

Cell Design Studio™

Endless Engineering Possibilities



Custom Engineering

Cell Design Studio at Sigma® Life Science works to provide solutions for your biological questions. Our experts have access to CRISPR and zinc finger nuclease (ZFN) technologies, giving us extensive flexibility in cell line engineering. Knockouts, knockins, tagging, and nucleotide-level gene editing – even at multiple alleles – are all possible. Let us show you what we can do.

Tagging Endogenous Alleles

Members of the JAK/STAT signaling pathway are important in cancer biology. Paradoxically, one STAT family member – STAT3 – is constitutively activated in numerous cancers, while another STAT family member – STAT1 – acts as a tumor suppressor. Because these proteins are related, identifying small molecules that inhibit STAT3 function while leaving STAT1 function intact has been challenging. We sought to engineer a solution to this problem by tagging each of these genes with a fluorescent protein, so their subcellular localization could be monitored in living cells. We also placed a luciferase reporter under the control of the endogenous Cyclin D1 promoter to act as a functional downstream readout of STAT3 activity.

Engineering Strategy

We employed CompoZr® ZFNs to target the C-terminus of STAT1, N-terminus of STAT3, and transcription start site of Cyclin D1 in A549 cells. Donor molecules with homology arms were designed to add an in-frame C-terminal GFP tag to STAT1 and an in-frame N-terminal RFP tag to STAT3 (Figure 1A), with each incorporating linker sequences to minimize the impact of the FP tag on function. For the Cyclin D1 promoter activity reporter, the luciferase donor included a 2A ribosomal skip sequence (Figure 1B).

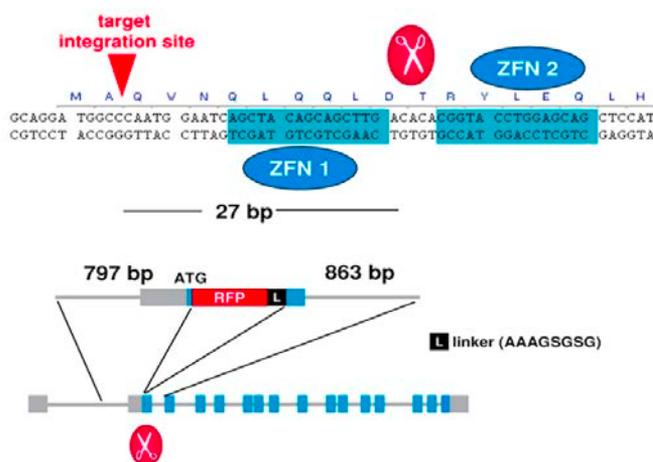


Figure 1A. Schematic of the STAT3 (Chr. 17) tagged locus, including the ZFN cut site, target integration site, and donor molecule with homology arms.

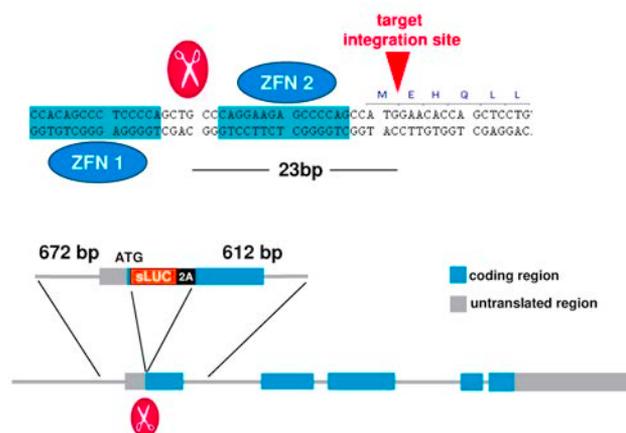


Figure 1B. Schematic of the Cyclin D1 (CCND1, Chr. 11) tagged locus, including the ZFN cut site, target integration site, and donor molecule with homology arms.

Functional Testing

Following isolation of single cell clones with all three alleles modified, we tested for normal activity of the tagged proteins. Cells were stimulated with IL-6 and IFN- γ and observed using fluorescence imaging. Consistent with the tagged proteins retaining normal activity, IL-6 specifically activated RFP-STAT3 while IFN- γ activated STAT1-GFP, both indicated by robust translocation of the tagged proteins into the nucleus (Figure 2).

