

**Microinjection of KOMP-CSD JM8A ES cell
clones into blastocysts
August 26, 2009**

Materials and Reagents:

Item	Vendor	Catalog Number
PMSG	National Hormone & Pituitary Program	1190
HCG	Sigma	CG-10
M2 medium	Speciality Media	MR-015
KSOM+AA	Specialty Media	MR-106-D
hepes buffered DMEM	Gibco	12430-054
FBS (ES cell tested)	Hyclone	SH300 70.03
Mineral Oil	Sigma	M-8410

Selection of Donor strain:

JM8A is derived from C57BL/6N strain. It has agouti gene engineered into the agouti locus. Inject these cells into blastocysts from B6D2F1 x C57BL/6 donors will get agouti and black chimeras.

Microtool Preparation:

Holding pipettes are prepared from glass capillaries (custom glass tubing #9-000-3000), injection pipettes from glass capillaries (custom glass tubing #9-000-2155) using a horizontal micropipette puller (Model: 97, Sutter Instrument co.). The holding pipette has an external diameter of 60-80 μm and an opening of 10-15 μm . The injection pipette has an external diameter of 12-17 μm and an internal diameter of 10-15 μm .

Superovulation of Donor Females:

1. Day 0: Give PMSG (5 I.U.) to 3-4 weeks old C57BL/6 females by I. P. injection at 2 pm.,
2. Day 2: Give HCG (5 I.U.) to the females at 1 pm by I. P. injection. Mate the females to B6D2F1 stud males.
3. Day 3: Check plugs.

Preparation of Blastocyst:

1. Day 6 - Sacrifice plugged female mice by CO2 asphyxiation or cervical dislocation at 3.5dpc at 8:00 am.
2. Dissect open the abdomen, locate the complete uterine horn and remove. Taking as much fat from the uteri off as possible.

3. Flush the uterine horn into a petri dish by inserting a 26g needle (attached to a 3ml syringe) into the oviduct/uterine junction and flush approximately 0.5ml of M2 through. Fluid should be visible flushing through the uterus and out of the cervical opening.
4. Collect all the eggs using a mouth transfer pipette and sort blastocysts, morulae and undeveloped embryos into separate 40 μ l drops of KSOM+AA covered with oil (pre-equilibrated) into the incubator (5% CO₂ at 37°C) Record the number of embryos.

ES cell preparation:

Follow "Thawing and Expansion of JM8A-derived KOMP Clones for Microinjection and Future Use" section under Culturing JM8A-derived KOMP Clones.

Blastocyst Microinjection:

1. Prepare a 60mm petri dish to place injected blastocysts into. Pipette four 40 μ l drops of KSOM+AA, cover with oil and place in a 5% CO₂ incubator to equilibrate.
2. Prepare a cavity slide by making a large (~40 μ l) drop of ES injection buffer into the center of the well and cover with mineral oil.
3. Place an appropriate number of blastocysts onto the slide, then add the prepared embryonic stem cells. Lower the holding pipette into the small drop of medium and fill to the shoulder, by capillary action. Place into the main drop.
4. Fill the tip of the injection needle with medium from the other small drop, by capillary action; place the needle into the main drop. Collect about 100 round and small ES cells with the injection pipette.
5. Hold a blastocyst using the holding pipette, with the inner cell mass closest to the holding pipette. Gently push the injection pipette into the blastocyst. Once inside release 10 to 15 ES cells and gently withdraw the needle. Continue until all blastocysts on the slide have been injected. Try not to exceed 30 min for each set of blastocysts.
6. Place injected blastocysts into the pre-equilibrated KOSM-AA drops in a 5% CO₂ incubator.

Embryo transfer:

Transfer 6 to 7 injected blastocysts into each uterine horn per 2.5 dpc pseudopregnant CD1 female.