

**Confidential**  
**Center for Functional Genomics**  
Transgenic Mouse Facility, Room 332

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**Mouse and ES Cell clone Screening Form**

Before the microinjections or electroporations can start, the CCFG requires that the investigator had made certain controls in order to detect their DNA construct. For transgenic mice, it could be by PCR, Dot blot or Southern blot analysis, unless the transgenic construct was made at the CFG. For ES cells, it could be by PCR or Southern Blot analysis.

[Dot Blot or Southern Blot Analysis](#)

**PCR Analysis**

To be able to detect transgenic founders by PCR, we will require the appropriate primers and conditions for the reaction.

5' primer name (max 8 characters) \_\_\_\_\_

5' primer length (bp), molar conc., total conc \_\_\_\_\_

3' primer name (max 8 characters) \_\_\_\_\_

3' primer length (bp), molar conc, total conc \_\_\_\_\_

Length of PCR product (bp) \_\_\_\_\_

Amount needed of each primer per reaction (ml) 5' \_\_\_\_\_ 3' \_\_\_\_\_

Denaturing Temp (°C): \_\_\_\_\_ Denaturing Time: \_\_\_\_\_

Annealing Temp (°C): \_\_\_\_\_ Annealing Time: \_\_\_\_\_

Extension Temp (°C): \_\_\_\_\_ Extension Time \_\_\_\_\_

# of Cycles: \_\_\_\_\_

Name of Molecular weight marker used \_\_\_\_\_

Amount in pgrs of amount used in 1 copy controls (see below) \_\_\_\_\_

Additional information (i.e. hot start, MgCl conc., dNTP conc., etc) \_\_\_\_\_

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The Investigator should supply a picture of the gel with the following controls:

1. primers with 100 ng of mouse genomic DNA
2. primers with 100 ng of mouse genomic DNA + 1 copy of the DNA construct
3. primers with 100 ng of mouse genomic DNA + 5 copies of DNA construct
4. primers with 100 ng of mouse genomic DNA + 10 copies of DNA construct
5. primers with 5 copies of DNA construct
6. Molecular weight marker

### Dot Blot or Southern Blot Analysis

To be able to detect the transgenic founders by Dot blot or Southern blot, we will require 1  $\mu\text{g}$  of purified probe in a concentration of 0.1  $\mu\text{g}/\mu\text{l}$ . Please follow [Instructions for DNA Cleaning and Shipping](#). The probe should be at least 300 bp in length. This is required for efficient labeling of the random priming reaction. In addition we would like the investigator's assurance that the probe does not cross hybridize with endogenous sequences (the investigator should run tests to proof this, see below). Also, we will need the hybridization and washing conditions to be used. If the investigator would require a Southern blot to detect the transgenic founders then a map should be included indicating the expected restriction enzymes to be used for digestion and the expected fragment sizes.

Probe name (max 8 characters) \_\_\_\_\_

Probe length (bp) \_\_\_\_\_

Probe concentration \_\_\_\_\_

Hybridization temperature \_\_\_\_\_

Hybridization buffer \_\_\_\_\_

Washing temperatures \_\_\_\_\_

Washing buffer \_\_\_\_\_

Size of detected band (for Southern blot) \_\_\_\_\_

The Investigator should supply a picture of the gel and autoradiograph with the following controls:

1. 5  $\mu\text{g}$  of mouse genomic DNA
2. 5  $\mu\text{g}$  of mouse genomic DNA + 1 copy of DNA construct
3. 5  $\mu\text{g}$  of mouse genomic DNA + 5 copies of DNA construct
4. 5  $\mu\text{g}$  of mouse genomic DNA + 10 copies of DNA construct
5. 5 copies of DNA construct
6. Molecular weight marker

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