Genome Editing

An outline of CRISPR/Cas9 mouse model generation services available through the VGER. Additional protocols and service forms are provided below. Contact Leesa.sampson@vanderbilt.edu for more information.

Keywords: Crispr, Cas9, Microinjection

Genome-Editing Custom Project General Description

CRISPR-Cas9 technology has emerged as a rapid, highly-precise method to generate knock-out and knock-in mouse models. We can perform your genome editing project for you from strategy design through founder genotyping. We successfully created 34 new mouse models at a 100% technical success rate in 2018. These included seven gene deletions, fourteen point mutations, six epitope tags or STOP codon insertions, four conditional alleles (floxed), one fluorescent reporter knock-in, and two site-directed transgenes inserted into the Rosa26 locus.

Basic steps for VGER mouse genome editing projects

1. Contact leesa.sampson@vanderbilt.edu to schedule an advisory meeting. Following the meeting, the investigator completes the "VGER Gene Editing Service Form" (see attachment below) and is provided with an estimate.
2. A genome editing strategy is devised and reviewed with the investigator. We now have four classes of CRISPR/Cas9 editing strategies based on the donor DNA format and length of the desired gene edit. See the attachment below for efficiency data for each type of project.
3. ctRNAs are ordered and tested in vitro in a ribonucleoprotein format with tracrRNA and Cas9 protein for on-target efficiency at the desired genetic locus. See the "MilliporeSigma 2018 Pricing" attachment below for current reagent pricing.
4. A donor ssDNA is designed for homology directed repair projects and reviewed with the investigator.
5. Typically, Cas9 protein, ctRNA(s), and a ssDNA oligo or dsDNA are injected into mouse zygotes at the one cell stage.
6. F0 pups are biopsied for genotyping and screened by a VGER-developed PCR based assay.
7. F0 Founder(s) are analyzed by Sanger Sequencing to identify those containing the predicted desired gene edit.
8. F0 Founder(s) is transferred to investigator for breeding to WT and validation of the desired genome edit in the N1 generation.
9. Breeding and screening of the N1 generation is also available by estimate upon request.

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 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 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-inducible transgenes into Rosa26, and are currently working to improve the efficiency of this approach by using 2-cell
homologous recombination.

We guarantee mouse model delivery for Type I and II projects in approved mouse strains with appropriate number of microinjection days for projects designed by the VGER.

Attachments

- Genome Editing Service Form 040219.docx - Added on April 2, 2019 at 1:43 PM by Jennifer Skelton

- MilliporeSigma 2018 Pricing TMESCSR.pdf - Added on February 20, 2018 at 2:30 PM by Leesa Sampson

- VGER CRISPR Editing Efficiencies.jpg - Added on February 21, 2019 at 4:24 PM by Leesa Sampson