Introduction to Transfection

What is transfection?

Broadly defined, transfection is the process of artificially introducing nucleic acids (DNA or RNA) into cells, utilizing means other than viral infection. Such introductions of foreign nucleic acid using various chemical, biological, or physical methods can result in a change of the properties of the cell, allowing the study of gene function and protein expression in the context of the cell.

In transfection, the introduced nucleic acid may exist in the cells transiently, such that it is only expressed for a limited period of time and does not replicate, or it may be stable and integrate into the genome of the recipient, replicating when the host genome replicates. Types of Transfection

Transfection terminology

The terminology used for various gene delivery systems has evolved to keep pace with technological advances in the field and further refined to distinguish various methods and cell types.

Transfection
Transfection commonly refers to the introduction of nucleic acids into eukaryotic cells, or more specifically, into animal cells. Classically, the term transfection was used to denote the uptake of viral nucleic acid from a prokaryote-infecting virus or bacteriophage, resulting in an infection and the production of mature virus particles. However, the term has acquired its present meaning to include any artificial introduction of foreign nucleic acid into a cell.

Transformation
Transformation is often used to describe non-viral DNA transfer in bacteria,

non-animal eukaryotic cells, and plant cells. However, transformation also refers to a particular event or a series of events that results in a permanent change in an animal cell's phenotype, and implies genetic instability and a progression to a cancerous state. Although transformation in this sense can arise from infection with a transforming virus or from gene transfection, it can also arise spontaneously or following external stressors such as ionizing radiation or chemical mutagens. As such, the term should be avoided for animal cells when describing introduction of exogenous genetic material.

Transduction
Transduction is used to describe virus-mediated DNA transfer. However, the term transfection is also used to refer to infecting a cell specifically with viral nucleic acid that is isolated either from a eukaryote virus or from a bacteriophage.

Applications

The two main purposes of transfection are to produce recombinant proteins, or to specifically enhance or inhibit gene expression in transfected cells. As such, transfection is a powerful analytical tool for the study of the function and regulation of genes or gene products, for the production of transgenic organisms, and as a method for gene therapy.

Gene expression

Transfection is most commonly performed to express a protein of interest in cultured cells (or an animal model) through the use of a plasmid vector or mRNA. Expression of the protein in eukaryotic cells allows the recombinant protein to be produced with proper folding and post-translational modifications required for its function. Further, introducing proteins with readily detectable markers and other modifications into cells allows the study of promoter and enhancer sequences or protein:protein interactions.

In addition, transfection can be used in various forms of bioproduction depending upon the transfection strategy. For example, delivery of reprogramming transcription factors enables the generation of induced pluripotent stem cell (iPSC). Stable transfection, on the other hand, provides the means for the bioproduction of various therapeutic molecules.

Gene inhibition

Another frequent use of transfection is in inhibiting the expression of specific proteins through RNA interference (RNAi). In mammalian cells, RNAi occurs through endogenously expressed non-coding RNA in the form of microRNAs (miRNAs), which are derived from a double-stranded RNA (dsRNA) precursor. The precursor is processed to a mature miRNA that becomes part of a RNA-induced silencing complex (RISC), which acts to inhibit translation of complementary target mRNAs.

Vector-based systems express miRNA precursors or short hairpin RNA (shRNA) precursors that are processed by endogenous machinery to produce miRNAs or

shRNAs, respectively, which then act to inhibit gene expression. These systems allow stable transfection of recombinant constructs, and can permit inducible expression of precursor molecules.

Chemically synthesized short/small interfering RNAs (siRNAs) can also be incorporated into a RISC and induce gene silencing by targeting complementary mRNA for degradation. Modifications to siRNAs help to prevent off-target effects, and also to ensure that the active strand of the dsRNA is loaded into the RISC.