PolyFect® Transfection Reagent Handbook

For optimized transfection of COS-7, NIH/3T3, HeLa, 293, and CHO cells

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QIAGEN

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QIAGEN Distributors

Please see the last page for contact information for your local QIAGEN distributor.

Contents

Kit Contents

1.0 ml of PolyFect Transfection Reagent (2 mg/ml), sufficient for 25–65 transfections in 60 mm dishes, or 50–100 transfections in 6-well plates.

Storage and Stability

PolyFect® Transfection Reagent is supplied as a ready-to-use solution and is shipped at ambient temperature without loss in stability. However, it should be stored at 2–8°C upon arrival. PolyFect Transfection Reagent is stable for 1 year at 2–8°C.

Quality Control

Endotoxin levels are ≤10 EU/ml determined using a Kinetic-QCL test (BioWhittaker, Inc.)

PolyFect Transfection Reagent is tested by transfection of pCMVβ (CLONTECH) into HeLa-S3 and COS-7 cells to ensure lot-to-lot consistency. Sterility tests guarantee absence of any contaminating bacteria or fungi.

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding PolyFect Transfection Reagent, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please call one of the QIAGEN Technical Service Departments or local distributors listed on the last page.

Introduction

PolyFect Transfection Reagent has been specifically developed and designed for transfection of commonly used cell lines. PolyFect Reagent protocols offer optimal conditions for transfection of COS-7, NIH/3T3, HeLa, 293, and CHO cells in three different plate formats. All protocols in this handbook have been optimized for each particular cell line, eliminating the need for optimization trials. In addition, the fast and easy protocols eliminate the need for removal of the complexes or a medium change after transfection. PolyFect Reagent combines excellent performance with a convenient transfection method.

PolyFect Transfection Reagent offers:

- Fast and easy protocol
- No removal of complex required
- Optimized protocols for COS-7, NIH/3T3, HeLa, 293, and CHO cells
- Transfection in the presence of serum
- Economical price
- High efficiency

The PolyFect Principle

PolyFect Transfection Reagent is an activated-dendrimer transfection reagent specifically designed for the transfection of commonly used cell lines. PolyFect Reagent offers significant advantages over many other transfection methods.

PolyFect Reagent possesses a defined spherical architecture, with branches radiating from a central core and terminating at charged amino groups. PolyFect Reagent assembles DNA into compact structures, optimizing the entry of DNA into the cell. PolyFect–DNA complexes possess a net positive charge, which allows them to bind to negatively charged receptors (e.g., sialylated glycoproteins) on the surface of eukaryotic cells. Once inside the cell, PolyFect Reagent buffers the lysosome after it has fused with the endosome, leading to pH inhibition of lysosomal nucleases. This ensures stability of PolyFect–DNA complexes and the transport of intact DNA into the nucleus.

NOTE: The amounts of DNA and PolyFect Reagent given in the protocols represent optimal conditions for transfection of the particular cell line. Therefore, we strongly recommend using the optimized amounts of DNA and PolyFect Reagent given in the appropriate protocol.

General Guidelines

Transfection efficiencies depend on a variety of parameters. The factors below should be taken into account when performing transfections.

Cell culture

A healthy cell culture lays the foundation for successful transfection. Different cells or cell lines have very specific medium, serum, and supplement requirements. Low passage number (<50 splitting cycles) ensures that the cell genotype has not become altered. Highest transfection efficiencies are obtained using the confluence levels indicated in the appropriate protocol sections. We recommend subculturing cells 24 h before transfection. This ensures normal cell metabolism and increases the likelihood of DNA uptake. Contamination with bacteria (e.g., mycoplasma) and fungi should be avoided, since this can drastically alter transfection results. Antibiotics can be included in the medium used for transfection with PolyFect Reagent and during subsequent incubation for gene expression.

