

Comparison of methods and protocols for routine DNA extraction in the DNA Bank Network

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Project

The DNA Bank Network comprises DNA banks from the Botanic Garden and Botanical Museum Berlin-Dahlem (BGBM), the Bavarian State Collection of Zoology Munich (ZSM), Zoological Researchmuseum Alexander Koenig Bonn (ZFMK), and the German Collection of Microorganisms and Cell Cultures Braunschweig (DSMZ).

Routine isolation of high quality genomic DNA from various organismic sources is a prerequisite for long-term storage of DNA as well as a crucial requirement for DNA bank costumers. Within the DNA Bank Network project we conducted parallel DNA extraction tests due to the DNA banks expertise on 12 representative taxa.

The network's DNA banks can be accessed for further information through our shared website www.dnabank-network.org.

Introduction

Preparation of genomic DNA from various sources can still be challenging since DNA-containing material varies in secondary compounds as well as in degradation products due to biological origin and storage conditions. Impurity of nucleic acid preparations can reduce the efficiency of downstream applications or result in DNA degradation during storage. Whereas extraction protocols have been adapted that are optimal for different taxonomical groups, comparisons among methods for more general use remain rare.

28 extraction protocols based on five extraction methods were compared (table 2) to obtain DNA from material of three different ages from 12 representative taxa (table 1).

The method of silica membrane binding for DNA recovery is widely used so that a variety of commercially available kits (EPICENTRE, INVITEK, MACHEREY & NAGEL, QIAGEN, ROCHE, SIGMA-ALDRICH, and VWR/Omega) was evaluated. Alternative methods based on magnetic beads binding (AGOWA[®] mag, AGOWA), anion exchange purification (Genomic Tip[®], QIAGEN), salting-out precipitation (Puregene Genomic DNA Purification[®], GENTRA/QIAGEN), contaminants and inhibitor binding by sorbent matrix (Nexttec[™] Genomic DNA Isolation, NEXTTEC), and adapted CTAB protocols were also compared.

The methods and protocols were evaluated for extraction efficiency, DNA quality, handling time and material cost. Here we present the results of our test series and propose the best group-specific protocols suitable for routine DNA extractions at the DNA Bank Network.

Materials and Methods

Samples were collected from the field or obtained from in-house collections of the DNA Bank Network partners. All extractions were performed by following the manufacturer's instructions. Modified CTAB extractions protocols were carried out at each partner lab.

Total DNA yield was calculated spectrometrically based on absorbance at 260 nm. DNA quality was estimated by using the OD 260/280 absorbance ratio and agarose electrophoresis. The complex fragment length results were condensed into three classes (score: green = high, yellow = medium, red = low).

Relative extraction efficiency was calculated for all extractions individually by dividing single DNA yields by the best yielding kit per set-up.

Total evaluation of all protocols was conducted by dividing all data into three quality classes (green = best, yellow = medium, red = poor), and combining these data into one value.

Results and Discussion

Total genomic DNA has been isolated successfully from 285 samples. Test results are summarized and demonstrated in table 2. In general, DNA could be separated from cell material more efficiently (resulting in higher DNA yields) by applying salting-out precipitation, anion exchange purification, and the CTAB based laboratory protocol. However, all of these methods are characterized by very high standard deviations. Extraction efficiency was lower but more consistent with silica membrane kits and with magnetic beads. In contrast, the salting-out precipitation reduces shearing of DNA molecules during extraction resulting in convenient DNA fragment length whereas DNA quality is poor.

Conclusion

Silica membrane based kits are superior for routine extractions of most taxa if all parameters evaluated are considered. Following the total evaluation (table 2) at the DNA Bank Network we prefer the NucleoSpin Plant II (MACHEREY & NAGEL) for plant samples, the DNeasy Blood & Tissue Kit (QIAGEN) for samples of animals and fungi, and the MasterPure DNA Purification Kit (EPICENTRE) for prokaryotes. The E.N.Z.A. Insect DNA Kit (VWR/Omega) provides good results for samples of insects.

If more knowledge of inhibiting compounds is available, tax-specific protocols are certainly to favour.

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Table 1. List of representative taxa tested

Group	Species
Archaea	<i>Halogeometricum borinquense</i> DSM 11551
Bacteria	<i>Actinosynnema mirum</i> DSM 43827
Bacteria	<i>Kribbella flavida</i> DSM 17836
Algae, Phaeophyta	<i>Laminaria digitata</i> (Hudson) Lam.
Monocots	<i>Hordeum murinum</i> L.
Dicots	<i>Cichorium intybus</i> L.
Fungi, Ascomycota	<i>Erysiphe alphytaides</i> U. Braun & S. Takam.
Arachnida	<i>Pholcus phalangoides</i> Fuesslin, 1775
Insecta	<i>Phalera bucephala</i> L., 1758
Mollusca	<i>Limax cinereoniger</i> Wolf, 1803
Reptilia	<i>Natrix natrix</i> L., 1758
Mammalia	<i>Apodemus sylvaticus</i> L., 1758

Table 2. Comparison of DNA extraction efficiency, DNA quality, handling time, and material cost by five methods and 28 protocols based on 285 extractions.

