Datasheet

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ForenSeq® Imagen Kit

Community-approved SNPs for appearance and biogeographic ancestry estimations.

Highlights:

- **Options to support regional regulations**
  Evaluate phenotypic SNPs with or without biogeographical ancestry.
- **High multiplexing for challenging samples**
  Acquire investigative lead generation data with as little as 32pg of DNA.
- **Interoperable with HIrisPlex-S software**
  Interpret NGS data with established tools like HIrisPlex-S.

Introduction

Recent advances in forensic genetics have enabled the analysis of new markers that help with forensic investigations. While short tandem repeats (STRs) or microsatellite loci are still the predominant forensic genetic marker for identity testing and kinship analysis, single nucleotide polymorphisms (SNPs) have emerged as a powerful tool for investigative lead generation.[1] SNPs are point mutations that encompass single-base substitutions and single-base insertions and/or deletions (InDel) and occur ubiquitously in coding and non-coding regions of the genome. SNPs represent the most common human genetic variation, occurring once every 1000 bp, having a lower mutation rate, and lending themselves to smaller amplicon sizes of 50–150 bp. This makes SNPs particularly suitable in cases of aged, degraded or low copy biological samples; in long-range kinship and paternity testing; and in population and evolutionary genetics research.[2]

Most SNPs are bi-allelic markers, and consequently, in the diploid human genome, there are only three possible genotypes (AA, BB, or AB) associated with each SNP. This lower discrimination power per SNP implies that numerous loci must be tested to yield the same discriminative power as STRs.[3]

Low cost next-generation sequencing (NGS) removes this limitation and enables the use of SNP-based forensic panels for differentiating individuals from one another, kinship/paternity testing and evolutionary studies, biogeographical ancestry (BGA), and estimation of visible traits or appearance, such as skin, hair or eye color, height, weight, facial morphology, etc., commonly known as External Visible Characteristics (EVCs).[3,4,5,6] The analysis of EVC and BGA could be especially useful in investigative cases where there are no potential suspects and no match between the evidence DNA sample under investigation and genetic profiles entered into criminal databases.

The ForenSeq Imagen workflow, comprising the ForenSeq Imagen kit, the MiSeq FGx Sequencing System with the MiSeq FGx Reagent Micro kit, and the ForenSeq Imagen analysis methods in the Universal Analysis Software (UAS), is a fully validated DNA-to-data workflow specifically designed for forensic genomics applications (Figure 1). Relying on established, peer-reviewed SNP marker sets that have been identified and tested by the forensic community, the ForenSeq Imagen kit is an optimized solution for operational forensic labs considering implementing an NGS-based lead generation workflow.[4,6,7]

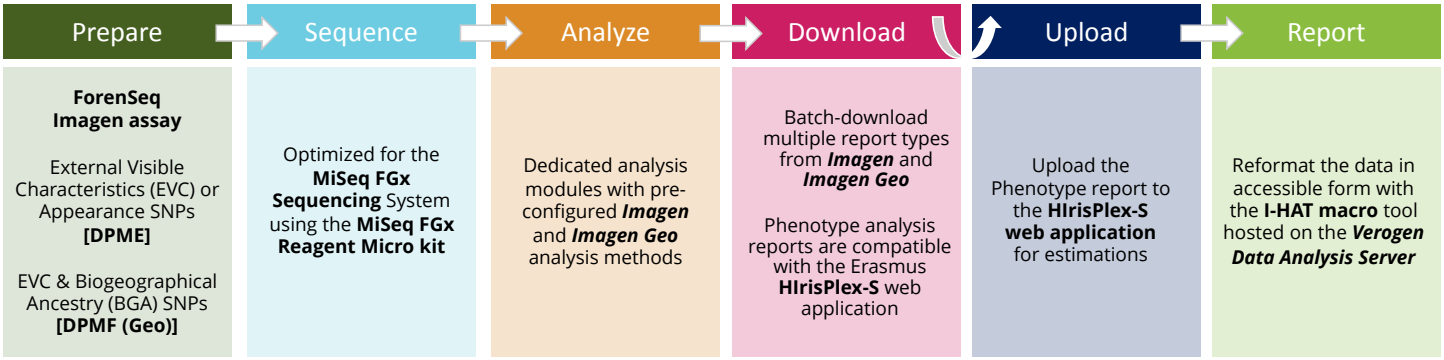

Figure 1: ForenSeq® Imagen Kit workflow.

