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Comparative analysis of Genomic DNA extraction protocols: Maxi-Preparation of Quality DNA for Genetic Evaluation

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Abstract: High yields of extracted DNA from tissues for a number of molecular research work are depended on acquiring tissues that should not highly degraded and must give a sufficient yield of DNA. Current experiments were performed on tissues using various methods, the DNA extraction can be done to get high quality and quantity of DNA. DNA was extracted from preserved tissues after specified intervals; quantification was performed using spectrophotometer and polymerase chain reaction amplifications through identification primers. Genomic DNA extraction method was used for good quantity and reproducibility for molecular markers studies. Phenol-chloroform method agreed clear, reproducible, and high quality of bands and the purity near was 1.7. For DNA isolation four DNA extraction methods were compared. The concentration, purity, and amplificability of DNA were tested. The results revealed the variability among extraction procedures in terms of DNA quality and quantity in *catla catla* muscle tissue processed under different processing technologies.

Keyword: *Catla catla*, Isolation, DNA, Methods

1. INTRODUCTION

Unwavering quality, plausibility and reproducibility of sub-atomic hereditary qualities studies are frequently restricted by the primer advance of DNA segregation. DNA segregation is an immediate, quick, capable organic path for in lessening the odds of pollution (Smith *et al.*, 2005). Finding the right DNA esteems that are of good quality are significant for hereditary material. Along these lines, the crucial and satisfactory convention must be painstakingly chosen. You have to utilize non-ruinous strategies for creature species (Wasko *et al.* 2003). DNA is accounted for to be the most suitable particle for the discovery and ID of fish species in handled sustenance items, offering various points of interest over the investigation of proteins (Chapela *et al.*, 2007). Proteins lose their organic movement soon after the fish has passed on, many are heat labile, and their quality and attributes rely upon the particular cell type being broke down (Cespedes *et al.*, 1999). DNA, then again, is available in all tissue types, has a more prominent steadiness at high temperatures, and the decent variety managed by the hereditary code permits separation of firmly related species (Pardo and Pérez Villarreal, 2004). Generally, DNA extraction conventions dependent on the expansion of natural solvents, for example, phenol and chloroform, have been much of the time used to confine genomic DNA from creature species (Lopera-Barrero *et al.*, 2008). Albeit such techniques produce adequate outcomes for tests of various roots, they are tedious and require the utilization of reagents that can artificially debase the extricated DNA, however which are likewise a wellbeing danger (Yue and Orban, 2001).The aim of this study was to identify the most feasible method for the extraction of high quantity and quality DNA from fish muscle tissue. With this objective, the efficiency of three published methods and two commercially available kits were compared for their ability to extract high yields of pure DNA suitable for PCR amplification.

2. MATERIALS AND METHODS

Fish samples

Specimens of freshwater fish species were obtained from river Chenab, Multan, Pakistan. Samples were identified morphologically by using taxonomist key on the basis of visible characteristics, their weight, lengths of dorsal and anal fin. Samples were stored at fisheries lab at -20 until further processing.

Tissue preparation

Muscle tissues samples were cut out from lateral part by removing skin at right side of specimen and were pounded by using sterile scissor or razor blades in order to done extraction process.

Method I: DNA isolation by modified Phenol chloroform extraction method:

Isolation of DNA takes place from the samples of different tissues usually caudal, pelvic, Pectoral, gills and Muscle were in used for the purpose of DNA

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extraction. Size of these numerous tissues were about 50 to 100 miligram with 600 to 800 µl buffer for extraction to homogenized the solution and instantly transferred into micro centrifuge tubes. Proteinase K was also used to add inside the tube it was mixed by vortex and then the tissue were kept for incubation at thirty seven degree for one hour and then fifty five degree for one hour. After that sample was used to centrifuge at 5000 rotation per minutes for ten minutes. Amass supernatant and then add it into the 25:24:1 ratio of phenol: chloro form: is amyl alcohol in tube and then mixed it into by repeated inversion for ten to fifteen minutes, further again centrifuge at 12000 rotation per minutes for 10 minutes. Amass supernatant and then add it into the 24:1 ratio of chloroform: isoamyl alcohol in tube, mix the solution through repeated inversion almost 15 min and again it become centrifuged at 12000 rotation per minute for 10 minutes, amass the upper layer and then add 10% 3 Molar sodium acetate and the same volume of 100% ethanol. Tubes were kept at -20 degree for 0ne to two hour and centrifuge at 1000 rpm for 10 minutes. Pellets of DNA been washed with 70% ethanol, kept it on air dry and resuspend it in appropriate volume of water in injection and then store it at -20 degree for more analysis.

