Plasmid Transformation

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1. **Experiments:**

   Transformation of Plasmid DNA

2. **Demonstration:**

   Plasmid extraction

Characteristics of plasmids

Plasmid is a type of DNA existence within bacteria

- extrachromosomal genomes
- self-replicating
- commonly used vectors

Some bacterial characteristics are determined by plasmids

1.Antibiotic resistance

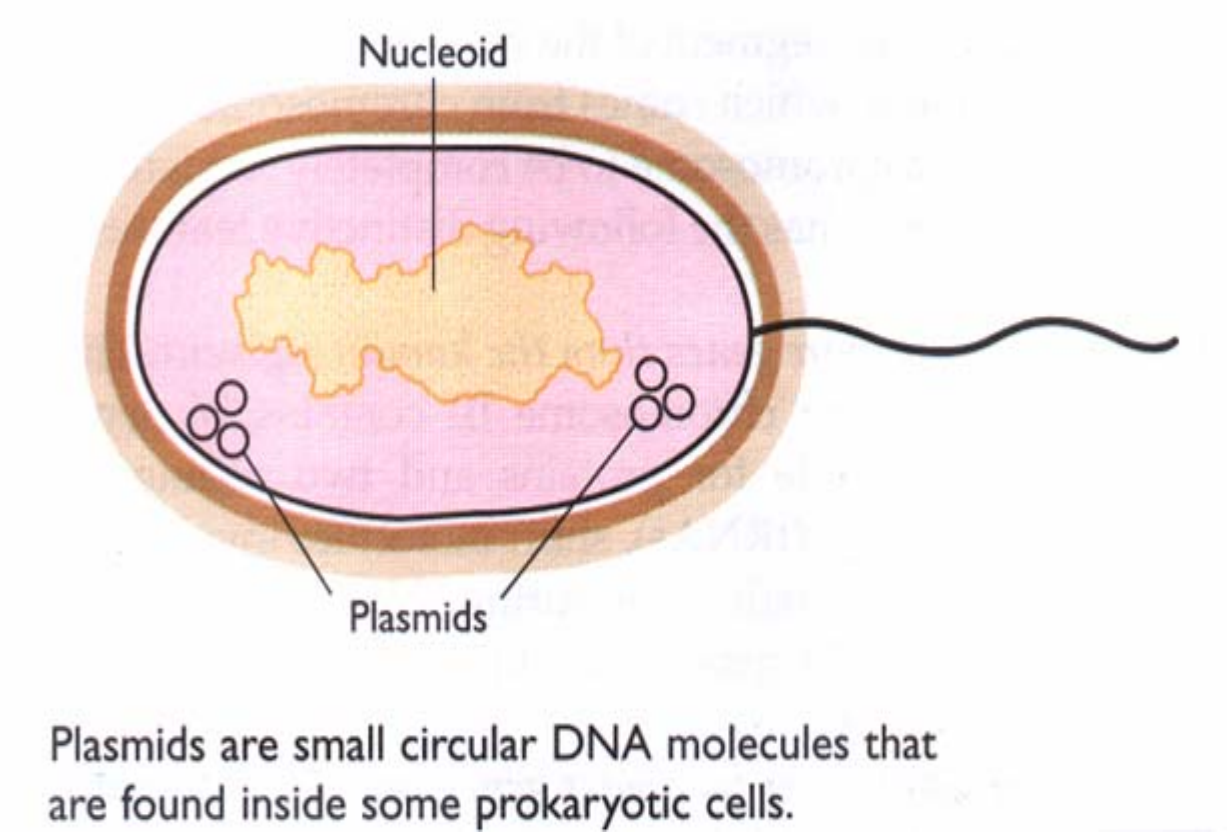
Many bacteria can become resistant to antibiotics by acquisition of a plasmid

2.Colicins and bacteriocins

Another property conferred by some plasmids that has been widely studied is the ability to produce a protein which has an antimicrobial action

3.Virulence determinants

In some bacterial species toxin genes are carried on plasmids



Plasmids are small circular DNA molecules that are found inside some prokaryotic cells.

schematic diagram of plasmid

Transformation

Gene Transfer

(1)Transformation, in which a cell takes up isolated

DNA molecules from the medium surrounding it.

