Automated TLA for Targeted Complete Transgene & Integration Sequencing in Pharmaceutical Cell Line Development & Quality Control

The pharmaceutical industry uses genetically engineered cells, such as Chinese Hamster Ovary (CHO) cells, for a wide variety of high impact applications including, but not limited to, the production of pharmaceutical proteins, cell line models, and gene as well as cell therapy products. Many techniques have been developed to create transgenic cell lines, however, these techniques can result in undesired (off-target) integrations, multiple integration sites, unexpected integrations of backbone sequences, undesired sequences and structural variants in the integrated transgene sequence. To analyse these cell lines researchers can use Southern blots, FISH, or PCR analyses but generated data using these methods is often incomplete or hard to interpret. Cergentis' proprietary Targeted Locus Amplification (TLA) technology, is a powerful tool to sequence transgenes, integration sites and gene editing events. TLA-based targeted sequencing accordingly presents a cost-effective and high-quality alternative to conventional transgenic cell line analysis techniques.

TLA Technology

The TLA technology constitutes a paradigm shift in targeted Next Generation Sequencing (NGS) by using the physical proximity of nucleotides within a locus of interest as the basis of selection. For the amplification of each genetic locus, only a few specific primer pairs are required. Any gene of interest can be amplified by TLA using the primer pair specific for the gene of interest. These generated amplicons can be processed using standard NGS library preparation techniques.

TLA enrichment in combination with NGS is a very flexible and easy approach to sequence regions of interest and identify all single nucleotide variants (SNVs) and structural variants which provides unique advantages in complete sequencing of targeted transgenes and their integration sites (Figure 1).

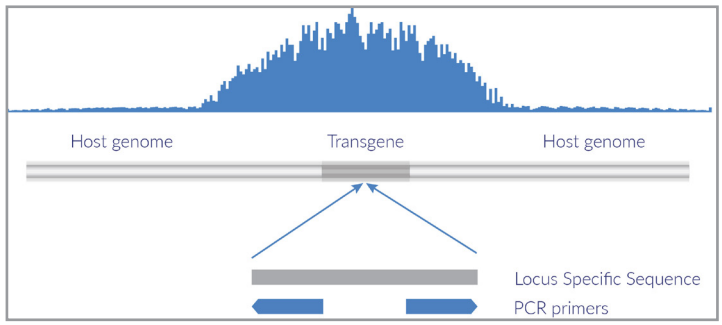

Figure 1: Overview of TLA-based transgene and integration site amplification and sequencing. TLA amplifications use one primer pair complementary to a short transgene-specific sequence. Generated NGS sequencing coverage (i.e. the number of NGS sequencing reads) spans both the entire transgene sequence and its integration site(s).

The TLA workflow

TLA technology workflow comprises these steps (Figure 2):

1. Physical DNA crosslinking,
2. Fragmentation of cross-linked DNA,
3. Circularization of DNA fragments by ligation,
4. Amplification of circularized DNA with primer pair(s) specific for the genetic locus of interest

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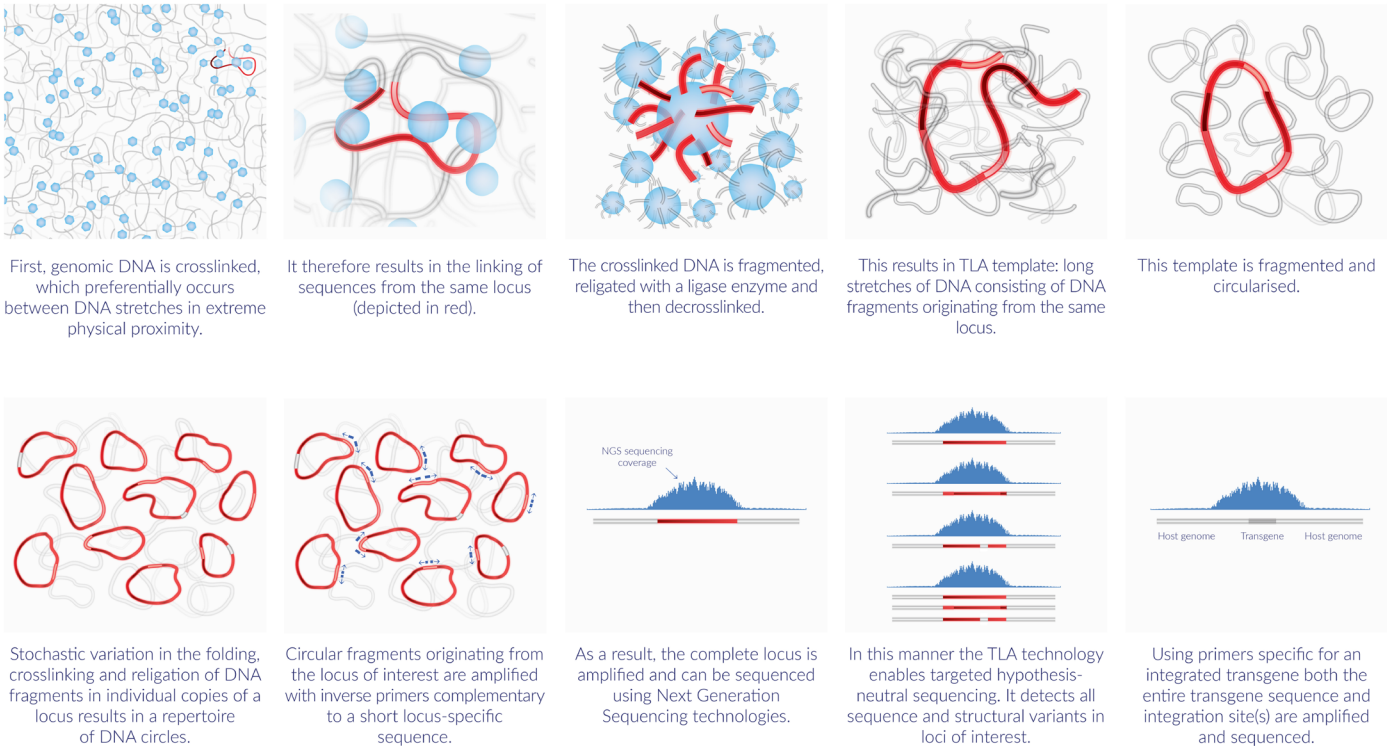

