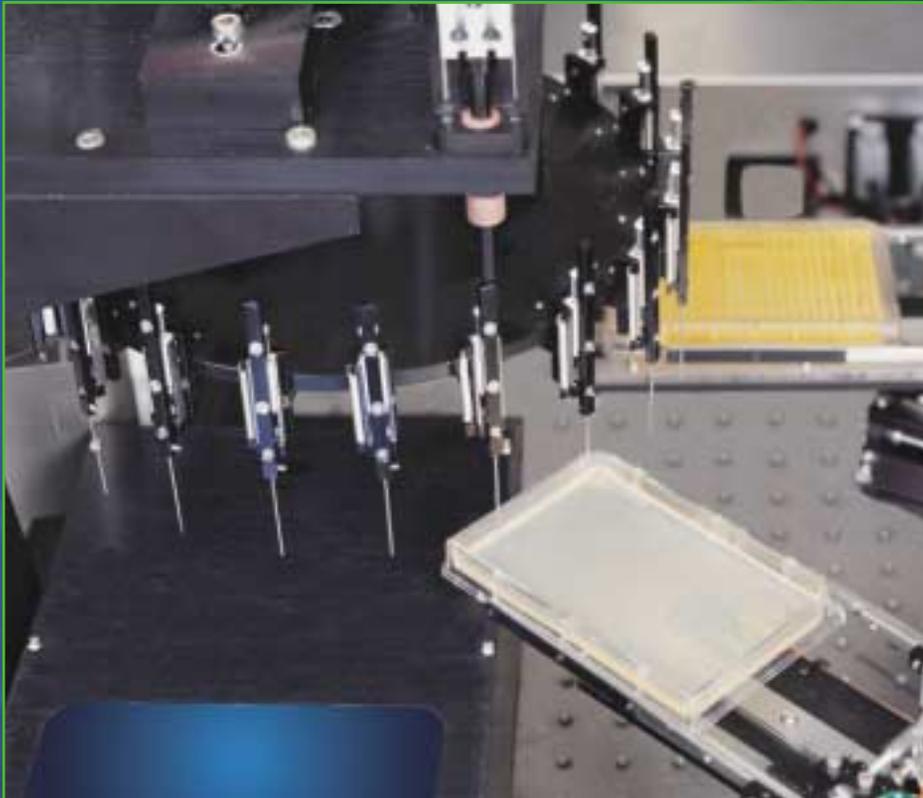




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An Automated Solution for Bacterial Colony and Plaque Picking



GeneMachines Mantis Colony and Plaque Picker

National Instruments LabVIEW, imaging hardware and software, and digital I/O products are integrated with GeneMachines robotic instrumentation to comprise a fully-automated, high-throughput instrument.

GeneMachines Mantis plaque and colony picker is a member of the GeneSuite product line that provides state-of-the-art automated solutions for the genomics and proteomics community. The Mantis picker is specifically designed to provide high-throughput picking of bacterial colonies used for DNA sample preparation and cell screening.

Bacterial cells are used as carriers to replicate and/or express specific genes or proteins in genomic research. When grown in petri dishes, these cells form bacterial colonies that are approximately 0.5-1 mm in diameter. Traditionally, each bacterial colony is hand-picked using a toothpick and placed in individual wells of a microwell plate for subsequent incubation. This manual picking process is very time-consuming and tedious.

Figure 1



By integrating National Instruments IMAQ and DAQ hardware, LabVIEW automation software, and IMAQ Vision software into Mantis's robotics, GeneMachines created an automated and functionally versatile means to selectively pick and evaluate colonies. A biologist can place up to 72 bacterial colony plates on the input side of Mantis to be picked. The output side holds the microwell plates (typically 96-well or 384-well) where the bacterial cells are deposited once picked. The input and output sides each use a three degree-of-freedom robotic arm, the GeneMachines Server Arm, to manipulate the individual plates. LabVIEW 6i software controls each of the arms and the entire robotic workstation.

Colony Discrimination

A petri dish containing bacterial colonies is picked up by the Server Arm and placed under a PULNiX 1300 CCD camera for imaging (Figure 1). Once the image is taken, IMAQ Vision 6 software is used to detect and analyze the location and properties of individual colonies in the dish. Using IMAQ Vision's blob analysis tools, each colony is scrutinized for size, roundness, and proximity to other colonies (Figure 2). During certain applications, colonies also are analyzed by color expression — either standard blue/white or various fluorescent proteins, GFP, RFP, and so on — and/or intensity, which can indicate the level of gene or protein expression. Once the colony analysis is complete, colonies meeting the user-defined specifications are selected by the software for “picking.” During the next stage of the Mantis protocol, the coordinates of these selected colonies will be translated into machine movements, resulting in precision picking of bacterial colonies.



