

次世代シーケンサの臨床応用

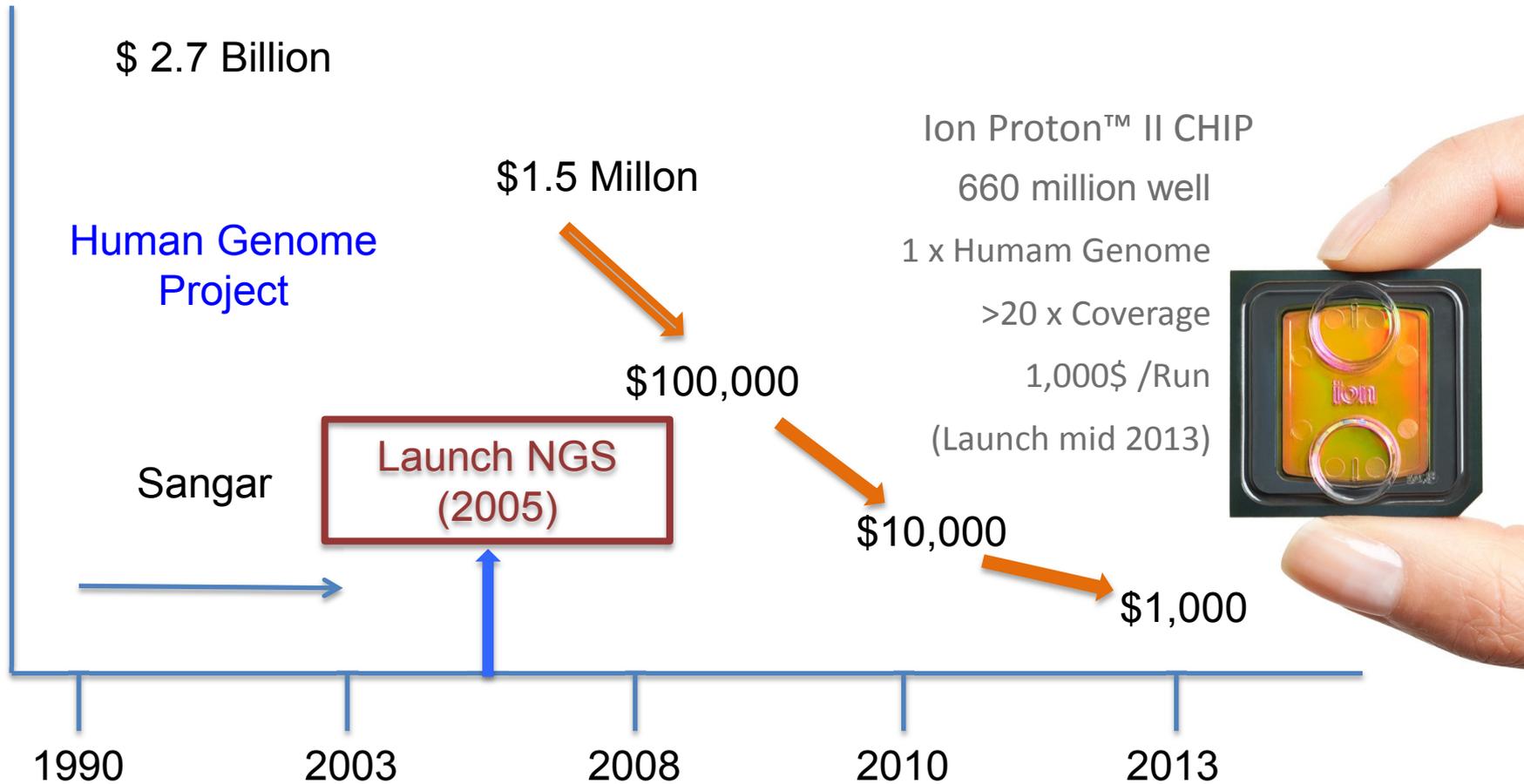
ライフテクノロジーズジャパン

片山 稔

For Research Use Only. Not for use in diagnostic procedures.

ion torrent
by life technologies™

Cost for Human genome sequence





次世代シーケンサが身近に

Ion PGM™



1Gbの、さらにその先へ

Ion Proton™



Gene to Genome by Next generation sequencer

“For genes”

“For exome & genomes”

