BioSpotlight

LASAGNA: A better taste for transcription factor analysis

Transcription factors (TF) play a critical role in cell homeostasis, signaling, and response to stimuli by binding gene regulatory regions in a sequence-specific manner and subsequently altering gene expression. Functional studies of individual TFs can provide specific details on the interactions between a TF and its binding site (TFBS), but such functional analyses are specific, expensive, and time-consuming. As an alternative, researchers frequently take advantage of computational tools to scan genomic sequences and make predictions regarding putative TFBSs. In addition to basic TF search functions, these currently available tools can also search variable length binding sites, retrieve promoter sequences, and predict binding specificities, among other functions. Although several useful programs are currently available to facilitate such computational studies of TFBS, none of these can complete all the possible analysis steps just described, requiring interested users to go between several programs for their searches. To improve the efficiency of TF analysis, Chih Lee and Chun-His Huang at the University of Connecticut describe the development of a web-based program they call LASAGNA-Search in the current issue of *BioTechniques*. LASAGNA-Search allows for complete TFBS search and visualization, including the ability to search variable-length TFBS, incorporation of five pre-computed models and 1726 TF models collected from user-curated TF databases, use of position dependence data for modeling, automatic promoter sequence retrieval, visualization of search results in the

User workflow for the LASAGNA-Search TF binding site analysis platform.

UCSC Genome Browser, and construction of gene regulatory networks, all without leaving the webpage. LASAGNA-Search currently supports promoter searches for seven species, and the authors extensively compared and contrasted their new pipeline with other available programs, demonstrating that LASAGNA-Search consistently outperforms MAPPER2, the most closely related program. LASAGNA-Search promises a more efficient and streamlined method for TFBS searches, with the added promise of expanded content and improved features in the future.

See “LASAGNA-Search: an integrated web tool for transcription factor binding site search and visualization” on page 141.

Optimal miRNA RT-qPCR

MicroRNAs (miRNAs) are key regulators of various biological processes, including development, cell proliferation, and apoptosis. Dysregulation of miRNA expression has been implicated in several disease states, driving recent interest in the suitability of miRNAs as biomarkers. In basic research and for clinical applications, it is essential to assure the accuracy and reproducibility of the techniques utilized for miRNA quantitation. Of these techniques, RT-qPCR is considered the gold standard based on superior sensitivity and specificity. In this issue, Redshaw et al., at LGC Ltd. (Middlesex, UK), examine several key issues potentially affecting the accuracy and repeatability of RT-qPCR measurements of miRNA. Firstly, although there are several widely used, commercially available RT-qPCR platforms for miRNA quantitation employing different approaches, these have not been extensively compared experimentally in the literature for measurement accuracy, precision, and linearity.

Redshaw et al. compared two of the most popular commercial RT-qPCR platforms, which use fundamentally different technologies: the Taqman miRNA assay and the miRCURY LNA Universal RT microRNA PCR assay. A second issue is that upstream procedures, such as RNA sample preparation, have not been carefully examined for their effects on downstream miRNA quantification either. Here, RNA samples are typically enriched for the short RNA fraction containing miRNAs, but the effect of this enrichment on miRNA quantitation by RT-qPCR has not been fully analyzed. Finally, the authors also compare external synthetic miRNA spikes to endogenous miRNAs for use in sample normalization to control for experimental variability during miRNA RT-qPCR. Overall, the results demonstrate that: i) although the two RT-qPCR platforms showed similar efficiencies, there was a difference in variability which could impact miRNA measurements; ii) the performance of synthetic *Arabidopsis* miRNAs spiked into human samples as controls for experimental

variability was comparable to that of the endogenous human miRNAs they examined; and iii) short RNA enrichment of samples can result in significant reductions in miRNA signal, indicating that miRNA quantitation using such pre-enriched samples can be highly problematic. The findings of Redshaw et al. should enable miRNA researchers to more properly design and interpret their miRNA RT-qPCR reactions to obtain the most accurate and reproducible measurements.

See “A comparison of miRNA isolation and RT-qPCR technologies and their effects on quantification accuracy and repeatability” on page 155.

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