ICS Standard operating procedure for EUCOMM mice production

1. Preparation of JM8 cells before microinjection

JM8 cells are plated one day before microinjection onto1 or 3 cm Petri dishes (depending upon the instructions from the ES clones production center) coated with gelatine and feeders.

Before preparing JM8 cells for blastocyst injection it is helpful to remove excess feeder cells by taking advantage of the different adhesive properties of ES cells and fibroblasts.

Using one 3-cm dish of overnight ES culture, change medium 1 hour before trypsinization. For trypsinization, rinse the cells with PBS 1X and add 500 µl trypsin/EDTA for 2 to 3 minutes.

Add 2 ml JM8 medium to inhibit the enzymatic reaction, and pipette up and down vigorously until a single cell suspension is obtained.

Add 5ml more JM8 medium and centrifuge at 1,200 rpm for 3 min.

Re-suspend the pellet in 2ml JM8 medium and place the 2 ml into a fresh 3-cm dish for 20 min into the incubator at 37°C; 7.5% CO_2.

Feeder cells will adhere tightly in this time, while the best ES cells will adhere weakly. Repeat this step one time taking the supernatant of floating ES cells.

Prior to microinjection, take off the floating ES cells in a tube, add 2 ml JM8 medium to the dish and agitate gently to detach the loosely adhering ES cells and transfer to the tube.
Centrifuge the cells (1,200 rpm for 3 minutes) and re-suspend the pellet in 1 ml of PBS-EGTA.
Centrifuge again.
Re-suspend the pellet into 25 to 30 µl injection medium and store the tube on ice at 4°C until microinjection is complete.

- ***Culture medium for JM8 cells grown on feeders***

Knockout DMEM (GIBCO: 10829)
15% FCS (PAA: tested batch)
2mM stable Glutamine (PAA M11-006))
40µg/ml gentamicin sulphate (Duchefa Biochemie)
0.1mM beta-mercaptoethanol (Sigma: M 7522)
LIF 10^3units/ml (Chemicon: ESG 1107) or ICS made batch

- ***Trypsin***

Dilute: vol/vol
Trypsin-EDTA 0.25% (GIBCO: 25200)
PBS 1 X (Eurobio: CS1 PBS01-01)
***Add** 1% chicken serum (GIBCO: 16110-082)*

JM8 cells are dissociated 2 to 3min after trypsin addition
Culture conditions as indicated by EUCOMM

2. Chimera Production

a. Balb/c females super-ovulation conditions for blastocysts production

Genotype: BALB/cAnN Crl or BALB/cAnN Tac
Age of females: 4 weeks
Female's weight: 11-12 g.
Light Cycle: 14/10

FSH hormone: SYNCRO-PART **PMSG** 500 UI BOVINS-OVINS
Source : CEVA santé animale
Amount: 5 UI

Time of administration (range): 11:30AM-12noon **(Day 1)**

Ovulation inducing hormone : CHORULON 1500 UI **(HCG)**
Source : INTERVET
Amount: 2.5 UI

Time of administration (range): 11:30AM-12noon **(Day3)**

Harvest time for blastocyst: 8:30- 9:30 AM **(Day 7)**

b. Injection and transfert condition

To prevent ES aggregates, ES cells are washed in the PBS-EGTA medium.
Microinjection is performed at room temperature.
10 to 15 JM8 cells are injected /BALB/cAnN blastocyst
Blastocyst with incorporatd ES cells are incubated into the incubation medium for 2 to 3 hours in at 37°C; 10% CO_2.
10 to 15 Blastocysts are then transferred into one uteri horn of NMRI pseudopregant females.

