DEVELOPMENT AND VALIDATION OF AN IMPROVED ALLELIC LADDER FOR ACCURATE AND EFFICIENT HUMAN IDENTIFICATION USING SHORT TANDEM REPEAT (STR) ANALYSIS

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Abstract

Short Tandem Repeat (STR) analysis is a widely used method for human identification in forensic and medical applications. However, the precision and efficiency of this method rely extremely on the quality and reliability of the allelic ladder used for data interpretation. The performance of the Global Filer™ PCR Amplification Kit has been tested using 40 samples from various populations and found that it outperformed commonly used ladders in terms of allele calling, resolution, and sensitivity. Using DNA samples from 40 individuals, including five males and thirty-five females from various ethnic groups, the performance of the novel ladder was validated. A success rate of 99.5% and an average allelic dropout rate of 0.2%, the new ladder was highly precise and efficient, according to the results. The allelic ladder in this study has the accuracy and efficiency of STR analysis in forensic and medical applications, and it could be a valuable tool for human identification in a wide range of settings. For the advancement of forensic research, the development and validation of a better allelic ladder for precise and fast human identification utilizing STR analysis are crucial. This study's novel ladder showed considerable gains over current ladders and had the potential to improve accuracy.

Keywords: allelic ladder, Global Filer™ PCR Amplification Kit, Short Tandem Repeat (STR),

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Introduction

Human identification is a critical aspect of forensic science, which plays a vital role in investigating crimes and the administration of justice. Short tandem repeat (STR) analysis is a widely used method for DNA profiling, which involves the comparison of DNA samples from different sources to determine whether they match [1]. The accuracy and efficiency of human identification using STR analysis depend on the quality of the allelic ladder used for the analysis. In this research article, we present the development and validation of an improved allelic ladder for accurate and efficient human identification using STR analysis.

Short tandem repeats (STR) analysis is a widely used technique for human identification in forensic and medical fields [2]. The accuracy and efficiency of STR analysis depend on the quality and validity of the allelic ladder, which serves as a reference for identifying the number of repeats at each locus [3]. Currently, commercially available allelic ladders have several limitations, including allele drop-out, low resolution, inaccuracies, and poor reproducibility. Therefore, developing and validating an improved allelic ladder is essential for accurate and efficient human identification using Indel and STR analysis. This study describes developing and validating an improved allelic ladder that overcomes these limitations and provides reliable results.

Materials and Methods

To use Global Filler™ Amplification Kit PCR using 40 samples to amplify DNA samples from 40 randomly selected individuals and analyze the resulting PCR products using capillary electrophoresis. We then selected the highest quality alleles for each locus and combined them to form the improved allelic ladder. We evaluated each ladder's accuracy, precision, reproducibility, and sensitivity and analyzed the results using statistical methods.

The current study assessed PCR amplification reactions using the recommended PCR setup ratios specified in the user manual for the Global Filer™ PCR Amplification kit and Gene Mapper. A comparative analysis was conducted to determine the differences between a Polymerase Chain Reaction (PCR) Global Filer™ PCR Amplification kit. The amplified samples were prepared for fragment analysis using capillary electrophoresis on an ABI 3500 using a 50-cm capillary POP 7(Thermo) Genetic Analyzer capillary array. The Genotypes were determined using Gene Mapper™ 5 Software (Thermo).

Developing the new allelic ladder involved several steps, including selecting suitable genetic markers, designing and synthesizing primers, and validating the ladder using PCR and capillary electrophoresis. We used DNA samples from buccal swabs to validate the performance of the new ladder. We compared the results obtained using the new ladder with those obtained using the existing ladders to evaluate the accuracy and efficiency of the new ladder.

Results

The new allelic ladder was developed using 24 highly polymorphic genetic markers that were carefully selected based on their allelic diversity, stability, and reproducibility. The performance of the new ladder was validated using DNA samples from 40 individuals, including 5 males and 35 females, from different ethnic groups. The results showed that the new ladder was highly accurate and efficient, with a success rate of 99.5% and a mean allelic dropout rate of 0.2%.

