Technical Note

VEROGEN

Verogen Representation in Forensic Genomics Literature

Verogen offers next-generation sequencing solutions that are the technologies of choice for modern forensic laboratories.

Summary: The Verogen portfolio is well represented in forensic genomics literature. Year after year, a growing number of studies apply MiSeq FGx sequencing and ForenSeq library prep and analysis, demonstrating rapid acceptance that cements Verogen as the preferred sequencing provider. In partnership with the forensic community, Verogen continues to expand possibilities for human identification.

Table of Contents

Preferred sequencing solution

The 2015 launch of the Verogen MiSeq FGx® Sequencing System introduced the field of forensic genomics to next-generation sequencing (NGS). Built on Illumina sequencing-by-synthesis (SBS) technology and featuring dedicated software and reagent kits, the MiSeq FGx System is the first and only NGS instrument designed and validated for forensic genomics applications. Additionally, the instrument is key to the National DNA Index System (NDIS)-approved MiSeq FGx Forensic Genomics Solution, an end-to-end platform for analyzing forensic samples.

Illumina SBS is the predominant NGS technology, powering 90% of all sequencing performed. Verogen combines this gold-standard technology with community input to deliver forensics-focused solutions worldwide. Every year, Verogen NGS is the foremost technology cited in forensic publications (Figure 1). Nearly 70% of publications describing commercial library prep methods use technologies designed for sequencing on the MiSeq FGx System while the ForenSeq® approach far outpaces other chemistries. This technical note highlights key papers and summarizes the representation of Verogen NGS technology in forensic genomics literature.

Community support across applications

The scientific community continues to embrace the Verogen portfolio, publishing more papers each year than the previous year while building academic and professional support for ForenSeq library prep, the MiSeq FGx System, and Universal Analysis Software (UAS). As of December 2020, the community has released nearly 400 peer-reviewed publications endorsing Verogen NGS technology across a broad range of forensic and human identification applications, including forensic genetic genealogy (FGG), analysis of challenging samples, missing persons and disaster victim identification (DVI), sequence allele implications, population analysis, and microhaplotypes. Figure 2 shows the rapid annual increases in the amount of literature that mentions the MiSeq FGx System or ForenSeq chemistry.

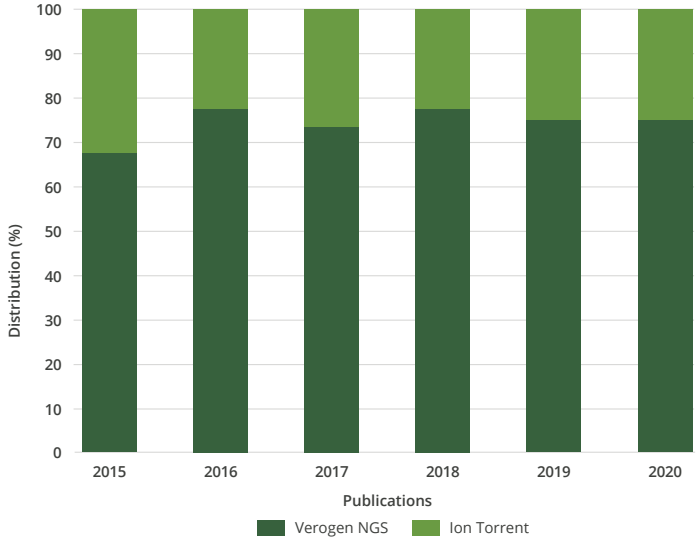

Figure 1: The annual distribution of papers supporting Verogen library prep shows Verogen as the dominant technology. Mentions of ForenSeq, Nextera, and TruSeq products supporting Verogen NGS publications and mentions of Precision ID and AmpliSeq products supporting Ion Torrent publications.

