QIAprep Spin Miniprep Kit Protocol (using a microcentrifuge)

1. Resuspend pelleted bacterial cells in 250 ul Buffer P1 and transfer to a microcentrifuge tube.

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

2. Add 240 ul Buffer P2 and gently invert the tube 4-6 times to mix.

Mix gently by invertin gthe tube. Do not vortex, as this will result in shearing of the genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 minutes.

3. Add 350 ul Buffer N3 and invert the tube immediately but gently 4-6 times.

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

4. Centrifuge for 10 Min. at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.

A compact white pellet will form.

5. Apply the supernatants from Step 4 to the QIAprep Spin Column by decanting or pippeting.

6. Centrifuge for 30-60 s. Discard the flow-through.

7. (Optional – not done): Wash the QIAprep Spin Column by adding 0.5 ml Buffer PB and centrifuging for 30-60 s. Discard the flow-through.

This step is necessary to remove trace nuclease activity when using endA+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohdyrate content. Host strains such as XL-1 Blue and DH5a do not require this additional wash step.

8. Wash QIAprep Spin Columd by adding 0.75 ml Buffer PE and centrifuging for 30-60 s.

9. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.

IMPORTANT: Residual wash buffer will not be completely removed unless teh flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. Place the QIAprep column in a clean 1.5 ml centrifuge tube. To elute the DNA add 50 ul Buffer EB (10 mM Tris-CL, pH 8.5) or water (usually used) to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuge for 1 min.