PHILIPS

Pathway Activity Profiling

Applications in Life Sciences

Unravelling the cell molecular phenotype

Coordinated activity of 10-15 cellular signal transduction pathways enables cells to communicate and adapt their function to control physiological processes. Measuring activity levels of these signaling pathways provides insights into the underlying functional mechanisms of the cell.

Philips Pathway Activity Profiling* introduces first-of-a-kind molecular phenotyping which translates transcriptomic data into quantified pathway activity of the key signal transduction pathways in a human sample [1-3]. Simultaneous quantitative profiling of the estrogen receptor (ER), androgen receptor (AR), PI3K, MAPK, JAK-STAT, NF-κB, Hedgehog (HH), Notch, TGFβ, and Wnt pathways is based on measurement of mRNA levels transcribed from direct target genes regulated by the pathway transcription factor. The expression of the target genes is translated by a knowledge-based Bayesian model into a quantitative pathway activity score, and activity of each pathway is reported on a normalized 0 to 100 scale. The test can be performed in any human tissue and cell type using RNA extracted from fresh frozen (FF), fixated, formalin-fixed paraffin-embedded (FFPE) tissue and cell culture samples as input.

Pathway Activity Profiling can support your research by providing a quantitative characterization of the cell molecular phenotype. The use of Pathway Activity Profiling is illustrated for selected applications:

1. Quantification and standardization of cell and tissue culture research
2. Measuring effects of experimental interventions
3. Ensuring that experimental in vitro models are optimally representative for human physiology and disease
4. Functional analysis of gene mutations
5. Assessment of pluripotency, differentiation state, and purity of stem cells and stem cell derivatives
6. Measuring functional activity state of immune cell types

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1. Quantification and standardization of cell and tissue culture research

Freezing/thawing procedures, cell passaging, and culturing cells under varying culture conditions (e.g. same cell line in different culture media, using a new batch of serum) may lead to variations in the phenotypic characteristics of cells and may interfere with experimental reproducibility and drug responses [4]. Similarly, passaging of cancer tissue or cell line grafts in cell line- or patient-derived xenograft (PDX) mice can lead to changes in cancer cell phenotype.

Pathway Activity Profiling enables quantitative comparison between cell lines or PDX mice of different passages, or between independent experiments, and may help to control cell culture conditions (e.g. choice of medium composition) to culture cells with required phenotypic characteristics in a reproducible manner.

The two following examples (figure 1.A,B) show that significant changes in signaling pathway activities result from cell culture passaging, which may cause changes in cell characteristics.

