Quantification of purified PCR samples

Introduction

In this note, we describe how to use the Purified PCR 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 Unmix app is specifically designed to report an accurate amplicon concentration. This applies to PCR samples purified using different methods or commercial kits.

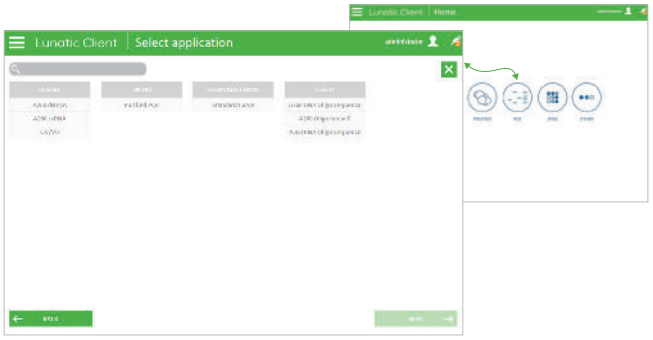

Figure 1: Illustration of the Lunatic "Select application" interface. The image in the back shows the Sample Type screen whereas the image in the front displays the available applications for the selected Sample Type.

App selection

On Lunatic, the Purified PCR application can be found in the "Unmix" column upon selection of "PCR" in the Sample Type screen (Figure 1). On Little Lunatic, the application can be found in 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 (Figures 3 and 4):

- **Amplicon** (green): molecule of interest. This profile is specific for amplified DNA. 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. These are dNTP's, guanidine-thiocyanate, EDTA, leachables and betaine.

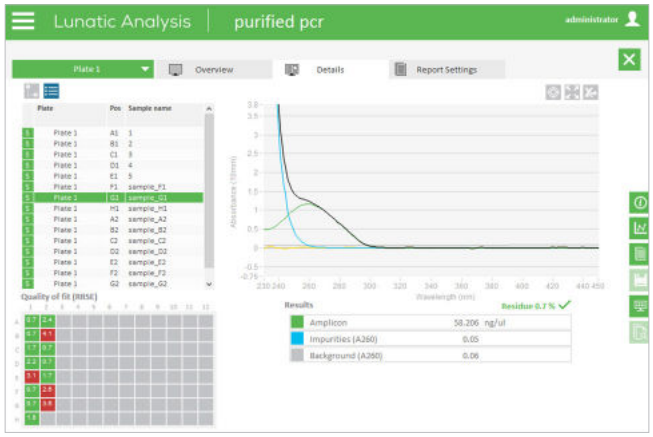

Figure 3: Illustration of the Results screen on the Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values.

- **Background** (gray): sample turbidity profile. Additional background detailing is reported as concentration of bead carry-over. 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 Little Lunatic, fixed report templates are used while the larger system allows full flexible selection of the content to be reported.

Case study

Figure 4 shows comparative data for a subset of samples (4 samples/ extraction kit) used for application development. Purified PCR app data was compared to a commercial available fluorescence kit and A260. Highest values are observed for A260 (gray). Data from Little Lunatic's Unmix app (blue) is comparable to quantification by fluorescence (green).

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.

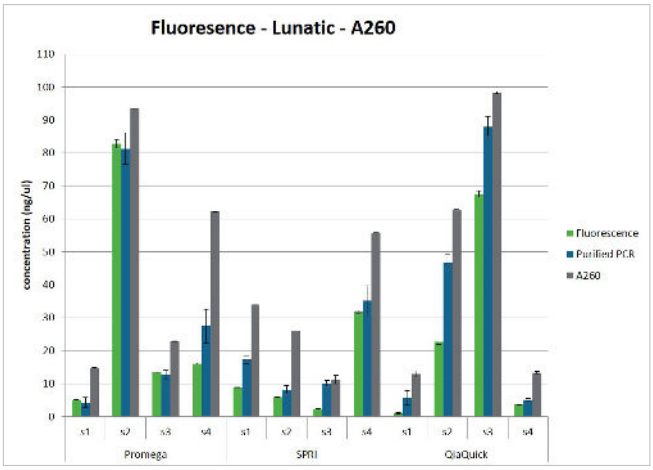

Figure 5: Comparison of Lunatic's Purified PCR results to fluorescence signal and A260 values.

The fluorescence method underestimates fragment lengths of less than 1000 bp if calibrated with a standard of different fragment length such as phage lambda DNA (Choppee Bortz PD, Wamhoff BR (2011), PLoS ONE 6(10): e26015. doi:10.1371/journal.pone.0026015). As many of our PCR amplicons were below 1000 bp this is a plausible explanation for differences between fluorescence and the Purified PCR app. This Unmix app is not sensitive for fragment length of PCR products.

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