Nucleic acid quantification on Lunatic using A260

Introduction

In this note, we describe how to use the classic A260 UV/Vis applications on the Lunatic systems. These are used for the quantification of dsDNA, ssDNA or RNA (ng/µL) based on the A260 peak height.

App selection

On the Lunatic, the classic A260 UV/Vis applications can be found under their respective sample type buttons, each time in the "Classic" column (Figure 1). On the Little Lunatic, these applications can be found on the applications screen (Figure 2). Aside from sample names, no additional user input is required. For proper use of the applications, always use the sample solution buffer as blank(s).

Results on screen

On Little Lunatic, A260 concentration values are shown in the overview tab and the slide thumbnail view respectively. For each sample, a more detailed analysis can be found in the Lunatic's details tab and below the graph on the Little Lunatic (Figures 3 and 4):

- **A260 concentration**: The baseline corrected UV/Vis spectrum (black curve on the Lunatic, white curve on the Little Lunatic) is used to calculate the A260 concentration in ng/µL. This is done by multiplying the A260 value with the nucleic acid-specific concentration factor (dsDNA = 50, ssDNA = 33, RNA = 40).
- **Background** (gray): sample turbidity profile. The background spectrum is subtracted from the measured spectrum, resulting in the content spectrum.

Also the classical spectrometry data are shown. The A260/A230 and A260/A280 ratios are used to determine the purity of the sample. On pure DNA samples, the A260/A280 ratio ideally is 1.8. For pure RNA samples this value is expected at 2.0. The A260/A280 ratio may indicate protein contamination when <1.8. The A260/A230 on the other hand should ideally be between 2.0 – 2.2 for pure nucleic

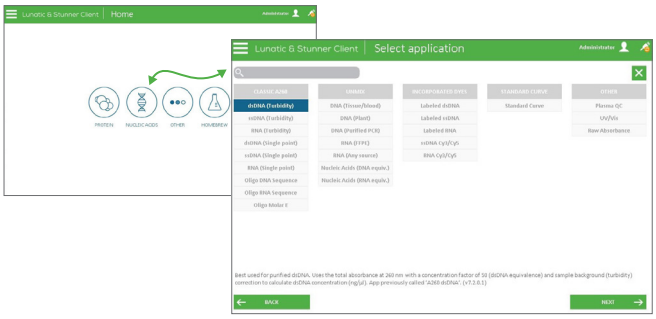

Figure 1: Illustration of the Lunatic interface. The image in the back shows the Sample Type screen whereas the image in the front displays the available applications for the selected Sample Type.

Figure 2: App buttons on the Little Lunatic app selection screen.

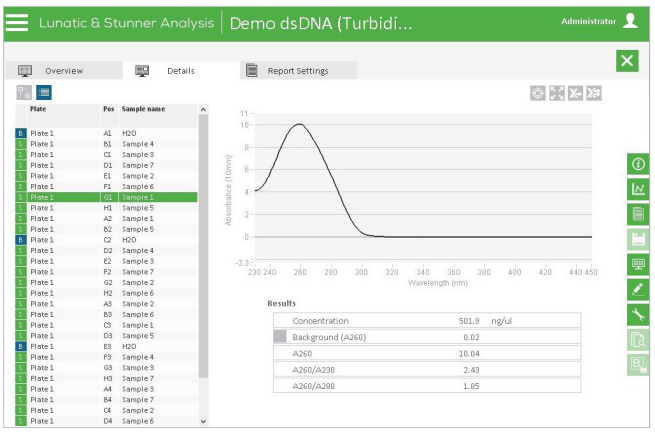

Figure 3: Illustration of the Results screen on the Lunatic (A260 dsDNA). In addition to the A260 and A260 concentration values, A260/A230 and A260/A280 ratios are shown.

acids, and lower values may indicate the presence of chemical contaminants such as salts or EDTA.

