Q5 High-Fidelity PCR

This protocol allows the amplification of a DNA fragment with Q5 High-Fidelity DNA polymerase.

Materials

- Forward primer (10 µM)
- Reverse primer (10 µM)
- Template DNA
- Q5 High-Fidelity 2X Master Mix (NEB #M0492S)
- Milli-Q water

Equipment

- Thermocycler (Eppendorf Mastercycler Nexus Gradient)
- PCR tubes
- Pipettes and pipette tips (0.5–10, 2–20 µL)

Volume Required per Reaction

Component	Volume (µL)
Q5 High-Fidelity 2X Master Mix	50
Milli-Q water	40
Forward primer (10 µM)	5
Reverse primer (10 µM)	5
Template DNA*	1

* 1 ng–1 µg genomic DNA, 1 pg–10 ng plasmid DNA

Divide the total 100 µL over 4 PCR tubes (25 µL each).

Thermocycling

Mix the entire reaction mixture and spin it down. Transfer the tubes to the thermocycler, which is set as follows:

Step	Time	Temperature	Cycles
Initial denaturation*	2 / 10 min	98 °C	
Denaturation	10 s	98 °C	
Annealing	10 s	varying**	30×
Extension	30 s/kb	72 °C	
Final extension	2 min	72 °C	
Hold	∞	4 °C	

* 2 min for plasmid, 10 min for genomic DNA

** Calculate the T_m with the New England Biolabs Tm calculator version 1.16.7

Colony PCR – OneTaq

This protocol allows the screening of bacterial colonies by PCR with OneTaq Hot Start DNA polymerase.

Materials

- Forward primer (10 µM)
- Reverse primer (10 µM)
- Bacterial colony
- OneTaq Hot Start 2X Master Mix with Standard Buffer (NEB #M0484)
- Milli-Q water

Equipment

- Thermocycler (Eppendorf Mastercycler Nexus Gradient)
- PCR tubes
- Pipettes and pipette tips (0.5–10, 2–20 µL)

Volume Required per Reaction

Component	Volume (µL)
OneTaq Hot Start 2X Master Mix	12.5
Forward primer	0.5
Reverse primer	0.5
Colony*	–
Milli-Q water	12

* Touch the colony with a 10 µL tip and mix it into the reaction mix

Thermocycling

The thermocycler is set as follows:

Step	Time	Temperature	Cycles
Initial denaturation	10 min	98 °C	
Denaturation	10 s	98 °C	
Annealing	10 s	varying	30×
Extension	60 s/kb	68 °C	
Final extension	5 min	68 °C	
Hold	∞	4 °C	

Error-Prone PCR – GeneMorph II

This protocol allows the random mutagenesis of a target sequence with the GeneMorph II kit (Mutazyme II DNA polymerase), for building libraries for directed evolution.

Materials

- Forward primer (10 µM)
- Reverse primer (10 µM)
- Target DNA (plasmid or purified fragment)
- GeneMorph II kit (Agilent #200550): Mutazyme II polymerase, 10X buffer, 40 mM dNTP mix
- Milli-Q water

Equipment

- Thermocycler (Eppendorf Mastercycler Nexus Gradient)
- PCR tubes
- Pipettes and pipette tips (0.5–10, 2–20 µL)

Volume Required per Reaction

Component	Volume (µL)
10X Mutazyme II reaction buffer	5
40 mM dNTP mix	1
Forward primer (10 µM)	1.25
Reverse primer (10 µM)	1.25
Target DNA*	variable
Mutazyme II DNA polymerase (2.5 U/µL)	1
Milli-Q water	to 50

* The amount of target sets the mutation frequency; see the table below

Mutation frequency (30 cycles)	Initial target
Low, 0–4.5 mutations/kb	500–1000 ng
Medium, 4.5–9 mutations/kb	100–500 ng
High, 9–16 mutations/kb	0.1–100 ng

Nanograms of *target*, not of plasmid: ng target = ng plasmid × (target bp / plasmid bp)

Thermocycling

The thermocycler is set as follows:

Step	Time	Temperature	Cycles
Initial denaturation	2 min	95 °C	
Denaturation	30 s	95 °C	
Annealing	30 s	varying*	30×
Extension	60 s/kb	72 °C	
Final extension	10 min	72 °C	
Hold	∞	4 °C	

* Calculate the T_m with the New England Biolabs Tm calculator, Taq setting

Keep the cycle number at 30, as fewer cycles also lower the mutation frequency. Run a no-template control and check the product on a gel