Topic Introduction

Introducing Genes into Cultured Mammalian Cells

Priti Kumar, Arvindhan Nagarajan, and Pradeep D. Uchil

Strategies for the delivery of genes into eukaryotic cells fall into three categories: transfection by biochemical methods, transfection by physical methods, and virus-mediated transduction. This introduction deals with the first two categories.

OVERVIEW OF TRANSFECTION METHODS

The choice of transfection method is determined by several experimental factors:

- whether gene expression or protein production is the desired end result
- the cell type to be used (adherent vs. suspension, culture-adapted vs. primary cells)
- the ability of the cell line to survive the stress of transfection
- the type of assay to be used for screening
- the culture conditions
- the nucleic acid to be transfected (DNA, RNA, siRNA, or oligonucleotides)
- the efficiency required of the system
- whether the application requires low, medium, or high throughput

Commercial transfection reagents are routinely tested for their efficacy with multiple cell types, different nucleic acids, and a variety of specific applications. Before starting, we recommend consulting the transfection reagent selection guide provided on many manufacturers' websites, and if time and resources permit, comparing the efficiencies of different transfection reagents.

Biochemical methods of transfection, including calcium phosphate-mediated and diethylaminoethyl (DEAE)-dextran-mediated transfection, have been used for more than 45 yr to deliver nucleic acids into cultured cells (see Introduction: **Transfection of Mammalian Cells with Calcium Phosphate-DNA Coprecipitates** [Kumar et al. 2019a] and Introduction: **DEAE-Dextran Transfection** [Kumar et al. 2018a], respectively). The work of Graham and van der Eb (1973) on transfection of mammalian cells by viral DNAs in the presence of calcium phosphate laid the foundation for the biochemical transfection of genetically marked mouse cells by cloned DNAs (Maitland and McDougall 1977; Wigler et al. 1977), for the transient expression of cloned genes in a variety of mammalian cells (e.g., see Gorman et al. 1983), and for the isolation and identification of cellular oncogenes, tumor-suppressing genes, and other single-copy mammalian genes (e.g., see Wigler et al. 1978; Perucho and Wigler 1981; Weinberg 1985; Friend et al. 1988). Nowadays, cationic/neutral lipid (liposome) reagents are the most popular reagents for better delivery of genes into a wide range of cell types (see

From the Molecular Cloning collection, edited by Michael R. Green and Joseph Sambrook.

© 2019 Cold Spring Harbor Laboratory Press

Cite this introduction as *Cold Spring Harb Protoc*; doi:10.1101/pdb.top095406

Introduction: Lipofection [Kumar et al. 2019b]). Here, the chemical agent forms a complex with the DNA, neutralizing or masking the negative charge (and sometimes even rendering a positive charge) on the DNA molecule, thus enabling interaction with the negatively charged cell membrane, which facilitates uptake.

In addition to biochemical methods, electroporation—a physical transfection method—is widely used (see **Introduction: Electroporation** [Kumar et al. 2019c]). Electroporation is a process in which brief electrical pulses create transient pores in the plasma membrane that allow nucleic acids to enter the cellular cytoplasm. Nucleofection, an improvement of the electroporation technology (developed by the company Amaxa), permits the introduction of nucleic acids directly into the nucleus using a combination of optimized electrical parameters with cell type-specific reagents. Finally, “optical transfection” exploits the ability of light to create small transient pores in the plasma membrane of mammalian cells (see **Introduction: Optical Transfection** [Kumar et al. 2018b]).

TRANSIENT VERSUS STABLE TRANSFECTION

Two different approaches are used to transfer DNA into eukaryotic cells: transient transfection and stable transfection. In transient transfection, recombinant DNA introduced into a recipient cell line generates a temporary but high level of expression of the target gene. The transfected DNA does not necessarily become integrated into the host chromosome. Transient transfection is the method of choice when large numbers of samples are to be analyzed within a short period of time. Typically, the cells are harvested 1 and 4 d after transfection, and the resulting cell lysates are assayed for expression of the target gene.

Stable or permanent transfection is used to establish clonal cell lines in which the transfected target gene is integrated into chromosomal DNA, from where it directs the synthesis of the target protein, usually in moderate amounts. In general (depending on the cell type), the formation of stably transfected cells occurs with an efficiency one to two orders of magnitude lower than the efficiency of transient transfection. Isolation of rare stable transfectants from a background of nontransfected cells is facilitated by the use of a selectable genetic marker. The marker may be present on the recombinant plasmid carrying the target gene, or it may be carried on a separate vector and introduced, together with the recombinant plasmid, into the desired cell line by cotransfection (for further details, see **Introduction: Selective Agents for Stable Transfection** [Kumar et al. 2018c]). In general, the methods described below are suitable for use both in transient transfection assays and for the generation of stable transfectants.

