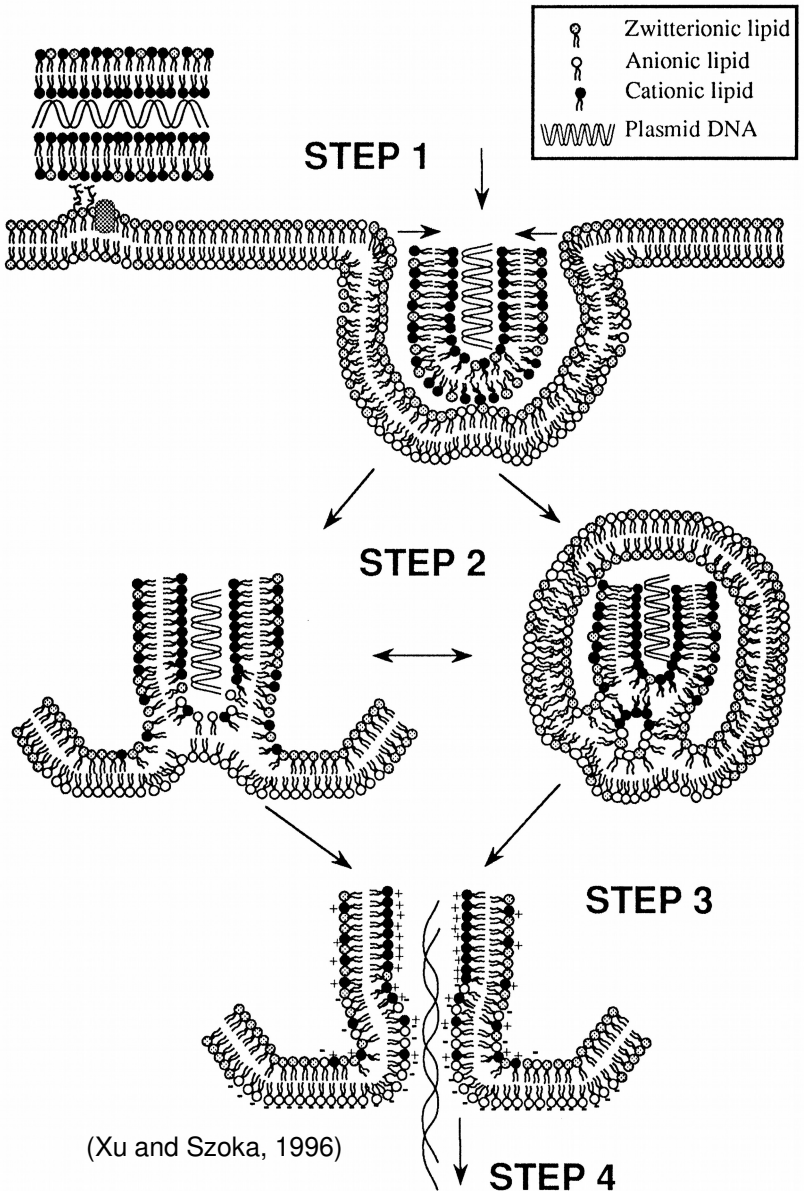
Exp5: Cell Transfection

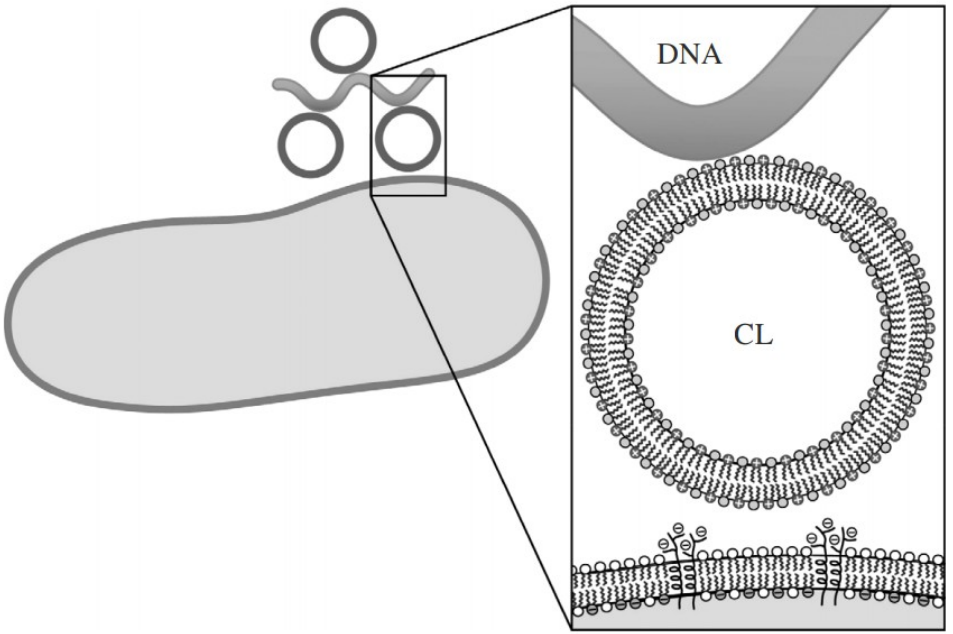
Transfer DsRed and