Effect of serum

In contrast to many liposome transfection reagents, the presence of serum during transfection with PolyFect Reagent significantly increases transfection efficiencies. Therefore, we recommend using the percentage of serum that cells have been adapted to in culture during incubation of cells with transfection complexes. However, we do not recommend using serum during complex formation between PolyFect Reagent and plasmid DNA, as serum may inhibit complex formation.

Vector construct

The type of transfection vector (plasmid DNA, RNA, PCR products, oligonucleotides) influences the transfection results. The configuration and size of the constructs determine the efficiency of transfection. Transient transfection is most efficient with supercoiled plasmid DNA. In stable transfection, linear DNA yields optimal integration of the DNA into the host genome, but results in lower DNA uptake by the cells, relative to supercoiled DNA.

Plasmid DNA quality

Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility and toxicity, as well as interpretation of results. Therefore only plasmid DNA of the highest purity should be used. DNA purified with QIAGEN, QIAfilter™ and HiSpeed™ Plasmid Kits is well suited for transfection of most cell lines. For best results with all cell lines, we recommend DNA purified with the EndoFree® Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.

Ratio of PolyFect Reagent to DNA

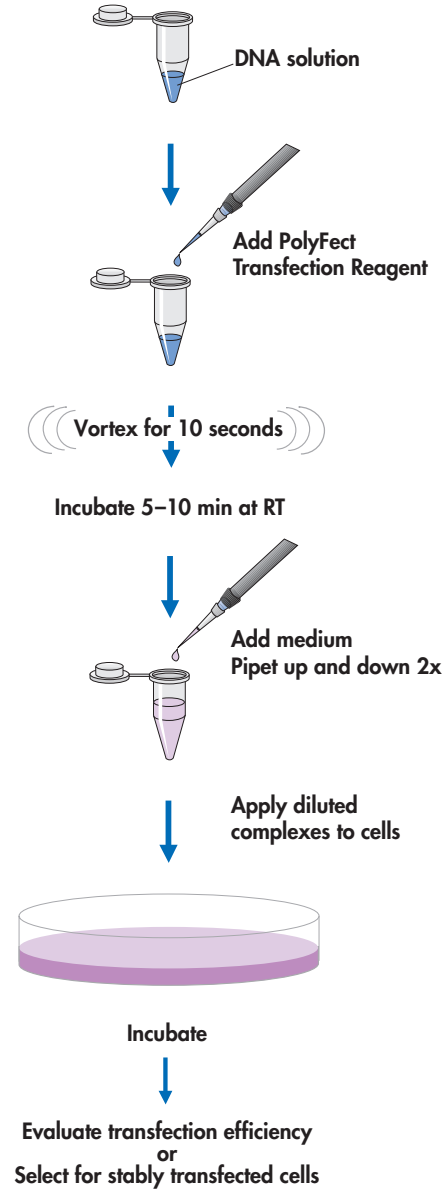
The overall charge of PolyFect–DNA complexes is determined by the ratio of PolyFect Reagent to DNA. Optimal binding of PolyFect–DNA complexes to the negatively charged groups (e.g., sialylated glycoproteins) on the cell surface requires a slightly positive net charge. To achieve an optimal ratio of PolyFect Reagent (µl) to DNA (µg), we strongly recommend using the optimized amounts of DNA and volumes of PolyFect Reagent indicated in the appropriate protocol.

Transfection of oligonucleotides or large vector constructs

For the transfection of oligonucleotides or large vector constructs (>20 kb), we recommend carrying out optimization trials to determine optimal amounts of DNA and PolyFect Reagent to be used. The amounts of DNA and PolyFect Reagent given in the protocols can be used as a starting point.

To determine the optimal amount of PolyFect Reagent, set up trial reactions using double and half the protocol-recommended amount, keeping the DNA amount as given in the protocol. In a second set of reactions, use double and half the protocol-recommended amount of DNA, keeping the PolyFect Reagent amount as given in the protocol. By following this optimization strategy you should be able to determine the optimal PolyFect Reagent/DNA ratio for your transfection.

