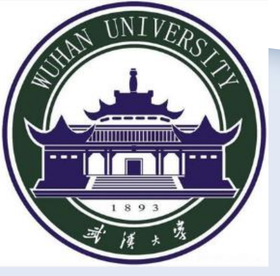


ISOLATION and PURIFICATION of Plasmid DNA

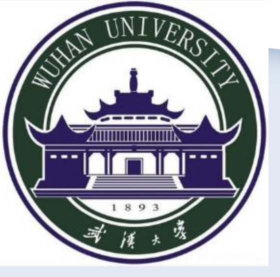
Contents

1 Preparation of Plasmid DNA (SDS-Alkaline Lysis Method) p138

2 Identification of DNA by UV-Spectrophotometry and gel electrophoresis p145

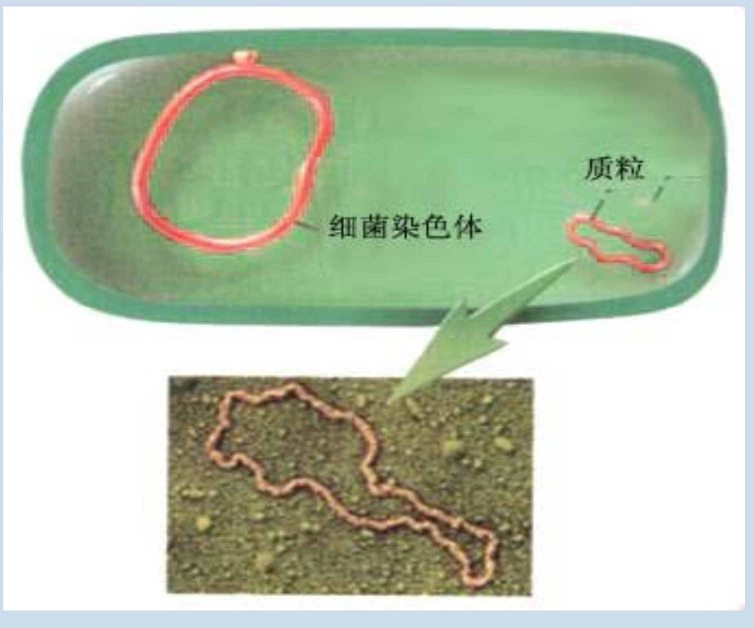
PREPARATION and ANALYSIS of DNA

1. Preparation of Nucleic Acids (DNA, RNA) is the most important first step in the research work of molecular biology, such as studying on the structure and function of gene through *genetic engineering*.
2. Identification of the purity and quality of DNA could use UV Spectrophotometry or gel electrophoresis.
3. Analysis of DNA has lots of methods, such as: DNA hybridization, PCR, DNA sequencing, et al.

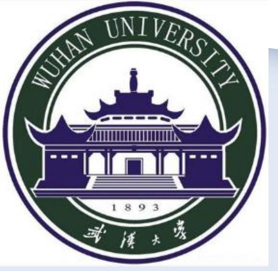


Isolation and Purification of Plasmid DNA

- A plasmid is **small**, **extra-chromosomal**, **circular** DNA molecule that replicates **from 2 to ~200 kb in size, which exist in multiple copies within the host cells.**

