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Combining in vitro Protein Expression with AlphaLISA Technology to Study Protein-Protein Interaction

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Abstract : The demand for a rapid and more efficient technique to identify protein-protein interaction particularly in the areas of therapeutics and diagnostics development is growing. The method described here is a rapid in vitro protein-protein interaction analysis approach based on AlphaLISA technology combined with Leishmania tarentolae cell-free protein production (LTE) system. Cell-free protein synthesis allows the rapid production of recombinant proteins in a multiplexed format. Among available in vitro expression systems, LTE offers several advantages over other eukaryotic cell-free systems. It is based on a fast growing fermentable organism that is inexpensive in cultivation and lysate production. High integrity of proteins produced in this system and the ability to co-express multiple proteins makes it a desirable method for screening protein interactions. Following the translation of protein pairs in LTE system, the physical interaction between proteins of interests is analysed by AlphaLISA assay. The assay is performed using unpurified in vitro translation reaction and therefore can be readily multiplexed. This approach can be used in various research applications such as epitope mapping, antigen-antibody analysis and protein interaction network mapping. The intra-viral protein interaction network of Zika virus was studied using the developed technique. The viral proteins were co-expressed pair-wise in LTE and all possible interactions among viral proteins were tested using AlphaLISA. The assay resulted to the identification of 54 intra-viral protein-protein interactions from which 19 binary interactions were found to be novel. The presented technique provides a powerful tool for rapid analysis of protein-protein interaction with high sensitivity and throughput.

Keywords : AlphaLISA technology, cell-free protein expression, epitope mapping, Leishmania tarentolae, protein-protein interaction
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