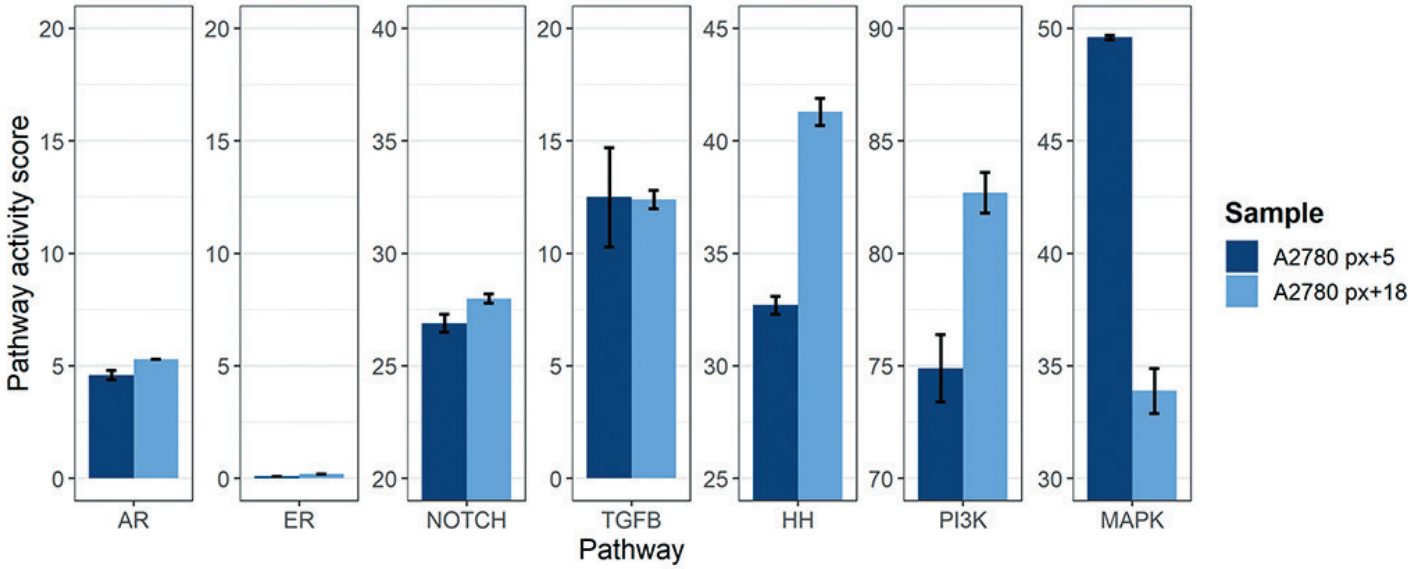

Figure 1.A: Ovarian cancer cell line A2780 was cultured under standard conditions and RNA was extracted from cells at passages px+5 and px+18. No changes are observed in pathway activity scores for the AR, ER, Notch and TGFβ pathways, whereas HH and PI3K pathway activity has increased, and MAPK pathway activity has decreased after several passages.

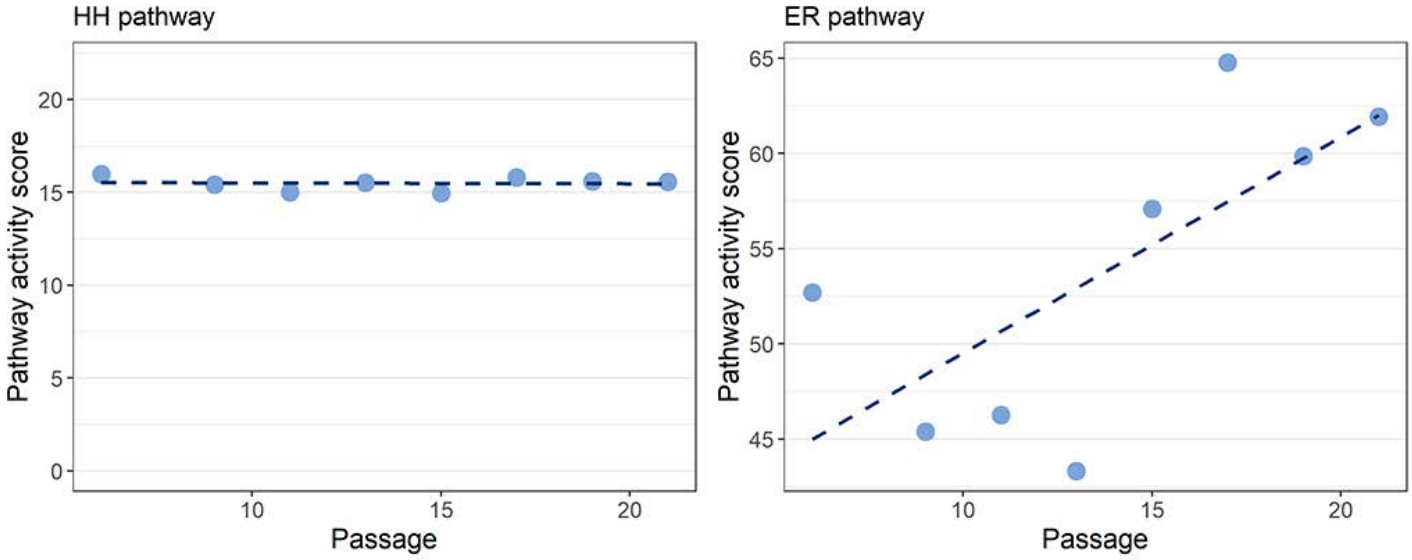

Figure 1.B: Breast cancer cell line MCF7 was cultured under standard conditions, and RNA was isolated at indicated passages (6,9,11,13,15,17,19,21). Hedgehog (HH) pathway activity remained stable over the different passages, whereas the estrogen receptor (ER) pathway activity increased with passaging (corr. 0.54).