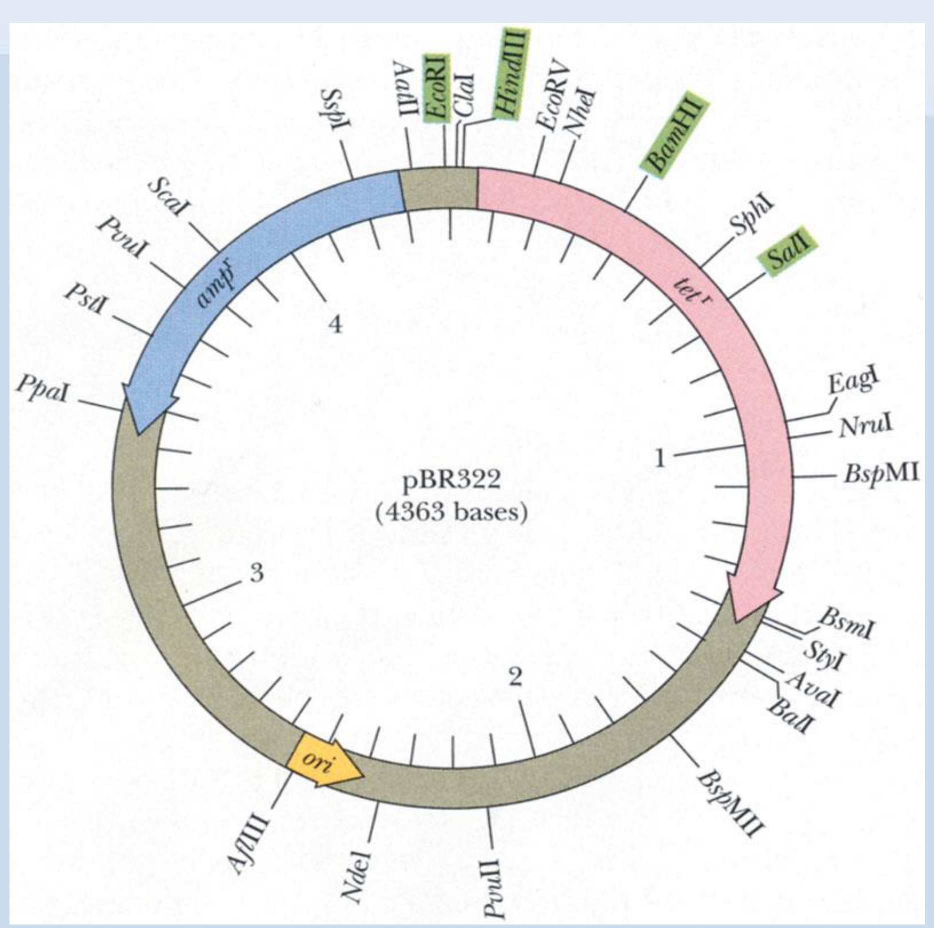


They carry genes specifying novel metabolic activities that are advantage to the host cell.



double-stranded, closed circular DNA molecules.

Contain an origin of replication, MCS and at least one selective marker (eg. amp^{r} gene encoding the enzyme which degrades ampicillin). The commonly used plasmid are small (~ 4 kb).



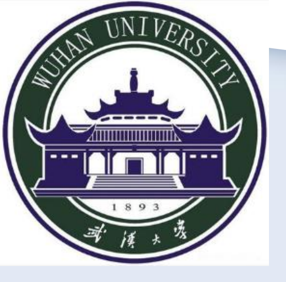
The structure Map of pBR322 (cloning vector)

Isolation and Purification of Plasmid DNA

- Plasmids can carry foreign DNA into bacteria for amplification or expression, so it is called **vectors** in genetic engineering.
- The isolation and extraction of plasmid DNA are the most popular techniques in molecular cloning.

Basic procedure

Step 1	Step 2	Step 3	Step 4
growth of bacteria containing the plasmid	collect the bacteria from the culture medium	Isolation and Purification	Concentration Storage



Isolation and Purification of Plasmid DNA

Methods for preparation of plasmid DNA include

SDS-alkaline denaturation method

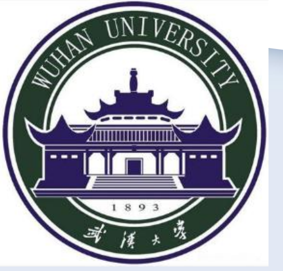
salt-SDS precipitation

Gradients centrifugation in CsCl-ethidium bromide,

Purification kits by adsorption , etc.

Notes of the preferred method

- Simple and rapid.
- Reduce degradation of DNA by mechanical damage, chemical degradation, such as vortex, strong stirring, repeatedly frozen storage.
- Prevent degradation of DNA by hydrolysis of nucleases. EDTA and citrate in buffers could inhibit the DNase activity.



