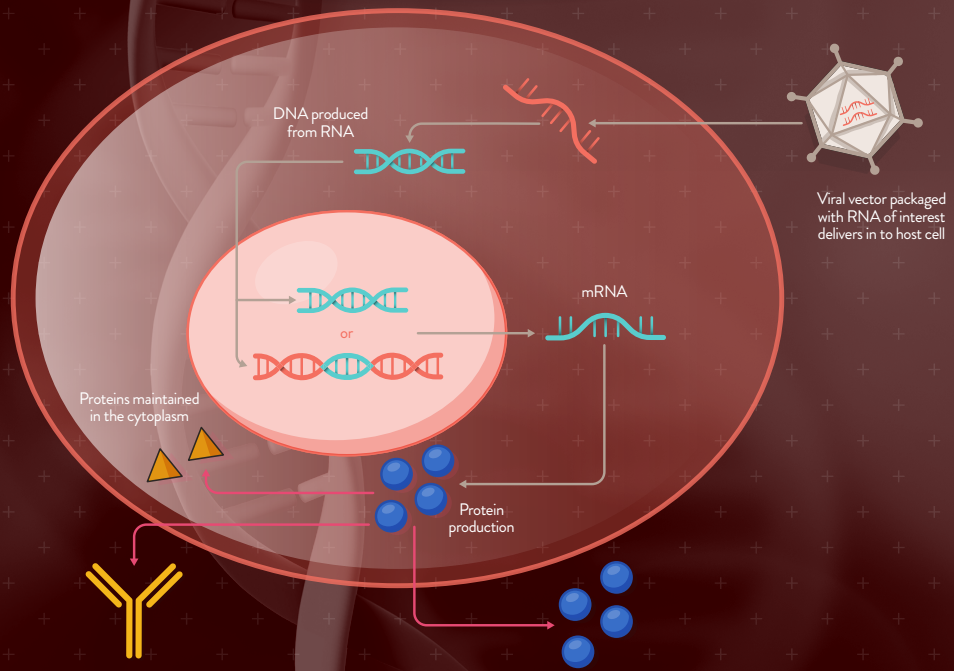
PACKAGE DELIVERY:
The Art of Transfection

Inserting genetic material into mammalian and insect cells without killing them can be a challenge, but scientists have developed several ways to perform this intricate task. Transfection is the process of introducing nucleic acids (plasmid DNA or messenger, short interfering, or micro RNA) into a cell. Researchers accomplish this with nonviral methods (chemical or physical transfection), or with viral methods, commonly referred to as transduction.

Researchers use chemical and physical transfection and viral transduction to explore gene expression and screening, for bioproduction of proteins and viruses, or for therapeutic purposes such as gene therapy. Successful nucleic acid delivery depends on the quality of DNA, ratio of DNA to a chemical reagent, cell passage number and confluency, and post-transfection incubation time. Non-viral transfection is commonly used for adherent immortalized cells, while transduction is often employed for the most difficult cell types, including primary, stem and immune cells. Transfection sometimes improves outcomes when using large DNA inserts because viral vectors have an insert limit of ~4.5 kb (adenoviral-associated vectors) to ~10 kb (lentiviral vectors).

TRANSDUCTION

Transduction is the process of using vectors including retroviruses, lentiviruses, adenoviruses, adeno-associated viruses, or hybrids to deliver genetic payloads into cells. Generally, a plasmid carrying genes flanked by viral sequences is first transfected into a producer cell with other virus-associated (packaging) plasmids. In the producer cells, virions form that contain the gene of interest. For safety, no plasmid used in the process contains all of the necessary sequences for virion formation, and only the plasmid carrying the gene of interest contains signals that allow it to be packaged into virions. Researchers then extract, purify, and use the virions from the producer cells to insert DNA into other cells to stably or transiently express the DNA of interest. The transferred genetic material, which lacks viral genes, cannot generate new viruses.



