Evaluation of eight protocols for genomic DNA extraction of *Hypostomus commersoni* (Valenciennes, 1836), Siluriformes: Loricariidae.

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Abstract

The principle and techniques applied in DNA extraction play a pivotal role in the obtention of a considerable and purified yield of genetic material. In this work, we evaluate the efficiency of seven protocols chosen from literature for the DNA extraction of *Hypostomus commersoni* (Valenciennes, 1836), an important component of South American freshwater ichthyofauna. We also proposed and evaluate an improved protocol, based on the combination of variables from the selected methodologies that demonstrated higher potential for extracting *H. commersoni* DNA. The quality of samples was assessed through spectrophotometry, gel electrophoresis and PCR-RAPD amplification. The efficiency of DNA extraction was influenced both by the method applied and target-tissue of choice. Higher concentrations and yield of DNA were obtained from ocular tissue, with a positive spectrum of incubation in lysis buffer for up to 36 hours after sample collection, without maceration, using fresh tissues and in the presence of high concentration of Proteinase K. In these conditions, samples were successfully amplified using a set of primers from Operon Technologies. To date, there is no record of description for the parameters analyzed in this work, neither the description of RAPD markers for *H. commersoni*, evidencing the importance of our findings.

Key-words: DNA extraction; PCR-RAPD; eye tissue; fresh fish tissue; proteinase K.

Introduction

A crucial step for high quality genetic analysis is considered to be a satisfactory DNA extraction. Nevertheless, this purpose is not always easy to achieve, since different biological compounds, such as lipids and proteins, can act as contaminants and interfere in the final quality of the product [1]. The principles and techniques applied in DNA extraction play a pivotal role in obtaining a considerable and purified yield of this molecule [2].

A variety of methods has been established to isolate DNA from biological materials [3,6]. Regardless the method of choice, the procedure usually consists of three steps: lysis, purification and DNA recovery, being the first the most critical stage, since is at this point that the chances of causing DNA damages are higher [7].

The quality and amount of available DNA strand template have a major influence on genetic analyzes based on PCR (Polymerase Chain Reaction), for example [8,9]. Therefore, an ideal extraction technique should optimize DNA yield, minimize DNA degradation, and be efficient in terms of cost, time, labor, and supplies [10].

With the availability of a plethora of molecular analysis machinery, there is an exponential demand for high quality samples, capable to generate great results with minimum of errors. To meet these requirements, commercial kits were developed, aiming for DNA extractions of excellence [11-13].

However, the market price of this products it is not always easily affordable to every laboratory [10, 14]. In the other hand, the extraction of genetic material with non-commercial reagents is less costly due to the use of homemade reagents and, therefore, such methodologies can be applied in laboratories with less financial resources.

Stablish a well succeed protocol for DNA extraction is a very important step in genetic analysis, considering that the same method may present variations between

different organisms [15]. Furthermore, the quality of isolated DNA through methods described for other taxa, may present unsatisfactory results when applied to fish species [2].

H. commersoni (Valenciennes, 1836), commonly known as suckermouth catfish, is an important component of freshwater ichthyofauna, inhabiting the Paraná and Uruguay basins, in South America [16, 17]. This taxon is marked by its ecological and commercial relevance [18-22], and, despite that, there is no record of description of well-succeed DNA extraction protocol nor PCR-RAPD markers to date.

In this work, we evaluated seven protocols chosen from literature in the extraction of genomic DNA of *H. commersoni* and, also, we propose an improved method, aiming to obtain high quality and purified DNA samples of this specie.

Materials and methods

Ethics Statement

This study was approved by the CEUA (Ethics Committee for Animal Use) of URI (Regional Integrated University). Written informed consent was obtained from the regulatory organ.

Sample collection

Specimens of *H. commersoni* were subjected to euthanasia with 0.1% clove oil in aquarium water, and the tissues selected for DNA extraction were removed and placed in a sterile 2 mL eppendorf tube for subsequent analysis according to each protocol particular necessity.

DNA Extraction

All DNA was extracted and quantified at the biochemistry and molecular biology laboratory of URI (Regional Integrated University, Erechim-RS/Brazil).

Protocols and variables analyzed in the DNA extraction

Seven protocols from literature were selected for DNA extraction of *H. commersoni*, whose methodologies had previously been described for fish species or whose parameters were similar to those intended to be analyzed in this work, such as the same extraction conditions and target tissues (Box 1). An eighth protocol, elaborated by the combination of the variables that presented better potential to extract *H. commersonii* DNA in each of the seven previously tested protocols, was proposed and evaluated too.

