Jump-In™ Parental Cell Lines

Overview

Generating Isogenic Cells with Jump-In™ Parental Cell Lines. Jump-In™ cell engineering is a novel gene targeting technology based on R4 integrase mediated site specific homologous recombination. This technology allows the targeted integration of genetic material into a specific pre-engineered site, which by design reduces the effort required for generation of stable cell lines compared to standard methods. Isogenic expression from a defined genomic locus provides the ideal solution for comparative analysis of gene families, isotypes or orthologs.

The Jump-In™ Parental Cell Lines were generated by genomic integration of an R4 acceptor site and a "promoterless" Blasticidin resistance gene (BSD) using homologous recombination via phiC31 integrase (Figure 1). Positive clones containing stable genomic integrations were selected based on their newly acquired Hygromycin resistance, picked by single-cell sorting, and then tested to determine the number of R4 sites present in the host cell genome by Southern blot and copy number analysis. Clones with a single R4 integration site were validated for retargeting by transfection with the pJTI™ R4 EXP CMV EmGFP pA and JTI™ R4 Int vectors, followed by antibiotic selection with Blasticidin for 2 to 3 weeks.

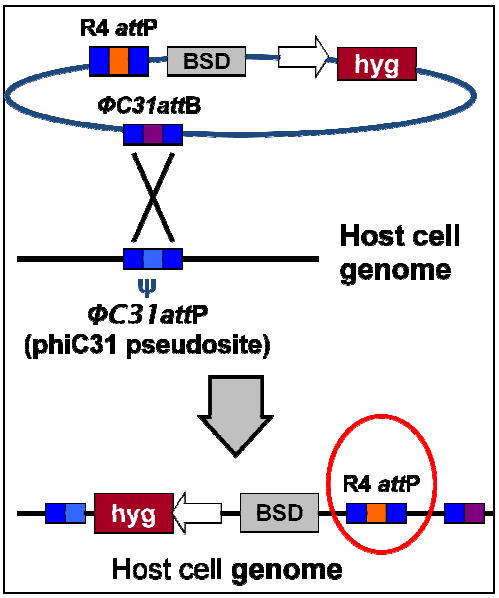

Figure 1: Schematic representation of Jump-In™ Cell Line Generation

The Jump-In™ Parental Cell Lines can be retargeted by co-transfection with a retargeting expression construct (generated from pJTI™ R4 DEST CMV pA using the Gateway® cloning technology) and the pJTI™ R4 Int vector expressing the R4 Integrase (Figure 3, below). During retargeting, the genetic elements of interest carried by the retargeting expression construct are site-specifically integrated into the genome of the platform cell line at the R4 *att*P target site. This integration event also positions the constitutive human EF1α promoter upstream of the "promoterless" Blasticidin resistance gene, thus allowing the selection of successfully "retargeted" transformants using Blasticidin (Figure 2).

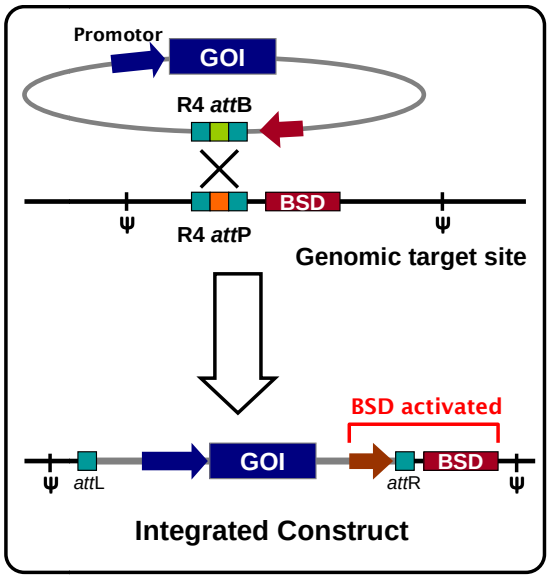

Figure 2: Targeted integration of the gene of interest (GOI) and a promoter for Blasticidin (BSD).

