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# Genetic variations among three major ethnic groups in Nigeria using RAPD

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## ABSTRACT

Genetically, every individual is unique; this may stem from inheritance, geographical locations, and/or environmental interactions. This study examined the possibility of developing a cheap and easy-to-use marker that can distinguish among the three ethnic groups in Nigeria using RAPD-PCR. Five RAPD primers, OPA1-3 and OPC1-2, were randomly selected and used to amplify DNA samples isolated from blood of eighteen human subjects representing the three major ethnic groups in Nigeria (six subjects each). Genomic DNAs were extracted using DNA isolation kit, RAPD-PCR amplification was performed and gel electrophoresis was done. Genetic similarity between the band polymorphism was evaluated as frequencies of occurrence and the phylogenetic tree constructed. Three of the five primers show various polymorphisms; the highest frequency band for primer OPA1 is 50% while that of primer OPA2 is 100% and for OPC2 is 83.33%. Although OPA2 has common bands in majority of the samples few of the bands are ethnic group specific. Bands 471 and 435 bp are specific for the Hausa ethnic group at 66.67% frequency. Similarly, in primer OPC2, band 320 can be used to distinguish the Hausas from the other two ethnic groups. Analysis of variance (ANOVA) and test for homogeneity showed that there is no significant difference in the polymorphism between and among the groups. In conclusion this research has given an insight into the possibility of developing RAPD primers that could be used to distinguish people of different ethnic groups.

**Keywords:** Nigerian ethnic; RAPD-PCR; Genetic Variation; PyElph; Phylogeny

## INTRODUCTION

Prior to advent of genetics, morphological and biochemical markers have been used to differentiate between and among species. Studies have shown that these types of differentiation are prone to errors [1, 2]. Molecular taxonomy has changed the ways organisms were classified, as many organisms, particularly the microorganism, that were put into wrong taxonomic groups

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using convectional morphological and biochemical attributes are now being correctly classified using molecular techniques [3]. In human, tracing the ethnic origin using morphological characteristics has also not provided reliable results, thus the importance of the use of genetic markers.

Molecular markers offer a lot of advantages over conventional, phenotype-based alternatives as they are stable and detectable in all tissues. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA [4]. Such DNA markers are of diverse types and are currently being used to investigate genetic variations. Prominent among these markers are microsatellites, also referred to as single sequence repeats (SSR), restriction fragment length polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD).

RAPD is a DNA fingerprinting technique based on polymerase chain reaction (PCR). In this technique, random fragments of genomic DNA are amplified with single short primers of arbitrary nucleotide sequences [4, 5]. The RAPD technique has been used to analyze the genetic diversity in various organisms: to discriminate between strains of different bacterial species [6, 7], molecular characterization of plant species [8], and in estimation of genetic diversity in various endangered plant species [9, 10]. In addition, RAPD technique has been used to successfully discriminate among several plant species of medicinal value from Saudi Arabian desert [8] and to investigate the genetic diversity in Nigerian bringal eggplant, *Solonum melongenal* [11]. Generally, characterization of organisms through identification and determination of random polymorphic markers have proved to be a powerful application of DNA technology [12]. Its use in human ethnic classification has however not been reported.

Nigeria, a federal republic country in West Africa, bordered in the west by Benin, in the east by Chad and Cameroon and in the north by Niger. It is a multinational state with over 500 ethnic groups of which Hausa, lgbo and Yoruba are the three main ethnic groups. It is hypothesized that RAPD-PCR analysis could be useful in identifying differences in the genetic makeup of individuals from the three major ethnic groups in Nigeria. Since RAPD–PCR detects sequence diversity of total DNA there is a high probability that the technique will display a good degree of sequence disparity among different ethnic groups. Moreover, the knowledge of the DNA sequence in the target individual is not required. It is also expected that RAPD-PCR will provide a cheap and fast means of distinguishing ethnic groups within a nation in the near future.

Previous work on blood groups showed that it can only be used independently to identify an individual [13]. Palmar and digital dermatogylphic of the three ethnic groups in Nigeria has been done, even though it showed differences, there were a lot of similarities among the ethnic groups [14], hence the need for molecular characterization.

