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A NON-DESTRUCTIVE DNS SAMPLIMG METHOD FOR FROM SALMONIDAE FISH SCALES FOR GENETIC ANALYSIS

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Salmonidae fish is among the most valuable biological resources in Latvia. Some species are introduced, bet some species (*Salmo trutta*, *Salmo salar*) are protected species in Latvia. The growth of salmonid aquaculture has raised concerns about the possibility of detrimental effects on the genetic integrity and diversity of wild population. Indigenous salmonid fish gene pools are affected by domesticated conspecifics, derived from aquaculture escapes and deliberate releases. Modern studies of population genetics rely increasingly on DNA markers amplified by polymerase chain reaction (PCR) for detecting genetic variations within and among populations. Such information is useful for planning conservation strategies for great number of species. Non-destructive sampling, that implies the use of tissues (blood, skin, scales, muscle biopsy) without critical damages to the animals, can exploit the full potential of DNA analyses. The dried scales are a valuable resource for population genetic. We are developed a simple and inexpensive method for dried and preserved fish scales of Salmonidae by combining steps of existing protocols with our slight modifications. *Salmo trutta* scale size is 2-3 mm, bet weigh is 1-2 mg, isolated DNA from *Salmo trutta* the weight was 100 to 300 ng mg^{-1}. The isolated DNA no sign of degradation and spectrophotometer comparision of absorbance at A260/ A280 nm provided a pure factor of 1.8-2.0 indicating its good quality. DNA samples isolated from scales by our method were successfully amplified by the *Salmo trutta* four specific microsatellite primers (Str151NRA, Str 731NRA, Str851 NRA, Str5431NRA) and provide reproducible results.

Key words: Salmonidae fish, non-destructive DNA sampling, microsatellite, scales

INTRODUCTION

Salmonidae fish is among the most valuable biological resources in Latvia. Some species are introduced, bet some species (*Salmo trutta, Salmo salar*) are protected species in Latvia. The growth of salmonid aquaculture has raised concerns about the possibility of detrimental effects on the genetic integrity and diversity of wild population. Indigenous salmonid fish gene pools are affected by domesticated conspecifics, derived from aquaculture escapes and deliberate releases. Modern studies of population genetics rely increasingly on DNA markers amplified by polymerase chain reaction (PCR) for detecting genetic variations within and among populations. Such information is useful for planning conservation strategies for great number of species. Liver and muscle are the most common tissues used as source of DNA, but for collection of liver the fish needs to be sacrificed. Non-destructive sampling, that implies the use of tissues (blood, fins, scales, buccal swabs) without critical damages to the animals, can exploit the full potential

of DNA analyses including individual determination, relatedness estimates, pedigree reconstruction, and determination of the effective population size and the level of genetic polymorphism within and between populations. However, this strategy usually results in a low quantity and poor quality DNA and also does not provide individual identification which limits its potential application. In this paper an improved DNA extraction method from fish scales has been described that used a modified lysis buffer. This DNA extraction method provides high-quality and high- quantity DNA that can serve as a template in polymerase chain reaction (PCR).

MATERIALS AND METHODS

Sample preservation

For DNA extraction were used the dried and frozen scales from sea trout *(Salmo trutta trutta)* and others *Salmonidae* taxa: *Oncorhynchus mykiss*, *Salmo salar*. Scales was used flesh from the top side point - the area between the dorsal fin and fat fin over the sideline (Figure 1) because in this region the fastest growing scales (Antoszek, 1999). The materials were used 1-3 scale on each individual. The weight of one scale was less than 1 mg.

DNA extraction

DNA was extracted from dried and frozen fish scales by Kumar et al. (2007) and Wasko et al. (2003) methods with our slight modification. One or two scales (1-2mg) were cut into small pieces and placed in 1.5 ml Eppendorf tube and homogenized in TNES-digestion buffer (10mM Tris-HCl, pH 8, 125mM NaCl, 10mM EDTA pH 8, 0.5% SDS, 4M urea) and 10 mkg ml^{-1} of Proteinase K was added. After then this homogenate was maintained 48°C for at least 40 min in Thermo-shaker. The contents in the tube were incubated at 48°C for 60 min in water bath. The DNA was then isolated by adding phenol: chloroform: isoamylalcohol (25:24:1) to the tubes. The tubes were rotated in Thermo-shaker 48°C for at least 40 min. Tube was then centrifuged for 10 min at 9.2 g. The top aqueous layer was transferred to a new Eppendorf tube leaving interphase and lower phase. The DNA was then precipitated by adding equal volume of isopropanol and 0.2 volumes of 10M ammonium acetate and precipitated -5°C overnight. The precipitated DNA was then pulled by centrifugation 13.5 g for 10 min. Further the DNA pellet was washed in 70% ethanol, air dried and suspended in an dd H_20. After completely solution the DNA was stored at -20°C.

