Rapid Dispatches Highlights

High-throughput, mass genotyping

In 2011, a group of researchers from Denmark reported in the pages of *BioTechniques* on their use of the Roche Genome Sequencer FLX to profile short tandem repeat (STR) loci for genotyping applications. The approach allowed interested researchers to move beyond the confines of traditional capillary electrophoresis (CE) STR profiling techniques to extend genotyping to a greater number of samples in a single instrument run. At the time, it looked as if the Genome Sequencer FLX was one of the few platforms capable of such STR profiling owing to the longer read lengths that can be attained using the sequencing methodology. But not wanting the short-read technology platforms and their higher-throughput to feel left out of the genotyping game, a team from the United States has now designed an STR genotyping pipeline for use with these sequencing platforms. Reporting in a May 2012 article appearing in *Rapid Dispatches*, the author's demonstrate STR genotyping with 99% confidence from less than 20,000 reads aligned to a reference genome using the Illumina GAII system. In addition to examining known STRs, the authors, just like the Denmark team from 2011, demonstrate the power of next-generation sequencing for STR profiling to also identify variant alleles and generate quantitative measurements to resolve mixed samples. This article closes a methodological gap, giving all researchers, regardless of sequencing platform, the opportunity to apply next-generation sequencing in their STR profiling efforts.

Simplifying complex isolation

Isolating proteins for downstream characterization is obviously a staple of any modern biochemistry lab. While capturing individual proteins can be a snap, when it comes to pulling down proteins that are aggregated in complexes, things can become a little tricky. But studying complexes is important -- identifying the proteins that make up various complexes is essential to understand how those complexes function in the cell. In a May article published through *Rapid Dispatches*, John LaCava and his team from The Rockefeller University

in New York, NY, detail a reliable methodology for capturing affinity-tagged proteins and associated complexes from human cell lines. Through experiments describing the isolation of protein complexes involved in RNA metabolism, LaCava and his co-authors detail the multiple optimization steps they went through that resulted in high yields and low background from human cells expressing tagged proteins at near endogenous-levels. This pipeline, and the detailed optimization steps, will be of great value to all researchers using protein purification to study protein biology.

Iso-heme

Multi-heme cytochrome C is of great biotechnology interest. To study the structure of heme containing proteins, the ability to label hemes presents an effect strategy to uncover structure-function relationships. Reporting in *Rapid Dispatches* in May, Ricardo Louro and colleagues describe a new methodology

for isotopically labeling hemes for spectroscopic analysis. Using a small tetraheme cytochrome from *Shewanella oneidensis*, the authors demonstrate the ability to efficiently express the heme containing protein and, in a single step, incorporate isotopically labeled hemes at specific atom positions. The authors suggest that their approach is generally applicable to heme proteins, opening a new door for structural characterization of this interesting and important class of proteins.

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Full articles can be found at biotechniques.com/rd

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Two are better than one

Imaging fluorescently-labeled antibodies within living animals can provide unique insights into basic biological processes as well as enhance our understanding of many antibody-based therapies. While visualizing multiple antibodies targeting different tissues or tumors is commonly performed, visualizing two different antibodies targeting the same tumor simultaneously has proven to be a far greater challenge owing to the difficulty in analyzing the overlapping fluorescence spectra. However, as David Krag and his colleagues from the University of Vermont report in *Rapid Dispatches* this does not always have to be the case. Using antibodies labeled with infrared fluorescent dyes of differing wavelengths, Krag's team describe an approach for assessing multiple antibody accumulation within single tumors as well as in normal body tissues. The authors suggest that this methodology will assist researchers in the design of therapeutic dosing strategies as well as identifying potential interactions between antibodies that could affect pharmacokinetics.

Representative images and signal quantification over time (30 min to 96 h) for infusion of a single antibody in two groups of mice.