DIPplex: Multiplex analysis of Deletion Insertion Polymorphisms for human identification

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Introduction

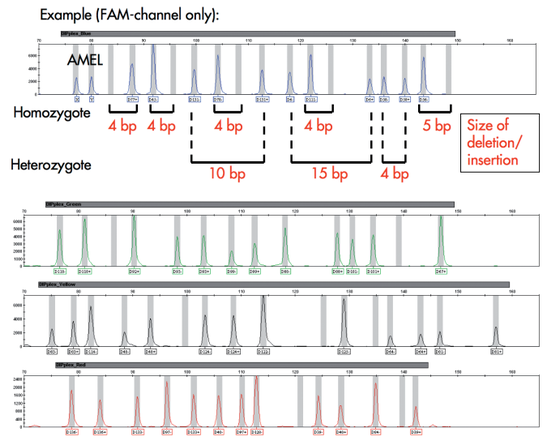
Often, compromised samples are the sole available source for retrieving genetic information for identification of persons. Nuclear DNA that can be obtained from such samples is typically of low concentration and strongly degraded due to adverse environmental conditions. Because of their high discriminatory power, short tandem repeats (STRs) have become the preferred type of genetic marker for human identification. However, STR loci require quite large fragments of genomic DNA to be amplified and drop-out of markers is frequently observed with degraded DNA. Reducing STR amplicon length as much as possible (so-called "mini-STRs") has been used as a strategy to deal with this limitation.

We have used an alternative approach for degraded DNA by choosing short deletion insertion polymorphisms (known as DIPs or Indels) to build up a multiplex assay that has a maximum amplicon size of ~150 bp. The Investigator™ DIPplex Kit combines amplification of 30 biallelic DIP markers and Amelogenin in a single PCR reaction. The assay follows the same workflow as STR assays and so may be performed by any forensic lab without the need for new instrumentation. A freely available software tool can be used for to conveniently interpret data. Selected DIP markers are distributed over 19 chromosomes and each one is at least 10 Mbp away from any commonly used STR marker. The assay provides a discriminatory power of 2.83 x 10^{-13} (combined probability of identity) based on a Caucasian population.

The DIPplex assay is highly sensitive and full profiles can be robustly obtained from only 63 pg of DNA. Artificially degraded DNA, as well as real-life samples, have been used to validate performance on compromised samples. Results show that the DIPplex assay returns more genetic information from samples such as old bones compared with a common STR assay.

Methods: DIPplex assay principle

The Investigator DIPplex Kit amplifies 30 biallelic DIP markers and Amelogenin. Deletion/insertion sequences of selected markers vary between 4 and 22 bp. All markers are arranged in 4 color panels (6-FAM™, BTG, BTY, BTR); corresponding amplicons range from 76 to 158 bp. Signals obtained from homozygous markers are of approximately double height compared to heterozygous markers. Amplified DIPs do not show stutter peaks.

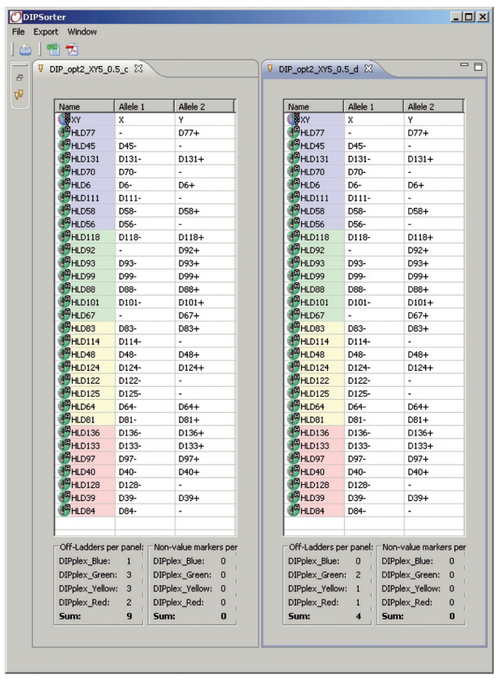


Example Investigator DIPplex electropherogram. 200 pg control DNA XY5 was amplified using the ABI GeneAmp 9700 thermal cycler. Analysis was performed on an ABI PRISM 3130 Genetic Analyzer. Allele assignment was performed using the GeneMapper® ID Software and the Investigator DIPplex Template files.

Assay workflow overview:

- USet up PCR reaction (automatable on QIAgility®): Optimal results are obtained with 200–500 pg DNA template.
- Run PCR on endpoint cycler: The assay has been validated on ABI GeneAmp® 9700, Eppendorf® Mastercycler® ep-S1, Biometra T1, Techne TC-512, and BioRad PTC-200 instruments.
- Set up samples for capillary electrophoresis (automatable on QIAgility): The procedure is the same as for STR analysis.
- Run capillary electrophoresis: The procedure is the same as for STR analysis using ABI PRISM® 310, 3100, 3130, and 3500 Genetic Analyzers.

