

Qiagen® Investigator® DIPplex Kit: Validation and Use With Parentage Section



Cases Involving Degraded/Inhibited Samples or Complex Relationships

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FORENSIC SCIENCE

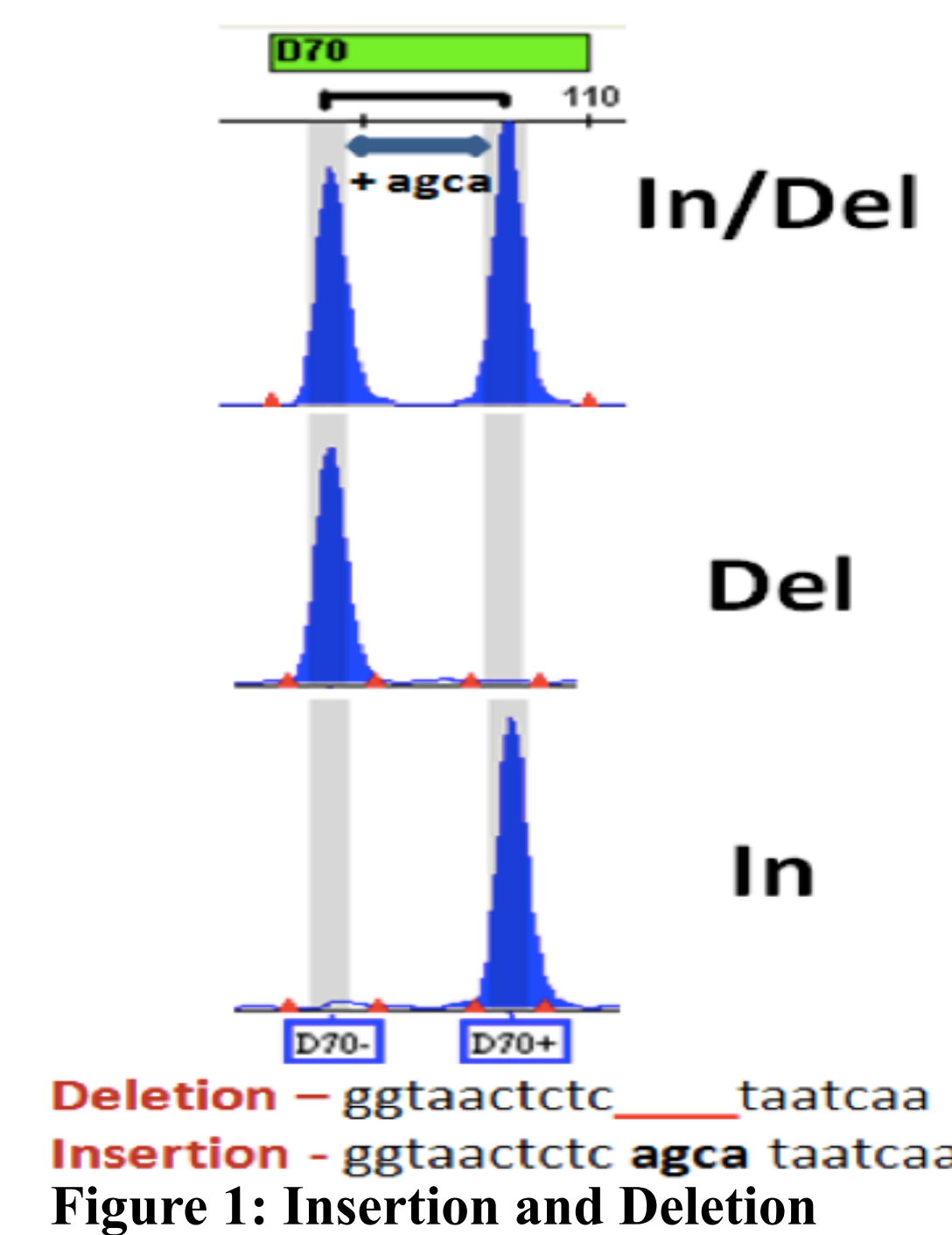
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Abstract

