Standardization in Next-Generation Sequencing
Combined CHARME, EMBnet and NETTAB Workshop

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NGS Technologies

Gb/Run

Read Length [b]
NGS Technologies
Problems in NGS Standardization

- Fast development of new platforms
- Simultaneously obsolescence of former systems
  - 454 and SOLiD: No development of new systems and ceased support
  - Expensive workflows and decreased cost of competitor platforms led to economic pressure
Problems in NGS Standardization

- Big market established in relatively short time
  - Sequencing service providers
  - Sequencing platform manufacturers
- Market leaders like Illumina are not willing to be forced by a general standard
  - 71% market share
  - 90% of all generated sequences by Illumina
  - Want to establish own standards
  - Considerable standardization efforts by Illumina
Problems in NGS Standardization

• Validity of standards across NGS applications
  ▫ Biggest effort in clinical diagnostics
  ▫ Oncology/precision medicine as a promising field
  ▫ Same standards are not applicable or reasonable in other applications and vice versa
Problems in NGS Standardization

- Distribution of NGS systems worldwide
- Highest amount in industrial countries
Problems in NGS Standardization

- Distribution of NGS standardization efforts
  - NGS originated in US
  - Majority of approaches are located overseas
  - No verifiable or published approaches currently in other countries
  - Initiatives should be found and encouraged to participate in the field (CEN)
Standardization Efforts

- NGS guidelines for somatic genetic variant detection by the New York State Department of Health – CLIA guidelines
  - Validation parameters/CLIA performance characteristics
    - Accuracy, robustness, precision, repeatability, reproducibility, analytical sensitivity and specificity, reportable and reference range
  - QC RM
    - NTC, - control, +/-sensitivity control
Standardization Efforts

- **NIST-GIAB**
  - Investigation of reference data, methods and standards for NGS
  - Well-characterized whole human genomes as RM
  - Methods for use of RM for understanding NGS performance

- **ABRF-NGS**
  - Identification of optimal methods and strategies for NGS projects
  - Performance evaluation of different NGS platforms

- **CAP-MOL**
  - Standards for documentation, validation, QA, confirmatory testing, exception logs, monitoring of upgrades, variant interpretation and reporting, incidental findings, data storage, version traceability and data transfer confidentiality

- **ERCC**
  - Development of RNA spike-in controls
Standardization Efforts

- **GSC-MIGS**
  - Remedy lack of incomplete genome descriptions for data submission
  - Depth of coverage, overall quality, taxonomy, trophic level, propagation

- **MAQC-III/SEQC and MAQC-IV/SEQC2**
  - Evaluation of technical performance between different NGS platforms by establishing benchmarks with reference samples

- **HGP-Bermuda Standards**
  - Standards for sequence fidelity
  - Q40 or 99.99% accuracy (1 error/10,000 bp)

- **Nex-StoCT**
  - Developed principles, guidelines, standards as well as recommendations for the implementation of NGS into diagnostic laboratories
  - Novel NGS PT system
    - Enables error identification (FMEA)
    - Indication of QC problems
    - Verification of test performance in laboratories
Possible Standards and *de facto* Standards

<table>
<thead>
<tr>
<th>Prepare sequencing library</th>
<th>Prepare and enrich template</th>
<th>Sequencing</th>
<th>Data analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Control input</td>
<td>(1) Prepare template</td>
<td>(1) Create a run</td>
<td>(1) Data quality check and analysis</td>
</tr>
<tr>
<td>Checkpoint – spectrophotometer, capillary gel electrophoresis (section 4.2, 4.3, 4.4)</td>
<td>(clonally amplified DNA on surface or beads)</td>
<td>(2) Clean and initialize the sequencer</td>
<td>Checkpoint – (section 5.1)</td>
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<tr>
<td>(2) Fragmentation and end-repair</td>
<td>Checkpoint – fluorometer (section 4.4)</td>
<td>(3) Start sequencing</td>
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<tr>
<td>Checkpoint – capillary gel electrophoresis (section 4.4)</td>
<td>(2) Enrich template</td>
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<td>(3) Adapter ligation and nick repair</td>
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<tr>
<td>Checkpoint – capillary gel electrophoresis (section 4.4)</td>
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<td>(4) Size selection</td>
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<tr>
<td>Checkpoint – capillary gel electrophoresis (section 4.4)</td>
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<td>(5) Library normalization / quantification</td>
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<tr>
<td>Checkpoint – fluorometer, qPCR, dPCR (section 4.4)</td>
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**Image:**
- A chart showing the steps involved in sequencing, including nucleotide lengths and quality metrics.
- A diagram of a sequencing protocol.
- Additional images of laboratory equipment and materials.
Possible Standards and *de facto* Standards

- Lack of documentation
- Development of procedure-, operating- and inspection instructions is required (SOP)
- Establishment of quality records respectively verification documents (quality proof of data)
- Quality records are suitable for providing quality certificates to customers
Remarks

- Enhancement of transparency, traceability and reproducibility of results
- Generation of reliable data
- Enhancement of financial and time efficiency
- Expansion of services (companies)
- Establishment of NGS into forensics and diagnostics, especially in consideration of emerging personalized medicine
- Acceleration of innovation process
Acknowledgements
Thanks for attention!