

QIAGEN Plasmid Maxi Prep

Things to do before starting

- Make sure RNase A solution has been added to Buffer P1.
- Check Buffer P2 for SDS precipitation
- Optional: Add LyseBlue reagent to Buffer P1; 1:1000 dilution; see notes below

Grow Bacterial culture

Inoculate 100mls (high copy number plasmids) to 500mls (low copy number plasmids-see note for chloramphenicol) from glycerol stock from -80. **DO NOT ALLOW GLYCEROL STOCK TO THAW AT ALL!!!!!!** Use sterile pipet tip to scrap a chunk of the glycerol stock for the culture. Be sure to include the appropriate antibiotic in your media. Use 500ml sterile Erlenmeyer flask for culture volume of 100-250mls)

LB EZ Mix: SIGMA L-7658

Ampicillin: SIGMA A9518-25g; Make a 1000x stock in water at 50mg/ml; freeze at -20 in aliquots; use 1ul/ml of LB

Kanamycin: SIGMA K4000-5g ; Make a 1000x stock in water at 30mg/ml. Only make 1ml at a time.

Chloramphenicol: SIGMA C3175-100mg (water soluble); Make 200x stock in water at 34mg/ml (final 170ug/ml); add to culture after 4 hours of growth (See notes below for reasons to use chloramphenicol) If not using water-soluble form, dilute in ethanol.

Procedure

A) Bacterial culture, harvest, and lysis

1. Pellet 100 ml overnight LB culture at 6000 x g for 15 min at 4°C (5000 rpm in Beckman or Sorvall High Speed Centrifuge). Use 250ml Nalgene bottles. Save the dirty culture flask for Procedure C6 below.
2. Homogeneously resuspend the bacterial pellet in 10 ml Buffer P1. Transfer to a 50ml oakridge tube.
3. Add 10 ml Buffer P2, mix thoroughly by vigorously inverting 4-6 times, and incubate at room temperature for 5 min. Do not let sit longer than 5 minutes or degradation will occur!!!!!!!
4. Add 10 ml Buffer P3, mix thoroughly by vigorously inverting 4-6 times, and incubate on ice for 20 min.

B) Bacterial lysate clearing

5. Centrifuge at $\geq 20,000$ x g for 30 min at 4°C (15,000rpm in Beckman or Sorvall high Speed centrifuge). Re-centrifuge the supernatant in a clean oakridge tube at $\geq 20,000$ x g for 15 min at 4°C.

C) Bind, wash, and elute plasmid DNA and QIAGEN-tip

6. Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT and allow column to empty by gravity flow. (You can use the original culture flask to hold the column. Columns can be used more than once for the same plasmid if re-equilibrated.)
7. Apply the supernatant (step 5) to the QIAGEN-tip and allow it to enter the resin by gravity flow.
8. Wipe out any excess debris from the column with a Kimwipe
9. Wash the QIAGEN-tip with 2 x 30 ml Buffer QC. Allow Buffer QC to move through the QIAGEN-tip by gravity flow. Note: the tip holds 30mls so you do not need to measure the wash amount.-just fill the tip.
10. Elute DNA with 15 ml Buffer QF into clean 50 ml oakridge tube.

D) Precipitate, wash, and redissolve plasmid DNA

10. Precipitate DNA by adding 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA and mix. Centrifuge at $\geq 15,000 \times g$ (15,000rpm in Beckman or Sorvall High Speed) for 30 min at 4°C. Carefully decant supernatant.
11. Wash DNA pellet with 1 ml room-temperature 70% ethanol. Transfer to a microfuge tube. Centrifuge for 10min at high speed in a microfuge. Carefully decant supernatant pipetting out an excess volume.
12. Air-dry pellet for 5-10 min and redissolve DNA in a suitable volume of TE (long-term storage) or sterile ddH₂O, typically 100ul.
13. After resuspended, quick spin 1 minute in microfuge to pellet anything that did not solubilize. Transfer supernatant to a clean tube.

NOTES:

Many of the currently used plasmid vectors replicate to such high copy numbers that they can be purified in large yield from cultures that have simply been grown to log phase in standard LB medium. In these cases, it is not necessary to amplify the DNA selectively. However, vectors of an earlier generation (eg, pBR322) which do not replicate so freely, need to be selectively amplified by incubating the partially grown bacterial culture in chloramphenicol for several hours. Chloramphenicol inhibits host protein synthesis and thus prevents replication of the bacterial chromosome. However, replication of relaxed plasmids continues and their copy number increases progressively for several hours.

In cases where plasmids grow poorly due to their large size or because of the foreign sequences they carry, it may be worthwhile to use Super Broth or Terrific Broth.

Low yield may also be due to poor lysis. Adding the Lyse-Blue to the P1 buffer will help you determine whether or not your Lysis was complete. Allowing the lysis reaction to go further than necessary will begin to denature the plasmid.

When adding Buffer P3, complete and thoroughly but gently mixed to ensure complete precipitation of the SDS otherwise the remaining SDS will inhibit the binding of DNA to the column.