

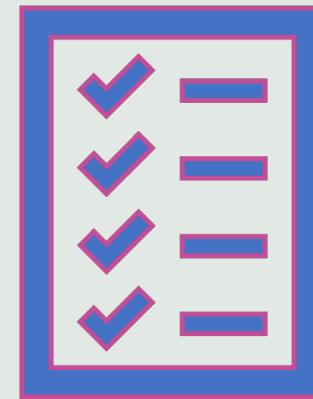
# **Design and Construction of Landing Pad Cell Lines targeting Specific Safe Harbor Sites**

Presenter: Irina Zhu

Supervisor: Michael Garton & Aaron Rosenstein

# Outline

- Overview
- Design Features
- Methodology
- Experiment Progress
- Potential Applications/General Protocol\*
- Future Improvements and Directions



# Overview

A landing pad cell line is a pre-engineered with integrated genetic elements that facilitate payload exchange with a donor plasmid coding for a specific promoter and gene of interest used by recombinase mediated cassette exchange(RMCE) [1].



[1]"Cell Engineering Safe Harbor Landing Pad Cell Lines," doi: 10.1534/genetics.111.131433.

# SHS231 Safe Harbor sites

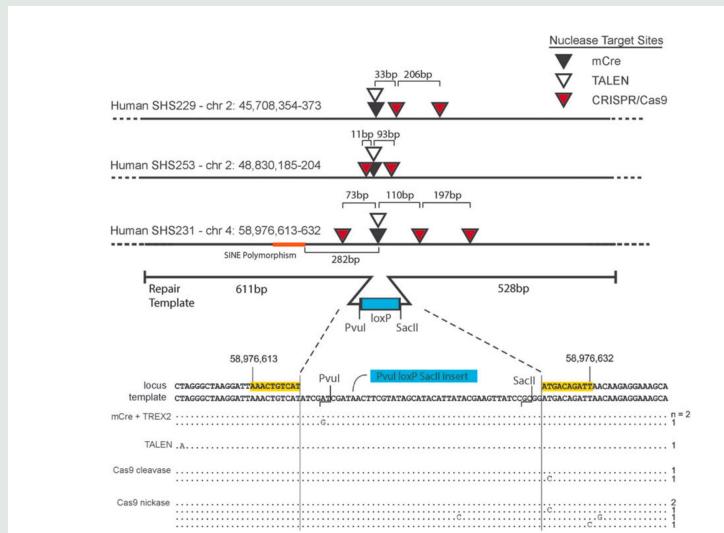


Figure 1. Structure of three representative new target sites indicating location of *mCre*, *Cas9*, and *TALEN* target sites. The top two sequence diagrams detail features of the chr2 SHS229 and SHS253, whereas the bottom diagram provides additional detail and results on the chr 4q SHS231 [2].

[2] S. Pellenz et al., "New Human Chromosomal Sites with 'Safe Harbor' Potential for Targeted Transgene Insertion," *Hum. Gene Ther.*, vol. 30, no. 7, pp. 814–828, Jul. 2019, doi: 10.1089/hum.2018.169.

# Design Features

An Interchangeable  
promoter and expression  
cassette

Unique Bxb1-attP sites  
flanking each module

Single copy integration into  
the SHS231 Safe Harbor  
locus

Red/Green/Blue  
Fluorescent protein – for  
identifying and sorting for  
successful integration

Puro/Hygro/Neo Antibiotic  
resistance markers – for  
identifying and sorting for  
successful integration

Human embryonic Kidney  
Cells (HEK 293)