Transfection Procedure for PolyFect Transfection Reagent



Protocol for Transient Transfection of COS-7, NIH/3T3, and CHO Cells

The following protocol is optimized for transient transfection of COS-7, NIH/3T3, and CHO cells in 60 mm dishes. Parameters for transfection using other culture formats are given in Table 1.

IMPORTANT: To ensure optimal results we strongly recommend using the optimized amounts of DNA and PolyFect Reagent given in the protocol and table below.

1. **The day before transfection, seed 8 x 10^5 cells per 60 mm dish in 5 ml of appropriate growth medium.**
2. **Incubate the cells at 37°C and 5% CO_2 in an incubator. The dishes should be 40–80% confluent on the day of transfection.**
3. **Dilute 2.5 µg of DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with cell growth medium containing no serum, proteins, or antibiotics to a total volume of 150 µl. Mix and spin down the solution for a few seconds to remove drops from the top of the tube.**

   IMPORTANT: Serum, proteins, and antibiotics present during this step interfere with complex formation and will significantly decrease transfection efficiency.

   Note: Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility and toxicity, as well as interpretation of results. Therefore only plasmid DNA of the highest purity should be used. DNA purified with QIAGEN, QIAfilter and HiSpeed Plasmid Kits is well suited for transfection of most cell lines. For best results with all cell lines, we recommend DNA purified with the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
4. **Add 15 µl of PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 sec.**

   Note: It is not necessary to keep PolyFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
5. **Incubate samples for 5–10 min at room temperature (20–25°C) to allow complex formation.**
6. **While complex formation takes place, gently aspirate the growth medium from the dish, wash cells once with 4 ml PBS, and add 3 ml of fresh cell growth medium (containing serum and antibiotics).**
7. **Add 1 ml of cell growth medium (containing serum and antibiotics) to the reaction tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately transfer the total volume to the cells in the 60 mm dishes. Gently swirl the dish to ensure uniform distribution of the complexes.**

   At this stage serum and antibiotics no longer interfere with transfection and will significantly enhance the transfection efficiency of PolyFect Reagent.

8. **Incubate cells with the complexes at 37°C and 5% CO_2 to allow for gene expression. Harvest cells and assay for reporter gene expression after an appropriate incubation time.**

   For example, cells transfected with *β-gal* or *cat* reporter constructs are typically incubated for 24–48 h after transfection to obtain maximal expression levels of the reporter gene.

Table 1. Parameters for transient transfection of COS-7, NIH/3T3, and CHO cells in different formats

Culture format	No. of cells to seed	Volume of medium‡ (ml)	DNA (µg)	Final volume of diluted DNA (µl)	Volume of PolyFect Reagent (µl)	Volume of medium to add to cells‡ (ml)	Volume of medium to add to complexes‡ (ml)
protocol step	**1**	**1**	**3**	**3**	**4**	**6**	**7**
6-well plate*	4×10^5	3.0	1.5	100	10	1.5	0.6
60 mm dish	8×10^5	5.0	2.5	150	15	3.0	1.0
100 mm dish†	1.6×10^6	8.0	4.0	300	25	7.0	1.0

** When working with 35 mm dishes use the amounts stated for 6-well plates.*

† When working with 85 or 90 mm dishes use the amounts stated for 100 mm dishes.

‡ Medium containing serum and antibiotics.

Protocol for Transient Transfection of HeLa Cells

The following protocol is optimized for transient transfection of HeLa cells in 60 mm dishes. Parameters for transfection using other culture formats are given in Table 2. **Please note that for HeLa-S3 cells the optimal amounts of DNA and PolyFect Reagent differ from the amounts given in the protocol below.** If you work with HeLa-S3 cells, please call one of the QIAGEN technical service departments (see inside front cover) or your local distributor (see inside back cover) for an optimized protocol.

