

I. Extraction of Genomic DNA

A. WLB (Worm lysis buffer): after Williams et al., 1994 Genetics 131:60

WLB (Worm lysis buffer):

No.	Chemicals	F.W.	Stock	Final	1 ml	2 ml	5 ml
1	KCl (Sigma, P-9541)	74.56g	1M	50 mM	50 μ l	100 μ l	250 μ l
2	Gelatin* (Dicto Bacto, 0143-02 ¼ lb)		1%	0.05 %	50 μ l	100 μ l	250 μ l
3	Tris pH 8.2 (BioRad, 161- 0719)	121.14g	1M	10 mM	10 μ l	20 μ l	50 μ l
4	Tween 20 (Fisher, FL-04-0796)	1227.54g	100%	0.45 %	4.5 μ l	9 μ l	22.5 μ l
5	Proteinase K (Roche, 0 092 766, 1gm)		20mg/ml	60 μ g/ml	3.3 μ l	6.6 μ l	16.5 μ l
6	MgCl ₂ (From PCR reagents)	95.21g	1M	2.5 mM	2.5 μ l	5 μ l	12.5 μ l
7	ddH ₂ O				880 μ l	1760 μ l	4400 μ l

(*: made fresh, 100mg gelatin in 10ml water and heat in microwave)

1. *Many worms*

- Add 50 μ l lysis buffer to tissue sample (approximately 0.5 cm) in a 0.5 ml PCR tube.
- Place at -70°C > 15min. Can store for several days. Or put in liquid nitrogen to 55°C water bath for 10 times to help break down the nematode body.
- Warm to room temperature and add 1 drop mineral oil.
- Incubate at 60°C > 1 hour. Vortex at least once during incubation to help breakup tissue.
- Heat to 95°C for 15 min. (Kills off Proteinase K).
- Cool to 4°C.
- Vortex briefly (2-3 sec).
- Spin at 6,000 rpm for 30 sec.
- Use 1 μ l supernatant as template for PCR amplification for 25 μ l reaction.

2. *Nematodes-for single worm*

- Add 15 μ l lysis buffer to worm in a 0.5 ml PCR tube.
- Place at -70°C > 15 minutes. Can store for several days.
- Warm sample to room temperature and add mineral oil.
- Incubate at 60°C > 1 hour.
- Heat to 95°C for 15 minutes
- Cool to 4°C.
- Pipet sample up and down to mix
- Use 2.5 μ l as template for PCR amplification.

B. DNA extraction using GeneClean III

- Turn on centrifuge to 4°C, turn on waterbath to 58°C, turn on rice cooker, take out Proteinase K from freezer to thaw.

1. Lysis

- a. Transfer droplet containing nematodes stored in 1M NaCl to clean slide (Cleaned with 70% ethanol and wiped with kimwipe).
- b. Transfer 1 nematode by a needle picker to 1.5ml microtube containing 18 µl Lysis Buffer.
- c. Crush nematode using pipette tip for about 40 times.
- d. Spin at 13,000 rpm for 1 min to bring solution down to bottom of the tube.
- e. Freeze at -20°C for 20 min.
- f. Boil for 5 min in rice cooker, after 1 min in boiling water bath, open tube caps and reclose to release pressure build-up.

2. Proteinase K digestion

- a. Burst spin.
- b. Add 2µl Proteinase K (20 mg/ml stock) and vortex.
- c. Incubate at 58°C for 3 hours.
- d. Turn on waterbath down to 51°C

3. Gene clean

- a. Burst spin.
- b. Add 60µl NaI solution and vortex.
- c. Add 0.8µl EZ-Glassmilk and vortex.
Incubate at room temperature for 5 min.
Spin for 30 seconds at full speed and remove supernatant.
- d. Add 60µl New Wash and vortex to resuspend all EZ-Glassmilk.
Burst spin and remove supernatant.
Repeat New Wash 2 more times.
Spin for 30 seconds at full speed and remove supernatant.
- e. Leave the cap open for 10 min at room temperature or place the tube under vacuum for 2-5 min.
- f. Add 10µl GCIII Elution and resuspend EZ-Glassmilk.
Incubate at 51°C for 3 min.
Spin for 30 seconds at full speed to make a solid pellet and collect supernatant into a new 0.5ml microtube.
- g. Add 5µl GCIII Elution and resuspend EZ-Glassmilk.
Incubate at 51°C for 3 min.
Spin for 30 seconds at full speed to make a solid pellet and collect supernatant into the previous microtube.
- h. Spin for 30 seconds at full speed again to get rid of the glass milk and collect supernatant into a new microtube
- i. Straight to PCR or freeze at -80°C until ready.

C. Non-manual lysis after Stanton et al., 1998 Australasian Plant Pathology 27:112.

1. Pick the single into microtube containing 10µl 0.25M NaOH, crash with pestle, then add 10µl 0.25M NaOH.
2. Incubate at 25°C for 24 hours.
3. Further incubate in a thermocycler at 99°C for 2 min.
4. 10µl 0.25M HCl, 5µl 0.5M Tris-HCl, pH8.0, and 5µl 2% Triton X-100 were added and incubated for another 2 min.
5. Straight to PCR or freeze at -80°C until ready.

Example SAMPLE TRACKING SHEET (simple format)

#	Species	PCR	Remark		#	Species	PCR	Remark	
1					11				
2					12				
3					13				
4					14				
5					15				
6					16				
7					17				
8					18				