EGFP to CHO-K1 cell

11510511 Yuejian Mo
11612218 Wenhao Zhang



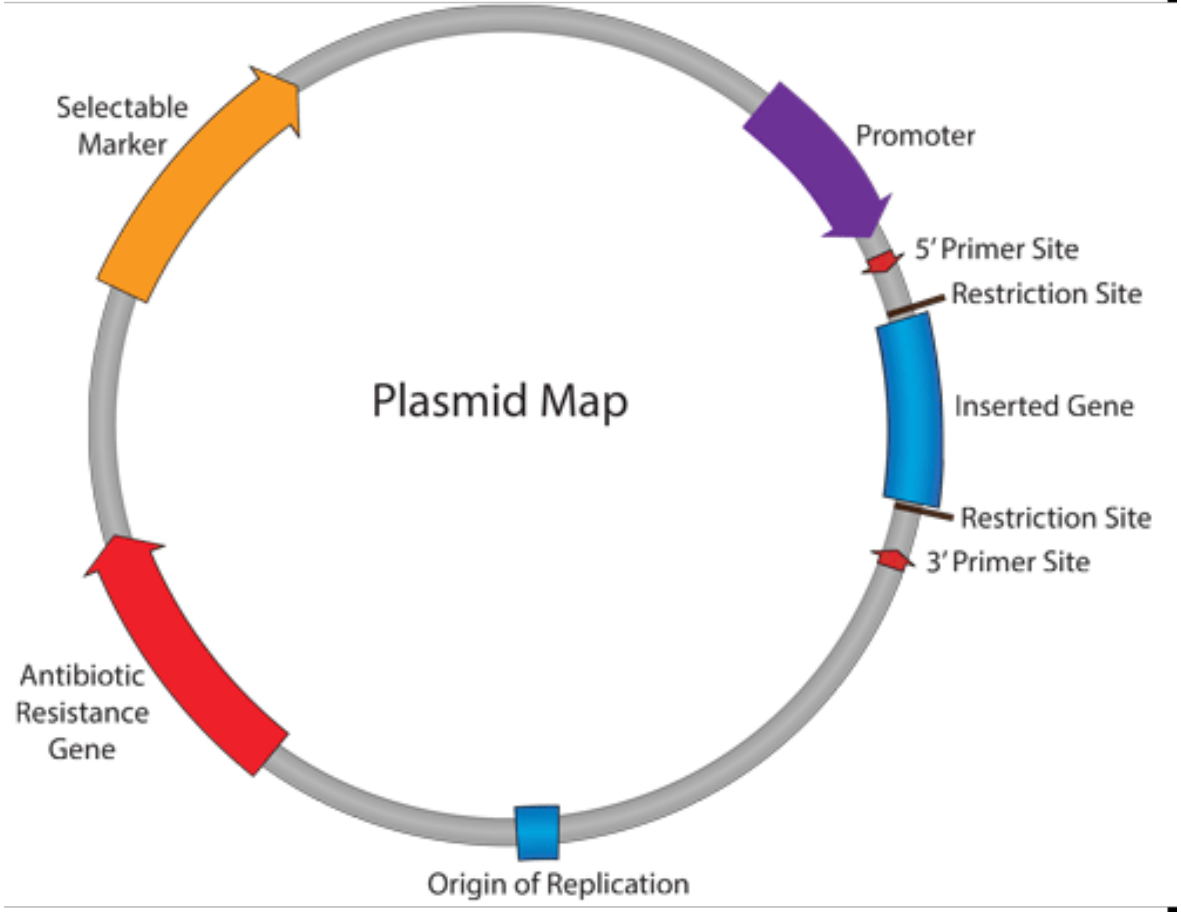
(Xu and Szoka, 1996)

