CHOSOURCE™ Transposon Technology: Enhancing Speed, Stability, and Productivity

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1 Abstract

Revvity's CHOSOURCE™ expression platform is a trusted global solution for biotherapeutics development. Built on a Chinese hamster ovary (CHO) CHO-K1 suspension-adapted host cell line with a Glutamine Synthetase (GS) knockout (KO), it provides a robust, industry-standard system for reliable selection and cell line development.

The platform has been further advanced with the introduction of **CHOSOURCE™ TnT transposon technology**, a powerful tool that leverages transposase activity to generate stable, high-expressing clones with far greater efficiency than traditional random integration.

By pairing the **CHOSOURCE TnT technology** with **CHOSOURCE cell lines**, researchers benefit from:

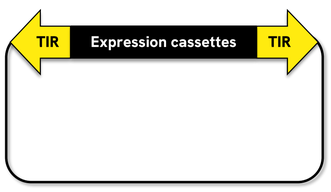
- **A streamlined, selection-agent-free workflow** - eliminating the need for methionine sulfoximine (MSX) or other agents.
- **Greater stability and consistency** - reduced clonal variation facilitates predictable performance at the clone level.
- **Accelerated timelines** - supports faster progression through cell line development; host-to-clone in virtually 12 weeks.

These improvements directly address common bottlenecks in biologics development, delivering an easy-to-implement tool to researchers and developers.

Data from the platform highlight its versatility and performance across diverse recombinant protein modalities, including monoclonal antibodies, bispecific antibodies, and challenging Fc-fusion proteins. With **CHOSOURCE TnT technology**, Revvity empowers scientists to advance their biologics programs with confidence, efficiency, and speed.

2 What is CHOSOURCE TnT Transposon Technology?

Revvity's CHOSOURCE TnT transposon technology is a two-component expression system (**Fig. 1**), compatible with both CHOSOURCE cell lines:



1) TnT Transposon Vector

- Two multiple cloning sites controlled by independent promoters for the expression of the gene of interest (GOI)
- Next-generation selection cassette
- Terminal inverted repeat (TIR) sequences

2) TnT Transposase mRNA

- Administered in 'trans' as mRNA
- Vector-to-chromosome
- Ready-to-use reagent

Fig. 1: CHOSOURCE TnT transposon vector and transposase mRNA.

Revvity's CHOSOURCE TnT transposon technology offers a proprietary vector design optimized for bioproduction applications. Following co-transfection of the two components, TnT transposase mRNA is translated and the resulting enzyme catalyzes transgene excision, followed by stable/permanent transgene integration in the host cell genome. Subsequent degradation of the transposase mRNA and protein ensures no transposition of the transgenes after their integration.

3 Random Integration vs. TnT

Characteristics of Random Integration (RI) Technologies

Traditional RI methods are non-catalyzed processes where multiple gene copies are often integrated at fewer loci, and in various rearrangements (**Fig. 2**). These loci could consist of inactive regions, potentially leading to GOI silencing. Due to these features, RI is known to be heterogenous, and genetic stability on a clonal level can be low (30-60%).

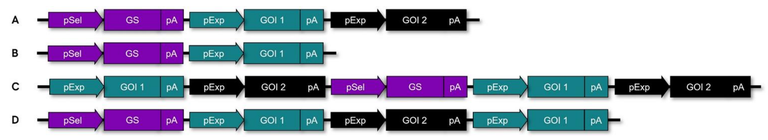


Fig. 2: Possible rearrangements of expression cassettes, when using RI. (A) wild-type, (B) fragmentation, (C) rearrangement, (D) concatemerisation.

Characteristics of CHOSOURCE Transposon Technology

CHOSOURCE TnT transposon technology is a catalyzed process whereby the TIR sequences enable integration in the host genome, in a manner where there is no concatemerisation, fragmentation or rearrangement (**Fig. 3**). Preferential integration at transcriptionally active sites leads to an overall homogenous population, where clonal stability is consistently over 95%.

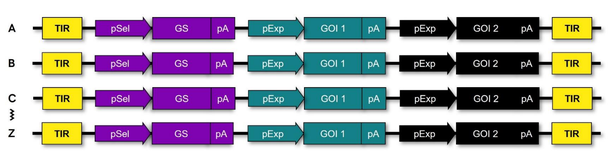


Fig. 3: Transposase-based technology enables stable and permanent integration of GOI in a consistent manner. (A) wild-type, (B-Z) unchanged genetic integration in host genome.

4 Monoclonal Antibody Cell Line Development

I- Bulk Pool Selection Profiles

Stable IgG-expressing pools were generated by transfecting CHOSOURCE GS KO cells with either CHOSOURCE TnT expression system or a RI vector. During selection, CHOSOURCE TnT pools (no MSX) consistently displayed faster recovery than pools transfected with RI vector (with MSX) (**Fig. 4**).

