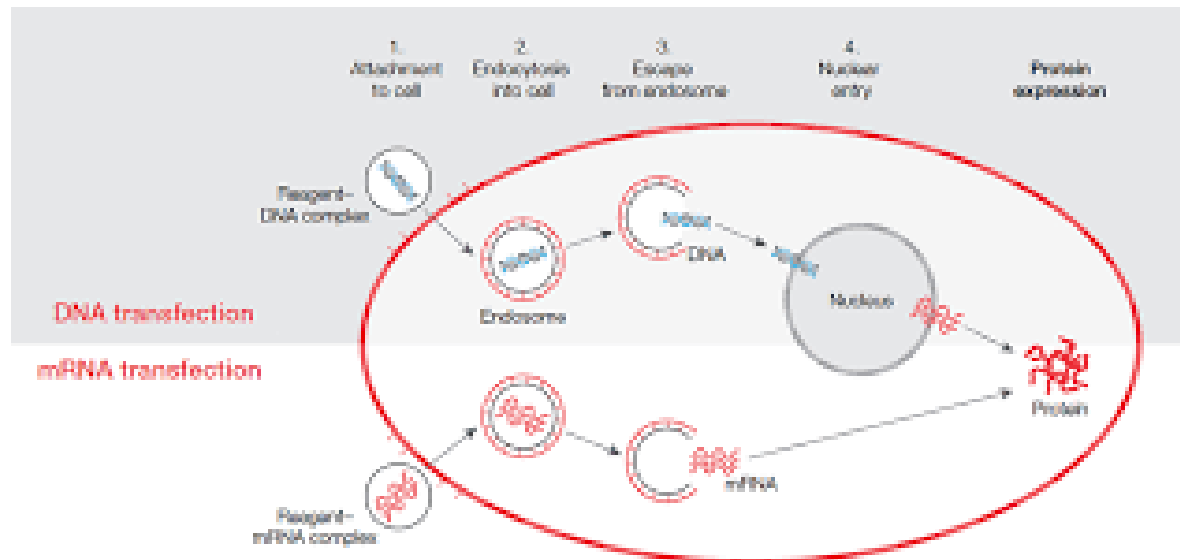


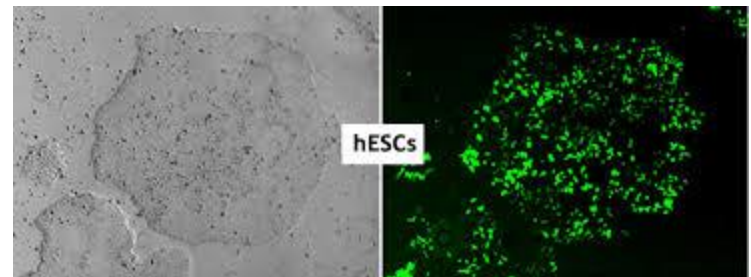
# TRANSFECTION

Mason Snead



# What is Transfection?

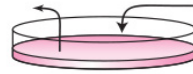
- Transfection is the process of inserting genetic material, such as DNA and double stranded RNA, into mammalian cells. The insertion of DNA enables the expression, or production, of proteins using the cells own machinery.
  - Either with endocytosis or phagocytosis.
- This is used in for virus production and gene variation.
- There are many ways to make this work.
  - Regent Based method
  - Instrument Based Method
  - Viral Tranfection



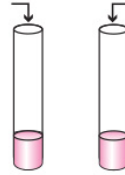
# Reagent Based Method

- For example - Calcium chloride is used.
  - This is mixed with DNA in a controlled manner to a buffered saline/phosphate solution; precipitate develops that is dispersed onto cells that is taken up via endocytosis or phagocytosis.
- Pros:
  - Inexpensive, highly efficient.
- Cons:
  - Reagent consistency is critical for reproduction, small pH changes can compromise efficiency, size and quantity of precipitate are crucial for successful transfection, does not work in RPMI.

1. Change media prior to transfection.



2. Add 2x viral plasmids to  
0.25 M  $\text{CaCl}_2$ .

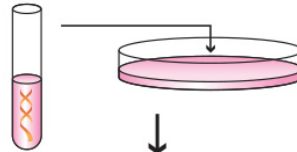


3. Add 2X HeBS or BBS  
Buffer to a tube.

4. Combine, mix and incubate.



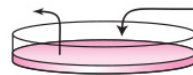
5. Add complexes directly to cells in serum containing media.



6. Incubate ~24 hours.



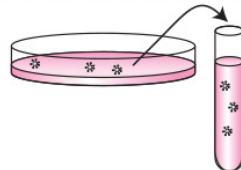
7. Change media post transfection.



