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Concordance Study between the AmpF*l*STR® SGM Plus™ and PowerPlex® 16 System Human Identification Kits in Bangladeshi Population

Shafiul Alam[2], Ahmad Ferdous[2], Mohammad Eunus Ali[2], Tania Hossain[2], Mahamud Hasan[2] and Sharif Akhteruzzaman[1]*

[1]*Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka -1000, Bangladesh*
[2]*National Forensic DNA Profiling Laboratory, Nuclear Medicine Building (10th Floor), Dhaka Medical College Campus, Dhaka- 1000, Bangladesh*

Abstract

In this study, we are reporting the concordance study between profiles from AmpFlSTR® SGM plus™ and that of PowerPlex®16 system kits in Bangladeshi Bangali population. DNA samples from 234 unrelated Bangladeshi Bangali individuals were evaluated employing both the SGM plus™ and PowerPlex®16 system human identification kits. Complete consistency was observed in 233 out of 234 profiles which represents substantial degree of concordance amounting 99.57% between these two kits. Only one discrepancy encompassing the dropout of allele 12 at D16S539 loci in PowerPlex®16 system was observed. Sequence analysis revealed two mutational events; one at 16 bp distal and another next to the 3′ end of the PowerPlex 16 reverse primer of D16S539 locus. This study has shown the significance of using of alternative set of primer to address the false homozygosity issue as well as of bringing attention regarding the fact of discrepant typing from different sets of primers to the scientific community during database handling and identity or kinship investigations.

Keywords: SGM plus™; PowerPlex®16; Concordance; Allele dropout; Mutation

Introduction

Short tandem repeat (STR) based DNA typing employing multiplex polymerase chain reaction (PCR) technique is worldwide familiar for forensic investigations and population genetics study [1-3]. SGM Plus™ and PowerPlex®16 system are two popular multiplexes in forensic community and can amplify concurrently 10 and 15 autosomal STR loci respectively with an additional sex determinant amelogenin locus [4-5]. In our laboratory we use SGM plus™ and PowerPlex®16 system for routine case works and have already performed forensic evaluation studies of these two kits for Bangladeshi Bangali population [6-7]. These two kits share eight common autosomal loci: D3S1358, vWA, D18S51, D21S11, D8S1179, D16S539, TH01 and FGA. Primer sequences for PowerPlex® 16 kit (Promega Corporation, USA) have been revealed [8], whereas the primer sequences for AmpFlSTR SGM Plus™ kits (Applied Biosystems, USA) are concealed by the manufacturer. The primer binding sites for SGM Plus™ and PowerPlex® 16 system loci are dissimilar which leads to difference in size of PCR products for same allele [9]. Mutation in primer binding site may lead to different genotype with these two different kits. Inconsistencies between commonly used commercial kits down to mutation in primer binding site have been reported in several studies [9-20]. As we use these two kits for routine case-works, we should have vast knowledge pertaining to the consistency between two kits and present study aims to achieve this purpose. To our knowledge, no concordance study between these two kits in Bangladeshi population has been reported and this study reports any study of it's kind for the first time.

Materials and Methods

Population

Liquid blood samples and buccal swab was collected from 234 unrelated Bangaldeshi Bengali individuals. It may be mentioned that there are four ethnic groups in Bangladesh; Dravidina, proto-Australina, Mogolians and Bengalis. The Bengalis are by far the largest group consisting about 98% of the total population. The Dravidian element of population is represented mainly the Oraons, a tribe of central Indian in origin. The proto-Australian group includes Khasia and Santals, mainly labourers in the tea garden at Sylhet district. The Mongolian group is confined to the hills along the southern spur of the Shilong plateau, in Chittagong hill tracts and Madhupur forest. Chakam, Tripura, Garo, Murang and Moghs are the major Mongolid tribes in Bangladesh. The individuals recruited in this study belongs to only the mainstream Bangali population.

