The Expanded Illumina Sequencing Portfolio
New Sample Prep Solutions and Workflow

Lukas Smink
Agenda

- Sequencing evolution
- Illumina sequencing technology
- Workflow
- Introduce applications
Illumina Sequencing Publications:

- **Data Analysis:** 18%
- **ChIP-Seq:** 20%
- **Transcriptomics:** 20%
- **DNA Methylation:** 20%
- **Genomics:** 20%
- **Sequencing Technology:** 2%

**Bar Graph: # of Publications**

- Q4, 2007: 50
- Q1, 2008: 100
- Q2, 2008: 150
- Q3, 2008: 200
- Q4, 2008: 250
- Q1, 2009: 300
- Q2, 2009: 350
- Q3, 2009: 400
- Q4, 2009: 450
- Q1, 2010: 500

**Pie Chart: Publications Distribution**

- **Data Analysis:** 18%
- **ChIP-Seq:** 20%
- **Transcriptomics:** 20%
- **DNA Methylation:** 20%
- **Genomics:** 20%
- **Sequencing Technology:** 2%
Key Developments Over the Last 12 Months

Substantial enhancements on the Genome Analyzer
  • >4x increase in output
  • 2x increase in read length
  • >2x the tags per run
  • Further streamlined workflow

May
  GA\textsubscript{IIx}
  Pipeline 1.4
  SCS 2.4

October
  V4 reagents
  SCS 2.5
  RTA/Pipeline 1.5

December
  SCS 2.6/RTA 1.6

January
  HiSeq 2000
  GA\textsubscript{IIe}

April
  HiScanSQ

23G
  2x75 bp
  RT base calling

33G
  2x100 bp
  Higher Q-values
  >30% faster

50G
  >500M reads

200G
  >2B reads

Arrays + Seq
An Illumina Sequencer to Cover all Needs

NGS made accessible.

Two proven technologies. One powerful platform.

Most widely adopted NGS platform.

Redefining the trajectory of sequencing.

Genome Analyzer IIe
HiScanSQ
Genome Analyzer IIx
HiSeq 2000
Illumina Sequencing Technology Evolution

- From 20Gb up to 200 Gb of high quality data / run
- From 1 up to 25 Gb / Day
- Up to 2B reads per paired-end run
- 2 x 100 bp supported read length
- Raw Accuracy: > 99.0% (2x50bp reads)
- Short-Insert PE and Long-Insert Mate Pair sequencing
Output Increase Per Run

Output (Gb)

Genome Analyzer

Genome Analyzer
Illumina 2010 Sequencing Portfolio

Instrument Price vs. Output

- **HiScanSQ**
- **GA IIx**
- **HiSeq 2000**

- Targeted Resequencing
- Small genome de novo
- Small genome resequencing
- ChIP-Seq
- Gene Expression – low sample #

Instrument Price

40G, 95G, 200G

Instrument Output
In one sequencing run you can…

SIMULTANEOUSLY
Run multiple applications requiring different read lengths

Whole genome sequencing
Targeted resequencing
Gene expression
Methylation
De novo
Metagenomics
ChIP-seq
Whole transcriptome

One Sequencing Run

Sequence one cancer & one normal genome
At 30x coverage

Profile 200 gene expression samples
In less than two days

Analyze two human methylomes
In one week
HiSeq 2000
Redefining the trajectory of sequencing

HIGHEST OUTPUT
Initially capable of up to 200 Gb per run

FASTEST DATA RATE
~25 Gb/day
7-8 days for 2 x 100 bp

HIGHEST NUMBER OF READS
One billion single-end reads*
Two billion paired-end reads*

*Based on one billion clusters passing filter
HiSeq 2000 Dual Flow Cell Design

*Instrument scalability and experimental flexibility*

**TWO INDEPENDENT FLOW CELLS**

Simultaneously run applications that require different read lengths
Run in single or dual flow cell mode

**SIMPLE FLOW CELL LOADING**

Flow cells held by vacuum
No oil needed
LED switch ensures correct connection
Introducing HiScanSQ

Technologies merge. Opportunities emerge.

