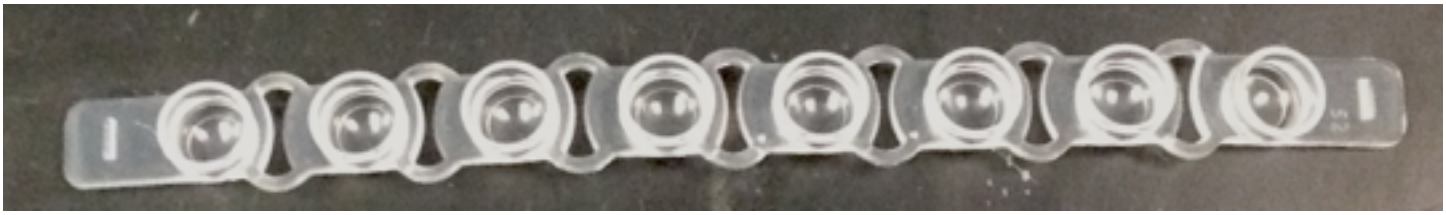
June 2013

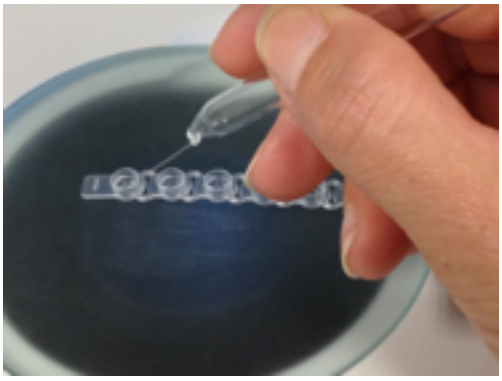
Genotyping Worms by PCR
(a.k.a. - “Lysis and PCR”)

1. Prepare lysis solution by combining 98 µl of 2X Lysis Buffer (recipe below) with 2 µl of 20 mg/ml Proteinase K (PK).
   a. Prepare enough lysis solution for the number of lyses you want to carry out.
      i. For example, if you want to do 8 lyses, 100 µl of lysis solution is enough. If you want to do 24 lyses, you will need 144 µl and you will need to make up 200 µl of lysis buffer.

2. Aliquot 6 µl of the lysis solution into each cap of an 8-tube strip cap using the Eppendorf repeat pipetter:

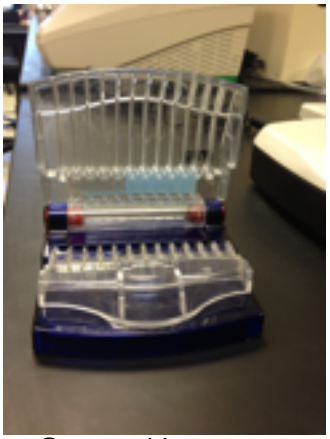


3. Pick desired number of worms into each well by picking the worms up from the plate and then shaking the pick in the lysis solution for the appropriate cap.

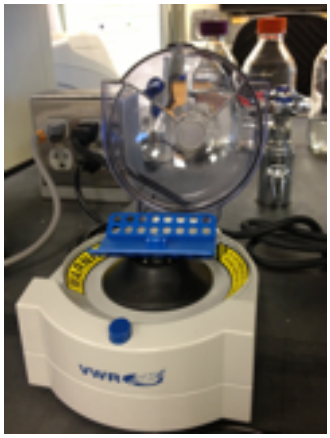


   a. You can watch the pick in the cap through the microscope to make sure that the worms come off the pick. **Note**: The microscope light is not on for the purpose of the picture!

4. Put the caps onto an appropriately labeled strip of tubes and close the caps with the capper/de-capper. Then, spin down the worms into the 8-strip tube using the minifuge.



Capper/de-capper



Minifuge

5. Turn on the thermocycler.

6. While the thermocycler is warming up, place your strip tubes into the ice in the -80°C freezer.

7. Set the thermocycler to run the following program (Lid at 105ºC):

Time	Temperature	Number of Cycles
1 hour	60°C	1
15 min	95°C	1
Hold	4°C	1

8. When there is about 20 minutes left on the lysis, set up your PCR mix:

For 1 reaction (total volume, 20 µL):

	Stock Concentration	Volume to Add	Final Concentration
Lysis Reaction	-	2 µL	-
Primer 1	4 µM	2 µL	0.4 µM
Primer 2	4 µM	2 µL	0.4 µM
dNTPs	2.5 mM	1.6 µL	0.2 mM
10X ThermoPol (Taq) Buffer	10X	2 µL	1X
Taq Polymerase	-	0.1 µL	-
dH_2	-	10.3 µL	-

a. Make a “master mix” that has everything ***except*** your lysis reaction. Add components in the following order: water, buffer, primers, dNTPs. Vortex and then add polymerase.
b. Make enough master mix for the number of reactions you want to do **PLUS 1 EXTRA REACTION.**
   i. e.g. - if you need to do 8 PCRs, make enough master mix for 9 PCRs.
c. Make up the reaction on ice.

9. Add 18 µL of Master Mix to each tube of a strip of tubes.

10. When the lysis is done, add 2 µL of the lysis to each reaction. **Keep reactions on ice until the thermocycler is ready.**

Step	Time	Temperature
1	3 min	95°C
2	15 sec	95°C
3	15 sec	60°C
4	1 min	72°C
5	Go to step 2, repeat 34 times	
6	5 min	72°C
7	hold	15°C

11. Turn on thermocycler and set to run the following program (Lid at 105ºC):

12. Run the PCR program.

13. While the PCR program is running, pour an agarose gel of the appropriate percentage to see your band.

14. When the thermocycler is done, add loading buffer to your samples and run gel!

<u>2x lysis buffer</u>
100 mM KCl
20 mM Tris pH 8.2
5 mM $MgCl_2$
0.9% IGEPAL
0.9% Tween 20
0.02% gelatin
Just before use add 20 μl 20 mg/ml proteinase K (in water, store in frozen aliquots) per ml of 2x lysis buffer.