DNA quantification using absorbance ($A_{260}$) and fluorescent methods (Qubit™ and Quant-iT™/PicoGreen™)

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- BMG LABTECH multi-mode plate readers are compatible with common DNA quantification methods
- Quantification of double-stranded DNA with hundreds of samples in parallel
- Detect down to 0.8 pg double-stranded DNA per well

Introduction

Double-stranded DNA not only holds all information to build a human but is also a popular molecule to study. Whether expression levels or infections that are measured by PCR, or next generation sequencing to generate sequence information and to detect mutations responsible for disease. DNA-based methods require the quantification of DNA: Here we present commonly used methods based on absorbance or fluorescence to quantify DNA at all concentrations, volumes and in any throughput.

Materials & Methods

- 384 well UV-transparent (Greiner #781801)
- black 384 well small volume (Greiner #784076)
- LVis plate (BMG LABTECH)
- CLARIOstar® Plus, FLUOstar Omega (BMG LABTECH)
- Qubit™ dsDNA BR (ThermoFisher #Q32850)
- Qubit™ dsDNA HS (ThermoFisher #Q32854)
- Quant-iT™ PicoGreen™ (ThermoFisher #P11469)
- Lambda DNA (ThermoFisher #SD0011)
- Roti® Stock 100x TE and H2O (Carl Roth)

Experimental procedure

Lambda DNA was diluted in 1x TE buffer to concentrations ranging between 1 pg/ml and 130 µg/ml. Qubit BR and Qubit HS assays were performed by diluting DNA 1:10 in the working solution. For 96 well plates, 20 µl DNA were mixed with 180 µl of dye solution, for 384 well plates, 2 µl of DNA were mixed with 18 µl of dye solution. In the Quant-iT/PicoGreen assay, DNA and working solution were mixed 1:1. A 96 plate well was filled with 100 µl of dye and 100 µl of DNA, a 384 well with 10 µl of each solution.

Absorbance-based measurements employ the DNA as it is and volumes of 100 µl, 50 µl and 2 µl were placed into a 96 well, 384 well or LVis plate, respectively. All measurements were done in triplicates and 12 replicates for blank measurements.

Results & Discussion

Absorbance-based dsDNA quantification
BMG LABTECH multi-mode microplate readers acquire absorbance spectra using an ultrafast UV/vis spectrometer. DNA spectra can be measured in volumes from 2-100 µl using different plate formats. The resulting absorbance spectra were automatically analyzed with the MARS analysis software. Next to DNA concentration, it calculates the $A_{260}/A_{280}$ ratio to determine protein contaminations (Fig 1).

![Fig. 1: DNA quantification by UV/vis absorbance in a 384 well plate. Spectra were acquired between 220-360 nm on a BMG LABTECH microplate reader.](image-url)
**Fig. 3:** Double-stranded DNA quantified with fluorescent assays and a CLARIOstar® Plus. A 384 well plate was used with a final volume of 20 µl.

**Fluorescence-based DNA quantification**

Fluorescent dyes for the quantification of DNA are used because, in contrast to absorption, they are more specific and detect lower DNA concentrations. Here, we compared three commonly used dyes: Qubit high sensitivity and low sensitivity kits and the Quant-iT PicoGreen dsDNA quantification kit. All kits were measured according to the instructions of use and with the same standards. Upon detection, the data were evaluated with the MARS analysis software. It calculated the concentration of unknowns, measured in parallel to the standard curve. The results and concentration ranges of the three assays are compared in Figure 3. It shows the Quant-iT and Qubit HS kits covering a large concentration range and being sensitive for low DNA concentrations. The Qubit BR kit covers high DNA concentrations.

**Comparison of plate formats and reader types**

Further, the limit of detection was calculated for all three assay kits and the absorbance method. The detection limit is the concentration at the blank value plus three times the standard deviation of 12 blanks (mean blanks + 3*SD blank). The results are shown in Table 1 and figure 4. The maximal measurable concentrations for fluorescent dyes are taken from the kits specifications.

**Fig. 4:** Comparison of dsDNA quantification methods analyzed on the FLUOstar Omega and CLARIOstar® Plus. Lowest detection limit was calculated based on measurements shown here. Upper detection limit was taken from kit specification (fluorescent dyes) and reader specification (absorbance).

**Table 1.** Comparison of microplate-based DNA quantification methods regarding sample volume and minimum DNA input (for CLARIOstar® Plus)

<table>
<thead>
<tr>
<th>Method</th>
<th>Plate</th>
<th>Sample volume</th>
<th>Minimum DNA input</th>
</tr>
</thead>
<tbody>
<tr>
<td>A260</td>
<td>LVis</td>
<td>2 µl</td>
<td>5000 pg</td>
</tr>
<tr>
<td>A260</td>
<td>96 well</td>
<td>100 µl</td>
<td>33000 pg</td>
</tr>
<tr>
<td>A260</td>
<td>384 well</td>
<td>50 µl</td>
<td>10500 pg</td>
</tr>
<tr>
<td>Qubit HS</td>
<td>96 well</td>
<td>1-20 µl</td>
<td>3.4 pg</td>
</tr>
<tr>
<td>Qubit HS</td>
<td>384 well sv</td>
<td>0.1-2 µl</td>
<td>0.8 pg</td>
</tr>
<tr>
<td>Qubit BR</td>
<td>96 well</td>
<td>1-20 µl</td>
<td>70 pg</td>
</tr>
<tr>
<td>Qubit BR</td>
<td>384 well sv</td>
<td>0.1-2 µl</td>
<td>13.6 pg</td>
</tr>
<tr>
<td>Quant-iT/PicoGreen</td>
<td>96 well</td>
<td>100 µl</td>
<td>3.5 pg</td>
</tr>
<tr>
<td>Quant-iT/PicoGreen</td>
<td>384 well sv</td>
<td>10 µl</td>
<td>2.3 pg</td>
</tr>
</tbody>
</table>

**Conclusion**

BMG LABTECH microplate readers reliably detect double-stranded DNA. The instruments cover quantification of any sample concentration and volume. The data shown above help to find the right instrument and quantification method for your needs.