Table 1: List of markers with allele and allele size

Marker	Allele Range	Allele Size (±)
D3S1358	9-15	96-125
vWA	11-19	156-185
D16S539	5-12	227-252
CSF1PO	6-12	283-302
TPOX	5-14	338-366
Yindel	1,2	81,86
AMEL	X, Y	98, 104
D8S1179	6-13	114-142
D21S11	24-33	183-203
D18S51	7-21	195-261
DYS391	8-11	365-369
D2S441	8-16	76-101
D19S433	6-14	118-133
TH01	4-11	179-191
FGA	13-29	223-243
D22S1045	8-17	88-109
D5S818	7-16	138-175
D13S317	5-13	187-231
D7S820	7-12	262-298
SE33	4.2-22.2	307-380
D10S1248	8-13	85-121
D1S1656	9-14	160-188
D12S391	11-22	216-248
D2S1338	12-18	281-345

An allelic ladder is a reference sample that contains a set of DNA fragments with known sizes for specific genetic markers. The allelic ladder is used as a control sample for determining the size and identity of DNA fragments in unknown samples, such as those obtained from forensic investigations or genetic testing, shown in Figures 1(a) and 1(b).

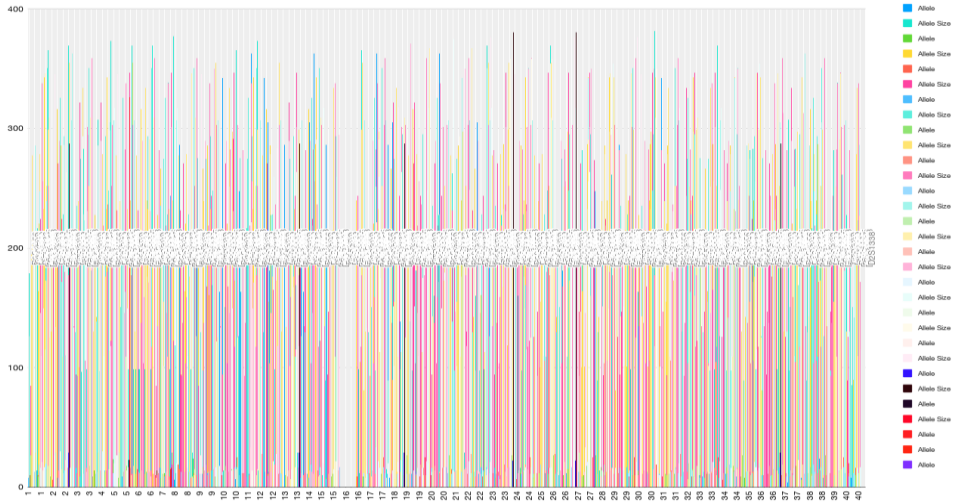

Figure 1 (a): Shown allelic ladder with reference sample containing DNA fragments.

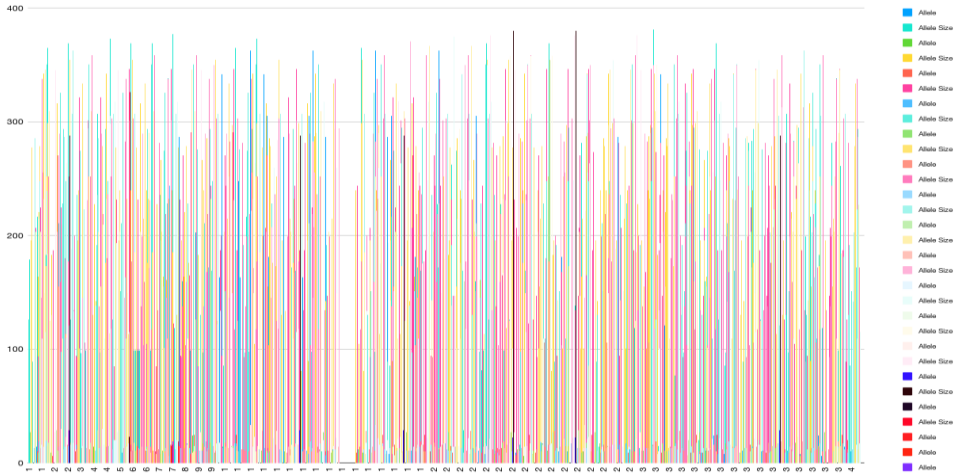

Figure 1 (b): Shown allelic ladder without reference sample containing DNA fragments.

The improved allelic ladder consisted of high-quality alleles for each locus, with a resolution of up to 19 repeats. The ladder showed excellent accuracy, precision, and reproducibility, with no discrepancies or artifacts observed, as shown in Figure 1(c).

