Molecular Guide

Creating Custom DNA Transposons for the TcBuster™ System

This guide is meant to be used as a resource to design DNA transposons (plasmids) for use with the TcBuster system. Bio-Techne does not guarantee the success of custom DNA transposons. It is the responsibility of the end user to design the transposon, assess the performance, and perform due diligence on any elements or sequences included in the construct design to determine freedom to operate.

What is a TcBuster DNA transposon?

- A TcBuster DNA transposon is a plasmid containing inverted terminal repeats (ITRs) that are compatible with the TcBuster transposase. The transposase binds to the ITRs and "cuts-and-pastes" the DNA to stably integrate genes into the host cell genome. Please visit the **non-viral gene engineering page** for more information.

Why is the Nanoplasmid™ Vector System from Aldevron™ recommended as the transposon backbone for cell therapy applications?

- T cells, NK cells and other cell types can be affected by DNA-mediated toxicity when high concentrations of plasmid DNA are used. Minimizing the plasmid backbone by using a Nanoplasmid, or similar technology, allows for a higher ratio of GOI sequence to µg of plasmid delivered. The Nanoplasmid can be delivered to cells at a lower concentration than a traditional plasmid with the same gene editing efficiency. Improved viability and cell growth are observed after editing with lower concentrations of DNA.
- Regulatory bodies, including the FDA, recommend that plasmids used in clinical applications do NOT contain antibiotic resistance genes for safety purposes. The Nanoplasmid does not contain a bacterial antibiotic resistance gene and is a good option for a TcBuster transposon.

What is the cost and timeline for a custom DNA transposon?

- The cost of ordering a transposon depends on the sequence complexity and size.
- For initial custom Nanoplasmid construct production, a **Nanoplasmid LULL** and a one time research license fee covering all preclinical work will be required.
- The timeline for generating a custom DNA transposon averages 7-10 weeks.

How do I order a custom DNA transposon for TcBuster?

- Bio-Techne and Aldevron have partnered to streamline the ordering process for custom DNA transposons. Bio-Techne has banked a TcBuster backbone, fully equipped with TcBuster compatible ITRs and a multiple cloning site, utilizing Aldevron's Nanoplasmid vector system.
- Once you have designed and built your custom DNA transposon, please contact Aldevron to perform gene synthesis, cloning, and manufacturing. We recommend a concentration of 2 mg/mL in nuclease-free water. Email your request to **nanoplasmids@aldevron.com**.

Can I use another vendor to manufacture my custom DNA transposon?

- The Nanoplasmid vector is licensed by Aldevron. You would need to negotiate your own license to manufacture the Nanoplasmid with another vendor.
- If you are using a pUC backbone, or similar alternative, you will be able to perform the cloning and manufacturing in your own lab, or with a vendor of your choice.

Is a plasmid map available to help design my custom DNA transposon?

- Included with this guide is a plasmid map of a pUC57 backbone containing the TcBuster compatible ITRs, Ef1α promoter sequence, multiple cloning site (MCS), and BGH PolyA signal sequence. See Table 01 for more information on these elements. The plasmid map file "TcBuster pUC57 backbone with MCS.dna" is openable with programs such as SnapGene. Double click the file below to open the plasmid map.

TcBuster pUC57 backbone with MCS

Custom DNA Transposon

Design and Ordering Process

Step 1: Design the Expression Cassette

The TcBuster-compatible Nanoplasmid vector banked at Aldevron includes the following elements:

- TcBuster-compatible ITRs
- Human elongation factor 1 alpha (Ef1α) or MND promoter sequence
- Multiple cloning site (see step 2)
- Bovine growth hormone (BGH) poly(A) signal sequence

The backbone may be further customized by Aldevron to meet your needs.

You must provide the following elements (if applicable) :

- Custom promoter sequence
- Kozak sequence
- Gene(s) of interest, separated by 2A elements if cargo is multicistronic
- Selection marker
- Custom poly(A) signal sequence

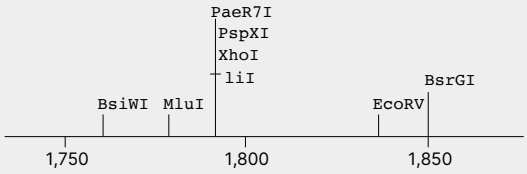
Step 2: Build the Linear Map

Build a linear map of the expression cassette in the molecular design software of choice, such as SnapGene or Benchling.

Design Tips:

- Ef1α and MND are strong constitutive promoters suitable for mammalian overexpression.
- GCCACC is generally considered the classic consensus Kozak sequence and is the standard sequence used for TcBuster overexpression vectors.
- Typically, inserts will be cloned into the MluI/EcoRV sites, unless a custom promoter sequence is required.
- Self-Cleaving peptides 2A element orientation: If the cargo is polycistronic, separate all genes from each other using GSG-2A elements.
  - For example: Gene 1-T2A-Gene 2-P2A-Gene 3-E2A-Gene 4-F2A.
  - Typically, a different 2A element is used between each gene of a polycistronic construct. This avoids potential recombination issues during cloning.
  - Data from a previous study suggests that the best 2A element order for optimal gene expression of a quad cistronic construct is T2A-P2A-E2A.

Multiple cloning site with unique restriction enzyme sites in the TcBuster-compatible Nanoplasmid vector:



Step 3: Review the Expression Cassette

Confirm that the following elements are present and correct:

- The expression cassette is in the proper orientation: Promoter-Kozak-GOI-Poly(A) signal
- The correct promoter is used
- Kozak sequence immediately precedes the GOI
- GOI is in-frame and includes:
  - Start codon
  - Stop codon
  - Properly placed GSG-2A elements, if applicable
- No premature transcriptional terminators (AATAAA or ATTAAA) are in the open reading frame

Run your expression cassette through the GenRCA Rare Codon Analysis Tool (optional).

- Analyze from start codon to stop codon.
- If codon adaptation index (CAI) is below 0.7, use GenSmart™ Codon Optimization or other equivalent codon optimization software to optimize heterologous expression of your GOI.

Step 4: Order Custom DNA Transposon From Aldevron

Contact Aldevron to perform gene synthesis, cloning, and manufacturing.

- Email requests to **nanoplasmids@aldevron.com**.
- In the email, inform them that you would like to use Bio-Techne's TcBuster Nanoplasmid Backbone. Specify which promoter you would like to use (EF1α or MND).

TABLE // 01

Plasmid Map Elements

Element	Sequence or Accession #	Region
TcBuster ITR upstream of cargo	Within plasmid map	1371-1623
Target site duplication	CTCTAGAG	Labeled TSD
EF1 alpha promoter	Sequence ID: PP944529.1	1677-2860
BGH Poly A signal	Sequence ID: MG437020.1	2971-3195
TcBuster ITR downstream of cargo	Within plasmid map	3332-3659
Self-cleaving 2A peptides	nature.com/articles/s41598-017-02460-2	See publication

Note: Region sequences are specific to the TcBuster pUC57 backbone with MCS plasmid sequence. The plasmid map of the TcBuster pUC57 backbone is available as a .dna file openable with programs such as SnapGene.

If you require further assistance, please email the technical support team at techsupport@bio-techne.com.