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Transfection by Electroporation

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Abstract

Electroporation—the use of high-voltage electric shocks to introduce DNA into cells—can be used with most cell types, yields a high frequency of both stable transformation and transient gene expression and, because it requires fewer steps, can be easier than alternate techniques. This unit describes electroporation of mammalian cells, including ES cells for the preparation of knockout, knockin, and transgenic mice,, describes protocols for using electroporation *in vivo* to perform gene therapy for cancer therapy and DNA vaccination, and outlines modifications for preparation and transfection of plant protoplasts.

Keywords

Molecular Biology; Introduction of DNA into Cells; Gene Regulation; Gene Expression; Transcription and Translation; Gene Therapy; DNA Vaccine

INTRODUCTION

Electroporation—the use of high-voltage electric shocks to introduce DNA into cells—is a procedure that is gaining in popularity for standard gene transfer and also allows the generation of genetically modified mice,. It can be used with most cell types, yields a high frequency of both stable transformation and transient gene expression, and, because it requires fewer steps, can be easier than alternate techniques (*UNITS 9.1, 9.2, 9.4* and introduction to Section I).

The basic protocol describes the electroporation of mammalian cells, including ES cells for the generation of transgenic and knockout/in mice. The *in vivo* protocols describe the use of electroporation to deliver plasmid DNA to muscle and skin. The alternate protocol outlines modifications for preparation and transfection of plant protoplasts.

BASIC PROTOCOL

ELECTROPORATION INTO MAMMALIAN CELLS

Electroporation can be used for both transient and stable (*UNIT 9.5*) transfection of mammalian cells. Cells are placed in suspension in an appropriate electroporation buffer and put into an electroporation cuvette. DNA is added, the cuvette is connected to a power

supply, and the cells are subjected to a high-voltage electrical pulse of defined magnitude and length. The cells are then allowed to recover briefly before they are placed in normal (non-selecting) cell growth medium. Factors that can be varied to optimize electroporation effectiveness are discussed in introduction to Section I, and protein expression strategies are discussed in Chapter 16. Selection for permanently transfected cells and for cells carrying targeted gene insertions by homologous recombination can be accomplished by modified media.

Materials—Mammalian cells to be transfected

Complete medium (*APPENDIX 3F*) without and with appropriate selective agents (*UNIT 9.5*)

Electroporation buffer, ice-cold

Linear or supercoiled, purified DNA preparation (see step 7)

Beckman JS-4.2 rotor or equivalent

Electroporation cuvettes (Bio-Rad #165-2088) and power source

Additional reagents and equipment for stable transformation in selective medium (*UNIT 9.5*) and for harvesting transfected cells (*UNITS 9.6–9.8 & 14.6*)

Prepare the cells for electroporation

1. Grow cells to be transfected to late-log phase in complete medium. Each permanent transfection will usually require 5×10^6 cells to yield a reasonable number of transfectants. Each transient expression may require $1\text{--}4 \times 10^7$ cells, depending on the promoter.

2. Harvest cells by centrifuging 5 min at $640 \times g$ (1500 rpm in a JS-4.2 rotor), 4°C.

Adherent cells are first trypsinized (introduction to Chapter 9) and the trypsin inactivated with serum.

3. Resuspend cell pellet in half its original volume of ice-cold electroporation buffer.

The choice of electroporation buffer may depend on the cell line used. See Critical Parameters for a complete discussion.

4. Harvest cells by centrifuging 5 min as in step 2.

5. Resuspend cells at 1×10^7 /ml in electroporation buffer at 0°C for permanent transfection. Higher concentrations of cells (up to 8×10^7) may be used for transient expression.

6. Transfer 0.5-ml aliquots of the cell suspension into desired number of electroporation cuvettes set on ice.

Add DNA and electroporate the cells

7. Add DNA to cell suspension in the cuvettes on ice.

For stable transformation, DNA should be linearized by cleavage with a restriction enzyme (UNIT 3.1) that cuts in a nonessential region and purified by phenol extraction and ethanol precipitation (UNIT 2.1). For transient expression, the DNA may be left supercoiled. In either case, the DNA should have been purified through two preparative CsCl/ethidium bromide equilibrium gradients (UNIT 1.7) followed by phenol extraction

and ethanol precipitation. The DNA stock may be sterilized by one ether extraction (UNIT 2.1); the (top) ether phase is removed and the DNA solution allowed to dry for a few minutes to evaporate any remaining ether.

