NanoGBS: A Miniaturized Procedure for High-Throughput Plant Genotyping-by-Sequencing Library Preparation

High-throughput sequencing-based genotyping methods to assist in agricultural breeding, such as genotyping-by-sequencing (GBS), have provided genotyping solutions in several species. This case study is based on a GenomeWeb webinar, sponsored by Beckman Coulter Life Sciences, in which Davoud Torkamaneh, assistant professor at Université Laval, presented NanoGBS — a protocol for GBS that employs an Echo Acoustic Liquid Handler from Beckman Coulter Life Sciences to reduce the volumes of reagents, plastic waste, hands-on time, and costs associated with GBS library preparation.

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Torkamaneh began by discussing genetic variation and its importance in agriculture, defining genetic variations as the differences in the DNA sequences amongst individuals within a population. There are many kinds of nucleotide variants, including structural variants like deletions, transmutations, inversions, and duplications of large (more than 50 base pairs) sections of the genome. More common, however, are single nucleotide polymorphisms (SNPs), of which one can be found in every thousand base pairs, on average, said Torkamaneh.

SNPs can cause phenotypic differences between individuals and thus can be used as molecular markers for phenotypic traits to assist in disease diagnosis and screening, as well as for selecting traits of interest in agricultural breeding. To do so, according to Torkamaneh, individuals must be genotyped to discover the SNPs associated with their phenotype. The most common methods of genotyping SNPs are with SNP microarrays, whole-genome sequencing, and GBS, each of which has benefits and drawbacks, Torkamaneh noted.

Microarrays require significant pre-investment to sequence a population and discover the relevant SNPs, then develop a microarray with which to genotype individuals. Microarrays cost about $100 to $150 per sample but provide genotyping results without requiring any significant bioinformatic analysis. Whole-genome sequencing does not require a pre-investment but involves sequencing the entire genome of each individual, costing about $500 to $5,000 per sample, depending on the genome size, and requiring the analysis of large datasets containing unneeded sequence information.

GBS is a targeted method by which DNA is fragmented and only specific regions of interest in the genome are sequenced. GBS lowers the cost of genotyping to about $20 to $30 per sample but also limits the amount of data acquired.

GBS Protocol

To begin the laboratory protocol for GBS, DNA samples are prepared in a well plate. A restriction/digestion enzyme mix is added, followed by a ligation mix and sequencing adapters with individual barcodes for each sample. The samples are then pooled and size-selected for appropriately sized fragments. The DNA is amplified by PCR, followed by quality control and sequencing. The sequencing data is then analyzed and can be used for genome-wide association analysis, genetic diversity analysis, or genomic selection for breeding.

In plant breeding without genomic selection, parent plants are bred to generate a population whose phenotypic traits are assessed for multiple generations, explained Torkamaneh. With genomic selection, the population is phenotyped and genotyped, and the information is used to train a statistical model used to predict phenotypic output of further breeding based on genotyping data. "We need to genotype thousands and sometimes millions of individuals," Torkamaneh said, "so we need a cost-effective genotyping platform."

The Costs of GBS

The costs of genotyping come from three elements: library preparation costs, sequencing costs, and data analysis costs. Sequencing costs and data analysis costs have seen huge, outsized reductions over the last two decades, but library preparation costs have not enjoyed the same reductions and have the greatest opportunity for improvement, Torkamaneh noted.

Previously, Torkamaneh's lab's GBS protocol used 10 µL of DNA, 20 µL of restriction enzyme mix, 15 µL of ligation mix, and 5 µL of adapter mix, creating a mixture of 50 µL in total volume. However, only 5 µL of the final mix was required for pooling, PCR, and sequencing, leaving 45 µL of waste. The lab's protocol could not accommodate smaller volumes because their pipettes could not reliably transfer volumes smaller than 5 µL. To make library preparation less costly and wasteful, Torkamaneh's lab employed an Echo Acoustic Liquid Handler from Beckman Coulter Life Sciences.

Echo Acoustic Liquid Handlers

The Echo Liquid Handlers transfer nanoliter volumes of liquids as droplets using acoustic energy and thus do not use pipette tips or otherwise contact the liquids. An acoustic energy transducer located below a source plate of reagents emits energy, ejecting precisely measured nanoliter-scale droplets from wells in the source plate into wells of an upside-down destination plate positioned above the source plate, where capillarity holds the liquids in place. The transducer can eject droplets hundreds of times per second to dispense liquids at different scales, and the plates can move relative to each other so liquid in any source well can be dispensed into any destination well.

"Echo systems are highly accurate and precise and preserve the integrity of liquids, including solutions containing DNA, RNA, enzymes, master mixes, polymers, proteins, and other small molecules," Torkamaneh said. His lab uses the Echo 525 system, which produces droplets of 25 nL each, can dispense up to 6 µL per second, and can dispense from 384-well source plates into destination plates with six wells and above.

NanoGBS

Using an Echo liquid handler, Torkamaneh explained, the team developed a new GBS protocol, called NanoGBS, that reduces opportunities for contamination, the need for plastic pipette tips, and volumes of reagents needed during library preparation. Using the Echo system, the GBS reactions can be performed using only 1 µL of DNA, 2 µL of restriction enzyme, 1.5 µL of ligation mix, and 0.5 µL of adapters, creating a final reaction volume of 5 µL — a 10-fold reduction from the traditional methods.

"In the NanoGBS method, we could save 90 percent in reagent usage," Torkamaneh said. "But the question was, 'Is the quality of the genotype the same when we reduce the reagent volume?'" To answer this question, his team performed NanoGBS and standard GBS on 96 soybean samples for which they have whole-genome sequencing data to compare results.

"We found more than 96 percent overlap between the SNP data derived from the NanoGBS and Standard GBS for the same samples," he said. "Most importantly, we found 100 percent agreement between these two methods. This means that by reducing the volumes of the materials for the library preparation, we can still generate exactly the same high-quality genotyping."

The team then compared the costs of the reagents and supplies of the two methods, finding that NanoGBS can save up to 72 percent of the cost, including labor and sequencing, depending on how many samples are being run at once. Additionally, hands-on time was reduced by 75 percent using NanoGBS as compared to standard GBS.

An additional benefit of NanoGBS is the reduction in plastic waste, added Torkamaneh. "The genomics lab is one of the sources of the production of plastics because we use tons of tips for manual pipetting," he said. "And the sad part is that most of these plastics that we use in the genomics labs, they're not recyclable." He cited a 2015 study that estimated that bioscience laboratories generated about 5.5 million tons of plastic waste in 2014, "roughly the combined tonnage of 67 cruise liners, and equal to 83 percent of the plastic recycled worldwide in 2012."

Standard GBS for one 96-well plate uses 63 grams of recyclable plastics and 59 grams of non-recyclable plastics: 122 grams in total. NanoGBS, Torkamaneh found, uses only 14 grams of non-recyclable plastics and no recyclable plastics, a reduction of 89 percent.

Lastly, Torkamaneh pointed out the health issues associated with prolonged pipetting. "In the lab, often you're pipetting 50,000 to 100,000 reactions per year. The quantity of the work is quite important," he said, noting that this can lead to repetitive strain injury and musculoskeletal issues. "But now, in the lab, we have the Echo (instrument) that we use for all the transferring work, and I think our personnel in the lab there are very happy with this machine too."

A downside of NanoGBS is that an Echo liquid handler may be too costly for some labs, noted Torkamaneh, but he added that the reduction in other costs can make up for the price within a few years. He said that his team plans on automating its library preparation protocols even further, possibly by integrating a Biomek liquid handler, which can work in tandem with the Echo instrument.