**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.