Competent cell preparation

1. Inoculate a 5ml starter culture in BSK II media from a glycerol stock of the *B. burgdorferi* strain that is to be transformed. Incubate at 35ºC until the culture density reaches 1-5 x 10^7 cells/mL (as determined by darkfield microscopy using a Petroff-Hausser chamber). Use this starter culture to then inoculate a larger volume of BSK II appropriate for preparing competent cells (100ml or more, see Step 2).

2. Grow *B. burgdorferi* cultures, at 35ºC, to mid- to late-exponential phase, approximately 5-7 x 10^7 cells/mL. Usually a 100 mL culture provides sufficient cell numbers for about 2 transformations (This is true in the case of low-passage strains. About 4 transformations can be done when preparing competent cells from a high passage strain).

3. Pellet cells by centrifugation (8,000 rpm/ 9,605xg, 10 minutes, 20ºC; see note) in a sterile centrifuge bottle. Decant the supernatant and wash the pellet by resuspension in 0.25 volumes ice-cold EPS, i.e. 25 mL EPS for a 100 mL culture. Transfer to a sterile 50ml centrifuge tube.

4. Pellet cells again (8,000 rpm/ 7,741xg, 10 minutes, 10-12ºC), decant the supernatant and resuspend in 0.1 volume ice-cold EPS i.e. 10 mL for an original culture volume of 100 mL. Transfer to a sterile 15ml centrifuge tube.

5. Pellet cells (as in Step 4), decant the supernatant and resuspend in ~0.0025 volumes ice-cold EPS, i.e. 250 µL EPS. Transfer to a sterile 1.5ml microfuge tube. Slowly pipette the cells up and down a few times, using a 100 or 200 µL pipette. The suspension should look milky white. Vortex the suspension for a few seconds and place 5µL on a microscope slide. Under darkfield microscopy, an ideal competence preparation should look like a continuous slow-flowing sheet of spirochetes. Problems arise when the cells clump or if the cells are too concentrated. If clumping occurs, try moderate vortexing and repeated pipetting. If the cells are too concentrated, then add more EPS until the cells begin to flow when viewed under darkfield microscopy. Cells may be used immediately

or frozen (high-passage strains only) at –80ºC. If using the cells immediately place them on ice until electroporation (Refer to protocol *Transformation of B. Burgdorferi cells*).

Note: The first centrifugation is done at 20ºC, as BSKII does solidify at cooler temperatures. The objective of the washes is to remove medium components from the cells that might cause arcing during the electroporation, while concentrating the bacterium in electroporation buffer. The number of washes and volumes of EPS described here were empirically determined and are a balance between efficient removal of the medium, yet limiting the mechanical damage caused to the *B. burgdorferi* cells during centrifugation and resuspension. More (3-4) or fewer washes (1) have also shown to be successful in preparing competent cells. The final volume that competent cells are resuspended in can be varied between 400 fold to 1000 fold less.