

24 Sept 2001

TO: Regan Group

FROM: Tom Magliery

RE: BioRad MicroPulser Electroporator

Our new electroporator arrived today and is set up opposite my bench in 323 (next to the 37 °C water bath). Since most of you use heat-shock transformation now, I wanted to tell you a little bit about why you might want to use electroporation instead.

1. It's faster. The actual process of transformation is to simply mix the cells with the DNA, pulse the cells, and grow them out. There is no pre-incubation period or heat-shock time. Additionally, if your selectable marker is Amp, you can plate the cells directly after the transformation, since ampicillin only kills growing cells.
2. The transformation efficiency is higher. It's quite easy to make electrocompetent cells with transformation efficiencies in the range of 10^9 cfu μg^{-1} . That's 10 to 1000 times better than commonly produced heat-shock cells. This improves your chances in cloning and is critical to library methods.
3. Juan is making and testing electrocompetent cells. We prepared DH10B (an excellent cloning strain, almost isogenetic with TOP10 and similar to DH5 α). You can also buy library-grade (10^9 μg^{-1}) electrocompetent cells (DH10B) in the Kline stockroom.

You can buy the cuvettes (BioRad) in the Kline Stockroom. Typically, you will want to use 0.2 cm cuvettes. The appropriate setting on the MicroPulser is Ec2 (2.5 kV), or set the device to 2.5 kV manually. If you use 0.1 cm cuvettes, use the Ec1 setting (1.7 kV). There is a manual available if you would like to transform other kinds of cells, like yeast.

I also wanted to note that for routine usage, the cuvettes can be recycled. Simply rinse them with distilled water and store them in ethanol until you'd like to dry them and re-use them. Obviously, you should use new cuvettes for library purposes.

Feel free to see me if you'd like help setting up your first electroporation. Also, tell me or Juan if you'd like a special strain of cells made electrocompetent.

Finally, I wanted to note that there is a quick protocol for making electrocompetent cells that produces sufficient cells for 1-4 transformations. If you only need to do a single transformation with some unusual strain, let me know and I can explain how to do this.

Attached is a protocol for making, testing and transforming electrocompetent cells. See me with any questions.