BioSpotlight

Gene capture touches down

Next-generation sequencing has been a major boon for evolutionary biologists interested in comparing the genomes of divergent species. In most cases, however, de novo whole genome sequencing of a previously uncharacterized species is impractical and often unnecessary given the time, labor, and cost. A far more efficient approach to exploring evolutionary relationships would be to sequence small groups of homologous genes using DNA hybridization capture technology (i.e. gene capture using bait sequences) or PCR. Gene capture is superior to PCR amplification for large-scale targeting of specific genes but tends to work best with closely related species or when baits are designed against highly conserved gene sequences within otherwise divergent genomes. Isolating genes whose sequences diverge significantly from the bait sequences has not been accomplished using current gene capture protocols. In this month's issue, Gavin Naylor and colleagues at the College of Charleston, (Charleston, SC) describe their new gene capture methodology that can isolate a predetermined set of protein-coding genes from species that have diverged by up to several hundred million years. The team first optimized the relaxed stringencies for the hybridization and washing steps from a previous touchdown hybridization gene capture scheme.

Baits targeting single-copy protein-coding genes shared across six different gnathosome vertebrate species (human, western clawed toad, green anole, zebrafish, and elephant shark) were designed with up to 39% dissimilarity and separately constructed in custom biotinylated RNA bait libraries. Baits from each species were then used to capture selected target genes from distantly related species within the same class, which were then sequenced. When using standard hybridization and washing conditions, very few target sequences were obtained, whereas Naylor and colleagues' touchdown scheme resulted in an eight-fold increase in captured sequences. Carrying out two rounds of capture further increased the number of targets obtained. This method also proved quite effective when applied to a diverse group of 13 chondrichthyan fishes, demonstrating that the technique was more consistent when used on a sample of more closely related taxonomic lineages. With this optimized gene capture protocol, researchers will be able to more effectively compare homologous genes across highly divergent taxa, further enhancing molecular phylogenetic studies.

See "Capturing protein genes across highly divergent species" on page 321.

Going digital

Since the concept of limiting dilution PCR was first introduced in *BioTechniques* in 1992, subsequent advances in methodology as well as microfluidic and array-based technologies have led to the digital PCR (dPCR) approaches available today. By partitioning samples into thousands of subreactions containing a single transcript each on a nanofluidic array, dPCR allows for binary reading of the results. A simple present or absent call for each subreaction allows near absolute quantification of DNA copy numbers, making dPCR especially attractive for transcript analysis in single cells where limited RNA content is a concern. Functional studies of single neurons have been conducted for years, relying on patch clamping techniques to record electrophysiological responses to particular stimuli. To detect gene expression changes accompanying those responses, researchers often harvest the cellular contents into the patch pipette. Subsequent RNA amplification allows for analysis of gene expression patterns, providing insight into the relationship between molecular and functional changes. In these experiments, transcript abundance is usually measured by real-time quantitative PCR (qRT-PCR), despite concerns about the sensitivity of this tech-

Application of spike-in template in dPCR analysis. Synthetic rps18 template (s_rps18) with subsequent dilution (0–81 copies per reaction).

nique. Although access to dPCR technology is increasing, it has not yet been applied to these single neuron studies. Now, in this issue of *BioTechniques*, Farago et al. present a combined digital PCR–patch clamp electrophysiology method for assessing mRNA and miRNA expression in single neurons. The accuracy and sensitivity of this approach was validated using spike-in templates of known concentration. Precise copy number counts were obtained for transcripts of interest, even when expression levels were very low. Although dPCR technology remains expensive, coupling it with patch clamp extraction of cellular contents enables the collection of accurate

and sensitive gene expression data for physiologically well-defined single neurons. The combination of dPCR and patch clamp promises improved accuracy for transcript analysis and will provide another option for researchers working with single cells.

See "Digital PCR to determine the number of transcripts from single neurons after patch-clamp recording" on page 327.

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