Advancing biotherapeutic development with transposon-based technologies

Stable transgene expression using plasmid-based transfection relies on recombination for integration of transgene(s) into the host cell's genome. Recombination is a chance event leading to random integration of the transgene and results in variability in transgene copy number and expression in the transfected cells. In contrast, transposon-based methods are a more reliable and reproducible way of integrating DNA into the host's genome.

What is transposon technology?

Transposons or transposable elements get their name from the term 'transpose' which means to move from one place to another and are often known as 'jumping genes'. They were first identified in maize and studied by Barbara McClintock in the 1940s. Transposons make up more than 80% of the genome of the maize plant[1]

Although they are referred to as 'jumping genes', the transposable elements are integrated in the genome with the capacity to copy-paste or cut-paste themselves to other positions, hence resulting in copies of themselves, leading to mutations at the site of their integration.

Generally, transposable elements are categorized into two classes

1. Class I (Retrotransposons or "copy-paste"):
   These replicate themselves via reverse transcription and have an RNA intermediate. They require reverse transcriptase enzyme to function.

2. Class II (DNA transposons or "cut-paste"):
   These transposons do not have an RNA intermediate. Most of them follow a cut-paste mechanism which requires a transposase enzyme[3].

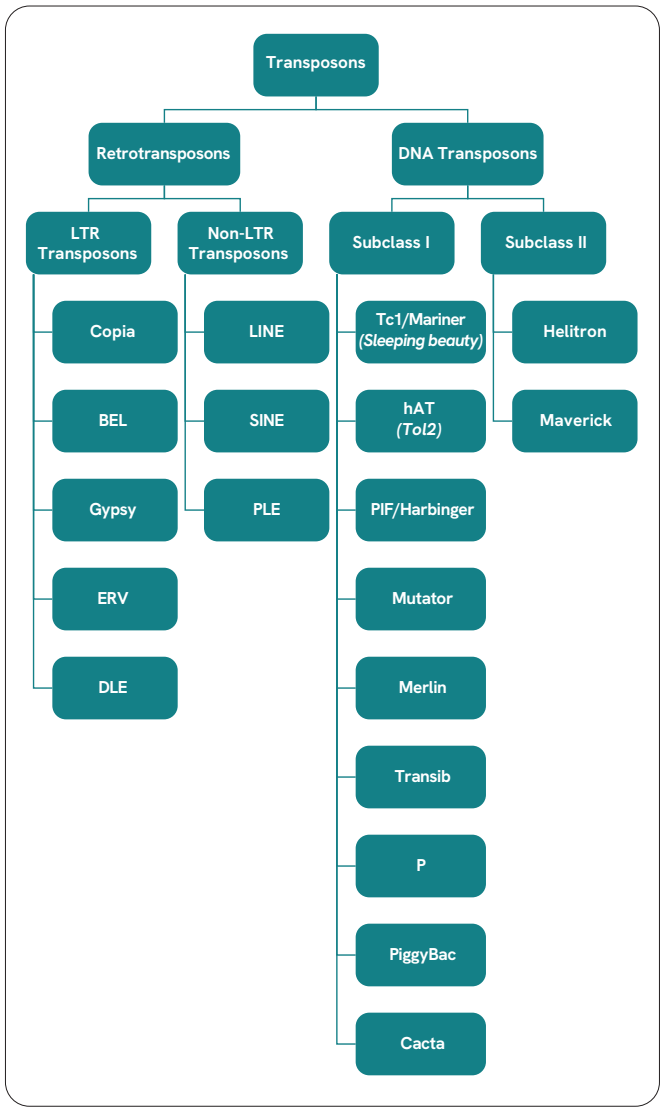

Figure 1: Classification of transposon elements. Retrotransposons (Class I) and DNA transposons (Class II).

Most DNA transposons move through a non-replicative, cut-paste mechanism. In nature, the DNA transposon consists of a transposon gene flanked by two Terminal Inverted Repeat (TIR) sequences. The transposase, once expressed, recognizes these TIRs to perform the excision of the transposon DNA, which is inserted into a new genomic location (Figure 2).

Transposons in most species undergo mutation and silencing over time and form part of the junk DNA of that species. Scientists have used reverse engineering to re-construct some of these transposons from invertebrates and lower vertebrates. These transposases are now functional and have been used as a genetic tool in non-native hosts[4].