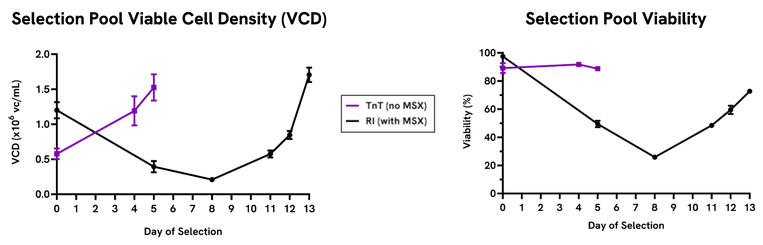


Fig. 4: Bulk pool selection profiles for CHOSOURCE GS KO cells when using CHOSOURCE TnT transposon technology (without MSX) or standard RI method (with MSX).

II- Pool Performance (Productivity)

On day of harvest, CHOSOURCE GS KO pools transfected using CHOSOURCE TnT transposon technology display product titers over 3.5-fold higher than titers achieved with RI vector (**Fig. 5**).

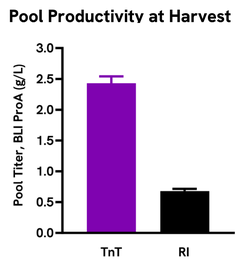


Fig. 5: CHOSOURCE TnT pool titer, analyzed by biolayer interferometry (BLI), is considerably higher, at day 14, compared to RI vector transfected pools (un-optimized fed-batch process).

III- Clone Performance (Productivity)

Clone titer was determined by enrolling a panel of CHOSOURCE TnT IgG-expressing clones into an unoptimized fed-batch process (**Fig. 6**). The top 30 expressing clones, originating from three biological pools, display titers above 4 g/L and comparable titers across the board.

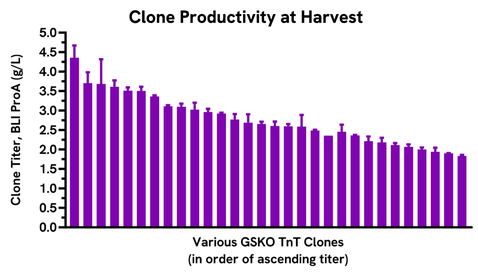


Fig. 6: Clone productivity from a panel of 30 clones originating from three biological pool populations (un-optimized fed-batch process).

IV- Clone Stability (Productivity)

Clone stability was defined by comparing performance of the top 15 expressing clones, over various generation (Gen) times. IgG titer assessment shows all 15 clones are stable up to at least Gen 90 (**Fig. 7**). Other clone stability studies using CHOSOURCE TnT transposon technology have consistently shown stability to be ≥95%.

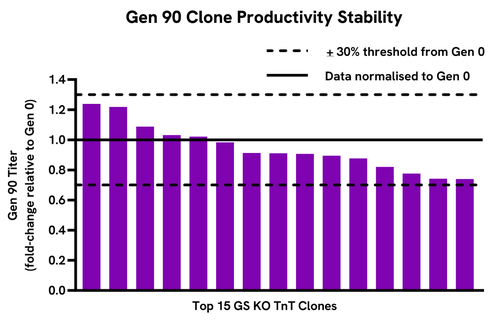


Fig. 7: Productivity stability profile of 15 clones, where Gen 90 titer is normalized to Gen 0 titer (un-optimized fed-batch process).

V- Clone Stability (Gene Copy Number Stoichiometry)

Copy number variation (CNV) analysis was performed for the three GOI- GS, IgG heavy chain (HC) and light chain (LC)- across the top 3 expressing clones at Gen 0, Gen 30, Gen 60 and Gen 90. 100% stability is shown across all samples, and a clear 1:1:1 stoichiometric ratio is shown between the three GOI, indicating intact integration of the transposon (**Fig. 8**).

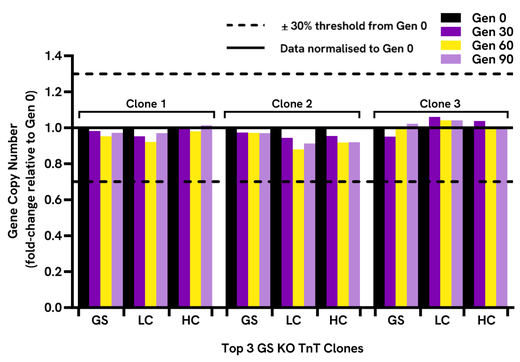


Fig. 8: GOI gene copy number for the top 3 expressing clones shows 1:1:1 stoichiometric ratio up to at least Gen 90.

VI- Pool and Clone N-Glycan Profiling

Revvity's LabChip™ GXII Touch™ System was used to detect the relative abundance of major glycoforms in several CHOSOURCE TnT IgG-expressing pools and clones, across Gen 0 to Gen 90. The overall relative glycan profile conveyed a similar glycan profile between Gen 90 clones and their Gen 0 counterparts, comparable glycan profiles between clones and their respective pool, and glycan profiles resembling the IgG standard (STD) used (**Fig. 9**).

