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The Fluidigm® SNPtrace™ Panel enables sample quality control by detecting sample contamination and accurately distinguishing related samples

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Introduction

Variable DNA sample quality and the lack of standardized sample tracking in biorepositories perpetuates sample ID errors caused by mishandling during sample collection, storage, or use. A study from the Center for Inherited Disease Research (CIDR) at Johns Hopkins University found that 3-4% of their biorepository samples were misidentified [B. Marosy et al., "Impact of sample pretesting in a high through-put genotyping facility," (poster presented at ASHG, Honolulu, HI, USA, October, 2009)]. Sample verification prevents erroneous use of misidentified samples in downstream experiments.

The Fluidigm® SNPtrace™ Panel facilitates quality control (QC) and tracking of DNA samples. The panel consists of 96 single-nucleotide polymorphisms (SNPs) selected by Dr. Andrew Brooks of Rutgers University Cell and DNA Repository to provide critical information regarding sample identity and quality.

In these experiments, we determined the limit of detection for sample contamination, distinguished closely related individuals, and paired tumor to normal samples using unique SNP profiles generated by the SNPtrace™ Panel. The SNPtrace™ Panel assays were run on the 96.96 Genotyping integrated fluidic circuit (IFC) in the BioMark™ HD System.

Conclusions

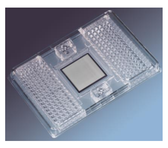
The SNPtrace™ Panel is an easy-to-use and cost-effective assay panel to track and QC human DNA samples in a biorepository or genomic core lab. The SNPtrace™ Panel generates a unique SNP profile for up to $2x10^{24}$ individual samples.

The unique SNP profile generated from the SNPtrace™ Panel distinguished closely related individuals from one another, identified sample similarities pairing tumor with normal samples, verified sample gender, and detected as little as 15% male contamination in a female sample.

Easy sample quality control

The BioMark™ HD System enables easy sample QC by high-throughput genotyping using the SNPtrace™ Panel and the 96.96 Genotyping IFC on the BioMark™ HD System.

1. Transfer your premixed SNPtrace™ assays and DNA samples in to the 96.96 Genotyping IFC (~10 min).



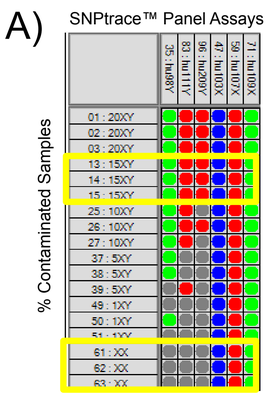
2. Place the IFC into the controller to create 9,216 individual reactions simultaneously (~90 min).

3. Place the loaded IFC into a BioMark™ HD System for PCR amplification and imaging (~90 min).

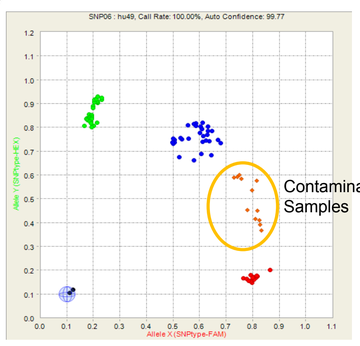
The SNPtrace™ Panel detected sample contamination

Verifying gender is a simple method for detecting contamination and sample quality control. The SNPtrace™ Panel contains 6 gender SNPs divided evenly between the X and Y chromosomes. To demonstrate that the SNPtrace™ Panel can verify the gender of known samples and determine the lowest percentage contamination detected, two 96.96 Genotyping IFCs were run with known samples and female samples contaminated with different percentages of male sample.

A)



B)

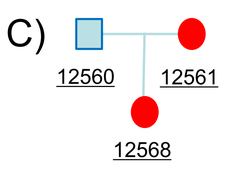


The SNPtrace™ Panel gender assays verified the gender of 56 individual samples. Analysis of the sample series suggested that the SNPtrace™ Panel detected as little as 15% male contaminant in female samples (A). Samples contaminated with 5-20% male DNA (orange) were also detected in autosomal assays when the male sample contained a distinguishing SNP (B).

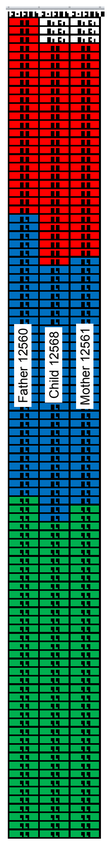
The SNPtrace™ Panel uniquely identified individuals

The 96 SNP assays of the SNPtrace™ Panel generate unique SNP profiles for every individual (C). In order to demonstrate that the SNPtrace™ Panel can distinguish closely related individuals, DNA (CEPH families from the Coriell Cell Repositories) from six mother/father/child trios and three generations of two families with 11 siblings were analysed with the SNPtrace™ Panel on a 96.96 Genotyping IFC in the BioMark™ HD System.

