Nucleic Acid Sample-Prep Tools Break New Ground

Josh Roberts, Ph.D.

Say "nucleic acid sample preparation" these days, and you could be talking about anything from a manual phenol-chloroform extraction of total RNA to a hands-free system that delivers a report of the organisms found in a sample of dirt.

Or perhaps it's a reference to what doesn't need to be done as, for example, when a sample can be processed directly from serum or whole blood.

A number of researchers will gather at the Knowledge Foundation Conference on "Integrating Sample Preparation: Techniques and Applications" in Baltimore later this month to address a host of sample preparation topics.

GEN recently spoke to several of these scientists whose talks will range from discussions on novel and improved methodologies to technologies that incorporate

Up- and Downstream Go Single Use

Vicki Glaser

Single-use bioprocessing product lines made their debut mainly in the form of disposable bags for mixing and buffer supply. They gradually moved into the heart of the bioprocess stream with the emergence of single-use bioreactor systems.

Single use has more recently made inroads in downstream process flows for separation, purification, and fill and finish applications. This evolution in the industry is clearly illustrated at bioprocessing conferences, in which presentations highlight the range of product areas and biopharmaceutical R&D and manufacturing applications targeted by single-use technologies.

Examples include IBC's recent "Single-Use Applications for Biopharmaceutical Manufacturing" see page 46

The FlexFactory flexible platform enables biomanufacturers to accelerate deployment of new manufacturing capacity while lowering risk, decreasing time to market, and reducing capital costs, according to Xcellerex, which reports that the approach enables the deployment of new production facilities in 9 to 18 months at a total cost less than 50% that of conventional plants.
these approaches for use in academic labs and for ready-market devices.

Traditionally, nucleic acid preps are designed to gather long stretches of RNA and/or DNA, with those less than 50 nucleotides considered purely unwanted fragments. Although that view has drastically changed in the past decade or so, most protocols for extracting RNA still purposefully get rid of diminishing species like the ~22-mer chloride mRNA.

These protocols do specifically include small nucleic acids typically include centrifugation and/or filtration steps, now—Bee-Ne Lee, Ph.D., senior applications scientist at Beckman Coulter Life Sciences (www.beckmancoulter.com).

“These methods usually do not produce consistent yield of mRNA for downstream applications, and they are not very amenable to high throughput.”

To rectify this and allow mRNA to be isolated from FFPE and cell cultures in an automated fashion, Dr. Lee modified the binding and re-binding buffer conditions used with Beckman Coulter's Agencourt FormaPure and RNAAdvace Cell v2 kits, respectively.

Lee utilized the company’s solid-phase reversible immobilization (SPRI) technology. SPRI's negatively charged carboxyl-coated magnetic beads would normally repel the negatively charged nucleic acids.

However, an aqueous pocket is created by using a “crowding reagent” which allows the nucleic acids to move to the polar phase and reversibly bind to the beads in the presence of binding buffer. After exposure to a magnetic field, the beads that bind nucleic acids will pull to form a ring at the bottom of a well.

“You can remove all the contamination. Aspirate everything out, touching the tip to the bottom,” explains Dr. Lee. The DNA or RNA is then eluted with buffer that is “mainly just water,” preventing rebinding of downstream applications that are used with other protocols.

For small RNA expression applications, “we recently will put the total RNA in … our yield is so high we don’t require enrichment,” she continues.

For this she created the SPRi technology, which in addition to proprietary reagents utilizes homogenous-sized beads that are slow to aggregate or sedent, alleviating the need to frequently re-aspirate. A large surface area-to-volume ratio allows for a high binding capacity, thus allowing the beads to rapidly respond to the magnetic field.

Taking on Sepsis

When looking for a needle in a haystack—or a single bacterium in a milliliter of blood—“the main problem becomes sample prep,” explains Sergey Dryga, Ph.D., vp of immunology at nanoMR (www.nanoim.org).

Because the concentration of microorganisms responsible for sepsis is low—on the order of one colony forming unit (CFU) per mL of infected blood in bacteremia, for example—rapid tests using small volumes of blood do not have the sensitivity to deliver a statistically significant result.

Currently only those starting from a positive blood culture can do so. However, the cultures must be grown for 12–48 hours before a pathogen might even be detected, and another 12–24 hours to identify the offending organism, with the delay impacting the ability and cost to successfully treat the infection, Dr. Dryga points out.

nanoMR's pathogen capture system (nanoMR PCS) uses antibody-coated magnetic nanoparticles to pull our pathogens from blood and deliver purified DNA in less than an hour, without the need to lyse the blood or purify the bacteria, Dr. Dryga continues.

“The method is old but nobody knows how to do it in blood, because blood is a very complicated matrix. Our innovation is that we basically developed bead chemistry, antibodies, and conditions that work in blood.”

To use the nanoMR PCS a 10 mL vacutainer of blood is delivered to a VCR tape.

