STABLER EXPRESSION IN STABLE CELL LINES

A major issue with stable cell lines generated by standard methods is the heterogeneous levels of recombinant protein expression in different cell clones due to the integration of variable numbers of the transgene and/or position effects caused by integration of the transgene into random locations in the genome. In addition, the use of strong heterologous promoters in transgenic vectors can drive expression to very high, non-physiological levels that harm the cells. In this month's issue, researchers led by Brian Chen at McGill University describe an efficient method for stable cell line generation that achieves controlled, physiological expression of a transgene. Using CRISPR/Cas9 gene editing, the team inserted a single copy of a transgene at a specific gene locus, placing it under the control of an endogenous promoter with the desired level of gene expression. Their transgenic vector also includes a protein quantitation reporter gene to produce an equimolar amount of a fluorescent protein, allowing easy quantitation of the recombinant protein.

See **"Generating stable cell lines with quantifiable protein production using CRISPR/Cas9 mediated knock-in"** on page 165.

TWO COLORS ARE BETTER THAN ONE FOR DNA METHYLATION ASSAY

The cytosine extension assay (CEA) assesses global DNA methylation levels by tracking the methylation of a CpG-containing restriction enzyme site, for example CCGG, which when methylated cannot be digested by *HpaII* but can be digested by its isoschizomer, *MspI*. The relative digestion of CCGG sites in a DNA sample by *HpaII* compared to *MspI* reflects the extent of DNA methylation and is assessed in CEA by the incorporation of a fluorescently labeled cytosine through end-filling by DNA polymerase of the 5' guanosine overhang at the *HpaII*/*MspI* cut site. In this issue of *BioTechniques*, Craig Parfett and his colleagues at Health Canada present an improved version of CEA. In standard CEA, the same fluorescently colored cytosine is used in the extension reactions following *HpaII* and *MspI* digestion. In their version, Parfett's team used a different fluorescently colored cytosine for the extension reaction of each digest, after which the reactions were combined for downstream purification and signal detection. This two-color CEA showed greater precision compared to the one-color CEA and is well-suited to high-throughput screening.

See **"Two-color fluorescent cytosine extension assay for the determination of global DNA methylation"** on page 157.

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

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ROOT PROBLEMS

Plant biologists have long been interested in understanding the interactions that occur in nature between microorganisms and plant roots. Plants both obtain information about their environment and send out signals to microbes through their roots. However, deciphering the interplay between plant roots and the microbial communities surrounding them has proven difficult. Now, researchers report in *PNAS* a new microfluidic-based device designed specifically for monitoring root-bacteria interactions over time. The device, which the authors named the tracking root interaction system (TRIS), is composed of 9 independent microfluidic chambers where roots can be established and into which bacteria are introduced for monitoring. The chambers are coupled to a microscope assembly that makes it possible to obtain both spatial and temporal information on root-bacteria interactions. In proof-of-concept experiments, the authors fluorescently labeled bacteria and were able to observe root colonization in real time over extended periods using their TRIS device.

Massalha, H. et al. 2017. Live imaging of root-bacteria interactions in a microfluidics setup. *Proc Natl Acad Sci USA*. doi: 10.1073/pnas.1618584114.

WESTERN BLOTTING GOES SMALL

Western blotting is a commonly used technique for studying cellular proteins. While effective when using large numbers of cells as starting material, western blot analysis has proven more difficult when scaled down for studies of single cells with their much lower protein concentrations. Seeking to overcome this limitation and extend the range of protein analysis, researchers describe a new rare-cell, single-cell western blotting procedure in *Nature Communications*. Building on previous work that established the feasibility of single-cell resolution western blotting, the authors sought to measure panels of proteins from extremely rare circulating tumor cell (CTC) isolates. For protein analysis, each CTC was placed into an individual microwell, where the cell was first lysed and then immediately electrophoresed into a polyacrylamide matrix. Following a miniaturized version of polyacrylamide gel electrophoresis (PAGE), in-gel immunoprobining was done on the size-separated CTC proteins. Using this method, the authors were able to successfully perform multiplexed protein analyses and report protein expression on a per-cell basis.

Sinkala, E. et al. 2017. Profiling protein expression in circulating tumor cells using microfluidic western blotting. *Nat Commun*. 8:14622.

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

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