Forensic DNA Phenotyping: a review on SNP Panels, Genotyping Techniques, and Prediction Models

Nuria Terrado-Ortuño[1]*, Patrick May[1]

[1]Genome Analysis, Bioinformatics Core, Luxembourg Centre for Systems Biomedicine, Esch-sur-Alzette, Luxembourg

*Corresponding author

Corresponding Author

Nuria Terrado Ortuño

Luxembourg Centre for Systems Biomedicine

Campus Belval - Bioinformatics Core, Genome Analysis

6, avenue du Swing, L-4367 Belvaux, Luxembourg

nuria.terrado@uni.lu

https://orcid.org/0000-0002-1896-206X

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Abstract

In the past few years, forensic DNA phenotyping has attracted a strong interest in the forensic research. Among the increasing publications, many have focused on testing the available panels to infer biogeographical ancestry on less represented populations and understanding the genetic mechanisms underlying externally visible characteristics. However, there are currently no publications that gather all the existing panels limited to forensic DNA phenotyping and discuss the main technical limitations of the technique. In this review, we performed a bibliographic search in Scopus database of phenotyping-related literature, which resulted in a total of 48, 43 and 15 panels for biogeographical ancestry, externally visible characteristics and both traits inference, respectively. Here we provide a list of commercial and non-commercial panels and the limitations regarding the lack of harmonization in terms of terminology (i.e., categorization and measurement of traits) and reporting, the lack of genetic knowledge and environment influence to select markers and develop panels, and the debate surrounding the selection of genotyping technologies and prediction models and algorithms. In conclusion, this review aims to be an updated guide and to present an overview of the current related literature.

Abbreviations

AIM/AISNP	Ancestry-informative marker/SNP
aSNP	Autosomal SNP
AUC	Area under the curve
B/MLR	Bi- or multinomial linear regression
BGA	Biogeographical ancestry
BMI	Body mass index
BRIM	Bootstrapped response-based imputation modelling
CE	Capillary electrophoresis
CRT	Classification and regression tree
DAPC	Discriminant PCA
EVC	Externally visible characteristic
FDP	Forensic DNA phenotyping
GDA	Genetic distance algorithm
GWAS	Genome-wide association study
HSR	Relative hand skill
HWE	Hardy-Weinberg equilibrium
InDel	Insertion and deletion
LDA	Linear discriminant analysis
LR	Likelihood ratio
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization - time-of-flight - mass spectrometry
MARS	Multi-variate adaptive regression splines
MDR	Multifactor dimensionality reduction
MDS	Multidimensional scaling
MH	Microhaplotypes
ML	Machine learning
MPB	Male-pattern baldness
MSE	Mean squared error
mtSNP	Mitochondrial DNA SNP
NB	Naïve Bayes
NGS	Next generation sequencing
NJ	Neighbour joining tree
NN	Neural networks
OR	Ordinal regression
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCR-REBA	PCR-reverse blot hybridization assay
PCR-RFLP	PCR-restriction fragment polymorphism
PISNP	Phenotype-informative SNP
PLRS	Partial least square regression

PO	Posterior odds
POI	Person of interest
RF	Random forest
SBE	Single base extension
SNP	Single nucleotide polymorphism
SR	Stepwise regression
STR	Short tandem repeat
SVM	Support vector machine
UVR	Ultraviolet radiation
Y-SNP	Chromosome Y SNP
XGB	Extreme gradient boosting

1. Introduction

In the forensic field, the use of human DNA has been mostly centred around individual identification using short tandem repeats (STR) [1–4]. This is achieved by "traditional matching", also called forensic DNA identification, which is based on the comparison of an unknown DNA profile, obtained from a biological sample found in the crime scene, with a known DNA profile [5–8]. However, in some cases there are no matches, or no known profiles from a person of interest (POI) to compare it with [6,9]. Thus, if other options are not feasible, such as using eyewitness statements, dragnets, or familial searching, these cases remain unsolved [7–12].

To overcome this, a new intelligence method emerged in the early 2000s, following the increase of genome-wide association studies (GWAS) that link common genomic variations, in particular single nucleotide polymorphisms (SNPs), with diseases and other phenotypic traits [9,12–17]. SNPs are base substitutions, insertions, or deletions, that are normally bi-allelic with low mutation rates and high heritability [18–21]. Moreover, the small size of their PCR amplicons makes them useful to analyse typically forensic degraded and low amount DNA samples [1,11,13,19,21–23]. These findings have a big forensic potential since the prediction of externally visible characteristics (EVC) and bio-geographical ancestry (BGA), together with sex and age estimation, can provide a somehow physical description of a sample's donor [7,8,13,17,18,24]. Hence, the so-called forensic DNA phenotyping (FDP) (or molecular photo-fitting) aims to act as a "biological witness" [2,25], providing new leads and reducing the pool of potential suspects [9,11,17]. FDP is also useful in missing persons' investigations and for the identification of human remains [16,22,26–32]. Even though it has already been applied in some forensic cases [33–35], it raises several ethical, legal, and social issues about the limits of its application, dividing the forensic community [7,8,10,11,36,37].

As mentioned before, STR profiling is a well-established and regulated technique owing to the great efforts from scientists and law enforcement to establish validated protocols in all forensic laboratories and to create police databases that contain profiles from criminals and missing persons [1,4,8] (more information available on the STRBase website [38]). On the contrary, due to the relatively new appearance of FDP, there is no standardization of methodologies [11,39,40]. For instance, several SNP typing techniques have been adapted to analyse a growing number of SNPs in a single run and to input forensic-type samples [40,41]. Although TaqMan® polymerase chain reactions (PCR) and single base extension (SBE) coupled with capillary electrophoresis (CE) (in particular, SNaPshot™ minisequencing) is extensively used, many efforts are now focused on implementing next generation sequencing (NGS) protocols [8,11,13,17,41].

In the last years, the available literature regarding FDP has grown exponentially: several reviews on new forensic developments started to include a small presentation of FDP [1–4,17,21,22,25,27,42–46]. Nonetheless, the number of articles exclusively dedicated to FDP is limited and not many evaluate in depth BGA [7,47–49] or EVC (e.g., pigmentation traits [7,24,36,50–52] and other characteristics such as weight, height, or facial morphology [8–12,14,53,54]). Phillip's review [49] is one of the few to include a comparison of BGA-informative markers and panels worth of consideration for forensic application, while Mehta's [13] and Schneider's [7] reviews describe the most informative panels for both BGA and EVC inference. The latest reviews on the current state of EVC prediction have been published by Tozzo et al. [8], Pośpiech et al. [12] and Dabas et al. [54]. All these include an extensive summary on newly found genetic markers and most common panels to predict continental, sub-continental and admixed ancestries, pigmentation traits (eye, hair and skin colour, hair greying), hair morphology (shape and thickness), eyebrow morphology (colour, thickness, monobrow), height, weight (BMI), facial morphology, presence of freckles, male-pattern baldness, and myopia. They also give a few comments on different prediction algorithms, genotyping technologies, and some limitations in FDP.

