Overview of Timeline for Generation of Transgenic Mice:

To generate transgenics, you MUST have an approved IACUC protocol prior to beginning this process. When your lab has verified the sequence of your transgenic construct you will either give us a bacterial stock or agar plate OR give us the purified DNA (2XCsCl preps):

Lab gives the TG facility DNA or bacterial stock to prepare DNA	Immediately
TG facility verifies concentration of the DNA by running a concentration gel	1 day
TG facility orders 5 F2 (C3Hf/HeRos X C57BL/10 Rospd) for "test" microinjection day (see Quality Control section of this website) and performs test microinjection day	1 week
If DNA kills embryos:	DNA must be remade by lab requesting microinjection or by us
If DNA is of good quality:	
TG facility orders mice from DLAR or outside vendor	1 week to 3 weeks depending on strain
TG facility sets up for microinjection day	1 week
TG facility performs microinjection	1.5 days
Birth of potential founders	3 weeks
TG facility weans, tags and tails potential founders	~ 3-4 weeks from birth (depends on size of pups)
TG facility prepares tail DNA via Laird's method	2 days
Investigator is notified that DNA is ready for pickup	Immediately
Investigator identifies founder mice and relates this information to TG facility via email and fax	ASAP
TG facility begins paperwork for transfer of animals to investigator's lab	Immediately
Mice are transferred	Dependent on Roswell's DLAR facility, and Institute where mice are shipped

Approximate time to identify founders:
8 weeks from microinjection day

1) Investigator:

- **Consult with Facility Assistant: Aimee Stablewski; aimee.stablewski@roswellpark.org**
- **Design transgenic vector and screening strategy**
- **Construct transgenic vector (you should include the appropriate promoter, gene(cDNA or genomic DNA) and a polyA tail minimally; you may also include reporter genes such as GFP or LacZ, or epitope tags such as HA or Myc)**
- **Test probes or primers**
- **Test transgene in vitro system (optional but simple control to tell if something is wrong with your construct)**
- **Use your preferred method for preparing DNA but be sure to do a 2X CsCl gradient****this is by far the MOST critical aspect of performing transgenic pronuclear injection**
- **Digest approximately 20ug of plasmid to release the transgene from the vector backbone (this is critical for expression of your transgene; excess additional vector sequence easily gets methylated and may shut down expression of your gene)**
- **Run digested DNA on agarose gel in TAE and gel purify using Qiagen's QiaQuick Gel Extraction kit (follow directions for plasmid preparation on website)**
- **Resuspend DNA in total of 40uL injection buffer**
- **Check concentration via running another gel next to concentration standards**
- **Give DNA to TG facility with the Transgenic Service Request Form**

1) Transgenic and Gene Targeting(TG/GT) Facility:

- **First, we will perform a "test" injection day with your prepared DNA by superovulating only a handful of mice to determine whether or not your prepared DNA is toxic to embryo development**
- **If the DNA is of good quality, we expect at least 50% to develop to the 2-cell stage overnight, if the DNA is toxic to the embryos, we highly suggest you consider having us prepare the DNA for you**
- **If DNA is good we will:**
- **Superovulate and harvest fertilized oocytes to inject**
- **Will inject between 80-100 fertilized oocytes/injection day**
- *****WE WILL PERFORM 2 MICROINJECTION DAYS UNLESS OTHERWISE SPECIFIED BY THE PI*****
- **Transfer the eggs which have developed to the 2-cell stage the following day**
- **Send a progress report to the investigator**

- **Wean, tag and tail pups born when they are 3 weeks old or are large enough (~6 weeks from injection day)**
- **Will prepare tail DNA using Laird's lysis method and provide DNA to the investigator**
- **If the DNA kills the embryos: we will need your lab to re-prepare it, or alternatively, we can do this for you(highly suggested)**

Investigator:

- **Screens DNA prepared by facility by PCR or Southern**
- **Notifies TG/GT facility of positive founder pups and returns genotyping analysis sheet to facility**

2) TG/GT facility:

- **Facility will arrange for transfer of founder pups (and any negative pups also) to investigators animal space**