The main goal of sample preparation is to confirm that the sample is in the best possible condition with the required standard of purity for subsequent analysis.

See Sample Prep on page 29

NEWS
Genomics & Proteomics

> Test Predicts Differential Treatment Benefit between Chemotherapy and Targeted Therapy for Lung Cancer Patients

Results from a VeriStrat analysis of 202 patients with non-small cell lung cancer (NSCLC) enrolled in a Phase II study of erlotinib, gemcitabine, and bevacizumab in elderly advanced non-small cell lung cancer patients showed that the VeriStrat test was able to identify patients more likely to have a slower progression of disease and longer overall survival when treated with either single agent chemotherapy, gemcitabine, or targeted therapy, erlotinib.

Biosideix developed VeriStrat as a blood-based test for patients with cancer to provide results on the patient's proteomic profile.

> Thermo Fisher Licenses Proteon's Metrics' Byonic Database Search Software

Thermo Fisher Scientific inked a nonexclusive agreement with Protein Metrics to license its Byonic software, a next-generation proteomics search engine. The database search software system will add next-generation capability to streamlined analysis of proteomics and other post-translational modification data, according to a Thermo official.

In addition, Thermo introduced its Proteomics Discoverer software version 2.0 for analyzing quantitative and qualitative proteomics data.

> CLC Bio Wins $2M for Pathogen Sequencing

Bioinformatics software firm CLC bio has been awarded $2 million in EU funding as part of the 510 million Pathseek project, a clinical microbiology initiative that aims to demonstrate the use of next-generation sequencing for detecting pathogens and drug resistance directly in clinical samples. CLC's involvement will focus on developing a user-friendly software package that will allow clinical laboratories to carry out pathogen laboratories to carry out pathogen identification, host biomarker identification, pathogen-variant characterization, and molecular epidemiology.

"Current platforms in diagnostic laboratories are time required for generating a result and by the limited sequence information available for pathogens," comments Rolf Forsberg, vp of R&D at CLC. "To overcome these limitations we're going to develop a disruptive diagnostic technological pathway which utilizes our world-leading bioinformatics expertise to enable scientists to go from a patient sample to a result, in less than 48 hours."

> Collaboration Focuses on Blood Biopsy Tumor Profiling

Transgenomic and NYU Langone Medical Center inked a collaboration focused on application of the firm's Ice Cold-PCR mutation detection technology to identify treatment-related mutations in the circulating tumor cells (CTCs) of patients with surgically resectable early-stage lung cancer as a means to help tailor therapy. The partnership follows on from Transgenomic's recent collaboration with researchers at the MD Anderson Cancer Center, which is focused on exploiting the Ice Cold-PCR to characterize tumor-derived DNA in blood and CTCs from patients with a variety of cancers.

The partnership will involve isolating CTCs from the blood of about 200 patients using ScreenCell's ScreenCell CTC capture system both before and after surgery to determine if CTC numbers change, or are linked with disease recurrence or progression. DNA from these cells and also cell-free DNA (cfDNA) will then be analyzed using the Ice Cold-PCR technology to identify mutations known to be linked with a response to targeted drugs.

> ShanghaiBio Partners with Ingenuity on Genomic Data Analysis

ShanghaiBio and Ingenuity Systems signed a collaboration agreement enabling ShanghaiBio to extend its current lab service offering to include Ingenuity solutions for downstream analysis and interpretation of genomics data.

ShanghaiBio officials say they will complement their sequencing, genotyping, and gene expression lab services by creating a bundled solution with Ingenuity's applications, which combine analytics and biomedical content to help get actionable insights from experiments.
Sample Prep

Field Work

While nanoMR’s initial primary market is likely to be hospital clinical microlabs, Integrated Nano-Technologies (INT; www.integratednano.com) aims to create a fully automated field laboratory using cartridges and a generic platform. Input can be a wide variety of samples: blood, tissue, insects, soil, or air filters.

“The fluidic cartridges and the devices that we’ve developed allow us to do a lot of the basic techniques you find in a laboratory,” explains INT’s president and CEO Michael Connolly, Ph.D. These include ultrasonic and chemical disruption, filtration, magnetic separation, washing and concentration of nucleic acids or proteins from a sample, small column desalting, or purification.

“And then we do PCR amplification in the cartridge, and then take that material to the detector in there.”

The company will initially produce two fully integrated units: one battery-powered, and plug-in (with battery back-up) capable of running ten tests simultaneously. Each has an integrated barcode reader and is GPS-, WiFi-, cellular-enabled, which allows them to be deployed on ships and in remote outposts.

Applications not requiring regulatory approval are expected to be available by year’s end. In one such application, the cartridge will contain a panel capable of recognizing the major mosquito-borne disease pathogens, including the alphavirus, flaviviruses, and bunyavirus; viruses, dengue, and malaria.

“So you can drop the mosquitoes in there and DNA will be taken out, cleaned, amplified, and taken to the sensor, and read. The results will then be reported to you,” says Dr. Connolly.

