Next-Generation Sequencing (NGS) in Cancer Genomics Research

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Field Application Scientist
Cancer is a genetic disorder

Inherited Predisposition  Acquired Somatic Mutations  Environmental/Epigenetic

Sustain Cellular Response
Sanger Sequencing vs Next-Generation Sequencing (NGS) in Cancer research

- Capillary electrophoresis (CE)-based Sanger sequencing has traditionally been the gold standard in cancer research.
- However, it has limitations in throughput, speed and resolution, also does not easily scale to projects with large numbers of samples.
- NGS, on the other hand, can massively sequence tens or even hundreds of genes in parallel.
- Hence, it provides a more comprehensive picture of the cancer being studied.

1 sample 1 gene vs 1 sample many genes
Ion Workflow Overview

1. DNA / RNA
2. Sample Preparation
3. DNA Sequencing
4. Data Analysis

- Prepare Library
- Clonal Amplification
- Isolate Positive Ion Sphere™ Particles
- Load Chip and Sequence
- Data Analysis
Ion Torrent™

Founded 2007 by Jonathan Rothberg
  Pioneered next gen sequencing
  Founded 454, CuraGen, Raindance
Ion Torrent™ by Life Technologies with offices in Connecticut and California
Global manufacturing sites
First PostLight™ sequencing technology

For Research Use Only. Not for use in diagnostic procedures.
Ion Torrent™ PGM sequencer

Next Generation Sequencing machine from Ion Torrent:
Personal Genome Machine (PGM) and Ion Proton

Main features:

- Scalability
- Simplicity
- Speed
Ion Technology Core Principles

Scalability

- Choice of throughput by chip selection

The Chip is the Machine™
Ion Technology Core Principles

**Scalability**

- Choice of throughput by chip selection

**Simplicity**

- Natural nucleotides
- No lasers
- No optics
- No camera
- No fluorescence
- No enzyme cascade

**Speed**

- Rapid detection of sequence extension

The Chip is the Machine™
DNA → Ions → Sequence

- Nucleotides flow sequentially over Ion semiconductor chip
- One sensor per well per sequencing reaction
- Direct detection of natural DNA extension
- Millions of sequencing reactions per chip
- Fast cycle time, real time detection

Rothberg J.M. et al Nature doi:10.1038/nature10242
 Ion Technology Core Principles

Scalability

- Choice of throughput by chip

Template Preparation, Sequencing, and Analysis Times (in Hours)

- No enzyme cascade

Speed

- Rapid detection of sequence extension

The Chip is the Machine™
Ion AmpliSeq™ product portfolio for cancer studies

Cancer Panel
- 50 genes
- 2,079 mutations
- Ion 314™ Chip

Comprehensive Cancer Panel
- ~409 genes
- Ion 318™ Chip

Ion Community Panels

Ion Custom AmpliSeq™ Kits

Ready to Use Panels

Custom Panels

www.ampliseq.com
Ion AmpliSeq™ Technology: As Simple As PCR

Ultra-high multiplex PCR for targeted resequencing

- >2000 Active Users
- From over 80 Countries
- >10,000 Designs Submitted

www.ampliseq.com

Simple web-based design tool for targeted resequencing panels

Pipeline based on >10 years Custom TaqMan® Assays experience

Requires just 10ng DNA from clinical samples (FFPE DNA)

DNA
Mutation Detection
RNA
Gene Expression
Ion Ampliseq single-day workflow

- Ion Ampliseq Cancer Hotspot Panel v2 for example:

<table>
<thead>
<tr>
<th>Construct Library</th>
<th>Prepare Template</th>
<th>Run Sequence</th>
<th>Analyze Data</th>
<th>Annotate Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5 Hours</td>
<td>4 Hours</td>
<td>1.5 Hours</td>
<td>0.5 Hours</td>
<td>0.5 Hours</td>
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</table>

