Evaluation of two MiniSTR loci mutation events in five Father-Mother-Child trios of Yoruba origin

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Abstract
The robustness of short tandem repeats for use in forensic and paternity depends on their high polymorphism and mutation rate. This study tried to determine the event of mutation of two miniSTR loci in the Yoruba population. Blood samples were collected from five father-mother-child trios of Yoruba origin. Two DNA extraction methods, an homemade method and Zymogen gDNA kit were tested for yield and purity for use in the STR assay. The DNA were amplified and resolved on 4% Agarose gel. The first DNA extraction method yielded an average DNA concentration of 1399 ng/µl and while the Kit yielded 984.1 ng/µl; absorbence quotient at 260/280 of 1.78 and 1.55 respectively. Locus D1GATA113 was detected in the father and mother of two families; A and C. D5S2500 was detected only in the male parent (father) in family D. DNA extracted using any of the two methods in this study is appropriate for use in STR mutation assay but the PCR condition for mutation miniSTR loci among the yoruba still requires extensive optimization.

Keywords: DNA extraction Methods, miniSTRs, mutation, Yoruba
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Introduction
Genetic identity testing involves identifying patterns of genetic material that are unique to almost every individual. Short tandem repeats (STRs), which according to Schoske et al., (2003) are repeating DNA sequences which are approximately 2-6 base pairs in length; are the most commonly used loci for human identification simply because of their high polymorphism, requirement of minimal template DNA and possession of a narrow size range that makes multiplexing easy (Butler, 2005; Opel et al., 2006).

Most STRs are found in the noncoding regions, while only about 8% locate in the coding regions (Ellengren, 2000). About 90% of STR existing in non-coding regions of the genome have prompted the question of what could be the function of these frequently repeating sequences. Over the last decades, several potential functions of some STR in the regulation of transcription of genes like the epidermal growth factor gene (Gebhardt, et al 1999), P-53 Inducible Gene-3 (Contente et al., 2002) and the regulation of gene expression (Heale and Pete, 1995), have been reported. The mutation rates in STR sequences are several orders of magnitude higher ranging from $10^{-8}$ – $10^{-7}$ nucleotides per generation in human (Fan and Chu, 2007), than in 'regular' DNA sequences in the genome (Ellengren, 2000). The chances of detecting germline genomic mutations have also increased with the use of STR markers due to their higher mutation rate when compared to other genetic markers. STR systems are nowadays the most commonly used systems in human identity and paternity cases. MiniSTRs are now been widely employed due to the limitations of STRs to produce full genetic profiles in degraded DNA samples in forensic and paternity case. MiniSTRs improve the chance of obtaining STR results
from samples with compromised DNA. This involves the redesigning of the primer binding sites of some STR loci to produce shorter amplicons (Gill et al., 2006). The miniSTR primer sets produce full genetic profiles in the majority of the samples containing degraded DNA (Butler, 2005); and have the potential to provide additional discrimination in complex paternity cases or missing persons cases (Goodwin et al., 2004).

There has been no data on miniSTR analysis on Nigerian populations most especially the Yorubas. This study tends to fill literature gap and provide foundational data on miniSTR analysis in Yoruba population of Nigeria, by evaluating the presence of two miniSTR loci and the event of mutation on the Yoruba population.

Materials and Method

For the second method using Genomic DNA extraction kit (Zymogen), the extraction was done with 100ul of each blood sample following the manufacturer’s instructions. The kit contained ZymoSpin IIC™ column, Genomic lysis buffer, pre DNA wash buffer, gDNA wash buffer and DNA elution buffer.

Spectrophotometric assay of DNA was performed to compare the density and concentration of DNA in the samples from the two methods of extraction using a Cary 60 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Absorbance was measured at wavelengths of 260 and 280 (A260 and A280 respectively) nm.

The integrity of genomic DNA of the two extraction methods were tested by resolving DNA extracts on a 1% agarose gel by electrophoresis (Bio-Rad, Hercules, CA, USA) ran at 70 volts for 45 minutes.

Table 1: MiniSTR Loci Primer Sequences.

<table>
<thead>
<tr>
<th>Locus Name</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1GATA113</td>
<td>F - TCTTAGCCTAGATAGATACCTTGTTCCC</td>
</tr>
<tr>
<td></td>
<td>R - GTCAACCTTTTGAGGCTATAGGAA</td>
</tr>
<tr>
<td>DSS2500</td>
<td>F - CTGGTGATACATAATAGGTAGGTAGG</td>
</tr>
<tr>
<td></td>
<td>R - GTCGTGGCCTACATAATATC</td>
</tr>
</tbody>
</table>

Hill et al. (2008)
System 9700 (Applied Biosystems) in a total reaction of 20µl containing 5x reaction master mix (Solis BioDyne), genomic DNA (2µl) and 2µl of each forward and reverse primer. The primers were run in singleplexes, using a pre-heating condition of 95°C for 10 mins, followed by 28 cycles of 45 secs denaturation at 94°C, 2 minutes annealation for each of the two primers D1GATA113 and D5S2500 at 60.25°C and 60.35°C respectively.