The Qiagen® Investigator® DIPplex kit is an amplification kit that targets thirty insertion-deletion polymorphisms (indels). Indels are ideal for the analysis of degraded samples and can be analyzed with the technology already present in most forensic DNA laboratories. The number of indels in the DIPplex kit and their low mutation rates make them suitable for relationship testing. An internal validation of the DIPplex kit was completed at the Marshall University Forensic Science Center (MUFSC) DNA Laboratory. At the MUFSC, the parentage section performs testing in body identification and questioned relationship cases. This study hypothesized that the use of an indel kit as a supplement to forensic STR profiling would provide additional information for degraded/inhibited samples and enhanced statistical significance in cases involving complex relationships. As hypothesized, the use of the DIPplex kit as a supplement to STR testing can provide more information in cases with degraded/inhibited samples and may improve the statistics of complex relationship cases.

Introduction

Short tandem repeat (STR) typing of DNA is considered the method of choice for human identification in the forensic field, but analysis of single nucleotide polymorphisms (SNPs) and insertion-deletion polymorphisms (indels) are options as well. The Qiagen® Investigator® DIPplex Kit allows for multiplex amplification of thirty biallelic autosomal indels plus amelogenin. Indels are biallelic DNA length polymorphisms characterized by the presence (insertion) or absence (deletion) of a certain DNA sequence made up of one or more nucleotides (Figure 1).



Indels combine the desirable characteristics of STRs and SNPs, including short amplicon sizes, low mutation rates, lack of stutter, and analysis using commercially available kits on instruments present in most forensic laboratories. Because of the short amplicons and low mutation rates, indels and SNPs are valuable in the analysis of highly degraded samples and complex relationship cases. As sequence polymorphisms, SNPs must be typed by complex sequencing methodologies that are not available in some forensic laboratories. With indels, the difference between alleles is based on size (like STRs) rather than detecting nucleotide substitution (like SNPs) and these size differences are readily resolvable using the same methods as STRs: simple end-labeled polymerase chain reaction (PCR) primers and capillary electrophoresis.

