Efficient And Versatile CRISPR/Cas9 Platform Facilitates Precise Genetic Modification In Mammalian Cell Lines
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Introduction
• CRISPR/Cas9 technology has revolutionized genome engineering and has emerged as a reliable and versatile tool for genome editing in various organisms and cells.
• Precise mutations, gene disruption, mutation corrections and insertions has enabled better understanding of the genetics and mechanism of diseases.
• CRISPR gene editing in cell lines, the workhorse of preclinical and biomedical research, enables the generation of unlimited in vitro models with precise gene modifications and advanced gene expression design that are physiologically relevant.
• To accurately model diseases, as well as for precise singe or biallelic manipulations, there is a strong dependency on homology directed repair (HDR) and gRNA selection strategies.
• Here, we present data demonstrating the versatility of the technology in editing cell lines:
  • Editing a variety of mammalian cell lines including hard-to-transfect blood lineage cells and stem cells.
  • Case studies highlighting complex modifications such as double gene knockout, large knock-in of transgenes into specific endogenous locus and safe harbor locus; gRNA selection strategies to ensure specific mono- and biallelic modifications.
  • Efficiency of generating knock-in, knockout and point mutations in various cell lines, including pluripotent stem cells.

Cas9 Mediated Genome Engineering

Cell Lines Amenable to CRISPR/Cas9 Gene Editing*

Knock-in of a Large “Fusion” Transgene into a Specific Locus in a Human Cancer Cell Line

Large Transgene into hH11 Safe Harbor Locus in Induced Pluripotent Stem Cells

Targeted Heterozygous and Homozygous Point Mutations in Human Embryonic Stem Cells

Efficiency of CRISPR-Mediated Genome Editing in Cell Lines

Conclusions
• CRISPR/Cas9 technology is a versatile gene editing technology and can be used for modifying a variety of cell lines including hard-to-transfect blood lineage cells such as Jurkat, bone marrow cell lines, and pluripotent stem cells.
• CRISPR can also be used to efficiently and precisely modify genes: knockout/ disruption, point mutations and transgene knock-in. As well, with proper gRNA and targeting design, off-target activity can be avoided or minimized.
• CRISPR technology also enables the generation of complex genetically engineered cell line models, double knockout, and inducible gene expression models, in addition to being easily manipulated to generate specific bi-allelic and mono-allelic clones.
• In summary, CRISPR gene editing in cell lines provides an unlimited source of physiologically relevant in vitro models for basic research, drug target discovery and initial stage drug screening, and has tremendous potential for cell replacement therapeutic applications.

*Selected list of cell lines successfully modified in Applied StemCell’s Cell Biology lab. CRISPR/cell editing is not limited to these cell lines.