SCRAPE DELIVERY OF MORPHOLINO OLIGOS

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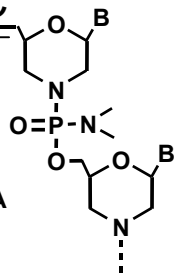

I. INTRODUCTION

A. Scrape Delivery

It is often desired to introduce antisense oligos, peptides, proteins, drugs, etc. into the cytosol of eucaryotic cells. However, most hydrophilic products are taken into eucaryotic cells via endocytosis and subsequently sequestered or degraded in lysosomes or exocytosed, with little or no intact product entering the cytosol.

Scrape delivery circumvents the delivery barriers inherent in the endocytosis pathway. Scrape delivery exploits the binding of adherent animal cells to their substrate via adhesion plaques. When adherent cells are scraped from a culture plate these plaques are pulled out of the plasma membrane, leaving holes which allow molecules in the extracellular medium to freely enter the cytosol of the scraped cell for up to about one minute, by which time most of the holes have resealed.

Partridge and coworkers have demonstrated that when antisense oligos are present in the culture medium when the cells are scraped, a substantial amount of oligo directly enters the cytosol of the cells during the first minute after scraping (Antisense & Nucleic Acid Drug Development **6**:169 (1996)).

Scrape delivery of antisense oligos entails:

1) Add your oligo to the culture medium bathing the cells.
2) Use a cell scraper to scrape cells from the plate or flask.
3) Transfer cells to a new plate or flask.

While there are a variety of cell scrapers on the market, some cause significant damage to the cells. **GENE TOOLS** offers Sarstedt cell scrapers which have been found to provide particularly good scrape delivery with minimal impact on cell viability. These optimal cell scrapers are priced at 20 for $50.

B. Cytosolic Concentration of Oligo Achieved by Scrape Delivery

In cell types which are well adhered to their substrate, such as HeLa cells, scrape delivery typically achieves a concentration of oligo in the cytosol about 20% of the concentration in the culture medium at the time of scraping. Thus, if you want to achieve a 2 microMolar cytosolic concentration of your oligo, we recommend you make the culture medium 10 microMolar in oligo prior to scraping the cells.

C. Advantages and Limitations of Scrape Delivery

a) Essentially any substance having a molecular weight up to about 50,000 daltons can be delivered into the cytosol of cells.
b) Delivery is equally effective in the presence or absence of serum.
c) The method is very simple, fast, and inexpensive.

d) Scrape delivery appears to have less detrimental impact on cells than other commonly used delivery methods - including particularly liposomes and streptolysin O.

While scrape delivery can be very effective, it should be appreciated that this method does have some limitations:

a) Cells in culture plates with small wells (ie., 24, 48 & 96-well plates) cannot be effectively scraped. For scrape delivery we recommend culturing cells in 6-well plates or larger, or in flasks.
b) Scrape delivery is only applicable to cell types which adhere well to their substrate.
c) Typically only about 85% of the cells are effectively loaded by this method - presumably the other 15% of the cells are in a stage of the cell cycle where they are less well adhered to the substrate. To increase delivery one can scrape the cells and then allow the cells to re-adhere to the surface for about 4 hours, and then rescrape. By this double-scrape tactic product can be effectively delivered into about 95% of the cells.

Alternatively, if you need to:

a) work with cells in small culture plate wells (eg., 48 and 96-well plates);
b) deliver product into poorly-adherent cells or into cells in suspension;
c) would like to achieve more effective and efficient delivery in a wide range of cell types;

We recommend you use **GENE TOOLS** Special Delivery System for your cytosolic delivery needs (for information about Special Delivery visit: www.gene-tools.com, call (541)929-7840, or FAX (541) 929-7841).

II. DELIVERING MORPHOLINO OLIGOS

A. Preparing Oligo Stock Solutions

Custom-Sequence Oligo: To one vial containing 300 nanoMoles of sterile freeze-dried custom-sequence Morpholino oligo add 1.5 ml of sterile water to give 1.5 ml of a 200 microMolar oligo stock solution.

Standard Control Oligo: To one vial containing 100 nanoMoles of sterile freeze-dried Standard Control Morpholino oligo add 0.5 ml of sterile water to give 0.5 ml of a 200 microMolar oligo stock solution.

B. Preparing Cells for Scrape Delivery

Cells can be cultured in plates or flasks. We find 6-well culture plates to be a convenient size for the scrape delivery method. Typically, 5×10^5 trypsinized cells in 1 ml of growth medium are seeded into each well of a 6-well culture plate. These are incubated at 37^0C for 16 to 48 hours, after which the depleted medium is removed and replaced with 1.0 ml of fresh medium in each well.

C. Scrape Delivery Procedure

PROCEDURE	NOTES
1. To the 1 ml of culture medium in the well add 53 microLiters of a 200 microMolar stock solution of antisense or control oligo (described above) and then swirl the plate for 10 seconds to mix. This gives a 10 microMolar Morpholino oligo concentration, which typically affords near quantitative inhibition of its targeted mRNA.	One may instead add the oligo to the culture medium before adding the medium to the cells. It is important to thoroughly mix the oligo with medium before scraping the cells**.**
2. Place the culture plate on a flat surface and gently scrape the cells off the surface of the well or flask with a sterile cell scraper.	Best results are achieved with a low-force sweeping motion using a scraper having a rubber blade. A rubber policeman will also work. Scrapers with plastic blades generally damage the cells. **GENE TOOLS** offers cell scrapers which have been found to give optimal results.
3. Gently pipet the scraped cell suspension up and down twice and transfer the cells to another culture plate or flask.	Gentle pipeting breaks up sheets of cells to give a more uniform distribution of cells in the new plate or flask. Oligo does not effectively enter the cytosol of cells which are not scraped from the surface. By transferring the cells, it is assured that the new plate or flask only contains cells which underwent scrape delivery.

There is no need to remove Morpholino oligo from the culture medium after scrape delivery. However, if you are using Phosphorothioate oligos you will need to centrifuge the cells, remove the medium, and replace it with medium lacking phosphorothioate oligo. This is because the polyanionic phosphorothioate oligos prevent cells from re-adhering to culture plates.

If you are running experiments involving induction of the targeted gene, the induction agent can be added immediately after the scrape delivery procedure.

The antisense-mediated phenotypic change can generally be assessed several hours to several days after delivery.

Special notes

The concentrations and method described herein have given excellent results with HeLa cells and with several other adherent cell types. However, we can provide no assurance that this scrape delivery method will be equally effective in delivering your product into your cells in your experimental system.

GENE TOOLS offers a fluorescein-labeled Std. Control oligo which allows visual assessment (via fluorescence microscopy) of the efficiency of cytosolic delivery by scrape delivery.