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Developmental validation of the HIrisPlex system: DNA-based eye and hair colour prediction for forensic and anthropological usage

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ABSTRACT

Forensic DNA Phenotyping or 'DNA intelligence' tools are expected to aid police investigations and find unknown individuals by providing information on externally visible characteristics of unknown suspects, perpetrators and missing persons from biological samples. This is especially useful in cases where conventional DNA profiling or other means remain non-informative. Recently, we introduced the HIrisPlex system, capable of predicting both eye and hair colour from DNA. In the present developmental validation study, we demonstrate that the HIrisPlex assay performs in full agreement with the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines providing an essential prerequisite for future HIrisPlex applications to forensic casework. The HIrisPlex assay produces complete profiles down to only 63 pg of DNA. Species testing revealed human specificity for a complete HIrisPlex profile, while only non-human primates showed the closest full profile at 20 out of the 24 DNA markers, in all animals tested. Rigorous testing of simulated forensic casework samples such as blood, semen, saliva stains, hairs with roots as well as extremely low quantity touch (trace) DNA samples, produced complete profiles in 88% of cases. Concordance testing performed between five independent forensic laboratories displayed consistent reproducible results on varying types of DNA samples. Due to its design, the assay caters for degraded samples, underlined here by results from artificially degraded DNA and from simulated casework samples of degraded DNA. This aspect was also demonstrated previously on DNA samples from human remains up to several hundreds of years old. With this paper, we also introduce enhanced eye and hair colour prediction models based on enlarged underlying databases of HIrisPlex genotypes and eye/hair colour phenotypes (eye colour: $N = 9188$ and hair colour: $N = 1601$). Furthermore, we present an online web-based system for individual eye and hair colour prediction from full and partial HIrisPlex DNA profiles. By demonstrating that the HIrisPlex assay is fully compatible with the SWGDAM guidelines, we provide the first forensically validated DNA test system for parallel eye and hair colour prediction now available to forensic laboratories for immediate casework application, including missing person cases. Given the robustness and sensitivity described here and in previous work, the HIrisPlex system is also suitable for analysing old and ancient DNA in anthropological and evolutionary studies.

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1. Introduction

When investigating a case that involves DNA, current forensic practice calls for short tandem repeat (STR) DNA analysis to identify the donor of a biological sample found at a crime scene. However, in certain circumstances an STR profile obtained from evidence material does not match that of a known suspect including any from a criminal DNA database, which often leads to progress in the investigation being halted (so-called cold cases). Due to this, and by taking advantage of scientific progress in the genetic understanding of human appearance, advancement has been made in the DNA prediction of externally visible characteristics (EVCs) [1–18]. This concept of DNA intelligence, also termed Forensic DNA Phenotyping (FDP), adds a new dimension to forensic DNA analysis, and provides a promising alternative to help with future investigations in cases where conventional STR profiling fails to identify a crime scene sample donor [19–21]. At this moment, only eye and hair colour DNA prediction is feasible with accuracies high enough to base police investigations on, while other group-specific EVCs such as skin colour, male baldness, hair morphology are under investigation to identify the underlying DNA variants and to estimate their phenotype predictive value (for recent reviews see [20,21]).

DNA prediction of eye colour is now achievable when it comes to broad categories such as blue or brown, with systems such as IrisPlex that uses model-based probability prediction [22–24] and that have been extensively tested [24] and forensically validated [25]. Liklihood association ratio methods [9], a bayesian classifier approach based on likelihood ratios [13] or lastly a prediction guide process [11], amongst others also currently exist for eye colour DNA prediction. Furthermore, DNA prediction of hair colour is already feasible via the HIrisPlex system [26] for parallel prediction of hair and eye colour from DNA, generating leading intelligence for two of the most obvious externally visible traits. Combining our previous eye colour prediction system IrisPlex with knowledge from early systems used for hair colour prediction [1,10,27], and considering knowledge from our previous study where we established the hair colour predictive value of a larger number of hair colour associated DNA variants [28], the HIrisPlex system was finally developed [26]. The HIrisPlex system consists of a single multiplex genotyping assay targeting 24 DNA variants identified in European population studies to be highly informative for eye and hair colour prediction [22,28], as well as two statistical prediction models, one for eye colour and one for hair colour and shade. These prediction models were previously developed from thousands (eye colour) [22] and hundreds (hair colour) [26] of individual genotype and phenotype datasets from European populations. Combined in this highly effective multiplex genotyping assay are the six most eye colour informative SNPs previously used in the IrisPlex system [23–25] and the twenty-two most hair colour informative DNA variants previously identified as carrying the most hair colour predictive information from a larger number of hair colour associated DNA variants [22,23,26,28]. According to previously established knowledge [22–24], the use of the IrisPlex eye colour prediction model within the IrisPlex or the HIrisPlex system can correctly predict human blue and brown eye colour with >90% precision, and the use of the HIrisPlex hair colour and shade prediction model and guide can predict on average hair colour accuracies of 79% [26].

