Centre for Regenerative Medicine

Mouse ES cell Electroporation and Colony picking User Instructions

Transfection of DNA constructs to make Transgenic animals is an important technique to make mouse models. These models give insight into the role of these genes *in vivo* which is important to our understanding of the role of these genes in development and disease. The transfection and colony picking of hundreds of clones is laborious and time consuming. Moreover great care must be taken to keep the cells in a high quality manner such they are suitable to pass through the mouse germline. The Transgenic Service can provide this as a service to reduce the workload and give the best opportunity for subsequent germline transmission. Although we can use the so called "96 well method" for freezing clones we tend to prefer the "24 well method" which although is more labour intensive provides the best long term storage, DNA quantity and best recovery.

1) **Come to see the unit manager and discuss the project well in advance:** The Transgenic Service manager will discuss with you the project and how the constructs have been designed. Drug selection will be discussed and the number of clones as well as the background genetic strain for the project.

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 cost for DNA transfection and colony picking are broken down so that you only pay the cost for each part as the project progresses.

3) **We will provide the mouse ES cells:**.We have very early passage mouse ES cells that we have tested for germline transmission. Our favourite is JU09 cells which are E14Tg2a cells that were rederived by the unit from mouse embryos. They are from the mouse 129 ola strain. They give high levels of chimerasim and excellent germline line transmission. If we inject three positive clones, usually two or more go through the germline. For some experiments a C57Bl6 genetic background is preferred. Backcrossing from 129 strain is time consuming even with accelerated backcrossing. We therefore have JM8 cells which are derived from C57B6 mice carrying an agouti repair so that coat colour chimerism is maintained. These have been tested for germline and although they are not as good as our JU09 cells, they do give germline transmission (around 1 in 3 clones will be germline).

4) **You will provide the construct:** We require from you 100-200ug of 1ug/ul DNA of you vector that is linearized and the final ethanol precipitation is done sterilely in a tissue culture flow hood. We do check the final DNA concentration by nanodrop before transfection but we do not linerise the DNA or run test gels.

5) **We will eletroporate the cells:** We use the Biorad machine in room 1.60 . We grow the mouse ES cells so that flask is just approaching confluency and cells look in a generally good condition (normally for such an electroporation the number of cells is between 2.0-10 x 10^7) so we grow a comfortable surplus. We gelatinise the required number of 9 cm diameter dishes and pipette Gmemb/10%FCS with LIF and PS into each plate so that final volume will be 10.0ml. We placing the plates at 37C for 1hour or greater to improve recovery and therefore transfection efficiency. We trypsinise and neutralise cells as normal by adding Gmemb/10%FCS from a narrow mouthed glass pipette and pipette several times to ensure single cell suspension. We wash twice with PBS and count the cell number on the second suspension. We adjust the cell density so that the required cell number is in 0.8ml PBS. Put DNA, (usually 100ug linearised into electroporation cuvette [0.4cm gap] and add 0.8 ml of ES cells,

resuspend once in cuvette to mix). We set up one cuvette with cells only as a mock electroporation. We leave the cell/DNA mixture for 3 minutes at room temperature (longer decreases transfection efficiency). We set the electroporation machine to 3mF / 0.80 kV and electroporate the DNA so we have a time constant of 0.1 to 0.2. Within 1 minute of electroporation we transfer cells to pre-warmed media (leaving the cells longer decreases transfection efficiency). We doo this by pipetting cells from the cuvette into a universal containing 9.2 ml of pre-warmed media using a plugged pasteur pipette. Then using some media from the universal, we rinse the inside of the cuvette once to recover as many cells as possible, mix cells by inversion to give a cell density of $1/10^{th}$ of the initial cell number. We then make further 1:10 dilutions until final plating densities are either 10^6 or 5×10^5 / 9cm petri dish. We swirl the dishes to distribute the cells and then leave in incubator for 30-40 hrs
Finally we add selection media (depending on the construct) and replace with fresh selection media every 48 hrs until mock plates are clear and colonies are of a suitable size for picking.

6) **We will pick the colonies:** Once individual colonies appear (usually 10-14 days) we wash the plates with PBS and place in PBS to cover the colonies. We place 10ul of trypsin in each well of two 96 well plates. We pick each colony using a Gilson and take the colony into 10ul PBS which we add to each of the trypsin wells. We work on a maximum of 24 colonies at a time. We place the 96 well plate into the incubator and wait three minutes. We use a multichannel pipette and plate each well into a gelatinsed 96 well dish containing 100ul of selection media. Once all wells from two 96 well plates has been filled we incubate overnight as normal and change the media daily until confluent. Once confluent we plate each well into two wells of a 24 well plate. One for freezing and one to make DNA.

7) **We will freeze the clones:** Once the 24 well plates destined for freezing are confluent we freeze each well into a cryotube. Each tube is labeled with the clone number. These are stored overnight at -80oC and then placed in LN2.

8) **We will perform a crude DNA prep:** For the 24 well copies of those clones that have been frozen we use a crude DNA lysis protocol. 100ul lysis Buffer (100 mM tris 5 mM EDTA 200mM NACL 2% SDS with proteinase K 10mg/ml) is used to cover the well and the next day is transferred into labelled tubes. Next 10% 3M NaOH is added and the DNA is precipitated with i2.5 vols of 100% EtOH. A 70% EtOH wash is performed and the DNA is dried and taken up into 50ul TE.

1) **You will perform a test digest and phenol extract the DNA if necessary:** Often the crude DNA preparations can be used directly for a digest without further purification. We recommend that you perform a nanodrop DNA concentration on a small number of samples and adjust DNA concentration accordingly and perform a test digest and run the digest on a minigel to confirm that the DNA is digesting properly. If not, then a phenol chloroform extraction is needed and you will need to perform this and re-precipitate and 70% Et OH wash the DNA ready for digestion. We will be able to provide protocols and advice if this is needed.

2) **You will perform the screening by Southern blot or PCR:** The Transgenic Service does not have the time or facilities to screen the DNA for positives. For targeting for example a 5' or 3' screen by Southern blot or PCR is needed and this should be designed and tested well in advance. We can however offer support and advice in the design of the screen and provide protocols to assist you. All colonies are frozen and stored in LN2 so there is no time limit to get the screen completed.

3) **We will recover the clones and make new stocks of cells:** Once you have successfully found positive clones we will recover these from LN2 and grow up a large flask and freeze around 5 vials of cells as a stock.

4) **We make DNA for you to confirm the identity of the clones:** In addition to making the stock freezes of positive clones we will make a crude DNA prep of the positives and provide these to you for confirmation of these clones. Sometimes potential positive bands are faint or artefacts and sometimes gel lanes are miss-read, so it is important to make sure that the clones that have been recovered are correct. As above the DNA prep is quite crude and test digests and phenol chloroform extractions should be performed if necessary.

5) **You will screen the cells and let us know which ones are suitable for making chimeras**: Prior to microinjection it is important that a good structural check is made on any targeting. This normally

includes confirming structural integrity both 5’ and 3’ of the mutation/insertion. Once full structural integrity has been assured the cells are ready for microinjection.