Box 1 – Protocols evaluated for DNA extraction.

Protocol	Reference[1]
1	[26] Doyle and Doyle (1987) *CTAB.
2	[27] Medrano et al. (1990) *STE/EDTA.
3	[29] Whitmore et al. (1992) *PHENOL.
4	[23] Aljanabi and Martinez (1997) *SALTS.
5	[28] Faleiro et al. (2003) *CTAB.
6	[24] Barrero et al. (2008) *SALTS.
7	[25] Coppini (2009) *STE/EDTA.
8	Developed in this work[2] *STE/EDTA.

[1] The full description of protocols 1 to 7 is available as supplementary material (S1).
[2] Described in full in the results section.
*Principle of the method.

In combination to the protocols, we also evaluate eight different variables that, based on literature findings and our previous analysis, was tough to have an influence on the purity and amount of DNA obtained, as follows:

1. **Preservation of specimens**: fresh tissues (1. collection, 2. euthanasia, 3. tissue extraction and immediate use) or stored in 70% and 95% ethanol at temperatures of 25 ° C, -10 ° C and -20 ° C;
2. **Maceration**: mechanic lysis (vortex), liquid nitrogen or manual lysis (with sterile glass stick);
3. **Target-tissue**: dorsal fin, caudal fin, muscle, gills, blood and ocular tissue;
4. **Time of incubation in extraction buffer**: 1 hour (55 ºC), 12 hours (27 ºC), 34 hours (27 ºC) and 36 hours (27 ºC);
5. **RNAse**: presence and absence, with gradients of concentration;
6. **Proteinase K**: presence and absence, with gradients of concentration;
7. **Final storage**: at 4 ºC, -10 ºC and -20 ºC;
8. **Integrity and quantification**: on the day of extraction, after 10, 20 and 30 days of storage.

Several tests aiming for experimental optimization were performed and for each variable, the factors capable to generate better concentration and purity of DNA were selected. The results of extractions for each variable were categorized into a qualitative matrix including three classes, according to the effect on the final extracted product:

(i) **Efficient**: satisfactory electrophoresis and spectrophotometry;
(ii) **Indifferent**: did not influence the parameters evaluated;
(iii) **Inefficient**: low concentration of genetic material, low purity or degradation of samples.

145 ## Analysis of DNA integrity

146 The integrity of the obtained samples was verified through agarose gel (0,8%)
147 electrophoresis, stained with ethidium bromide (1 mg/ml). The reaction run for 150
148 minutes at 90 volts and the visualization and image caption of the gel were obtained under
149 ultraviolet (UV) light.

150

151 ## Evaluation of concentration and purity of DNA

152 The samples whose extraction was successful and did not result in DNA degradation
153 according to the electrophoresis, where submitted to spectrophotometry and analyzed for
154 protein contamination (purity). Samples were quantified in a spectrophotometer (model NI
155 1600UV - Nova Instruments), at A_{260} and A_{280} nanometers (nm). The purity of the DNA
156 was confirmed by the ratio of absorbance values at the two wavelengths measured [33].
157 The DNA concentration of each sample was determined by the equation: [DNA] = 50
158 µg/ml x D (dilution factor) x A_{260} (read value obtained in the wavelength of 260 nm).
159 Satisfactory purity values were considered to be ranging between 1.7-2.0.

160

161 ## DNA amplification by PCR-RAPD (Polymerase Chain Reaction
162 ## - Random Amplified Polymorphic DNA)

163 Samples that reached the aforementioned parameters of DNA concentration and yield were
164 submitted to amplification with different primers of PCR-RAPD. In total, 32 primers from
165 Operon Technologies kits OPA (01, 02, 03, 04, 05, 06, 07, 08, 09), OPB (03, 05, 07, 10,
166 11, 15), OPF (10, 11, 12 e 13), OPW (01, 02, 03, 04, 05, 06, 07, 08, 09, 10) and OPY
167 (01,02,03) were tested. Ten nanograms of genomic DNA was amplified in PCR buffer (20
168 mM Tris-Hcl pH8.4, 50 mM KCL), 4 mM MgCl2, 0.7 mM dNTPs, 0.3 µM primers e 1

169 unity of Taq DNA polymerase enzyme (Invitrogen). The reaction was conducted in a final
170 volume of 25 µL, in the following conditions: denaturation at 95ºC for 30 s, annealing at
171 50ºC for 1 min e extension at 72ºC for 5 min, totaling 40 cycles. The amplification
172 products were applied in agarose gel (1.5%), stained with ethidium bromide (1 mg/ml).
173 The reaction run for 150 minutes at 150V. Visualization and image caption of band
174 patterns were obtained under ultraviolet (UV) light.

