E14Tg2a Medium:

- 500 ml GMEM (Sigma #G5154)
- 50 ml FBS (Invitrogen)
- 5 ml 100X GIBCO L-Glutamine (Invitrogen #25030-024)
- 5 ml 100X beta-mercaptoethanol (360 microliters/500 ml PBS; filtered, stored at -20°C)
- Murine LIF (ESGRO,Millipore ESG1107, dilute as directed)

Freezing Medium:

- Add dimethylsulphoxide (DMSO) (Sigma # D2650) to E14Tg2a medium to a final concentration of 10% (v/v)
- Filter sterilize

Make fresh before use

PBS:

- Dulbecco's phosphate-buffered saline (PBS) without calcium and magnesium (Invitrogen # 14190-094)

Trypsin

- To 500ml PBS (Gibco cat # 14190-144) add 0.1g EDTA (Sigma cat # 02650) and 0.5g D-glucose (Sigma cat # G7528 1 kilo)
- Filter sterilize (0.22 µm)
- Add 5ml Chicken Serum (Gibco cat # 16110-082)
- Add 10ml 2.5% Trypsin (Gibco cat # 151090-046)
  Store in 20 ml aliquots at -20°C

Geneticin (G418; Invitrogen Cat No. 11811-031)

129 / E14 ES cells: 100 micrograms (active)/ml Geneticin is recommended for trapping experiments and expansion of existing ES cell clones. (150 micrograms (active)/ml only for targeting experiments).
The concentration of each Lot of geneticin should be titrated to determine the minimum concentration that will kill non-transfected ES cells in 5 days. While ordering Geneticin, request several bottles from the same Lot.
Dissolve the powder in the appropriate amount of PBS to make a 100mg/ml stock solution (active concentration).
For example; 5g of powdered geneticin with an active concentration of 740 µg/mg would require a final volume of 37ml of PBS

$$\frac{5000\ \text{mg} \times 740\ \mu\text{g}}{\quad} = 37\ \text{ml of PBS}$$

100 µg/ml 1000 µg

Add approximately half the final volume of PBS to the Geneticin; swirl bottle until powder is fully dissolved
Aspirate the solution and transfer to a 50ml tube
Add approximately quarter of the final volume of PBS to the Genticin bottle to ensure residue is collected.
Aspirate the solution and add to the geneticin solution. Mix well. Add PBS to final volume (i.e. 37 ml)
Filter sterilize (0.22 µm filter)
Aliquot and store at -20 ºC
Store at 20ºC

0.1% Gelatin

- Add 25ml of 2% Gelatin solution (Sigma #g1393) to 500ml PBS (Gibco cat # 14190-144)

Gelatin solution needs to be warmed in the water bath prior to mix.

Thawing and Passaging E14Tg2-ES cells

Before you start

- See Medium & Reagents for E14Tg2a cells' protocol for all reagents
- Thaw and pre-warm Trypsin
- Pre-warm PBS
- Pre-warm required media
- Pre-warm gelatine
- Prepare **gelatine-coated plates** (see table below) , leave the gelatine at least 10 minutes on the plates (better is about 2h)

Dish/Flask/Plate	Volume of Gelatin/PBS	Volume of Trypsin	Volume of Media
96 well plate	100 µl	25 µl	200 µl
6 well plate	2ml	500µl	3 ml
10cm Petri dish	8ml	1.5 ml	10 ml

Thawing gene trap ES cell clones

- Aspirate off the gelatine from one well of a 6-well plate
- Transfer 3ml of **E14Tg2a medium** into this well
- Thaw one vial of E14Tg2a-ES cells in a 37°C water bath
- Remove the oil on top of the cells
- Resuspend the cells by gently pipetting the cells two times
- Transfer the whole amount of cells (200 µl) directly into 3ml medium of the prepared one 6-well dish
- Change the medium early the next day

- Change the ES cell medium once each day until the cells are confluent (2-4 days)

Passaging E14Tg2a ES cells

- Prepare **gelatine-coated plates**, leave the gelatine at least 10 minutes on the plates (better is about 2h)

- Remove the parent 6-well plate from the incubator and observe under the microscope. Check cells are healthy and confluent
- Aspirate old media
- Wash with 2 ml pre-warmed PBS , *Add PBS to the side of the dish, and slowly tilt dish to gently wash the cells*
- Add 0.5 ml pre-warmed trypsin.
- Gently swirl the dish to cover all cells with trypsin
- Incubate at 37°C for 5 minutes
- While cells are incubating, remove medium from fresh gelatine-coated plates and add fresh, pre-warmed medium
- After incubation, gently swirl the plate again and add 2.5 ml medium to inactivate the trypsin.
- Pipette up and down gently 3 to 4 times to disperse cells (you may split those cells 1:5)
- Transfer 0.6 ml of the cell suspension to another 6-well of a 6 well plate
- Swirl the plate to distribute the cells evenly across the plate
- Incubate the plates in an incubator at 37ºC with 5% CO2.
- Check cells the following day and media change to remove all traces of trypsin and dead cells.