Speeding Medical Innovation: Clinical Next Generation Sequencing and the Path to Whole Genome Analysis

Paul R. Billings MD, PhD
Chief Medical Officer
Life Technologies, Inc.

2012 Executive War College
May 3, New Orleans, LA
Contents

Introduction

Clinical innovation...who cares?

What is needed to speed sequencing in to clinical use?

Conclusions
Life Technologies R&D

**People**
- ~1,200 R&D professionals, multiple disciplines
  - Engineering
  - Molecular Biology
  - Cell Biology
  - Chemistry
  - Software & Bioinformatics
- 14 locations
- Over $300 million annual investment in R&D

**Technologies**
- Genetic Analysis
  - Sequencing
  - qPCR
- Molecular & Cellular Biology
  - Nucleic acid & protein technologies
  - Synthetic biology
  - Cell engineering & analysis
  - Primary & stem cells
  - Biochemical & cellular screening

**Products**
- Invitrogen
- Applied Biosystems
- Gibco
- Molecular Probes
- Taqman
- Ambion
- Novex
- Ion Torrent

**Triumphs**
- With the Ion Torrent Proton, first to enable human genome sequencing in a single day for $1,000
- Part of *MIT Technology Reviews’* Top 50 Most Innovative Companies in 2012
- Named #1 Innovative Biotech Company by *Fast Company* in 2012
- Over 4,000 patents & exclusive licenses
Developing, manufacturing and commercializing IVD Products

• Variety of IVD instruments and reagent technologies
  – Real-Time PCR – 7500 DX, Via7
  – Transplant diagnostics – 3500 Dx
  – Pathogen detection
  – Molecular Pathology

• Quality systems and manufacturing infrastructure compliant with ISO 13485:2003

• Global infrastructure of Field Service and Technical Support personnel

• Assay Partner Program – Asuragen, Quidel
Alphabet Soup

NGS
WGS
RUO
Contents

Introduction

Clinical innovation...who cares?

What is needed to speed sequencing in to clinical use?

Conclusions
Unlock the secrets of your DNA for a healthier, more vibrant YOU!

Feel better and live better — the secret is in YOU!
By decoding your DNA, you’ll have the answers to unlock the secrets of 12 KEY GENES relating to cardio health, bone health, oxidative stress, immune health, defense against environmental pollutants, and more — ALL ESSENTIAL to nourishing and supporting your health and wellness, and helping you BE BETTER than you thought possible!

Using patented technology to assess your unique DNA is the geneME™ difference...
You can learn what your body needs — based on YOUR DNA. Assessing and harnessing this uniquely-personalized information (from your DNA sample) is exactly what geneME does best! The powerful knowledge established in the Human Genome Project about how small DNA variations can influence your health allows us to make products CUSTOMIZED for your unique DNA. We create PERSONAL FORMULAS that fill in and support the gaps of your specific genetics, giving you more energy, efficiently fueling your body and HELPING YOU FEEL TRULY FANTASTIC!

Balance your body with custom-made nutrition specified by your own DNA!
Imagine the edge you’ll have when you know the proper building blocks your body needs to get your healthiest glow — based on YOUR UNIQUE DNA. You’ll know the deficiencies present in your individual genetic makeup and geneME DNA CUSTOMIZED NUTRITIONAL SUPPLEMENTS will provide a custom-made formula to support your body with the optimal nutrients you need.

Enjoy cutting-edge science and high-quality ingredients formulated especially for you!
Supply your body and genetic machinery with the best! You’ll love the customized, personalized, superior nutrition you get with your one-of-a-kind formula, carefully designed to provide “key essentials” PLUS — you get the addition of your unique nutritional ingredients to better support YOUR genetics!

Be your BEST YOU, with geneME DNA CUSTOMIZED NUTRITIONAL SUPPLEMENTS MADE JUST FOR YOU!

Science by GENELINK. ©2012 geneME, LLC. All Rights Reserved.
These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.
The Doctor by Sir Luke Fildes
“Doctors are men who prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing”

Voltaire, French philosopher (d.1778)
20th Century Medicine: An Imperfect Art

Response Rate (%)

Given limited ability to predict responders, doctors practice trial-and-error medicine

Adapted from: Spear et al. TRENDS in Molecular Medicine Vol.7 No.5 May 2001; PMC Nov 2006
Rise in Drug-Related Adverse Outcomes

Of more than 2.7 million medication-related hospital visits, these top five drug categories accounted for 45% of hospital admissions and 19% of treat-and-release visits to the emergency department.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Hospital Admissions</th>
<th>Treat-and-release ED Visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>283,700</td>
<td>13,300</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>218,800</td>
<td>29,200</td>
</tr>
<tr>
<td>Antineoplastic and immunosuppressive drugs</td>
<td>217,700</td>
<td>11,400</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>131,300</td>
<td>95,100</td>
</tr>
<tr>
<td>Opiates</td>
<td>121,200</td>
<td>44,300</td>
</tr>
</tbody>
</table>

Source: “Medication-Related Adverse Outcomes in U.S. Hospitals and Emergency Departments, 2008,” Healthcare Cost and Utilization Project Statistical Brief #109, Agency for Healthcare Research and Quality, April
Error Rates in Health Care are Higher than Other Industries

- Mammography screening
- Inpatient medication accuracy
- Low back (TX)
- Post heart attack medications
- Airline baggage handling
- U.S. industry best-in-class
- Domestic airline flight fatality rate (.43 PPM)
The Cost Of A Wrong Answer

Wrong drug selected
Find out 6 months later

Note: metastatic basal cell carcinoma
Human Genomic Sequencing: Unparalleled Productivity

- Cost to sequence genome
- Information Value
- Moore's Law
- Clinical Value

- 1999
- 2005
- 2011

- $3B
- $5K
Eroom’s Law

a Overall trend in R&D efficiency (inflation-adjusted)

- FDA tightens regulation post-thalidomide
- FDA clears backlog following PDUFA regulations plus small bolus of HIV drugs
- First wave of biotechnology-derived therapies

b Rate of decline over 10-year periods

c Adjusting for 5-year delay in spending impact

Nature Reviews | Drug Discovery
Genomic Classification is coming

Forbes et al. Nucleic Acid Res (2011); 39:D945-50
A Collision Course with Complexity

- 500 compounds on the way
- Targeting more than 100 biomarkers
- $6 million & 2 years to develop each
- Multiple Dx tests for each marker
Today's Model is Unsustainable

- **Sample 1**: Cost - $1,000, Days - 5
- **Sample 2**: Cost - $2,500, Days - 5
- **Sample 3**: Cost - $4,000, Days - 15
- **Sample 4**: Cost - $0, Days - 15

**Total Cost**: $7,500

**Patient Health**

**Total Days**: 40

**Physician Frustration**

- Hospital
- Reference Lab
- Esoteric Lab
- Pharma (CRO)
Relationship between what clinician selected as what she/he would use to treat the patient before knowing what Molecular Profiling (IHC/Microarray) results suggested (n=66)
Speed Matters, Especially in Oncology

