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Rapid Identity DNA Matching using a 2-Phase PCR System Coupled with High Resolution Melting

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Introduction

The ability to rapidly match or distinguish 2 DNA samples from each other has many forensic uses. We have developed a method to rapidly determine if 2 DNA samples are identical within statistical probability. We use 2 Short Tandem Repeat (STR) markers and Human Leukocyte Antigen (HLA) A, B and C exons 2 as targets in a triplex PCR reaction. The two DNAs being tested are amplified and melted in 2 separate reaction phases within the same reaction tube. The PCR reactions are separated by a wax plug. After the amplification and melting of the separated reactions, the 2 phases are mixed and melted a second time. The second melt can then more easily distinguish sequence differences between the 2 DNAs due to the formation of heterozygous DNA pairs[1].

Materials and Methods

Primers and Reagents:

CSF1PO 118 bp 32%GC

F	ACAGTAACTGCCTTCATAGATA	0.5uM
R	TGTGTCAGACCCTGTTCTAAGTA	0.5uM

TH01 89 bp 46%GC

F	CCCATTGGCCTGTTCCTCCCTTATTTCC	0.5uM
R	GCAGGTCACAGGGAACACAGACTCCATGGTGA	0.5uM

HLA-ABC exon 2 117bp High %GC (highly variable)

F	CACTGCTCGYCCCCAG	1.0uM
R	GTCGTGTCGTCCASGTAGC	1.0uM

3mM $MgCl_2$ (Sigma), 200μM each dNTP (Bioline), 0.16U KlenTaq (DNA Polymerase Technology), 0.07U Anti-Taq Antibody (eEnzyme), 500μg/ml BSA (Sigma), 20ng DNA

Loading Capillaries

4μL of PCR reaction mix with DNA α is loaded into a LightCycler capillary tube (Roche) and centrifuged down. 2μL of Chill-Out Liquid Wax (BioRad) is then pipetted into the capillary and centrifuged down. The capillary is then chilled on ice in an aluminum LightCycler Centrifuge Adapter (Roche). This cooling step solidifies the wax layer above the 1st reaction mix, DNA α. 4μL of the 2nd PCR reaction mix with DNA β is then pipetted into the capillary tube and centrifuged down using the chilled centrifuge adaptor. This allows the 2nd reaction mix, DNA β, to be layered on top of the solid wax plug thereby separating the 2 DNA mixes.