Principle: Cationic Liposome



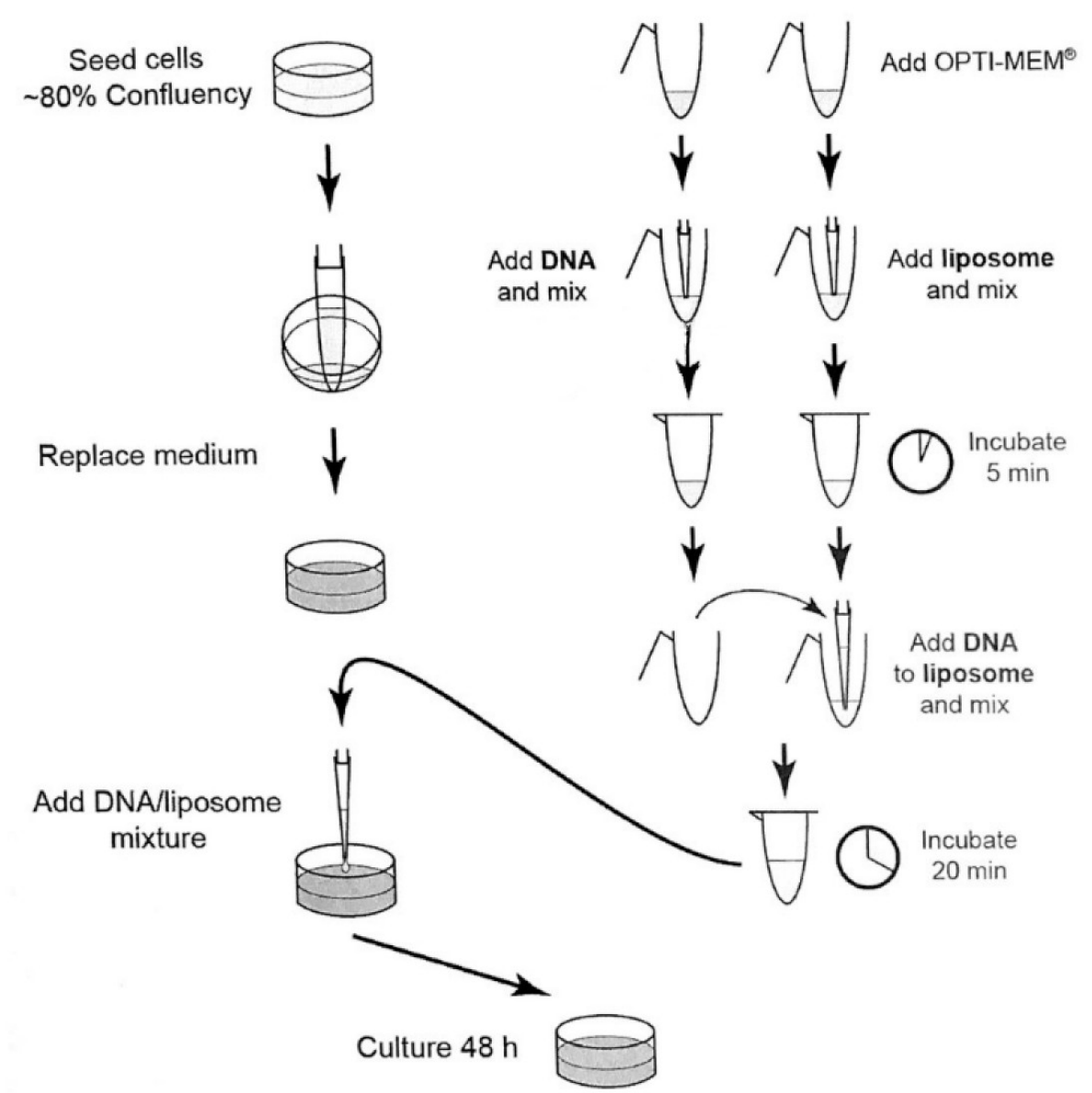
(Safinya et al., 2006)

Principle: Vector Design



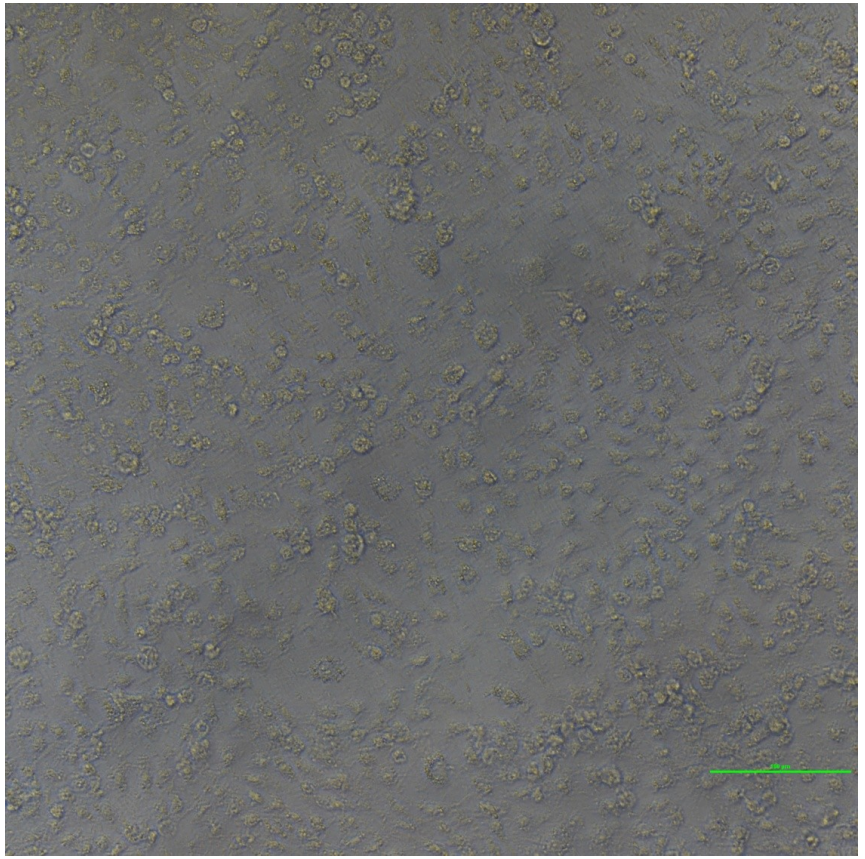
- Reporter Gene vs Marker
- Fluorescence Protein
- Nuclear Localization Signal or Sequence (NLS)