# Methodology

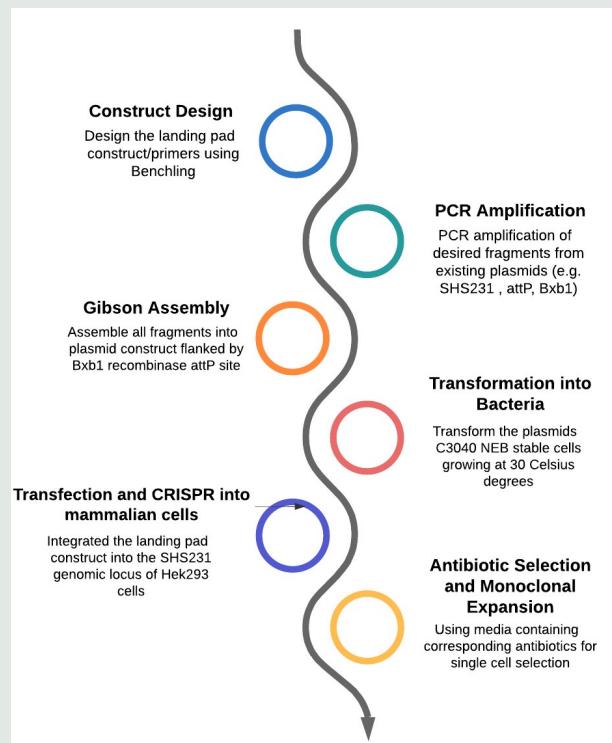


Figure 2. Method Workflow

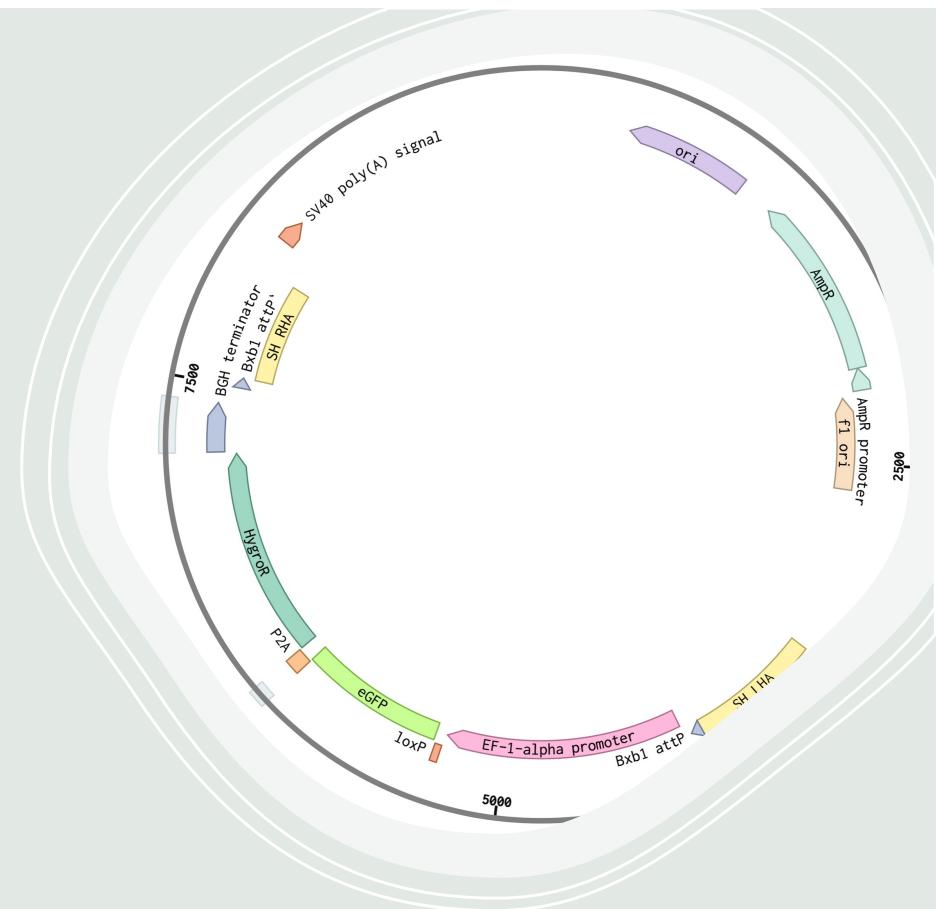


Figure 3. Sample Plasmid Design of SHS231-EF1-RFP-HYGRO from Benchling

# Experiment Progress/Result

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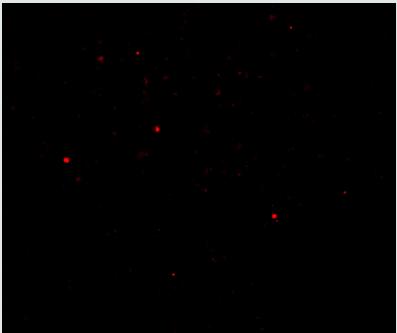
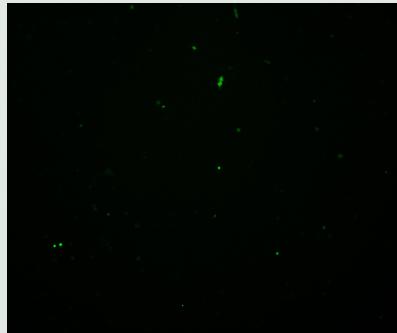


