**PCR Mix:**

2.3575µl  H₂O  
1.15µl  10x Perkin-Elmer PCR Buffer  
0.92µl  10mM dNTP  
0.69µl  25mM MgCl₂  

We make this in large batches and freeze in 500µl aliquots at –20°C.  

When you are ready to set up your PCR make a cocktail:

**Cocktail:**

5.1175µl  PCR mix  
0.575µl  primers (5µM each)  
0.08µl  Taq (we use Taq Gold routinely as some markers need it)  

When you make the cocktail multiply by exactly how many reactions you have.  The extra cocktail you need due to pipeting error has already been calculated in.  Next set up your PCR reaction:

**PCR Reaction:**

5µl cocktail  
5µl DNA (see below)  

Run PCR, our standard program is:

94°C, 12:00  1X  
94°C, 0:20; 55°C, 0:30; 72°C, 0:45  55X  
72°C, 7:00  1X  

Note on the DNA:

The DNA for the genome scan is prepared more carefully than the DNA we use to genotype daily in the colony.  It is a phenol prep and the DNA is diluted to 10ng/µl so that 50ng go in each reaction.  

For ear punches:

Use 3% proteinase K in 1 X PCR buffer.  Put at 55°C overnight.  Inactivate PK with 10 minutes at 95°C.  Vortex 5 minutes.  Dilute DNA 1:10 (we usually do 5µl in 45µl H₂O in 96 well plate).