Centre for Regenerative Medicine

Kidney Grafting User Instructions

Kidney grafting of cells or tissue under the kidney capsule is an essential tool for many experiments with no *in vitro* alternative. This includes transfer of stem cells to look for their differentiation into cells of all three germ layers (teratoma assay) or to study functionality of cell constructs as mini organs (e.g thymic cell engraftment). Below are the steps that are needed to request and conduct the assays.

1) **Come to see the unit manager and discuss the project well in advance:** This is to confirm that the project complies with the Home Office License we will use, and that the experiment is appropriate to address the biological question. We will discuss the time scale, the numbers and the potential outcomes and challenges.

2) **Obtain a quote for the work:** All Transgenic Services work on a cost recovery basis that is chargeable to a grant. All students and post docs should confirm with their PI that they have funds to recover this cost. Typically the costs animal costs are billed directly from the animal facility however estimates of this part of the cost of the experiment can be provided.

3) **We will order the animals:** Kidney grafting assays are predominately conducted on male mice at around 6 weeks of age. The starting date of the grafting will depend on the availability of the strain required. For human cell ES or iPS cell engraftment for example we would use NOD/SCID gamma mice, which we may have to wait 6 weeks or more to have sufficient numbers.

4) **We will make fill out an Experimental Protocol Sheet:** This is required by the Vet before we conduct the experiment. This form will explain the numbers and experimental design and confirm that this is covered by both personal and project home office licences.

5) **You will provide evidence that the cells have been screened:** The level of screening is dependent on the origin of the cells and the species. So for example all human cells should have been screened for both mycoplasma and human viruses. This is to protect both the technicians as well as the recipient animals.

6) **You will need to provide the cells in a timely manner:** Cells (mouse/human ES/iPS) should be grown under usual conditions to provide greater than 1 x 10^7 cells in total. Cells should be tryspinised or collagenase treated (whatever is the standard protocol) to provide a single cell suspension (note for human iPS or ES cells use accutase and keep clumped as per standard passage- speak to Joe Mee if there is an issue) that should be spun and washed in standard serum containing media and re-suspended at 5 x 10^6 cells in 100µl in HEPES buffered DMEM containing media (with FCS and glutamine -can be obtained from Transgenic unit) in a universal or bijou. Approximately 10-20ul of this cell slurry is used per transfer. (see below for recommendations for human ES cells and iPS cells)

7) **The experiment should be designed such that no more that 20 transfers are done in a day:** Each operation to place the cells or tissue into the recipients takes a finite amount of time. Cells need to be ready early in the morning if many transfers are to be done in a day.

8) **We will perform the operations and after care:** We make sure all surgical instruments are sterile and all glass capillaries and mouth pipettes are in the room the day before transfers. We anaesthetise each mouse and place in a flow hood. We remove fur at the site of the operation and follow the line of hip to make surgical incision and expose body wall. We open body wall over the kidney. We push firmly to expose kidney by pushing out of the body wall. We take up approximately 20ul of the cell slurry in a

glass capillary and position this next to scope. Under the microscope we make a small tear in the kidney capsule using ultra fine tweezers. We position the glass capillary to enter the capsule at an angle to enter the capsule in such a manner as not to damage the kidney. We gently expel the cells between kidney and the capsule. We return the kidney inside body wall and stitch the body wall and staple the skin. We leave the animals on a heat pad and monitor them until they recover. We then place the animal in cage ready to return to isolator. We keep no more than 3-5 animals per cage. We label the cages with cell line information.

9) **Your animals will be monitored daily:** The animal facility has day sheets that they sign when they check your mice on a daily basis. A member of the Transgenic team is available at all times to deal with any animals displaying signs of a tumour. An excell database is used to record the animals and when they are culled and the size and type of any tumour.

10) **We can remove the tumours and provide histological analysis:** We will remove tumours from any animals displaying signs. We will arrange with Ron Wilkie to have these PFA fixed and sectioned (Ron will bill for this separately). We can then either provide a full histological report on the tumours or provide training for histological examination.

hES cell dissociation

Usually when we passage these ES lines we use collagenase and scrape so that we re-seed small-medium sized colonies. However, as I understand it, your technician will be injecting the cells under the kidney capsule. In this case, the cells will need to be completely disaggregated so I would:

1. Remove medium
2. Wash once with PBS (no Ca, Mg)
3. wash once with a small volume of trypsin/EDTA
4. Add fresh trypsin to just cover the surface of the well.
5. Place at 37 degrees for about 3 mins, check under microscope and shake a little. When the cells are beginning to lift off, the plate can be given a careful knock - "careful" really just so the trypsin does not hit the plate lid.
6. Add about 2mL per well (6wp) of serum-containing medium (to slow down the effect of the trypsin).
7. Pipette the cells up and down and pool in a Falcon tube. There will be 12 wells (2 x 6wp). Pipette up and down again to obtain single cells - if required by pressing the pipette against the bottom of the tube and tilting slightly to allow gentle pipetting.
8. Prepare cell slurry at 5×10^6 cells in 100µl in HEPES buffered DMEM containing media with FCS and glutamine (can be obtained from Transgenic unit).