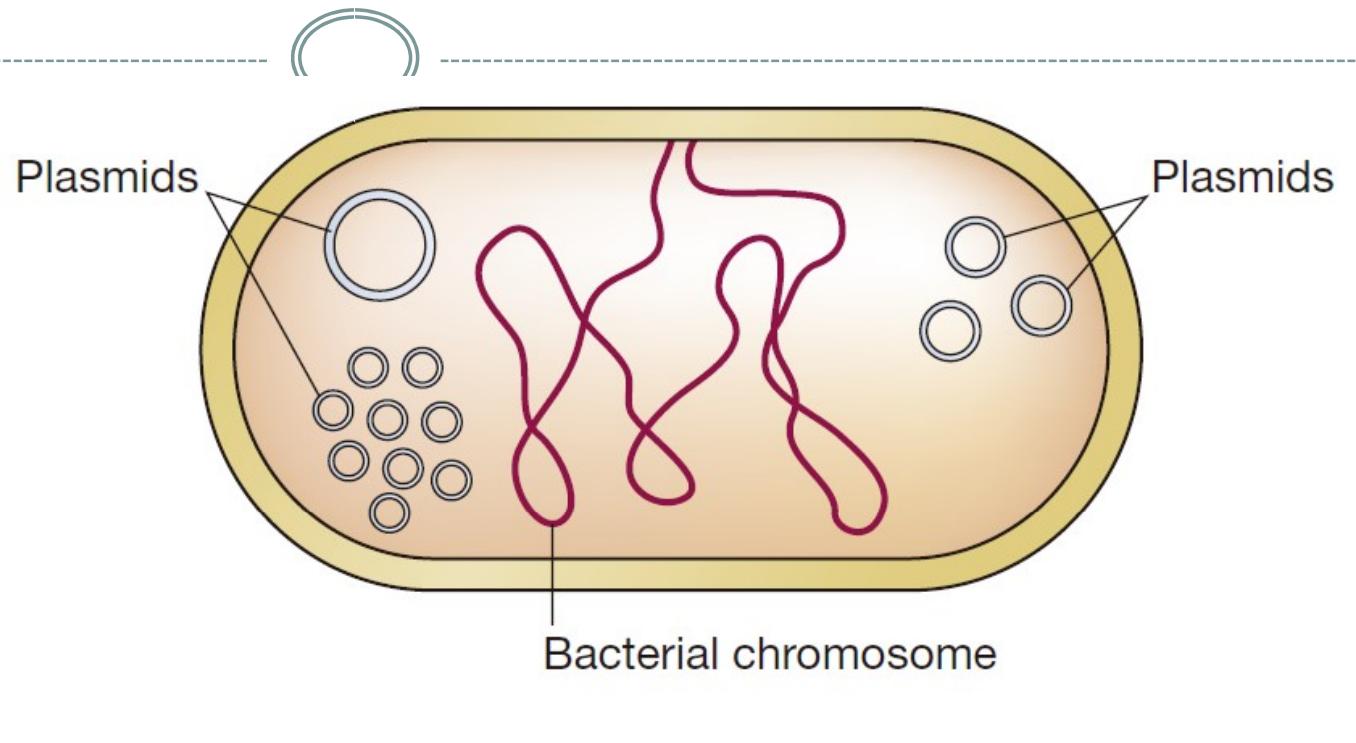


Plasmid Extraction

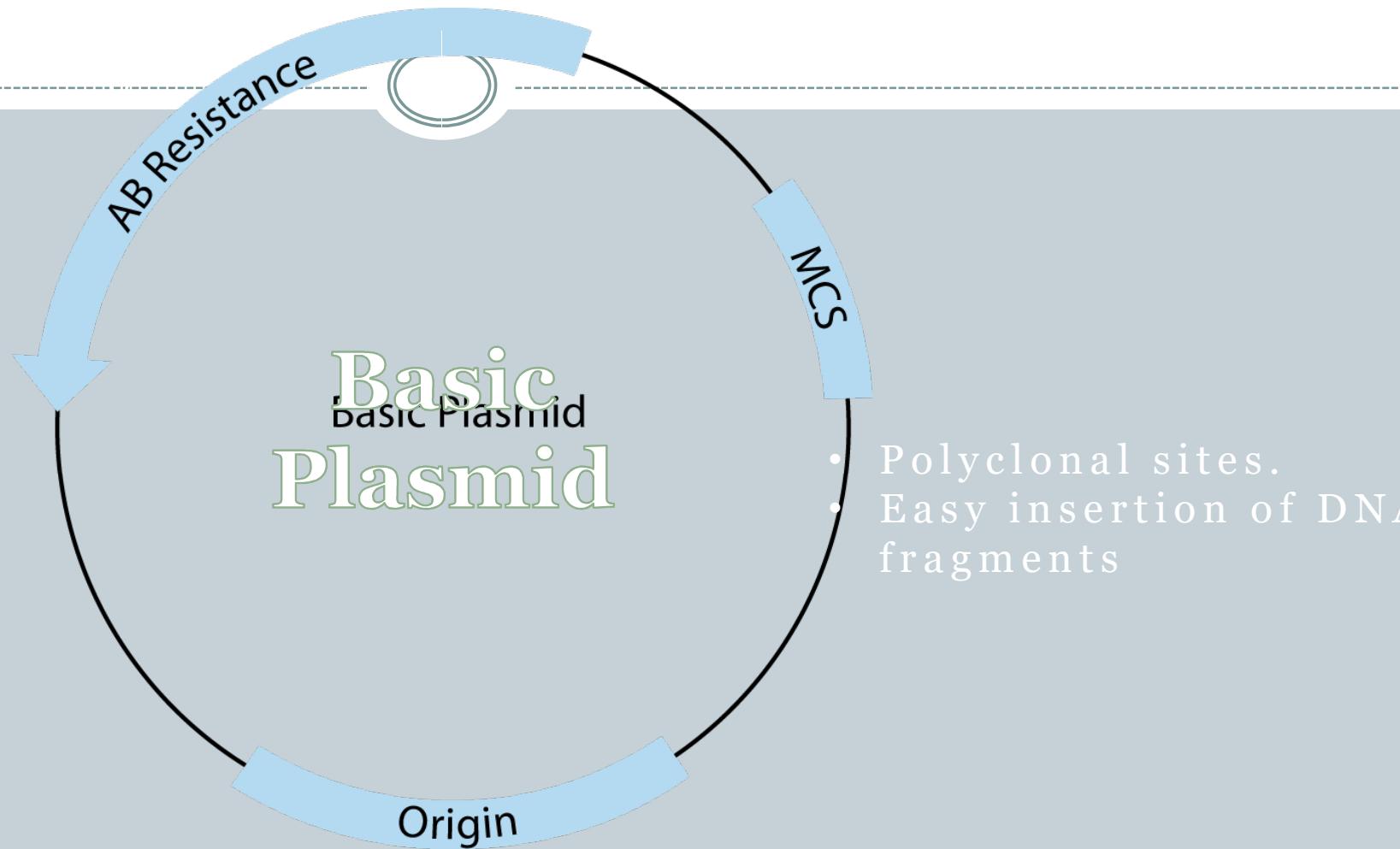
Nanjing_NFLS

质粒 (Plasmids)



- Plasmids are loops of DNA **outside the genome**.
- Plasmid size ranged from 1 kb-200 kb, much smaller than the E. coli genome (4.6 Mb).
- Plasmids are communication tools for microbes.

