DNA & RNA Measurement
-resources in our lab

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Resources in our lab

• NanoDrop ND-1000 Spectrophotometer
• Quant-iT™ PicoGreen® dsDNA Assay Kit
• Qubit® 3.0 Fluorometer
• 4200 TapeStation
NanoDrop ND-1000

- Absorbance of all molecules in the sample that absorb at the wavelength of interest.
- 260nm - nucleotides, RNA, ssDNA, and dsDNA.
- 280nm - protein, phenol or other contaminants.
- The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as “pure” for DNA; a ratio of ~2.0 is generally accepted as “pure” for RNA.
- This 260/230 ratio is used as a secondary measure of nucleic acid purity. The 260/230 values for “pure” nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorb at 230 nm.
- Range – 2ng/ul to 3700ng/ul (dsDNA), 3000ng/ul (RNA).
Quant-iT™ PicoGreen® dsDNA Assay Kit

- Ultra sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solution.
Qubit® 3.0 Fluorometer

- The Qubit® Fluorometer utilizes fluorescent dyes that are specific to the target of interest. These fluorescent dyes emit only when bound to the target molecules, even at low concentrations.
- Assays for dsDNA, oligos, total RNA, microRNA, and protein.
Qubit® 3.0 Fluorometer

What kits we have in lab currently:

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantitation range</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qubit dsDNA BR Assay Kit</td>
<td>2–1,000 ng</td>
<td>Quantitation of genomic and miniprep DNA samples</td>
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<tr>
<td>Qubit dsDNA HS Assay Kit</td>
<td>0.2–100 ng</td>
<td>Quantitation of PCR products, viral DNA, or samples for NGS</td>
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<tr>
<td>Qubit RNA BR Assay Kit</td>
<td>20–1,000 ng</td>
<td></td>
</tr>
<tr>
<td>Qubit RNA HS Assay Kit</td>
<td>5–100 ng</td>
<td>Quantitation of samples for microarray, RT-PCR, and northern blot procedures</td>
</tr>
</tbody>
</table>
Qubit® 3.0 Fluorometer

Workflow:

Figure 5. The Qubit® quantitation assay workflow. When paired with the Qubit® 3.0 Fluorometer, the Qubit® Quantitation Assay Kits provide a seamless workflow and easy data collection. Each Qubit® Assay Kit provides concentrated assay reagent, dilution buffer, and prediluted standards. Simply dilute the reagent using the buffer provided, add your sample (any volume between 1 µL and 20 µL is acceptable), and read the concentration on the Qubit® 3.0 Fluorometer. The assays are performed at room temperature, and the signal is stable for 3 hours.

4200 TapeStation

• The Agilent 4200 TapeStation system offers automated sample processing for quick and reliable sample quality control within any Next Generation Sequencing (NGS), microarray (aCGH) or quantitative PCR (qPCR) workflow.
4200 TapeStation

What kits we have in lab currently:

• Genomic DNA ScreenTape
  – For the analysis of genomic DNA from 200 to > 60,000 bp.

• RNA ScreenTape
  – For the analysis of total RNA from 25 to 500ng/µL.

• High Sensitivity RNA ScreenTape
  – For the analysis of total RNA from 500 to 10,000 pg/µL.
4200 TapeStation-RNA example

Total RNA analysis with the RNA ScreenTape assay

http://www.genomics.agilent.com/article.jsp?crumbAction=push&pageId=900109
4200 TapeStation-DNA example

4200 TapeStation

Heat denature is necessary to get real RIN!

TapeStation data from our lab

Bioanalyzer data from functional genomics
Take home message

• NanoDrop first for all samples to decide purity and range of concentration.
• Pick correct Qubit assay to run if <20 samples, otherwise Quant-iT™ Technology.
• Run correct TapeStation assay if samples are for NGS projects etc.
• Understand core facility procedure if send out samples.
Thank you!