

# **Transgenic Mouse Production**

Transgenic core, Gensat Project(July, 2007)

Embryos from gonadotropin-treated FVB/N female mice (Taconic) are obtained by intraperitoneally injecting females with pregnant mare's serum gonadotropin (PMS, 5 IU per female, Calbiochem 367222) followed 46 hours later by an injection of human chorionic gonadotropin (HCG, 5 IU per female, Sigma C1063). PMS injections are done at 15:00 hours on day one and are followed by an HCG injection at 13:00 on day three. Females are paired with FVB/N stud males immediately after HCG injections. The next morning, females are selected for ova donation by checking for copulation plugs. After oviducts are removed from the donor females, one cell embryos are collected and microinjected with BAC DNA in injection buffer (10 mM Tris, pH 7.5, 0.1 mM EDTA, 100 mM NaCl). Standard microinjection protocol is used as described in *Manipulating the Mouse Embryo*<sup>1</sup>. Injected ova are surgically transferred into the infundibulum of pseudopregnant recipient Swiss Webster females on the day of injection. Pseudopregnant mice are produced by mating females in estrus with vasectomized Swiss Webster males that have been proven sterile.

## ***Preparation of BAC DNA for microinjection***

The BAC DNA is stored at 4°C and injected into fertilized ova within 10 days after preparation. Concentrated DNA stock (2-10 ng/ul) is diluted for microinjection with injection buffer (recipe above) to 0.5ng/ul on the day of injection. If this concentration (0.5ng/ul) is too sticky or viscous for injection, the DNA is diluted to a concentration that allows ease of flow (no lower than 0.125 ng/ul). If the DNA solution remains too sticky or particulate at the most dilute concentration, it is prepared again starting with step 2 of the protocol, i.e. BAC DNA Purification. In our experience, the ease with which DNA can be injected is linked to the quality of the molecule. Taking adequate time to produce clean and non-sticky DNA assure that the greatest number of founders will be produced.

Swiss Webster females have a gestational period of 20-21 days. Pups are genotyped 10-14days after birth for the presence of EGFP. Beta actin is assayed as a control.

## ***Genotyping of putative transgenic mice***

Individual tail samples are incubated overnight at 55°C in 400 ul of lysis buffer (recipe below) to which 1.5 ul of proteinase K has been added. After incubation, the tubes are vortexed for 5 sec and the lysate spun at

13,200 rpm for 1 min before being placed on a heating block for 10 min at 100°C. A PCR reaction, using Qiagen Taq DNA polymerase (Cat # 201205), is set up as follows:

<u>Component</u>	<u>Volume</u>	<u>Final concentration</u>
H2O	11.8 ul	
10x Buffer	2.0 ul	
dNTP (2.5 mM)	0.5 ul	
EGFP 5'	0.5 ul	25 pmol
EGFP 3'	0.5 ul	25 pmol
Actin 5'	0.5 ul	25 pmol
Actin 3'	0.5 ul	25 pmol
Taq	0.2 ul	1.0 U/Rx
MgCl <sub>2</sub> (5mM)	0.5 ul	
<u>Genomic DNA</u>	<u>3.0 ul</u>	
Total volume	20 ul	

EGFP primers (Bio-Synthesis Inc)

Forward (5') primer: 5'-CCT ACG GCG TGC AGT GCT TCA GC-3'

Reverse (3') primer: 5'-CGG CGA GCT GCA CGC TGC CGT CCT C-3'

Actin primers (Bio-Synthesis Inc)

Forward (5') primer: 5'-GAT GAC GAT ATC GCT GCG CTG GTC G-3'

Reverse (3') primer: 5'-GCC TGT GGT ACG ACC AGA GGC ATA CAG-3'

PCR cycle

1. 94°C 2 minutes
2. 94°C 30 seconds
3. 62°C 45 seconds
4. 72°C 45 seconds
5. Go to step 2, 30 times
6. 72°C 10 minutes

The PCR samples are run on a 2.1% agarose gel with ethidium bromide. The EGFP PCR product is identified as a band at 300 base pairs and the beta actin PCR product as a band at 1k base pairs.

Lysis buffer

50 ml 1 M Tris (pH 8.8)

2 mL 0.5M EDTA

50 mL Tween 20 (10%)

898 ml dH<sub>2</sub>O

## *References*

- 1 Brigid Hogan, Rosa Beddington, Frank Costantini, Elizabeth Lacy. *Manipulating the Mouse Embryo*, 2<sup>nd</sup> ed. Cold Spring Harbor Laboratory Press, 1994.