SEQUENCING APPLICATIONS →

SMALL GENOMES

SETS OF GENES

GENE EXPRESSION CHIP SEQ

WHOLE TRANSCRIPTOME

HUMAN EXOMES

HUMAN GENOMES

ION PGM™ SEQUENCER
for genes



+



ION PROTON™ SEQUENCER
for genomes



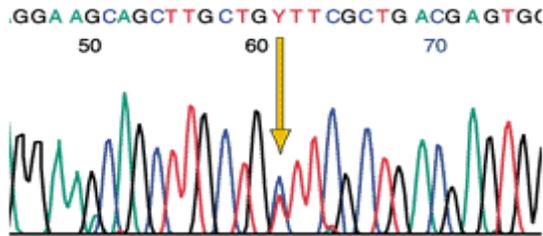
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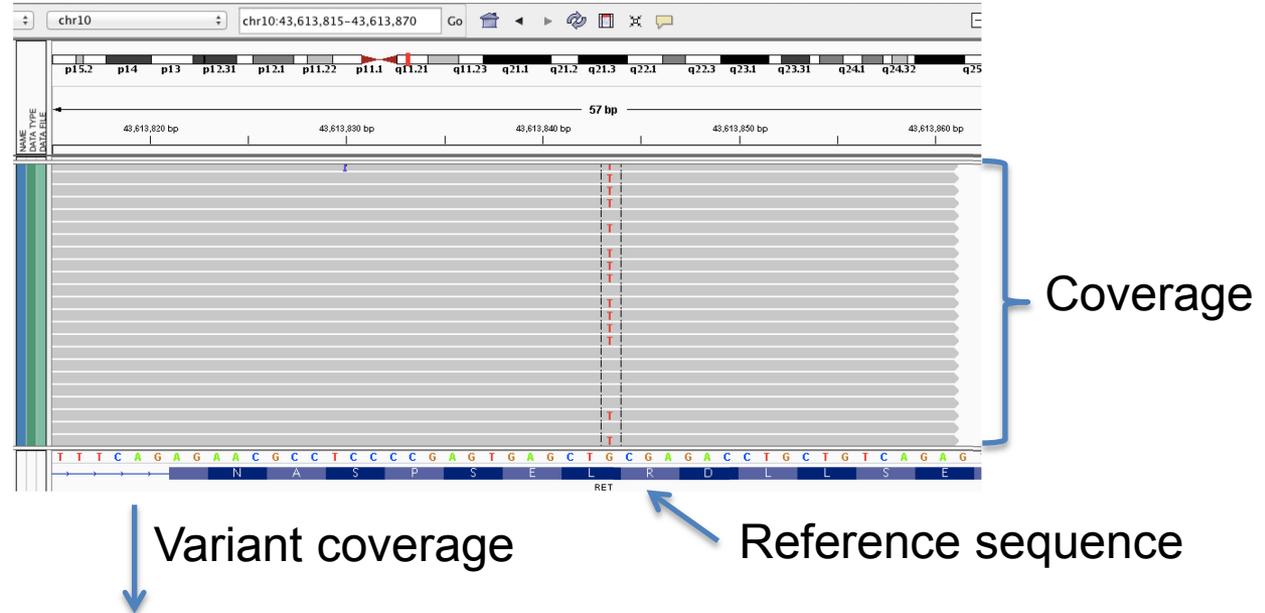
SEQUENCING CHIPS →



NGS potential as a diagnostic tool:



