Focus Assays in Rat1 cells using Electroporations

(David Dankort)

This protocol has been worked out for Rat1 cells using supercoiled DNA. I have shown that using a MMoLV LTR driven Neu oncogene the conditions described herein are within the linear range. We electroporate 10^7 cells, plating at a density of 10^6 per plate and typically obtain 200-500 foci per plate using 5ug/plate. I have not scaled this down as it is convenient to save six plates for counting and 2-3 plates for deriving cell lines or to normalize for transformation efficiency. All electroporations are carried out in a final volume of 800ul/cuvette.

Required Reagents:

BioRad GenePulsar II
1xPBS

Per electroporation one will require the following:

50ug transforming DNA / electroporation
2ug selectable marker / electroporation
100mm tissue culture dishes, 6-9 / electroporation
100ml DMEM containing 10% FBS, Penn/Strep, Fungizone
BioRad GenePulsar Cuvette with 0.4cm electrode gap (cat#165-2088)

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i) Quantify DNA and aliquot DNA into a sterile eppendorf. Aliquot 50ug (5ug/plate) of the transforming plasmid and 2ug/electroporation of a selectable marker to derive lines and/or normalize for efficiency. Bring this to a final concentration of 1xPBS with the addition of 10xPBS (e.g. if two DNAs are in a total volume of 180ul add 20ul of 10xPBS). Note: we do not sterilize our DNAs by precipitation prior to use and have thus far found no problems with DNA-derived contamination.

ii) For each electroporation, aliquot exactly 100mls of prewarmed DMEM containing 5-10% FBS and anti- bacterial and fungal agents into a sterile 100ml tissue culture bottle and put each bottle in the 37°C incubator. We "measure" out 100mls using 50ml Falcon tubes. At this point label the bottles.

iii) Rat1s are grown to ~80% confluence in 150mm tissue culture dishes in DMEM containing 10% FBS and antifungal/ bacterial stuff. Keep in mind that you will require approximately 5.5 dishes for each electroporation.

iv) Remove media and trypsinize cells in the smallest possible volume. This can be done by adding 2ml of 1xtrypsin, swirling it around to cover the plate

and then aspirating it off. These plates are then put into 37°C for a few minutes. Cells are removed via the addition of DMEM/FBS and pooled. It is important to obtain single cells to maximize efficiency.

v) Count the harvested cells to determine the concentration and total number. Assuming 1x10^7 cells / electroporation, aliquot the required volume of Rat1s into a 50ml Falcon tube(s) and spin down at 1000rpm at room temp for 7-10 min.

vi) Aspirate off the media and GENTLY resuspend the cells in 1xPBS (room temp) pooling cells from several 50ml Falcon tubes if necessary. Bring volume up to 40-50ml with 1xPBS and spin down at 1000rpm at room temp for 7-10 min. (the purpose of this step is to remove all traces of media)

vii) Repeat step (vi) two more times. Following the third PBS wash, resuspend cells in the appropriate volume of 1xPBS for electroporation. Using the example above this would mean GENTLY resuspend the cells in 600ul for each electroporation, e.g. 3mls for five of these electroporations.

viii) Transfer 10^7 cells (here 600ul) to the eppendorf containing DNA+PBS, mix and transfer to a labeled electroporation cuvette.

ix) Set electroporator to 1000V at 25uF. Put cuvette containing 800ul of cells and DNA in 1xPBS into the chamber and ZAP. One should expect a time constant of apprxly 0.4-0.5msec. Electroporate one or two at a time.

x) Moving quickly, remove the cells to the prewarmed 100ml bottle of DMEM/FBS using a pipette and rinse out the cuvette.

xi) Repeat steps (ix) and (x) until all electroporations have been completed.

xii) From the 100ml bottles plate 10ml per plate into 100mm tissue culture dishes (we find Corning are superior to Nunc). These should form a monolayer within two days following electroporation.

xiii) Change media every third day.

xiv) One the 14th day after a monolayer has been formed, rinse the plates twice in 1xPBS, fix the monolayer with 10%phosphate buffered formalin for at least one hour and stain with Giemsa following manufacturer's instructions.

xv) count and publish at your leisure

To select for neomycin/G418 or puromycin resistance add 400-800 ug/ml geneticin or 3.0ug/ml puromycin respectively to the media. Neomycin selection can begin the day after electroporation whereas puromycin is quite brutal thus we wait 3-4 days before adding it.