For transient expression, 10 to 40 μ g is optimal. For stable transformation, 1 to 10 μ g is sufficient. Cotransfection (UNIT 9.5), although not recommended because of the work required to select and test transformants, can be done with 1 μ g of a selectable marker containing DNA and 10 μ g of the DNA containing the gene of interest.

8. Mix DNA/cell suspension by holding the cuvette on the two “window sides” and flicking the bottom. Incubate 5 min on ice.

9. Place cuvette in the holder in the electroporation apparatus (at room temperature) and shock one or more times at the desired voltage and capacitance settings.

The number of shocks and the voltage and capacitance settings will vary depending on the cell type and should be optimized (critical parameters; see also introduction to Section I).

10. After electroporation, return cuvette containing cells and DNA to ice for 10 min.

Culture and harvest the transfected cells

11. Dilute transfected cells 20-fold in nonselective complete medium and rinse cuvette with this same medium to remove all transfected cells.

12a. For stable transformation: Grow cells 48 hr (about two generations) in nonselective medium, then transfer to antibiotic-containing medium.

Selection conditions will vary with cell type. For example, neo selection generally requires ~400 μ g/ml G418 in the medium. XGPRT selection requires 1 μ g/ml mycophenolic acid, 250 μ g/ml xanthine, and 15 μ g/ml hypoxanthine in the medium (see UNIT9.5).

It is often convenient to plate adherent cells at limiting dilution (see UNIT 11.8) immediately following the shock, or suspension cells at the time of antibiotic addition.

12b. For transient expression: Incubate cells 50 to 60 hr, then harvest cells for transient expression assays.

Transfected cells can be visualized by standard transient expression assays (UNITS 9.6A–9.7).

IN VIVO PROTOCOL

ELECTROPORATION INTO MUSCLE OR SKIN

Electroporation has been used successfully to deliver plasmid DNA to a variety of tissues in vivo (Heller et al., 2006a). Because of its physical nature, EP can be applied to practically any cell or tissue. Plasmid DNA in the appropriate diluent is injected into the tissue. Electrodes are then placed around the injection site and the cells within the tissue are subjected to a high-voltage electrical pulse of defined magnitude and length. The animals are then allowed to recover and the tissue is evaluated at specified time points following delivery. Factors that can be varied to optimize electroporation effectiveness are pulse width, number, amplitude and electrode configuration.

Materials—Animals to undergo procedure

Syringe (1 CC) and needle size - 25–30 gauge

Linear or supercoiled, purified DNA preparation (see step 1)

Electrodes for administering the pulses

Electroporation power source

Additional reagents and equipment for harvesting tissue or evaluating expression levels and efficiency.

Prepare DNA for procedure

1. Amplification of DNA: For in vivo procedures, DNA used will typically be supercoiled. The plasmid to be delivered will need to be amplified and stock solution should be at a concentration of 2–5 $\mu\text{g}/\mu\text{l}$.

There are several commercially available kits for performing amplification as well as commercial entities that will prepare the plasmid at the appropriate concentration and quantity. DNA should be prepared with low endotoxin levels. While tissue specific promoters can be used, for both muscle and skin plasmids containing the CMV promoter are often used and are very effective.

2. DNA should be suspended in appropriate concentration. Typically for muscle this will be 0.5–1.0 $\mu\text{g}/\mu\text{l}$ and skin 1.0–2.0 $\mu\text{g}/\mu\text{l}$. The diluent is typically sterile 0.9% saline. Both sterile phosphate buffered saline and sterile water have also been used successfully. DNA should be stored cold.

In vivo Delivery procedure

3. Remove hair from area (skin or skin above muscle) to be transfected. This can be done with an electric razor, disposable razor or hair removal product.

