

Obtaining Probative Results from Human Remains Recovered from Conflict Regions Using Multiple Systems in Parallel

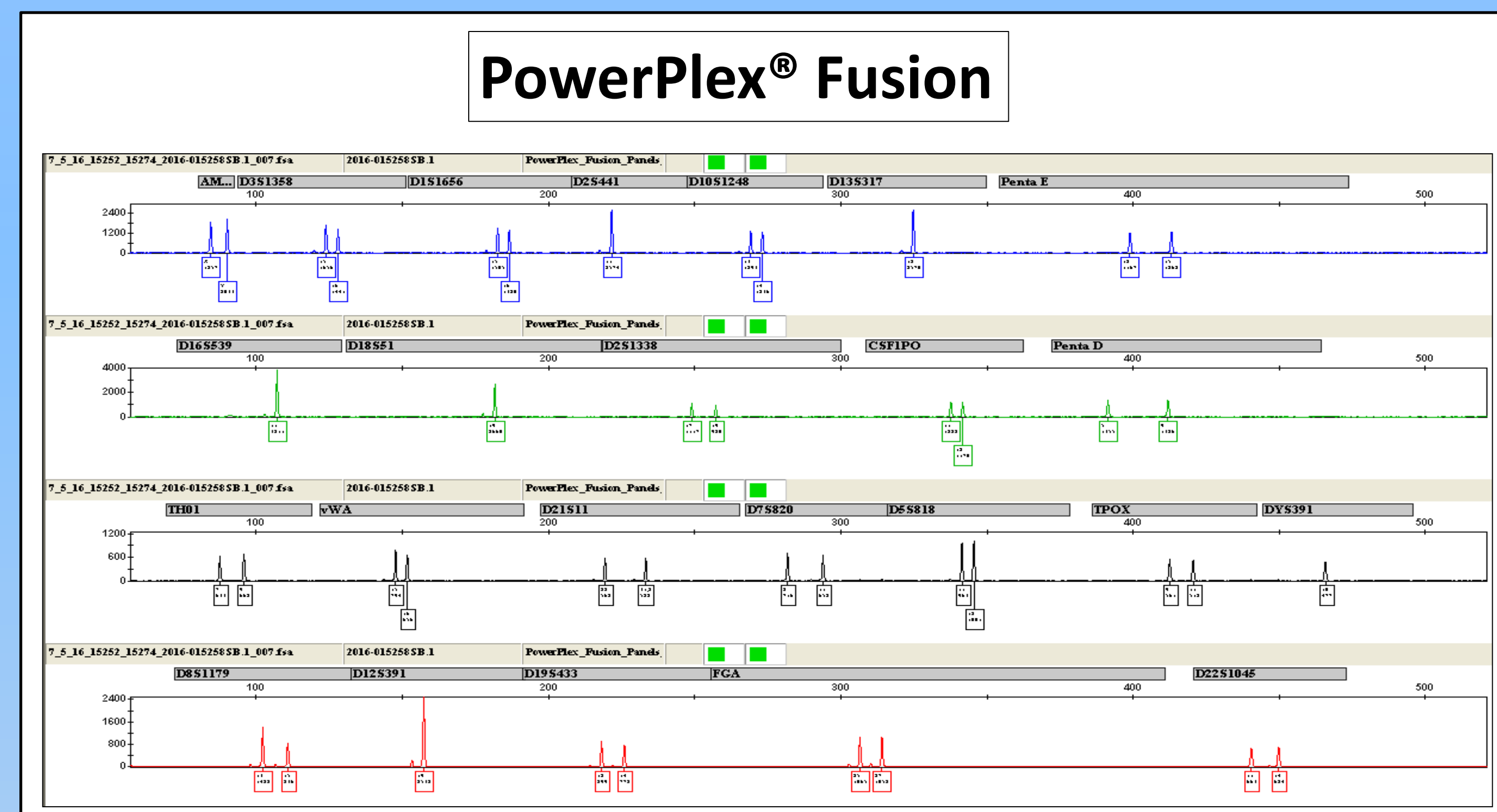
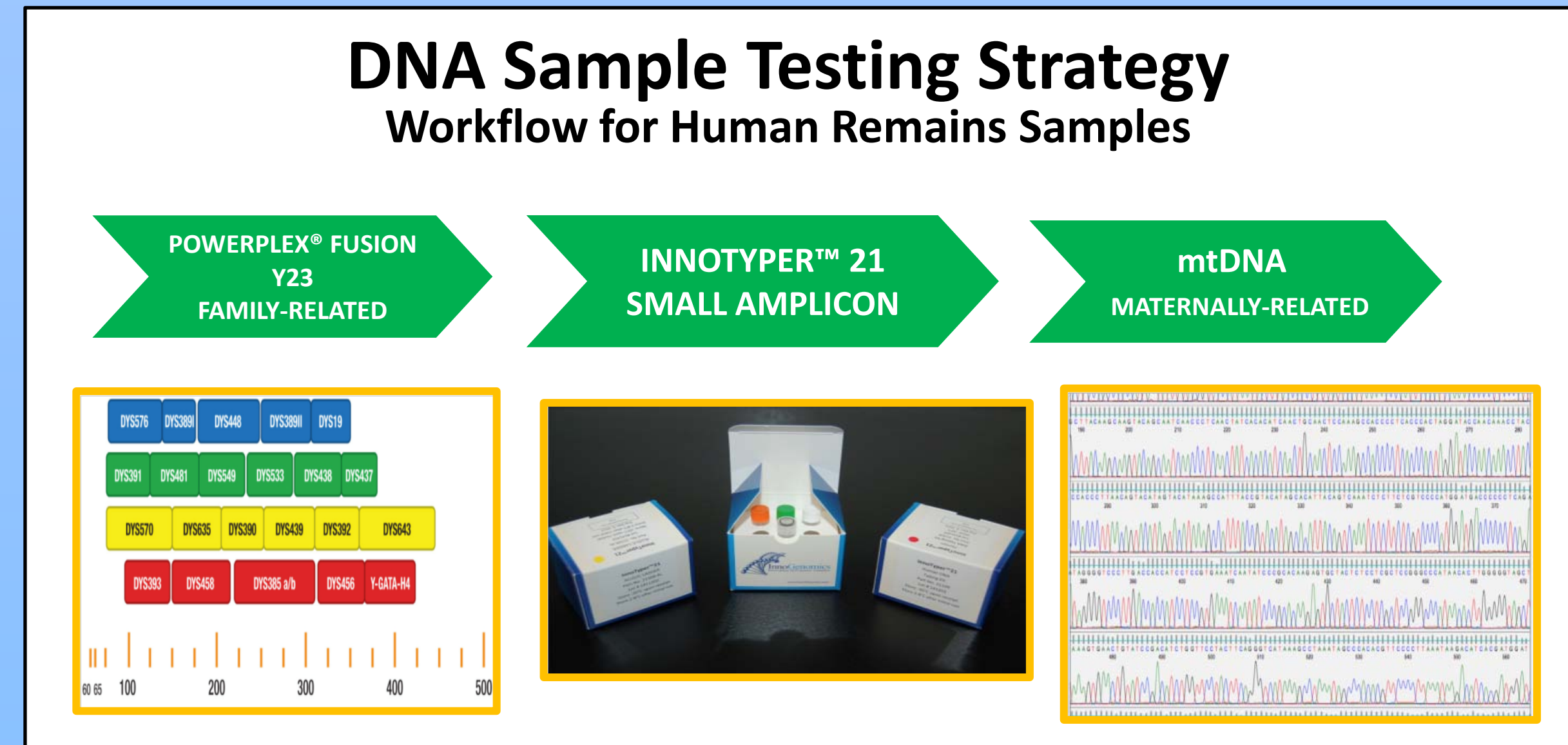
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Abstract

