

The Generation of *Alu*-Based DNA Profiles Using EPICENTRE's FailSafe™ PCR System

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Roughly 45% of the human genome consists of interspersed repeat sequences, with the 300 basepair *Alu* element representing the predominant family of Short Interspersed Elements (SINEs). Approximately 5000 of the over one million *Alu* elements in the human genome have integrated into the human genome relatively recently, following the human-chimpanzee split.¹ Furthermore, about 25% of this small subset are not fixed in human populations and therefore provide polymorphic markers in the form of presence/absence variants, referred to as *Alu* dimorphisms. These *Alu*-based variants provide useful DNA markers for human population studies,² forensic studies³ and paternity analyses.⁴ Additionally, an *Alu* variant has been commonly used as a pedagogical tool to study genetic variation and population genetics.⁵ A locus consisting of presence and absence variants of an *Alu* element yields three possible genotypes. Assaying additional *Alu* variants exponentially increases the number of possible genotypes (3ⁿ).

Simultaneously analyzing several *Alu*-based loci is technically more difficult than other multiplex amplification reactions as *Alu* elements from the different loci can form heteroduplexes. Although amplification of single reactions works well using native *Taq*, difficulties begin with duplex reactions. Developing triplex reactions of *Alu* variants therefore is further problematic.⁶ Commencing

with the guidelines of Henegariu, *et al.*,⁷ for efficient multiplex PCR, and Thomas and Herrera⁶ for *Alu* multiplexing, only minor improvements were obtained through various alterations of PCR conditions. However, the use of EPICENTRE Biotechnologies' FailSafe™ PCR System immediately established markedly improved results, with only slight modifications necessary for further refinement, as well as permitting the production of high quality, reproducible *Alu*-based tetraplex DNA profiles.^{8,9}

Methods

A scheme for generating *Alu*-based profiles is shown in FIG 1. *Alu* dimorphisms were selected in which both presence and absence alleles are common and hence more informative. Information on these intermediate frequency (IF) *Alus* is available in the literature^{1,10} including primer sets, expected amplicon sizes, and frequencies among various ethnic groups. The expected amplicons for multiplexing should be resolvable on a 2% agarose gel. Upon identification of individuals

heterozygous for variants (generating all the bands would be more difficult than amplifying a lesser number of bands), duplex and triplex reactions are initially performed with these samples either by optimization using EPICENTRE's FailSafe PCR System or by directly using FailSafe PreMixes E or I, which have thus far been the optimal PreMixes.^{8,9} Only fine-tuning (FIG 1) seems necessary for obtaining optimal results since the best FailSafe PreMix (already containing dNTPs, MgCl₂, and PCR Enhancer with Betaine*) has previously been determined. This is then followed by incorporating a third or fourth primer set and further fine tuning. Confidence in the system is attained by verifying genotypes of a sample of individuals through analyzing each locus individually.

Results

I have thus far developed two tetraplex profiles with the FailSafe System that are highly reproducible (FIG 2), the specific conditions of which are available.^{8,9} These provide a rapid tool for human population studies. Additionally, I have

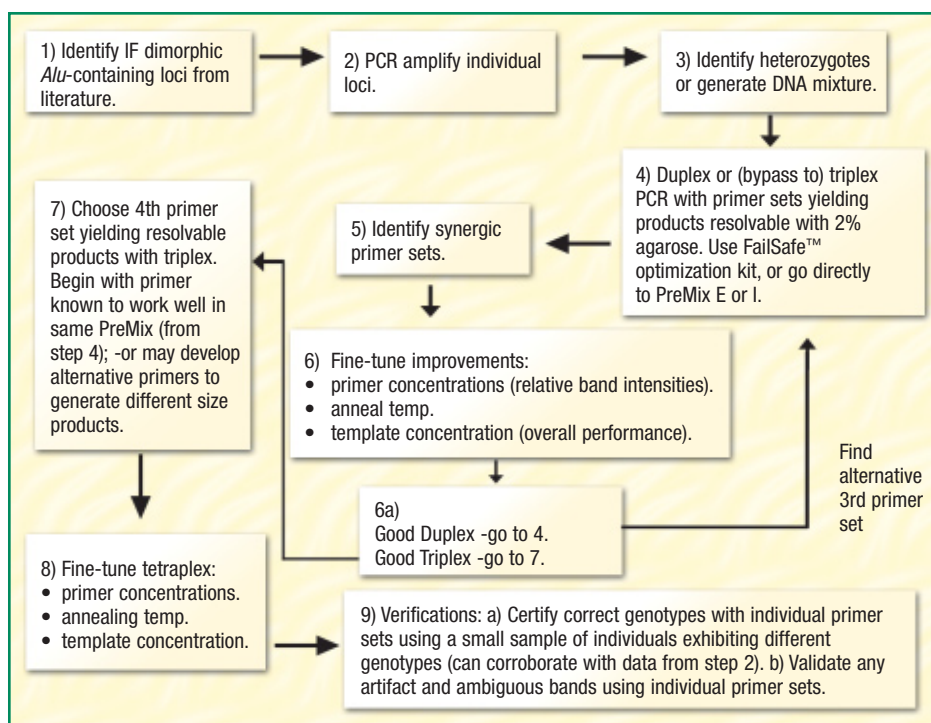


FIG 1. Flow chart for the development of an *Alu*-based multiplex system.

incorporated these profiles into the classroom in an undergraduate genomics laboratory course.⁹ These are highly effective for demonstrating applications in forensics, paternity analysis, and population genetics studies.

