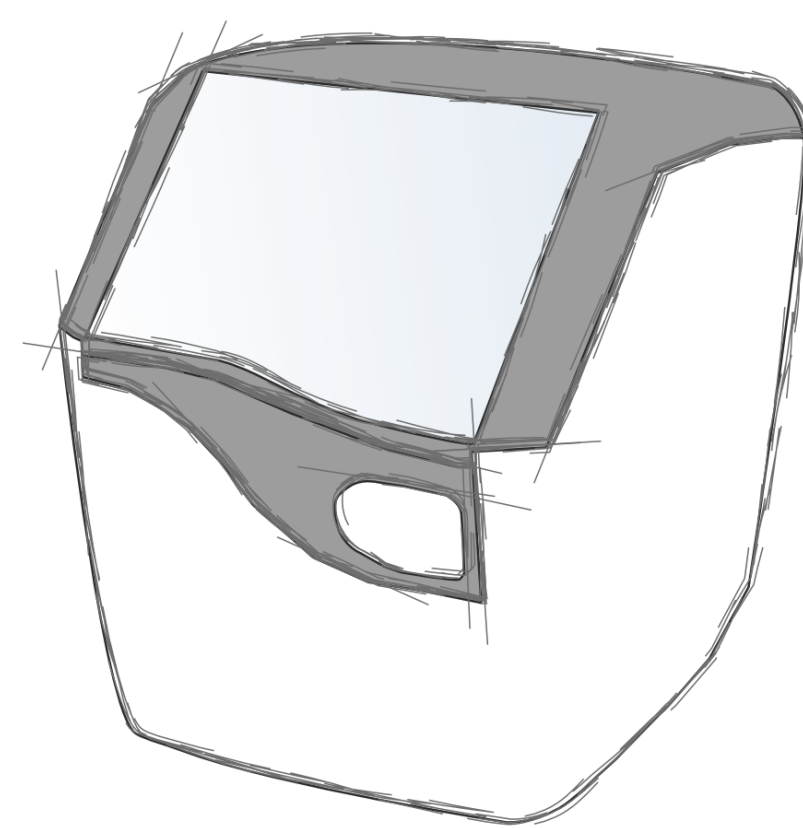


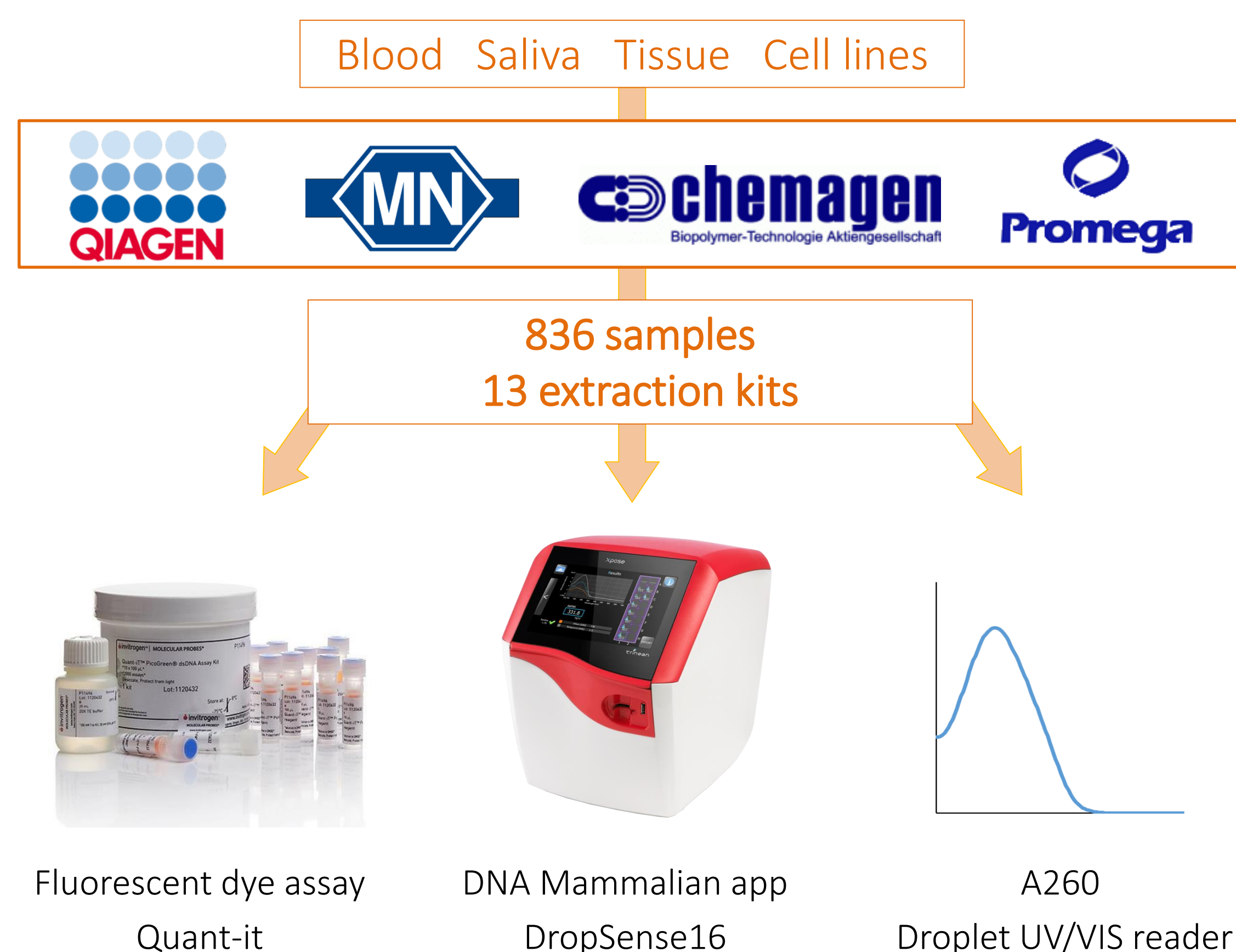


Introduction

Genetic tests become ever more powerful, yet also more complex and costly, which is why it is prudent to include quality control steps throughout the preparation process to ensure the quality of the downstream data obtained. To make an informed go/no go decision and avoid analytical failures, a good assessment of the yield and purity of DNA/RNA isolations is essential, including the detection of carry-over constituents that may interfere with downstream tests. Here, we validate a novel approach for DNA quality assessment using a large DNA sample set derived from a variety of human tissues combined with a wide scale of extraction methods. This new QC tool employs the micro-volume spectroscopy on the DropSense16 'Touch & Go' reader by Trinean combined with its cDrop software to specifically quantify the isolated DNA as well as identify each contaminating constituent in the sample contributing to the measured UV/VIS spectra.

