PCR – a general protocol

1. Mix the following reagents in a 0.2-mL thin-walled PCR tube:

   50 ng of template DNA[1]
   50 pmol forward primer[2]
   50 pmol reverse primer[2]
   5 μL 10X PCR Buffer without $MgCl_2$[3]
   4 μL 25 mM $MgCl_2$
   1 μL 10 mM dNTP mixture
   0.2 μL Taq DNA polymerase[4,5]
   sterile water

   total volume: 50 μL

2. Perform PCR as follows:

	1 cycle:	**94˚C for 3 min**
	25 – 35 cycles:	**94˚C for 30 sec** **55˚C for 30 sec** **72˚C for 1 min per kb product size[5]**
	1 cycle:	**72˚C for 10 min**
	1 cycle:	**4˚C indefinitely**

3. Analyze 5 μL of PCR by agarose gel electrophoresis.

*If many templates are to be subjected to PCR, prepare a "master mix" containing all of the reagents except the DNA and/or primers (if different templates or primer sets are required). Add aliquots of the master mix to an aliquot of each DNA and/or primer set that has been aliquoted into a PCR tube. To account for volume loss, the master mix should contain enough reagents for all of the samples plus an additional 2 – 3 samples.

For example, if 8 different templates are to be examined using the same set of primers, and all of the templates consist of miniprep DNA that has been diluted 1:150 in sterile water:

reagent:	**for 1 sample:**	**master mix for 10 samples:**
25 μM forward primer	2 μL	20 μL
25 μM reverse primer	2 μL	20 μL
10X PCR Buffer	5 μL	50 μL
25 mM MgCl2	4 μL	40 μL
10 mM dNTP mix	1 μL	10 μL
Taq DNA polymerase[4]	0.2 μL	2 μL
sterile water	34.8 μL	348 μL
total volume:	49 μL	490 μL

Vortex master mix briefly, then pulse-spin at full speed for 5 seconds. Dispense 49 μL of the master mix to PCR tubes in which 1 μL of each template has been aliquoted.

Notes:

[1]If plasmid miniprep DNA is to be used as a template, dilute the DNA 1:150 in sterile water and use 1 μL (~50 ng). Use 5 μL of template consisting of genomic DNA harvested from 5 mL of an overnight saturated culture of yeast cells and resuspended in 500 μL TE.

[2]A convenient primer stock concentration is 25 μM in sterile water.

[3]Some manufacturers include $MgCl_2$ in their 10X PCR buffer. If so, do not add more $MgCl_2$.

[4]Add Taq to the reaction last.

[5]A high-fidelity polymerase (e.g., Pfu) can be added together with Taq or instead of Taq. In either case, increase the extension time at 72˚C to 2 min per kb product size.

12/10/04