bio-techne®

Flyer

De-Risk Cell Therapy Manufacturing

With the TcBuster™ System for Non-Viral Gene Transfer

Gene transfer is a critical step in manufacturing genetically modified cell therapies. However, traditional vector-based delivery systems are costly, with long manufacturing lead times, batch-to-batch variability, and insertional preferences for transcriptional units[1].

To address these challenges, manufacturers are exploring non-viral transposon-based solutions[2], such as the TcBuster system. As of 2024, after being studied in clinical trials for over a decade[3], more than 50 clinical trials have utilized transposon-based gene transfer technologies, including TcBuster[4].

Genome engineering significantly impacts cell therapy potency & safety, and must therefore be carefully assessed. Valuable insights can be gained through integration site analysis (ISA) and vector copy number (VCN).

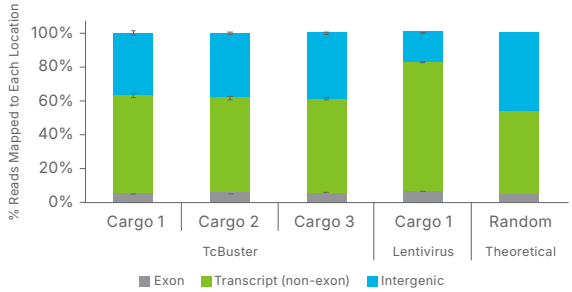
ISA reveals that T cells transposed with TcBuster exhibit an insertional profile nearly consistent with a theoretical unbiased (random) control, while lentiviral vectors show a bias towards cargo integration into non-exon transcript regions (**Figure A**), which is consistent with what has been observed in literature[1].

VCN analysis of T cells from three donors, modified with three different DNA cargos, reveals average VCN of < 3 for lentivirus and < 8 for TcBuster (**Figure B**). FDA published cellular and gene therapy guidance recommends VCN justification based on a risk assessment approach[5]. Because transposon-based methods lack the same insertional bias of lentiviral vectors, they are expected to have a de-risked integration profile[6,7] and tolerate modest increases in VCN compared to viral vectors.

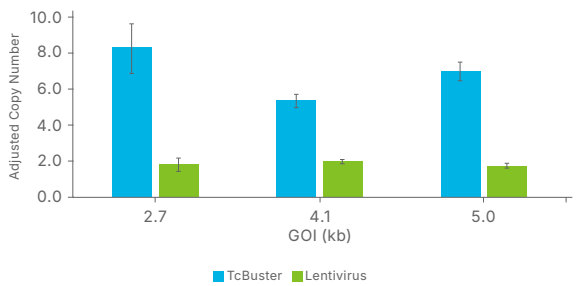
Persistent transposase expression is an additional concern in transposon systems[8], as it could increase the average VCN and raise the risk of insertional mutagenesis. Delivery by mRNA minimizes these extended editing events[7], thus TcBuster transposase is provided in this form.

To understand persistence, TcBuster mRNA and enzyme clearance was evaluated in primary human T cells. RT-qPCR showed that mRNA is undetectable after 4 days post-electroporation (**Figure C**), while the transposase enzyme is nearly undetectable on the Simple Western™ Jess after 2 days (**Figure D**). This suggests that editing with the TcBuster system is transient and does not persist over an extended period.

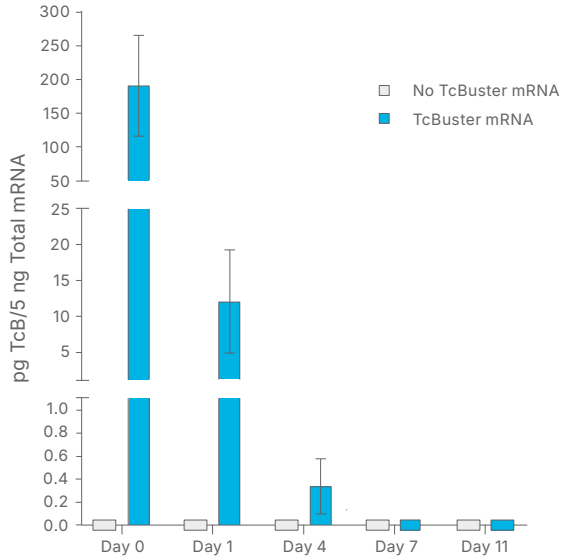
A Integration Site Analysis



B Average Adjusted Vector Copy Number

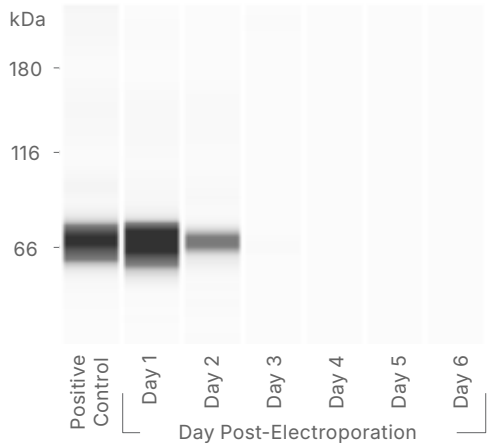


C mRNA Detection Post-Electroporation



Method:	RT-qPCR
Detecting:	Transposase mRNA
Clearance:	Undetectable by Day 7

D Transposase Enzyme Detection Post-Electroporation



Method:	Simple Western (Jess)
Detecting:	Transposase Protein, detected by TcBuster-M Transposase Antibody (Catalog # MAB11511)
Clearance:	Undetectable by Day 3

Conclusion

In summary, the TcBuster system is an efficient non-viral gene editing system that allows for de-risked, stable integration of multicistronic DNA cargo for cell therapy applications. This DNA transposon-based system exhibits a superior insertional profile compared to lentivirus, while maintaining low VCN. The mRNA format of the transposase ensures the expression of TcBuster enzyme is a transient event, with both TcBuster mRNA and transposase enzyme undetectable a few days post-electroporation.

In addition to de-risking gene transfer, other benefits of implementing the TcBuster system into your cell therapy process include minimal lead times for GMP material, reduced COGS, effortless scalability, and the ability to deliver DNA cargo non-virally. Bio-Techne offers electroporation protocols for transposing T cells, with troubleshooting tips to achieve a sufficiently low VCN.

Questions? Contact our team at **techsupport@bio-techne.com**.

References

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