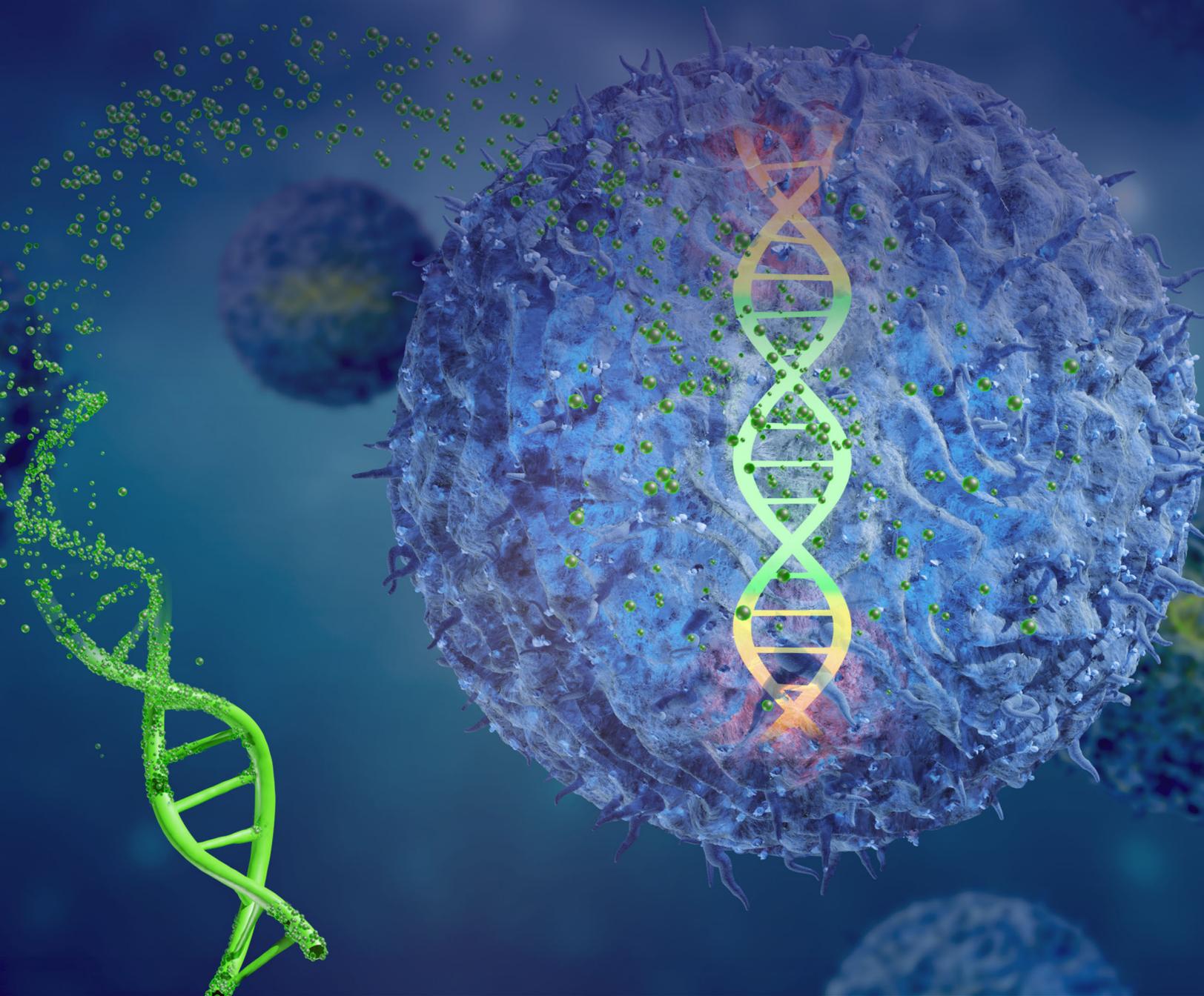


# GENOME ENGINEERING SERVICES FOR CELL THERAPY MANUFACTURING



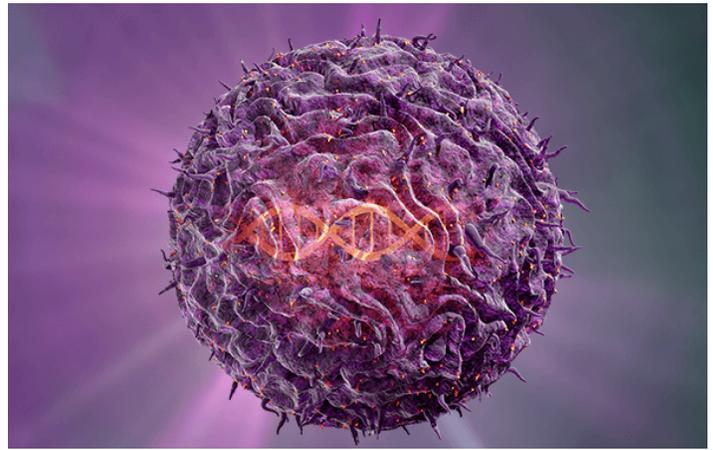
**biotechne<sup>®</sup>**

# 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:

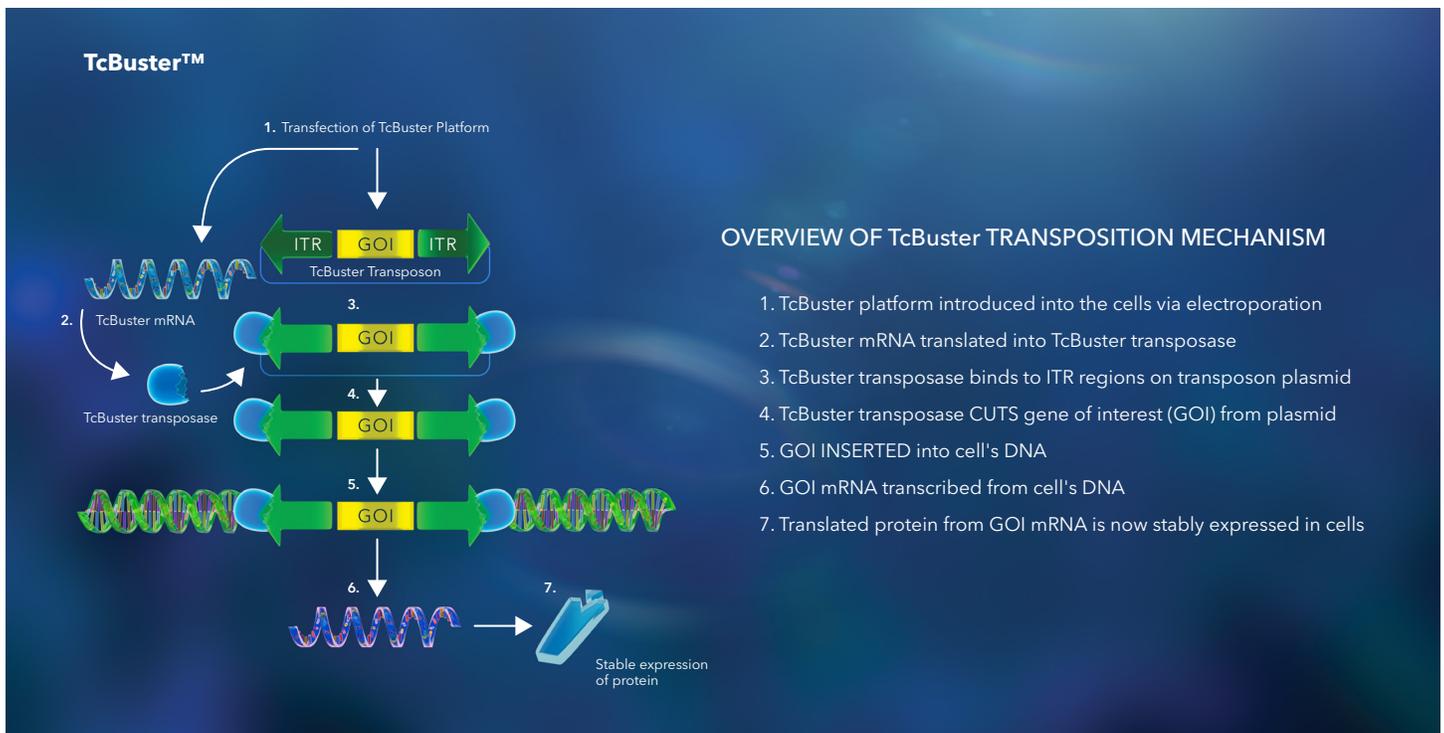
- Express proteins that specifically target tumor cells, e.g. CAR-T cells, TCR
- Suppress immune responses in autoimmune disease and chronic inflammation
- Mask cell therapies to avoid host immunity
- Enable cells to infiltrate the target tissue
- Deliver genes and correct genetic defects for gene therapy



