PCR

Aim of the experiment

This experiment can be used for exponential amplification of a DNA of interest. There are different existing variations and applications of the reaction which can be used for special functions (i.e. addition of certain short sequences at 3 or 5 point end, insertion of point mutation etc.) Use NEB Tm Calculator to calculate the annealing temperature of the primers.

Materials

- Reverse Primer
- Template DNA
- 2×Pfu MasterMix（Dye）
- Forward Primer
- ddH2O

Procedure

1. To a PCR tube add following reagents:

Table : PCR-Mix

Chemicals	Concentration
2×Pfu MasterMix（Dye）	1×
Forward Primer，10 μM	0.4uM
Reverse Primer，10 μM	0.4uM
Template DNA	<0.5 μg/50 μl
ddH2O	up to 50 μl

Transfer tube to a Thermocycler and run following program:

Step	Temperature	Time
Initial denaturation	94℃	2min

denaturation	94℃	30s	30 cycles
annealing temperature	60℃	30s	
extension	72℃	10s	
Final extension	72℃	5min	

Possible follow-up protocols

The following protocols are the next steps of a possible cloning cycle after a Polymerase Chain Reaction (PCR):

1. Restriction digest
2. Agarose-Gel-electrophoresis
3. PCR clean-up