DNA Extraction and PCR amplification

DNA was obtained from blood or buccal swab of 234 unrelated Bangladeshi Banglai Individuals using the Chelex-100 method described by Walsh et al. [21]. Approximately 1–2 ng of DNA was co-amplified using SGM Plus™ and PowerPlex® 16 PCR amplification kits and following the protocol provided by the manufacturer. The PCR reaction was carried out in a GenAmp PCR system 2700 (Applied Biosystems).

Electrophoresis and typing

PCR amplified fragments were separated and analyzed on ABI Prism 3100-avant Genetic Analyzer (Applied Biosystems) using POP-4 polymer and Data Collection Software version 1.1. Data were sized using GeneScan Software version 3.7. Tabular data from GeneScan was converted to genotype calls using Genotyper version 3.7 NT with the help of Kazam macro. In cases where samples were amplified by PowerPlex® 16 PCR amplification kit, Power Typer macro was used.

***Corresponding author:** Sharif Akhteruzzaman PhD., Professor, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh, Tel: +88-01714111267; Fax: +8802-8615583; E-mail: sharif_akhteruzzaman@yahoo.com

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Results and Discussion

In this study, a total 234 blood or buccal scrape samples from unrelated Bangladeshi Bangali individuals were investigated with the SGM Plus™ and PowerPlex®16 kits. A high degree of harmony between these two kits was observed. Complete consistency was ascertained in 233 (99.57%) out of 234 profiles. Out of 1872 STR loci profiles (eight in each individual's profile) 1871, or 99.95%, were compatible with these two kits. Only one discrepancy was noticed and it encompassed the locus D16S539 (Figure 1). Amplification employing PowerPlex®16 system resulted in homozygous allele call 9,9 at the discrepant D16S539 locus , whereas heterozygous allele call 9,12 was resulted in case of SGM plus™ kit. Our finding pertaining to the level of concordance between two kits for Bangladeshi Bangali population is very much consistent with the findings of other studies. Vanderheyden et al. [10] reported 99.42% concordance in a comparison study between SGM Plus™ and PowerPlex®16 system kits. Study conducted by Delamoye et al. [9] revealed the concordance of 99.37% when comparing the profile from SGM plus™ or Profiler plus™ to the PowerPlex®16system kit. Using PowerPlex®16 and The Profiler Plus™/Cofiler™ kits, Budowle et al. [17] compared population database samples covering African-Americans, Bahamians, and Southwestern Hispanics and found only one inconsistency due to allele dropout which led to a high concordance of 99.81%. Consistency between the profiles from PowerPlex®16 and that from Profiler/Cofiler was approximately 98.63% as explored by Budowle et al. [15] where D8S1179 had shown higher population specific allele dropouts comprising 62% among all the dropouts detected.

The prime reasons for inconsistent typing between kits are allele dropout [9-20] and deletion of DNA segment out side the flanking region [20]. There are several reasons for allele dropout such as inadequate template DNA [22], substandard conditions in the DNA amplification process [23] and mutational event inside the primer binding region [9-20] or at distal position to the hybridization site of primer and template [17]. Mismatch of the primer to the template near the 3′ end of primer due to mutational event leads to the prevention of primer extension process during the PCR [15] where as mutational

