DR. SULEKHA RANI

Paper BT-121 Animal Biotechnology

Lesson Plan

- **Topic-** Methods available for DNA transfer into mammalian cells, their advantages ,limitations and latest advancements

There are various methods of introducing foreign DNA into a eukaryotic cells some rely on physical treatment (electroporation, cell squeezing, nanoparticles, magnetofection), other on chemical materials or biological particles (viruses) that are used as carriers.

Chemical methods

- One of the cheapest methods uses calcium phosphate, originally discovered by F. L. Graham and A. J. van der Eb in 1973. A buffered saline solution containing phosphate ions is combined with a CaCl2 solution containing the DNA to be transfected. When the two are combined, a fine precipitate of the positively charged calcium and the negatively charged phosphate will form, binding the DNA to be transfected on its surface. The suspension of the precipitate is then added to the cells to be transfected (usually a cell culture grown in a monolayer). By a process not entirely understood, the cells take up some of the precipitate, and with it, the DNA. This process has been a preferred method of identifying many oncogenes.
- Other methods use highly branched organic compounds (dendrimers) to bind the DNA and get it into the cell.
- A very efficient method is the inclusion of the DNA to be transfected in liposomes i.e. small, membrane-bounded bodies that are in some ways similar to the structure of a cell and can actually fuse with the membrane releasing the DNA into the cell. For eukaryotic cells, transfection is better achieved using cationic liposomes (or mixtures), because the cells are more sensitive.
- Another method is the use of cationic polymers such as DEAE -dextran.

<u>Recommended Books:</u>

1. Molecular Biotechnology: Principles and Applications of Recombinant DNA

By- Glick, B.R. and Pasternak, J.J., ASM Press, USA.

2. Principals of Gene Manipulation and Genomics

By- Primose, S. B and Twyman, R.M., Blackwell Publishing.

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Topic- Non-chemical methods available for DNA transfer into mammalian cells

Non-chemical methods

Electroporation is a popular method, where transient increase in the permeability of cell membrane is achieved when the cells are exposed to short pulses of an intense electric field.

- Cell squeezing is a method invented in 2013 .It enables delivery of molecules into cells by a gentle squeezing of the cell membrane. It is a high throughput vector-free microfluidic platform for intracellular delivery. It eliminates the possibility of toxicity or off-target effects as it does not rely on exogenous materials or electrical fields.

- Sonoporation uses high-intensity ultrasound to induce pore formation in cell membranes. This pore formation is attributed mainly to the cavitation of gas bubbles interacting with nearby cell membranes since is enhanced by the addition of ultrasound contrast agent, a source of cavitation nuclei.

- Optical transfection is a method where a tiny (~1 μm diameter) hole is transiently generated in the plasma membrane of a cell using a highly focused laser. This technique was first described in 1984 by Tsukakoshi et al., who used a frequency tripled Nd:YAG to generate stable and transient transfection of normal rat kidney cells.[10] In this technique, one cell at a time is treated, making it particularly useful for single cell analysis.

- Protoplast fusion is a technique in which transformed bacterial cells are treated with lysozyme in order to remove the cell wall. Following this, fusogenic agents (e.g., Sendai virus, PEG, or electroporation) are used in order to fuse the protoplast carrying the gene of interest with the target recipient cell. A major disadvantage of this method is that bacterial components are non-specifically introduced into the target cell as well.

- Impalefection is a method of introducing DNA bound to a surface of a nanofiber that is inserted into a cell. This approach can also be implemented with arrays of nanofibers that are introduced into large numbers of cells and intact tissue.

Recommended Books:

1. Molecular Biotechnology: Principles and Applications of Recombinant DNA

By- Glick, B.R. and Pasternak, J.J., ASM Press, USA.

2. Principals of Gene Manipulation and Genomics

By- Primose, S. B and Twyman, R.M., Blackwell Publishing.

3. Recombinant DNA

By- Watson, J.D., Gilman,M., Witkowski,J. and Zoller,M., Scientific American Books.

DR. SUNITA DALAL

Lesson plan for feb 2015:paper Bioinformatics.

Retrival systems for databases.
Pairwise sequence alignment.
BLAST,FASTA
MSA

Name of the Teacher – **Dr. Ritu Mahajan**

For M.Sc. Students

Subject taken – **Enzyme Technology (BT110)**

Outline of the lectures to be delivered this week – i.e. February 9 to February 14, 2015

- Monomeric and oligomeric enzymes
- Multienzyme complex
- Isoenzymes
- Denaturation and renaturation
- Enzyme specificity

Books for reference:-

Fundamentals of Enzymology by Price and Stevens
Enzymes by Palmer and Bonner
Enzymes by Devasena
Biochemistry by Lehninger
Fundamentals of enzymology by Meena and Chauhan
Biochemistry by U Satyanarayan

For Ph.D. Students

Outline of the lecture to be delivered to Ph.D. students

- Extraction and purification of enzymes from Microbial sources