Human Mixture Sample Analysis on the Spectrum Compact CE System

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1. Introduction

Human sample identification is used for confirming twin zygosity, biological relationships of research subjects, and sample provenance of archival tumor/normal samples. Human sample identification may also be required to identify human mixtures, including cell line contamination, histological sample contamination, mixtures of maternal and fetal samples, or tracking of xenografts.

The *GenePrint*® 24 System is a multiplex PCR-based genotyping method for research use only. The *GenePrint*® 24 System includes 22 polymorphic STR markers and two sex markers for sensitive human identification and mixture detection. After amplification, samples can be analyzed using the Spectrum Compact CE System for capillary electrophoresis. With this method, half of the alleles unique to a minor contributor were detected from a mixture containing only 3% minor contributor DNA. The degree of contamination or chimerism can also be estimated from this data based on the peak heights of alleles using ChimerMarker™ Software. This study demonstrates these sensitive applications of human mixture analysis on the Spectrum Compact CE System.

2. Methods

1 Human sample mixtures
Male and female DNA were quantified and mixed at ratios down to 1% male: 99% female

2 DNA genotyping by multiplex STR amplification
22 polymorphic STR markers and 2 sex markers were amplified in a single multiplex PCR reaction using the *GenePrint*® 24 System. All DNA mixtures and controls were amplified in triplicate using 2.5ng and 5.0ng DNA input.

3 Preparing samples for electrophoresis
Fluorescently-labeled PCR products were mixed with an internal sizing standard and heat-denatured with Hi-Di™ Formamide.

4 Preparing the CE instrument for electrophoresis
Spectral calibration was performed on the Spectrum Compact CE System using the *GenePrint*® 5C Matrix Standard and Polymer7.

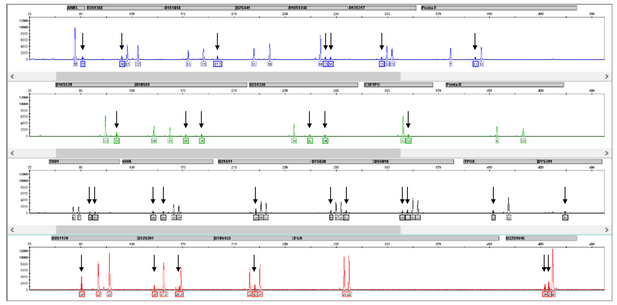
5 Capillary electrophoresis
Prepared samples were injected on the Spectrum Compact CE System using the pre-loaded fragment analysis conditions for Promega's 5-dye chemistries with Polymer7.

Injection voltage	1.5 kV
Injection time	9 seconds
Run voltage	13 kV
Run time	1290 seconds
Oven temperature	60°C

6 Data analysis
Qualitative data analysis was performed using GeneMapper™ Software v6 (ThermoFisher). Quantitative data analysis was performed using ChimerMarker™ Software v3.1.5 (SoftGenetics).

3. Human mixtures result in additional genotyping peaks

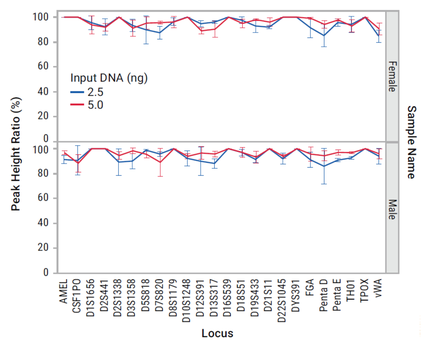
Human male and female DNA was mixed at various ratios down to 1% male:99% female, amplified using the *GenePrint*® 24 System and analyzed on a Spectrum Compact CE System. A representative electropherogram is shown of a 20% male:80% female mixture. All 30 unique male alleles were detected (indicated with arrows).



