WHAT DO YOU WANT YOUR CELLS TO DO?
TRANSFECTION 101

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Changing the Code: What is Transfection?

The ability to manipulate the genetic composition of a cell has been an integral part of life sciences research for the past several decades. It has furthered our characterization of the relationship between both gene sequences and functions and between genes and proteins. It has allowed scientists to create new experimental models, explore cell signaling, and harness transcriptional and translational machinery to produce specific biological agents. Most importantly, it has helped shape our understanding of etiological factors underlying human pathogenesis and the therapeutic approaches that we must adopt to combat disease.

Transfection, the process of introducing foreign genetic material into a cell using non-viral methods, has been at the forefront of these advances. The success or failure of any transfection experiment depends on its ability to deliver its payload, intact, to a place where the cell can transcribe and/or translate the foreign genetic material. To accomplish this, in contrast to transduction, which uses the natural infectiousness of viral vectors to penetrate the cell and deliver a desired payload, transfection utilizes an array of different nonviral approaches to bring nucleic acids into contact with cellular machinery.

Unlocking the Genome: Bypassing Cellular Barriers

Nucleic acids are generally unable to cross phospholipid bilayer membranes unaided, and the uptake of naked nucleic acids is a relatively rare phenomenon in eukaryotic cells. Moreover, eukaryotic cells are protected from potential compromise of genomic integrity by extracellular enzymes and intracellular organelles (e.g., lysosomes, endosomes), both of which target unprotected nucleic acids for sequestering and degradation. Therefore, the main obstacles to successful transfection lie in overcoming these physical and chemical obstacles, and many transfection methods incorporate elements designed to bypass physical impediments while shielding nucleic acids from chemical threats. For example, lipofection involves the packaging of genetic material into lipid vesicles, allowing for uptake via endocytosis or integration with the cell membrane. Alternatively, electroporation and sonoporation use current and sound, respectively, to increase cell membrane permeability to a point where nucleic acids can pass through.

Transient or Stable: What’s the Difference?

Genetic material introduced during transfection can persist in the cell for variable lengths of time. In stable transfection, the delivered foreign nucleic acids integrate within the cellular genome, potentially persisting across generations and allowing researchers to generate a large population of transgene-possessing cells from the transfection of a smaller number. In transient transfections, no genomic integration takes place, meaning that transgene expression persists for a much shorter span of time. Additionally, cell division and environmental factors can prematurely result in the cessation of transgene expression. However, transient transfection allows for higher expression levels of the transgene during that initial window than stable transfection. Ultimately, the decision to use stable or transient transfection depends on the objective of the experiment.

Beyond DNA: Modulating Cellular Function using RNA

While DNA is arguably the most common type of genetic material delivered during transfection, RNA transfection has also proven to be useful in many circumstances. The introduction of small interfering RNA (siRNA), for example, can create a transient gene silencing or knock-down model, while messenger RNA (mRNA) transfection potentially offers better control over gene expression levels than DNA transfection. Since mRNA does not require access to the nucleus, its introduction can result in expression much more rapidly relative to DNA transfection. mRNA has proven suitable for targeted subcellular transfection, where nucleic acids are introduced only to specific domains within a cell, offering researchers a way to examine the effects of localized gene expression modulation, especially in cells known to have specialized regions with unique functions such as neurons.

Transfection and CRISPR: Guiding the Future

The discovery and adaptation of the CRISPR-Cas system (CRISPR-Cas9 in particular) for genome alteration and genetic engineering has transformed biomedical research, and CRISPR-Cas-mediated gene editing has already proven its utility for creating new experimental models and for treating pathogenic mutations in translational studies. Transfection, in turn, has proven essential for the delivery of CRISPR-Cas system-associated genetic material (and even proteins) to eukaryotic cells, which naturally lack the components of a mechanism first discovered as a prokaryotic immune mechanism. As scientists use CRISPR-mediated gene editing on a greater and more diverse range of cell types, transfection methods too will need to adapt for the efficient delivery of CRISPR machinery to these cells.