Thermocycling Protocol

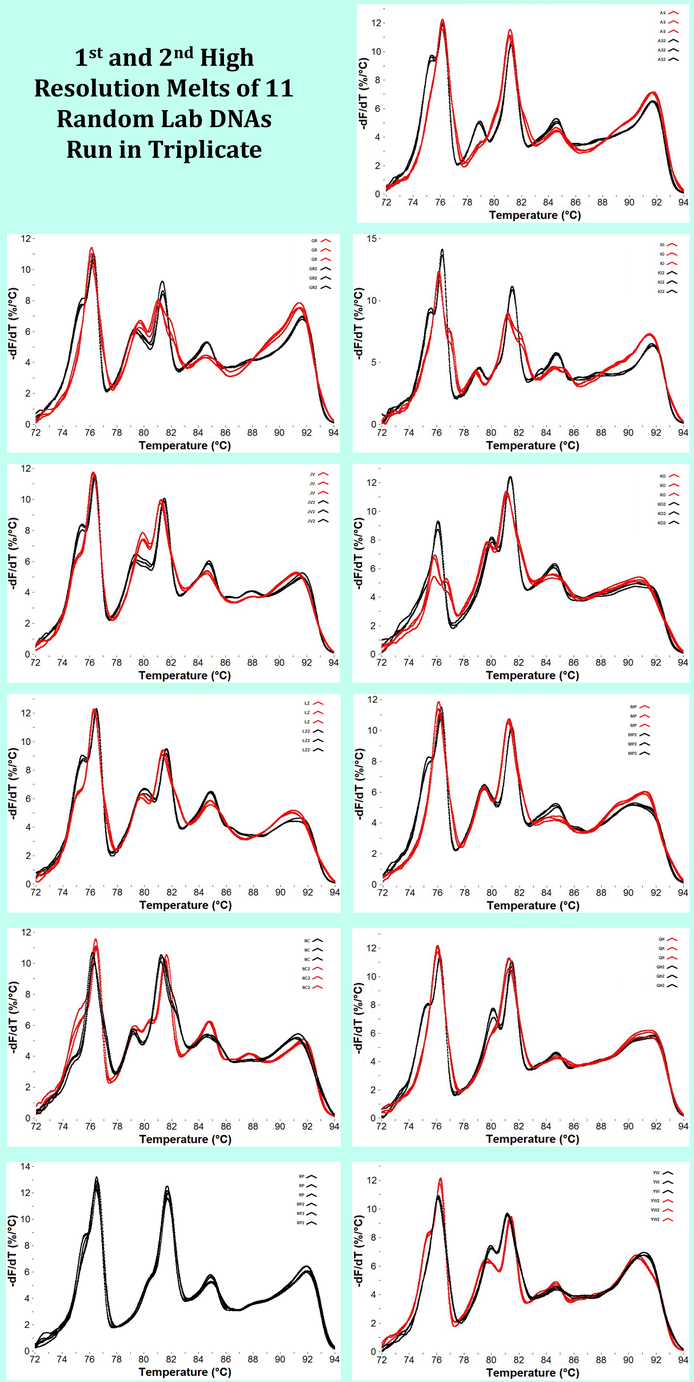
Using a LightScanner 32 (BioFire): Hot Start of 95ºC for 10s. Then 45 cycles of 96ºC for 1s, 58ºC for 1s, and 72ºC for 5s. One extra extension of 72ºC for 20s. Followed by a denature-cool cycle of 95ºC for 10s and 60ºC for 0s. High resolution melting is then performed, ramping from 68ºC to 98ºC @ 0.3ºC/s.

After the 1st high resolution melt, the capillary tubes are inverted and centrifuged to mix the 2 phases. The mixed phases are then re-centrifuged back down into the capillary tubes and place back into the LightScanner 32. The samples are denatured and cooled with 1 cycle of 95ºC for 10s and 60ºC for 0s. This forms heterozygous DNA pairs. High resolution melting is then performed on the samples again for the 2nd melt by ramping from 68ºC to 98ºC @ 0.3ºC/s.

