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Research Article

# DNA Research on Morphological Traits

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## Abstract

In police investigation, description of the morphology of the suspect can be deceptively misdescriptive.

Also in 2015 the Laboratory of Hematology Forensic has proposed a new analysis to assist forensic investigation: Highlighting morphological characters to a person from a biological trace found on the crime scene (eye, skin and hair color).

This analysis is based on Study of SNPs (Single Nucleotide Polymorphism) in genes involved in pigmentation mechanisms.

For this study the laboratory selected three hundred thirty-seven volunteers with different phenotypic traits. All samples were collected according to the guidelines approved by ethics committees in France.

**In this method we built classification:**

- Three categories with SNPs of known association with eye color phenotypes: blue, brown and intermediate.
- Six models with SNPs of known association with skin color phenotypes between fair skin and dark skin
- Seven categories with SNPs involved in hair color: Red hair vs. dark hair

The analysis of SNPs was undertaken using Snapshot chemistry and capillary electrophoresis. A set of twenty-six SNPs from twelve different genes was selected to perform it.

The laboratory has set up a database (Filemaker Software) for storing the genetic and phenotypic data of all those volunteers. This developed tool helps to predict morphological traits to a person from biological material found at crime scenes.

## Introduction

In the middle 90s, STRs (Short Tandem Repeat) nuclear DNA was introduced in France in the forensic. STRs are especially suitable for human identification. These DNA markers exhibit highest possible variation in order to discriminate between the individuals. STRs analysis is a good forensic application with a focus on degraded DNA or mixed, such as in a sexual assault case. STRs with higher power of discrimination are chosen for human identification in forensic cases on a regular basis. It is used to identify victim, perpetrator, missing persons, and others.

In 2001, France set up its DNA database (FNAEG), based on legislation adopted in 1998, to collect DNA profile from suspects and persons sentenced. This database can be used by the judge and the criminal investigation police to identify unknown DNA from biological material found at crime scene. However, DNA profile does not allow obtaining information on the portrait of the face of the unknown criminal. In the 2000s after sequencing the whole human genome, the detection of specific DNA variations: SNPs (Single Nucleotide Polymorphism) promotes the development of new research tools. Studies of SNPs in genes involved in pigmentation mechanisms carried out by scientific groups: IFSG (International Forensic Science Genetics) and EUROFORGEN-NoE (European Forensic Genetics Network of Excellence) allow developing new analyses.

Also the Laboratory of Hematology Forensic has developed a new analysis to assist forensic investigation. Approved by the Supreme Court on 25 June 2014 and accredited by COFRAC in March 2016: Highlighting morphological characters to a person from a biological trace found on the crime scene (eye, skin and hair color).

This project consisted to perform a multiplex analysis, to predict skin, eye and hair color, including six SNPs proposed as the accurate prediction of blue and brown eye color by S. Walsh, et al [1] to which we included 20 other SNPs.

Also to help to predict morphological traits to a person from biological material found at crime scenes, a specialized software tool was developed.

## Materials and Methods

### Sample collection and data acquisition

337 samples from non-related individuals are collected. Before donating a sample, each volunteer

**Table 1:** Fitzpatrick photo typing scale is a numerical classification schema for human skin color. It was developed in 1975 by Thomas B. Fitzpatrick, a Harvard dermatologist, as a way to estimate the response of different types of skin to Ultraviolet (UV) light.

| Fitzpatrick Phototyping Scale | | |
|---|---|---|
| **Categories** | **Characteristics** | **Type of people** |
| Type-I | Always burns, never tans | Pale white; blond or red hair; blue eyes; freckles |
| Type-II | Usually burns, tans minimally | White; fair; blond or red hair; blue, green, or hazel eyes |
| Type-III | Sometimes mild burn, tans uniformly | Cream white, fair with any hair or eye color |
| Type-IV | Burns minimally, always tans well | Moderate brown |
| Type-V | Very rarely burns, tans very easily | Dark brown |
| Type-VI | Never burns, never tans | Deeply pigmented dark brown to darkest brown |

read and signed the consent form, according to the guidelines approved by ethics committees in France. For data acquisition, each volunteer filled out a questionnaire which asked for detailed information on eye, hair, and skin coloration. Various qualitative measures of skin color were established by comparing to the Fitzpatrick photo-typing scale (Table 1). Collected eye and hair information was assigned into several groups, blue, brown and intermediate (green, hazel, etc.,) for eye color and red, light blond, blond, dark blond, brown, dark brown and black for hair color.