Figure 4. Images of HEK293 after CRISPR/Cas9 transfection under FL(day1)

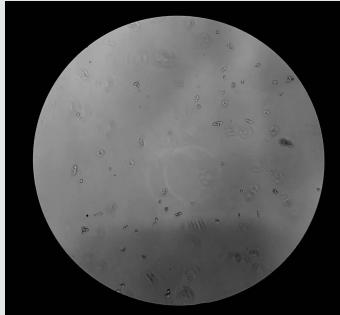


Figure 5. Image of HEK293 after transfection under BF (day4)

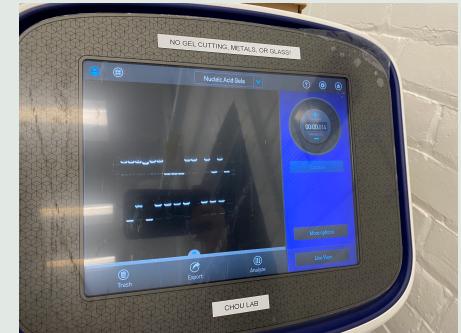
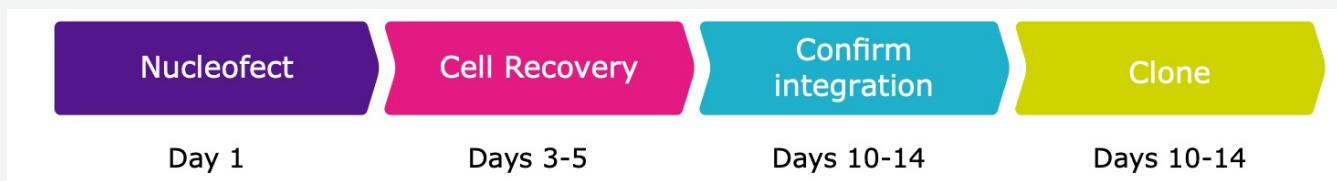


Figure 6. Colony PCR check where recombinases occur (the bottom line)