Limitation:
15~20 % mutation



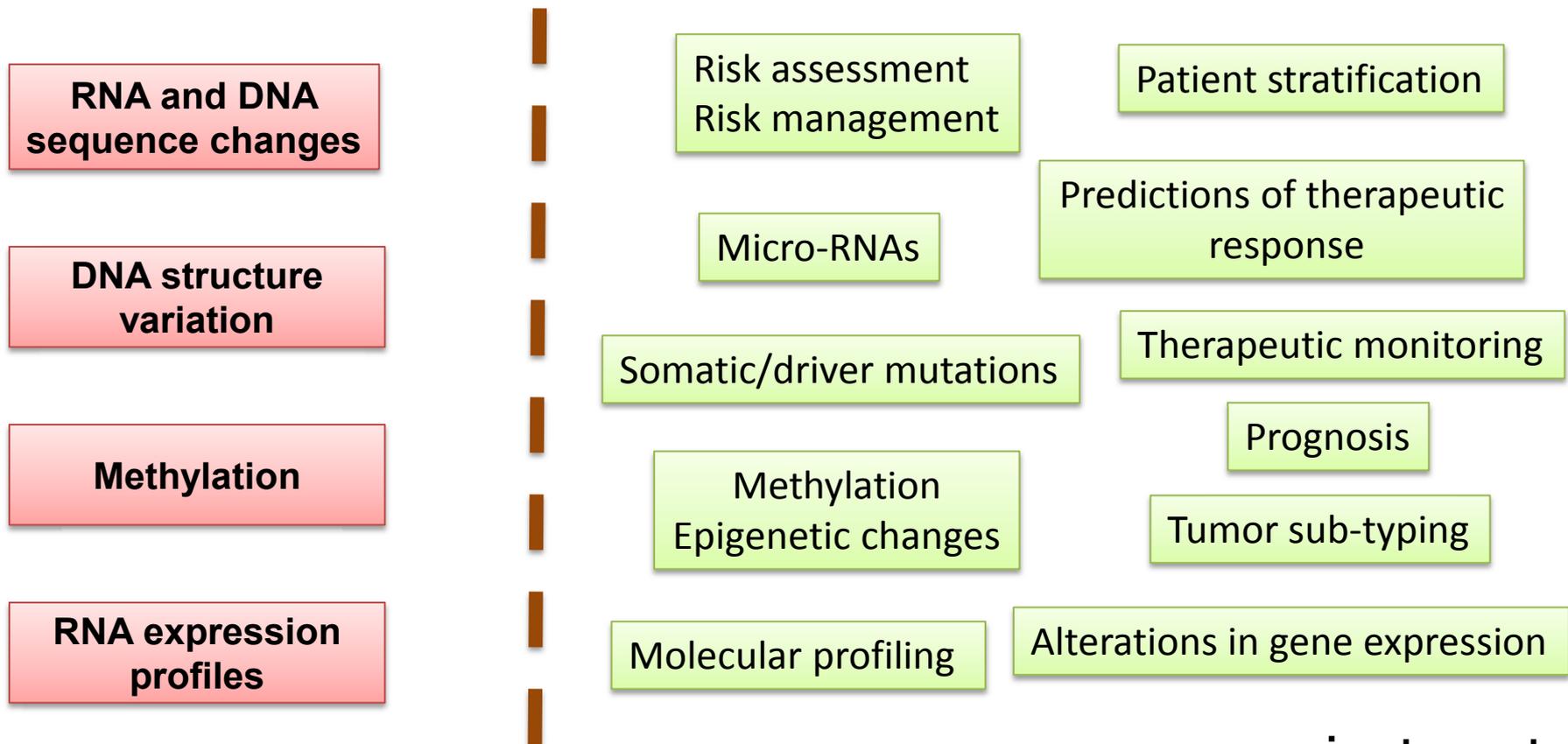
Ref	Variant	Var Freq	Coverage	Ref Cov	Var Cov
G	T	59.66	24152	9739	14409

On sequence variant detection

NGS can get both of qualitative and quantitative data in same time!
AND low mutation can detect than Sanger sequence!!

Examples for NGS Application

Sequence application  Application for neoplastic condition

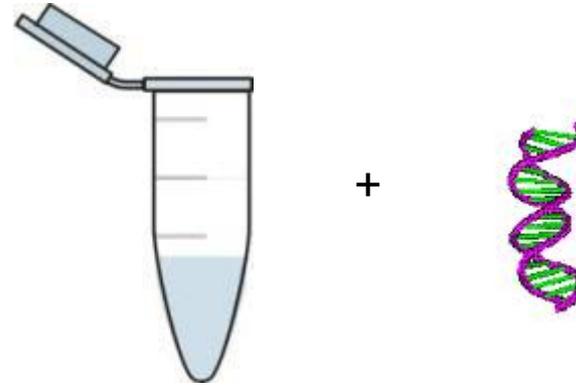


Ion AmpliSeq™ Technology: As Simple As PCR

Single-tube, ultra-high multiplex for Targeted Resequencing

Up to 3072 primer pairs

10ng DNA



Multiplex PCR: Maximum 3072 amplicon from 1 tube reaction



3.5 hours

AmpliSeq™ panels



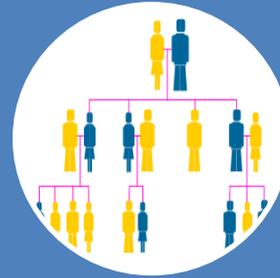
Cancer Primer Pool

46 genes
739 mutations



Comprehensive Cancer Panel

409 genes



Inherited Disease Panel

328 genes
>700 diseases



Ion AmpliSeq™ Designer

www.ampliseq.com
24 to 3,072
amplicons/tube

Example for AmpliSeq Custom panel

Knight cancer institute



KNIGHT DIAGNOSTIC LABORATORIES

Pioneering Personalized Diagnostics

About Us

Genetic Disorders

Infectious Diseases

Oncology

Research & Development

Client Services

Search All Tests

Search Tests

Alphabetical

by Department

Home / Search Results

Test Search Results - *ampliseq*

GeneTrails GIST Gene Panel

Test Code: 4500 • **Department:** Solid Tumors

Test Synonyms: GIST Cancer Gene Panel • **AmpliSeq** GIST Panel • Ion Torrent GISH Panel • AKT1 • AKT2 • AKT3 • ATM • BRAF • CDKN2A • HRAS • KIT • KRAS • MAP2K1 • NF1 • NRAS • PDGFRA • PIK3CA • PTEN • PTPN11 • SDHA • SDHAF1 • SDHAF2 • SDHB • SDHC • SDHD • TP53 • RAS • RAF