TRANSFECTION METHODS

In the past, cloned DNA was introduced into cultured eukaryotic cells chiefly by biochemical methods using either calcium phosphate or DEAE-dextran. Lipid reagents are now preferred because of the high efficiency of transfection that can be obtained and because of the ability of this class of reagents to mediate transfection of all types of nucleic acids into a wide range of cell types. Additional advantages include their ease of use, reproducibility, low toxicity, and ability to mediate effective transfection of suspension cultures. Physical methods, such as electroporation and nucleofection, are options to consider with cell lines that are resistant to transfection by other means. A brief summary of transfection methods is given in Table 1.

TRANSFECTION CONTROLS

All transfection experiments should include controls to test individual reagents and plasmid DNA preparations, and to test for toxicity of the gene or construct being introduced.

TABLE 1. Transfection methods

Method	Expression Transient	Stable	Cell toxicity	Cell types	Comments	Pros	Cons
Lipid-mediated	Yes	Yes	Varies	Adherent cells, primary cell lines, suspension cultures	Cationic lipids are used to bind negatively charged DNA molecules, sometimes creating artificial membrane vesicles (liposomes). The resulting stable cationic complexes adhere to and fuse with the negatively charged cell membrane (Felgner et al. 1987, 1994).	Very low immunogenicity and cytotoxicity. They can be used with large plasmids. Low safety risk, simple handling procedure, single protocol for all nucleic acids (DNA, mRNA, siRNA, etc.), rapid procedure, comparatively low costs, and adaptable to high-throughput systems.	Low-to-variable efficiency in primary and suspension cells
Calcium phosphate- mediated	Yes	Yes	No	Adherent cells (CHO, 293); suspension cultures	Calcium phosphate forms an insoluble coprecipitate with DNA, which attaches to the cell surface and is absorbed by endocytosis or phagocytosis (Graham and van der Eb 1973).	Low cytotoxicity, can be used with large plasmids, low safety risk, simple handling procedure, very inexpensive.	Reagent consistency is critical to achieve transfection efficiency; small changes in pH (± 0.1) can greatly affect transfection efficiency. The size and quality of the precipitate are crucial for efficient transfection. Sometimes can prove to be immunogenic. The protocol may require tweaking for different nucleic acids and may result in variable efficiencies, and variable efficiency for adherent and suspension cells. Ineffective for primary cells. Complexes cannot be made in culture medium because of the high concentration of phosphate in the medium.
DEAE-dextran- mediated	Yes	No	Yes	BSC-1, CV-1, and COS	Positively charged DEAE-dextran binds to negatively charged phosphate groups of DNA, forming aggregates that bind to the negatively charged plasma membrane. Uptake into the cell is believed to be mediated by endocytosis, which is potentiated by osmotic shock (Vaheri and Pagano 1965).	Can be used with large plasmids, low safety risk, simple handling procedure, inexpensive.	Can prove immunogenic and cytotoxic. Can only be used for transient transfections. The protocol may require tweaking for different nucleic acids with highly variable efficiencies and variable efficiency for adherent and suspension cells. Ineffective for primary cells.

(continued)

TABLE 1. Continued

Method	Expression Transient	Stable	Cell toxicity	Cell types	Comments	Pros	Cons
Electroporation	Yes	Yes	Yes	Many; including cells refractory to lipofection	Application of brief high-voltage electrical pulses to a variety of mammalian and plant cells leads to the formation of nanometer-sized pores in the plasma membrane (Neumann et al. 1982; Zimmermann 1982). DNA is taken directly into the cell cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies the closure of the pores.	Can be used with large plasmids, low safety risk, fairly simple handling procedure, single protocol for all nucleic acids (DNA, mRNA, siRNA, etc.), rapid procedure, nonchemical method that does not alter the biological structure or function of cells.	Can be highly cytotoxic, poor efficiencies for suspension and primary cells, moderate costs, requires specialized equipment. Experimental settings vary based on the cell type used.
Nucleoporation	Yes	Yes	Varies	Many; including cells refractory to lipofection	Based on the physical method of electroporation. Nucleofection uses a combination of electrical parameters, generated by a device called a Nucleofector, with cell-type-specific reagents to transfer nucleic acids directly into the cell nucleus and the cytoplasm (Greiner et al. 2004; Johnson et al. 2005).	Fairly low cytotoxicity for many cell types; can be used with large plasmids; low safety risk; good efficiencies with adherent, suspension, and primary cell types; fairly simple handling procedure; single protocol for all nucleic acids (DNA, mRNA, siRNA, etc.); rapid procedure.	Moderate costs, requires specialized equipment. Experimental settings vary for each cell type and require specialized buffers provided by a single manufacturer.

