Vanderbilt Genome Editing Resource

New services

We are pleased to offer four new services to support the characterization and expansion of CRISPR-edited mice.

1. **Allele validation in the N1 generation.** CRISPR mutations in the F0 generation can often be a problem to sort out. Thus, for new gene editing projects we now offer the option of having the VGER breed and genotype N1 generation offspring for you. This allows a repeat validation of the induced mutation in a heterozygous animal.
2. **Off-Target Analysis:** CRISPR has the potential to generate off target mutations. If this is a concern for you, we can design and perform PCR assays to detect editing at predicted off-target sites.
3. **Random Insertion Analysis:** DNA donor sequences, particularly longer ones, can insert randomly in the genome. We can design and perform assays to detect random insertions and use this information to help you decide which founders to breed.
4. **Rapid Colony Expansion and Cryopreservation**. It can be very time consuming to establish a colony of experimental animals from a single founder. If time is critical for you, we can harvest sperm from N1 heterozygous mice and use it to fertilize multiple isogenic wild type embryos. Aggressive breeding of the resulting heterozygous mice can accelerate experimental analysis by saving two or more generations of natural mating.

Two categories of CRISPR edits are now guaranteed

After tracking our results for the past two years, we now have good historical data that predicts the gene editing efficiency based on the nature of the desired edit and the donor DNA. The gene editing efficiencies we achieve now allows us to guarantee* that you receive the mouse you want for two of the four experimental categories described below.

Type I – Non-Homologous End-Joining (NHEJ). This category is for imprecise DNA deletions that cause frameshifts or other small alterations. In our experience, 4.2% of the potential founder mice will have the desired mutation. This enables us to guarantee* model delivery after two injection days in an approved mouse strain.

Type II – Homology Directed Repair (HDR) with short single stranded DNA (ssDNA). This category includes mice with point mutations, epitope tags, or precise deletions that are 80 bp or less. On average, 2.4% of the injected embryos will have the desired mutation. This enables us to guarantee* model delivery after three injection days in an approved mouse strain.

Type III – HDR with long ssDNA (< 5 kb). This approach works very well for altering longer stretches of DNA segments, and is being used to create floxed alleles and other more complex modifications. Our average efficiency is 1.6% founders per injected embryo. Although the efficiency is a bit lower for this category, these projects are typically successful within three days of microinjections.

Type IV – HDR with double stranded DNAs (> 5 kb). The use of a double stranded DNA (dsDNA) template may be necessary for longer sequence alterations, or when the desired edit contains repetitive elements. Since one percent or fewer of the founders generated will have the desired mutation, three or more microinjection days may be required. We are currently working to improve the efficiency of this category using some recently described technology enhancements.

*Guarantees apply only to VGER full service projects that include design through F0 screening.

To initiate a genome editing project, contact Leesa Sampson at leesa.sampson@vanderbilt.edu.

For other information, please visit our website: https://labnodes.vanderbilt.edu/vger or contact jennifer.skelton@vanderbilt.edu.

Have a great summer!

Mark A. Magnuson
Jennifer Skelton
Leesa Sampson
Linda Gower

Vanderbilt Center for Stem Cell Biology