(2) Conjugation

(3) Transduction

The developing prosess of transformation

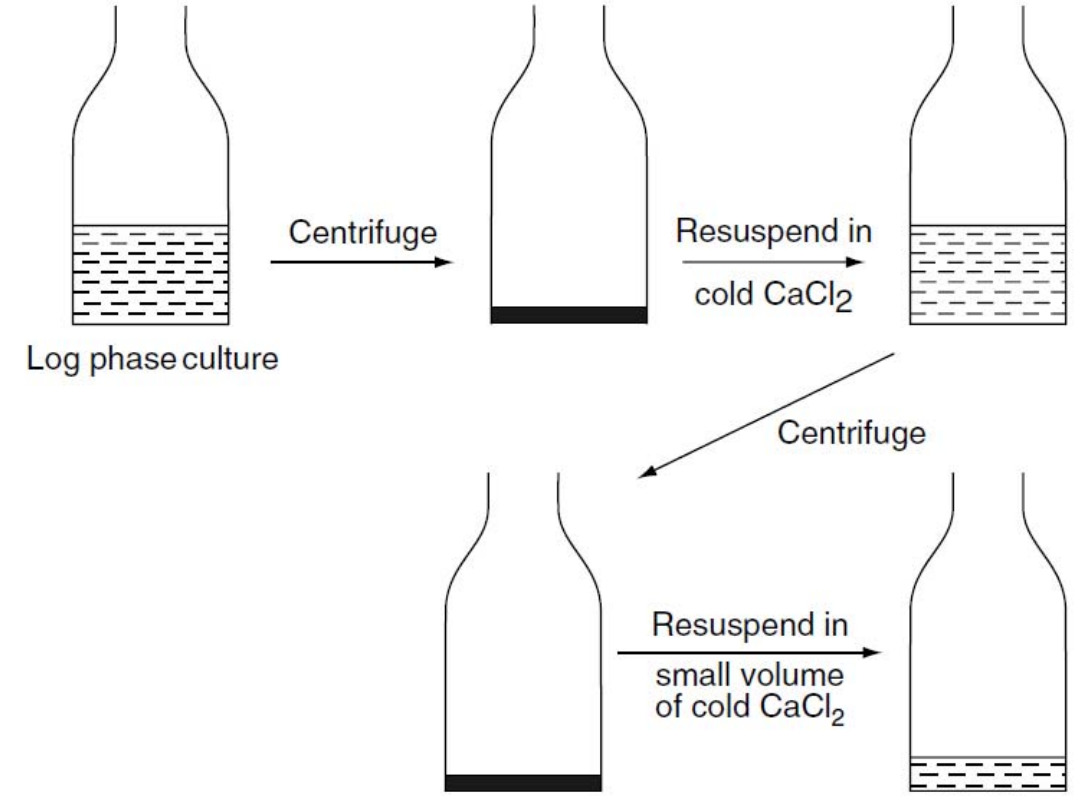
1928，Fred Griffith, working with pneumococcus(肺炎链球菌) avirulent strains could be restored to virulence by incubation with an extract from killed virulent cells.

1944，Avery, MacLeod and McCarty demonstrated that the ‘transforming principle’ was DNA.

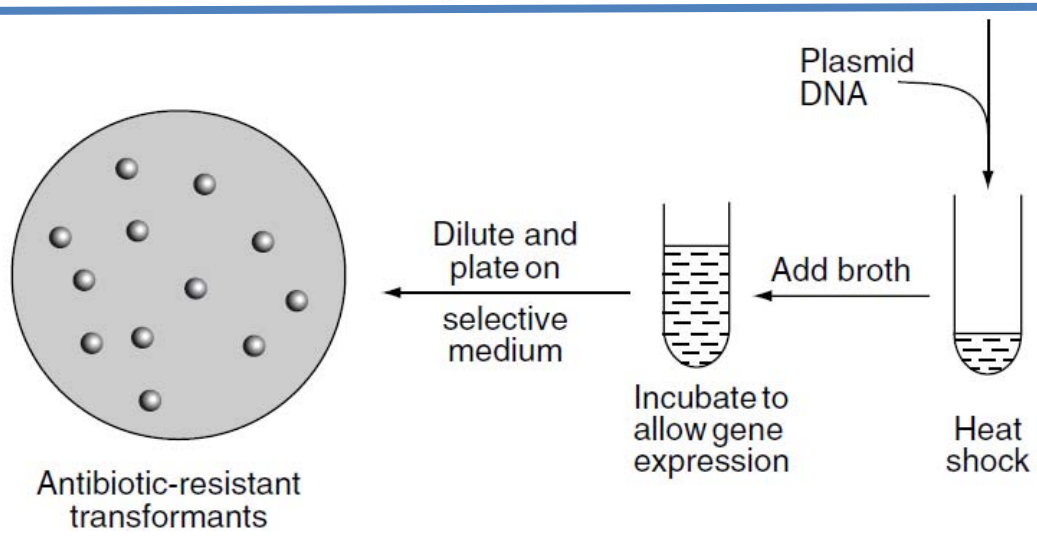
1970，Mandel and Hlga did the transformation by competent cells（chemical method，CaCl，deal with cells）

Now， transformation is very important because of its key role in gene cloning.

