PCR

Vent PCR

1. Prepare 50µl reaction in 0.5 ml PCR tube on ice

	Volume (µl)	Final concentration
ddH_2O	Fill up until the final volume is 50µl	
ThermoPol Reaction Buffer (10X)	5	1X
Deoxynucleotide (dNTP) Solution Mix (10mM)	1	200µM
Forward Primer (10µM)	0.5 - 2.5	0.1 – 0.5 µM
Reverse Primer (10µM)	0.5 - 2.5	0.1 – 0.5 µM
DNA tempelate	± 10ng	
Vent DNA Polymerase	0.5	

2. Mix the reaction and spin down in microcentrifuge
3. Put the tube in the PCR machine using below conditions

Step	Temp	Time
Initial Denaturation	95°C	2-5 minutes
20-30 Cycles	95 °C 55-65 °C 72 °C	15-30 seconds 15-30 seconds 1 minute per kb
Final Extension	72 °C	5 minutes
Store	4-10 °C	

Taq PCR

1. Prepare 50µl reaction in 0.5 ml PCR tube on ice

Component	20µl Reaction	50µl Reaction	Final Concentration
ddH_2O	up to 20µl	up to 50µl	
10X ThermoPol or Standard Taq Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05-1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05-1 µM)
DNA	Depend on the concentration	Depend on the concentration	< 1000ng
Taq DNA Polymerase	0.125 µl	0.25 µl	1.25 units/50 µl PCR

2. Mix the reaction and spin down in microcentrifuge
3. Run the PCR machine using below conditions

Step	Temp	Time
Initial Denaturation	95°C	30 seconds
25-35 cycles	95 °C 45-68 °C 68 °C	15-30 seconds 15-60 seconds 1 minute per kb
Final Extension	68 °C	5 minutes
Hold	4-10 °C	

Phusion PCR

1. Prepare the mixture in PCR tubes on ice

Component	20µl Reaction	50µl Reaction	Final Concentration
ddH_2O	up to 20µl	up to 50µl	
5X Phusion HF or GC Buffer	4 µl	10 µl	1X
10 mM dNTPs	0.4 µl	1 µl	200 µM
10 µM Forward Primer	1 µl	2.5 µl	0.5 µM
10 µM Reverse Primer	1 µl	2.5 µl	0.5 µM
DNA	Depend on the concentration	Depend on the concentration	< 250 ng
Phusion DNA Polymerase	0.2 µl	0.5 µl	1.0 units/50 µl PCR

2. Mix the solution and moves the tubes from ice to PCR machine
3. Run the PCR machine using below conditions

Step	Temp	Time
Initial Denaturation	98°C	30 seconds
25-35 cycles	98 °C 45-72 °C 72 °C	5-10 seconds 10-30 seconds 15-30 seconds per kb
Final Extension	72 °C	5-10 minutes
Hold	4-10 °C	