# Polymerase Chain Reaction (PCR)

## Materials

- **Sterile $ddH_2O$**
- **10X PCR Buffer**
- **$MgCl_2$, 50 mM**
- **dNTPs, 2.5 mM**
- **5' and 3' Primers, 2.5 µM**
- **DNA Taq Polymerase, 5 u/µl (Bioline, Cat # BIO-21042)**

## Methods

- **PCR Master Mix**

1. Keep all reagents on ice as well as the tubes for PCR.

2. Make a PCR master mix by adding the following ingredients in the order shown. Add the Taq Polymerase just before using. Mix well by vortexing.

| Solution | Volume (µl) | Stock concentration | Final concentration |
|---|---|---|---|
| $ddH_2O$ | 35.5 | | |
| dNTPs | 2 | 2.5 mM | 0.1 mM |
| Primer 1 | 2 | 2.5 µM | 0.1 µM |
| Primer 2 | 2 | 2.5 µM | 0.1 µM |
| $MgCl_2$ | 1.5 (0.5-5) | 50 mM | 1.5 (0.5-5) mM |
| PCR Buffer | 5 | 10X | 1X |
| Taq Polymerase | 0.5 | 5 u/µl | 2.5 u/reaction |
| Total | 50 | | |

The $MgCl_2$ concentration in the final reaction mixture is usually between 0.5 to 5.0 mM, and the optimum concentration is determined empirically (typically between 1.0 - 1.5 mM) $Mg^{2+}$ ions**.** Generally, low Mg2+ leads to low yields (or no yield) and high $Mg^{2+}$ leads to accumulation of nonspecific products (mispriming).

3. Add 1-5 µl DNA to PCR tube then add 20-24 µl master mix so that the total volume is 25 µl. The amount of DNA needed will depend upon the concentration; 10-100 ng of genomic DNA or 1-10 ng of plasmid DNA is sufficient.

4. Briefly spin the tubes to make sure all liquid is at the bottom.

### ⌘ PCR Reaction

1. Set up a PCR template such as;

| Step | Temperature (°C) | Duration (minutes) | Number of cycles |
|---|---|---|---|
| Initial Hold | 94 | 3 minute | 1 |
| Denaturation | 94 | 30-90 seconds | 25-40 |
| Annealing | 50-60 | 30-90 seconds | |
| Extension | 72 | 30-90 seconds | |
| $2^{nd}$ Hold | 72 | 10 | 1 |

- Primers are usually 18-30 bp long with similar $T_m$ values.
- 30 cycles is typical, too many can result in a high amount of nonspecific background.
- The initial hold step may be as long as 5 minutes.
- The denaturation, annealing, and extension time will depend upon on the length of the PCR fragment.
- The annealing temperature is usually 5°C lower than the true $T_m$ of the primers.

## References

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