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Increasing Yield in Nucleic Acid Sample Prep

Researchers Discuss Ways to Combat Sample Diversity and Degradation Issues

- Vicki Glaser

Extraction and purification of nucleic acids from biological materials, whether for research use such as genomic analysis, gene-expression studies, and genotyping or clinical applications like pharmacogenomics, diagnostic and prognostic use, or forensics, involve many of the same challenges. These include the diversity of sample types, the susceptibility of DNA and RNA to nucleases present in cell lysates, and the demand for high-quality, pure nucleic acid suitable for amplification and quantification, use in microarrays, and storage/sample archiving.

Samples may range from fresh or frozen whole blood to plasma, buccal scrapes, cell culture, biopsy material, paraffin-embedded tissue, or plant tissue. Researchers at next month's "GOT Summit" CHI meeting, will be discussing new tools and techniques for improving sample prep.

"For a while now, the trend in nucleic acid sample prep has been the more simple the kit and more simple the protocol, the better for the user," says Steven Dodsworth, Ph.D., director of molecular genetics and genotyping at [Tepnel Life Sciences](#). "If things go wrong in downstream processes, whatever technique you are using, sample prep is something you should automatically suspect. Yet if the user knows little about the extraction and purification process, it may be difficult to troubleshoot the problem."

From a product-development perspective, the robustness of an extraction method is paramount, as the more robust the method, the simpler the protocol can be. As users will vary in their technical abilities, ensuring robustness means that even with variation in volume, for example, of as much as $\pm 10\%$, the prep should still be salvageable. Trying to save on costs upstream during sample prep may have an effect not only on process quality and outcomes but, ultimately, on overall cost.

Tepnel's nucleic acid prep kits are based on the company's Nucleon™ extraction chemistry. The Nucleon HT DNA extraction system can be used to extract DNA from paraffin-embedded tissues or other hard tissues such as mouse tail that require proteinase K digestion for cell lysis. The protocol takes less than 40 minutes and yields more than 40 μg of high purity ($A_{260/280}=1.8-1.9$) and high molecular weight (>50 kb) DNA per cm of mouse tail, according to Dr. Dodsworth.

The Nucleon BACC DNA extraction system can isolate high molecular weight DNA from whole blood and cultured cells, with yields of 370–440 $\mu\text{g}/10$ mL of blood in less than 30 minutes, reports Dr.

Dodsworth. The kit is scalable from 1.5 to 10 mL.

Kevin Krenitzky, M.D., CEO of [BioServe](#), highlights the company's capabilities for extracting DNA, RNA, and protein at the same time from a biological sample to help customers go from "biomaterial to validated data."

BioServe recently moved into its new headquarters in Beltsville, MD, and also maintains a facility in Hyderabad, India. This new, larger home has the capacity to store the Global Repository®, which is composed of more than 600,000 DNA, tissue, and serum samples together with associated clinical data, which BioServe gained as part of its acquisition of Genomics Collaborative one year ago.

"Extraction of nucleic acids is a core competency of the company," says Dr. Krenitzky. "The first step in mining this data is extraction, purification, and QC of the nucleic acid. We have already completed this on about 100,000 samples. Using our multianalyte purification system, we can extract DNA, RNA, and protein."

Dr. Krenitzky has seen a shift in the market over the past 12 months or so from DNA extraction to isolating RNA from biological samples, which yields information about differential gene expression.

In the area of breast cancer diagnostics, for example, while genetic analysis provides a picture of the DNA one inherits, which is basically static except for mutation events, identification and quantification of RNA transcripts enables a comparison of differences in gene expression between normal and tumor tissue.

Dr. Krenitzky describes a trend in which, by comparing the RNA present in tumor cells with the DNA from the peripheral blood of the same person, researchers can begin to understand how an individual's genetic profile combined with environmental risk factors affect changes in gene expression.

BioServe has developed processes and reagents for nucleic acid extraction and optimizes those for each type of sample. For example, it has developed a protocol for isolating DNA from breast tissue, which is a particularly challenging sample to work with because of its high fat content. The company has also optimized an extraction process for purifying DNA from blood clots.

All processes are done manually. "When you use a robot, you lose some control over the process, and we want to maximize yield at each step," explains Mike Seddon, laboratory director at BioServe. Plans are also under way to commercialize the DNA Qwik™ line of DNA extraction kits, which will be followed with an RNA extraction kit.

Improving Recovery

Strategies to maximize target recovery for nucleic acid detection in clinical specimens will be the topic of a "GOT Summit" talk to be given by Bernhard Kaltenboeck, D.V.M., Ph.D., professor and director of the molecular diagnostics lab, department of pathobiology, at Auburn University.

