

# ***Scientific Analysis on DNA Analysis Methods (SWGDM) January 2014 Report***

*January 7-9, 2014*

*FBI Academy at Quantico, Virginia*

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## **I. Agenda:**

Please see Attachment 1.

## **II. Meeting Synopsis:**

### **Tuesday, January 7, 2014**

SWGDM Chairman, Anthony Onorato, welcomed the attendees who introduced themselves. The Chair noted that the dates for the July meeting are: July 15-17, 2014. The Chair reviewed the agenda for the January 2014 meeting and recognized Peter Vallone (National Institute of Standards and Technology; NIST) for his assistance in assembling speakers for the technical session on Next Generation Sequencing.

With respect to membership, the Chair noted that Cathy McCord has resigned as she was promoted to Laboratory Director and that Ken Konzak would be retiring before the July meeting and this would be his last meeting.

In introducing the morning presentations, the Chair explained that the technical session would be devoted to a background on Next Generation Sequencing (NGS) and introduced Dr. Thomas Callaghan from the FBI Laboratory to provide a history of DNA sequencing.

Dr. Callaghan's presentation included a timeline on DNA sequencing beginning in 1972 with the first gene being sequenced. Dr. Callaghan explained the Sanger method of sequencing. In determining whether Next Generation Sequencing (NGS) is suitable for human identification, Dr. Callaghan raised the following questions: (1) Can NGS technology increase sensitivity of analysis on limited and/or degraded samples (crime scene application)? (2) Can NGS produce mtDNA and STR data that matches today's quality and reliability?; and (3) When the person of interest is not in the DNA database, can we take advantage of advances in genetic analysis to provide investigative leads on the person of interest? Dr. Callaghan noted that before the FBI would apply NGS to crime scene sample analysis, it would have to gain approval from FBI and DOJ executive management and establish well thought out rules and policies in order to maintain public

trust in forensic DNA analysis. Dr. Callaghan concluded that before NGS was used in routine human forensic DNA analysis, the following would need to be addressed: sensitivity, platform and chemistry limitations (read lengths), alignment/assembly, standards, and quality assessment and assurance.

Dr. Peter Vallone of NIST presented the current technology and applications of NGS. Dr. Vallone explained that a variety of different terms are used for NGS such as second generation sequencing, whole genome sequencing, third generation sequencing, high throughput sequencing and next generation genomics. His review of the scientific literature indicated NGS articles started to appear in 2007 and the number of articles have increased over the past several years and such articles appear in connection with cancer, clinical, microbial, virus, prenatal, transplant, forensic, (in order of frequency). Dr. Vallone explained that NGS has many non-forensic applications such as, clinical, reproductive health, cancer, transplant, public health, etc... Dr. Vallone contrasted whole genome sequencing with targeted sequencing (for example, specific regions, such as CODIS STRs or SNPs). Dr. Vallone explained the various formats (FASTQ, SAM/BAM) as well as current and future platforms. To determine what instrument platform is best for your application: markers, coverage, samples and cost should be considered as well as input amounts, level of accuracy, integrity of DNA and mixtures. Dr. Vallone offered the following topics for further thought: additional genetic markers, data interpretation and review, STR nomenclature, ethical considerations with coding region markers, and validation of NGS systems/methods.

Dr. Katherine Gettings with the Applied Genetics Group at NIST presented on the forensic applications of NGS. Dr. Gettings described three forensic applications: whole mitochondrial genome analysis, forensically relevant SNPs and going in depth into STR loci. Dr. Gettings identified the following challenges for NGS forensic applications: sample input requirements, library preparation methods, read length, data analysis, cost and time per unit of information, privacy re: disease related markers, and validation and court acceptance. Dr. Gettings reviewed the various forensic applications in detail. With respect to whole genome mtDNA sequencing, Dr. Gettings noted that it is amenable to library preparation, sequencing platforms and bioinformatics and that it is an improvement over current methods as it will allow for multiplexing of many samples and facilitate whole genome sequencing which is labor intensive. Although forensic SNP typing is not commonly used by forensic DNA laboratories, it is amenable to library preparation, sequencing platforms and bioinformatics. Dr. Gettings described how the STR loci are amenable to library preparation and sequencing platforms and that the bioinformatics have challenges relating to single source and mixture samples. Additionally, using NGS sequencing for the STR loci would not be an improvement over

the current methods because the CE-based processing is already streamlined. Dr. Gettings concluded by describing a hypothetical multimarker multiplex for 96 samples: 20 STR loci, 100 SNPs, and mtDNA genome.