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Forensically optimized SNP multiplex modularized for regional regulatory compliance

The ForenSeq Imagen kit is a fully kitted solution that includes all the reagents and amplification controls required for the generation of 96 unique DNA libraries for sequencing on the MiSeq FGx Sequencing system using the MiSeq FGx Reagent Micro kit. To ensure compatibility with existing and emerging regulations, two primer pools, one comprised of markers for EVC, also referred to as DPME, and another comprised of markers for a combination of EVC and BGA, also referred to as DPMF-Geo. Both primer sets encompass 41 SNPs for the phenotypic estimations that include 22 SNPs for the estimation of hair color, 6 SNPs for the estimation of eye color, 36 markers for the estimation of skin color, with some SNPs common to more than one phenotype. Both primer sets encompass 14 Y-SNPs to enable robust estimation of biological sex. In addition, 56 SNPs for the estimation of biogeographic ancestry are included in one of the primer sets. Labs have the flexibility to use either of the two primer sets based on the regulatory approaches associated with their geography. Table 1 provides a summary of the number of markers associated with both primer sets in the ForenSeq Imagen kit.[8]

Leveraging the established ForenSeq chemistry[9] used by all Verogen library preparation products, ForenSeq Imagen is a six-step method that leads to the generation of high-quality sequencing libraries in 7 hours and 15 minutes, with just 1 hour and 30 minutes of hands-on time. Optimized for the most common sources of forensic DNA and utilizing 8 µl of DNA extract input volume, this sensitive, PCR-based assay is designed to generate short amplicons and is integrated with Unique Dual Indices (UDIs), maximizing the likelihood of generating DNA profiles with degraded and low-input samples. Optionally, the kitted buffer or the ForenSeq Enhanced PCR1 Buffer System can be used for challenging bone samples to enable support for inhibited forensic samples. The workflow includes five safe stopping points and a pre-mixed adapter plate that increases library preparation efficiency and ease.

High multiplexing capability coupled with a one-year shelf life enables sequencing while maximizing sample throughput and minimizing cost per sample. A low gDNA input recommendation of 1 ng enables reliable and reproducible recovery of full profiles from high-quality single-source samples all the way down to 32 pg low input and difficult samples using the EVC+BGA (DPMF-Geo) primer set and 16 pg for the EVC (DPME) primer set. ForenSeq Imagen enables easy and automatable access to an operational workflow for EVC and BGA. Table 2 provides a complete list of kit specifications.

Table 1: Categories and number of SNP markers in the ForenSeq Imagen kit.

SNP Category	Number of Markers	Amplicon Size Range (bp)	Included in DNA Primer Mix E	Included in DNA Primer Mix F
Y-SNPs	14	88-130	Yes	Yes
Phenotypic SNPs[1]	41	69-227	Yes	Yes
Hair color	22	92-227	Yes	Yes
Eye color	6	73-119	Yes	Yes
Skin color	36	69-213	Yes	Yes
Biogeographical[2] **ancestry SNPs**	56	67-200	No	Yes

1. 4 SNPs overlap across eye, skin, and hair color phenotypes; 13 SNPs overlap across hair and skin color phenotypes; and 2 SNPs overlap across eye and skin color phenotypes.
2. Only available in the EVC+BGA SNP panel (DPMF-Geo).

Table 2: ForenSeq Imagen Kit specifications.

Sample Type	Extracted gDNA from bones, blood and teeth, punches from storage cards (FTA®)
Recommended input	1 ng per sample (gDNA) 1.2 mm per samples (FTA card punch)
Kit configuration	96 reactions
Primer sets	2
Marker composition	DPME: 41 EVC markers, 14 Y-SNPs DPMF (Geo): 41 EVC, 56 BGA markers, 14 Y-SNPs
Amplicon size (Mean)	Imagen (DPME) - 112 bp Imagen (DPMF-Geo) – 111 bp
Recommended sequencing plexity	8 to 96 samples on a MiSeq FGx Reagent Micro Kit
Library prep time	7 hours 15 minutes (total) 1 hour 30 minutes (hands on)