[View Test >](#)

GeneTrails NSCLC Panel

Test Code: 5120 (Sequencing) 7600 (FISH Panel) • **Department:** Solid Tumors

Test Synonyms: Lung Cancer Panel • **AmpliSeq** NSCLC Panel • Ion Torrent Lung Panel • AKT1 • ALK • BRAF • CDKN2A • DDR2 • EGFR • ERBB2 • HRAS • JAK2 • KDR • KRAS • MAP2K1 • NOTCH1 • NRAS • NTRK1 • NTRK2 • NTRK3 • PIK3CA • PIK3R1 • PIK3R2 • PTEN • PTPRD • TP53 • ROS1 • RET • MET • RAS • RAF

[View Test >](#)

Contact Us:

855-535-1522

<http://www.knightdxlabs.com/home/search-results?q=ampliseq>

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Diagnostic Spectrum

Sanger

NGS

Single gene
To
Several genes

**Targeted
Gene/Variant
Analysis**

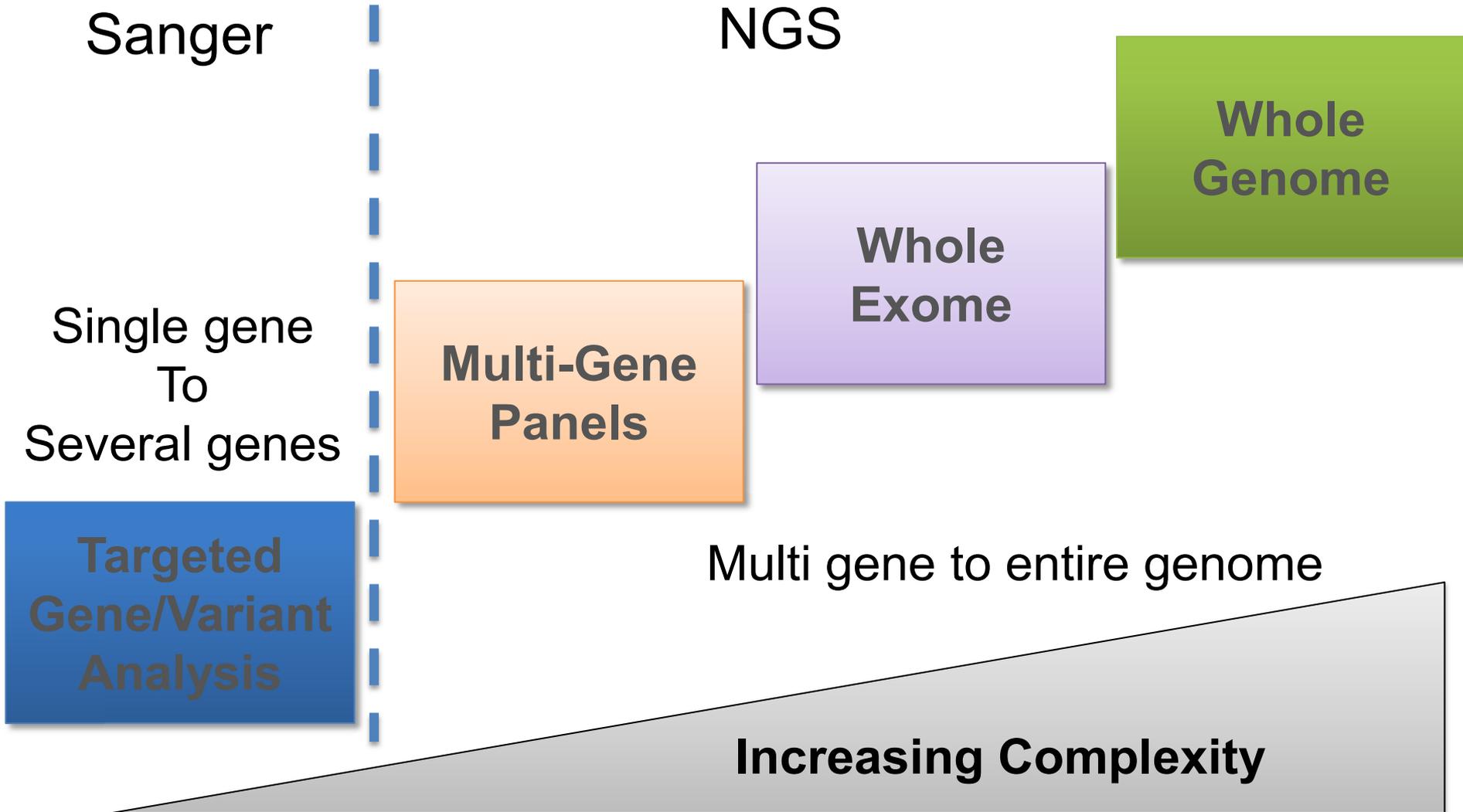
**Multi-Gene
Panels**

**Whole
Exome**

**Whole
Genome**

Multi gene to entire genome

Increasing Complexity



Clinical implementation of NGS

- Division of Laboratory Science and Standards (CDC)
- Genetic Testing Reference Material Coordination Program (Get-RM) (CDC)
<http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx>
- Clinical Laboratory Standards Institute (CLSI)
- American College of Medical Genetics (ACMG)
- College of American Pathologists (CAP)
- Association For Molecular Pathology (AMP)

Clinical decision support systems

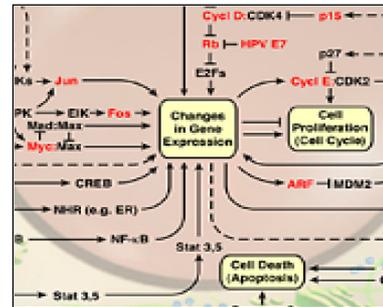
Example

- Expert interpretation and guidance
- Proficiency testing and alternative assessment are challenging

Ion Torrent PGM*



From sequences...



... to biological understanding...



Medical Laboratories

CELL ID # XXX-000-XXX
M.D.

Patient name:	Bowman, Mary Louie	Case number:	808-0006				
Patient ID:	383210593	Collection date:	8/12/2008 08:43				
Date of birth:	08/09/1978	Age:	30	Sex:	F	Delivery date:	8/12/2008 08:43
						Approval date:	8/16/2008 08:02

Provider: Patricia Hedling, MD

Bone Marrow Pathology Report

FINAL DIAGNOSIS

WHO Acute Myeloid Leukemia Not Otherwise Specified: FAB Acute Myelomonocytic Leukemia (M4)

