Electroporation of U2OS Cells

Establishing efficient electroporation conditions for a new cell line is time consuming due to the large number of parameters that must be dealt with (we have, however, outlined a fairly simple approach for optimization in the "Electroporation Optimization" section). Outlined below, are conditions that we have established for electroporation of U2OS cells.

Experimental Design Considerations

- Like most transfection methods, the condition and health of the recipient cells appears to be important to obtain efficient gene transfer. For other transfection methods, efficient cell cycling is important because DNA can only enter nucleus during mitosis (see Rodriguez A, and Flemington EK. Transfection mediated cell cycle signaling. Anal Bio 1999; 272:171-181). While we don't know specifically whether mitosis is required for entry of DNA into the nucleus using electroporation, we have found that the health of the cells is important for obtaining efficient gene transduction and rapidly cycling cells yield significantly better results.
- The total amount of DNA used is important for obtaining a high transfection efficiency. However, large amounts of plasmids containing potent promoters like the CMV early promoter are toxic to cells (see Rodriguez A, and Flemington EK. Transfection mediated cell cycle signaling. Anal Bio 1999; 272:171-181). Therefore, we use between 100ng – 5ug (usually between 250ng and 1ug) of a CMV based expression vector and make up the remainder of the total (30ug) with a plasmid that doesn't contain a eukaryotic promoter [we typically use the Promega vector, pGL3Basic (Cat# E1751, Genebank Accession Number=U47295)].
- Expression of genes introduced by electroporation is highest within 12hrs and expression falls off quite rapidly thereafter. This is in contrast to other transfection methods where high level expression can be observed for many days following transfection. This distinction is probably because in other methods, the DNA is encapsidated and is therefore degraded more slowly than electroporated DNA, which is naked. This issue should be taken into consideration in the design of electroporation experiments and in general, experiments should be completed by 32 hours following electroporation (we typically harvest cells at 24 or 32 hrs for most experiments).

Protocol

General Parameters:

Medium Resistivity Medium	DME complete media (Life Technologies)
Volume in the chamber	0.7 ml
Gene Pulser Cuvette	0.4 cm electrode gap (Bio-Rad)
Operating temperature	Room temperature

Method:

- The day before electroporating cells, split so that they will be approximately 80-85% confluent on the day of use.
- The next day, typsinize cells, neutralize with DME (+10%FBS, + Pen/Strep), and count.
- Spin down enough cells to allow for 3.5 x 10^6 cells per sample.
- Resuspend cells in 0.7ml DME (+10%FBS, + Pen/Strep) per sample (i.e 3.5 x 10^6/0.4ml).
- Transfer 0.7ml of cells to each cuvette.
- Add DNA [10ug/cuvette (see **Note)].
- Set up Electrocell Manipulator (ECM 600 BTX):
  Voltage=250 V Capacitance=975 uF Resistance=186 Ohms
- Place cuvette into the chamber and discharge! (should observe voltage readout of between 235-250 volts and a pulse length of 13 msec).

- Add 1.0ml DME(+10% FBS, +Pen/Strep), pipette up and down, and plate immediately in a T75 flask or a 100mm plate containing media.
- Incubate 24-32 hrs in a tissue culture incubator prior to harvesting.

**Note:

Total amount of DNA added per sample should be 10ug.

- For experiments designed to assess the function of a gene product using expression vectors, we use between 100ng and 5ug of the expression vectors (usually between 100ng and 1ug of CMV based expression vectors) and make up the remainder with a carrier plasmid that contains no eukaryotic promoters [such as pGL3Basic (Cat# E1751, Genebank Accession Number=U47295)] (see experimental design considerations).
- For cell cycle analysis of transfected cell populations we typcially transfect co-transfect with between 0.2 and 1ug of the GFP plasmids, Us9-GFP [described by Kalejta et al, Exp. Cell Res. 248; 322 (1999)] or GFP-SP [described by Kalejta et al, Cytometry 29;286 (1997)] (for further information on the use of GFP for cell cycle studies, see “Preparing Cells for FACS/PI”).