DYS SEE

PCR - Q5.

iGEM Thessaly

Protocols

PCR protocol for NEB's *Q5 High-Fidelity 2X Master Mix*

Reaction setup according to NEB

Component	25 µl Reaction	50 µl Reaction	Final Concentration
Q5 High-Fidelity 2X Master Mix	12.5 µl	25 µl	1X
10 µM 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
ddH_2O	to 25 µl	to 50 µl	

- Prepare all reactions on ice.
- Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.
- Transfer PCR tubes to a PCR machine and begin thermocycling.

Thermocycling Conditions for a Routine PCR

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
35 Cycles	98°C	10 seconds
	*50–72°C	15 seconds
	72°C	10 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

Protocols