Despite this, there are currently no publications that gather all the existing research limited to FDP. Thus, this review will include only those articles that have specifically developed and/or applied panels with the aim to use it as an intelligence tool to reduce the set of suspects or to identify human remains, excluding those regarding the discovery of markers in a non-forensic/clinical setting. This will allow us to have a general view of all the commercial, commonly used, and other customized sets of markers and to provide an evaluation of the FDP field. In particular, the focus will be on the lack of harmonisation concerning classification algorithms and methodologies, and limitations in terms of reference datasets, informative SNPs, environmental influences, and lack of a common lexicon, among others. To do so, we will take as reference the following reviews [7,8,12,13,52,54] and present a more detailed summary that will include the panels' markers (type and number), genotyping technology,

statistical methods, traits, and related literature. Hence, the aim of this scoping review is to present an overview of the current FDP-related literature, so it serves as an updated guide of the global aspects of FDP which can redirect readers to further specific reading.

2. Materials and methods

This review followed the Preferred Reported Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines [55].

Any published paper, written in English, between 2000 and 2022, and whose focal point was FDP (in particular, EVCs and BGA) were eligible for inclusion. It is important to notice that only those papers that researched genetic human variation for FDP applications using SNPs were considered, whilst those that referred to the ethical, legal, and social implications, were not because they are beyond the scope of this review.

Four separate searches were performed on Scopus database (last search in January 2023). First, a generalized search was carried out as follows: "forensic DNA phenotyping" OR "forensic DNA intelligence" OR "molecular photo-fitting". To obtain a more specialized search on the topic, the other four searches were conducted with the following combination of keywords: 1) "external visible characteristics" OR "physical appearance" OR "physical trait" OR "physical characteristic" AND "forensic"; 2) "biogeographical ancestry" AND "forensic"; and 3) "SNP typing" OR "prediction model" AND "forensic". A total of 1016 records were obtained from Scopus (FDP=376, EVC=241, BGA=97 and methods=302). After removal of duplicates (n=77), a manual selection of documents was first performed based on title and abstract and after on a full text evaluation. The following criteria was used to select the articles: if they inferred BGA or/and EVC, if they specified their aim was for forensic phenotyping and not identification purposes, if the analysis was performed with human DNA samples/data, if the main marker type was SNPs and if the manuscript was available and not retracted. It concluded with the inclusion of 201 articles. Finally, 101 records were identified from the chosen papers' references. For each article, author(s), title, year of publication, publication journal and details on their studied FDP trait can be found in the supplemental material.

3. Discussion

3.1. BGA

Bio-geographical ancestry (BGA) describes the most likely continental and/or sub-continental regions of origin of an individual's ancestors. Despite it being based on the genomic differences and similarities among populations [1,56], it should not be confused with the notion of ethnicity, nationality, or religious affiliations since it does not represent the place of birth or where one lives [7,57,58].

Although STRs were the first markers proposed to infer someone's origins [59,60], SNPs show greater inter-population differences and a positive association with ancestral populations [3,18]. Current research is focused on combining different types of markers, such as InDels (i.e., insertions and deletions) [61] and microhaplotypes (MH) [62] with SNPs, especially for the analysis of DNA mixtures and admixed individuals, respectively [48]. However, in this review, only those panels including SNPs will be considered.

There are three types of SNPs considered as ancestry-informative markers (AIMs or AISNPs): Y-chromosome SNPs (Y-SNPs), mitochondrial SNPs (mt-SNPs) and autosomal SNPs (aSNPs). The first two define paternal and maternal haplogroups and they have been historically used for evolution studies because of their low recombination rates, their non-random geographical distribution, and their well-known global frequency distribution [63,64]. Interestingly, Y-SNPs show a better genetic differentiation with geographical distance than mtDNA or autosomal SNPs due to patrilocality [5]. The main issue when inferring ancestry using non-autosomal markers is that although being highly accurate when recent ancestors were from the same region [65], they only represent half of the lineage [48] and they can lead to misinterpretation of complex origins [49]. Therefore, autosomal SNPs are proposed in combination with parental SNPs to infer admixed ancestries [7,21,35,48,66,67].

3.1.1. BGA-related literature

In 2001, Jobling published the first review that considered Y-SNP haplogroup inferring as an exclusion tool to target an initial suspect [5]. Many reviews that discuss the aspects of BGA inference such as the development of panels and selecting classification algorithms are available [47,48,67–70]. However, only few discuss the different panels for FDP application [7,13,49], and they are usually centred around the most used ones. In this review, a total of 48 sets of markers that have been developed and/or applied in forensic genetics can be found in the Tables 1 and 2.

The first commercially available tools for forensic inference of BGA were launched in 2003 by DNAPrint Genomics: DNAWitness-Y™ and DNAWitness-Mito™ for parental lineages, and DNAWitness™ to infer sub-Saharan African, Native American, East Asian, and European ancestries

(the latest also can be sub-divided into North-western European, South-eastern European, Middle Eastern and South Asian using the EUROWitness™ panel). These panel had already been applied to solve real forensic cases, such as the Louisiana rapist or the Night Stalker [33]. After they were discontinued, a well-known NGS-based commercial solution was presented by ThermoFisher Scientific: The Precision ID Ancestry Panel (before known as HID-Ion AmpliSeq™ Ancestry Panel) [146], which includes 165 aAISNPs, allowing the differentiation of African, European, American, East Asian, South Asian, Southwest Asian, and Oceanian populations.

Concerning the non-commercial panels, one of the first applied panels in forensic research was proposed by the SNPforID Consortium. The SNPforID 34-plex panel [89] allows differentiation among sub-Saharan Africans, Europeans and East Asians and is suitable to use with SNaPshot™ technology. This panel is included in the online webtool Snipper App, developed by the University of Santiago de Compostela (USC) [147] and it allows to use the panel to infer three to five populations and to choose a classifier among naïve Bayes (NB; applying or not Hardy-Weinberg equilibrium (HWE)), multinomial logistic regression (MLR) or genetic distance algorithm (GDA) (according to allele and genotype frequency).

The following years, three population-specific panels were developed to be used in combination with the 34-plex: a 23-plex called Eurasiaplex [105], which enhances differentiation between Europeans and South Asians; the Pacifiplex [125], a panel of 29 AIMs for differentiating Oceanian populations; and the 26-plex Population Informative Multiplex for the Americas (PIMA) dedicated to Indigenous American populations [122]. Other most used sets in this field are the Kidd's lab panel, containing 55 SNPs that can distinguish seven to eight continental regions [108], and the EUROFORGEN Global AIM-SNP set which is composed of 128 autosomal SNPs to differentiate the main five global groups (Africa, Europe, East Asia, Native America, and Oceania) [95]. This last panel was reduced to a 31-plex, the Global AIMs Nano, and can be combined with the EUROFORGEN NAME [119], which uses 111 aAISNPs to enhance differentiation of Middle Eastern and North Africans.

3.2. EVC

Externally visible characteristics (EVC) are described as physical traits that are apparent at view (i.e., pigmentation, height, weight, and facial morphology). Genetically, they are considered complex traits due to their multigenic and multifactional nature [11,14,148], since they are influenced by environmental factors [11,17], such as climate, altitude, and nutrition [1,6,14,36]. The markers used to infer EVC are commonly referred to as phenotypic-informative SNPs (PISNPs) and can be both present in the coding and non-coding regions of the DNA [21].