In addition, with the presence of over 2 million internally-displaced persons in Nigeria, most of which are children, developing a cheap and fast means of identifying ethnic origin of such people may help trace the origin and then the family of such children. Reports on molecular characterization of ethnics are scarce, to the best of our knowledge, there is no report on the molecular characterization of the ethnics using RAPD-PCR. The result of this study will provide a template from which cheap and fast molecular basis of differentiating the three major ethnic groups in Nigeria could emerge, thus it can be used to trace the origin of displaced people and, if expounded, in the enforcement of immigration laws. Furthermore, the study can find applications in forensic identification of corpse and crime detection. However, the basic aim of this study is to investigate the possibility of using RAPD marker to differentiate between the three different ethnic groups in Nigeria.

## MATERIALS AND METHODS

**Materials:** Genomic DNA isolation kits were purchased from Norgen Biotek Corporation, (Thorold, Canada). Agarose from Cleaver Scientific Limited, Quickload 100bp DNA ladder from New England Biolabs Inc., PCR Master-mix with standard buffer (Quick-Ld2X) containing Taq polymerase was also from New England Biolabs Inc. and the oligonucleotide primers were from Inqaba, South Africa. All other chemicals were of molecular biology grade.

**Sample Collection:** Human blood samples were collected from eighteen donors; six from each of the common tribes. Five millimeters of blood were obtained from each donor, placed in tubes containing anti-coagulant (EDTA) and stored at -20°C prior to DNA extraction.

**Extraction of Genomic DNA and RAPD-PCR Amplification:** Genomic DNA was extracted from the blood samples following the instructions from the Genomic DNA Isolation Kit, obtained from Norgen Biotek Corporation (Thorold, Canada). Agarose gel electrophoresis was used to determine the purity and integrity of the DNA. The 5 RAPD primers from eurofins RAPD 10mers kits were used in this study. All primers used were re-suspended in nuclease free water to make 100 µM working solutions and stored at -20°C while not in use. The DNA sequences of the RAPD primers are in Table 1.

**Table 1:** Primers used for the RAPD analysis, their sequences and the amplification patterns

| Primers | Annealing Temperature | Sequence 5'-3' | Amplification pattern |
|---|---|---|---|
| OPA1 | 43.6 | CAGGCCCTTC | Polymorphic |
| OPA2 | 43.6 | TGCCGAGCTG | Polymorphic |
| OPA3 | 39.5 | AGTCAGCCAC | No amplification |
| OPC1 | 39.5 | TTCGAGCCAG | No amplification |
| OPC2 | 43.6 | GTGAGGCGTC | Polymorphic |

The RAPD mixtures were processed in 25 µl reactions containing 0.2 mM of each dNTP, 0.3 µM primer, 25 U/ml of Taq DNA polymerase (Biolabs), 10 x buffer (supplied with the enzyme; 20 mM Tris-HCl (with pH8.9), 1.8 mM $MgCl_2$, 22 mM KCl, pH 8.9), and 1-2ul of genomic DNA. Amplification was performed in a thermocycler (2720 Thermal Cycler, Applied Biosystems, Waltham, USA) using 1 cycle at 94°C for 5 min, followed by 35 cycles at 94°C for 1 min, 43.6/39.5°C for 1 min, 72°C for 2 min, and final extension at 72°C for 10 min. A negative control (containing water and no DNA) was included to detect contamination.

Whole genome purity and integrity were done in 1% agarose gels while the amplified products were analyzed on 2% agarose gels run in 1 × TBE buffer stained with either Gel Red® or ethidium bromide. All gels were visualized under UV transilluminator (Cleaver Scientific, UK) and photographed with AlphaImager Gel Documentation System, (Cell Biosciences, California, USA). A 1 kb DNA ladder was used as a molecular marker for whole genome while 100 bp (New England Biolabs, UK) was used for the amplified products.

**Data Analysis:** Genetic similarity between the band polymorphism was examined using AlphaView® and evaluated through multiple variations, frequency and regression analysis (IBM SPSS Statistics version 20); genetic distances were calculated and phylogenetic tree was constructed using PyElph [15].

**Ethical Clearance:** Ethical clearance for this research study was obtained from the Nigerian Institute of Medical Research Institutional Review Board (NIMR-IRB) Yaba, Lagos.