Robust inhibition tolerance and high sensitivity enable accurate SNP detection

Forensic DNA samples are frequently challenged by a variety of environmental factors and PCR inhibitors such as hematin, humic acid, tannic acid, and indigo. The presence of these inhibitors reduces the amplification efficiency of the workflow.[2,9,10] The ForenSeq Imagen kit contains a robust inhibition buffer (PCR1) that enables efficient amplification in the presence of these commonly occurring inhibitors. For severely challenged samples such as interred bones, the ForenSeq Imagen kit is also compatible with the ForenSeq Enhanced PCR1 Buffer System (ePCR1). The ForenSeq Imagen kit demonstrates high call rates of 100% with 0.63 ng/µL of humic acid, 1 µM of tannic acid, and 133 µM of Indigo, and 97% with 50 µM of Hematin, when processed with the DPMF (Geo) primer mix. The enhanced buffer system demonstrates a 20X higher tolerance for humic acid, 6X higher tolerance for hematin, and 4X higher tolerance for tannic acid compared to PCR1 buffer. Figure 2 summarizes studies on inhibition tolerance with DPMF. Inhibition studies with DPME show similar results.

The ability to generate accurate SNP calls across a range of DNA inputs was evaluated using serially diluted gDNA in template amounts of 2 ng, 1 ng, 500 pg, 250 pg, 125 pg, 62.5 pg, 31.25 pg, 15.625 pg, and 7.82 pg, in triplicate. 96 libraries were simultaneously sequenced using the MiSeq FGx Reagent Micro Kit. The ForenSeq Imagen Kit offers a high level of sensitivity, generating 100% call rate with as little as 32 pg of input DNA with both primer sets and 94% call rate with 16 pg of input DNA using the DPMF, when 96 libraries were simultaneously sequenced. Figure 3 summarizes studies of the sensitivity.

Reproducible high-quality results with mock casework and degraded samples

The ForenSeq Imagen kit enables generation of high-quality SNP profiles from mock casework and degraded samples. Evaluation of profiles from 1 ng of DNA extracted from saliva-FTA cards, blood, buccal swabs, plucked hair, teeth, and bones subject to a variety of insults demonstrated an average call rate of 100%. Similar studies were performed on 1 ng of DNA extracted from a blood sample subjected to artificial degradation to generate a degradation series spanning a degradation index (DI) of 1 to 50. Samples processed with the DPME primer mix showed an average call rate of 100% up to a DI of 26 and a 98% call rate for a DI of 50. The DPMF (Geo) primer mix showed a call rate of 100% up to a DI of 8 and a 98% call rate for a DI of 50. The ForenSeq Imagen kit enables forensic laboratories to generate high quality SNP markers across a range of sample sources and degradation levels. Table 3 and Figure 4 summarize studies with mock case work and degraded samples, respectively.[11]

