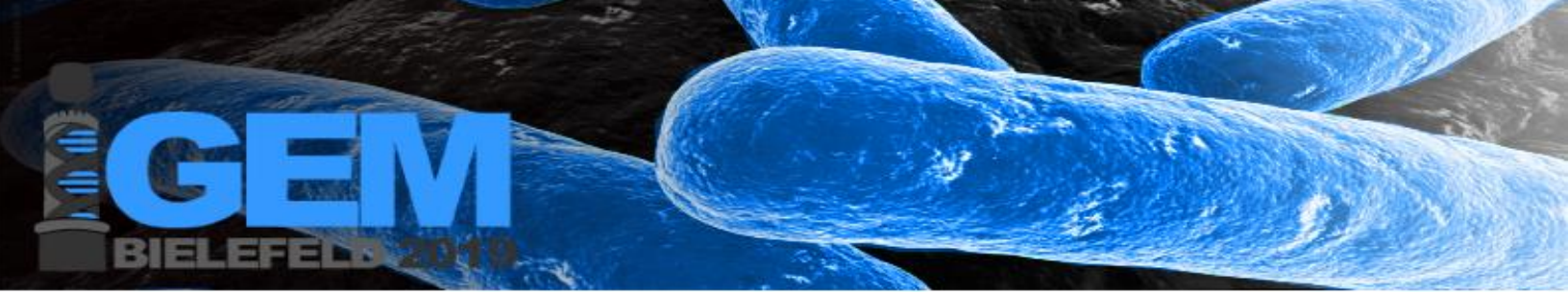

Colony PCR Using LongAmp® *Taq* DNA Polymerase (M0323)

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 (94°C).

Component	25 µL Reaction	50 µL Reaction	Final Reaction
5X LongAmp *Taq* Reaction Buffer	5 µL	10 µL	1X
10 mM dNTPs	0.75 µL	1.5 µL	300 µM
10µM Forward Primer	1 µL	2 µL	0.4 µM (0.05-1 µM)
10µM Reverse Primer	1 µL	2 µL	0.4 µM (0.05-1 µM)
Template DNA	variable	variable	<1,000 ng
LongAmp *Taq* DNA Polymerase	1 µL	2 µL	5 units/50 µL PCR
Nuclease-Free Water	To 25 µL	To 50 µL	

All the components are applied according to the amount written on the table except for the DNA polymerase, of which only half of the advised amount is applied. 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	94°C	30 seconds
30 Cycles	94°C	15-30 seconds
	*45-65°C	15-60 seconds
	65°C	50 seconds/kb
Final Extension	65°C	10 minutes
Hold	4-10°C	

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

From: iGEM Bielefeld-CeBiTec

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