

Supplementary material for the paper:

Ivanova NV, deWaard J, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, **6**, 998-1002.

CCDB - DNA Extraction

Simple methods of DNA extraction, such as a proteinase K digestion and/or Chelex extraction are usually sufficient especially while dealing with fresh specimens, but give poor quality DNA extracts.

In 2006 CCDB directed its efforts towards the development of an automation-friendly inexpensive method yielding high-quality DNA extracts. This resulted in a major advance – the elaboration of a membrane-based protocol that matches the performance of the best commercial kits, but is 75% less expensive (\$0.50 versus >\$2.00 per sample). Currently this protocol replaced previously used Chelex extraction for fresh specimens and commercial kits – GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma) and Genomic DNA NucleoSpin® 96 Tissue Kit (Machery-Nagel) used for archival specimens.

We have now employed both manual and robotic versions of our DNA extraction protocol on more than 50000 vertebrate and invertebrate specimens (>5000 species) with highly positive results.

In September 2006 CCDB purchased two Biomek® FXP liquid handling stations both equipped with 96 multichannel head and Thermo Cytomat hotel. Currently our Biomek® FXP is able to process 20 96-well plates in ~5 hours of unattended operation.

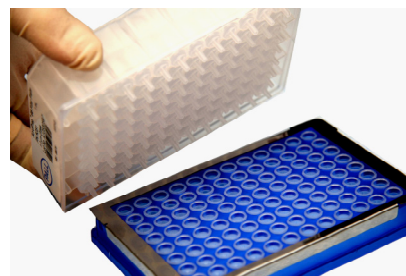
Manual Protocol: Centrifugation Method



AcroPrep™ PALL
Glass Fiber plate



Bind & wash on a square-well block.



Position GF plate for elution step with a PALL collar.

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 mm³ 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°C 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. Cover plate with cap strips. 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 cover.

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 cover 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 cover film. 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 ddH₂O (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 or aluminum PCR foil. DNA can be temporarily stored at 4°C or at –20°C 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 ₂ O	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 ₂ O	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 Scientific® 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 cover	Axygen Scientific® PCR-SP
Eppendorf® twin.tec 96-well microplates	microplate	Fisher Scientific® E951020427
Matrix® Impact2 pipettor, 15 µl-1250 µl, 8-channel	multichannel pipette	Matrix® 2004
Matrix® 1250uL Talltip (102mm) Filter tip.		Matrix® 8245
PP MASTERBLOCK®, 96 Well, 2 ml	square-well block	Greiner Bio-One® 780271
SBS Receiver Plate Collar	PALL collar	PALL® 5225

Stock solutions:

Description	Reagents & Weight	Final Volume
1M Tris-HCl, pH 8.0		500 ml
	Trizma® base	26.5 g
	Trizma® HCl	44.4 g
1M Tris-HCl, pH 7.4		500 ml
	Trizma® base	9.7 g
	Trizma® HCl	66.1 g
0.1M Tris-HCl, pH 6.4		500 ml
	Trizma® base	6.06 g
	Note: Adjust pH with HCl to 6.4-6.5	
1M NaCl		500 ml
	NaCl	29.22 g
0.5 M EDTA pH 8.0		1000 ml
	EDTA	186.1 g
	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 ₂ O in a separate glass before dissolving them.		
Proteinase K (20mg/ml)		5 ml
	Proteinase K	100 mg
Note: Add 5 ml of ddH ₂ O to a 100 mg package of Proteinase K; aliquot by 0.5 ml. Store at – 20°C and do not freeze-thaw aliquots.		

Additional note: Thoroughly wash labware with ELIMINase®, rinse with dH₂O. Weigh reagents using a clean spatula, fill up with the molecular grade ddH₂O 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 4°C.

Working solutions for DNA extraction:

Description	Volume from 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 56°C 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 -20°C.		
Binding Mix (BM)		100 ml
	Binding Buffer 50 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 ddH₂O to the final volume. No filtering is required.

References

- Ivanova NV, deWaard JR, Hebert PDN (2006). An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, **6**, 998-1002.
- Ivanova N, Grainger C, Hajibabaei M (2006) Glass fiber DNA extraction: A new inexpensive method for high throughput DNA isolation. *CCDB Advances, Methods Release No. 1*, November 3rd, 2006.