EXPERIMENT 5 – EVOLUTION OF THE HUMAN GENOME – BLOND HAIR AND BLUE EYES

Introduction

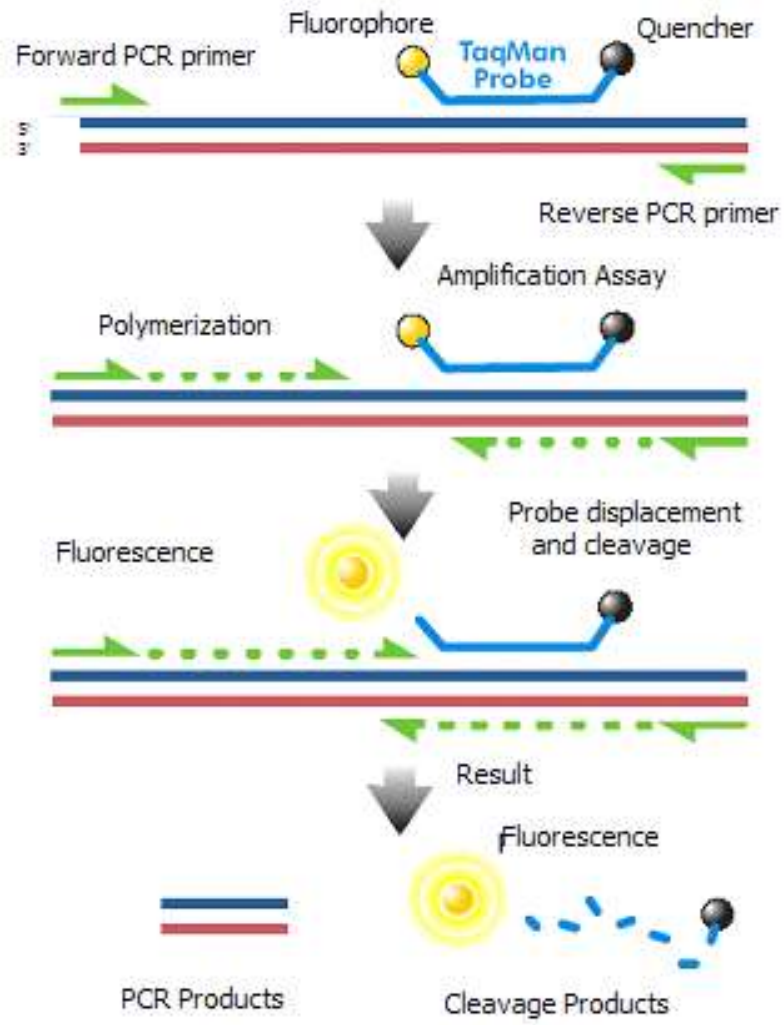
Human hair and eye colour are complex phenotypes that depend on over 100 different genes. Yet a large component of the observed variability has been identified to be associated with several different SNPs that map to within or near the human *OCA2 (*oculocutaneous albinism type 2) and *MC1R* (melanocortin-1 receptor) genes, with some additional association with SNPs in the *SLC45A2/MATP* (membrane-associated transporter protein) and *TYR* (tyrosinase) genes. Polymorphism of these genes is responsible for variations seen in pigmentary phenotypes both within and between human populations, but rarer non-functional mutations also occur which are associated with dramatic changes in pigmentation such as albinism. The genotype of the *MC1R* gene in the Neanderthal showed that at least some of these human forerunners likely had red hair and green eyes[1].

Source: Public Domain Creative Commons. https://commons.wikimedia.org/wiki/File:Eye_color.jpg.

Recent genome wide studies have evaluated the accuracy of predicting human eye and hair colour in heterogenous populations of Northern European, as well as in African American, African Caribbean and Japanese populations using a limited number of SNPs within intron 1 of the *OCA2* gene (rs4778138, reference [2]) and within intron 86 of the *HERC2* gene (rs12913832, reference [3]). The rs4778138-C allele is associated with darker hair and brown eye colour. This SNP also contributes to a 3 SNP haplotype of TGT (rs7495174-T/C, rs6497268-G/T and rs4778138-T/C) which is associated with blue or green eye colour[2].

