Rapid Dispatches Highlights

Kinase discrimination

Determining the target site for a kinase can help in understanding protein biology. While in most cases, serine/threonine protein kinases are basophilic, a small number are in fact acidophilic. One such acidophilic kinase is casein kinase 2 (CK2), which has been implicated in cell cycle control and DNA repair. Polo-like kinase (PLK) family members PLK2 and PLK3 were recently found to recognize a consensus sequence similar to CK2, making the discrimination of acid phosphosites generated by CK2 or PLK2 and PLK3 difficult. In a recent *Rapid Dispatches* Report, Mauro Salvi and his colleagues from University of Padova in Colombo, Italy and Washington University Medical School in St. Louis, Missouri describe a series of tools that can be used to accurately differentiate these acidophilic kinases. To begin with, the authors provided the first determination of the optimal conditions and co-factors for PLK2 and PLK3 kinase activity. Since conditions for CK2 activity are well established, the authors could compare and contrast these optimal conditions to identify cofactors and conditions that could be used for discrimination. CK2 is able to use GTP as a phosphodonor substrate almost as well as ATP in phosphorylation reactions. However, when the authors tested the ability of either PLK2 or PLK3 to use GTP, they found it could not substitute for ATP as a phosphodonor and therefore GTP could be used to discriminate between the activity of CK2 and PLK2 and PLK3. In addition, two inhibitors, CX-4945 (a CK2 inhibitor) and BI 2536 (a PLK2 and PLK3 inhibitor), were tested for their ability to discriminate between these kinases. In both cases, the inhibitors were effective against their previously described targets, but neither could inhibit the other acidophilic kinase, making CX-4945 and BI 2536 a second tool that can be used to discriminate CK2 from PLK2 or PLK3 activity. Taken together, Salvi and his team have identified a series of tests that can be used to effectively discriminate the similar target specificities of CK2 and PLK2 and PLK3 acidophilic kinases. **See: “Tools to discriminate between targets of CK2 vs PLK2/PLK3 acidophilic kinases.”**

Category: Biochemistry

SUMOnation

Small ubiquitin-related modifiers (SUMOs) are ubiquitin-like biomolecules that are able to conjugate to target proteins. Three SUMO

Cell surgery

Studying isolated, single cells is yielding new insights into transcriptional regulation and protein production patterns. However, working with single cells can be a challenge, especially when it comes to their isolation from complex tissues. In a recent *Benchmark* article available at *Rapid Dispatches*, Zhifeng Shao and colleagues from Shanghai, China describe an approach to microdissect single nuclei from solid tumors for whole genome sequencing applications. For the authors, the first challenge was to identify single nuclei of interest from cryosectioned tissue samples that could be dissected using laser capture microdissection techniques. Shao and co-authors found their answer through the use of optimal thickness and through-focus evaluations of the sample. First the authors DAPI-stained cells such that single nuclei could be located in three-dimensions. The authors next evaluated the maximum number of intact nuclei in sections of varying thickness. Here, the authors found that, for their tissue of interest, below 14 μm resulted in non-intact nuclei and above this thickness, overlapping nuclei were observed. Following preparation of sections of the correct size, the author located nuclei of interest using the through-focus procedure and then cut the nuclei of interest with the laser. Using the isolated material, Shao's team performed whole genome amplification and then tested the resulting amplified product to demonstrate the effectiveness of SNP analysis and PCR using the collected single nuclei sample. This approach, and others like it, will surely facilitate the genetic exploration of single cell samples in the years to come. **See: “Microdissection of spatially identified single nuclei in a solid tumor for single cell whole genome sequencing.”**

Category: General Methods

DAPI-stained single, complete nuclei in a cryosection

isoforms exist—SUMO-1, SUMO-2, and SUMO-3—which are involved in cellular processes such as DNA repair, transcription regulation and subcellular protein localization. While assays are available to examine attachment of SUMO isoforms to proteins (a process called SUMOylation), there is still a need for additional tools to examine global patterns of SUMOylation in cells. In a new article at *Rapid Dispatches*, Castoralova et al. from the Institute of Chemical Technology in Prague, describe the application of dot blotting to the rapid detection of global changes in SUMO-2/3 conjugation. The authors demonstrate the ability to assess SUMO-2/3 conjugation globally by examining the increase in signal intensity on the dot blot. They also provide data showing the linear relationship between dot blot signal and levels of global SUMO-2/3 conjugation following heat shock. While Western blotting has been used in the past to assay such conju-

gation events, the availability of this new dot blot approach could save time and present an opportunity to assay a far greater number of samples for interested researchers. **See: “Using dot blots with immunochemical detection to evaluate global changes in SUMO-2/3 conjugation.”**

Category: Molecular Biology

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