A new method for the rapid detection of Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*), Alaska pollock (*Gadus chalcogrammus*) and ling (*Molva molva*) using a lateral flow dipstick assay

Running title: A new method for the rapid detection of gadoids using LFD

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ABSTRACT

Species-specific lateral flow dipstick (LFD) assays for the identification of Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*), Alaska pollock (*Gadus chalcogrammus*) and ling (*Molva molva*) in food products were developed. The method comprises a PCR system with four sets of specific primers, for each target species. This step was also devised to dual-labeling of PCR products with biotin and 6-FAM, which are then easily read on a lateral flow dipstick, upon which these products are immobilized by a fixed biotin-ligand and visualized with anti-FAM antibody-coated gold nanoparticles. Sensitivity and selectivity were determined for each of the developed assays. Validation of the assays was performed with DNA extracted from commercial fish products, the identification of all samples by PCR-LFD was coherent with the results found with DNA sequencing. Target species were successfully detected in analyzed commercial samples, demonstrating the applicability of this method to the rapid analysis of food products.

Keywords: Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*), Alaska pollock (*Gadus chalcogrammus*), ling (*Molva molva*), Cytochrome b, lateral flow dipstick.

1. Introduction

Seafood fraud is an important concern and involves several aspects of the industry, including economically motivated fraud, e.g., using cheaper species in processed products that are labeled with the names of higher quality and more expensive species (Everstine, Spink & Kennedy, 2013); consumer safety (Miller & Mariani, 2010 and Sakaguchi, 2000); and the sustainability of fisheries (e.g., hiding illegal fishing practices or skipping importation taxes by mislabeling seafood) (Galal-Khallaf, Ardura, Mohammed-Geba, Borrell & Garcia-Vazquez, 2014; Triantafyllidis et al 2010 and Jacquet & Pauly, 2008). During last five years, there have been several reports in the media about the existence of seafood fraud in different geographic areas (Mariani et al., 2015). Seafood labeling is an important tool for helping stakeholders, administration and consumers to identify and verify characteristics of particular seafood products along the seafood value chain. In fact, labeling seafood is regulated by guidelines and directives that specify commercial and scientific names, which should be included on the labels of seafood products (in the case of the European Union [EU]: EC 104/2000, CR 2065/2001, and EU 1397/2013). However, legislation is useful only when oversight is adequately and regularly exercised by authorities or when consumer associations campaign to evaluate and disseminate the observance of labeling regulations for different food commodities. Analytical techniques have demonstrated key elements for the identification and authentication of fish in seafood (Griffiths et al., 2014), and a growing variety of analytical tools have been effectively developed to uncover or reduce the existence of seafood fraud throughout the world (Mariani et al., 2015).

Gadoids are a group of fish with historical importance in the fishing industry, especially in the case of species such as Atlantic cod (*Gadus morhua*), which is in high demand as a result of its fisheries being regulated to recover from over-exploitation issues (Brander, 2010).

This high demand is one of the reasons why cod products are often mislabeled, and there have been numerous observations of the replacement of Atlantic cod (*Gadus morhua*) by cheaper species (Helyar et al., 2014)or its illegal capture being hidden by the use of other species names (Miller & Mariani, 2010).

Although DNA testing is the most appropriate method for authenticity testing (Griffiths et al., 2014) and many DNA-based methods have been developed for the detection and identification of fish species in food products, such as PCR (Hubalkova; Kralik; Kasalova & Rencova, 2008 and Moran & Garcia-Vazquez, 2006), PCR-RFLP (Di Finizio, Guerriero, Russo & Ciarcia, 2007), RT-PCR, (Bertoja, Giaccone, Carraro, Mininni & Cardazzo, 2009; Hird et al, 2012; Hird et al, 2005; Sanchez, Quinteiro, Rey-Mendez, Perez-Martin & Sotelo, 2009 and Taylor, Fox, Rico & Rico, 2002), FINS (Primrose, Woolfe & Rollinson, 2010 and Cutarelli et al, 2014), SSCP (Chapela, Sanchez, Suarez, Perez-Martin & Sotelo, 2007), SNPs (Maretto, Reffo, Dalvit, Barcaccia & Mantovani, 2007), PCR-ELISA (Asensio et al, 2004 and Taboada et al, 2014) and RT-NASBA (Ulrich et al, 2015), most current methods involve the use of expensive specialized equipment, such as DNA sequencers, and the need for skilled personnel to analyze and interpret the results.

The present work describes the development, optimization and validation of an innovative and rapid specific molecular technique to identify four species of the Gadiformes order in seafood products. The method is an Lateral Flow Dipstick(LFD)-PCR-based analysis of the mitochondrial cytochrome b gene that offers a direct detection (one-step) tool for species identification. Disposable LFD-type DNA biosensors are particularly useful for DNA authentication in small facilities or field analysis due to their simplicity, low cost and portability.

This method also enables visual genotyping without the need for specialized instruments (i.e. DNA sequencers).

One of the advantages of the LFD-PCR methodology is that, due to its simplicity, the method does not require highly qualified personnel. The procedure comprises two steps: (i) PCR amplification of the DNA segments and (ii) detection of the products of the amplification reaction within a few minutes by the naked eye employing the LFD. No purification is required prior to application of the amplification products to the LFD. Furthermore, the method can be applied to fresh, frozen or processed products, and it only requires a conventional PCR system (thermo-cycler), as the results can be evaluated with the naked eye. Molecular assays in LFD format have been used in the detection of nematodes (Niu et al, 2011), viruses (Arunrut, Prombun, Saksmerprome, Flegel & Kiatpathomchai, 2011 and Ge et al, 2013), bacteria (Surasilp et al, 2011; Kalogianni et al, 2007 and Chua, Yean, Ravichandran, Lim & Lalitha, 2011), GMOs (Kalogianni., Koraki, Christopoulos & Ioannou, 2006 and Huang, Zhai, You & Chen, 2014) and coffee (Trantakis et al, 2012), but, as far as the authors know, this is the first time that this technique has been used to identify fish species in food, introducing a new methodology for seafood authentication in the field.

2. Materials and Methods

2.1. *Fish samples*

Fifteen specimens of each of the following species—*Gadus morhua, Gadus chalcogrammus* and *Molva molva*— and seven specimens of *Gadus macrocephalus* were collected. The identity of all of them was confirmed by morphological identification and FINS.