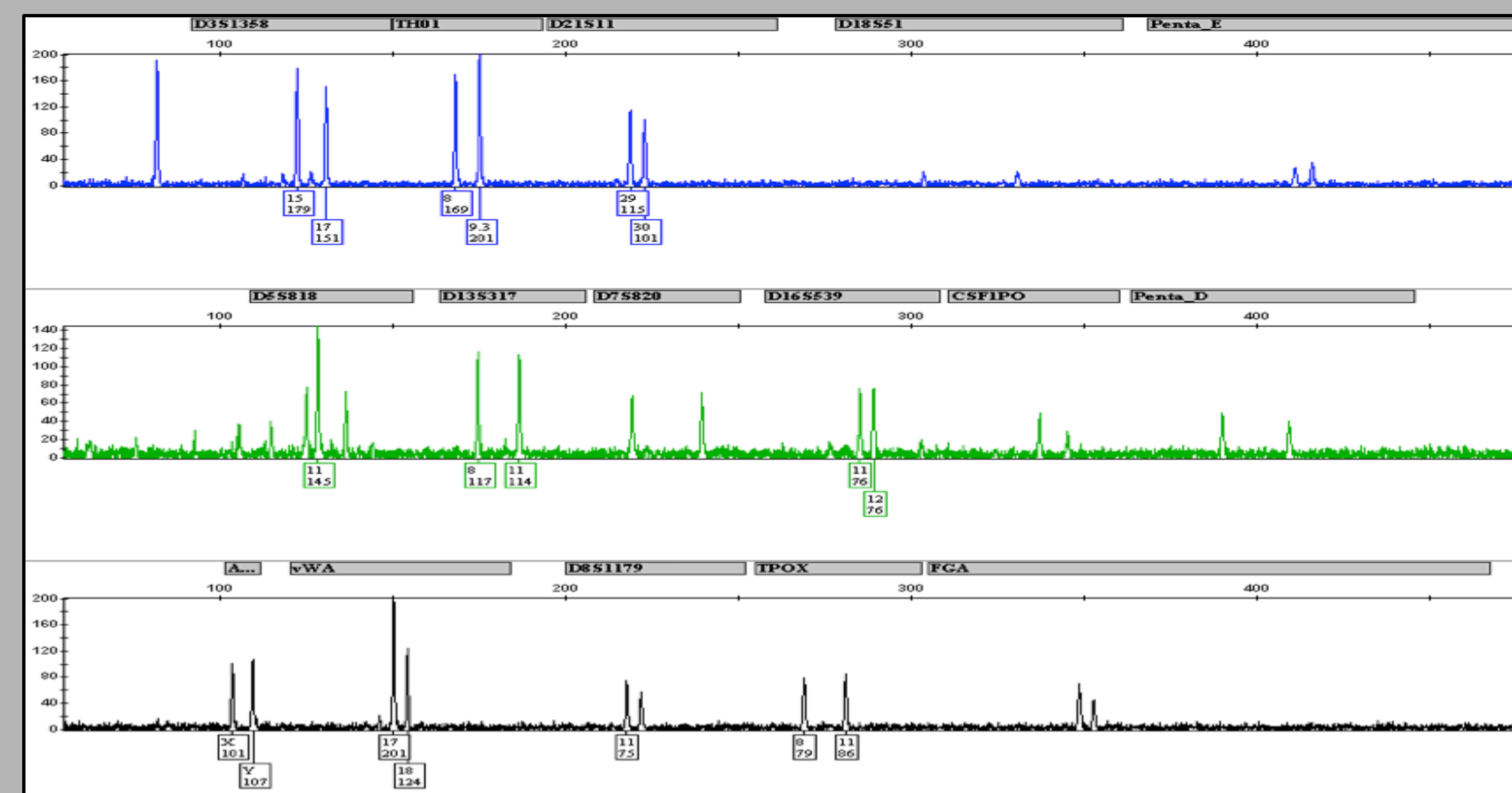
At the Marshall University Forensic Science Center (MUFSC) DNA Laboratory, the parentage section routinely encounters complex relationship cases or degraded/inhibited bone in body identification cases. It is hypothesized that an indel kit, such as DIPplex, can serve as a supplement to STR testing by providing more information in cases with degraded/inhibited samples and improving the statistical analysis in complex relationship cases. These statistics include the combined relationship index (CRI) and probability of paternity (POP). This hypothesis was evaluated during an internal validation study of the DIPplex kit at the MUFSC.

