Qiagen Midi Prep Protocol

Procedure

1. Typically you would start by inoculating a 50-100 ml culture with .5-3mls of starter culture and incubating overnight at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm)

2. The next day your culture should be turbid. Centrifuge the bacteria in 50 ml conical tubes (centrifugation at 7000 x g for 15 min at 4°C.

3. Qiagen recommends 25 mls of culture for a high copy plasmid and 100mls of culture for a low copy plasmid. **We routinely use 50 mls of with good success. So use 50 mls of culture to do your midiprep unless you know your copy is a low copy# plasmid.**

4. If you grew up 100 mls of culture, freeze the other pellet at -80 in a labeled conical tube (just in case something goes wrong with the first). Don't so more midi preps than needed. Save time and money!

5. Resuspend the bacterial pellet in ▲ 4 ml of Buffer P1.

Ensure that RNase A has been added to Buffer P1.

The bacteria should be resuspended completely by vortexing

6. Add ▲ 4 ml of Buffer P2, mix thoroughly by inverting the sealed

tube 4–6 times, and incubate at room temperature (15–25°C) for 5 min.

Do not vortex, as this will result in shearing of genomic DNA. The lysate should

appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After

use, the bottle containing Buffer P2 should be closed immediately to avoid

acidification from CO 2 in the air.

If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after

addition of Buffer P2. Mixing should result in a homogeneously colored suspension.

If the suspension contains localized colorless regions or if brownish cell clumps are

still visible, continue mixing the solution until a homogeneously colored suspension

is achieved.

6. Add ▲ 4 ml **of chilled Buffer P3**, mix immediately and thoroughly by

inverting 4–6 times, and incubate on ice for ▲ 15 min.

Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris.

The lysate should be mixed thoroughly to ensure even potassium dodecyl sulfate precipitation. If the mixture still appears viscous, more mixing is required to completely neutralize the solution.

If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

7. In our Centrifuge, centrifuge at 7,000 x g for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.

Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in polypropylene 50 ml conical tubes. After centrifugation the supernatant should be clear.

8. After Centrifugation remove the clear supernatant (this contains your plasmid DNA). Filter this supernatant through a .2 micron filter to remove any residual precipitate.

9. Equilibrate a ▲ QIAGEN-tip 100 by applying ▲ 4 ml of Buffer QBT, and allow the column to empty by gravity flow.

Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

10. Apply the supernatant/filtered lysate from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow.

The supernatant should be loaded onto the QIAGEN-tip promptly.

11. Wash the QIAGEN-tip with ▲ 2 x 10 ml of Buffer QC.

Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in the majority of plasmid DNA preparations. The second wash is especially necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

12. Elute DNA with ▲ 5 ml of Buffer QF.

Collect the eluate in a sterile/new/clean 50 ml tube (not supplied). Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

13. Precipitate DNA by adding ▲ 3.5 ml of (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at 7,000 x g for 30 min at 4°C. Carefully decant the supernatant.

All solutions should be at room temperature in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample.

Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant. Mark the location of the pellet.

14. Wash DNA pellet with ▲ 2 ml of room-temperature 70% ethanol, and

centrifuge at 7,000 x g for 10 min. Carefully decant the supernatant without

disturbing the pellet.

The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

15. Air-dry the pellet for 5–10 min. Can place tubes on a 50 Degree heat block for 3-6 minutes to aid drying. Once all traced of water and ethanol are gone redissolve the DNA in a suitable volume of buffer

(e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).

Redissolve the DNA pellet by rinsing the area the DNA pellet was located. Pipetting the DNA up and down to promote. Usually 50-100uls of TE buffer is an appropriate amount to resuspend the DNA pellet with.

Resuspension may cause shearing and should be avoided. Overdrying the pellet will

make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline

conditions; it does not easily dissolve in acidic buffers.

16. Add your solubilized DNA to a new/clean/sterile 1.5 ml centrifuge tube. You should solubilize your DNA pellet in 50uls of TE buffer. Then measure on the nanodrop. If the concentration is above 1.5 ugs/ul then add another 25+ uls of TE Buffer. Then measure on the nanodrop again. This kit is designed to purify 100ugs of DNA. We aim to have DNA at a concentration ~ 1 ug/ul.

17. Label your tubes appropriately and freeze at -20