The rs12913832 SNP is located 21 kbp upstream of the *OCA2* gene in a region highly conserved in animals. Genotype-phenotype correlations reveal that this SNP exhibited strong associations with skin colour ($P = 0.003$), hair colour ($P = 0.0001$) and eye colour ($P = 5 \times 10^{-23}$) in European populations[4]. The rs12913832-T allele, associated with darker pigmentation and non-blue eye colour, appears to act as an enhancer of *OCA2* expression, by facilitating transcription factor binding and chromatin looping between the enhancer site and the *OCA2* promoter, leading to elevated *OCA2* expression[5]. In contrast, the blue eye colour and lighter pigmentation allele, rs1291382-C, displays lower levels of transcription factor binding and less chromatin looping, resulting in lower *OCA2* expression. This is readily observed in cultured melanocyte cells genotyped for this SNP[4]. The P-protein, encoded by the OCA2 gene, is a proton transporter required for acidification of the melanosome, the organelle within melanocyte cells responsible for pigment synthesis. A lower pH is required for optimal activity of the enzyme tyrosinase, the rate-limiting enzyme for pigment synthesis, and which is mutated in *OCA1* [6].

In this experiment, you will determine your genotype for the rs12913832 and rs4778138 SNPs, and correlate this with your pigmentary phenotype. This will be done using a probe-based realtime PCR assay. Like other PCR reactions you have done this week, this method uses a forward and reverse primer, but the PCR reaction also contains two related probes, that have the same nucleotide sequence except for a single base difference that corresponds to the SNP being genotyped. In addition, each probe has a covalently bonded quencher (Q) and an allele specific fluorophore (F), which when in close proximity to the fluorophore, will absorb the light energy emitted by the fluorophore and no signal (meaning no amplification) will be detected. As the PCR proceeds, the exonucleoase activity of DNA polymerase cleaves the quencher from the fluorophore, allowing fluorescence signal to be detected. Because binding of each probe occurs in a sequence-specific manner, and each allele-specific probe is bonded to a different fluorophore, the presence of each allele can be specifically detected by comparing the intensity of emitted fluorescence at specific wavelengths. This is often called TaqMan chemistry, but this is a trade-name.



Source: Public Domain Creative Commons:
https://commons.wikimedia.org/wiki/File:Taqman.png

TaqMan PCR

Equipment required (per class)

StepOne PCR Thermal cycler
Tomy HF-120 microfuge
Centrifuge capable of holding 96-well plates.
96-well PCR plate with barcode (to fit StepOne PCR machine)

Equipment required (per group)

0.5 - 10 µL LabServ pipette and filter tips

Procedure:

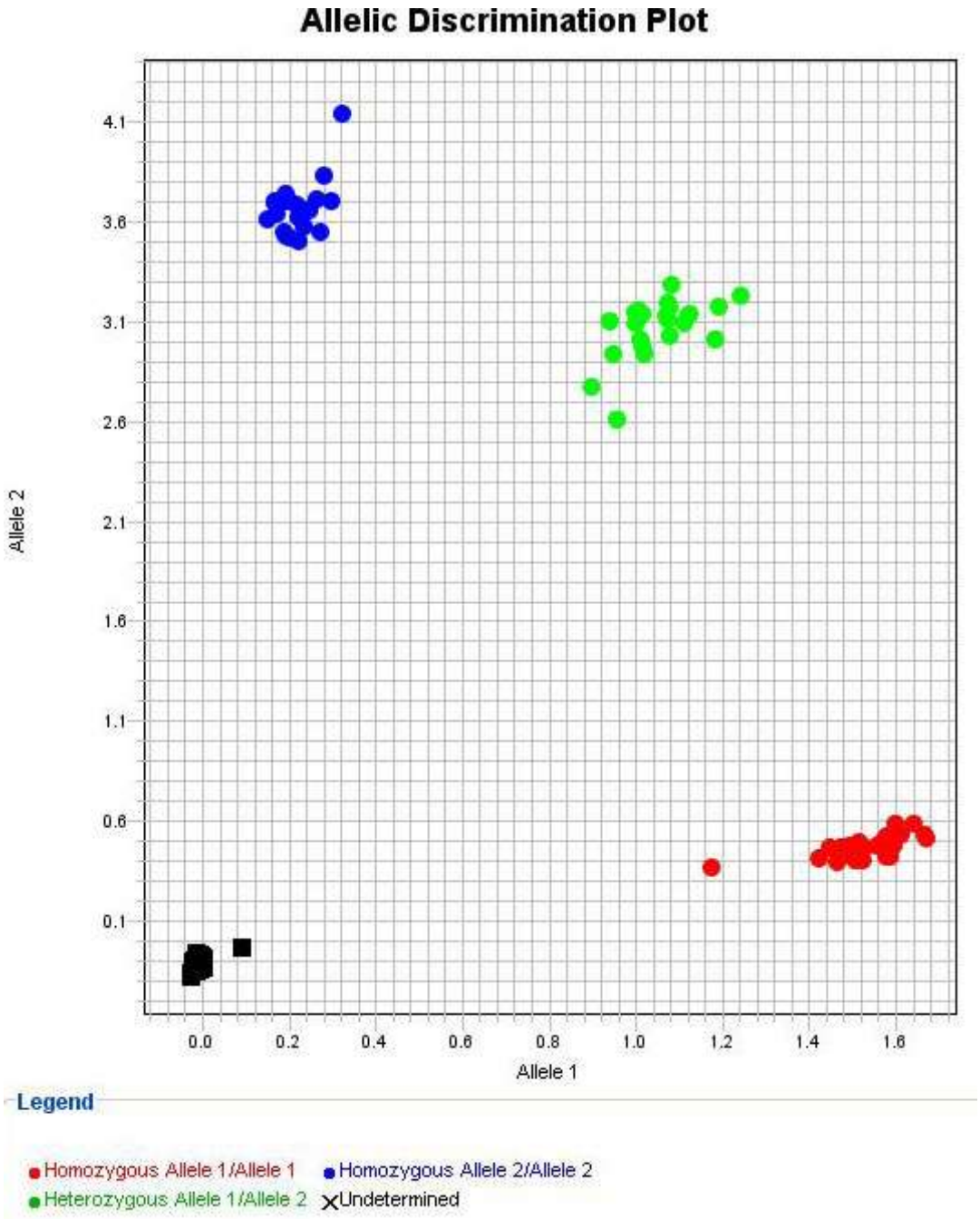
1. (*done for you*) For each SNP, prepare TaqMan genotyping master mix:

