STR Typing Workflows on the Ion Torrent S5 XL Sequencing Platform

Kevin Kessler, M.S.
Research Biologist, Applied Genomics Group, NIST
Green Mountain DNA Conference
August 1, 2018

Outline

• Using NGS
• Sequencing STR loci
• S5-Ion Chef workflows
• Using Converge software for CE-NGS data comparisons

Sequencing STRs: Plusses and Minuses

+ Plusses
  • Smaller amplicons
  • Not constrained by CE size separation
  • Better for degraded DNA
  • Improved mixture decompositional potential
  • Discriminate shared alleles & stutter masking by sequence differences

- Minuses
  • Higher cost vs CE
  • More complex procedure
  • Novel analysis methods, nomenclature
  • Longer time to result
  • New methods require training, validation, implementation

NIST’s Experiences Sequencing STRs at Scale

• 2014 Promega PowerSeq (24-plex)
  • 183 samples from NIST population set
  • African American, Asian, Caucasian, Hispanic
  • Collaboration with Battelle Institute
  • Wet lab time: ~ two weeks
  • Goal: initial characterization of sequence variation

• 2016 ForenSeq Signature Kit
  • 1386 samples from NIST population set
  • Goal: U.S. population allele frequencies

Why Use S5 XL & Ion Chef?

• Modular panels -vs- megaplex
  • Lower complexity = improved balance & sensitivity
  • Large multiplexes give more information, but tricky to optimize

• Thermo-Fisher Panels
  • STR
    • Precision-ID GlobalFiler NGS (v2) panel for S5 instrument
  • SNP
    • Precision-ID Ancestry Panel
    • Precision-ID Identity Panel (includes Y- and X-chromosomes)
  • Mitochondrial DNA
    • Precision-ID Whole Genome Panel
  • Precision-ID Control Region Panel

• Ion Chef
  • Full automation of library construction and templating procedures
  • Developed and supported by ThermoFisher

NIST’s Experiences Sequencing STRs at Scale

• 2017 Promega PowerSeq 466Y – collaboration with Promega
  • 665 samples from NIST population set
  • Time to complete sequencing: ~ two months
  • Goal: assist with assay development, informatics

• 2017 Precision ID GlobalFiler NGS (v2) – collaboration with ThermoFisher
  • 88 Japanese from 5 prefectures
  • Time to complete sequencing: ~ one month
  • Goal: population allele frequencies - Japanese

• 2018 Precision ID GlobalFiler NGS (v2) – collaboration with ThermoFisher
  • 235 samples (n = 123 Asian, 98 Hispanic, 14 “Challenging”)
  • Time to complete sequencing: ~ two months
  • Goal: population allele frequencies – U.S. Asian & Hispanic, also assay development
Templating Process

Data Analysis Workflow

Profile Comparison: Concordance Testing

Sequencing Eliminates Single Nucleotide Resolution Issues
Conclusions/summary

- **STR Sequencing**
  - Increased information – variation in STR, flanking SNPs
  - Useful with difficult casework samples
  - Mixtures, degraded DNA
- **Ion Chef**
  - Saves labor
  - Cost vs. time trade-off
  - Increases reproducibility, reliability
- **Converge**
  - Visualize & edit data
  - Data comparison (CE/NGS)

Thank You!

Kevin.Kiesler@NIST.gov

- **Funding**
  - NIST Special Programs Office: Forensic DNA
  - FBI Biometrics Center of Excellence: Forensic DNA Typing as a Biometric tool.

- **Disclaimer**: Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Commerce or the Department of Justice. Certain commercial equipment, instruments, and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by NIST, nor does it imply that any of the materials, instruments, or equipment identified are necessarily the best available for the purpose.

- All work presented has been reviewed and approved by the NIST Human Subjects Protection Office.