Experimental setup

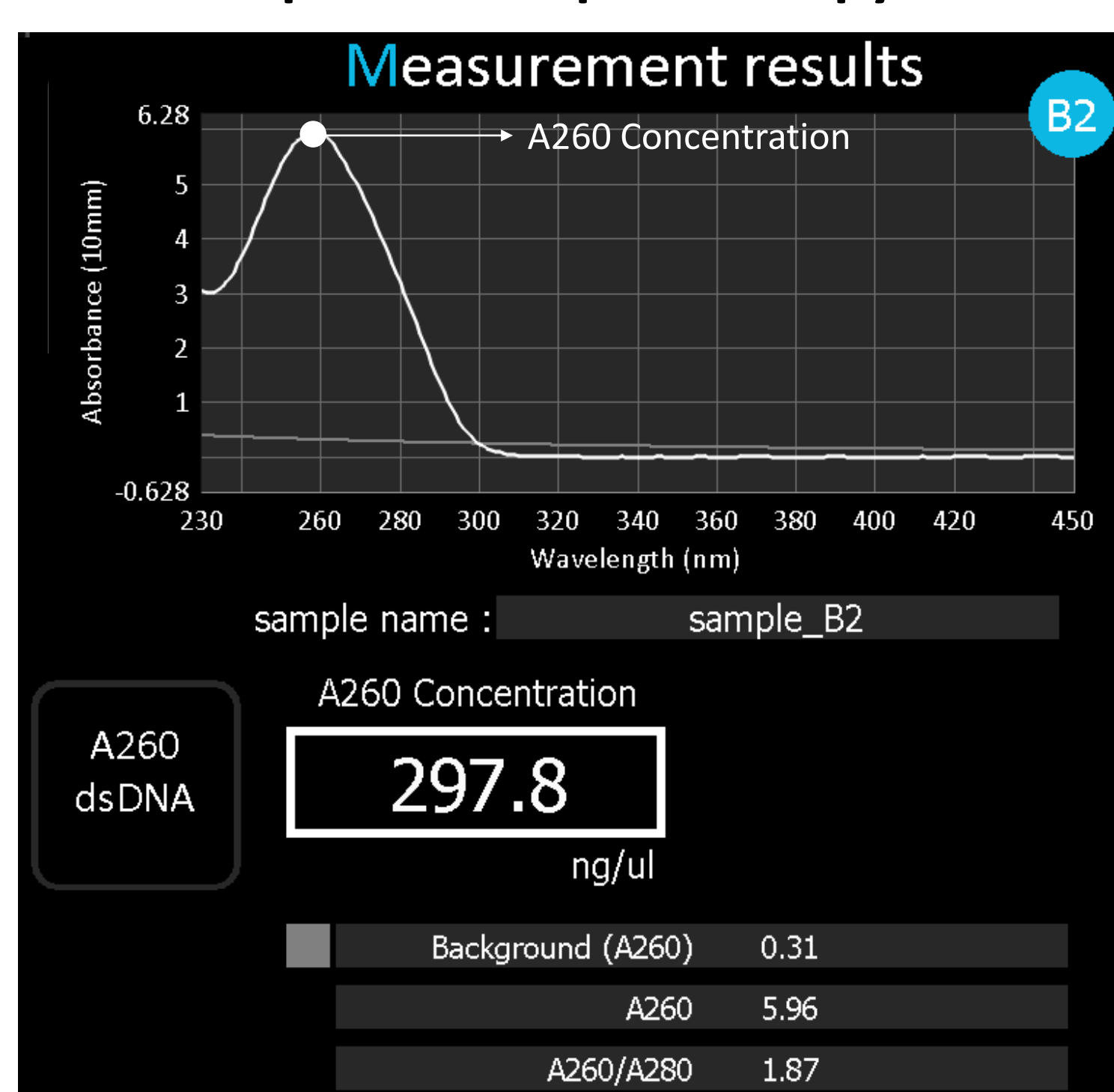
DNA samples were provided by cooperating labs employing Trinean platforms, amounting to a total of 836 samples from different mammalian sources (blood, tissue, saliva, cell lines) using 13 different extraction kits, ranging from bead-based to column-based and salting-out extractions. DNA concentration of all samples was determined by 2 direct quantification methods, droplet A260 spectrophotometry and Trinean's 'DNA mammalian' app on the DropSense16 reader, next to one fluorescence-based assay, Quant-It PicoGreen (Thermo Fisher Scientific).