- Ion AmpliSeq Panels
- Ion GenTouch System
- Ion PGM Sequencer
- Torrent Server
- Ion Reporter Software
Ion AmpliSeq Ready To Use Panels

**Cancer Hotspot Panel v2**
- 50 genes
- >2,800 COSMIC Mutations
- 207 Amplicons

**RNA Cancer Panel**
- 50 genes
- Corresponds to genes in Ion AmpliSeq™ Cancer Hotspot v2

**Comprehensive Cancer Panel**
- 409 Genes
- 16,000 Amplicons

**RNA Custom Panel**
- Target any gene
- 300 genes in single tube
# Ion AmpliSeq™ Cancer Hotspot Panel v2

**50 genes, ~2,800 COSMIC mutations, one tube, one day**

The Ion AmpliSeq™ Cancer Panel targets 50 genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
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<tbody>
<tr>
<td>ABL1</td>
<td>EZH2</td>
<td>JAK3</td>
<td>PTEN</td>
</tr>
<tr>
<td>AKT1</td>
<td>FBXW7</td>
<td>IDH2</td>
<td>PTPN11</td>
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<tr>
<td>ALK</td>
<td>FGFR1</td>
<td>KDR</td>
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<td>KRAS</td>
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<td>MET</td>
<td>SMARCB1</td>
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<td>CDH1</td>
<td>GNA11</td>
<td>MLH1</td>
<td>SMO</td>
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<tr>
<td>CDKN2A</td>
<td>GNAS</td>
<td>MPL</td>
<td>SRC</td>
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<table>
<thead>
<tr>
<th>Specification</th>
<th>Observed performance (Ion 314™ Chip)</th>
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<tr>
<td>Coverage uniformity*</td>
<td>≥95%</td>
</tr>
<tr>
<td>On-target reads†</td>
<td>≥90%</td>
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<tr>
<td>Average depth of coverage</td>
<td>NA</td>
</tr>
<tr>
<td>SNP detection sensitivity</td>
<td>NA</td>
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</table>
Ion AmpliSeq Community Panels

**BRCA 1&2 Panel**
- 2 genes
- 167 amplicons

**Colon and Lung Cancer Panel**
- 22 genes
- 90 amplicons

**AML Panel**
- Coding regions of known mutations
- 21 genes

**CFTR Panel**
- All exons, intron-exon boundaries, and UTRs
- 1 gene

**Cardio Panel**
- All exons and UTRs
- 62 genes

**TP53 Panel**
- All exons and UTRs
- 1 gene
AmpliSeq™ Designer: www.ampliseq.com
Suitable with low DNA input with high accuracy

- 10 ng of DNA from 31 samples were tested in an Ampliseq Cancer Hotspot panel
- **100% concordance** between next-generation sequencing and conventional test platforms for all previously known point mutations
- Besides, new variants in 19 of the 31 (61%) patient samples were detected but not by traditional platforms, thus increasing the utility of mutation analysis

"The rationale for selection of the Ion PGM compared with other current NGS platforms such as Illumina Miseq (Illumina Inc., San Diego, CA) was mainly the differences in the DNA input. The DNA requirements for a 46-gene AmpliSeq panel on Ion PGM was markedly less (10 ng) compared with a 48-gene TruSeq panel on Illumina Miseq (250 ng). As most of our solid tumor specimens in our laboratory are FNA smears, FFPE cell blocks and core needle biopsies, we were unable to obtain a yield of 250 ng of DNA."
Deep coverage that potentially enables routine use

Samples used: fresh frozen cell line DNA controls, FFPE reference standard engineered cell lines, lung, colon, melanoma, rectal and ovarian adenocarcinoma FFPE samples from surgical resections, biopsies, fine needle aspirates and pleural fluid

">100 × coverage is needed to identify somatic mutation results with confidence."