- ***Media for micro-injection***

Flushing medium:

DMEM-Glutamax (Invitrogen: cat n°: 31966-021)
40µg/ml Gentamicin sulphate
10% FCS (tested batch)

Blastocysts injection medium: filtered on 22µ

DMEM - HEPES modification (Sigma : D-6171)
Gentamicin 40µg/ml
2mM stable Glutamine
10% FCS

Blastocysts incubation medium: filtered on 22µ

DMEM-Glutamax
40µg/ml Gentamicin
1X non essentiel aminoacids (MEM NEAA 100X GIBCO 1140)
10% FCS

LIF Esgro: 10^3 units/ml or batch produced and tested at ICS

c. Scoring of chimera

Chimeras are identified by coat color (mix of white and brown hairs, see fig 1).
Chimeras are weaned and the degree of chimerism is assessed by coat color.

Fig 1: Chimera from JM8 ES cells into injection into Balb/c blastocysts

3. Chimera breeding and establishment of EUCOMM mouse lines

At 7weeks of age, the eight strongest chimeras for each EUCOMM clones are mated with C57BL/6NTac females to determine germ line transmission (GLT) according to the breeding scheme below (fig2).
Each chimera is mated with 2 females.
Up to 5 litters or 40 pups are analyzed per chimeric male. Agouti pups are culled. Black pups (coat color transmission) is meaning of putative GLT.
GLT is confirmed by PCR genotyping on Tail biopsies.
Once the GLT is confirmed and the mouse line established (at least 10 F1genotyped mice), all the chimeras breedings are stopped and chimeras are culled.

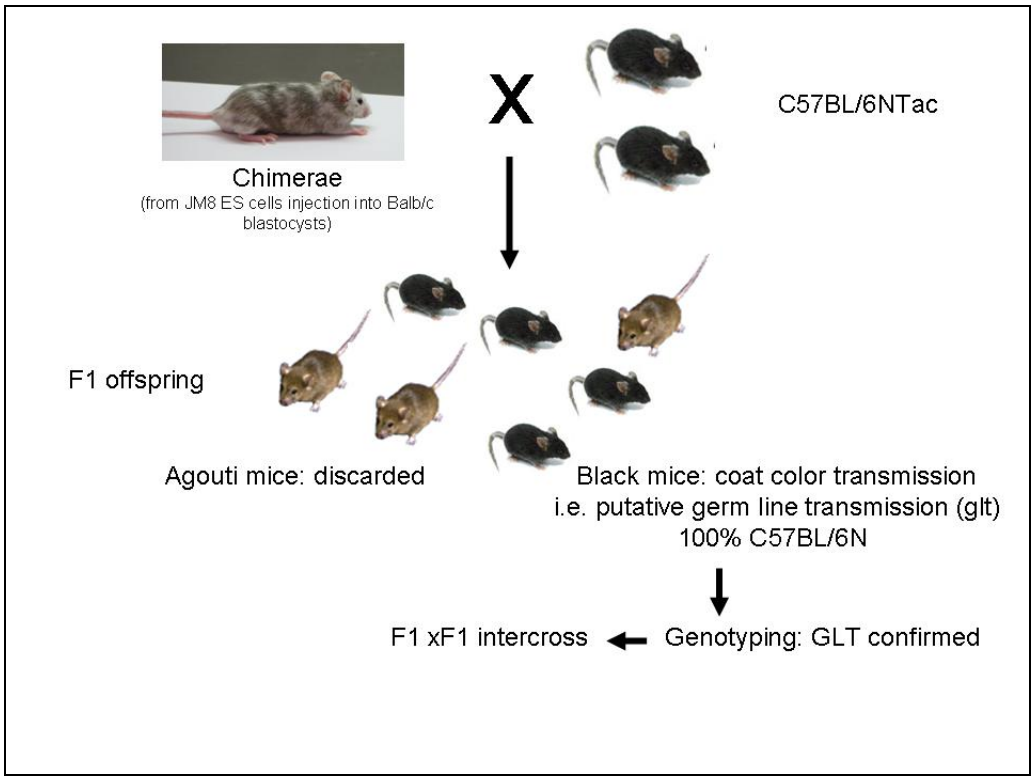

Fig 2: ***EUCOMM breeding scheme***