DNA quantity and quality

The quantity, quality and suitability of the

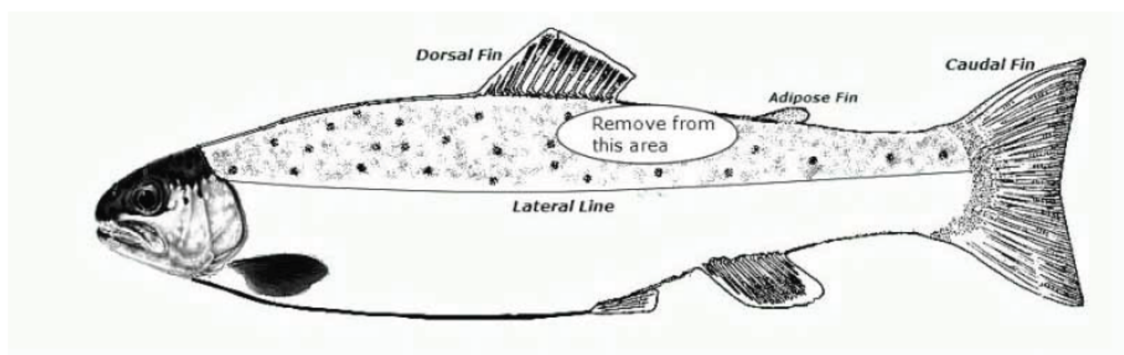

Figure 1. The place on fish, from which the scales were taken to DNA extraction (Manual …..Methodology, 2011).

isolated DNA samples for PCR were checked by using spectrophotometric measurements by Bio Spec-nano spectrometer. The ratio of the absorbance at 260 and 280 nm (A260/280>1.8) and A260/A230 were used to assess the purity of nucleic acids. The quality and suitability of the isolated DNA samples for PCR were checked on 2 % agarose gel stained with ethidium bromide.

PCR amplification

Polymerase chain reactions for amplification of DNA samples extracted from *Salmo trutta* scales as templates on PCR were carried out with four specific microsattelites primers Str151NRA, Str 731NRA, Str851 NRA, Str5431NRA after recommendation (Gross, 2009.)

The amplification was carried out in the Eppendorf Master Cycler-Pro and annealing temperature was optimized for each primers (Figure 2): the annealing temperature was 55ºC for primers Str15 and Str73, but annealing temperature was 58.5ºC for primers Ssa197 and Ssa85 PCR reaction contained 40-80 mg genomic DNA, PCR buffer (Fermentas), 20 mM MgCl 2, 0.2 mM dNTPs mix (Fermentas), 0.25 U Taq DNA Polymerase (Fermentas). PCR condition were initial denaturation 94ºC for 3 min, followed by 35 cycles of denaturation 94ºC for 40 s, annealing temperature for each primers 40 s and extension at 72ºC for 10 min. All amplification products were visualized by 10 % polyacrilamid gels (PAAG) (Figure 3) compared with a standard marker 100bp DNA Ladder (GeneRuler), (Fermentas).

RESULTS AND DISCUSSION

Modern studies of population genetics rely increasingly on DNA markers amplified by polymerase chain reaction (PCR) for detecting genetic variations within and among populations. Such information is useful for planning conservation strategies for great number of species. The isolation of high-quality DNA for genetic analysis has become one of the major concerns for DNA based techniques, especially when a large number of samples must be processed. In fish most of the DNA isolation is done from blood. But some Salmonidae fishes are protected species and the DNA isolation from blood is not desirable to extract blood. In such situation collection of scales in paper or plastic tube can serve the purpose of DNA extraction. Since the collection of few scales does not harm the fish.

Fish fins and scales are a reliable non-destructive source of DNA and these materials have been used to isolate DNA from some species Astorga et al. (2008), Many publications have described methods of DNA extraction from fish scales (Yue & Orban, 2001), but some methods can

Table 1.
The characteristic of used microsattelites primers.