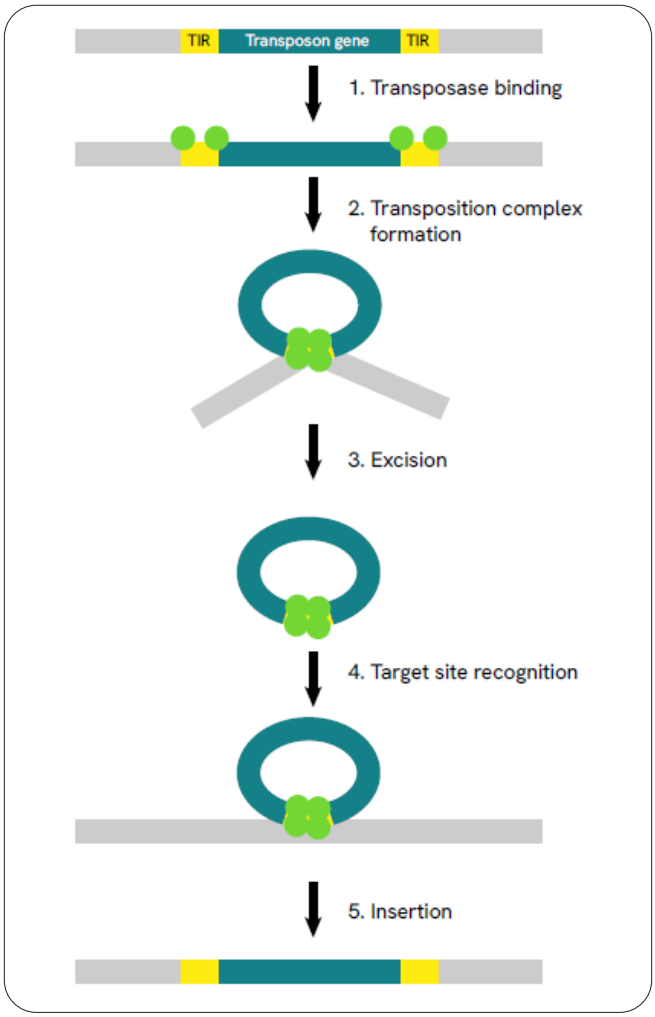

Figure 2: Schematic overview of the cut -paste mechanism mediated by transposases. (1) Transposase recognizes and binds the TIRs flanking the transposon, (2) transposition complex is formed, (3) transposition complex is excised, (4) transposition complex recognizes a target site in the genome, (5) the transposon is inserted into the genome. Image adapted from Skipper, K.A., et al, 2013[2].

Use of transposons in recombinant protein production and cell and gene therapy

Transposons have a natural propensity to be mobile, hence, they have served as a very important genetic tool. Most DNA transposons carry their own transposase gene flanked by TIR sequences which aid the transposition of the DNA. The basic vector design for using transposons to deliver recombinant genes consists of the gene of interest (GOI) and selection marker flanked by the TIRs, and a transposase gene located on a separate vector in trans. However, to control the activity of the transposase gene, and hence the mobility of the gene cargo, the transposase is often utilized in the form of mRNA[5] (Figure 3).

Due to its multiple advantages, this non-viral method of gene transfer has become quite popular in the field of recombinant protein expression, as well as in cell and gene therapy. Transposon-based vectors have been adapted from the natural elements of gene transfer, like viral vectors. However, transposon-based vectors have several advantages over viral vectors. Primarily, since transposons are non-viral and non-infectious, they are safer to use and do not require special biocontainment protocols[6]. Transposon vectors can be manufactured like traditional DNA plasmids, but unlike traditional random integration vectors, transposons aid in the high copy, semi-targeted integration of intact expression cassettes into open chromosomes of the host cells. The transpositioning of the GOI, from the donor vector to the host genome, is led by the transposase that enables the integration of intact transgene structures and prevents the formation of concatemers or truncated genes. Additionally, transposons have a very high cargo size capacity. Most transposon systems can effectively translocate hundreds of kilobases into the genome of host cells. Rostovskaya et al[7] transposed a bacterial artificial chromosome (BAC) with a size of approximately 100 kb into embryonic stem cells. Furthermore, the use of conventional DNA vectors that are propagated and isolated from bacteria may raise safety concerns for broader clinical applications. In fact, bacterial elements in the backbone DNA, including antibiotic resistance gene and bacteria origin of replication, can be integrated in the host genome with random integration methods.