The pigmentation variation depends on the amount, type, and distribution of melanin and eumelanin and it varies depending on the sex, age, ultra-violet ray (UVR) exposure and body site [24,50,148–150]. It is said that their genetics follow a semi-Mendelian inheritance [11,24], and their heritability is between 60 and 90% [7,12,14]. For this very reason, they have been the focus of FDP - since their genetics is extensively studied in clinical research - and they were the first ones to be predicted. The eye colour is the most successfully predicted EVC and is highly variable in European ancestries [43,151]. It is normally categorized in two or three groups: blue (non-brown) vs brown (non-blue), or blue vs intermediate (green/hazel) vs brown/black. Additionally, they are divided into light (blue, green) and dark categories (brown, black). Instead, hair colour is usually divided into blond, brown, red, and black groups. This trait is highly influenced by age, since individuals with red and blond hair in their childhood usually transition to blond and brown – respectively –, and hair whitens/greys when older [6,9,11,24,43]. In addition, the categorization of skin colour in humans is the most complex and the most varying among studies. Usually, they are divided according to the Fitzpatrick Scale [152] as very pale vs pale/light vs intermediate/olive/light-brown vs brown vs dark/black. For this reason, skin colour is the most complicated trait to predict among the pigmentation phenotypes [21,43]. Another unique pigmentation feature is the presence of ephelides - or freckles – which is also affected by URV exposure and age [153,154]. Their prediction can be based on a two- and four-categorical model: non-freckled vs freckled, or light-freckled vs mild-freckled vs severe-freckled vs non-freckled.

Two of the most interesting quantitative traits are height and weight. On one hand, stature is an easy to measure trait, leading to homogeneous, reliable, and accurate data [12,14]. It is highly polygenic and greatly influenced by environmental factors (e.g., social class, income, education, family size, housing, urban locations, etc.) [9,14]. Although it has been immensely studied in clinical research, few studies are directed to its incorporation to FDP. On the other hand, an individual's weight is usually measured using the body mass index (BMI), and it is said to have a heritability around 60 to 70%, despite knowing that epigenetic factors have more influence that genetic ones [155].

The most ambitious and challenging phenotype to incorporate is facial morphology. Even though environment has a small effect, and the genetic component is strong, it is highly polygenic, and the knowledge of their underlying genetic mechanisms is scarce [8,9,11,12,156]. The FaceBase Consortium (see [157]) and the International Visible Trait Genetics (VisiGen) Consortium (see [158]) have discovered many markers associated with the actual human morphology and researchers tend to first focus on single facial features to later apply it to whole-face predictions [11,16,156,159]. Some of the individual traits that have been investigated are related to eye morphology, such as eyelid fold, epicanthal index and palpebral fissure distance and inclination.

Another trait that could be incorporated in FDP is hair morphology, including hair shape, which can be grouped into three categories: straight, wavy, and curly; hair thickness from the scalp hair;

eyebrows (e.g., monobrows) and beard; and hair loss, in particular male-patterned baldness (MPB). Moreover, an interesting phenotype that has only been suggested once [160] is the relative hand skill (HSR) or handedness, which is based on the preference of using the right or left hand to perform complex tasks.

3.2.1. EVC-related literature

The prediction of physical characteristics is being extensively researched, in comparison with BGA. There are many reviews solely focused on the current knowledge on genetic mechanisms of pigmentation traits and facial morphology, as well as discovering new and more informative markers [161–174], and few include other traits that have potential to be included as part of FDP (freckles [164,171,175,176], facial morphology [177–181], high myopia [182], handedness [160], hair greying [183,184] or hair morphology [26,184–186]). In terms of prediction panels, there are seven reviews that include detailed descriptions on traits and their associated SNPs [7,8,12,13,15,52,54]. This review includes a total of 43 sets of markers to infer EVC (Table 3).

The only commercial test purely focused on EVC inference was **RETINOME™**, also developed by DNAPrint™ Genomics who guaranteed a 97% of correct eye colour predictions [264]. Nonetheless, the most currently used free online tools to predict pigmentation traits are the **IrisPlex** system and its updated versions (**HIrisPlex** and **HIrisPlex-S**), created by the Erasmus University Medical Centre Rotterdam [151,219,234,265]. IrisPlex uses six SNPs to predict blue, intermediate, and brown colours with an average accuracy of 0.94 AUC, 0.74 AUC and 0.95 AUC, respectively. The HIrisPlex system allows the inference of eye and hair colour by simultaneously targeting 23 SNPs and 1 InDel. It is possible to obtain accuracies of 0.92 AUC for red, 0.83 AUC for black, 0.80 AUC for blond and 0.72 AUC for brown. Moreover, the final HIrisPlex-S system can predict 5 skin pigmentation categories together with eye and hair colour using 41 SNPs with an accuracy of 0.74 AUC for very light, 0.72 AUC for light, 0.73 AUC for intermediate, 0.88 AUC for dark and 0.96 AUC for dark to black categories.

Other panels, more precisely the **SHEP** panels [212,233,253] to infer pigmentation traits, have been included in the **Snipper App** [147]. Regarding eye colour, this webtool allows to select between 7, 13 or 23 SNPs to infer blue, green/hazel, or brown eyes. Hair colour can be classified in two or four categories (light vs dark, or red vs blond vs brown vs black) when genotyping 12 markers, whereas skin colour is categorized as light, intermediate, or black typing 10 SNPs. These traits can be predicted using NB, MLR, or GDA.

3.3. BGA and EVC

As observed, some physical characteristics, in particular pigmentation traits, vary according to continental populations [8,11,66,148]. Thus, it is important to always consider both when interpretating the results. A total number of 15 panels that infer simultaneously BGA and EVC have been included in this review (Table 4).

The VISAGE Consortium presented their first appearance and ancestry single assay, referred as **VISAGE Basic Tool (BT)** [292]. It consists of a total of 153 AISNPs for continental origin inference, most of them part of the EUROFORGEN Global AIM-MPs ancestry panel [95], two SNPs from Kidd's panel [108,146] and 11 from the Precision ID ancestry panel [146], and the 41 SNPs from the HIrisPlex-S panel [265] for pigmentation inference.

In terms of commercial solutions, the **MiSeq FGx™ Forensic Genomics System** (which includes the ForenSeq™ DNA Signature Prep Kit and ForenSeq™ Universal Analysis Software) (Illumina S.A., USA) [301] is one of the most complete forensic tools since it contains two panels: the first one including 27 autosomal, 7 X- and 24 Y-chromosomal STRs and 94 identity-SNPs for identification purposes, while the second panel contains 56 ancestry- (to classify four populations (European, American, African, and East Asian)) and 22 phenotype-informative SNPs (for eye and hair colour). Moreover, the VisiGen Consortium developed another commercial solution, which included **Identitas v1 Forensic Chip** and **Identify software** [287,302], which allows inference of bi-parental BGA, eye and hair colour, relatedness, and sex by interrogating 201,173 genome-wide autosomal (192,658), Y- (3,012), X- (5,075) and mt-SNPs (428). Finally, Parabon Nanolabs offers the **Snapshot™ DNA Phenotyping Service** [303], which they deem capable of creating a complete profile, including genetic ancestry, eye, hair, and skin colour, freckling and face shape.

4. Findings

The relatively new appearance of FDP and its debated implementation [304–308] translates into a complicated harmonization of its methodology, which is clear after inspecting all the SNP panels included in this review. It can be concluded that the factors that will influence the accuracy of the prediction are the genetic heritability of the trait, the method of SNP selection and genotyping, the informativeness of the SNP, the reference dataset, and the mathematical approach [7,9,12,103]. Thus, before FDP methods can be used in forensic investigations, they need to be standardized and forensically validated, according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines [309], to finally provide reliable and reproducible results. To do so, all the technical advantages and limitations of FDP must be considered. In addition, a consensus between

researchers and field experts is needed to prepare protocols and directives to meet all ethical, social, and legal requirements (reviewed in [310]).