## Potential Application – Stable Transfection

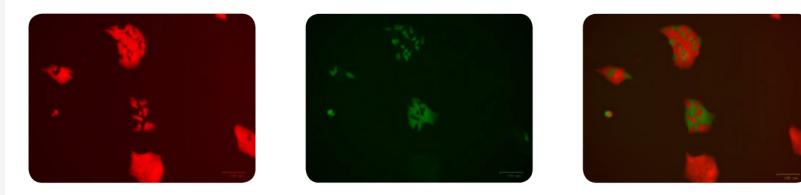
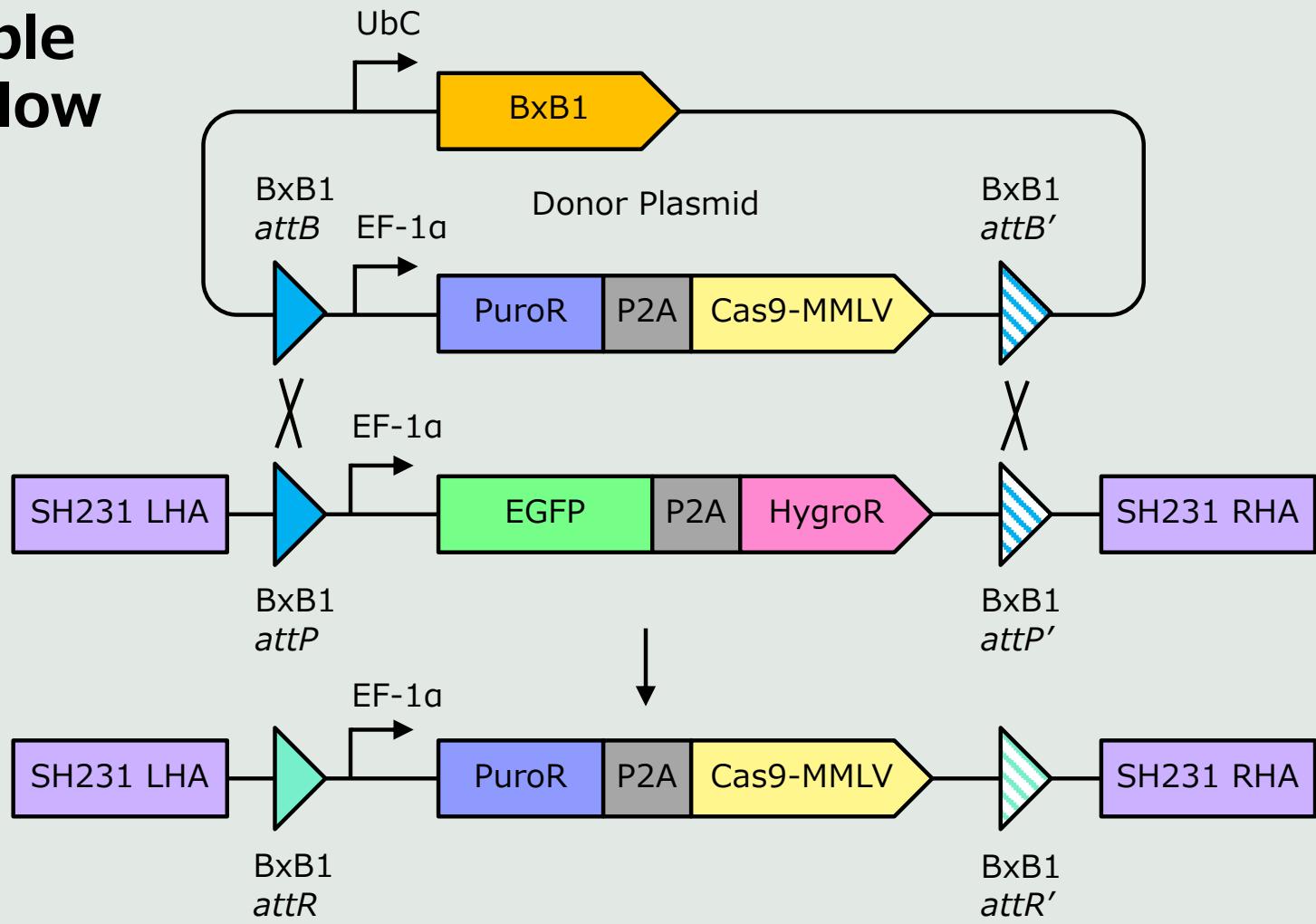


Figure 7. Exchange of Landing Pad payload exchange using Cre recombinase and targeting vector with appropriate LoxP elements [3]

[3] M. C. Inniss et al., "A novel Bxb1 integrase RMCE system for high fidelity site-specific integration of mAb expression cassette in CHO Cells," *Biotechnol. Bioeng.*, vol. 114, no. 8, pp. 1837–1846, Aug. 2017, doi: 10.1002/bit.26268.

# Example Workflow



# Future Improvement

- Fluorescence Marker Choice: Brighter red fluorescence protein
- Antibiotic Choice: Perform Antibiotic Kill Curve to measure the appropriate concentration
- Technological Improvements:
  - 1) Mammalian electroporation for transfection greatly improve efficiency comparing to lipofectamine chemical transfection
  - 2) Flow cytometry to perform cell selection according to fluorescence markers makes generating new landing pad cell variants faster.
  - 3) Using TK negative selection for unrecombined cells.

## **Future Direction**

- Landing Pad Cell lines targeting different safe harbor sites for specific applications
- Landing Pad Cell lines with different cells



**Thank you!**