Genome Editing

An outline of CRISPR/Cas9 mouse model generation services available through the TMESCSR. Additional protocols and service forms are provided below. Contact Jennifer.skelton@vanderbilt.edu or Leesa.sampson@vanderbilt.edu for additional 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.

Basic steps for TMESCSR CRISPR/Cas9-mediated mouse mutagenesis projects

1. Contact leesa.sampson@vanderbilt.edu to schedule an advisory meeting. Following the meeting the investigator completes the "TMESCSR Gene Editing Service Form" (see attachment below) and is provided with an estimate.
2. A gene targeting strategy is devised and reviewed with the investigator.
3. Targeting guide RNAs are ordered and tested in vitro for cutting at the desired genetic locus/loci. See the "MilliporeSigma 2018 Pricing TMESCSR" attachment below for current reagent pricing.
4. A donor oligo is designed for homology directed repair projects and reviewed with the investigator.
5. Typically, Cas9 protein, guide RNA(s), and a 180 bp donor ssDNA oligo are injected into mouse zygotes. Delivery via plasmid or RNA is also available.
6. F0 pups are biopsied for genotyping and screened by a custom designed method.
7. Founder(s) undergoes verification of desired gene-edit by Sanger Sequencing.
8. Founder(s) is transferred to investigator.
9. Genotyping and screening of the F1 generation is also available as needed.

Current recommended starting concentrations for CRISPR/Cas9 reagents for various formats:

<table>
<thead>
<tr>
<th>CRISPR Cas9 Reagents</th>
<th>gRNA concentration</th>
<th>Cas9 concentration</th>
<th>Donor DNA concentration (ssDNA 120-180 bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>sgRNA and Cas9-expressing plasmid at 5 ng/µl</td>
<td>10 ng/µl</td>
<td></td>
</tr>
<tr>
<td>RNA</td>
<td>50 ng/µl</td>
<td>100 ng/µl</td>
<td>50 ng/µl</td>
</tr>
<tr>
<td>RNP</td>
<td>50 ng/µl</td>
<td>100 ng/µl</td>
<td>100 ng/µl</td>
</tr>
</tbody>
</table>

Click here to contact the core

Attachments

- [Genome Editing Service Form 2018.docx](#) - Added on January 5, 2018 at 1:24 PM by Jennifer Skelton
- [MilliporeSigma 2018 Pricing TMESCSR.pdf](#) - Added on February 20, 2018 at 2:30 PM by Leesa Sampson

General service form for CRISPR and TARGATT gene editing projects