## 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 transposon 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.

TcBuster supports the rapid generation of transgenic mammalian cells by allowing for multigene transfer and CRISPR-mediated knockouts in one operation. TcBuster is a valuable tool for both proof-of-concept studies and manufacturing scale process development.





## THE RED FLOUR BEETLE (TRIBOLIUM CASTANEUM)

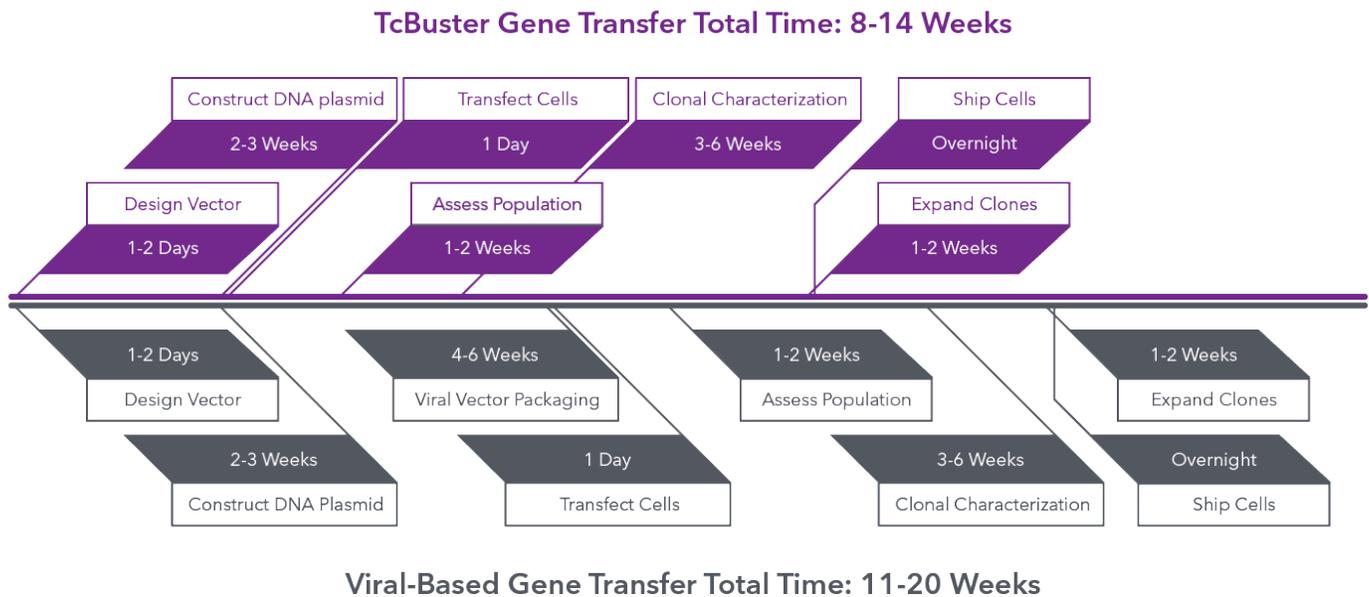
The TcBuster transposon is derived from this beetle that feeds on broken grain kernels and flour in food storage areas. The beetle is dark red or brown, and it can fly and live for over a year. For the laboratory-minded, the red flour beetle is easy to maintain in culture. Over their lifetime, females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

Learn about Genome Engineering Services | [rndsystems.com/products/tcbuster-gene-transfer-system](https://rndsystems.com/products/tcbuster-gene-transfer-system)

