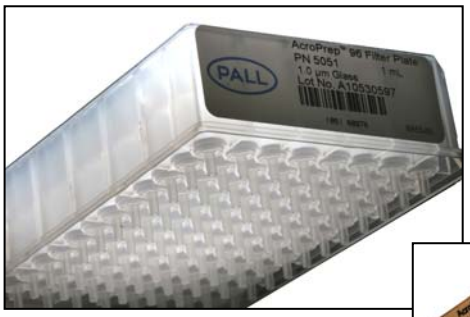
Glass Fiber Plate DNA Extraction Protocol:

Manual Protocol Employing Centrifugation Method

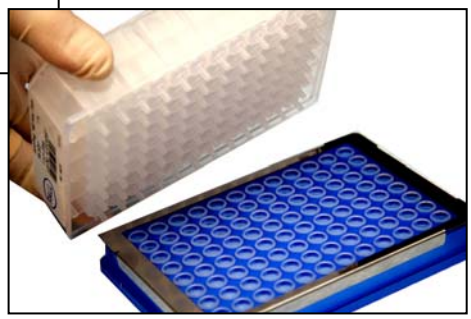
Natalia V. Ivanova, Jeremy R. deWaard & Paul D.N. Hebert

PALL Glass Fiber plate



Bind & wash on a square-well block

Positioning GF plate for elution step with a PALL collar



July 6, 2006

Glass fiber (GF) plate DNA isolation for recent and archived specimens:

Note: Use **Insect Lysis Buffer** and **PALL2** plate for DNA extraction from Arthropods and centrifuge **PALL2** plates at 6000 g.

1. For 1 plate mix 5 ml of **Vertebrate Lysis Buffer** and 0.5 ml of **Proteinase K,** 20 mg/ml in a sterile container. Add 50 µl of Lysis Mix to each well of 96-well Eppendorf plate.

2. Add a small amount of tissue (e.g. 2-4 mm of insect leg or 2-3 mm^3 of ethanol preserved tissue) to each well of 96-well solid skirted microplate (flame sterilize instruments between samples) cover plate with caps.

3. Incubate at 56 ºC for a minimum of 6 hours or overnight to allow digestion.

4. Centrifuge at 1500 g for 15 sec to remove any condensate from the cap strips.

5. Add 100 µl of **Binding Mix** to each sample using multichannel pipette. Shake vigorously for 10 – 15 sec and centrifuge at 1000 g for 20 sec to remove any sample from the cap strips.

6. Remove cap strips and transfer the lysate (about 150 µl) from the wells of microplate into the wells of the GF plate (PALL1) placed on top of a square-well block using multichannel pipette. Seal the plate with self-adhering foil.

7. Centrifuge at 5000 g for 5 min to bind DNA to the GF membrane.

8. First wash step: Add 180 µl of **Protein Wash Buffer (PWB)** to each well of GF plate. Seal with a new cover and centrifuge at 5000 for 2 min.

9. Second wash step: Add 750 µl of **Wash Buffer (WB)** to each well of the GF plate. Seal with a new self-adhering foil and centrifuge at 5000 for 5 min.

10. For PALL2 plates only (to avoid incomplete Wash Buffer removal): Open the sealing cover, close it and centrifuge the GF plates again for 5 min at 6000 g.

11. Remove the self-adhering foil. Place GF plate on the lid of a tip box. Incubate at 56 °C for 30 min to evaporate residual ethanol.

12. Position a PALL collar on the collection microplate and place the GF plate on top. Dispense 30 – 60 µl of ddH_20 (prewarmed to 56°C) directly onto the membrane in each well of GF plate and incubate at room temperature for 1 min. Seal plate.

13. Place the assembled plates on a clean square-well block to prevent cracking of the collection plate and centrifuge at 5000 g for 5 min to collect the DNA eluate. Remove the GF plate and discard it.

14. Cover DNA plate with cap strips. DNA can be temporarily stored at 4 ºC or at –20 ºC for long-term storage.

15. Use 1-5 µl of the DNA for PCR.

Additional note: square-well blocks could be washed with ELIMINase (or with any other DNA removing detergent), autoclaved and re-used.

Reagents and Stock Solutions for DNA Extraction Using Glass Fiber Plates

Reagents:

Description	Abbreviation	Supplier & Catalogue #
Disodium ethylenediamine tetraacetate · $2H_2O$	EDTA	Fisher Scientific S311-500
ELIMINase		Decon Labs Inc. 1102
Ethyl alcohol (anhydrous)	EtOH 96%	Commercial Alcohols Inc. 472-06-02
Guanidine thiocyanate	GuSCN	Sigma G9277-500g
Molecular biology grade water	ddH_2O	HyClone SH30538.02
Polyethylene glycol sorbitan monolaurate	Tween-20	Fluka 93773
Proteinase K		Promega V3021
Sodium chloride	NaCl	Fisher Scientific S271-3
Sodium dodecyl sulfate	SDS	GibcoBRL 15525-025
Sodium hydroxide	NaOH	Fisher Scientific S318-3
t-Octylphenoxypolyethoxyethanol	Triton X-100	Sigma T8787-100ML
Tris(hydroxymethyl)aminometane	Trizma base	Sigma T6066-100g
Tris(hydroxymethyl)aminometane hydrochloride	Trizma HCl	Sigma T5941-100g

