NGS Sample QC with the Agilent 2100 Bioanalyzer and Agilent 2200 TapeStation

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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
- Industry-Standard for the analysis of RNA (RIN)
- Standard for sample QC in Next-Generation Sequencing workflows

Nov 2010: >15,000 citations!
2100 Bioanalyzer Instrument & the 2200 TapeStation Instrument

Agilent 2100 Bioanalyzer Instrument
• Industry standard
• Integrated in many protocols and extensively cited
• Widest range of applications

Agilent 2200 TapeStation Instrument
• Easy to use
• Scalable throughput at constant cost
• Core Applications
• Familiar software interface
Agilent 2200 TapeStation – Extension of Agilent’s Market Leading NGS QC System

Ease of use

2100 Bioanalyzer

Gold Standard for NGS sample and library QC

Automation & Throughput

2200 TapeStation

• 96-well compatible
• 1min/sample run time
• Fully scalable throughput
• Ready-to-use ScreenTape
• 1µl - 2µl of sample

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Agilent 2200 TapeStation System
Agilent 2200 TapeStation System

1. Place ScreenTape and some tips in the TapeStation

2. Place your prepared samples in the TapeStation and press ‘Start’ on the instrument controller software

3. View your analysed results in around 1 min per sample
Agilent ScreenTape Overview

- **Ready to use:** ScreenTape is prefilled with separation matrix and running buffer. The design also incorporates the electrodes. This means there is no need to prime!
- **No carry over:** 16 individual separation channels means that samples can be run with no cross contamination or carry over.
- **Variable throughput:** 2 to 96 samples can be run per experiment
- **Constant cost, regardless sample number:** unused lanes can be kept for up to two weeks
# Agilent ScreenTape Portfolio

<table>
<thead>
<tr>
<th>High Sensitivity D1K* ScreenTape &amp; Reagents</th>
<th>D1K ScreenTape &amp; Reagents</th>
<th>High Sensitivity R6K# ScreenTape &amp; Reagents</th>
<th>R6K# ScreenTape &amp; Reagents</th>
<th>P200 ScreenTape &amp; Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 to 1000bp</td>
<td>35 to 1000bp</td>
<td>50 to 6000nt</td>
<td>50 to 6000nt</td>
<td>10 to 200kDa</td>
</tr>
<tr>
<td>2µl of sample</td>
<td>1µl of sample</td>
<td>2µl of sample</td>
<td>1µl of sample</td>
<td>2µl of sample</td>
</tr>
</tbody>
</table>

- **Limit of Detection**
  - 5 pg/µL
  - 50 pg/µL
  - 100 pg/µL
  - 5 ng/µL
  - 5 ng/µL

*Bioanalyzer sensitivity on Nucleic Acid TapeStation

*R6K ScreenTape & Reagents

*TapeStation Software: RIN® for RNA QC

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Agilent 2200 TapeStation Instrument Overview

Scalable throughput
The 2200 TapeStation instrument distinguishes automatically between tube strips and 96-well plates

Zero carryover
The liquid handler uses a new filtered pipette tip to load each individual sample onto the ScreenTape eliminating contamination and carryover

Easy to use
The integrated barcode scanner automatically identifies which ScreenTape you have loaded facilitating transfer of assay specific parameters to the 2200 TapeStation software without any manual intervention. Both sample and ladder are loaded automatically.

Excellent reproducibility
The tape holder aligns the ScreenTape with the built-in electrodes and imaging system.
Agilent 2200 TapeStation Instruments

Nucleic Acid System (G2965AA)

DNA and RNA analysis only

TapeStation System (G2964AA)

DNA, RNA and Protein Analysis
2200 TapeStation Controller Software Overview

Select samples from:
- 96 well plate
- 8 way 0.2ml strips

Required loading tips are highlighted.

Lanes are highlighted, unused lanes not available to select.

Screen Tape type - a “deck” appears if more than 1 tape required (96 well only).

Enter sample descriptions, copy/paste from Excel or LIMS.

Notes are entered manually.

User name is taken from Windows account and is editable.

