Transfection of B- and T-cell lines by electroporation

The following are general guidelines for transfection of non-adherent B and T cell lines that I have done in the past. Optimization of conditions for each cell line is essential. You need to pre-optimize **(1)** the electroporation parameters and **(2)** the selection paratmers. I also will try to point out where other protocols differ.

1. Grow desired cells in the absence of **any** antibiotics for at least one passage.

2. Prepare DNA.

- generally, I use 20 - 25μg of the DNA expressing what I want to express and 1.0 - 1.25μg of selecting DNA (such as pSV2Neo, RSVneo, RSVhygro, etc.). If the selecting fragment is on the same plasmid, just use 25μg per shot.
- minimally, you should ethanol precipitate the DNA (together, if using two plasmids), and remove the ethanol, wash with 70%, dry, and resuspend the DNA in the tissue culture hood to maintain sterility.
- most people suggest linearizing the DNA before electroporation. The idea is that it is going to be linearized in the cell anyway, and by doing it first, you get to select where; thus, you decrease the chance that the cut will be in the middle of your expression cassette. While this makes a lot of sense and should be a good idea, in practice I have seen very little difference between linearizing and not linearizing. The disadvantage is that you need to get rid of the restriction enzyme, and if you do this by phenol/chloroform extraction, you run the risk of leaving some behind - this will also ruin your transfection. It's a judgement call - linearize, or not. In either case, purify the DNA afterwards by ethanol precipitation.

3. Prepare transfection buffer with desired amount of DNA. You will be resuspending each 10^7 cells in 0.5 - 1ml of transfection buffer, so take that into account.

- I have used regular old medium in the past, and had good success. Some people suggest using serum free medium or 1X HBS (recipe below), after giving cells one wash in it. You can try either/or. I have in the past gotten stable transfectants of Jurkat with just regular serum-full (10% FBS) medium.

4. Harvest 5x106 - 1x107 of the desired cells for each transfection that you plan to do. Cells should be in log phase and should be healthy (i.e., no mycoplasma and few dead cells). Mycoplasma is a definite killer of transfection.

5. Let cells sit on ice for 10 minutes. During this time, transfer to electroporation cuvettes, also on ice.

- use the 0.4cm cuvettes. The source of the cuvettes does not seem to be critical. I have used BTX and Bio-Rad cuvettes interchangeably on BTX and Bio-Rad electorporators.

6. Zap cells under conditions pre-determined to be good on *your* electroporator. Put cells back on ice for 10-15 minutes.

7. Check viability of cells. As discussed below, transfection efficiency is usually optimal when 50-70% of the cells die. Plate out cells in warm medium in the absence of the selection agent.

- cells need a day or two to recover before you start whacking them with selection agent. I usually plate them in bulk after the electroporation, and transfer to 96well plates the next day with selective agent. Of course, I am running the risk of some of the cells dividing this way, so you're probably better off plating into 96 well plates right away; it's less convenient, though.

8. After 24-48hrs, apply selective agent. Feed cells weekly until clones expand enough for transfer and determination of expression of your transgene.

Optimization

Selection This is straightforward - just grow cells in varying amounts of selection agent (e.g. G418 or hygromycin), and determine at which concentration cells die. I have had best luck when I chose to start the selection with a concentration that *really* wiped the cells out . For example, with Jurkat (if I remember right), I started seeing an effect at 800μg/ml active G418 (most cells died), but they weren't completely anihilated until 1.6mg/ml. I do the selection with 1.6mg/ml, but once individual clones are chosen and expanded, I maintain them in the presence of 800μg/ml. Cells vary greatly in their resistance, particularly to G418, and clones of things like Jurkat or HeLa vary from lab to lab, so it is worth checking your own cells. Mouse cells tend to be less resistant, (require less to kill) and human lymphoid cells tend to be more resistant.

Be aware that people talk of *active* G418 as opposed to *total* G418: G418 is impure, and when you get the powder from GIBCO/BRL, they tell you that only 748μg/mg is active, or something like that. I always use the active amount, but some people just weigh it out and use that number. So if you are relying on somebody else's titration, be sure you know which number that person used.

Electroporation parameters You can vary both the voltage and the capacitance of the jolt given to the cells, and the settings will vary for optimal transfection from cell to cell. Furthermore, I am told that the settings will vary from machine to machine as well, and my experience supports this notion. Thus, you are best to optimize the conditions for your cells on your machine before doing a real electroporation. The idea is basically to do a bunch of test samples, and test a few different voltages or capacitance levels; after you see initial results, you might want to fine tune the parameters in subsequent trials, or just go with something that looks OK.

You can be partially anal or really anal here. The *best* way to determine the conditions is to transfect with a reporter, such as CMV-luciferase, and measure luciferase activity in bulk transfected cells the next day. This could be a pain in the ass if you're not set up to do it. The other way, which is what I do, is to simply measure cell survival. Generally,

transfection is optimal when you wind up killing 50-70% of the cells. The idea is that you have to give a big enough jolt to disrupt the membrane and allow things, like DNA, to get into the cells; if you don't kill enough of the cells, you probably haven't delivered a big enough jolt. On the other side, if you kill too many cells, you won't have anything to grow out. Like I said, this is a generality, and some cells don't fit the bill - thus, the reporter assay is always favorable, if it is not too hard. If you choose to do the death, simply jolt at a few settings, then after the cells recover for a while (I wait the 10 minutes on ice, then give them a chance to recover in warm medium), count live vs. dead cells after staining with trypan blue. I have found this to work OK with Jurkat.

Generally, most cells that I have used prefer the 960μF setting for capacitance, and vary from 190-350V for the voltage. Jurkat was around the middle, if I remember right. This should serve as a good starting point.