DNA mammalian app on DropSense16 reader

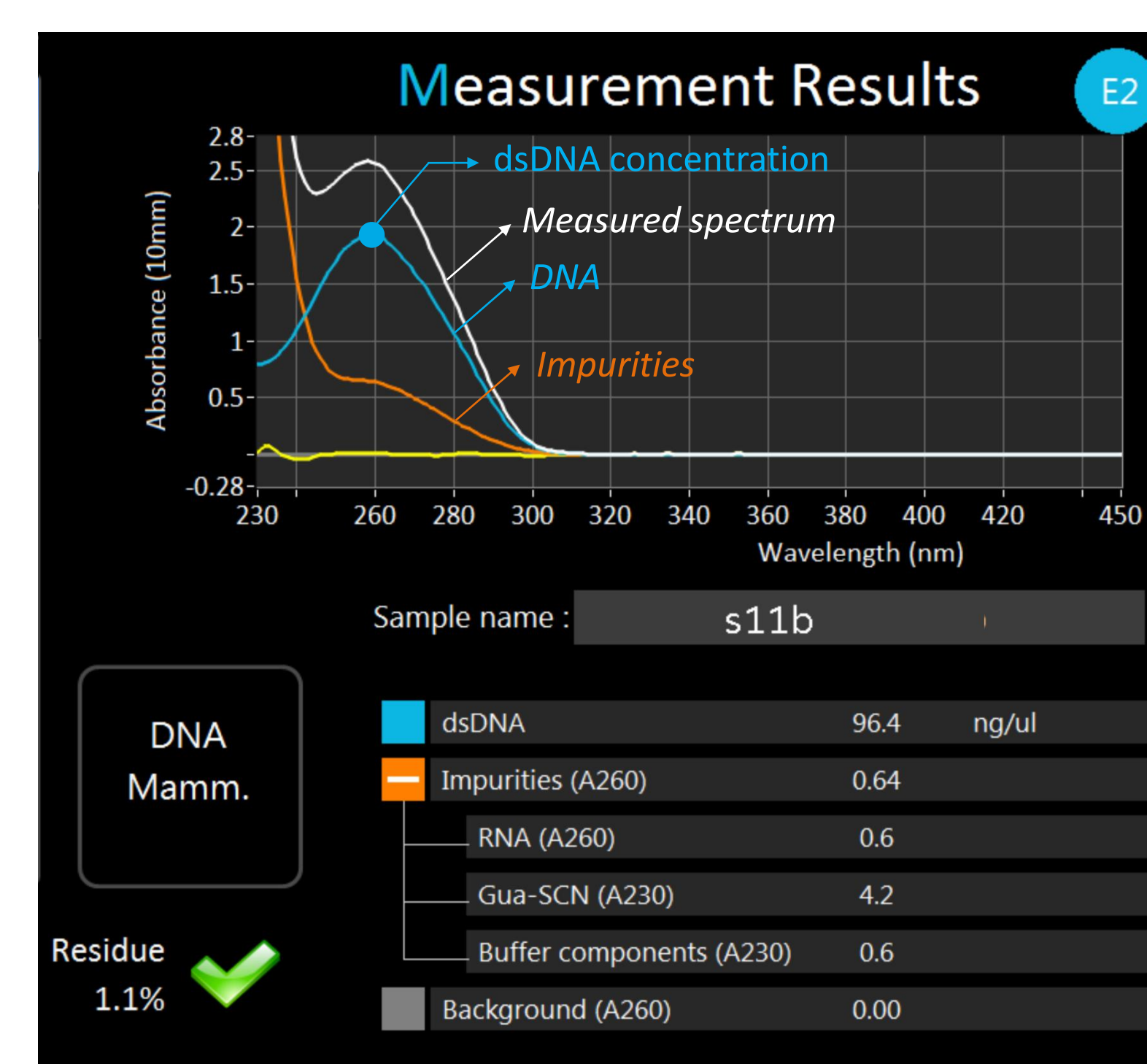
The DropSense16 reader measures the full UV/VIS spectrum of native DNA samples between the concentration range of 1.5 – 10,000 ng/μl. With the 'DNA mammalian' app, an additional analysis of the spectral shape is performed by Trinean's proprietary cDrop algorithms. By consulting a database with UV/VIS-spectra of putative contaminants, the software determines of which light-absorbing components the UV/VIS spectrum is composed, and provides a DNA concentration corrected for any A260-absorbing impurities that might be present in the sample, including RNA, salts etc.

