

PCR Reaction Protocol

- **PCR Recipe**

Components	Liquid PCR (in μL)	Colony PCR (in μL)	Master Mix (for 4)
10X Standard <i>Taq</i> Reaction Buffer	2	2	8
10mM dNTPs	0.5	0.5	2
10 μM Forward Primer	0.5	0.5	2
10 μM Reverse Primer	0.5	0.5	2
Nuclease-free Water (ddH ₂ O)	10	10	40
<i>Taq</i> DNA Polymerase	0.125	0.125	0.5
Variable: Template DNA	7 μL	-	-
Total Reaction Mixture:	20	20	80

Quantity of DNA Template depends on DNA Concentration.

Maximum DNA load: >1000ng/ μL

The DNA Concentration used for this protocol is 95.6 $\mu\text{mol}/\mu\text{L}$

- **PCR Reaction**

PCR Reaction	Conditions for Liquid/Regular PCR (Time, Temperature)	Conditions for Colony PCR
Initial Denaturation	1', 95°C	5', 95°C
Denaturation	15", 95°C	15", 95°C
Annealing	30", 95°C	30", 95°C
Extension	1', 72°C	1', 72°C
Final Extension	5', 72°C	5', 72°C
Storage	10°C	10°C

Liquid/Regular PCR and ddH₂O goes through 1 minute of denaturation, while Colony PCR goes through 5 minutes of denaturation.