### Genotype screening

A total of 26 genetic variants from 13 different genes were included: HERC2 gene-rs12913832, rs1129038, rs916977, rs1667394, rs125593929; OCA2 gene-rs7495174, rs4778138, rs4778241, rs rs1800401, rs1800407, rs1545397, rs1800414; MC1R gene-rs1805007, rs1805008; SLC45A2 gene-rs26722, rs16891982; SLC24A5 gene-rs1426654; SLC24A4 gene (not located within SLC24A4, flanking position)-rs12896399; TYR gene-rs1393350, rs1042602; IRF4 gene-rs12203592; DCT gene-rs2031526; intergenic between IRF4 and EXOC2 gene-rs4959270; rs1540771; BNC2 gene-rs2153271 and EDNRB gene - rs9544611 [1-7] (This SNP selection includes all of the SNPs employed in the validated IrisPlex). The genetic variants were selected based on correlation with human eye, skin and hair coloration).

### DNA Analysis

DNA samples were extracted using the EZ1 DNA Investigator kit, and purified with the EZ1 Advanced XL (QIagen, Germany) according to the manufacturer's protocol.

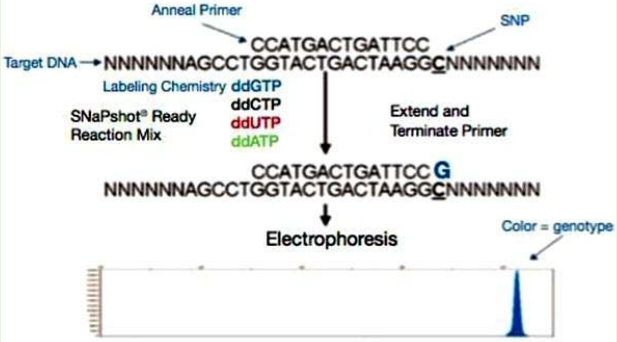


**Figure 1:** Snapshot labeling chemistry relies on single-base extension and terminaision.

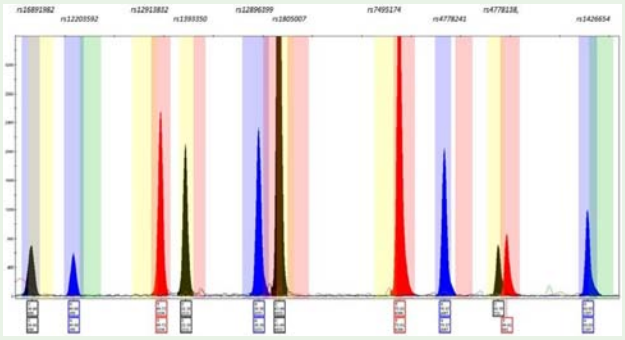

**Figure 2:** Electropherogram of PANEL 1-1 multiplex extension. Plot of results from separation done by electrophoresis using ABI Prism 3500XL Genetic Analyzer.

The PCR primers pairs were designed using the free web-based design software Primer3Plus. Each PCR fragment size was limited to less than 170 bp to be suitable for degraded DNA samples.

For the four multiplex PCR, a total of 8 µl (0,1ng - 1ng) genomic DNA extract was amplified in 15 µl PCR reaction.

The design of four Multiplex PCR has been carried out to obtain perfect separation of DNA sequences.

Each cleaned multiplex PCR product has been sequenced using Snapshot multiplex kit (Thermo Fisher Scientific) and multiplex SBE (Single Base Extension) primers (Figure 1).

SBE primers were spaced so as to enable multiplexing into each assay; poly-(T) tails were added to primers to increase the length of the extension.

Each purified multiplex extension product was separated by capillary electrophoresis (Figure 2) on an ABI Prism 3500xl Genetic Analyzer (Thermo Fisher Scientific) following the ABI Prism Snapshot standard protocol. Allele calling was performed using Gene Mapper v5 software (Thermo Fisher Scientific).

## Data Analysis-Results

### Analysis of phenotypic information's

The information's of the phenotypic characteristics from 337 samples of each volunteer were defined in two tables (Tables 2 and 3).

In this sample of population, 76, 5% (258 people) have light skin color (Type-I to Type-III) and 23, 5% (79 people) have brown skin color (Type-IV to Type-VI).

