Customer Success Story | Forensic DNA Service

Genotyping of degraded and ancient DNA samples using AmpFℓSTR® MiniFiler™ PCR Amplification Kit

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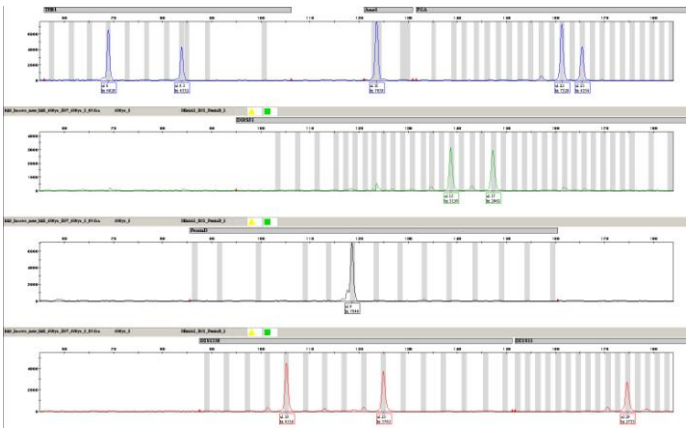
Application:
Human identification
Research and development

Applied Biosystems Technology:
GeneAmp® PCR System 9700
ABI PRISM® 310 Genetic Analyzer
AmpFℓSTR® PCR kits
ABI Prism GeneMapper® Software

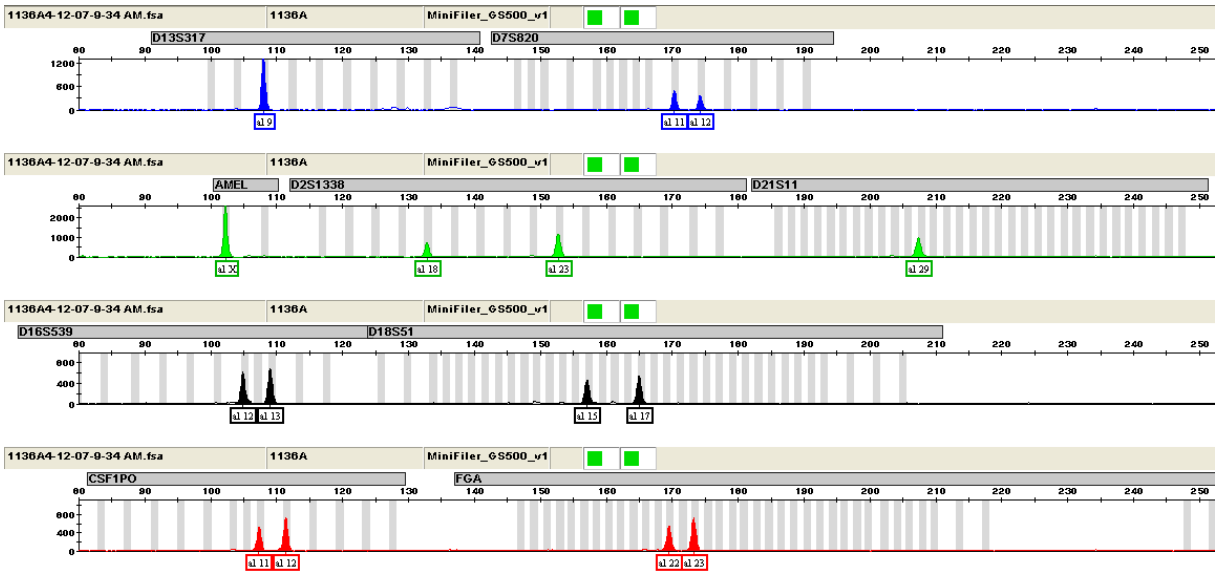
This story started back in 2002 at the ICMP (International Commission on Missing Persons) laboratory in Sarajevo, Bosnia and Herzegovina when Jon Davoren and I started an extensive work on developing the best possible DNA extraction protocol for bones from the mass graves. Nights spent in a lab were finally repaid with a procedure (Davoren J., et.al., CroMedJournal, 2007, Vol.48, No.4) that highly overpowered the standard phenol-chloroform extraction and enabled us to get full DNA profiles from more than 90% of mass grave samples submitted to the laboratory. Stimulated by this success we greedily tested the procedure on ancient bones that I got from anthropologists working on Bronze Age settlement excavation in Knezeves near Prague. We started extractions on 5 different samples 832A-C, 1136A, 1136D) but managed to measure DNA quantity (Quantifiler) only in one of the samples (1136A) and the others have shown strong inhibition even after extract dilution. The brownish sediment in the tube with DNA extract suggested that we co-purified one of the biggest enemies you can face while working with aged bone specimen – humic acids. However Jon D. tried to DNA type all extracts using the lab-made kit with short STR amplicons (TH01, Amelogenin, FGA, D18S51, Penta D, D2S1338, D21S11) and we got, not surprisingly, DNA profile just from the sample we managed to quantify (see picture No.1).