Mr. Kevin Kiesler of the Applied Genetics Group at NIST presented on NGS workflow and infrastructure requirements. Mr. Kiesler explained that the proper instrumentation will depend on the throughput requirements of the laboratory. He recommended separate areas for pre-PCR, post-PCR and post library amplification and sequencing. Additional infrastructure recommendations included network connectivity, computational facilities and data storage. All presenters commented on the large amount of data generated in the sequencing process. Specialized equipment recommendations included, but were not limited to, ultrapure water system, sonicator, size selection system, and quantitative PCR instrument. Mr. Kiesler explained the workflow from the perspective of front end enrichment, library preparation and sequencing on several instruments, data analysis and cost analysis. Mr. Kiesler concluded with the following observations: library preparation is complex and requires well-trained and skilled personnel; the costs associated with NGS are better suited to high-throughput applications; and the informatics approach can have a significant effect.

The afternoon session included user experiences on NGS by Dr. Jodi Irwin of the FBI Laboratory, Dr. Toni Diegoli from the Emerging Technologies Section of the Armed Forces DNA Investigation Laboratory and Dr. Seth Faith from Battelle Memorial Institute.

### **Wednesday, January 8, 2014**

The morning session began with updates from John Tonkyn from the Missing Person Committee and Tamyra Moretti from the Y-STR Committee. Each described the status of their documents and revisions. Members and guests were encouraged to provide any comments on the two documents under consideration to the Committees for consideration on Wednesday.

After the updates, the following Committees met for the remainder of the day:

Autosomal STR Committee

CODIS Committee

Enhanced Detection Methods and Interpretation Committee

Missing Person and Mass Disaster Committee

Quality Assurance Committee

Rapid DNA Committee

Y-STR Committee

A round table discussion was held Wednesday evening that began with a tribute to Ken Konzak of the California Department of Justice, who has been a participant in SWGDAM since its inception (when it was known as the Technical Working Group on DNA Analysis Methods) by Gary Sims. Ken followed with a retrospective on TWGDAM/SWGDAM.

Russell Gettig from the New York State Police Forensic Investigation Center described his laboratory's validation and implementation of TrueAllele® for casework mixtures/probabilistic genotyping. Members and Invited Guests raised additional technical issues, including, but not limited to: trend to require that analysts are certified in their disciplines; obtaining DNA from latent fingerprints; scraping v. swabbing to obtain DNA; use of A2LA for accreditation and audits; and use of mixture analysis tools.

#### **Thursday, January 17, 2013**

The Committees continued their individual meetings for the morning session.

The Chair opened the Thursday afternoon session with a brief update by Jennifer Wendel on the Rapid DNA Committee.

Mr. John Tonkyn, Chair of the Missing Person Committee, described the revisions made to their document – Guidelines for Missing Persons Casework. A motion was made and seconded to approve the Guidelines as revised, including the revision of Figure 2B and the Guidelines were unanimously approved.

Dr. Tamyra Moretti, joined by the other members of the Y-STR Committee present, reviewed the comments received and revisions to the Interpretation Guidelines for Y-Chromosome STR Typing by Forensic DNA Laboratories. A motion was made and seconded to approve the Guidelines as revised and the Guidelines were approved, with one vote against approval and four abstentions.

The afternoon update session began with an update on the National Institute of Justice presented by Minh Nguyen and Chad Ernst. They described the priorities identified by the Forensic Technical Working Group available at <http://nij.gov/topics/forensics/Documents/fy14-forensic-twg-table.pdf>.

Dr. Douglas Hares of the FBI Laboratory's CODIS Unit presented an update on the CODIS Core Loci Working Group. Dr. Hares announced that NDIS had reached the milestone of over 13 million DNA profiles. Dr. Hares noted that the NDIS Operational Procedures Manual is available on the FBI's Internet site at [www.fbi.gov/about-us/lab/biometric-analysis/codis](http://www.fbi.gov/about-us/lab/biometric-analysis/codis) and the next revision will be available at the end of the month. Dr. Hares explained that the CODIS core loci project's database validation began in December 2012. The casework validation began July 2013. Once all the data is compiled and reviewed, the selection of new core is anticipated for mid to late 2014 with implementation of the additional core loci expected to occur 24 months after selection. Dr. Hares emphasized that it is important to re-evaluate existing analytical thresholds, stochastic thresholds and interpretation guidelines during validation and implementation of the new megaplex kits. Dr. Hares acknowledged the efforts of the National Institute of Standards and Technology (NIST) team in performing data analysis of the validation studies completed thus far and reviewed some examples from that data.