From (Table 4), we could observe than blue eye color is associated with light skin color between Type-I and Type-III (Figure 3). However, it was more likely than people with blue eye have skin Type-II. People

**Table 2:** Eye and Skin color in the volunteer sample.

| Eye color | Skin Color | | | | | |
|---|---|---|---|---|---|---|
| | **Type-I** | **Type-II** | **Type-III** | **Type-IV** | **Type-V** | **Type-VI** |
| Blue | 10 | 41 | 14 | | | |
| Brown | 6 | 40 | 55 | 28 | 22 | 20 |
| Intermediate | 6 | 54 | 34 | 4 | 3 | |

**Table 3:** Eye, Hair and Skin color in the volunteer sample.

| Eye Color Skin Color | Hair Color | | | | | | |
|---|---|---|---|---|---|---|---|
| | Red | Light blond | Blond | Dark blond | Brown | Dark brown | Black |
| Blue-Type-I | | 3 | 2 | 3 | 1 | | |
| Blue-Type-II | 1 | 12 | 7 | 17 | 3 | 1 | |
| Blue-Type-III | | | 1 | 3 | 5 | 3 | |
| Brown-Type-I | | | | 2 | 4 | | |
| Brown-Type-II | | | 1 | 14 | 11 | 14 | |
| Brown-Type-III | 2 | | | 12 | 16 | 21 | 4 |
| Brown-Type-IV | | | | | | 1 | 13 |
| Brown-Type-V | | | | | | 1 | 15 |
| Brown-Type-VI | | | | | | | 20 |
| Intermediate-Type-I | | | 1 | 2 | 1 | 3 | |
| Intermediate-Type-II | | 4 | 6 | 22 | 9 | 13 | |
| Intermediate-Type-III | | | 5 | 11 | 8 | 11 | 1 |
| Intermediate-Type-IV | | | | | | 3 | 1 |
| Intermediate-Type-V | | | | | 1 | 1 | 1 |

with intermediate eye color have more likely light skin, Type-II or Type-III (Figure 5) and people with brown eye color have unlikely light skin Type-I (Figure 4).

The same observation could be applied to the hair color (Table 3). Red, light blond and blond hair (Figure 6) is never associated with brown skin color (Type-IV to Type-VI). Dark blond hair color independently of eye color was often correlated with fair skin color, Type-II or Type-III (Figure 7), while brown and dark brown hair color was dependent eye and skin color (Figure 8). Black hair was depending of dark skin color (Type-IV to Type-VI).

So we observed a correlation phenotypic between the pigmentation of eyes, hairs and skin to the individuals.

However from extracted DNA of a biological trace found on a crime scene, it is impossible to predict the phenotypic traits of the suspect.

But our phenotypic characters are defined into our genes and we can establish morphological characters to a person by analyzing genetic variants in genes implicated in pigmentation mechanisms.

Also we searched associations between SNPs to predict eye, hair and skin color of the person. The study was developed by using a training set consisted of 337 samples.

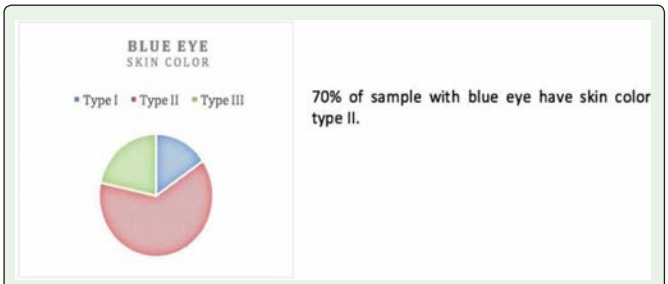


**Figure 3:** Phenotypic correlation observed in the population of volunteers between blue eye and skin color.

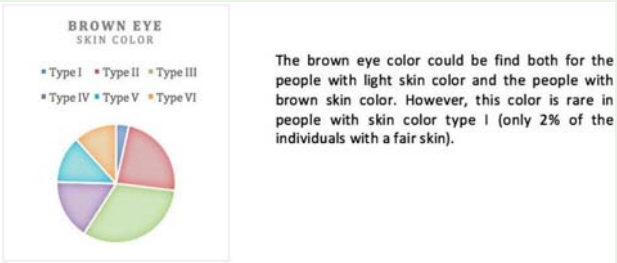


**Figure 4:** Phenotypic correlation observed in the population of volunteer between brown eye and skin color.

## Analysis results of genotypes

A classification has been established to allow us to predict eye, hair and skin color. Two SNPs, rs12913832 in HERC2 gene and rs16891982 in SLC45A2 gene, for their influences in eye (Table 4) [8], and skin (Table 5) [9-15] color have been selected to filter the prediction.