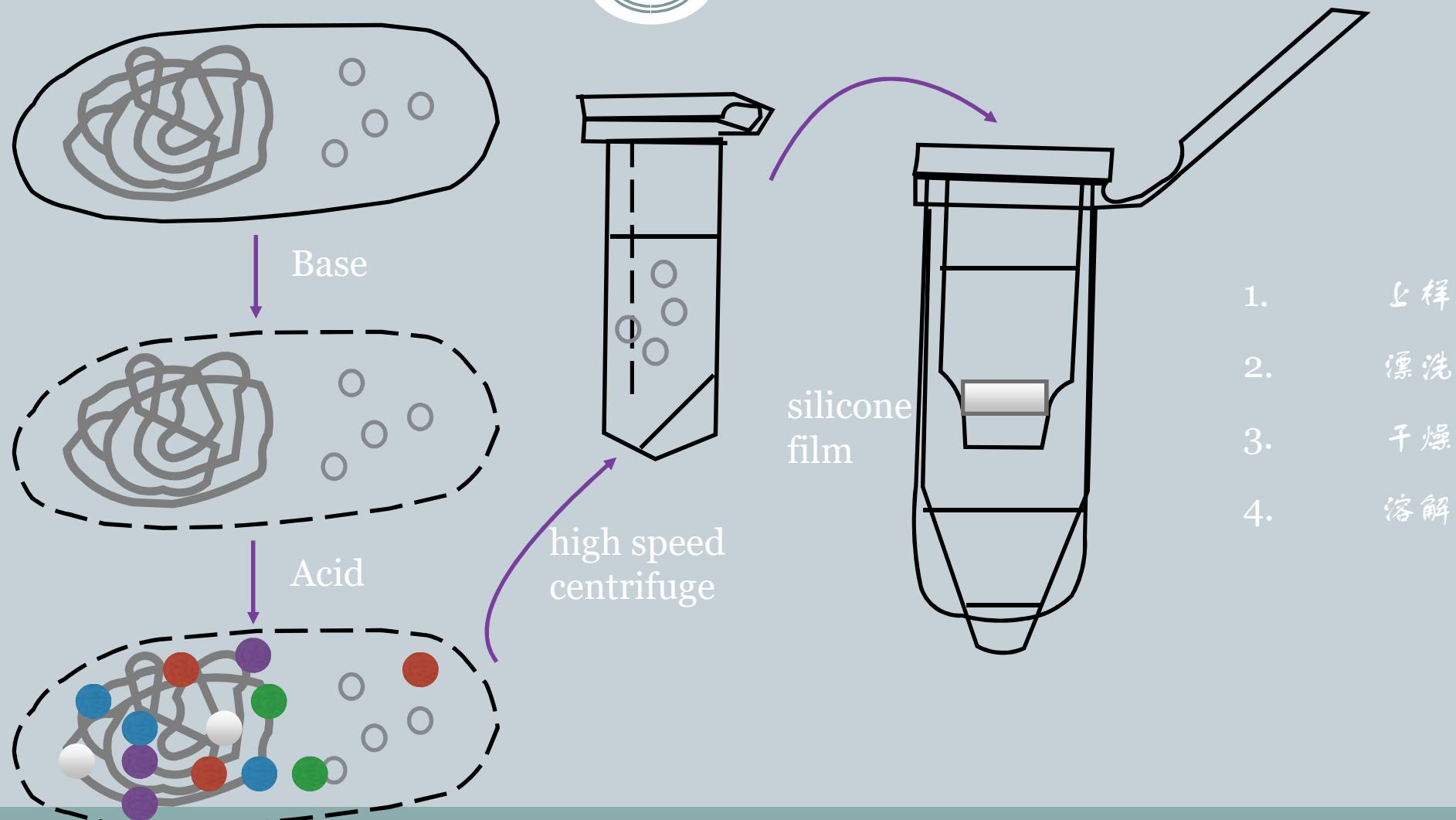
monoclonal



- Replication start site: determines the number of copies of the plasmid

- Polyclonal sites.
- Easy insertion of DNA fragments

Plasmid Extraction: Base Cleavage, Over-Column Purification



Practice using a pipette



- 1 step for sampling, 2 stop for adding samples
- Tune a gun.
- Put a gun on the head.
- Liquid extraction.
- Hold the tip of the gun against the wall of the barrel to sample, or stick the tip below the liquid surface to sample.
- Immediately after the addition of the sample, leave the nozzle from the liquid surface, do no suction back.

Practice adding liquid to the walls of the tube: 300 µL, 30 µL, 3 µL.

Plasmid Extraction



1. Take 2mL of the overnight culture, centrifuge at 12000rpm for 1min, discard the supernatant. 加入250ul RB，盖上盖子，之后用vortex震荡混匀细菌和buffer，直至无任何块状菌体残留。
2. 加入250ul蓝色溶液 LB，盖上盖子，温柔地上下颠倒混匀6-8次，使菌体充分裂解(不宜超过5分钟)。
3. 立即加入350ul NB，盖上盖子，上下颠倒混匀15-20次，由慢到快，直至充分混匀（但不要产生气泡）。观察溶液的变化。室温静置2min。
4. 13000rpm离心5分钟，分离质粒DNA和杂质。