Isolation and Purification of Plasmid DNA

1. To lyse the bacteria
2. To remove contaminating proteins, RNA, chromosomal DNA and other macromolecules by basic enzymatic or chemical methods.
3. Solubilize the DNA

Isolation of Plasmid DNA by SDS-Alkaline Lysis using purification Kit

Principle

- This method are **based on denaturation and renaturation** characteristics of covalently closed circular plasmid DNA and chromosomal DNA fragments.
- Under alkaline conditions (at pH 12), both plasmid and chromosomal DNA are efficiently denatured.

Isolation of Plasmid DNA by SDS-Alkaline Lysis using purification Kit

Principle

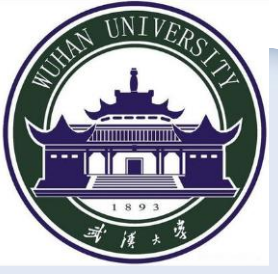
- Rapid neutralization with a high-salt buffer such as potassium acetate in the presence of SDS has two effects： First, the large, chromosomal DNA and protein form insoluble aggregates that precipitates out of solution, while the smaller, circular plasmid DNA rehybridization, remaining in solution.
- After centrifugation, Separation of soluble and insoluble material is accomplished by a clearing method, then the soluble plasmid DNA is further purified (e.g., precipitation, adsorption by column).

Procedure

1. Pipette 1~4 ml of *E. Coli* to a 1.5 ml Ep tube, then centrifuge at 10 000 rpm for 1 min. Repeat one time and discard the supernatant.
2. Add 250μl of Buffer S1. Votex it for 20 sec to resuspend bacteria.
3. Add 250 μl of Buffer S2 (SDS, NaOH, pH12), Mix it gently for 20 sec and keep for 3~5 min (less than 5 min).
4. Add 350 μl of Buffer S3 (NaAc, pH4.8). Mix it by hand gently for 20 sec, and then centrifuge at 12 000 rpm for 12 min.
5. Transfer the supernatant carefully to a column tube within a 2 ml collecting tube.
6. Centrifuge at 12 000 rpm for 1 min and discard the elution.

Procedure

7. Add 500 μl of Washing Buffer W1. Centrifuge at 12 000 rpm for 1 min and Discard the elution.
8. Wash the column tube by adding 700 μl of Washing Buffer W2. Centrifuge 1 min at 12000 rpm, discard the elution. Then centrifuge 2 min at 12000 rpm for emptying Ep tube).
9. Put the column tube to a new 1.5 ml Ep tube, add 60-80 μl Eluent buffer (65 ℃) to dissolve DNA for 5 min.
10. Centrifuge 1 min at 12000 rpm, transfer elution to the upper column tube, centrifuge 1 min again,then discard the column.
11. Employ UV Spectrophotometry and gel electrophoresis to determine the purity and yield of DNA isolation or store the samples at -20 ℃.



