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EXPLORING UNCHARTED INTERACTIONS WITH

CELL SIGNALING PATHWAY ANALYSIS

FROM PROTEIN TO PHENOTYPE

Cellular signaling is an elaborate, dynamic, and interactive system of inter- and intracellular communication, responsible for governing and coordinating activities, from the basic to the complex. Amidst the intricate signaling pathways and their effector molecules, these complex interactions require detailed analyses to parse the pathways and unlock the unknown.

PHENOTYPIC SCREENING

Phenotypic assays explore the behavioral responses of physiologically relevant cell types, including stem cells, 3-D cultures, and organoid cultures, at the whole-cell level. This strategy is particularly efficient at identifying chemicals and conditions that globally modulate a cell's behavior. Typically, this involves looking at disease- or stimulus-driven biological responses, followed by target deconvolution, to identify the molecular target. However, due to phenotypic screening's reliance on optical analysis, accurate and reproducible cellular imaging is an absolute requirement for consistent, quantifiable data.

Multiparametric phenotypic profiling enables the simultaneous analysis of a variety of phenotypic changes (mRNA, protein, morphology, etc.) in response to the addition of agents of known composition, providing key insights into cellular behavior. This offers the many benefits of high-throughput analysis, although, the level of throughput is negatively correlated with the ability to multiplex.[1]

HCA (high-content analysis) combines high-throughput, automated imaging with analysis to extract data at the single-cell level. From 3-D microtissue imaging and live-cell imaging to protein-protein interactions, this cutting-edge technology has a myriad of applications.

References:

1. Y. Feng et al., "Multi-parameter phenotypic profiling: using cellular effects to characterize small-molecule compounds," *Nat Rev Drug Discov*, doi: 10.1038/nrd2876, 2009.

2. A.J. Hughes et al., "Single-cell western blotting," *Nat Methods*, doi: 10.1038/nmeth.2992, 2014.

3. M.F. Elshal et al., "Multiplex bead array assays: Performance evaluation and comparison of sensitivity to ELISA," *Methods*, doi: 10.1016/j.ymeth.2005.11.010, 2006.

PHENOTYPIC SCREENING

TARGET-BASED SCREENING

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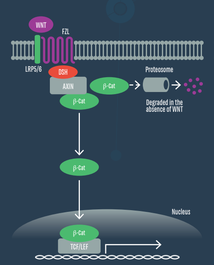
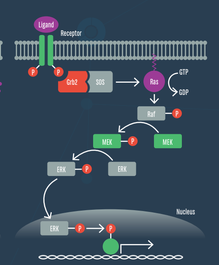
CANONICAL WNT SIGNALING



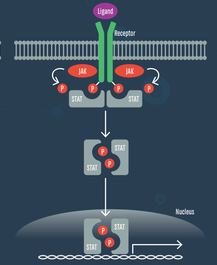
Canonical WNT signaling regulates gene transcription by modulating the concentration of β-catenin. In the absence of WNT, a complex including axin and GSK-3β constitutively phosphorylates β-catenin, leading to its proteosomal degradation. β-catenin levels and β-catenin phosphorylation levels can be monitored by Western blot, IHC, ELISA, and no-wash proximity assays.

MAPK SIGNALING



MAPK signaling is involved in the regulation of cellular homeostasis, including the proliferation, differentiation, migration, and survival of cells. MAPK signaling can be assessed by quantifying changes in MAPK phosphorylation (such as JNK, p38, or ERK) by Western blot, ELISA, or no-wash proximity assays. Additionally, live-cell HCA is a useful tool to study MAPK-induced cell migration and cell proliferation.

JAK/STAT SIGNALING



JAK/STAT signaling controls the expression of genes associated with immunity, proliferation, apoptosis, and oncogenesis. Changes in STAT levels can be detected and quantified using a variety of assays including Western blot, IHC, ELISA, and no-wash proximity assays.

TARGET-BASED SCREENING

Target-based screening is a directed methodology that measures the effect of selected compounds on a target protein or nucleic acid sequence via biochemical or cell-based in vitro assays. This approach delivers a high level of precision and is well suited to high-throughput screening (HTS), however, the narrow scope of target-based screening may limit new discovery and overlook modulators that globally regulate related signaling networks not under investigation.

Western blots detect changes in protein expression or modification. While this technology typically looks at a population of cells, new technologies have facilitated the analysis of protein changes on a deeper, single-cell level.[2]

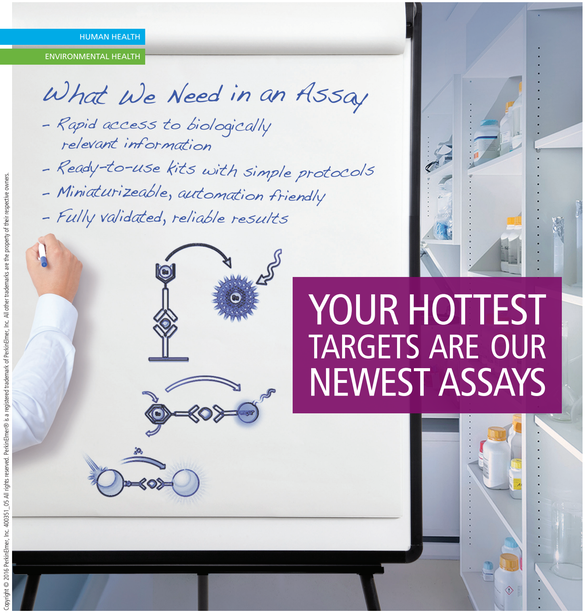
ELISAs (enzyme-linked immunosorbent assays) are useful tools for detecting and quantifying a protein of interest. ELISAs are highly quantitative and generally reproducible, however, their dynamic range is narrow in relation to other technologies, like multiplex assays.[3]

No-wash proximity assays, including bead-based, amplified luminescent proximity assays, and TR-FRET (time-resolved fluorescence resonance energy transfer), are considered to be superior to conventional ELISAs because of their high sensitivity, ease of use, ability to be miniaturized, and wide dynamic range, which makes them ideal assays for HTS. Not only are these robust technologies useful for antibody-based assays, but they can also be applied to protein-protein and protein-DNA/RNA interaction assays.

IHC (immunohistochemistry) characterizes the subcellular distribution and localization of pathway-signaling partners and differentially expressed proteins, allowing researchers to identify deleterious aberrations present in diseased or abnormal cells.

HUMAN HEALTH

ENVIRONMENTAL HEALTH



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HUMAN HEALTH

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ON THE PATHWAY TO DISCOVERY
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