Method II: High Salt protocol:

DNA was extracted by all water standards according to salts extraction method, which was originally to extract the DNA of the fish (Aljanabi & Martinez, 1997). Make water bath to swim up to 55 °C. Take a small amount of sample and pour it with scissor. Transfer samples to the 1.5mililiter micro eppendorf tube. 600 micro liters of TNES buffer solution and 35 micro liters of Protein Kinase. Mix the sample and inverted the tube several times. Make samples for overnight at 50 degree. You can increase protein K to speed up the exercise and reduce the duration of 2 to 4 hours. Add 6 molar of 166.7 of solution samples for 20 minutes. Move the samples at a reasonable speed (12 to 14000 rotations per minutes) for 5 to 10 minutes. 100 % ethanol added to wash it and gently mixes by diverting the tube several times you need to see the clump so does not mix up the solution. Centrifuge sample again 12-14,000 rotations per minutes for 10 to 20 minutes at four degree. Wash in the pallet of DNA with 200-700 µl of ethanol 100%, cover tube and ask for warmth, or gently mix the tube. Keep ethanol in a short time to keep the pellet dry the tube. Again wash DNA pellet with 70% ethanol above. After removing the ethanol 70% for a short time then remove the ethanol. Keep the sample for dry air for 10 to 30 minutes depending on the temperature. Once the sample become dry, place the DNA 100 to 200 µl of contaminated water or Tris-EDTA.

Method III: TNES extraction protocol:

Twenty milligram of dried tissue sample is used to extract the genomic DNA. Cut the small pieces of various tissues with the help of a delicious scissor. Dried crumbs on the filter paper and put in and homogenize it into 800 µl of buffer. Add 10µl of RNase inside the tube and kept it in forty two degree at one hour. After that 10µl of Protein Kinase was added, compactly combined with forty two degree for overnight. Then add 800 µl of 25:24:1 ratio of phenol: chloroform: isoamyl alcohol in tube, with a simple inversion tube and then used to centrifuge 10000 rotation per minutes for fifteen minutes. Add 1 molar sodium chloride and two volume of chilled ethanol in a tube and centrifuge it. Pallets clean by 70% ethanol, dried in the air with distilled water 60µl and stored it on -20 for further usage.

Method IV: UREA extraction protocol:

50 milligram of dried tissue sample is used to extract the genomic DNA. Cut the small pieces of various tissues with the help of a delicious scissor. Dried crumbs on the filter paper and put in 2mililiter of lysis buffer in 15 milliliter tube. Add 30µl of RNase inside the tube and kept it in forty two degree at one hour. After that 30µl of Protein Kinas was added, compactly combined with forty two degree for ten hours. Then add it into the 25:24:1 ratio of phenol: chloroform: isoamyl alcohol in tube, with a simple inversion tube and then used to centrifuge 13000 rotation per minutes for fifteen minutes. Add 1 molar sodium chloride and 2 to 3 volume of chilled ethanol in a tube. Put it on -20 for 1 to 2 hours and then again centrifuge it 13000 rotations per minutes for fifteen minutes. Pallets wash briefly by 70% ethanol, dried in the air and rebuilt with the correct sound of water with distilled water and stored it on -20 for further usage.

Statistical analysis

All figures were done in the form of R. (R: Language and environment for statistics. R Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Compared to the DNA methods extracted from all types of methods and methods of the DNA of devices, the analysis of the difference (ANOVA) was done. ANOVA was also used to compare DNA hygiene from all sorts of ways and different forms of DNA. It was assumed to be different in terms of 5% ($P < 0.05$). The Tukey fishermen's and dannett method was used when their partnership was important to explain what the various effects were. The distinction between the different stages of the DNA from the different stages was also compared to many different approaches, excluding different types between different types, and taking independent products.

3. RESULTS

In the course yield, DNA is implicit to be exposing by physical and chemical treatment or by the heat that mainly can influence the DNA quantity and quality, most probably by ensuing the disintegration of molecules of DNA. To prefer an optimum procedure of extraction, numerous factor that taken into description. DNA should restrain as slight as protein slightly, organic compound, RNA or other polymerase chain reaction inhibitors. Purity and concentration of DNA was determined through nanophotometer absorbance measuring of DNA $A_{260/A280}$ and $A_{260/A230}$ ratios.