4.1. Terminology and reporting

The first most important issue is the terminology employed to identify FDP research. Although the word 'FDP' was already introduced in 2008 [10,47], not all articles on BGA or EVC inference identify it as such and simply refer it as an intelligence tool. For instance, only 78 articles included in this review identify FDP (two of them as molecular photofitting). Thus, the correct identification of the term as keyword and in the text would allow a more congruent literature search.

Similarly, the second issue is the definition, categorization, and measurement of traits. On one hand, considering the nature of the traits (i.e., quantitative, like height and BMI; or qualitative, such as pigmentation traits and BGA), encasing the latter into categories, may lead to oversimplification [24], irreproducible results, and incomparable studies [287]. This especially becomes challenging when analysing data from multiple sources. Moreover, these categories are usually mistaken with stereotypes or sense of nationality [33,287]. Although categorization in forensics is preferred [39,205,214,311] – since the application in casework implies human interpretation (i.e., investigators) -, some researchers recommend using a continuous and quantitative spectrum instead [9,188,195]. On the other hand, measurements tend to be quite subjective, with most studies based on self-reported EVCs data via questionnaires or reported by simple observation of a non- or medical expert. For example, even when pigmentation traits are usually recorded via digital photographs they are later interpreted and put into categories by researchers. To avoid errors due to different perceptions of a trait [24,197], several studies suggested applying specialized equipment and reflectance, bioimaging and biochemical technologies [194,210] to find stronger genotype-phenotype associations [214]. In the case of BGA, information on up until a third-degree familial ancestry is usually reported and accompanied with a family pedigree.

The same issue arises when FDP results are being reported. For instance, Atwood et al. [34] compared different service providers in terms of prediction accuracy, clarity of reporting and consistent terminology, limitations, cost, and time. The authors concluded that it is imperative that guidelines are created for a shared methodology, and clear reporting and easy interpretation of the analysis for non-experts. Interestingly, results were shown in many ways, from simple verbal "not-/likely" to highlighting -or not- the highest probability for each trait variation or ancestry, or finally with a visual map representing where the individual falls on the represented population clusters.

4.2. Development of panels

Before developing a panel for a certain trait or combinations of traits, researchers concentrate on finding the most informative set of markers for each trait. Usually, the discovery is performed via GWAS and later, confirmed by association studies [9,13,287]. This allows to avoid false positives and to find genes with weaker effects that may have been ignored [10,14]. Even so, these studies are usually carried out with small sample size and are not extensively replicated, creating some scepticism on the validity of the found associations [14]. Ideally, a worldwide population scan would be key to find candidate genes [312], considering that normally sub-populations are less represented in exploratory panels [266]. Other studies find SNPs by comparing allele frequencies found in genetic population databases (e.g., HapMap, 1000 Genomes, CEPH Human Genome Diversity Panel, Complete Genomics) with specialized tools (e.g., SPSmart, FROG-kb [313,314]).

In the case of BGA inference, it is important to select those variants with extreme allele frequency differences between populations [65,69,89,102,315] and obtain marker combinations to have equivalent levels of differentiation among those [95]. On the contrary, the genetic complexity of EVCs, due to pleiotropies (i.e., a single SNP influencing multiple traits) [11,14], epistasis (i.e., several SNPs influencing a single trait) [11,197,245], allelic heterogeneity [151,232,257], phenotypic variability, and gene-environment interactions, need to be assessed before selecting the candidate markers. However, these genetic mechanisms are still not fully understood [9], and it is possible that many other implicated and more informative genes are being ignored [15].

One of the first debates is centred around the number of SNPs needed in a panel to obtain reliable predictions. On one hand, small SNP panels must contain the most informative and differentiating markers and are ideal for the current available SNaPshot™ technologies and to obtain lesser partial profiles when typing low DNA samples [90,112,291]. On the other side, increasing the number of SNPs improves the accuracy, especially with missing data [80,136,287,315]. However, the number of SNPs will also depend on the analysis' purpose and the genetic complexity of the trait. For instance, the four or five main continental populations can be distinguished with ease using less than 40 markers [39,291], and eye colour can be distinguished with only six SNPs [151]. Conversely, even though the heritability of height and eye pigmentation is similar, the number of SNPs needed to infer stature is increasing by hundreds as its molecular mechanisms are discovered [262,263]. In this sense, several authors believe that it is better to have markers with a strong influence [110,312], due to the scare amount of DNA in the samples, while others suggest finding genes with weak effects to complement the inference [254,258,316]. Also, in the case of BGA, researchers recommend using a two-tier approach: first, a panel with maximum 100 markers to infer at least 12 global populations, and later other panels to refine sub-population inference [39,58,121,131]. That is the case of the SNPfor ID 34-plex [90] and its EurasiaPlex [105], Pacifiplex [125] and PIMA's [122] sub-panels.

Even though some researchers evaluated the capacity of EVC-associated variants to be used as AISNPs [100,104,110,165–167,315,317,318], making indirect inferences based on either BGA or EVC is a highly debated practice. Indeed, some authors made assumptions about individuals' appearance using only BGA data [34,129], or vice-versa [16,36,67,148,319]. Nonetheless, most researchers discourage this practice, especially with the increasing population admixture [9,33,43,312] and the fact that some shared alleles may not be related to ancestry but to environmental exposures that are the same in different populations [33]. Despite this, it is still important to infer BGA, as well as biological age and sex, together with EVCs, especially if a trait is restricted to a population, sex, or age group [10,165,320], to avoid any misleading interpretations.

Extensive lists of markers associated with EVCs are available [8,15,54] and they have been combined in multiple ways, yet the number of overlapping of unique markers is minimal. Soundararajan et al. [39] reported this same fact on BGA panels and emphasised the need for a collaboration among researchers to find the "best" markers and test them on a large data set representative of all global populations. Therefore, validation and inter-laboratory testing of panels is important to meet the specific quality requirements typical of forensic DNA analysis. Only few systems have been validated for forensic use [6,7,9,11,27]. Furthermore, panels are commonly developed using homogeneous and European reference data, and then validated in other populations; and they are replicated and validated adopting different methodologies, generating a more complicated comparison exercise [39]. The best outcomes would be to adapt the panel to each individual population [321] or obtain a complete allele frequency data for all existing populations and subpopulations [131].

Another factor that influences this choice is if SNPs are found in 'coding' or 'non-coding' genes, and their informativeness of other health-related phenotypes. This first differentiation follows the legal regulations that have been used for STR identification and although scientist have discussed that these categories do not reflect the reality, it is still used as a reason to include or discard markers. However, FDP implies the use of 'associative' markers that can be found in both non- and coding regions. The fear of including coding markers is based on their higher potential to provide health information [9,148,197], although non-coding genes can provide similar information if they are in linkage disequilibrium with the implicated coding genes [10] or regulatory regions [322]. Moreover, many disease- or trait-related candidate genes are first discovered when researching pathological or extreme variations, and other mutation within are found to be associated with normal variation instead [15,188]. For example, *OCA2* gene mutations are associated with eye colour and oculocutaneous albinism [15,24,164]. Regarding these off-target phenotypes, Bradbury et al. [323] studied the possibility to reveal health information while predicting EVC, and only 27 out of 1766 FDP-related markers were associated with risk of having cancer, induced asthma or risk of alcoholism. However,

these associations do not mean that an individual is suffering from these diseases and a single marker cannot be used to predict or confirm these risks.