IMPORTANT: To ensure optimal results we strongly recommend using the optimized amounts of DNA and PolyFect Reagent given in the protocol and table below.

1. **The day before transfection, seed 8 x 10^5 cells per 60 mm dish in 5 ml of appropriate growth medium.**
2. **Incubate the cells at 37°C and 5% CO_2 in an incubator. The dishes should be 40–80% confluent on the day of transfection.**
3. **Dilute 3 µg of DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with cell growth medium containing no serum, proteins, or antibiotics to a total volume of 150 µl. Mix and spin down the solution for a few seconds to remove drops from the top of the tube.**

   IMPORTANT: Serum, proteins, and antibiotics present during this step interfere with complex formation and will significantly decrease transfection efficiency.

   Note: Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility and toxicity, as well as interpretation of results. Therefore only plasmid DNA of the highest purity should be used. DNA purified with QIAGEN, QIAfilter and HiSpeed Plasmid Kits is well suited for transfection of most cell lines. For best results with all cell lines, we recommend DNA purified with the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
4. **Add 25 µl of PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 sec.**

   Note: It is not necessary to keep PolyFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
5. **Incubate samples for 5–10 min at room temperature (20–25°C) to allow complex formation.**
6. **While complex formation takes place, gently aspirate the growth medium from the dish, wash cells once with 4 ml PBS, and add 3 ml of fresh cell growth medium (containing serum and antibiotics).**

7. **Add 1 ml of cell growth medium (containing serum and antibiotics) to the reaction tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately transfer the total volume to the cells in the 60 mm dishes. Gently swirl the dish to ensure uniform distribution of the complexes.**

   At this stage serum and antibiotics no longer interfere with transfection and will significantly enhance the transfection efficiency of PolyFect Reagent.

8. **Incubate cells with the complexes at 37°C and 5% CO_2 to allow for gene expression. Harvest cells and assay for reporter gene expression after an appropriate incubation time.**

   For example, cells transfected with *β-gal* or *cat* reporter constructs are typically incubated for 24–48 h after transfection to obtain maximal expression levels of the reporter gene.

Table 2. Parameters for transient transfection of HeLa cells in different formats

Culture format	No. of to cells seed	Volume of medium‡ (ml)	DNA (µg)	Final volume of diluted DNA (µl)	Volume of PolyFect Reagent (µl)	Volume of medium to add to cells‡ (ml)	Volume of medium to add to complexes‡ (ml)
protocol step	**1**	**1**	**3**	**3**	**4**	**6**	**7**
6-well plate*	4×10^5	3.0	1.5	100	12	1.5	0.6
60 mm dish	8×10^5	5.0	3.0	150	25	3.0	1.0
100 mm dish†	1.6×10^6	8.0	6.0	300	50	7.0	1.0

** When working with 35 mm dishes use the amounts given for 6-well plates.*

† When working with 85 or 90 mm dishes use the amounts given for 100 mm dishes.

‡ Medium containing serum and antibiotics.

Protocol for Transient Transfection of 293 Cells

The following protocol is optimized for transient transfection of 293 cells in 60 mm dishes. Parameters for transfection using other culture formats are given in Table 3.

IMPORTANT: To ensure optimal results we strongly recommend using the optimized amounts of DNA and PolyFect Reagent given in the protocol and table below.

1. **The day before transfection, seed 1.2 x 10^6 cells per 60 mm dish in 5 ml of appropriate growth medium.**
2. **Incubate the cells at 37°C and 5% CO_2 in an incubator. The dishes should be 40–80% confluent on the day of transfection.**
3. **Dilute 4 µg of DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with cell growth medium containing no serum, proteins, or antibiotics to a total volume of 150 µl. Mix and spin down the solution for a few seconds to remove drops from the top of the tube.**

   IMPORTANT: Serum, proteins, and antibiotics present during this step interfere with complex formation and will significantly decrease transfection efficiency.