Procedures

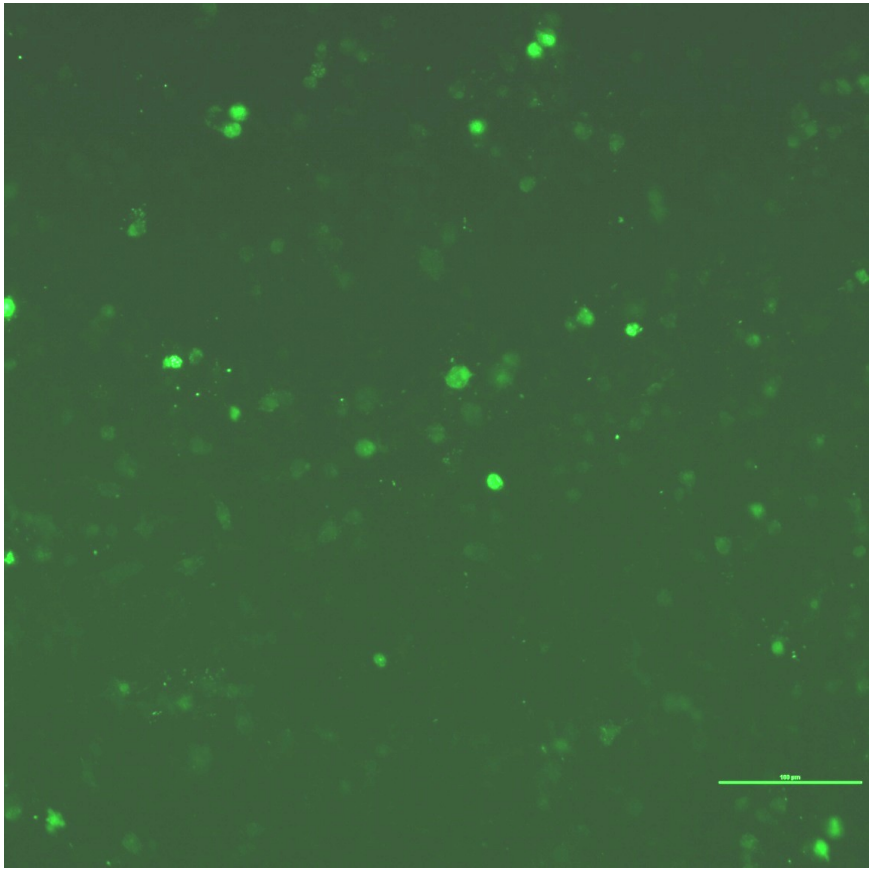


pDsRed 2-Nuc	pEGFP-Actin	Double Transfection
0.25µl	0.25µl	0.125µl+0.125µl
0.5µl	0.5µl	0.25µl + 0.25µl