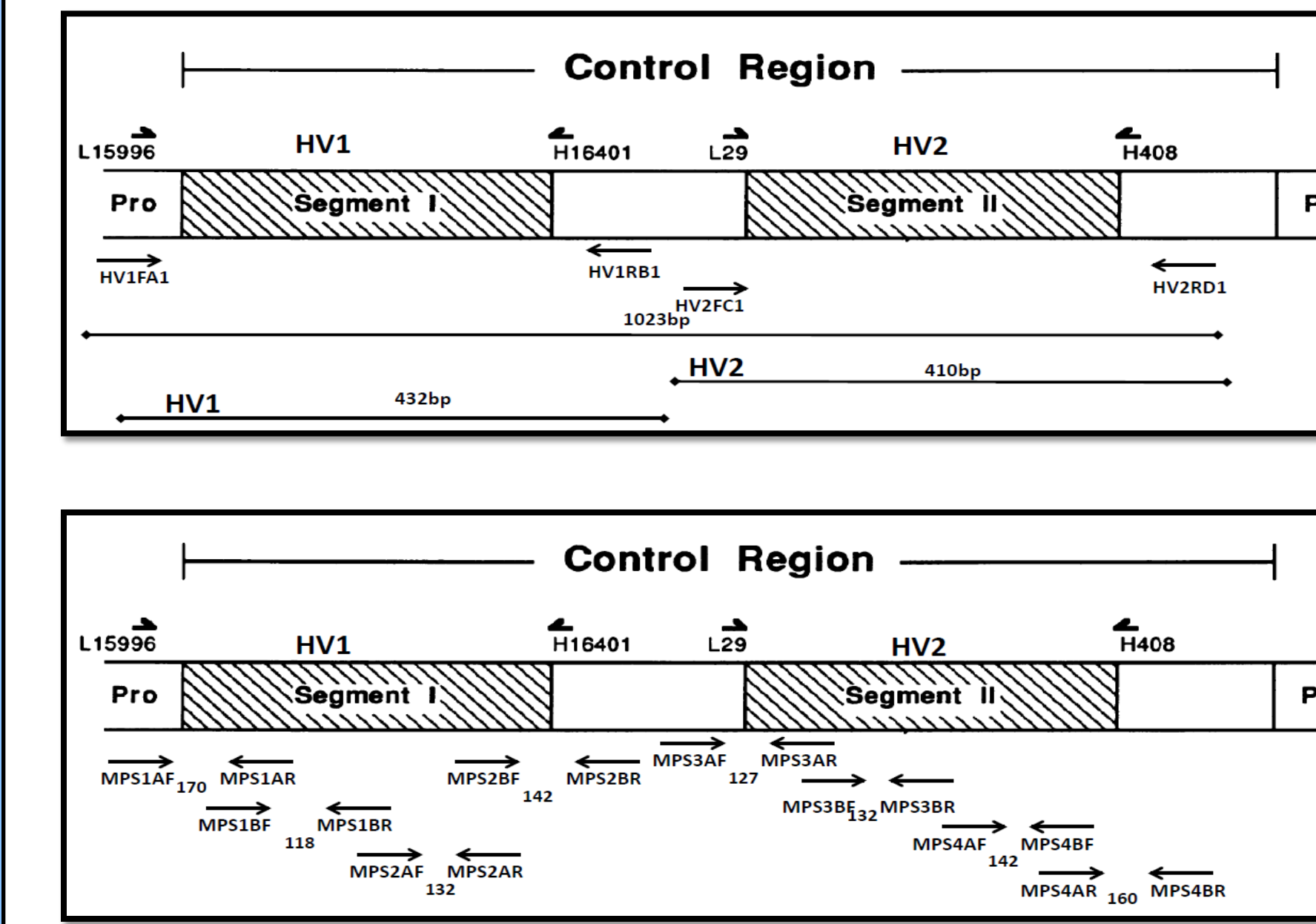
DNA Solutions has developed a specialized workflow for processing human remain samples recovered from multiple conflict regions around the world. This process yields maximum data from downstream systems, including the PowerPlex® Fusion System, a 24-locus autosomal multiplex for human identification, the PowerPlex® Y 23 System, a 23-locus Y chromosomal multiplex for human male identification, the InnoTyper™ 21 bi-allelic, small amplicon DNA typing system, and the mitochondrial sequencing of regions, HV1 and HV2. These samples are highly degraded and have been exposed to multiple PCR inhibitors, including salt, soil, and sewage. To date, multiple human remains' samples from different conflict areas have been processed using this workflow, obtaining informative results.

