Integration of next-generation transposon vectors with state-of-the-art host cell lines

Tanaya Surve, Ana Rebocho, James Fleming, Nuno Pinto, Tulshi Patel, Karen Sandeman, Delphine Cougot, Holly Astley, Jesús Zurdo

Contact: bioproduction@horizondiscovery.com

Revvity, Cambridge Research Park, 8100 Beach Dr, Waterbeach, Cambridge, CB25 9TL, UK

1 Abstract

Revvity's CHOSOURCE™ expression platform is used globally for development of biotherapeutics. The platform consists of the Chinese hamster ovary (CHO) CHO-K1 suspension adapted host cell line with the Glutamine Synthetase (GS) gene knocked out (KO), to provide a robust industry standard selection system.

The CHOSOURCE™ expression platform has been recently improved by the introduction of the CHOSOURCE™ TnT transposon technology. This technology uses a transposase to generate, far more effectively, stable expressing clones. The partnership of CHOSOURCE™ TnT transposon technology with CHOSOURCE™ cell lines enables the implementation of a robust and safe (no methionine sulfoximine (MSX) or other selection agents required) cell line development pipeline with low variation and high stability at clone level, contributing to the acceleration of biologic development programs when compared to traditional random integration methods.

The data presented shows the performances of CHOSOURCE™ TnT transposon technology with the CHOSOURCE™ GS KO cells when expressing a non-optimized immunoglobulin G (IgG) reference molecule or non-optimized Fc-Fusion protein.

2 Why use transposons?

Over the years, transposon-based technologies have been deemed as industry standard for the manufacture of biologics, vaccines and diagnostics, with many proven benefits[1,2]:

- **Established technology** for use in bioproduction and cell and gene therapy, with consistent performance in diverse platforms.

- **Safe** to use, actively used in multiple clinical programmes and in the design of novel cell therapies.

- **Efficient** system favours gene integration in transcriptionally active regions, supporting genetic integrity.

- **Easy to use** as no vector digestion/purification needed, making transfection simple.

- **Predictable** results as high pool-to-pool reproducibility is observed. Comparable titers across top clones from same panel means faster/simplified screening practises.

- **Proven performance** due to high viability, high productivity and high clonal stability.

- **Future-proof** for new development and manufacturing paradigms, redefining therapeutic program timelines by reducing time to clinic.

(1) Schmieder, V. *et al*. J.Biotechnol. (2022) 349: 53-64

(2) Sandoval-Villegas et al. Int J Mol Sci (2021) 22(10): 5084

3 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 optimised for bioproduction applications. Following co-transfection of the two components, TnT transposase mRNA is translated and the resulting enzyme catalyses 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.

4 Random integration vs. TnT

Characteristics of Random Integration (RI) Technologies

Traditional RI methods are non-catalysed 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%).
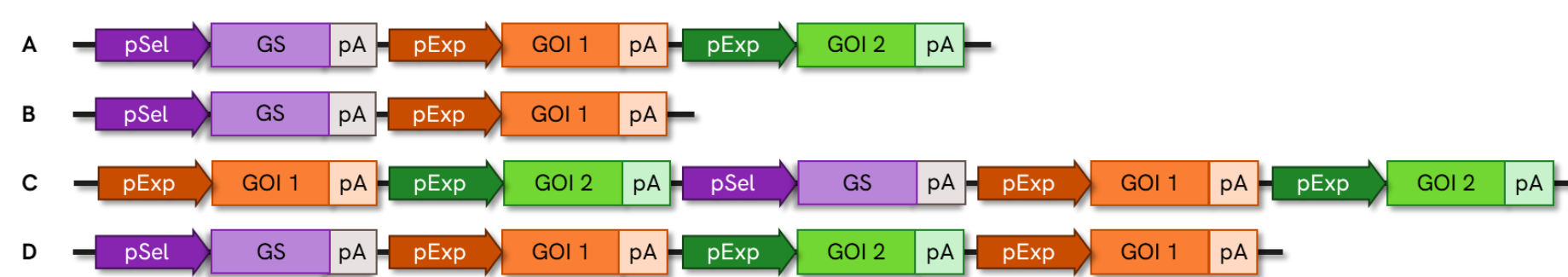

Fig. 2: Possible rearrangements of expression cassettes, when using RI. (A) wild-type, (B) fragmentation, (C) rearrangement, (D) concatemerization.