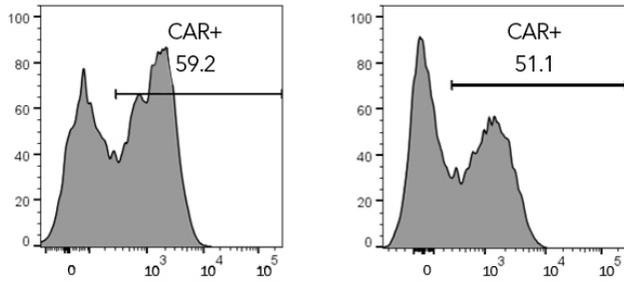
## HOW DOES THE TCBUSTER SYSTEM COMPARE TO VIRUS-BASED SYSTEMS?

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:

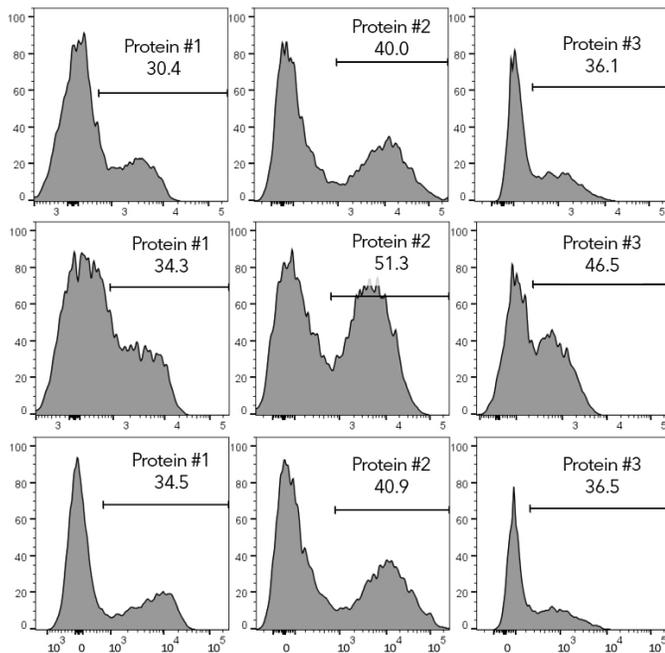
- Reducing the time required and the cost of introducing GOI
- Increasing the practical GOI cargo capacity compared to virus-based methods
- Avoiding inconsistent reagent availability