Locus	Annealing temperature	Alleles	Size (bp)	Repeat	Primers sequences
Str15INRA	58ºC	10	193-225	CT	5’-TGCAGGCAGACGGATCAGGC-3 5’-AATCCTCTACGTAAGGGATTTGC-3’
Str73INRA	58ºC	11	138-162	GT	5’-CCTGGAGATCCTCCAGCAGGA-3’ 5’-CTATTCTGCTTGTAACTAGACCTA-3’
Str85INRA	55ºC	19	146-200	CT	5’-GGAAGGAAGGGAGAAAGGT-3’ 5’-GGAAAATCAATACTAACAA-3’
Str543INRA	55ºC	24	119-169	CT	5’-ATTCTTCGGCTTTCTCTTGC-3’ 5’-ATCTGGTCAGTTTCTTTATG-3’

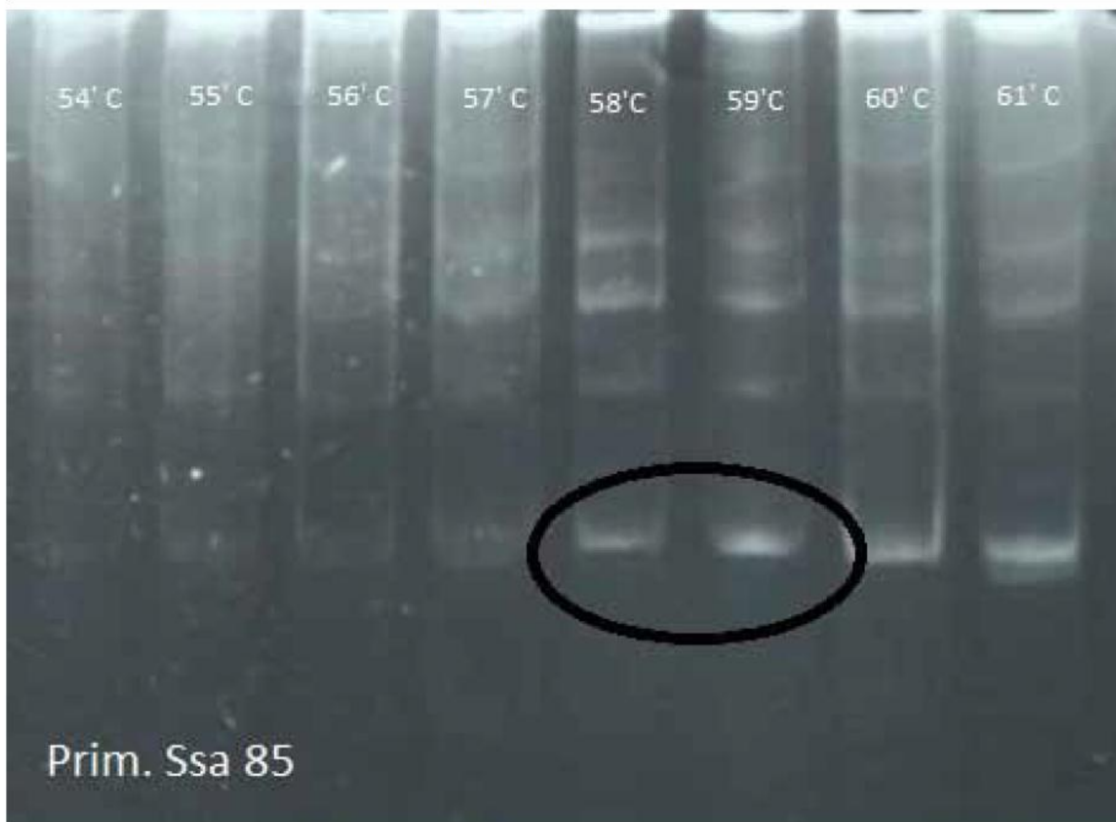

Figure 2. Electrophoretic profile of PCR amplification gradient product from 54°C – 61°C, temperature was optimized for primers Ssa197 and Ssa85 was 58.5°C.

