DETECTING RARE T-CELLS

The specificity of a naïve T cell is determined by its T-cell receptor (TCR), which binds to antigenic peptides presented on the surfaces of target cells by major histocompatibility complex (MHC) proteins. To identify antigen-specific T cells, researchers label them using fluorescent peptide–MHC (pMHC) molecules, but given the weak affinity of the TCR for pMHC monomers, pMHC multimers must be used. Detecting T cells possessing very low-affinity TCRs requires the use of very high-valency pMHC multimers such as dextramers, which are assembled by binding biotinylated pMHC monomers to dextran functionalized with streptavidin and a fluorescent label. These dextramers, however, are expensive and difficult to produce in-house. In this issue of *BioTechniques*, a team led by David Baltimore at the California Institute of Technology describes a simple and inexpensive protocol for producing pMHC dextramers. Sub-stoichiometric amounts of biotinylated pMHC monomers are first bound to streptavidin-fluorochrome, and biotinylated dextran is added to bind the remaining streptavidin sites. The authors successfully used these dextramers to detect low-affinity tumor antigen–reactive T cells from patients.

See “Preparation of peptide–MHC and T-cell receptor dextramers by biotinylated dextran doping” on page 123.

THE TIP SNIP WAY OF SUBCLONING

De novo DNA synthesis of an entire construct consisting of a gene (or genes) and a vector is still prohibitively expensive. For this reason, synthetic biologists continue to clone PCR products or synthetic genes into plasmids; however, this method is labor-intensive and difficult to automate. In this issue, Ichiro Matsumura at Emory University describes his Tip Snip method for automated restriction enzyme/T4 ligase subcloning that does not require PCR or gel purification and works with almost any plasmid polylinker. In this scheme, the cohesive ends of undesired restriction fragments are snipped off by digestion with other restriction enzymes, and the resulting short “snippet” fragments are removed by hybridization with complementary “anti-snippet” oligonucleotides and size-selection spin chromatography. The cohesive ends of the unwanted linear donor vector are then ligated to unlinkers to form blunt ends that prevent vector re-circularization. These steps allow only the donor insert and the recipient plasmid to ligate together, making subcloning efficient and suitable for automation.

See “Semi-automated Tip Snip cloning of restriction fragments into and out of plasmid polylinkers” on page 99.

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miRNA FINDS THE PERFECT SPACE

One curious question surrounding the way in which microRNAs (miRNAs) regulate gene expression has to do with abundance—often there are more potential mRNA targets in cells than miRNAs. Sambandan et al. provide a potential explanation for this discrepancy through a series of experiments using inducible fluorescent probes attached to precursor miRNAs (pre-miRNAs). The authors tested whether or not miRNA maturation takes place in spatially and temporally localized regions of neurons. Fluorescent probes were first attached to pre-miRNAs in a quenched state. Following cleavage by Dicer, the enzyme that activates conversion of pre-miRNAs to miRNAs, fluorescence is induced, which enables imaging and localization of miRNAs within the neurons. The results showed that miRNA maturation did occur in a localized manner, and protein levels in those regions subsequently decreased, providing new insight into the spatial and temporal mechanisms miRNAs use to repress protein expression in cells.

Sambandan et al. 2017. Activity-dependent spatially localized miRNA maturation in neuronal dendrites. *Science*. 355:634–637.

MAX-ING OUT POOLED SCREENS

Although pooled screens are efficient and scalable, they yield only basic readouts (cell growth for example). Dixit et al. expand the capabilities of pooled-screen approaches with their new method called Perturb-seq. Perturb-seq is a combination of single-cell RNA sequencing and CRISPR-based genetic perturbations. Initially, pools of barcoded single-guide RNA (sgRNA) libraries that encode specific perturbations are transduced into cells. Following single-cell selection and mRNA capture, next-generation sequencing libraries are constructed and analyzed to assess gene expression changes. To test their method, the authors analyzed 200,000 cells, focusing on the activity of transcription factors involved in the response of dendritic cells to lipopolysaccharides. By sequencing gene transcripts from these cells and then using a computational framework to estimate the impact of each perturbation on gene expression, the authors were able to identify individual gene targets, gene signatures, and affected cell states.

Dixit et al. 2016. Perturb-Seq: Dissecting molecular circuits with scalable single cell RNA profiling of pooled genetic screens. *Cell*. 167:1853–1866.

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

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