How to get competent cells?



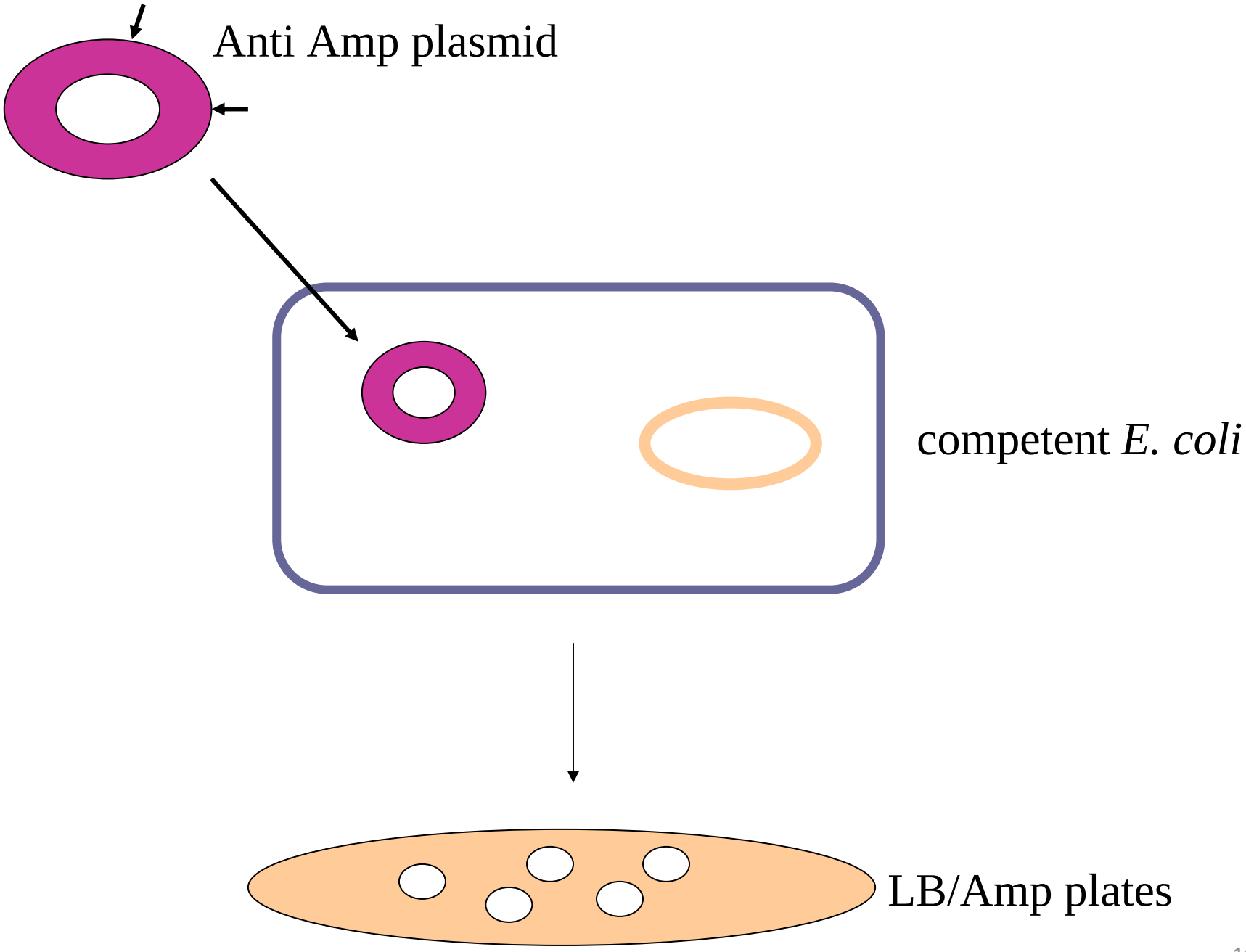
transformation



Experiment:
Transformation of Plasmids into *E. coli*

Purpose:

To utilize competent *E. coli* bacteria to replicate a specific DNA fragment.