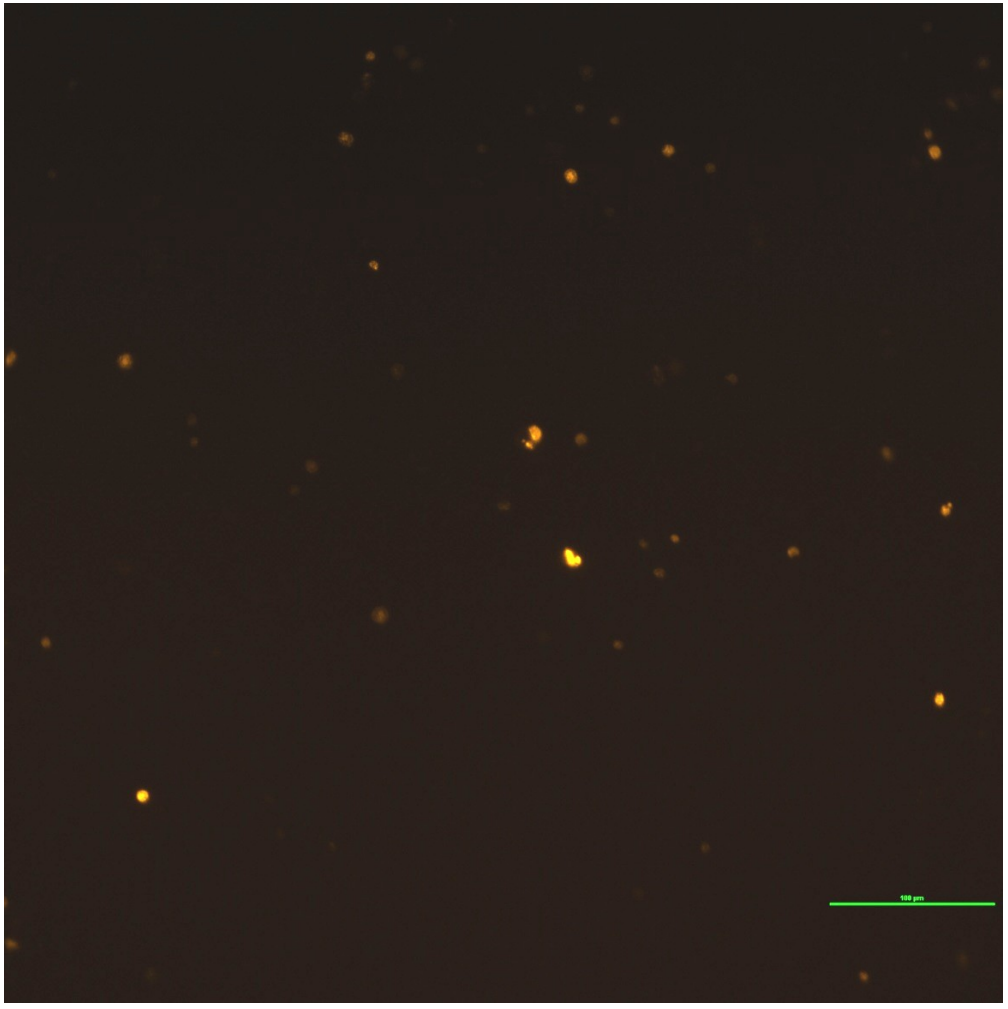
Result: Cotransfection CHOK1



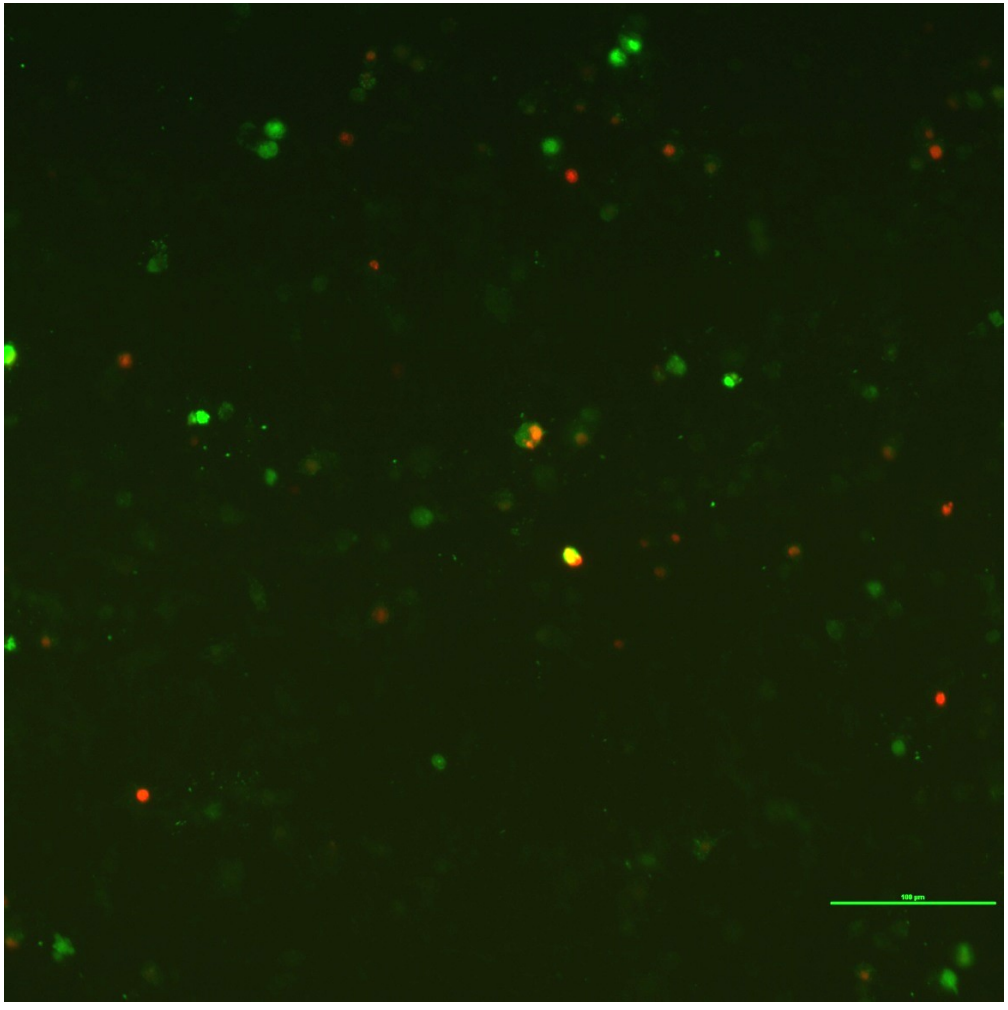
BF(Scale Bar 100micron)



Green Fluorescence



Red Fluorescence



Merged Green and Red Fluorescence

Notice

- Incubate mixture for 20 min
- Add mixture drop by drop
- Mix well

Efficiency factors

- Cell condition
- DNA quality
- Avoid serum and antibiotics
- Time exposed to transfection reagent