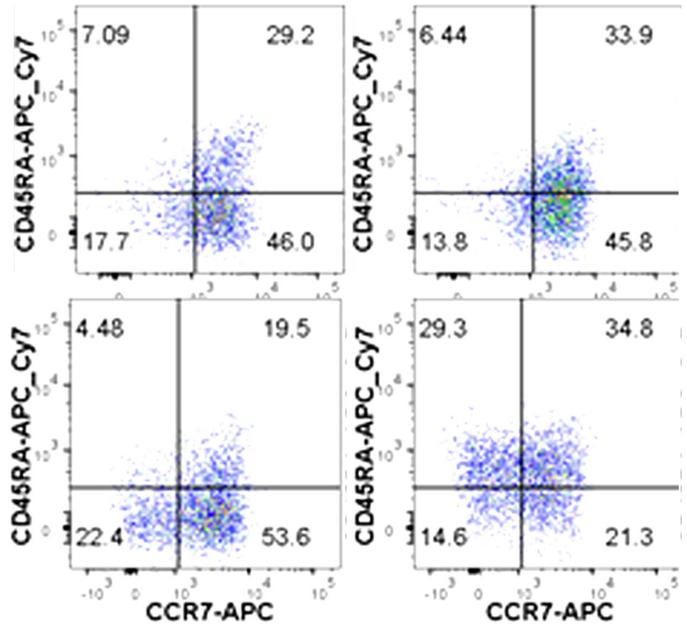
# TCBUSTER EDITS PRIMARY HUMAN T CELLS SIMILAR TO LENTIVIRUS



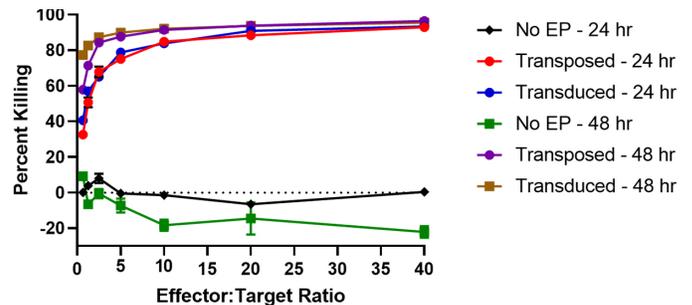
Primary human T cells were modified by introducing a CD19 CAR with TcBuster (left) and lentivirus (right). Flow cytometry analysis shows that TcBuster modified a greater percentage of the target cells and led to higher expression of the CAR compared to lentivirus transduction.



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



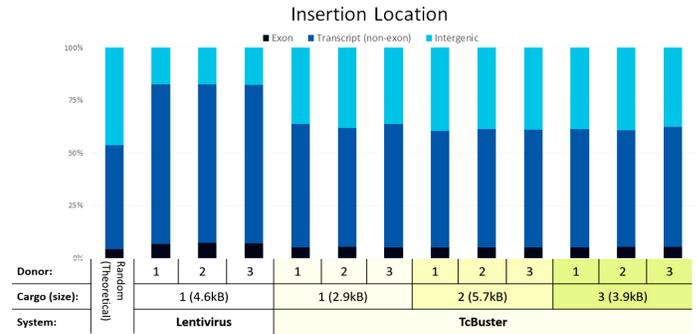
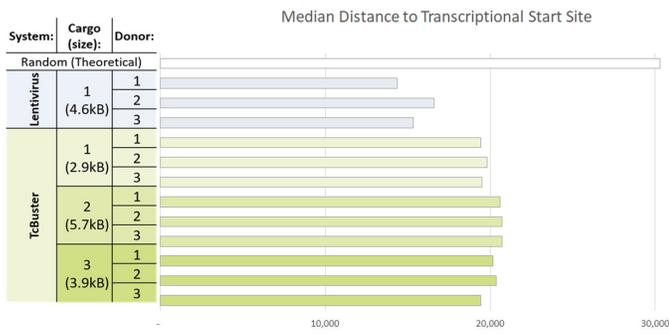
Flow cytometry analysis of CCR7 CAR insertion following transposition with TcBuster (A, B) or transduction with lentivirus (C, D). Cells were gated for CD4 (A, C) or CD8 (B, D) expression. TcBuster modified cells had a greater percentage of CD45RA<sup>+</sup> cells (naïve T cells) compared to lentivirus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



\*Note: E:T ratio was total T cells to targets.

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

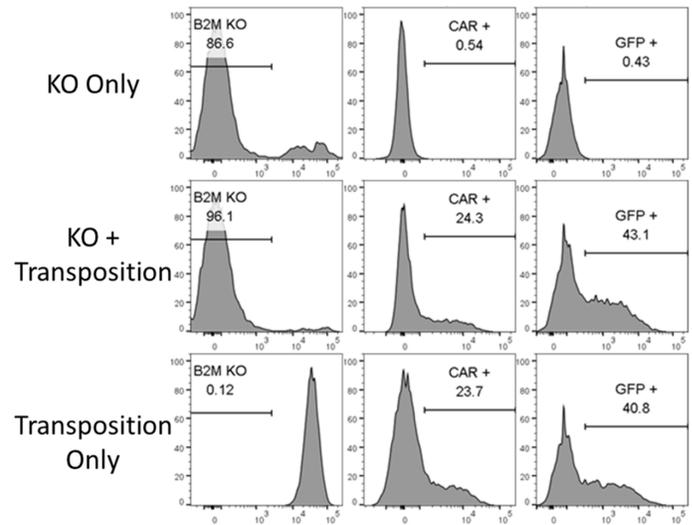


TcBuster inserts genes into the genome in a nearly random pattern. It shows a lower preference for active genes than seen with viral methods, and this translates to a reduced possibility that transposition will disrupt critical cellular genes.

