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Transgene Mapping Analysis

BY TARGETED LOCUS AMPLIFICATION TECHNOLOGY

WHY MAP A TRANSGENE?

The production of transgenic rodent models by traditional pronuclear injection results in random integration of single or multiple copies of the transgene in the genome. The site of transgene integration can have a profound effect on the expression of the transgene and endogenous gene functions, a phenomenon known as **position effect**. When the transgene insertion site is unknown, zygosity is determined by expensive quantitative PCR-based approach. These limitations often force investigators to maintain transgenic models in a hemizygous state, which may lead to less than desired expression levels of the transgene and make breeding less efficient and more costly.

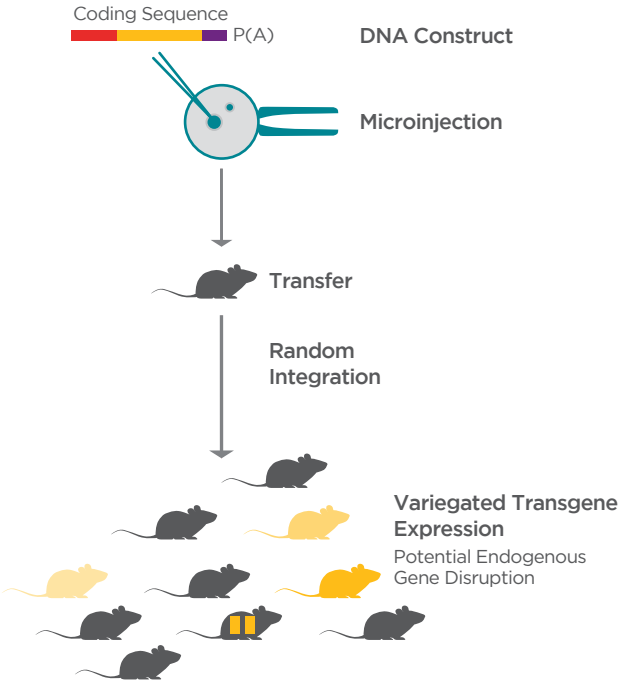
BENEFITS OF CHARACTERIZING A TRANSGENE INSERTION SITE

- Better correlation of phenotypes with transgene expression
- Ability to determine zygosity by genotyping assay and more cost effective management of transgenic strains
- Enhanced predictability of transgene segregation when breeding and intercrossing
- Awareness of any potential disruption of the regulatory or coding region of a critical endogenous gene

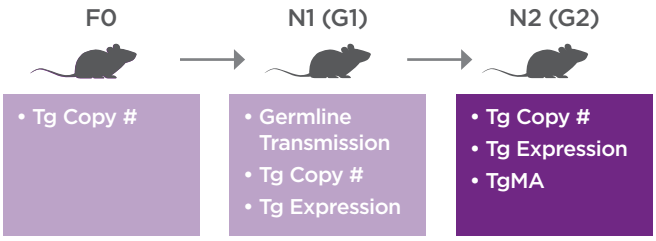
HOW DOES IT WORK?

Transgene Mapping Analysis (TgMA) uses Targeted Locus Amplification (TLA) technology[1] developed by Taconic Biosciences' partner, Cergentis B.V.[2], to precisely determine the transgene integration site(s) and transgene integrity in genetically engineered rodent models. This technology combines the principle of proximity ligation with next generation sequencing to selectively amplify and sequence the transgene and surrounding genomic region of sizes ranging from tens to hundreds of kilobases. Requiring only one primer pair complementary to a short sequence unique to the transgene, TgMA not only uncovers insertion sites and sequences of integrated transgenes, but also enables detection of single nucleotide variations, structural variations, and transgene-transgene fusions. The integration sites are detected by analyzing the coverage profile of sequencing reads and by identifying breakpoint sequences.

TRANSGENE INTEGRATION IS RANDOM



WHEN TO UTILIZE TgMA?



TgMA can be applied at any generational stage of the transgenic project, but is ideally suited for new or ongoing projects at N2 (G2) generation or greater where germline transmission and stale integration have been confirmed.

