

'Smart-pooling' by design

Large-scale protein-protein interaction experiments are increasing in size and complexity as scientists try to elucidate signaling networks in cells. New high-throughput screening platforms can help ease the burden, but the task of mapping these networks is still daunting. The use of pooling in initial screens can improve efficiency, but limitations in the coverage during an initial screen can't be corrected in later secondary screens. Moreover, the requirement for experimental deconvolution of all hits in secondary screens has complicated previous pooling strategies. 'Smart-pooling' has the potential to overcome these problems by guiding the design of carefully chosen pools in which each probe is present in multiple pools. This provides built-in redundancy and error checking. Huang and colleagues describe a simple-to-use smart-pooling strategy that promises to greatly increase screening efficiency while providing better accuracy and coverage. Encouragingly, they demonstrate its performance in three different large-scale screens.

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Eliminating off-targets

Users of the popular gene knockdown technique, RNA interference, are becoming increasingly aware that small interfering RNA (siRNAs) can target both their gene of interest and unrelated genes referred to as 'off-targets'. In a systematic analysis of predicted and experimentally validated off-targets, Birmingham *et al.* demonstrate that the current practice of using overall identity-based algorithms to minimize off-targeting is grossly ineffective. Instead, off-targeting is associated with one or more perfect matches between the seed region of an siRNA (antisense nucleotides 2–8) and the 3' untranslated region (UTR) of the off-targeted genes. To better predict future off-target effects, the authors provide an online tool that allows users to identify matches between siRNA seed regions and the 3' UTRs of the human genome.

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Gene annotation out of the CAGE

To fully annotate a genome, information about the location of transcription start sites and promoters is crucial. Carninci and colleagues now present a comprehensive protocol for cap analysis of gene expression (CAGE) that describes the essential

steps for enrichment of full-length cDNA and improved cDNA library construction followed by high-throughput sequencing of concatenated tags. In addition to genome annotation, this protocol will be useful for expression profiling as well as for discovery of new genes.

Protocol p211

Illuminating protein topology

The topology of a protein in the cell dictates what proteins it can interact with. There is, however, no reliable and easy-to-use method for determining the topology of proteins located in intracellular organelles. The Lippincott-Schwartz laboratory has used fluorescent proteins to probe the function of cellular organelles for a decade. They now show that by using GFP fusion proteins in combination with selective membrane permeabilization and protease treatment it is possible to determine whether a protein is cytoplasmic, luminal or associated with a membrane. For transmembrane proteins, the method can also determine what portions of the protein are luminal and cytoplasmic. This fluorescence protease protection assay is likely to replace many of the more demanding methods currently used to answer such questions.

Article p205

Retro remake for TCR transgenic mice

T cells, the key players in our immune system, derive from precursors generated in the thymus and are selected on the basis of the

recognition by their surface receptor of self and foreign antigens. The selection process is best studied *in vivo*, using transgenic mice in which all the precursors carry the same T cell receptor, a simplification of the natural heterogeneity necessary for experimental investigation. Using a retroviral vector that they previously developed, Vignali and colleagues have now optimized an alternative method to generate such mice without the time-consuming breeding involved in deriving new transgenic lines, but relying rather on a bone-marrow transplant procedure. Although it requires some expertise, this alternative technique offers higher throughput and a new level of flexibility for the generation of TCR mice with diverse genetic backgrounds.

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