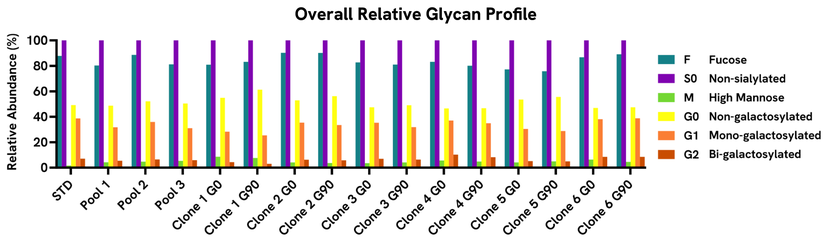


Fig. 9: Overall relative glycan profile showing three biological expressing pools (Pool 1-3) and six biological clones (Clone 1-6) across two time-points (Gen 0 and Gen 90).

5 In-house Study: Various Molecule Expression

I- Bulk Pool Selection Profiles (Un-optimized molecule sequences)

CHOSOURCE GS KO cells stably expressing a panel of molecules, ranging in complexity of expression, all display fast selection recovery following transfection using CHOSOURCE TnT technology (**Fig. 10**).

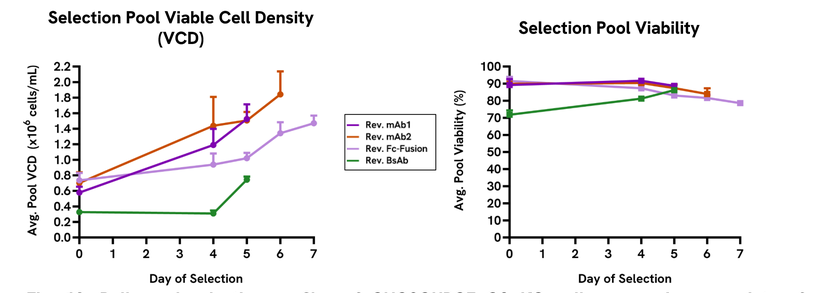


Fig. 10: Bulk pool selection profiles of CHOSOURCE GS KO cells expressing a variety of recombinant proteins all show quick recovery (within 7 days) and low pool-to-pool variation. mAb: monoclonal antibody, BsAb: bispecific antibody.

II- Pool Productivity (Un-optimized process)

CHOSOURCE TnT technology and CHOSOURCE GS KO cell line demonstrate robust performance as high pool titers are achieved following stable expression of various recombinant proteins, under unoptimized fed-batch conditions (**Fig. 11**).

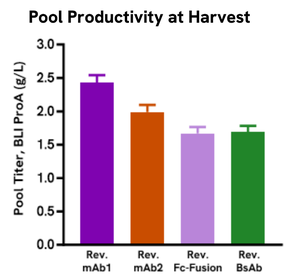


Fig. 11: CHOSOURCE TnT can be employed for the expression of several biologic types.

6 Customer Study: Various Molecule Expression

Stable transfections were performed using CHOSOURCE TnT technology and CHOSOURCE GS KO cells for the expression of several customer molecules which varied in their complexity to be expressed (mAb, BsAb and Fc-Fusion). Production assessments were conducted using the customer's protocol. Stable pools were shown to reach up to 6 g/L (in the case of Fc-Fusion expressing cells), and stable clones were shown to reach top titers of > 5 g/L (in the case of mAb1) (**Fig. 12**).

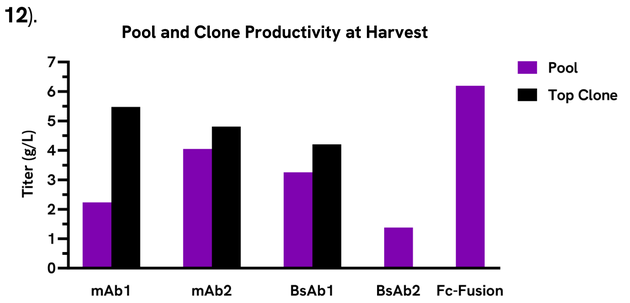


Fig. 12: CHOSOURCE Expression Platform demonstrates robust performance for the expression of difficult to-express molecules and has proven adaptability for different process strategies.

7 Conclusion

- **CHOSOURCE TnT transposon technology offers multi-copy and non-fragmented gene cassette integration, leading to high clonal stability (> 95%) at both genetic and phenotypic levels, thus supporting alternative approaches to cell line development (*i.e.* stability studies are not on the critical path).**
- **CHOSOURCE expression platform requires no addition of MSX and displays rapid pool recovery across molecule types. Accelerated selection recovery can shorten developmental timelines, reducing bioprocessing costs.**
- **The platform facilitates steady productivity at pool level, simplifying process development by avoiding delays and failures during development.**
- **Comparable titers between the top clones from different clone panels reduces screening efforts when searching for desirable clones.**
- **CHOSOURCE TnT transposon technology offers a well-established and robust technology with the potential to accelerate therapeutic development, whilst helps de-risking manufacturing.**