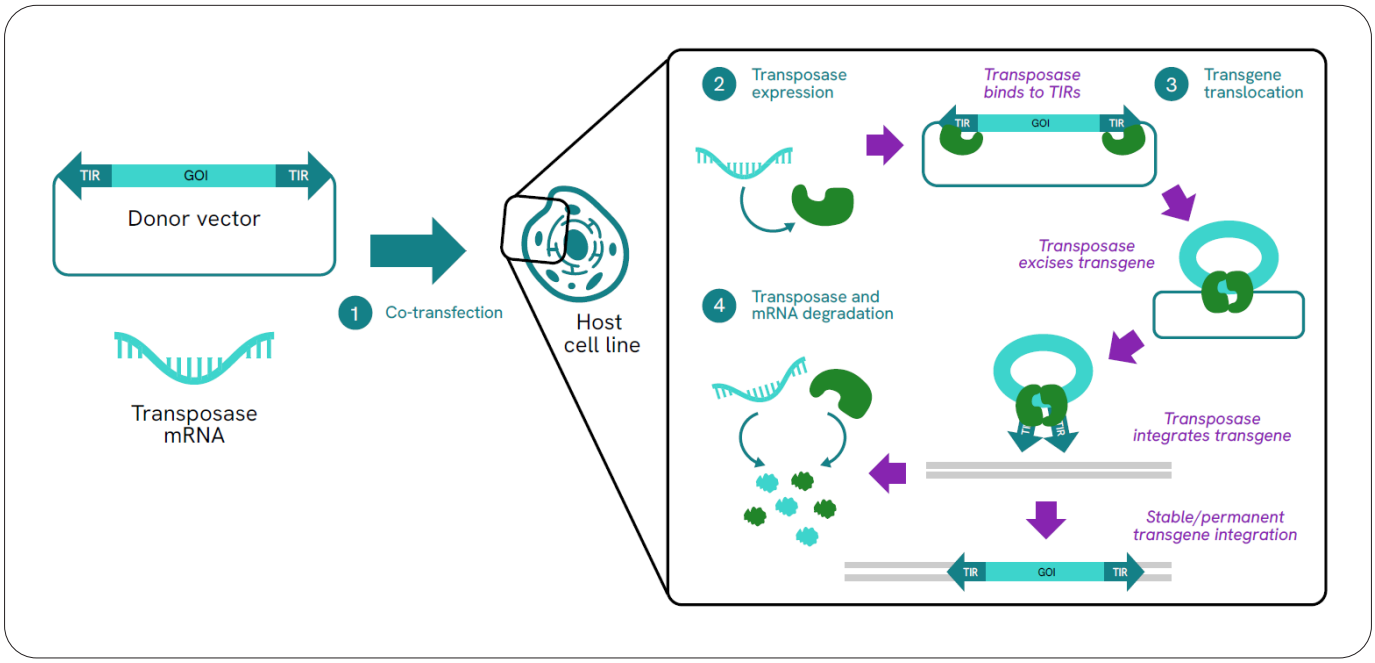

Figure 3: Schematic overview of transposase-mediated cut-and-paste transposition. After co-transfection of the donor plasmid and the transposase mRNA into the host cell, the transposase excises the GOI from the donor vector and inserts the GOI into a locus of the host genome.

The use of transposon-based vectors eliminates these risks since the integration of the transgene is catalyzed by the transposase that specifically recognizes the TIRs flanking the GOI and selection cassette. In this way, only the GOI and selection cassette are integrated into the host genome, thus eliminating the safety concerns associated with integration of bacterial elements. Integration in this catalyzed manner enables consistency and reproducibility, with regards to the integration of the transposon, between different transfections, and so improves clonal stability[8].

Table 1: Comparison of gene delivery systems.

	DNA plasmid (Random Integration)	Viral vectors	Transposon-based vectors
Safe to handle	+++	+	+++
Ease of vector preparation	+++	++	+++
Ease of transfection	+++	+++	+++
Integration efficiency	+	++	+++
Transgene integrity	+	+++	+++
Cargo size	+	+	+++
Universality of target cell line	+++	+	+++

Transposon technology and safety

Gene and cell therapy have extended the application of ex-vivo engineered cells into the clinic, thus raising a need for safer and more controlled methods of gene delivery. Chimeric Antigen Receptor (CAR)-T cell therapy is one such major breakthroughs in cancer therapy. Traditionally, viral vectors are used for the transfer of CAR genes to T-cells since they can transduce T-cells with high efficiency. Transposons have been used as an alternative method for gene transfer in CAR-T cell therapy. The first-in-human clinical trial with SLAMF7 CAR-T cells (CARAMBA) utilized the SB100X (Sleeping Beauty) transposase as a method for gene transfer[9]. The use of transposase in the form of an mRNA enables a controlled single event of integration within the CAR-T cells, hence making them safer for use in the clinic. In another instance, a Sleeping Beauty based system was used to genetically modify T-cells with 84% CAR expression[10]. These clinical trials have shown comparable efficiency with improved safety and reduced costs for clinical application of cell and gene therapy[11].