BLEND TWO PROVEN TECHNOLOGIES

HiSeq2000 optics for industry leading sequencing data quality

iScan leading array throughput and applications flexibility

EASILY EXPANDABLE

Scale up with automation options

Add array genotyping and GEx modules
Combo Drawer
Application integration and ease of flow cell loading

RUN ARRAYS OR SEQUENCING
Run in array or scanner mode
 Seamlessly switch between applications
Seamlessly Transition Between Technologies.

*Go where the biology takes you.*

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**THE ABILITY TO EXPLORE THE GENOME, TRANSCRIPTOME AND EPIGENOME DISCOVERY AND SCREENING ON THE SAME SAMPLE, ON THE SAME INSTRUMENT**

**The power of next-gen sequencing**

<table>
<thead>
<tr>
<th>Whole-Genome Analysis</th>
<th>Whole-Genome SNP Discovery</th>
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<tbody>
<tr>
<td>Copy Number Variation (CNV)</td>
<td>CNV Discovery</td>
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<tr>
<td>Targeted Genome Analysis</td>
<td>Targeted Resequencing</td>
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</tbody>
</table>
| Gene Regulation and Epigenetic Analysis | • Whole-Genome DNA Methylation Discovery and Analysis  
• Chromatin Immunoprecipitation (ChIP-Seq)  
• Small RNA Discovery and Analysis |
| Gene Expression | Transcriptome Discovery and Profiling |
| Cytogenetics | Digital Karyotyping |
Genome Analyzer\textsubscript{IIx} Roadmap

World’s most adopted and proven next-gen sequencer

**ON PATH TO 95GB**

Internal runs have generated >95Gb

**New RTA 1.8** - detects more clusters

**New Version 5 reagents** - 2 x 150 bp reads

<table>
<thead>
<tr>
<th>GA\textsubscript{IIx} (at 50G)</th>
<th>GA\textsubscript{IIx} (at 95G)</th>
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<tbody>
<tr>
<td>Gb per run</td>
<td>50</td>
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<tr>
<td>Gb per day</td>
<td>5</td>
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<tr>
<td>Cluster density in KClusters/mm\textsuperscript{2}**</td>
<td>490</td>
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<tr>
<td>Read length</td>
<td>2 x100</td>
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<td>Available surface area (mm\textsuperscript{2})*</td>
<td>510</td>
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</table>
RTA 1.8 Enables Higher Cluster Density
95G Example Data

*Improved accuracy at all read lengths*

### Q scores are accurate

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<th>Cycle</th>
<th>Predicted Q score</th>
<th>Empirical Q score</th>
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### Average accuracy (aligned PF data)

<table>
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<tr>
<th>Cycle</th>
<th>Average accuracy (%)</th>
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<tbody>
<tr>
<td>35</td>
<td>99.95%</td>
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<tr>
<td>50</td>
<td>99.93%</td>
</tr>
<tr>
<td>75</td>
<td>99.90%</td>
</tr>
<tr>
<td>100</td>
<td>99.79%</td>
</tr>
</tbody>
</table>

### % PF bases > Q30 (99.9% accuracy)

<table>
<thead>
<tr>
<th>Cycle</th>
<th>% PF bases &gt; Q30 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>96.5%</td>
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<tr>
<td>50</td>
<td>95.9%</td>
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<tr>
<td>75</td>
<td>94.6%</td>
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<tr>
<td>100</td>
<td>92.3%</td>
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</table>
Introducing the Genome Analyzer IIe

MARKET LEADING TECHNOLOGY, LOWER PRICE
Accessibility for labs of all sizes

PROVEN SYSTEM, ASSURED RESULTS
Leverage proven SBS chemistry and hardware
Same simple workflow, same reagents
Range of Illumina and 3rd party analysis solutions
Upgradeable to GA IIx

