<u>Bio 181: Internet-based exercise:</u> Week 3
DUE: *Monday, October 1st*

Gene delivery systems. In lab we have discussed, and performed, transformation of chemically competent bacteria to get DNA into cells. Many other methods of delivering DNA into cells are also used (particularly for eukaryotic cells).
Following text is an excerpt from http://www.promega.com/guides/transfxn_guide/chapter_one.pdf; read it here, or at the actual website.

Historical Background

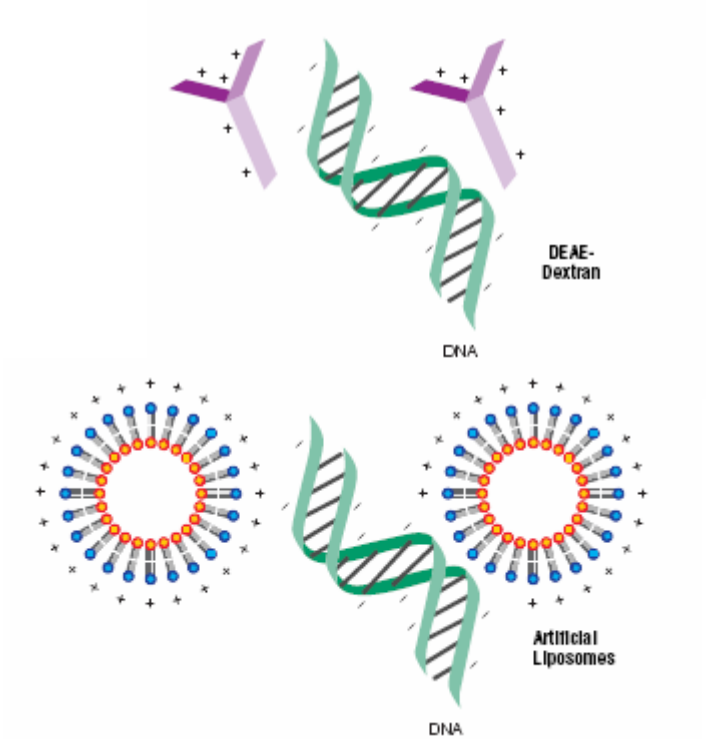
The ability to introduce nucleic acids into cells has enabled the advancement of our knowledge of genetic regulation and protein function within eukaryotic cells, tissues and organisms. The successful pioneering studies of Vaheri and Pagano (1), and Graham and van der Eb (2) with DEAE-dextran and calcium phosphate-mediated transfection techniques, paved the way for future experiments necessitating DNA transfer into cultured eukaryotic cells. The process of introducing nucleic acids into cells by non-viral methods, such as the DEAE-dextran and calcium phosphate techniques, is defined as "transfection". This process is distinct from "infection", which is a viral method of nucleic acid introduction into cells.

Transfection Technologies

Many transfection techniques have been developed. Desirable features include high efficiency transfer of nucleic acid to the appropriate cellular organelle (for example, DNA into the nucleus), minimal intrusion or interference with normal cell physiology, low toxicity, ease of use, reproducibility, successful generation of stable transfectants, and *in vivo* efficacy. The techniques developed for gene transfer can be broadly classified as either chemical reagents or physical methods.

Chemical Reagents

DEAE-dextran was one of the first chemical reagents used for transfer of nucleic acids into cultured mammalian cells (1,9). The ProFection® Mammalian Transfection System-DEAE-Dextran provides reagents for this transfection technique (see Chapter 4 for further information). DEAE-dextran is a cationic polymer that associates with negatively charged nucleic acids. An excess of positive charge, contributed by the polymer in the DNA/polymer complex allows the complex to come into closer association with the negatively charged cell membrane. Uptake of the complex is presumably by endocytosis. This method is successful for delivery of nucleic acids into cells for transient expression; that is, for short-term expression studies of a few days in duration. However, this technique is not generally useful for stable transfection studies that rely upon integration of the transferred DNA into the chromosome (10). Other synthetic cationic polymers have been used for the transfer of DNA into cells, including polybrene (11), polyethyleneimine (12) and dendrimers (13,14).



By 1980, **artificial liposomes** were being used to deliver DNA into cells (5). The next advancement in liposomal vehicles was the development of synthetic cationic lipids by Felgner and colleagues (16). Liposome-mediated delivery offers advantages such as relatively high efficiency of gene transfer, ability to transfect certain cell types that are intransigent to calcium phosphate or DEAE-dextran, successful delivery of DNA of all sizes from oligonucleotides to yeast artificial chromosomes (16-20), delivery of RNA (21), and delivery of protein (22). Cells transfected by liposome techniques can be used for transient and for longer term experiments that rely upon integration of the DNA into the chromosome or episomal maintenance. Unlike the DEAE-dextran or calcium phosphate chemical methods, liposome-mediated nucleic acid delivery can be used for *in vivo* transfer of DNA and RNA to animals and humans (23).

Physical Methods

Direct microinjection into cultured cells or nuclei is an effective, although laborious technique to deliver nucleic acids into cells. This method has been used to transfer DNA into embryonic stem cells that are used to produce transgenic organisms (25). However, this technique is not appropriate for studies that require a large number of transfected cells.

Electroporation was first reported for gene transfer studies in 1982 (4). This technique is often used for cell types such as plant protoplasts that are particularly recalcitrant to milder methods of gene transfer. The mechanism for entry into the cell is based upon perturbation of the cell membrane by an electrical pulse, which forms pores that allow the passage of nucleic acids into the cell (26). The technique requires fine-tuning and optimization for duration and strength of the pulse for each type of cell used. A critical balance must be achieved between conditions that allow efficient delivery and conditions that kill cells.

Another physical method of gene delivery is **biolistic particle delivery**. This method relies upon high velocity delivery of nucleic acids on microprojectiles to recipient cells (27). This method has been successfully employed to deliver nucleic acid to cultured cells, as well as to cells *in vivo* (28).

1. How do DEAE-dextran/DNA complexes enter cells?

2. What is <u>transient</u> transfection?

3. What chemical method is useful for long-term transfections (with integration of foreign DNA into chromosome)?

4. Electroporation involves shocking cells with electricity. Longer and stronger electrical pulses increase the efficiency of gene delivery, but conditions must be balanced to prevent what?

Go to **www.bio-rad.com.** Click on "**Life Science Research**". Next, under "Catalog Index" choose "**Gene Transfer**". Next, on the upper left (under 'associate pages'), click "**Applications**".

5. You need to get DNA into each of the following cell types. Which gene transfer technology would you choose for each cell type?
    a. Gram negative bacteria (*E. coli)*
    b. Suspension cell culture
    c. Yeast
    d. Plant cells

From that page, click on Biolistic Particle Delivery; then choose Helios Gene Gun.

6. The "bullets" (microcarriers) of the Gene Gun (biolistic particle delivery) are coated with DNA or RNA. What are the bullets themselves made of?