The influence of DNA extraction methods on species identification results of seafood products

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INTRODUCTION

In terms of species identification, the final aim of isolating DNA is the subsequent amplification of the gene, so the quality and the quantity of the extracted DNA must be sufficient for subsequent PCR-based methods. The purpose of this study is to compare five DNA extraction methods according to parameters of quantity, quality, and work simplicity among others, in order to find the most suitable for three relevant groups of species (Cephalopoda, Gadiformes and Pleuronectiformes). Wizard DNA Clean-up System Kit (Promega), Automated Nucleic Acid Purification System MPure-12™ (MP Biomedicals), Chelex 100 resin (Biorad), DNeasy Blood and Tissue Kit (Qiagen) and Swab method were examined.

MATERIAL AND METHODS

1. Samples

Cephalopoda *(Loligo reynaudii*, *Sepia officinalis*), Gadiformes (*Merluccius merluccius*, *Gadus morhua*) and Pleuronectiformes (*Scophthalmus maximus*, *Lepidorhombus boscii*) were purchased fresh at a local fish market in Vigo (Spain). Three specimens per species were obtained, making a total of 18 samples. Specimens were visually identified and photographed before further processing.

2. DNA extraction

	Wizard DNA Clean-up System Kit (Promega)	**Chelex 100 resin** (Biorad)	**DNeasy Blood and Tissue Kit** (Qiagen)	**Automated Nucleic Acid Purification System Mpure-12™** (MP Biomedicals)	**Swab method**
Method principles	Biding resin	Chelating ion exchanger	Spin-column based	Magnetic bead separation	Sterile cotton

3. DNA quantity, purity and quality determination

- The extracted double stranded DNA was quantified with the Invitrogen Qubit 4 Fluorometer (ThermoFisher).
- Purity was determined with the spectrophotometer Nanodrop 2000 (Thermo Scientific) with the ratios of absorbance A260/A280 and A260/A230.
- The determination of the extracts quality in terms of DNA fragmentation was evaluated by running extracts in a 1% (w/v) agarose gel.

4. Amplification and sequencing

PCR amplifications of 720 bp fragments of cytochrome c oxidase I (COI) in Cephalopods (Folmer 1994) and 465 bp fragments of cytochrome b in Gadiformes and Pleuronectidae (Burgener 1997).

5. Species authentication

The authentication of the species was carried out by FINS (Forensically Informative Nucleotide Sequencing) and BLAST (NCBI).

6. Sequence quality analysis

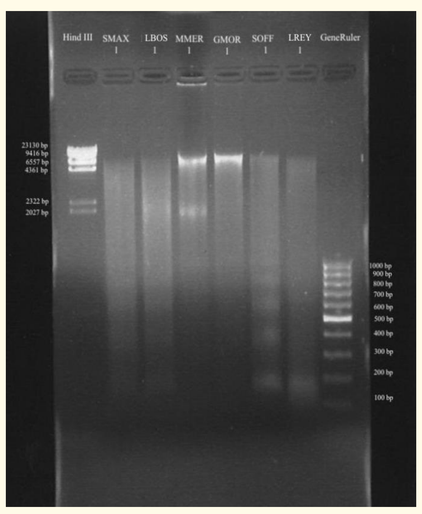
Sequences quality was estimated with the bioinformatics software (Geneious).

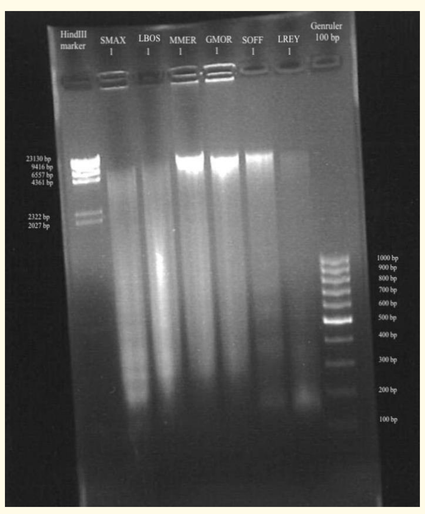
RESULTS

Method	**Digestion time**	**Handling time**	**Total extraction time**	**Yield (ng)**	**Method efficiency (ng DNA/mg wet tissue)**
Wizard [1]	2h	3h	5h	11404,72±4307,16	38,108±14,943
Chelex	-	1h	1h 30 min	194,66±111,16	68,393±43,454
DNeasy	2h	2h	4h	787,64±721,96	36,523±33,292
MPure	2h	1h	5h	2653,06±1372,63	66,843±36,349
Swab	-	1h	1h	892,92±336,08	17,505±7,098

