PIQMIe: a web tool for semi-quantitative proteomics applied to dissect the mechanism of cancer therapy based on interference with DNA repair

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Summary

Stable isotope labeling with amino acids in cell culture (SILAC)-based mass spectrometry is a mature technique in semi-quantitative proteomics. In a typical SILAC proteome experiment, tens of thousands of peptides and thousands of (non-redundant) proteins are reliably identified and quantified using e.g., the widely-used MaxQuant software. Further downstream analyses of the result files are then facilitated using (dedicated) spreadsheet-based tools such as MS Excel or Perseus. Although these tools are useful they do not provide full control over the data as compared to a database management or information retrieval system. Moreover, they are prone to data-manipulation errors, lack interoperability and scalability. To remedy this, we have developed a web-based tool called PIQMIe (Proteomics Identifications Quantitations data Management and Integration service), and deployed it on a Cloud computing infrastructure. Here we used PIQMIe to mine and visualize the results from mass spectrometry experiments that not only reveal new DNA repair relevant interactors of the BRCA2 protein but also reveal changes in the BRCA2 complex induced by anti-cancer therapy.

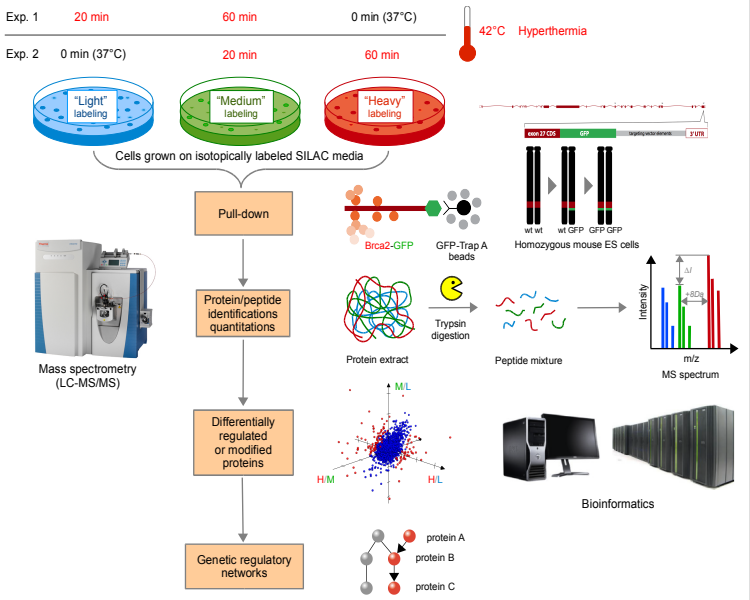

Figure 1. Triplex SILAC proteomics experiments with BRCA2-GFP pull-down to study hyperthermia-induced changes of the BRCA2 complex.

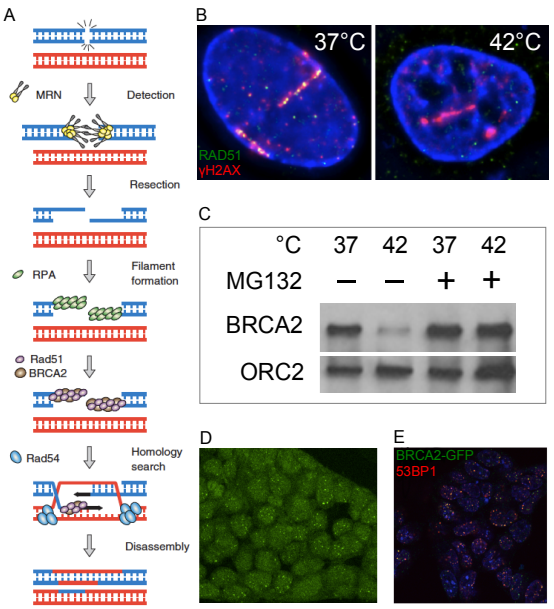

Figure 2. Homologous recombination is an essential DNA repair pathway mediated by BRCA2 and RAD51 (A). Clinically used mild hyperthermia leads to mislocalization of RAD51 to DNA breaks, thereby augmenting DNA damage-based anti-cancer therapies (B). The mislocalization occurs due to hyperthermia-triggered proteasomal degradation of BCRA2 (C). BRCA2-GFP fluorescence can be detected in IR (8 Gy) induced DNA repair foci (D). BRCA2-GFP colocalizes with sites of DNA damage marked by 53BP1 (E).

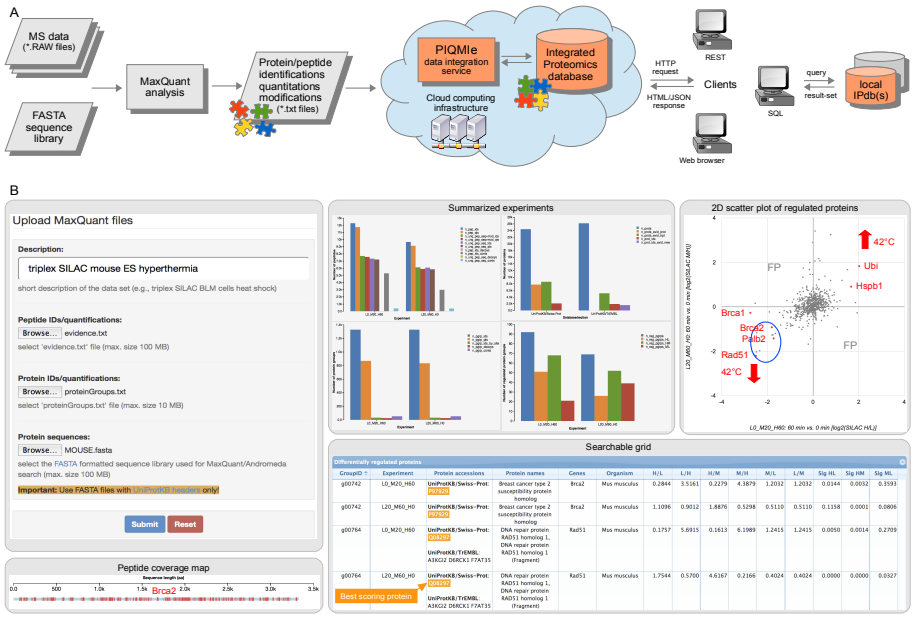

Figure 3. Computational proteomics workflow including the PIQMIe data integration service. After MaxQuant analysis users upload the used protein sequence library and result files to the server, which then populates an embedded relational database from the input data. The indexed database(s) can be accessed i) locally via Structured Query Language (SQL), and ii) remotely via programmatic (RESTful) web service or (iii) web-based user interface (A). The web interface consists of data submission page and results pages with barcharts, 2D scatterplot, searchable grid and peptide coverage map (B). Note: In the scatterplot, potential new interactors of the BRCA2 complex are delineated by blue ellipse.