Automation accelerates crop improvement

Scientists at the Institute of Plant Breeding of the University of Kiel, Germany, are developing TILLING® (Targeting Induced Local Lesions in Genomes) programs in rape seed and sugar beet, relying on their Freedom EVO® liquid handling workstation for high throughput DNA purification and normalization using MACHEREY-NAGEL NucleoSpin® 96 Plant DNA extraction kits.

The Institute of Plant Breeding, part of the Institute of Agronomy and Crop Science at the University of Kiel, is a research and teaching facility, conducting various plant breeding projects in crop species like sugar beet, barley, rape seeds and asparagus. Some of the projects are funded by the German national genome program, Genome Analysis of the Plant Biological System (GABI), including rape seed and sugar beet TILLING projects. Tecan's Freedom EVO 200 liquid handling workstation is being used for automated DNA extraction and normalization for establishing the TILLING projects in which, typically, there are 4,000 or more plants in a population from which DNA needs to be individually extracted.

"We chose Tecan's workstation because we needed an automated, high throughput system to extract DNA," explained Professor Christian Jung, Director of the Institute of Plant Breeding.

"Our Freedom EVO platform has an integrated centrifuge, so it is able to perform DNA purification using MACHEREY-NAGEL's NucleoSpin 96 Plant kit, automating the whole procedure after homogenization of leaf material with a SPEX SamplePrep 2000 Geno/Grinder®, which uses small steel beads to process simultaneously up to 48 samples in 2 ml reaction tubes, or 192 samples using 8-strip microtubes in a 96-well format. The Freedom EVO also normalizes the purified DNA, using the integrated GENios™ fluorescence reader to measure the DNA concentration in 96-well microplates and dilute the DNA in each well to the right concentration for PCR amplification. This is a huge help for our projects, because it is very tedious to normalize by hand, and almost impossible in micro plates, so the automation makes life much easier for the technician faced with normalizing thousands of DNA samples. The DNA needs to be of good quality, because it will be stored and used in PCR reactions for the next 10 to 20 years. DNA extracted manually may not be of sufficient quality to withstand long-term storage whereas, in combination with the MACHEREY-NAGEL extraction kits, we can obtain highly purified DNA that we can store for long periods. The greatest advantage of this system is that the entire purification and normalization procedures are automated, and this offers the technician time to perform other tasks."

The institute has had the Freedom EVO since 2006, and Tecan and MACHEREY-NAGEL worked together to adapt and optimize the protocol for automated DNA extraction and normalization. Prof Jung added: "We have been using it routinely for about a year now, and our technician, Gislind Bräcker, is happy with its performance. The yield of DNA has

Prof Christian Jung and Mrs Gislind Bräcker

(l) The Institute of Plant Breeding at the University of Kiel

(r) The Freedom EVO platform is used for high throughput DNA purification and normalization

been improved, and we can consistently purify several micrograms of DNA from each sample, processing up to 192 samples in 2 ml reaction tubes in a day. The complete DNA extraction process takes about 6.5 hours, including the four hours spent to prepare the starting material by homogenization of leaf tissue, removal of the steel beads, and a two-hour incubation and centrifugation. In the future, we are planning to switch to using 8-strip microtubes in 96-well format, with which we expect the procedure to be quicker. The PCR set-up will also be automated on the Freedom EVO in the future, to further streamline our workflow. For DNA normalization, 384 samples can be measured and adjusted in one day."

TILLING involves the identification of mutants within specific genomic regions, among huge populations that have been mutagenized by ethylmethane sulfonate (EMS). M1 generation plants are mutagenized and self-pollinated to produce the M2 population, and the DNA from the leaves of each individual M2 plant is extracted and stored, while the plants are self-pollinated again and M3 generation seeds are produced. The DNA is analyzed by PCR in specific genomic regions of interest, and the amplified fragments are treated with endonuclease *Cell*, which cleaves mis-matches, yielding additional fragments when there is a base substitution in the selected region. When the mutated sequence indicates the possibility of an altered phenotype, the corresponding batch of M3 seeds is analyzed, and the plants carrying the mutation are isolated for further study. "Plant breeders need new genetic variation and, because of the unpopularity of transgenic technology in Europe, mutation technology and TILLING is a new source for genetic variation. Breeders, particularly in Europe, are very interested in this technology. Identifying sequence variants or mutations in this way, by genotype rather than by phenotype, is now possible because more and more sequence information of crop species is available. That is the reason why a number of these TILLING platforms have been established in different countries around the world for various crop species."

Prof Jung continued: "We are currently developing TILLING platforms for rape seed and sugar beet, in collaboration with commercial plant breeders. For one project in particular, we are looking at a deleterious component in rape seed, called sinapin, that makes it unsuitable as animal fodder. We are looking for plants that have a mutation in one of the genes for sinapin metabolism, and we expect that if one of these major genes is mutated, the plant will have a reduced amount of sinapin, and this mutant would then be suitable for feeding to animals. This is a clear practical breeding purpose, where the yield of our project can be used by commercial plant breeders."

"Very recently, the animal breeding unit of the university has started to use the Freedom EVO for automated DNA extraction from cows for a program on mapping, genotyping and haplotyping cattle DNA. Although between us we have huge numbers of DNA extractions to perform, the Freedom EVO has the capacity to cope with these sample numbers," concluded Prof Jung.