

A complex network diagram with numerous nodes of various colors (blue, green, red, purple, grey) connected by thin grey lines. The nodes are scattered across the frame, with a higher density on the right side. A large, faint circular graphic is visible on the left side of the background.

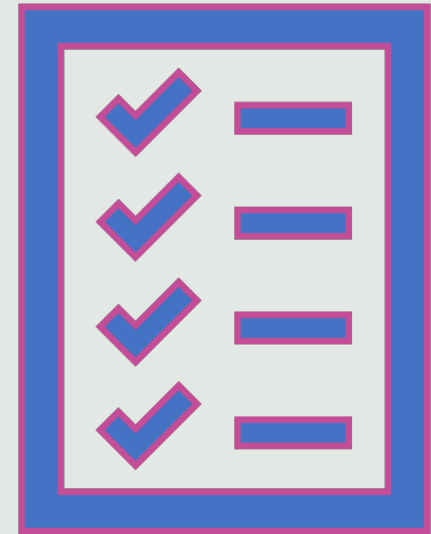
# **Design and Construction of Landing Pad Cell Lines targeting Specific Safe Harbor Sites**

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# Outline

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



# Overview

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



# SHS231 Safe Harbor sites

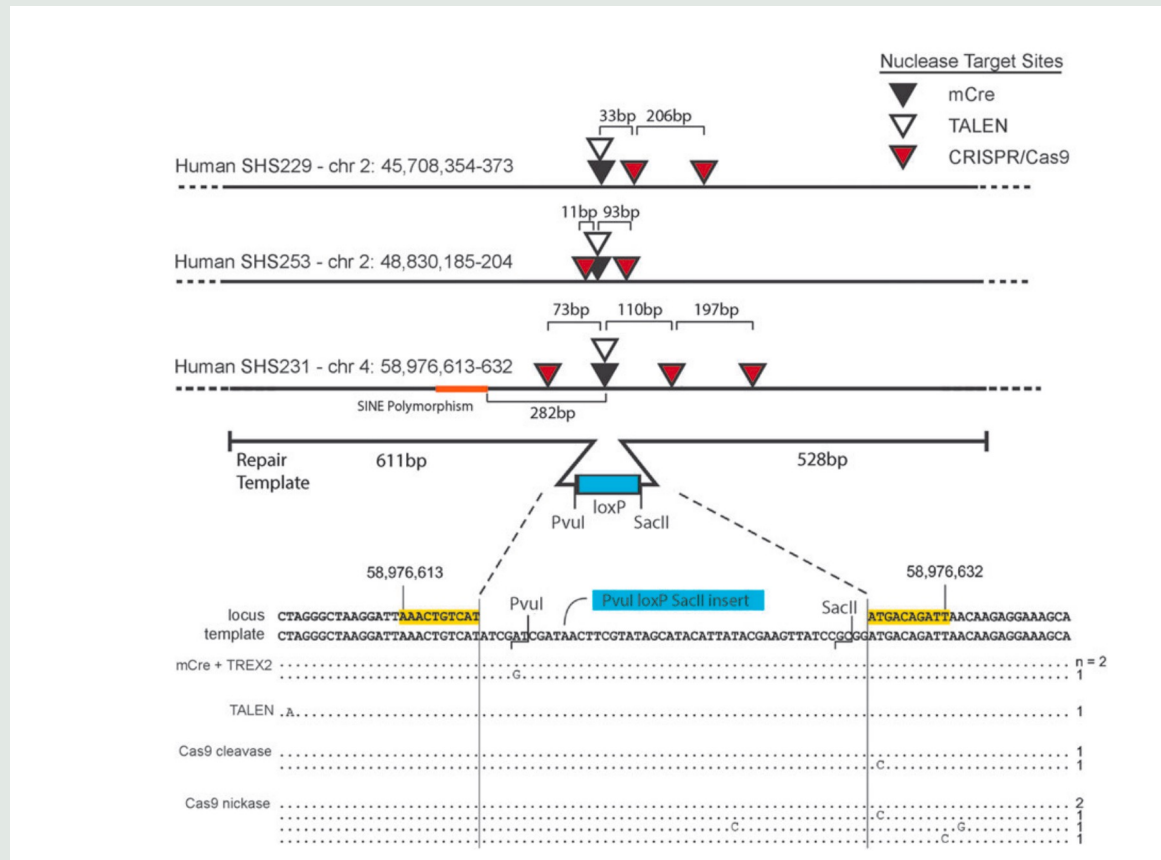


Figure 1. Structure of three representative new target sites indicating location of mCreI, Cas9, and TALEN target sites. The top two sequence diagrams detail features of the chr2 SHS229 and SHS253, whereas the bottom diagram provides additional detail and results on the chr 4q SHS231.

