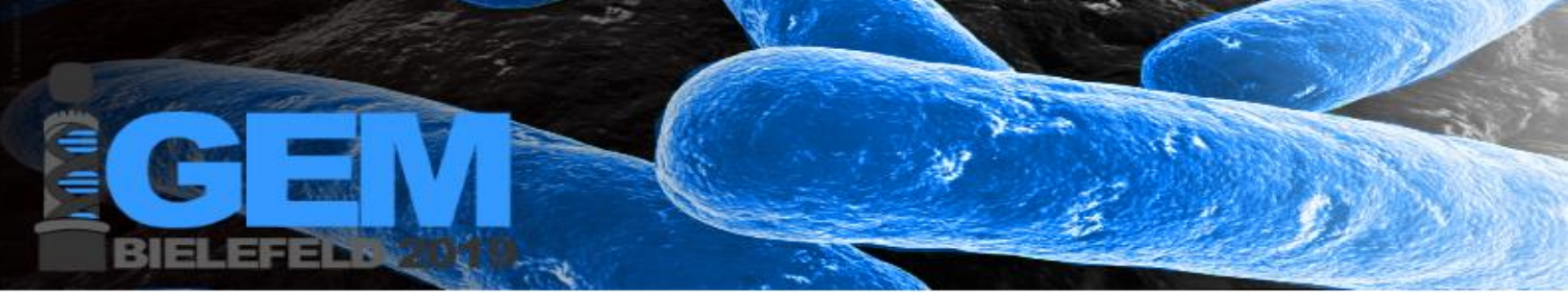

Colony PCR Using Go*Taq*® DNA Polymerase with Go*Taq*® Flexi Buffer from NEB

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
5X Colorless Go*Taq*® Flexi Buffer OR 5X Green Go*Taq*® Flexi Buffer	5 µL	10 µL	1X
10 mM dNTPs	0.5 µL	1 µL	200 µM
Forward Primer	2.5-25 pmol	0.5-50 pmol	0.1-1.0 µM
Reverse Primer	2.5-25 pmol	0.5-50 pmol	0.1-1.0 µM
Template DNA	variable	variable	<0.25µg/25µl <0.5µg/50µl
Go*Taq*® DNA Polymerase, 5u/ **µL**	0.125 µL	0.25 µL	0.625u/25 µL 1.25u/50 µL
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	2 minutes
30 Cycles	95°C	0.5-1 minute
	*42-65°C	0.5-1 minute
	72°C	1 minute/kb
Final Extension	72°C	5 minutes
Hold	4°C	

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

From: iGEM Bielefeld-CeBiTec

iGEM
BIELEFELD 2019