Notes are entered manually.

Invalid dates are indicated.

Notes are entered manually.
Agilent 2200 TapeStation Software
2100 Bioanalyzer Expert Software look and feel

Analysis Functions
Functions are navigated through a context sensitive ribbon.

Navigation pane
Navigate through experiments and individual lanes. Lanes can be selected in any order from the Plate Selector.

Result Presentation
Customisable views of gel image, electropherogram and data table.
## Agilent D1K ScreenTape Specifications

<table>
<thead>
<tr>
<th>Analytical Specification</th>
<th>High Sensitivity D1K ScreenTape</th>
<th>D1K ScreenTape</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sizing Range</strong></td>
<td>35 - 1000 bp</td>
<td>35 - 1000 bp</td>
</tr>
<tr>
<td><strong>Resolution</strong></td>
<td>35-300 bp: 15%</td>
<td>35-300 bp: 15%</td>
</tr>
<tr>
<td></td>
<td>300-1000 bp: 10%</td>
<td>300-1000 bp: 10%</td>
</tr>
<tr>
<td><strong>Sensitivity (fragments)</strong></td>
<td>5 pg/µL</td>
<td>0.05 ng/µL</td>
</tr>
<tr>
<td><strong>Sizing Precision</strong></td>
<td>5 % CV</td>
<td>5 % CV</td>
</tr>
<tr>
<td><strong>Sizing Accuracy</strong></td>
<td>± 10 %</td>
<td>± 10 %</td>
</tr>
<tr>
<td><strong>Quantitative Precision</strong></td>
<td>15% CV</td>
<td>10% CV</td>
</tr>
<tr>
<td><strong>Quantitative Accuracy</strong></td>
<td>± 20 %</td>
<td>± 20 %</td>
</tr>
<tr>
<td><strong>Qualitative Range / Linear concentration range (fragments)</strong></td>
<td>75 - 1000 pg/µL</td>
<td>0.1 - 50 ng/µL</td>
</tr>
<tr>
<td><strong>Carry Over</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Physical Specification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Analysis Time</strong></td>
<td>16 samples &lt; 20 minutes</td>
<td>16 samples &lt; 20 minutes</td>
</tr>
<tr>
<td></td>
<td>96 samples &lt;100 minutes</td>
<td>96 samples &lt;100 minutes</td>
</tr>
<tr>
<td><strong>Samples per consumable</strong></td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td><strong>Sample Volume Required</strong></td>
<td>2 µL</td>
<td>1 µL</td>
</tr>
<tr>
<td><strong>Shelf Life</strong></td>
<td>4 months</td>
<td>4 months</td>
</tr>
<tr>
<td><strong>Box/Kit size</strong></td>
<td>112 samples/box</td>
<td>112 samples/box</td>
</tr>
</tbody>
</table>

1. Resolution is defined as the separation of fragments at half peak height or better
2. Signal:noise ratio > 3 for a single peak
3. 2200 TapeStation Nucleic Acid System (G2965AA)
4. Determined using the D1K ladder as sample
Agilent D1K ScreenTape Sample Preparation

- Easy sample preparation
- No preparation of the “chip”
- Sample to result in approximately 1 minute per sample
High Sensitivity D1K ScreenTape

5pg/μl peak on 2200 Nucleic Acid TapeStation
System Features For Next Generation Sequencing

- **96 well plate capability**: Generate results from a 96 well plate, meeting higher throughput needs.

- **Regions easily defined**: Regions are applied either manually or automatically.

- **Region Table**: Data presented in a table, includes average size, concentration and molarity.