## RESULTS

Eighteen samples, six each of Igbo, Yoruba and Hausa, were screened using RAPD-PCR in the search for ethnic distinguishing markers in human samples living in Lagos Nigeria. This preliminary study investigates the possibility of RAPD markers to distinguish among the three common ethnic groups in Nigeria. The purity and integrity of the extracted DNA was verified using electrophoresis. Samples with clear and distinct bands were used for RAPD-PCR amplification. Table 1 lists the 5 primers used in this research, their sequences, and the amplification products. No bands were observed after PCR for OPA3 and OPC1 Primers; the remaining primers however gave polymorphic bands for most of the samples.

The polymorphic profile of the RAPD amplification of human blood samples of the three ethnic groups using OPA1 is shown in figure 1A. The bands appear with different frequency, of which bands 389, 218, 179 and 137 bp show the highest frequency of 50% for Igbo. The same frequency was recorded for Hausa by bands 331, 256 and 142 bp. None of the bands can attain the same frequency with Yoruba ethnic group.

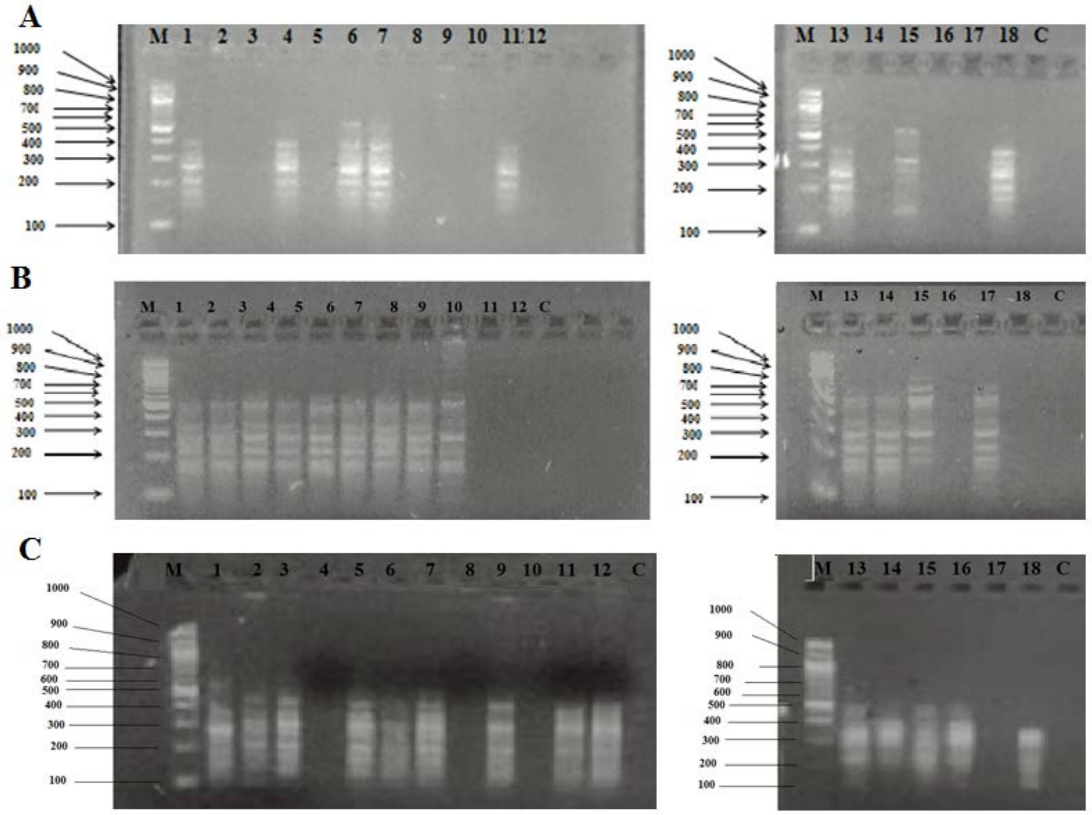


**Figure 1:** RAPD amplification products generated by primer OPA1 (A), OPA2 (B), and OPC2 (C) resolved by electrophoresis in 2% agarose gel, M: 100 bp ladder; lanes 1-6 DNA samples from Igbo; lanes 7-12 DNA samples from Yoruba; lanes 13-18 DNA samples from Hausa and C, negative control.