   Note: Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility and toxicity, as well as interpretation of results. Therefore only plasmid DNA of the highest purity should be used. DNA purified with QIAGEN, QIAfilter and HiSpeed Plasmid Kits is well suited for transfection of most cell lines. For best results with all cell lines, we recommend DNA purified with the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
4. **Add 40 µl of PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 sec.**

   Note: It is not necessary to keep PolyFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
5. **Incubate samples for 5–10 min at room temperature (20–25°C) to allow complex formation.**
6. **While complex formation takes place, gently aspirate the growth medium from the dish and add 3 ml of fresh cell growth medium (containing serum and antibiotics).**
7. **Add 1 ml of cell growth medium (containing serum and antibiotics) to the reaction tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately transfer the total volume to the cells in the 60 mm dishes. Gently swirl the dish to ensure uniform distribution of the complexes.**

   At this stage serum and antibiotics no longer interfere with transfection and will significantly enhance the transfection efficiency of PolyFect Reagent.

8. **Incubate cells with the complexes at 37°C and 5% CO_2 to allow for gene expression. Harvest cells and assay for reporter gene expression after an appropriate incubation time.**

   For example, cells transfected with *β-gal* or *cat* reporter constructs are typically incubated for 24–48 h after transfection to obtain maximal expression levels of the reporter gene.

Table 3. Parameters for transient transfection of 293 cells in different formats

Culture format	No. of cells to seed	Volume of medium‡ (ml)	DNA (µg)	Final volume of diluted DNA (µl)	Volume of PolyFect Reagent (µl)	Volume of medium to add to cells‡ (ml)	Volume of medium to add to complexes‡ (ml)
protocol step	**1**	**1**	**3**	**3**	**4**	**6**	**7**
6-well plate*	6×10^5	3.0	2.0	100	20	1.5	0.6
60 mm dish	1.2×10^6	5.0	4.0	150	40	3.0	1.0
100 mm dish†	2.4×10^6	8.0	8.0	300	80	7.0	1.0

** When working with 35 mm dishes use the amounts given for 6-well plates.*

† When working with 85 or 90 mm dishes use the amounts given for 100 mm dishes.

‡ Medium containing serum and antibiotics.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see last page for contact information).

Observation	Possible Cause	Comments and suggestions
Low transfection efficiency	PolyFect Reagent or DNA amount is suboptimal	The amounts of DNA and PolyFect Reagent given in the protocols represent optimal conditions for the transfection of the particular cell line. To ensure optimal results use the amounts of PolyFect Reagent and DNA given in the appropriate protocol. However, if you are working with oligonucleotides or large vector constructs (> 20 kb) please follow the guidelines given on page 8 under "Transfection of Oligonucleotides or large vector constructs".
	Incubation time for gene expression is suboptimal	Different cell types achieve maximal expression levels at different times post-transfection. This should be kept in mind when determining the length of incubation with the complexes. If the time point of maximal expression is not known for a particular cell line, a time course experiment may be necessary.
	Cell density at the time of PolyFect-DNA complex addition is too high	If the cell density is too high during complex addition cells may not be at the optimal phase of growth. This can lead to insufficient uptake of the complexes into the cells or to insufficient expression of the gene of interest. Be sure to use the confluence levels indicated in the appropriate protocol.
	Reporter assay problem	Include positive controls to ensure that the reporter assay is working properly.

