PCR protocol using MyTaq Red Mix

Description

Polymerase chain reaction (PCR) is a common molecular technique used to amplify DNA. In this course, we will use MyTaq Red Mix to perform fast and highly-specific PCR. This master mix contains all the reagents needed for a trouble-free PCR set up. The reaction mixture only requires the addition of the DNA template, primers and water. Furthermore, the users can load samples directly onto a gel post PCR without adding any loading buffer.

Materials

Reagent	Vol/rxn (uL)	x ____ rxns (uL)
PCR grade molecular water	16	
MyTaq Red Mix (BIOLINE cat. no. BIO-25043)	25	
Forward primer	2	
Reverse primer	2	
DNA template	5	NA
Total volume	**50**	

Procedure for setting up PCR

1. Calculate how much of each reagent you will need for ***n*** reactions. **Remember to include positive and negative controls**.
2. Prepare a master mix by adding the above reagent in order starting with the water. **Do not add DNA**.
3. Aliquot 45uL of the master mix into each PCR tube.
4. Next, add 5uL of DNA template to the labeled tubes.
5. Tightly close the tubes, spin down, quickly flick the tubes to mix the contents and spin down before putting the tubes in the thermocycler.

PCR conditions

1. Configure the thermocycler with the following PCR cycling conditions.

Step	Temperature	Time (mm:ss)	Cycles
Initial denaturation	94°C	3:00	1
Denaturation	94°C	0:45	35
Annealing	50°C	1:00	
Extension	72°C	1:30	
Final extension	72°C	10:00	1
Hold	4°C	∞	NA

2. After the PCR is completed, perform gel electrophoresis to confirm the presence of a PCR product. Use 5uL of the product for running the gel.