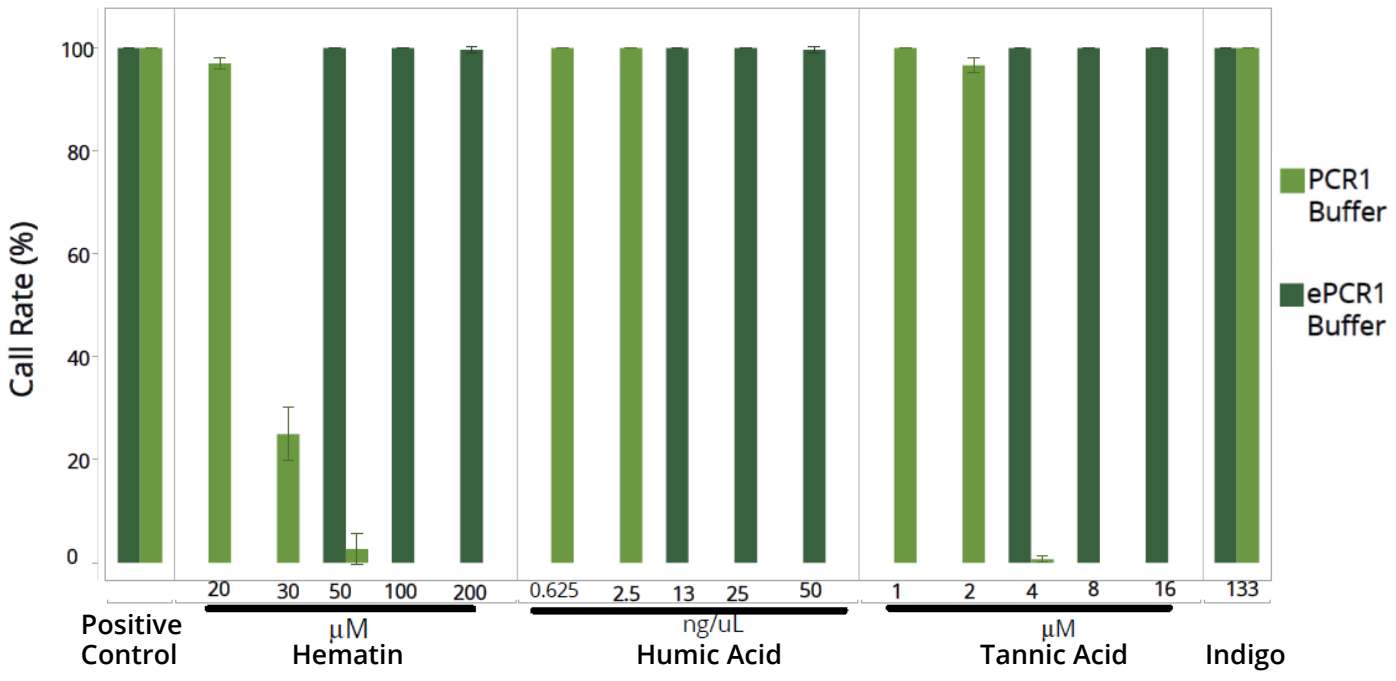

Figure 2: Inhibition study. 1 ng gDNA samples treated with common forensic inhibitors, processed with both PCR1 and ePCR1 buffers and the DPMF (Geo) primer mixes in triplicate, show high average call rates.

The ForenSeq Imagen kit uses community-approved SNP marker sets for the estimation of phenotypes and biogeographic ancestry. These established markers have been previously tested by multiple academic and operational forensic laboratories. To supplement these studies, 60 samples with known genotypes were sequenced with both DPME and DPMF as part of 96 sample runs. Data from the 1000 Genomes Project served as the truth set for concordance and accuracy studies.[12] A high concordance rate of 99% and accuracy rate of 99% was observed with more multiplexes. Precision was calculated using data from 121 positive controls generated by 10 users. High precision rates of 99% were observed with both primer multiplexes. In addition, accuracy of EVC or appearance predictions were made by the HIrisPlex-S web application using SNP profiles generated for 1 ng of 11 reference samples that were processed using DPMF and sequenced in triplicate by three operators.[5]

Data from the 1000 Genomes Project was used to evaluate the accuracy of predictions. All samples demonstrated 100% call rates and 100% accuracy of predicted phenotypes (Table 4). Accuracy of ancestry predictions made by the UAS was evaluated using DPMF (Geo) SNP profiles generated from 1 ng DNA across 58 samples that were part of the 1000 Genomes Project. All samples demonstrated 100% call rates and 100% accuracy of ancestry predictions across four population groups of Caucasian, African, admixed American, and Asian populations.

Table 3: Mock case-work studies.

Sample	Treatment	Average Call Rate (%) DPMF	DPMF (Geo)
Blood Chelex	No Heme	100%	100%
	Moderate Heme	100%	100%
	Light Heme	100%	100%
	Heavy Heme	100%	100%
Bone	Burned (2 samples)	100%	100%
	Cremated (2 samples)	100%	100%
	Enbalmed (2 samples)	100%	100%
Saliva	FTA Card (1 sample)	100%	100%
Buccal	Swab (5 samples)	100%	100%
Hair	Plucked (5 samples)	100%	99.50%
Teeth		100%	99.80%

Table 4: ForenSeq Imagen generates highly concordant genotypes when compared with orthogonal technologies, as well as high precision and accuracy rates with both DPME and DPMF (Geo).