After the first interim evaluation, the level of prediction has been increased by associating other SNPs of color genes.

For this analysis, we will take an example of eye color with CC genotype of rs12913832 (variant of HERC2 gene). We will associated the genetic variants rs1800407, rs7495174, rs4778241, rs4778241, rs1545397, rs1800414, rs4778138, rs1800401 of OCA2 gene [8,12-14] with rs12913832.

We observed a similar genotype between two phenotypes (Table 6) with the proportions following:

- **Probability blue eyes:** 81, 25%.
- **Probability intermediate eye color:** 18.75%.

We completed this analysis associating genetic variants of genes, SLC45A2 (rs16891982, rs26722), SLC24A4 (rs12896399) and SLC24A5 (rs1426654) to define different genotypes between blue eye and intermediate eye color.

This panel of SNPs showed concluding results and clearly allows defining two genotypes associated with blue eye color (Table 7). But three genotypes were always common for both phenotypes.

A new set of SNP markers was used to predict eye color completing the first sets of genetic variants. This novel association of SNPs should allow differentiating by genetic analysis blue and intermediate eye color (Table 8).

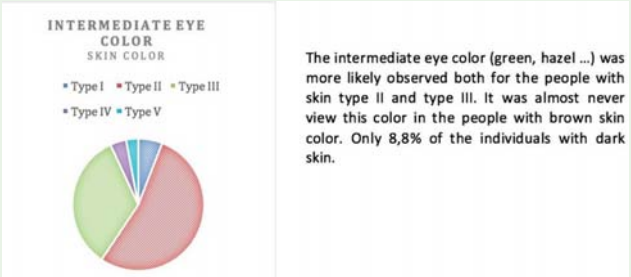


**Figure 5:** Phenotypic correlation observed in the population of volunteers between intermediate eye color and skin color.

**Table 4:** The genetic variant (rs16891982) in SLC45A2, influence the gene penetrance of allele and causes of light skin color.

| Gene | SLC45A2 | Phenotypic Characters Existing in Sample of Volunteers | | | | | | Skin Color Prediction After Genetic Analysis of Extracted DNA |
|---|---|---|---|---|---|---|---|---|
| SNP | rs16891982 | Type I | Type II | Type III | Type IV | Type V | Type VI | |
| GenoType | CC | | 4 | | 8 | 16 | 12 | Moderate brown to dark brown skin color more likely |
| | GC | | 6 | 35 | 22 | 8 | 7 | Cream white or moderate brown skin color more likely |
| | GG | 22 | 124 | 68 | 2 | 1 | | Light skin color more likely |

**Table 5:** The genetic variant rs16891982 influence HERC2 gene penetrance of allele and causes blue eye color.

| Gene | HERC2 | Phenotypic Characters Existing in Sample of Volunteers | | | Eye Color Prediction After Genetic Analysis of Extracted DNA |
|---|---|---|---|---|---|
| SNP | rs12913832 | Blue | Intermediate | Brown | |
| GenoType | TT | | 16 | 100 | Brown eye color more likely |
| | CT | 4 | 73 | 70 | Brown or intermediate eye color more likely |
| | CC | 61 | 12 | | Blue eye color more likely |

**Table 6:** The different genoTypes of OCA2 variants observed in sample of volunteers with CC genoType of rs12913832.

| Genes | HERC2 | OCA2 | | | |
|---|---|---|---|---|---|
| SNPs | rs12913832 | rs1800407, rs7495174, rs4778241, rs4778241, rs1545397, rs1800414, rs4778138, rs1800401 | | | |
| | | Phenotypic Characters Existing in Sample of Volunteers | | | |
| | | Blue | Nb | Intermediate | Nb |
| GenoTypes | CC | CC-CT-TT-CC-TT-AA-CC | 1 | | |
| | | CC-TT-GG-CT-TT-AA-CC | 3 | | |
| | | CC-TT-GG-TT-AT-AA-CC | 5 | | |
| | | CC-TT-GG-TT-TT-AA-CC | 39 | CC-TT-GG-TT-TT-AA-CC | 9 |
| | | CC-TT-GG-TT-TT-AA-CT | 4 | | |
| | | CC-TT-GT-CT-TT-AA-CC | 2 | CC-TT-GT-CT-TT-AA-CC | 1 |
| | | CC-TT-GT-TT-TT-AA-CC | 2 | | |
| | | CT-TT-GG-TT-TT-AA-CC | 4 | | |
| | | CT-TT-GT-TT-TT-AA-CC | 1 | | |
| | | | | CT-TT-GG-TT-TT-AA-CC | 2 |