Procedure

| 250 μl Buffer S1

| 250 μl Buffer S2

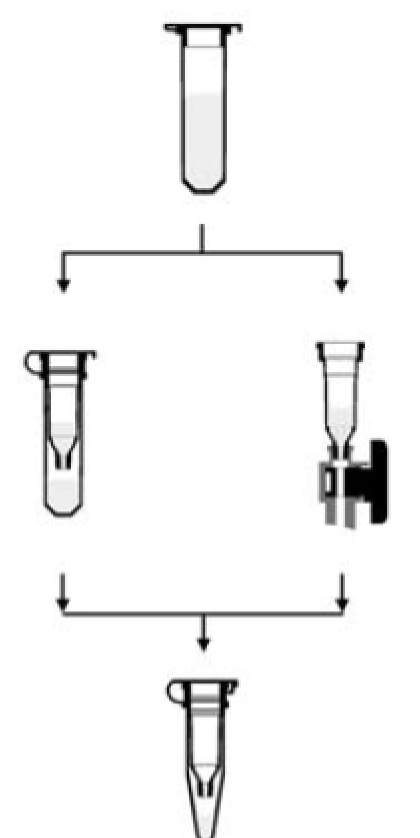
| 350 μl Buffer S3

| 500 μl Buffer W1

| 700 μl Buffer W2

| 700 μl Buffer W2

|60-80 μl Eluent 或去离子水



Lyse bacteria

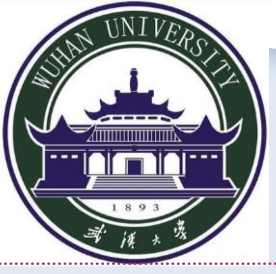
Denaturation

neutralization

Binding column

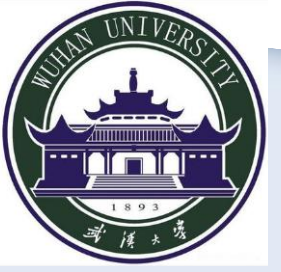
Washing (Centrifugation)

Elution (collection)



Identification and Analysis of DNA

1. Quantitative and Qualitative Analysis P145
2. Gel Electrophoresis P145
3. Hybridization of Nucleic Acid
4. Polymerase Chain Reaction, PCR
5. DNA Sequencing



DNA Determination by UV Absorption

- With the use of UV spectrophotometry, the identification and quantitative analysis of nucleic acids has established as a routine method in many labs.

- DNA, RNA, oligonucleotides and even mononucleotides absorb UV light very efficiently and they can be measured directly at 260 nm in solutions, the sensitive quantification can be at concentrations as low as 2.5 ng/μl.

DNA Determination by UV Absorption

- Purity determination of DNA interference by contaminants **can be recognized by the calculation of “ratio”.**

DNA Purity = A_{260}/A_{280}

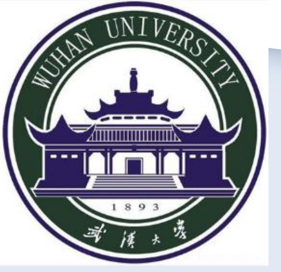
1. Pure DNA should have a ratio of ≈ 1.8 .
2. If $A_{260}/A_{280} < 1.7$, it means the sample still exist protein, Use phenol extractions to remove protein.
3. If $A_{260}/A_{280} > 1.8$, it means still exist RNA, treat it with RNase then remove RNA.

DNA Determination by UV Absorption

- If DNA solution is pure, then calculate
- 1 OD (A) ≈ 50 μg/ml dsDNA,
- 1 OD (A) ≈ 40 μg/ml RNA
- 1 OD (A) ≈ 30 μg/ml oligonucleotides.
- You can calculate the concentration of the pure DNA in your sample as followings:

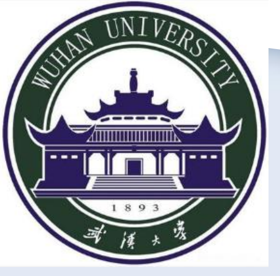
DNA concentration (μg/ml)

= (A_{260}) x (dilution factor) x (50 μg/ml)



Agarose gel electrophoresis of DNA

- DNA is negatively charged molecule, and is moved by electric current though Agarose matrix.
- Agarose gels are often run in a horizontal configuration in an electric field of constant strength and direction.
- The Impact factors include: **Character of DNA (size, conformation, base composition), Pore size of gel, electric field strength and Buffer.**



Agarose gel electrophoresis of DNA

- Agarose gel electrophoresis is usually employed to determine the yield and purity of DNA, and to size fractionate DNA molecules.

  The percentage of Agarose in the gel varied and gels should be prepared depending on the expected sizes of DNA fragments.

  (table 6-1) P126

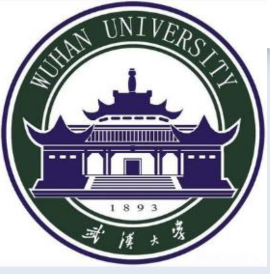
  Agarose gels have a greater range of separation so we must choose proper concentration.

