Quantification of Cy3/Cy5 labeled RNA and ssDNA

Introduction

In this note, we describe how to use the RNA Cy3/Cy5 and ssDNA Cy3/Cy5 applications on the Lunatic systems. These Unmix applications are 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. These Cy3/Cy5 Unmix apps isolate the RNA or ssDNA profile as well as specific Cy3 and Cy5 profiles from the measured UV/Vis spectrum, thereby distinguishing them from other absorbing sample contaminants. As such, they determine both the concentration of the nucleic acids as well as the amount of dye present, and calculate the degree of labeling (DoL).

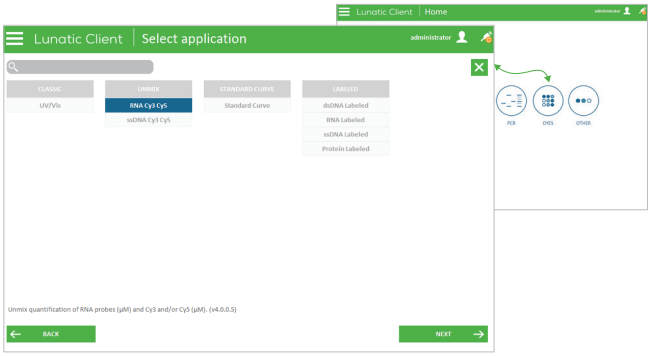

Figure 1: Illustration of the Lunatic "Select application" interface. The image in the back shows the Sample Type screen, whereas the image in the front displays the applications that are available for the selected Sample Type.

App selection

On Lunatic, the Cy3/Cy5 applications can be found under the "Dyes" sample type button, in the "Unmix" column (Figure 1). On the Little Lunatic, this application can be found on the applications screen (Figure 2). For proper use of the Unmix applications, always use pure water as blank(s).

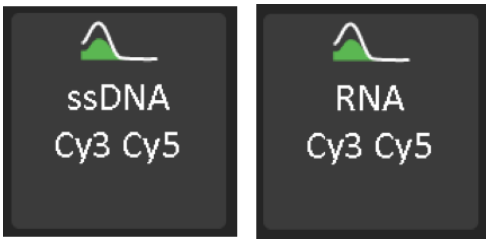

Figure 2: App buttons on the Little Lunatic app selection screen.

Results on screen

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

- **ssDNA or RNA (green)**: molecule of interest (ng/µL and pmol/µL)
- **Cy3 (green)**: Cy3 label present in sample
- **Cy5 (red)**: Cy5 label present in sample
- **Impurities (blue)**: non-DNA molecules that also absorb in the UV/Vis-region. These are proteins, guanidine-thiocyanate, azide, EDTA and citrate for both applications. The RNA application also includes phenol as additional impurity.

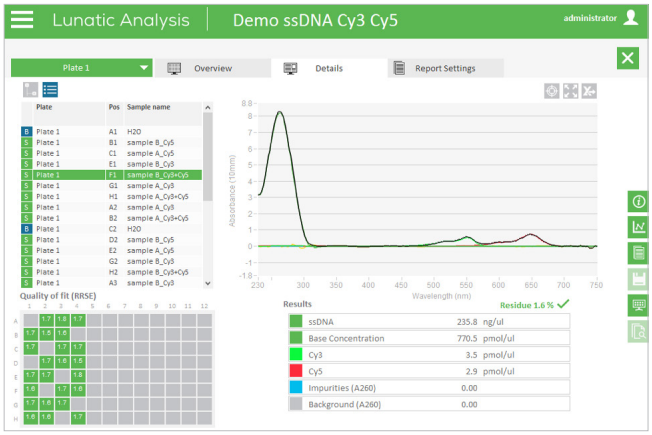

Figure 3: Illustration of the Results screen on the Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values.

- **Background (gray)**: profile combining sample turbidity, bead carry-over and hemoglobin/ heme absorbance. The background spectrum is subtracted from the raw 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 RNA or ssDNA 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 RNA (=40) or ssDNA (= 33) in their respective apps.

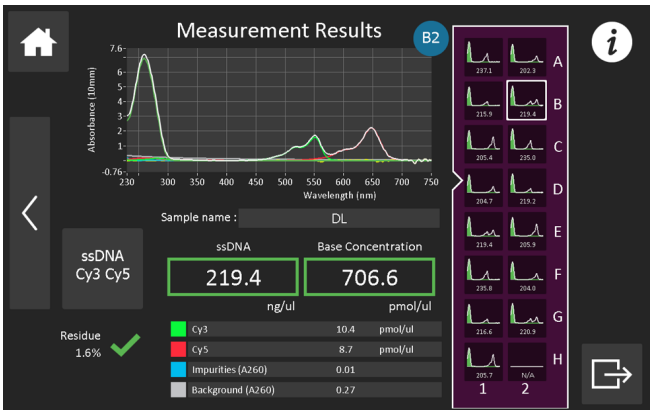

Figure 4: Illustration of the Results screen on the Little Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values.

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.

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