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 before 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.

LYSOZYME-TRITON PLASMID PREP

1) **Grow up 5ml culture during the day; inoculate O/N culture – 500 ml and LB Broth in a 2L flask. Add appropriate antibiotic.**
2) **Harvest cells, 5K, 15 minutes. Beckman JA-10 rotor.**
3) **Resuspend into 12.0 ml, 25% sucrose, 0.05M tris pH 8.**
4) **Make fresh - add 2.0 ml, 10mg lysozyme/ml.**
   - ✍ **0.05M tris. pH 8**
   - ✍ **Incubate on ice for 15 minutes.**
5) **Add 4.0 ml 0.25M EDTA, pH 8. Incubate on ice for 15 minutes.**
6) **Add 16.0 ml 2% triton.**
   - ✍ **0.05M tris, pH 8**
   - ✍ **0.0625M EDTA, pH 8**
   - ✍ **Incubate on ice for 15 minutes**
7) **Pour into screw-top bottle.**
   - ✍ **Boil 10 minutes**
   - ✍ **Tighten lids**
   - ✍ **Spin 18K (30 minutes) in Beckman JA-10 rotor**
   - ✍ **30 minutes**
8) **Recover supernatant into 50ml plastic tube. (*Should produce 28-30 ml, if not, re-boil and re-spin).***
9) **Add 0.96 g CsCl/ml of lysate recovered.**
10) **Add 0.036 ml of 10 mg EtBr/ml per ml of lysate recovered.**
11) **Pour into screw-top bottle, spin 18K, 30 minutes (to clear).**
12) **Filter through glass wool into Beckman Quick Seal (30 ml size) tubes. (12 cc syringe & 18 g needle, a bit of glass wool).**
13) **Balance tubes to within 0.01g, add oil (light mineral oil) and seal.**
14) **Spin in VTi 50 rotor, 48K, 16 hours.**
15) **Recover the bottom band and place into Beckman Quick Seal (5ml) tube, spin again in Vti 65, 55K, 5 hr or 48K O/N.**
16) **Recover bottom band and extract 4 times with Butanol/water saturated.**
17) **Add 2 volumes of TE to the bottom layer (contains DNA).**
18) **Add 2 volumes of 100% EtOH and precipitate O/N. (Don't add salt, want to get rid of CsCl).**
19) **Spin 7.5K, 15 minutes in JS-13 rotor.**
20) **Wash 2 times with 70% EtOH and then dry.**
21) **Re-suspend in 200-400 ?l of TE.**
22) **Add 3M NaOAc to make a 1:10 dilution of the NaOAc.**
23) **Transfer to Eppendorf tubes and do 2 PCI extractions (25:24:1) and 2 CIA (24:1) extractions.**
24) **Precipitate DNA with 2 volumes of 100% EtOH O/N.**
25) **Wash 2 times with 70% EtOH and then dry.**
26) **Resuspend in 200-400?l of TE and take ODs.**

Purification of Plasmid DNA for Microinjection

1) Prepare plasmid from bacteria using your preferred method (alkaline lysis, lysozyme-triton or other methods), but **be sure to perform 2 CsCl gradients**. Also note that phenol kills injected embryos so an ethanol precipitation and ethanol wash MUST follow all phenol extractions.
2) Set up an overnight digest using 20 ?g of plasmid DNA with the appropriate restriction enzyme to cut the insert from the vector (use 100 ?l total volume for digest mix).
3) Prepare a 0.8% agarose minigel (1X TAE buffer with ethidium bromide) using a comb with ~6 mm wide teeth.
4) Add 20 ?l of bromophenol blue/sucrose loading dye to the digest mix and load evenly over 4 wells. Don't use a size or concentration standard on this gel in order to prevent possible contamination of the insert DNA.
5) Run in 1X TAE + ethidium bromide at a low voltage (~35) for several hours or until the insert and vector bands are clearly separated.
6) Photograph the gel in its tray in order to prevent contamination from the transilluminator surface. Limit the time of UV light exposure so the DNA does not become damaged.
7) With a new, sterile scalpel blade, excise the insert band from the gel. Trim away excess agarose and avoid dragging the blade or the excised bands across the area of the gel where the vector bands are.
8) For transgenes less than 10 kbp, purify the insert DNA using a Q1Aquick Gel Extraction Kit (Qiagen, 800-426-8157, www.qiagen.com; Cat. No. 28704). Use the vendor's protocol (http://www.qiagen.com/literature/handbooks/qqspin/qqspin_gelextract.pdf or get the protocol from us) using the following modifications:
    a) carry out step 3 at room temperature, or 37ºC if necessary, for dissolution of the agarose and mix gently by hand instead of vortexing.
    b) apply equal volumes of the DNA solution into two columns at step 6.
    c) include the optional step 9 and repeat for a total of two Buffer QX1 rinses.
    d) repeat step 10 twice for a total of 3 Buffer PE washes, letting the column stand for 5 min before centrifugation after the first addition of Buffer PE.
    e) at step 13, add 30 ?l of injection buffer to one of the columns and let stand 1 min before centrifugation. Use the 30 ?l of collected sample for elution from the remaining column. Do a second rinse on this column with 10 ?l of fresh injection buffer for a total of 40 ?l.
1) On a 0.8% agarose gel, run 2 and 5 ?l aliquots of the purified, eluted DNA next to size and concentration standards. Desired injection concentration for most constructs in 2.5 ng/?l. Do not dilute all of the stock. After dilution of a portion of the stock with injection buffer, filter using a Millipore HV 0.45 ?m unit (attaches to a 1 cc syringe). Particle free ultra clean DNA is a must!

*Injection Buffer is 10mM Tris C1 pH 7.5 and 0.1mM EDTA made from sterile millipore system purified water.