Procedure of Agarose gel electrophoresis

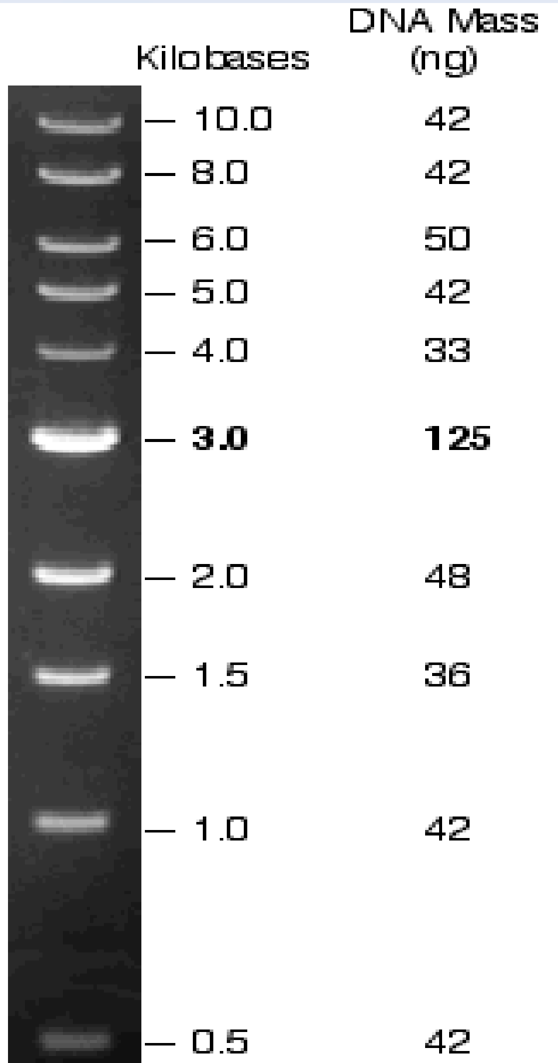
1. **Assemble** the gel casting tray and comb.
2. **Melt** the agarose gels using a microwave oven. When the agarose solution cooled to about 50 ~60°C, **poured gel** into the tray.
3. Carefully remove the comb, **adding** 0.5×TBE **buffer** to cover the gel.
4. **Loading** 10-15 μl DNA samples after mixing DNA loading buffer and turn on the power supply, and **running** 1h at 100 V.
5. Turn off the power supply and transfer the gel into EB **staining** solution for 10-20 min.
6. **Visualize** the DNA fragments under UV light.

Notes of Agarose gel electrophoresis of DNA

- Electrophoresis usually is less than 10 V/cm gel for running 1 hour.
- Size markers are co-electrophoresed with DNA samples, when appropriate for fragment size determination.
- DNA is to enable fluorescent visualization by staining with ethidium bromide (EB) or SYBR gold.
- The minimum amount of DNA detectable by EB on a 3-mm-thick gel and a 5-mm-wide lane is 1 ng.
- Migration of DNA is retarded and band distortion can occur when too much buffer covers the gel.



Agarose gel electrophoresis of DNA



1. **Plasmid**
2. **1kb DNA Markers**

If you see faint or no bands on the gel

1. There was insufficient quantity or concentration of DNA loaded on the gel. Increase the amount of DNA .
2. The DNA was degraded.
3. The DNA was electrophoresed off the gel.
4. Improper UV light source was used for visualization of EB-stained DNA. Use a short wavelength (254 nm) UV light for greater sensitivity.

If you see smeared DNA bands

1. The DNA was degraded.
2. Too much DNA was loaded on the gel.
3. There was too much salt in the DNA.
4. The DNA was contaminated with protein.
5. Small DNA bands diffused during staining.
6. Improper electrophoresis conditions were used. Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30℃ during electrophoresis.