4. Anesthetize the animals. Using an induction chamber, animals can be anesthetized in 2–4% isoflurane in oxygen. Once animal is anesthetized, they are fitted with an appropriate mask and kept under general anesthesia (2–3% isoflurane in oxygen) for the entire procedure.

5. Inject DNA into tissue. A standard injection volume is 50 μl , although volumes between 10–100 μl have been used. For muscle, concentration of DNA should be between 0.5–1.0 $\mu\text{g}/\mu\text{l}$ and for skin the concentration should be between 1.0–2.0 $\mu\text{g}/\mu\text{l}$.

Several muscles have been used successfully with in vivo electroporation including tibialis anterior, gastrocnemius and rectus femoris. Muscle chosen will be dependent on animal species utilized and specific application. For skin, delivery is via an intradermal injection and is typically on the flank, abdomen or base of tail. The concentration and injection volume will also be dependent on the specific application. For vaccines and immunotherapy the volumes and concentrations may be lower than for protein replacement therapies.

6. Placement of electrodes. Electrodes are placed around the injection site.

There are two types of electrodes that can be utilized - penetrating and nonpenetrating. Commercially available penetrating electrodes consist of two parallel needles of various sizes. The two critical dimensions of these electrodes are the length and the distance between them. For mouse or rat muscle electrodes 5 mm long and a gap of 5 mm will function well. The needles can be placed at either end of the injection site along the long axis of the muscle (through the skin and into the muscle) or under the skin and parallel to the muscle. An additional configuration is two rows of short needles. This configuration has been used for skin delivery. There are a variety of commercially

available nonpenetrating electrodes. These are typically two parallel plates of different sizes with different distances between the electrodes that are fixed or adjustable. This type of electrode is typically used for skin delivery and can also be utilized for muscle. The two plates are placed on either side of the injection.

7. Administer electroporation pulses.

The number of applied pulses, pulse width and applied voltage will vary depending on the tissue and the type of electrode utilized. (critical parameters).

8. Remove animal from anesthesia and allow them to recover.

Evaluation of transfection procedure

9. Protein expression can be assayed 24 hours after delivery procedure.

Time course of expression varies dependent on the tissue, protein expressed and plasmid construct. Muscle expression has been observed for several months. Skin expression has typically been observed for 2–3 weeks.

10. For secreted proteins. Blood samples can be obtained at various time points and assayed for protein levels.

11. For non secreted proteins. Tissue sample from the site of delivery can be taken, tissue homogenized and assayed for presence of expressed protein. Alternatively, if the secreted protein is fluorescent or luminescent, levels can be assayed by in vivo imaging. There are several instruments available now for doing this analysis (i.e. Caliper Life Sciences, IVIS instruments).

12. Biological effect can be utilized to determine the result of delivery procedure. This is dependent on the protein being expressed. For example, delivery of a plasmid encoding erythropoietin can be evaluated by measuring hematocrit.

ALTERNATE PROTOCOL

ELECTROPORATION INTO PLANT PROTOPLASTS

This is a modification of the basic protocol that is intended for use with plant cells. Plant cells are stripped of their cell walls and DNA is introduced into the resulting protoplasts.

Additional Materials—5-mm strips (1 g dry weight) sterile plant material

Protoplast solution

Plant electroporation buffer

80- μ m-mesh nylon screen

Sterile 15-ml conical centrifuge tube

Additional reagents and equipment for plant RNA preparation (*UNIT 4.3*)

1. Obtain protoplasts from carefully sliced 5-mm strips of sterile plant material by incubating in 8 ml protoplast solution for 3 to 6 hr at 30°C on a rotary shaker.
2. Remove debris by filtration through an 80- μ m-mesh nylon screen.
3. Rinse screen with 4 ml plant electroporation buffer. Combine protoplasts in a sterile 15-ml conical centrifuge tube.

4. Centrifuge 5 min at $300 \times g$ (1000 rpm in a JS-4.2 rotor). Discard supernatant, add 5 ml plant electroporation buffer, and repeat wash step. Resuspend in plant electroporation buffer at $1.5\text{--}2 \times 10^6$ protoplasts/ml.