Characteristics of CHOSOURCE™ Transposon Technology

CHOSOURCE™ TnT transposon technology is a catalysed process whereby the TIR sequences enable integration in the host genome, in a manner where there is no concatemerization, 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.

5 Results

I- Bulk Pool Selection Profiles

Stable IgG-expressing pools were generated by transfecting CHOSOURCE™ GS KO cells with either CHOSOURCE™ TnT expression system with or without the TnT transposase or a RI vector. During selection, CHOSOURCE™ TnT pools (no MSX) consistently displayed faster recovery than pools transfected with RI vector (with MSX). In the absence of transposase, the cells take longer to recover (**Fig. 4**).
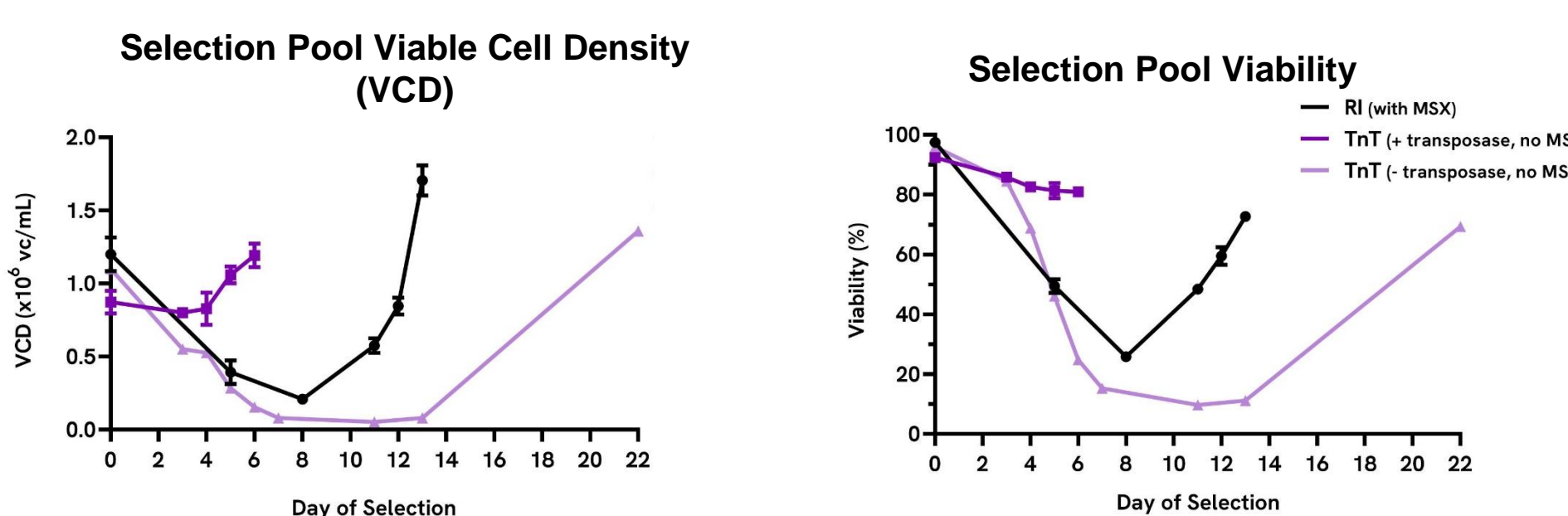

Fig. 4: Bulk pool selection profiles for CHOSOURCE™ GS KO cells when using CHOSOURCE™ TnT transposon technology with or without the TnT transposase (without MSX), or standard RI method (with MSX).