Component	**× 1**	**× 50**
2 × TaqMan PCR master mix	10 µL	500 µL
Nuclease-free water	4.5 µL	225 µL
40 × SNP Genotyping probe	0.5 µL	25 µL
Total	15 µL	750 µL

2. (*done for you*) Aliquot 15 µL of TaqMan genotyping master mix into wells of 96-well plate.
    a. Use rows A-D for rs12913832
    b. Use rows E-H for rs4778138
3. Dilute template DNA to 1 ng/ µL with molecular grade water.
4. Add 5 µL DNA to two wells for each genotyping assay.
   Record which wells correspond to your DNA samples on the log sheet next to the 96-well plate, ***AND*** record them here or in your lab book:
    a. rs12913832 (*HERC2*) Well 1: Well 2:
    b. rs4778138 (*OCA2*) Well 1: Well 2:
5. (*done for you*) The plate is sealed with film, centrifuged briefly, and loaded into the StepOne PCR machine (well A1 top left).
6. (*done for you*) Start the StepOne software and run an Allelic Discrimination assay.

7. (*done for you*) After the PCR cycles are completed, conduct an Allelic Discrimination analysis to generate an Allelic Discrimination plot for each SNP assayed.

Analysis of Results:



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You should now have an Allelic Discrimination plot for each SNP that resembles the one shown. Note that the Non-Template Control (NTC) reactions are in the lower left-hand corner of the graph, and each allele of the SNP is represented along each axis. The first nucleotide in the brackets is the VIC dye and is shown on the x-axis, the second is the FAM dye and is shown on the y-axis. Because these

fluorophores emit light at different wavelengths, it allows the different alleles to be distinguished based on the intensity of each colour of light produced in the reaction.

Samples that are homozygous for allele 1 (on the x-axis) are shown as a cluster of crosses in the bottom right of the graph, indicating that 'allele 1' fluorescence signal, but not 'allele 2' fluorescence signal has increased. Similarly, samples homozygous for allele 2 are shown in the top left. Heterozygous samples are present in the top right corner, indicating that both alleles have been amplified.

Based on the data provided to you, you should now be able to determine you own genotype for each SNP, and explain how your genotype is responsible for your phenotype.

SNP ID	**Gene**	**Context sequence**
rs4778138	*OCA2*	GTGAAAATATAACATATCAAAATTG**[A/G]**CAGAACACAGCTAAATCAGTGATGG
rs12913832	*HERC2*	GAGGCCAGTTTCATTTGAGCATTAA**[A/G]**TGTCAAGTTCTGCACGCTATCATCA

1 Lalueza-Fox. Science **318**, 1453 (2007).
2 Duffy. Am J Hum Genet **80**, 241 (2007).
3 Sturm. Am J Hum Genet **82**, 424 (2008).
4 Cook. J Invest Dermatol **129**, 392 (2009).
5 Visser. Genome Res **22**, 446 (2012).
6 Sturm. Hum Mol Genet **18**, R9 (2009).

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