

The target of personalized medicine moves ever closer

Researchers at Genomic Health Inc., California, are using several Freedom EVO® 200 workstations and a Genesis platform to automate the development and routine screening of gene panels for predicting the risk of recurrence and response to treatment of breast cancer.



Scientists now firmly believe that an individual's genetic makeup has a strong influence on susceptibility to disease onset or recurrence and response to different treatments, and, as a consequence, understanding these genetic variations is seen as central to creating personalized drugs, tailored to the individual, with greater efficacy, safety and fewer adverse side-effects.

Screening genomes for these variations is logistically awesome as there are potentially many candidate genes that are likely to be involved. The focus of research and development at life science and clinical diagnostics company Genomic Health Inc. is to do just that

and current efforts are concentrated on developing a gene panel which serves as an initial screen of many genes.

In 2004, Genomic Health launched the Oncotype DX™, the first diagnostic multi-gene expression test commercially available that has clinical evidence validating its ability to quantify the likelihood of breast cancer recurrence in women with newly diagnosed, early stage, node-negative, estrogen receptor-positive breast cancer. The Oncotype DX™ assay analyzes the expression of a panel of 21 genes from a tumor specimen using RT-PCR, and the results are presented as a Recurrence Score™ that estimates the risk of cancer recurrence within ten years, on

a scale from 0 to 100. These scores are more accurate and reproducible than the variables that clinicians currently rely on, like tumor size and grade, and patients' ages.

The assay has five steps, starting with manual RNA extraction from formalin-fixed, paraffin-embedded tumor tissue (FPET) samples. All the subsequent steps are automated, from RNA quantification by fluorescence assay, to a quantitative PCR assay that checks for DNA contamination, and reverse transcription (RT) which produces complementary DNA from the mRNA. The final step is quantitative TaqMan® RT-PCR using the complementary DNA as

the template in 384-well plates, with the expression of each of 16 genes measured in triplicate and then normalized relative to a set of five reference genes. RT-PCR is highly reproducible as it is sensitive, specific, unaffected by inherent variability in the original tumor blocks, and amenable to high throughput and real-time quantification, with successful amplification even from degraded starting RNA.

The work to develop the Oncotype DX™ assay down to a much more manageable 21 genes has relied on three independent clinical studies and the assay has been validated in a large, independent multi-center clinical trial (NSABP Study B-14) and in a large population-based case-control study in breast cancer patients at the Northern California Kaiser Permanente. The magnitude of these and potentially more studies in the future made the use of automation in the development work essential, as Jay Snable, Director of Process Automation, explained:



From left to right: Jay Snable, Darrin Chan, Keith Volk, Kenneth Hoyt, Anhthu Nguyen and Ian Storer from Genomic Health.

“We needed automated systems for our ongoing assay development and, although we chose Tecan for several reasons, the primary reason was the highly flexible technology, offering so many accessories that we can basically do whatever we need to. As a clinical reference laboratory, we receive the patient samples in tubes, and Tecan provided the best solutions for handling all the different formats we need, from single tubes through to 384-well microplates, in a highly integrated system with quality controls all the way. It incorporates integral barcode scanning and identification, enabling tracking of individual samples through all the assay steps.”

“A big labor-saving feature of the platform comes from the barcode information that the system generates during assembly of samples into assay plates. We can put samples on in any order; they are scanned and information is sent to our LIMS where the tube and plate barcodes are married, eliminating manual batching steps. This was just one of the benefits coming from the successful integration of the equipment with our LIMS. For example, we also use the LIMS for normalization. Tubes are placed on the robot and scanned. The resulting file is exported to the LIMS and a Gemini™ script for adjusting sample volumes and concentrations is generated and sent back to the platform. The samples are then normalized for use in some of the intermediate steps of the Oncotype DX™ assay, the genomic DNA assay and reverse transcription.”

Most of the Tecan workstations at Genomic Health are configured with Tecan’s Multichannel Pipettor, Te-MO™. The PCRs are set up on the Freedom EVO workstations using Te-MO 3/5 multipipettors with 96-channel pipetting heads (Te-MO 96). These workstations are also equipped with a PosID™ System for automated barcode reading. Applied Biosystems’ 384-well thermocyclers (7900HT Fast Real-Time PCR systems) are used for the RT-PCR and resulting quantification. The data are reduced and analyzed using custom software developed in-house and, after passing a series of quality control steps, a Recurrence Score™ is calculated. A report is generated, reviewed by clinical staff, and released to doctors who advise the patients.

Jay concluded: “In 2005, we ran about 7,000 RNA samples in the clinical laboratory with a targeted turnaround time of 10 to 14 days per sample, including review and quality control processes. We continued to scale up through 2006 and processed approximately 13,500 patient samples. Genomic Health is also now scaling up the development process. For example, we are now looking at 761 genes in an initial screen to create a gene profile for an assay to test for risk of recurrence of another cancer type, and this is where the Tecan technology will prove especially useful for the high throughput we need to achieve.”

Not all options discussed in this article have been cleared for use in all markets. Contact your local sales office for specific information.

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