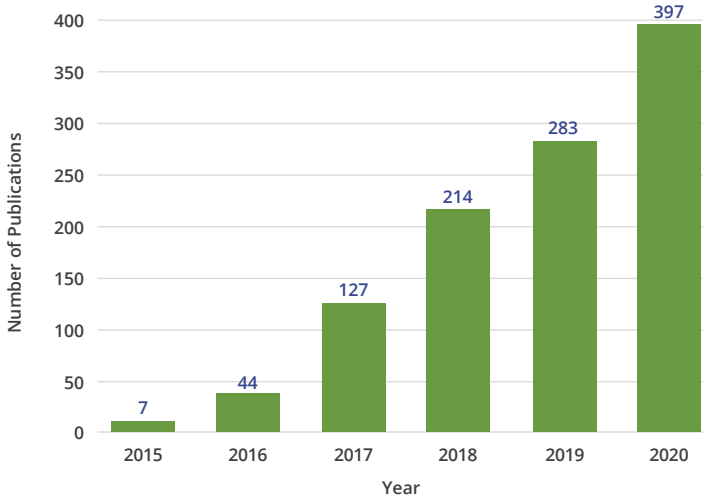

Figure 2: Cumulative publications referencing products in the Verogen portfolio demonstrate consistent annual increases in the amount of literature that mentions the MiSeq FGx System or ForenSeq library prep. The rapid acceptance of the Verogen portfolio is attributable to robust workflows, ease of use, and high data quality.

Key peer-reviewed papers

With the simple, fully kitted assays of ForenSeq library prep kits, the high resolution and unmatched accuracy of the MiSeq FGx System, and the user-centric library management, data analysis, reporting, and visualization of UAS, Verogen NGS technology transforms the most fragile, degraded, or mixed samples into powerful results. The following selection of peer-reviewed papers highlights the research supporting the Verogen portfolio and demonstrates how NGS technology is powering modern forensic laboratories and advancing human identification. To access all papers, visit verogen.com/publications.

Age estimation

Aliferi, Anastasia, David Ballard, Matteo D. Gallidabino, et al., "DNA methylation-based age prediction using massively parallel sequencing data and multiple machine learning models," *Forensic Science International: Genetics* 37 (November 2018): 215–226, https://doi.org/10.1016/j.fsigen.2018.09.003.
Statistical modeling approach to analyze NGS data for age-correlated CpG sites, with accuracy retained down to 2 ng input DNA.

Heideggera, A., C. Xaviera, H. Niederstätter, et al., "Development and Optimization of the VISAGE basic prototype tool for forensic age estimation," *Forensic Science International: Genetics* 48 (June 2020): 102322. https://doi.org/10.1016/j.fsigen.2020.102322.
Presentation of the VISible Attributes through GEnomics (VISAGE) prototype for age estimation targeting 32 CpG sites using targeted bisulfite sequencing on the MiSeq FGx System.

Naue, Jana, Timo Sänger, Huub C.J. Hoefsloot, et al., "Proof of concept study of age-dependent DNA methylation markers across different tissues by massive parallel sequencing," *Forensic Science International: Genetics* 36 (September 2018): 152–159, https://doi.org/10.1016/j.fsigen.2018.07.007.
Pilot study showing the potential of blood DNA methylation (DNAm) markers for age estimation to evaluate tissues other than blood.

Parson, Walther, "Age Estimation with DNA: From Forensic DNA Fingerprinting to Forensic (Epi)Genomics: A Mini-Review," *Gerontology* 56, no. 4 (June 2018): 326–332, https://doi.org/10.1159/000486239.
Review tracing developments in age estimation methods, contextualizing them with forensic goals and needs while highlighting a path forward.

Vidaki, Athina, David Ballard, Anastasia Aliferi, et al., "DNA methylation-based forensic age prediction using artificial neural networks and next generation sequencing," *Forensic Science International: Genetics* 28 (February 2017): 225–236, https://doi.org/10.1016/j.fsigen.2017.02.009.
Study assessing DNA methylation profiles from 1156 individuals to create an accurate model for chronological age estimation from whole blood data, combining NGS and machine learning.

Ancestry-informative marker set

Phillips, Christopher, "Forensic genetic analysis of bio-geographical ancestry," *Forensic Science International: Genetics* 18 (September 2015): 49–65, https://doi.org/10.1016/j.fsigen.2015.05.012.
Outline of past human population structure and how it influenced the distribution of contemporary diversity, guiding selection of ancestry-informative marker (AIM) sets and level of geographic resolution.