<table>
<thead>
<tr>
<th></th>
<th>GA IIe (at launch)</th>
<th>GA IIe (at 40G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gb per run</td>
<td>18-20</td>
<td>~38</td>
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<tr>
<td>Gb per day</td>
<td>2</td>
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<tr>
<td>Cluster density in KClusters/mm²</td>
<td>490</td>
<td>620</td>
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<tr>
<td>Read Length</td>
<td>2x100</td>
<td>2x150</td>
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<tr>
<td>Run time</td>
<td>9.5 days</td>
<td>~14 days</td>
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</table>
Simplest Sequencing Workflow

SIMPLIFIED SAMPLE PREP
- Parallel sample processing

cBot CLUSTER GENERATION
- Automated cluster generation

SEQUENCING
- Automated sequencing

DATA PROCESSING & ANALYSIS
- Simple, efficient data analysis
New Simplified Sample Prep Kits*

SIMPLIFIED, AUTOMATION-FRIENDLY WORKFLOW
Reduced hands-on time
Master-mixed reagents, 50% reduction in tubes
75% of clean-up and 90% of gel steps eliminated

PARALLEL SAMPLE PROCESSING
Run 96 samples at a time
Simplified indexing using adapters
Built-in quality control sequences
Lower cost of sample prep

*Expected availability Q3 2010
The flow cell design

Surface of flow cell coated with a lawn of oligo pairs

- 8 channels

- Contained environment
- No need for clean rooms
- Sequencing performed inside the flow cell
Cluster Generation

*Hybridize Fragment & Extend*

- Single molecules hybridize to the lawn of primers
- Molecules are extended by polymerases
- Newly synthesized covalently attached to the flow cell surface in a random pattern
- Double-stranded denaturation
- Original template is washed away
- Newly synthesized strand
Cluster Generation

*Bridge Amplification*

- Single-strand flips over to hybridize to adjacent primers to form a bridge
- Hybridized primer is extended by polymerases
- Bridge is denatured
Cluster Generation

*Bridge Amplification*

- Bridge amplification cycle repeated until multiple bridges are formed
- Bridges denaturation
- Reverse strands cleaved and washed away
Evolution of Automated Cluster Generation
Evolution of Automated Cluster Generation
cBot Performance Specifications

- Compatible with GA, HiSeq, HiScanSQ
- User installable
- Total hands-on time: <10 min
- Total run time: ~4 hrs
- Integrated barcode scanner
- Integrated touch-screen monitor
Sequencing by Synthesis Chemistry

Add 4 Fl-NTP’s + Polymerase

Incorporated Fl-NTP is imaged

Terminator and fluorescent dye are cleaved from the Fl-NTP

X 36 - 100
Broader range of applications

Optimized, streamlined and easy-to-use reagent solutions

Sample Prep

Whole genome
- Resequencing
- De-novo
- Targeted

Transcriptome
- RNA-Seq
- Small RNA
- miRNA
- Directional Seq

Regulation
- Methylation
- ChIP-Seq

Automated Cluster Generation

Sequencing
Broadest range of applications

*Optimized, streamlined and easy-to-use reagent solutions*

### Sample Prep

**Whole genome**
- Resequencing
- De-novo
- Targeted

**Transcriptome**
- RNA-Seq
- Small RNA
- miRNA
- Directional Seq

**Regulation**
- Methylation
- ChIP-Seq

### Automated Cluster Generation

### Sequencing
Driving down the cost of human genome sequencing

Reagents price per 30x human genome

 Genome Analyzer

 HiSeq 2000
Addressing Breadth of Customer Needs

<table>
<thead>
<tr>
<th>Discovery</th>
<th>Focused Research/Validation</th>
<th>Screening</th>
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<tbody>
<tr>
<td>&gt;10⁹</td>
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Whole Genome

- WG Resequencing
- WG Gene Expr.
- miRNA Discovery & Profiling
- Candidate Resequencing
- WG ChIP-Seq

Sequencing

- Gene Expr.
- WG GT
- WG CNV
- Biomarker Discovery
- HT Biomarker Validation
- Custom GT
- Focused Expr.
- FFPE

HiScan SQ

- Low/mid density GT/GEX
- Protein applications
- Biomarker screening
- Biomarker panels
Thank you!