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

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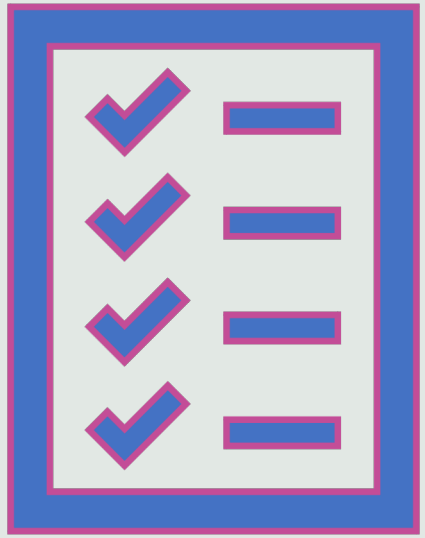
Outline

- 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

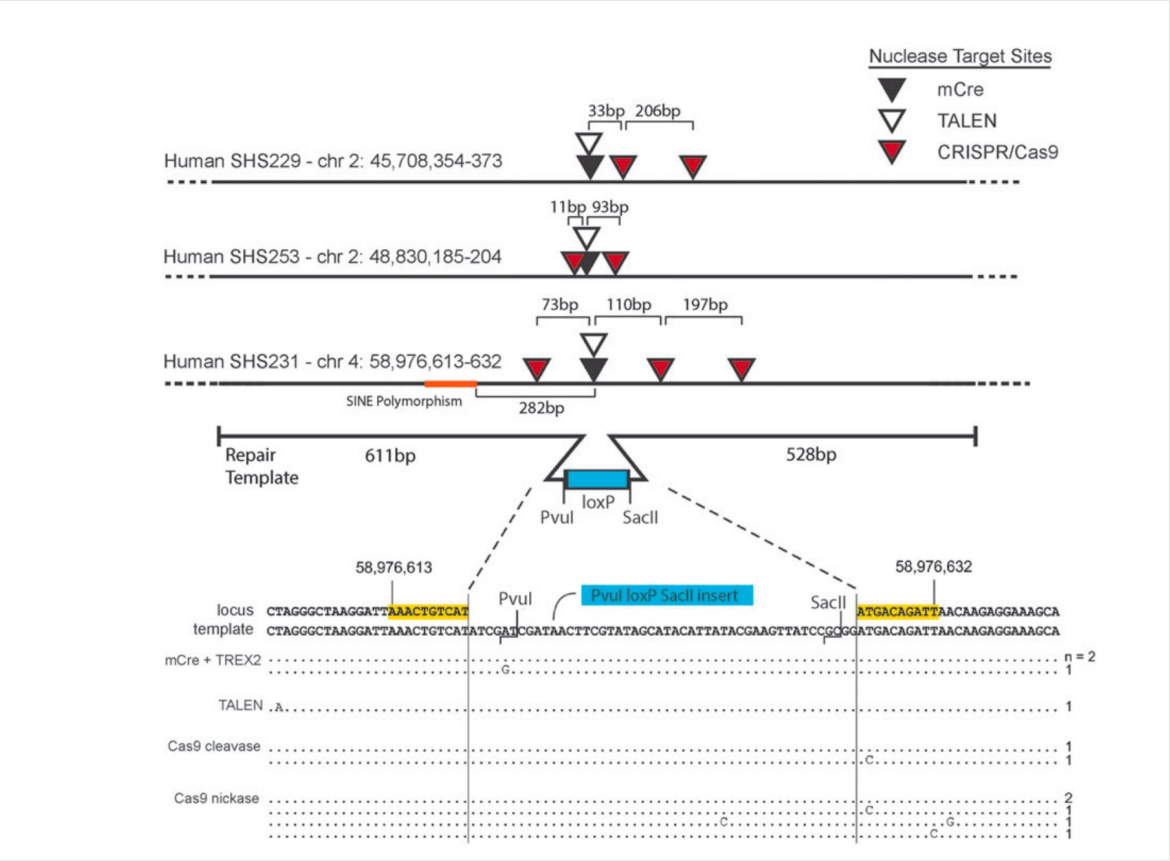


Figure 1. Structure of three representative new target sites indicating location of mCreI, Cas9, and TALEN target sites. The top two sequence diagrams detail features of the chr2 SHS229 and SHS253, whereas the bottom diagram provides additional detail and results on the chr 4q SHS231.