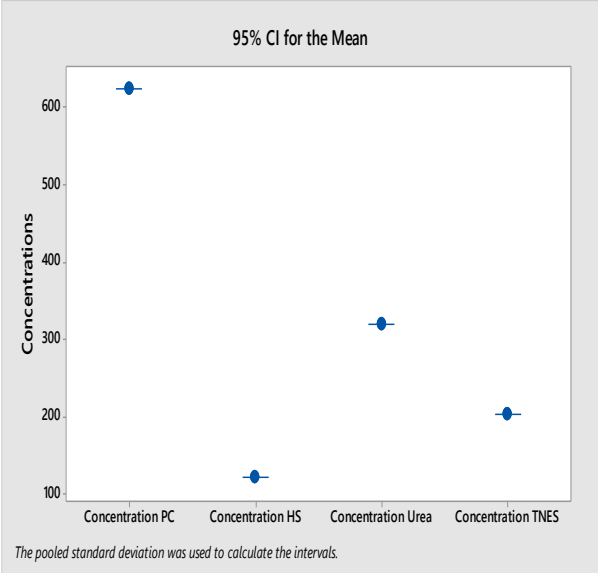
Based on the DNA fortitude and its concentration through spectrophotometer, it is also helpful to identify the differences in effectiveness by using different analysis methods. Generally, the highest concentration was detected in phenol chloroform method extraction method, followed by other studied methods. Lower amounts of DNA were observed in Urea and TNES method and concentration was found lowest in High salt samples. For determinination of DNA quality by calculation of the A260/280 and A230/260 ration of absorbance, the majority of the samples in the particular range of 1 to 1.7. Overall 23 samples were used for study.

Concentration of extracted DNA with different method

Concentrations of the extracted DNA were assessed by measuring the absorbance at by using the extracted DNA from phenol chloroform, Urea, High salt and TNES quality were founded rangers from 168-1089, 195-473, 4.5-332, and 122-302 respectively the mean and standard deviation of these method were found 622.86 + 292.482, 319 +118.832, 121.44 + 99.740 and 203 + 75.657 respectively.

In study DNA obtain from *Catla catla u*se the four extraction method of DNA. Analysis employ to identified the maximum DNA purity and yields differ significantly amongst the various method used in the study, showed a significantly interaction exist among different method for DNA extraction and among the individual fish specie.

Extraction of DNA using various methods, the Phenol chloroform method constantly extract the higher DNA yield from all numerous methods of species in fish. DNA extraction method need 50 to 100 mg of muscle then other method used differ amount. The higher DNA concentration and yields obtained with phenol chloroform extraction method .*Catla catla* DNA concentration and yield obtain with the Phenol chloroform method found significantly higher than other with Urea, High Salt and TNES method respectively as shown in figure .Comparison of *Catla catla* as per methods respectively according to DNA concentration shown in Table and figure. DNA concentration shows a significant concentration in all using methods comparatively in Phenol chloroform; High salt; Urea, TNES.



Method wise Comparison of concentration

Different Methods	**Mean**	*Range*
Phenol chloroform	622.86 + 292.482	168-1089
High Salt	121.44 + 99.740	4.5-332
Urea	319 +118.832	195-473
TNES	203 + 75.657	122-302

Mean values and range of DNA concentration by different extraction methods of catla catla
n = 23

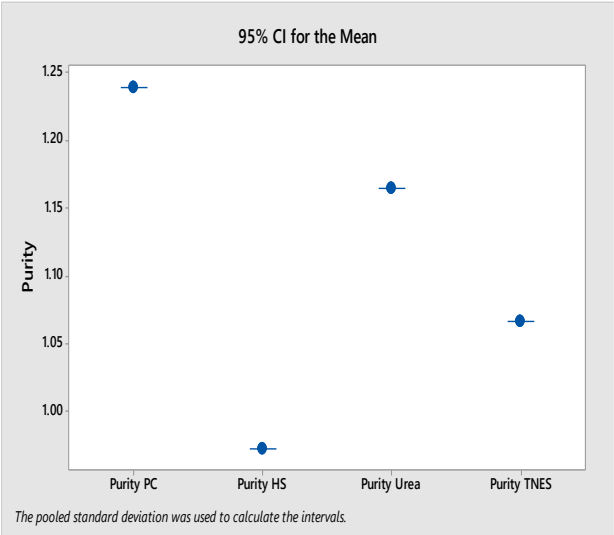
Quality of extracted DNA

$A_{260/280}$ quality and $A_{260/230}$ quantity of DNA extracted were asses through absorbance measuring at wavelengths of 260/280nm and 260/230nm by using the extracted DNA on nanophotometer. 260/230 ratio and the concentration of the extracted DNA for the phenol chloroform, High salt, urea and TNES quality were founded rangers from 0.661-1.667, 0.551-0.847, 0.898-1.211 and 0.776-1.011 respectively the mean and standard deviation of these method were found 1.157 + 0.251, 0.678 + 0.093, 1.060 + 0.105 and 0.892 + 0.096 respectively.260/280 ratio and the concentration of the extracted DNA for the phenol chloroform, Urea,

High salt and TNES quality were founded rangers from 0.989-1.311, 0.989-1.311, 0.421-1.317 and 0.811-1.211 respectively the mean and standard deviation of these method were found 1.239+0.261, 0.972+0.358, 1.164+1.119 and 1.066 + 1.175 respectively.

