

An outline of CRISPR-Cas9 mouse model generation services available through the VGER. Contact Leesa.sampson@vanderbilt.edu for more information.

Keywords: [Crispr](#) [Cas9](#) [Microinjection](#)

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CRISPR-Cas9 technology has emerged as a rapid and precise method to generate genome edited mouse models. We offer full service mouse projects from genome editing strategy design through validation of the heterozygous N1 generation. We also offer injection services, CRISPR reagent sourcing, and design review for investigators wishing to design their own models using CRISPR-Cas9 technology.

Basic steps for VGER mouse genome editing projects

1. Contact leesa.sampson@vanderbilt.edu to schedule an advisory meeting. As of March 23, 2020, all advisory meetings will be held by phone or ZOOM. Following the meeting, the investigator completes the service request through iLab: https://vanderbilt.corefacilities.org/service_center/show_external/5102
2. A genome editing strategy is devised and reviewed with the investigator. We have four classes of CRISPR-Cas9 genome editing projects based on the donor DNA format, length of the desired gene edit, and whether the edit is an insertion or replacement.
3. Guide RNAs are sourced in a crRNA + tracrRNA format from MilliporeSigma and tested for activity in a test tube assay. Guides that pass the test tube activity assay are usually determined to be sufficiently active in vivo, but more complex projects that require multiple guides may benefit from pre-screening in vivo.
4. CRISPR editing reagents and a donor DNA, if required, are delivered into the pronuclei of one-cell fertilized mouse zygotes. Our standard strain options are C57BL/6J and C57BL/6N. Other strains may also be used for genome editing. Inquire for more information.
5. Resulting pups are biopsied and screened by PCR and Sanger sequencing.
6. Predicted founders are transferred to the investigator for breeding and validation of the desired genome edit in the N1 generation. Alternatively, breeding and screening of the N1 generation can be performed by VGER.
7. Investigators are provided with a validated genotyping protocol and a genome editing report that includes a description of what the model is, how it was designed and produced, and additional critical information such as correct nomenclature for the new allele.

Vanderbilt Genome Editing Resource – Genome Editing Efficiencies

As of March 2019, VGER has performed embryo microinjections for a total of 82 successful CRISPR gene editing projects.

100% of the last 26 VGER-designed and executed projects have been technical successes (as defined by the desired modification being introduced into a mouse). However, one project caused embryonic lethality. Thus, live mice have been delivered for 25/26 projects.

Our projects fall into four categories:

- I. **Non-Homologous End Joining (NHEJ)**. We recommend the use of this high-efficiency editing strategy to create large deletions when precise breakpoints are not required.
- II. **Homology Directed Repair (HDR) with Single-Stranded DNAs (≤ 180 nucleotides)**. We recommend the use of this strategy to introduce small edits, such as point mutations or small protein tags (e.g., HA or Flag), and for the creation of precise DNA deletions.
- III. **HDR with Single-Stranded DNAs (181-5,000 nucleotides)**. This approach enables the modification of longer DNA segments up to approximately 5 kb. It is currently being used to insert loxP sites around one or more exons, to introduce multiple point mutations, and to insert exogenous coding sequences encoding fluorescent proteins or Cre.
- IV. **HDR with Double-Stranded DNAs (generally > 5 kb)**. We recommend using double-stranded (ds) when the desired genome edit exceeds 5 kb, or when commercial projection of a long ssDNA is not feasible. We have used this approach to insert two cre-

