Modified Qiagen plasmid mini/midi prep Protocol

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Qiagen columns actually have a much higher capacity than advertised. This prep gives about 50-75 µg of pUC or bluescript plasmid from a 20 ml culture, is fairly rapid, and is high enough quality for double stranded sequencing, microinjections into worms, or long term storage at 4°. This also gives adequate yields of cosmid or other low copy plasmid DNAs.

1. Grow a 20 ml culture overnight (use a large culture tube).

2. Spin down 5K, 5 min in an appropriate tube.

3. Carefully remove all the supernatant (if even a couple hundred µl are left, the prep won't end up fitting in the tube!)

4. Resuspend in 1200 µl buffer "P1". Transfer 600 µl to each of two 2 ml eppendorf tubes. Note that P1 can be kept at 4° for over 2 months, not <1 month like the Qiagen protocol says.

5. Add 600 µl P2 to each tube and immediately but gently mix by inversion. Wait 5 min.

6. Add 600 µl P3 and mix by inversion until solution is clear with floating white stuff. Spin 15 min in microfuge. Remove sup.

7. Equilibrate a Tip-20 column by running 1 ml of QBT over it.

8. Load the entire supernatant from both tubes on the column.

9. Wash with 2X 1 ml QC buffer.

10. Elute into an eppendorf tube with 0.8 ml QF buffer. Force out the remaining liquid from the column with a 1 ml pipettman.

11. Add 600 µl isopropanol, mix, and microfuge 30 minutes to pellet the DNA. For high copy plasmids, the DNA pellet should be visible.

8. Wash with 70% EtOH, dry briefly, and resuspend in 50 µl TE (30µl for cosmids). For pUC plasmids, expect a final concentration of ~1-1.5 mg/ml.

I found that deviations from this protocol have given yields that are quite variable; I stick to this protocol exactly and have been getting reproducible yields.

Solutions for running Qiagen columns

1. You can buy these solutions, but it's much cheaper to make them yourself. The recipes that Qiagen recommends for these buffers have changed a few times in recent years. These recipes are from the Spring 1992 protocol.

(previous recipes tended to use buffers at pH's that were very far from their pKa's; the new recipes were probably intended to correct this).

2. It is important that these solutions be made accurately: if the NaCl concentration is off by a few percent, this could mess things up.

Stock solutions:

0.5 M MOPS pH 7.0

209.27 g MOPS (use the free acid form that USB sells)

~750 ml dH20

pH to 7.0 with 10 N NaOH

make up to 2 liters with dH20

3M NaCl

350.6 g Nacl

make up to 2 liters with dH20

1 M Tris pH 8.5

121.1 g Tris base

~750 ml dH20

pH to 8.5 with concentrated HCl (takes ~20 ml)

make up to 1 liter with dH20

store these solutions at room temp.

QC buffer

666 ml 3M NaCl

200 ml 0.5 M MOPS pH 7.0

300 ml EtOH

dH2O to 2 liters

check the pH and adjust to 7.0 (it should be close) with NaOH or HCl

QF buffer

833 ml 3M NaCl

100 ml 1M Tris pH 8.5

300 ml EtOH

dH2O to 2 liters

check the pH and adjust to 8.5 (it should be close) with NaOH or HCl

store QC and QF in tightly capped containers at room temp.

QBT buffer

500 ml 3M NaCl

200 ml 0.5 M MOPS pH 7.0

300 ml EtOH

3 ml Triton X-100

dH2O to 2 liters

P1 buffer

1M Tris HCl pH 8.0 2.5 ml

0.5 M EDTA pH 8.0 1 ml

10 mg/ml RNAseA, boiled 0.5 ml

dH2O 46 ml

50ml total

P2 buffer

2M NaOH 5 ml

20% SDS 2.5 ml

dH2O 42.5 ml

50 ml total

Apparently this solution can go bad due to oxidation: keep the container tightly capped.

P3 buffer

potassium acetate 25 g

glacial acetic acid 13 ml

H2O 87 ml

100 ml, total final pH should be ~5.5