Controls for Transient Expression

Negative Controls

In transient transfection experiments, one or two dishes of cells should be transfected with the carrier DNA and/or buffer used to dilute the test plasmid or gene. Typically, salmon sperm DNA or another inert carrier such as the empty vector used to construct the recombinant is transfected into adherent cells in the absence of the test gene. After transfection, the cultured cells should not detach from the dish nor become rounded and glassy in appearance.

Positive Controls

One or two dishes of cells are transfected with a plasmid encoding a readily assayed gene product such as chloramphenicol acetyl transferase, luciferase, *Escherichia coli* β -galactosidase (see Protocol: **Histochemical Staining of Cell Monolayers for β -Galactosidase** [Kumar et al. 2019d]), or fluorescent proteins such as green fluorescent protein (GFP) and its derivatives, whose expression is driven by a panspecific promoter such as the human cytomegalovirus immediate early gene region promoter and its associated enhancer. Tracer plasmids of this kind are available from commercial suppliers who sell kits containing the enzymes and reagents needed for detection of the encoded protein. Because the endogenous levels of these reporter activities are typically low, the increase in enzyme activity or fluorescence provides a direct indication of the efficiency of the transfection and the quality of the reagents used for a particular experiment. This control is especially important when comparing results of transfection experiments performed at different times. Cotransfecting the reporter plasmid with the test plasmid or genomic DNA also provides a control for nonspecific toxicity in the overall transfection process.

Controls for Stable Expression

Negative Controls

One or two dishes should be transfected with an inert nucleic acid such as salmon sperm DNA, in the absence of the selectable marker. After culturing for 2–3 wk in the presence of the appropriate selective agent (e.g., G418, hygromycin, mycophenolic acid), no colonies should be visible.

Positive Controls

One or two dishes of cells should be transfected with the empty plasmid encoding the selectable marker. The number of viable colonies detected at the end of a 2–3-wk selection period is a measure of the efficiency of the transfection/selection process. A similar number of colonies should be present on dishes into which both the selectable marker and the test plasmid or gene were introduced. A marked discrepancy in the number of colonies on these two sets of dishes can be an indication of a toxic gene product (or, in rare instances, of a gene product that enhances survival of the transfected cells). If a particular cDNA or gene proves toxic to recipient cells, consider the use of a regulated promoter such as metallothionein (a Zn^{2+} - or Cd^{2+} -responsive DNA), the mouse mammary tumor virus long terminal repeat promoter (a glucocorticoid-responsive DNA), or a tetracycline-regulated promoter (Gossen and Bujard 1992; Gossen et al. 1995; Shockett et al. 1995).

OPTIMIZATION AND SPECIAL CONSIDERATIONS

Irrespective of the method used to introduce DNA into cells, the efficiency of transient or stable transfection is determined largely by the cell type that is used (see Table 1). Different lines of cultured cells vary by several orders of magnitude in their ability to take up and express exogenously added

DNA. In addition, a method that works well for one type of cultured cell may be useless for another. Many of the protocols described in this introduction have been optimized for the standard lines of cultured cells. When using more exotic lines of cells, it is important to compare the efficiencies of several different methods. Protocol: **Transfection Mediated by DEAE-Dextran** (Kumar et al. 2018d), Protocol: **DNA Transfection Mediated by Cationic Lipid Reagents** (Kumar et al. 2019e), Protocol: **Calcium-Phosphate-Mediated Transfection of Eukaryotic Cells with Plasmid DNAs** (Kumar et al. 2019f), Protocol: **Calcium-Phosphate-Mediated Transfection of Cells with High-Molecular-Weight Genomic DNA** (Kumar et al. 2019g), Protocol: **Calcium-Phosphate-Mediated Transfection of Adherent Cells or Cells Growing in Suspension: Variations on the Basic Method** (Kumar et al. 2019h), and Protocol: **DNA Transfection by Electroporation** (Kumar et al. 2019i) describe commonly used transfection techniques as well as methods that have been successful with cell lines that are resistant to transfection by standard techniques. Commercial kits are available that provide collections of reagents for many types of transfections.