DNA purity obtain from *Catla catla u*se the four extraction method of DNA. Analysis employ to identified the maximum DNA purity and yields differ significantly amongst the various method used in the study, showed a significantly interaction exist among different method for DNA extraction and among the individual fish specie. Extraction of DNA using various methods, the Phenol chloroform method constantly extract the higher DNA yield from all numerous methods of species in fish. DNA extraction method need 50 to 100 mg of muscle then other method used differ amount. The higher DNA purity and yields obtained with phenol chloroform extraction method .*Catla catla* DNA purity and yield obtain with the Phenol chloroform method found significantly higher than other with Urea, High Salt and TNES method respectively as shown in figure .Comparison of Catla catla as per methods respectively according to DNA purity. DNA purity shows a significant purity in all using methods comparatively in Phenol chloroform; High salt; Urea, TNES.

Comparison of *Catla catla* as per methods respectively according to yields shown in Table and figure. DNA concentration shows a significant concentration in all using methods comparatively in Phenol chloroform; High salt; Urea, TNES.



Method wise Comparison of purity

Mean values and range of DNA purity (A280/260) by different extraction methods of catla catla

n = 23

Different Methods	**Mean**	***Range***
Phenol chloroform	1.239 + 0.261	0.521-1.701
High Salt	0.972+0.358	0.421-1.317
Urea	1.164+1.119	0.989-1.311
TNES	1.066 + 1.175	0.811-1.211

Mean values and range of DNA purity (A260/230) by different extraction methods of catla catla

n = 23

Different Methods	**Mean**	***Range***
Phenol chloroform	1.157 + 0.251	0.661-1.667
High Salt	0.678 + 0.093	0.551-0.847
Urea	1.060 + 0.105	0.898-1.211
TNES	0.892 + 0.096	0.776-1.011