Plasmid Extraction



1. 取过夜培养的菌液2mL, 12000rpm 离心1min, 弃去上清。
2. Add 250ul RB, cover with a lid, and afterwards mix the bacteria and buffer with vortex shaking until no lumps of bacteria remain. 加入250ul 蓝色溶液 LB, 盖上盖子, 温柔地上下颠倒混匀6-8次, 使菌体充分裂解(不宜超过5分钟)。
3. 立即加入350ul NB, 盖上盖子, 上下颠倒混匀15-20次, 由慢到快, 直至充分混匀(但不要产生气泡)。观察溶液的变化。室温静置2min。
4. 13000rpm 离心5分钟, 分离质粒DNA和杂质。

Plasmid Extraction



1. 取过夜培养的菌液2mL，12000rpm离心1min，弃去上清。
2. 加入250ul RB，盖上盖子，之后用vortex震荡混匀细菌和buffer，直至无任何块状菌体残留。
3. Add 250ul of the blue solution LB, cover and gently mix upside down 6-8 times to fully lyse the bacteria (should not take more than 5 minutes). 立即加入350ul NB，盖上盖子，上下颠倒混匀15-20次，由慢到快，直至充分混匀（但不要产生气泡）。观察溶液的变化。室温静置2min。
4. 13000rpm离心5分钟，分离质粒DNA和杂质。

Plasmid Extraction



1. 取过夜培养的菌液2mL，12000rpm离心1min，弃去上清。
2. 加入250ul RB，盖上盖子，之后用vortex震荡混匀细菌和buffer，直至无任何块状菌体残留。
3. 加入250ul蓝色溶液 LB，盖上盖子，温柔地上下颠倒混匀6-8次，使菌体充分裂解(不宜超过5分钟)。
4. Immediately add 350ul of NB, cover and mix 15-20 times upside down, slowly to quickly, until well mixed (but do not create bubbles). Observe the change in the solution. 室温静置2min。
5. 13000rpm离心5分钟，分离质粒DNA和杂质。

Plasmid Extraction



1. 取过夜培养的菌液2mL，12000rpm离心1min，弃去上清。
2. 加入250ul RB，盖上盖子，之后用vortex震荡混匀细菌和buffer，直至无任何块状菌体残留。
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4. 立即加入350ul NB，盖上盖子，上下颠倒混匀15-20次，由慢到快，直至充分混匀（但不要产生气泡）。观察溶液的变化。室温静置2min。
5. Centrifuge at 13,000 rpm for 5 minutes to separate plasmid DNA and impurities.

Plasmid Extraction



6. Label the adsorption column, mount the blue adsorption column on a collection tube, transfer all the supernatant obtained in step 4 into the column, and centrifuge at 13,000 rpm for 1 minute. **弃掉收集管中的液体，取25oul TB buffer 加入吸附柱，12000rpm 离心1分钟。**
7. **弃掉收集管中的液体，取65oul WB 加入吸附柱，12000rpm 离心1分钟。**

质 Plasmid Extraction



6. 标记好吸附柱，将蓝色的吸附柱装在收集管上，将步骤4得到的上清液全部转移在吸附柱内，13000rpm 离心1分钟。
7. Discard the liquid in the collection tube, take 650ul of WB and add it to the column and centrifuge at 1 minute at 12000 rpm.

Plasmid Extraction



9. The liquid in the collection tube was discarded, and after loading back into the adsorption column, the column was idled at 13,000 rpm for 2 minutes to remove ethanol impurities.
10. 取一只干净的1.5ml离心管，标记pV和你的序号，将步骤8后的吸附柱装进离心管，打开盖子，室温放置5分钟，挥发酒精。
11. 向吸附柱内添加50ul Elution buffer，室温静置5分钟，12000rpm离心2分钟。

质 Plasmid Extraction



9. 弃掉收集管中的液体，装回吸附柱后，13000rpm室转2分钟，去除乙醇杂质
10. Take a clean 1.5 ml centrifuge tube, label the pV and your serial number, load the adsorption column after step 8 into the centrifuge tube, open the lid and leave it at room temperature for 5 minutes to evaporate the alcohol.
向吸附柱内添加50ul Elution buffer，室温静置5分钟，12000rpm离心2分钟

Plasmid Extraction



9. 弃掉收集管中的液体，装回吸附柱后，13000rpm室转2分钟，去除乙醇杂质。
10. 取一只干净的1.5ml离心管，标记pV和你的序号，将步骤8后的吸附柱装进离心管，打开盖子，室温放置5分钟，挥发酒精。
11. Add 5oul Elution buffer to the adsorption column and leave it to stand for 5 min at room temperature, then centrifuge at 12000 rpm for 2 min.