2. Measuring the effect of experimental interventions

Pathway Activity Profiling can be used to quantitatively measure the effect of genetic engineering or of drug compounds on pathway activity, e.g. to generate dose-response curves and to determine the relation between pathway activity and cell growth.

In figure 2.A the effects of PI3K, mTOR and Akt inhibitors on PI3K pathway activity and cell culture growth in an MCF7 breast cancer cell line are shown. The effects of pathway inhibition with the different compounds can be directly related to cell growth inhibition.

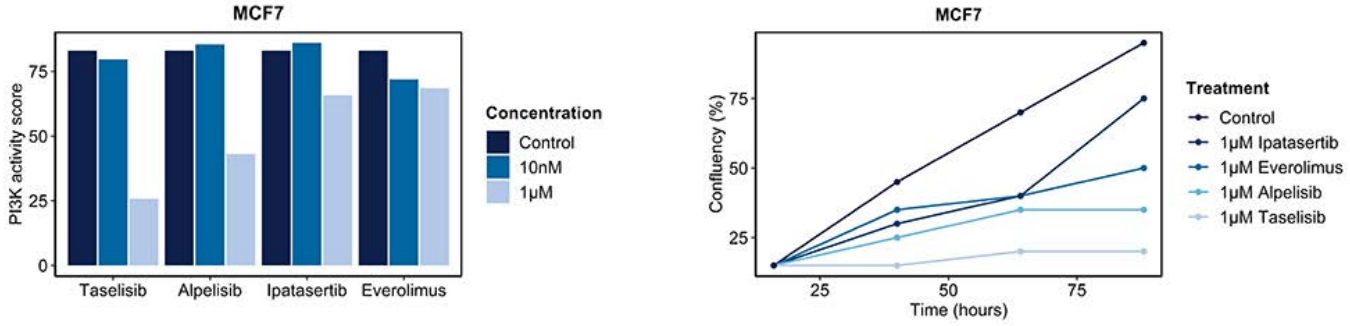

Figure 2.A: Left: PI3K pathway activity scores for MCF7 breast cancer cells treated with two concentrations of taselisib (PIK3CA inhibitor), alpelisib (PIK3CA inhibitor), ipatasertib (AKT inhibitor), everolimus (mTOR inhibitor) and untreated MCF7 cells (control).
Right: Corresponding effects of the drugs on MCF7 cell confluency.

Figure 2.B represents an example of pathway activity profiling of the cell line MDA-MB-231 containing an inducible FOXO-A3 construct, and upon induction with doxycycline a clear decrease in PI3K pathway activity compared to the control cell line (untreated) is seen; the PI3K pathway activity score is derived from the inverse reading of the FOXO transcription factor. RNA samples were tested five times using the RT-qPCR-based test. The results demonstrate high reproducibility of the pathway activity scores.

Sample	Number of replicates	ER Score ± SD	AR Score ± SD	PI3K Score ± SD	MAPK Score ± SD	HH Score ± SD	NOTCH Score ± SD	TGFβ Score ± SD
MDA-MB-231 cells 18 hrs doxycycline	5	8.4 ± *3.3*	17.7 ± *0.9*	31.8 ± *3.9*	65.2 ± *1.1*	26.6 ± *2.5*	14.6 ± *1.3*	22.2 ± *1.4*
MDA-MB-231 cells untreated	5	7.0 ± *1.1*	17.2 ± *0.7*	73.7 ± *0.9*	61.6 ± *1.4*	31.4 ± *2.3*	16.7 ± *1.2*	20.3 ± *1.4*

Figure 2.B: Pathway activity scores of the MDA-MB-231 cell line with induced FOXO-3A construct and the MDA-MB-231 control cell line

3. Ensuring that experimental *in vitro* models are optimally representative for human physiology and disease

Cell- and tissue culture-based *in vitro* disease models are often not representative for the disease in human patients, making it difficult to extrapolate *in vitro* results to the human *in vivo* situation.