8. Incubate 24-48 more hours.



9. Harvest media containing virus.



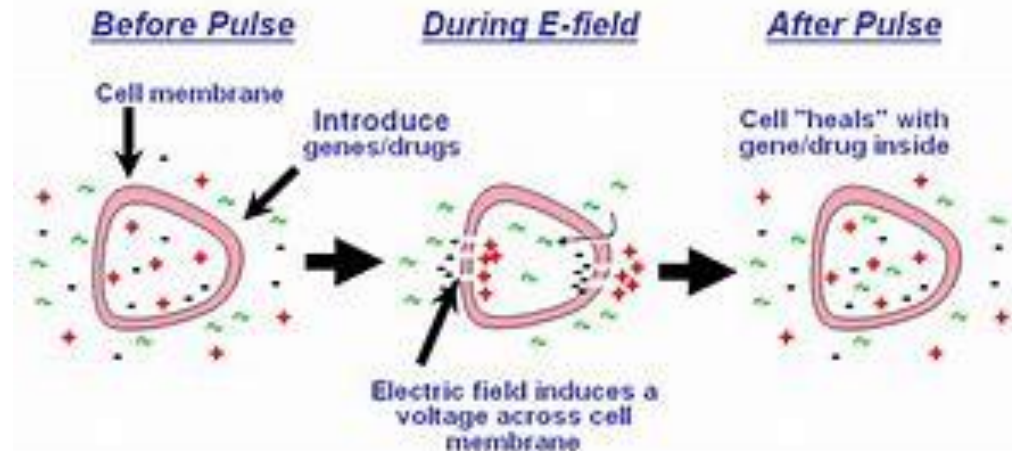
# Instrument Based Method

- Step-by-Step process of Electroporation.
- Step 1
  - Expose the cells to high intensity electrical field which temporarily destabilizes pores from the cell membrane.
- Step 2
  - The destabilization of membrane pores .
- Step 3
  - Causes the cell membrane to be highly permeable to exogenous molecules present within the media; DNA can move freely into the cell through the pores.



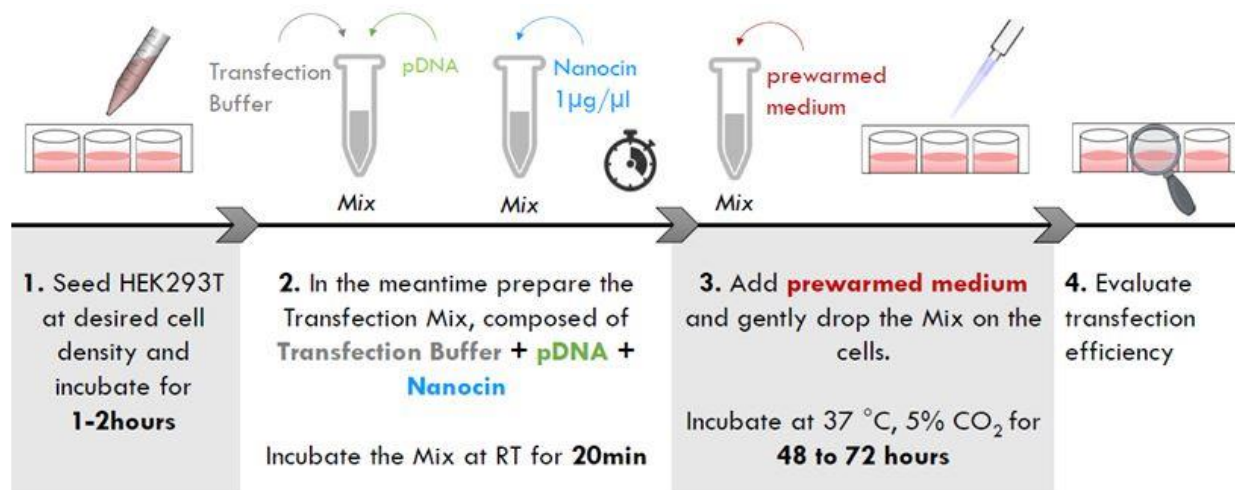
# Continued...

- Step 4 of electroporation
  - When the electrical field is removed, the pores seal and trap the exogenous DNA within the cell.
- Pros:
  - Nonchemical method which appears to not alter biological structure or function of cells, easy, efficient, applicable to variety of cell types.
- Cons:
  - Cell mortality



# Viral Transfection

- DNA can also be introduced into cells using viruses as a carrier.
  - In such cases, the technique is called viral transduction, and the cells are said to be transduced.
- AAV vectors can be useful for viral transfection methods because they can transfer genes into a wide variety of human cells and have high transfer rates



pAAV-GOI Vector  
pRC2-mi342 Vector  
pHelper Vector

Transfection

Transcription and Translation

Replication

AAV ssDNA

293 Cell

AAV Extraction Solution

AAV2 Particles