Phenol chloroform

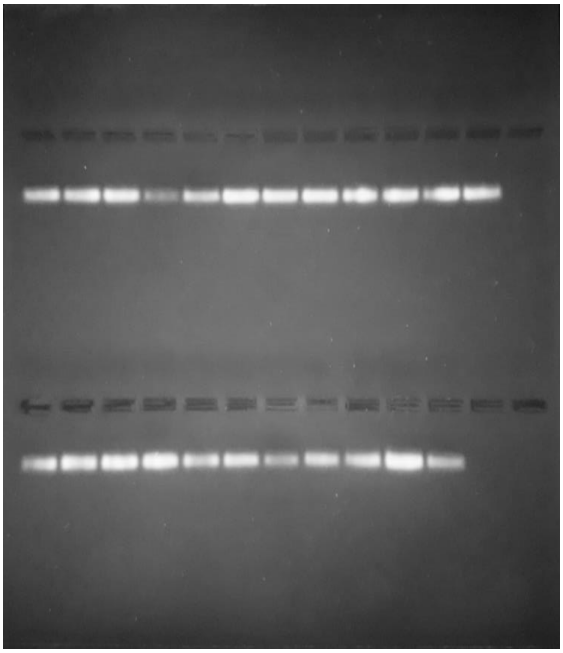


Fig. 1. Gel pic of sample 1-23 of Phenol chloroform method

High Salt method

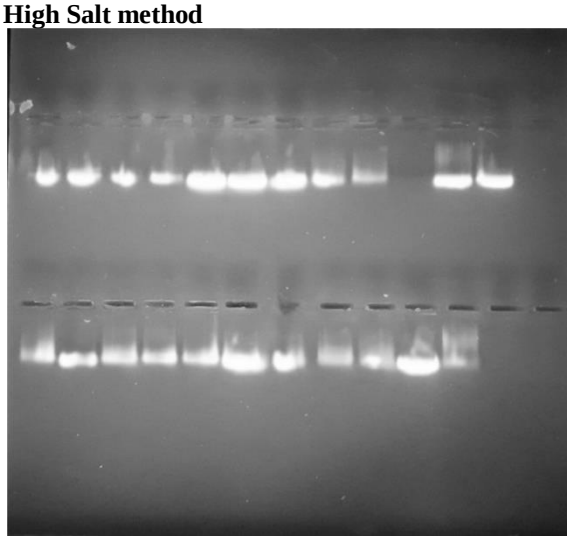


Fig.2. Gel pic of sample 1-23 of High salt method

TNES Method

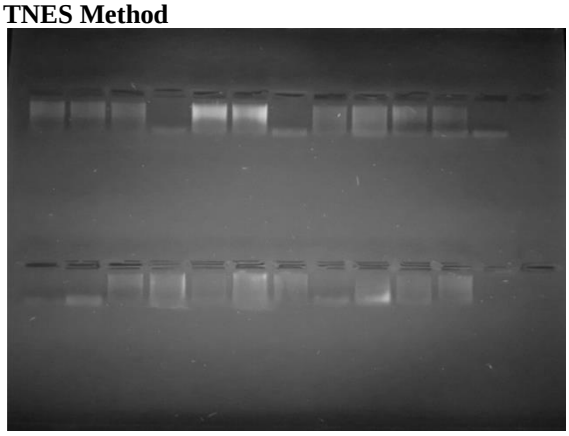


Fig. 3. Gel pic of sample 1-23 of TNES method

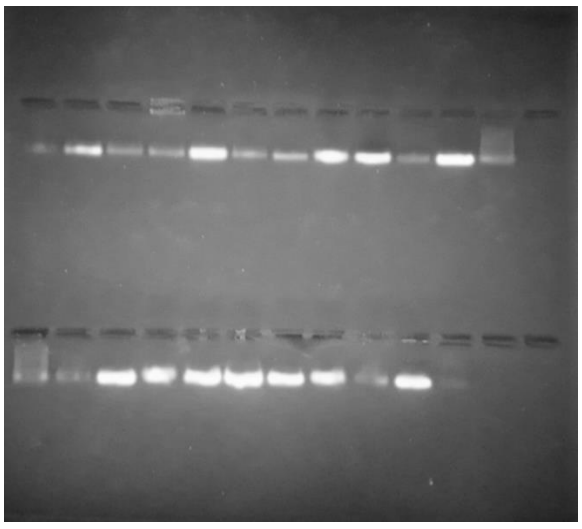


Fig.4 Gel pic of sample 1-23 of Urea method

4. DISCUSSION

It is clear that when DNA extraction methods were used, significant differences in DNA yield and concentration of individual species were found. Four methods for the extraction of DNA by using 50 to 100 milligram starting material the used of Phenol chloroform method delivered significantly higher yield of DNA than other methods. The accuracy of this method of extracting DNA depend on whether the process needs any technique that can provide the same product of DNA (generating high quality) or how to obtain high quality DNA products. (Biase *et al.,* 2002) have confirmation for DNA products derived from swine muscles.

The best way to extract, there are a number of factors that need to be carefully considered. DNA should have protein, RNA, ingredients of organic, or any other polymerase chain reaction inhibitors. DNA was determined to concentrate and clean up the product of DNA and check its A_{260} / A_{280} values. DNA considers to be purely satisfactory when the A_{260} / A_{280} standards ranges were within 1.7. The contamination of DNA and protein often reduces the quality and quantity values as shown by the A_{260} / A_{280} less than 1.7 (Cawthorn *et al.*, 2011). A recent study reveals that the concept of DNA and its purity Phenol chloroform is found in consistent and general agreement with the study. The purity of DNA is derived from a variety of methods, which was estimated at a fraction of A_{260} / A_{280}. In the case of DNA, statistics show that there is a big difference between the different ways of doing it. The DNA was considered satisfactory when the average A_{260} / A_{280} ratio was within 1.6 to 2.0.

To recognize several fact of different starting material amounts of DNA to extract by different ways, an attempt was made to examine DNA extraction using the same tissue for DNA. The phenol chloroform method providing great products when done in the protocol was repeated by 50-100 mg muscles as the first thing. This was to allow direct comparison with other methods. The products of DNA extracted from all methods was used. These results show that the Phenol chloroform method is suitable to provide more feasible DNA products than other methods available. In general, the highest frequency is found in Phenol chloroform, followed by Urea. The lower DNA was observed by the TNES and the lowest yields were obtained with high salt content. If there is a value of DNA by measuring the parts of the $A_{260/280}$, most of the samples were in a 1-1.7. According to the criteria listed in the list, the Phenol chloroform method. As can be seen it showed a positive polymerase chain reaction analysis (Ram *et al.*, 1996). This study has found a comprehensive agreement with our study as these are the ones that provide the

cleanliness for high quality DNA. This study found general agreement with the study as these are the influence the concentration and purity for quality DNA.

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