Quantification of mammalian gDNA

Introduction

In this note, we describe how to use the DNA mammalian application on the Lunatic systems. This Unmix application is used to analyze the UV/Vis spectral shape of the sample to isolate the fraction of the molecule of interest from co-absorbing entities contributing to the total UV/Vis absorption spectrum. Accurate quantification of the molecule of interest is established using its isolated spectrum fraction. This applies to genomic DNA (gDNA) from different mammalian sample sources (human or animal blood, saliva, tissue or cell line) that are extracted using different extraction methods or commercial kits for DNA isolation.

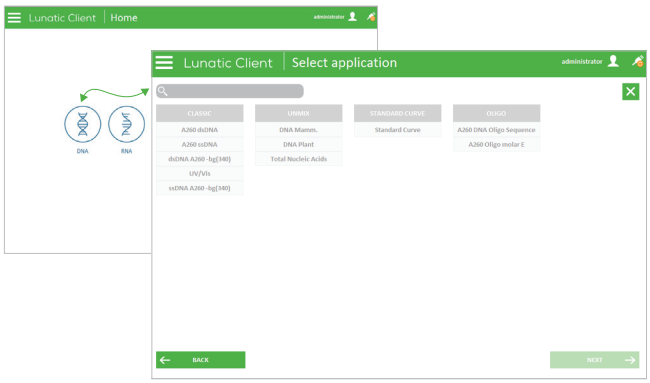

Figure 1: Illustration of the Lunatic "Select application" interface. The image in the back shows the Sample Type screen, whereas the image in the front displays the applications that are available for the selected Sample Type.

App selection

On Lunatic, the DNA mammalian application can be found in the "Unmix" column upon selection of "DNA" in the Sample Type screen (Figure 1). On Little Lunatic, this application can be found on the applications screen (Figure 2). Aside from sample names, no additional user input is required. For proper use of Unmix applications, always use pure water as blank(s).

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

Results on screen

The Unmix app will analyze the measured UV/Vis spectrum to detect the presence of specific component groups (Figure 3 and 4):

- **dsDNA** (green): molecule of interest. This profile is specific for gDNA (40-45 %GC). The concentration is calculated using the A260 peak value of this profile multiplied by the concentration factor of dsDNA (= 50).
- **Impurities** (blue): non-DNA molecules that also absorb in the UV/Vis-region. Additional impurity detailing is reported as concentration of RNA (ng/uL), thiocyanate salts (mM), buffer components (OD230) and phenol (mM).

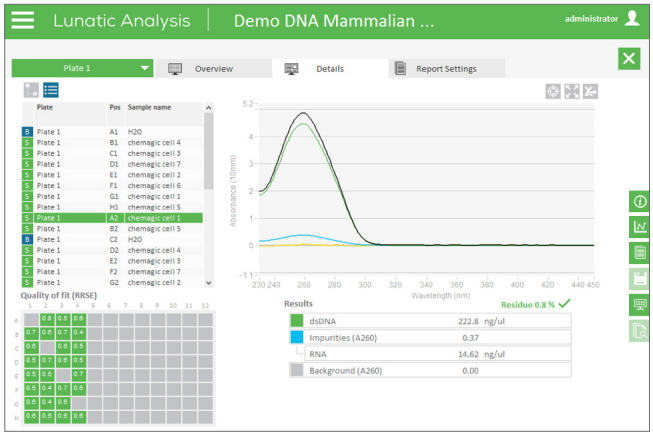

Figure 3: Illustration of the Results screen on Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values. Additional impurity and/or background detailing will be reported if possible.

- **Background** (gray): sample turbidity profile. Additional background detailing is reported as concentration of bead carry-over and hemoglobin/heme (absorbance max at 405 nm). The background spectrum is subtracted from the measured spectrum, resulting in the content spectrum (black curve on Lunatic, white curve on Little Lunatic).

The Residue or 'Quality of fit' value (RRSE) is the % of the measured spectrum which could not be annotated, representing the quality of fitting. This parameter is displayed as a yellow curve as well as a percentage value below the graph. A warning sign (red cross) will appear for samples with a residue value above 2.5% due to (1) too high turbidity of the sample, (2) presence of an unknown chemical, (3) low-concentrated samples. When this warning sign appears or when samples have an A260 below 0.5 OD, the Unmix app isn't able to show a DNA specific profile but will quantify all nucleic acids collectively shown as a purple 'total nucleic acids' spectrum. The nucleic acid concentration is calculated using the A260 peak value of this profile multiplied by the concentration factor of dsDNA (= 50).