ASSESSING CELL VIABILITY IN TRANSFECTED CELL LINES

In determining the best option for transfecting a particular cell line, it may be worthwhile to assess the effects of transfection on cell viability. Assessment of cellular health requires accurate quantitative and qualitative measurements over a period of time. Assays are available to measure a variety of different markers that indicate the number of dead cells (cytotoxicity assay), the number of live cells (viability assay), the total number of cells, or the mechanism of cell death (e.g., apoptosis vs. necrosis). The DNA content, the presence or absence of enzyme activity within the cell, the amount of ATP within cells, membrane integrity, and metabolic activity are all indicators of cellular integrity. Assays (e.g., ATP quantitation or LDH-release assays) that generate a measurable signal quickly enough to provide information representing a snapshot in time have the advantage of speed over assays that require several hours of incubation to develop a signal (e.g., MTT or resazurin). The latter are useful for measurements in unsynchronized and heterogeneous cell populations that respond over the course of several hours or days.

Detection sensitivity, a parameter of great significance when measuring the toxicity of drugs, is of limited concern when optimizing transfection reagents, because one measures a change in the viability of a large population of cells. In addition, assaying a large number of samples requires a high-throughput method that uses a simple, stable, nonradioactive reagent that is compatible with robotic handling. The method must also be relatively inexpensive, not labor intensive, and capable of determining the kinetics of cell growth in multiple cell types without interfering with basic cellular functions. It is for these reasons that when dealing with a large number of samples, the classical Trypan Blue exclusion test, used for estimating the number of dead cells, is less desirable than the many other easy-to-perform assays that provide an accurate quantitative and qualitative indicator of cell viability.

Protocol: **Analysis of Cell Viability by the alamarBlue Assay** (Kumar et al. 2018e), Protocol: **Analysis of Cell Viability by the Lactate Dehydrogenase Assay** (Kumar et al. 2018f), and Protocol: **Analysis of Cell Viability by the MTT Assay** (Kumar et al. 2018g) detail the use of three reagents: alamarBlue, lactate dehydrogenase (LDH), and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole], respectively. The alamarBlue and MTT assays measure cell viability directly, whereas the LDH assay measures cell death and is hence a cytotoxicity assay. In addition, although all three assays provide time-dependent quantitation of the cell death mechanism (i.e., apoptosis vs. necrosis), only the LDH assay can distinguish between the two mechanisms in single-time-point measurements. The LDH assay measures the release of a cytoplasmic enzyme, a process that occurs only when necrosis causes loss of cellular membrane integrity. Because all three assays can be adapted for high-throughput measurements, they have largely replaced older radioactive assays such as incorporation of [^3H] thymidine into cellular DNA.

ACKNOWLEDGMENTS

The authors thank Michelle Collins of Bio-Rad, Kevin Smoker of Life Technologies, and Daniela Bruell of Lonza Cologne AG for technical and editorial support.

