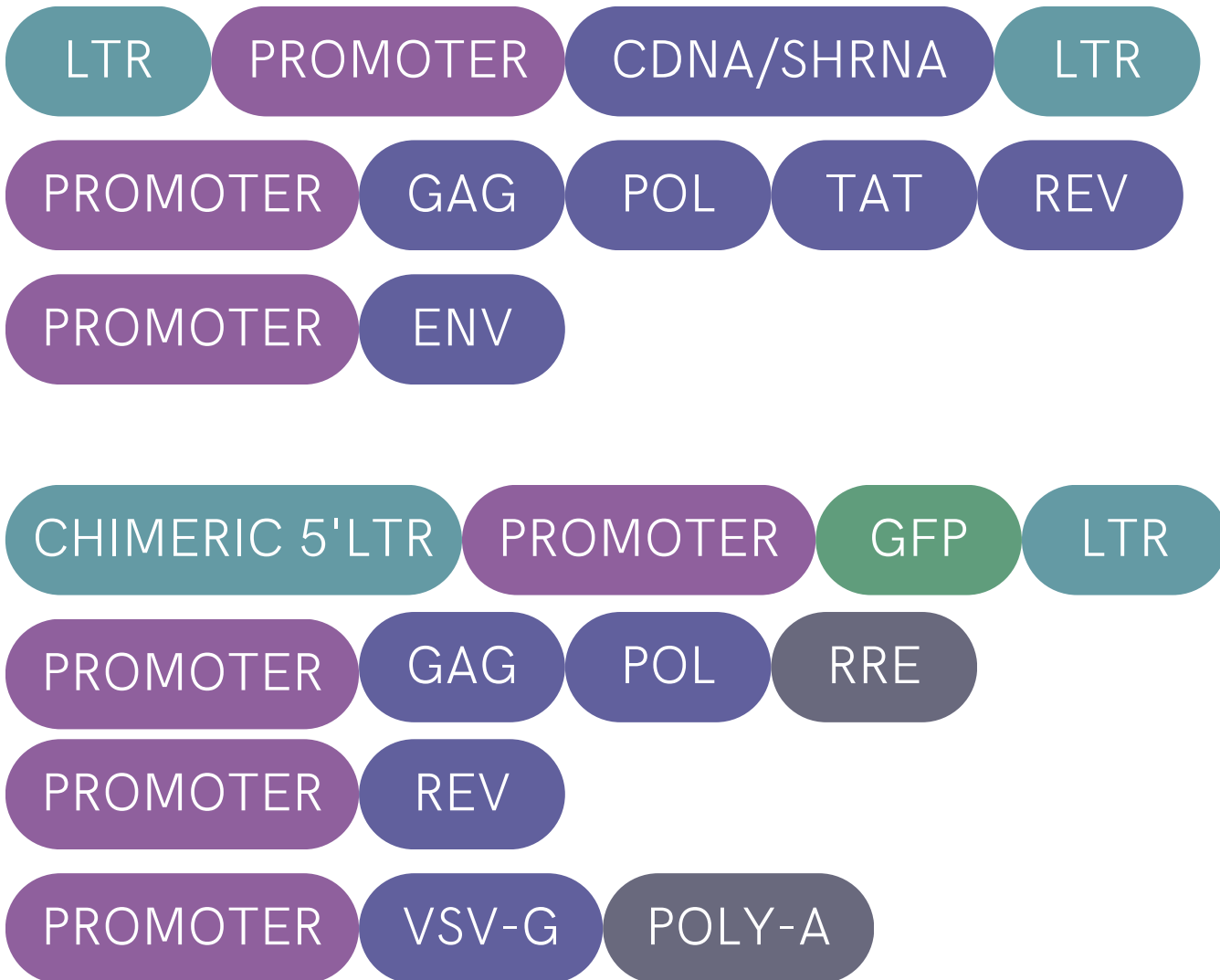


PROGRESS IN THE DEVELOPMENT OF AN IN-HOUSE 3RD GENERATION LENTIVIRAL SYSTEM

Esme **ALL GRAPHICS PREPARED BY STUDENT RESEARCHER**

INTRODUCTION

- Lentiviral vector is a retrovirus used for gene delivery
- 2 generations developed
 - 2nd gen: cancer causing
 - 3rd gen: low viral titer, but better gene expression



OBJECTIVE

This study aims to assess the progress of proprietary of a lab-manufactured 3rd generation lentiviral vector, by comparing viral titers to a 2nd generation system as a control.

