

General protocol for optimization of electroporation parameters



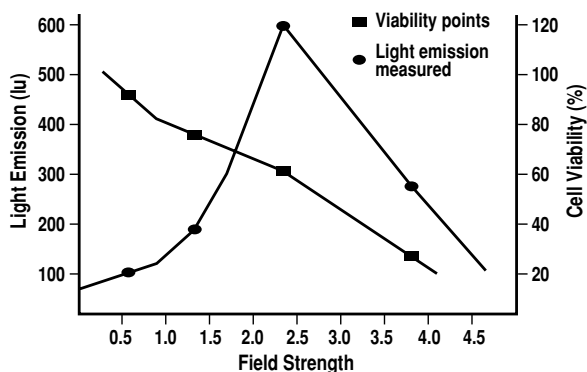
Electroporation and electrofusion processes are replacing the chemical methods traditionally used for cell transformation and cell fusion. Even as many bacteria, mammalian, plant, yeast and insect cells have been successfully electroporated, researchers are still improving the process. The variability of the cell line, media and plasmid compels researchers to optimize parameters for their own specific electroporation and electrofusion applications. Once these parameters are obtained, production of large scale transformed cells or hybridomas can be an easy task.

The two primary parameters that need to be optimized are field strength and pulse length. Field strength is measured as voltage delivered across an electrode gap and is expressed as kV/cm. This relates to the potential difference experienced by the cell membrane in the electric field. When the induced potential reaches a critical value, a reversible breakdown of the cell membrane occurs. In general, the voltage required to cause membrane breakdown is inversely proportional to the cell size. The membrane breakdown results in the formation of multiple pores through which macromolecules can pass. Field strength can be manipulated by adjusting the delivered voltage of the pulse generator, or by varying the distance between the sample electrodes.

The following protocol is similar to that recommended by D. Chang in Guide to Electroporation and Electrofusion published by Academic Press (1992), Chapter 26. The pulse length is obtained from the BTX protocol library and it is based on the value of pulse lengths for most cell lines. The value of the pulse length depends on the conductivity of the media used. In general, the more conductive the media is, the lower the pulse length which should be used.

General Electroporation Protocol

The field strengths necessary to transform bacteria fall between 3-24 kV/cm. Mammalian and plant cells usually require lower field strengths, 0.25-3 kV/cm. The use of the luciferase gene, FITC (fluorescent), or lucifer yellow (fluorescent) will help the researcher to optimize the parameters for cell permeability and re-sealing. Use a non-conductive media (low ion concentration) whenever possible. Place the media containing cells and luciferase gene into the cuvette. Use 5 msec as the pulse length for bacteria, 3 msec for mammalian and plant cells. Start with the lower field strength and change gradually to the highest field strength of the applicable cell type.



Measure the viability and expression of the cell after each electroporation. Plot the points. Based on the viability and the expression, the researcher can choose the correct field strength. Then, the second set of experiments will vary the pulse length while measuring viability and efficiency.

General Electrofusion Protocol

The ECM 2001 has AC and DC pulses. The AC field is used for the alignment of the cells and it has two variables, amplitude and pulse length. The frequency of ECM 2001 is fixed at 1 MHz. In order to optimize the AC setting, the cells are first placed on the microslide and observed under the microscope. In manual mode, set a certain low amplitude and watch the cells. They should form a pearl-chain or dimers. If the cells are lining up quickly (a few seconds, the amplitude is too high. Turn down the amplitude. Once a good number of hybridomas are formed, the DC pulse(s) is applied in order to fuse the cells.

To optimize the fusion parameters, the researcher can try various field strengths keeping the pulse length constant. Low conductive media (low salt) are used to reduce the heating effect. Most often, bacteria need 4-20 kV/cm and a pulse length of 5-15 μ sec. Mammalian cells need 0.5-9 kV/cm and pulse length of 3-50 μ sec. Using multiple numbers of pulses can also increase the yield of hybrids formed in the fusion process.