In addition, 17 representative specimens of orders Gadiformes, Lophiiformes, Clupeiformes and Perciformes, were included in this study. The scientific and common names of these species are listed in Table 1

Thirty-one commercial samples from different local markets were used for the validation step of the developed methodology (Table 2).

2.2. *DNA extraction*

DNA was extracted from 0.3 g of thawed muscle and tissue of commercial samples, which were digested for 3 h in a thermoshaker at 56°C with 860 µL of lysis buffer (1% SDS, 150 mM NaCl, 2 mM EDTA, and 10 mM Tris-HCl at pH 8), 100 µL of 5 M guanidinium thiocyanate (Sigma-Aldrich, Missouri, USA), and 40 µL of proteinase K (20 mg/mL; Gibco Invitrogen, Life Technologies). Then, extra proteinase K (40 µL) was added to the solution and it was left overnight. After digestion, DNA was isolated employing the Wizard DNA Clean-Up System kit (Promega) by following the manufacturer's instructions. DNA was quantified with a NanoDrop 2000 Spectrophotometer (Thermo Scientific) at 260 nm. The 260/280 nm ratio was between 1.8 and 2.0. The DNA concentration was adjusted to 50 ng/µL with molecular biology grade water. The purified DNA was stored at -20°C.

2.3. Design of LFD assay system

Four specific sets of primers were designed with Oligo Analyzer v. 1.0.3 (Freeware, Teemu Kuulasmaa, Finland) to amplify four small fragments of the *Cytochrome b* gene containing the previously identified specific polymorphisms (Figure S1). Forward primers were labeled with biotin at the 5'end and reverse primers were labeled with 6-FAM at the 5' end.

120 To confirm the absence of false negatives, a test with universal primers (FishDC-F and
121 FishDC-R) was included. Primer sequences are listed in Table 3.

122 *2.4. Amplification and sequencing of DNA fragments*

123 PCR reactions were performed in a final volume of 25 µL using PureTaq™ Ready-to-Go™
124 PCR beads (GE Healthcare UK Ltd) with the addition of molecular-grade water, primers (final
125 concentration 0.2 µM) and 50 ng of DNA template. The thermal cycling parameters were as
126 follows: 95°C for 5 min, followed by 25 cycles of 95°C for 20 s, 69°C for 30 s, 72°C for 30 s
127 and a terminal extension step of 72°C for 5 min. Negative controls (molecular-grade water) were
128 included in each set of reactions. These PCR reactions were carried out in an Applied
129 Biosystems 2720 Thermocycler.
130 The amplification products were tested in a 2% agarose gel (Pronadisa), containing RedSafe™
131 1X (iNtRON Biotechnology) in 0.5X TBE buffer (Sigma). DNA fragments were visualized
132 using the Gel Doc XR System and the software Quantity One® v 4.5.2 (Bio-Rad).
133 Sequencing was used to verify the correct species assignation of the specimens used for the
134 LFD method set-up. L14735 and H1549D (Kocher et al, 1989) were used for the amplification
135 and subsequent sequencing on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The
136 resulting *Cytochrome b* nucleotide sequences were analyzed using *Megablast* with those present
137 in the NCBI database.

138 *2.5. Detection of PCR products using the LFD assay*

139 A 5 µL aliquot of the labeled PCR product was applied at the sample application location of
140 commercially prepared Milenia GenLine HybriDetect 2T (Milenia Biotec GmbH, Germany)

where the anti-FAM antibody-coated gold nanoparticles are included. The sample application location of the sensor was then dipped into a microcentrifuge tube containing 100 µL of hybridization buffer (2X SSC, 10 mL/L Tween 20, 4 mL/L glycerol, 0.5 g/L SDS), to allow the upward capillary flow which carries conjugated gold nanoparticles through the LFD different sections. Figure S3 shows schematically the entire process. The visual detection of PCR products was complete within 5 minutes.

2.6. Verification of LFD assay detection specificity

To confirm the specificity of the four designed systems, the four primer pairs were tested using 50 ng of DNA from all species listed in Table 1.

2.7. Verification of LFD assay detection sensitivity

The LFD assay sensitivity and the limit of detection were assessed by comparing them with detection in an agarose gel. Six serial 10-fold dilutions of DNA extracted from the positive control sample (50 ng/µL) were used as templates in an LFD assay and a gel-based assay.

2.8. Validation of the LFD assay with commercial samples

Once the method was tested, commercial samples purchased in markets and shops from different places in Spain were used for testing the suitability of the LFD assay to identify the species present in these products.

3. Results and Discussion

159 *3.1. LFD assay system design*

160 The aim of this work was the development of a simple, rapid and inexpensive DNA-based
161 assay for the identification and authentication of Atlantic cod (*Gadus morhua*), Alaska pollock
162 (*Gadus chalcogrammus*), Pacific cod (*Gadus macrocephalus*) and ling (*Molva molva*). The
163 adequate design of the specifically labeled primers that are linked through an antigen-antibody
164 reaction to gold nanoparticles, gave as a result a successful assay system. Gold nanoparticles
165 were employed as reporters that enabled visual detection with good sensitivity even for
166 processed samples. Furthermore, detection was performed with a low-cost, disposable, LFD-type
167 device that incorporated most of the required reagents in dry form, requiring only the pipetting of
168 labeled PCR product and then dipping the LFD in hybridization buffer, thereby avoiding
169 multiple pipetting and incubation steps.

170 Mitochondrial DNA is relatively more abundant than nuclear DNA in cells, evolves much
171 faster, and thus contains greater sequence diversity compared to nuclear DNA; therefore, most
172 DNA-based detection of fish species preferably relies on mitochondrial DNA markers
173 (Teletchea, 2009; Wilson & Turner, 2009 and Rastogi et al, 2007). In our experience in the case
174 of gadoids, cytochrome b provides sufficiently significant interspecific nucleotide differences to
175 enable the design of specific primer systems (Teletchea, 2009; Wilson & Turner, 2009 and
176 Rastogi et al, 2007). The developed method is based on the existence of SNPs in small segments
177 of the cytochrome b mitochondrial DNA sequence from the four target species. Specific primer
178 systems for specific amplification are graphically shown in Figure S1. These systems were
179 selected based on one essential condition that no intraspecific variability was found in the primer
180 sequence; otherwise, some individuals of the target species could show up as false negatives.
181 Another condition was that the system be designed using a sequence region where the number of

nucleotide interspecific differences was highest, thus exhibiting almost no cross-reaction possibilities (false positives). The specifically amplified and labeled DNA was then detected by LFD assay.