Body fluid identification

Chirnside, Olivia, Anna Lemalu, and Rachel Fleming, "Identification of nasal mucosa markers for forensic mRNA body fluid determination," *Forensic Science International: Genetics* 48 (May 2020): 102317. https://doi.org/10.1016/j.fsigen.2020.102317.
Report on the use of RNA sequencing to identify promising mRNA markers for nasal mucosa and help interpret possible false positives.

Hanson, Erin, and Jack Ballantyne, "Human Organ Tissue Identification by Targeted RNA Deep Sequencing to Aid the Investigation of Traumatic Injury," *Genes* 8, no. 11 (November 2017): 319, https://doi.org/10.3390/genes8110319.
Description of a prototype NGS mRNA profiling assay that can detect RNA mixtures from different tissues and identify the tissue source of origin.

Ingold, Sabrina, Guro Dørum, Erin K. Hanson, et al., "Body fluid identification using a targeted mRNA massively parallel sequencing approach – results of a EUROFORGEN/EDNAP collaborative exercise," *Forensic Science International: Genetics* 34 (May 2018): 105–115, https://doi.org/10.1016/j.fsigen.2018.01.002.
Collaboration to analyze dried body fluid stains with an mRNA panel for body fluid and tissue identification and a coding region SNP (cSNP) panel for assigning body fluids and tissues to donors.

Forensic genetic genealogy

Tillmar, Andreas, Peter Sjölund, Bo Lundqvist, et al., "Whole-genome sequencing of human remains to enable genealogy DNA database searches – A case report," *Forensic Science International: Genetics* 46 (May 2020): 102233. https://doi.org/10.1016/j.fsigen.2020.102233.
Report demonstrating the success of sequencing forensic samples to create SNP genotypes for searches in genealogical databases (i.e., GEDmatch) to generate leads to identify missing or unknown individuals.

Nomenclature

Bodner, Martin, Ingo Basisch, John M. Butler, et al., "Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on quality control of autosomal Short Tandem Repeat allele frequency databasing (STRidER)," *Forensic Science International: Genetics* 24 (September 2016): 97–102, https://doi.org/10.1016/j.fsigen.2016.06.008.
Presentation of STRiDER, a free, curated forensic autosomal STR (aSTR) database that enables reliable frequency estimates from high-quality data and offers quality control for aSTR data.

Gettings, Katherine Butler, David Ballard, Martin Bodner, et al., "Report from the STRAND Working Group on the 2019 STR sequence nomenclature meeting," *Forensic Science International: Genetics* 43 (November 2019): 102165. https://doi.org/10.1016/j.fsigen.2019.102165
Report summarizing topics discussed at the STR sequence nomenclature meeting the STRAND Working Group, a forum for presenting and discussing ideas.

Just, Rebecca S., and Jodi A. Irwin, "Use of the LUS in sequence allele designations to facilitate probabilistic genotyping of NGS-based STR typing results," *Forensic Science International: Genetics* 34 (May 2018): 197–205, https://doi.org/10.1016/j.fsigen.2018.02.016.
Proposal to use the longest uninterrupted stretch (LUS) in allele designations as a straightforward method to represent sequence variation in STR repeat regions and facilitate probabilistic interpretation of NGS typing results.

Phillips, Christopher, Katherine Butler Gettings, Jonathan L. King, et al., "'The devil's in the detail': Release of an expanded, enhanced and dynamically revised forensic STR Sequence Guide," *Forensic Science International: Genetics* 34 (May 2018): 162–169, https://doi.org/10.1016/j.fsigen.2018.02.017.
Comprehensive revision to the sequence template file that expands the forensic STR list, adds annotations, and makes the file available as an FTP download with dynamic revisions and change log.

Population data

Borsuk, Lisa A., Carolyn R.Steffen, Kevin M. Kiesler, "Sequence-based U.S. population data for 7 X-STR loci," *Forensic Science International: Reports* 2 (December 2020): 100160. https://doi.org/10.1016/j.fsir.2020.100160.
National Institute of Standards and Technology (NIST) X-STR population data that provides high-confidence sequence-based allelic and linkage group frequencies to support forensic casework and kinship analysis.

