

ᄬ Bacteria Colony/Liqiud PCR

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Abstract

Colony PCR using DreamTaq Green PCR mastermix (based on manufacturer ThermoFisher Scientific protocol).

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Protocol

Step 1.

For 1 PCR reaction, mix the following - multiply the amounts for the number of reactions you need, prepare 1.1x the final volume needed for your reactions:

Total	20 ul
ddH ₂ 0	7 ul
Bacteria Colony or Liqiud	1ul
reverse primer (10 uM	1 ul
forward primer (10 uM)	1 ul
Taq Mix (2X)	10 ul

Step 2.

PCR cycling protocol: (Use the lower Tm value of your primer pair)

step	temperature	time	number of cycles
initial denaturation	94°C	10min	1
denaturation	94°C	30s	
annealing	Tm-5°C	30s	25-35
extension	72°C	1min/kb	
final extension	72°C	10min	1
hold	4°C	∞	1