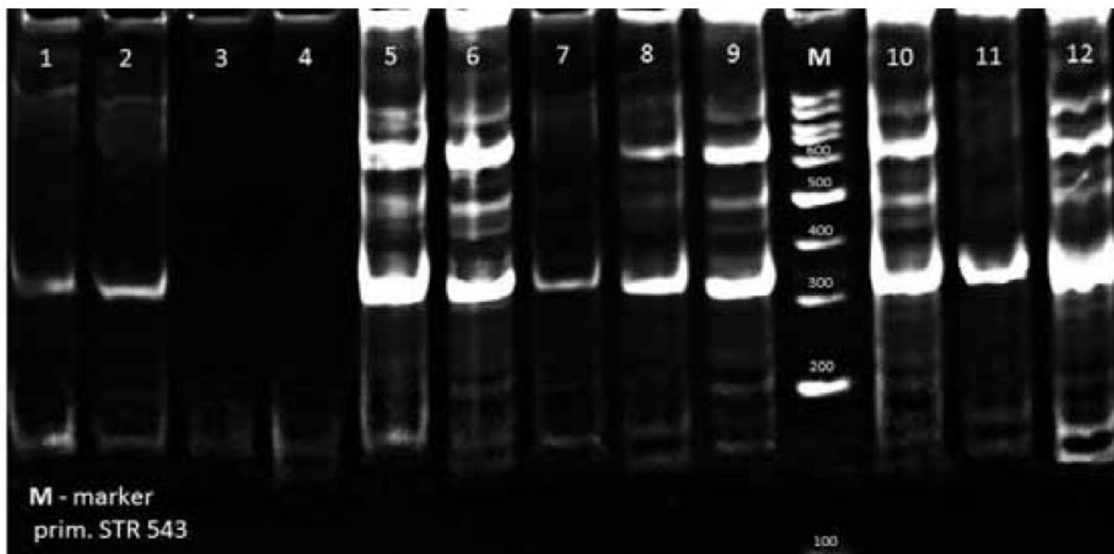

Figure 3. 10% polyacrylamide gel electrophoretic profile of specific microsatellite primers Str5431NRA of *Salmo trutta* PCR amplification. M- marker 100-bp ladder (Fermentas). 1-12 – *Salmo trutta* examles.

used for large number of individuals or large size scales, others methods can't used for PCR amplification using primers. There are some difficulties due to the consistency and small size of these tissues which can lead to a low amount and poor quality of total recovered DNA. There is a view, that the classical phenol-chloroform DNA extraction usually gives poor results. Initially therefore DNA extraction was a limit factor to the use of noninvasive sampling but this technical difficulty has been largely overcomes. The Chelex method has proved to be efficient for fresh scales. The silica methods used either published protocol or commercial kits (e.g. QIAampKit, QIA gene) is very effective. As stated by some authors (Pinto, 2011), tissue homogenization in liquid nitrogen can be an efficient method to isolate significant amounts of DNA, especially on hard consistent tissues. However, in others experiments the use of nitrogen maceration with fins and scales did not give any further improvement in the DNA isolation (Yue & Orban, 2001).

We are developed a simple and inexpensive method for dried and preserved fish scales of Salmonidae by combining steps of existing protocols with our slight modifications. In first step of our methodical approach we used the homogenization of scales with 4M urea buffer (Kumar, 2007). It was known that good results was achieved mixing the small pieces of the fins with a cell lysis solution containing urea. The initial 8M urea concentration of the buffer, suggested by Asahida (Bayani et al., 2009), was gradually decreased to 4M, which allowed a better preservation of the material and a non-degraded isolated DNA. Urea treatment seems to be a necessary step to breakdown hard tissues and at least it disrupts most likely any protein multicomplexes. We used the homogenization of one scales with silicon powder. This methodical approach increased the DNA amount from average 70 ng μl^{-1} to average 250 ng μl^{-1}. According with Wasko (Wasko et al., 2003) RNAse treatment was used. But we have a good result in DNA quantification without RNAse pretreatment according with Kumar (Kumar, 2007).

Some of protocols of DNA isolation from scales used the incubation of cells at 37°C overnight, which increased the duration technique for scale (Yue & Orban, 2001). We used not only incubation at 48°C according with Kumar method (Kumar, 2007), but also maintained in Thermo-shaker, after then the contents of the tube were incubated at 48°C for 60 min in water bath. This incubation temperature was found to be appropriate for quick digestion of scales, without compromising for DNA quality and quantity.

There is a view, that the classical phenol-chloroform DNA extraction usually gives poor results. But we successfully isolated DNA from dried scales by the traditional phenol-chloroform procedure. After scales digestion, a phenol-chloroform-isoamyl alcohol purification step was utilized as suggested by Taggart et al. (2005) and by Kumar (2007). The use of phenol-chloroform-isoamyl alcohol was found to be essential in obtaining pure DNA samples from fish scales.

The yield of DNA isolation from scales of different fish species (*Cirrhinus mrigala, Channa punctatus*, *Cyprinus carpio, Catla catla, Labeo bata* and others) is from 100 to 400 ng/mg of scales approximately (Kumar 2007). But *Salmonidae* scales size and weight is very small: for example, *Salmo trutta* scale size is 2-3 mm, bet weigh is 1-2 mg. However, the weight of isolated DNA from *Salmo trutta* was 100 to 300 ng mg^{-1} in our procedure. The isolated DNA no sign of degradation and spectrophotometer comparison of absorbance at A260/A280 nm provided a pure factor of 1.8-2.0 indicating its good quality. The DNA extracted with this method was very stable and could be stored at 4°C temperature for months together without any adverse effect on its concentration and its use for PCR. DNA samples isolated from scales by our method were successfully amplified by the *Salmo trutta* specific four microsatellite primers (Str151NRA, Str 731NRA, Str851 NRA,

Str543NRA) and provide reproducible results (Figure 3). The amount of DNA is enough to serve as template for a minimum 30 PCR reaction.

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