Report

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

Case study

In this case study, DNA from different sample sources and extracted using different chemistries is analyzed using the DNA mammalian application. The Quant-It PicoGreen (Thermo Fisher Scientific) assay was used as reference method, as this fluorescence-based assay specifically measures the dsDNA content.

The first sample set contains DNA extracted from blood samples using Qiagen's QIAsymphony DNA extraction chemistry. When analyzing these samples, a high absorption peak around 230 nm can be observed due to the presence of buffer salts (Figure 6). The

Figure 4: Illustration of the Results screen on the Little Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values. Additional impurity and/or background detailing will be reported if possible.

second sample set was obtained extracting DNA from cultured cells using the chemagen chemagic DNA cell kit by Perkin Elmer. As cultured cell samples typically contain high RNA amounts (Figure 7), the A260 concentration values will be an overestimation of the actual DNA content present in the sample.

In both examples, Unmix app profiling results in more accurate DNA concentration determination as the measured spectrum is corrected for possible UV/VIS-absorbing impurities. Indeed, Unmix app DNA values show a closer match with PicoGreen data (Figure 5 and Figure 7).

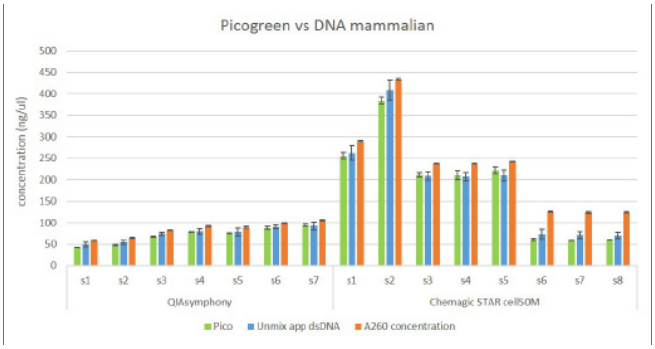

Figure 5: Comparison of DNA mammalian dsDNA values with classic A260 concentrations and PicoGreen assay results. Shown are the average values of 3 replicate measurements, error bars denote the standard deviation. Whereas the A260 concentration tends to overestimate the PicoGreen concentration, DNA mammalian dsDNA values more closely approximate the PicoGreen values.

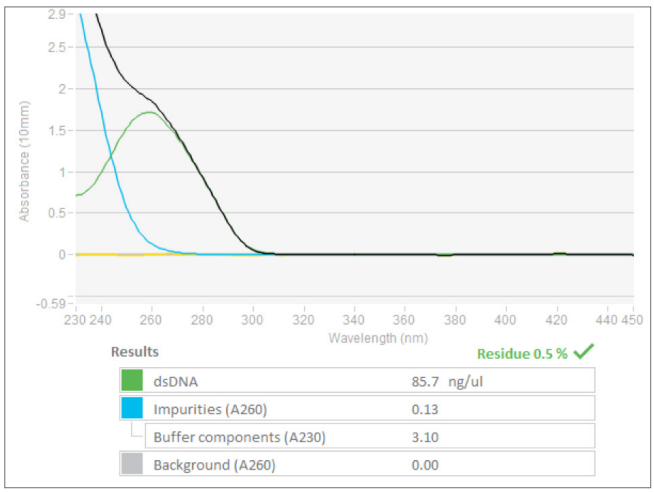

Figure 6: Illustration of a sample extracted from blood using the QIAsymphony chemistry. The Unmix app detects absorption of residual buffer salts at A230.

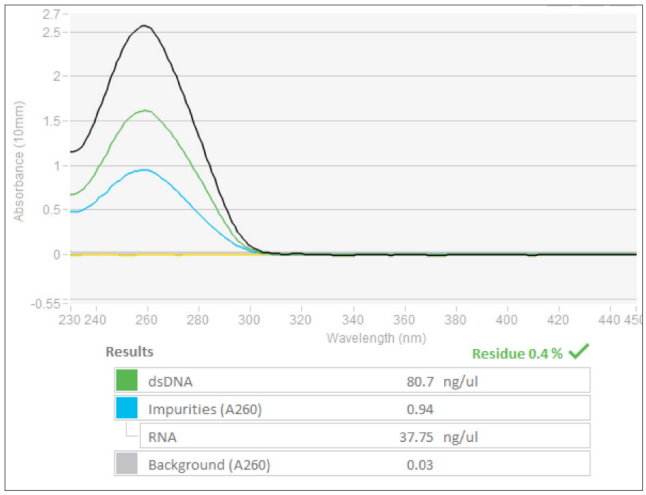

Figure 7: Illustration of a sample extracted from cell pellets using the chemagen chemistry. The Unmix app is able to discriminate residual RNA.

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