Cases

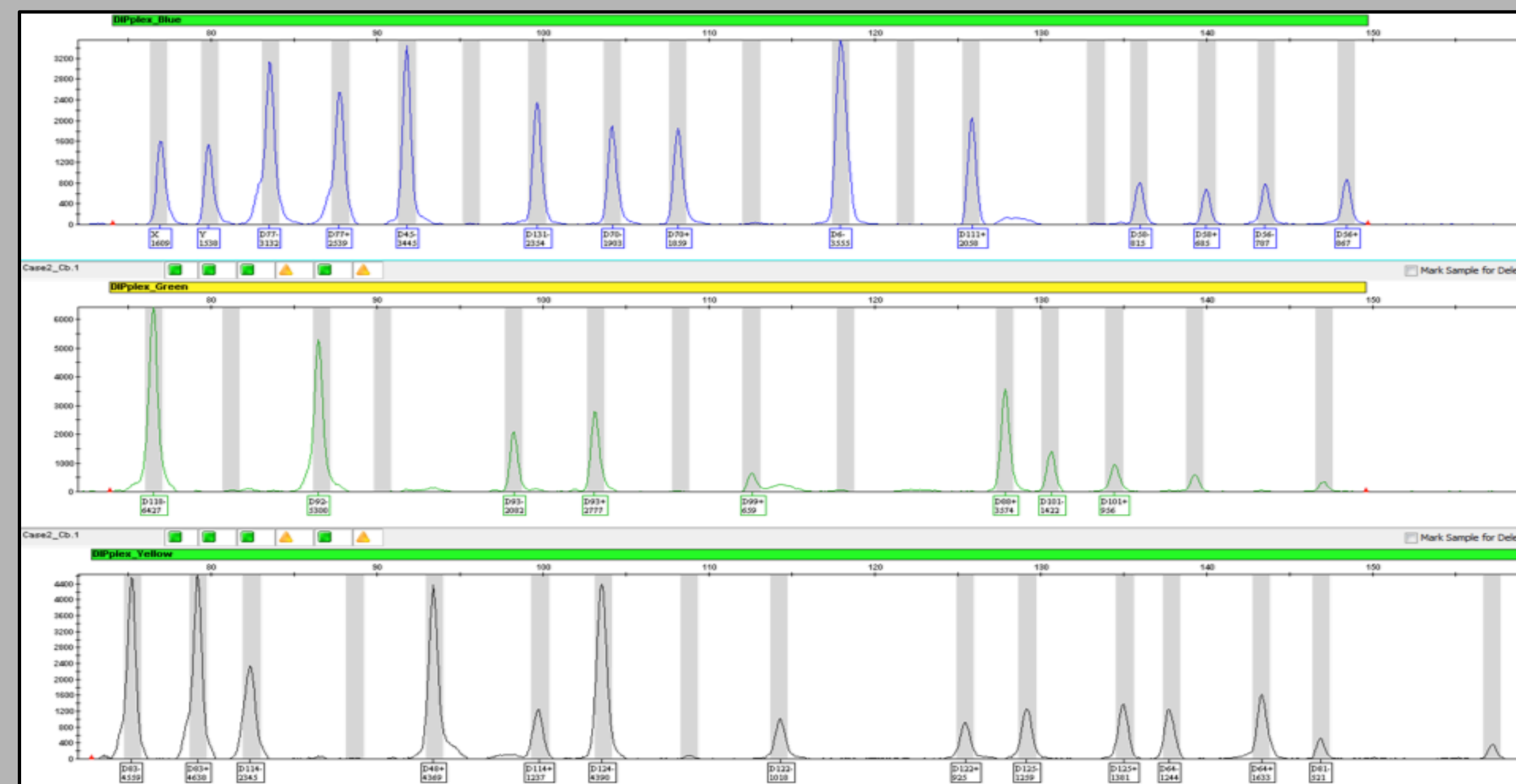
- **Case 1:** Degraded/inhibited bone from missing person case
- **Case 2:** Paternity analysis with multiple alleged fathers (AF) related to each other and the victim
- **Case 3:** Half sibship analysis
- **Case 4:** Product of conception (POC) with no identifiable parts that resulted in an STR mixture

Materials & Methods

- **Quantitation**
 - Applied Biosystems® 7500 Real-Time PCR System
 - Qiagen® Investigator® Quantiplex HYres Kit
- **Amplification**
 - Applied Biosystems® GeneAmp® PCR System 9700 Thermal Cycler
 - Qiagen® Investigator® DIPplex Kit
- **Capillary Electrophoresis**
 - Applied Biosystems® 3130xL Genetic Analyzer
 - Applied Biosystems® 3500 Genetic Analyzer
- **Analysis**
 - Applied Biosystems® GeneMapper® ID Version 3.2.1
 - Applied Biosystems® GeneMapper® ID-X Version 1.4



Case 1: STR Results- Dropout rate of 40%



Case 1: DIPplex Results- Dropout rate of 0%

Case 2: STR Results

- AF1- Victim's brother
 - No inconsistencies
 - CRI= 5,948,000
 - POP= 99.9999%
- AF2- Victim's father
 - 2 inconsistencies
 - CRI= 0.1107
 - POP= 9.9702%
- AF3- Victim's brother
 - 4 inconsistencies

