Transfection Technologies

The introduction of nucleic acids into cells is one of the most valuable and frequently used tools of biological science. Transfection methods are used for a range of applications, including gene function studies, modulation of gene expression, biochemical mapping, mutational analysis and production of recombinant proteins. The transfection techniques which are commonly used today can be classified into three groups:

1. Methods that make use of genetically engineered viruses
2. Chemical methods or methods that rely on carrier molecules
3. Physical methods or methods that deliver nucleic acids directly to the cytoplasm

Not all transfection methods can be applied to all types of cells or experiments, and there is wide variation with respect to the achieved transfection efficiency, viability, level of gene expression etc. Determination of the best method for a specific application depends on several factors like cell type (primary cells or cell line), cellular context (in vitro, in vivo, ex vivo), transgene capacity, general safety, desired efficiency, cost, time etc.

Viral Gene Transfer

Particular viruses have been selected as gene delivery vehicles because of their capacities to carry foreign genes and their ability to efficiently deliver these genes associated with efficient gene expression.

Principle

- Generation of recombinant virus containing the transgene via gene cloning
- Amplification of viral particles in packaging cell line and virus isolation
- Purification and titration of viral particles
- Infection of cell type of interest
- Depending on the virus, integration of transgene in host genome

In general, the achieved transfection efficiencies in primary cells and cell lines are high. However, you have to keep in mind that you can only transfect cell types carrying a viral specific receptor. The first step in the infection cycle of a virus is the interaction between the virus and a cellular receptor on the surface of a target cell, resulting in the fusion of the viral...
and the cellular membrane. Cells which are not carrying such a receptor cannot be infected by the virus. Furthermore, you have to consider that the generation of recombinant viruses requires DNA transfection of cultured cells (packaging cells) by one of the non-viral transfection methods described below.

Other limitations of viral gene transfer are the time consuming and laborious production of vectors, elevated laboratory costs due to higher biosafety level requirements, limitation of insert size (~10 kb for most viral vectors versus ~100 kb for non-viral vectors), variability in infection potencies of the generated virus particle preparations and possible immunogenic reaction in animal or clinical trials. If you want to perform co-transfections using viral vectors, you can possibly insert two genes in a double expression cassette of the viral transfer vector. But you have to consider that the maximum insertion size is limited. Alternatively, using different recombinant viruses each expressing a different protein, a co-infection of the desired cell lines can be performed. However, this is very time consuming and laborious.

Well-known examples for viral gene transfer vectors are recombinant adenoviruses, retroviruses, adenoassociated viruses, herpes simplex viruses and vaccinia viruses.

**Adenoviruses** have a broad cell tropism and can infect both dividing and non-dividing cells. Exceptions are some lymphoid cells, which are more resistant to adenoviral infection than other cell types. The packaging capacity of adenoviral vectors is 7 to 8 kb. Unlike retroviruses, adenoviruses allow production of $10^{10}$ to $10^{11}$ viral particles/ml which can be concentrated up to $10^{13}$ viral particles/ml. One disadvantage of adenoviral vectors is their episomal status in the host cell, allowing only transient expression of the transgene. This also means that adenoviruses do not interfere with the host genome. Furthermore, expression of adenoviral proteins like E2 provokes inflammatory reactions and toxicity that limit the repeated application of adenoviral vectors for gene therapy.

**Retroviruses** make excellent gene therapy vectors because they have the ability to integrate their genome into a host cell genome, thus enabling stable expression of the transgene. Most retroviruses are limited by the requirement of replicating cells for infection. An exception are the lentiviruses (subgroup of retroviruses), which have the ability to infect and integrate into non-dividing cells. Based on this feature, the use of lentiviral-based vectors could be of great value for gene delivery to tissues of non-dividing, terminally differentiated cell populations, such as neuronal tissue, hematopoietic cells, myofibers etc.