Pathway Activity Profiling enables quantitative characterization of the pathophysiology (mechanism) of a disease. For example, a quantitative comparison between a pathway activity profile in a pathology slide from a patient and a corresponding *in vitro* disease model can be performed to investigate similarity. In addition, obtained pathway activity profiles can be used to adapt cell culture conditions to improve the similarity between *in vitro* and *in vivo*.

In figure 3 pathway activity of the androgen receptor (AR) and PI3K pathway in primary prostate cancer patient tissue, prostate cancer cell line LNCaP and prostate cancer PDX tissue was assessed. While AR pathway activity shows slightly lower activity in preclinical models when compared to in vivo human disease, activity of the PI3K pathway is much higher in the preclinical models. Activity of the PI3K pathway is dependent on the presence of growth factor ligands in the cancer cell microenvironment and is therefore influenced by culture conditions (e.g. percentage of serum), and the host mouse in case of PDX models..

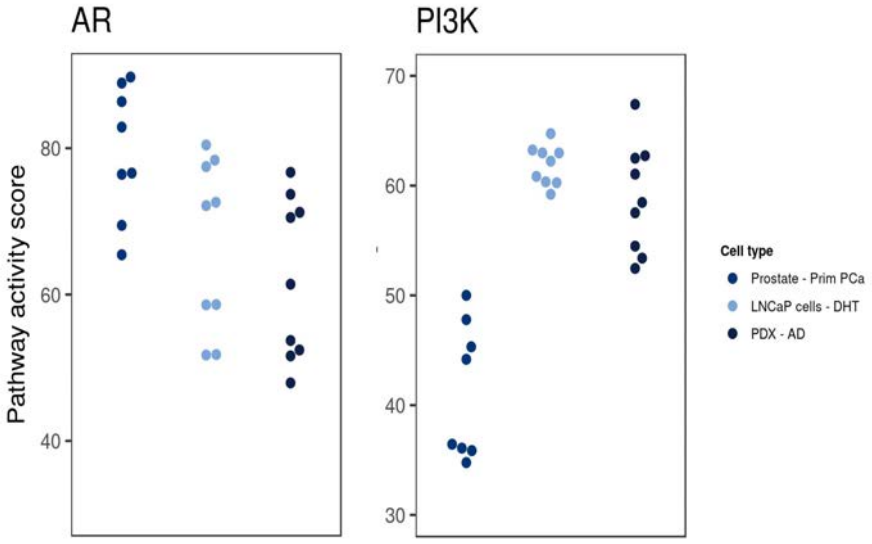

Figure 3: AR and PI3K pathway activity scores in primary prostate cancer tissue samples (Prostate – Prim PCa), prostate cancer cell line LNCaP cells stimulated with dihydroxytestosterone (LNCaP cells – DHT) and patient derived xenograft samples (PDX – AD). Analyzed datasets: GSE7708, GSE21887, GSE3325.

4. Functional analysis of gene mutations

Pathway Activity Profiling facilitates analysis of functional consequences of gene mutations and can be used to determine if a mutation drives pathway activity.

In the following example (figure 4), functional characterization (gain-of-function and loss-of-function) of Wnt pathway mutations in PDX mice was assessed. Wnt pathway activity scores were measured in ovarian and breast cancer PDX (xenograft) mice. Loss of APC and gain of beta catenin protein function are known to result in increased Wnt pathway activity. Wnt pathway activity scores were normal in the reference (WT), increased in loss-of-function APC-mutated ovary cancer and in gain-of-function CTNNB1 Asp32Tyr mutated breast cancer, and normal in non-functionally mutated CTNNB1 Asp665Glu breast cancer.

