

## GENERAL PCR PROTOCOL:

### Ex. Candidate Gene Search or Genotyping

#### Reagents and Materials:

96 deep well plate	dNTPs
Taq Polymerase	dH <sub>2</sub> O
DNA or cDNA template	DMSO
Primers	

#### Technique notes:

- CHILL PRE-MIX TUBE and PCR PLATE ON ICE WHILE WORKING!
- Not necessary to wear gloves.
- When making pre-mix: make one more vol than needed (4x->5x).

#### MIX:

If testing different primers on one type of template, add template to pre-mix but obviously not primers.

Ex. Candidate gene search: test different primers on mutant and wt

If testing one type of primer pair on different templates, add primers but not templates.

Ex. Genotyping for mapping: test different mouse genomes using 2 pairs of microsatellite marker primers

#### 1X:

20 $\lambda$	10 X Promega PCR Buffer
0.2	Taq Pol ( <b>Add last</b> )
0.8	dNTPs
0.5	DMSO (If expecting long or GC rich amplicons)
0.5	Primer 1 (f)
0.5	Primer 2 (r)
?	Template 2 $\mu$ g
$\frac{?}{20 \lambda}$	dH <sub>2</sub> O

1. Thoroughly mix above by flicking upon addition of Taq.
2. Load 20  $\lambda$  minus 1 X volume of primers (= 19 $\lambda$ ) of pre-mix into designated wells if omitted primers from pre-mix.  
Load 20 $\lambda$  minus volume of 1X template required if omitted template from pre-mix.
3. Cover plate with mat before putting on PCR machine. Go to PCR with plate still on ice to do a "HOT START". (This is to minimize primer dimers.)  
Do not put plate onto block until lid is heated and block has warmed up to 70°C.

**Do not edit a cycle someone is already running!!!** Be sure to check other blocks!