Plasmid minipreps for sequencing

Adapted from *FLC Protocols Plasmid minipreps 02/09/2004*

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Reagents

Below are reagents you will need to prepare before starting the protocol and how to prepare them.

Solution I: Re-suspension buffer

50 mM Tris-Cl, pH 8.0, 10mM EDTA, 100 μg/mL RNaseA.

1. Dissolve 6.06 g Tris base, 3.72g EDTA-2H_20 in 800mL npH_20. Adjust the pH to 8.0 with HCL.
2. Adjust the volume to 1 liter with npH_2O. Add 100 mg RNaseA per liter of Solution I. Store at -4 °C after adding RnaseA.

Solution II: Lysis buffer

200mM NaOH, 1% SDS.

1. Dissolve 8.09 g of NaOH pellets in 950mL npH_2O.
2. Add 50 mL 20% SDS solution. If making SDS solution stock be sure to add SDS to water slowly. Store at room temperature.

Solution III: Neutralization Buffer

3.0 M potassium acetate, pH 5.5 .

1. Dissolve 294.5 g potassium acetate in 1 L npH_2O.
2. Adjust the pH to 5.5 with glacial acetic acid (about 110 mL). Store at -4°C or room temperature

10 M LiCl

1. Dissolve 42.4 g of LiCl in 90 ml npH_2O.
2. Adjust volume to 100 ml. Store at room temperature.

10 mM Tris HCL pH 7.5

If stock is not on hand make a 1 M Tris-HCL solution, otherwise skip to step 3.

1. Dissolve 121.1 g Tris base in 800 ml npH_2O.
2. Adjust the pH to 7.5 using HCL.
3. Add 5 ml of 1 M Tris-HCL pH 7.5 to 495 ml npH_2O.

If making RnaseA solution, add RnaseA stock to a final concentration of 20 μg / ml.

Other items

- 100% ethanol.
- 70% ethanol.
- Lots of clean (autoclaved) 2 ml tubes.
- 3-5 hours of time to complete the protocol.

Before beginning

Place small bottle 50 - 250 ml 100% EtOH in the freezer. Wipe all surfaces with 10% bleach solution. Work near lite Bunsun burner when transfering bacterial cultures between tubes if necessary.

Protocol

1. Begin with a minimum of 2 and a maximum of 6 ml of overnight bacterial culture (if ampicillin is used as the selective medium use 100 μ/ml). If volume of culture is greater than 2 ml proceed to step 2, otherwise skip to step 3.
2. Transfer culture to a 12 ml tube. Spin down bacteria at 4500 rpm at 23 °C for 10 minutes using the large bench-top centrifuge and the swing bucket rotor [1].
3. Transfer culture to a 2ml centrifuge tube and spin down bacteria at 6,000 rpm at 23 °C for 6 minutes.
4. Remove supernantent throughly with a vacuum trap.
5. Add 200 μl solution I [2]. Re-suspend pellets by vortexing vigorously. If using $>$ 2 ml of culture (see step 2) transfer re-suspended bacteria to a 2 ml tube. Incubate samples at room temperature for 10 minutes. While you are waiting cool down the 2 ml bench-top centrifuge to 4 °C.
6. Add 400 μl of solution II. Mix the samples by gently inverting 6 times. **Do not vortex**. Place the tubes on ice for exactly 5 minutes.
7. Add 300 μl of cold solution III. Mix well by inverting several times. Incubate samples on ice for 5 minutes.
8. Centrifuge for 5 minutes at max speed (15,000 rpm) at 4 °C.
9. Transfer the supernatant to a clean 2 ml tube. Add 1 volume (900 μl) of isopropanol. Mix the samples by inverting several times.
10. Centrifuge samples for 15 minutes at max speed at 4 °C.

[1] Plastic 12 ml tubes will shatter if spun at too high a speed with too great a volume. If you have doubts about your tubes or have not used them in the centrifuge before test them by spinning a few with 11 μl H_2O at 4200 rpm.

[2] I highly recommend using the serial pippetter for adding all solutions at this point. It will save you hours over traditional pipetting. If you do not know what this is or where to find it ask someone.

11. Discard supernatent by decanting [3] or with vacuum trap. Wash pellet with 500 μl of 70% ethanol. Remove ethanol wash using a vacuum trap.

12. Add 200 μl 10mM Tris HCL RnaseA solution to each sample [4]. Vortex to disolve the pellet. Incubate samples at 37 °C for 10 minutes.

13. Add 200 μl of 10M LiCl. Mix solutions by inverting 6 times.

14. Incubate samples for $\geq$ 20 minutes at -20 °C.

15. Centrifuge samples at max speed for 10 minutes at 4 °C. Transfer the **supernatent** to a clean 2 ml tube.

16. Add 2.2 volumes of ice cold 100% ethanol. Mix samples by inverting several times.

17. Add 0.5 μl molecular grade glycogen (stock is 20 mg / ml) to each sample.

18. Centrifuge samples at max speed for 10 minutes at 4 °C. Remove supernatent with a vacuum trap. [5]

19. Wash the pellet up to two times with 500 μl of 70% ethanol [6]. Remove ethanol using a vacuum trap. Washing can sometimes dislodge the pellet and it can be helpful to spin samples at max speed for 2 minutes prior to removing the wash to secure the pellet.

20. Dry the pellet for 10-15 minutes and dissolve in 40 μl TE.

21. Check sample concentration using the nanodrop. If concentration is too high dilute sample in 40 μl steps of 10mM Tris HCL pH 7.5 until you reach a reasonable concentration (200-400 ng/ul).

22. Disinfect surfaces and vacuum trap intake with 10% bleach solution to avoid bacterial contaimination.

23. Run between 200 and 600 ng per lane of each sample on an agarose gel and image.

Other relevant information

Washing glass culture tubes and caps

1. Place culture tubes upwards in holding rack. Rinse tubes and caps with 10% bleach solution.

[3] Generally I decant the isopropanol by placing samples in the front row of a tube rack, facing them so the front of the tube faces away from the rows of the rack and using this configuration to decant all the samples in the rack at once. It helps to find a tube rack that is older and slightly deformed as these will hold onto your tubes better.

[4] This is a potential stopping point. If you intend to stop here add 200 μl 10mM Tris HCL without RNasA and freeze samples. When you come back add RnaseA to a final concentration of 20 μg/μl to each of your samples

[5] Remember to use a pipette tip over the end of the vacuum tube.

[6] The 2/9/04 protocol recommends washing twice. I haven't noticed a difference between single and double wash samples but if you are being extra careful it doesn't hurt to wash twice.

2. Flush caps and tubes with water.

3. Place glass tubes upside down to square metal washing rack.

Measuring sample concentration

The nanodrop can only measure samples up to around 2 μg/μl. If your sample is too concentrated the concentration will read "—-". Dilute your sample and re-measure.