GENERATION OF A LANDING PAD T CELL LINE USEFUL FOR T CELL RECEPTOR CUSTOMIZATION

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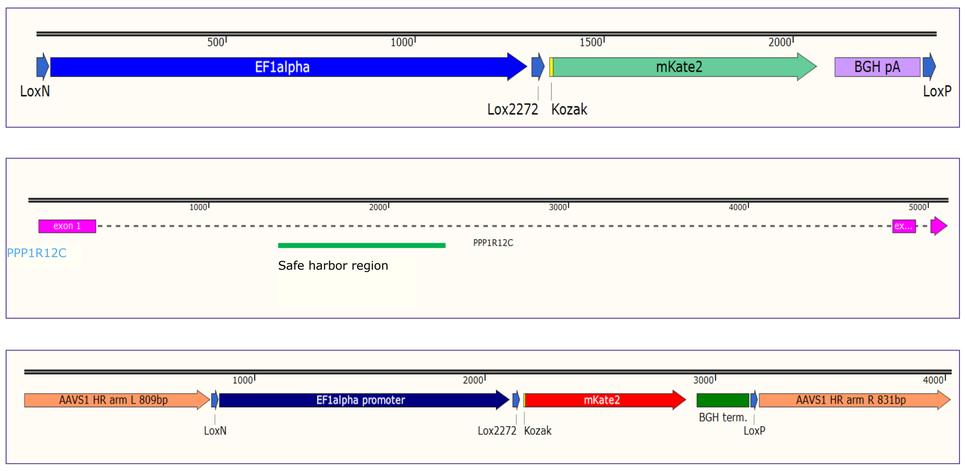
Introduction

T cell biology is integral to the study of normal immune regulation as well as cancer biology, CAR-T cells, epitope specificity and antigen presentation. However, primary T cells can be difficult to propagate in culture for the length of time necessary for functional assays. In addition, populations of primary T cells express variant T cell receptor (TCR) heterodimers that can be challenging to identify and may not be optimal for downstream studies. We sought to simplify this system using transformed T cells which can be grown in culture for extended periods of time. We engineered a floxed landing pad sequence into the safe harbor AAVS1 locus using CompoZr® zinc finger nucleases. Both the promoter and landing pad expression cassette are flanked by unique lox sites, allowing swapping of the promoter and/or expression cassette as needed. We ensured that only one copy of this sequence was found within the genome to avoid any complications associated with random insertion events. We also generated a landing pad cell line null for the endogenous TCR using targeted nucleases. Both the TCR alpha and beta loci were rendered null due to non-homologous end joining and the presence of insertions and deletions culminating in premature stop codons were genotyped using next generation sequencing. The absence of a functional TCR was validated using flow cytometry staining for surface TCR and CD3. This cell line was then used to generate a knock-in of the desired exogenous TCR heterodimer to the landing pad locus, verified using flow cytometry staining. These lines will be very useful for a multitude of studies where a researcher needs to express a gene of interest in a discrete genetic locus or wants to generate a panel of TCR expressing cell lines.

Landing pad design

- **Interchangeable promoter and expression cassette**
- **Unique lox sites flanking each module**
- **EF1α promoter is active in a wide variety of cell types**
- **mKate2 expresses a red fluorescent protein**
- **Integrated into the hAAVS1 safe harbor locus**



Methods

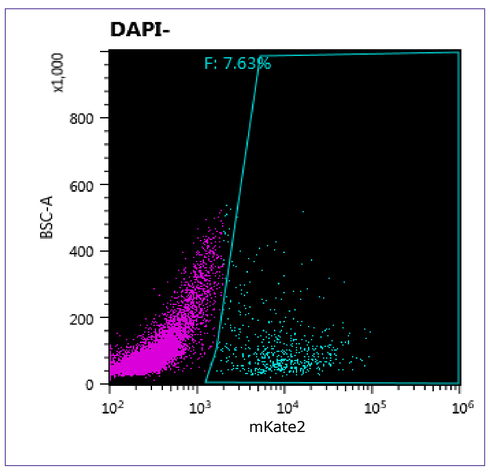


Figure 1
Top, flow cytometry dot plot of cells nucleofected with hAAVS1 ZFN RNA and landing pad donor DNA. Bottom, agarose gels indicating 5' and 3' junction PCR reactions. Lanes 1 and 4, donor DNA nucleofected cells alone. Lanes 2, 3, 5 and 6, ZFN+donor DNA nucleofected cells.

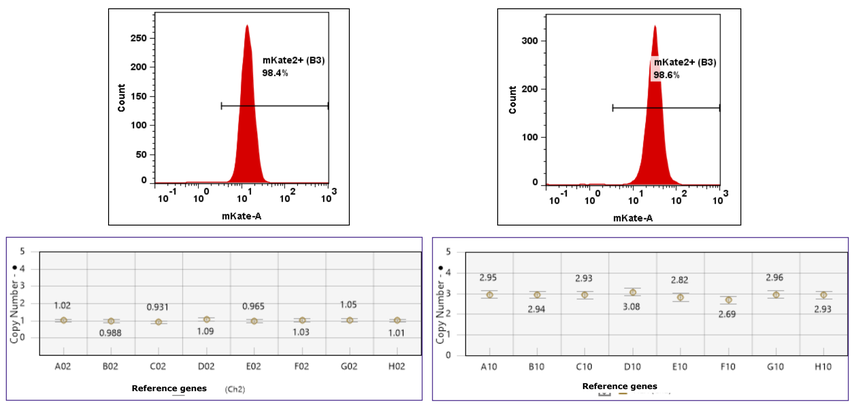


Figure 2
Copy number analysis of two representative clones. Left, copy number 1. Right, copy number 3. Top histograms are MACSQuant analysis for mKate2 expression, bottom graphs are calculated copy number of mKate2 normalized to eight different reference genes.0

