

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

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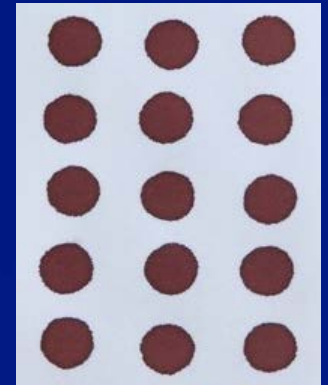
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# Study Objectives

- ❑ 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

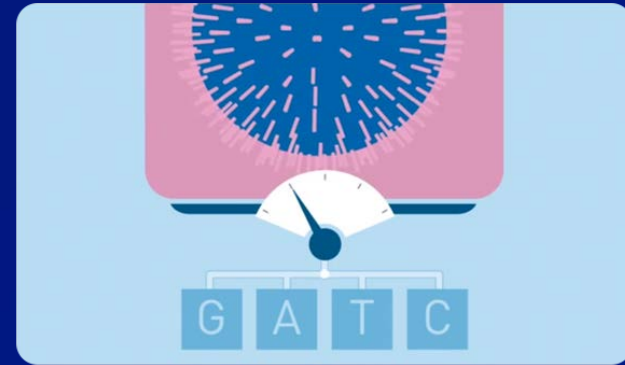
- ❑ Scalable sample throughput (different chip sizes)
- ❑ Multiplex up to 96 individuals on one chip
- ❑ Massively parallel sequencing to more easily evaluate small insertions or deletions (indels)
- ❑ Automated data analysis
- ❑ Potential for cost savings as multiplexing larger numbers of samples

# Ion Torrent Technology

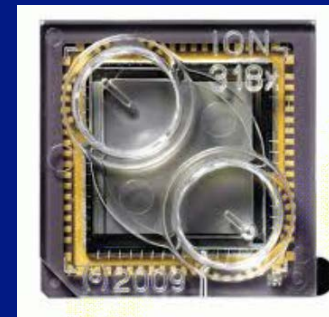
Direct conversion of released  $H^+$  into digital information



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

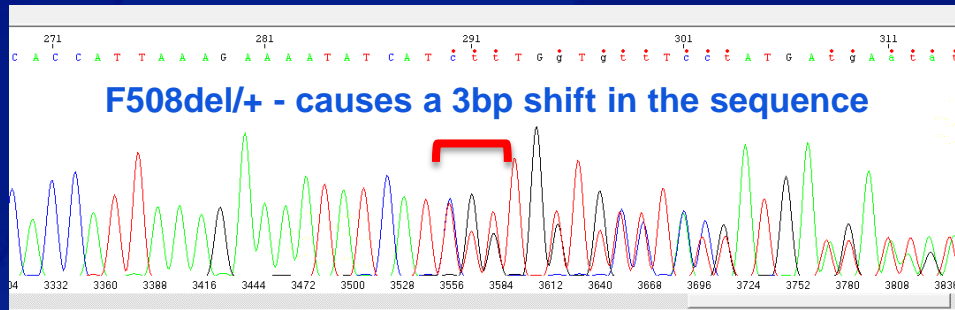
- ❑ Requires >220 ng of gDNA
- ❑ Single-plex
  - 42 PCR rxns/sample followed by 86 seq rxns
- ❑ Manual Analysis using SeqScape software
- ❑ ~ 5 weeks to sequence and analyze 32 samples

## Ion Torrent Sequencing

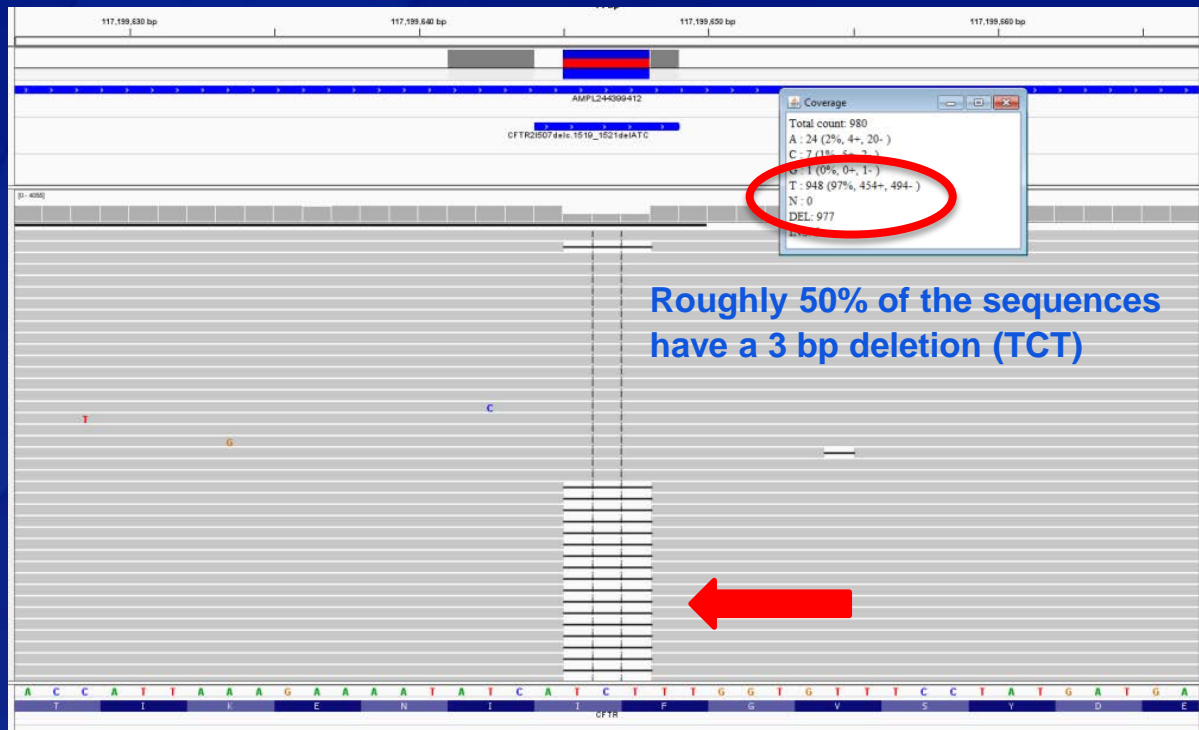
- ❑ Requires 10 ng gDNA per pool\*
- ❑ Multiplex
  - 8 - 96 library preps pooled for sequencing
- ❑ Automated Analysis (hotspot file contains 240 variants)
- ❑ <1 week to sequence and analyze 32 samples