Like adenoviruses, retroviruses can carry foreign genes of around 8 kb. Among the disadvantages are the instability of some retroviral vectors and possible insertional mutagenesis by random integration into the host DNA.

**Adenoassociated viruses** need helper viruses like adenovirus or herpes virus for lytic infection. This causes difficulties in obtaining high quality viral stocks free of helper viruses. Moreover, the adenoassociated viruses have only limited capacity for insertion of foreign genes ranging up to 4.9 kb. Wildtype viruses have the ability to integrate into a specific region of the human chromosome, thus avoiding insertional mutagenesis. Another advantage is the low immunogenicity of adenoassociated viruses, which is important for the application in human gene therapy. It has been shown that recombinant adenoassociated vectors are suitable for in vitro and in vivo gene transfer into e.g., muscle, brain, hematopoietic cells, neurons and liver cells.

Besides retroviruses, adenoviruses and adenoassociated viruses, herpes simplex viruses and vaccinia viruses are frequently used for viral gene transfer. They have the ability to carry large inserts up to 50 kb. Herpes simplex viruses have been used for gene transfer into neurons, brain tumors, various tumor cells and B cells. One disadvantage of herpes simplex viruses is that they may become latent in neural cells and that there is so far little information of the fate or stability of the vector. On the other hand, latency may be an advantage for stable gene expression in chronic diseases.

### A Comparison of Different Viral Systems

<table>
<thead>
<tr>
<th>Viral Vector</th>
<th>Size</th>
<th>DNA/Insert Size</th>
<th>Maximum Titer (partic. ml⁻¹)</th>
<th>Infection</th>
<th>Expression</th>
<th>Potential Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovirus</td>
<td>7–11 kb (ssRNA)</td>
<td>8 kb</td>
<td>$1 \times 10^7$</td>
<td>Dividing cells</td>
<td>Stable</td>
<td>Insertional mutagenesis</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>8 kb (ssRNA)</td>
<td>9 kb</td>
<td>$1 \times 10^7$</td>
<td>Dividing and non-dividing cells</td>
<td>Stable</td>
<td>Insertional mutagenesis</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>36 kb (dsDNA)</td>
<td>8 kb</td>
<td>$1 \times 10^{13}$</td>
<td>Dividing and non-dividing cells</td>
<td>Transient</td>
<td>Strong antiviral immune response limits repeat administration</td>
</tr>
<tr>
<td>Adenoassociated Virus (AAV)</td>
<td>8.5 kb (ssDNA)</td>
<td>5 kb</td>
<td>$1 \times 10^{11}$</td>
<td>Dividing and non-dividing cells</td>
<td>Stable: integration in one spot of host genome</td>
<td>Helper virus required for replication. Difficult to produce pure stocks of AAV free of helper virus</td>
</tr>
<tr>
<td>Herpes Simplex Virus</td>
<td>150 kb (dsDNA)</td>
<td>30 – 40 kb</td>
<td>$1 \times 10^7$</td>
<td>Dividing and non-dividing cells</td>
<td>Transient</td>
<td>Lack of gene transcription after latent infection</td>
</tr>
<tr>
<td>Vacciniairus</td>
<td>190 kb (dsDNA)</td>
<td>25 kb</td>
<td></td>
<td>Dividing cells</td>
<td>Transient</td>
<td>Potential cytopathic effects</td>
</tr>
</tbody>
</table>
Chemical Methods of Gene Delivery

Chemical methods are transfection techniques that make use of carrier molecules to overcome the cell-membrane barrier. Different from what the name implicates, there are no chemical reactions taking place between the carrier molecule and the nucleic acid or any cellular component. The principle consists of the interaction of negatively charged nucleic acids with positively charged carrier molecules, like polymers or lipids, enabling the nucleic acid to come into contact with the negatively charged membrane components and incorporating the gene into the cell by endocytosis and later releasing it into the cytoplasm.

DEAE (Diethylaminoethyl)-dextran
DEAE-dextran was the first non-viral transfection method verified by Vaheri and Pagano in 1965.