A universal LFD system operates in three zones: the sample application zone, the test zone and a control zone (Figure S2). The previously labeled PCR product was applied to the sample application zone of the LFD strips where the anti-FAM-coated gold nanoparticles were also present, these specifically bind the FAM label present in the specific PCR product. The sample application zone of the sensor was then dipped into the hybridization buffer that migrated upward. Specific amplification products were labeled with FAM at the 5' end, allowing for their union with anti-fluorescein antibody-coated gold nanoparticles. Because forward primers were also labeled with biotin at the 5' end, the biotin-ligands which are immobilized in the test zone resulted in the trapping of gold-bound specific PCR products. The excess of non-bound nanoparticles was captured by immobilized anti-rabbit antibodies in the control zone of the strip, forming a band, which confirmed the correct performance of the strip (Figures 2C and 2D). The visual detection of specific PCR products was complete within 5 minutes.

3.2. Detection and confirmation of labeled PCR products by LFD

The designed test relies on the four specific primer pairs designed, and the first step was to investigate and confirm the specific amplification of PCR-labeled products from *M. molva,* Alaska pollock (*Gadus chalcogrammus*)*,* Pacific cod (*Gadus macrocephalus*) and Atlantic cod (*Gadus morhua*). Table 3 shows the details of the primers designed and the size of each of the amplicons produced, together with the optimal temperature for the annealing, which was 69°C in the four cases.

All inclusivity tests were positive for each species, whereas all non-target species tested were negative. Figure 1 shows that positive reactions were determined by the naked eye as a colored band appearing in the test zone of the strip, also showing color in the control zone, indicating that gold nanoparticles migrated adequately through the strip. The specificity of the amplification products was also confirmed with a gel-based assay, and the results were in agreement (data not shown).

The possible occurrence of false-negative amplifications was checked by the use of a parallel amplification with a universal primer system, i.e., *FishDC,* which was designed to be within the 16S rDNA. Amplification with this primer pair was always positive in fish samples and should be performed to discard the occurrence of negative results due to problems associated with the samples, such as the presence of inhibitors or low-quality DNA associated with seafood processing.

The PCR-LFD procedure is faster than other molecular detection technologies such as PCR-ELISA (Taboada et al, 2014), and results can be obtained in less than five minutes after amplification. Moreover, the results can be visually read, eliminating the need for expensive equipment, thus reducing costs and minimizing the space required. This method also allows some portability because once the PCR products are obtained, the detection reaction can be performed without the involvement of sophisticated lab equipment.

3.3. Determination of the sensitivity of the LFD assay

The sensitivity of the LFD assay was assessed by comparing it with detection in an agarose gel. The limit of detection was determined via PCR amplification of six serial 10-fold dilutions of DNA extracted from the positive control sample (50 ng/μL; Figure 2). The limit of detection

for the LFD assay with the *GmorD* and *MmolD* primer pairs was $50x10^{-3}$ ng, and that with the *GchalD* and *GmacD* primer pairs was $50x10^{-2}$ ng. With the conventional agarose gel electrophoresis method, the minimum amount of DNA template produced an easily visible band, which was $50x10^{-2}$ ng for the ling (*Molva molva*) identification system, $50x10^{-1}$ ng for the Atlantic cod (*Gadus morhua*) system and 50 ng for the Pacific cod (*Gadus macrocephalus*) and Alaska pollock (*Gadus chalcogrammus*) systems. Therefore, the data presented here indicate that the LFD technique, based on the detection of *Cytochrome b* gene products, is more sensitive than the gel-based detection method.

3.4. Application to commercial samples: Validation

The developed PCR-LFD methodology was tested for use in the authentication of four gadoid species in commercial products. Table 2 shows information on the 31 samples collected and analyzed from different Spanish retailers.

DNA from each commercial product was amplified with L14735 and H1549D (Kocher et al, 1989) primers, for verifying by FINS the species assignation made with the PCR-LFD methodology.

As shown in Table 2, the identification of all samples by PCR-LFD was coherent with the results found with DNA sequencing. The results were completely visual and obtained in less than 5 minutes after the application of the labeled PCR product to the LFD (Figure 3).

PCR-LFD was useful for the authentication of all samples, the results shows that mislabeling of this commercial sample set was 22.58% (7 out of 31 samples), and there were a variety of reasons for this mislabeling rate. The LFD results showed that 3 out of 31 commercial samples were negative for the four LFD assays (samples 13, 22 and 31), two of them turned out to be

Brosme brosme but labeled as ling and cod, respectively. In the other case which no positive LFD signal was found, sample 13, the label declared to be ling (*Molva molva*), however, FINS showed that *Molva dypterygia* was present. In some other cases, the positive signal did not match the commercial name, as in sample number 5, where the label stated that Atlantic cod (*Gadus morhua*) was present and instead ling (*Molva molva*) was found by the LFD assay.

Another important result is the potential for this methodology to detect mixtures of these 4 species; one of the analyzed samples (sample 14) contained a mixture of Atlantic cod (*Gadus morhua*) *and* ling (*Molva molva*), and this mixture was not detected using DNA sequencing, as the DNA sequence was impossible to analyze (Table 2).

Previously published methodologies for the identification of gadoids relied on performing DNA sequence analysis with two cytochrome b fragments (Lago, Vieites & Espiñeira, 2012), although the method was able to detect mixtures, the time required was much longer and the method required a DNA sequencer. Another methodology has been published for the identification of Atlantic cod (*Gadus morhua*) via real-time PCR. In this case, a similar amount of time as the technique described here was needed for identification; however, this method was not able to detect mixtures, as only a Atlantic cod (*Gadus morhua*) probe was employed, and it required a real-time thermocycler (Herrero, Madrinan, Vieites & Espineira, 2010).

The protocol described here is faster and simpler to perform than any of the techniques described above and, consequently, may prove useful as a substitute or even as complementary to methods published previously. This method relies on species-specific positive results that show up only if the product contains the correct nucleic acid.