Protoplasts can be counted with a hemacytometer (UNIT 1.2).

5. Carry out electroporation as described for mammalian cells (steps 6 to 11 of the basic protocol). Use one or several shocks at 1 to 2 kV with a 3- to 25- μF capacitance as a starting point for optimizing the system.

Alternatively, use 200 to 300 V with 500 to 1000 μF capacitance if the phosphate in the electroporation buffer is reduced to 10 mM final.

6. Harvest cells after 48 hr growth and isolate RNA, assay for transient gene expression, or select for stable transformants.

Protoplasts can also be selected and grown into full transgenic plants (Rhodes et al., 1988).

REAGENTS AND SOLUTIONS

Electroporation buffers—Choice of electroporation buffer depends on the cells being used in the experiment (see Critical Parameters). The following buffers (stored at 4°C) can be used:

1. PBS (*APPENDIX 2*) without Ca^{++} or Mg^{++}
2. HEPES-buffered saline (HeBS; *UNIT 9.1*)
3. Tissue culture medium *without* FCS (introduction to Chapter 9)
4. Phosphate-buffered sucrose: 272 mM sucrose/7 mM K_2HPO_4 (adjusted to pH 7.4 with phosphoric acid)/1 mM MgCl_2

Plant electroporation buffer—*Prepare in PBS (APPENDIX 2)*

0.4 M mannitol

5 mM CaCl_2

Store at 4°C

Protoplast solution—2% (w/v) cellulase (Yakult Honsha)

1% (w/v) macerozyme (Yakult Honsha)

0.01% (w/v) pectylase

0.4 M mannitol

40 mM CaCl_2

10 mM 2-[*N*-morpholino]ethanesulfonic acid (MES), pH 5.5

Prepare fresh before use

COMMENTARY

Background Information

DNA transfection by electroporation is an established technique that is applicable to perhaps all cell types. It yields a high frequency of stable transformants and has a high efficiency of transient gene expression. Electroporation has now been shown to be effective at delivering plasmid DNA *in vivo* to a variety of tissue types. Electroporation makes use of the fact that the cell membrane acts as an electrical capacitor that is unable to pass current (except through ion channels). Subjecting membranes to a high-voltage electric field results in their temporary breakdown and the formation of pores that are large enough to allow macromolecules (as well as smaller molecules such as ATP) to enter or leave the cell. The reclosing of the membrane pores is a natural decay process that is delayed at 0°C.

During the time that the pores are open, nucleic acid can enter the cell and ultimately the nucleus. Linear DNA with free ends is more recombinogenic and more likely to be integrated into the host chromosome to yield stable transformants. Supercoiled DNA is more easily packaged into chromatin and is generally more effective for transient gene expression.

The use of high-voltage electric shocks to introduce DNA into cells was first performed by Wong and Neumann using fibroblasts (Wong and Neumann, 1982; Neumann et al., 1982). The technique was then generalized (Potter et al., 1984) to all cell types—even those such as lymphocytes that, unlike fibroblasts, cannot be transfected with other procedures (e.g., calcium phosphate or DEAE-dextran DNA coprecipitates).

Oliver Smithies and colleagues then used electroporation to introduce DNA into embryonic stem (ES) cells and designed targeting vectors that allowed the introduced DNA to recombine with homologous regions in the genome and either introduce an altered gene or a disrupting sequence to generate ES cells with a specific gene ‘knocked in’ or ‘knocked out’. The altered ES cells were then used to generate the corresponding knockin or knockout mice. Electroporation was needed for these gene transfer applications because it introduces DNA into cells in a naked form that can easily participate in homologous recombination. This extension of electroporation led to Dr. Smithies sharing the 2007 Nobel Prize for Medicine or Physiology. The methodology for electroporating ES cells is essentially the same as for other mammalian cells. If homologous gene replacement is desired, then vectors that allow “positive-negative” screening must be designed (Bronson and Smithies, 1994; Joyner 2000) such that one selection recovers all cells in which the electroporated DNA has inserted into the genome, and the second selection is against ES clones in which the DNA has inserted randomly. Knockin/out mice can then be generated by fusing the selected cloned ES cells with embryos, reimplanting to allow development, and breeding the resulting chimeras to generate mice in which all cells carry the altered gene.