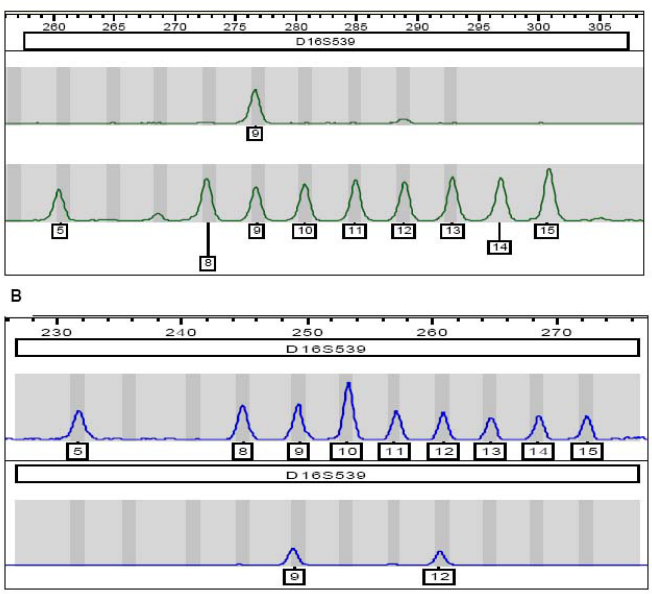

Figure 1: Panel (A) displays the homozygous allele call at D16S539 locus using PowerPlex® 16 kit. Panel (B) displays heterozygous allele call at the same locus using the SGM plus™ kit.

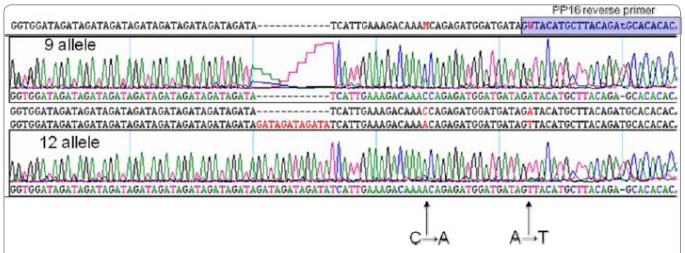

Figure 2: Sequence analysis of D16S539 loci. Allele 12 contain tow novel mutations; one C→A mutation and another A→T mutation located 16 bp distal and next to the 3′ end of PowerPleax 16 reverse D16S539 primer binding site respectively. GATA is the repeating unit.

event at distal to the primer binding site may instigate a secondary structure that mask the hybridization site [9]. Above all, mutational event at the primer binding site is proved to be highest attributor for the cause of allele dropout. Delamoye et al. [9] revealed that among conclusive analyses, 100% dropouts were attributed to the mutational event inside primer binding region and same finding was explored by Vanderheyden et al. [10].

In this study, the technical factors including inhibition and stochastic effect as causes of dropout at D16S539 was ruled out as we analyzed a second sample from the same individual and purity of DNA was confirmed by 260/280 ratio using Nano Drop-1000 (Thermo Scientific) and concentration of DNA was evaluated by Quantiblot method (Applied Biosystem).

Allele dropouts at D16S539 were also identified in several other studies. Nelson et al. [18] reported the African–American population specific allele dropout at D16S539 in PowerPlex1.1 kit due to a T → A mutation corresponding to the next to the last base in the 3′ primer binding sequence and this mutational effect was reversed by degenerative primer and also by the primer of PowerPlex® 16 which included additional five bases at 3′ end when compared the same primer of Powerplex1.1. Additional five bases were proved to have effect to stabilize the primer- template hybrid. So from the findings of Nelson et al. [18] and that of our study we inferred that allele dropout at D16S539 of PowerPlex®16 in our study presumably is due to the mutational event that encompasses the additional five nucleotides, though we can not rule out the possibility of mutation at the distal region to the primer binding site.

To confirm our prediction we conducted a sequence study with the help of NIST laboratory, USA. (Figure 2). DNA sequence analysis revealed that allele 12 harbored two SNP; a C→A mutation at 16 bp upstream to 3′ end of PowerPlex16 reverse Primer at D16S539 locus, and a A→T mutation at the 3′ end of reverse primer binding region. Among these two mutations, mutation near the 3′ end of primer binding site might be the highest. Full profile using SGM plus primer further support that mutation at the distal region from the primer binding site might have little effect for allele12 dropout using PowePlex 16 primer. Though very low frequency of inconsistency between SGM plus™ and PowerPlex®16 exists in Bangladeshi Bangali population, simultaneous use of both of the kits having different sets of primers could demonstrate the value to address the false homozygosity issue.

Conclusion

This study has conspicuous worth in bringing attention regarding the fact that, inconsistent typing from different sets of primers, to the scientific community at the time of routine paternity test, database managing as well as identity or kinship investigations.

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