<table>
<thead>
<tr>
<th>Application</th>
<th>Current</th>
<th>Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis</td>
<td>4-6 weeks</td>
<td>Days</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Days</td>
<td>Hours</td>
</tr>
<tr>
<td>HIV/HCV/HBC Genotyping</td>
<td>Weeks</td>
<td>Days</td>
</tr>
<tr>
<td>Pharmacogenomics</td>
<td>Weeks</td>
<td>Days</td>
</tr>
</tbody>
</table>

Why Speed Matters in Oncology

- **Research**: lead to target, enrollment, to termination, to approval
- **Diagnosis**: Wasted work-ups (medical odysseys), proper level of care and hospitalization, reversible disease
- **Treatment**: More options, better outcomes, less toxicity and wasted cycles
- **Reporting**: Comparative databases (case studies aggregated), standardization and SOP development, more uniform quantitative care
"You’re fifty-seven years old. I’d like to get that down a bit."
Contents

Introduction

Clinical innovation...who cares?

What is needed to speed sequencing in to clinical use?

Conclusions
1. A fast, improving and cost-effective technology
An integrated semiconductor device enabling non-optical genome sequencing

Rothberg J.M. et al Nature doi:10.1038/nature10242

(available at www.iontorrent.com)
The Chip is the Machine™

Scalability

Simplicity

Speed
Life Technologies’ Disruptive Technology

Main Frame

Mini Computer

Personal Computer

Sanger Sequencing

Next-Gen Sequencing

Ion Semiconductor Sequencing
Technology Summary
Simple Natural Chemistry
Ion Semiconductor Sequencing
Rapid, Benchtop Sequencing for All

PGM™ for genes.
Proton™ for genomes.

Ion PGM™ Sequencer
Ion Proton™ Sequencer

The content provided herein may relate to products that have not been officially released and is subject to change without notice.
Unprecedented Scalability...
Rapidly Improving Read Length

Nothing gets better faster
Rapid Customer Performance Improvement

Ion 314™, Ion 316™ Ion 318™ Chips – User runs at Ion Community Leader Board

AQ20 Mb

<table>
<thead>
<tr>
<th>Month</th>
<th>314</th>
<th>316</th>
<th>318</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>38.41</td>
<td>41.75</td>
<td>60.75</td>
</tr>
<tr>
<td>July</td>
<td>41.75</td>
<td>82.94</td>
<td>155.41</td>
</tr>
<tr>
<td>August</td>
<td>62.25</td>
<td>220.72</td>
<td>250.55</td>
</tr>
<tr>
<td>September</td>
<td>50.74</td>
<td>372.97</td>
<td></td>
</tr>
<tr>
<td>October</td>
<td>71.8</td>
<td>589.74</td>
<td>1087.76</td>
</tr>
</tbody>
</table>

Invitrogen™, Applied Biosystems®, Gibco®, Molecular Probes®, Novex®, TaqMan®, Ambion®, Ion Torrent™
$500 Exome and $1,000 Genome

Sequencing in a few hours on the benchtop

**Ion Proton™ I Chip**
- 165 million wells
- 2 human exomes
- Up to 10 Gb
- $1,000 per run

**Ion Proton™ II Chip**
- 660 million wells
- 1 human genome
- >20x coverage
- $1,000 per run

Highest throughput
Ion Proton™ System
The Benchtop Genome Center

- Supports Ion Proton™ I and Proton™ II chips from raw signal to variant calling
- Benchtop system with state-of-the-art electronics to support highest throughput
...While Seamlessly Scaling Ion’s PostLight™ Sequencing Chemistry

Ion 314™ Chip signal

Ion Proton™ I Chip signal

Same signal, same speed

Internally generated R&D data shown.
The content provided herein may relate to products that have not been officially released and is subject to change without notice.
50+ Base Perfect Reads – November 12, 2011

Single-Well Ionogram for (19,805)
200+ Base Perfect Reads – January 14, 2012

Single-Well Ionogram for (21.86)

Internally generated R&D data shown.
The content provided herein may relate to products that have not been officially released and is subject to change without notice.
5 Gb Exome Run on Proton (March 2011)

<table>
<thead>
<tr>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Addressable Wells</td>
<td>164,822,784</td>
</tr>
<tr>
<td>* Wells with ISPs</td>
<td>88,337,524</td>
</tr>
<tr>
<td>* Live ISPs</td>
<td>82,823,169</td>
</tr>
<tr>
<td>* Test Fragment ISPs</td>
<td>1,371,506</td>
</tr>
<tr>
<td>* Library ISPs</td>
<td>81,451,663</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AQ17</th>
<th>AQ20</th>
<th>Perfect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number of Bases [Mb]</td>
<td>4,910.32</td>
<td>3,548.10</td>
</tr>
<tr>
<td>Mean Length [bp]</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td>Longest Alignment [bp]</td>
<td>186</td>
<td>181</td>
</tr>
<tr>
<td>Mean Coverage Depth</td>
<td>1.60×</td>
<td>1.10×</td>
</tr>
<tr>
<td>Percentage of Library Covered</td>
<td>8%</td>
<td>7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Read Length [bp]</th>
<th>Reads</th>
<th>Unmapped</th>
<th>Excluded</th>
<th>Clipped</th>
<th>Perfect</th>
<th>1 mismatch</th>
<th>≥2 mismatches</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>76,393,135</td>
<td>6,734,815</td>
<td>34,210</td>
<td>0</td>
<td>23,620,148</td>
<td>17,908,025</td>
<td>28,095,937</td>
</tr>
<tr>
<td>100</td>
<td>74,350,840</td>
<td>6,392,425</td>
<td>29,181</td>
<td>0</td>
<td>8,334,208</td>
<td>12,216,237</td>
<td>47,378,789</td>
</tr>
<tr>
<td>150</td>
<td>39,009,493</td>
<td>5,850,706</td>
<td>20,325</td>
<td>0</td>
<td>20,528</td>
<td>60,001</td>
<td>33,057,933</td>
</tr>
<tr>
<td>200</td>
<td>2,589,102</td>
<td>2,065,994</td>
<td>2,066</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>521,042</td>
</tr>
<tr>
<td>250</td>
<td>347,596</td>
<td>330,761</td>
<td>120</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16,715</td>
</tr>
<tr>
<td>300</td>
<td>84,535</td>
<td>82,957</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1,553</td>
</tr>
<tr>
<td>350</td>
<td>38,425</td>
<td>38,059</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>357</td>
</tr>
<tr>
<td>400</td>
<td>22,807</td>
<td>22,682</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>121</td>
</tr>
</tbody>
</table>

The content provided herein may relate to products that have not been officially released and is subject to change without notice.
**Perfect Clinical Fit**