- **Highest Sensitivity**: Single band sensitivity is as low as 5pg/µL for High Sensitivity D1K

- **Report Generation**: Simple reporting tool generates reports to pdf and Word formats with the region table incorporated with every sample.
# Agilent R6K ScreenTape Specifications

<table>
<thead>
<tr>
<th>Analytical Specification</th>
<th>High Sensitivity R6K ScreenTape</th>
<th>R6K ScreenTape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality Score</td>
<td>RIN⁶</td>
<td>RIN⁶</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100 pg/µL</td>
<td>2 ng/µL</td>
</tr>
<tr>
<td>Quantitative Precision¹</td>
<td>20% CV</td>
<td>15% CV</td>
</tr>
<tr>
<td>Qualitative Range</td>
<td>100 – 10,000 pg/µL</td>
<td>2 - 500 ng/µL</td>
</tr>
</tbody>
</table>

## Physical Specification

<table>
<thead>
<tr>
<th>Analysis Time</th>
<th>16 samples &lt; 15 minutes 96 samples ~ 100 minutes</th>
<th>16 samples &lt; 20 minutes 96 samples ~ 100 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples per consumable</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Sample Volume Required</td>
<td>2 µL</td>
<td>1 µL</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>4 months</td>
<td>4 months</td>
</tr>
<tr>
<td>ScreenTape box size</td>
<td>112 samples/box</td>
<td>112 samples/box</td>
</tr>
</tbody>
</table>

¹ Within a ScreenTape
Agilent R6K ScreenTape Sample Preparation

**Std. Sensitivity:**
- Sample 1µl
- + 4µl sample buffer
- 72ºC – 3min
- Total volume 5µl
- Ice for 2min
- Place tubes in TapeStation

**High Sensitivity:**
- Sample 2µl
- + 1µl sample buffer
- 72ºC – 3min
- Total volume 3µl
- Ice for 2min
- Place tubes in TapeStation

- Easy sample preparation
- No preparation of the “chip”
- Sample to result in approximately 1 minute per sample
RNA electropherogram
Main features 28S, 18S can be manually assigned if misidentified and RIN\(^e\) recalculated

RIN\(^e\) optional colour coding
Values for RIN\(^e\)s represented below the gel image can be colour coded for rapid identification of RNA quality

R6K data table
RIN\(^e\) Colour coding if implemented exported to a report along with 28/18S ratios, concentration and description.

• Equivalent quality metric to Bioanalyzer RIN: Validated against over 400 samples, RIN\(^e\) is equivalent to the industry leading RNA quality metric RIN.

• 96 well Compatible: Accelerate RNA QC to unprecedented levels. Full 96 analysis delivered in around 100 minutes with no instrument set up time!
• **RIN\textsuperscript{e}** validated against RIN
• Over 400 whole RNA samples from 40 different sources have validated that RIN\textsuperscript{e} is equivalent to RIN
• Median error < ±0.4 RIN units

• **NOTE: RIN\textsuperscript{e} does not use the same algorithm as RIN**
• RIN\textsuperscript{e} requires correct identification of 18S and bottom marker
• Outlying differences between RIN and RIN\textsuperscript{e} accounted for by anomalous profiles (included in validation study)
## Next Generation Sequencing Workflow

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNA Purification</strong></td>
<td>Extracting &amp; purifying native or synthesised dsDNA from various sources</td>
</tr>
<tr>
<td><strong>Library Creation</strong></td>
<td>Fragmenting the DNA and attaching sequences that enable amplification &amp; downstream processing</td>
</tr>
<tr>
<td><strong>Target Enrichment</strong></td>
<td>Selecting &amp; capturing genomic regions of interest</td>
</tr>
<tr>
<td><strong>Amplification</strong></td>
<td>Increasing the amount of DNA available for sequencing by cluster generation (bridge amplification) or emulsion PCR</td>
</tr>
<tr>
<td><strong>Sequencing &amp; Data Analysis</strong></td>
<td>Determining &amp; mapping the sequence of original source material</td>
</tr>
</tbody>
</table>
Sample Preparation for Illumina Sequencing using SureSelect

- gDNA extraction & fragmentation
- End Repair & 3’ A addition
- Ligate Adaptors
- PCR Enrich Library

SureSelect

- Library Hybridization
- Magnetic Bead Capture
- Amplification

Illumina Cluster & Sequencing
Sample Preparation for Illumina Sequencing using SureSelect