Anti Amp plasmid
competent *E. coli*
LB/Amp plates

Materials required:

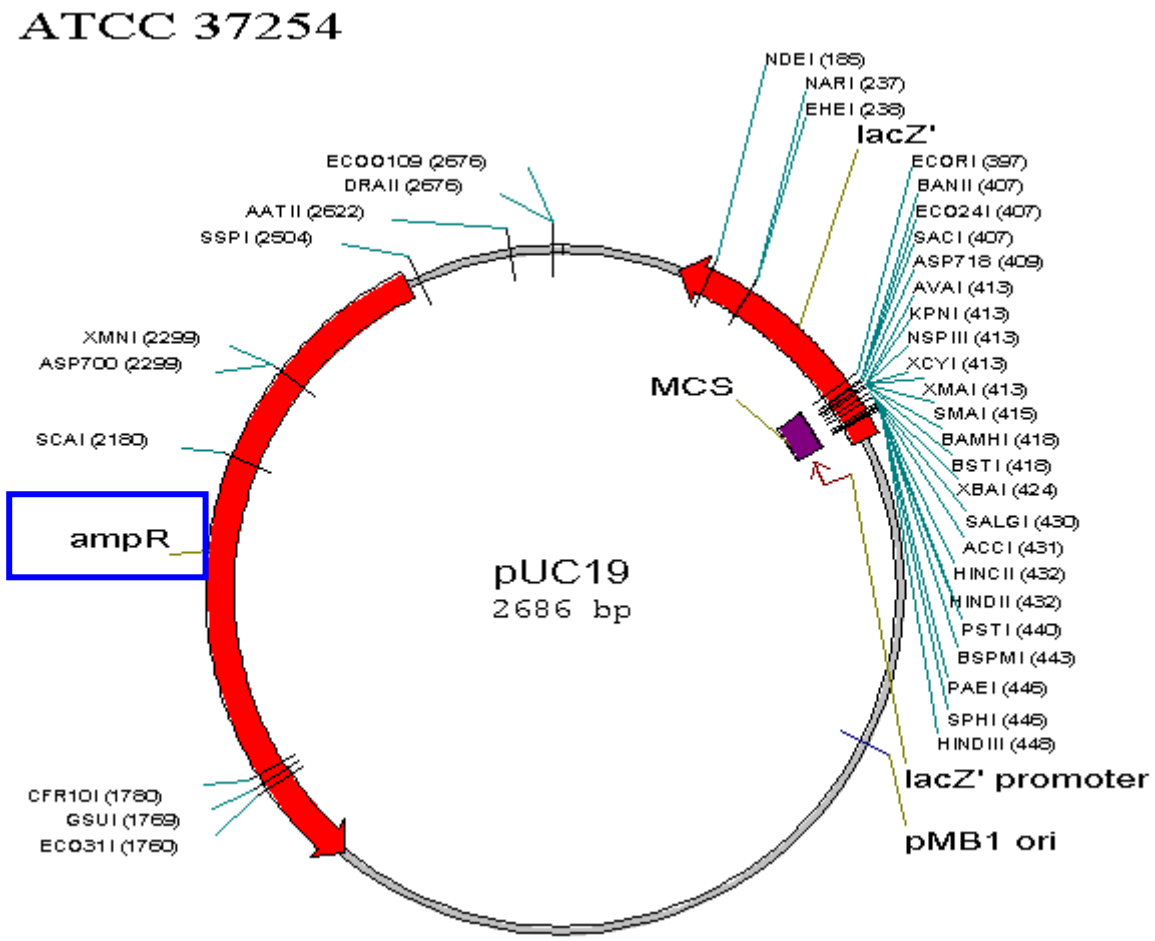
Competent Cells (DH5- α cells)

Anti Amp Plasmid

LB/Amp plates

LB plates

Lauria-Bertani (LB) culture



Ampicillin 2486 - 1626

Time required:

Transformation: about 1 hour (Day 1)

Growth: approximately 16 hours for visible colonies (Day 2)