Finally, there is a continuous debate on using commercial or non-commercial panels. While commercial houses' strongest point is their constant supply of ready to use kits, they claim the kit's technical information (e.g., markers, accuracy, statistical model, etc.) as their intellectual property. Hence, researchers cannot ensure a truthful validation and reproducibility of the kit. Consequently, some companies have been discontinued, like DNAPrint [33]; while others, such as Parabon Nanolabs have been criticised by many FDP-experts [53].

4.3. Genotyping technology

All available SNP typing methodologies have already been evaluated for forensic application ([18–20,22,27]). These techniques are known to be very versatile, allowing the combination of different chemical reactions, assay formats and detection methods [19,20]. Then again, not all techniques are suitable as FDP faces similar problems to STR identification when analysing forensic samples (i.e., low quantity and degraded DNA and often mixtures). The selection of methodology will be based on its accuracy, multiplexing and automation capacity, high-throughput, cost, and time; as well as the purpose of the analysis (e.g., the number of traits and markers to be included).

A great number of genetic techniques have been used to infer BGA or EVCs [13,40,196,242,324]: PCR assays (e.g., PCR-RFLP [171], PCR-REBA [82], and most commonly TaqMan® SNP genotyping assay), microarrays (e.g., GeneChip™ [102,287]), minisequencing (e.g., SNPlex™), MALDI-TOF (matrix-assisted laser desorption/ionization – time-of-flight) together with mass spectrometry (MS) detection (e.g., Sequenom® MassARRAY®) [107,116,120] and high-resolution melting (HRM) [65,196,324]. While some techniques like Sequenom® MassARRAY® or HRM do not reach the sensitivity requirements for forensic samples [107,115,325], others have been developed but discontinued, such as Genomelab™ SNPstream® [66,156,159,214] and Genplex®. Nonetheless, the golden standard is still SNaPshot™ (SBE-CE assay) due to its robustness, simplicity, and efficiency, but more precisely because the instrument is already present in forensic laboratories and great efforts were invested in their standardization [2,13,40,196,325].

Despite this, SNaPshot™-CE has a higher risk of contamination and error, and more importantly is limited to analyse one single trait inferred with 30 to 40 markers at a time and hence, it cannot keep up with the increasing number of markers needed for FDP [98,99,238,287]. For this reason, researchers are shifting to NGS techniques, in particular Ion Torrent™ (Thermofisher Scientific) and Illumina® [41,98,326]. They allow higher throughput, multiplexing capacity and sequencing accuracy

[15], as well as the possibility to automate and sequence different markers in the same run (e.g., STR, SNPs, InDels, microhaplotypes) [23,143]. However, this implies a longer preparation, sequencing, and analysis time [271]. As a result, the current focus is on testing SNaPshot™ panels using NGS instruments [98,99,237,238,243,320], applying single cell sequencing and NGS to analyse mixtures and touch DNA samples [136,142,241,297], and automating analysis and result interpretation to reduce analysis time [23]. This last one would allow a better handling of the samples, increase simple size, and reduce costs and time.

All these techniques have their advantages and limitations, making it harder to choose one to proceed with their standardization. Moreover, the methodology will be chosen depending on the investigation requirements and purpose [8,14,19,98] and any new one such as MPS needs to be extensively validated in larger datasets and optimized before being incorporated [35,112,271]. Other factors that restrain technological advancement in the field are the costs to renovate workspaces, to train the staff, and to increase bioinformatic support and storage capacity [13,44,45,291].

4.4. Prediction models and algorithms

Prediction models are created to support and understand the relationship between genotype and phenotype [14,15]. There are two type of algorithms that can be used to predict BGA or EVC outcomes: statistical and machine-learning (ML). Statistical algorithms, such as MLR, work better when the predictors are dependent from each other, while ML algorithms usually assume independence among predictors [15] and detect in a linear or more complex way the dependency between variable and attributes [217]. Both methods may provide similar accuracy when the same SNP panel is used [15] although ML methods require a higher computational cost and expertise. Indeed, several articles compared and introduced different classifiers for FDP analysis [12,48,67,68,70,117,205,206,217,227,252,299,327].

Two of the most used programs, STRUCTURE and Snipper, are based on the NB algorithm. This algorithm calculates how likely a trait belongs to a class comparing it with the allele frequencies that are observed in each cluster and make assumptions on unknown profiles. [68,90]. It is also capable of incorporating missing data [68]. The gold standard for BGA inference is the STRUCTURE software (and its updated version, ADMIXTURE), because of its “efficient clustering based on similarities or dissimilarities with the other samples” [48,49,95] and thus, good inference of admixture proportions, but only if the populations are well differentiated in the reference data [90,117]. Its main disadvantages are assuming HWE, which is not compatible with BGA nor EVC inference [68], and its long and computationally intensive run times when classifying single profiles with large datasets - since the parental data and the unknown profile need to be analysed simultaneously and missing data needs to be

imputed. Otherwise, Snipper can solve some of the issues STRUCTURE presents, providing a faster analysis [89,90], allowing the incorporation of one's own reference dataset [105] and being able to classify single profiles in real time [105]. The later has been both used for BGA and EVC inference.

Other alternative methods have also been tested. For example, GDA provides a continuous clustering by evaluating the informative proportions of each component, it doesn't assume HWE, and it can be used as input for hierarchical clustering, like neighbour joining trees. Although it is highly sensitive to noise [48], it has been proven better for admixture classification [67,117]. On another note, visual representations of individual and population structure like principal component analysis (PCA), discriminant analysis of principal components (DAPC) [290] or multidimensional scaling (MDS) are helpful to interpretate the outcome [68]. However, since they are reduced to the two or three most important components, it may lead to misclassification [48]. In addition, logistic regression (bi- or multinomial LR) is perfect for assessing categorical outcomes, even though it tends to misclassify partial profiles [68]. It has been traditionally applied to infer pigmentation colours [151,219]. Also, multifactor dimensionality reduction (MDR) is used in small sample size studies to better detect epistatic effects [233,245,328]. Other available and tested ML methods are linear discriminant analysis (LDA), support vector machine (SVM) [110,217,316], partial least square regression (PLRS) [156], extreme gradient boosting (XGB) [217,246], classification and regression trees (CRT) [204,217,218,254], multi-variate adaptive regression splines (MARS) [217], bootstrapped response-based imputation modelling (BRIM), ordinal and stepwise regressions (OR and SR) [209,246], and deep learning approaches such as neural networks (NN) and random forest (RF) [67,117,155,183,217,246,252,316]. NN are proposed as an alternative to LR as it recognises the patterns of complex data typical from EVC inference [156,254].

Hence, not all algorithms are appropriate, and will need to be selected depending on several aspects. First, the amount and type of data [217], as well as the impact of missing/partial profiles in the classification performance [68,89]. Second, the reference population, which not only affects the selection of SNPs but also the training of the classifiers. These must be representative of all variations and ancestries, especially when estimating admixed individuals [35,67,68,117,329]. Third, with the inability to incorporate environmental factors to the prediction, only sex and age can be incorporated as covariates. In the same way, the accuracy of the model will increase when considering both BGA and EVC if there is population dependency [188,189]. Some researchers defend that "when all the causing factors of a trait will be accounted for in the model, then the accuracy will be the same in all populations" [330].