Although whole plants or leaf tissue have been reported to be transfectable by electroporation, plant cells must generally be made into protoplasts before DNA can be easily introduced into them (alternate protocol; Fromm et al., 1985; Ou-Lee et al., 1986). Like mammalian cells, plant protoplasts may be electroporated under a variety of electrical conditions (critical parameters). Both high voltage with low capacitance (short pulse duration) or low voltage with high capacitance (long pulse duration) have been used to achieve successful gene transfer (Chu et al., 1991). *In vivo* EP was originally utilized to deliver chemotherapeutic agents to solid tumors and progressed from preclinical studies through to clinical trials (Gothelf, et al., 2003). The *in vivo* delivery of plasmid DNA using electroporation was first reported in the early to mid 1990s (Titomarov, et al., 1991; Heller, et al., 1996; Nishi, et al., 1996) and was a logical advance based on the success of *in vitro* transfections with electroporation and the demonstration that the procedure could be

performed safely in vivo when delivering small molecules such as chemotherapeutic agents. The use of in vivo electroporation for delivery of plasmid DNA has seen tremendous growth in the number of preclinical studies being conducted and has recently been translated into the clinic (Heller, et al., 2006a and Bodles-Brakhop, et al., 2009).

The wide use of electroporation has been made possible in large part by the availability of commercial apparatuses that are safe and easy to use and that give extremely reproducible results. Designs of these machines vary substantially, but fall into two basic categories that use different means of controlling pulse duration and voltage (the two electrical parameters that govern pore formation). One kind uses a capacitor discharge system to generate an exponentially decaying current pulse, and the other generates a true square wave (or an approximation thereof). The capacitor discharge instruments charge their internal capacitor to a certain voltage and then discharge it through the cell-DNA suspension. Both the size of the capacitor and the voltage can be varied. Because the current pulse is an exponentially decaying function of (1) the initial voltage, (2) the capacitance setting of the instrument, and (3) the resistance of the circuit (including the sample), changing the capacitor size to allow more (or less) charge to be stored at the voltage will result in longer (or shorter) decay times and hence a different effective pulse duration. In contrast, square wave generators control both the voltage and pulse duration with solid-state switching devices. They also can produce rapidly repeating pulses. For in vivo applications, square wave generators are preferred. In addition to pulse duration and amplitude, pulse number and electrode configuration also influence efficiency of delivery.

Most of our in vitro electroporation experiments have used the Bio-Rad Gene Pulser, a capacitor discharge device, but are directly applicable to other capacitor discharge devices, and with some adjustment to square wave generators. Capacitor discharge devices are also available from GIBCO/BRL, BTX, Hoeffler Scientific, and International Biotechnologies (see *APPENDIX 4* for suppliers' addresses). These machines, either in a single unit or through add-on components, can deliver a variety of electroporation conditions suitable for most applications. Square wave generators are available from BTX, Baekon, CytoPulse Sciences, Sonidel, Bio-Rad, Jouan and IGEA and offer great control over pulse width, allow multiple, rapid pulses, and can be more effective for cells that are very sensitive or otherwise difficult to transfect. Using electroporation to accomplish gene therapy in living animals or humans also requires good control over electroporation parameters to assure efficient DNA transfer with minimal tissue damage and this generally requires square wave generators. Generators are available to administer pulses as either constant voltage or constant current. In addition to supplying square wave generators, electrodes suitable for in vivo electroporation are also available from these suppliers. These machines are generally more expensive. It has become apparent that alternating current pulses at ~100 kHz may be the most effective wave form for electroporation and possibly electrofusion (Chang, 1989).

