PCR protocol

Emphasis:

- Avoid contamination! Work with clean gloves. Clean the pipettors and working area with ethanol. Cover the working area with paper.
- The polymerase enzyme must be kept at -20 °C at all times. When adding it to the reaction mix, take it out, put it on ice, bring to your working area, draw the required amount and return the enzyme to the -20 °C freezer immediately.
- You will be making a 20- 50 µl reaction for each tube.

You need to fill the table below on each PCR:

Clone name:_____ date:_____

Template name:_____

Tm: _____

If using Phusion Hot Start – this is your protocol

	component	Volume
-20°C	Buffer (X10)	5 µl
-20°C	dNTPs (10 mM)	1 µl
-21°C	Forward Primer [10 µM]	2.5 µl
-21°C	Reverse Primer [10 µM]	2.5 µl
-20°C	Template [~2-10ng]	X µl
-20°C	PCR enzyme	0.5 µl
	Ultra pure water	Y µl
	Total Volume	**50 µl**

Before you start the procedure:

- Fill reaction table for each reaction and paste it into your group lab notebook.
- If working with plasmid DNA (and not genomic DNA), the final amount of DNA in the reaction tube should be 2-10 ng. You need to calculate the required dilution of your minipreped/PCRed plasmid and the volume that you will use for the reaction. Typically you will need to dilute the minipreped plasmid by 100 or by 50 times to a final volume of 100 µl in an ependorf. Keep this diluted solution at -

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20 °C for future use (mark the ependorf with the plasmid name, insert and concentration).

- Write down the size of your amplified DNA in kb.
- Write down the Tm for each primer (according to the IDT OligoAnalyzer or NEB Tm calculator). The annealing temp should be use 3°C above the Tm of the lower Tm between the two primers used

If a mix for multiple reactions is prepared, keep the following in mind:

- The enzyme is expensive so prepare enough mix for the exact number of reactions that you are going to run (including control).
- The mix should include: ultra pure water, buffer, primers, template, DNTPs and the enzyme, once you add the enzyme, the mix tube must be on ice!
- In general when preparing the mix, add the components in order from the biggest to smallest volume.
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- After adding all the ingredients to the mix, do spin down (NOT VORTEX - the enzyme is very sensitive to harsh vortex).
- Divide the total mix volume by the number of reactions (including control) that you are going to prepare. This will be the volume of mix to be added to each reaction.
- The control reaction should be the regular reaction without template (to check for DNA contaminations). In this case replace the template with the same volume of ultra pure water

The PCR program:

In the PCR machine, there is a folder named iGEM 2014 , within this folder all of your PCR programs will be saved.

There are two fields to be adjusted each time you run the program:

1. Annealing temperature - Typically, for primers > 20 nt, use 3°C above the Tm of the lower Tm primer. Also, if needed, you can determine a range of different Tm's,
2. Time for the extension step 30 seconds per kb.

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3. If you are changing polymerase look at the specific protocol and change the program accordingly.
4.

The program is as follows (change only the 2 bold underlined steps):

Initial denaturation:

98°C 10-30 seconds

98°C 10 seconds

Annealing temperature **(Y °C)** 15-30 seconds

72°C 15-30 seconds/1 kb → Extension step: **X seconds**

72°C 2-10 minutes

4°C hold

Final notes

- The PCR products are kept at -20 °C.
- Run 5ul from the reaction tube on agarose gel.
- Take a picture of the resulting bands on the gel and paste it into your notebook- regardless to the result!
- Purify the products from gel/PCR, based on the gel results.
- After the purification, the concentration of the PCR products should be measured in Nanodrop and documented in your notebook.

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