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# PCR

*Before starting your PCR you will need to design Primers. Make sure that the annealing temperatures of you forward and your reverse primer have a difference of not more than 5°C. To calculate the annealing temperature use the website from the company you ordered your PCR components from. We used Biolabs, where they have an application that directly calculates you annealing temperatures.*

*It is also important to keep all the components and the reaction on ice. Make sure to add the polymerase last to avoid unspecific amplifications and/or denaturation of the protein. If you want to make several PCR, it is better to do a master mix in advance. And of course do not forget your negative control, for example:*

*-Add Master mix without DNA*

*-Add Master mix with DNA but without polymerase*

*We used two different cycling programs. One that is standard and the other one that runs the first ten cycles 10°C bellow the normal annealing temperature. This is very useful if your PCRs do not work. If you use a plasmid as template you can also linearized it first with a Restriction Digest, just make sure you do not cut within your amplicon.*

## MATERIALS:

Component	50ul Reaction	Final Concentration
Nuclease free water	_____ ul (up to 50ul of total volume)	
5X HF Buffer	10ul	1X
10mM dNTP's	1ul	200 uM
10uM Forward Primer	2.5ul	0.5 uM
10uM Reverse Primer	2.5ul	0.5 uM
Template DNA	100ng	<250 ng
Phusion DNA polymerase	0.5 ul	1.0 units/50ul PCR

## METHOD:

*Add the components in decreasing order of volume with the Phusion polymerase last.*

Cycling conditions (standard)

Cycle step	Temp	Time	#of cycles
Initial Denaturation	98°C	30s	1
Denaturation	98°C	10s	35
Annealing	Primer temp	30s	
Extension	72°C	30s/kb	
Final extension	72°C		1
	4°C		

Cycling conditions (special)

Cycle step	Temp	Time	#of cycles
<b>Initial Denaturation</b>	98°C	30s	1
<b>Denaturation</b> <b>Annealing</b> <b>Extension</b>	98°C Primer temp-10°C 72°C	10s 30s 30s/kb	10
<b>Denaturation</b> <b>Annealing</b> <b>Extension</b>	98°C Primer temp 72°C	10s 30s 30s/kb	25
<b>Final extension</b>	72°C 4°C		1 1

