



Introducing Antisense Oligonucleotides into Cells Quick Look

This is a modified, quick look version of the full Technical Report *Introducing Antisense Oligonucleotides into Cells*. Please see the full version for a more comprehensive explanation.

Antisense experimental design

A successful antisense experiment depends on the following characteristics [1]:

- Unique DNA sequence
- Efficient cellular uptake
- Minimal nonspecific binding
- Target specific hybridization
- Non-toxic antisense chemistry
- Nuclease resistant chemistry to protect the antisense oligonucleotide (ASO)
- Minimal inflammatory or immune response (CpG effects)
- Demonstration of reduction in target mRNA
- Appropriate controls

Controls

Antisense oligonucleotides can incite unexpected biological and pharmacological effects and so, good controls are imperative. Using a variety of controls strengthens confidence in the interpretation of antisense experiments. Controls are divided into four main types:

- Oligonucleotide sequences that maintain structural features such as hairpins but have different base compositions from the antisense.
- Scrambled oligonucleotide sequences that have the same base compositions as the antisense but not the same structural features, such as hairpins.
- Antisense oligonucleotide sequences with one or more mismatches to show if the target is selectively hybridized.
- Use of cell lines in which the target gene is mutated or deleted or rescue of phenotype with transfection of a codon varied version of the target gene that the ASO cannot target

Oligonucleotide Uptake

Oligonucleotides may be introduced to cells by a variety of methods:

- Receptor-mediated endocytosis
- Microinjection

- Cationic Lipids/Liposomes
- Electroporation
- Other methods of facilitated entry
- *In vivo* delivery systems

Receptor-mediated endocytosis occurs when the plasma membrane of a cell buds inward to form a vesicle which contains receptor sites. Molecules from outside the cell specifically bind to these receptor sites and are internalized into the cell.

Advantages:

- Small oligonucleotides are taken up rapidly
- Uptake is temperature dependent and will occur more rapidly at 37°C than 4°C
- Phosphorothioate-modified oligonucleotides are internalized better by tissue culture cells [2].

Disadvantages:

- Large oligonucleotides are taken up more slowly and can be competitively inhibited by other small oligonucleotides, especially if they contain a 5' phosphate
- Modifications on oligonucleotides can change uptake efficiency
- Phosphodiester oligonucleotides degrade and neutral modifications, such as methylphosphonates, do not fair well in tissue culture cell internalization [2]
- Oligonucleotide uptake is inefficient and seems to deposit the oligonucleotides into non-nuclear intracellular compartments that are largely inaccessible to RNA [3].

Microinjection is the process whereby a micropipette is used to deliver materials into a single living cell.

Advantages:

- Results in rapid accumulation of the oligonucleotide in the nucleus [5, 6].
- Toxicity can be limited by controlling the purity of the preparation

Disadvantages:

- Cannot be used in many *in vivo* studies
- Can only treat a limited number of cells

Cationic Lipids are positively charged lipids which interact with the negatively charged DNA and cell membranes. Through these interactions, they are able to internalize the negatively charged DNA into the cells.

Advantages:

- Oligonucleotides that have been introduced through lipofectin-induced uptake can be diffusely distributed in the cytoplasm and the nucleus [7]. This distribution presumably results in greater oligonucleotide bioavailability and subsequent enhancement in antisense effect.
- Encapsulation of concentrated oligonucleotides in lipid vesicles by the minimum volume entrapment method can protect oligonucleotides from attack by nucleases in serum and deliver them intact into cells [8].
- Liposomes can be modified in a number of ways that enhance their ability to deliver nucleic acids into living cells

Disadvantages:

- Some oligonucleotides may be distributed in a punctuate manner rather than diffusely.
- Different cell lines and different forms of DNA (single-stranded oligonucleotides, circular plasmid, etc.) may each have a different optimal lipid agent.
- Lipids do not work at all for some cell types
- Different chemically modified forms of DNA may require re-optimization of the transfection procedure [9].
- Even with the use of lipid agents to promote transfection, the efficiency of oligonucleotide entry into individual cells can vary dramatically.
- High toxicity with a large off target effect profile

Available at IDT: TriFECTin™ Transfection Reagent. Trifectin is a proprietary cationic lipid formulation that has been optimized for delivery of IDT's Dicer-Substrate siRNAs into a wide variety of cell types with minimal toxicity. For more information and to order TriFECTin, please visit the IDT website.

Electroporation is a process used to transform or transfect a wide variety of cell types through the use of an externally applied electrical field. The charge affects the permeability of the cell plasma membrane which allows for the entrance of the intended insert.

Advantages:

- Efficient at delivering a large amount of nucleic acid to a cell
- Useful for delivering to cells which are difficult to transfect

Disadvantages

- Requires a large amount of delivered material (typically 500 nM – 2 μ M) in order to work efficiently
- Cells must be treated very carefully following the applied electrical field in order to survive
- Increased risk of toxicity to cells

Other Methods of Faciliated Entry

- Pretreatment of cells with streptolysin *O* led to a 100-fold increase in oligonucleotide permeation with minimal cellular toxicity [10].
- Cell Penetrating Peptides (CPPs): The antennapedia homeodomain protein is translocated through cell membranes and targeted to nuclear localization. A 16 amino acid peptide fragment from the third helix has been shown to confer this property to the protein [11]. Investigators have used this peptide coupled to an antisense oligonucleotide to facilitate direct entry of the oligonucleotide into the nucleus, giving high efficiency of penetration with low dosing.
- Small molecule tags used to modify oligonucleotides improve their uptake efficiency. These types of tags include cholesterol-modification, membrane-permeant peptides, folate, antibiotics, VITE, and VITA [12].
- Cationic polymers can bind to large nucleic acids and condense them into stable nanoparticles and can, thus, serve as efficient transfection agents [12].

In vivo delivery systems

- Proteins derived from the coat of Sendai viruses are known to promote fusion of lipid bilayers. In one series of experiments, oligonucleotides were packaged in liposomes complexed with coat proteins derived from the hemagglutinating virus of Japan (HVJ, a Sendai family virus) and were infused into rat carotid arteries where they fused with the vascular endothelium and neointima. The oligonucleotides were able to curtail intimal hyperplasia following vascular injury [13, 14].
- It is possible to use fluorescein-conjugated antisense oligonucleotides to track their fate *in vivo*. Oligonucleotides delivered via liposome encapsulation can be detected in the nucleus for up to two weeks after administration [13].
- Antisense uptake into tissues in living organisms using intravenous, subcutaneous, or intraperitoneal injections has been higher than expected based on previous experience with cell culture [15].
- Certain tissues are accessible to topical or localized administration of siRNA including the eye, mucus membranes, and local tumors [12].
- Phosphorylated DNA antisense oligonucleotides have appreciable uptake *in vivo*.

References

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