7.4 Experiment Report（A）

Experiment : Transient transfection of plasmids in the B138 cell line

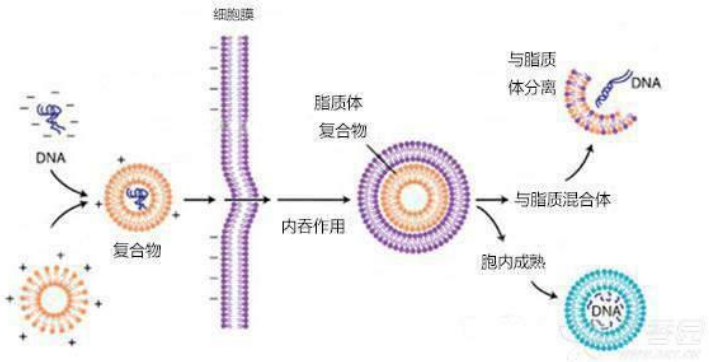
I. **Experimental purpose:** Expression of S1-his and RBD-his proteins required for subsequent experiments

II. **Experimental principle:** Transient transfection of mammalian cells is widely used because of its ability to rapidly express highly active proteins in a short period of time.

Mammalian cells have their own protein folding and post-translational modifications to obtain proteins with more natural activity. Mammalian cells produce proteins in two ways: transient transfection and stable transfection. Transient transfection is characterized by rapid short-term expression and satisfies the preparation of small amounts of proteins. Stable transfection, on the other hand, is capable of satisfying large and long-term production of proteins by constructing stable cell lines.

Transient transfection refers to the introduction of a constructed plasmid into mammalian cells (mainly HEK293 cells) by some means, and the exogenous genes on the plasmid are not integrated into the cell's own genome. As the cell grows and divides, the exogenous genes are gradually lost. The plasmid is able to exist inside the cell for 3-4 days, during which time the plasmid exogenous gene is transcribed and translated inside the cell, resulting in a very small amount of protein. This whole process of rapid transfection to obtain protein is called transient transfection expression.

Lipo liposome transfection principle:



Principle of PEI transfection: The principle of PEI transfection reagent is that the positively charged cationic polymer forms a positively charged complex with the negatively charged phosphate group in the nucleic acid, which then interacts with the negatively charged proteoglycan on the cell surface and enters the cell through cytokinesis of the cell.

III. Experimental procedure:

1. Preparation of transfected cells:

(i) Place HEK293-B138 cells in a 5% CO2 constant temperature shaker and incubate at 37℃ and 120rpm with constant temperature shaking to determine their cell density and viability. To ensure the transfection effect, it is recommended to use cells in exponential growth phase (density of about 3-6×10^ 6 cells/ml) with a survival rate of more than 97% for transfection.

(ii) Cell density was measured by flow cytometry, and the data showed that the cell density of the first group of cells was 1.03×10^6, and the survival rate was 96.4%; the cell density of the second group of cells was 3.31×10^6, and the cell survival rate was 98.6%.

(iii) The cells were directly mixed into CD05 medium without centrifugation (the medium could not be pre-warmed), and the cell density was diluted to 3×10^6 cells/ml.

(iv) Place the shake flask in a 5% CO2 shaker, incubate at 37℃ and 120rpm with constant temperature shaking for 10min and then transfect (at this time you can incubate the transfection reagent-plasmid complex).

(v) Prepare 126ug/ul of S1 protein (Subunit 1) solution and 220.9ug/ul of RBD protein (Receptor Binding Domain) solution, extract 25ug each and put them into the constant temperature shaker.

2. Transient transfection

(i) Prepare culture medium (50 ml DMEM + 10% serotonin).

(ii) Take out the prepared (cell density of 3×10^ 6 cells/ml) two bottles of B138 cell suspension from the thermostatic shaker

(iii) Add PEI-plasmid complex drop by drop and place in a constant temperature incubator (37℃, 5% CO2, 120rpm).