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Automation of DNA Sequencing

Workflow sequencing

Integration of robot in genomic lab

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GATC Biotech, a leading genomics service provider located in Constance, was faced with the challenge of automating all routine tasks involved in DMA sequencing. The automation should minimise costs and be scalable to serve the ever increasing demand for custom and high-throughput sequencing projects.These tasks require very different protocols for the efficient and flexible handling of huge numbers of daily samples.

The principal part of this automation was the application of a highly flexible pipetting robot, which allowed easy integration into the existing work processes. The ML-STAR robot produced by the company Hamilton was selected for this task.

An important feature is the robot's ability to operate with disposable tips as well as with washable steel needles. The tips are taken up by the pipetting channels using a unique technique (CO-RE, i.e. compression-induced o-ring expansion) and are available in lOul, 300 ul and 1000 ul sizes. During a running process the autoload option allows carriers holding microtiter plates, or other ,_ containers, to be taken from or placed ' on the robot's work table manually or by means of the robot's arm (SWAP Hamilton).

Linkage of LIMS and Robot

In order to efficiently automate the sequencing laboratory, GATC developed its own laboratory-information-management-system (LIMS) in 1998 to manage all data generated in the lab.This proprietary LIMS consists of an internal module (internal workflow and sample management) and an external interface called MaxControl. This interface allows customers to place orders on-line via the Internet, to track the production status and to download the sequencing results from a customised web site (WatchBox). The data transfer through the World Wide Web is securely encoded by SSL-Encrypt Technology.

The pipetting robot could be optimally linked to the LIMS and guarantees smooth data exchange (in the form of function lists and log files). All function lists needed for the execution of certain processes are automatically generated by the LIMS and contain all the necessary information for the robot to process certain work protocols smoothly and error-free. For example, the robot scans a barcode on the microtiter plate and transfers the data to the LIMS. Function lists based on the data are generated and transferred back to the robot.

Another reason for choosing the ML-STAR was the easy and trouble-free programming of the required work protocols using the unit's internal software. Apart from easy control of the robot the software guarantees easy data input / output as well as an uncomplicated interface for control of other external equip-mentThe software also offers high flexibility and safety guaranteed by the protocols' data structures. It provides the user with all the necessary information regarding work procedures and instructions for loading the robot with samples. The robot controls and documents the work procedures and transfers the information (log files) to the LIMS. If a protocol is for some reason interrupted, the log files are used to resume work at the next sample. It is not necessary to start again from the beginning.

High Capacity in HT Sequencing

For high-throughput sequencing (HT sequencing) large numbers of microtiter plates (96 well and 384 well) have to be processed quickly and efficiently. The pipetting robot has to provide high capacity and speed.The ML-STAR guarantees these requirements as it is equipped with a large work table and an autoload option.

After purification of the plasmid DNA, the quality and quantity of the isolated DNA is determined photometrically. If necessary the sample is diluted so that all samples have the same concentration. This ensures optimal sequence quality and read lengths. Using the ML-STAR's software, a quality control protocol was created to automatically check the DNA quantity and quality and for parallel processing of samples. The quality control data are automatically transferred to the LIMS and stored for later use (i.e. finishing).

A second protocol is used for the preparation of sequencing reactions. Depending on the sequencing project multiple primers (forward or reverse) can be used. The LIMS provides all necessary data (required DNA quantity, primer, number of microtiter plates).

High Flexibility for Custom Sequencing

More flexibility is required for custom sequencing because large numbers of different DNA templates are processed in parallel. The required function lists are generated individually and up-to-the-minute for the daily sequencing reactions. The work steps processed by the robot are documented, transferred to the LIMS and used to create on-line comments. Each customer can monitor the production status on a customized web site (WatchBox). These data are also used to update the database of the LIMS and to generate new function lists for subsequent processes.

The pipetting robot transfers the different DNA templates into microtiter plates for efficient processing. Although each sequencing reaction differs in DNA quantity and primer, all reactions can be processed simultaneously. GATC is able to prepare the reactions with individual customer primers as well as with over 150 different standard primers in parallel in the same microtiter plate.

Conclusion

Due to the technical superiority of the pipetting robot ML-STAR and the cooperation and excellent technical support by Hamilton, GATC was able to quickly and efficiently automate its DNA sequencing laboratory. The robot has proved to be highly flexible and scalable. The interface with external laboratory hardware to increase lab automatisation is straightforward and uncomplicated. The ML-STAR had enabled GATC to automate other molecular biology services, as well, i.e. genomic research services.

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