Molecular cloning and genetic engineering - Ultrasonic Crushing

Aim

There are many methods to break E. coli cells, such as repeated freeze-thaw method, osmotic impact, ultrasonic breaking, pressure breaking, etc. Ultrasound crushing and pressure crushing are common methods in our experiment. In this protocol, we document ultrasound crushing method.

Material

PBS or Tris-NaCl

Procedure

In the process of establishing expression conditions, a small amount of samples can be prepared by a miniature ultrasound cell disintegrator. After centrifugation for 1.0-1.5 ml, the bacteria were collected and suspended in a buffer of 300-500 ml depending on the amount of the bacteria. The cells were then broken by ultrasound on ice.

1. 100 ml induced bacterial solution (OD_{600} = 1.2), 12000 rpm, centrifuged at 4 for 2min, collected bacteria.

2. Add 50 ml of pre-cooled buffer, wash the bacteria with suspension cells once, 12000 rpm, centrifuge at 4℃ for 2 min, and collect the bacteria; PBS or

Tris-NaCl are the most commonly used buffers in routine operation.

3. Add 15 ml buffer to the sediment as above (if the bacterium is too thick, it can be doubled) and suspend sufficiently. Place the bacterium suspension in a small glass beaker and place it in a mixture of ice and water.

4. Crushing: Put the ultrasonic probe washed with buffer into the bacterium solution, do not touch the bottom of beaker. Cool the bacterium solution every 30sec interval for 1minute. The output strength is 5-6 and the frequency is 60-70%.

5. Separation supernatant and precipitation: 12000 rpm, centrifugation at 4℃ for 20 min, separation supernatant and precipitation.

6. SDS-PAGE analysis of protein expression.

7. Preparing to purify the protein.

Note

1. The judgement of complete fragmentation: the suspension of bacteria before ultrasound is turbid, and after ultrasound is complete, it becomes transparent and clear.

2. If black precipitation occurs during ultrasound, the power of ultrasound is too strong.

3. Too long ultrasound time and too high power will definitely affect the

activity of protein.

4. Prevent foam as far as possible.

5. When a large amount of bacterial liquid is broken, it is placed in a glassware and ice-water mixture to increase heat dissipation and reduce the damage to protein.

6. Pretreatment before crushing, lysozyme (100 ug/ml) can be used to treat the broken cell wall (10min, 30℃) before crushing to increase cell permeability.