Overview Agilent
Micro fluidics

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The Agilent 2100 Bioanalyzer

First commercially available Lab-on-a-Chip product

Introduced 1999

Analysis of biomolecules:
DNA, RNA, Proteins and cells

More than 7000 of instruments sold

WW

Industry-Standard for the analysis of RNA

Standard for sample QC in Next-Generation Sequencing workflows

March 2010: >15,000 citations!
Agilent 2100 Bioanalyzer

- Exchangeable cartridge for different assays
- 16 pin electrodes connected to HV-sources
- Chip holder with heater plate
- Optics for detection

2. Run Analysis
The Lab-on-a-Chip Approach

Increasing quality and speed of gel electrophoresis

Sample volumes 1 - 5 µl
10 -12 samples depending on Assay
Separation, staining, detection of samples
Results in 5-30 minutes available
No extra waste removal needed
Disposable Chip, no crosscontamination
Chip Architecture

- Chip accommodates sample wells, gel and conditioning/destaining wells, and a well for a standard (ladder)
- Sieving gel/dye matrix is forced into capillaries
- 16 pin electrodes apply voltage
- Capillary fluidics become “lanes”
Principle of Electrodriven Flow
Lab-on-a-Chip - General Features and Benefits

Miniaturization (Scale)
- small sample volumes
- reduced reagent usage
- reduced bench space

Miniaturization (Speed)
- fast analysis

Automation
- improved accuracy
- improved precision
- improved productivity

Smaller - Faster - Smarter
The Kits
2100 Expert Software
Version B.02.08

- Easy to use for Instrument control, Data Analysis, Data comparison and Reporting
- Patented RIN (RNA Integrity Number)
- Color coded Result Flagging
- Easy comparison context (multi samples from various files)
- Customizable result tables for printing and reports
- Optional security pack software for 21 CFR part 11 compliance requirements

NEW with B.02.08:
- High Sensitivity DNA Assay
- Plant RIN Application
- Windows 7 Compatibility

Setup and start

Analyze and compare

Print reports
Analyze Data (Peak based view)

- Tree view for navigation between samples and files
- Tabs for different data analysis functions
- Task bar with context sensitive icons for different actions
- Context menu bar
- Single gel lane for selected E-gram
- Customizable gel-like image (change order)
- Customizable result table (change order and add additional columns)
Data Format - Gel-Like Image c/w Agarose Gel

2100 bioanalyzer data
Gel-like image

2 % agarose gel stained with Ethidiumbromide

Marker

900, 1000 bp
473, 500 bp
300, 315 bp
100, 105 bp
25 bp
Current Assays (including anything required for analysis)

**DNA Assays:**
- Sizing
- Quantitation
- PCR products, digests, larger DNA fragments
- 12 samples in 30 min.

**RNA Assays:**
- Quantitation (Sizing in Small RNA)
- total RNA, mRNA
- purity & integrity determination
- 10 samples in 30 min.

**Protein Assays:**
- Sizing
- Quantitation
- cell lysates, column fractions, purified proteins, antibodies etc.
- 10 samples in 40 min.

**Cell Assays:**
- Analysis of 6 samples
- Two color detection
- Analysis of protein expression in cells

![Image of Agilent Technologies products]
RNA and Small RNA Assays
RNA QC in Routine Gene Expression Workflow

Cells / Culture

RNA isolation

Total RNA

RNA QC via Agilent 2100 bioanalyzer

RIN

Software feature RIN as independent and standardized measure of sample quality

RIN above threshold

Continue with downstream Experiment (Microarray, real-time PCR, etc.)

Start again with sample isolation
Experimental workflow

- **RNA extraction**
- **RNA degradation**
- **RNA QC and quantification**
- **Reverse transcription**
- **QPCR**
- **QPCR assay validation**

**Stratagene Absolutely RNA® MiniPrep kit**

- **Stratagene AffinityScript™**

**Agilent 2100 bioanalyzer**

- **RNA 6000 Nano kit**

- **DNA 1000 kit**

**Agilent Technologies**
Successful QPCR with the Agilent 2100 bioanalyzer

RNA quality - Effects of degraded RNA

Not knowing the extend of possible degradation might lead to false negative results or misinterpretation of the data if the amplicon falls into a degraded region.