1. Targeted Locus Amplification (TLA) technology: **nature.com/nbt/journal/vaop/ncurrent/full/nbt.2959.html**
2. Taconic's partner, Cergentis B.V., provides services and kits on the basis of its proprietary TLA Technology. Key applications include transgene and transgene integration in animal models and mammalian cell-lines, and the targeted complete sequencing of genes relevant for genetic diagnostics and personalized medicine. Learn more at **cergentis.com**

ADVANTAGES OF TgMA TO CONVENTIONAL METHODS OF TRANSGENE MAPPING

■ LOW
■ ■ ■ ■ ■ HIGH

	Fluorescent In-Situ Hybridization (FISH)	PCR-based Methods (e.g., Inverse PCR)	Whole Genome Sequencing (WGS)	TgMA by Targeted Locus Amplification
Resolution	■	■ ■	■ ■ ■	■ ■ ■ ■ ■
Reliability	■ ■	■ ■	■ ■ ■	■ ■ ■ ■ ■
Flexibility	■	■ ■	■ ■ ■	■ ■ ■ ■
Convenience	■	■ ■	■ ■ ■	■ ■ ■ ■
Cost	■ ■ ■ ■	■ ■	■ ■ ■ ■	■ ■ ■

Traditional methods for mapping transgene insertion sites are expensive, cumbersome, and offer low resolution. Moreover, the presence of multiple or highly complex insertion events, truncated transgene sequences, and repetitive sequences of host genomes can all confound the results. Although some of these limitations can be overcome by whole genome sequencing (WGS), such an approach is often bioinformatics intensive and cannot reliably detect all structural variations in and around the transgene insertion site such as unknown sequences introduced by chromosomal rearrangement.

Transgene Mapping Analysis by Targeted Locus Amplification technology offers an alternative to traditional methods that yields unmatched clarity about the integrated transgene sequences and their integration sites without detailed prior locus information.

This partnership brings Cergentis's best in class transgene mapping methodologies with Taconic Biosciences' industry-leading transgene model generation and breeding services.

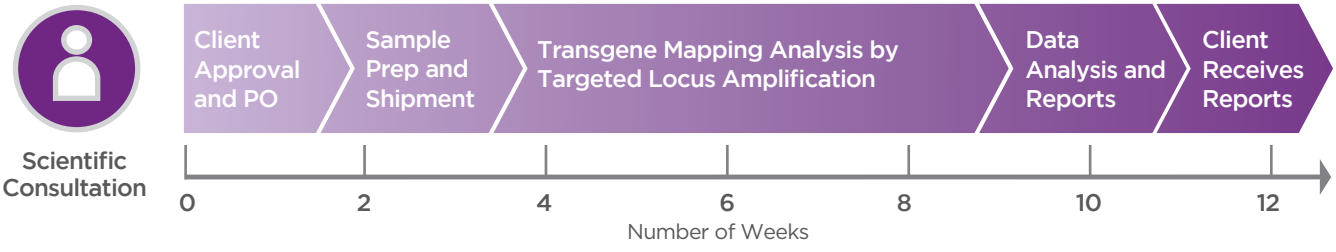
DELIVERABLES

- Full scope support service—from sample preparation through to the final report
- Detailed report of insertion and transgene position and characterization of structural changes and/or Single Nucleotide Variations (SNVs)
- Expert interpretation specific to a new transgenic line
- Genotyping assay to differentiate homozygous versus heterozygous (eliminating zygosity testing by quantitative PCR)
- Full design of optimized breeding strategies that account for transgene integration site

INPUTS AND MATERIALS REQUIRED

- Transgene sequence (prefer a .gb file with different sequence elements annotated) and current genotyping primer information
- Description of line (e.g., age, gender, zygosity if known) and observations from breeding
- Splenocytes obtained from a minimum of two transgenic animals per line at 6–8 weeks of age, shipped frozen at 10 million cells per vial

PROJECT TIMELINE



LEARN MORE AT: TACONIC.COM/TRANSGENE

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