<table>
<thead>
<tr>
<th></th>
<th>Ion Torrent</th>
<th>Clinical Need</th>
<th>Match?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cost of Sequencer</strong></td>
<td>$50k</td>
<td>$50-100k</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>Yearly Operating</strong></td>
<td>$50-100k</td>
<td>Low</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>Costs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Turn Around Time</strong></td>
<td>&lt;1 day</td>
<td>Hours</td>
<td>✔️</td>
</tr>
<tr>
<td><strong>(TAT)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical Footprint</strong></td>
<td>Small</td>
<td>Small</td>
<td>✔️</td>
</tr>
</tbody>
</table>

- Simplicity of use, improved work flow, and fast turn around time (TAT) makes the ION Torrent ideally suited for clinical applications
High Data Quality

Superior accuracy where it counts: Superior homopolymer performance

substitutions and long reads

better for 4mers and longer

The content provided herein may relate to products that have not
been officially released and is subject to change without notice.
# Targeted Resequecing on PGM

## Target region size

<table>
<thead>
<tr>
<th></th>
<th>1kb</th>
<th>10kb</th>
<th>100kb</th>
<th>1Mb</th>
<th>10Mb</th>
</tr>
</thead>
<tbody>
<tr>
<td>314 (10Mb)</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>316 (100Mb)</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>318 (1Gb)</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## # of genes

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1,000</th>
<th>10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>314 (10Mb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>316 (100Mb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>318 (1Gb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Number of Samples per Chip (@100x coverage)

<table>
<thead>
<tr>
<th></th>
<th>314 (10Mb)</th>
<th>316 (100Mb)</th>
<th>318 (1Gb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1kb</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10kb</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>100kb</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1Mb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10Mb</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Samples Per Day Per PGM

<table>
<thead>
<tr>
<th></th>
<th>314 (10Mb)</th>
<th>316 (100Mb)</th>
<th>318 (1Gb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1kb</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>10kb</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>100kb</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1Mb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10Mb</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Per Sample Cost*

<table>
<thead>
<tr>
<th></th>
<th>314 (10Mb)</th>
<th>316 (100Mb)</th>
<th>318 (1Gb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1kb</td>
<td>$50</td>
<td>$50</td>
<td>$55</td>
</tr>
<tr>
<td>10kb</td>
<td>$90</td>
<td>$118</td>
<td>$118</td>
</tr>
<tr>
<td>100kb</td>
<td></td>
<td>$118</td>
<td>$738</td>
</tr>
</tbody>
</table>

*Excluding Primer and Target Enrichment Costs

---

Some products have not yet been officially released and information about those products is subject to change without notice.
Ion PGM™ Path to CE-IVD and 510(k) in 2012

Product Lock Down by end of 2011

Ion Dx technology

- Will be accessible to all clinical labs
- 2-4 hour sequencing runs
- Low cost sequencer and kits
- Millions of 200+ base pair long reads
- Field proven robustness (100s in the field)

Core product completion
Low cost, starting at < $299 per run

Regulatory filings
Builds on Life’s IVD experience: 7500 Fast Dx & 3500Dx

2011  2012
2. Integration in to the workflow
Speed – Single Day Workflow

• ~2 hour sequencing runs – enabled by PostLight™ Sequencing

• Innovative automated template preparation for PGM sequencer matches the speed of semiconductor sequencing

• Complete end-to-end workflow within 1 day or multiple samples per day
“We’re pleased the Proton installation went so quickly and smoothly. We are now generating all the raw data needed for full exomes in just a few hours, and it’s exhilarating to see what’s to come.”

Dr. Richard Gibbs
Director, Human Genome Sequencing Center
Baylor College of Medicine

http://www.youtube.com/watch?feature=player_embedded&v=uVevXBWvlTo
From Bench to Bedside: New Clinical Workflow

Patient Presents

Tests

Interpretation

Treatment & Clinical Trial Options

Personalized Treatment & Monitoring Plans

Ion Torrent fits seamlessly into current clinical workflow
Workup: Biopsy to Treatment Options in One Week

Monday
- Biopsy & Pathology

Tuesday
- Sequencing
- Bioinformatics & Interpretation

Wednesday
- Wednesday

Thu/Friday
- Confirmatory Testing

Monday
- Results with Treatment Options

Surgery/Oncology
- Molecular Dx Lab
- Molecular Pathology
- Molecular Dx Lab
- Oncology

The means of finding treatment options will forever change.... Pioneers will seize the moment to grow their business.

Invitrogen™  Applied Biosystems®  Gibco®  Molecular Probes®  Novex®  TaqMan®  Ambion®  Ion Torrent™
3. Confirmatory and Supplementary Data
Digital PCR Overview

Digital PCR is an analytical technique for absolute quantification of nucleic acid samples based on clonal PCR amplification of single template molecules.

Use the ratio of positive (red) to negative (Black) PCR reactions to count the number of target molecules.
castPCR™ Technology

**Competitive Allele-Specific TaqMan PCR**

- **Performance**
  - High success rate (>90%)
  - Higher specificity: 1 in 100,000,000
  - Detection sensitivity: up to 1 copy

- **Key applications**
  - Early cancer detection
  - Prenatal analysis
  - Virus typing/rare pathogen detection
  - Cell line QC
Biomarker Monitoring

Circulating Tumor Cells have significant potential yet broad clinical adoption remains challenging.

Detect/Quantify

Imaging – more sensitive/specific markers for detection, quantitation and separation.

Genomic profiling

Global characterization to determine relationship to primary tumor and evolution to resistance/metastatic clones

Biomarker Monitoring

Monitoring of biomarkers through treatment
Cellular analysis
The Attune® Acoustic Focusing Flow Cytometer

- Accoustic focusing of cells
- Higher sensitivity/Higher precision
- Faster experiments
- Open access software
- No wash protocols

25 µl/min  200 µl/min  1000 µl/min

CV 3.22%  3.17%  4.21%

0.542% of Live Cells

232 CD34 / CD31+ events
0.0059% of Live Cells
FLoid™ cell imaging station

- Point & shoot microscopy
- 3 color fluorescence
- Printer
- Focus Assist
- Image in room light

10 years & 70 seconds
Patient to ‘Diseases in a Dish’: Enhancing the iPSC Workflow

Bringing together innovative technologies to create relevant cellular models for discovering new medicines
Steps to clinical journey: ‘Right first time’ translational tools

- FDA DMF or 510(k)
- ISO & GMP manufacturing
- Animal origin-free or xeno-free
- Certificates of Origin
- Certificates of Analysis
- Adventitious agent testing
- Sterility testing
- Mycoplasma testing
- Endotoxin testing

Introducing Gibco® Cell Therapy Systems (CTS™)
A simple choice in a complex field
Steps to clinical journey: Academic & industrial endeavors

Stem Cell-Derived Astrocyte Precursor Transplants in Amyotrophic Lateral Sclerosis

Multiple hESC Lines

Expansion & Banking

Astrocyte progenitor cells

Animal efficacy studies

IND submission
4. Informatics
Streamlined Bioinformatics Infrastructure

Server room & informaticists in a box and in the cloud

Ion Reporter™ Solution

Proton™ Torrent Server and Torrent Suite Software
Torrent Browser Runs on Proton Torrent Server