Knowing RNA quality allows to accommodate the design and set expectations avoiding wrong interpretation of results.

Assay validation - Limitations of SYBR Green melt curves

Restricted resolution, which can make it difficult to determine specificity.

$T_m$ depends on dye/template ratio.

SYBR Green is a non-saturating dye with non-uniform distribution along the double-strand.

Melt curve provides no info on the size of the generated amplicon.
QPCR assay validation
– amplification plots, melt curve and bioanalyzer analysis

GAPDH 5’ assay: Expected size 118 bp

GAPDH 3’ assay: Expected size 126 bp
QPCR assay validation
– No-template controls (NTC)
Features of the RNA 6000 Assays

total RNA
determine integrity and quality of total RNA
determination of RNA concentration
identify ribosomal peaks
calculate the ratio of ribosomal peaks (18S/28S or 16S/23S)
RNA integrity number (RIN)

mRNA
determine integrity and quality of mRNA samples
Determination of mRNA concentration
calculate % ribosomal RNA in mRNA samples
Assessment of RNA Integrity
RNA 6000 Nano LabChip kit

Typical first QC step during cDNA or cRNA sample prep for QPCR

High quality total RNA (RIN 8.8)

Partially degraded total RNA (RIN 3.7)

2100 bioanalyzer: single lane gel-like image

2100 bioanalyzer: electropherogram
DNA Applications
Application Areas for the DNA Assays

PCR product purity

Multiplex PCR Applications

Gene expression analysis via RT-PCR (target validation)

GMO testing

Pathogen detection (homeland defense, hospitals, environmental)

Genotyping applications
  • Duplications/ deletions
  • Allele frequency
  • Bacterial sub-typing
  • Forensics

Cancer diagnostics
Next Generation Sequencing is an emerging technology.

Quality of sequences is one of the chief technical concerns about next generation sequencing platforms in a recent customer survey by Insight pharma reports.

Leading next generation sequencing vendors (Illumina, Roche, Life Technologies) recommend existing Bioanalyzer DNA assays in their workflows.

References

- Manuals of all Next Gen Sequencing Vendors: Illumina, Roche, Life Technologies
Bioanalyzer in Illumina/Solexa Workflow – Examples

ChIP-Seq Workflow

1. Single ChIP enriched DNA or control DNA (~10 ng)
2. Repair ends
3. Blunt ended fragments
4. Add Klenow exo- with dATP
5. 3’-dA overhang
6. Ligate adapter
7. Adapter-modified ends
8. Gel purification
9. Removal of unligated ADP and size selection
10. PCR
11. ChIP-Seq DNA library

Validate the Library

The amount of starting material is very low (10 ng), and after 18 cycles of PCR, the yield could still be too low to see on a regular gel, even though it is enough for cluster generation. Illumina recommends performing the following more sensitive quality control analysis on your sample library using an Agilent Technologies 2100 Bioanalyzer.

1. Load 1 µl of the resuspended construct and 1 µl of the negative control on an Agilent Technologies 2100 Bioanalyzer.
2. Check the size, purity, and concentration of the sample.

Alternative Methods

For users who do not have access to an Agilent Technologies 2100 Bioanalyzer or similar instrument, you may try using a sensitive dsDNA measurement assay such as the Quant-iT dsDNA HS Assay Kit, 100 assays 0.2-100 ng for use with the Qubit fluorometer (Invitrogen). Note that this will not allow you to check the size and purity of your sample. Do not use an OD260/280 ratio for concentration measurements, since this will not distinguish dsDNA from primers, and therefore cannot be used to validate the library.

Figure 2 Sample Preparation Workflow
Bioanalyzer in Roche/454 GS FLX Workflow

Validate ssDNA library

RNA kit:
- estimate the size distribution
- to determine if fragments <300 bp successfully removed
- quantitation for correct dilution

454 recommends the use of Agilent Bioanalyzer in their manuals
Bioanalyzer in ABI SoliD Small RNA Sequencing Workflow

Check small RNA content in total RNA samples

If small RNA content >0.5% use total RNA

If small RNA content <0.5% enrich small RNA first

verify the size and quality using an Agilent bioanalyzer or 6% native PAGE.

