

## **The HirisPlex System for Simultaneous Prediction of Hair and Eye Colour from DNA**

Susan Walsh, Fan Liu, Andreas Wollstein, Leda Kovatsi, Arwin Ralf, Agnieszka Kosiniak-Kamysz, Wojciech Branicki, and Manfred Kayser  
Erasmus MC, Forensic Molecular Biology, Rotterdam, The Netherlands

Recently, the field of predicting phenotypes of externally visible characteristics (EVC's) from DNA genotypes with the final aim of concentrating police investigations to find persons completely unknown to investigating authorities, also referred to as Forensic DNA Phenotyping (FDP), has started to become established in forensic biology. We previously developed and forensically validated the IrisPlex system for accurate prediction of blue and brown eye colour from DNA, and recently showed that all major hair colour categories are predictable from carefully selected DNA markers. Here, we introduce the newly developed HirisPlex system, which is capable of simultaneously predicting both hair and eye colour from DNA. HirisPlex consists of a single multiplex assay targeting 24 eye and hair colour predictive DNA variants including all 6 IrisPlex SNPs, as well as two prediction models, a newly developed model for hair colour categories and shade, and the previously developed IrisPlex model for eye colour. The HirisPlex assay was designed to cope with low amounts of template DNA, as well as degraded DNA, and preliminary sensitivity testing revealed full DNA profiles down to 63 pg input DNA. The power of the HirisPlex system to predict hair colour was assessed in 1551 individuals from three different parts of Europe showing different hair colour frequencies. Using a 20% subset of individuals, while 80% were used for model building, the individual-based prediction accuracies employing a prediction-guided approach were 69.5% for blond, 78.5% for brown, 80% for red and 87.5% for black hair colour. Results from HirisPlex analysis on worldwide DNA samples imply that HirisPlex hair colour prediction is reliable independent of bio-geographic ancestry (similar to IrisPlex findings). We furthermore demonstrate that it is possible to infer with a prediction accuracy of >86% if a brown-eyed, black-haired individual is of non-European (excluding regions nearby Europe) versus European (including nearby regions) bio-geographic origin solely from the strength of HirisPlex eye and hair colour probabilities, which provides extra intelligence for future forensic applications. The HirisPlex system introduced here, together with an interactive tool and prediction guide for the most accurate interpretation and reporting of hair colour, represents the first system for simultaneously establishing categorical eye and hair colour of a person from DNA. The practical forensic application of the HirisPlex system is expected to benefit cases where other avenues of investigation, including STR profiling, provide no leads on who the unknown crime scene sample donor or the unknown missing person might be.