Observation	Possible Cause	Comments and suggestions
	Poor DNA quality	Plasmid DNA quality strongly influences several transfection parameters such as efficiency, reproducibility and toxicity, as well as interpretation of results. Therefore only plasmid DNA of the highest purity should be used. DNA purified with QIAGEN, QIAfilter and HiSpeed Plasmid Kits is well suited for transfection of most cell lines. For best results with all cell lines, we recommend DNA purified with the EndoFree Plasmid Kit. This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
Excessive cell death	Amount of PolyFect Reagent or DNA is too high	The amounts of DNA and PolyFect Reagent given in the protocols represent optimized conditions for the transfection of the respective cell line. To ensure optimal results use the amounts of PolyFect Reagent and DNA given in the appropriate protocol.
	Cells are stressed	In general, avoid stressing cells with temperature shifts and long periods without medium during washing steps. We recommend performing transfection experiments in the presence of serum, so that cells are not deprived of necessary growth factors and nutrients.
	Vector related influences	Toxic effects may arise if a plasmid encoding a toxic protein is used or if too much plasmid with a high expression rate is used. Conversely, if insufficient plasmid with low expression rate is used, transfection efficiency may be too low. When performing transfection experiments, it is useful to perform controls. A known reporter construct can help determine if the promoter works in the target cell line. In addition, using a plasmid with no insert can help determine if the gene of interest is toxic.

Observation	Possible Cause	Comments and suggestions
Variable transfection efficiencies in replicate experiments	Inconsistent cell confluency in replicate experiments	Count cells prior to seeding to ensure that the same number of cells is seeded for each experiment. Keep incubation times between seeding and complex addition consistent between experiments.
	Possible mycoplasma contamination	Mycoplasma contamination influences transfection efficiency. Variations in the growth behavior of mycoplasma-infected cells will lead to different transfection efficiencies between replicate experiments.
	Cells have been passaged too many times	Cells that have been passaged for an extended number of times tend to change their growth behavior, morphology and transfectability. When cells with high passage numbers are used for replicate experiments, decreased transfection efficiencies may be observed in later experiments. We recommend using cells with low passage number (<50 splitting cycles).
	Serum variability	Variations in serum quality can lead to variations in transfection efficiency. In general, it is advisable to test a small lot of serum from a reputable supplier with a control cell line before performing transfection experiments. Once a given lot has yielded satisfactory and reproducible results, additional sera from the same lot should be purchased.

Appendix A: Composition of Buffers

Buffer	Composition	Storage
1x PBS (phosphate buffered saline)	137 mM NaCl 2.7 mM KCl 4.3 mM Na_2HPO_4 1.47 mM KH_2PO_4 Adjust to a final pH of 7.4	Room temperature
1x TE buffer, pH 7 to pH 8	10 mM Tris·Cl 1 mM EDTA Adjust to a pH between 7 and 8	Room temperature

Appendix B: Background Information

Transfection Principle

Transfection — delivery of foreign molecules such as DNA into eukaryotic cells — has become a powerful tool for the study and control of gene expression, for example in biochemical characterization, mutational analyses, or investigation of the effects of regulatory elements or cell growth behavior. Two principally different transfection techniques can be used; transient transfection and stable transfection. For further background information on transfection, please refer to current molecular biology manuals (1, 2).

Transient transfection

When cells are transiently transfected, the DNA is introduced into the nucleus of the cell, but does not integrate into the chromosome. This means that many copies of the gene of interest are present, leading to high levels of expressed protein. Transcription of the transfected gene can be analyzed within 24 to 96 hours after introduction of the DNA depending on the construct used. Transient transfection is most efficient when supercoiled plasmid DNA is used.

Stable transfection

With stable or permanent transfection, the transfected DNA is either integrated into the chromosomal DNA or maintained as an episome. Stable transfection involving integration of the DNA is most efficient when linearized plasmid DNA is used, since linearization facilitates recombination of the DNA with the host cell chromosome. Cells which have successfully integrated the DNA of interest or have maintained episomal plasmid DNA can be distinguished by using selectable markers. Frequently used selectable markers are the genes encoding aminoglycoside phosphotransferase (APH; *neo*^{R} gene) or hygromycin B phosphotransferase (HPH). Other selectable markers are the genes encoding adenosine deaminase (ADA), dihydrofolate reductase (DHFR), thymidine kinase (TK) or xanthine-guanine phosphoribosyl transferase (XGPRT; *gpt* gene).

