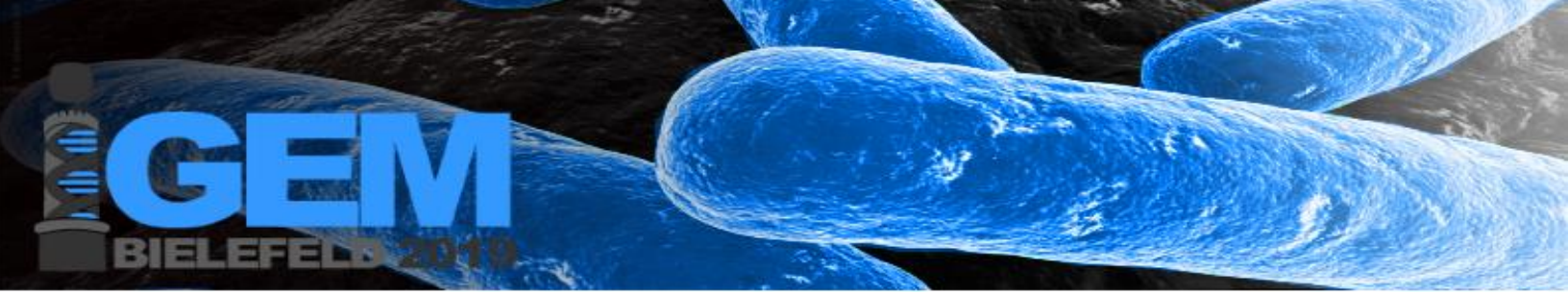

Colony PCR Using *Taq* DNA Polymerase with Standard *Taq* Buffer (M0273) from New England BioLabs® Inc.

1. Reaction setup:

It is recommended to assemble all reaction components on ice and transfer the reactions to a thermocycler preheated to the denaturation temperature (95°C).

Component	25 µL Reaction	50 µL Reaction	Final Reaction
10X Standard *Taq* Reaction Buffer	2.5 µL	5 µL	1X
10 mM dNTPs	0.5 µL	1 µL	200 µM
10 µM Primer Forward Primer	0.5µL	1 µL	0.2 µM (0.05-1 µM)
10 µM Reverse Primer	0.5µL	1 µL	0.2 µM (0.05-1 µM)
Template DNA	variable	variable	< 1.000 ng
Taq DNA Polymerase	0.125 µL	0.25 µL	1.25 Units/50 µL PCR
Nuclease-Free Water	To 25 µL	To 50 µL	

Gently mix the reaction in a PCR tube and transfer the tubes to a PCR machine and begin thermocycling.

2. Thermocycling conditions:

Step	Temperature	Time
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C	15-30 seconds
	*45-68°C	15-60 seconds
	68°C	1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

*Adjust accordingly to the annealing temperatures of the used primer.

From: iGEM Bielefeld-CeBiTec