

Materials List for:

Laboratory Protocol for Genetic Gut Content Analyses of Aquatic Macroinvertebrates Using Group-specific rDNA Primers

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Materials

Name	Company	Catalog Number	Comments
liquid nitrogen	Any	NA	For transport of field samples of macroinvertebrates to the laboratory.
1.5 ml reaction tubes - Safelock	Any	NA	For collection of consumer specimens in the field and subsequent conservation in liquid nitrogen reaction tubes do not necessarily need to be PCR clean. To avoid exploding of the tubes, the lid of the tubes should be punctured with a needle.
Stereomicroscope	Any	NA	Make sure magnification is suitable to prepare the gastro-intestinal tract of the size of consumer of interest.
Petri dishes	Any	NA	To dissect the gastro-intestinal tract of consumer specimens under the stereomikroskop.
Stainless steel forceps: Dumont forceps No. 7	Bioform	B40d	This curved stainless steel forceps can sometimes be very helpful to hold specimen fixed inbetween the curved part without puncturing the gastro-intestinal tract.
Stainless steel forceps: Dumont forceps Electronic No. 5	Bioform	B40b	This fine stainless steel forceps is ideally suited for the preparation of the gastro-intestinal tract of consumer specimens.
DNA-ExitusPlus	AppliChem	A7409,1000RF	Decontamination solution for the removal of DNA and RNA contaminations
fuzz-free paper towel	Any	NA	
Lab scale	Any	NA	Precision needs to be at least 0.01 g.
Microlitre pipettes (0.5-10/10-100/50-200/100-1000 µL)	Any	NA	
Safe-Lock Tubes 2.0 ml	VWR	211-2165	It is crucial that these reaction tubes are free of DNA, DNase, RNase and PCR inhibitors (PCR clean)!
Safe-Lock Tubes 1.5 ml	VWR	211-2164	It is crucial that these reaction tubes are free of DNA, DNase, RNase and PCR inhibitors (PCR clean)!

Sodium chloride ≥99.5%, p.a., ACS, ISO	carlroth	3957.3	For salt extraction buffer (SEB) and 5M NaCl-solution required for extraction of DNA.
Tris(hydroxymethyl)-aminomethane Tris	carlroth	4855.2	For salt extraction buffer (SEB) and TBE buffer
Microtitre pipettes (0.5-10/10-100/50-200/100-1000 µL)	carlroth	8043.1	For salt extraction buffer (SEB) and TBE buffer
Boric acid ≥99.8 %, p.a., ACS, ISO	carlroth	6943.2	For TBE buffer. Not needed if commercially available TBE buffer is obtained.
Sodium dodecyl sulfate (SDS)	carlroth	4360.1	For extraction of DNA
Proteinase K lyophil.	carlroth	7528.1	Prepare a working solution containing 10 mg/ml.
TissueLyser II	Quiagen	85300	Bead mill for homogenization of the samples.
Stainless Steel Beads, 5 mm	Quiagen	69989	For homogenization of samples.
Heating Thermoshaker	Any	NA	
Vortex Mixer	Any	NA	
Refrigerated centrifuge: himac CT 15 RE	Hitachi	NA	Refrigerated centrifuge used for centrifugation during the extraction of DNA (Protocol 1).
Isopropanol	carlroth	6752.5	
Ethanol 99.8 %, p.a.	Any	NA	
Water Molecular biology grade (ddH ₂ O)	AppliChem	A7398,1000	nuclease-free (stream sterilized and DEPC treated) water used for re-suspension of DNA and whenever referred to ddH ₂ O within the manuscript
Unmodified Olegonucleotides	Eurofins MWG Operon	NA	Primers unlabeled
Modified olegonucleotides	Metabion International AG	NA	Primers labelled
peqGOLD Taq all inclusive	VWR Peqlab	01-1000	Taq all inclusive for PCR reaction mixtures (reaction buffer Y and S, dNTPs and MgCl ₂)
Primus 96 advanced gradient thermocycler or peqStar96x Universal Gradient	Peqlab	NA	Primer sets were originally established on a Primus 96 advanced gradient thermocycler. As this cyclor was quite old, we than checked if our primer sets are efficient and specific under the same conditions using the peqSta96x (which also could simulate the older model). However, tests showed that our primers work well and are still specific (see Koester et al. 2013) without using the simulation of the old machine.
Standard 96 Well PCR Plates	VWR Peqlab	732-2879	Used for PCR reactions.
Low Profile Tube Stripes 0.15 ml with Flat Cap Stripes	VWR Peqlab	732-3223	Cap stripes used to close the PCR plates.
Agarose	Peqlab	35-1020	Used for gel electrophoresis.
Microwave	Any	NA	
Conical flask	Any	NA	
Measuring cylinder	Any	NA	