Case 2: DIPplex Results

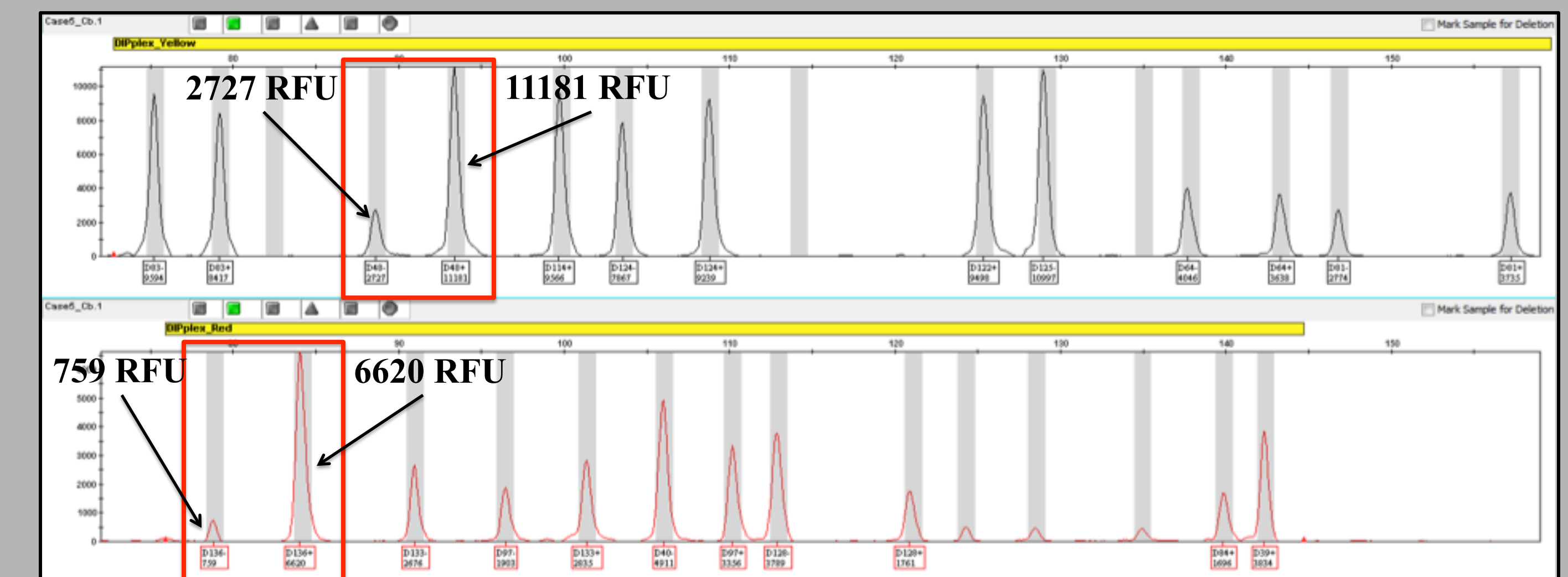
- AF1- Victim's brother
 - No inconsistencies
 - RI= 2368.1091
 - POP= 99.9578%
- AF2- Victim's father
 - 2 inconsistencies
- AF3- Victim's brother
 - 3 inconsistencies

Case 3: STR Results

- CRI= 38.9024
- POP= 97.4939%

Case 3: DIPplex Results

- CRI=1707.4460
- POP= 99.9415%



Case 4: DIPplex Results Exhibiting Presence of a Mixture

Case 4: STR Mixture Ratios

- Average= 3.04:1
- Range= 1.75:1 to 4.81:1

Case 4: DIPplex Mixture Ratios

- Average= 4.97:1
- Range= 1.93:1 to 11.73:1

Discussion and Conclusions

Whether the DIPplex kit is used as an alternative or a supplement to STR testing, it provides an additional method of DNA analysis for forensic laboratories. Analysis of a degraded/inhibited sample using DIPplex yielded a more robust profile compared to the STR profile. Complex relationship cases analyzed using this kit resulted in improved statistics for the cases. This kit was able to identify when a sample contains a mixture of DNA. In the future, additional degraded/inhibited samples and complex relationship cases should be analyzed using DIPplex in order to enhance the understanding of the advantages and disadvantages of the kits implementation.

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