BioSpotlight

Tuning Out

Rapid size fractionation of nucleic acids (e.g., using silica mini spin columns to remove primers from a PCR reaction) is often required prior to downstream applications. However, silica mini spin columns typically have a cutoff of 100 bp, restricting their usefulness when it comes to removing larger DNA fragments and artifacts. In order to remove these larger DNA fragments, M. Salimullah, P. Carninci, and colleagues at the RIKEN Yokohama Institute (Kanagawa, Japan) have devised a new method for the tunable size fractionation of DNA from spin columns. They substituted a solution of cetyltrimethylammonium bromide (CTAB), a cationic detergent, and urea for the chaotropic salt solution commonly used to capture DNA in a glass fiber spin column, while varying the NaCl concentration in the CTAB-urea solution to obtain different size cutoffs for purified DNA. The authors tested their method by mixing a 50-bp DNA marker ladder with a CTAB-urea solution containing various final concentrations

of NaCl ranging from 0.4 to 0.6 M. In general, increasing the salt concentration resulted in an increase in the cutoff of the size of DNA fragments isolated from the column. The authors found that with a 0.6 M NaCl final concentration the cutoff increased to 150 bp. Although there was a reduction in the overall amount of DNA recovered at this concentration, the authors had sufficient material for library preparation for cap analysis of gene expression (CAGE), a procedure that requires removal of DNA fragments 150 bp and less in size. Aside from tunable size selection, another benefit of the new method is the substitution of the CTAB-urea solution for the chaotropic salt, since the latter is expensive, hazardous, and can interfere with downstream reactions if carried over. This simple, rapid, and inexpensive technique for tunable size purification of DNA should prove useful in a great number of molecular biology protocols.

(See “Tunable fractionation of nucleic acids” on page 1041.)

ALISSA

Methods for live-cell imaging have advanced substantially over the past several years with the introduction of new imaging technologies and fluorescent sensors designed to detect a variety of cellular events. But challenges still remain, particularly in the use of confocal time-lapse imaging to study single cells undergoing proliferation or apoptosis. These events often occur rapidly after prolonged lag periods, making them easy to miss when using standard experimental imaging schedules. Additionally, the extended time frames necessary for these experiments can lead to phototoxicity or bleaching of fluorescent probes, resulting in the loss of resolution or unusable image stacks. To address this problem, J. Wenus, H. Düssmann, and colleagues at the Royal College of Surgeons (Dublin, Ireland) and National University of Ireland, Maynooth developed a method to time laser excitation and imaging with specific experimental stages, which would result in the most useful data. The method, automated live-cell imaging system for signal transduction analyses (ALISSA), consists of three software modules that work together to perform threshold-based automated live-cell microscopy. One component is specific to image analysis, a second is for control of the microscope, and a third depends on the particular biological application and controls

communication between the modules. According to the authors, separating these processes into different modules provides maximum flexibility when it comes to using different microscopy equipment in a broad spectrum of signal transduction applications. The system allows for parallel evaluation of different fluorescent probes, and can be either fully automated or allow the user to select the specific regions or cells on the slide for study. Cell shapes and locations are tracked by differential interference contrast microscopy between each measurement, so cell migration can be imaged easily while the effects

of photobleaching are minimized. The technology is applicable to all microscopes where vendors provide open application program interfaces or software libraries for control of their hardware. The authors are encouraging other researchers to add on to the ALISSA software with their own applications and expect this expansion to create a demand for vendors who do not currently release their software libraries to do so in the future.

(See “ALISSA: an automated live-cell imaging system for signal-transduction analyses” on page 1033.)

Contaminated Shellfish

Glycogen is commonly used as a carrier molecule for the precipitation of very small amounts of nucleic acids. Given the increasingly sensitive nature of many downstream nucleic acid analysis techniques, it is becoming critical to establish that non-nucleic acid coprecipitants are completely free of contaminating nucleic acids. In this issue, A. Bartram, C. Poon, and J. Neufeld at the University of Waterloo (Ontario, Canada) describe their examination of potential nucleic acid contamination in two different carrier molecules: commercially obtained molecular-grade glycogen, which is commonly isolated from mussels or oysters, and a synthetic coprecipitant, linear polyacrylamide (LPA). Initially, nine glycogen and four LPA samples were tested for amplification of a bacterial 16S rRNA gene by PCR. When examined using agarose gel electrophoresis, two glycogen samples (A and D) were positive for a PCR product, while an additional two glycogen samples (C and F) were determined to be positive by denaturing gradient gel electrophoresis (DGGE). None of the LPA samples were found to be positive by either electrophoretic method. Agarose gel electrophoresis of aliquots of glycogen samples A and D clearly demonstrated the presence of both genomic DNA and rRNA bands. Subsequent sequencing of the 16S rRNA PCR products amplified from those two glycogen samples revealed that both products were derived from *Acinetobacter lwoffii*, a known microbiotic inhabitant of mussels. Given the lack of contamination of the LPA samples, the authors next assessed the efficiency of LPA in comparison to glycogen for precipitation of genomic DNA, showing that it is not significantly different regardless of the starting amount of genomic DNA (5 or 50 ng) or the precipitant used (polyethylene glycol solution or isopropanol). Based on their findings, the authors recommend the use of LPA in preference to glycogen as a carrier due to its lack of contaminating nucleic acids and comparable efficiency and cost. But if glycogen is to be used, they recommend that it be exposed to UV illumination, which they show destroys any contaminating nucleic acids.

(See “Nucleic acid contamination of glycogen used in nucleic acid precipitation and assessment of linear polyacrylamide as an alternative co-precipitant” on page 1019.)

Mycoplasma and Microarray

Mycoplasma contamination of cultured cells alters gene expression by adversely affecting cellular physiology. Because the presence of mycoplasma in cultures can be difficult to detect, contaminated cell lines may unintentionally be used in microarray evaluation, leading to confounded data analysis and erroneous conclusions regarding the patterns of gene expression. In a letter to the editor, M. Arno and colleagues at King's College London describe their identification of a probeset on the Affymetrix HG-U133 Plus 2.0 human microarray (1570561_at) that unexpectedly mapped to the 16S–23S intergenic transcribed spacer (ITS) region of multiple mycoplasma species. This sequence was originally submitted as an unknown *Homo sapiens* expressed sequence tag acquired during a full-length cDNA sequencing project, which the authors suspect might explain its inclusion on the human microarray. FASTA alignments of all other target sequences contained on the array showed that only this probeset mapped to the mycoplasma genome. For the authors, the unexpected presence of this mycoplasma probeset raised the question of whether these probes could be used to indicate mycoplasma-contaminated samples. To test this possibility, the authors first searched through publicly available microarray datasets in the Gene Expression Omnibus (GEO) database to find samples showing high expression levels for this probeset and then determined whether those samples came from cultured cells or from non-cultured cells or tissues. Although cultured cells represented only 34% of samples in the entire database, 94% of samples with high expression signals for that particular probeset were from cultured cells, indicating that these samples may have been contaminated with mycoplasma. The authors conclude that while the 1570561_at probeset on the Affymetrix HG-U133 Plus 2.0 array was most likely derived from mycoplasma, this probeset may be useful for detecting contaminating mycoplasma RNA in human microarray samples and high expression values returned from this probeset may indicate mycoplasma infection of a sample.

(See “Unexpected presence of mycoplasma probes on human microarrays” on page 1013.)

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