In figure 1B, the amplification pattern produced by primer OPA2 showed a high but non-significant frequency of 100% among Igbo, with five bands recording the maximum frequency: bands 519, 347, 280, 220, and 175 bp. Both Yoruba and Hausa tribes recorded 66.67% for these bands except the 347 band which is 50% for Hausa and 519 which is completely absent in the same tribe. Bands 471 and 435 bp are however unique to the Hausa ethnic group but at 66.67% frequency. The RAPD amplification products generated by primer OPC2 are presented in figure 1C. It shows a unique fingerprint pattern for the Hausa ethnic group, with 66.67% frequency for

band 320 which is found only in the tribe. The bands with the highest frequencies are bands 283 bp and 205 bp each, with 83.33 % for Igbo and Hausa respectively.

The results of the analysis of variance (ANOVA), comparing OPA1 bands with the ethnic groups, showed f values of 1.686, 1.102 and 1.111 and p-values of 0.117, 0.379 and 0.372 for Igbo, Hausa and Yoruba, respectively. Similar results obtained for the test for homogeneity using Turkey post hoc, p values 0.623, 0.656 and 0.429 for Igbo, Hausa and Yoruba respectively. The result of the phylogenetic tree using OPA2 is presented in figure 2. It showed some level of homogeinity among the Hausas while the Yoruba appears to be dispersed through the tree. that using the entire RAPD bands will not be discriminative enough to distinguish the three ethnic groups. In general, polymorphic bands produced by OPA1, OPA2 and OPC2 primers have different frequencies among the three ethnic groups (Table 2).

**Table 2:** Frequency of polymorphic groups in the three ethnic groups in Nigeria using OPA1, OPA2 and OPC2 RAPD primers

| Primer | Polymorphic band size (bp) | Frequency of polymorphic bands (100%) | | |
|---|---|---|---|---|
| | | Igbo | Yoruba | Hausa |
| **OPA1** | 530 | 0.00 | 0.00 | 16.67 |
| | 400 | 16.67 | 16.67 | 16.67 |
| | 389 | 50.00 | 0.00 | 33.33 |
| | 331 | 33.33 | 33.33 | 50.00 |
| | 282 | 50.00 | 33.33 | 0.00 |
| | 256 | 0.00 | 0.00 | 50.00 |
| | 218 | 50.00 | 33.33 | 33.33 |
| | 179 | 50.00 | 33.33 | 33.33 |
| | 142 | 0.00 | 0.00 | 50.00 |
| | 137 | 50.00 | 16.67 | 0.00 |
| **OPA2** | 572 | 0.00 | 0.00 | 16.67 |
| | 519 | 100.00 | 66.67 | 0.00 |
| | 471 | 0.00 | 0.00 | 66.67 |
| | 435 | 0.00 | 0.00 | 66.67 |
| | 347 | 100.00 | 66.67 | 50.00 |
| | 280 | 100.00 | 66.67 | 66.67 |
| | 220 | 100.00 | 66.67 | 66.67 |
| | 175 | 100.00 | 66.67 | 66.67 |
| | 155 | 0.00 | 0.00 | 50.00 |
| **OPC2** | 522 | 16.67 | 0.00 | 0.00 |
| | 474 | 0.00 | 0.00 | 33.33 |
| | 442 | 66.67 | 50.00 | 0.00 |
| | 366 | 66.67 | 50.00 | 33.33 |
| | 320 | 0.00 | 0.00 | 66.67 |
| | 283 | 83.33 | 66.67 | 0.00 |
| | 205 | 66.67 | 16.67 | 83.33 |
| | 155 | 83.33 | 33.33 | 0.00 |
| | 129 | 50.00 | 66.67 | 0.00 |

## DISCUSSION

Often, it is important to trace the accessorial link of the people living in a country. In periods of war and natural disasters, most people, particularly the children are displaced and some even dead. In other instances, immigrants may throw away their passport with views of claiming their host countries. Identifying these displaced persons or immigrants, who are playing pranks, often poses some challenges. The use of genetic variation thus became an invaluable tool. While many methods tried to differentiate tribes and trace people's origin using expensive and high-flown techniques, this study investigated the possibility of using cheap and easy method of RAPD-PCR to distinguish the three common tribes in Nigeria.