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. In the absence of transposase, very low titer is observed, demonstrating negligible levels of vector insertion by random integration (**Fig. 5**).
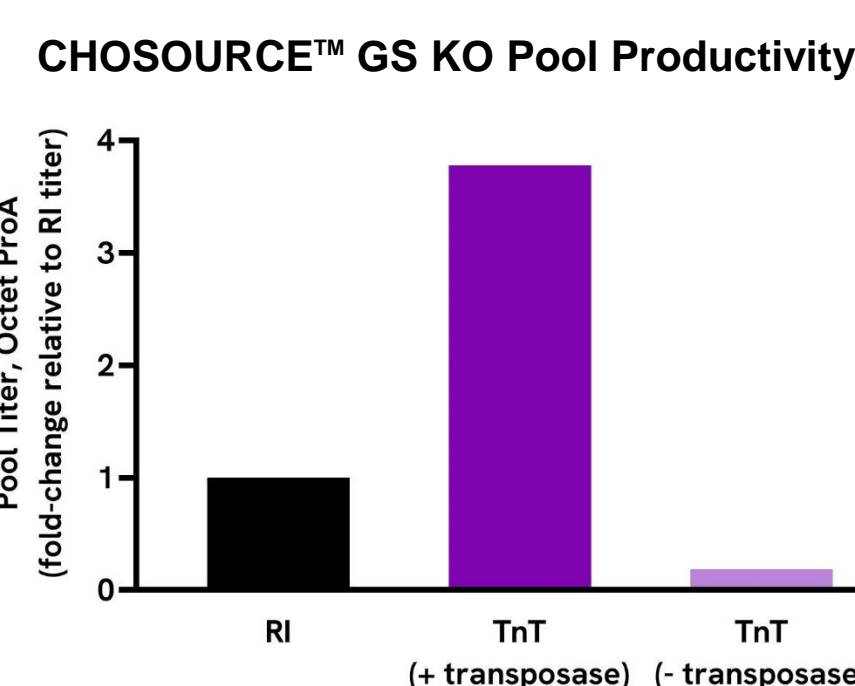

Fig. 5: CHOSOURCE™ TnT pool titer at day 14 is considerably higher compared to RI vector transfected pools (unoptimized fed-batch process).

III- Pool Performance (Gene Copy Number)

Copy number variation (CNV) analysis shows the presence of CHOSOURCE TnT transposase leads to specific integration of the region present between the TIRs (e.g. GOI) (**Fig. 6A**). The ampicillin resistance (AmpR) gene, used for bacterial selection and present outside of the TIRs, shows much lower integration in the transposase transfected pools, compared to the GOI. This suggests almost negligible random integration of the CHOSOURCE TnT vector in the presence of the CHOSOURCE TnT transposase. Finally, the pool copy number is shown clearly as a 1:1:1 ratio between the three genes of interest, indicating intact integration of the transposon (**Fig. 6B**).
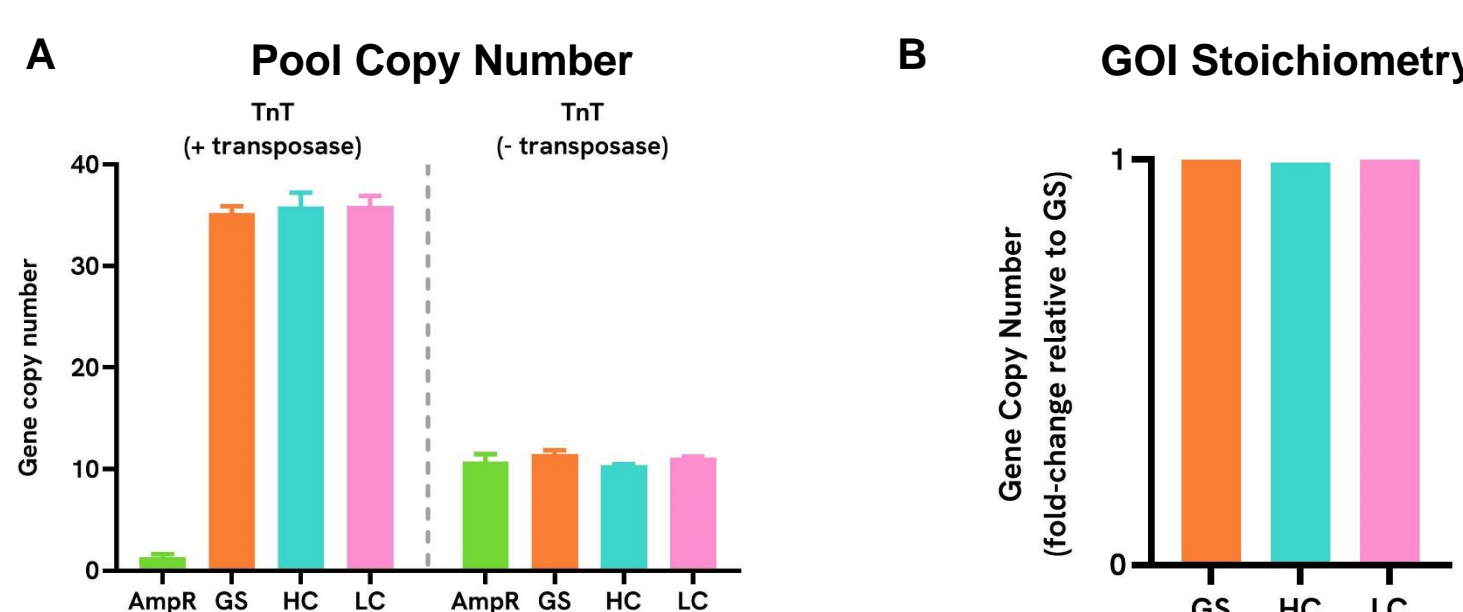

