RAPID DIRECT PCR, A SIMPLE METHOD FOR PRODUCING GENOTYPES IN 20 MINUTES

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There are situations in which it becomes important to quickly identify a suspect or detainee held at police stations or border entry points. The problem is that standard methods used to produce a DNA type, including extraction, amplification and genotyping, can take many hours. The ability to perform direct PCR coupled with high speed thermal cycling can change all this. Direct PCR permits sample amplification straight off of a paper punch and when coupled to high speed thermal cyclers the entire process from buccal swab to amplified product can be performed in under 16 minutes. To achieve this result we have utilized a high speed thermal cycler, (Streck Philisa) high speed polymerases (Takara) and specially modified direct amplification buffers (Bioquest). When prepared with appropriate enzymes, we can utilize a ramp rate of 15 degrees per second with each amplification cycle taking 25 seconds including denaturation (5 sec), annealing (5 sec) and extension (15 sec.). In our optimization studies we have compared a variety of high speed polymerases and direct buffers. These were further modified by examining the effects of polymerase, primer, dNTP and Mg concentration.

The resultant amplified product can then be separated and analyzed using a modified Agilent Bioanalyzer that permits high resolution ssDNA separations. The analysis is performed on a subset of 7 CODIS loci, CSF1PO D16S236, D7S820, D13S317, D5S818, Amelogenin and Penta D and takes under 80 seconds. Thus, the entire time from sample collection to genotype takes under 20 minutes with the Agilent microfluidic device or under 45 minutes with a standard ABI 310. In the presentation, we will discuss our initial development of this method and show some results of our current validation studies. Future work will include expansion of the number of loci and full developmental validation of the process. ⌘