

Plating *Borrelia burgdorferi* for single colonies or after transformation

Basic protocol—for 16 plates.

1. Equilibrate 300 ml of 1.5x BSK⁺ (stored frozen; see Recipes) and 200 ml 1.7% agarose (Seakem LE, autoclaved in deionized water, stored at RT, and microwaved before use) to 55°C.
2. Mix BSK and agarose. When plating transformation, either place enough medium to assess bacterial survival and outgrowth (at least one plate, i.e., 30 ml, per transformation) in a separate small bottle, or pour no-drug plates before adding antibiotic. Add antibiotics (200 mg/ml kanamycin, 40 mg/ml gentamicin, or 50 mg/ml streptomycin) to remainder of medium for selecting transformants.
3. Pipet 13-15 ml of mixture into 16 Petri dishes (tilt plate to cover bottom) and replace rest of medium in 55°C water bath.
4. Count cultures and dilute to desired density in BSK-H. After bottom plate is solidified, add enough bacteria for one, two, or three plates to 50 ml plastic tube (or other sterile disposable tube), pour in 15, 30, or 45 ml medium, pour onto one, two, or three plates. The same 50 ml tube can be used for multiple sets of plates, if, for example, a transformation outgrowth is to be spread over many plates. Since Bb is sensitive to high temperature, care should be taken to ensure that the bacteria are not exposed to 55°C for an extended period.
5. Colonies should appear 5-7 days after plating (when incubated at 35°C in 2.5% CO₂). Transformants growing in the presence of antibiotic selection often appear one to two days after the colonies on no drug plates.

Variations

1. Single pour method. The entire 30 ml can be added at one time to the bacteria to be plated, and then poured into an empty Petri dish. This method saves time and trouble. As mentioned above, Bb is sensitive to high temperature and plates poured all at once cool more slowly, so some cooling from 55°C is advisable. Also, resultant colonies are distributed throughout the plate, so they will be somewhat more heterogeneous.
2. Thinner plates. Plate volume can be reduced as low as 20 ml when more plates are needed.

Assessing spirochete load in ticks, blood, and other tissues

1. Plating ticks. Individual ticks in Eppendorf tubes are surface sterilized by washing in 3% hydrogen peroxide, followed by 70% ethanol. After drying, ticks are crushed with a plastic pestle in 500 µl BSK-H and an additional 500 µl BSK-H is added. Depending on the number of spirochetes expected, some combination of 1, 10, 100, and 900 µl of the triturate is plated in solid medium containing Bb antibiotics (see recipe below).
2. Plating blood. 100 µl of fresh mouse blood is diluted immediately into 900 µl of BSK-H. 100 µl and 900 µl are plated in solid medium containing Bb antibiotics.
3. Plating tissues. Tissues can be plated as described for blood after collagenase treatment and/or pushing through a tissue strainer or disrupting by some other mechanical means. Contamination with blood should be avoided, if possible. Removing hearts after other organs will help. Otherwise, tissues should be rinsed in medium before disruption and plating.