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	

Isolation of gDNA from Mamalian Cells

Materials and Solutions

1. Cell Resuspension Buffer TE Buffer (100ml)

- 1 ml of 1 M Tris-Cl (10mM Tris-HCl pH 8.0)
- 1 ml of 0.5 M EDTA (10mM EDTA)
- Add ddH_2O to make a final volume to 100ml
- Keep at 4^oC

2. Proteinase K Solution (10 mg/ml)

- 1 mg of Proteinase K
- 1 ml ddH_2O
- Stored at -20 ^oC

Procedures

1. Trypsinize, harvest and resuspend cells at 10^7 cells/ ml in Cell Resuspension Buffer (TE Buffer). No more than 500ul.
2. Add 10% SDS and Proteinase K to a final concentration of 0.5% and 200 µg/ml, respectively.
3. Mix and incubate at 55°C for 2 hours.
4. Add NaCl to a final concentration of 0.2M.
5. Extract twice with equal volumes of phenol:chloroform (1:1), centrifuge at full speed for 5 min, then extract supernatant and transfer in to a clean tube then add once with initial volume of chloroform. Centrifuge at full speed for 10 min.
6. Transfer supernatant to a clean tube.
7. Add RNase A(DNase-free and protease-free) to a final concentration of 25 µg/ml and incubate for 1 hour at 37°C. The concentration of the enzyme may vary for different cell types.
8. Extract twice with equal volumes of phenol:chloroform (1:1), centrifuge at full speed for 5 minutes, then extract supernatant and transfer in to a clean tube then add once with initial volume of chloroform. Centrifuge at full speed for 10 minutes.

9. Precipitate DNA by adding 1.5 volumes of 100% Ethanol.
10. Centrifuge at 10,000x g for 8 minutes to pellet the DNA. Do not over-dry the DNA pellet.
11. Resuspend pellet with 75% Ethanol then centrifuge at full speed for 10 minutes, remove the supernatant and dry remaining ethanol do not over dry the pellet, it will be very difficult to dissolve the DNA in TE buffer.
12. Resuspend the pellet in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.