Figure 2: (above) Image Analysis Screen. An image of the input colony plate is presented on the left. The two images on the right denote close-up views of colonies and indicate colony selection and rejection. Selection criteria are configured with the controls in the lower right-hand corner.

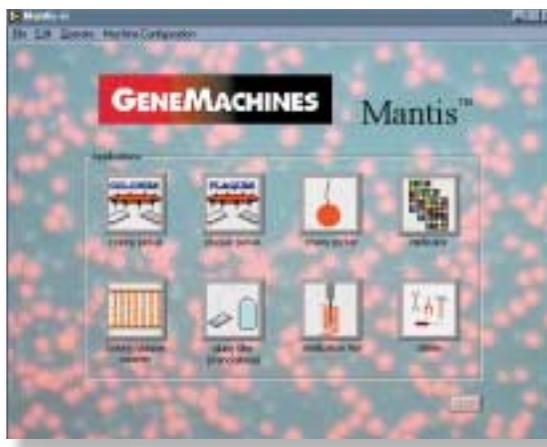


Figure 3: (left) Front Panel Function Selection Screen. Each button denotes a different core capability of the Mantis picker.

Picking and Transfer

LabVIEW software interfaces with an NI PCI-DIO-96 I/O card to control all the components involved in sterile picking and transfer, including individual pneumatic actuators that plunge the picking needles, pneumatic washpads, a thermal sterilization coil, and several other components. The VIs written to control the Mantis picker are devised to parallel task these functions. In other words, while one needle is being sterilized, another can be picking a colony, and yet

another can be depositing a colony.

Twenty of these pneumatically-controlled picking needles are mounted underneath a round plate to create a rotary needle array that is driven by a stepper motor. First, a needle is sterilized by plunging into washpads that clean its surface and then into a thermal sterilization coil. The sterilized needle is then ready to pick bacterial cells. Using the machine coordinates obtained with IMAQ/Vision for the selected colonies, the Server Arm places the imaged plate under the needle so that, when actuated, the

needle plunges into a selected colony. Bacterial cells adhere to the tip of the plunged needle. The needle is then lifted, rotated to the next actuator, and plunged again to deposit the colony into an individual well of a microwell plate. These microwell plates reside on the output side of the Mantis picker and are handled by the second Server Arm. The user can choose to deposit the picked bacterial colonies into either 96- or 384-well plates. The precision enabled by the combination of the LabVIEW 6i soft-

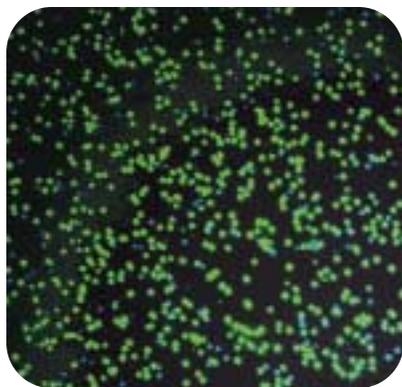
defined controls to conduct tests for contamination between wells in a microwell plate.

The versatility of the Mantis LabVIEW application also allows it to perform other functions and techniques commonly used by biologists in genomic research. These functions include a rearrangement protocol to transfer a subset of wells into an output plate, called “cherry picking” or rearraying; a colony counter that allows the number of colonies to be counted on a given plate; and com-

understand how to manipulate the different functions of the Mantis picker without the need to understand engineering or the LabVIEW programming language.

Conclusion

The integration of NI hardware, drivers, and software into the GeneMachines Mantis plaque and colony picker resulted in a powerful, flexible, and user-friendly robotic platform. The Mantis picker delivers 2000 colonies per hour and unat-



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ware and the Server Arm allows incremental movements so that sequential wells can each be inoculated by sequential needles.

Along with the basic functionalities of colony picking, additional features ensure an efficient, reliable, and flexible scheme. Efficiency-boosting features include an option to automatically de-lid the plates, a plate filler to fill the 96-well or 384-well plates with growth media, and bar-code readers to catalog the location of transferred colonies in the output plates. Due to the strict control of components that LabVIEW provides, the validity and reliability of the colony picking process can be easily verified. Sterilization protocols can be initiated along with user-

pression/expansion function where the needles are submerged into individual wells of microwell plates in the input side and are transferred to inoculate specific user-defined wells on the output side.

The End User

While LabVIEW provides a powerful medium to integrate all of the sophisticated functions and protocols, perhaps its most important feature is the capability to create an easy-to-use graphical user interface (GUI) (Figures 2 and 3). The end user of a Mantis picker is typically not an engineer, but a biologist with little or no engineering background. The PC-based GUI generated by LabVIEW allows biologists to

tended run times in excess of 14 hours. In order to keep the GeneMachines Mantis picker on the cutting edge of plaque and colony screening, future enhancements are planned to implement additional image analysis capabilities to enable simultaneous multiple fluorescent protein detection.

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