



Applied StemCell

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PROTOCOL



Transgenic Kit, Mouse Embryos and Genotyping

Cat. Numbers:

AST-0001	AST-0003	AST-1001	AST-1011	AST-1013
AST-0002	AST-0004	AST-1002	AST-1012	AST-1014

Steps

1. Clone your gene of interest into the **TARGATT™ vector**
2. Prepare DNA for microinjection
3. Microinject DNA into the pronucleus of **TARGATT™ embryos** (H11 or Rosa26)
4. Transfer embryos into pseudo-pregnant female mouse
5. Genotype the pups with the **TARGATT™ Mouse Tail Genotyping Kit**

Preparation of DNA for microinjection

1. Prepare plasmid DNA using Qiagen mini-preps.
2. Add one equal volume of phenol:chloroform (50:50) mix to the DNA solution. Mix gently, spin for 5 minutes at max speed.
3. Transfer aqueous layer (top layer) to a new tube. Decant the bottom layer into phenol:chloroform liquid waste container.
4. Repeat step 2 and 3 once.
5. Add an equal volume of chloroform to the DNA solution. Mix gently and spin for 5 minutes at max speed.
6. Transfer aqueous layer (top layer) to a new tube. Decant the bottom layer into phenol:chloroform liquid waste container.
7. Repeat step 5 and 6 once.
8. Add 1/10 volume of 3M sodium-acetate (pH 5.2) and 2.7 volumes of ethanol to DNA solution. Mix well and let DNA precipitate in a -20°C freezer for 20 minutes. Spin for 10 minutes at 4°C.

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9. Wash with 500 μ l of 75% ethanol. Spin for 5 minutes at room temperature.
10. Aspirate supernatant. There will be a clear pellet on the bottom of the tube. It may be difficult to see.
11. Dry the pellet and dissolve it in an appropriate amount of miTE Buffer.
12. Filter DNA solution through a 0.2 μ m filter and measure the concentration. The DNA should be tested to be RNase-free by incubation with **control RNA** at 37°C for 1h and running the mixture on a 1% agarose gel.
13. Dilute the DNA to 6 ng/ μ l with sterile miTE and store it at -80°C until the injection.
14. Mix equal volume of 6 ng/ μ l DNA solution and **TARGATT™ Integrase** solution before injection.

Handling of TARGATT™ Embryos

Fresh embryos:

- Each vial contains 50 fresh embryos (E3.5) in KSOM medium and is shipped overnight at ambient temperature.
- Embryos need to be transferred into the uterine of day 2.5 pseudo-pregnant recipient mothers immediately upon arrival.

Cryopreserved embryos:

- Each cryovial contains 50 cryopreserved embryos (E2.5) and is shipped in a dry shipper containing liquid nitrogen.
- Embryos need to be stored in liquid nitrogen (submerged in LN2) upon arrival.

Please be aware that our TARGATT™ Embryos allow you to generate your own TARGATT™ mouse colony; however, you cannot use them for pronuclear injection directly.

Genotyping of TARGATT™ Transgenic mice

1. Tail Collection and Lysis

- 1.1. Collect approximately 2 mm of tail tissue in a 1.5 ml microcentrifuge tube.
- 1.2. Add 200 μ l 1X **Tail lysis buffer** and 2 μ l **Proteinase K** solution to each sample
- 1.3. Incubate in a 55°C waterbath with occasional vortexing until tissue is degraded (1-3 hours).
- 1.4. Heat the samples to 95°C for 10 min to inactivate Proteinase K.
- 1.5. Proceed with the PCR reaction or store at -20°C

2. Genotyping PCR Reaction

2.1. Set up the PCR reaction according to following table on ice. (The PCR mix may vary depending on the Taq polymerase. Please refer to the manufacture's instruction.)

DNA	1 μ l
10X Tail lysis buffer	2 μ l
dNTPs (10 mM)	0.4 μ l
Primer set (10 uM)	1 μ l
Taq Polymerase (5 U/ul)	0.2 μ l
Nuclease-free water	15.4 μ l
Total Volume	20 μl

2.2. Perform PCR amplification according to following protocol. Repeat steps 2-4 for 30-35 cycles.

1	94°C	3 min
2	94°C	20 s
3	58°C	25 s
4	72°C	45 s
5	72°C	5 min

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