Local compute and storage with an integrated web interface

- Primary analysis to base calls & alignment
- Hardware appliance – Torrent Server
- Easy web access to Ion data - Torrent Browser
Torrent Server and Ion Reporter

Local compute and cloud compute

- Base calls generated using local compute
- Secure transfer & storage from local to cloud
- Cloud compute to variants
Ion Reporter Analysis and Report

A secure, hosted informatics infrastructure for routine assays

Automated informatics:
- Reads
- Mapped Reads
- Variants
- Annotated Variants
- Confident Variants
- Relevant Variants
- Interpretive Report

User interpretation:
- Confident Variants
- Relevant Variants
- Interpretive Report

Life Technologies™ Proprietary
Three Views in Ion Reporter Software

Track Analysis Progress, Find Variants, & Report Relevant Information
5. Content and Evidence
...To Enable All Applications
Ion AmpliSeq™ Product Portfolio

- **Cancer Panel**
  - 46 genes
  - 739 mutations
  - Ion 314™ Chip

- **Inherited Disease Panel**
  - ~100 diseases
  - Ion 316™ Chip

- **Comprehensive Cancer Panel**
  - ~400 genes
  - Ion 318™ Chip

- **Ion AmpliSeq Kit v2.0**
  - Core reagent kit for amplification and library construction

- **Ion AmpliSeq™ Kit Designer Software**
  - www.ampliseq.com
  - Up to 1,536plex per tube

- **Custom Panels**

- **Ready to Use Panels**

- **Now**
  - Cancer Panel
  - Inherited Disease Panel
  - Comprehensive Cancer Panel
  - Ion AmpliSeq Kit v2.0

- **Q2**
  - Custom Panels

- **Q2.**

- **Available**

For research use only. Not intended for any animal or human therapeutic or diagnostic use.
Supported Applications

**Microbial sequencing**
- Accurate, fast bacteria and virus de-novo & resequencing

**Mitochondrial sequencing**
- Highly multiplexed mitochondrial sequencing for research, clinical, and forensic applications

**Amplicon sequencing**
- Multiplexed amplicon sequencing for rapid detection of germline and somatic mutations

**Custom or fixed content amplicon panels for targeted resequencing by ultra-high multiplex PCR**
- Revolutionary Ion AmpliSeq™ Target Selection technology simplifies targeted resequencing for research and clinical applications

**Custom targeted resequencing by target enrichment**
- Fast and simple workflows optimized for all major target enrichment providers

**Validation of whole genome and whole exome mutation**
- Orthogonal technology to validate SOLiD® System/Illumina whole genome/whole exome results

**Library Assessment**
- Rapid library complexity validation/QC prior to run on high throughput sequencing platforms

**RNA-Seq**
- Affordable, fast and simple RNA-Seq solution *(Initially focused on small RNAs & low complexity transcriptomes)*
Supported Applications

Whole-transcriptome human RNA-Seq
- New RNA-Seq kits featuring faster workflow and lower RNA input for human whole transcriptome analysis
- Simplified and intuitive data analysis tools to make seamless transition from microarrays

Chip-Seq
- Fast and affordable analysis of DNA binding proteins target sequences

Copy number detection
- Accurate Targeted copy-number detection for basic and clinical research application
Clinical Applications of NGS

- Infectious Diseases
- Genetic Diseases
- Oncology
Infectious Diseases

- Rapid identification of emerging strains
- Characterize strains

- Drug-susceptibility profiling
- Optimal antibiotic selection

- Screen for colonization

- Identification of virulence factors as new therapeutic targets
- Develop new preventive strategies

- Optimal anti-retroviral drug selection
- Low level resistant clone detection

Chart adapted from: NEJM 2011
Recent Publications

**Enterohemorrhagic E. coli**

*Outbreak Detectives Embrace The Genome Era*

Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology

Alexander Mellmann, Dag Harmsen, Craig A. Cummings et al.

**Shiga-Toxin–Producing E. coli**

*Open-Source Genomic Analysis of Shiga-Toxin–Producing E. coli O104:H4*

Mark Pallen, Junjie Qin, Ph.D et al.

See links at www.iontorrent.com/community
# Healthcare Application

## German E. coli Outbreak Characterized on Ion PGM™ in 3 days

"The biggest advantage [of the PGM] from my point of view as a public health official is that it's speedy, and speed is what is needed at the moment," Prof. Dr. Med Dag Harmen, University Hospital Muenster

"[The PGM] takes the shortest time to generate genomic data." Junjie Qin, BGI

### Rapid sequencing, de novo assembly & identification of novel microbial strains.

<table>
<thead>
<tr>
<th>Date</th>
<th>Activity</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monday May 30</strong></td>
<td>Library preparation</td>
<td>O104:H4 and HUSC41 samples (reference) strain libraries prepared</td>
</tr>
<tr>
<td><strong>Tuesday May 31</strong></td>
<td>Sequencing runs</td>
<td>0104:H4 amplified and sequenced 2 x 2 runs (Ion 314)</td>
</tr>
<tr>
<td><strong>Wednesday June 01</strong></td>
<td>Sequencing runs</td>
<td>0104:H4 sequenced 3 x 2 runs (Ion 314)</td>
</tr>
<tr>
<td><strong>Thursday June 02</strong></td>
<td>Assembly</td>
<td>Draft Genome identified, Assembled, Submitted and Released from NCBI</td>
</tr>
<tr>
<td><strong>Friday June 03</strong></td>
<td>Assay Design</td>
<td>TaqMan Assays Designed</td>
</tr>
</tbody>
</table>

*May 22 CEDC reports significant increase in patients with hemolytic uremic syndrome

---

**Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology**

Alexander Mallmann1,2, Dag Harmsen1,2, Craig A. Cummings3, Emily B. Zant4, Shana R. Leopold5, Alain Rico6, Karola Priez7, Rafał Szczerpanski8, Yongmei Ji9, Wenzian Zhang9, Stephen F. McLaughlin1, John R. Henkhaus1, Benjamin Leopold9, Martina Bialaszewski9, Rita Prager9, Pius M. Brozonska10, Richard L. Moore9, Simona Guenther9, Jonathan M. Rothberg9, Holge Karch1

1 Institute of Hygiene, University Münster, Münster, Germany, 2Department of Paediatrics, University Münster, Münster, Germany, BGI technologies, Foster City, California, United States of America, 3Life Technologies, Carlsbad, California, United States of America, 4Life Technologies, Ebersberg, Germany, 5BGI Shenzhen Co., Ltd, Nanshan District, Shenzhen, Guangdong, China, 6BGI Technologies, Shenzhen, Guangdong, China, 7BGI Technologies, Hannover, Germany, 8BGI Technologies, Düsseldorf, Germany, 9BIOMIN Test Institute, Wiesbaden, Germany, 10Life Technologies, Guilford, Connecticut, United States of America