In conclusion, the purpose of this study was the development and evaluation of a PCR-LFD technique for the detection of Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*),

Alaska pollock (*Gadus chalcogrammus*) *and* ling (*Molva molva*) in fish products, which has been successfully accomplished. The PCR-LFD technique described in this article was shown to be highly sensitive and specific for rapid identification of the four target species in commercial products. Thus, this technique is a good alternative to other molecular assays.

This methodology represents a useful tool for enforcing labeling regulations in the authentication of fresh or elaborated fish products and could be used for routine analysis in food control laboratories.

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Appendix A. Supplementary data

Conflict of interest

Authors declare not having any conflict of interest related with the research described here

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406 **FIGURE CAPTIONS**

407 **Figure S1.** Location and position of the designed primers within cytochrome b gene.

408 **Figure S2.** LFD assay test principle. A. Elements of the LFD aasay. B. Scheme of a LFD strip
409 showing the three sections. C. Positive LFD strip. D. Negative LFD strip.

410 **Figure S3.** Workflow diagram showing the complete LFD assay steps: DNA extraction, PCR
411 amplification and LFD detection of specific PCR amplicons.

412 **Figure 1.** Specific detection of PCR products derived from amplifications with a PCR-LFD
413 assay. (A) GmacD system, (B) GmorD system, (C) GchalD system and (D) MmolD system.
414 DNA templates: 1: *G. macrocephalus*; 2: *G. morhua*; 3: *G. chalcogrammus*; 4: *M. molva*; 5: *M.*
415 *aeglefinus*; 6: *M. poutassou*; 7: *P. pollachius*; 8: *P. virens*; 9: *T. minutus*; 10: *M. magellanicus*;
416 11: *M. bilinearis*; 12: *M. capensis*; 13: *M. hubbsi*; 14: *M. merluccius*; 15: *M. paradoxus*; 16: *B.*
417 *brosme*; 17: *M. dypterygia*; 18: *L. piscatorius*; 19: *E. encrasicolus*; 20: *S. pilchardus*; 21: *T.*
418 *alalunga*; 22: negative control.

419 **Figure 2.** Sensitivity of detection of PCR products derived from amplifications with a PCR-LFD
420 assay and agarose gel electrophoresis of (A) G. macrocephalus DNA templates, (B) G. morhua
421 DNA templates, (C) G. chalcogrammus DNA templates and (D) M. molva DNA templates. 1: 50
422 ng; 2: $50x10^{-1}$ ng; 3: $50x10^{-2}$ ng; 4: $50x10^{-3}$ ng; 5: $50x10^{-4}$ ng; 6: $50x10^{-5}$ ng; 7: negative control.

423 **Figure 3.** PCR-LFD results for commercial products. Positive and negative results are clearly
424 shown.

Table 1. List of reference species used in the study

Species	N	Common name	Source
Order Gadiformes			
Family Gadidae			
Gadus macrocephalus/ogac	7	Pacific or Greenland cod	University of Kansas (United States)
Gadus morhua	15	Atlantic cod	Instituto de Investigaciones Marinas, CSIC (Spain)
Gadus chalcogrammus	15	Alaska pollock	Max Rubner-Institute (Germany) and Instituto de Investigaciones Marinas (Spain)
Melanogrammus aeglefinus	1	Haddock	Max Rubner-Institute (Germany)
Micromesistius poutassou	1	Blue whiting	Instituto de Investigaciones Marinas, CSIC (Spain)
Pollachius pollachius	1	Pollack	Max Rubner-Institute (Germany)
Pollachius virens	1	Saithe	Instituto de Investigaciones Marinas, CSIC (Spain)
Trisopterus minutus	1	Poor cod	Max Rubner-Institute (Germany)
Family Merlucciidae			
Macruronus magellanicus	1	Patagonian grenadier	Vigo, Border Inspection Posts (Spain)
Merluccius bilinearis	1	Silver hake	Instituto de Investigaciones Marinas, CSIC (Spain)
Merluccius capensis	1	Shallow-water cape hake	Marine and Coastal Management (South Africa)
Merluccius hubbsi	1	Argentina hake	Pescapuerta, Fishing Company (Spain)
Merluccius merluccius	1	European hake	Instituto de Investigaciones Marinas, CSIC (Spain)
Merluccius paradoxus	1	Deep-water cape hake	Marine and Coastal Management (South Africa)
Family Lotidae			
Brosme brosme	1	Tusk	Max Rubner-Institute (Germany)
Molva dypterygia	1	Blue ling	Max Rubner-Institute (Germany)
Molva molva	15	Ling	Max Rubner-Institute (Germany) and Instituto de Investigaciones Marinas (Spain)
Order Lophiiformes			
Family Lophiidae			
Lophius piscatorius	1	Angler	Instituto de Investigaciones Marinas (Spain)
Order Clupeiformes			

Family Engraulidae			
Engraulis encrasicolus	1	European anchovy	Alfageme, Fishing Company (Spain)
Family Clupeidae			
Sardina pilchardus	1	European pilchard	Instituto de Investigaciones Marinas (Spain)
Order Perciformes			
Family Scombridae			
Thunnus alalunga	1	Albacore	Connorsa, Fishing Company (Spain)

Table(s)

Table 2. Commercial products used in the study, results of the PCR-LFD and Cytochrome b sequence identification of commercial products**.** A "+" means a confirmation of the species; highlighting indicates mislabeled samples.