Primary cells and cell lines

Depending on their origin, cell cultures or cell lines grow as an adherent monolayer or in suspension. Cells or cell lines vary greatly with respect to their growth behavior and nutritional requirements (1). Optimization of the cell culture technique is therefore necessary to ensure that cells are healthy and in optimal condition for transfection. For extensive information on culturing of cells, please refer to the manual *Culture of Animal Cells* (1).

Adherent cells

Adherent cells are anchorage-dependent and propagate as a monolayer attached to the culture vessel. This attachment is essential for proliferation. Most cells derived from tissues are anchorage-dependent with the exception of hemopoetic cells (cells derived from blood).

Suspension cells

Suspension cells are able to survive and proliferate without attachment. Hemopoetic cells, transformed cell lines, and cells from malignant tumors can be grown in suspension.

Primary cell cultures

Primary cell cultures arise from the outgrowth of migrating cells from a piece of tissue or by enzymatic, chemical, or mechanical dispersal of the tissue. Primary cell cultures are morphologically most similar to the parent tissue.

Finite cell lines

Finite cell lines are formed after the first subculturing (passaging) of a primary cell culture, and can be propagated and subcultured several times.

Continuous cell lines

There is a limit to the number of generations that a finite cell line can be propagated. After that it will either die out or acquire a stable, heritable alteration, giving rise to a continuous cell line. This alteration is commonly known as in vitro transformation or immortalization, and frequently correlates with tumorigenicity.

Transfection considerations

Media and supplements

Media are composed of a mixture of essential salts, nutrients, and buffering agents. Sterile media are usually purchased in solution. Alternatively, packaged premixed powders are available. Most media purchased are guaranteed to be mycoplasma- and endotoxin-free. Supplements to the media must include glutamine and can include nonessential amino acids, sodium pyruvate, and antibiotics. Some common media include DMEM, F12, DMEM/F12, RPMI 1640, MEM, and S-MEM.

Serum

In most cases media are supplemented shortly before use with serum. Fetal calf serum (FCS) is often used, but for some applications less expensive sera like horse- or calf serum can be used. Generally serum is a partially undefined material, which contains growth- and attachment factors and may show considerable variation in the ability to support growth of particular cells. Variations in the serum quality can also lead to variation in transfection efficiency. In general, it is advisable to test a small lot of serum from a reputable supplier with a control cell line and assay before performing transfection experiments. Once a given lot has been shown to yield satisfactory and reproducible results, additional sera from the same lot should be purchased.

Transfection methods

Of the variety of different transfection methods described in literature (2, 3), the DEAE-dextran method, the calcium-phosphate method, electroporation, and liposome-mediated transfection are the most commonly used. Each individual method has its characteristic advantages and disadvantages and the choice of transfection method strongly influences transfection results. PolyFect Transfection Reagent represents a transfection reagent based on activated-dendrimer technology, and has been designed to offer very high transfection efficiencies, good reproducibility, and reduced cytotoxic effects. In addition, optimized protocols for commonly used cell lines minimize optimization time.

Plasmid DNA quality

The quality of the plasmid DNA strongly influences the results of transfection experiments. Therefore only plasmid DNA of the highest quality, which is completely free of contaminating RNA, genomic DNA, and proteins, should be used. DNA purified with QIAGEN, QIAfilter and HiSpeed Plasmid Kits is ideally suited for transfection of most cell lines. For transfection of endotoxin sensitive cells, we recommend using DNA purified with the EndoFree Plasmid Kit. This kit efficiently removes bacterial lipopolysaccharide molecules during the plasmid purification procedure, ensuring optimal transfection results.

