Olink®

Technical note

High consistency between qPCR readout instruments for Olink data

Introduction

Olink® Signature Q100 is a dedicated solution for the readout of Olink® Target 96, Target 48 and Focus panels. Unlike the Biomark™ HD system from Standard BioTools™ (formerly Fluidigm), which can also be used for the readout of the same qPCR-based panels, Signature Q100 is less than one sixth the size of a Biomark and does not require a separate instrument for the Integrated Fluidic Circuit (IFC) priming and loading steps. Signature Q100 has a low investment threshold, broadening access to proteomic profiling to more researchers than ever before.

To demonstrate that both instruments produce equally high quality data when used with Olink panels, a comparative study was performed.

Study design

A plate of 8 different plasma samples was set up, each in 10 replicates together with a set of Negative Controls and Inter-Plate Controls (IPCs). See Figure 1 for the plate layout. The plate was run according to the Olink Target 96 User Manual through the Incubation and Extension and amplification steps using the Olink® Target 96 Inflammation kit. The resulting extension product went through five separate detection runs on both Olink Signature Q100, and on the Biomark™ HD system.

The same extension product was used for all runs to minimize variation caused by any other factor than the instrumentation. For the same reason, all runs were performed by the same operator.

The run data went through data pre-processing and quality control (QC) in the Fluidigm Real-Time PCR Analysis Software and the Olink® NPX Signature software as outlined in the Olink® NPX Signature User Manual. IPC-normalized NPX data exported from Olink NPX Signature was used for the subsequent data analysis. Data below Limit of Detection (LOD) or from sample replicates that did not pass QC were excluded.

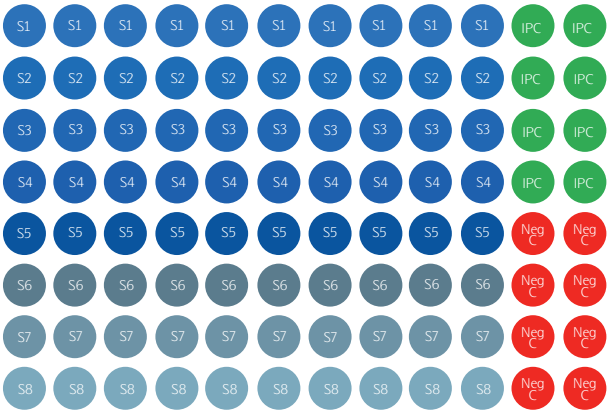

Figure 1. Plate layout.

Deviations

During the QC, it was discovered that one of the runs performed on the Biomark™ HD system (run "BM1") had some severe technical issues. This run was therefore excluded from all of the subsequent analyses. Similarly, one replicate each of sample S4 in run Q4 (the fourth run on Olink Signature Q100) and BM4 (in both cases in well D4) did not correlate well with other replicates of this sample. However, these two replicates correlated well with each other. The runs BM4 and Q4 were prepared in parallel, and the same set of 8 pipette tips was used in the setup of both plates. It was assumed that the deviating signal for these replicates stems from some technical issue at this stage and the replicates

Table 1. Number of samples/replicates per run, with and without QC warnings. BM=Biomark, Q=Signature Q100.

QC result	BM2	BM3	BM4	BM5	Q1	Q2	Q3	Q4	Q5
Pass	96	96	95	96	90	95	94	94	90
Warning	0	0	1	0	6	1	2	2	6

were therefore excluded from the analysis so as to not mask or exaggerate device dependent variation.

One part of the QC of the runs, is the sample QC. For that purpose two of the internal controls spiked into each well at the same concentration is used. For each plate and internal control, a plate median is calculated. If any sample falls outside of the plate median (+/-0.3 NPX for either or both of the internal controls), the sample will be marked with QC Warning. The number of sample replicates marked with QC Warning for each run can be found in Table 1. In the majority of cases, the samples that did get marked with a QC Warning were samples/replicates that were very close to the +/-0.3 NPX border in all runs, but due to minor variations fell either just inside or just outside the set limit.

Results

To quantify the effect that the instrument type (Biomark™ HD or Signature Q100) has on the data, a linear mixed effect model was used to quantify how much the NPX value changes when using different instruments, controlling for the effect of Olink IDs (Assays), and Samples (within run):

$$NPX \sim 1 + Instrument + (1|run:Sample) + (1|OlinkID)$$

This model estimates the effect of device type (difference in NPX between the Biomark™ HD or Signature Q100). To account for non-independence of datapoints belonging to the same assay, sample, or run, we used a hierarchical structure. Each OlinkID (unique assay identifier) has its own random effect on baseline NPX, as does each sample. Furthermore, samples are clustered within runs.

Only samples and not the IPC or Negative Control data were used in this model. While sample ID, assay and run are expected to influence the NPX value, the instrument type is expected to have a minimal effect on NPX values which would allow the devices to be used interchangeably.

The results from the linear mixed effect model do indicate that the effect of instrument type on NPX is minimal, with an estimate of -0.03 and a 95% confidence interval between -0.17 and 0.12. With 95% probability the true effect of type of instrument lies within this interval, and given the data within this data set, it can be considered unlikely that the true effect of instrument type on the NPX value is outside of this narrow range that includes 0. The p-value of 0.7 also indicates that there was no evidence that the effect of "instrument type" on NPX is different from 0. This can also be seen directly from the data by comparing the distribution of NPX values between the instruments. As seen in Figure 2, the distribution of values is extremely similar, indicating that there is no overall effect of instrument type on NPX values.

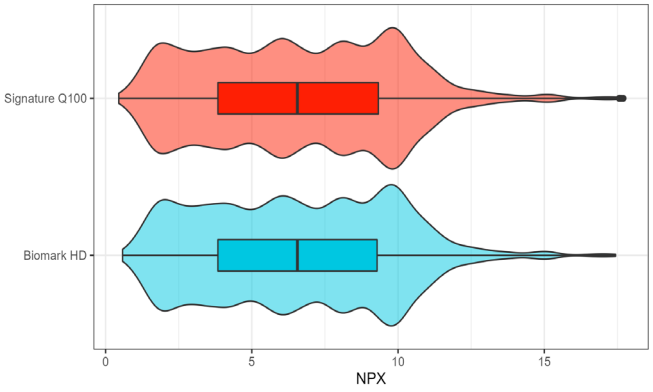

Figure 2. Distribution of NPX values by instrument type.

Conclusion

The data analyzed here show no evidence of differences in generated NPX values between the Biomark™ HD and Olink Signature Q100.

In conclusion, these results indicate with confidence that we can safely recommend to our customers to run samples in the same study on both Biomark™ HD and Olink Signature Q100.

Table 2. Estimated effects on NPX from the linear mixed effect model. The additive effect on NPX value that can be attributed to the device type was found to be non-significant (-0.03 ± 0.07, p > 0.7).

	Estimate	Std .error	df	t value	Pr(>|t|)	2.5 %	97.5 %
Signature Q100 relative to Biomark HD	-0.02757	0.07288	69.93419	-0.37822	0.70641	-0.17134	0.11619

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