Delest, Anna, Dominique Godfrin, Yann Chantrel, et al., "Sequenced-based French population data from 169 unrelated individuals with Verogen's ForenSeq DNA signature prep kit," *Forensic Science International: Genetics* 47 (July 2020): 102304. https://doi.org/10.1016/j.fsigen.2020.102304.
Study using the ForenSeq DNA Signature Prep Kit to obtain sequences from unrelated French individuals, helping forensic laboratories increase discrimination power for human identification, kinship analysis, and mixture interpretation.

Devesse, Laurence, David Ballard, Lucinda Davenport, et al., "Concordance of the ForenSeq system and characterisation of sequence-specific autosomal STR alleles across two major population groups," *Forensic Science International: Genetics* 34 (May 2018): 57–61, https://doi.org/10.1016/j.fsigen.2017.10.012.
Concordance assessment of autosomal STRs and population variability using commercial STR kits, capillary electrophoresis (CE), and Verogen NGS technology to type 400 samples. Results demonstrate high concordance.

Khubrani, Yahya M., Mark A. Jobling, and Jon H. Wetton, "Massively parallel sequencing of sex-chromosomal STRs in Saudi Arabia reveals patrilineage-associated sequence variants," *Forensic Science International: Genetics* 49 (November 2020): 102402. https://doi.org/10.1016/j.fsigen.2020.102402.
NGS-based analysis demonstrates a marked increase in allele diversity compared to CE. Combined with the simultaneous testing and reduced sample consumption ForenSeq DNA Signature Prep enables, this research suggests a significant role in tough cases.

Novroski, Nicole M.M., Jonathan L. King, Jennifer D. Churchill, et al., "Characterization of genetic sequence variation of 58 STR loci in four major population groups," *Forensic Science International: Genetics* 25 (November 2016): 214–226, https://doi.org/10.1016/j.fsigen.2016.09.007.
Characterization using the MiSeq FGx System and other NGS tools. The resulting population data illustrate the genetic variation in common STR markers for the selected population samples and provide allele frequencies for statistical calculations related to STR profiling.

Peng, Dan, Yinming Zhang, Han Ren, et al., "Identification of sequence polymorphisms at 58 STRs and 94 iiSNPs in a Tibetan population using massively parallel sequencing," Scientific Reports 10 (July 2020): 12225. https://doi.org/10.1038/s41598-020-69137-1.
Study providing genotype and frequencies data of two types of genetic markers for forensic applications. Also demonstrate that ForenSeq DNA Signature Prep is highly polymorphic and informative in the Tibetan population, showing promise for kinship and identification.

Sanne E. Aalbers, Michael J. Hipp, Scott R. Kennedy, et al., "Analyzing population structure for forensic STR markers in next generation sequencing data," Forensic Science International: Genetics 49 (November 2020): 102364. https://doi.org/10.1016/j.fsigen.2020.102364.
Assessment of population structure for NGS-based data applied to 27 loci in five different groups. Matching proportions obtains locus-specific estimates, estimates per geographic group, and a global measure.

Simayijiang, Halimureti, Niels Morling, and Claus Børsting, "Sequencing of human identification markers in an Uyghur population using the MiSeq FGx Forensic Genomics System," *Forensic Sciences Research* (September 2020): https://doi.org/10.1080/20961790.2020.1779967.
Typing of 264 Uyghurs to assess MiSeq FGx System performance, compare results to CE, analyze sequence diversity, analyze sequence variation in STR flanking regions, and generate allele frequency data for the Uyghur population.

Wu, Riga, Dan Peng, Han Ren, et al., "Characterization of genetic polymorphisms in Nigerians residing in Guangzhou using massively parallel sequencing," *Forensic Science International: Genetics* 48 (June 2020): 102323. https://doi.org/10.1016/j.fsigen.2020.102323.
A study examining the genetic diversity of 85 Nigerians residing in Guangzhou, China. Genotyping forensically relevant markers uncovered the population's genetic features to provide valuable frequency data for forensic applications.

