This service provides an efficient means of introduction foreign DNA, RNA, and ribonucleoproteins into the mouse genome. DNA or RNA is microinjected into the pronucleus or cytoplasm of 0.5 day fertilized mouse embryos. Surviving embryos are transferred to the oviduct of a pseudopregnant recipient and the animals are housed under the protocol number of the investigator but husbandry is provided by the TMESCSR and DAC. The gestation period of the mouse is 19 to 21 days. Pups are born, and will be weaned, tailed and numbered by core personnel at 3-4 weeks of age. The investigator will need to perform DNA isolation and analyze the DNA by PCR or Southern blotting to identify transgenic founders.

Several factors influence the production of transgenic mice. Of utmost importance for successful outcome is the quality and size of the DNA injected, thus no guarantee is made by the Transgenic Mouse/Embryonic Stem Cell Shared Resource for the number of transgenic mice produced. However, provided that the DNA has been prepared in the recommended manner, our records indicate that 10 to 20% of the pups born will usually carry the transgene.

The TMESCSR has joined the Sigma core partnership program (http://www.sigmaaldrich.com/programs/crispr-core-partnership-program.html). The TMESCSR has a discount pricing agreement with Sigma-Aldrich on CRISPR reagents. To use the discount, contact Christopher Lemke at Sigma-Aldrich to generate a quote (christopher.lemke@sial.com). Once you have the quote, contact the Core and we will place the order for you and bill you at cost. At the bottom of this page you will find a link where you can download the current discount price sheet for Sigma-Aldrich CRISPR reagents. For more information on their CRISPR products, consult the Sigma-Aldrich web site: www.sigmaaldrich.com/catalog/product/sigma/crispr?lang=en&region=US

Link to our recommended protocol for preparing plasmid DNA fragments for pronuclear injection

The following data may be useful in estimating the number of injection days required to generate transgenic founders. Typically, ~100 embryos are injected per "injection day".

TMESCR Pronuclear Injection Statistics (October 2008 - December 2015)

<table>
<thead>
<tr>
<th>DNA type</th>
<th>Embryonic Strain</th>
<th># constructs injected</th>
<th>Total # transgenic pups</th>
<th>% Transgenic pups / total born</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid or BAC</td>
<td>B6D2</td>
<td>91</td>
<td>271</td>
<td>16%</td>
</tr>
<tr>
<td>Plasmid or BAC</td>
<td>C57BL/6</td>
<td>55</td>
<td>126</td>
<td>15%</td>
</tr>
</tbody>
</table>

Experimental Flow Chart:

1. PI submits frozen DNA and appropriate service form to TMESCSR for microinjection.
2. Service forms are reviewed and approved by the Co-Directors of the facility, DNA is quantitated, quality controlled, and diluted by Tg Mouse/ESC staff.
3. Injections are scheduled, mice are ordered.
4. Injections are performed, surviving embryos are transferred to pseudopregnant females.
5. Pups born 19 to 20 days post injection.
6. Pups weaned, tailed and ear punched by TMESCSR staff at 3 weeks of age.
7. Dams are serology tested at weaning.
8. PI identifies transgenic founders from tail DNA using PCR or Southern blotting.
9. PI notifies TMESCSR staff about transgenic founders identifying individual mice
10. DAC staff review serology results, approve movement of mice, and transfer transgenic founders to PIs mouse housing room.

Attachments

Injection_Sequence_13.jpg - Added on March 29, 2016 at 1:49 PM by Jennifer Skelton

Pronuclear microinjection

Pronuclear DNA injection video with BAC DNA and SJL/J embryos.

Pronuclear_DNA_Form_1017.docx - Added on October 4, 2017 at 2:10 PM by Jennifer Skelton

pronuclear_injection_videos_10.avi - Added on September 28, 2011 at 1:42 PM by Jennifer Skelton