Other methods

- Stable transfection
- Viral transduction

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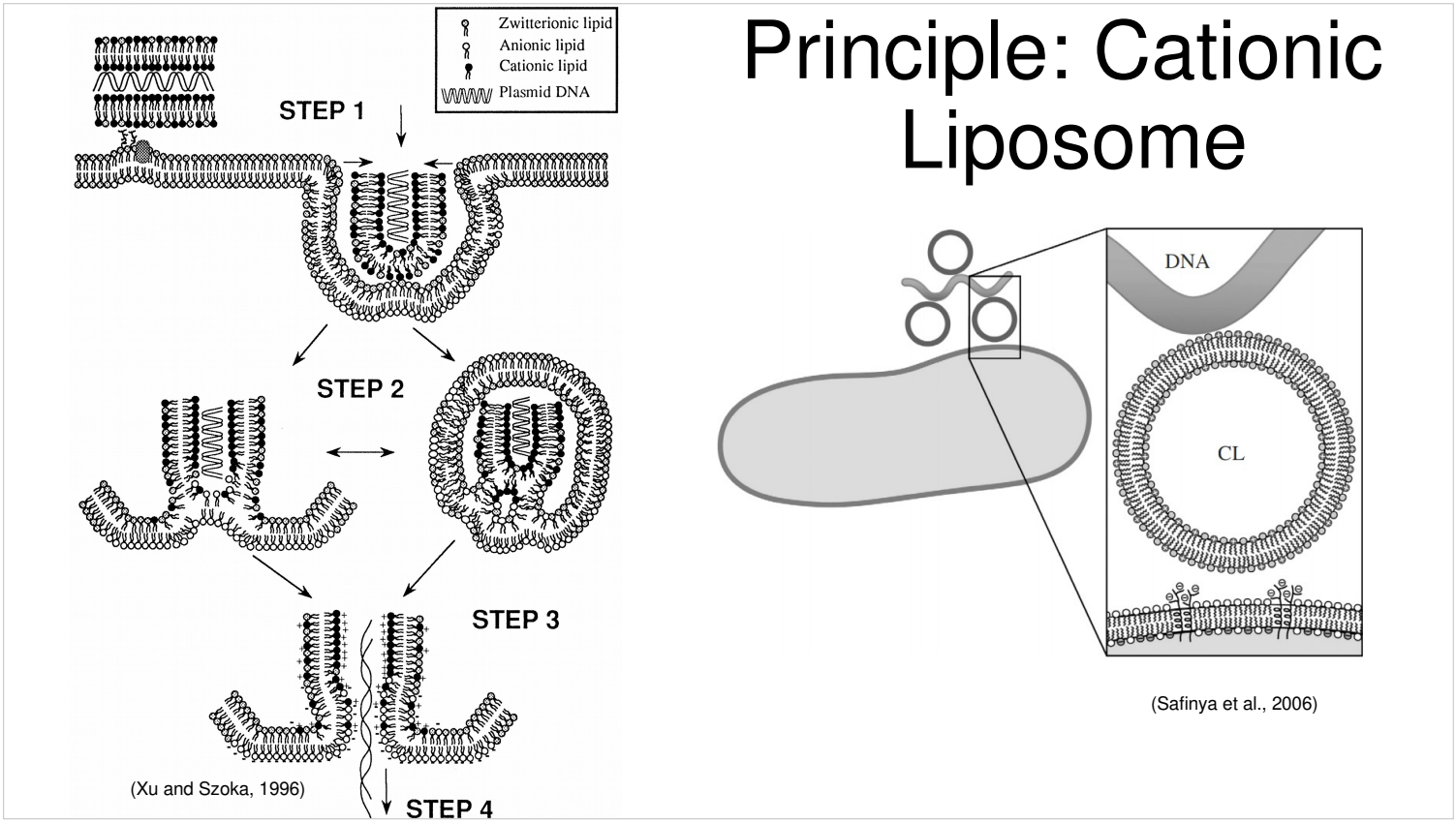


F IGURE 5: Mechanism of uptake and release of plasmid DNA from the complex. (Step 1) After electrostatic interaction with the cell membrane, cationic liposome/DNA complexes are endocytosed. (Step 2) In the early endosome, membrane destabilization results in anionic phospholipid flip−flop. (Step 3) The anionic lipids diffuse into the complex and form a charge neutral ion pair with cationic lipids. (Step 4) The DNA dissociates from the complex and is released into the cytoplasm.

Cartoon of a ‘beads−on−a−string’ (i.e. CLs attached to a string of DNA) complex electrostatically bound to the surface of an animal cell. The blow−up shows a CL electrostatically binding a section of negatively charged DNA on one side and the plasma membrane containing cell surface proteoglycans with negatively charged sulphated groups on the other side. X−ray diffraction studies have shown that the equilibrium phases of mixtures of CLs and DNA are composed of higher ordered self−assembled liquid crystalline structures, two of which are depicted in figures 2 and 8.