Droplet A260 spectroscopy



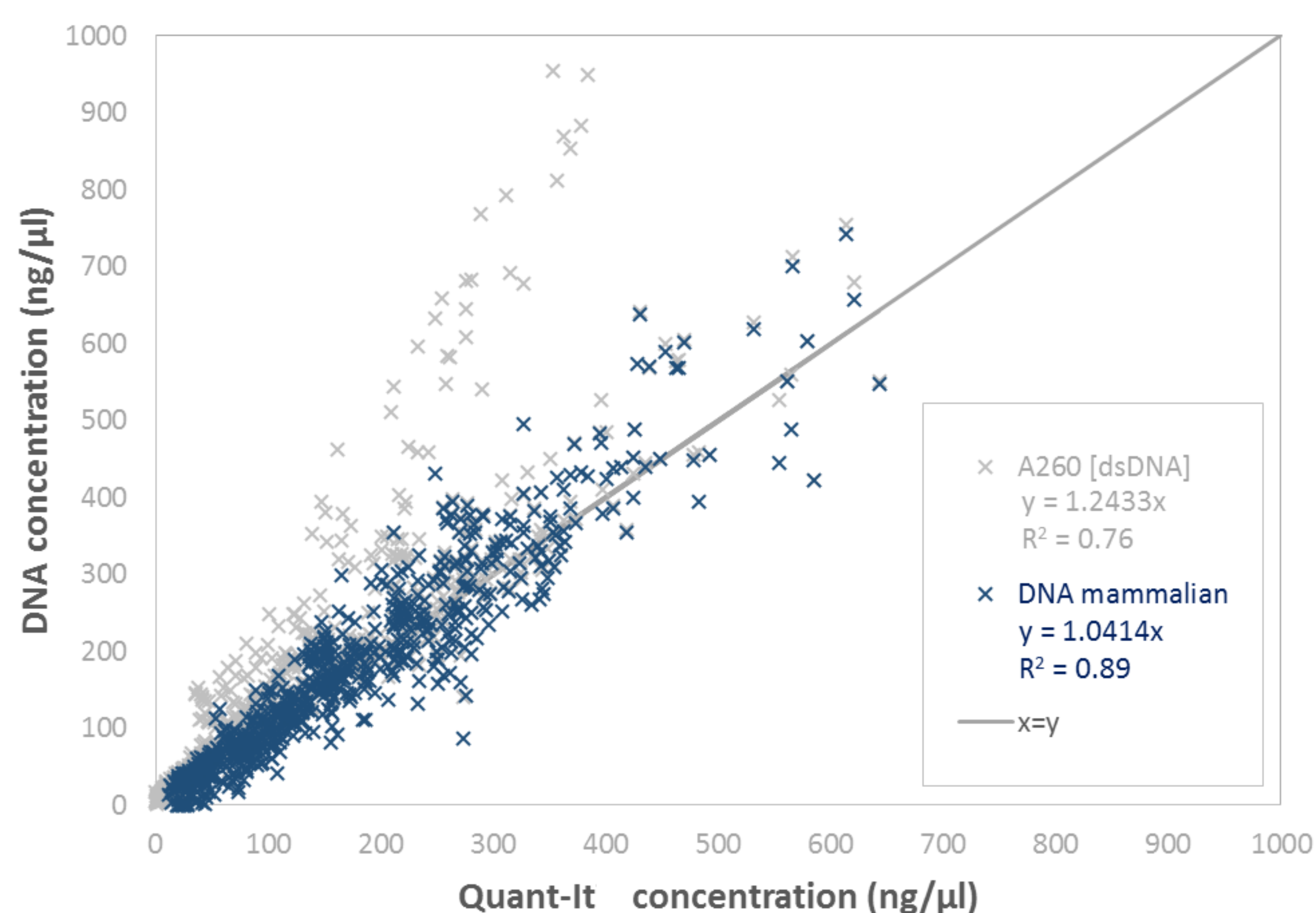
- DNA concentration** based on the absorbance (10 mm) at 260 nm and corresponding concentration factor of 50.
- Purity** indicated by A260/A280 and A260/A230 ratio's.

