Guidelines - Mouse Genome-Editing Considerations

Some basic information to consider for your mouse genome-editing project

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Mouse Genome Editing at the Vanderbilt Transgenic Mouse Shared Resource

I have an idea for a new mouse model. How do I get started?

We can do as much or as little as you require. All services are priced á la carte:

**Option 1:** Are you confident in your own genome editing skills? If so, you can design, produce, and prepare all genome editing reagents and simply order injection services by our skilled team. Resulting pups are tailed and provided to you for genotyping and identification of founders.

**Option 2:** Do you feel okay about some steps, but not everything? We can do some of the work for you. For instance, you can design your experiment, but let us handle the molecular biology, injections, and genotyping. You can provide input and/or perform your own experiments as you wish.

**Option 3:** Do you have no idea how to even begin? We can do it all. We’ll design the experiment with your input and approval; order, prepare, and validate all reagents; perform injections; and screen the pups. Any founders will be transferred to your mouse room.

**Based on my desired genome edit, what type of genomic engineering method should I choose?**

The core has success using CRISPR/Cas9 to produce mice with small deletions, mutations, and small insertions such as Lox and Flag tags. Larger insertions, such as EGFP, have been obtained by Vanderbilt investigators, but the efficiency is usually lower and more injections may be required. As an alternative for some larger knock-in projects, we recommend the published C31 integrase-mediated transgene insertion strategy for knock-in in the “safe-harbor” H11 locus (described in more detail later). Note that we no longer offer conventional gene-targeting by mouse ESC homologous recombination due to reduced use of the technology upon the advent of CRISPR/Cas9.

**Say I want to generate a simple KO or small KI using CRISPR/Cas9. What basic steps are involved?**

1. **The first step is universal to all gene-editing projects:** Think very carefully about what type of mouse model you need to test your hypothesis. This is the most critical step. Making a transgenic mouse is cheaper, faster, and easier than in the past, but it is still not trivial and significant time and effort go into genome-editing projects. Consider what type of data you will want to collect and ensure your design would fulfill your needs. Discuss with colleagues who have experience using mouse models in your particular area of research. After you decide on your desired model, contact us for a consultation to discuss the technical aspects of your project. We have extensive experience and can help optimize your design.

2. **There are many published protocols detailing how to design a CRISPR/Cas9-mediated transgenic mouse.** Variations and optimizations on the basic protocols are being published continuously. As such, we do not provide a specific protocol for generating mice through the TMESCSR. Rather, if you plan to design your own strategy, we suggest you read a few reviews and some recent primary literature to decide what should work best for your particular project. (Note that the TMESCSR is hoping to soon offer zygote electroporation as an alternative to pronuclear injections, but the protocol is still in development.) Some select protocols are listed below:


One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. Wang et al., Cell,


Additional resources, including informational webinars, can be found at commercial vendor websites, such as IDT, Clontech, Sigma, and JAX. The core staff are happy to discuss any remaining questions. **Make sure your design includes a detailed strategy for genotyping and identification of founders!**

3. **Generate CRISPR/Cas9 reagents (DNA plasmid, RNA, or ribonucleoproteins).** CRISPR/Cas9 plasmids can be obtained through Addgene. gRNAs can be produced by *in vitro* translation or ordered through vendors such as Sigma or IDT. Cas9 protein can be ordered commercially. We suggest that if you generate your own CRISPR/Cas9 reagents that you consult with the core staff to agree upon the final concentrations for pronuclear injections. These concentrations are dependent upon your strategy (desired outcomes, number of reagents co-injected, etc.). Note that your injection materials have to be highly pure: DNA purified with commercial kits and phenol/chloroform purified and all injection solutions prepared in a cell culture hood. Even small amounts of impurities can be highly toxic to a developing embryo.

4. **Order cytoplasmic or pronuclear injections by the core staff.** Our experienced staff have been doing zygotic injections since the 1990’s and have consistently high survival rates. Depending on your strategy and the mouse background you desire we can help you decide how many days of injections you should plan for your best chance of obtaining a gene-edited founder.

5. **Genotyping/screening of F0 pups.** Your genotyping strategy is going to be project dependent. If you are generating a simple knock-out, you will need to perform a surveyor assay (commercial kits are available) and confirm deletions by cloning and sequencing. If you generated a knock-in, then you may have designed a restriction site that will permit screening or you can make construct-specific primers for screening.

*I want to generate a reporter or overexpression transgenic mouse. What are my options?*

Traditional transgenesis involves the pronuclear injection of your transgene insert into zygotes. This technique has long served to generate transgenic mice, but improvements in transgenic technology have been described. You could consider using Tol2-mediated transgenesis to insert your DNA sequence into the genome—its advantages are high rates of transgenesis and the ability to titrate enzyme concentration to have some control over transgene copy numbers. However, if you want to avoid positional effects and/or multiple insertions, there is a C31 integrase method that will drop a single copy of your transgene into a safe-harbor locus. We can obtain live H11 mice through Charles Rivers for this method and Rosa26 mice are also available as a frozen line.

**Note that all project-specific notes and results will be made available through our website at:**
https://labnodes.vanderbilt.edu/tmescsr for viewing by the investigator for the duration of their genomic editing project.

The Transgenic Core is dedicated to providing a high quality genome editing service to the Vanderbilt community. If you have any questions or concerns before or during the production of your transgenic model, **please contact us:**

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