They found that the limit of detection was found to be 5% for SNVs and 20% for indels

With the deep sequencing results, they were able to identify two additional actionable EGFR mutations (T790M) from a cohort of 45 lung samples

“We have found the Ion Torrent AmpliSeq Cancer Hotspot v2 assay and the PGM to be suitable for use in a routine clinical setting”
Flexibility to design and confine your target with a custom panel

- Discovery of JAK2 mutation in Myeloproliferative neoplasm (MPN)
- A two-tiered study, first evaluating the somatic mutation status of a mostly inclusive list of known cancer-related genes in a 20 (MPN) sample learning set
- A custom panel was made to evaluate the truly somatic variants in 189 MPN patients
- 141 genuine novel somatic mutations were found at this stage
- Demonstrated a mutation frequency of 3-8% for genes targeted by the panel
- They also found NRAS mutation frequency of 4.7%, which was associated with a worse outcome for primary myelofibrosis patients

“this NGS study presents new data that contribute to elucidating the very high genomic complexity in MPN disorders and identifies new variants in cancer-related genes that are potentially involved in the pathogenesis of the disease”
Extended genetic composition helps to differentiate primary and metastatic tumors

- Subject background: a multifocal colonic adenocarcinoma. Later two lesions were detected in the left and right lung.
- Bilateral metastatic bronchopulmonary adenocarcinoma of the lung were diagnosed by conventional morphology.
- Metastatic colonic carcinoma was favored after initial molecular genetic analysis.
- Panel results revealed that all 4 carcinomas carried completely different mutations, indicating 4 individual primary carcinomas of the colon and lung, which will lead to different set of clinical treatments.

"in a clinical setting, targeted-NGS has the capacity to differentiate between primary and metastatic carcinoma with a major impact on tumour classification, prognosis and therapy"
Insight from cancer panel on a single drug treatment

- **FBXW7** is a tumor suppressor gene that is mutated in various human tumors, which increases the level of total and activated mTOR.
- The mutational status of **FBXW7** in cancer patients in a phase I clinical trial was examined.
- 10 patients positive for **FBXW7** mutations were treated with mTOR inhibitors.
- A median time to treatment failure of 2.8 months (range, 1.3-6.8). 1 patient with liver cancer continues to have a prolonged stable disease for 6.8+ months.
- As **FBXW7** is usually occur with other simultaneous molecular aberrations in advanced tumors, the therapeutic efficacy of mTOR inhibitors single treatment is limited.

“The concomitance of other oncogene mutations provides challenges to targeting tumors harboring **FBXW7** abnormalities.”

http://dx.doi.org/10.1371%2Fjournal.pone.0089388
Ion Reporter™ Software

Simple push-button informatics enables any lab to do next-gen sequencing

- Direct integration with the sequencer
- Automated analysis from data generation to annotated variants
- Simple setup, analysis, interpretation
- Two solutions, local and hosted, optimized to fit your needs
Ion Reporter™ Software

For discovery or routine assays of variation Ion Reporter™ Software delivers the functionality you need

**Integration w/ TS**
- Select Ion Reporter workflows directly from within Torrent Suite

**Simple User Interface**
- No need for command-lines
- New UI coming with IR 4.0

**Annotation Content**
- Rich annotation content integrated (dbSNP, DrugBank, ClinVar, and more) or import custom annotations

**Variant Detection**
- Quickly identify somatic or germline SNP, InDels, and CNVs with one assay and one workflow

**Aneuploidy Workflow**
- Detect large chromosomal abnormalities from low-pass whole genome sequencing (0.01X)

**Filter Variants**
- Quickly filter variants to find those that are biologically relevant

**16S Metagenomics**
- Taxonomic classification of your 16S samples
- Interactive taxonomy visualization

**Broad’s IGV**
- One click access to data visualization (SNPs, InDels, CNVs, etc)
- Customized karyotype view

**Data Security**
- Role-based logins control access to data
- Audit logs monitor who does what / when
For Research Use Only; Not for use in diagnostic procedures.

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