METHODOLOGY

Plasmid production:

- Transforming E.coli in penicillin broth, extraction with Maxi-prep
- Plasmid analysis using spectrophotometry to measure purity/concentration, and gel electrophoresis to gauge size

Cell culture:

- HEK293T

Transfection Efficiency:

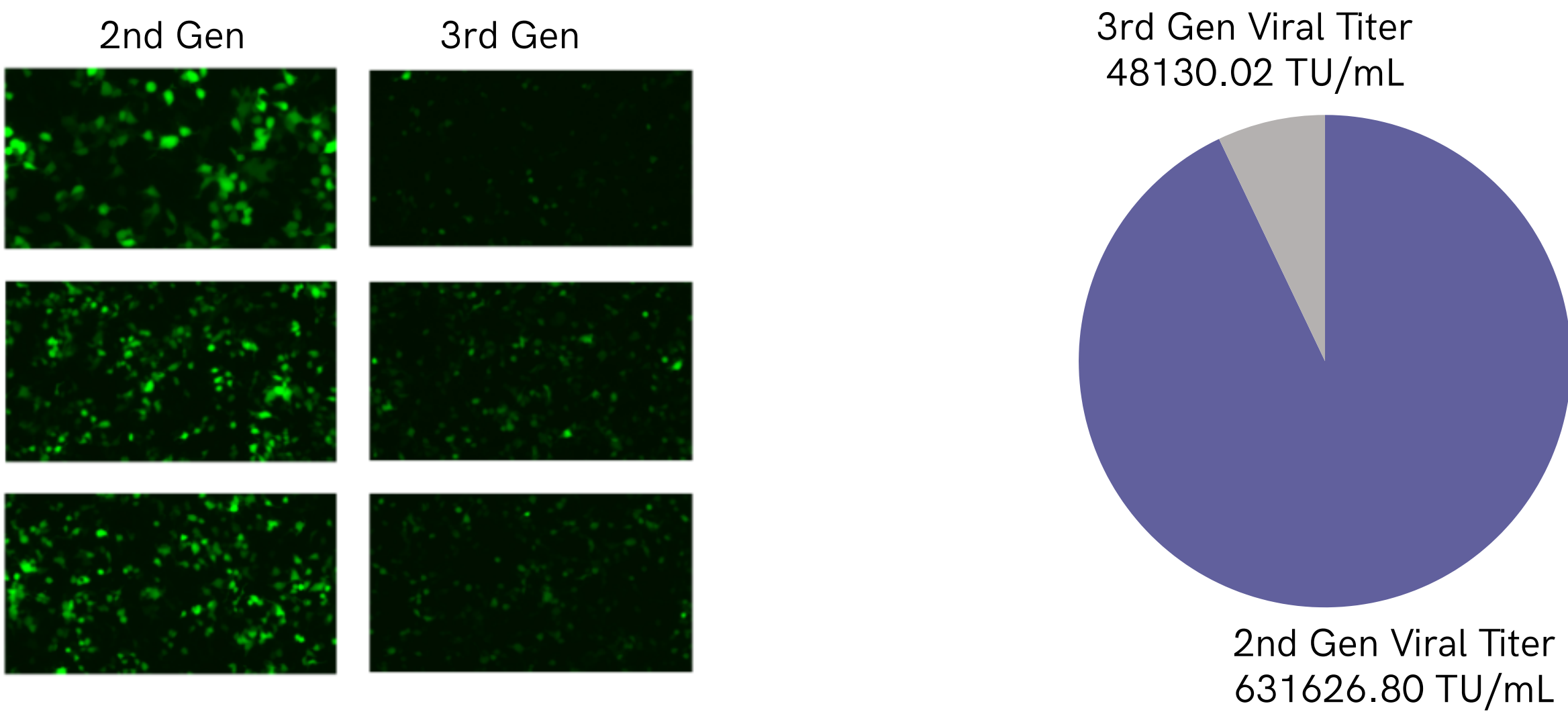
- FITC fluorescence imaging, Takara Lenti-X Go-Stix, and qPCR for quantification of viral titer

