

Transformation of B. burgdorferi cells

1. Vortex the cells for a few seconds (to prepare competent cells refer to protocol on competent cell preparation) and transfer 100 μ L into a sterile 1.5ml microfuge tube containing the plasmid DNA (10-20 μ g in a 5-10 μ L volume of sterilized H₂O).
2. Transfer the cell/DNA mix to a pre-chilled disposable 0.2cm-gapped cuvette. (cuvettes and cuvette holder are kept at -20°C until use). Tap the cuvette on the side or on its bottom to get all of the cell/DNA mix settled down. Make sure to remove any condensation on the outer surface of the cuvette before electroporation.
3. Electroporate the cell/DNA mix using the following settings for the electroporator: 2.5 kV, 25 μ F, and 200 Ω . A good time constant is 4.8ms or above.
3. Immediately after transformation, quickly resuspend the cells in a final volume of 5 mL BSK II medium. This is done by taking a small amount of BSK II, with a Pasteur pipette, and resuspending the electroporated cells from the cuvette and transferring them into the 5ml of BSK II. Transformed cells are generally allowed to recover overnight at 35°C before selection is imposed. The length of the recovery period can be shortened to 8 hours, which allows transformation and plating to occur in the same day. (refer to protocol on plating for *Borrelia burgdorferi*)