December, 2020

Vanderbilt Genome Editing Resource

CRISPR-mediated collateral damage and the need for careful validation

While CRISPR-Cas9 is a marvelous new addition to our gene editing toolbox, undesired mutations may occur. In mice, off-target editing can be largely managed by choosing high specificity guide RNAs and segregating any off-targets that occur by breeding over several generations. However, mutations at the target locus, such as large deletions, inversions, and chromosomal translocations, can also occur (Shin et al., 2017; Burgio and Teboul, 2020). Recently, donor DNAs, a necessary component for introducing specific sequences into the genome by homology directed repair, have been shown to sometimes integrate in a head-to-tail, or "multiplexed" manner. This type of on-target mutation is often impossible to detect in the F0 generation (Skryabin et al., 2020). For this reason, we now offer a F0 founder breeding and allele sequence validation service that includes testing for the multiplexed integration of donor DNA. We encourage you to use our expertise in validating genome-edited alleles in your next mouse project.

Genome editing projects: differing degrees of difficulty

Over the past three years we have performed sixty-two full service mouse genome editing projects using CRISPR-Cas9. In 84% of these projects we have achieved the desired gene editing outcome. We can now more accurately predict which projects are likely be straightforward, and which projects will be challenging.

- DNA deletions under 10 kb and DNA insertions under 1.5 kb are generally very efficient. These manipulations almost always work, but efficiencies vary by locus and desired edit.
- Large DNA deletions (10 kb or more), DNA insertions longer than 1.5 kb, and editing of genes with high sequence identity with other genes can be challenging, but often succeed. However, they require more effort to design, screen, and validate.
- Conditional inversion alleles have uniformly failed.

We are always pleased to share our experience, discuss your ideas, and offer advice on how to both maximize your chances of success and to minimize expense.

Cryopreserve to save time, money, and space

We understand that budgets are limited, often leading investigators to avoid cryopreserving lines of mice to save money. However, consider for a moment that maintaining three cages of mice for a year costs $1,193.55, not including genotyping costs and staff effort. Since it currently costs $1,582.74 to cryopreserve a line of mice, it is possible to break even in terms of cost in as little as a year. Remember, cryopreservation of valuable lines will protect you from accidental loss or major breeding mishaps.

Thank you to our VGER users

As we reach the end of this challenging year, we want to thank you for continuing using this resource during an uncertain time. We hope you stay healthy and safe this holiday season!

Sincerely,

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