Fig. 6: Pool gene copy analysis (A) CNV of genes in cells transfected with/without TnT transposase, (B) stoichiometric GOI CNV. AmpR; ampicillin resistance, HC; heavy chain, LC; light chain, GS; glutamine synthetase.

IV- Clone Performance & Stability

Clone titer was determined by enrolling a panel of CHOSOURCE™ TnT IgG-expressing clones into an unoptimized fed-batch process (**Fig. 7**). The top 15 expressing clones, originating from three biological pools, display titers above 3 g/L and comparable titers across the board.
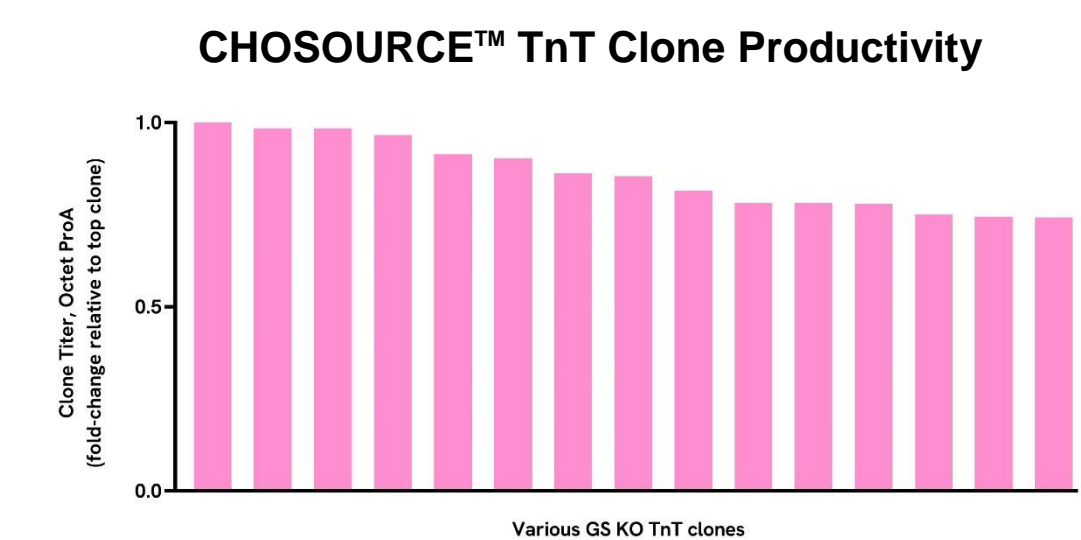

Fig. 7: Clone productivity from a panel of 15 clones originating from three biological pool populations (unoptimized fed-batch process).

V- Clone Performance & Stability

Clone performance was determined by analysing a panel of CHOSOURCE™ TnT IgG-expressing clones.