The majority of our in vivo experiments have utilized BTX T820 or T830 square wave generators. These experiments have utilized commercially available electrodes such as a 2-needle array, caliper electrodes and forceps electrodes as well as custom designed electrodes. As mentioned above, major suppliers of electroporation equipment have a variety of penetrating and nonpenetrating electrodes available. Square wave generators afford better control of pulse parameters which is particularly important when performing in vivo delivery. The growth of the use of in vivo electroporation is directly related to its effective delivery into muscle [Andre, et al., 2004]. The application of intramuscular delivery of genes using electroporation has been particularly important for vaccination purposes (Abdulhagg, et al., 2008). Muscle has also been demonstrated to be an excellent depot for gene-based protein replacement applications (Trollet, et al., 2006). Delivery to muscle can also be used for delivery of anti-cancer vaccines (Bodles-Brakhop, et al., 2009). Delivery to

the skin has also gained acceptance as a versatile target. Delivery to the skin can be used to treat cutaneous diseases directly, delivering proteins to the circulation for systemic therapy, cancer therapy and for delivering DNA vaccines (Hirao, et al., 2008, Roos, et al., 2006, Glasspool-Malone, et al., 2000).

Electroporation can be easier to carry out than alternative techniques, which is why it is becoming increasingly utilized. Its drawback for use with transient analysis is that almost fivefold more cells and DNA are needed than with either calcium phosphate- or DEAE-dextran-mediated transfection (*UNITS 9.1, 9.2 & 16.12*). The main difference between electroporation and calcium phosphate coprecipitation procedures is the state of the integrated DNA after selection in appropriate antibiotic media. In the case of calcium phosphate, the amount of DNA taken up and integrated into the genome of each transfected cell is in the range of 3×10^6 bp. As a result, the transfected DNA often integrates as large tandem arrays containing many copies of the transfected DNA. This would be an advantage when transfection of genomic DNA into recipient cells and selection for some phenotypic change such as malignant transformation is desired; here a large amount of DNA integrated per recipient cell is essential. In contrast, electroporation can be adjusted to result in one to many copies of inserted DNA per recipient cell. This would be an advantage for gene expression studies, as the identity of the particular copy responsible for the gene expression can be controlled, and, as discussed above, is essential for gene targeting of ES cells to generate transgenic mice.

Critical Parameters

As discussed above, the two parameters that are critical for successful in vitro electroporation are the maximum voltage of the shock and the duration of the current pulse (see also introduction to Section I). The voltage and capacitance settings must be optimized for each cell type, with the resistance of the electroporation buffer being critical for choosing the initial instrument settings. The guidelines presented in this unit are meant to be adapted according to the manufacturers' instructions and the individual investigator's needs. Optimal stable and transient transformation occur at about the same instrument settings, so transient expression can be used to optimize conditions for a new cell type.

For low-resistance (high-salt) buffers such as PBS, HeBS, or tissue culture medium, start with a capacitor setting of 25 μ F and a voltage of 1200 V for 0.4-cm cuvettes, then increase or decrease the voltage until optimal transfection is obtained (usually at ~40% to 70% cell viability as detected by trypan blue exclusion; *UNIT 11.5*). For many cell types, the choice between PBS, HeBS, and tissue culture medium is arbitrary. However, some cells (especially primary cells) are very easily killed and thus electroporate poorly at the high voltages needed for PBS or HeBS electroporation buffers. Particularly sensitive cells seem to prefer tissue culture medium, though it has been shown that the calcium and magnesium ions in the medium lower the electroporation efficiency (Neumann et al., 1982). Phosphate-buffered sucrose has the advantage that it can be optimized at voltages several hundred volts below those used with PBS or HeBS. Alternatively, Chu et al. (1991) found many sensitive cells were electroporated more effectively in HeBS with a low voltage/high capacitance setting that resulted in at least 10-fold longer pulse duration. For these conditions, start at 250 V/960 μ F and change the voltage up to 350 V or down to 100 V in steps to determine optimal settings.

Keeping cells on ice (at 0°C) often improves cell viability and thus results in higher effective transfection frequency, especially at high power which can lead to heating (Potter et al., 1984). However, Chu et al. (1991) found that under low voltage/high capacitance conditions, some cell lines electroporate with higher efficiency at room temperature.