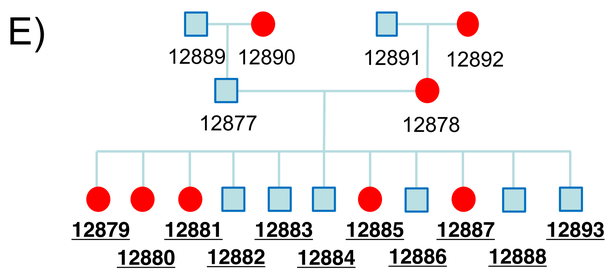
C)



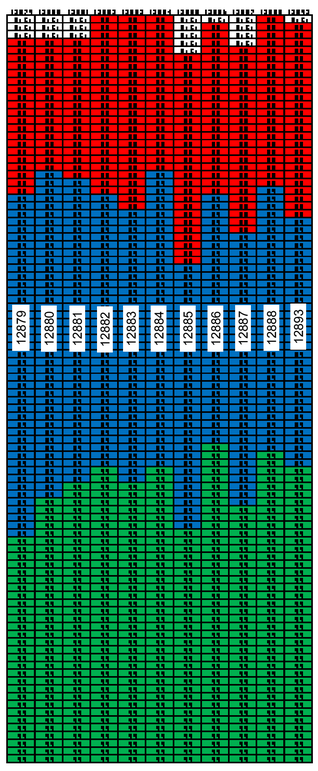
D)



E)



F)

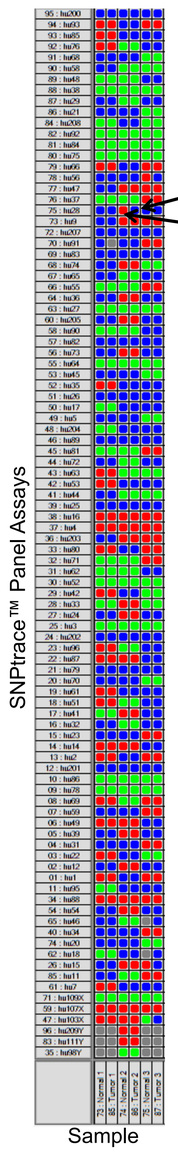


Color blocking of SNP profiles from the SNPtrace™ Panel uniquely identified closely related individuals from the trios and the two families. Underlined individuals from trio 12 (C) were identified (D), where the child is flanked by parents. The 11 underlined siblings in family 1463 (E) were also identified (F).

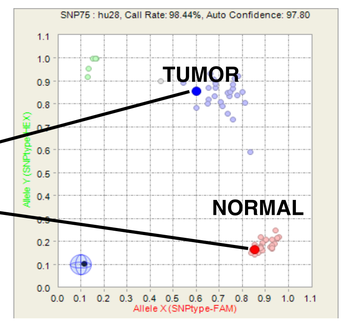
The SNPtrace™ Panel correctly paired tumor and normal samples

In order to demonstrate the ability of the SNPtrace™ Panel to accurately pair tumor to normal tissue, DNA from three tumor and normal tissue pairs were analyzed by the SNPtrace™ Panel.

G)



H)



Comparison of tumor and normal SNP profiles (G) demonstrated that the SNPtrace™ Panel paired a tumor sample to the correct normal sample. Tumor samples were 94.8-98.9% match to normal. Any mismatched genotypes were reviewed and determined to be true calls (H) possibly caused by genomic instability or change in the tumor sample.

Discussion

The SNPtrace™ Panel run in the 96.96 Genotyping IFC on the BioMark™ HD System enables high-throughput sample quality control in facilities that use or store human biospecimens.

This poster demonstrated that the SNPtrace™ Panel:

- Generated unique SNP profiles that distinguished closely related individuals
- Enabled tumor-to-normal sample pairing
- Verified the gender of samples and detected as little as 15% male DNA contamination in female samples

These findings suggest that the SNPtrace™ Panel is an ideal tool for providing and preserving sample quality control in biorepositories.

Lack of sample quality control results in expensive time-consuming mistakes and damaged reputations. For example, confounding data produced by sequencing a single mismatched tumor/normal pair can result in at least $10,000 ($5,000/sample) of preventable cost and months of wasted data analysis time. Conversely, systematic sample ID and QC testing to prevent sample misidentification costs as little as $10 per sample. These mistakes can be prevented with periodic sample quality control analysis with the SNPtrace panel.

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