# Biol446, Cell Biology Laboratory
# Laboratory Exercise 6

## Transfection

**Objective:** To gain experience in delivering genes into cultured mammalian cells by transient transfect and determine the transfection efficiency using fluorescence microscope.

**Background:**

Transfection, the delivery of DNA into a eukaryotic cell is now known to the general public for its use in treating diseases of genetic origin. Gene therapy holds high hopes for providing the missing genetic material, correcting defective genes, or increasing the expression of genes already present. For biological scientist, transfection is now the standard methodology for studying gene expression and function using primary or cultured cells in the laboratory. However, for both its use in clinic and its use in basic research, better transfection methods still need to be developed. The major barrier to the development of gene therapy is low permeability of the cell membrane to nucleic acids. It is also challenging to design transfection reagents that improve intracellular trafficking of the nucleic acids and nuclear delivery of genes into the target cells without toxicity to normal cells.

At present, viral transfections are the most advanced of current transfection technologies and are being used in *in vitro* transfection of cultured cells and are being tested in human subjects. The construction of viral vectors involves the insertion of therapeutic DNA into the viral genome. The virus is then administered to infect the patient with the hope that it will deliver the precious cargo to a diseased cell nucleus.

Non-viral delivery systems are also well developed. One group of non-viral system uses cationic lipids. These lipid systems feature molecules that are generally composed of three parts: a hydrophobic anchor, a linker, and a cationic group. The molecules self-assemble in water to form micelles with hydrophobic end of the molecule in the inside of the micelles and the charged group exposed to the highly polar water molecules. These cationic lipids micelles associate with DNA by interacting with its negative charges to form a complex structure called lipoplexes. The lipoplex almost totally protects the DNA and carries the DNA into the cell due to the interaction between the hydrophobic anchor of the lipids and the cell membrane.

Another group of non-viral delivery systems are the Peptide-based systems. These have been developed in recent years. An idea peptide-based system would contain self-assembly peptide(s) that has the following properties: (1) Binds to DNA and condenses DNA so that it can act as a carrier for the DNA. (2) Associates and destabilizes the cell membrane so that it car carry DNA to the cell. (3) Transport the DNA to the cell nucleus by a nuclear localization sequence or domain so that the "gene" contained in the DNA delivered can be expressed. Transfection systems utilizing reagents with other molecular properties have also be been developed.

In this lab exercise, we will use the commercial transfection reagent Lipofectamine™ LTX Reagent from Invitrogen to transfect HeLa cells with pEGFP-C4.. **HeLa (ATCC #CCL-2) cells are derived from cervix cancer tissue and may contain poliovirus 1, 2, 3; adenovirus 3, encephalomyocarditis; coxsackievirus B serotypes. Handle with extreme care and follow the sterile procedures as these cells are potentially biohazardous material.** The vector pEGFP-C4 is a derivative of the pEGFP-C series from BD Biosciences Clonetech with a

modified MCS. It encodes the engineered green fluorescence protein with enhanced fluorescence. Transfected cells will express EGFP that can be detected in live cells under a fluorescence microscope. We will use a Nikon TE2000 U microscope to count the transfected cells and the untranstected cells in a field containing at least 100 cells to determine the transfection efficiency (the percentage of cells that are transfected). Follow the microscope usage instructions when using the TE2000.

**Protocol:**

**Preparing the cells:**

HeLa cells will be grown by the instructor well before the lab day. Steps 1-8 will be performed by your TA(s).

**1.** 24 hours before the lab time, warm up the media, trypsin solution, and the wash solution in a water bath at 37°C. Wash the cover and the joints with EtOH and put them in a biosafty hood. **DO NOT HEAT TRYPSIN and NEVER OPEN A BOTTLE OUTSIDE THE HOOD.**

2. Add 5 ml 100X Penicillin/Streptomycin stock (5,000 units/ml penicillin and 5,000 μg/ml streptomycin) and 50 ml FCS (Fetal Calf Serum) in 500ml DMEM. FCS has lots of Growth Hormones that will keep the cells growing. Write the date and what you have added to the DMEM.
3. Take a preexisting culture growing in a 100 mm culture plate to the hood and use an aspirator to suck up the old media from the plate.
4. Add 4 ml HBSS without $Ca^{+2}$ and $Mg^{+2}$ and aspirate the HBSS after briefly swirling the plate.
5. Add 3ml Trypsin-EDTA solution and wait for minutes for the cell to detach. Do not trypsinize for a long time, since this would lyse the cells. Check the cells occasionally and stop the trypsinization as soon as the cells detach.
6. Pipette 2-3 times to separate clumps of cells.
7. If the cell is close to confluent before trypsinization, plate the cells in 1:3 dilution. This means that you will plate three 100 mm plates. So, add 23 ml of medium to the plate, mix, and add 1.5 ml of the culture to each 35 mm plate. (The surface area of a 100 mm plate is about 8 times that of a 35 mm plate). (The aim is to get the cell to 50%-80% confluent at the time of transfection).
8. Incubate the 35 mm plates with HeLa cells in a $CO_2$ incubator.

**Transfection:**

**9.** Clean up the biosafty hood by wiping it with 70% ethanol or 100% methanol.

**10.** Add 0.75 μg of DNA to 300 μl of serum-free medium (e.g. Opti-MEM from Gibco) and mix thoroughly by inverting.

**11.** Add 2.7 μl of Lipofectamine LTX reagent into the DNA solution. Mix gently by inverting and incubate for 25 minutes at room temperature to form DNA-Lipofectamine LTX complexes.

**12.** Remove medium from the plate prepared in step 8 and replace it with 1.5 ml per well fresh complete growth medium.

**13.** Add the transfection reagent-DNA complex from step 11 to the plate. Gently rock the plate back and forth and from side to side.

**14.** Incubate the cells at 37°C in a $CO_2$ incubator for 18-24 hours.

**Lab day 2:**

**15.** Analyze your transfection by visualizing the fluorescence signal under a fluorescence microscope. Take a snapshot of the transfected cells using a filter for GFP and take a snapshot of the total cells in the same field with phase contract. Count the number of transfected cells vs. total cells and determine the transfection efficiency. Ideally, this is to be done 24 hours after transfection.