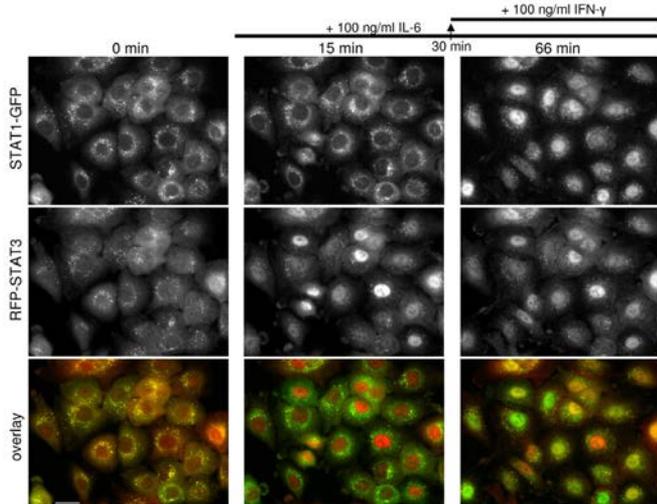


Figure 2. Live cell imaging of modified A549 cells following treatment with IL-6 and IFN- γ .

Furthermore, we found that inhibition of STAT3 using the small molecule Cpd3 not only prevented RFP-STAT3 translocation into the nucleus, but also significantly inhibited activation of the downstream target Cyclin D1, as indicated by luciferase activity derived from the sLuc-CCND1 reporter allele.

iPS Cell Engineering

Our cell engineering projects are not limited to immortalized cell lines. We have extensive experience using CRISPR and ZFN technologies to modify iPS cell lines. As an example of this ability, we engineered a Sigma-Aldrich® in-house iPS cell line (iPSC0028) with a GFP coding sequence at the 3' end of the NKX2-5 allele following a 2A ribosomal skip sequence. The Nkx2.5 protein is expressed in heart lineages from embryonic progenitors to mature cardiomyocytes. It is essential for cardiac development as evidenced by the association of numerous NKX2-5 polymorphisms with human congenital heart defects.

Following confirmation of the genetic modification, cardiomyocytes were generated by directed differentiation of NKX2-5-2A-GFP iPSC0028 cells. Unlike the still-pluripotent modified cells, which showed no expression of GFP, modified cells exhibited robust GFP expression once differentiated to the cardiomyocyte lineage (Figure 3A). Modified cells also showed GFP expression, indicating the presence of cardiac progenitor cells, when formed into embryoid bodies (Figure 3B).

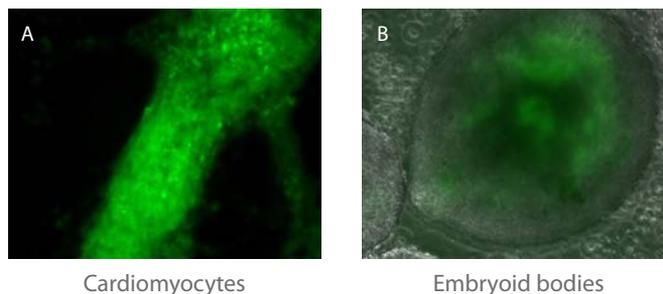


Figure 3. Live cell imaging of (A) cardiomyocytes and (B) embryoid bodies derived from modified iPSC0028 cells.

Additional CDS Capabilities

Tagged endogenous alleles in immortalized and iPS cell lines are just the beginning. In addition to these highlighted projects showing the use of targeted integration, we have experience with the following types of projects:

- Traditional knockouts
- Targeted knockins/transgenics
- SNP introduction or correction

We are also capable of targeting multiple alleles and multiple genes, and offer services using either your cells or ours. Whether you would like a disease-associated SNP corrected in a patient-derived iPS cell line, or a knockout produced in an aneuploid cancer cell line, we have the tools to meet your needs. Contact us to find out how Cell Design Studio at Sigma-Aldrich® can help you answer your most challenging scientific questions.

Email CDSInquiries@sial.com to contact us and get a custom proposal.

Note: First and second figures from: Samsonov et al. (2013). Tagging of Genomic STAT3 and STAT1 with Fluorescent Proteins and Insertion of a Luciferase Reporter in the Cyclin D1 Gene Provides a Modified A549 Cell Line to Screen for Selective STAT3 Inhibitors. PLoS ONE 8(7):e68391.

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World Headquarters
 3050 Spruce St.
 St. Louis, MO 63103
 (314) 771-5765
sigma-aldrich.com

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