SOMEONE'S KNOCK-IN AT THE DOOR

CRISPR/Cas9-mediated genome engineering has changed the face of molecular biology and genetics. In this month's issue of *BioTechniques*, Jia-Wang Wang from the University of South Florida and his colleagues report another powerful enhancement of the CRISPR/Cas9 system—a highly efficient gene knock-in strategy aimed at mouse stem cells and zygotes. CRISPR works by creating double-strand DNA breaks that are most often repaired through the non-homologous end-joining repair (NHEJ) pathway. However, when it comes to precisely inserting or deleting a DNA sequence using CRISPR, the homology-directed repair (HDR) pathway is more effective. The trouble with exploiting the HDR pathway has been that researchers needed to inhibit the NHEJ pathway for optimal targeting efficiency. Wang and his team developed and validated an approach for inserting large DNA fragments (up to 7.4 kb) into specific genomic regions without the need for NHEJ inhibitors. Their data demonstrate that the new method not only possesses efficiencies rivaling earlier techniques using NHEJ inhibitors, but that it can also be accomplished using only a quarter of the regular concentrations of CRISPR reagents.

See “Highly efficient CRISPR/HDR-mediated knock-in for mouse embryonic stem cells and zygotes” on page 201.

BAGGING A LOW OXYGEN ENVIRONMENT

Cells experience different oxygen levels depending on the tissue in which they reside as well as their location within that tissue. Recreating these different oxygen environments in the lab can be a challenge, especially those with low oxygen (hypoxic) concentrations. In addition, maintaining hypoxic conditions inside cell incubators requires specialized and expensive equipment. In this issue, a team of researchers from Australia led by Supun Bakmiwewa presents an attractive, low-cost alternative for creating hypoxic cell environments in the lab: food storage vacuum bags. This technique is straightforward: flasks of cells are placed into sealable food bags that are only partially sealed at both ends. Gas with the desired oxygen concentration is blown into the vacuum bag, which is then fully sealed and placed into a standard incubator. To validate their approach, the authors conducted a series of experiments aimed at assessing both the experimental reproducibility and overall robustness of the technique for maintaining low oxygen levels over an extended period of time. The use of inexpensive food storage bags for hypoxia studies should allow a greater number of researchers to study their cells under oxygen conditions more reflective of *in vivo* microenvironments.

See “An effective, low-cost method for achieving and maintaining hypoxia during cell culture studies” on page 223.

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

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IDENTIFYING DNA BOUND BY PROTEIN COMPLEXES

The go-to method for revealing genomic sites bound by transcriptional regulators, ChIP-Seq, isn't suitable for studying proteins active in only a few cells or for brief periods of time. Using Notch signaling as a case study, Hass et al. introduce a system for sensitive analyses of combinatorial protein–DNA interactions. Previously, bacterial DNA adenine methyltransferase (DAM) has been fused to DNA-binding proteins so that DNA recognition sites could be identified by their sensitivity to digestion by the methylation-dependent restriction enzyme *DpnI*. The new approach, spDamID (split DAM identification), adds protein complementation to traditional DamID (DNA adenine methyltransferase identification), enabling identification of DNA sequences bound simultaneously by protein pairs. The reconstituted DAM activity can be detected by target-specific PCR or, at a genome-wide level, by microarray hybridization or next-generation sequencing of *DpnI*-released fragment libraries. Unlike ChIP, spDamID requires no antibodies, can distinguish monomeric from dimeric binding, and can detect proteins that bind within a 100-bp window even if they do not physically associate.

M.R. Hass et al. 2015. SpDamID: marking DNA bound by protein complexes identifies Notch-dimer responsive enhancers. *Mol Cell*. 59:685-97.

SINGLE-CELL KINASE ACTIVITY MONITORING

The usual approach to measuring kinase activity dynamically in single cells is to detect phosphorylation-dependent FRET between two fluorescent proteins. However, FRET can be tricky to optimize, and the requirement for two fluorescent proteins constrains the number of kinases that can be monitored simultaneously. In the context of mitogen-activated protein kinase (MAPK) pathways in yeast, Durandau et al. offer a detailed methodology for quantifying kinase activity. The readout is via synthetic kinase activity relocation sensors (SKARS). These tripartite fusion proteins contain the docking site for the kinase of interest, two instances of a phosphorylation-sensitive nuclear localization signal (NLS), and a fluorescent marker. Once phosphorylated, the NLSs are obscured, and the SKARS are no longer imported into the nucleus, causing the ratio of nuclear to cytoplasmic fluorescence signal to drop measurably. The authors show that two SKARS can be used simultaneously and that they can be combined with other fluorescent sensors.

E. Durandau et al. 2015. Dynamic single cell measurements of kinase activity by synthetic kinase activity relocation sensors. *BMC Biol*. 13:55.

Selected and written by Nijse Dorman, Ph.D.

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