Clone stability was defined by comparing performance over various generation (Gen) times. IgG titer assessment shows 29 out of 30 clones are stable at Gen 90 (**Fig. 8**). Clone stability studies using CHOSOURCE™ TnT transposon technology have consistently shown stability to be ≥95%.
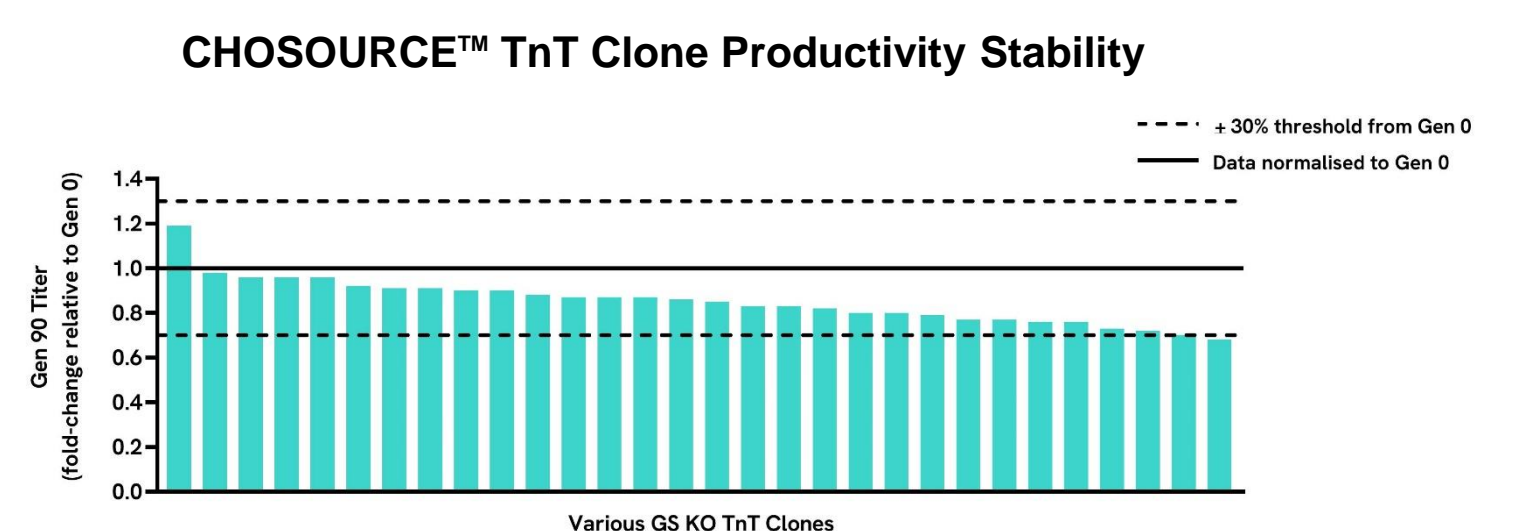

Fig. 8: Productivity stability profile of 30 clones, where Gen 90 titer is normalised to Gen 0 titer (unoptimized fed-batch process).

CNV analysis of top 15 expressing clones, from Gen 0 to Gen 90, conveys 100% stability across all clones (**Fig. 9**).
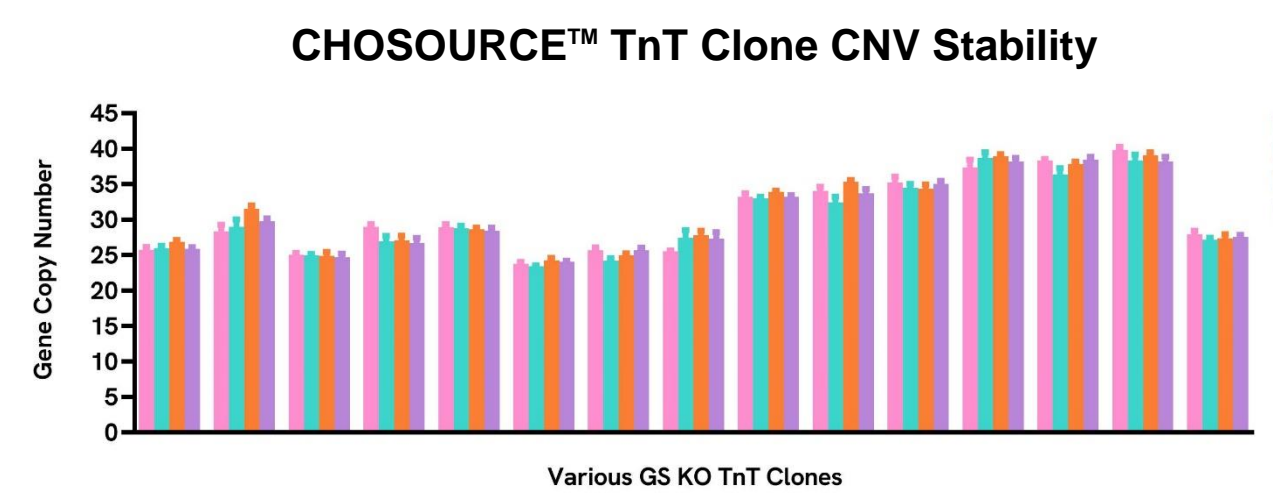

Fig. 9: Gene copy number for top 15 expressing clones shows all clones are consistently stable up to at least Gen 90.

