SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPING IN PHARMACOGENOMICS AND DRUG DISCOVERY USING THE BECKMAN COULTER GenomeLab™ SNPstream SYSTEM

Suresh Jivan Gadher

Beckman Coulter International S.A., 22, rue Juste Olivier, Casa Postale 1059, CH – 1260 Nyon 1, Switzerland; sgadher@beckman.com

Abstract

Single nucleotide polymorphism (SNP) is the most common type of genetic variation occurring every 300 to 1000 bases in the human genome and many other species. By examining SNPs, investigators can better understand the genetic variances related to certain disorders and diseases. Hence, SNP identification plays a powerful role in discovering genetic factors by examining one's genotype which can ultimately predict one's risk to developing certain types of diseases. Clinical genotyping instrumentation and kits play a vital role in today's Pharmacogenomic laboratories. To this end, Beckman Coulter developed an automated, ultra-high throughput system, called the 'GenomeLab™ SNPstream Genotyping System'. It utilises the single-base primer extension technology together with multiplexed PCR in conjunction with tagged-arrays. Today, this System can be seen at the cutting edge of Pharmacogenomics and Drug Discovery Research throughout the World. Examples of such system utilisation include projects such as the LRRK2 gene in Parkinson Disease (C. Paisan-Ruiz, et al 2005. Neurology 65: 696–700), Measles Vaccine Immunity and SNP in Cytokine and Cytokine Receptor Genes (Neelam Dhiman, et al. 2007. J. of Infectious Diseases 195: 21–29) and numerous other studies being carried out world wide.*

The 'GenomeLab SNPstream Genotyping 48-plex System delivers between 4,000 to over 3 Million Genotypes per day and is an automated, multiplexed system that can process up to 48 SNPs in each well of an arrayed 384-well plate. SNPstream operates at a consistent cost regardless of run throughput, allowing users to cost-effectively home in on targets with low-throughput or high-throughput analyses. This unique opportunity is achieved by lowering PCR expenses and improving efficiency, thus lowering the cost per genotype and per sample. The system features a simple protocol with just three steps, compared to more than 15 steps that are often required by other similar systems. Firstly, the SNP primer is hybridized to the pre-amplified DNA and then the primer is extended by a labeled terminating base at the target SNP site. Finally, the extended primer is captured on the SNPware Tag Array plate and detected. With millions of genotypes generated, using single-base primer extension, the GenomeLab SNPstream Genotyping System has been proven to deliver the accuracy (99 %) and reliability of data that today's researchers demand. Due to the high sensitivity of the system, accurate results can be obtained by using as little as 2 ng of genomic DNA for either the 12-plex or 48-plex assay. This equates to 0.04 ng DNA required per genotype. The Multi-well plates are compatible with Biomek® series of liquid handlers, for fast pre-and post-PCR set-up. Multiplexing within the system's 384-well plate enables the user to run a range of genomic samples on the same plate. The system can be run manually or integrated with plate-handling robotics for a walk away automation of up to 72 plates. Data collection, analysis and QC from raw data to genotype are fully automated and a complete summary report is provided. »The GenomeLab SNPstream systems are validated on multiple sources and species, including genomic DNA from blood, tissue, cell culture or buccal swab, and from human, mouse, cow, microbial and plant species. SNPstream users have access to Beckman Coulter's Autoprimer.com, a unique web-based tool that automatically provides primers and tag assignments in a matter of seconds, using proprietary algorithms. This innovative, on-line resource takes the uncertainty out of assay design by providing fast, easy-to-use primer design support and tag assignments. Just by uploading or entering a sequence at www.autoprimer.com and the state-of-the art algorithms instantly design robust, best-fit multiplex PCR and extension primers. By designing panels to work*

* The PCR process is covered by patents owned by Roche Molecular Systems, Inc & F. Hoffmann-La Roche Ltd. Key Words: SNP, GenomeLab™, SNPstream, Genotyping.

under a single PCR condition as well as extension conditions, Autoprimer.com software takes care of the optimisation for the end user.
In summary, Beckman Coulter's 48-plex GenomeLab SNPstream Genotyping System provides a scalable solution to meet medium to high-throughput needs where assay automation, automated data collection, data analysis, and auto QC provides a flexible and fully automated solution from DNA to genotype calls. Additionally, the GenomeLab CEQ™ 8000 Genetic Analysis System also provides automated sequencing for SNP discovery and fragment sizing with size / colour allele identification for single or multiplexed SNP scoring by utilizing the 'GenomeLab SNPStart Kit' and the single base extension technology.
To add to the whole, the recent acquisition of 'Agencourt Bioscience Corporation' and its portfolio of products, should stand the user in good stead to enable identification of new markers of biological, diagnostic and therapeutic interest.