Bone Marrow Biopsy, Aspirate and Particle Preparation:

1. Acute Myeloid Leukemia with marked hypercellularity, numerous blasts (67%) and eosinophils (21%).
2. Reduced T-lymphocyte Hematopoiesis.

Peripheral Blood:

1. Acute Myeloid Leukemia with leukocytosis including numerous blasts (48%), monocytosis (25%), and eosinophils (16%).
2. Anemia and Hemorrhagic spots.

Genomic Analysis Interpretation

Fluorescence immunophenotyping studies performed on bone marrow demonstrated numerous CD34 positive CD17 positive myeloid blasts (14/22% positive); these cells coexpressed the myeloid markers CD13/33. Many expressed HLA-DR and TdT, also markers of myeloid immaturity. Also, there was a distinct population of cells that expressed the monoclonal marker, CD34.

Clinical History: A 30-year-old female without any significant past medical history, developed symptoms of sinus pressure and headache for approximately three weeks. These were thought to be sinusitis and treated with oral antibiotics (Skeetricin) and antihistamines. Subsequently she developed gingival hyperplasia and was found to have a white blood cell count of over $70 \times 10^9/L$.

Microscopic Examination

Bone Marrow biopsy and aspirate were performed with the following remarkable differential counts:

WBCs:	67.0%	(normal 0.0 - 2.0)
Eos Myelo/Mon:	15.0%	(normal 1.0 - 4.0)
Eos Blast:	2.7%	(normal 1.0 - 2.0)
Eos Seg:	2.3%	(normal 1.0 - 2.0)

The marrow was markedly hypercellular (approximately 100%). The predominant cells were blasts but eosinophils also appeared markedly increased. The blasts in the marrow were generally large with many having a moderate amount of

Case number: 808-0006
This report continues... (Preliminary)

Reviewed by: _____

...to actionable information



Ion Semiconductor Sequencing

Rapid, Benchtop Sequencing for All

PGM™ for genes.
Proton™ for genomes.
Sequencing for all.



Ion PGM™ Sequencer



Ion Proton™ Sequencer

For more information, visit <http://lifetechnologies.com/proton>