[2]

# Design Features

An Interchangeable promoter and expression cassette

Unique Bxb1 sites flanking each module (after EF1A as modification on the original plasmid)

Single copy integration into the SHS231 Safe Harbor locus

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

Puro/Hygro/Neo Antibiotics – for identifying and sorting for successful integration

Human Hek293 Cells

# Methodology

**Construct Design**

Design the landing pad construct/primers using Benchling

**PCR Amplification**

PCR amplification of desired fragments from existing plasmids (e.g. SHS231 , attP, Bxb1)

**Gibson Assembly**

Assemble all fragments into plasmid construct flanked by Bxb1 recombinase

**Transformation into Bacteria**

Transform the plasmids C3040 NEB stable cells growing at 30 Celsius degrees

**Transfection into Mammalian Cells**

Integrated the landing pad construct into the SHS231 genomic locus of Hek293 cells

**Antibiotic Selections**

Using media containing corresponding antibiotics for single cell selection



Figure 2. Method Workflow

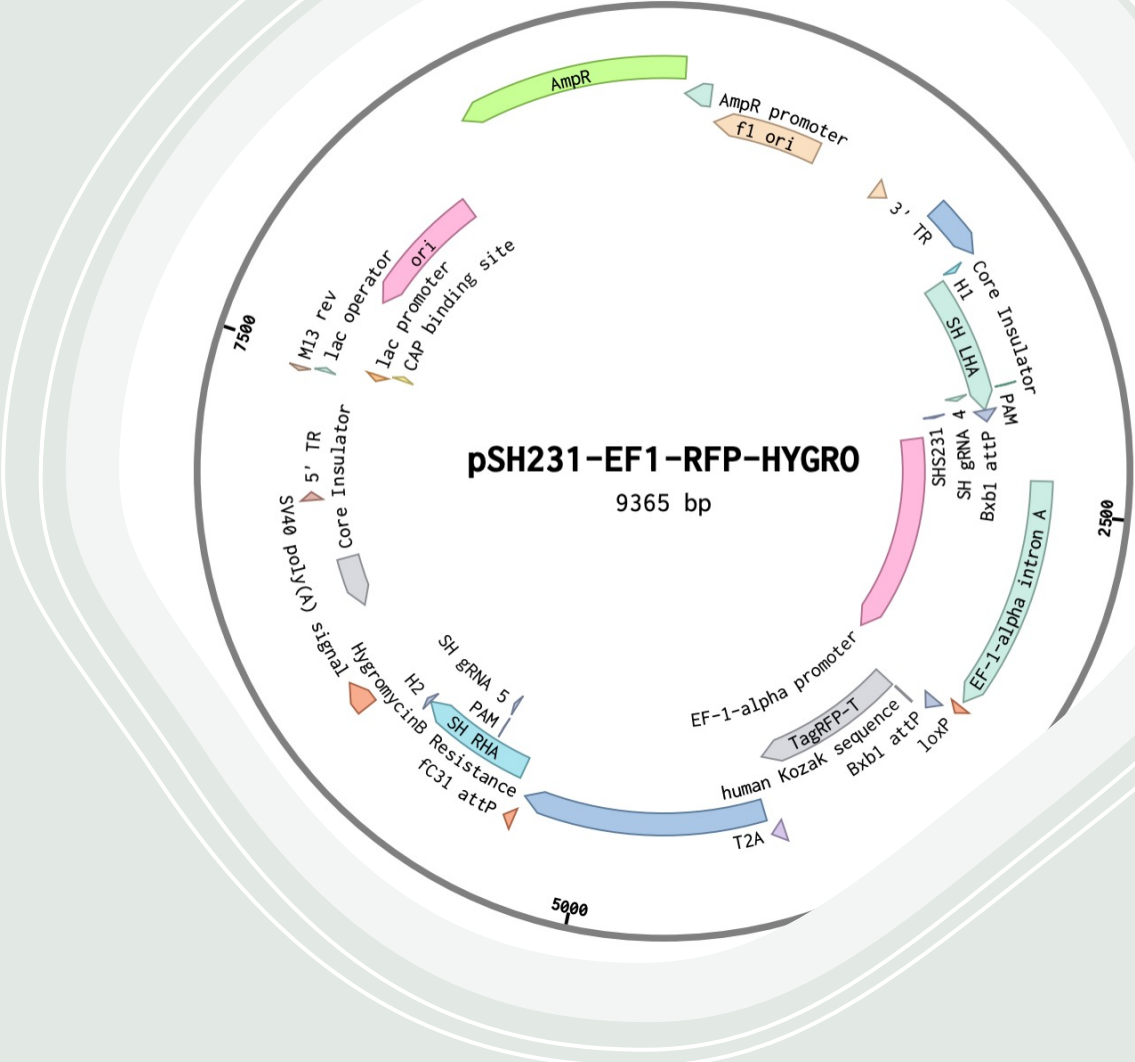