175

176 Statistical analysis

177 Statistical analysis was performed using Python (version 3.6.0). Differences between DNA
178 extraction methods in total DNA concentration were determined using ANOVA with
179 Tukey HSD post-hoc test.

180

181 Results

182 DNA extraction method impacts DNA yield

183 Our work corroborated that even a method that uses in-house produced reagents instead of
184 commercial kits is capable to extract DNA with sufficient quality to be used in molecular
185 analysis, as PCR-RAPD. The amount of DNA extracted was dependent on the DNA
186 extraction technique and the target-tissue of choice. For all variables examined, the
187 improved extraction method developed and proposed at this work, produced the greatest
188 total DNA mean yields, for all tissues, resulting in significantly higher absorbance ratios
189 when compared to the seven other extraction methods. Different variables also affect in
190 different ways the extraction of DNA, as showed in Table 1.

191

192

193 **Table 1.** Effect of different variables in the DNA extraction of *H. commersoni*.

Variables	Efficient[1]	Indifferent[2]	Inefficient[3]
Specimens conservation	Fresh (27 ºC)	-	Fresh (-10 ºC and -20 ºC) 70% and 95% ethanol (4 ºC, -10 ºC and -20 ºC)
Maceration	Lysis with buffer	Liquid nitrogen	In vortex Manual lysis
Target-Tissue	Ocular tissue	–	Caudal fin Dorsal fin Muscle Gills Blood
Time of incubation	1h (55 ºC) 12h, 24h and 36h (27 ºC)	–	–
RNAse	–	Presence	–
Proteinase K	Presence in high concentration (≥ 20 mg.ml^{-1})	–	–
Storage	-10 ºC -20 ºC	–	4 ºC
Quantification	In the extraction day After 10, 20 and 30 days of storage	–	–

194 [1]Efficient: satisfactory electrophoresis (integrity) and spectrophotometry (quantification);
195 [2]Indifferent: did not influenced the final amount and the purity of DNA extracted;
196 [3]Inefficient: sample degradation and low purity observed.
197

198 # Improved protocol for DNA extraction of *H. commersoni*

199 The eighth protocol evaluated, improved and proposed in this work, in general, presented
200 the highest efficiency in the extraction of *H. commersoni* DNA. The samples submitted to
201 extraction through this protocol were the only ones that amplified with PCR-RAPD
202 primers, since they had the best DNA concentration and yield of purify DNA in the
203 samples (according to the absorbance ratio of 260/280 nanometers).

204 The complete methodology of protocol 8, including reagents and steps of
205 extraction, is describe in Box 2.

206
207

208 **Box 2.** Description of reagents and steps for DNA extraction of *H. commersoni* through the
209 method proposed in this work.

Reagents	
STE Buffer	0,1 M NaCl + 10 mM Tris + 1 mM EDTA (pH 8,0)
Lysis Buffer	2,5 mM Tris-HCl pH 8,0 + 0,1 mM EDTA pH 8,0 + 0,1% SDS + 8,0 mM NaCl
Proteinase K	20 $mg.mL^{-1}$
CIA	Chloroform + isoamylic alcohol (1:24 v/v)
TE Buffer	10 mM Tris pH 8,0 + 1 mM EDTA pH 8,0
TBE Buffer	500 mM Tris-HCl + 60 mM boric acid + 83 mM EDTA
Steps of extraction	
1. Collect 20 mg of fresh tissue.	
2. Wash in 500 µl of STE (cold) and discard.	
3. Add 500 µl of lysis buffer, 2 µL of β-Mercaptoetanol and 13 µl of proteinase K, in this order, and incube at 55 ºC for 1 hora.	
4. Add 700 µl of CIA and homogenize by inversion.	
5. Centrifuge for 10 minutes at 9,000 rpm and transfer the supernatant to a new tube.	
6. Repeat the steps 4 and 5 (3x).	
7. Transfer the aqueous phase to a new tube containing 900 µl of absolute isopropanol (cold), homogenize by inversion.	
8. Incubate for 2 hours at –20 ºC.	
9. Centrifuge for 15 minutes at 9000 rpm and discard the supernatant.	
10. Wash the resulting *pellet* with ethanol analytical grade (cold).	
11. Discard all the volume and incube at 37 ºC for 15 minutes.	
12. Add 100 µL of TE buffer and incube for 1 hour at ≈ 25 ºC.	
13. Storage at – 10 ºC until required.	