**Abstract**

An ongoing outbreak of exceptionally virulent Shiga toxin (Stx-producing *Escherichia coli* O104:H4) centered in Germany, has caused over 130 cases of hemolytic uremic syndrome (HUS) and 46 deaths since May 2011. *Shiga toxin* O104:H4, which has not been detected in animals, has rarely been associated with HUS in the past. To prospectively elucidate the unique characteristics of this strain in the early stages of this outbreak, we applied whole-genome sequencing on the Life Technologies Ion Torrent™ sequencer and Optical Mapping to characterize one outbreak isolate (S35226600) and a historic O104:H4 HUS isolate from 2001 (S0100591). Reference-guided draft assemblies of both strains were completed within 62 hours. The HUS-associated strain both carried genes typically found in two types of pathogenic *E. coli*, enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). Phylogenetic analyses of 1,144 conserved *E. coli* genes indicate that the *HUS-causing O104:H4* strain and the previously published sequence of the S35226600 show a close relationship but are only distantly related to common EHEC serotypes. Though closely related, the outbreak strain differs from the 2001 strain in plasmid content and fimbrial genes. We propose a model in which EABE, S35226600, and EHEC O104:H4 strains evolved from a common EHEC O104:H4 progenitor, and suggest that by stepwise gain and loss of chromosomal and plasmid-encodedulence factors, a highly pathogenic hybrid of EABE and S35226600 emerged as the current outbreak clone. In conclusion, rapid next-generation technologies facilitated prospective whole genome characterization in the early stages of an outbreak.
Clinical Applications of NGS

- Infectious Diseases
- Genetic Diseases
- Oncology
Genetic Basis of Disease

Types of Disease
- Prenatal: Trisomies
- Newborn: Metabolic, Storage, Hemoglobinopathies
- Pediatric: Cardiac, Immunological, Neurological
- Adult: Multifactorial

Types of Mutations
- Chromosomal Number & Structure Aberrations
  - Prenatal
  - Newborn
  - Pediatric
  - Adult
- Mendelian (biochemical)
  - Newborn
  - Pediatric
- Mendelian
  - Pediatric
- Multifactorial
  - Pediatric
  - Adult

Current Test Methods
- Cytogenetics: Prenatal
- Biochemical: Newborn, Pediatric
- PCR, Sequencing: Pediatric
- No Definitive Method Accepted: Adult

Start with Pediatric Applications
Preconception Screening

- Mendelian diseases account for:
  - 20% infant mortality
  - 10% of pediatric hospitalizations

- Recommended tests today:
  - Fragile X, Cystic Fibrosis, TSD, Canavan Disease, Fam Dysautonomia

- Costs for just Tay-Sachs:
  - $130 (enzyme activity)
  - $225 (targeted mutations)

---

**Carrier Testing for Severe Childhood Recessive Diseases by Next-Generation Sequencing**


NGS for Carrier Mutation Testing

- Kingsmore et al, used NGS to develop an assay for preconception screening of 448 severe recessive mutations
- Identified mutations confirmed with Sanger Sequencing
- 95% Sensitivity and 100% Specificity
- Analytical cost of $378 per patient
Chromosomal Copy Number Analysis

Two known cell lines with chromosomal abnormalities (47,XY+13 & 47,XY+18) were analyzed with an amplicon sequencing approach using a single Ion 316™ Chip.

<table>
<thead>
<tr>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reads mapped to chromosome</td>
<td>% reads/total</td>
</tr>
<tr>
<td>6521</td>
<td>5</td>
</tr>
<tr>
<td>3651</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chr</th>
<th>A</th>
<th>B</th>
<th>A/B</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>5</td>
<td>5</td>
<td>95%</td>
<td>105%</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>4</td>
<td>66%</td>
<td>151%</td>
</tr>
</tbody>
</table>

Trisomy 18

Trisomy 13
NGS: Rare and Idiopathic Single-Gene Disorders

• Historically required identifying affected pedigrees, followed by linkage analysis

• Recent Examples in Literature
  - Univ of Washington scientists identify gene mutation responsible for Miller syndrome using just four probands
  - Wisconsin researchers report identification of genetic lesions responsible for severe pediatric IBD from a single patient
**Mental Retardation: Customer Verified Performance**

**Patient**

Coding mutation covered 21 reads:
- 8 G (ref)
- 8 C (alternate)
- 5N
  *excluding reads close to mutation (<6 bp)*

Codon: CCA -> CGA (Proline to Arginine)

**Mother**

**Father**

Target region: GRIN2A – Glutamate Receptor, Ionotropic (NMDA2)
Exon 9, chr16:9,927,961-9,928,087, Encoding PBPe functional domain

---

**A de novo paradigm for mental retardation**

Lisonka E L M Visser1,2, Joep de Loyt1,2, Christiana Gilissen1, Irene Janssen1, Marloes Steenhouwer1, Petra de Vries1, Bart van Lier1, Peer Arts1, Nienke Wessink1, Marisol del Rosario2, Bregje W M van Bon1, Alexander Holscher1, Bert B A de Vries1, Han G Brunner1,2 & Joris A Veltman1,3

The per-generation mutation rate in humans is high. De novo mutations may compensate for allele loss due to severely reduced fertility in common neurodevelopmental and psychiatric diseases, explaining a major paradox in evolutionary genetic theory. Here we used a family-based exome sequencing approach to test this de novo mutation hypothesis in ten individuals with unexplained mental retardation. We identified and validated unique non-synonymous de novo mutations in nine genes. Six of these, identified in six different individuals, are likely to be pathogenic based on gene function, evolutionary conservation and mutation impact. Our findings provide strong experimental support for a de novo paradigm for mental retardation. Together with de novo copy number variation, de novo point mutations of large effect could explain the majority of all mental retardation cases in the population.
Ion AmpliSeq™ Inherited Disease Panel

- 30ng DNA total required (10ng DNA per primer pool)
- Broad survey of disease genes
  - Average coverage ~150X on single 316 chip
  - Multiplex on 318
- Ion AmpliSeq™ Custom Panels for follow-up
  - Neuromuscular
    - 27 disorders
    - 140 genes
  - Heart disease
    - 12 disorders
    - 60 genes
  - Developmental
    - 33 disorders
    - 37 genes
  - Metabolic
    - 21 disorders
    - 28 genes
  - Other (inherited cancer, blindness, etc)
    - 83 disorders
    - 37 genes
- 176 disorders across most frequent Mendelian diseases
Clinical Applications of NGS

