Genome Editing

An outline of CRISPR/Cas9 and TARGATT-mediated 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 on the genetic background of your choice. We can perform your genome editing project for you from project design to founder identification and grant/manuscript preparation.

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

1. Contact leesa.sampson@vanderbilt.edu for an advisory meeting. Following the meeting the investigator completes the "TMESCSR Gene Editing Service Form" (see attachment below).
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 2017 Pricing TMESCSR" attachment below for current reagent pricing.
4. A donor oligo is designed for knock-in projects and reviewed with the investigator.
5. Cas9 protein, guide RNA(s), and donor DNA oligo are injected into mouse zygotes. Delivery via plasmid or RNA is also available.
6. F0 pups are biopsied for genotyping and screened by designed method.
7. Founder(s) undergoes verification of desired gene-edit by Sanger Sequencing.
8. Founder(s) is transferred to investigator.

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>
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</tbody>
</table>

ΦC31 Integrase/TARGATT

Traditional mouse transgenesis, whereby DNA is introduced by pronuclear injection, has limitations: transgene landing sites, copy number, and integrity cannot be controlled. ΦC31 integrase-mediated site-specific transgenesis allows specific integration of a single transgene into a "safe-harbor" genomic locus, either H11 or Rosa26, with high efficiency. See original manuscript for more information: Site-specific integrase-mediated transgenesis in mice via pronuclear injection. This method is particularly useful for generating overexpression, knock-down, humanized, and Cre mouse lines.

The TMESCSR can design and complete your entire ΦC31 integrase-mediated transgenic mouse project or you can complete some of the steps in your own laboratory.

Basic steps:

1. Clone your transgene into an appropriate plasmid: Plasmid List
2. Choose either the H11 (live mice) or Rosa26 (frozen stock) landing-site mouse line and the TMESCSR will order from Charles...
Rivers: TARGATT mouse

3. Order pronuclear injections of your constructed plasmid and ΦC31 Integrase into desired zygotes. See the TARGATT plasmid preparation protocol attached below for preparing your DNA for microinjections.

4. Resulting pups are genotyped and founders identified.

Attachments

- [Genome Editing Service Form 2017.docx](#) - Added on July 5, 2017 at 10:43 AM by Leesa Sampson
  General service form for CRISPR and TARGATT gene editing projects

- [MilliporeSigma 2017 Pricing TMESCSR.pdf](#) - Added on August 22, 2017 at 8:32 AM by Leesa Sampson
  MilliporeSigma contract prices for CRISPR/Cas9 reagents through the TMESCSR are valid through December of 2017.

- [TARGATT plasmid preparation protocol.docx](#) - Added on August 18, 2017 at 2:19 PM by Leesa Sampson
  A protocol detailing how to prepare TARGATT plasmids for zygote microinjections