## COMPLEX GENE EDITING WITH TCBUSTER

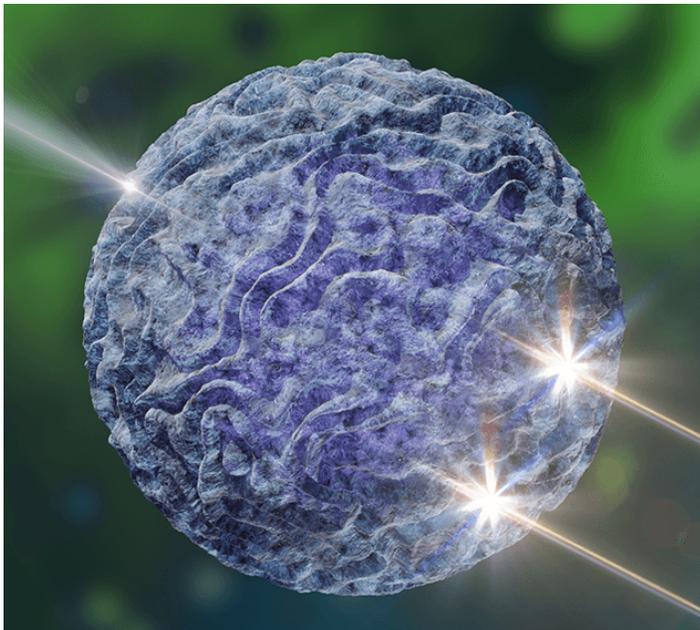
With the TcBuster system, a one-step process can be used to engineer knockouts and TcBuster-mediated gene delivery without sacrificing cell growth.

The transposition of a CD19-GFP CAR was successfully completed in combination with knockout of beta 2-Microglobulin.



## ADDITIONAL PRODUCTS AND SERVICES FOR CELL THERAPY MANUFACTURING

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
- 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