For references, please see page 7.
Breaking Through: Selecting a Transfection Method

The cell membrane is generally impervious to nucleic acids. As such, additional steps need to be taken to bring genetic material into the cell (and possibly the nucleus) for transfection. Many different transfection methods, incorporating distinct mechanisms of action, have been devised, each with its own pros and cons.

**Electroporation**
- Uses an electric field to destabilize the cell membrane, increasing permeability
- Applicable to a wide range of cell types; useful for cells resistant to other transfection methods
- Can deleteriously impact cell health and viability

**Lipofection**
- Conjugates genetic material to liposomes to facilitate easy passage through phospholipid bilayers
- High efficiency; applicable to high-throughput systems
- Not applicable to all cell types; susceptible to interference from culture media components

**Sonoporation**
- Uses ultrasound to form pores in the cell membrane
- Non-invasive nature makes it useful for in vivo applications; can complement and augment other transfection techniques
- Lower gene transfer efficiency; can damage cells

**Chemical Transfection**
- Conjugates nucleic acids with cationic polymers or calcium phosphate, facilitating uptake by endocytosis or phagocytosis
- Simple, flexible, and inexpensive
- Highly susceptible to changes in reagent composition, pH, and nucleic acid quality

References
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The Right Tools for the Job? Transfection Reagents and Cell Types

Transfection efficiency is determined by a great number of variables pertaining to the cell type, the method, the desired genetic modification, or a combination thereof. As such, there is no one-size-fits-all solution or protocol when it comes to transfection. For the scientist, this begs the question: how can I improve the odds of successful transfection while maximizing my transfection efficiency?

Reagent Choices Matter!

Don't dismiss the importance of the reagents used during the transfection process as immutable features of your chosen method. While transfection method does play a leading role in dictating reagent selection, it is only one factor out of many. When the performance of two synthetic and two natural transfection reagents used in the lipofection of primary mesenchymal cells was evaluated, differences were observed in not only efficiency, but also cell viability, along with a myriad of other non-specific effects.¹

Unsurprisingly, given that different cell types possess different ideal culture conditions, growth characteristics, and nutrient requirements, they respond differently to the same transfection reagent. For example, a study that examined the capabilities of various lipid-based transfection reagents in three different cell lines found that none of the reagents demonstrated similar efficiencies across all three cell lines, and that one reagent demonstrated considerably different efficiencies across all three cell lines.²

A Fine Touch: Minimizing Cellular Damage and Death

How a cell responds to treatment with a transfection reagent plays a significant role in whether that reagent can be used or not. Researchers need to remember that transfection protocols are intended to compromise the cell membrane and thus transfection reagents are cytotoxic to varying degrees—the extent of which depends on the susceptibility of the cell type in question, the baseline toxicity of the reagent itself, and the concentration employed. In general, reagent concentrations are positively correlated with cytotoxicity, although exceptions do exist.³ Therefore, researchers must carefully balance the desire to maximize transfection efficiency with the desire to minimize cellular disruptions and death.

A Different Reagent for a Different Nucleic Acid

The type of genetic material being transfected plays a role in determining reagent selection. Efficient RNA delivery presents different challenges than does DNA delivery, and therefore requires a different array of transfection reagents. Indeed, specific individual reagents have been developed for the optimized transfection of mRNA, siRNA, and short hairpin RNA (shRNA). Nucleic acid quality also plays a role in determining reagent performance, especially in terms of affecting the relationship between concentration and efficiency.

Opening the Path to the Future with the Guidance of the Past

Unfortunately, it’s difficult to determine the best reagents, and their optimal concentrations, for any given transfection experiment without a degree of trial and error. Fortunately, researchers today are able to refer to a treasure trove of reference material on the subject. While no singular prior study or reference sheet will perfectly mimic a researcher’s individual circumstances in their own laboratory, the collective experiences of both the academic and industry sectors provide scientists with a firm understanding of the factors impacting transfection efficiency, as well as a logical starting point from which a shortlist of potential reagents can be selected for further optimization. In particular, reference materials from reagent manufacturers offer a comprehensiveness and scale in terms of experimental combinations attempted that academic studies are hard pressed to match.