Figure 2: The TLA Protocol

Automating TLA Protocols

TLA sample preparation, like all NGS applications, necessitates robust template preparation to yield high-quality DNA samples. Errors can lead to sample mix-ups, the loss of precious samples, wasted reagents, and sequencing delays. PerkinElmer has standardized and tested protocols automating the Cergentis® TLA protocol on our suite of liquid handing workstations (Figure 4). With flexibility in throughput, capacity and dynamic volume range, high quality manufacturing standards, and outstanding customer service and support, PerkinElmer offers automated liquid handling solutions optimized to meet your laboratory's needs. All of the PerkinElmer liquid handling workstations increase throughput and walkaway time, while eliminating inter-operator variability in sample preparation performance as well as sample tracking errors.

Automating the Cergentis® TLA protocol on the reduces the hands-on time by 75%. The total hands-on time of the manual and automated protocols (including and excluding automated centrifugation and DNA concentration measurements) is specified in Figure 3.

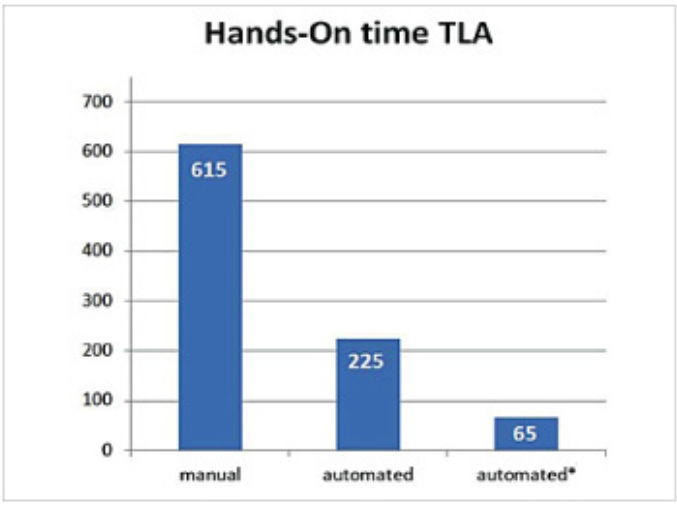

Figure 2: Hands-on time of the manual protocol, current automated with and without automated centrifugation and DNA concentration measurement.

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"TLA/NGS is a robust screening method useful for routine clone analytics during cell line development with the potential to process up to 24 CHO clones in less than 7 workdays."

Aeschlimann, et al., (2019) Biotechnology Journal, 14(7).

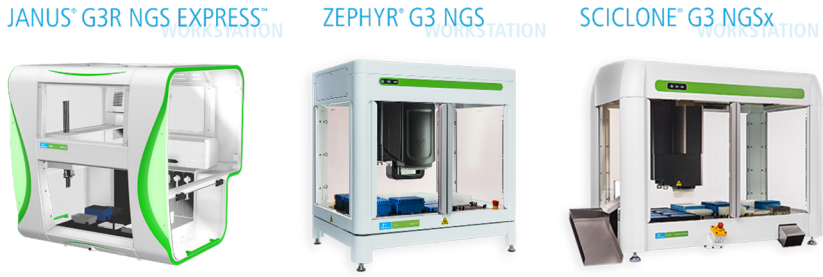

Figure 4: PerkinElmer automated solutions

The publication "Enhanced CHO Clone Screening: Application of Targeted Locus Amplification and Next-Generation Sequencing Technologies for Cell Line Development" provides a detailed overview of the utility of the TLA Technology automated on the Sciclone® G3 NGSx workstation in CHO cell line development. Cergentis' targeted locus amplification (TLA) strategy, automated with PerkinElmer's liquid handlers, offers an efficient NGS workflow for the genomic screening of antibody expressing CHO clones. As such, the automation of TLA Technology is a robust screening method useful for routine clone analytics during cell line development.

References

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