The RAPD-PCR analysis has been used in various studies such as in plant characterization, phylogenetic analysis of HIV patients and in typifying various diseases [12, 16]. Several of such studies showed DNA polymorphisms in human genomic DNA. Similar Polymorphisms

were seen when human genome were amplified using OPA1, OPA2 and OPC2 primers. Sharma *et al*. [12] has reported that primers of high polymorphic information content are useful for estimating relationship between genotypes. The primer ability to show polymorphisms in comparison to polymorphisms shown by all primers is known as primer discriminator power [17].

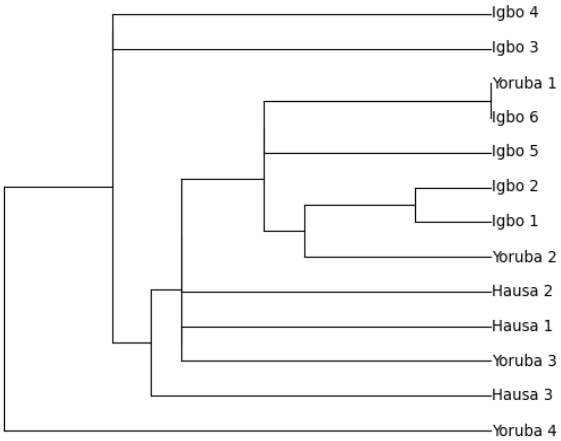


**Figure 2:** Phylogenetic Clustering of the three ethnic groups RAPD profiles produced by primer OPA2 using PyElph.

Although OPA2 produced the bands with the highest frequency of 100% for Igbo, it is important to note that the same set of bands have 66.64% for Yoruba and Hausa at various points. The fact that some Hausa and Yoruba samples were not amplified by the primers further suggested that the primer may not be good enough for distinguishing Igbo ethnic group as it may be presumed from the result. The primer may however found use in distinguishing human from other organisms. Further studies must be carried out to establish this.

The presence of bands such as 283 and 115 bp in the polymorphic profile of Igbo at 83.33% showed that OPC2 primer could be an important tool in distinguishing Igbo from Hausa, in which all samples lack the 115 bp band. The same primer can be used to distinguish Hausa, since the 320 bp band occurs only in Hausa ethnic group at the frequency of 66.67%. In fact, the finger printing pattern of Hausa using OPC2 is a great variant to those of Igbo and Yoruba.

Although there is no statistically significant variation among the ethnic groups with regards to the specific primers, there are however specific bands that are unique to specific tribe and such could be used in characterizing the group, for instance by developing Sequenced Characterized Amplified Region Marker (SCAR). Ismaeel [18] has reported the possibility of using such bands in diagnosis of tumors. The non-statistical significant variation may be as a result of non- amplification of some samples cum the small primer and sample numbers used in the study. In addition, the ethnic groups in Nigeria are occasionally fusions created by intermarriage, intermingling and, or assimilation. In such fusions the groups of which they are composed maintain a limited individual identity. The some-worth homogeneity observed in Hausas might also being associated with the reduced intermarriage with other tribes. The statistical values of the study could be improved on if non- amplified samples are excluded, which may require large sample size. Bryc *et al*. [19] has reported the use of 568 samples in the population studies of African-American.

The RAPD primers OPA2 and OPC2 produced two DNA fragments (155 and 283 bp) in genomic DNA of Samples of Igbo indigene, which can distinguish Igbo from Hausa. The same primer also produced a 320 bp band that is specific for Hausa and thus can be used to distinguish Hausa from Yoruba. Thus, the sequence of these amplified DNA fragments might be

useful in the development of Sequenced Characterized Amplified Region Marker (SCAR) for distinguishing among common ethnic groups in Nigeria. This preliminary study gives an insight into the possibility of developing a cheap molecular marker for ethnicity detection, immigration control and forensic science as a whole. However, it is important to report here that the study was limited by sample size; mainly because getting willing subjects from the northern part of the country was not easy and in addition to lack of fund. We hope to conduct more research works in the nearest future study.

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**Conflict of Interest:** No conflicts of interest exist.

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