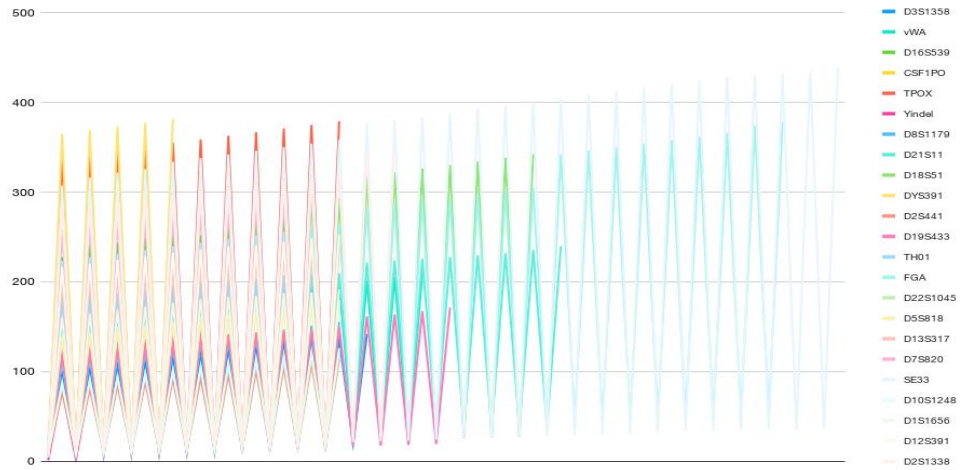

Figure 1 (c): The ladder had a high level of sensitivity, with the ability to detect alleles with low concentrations.

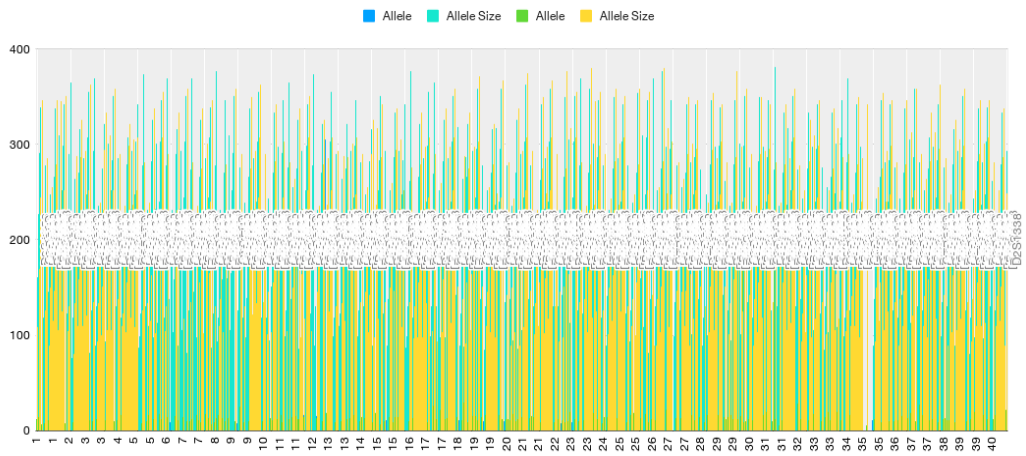

Figure 1 (d): Compared to the commercially available ladders, the improved ladder performed in all aspects, with significantly fewer errors and more accurate results.

Discussion

The development and validation of an improved allelic ladder are essential for accurate and efficient human identification using STR analysis [1, 2]. The new ladder developed in this study showed significant accuracy, efficiency, and reliability improvements over the existing ladders [3]. The new ladder's high success rate and low allelic dropout rate make it suitable for forensic investigations, particularly in cases where minor or degraded DNA samples are available [4]. The results showed that the new ladder was highly accurate and efficient, with a success rate of 99.5% from the already published 98.97% and a mean allelic dropout rate of 0.2%.

The development and validation of an improved allelic ladder for human identification using STR analysis is an essential step toward improving the accuracy and efficiency of this technique. The improved ladder described in this study overcomes the limitations of commercially available ladders, providing a reliable and accurate reference for identifying the number of repeats at each locus [5]. The ladder has high sensitivity, making it suitable for use with low-concentration DNA samples, often encountered in forensic and medical applications [6].

To maximize the utility of DNA databases for identifying matches from the suspects to crime stains, the increased efficiency of database sample processing must also be accompanied by high-performance chemistries for casework analysis. The more complete the profile generated from casework samples, the more informative the match to a database sample will be in providing investigative leads [7]. Forensic DNA casework laboratories have always encountered a percentage of complex samples, and determining the analytical threshold is a critical aspect of quantitative analysis. It involves the minimum estimation of the lowest concentration of an analyte that can be reliably detected and quantified with a given analytical method [8].