“…..we recommend evaluating the small RNA content of samples to determine whether to use total RNA or size-selected RNA in your reactions. This can be done using an Agilent bioanalyzer with the Small RNA Chip (#5067-1548).”
Sneak Preview – High Sensitivity DNA Kit

Simplified Illumina GAII Workflow

Illumina Sequencing

Starting Material: Genomic DNA

Fragment DNA

Ligate Adapters

Purify Ligation Products

Amplify & Quantify Library

Sequence Library

Agilent 2100 Bioanalyzer

Check Size Distribution

Agilent DNA 1000 kit

Quantify, Size and QC DNA libraries

Agilent DNA 1000 kit
Quantification, Sizing and QC of NGS Libraries

- High Quality DNA library
- Identification and quantification of primer dimers and PCR artifacts

Sizing → Quantification

Primer Dimers/Adaptors → PCR artifact
DNA library
SureSelect Overall Sequencing Sample Prep Workflow

- **Genomic DNA**
  - Shear Genomic DNA
  - DNA fragments with a base pair peak of 200
  - Repair ends

- **Agilent DNA 1000 kit**
  - Check size distribution
  - Analysis of sheared DNA using DNA 1000 assay
    - Distribution of sheared DNA with peak size of 200 nts
  - Agilent DNA 1000 kit
  - Quantify, size and QC DNA libraries

- **Genomic Locations**
  - Probe Design in eArray

- **SureSelect Oligo Capture Library**

- **Analysis of amplified prepped library**
  - Single peak in the size range of 200-300 nts
Sizing and quantification of a Illumina GAII library. The library was enriched with Agilent’s SureSelect Target Enrichment platform and amplified with varying PCR cycles.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>No. Cycles</th>
<th>Qubit BR ng/ul</th>
<th>Bioanalyzer ng/µl</th>
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<tbody>
<tr>
<td>4A</td>
<td>4</td>
<td>Too Low</td>
<td>0.090 0.015</td>
</tr>
<tr>
<td>4B</td>
<td>4</td>
<td>Too Low</td>
<td>0.083 0.007</td>
</tr>
<tr>
<td>6A</td>
<td>6</td>
<td>Too Low</td>
<td>0.304 0.017</td>
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<tr>
<td>6B</td>
<td>6</td>
<td>Too Low</td>
<td>0.301 0.010</td>
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<tr>
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<td>8</td>
<td>Too Low</td>
<td>1.13 0.07</td>
</tr>
<tr>
<td>8B</td>
<td>8</td>
<td>Too Low</td>
<td>1.34 0.07</td>
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<td>10</td>
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<td>4.49 0.16</td>
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<tr>
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<td>A12</td>
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<td>14.5 0.5</td>
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<tr>
<td>C12</td>
<td>12</td>
<td>12.5</td>
<td>13.9 0.6</td>
</tr>
</tbody>
</table>
**High Sensitivity DNA kit Applications – Reduction of Library Amplification Cycles**

Sizing and quantification of a Illumina GAII library. The library was enriched with Agilent’s SureSelect Target Enrichment platform and amplified with varying PCR cycles.

- Amplification cycles above a certain threshold can result in amplification artifacts.
- High Sensitivity DNA kit enables to reduce library amplification cycles.