	EVC panel (DPME)	EVC + BGA panel (DPMF-Geo)
Average concordance rate	99.8%	99.8%
Accuracy (121 samples)	99.8%	99.8%
Precision (58 samples)	99.9%	100%

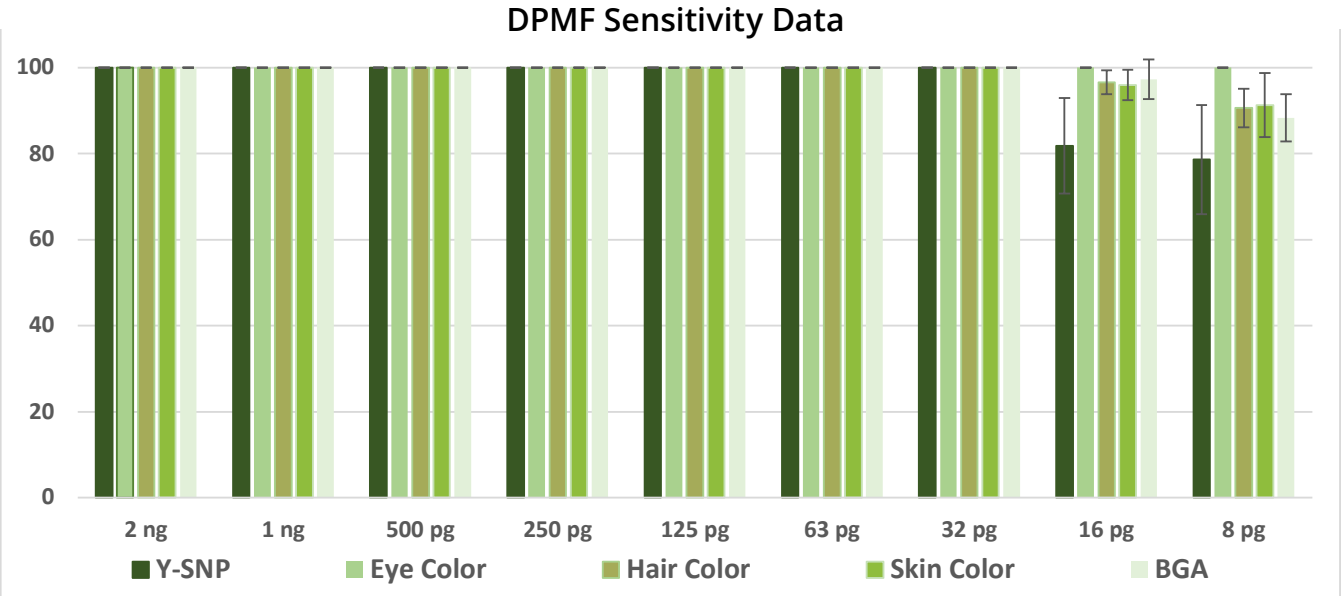

Figure 3: Sensitivity Study. Serially diluted gDNA samples processed with the DPMF (Geo) primer mix in triplicate show high average call rates.

End-to-end workflow integration across analysis and interpretation

Data generated by the ForenSeq Imagen kit is optimized for analysis using the Universal Analysis Software. Two fit-for-purpose analysis methods enable labs to analyze data from the DPME or DPMF (Geo) primer mixes separately. Robust out-of-the-box thresholds and settings allow easy validation and implementation. Rich data visualization capabilities enable labs to quickly gauge the quality of their sequencing run and SNP calls. Extensive filtering and sorting capabilities enable manual review of markers that require inspection. The UAS generates SNP reports that can be uploaded to the HIrisPlex-S web-application for predictions that are powered by solutions that have been described in multiple peer-reviewed publications.[13] To allow easy interpretation of complex statistical information, reports from the HIrisPlex-S web-application[14] can be parsed by the Imagen-HIrisPlex-S Analysis Tool (I-HAT) application on the UAS data server. The I-HAT macro generates human-readable reports that summarize the predictions and probabilities associated with typed SNPs. Automation of the lab workflow allows labs to onboard the entire ForenSeq Imagen workflow from lab to report with confidence.

