Lentivirus Vectors Perivitelline Injection

Lentiviruses carring interested DNA constructs are microinjected directly into the perivitelline space of mouse embryos at embryonic day 0.5 to generate transgenic mice.

Materials:

Mice: Egg donor and stud male: B6SJL Egg recipient: CD1 Vasectomized male: B6D2 Lentivirus M2, M16, Hyaluranidase, 2.5% Avertin Microscope with micromanipulator and microinjector Surgical tools

Methods:

1. Superovulation and set up mating of the donor female mice PMSG 10iu i.p. to B6SJL female (3-5 weeks old) around 3pm; Followed by hCG 10iu i.p. 46~48 hours later. Set up mating with B6SJL stud male. 2. Set up mating of the recipient female After set up mating of donor female, put CD1 female in estrus to vasectomized male. The ratio can be 1:1 or 2:1. Ideally, the CD1 females are not older than 12 weeks since CD1 females are easily getting fat after this age, which can make the embryo transfer more difficult. 3. Check up plugs Both of egg donor and embryo recipient females are checked for plugs the following morning after setting up mating; record the plug rate for check the performance of stud males and vasectomized males. 4. Prepare Mediums M16 is used to culture the embryos, which need to be equilibrated in 5%, 37C incubator for at least 2 hours before use. M2, which contains Hepes, is used for embryo handling out of incubator for longer time, such as collecting embryos and microinjection. If embryos needs to be cultured to blastocyst, KSOM can be used. 5. Harvest Embryos On e0.5, sacrifice female donors and collect oviducts and place them into a drop of M2 with Hyaluronidase; Using two needles (25G) to gently tear open the oviducts that have obviously swallen ampulla, release the eggs. At that time, eggs are covered with cumulus cells. Or can flush the oviduct with M2 from the opening. Transfer the eggs to new drops M2 4 to 5 times until all the debris are removed. Transfer the eggs to M16 in 5%, 37C incubator. 6. Lentivirus loading Pull needles and insert needles into the tube with lentivirus 7. Microinjection Set up the needles and holding pipette, adjust them to be parallel to each other; Turn on the microinjector; Place eggs into a M2 drop covered by mineral oil; Use the holding pipette to stabilize one egg, focus the Zona pellucida; Insert the loaded needle into the perivitelline space of an egg with two pronucleus to see an obvious swelling of the perivitelline space. Release the injected egg, repeat the upper step to get all the fertilized eggs injected. 8. Embryo transfer, Select the good quality embryos for transfer; From the opening of oviduct, transfer 20~25 eggs to each pseudo-pregnant female.

Comments:

1. The required titer of lentivirus should be at least 5x10^8 to 5x10^9; 2. Clean, high quality of lentivrus makes microinjection easier; 3. A good needle with right diamete, length is very helpful; 4. The eggs can be transfered at e0.5, or can cultured to 2-cell stage or even blastocyst. 5. The leftover of lentivirus, including loaded needles, should be disposed in a biohazard bag.