The Chair introduced Dr. Mike Coble and Dr. John Butler from the National Institute of Standards and Technology (NIST) to provide an update on the Applied Genetics Group activities and the National Commission on Forensic Science. Dr. Coble provided an update on the following items: research underway on digital PCR for the next generation quantification standard; continued testing of Rapid DNA instruments in order to provide feedback to vendors; interlaboratory study of the Rapid DNA instruments; Next Generation Sequencing studies as described on Tuesday; sequencing of variant alleles (sequencing of SRM 2391c to further characterize and determine interesting genomic characteristics within STR fragments in support of Next Generation Sequencing); finalized agreement to test a new 27 Y-STR multiplex kit; providing training on mixtures (including DNA Technical Leader Summit); description of scenarios/results from the interlaboratory mixture study; and upcoming forensic webcasts on mixtures, probabilistic genotyping and new STR kits.

Dr. John Butler updated the participants on the National Commission on Forensic Science, a Federal Advisory Committee for the U.S. Department of Justice (DOJ). Dr. Butler reviewed the duties of the Commission that include recommending priorities for standards development, recommending that the Attorney General endorse guidance identified/developed by subject matter experts; and developing guidance for the intersection between forensic science and the courtroom. The Commission will have 37 members who will be named in the near future. There will be Co-Chairs and Vice-Chairs from the Department of Justice and the NIST; Dr. Butler is the NIST Vice-Chair. The Commission's first meeting is scheduled for February 3-4, 2014 and they will discuss

accreditation, certification and proficiency as well as NIST's progress with the guidance groups. The comments submitted in response to NIST's Notice of Inquiry will be presented to the Commission at this meeting.

**III. Attendees:**

Please see Attachment 2.

**IV. Next Meeting:**

The next meeting will be held July 15-17, 2014.



## SWGDM REGULAR MEETING

January 7 - 9, 2014

### Tuesday, January 7, 2014

- 8:00 AM Chair and Vice Chair Meeting  
8:30 AM Welcome and Business Matters

### TECHNICAL SESSION: NEXT GENERATION SEQUENCING (NGS)

- 9:00 AM History of DNA Sequencing – Thomas Callaghan  
9:45 AM NGS: Technology and Current Applications – Peter Vallone  
10:45 AM BREAK  
11:00 AM NGS: Forensic Applications – Katherine Gettings  
12:00 PM LUNCH  
1:00 PM NGS: Workflow Considerations and Infrastructure Requirements- Kevin Kiesler  
2:00 PM NGS: User Experiences  
FBI – Jodi Irwin  
AFDIL – Toni Diegoli  
Battelle – Seth Faith  
3:30 PM BREAK  
3:45 PM Continue User Experiences  
5:30 PM ADJOURNMENT



**Wednesday, January 8, 2014**

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| 8:00 AM  | Review of the Recommendations for Missing Person Casework – John Tonkyn   |
| 8:15 AM  | Review of the proposed revisions to the SWGDAM Y-STR Interpretation Guidelines – Tamyra Moretti   |
| 8:30 AM  | Committee Breakout Sessions<br>Autosomal STR – John Butler<br>CODIS – Douglas Hares<br>Enhanced Detection Methods – Eugene Lien<br>Missing Persons/Mass Disaster – John Tonkyn<br>QA – Jodi Dahl<br>Rapid DNA – Jennifer Wendel<br>Y-STR – Tamyra Moretti |
| 12:00 PM | LUNCH   |
| 1:00 PM  | Committee Break-Out Sessions  |
| 4:30 PM  | ADJOURNMENT   |

**ROUND TABLE SESSION**

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| 6:00 PM | TBD - Moderator: Russell Vossbrink |
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SCIENTIFIC WORKING GROUP

DNA ANALYSIS METHODS

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**Thursday, January 9, 2014**

