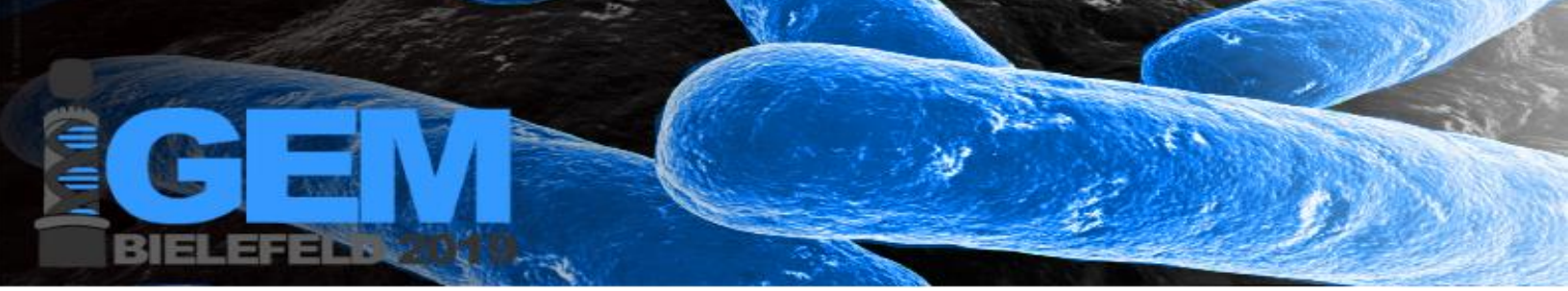

PCR Using Q5® High-Fidelity DNA Polymerase (M0491) from New England BioLabs® Inc.

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 (98°C). All components should be mixed prior to use.

Component	25 µL Reaction	50 µL Reaction	Final Reaction
5X Q5 Reaction Buffer	5 µL	10 µL	1X
10 mM dNTPs	0.5 µL	1 µL	200 µM
10 µM Primer Forward Primer	1.25 µL	2.5 µL	0.5 µM
10 µM Reverse Primer	1.25 µL	2.5 µL	0.5 µM
Template DNA	variable	variable	< 1.000 ng
Q5 High-Fidelity DNA Polymerase	0.25 µL	0.5 µL	0.02 U/µL
5X Q5 High GC Enhancer (optional)	(5 µL)	(10 µL)	(1X)
Nuclease-Free Water	To 25 µL	To 50 µL	

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

2. **Thermocycling conditions:**

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
25-35 Cycles	98°C	5-10 seconds
	*50-72°C	10-30 seconds
	72°C	20-30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4-10°C	

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

From: iGEM Bielefeld-CeBiTec