Therefore, steps 6 to 10 of the basic protocol should be carried out separately at both temperatures to determine the optimum conditions for a new cell line.

Another factor contributing to cell death appears to be the pH change that results from electrolysis at the electrodes. This problem can be reduced by replacing some of the ionic strength of the PBS with extra buffer (e.g., 20 mM HEPES, pH 7.5).

Optimal parameters for plant electroporation differ depending on whether tissue culture cells or various parts of the whole plant are used as a source of protoplasts. In particular, the high salt in PBS can be damaging to protoplasts freshly produced from plant tissue. Replacing the NaCl in PBS with 135 mM LiCl may increase CAT transient gene expression (*UNIT 9.6A*) in electroporated plant protoplasts 4- to 70-fold (Saunders et al., 1989). Alternatively, an electroporation buffer of 0.6 M mannitol/25 mM KCl for leaf cells, or 0.7 M mannitol/40 mM KCl/4 mM MES (pH 5.7)/1 mM 2-ME added for root and stem cells, is recommended (Sheen, 1990). In addition, 0.1% BSA/15 mM 2-ME/1 mM MgCl₂ can be added to either protoplast isolation buffer and the CaCl₂ reduced to 1 mM final. A low salt concentration in the electroporation buffer reduces the optimal capacitance setting to 200 μ F.

Optimal parameters for in vivo delivery differ dependent on tissue and specific application. Expression levels can be controlled by selection of the appropriate parameters including electrode configuration and can result in obtaining high, low, long-term or short-term expression. This versatility and relative control in choosing the type of expression obtained can facilitate the success or failure of a particular therapeutic application. Versatility can be advantageous in selecting the appropriate expression, but it also means that when a new application utilizing electroporation is initiated it is important to consider all the variables to develop the right delivery protocol for that specific application. For muscle delivery utilizing two parallel plates, electroporation parameters that will achieve high, long-term expression are 200 V/cm, 20 ms and 8 pulses (Mir, et al, 1999). To achieve similar expression with needle electrodes, the parameters would be 100 V/cm, 20 ms and 8–12 pulses (Lucas, et al., 2001). For delivery to the skin, using plate electrodes, successful electroporation parameters are 100 V/cm, 150 ms and 8 pulses (Heller, et al., 2006b). With needles electrodes, parameters were 275 V/cm, 10 ms and 8 pulses (Roos, et al., 2006).

Anticipated Results

The efficiency of transfection by electroporation is dependent upon cell type. For fibroblasts, which are easily transfected by calcium phosphate or DEAE-dextran coprecipitation (*UNITS 9.1 & 9.2*), electroporation gives a stable transformation frequency of 1 in $\sim 10^3$ to 10^4 live cells—approximately that obtainable by the above traditional procedures. For cells refractory to traditional methods, electroporation gives a stable transformation frequency between 1 in 10^4 to 10^5 for most cell types. Occasionally a cell line (e.g., some T lymphocytes) will transfect poorly under our standard conditions (1 in 10^6), and even this frequency is sufficient to obtain significant numbers of transfectants. In general, cells that transfect efficiently for stable transformants also do so for transient gene expression. Increasing the number of cells and the amount of DNA used in the electroporation for studying transient gene expression can circumvent problems of low transfection efficiency and low promoter/enhancer efficiency.

For plant protoplast electroporation, the frequency of stable transformants is between 1 in 10^2 and 1 in 10^3 dividing cells.

Efficiency of in vivo electroporation is dependent on tissue type, protein being expressed, plasmid size and promoter. Skin delivery efficiency has been reported as high as 32%

(Heller et al., 2006b). Muscle has also been demonstrated to achieve efficient delivery of greater than 30% (Mir, et al, 1999).

Time Considerations

The entire process of electroporation of mammalian cells will take <1 hr. Electroporation of plant cells requires ≤6 hr to prepare the protoplasts and <1 hr for the actual electroporation process. As with other transfection procedures, the experiment should be planned to allow for harvest or splitting of the cells 1 to 2 days after transfection. For in vivo electroporation the procedure can be done in < one hour. Evaluation of expression following delivery can be for hours, days, weeks or months.

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