Review

Ballard, David, Jakub Winkler-Galicki, and Joanna Wesoły, "Massive parallel sequencing in forensics: advantages, issues, technicalities, and prospects," *International Journal of Legal Medicine* (May 2020): 134, 1291–1303, https://doi.org/10.1007/s00414-020-02294-0.
A discussion of the utility NGS offers forensics, with an emphasis on advantages, lingering issues, technical aspects, commercial solutions, and interesting applications.

Bleka, Øyvind, Mayra Eduardoff, Carla Santos, et al., "Open source software EuroForMix can be used to analyse complex SNP mixtures," *Forensic Science International: Genetics* 31 (November 2017): 105–110, https://doi.org/10.1016/j.fsigen.2017.08.001.
Quantitative likelihood ratio mixture calculation of a SNP panel demonstrating that uncertainty about the number of contributors to a mixture has little effect on the likelihood ratio, removing a barrier to widespread adoption of SNP crime-stain analysis.

de Knijff, Peter, "From next generation sequencing to now generation sequencing in forensics," *Forensic Science International: Genetics* 38 (January 2019): 175–180, https://doi.org/10.1016/j.fsigen.2018.10.017.
Appeal for wider adoption of NGS in forensic genomic applications with a focus on issues essential to successful implementation in the laboratory and in court.

England, Ryan, and Sallyann Harbison, "A review of the method and validation of the MiSeq FGx Forensic Genomics Solution," *WIREs Forensic Science* 2, no. 1 (January/February 2020): e1351. https://doi.org/10.1002/wfs2.1351.
Methods to prepare libraries with the ForenSeq DNA Signature Prep Kit, sequence with the MiSeq FGx System, and analyze data with ForenSeq Universal Analysis Software. Includes an assessment cementing these products as reliable and robust.

Sequencing characterization

Cheng, Kevin, Jessica Skillman, Stephanie Hickey, et al. "Variability and additivity of read counts for aSTRs in NGS DNA profiles," *Forensic Science International: Genetics* 48 (September 2020): 102351. https://doi.org/10.1016/j.fsigen.2020.102351.
Investigation of NGS analyte signal variation using the ForenSeq DNA Signature Prep Kit. Describes model to help inform a continuous method for interpreting DNA profile data.

Devesse, Laurence, Lucinda Davenport, Lisa Borsuk, et al., "Classification of STR allelic variation using massively parallel sequencing and assessment of flanking region power," Forensic Science International: Genetics 48 (September 2020): 102356. https://doi.org/10.1016/j.fsigen.2020.102356.
Genotyping of 1007 samples from five population groups that demonstrates concordance between the MiSeq FGx System and CE. Provides sequence-based frequencies to enable statistical calculations.

Gettings, Katherine Butler, Lisa A. Borsuk, David Ballard, et al., "STRSeq: A catalog of sequence diversity at human identification Short Tandem Repeat loci," *Forensic Science International: Genetics* 31 (November 2017): 111–117, https://doi.org/10.1016/j.fsigen.2017.08.017.
Summary of the STR Sequencing Project (STRSeq), a collaborative, international effort to facilitate the description of sequence-based alleles at the STR loci targeted in human identification assays.

Gill, Peter, Rebecca Just, Walther Parson, et al., *Forensic Practitioner's Guide to the Interpretation of Complex DNA Profiles* (2020), chap. 13, https://doi.org/10.1016/B978-0-12-820562-4.00021-3.
An interpretation of complex DNA profiles generated by NGS, using a method applicable to regression analyses to establish general trends with data sets.

Gorden, Erin M., Kimberly Sturk-Andreaggi, Julia Warnke-Sommer, et al., "Next generation sequencing of STR artifacts produced from historical bone samples," *Forensic Science International: Genetics* 49 (September 2020): 102397. https://doi.org/10.1016/j.fsigen.2020.102397.
Presentation of data demonstrating the ability of NGS to distinguish between authentic human alleles and nonhuman PCR artifacts.