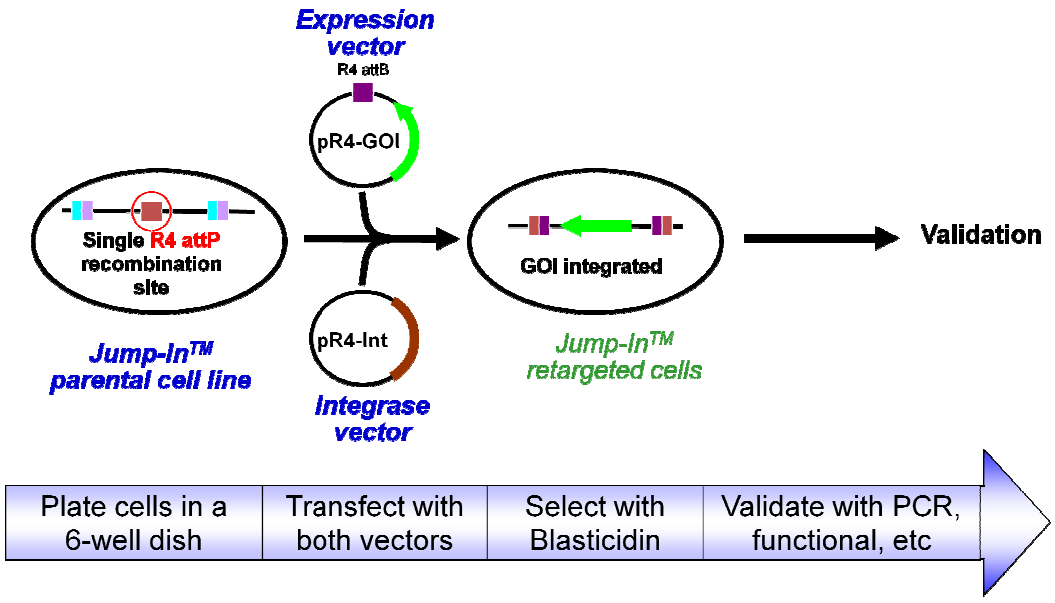

Figure 3: Workflow for the retargeting of a Jump-In™ parental cell line with your gene of interest (GOI)

Advantages of using Jump-In™ Parental Cell Line Kits

- **Rapid and efficient generation of engineered cell lines**
  - Functional cell pools can be generated in as little as 2-3 weeks
  - No laborious clone isolation and analysis

- Accepts large multi-gene inserts
- Generate an unlimited number of cell lines without complicated licenses or restrictions to interpret

- **Isogenic expression**
  - All cell lines derived from a given parental Jump-In™ cell line express the gene of interest from the same genomic locus.
  -
- **Ideal solution for**
  - Cells and assays problematic for transient engineering technologies
  - Difficult to "engineer" cell lines
  - Expression of target panels (families/isoforms/orthologs)

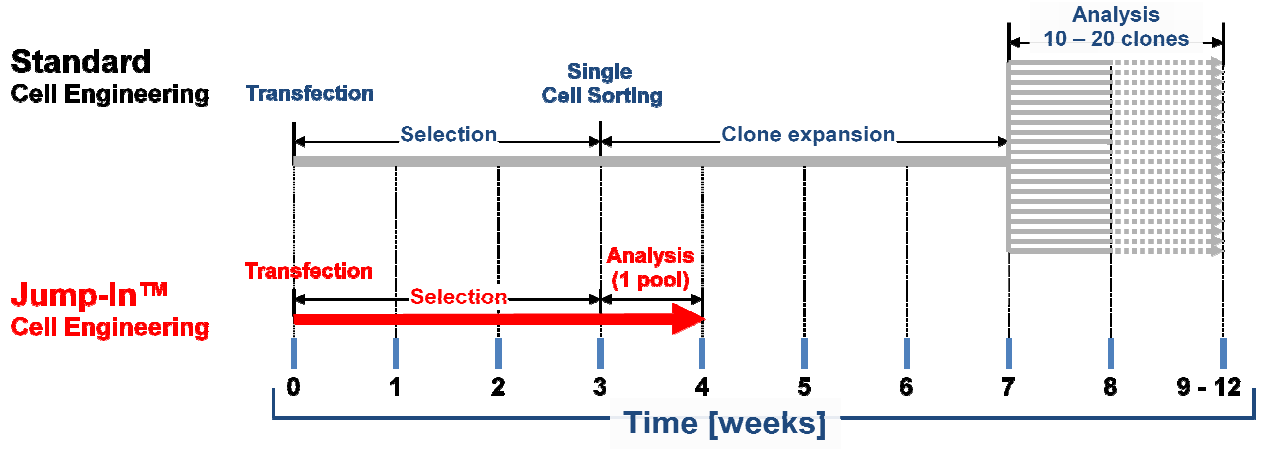

Figure 4: Time savings comparsion of Jump-In™ cell engineering vs standard cell engineering shows that the Jump-In™ approach provides isogenic cells in approximately half the time as traditional clonal cell line engineering.