Figure 3. Sample Plasmid Design of SHS231-EF1-RFP-HYGR0 from Benchling

# Experiment Progress / Result

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Figure 4. Images of HEK293 after CRISPR/Cas9 Transfection (day 1)

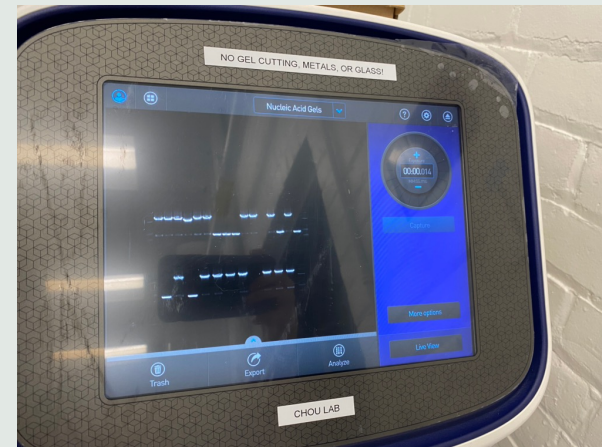


Figure 5. Colony PCR check where recombinases occur (the bottom line)



## Potential Application – Stable Transfection

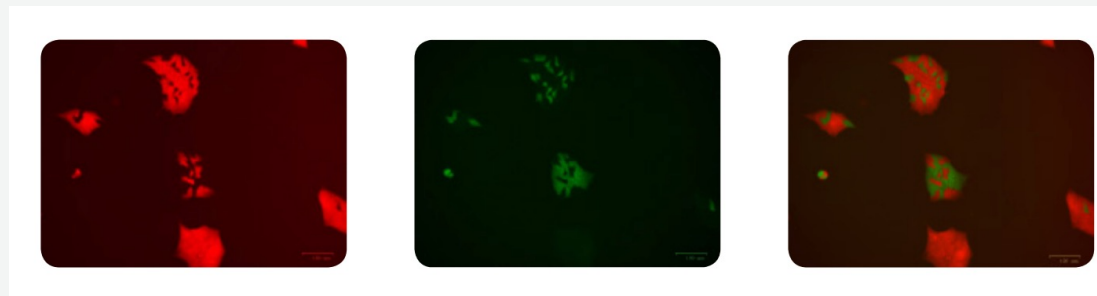


Figure 6. Exchange of Landing Pad payload exchange using Cre recombinase and targeting vector with appropriate LoxP elements [3]



# Future Improvement

- Florescence marker choice: Brighter red florescence protein
- Antibiotic Choice: Perform antibiotic kill curve to ensure the appropriate concentrations
- Technological Improvements:
  - 1). Mammalian electroporation for transfection greatly improve efficiency comparing to lipofectamine chemical transfection
  - 2). Flow cytometry to perform cell selection according to florescence markers

# Future Direction

- Landing Pad cell lines targeting different safe harbor sites for specific applications



**Thank you!**