[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

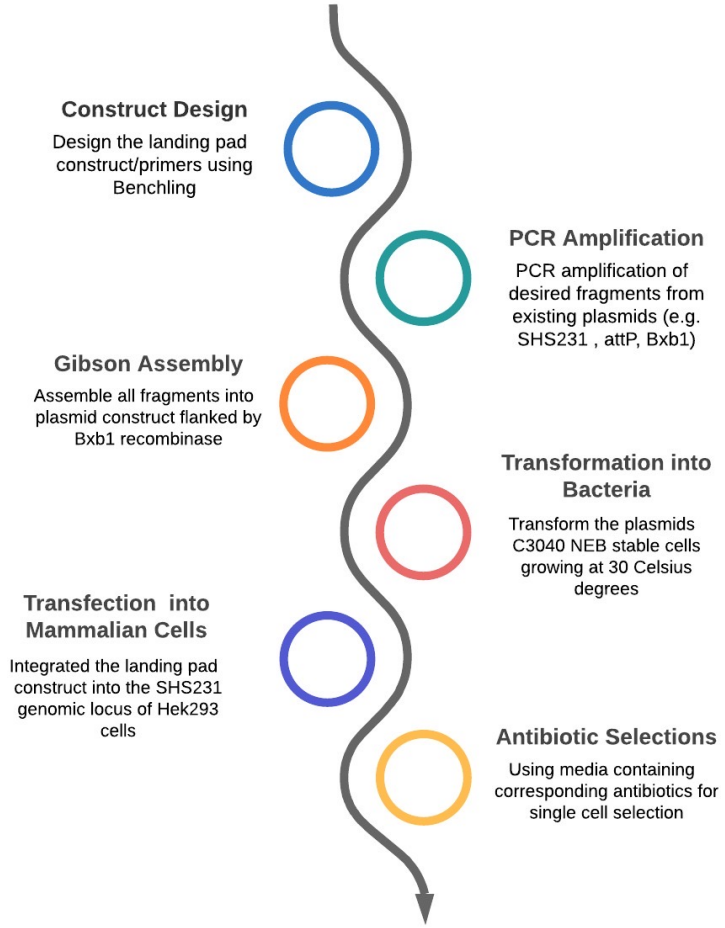


Figure 2. Method Workflow

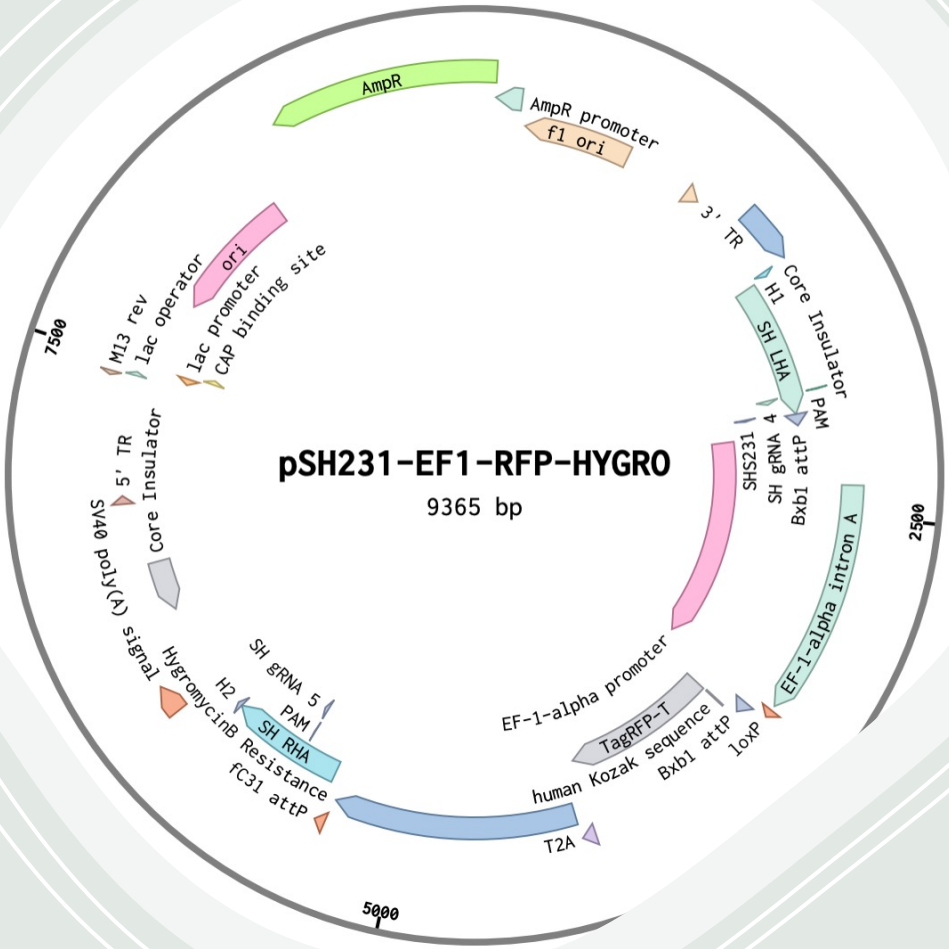


Figure 3. Sample Plasmid Design of SHS231-EF1-RFP-HYGRO from Benchling

Experiment Progress/Result

Figure 4.Images of HEK293 after CIRSPR/Cas9 Transfection(day1)

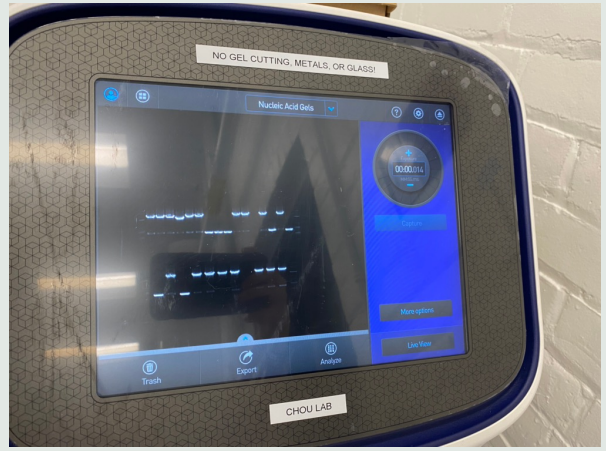


Figure 5.Colony PCR check where recombinases occur (the bottom line)

Potential Application – Stable Transfection

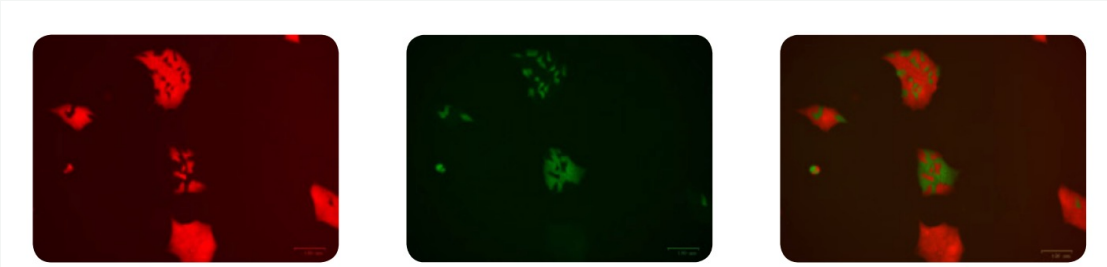


Figure 6. Exchange of Landing Pad payload exchange using Cre recombinase and targeting vector with appropriate LoxP elements [3]

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!