Problems with traditional methods

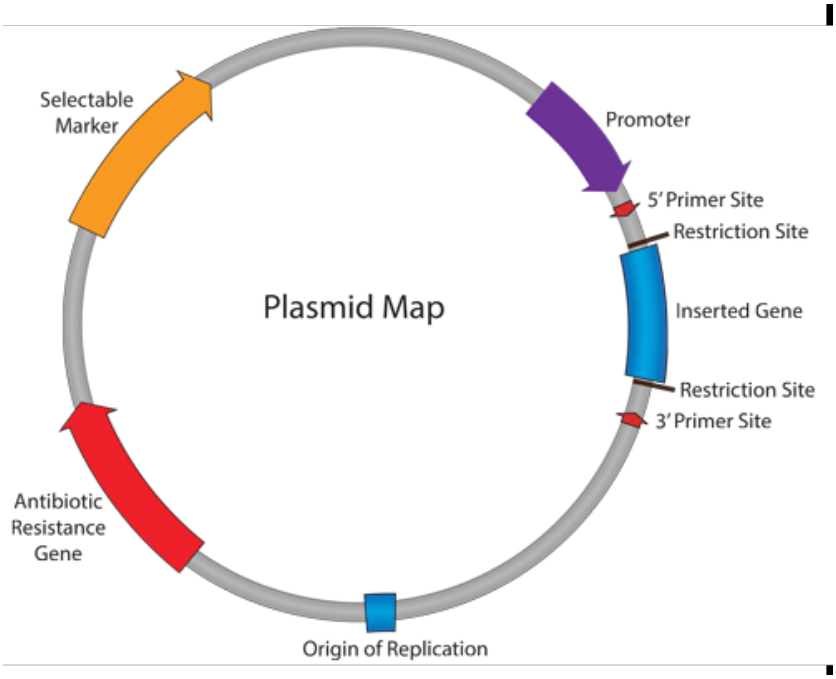
Methods like calcium phosphate co-precipitation, DEAE-dextran, polybrene, and electroporation include problems such as:

- low efficiency of DNA delivery
- poor reproducibility
- cell toxicity
- inconvenience

In contrast, lipid me transfection:

- Yields high and previously transfection efficiencies
- Works in a wide variety o
- Is simple to perform
- Ensures consistently repr

Moreover, a number of cell li to transfection by other meth



Principle: Vector Design

- Reporter Gene vs Marker
- Fluorescence Protein
- Nuclear Localization Signal or Sequence (NLS)

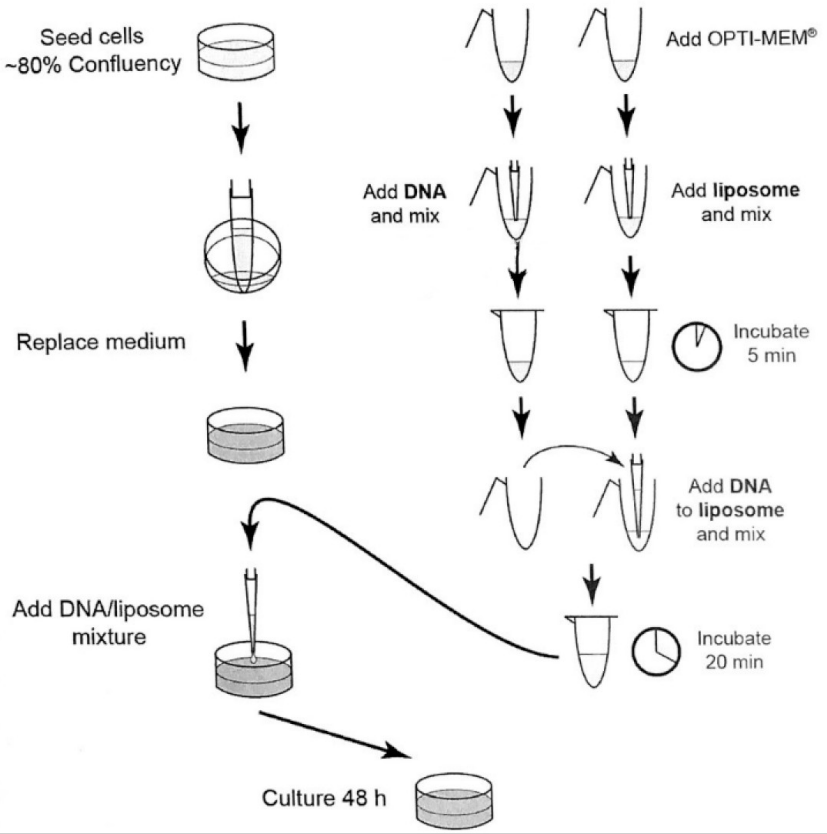
A marker gene is a gene used in molecular biology to determine if a nucleic acid sequence has been successfully inserted into an organism's DNA. There are two types of marker genes: a selectable mar ker (Antibiotics) and a marker for screening(Green fluorescent protein).

a reporter gene (often simply reporter) is a gene that researchers attach to a regulatory sequence of a nother gene of interest in cell culture, animals or plants. Certain genes are chosen as reporters becau se the characteristics they confer on organisms expressing them are easily identified and measured(G reen fluorescent protein), or because they are selectable markers(Antibiotics).

I think they are almost similar. depends on the goal of your project definition will be different

https://www.researchgate.net/post/What_is_the_main_difference_between_marker_and_reporter_gene.

Procedures



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Reference to manual

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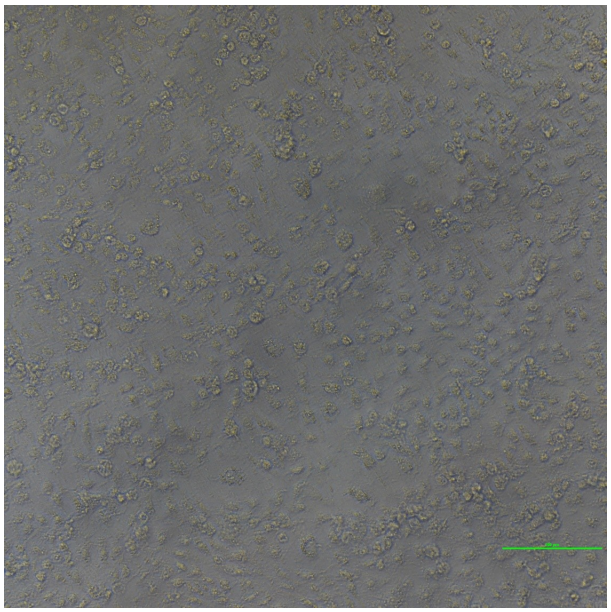

