Automating NGS protocols for a wide range of sample types

PTP's Genomics Platform has automated all the steps from DNA/RNA extraction to the preparation of sequencing pools. Using three Freedom EVO® platforms, comprehensive automated workflows ensure high quality data and complete sample traceability, with a daily throughput of up to 288 genomic DNA samples.

PTP's Genomics Platform facility

Parco Tecnologico Padano

Parco Technologico Padano (PTP) in Lodi, in the north of Italy, is a science park serving universities and publicly-funded research centers in Italy – as well as other private companies and institutes across Europe – in the agriculture, food, animal feed and biotechnology sectors. PTP's Genomics Platform (PGP), headed by Valentina Gualdi, was set up in 2005 and carries out genotyping, Sanger sequencing, next generation sequencing (NGS) and high throughput genomic analyses. Established in 2012, the NGS laboratory performs a mixture of genotyping services on plant, animal and human samples for in-house research groups, dietary and fitness counseling, and several clients in the food industry, including the analysis of durum wheat, herbs, rice and tomatoes, and checking kosher foods for the presence of pork.

Valentina explained: "Our three Freedom EVO platforms were installed 10 years ago, and have been reconfigured many times since as our needs have changed. We first turned to automation to manage the large numbers of samples from our collaborative Italian and European projects more easily. Automation saves us money by allowing us to process smaller volume samples – as low as 5 µl – while avoiding errors and maintaining full sample traceability, which is essential to our laboratory's European ISO 9001 and Italian Accredia quality control regulations. Having one manufacturer also makes transferring samples and protocols between the instruments much easier."

Samples received by the laboratory are aliquoted into 96-well plates by a Freedom EVO 100 platform equipped with a four-channel Liquid Handling (LiHa) Arm and disposable tips. These plates are then transferred to a Freedom EVO 150 workstation for extraction and fluorimetric quantification on an integrated GENios™ microplate reader. After quantification, this instrument is also used to set up PCR reactions, including KASP™ genotyping assays.

Post-PCR processing is performed on a second Freedom EVO 150 platform configured with an eight-channel LiHa Arm using fixed tips, a

MultiChannel Arm™ (MCA) 96 and a Robotic Manipulator Arm – as well as a cooling system, a shaker and an integrated Infinite® M1000 microplate reader. The post-PCR steps include automated magnetic bead PCR and Sanger sequencing dye clean-ups, microsatellite analysis, pooling and NGS library preparation with Nextera® XT or TruSeq™ kits for DNA or RNA analysis.

PTP's NGS facility is also a Certified Service Provider for Illumina, and the Company recommends double quantification with both PicoGreen® dye labeling and quantitative PCR with KAPA kits. For PicoGreen quantification, the Freedom EVO 150 sets up the 96-well plates, builds the standard curve, makes sample replicates, and runs fluorimetric readings on the Infinite M1000 reader. Data is then exported into an Excel® file, from which the workflow template is generated, to ensure all samples are represented equally in the pooled libraries loaded onto the Illumina sequencers (HiSeq™ 2000 and MiSeq™).

"For each project, we do all the DNA or RNA extractions first, which can take up to a month," Valentina continued. "Extraction of each 96-well plate takes two to three hours, with a maximum daily output of 288 samples. Accurate quantification of DNA or RNA for library preparation is critical for good quality NGS data and so, once extraction of all the samples is complete, we perform all the DNA/RNA quantifications and quality checks on the same day. We then verify results and normalize the samples before beginning the PCR set-up. For genotyping set-ups, we can perform 10 or more PCRs per plate in one day, generating as many as 100 data points per sample – the only limiting factor is the amount of DNA/RNA we can extract. For NGS analysis with TruSeq DNA, one sample is generally loaded per lane, with up to 16 per run, whereas the Nextera XT protocol for amplicon sequencing or metagenomics allows the pooling of up to 96 or 384 samples."

"We have always been satisfied with Tecan's products and service. We usually develop our own scripts on the Freedom EVO platforms, but help from Tecan's specialists is always available by telephone or email. I attended Tecan's training course when we first had the platforms, and, in turn, trained PGP's technicians; with experience the Freedom EVOware® software is very intuitive, and they can all write and edit scripts on the platforms. This flexibility is essential for handling the wide range of samples which arrive in the laboratory daily," Valentina concluded.

To find out more about Tecan's genomics solutions, visit **www.tecan.com/genomics**

To read about the PTP Genomic Platform, visit **www.tecnoparco.org/index.php?option=com_content&view=article&id=103&Itemid=244&lang=en**

"Flexibility is essential for handling the wide range of samples which arrive in the laboratory daily."

Valentina Gualdi, Laboratory and Quality Manager at the Genomics Platform

The NGS laboratory team