

Colony PCR for cloning confirmation

1. Put 15uL of sterile water in a PCR tube
2. Touch slightly the colony to test with a tip and resuspended in water
3. Incubate in the PCR machine for 20 min at 95°C
4. Centrifuge 4 min at 15000 rpm
5. Transfer 2 ul of supernatant in a new PCR tube containing the PCR mix

6. PCR mix:

2.5 ul	10X Bioshop PCR Buffer
1.5 ul	25mM MgCl ₂
0.5 ul	10mM dNTP mix
0.5 ul	10 uM OligoF
0.5 ul	10 uM OligoR
0.15 ul	5U/ul Bioshop Taq DNA polymerase
17.35 ul	Water

7. Conditions:

5 min at 94°C

35 cycles of:

30 s at 94°C

30 s at 52°C (or the appropriate T_m for your Oligos)

1 min at 72°C (1 min/kb)

3 min at 72°C

hold at 10°C