REFERENCES

- Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, Northrop JP, Ringold GM, Danielsen M. 1987. Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci* **84**: 7413–7417.
- Felgner JH, Kumar R, Sridhar CN, Wheeler CJ, Tsai YJ, Border R, Ramsey P, Martin M, Felgner PL. 1994. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J Biol Chem* **269**: 2550–2561.
- Friend SH, Dryja TP, Weinberg RA. 1988. Oncogenes and tumor-suppressing genes. *N Engl J Med* **318**: 618–622.
- Gorman C, Padmanabhan R, Howard BH. 1983. High efficiency DNA-mediated transformation of primate cells. *Science* **221**: 551–553.
- Gossen M, Bujard H. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci* **89**: 5547–5551.
- Gossen M, Freundlieb S, Bender G, Müller G, Hillen W, Bujard H. 1995. Transcriptional activation by tetracyclines in mammalian cells. *Science* **268**: 1766–1769.
- Graham FL, van der Eb AJ. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**: 456–467.
- Greiner J, Wiehe J, Wiesneth M, Zwaka TP, Prill T, Schwarz K, Bienek-Ziolkowski M, Schmitt M, Döhner H, Hombach V, et al. 2004. Transient genetic labeling of human CD34 positive hematopoietic stem cells using nucleofection. *Transfus Med Hemother* **31**: 136–141.
- Johnson BD, Gershan JA, Natalia N, Zujewski H, Weber JJ, Yan X, Orentas RJ. 2005. Neuroblastoma cells transiently transfected to simultaneously express the co-stimulatory molecules CD54, CD80, CD86, and CD137L generate antitumor immunity in mice. *J Immunother* **28**: 449–460.
- Kumar P, Nagarajan A, Uchil PD. 2018a. DEAE-dextran transfection. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top096263.
- Kumar P, Nagarajan A, Uchil PD. 2018b. Optical transfection. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top096222.
- Kumar P, Nagarajan A, Uchil PD. 2018c. Selective agents for stable transfection. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top096230.
- Kumar P, Nagarajan A, Uchil PD. 2018d. Transfection mediated by DEAE-dextran. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095463.
- Kumar P, Nagarajan A, Uchil PD. 2018e. Analysis of cell viability by the alamarBlue assay. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095489.
- Kumar P, Nagarajan A, Uchil PD. 2018f. Analysis of cell viability by the lactate dehydrogenase assay. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095497.
- Kumar P, Nagarajan A, Uchil PD. 2018g. Analysis of cell viability by the MTT assay. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095505.
- Kumar P, Nagarajan A, Uchil PD. 2019a. Transfection of mammalian cells with calcium phosphate–DNA coprecipitates. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top096255.
- Kumar P, Nagarajan A, Uchil PD. 2019b. Lipofection. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top096248.
- Kumar P, Nagarajan A, Uchil PD. 2019c. Electroporation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top096271.
- Kumar P, Nagarajan A, Uchil PD. 2019d. Histochemical staining of cell monolayers for β -galactosidase. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095422.
- Kumar P, Nagarajan A, Uchil PD. 2019e. DNA transfection mediated by cationic lipid reagents. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095414.
- Kumar P, Nagarajan A, Uchil PD. 2019f. Calcium-phosphate-mediated transfection of eukaryotic cells with plasmid DNAs. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095430.
- Kumar P, Nagarajan A, Uchil PD. 2019g. Calcium-phosphate-mediated transfection of cells with high-molecular-weight genomic DNA. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095448.
- Kumar P, Nagarajan A, Uchil PD. 2019h. Calcium-phosphate-mediated transfection of adherent cells or cells growing in suspension: Variations on the basic method. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095455.
- Kumar P, Nagarajan A, Uchil PD. 2019i. DNA transfection by electroporation. *Cold Spring Harb Protoc* doi: 10.1101/pdb.top095471.
- Maitland NJ, McDougall JK. 1977. Biochemical transformation of mouse cells by fragments of herpes simplex DNA. *Cell* **11**: 233–241.
- Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH. 1982. Gene transfer into mouse lymphoma cells by electroporation in high electric fields. *EMBO J* **1**: 841–845.
- Perucho M, Wigler M. 1981. Linkage and expression of foreign DNA in cultured animal cells. *Cold Spring Harb Symp Quant Biol* **45**: 829–838.
- Shockett P, Difilippantonio M, Hellman N, Schatz D. 1995. A modified tetracycline-regulated system provides autoregulatory, inducible gene expression in cultured cells and transgenic mice. *Proc Natl Acad Sci* **92**: 6522–6526.
- Vaheri A, Pagano JS. 1965. Infectious poliovirus RNA: A sensitive method of assay. *Virology* **27**: 434–436.
- Weinberg RA. 1985. Oncogenes and the molecular basis of cancer. *Harvey Lect* **80**: 29–136.
- Wigler M, Pellicer A, Silverstein S, Axel R. 1978. Biochemical transfer of single-copy eukaryotic genes using total cellular DNA as a donor. *Cell* **14**: 725–731.
- Wigler M, Silverstein S, Lee LS, Pellicer A, Cheng VC, Axel R. 1977. Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. *Cell* **11**: 223–232.
- Zimmermann U. 1982. Electric field-mediated fusion and related electrical phenomena. *Biochim Biophys Acta* **694**: 227–277.

Cold Spring Harbor Protocols

Introducing Genes into Cultured Mammalian Cells

Priti Kumar, Arvindhan Nagarajan and Pradeep D. Uchil

Cold Spring Harb Protoc; doi: 10.1101/pdb.top095406 originally published online July 8, 2019

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

[Cell Biology, general](#) (1382 articles)
[DNA Delivery/Gene Transfer](#) (344 articles)
[DNA Delivery/Gene Transfer, general](#) (341 articles)
[Non-Viral Methods](#) (226 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>