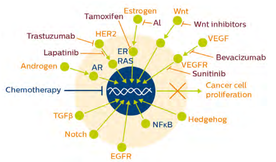
# Assays for measurement of functional signal transduction pathway activity in any cell or tissue sample

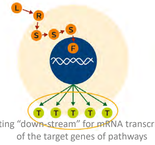
Anja van de Stolpe, Henk van Ooijen, Laurent Holtzer, Wim Verhaegh

[1] Philips Research, Eindhoven, The Netherlands
contact: anja.van.de.stolpe@philips.com

## Summary

A novel *biologically validated* method is presented which enables quantitative measurement of activity of the ER, AR, FOXO-PI3K, Wnt, TGFbeta, Hedgehog, Notch, and NFkB signal transduction pathways in any cell or tissue type.
Bayesian models interpret measurements of target mRNAs of pathway-associated transcription factors to provide activity scores.

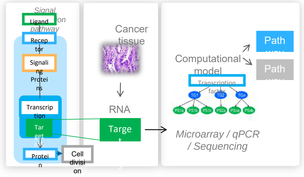



Testing "down-stream" for mRNA transcription of the target genes of pathways

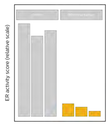
## Knowledge-based models for quantitative measurement of signal transduction pathway activity

Models infer signal transduction pathway activity from mRNA expression levels of the direct target genes of the pathway-associated transcription factor. Input for the measurement is Affymetrix HG-U133Plus2.0 microarray data or qPCR [Verhaegh et al., Cancer Res 2014;74(11):2936-45]. Bayesian network m,odel with three types of nodes: a transcription complex, target genes and probesets. The model describes (i) how the expression of the target genes depends on the activation of the respective transcription complex, and (ii) how probeset intensities depend in turn on the expression of the respective target genes.

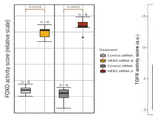


Models can be used to quantitatively measure pathway activity in an individual test sample by entering mRNA measurements, and inferring backwards in the model the probability (or log2odds) that the active transcription complex must have been present.

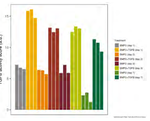
## Pathway model calibration on one cell type; biological validation on different cell types (examples)



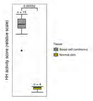

ER (Estrogen receptor). Calibration: Breast cancer cell line. GSE37820, Alpha (ESR1) knockdown by siRNA in ER positive MCF7



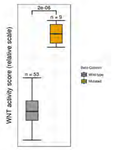

FOXO-PI3K. Calibration: HUVEC. GSE71347, HER2-siRNA knockdown in breast cancer cell line (BT-474)



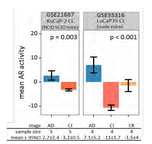

TGFbeta. Calibration: lung cancer cell line. GSE84500, Mesenchymal stem cells treated with BMP or BMP+TGFbeta (1,2,3,7 days)



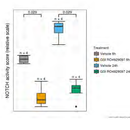

HH (Hedgehog). Calibration: basal cell cancer. GSE29316, Sonic Hedgehog (SHH) treatment of fibroblasts in a co-culture of HUVEC cells and colon fibroblasts



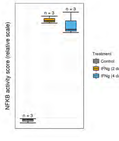

Wnt, Calibration: colon cancer cell line. GSE10327, medulloblastoma with beta-catenin activating mutation.




AR. Calibration: prostate cancer cell line. GSE21887, KUCaP-2 cell grafted in NOD SCID mice, androgen dependent tumor growth (AD) and castration-induced tumor regression (CI); GSE33316, LuCaP35 cells grafted in nude mice, AD, CI and castration resistant (CR) regrowth

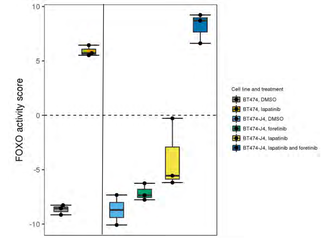



Notch. Calibration: ovarian cancer. GSE6495, ALL MOLT4 cell line treated with gammaSI

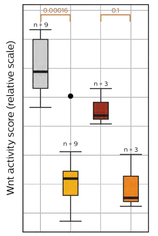



NFkB. Calibration: *activated B cell-like* (ABC) subtype of diffuse large B-cell lymphoma DLBCL1 GSE58096, THP-1 monocytic cells stimulated with IFNgamma or vehicle for indicated time periods.