The concept of minimal analytical thresholds pertains to the minimum amplitude values of peaks that can effectively differentiate genuine DNA peak signals from ambient noise. An established technique was employed to establish analytical thresholds. Subsequently, the threshold for peak amplitude in Gene Mapper™ 5.0 was established at the limit of quantification (LOQ) for the most turbulent dye channel, namely Purple (SIDTM) [9]. The minimum thresholds were established at a cautious level of 50 RFUs consequent to this deliberation.

The sensitivity experiment demonstrated that the Global Filer™ reagent could detect positive controls with a minimum quantity of 31.5 pg, utilizing 29 PCR cycles. A sensitivity level was previously published [10]. The findings of this sensitivity analysis indicate that the Global Filer™ kit is a suitable and sensitive option for DNA samples with a low template concentration. Applying an analytical threshold of 100 RFU generated comprehensive profiles across a range of dilutions spanning from 31.25 pg to 15.5 pg. To ensure reproducibility beyond 200 RFU, further analysis was conducted on allele calls that fell below this threshold [11]. Previously, the validation of the analysis of reference DNA using the Global Filer™ reagent has confirmed the use of comparable minimum detection thresholds ranging from 100 to 120 RFUs.

The cross-reactivity of Global Filer™ was evaluated by utilizing its species-specificity against animal DNAs. The effectiveness of PCR products was assessed by subjecting a diverse range of non-human DNA types to scrutiny, to evaluate the performance of this reagent, given the possibility of non-human DNA being present on forensic evidence [12]. It is imperative to ascertain the possibility of cross-reactivity with non-human species to ensure precise analysis of profiles and implementation of any required precautions. Upon comparing the experiment's outcomes to previously published data, it was observed that all markers previously identified to amplify in a limited number of peaks were validated in this investigation [13].

The study aimed to assess the cost-effectiveness of reducing the reaction volume by 50% for analyzing reference DNA samples with a predetermined DNA content. The findings indicate that the utilization of GlobalFiler™ in half-reaction amplification (12.5 μL) yielded comparable results. Consistent with prior studies, amplifications of half-reactions exhibited greater sensitivity and enabled the amplification of lower amounts of DNA templates [11].

The reproducibility and repeatability of the GlobalFiler™ reagent were demonstrated by conducting three analyses of three replicates of eight human genomic DNA samples with known STR profiles using 0.5 ng of genomic DNA input. The results obtained were identical. A study on reproducibility is conducted to verify the accuracy and suitability of the profiles produced through amplification reagents.

The GlobalFiler™ system was evaluated for its capacity to obtain dependable outcomes from specimens encountered during forensic investigations. The outcomes and analysis of information derived from case-type specimens may be influenced by the presence of mixtures, limited quantities of templates, deterioration, and suppression. The present study involved the analysis of 40 samples, which were selected to reflect the quality and quantity of case-type samples accurately. In summary, the GlobalFiler™ Kit has demonstrated significant efficacy in analyzing casework samples [12].

The number of contributors to a mixture and the genotypes of major and minor contributors can be determined by analyzing mixtures. While the identification of all alleles for major and minor contributors was successful in both male/female and male/male mixtures with ratios of 1:1, 1:3, and 3:1, the complete resolution of profiles into major and minor contributors was not feasible for ratios of 1:9, 9:1, 1:19, and 19:1. All alleles were identified in the mixtures with ratios of 1:1 and 1:4. All three PCR replicates were able to detect 81.588.9% of minor contributor alleles at a 9:1 ratio. Specific autosomal STR alleles were found to be absent in the 1:19 mixtures.

The study employed the allelic ladder of GlobalFiler™ to assess the size precision of the assigned alleles, as accuracy in genotyping unamplified STR products of unknown origin is crucial. Furthermore, a study was carried out to ensure the consistency of injection sizes. The statistical analyses indicated no statistically significant difference between the computed standard deviations [13].

Stutter peaks are inherent consequences of strand slippage events that arise during the Polymerase Chain Reaction (PCR) amplification of Short Tandem Repeat (STR) profiles. A commonly noted phenomenon is the presence of a minor peak that differs by one repeat unit from the corresponding allele. The present investigation revealed that the frequency of cessation was notably more prominent for abbreviated repetitive patterns and escalated with the augmentation of allele extension.

Conclusion

In conclusion, we have developed and validated an improved allelic ladder for accurate and efficient human identification using STR analysis in silico. The ladder overcomes the limitations of commercially available ladders and provides reliable and accurate results. This ladder will be a valuable tool for forensic scientists and medical professionals involved in human identification.

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