Discussion

Currently the PV92 *Alu* insertion polymorphism and the D1S80 variable number of tandem repeats (VNTR) locus are the mainstay pedagogical DNA profiling tools. Although highly variable, the D1S80 variants are not all easily resolvable on an agarose gel. The single *Alu* variant is not overly informative as it is a two-allele system (three genotypes). The two tetraplex reactions of *Alu* variants generate 6,561 possible genotypes. Therefore, each student in a class would most probably have their own unique profile, which would demonstrate forensic applications. Additionally, a family volunteering DNA with two unrelated individuals provides a mechanism for students to analyze paternity. Lastly, Robert LaRoe (an Eastern Michigan University computer science graduate student) and I have been developing a web-based analysis system (www.emudnaprofiledatabse.org) in the style of the Dolan DNA Learning Center Allele Server (www.bioservers.org/bioserver). This allows for population studies within and among classes that incorporate their data determining Hardy-Weinberg equilibrium, genetic distance, etc. Additionally, we provide a mechanism whereby as profiles get incorporated, the frequencies of each of the 6,561 genotypes are determined. Therefore, an individual can readily determine the frequency of his/her profile in a population.

Conclusion

A DNA profiling system has been developed predominantly powered by EPICENTRE Biotechnologies FailSafe™ PCR System. The ease of use, high number of profiles, and web-based analysis presents this tool as a unique alternative to current methodology for teaching labs, and provides a labor saving alternative for human population studies.

Acknowledgements

I wish to acknowledge Robert LaRoe for his dedicated work in building a web-based tool for utilizing the *Alu*-based DNA profiling tool.

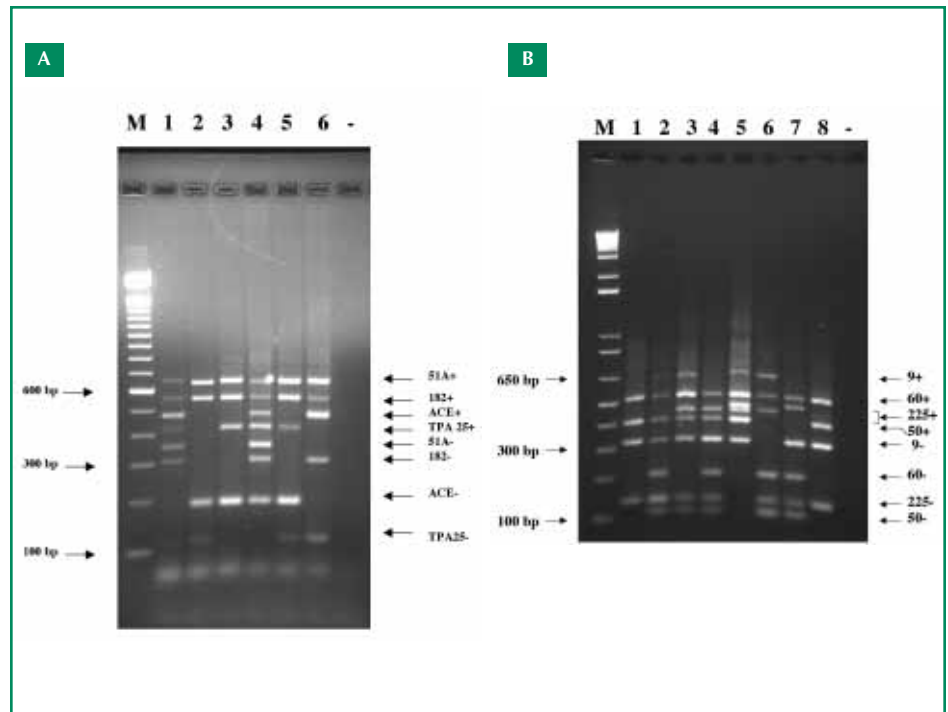


FIG 2. Two *Alu*-based tetraplex DNA profiles analyzed on ethidium bromide-stained 2% agarose gels. Lane M, refers to molecular weight markers; numbers above lanes refer to individual human DNA samples, numbers to the right of the gels refer to *Alu* loci; Lane - refers to the negative control (no DNA). Conditions for the two profiles A and B, can be found in references 8 and 9, respectively. [Gel 2A reprinted with permission from Elsevier.⁸]

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*Use of Betaine for DNA Polymerase Reactions, including, but not limited to, use for PCR or DNA Sequencing, is covered by U.S. Patent No. 6,270,962, European Patent No. 0742838, German Patent No. DE4411588C1, and other issued or pending applications in the U.S. and other countries that are either assigned or exclusively licensed to EPICENTRE. These products are accompanied by a limited non-exclusive license for the purchaser to use the purchased products solely for life science research. Contact EPICENTRE for information on licenses for uses in diagnostics or other fields.

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