PTMScan® Direct: Quantitative, Multiplexed Monitoring of Signaling Pathways

Matthew P. Stokes, Jeffrey C. Silva, Charles L. Farnsworth, Xiaoying Jia, Kimberly A. Lee, Roberto D. Polakiewicz, Michael J. Comb • Cell Signaling Technology, Danvers, MA

Introduction

PTMScan® Direct is an immunoaffinity-based method that allows identification and quantification of peptides from selected signaling pathways or protein types. This strategy allows focused, multiplexed analysis of hundreds of post-translationally modified peptides from proteins known to be critical to cellular signaling events, bypassing the lack of specificity inherent to traditional data-dependent LC-MS/MS analysis. Six PTMScan Direct reagents have been validated to date (Multipathway Reagent, Ser/Thr Kinases Reagent, Tyr Kinases Reagent, Akt/PI3K Pathway Reagent, DNA Damage/Cell Cycle Reagent, and Apoptosis/Autophagy Reagent). Reagents were validated using both human cell lines and mouse tissues. Cell lines, tissues, xenografts, or any other starting material from a species for which a genomic database exists can be used as starting material for PTMScan Direct studies, and the method is amenable to both label-free quantification of chromatographic peak areas as well as isotopic labeling methods such as SILAC.

PTMScan® Direct Method

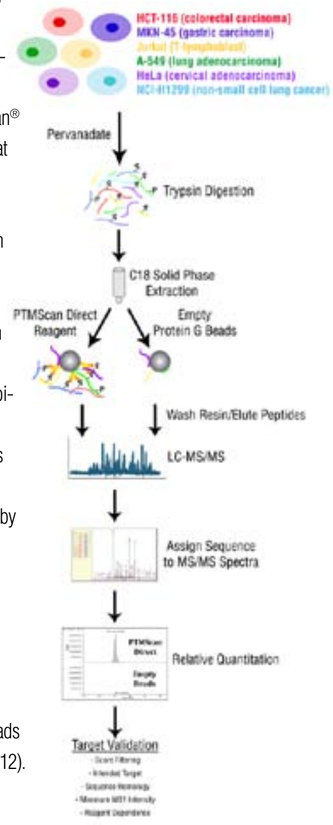

Figure 1: PTMScan® Direct Reagent validation strategy. PTMScan Direct is a published method (Stokes et al, 2012) adapted from the original PhosphoScan® method (Rush et al, 2005) developed at Cell Signaling Technology. PTMScan Direct Reagents are validated using mixtures of pervanadate treated human cancer cell lines digested with trypsin. Peptides are desalted over C18 columns and immunoprecipitated with either the PTMScan Direct reagent or empty Protein G beads. Immunoprecipitated peptide mixtures are analyzed by LC-MS/MS and relative quantitation is performed. Validated peptides meet several strict criteria: they are targeted by the reagent or are homologous to a target, they must pass score filtering (Lundgren et al. , 2009) and signal intensity thresholds, and they must be present in higher abundance in the PTMScan Direct reagent immunoprecipitation than the empty Protein G beads immunoprecipitation. (Stokes et al., 2012).

PTMScan® Direct Reagents

1. **Multipathway Reagent**
2. **Ser/Thr Kinases**
3. **Tyr Kinases Reagents**
4. **Akt/PI3K Reagent**
5. **DNA Damage/ Cell Cycle Reagent**
6. **Apoptosis / Autophagy Reagent**

Interaction maps were generated from the STRING database (string.embl.de) using high confidence scores (>0.700) from experimental, database, and text mining lines of evidence. Interactions were also defined from the substrate search page of PhosphoSitePlus® (**www.phosphosite.org**). Node colors and shapes denote different protein classes. Edge color denotes interaction type.

Multipathway Reagent

Monitoring phosphorylation of critical proteins from many signaling pathways.