Table 2: Summary of current clinical trials employing transposon-based methods.

Clinical trial ID	Disease	Clinical phase	Status	Estimated start year	Country
NCT04102436	Cancer	II	open	2022	USA
NCT01163825	Alzheimer's disease	I	open	2008	Sweden
UMIN000030984	B-cell chronic lymphocytic leukaemia	I	open	2018	Japan
NCT04289220	B-cell chronic lymphocytic leukaemia	I	open	2020	China
NCT04249947	Prostatic cancer	I	open	2020	USA
NCT05292859	Cancer	I/II	No yet started	TBC	USA
NCT05194735	Solid tumour	I/II	open	2020	USA

Disruptive potential of transposons in bioproduction

The cell line development (CLD) process is often slow with most time spent in pool selection, pool screening, clone generation, clone screening and stability study.

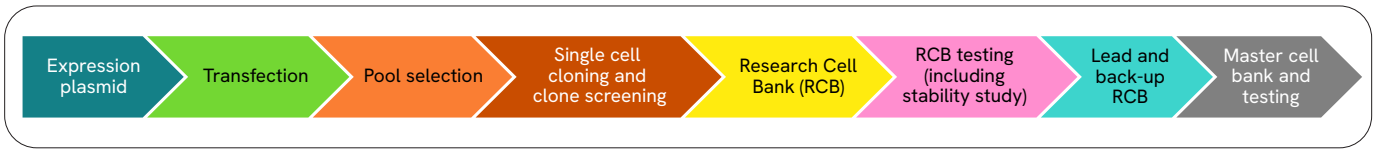

Figure 4: Standard CLD and process workflow. Image adapted from Bolisetty et al.[14].

Product variability can be introduced at various stages of the CLD process and includes, but is not limited to, variability in transfection efficacy, randomness of genetic integration in host cells that can lead to clonal heterogeneity, and instability and/or silencing of the transgene(s). Consequently, stable clonal cell banks are used for GMP production to minimize the variability of the final product. However, with the current race to clinic, more groups are striving to achieve homogeneity in product quality earlier in the process[15,17], hence enabling pre-clinical studies using material produced from transfected CHO pools. A recent review has highlighted the efforts to accelerate production of COVID-19 monoclonal antibodies during the pandemic, to enable faster access to patients. Strategies employed included using material derived from GMP-grade stable CHO pools for first-in-human clinical trial, thereby shortening traditional timelines. The pandemic paved a new path for biopharma to consider applying 'fast-to-clinic' strategies to other non-COVID-19 biologics. With access to newer technologies such as transposon system, biologics can be produced faster, with homogeneous product quality and reduced variability during the CLD process. Such technologies are currently being employed widely by biologics developers to bring therapies to patients faster- in less than 3 months from transfection to GMP-grade material production[18].

Combination of robust manufacturing cell lines, transposon-based technologies and optimized processes, results in a platform capable of supporting accelerated biologics development. In a constantly changing world with evolving therapeutic needs, rethinking the current concept of biotherapeutic development process becomes even more vital in situations with high medical needs or high mortality risks for patients. Pseudo-clonal cell lines and well-characterized stable pools can indeed provide adequate material for early clinical validation of new therapeutics, drastically reducing time to the clinic[19].

CHOSOURCE TnT transposon technology

Revvity's CHOSOURCE TnT transposon technology is a transposon-based vector which enables faster, reliable, and stable integration of recombinant genes into glutamine synthetase knockout (GS KO) Chinese hamster ovary (CHO) cell lines.