210 *This protocol described the sample incubation in lysis buffer for 1 hour (step 3) but can also be
211 applied for the spectrum of 6, 12 or 36 hours previous to the continuity of extraction (steps 4-13).
212

213 Analysis of DNA integrity in electrophoresis gel

214 All samples from the six tissues and the eight applied conditions were submitted to gel
215 electrophoresis in triplicate, resulting in a total count of 144 samples. The ocular tissue
216 presented the highest integrity of extracted DNA, in all combinations of variables and
217 protocols. For all tissues, the main bands of DNA were around 46-48 kb in size.
218 Comparing protocols, the EDTA methods (P2, P7 and P8) showed relatively lighter smear
219 tails, indicating less DNA degradation.
220

221 Quantification and assessment of purity

222 The resulting values of quantification for each DNA sample extracted under the different
223 conditions and through the different protocols evaluated, are presented in Table 2 and 3

224 and Fig 1A–1F. The amount of DNA obtained, and final yield mean values varied between
225 different tissue samples, as well as between samples of the same tissue but extracted using
226 different protocols.

227 **Table 2.** Mean of total DNA amount (ng/µl) extracted using eight different methods for six
228 different tissues.

Method (Protocol)	CF	DF	MC	GL	BD	OT	
	MEAN OF TOTAL DNA AMOUNT (ng/µl)						Methods Mean (ng/µl)
	Mean ± SD						
1. Doyle & Doyle (1987)	120 ±21.2	139 ±26.9	198 ±10.6	110 ±22	365 ±60.4	400 ±26.8	222
2. Medrano et al. (1990)	175 ±8.6	114 ±23.7	155 ±32.8	137 ±27.8	375 ±115.2	520 ±62.8	246
3. Whitmore et al. (1992)	116 ±18.0	74 ±19	173 ±35.3	125 ±6.5	335 ±70.3	455 ±48.8	216
4. Aljanabi & Martinez (1997)	181 ±17.3	90 ±12.3	120 ±33.2	110 ±12.6	455 ±60.1	465 ±50.9	237
5. Faleiro et al. (2003)	168 ±39.9	150 ±24.5	205 ±28.1	155 ±10.6	425 ±20.4	505 ±63.9	268
6. Barreto et al. (2008)	157 ±29.0	97 ±9.6	207 ±32.6	175 ±18.7	460 ±51.9	555 ±47.3	275
7. Coppini (2009)	186 ±6.6	160 ±44.4	170 ±19.1	135 ±1.6	330 ±17.1	516 ±22.2	250
8. Improved protocol (2019)	209 ±15.7	220 ±33.5	216 ±0.8	210 ±4.1	463 ±9.7	712 ±18.8	438
Tissues Mean	**164**	**131**	**180**	**145**	**404**	**516**	-

