COUPLES THERAPY FOR TOXIC PROTEIN EXPRESSION

While the use of *E. coli* to express recombinant proteins remains popular, problems often arise when expressing toxic proteins from inducible, high-expression plasmid vectors. These vectors can have significant levels of uninduced expression, leading to plasmid instability because cells with plasmids that have lost expression of the toxic protein due to mutations in the inducible promoter have a selective growth advantage. This allows cells containing the mutant plasmids to rapidly take over the liquid culture, resulting in drastically reduced protein yields. In this issue of *BioTechniques*, Marcello Tagliavia and Angela Cuttitta from the National Research Council in Torretta Granitola, Italy, describe their method for preventing plasmid instability by cleverly exploiting translational coupling, a prokaryotic mechanism for regulating gene expression in polycistronic mRNAs. The authors constructed an inducible expression vector in which the translation of a selective marker gene depends on translation of the upstream cloned gene to which it is coupled. Under selection for marker gene expression, plasmid instability was suppressed, and the cells that grew contained only plasmids expressing the cloned gene.

See “Exploiting translational coupling for the selection of cells producing toxic recombinant proteins from expression vectors” on page 113.

THE END IS NEAR

mRNA 3'-end formation depends on *cis*-acting sequences, including the AUUAAA polyadenylation signal, the upstream sequence element (USE), the downstream sequence element (DSE), and the polyadenylation cleavage site. Mutations in these sequence elements dysregulate 3'-end formation and are implicated in various diseases. In order to facilitate the identification and analysis of these mutations, Natoya Peart and Eric Wagner from the University of Texas Medical School at Houston present in this issue their novel GFP expression-based cellular reporter construct for the study of mRNA 3'-end formation. In this transcriptional readthrough reporter, sequences of interest are cloned upstream of a GFP gene; if a test sequence contains a functional cleavage and polyadenylation signal, the nascent transcript will be cleaved before GFP is transcribed, thus blocking GFP expression. The authors demonstrated that the insertion of a sequence containing the late SV40 PAS, cleavage site, and DSE prevented GFP expression, while mutations in the PAS or DSE of that sequence resulted in GFP expression.

See “Gain-of-function reporters for analysis of mRNA 3'-end formation: Design and optimization” on page 137.

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AUTOMATING NEOANTIGEN IDENTIFICATION

The goal of cancer immuno-oncology is to harness the body's immune system in the quest to fight tumor cells. The ability to rapidly identify tumor-specific mutant peptides—so-called neoantigens—that are capable of inducing anti-tumor T-cell responses, could enhance efforts to develop more effective cancer immuno-therapies. While high-throughput DNA and RNA sequencing are often used to identify tumor somatic mutations that give rise to neoantigens, integrated computational workflows capable of analyzing these data to identify candidate neoantigen peptides for personalized vaccine development have been lacking. Now, Hundal et al. present a new software tool called pVAC-Seq (personalized Variant Antigens by Cancer Sequencing) that is capable of comparing epitopes found in cancer cells to those in normal cells to guide development of personalized immuno-therapies. pVAC-Seq uses sequence read coverage, gene expression information, and peptide binding affinities in normal and mutant samples to predict which neoantigen peptides will be most effective when used in dendritic cell-based vaccines.

Hundal, J. et al. 2016. pVAC-Seq: A genome-guided in silico approach to identifying tumor neoantigens. *Genome Biol.* 8:11.

CHROMOSOME DYNAMICS IN MULTI-COLOR

In recent years, it has become clear that understanding chromosome structural dynamics is one key to demystifying the process of gene regulation. The ability to directly label and visualize different chromosomal regions can be critical in efforts to elucidate chromosome structure and organization. To this end, Shao et al. report a new dual-tracking strategy for genomic loci based on use of the CRISPR/Cas9 system. They have modified the traditional single-guide RNA (sgRNA) to include either an MS2 or PP7 RNA aptamer, which acts to recruit and bind effector proteins tagged with fluorescent proteins. After optimizing the sequences of their modified sgRNAs, the authors validated the new method by using it to perform multi-color, live-cell chromosome loci imaging. Given the flexibility in designing sgRNAs for the CRISPR/Cas9 system and the fast exchange kinetics of the observed fluorescence, this new system should enable easier, dual-color chromosome labeling for long-term tracking of chromosome structural dynamics.

Shao, S. et al. Long-term dual-color tracking of genomic loci by modified sgRNAs of the CRISPR/Cas9 system. *Nucleic Acids Res.* [Epub ahead of print, February 4, 2016; doi: 10.1093/nar/gkw066.]

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