Conclusion

ForenSeq Imagen offers an easy solution for the inclusion of NGS-based investigative intelligence into operational workflows. The ForenSeq Imagen Kit contains reagents to sequence SNP markers that predict hair, eye, skin color, and biogeographic ancestry. Modular components ensure that labs can pick the markers that are approved by their regional regulatory body. Low input recommendations of 1 ng gDNA deliver a reproducibly high call rate regardless of inhibition or degradation. Seamless integration of the data with the Imagen Analysis Module across the HIrisPlex-S web-application and the I-HAT macro tool allows laboratories to easily sequence, analyze, interpret, and report data in less than 1.5 days.

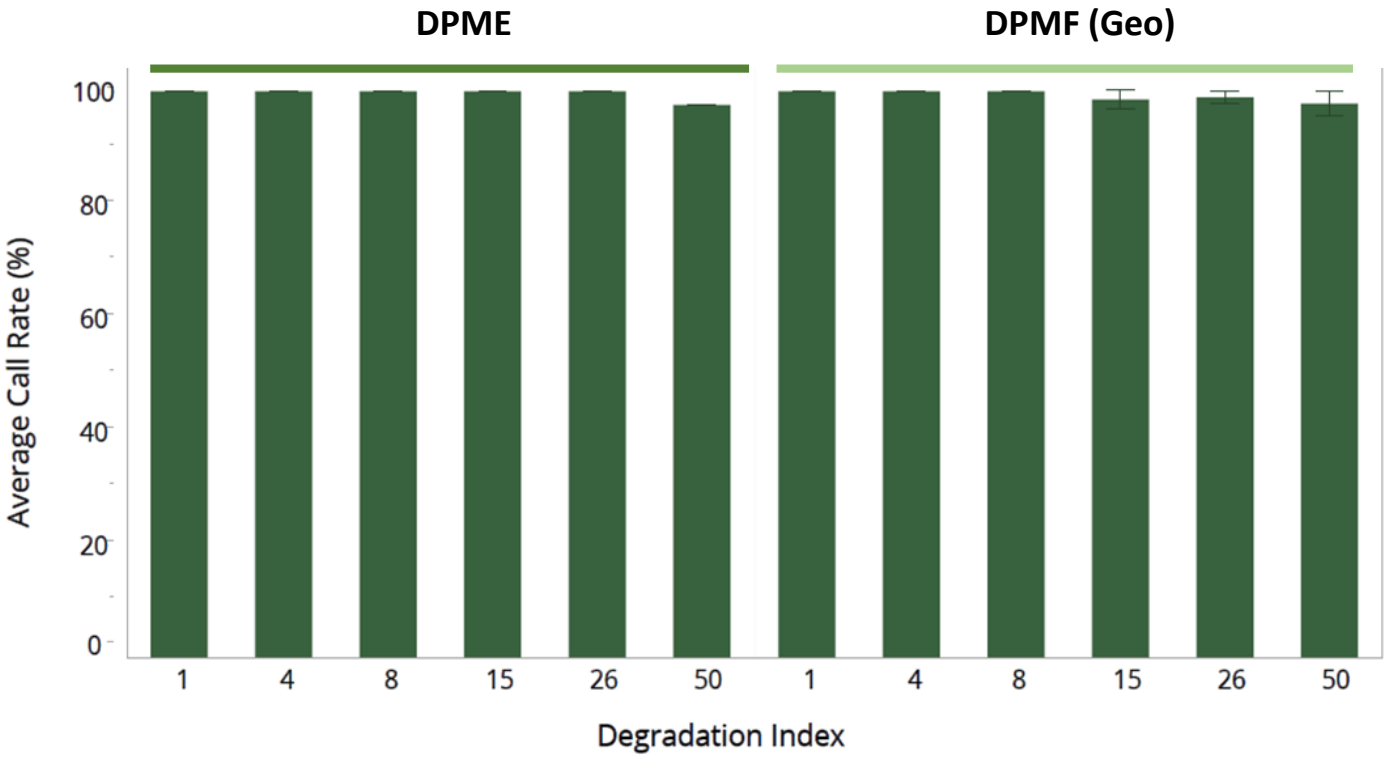

Figure 4: Degradation study. Serial degradation of 1 ng gDNA samples processed with DPMF (Geo) primer mix in triplicate show high average call rates.

Product documentation is available for download at www.verogen.com/support

Ordering information

Product	Part #
ForenSeq Imagen Kit (96 Reactions)	V16000189
ForenSeq Enhanced PCR1 Buffer System (96 Reactions)	V16000137

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