The company will pursue three market segments. Much of their funding has come from the U.S. Department of Defense for military/security applications, and the company has a multiplexed test for bioterrorism including anthrax in the offing, for example. They also plan to pursue the veterinary market and, as a longer-term goal, human diagnostics. The latter, Dr. Connolly points out, overlaps with the military market in that applications will be designed to test deployed soldiers for endemic infections.

Diving into mRNA Detection

Signs of organ disease may be in the blood long before other phenotypic signs are evident. The kidney and liver, for example, release abundant amounts of mRNA indicating the organ is no longer healthy, explains Martin Hegner, Ph.D., professor in the Centre for Research on Adaptive Nanostructures and Nanodevices at Trinity College Dublin.

Similarly, such miRNAs could be used to confirm a diagnosis in an emergency situation. Dr. Hegner has been working for the past decade on ways to detect soluble macromolecules with small cantilevered array sensors.

See Sample Prep on page 30

The disposable fast cartridge for the Palladium field diagnostic system automates all steps of sample preparation, amplification, and detection in a single low-cost disposable, according to Integrated Nano-Technologies.
At the Knowledge Foundation conference he will describe work undertaken in collaboration with Hoffman LaRoche (www.roche.com) using these springboard-like nanomechanical sensors to specifically detect small RNA from serum using cantilever array sensors within 10–15 minutes.

"You have a sample from cells, you lyse the cells, then you sediment the debris and inject the supernatant." Because mRNA in blood may be present at concentrations of up to 200 million per milliliter and quite stable (as opposed to its longer RNA cousins), it should in principle be quite easy to measure, "This is not something where you’re going for single molecule detection," he notes.

The sensors are coated with matching sequences which detect bound complimentary mRNA in a couple of ways, with no labeling or modification of the target necessary, either by binding of bait, or by changing the bait’s oscillation. The technology was initially derived from scanning probe microscopy, and delivers sensitivity at the Angstrom level that can be read out using laser optics.

Any kind of small mechanical sensor will react to its environment and so it’s mandatory to include reference sensors "which are able to decouple any kind of background non-specific binding from the real signal we are looking at," explains Dr. Hegner. "We always have a minimum of two sensors. It’s a differential readout." The team is also collaborating with the California Institute of Technology to develop a version using integrated nanoelectronics in the springboard itself “where we don’t need an optical readout," he says, with the aim being the creation of an entire system in a handheld device, perhaps even for use in an ambulance.

SNP

Preparing samples for Eureka Genomics’ (www.eurekagénomics.com) Mass Genotyping by Sequencing Technology methodology starts with heating DNA to melt it apart and break it into smaller pieces, "which makes the hybridization of the next set of probes onto it much easier," explains John Curry, Ph.D., senior scientist.

For each single nucleotide polymorphism (SNP) site on the DNA, three barcoded probes are added: a phosphorylated right hybridization sequence, and two left hybridization sequences, which differ by a complementary SNP and permit discrimination between the two different alleles in the genomic sequence. The hybridized probes are then ligated and act as a template for the subsequent PCR reaction, which further adds sample specific indexes.

The assays (one sample, but hundreds of loci, per well) begin in 384-well plates and are combined and spun down into a small library "so we’re really taking a few milliliters of PCR products and reducing it down to 100 pL of library," explains Dr. Curry. "And a portion of that library goes into the sequencer."

This type of ligase discrimination for SNPs has been done for 20 years. Yet "whereas before people would do this assay one at a time, or 40 at a time, and resolve it on a PAGE gel, we’re resolving it on a next-gen sequencing platform," he continues.

"So we’re able to put thousands of samples, with hundreds of loci, into a single tube, onto a single lane of an instrument, and get the information back, and decipher it, and determine the genotypes for basically 1,000 x 100 SNP sample combinations."

The assay never actually has to read the biological information itself "because they’re all on the probes that are designed from the biological information," notes Dr Curry. This allows for shorter, more economical reads. In addition the barcodes can be multiplexed.

"We’re able to drive the cost down to fractions of a cent per animal SNP- combination, and do them all at once," Eureka Genomics has already commercialized this process for agricultural and clinical applications.

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The new CELLreactor™ from Greiner Bio-One is an innovative 50 ml polypropylene tube which has a filter screw cap with a USP Class VI certified capillary pore membrane. With a pore size of 0.2 μm, the membrane not only keeps the contents of the tube completely sterile, but also ensures an excellent gas exchange. This can be reduced by closing individual openings.

The tube enables the miniaturisation of large-scale setups while simultaneously maximising the number of parallel experiments. It can therefore be used as a small bioreactor for the cultivation of cells. Another advantage of the CELLreactor™ is that no transfer is required for cell harvest. Due to its conical design, the tube fits into all standard 50 ml centrifuge rotors and the cells can be sedimented directly within the tube.

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