DNA mammalian

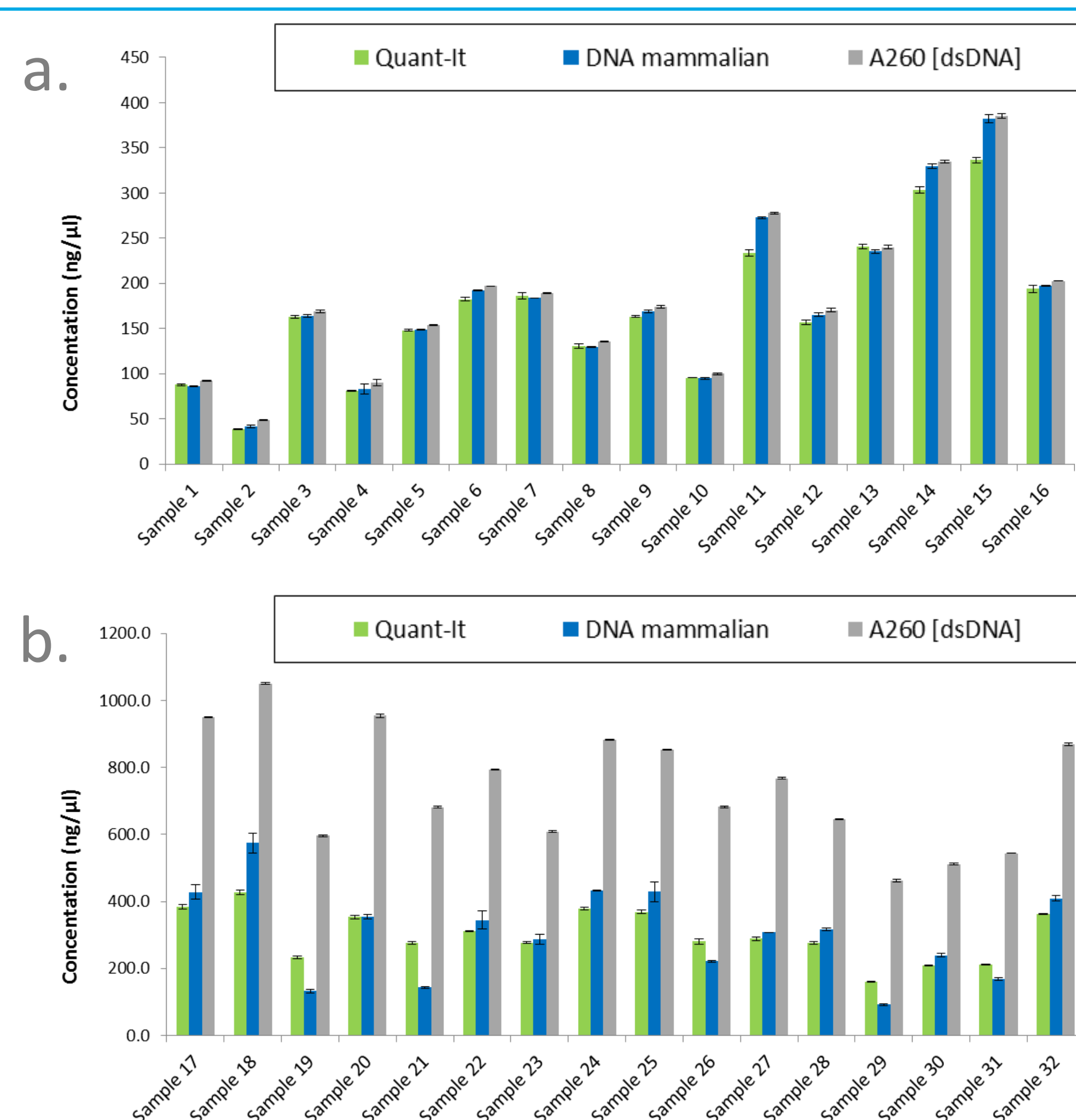


- White curve** = Measured UV/VIS spectrum.
- Blue curve** = DNA profile. Concentration (ng/μl) corrected for A260 absorbing contaminants.
- Orange curve** = Impurities profile. Detailed info on each contaminant is provided,

Results



The scatterplot above demonstrates that for 836 samples, the DNA concentrations determined by the 'DNA mammalian' app (blue crosses) correlate to values obtained with Quant-It PicoGreen ($y = 1.04x$), a standard technique for dsDNA quantification. Classic A260 spectrophotometry (gray crosses), however, tends to overestimate these ($y = 1.24x$), due to the presence of UV-absorbing impurities in the sample contributing to the A260 signal such as RNA, heme, turbidity scatter, etc.



The top bar chart (a) compares DNA concentrations extracted from blood samples using a bead-based kit. The samples appear pure, as A260 (gray), Quant-It (green) and **DNA mammalian** (blue) concentrations correlate nicely. The bottom chart (b) compares concentrations of DNA extracted from a lymphoblastoid cell line. Here, however, A260 overestimates DNA concentration compared to Quant-It, likely due to RNA contamination, whereas **DNA mammalian** is able to discriminate DNA from RNA.

Conclusion

In this study, DNA was extracted from different sources using a variety of extraction kits ranging from bead-based and column-based kits to salting-out extractions. DNA concentrations were determined with a fluorescence-based Quant-It PicoGreen assay (Thermo Fisher Scientific), Trinean's 'DNA mammalian' app on the DropSense16 'Touch & Go' reader and droplet A260 spectrophotometer. We demonstrate the tendency of A260 spectrophotometry to overestimate DNA concentration due to the presence of UV/VIS absorbing impurities in the sample. The 'DNA mammalian' app, notwithstanding being a direct quantification approach, accounts for these impurities and shows a corrected DNA concentration, which corresponds to values found with the more labor-intensive Quant-It fluorescence assay.