Quechee, Vermont
1. Molecular infectious disease testing
2. Bringing new tests into the lab
3. Molecular testing with FFPE and cytology samples
4. Targeted therapy
5. Molecular hematology
6. Validation/Verification
7. Molecular genetics and PGx
8. FISH
9. QA
**What are we trying to do?**
The Universal Genetic Code

<table>
<thead>
<tr>
<th>First Position (5´-end)</th>
<th>Second Position</th>
<th>Third Position (3´-end)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>C</td>
</tr>
<tr>
<td>U</td>
<td>Phe</td>
<td>Ser</td>
</tr>
<tr>
<td>U</td>
<td>Phe</td>
<td>Ser</td>
</tr>
<tr>
<td>U</td>
<td>Leu</td>
<td>Ser</td>
</tr>
<tr>
<td>U</td>
<td>Leu</td>
<td>Ser</td>
</tr>
<tr>
<td>C</td>
<td>Leu</td>
<td>Pro</td>
</tr>
<tr>
<td>C</td>
<td>Leu</td>
<td>Pro</td>
</tr>
<tr>
<td>C</td>
<td>Leu</td>
<td>Pro</td>
</tr>
<tr>
<td>C</td>
<td>Leu</td>
<td>Pro</td>
</tr>
<tr>
<td>A</td>
<td>Ile</td>
<td>Thr</td>
</tr>
<tr>
<td>A</td>
<td>Ile</td>
<td>Thr</td>
</tr>
<tr>
<td>A</td>
<td>Ile</td>
<td>Thr</td>
</tr>
<tr>
<td>A</td>
<td>Met</td>
<td>Thr</td>
</tr>
<tr>
<td>G</td>
<td>Val</td>
<td>Ala</td>
</tr>
<tr>
<td>G</td>
<td>Val</td>
<td>Ala</td>
</tr>
<tr>
<td>G</td>
<td>Val</td>
<td>Ala</td>
</tr>
<tr>
<td>G</td>
<td>Val</td>
<td>Ala</td>
</tr>
</tbody>
</table>
How are we trying to do this?
The Structure of DNA

- Nucleosides:
  - G; Guanosine
  - C; Cytosine
  - A; Adenosine
  - T; Thymine
  - U; Uracil

- Sugar-phosphate backbone
- Nucleotide base pairs
- Phosphate backbone

5’ 3’
What tools do we have?
DNA/RNA Extraction Technologies in the Clinical Lab

1980- Manual Extractions:
1. Make your own buffers
2. pH your own buffers
3. Lab math
4. TAT = 1.5 days

2000- Automated Extractions:
1. Reagent contracts
2. Consumables
3. Maintenance contracts
4. TAT = 20-45 minutes
1980- Southern blot:
1. Fresh or frozen tissue
2. Large amounts of HMW DNA
3. Restriction digests and transfer
4. TAT = 7-20 days

1990- PCR-RFLP:
1. FFPE tissue and others
2. Small amounts of DNA
3. Restriction digests and gels
4. TAT = 2-3 days
PCR-mediated RFLP Analysis for Hereditary Hemochromatosis
2000- Real Time PCR:
1. All specimen types
2. Increased sensitivity and specificity
3. Quantification possible
4. TAT = 2-3 hours
Figure 3. ABI 7500 Fast TAQman™ assay

Allelic Discrimination C282Y

H63D Allelic Discrimination

Automating Real Time PCR - 2004
The Automation Revolution Continues - 2011

BD MAX™ System (HandyLab Jaguar)

1. Fully automates cell lysis, nucleic acid extraction, PCR set-up, amplification and detection
2. 24 samples per run

Enigma Diagnostics
Enigma ML
1. Portable
2. Self-contained
3. Ultra-rapid, laboratory-standard results
4. Point-of-care testing
Smaller is Better

Idaho Technologies
Film Array

IQuum
Liat Analyzer
Where do we go from here?
Multiplexing and New Instruments
Primera Dx ICEPlex

Oncology Multi-Modal Panel:
- Fusion gene variants
- Oncogene Gain-of-function SNPs
- Gene Expression Signature
- MicroRNAs