\*note ~ 5 ng/pool was used in this study

# Calling Indels - Sanger vs. Ion Torrent



SeqScape software  
(LifeTech/ThermoFisher)



Integrated  
Genome Viewer  
(Broad Institute)

**F508del/+ Sample**

# Automated Ion Torrent Mutation/Variant Calls

Library type: AmpliSeq  
 Targeted regions: CFTR<sub>Exon.20131001.designed</sub>  
 Hotspot regions: CFTR\_hotspots\_1126\_2013  
 Configuration: Custom

Download all barcodes:

Barcode Name	Sample Name	Variants	Hotspot Variants
<a href="#">IonXpress_041</a>	None	9	8
<a href="#">IonXpress_042</a>	None	7	6

## Variant Calls by Allele

Chrom  Position  to  Allele Name  Gene ID  Rec

Type  Allele Call  Var Freq  to  Total Cov ≥

	Position	Ref	Variant	Allele Call	Frequency	Quality	Variant Type	Allele Source	Allele Name
<input type="checkbox"/>	<a href="#">chr7:117308413</a>	C	T	Heterozygous	34.5 %	35.4	SNP	Hotspot	CDC45751251CT15751251CT
<input type="checkbox"/>	<a href="#">chr7:117227860</a>	G	A	Heterozygous	50.0 %	223.8	SNP	Hotspot	CFTR2G551Dc.1652GA
<input type="checkbox"/>	<a href="#">chr7:117199457</a>	A	G	Heterozygous	48.9 %	743.5	SNP	Hotspot	CDC1525-81AGc.1393-81AG
<input type="checkbox"/>	<a href="#">chr7:117143718</a>	A	G	Heterozygous	48.0 %	963.5	SNP	Novel	---
<input type="checkbox"/>	<a href="#">chr7:117176569</a>	GATT	-	Heterozygous	50.9 %	1174.3	DEL	Hotspot	CDCTTGA5or8TTGA5or8
<input type="checkbox"/>	<a href="#">chr7:117307108</a>	G	A	Heterozygous	47.3 %	1306.6	SNP	Hotspot	CDC4521GAc.4389GA
<input type="checkbox"/>	<a href="#">chr7:117267511</a>	C	A	Heterozygous	44.4 %	1571.8	SNP	Hotspot	CDC3601-85CAc.3469-85CA
<input type="checkbox"/>	<a href="#">chr7:117176738</a>	C	T	Heterozygous	49.5 %	1665.9	SNP	Hotspot	CDC100111CTc.86911CT
<input type="checkbox"/>	<a href="#">chr7:117254527</a>	A	C	Heterozygous	51.7 %	2233.5	SNP	Hotspot	CDC3509-149Ac.3389-149AC
<input type="checkbox"/>	<a href="#">chr7:117199646</a>	CTT	-	Heterozygous	51.8 %	2894.2	DEL	Hotspot	CDC.CFTR2F508delc.1521_1523delCTT
<input type="checkbox"/>	<a href="#">chr7:117188680</a>	G	-	Homozygous	42.8 %	3596.5	DEL	Novel	---
<input type="checkbox"/>	<a href="#">chr7:117229537</a>	T	A	Homozygous	100.0 %	3933.0	SNP	Novel	---
<input type="checkbox"/>	<a href="#">chr7:117235055</a>	T	G	Heterozygous	48.3 %	4692.4	SNP	Hotspot	CDC2694TGc.2562TG
<input type="checkbox"/>	<a href="#">chr7:117199533</a>	G	A	Homozygous	100.0 %	12811.4	SNP	Hotspot	CDC.CFTR2M470Vc.1408AG

G551D

F508del

Hotspot file contains 240 unique variants



# CFTR Mutation/Variation Comparison

## Ion Torrent vs. Sanger Data

	Mut/ Var* N=108	Ion Torrent Precision	Ion Torrent Sensitivity	Ion Torrent Technical Issues
Mutations	162 (57 unique)	100%	100%	2
Variants	539 (39 unique)	100%	99.3%	4 No-Calls

\*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!



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***Saving Lives.***

***Promoting Healthier Babies.***

***Protecting our Future.***



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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.

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