Structuring your workflow

Chromosomal structural rearrangements have are associated with human cancers and other genetic diseases. But interrogating large numbers of structural variants can be a challenge for researchers, especially since traditional approaches rely on lower-throughput Sanger sequencing and manual sample preparation methodologies. Now, structural analysis is getting a much needed update in this issue of *BioTechniques* as Nic Waddell, Sean Grimmond, and their colleagues describe a new automated workflow focused on the verification of chromosomal rearrangements that requires half the time of the traditional approach while processing more events. The two keys to this new workflow are (i) the use of dedicated computational tools to design primers and assemble sequence contigs and (ii) moving from Sanger sequencing to higher-throughput next-generation platforms. Using their pipeline, the authors were able to examine more than 300 breakpoints in a single experiment, identifying more than 80% of the breakpoints at single nucleotide resolution without any loss in accuracy.

See “A workflow to increase verification rate of chromosomal structural rearrangements using high-throughput next-generation sequencing” on page 31.

Find the link

In protein biochemistry, the ability to attach a specific tag to a protein of interest, or even attach two proteins together, is crucial for purification experiments, localizing a protein in a cell, or studying how a protein of interest interacts with other proteins. Amino acid linkers are used to separate these proteins and tags, yet finding the optimal length of an amino acid linker can be a cumbersome and time-consuming task. This month, Ulrich Schwaneberg and his colleagues present a ligase-free, restriction enzyme independent method to generate multiple protein fusions possessing different linker lengths in a single reaction. The authors took advantage of their previously developed cloning approach, called PLICing, to develop the new P-Link system, which makes use of phosphorothioate primers for amplification. In a single cloning and transformation step, P-Link is able generate protein fusions with linkers varying from 1 to 16 amino acids in length that users can then test for activity and function. In one proof-of-concept test, Schwaneberg and his co-authors fused the P450cin monooxygenase (CinA) to a flavodoxin shuttle protein (CinC) and then screened for production of 2-β-hydroxy-1,8-cineole. Their results showed that fusions with linkers 10 amino acids long had optimal enzymatic activity in this system.

See “P-Link: A method for generating multicomponent cytochrome p450 fusions with variable linker length” on page 13.

Selected and written by Nathan Blow, Ph.D.

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Cell-specific proteomic analysis of long-term co-cultures

Studying cell-cell communication using proteomics requires determining which proteins belong to which cells. While SILAC (stable isotope labeling with amino acids in cell culture) disaggregates mass spectrometry data of cell mixtures when one cell type has been labeled, separation fidelity diminishes with ongoing co-culture. Cell-type specific labeling with amino acid precursors (CTAP) enables long-term co-culture by stably expressing a non-mammalian enzyme capable of converting a lysine precursor to L-lysine. In co-cultures where one precursor is isotopically labeled, cells able to convert that particular substrate are labeled, unlike cells needing the other (unlabeled) precursor. Unfortunately, suboptimal performance of CTAP necessitates daily media exchange, interrupting intercellular communication. But after testing and engineering a variety of enzymes, Tape et al. improved CTAP, achieving 90% fidelity of cell-specific labeling after 10 days of co-culture without the need for media exchange. This new method, which detected phosphorylation events missed in interrupted co-culture, should enhance studies of intercellular communication.

Tape et al. Cell-specific labeling enzymes for analysis of cell-cell communication in continuous co-culture. [Epub ahead of print, May 12, 2014; doi:10.1074/mcp.O113.037119].

Imaging protein-protein and RNA-protein binding in vivo

Fluorescence complementation excels at assaying protein-protein and RNA-protein interactions in cell culture, but this method fails in vivo due to tissue opacity at excitation wavelengths below 600 nm. Han et al. solve this problem with mNeptune, a fluorophore with excitation and emission peaks at 600 and 650 nm, respectively. For bimolecular assays, mNeptune is split at amino acid 155, with each fragment expressed in fusion with potential protein interactors. For trimolecular assays, one half of mNeptune is fused with MS2 binding protein and the other with the RNA binding protein under study. An RNA containing the MS2 hairpin and the ligand sequence under study draws the mNeptune halves together, if the anticipated RNA-protein interaction occurs. Complementation could be detected in mice injected subcutaneously with cells expressing Jun and Fos constructs or influenza NS1 protein and RNA partners. No correction for tissue autofluorescence was required, confirming the suitability of this new system for in vivo monitoring of protein and RNA binding events.

Han et al. In vivo imaging of protein-protein and RNA-protein interactions using novel far-red fluorescence complementation systems. [Epub ahead of print, May 9, 2014; doi:10.1093/nar/gku408].

Selected and written by Nijsje Dorman, Ph.D.

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