Drug Discovery & Development
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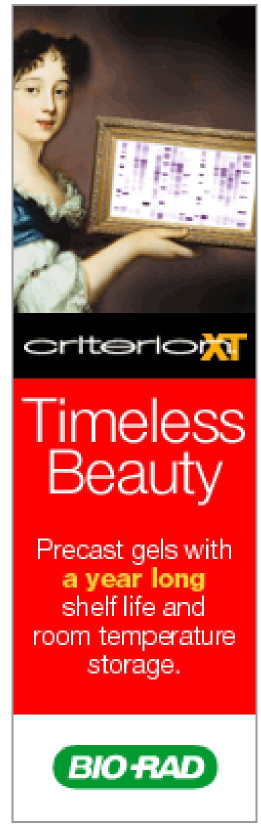
May 15, 2003



Feature Stories

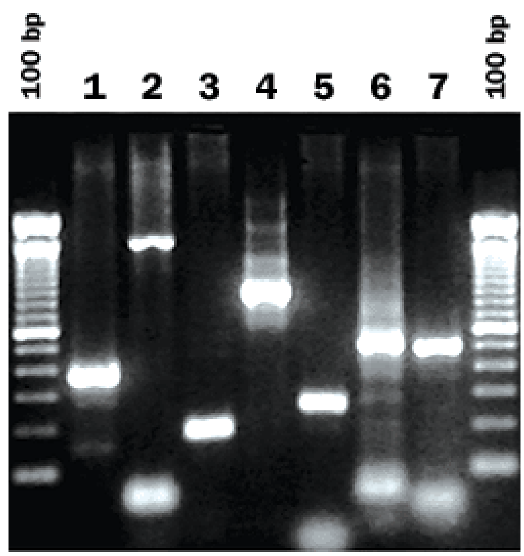
January 2002

Fresh Fuel for the Genomics Fire

Automation has revolutionized the genotyping process, allowing researchers to streamline genomic DNA purification.

Today, scientists use automated genotyping to sift through thousands of gene variants in literally millions of people. In order to supply human DNA for these projects, automation is required.

Among the first researchers to automate DNA extraction is Tim Howard of Wake Forest Univ. School of Medicine, Winston-Salem, N.C. Howard is a member of the university's new Center for Human Genomics and also works with researchers at three other universities as part of the Collaborative Study on the Genetics of Asthma (CSGA). Howard and his colleagues are searching for genes governing predisposition to asthma and related allergic diseases.



DNA purified from human whole blood on the Autopure LS was analyzed by polymerase chain reaction amplification. The DNA was amplified with primers specific for seven different loci, including HLA-H, CYP2D6, MTHFR, HLA-H, Factor V, D1S80, and Factor II (lanes one through seven respectively).

Howard's urge to remove bottlenecks led him to automated DNA purification. His center extracts genomic DNA from approximately 4,000 blood samples a year. Manual purifications used to absorb the efforts (and most of the salaries) of three technicians.

Initially, Howard was looking for an instrument that would purify DNA from 10-mL blood draws with minimal human intervention.

The first instruments he looked at either made DNA from small volumes, which were usually only 1 mL or less, or couldn't purify DNA from blood. Then in 2000, Howard's interest was peaked when Gentra Systems Inc., Minneapolis, introduced the Autopure LS.

The Autopure LS was automatic, purified genomic DNA from 10-mL blood samples, and processed in batches of eight or 16. It yielded up to 500 µg of DNA/sample. The average molecular weight was about 100 to 200 kb.

Today, one technician operates the Autopure LS, usually doing 64 preps a day. In addition, in eight hours, the instrument can finish 96 preps, beginning with blood and ending with pure DNA.

Archiving DNA

The 10-mL blood draw is popular because that much blood assures a good yield of DNA. Some investigators are satisfied with the DNA they can get from cheek swabs. But if they run out of DNA, it's often expensive or impossible to go back to a subject for more. The wiser practice is to make enough DNA to archive.

As part of the CSGA study, Howard and his collaborators collect three tubes of blood from each patient or family member. They use the Autopure LS to isolate DNA from each blood vial independently, for three DNA stock tubes per subject. From one stock tube, a dilution tube is made and used repeatedly until it becomes necessary to go back to the stock.

