User-Developed Protocol:

Isolation of plasmid DNA from *Staphylococcus* spp. using the QIAGEN® Plasmid Midi Kit

This procedure has been adapted by customers from the QIAGEN® Plasmid Midi Kit Protocol. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully for isolation of a variety of medium-copy-number shuttle vectors from *S. xylosus, S. carnosus, S. epidermidis,* and *S. aureus*. Yield of plasmid DNA was typically 2–10 µg from 50 ml culture.

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

Procedure

1. **Inoculate 50 ml Brain Heart Infusion (Difco, available from Becton Dickinson) 1:50 with an overnight pre-culture. Grow the culture at 37°C for 6–7 hours.**

   Supplement the growth medium with the appropriate selective agent, e.g., chloramphenicol, erythromycin, or tetracycline. It is not advisable to use a higher culture volume, since this will lead to problematic lysis. The A_{578} of the culture should be 6–7. Do not grow the culture for longer than 6–7 hours as excessive cell densities result in inefficient lysis.

   Alternatively cultures can be grown in 50 ml LB medium for 16 hours. Cultures grown in LB medium are less dense than cultures grown in Brainheart Infusion.

2. **Harvest the cells by centrifugation at 6000 x *g* for 15 min.**

3. **Resuspend the bacterial pellet in 4 ml Buffer P1 containing 200–400 µl lysostaphin 0.5 mg/ml.**

   Ensure that RNase A (100 µg/ml) has been added to Buffer P1.

   Lysostaphin can be obtained from suppliers such as Sigma. The range is given because batches of lysostaphin vary in their activity. As lysostaphin is expensive it is a good idea to start with a lower amount and visually check if lysis occurs. If is does not, more lysostaphin can be added.

4. **Incubate at 37°C for 20–30 min. Cool to 20–25°C.**

5. **Add 4 ml Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature for 5 min.**

   Check Buffer P2 before use for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.

6. **Add 4 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 15 min.**

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

8. **Centrifuge again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly**

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

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

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

12. **Elute DNA with 5 ml Buffer QF.**

13. **Precipitate DNA by adding 3.5 ml room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**

14. **Wash DNA pellet with 2 ml of room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

15. **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).**