Lastly, there are many options to interpretate the results obtained from the prediction model. It is key that field and legal experts easily understand and apply the findings. Logically, one may recommend continuing using likelihood rations (LR), since it already used in STR identification [133,188,195,272].

Nonetheless, as Caliebe et al. observed [321], since FDP does not apply the same principle of comparing two hypotheses (i.e., sample belonging to a random individual vs the suspect), and the highest value may not represent the correct category [35]. Hence, it will be more appropriate to use statistical probability, represented as posterior odds (PO), but unfortunately, statistics are often harder to understand by the plain audience. Other ways to represent accuracy have been incorporated: area under the curve (AUC) for categorical predictions – that vary from 0.5 (random phenotype) to 1 (exact phenotype) [7,11,12,15,17]; and correlation (R or R^2) or mean squared error (MSE) for quantitative measurements [15].

5. Conclusions, Limitations and Recommendations

The expectations that the forensic experts have on FDP reveals the need to provide accurate and tangible results to solve more complicated investigations. In this review, we investigated those panels that had been developed precisely for FDP and analysed the limitations to have in mind before an agreed application of the technique in the forensic workload. Among the available bibliography, 304 publications were strictly related to FDP inference and only 80 of them clearly identified that the research was for FDP inference. A total of 48 panels have been developed for BGA inference, six being commercial tests; while only one of the 43 panels available to infer several EVCs is from a commercial vendor. In addition, BGA and EVC can be simultaneously inferred with 15 panels, two of which are wildly used commercial solutions.

Throughout the literature, there is a recurrent stance from researchers: FDP is not to use in trial, but during the investigation step. This reasoning is because FDP cannot reach the level of "scientific certainty" that has been attributed to STR identification. Hence, although the justice seeks for an "absolute truth", there needs to be a shift regarding the expectations on the results' conclusiveness [2]. Realistically, in the near future of FDP, accuracy will not improve drastically. This is because even if more genetic and environmental interactions are found, the fully understanding of the effects on phenotypes complicates at the same time. There are a few things that can be done to increase the results accuracy, such as using quantitative and continuous predictions, promoting validation on all possible human populations and sub-populations, and investigating the incorporation of prior knowledge in the models [206]. The same can be said about incorporating other traits into the FDP profile, since the current extensive research (on height, weight, and facial morphology, among others) does not provide enough weight to obtain acceptable prediction accuracies. Moreover, there is an increasing interest in combining FDP with epigenetic information, not only to infer age, but to infer traits that are age-dependent like hair greying, and with other types of analysis, such as investigative genetic genealogy (IGG) or behavioural tendencies. These last two come with many ethical implications, such as the

violation of genetic information of family members or whether a tendency such as aggression or depression is more influenced by physiological, rather than genetic factors and thus, considered medical information.

Finally, a decision concerning methodology advancement must be made by forensic services, either MPS is incorporated to laboratories to keep up with the increasing demand of high number of markers and traits - that current SNaPshot™ methods cannot, or either, if FDP is considered as a tool that will not be used regularly and only in "desperate times", this task is to be entrusted to specialized external centres. Nonetheless, the advancement of FDP application will rest on the efforts of the forensic community on creating guidelines and standards for EVC and BGA inference, from their measurement and categorization to their genotyping and prediction models.

Supplementary Material

All articles from the Scopus search can be found in the supplementary table, containing information about the author(s), title, year of publication, publication journal and details on their studied FDP trait(s).

Author contributions

Nuria Terrado Ortuño carried out the conceptualization and drafted the manuscript. Patrick May participated in its design and critical review of this manuscript. Both authors contributed to the final text and approved it.

Compliance with ethical standard

The opinions expressed in this paper belong to the authors and do not necessarily reflect the opinion of PCI. No human participants were involved in this review paper.

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Table 1. Non-commercial BGA panels proposed in the literature, including their reference article, number and AISNPs type, first used genotyping technology and prediction model, inferred populations and related articles. EUR: European (NEU: north), AFR: African (WAF: west, NAF: north, NEAF: north-east), ASN: Asian (EAS: east, WAS: west, WEAS: west-east, SAS: south, SWAS: south-west, SEAS: south-east, central-south, NAS: north), EURAS: Eurasian, NAM: Native American, AUS: Australian, OCE: Oceanian (NOCE: near), PAC: Pacific, NES: near east, MES: Middle East, MED: Mediterranean.

	AISNPs	Genotyping technology	Statistical model	Inferred BGA	Related articles
Y-AISNPs Panel					
Major Y-chromosome haplogroup typing kit. [71,72]	29 Y-SNPs	SNaPshot™ + CE	MDS	31 major global Y-haplogroups	[73]
[74]	30 Y-SNPs	SNaPshot™ + CE	MDS	32 major EUR Y-haplogroups	NA
[75]	37 Y-SNPs	SNaPshot™ + CE	CRT	Major EUR Y-haplogroups	NA
[76]	13 Y-SNPs	SNaPshot™ + CE	GDA CRT NB (Snipper)	Major ASN Y-haplogroups	NA
[77]	12 Y-SNPs	SNaPshot™ + CE	CRT	Venezuelan Y-haplogroups	NA
[78]	28 Y-SNPs	SNaPshot™ + CE	CRT	Macedonian Y-haplogroups	NA
[79]	28 Y-SNPs	SNaPshot™ + CE	CRT	Major global Y-haplogroups	NA
[80]	7 Y-SNPs	SNaPshot™ + CE	CRT	EUR, EAS, AFR Y-haplogroups	NA
[81]	859 Y-SNPs	NGS	CRT	640 Y-haplogroups	NA
[82]	9 Y-SNPs	PCR-REBA + Sequencing	CRT	Major global Y-haplogroups	NA
mt-AISNPs Panel					
[83]	11 mt-SNPs 1 mt-InDel	SNaPshot™ + CE	CRT	15 mt-haplogroups	NA
[84]	36 mt-SNPs	SNaPshot™ + CE	CRT	43 mt-haplogroups	NA

				(AFR, west and east EURAS, NAM)	
[85]	26 mt-SNPs	SNaPshot™ + CE	CRT	20 OCE and 10 AFR, EUR and ASN mt-haplogroups	NA
[86]	62 mt-SNPs	SNaPshot™ + CE	CRT	70 global mt-haplogroups (AFR, NAM, WEAS, EAS, AUS, OCE)	NA
[87]	52 mt-SNPs	SNaPshot™ + CE	CRT	Major global mt-haplogroups	[87]
aAISNPs Panel					
[88]	6 aSNPs	SNaPshot™ + CE	NJ	Major AUS sub-populations	NA
SNPforID 34-plex [89–91]	34 aSNPs	SNaPshot™ + CE	NB (Snipper and STRUCTURE)	3 populations (AFR, EUR, EAS)	[91–99]
[66]	176 aSNPs	SNPstream + CE	NB (STRUCTURE) ML (unspecified)	4 populations (EUR, WAF, NAM, EAS)	NA
[100,101]	16 aSNPs	SNaPshot™ + CE	NB (Snipper)	6 AUS sub-populations	NA
[102]	47 aSNPs	GeneChip® array TaqMan® SNP genotyping	NB (STRUCTURE)	4 populations (AFR, EURAS, EAS, NAM)	NA
Seldin set. [103]	128 aSNPs	TaqMan® SNP genotyping	NB (STRUCTURE)	4 populations (AFR, EUR, EAS, NAM)	NA
[104]	16 aSNPs	SNaPshot™ + CE	MLR	7 populations (WAF, NAF, Turkey, NES, Balkan states, NEU, Japan)	NA
EurasiaPlex [105]	23 aSNPs	SNaPshot™ + CE	NB (Snipper and STRUCTURE)	2 sub-populations (EUR and EAS, MES, and SAS)	[91,98,99]
EUROFORGEN Global AIM-SNP [95]	128 aSNPs	Sequenom® MassARRAY® Sanger sequencing	MDS NB (Snipper and STRUCTURE)	5 populations (AFR, EUR, EAS, NAM, OCE)	[106,107]
Kidd Lab [108]	55 aSNPs	TaqMan® SNP genotyping	MDS NB (STRUCTURE)	7 to 8 populations (sub-Saharan AFR, admixed and NEAF, SWAS, EUR, Siberian, SAS, EAS, SEAS, PAC, NAM)	[109]
[110]	14 aSNPs	SNaPshot™ + CE	NB (Snipper) SVM	3 populations (EUR, AFR, EAS)	NA

