Technical Note

VEROGEN

Representation of Verogen Technology in Forensic Genomics Literature

MiSeq FGx sequencing and ForenSeq library prep and analysis are the technologies of choice for modern forensic laboratories.

Highlights

- **Preferred sequencing technology**
  The versatility and superior data quality of the MiSeq FGx System make it the platform of choice in 70% of peer-reviewed publications.
- **Rapid acceptance of the Verogen portfolio**
  Nearly 350 peer-reviewed studies have employed Verogen platforms and applications to date—a doubling of net new papers every year.

Preferred sequencing technology for forensic genomics

The 2015 launch of the Verogen MiSeq FGx® Sequencing System introduced the industry to next-generation sequencing (NGS), also called massively parallel sequencing (MPS). Since then, Illumina sequencing-by-synthesis (SBS) technology has been the preeminent force driving Verogen technology in the forensic NGS space. The Illumina SBS technology underpinning the MiSeq FGx System now powers 90% of the sequencing data generated worldwide. Nearly 70% of publications describing commercial library prep methods use technologies specifically designed for sequencing on a MiSeq FGx System, with the ForenSeq™ approach far outpacing other chemistries. Every year, Verogen NGS is the dominant technology cited in forensic genomics publications. Figure 1 presents this finding, with mentions of ForenSeq, Nextera, and TruSeq products supporting Verogen NGS publications and mentions of Precision ID and AmpliSeq products supporting Ion Torrent publications. This technical note summarizes the pertinent publications and highlights key papers.

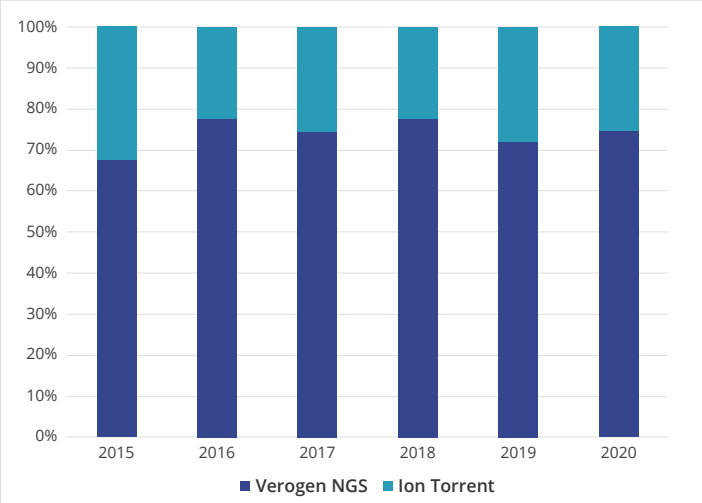

Figure 1: Annual distribution of papers supporting Verogen library prep

Community support across applications

Year after year, the scientific community has embraced the Verogen portfolio, publishing more papers each year than the previous year and building academic and professional support for ForenSeq library prep, the MiSeq FGx System, and ForenSeq Universal Analysis Software (UAS). As of March 2020, the community has released 349 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 ForenSeq chemistry or the MiSeq FGx System.

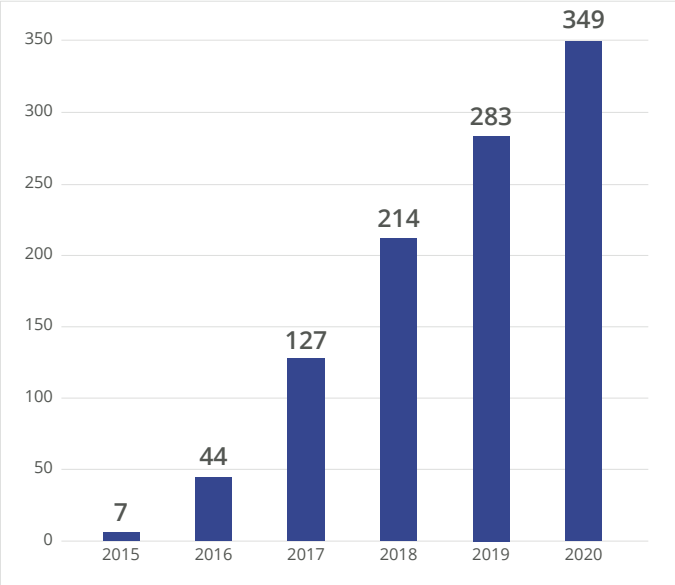

Figure 2: Cumulative publications referencing Verogen products

Complete sample-to-answer solution

The ForenSeq workflow provides the first fully validated sample-to-answer sequencing solution developed for forensic applications. 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, and visualization of ForenSeq UAS, Verogen NGS technology transforms the most fragile, degraded, or mixed samples into powerful results.

Key peer-reviewed papers

Verogen offers flexible solutions for a diverse range of forensic studies, from STR analysis for routine casework to FGG that helps further cold cases. The following selection of peer-reviewed papers highlights the research behind the Verogen portfolio and demonstrates how NGS technology is powering modern forensic laboratories and advancing human identification.

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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: Collaborative exercise to analyze dried body fluid stains with two panels: 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.
Summary: 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.
Summary: 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
Summary: 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.
Summary: 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.
Summary: Comprehensive revision to the sequence template file. The update expands the forensic STR list, adds annotations, and makes the file available as an FTP download with dynamic revisions and a date-stamped change log.

Population data

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.
Summary: 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.
Summary: 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.

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.
Summary: 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.

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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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

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: 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.

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.
Summary: 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.

STR analysis

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.
Summary: 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.

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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.
Summary: 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.

To learn more about Verogen applications, visit **www.verogen.com/applications**.

 | Document # VD2019011 Rev. C | September 2020