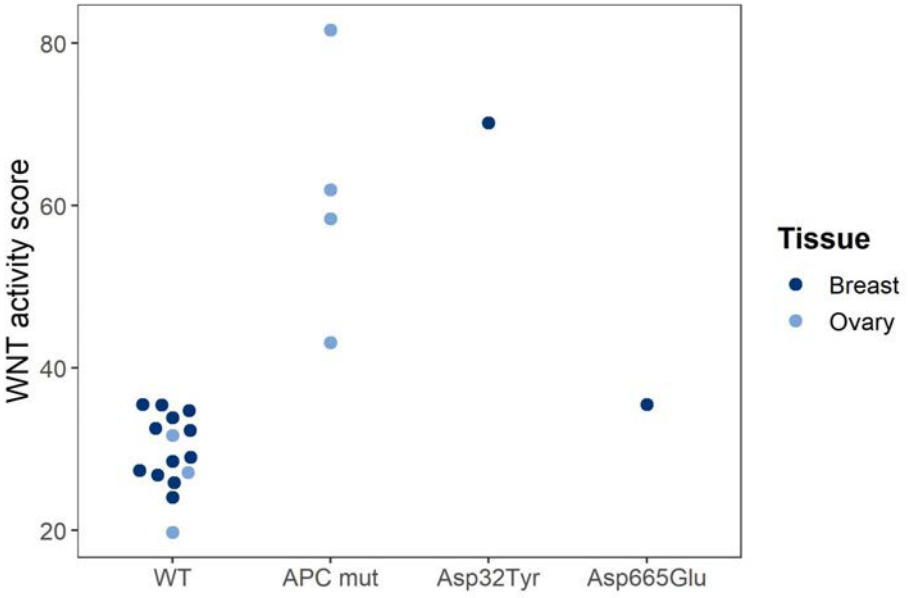

Figure 4: Wnt pathway activity scores were determined in patient derived breast cancer xenografts and patient derived ovarian cancer xenografts. Pathway tests were adapted to use in mouse PDX models (to exclude interference of mouse model microenvironment). Tissue samples from human breast cancer tissue grafts were analyzed in a collaboration with Charles River Labs (CRL).[5]

5. Assessment of pluripotency, differentiation state, and purity of stem cells

Pluripotent iPS (and HES) stem cell lines have not all been derived in the same manner and contain genetic variations. As a result, they may vary in pluripotency and capability to differentiate to cell types of the three germ layers. During stem cell differentiation it is often difficult to assess the exact differentiation state and purity of the differentiated cell population without using reporter genes. In addition, for regenerative medicine purposes it is necessary to exclude malignant potential of the cultured cells.

Pathway Activity Profiling enables quantification of stem cell pluripotency. Characteristics of the stem cell line can be adjusted for example by changing component concentrations in culture (e.g. FGF), guided by measuring the pathway activity profile. Pathway Activity Profiling can also be used to measure the required differentiation state of the cell population, and to assess its purity.

In the following example (figure 5), pathway activity profiles from pluripotent human embryonic stem cells (hES-T3), cultured under different conditions are shown. The embryonic stem cells differ with respect to pathway activities which determine pluripotency and differentiation capability, depending on culture conditions.

Sample	MAPK	PI3K	HH	NOTCH	TGFβ	NFκB	JAK-STAT3	Wnt
hES-T3 cells, feeder-free Matrigel, T3HDF-conditioned medium	30.2	61.7	62.9	48.2	33.5	25.9	52.1	25.6
hES-T3 cells, feeder-free Matrigel, T3HDF-conditioned medium	29.0	61.2	63.7	48.0	35.4	26.5	52.7	25.9
hES-T3 cells, feeder-free Matrigel, MEF-conditioned medium	13.7	85.5	55.7	28.2	23.9	23.0	47.4	21.8
hES-T3 cells, feeder-free Matrigel, MEF-conditioned medium	14.7	86.6	55.2	26.8	23.1	22.6	47.8	22.1
hES-T3 cells, T3HDF feeder	30.5	84.4	71.1	47.1	31.3	28.2	62.8	22.7
hES-T3 cells, T3HDF feeder	28.7	85.7	70.0	45.7	33.5	27.8	62.6	23.2
hES-T3 cells, MEF feeder	13.1	84.4	49.8	22.7	22.3	21.8	48.7	26.1
hES-T3 cells, MEF feeder	11.6	83.9	50.4	21.3	21.2	22.6	48.3	25.9

Figure 5: Pluripotent human embryonic stem cells (hES-T3), cultured under different conditions: on a mouse feeder layer (MEF), with MEF-conditioned medium, on a human feeder layer (T3HDF) and with T3HDF-conditioned medium. Analyzed dataset: GSE19902.