**Figure 6:** Phenotypic correlation observed in the population observed in light hair color and eye and skin color.

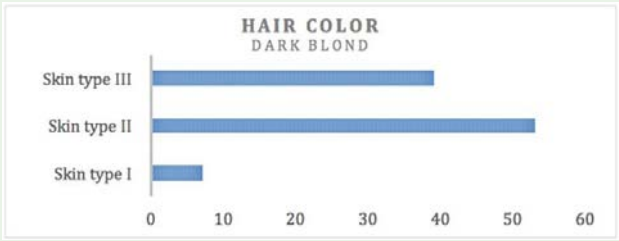


**Figure 7:** Phenotypic correlation observed in the population of volunteers between dark blond hair color, independently of eye color and fair skin color.

This association of sixteen SNP was extremely conclusive. The results had shown no cross genotypes. We have obtained seventeen genotypes and each genotype was associated to a single phenotype. Five genotypes for intermediate eye color and twelve genotypes for blue eye color.

A first study with a single SNP (rs rs12913832) allowed to direct the prediction eye color (Figure 9) [16].

The result of fifteen SNPs panel from height color genes (HERC2, OCA2, HERC2, OCA2, SLC45A2, SLC24A4, SLC24A5, IRF4, TYR, and BNC2) was including to the genotype reading to establish a correlation between the obtained genotype and the phenotype of each volunteer.

All interpretations of eye, hair and skin color were performed on same pattern allowing building software to predict morphological trait of an individual from extracted DNA of a biological fluid found on a crime scene.

## Application

This method to predict eye, hair and skin color was used in criminal case for the first time in France, on May 27, 2013.

**Table 7:** The different genoTypes including four new genetic variants.

| Genes | HERC2, OCA2 | SLC45A2, SLC24A4, SLC24A5 | | | |
|---|---|---|---|---|---|
| SNPs | rs12913832 rs1800407 rs7495174 rs4778241 rs1545397 rs1800414 rs4778138 rs1800401 | rs16891982, rs26722, rs12896399, rs1426654 | | | |
| | | Phenotypic Characters Existing in Sample of Volunteers | | | |
| | | Blue | Nb | Intermediate | Nb |
| GenoTypes | CC CC-TT-GG- TT-TT-AA- CC | GC-CC-GG-AA | 2 | GC-CC-GG-AA | 2 |
| | | GC-CC-GT-AA | 1 | | |
| | | GC-CT-GT-AA | 1 | | |
| | | | | GC-CT-GT-GA | 1 |
| | | GG-CC-GG-AA | 11 | GG-CC-GG-AA | 6 |
| | | GG-CC-GT-AA | 19 | | |
| | | GG-CC-TT-AA | 5 | | |
| | CC CC-TT-GT- CT-TT-AA- CC | GC-CC-GT-AA | 2 | GC-CC-GT-AA | 1 |

**Table 8:** The different genoTypes including four new genetic variants of genes implicated in pigmentation mechanisms.

| Genes | HERC2, OCA2 SLC45A2 SLC24A4 SLC24A5 | IRF4, TYR, BNC2 | | | |
|---|---|---|---|---|---|
| SNPs | rs12913832 rs1800407 rs7495174 rs4778241 rs1545397 rs1800414 rs4778138 rs1800401 rs16891982 rs26722 rs12896399 rs1426654 | rs122003592, rs1393350, rs1042602, rs2153271 | | | |
| | | Phenotypic Characters Existing in Sample of Volunteers | | | |
| | | Blue | Nb | Intermediate | Nb |
| GenoTypes | CC CC-TT-GG- TT-TT-AA- CC GG-CC-GG- AA | | | GG-CT-CC-AA | 1 |
| | | | | GG-CC-AA-GG | 1 |
| | | GG-CT-CC-GG | 1 | | |
| | | GG-CT-CC-GA | 1 | | |
| | | GG-CC-CC-GA | 1 | | |
| | | GG-CC-CC-AA | 2 | | |
| | | GG-CC-CA-GG | 3 | | |
| | | GG-CC-CA-AA | 1 | | |
| | | GA-CT-CC-GG | 1 | | |
| | | GA-CC-CA-GA | 1 | | |
| | CC CC-TT-GG- TT-TT-AA- CC GC-CC-GG- AA | GG-CC-CC-AA | 1 | | |
| | | GG-CC-CA-GA | 1 | | |
| | | | | GG-CT-CC-AA | 1 |
| | | | | GG-CC-AA-GG | 1 |
| | CC CC-TT-GT- CT-TT-AA- CC GC-CC-GT- AA | | | GG-CC-CA-AA | 1 |
| | | GA-CT-AA-GG | 1 | | |
| | | GA-CC-AA-GA | 1 | | |