VI- Fc-Fusion Pool Performance

CHOSOURCE™ GS KO cells transfected with a non-optimized difficult-to-express Fc-Fusion molecule, using CHOSOURCE™ TnT transposon technology, displayed comparable performance to the expression of a standard IgG molecule (**Fig. 10**).
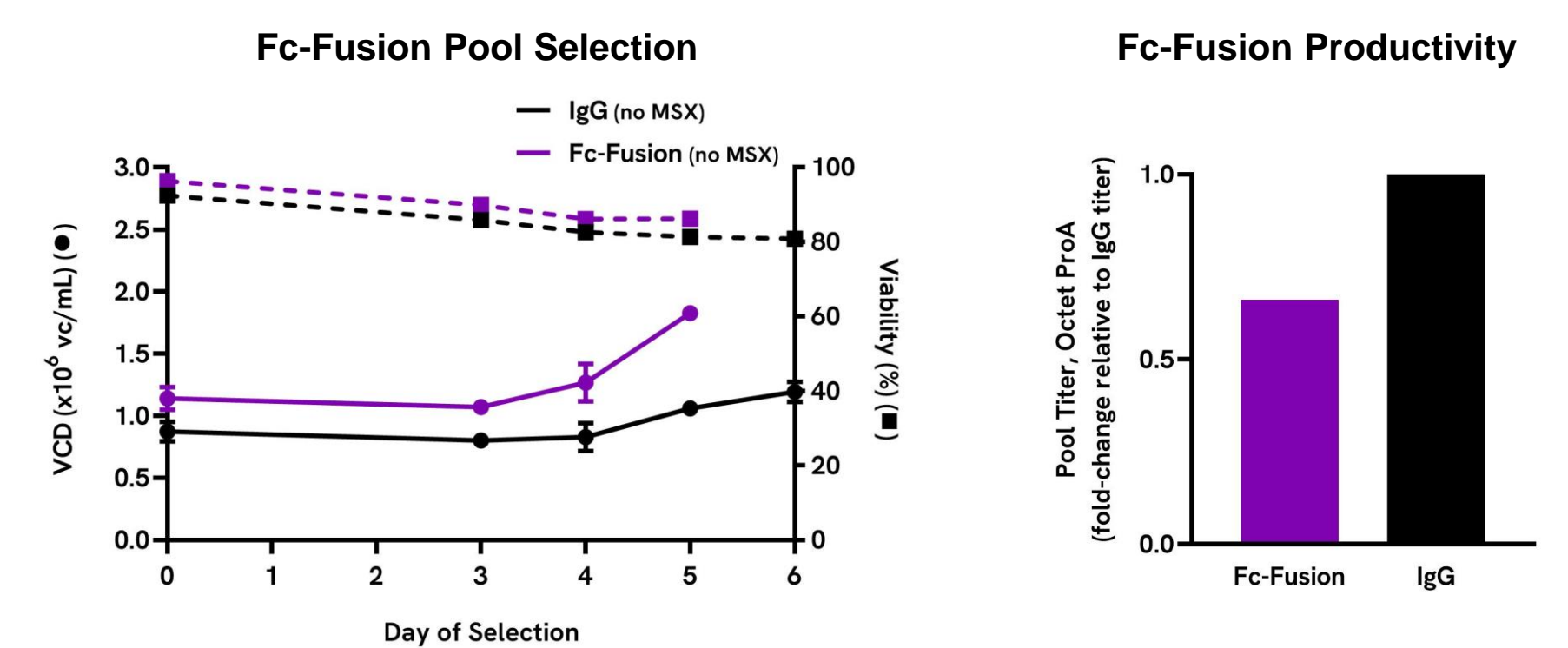

Fig. 10: Performance demonstrated with difficult-to-express proteins.

6 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, may enable alternative approaches to cell line development (*i.e.* stability studies are not on the critical path).

- The controlled integration of the CHOSOURCE TnT transposon vector by the CHOSOURCE TnT transposase enables specific integration of the region between the TIRs which includes the GOI and avoids integration of unwanted bacterial elements in the host genome.

- CHOSOURCE™ expression platform requires no addition of MSX, and displays fast pool selection recovery when expressing non-optimized IgG and Fc-fusion protein. Selection recovery has shown to be accelerated, compared to standard random integration technologies.

- The platform enables 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 minimizes 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 de-risking manufacturing.