Citations

Illuminating Dark Matter

Of the analyzed human genome, only ~1% codes for proteins, but the vast majority is transcribed. Interest in understanding the function of this RNA “dark matter” has surged, and initial studies have suggested involvement of noncoding RNAs (ncRNAs) in controlling chromatin architecture and directing developmental pathways. One way that ncRNAs might fulfill this role is by serving as cofactors that act to target chromatin modifiers to particular locations in the genome. A better understanding of these pathways will require dedicated methods and resources. In an article in *Molecular Cell*, Zhao et al. describe a genome-wide fishing strategy to identify ncRNA cofactors for the histone methylation factor Polycomb repressive complex 2 (PRC2). Although the technique could be used with any chromatin modifier, PRC2 is a logical target because of the importance of Polycomb proteins in normal development, and evidence suggesting that they are deranged in cancer. To tackle the PRC2-associated noncoding transcriptome, Zhao et al. combine native RNA immunoprecipitation (RIP) with RNA-seq, a method for massively parallel sequencing of random RNA libraries. In the new hybrid strategy, RIP-seq, nuclear lysate is immunoprecipitated with an antibody against a PRC2 component, followed by RNA extraction for preparation of a cDNA library, sequencing, and data analysis. After pilot studies to determine filtering and threshold criteria, the

authors’ final data set comprised some 10,000 sequences from the mouse embryonic stem cells they used as starting material. Over one-third of the hits were antisense transcripts and 19% were previously unannotated transcripts; in all, the authors estimate that the PRC2 noncoding transcriptome accounts for 5–25% of all murine transcripts. For researchers interested in browsing the data set—which includes many oncogene, tumor suppressor, and stem-cell related loci—the complete results are available online. For those seeking to analyze other chromatin modifiers for ncRNA partners, the authors walk through their many validation experiments, which include using PCR after RIP to quantify enrichment of candidate RNAs, confirming direct RNA-protein interactions by gel mobility shift assays, and using chromatin immunoprecipitation to address whether knocking down a candidate ncRNA cofactor influenced chromatin recruitment of PRC2. Given the sheer number of hits they obtained, the authors could validate only a small number of transcripts picked up by RIP-seq. Investigating the uncharacterized hits and repeating RIP-seq with other cell types and baits will illuminate the still-shadowy world of ncRNA control of genome dynamics and transcriptional regulation.

Zhao et al. 2010. Genome-wide identification of polycomb-associated RNAs by RIP-seq. *Mol Cell*. 40:939–953.

Package Deal

Trying to coordinate too many items at once may lead to headaches, not harmony. Case in point is the difficulty of coaxing mammalian cells to coexpress several transgenes. Sequential transfection is tedious and slow, stable cotransfection would require a sobering number of selectable markers, and coinfection of viral vectors can introduce biosafety complications, to name a few techniques and their limitations. Even if these or similar methods were convenient and efficient, the chances of getting all transgene to express at once is an unlikely proposition. What, then, is the solution for researchers looking to track multiple fluorescently labeled proteins in a single cell or coexpress a slew of exogenous factors? According to an article from Kriz et al. in *Nature Communications*, the new “MultiLabel” system is just what’s needed. In keeping with modern expression platforms, the vectors used in the system are highly modular for easy assembly from component elements such as resistance markers and promoters. To prepare a MultiLabel plasmid from these expression vectors, an “acceptor” is fused by recombination to one to four “donor” expression vectors. Each donor plasmid has a condi-

Homogeneity of expression with MultiLabel versus cotransfection of separate plasmids. Reprinted with permission © 2010 NPG.

tional origin of replication. The product of the recombination reaction is therefore transformed into a nonpermissive bacterial strain in order to weed out any donors that have not fused with an acceptor vector, which has a functioning origin and a resistance marker appropriate for selection in mammalian cells. Whereas cotransfection of five separate vectors expressing different fluorescent proteins resulted in cells that lacked expression of one or more markers, cells transfected with a single MultiLabel plasmid containing the same expression cassettes in one big construct had comparable levels of expression of all five proteins. For stable transfectants, a

MultiLabel plasmid with a Flp-In cassette facilitated recombinational integration, provided that the total size of the plasmid was kept to 15 kb. Larger plasmids (20–25 kb) can also integrate successfully if they are linearized at a homing endonuclease site prior to transfection. As would be expected, the transfection efficiencies of the largest MultiLabel plasmids are low (1–20%, depending on the cell type); however, because each transfected cell reliably expresses all the transgenes in the MultiLabel plasmid, even this modest success rate is sufficient for observing cellular phenomena and performing biochemical analysis.

Kriz et al. 2010. A plasmid-based multigene expression system for mammalian cells. *Nat Commun*. 1:120.

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