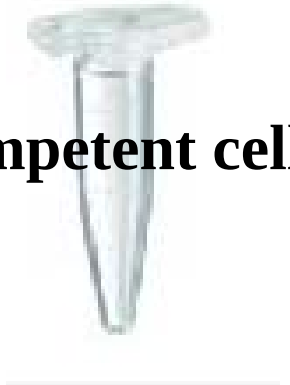
Control

Transfer same volume H_2O to competent cells

Spread on **LB/Amp plate or LB plate**

Basic procedure for plasmid transformation of E. coli

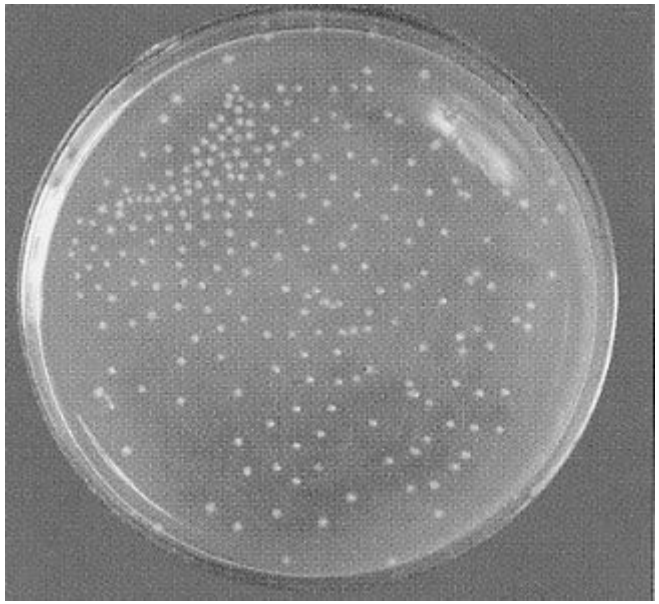
1. **1ul Plasmid**
   1ul H_2O(control)
   add gently → **competent cells**



2. **on ice ,30 minutes**
3. **42** ℃ water bath heat shock, **90 seconds**
4. **on ice ,2 minutes**
5. add 800 μl of LB, **37**℃ shaker ,**30 minutes**
6. **spread** 200 ul competent cells on **LB/Amp plate**
7. **spread** 200 ul control on **LB/Amp or LB plate**
8. **room temperature** until the liquid has been absorbed
9. invert the plates and **incubate at 37**℃

Day 2

Examine the plates and determine the efficiency of transformation.



Transformation ratio:

Sum of converter =number of colonies × multiple of dilution × volume of transformation reaction /volume of bacterium transfer to plate

Sum of competent cell= colonies on control 2 × multiple of dilution × total volume of bacterium/volume of bacterium transfer to plate

Transformation ratio of competent cell= Sum of Converter/ sum of competent cell

Question or discussion:

What do the results look like on the control plates?

Can transformation happen naturally ?

Alkaline Lysis Minipreps of Plasmid DNA

Principle:

Alkaline Lysis procedure is to take advantage of the alkaline denaturation of plasmid and chromosomal DNA and of the selective renaturation of plasmid DNA following neutralization of the solution.

Reagents

- Alkaline lysis solution I

  glucose, EDTA, Tris-HCl

- Alkaline lysis solution II (Lysis Solution)