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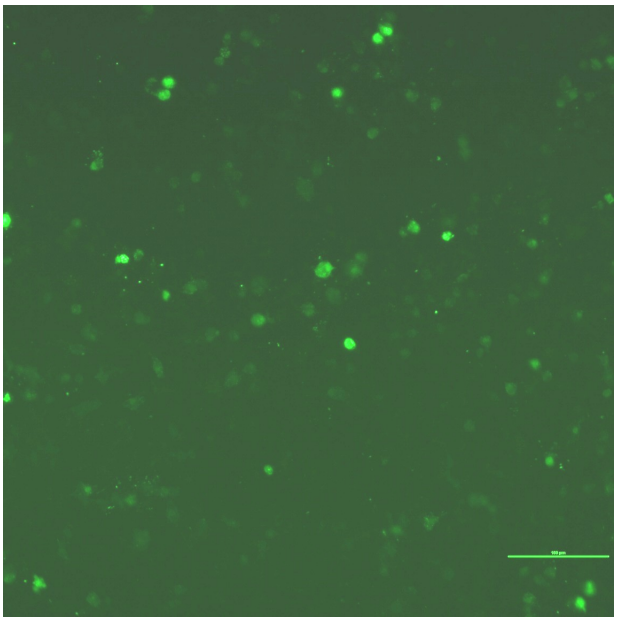
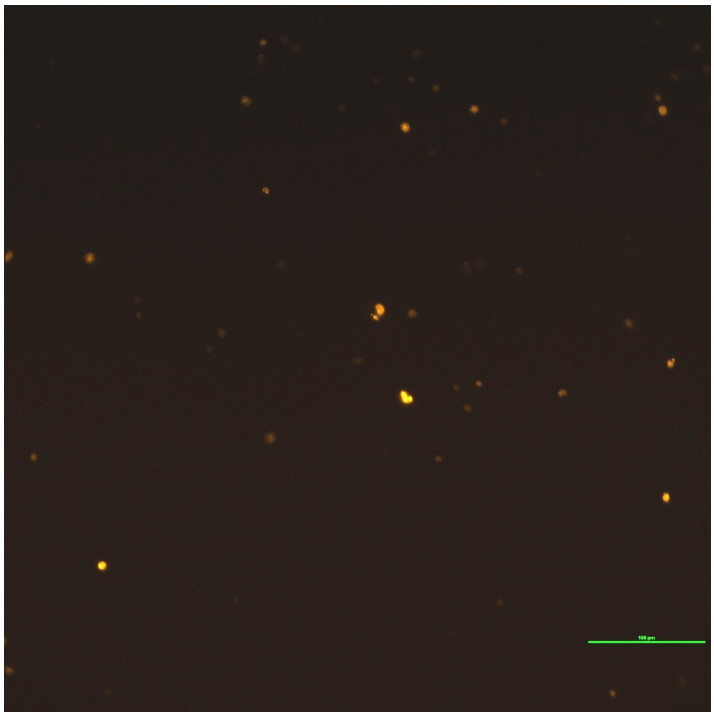


Green Fluorescence



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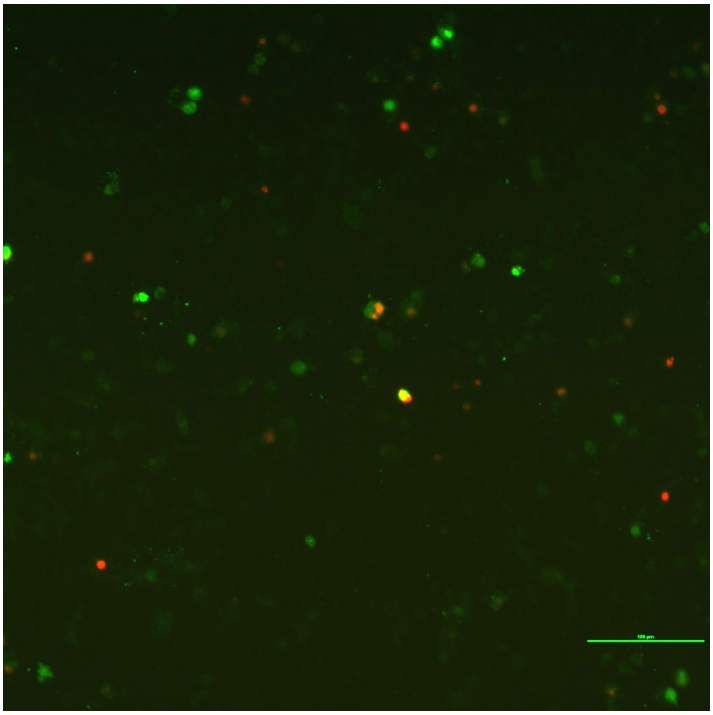

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