Electroporation of Burkitt's Lymphoma cell lines

Although several different methods can be used to introduce DNA into Burkitt's lymphoma (BL) cells, we have found that electroporation is much more efficient than most other methods (20-50% transfection efficiencies can be routinely obtained depending on the cell line).

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). Below, are conditions that we have established for electroporation of Burkitt's Lymphoma (BL) B-lymphocytes. These conditions were optimized using two BL lines but these conditions have so far been broadly applicable to every BL line that we've tested.

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 (at least 30ug). 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	RPMI 1640 Life Technologies complete media
Volume in the chamber	0.4 ml
Gene Pulser Cuvette	0.4 cm electrode gap (Bio-Rad)
Operating temperature	Room temperature

Method:

- Culture cells to near saturation in RPMI (+10% FBS, +Pen/Strep) (i.e. until the media has become somewhat yellow).
- Dilute cells 1:3 in fresh media (i.e. add 2 volumes of fresh media).
- The next day, the cells should be ready for electroporation.
  [Optimal results will be obtained if density is between $0.5\text{-}1.0 \times 10^6$ cells/ml]
- Count cells and spin down enough to allow for 1.5×10^7 per sample.

- Suspend cells in 0.4ml RPMI (+10% FBS, +Pen/Strep) per sample (i.e. 1.5 x 10^7/0.4ml).
- Transfer 0.4ml of cells to each cuvette.
- Add DNA [total of 30ug/cuvette (see **Note)].
- Set up Electrocell Manipulator (we use a BTX, ECM600).
  Voltage=265 Capacitance=975uF Resistance=720 Ohms
- Place cuvette into chamber and discharge! (should observe voltage readout of between 248-256V and a pulse length of 29-30msec)
- Add 0.6ml RPMI(+10% FBS, + Pen/Strep), pipette up and down, and transfer immediately to a T25 flask containing 10ml RPMI (+10%FBS, + Pen/Strep) (After shocking the cells, the media becomes very acidic, so don't leave very long before adding 0.6ml media and transferring to the flask. If you work relatively quickly, you can shock 4 samples at a time).
- Incubate 24-32 hrs in tissue culture incubator prior to harvesting.

**Note:

Total amount of DNA added per sample should be 30ug.

- 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 co-transfect with 0.2-1ug of the GFP based 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").