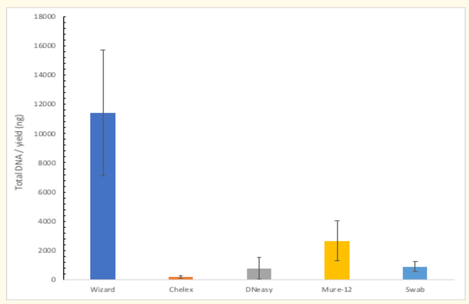
Method	**Ratio 260/280**	**Ratio 260/230**	**Yield (ng) per PCR reaction**	**Amplification success %**	**HQ% Kocher primers**	**HQ% Folmer primers**
Wizard [1]	1,967±0,054	0,667±0,748	100	94,44	99±0,2	95,1±2,70
Chelex	1,843±0,526	0,481±0,168	9,733	33,33	89,2	-
DNeasy	2,177±0,263	2,204±0,691	19,691	100	97±3,3	89,1±7,21
MPure	2,031±0,184	2,257±0,383	100	94,44	99,3±0,28	95,35±1,48
Swab	1,547±0,159	0,462±0,126	8,929	5,55	-	-

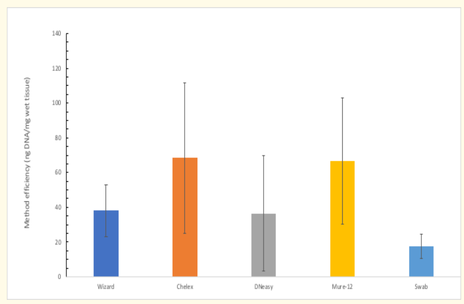
DNA quantity and extraction time (top table), quality (bottom table): Comparison among different extraction methods of DNA. Mean ± SD of samples per method. [1] Wizard method is standardized to 10 samples.

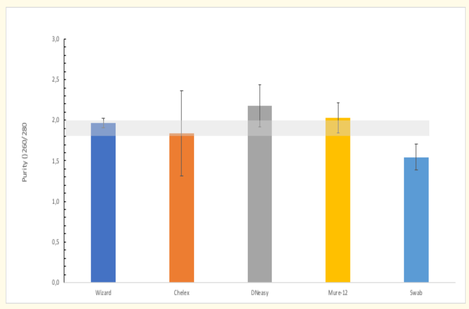


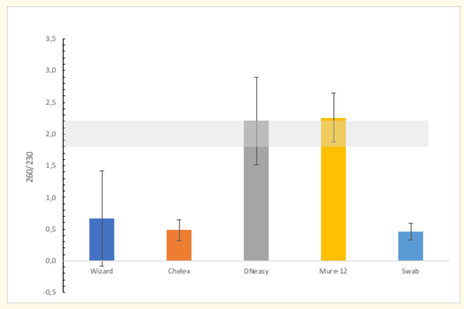


1% agarose electrophoresis gel of Wizard (left) and Mpure-12 (right) extracts.









Yields (ng)(top left), method efficiency (ng DNA/ mg wet tissue) (top right), purity (bottom left) and 260/230 ratio (bottom right) of the different DNA extraction methods. Mean ± SD of the different samples per method.

- Method with higher yield: Wizard
- Method with higher efficiency: Chelex
- Method that extracted DNA of better quality: Wizard
- Method without a significant concentration of contaminants: Dneasy and MPure-12
- Highest amplification capacity method: Wizard
- Method with best high qualities of sequencing: Wizard and Mpure-12

Significant differences between yield according type of method and tissue ($p < 0.05$),($n= 6$). No significant differences between purity and efficiency and tissue ($p < 0.05$), ($n = 6$).

	Wizard	**Chelex**	**DNeasy**	**MPure-12**	**Swab**
Technical simplicity	+	+ + +	+ +	+ +	+ + + +
Automation	-	-	+ + + +	+ + + +	-
Rapidness (Extraction time)	5h*	1h 30’	4h	5h	1h
Efficiency (ngDNA/mg wet tissue)	38,108 ± 14,943	68,393 ± 43,454	36,523 ± 33,292	66,843 ± 36,349	17,505 ± 7,098
Yield (Total DNA (ng))	11404,72 ± 4307,16	194,66 ± 111,16	787,64 ± 721,96	2653,06 ± 1372,63	892,92 ± 336,08
Purity	1,967 ± 0,054	1,843 ± 0,526	2,177 ± 0,263	2,031 ± 0,184	1,547 ± 0,159
PCR amplification success %	94,44	33,33	100	99,44	5,55
Affordability (Reagents cost per prep)	2,22€	0,001918€	4,26€	5,65€	0,051€
Safety of components	+ +	+ + +	+	+ + +	+ + + +
Affordability (Specific equipment value)	Vacuum manifold	Not required	Not required	MPure-12™ Automated Nucleic Acid Purification System	Not required

Parameters measured for the comparison of the different DNA extraction methods. In the case of non-quantitative variables, the measurement scale goes from + to ++++ (minimum to maximum, respectively). The measurements have been highlighted in a color scale (green, yellow, orange, and red) indicating a scale of values, from best to worst respectively