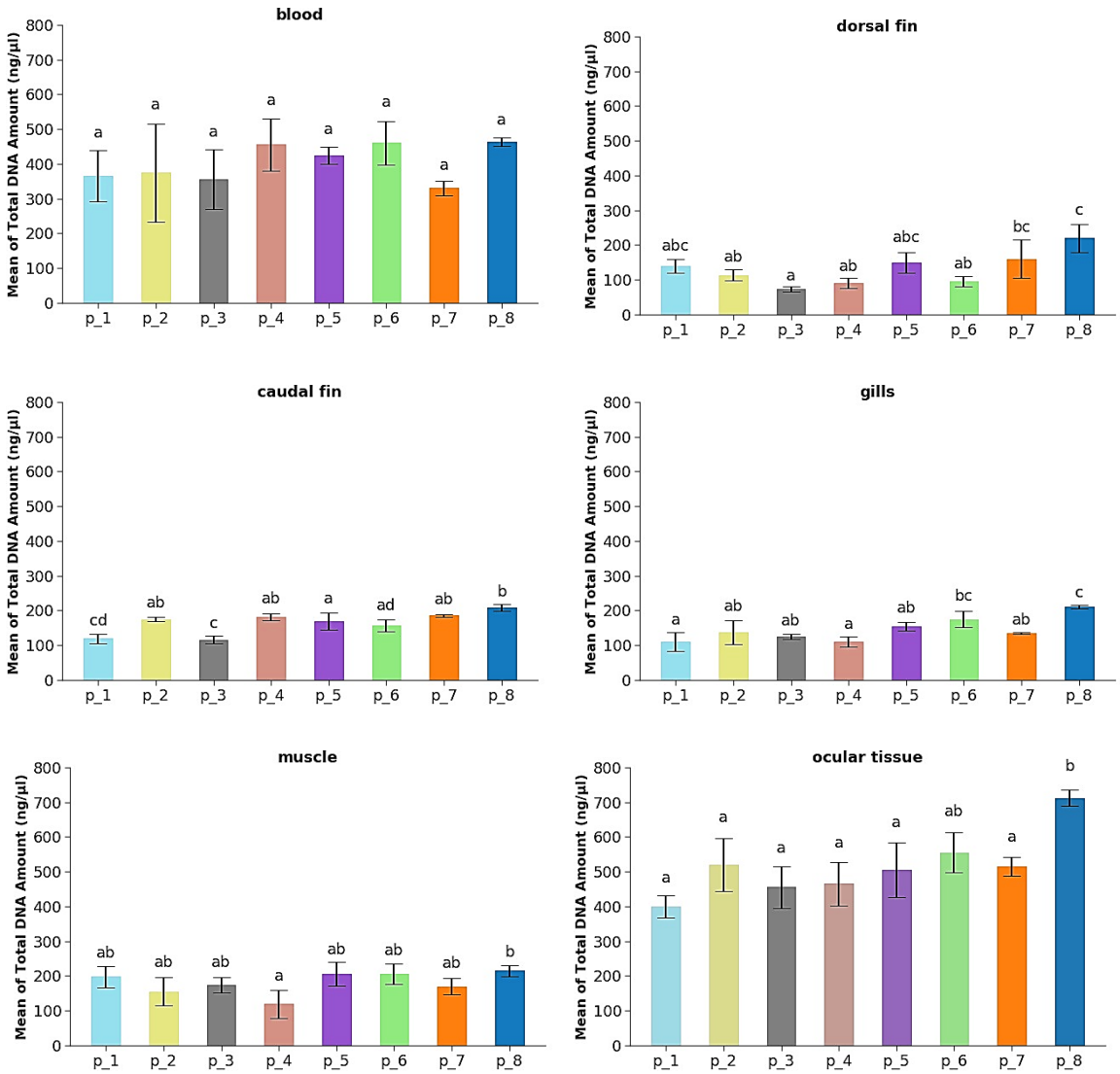
229 ***Tissues:** CF: caudal fin. DF: dorsal fin. MC: muscle. GL: gills. BD: blood. OT: ocular tissue.
230 **Sample values from read in the wavelength of 260 nm where applied to the equation [DNA] = 50
231 µg/mL x D (dilution factor) x A_{260} to obtain its concentration in ng per µl.
232

233 **Table 3.** Mean of DNA amount from ratio 260/280 nm, extracted using eight different
234 methods for six different tissues.

Method (Protocol)	CF	DF	MC	GL	BD	OT	
	ABSORBANCE RATIO (OD 280/260 nm)						Methods Mean
	Mean ± SD						
1. Doyle & Doyle (1987)	1.5 ±0.016	0.8 ±0.08	1.4 ±0.08	1 ±0	1.9 ±0.08	1.8 ±0.12	1.4
2. Medrano et al. (1990)	1.8 ±0.08	0.6 ±0.08	1.6 ±0.08	1 ±0.08	1.4 ±0.08	1.6 ±0.08	1.3
3. Whitmore et al. (1992)	1 ±0.14	0.7 ±0.08	1.6 ±0.08	1.2 ±0	1.6 ±0.08	1.8 ±0.08	1.3
4. Aljanabi & Martinez (1997)	1.1 ±0.16	0.8 ±0.08	1 ±0.08	1 ±0	1.7 ±0.08	1.6 ±0.08	1.2
5. Faleiro et al. (2003)	1.1 ±0.08	0.9 ±0.08	1.6 ±0.08	1.1 ±0.14	1.6 ±0	1.9 ±0.08	1.4
6. Barreto et al. (2008)	1.1 ±0.08	0.9 ±0.08	1.3 ±0.08	1.2 ±0	1.8 ±0.08	1.7 ±0.08	1.3
7. Coppini (2009)	1.5 ±0.08	1.1 ±0.12	0.8 ±0.08	1.1 ±0.08	1.6 ±0	1.8 ±0.08	1.3
8. Improved protocol (2019)	2 ±0	1.5 ±0.08	1.7 ±0.08	1.5 ±0.08	1.9 ±0.08	2 ±0.08	1.8
Tissues Mean	**1.4**	**0.9**	**1.3**	**1.1**	**1.7**	**1.8**	-