The PCR products were resolved in 4% agarose gel at 70 volts for 45 minutes. Twenty base pair (20bp) DNA ladder was loaded as DNA size marker to monitor the bands sizes.

**Results**

The Iranpur and Esmailizadeh (2014) extraction method yielded an average DNA concentration of 1399 ng/µl, while the Zymogen DNA Kit yielded 984.1 ng/µl yield (Table 3). The absorbance quotient at 260/280 of the Iranpur and Esmailizadeh (2014) is 1.787? while the kit produced an average of 1.547?.

**Table 2**: DNA concentration summary in ng/ul.

<table>
<thead>
<tr>
<th>DNA Extraction methods</th>
<th>N</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iranpur and Esmailizadeh</td>
<td>15</td>
<td>20984</td>
<td>1399</td>
<td>209.0</td>
</tr>
<tr>
<td>Zymogen DNA Kit</td>
<td>15</td>
<td>14762</td>
<td>984.1</td>
<td>156.3</td>
</tr>
</tbody>
</table>

**Table 3**: Summary of Absorbance quotients at 260/280 in ?

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iranpur and Esmailizadeh</td>
<td>15</td>
<td>26.800</td>
<td>1.787</td>
<td>0.09605</td>
</tr>
<tr>
<td>Zymogen DNA Kit</td>
<td>15</td>
<td>23.206</td>
<td>1.587</td>
<td>0.1536</td>
</tr>
</tbody>
</table>

Smear - which indicates DNA fragmentation

Fig 1: A Gel representing DNA concentration of samples extracted using Kit in agarose gel electrophoresis.
The result of the PCR amplification after running in 4% agarose detected loci D1GATA113 (Fig 3) in two families though not up to a trio set in each family and loci D5S2500 was detected in one family (Fig 4).

The few amplicons produced also couldn’t separate very well for us to see the alleles of the band and this improper separation is the same of the ladder marker that was used in the electrophoresis (fig 4).

Absorbance quotient value ranging from 1.8 - 2.0?, is indicative of good and purified DNA (Ghatak et al. 2013). This implies that Iranpur and Esmailizadeh extraction method yielded the purest DNA of the two methods. Though, on the overall, the amplicon frequency was low in both methods, which probably means the methods have nothing to do it the frequency of amplicons recorded. Another observation of interest that featured in our result is the presence of smear in DNA fingerprinting has been very helpful in forensic identification especially the use of miniSTR. However, the marker to use must first be validated for its polymorphism within the population. The DNA concentration average value of 1399 ng/ml and 984.1ng/ml obtained from the two extraction methods for this study cannot be said to be too low for STR mutation analysis. Though, the amount of DNA obtained using the Iranpur and Esmailizadeh’s (2014) method is higher than the yields from the extraction kit, which may be as a result of the difference in the amount of blood used to get a 50 ul of dissolved DNA. The Iranpur and Esmailizadeh, method requires 500 ul of blood, while the extraction kit used up 100 ul of blood in each sample to give 5ul of DNA in solution. STR was specifically designed because many samples recovered from crime scenes yield only few nanogram or picogram amounts of DNA that is sometimes degraded (Edwards, et al., 1992). Based on amount of DNA, both methods are still proficient for use in STR mutation assays. However, purity of DNA is of greater concern as contamination of samples (Lorenz, 2012) by RNA, protein(s), enzymes like nuclease(s) may yield poor amplification.

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Discussion
DNA fingerprinting has been very helpful in forensic identification especially the use of miniSTR. However, the marker to use must first be validated for its polymorphism within the population. The DNA concentration average value of 1399 ng/ml and 984.1ng/ml obtained from the two extraction methods for this study cannot be said to be too low for STR mutation analysis. Though, the amount of DNA obtained using the Iranpur and Esmailizadeh’s (2014) method is higher than the yields from the extraction kit, which may be as a result of the difference in the amount of blood used to get a 50 ul of dissolved DNA. The Iranpur and Esmailizadeh,
agarose gel. The improper separation of the 20 bp ladder used as rule marker confirmed this. This result contradicts White and Kuskawa, (1997) reports that agarose gel is sufficient for analysis of samples for several commonly used STR loci. This probably can also be because, the miniSTR are rather shorter compared to regular STR sizes. The relative ‘ease’ or ‘difficulty of electrophoretic discrimination between different alleles is determined by the size of the repeating units (White and Kuskawa, 1997). Many research studies on STR in the recent years have been run on polyacrylamide gel (Hill, et al. 2008; Iranpur and Esmailizadeh, 2014). Since the alleles didn’t come up probably because inappropriate PCR conditions and improper separation of the agarose gel used, the allele bands could not be scored.

This study reveals that DNA extracted using any of the two methods in this study is appropriate for use in STR mutation assay. However, the PCR condition for detecting the type and frequency of mutations of miniSTR loci among the Yoruba population still requires extensive optimization. Most importantly, this is the first study to detect miniSTR loci in a Yoruba population.

References


