A NEED TO COMPENSATE

Gene editing using the CRISPR/Cas9 system has been a great boon for the analysis of gene function in cells, but it can be problematic when applied to genes for essential proteins since mutations in these genes could kill the cell. In this month's issue of *BioTechniques*, Marc Vigneron and his colleagues at the University of Strasbourg describe their new complementation method that facilitates mutational analysis of essential protein genes in human cells using CRISPR/Cas9. To compensate for the CRISPR/Cas9-mediated inactivation of the chromosomal copy of an essential protein gene, a plasmid expressing the same gene is transfected into the cell. The gene on the compensatory plasmid is engineered so as to prevent its cleavage by the CRISPR/Cas used to inactivate the chromosomal gene, allowing the plasmid gene to complement the knocked-out endogenous gene. The team demonstrated that their method could be used to analyze the effects of different missense mutations in the proliferating cell nuclear antigen gene (*PCNA*) when they were introduced into the plasmid-encoded copy of that gene used to complement inactivated chromosomal *PCNA*.

See “A fast method for analyzing essential protein mutants in human cells” on page 80.

DOUBLE DUTY FOR TAQMAN PROBES

Methods to detect and quantify in tumor DNA the levels of specific oncogene mutations that drive tumor formation are critical for cancer diagnostics. Among these methods, DNA melting analysis (DMA) using TaqMan probes is simpler and higher-throughput than costlier, more laborious methods such as pyrosequencing; however, DMA is also less sensitive than other available methods. In this issue, Anatoly V. Lichtenstein and his team at the N.N. Blokhin Cancer Research Center in Moscow, Russia demonstrate that they can significantly increase the sensitivity of DMA for mutant allele detection through a simple but clever modification of the assay. In standard DMA, asymmetric PCR is used to generate sufficient quantities of one strand of the target sequence that is subsequently hybridized with a TaqMan probe in order to quantify the amount of a specific allele by melting curve analysis. In the modified DMA approach, the TaqMan probe is also used during the asymmetric PCR step as a blocking agent for the wild-type allele, thereby enhancing amplification of the mutant allele. This allowed Lichtenstein's team to obtain a 10-fold increase in the sensitivity of detection of *BRAF* and *NRAS* mutants.

See “TaqMan probes as blocking agents for enriched PCR amplification and DNA melting analysis of mutant genes” on page 62.

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“Y” MARKS THE SPOT FOR tRNA SEQUENCING

There are >600 different tRNAs that play important roles in the translation of mRNA into protein. Because tRNAs are relatively short (60–70 bases), sequencing them and assessing their abundances in a cell is challenging. In the latest issue of *Nucleic Acids Research*, Shigematsu and colleagues present a new approach for tRNA sequencing by making use of T4 RNA ligase and a Y-shaped adaptor (Y-AD) molecule to generate tRNA templates more amenable to RT-PCR and subsequent sequencing. Previous efforts to attach adaptors to tRNAs resulted in incomplete ligation reactions, low yields of tRNA sequence data, or both. Here, the authors turned to T4 RNA ligase 2 rather than T4 RNA ligase 1, which has been used previously. This modification made the tRNA ligation reaction significantly more effective at attaching Y-ADs to tRNA molecules. Since the Y-AD contains both 5' and 3' Illumina adapter sequences, Illumina small RNA sequencing kits can be used to generate sequencing libraries. This new approach, which the authors named YAMAT-seq (Y-shaped Adapter-ligated Mature tRNA sequencing), should enhance experiments aimed at understanding the roles of tRNAs in cells.

Shigematsu, M. et al. 2017. “YAMAT-seq: an efficient method for high-throughput sequencing of mature transfer RNAs.” *Nucleic Acids Res.* doi: 10.1093/nar/gkx005

NEXT-GEN SEQUENCING AND FIRE DON'T MIX

Molecular analyses of ancient archeological materials can be technically challenging. Identifying the best starting material or sample type is critical to success. When it comes to ancient plant samples, these are often found as charred remains, meaning the samples have undergone extremes of temperature, environmental exposure, and oxidation. To determine if charred samples could be used for DNA analysis, Nistelberger et al. tested four plant species by extracting DNA for next-generation sequencing (NGS) and target enrichment. The samples ranged in age from 4450 to 550 years old. Sequencing results showed that for 3 out of 4 samples, there was a definite lack of endogenous DNA present—only in the case of a lightly charred maize cob was appreciable DNA recovered. The use of target enrichment with these samples also did not result in adequate data for the researchers to make meaningful conclusions. In the end, the authors recommend that charred archeological plant material is not suitable for DNA analysis using either target enrichment or NGS approaches.

Nistelberger, H.M. et al. 2016. The efficacy of high-throughput sequencing and target enrichment on charred archeobotanical remains. *Sci Rep.* 6:37347

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

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