In the present study, we performed the developmental validation of the HIrisPlex genotyping assay following the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines [29]. These guidelines allow an assessment of the assay for use in certified forensic laboratories. It examines the assay's performance quality and limitations under differing conditions such as sensitivity, reproducibility and concordance as well as non-human species amplification, mixtures, degraded DNA and simulated casework samples. In completing these evaluations, and by demonstrating that the HIrisplex assay fully meets all SWGDAM requirements, we provide a necessary step towards the implementation of the HIrisPlex system in forensic (and other) laboratories to be used for enhancing police investigations in suitable cases. We also introduce here an enhanced prediction model for hair colour, which includes additional individuals to the underlying prediction databases relative to our previously introduced eye and hair colour prediction models. Furthermore, we developed an online web-based tool freely available under www.erasmusmc.nl/fmb/resources for eye and hair colour DNA prediction from complete and partial IrisPlex/HIrisPlex genotype profiles.

2. Materials and methods

2.1. Human samples

A selection of human body fluid and tissue samples were collected from donors with informed consent. The hair and eye colour phenotype of these sample donors was also recorded. Test samples included single and multiple source samples, and simulated casework samples (blood, saliva, semen, hair with roots, touched items). DNA was extracted from the samples using the QIAamp DNA Mini kit (Qiagen, Hagen, Germany) according to the manufacturer's guidelines or an in-house extraction protocol (unpublished) and quantified using the Quantifiler Human DNA Quantification Kit (Applied Biosystems Inc., Foster City, USA) following manufacturers guidelines.

2.2. Multiplex protocol

As outlined previously [26], the HIrisPlex assay consists of 24 SNPs, 6 of these make up the eye colour prediction portion of the tool, also termed IrisPlex [22–25], these are rs12913832 (*HERC2*), rs1800407 (*OCA2*), rs12896399 (*SLC24A4*), rs16891982 (*SLC45A2* (*MATP*)), rs1393350 (*TYR*) and rs12203592 (*IRF4*), while the other 18 (including IrisPlex SNPs 1, 2, 4 and 6), are used for hair colour and shade prediction. These 18 DNA variants are 1 insertion/deletion (INDEL) polymorphism N29insA and 10 SNPs from the *MC1R* gene, rs11547464, rs885479, rs1805008, rs1805005, rs1805006, rs1805007, rs1805009, Y152OCH, rs2228479, and rs1110400, rs28777 (*SLC45A2*), rs12821256 (*KITLG*), rs4959270 (*EXOC2*), rs1042602 (*TYR*), rs2402130 (*SLC24A4*), rs2378249 (*ASIP*/*PIGU*), and rs683 (*TYRP1*). All marker details and primer sequences, including a redesign of the extension primer for N29insA and slight alterations to the previously published protocols concentrations [26], can be found in Table 1. The protocol consists of a single multiplex two step PCR using 1 μl genomic DNA extract (varying concentrations) and primers in a 10 μl reaction which includes 1× PCR buffer, 2.5 mM $MgCl_2$, 220 μM of each dNTP and 1.75 U Amplitaq Gold DNA polymerase following published thermocycling conditions [26]. This is followed by product purification and a further multiplex single base extension (SBE) reaction using 2 μl cleaned product with 1 μl ABI Prism® SNaPSHOT chemistry (Applied Biosystems) using primer concentrations found in Table 1 and our previously published thermocycling conditions [26]. Lastly, all cleaned products were analysed on the ABI 3130*xl* Genetic Analyser (Applied Biosystems) with POP-7 on a 36 cm capillary length array. Run parameters were optimised to increase sensitivity, with an injection voltage of 2.5 kV for 10 s, and run time of 500 s at 60 °C.