A MORE SENSITIVE MAMMALIAN TWO-HYBRID ASSAY

The two-hybrid technique is a well-established cell-based method for analyzing protein–protein interactions. Originally developed for use in yeast, the system was later adapted for mammalian cells. The mammalian two-hybrid (M2H) assay allows analysis of interactions between mammalian proteins that require post-translational modifications to function properly. A major issue with the M2H system, however, is its low sensitivity for weak interactions. In this month's issue, Thomas Czerny and his colleagues at the University of Applied Sciences in Vienna describe an inducible M2H (iM2H) method with greatly improved sensitivity. They used a stronger DNA-binding domain (DBD) than the standard yeast GAL4 DBD and optimized the number of target binding sites in the reporter construct. Since prior attempts to boost the activity of the prey protein-activation domain (AD) fusion by attaching multiple copies of the AD to the prey protein resulted in rapid degradation, the authors instead induced the multimerization of separate ADs to each prey protein using rapalog, a small-molecule dimerizer. These improvements together resulted in up to a 100-fold increase in sensitivity compared to standard M2H approaches.

See “A sensitive, semi-quantitative mammalian two-hybrid assay” on page 206.

THE NTIRE WAY OF ORGAN DECELLULARIZATION

The persistent shortage of organs for transplantation has prompted efforts to create artificial organs through tissue engineering using 3-D tissue scaffolds that exogenous cells can be grown on. A promising approach for producing tissue scaffolds is whole-organ decellularization, which results in a scaffold containing native extracellular matrix (ECM) and an intact microvasculature, ideal for growing an artificial organ. In this issue, Tammy Chang and her colleagues at the University of California, San Francisco describe a new approach for the *in vivo* decellularization of an organ. They used non-thermal irreversible electroporation (NTIRE), a tissue and tumor ablation technique involving the application of very brief, high electric field pulses to irreversibly permeabilize cell membranes, causing cell death without scar tissue formation. The authors treated part of the liver in a live mouse with NTIRE and then implanted exogenous hepatocytes into the cell-ablated region, resulting in the subsequent integration of the implanted cells into the host liver parenchyma.

See “Using non-thermal irreversible electroporation to create an *in vivo* niche for exogenous cell engraftment” on page 229.

Selected and written by Patrick C.H. Lo, Ph.D.

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EXPANSION MICROSCOPY GETS BIGGER

In 2015, Massachusetts Institute of Technology researcher Ed Boyden and his colleagues introduced a new method for high-resolution imaging of biological samples called expansion microscopy. In expansion microscopy, a sample is physically enlarged for imaging, a direct contrast to the more conventional approach where technical imaging capabilities are improved. Expansion microscopy works by first embedding a sample in a polyelectrolyte gel where labels or biomolecules can be anchored. From there, the sample is homogenized and then swelled, effectively increasing its physical size 4.5 times. In a new article, Boyden's team shows that it is possible to perform a second round of expansion to increase the sample's size even more, 20 times, allowing for nearly 25 nm resolution imaging of tissues and cells using conventional microscopy systems.

Chang et al. 2017. Iterative expansion microscopy. *Nat. Methods*. [Epub ahead of print, April 17, 2017; doi: 10.1038/nmeth.4261].

BETTER SEQUENCING COVERAGE

In next-generation sequencing, library construction is a key element to success. Many library construction protocols involve the sheering of genomic DNA to a specific size, followed by the attachment of adaptors possessing the primer sites needed for sequencing. Another approach is the Nextra method, which takes advantage of transposons with primer sites incorporated at the ends to insert into genomic DNA for generation of sequencing libraries. Although the Nextra approach uses a streamlined workflow, the main drawback with this transposon-based method has been the insertion bias that can occur, necessitating deeper sequencing, especially for GC-rich genomes. Kia et al. now present a solution to this bias dilemma with an engineered transposase that possesses lower GC insertion bias. In proof-of-concept experiments, the mutant transposase reduced AT dropout while increasing the uniformity of coverage in both human and bacterial genomes.

Kia et al. 2017. Improved genome sequencing using an engineered transposase. *BMC Biotechnology*. 17:6.

Selected and written by Nathan S. Blow, Ph.D.

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