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

Robert J. Pryor1, Luming Zhou1, Bob Palais1,2, Yotam Ardon1 and Carl T. Wittwer1
1. Department of Pathology, University of Utah, 2. Math Department, Utah Valley University

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.

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

Materials and Methods

Primers and Reagents:
- CSF1PO 118 bp 5’/3’GC AGAAAATACCTTCTTAATAATA 0.5µm
- TH01 69 bp 5’/3’GC CACTGCTCGYCCCCAG 1.0µm
- HLA-ABC exon 2 117bp 5’/3’ GCAGGTCACAGGGAACACAGACTCCATGGTGA 0.5µm
- TH01 89 bp 5’/3’GC TGTGTCAGACCCTGTTCTAAGTA 0.5µm
- TH01 89 bp 5’/3’GC TGTGTCAGACCCTGTTCTAAGTA 0.5µm
- HLA-A, B and C exons 2 as targets in a triplex PCR reaction

PCR Loading Capillaries

4µl of PCR reaction mix with DNA α is loaded into a LightCycler capillary tube (Roche) and centrifuged down. 2µl of Chlo-Init 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 separating the 2 DNA mixes.

Thermocycling Protocol

Using a LightScanner 32 (BioFire): Hot Start of 55oC for 10s. Then 45 cycles of 95oC 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 1s and 60oC for 5s. High resolution melting is then performed, ramping from 68oC to 98oC @ 0.3°C/s.

After the 1st high resolution melt, the capillary tubes are inverted and centrifuged back down into the capillary tubes and placed back into the LightScanner 32. The samples are denatured and cooled with 1 cycle of 95oC for 10s and 60oC for 5s. This forms heterozygous DNA pairs. High resolution melting is then performed on the samples again for the 2nd melt by ramping from 68°C to 88°C @ 0.3°C/s.

High Resolution Melting Analysis

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

Results

The lowest melt region of 72°C to 77°C on the plotted data is the STR CSF1PO amplicon. The middle melt region of 83°C to 89°C 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 0.6%. To further test the 2 phase technique, we will conduct a double blinded study.

References


Contact

Rob.Pryor@path.utah.edu
University of Utah/Pathology 883 Coleman Dr.
Salt Lake City, Utah 84108
Rob.Pryor@path.utah.edu