

**PCR (15ul reaction)**

1. Reserve a PCR machine by signing up the sign sheet.
2. Bring ice in a bucket from the ice machine.
3. Take out the required stuff from -20°C freezer i.e. DNA, 10X buffer, MgCl<sub>2</sub>, dNTP. Allow them to thaw /melt and then place them on ice.
4. Decide on the primers you want to work with and take them out of the freezer and let them thaw/melt and then place them on ice.
5. Remove TAQ polymerase at last and place on ice.
6. **Make a plan in your note book** of how you want to perform the PCR reaction today and then start.

Chemicals	Conc.	1X	12X	30X	50X	100X	110X
10X Buffer	-	1.5ul	18ul	45ul	75ul	150ul	165ul
dNTP	2.5mM	1.5ul	18ul	45ul	75ul	150ul	165ul
MgCl <sub>2</sub>	50mM	0.45ul	5.4ul	13.5ul	22.5ul	45ul	49.5 ul
Taq Poly	5U/ul	0.06ul	0.72ul	1.8ul	3.0ul	6.0ul	6.6 ul
Primer	4uM	1.5ul	18.0ul	45ul	75ul	150ul	165 ul
DNA	20ng/ul	5.0ul	60ul	150ul	250ul	500ul	550ul
ddH <sub>2</sub> O	-	4.99ul	59.9ul	149.7ul	249.5ul	499ul	548.9ul
<b>Total</b>		<b>15.0ul</b>					

**Note:** Out of the Primer and DNA conc. given, only one can be used in one experiment. if primer conc. applies in your expt. then DNA conc. have no meaning **and** if DNA conc. applies in your experiments, then primer conc. have no meaning (these are **mutually exclusive**)

7. Make the PCR cocktail mix (MASTER MIX) in 1.5-2.0 ml centrifuge tube by adding each component in the order: water, 10x buffer, dNTPs, MgCl<sub>2</sub>, then primers in the required amount (if no. of reactions is very small (6 or less) you can directly add the components to the PCR tube).
8. Add Taq Polymerase to the MASTER MIX.
9. Vortex the MASTER MIX to mix all components.
10. Add the cocktail mix in a PCR tube or PCR plate in required amount (that varies from 10-13.5 ul per reaction well).
11. Seal the tube with a cap or the PCR plate with the film.
12. Gently vortex the sample/plate and then briefly centrifuge to collect all drops from walls of tube/plate.

**Reaction in PCR machine:**

1. Turn on the machine.
2. Open the folder and select the file to check its steps are **ok!**

3. **Run the desired program.** (The PCR reaction is divided into three repeated cycles)

- Step1** Denaturation at **95°C for 5-6 mins.**
- Step2** Denaturation at **95°C for 30-60 secs**
- Step3** Annealing at **50°C to 65°C for 30-60 secs**
- Step4** Extension at **72°C for 1.30-2.00 mins**
- Step5** **GO to Step 2 for 34 cycles**
- Step6** Final Extension at **72°C for 8 mins**
- Step7** Stay/Hold at **4°C for infinite time**
- Step8** END

13. Remove the samples after the reaction is finished and store at **4°C**.

Notes:

1. Use double distilled autoclaved water for reactions.
2. Always keep everything on ice.
3. Always make notes of your PCR reaction.
4. Label the PCR plates and tubes correctly with date and annealing temperature on it.
5. **Add template DNA in the plates (5-6 ul) before you start the PCR reaction. It is better if you make multiple copies of PCR plates with template DNA so that you can use them for 2-3 days.**