

Ion Torrent

Semiconductor Sequencing for Life™

ion torrent



by life technologies™

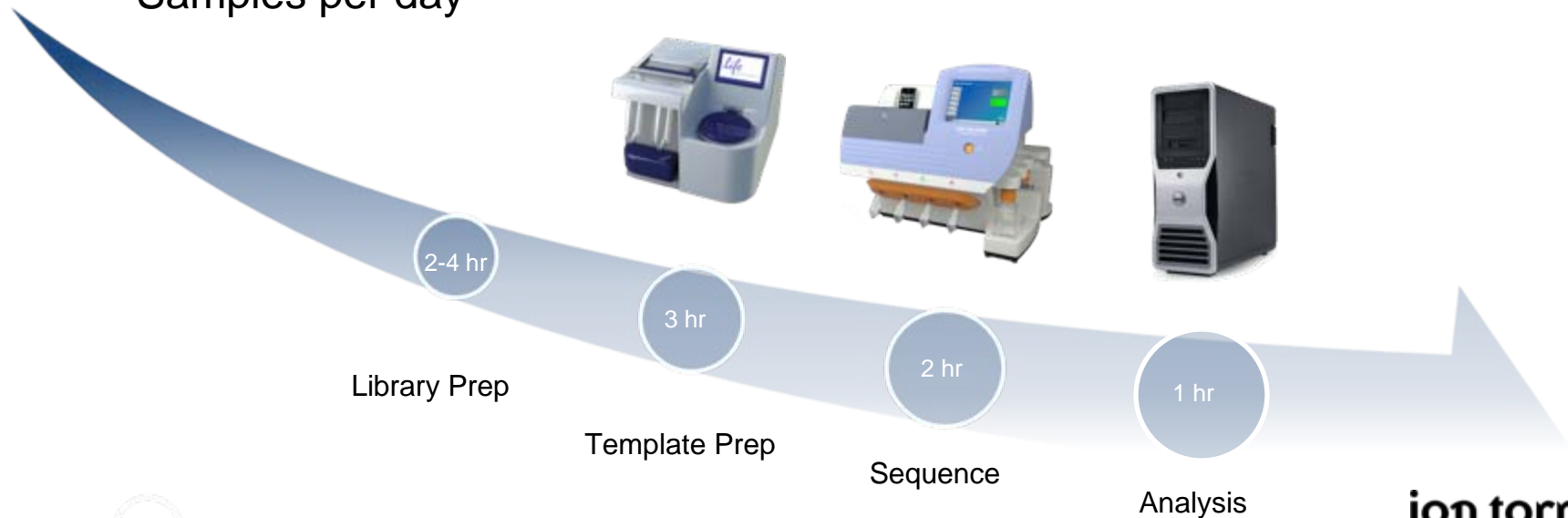
Resequencing Amplicons > 100bp on PGM

- Most resequencing assays are based on PCR products (amplicons) using Sanger sequencing chemistry and capillary electrophoresis
- Amplicons designed for Sanger resequencing assays are typically longer than current read length of PGM™ sequencer (100 bp)
- In the anticipation of rapidly increasing read lengths it would be nice not to have to redesign the amplicons
- We hereby demonstrate how customers can sequence any established PCR assay on PGM™ sequencer now

Speed

10X Faster Workflow for Any Project

- 2 hour sequencing runs
- Innovative automated template preparation for PGM matches the speed of semiconductor sequencing
- Complete end-to-end workflow within 1 day or Multiple Samples per day



Sanger Sequencing Compatible Workflow

Streamlined compatibility

Use Sanger sequencing primers/amplicons as-is, no need to redesign
Fast sequencing run at 2 hours

Superior data quality

Detection of mutations at low frequency
Point mutations, insertions, and deletions can be detected

Cost-effective genotyping

Run multiple samples on a single chip with barcodes

c.1521_1523delCTT



FALCON Application Development Team, Life Technologies
34 amplicons multiplexed in single run.
One amplicon shown with insertion/deletion detected



Prepare amplicons, with CE primers
Concatenation and mechanical shearing or
Direct fragmentation via enzymatic shearing

Detecting mutations of CFTR gene*

- The human Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein is encoded by the CFTR gene, ~200 kbp, 27 exons
- CFTR is a ABC transporter-class ion channel that transports chloride and thiocyanate ions across epithelial cell membranes
- Research on CFTR gene include detection of point mutations or insertions and deletions.