- 8:30 AM      Committee Break-Out Sessions
- 12:00 PM      LUNCH
- 1:00 PM      Committee Progress Updates (including review of Committee work products)
- Missing Person/Mass Disaster – John Tonkyn
- Y-STR – Tamyra Moretti

**UPDATE SESSION**

- 2:30 PM      NIJ Update – Minh Nguyen
- 3:30 PM      CODIS Core Loci Expansion Update – Douglas Hares
- 3:45 PM      BREAK
- 4:00 PM      NIST Update – Mike Coble
- 4:30 PM      National Commission on Forensic Science Update – John Butler

**BUSINESS SESSION**

- 4:45 PM      Chair Report – Tony Onorato
- 5:00 PM      ADJOURNMENT

## SWGDM January 2014 Meeting Members and Invited Guests

### SWGDM Chair

**Anthony Onorato**  
FBI Laboratory

### SWGDM Vice Chair

**Russell Vossbrink**  
Scottsdale Police Department

### SWGDM Executive Secretary

**Dawn Herkenham (IG)**  
Leidos

**Patricia Aagaard (IG)**  
FBI Laboratory

**Angelo Della Manna**  
Alabama Department of Forensic Sciences

**Jack Ballantyne (IG)**  
University of Central Florida

**Suzanne Barritt-Ross (IG)**  
Armed Forces DNA Identification Lab

**Todd Bille (IG)**  
Bureau of Alcohol, Tobacco and Firearms

**John Butler (IG)**  
National Institute of Standards and Technology

**Jocelyn Carlson (IG)**  
FBI Laboratory

**Amber Carr (IG)**  
FBI Laboratory

**Chris Carney (IG)**  
FDLE – DNA Database

**Michael Coble (IG)**  
NIST

**Jerrilyn Conway (IG)**  
FBI Laboratory

**James Corcoran (IG)**  
FBI Laboratory

**Rhonda Craig (IG)**  
FBI Laboratory

**Jodi Dahl (IG)**  
FBI Laboratory

**Tina Delgado (IG)**  
FBI Laboratory

**Neil Fernandopulle (IG)**  
Centre of Forensic Sciences

**Russell Gettig (IG)**  
NYS Police Forensic Investigation Center

**Ann Marie Gross**  
Minnesota Bureau of Criminal Apprehension

**Douglas Hares (IG)**  
FBI Laboratory

**Bruce Heidebrecht**  
Maryland State Police

**Brian Hoey**  
Missouri State Highway Patrol

**Deedra Hughes**  
Mississippi Crime Laboratory

**Clark Jaw (IG)**  
FBI Laboratory

**Elizabeth Johnson (IG)**  
U.S. Army Crime Lab

**Ken Konzak**  
California Department of Justice

**George Li**  
Virginia Department of Forensic Science

**Eugene Lien**  
NYC Office of the Chief Medical Examiner

**Beth Ann Marne**  
Pennsylvania State Police

**Amy McGuckian**  
Palm Beach County Sheriff's Office

**Scott McWilliams (IG)**  
Wyoming State Crime Laboratory

**Gary J. Molina (IG)**  
Texas Dept. of Public Safety Crime Lab – CODIS

**Tamyra Moretti (IG)**  
FBI Laboratory

**Steven Myers**  
California Department of Justice

**Jeffrey Nye (IG)**  
Michigan State Police

**Thomas Parsons (IG)**  
International Commission on Missing Persons

**Dixie Peters (IG)**  
University of North Texas Health Science Center

**John Planz**  
University of North Texas Health Science Center

**Eric Pokorak**  
FBI Laboratory

**Juliet Rolando (IG)**  
FBI Laboratory

**Taylor Scott**  
Illinois State Police

**Gary Sims**  
California Department of Justice

**Joel Sutton**  
USA CIL

**John Tonkyn**  
California Department of Justice

**Peter Vallone (IG)**  
National Institute of Standards and Technology

**Stacy Warnecke (IG)**  
Kentucky State Police

**Jennifer Wendel**  
FBI Laboratory

**Ray Wickenheiser (IG)**  
NYS Police Forensic Crime Lab System

**Tim Zolandz (IG)**  
FBI Laboratory

Presenters:

**Erica Butts**  
NIST

**Toni Diegoli**  
AFDIL

**Seth A. Faith**  
Battelle

**Katherine Butler Gettings**  
NIST

**Kevin Kiesler**  
NIST

**Minh Nguyen**  
NIJ