

Purpose: To amplify a sequence of DNA

PCR Protocol

20 μ L Reaction

1. Prepare a PCR concentration cocktail with the following proportions: 7 μ L of diH₂O, 10 μ L PCR Mastermix, 1 μ L of the forward primer, and 1 μ L of the reverse primer.
2. Add 19 μ L of the concentration cocktail into a PCR tube along with 1 μ L of the DNA.
2. Place PCR tube in the thermocycler at the following generic settings:
 1. 95° C for 3:00 minutes
 2. 95° C for 1:00 minute
 3. 52° C for 1:00 minute *Annealing temperature varies depending on primer
 4. 72° C for 1:00 minute
 5. 30X (Go to Step 2)
 6. 72° C for 5:00 minutesLid Temperature: 105° C

Colony PCR Protocol

20 μ L Reaction

1. Prepare a PCR concentration cocktail with the following proportions: 7 μ L of diH₂O, 10 μ L PCR Mastermix, 1 μ L of the forward primer, and 1 μ L of the reverse primer.
2. Add 19 μ L of the concentration cocktail into a PCR tube.
3. Using a 10 μ L micropipette, touch the tip onto the selected colony and swirl around in the PCR tube.
4. Place PCR tube in the thermocycler at the following generic settings:
 1. 95° C for 3:00 minutes
 2. 95° C for 1:00 minute
 3. 52° C for 1:00 minute *Annealing temperature varies depending on primer
 4. 72° C for 1:00 minute
 5. 30X (Go to Step 2)
 6. 72° C for 5:00 minutesLid Temperature: 105° C