- gDNA extraction & fragmentation
- End Repair & 3’ A addition
- Ligate Adaptors
- PCR Enrich
- Library Hybridization
- Magnetic Bead Capture
- Amplification
- Illumina Cluster & Sequencing
Post Shear and Clean-up Samples on 2100 Bioanalyzer & 2200 TapeStation
Electropherograms of Post Shear Samples

2100 Bioanalyzer DNA 1000 Assay

2200 TapeStation D1K ScreenTape

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Post Shear Quantification & Sizing - D1K

**Concentration Values**

<table>
<thead>
<tr>
<th>Bioanalyzer Mean</th>
<th>TapeStation Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quantification Median Deviation 8.8%

**Sizing Values**

<table>
<thead>
<tr>
<th>Bioanalyzer</th>
<th>TapeStation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sizing Median Deviation 4.5%

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Pre-Hybridisation Amplification Samples

2100 Bioanalyzer

2200 TapeStation
Pre-Hybridisation Amplification Electropherograms

2100 Bioanalyzer DNA 1000 Assay

2200 TapeStation D1K ScreenTape
Pre-hybridisation sizing and quantification – D1K

Concentration Values

- Bioanalyzer Mean
- TapeStation Mean

Sizing Values

- Bioanalyzer
- TapeStation

Quantification Median Deviation 10%

Sizing Median Deviation 2.5%
Post-Hybridisation Amplified Samples, 50Mb

2100 Bioanalyzer

2200 TapeStation
Post-Hybridisation Amplification Electropherograms

2100 Bioanalyzer High Sensitivity DNA Assay

2200 TapeStation High Sensitivity D1K ScreenTape

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Post Hybridisation Amplification sizing and quantification - High Sensitivity D1K

**Concentration Values**
- Bioanalyzer Mean
- TapeStation Mean

**Sizing Values**
- Bioanalyzer
- TapeStation

Quantification Median Deviation 11.3%
Sizing Median Deviation 1.6%

**Bioanalyzer Mean**

**TapeStation Mean**

**Concentration Values**

**Sizing Values**

**Bioanalyzer**

**TapeStation**

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Ion Torrent QC, 2200 TapeStation & 2100 Bioanalyzer

Post Shear

Post Gel Size Selection

Pre Capture

Post Capture

Bioanalyzer

TapeStation
Benefit from the scalability, speed and simplicity of the Agilent 2200 TapeStation system:

**Scalable throughput**
Individual channels facilitate variable throughput at a constant cost per sample.

**Fast results**
Obtain results in approximately one minute per sample, even for 96 samples.

**Easy to use**
Simplify your workflow with ready-to-use ScreenTape consumables.

**Excellent reproducibility**
Achieve user-independent results through minimal manual intervention and pre-packaged reagents.

**Low sample need**
Use no more than 2 μL of your precious samples per run – even for high sensitivity analysis.

**Highest flexibility**
Switch with ease between DNA, RNA and Protein ScreenTape for greater flexibility.

**Absolute robustness**
The system does not contain fragile or other error-prone features and procedures.

**Gold standard for RNA QC**
Rely on the market-leading RNA quality standard (RIN) by using the Agilent-developed RINe.

**Zero carryover**
In addition to individual loading tips for each sample, the ScreenTape analyses each sample in a separate lane.

**Protect sample performance**
Avoid sample evaporation by covering your 96-well plates with a foil seal.

**Small footprint**
Save valuable benchspace in your lab as there is no need for gel tanks, buffer bottles or documentation systems.
Comparison of systems for Sample QC in NGS

2100 Bioanalyzer
- High sensitivity
- Industry standard in xNA QC → proven performance
- Low entry price
- Best-in-class software
- Versatile kit portfolio
- Matches well with automated DNA purification systems from Sage or Caliper

2200 TapeStation
- 96-well plate compatible for higher throughput
- Shorter hands-on-time and reduced user interaction due to higher degree of automation
- Very fast time-to-result due to parallel sample processing
- Scalable throughput at constant cost per sample
More information available online…

For more information on pricing, specifications as well as access to application and technical notes, please visit the TapeStation pages on Agilent.com

www.agilent.com/genomics/TapeStation