Sample	Commercial name	Declared Species	Presentation	Capture zone	Species identified by FINS	Dipstick signal			
						Gmor	Gchal	Mmol	Gmac
1	cod roe	*not available*	canned	unidentified	*G. morhua*	+	-	-	-
2	cod liver	*not available*	canned	unidentified	*G. morhua*	+	-	-	-
3	cod fritter	*G. morhua*	frozen	Atl. Northeast/ FAO 27	*G. morhua*	+	-	-	-
4	nordic cod	*G. morhua*	smoked	Atl. North	*G. morhua*	+	-	-	-
5	minced cod	*G.morhua*	salted	Atl. Northeast/ FAO 27	*M. molva*	-	-	+	-
6	cod	*G. morhua*	salted	Atl. Northeast	*G. morhua*	+	-	-	-
7	cod cheeks	*G. morhua*	desalted	Atl. Northeast/ FAO 27	*G. morhua*	+	-	-	-
8	cod croquettes	*not available*	frozen	unidentified	*G. morhua*	+	-	-	-
9	cod	*G. morhua*	frozen	Atl. Northeast/ FAO 27	*G. morhua*	+	-	-	-
10	iceland cod	*G. morhua*	salted	Atl. Northeast/ FAO 27	*G. morhua*	+	-	-	-
11	cod	*Gadus spp*	salted	Atl. Northwest	*G. morhua*	+	-	-	-
12	cod liver	*not available*	canned	unidentified	*G. morhua*	+	-	-	-
13	ling loins	*M. molva*	salted	Atl. Northeast/ FAO 27	*M. dypterigia*	-	-	-	-
14	cod omelette	*not available*	precooked	unidentified	*Mixed DNA*	+	-	+	-
15	minced Alaska pollock	*G.chalcogrammus*	salted	Pacific/FAO 67	*G.chalcogrammus*	-	+	-	-
16	Alaska pollock loins	*G.chalcogrammus*	frozen	unidentified	*G.chalcogrammus*	-	+	-	-
17	minced	*G.chalcogrammus*	salted	Pacific/ FAO 61-FAO 67	*G.chalcogrammus*	-	+	-	-
18	Alaska pollock loins	*G.chalcogrammus*	frozen	unidentified	*G.chalcogrammus*	-	+	-	-
19	ling roe	*Molva molva*	dry-salted	Atl. Northeast/ FAO 27	*Molva molva*	-	-	+	-
20	minced cod	*G. morhua*	salted	unidentified	*G. macrocephalus*	-	-	-	+
21	ling roe	*Molva molva*	dry-salted	Atl. Northeast / FAO 27	*Molva molva*	-	-	+	-
22	ling loins	*Molva molva*	salted	unidentified	*Brosme brosme*	-	-	-	-
23	ling roe	*Molva molva*	dry-salted	unidentified	*Molva molva*	-	-	+	-
24	ling loins	*Molva molva*	frozen	unidentified	*G. morhua*	+	-	-	-
25	minced cod	*not available*	salted	unidentified	*G. macrocephalus*	-	-	-	+
26	ling loins	*Molva molva*	refrigerated	unidentified	*Molva molva*	-	-	+	-
27	ling loins	*Molva molva*	refrigerated	Atl. Northwest	*Molva molva*	-	-	+	-
28	ling loins	*Molva molva*	refrigerated	Atl. Northwest	*Molva molva*	-	-	+	-
29	ling loins	*Molva molva*	refrigerated	Atl. Northwest	*Molva molva*	-	-	+	-
30	cod loins	*G. morhua*	salted	Atl. Northeast / FAO 27	*Molva molva*	-	-	+	-
31	cod fritter	*G. morhua*	frozen	unidentified	*Brosme brosme*	-	-	-	-

Table(s)

Table 3. Primers Used in This Work

Primer/Probe name	*PCR amplicon (pb)*	*Molecular marker*	*Target species*	*Sequence 5′- 3′*	*Size (bp)*	*T^a annealing/hybridization*
GmorD-F	*Gmor (91)*	*Cyt b*	*G. morhua*	5′-[6-FAM]CAGGTGGCGTACTTGCACTCCTATTCTCG-3′	30	69
GmorD-R				5′-[Biotin]GAATGTTAAACCTCGTTGTTTTGACGTATGGAGA-3′	34	
GchalD-F	*Gchal (92)*	Cyt b	*G. chalcogramma*	5′-[6-FAM]CATCTTACGCTCTATTCCTAATAAACTAGGCGGTG-3′	35	69
GchalD-R				5′-[Biotin]GAAGTATGGAGAAAGGGTACAACCATAAGGACTAGAATG-3′	39	
GmacD-F	*Gmac (74)*	Cyt b	*G. macrocephalus*	5′-[6-FAM]CTCTACGCTCTATTCCTAATAAATTAGGTGGC-3′	32	69
GmacD-R				5′-[Biotin]AGTGTGGAGGAAGGGCACAACT-3′	22	
MmolD-F	*Mmol (82)*	*Cyt b*	*M. molva*	5′-[6-FAM]TTTGCCTACGCTATCCTACGATCTATTCCCAAC-3′	33	69
MmolD-R				5′-[Biotin]TATGAGAACTAGAATTGAAAATAGAAGTGCGAGAACC-3′	37	
FishDC-F	153 bp	16S rDNA		5′-[6-FAM]CCYAGGGATAACAGCGCAATC-3′	21	69
FishDC-R				5′-[Biotin]TCCGGTCTGAACTCAGATCAC-3′	21	
L14735	*Kocher (460)*	*tRNAglu-cytb*		5′-GCICCTCARAATGAYATTTGTCCTCA-3′	26	55
H15149D				5′-AAAAACCACCGTTGTTATTCAACTA-3′	25	

Figure(s)

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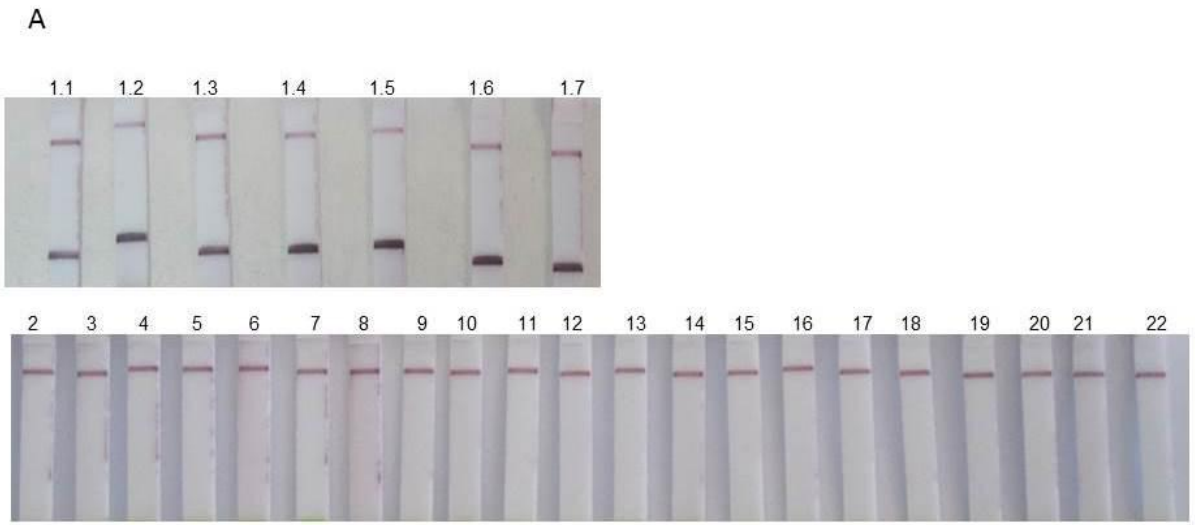

