PCR Amplification using Phusion Polymerase

Aim

To amplify gene of interest and the plasmid for further analysis and/or experiments.

Procedure

Recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C).

1. Thaw 5x Phusion GC or HF buffer, dNTP mix and primer solutions on ice. Keep solutions on ice after complete thawing and mix thoroughly before use to avoid localized differences in salt concentration.

2. Prepare a master reaction mix according to Table 1. Mix will contain all components needed except the template DNA. Calculate the appropriate volumes yourself - it is recommended to add the volumes for one extra reaction for each 8 reactions, e.g. for 16 reactions prepare a master mix sufficient for 18 reactions.

Table 1: Reaction Mix for total reaction volume of 20μl

Component	Volume/Reaction [μl]	Final concentration
5X Phusion HF or GC Buffer	4	1x
dNTP mix (2 mM)	2	200 mM
Phusion DNA polymerase	0.2	1.0 units/ 50 [μl] reaction
Forward Primer	See end of protocol	0.5 μM
Reversed Primer	See end of protocol	0.5 μM
Template DNA	variable	$<$ 250 ng
DMSO (optional)	(0.6 [μl]) 3%	
RNAse free water	Fill up to 20	

Table 2: PCR Thermocycler Program

Step	Temperature [°C]	Time
Initial Denaturation	98	30s
25-35 Cycles	98	5-10s
	45-72	10-30s
	72	15-30s per kb
Final Extension	72	5-10 min
Hold	4-10	N/A

3. Mix the reaction mix gently but thoroughly, by pipetting up and down a few times.

4. Place a 96 well plate into a ice bucket as a holder for the 0.2 ml thin walled PCR tubes. Transfer reaction mix to a PCR tube. Allowing the PCR reaction mix to be added into cold 0.2 ml thin walled PCR tubes will help prevent nuclease activity and nonspecific priming.

5. For each PCR tube add 1 μg of your DNA template, i.e. 1 μg DNA template/ reaction. For each reaction set up, make a negative and positive control with a PCR tube containing:
   a) all PCR components except the DNA template, our **negative control** (No template control, NTC), fill the missing volume with water.
   b) all PCR components with a DNA template you know will be amplified if reaction goes as planned with same primers as in your sample, our **positive control** (if standard template DNA is available)

6. Program the thermocycler according to the conditions in Table 2. Annealing temperature should Approximately be 5 °C below Tm of primers. For PCR products longer than 1 kb, use an extension time of 1 min per kb DNA. Put caps on the 0.2 ml thin walled PCR tubes and place them into the thermocycler. Once the lid to the thermocycler is firmly closed, start the program.

7. When the program has finished, the 0.2 ml thin walled PCR tubes may be removed and stored at 4 °C.

8. PCR products can be detected by loading aliquots of each reaction into wells of an agarose gel then staining DNA that has migrated into the gel following electrophoresis.

Primers

Amount of Primers volume/reaction for primers calculate based on primer concentration. If VF2_R and VF2_F **working stock** are used, it is 2 μl per reaction for each of the primers.

Sources

PCR Protocol for Phusion High-Fidelity DNA Polymerase (M0530).