Introduction

The concept of our military's "leave no man behind" has been around a long time. Even in death, the idea of "no man left behind" is there. As search and rescue technology has improved so has our ability to identify and recover remains from fallen soldiers. Now using DNA technology and familial genetic matching, it is possible to positively identify remains of individuals lost during battles decades ago. The challenge has been to recover enough genetic material from the decayed remains to allow for the most advanced DNA testing technologies to yield results for comparison to living relatives. Often only partial data from nuclear DNA autosomal analysis can be obtained. Maternal relations can be confirmed using mitochondrial sequencing and male lineages can be confirmed using the Y-STR analysis strengthening the final genetic analysis to the point of a positive identification. The application of our workflow has resulted in the successful repatriation of a number of soldier's remains who have subsequently been given proper military burials.

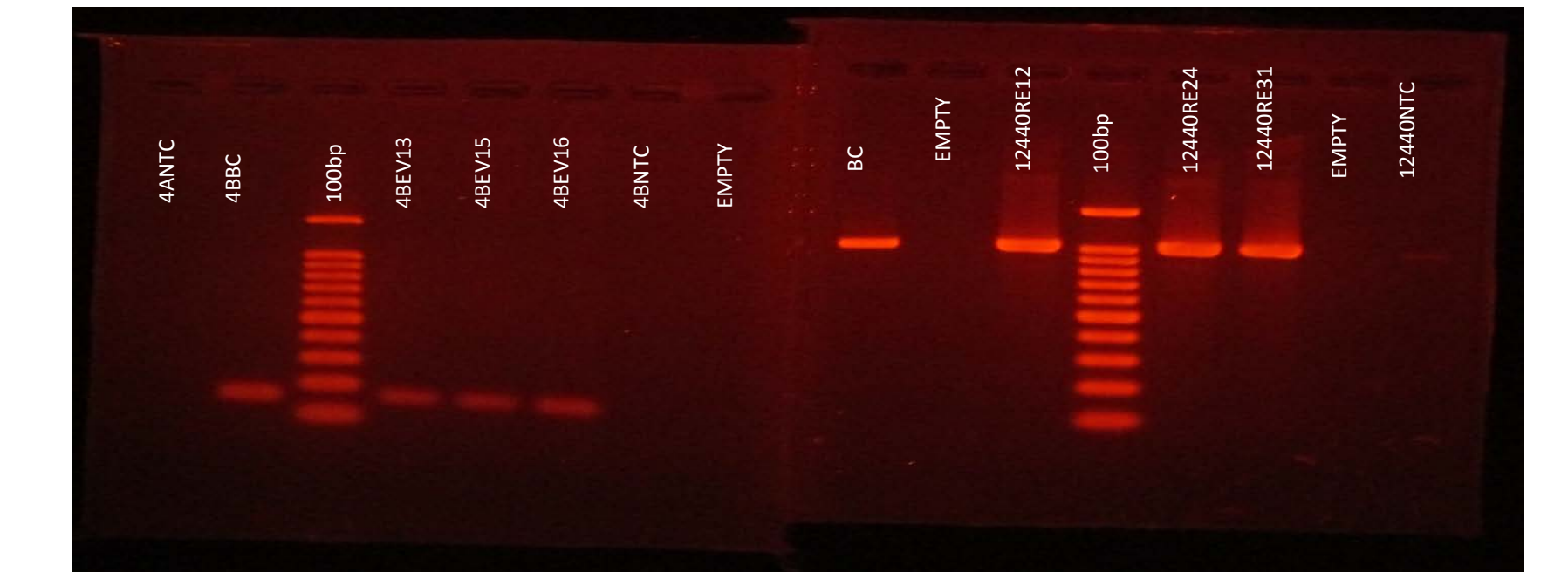


Mitochondrial Sequencing Strategies



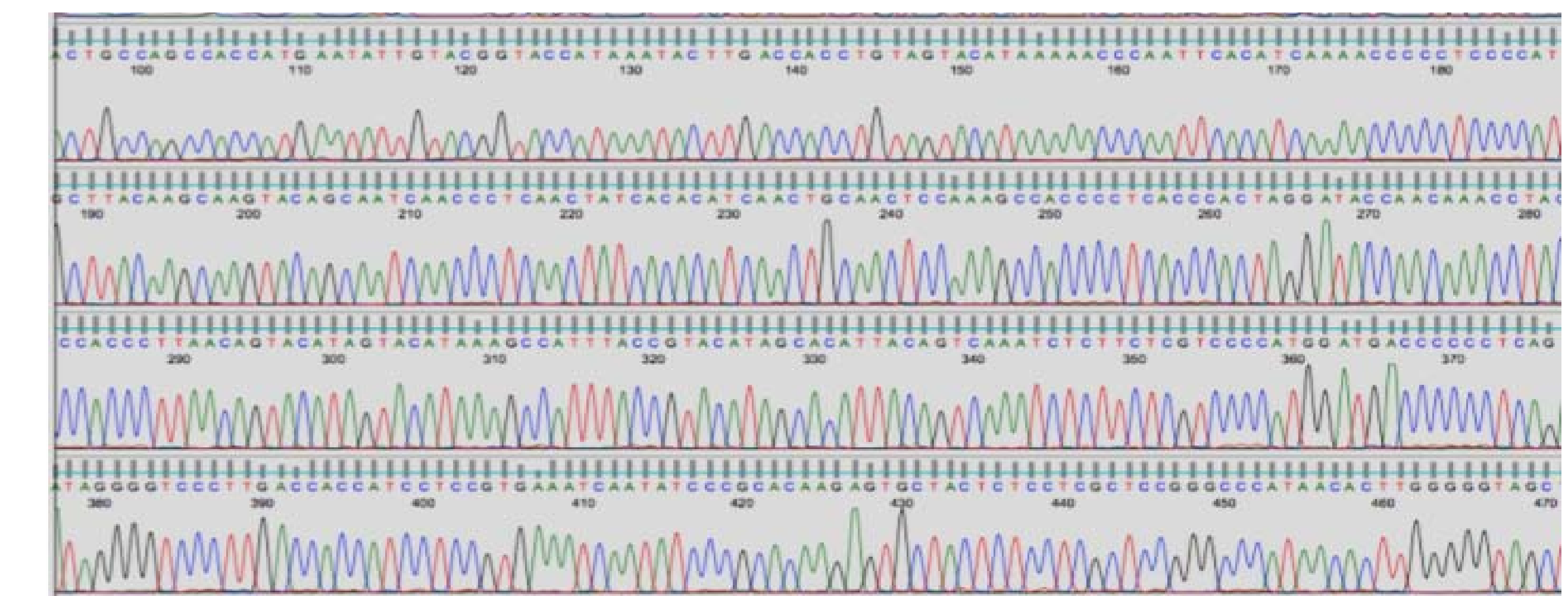
Mini Primer Amplification

Control Region Primer Amplification



Compromised Bone Samples

Reference Samples

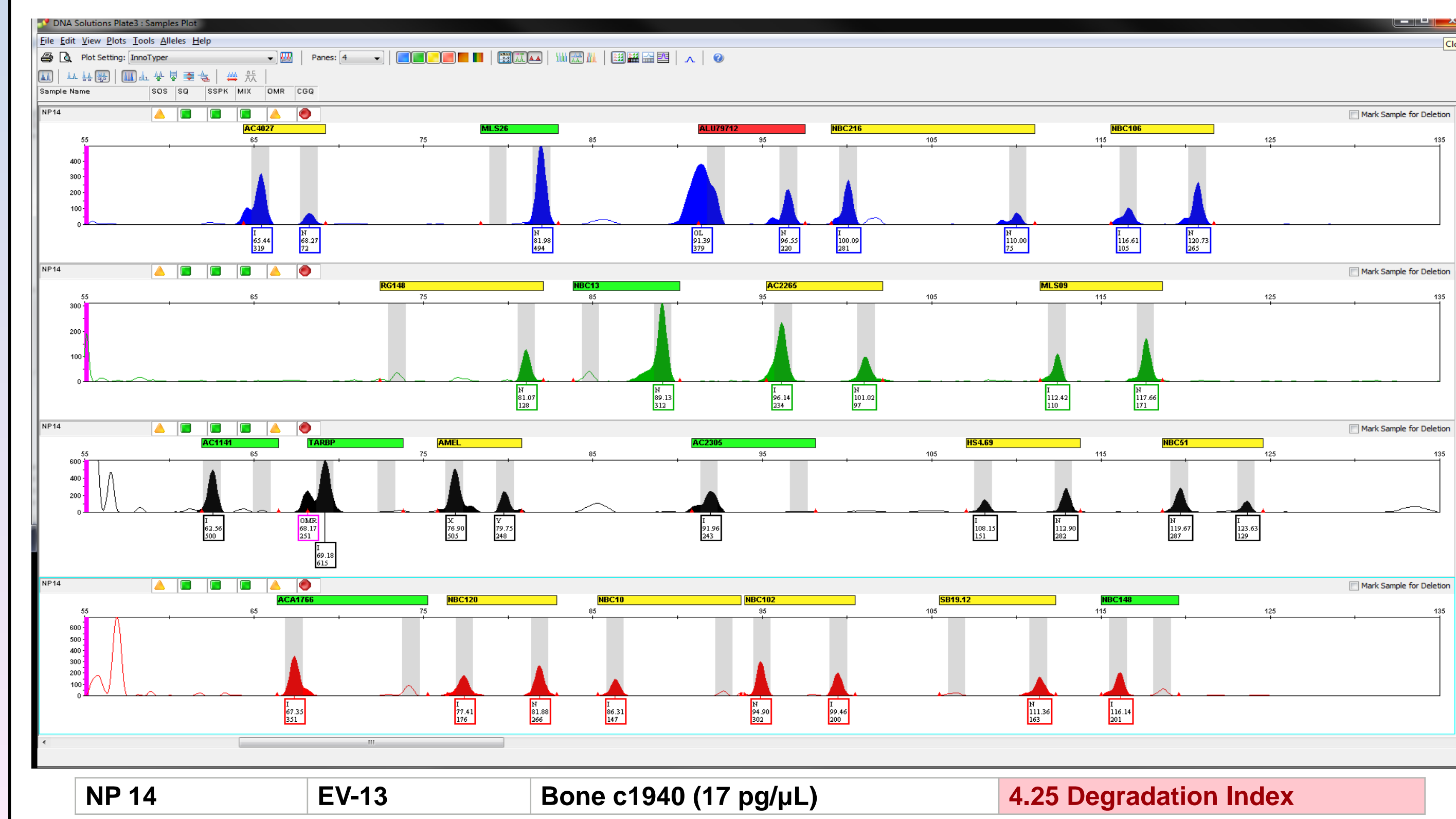
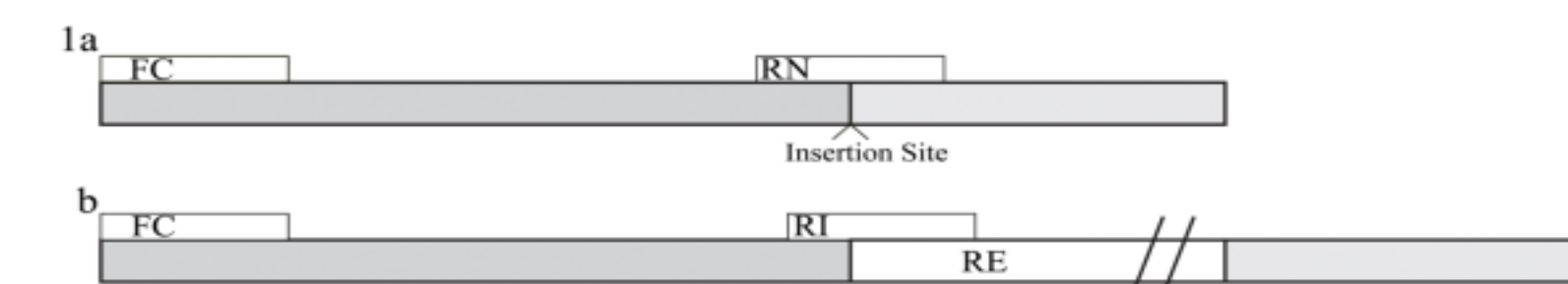


