The Dive into Next Generation Sequencing: From Validation to Implementation and Beyond!

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ABSTRACT

For almost a quarter of a century, DNA analysis has been dominated by capillary electrophoresis-based short tandem repeat (STR) testing. Now is the time to dive into a new era of DNA technology! Utilizing next generation sequencing (NGS), hundreds of forensic loci are targeted and amplified including the Combined DNA Index System (CODIS) core locations that have been used in DNA testing for years. This is important as it allows for backwards compatibility between the current DNA database formats and NGS technology. Additionally, NGS can be employed in multiple platforms which provides the ability to target other sections of DNA, including ancestry markers, phenotypic markers, and whole genome mitochondrial DNA. This technology may heat up cold cases by providing new leads, especially in unidentified humans remains cases, instances where a comparable mtDNA profile may establish maternal lineage, or when genealogy can determine a potential family member leading to identification.

With these eye-opening benefits, three different platforms of NGS have been validated at DNA Labs International including the Verogen® ForenSeq DNA Signature Prep Primer Set B Kit, ForenSeq mtDNA Whole Genome Kit, and ForenSeq Kintelligence kits. In addition to performing internal validation studies in accordance the FBI Quality Assurance Standards (QAS), at the recommendation of the Scientific Working Group on DNA Analysis Methods (SWGDAM) an additional study was performed to evaluate the effect of processing less samples on a single run in order to obtain more reads per sample. A disadvantage to NGS, as opposed to traditional capillary electrophoresis based STR testing, is the amount of hands-on time that is required by the scientist. To provide a high throughput option for the NGS setup, the Aurora Biomed® Versa 1000 robotic platform was also evaluated as an alternative to a manual setup. This presentation will review the internal validation process used to bring online three different next generation sequencing platforms, the implementation process in the laboratory, and cases that have already benefitted from these new kits!

DNA LABS INTERNATIONAL

ISO/IEC 17025:2017 and FBI QAS DNA Laboratory

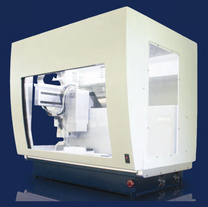
Why Not Use Existing Technology?

- CE based systems are outdated technology
- Current commonly used commercial CE-based systems cannot analyze other marker types such as single nucleotide polymorphisms (SNPs)
- CE cannot offer extended tools such as FGG and large multi-marker systems due to limitations in the dye channels
- Keeping it all 'in the lab'
- The need to bring FGG in-house opposed to sending to non-forensic laboratories

Automated Platform for Setup

Aurora Biomed Versa 1000 robotic platfor

- Alternate Option as opposed to Manual Setup
- Increases throughput
- Reduces chances of pipetting errors
- Can process up to 96 samples at a time on a plate
- Used for post-PCR sample setup



Standards, Guidelines, and Recommendations

- FBI QAS
- ISO 17025
- SWGDAM
- ANAB Guidance Doc.
- DOJ interim policy
- FTDNA (Family Tree) Terms of Service
- GedMatch Terms of Service

Accreditation Requirements

- Must adhere to FBI QAS
- Must adhere to ISO/IEC 17025:2017
- No reports issued with logo until scope of accreditation has been changed
- MPS/XSTR/SNP/mtDNA all need to be added
- All of the typical studies must be covered; accuracy, precision, reproducibility, mixtures, etc.

Development of Protocols

Quality Manual

Expansion of scope for accreditation?

Standard Operating Procedure

Methods

Manual/Robotic?

What did the validation teach about the limitations and expectations for data?

- Lab Processing
- Thresholds
- Pooling Strategies

Interpretation

Case File & Reporting

Validation

Testing Scheme	NGS Implementation vs Kintelligence Implementation	How Do You Demonstrate Concordance?	What About Mixtures?
Tying in Other Validations	FBI and ISO Requirements	Who Trains?	Considerations for Cost

Validation Setbacks

Factors that led to validation delays:

Social Distancing

- Half of our staff converted to remote work
- On-site staff had to shift to a casework focus
- Less people = less validation

Reduced Stock of Reagents/Supplies

- Masks
- Lab coats

Pipette Tip Shortage

- Had to direct our resources towards the casework team to ensure cases were still completed.

Implementation Full Workflow

MiSeq FGx Forensic Genomics System

Verogen® ForenSeq DNA Signature Prep Kit (B)

- 27 autosomal STRs
- 24 Y-STRs
- 7 X-STRs
- 94 Identity SNPs
- 56 Ancestry SNPs
- 22 Phenotypic SNPs

ForenSeq mtDNA Whole Genome Kit

- Entire mtDNA Genome
- Shorter amplicons
- Optimized library

ForenSeq Kintelligence Kit

- Minimizes privacy concerns
- SNP selection for forensic relevance
- Generates a GEDmatch compatible report

Call Rates

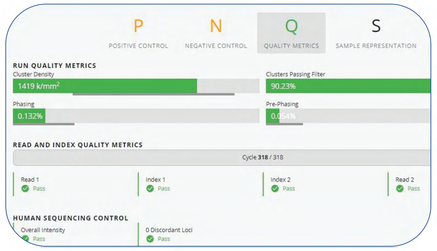
SS1_Female1_0.75ng | Select an Analysis Method: Verogen Kintelligence Analysis Method | State: Completed | Sample Type: Sample | Date Created: Sep 10, 2021, 11:48:42 AM | Date Completed: Sep 12, 2021, 5:05:35 AM | Other Options

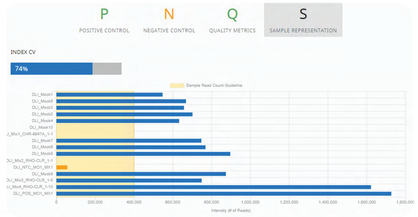
Sample Overview — CREATE REPORT

Sample Locus Call Rate	Total Sample Reads	Autosomal Heterozygotes	X Heterozygotes	Autosomal Intralocus Balance	X Intralocus Balance	Gender	Contributor Status
98.14% 10040 / 10230	11,862,439	46.5% 4621 / 9935	34.3% 36 / 105	73.9%	73.7%	XX	Single-Source

	X-SNP		Y-SNP		Ancestry SNP		Phenotype SNP		Identity SNP		Kinship SNP	
Loci Typed	99.06%	105 / 106	0.00%	0 / 85	100.00%	54 / 54	100.00%	24 / 24	98.94%	93 / 94	98.96%	9764 / 9867
< 150 bp Loci Typed	99.04%	103 / 104	0.00%	0 / 83	100.00%	49 / 49	100.00%	13 / 13	98.70%	76 / 77	99.09%	9585 / 9673
>= 150 bp Loci Typed	100.00%	2 / 2	0.00%	0 / 2	100.00%	5 / 5	100.00%	11 / 11	100.00%	17 / 17	92.27%	179 / 194

Phenotype Estimation Report





Where Are We Now?

Verogen® ForenSeq DNA Signature Prep Kit (B)

- All validation study runs completed by DLI
- Write-up from Verogen
- Supplemental write-ups from DLI
  - XSTR/SNP/Phenotyping Stats
  - Stochastic Threshold
  - Versa

ForenSeq mtDNA Whole Genome Kit

- All validation study runs completed
- Write-up from DLI

ForenSeq Kintelligence Kit

- All validation study runs completed
- Write-up from DLI