

**Lab Prep**  
**Worm PCR lab**  
**By Lynn Boyd at University of Alabama in Huntsville**

**Lab day #1:**

Each Group

- dissecting scopes
- 1.5 ml tube with 95% EtOH
- plate with dpy-5 heterozygotes
- plate with bacteria (no worms)
- paintbrushes and green fishing line

**Lab day #2:**

Each Group

- dissecting scopes
- 1.5 ml tube with 95% EtOH
- worm plate from previous lab
- $\mu$ fuge tube rack
- three small  $\mu$ fuge tubes (0.6ml)
- pipettors and tips
- waste beaker for tips
- paintbrushes and green fishing line

Common supplies:

- ice
- lysis buffer (should be stock in -20° freezer)
- add proteinase K to lysis buffer immediately prior to lab  
(proteinase K in -20° freezer; thaw on ice)

**Lab day #3:**

Each Group

- $\mu$ fuge tube rack
- pipettors and tips
- waste beaker for tips

Common supplies:

- ice
- PCR master mix  
must be made immediately prior to lab  
make at least this much: # of groups times 75  $\mu$ l
- thermocycler

### Recipe for PCR Master Mix

Stock conc.	Ingredient	Amount	Final conc.
10X	Taq buffer	110 $\mu$ l	1.1 X
10 mM	dNTP's	25 $\mu$ l	250 $\mu$ M
20 $\mu$ M	Primer 1	100 $\mu$ l	2 $\mu$ M
20 $\mu$ M	Primer 2	100 $\mu$ l	2 $\mu$ M
	H <sub>2</sub> O	659 $\mu$ l	
5 u/ $\mu$ l	Taq DNA pol	6 $\mu$ l	0.03 u/ $\mu$ l
		Total=1000 $\mu$ l	

#### Lab day #4:

Each Group

- $\mu$ fuge tube rack
- pipettors and tips
- waste beaker for tips
- agarose gel and power supply

Common Supplies

- 6X loading dye
- DNA MWM's
- EtBr bath
- containers for rinsing gels after EtBr
- gloves