4. Peak heights are balanced in single source samples

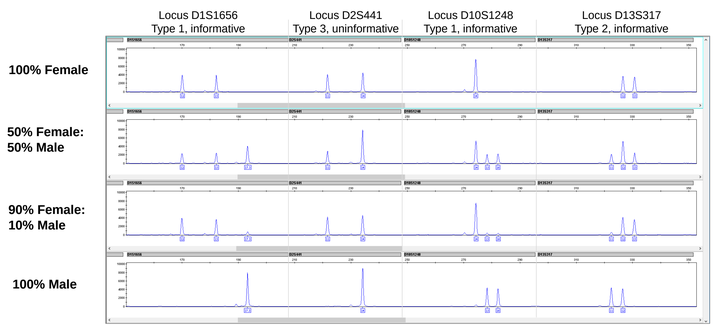
Peak height ratios are shown for single source female (top) and male (bottom) DNA samples amplified with the *GenePrint*® 24 System and injected on a Spectrum Compact CE System. Peak height ratios for all heterozygous autosomal loci were calculated as the lesser peak height divided by the greater peak height and expressed as a percent (mean ± stdev of n=3). Homozygous markers are shown as 100% by default.

These data demonstrate heterozygous peak height balance and support the use of relative peak height for estimating percent representation of an allele in a mixture.



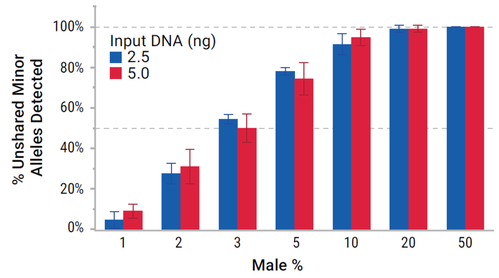
5. Peak height balance is proportional to allele frequency

Representative electropherograms in the blue (FAM) channel showing detection of alleles in a mixture. Loci are classified as Type I (both contributors have entirely unique alleles), Type II (contributors are heterozygous and have one shared and one unique allele), or Type III (at least one contributor has no unique alleles).[1] Loci are also classified as informative/uninformative based on the presence of unique alleles.[2] Vertical gray lines have been added to separate the loci for viewing purposes.

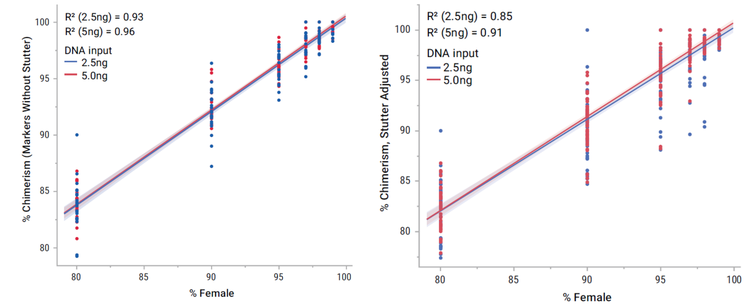


6. Mixtures can be detected with high sensitivity

Unique male alleles were detected in mixtures of male and female DNA with 1-50% male DNA. Mixtures were amplified with the *GenePrint*® 24 System and analyzed on a Spectrum Compact CE System. The percent of unique male alleles detected out of 30 possible (relative to the female sample) is shown as the mean ± StdDev, n=3.



7. Quantitative detection of human mixtures



Percent chimerism was calculated for mixtures of male and female DNA using ChimerMarker™ Software. Only informative Type I and Type II markers are included, using the 7 markers not affected by stutter (left) or 14 markers with stutter adjustment algorithms (right). Percent chimerism is plotted for each marker for triplicate reactions using either 2.5ng or 5.0ng DNA input. A linear regression with 95% confidence intervals was fitted to the data. R^2 values are reported.

8. Conclusions

The Spectrum Compact CE System provides excellent sensitivity for detection of human mixtures when paired with the *GenePrint*® 24 System

- Peak height balance was reproducibly high and provides a quantitative measure of allele representation in a mixture
- Half of the alleles unique to a minor contributor were detected from a mixture containing only 3% minor contributor DNA
- Detection of unique minor contributor alleles is linearly proportional to the sample representation in the DNA mixture using ChimerMarker™ Software

The Spectrum Compact CE System offers flexible benchtop access to labs interested in controlling their own capillary electrophoresis applications

- Human mixture analysis and contamination detection
- Cell line authentication
- Fragment analysis
- Sanger sequencing

1. Clark, J.R., et al. (2014) Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *British Journal of Haematology* 168, 26–37.
2. Thiede, C., et al. (1999) Rapid quantification of mixed chimerism using multiplex amplification of short tandem repeat markers and fluorescence detection. *Bone Marrow Transplantation* 23, 1055–60.