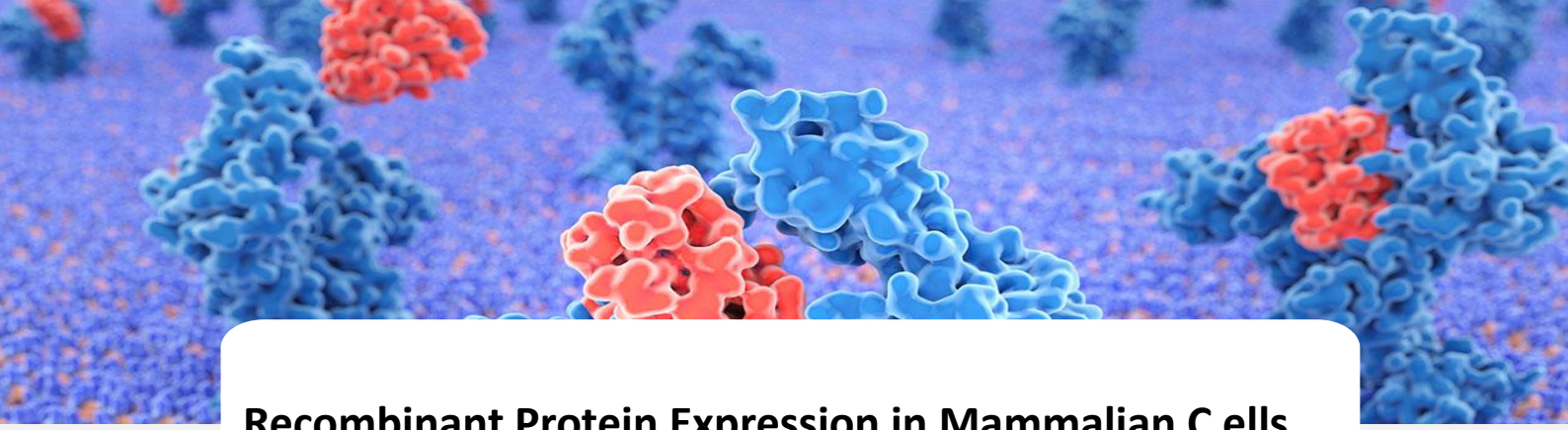

Recombinant Protein Expression in Mammalian C ells

Alpha Lifetech

1. Introduction

Mammalian cells require an transfection reagent, such as lipofectamine 2000, lipofectamine 3000, etc., for protein expression. Mammalian cell expression system, as Eukaryotic expression system, which is selected by most researchers due to its expressed protein has correct high-level structure. The basic flow of recombinant protein expression in mammalian cells is shown in Figure 1.

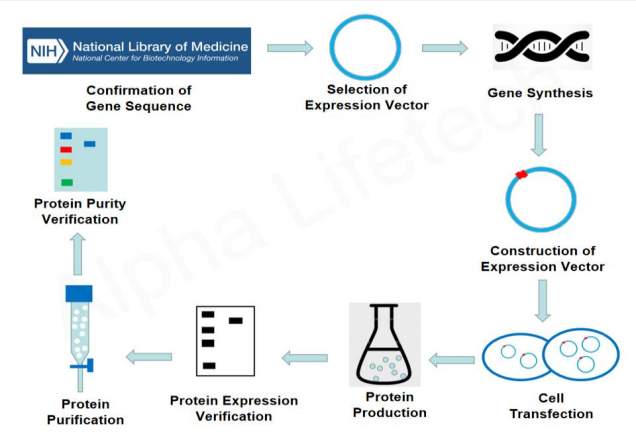

Fig 1 Recombinant Protein Expression in Prokaryotic Cells

2. Q&As for Recombinant Protein Expression

Q1: When are mammalian expression systems chosen?

A1: Proteins produced in mammalian cells have the advantage of containing all post-translational modifications (e.g., proper folding, glycosylation, phosphorylation, etc.) of the expressed protein. However, mammalian expression levels tend to be lower and therefore more costly to achieve large amounts of protein expression (> 10mg) is more difficult.

Q2: Low transfection efficiency

A2: Possible causes:

(1) Plasmid DNA, siRNA, or transfection reagents, diluted in medium containing serum, or formed complexes in the presence of serum.

The measures:

Serum-free medium was used to dilute plasmid DNA, siRNA, and transfection reagents.

(2) The dilution time or compound culture period is too short.

The measures:

We recommend that DNA and transfection reagents be diluted and incubated for 5 minutes. Diluted reagents were added together and incubated for 20 min for optimum performance.

(3) The transfected plasmid DNA or siRNA was degraded or of poor quality.

The measures:

Ensure that the plasmid DNA or siRNA used for transfection is of high quality.

(4) Inhibitors are present in the culture medium.

The measures:

Do not use antibiotics, EDTA, citrate, phosphate, RPMI, chondroitin sulfate, hyaluronic acid, glucan sulfate, or other sulfated proteoglycans in growth media or in media used to prepare DNA: transfection reagent complexes.

(5) Cells do not recognize promoters/enhancers on the vector.

The measures:

Verify that the promoter/enhancer on the vector construct is compatible with the target cell type.

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Q3: The cell viability after transfection was low

A3: Possible causes:

(1) During transfection, antimicrobial agents were added to the growth medium.

The measures:

Do not use antibiotics such as chloroquine, penicillin, or streptomycin in the growth medium, as cells are more easily permeated by antibiotics during transfection and may become toxic.

(2) Improper storage of transfection reagents.

The measures:

We recommend that transfection reagents be stored at 4°C. Freezing or storing at room temperature may reduce the activity of the reagent.

(3) The complex was not adequately mixed in the growth medium.

The measures:

After addition of the transfection complex to the medium, ensure that the plates or Wells are thoroughly mixed.

(4) The cationic lipid reagent is oxidized.

The measures:

Do not over vortex or agitate cationic lipid reagents, which may form cationic lipid reagents peroxides.

(5) Selective antibiotics are added too quickly.

The measures:

When stable cell lines are established, cells are allowed to express resistance genes at least 72 h before addition of selective antibiotics.

Q4: Transfection results were not reproducible

A4: Possible causes:

(1) Over time, the cell changes, or the conditions of division change.

The measures:

If the transfection performance suddenly decreases, it may be related to the cell. We recommend dividing and plating cells on a consistent schedule and keeping the cell density not too sparse or too dense. Over-division also reduced transfection performance.

(2) Transfection was performed with different cell confluence or with different ratios of DNA to transfection reagents.

The measures:

The reproducibility of transfection performance depends on the consistency of cell division, plating, and transfection using a consistent protocol (the same ratio of DNA to transfection reagents). The transfection performance may also be altered by changes in different DNA preparations or media.

3. Characteristics of mammalian expression systems

(1) Unique advantages in protein initiation signal, processing, secretion and glycosylation.
(2) Suitable for expressing complete macromolecular proteins.
(3) The activity of the expressed foreign protein is closer to that of the native protein.
(4) The structure is complex and the operation technology is high.
(5) The expression yield is low, and sometimes leads to virus infection.