Colony screening for *B. burgdorferi* transformants

1. Allow colonies to grow large enough that they are clearly visible so a toothpick poke will not destroy entire colony.

2. PCR for a gene specific to the transformed DNA using the following reaction mixture per colony:

   2 μL 10X PCR buffer
   3.2 μL dNTP mix (working stock containing 1.25 mM/each dNTP)
   1 μL forward primer (20 pmols)
   1 μL reverse primer (20 pmols)
   0.2 μL Taq polymerase (1 U)
   0.2 μL 150 mM $MgCl_2$
   12.4 μL ddH_2O
   20 μL total

3. Make a master mix for the number of colonies you need to screen, plus two addition wells for positive and negative controls and 10% extra to account for pipetting error. For example, if you need to screen 10 colonies, your master mix would contain enough of each reagent for 14-20 μL reactions:

   Master Mix:
   28 μL 10X PCR buffer
   44.8 μL dNTP mix (working stock containing 1.25 mM/each dNTP)
   14 μL forward primer (20 pmols/μL)
   14 μL reverse primer (20 pmols/μL)
   2.8 μL Taq polymerase (5 U/μL)
   2.8 μL 150 mM $MgCl_2$
   173.6 μL ddH_2O
   280 μL total

4. Add 20 μL of the master mix to each well of a PCR plate for each colony, plus two addition wells for positive (DNA containing what you are screening for) and negative (no DNA or an agar stab from an area of the plate without a colony) controls.

5. Poke colony with toothpick and place into PCR well. Twist toothpick in the well a few times and then discard (toothpicks work best for this procedure, as opposed to pipet tips, etc.)

6. Run PCR reaction under the following conditions:

    1. 94°C for 1 min.
    2. 94°C for 45 seconds
    3. 55°C for 1 min.
    4. 68°C for 2 min.
    5. Cycle to step 2 for 29 more times

7. Run reactions on agarose gel.