Principle
- DNA is mixed with DEAE-dextran (polycationic derivative of dextran, a carbohydrate polymer)
- DNA/polymer complex comes into contact with negatively charged membrane due to excess of positive charge contributed by polymer
- Uptake presumably by endocytosis
Among the advantages of the DEAE-dextran transfection method are its simplicity and low costs. A major drawback is that the achieved transfection efficiency is low for a range of cell types. Additionally, this method is not suitable to generate stable lines and cytotoxicity must be considered.

Calcium-Phosphate
This transfection method has been verified by Graham and van der Eb in 19731.

Principle
- DNA is mixed with calcium chloride
- Addition to buffered saline/phosphate solution and incubating
- at room temperature
- Formation of DNA-calcium phosphate coprecipitates which adhere to surface of cells
- Uptake presumably by endocytosis
Calcium phosphate co-precipitation is widely used because the components are easily available and reasonable in price. Another advantage is its applicability to generate stably-transfected cell lines, allowing for long-term gene expression studies. The disadvantages include its toxicity, especially to primary cells, and its sensitivity to slight changes in pH, temperature and buffer salt concentrations, as well as its relatively poor transfection efficiency compared to other chemical transfection methods like lipofection. Like all other methods achieving delivery into the cytoplasm, expression is dependent on cell division after transfection.

Lipofection
Among the lipofection transfection reagents, you can distinguish between three generations. The first generation is comprised of cationic liposomal reagents, and the second generation multicomponent liposomal reagents, consisting of lipids, polymers and combinations thereof. The third generation consists of multicomponent liposomal reagents conjugated with antibodies or ligands which enable a specific targeting.

Lipofection is the most commonly used chemical gene transfer method. Cationic transfection lipids consist of a positively charged head group, such as an amine, a flexible linker group such as an ester or ether, and two or more hydrophobic tail groups.

Principle (Liposome Mediated Gene Transfer)
- A cationic lipid is mixed with a neutral lipid/helper lipid (e.g., DOPE) and unilamellar liposome vesicles are formed carrying a net positive charge
- Nucleic acids adsorb to these vesicles/packed structure
- Ionic absorption to the cellular membrane occurs
- Uptake presumably by endocytosis
- Neutral “helper” lipids, such as DOPE, allow entrapped DNA to escape the endosomes by fusion of the liposome with the membrane
The main advantages of cationic lipid transfection reagents are their ability to transfect a wide range of cell types (mainly adherent cell lines) with high efficiency, and their relatively low costs. Additionally, lipofection offers advantages like the successful delivery of DNA of all sizes, delivery of RNA and protein, as well as the applicability to use this technique for both transient and stable protein production. Despite these advantages, there are several drawbacks, including low efficiencies in most primary cells, as well as suspension cell lines, linked to the dependence on endocytotic activity, its cytotoxicity and its dependence on cell division. The second generation makes use of non-liposomal lipids and polymers which complex with DNA or RNA and form micelles. The reaction is usually performed under aqueous conditions to allow the lipophilic sections of the original amphiphilic compound to form the micelle core. Dendrimers are three-dimensional globular macromolecules that are capable of condensing DNA in small complexes, and therefore increase plasmid transfection efficiency. Dendrimers are typically stable in serum and not temperature sensitive, providing high transfection efficiency in several tissue culture models. However, dendrimers are also non-biodegradable and may cause significant cytotoxicity.
Physical Methods of Gene Delivery

Physical methods enable the direct transfer of nucleic acids into the cytoplasm, or nucleus by physical or mechanical means and without the usage of foreign substances like lipids.

Microinjection

This physical method is mainly used for manipulation of single cells, such as oocytes, by injection of DNA, mRNA, and proteins. It can also be used for the transfer of DNA into embryonic stem cells to generate transgenic organisms.