235 ***Tissues:** CF: caudal fin. DF: dorsal fin. MC: muscle. GL: gills. BD: blood. OT: ocular tissue.
236 **Sample values from ratio of the reads in the wavelength of 260 and 280 nanometers, to quantify
237 the purity of DNA in the samples.
238

239



240 **Fig 1. Mean of total DNA amount (± standard deviation) extracted from six different**

241 **tissues using eight different protocols.** Samples were extracted and total DNA was measured

242 by spectrophotometry. Statistical significance was determined using one way ANOVA with Tukey-

243 HSD post-hoc test. Significance defined by $p \leq 0.05$ and denoted by lower case letters (samples

244 marked with the same letter are significantly equal). Whiskers denoting lower standard deviation

245 are cropped at zero.

246

247 When comparing samples, the ocular tissue had the highest mean yield of extracted DNA

248 (516 ng/µl), while the lowest concentration was for the dorsal fin (131ng/µl). In relation to

249 the protocols, means between different tissues indicated that the lowest value was for

250 extractions performed using protocol 3 (216 ng/µl), while the highest mean concentration
251 of DNA was obtained with protocol 8 (438 ng/µl). Statistically, as indicated by the lower
252 case letters, there is no significant difference between most of the samples whose DNA
253 was extracted from the tissues dorsal fin, caudal fin, gills and muscle. The major
254 exceptions were blood and ocular tissue, which had similar p value for most of the
255 evaluated protocols.

256 Although the total amount of DNA present high values and several samples were
257 statistically similar, the mean values of the absorbance ratio are the important data, once
258 concerns the purity of samples and DNA amount. The absorbance ratio values were 1.2
259 (protocol 4), 1.3 (protocols 2, 3, 6 and 7), 1.4 (protocols 1 and 5) and 1.8 (protocol 8),
260 confirming its higher efficiency in comparison to the others, indicated also by the lowest
261 observed standard deviation.

262

263 DNA extraction method influences PCR-RAPD amplification

264 All samples were subject to PCR-RAPD analysis, but only the ones extracted with protocol
265 8 amplified with the selected primers. Of the 34 primers tested, 9 amplified the DNA of the
266 evaluated specie in the concentrations of 40 and 20 ng/µl and were described in this work.
267 These markers present potential to be used in studies evaluating the genetic structure of
268 ocular tissue samples of *H. commersoni* through PCR-RAPD markers.

269 The score of bands varied between the amplifications using different concentrations
270 of DNA (Table 4). The concentration of 20 ng/µl generated the major count of fragments,
271 followed by the concentration of 40 ng/µl. The concentration of 10 ng/µl of DNA did not
272 amplify with the primers OPW05, OPW06, OPW07, OPW08 and OPW09, and, therefore,
273 this concentration is the less indicated for use in the PCR-RAPD reaction with this set of
274 primers and specie, according to our findings.

275

276 **Table 4.** Score of DNA bands amplified for each primer.

