Quantitative Profiling of Post-Translational Modifications by SILAC approach with PEAKS®

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Introduction

Protein post-translational modifications (PTMs) play critical roles in diverse biological processes. LC-MS/MS-based isotope labeling strategies such as SILAC, iTRAQ and TMT has gained popularity to identify and quantify PTMs and their dynamics. The challenge of PTM quantitation by data-dependent acquisition is partially caused by the presence of missing values of low-abundant peptides, which decreases accuracy and sensitivity. To recover quantitation information of low-abundant peptides, a new algorithm was developed for PTM analysis. Initial tests showed increased sensitivity and accuracy for PTM profiling across complex biological samples.

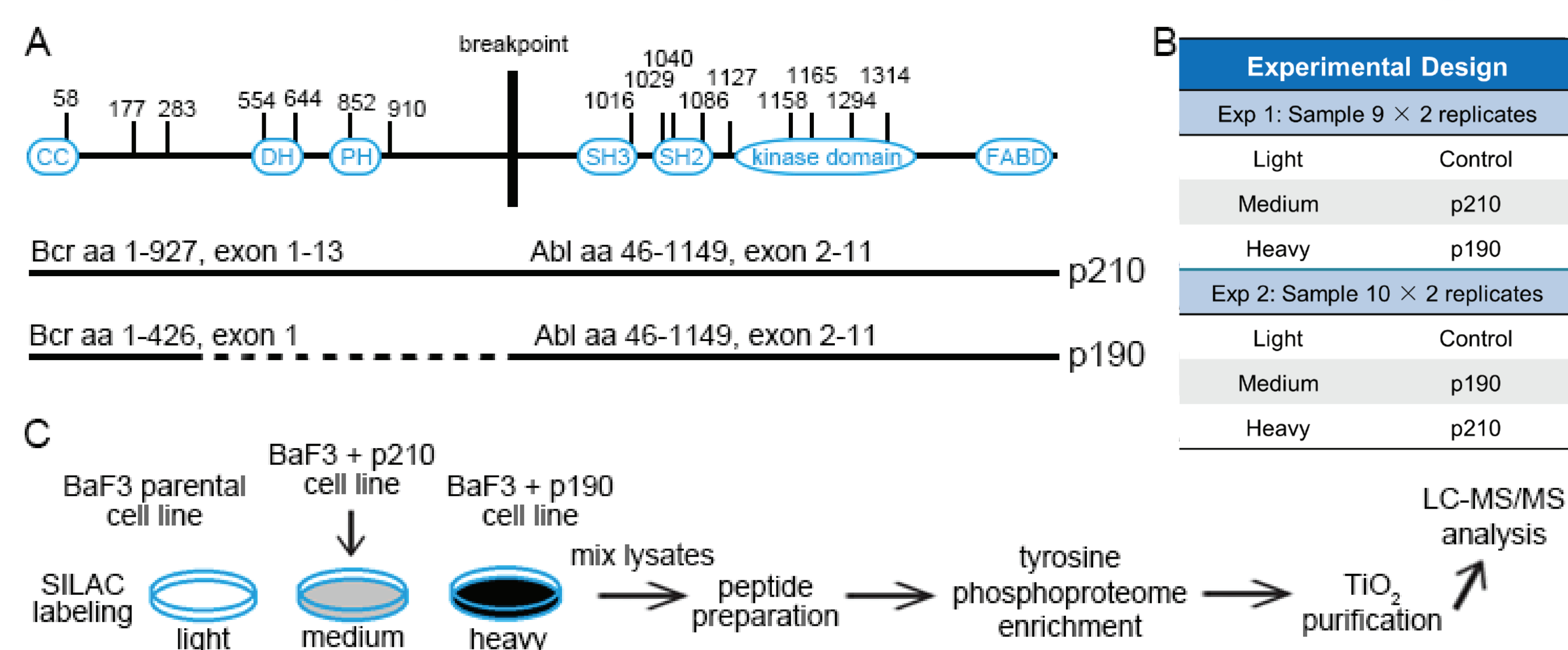
Methods

PTM Profile function for SILAC, TMT and iTRAQ types of MS data in PEAKS® Studio is performed as below:

1. Peptide features were extracted from MS1 data. MS2 data were searched against databases for peptide (unmodified and modified) identification. Alignment of multiple LC-MS runs was used to increase sensitivity and accuracy. Missing data were replaced by paired feature intensities by matching within tight retention time, isotopic envelops and mass windows to enable quantification without identification.
2. For each modification site identified, peptides that contain the PTM site are categorized into either unmodified or modified group based on whether the specific site is confidently modified.
3. Unmodified and modified peptide intensities in different samples/labeling channels are calculated based on:
   1) Precursor ion labeling (e.g. SILAC): the summed peptide feature area from MS1 extracted-ion chromatograms (XICs) of unmodified and modified groups for different labeling channels separately;
   2) Reporter ion labeling (e.g. TMT and iTRAQ): the summed peptide feature area from MS1 XICs of unmodified and modified groups that are distributed to different samples/labeling channels respectively based on the ratios of their reporter ion intensities.

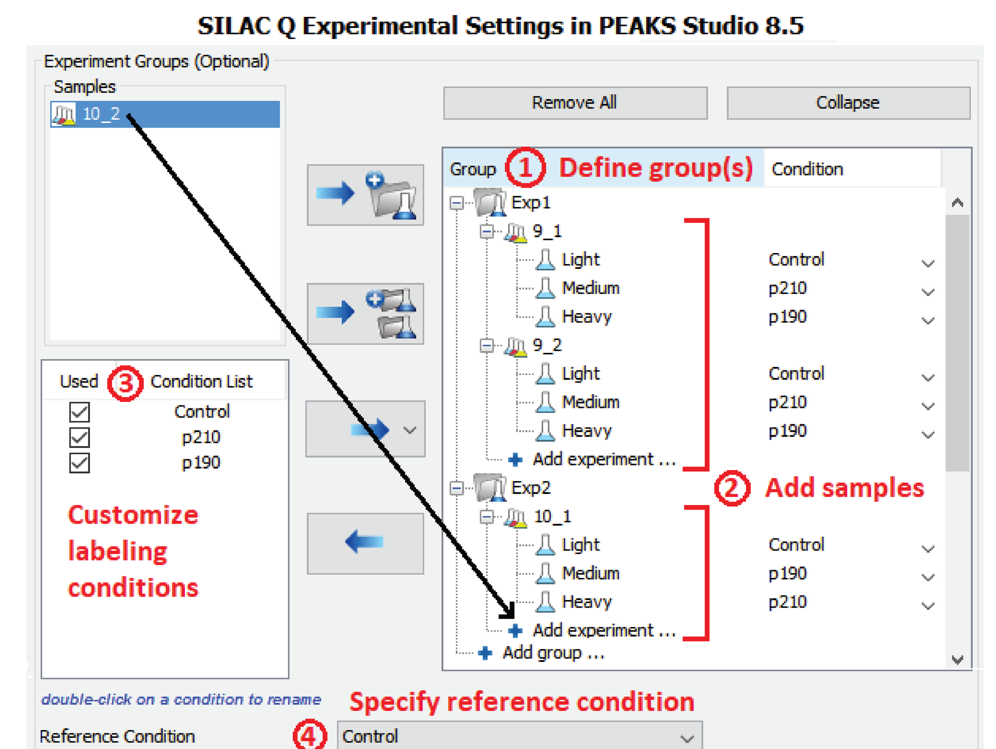
Furthermore, PTM quantification results can be normalized by protein expression levels automatically.

Case Study:SILAC-based PTM Profiling



Bcr-Abl protein domain organization (A) and overview of the phosphoproteome experiments (B, C). Data analyzed from [1]. Study aim: to map kinase activation state of Bcr-Abl tyrosine kinase (two major isoforms: p210 and p190) in leukemia.