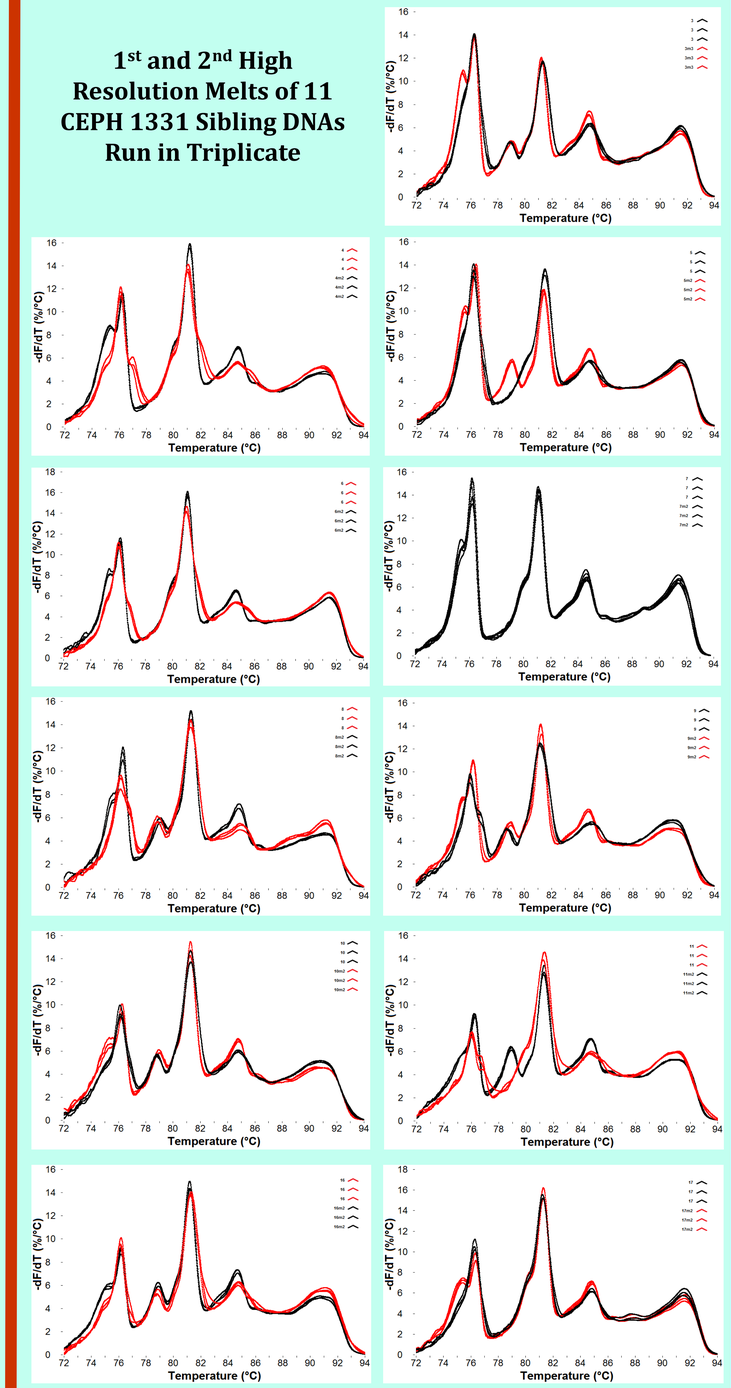
High Resolution Melting Analysis

The 1st and 2nd high resolution melts for each DNA reaction pair are analyzed together using LabView software[2]. The melt curves are normalized for background. The negative derivative of fluorescence with respect to temperature is calculated and then plotted verses temperature. The melt curves are then automatically clustered.

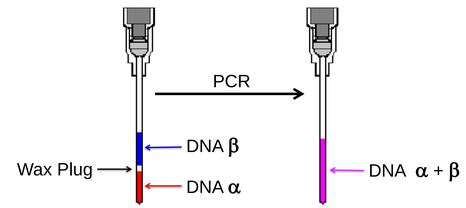
1st and 2nd High Resolution Melts of 11 Random Lab DNAs Run in Triplicate



1st and 2nd High Resolution Melts of 11 CEPH 1331 Sibling DNAs Run in Triplicate



2 Phase PCR



Results

The lowest melt region of 72ºC to 77ºC on the plotted data is the STR CSF1PO amplicon. The middle melt region of 78ºC to 82ºC is the STR TH01 amplicon. The high melt region of 83ºC to 93ºC is the HLA-ABC exon 2 amplicon. The analyzed data for each DNA set shows that the high resolution melts are distinguishable from the randomly chosen "β" DNA from that set. The same "β" DNA was used within each DNA set (lab DNAs or CEPH 1331 siblings) so that 1 of the 11 DNAs in the data set is a match.

Conclusions

This method can be used to rapidly (< 30 min.) confirm whether a pair of DNA samples are identical. The probability of sibling DNAs matching the 3 alleles is 1 in 64. The probability of 2 unrelated DNAs matching is considerably lower but difficult to calculate due to the exceptional variability of HLA-A, B and C. The probability of a match using just the 2 STRs on 2 unrelated DNAs would be approximately 6.6%. To further test the 2 phase technique, we will conduct a double blinded study.

References

1. Erali M, Wittwer CT., Methods. 2010 Apr;50(4):250-61.
2. Palais R, Wittwer CT., Methods Enzymol. 2009;454:323-43.

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