DNA Sample Testing Strategy Workflow for Human Remains Samples



InnoTyper® 21 Kit

- a small amplicon (~60-125 bp) DNA typing system for challenging forensic samples that is compatible with currently used instrument platforms.
- results from as little as 10 picograms
- for degraded samples such as old remains and hair shafts
- use of retrotransposable elements (RE)
- biallelic system – ave. POD 1X10⁸



Discussion:

Our use of multiple systems in parallel has proven effective in the identification of remains from conflicts dating back to WWII. Maximizing the recovery of DNA from highly compromised bones followed by a sensitive quantitation system that provides gender identification and a degradation index allows us to select the best method for downstream processing. We have demonstrated that autosomal/Y STRs, InnoTyper® 21 and mitochondrial D loop sequencing in combination yields sufficient genetic information to confirm relationships using multigenerational family reconstructions. Using the Brutus pedigree builder software, designed by Genetic Technologies, Inc, we are able to test relationship hypotheses using unlimited reference genetic data to maximize the probability of returning a high value for a true relationship.

Conclusions

- 1) Even with an optimized bone extraction protocol, each bone is unique and there is no way to know if it is going to yield good results or not.
- 2) Highly compromised bone sample rarely produce full autosomal STR profiles. Additional genetic analysis techniques are needed.
- 3) InnoTyper® 21 can produce full profiles when other autosomal STR systems fail.
- 4) Most remains will produce some mitochondrial results. Extreme caution must be used to avoid contamination of extracts from highly compromised samples.

Acknowledgements: Thank you to InnoGenomics™ for providing InnoTyper® 21 and the staff at DNA Solutions for their work to complete these studies.

Bone Extraction Procedure Optimization

Method

- Cleaning process (sample and environment)
- Decalcification
- Proteinase K digest
- SDS
- Ratio of bone to buffer important
- Magnetic bead purification

Metatarsal