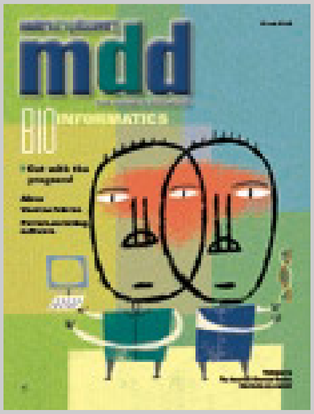
Archiving DNA requires excellent purification chemistry plus precautions against degradation. The Autopure LS uses Gentra's Puregene chemistry, which routinely renders contaminant-free, high molecular-weight genomic DNA. And unlike the phenol-chloroform method still in use for manual DNA preps, Puregene chemistry produces no toxic wastes.

In addition, Gentra's data demonstrates that genomic DNA prepared by Puregene chemistry and stored at 4ºC remains intact for at least nine years.

Another Puregene and Autopure LS user, Markus Perola, who studies cardiovascular disorders at the National Public Health Institute, Helsinki, Finland, found an occasion to reuse human DNA samples frozen five years ago. He and his collaborator Leena Peltonen of the Univ. of California, Los Angeles, had a hunch that their genomics data on Finnish families with rare genetic disorders could be reanalyzed to pinpoint genes for traits entirely unrelated to those diseases.

During the course of their investigation, they needed to retrieve DNA samples archived five years before. Without these archives, it would have been difficult to confirm their discovery of a significant locus for height on chromosome 7.

Already, Howard credits the Autopure LS with giving him more time for the things he truly cares about. "Before, one of our main focuses was DNA isolation," he says. Now, DNA isolation runs continuously in the background and he is free to concentrate on science.



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Vol. 5, No. 3, pp 25–26.

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High-throughput DNA purification

Automation is the buzz that's busting bottlenecks in large-scale population studies

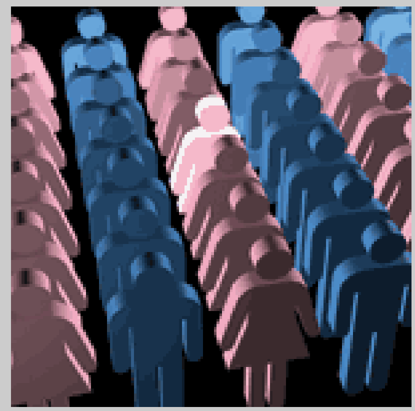

ILLUSTRATION: TONY FERNANDEZ

With so much genomics research going on in the United States, it is easy to miss the hum of initiatives around the world. Researchers eager to lay hands on genes controlling disease have launched genomics projects in lands as distant and different as Iceland, Estonia, Sardinia, Newfoundland, and the South Pacific island kingdom of Tonga. Together, these population projects account for millions of large-volume patient samples, yet the idea of purifying DNA from even a few thousand samples remains a daunting prospect.

Scientists want to use automated genotyping to examine thousands of gene variants in millions of people, tracking down single genes controlling the rarest of diseases and locating the many genes that subtly influence our most widespread and complex diseases. Genomics in a faraway place can make those tasks easier: Quirks in isolated gene pools sometimes give researchers piercing insight into the genetic aspects of complex diseases. Genomic research in Icelandic populations has already resulted in the identification of genes linked to obesity, anxiety, diabetes, schizophrenia, Parkinson's disease, and rheumatoid arthritis.

Genomics is also spreading for other reasons. Iceland's genomics project is aided by family trees that trace as far back as the 9th century, when ties of blood were recorded with the ink of blood, and animal skins served in place of paper. Estonia drives its project forward on sheer ambition. Its goal is to own the world's largest genome project within five years, holding DNA samples from 1 million people–2 of every 3 Estonians.

The buzz behind this genetic land rush is that population geneticists must find faster methods than preparing human genomic DNA by hand. Think of it this way. Which of the following phrases sounds out of place in a genomics laboratory?

- Thousands of bases sequenced per day.
- Thousands of genotypes per day.
- Dozens of manual DNA preps per day.

The fact is that population genomics requires automation wherever possible, including DNA purification. Manual preps are fine for small projects, but competing in genomics by using hand-prepped DNA is like filling the tank at a NASCAR pit stop by using a teaspoon.

First to Finnish

One researcher who understands this is Markus Perola, who conducts genomics research in the human genetics laboratory of Leena Peltonen at the University of California, Los Angeles (UCLA). While working with Peltonen as a graduate student at the National Public Health Institute in Helsinki, Perola studied rare recessive disorders in Finnish families, mapping and isolating the responsible genes. "Many people call Finland ideal for studying genetic disorders," Perola comments, "because we are sort of isolated, separated for centuries without much immigration."