The limited sensitivity of many current assays designed to detect pathogenic agents present in low numbers in biological samples is an ongoing challenge for nucleic acid-based diagnostics, despite the ability of PCR and other nucleic acid amplification techniques to detect single target copies. The main problem, according to Dr. Kaltenboeck, is the lack of robust extraction methods capable of recovering

rare copies of target DNA from large sample volumes and concentrating them for use in assay protocols.

Dr. Kaltenboeck will present an approach for the efficient extraction and concentration of low copy number DNA targets that can increase assay detection sensitivity by as much as 100-fold compared to conventional techniques, he says.

Chlamydia is described by Dr. Kaltenboeck as an example of a sneaky pathogen that causes low-level inflammation but does not kill cells. It has been implicated in heart disease and other chronic degenerative inflammatory diseases. The pathogen is present in very low copy numbers in affected tissue and is difficult to cultivate. The diagnostic challenge lies in capturing the few copies of *Chlamydia* that are present for detection with PCR in a 20 μ L reaction volume, of which only 5–10 μ L is sample.

Mastitis in cows may be caused by *Chlamydia*. To test their nucleic acid extraction method, Dr. Kaltenboeck's group spiked cow's milk, a particularly difficult diagnostic medium due to its high fat and sugar content, with *Chlamydia* and removed the DNA from solution by binding to a glass fiber matrix. Following multiple wash steps to remove the proteins, carbohydrates, lipids, and other cell matrix components, they then eluted the DNA by increasing the pH and collected the sample reservoir.

While DNA extraction kits that perform these functions are commercially available, "the standard protocol for their use will result in the recovery of a dilute DNA sample, so that five microliters of the eluted DNA (the PCR input volume) will be equivalent to about one microliter of original sample volume," says Dr. Kaltenboeck. "It is obvious that this limits the detection by even the best PCR method to about 1,000 DNA copies per mL of original sample. This is not sufficiently sensitive for detection of low-level, chronic infections."

Dr. Kaltenboeck improved the overall DNA extraction efficiency by combining multiple steps that stabilized nucleic acids, bound more nucleic acids to the extraction matrix, and recovered those nucleic acids in a lower eluate volume. These steps are multiplicative in their effect on improving nucleic acids recovery.

A frequently overlooked factor limiting detection sensitivity is the rapid degradation of nucleic acids after thawing of frozen specimens. "This can reduce recoverable nucleic acids by as much as 50 percent within five minutes at room temperature," explains Dr. Kaltenboeck.

Addition of an equal volume of guanidinium-based sample-preservation buffer immediately after sample collection stops nucleic acid degradation and allows storage and shipment of samples at ambient temperature, with less than 30% target loss over a one-year storage time, according to Dr. Kaltenboeck. Furthermore, repeated addition of up to seven 200 μ L aliquots of the sample-preservation buffer mix, instead of a single aliquot, increases nucleic acid binding by up to sevenfold. Elution in a 20 μ L reaction volume instead of 200 μ L increased nucleic acid concentration by another 10-fold.

"When combined, these steps amounted to 100-fold higher DNA input per PCR than by using standard nucleic acid extraction procedures and increased PCR sensitivity by this amount," says Dr. Kaltenboeck.

At the "GOT Summit," Huimin Kong, Ph.D., president and CEO of [BioHelix](#), will describe the company's Rapisome™ pWGA kit, a primase-based whole-genome amplification (WGA) system that can be used for nucleic acid sample prep. WGA is a valuable method of generating the starting material needed for real-time PCR and of expanding a limited genetic sample for archiving, genotyping, forensic analysis, aCGH for genome-wide copy number analysis, or other downstream applications, according to Dr. Kong.

“pWGA can achieve 1,000-fold amplification,” reports Dr. Kong. The technology utilizes a primase enzyme to synthesize primers directly on the DNA template, rather than adding random, ready-made primers to the sample. It performs rapid isothermal DNA amplification without the need for thermocycling or heat denaturation, explains Dr. Kong.

Rapisome also incorporates helicase activity to unwind the DNA to enable primer synthesis. “We have reconstituted the in vivo DNA replication system and can do it in vitro in one step in one hour,” explains Dr. Kong.

The Rapisome pWGA kit’s primary research application has been in preamplification for downstream analysis, but Dr. Kong envisions a growing role for the technology in clinical diagnostics. For example, Dr. Kong points to cystic fibrosis diagnostics, which at present rely on simultaneous scoring of 20 mutations. The patient sample must first undergo DNA amplification to generate enough DNA to perform multiplexed SNP genotyping. Multiplex PCR-based amplification has limitations that Dr. Kong believes can be overcome using WGA. BioHelix is working to enhance the amplification power and sensitivity of WGA to enable its future development for highly multiplexed diagnostic applications.

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