A TEST FOR HOT-START POLYMERASES

Hot-start polymerases for PCR are engineered to be inactive until heated to a temperature at which oligonucleotides can no longer anneal to the template DNA. These polymerases were designed to prevent non-specific amplification and primer-dimer formation—but how well do they work in different experiments? In this issue of *BioTechniques*, Aaron Stevens and his colleagues at the University of Otago in Christchurch, New Zealand report that several commercially available hot-start polymerases mediated primer-dimer formation during a telomere PCR assay. To test different hot-start polymerases, the authors designed a simple assay to detect polymerase activity prior to heat denaturation. Their assay uses 2 different primer pairs, each possessing a 3-bp overlap between the forward and reverse primers that will anneal at room temperature. When a polymerase extends either primer pair prior to thermal activation, an ~79-bp primer-dimer product is generated. The authors tested 17 different hot-start enzymes using their assay, finding that many did exhibit polymerase activity at ambient temperature prior to thermal activation.

See “Many commercial hot-start polymerases demonstrate activity prior to thermal activation” on page 293.

TARGET CAPTURE GOES LONG

Targeted capture of DNA sequences is an effective means of selecting specific genes or genomic regions for direct sequencing. In the past, targeted capture strategies have focused on smaller DNA fragments—such as single exons or short regions of genomic sequences. However, with advances in single-molecule and long-read sequencing technologies, there is a growing interest in capturing and sequencing larger DNA fragments, which would allow direct sequencing of whole genes, introns, and intergenic sequences. In this issue, Michael Giolai and his colleagues detail an improved methodology that enables the capture and isolation of DNA molecules as large as 7 kb. Their method relies on a shearing device to obtain large DNA fragments and hot-start polymerase PCR to improve the amplification yields of longer targets. The authors showed that these two steps resulted in efficient removal of smaller DNA molecules and capture of longer targets, yielding longer average sequencing reads with high accuracy.

See “Targeted capture and sequencing of gene-sized DNA molecules” on page 315.

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

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ABSORBANCE-BASED ENZYME EVOLUTION SCREENS

Protein evolution experiments are important for understanding protein structure–function relationships, as well as for the discovery of new catalysts. Microfluidic devices incorporating water-in-oil emulsion droplets have made screening protein libraries faster and more economical. One limitation of these systems, however, is that fluorescence has been the only available means of detection, restricting assay development. Now, Gielen et al. report their design of a new microfluidic system, the absorbance-activated droplet sorter (AADS), that enables the assessment of droplets based on UV/vis absorbance measurements. The authors validated the technology by evolving a dehydrogenase through the screening of half-a-million library members. The key to AADS lies in the large droplet volumes used, which boost sensitivity, and the high sample/volume ratio. These modifications allow for the greater sensitivity needed when performing absorbance-based detection.

Gielen F. et al. 2016. Ultrahigh-throughput-directed enzyme evolution by absorbance-activated droplet sorting (AADS). *Proc Natl Acad Sci U S A*. 113:E7383–E7389.

RESCUING LETHAL PHENOTYPES

Studying genes that induce lethal phenotypes when mutated can be very challenging because such experiments tend to be cumbersome and technically demanding. In a new article in *Scientific Reports*, Sakata et al. present a clever solution for rescuing a lethal phenotype—integrate a copy of the target gene into the X chromosome. In females, X chromosomes undergo the process of X-inactivation, where gene expression from one of the two X chromosomes is randomly turned off. During their experiments, the authors found an embryonic stem cell line with a gene trap vector on one X chromosome that could be used to efficiently integrate a target gene, thus creating a mosaic phenotype in mice. They used their new approach to examine the role of serine protease inhibitor, Kazal type 1 (SPINK1), which is associated with chronic pancreatitis. Mice lacking the murine *SPINK1* homolog die early, but by targeting a copy of *SPINK1* to the X chromosome and utilizing X-inactivation, the authors produced live mice with partially restored *SPINK1* expression that developed spontaneous pancreatitis.

Sakata, K. et al. 2016. Novel method to rescue a lethal phenotype through integration of target gene onto the X-chromosome. *Scientific Reports*. doi: 10.1038/srep37200

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