- * Source: wikipedia

Life Technologies Products

- T4 DNA Ligase
- PureLink® PCR Purification Kit
- Ion Xpress™ Fragment Library Kit
- Qubit® dsDNA HS Assay Kit
- Qubit® 2.0 Fluorometer
- Ion OneTouch™ System
- Ion Template 314™ Kit
- Ion Sequencing 314™ Kit
- Personal Genome Machine™ (PGM™) Sequencer
- Torrent Server and Torrent Suite Software

CFTR Amplicons Resequencing Workflow

Sample: Coriell gDNA

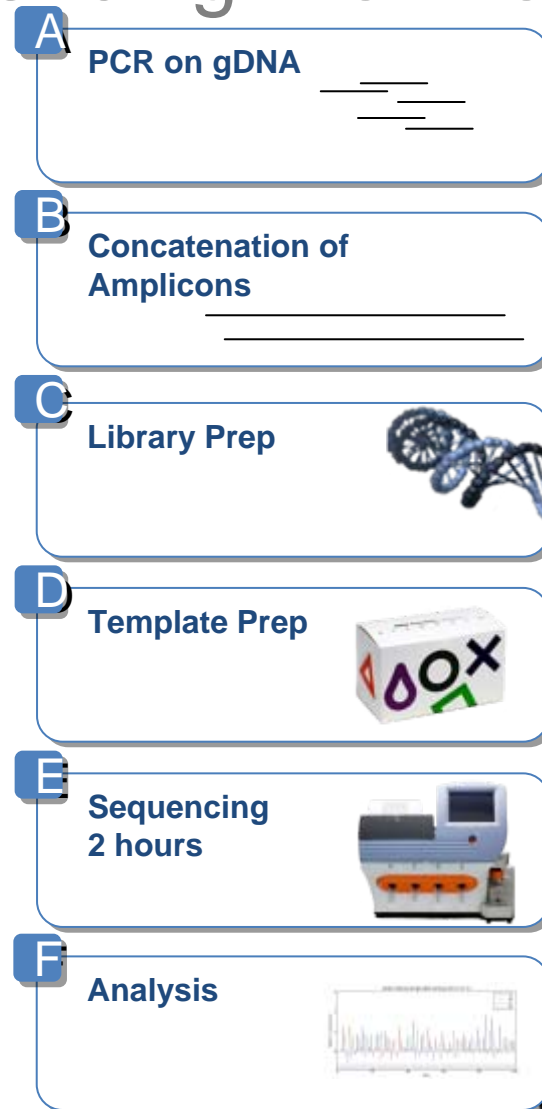
- Known mutations of CFTR gene

Amplification:

- MASTR CFTR kit; Multiplicon N.V.
- 34 amplicons; 2 multiplex PCRs
- 8,947 bases amplified
- Amplicons by MVZ genteQ GmbH

Sequencing: Ion 314™ Kit

- 2 hours
- 9.65 Mbp*
- 92,589 reads
- 104 bp average read length
- * excluding primers from CFTR MASTR kit



