Next Generation Sequencing of CFTR from dried blood spots using the Ion Torrent PGM

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Study Objectives

- Evaluate Ion Torrent Next Gen Sequencing of *CFTR* on DBS DNA extracted from CDC’s Newborn Screening Quality Assurance Program (NSQAP) PT samples

- Compare traditional Sanger sequencing data with the highly parallel sequencing data from Ion Torrent

- Determine if Ion Torrent Next Gen Sequencing assay can be used as a primary method with Sanger sequencing confirmation for PT specimen characterization
CF DNA Proficiency Testing Program

- PT pilot program began in early 2007
- CF Patient and Family PT Samples
  - Collaborations with Phil Farrell, U of WI School of Med, Marty Kharrazi, CA Dept. of PH and Charlene Sacramento, Sequoia Foundation
- 25 participants in 2007 and today 65 enrolled laboratories in 19 countries
- NSQAP Repository contains all of the ACMG 23 recommended mutations as well as 41 additional mutations
Advantages of the Ion Torrent Method

- Scalable sample throughput (different chip sizes)
- Multiplex up to 96 individuals on one chip
- Massively parallel sequencing to more easily evaluate small insertions or deletions (indels)
- Automated data analysis
- Potential for cost savings as multiplexing larger numbers of samples
A H⁺ ion is released as each nucleotide is incorporated into the DNA strand causing a transient pH change.

Ion Torrent semiconductor sequencing chip is able to detect this change in pH and convert it directly to base calls.

Slide compliments of Life Technologies
Comparison of Sanger and Ion Torrent CFTR Sequencing

Sanger Sequencing

- Requires >220 ng of gDNA
- Single-plex
  - 42 PCR rxns/sample followed by 86 seq rxns
- Manual Analysis using SeqScape software
- ~ 5 weeks to sequence and analyze 32 samples

Ion Torrent Sequencing

- Requires 10 ng gDNA per pool*
- Multiplex
  - 8 - 96 library preps pooled for sequencing
- Automated Analysis (hotspot file contains 240 variants)
- <1 week to sequence and analyze 32 samples

*note ~ 5 ng/pool was used in this study
Calling Indels - Sanger vs. Ion Torrent

F508del/+ - causes a 3bp shift in the sequence

Roughly 50% of the sequences have a 3 bp deletion (TCT)

SeqScape software (LifeTech/ThermoFisher)

Integrated Genome Viewer (Broad Institute)
Automated Ion Torrent Mutation/Variant Calls

### Library type
AmpliSeq

### Targeted regions
CFTRexon.20131001.designed

### Hotspot regions
CFTR_hotspots_1126_2013

### Configuration
Custom

<table>
<thead>
<tr>
<th>Barcode Name</th>
<th>Sample Name</th>
<th>Variants</th>
<th>Hotspot Variants</th>
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</thead>
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<tr>
<td>IonXpress_041</td>
<td>None</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>IonXpress_042</td>
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</table>

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### Variant Calls by Allele

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<tr>
<th>Chrom</th>
<th>Position</th>
<th>Type</th>
<th>Allele Call</th>
<th>Ref</th>
<th>Variant</th>
<th>Frequency</th>
<th>Quality</th>
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<td>CDC:CFTR2F508del:1521_1523delGTT</td>
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</table>

Hotspot file contains 240 unique variants

- **G551D**
- **F508del**
<table>
<thead>
<tr>
<th>Mut/ Var*</th>
<th>Ion Torrent Precision</th>
<th>Ion Torrent Sensitivity</th>
<th>Ion Torrent Technical Issues</th>
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</thead>
<tbody>
<tr>
<td>Mutations</td>
<td>162 (57 unique)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Variants</td>
<td>539 (39 unique)</td>
<td>100%</td>
<td>99.3%</td>
</tr>
</tbody>
</table>

*N=108

*excludes IVS8 variant - TGxTy
Conclusions

- Ion Torrent is Faster than Sanger for CFTR
  - <1 week vs 5 for sequencing 32 samples
  - Automated data analysis significantly faster than manual

- Ion Torrent Precision and Sensitivity is >99%
  - Mutation Detection Precision - 100%; Sensitivity – 100%
  - Variant Detection Precision - 100%; Sensitivity – 99.3%
  - IVS8 (TGxTy) 5T can be distinguished from the 7T and 9T

- Ion Torrent is Scalable from 8 – 96 samples
  - 3 different density chip sizes allow flexibility

- Ion Torrent Can Save Money
  - Cost savings over Sanger when using a 316 or 318 chip at 500x

- Ion Torrent Can Potentially Replace Sanger
  - Sanger should be used for IVS8 characterization
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Thank you!

Newborn Screening

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Promoting Healthier Babies.

Protecting our Future.

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E-mail: cdcinfo@cdc.gov  Web: www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.