**Footprint-Free Gene Editing Using CRISPR/Cas9 and Single-Cell Cloning of Edited Human iPS Cells**

Montse Morell
Takara Bio USA, Mountain View, California, USA

CRISPR/Cas9-based gene editing has revolutionized the field of cell biology and is quickly being incorporated into the toolboxes of many laboratories. This system uses a relatively robust RNA-guided endonuclease for targeting and cleaving desired genomic loci. In practice, only two elements are required for genome editing, the Cas9 protein combined with a single guide RNA (sgRNA) that determines its target specificity.

The combination of two powerful technologies—human induced pluripotent stem (hiPS) cells and precise, footprint-free editing using CRISPR/Cas9—allows for a new level of sophistication in cell biology research and disease model development. The ability to create hiPS cell lines from different donors and to determine the effects of specific mutations created via gene editing within a donor-specific genetic background will enable discoveries with a new level of granularity.

While CRISPR/Cas9 is a powerful technique for gene manipulation, two significant challenges remain: obtaining efficient delivery of Cas9 to all cell types and achieving fewer off-target effects. It has been previously demonstrated that genome editing via direct delivery of Cas9/sgRNA ribonucleoprotein (RNP) is as effective as plasmid-based delivery, but with the added benefit of decreased off-target effects due to the short duration of the RNP in the cell.

hiPS cells have been traditionally grown and passaged as colonies, which means that obtaining single-cell clones of edited hiPS cells has been a major bottleneck for researchers. In order to obtain single cells for cloning purposes, the cells must first be dissociated into a single-cell suspension, which often results in cell death or premature differentiation. In contrast to traditional methods, the Cellartis® DEF-CS™ 500 Culture System allows culturing of hiPS cells in a monolayer and permits the isolation of single cells. The specific compositions of the DEF-CS culture media and coat allow for a very high rate of single-cell survival and clone expansion.

We have developed a complete workflow for editing and cloning hiPS cells, starting with CRISPR/Cas9-mediated editing using Cas9/sgRNA RNP complexes delivered to hiPS cells via either electroporation or cell-derived nanoparticles called gesicles, followed by successful single-cell cloning of these edited hiPS cells. Non-DNA-based delivery methods were chosen in order to guarantee footprint-free editing of the hiPS cells. We achieved endogenous gene knockout efficiencies of up to 65% for the membrane protein CD81 in a hiPS cell population. Single, edited hiPS cells were seeded onto a 96-well plate and were expanded to clonal cell lines that were still pluripotent.

The data show that the workflow described here resulted in a high number of edited and expandable hiPS clones which maintained the hallmarks of pluripotency.