Report

A variety of report types are generated: an HTML, XML, TXT and a CSV file are created on both systems. In addition, the Lunatic also creates XLSX and PDF report files. On the Little Lunatic fixed report templates are used while the larger Lunatic system allows full flexible selection of the content to be reported.

Case study

In this case study a comparison was made between the NanoDrop 2000 and the Lunatic. Therefore, two gravimetric dilution series were measured on both instruments in octuplicate. The results of the DNA and RNA gravimetric dilution series can be found in Figures 5 and 6 respectively. In every case, the measured A260 concentrations are plotted against the predetermined target concentration.

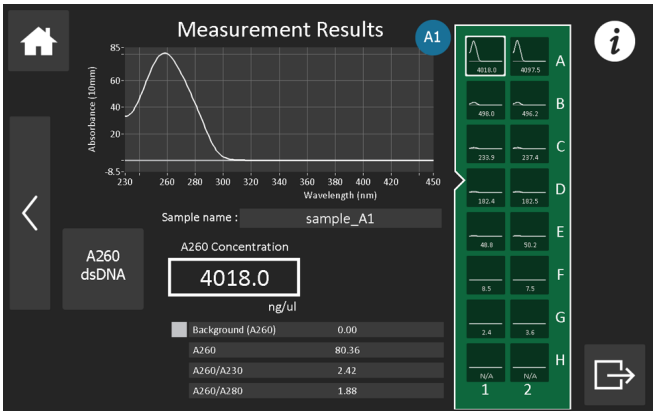

Figure 4: Illustration of the Results screen on the Little Lunatic (A260 RNA). In addition to the A260 and A260 concentration values, A260/A230 and A260/A280 ratios are shown.

The results are very comparable and close to the target value (gray dotted line). In terms of linearity, the R^2 values and equations are also shown, indicating that not only results but also linearity is very similar.

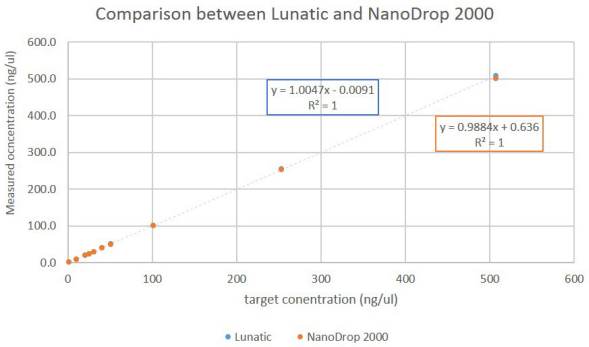

Figure 5: In this graph, octuplicate measurements of a Calf Thymus DNA gravimetric dilution series are plotted against the target values. Measurements on the Lunatic are shown in blue, NanoDrop 2000 measurements are shown in red. The gray dotted line represents the y=x line.

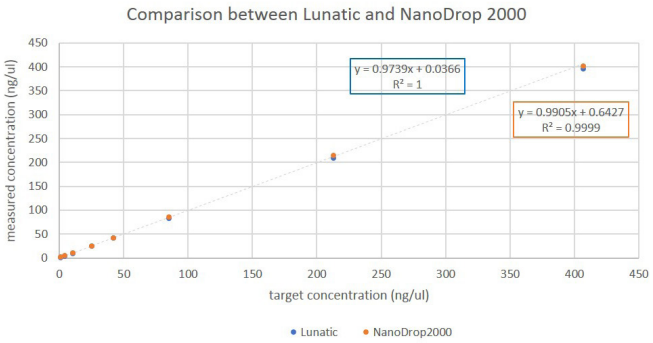

Figure 6: In this graph, octuplicate measurements of an RNA gravimetric dilution series are plotted against the target values. Measurements on the Lunatic are shown in blue, NanoDrop 2000 measurements are displayed in red. The gray dotted line represents the y=x line.

Unchained Labs
6870 Koll Center Parkway
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

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