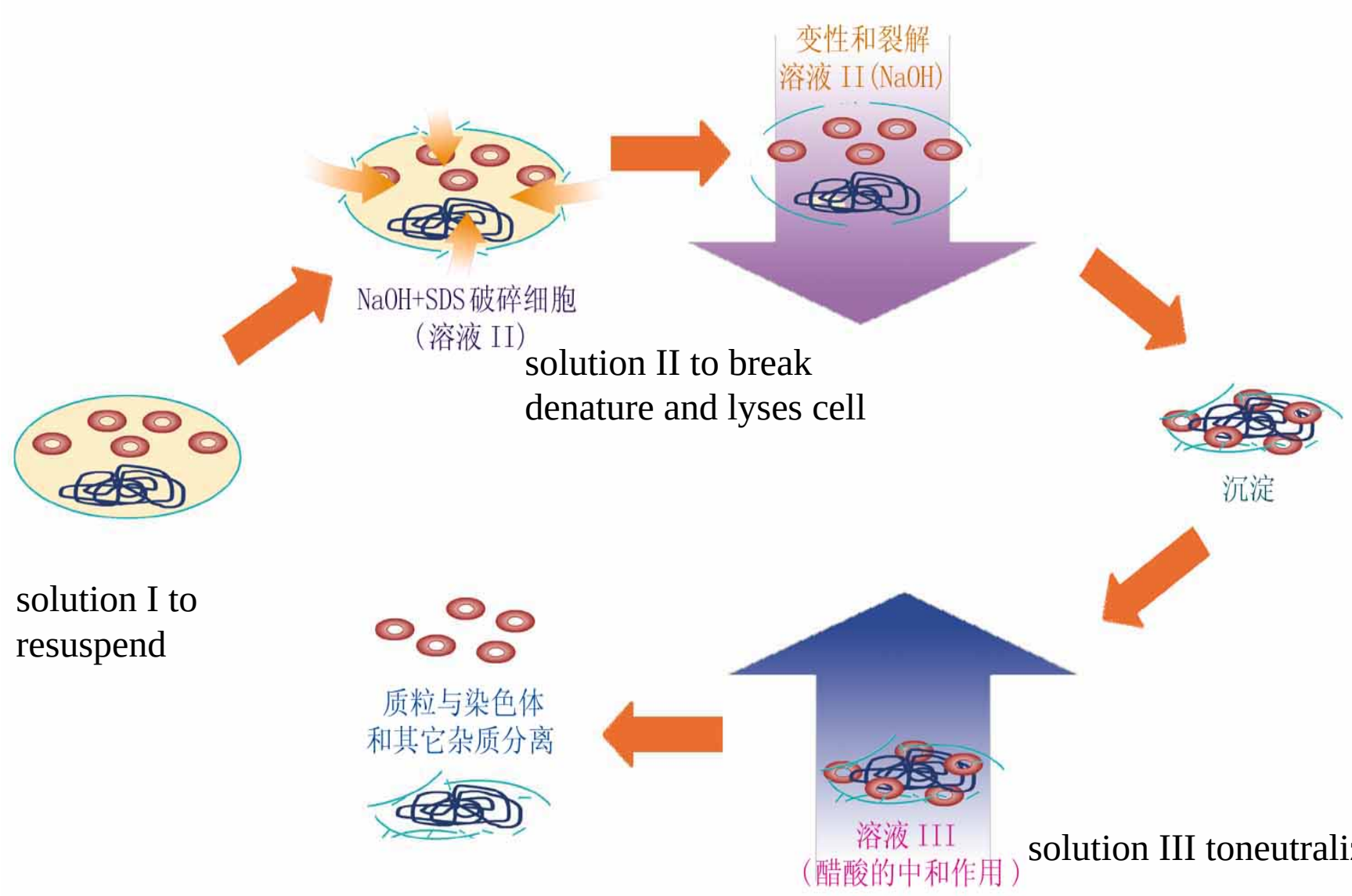
  NaOH, SDS

- Alkaline lysis solution III

  potassium acetate, glacial acetic acid

- Ethanol

Principle



solution II to break denature and lyses cell

solution I to resuspend

solution III toneutralize

Day 1

Inoculate 4 ml of LB medium containing 50 ug /ml ampicillin with a single bacterial colony containing the desired plasmid. Incubate at 37 ℃ overnight with vigorous shaking.

Laboratory manual page 31

1. Pour 1.5 ml of the culture into a microfuge tube. Centrifuge at maximum speed for 30 seconds at 4 ℃ in a microfuge.
2. Remove the medium by aspiration, leaving the bacterial pellet as dry as possible.
3. Resuspend the bacterial pellet in 100 µl of ice-cold Alkaline lysis solution I by vigorous vortexing.
4. Add 200 µl of freshly prepared Alkaline lysis solution II to each bacterial suspension. Close the tube tightly, and mix the contents by inverting the tube rapidly five times. Store the tube on ice.
5. Add 150 µl of ice-cold Alkaline lysis solution III. Close the tube and disperse Alkaline lysis solution III through the viscous bacterial lysate by inverting the tube several times. Store the tube on ice for 3-5 minutes.

6. Centrifuge the bacterial lysate at maximum speed for 5 minutes at 4 ℃ in a microfuge. Transfer the supernatant to a fresh tube.
7. Precipitate nucleic acids from the supernatant by adding 2 volumes of ethanol at room temperature. Mix the solution by vortexing and then allow the mixture to stand for 2 minutes at room temperature.
8. Collect the precipitated nucleic acids by centrifugation at maximum speed for 5 minutes at 4 ℃ in a microfuge.

9.Remove the supernatant by gentle. Stand the tube in an inverted position on a paper towel to allow all of the fluid to drain away.

- Add 1 ml of 70% ethanol to the pellet and invert the closed tube several times. Recover the DNA by centrifugation at maximum speed for 2 minutes at 4 ℃ in a microfuge.
- Remove all of the supernatant by gentle .
- Dissolve the nucleic acids in 50 µl of TE (pH 8.0) containing 20 µg/ml DNase-free RNase A. Vortex the solution gently for a few seconds. Store the DNA solution at -20 ℃ .