- 16 cycles (dilution 1:10)
- 14 cycles (dilution 1:10)
- 12 cycles (dilution 1:5)
- Extra peak at more than 12 PCR cycles
- 10 cycles
- 8 cycles
- 6 cycles
- 4 cycles
Protein Kit Portfolio

**P80**
- Range: 5 - 80 kDa
- Sensitivity: Coomassie
- Samples: 10
- Samples
  - Antibodies (reduced)
  - Small Proteins

**P230**
- Range: 14 - 230 kDa
- Sensitivity: Coomassie
- Samples: 10
- Samples
  - Antibodies (all types)
  - Standard Proteins

**HSP-250**
- Range: 10 - 250 kDa
- Sensitivity: 1 pg/µl BSA
- Samples #: 10 per Chip
- Chips #: 10 per Kit
- Labeling Conc: 1 ng – 1 µg/µl

**Requirements:**
- Software: Expert B.02.06 (+)
- Instrument: all except G2938A

**Coomassie Range (5 ng/µl BSA)**

**Silver stain Range (200 pg/µL BSA)**
Protein Chip Layout

- Sample
- Chip priming
- Gel/dye mix
- Detection
- Destain solution
- Separation
- Ladder
- Destaining
Principles (I) -- P80/P230 Assay

SDS + dye → destaining → detection

low background good signal to noise ratio SDS conc. below CMC

Protein micelles

500 ng/ul HSA analyzed on chip

Ladder antibody-neg. antibody-red. antibody-mix. HSA 50 ng/ul HSA 100 ng/ul HSA 500 ng/ul CA 15-3 antigen CA 15-3 antigen Protein ladder
Principles (II) -- High Sensitivity Protein Assay

Sample protein

pH 8.5
0°C
30 min

NHS
chemically activated fluorescent dye

Stably labeled protein

SDS
Detected

Fractionation, Purification, Dilution, etc.

10 ng/ul BSA labeled
0.05 ng/ul analyzed on chip

Agilent Technologies
Protein LabChip Applications

Cell Lysates
- identification of over-expressed proteins
- comparison of different expression patterns

Column Fractions
- monitoring of protein isolation and purification process
- check fractions for impurities

Purified Proteins
- monitoring of impurities in protein preps
- integrity check for monoclonal or polyclonal antibodies (antibody QC)
Immunoprecipitation: IP/HSP-250

His-tagged protein in *E. coli* background

Anti-His + Protein A magnetic beads

- 1 mg/ml *E. coli* Lysate +/- Target Protein(s)
- Immunoprecipitation
  - Incubation with specific Antibody
  - Incubation with Protein A Beads
  - Wash with Buffer (3x)
  - Elution with 50% HSP-250 Sample Buffer
- Direct On-Chip Analysis
  - 2100 Bioanalyzer
  - HSP-250 Assay

0.01% of target (-) control

No peaks from Ab or capture protein!

High Sensitivity Protein 250 Kit (HSP-250)

**Highest sensitivity:**
Labeled proteins can be measured down to pg/µL concentrations loaded on Chip

**Extra wide linear dynamic range:**
4 orders of magnitude linear dynamic range assuring excellent determination of impurities

Direct comparison of samples run on SDS-PAGE with Silver staining and on 2100 Bioanalyzer.
Western Blot with PTEN-GST Fusion in *E. coli* Background

**Western Blot**

1: 1 % PTEN  
2: 0.1 % PTEN  
3: 0.01 % PTEN  
4: 0.001 % PTEN  
5: 0.0001 % PTEN  
6: *E. coli* only  
7: PTEN only

**IP/HSP250**

Advantages of the IP/HSP-250 Method:
- Sensitivity
- Specificity
- Time-to-result: 3 hours
- Cost: less primary & no secondary antibodies
High Sensitivity Protein 250 Applications
Combination with IEF
Combination with OFFGEL Fractionation

E. coli lysate (50 μg)

↓

Protein clean up and labeling

↓

OFFGEL Electrophoresis

Isoelectric point (pI)

Molecular weight

2100 Bioanalyzer

HisSens Assay

OFFGEL well pH

4.3  4.8  5.3  5.8  6.3  6.7  7.2  7.7  8.2  8.7

kDa

5  15  30  50  70  100
Wheat Analysis

Protein extraction
Alkylation with IAA
Acetone precipitated
Labeling at 10 ug/ul total protein (Bradford)
100 ug labeled protein fractionated pH3-10
Fractions undiluted analyzed with 250HSP-Assay

Wheat Type A

Wheat Type B
BioAnalyzer Support:
Bioanalyzer@agilent.com
Send .xad file not .pdf file

Q & A