Poster #740

A robust rapid mRNA based test to profile simultaneously ER, AR, PI3K and MAPK functional signaling pathway activity for precision oncology

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Introduction

OncoSignal test was developed to determine and quantify functional signal transduction pathway activity levels of 4 key oncogenic signaling pathways (ER, AR, PI3K-FOXO and MAPK-AP1), based on the quantitative measurement of mRNA expression levels of the direct target genes of the transcription factors. Here we present data of the 4 pathway qPCR test, that can be used in standard molecular labs and enables easy measurement of pathway activities in tumor tissue samples, potentially providing important information for understanding tumor behavior and help optimal therapy selection for treatment of cancer.

Methods

The performance of the OncoSignal ER AR PI3K MAPK Pathway Activity Profiling Test was compared between 2 reference laboratories; the Philips Service Lab in the Netherlands and the Protean BioDiagnostics lab (a US CAP-certified CLIA service lab), according to the scheme in figure 1

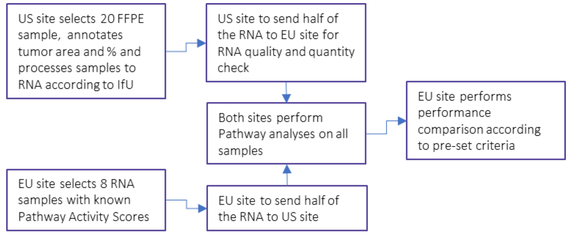

Figure 1: Process flow for technical performance evaluation of the OncoSignal test

Pre-defined performance criteria:

- ≥ 95% of the analyses should lead to a valid result (QC pass)
- ≥ 90% of the duplicate scores have an overlap of the 95% Confidence interval (CI)
- In case the difference between duplicates is not within 95% CI, (max 10% of all duplicates) scores should be less then 10 activity points apart (on a 0-100 scale)

When all criteria set for reproducibility were met, mRNA was isolated from an additional set of unstained FFPE tissue sections of 54 breast cancer samples (retrospective analyses) of different subtypes compared to 10 normal adjacent tissue samples to show a use case of the OncoSignal Test. Annotated areas with at least 50% tumor cells were used for tumor samples (total tissue volume about 0.25mm^3). IHC staining for ER, PR and HER2 was available for all samples, enabling tumor categorization into breast cancer subtypes. HER2 positive samples were characterized by 3+ IHC staining. KI67 staining was not available for all samples.

Results

Excellent inter-lab concordance was found with correlation coefficients of 0.99 for all 4 Pathways tested: ER, AR, PI3K and MAPK, respectively. Average absolute difference between pathway activities was 3.6 activity points on a 0-100 activity score scale. All 28 cases passed the criteria set for reproducibility. Only one (1.8%) out of 56 analyses resulted in a QC fail, which was well within specification.

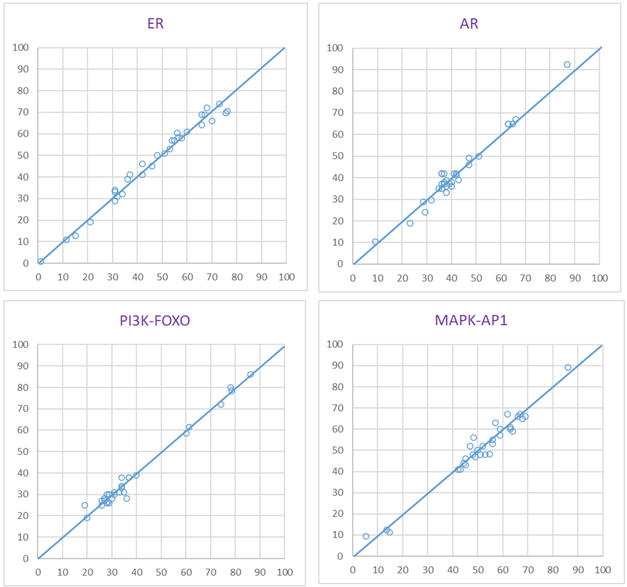

Figure 2: Correlation plot for each pathway show that Activity scores between labs are very well correlated

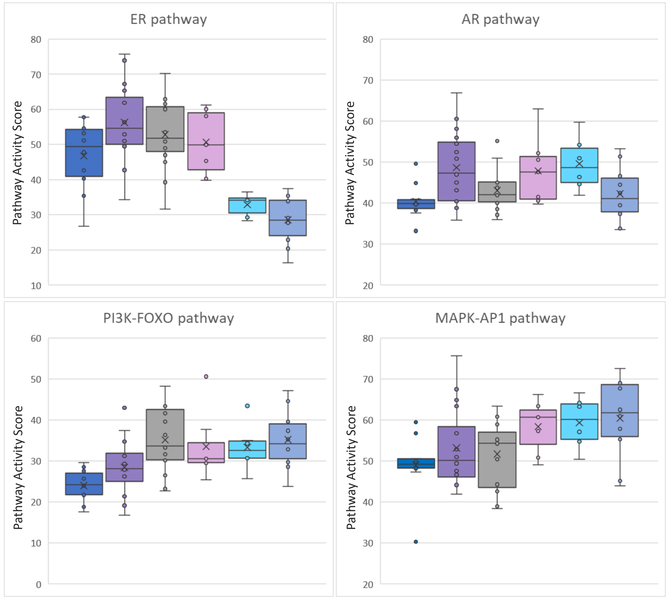

Figure 3: Pathway activity score distribution of different breast cancer subtypes and normal adjacent tissue

All 54 additional breast cancer samples were subtyped based on IHC, confirmed by mRNA expression of ESR1, PGR, ERBB2 and mKI67. Since KI67 stain was not available for all samples, the mRNA expression of mKI67 was used as marker. This resulted in 29.6% luminal A samples (n=16), 27.8% luminal B samples (n=15), 13% HER2 positive luminal B samples (n=7), 11.1% ER neg/HER2 enriched samples (n=6) and 18.5% Triple Negative Breast Cancer samples. NAT contains 5 pre- and 5 postmenopausal samples..

Observations

Of interest, ER (and/or PR) positive breast cancer showed high variation in ER pathway activity between cases, suggesting that the ER signaling pathway is not the tumor driving pathway in a subset of ER positive tumors. As expected, the HER2-enriched and TNBC subgroups predominantly had a very low ER pathway activity scores. PI3K pathway activity was most prominent in the luminal B and TNBC tumor groups, while MAPK pathway was most active in TNBC. AR pathway had the highest median activity in the HER2 subtype.

NAT
LumA
lumB
lumB/HER2
HER2
TNBC

The results obtained with the OncoSignal qPCR test are in line with the results found with OncoSignal results of an Affymetrix based dataset (see poster **#533**)

Conclusion

The Philips OncoSignal ER AR MAPK PI3K Pathway Test is robust and has been shown to be easily implemented in a molecular lab. Correlation between 2 different labs show excellent correlation results. The results of the pathway activity analysis in a set of FFPE BrCa samples are in line with breast subtype classification based on IHC (ER activity highest in luminal types, PI3K higher in more aggressive types). But also and especially, the assay has the capability to uncover specific pathway activation independent of subtype which may have significant value for precision oncology and targeted therapy selection. The test has potential for wide utilization as it can be performed on conventional FFPE prepared specimens of multiple tumor types and may be complementary to conventional mutational and IHC analysis for classification of tumors.

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