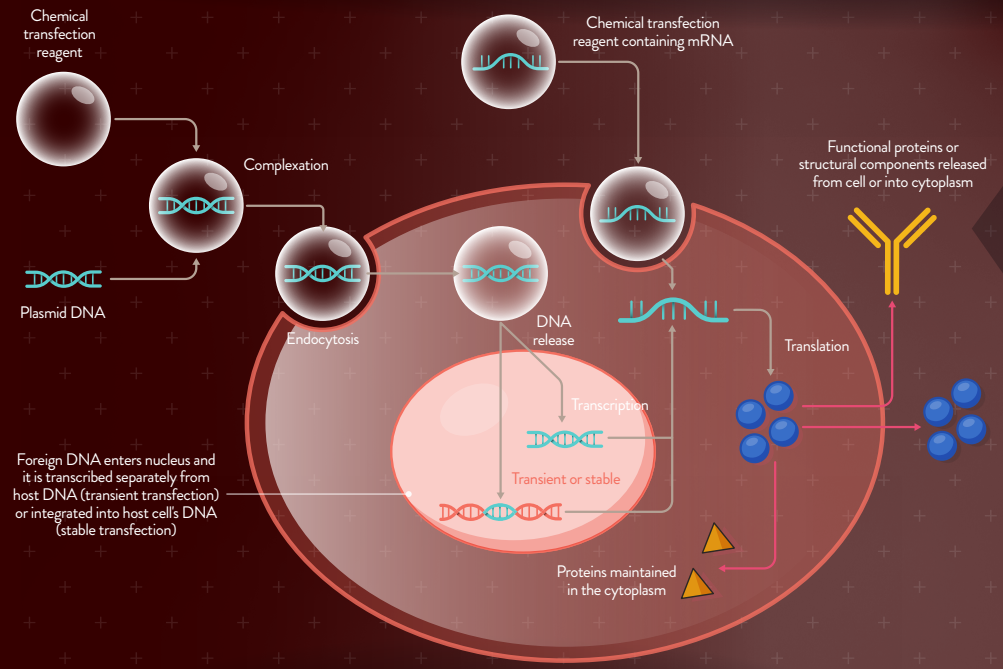
TRANSIENT VS. STABLE TRANSFECTION

DNA transfection can be classed as stable—where the foreign gene integrates into the host genome—or transient—where the gene does not integrate into the genome. Transfection leads to transient or stable expression of DNA in cells, depending on the method or the viral tool used. Transfection of RNA, however, is always transient. Whether a scientist uses stable or transient transfection depends on the desired experimental outcome.

TRANSIENT	STABLE
Foreign DNA does not integrate into the host genome	Foreign DNA integrates into the host genome
Short-term expression	Long-term expression
Usually chemical or electroporation-based	Usually involves viral vectors or targeted endonucleases
No potential for non-specific integration	Potential for non-specific integration
Useful for manipulating specific gene activity in cell culture	Useful for gain- or loss-of-function studies
GFP or other co-expressed markers can be used to check for successful transfection/transduction	Co-expressed selection markers can be used to produce a selectable advantage, or lentiviral vectors can insert DNA randomly into the host genome

Mirus

NON-VIRAL TRANSFECTION

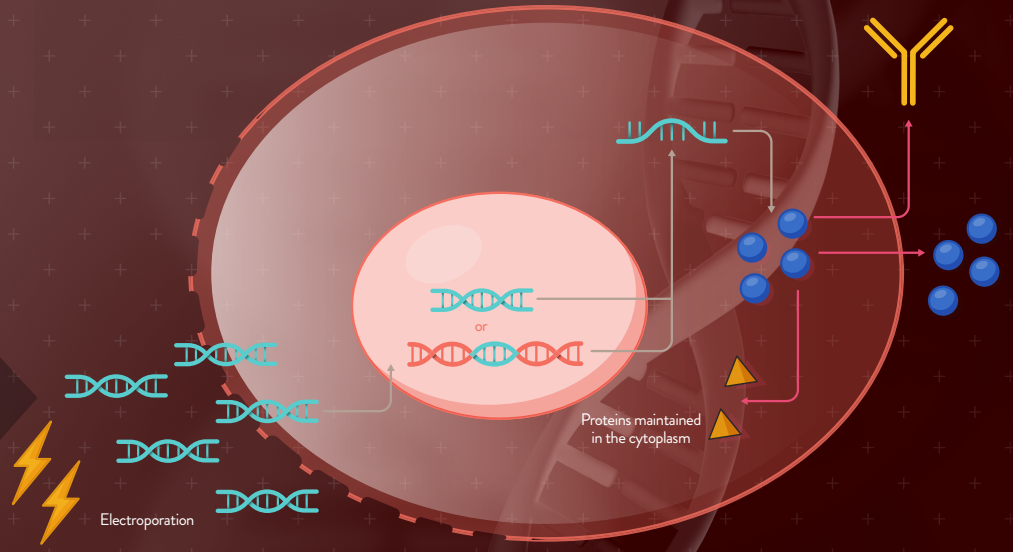


CHEMICAL TRANSFECTION

Chemical carriers represent the most straightforward and widespread tools for gene delivery experiments in mammalian cells. Chemical transfection experiments follow a simple workflow and provide high efficiency nucleic acid delivery for the most commonly used cells as well as many hard-to-transfect cell lines. One of the oldest chemical transfection approaches was the use of calcium phosphate, which in the hands of a skilled technician, could be used to co-precipitate DNA for subsequent delivery to certain permissible cell types. More modern approaches achieve higher transfection efficiencies in a broad array of cell types and utilize proprietary commercial lipids and polymers that are precisely engineered for optimal DNA condensation, electrostatic interaction with target cells, cellular uptake and endosomal release.

ELECTROPORATION

Electroporation is the most common physical transfection method and is often used for primary, progenitor, and stem cells. Electroporation causes cell membrane permeability via short pulses of an intense electric field. During the pulse, temporary physical channels are created in the membrane that allow the desired cargo to enter the cells. Electroporation is easy, quick, and reliable, but can be stressful for cells, rendering the techniques unsuitable for difficult-to-culture or sensitive cells due to high cell-death numbers. One of the primary advantages of electroporation is its versatility—it can be adapted to deliver diverse types of nucleic acids and other molecules to virtually any mammalian cell type.



THE TRANSFECTION EXPERTS