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Optimizing Transfection: Tips and Tricks

Despite its relatively long history, transfection can still be challenging due to the sheer volume of options and variables facing researchers. Instead of hunting for universal tips and tricks, it's better to identify potential trouble points. This is best accomplished using an understanding of the mechanisms underlying the respective transfection methods available, as well as the properties, conditions, and variables that can affect the transfection process.

Your Lab, Your Conditions, Your Protocol

While it is well-advised to use prior experiences—whether personal or found in the literature—when designing a transfection protocol, it is vital to adapt any protocol to the parameters of your own laboratory and experiment. For example, cell culture conditions can both directly and indirectly affect transfection efficiency. The size and shape of the containers housing the cells being transfected directly affects the optimal concentrations for transfection reagents, as well as the optimal genetic material:transfection reagent ratio. The composition of the culture media—and, in particular, the presence of serum and/or antibiotics—inhibit the functionality of some transfection reagents. Finally, cell density and confluence not only directly affect reagent and nucleic acid volume requirements, but can indirectly impact transfection efficiency by modulating cellular function and health. Overcrowding can result in contact inhibition, which inhibits cell division and affects cellular metabolism. Conversely, undercrowding can lead to phenotypic alterations and reduced cell growth due to a lack of cell-cell interaction.

Back to the Start: The Importance of Nucleic Acid Quality

Another way to increase the odds of transfection success is to improve the quality of the nucleic acids being used. Low quality nucleic acids have a lower chance of properly integrating within the cellular genome, reducing transgene presence during selection and lowering the efficiency of stable transfections. Even if the nucleic acids are processed by cellular transcriptional/translational machinery, damaged sequences can result in truncated, misfolded, or otherwise altered proteins that do not function as intended. If cell transfection attempts are not yielding expected results, making adjustments to the nucleic acid extraction and purification protocol may remedy the situation. It must also be noted that nucleic acid quality can be impacted post-extraction, as care must be taken that the transfection payload is not exposed to DNases or RNases present in the culture media or transfection reagents.

Balancing Cellular Health with Transfection Efficiency

In every transfection experiment, the need to deliver genetic material should be balanced with the fact that transfection methods disrupt cellular homeostasis to varying degrees and can be deleterious to cell health. In particular, methods that modulate membrane integrity such as electroporation are generally more cytotoxic than methods that do not such as lipofection. While the ultimate goal is always to limit cytotoxicity as much as possible, the amount of cytotoxicity that a scientist is willing to tolerate depends on their research aims. Whereas the use of transient transfection to generate a receptor overexpression cellular model for immediate calcium imaging may prioritize gene translation and protein abundance over cell health and survival, the creation of a stable transgenic cell line necessitates that transfected cells survive in good health.

Controlling for Error

Finally, the use of controls during the transfection process is critical for every researcher. Having the right controls for individual stages of transfection not only allows the scientist to separate intended effects from non-specific effects, but can also isolate the specific step and/or procedure resulting in the unintended effect. Researchers should use positive controls (e.g., housekeeping genes) to examine the functionality and efficiency of their transfection conditions, negative controls to examine the effects of introducing foreign genetic material into the cell and/or genome, mock transfections containing no genetic material to examine the effects of the process itself, and an untreated control to examine whether any observed effects may stem simply from culturing the cells.

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X-tremeGENE™ transfection reagents benefits include:

- Efficient delivery of siRNA/miRNA, plasmid DNA, and CRISPR/Cas9 materials
- Transient and stable transfection that is animal-derived component free
- Suitable for eukaryotic, insect, and hard-to-transfect cell lines, including primary and tumor cells

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