Citations

It's Elementary

Bead-based assays for nucleic acids can be multiplexed to the extent that the differently labeled microbeads can be reliably distinguished from one another. In the optical detection space, fluorescence dyes or quantum dots are popular tags but spectral overlap and intensity variations mean that problems arise once several targets need to be differentiated. Color-coded microparticles offer greater scope for multiplexing, but even this approach is limited by dye compatibility and microsphere polydispersity. In proteomic assays, labeling with an element tag enables nonoptical detection by mass spectrometry (MS). However, the few attempts to extend this strategy to nucleic acid detection have been hampered by unwieldy labeling and pre-MS separation procedures. Now, in *Angewandte Chemie International Edition*, Han et al. offer a simpler alternative. Their elemental labeling and detection strategy uses rare-earth element tags and inductively coupled plasma (ICP)-MS for quantitation. The reporter tags are made by functionalizing an oligonucleotide probe with a thiol group, then reacting the sulfhydryl DNA with the maleimide group of MMA-DOTA, which can chelate rare earth elements. Thus one oligonucleotide probe might be labeled by yttrium, another by europium, and so on (up to 15-plex in this publication). At the same time, a magnetic microparticle is

functionalized with capture oligonucleotides recognizing the targeted nucleic acids. After sandwich hybridization and washing to remove the unbound probes, the element-tagged probes are eluted and measured by ICP-MS. One of the probes recognizes a spiked-in sequence, allowing relative quantification. For absolute quantification, the authors developed a strategy involving an additional reporter oligonucleotide, the dilution probe. Instead of being labeled with a natural rare earth element, the dilution probe is tagged with an artificially enriched isotope of that element, and it is directed against the magnetic microparticle-conjugated capture probe, not the DNA target. Using isotope dilution calculations, the absolute DNA amount can be derived. In a duplex assay using dysprosium and erbium and ^{161}Dy - and ^{168}Er -enriched isotopes, respectively, the authors arrived at target quantifications that corresponded within 6.5% of values determined by UV spectroscopy. Although this work is still in the initial stages, the independence of ICP-MS from sample matrix effects bodes well for real-world samples

G. Han et al. 2013. Absolute and relative quantification of multiplex DNA assays based on an elemental labeling strategy. *Angew. Chem. Int. Ed. Engl.* 52:1466-71.

2-Hybrid 2.0

Even the most math-averse biologist would concede that quantitative approaches are gaining ground in the field. Now it's the turn of the yeast 2-hybrid assay to get a quantitative makeover. In the usual method, readout of bait-prey interactions is based on colorimetric screening of colonies on selective media plates, with the identity of hits determined by sequencing. More recently, matrix-based assays, in which arrays of annotated ORF libraries are screened in replicate, have enabled high-throughput data collection appropriate for interactomics. As an alternative to the yes/no or count-based data these methods produce, Suter et al. envisioned a protein-protein interaction (PPI) score as output; they describe their quantitative twist in *Nucleic Acids Research*. Their method recruits the statistics of microarray analysis for detection of PPIs. The initial experimental steps are the same as traditional yeast 2-hybrid: in this case the authors prepared a prey library of some 14,000 ORFs, with the goal of identifying interactors with the neurodegenerative disease proteins huntingtin and ataxin 1, which were cloned into separate bait vectors. Pooled yeast containing the prey collection were mated with bait-harboring strains, and the cells were placed in selective medium. However,

Microarray-based yeast two-hybrid, showing preparation and comparison of pool control, experimental, and vector control cultures, respectively.

instead of plating out the cells, the authors purified plasmid DNA from the culture and PCR amplified the cloned ORFs. These PCR products were then labeled and hybridized to Affymetrix arrays, and compared with a "pool" control (the unselected prey collection, showing the library's background signal) and an empty vector control (indicating bait-independent reporter activation). As a first analysis, Suter et al. arbitrarily defined cutoffs defining differential enrichment compared to the two controls. Then, with a statistical tool they developed previously,

the authors used data from a PPI database to develop empirical cutoffs that gave, for an amino-terminal huntingtin fragment bait, a set of 44 high-confidence PPIs, 61% of which were confirmed either by co-immunoprecipitation or by published data. For ataxin 1, the authors showed how microarray-based yeast 2-hybrid could engender parallel comparison of PPI patterns for mutant and wild-type bait proteins, leading to inferences about the PPI consequences of particular mutations. The authors acknowledge that this method may miss ORFs that PCR amplify poorly, but conclude that quantitation offers unprecedented opportunities for integration with data mining approaches to elucidate protein interactomes.

B. Suter et al. Development and application of a DNA microarray-based yeast two-hybrid system. [Epub ahead of print, December 28, 2012; doi:10.1093/nar/gks1329].

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