Horizontal electrophoresis unit and respective combs with teeth	Any	NA	For agarose gel electrophoresis.
Electrophoresis Power Supply PS3002	Life Technologies	NA	
Ethidium bromide solution 1 %	carlroth	2218.1	Used to prepare staining bath for agarose gels.
Formamide	carlroth	6749.1	Needed for the denaturation buffer for SSCP analyses.
Bromphenol blue	AppliChem	A3640.0010	Needed for the loading dye for agarose gel electrophoresis.
Sucrose	carlroth	4621.1	Needed for the loading dye for agarose gel electrophoresis.
100 bp-DNA-Ladder extended	carlroth	T835.1	As size standard for agarose gel electrophoresis.
UV-Documentation system	Any	NA	To visualize gel electrophoresis results.
Rotiphorese NF-Acrylamide/Bis-solution 40 % (29:1)	carlroth	A121.1	For the preparation of polyacrylamid gels.
Ammonium peroxydisulphate	carlroth	9592.2	For the preparation of polyacrylamid gels.
foldback clips (32 mm)	Any	NA	For the preparation of polyacrylamid gels.
square-shaped plastic box (21 × 6.5 × 5 cm)	Any	NA	For the preparation of polyacrylamid gels.
Tetramethylethylenediamine (TEMED)	carlroth	23.67.1	For the preparation of polyacrylamid gels.
Beaker	Any	NA	
RC 10 Digital chiller	VWR	462-0138	For SSCP analyses using polyacrylamide gel electrophoresis.
Vertical electrophoresis unit P10DS (OWL Separation Systems)	VWR	700-2361	For SSCP analyses using polyacrylamide gel electrophoresis.
Notched glass plate	VWR	730-0261	For SSCP analyses using polyacrylamide gel electrophoresis.
Blank glass plate	VWR	730-0260	For SSCP analyses using polyacrylamide gel electrophoresis.
Spacers	VWR	730-0262	For SSCP analyses using polyacrylamide gel electrophoresis.
Comb with 25 teeth	VWR	730-0294	For SSCP analyses using polyacrylamide gel electrophoresis.
Shaking platform	Any	NA	For constant shaking of the staining bath.
GelStar Nucleic Acid Stain (Lonza Rockland, Inc.)	Biozym	850535 (50535)	For staining of polyacrylamide gels.
GenomeLab DNA Size Standard Kit 400bp	AB Sciex	608098	For automated fragment analyses. Size stanard for Batch B.
GenomeLab DNA Size Standard Kit 600bp	AB Sciex	608095	For automated fragment analyses. Size stanard for Batch A.
Sample Loading Solution (SLS)	AB Sciex	608082	For automated fragment analyses.
Sequencing plate - 96 Well Polypropylene PCR Microplate	Costar	6551	For automated fragment analyses.
Plate centrifuge, PerfectSpin P	VWR Peqlab	NA	mini plate centrifuge used to spin down PCR products.
Buffer plate - 96 Well EIA/RIA Clear Flat Bottom Polystyrene Not Treated Microplate	Corning	9017	For automated fragment analyses.

GenomeLab Separation buffer	AB Sciex	608012	For automated fragment analyses.
Separation Gel - LPAI	AB Sciex	608010	For automated fragment analyses.
Sequenzer - CEQ8000 Genetic Analysis System	Beckman Coulter	NA	For automated fragment analyses.
GeneMarker V1.95	Softgenetics	NA	To analyze results of automated fragment analyses.