Automating clinical cytogenetics

The Necker Hospital for Sick Children has automated its comparative genomic hybridization workflow on a Freedom EVO® workstation, increasing throughput more than 10-fold while significantly improving reproducibility and process security.

The Necker Hospital for Sick Children in Paris, France, was founded over 200 years ago, and remains at the forefront of pediatric medicine today. Acting as a European reference center for rare childhood illnesses, the Necker uses a wide range of cutting-edge genomic technologies for both its research and diagnostics activities, using current and historic samples to identify genetic markers that indicate disease or susceptibility. One of the diagnostic tools used at the Hospital is comparative genomic hybridization (CGH). This cytogenetic technique uses a competitive *in situ* hybridization method to compare DNA from a patient to a reference sample, providing a rapid method of identifying genetic variations which can affect prenatal or childhood development.

CGH can be used to identify both physical and cognitive problems, as well as a variety of cancers, as Jean-Michel Lapierre, a biology engineer for the Historic Embryology Cytogenetic Service at the Necker, explained:

> "Our Tecan equipment has given us the reproducibility and process security which we need."

"In cytogenetics, we predominantly use CGH to study the genomic DNA of patients born with mental retardation and/or facial dimorphism, looking for any underlying DNA reshuffles causing imbalances in their genomes. CGH is also used for prenatal diagnostics where there is a risk of chromosomal abnormality due to a history of mental retardation in siblings, or if an ultrasound scan reveals physical abnormalities. Outside of this area, we use CGH for oncology and hematology – for small projects looking at imbalances in the genomes of various cancers – so we work with a variety of sample types, including blood, amniotic fluid, fresh and formalin-fixed paraffin-embedded (FFPE) tissues and bone marrow."

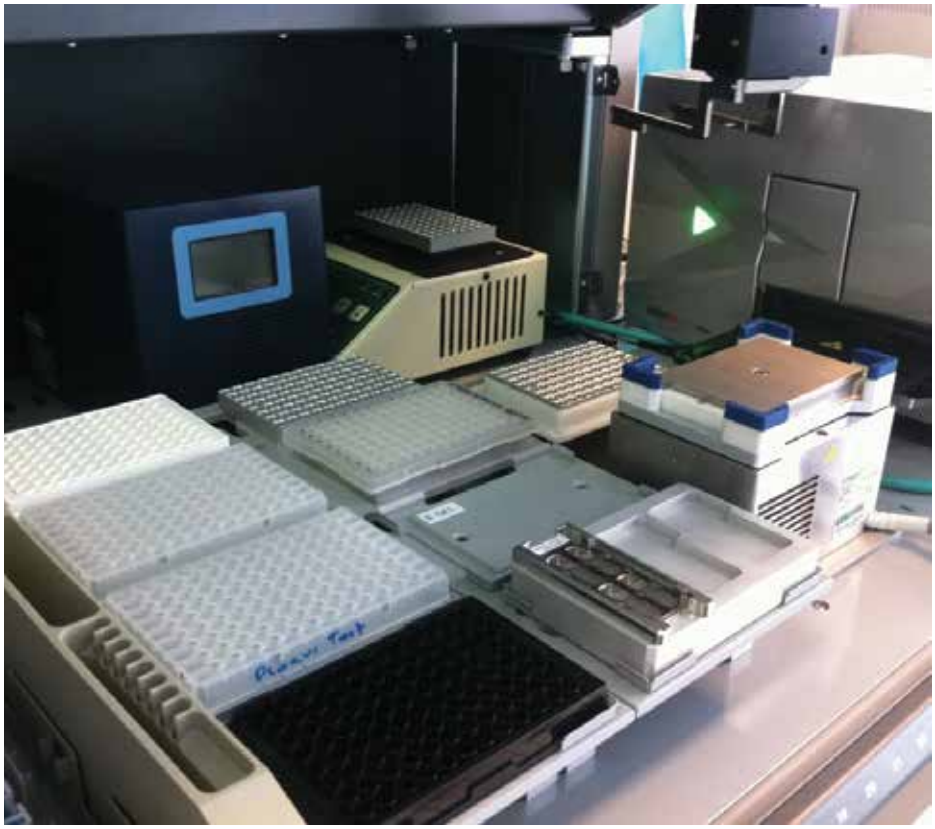
"My role is to transfer our manually-developed CGH protocols onto our automated systems, which include a Freedom EVO 100 liquid handling platform and an integrated Infinite® 200 PRO microplate reader, ensuring that transfer of the technology follows good biological practice and satisfies the high quality standards necessary for our work. When samples arrive in the hospital, we automatically extract the genomic DNA with a Qiagen Autopure LS® instrument, and measure the optical density ratios (OD_{260}/OD_{280} and OD_{260}/OD_{230}) using our Infinite 200 PRO multimode reader. We then normalize the DNA concentrations based on these ratios, and check the quality of the extracted DNA by gel electrophoresis using the Freedom EVO 100 platform to ensure that it is not too degraded to analyze. Samples meeting the quality criteria for CGH are loaded onto the Freedom EVO platform in 96-well plates. Each patient's DNA is then labeled with a fluorophore using a random

From left to right: Jean-Michel Lapierre, Sylvie Nusbaum, Sophie Fontaine and Catherine Ozilou

priming technique, and transferred back to the Infinite reader to check the labeling efficiency by fluorescence analysis."

Jean-Michel continued: "The principle of CGH is to compare the fluorescence intensities of the patient DNA with control DNA that has been labeled with a different fluorophore. The patient and control samples are therefore hybridized on microarrays representing the entire human genome; the more oligonucleotide genomic DNA sequences that are deposited on the microarray slide, the more the resolution of the technique increases. After hybridization, there is a wash step to remove any unbound genetic material from the arrays, then the slides are transferred to our scanner for analysis. This instrument determines the fluorescence intensities of the two fluorophores at each point on the array, and our software compares the fluorescence patterns, showing any copy number variations (losses or gains) in the patient genome relative to the control. This data is then used to try to determine the genotype-phenotype relationship between the genomic abnormalities detected by CGH and the clinical signs; if there is no clear correlation, we continue investigating with a higher resolution or with other molecular techniques. If a copy number variation is detected, we confirm this result using a complementary technique such as fluorescence *in situ* hybridization (FISH) or quantitative PCR, and use the Freedom EVO platform to generate the library of hybridization probes."

"We chose Tecan's Freedom EVO platform because its open architecture gave us the flexibility to perform many different protocols on a single instrument. Both this platform and the Infinite 200 PRO are now essential in our work; they are easy to use and we can trust them to perform routinely without any problems. Before automation, we had to do everything by hand, resulting in a low sample throughput of around 500 to 700 samples annually, as well as issues with errors and reproducibility. Since we began using the Freedom EVO platform, we now handle approximately 1,800 samples annually, including postnatal (75 %), prenatal (20 %) and onco-hematology diagnosis (5 %), with a team of three technicians. Our Tecan equipment has given us the reproducibility and process security that we need, with the potential to process 2,000 to 2,500 samples a year without increasing the size of our team."



The Freedom EVO offers high throughput and excellent process security

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

To learn more about Necker Children's Hospital, go to **www.hopital-necker.aphp.fr**