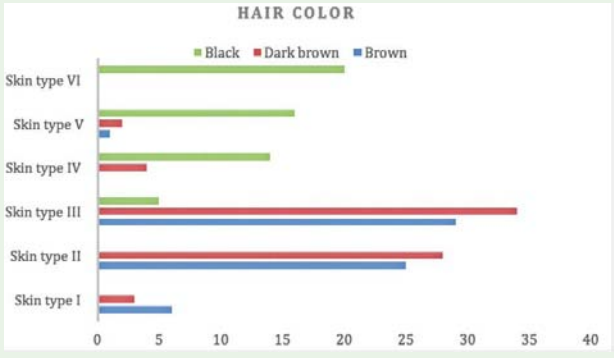


**Figure 8:** Phenotypic correlation observed in the population of volunteers between dark hair color and skin color.

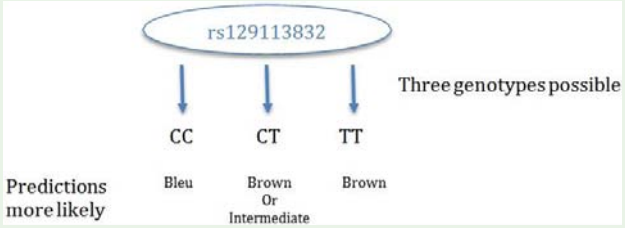


**Figure 9:** Prediction eye color more likely only with analysis rs129113832.

The analysis was performed from extracted DNA of a semen sample from a vaginal swab.

A differential extraction was realized to separate epithelial cells of the victim and sperm of the suspect in order to avoid mixed sample.

The cleaned DNA sample has been quantified and amplified with four multiplex PCR.

We sequenced the purified PCR product using Snapshot multiplex kit and multiplex SBE primers.

**Table 9:** Conclusion on eye, skin and hair color.

| Color More Likely | Laboratory Proposition |
|---|---|
| EYE | BROWN |
| SKIN | MODERATE BROWN-Type-IV |
| HAIR | DARK BROWN - BLACK |

Each purified multiplex extension product was separated by capillary electrophoresis on an ABI Prism 3500 x l Genetic Analyzer.

The results of analysis had been interpreted with the developed software and the laboratory conclusions were as follows (Table 9):

After the suspect's arrest, his picture was shown on TV. The result from the laboratory corresponded exactly with the apparent morphological characters of suspected person.

## Conclusion

Human pigmentation is under genetic control with multiple genes involved. Many studies on pigmentation traits revealed the implication of multiple genes and a high level of polymorphism. Be able easily to predict eye, skin and hair coloration by analyzing regions SNPs of extracted DNA could be very interesting in the forensic context. Provide information on physical appearance in suspect cases or cases concerning identification of human remains could speed up investigation by narrowing down a group of potential suspects.

Also the laboratory developed tool helps to predict morphological traits to a person from biological material found at crime scenes, to assist the investigation.

Through the literature search twenty six SNPs in twelve genes were selected which have been associated with human eye, skin and hair color variation. The genes included in our development comprised HERC2, OCA2, SLC45A2, SLC24A4, SLC24A5, MC1R, IRF4, TYR, DCT, EDNRB, EXOC2, and BNC2.

Before to use this method for forensic examination, blind tests were executed to verify the reliability to predict eye, skin and hair color. The tests showed an efficiency of developed software with a high reliability for eye, hair and skin color. However prediction accuracies for blond and brown colors were lower. Less precision may be reported to age-dependent hair color change during adolescence.

This novel analysis, carried out for first time in 2013 in criminal case, was approved by the Supreme Court on 25 June 2014.

Since 2015, the laboratory performs in routine the study of eye, hair, skin color by analyzing genetic variants from gene implicated in pigmentation mechanisms and by using home software.

This analysis has been authorized in France in 2014 and was accredited by COFRAC in March 2016 in the laboratory.

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