QIAGEN Supplementary Protocol:

Isolation of single-stranded DNA from M13 phage using QIAGEN® Plasmid Kits

This procedure is an adaptation of the QIAGEN® Plasmid Mini, Midi and Maxi Kit Protocols and is designed for the preparation of ultrapure single-stranded DNA from M13 phage or phagemid. The amount of M13 DNA that can be isolated depends on the type of vector, the *E. coli* strain, the efficiency of inoculation and the culture media. Isolated DNA is free of DNase and RNase activity, and PEG.

Throughout this protocol, single-underlined text denotes information for QIAGEN-tip 20 (miniprep), double-underlined text denotes information for QIAGEN-tip 100 (midiprep), and dotted-underlined text denotes information for QIAGEN-tip 500 (maxiprep).

Please be sure to read the *QIAGEN Plasmid Purification Handbook* and the detailed QIAGEN Plasmid Midi and Maxi Kits Protocol carefully before beginning this procedure.

Important notes before starting

- Prepare M13 cultures according to: Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989), *Molecular Cloning: A Laboratory Manual*, 2nd Edition. New York: Cold Spring Harbor Laboratory Press. Additional information about M13 may be found in the *QIAprep® M13 Handbook*.
- The best M13 yields are obtained when the culture is grown for approximately 4–5 hours.
- Select the appropriate QIAGEN-tip:

Culture volume	QIAGEN-tip size	Maximum yield
3 ml	QIAGEN-tip 20	20 µg DNA
25 ml	QIAGEN-tip 100	100 µg DNA
100 ml	QIAGEN-tip 500	500 µg DNA

- Buffers M1 and M2 are not supplied in QIAGEN Plasmid Kits and must be prepared by the user. Preparation notes for Buffers M1 and M2 appear at the end of the protocol.

Procedure

1. **Spin down bacterial cells at 10,000 x *g* for 5 min at room temperature.**
2. **Transfer supernatant containing M13 bacteriophage to a fresh reaction tube.**

   Be careful not to disturb the bacterial pellet. Any carryover of bacterial cells will result in contamination of the M13 preparation with bacterial chromosomal DNA or double-stranded bacteriophage RF DNA.
3. **Add 0.2 volumes of chilled Buffer M1 and mix. Incubate on ice for 60 min.**

   Buffer M1 should be cooled to 4°C for optimal precipitation of phage.

4. **Pellet phage at 10,000 x *g* for 10 min at room temperature. Discard the supernatant.**

   It is essential that the PEG from Buffer M1 is completely removed. After decanting the supernatant, spin the tube again for 20 sec and remove the accumulated supernatant with a pipette. Alternatively, the tube may be drained by inverting for one minute. Do not wipe the tube to remove excess PEG as the phage pellet is clear and may be distributed over the walls of the tube. Spinning in a swing-arm rotor may result in a more compact pellet.

5. **Resuspend the pellet in 1 ml, 10 ml or 30 ml of Buffer M2.**

   Vortex well to resuspend the pellet completely.

6. **Incubate at 80°C for 20 min, 30 min, or 40 min to lyse the phage particles.**

   Incubation at 80°C is necessary for complete lysis of phage particles.

7. **Mix lysed phage particles by inversion, and cool to room temperature.**

   The Triton® X-100 in Buffer M2 may form a separate phase. If this occurs, invert to mix.

8. **Equilibrate a QIAGEN-tip 20, -tip 100 or -tip 500 by applying 1 ml, 3 ml or 10 ml 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.

9. **Apply the lysed phage sample from step 7 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

   The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be re-centrifuged or filtered before loading to prevent clogging of the QIAGEN-tip.

10. **Wash the QIAGEN-tip with 2 x 1 ml, 10 ml or 30 ml of Buffer QC.**

    Allow Buffer QC to move through the QIAGEN-tip by gravity flow.

11. **Elute DNA with 0.8 ml, 5 ml or 15 ml of Buffer QF**

    Collect the eluate in a 1-ml, 10-ml or 30-ml tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

12. **Precipitate DNA by adding 0.6 ml, 3.5 ml or 10.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately for 30 min. Carefully decant the supernatant.**

    Precipitation should be carried out at room temperature to minimize salt precipitation.

13. **Wash DNA pellet with 1 ml, 2 ml or 5 ml room-temperature 70% ethanol, and centrifuge 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. A second wash with 70% ethanol may improve results in more sensitive applications, such as sequencing.

14. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve the DNA pellet by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing. 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.

Composition of buffers

Buffer M1: 30% polyethylene glycol (PEG 6000); 3 M NaCl. Store at 4°C

Buffer M2: 1% Triton X-100; 500 mM Guanidine-HCl; 10 mM MOPS, pH 6.5. Store at room temperature.