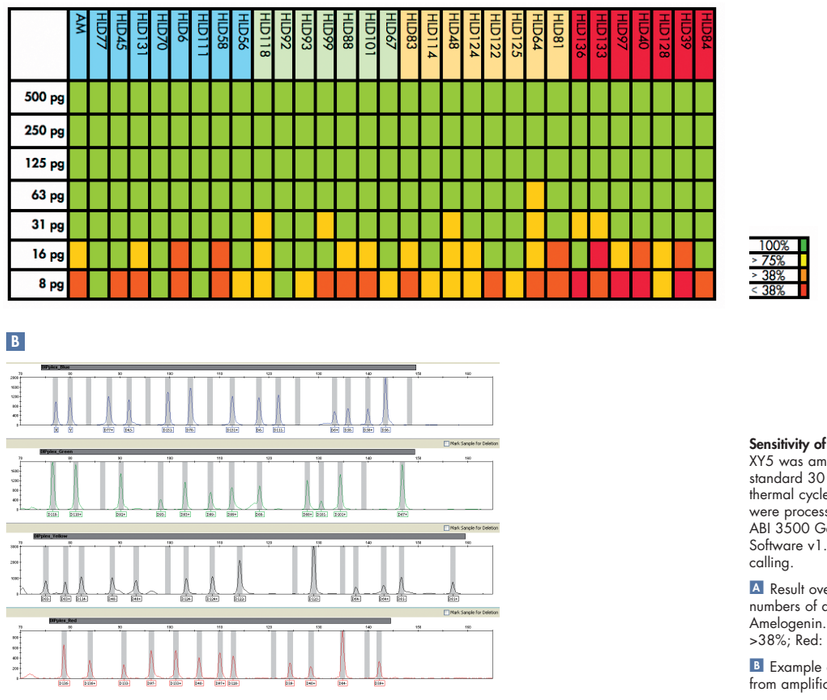
Methods: DIPSorter analysis tool



Example of Investigator DIPSorter genotype tables. Two sample editors are opened for comparison.

DIPplex data can be analyzed by standard software used for STR analysis, such as GeneMapper® ID, GeneMapper ID-X, or Genotyper® Software. For straightforward interpretation of data, it is however useful to rearrange genotype information. The DIPSorter analysis tool (available as freeware) allows genotype tables to be imported from the analysis software mentioned above. Allele information for each DIP marker is sorted into one line of a genotype table, which than can be exported again in *PDF or *CSV format. Single samples, as well as databases of imported samples, can be exported. Off-ladder alleles and markers with no allele detected are counted for each panel and displayed in the summary. Two sample editors can be opened at the same time to compare genotypes.

Results: Sensitivity

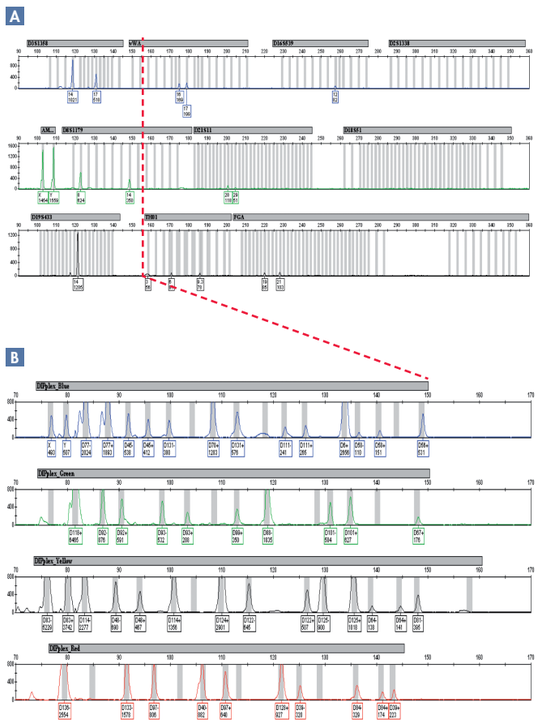


Sensitivity of the Investigator DIPplex Kit. Control DNA XY5 was amplified using 8–500 pg as template in a standard 30 cycle protocol on an ABI GeneAmp 9700 thermal cycler. 4 replicates of each DNA concentration were processed. PCR products were analyzed on an ABI 3500 Genetic Analyzer and GeneMapper ID-X Software v1.2, using 50 rfu as threshold for allele calling.

A Result overview. Color-coded representation of numbers of detected alleles for all 30 DIP markers and Amelogenin. Green: 100%; Yellow:>75%; Orange: >38%; Red: <38% of expected alleles.

B Example electropherogram. Full profile obtained from amplification of 63 pg control DNA XY5.

Results: Profiling of compromised bone samples



Electropherograms of an old bone sample amplified using **A** the AmpF/STR SGM Plus PCR Amplification Kit and **A** the Investigator DIPplex Kit. The red line shows the difference in amplicon size range between the two assays.

A femur bone sample from a World War II victim that had been buried for more than 60 years was analyzed using the Investigator DIPplex and the ABI AmpF/STR® SGM Plus® PCR Amplification Kit.

DNA was extracted using a modified phenol/chloroform protocol and further cleaned up using the QIAGEN QIAquick® PCR Purification Kit.

DNA was amplified according to manufacturer's instructions. All markers were successfully amplified using the DIPplex assay. As expected, due to the advanced DNA degradation, long STR markers dropped out when the AmpF/STR SGM Plus PCR Amplification Kit was used.

Conclusions

- The Investigator DIPplex Kit can be used with standard forensic PCR and CE equipment.
- Easy interpretation of results using DIPSorter Freeware software.
- Highly sensitive: Standard reactions only require 200 pg DNA, and full profiles are routinely obtained with less than 100 pg DNA.
- Highly suited for analysis of degraded DNA: Maximum amplicon length is approximately 150 bp.

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