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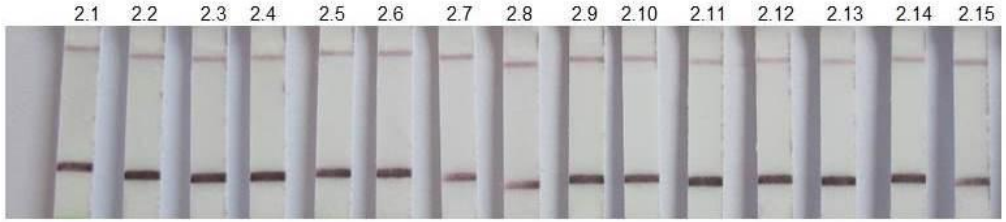
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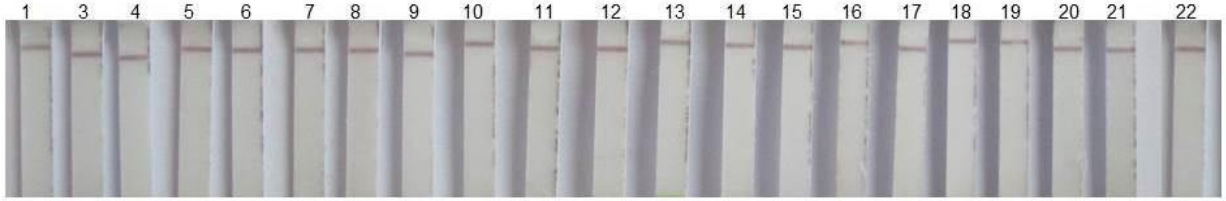
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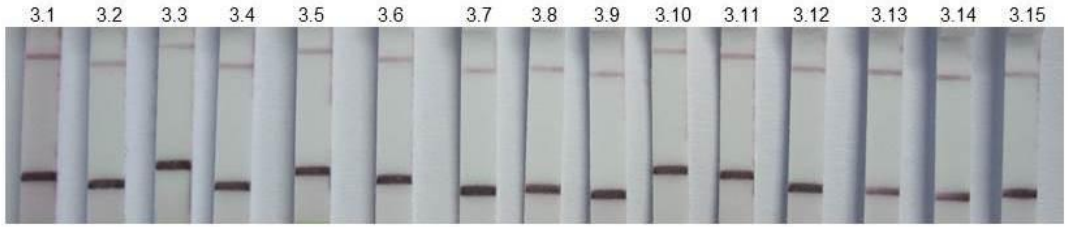
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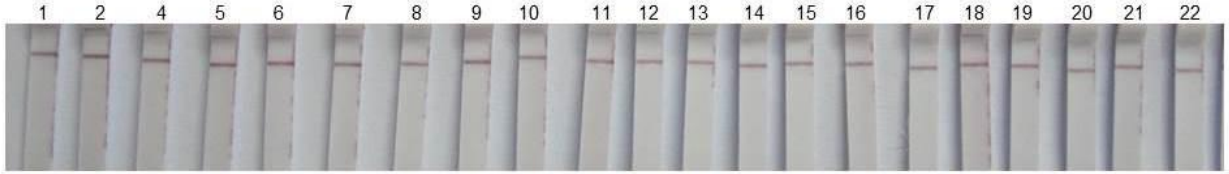
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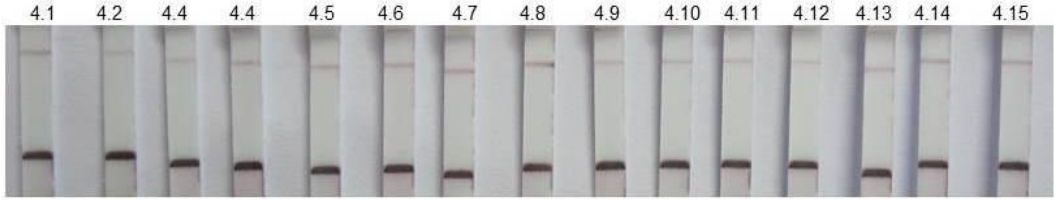
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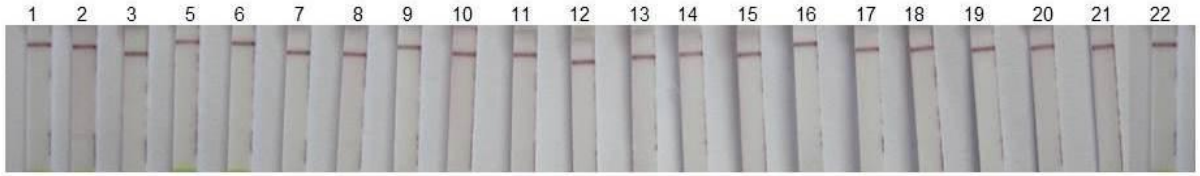
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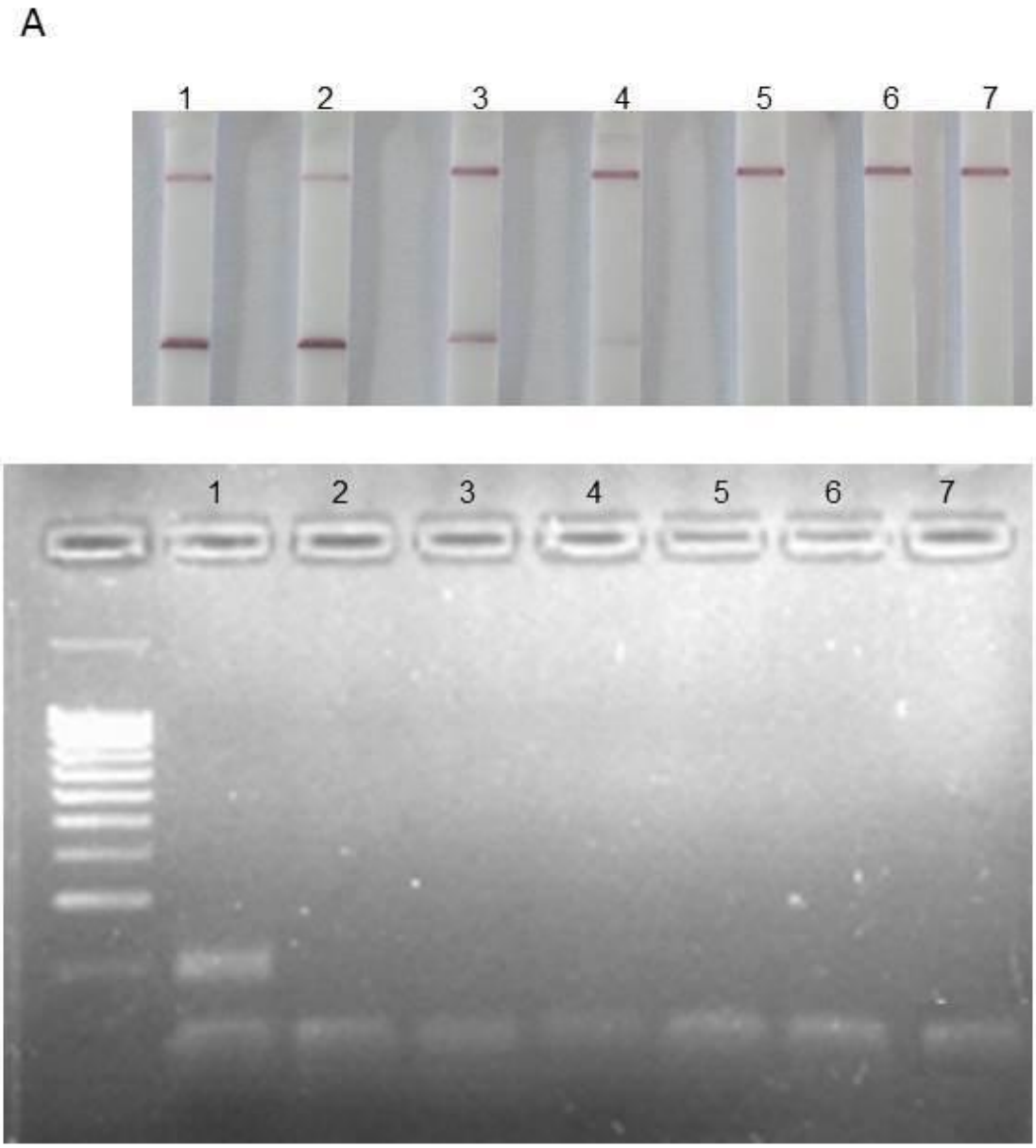