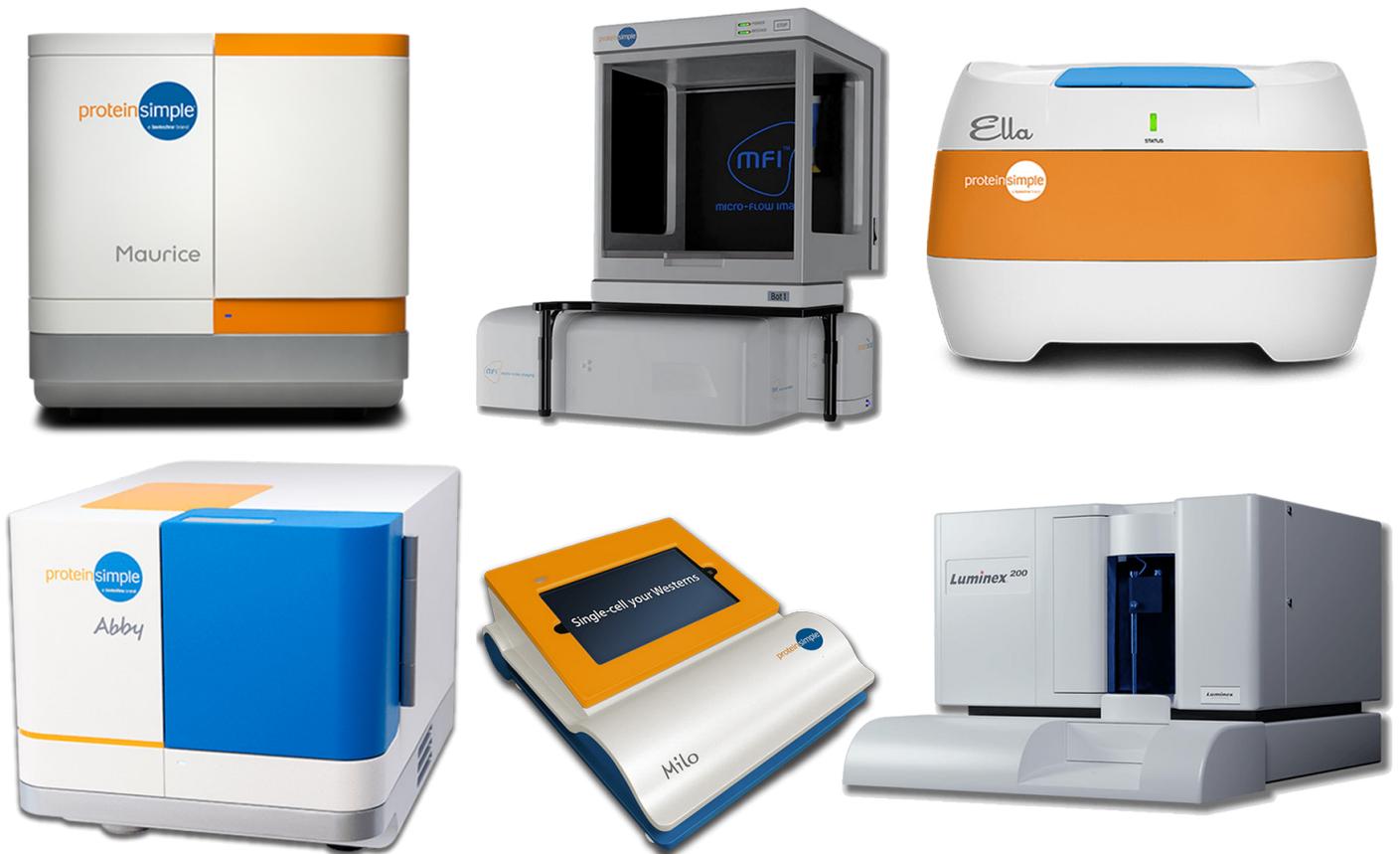
## CELL MANUFACTURING



- GMP Proteins - cytokines and growth factors for *ex vivo* use in cell manufacturing processes
- GMP Small Molecules - reprogramming stem cells for regenerative medicine processes
- Cloudz™ Cell Activation Kits - GMP and RUO immune cell expansion with dissolvable microspheres
- GMP ProDots™ Proteins - pre-aliquoted, rapidly dissolvable cytokine microspheres for closed system cell culture
- ExCellerate™ Media - xeno-free formulations for defined composition culture of immune cells

## 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
- Luminex® - 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.



To learn more | [scaleready.com](https://scaleready.com)

## WHAT CAN I EXPECT FROM GENOME ENGINEERING SERVICES?

Whether you are new to gene engineering, have limited experience, or represent a gene engineering core group with limited bandwidth, we provide the same level of responsive partnership and customer service. We will connect scientist-to-scientist and walk through the entire project with you. For projects ready for transition to the clinic, we will discuss proof of concept projects and process development work. For all projects, we will work with you to:



- Identify the scope of the request
- Define the best engineering and cell assays that align with your project
- Refine the project specifics, milestones, and deliverables
- Review a statement of work
- Deliver a timely quote
- Deliver regular project updates
- Deliver your customized products

## OTHER CELL ENGINEERING SERVICES

### CELL LINES AND PRIMARY CELLS

No cell line is too difficult for us to engineer, and our highly efficient genome editing process speeds up delivery timelines. Regardless of the project, we can customize all deliverables including the number of cells/vial, validation testing, datasheet preparation, and batch record documentation.

- RUO, GMP, and xeno-free capabilities
- Gene engineering with any platform for KO, KI, or multiplex edits
- Correction or introduction of single nucleotide polymorphisms (SNPs)
- Isolation of primary cells at small or large scale
- Validation of engineered cells - genetic, protein expression, and custom functional analysis
- Banking of wild-type clones and engineered cells

### 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

## OUR CAPABILITIES FOR GMP MANUFACTURING

Our Contract Development and Manufacturing Organization (CDMO) services cover large scale isolation, cell engineering and activation, and final fill and finish. Our services include optimization of process cost and performance repeatability, dual sourcing of critical components, and incorporation of key flexibility points to avoid non-conformity.



- Prepare CAR-T, TCR, or iPSC processes for GMP manufacturing
- Sample processing for Phase I and Phase II clinical trials
- GMP manufacturing, culturing, and banking of iPSC cell lines

### CLEAN ROOM FACILITIES

- ISO Class 7 certification (Class 10,000) meeting U.S. requirements
- 2 cell processing suites
- 6,000 sq. ft. GMP manufacturing space in Minneapolis, MN, USA
- 2 development labs and one in-house quality control lab





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