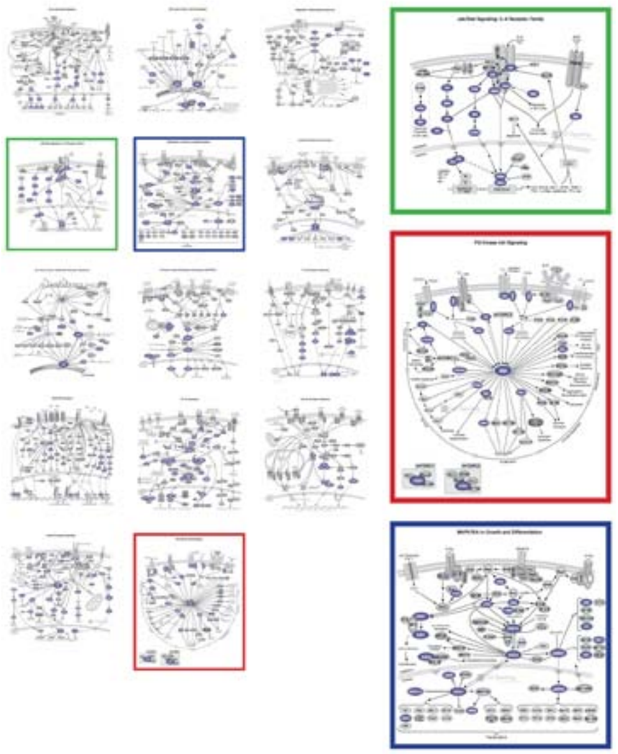

Figure 2: Multipathway Reagent coverage of selected signaling pathways. Multipathway reagent targets are shown in **purple**.

Ser/Thr Kinases and Tyr Kinases Reagents

Profiling sites of activation and inhibition on protein kinases.

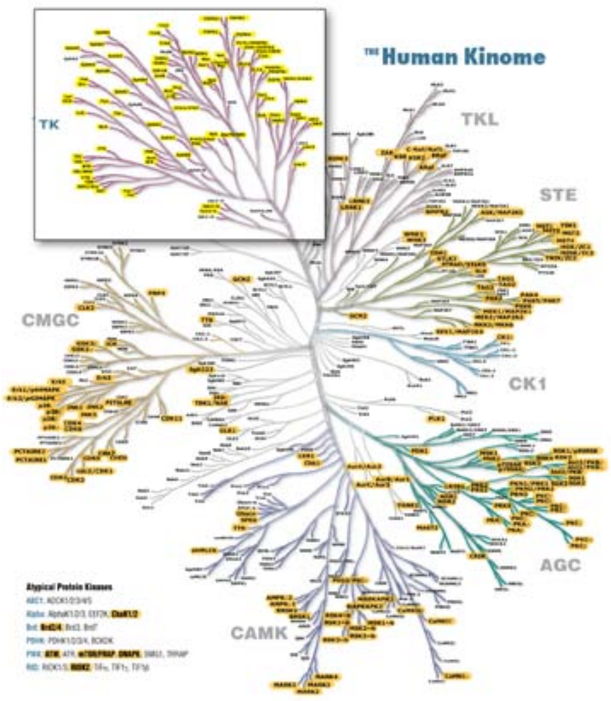

Figure 3: Ser/Thr Kinases Reagent and Tyr Kinases Reagent targets mapped onto the human kinome tree. Yellow highlighting indicates kinases for which peptides are identified using the reagents. Tyr Kinases Reagent coverage is shown in the inset ("TK").

Akt/PI3K Pathway Reagent

In-depth analysis of PI3K/Akt and associated signaling pathways.

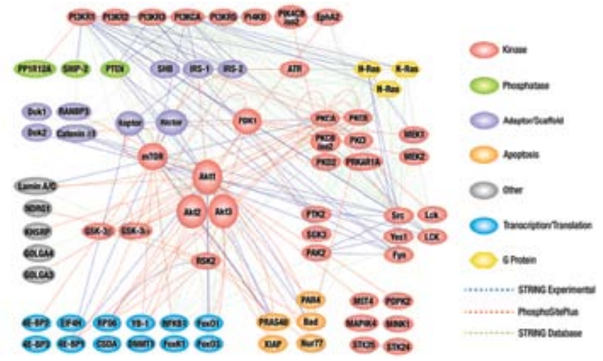

Figure 4: Akt/PI3K Pathway Reagent interaction map.

MOUSE TISSUE STUDY

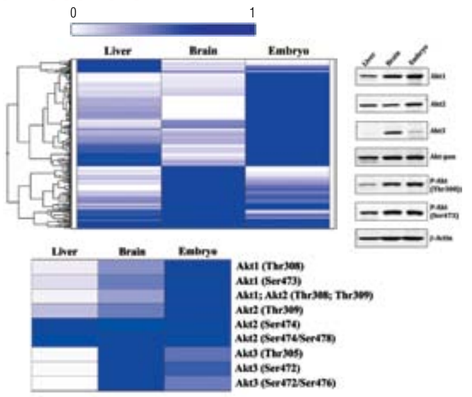

Figure 5: Akt / PI3K Pathway Reagent mouse tissue profiling. Hierarchical clustering of relative intensities for mouse liver, brain, and embryo. Each row represents a different validated Akt/PI3K pathway peptide. The maximum intensity across the three tissues was set to 1 and the other two intensities normalized to the max. **Blue** indicates higher intensity.