Perola was given a thesis project on the genetics of hypertension. The complexity of the disease required DNA samples from many people, and he quickly became expert at isolating and purifying genomic DNA. Around the same time, epidemiologists at the institute began studies calling for enormous numbers of DNA samples. Perola was enlisted to help them. Soon, he found himself acting as the head of a team of eight, preparing DNA from 60,000 Finns entirely by hand, using the old phenol–chloroform method (see box, "DNA preparation: Old and new").

After receiving his doctorate, Perola followed Peltonen to her new laboratory in California, where he began using DNA microarrays to study the genetics of psychiatric and cardiovascular problems. Soon after his arrival, UCLA's human genetics department asked him to advise it on the best way to handle DNA preparation. He never doubted that the answer was to automate. The question was how.

The standard source of human DNA is blood drawn in 10-mL aliquots. Perola needed an instrument that would work with minimal human intervention, extracting DNA pure enough to store for years without degradation. Ideally, the preparation chemistry would not generate toxic wastes. He decided on the Autopure LS, introduced in 2000 by Gentra Systems, Inc. (Minneapolis; www.gentra.com).

Archival-quality DNA

It should be possible to put genomic DNA in cold storage and forget about it, without wondering if it will be there when you need it. But as Perola knows, unless DNA is prepared correctly it will not be of archival quality, and researchers' hopes of meaningful findings will fragment and degrade along with their precious DNA samples. For even in the cold, incorrectly prepared DNA can break down.

DNA preps degrade for three reasons. They can be contaminated by bacteria, but storage at 4 °C or lower prevents this, says Perola. More often, degradation is due to contamination of the sample with DNase from skin. Wearing gloves while preparing DNA can solve this. Generally overlooked, however, is degradation caused by continual cycles of freezing and thawing. Perola minimizes the problem by planning ahead.

"We have a one-usage tube, as we call it, for aliquoting," he says, "and a stock tube that is used once every few years." As an extra precaution, each sample has two stock tubes, which he stores at –20 °C. A better temperature would be –80 °C, which avoids slow evaporation and changes in DNA concentration, but freezing at –20 °C is less expensive.

Perola strongly advises preparing DNA as soon as possible after drawing blood. Storing blood first and preparing DNA later sounds convenient but creates unnecessary work. His experience is consistent with findings that Gentra has published for some years: Storing blood results in lower yields (often substantially lower), no matter what the purification method.

Productivity

UCLA's human genetics department organizes genomics with core laboratories. Instead of each faculty member's laboratory doing its own genotyping, sequencing, and DNA extraction, core laboratories boost department productivity by handling these functions as services. UCLA already has core laboratories for genotyping and sequencing. Perola set up an automated DNA extraction service before returning to Finland, where he will continue his work in human genetics.

What Perola did for UCLA, he hopes to repeat in his laboratory back home. He sees the difference that automation makes. "At UCLA, one person can do about all that needs to be done," says Perola. "The tech puts the samples on and comes back later. There's the DNA." Another bonus is not having to worry about phenol and chloroform. "This is important for us because both phenol and chloroform are carcinogenic and toxic," he says. "And they also break DNA."

Frozen treasure

Recently, Perola found occasion to reuse DNA samples frozen five years earlier. He and Peltonen decided to reanalyze genomics data from earlier studies on Finnish families with hypertension, obesity, osteoarthritis, migraine, and familial combined hyperlipidemia. This time, they hoped that the data could be used to pinpoint genes controlling traits unrelated to those diseases. Fortunately, a great deal of patient information was collected with each blood sample used for DNA purification, giving the researchers the opportunity to use the samples retrospectively for analysis of linkage to other diseases and traits. Soon, they found something. But to be sure of what they had, they needed to retrieve the stored DNA samples and genotype them one more time.

"That's an example of the kind of treasure you have once you have these materials," Perola says with enthusiasm. "One analysis is not enough. You have to reanalyze in order to get all the information out." The discovery confirmed by their return to the archives—that a gene on chromosome 7 is linked to height—was published in the July 2000 issue of the *American Journal of Human Genetics*.

With the help of heavy-duty nucleic acid purification systems that can produce archival-quality DNA, investigators like Markus Perola can look forward to more successes. Each one is a part of the quiet hum of progress in international research, which is punctuated occasionally by the sound of a geneticist busting through another bottleneck. And in genomics research, busting barriers is the buzz.