## Use example: Measuring and quantifying drug response in cell culture or PDX mice

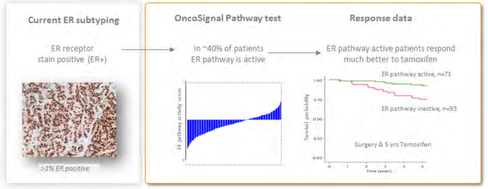



GSE16179: FOXO activity increased after lapatinib (anti HER2) treatment of ER+/HER2+ cell line BT474. The increase is less on lapatinib-resistant derived clone BT474-J4, but large again after restoring lapatinib sensitivity with foretinib. (n= too low for appropriate statistics)




GSE73906. Colon cancer PDX mice were treated with antiRSPO3 (Wnt inhibitor) and/or chemotherapy (Irinotecan). Decrease in WNT pathway activity after antiRSPO3 treatment. P (control vs irinotecan)= 0.036; P (control vs antiRSPO3)= $1.6 \cdot 10^{-4}$; P (control vs irinotecan+antiRSPO3)= 0.009.

## Use example: Prediction of therapy response in cancer patients




GSE6532/GSE9195, n=164. ER pathway active in one third of ER-positive patients; ER active have improved clinical outcome (less recurrence) upon tamoxifen adjuvant treatment.

## Conclusion

Our mRNA-based pathway assays measure signal transduction pathway activity in multiple cell and tissue types, and can be used as readout for organ-on-chip cell/tissue culture models.
Potential applications: comparison between cultured diseased tissue and in vivo pathology; quantitative assessment of drug efficacy/toxicity; differentiation/maturation status of stem cell derivatives.
Assays have been adapted to qPCR, enabling use on FFPE tissue and small samples.

*Ref:* Verhaegh, et al. Cancer Res. 2014;74(11):2936-45

## Pathway activity in human stem cells

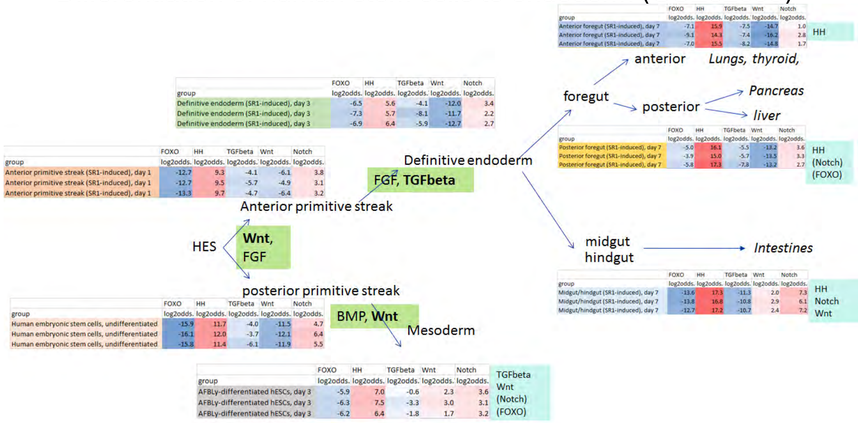
*Pathway activity score indicated as log2odds, colour indicates activity, ranging from blue (low) to red (high)*

Human embryonic stem cells (HES) cultured under different conditions (GSE19902).

| group | FOXO log2odds. | HH log2odds. | TGFbeta log2odds. | Wnt log2odds. | Notch log2odds. |
|---|---|---|---|---|---|
| hES-T3 cells grown on feeder-free Matrigel in T3HDF-conditioned medium | -0.6 | 13.8 | 1.2 | -15.2 | 5.9 |
| hES-T3 cells grown on feeder-free Matrigel in T3HDF-conditioned medium | 0.8 | 13.9 | 2.3 | -14.9 | 6.8 |
| hES-T3 cells grown on feeder-free Matrigel in MEF-conditioned medium | -11.8 | 14.1 | -12.2 | -19.1 | -1.3 |
| hES-T3 cells grown on feeder-free Matrigel in MEF-conditioned medium | -11.9 | 14.6 | -11.9 | -18.8 | -1.7 |
| hES-T3 cells grown on T3HDF feeder | -9.6 | 16.6 | -4.1 | -18.2 | 9.3 |
| hES-T3 cells grown on T3HDF feeder | -9.9 | 15.5 | -1.1 | -17.7 | 8.2 |
| hES-T3 cells grown on MEF feeder | -11.4 | 14.7 | -14.3 | -14.5 | -3.8 |
| hES-T3 cells grown on MEF feeder | -11.4 | 14.8 | -15.1 | -14.8 | -3.7 |