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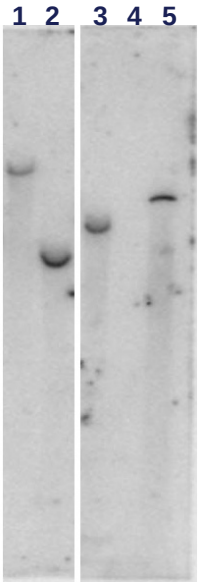
Figure 5: Validation of the Jump-In™ Parental Cell Lines to contain a single R4 site

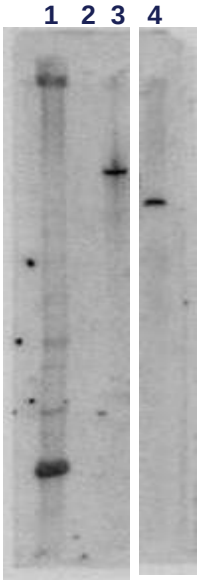
Jump-In™ GripTite™ 293 Southern



Lane 1:
Jump-In™ HEK293 GripTite / BamHI
Lane 2:
Jump-In™ HEK293 GripTite / Hind III
Lane 3:
Jump-In™ HEK293 / BamHI (positive control 1)
Lane 4:
parental (negative control)
Lane 5:
hygro (R4-vector, positive control 2)

Note:
Image shows different lanes reassembled from the same southern blot

Jump-In™ CHO-K1 Southern

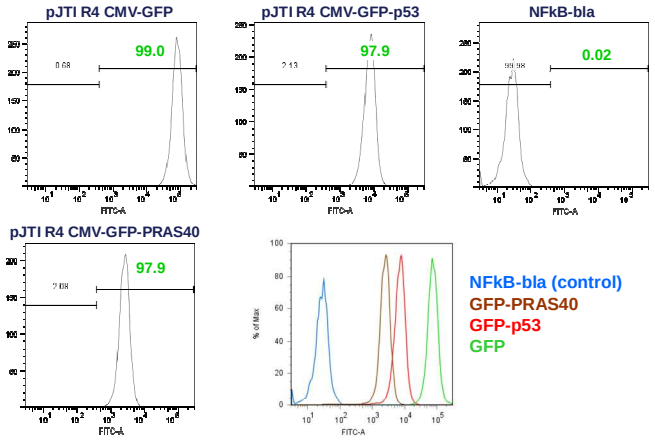


Lane 1:
1 Kb Plus Ladder
Lane 2:
parental (negative control)
Lane 3:
Jump-In™ CHO-K1 / BamHI
Lane 4:
hygro (R4-vector, positive control 2)

Note:
Image shows different lanes reassembled from the same southern blot

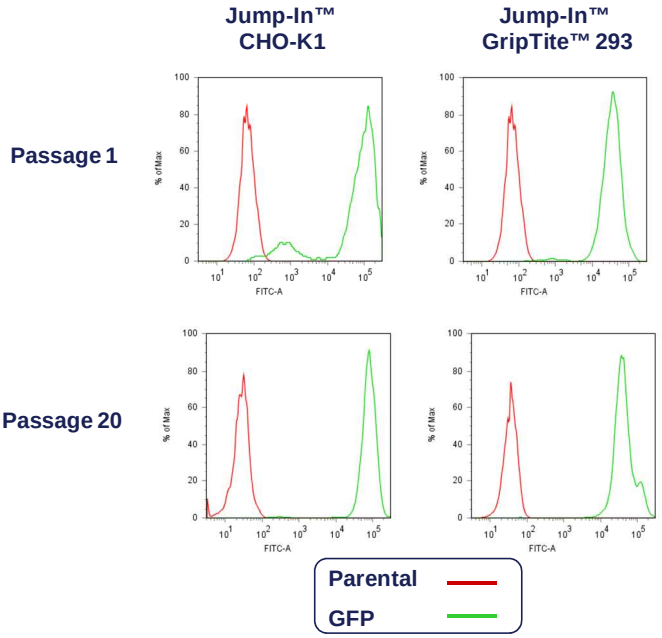
The number of genomic R4 sites was determined by Southern Blot using genomic DNA from Jump-In™ GripTite™ 293 cells and Jump-In™ CHO-K1 cells. One R4 site was found in both Jump-In™ Parental Cell Lines.

