Genome Engineering for Cell Therapy Manufacturing

HARNESS THE POWER OF CELL ENGINEERING FOR YOUR THERAPY DEVELOPMENT

Gene engineering is a powerful tool for fine tuning cell phenotype in immune cell and regenerative medicine therapies. It allows the introduction of new genes into cells and very precise alteration of their existing genes. Cell engineering can augment cell therapy effectiveness by increasing cell potency and persistence in vivo. It has recently been used to:

- Express proteins that specifically target tumor cells, e.g. CART cells, TCR and CAR-NK
- Suppress immune responses in autoimmune disease and chronic inflammation
- Mask cell therapies to avoid host immunity
- Enable cells to infiltrate the target tissue
- Deliver genes and correct genetic defects for gene therapy
Rapid and Flexible Gene Delivery with TcBuster

TcBuster is a non-viral gene delivery system that enables stable gene transfer into any cell type. It is based on a transposition from the red flour beetle. The TcBuster system includes a transposon plasmid containing the gene of interest (GOI) to be inserted in the target cell's genome.

**OVERVIEW OF TcBuster TRANSPOSITION MECHANISM**

1. TcBuster platform introduced into the cells via electroporation
2. TcBuster mRNA translated into TcBuster transposase
3. TcBuster transposase binds to ITR regions on transposon plasmid
4. TcBuster transposase CUTS gene of interest (GOI) from plasmid
5. GOI INSERTED into cell's DNA
6. GOI mRNA transcribed from cell's DNA
7. Translated protein from GOI mRNA is now stably expressed in cells

**THE RED FLOUR BEETLE (TRIBOLIUM CASTANEUM)**

The TcBuster transposon is derived from this beetle that feeds on broken grain kernels and flour in food storage areas. The beetle is dark red or brown, and it can fly and live for over a year. For the laboratory-minded, the red flour beetle is easy to maintain in culture. Over their lifetime, females may lay up to 1,000 eggs which hatch in 5-12 days.

**How Does the TcBuster System Compare to Virus-Based Systems?**

While both these approaches are designed to deliver genetic material to a target cell, viral methods for genetic modification (e.g., with lentivirus or AAV) involve transduction of the target cells, while non-viral transposons are integrated into target cells using standard transfection methods (e.g., electroporation). Both approaches provide a means of cell entry for the gene of interest (the cargo) and a mechanism for integrating the GOI into the cellular genome. The advantages of TcBuster for cell therapy manufacturing include:

- Reducing the time required and the cost of introducing GOI
- Increasing the practical GOI cargo capacity compared to virus-based methods
- Avoiding inconsistent reagent availability
TcBuster Edits Primary Human T Cells Similar to Lentivirus

Primary human T cells were modified by introducing a CD19 CAR with TcBuster (left) and lentivirus (right). Flow cytometry analysis shows that TcBuster modified a greater percentage of the target cells and led to higher expression of the CAR compared to lentivirus transduction.

Flow cytometry analysis of CCR7 CAR insertion following transposition with TcBuster (A, B) or transduction with lentivirus (C, D). Cells were gated for CD4 (A, C) or CD8 (B, D) expression. TcBuster modified cells had a greater percentage of CD45RA+ cells (naïve T cells) compared to lentivirus transduction.

Three genes were transposed into T cell cultures of 10 x 10^6, 80 x 10^6, or 400 x 10^6 cells. All genes were delivered in a single reaction. At all scales, transposition efficiency remains above 30%.

CD19 CAR-T cells engineered with TcBuster (red and purple) or lentivirus (blue and brown) show similar cytotoxicity against CD19-expressing Nalm6/luc cells. The non-electroporated (EP) controls (black and green) do not kill cells.

Genomic Locus Insertion Preferences

TcBuster inserts genes into the genome in a nearly random pattern. It shows a lower preference for active genes than seen with viral methods, and this translates to a reduced possibility that transposition will disrupt critical cellular genes.

Complex Gene Editing with TcBuster

With the TcBuster system, a one-step process can be used to engineer knockouts and TcBuster-mediated gene delivery without sacrificing cell growth.

The transposition of a CD19-GFP CAR was successfully completed in combination with knockout of beta 2-Microglobulin.
To complement your genome engineering program, our comprehensive portfolio of high-quality products will enable you to develop and rigorously characterize engineered cells. We also offer custom development and GMP manufacturing services so we can meet your particular requirements.

Reagents and Assays

- Flow Cytometry Reagents and Kits – over 15 fluorophores with thousands of antibodies
- Fluorokines™ Fluorescent-Labeled Proteins – for ligand-based analysis of CAR-transposed cells
- RNAscope™ ISH – confirmation of genetic modification in intact cells and tissue biopsies
- Immunoassays – multiple formats for quantitative, precise, and reproducible results
- Custom Services for Cell and Gene Therapy including custom vialing of GMP proteins, GMP small molecules, and GMP antibodies

Cell Manufacturing

- GMP Proteins – cytokines and growth factors for ex vivo use in cell manufacturing processes
- GMP Small Molecules – reprogramming stem cells for regenerative medicine processes
- ExCellerate Media - serum-free formulations for immune cell and stem cell culture
Analytical Instruments

- iCE Maurice™ – automated capillary electrophoresis with pre-assembled cartridges and onboard sample mixing
- Micro-Flow Imaging™ – analysis of protein aggregation and particulate contamination
- Simple Plex™ – automated cartridge-based ELISAs with 4-5 log dynamic range
- Simple Western™ – automated capillary-based immunoassays for size- and charge-based complex sample screening
- Single-Cell Western™ – analysis of protein expression in ~1,000 single cells in parallel
- Lumines® - for multiplex immunoassays to screen cell culture samples for secreted proteins

For Applications of TcBuster in Immune Cell Therapy

To complement your genome engineering program, our comprehensive portfolio of high-quality products will enable you to develop and rigorously characterize engineered cells. We also offer custom development and GMP manufacturing services so we can meet your particular requirements.

Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) are produced by the reprogramming of differentiated somatic cells such as PBMCs, fibroblasts, and patient-specific samples. They can be engineered to address genetic defects, promote the rebuilding of damaged tissues, and avoid immune rejection after implantation.

- iPSC reprogramming, expansion, and master cell banking – all performed in-house
- Feeder, feeder-free, and xeno-free cell culture expertise
- Customizable validation assays and documentation

Learn about Genome Engineering Services | bio-techne.com/services/gene-engineering-services

Learn more | ndsystems.com/proteins
Where Science Intersects Innovation™