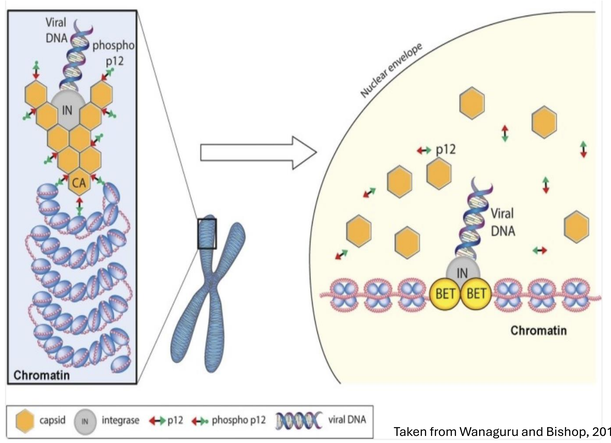
Tracking DNA-Virus Interactions Throughout the Cell Cycle

Joshua Glick, Michelle Kortyna, and Dr. Ashwanth C. Francis
Department of Biological Sciences, Institute of Molecular Biophysics
Florida State University, Tallahassee, FL 32306

FSU UNDERGRADUATE RESEARCH OPPORTUNITY PROGRAM
CENTER FOR UNDERGRADUATE RESEARCH & ACADEMIC ENGAGEMENT

INTRODUCTION

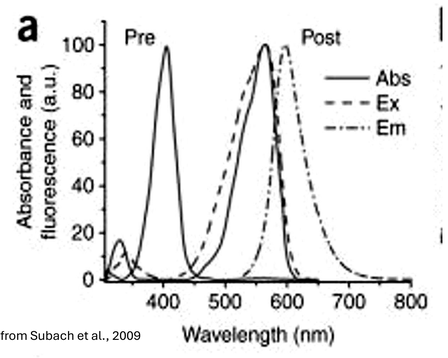
Murine Leukemia Virus (MLV) is a gammaretrovirus which means it cannot enter the nucleus during interphase. During Mitosis, the virus attaches to host chromatin then detaches once the daughter cells are fully formed (Fig. 1). The virus then moves rapidly throughout the nucleus before reattaching and integrating its vDNA into the host genome.



Taken from Wanaguru and Bishop, 2018

Fig. 1. MLV Accesses the host genome during mitosis

2. Photoactivatable fluorescent proteins do not emit fluorescence unless they are exposed to light of a shorter wavelength. Thus, they require 2 wavelengths of light, one which produces a conformational change in the protein and a second wavelength that can excite their fluorescence (Fig.2). By using a photoactivatable fluorophore, we could specifically activate fluorescence in a given region of interest and visualize specific processes, as opposed to viewing a large section at one time.



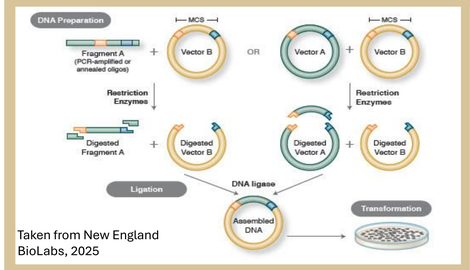
Taken from Subach et al., 2009

Fig. 2 A 405nm light is required to activate PAmCherry

PROJECT GOALS

- Cloning a pLenti-PAmCherry-H2B plasmid
- Using the plasmid to make vectors
- Transduce stable cell line

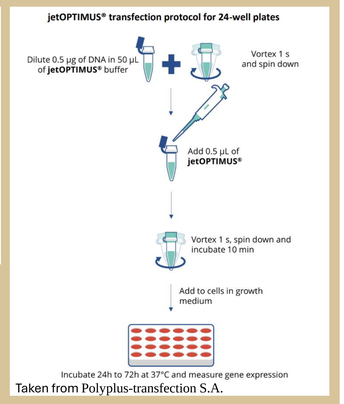
METHODS



Taken from New England BioLabs, 2025

Fig. 3A Schematics of Cloning procedure

- PAmCherry2 enzymes
  - Xho1
  - BamH1(BSTH1)
- H2B enzymes
  - Age1
  - Xho1



Taken from Polyplus-transfection S.A.

Fig. 3B Schematics of plasmid transfection

RESULTS

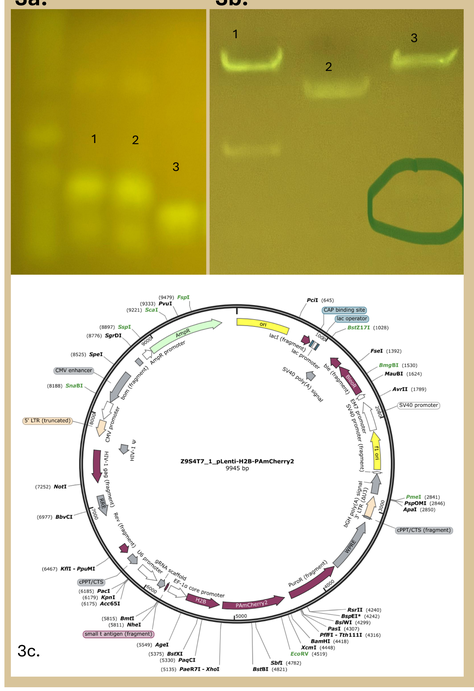


Image 3a.
PCR Products
1. PAmCherry1~(700bp)
2. PAmCherry2~(700bp)
3. H2B~(200bp)

Image 3b.
1. Cut pLenti-ebfp2-Lamin
2. Uncut pLenti-H2B-PAmCherry2
3. Cut pLenti-H2B-PAmCherry2

Image 3c.
This is a map of the ligated pLenti-H2B-PAmCherry2 plasmid

VALIDATION

Future Directions

- Test photoactivation in live cells
- Establish stable cell line expressing photoactivatable mCherry-H2B
- Infect that cell line with fluorescent MLV and track virus-DNA interactions
- Future implications include pharmaceutical use and further inhibition of MLV

ACKNOWLEDGEMENTS

I would like to thank Dr. Ashwanth C. Francis, Graduate Student Michelle Kortyna, and the Francis-Lab team as a whole for having helped me through this project.

REFERENCES

Polyplus-transfection S.A. (2020). *jetOPTIMUS® in vitro DNA transfection reagent protocol (CPT117 vD).* Polyplus-transfection S.A. https://www.polyplus-transfection.com/

Subach, F. V., Patterson, G. H., Manley, S., Gillette, J. M., Lippincott-Schwartz, J., & Verkhusha, V. V. (2009). Photoactivatable mCherry for high-resolution two-color fluorescence microscopy. *Nature Methods*, *6*(2), 153–159. https://doi.org/10.1038/nmeth.1298

Tirabassi, R. (n.d.). *Foundations of molecular cloning: Past, present, and future*. New England Biolabs. Retrieved March 3, 2025, from https://www.neb.com/en-us/tools-and-resources/feature-articles/foundations-of-molecular-cloning-past-present-and-future

Wanaguru, M., & Bishop, K. N. (2018). Gammaretroviruses tether to mitotic chromatin by directly binding nucleosomal histone proteins. *Microbial Cell*, *5*(8), 385-388. https://doi.org/10.15698/mic2018.08.643