All other commercial "CODIS" kits provided negative, "no amplification" results in all cases. Even if this was a partial success, the main question of the trial on the Bronze Age bones remained, because we wanted to test the family relations between the skeletons plus to proof that mini-STRs can be routinely used even on such an old specimen. And we also needed to verify the results from sample 1136A in an independent study some time in the future. Even if Jon and I both left ICMP and our paths led to different places



Picture no.1 - EPG 1136A using Jon´s MiniPlex

we somehow felt the story will continue. Jon returned to North America and joined Bode Technologies, I moved back to Prague and realized my big dream of having my own research DNA laboratory. The breakthrough came at the beginning of 2007 when we finally got reasonable results with a new buffer formula for removing humic acids (HARB) from aged bone samples. What helped me the most was to review my notes from the early experiments done during the development of the DNA extraction protocol in Sarajevo. Notes that gave me no sense 5 years ago were now clear and understandable. In certain cases we received bones that were artificially coloured (blue, pink, sea-green, etc.) but even if we expected no DNA profiles those samples worked well. The explanation was quite simple when we realized the chemical structure and nature of humic acids and its strong affinity to certain iron ions. Having HARB in our hands, helping to remove a significant amount of humic inhibitors, was only a half of the success as we needed to get STR profiles out of our bone DNA extracts. Not only to finish the 1136A study from the bronze age sample but also to get results for numerous samples from other projects and cases we were working on. The second stroke of a good luck that year was the possibility to get from ABI the new AmpFℓSTR® MiniFiler™ even before its official release to the market. After a short validation, sensitivity and concordance study we tested MiniFiler on the first set of real samples – DNA extracted from latent fingerprints - where most of the epithelial cells captured on the lift already went through the programmed cell death cycle and the small amount of fragmented DNA in the final extract gave very poor results with standard identification kits. The results we got from fingerprint samples we much better than our expectations so we quickly moved to bone DNA extracts. First of all we re-tested older forensic cases where we got just partial STR profiles. Once we got full set of MiniFiler loci from all previously difficult samples we decided that now is the right time to continue with the 1136A study. The worst case scenario – no results or different STR profile – was our nightmare while waiting for the results. But this story ended up as a fairy-tale as we got a nice electropherogram (see picture 2) and when we compared the 1136A DNA profile made by Jon and with our results we saw a clear match in all overlapping loci (see table 1).



Picture no.2 – EPG 1136A using MiniFiler

Locus	Jon´s MiniPlex	MiniFiler
D13S317	-	9
D7S820	-	11,12
Ame	**XX**	**XX**
D2S1338	**18,23**	**18,23**
D21S11	**29**	**29**
D16S539	-	12,13
D18S51	**15,17**	**15,17**
CSF1PO	**-**	11,12
FGA	**22,23**	**22,23**
THO1	6, 9.3	-
Penta D	9	-

Table 1 - comparison of results from 2 independent studies on Bronze Age bones

After that success we were even able to get MiniFiler STR profiles from other Bronze Age samples (Knezeves burial site) (picture 3) and also from other excavation sites like Podlazice (12th century cemetery). But this story is to be continued as we started to work on a very ambitious project named Archeosteon where the main goal is DNA identification of Premyslid dynasty skeletal remains on Prague Castle. We will deal with samples of incalculable historical value, as Premyslids are credited as the founders of Czech statehood and the dynasty ruled over Bohemia from the ninth century until 1306.

What we learned during the months of testing MiniFiler is that we got very useful tool that will help up not only in forensic practice but also in DNA profiling of ancient bones and we really acknowledge its resistance to humic acids, ability to process fragmented DNA and to work with very low DNA concentrations. But one has to be careful, as due to MiniFiler´s high sensitivity it is necessary to routinely check the presence of even minute amounts of contaminating DNA in the laboratory and be able to identify the source of contamination.