Infectious Diseases

Genetic Diseases

Oncology
**Ion AmpliSeq™ Cancer Panel:**

46 genes, 739 mutations, one day

<table>
<thead>
<tr>
<th>KRAS</th>
<th>BRAF</th>
<th>EGFR</th>
<th>TP53</th>
<th>PIK3CA</th>
<th>CSF1R</th>
<th>JAK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS</td>
<td>PTPN11</td>
<td>ERBB2</td>
<td>SRC</td>
<td>FGFR3</td>
<td>NPM1</td>
<td>CDKN2A</td>
</tr>
<tr>
<td>RET</td>
<td>HNF1A</td>
<td>SMAD4</td>
<td>GNAS</td>
<td>PDGFRA</td>
<td>MPL</td>
<td>ABL1</td>
</tr>
<tr>
<td>PTEN</td>
<td>FLT3</td>
<td>STK11</td>
<td>SMARCB1</td>
<td>KIT</td>
<td>MET</td>
<td>NOTCH1</td>
</tr>
<tr>
<td>FGFR2</td>
<td>RB1</td>
<td>JAK3</td>
<td>VHL</td>
<td>KDR</td>
<td>SMO</td>
<td></td>
</tr>
<tr>
<td>HRAS</td>
<td>AKT1</td>
<td>ALK</td>
<td>MLH1</td>
<td>FBXW7</td>
<td>ERBB4</td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td>CDH1</td>
<td>IDH1</td>
<td>CTNNB1</td>
<td>APC</td>
<td>FGFR1</td>
<td></td>
</tr>
</tbody>
</table>

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.
Ion AmpliSeq™ Cancer Panel v2

- Same content as Ion AmpliSeq™ Cancer Panel
- Improved primer designs
- Optimized with most up-to-date chemistry

<table>
<thead>
<tr>
<th>KRAS</th>
<th>BRAF</th>
<th>EGFR</th>
<th>TP53</th>
<th>PIK3CA</th>
<th>CSF1R</th>
<th>JAK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS</td>
<td>PTPN11</td>
<td>ERBB2</td>
<td>SRC</td>
<td>FGFR3</td>
<td>NPM1</td>
<td>CDKN2A</td>
</tr>
<tr>
<td>RET</td>
<td>HNF1A</td>
<td>SMAD4</td>
<td>GNAS</td>
<td>PDGFRA</td>
<td>MPL</td>
<td>ABL1</td>
</tr>
<tr>
<td>PTEN</td>
<td>FLT3</td>
<td>STK11</td>
<td>SMARCB1</td>
<td>KIT</td>
<td>MET</td>
<td>NOTCH1</td>
</tr>
<tr>
<td>FGFR2</td>
<td>RB1</td>
<td>JAK3</td>
<td>VHL</td>
<td>KDR</td>
<td>SMO</td>
<td></td>
</tr>
<tr>
<td>HRAS</td>
<td>AKT1</td>
<td>ALK</td>
<td>MLH1</td>
<td>FBXW7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td>CDH1</td>
<td>IDH1</td>
<td>CTNNB1</td>
<td>APC</td>
<td>FGFR1</td>
<td></td>
</tr>
</tbody>
</table>
Ion AmpliSeq™ Comprehensive Cancer Panel (CCP)

- Truly comprehensive
  - Full exon coverage of ~400 key cancer genes
  - Includes content from Ion AmpliSeq™ Cancer Panel
- Signature Ion AmpliSeq™ simplicity
  - Ready-to-use, no primer design
  - Low DNA input of 10ng per pool
  - FFPE compatible
- Deep sequencing of key cancer genes
  - Detection of somatic variants at ~10% allele frequency

Forbes S A et al. Nucl. Acids Res. 2011;39:D945-D950

For research use only. Not intended for any animal or human therapeutic or diagnostic use.
## Updated list

### Summary of changes:
- Addition of NRAS (C, M)
- Removal of germline SNPs
- Removal of expression markers
- Extended TP53 tests (O)
- Extended BRAF tests (L)
- Extended KRAS tests (L)
- Extended PI3KCA tests (M)

### Potential late additions:
- Extend BRAF testing to all
- BRAF e11 & e15 sequencing
- DDR2 sequencing

### Table

<table>
<thead>
<tr>
<th>Indication</th>
<th>Gene</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>KRAS</td>
<td>Codons 12, 13, 61 and 146* Exon 15/ codons 599, 600, 601</td>
</tr>
<tr>
<td></td>
<td>BRAF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NRAS</td>
<td>Codons 12, 13, 61 Exons 9 and 20</td>
</tr>
<tr>
<td></td>
<td>PI3KCA</td>
<td>Exons 4-9</td>
</tr>
<tr>
<td></td>
<td>TP53</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>PI3KCA</td>
<td>Exons 9 and 20</td>
</tr>
<tr>
<td></td>
<td>TP53</td>
<td>Exons 4-9</td>
</tr>
<tr>
<td></td>
<td>PTEN**</td>
<td>Exons 2-10</td>
</tr>
<tr>
<td>Prostate</td>
<td>PTEN**</td>
<td>Exons 2-10</td>
</tr>
<tr>
<td></td>
<td>TMPRSS-ERG</td>
<td>FISH***</td>
</tr>
<tr>
<td>Lung</td>
<td>EGFR</td>
<td>Exons 18-21</td>
</tr>
<tr>
<td></td>
<td>KRAS</td>
<td>Codons 12, 13, 61 and 146* FISH***</td>
</tr>
<tr>
<td></td>
<td>EML4-ALK</td>
<td>Exon 15/ codons 599, 600, 601</td>
</tr>
<tr>
<td></td>
<td>BRAF</td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>TP53</td>
<td>Exons 4-9</td>
</tr>
<tr>
<td></td>
<td>PTEN**</td>
<td>Exons 2-10</td>
</tr>
<tr>
<td></td>
<td>PI3KCA</td>
<td>Exons 9 and 20</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF</td>
<td>Exon 15/ codons 599, 600, 601</td>
</tr>
<tr>
<td></td>
<td>CKIT</td>
<td>Exons 11, 13 and 17</td>
</tr>
<tr>
<td></td>
<td>NRAS</td>
<td>Codons 12, 13, 61</td>
</tr>
<tr>
<td></td>
<td>PI3KCA</td>
<td>Exons 9 and 20</td>
</tr>
</tbody>
</table>

* Codon 146 added to KRAS testing  
** PTEN – sequencing and LOH  
***TMPRSS2-ERG & EML4-ALK by FISH initially. RT-PCR to be developed.
# Solid Cancers Gene Panel