Disposables & equipment:

Description	Abbreviation	Supplier & Catalogue #
ABGene (Fisher) 8-Strip flat PCR caps	cap strips	Fisher AB-0783
AcroPrep 96 1 ml filter plate with 1.0 µm Glass Fiber media, natural housing	PALL1	PALL 5051
AcroPrep 96 1 ml filter plate with 3.0 µm Glass Fiber media over 0.2 µm Bio-Inert membrane, natural housing	PALL2	PALL 5053
Axyseal sealing film	self-adhering foil	Axygene PCR-SP
Eppendorf twin.tec 96-well microplates	microplate	Fisher E951020427
Matrix Impact2 pipettor, 15 µl-1250 µl, 8-channel	multichannel pipette	Matrix 2004
Matrix Technologies 1250uL Talltip (102mm) Filter tip.		Matrix 8245
PP MASTERBLOCK, 96 Well, 2 ml	square-well block	Greiner 780271
SBS Receiver Plate Collar	PALL collar	PALL 5225

Stock solutions:

Description	Reagents & Weight		Final volume
1M Tris-HCl, pH 8.0			
	Trizma base	26.5 g	**500 ml**
	Trizma HCl	44.4 g	
1M Tris-HCl, pH 7.4			
	Trizma base	9.7 g	**500 ml**
	Trizma HCl	66.1 g	
0.1M Tris-HCl, pH 6.4			
	Trizma base	6.06 g	**500 ml**
Note: Adjust pH with HCl to 6.4-6.5.			
1M NaCl			
	NaCl	29.22 g	**500 ml**
0.5 M EDTA pH 8.0			
	EDTA	186.1 g	**1000 ml**
	NaOH	~20.0 g	
Note: Vigorously mix on magnetic stirrer with heater. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to ~8.0 by the addition of NaOH. Useful tip – give a brief rinse to NaOH granules with ddH_20 in a separate glass before dissolving them.			
Proteinase K (20mg/ml)			
	Proteinase K	100 mg	**5 ml**
Note: Add 5 ml of ddH_20 to a 100 mg package of Proteinase K; aliquot by 0.5 ml. Store at – 20ºC and do not freeze-thaw aliquots.			

Additional note: Thoroughly wash labware with ELIMINase, rinse with dH_20. Weigh reagents using a clean spatula, fill up with the molecular grade ddH_20 to the final volume. Filter buffers through 0.2 µm filter into a clean bottle; make smaller volume working aliquots (e.g. 100 ml). Store stock solutions and working aliquots at 4ºC.

Working solutions for DNA extraction:

Description	Volume from a stock solution (ml) or weight (g)		Final volume
Vertebrate Lysis Buffer (VLB)			**200 ml**
100 mM NaCl	1M NaCl	20 ml	
50 mM Tris-HCl, pH 8.0	1M Tris-HCl, pH 8.0	10 ml	
10 mM EDTA, pH 8.0	0.5M EDTA, pH.8.0	4 ml	
0.5% SDS	SDS	1.0 g	
Insect Lysis Buffer			**200 ml**
700 mM GuSCN	GuSCN	16.5 g	
30 mM EDTA pH 8.0	0.5M EDTA, pH.8.0	12 ml	
30 mM Tris-HCl pH 8.0	1M Tris-HCl, pH 8.0	6 ml	
0.5% Triton X-100	Triton X-100	1 ml	
5% Tween-20	Tween-20	10 ml	
Note: Vigorously mix on magnetic stirrer with heater.			
Binding Buffer (BB)			**500 ml**
6M GuSCN	GuSCN	354.6 g	
20 mM EDTA pH 8.0	0.5M EDTA, pH.8.0	20 ml	
10 mM Tris-HCl pH 6.4	0.1M Tris-HCl, pH 6.4	50 ml	
4% Triton X-100	Triton X-100	20 ml	
Note: Vigorously mix on magnetic stirrer with heater. If any re-crystallization occurs, pre-warm at 56ºC to dissolve before use.			
Wash Buffer (WB)			**475 ml**
60 % EtOH	EtOH 96%	300 ml	
50 mM NaCl	1M NaCl	23.75 ml	
10 mM Tris-HCl, pH 7.4	1M Tris-HCl, pH 7.4	4.75 ml	
0.5 mM EDTA, pH 8.0	0.5M EDTA, pH 8.0	0.475 ml	
Note: mix well, store at –20ºC.			
Binding Mix (BM)			
	Binding Buffer	50 ml	**100 ml**
	EtOH 96%	50 ml	
Note: stable at room temperature for 1 week.			
Protein Wash Buffer (PWB)			**100 ml**
	Binding Buffer	26 ml	
	EtOH 96%	70 ml	
Note: stable at room temperature for ~ 1 week, discard if any crystallization occurs.			

Additional note: Weigh the dry components (e.g. SDS or GuSCN) first, then add required volumes of the stock solutions, and fill up with the molecular grade ddH_20 to the final volume. No filtering is required.