

I. Single worm PCR

A. Make worm lysis buffer

1. Make a solution of 30mM Tris at pH8 with 1mg/mL proteinase K
 - a) *Good to have a stock of 30mM Tris*
 - b) *Good to have a stock of aliquoted 10 or 20 mg/mL proteinase K (10 or 5 uL aliquots are common for making 100uL of lysis solution)*

B. Put 10uL of lysis solution into a PCR tube

1. Place an individual adult worm into the PCR tube using a bent pick
 - a) *a right angle (elbow) on your pick often works well here*
 - b) *use the stereoscope optics to help guide or confirm the placement of the worm into the tube*
 - c) *5ul volume can be used for smaller worms*
 - d) *PCRs from 10 larval worms in 10 uL lysis solution work*
2. Use a PCR machine to incubate the tube at 60° (centigrade) for one hour, followed by a 20 minute incubation at 95° to heat kill the proteinase K
 - a) *Failure to heat kill the proteinase K will cause PCR to fail because proteinase K will digest the DNA polymerase*
3. Screen for genotype using Invitrogen platinum blue supermix
 - a) *This mix works well for "dirtier" DNA and the reactions can be loaded directly into gels because they are already viscous/denser than buffer*