EurEAs_Gplex [111]	14 aSNPs	SNaPshot™ + CE	MDS NB (Snipper and STRUCTURE)	3 populations (EUR, AFR and EAS)	NA
Global AIMs Nano [112]	31 aSNPs	SNaPshot™ + CE	NB (Snipper and STRUCTURE)	5 populations (AFR, EUR, EAS, OCE, NAM)	NA
[113]	32 aSNPs	TaqMan® SNP genotyping	NB (STRUCTURE)	MED and SWAS	NA
[114]	74 aSNPs	TaqMan® SNP genotyping Sequenom® MassARRAY®	NB (STRUCTURE)	10 populations (sub-Saharan AFR and NAF, EUR, SWAS, NAS, SAS, EAS, SEAS, OCE, NAM)	[115]
[116]	130 aSNPs	Sequenom® MassARRAY®	MLR	EUR and 5 ASN sub-populations	NA
[68]	142 aSNPs	Not used	NB (Snipper and STRUCTURE) MLR GDA	4 populations (AFR, EUR, EAS, NAM)	[117]
[67]	93 aSNPs	Not used	NN	7 populations (AFR, EUR, CSAS, MEA, EAS, NAM, OCE)	NA
JapanesePlex [118]	60 aSNPs	SNaPshot™ + CE	NB (Snipper)	EAS sub-populations	NA
SWA AISNP panel [58]	86 aSNPs	TaqMan® SNP genotyping	NB (STRUCTURE)	SWAS and MED sub-populations	[109]
EUROFORGEN NAME [119]	111 aSNPs	Sequenom® MassARRAY®	NB (Snipper and STRUCTURE)	NAF and MES	[120]
[121]	48 aSNPs	Not used	NB (ADMIXTURE) MLR	Chinese sub-populations (Uygur, Han, Mongolian)	NA
Population Informative multiplex for the Americas (PIMA) [122]	26 aSNPs	SNaPshot™ + CE	PCA, NB (Snipper)	NAM sub-populations	[123]
Multiple AISNPs Panel					
[65]	7 Y-SNPs 12 mt-SNPs 6 aSNPs	SNaPshot™ + CE HRM	NB (STRUCTURE)	2 populations (ASN and EUR)	NA
[124]	31 aSNPs 21 InDels	SNaPshot™ + CE	NB (Snipper and STRUCTURE)	5 populations (AFR, EAS, MES, EUR, CSAS)	NA
Pacifiplex [125]	27 aSNPs 2 X-SNPs	SNaPshot™ + CE	NB (Snipper and STRUCTURE)	OCE sub-populations	[96,98,99]

MAPlex [126,127]	144 aSNPs 20 MH	NGS	NB (Snipper and STRUCTURE)	3 populations (EAS, SAS, NOCE)	[128]

Table 2. Commercial BGA panels proposed in the literature, including their reference article, number and AISNPs type, first used genotyping technology and prediction model, inferred populations and related articles. EUR: European (NWEU: north-west, SWEU: south-west), AFR: African, ASN: Asian (EAS: east, SAS: south, SWAS: south-west), NAM: Native American, OCE: Oceanian, MES: Middle East.

	SNPs	Genotyping technology	Statistical model	Inferred BGA	Related articles
Signet™ Y-SNP kit (Marligen Bioscience Inc.)	42 Y-SNPs Amelogenin	Multiplex PCR + flow cytometry	CRT	6 major global Y-haplogroups	[129]
Precision ID Ancestry panel (Thermofisher Scientific)	165 aSNPs	NGS	HID-SNP Genotyper PlugIn (undisclosed)	7 populations (EUR, AFR, NAM, EAS, OCE, SAS, SWAS)	[35,57,130–145]
DNAWitness™ (DNAPrint Genomics)	178 aSNPs	SNPstream®	NA	4 populations (sub-Saharan AFR, NAM, EAS, EUR)	NA
DNAWitness-Y™ (DNAPrint Genomics)	NA	SNPstream®	NA	Y-haplogroups	NA
DNAWitness-Mito™ (DNAPrint Genomics)	NA	SNPstream®	NA	mt-haplogroups	NA
EUROWitness™ (DNAPrint Genomics)	NA	SNPstream®	NA	4 EUR sub-populations (NWEU, SWEU, MES and SAS)	NA

Table 3. Commercial and non-commercial EVC panels proposed in the literature, including their reference article, number of PISNPs, first used genotyping technology and prediction model, inferred traits and related articles.

	SNPs	Genotyping technology	Statistical model	Inferred traits	Related articles
Pigmentation traits					
RETINOME™ (DNAPrint Genomics)	NA	NA	NA	Eye colour	NA
IrisPlex [151,187]	6 PISNPs	SNaPshot™ + CE	MLR	Eye colour	[35,98,99,123,149,150,172,188–211]

SHEP 1 [212]	13 PISNPs	SNaPshot™ + CE	NB (Snipper)	Eye colour	[97,123,194,201, 202,204]
[213]	19 PISNPs	TaqMan® SNP genotyping	CRT	Eye colour	[204]
[214]	23 PISNPs 2 InDels	TaqMan® SNP genotyping SNaPshot™ + CE	LR	Eye colour	NA
[215]	2 PISNPs	TaqMan® SNP genotyping	LR	Eye colour	NA
[216]	5 PISNPs	Sanger sequencing	MLR	Eye colour	NA
[217]	137 PISNPs	NGS	LR, CRT, RF, XGB, MARS, NN, SVM and NB	Eye colour	NA
EC11 [218]	11 PISNPs	Sequenom® MassARRAY®	LR, CRT	Eye colour	NA
HIrisPlex [219,220]	23 PISNPs 1 InDel	SNaPshot™ + CE TaqMan® SNP genotyping	MLR	Eye colour Hair colour	[31,136,142,205, 206,221–226]
[227]	12 PISNPs	SNaPshot™ + CE	MLR, BLR, NB	Eye colour Hair colour	[208]
[228]	10 PISNPs 2 InDels	Solid-phase fluorescent minisequencing	GDA	Hair colour	NA
[229,230]	5 PISNPs	SNaPshot™ + CE	GDA	Hair colour	NA
[231]	11 PISNPs Amelogenin	SNaPshot™ + CE	BN	Hair colour	NA
[232]	13 SNPs	Sequenom® MassARRAY® SNaPshot™ + CE	MLR, LASSO regression	Hair colour	[209]
SHEP 4 [233]	12 PISNPs	SNaPshot™ + CE	LR NB (Snipper, iterative NB)	Hair colour	[97]
[183]	12-14 PISNPs Amelogenin	NGS	NN	Hair greying	NA
HIrisPlex-S [234]	41 SNPs	SNaPshot™ + CE	MLR	Eye colour Hair colour Skin colour	[109,141,166,205 ,206,235–244]
[245]	13 PISNPs	SNaPshot™ + CE	MDR MLR	Eye colour Hair colour Skin colour	NA
[209]	12 PINSPs	SNaPshot™ + CE	LR, OR	Eye colour Hair colour Skin colour	NA
CAN-E, CAN-S and CAN-H [246]	277 PISNPs	Not used	LR, MLR, RF, XGB, ANN, OR and SR	Eye colour Hair colour Skin colour	NA
[247]	12 PISNPs	TaqMan® SNP genotyping	MLR	Eye colour Hair colour Skin colour	NA
[248]	7 PISNPs	TaqMan® SNP genotyping	GDA	Eye colour Skin colour	[193,249]