- Active HH
- Activity of TGFbeta, Notch and PI3K pathway activity depend on culture conditions

Differentiation of human stem cells to endoderm (GSE52658)




| group | FOXO log2odds. | HH log2odds. | TGFbeta log2odds. | Wnt log2odds. | Notch log2odds. |
|---|---|---|---|---|---|
| Human embryonic stem cells, undifferentiated | -15.9 | 11.7 | -4.0 | -11.5 | 4.7 |
| Human embryonic stem cells, undifferentiated | -16.1 | 12.0 | -3.7 | -12.1 | 6.4 |
| Human embryonic stem cells, undifferentiated | -15.8 | 11.4 | -6.1 | -11.9 | 5.5 |
| Anterior primitive streak (SR1-induced), day 1 | -12.7 | 9.3 | -4.1 | -6.1 | 3.8 |
| Anterior primitive streak (SR1-induced), day 1 | -12.7 | 9.5 | -5.7 | -4.9 | 3.1 |
| Anterior primitive streak (SR1-induced), day 1 | -13.3 | 9.7 | -4.7 | -6.4 | 3.2 |
| Definitive endoderm (SR1-induced), day 3 | -6.5 | 5.6 | -4.1 | -12.0 | 3.4 |
| Definitive endoderm (SR1-induced), day 3 | -7.3 | 5.7 | -8.1 | -11.7 | 2.2 |
| Definitive endoderm (SR1-induced), day 3 | -6.9 | 6.4 | -5.9 | -12.7 | 2.7 |
| Definitive endoderm, day 3 | -7.3 | 5.7 | -8.1 | -11.7 | 2.2 |
| Definitive endoderm, day 3 | -6.9 | 6.4 | -5.9 | -12.7 | 2.7 |
| AFBLy-differentiated hESCs, day 3 | -5.9 | 7.0 | -0.6 | 2.3 | 3.6 |
| AFBLy-differentiated hESCs, day 3 | -6.3 | 7.5 | -3.3 | 3.0 | 3.1 |
| AFBLy-differentiated hESCs, day 3 | -6.2 | 6.4 | -1.8 | 1.7 | 3.2 |
| Serum-differentiated hESCs, day 3 | -9.0 | 11.1 | -4.2 | -9.3 | 5.6 |
| Serum-differentiated hESCs, day 3 | -8.7 | 10.9 | -4.3 | -9.6 | 3.6 |
| Serum-differentiated hESCs, day 3 | -9.3 | 11.0 | -3.5 | -11.1 | 4.3 |
| Anterior foregut (SR1-induced), day 7 | -7.1 | 15.9 | -7.5 | -14.7 | 1.0 |
| Anterior foregut (SR1-induced), day 7 | -9.1 | 14.3 | -7.4 | -16.2 | 2.8 |
| Anterior foregut (SR1-induced), day 7 | -7.0 | 15.5 | -8.2 | -14.8 | 1.7 |
| Posterior foregut (SR1-induced), day 7 | -5.0 | 16.1 | -5.5 | -13.2 | 3.6 |
| Posterior foregut (SR1-induced), day 7 | -3.9 | 15.0 | -5.7 | -13.5 | 3.3 |
| Posterior foregut (SR1-induced), day 7 | -5.8 | 17.3 | -7.8 | -13.2 | 2.7 |
| Midgut/hindgut (SR1-induced), day 7 | -13.6 | 17.3 | -11.3 | 2.0 | 7.3 |
| Midgut/hindgut (SR1-induced), day 7 | -13.8 | 16.8 | -10.8 | 2.9 | 6.1 |
| Midgut/hindgut (SR1-induced), day 7 | -12.7 | 17.2 | -10.7 | 2.4 | 7.2 |

- Differentiation to midgut/hindgut inhibits TGFbeta
- Wnt active in hindgut; inactive in midgut/foregut