Genetic Reporter Systems

After cloning a gene of interest, transfection is a useful tool to determine how *cis*-acting sequences, such as promoters and enhancers, and *trans*-acting factors, such as transcription factors, act together to control eukaryotic gene expression. Common methods to monitor gene expression involve using techniques such as northern blot analysis or nuclease protection assays to quantitate the specific mRNAs transcribed from the gene of interest. Since these procedures are time-consuming and inconvenient for multiple samples (resulting from multiple constructs), an alternative approach is to link the presumed *cis*-acting sequences from the gene of interest to the coding sequence of an unrelated reporter gene (see examples below) (2, 3). The reporter gene provides an indirect way of measuring how such regulatory sequences influence gene expression. Reporter genes are also useful

in serving as controls. Transfection efficiencies between transfection experiments can be standardized by comparing the expression of the reporter gene product. Further information on genetic reporter systems can be obtained from current molecular biology manuals (2, 3). In choosing a suitable reporter system, several considerations should be taken into account. First, the reporter gene should be absent from the cells used in the study or easily distinguished from the native form of the gene. Second, the assay for the reporter gene product should be quick, easy, sensitive, and inexpensive. In particular, a broad linear range is important to enable detection of both small and large changes in reporter gene expression. Finally, the presence of the reporter gene should not affect the physiology of the cells being used.

Chloramphenicol acetyltransferase

The prokaryotic enzyme chloramphenicol acetyltransferase (CAT) is commonly used as a reporter. This enzyme catalyzes the transfer of acetyl groups from acetyl-coenzyme A to chloramphenicol. In the common CAT assay, cell lysates prepared from transfected cells are incubated with ^{14}C-labeled chloramphenicol. The resulting acetylated and unacetylated forms of chloramphenicol are separated by thin-layer chromatography. A qualitative estimate of CAT activity can be obtained simply by exposing the plates to X-ray film. For quantitative analysis, the separated bands can be scraped from the thin-layer plate and the levels of radioactivity measured in a scintillation counter. Currently, a CAT ELISA is also often used. In this assay the **total expression** of the chloramphenicol acetyltransferase is measured via antibody detection, in contrast to the classic CAT assay described above, which determines only the **active protein**.

Firefly luciferase

Luciferase catalyses a bioluminescent reaction involving the substrate luciferin, ATP, Mg^{2+}, and molecular oxygen. When these components are mixed with cell lysates containing luciferase, a flash of light is emitted. Light signals are detected using a luminometer or a liquid scintillation counter.

β-Galactosidase

The prokaryotic enzyme β-galactosidase can be assayed colorimetrically using the substrate o-nitrophenyl-β-D-galactopyranoside (ONPG). The hydrolysis of ONPG by β-galactosidase yields a yellow-colored product, o-nitrophenol, which can be measured photometrically.

Human growth hormone (hGH)

The assay for human growth hormone is based on immunological detection of hGH secreted by transfected cells. Specific ^{125}I-labeled antibodies against hGH are used and results are monitored in a scintillation counter. Currently, a sandwich-ELISA is also often used, which involves an antibody coupled ELISA plate. The hGH protein binds to the antibody on the plate, a digoxygenated antibody binds to hGH, and a secondary antibody coupled to alkaline phosphatase is used for detection.

Green fluorescent protein

Green fluorescent protein (GFP), originally isolated from the jellyfish *Aequorea victoria* (2), has the ability to absorb blue light and emit green light. This unique protein can be expressed in mammalian cells and protein expression can be visually monitored in living cells. Although the system provides a convenient way to detect protein expression without a specific assay, quantitative analysis is limited. This reporter gene system is best suited for *in situ* detection of gene expression, such as localization studies of fusion proteins within cells.

References

1. *Freshney, R.I.,* Culture of Animal Cells, A Manual of Basic Technique*, 3rd Ed., Wiley-Liss.*
2. *Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds. (1991)* Current Protocols in Molecular Biology*, New York: Wiley Interscience.*
3. *Sambrook, J., Fritsch, E.F., and Maniatis, T., eds. (1989)* Molecular cloning — a laboratory manual*, 2nd Ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.*

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