[249]	8 PISNPs	TaqMan® SNP genotyping	GDA	Eye colour Skin colour	[204,250]
[251]	5 PISNPs	NGS	GDA	Eye colour Skin colour	NA
[252]	14 PISNPs	SNaPshot™ + CE	LR, RF and NN	Skin colour Tanning Freckles	NA
[154]	5 PISNPs	KASP Genotyping Chemistry TaqMan® SNP genotyping	MLR	Freckles	NA
[153]	12-14 PISNPs	NGS	LR	Freckles	[206]
SHEP 1 [253]	110 PISNPs	SNaPshot™ + CE	NB (Snipper)	Skin colour	[97]
Other hair-related traits					
[254]	6 PISNPs	SNaPshot™ + CE NGS	LR, CRT and NN	Hair morphology	NA
[185]	32-33 PISNPs	NGS Sequenom® MassARRAY®	LR	Hair morphology	[206]
[255]	14 PISNPs	Microarray	MLR	Hair morphology	NA
[256]	4-21 PISNPs	NGS	LR	Hair morphology	NA
[257]	5-20 PISNPs	SNaPshot™ + CE NGS	LR	Male-pattern baldness	NA
[258]	25 PISNPs	SNaPshot™ + CE PCR-RFLP	LR	Male-pattern baldness	NA
Facial traits					
[156,159]	24 PISNPs 68 AISNPs Amelogenin	SNPStream™	PLSR, BRIM	Facial morphology	NA
[259]	~ 90.000 PISNPs	Microarray	PCA, LR	Facial morphology	NA
[181]	1 PISNPs	NGS	LR	Eyelid	NA
[260]	4 PISNPs	Microarray	PCA	Facial morphology	NA
[261]	21 PISNPs	SNaPshot™ + CE	OR MLR	Ear morphology	NA
Other traits					
[155]	8 PISNPs 4 CpG sites	SNaPshot™ + CE Pyrosequencing	RF	BMI	NA
[262]	180 PISNPs	Microarray	LR	Height	NA
[263]	412-689 PISNPs	Microarray	LR	Height	NA

Table 4. Commercial and non-commercial BGA and EVC panels proposed in the literature, including their reference article, number, and SNPs type, first used genotyping technology, prediction model and inferred populations and traits. EUR: European, AFR: African (NAF: north, AFR-AME: American), ASN: Asian (EAS: east, CAS: central, SAS: south, SWAS: southwest), NAM: Native American, OCE: Oceanian, MED: Mediterranean, HIS: Hispanic.

	SNPs	Genotyping technology	Statistical model	Inferred BGA and traits	Related articles
MiSeq FGx™ Forensic Genomic System (includes ForenSeq™ Signature kit B)	22 PISNPs 56 aAISNPs	NGS	Illumina ForenSeq Universal analysis Software™ (Undisclosed)	BGA (Undisclosed) Eye, hair, and skin colour	[28,143,242,266–283]
Parabon™ Snapshot®	Undisclosed	NGS	Undisclosed	BGA (EUR, MED, EAS, CAS, AFR) Eye, hair, and skin colour Freckling Face shape	NA
[284]	6 PISNPs 4 AISNPs	SNaPshot™ + CE	NB (STRUCTURE)	BGA (EUR, AFR, ASN) Eye, hair, and skin colour	NA
[285]	60 PISNPs 43 AISNPs	SNaPshot™ + CE	NB (STRUCTURE)	BGA (AFR, AFR-AME, EUR, SAS, ASN, NAM, HIS) Eye, hair, and skin colour Hair morphology Male-pattern baldness	NA
[286]	21 mt-AISNPs 28 Y-AISNPs 14 AI-/PISNPs	SNaPshot™ + CE	GDA	BGA (AFR, EUR, NAF/MED, ASN, EAS) Eye, hair, and skin colour	NA
Identitas v1 Forensic Chip [287]	192,658 aSNPs 3,012 Y-SNPs 5,075 X-SNPs 428 mt-SNPs	Microchip	MLR	BGA (EUR, AFR, EAS, SAS, NAM) Eye and hair colour Kinship Sex	NA
[288]	31 PISNPs 19 AISNPs	SNaPshot™ + CE	MLR NB (Snipper)	BGA (EUR, AFR-AME, NAM/HIS, ASN) Eye colour	NA

32-plex [289,290]	10 PISNPs 22 AISNPs	SNaPshot™ + CE	NB (STRUCTURE and Snipper) DAPC	BGA (AFR, EUR, SAS, EAS, NAM) Eye, hair, and skin colour	[124]
MiniPlex [291]	5 mt-AISNPs 4 Y-AISNPs 1 Y-AI InDel 5 aAISNPs 3 PISNPs	SNaPshot™ + CE	MLR, NB (Snipper)	BGA (5 global mt- and Y-haplogroups, AFR, EUR, EAS, OCE, NAM) Eye colour Lineage	NA
VISAGE Basic Tool for Ancestry and Appearance (BT A&A) [292]	41 PISNPs 153 AISNPs	NGS	NB (Snipper)	BGA (AFR, EUR, EAS, NAM, OCE, SAS) Eye, hair, and skin colour	[293–295]
Ion AmpliSeq™ PhenoTrivium Panel [296]	41 PISNPs 163 AISNPs 120 Y-AISNPs	NGS	NB (Snipper)	BGA (AFR, EAS, SAS, SWAS, EUR, NAM, OCE) Eye, hair, and skin colour	[297]
[298]	67 AISNPs 23 PISNPs 35 Y-AISNPs	NGS	NB (Snipper) CRT	BGA (Pakistan pub-populations) Eye, hair, and skin colour	NA
[299]	2 AISNPs 3 PISNPs	TaqMan® SNP genotyping	BN	BGA (EUR, ASN) Eye colour	NA
[109]	41 PISNPs 141 AISNPs	NGS	LR MLR	BGA (AFR, EUR, ASN, NAM, SWAS, MED) Eye, hair, and skin colour	NA
Phenotype Expert [300]	41 PISNPs 14 Y-ASNPs Amelogenin 4 ABO blood group SNPs	Microchip	MLR CRT	BGA (Slavic Y-haplogroups) Eye, hair, and skin colour	NA