Figure 6: Stability of the Jump-In™ Parental Cell Lines



Jump-In™ HEK293 GripTite™ cells were propagated for 20 passage before retargeting with GFP, GFP-PRAS40, GFP-p53 or NFkB beta lactamase reporter and analyzed by fluorescent activated cell sorting (FACS). The NFkB negative control showed no fluorescent green cells and all of the other retargeted cells showed >97% green fluorescent cells indicating the Jump-In™ parental cells are stable up to at least 20 passages

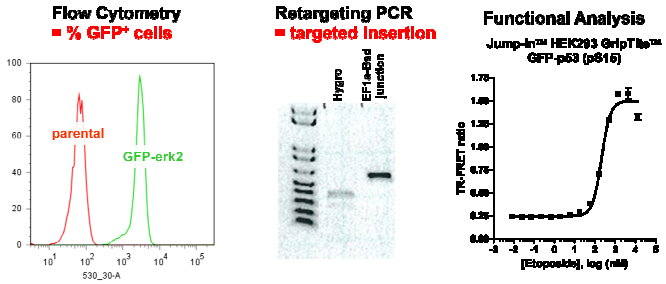
Figure 7: Stability of Jump-In™ retargeted cells



Jump-In™ Parental Cells were retargeted with GFP. Analysis of GFP expression by flow cytometry at passage 1 (after selection with blasticidin for 2-3 weeks) and passage 20 showed that at passage 20 the cells were still stably expressing GFP.

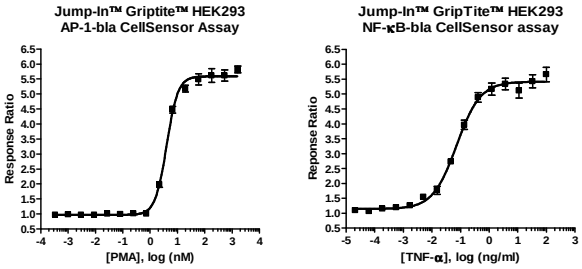
Representative Data for Jump-In™ GripTite™ HEK 293 and CHO-K1 Parental Cell Lines

Figure 8: Validation of retargeted cells



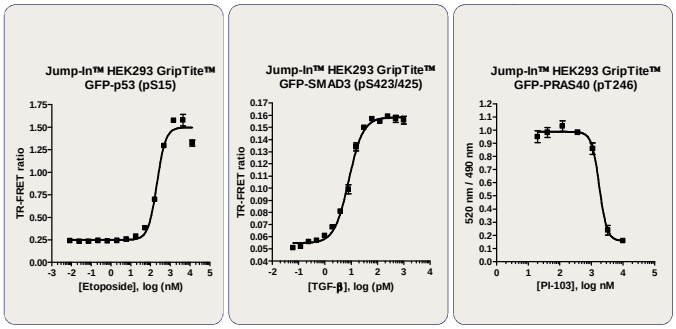
Retargeted cells can be validated in a number of different ways, depending on the genetic material used. If there is a fluorescent tag, flow cytometry can be used to determine the percent of successfully integrated cells. PCR with primers for the hygromycin resistant gene or the EF1a-blasticidin resistant gene junction can be used. When possible, a functional readout can be used, such as the LanthaScreen® p53 cellular assay for detecting phosphorylation at serine 15.

Figure 9: Performance of retargeted cells in CellSensor® Reporter Gene Assays



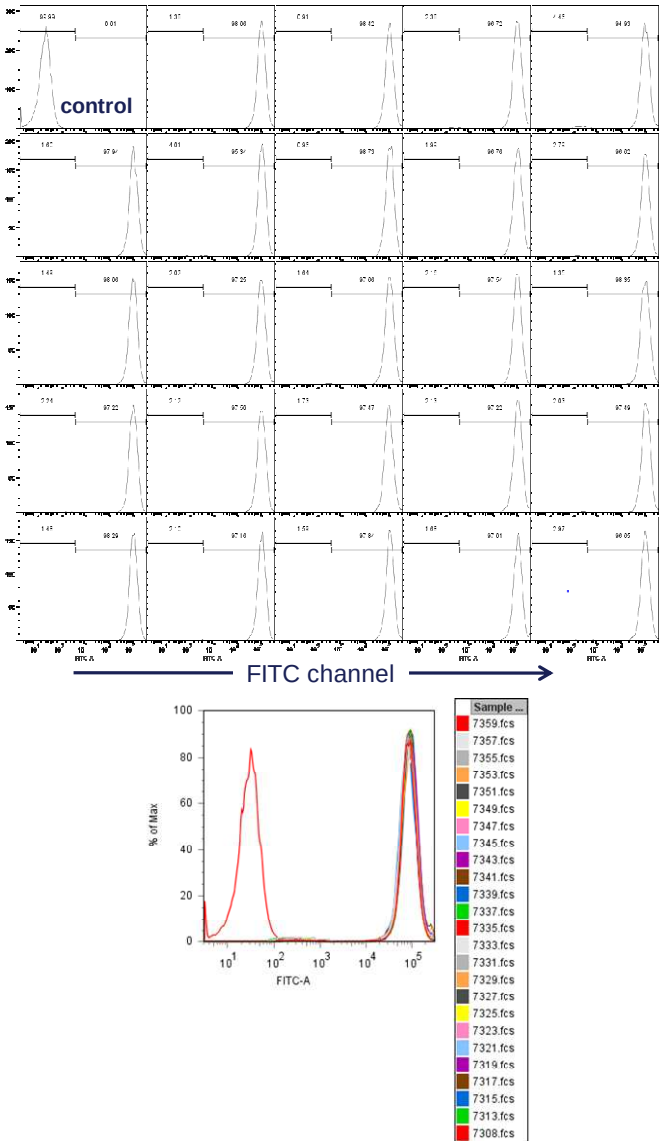
Jump-In™ GripTite™ HEK293 cells were retargeted with either the AP-1 beta lactamase reporter or the NF-κB beta lactamase reporter. After antibiotic selection, CellSensor® Assays were run and demonstrated expected assay windows and EC_{50} values.