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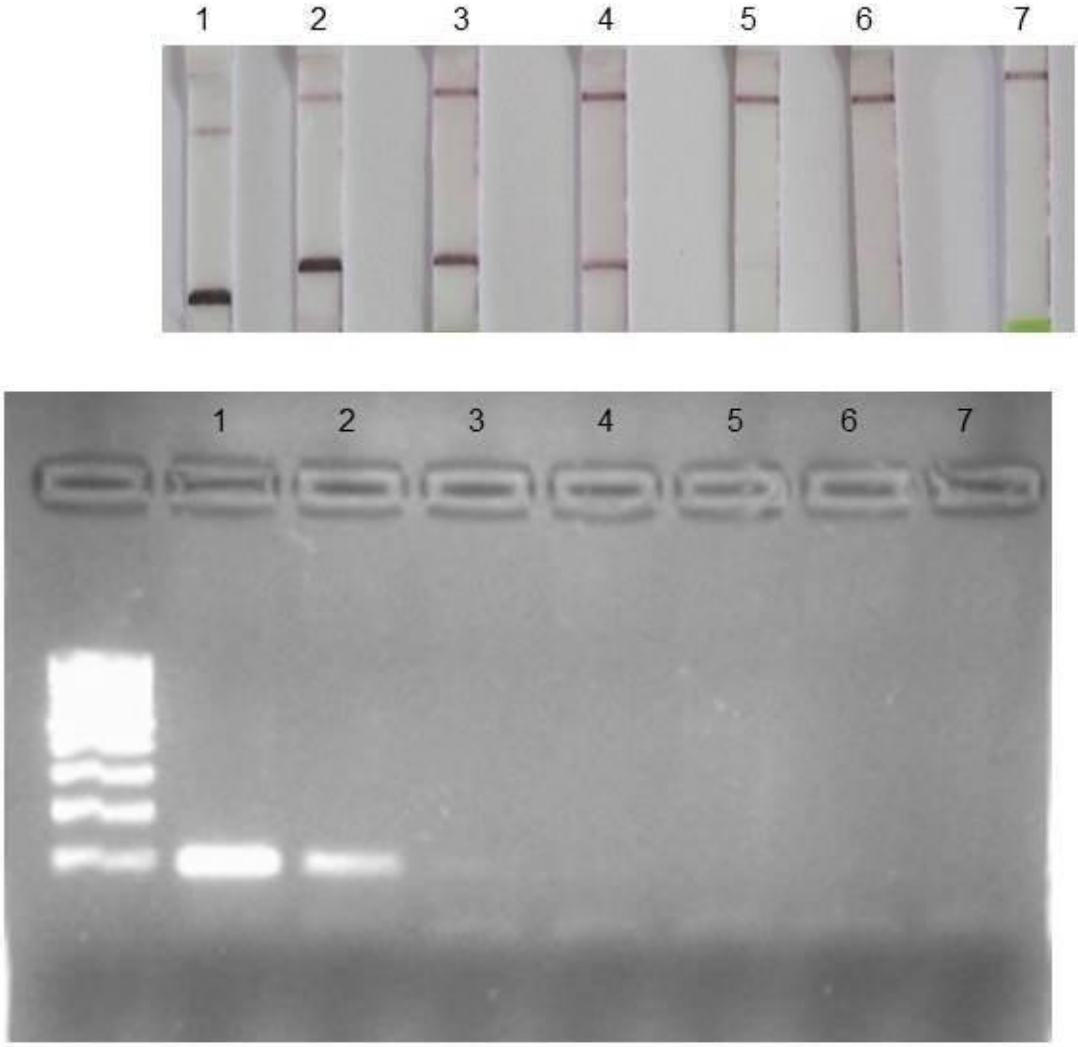