Results

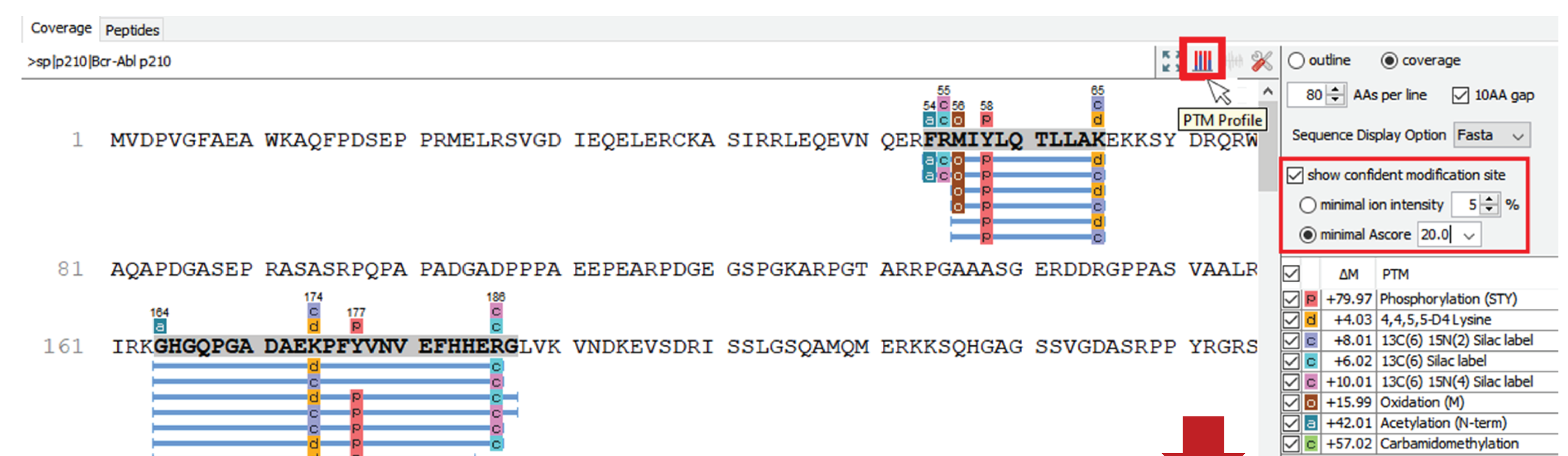


- **Experimental Setting**

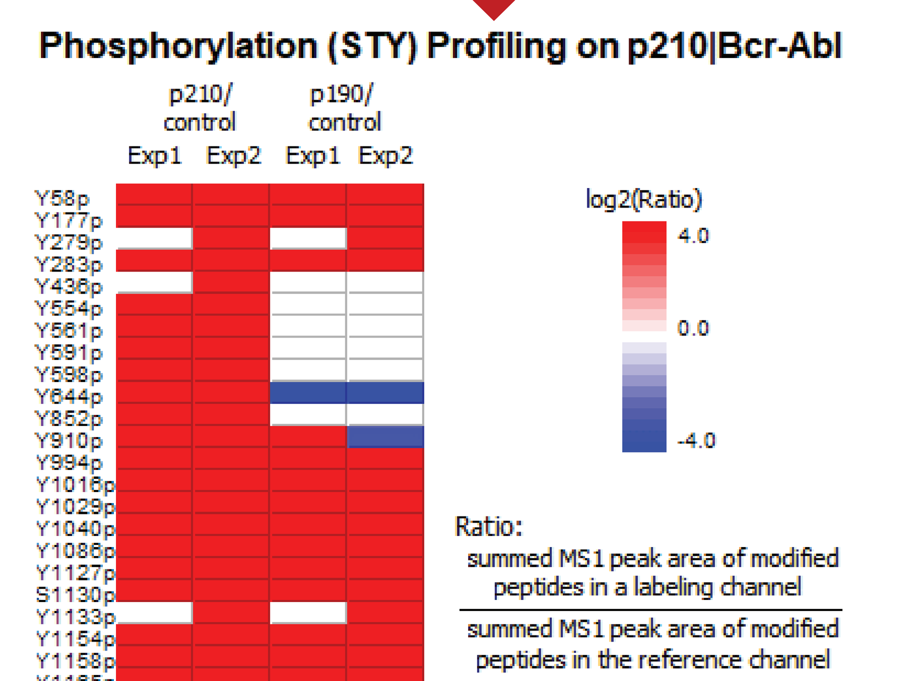
Murine BaF3 cells were transduced with human Bcr-Abl p210 and p190 cDNAs. Parental (untransduced) BaF3 cells were used as a control and grown in SILAC light media. Cells that expressed human p210 and p190 were labeled with SILAC medium and heavy media and swapped between 2 experiments (Exp 1 and 2). Phosphotyrosine peptides were enriched and analyzed by high-resolution LC-MS/MS. Experimental settings in PEAKS Q is shown accordingly.