<table>
<thead>
<tr>
<th>29 Targets + 9 Controls</th>
<th>Real-Time Instrument</th>
</tr>
</thead>
</table>

| 96 Patient Samples | 6 days |

| 6 days | 1 morning |
ICEPlex Technology

1. PCR: Fluorescently labeled amplicons of differing sizes.
2. Capillary Electrophoresis: Real-time detection of PCR products fully separates all amplicons by size.
3. Amplification Curves: Required for accurate quantification in PCR.
4. Clinically Actionable Results
Infectious Disease – Automated Multiplex Results

Background
Simultaneously detection and quantification of CMV, EBV, BKV, HHV-6 and HHV-7 in whole blood. The high level of multiplexing available on the ICEPlex instrument enables the incorporation of important quantification standards as well as quality controls for extraction efficiency into one test. These important features can decrease the occurrence of false negatives and increase confidence in results.
GenMark eSensor® Multiplex Cartridge

Capture Probe
Electrodes are coated with a capture probe specific for an allele or viral target

Sample Loading Reservoir

Pneumatic Pump Membrane
Pumps sample solution through the cartridge chambers

EEPROM
Pre-programmed memory chip contains the test protocol, lot number, and expiration date
GenMark Technology

Hybridization Produces Electrochemical Signal
Workflow

Add proprietary label

Load mixture into cartridge

Insert cartridge into XT-8

Patient results reported
Copy Number Variants and Constitutional Genetics

The highest resolution across the genome
CytoScan™ HD Array

Simplified, streamlined, and quality assurance
Kitted reagents

Designed by cytogeneticists for cytogeneticists
Chromosome Analysis Suite (ChAS) Software

GCS 3000 or the 510(k) cleared GCS 3000Dx2
Instrumentation
Hemizygous loss on CytoScan HD
Capillary Electrophoresis and DNA Sequencing
Sanger Method Sequencing Gel
Capillary Electrophoresis
Ion Torrent
The Chip is the Machine™

Scalability
Simplicity
Speed
Scalable Semiconductor Technology

Wafer
Semiconductor Manufacturing

Chip
Semiconductor Packaging

Chip Cross Section
Semiconductor Design
Simple Natural Chemistry
01 Construct Library

02 Prepare Template

03 Run Sequence

04 Analyze Data

+ ION KITS

+ ION ONE-TOUCH™ SYSTEM

+ ION PGM™ SEQUENCER

+ TORRENT SERVER
What if............
...you could:

- Miniaturize the instrument
- Have a disposable cartridge
- Have high performance
- Have TAT <20 minutes
- Run off of a 9V battery
- Program it from your cell phone
- Not pay a lot for it

Thinking outside of the box
Lessons Learned From This Project So Far

• There are a lot of much smarter people in the world
• Never say “Can’t”
• There are still many opportunities for success
• There are many, many unmet needs
The Two Most Common Questions Asked to Me This Week

• Do you want to eat?
• Do you need to use the “Gents” room?
Many thanks to all of you!
DHMC Molecular Pathology Laboratory and Translational Research Program

Collaborators

David Axelrod
David Baltimore
Jeoffry Brennick
David Bucci
Mark Cervinski
Michael Chobanian
Neil Cornell
Thomas Davis
Mary Beth Dinulos
Alan Eastman
Camilo Fadul
Richard Freeman
Tim Gardner
Jorge Gonzalez
Alan Green
William Hickey
John Hill
Margaret Karagas
William Kinlaw
Frederick Lansigan
Hong Kee Lee

Norman Levy
Lionel Lewis
George Maltezos
Thomas McAllister
Ken Meehan
John Moeschler
Deborah Ornstein
Kim Ornvold
Cocav Rawerdink
C. Harker Rhodes
William Rigby
Axel Scherer
Alan Schned
Kerrington Smith
Michael Tsapakos
Heather Wishart
Shaofeng Yan
Bassem Zaki
Richard Zuckerman

Samantha Allen
Betty Dokus
Susan Gallagher
Carol Hart
Arnold Hawk
Claudine Lefferts, Ph.D.
Joel Lefferts, Ph.D.
Rebecca O’Meara
Elizabeth Reader
Mary Schwab
Heather Steinmetz
Laura Tafe, M.D.
Brian Ward
Brendan Wood
Eric York

Claudine Lefferts, Ph.D.
Joel Lefferts, Ph.D.
Rebecca O’Meara
Elizabeth Reader
Mary Schwab
Heather Steinmetz
Laura Tafe, M.D.
Brian Ward
Brendan Wood
Eric York