User-Developed Protocol:

Isolation of plasmid DNA from *Borrelia* 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 linear plasmids from *Borrelia burgdorferi* sensu lato species, which include *Borrelia burgdorferi* sensu stricto, *Borrelia afzelli,* and *Borrelia garinii.*

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 a *Borrelia* colony into 100 ml Modified Kelly Medium (MKP), and grow at 33°C for 4–5 days, to a cell density of 10^8 cells per ml.**
   Use only strains that have been subcultured less than 6 times.
2. **Harvest the cells by centrifugation at 6000 x *g* for 15 min.**
3. **Wash the pellet 3 times with TN buffer.**
4. **Resuspend the bacterial pellet in 8 ml Buffer P1.**
   Ensure that RNase A has been added to Buffer P1.
5. **Add 8 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 8 ml chilled Buffer P3. Mix immediately by gently inverting 4–6 times, and incubate on ice for 40 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 allow 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).**

Media and buffer compositions

MKP Basic Medium (1 liter)

100 ml CMRL-1066 (10x) without glutamine; 3.0 g neopeptone; 6.0 g Hepes; 0.7 g citric acid; 3.0 g glucose; 1.8 g pyruvic acid; 0.4 g N-acetylglucosamine; 2.0 g sodium bicarbonate. Adjusted to pH 7.6 with 5N NaOH and sterilized by filtration. Basic medium can be stored for 3 months at –20°C.

MKP Medium (2 liters)

1.6 liters of basic medium, 200 ml 7% gelatine (autoclaved at 115°C for 15 min), 100 ml partially hemolyzed rabbit serum (inactivated at 56°C for 30 min), and 100 ml bovine albumin (35%). MKP medium is stored in 6.5 ml aliquots at 4°C for up to 3 weeks.

TN Buffer

50 mM Tris·Cl, pH 7.6; 150 mM NaCl.