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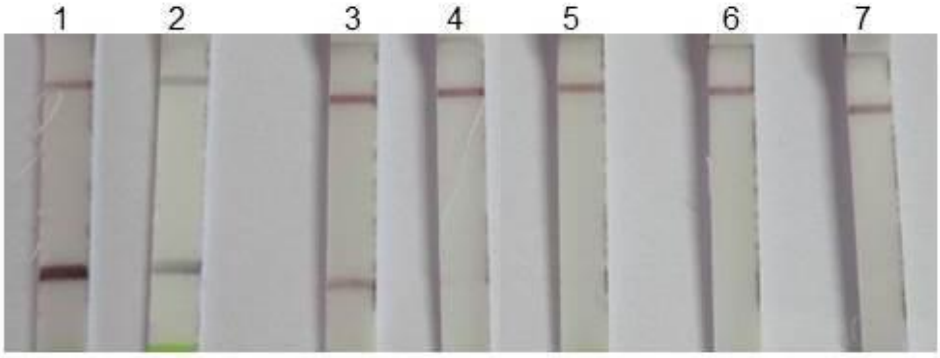

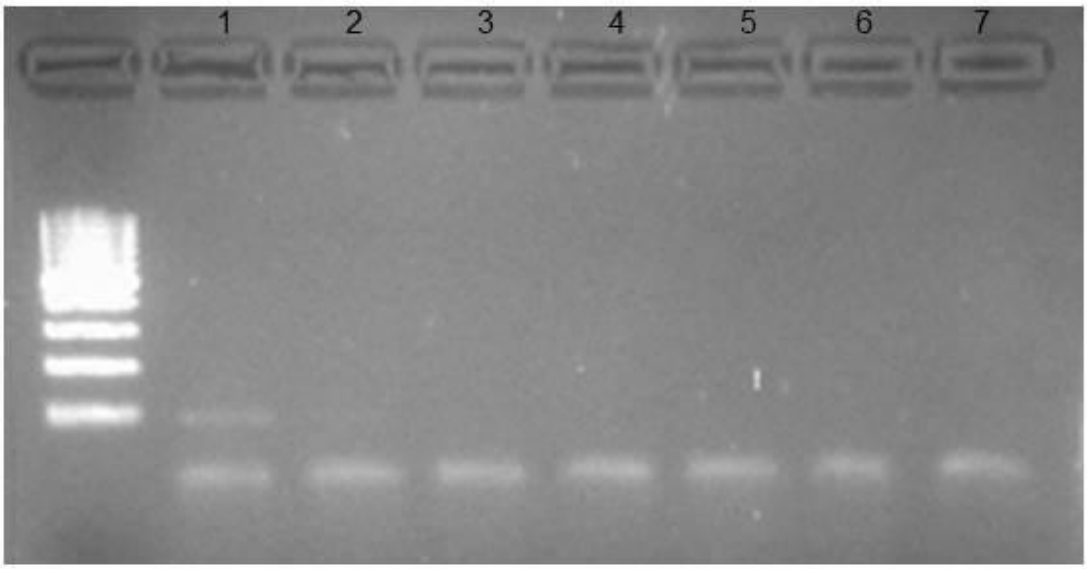

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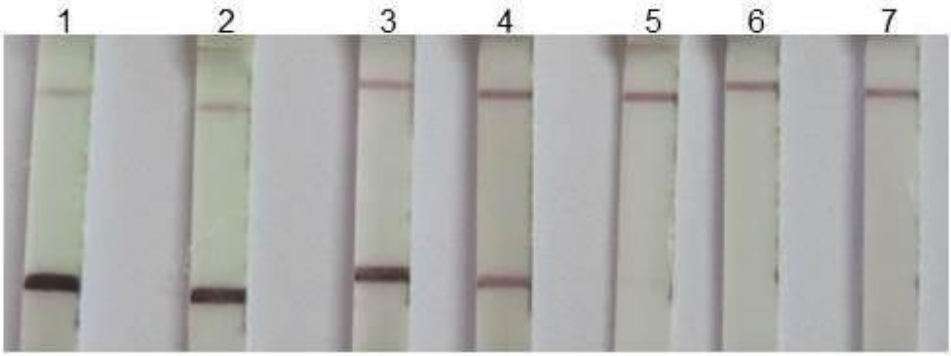

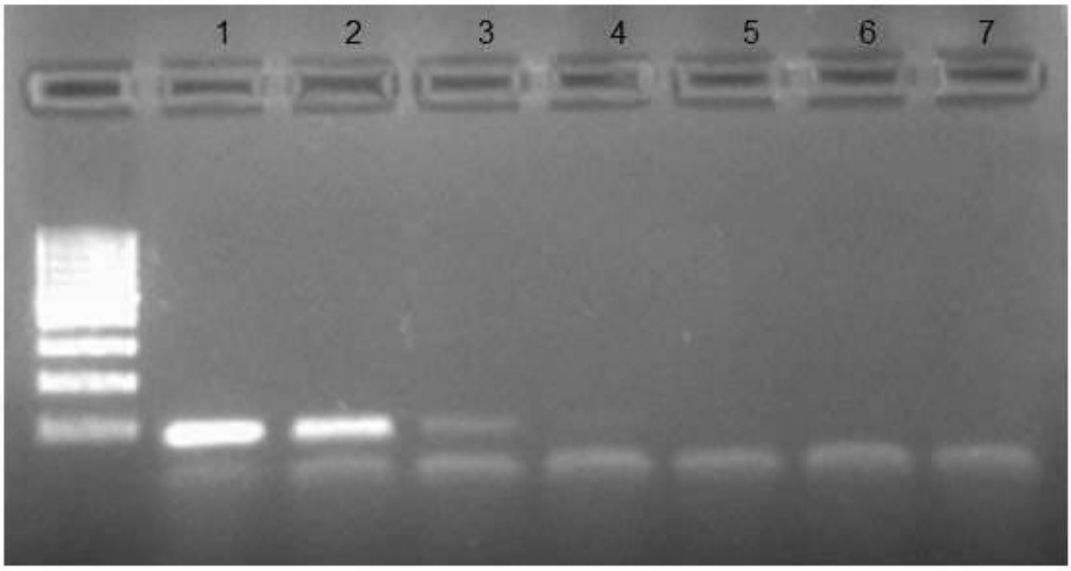

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