

Lentiviral Vectors Perivitelline Injection Protocol

Purpose: To generate transgenic mice.

Engineered lentiviral particles are microinjected directly into the perivitelline space of mouse embryos 0.5 days after fertilization.^{a,b} The viral particles are comprised of a self-inactivating viral vector containing a gene of interest and a promoter, as well as a marker gene in some cases. The particles also contain reverse transcriptase to catalyze the incorporation of the vector sequence into the genome and the viral particle itself has a glycoprotein coat that mediates its adherence to the embryo. In some percentage of cases, the viral vector incorporates into the genome of the one-celled embryo, carrying the gene of interest with it. Once integrated, the viral sequences cannot be replicated, due to a deletion in the requisite sequence. Embryos are incubated at 37°C overnight. Two-cell embryos are implanted into the oviduct of pseudopregnant female mice the following day. Pups resulting from this procedure are genotyped to test for the presence of the transgene.

Superovulation and mating of the donor female mice

- 1) Three days prior to lenti-viral injections, administer 0.1 ml PMS by intraperitoneal injection to female donor mice (C57BL/6 or FVB).
- 2) Forty-seven hours after the PMS injection, administer 0.1 ml hCG by IP injection and pair the females with stud males.
- 3) Check for plugs the following morning.

Media preparation

- 1) The afternoon prior to injection day, set up two 60 mm culture dish (Corning catalogue #25382-381) with four 50 µl microdrops of KSOM (Cell and Molecular Technology, catalogue #MR-101-D) covered with oil, and one 35 mm dishes (Corning catalogue # 25382-348) with three 50 µl microdrops covered with oil. Label each dish with your name and the date, then place into the CO₂ incubator. The media is incubated overnight to allow the temperature, and more importantly, the pH to equilibrate.

- 3) Just prior to egg harvest on the morning of the injections, add 4 ml of FHM (Cell and Molecular Technology catalogue #MR-025-D) to a 15 ml conical tube containing 2.6 mg of hyaluronidase (aliquots stored in the -20 freezer). Invert gently to prevent foam, do not shake.

Embryo collection:

- 1) On the morning of injection, sacrifice female donors in groups of five or less, using cervical dislocation.
- 2) Collect oviducts and place them into a drop of FHM/hyaluronidase on the bottom of a 60 mm culture dish.
- 3) Make another FHM/hyaluronidase drop in the same dish.
- 4) Using two pairs of forceps, gently tear open only those oviducts that have an obviously swollen ampulla, releasing the egg/cumulus cell bunch into the drop.
- 5) The remaining oviducts can be flushed using the FHM/hyaluronidase mixture, a 1 cc syringe and a 32 gauge flushing needle.
- 6) Transfer the embryos to the 60 mm dish of four drops of KOSM, set out the night before. Rinse the embryos through the drops until all debris are removed.
- 7) On Mondays, set aside five embryos as controls. They should remain unmanipulated in the incubator to ensure that the media, incubator etc. are adequate. In most cases, all five embryos should develop into blastocysts by Friday. Results are recorded in a log.

Viral loading:

- 1) Viral aliquots provided by the investigator are kept at -80 C. Take one aliquot of 5-10 µl and thaw within a 50 ml conical tube filled with ice. Keep the conical tube in an ice bucket. Any direct contact with the virus should be done under the hood while double gloved.
- 2) If dilution is necessary, this should be performed in the hood in 0098. The viral titer should be 5×10^8 to 5×10^9 . If high titer prep is too viscous, it may be diluted 1:1 or 1:2 with ice-cold PBS.
- 3) Complete a project record sheet, filling in the viral vector, the gene construct, the date of injection, the date of preparation, the viral preparer, the injector, and the project number and replicate number.
- 4) In the hood, back-load the injection needle using a long, sterile micropipette tip. Attach the needle to the needle-holder and, with the needle-holder in the hood, press the flush button on the injector until the media fills the tip of the needle.

- 5) Attach the needle-holder with the loaded needle to the micromanipulator and then exterior gloves prior to continuing with the injection procedure.

Setting up the microscope:

- 1) Turn on the nitrogen tank
- 2) Turn on the microinjector (Green switch)
- 3) Place a set of eggs into a drop of oil-covered FHM in the cooled (4C) injection dish (~30)
Note: Time of egg exposure to FHM (including harvest time) should be no more than one hour.
- 4) Place the injection dish in the center of the microscope stage.
- 5) Focus the microscope at the lowest power on the embryos.
- 6) Place a holding pipette onto the opposite side of the scope, (e.g., if you are right handed, the holding pipette should be on the left and the injection needle on the right).
- 7) Lower both pipettes using the course controls into the injection dish.
- 8) Maneuver needle and holding pipette so that they are parallel to each other and visible at the lowest magnification.

Injections:

- 1) Use the holding pipette to stabilize eggs for injection.
- 2) Focus on the zona pellucida at the highest power, and inject into the perivitelline space with adequate volume to see an obvious swelling of the perivitelline space.
- 3) After ~30 eggs have been injected, place eggs into the 35 mm dish with three drops of warmed KSOM: Use the first two drops for rinsing to remove residual injection media.
- 4) Incubate all injected eggs overnight.
- 5) Unused virus, the injection needles, and the injection dishes should be discarded in biohazardous waste. The needle holders, the injection dish tray, and all pipetmen used should be placed in the hood under UV light.

Oviduct implant surgeries:

- 1) The next morning count the number of one cell and two cell embryos. The ratio should be 80/20, two cell to one cell; an indication that the virus was at an optimal concentration. If the ratio is lower than 80:20, then the virus should be diluted further at the next injection.
- 2) Transfer the two-cell embryos bilaterally into the oviducts of a 0.5 day pregnant recipient (See SOP for oviduct embryo transfer).

3) A maximum of 26-30 two cells should be placed into one recipient.

^aLois, C., Hong, E.J., Pease, S., Brown, E.J., and Baltimore, D. (2002) Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. *Science*. 295:868-872.

^bRubinson, D.A., Dillon, C.P., Kwiatkowski, A.V., Sievers, C., Yang, L., Kopinja, J., Zhang, M., McManus, M.T., Gertler, F.B., Scott, M.L., and Van Parijs, L. (2003) A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells, and transgenic mice by RNA interference. *Nat Genet*. 33:401-406.