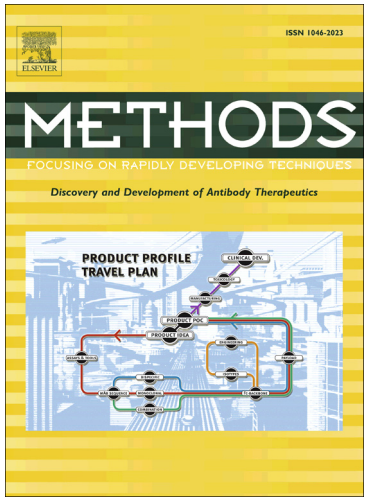
Accepted Manuscript



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PII: S1046-2023(15)30066-9
DOI: http://dx.doi.org/10.1016/j.ymeth.2015.08.022
Reference: YMETH 3779

To appear in: *Methods*

Received Date: 30 June 2015
Revised Date: 26 August 2015
Accepted Date: 27 August 2015

Please cite this article as: D.G. Carneiro, T. Clarke, C.C. Davies, D. Bailey, Identifying novel protein interactions: proteomic methods, optimisation approaches and data analysis pipelines, *Methods* (2015), doi: http://dx.doi.org/10.1016/j.ymeth.2015.08.022

Identifying novel protein interactions: proteomic methods, optimisation approaches and data analysis pipelines

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Abstract:

The technological revolution in high-throughput nucleic acid and protein analysis in the last 15 years has launched the field of 'omics and led to great advances in our understanding of cell biology. Consequently the study of the cellular proteome and protein dynamics, in particular interactomics, has been a matter of intense investigation, specifically the determination and description of complex protein interaction networks in the cell, not only with other proteins but also with RNA and DNA. The analysis of these interactions, beginning with their identification and ultimately resulting in structural level examination, is one of the cornerstones of modern biological science underpinning basic research and impacting on applied biology, biomedicine and drug discovery. In this review we summarise a selection of emerging and established techniques currently being applied in this field with a particular focus on affinity-based purification systems and their optimisation, including tandem affinity purification (TAP) tagging, isolation of proteins on nascent DNA (IPOND) and RNA-Protein immunoprecipitation in tandem (RIPiT). The recent application of quantitative proteomics to improve stringency and specificity is also discussed, including the use of metabolic labelling by stable isotope labelling by amino acids in cell culture (SILAC), localization of organelle proteins by isotope tagging (LOPIT) and proximity-dependent biotin identification (BioID). Finally, we describe a range of software resources that can be applied to interactomics, both to handle raw data and also to scrutinise its broader biological context. In this section we focus especially on open-access online interactomic databases such as Reactome and IntAct.

Keywords: interactomics, protein-protein interaction, RNA-protein interaction, DNA-protein interactions, affinity purification, TAP-tagging