- **Identification and Quantification of Phosphorylated Proteins with PEAKS PTM Profile**

MS data was analyzed in PEAKS® Studio 8.5 using the built-in SILAC-3plex (R6, K4|R10, K8) method in PEAKS Q for quantification. The phosphorylation profile of Bcr-Abl protein was analyzed with PTM Profile function.



25 phosphorylation sites of Bcr-Abl were quantified (modified peptides filtered by Ascore [2] > 20). Ratios of summed MS1 peak area of modified peptides containing a certain confident PTM site in p210 and p190 relative to the control are colored in the heatmap and detailed in the PTM profile table.



PTM Profile Table

Protein Position	PTM	-10logP	AScore	p210: Exp1 Ratio	p210: Exp2 Ratio	p190: Exp1 Ratio	p190: Exp2 Ratio
Y58	Phosphorylation (STY)	37.34	83.87	82.81	68.02	117.61	118.53
Y177	Phosphorylation (STY)	61.57	1000.00	102.94	123.30	98.12	146.38

Modified peptides containing the confident PTM site are listed in Peptides table.

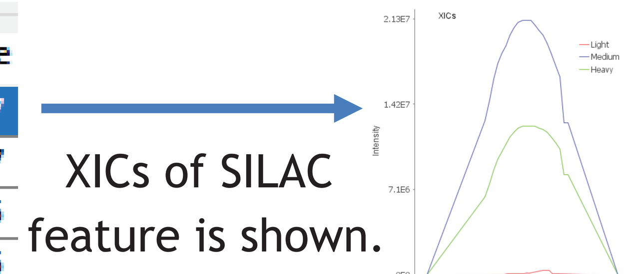
Peptides

Peptide	Peptide Position	AScore	p210: Exp1	p210: Exp2	p190: Exp1	p190: Exp2
F(+42.01)R(*)M(+15.99)IY(+79.97)LQTLLAK(*)	5	38.15	256.00	256.00	256.00	256.00
M(+15.99)IY(+79.97)LQTLLAK(*)	3	83.87	60.34	54.09	87.11	91.07
MIY(+79.97)LQTLLAK(*)	3	83.87	256.00	256.00	256.00	256.00

SILAC feature pairs associated with each peptide are listed in Feature Vectors table.

Feature Vectors

Sample	Fraction	Charge	Quality	Control Area	p210 Area	p190 Area	AScore
10_1	F1	2	54.48	2.71E4	1.67E6	2.84E6	83.87
10_2	F2	2	54.05	4.67E4	2.17E6	3.61E6	83.87
9_1	F3	2	55.83	5.32E4	2.31E6	3.44E6	78.06
9_2	F4	2	54.76	2.67E4	2.07E6	2.93E6	78.06



XICs of SILAC feature is shown.

References

[1] Reckel, S. et al, Leukemia 2017 Jul;31(7):1502-1512.
[2] Neubert, T. A. et al., Nature methods 2010, 7, 361-362.

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