QIAGEN Supplementary Protocol:

Isolation of plasmid (2 x 96) using the Sigma Centrifuge 4-15

This protocol is designed for 96 parallel plasmid DNA preparations from 1.3 ml overnight cultures. **It has not been thoroughly tested and optimized by QIAGEN.** Please read the protocol thoroughly before starting.

Please be sure to read the *QIAprep® Miniprep Handbook* and the detailed QIAprep 96 Turbo Miniprep Kit Protocol carefully before beginning this procedure.

Equipment to be supplied by the user

- Sigma Centrifuge 4-15, 4K15, 6-10, or 6K10
- Plate Rotor 2 x 96
- Square-well blocks

Important notes before starting

- Centrifugation of QIAprep 96 plates is performed at 6000 rpm (5788 x *g*). The speed limit of the centrifuge is programmed so that the given *g*-force will not be exceeded. All centrifugation steps are performed at room temperature.
- It is useful to perform 2 x 96 preparations at one time since in all cases the rotor must be balanced.
- Add the provided RNase A to Buffer P1, mix, and store at 4°C.
- Add ethanol (96–100%) to concentrated Buffer PE before use (see bottle label for volume).
- For information about buffers and elution conditions, please refer to Important Notes for QIAprep Procedures in the *QIAprep Miniprep Handbook*.
- For information about growth of 1.3 ml overnight cultures in a 96-well block, please refer to the protocol in the *QIAprep Miniprep Handbook*.
- A repeating pipet and a multichannel pipet facilitate liquid handling during the QIAprep 96 Plasmid Miniprep procedure.

Procedure

For growth of 1.3 ml overnight cultures in a 96-well block, please refer to the protocol in the *QIAprep Miniprep Handbook*.

Before starting, please read the Important Notes on the previous page and the Important Notes in the *QIAprep Miniprep Handbook*.

1. **Resuspend pelleted bacterial cells in 250 µl of Buffer P1 and transfer to a 96-well block. Mark the block for later identification.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellets.

2. **Add 250 µl of Buffer P2 to each sample, seal the block with the tape and gently invert the block 4–6 times to mix.**

   It is important to mix gently by inverting the block. Do not shake vigorously, as this will result in shearing of genomic DNA. If necessary, continue inverting the block until the solution becomes viscous and slightly clear.

3. **Remove the tape from the block. Add 350 µl of Buffer N3 to each sample and seal the block with a new tape. Gently invert the block 4–6 times.**

   To avoid localized precipitation, mix the samples gently but thoroughly, immediately after addition of Buffer N3. The solutions should become cloudy.

4. **Load each block onto the carrier then place in the rotor bucket. Centrifuge at 6000 rpm for 10 min.**

5. **Place QIAprep 96 plate on top of a 96-well block (e.g., a square-well block). Mark the QIAprep 96 plate for later identification.**

6. **Carefully transfer the supernatants from step 4 to the wells of the QIAprep 96 plate.**

   The supernatants should be loaded onto the QIAprep 96 plate promptly. If they are left too long and become cloudy, they must be centrifuged again before loading to prevent clogging of the QIAprep 96 plate. Avoid disturbing the pellet and avoid transferring any of the lipid layer floating on the surface of the cleared lysate.

7. **Load each block and QIAprep 96 plate onto the carrier then place in the rotor bucket. Centrifuge at 6000 rpm for 4 min.**

8. **Empty the block. Add 1 ml of Buffer PB to each well. Centrifuge at 6000 rpm for 4 min.**

   This step is necessary to removes trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB 101 and its derivatives, or any wild-type strains that have high levels of nuclease activity or high carbohydrate content. If the plasmid DNA will be used for *in vitro* transcription assays the PB wash step is required for all strains.

9. **Empty the block. Add 900 µl of Buffer PE to each well. Centrifuge at 6000 rpm for 4 min.**

10. **Repeat step 9.**

11. **Place QIAprep 96 plate on top of a collection-microtube rack containing 1.2 ml collection microtubes. Incubate for 10 min at 70°C in an incubator or oven to dry the membrane.**

    IMPORTANT: This step removes residual Buffer PE from the membrane. Residual ethanol, which is in Buffer PE, may inhibit downstream enzymatic reactions such as sequencing.

12. **To elute, add 100 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each well of the QIAprep 96 plate, leave to stand for 1 min, and centrifuge at 6000 rpm for 4 min.**