WORKING WITH MULTIPLE DATA SETS

Much recent effort has gone into identifying and characterizing DNA binding motifs, and a number of computer programs have been developed specifically to locate DNA binding sites within sequence data. However, comparing results amongst these various programs can be difficult. In this issue of *BioTechniques*, Ngoc Tam L. Tran and his colleagues at the University of Connecticut describe their development of MOTIFSIM, a web-based tool enabling comparisons and similarity detection between multiple DNA motif data sets. With MOTIFSIM, researchers can locate binding motifs that are common between different data sets and then compare the outputs of different programs. The authors tested MOTIFSIM using three experimental data sets that had each been analyzed using multiple motif finding tools. MOTIFSIM was able to identify binding sites that were in agreement with STAMP, an established program capable of performing pairwise comparisons between DNA binding motif data sets. Taken together, MOTIFSIM provides a validated tool for identifying biologically significant DNA binding motifs or discovering motifs that a single tool might have missed.

See “MOTIFSIM: A web tool for detecting similarity in multiple DNA motif datasets” on page 26.

CAN WE TURN BACK?

As any molecular biologist knows, nucleic acids can be damaged by environmental or chemical conditions. Normally, living cells possess repair pathways capable of fixing such hydrolysis or oxidation damage to DNA. Once cell death occurs, however, the damage cannot be reversed naturally, creating a major challenge for researchers in the fields of ancient DNA and forensics. One strategy to overcome this problem is to incubate damaged DNA with a combination of enzymes and biomolecules to reverse post-mortem damage and therefore improve DNA quality for downstream applications. In this issue, Natalie Mouttham and her colleagues at McMaster University test this idea by examining the ability of two available repair protocols to enhance sequence data obtained from four ancient mammoth DNA samples. Unfortunately, neither protocol appreciably repaired ancient DNA samples from mammoth bones. The authors do suggest, however, that optimizing enzymatic repair will be critical for genetic analysis of heavily damaged fossil DNA extracts in the future; thus, detailed experiments and analysis, such as performed here, could provide a launching point for future studies of repair protocols.

See “Surveying the repair of ancient DNA from bones via high-throughput sequencing” on page 19.

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

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AFFINITY PURIFICATION OF EXTRACELLULAR VESICLES

Extracellular vesicles (EVs) are of interest in intercellular communication and biomarker studies, but EV purification methods are still relatively nonspecific, laborious, or small-scale. Since heparin can interfere with EV uptake, Balaj et al. tested heparin affinity purification of EVs. For cell culture samples, concentrated conditioned medium is incubated with heparin-coated beads, and EVs are then eluted with high salt. Depending on the cell type, EV recovery ranges from 30%–60%. Heparin-isolated EVs have substantially less BSA contamination than ultracentrifuged samples and have higher levels of the EV-associated protein Alix. RNA profiles are comparable to those of sucrose gradient-purified EVs. Affinity-purified EVs have a normal appearance as assessed by electron microscopy and function as expected in internalization assays. For applications that don't require intact EVs, the agarose bead/high-salt elution method can be replaced by magnetic beads and kit-based RNA extraction. This convenient, high-yield method for isolating EVs from cell culture or biofluids should accelerate functional assays and biomarker identification.

L. Balaj et al. 2015. Heparin affinity purification of extracellular vesicles. *Sci Rep.* 5:10266.

NONCODING RNA TOOL ENHANCES TRANSLATION

Move over, RNAi, there's a new RNA-based tool for regulating the expression of a target gene. RNAe (RNA enhancement) uses long noncoding RNAs containing sequences that base pair in the vicinity of a target mRNA's initiation codon and ramp up translation. Yao et al. show that RNAe works with various cell lines and targets, boosting expression up to 10-fold. Endogenous transcripts can be targeted, and RNAe may be delivered via RNAe-expressing plasmids or as in vitro-produced transcripts. For researchers preferring the latter, Yao et al. have constructed a plasmid for co-expression of a GFP- or HA-tagged target gene with an anti-tag RNAe. For those favoring a custom approach, the authors describe the minimal RNAe, which comprises a 72-nucleotide antisense sequence, a 167-nucleotide inverted SINEB2 sequence, and a poly(A) tail. RNAe should be useful for protein production and in screening assays as a complement to RNAi knockdown.

Y. Yao et al. 2015. RNAe: an effective method for targeted protein translation enhancement by artificial non-coding RNA with SINEB2 repeat. *Nucleic Acids Res.* 43:e58.

Selected and written by Nijsje Dorman, Ph.D.

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