# Single multiplex system of twelve SNPs: validation and implementation for association of SNPs with human eye and hair color

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### Introduction

Human pigmentation, more accurately, pigmentation of the eyes and hair, is one of the most obvious externally visible characteristics for each individual. The possibility of predicting these phenotype characteristics, based solely on biological deposits found at a crime scene, is one of the purposes of forensic genetic investigations in the near future. Various studies have revealed that single nucleotide polymorphisms (SNPs) within the *HERC2*, *OCA2*, *MCIR*, *SLC24A5*, *SLC45A2* and *TYR* genes have been strongly associated with pigmentation trait variations in Caucasian populations. According to current knowledge, the highest prediction value for eye color variation is based on SNPs inside the *OCA2* and *HERC2* genes. Other genes were also identified as contributing to eye color variations such as *SLC24A5*, *SLC45A2* and *TYR*, but to a much lesser degree [1, 2, 3]. For hair color prediction, we consider all the aforementioned genes to be informative, in addition to the *MCIR* gene which is, according to previous work performed, strongly associated with red/blond hair color [2]. In light of the aforementioned studies in the genetics of human eye and hair color, our research was focused on a single multiplex genotyping assay including twelve SNPs markers in order to better understand the predictive potential of SNPs marker within the Slovene population.

### Materials and methods

- the buccal epithelia cells from 105 Slovene volunteers were colected
- DNA was extracted using Chelex extraction method
- DNA concentrations were determined by real-time PCR
   a total of 12 autosomal SNPs, were amplified by PCR in 25 μl reactions:
- 0,03125-4.0 ng DNA
- AmpFISTR PCR reaction mix (AB)
- 8 mM MgCl2
- 0,1-1,7 µM of each primer
- 2-5U AmpliTaq Gold DNA polymerase (AB)
- samples were amplified through <u>35 cycles</u>:
   30 s at 95°C, 30 s at 60°C and 30 s at 72°C,
   previous denaturation at 95°C for 10 min
   final incubation at 72°C for 7 min

excess primers and dNTPs were removed

- SBE reactions were performed in 8 μL reaction:

   1 μL purified PCR products
   4 μL SNaPshot® reaction mix (AB)
   0.01-1.7 μM of each SBE primer
- thermal cycling for SBE was performed as <u>30 cycles</u>
   96°C for 10 s, 50°C for 5 s and 60°C for 30 s
- excess nucleotides were removed

• the SBE products were analyzed by capillary electrophoresis using ABI Prism 3130 Genetic Analyser (AB) with POP-4 and 36 cm capillary length array.

analysis was made using GeneMapperID ver. 3.2



## **Results**

- our assay (Slplex) works optimally between 1.0 4.0 ng of template DNA
  the drop-out appears at only 62.5 pg of DNA, when using 5U AmpliTaq Gold DNA polymerase (AB)
- the allele frequencies for all SNPs based on Slovene samples were comparable to those of HapMap CEU subjects
   the SNP rs1426654 was the only one monomorphic, what indicates that it is fixed in European population
- all SNPs were in Hardy-Weinberg equilibrium
- we established to confirm that SNPs rs1129038 and rs12913832 are in perfect linkage disequilibrium (LD) and could be considered as single haplotype
   two SNPs rs1129038/rs12913832 are in LD with SNP rs1667394
- the SNPs rs1667394 and rs1800407 are in LD
- the HERC2 genes (rs1129038, rs12913832) carries most of the eye color predictive information
- SNPs rs1129038/ rs12913832 have probability value 0.96 for blue (rs1129038 AA/TT) or brown (rs12913832 GG/CC) eyes, respectively
- the additional four SNPs just slightly increased the prediction accuracy
   for brown eyes: rs1393350 GG/TT
   for blue eyes: rs1667394 AA/TT, rs16891982 GG/TT and rs7170989 GG/CC)
- seven SNPs which could predict hair color with more or less powerful predictive information:
   more accurate predict darker hair color: rs1129038 AA/TT, rs1291383 GG/CC, rs1667394 AA/TT, rs1805005 GG/CC and rs7495174 AA/TT
   more accurate predict lighter hair color rs1393350 GG/CC and rs7170989 GG/CC







## Conclusions

The SNP assay presented is a robust and sensitive DNA tool regarding amplification and regular determination of the homozygosity/ heterozygosity for each SNP included, based on a small DNA input. On the basis of these facts, the SIPlex assay could be suitable for use in forensic casework. Although all the SNPs included in SIPlex are not particularly statistically significant regarding color prediction, they may still be considered relevant for further validation – either in the population of Slovenia or elsewhere.