

DropSense16 App – Purified PCR

Quantification of purified PCR samples using cDrop on DropSense16



Fig 1. App button on the DropSense16 app selection screen

PCR purification kit	Brand	Type
QIAquick PCR purification kit	QIAGEN	Column
Wizard PCR preps DNA purification system	Promega	Column
Agencourt SPRI beads	Beckman Coulter	Beads

Table 1. Overview of used extraction kits

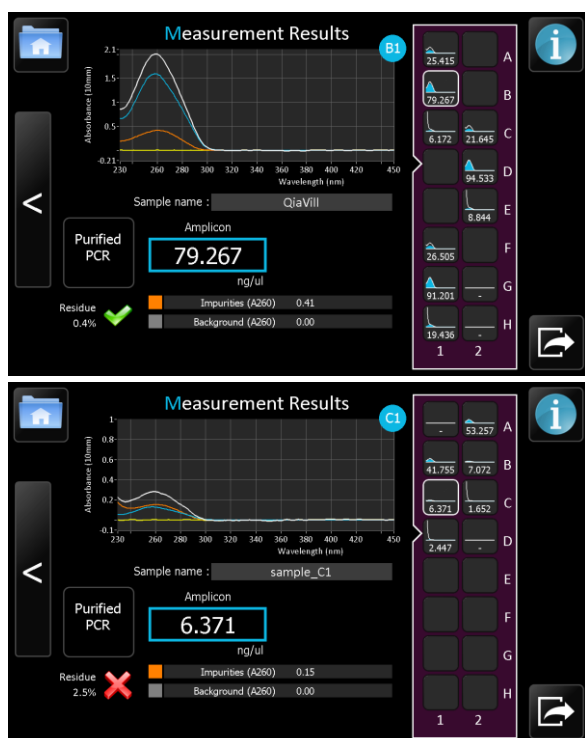


Fig 2. Illustration of the results screen on DropSense16.

Introduction

An app for specific quantification of DNA amplicon in purified PCR samples is available on the DropSense16. Trinean's proprietary cDrop software is used to isolate the profile of the desired molecule (here DNA) from the measured UV/VIS spectrum, thereby distinguishing it from absorbing sample contaminants. The specific DNA profile is then used to determine the DNA concentration. In this note we describe how to use this application and how to interpret its results.

App description

This cDrop app is specifically designed to quantify amplified DNA in purified PCR samples. This app is tested on PCR samples, kindly provided by Geert van Loo and Tom Vanden Berghe, VIB Ghent. Samples were purified with 2 column-based kits and 1 bead-based kit (see table 1). For proper use of this application, always use pure water as blank(s) on DropSense16 slides.

App Profiles

The cDrop algorithm in this app will analyse the measured UV/VIS spectrum (white curve) to extract specific profiles (see figure 2):

- **DNA (blue)** = molecule of interest
- **Impurities (orange)** = molecules except DNA with absorbance in UV/VIS. These are: dNTP's, guanidine-thiocyanate, EDTA, leachables and betaine.
- **Background (grey)** = profile combining intrinsic sample turbidity and scattering from bead contamination. Background is subtracted from the measured spectrum prior to cDrop analysis.
- **Residue (Yellow)** = unidentified part of the measured spectrum, also shown as % of the measured spectrum. This is a cDrop quality control parameter.

In certain cases, amplicon quantification by this app is less reliable when: **(1)** amplicon concentrations are below 10 ng/μl, as the standard deviation of this application is 3 ng/μl. or **(2)** when the cDrop residue is above 2,5% due to an unknown component. This is shown on the screen by a red cross next to the residue percentage.

Position	Sample name	Amplicon (ng/ul)	Nucleic Acids (ng/ul)	Impurities (A260)	Background (A260)
A1	SPRIH	25.415	56.4	0.62	0.00
B1	QiaVIII	79.267	99.6	0.41	0.00
C1	Prom11	6.172	19.2	0.32	0.30
F1	SPRIH	26.505	57.1	0.61	0.00
G1	QiaVIII	91.201	99.6	0.17	0.00
H1	Prom11	19.436	21.3	0.10	0.52
C2	SPRIH	21.645	55.2	0.67	0.00
D2	QiaVIII	94.533	99.5	0.11	0.00
E2	Prom11	8.844	18.1	0.24	0.39
G2	0	-	-	-	0.00
H2	BL	-	-	-	0.00

Residue (%)	A260	A260/A280
1.0	1.13	1.81
0.4	2.00	1.87
0.7	0.45	2.03
1.3	1.14	1.81
0.4	2.00	1.86
0.8	0.49	1.82
1.3	1.11	1.80
0.6	1.99	1.87
0.6	0.42	2.05
-	0.00	-1.08
-	-0.00	-1.08

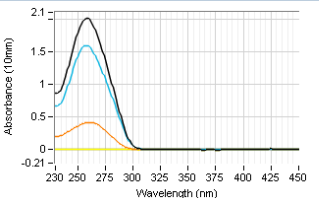


Fig 3. Example of HTML report

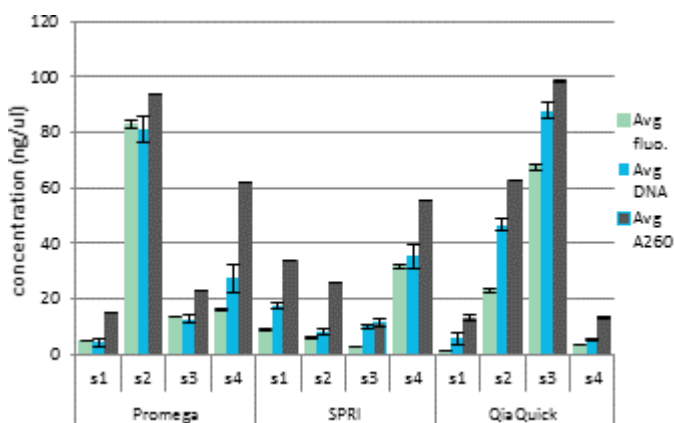


Fig 4. DropSense16 data compared to fluorescence signal and A260

App Report

Four report types are generated: an HTML file, XML, txt and a CSV file (for import in MS Excel). All reports include a table containing cDrop results as well as classic spectrometry data like A260, 260/230 and 260/280 (Note that these values are calculated after subtraction of the background profile).

In addition, for each separate measurement, the HTML report contains graphs with cDrop curves. Headings of the table columns are underlined with the colours matching the profile in the graphs (see figure 3). These reports are easily exported from the DropSense16 to a USB key, network folder or a smart device (using the QR code).

An additional DropSense16 specific measurement file (.bin) is also exported.

Results

Figure 4 shows comparative data for a subset of samples (4 samples/ kit) used for application development. DropSense16 data was compared to a commercial available fluorescence kit and A260. Highest values are observed for A260 (yellow). DropSense16 data (blue) is comparable to quantification by fluorescence (green).

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 DropSense16 Purified PCR app. The DropSense16 app is not sensitive for fragment length of PCR products.



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