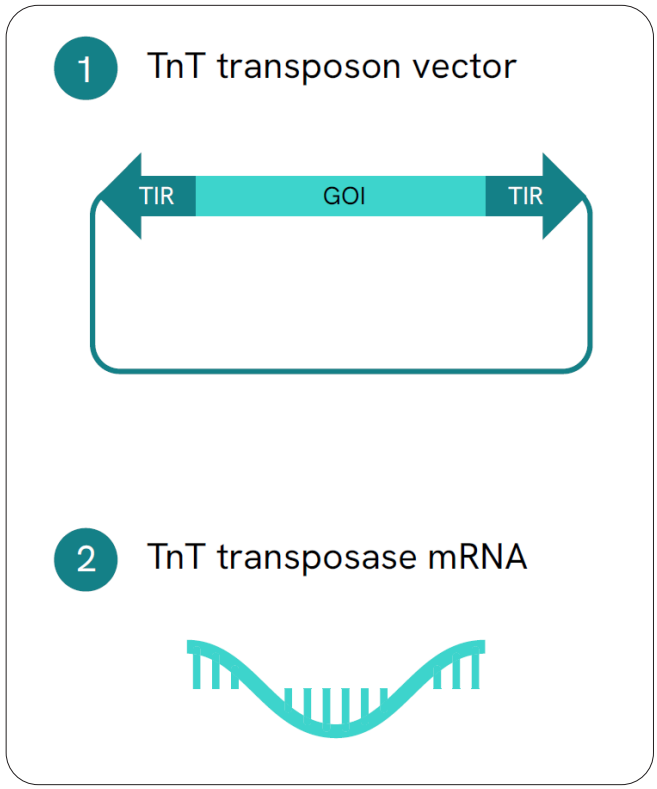

Figure 5: Revvity's CHOSOURCE TnT comprises of (1) TnT transposon vector, and (2) TnT transposase in the form of mRNA.

Revvity's CHOSOURCE TnT transposon technology consists of a TnT transposon vector, with optimized selection marker for higher productivity, and an mRNA coding for the transposase, which aids in the stable integration of the cassette into the host genome (Figure 5).

The optimized CHOSOURCE TnT vector includes:

- Dual expression cassette: Dual cassette vector aids in the easy cloning of GOI into the two multiple cloning sites (MCS) required for expression of two- or multiple-chain proteins[20]
- TIRs: The left and right TIRs flank the dual MCSs and selection gene, and are recognized by the transposase, helping in the excision of the cassettes from the vector and integration of the intact cassettes into the host genome.
- Selection cassette: A next-generation GS selection cassette enables the stringent selection of transfected CHOSOURCE cells.

The transposase, required for integration of the cassette (cut-paste mechanism) containing the GOI and the selection gene, is provided in the form of mRNA. This enables a single, non-reversible integration event into the host genome.

CHOSOURCE TnT transposon technology offers many benefits over the traditional random integration methods of gene transfer and can help advance CLD programs with:

- **Safe and robust genetic modification of host cells**

  CHOSOURCE TnT is designed to maximize host cell genetic modification whilst minimizing the risk of integrating unwanted bacterial sequences. The TnT transposase catalyzes semi-targeted integration of intact expression cassettes into the host genome, generating pools and clones with high and uniform gene copy numbers.

- **Faster development timelines**

  Faster recovery time during pool selection and high reproducibility across pools and clones can help to accelerate CLD programs. Selection timelines are often long and unreliable with random integration vectors, causing variability in the gene copy number of the selection gene, leading to diverse recovery times of cells in a pool. CHOSOURCE TnT transposon technology aids in the generation of more homogenous pools, with selection recovery being completed in as little as five days (from the point of transfection). In addition, higher reproducibility in GOI expression implies lesser screening efforts of pools and clones thus reducing the burden on workforce, costs and time. CHOSOURCE TnT shows consistently higher productivity (2.5-fold increase) when compared to a random integration vector, expressing the same GOI, across multiple transfections.

- **Streamlined CLD**

  CLD becomes simple and predictable with CHOSOURCE TnT. The platform eliminates the need for problematic methionine sulfoximine (MSX) mediated GS inhibition, without compromising selection stringency during CLD. The TnT transposase promotes multiple gene integrations in transcriptionally active regions of the genome, and this is reflected in high clonal stability levels (>95% for 90 generations) even under very stringent conditions.

Conclusions

Transposon-based technologies have gained increased recognition in the past years due to their versatility and effectiveness in modifying cells for diverse applications including CLD, cell and gene therapy, amongst others. They are safe and have been extensively used in diverse clinical interventions with many ongoing clinical trials. In this constantly changing world with evolving therapeutic demands, the industry requires novel, robust, and fast approaches for the development of therapeutics capable of shortening time to clinic and to market. Transposon-based technology has the potential to unlock new ways of therapeutic development, particularly in clinical emergency situations with high risks to patients. Simpler, faster, and consistent generation of highly producing pools enable the production of representative material very early during the program, enabling early downstream processing development, formulation development and even generation of adequate material for early preclinical studies.

The robustness and reliability of CHOSOURCE TnT transposon technology makes it a great tool to streamline development and manufacturing efforts, potentially enabling new development paradigms to expedite preclinical (and potentially clinical) development. The resourcefulness of transposon technology can dramatically change how development programs are conducted, thus improving therapeutic development throughput whilst expediting time to clinic.

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