Transduction Efficiency:

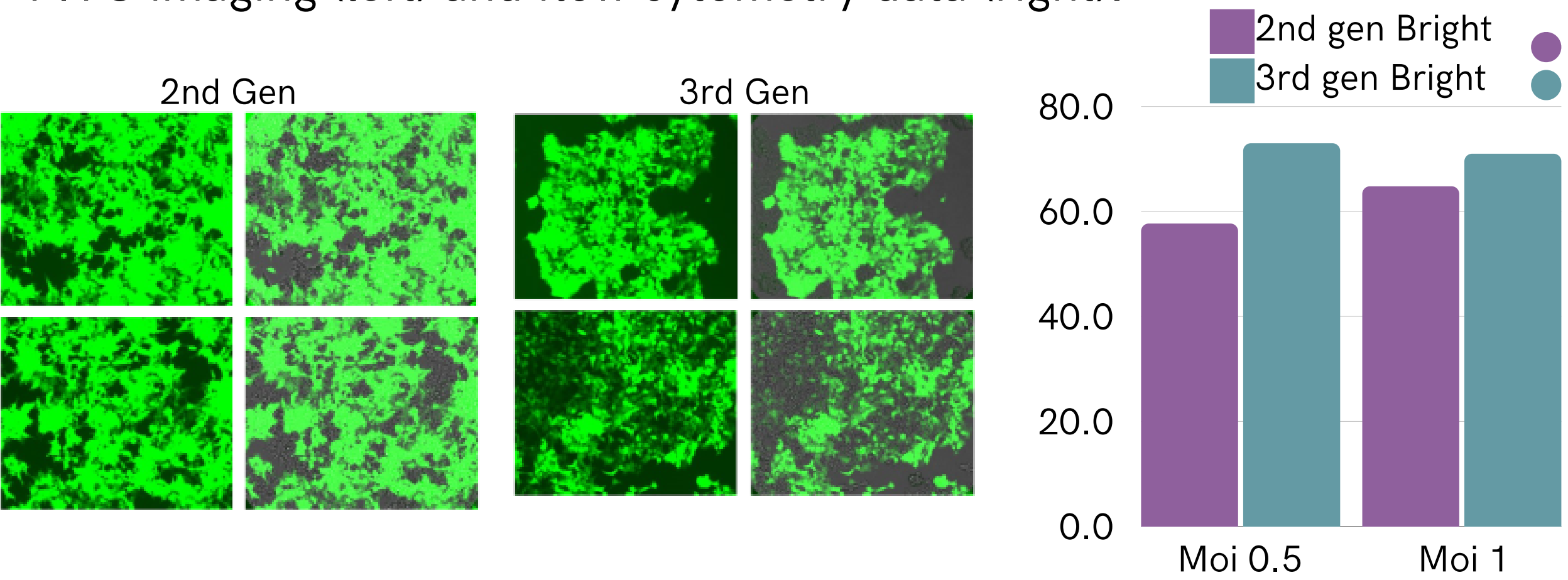
- FITC fluorescence imaging, and flow cytometry for infection rate

RESULTS/FINDINGS

After transfection, the 2nd generation produced 13x more viral titer than the 3rd generation. This is shown by the FITC imaging (left) and qPCR data (right).



After transduction, the 2nd and 3rd generation viruses were infected at a similar rate, with the 3rd generation showing more gene expression longevity. This is shown by the FITC imaging (left) and flow cytometry data (right).



CONCLUSION

The 3rd generation produced unsatisfactory results when measuring its viral titer. This means that the viral plasmids need further development, which is corroborated by the packaging REV plasmid's high contamination when tested before experimentation. However, its ability to infect cells as a completed virus is on par with the 2nd generation, reinforcing its ability to express target genes better after infecting a host cell. Future research will have to be conducted on plasmid design and transfection protocol.