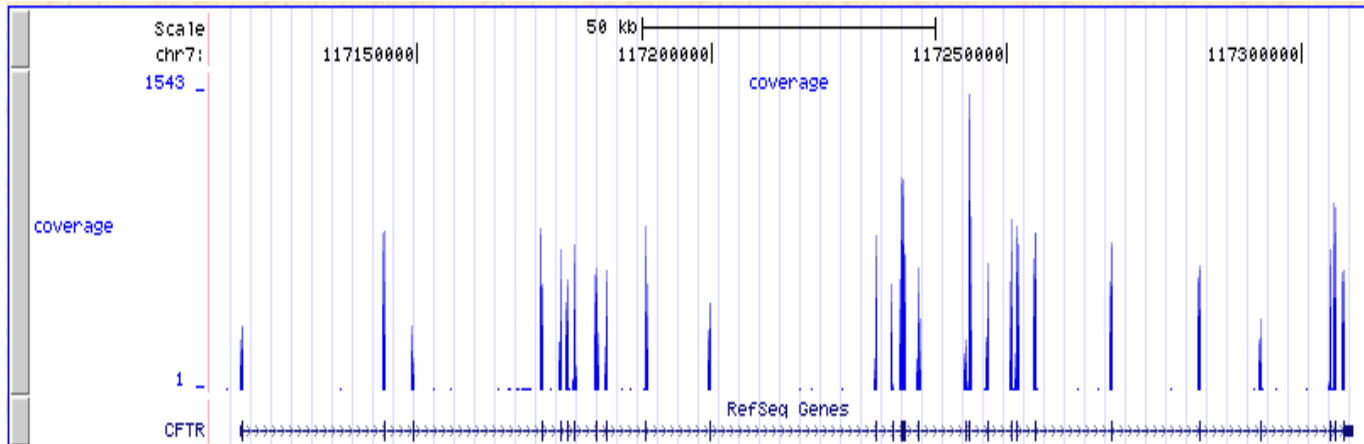
Analysis

- PGM generated raw data generated
 - Type: bases
 - Format: fastaq
- Reference: CFTR gene sequence
 - NCBI gi|287325315|ref|NG_016465.1|, 195703 bp
- Analysis 1: full data set
 - Estimation of maximum coverage
- Analysis 2: subset of 4,001 reads only
 - Suitable for multiplexing of samples within a single chip*
- Mapping: Targeted Resequencing pipeline of SeqMan® NGen®, DNASTar Inc.
- Visualization: SeqMan® Pro, DNASTar Inc.

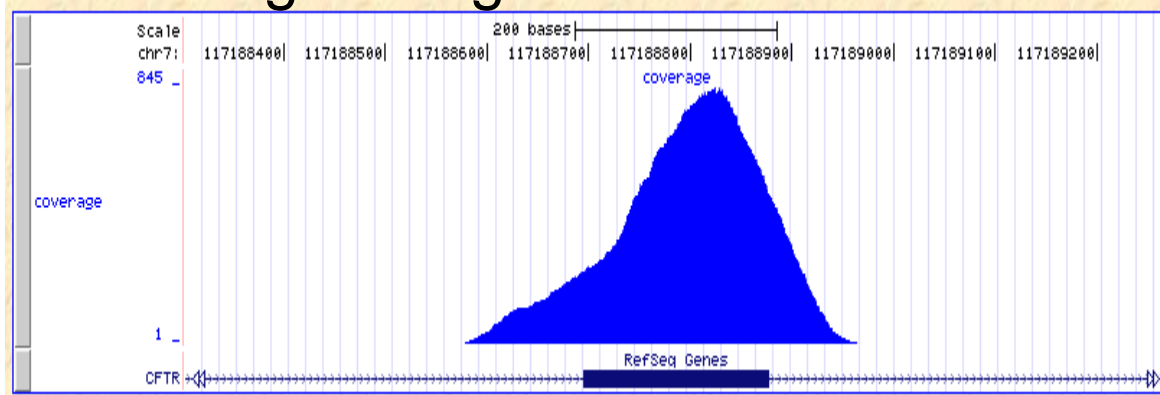
- * available Q2 2011

Coverage plots, UCSC Genome Browser

- CFTR MASTR regions covered at 100%



- Typical coverage using full data set



Detection of Variants

- Evaluation of detection at low coverage (4,001 reads)
- 5 point mutations and 1 insertion/deletion were detected
 - c.869+11C>T
 - c.1408A>G
 - c.2562T>G
 - c.4389G>A
 - c.1-8G>C
 - c.1521_1523delCTT
- Mutation pattern allowed correct identification of the blinded sample

About the project

- Scientific contributors. Thanks to:
 - Dr Achmann, Dr Auber, genteQ, Hamburg, Germany
 - Geoffrey Henno, Jurgen Del-Favero, Multiplicon N.V., Belgium
- This project was led by Alain Rico, FALCON team, Europe, Life Technologies
- Acknowledgements
 - Sarah O'Meara, Thierry Scarcez, Liqun He, Jason Myers (all Life Technologies)

ion torrent



by *life* technologies™

© 2011 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owner.

SeqMan and NGen are registered trademarks of DNASTAR, Inc.

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.