B99 Ultrahigh-Speed Polymerase Chain Reaction (PCR): A Method for Obtaining Short Tandem Repeat (STR) -Based Genotypes in Eight Minutes or Less

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After attending this presentation, attendees will better appreciate new methods to accelerate the PCR reaction using a specially designed seven-loci STR multiplex that permits complete amplifications in eight minutes or less, thus aiding in the analysis of time-sensitive forensic casework.

This presentation will impact the forensic science community by demonstrating an approach toward optimizing rapid, multiplex PCR amplifications, which can be easily implemented in their own laboratories. This procedure uses small, high-speed thermal cyclers and fast polymerases with high processivity and inhibition resistance. The loci used in this multiplex are a small subset of the standard Combined DNA Index System (CODIS) set, making them readily compatible with current statistical approaches. This new procedure should be particularly useful for situations in which screening of suspects and crime scene samples is necessary.

It is often extremely important to rapidly screen suspect samples at border controls and police stations where the individuals in question can only be detained for short periods of time. Current DNA typing methods provide the best biometric information yielding identity, kinship, and geographical origin, but they are not sufficiently fast to permit identity of a suspect DNA in real time. Current rapid DNA systems take approximately 90 minutes, and full laboratory DNA analysis is even slower.[1,2]

Ultra-high speed PCR coupled with rapid polymerases can greatly reduce the processing time from sample to STR genotype.[3] Coupling these advances with new thermal cycler designs can make the analysis even faster. Using Peltier and flow-based thermal cyclers, PCR reactions have been optimized, resulting in the completion of singleplex reactions in four minutes and complete multiplex reactions in eight minutes. When coupled with microfluidic electrophoresis, complete genotypes can be produced in 15 minutes or less.[3]

To accomplish this, specially engineered enzymes and buffer systems have been tested to rapidly amplify autosomal and Y-chromosomal Short Tandem Repeat (Y-STR) multiplexes.[3,4] The designed multiplex includes D5S181, D13S317, D7S820, CSF1PO, D16S539, Penta D, and Amelogenin, which have sizes between 106bp and 454bp. A complementary rapid Y-STR multiplex has also been developed. By using off-the-shelf components and commercially available enzymes, it is possible to create a procedure that acts as a quick, highly informative sample screening process that also retains sufficient DNA for later manual processing using standard STR or Y-STR kits.

The first phase of this study further accelerated a previously reported amplification by increasing DNA concentration and reducing cycle number in order to produce a 7-locus multiplex amplification in eight minutes. Two different non-hotstart polymerases were compared in these studies. Additional work was performed on a novel flow-based thermal cycler in which the sample moves fluidically between two thermally isolated regions. This system was able to produce singleplex PCR reactions in four minutes. This work is currently being expanded to permit multiplex amplifications. In the current research, control DNA standards 9948 M DNA and K562 F DNA were used, as well as donated saliva samples from five adults. Samples were analyzed using gel, capillary, and microfluidic electrophoresis separation approaches.

The results of this study demonstrate the application of ultra-high speed PCR for the successful amplification of two different PCR multiplexes. With such a procedure in place, any crime laboratory can produce a nearly instantaneous genotype from buccal samples.

Reference(s):

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