

### Single worm PCR

Eric Greer 2/21/12

Make worm lysis buffer

-50mM KCl	1.25ml of 2M stock
-10mM Tris pH8.3	500 $\mu$ l of 1M stock
-2.5mM MgCl <sub>2</sub>	125 $\mu$ l of 1M stock
-0.45% NP40	225 $\mu$ l of 100% stock
-0.45% Tween-20	225 $\mu$ l of 100% stock
-0.01% gelatin (w/v)	<u>5<math>\mu</math>g</u>
	50ml

store this at -20° (make 1 ml or 500 $\mu$ l aliquots of part of it)

+ 5 ul of proteinase K to 500  $\mu$ l of worm lysis buffer

put 5  $\mu$ l in per tube well

+ single worm to 5  $\mu$ l liquid

put at -80° for 1 hour

run worm PCR #1

-60° 1 hour

-95° 15 minutes

-4° forever

add PCR master mix to 5 $\mu$ l WLB + worm

-12.5  $\mu$ l Platinum PCR supermix (Invitrogen)

-1 $\mu$ l primer mix

-6.5  $\mu$ l dH<sub>2</sub>O

25ul

run worm PCR #2

-94° 5 minutes

-94° 45''

-56° 45''

-72° (1' per kb)

-72° 10'

-4° forever

run on 1% gel

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} x40  
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