DNA Damage/Cell Cycle Reagent

A comprehensive view of the DNA damage response.

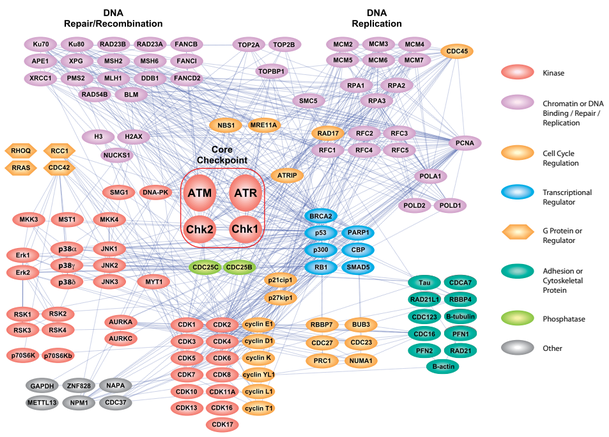

Figure 5: DNA Damage / Cell Cycle Reagent interaction map.

HeLa -/+ UV DAMAGE

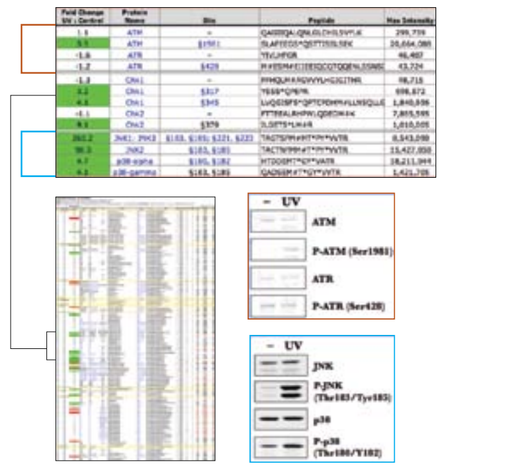

Figure 7: DNA Damage/Cell Cycle Reagent profiling of response to UV damage. Hela cells were untreated or treated with 500mJ/cm² UV light and harvested at 2hr post treatment. Fold changes were calculated from chromatographic peak heights/areas. **Green** cells indicate peptides more abundant with UV damage, **Red** cells indicate peptides less abundant. Selected DNA damage response proteins are shown in detail with accompanying western blots.

Apoptosis/Autophagy Reagent

Monitoring activity of apoptotic proteins and autophagolytic pathways.

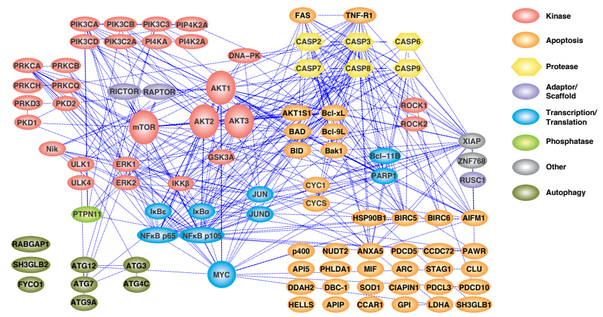

Figure 8: Apoptosis / Autophagy Reagent interaction map.

HeLa -/+ UV DAMAGE

Figure 9: Apoptosis/Autophagy Reagentprofiling of response to UV damage. Quantitative data for caspase peptides identified with the Apoptosis / Autophagy reagent used to probe UV damaged HeLa cells (500mJ/cm², harvest 2hr post treatment). **Green** indicates increased abundance relative to control.

Summary

PTMScan Direct is a recently published method that allows identification and quantification of hundreds of peptides from selected protein types or signaling pathways. This approach allows focus on proteins of interest instead of the random sampling of peptides that occurs in traditional data-dependent proteomic analysis. PTMScan Direct is widely applicable in drug development and discovery, as well as in any application where monitoring of known signaling pathways is desired.

References

1. Stokes M.P.et al. (2012) *Mol. Cell Proteomics.* 11(5): 187–201
2. Rush J. et al. (2005) *Nat. Biotechnol.* 23: 94-101
3. Lundgren, et al. (2009) *Curr. Protoc. Bioinformatics.* 13: Unit 13–3

Contact Information

Matthew P. Stokes
Cell Signaling Technology, Inc.
Tel: (978) 867-2467
Email: mstokes@cellsignal.com

PTMScan Services Department
Email: ptmscan@cellsignal.com
Visit www.cellsignal.com/services/index.html for more information regarding MethylScan

www.cellsignal.com/services