Figure 10: Performance of retargeted cells in LanthaScreen® Assays



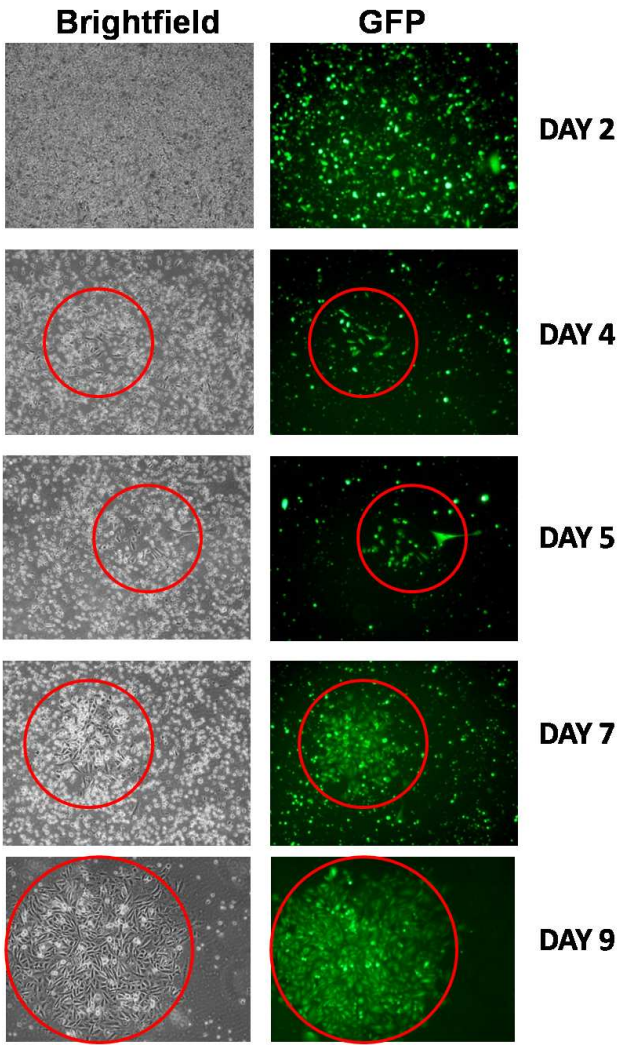
Jump-In™ GripTite™ HEK293 cells were retargeted with GFP-p53, GFP-SMAD3, or GFP-PRAS40. After antibiotic selection, Cellular LanthaScreen® Assays against the indicated modifications were run and results showed expected assay windows and EC_{50} values.

Figure 11: Reproducibility of the retargeting



Jump-In™ HEK293 GripTite™ cells were retargeted with GFP in 24 independent transfections (24-well plate). Analysis by flow cytometry indicated that 100% of wells were retargeted and cell pools were 94.9 – 98.7% GFP positive.

Figure 12: GFP expression from retargeting with the control vector



Jump-In™ CHO-K1 cells were imaged following transfection with pJTI™ R4 Exp CMV-GFP-pA. Brightfield and fluorescence microscopy (GFP) images (10 x objective) were taken at the indicated day of antibiotic selection with blasticidin. The red circles indicate colonies of blasticidin resistant retargeted Jump-In™ CHO-K1 cells.