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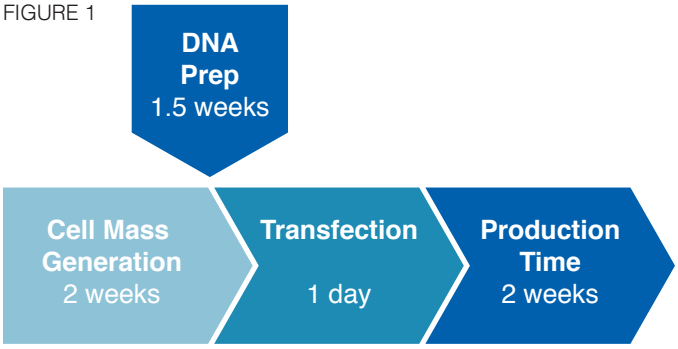
Making the Right Choice: Transient vs. Stable Pool Expression in CHO Cells

Historically, transient and stable transfections have been performed for different purposes. Transient transfections can rapidly provide reasonable amounts of recombinant proteins (mg quantities) for early evaluation of their activity and typically transient transfections were used in the screening stages of a project when potential clinical candidates were being selected. Due to the time that it took to generate stable clonal cell lines (7–12 months), they were generated only when clinical candidates had been selected. Over the past 20 years, however, technological advances in the generation of stable and transient transfectants have improved productivity and reduced timelines, leading to some debate over whether it is better to use transiently or stably transfected cell lines during development.

Although transient transfection procedures for CHO suspension cell lines have improved, they still typically yield only between 20–100 mg/L of material. Furthermore, transiently transfected genes are not stably integrated into the genome and the plasmids are usually lost within 1–2 weeks. For example, in order to generate material for a 10-L run, the first 10 liters' worth of cells must first be grown, which takes approximately 2 weeks, and massive quantities of plasmid(s) need to be generated in order to transfect the entire bioreactor (see Figure 1). Following the transfection, the bioreactor will be productive for 2 weeks and will typically yield between 0.2–1 grams of material, with no research cell banks to refer to for any follow-up experiments or any generation of a master cell bank. Additionally, there is no guarantee that during the next transient transfection that the post-translational modifications will be the same as those in the initial transient transfection.

FIGURE 1



By comparison, using the SURE*technology* Platform™ (stable transfection) for the same 10-L run with and the same starting plasmid, the amount of starting material in terms of plasmid and cells is much smaller, and the transfection is performed by day 3. Two weeks later, the first round of expression pools are selected based on production levels (g/L), and at 4 weeks, the second round of expression pools are selected based on production level (g/L) and product activity and the top pools are banked. By this point, even though the culture has not reached 10L, there is usually sufficient material to conduct significant testing. The remaining 4 weeks of development in the stable transfection approach is used to increase cell mass for the 10L bioreactor (2 weeks), plus an additional 2 weeks to maximize production, resulting in typical yields of 0.8 – 3.0 g/L (8–30 grams total), with cell banks that can be thawed, expanded, and used as needed. Not only does the stable pool approach yield between 5-30 times more material than the transient transfection method, but the availability of banked pools means that the material will be more consistent and reproducible from batch to batch. The investment of the additional 4 weeks upfront will save months on the back-end by eliminating the time that is needed for additional transfections, results in the availability of consistent protein material throughout the development process and allows for direct cloning from pool material to generate stable clones for research cell banking.

Conculusion:
Overall, a transient transfection strategy can be useful in early development when only small quantities of material are needed, and its associated heterogeneity can be tolerated, as in screening campaigns, when most of the candidate recombinant proteins will be eliminated from development. However, in all other situations, given the relatively short timelines that are now achievable with the generation of stable pools, and its high reproducibility, the low variability in production levels, low batch-to-batch cellular heterogeneity, and the 5–20 fold higher productivity compared to transient transfectants, stable transfections are typically the best choice for biologic development programs.

	Transient Method	Stable Pools Method
Low Variability	-	✔✔
Low Heterogeneity	-	✔✔
Productivity	✔	✔✔
Speed	✔✔	✔
Reproducibility	-	✔✔
Commercial Viability (>2g/l)	-	✔✔