B70 The Application of Reduced Size STR Amplicons in the Analysis of Degraded DNA

Kerry L. Opel, MA, Denise T. Chung, BS*, Bruce R. McCord, PhD, Department of Chemistry and Biochemistry, Ohio University, Clippinger Laboratories, Athens, OH*

The objective of this presentation is to develop STR markers with re-designed primer sequences to produce smaller amplicons. These new markers can be utilized in the analysis of degraded DNA and provide a useful alternative to mtDNA sequencing with the added benefit of complete compatibility with the CODIS STR set. These markers have been combined to produce subsets of 3 to 6 loci and are entitled "Miniplexes." The effectiveness of these miniplex sets was tested on enzymatically degraded DNA and DNA extracted from human skeletal remains from which previous attempts using standards primers had not yielded usable results.

In heavily degraded DNA, poor amplification of the larger sized amplicons in standard multiplex typing kits (300-500 base pairs) is common. This is because as the sample decomposes, the DNA template can become highly fragmented, and the yield of template fragments having a complete target sequence is greatly reduced. Thus in multiplex kits with a wide range of amplicon sizes, a "decay curve" is seen, in which the larger loci have much lower intensity, and often drop out or fall below the detection threshold. The new "Miniplex Primers" have been re-designed so that the target sequence is much closer to the repeat region and will therefore produce smaller amplicons.

The re-designed primers were developed through a collaborative arrangement between Dr. John Butler at the National Institute of Standards and Technology and the McCord research group at Ohio University. The primers include the majority of the 13 CODIS STR loci (TH01, CSF1PO, TPOX, D5S818, D8S1179, D16S539, FGA, D21S11, D7S820, vWA, D18S51, D13S317), as well as 3 non-CODIS loci (Penta D, Penta E, D2S1338). Five sets of loci were designated as miniplexes. To avoid overlap, the markers were amplified with one locus in each dye lane and typically contain only 3 STRs per set. The primers were labeled with 6-FAM (blue), VIC (green) and NED (yellow). Miniplex 1 and Miniplex 3 differed in the size ranges and can be multiplexed together to create a six-loci set entitled "Big Mini." These miniplex sets allow for a reduction in product size up to 299 base pairs, with most amplicons ranging in size from 60-200 base pairs. The allele designation and profile remains unaltered. Because these primers produce small amplicons, they allow for a more complete profile from degraded DNA. Degraded DNA was amplified using these primers and compared to the larger primers from commercial kits.

To determine the effectiveness of the miniplex kits in the analysis of degraded DNA, genomic DNA was enzymatically digested using DNase I. The DNA was incubated with DNase I for a range of time periods: 2, 5, 10, 15, 20, and 30 minutes. The degraded DNA was separated by gel electrophoresis using 2% agarose and stained with ethidium bromide for detection. Different regions of the gel corresponding to different fragment sizes were excised from the gel and purified using the QIAquick Gel Extraction Kit. The different fragment sizes were then amplified by PCR using the "Big Mini" primer set and a commercial primer set. The amplified DNA was analyzed using an ABI Prism 310 capillary electrophoresis with four-channel fluorescence detection and the GS 500 ROX size standard. The Big Mini primer set was capable of producing more complete profiles from the smaller fragment samples.

Bone samples were prepared by extracting DNA from femur sections that had been cleaned with ethanol and distilled water, cut with a rotary tool, cleaned again, and pulverized under liquid nitrogen to produce a powder. The powder (0.1g) was decalcified in EDTA, digested using a stain extraction buffer and proteinase K, and purified and concentrated using the QIAgen MiniAmp Blood Kit. The DNA was amplified by PCR using the miniplex kits and a commercial kit. The amplified DNA was analyzed using an ABI Prism 310 Capillary electrophoresis with four-channel fluorescence detection and the GS 500 ROX size standard. The miniplex primer sets were capable of producing more complete profiles from the DNA extracted from bone samples.

In both types of degraded DNA, the miniplex primer sets were capable of producing more complete profiles. These re-designed primers can provide a new tool for the analysis of degraded DNA and increase the probability of obtaining a usable profile from degraded DNA.

Degraded DNA, STR, Miniplex

 ** Presenting Author*