B8 Eye and Skin Color Identity Single Nucleotide Polymorphisms (SNPs) Screening Using Polymerase Chain Reaction (PCR) High Resolution Melt (HRM) Assays

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Learning Overview: After attending this presentation, attendees will understand the advantages of using a single-step assay to screen eye and skin color phenotype characteristics for unknown samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing screening tools that can be used post-PCR, and potentially within a quantitation assay, to determine phenotypic traits of eye and skin color, potentially eliminating the need for additional post-PCR assays. The screening assays can also be used to check sequencing results that are difficult to interpret.

Determining phenotypic characteristics can be extremely beneficial in forensic investigations, providing information to aid in identifying unknown suspects or missing persons or in corroborating eyewitness testimony.[1,2] This tool, commonly called Forensic DNA Phenotyping (FDP), can also aid in forensic facial reconstruction by providing phenotypic traits or pigment-related features of unidentified skeletal remains that would previously not be known.[1-3] SNPs are the key to FDP and are used to predict eye and skin color using SNPs that have been previously linked to phenotypic gene expression.

One forward and two reverse PCR primers were designed for each of six SNPs. Each SNP has a major and minor allele, and was differentiated by the two reverse primers, one with a GC-clamp to shift the melt temperature higher and one without. Proper PCR amplification was checked using several DNA standards including 2800M, K562, 9948 that were previously sequenced using the Verogen ForenSeq™ DNA Signature Prep Kit using Primer Set B on the MiSeq® FGx through Massively Parallel Sequencing (MPS). The targeted SNPs included rs12913832 (HERC2 gene), rs12203592 (IRF4 gene), rs12896399 (SLC24A4 gene), rs16891982 (SLC45A2 gene), rs1426654 (SLC24A5 gene), and rs885479 (MC1R gene). Following PCR, an HRM assay was performed using a Rotor-Gene® Q real-time PCR instrument. The QIAGEN® ScreenClust® software was used to identify allele(s) present based on their differing melting temperatures and clustering. After the primers were optimized for accurate identification of the SNPs for the standards, buccal swabs were collected from volunteer donors as approved by the Towson University Institutional Review Board. Following DNA extraction and quantitation, each sample was analyzed with the optimized primer sets for each SNP, and the results were used to make phenotypic predictions of eye and skin color using decision tree models for each. The allele(s) present at the six SNPs was confirmed for each donor using Sanger sequencing. PCR HRM assays can be used as preliminary screening tools to determine eye and skin color and are much more cost-effective than MPS. PCR HRM can also be used to determine allele calls when the number of MPS reads at the SNP does not clearly support a determination of heterozygote or homozygote.

Reference(s):

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2. Kayser M., Schneider P.M. DNA-based prediction of human externally visible characteristics in forensics: Motivations, scientific challenges, and ethical considerations. *Forensic Sci Int Genet* 2009;3(3):154–61.
3. Walsh S., Chaitanya L., Clarisse L., Wirken L., Draus-Barini J., Kovatsi L., et al. Developmental validation of the HIrisPlex system: DNA-based eye and hair colour prediction for forensic and anthropological usage. *Forensic Sci Int Genet* 2014;9:150–61.

Forensic Science, SNPs, DNA Typing

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