**Inclusion Criteria**

- for which there is an approved therapeutic
- for which there is a drug in early or late stage clinical trials
- which are relevant to multiple solid tumour types
- which have been recommended by more than one investigator
- have a detectable mutation frequency using platform (>1%)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>AURKA</td>
<td>CSF1R</td>
<td>ERCC2</td>
<td>FOXL2</td>
<td>IDH2</td>
<td>MAP2K2</td>
<td>NOTCH2</td>
<td>PIK3R1</td>
<td>RPTOR</td>
<td>STK11</td>
<td>UBR5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABL2</td>
<td>AURKB</td>
<td>CSMD3</td>
<td>EZH2</td>
<td>GATA3</td>
<td>IGF2R</td>
<td>MAP2K4</td>
<td>NOTCH4</td>
<td>PIK3R2</td>
<td>RRM1</td>
<td>TAF1</td>
<td>UGT1A1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACVR2A</td>
<td>AURKC</td>
<td>CTNNB1</td>
<td>FANCA</td>
<td>GNA11</td>
<td>IKBKB</td>
<td>MAP3K1</td>
<td>NPM1</td>
<td>PKHD1</td>
<td>RUNX1</td>
<td>TAF1L</td>
<td>VHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKT1</td>
<td>BAI3</td>
<td>CYP2D6</td>
<td>FANCC</td>
<td>GNAQ</td>
<td>JAK1</td>
<td>MDM2</td>
<td>NRAS</td>
<td>POT1</td>
<td>SDHA</td>
<td>TET2</td>
<td>WT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKT2</td>
<td>BRAF</td>
<td>DDR2</td>
<td>FANCD2</td>
<td>GNAS</td>
<td>JAK2</td>
<td>MEN1</td>
<td>PARP1</td>
<td>PPP2R1A</td>
<td>SDHB</td>
<td>TGFBR2</td>
<td>XRCC2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKT3</td>
<td>BRCA1</td>
<td>EGFR</td>
<td>FBXW7</td>
<td>GRM8</td>
<td>JAK3</td>
<td>MET</td>
<td>PAX5</td>
<td>PTCH1</td>
<td>SDHC</td>
<td>TLR4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALK</td>
<td>BRCA2</td>
<td>EML4-ALK</td>
<td>FGFR1</td>
<td>HDAC1</td>
<td>KDR</td>
<td>MLH1</td>
<td>PBRM1</td>
<td>PTEN</td>
<td>SDHD</td>
<td>TNKS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>CCND1</td>
<td>EMT</td>
<td>FGFR2</td>
<td>HDAC2</td>
<td>KEAP1</td>
<td>MRE11A</td>
<td>PDGFR1</td>
<td>PTPN11</td>
<td>SETD2</td>
<td>TNKS2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>CDH1</td>
<td>EPHA3</td>
<td>FGFR3</td>
<td>HDAC3</td>
<td>KIT</td>
<td>MSH2</td>
<td>PDGFRB</td>
<td>RAD51</td>
<td>SMAD4</td>
<td>TOP1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARID1A</td>
<td>CDK4</td>
<td>ERBB2</td>
<td>FGFR4</td>
<td>HIF1A</td>
<td>KRAS</td>
<td>MSH6</td>
<td>PIK3C2B</td>
<td>RB1</td>
<td>SMARCA4</td>
<td>TP53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td>CDKN2A</td>
<td>ERBB3</td>
<td>FLT1</td>
<td>HNF1A</td>
<td>LPHN3</td>
<td>MTO1</td>
<td>PIK3CA</td>
<td>RET</td>
<td>SMARCB1</td>
<td>TSC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATR</td>
<td>CHEK1</td>
<td>ERBB4</td>
<td>FLT3</td>
<td>HRAS</td>
<td>LRP1B</td>
<td>NF1</td>
<td>PIK3CB</td>
<td>RICTOR</td>
<td>SMO</td>
<td>TSC2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATRX</td>
<td>CHEK2</td>
<td>ERCC1</td>
<td>FLT4</td>
<td>IDH1</td>
<td>MAP2K1</td>
<td>NOTCH1</td>
<td>PIK3CG</td>
<td>ROS1</td>
<td>SRC</td>
<td>TSHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Clinical Cancer Sequencing Volume

- Due to lower costs, exome sequencing studies have dominated the clinical cancer sequencing landscape.
- However, WGS studies rapidly gaining in popularity with percentage of cancer sequencing volume increasing from 5% in 2010 to a projected 15% in 2012.

*Selected high quality published clinical studies utilizing human tissue*
Huge Explosion in Next Gen Research

Cancer Becoming a Major Driver

Next Gen Publications per Year

- Exponential increase in Next Gen publications year over year
- Cancer sequencing publications increasing as percent of total

Percent Cancer Related

- 2010: 0%
- 2011: 5%
- 2012: 20%
<table>
<thead>
<tr>
<th>Title</th>
<th>Journal</th>
<th>Cancer Type</th>
<th>Number of Patients</th>
<th>Study Type</th>
<th>Key Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-Cell Exome Sequencing and Monoclonal Evolution of a JAK2-Negative Myeloproliferative Neoplasm</td>
<td>Cancer Discovery</td>
<td>Myeloproliferative Neoplasm</td>
<td>1</td>
<td>Exome, single-cell</td>
<td>First study showing feasibility of single cell sequencing</td>
</tr>
<tr>
<td>Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies</td>
<td>Genes Chromosomes Cancer</td>
<td>Colon, NSCLC</td>
<td>64</td>
<td>Panel (145 genes)</td>
<td>Validated panel of genes for diagnosis of common and actionable cancer mutations</td>
</tr>
<tr>
<td>Next-generation sequencing for minimal residual disease monitoring in acute myeloid leukemia patients with FLT3-ITD or NPM1 mutations</td>
<td>Cancer Discovery</td>
<td>Acute Myeloid Leukemia</td>
<td>20</td>
<td>Targeted Sequencing, Disease Monitoring</td>
<td>NGS can be used to monitor disease burden/recurrence</td>
</tr>
<tr>
<td>High-Throughput Detection of Actionable Genomic Alterations in Clinical Tumor Samples by Targeted, Massively Parallel Sequencing</td>
<td>Cancer Discovery</td>
<td>Breast/Colon Proof of Concept</td>
<td>10</td>
<td>Panel (137 genes)</td>
<td>Proof of concept study that NGS Panel sequencing possible on FFPE tumor samples. High sensitivity and specificity. Turnaround time 1-2 weeks, inline with other molecular tests. Higher sensitivity than OncoMap (Sequenom, mass spec)</td>
</tr>
<tr>
<td>Personalized oncology through integrative high-throughput sequencing: a pilot study</td>
<td>Sci Transl Med</td>
<td>Prostate, Colon</td>
<td>4</td>
<td>WGS/Exome, Treatment Selection</td>
<td>Mutations found through sequencing can be utilized for clinical decision making. Turn around time in their study was 3-4 weeks.</td>
</tr>
</tbody>
</table>
6. The Genomic Physician(s)
Turning Genetic Information Into Treatment Decisions: Role of Molecular Pathologist

Medical Knowledge
Delivered in Life Tech Reports
- Medical guidelines
- Drug trial matching rules
- Research literature

Sequence Data From Instrument

Molecular Pathologist
Applies experience, judgment, and education

Network Patient Data From Participating Centers
- For genetically similar patients:
  - Basic diagnosis
  - Treatment selection
  - Outcome information
- Population-based treatment options

Rules-based treatment options

Report to Treating Oncologist
Interpretation & Reporting Solutions

Interpretation: For Pathologist/Lab

Features
- Filters: degree of amino acid change, frequency in population, etc.
- Links to literature: structure/function, implications to drug response
- Drug trial options
- Ability to flag/prioritize mutations of interest

