

## PCR

Last updated: December 10, 2010

Description: PCR involves using genomic DNA along with the appropriate primers to amplify a gene or other region of interest

Total time: 3hr      Lab time: 0.5-1hr      Wait time: 2hr

\*Make sure the PCR machine is not being used or other lab members are not planning to use it

1. Primers will come in small aliquots. Re-suspend primer in ultrapure H<sub>2</sub>O to a concentration of 100uM

Quick calculation: for example, if you have 27.95 nM of primer, you will resuspend it in 279.5ul of water (multiply the #nM by 10)

2. Mix PCR reactant with DNA of interest:

\*It is easier to make a **master mix** of PCR reactants. When making a master mix, make enough for 1 extra tube to account for pipetting error.

For EACH sample (**TAQ**) \*use for PCR checks

20ul water  
3ul Taq buffer (10x)  
0.5ul dNTPs

or

For Each sample (**PHUSION**) \*use for initial PCR from genomic DNA or cDNA

18ul water  
5ul Phusion buffer (5x)  
0.5 ul dNTPs

Then add the following:

0.25 - 0.5ul DNA  
0.5ul 5' primer  
0.5ul 3' primer  
0.25 - 0.5ul Taq/Phusion polymerase (add LAST!! only take out of freezer when needed. RETURN TO FREEZER after use!)

3. After ALL of the above are added in the PCR tubes, add a drop of mineral oil to the top of each PCR tube (~10-20 ul)
4. Stick tubes in PCR machine. Run PCR on correct program for 1.5-2hr.  
Run Taq on JERTAQ                      Run Phusion on JTPHUS

\*make sure the annealing temperature and the extension time are correct for your PCR run (see next page!)

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**JERTAQ**

<u>Step</u>	<u>Temp</u>	<u>Time</u>
1	95	3min
2	95	15sec (DENATURE)
3	*	30sec (ANNEALING)
4	72	** (EXTENSION)
5	"28 times to 2"	
6	72	5min
7	4	DONE

\* primer annealing temp (usually 60)

\*\* extension time = 1 min per 1 kb length

**JTPHUS**

<u>Step</u>	<u>Temp</u>	<u>Time</u>
1	98	2min
2	98	10sec (DENATURE)
3	*	1min (ANNEALING)
4	72	** (EXTENSION)
5	"28 times to 2"	
6	72	5min
7	4	DONE

\* 3 degrees above primer annealing temp (usually 63)

\*\* extension time = 30 sec per 1 kb length