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An Introduction to Transfection

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Abstract

Transfection is the process of inserting genetic material, such as DNA and double stranded RNA, into mammalian cells. The insertion of DNA into a cell enables the expression, or production, of proteins using the cells own machinery, whereas insertion of RNA into a cell is used to down-regulate the production of a specific protein by stopping translation. While the site of action for transfected RNA is the cytoplasm, DNA must be transported to the nucleus for effective transfection. There, the DNA can be transiently expressed for a short period of time, or become incorporated into the genomic DNA, where the change is passed on from cell to cell as it divides.

This video describes the basics behind chemical mediated transfections and introduces some of the most commonly-used reagents, including charged lipids, polymers, and calcium phosphate. Each step is described from the preparation of cells for transfection through analysis of transfection efficiency. Additionally, the applications section of this video-article describes the use of electroporation and a biolistic transfection as alternative methods for introducing nucleic acid into mammalian cells. It also describes an advanced use of transfection where co-transfection of interfering RNA and DNA are introduced as a way to down-regulate a naturally occurring protein while at the same time producing a mutant variant of it within the same cell.

Transcript

Transfection is the process of inserting genetic material, such as DNA and double stranded RNA, into mammalian cells. The insertion of DNA enables the expression, or production, of proteins using the cells own machinery. Whereas insertion of double-stranded RNA is used to shut down the production of a specific protein by stopping translation. This powerful tool has allowed researchers better study gene function and expression, protein function, and genetic mutations.

No single transfection reagent or method works for all cell types. Fortunately, many methods and reagents have been developed in the past few decades to facilitate transfection of a wide variety of cells. These methods can be separated into two main groups: chemical and physical transfections.

In this video, we will focus on the different chemical delivery systems, as they have become increasingly common in recent years. These methods include lipid-based approaches, calcium phosphate mediated transfection, and the use of cationic polymers to name a few.

The underlying principle of all the chemical transfection methods are similar. They all make use positively charged carrier molecules that complex with nucleic acids to package them for cellular delivery. These carrier molecules overcome the negative charge of the cell-membrane barrier which allows them to pass through the membrane to deliver their contents.

In lipid transfection, cationic lipids form a liposome which then combines with nucleic acids to form a “transfection complex”. Whereas calcium phosphate simply condenses the DNA and gives it a net positive charge. Additionally, cationic polymers such as polyetheleneimine condense the DNA into positively charged particles.

The next step in chemical mediated transfection is attachment of the positively charged complexes to the negatively charged cell membrane through simple electrostatic attraction.

Then, the complex enters the cell via endocytosis - a process by which molecules enter the cell via membrane-bound vesicles called endosomes.

Once inside the cell, the nucleic acids must escape from the endosome by a process that is still unknown. Once outside the endosome, the nucleic acids will find themselves in the cell’s cytoplasm and then ultimately the nucleus, where the cell’s machinery is able to make mRNA and then protein from it. The cytoplasm is the site of action for the small interfering RNA, or siRNA where it reduces the production of protein by interfering with a part of the cell’s protein producing machinery.

Transfected DNA can exist either stably or transiently. Stable transfections occur when transfected DNA is introduced into the genome and therefore persists as the cell divides. Transient transfections occur where the DNA is not incorporated into the genome and expression of the coded protein is lost during a span of about 24-96 hours.

The efficiency of DNA transfection is typically measured through reporter systems that are tethered to the inserted gene. These are systems that can be easily detected either by directly observing the reporter protein itself, such as in the case of green fluorescent protein, or by its measuring its enzymatic activity using a colorimetric assay, as in the case of a luciferase enzyme reporter. Stable DNA transfection is best measured by genomic analysis such as RT-PCR.

To measure the success of siRNA silencing, the targeted protein levels in each sample can be determined by Immunoblot. Successful siRNA transfection should decrease the expression of the target protein within the cells while levels of the housekeeping gene, GAPDH, remain stable.

To maximize transfection efficiency, cells should be maintained in log phase growth and be between 40 and 80% confluent, at the time of transfection. In order to accomplish this, cells in culture should be harvested the day before… counted… and seeded into a multi-well plate at a concentration that will yield the correct level of confluencey at the time of transfection.

Next, the chemical reagents and nucleic acids are mixed and given time to form the nucleic acid-reagent complexes. For each chemical delivery system the specific concentrations of each component must be optimized.

The nucleic acid-reagent complexes are then added to the plated cells and incubated often overnight to give plenty of time for the complexes to attach to the cells and mediate transfection. After 24 hours, the media should be removed and replaced with fresh culture media.

Many variations and applications of transfection exist. Co-transfection can allow a researcher to study the effect of missense mutations on the function of cellular proteins. Here, RNAi was transfected into HeLa cells in order to down-regulate the endogenous BRCA1 protein, which causes a reduction in the number of GFP positive cells. At the same time a mutant BRCA1 protein was also transfected and produced by the cell. If the mutated protein was fully functional it caused a recovery in the number of GFP positive cells, but if the mutation negatively affected function, then the number of GFP positive cells stayed low.

As an alternative to chemical transfection methods, a researcher, shown here, uses a gene gun to fire gold particles laced with DNA at cells in culture. Cells that end up with the small DNA coated bullets within their cytoplasm have a good chance for becoming transfected.

Another alternative method for transfection is electroporation. Electroporation is the use of electrical current to damage the cell's membrane allowing DNA or RNA to enter the cell for a short time before the cells have time to repair. Here, tweezer electrodes are placed around a mouse brain and short pulses of electricity are passed through the brain to initiate ex-vivo transfection of the injected RNAi molecules within the blue solution. The effects of gene silencing on the structure of the developing cortex is then observed.

You've just watched JoVE's video on transfection. As always, thanks for watching!