Medical Options: For Oncologist

Features
- Mutations flagged by pathologist/lab
- Medical options to address flagged mutations
  - Medical guidance
  - Off-label options
  - Drug trial options
Personalized Medicine Care: Professionalization

• Genomic Medicine Specialty: AMA, ACP, ACMG and AMP
• Role of non-MDs in the process
• Std of Care development and EBM stds
• Patient empowerment
7. Business Models
Key Technology Features

• Custom or vetted content for unmet needs and branding
• Low capital and consumable costs
• Low labor costs
• Easy integration
• Community of users
• A new technology that saves money!
Unmatched Speed and Throughput

Targeted Reseq of 400 Genes

- **Comp A**
  - 10 Samples per week

Whole Genome Sequencing

- **Comp A**
  - 20 Genomes in 10 days
  - 6 Genomes in 10 days

Rapid time to results supports highly iterative science
Enabling High-Value Genomic Medicine Services in Health Care

Specimen Collection & Testing*
- Sample collected
- Sequencing Performed

Analysis
- Variants reviewed with knowledge apps
- Test data deposited in index
- Findings reported to physician

Follow-up
- Treatment or trial selected
- Disease monitoring & outcome reporting
- Patient engagement
  - Expert consultations
  - Social networking

- Premium service
- Highly visible
- Engages patients

*Under IRB Supervision with Patient Consent.
Next Generation Translational Trials

Patient or Grant Pays

Variant File
To Molecular Pathologist

Gene Panel Test

Medical knowledge

Mutations of Interest

Confirmatory Testing

Report to Treating Physician

Drug Trial

Pharma Pays

Insurance Pays

Evidence accrues: patient benefit, cost effectiveness

Payer adoption
Personalized Medicine Care: Reimbursement

• Coding
• TC versus PC
• Health economic data
• Adoption by payers including role of FDA, CMS, professional societies and proprietary factors
Reimbursement & Regulation

- Currently done via “Code-stacking”
- CPT code series 83890 - 83914
  - First six codes introduced in 1993
  - Expanded to 21 codes by 2007
- Codes are per procedure versus analyte/analysis
- Most sequencing tests currently fall under CLIA LDT guidelines
  - Regulation and oversight currently not well defined
  - FDA signaled that will provide additional guidance for regulation of LDTs in coming year

Coverage of Select MDx Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>FDA Cleared</th>
<th>Aetna</th>
<th>Regional CMS</th>
<th>Cigna</th>
<th>Regional BCBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlloMap</td>
<td>510k</td>
<td>YES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oncotype DX</td>
<td>LDT</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>BRACAnalysis</td>
<td>LDT</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>KRAS</td>
<td>LDT</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>MammaPrint</td>
<td>510k</td>
<td>YES</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NGS: Pan Cardiomyopathy Panel (46 Genes)

<table>
<thead>
<tr>
<th>CPT Code</th>
<th>Reimbursement</th>
</tr>
</thead>
<tbody>
<tr>
<td>83891</td>
<td>$5.74</td>
</tr>
<tr>
<td>83898</td>
<td>24.01 x46</td>
</tr>
<tr>
<td>83904</td>
<td>24.01 x46</td>
</tr>
<tr>
<td>83912</td>
<td>5.74</td>
</tr>
</tbody>
</table>

Total $2220
Contents

Introduction

Clinical innovation...who cares?

What is needed to speed sequencing in to clinical use?

Conclusions
PGM for genes.
Proton for genomes.
Sequencing for all.
Technology Only Part Of Solution...

Personalized Medicine

Ethical

Economics

Privacy

Technology

Medical

Regulatory

Clinical outcome improvements
TNBC Trial at TGEN and US Oncology

• Supported by Life Technologies
• Utilized WGS sequencing of germline and somatic samples as well as whole transcriptome analysis
• 14 patients with relapsed TNBC
• Individualized analysis including mutational confirmation (Caris) and multidisciplinary tumor board
• Many novel mutations and rearrangements noted
• Druggable targets (MEK, MTOR, others) identified
• Clinical trial referrals initiated
• Manuscript in preparation
The Oxford Trial

• Oxford University, CRUK and TSB
• Do panels improve care?
• 9K patients
• Std of care change outcome
• Panel established and protocols under review
Early Observations: Cancer Sequencing Panels

• Rapid high fidelity sequencing accomplished
• Easy, one day workflow for large sequencing panels
• 100% concordance with known mutations during validations
• Deep sequencing reveals novel mutational findings and heterogeneity including unsuspected therapeutic targets
• Actionability includes clinical trial referrals
• Data standardization and sharing should speed comparative genomic medicine and improve care
• Research participation is unacceptably low
Research Medicine is the Best Medicine!

• Low participation rates serve no one

• Properly conducted and vetted protocols are:
  > Good for the patient
  > Their family
  > Their neighbors
  > Their community
  > Their health providers

PARTICIPATION WILL SPEED CANCER CURES
“Yeah, but good luck getting it peer-reviewed.”
NGS needs NGEBM to optimize translation

Next Generation Sequencing (NGS) needs new validation paradigm:

• Prospective RCT: Gold standard but too costly and impractical

• Cases and Case Studies: Long history but not powerful

• Comparative Genomic Medicine: reliable genomic databases linked to EMR based clinical descriptions and outcomes yielding powerful case based inferences

A Next Generation of Evidence Based Medicine (NGEBM)
Conclusions

• Life Technologies is a leader in all phases of the molecular medicine transformation of healthcare and personalized medicine

• NGS is ready for adoption in clinical settings and practical, rapid methods for WGS are being introduced

• New methods for data gathering and trials will be needed to *speed* applications of genomic medicine

• Research participation is crucial to the value proposition of genomic medicine

• Technology, informatics and translational medicine are rapidly creating the Personalized Medicine world.
Partial List of Collaborators

• ION: Jon Rothberg, Gregg Fergus, Manesh Jain, Mark Anderson, Garrick Peters, John Stark, Brian McKelligon and Glen Powell

• Data: Martin Naley, Asim Siddqui, Ellen Beasley, Mike Lelivelt and Alan Williams

• TGEN: David Craig, John Carpten, Spryo Mousses, and Jeff Trent

• US Oncology: Dan von Hoff and Jacqueline O’Shaughnessy

• Others: Sir John Bell, Brian Druker and many others
The Interpreting Physician Command Center

Driving toward a patient-centric, technology-agnostic vision of the future requires use of multiple biomarker types, access to comparative medicine database and clinical judgment

A Glimpse Into the Future Of Molecular Medicine
The Treating Physician Portal

• Allows real time access to patient information and education to drive better management of complex diseases
Changing Medicine Forever

Empiric Medicine

Evidence Based Medicine

Personalized (Sequencing) Medicine

Real Personalized Medicine is here ... TODAY
SPEED KILLS

NOW, SPEED CURES!
Thank You!