Primer	Sequence 5' - 3'	Score of Amplified DNA bands		
		40 ng/µl	20 ng/µl	10 ng/µl
OPW-01	CTCAGTGTCC	6	6	6
OPW-02	ACCCCGCCAA	6	8	8
OPW-03	GTCCGGAGTG	4	8	7
OPW-04	CAGAAGCGGA	7	9	7
OPW-05	GGCGGATAAG	4	4	-
OPW-06	AGGCCCGATG	7	8	-
OPW-07	CTGGACGTCA	7	8	-
OPW-08	GACTGCCTCT	5	5	-
OPW-09	GTGACCGAGT	6	6	-

277

278 # Discussion

279 The relevance in evaluating different protocols for DNA extraction and the description of
280 an efficient method for the specie *H. commersoni* is justified by the fact that obtaining high
281 quality samples is crucial for studying organisms at genetic level. Specifically, in the case
282 of *H. commersoni*, there are few molecular, genetic and/or phylogenetic data available in
283 the literature. The information compilated at this work may allow future analyzes of
284 molecular characterization of the group and, nevertheless, the techniques described here
285 have potential to be used to study other organisms or fish species

286 In this work, we proposed a methodology based in the DNA extraction from ocular
287 tissue and, to our knowledge, there is no record of this parameter for any fish species to
288 date. The use of this tissue may present some limitations, such its availability in number
289 and size, but our results indicate that this organ have a great potential as source for extract
290 good quality genomic DNA in *H. commersoni* and possibly in other fish species.

291 This technique can be useful in cases where DNA extraction is performed using
292 samples belonging to scientific collections, where the specimens have already bein
293 euthanized, or when an alternative model of extraction source is required, once the
294 classical ones have been exhausted without present a positive result.

295 During the test period, all the steps that presented promising results regarding the
296 primary objective of our study were selected, aiming to group them into a single protocol,
297 considered the "ideal" for the *H. commersoni* species. In this way, it is possible to discuss
298 some of the characteristics that presented better efficiency in terms of quantity and quality
299 of isolated DNA.

300 The use of liquid nitrogen for tissue maceration in DNA isolation, especially in
301 solid tissues, is a method described as efficient according to several findings [35].
302 However, in this work, it has not been shown to be a factor influencing the quality or
303 quantity of product obtained. This data is important because it results in a protocol of lower
304 cost and greater ease of execution, facilitating the application of this methodology in a
305 larger number of laboratories and research groups.

306 The addition of RNAse is suggested as important in protocols for extracting fish
307 DNA, being used in the steps of breaking tissues and flakes, in order to eliminate the
308 contamination of the samples by RNA and to increase DNA yield [30]. However, in this
309 study the use of RNAse had no effect on DNA extraction. For all protocols and variables
310 tested, RNAse did not involve more pure or concentrated samples, even when evaluated in
311 several gradients and at different stages of each protocol. The possibility of eliminating the
312 use of RNAse during DNA extraction also results in the development of a lower cost
313 protocol.

314 Other variables, however, corroborated with information found in the literature,
315 such as the use of proteinase K to increase extraction efficiency, especially at temperatures
316 of sample incubation up to 55 ºC. Proteinase K digestion is characterized by the
317 purification of quality DNA for genetic analyzes such as PCR [31, 32].

318 One of the most promising results was the possibility of exploring different
319 incubation times of the samples in the extraction buffer, with a proven spectrum of
320 efficiency in 1, 12, 24 and 36 hours, using the ocular tissue of *H. commersoni*. The

advantages related to this discovery are the possibility of extracting the DNA in a single day (1 hour at 55 ºC), incubating the samples overnight, or, furthermore, being able to postpone extraction after its incubation in lysis buffer, until 36 hours at room temperature of approximately 25 ° C. This result is also important because it facilitates the transport of material where the specimens can be euthanized in the field and the ocular tissue maintained in lysis buffer for up to 36 hours without the need for refrigeration or any other form of preservation until the DNA can be extracted in adequate conditions.

In general, all 7 protocols chosen from the literature and evaluated in this work presented relative efficiency in the extraction of *H. commersoni* DNA, despite some limitations, when evaluating the concentration indices obtained from each sample. However, when the values were submitted to the absorbance ratio equation, it is evident that the protocol proposed in this paper was the one that obtained the highest DNA purity values in the samples.

The protocol that presented higher efficiency is a simple and economic method to obtain DNA from *H. commersoni*, with the potential to be applied in other similar species. Following the methodology described in our improved protocol, it was possible to submit the samples to the amplification reaction, using primers for PCR-RAPD. The samples from the other protocols were submitted to the same analysis, but without success.

Under optimal conditions, a single cell contains broadly DNA to serve as a template for PCR reactions. The usefulness of this protocol in PCR-based genetic studies was confirmed through the amplification of samples using PCR-RAPD markers. Our findings brought unprecedented data for *H. commersoni*, and this information can be applied in further studies of genetic characterization of this taxon.

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