GOING ANALOG

PCR amplification of GC-rich sequences is often tricky because these templates can form secondary structures that prevent primer annealing and block extension by DNA polymerase. Methods for disrupting these secondary structures include: (i) PCR protocols such as stepdown or slowdown PCR; (ii) use of organic additives such as DMSO, formamide, or betaine; and (iii) incorporation of nucleotide analogs of cytosine or guanine to weaken G:C base pairing. In this month's issue, Carlos Pless and his colleagues examine the use of the dCTP analog N⁴-methyl-2'-deoxycytidine 5'-triphosphate (N⁴me-dCTP) for PCR amplification of GC-rich DNA sequences. Replacing N⁴me-dCTP with dCTP in the PCR mixture produces amplicons containing N⁴-methylcytosine (N⁴meC) instead of unmodified cytosine, which reduces the stability of G:C base pairs to that of A:T base pairs. Complete or partial substitution of N⁴me-dCTP for dCTP allowed the correct amplification of a very GC-rich sequence that produced spurious amplicons with dCTP. N⁴me-dCTP was also superior to organic additives for amplification of problematic GC-rich sequences.

See "PCR amplification of GC-rich DNA regions using the nucleotide analog N⁴-methyl-2'-deoxycytidine 5'-triphosphate" on page 175.

NEW qRT-PCR DYE TAKES THE DIAMOND

A number of fluorescent DNA-binding dyes can be used for quantitative real-time PCR (qRT-PCR), including SYBR Green (SG), EvaGreen (EG), LCGreen, and BRYT Green (BG). SG has been the most popular of these, but it is fairly toxic and also inhibits PCR amplification at high concentrations. In the ongoing search for alternative dyes for qRT-PCR, Adrian Linacre and his team at Flinders University describe their evaluation of Diamond Nucleic Acid Dye (DD) in this issue of *BioTechniques*. Until now, this fluorescent DNA-binding dye had only been used for the visualization of DNA in agarose gels, but it had been shown to be less mutagenic than DNA-binding dyes such as ethidium bromide. Linacre's group observed that when DD was used for qRT-PCR, it demonstrated comparable sensitivity, efficiency, and linearity of standard curves as compared with the use of SG, EG, and BG. Since it is safer and cheaper (by ~5-fold), DD should prove to be an attractive alternative to those other dyes for use in qRT-PCR.

See "Optimization of Diamond Nucleic Acid Dye for quantitative PCR" on page 183.

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MICROBIAL TYPING IN METAGENOMICS

Even though next-generation sequencing (NGS) allows researchers to generate large volumes of metagenomic data, identifying the bacterial strains represented in these data remains challenging. At the moment, multilocus sequence typing (MLST) can be performed in low-throughput on samples where a bacterial species has been isolated. To expand this capability for use on mixed bacterial cultures, Zolfo et al. propose a new computational pipeline, MetaMLST, for strain-level typing of bacteria in large metagenomic data sets. MetaMLST generates *in silico* allelic profiles of microbial strains based on consensus sequence assemblies, which can then be compared with available MLST databases. The authors validated their pipeline using synthetic and spiked-in metagenomes as well as real samples, reconstructing MLST sequences with >98% accuracy and a higher sensitivity than previous assembly-based methods.

Zolfo et al. MetaMLST: multi-locus strain-level bacterial typing from metagenomic samples. *Nucleic Acids Res.* doi: 10.1093/nar/gkw837

SEEING HIGH-THROUGHPUT TRANSCRIPTOMICS

Imaging cell transcriptomes provides a unique complement to sequencing-based transcriptomics. With imaging, spatial information on specific RNAs can be obtained, something lost when sequencing. One such imaging approach is called multiplexed error-robust fluorescence *in situ* hybridization (MERFISH), where RNAs are labeled with fluorescent barcodes that can then be visualized using sequential rounds of single-molecule FISH. The original MERFISH technique was low-throughput, permitting RNA profiling in only a limited number of cells. Now Moffitt et al. describe a series of optimization steps that enable gene expression profiling of tens of thousands of cells in a single day. A key modification was switching to chemical cleavage of barcode tags rather than the photobleaching used in the original protocol. Chemical cleavage makes it possible to extinguish the entire sample simultaneously and immediately start another round of imaging. This modification, along with improved analysis tools, substantially increases both throughput and speed.

Moffitt et al. 2016. High-throughput single-cell gene-expression profiling with multiplexed error-robust fluorescence *in situ* hybridization. *Proc Natl Acad Sci U S A.* doi: 10.173/pnas.1612826113

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