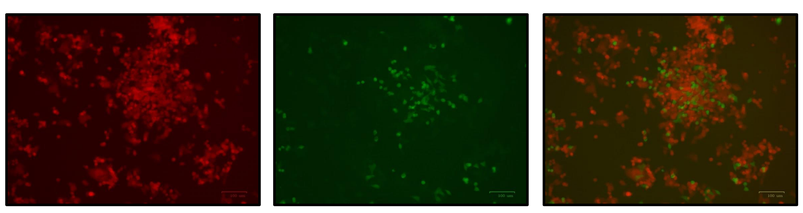


Figure 3
Fluorescence microscopy pictures of a landing pad integrated cell line Cre exchanged with a GFP donor cassette. Cells were nucleofected with Cre mRNA and a floxed GFP donor DNA. Left panel, mKate2 expression, middle panel, GFP expression, right panel, merged image.

T cell receptor swapping

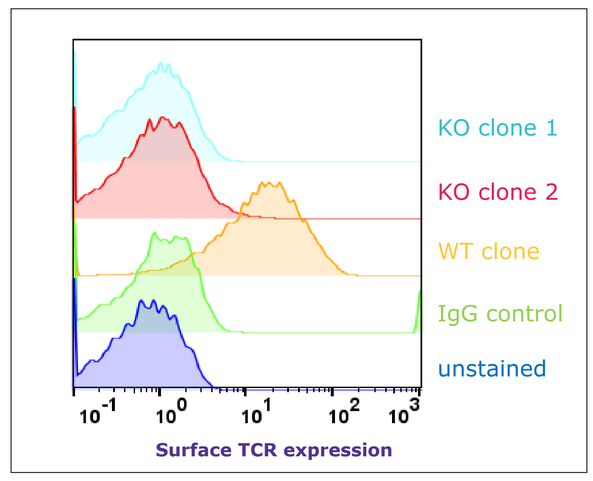
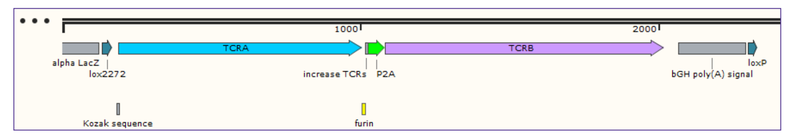
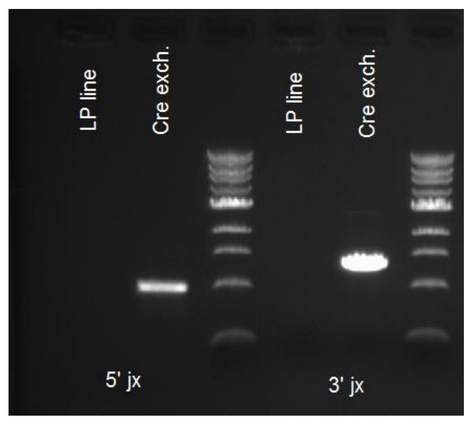


Figure 4
Verification of TCR knockout in Jurkat landing pad line. TCRα and β genes were knocked out using targeted nucleases and cells were stained with a pan-TCR antibody.





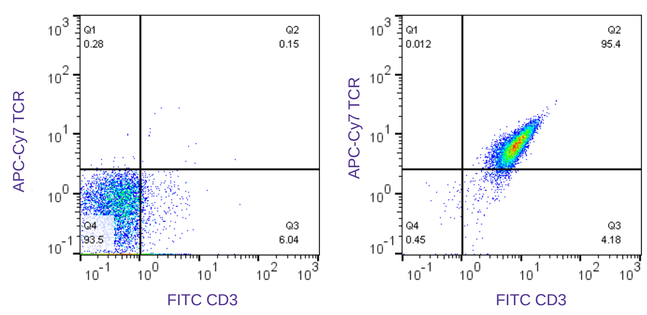


Figure 5
Cre-mediated TCR exchange. Jurkat landing pad/TCR knockout cells were nucleofected with Cre mRNA and floxed TCR donor. Genetic recombination was evaluated using junction PCR (top panel) and phenotyped using flow cytometry (bottom panel; TCR KO landing pad cells left plot, exchanged clone right plot).

Acknowledgements

Merck & Co. Palo Alto

- Sabine Le Saux
- Omar Nourzaie
- Aarron Willingham
- Rene de Waal Malefyt

Summary

Cell lines with integrated landing pad

- Jurkat human T lymphocytes
- A549 human lung epithelium
- HCT-116 human colon cells
- A375 human melanoma cells
- THP-1 human monocytes

Applications

- TCR screening
- Reporter Activation/Deactivation
- Antigen expression
- Promoter transactivation studies
- Your imagination is the limit!

Have Questions? Have Ideas?

Check out our webpage for the Safe Harbor Landing Pad Lines using the QR code or contact us Cellengineering@sial.com