Genomic Medicine: What every pathologist needs to know

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Genomics and Genetics in 21st Century Pathology

- Diagnosis
  - Genomic strategies can aid in the proper identification of cancer types

- Prognosis
  - Genomic strategies can improve prognosis for patients

- Prediction
  - Genomic strategies can make correct predictions about therapeutic strategies?

But first, how the heck do these machines work?

- Sequencing by synthesis
  - An elegant and brute force approach to obtaining sequence data

- Alignment to reference genomes
  - What, or whom, are the reference genomes
  - When do we need them, and when do we not?

- Short read versus long read sequencing
  - The evolution of sequencing technology
Massively parallel DNA sequencing:
Next-Gen sequencing

- Replaces old-school Sanger Sequencing
- Replaces PCR-based sequencing
- Allows for rapid sequencing of
  - Whole genomes
  - Whole exomes
  - Targeted gene panels
  - DNA or RNA
  - DNA or RNA complexes
- Species agnostic

Sequencing Workflow

Library Preparation → Cluster Generation → Sequencing → Data Analysis

Library Prep

Cluster generation
Sequencing by Synthesis

- Cycle 1: Add sequencing reagents
- First base incorporated
- Remove unincorporated bases
- Wait a minute
- Cycle 2-4: Add sequencing reagents and repeat

- All four labelled nucleotides in one reaction
- Base-by-base sequencing

Slide made by Christian Zeeh
Discovering genetic variation

Single nucleotide change

The reference genome is key in telling a mutation from a SNP

This is why they call it massively parallel sequencing...

Deletion

Read length is key!

Platform Features

<table>
<thead>
<tr>
<th>Feature</th>
<th>Illumina HiSeq2500 High output</th>
<th>Illumina HiSeq2500 Real-time mode</th>
<th>MiSeq</th>
<th>PacBio RS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of reads</td>
<td>150-180M/lane</td>
<td>150-150M/lane</td>
<td>15-55M (v2)</td>
<td>50-80K/SMRT cell</td>
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<tr>
<td>Read length</td>
<td>2 x 100 bp</td>
<td>2 x 150 bp</td>
<td>2 x 100 bp (v3)</td>
<td>~10-20 kb</td>
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<tr>
<td>Yield per lane (Pf)</td>
<td>up to 35 Gb</td>
<td>up to 45 Gb</td>
<td>up to 35 Gb</td>
<td>up to 0.4 Gb</td>
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<tr>
<td>Instrument Time</td>
<td>~32-14 days</td>
<td>~2 days</td>
<td>~2 days</td>
<td>~2 hours</td>
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<tr>
<td>Pricing per Gb</td>
<td>$591 (PE100)</td>
<td>$531 (PE150)</td>
<td>$1088 (PE300)</td>
<td>$697</td>
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</tbody>
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Pair end sequencing allows identification of splice isoforms and...

....the presence of gene rearrangements
Expression profiling for diagnosis

Gene expression profiling by RNA-Seq

RNA-Seq analysis can provide data on:

• Gene expression level
• Expression level of different isoforms of the same gene
• Chimeric genes that result from gene rearrangements
• Gene loss or gene deletion
• miRNA
• IncRNA
• Not limited by what’s on an array
Expression profiling for prognosis
Identification of breast cancer subtypes using the PAM50 gene set

Oncotype DX® 21-Gene Recurrence Score® (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

PROLIFERATION
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

ESTROGEN
- ER
- PR
- SU2
- SCUBE2

INVASION
- Stromelysin 3
- Cathespin L2

HER2
- HER2

REFERENCES
- Beta-actin
- GAPDH
- RPLPO
- GUSB
- TFRC

The Oncotype DX test measures the expression levels of 20 genes in a breast cancer specimen.
Genomic analysis of cancer DNA for therapeutics

- Cancer is a genomic disease
- Oncogenes are amplified, mutated, or rearranged versions of normal cellular genes
- Activated oncogenes drive the development of cancer
- Activated oncogenes can be diagnosed using genomic technologies
- Activated oncogenes are often the direct targets of new cancer drugs

Old School: Karyotype

Comparative Genomic Hybridization
Chromosome CGH: FGFR2 as a target gene for 10q26 amplification

Ideogram Summary of Gains (Green) and Losses (Red) in Eleven SUM Breast Cancer Cell Lines

Array CGH: It’s all about resolution
Onco\-gene addiction and onco\-gene progression

Druggable breast cancer onco\-genes activated by gene amplification

- HER-2 (ERBB2)
- FGFR2
- FGFR3
- AKT
- CCND1
- CCNE1
- EGFR
- BCL2L1
Oncogenes activated by point mutations: EGFR, PIK3CA, BRAF

- EGFR mutated in 10% of non-small cell lung cancer. Erlotinib targets mutant EGFR
- PIK3CA mutated in 20% of breast cancers. Several drugs in clinical trials that target this enzyme
- B-RAF mutated in 50% of melanoma targeted by Vemurafenib

Patient with metastatic melanoma following 15 days of treatment with PLX4032

Evolution of Genomic Sequencing for Patients

Real-Time PCR

Massively Parallel Sequencing

EGFR, KRAS, BRAF

29 variants

11 variants

Send-Out cKIT analysis, 4 exons by pyrosequencing

80 exons covered in 26 genes, 21,000 bases of DNA

Exons 18, 19, 20, 21

2, 3, 4, 5

9, 11, 13, 17, 18

NGS Cancer Theranostics Panel

Original MUSC 26 gene panel

Current MUSC 50 gene panel

Don't forget about copy number/gene amplification!
Memorial Sloan Kettering IMPACT panel

400-gene panel developed by Dr. Michael Berger at MSK. This test measures amplification, point mutation and rearrangement of 400 candidate oncogenes.

Precision medicine applications

Basket trials of targeted drugs

The NCI MATCH trial, a BASKET trial

- Molecular Analysis for Therapy Choice
- Advanced solid tumors and lymphomas
- 24 arms!
- 5000 patients, 143 genes, 4000 variants included so far
- Arms can be dropped and replaced adaptively
- Overall Response Rate (ORR, radiologic) and PFS (Progression-Free Survival) endpoints (16% minimum)
- Patients with MMR (Mismatch Repair) deficits assigned to nivolumab (more intrinsic mutations, more neoantigens)

NCI MATCH SCHEMA

1 CR, PR, SD, and PD as defined by RECIST
2 Refractory: if additional mutations, offer new targeted therapy
What’s next? Where do we go from here?

Oncogene Signatures: The full complement of activated oncogenes that drive the phenotypes of human cancers

Genomic analysis of cancer is the gateway to combinatorial targeted therapy!

- Combining a targeted agent with conventional chemotherapy
- Combining multiple targeted agents based on oncogene signature
- Targeting multiple oncogenes to prevent recurrence
Genomic Tumor Boards

Invasive Breast Carcinoma as diagnosed in the 20th century

Invasive Breast Carcinoma as diagnosed in the 21st Century

SUM-185
MCF10A

PD174170 plus Navitoclax induces rapid apoptosis in SUM-185 cells

hr: 0 6 12 24
Pre-Caspase 3
Claved Caspase 3
**BH3 Mimetic Combination Therapy in Vivo**

- Tumor growth
- Enroll into 1 of 4 treatments
- Monitor tumor size

FGFR inhibitor was highly effective at shrinking tumors on its own

**Regression of large tumors following combined inhibition of FGFR and BCL2L1**

- ½ dose FGFRi + ½ dose BH3m