Extraction efficiency, OD ratio, and gDNA fragment length was calculated as described above. Quality classes are indicated by green (high quality; extraction eff. 1 to 0.4; OD 1.8-2; handling time <1.5h, material cost <200 €), yellow (medium quality; extraction eff. 0.4-0.1; OD 1.6-1.8 and 2.0-2.2; handling time 1.5h to <3.3 h; material cost <200 to 400€), and red (poor quality extraction eff. <0.1; OD <1.6 and >2.2; handling time >3.3 h; material cost > 400€). The total evaluation colour sums up all evaluated parameters equally. * Value could not be determined. AGOWA – commissarial extractions, cost includes material and handling - grey highlighted.

Taxon	Laboratory	Extraction method	Kit	Company	Relative Extraction Efficiency	OD ratio 260/280 (Ø)	Fragment length (Ø)	Handling Time [in h, 20 preps]	Material Cost [in €, (100 preps)]	Total Evaluation [Best Kits, 1 to 3]
Prokaryotes	DSMZ	Silica membrane binding	MasterPure DNA Purification	EPICENTRE	0.46	1.85	1.5	113	1	
Prokaryotes	DSMZ	Magnetic beads binding	Mag DNA Isolation	AGOWA	0.23	1.92	1	1050	2	
Prokaryotes	DSMZ	Silica membrane binding	DNeasy Blood & Tissue	QIAGEN	0.18	1.76	1.5	240	3	
Prokaryotes	DSMZ	CTAB lysis	Laboratory protocol	(DSMZ)	0.99	1.84	4	100		
Prokaryotes	DSMZ	Anion exchange	Genomic Tip 500/G	QIAGEN	0.33	1.93	7.5	1770		
Plants	BGBM	Silica membrane binding	NucleoSpin Plant II	MACHEREY & NAGEL	0.15	1.91	2.5	202	1	
Plants	BGBM	Silica membrane binding	GeneElute Plant Genomic DNA	SIGMA	0.16	2.05	2.5	167	2	
Plants	BGBM	Magnetic beads binding	Mag DNA Isolation	AGOWA	0.19	1.77	1	1050	3	
Plants	BGBM	Silica membrane binding	Invisor Spin Plant Mini	INVITEK	0.36	1.60	2.5	199		
Plants	BGBM	Silica membrane binding	Power Plant DNA Isolation	MOBIO	0.28	2.08	2.5	377		
Plants	BGBM	Silica membrane binding	DNeasy Plant Mini	QIAGEN	0.16	1.51	2.5	238		
Plants	BGBM	Guanidine detergent lysis	Plant DNAzol	INVITROGEN	0.64	1.66	3.5	120		
Plants	BGBM	Salting-out precipitation	Puregene DNA Tissue	GENTRA/QIAGEN	0.87	1.49	5	105		
Plants	BGBM	Anion exchange	Genomic Tip 20/G (adapted)	QIAGEN	0.63	1.93	8	924		
Plants	BGBM	CTAB lysis	Laboratory protocol	(BGBM)	0.44	1.59	3	140		
Plants	BGBM	Inhibitor binding by sorbent	Genomic DNA Isolation Plants	NEXTTEC	*	*	1	215	*	
Animals, fungi	ZFMK, ZSM	Silica membrane binding	DNeasy Blood & Tissue	QIAGEN	0.63	1.83	1.5	274	1	
Insects	ZSM	Silica membrane binding	E.N.Z.A. Insect	VWR/OMEGA	0.62	1.97	2.5	215	(1)	
Animals, fungi	ZFMK, ZSM	Silica membrane binding	NucleoSpin Tissue	MACHEREY & NAGEL	0.36	2.02	1.5	265	2	
Animals, fungi	ZFMK, ZSM	CTAB lysis	Laboratory protocol	(ZSM, ZFMK)	0.24	1.88	4	100	3	
Animals, fungi	ZFMK, ZSM	Salting-out precipitation	Puregene DNA Tissue	GENTRA/QIAGEN	0.39	1.51	4	320		
Animals, fungi	ZFMK, ZSM	Magnetic beads binding	Mag DNA Isolation	AGOWA	0.10	2.51	1	1050		
Animals, fungi	ZFMK, ZSM	Silica membrane binding	DNA Isolation Cells and Tissue	ROCHE	0.38	1.39	4	342		
Molluscs	ZSM	Silica membrane binding	E.N.Z.A. Mollusc	VWR/OMEGA	0.06	2.21	2.5	215		
Animals, fungi	ZFMK, ZSM	Anion exchange	Genomic Tip 20/G	QIAGEN	0.12	1.09	3.25	924		
Animals, fungi	ZFMK	Silica membrane binding	NucleoSpin Tissue XS	MACHEREY & NAGEL	0.05	1.53	1.5	307		
Fungi	ZSM	Silica membrane binding	E.N.Z.A. Fungal	VWR/OMEGA	0.03	1.18	2.5	215		
Animals, fungi	ZFMK, ZSM	Inhibitor binding by sorbent	Genomic DNA Isolation Tissue	NEXTTEC	*	*	1	232	*	