**Principle**

- Using a micromanipulator and microscope, a very fine tipped pipet is inserted into the cytoplasm or directly into the nucleus.

A major advantage is the high efficiency of this method (nearly 100%). However, the method is not appropriate to transfect a large number of cells and the method requires certain operator skills. Microinjection is also very timeconsuming and expensive.

Biolistic Particle Delivery

This method has been successfully employed to deliver nucleic acid to cultured cells, as well as to cells in vivo. It is mainly used for genetic vaccination and agriculture application, where cells on the surface of whole organs can be transfected.

**Principle**

- Transfer of DNA that is coated on the surface of microparticles such as gold or tungsten
- Particles are accelerated by a particular driving force, e.g., by establishing a high voltage discharge between two electrodes or gas pressure

This technique is fast and simple and enables transfection of dividing and non-dividing cells. Also, there appears to be no limit to the size or number of genes that can be delivered. However, the mortality is very high and therefore you need high cell numbers.

Electroporation

Electroporation is a frequently used physical gene transfer method.

**Principle**

- Cells and DNA are suspended in an electroporation buffer
- High voltage pulses of electricity are applied to the cells
- Electrical pulse creates a potential difference across the membrane, as well as charged membrane components, and induces temporary pores in the cell membrane for DNA entry

It is possible to transfect large DNA fragments and the efficiencies achieved in cell lines are good. Unlike liposomal reagents, there is no reagent-induced cytotoxicity towards the cells. Drawbacks are low efficiency in primary cells and high mortality rates, caused by the high voltage pulses or only partially successful membrane repair. The technique requires fine-tuning and optimization for duration and strength of the pulse for each type of cell used. As a consequence of the compromise between efficiency and mortality, usually 50% of the cells are lost.

Nucleofector™ Technology

Lonza offers the Nucleofector™ Technology, which is a highly efficient, non-viral method for transfection of difficult-to-transfect cell lines and primary cells. It is based on two components: The Nucleofector™ Device that delivers unique electrical parameters, and Nucleofector™ Kits which contain cell type-specific Nucleofector™ Solutions, Cuvettes and Pipettes. With Nucleofection™ Technology the DNA is transported directly into the nucleus of the target cell.

With other substrates, such as siRNA or miRNA, Nucleofector™ Technology also offers highly efficient and robust transfection with high cell viability. The combined effect of electrical parameters and cell type-specific solutions of the Nucleofector™ Technology leads to better performance than other commercially available electroporation devices. Each electrical setting is displayed as a distinct program which has been adapted to the requirements of a particular cell type. As many electrical settings are pre-programmed into the Nucleofector™ Device, optimization of the electrical parameters by the user is not necessary.

Please see our cell database for Optimized Protocols and data on more than 500 cell types: [www.lonza.com/cell-database](http://www.lonza.com/cell-database).
## Comparison of Different Gene Transfer Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>No Immunogenicity</th>
<th>No Cytotoxicity</th>
<th>Carrying Large Inserts</th>
<th>Higher Biosafety Level Required</th>
<th>Simple Handling</th>
<th>One Protocol for DNA, siRNA, mRNA etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleofection™</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Viral Transfection</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipofection</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
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<tr>
<td>DEAE-dextran</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Calciumphosphate</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
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<tr>
<td>Electroporation</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Microinjection</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Biolistic Particle Delivery</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>High Efficiency in Primary Cells</th>
<th>High Efficiency in Adherent Cell Lines</th>
<th>High Efficiency in Suspension Cell Lines</th>
<th>Fast</th>
<th>High Viability</th>
<th>Costs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleofection™</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>moderate</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Lipofection</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>low</td>
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<tr>
<td>DEAE-dextran</td>
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<td>+/-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>low</td>
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<tr>
<td>Calciumphosphate</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>low</td>
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<tr>
<td>Electroporation</td>
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<td>-</td>
<td>+</td>
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<td>moderate</td>
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<td>Biolistic Particle Delivery</td>
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## References