6. Measuring functional activity state of immune cell types

For immunology research it may be necessary to measure the functional status of the immune cell type of interest, e.g. naive, resting, activated, or immune suppressed. The current standard of immune cell testing is assessing surface membrane antigens known to be associated with certain immune cell types, e.g. CD4 or CD8 antigen. Expression of such membrane antigens is not directly related to the functional activity state of the cells.

Pathway activity profiling enables characterization of the functional activity state of immune cell types. The following example (figure 6) demonstrates that withdrawal of IL-2 from IL2-activated PBMCs leads to inactivation of the immune cells (predominantly lymphocytes). Inactivation was associated with a decrease in PI3K, NF-KB, JAK-STAT3, and TGFβ pathway activity.

sample category	annotation per sample		PI3K	NFκB	NOTCH	JAK-STAT1/2	JAK-STAT3	TGFβ
Lymphocyte PBMCs	Before IL-2 withdrawal	donor 1	65.4	50.6	3.7	30.4	53.0	13.3
	24h after IL-2 withdrawal	donor 1	48.7	41.4	4.4	25.8	36.0	5.3
	Before IL-2 withdrawal	donor 2	70.1	45.3	4.1	25.4	48.7	8.3
	24h after IL-2 withdrawal	donor 2	52.2	41.6	5.6	26.3	32.1	7.3

Figure 6. Pathway activity scores in PBMCs from two donors before and after IL-2 withdrawal. Analyzed dataset: GSE7345.

OncoSignal products and services

Pathway Activity Profiling Test

- ER, AR, PI3K, MAPK pathways
- Compatible with FFPE samples from human origin
- PCR testing plates including quality checks on sample input and correct plate filling
- Result overview in dedicated Pathway Activity Profiling Report
- For in-house testing using standard laboratory equipment
- Developed under ISO13485

Pathway Activity Profiling Service

- ER, AR, PI3K, MAPK, Hedgehog, TGFβ, Notch pathways
- Compatible with FFPE tissue samples and RNA extracted from cell lines and tissue of human origin
- Extensive quality checks on samples, laboratory process and results
- Result overview in dedicated Pathway Activity Profiling Report
- ISO13485 certified

Pathway Activity Profiling Data Service

- ER, AR, PI3K, MAPK, Hedgehog, TGFβ, Notch, Wnt, JAK-STAT1/2*, JAK-STAT3*, NF-KB* pathway
- Compatible with Affymetrix Microarray gene expression data and RNA sequencing data
- Extensive quality checks on samples and results
- Result overview in dedicated Pathway Activity Profiling Report

* Under development.

References

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[2)] van Ooijen H et al. Assessment of Functional Phosphatidylinositol 3-Kinase Pathway Activity in Cancer Tissue Using Forkhead Box-O Target Gene Expression in a Knowledge-Based Computational Model. Am J Pathol. 2018 Sep;188(9):1956–72.

[3)] Stolpe A van de et al. Enabling precision medicine by unravelling disease pathophysiology: quantifying signal transduction pathway activity across cell and tissue types. Sci Rep. 2019 Feb 7;9(1):1603.

[4)] Ben-David U, et al. Genetic and transcriptional evolution alters cancer cell line drug response. Nature. 2018;560(7718):325–30.

[5)] Verhaegh W et al. Identification of signal transduction pathway activity in patient-derived xenograft models in comparison with their originating clinical samples of a variety of human cancer types. Cancer Res. 78, 2018;1052–1052.5.

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