Phillips, Christopher, Laurence Devesse, David Ballard, et al., "Global patterns of STR sequence variation: Sequencing the CEPH human genome diversity panel for 58 forensic STRs using the Illumina ForenSeq DNA Signature Prep Kit," *Electrophoresis* 39 (August 2018): 2708–2724, https://doi.org/10.1002/elps.201800117.
A detailed population study of sequence variation with ramifications for coordinating the compilation of sequence variation on a much larger scale than was required before forensic laboratories started adopting NGS.

So Yeun Kwon, Hwan Young Lee, Sun Hye Kim, et al., "Investigation into the sequence structure of 23 Y chromosomal STR loci using massively parallel sequencing," *Forensic Science International: Genetics* 25 (November 2016): 132–141, https://doi.org/10.1016/j.fsigen.2016.08.010.
Study sequencing samples from 250 unrelated Korean males. Results indicated that the MiSeq platform-based analysis system used in the study can facilitate forensic laboratories' investigation into the sequences of the 23 Y-STRs.

Unidentified remains

Finaughty, Chandra, Kate Megan Reid, Iekram Hoosen Alli, et al., "A first for forensic genetics in Africa: successful identification of skeletal remains from the marine environment using massively parallel sequencing," *Forensic Science International: Genetics* 49 (November 2020): 102370. https://doi.org/10.1016/j.fsigen.2020.102370.
The successful identification of human remains in Africa using the MiSeq FGx Forensic Genomics Solution. Also, the first case report on successful use of NGS in a marine decomposition case.

Validation

England, Ryan, Gemma Nancollis, Janet Stacey, et al., "Compatibility of the ForenSeq™ DNA Signature Prep Kit with laser microdissected cells: An exploration of issues that arise with samples containing low cell numbers," *Forensic Science International: Genetics* 47 (May 2020): 102278. https://doi.org/10.1016/j.fsigen.2020.102278.
Work establishing NGS compatibility with laser microdissection cell collection, including adding magnesium chloride to increase amplification efficiency. From 50 epithelial and 100 sperm cells, the authors obtained full aSTR profiles.

Jäger, Anne C., Michelle L. Alvarez, Carey P. Davis, et al., "Developmental validation of the MiSeq FGx Forensic Genomics System for Targeted Next Generation Sequencing in Forensic DNA Casework and Database Laboratories," *Forensic Science International: Genetics* 28 (May 2017): 52–70, https://doi.org/10.1016/j.fsigen.2017.01.011.
Methods for sequencing using Verogen NGS technology. Highlights include sequencing many forensic loci in one multiplex reaction with semi-automated genotyping, successful SWGDAM developmental validation, and generating more actionable information.

Köcher, Steffi, Petra Müller, Burkhard Berger, et al., "Inter-laboratory validation study of the ForenSeq™ DNA Signature Prep Kit," *Forensic Science International: Genetics* 36 (September 2018): 77–85, https://doi.org/10.1016/j.fsigen.2018.05.007.
Inter-laboratory validation assessing concordance, reproducibility, sensitivity, and mixtures with a statistical comparison of inter-locus balance between CE and NGS.

Laurent, F.X., L. Ausset, M. Clot, et al., "Automation of library preparation using Illumina ForenSeq kit for routine sequencing of casework samples," *Forensic Science International: Genetics Supplemental Series* 6 (December 2017): e415–e417, https://doi.org/10.1016/j.fsigss.2017.09.156.
Development and validation of a fully automated workflow with the ForenSeq DNA Signature Prep Kit and NGS STARlet. Automation improves reproducibility and reduces hands-on time, facilitating NGS adoption in forensic laboratories.

Müller, Petra, Christian Sell, Thorsten Hadrys, et al., "Inter-laboratory study on standardized MPS libraries: evaluation of performance, concordance, and sensitivity using mixtures and degraded DNA," *International Journal of Legal Medicine* 134 (January 2020): 185–198, https://doi.org/10.1007/s00414-019-02201-2.
Inter-laboratory study evaluating forensically relevant parameters in the framework of the SeqForSTRs project. Eight laboratories sequenced a shared ForenSeq DNA Signature Prep library on MiSeq FGx Systems. All obtained quality metrics, spotlighting NGS as a promising tool for human identification.

Find more than insights. Learn more at **www.verogen.com/capabilities**.

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 | Document # VD2020011 Rev. D | February 2021