Gene Targeting and Transgenic Resource
Roswell Park Cancer Institute
Transgenic Service Request Form

Please fill out as completely as possible. Provide as many of the details in section II that apply to your construct. Return to Aimee Stablewski (aimee.stablewski@roswellpark.org or fax 845-5908; tel 845-5843).

I. Investigator Name: _____

Institution: ☐ Roswell Park ☐ SUNYAB

II. DNA construct to be injected:

1. Construct name: _____
2. Gene: _____
3. Promoter: _____
4. Poly A site: _____
5. Splice sites (*Needed at the 5' end of your gene for good expression)*: _____
6. Epitope tags (*Optional but useful for detecting transgene expression)*: _____
7. Cloning vector: _____
8. Insert size: _____
9. Restriction enzymes used to remove the insert from the vector (*Note that successful expression of transgenes requires that they are removed from surrounding vector sequences!):*

   _____
10. Any other features that are relevant to the transgene (e.g., Locus control regions, enhancers, etc.):

    _____
11. Please attach a detailed map of the clone.

III. Success Issues:

1. Has a construct like this been previously used, anywhere, to make transgenic animals?
   ☐ Yes ☐ No Reference: _____
2. Have you tested the construct for expression in mouse cells?
   In many cases, this is a simple control that is very easy to do and can tell you ahead of time if there is a problem with your construct!
   ☐ Yes ☐ No
3. If expression has been tested, how was this done and what was the result? If it was not tested, is there a reason this cannot be done?

   _____

   _____

   _____

4. Do you have any reason to anticipate lethality problems in utero, or implantation problems?
☐ Yes ☐ No

5. Do you have any reason to anticipate health problems with positive pups?
☐ Yes ☐ No

IV. Founder Detection

1. How will transgene integration be confirmed in of potential founder mice?
Typically, Southern or PCR analysis is done using tail DNA, but other methods are possible as well.
☐ Southern ☐ PCR ☐ Other (describe below)

_____

2. Have the probes or primers already been tested using mouse DNA only as a negative control and mouse DNA spiked with single copy amounts of transgene-containing plasmid as a positive control? For this control, use about one picogm. linearized transgenic construct per microgm of mouse DNA.
A robust founder detection method is critical to set up <u>before</u> we begin to make mice! If you need mouse DNA to use as a control, we can provide this. Check all the boxes that apply.
☐ Yes, testing was done ☐ The probes work well (*we can show the results)*
☐ Testing in progress ☐ No, testing was not done ☐ Please send control DNA

3. How will transgene expression be confirmed in founder mice? Describe below.
This typically involves RNA or protein detection with a transgene-specific assay.

_____

_____

V. Animal Issues:

1. What protocol number will the mice be under when they are transferred to you? _____
Approval date _____

2. Do you have approved space to house these animals from Lab Animal Resources? _____

VI. Getting us going on your microinjections

1. Use your preferred method for preparing DNA *but be sure to do 2 CsCl gradients.*
2. Digest approximately 20 µgm of plasmid and use half for isolating the fragment for microinjection. *Use OUR protocol for preparing fragments (next page).* Do not gel purify the remaining half of your digested DNA, give it to us as is.
3. Provide a concentrated stock solution of DNA dissolved in sterile microinjection buffer (10 mM Tris pH 7.5, 0.1 mM EDTA) that we can dilute.
4. Provide a photo of the gel showing the DNA with concentration standards and size markers.
5. Unless you have a particular need for a different mouse strain, we will do microinjections in F2(C3Hf/HeRos X C57BL/10Rospd) embryos. Note that the requirement for different mouse strains may incur additional costs.