

The importance of high throughput SNP genotyping for complex disease research in Spain

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The technique of SNP analysis has become more widely used over the last four to five years because the high density maps it provides make it easier to locate small but potentially significant changes in the genome. It is useful for a wide range of applications – from biomedical investigations in hospitals and academic institutes to drug development studies for biotech or pharmaceutical companies. Three laboratories in Barcelona, Madrid and Santiago de Compostela, collectively the Centro Nacional de Genotipado (National Genotyping Centre)(CeGen), were established in 2003/4 to provide a flexible and high throughput facility for medium to large scale SNP genotyping research projects throughout Spain and beyond.

The Santiago de Compostela division of CeGen, based at the university, has concentrated on establishing reliable and fast systems to meet its role as a highly efficient contract services laboratory. Two automated liquid handling workstations from Tecan were chosen to achieve a high throughput and prevent bottlenecks in certain parts of the process; a Freedom EVO[®] 150 platform deals with pre-PCR procedures and an Aquarius[™] multichannel pipettor with a 96-channel head handles post-PCR manipulations, both systems running alongside two genotyping platforms, an Applied Biosystems SNPlex[™] Genotyping System and a Sequenom MassArray[®] DNA analysis platform. The Freedom EVO is equipped with a number of additional features, including a Te-MO[™] multichannel pipetting option with a 96 channel head, an eight channel liquid

handling (LiHa) arm and a robotic manipulator (RoMa) arm. The complete system automates extraction of genomic DNA from sets of 96 fresh or frozen blood samples with a NucleoMag 96 Blood kit (Macherey-Nagel[®]). This magnetic bead-based technology yields 1-4 µg of high purity DNA from 100 µl blood. Following extraction, the DNA concentration is determined using PicoGreen[®] (Molecular Probes), a fluorescent dye that binds the DNA and can be quantified using the laboratory's Tecan GENios[™] plate reader. Measurements are obtained for two serial dilutions for each sample and this highly sensitive technique allows the detection of up to 25 pg/ml of DNA. Five 96-well plates are processed per day by the GENios reader, equating to 480 samples. Using SNPlex, the DNA can then be genotyped on a medium to large scale at low cost and, using 384-well plates, up to 48 SNPs can be simultaneously genotyped in one sample, which equates at present to 70,656 genotypes carried out per week.



Dr Chris Phillips and his team at the Santiago de Compostela division of the Centro Nacional de Genotipado (National Genotyping Centre)(CeGen) in Spain

Throughput is no doubt a major consideration for the laboratory and the current operation has a potential capacity of 45,000 genotypes per week. The Tecan workstations have achieved the high workflow with reliability and, very importantly for a contract services laboratory such as this, with flexibility. Flexibility and ease of programming is hugely important because often the laboratory has to tailor its processes to suit a project's needs, for example, researchers might send samples in unusual plates or formats or, even more likely, will need the optimization of an entirely new set of assays. SNP analysis can be very straightforward when you are only dealing with, for example, six markers that remain constant; you know what causes problems and what works well. For the laboratory in Santiago, however, there is normally a completely new set of markers every few weeks and, each time this happens the assays and techniques need to be re-optimized which takes a significant amount of time. On occasion, the set of markers may only be for a small number of samples, say 400, and the analyses might be complete within just a couple of days but the optimization may have taken a week or more. This is a challenge compared to a diagnostic set-up, for example, where the markers are known and similar samples are regularly received.

Under these circumstances, flexibility and easy programming is very important and is undoubtedly met by Tecan's instruments, especially the Freedom EVO workstation. A new workflow for handling different volumes; a five-tip head for cherry picking and the 96-tip head working well together; features like this are important advantages of the Freedom EVO that have helped the Santiago laboratory to be prepared for whatever project comes round the corner.

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