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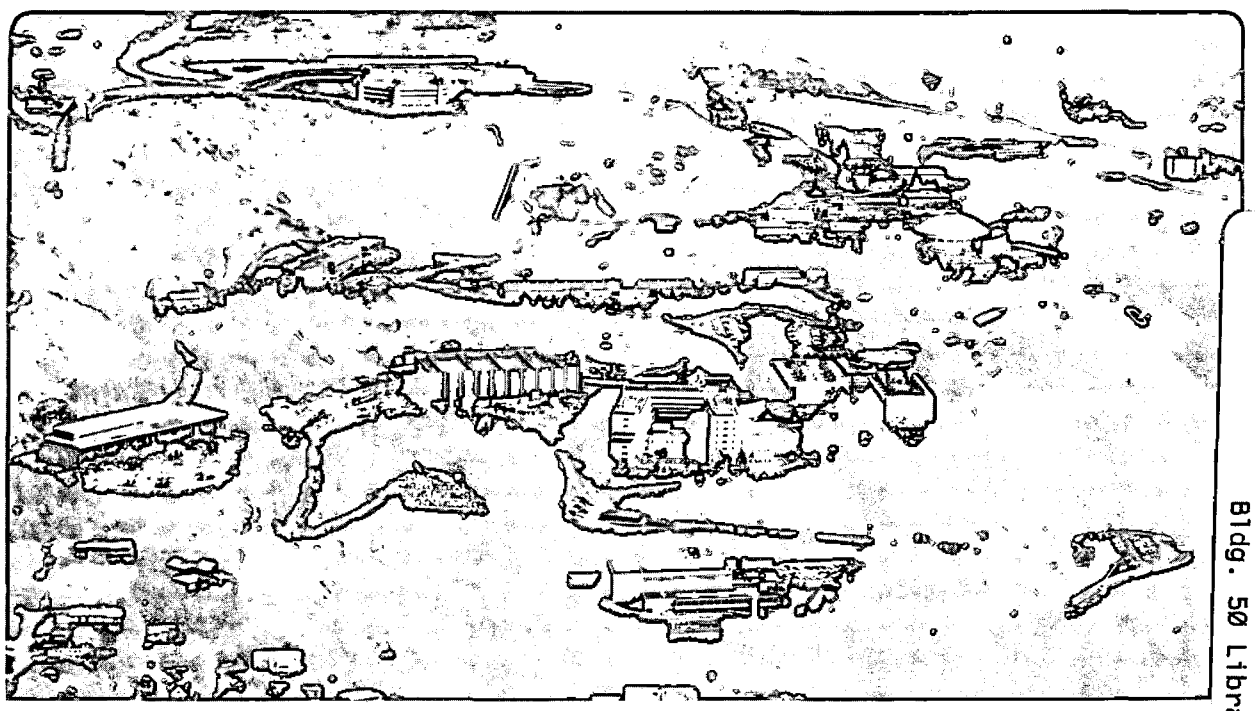
October 1993



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Application of Robotics-based Automation to the Replication of Cloned DNA Libraries

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The term "DNA library" refers to a set of altered host cells containing DNA fragments from one or more chromosomes of a single individual of a species under study. DNA libraries are a primary resource for gene mapping and other genetic studies, and their replication by cloning provides a virtually unlimited supply of these materials for research purposes. To create such libraries, restriction enzymes are employed to cut the chromosomes into many small overlapping DNA fragments which are inserted into single-cell host organisms such as yeast or bacteria and grown up as colonies (Fig. 1).

Physically, DNA libraries are housed in 96-well microtiter plates (8.5 cm X 12.5 cm X 1.5 cm), with each clone colony occupying one 200-µL well. A typical library of 50,000 to 60,000 unique cloned fragments occupies 500 to 600 plates. Distribution of DNA libraries to researchers is an important activity in support of the Human Genome Program.* Manual replication of DNA libraries is a tedious, time consuming task and is inadequate to meet the demand for library copies. The introduction of appropriate automation is seen as a way to make library replication more efficient and cost-effective as well as speeding access to these valuable materials by molecular biologists, geneticists, and medical researchers. Anticipated collateral benefits resulting from the automation of DNA library replication include greater product uniformity and the redeployment of skilled personnel from the routine and error-prone tasks associated with manual replication to more productive activities.

Robotics-Based Laboratory Automation

Although laboratory instrumentation and associated data processing have become increasingly automated, it is only recently that laboratory robots with the flexible geometry of movement and dexterity needed to replicate laboratory procedures have become available. When linked with an appropriate computer, such robots can be programmed to carry out complex object manipulations. By integrating and sequencing these manipulations with appropriate workstations and instrumentation, it is possible to build an automated laboratory system that can run with little human intervention.[1] Replication of DNA libraries is a task with a level of complexity, procedural diversity, and integration requirement that would be well-served by laboratory robotics.

A robot must have the necessary flexibility to fit the laboratory environment without requiring significant modification of either machine or work space. This characteristic speeds robot installation and holds implementation costs to a practicable level. In

* Funded primarily by the U.S. Department of Energy (DOE) and the National Institutes of Health, the objective of the Human Genome Program (HGP) is the mapping and sequencing of the three billion DNA base pairs of the human genome plus those of candidate model organisms. The bulk of DOE-sponsored HGP research is being carried out at national laboratory sites in Livermore and Berkeley, California, and Los Alamos, New Mexico.

addition, to fulfill its role as an efficient and productive substitute for a human operator, a robot should be able to address the entire work surface of a laboratory bench, access laboratory instrumentation, and perform appropriate manipulations. In general, tasks required of a laboratory robot include the introduction, removal, and transport of samples, and the manipulation of objects such as sample containers, apparatus, tools, and instruments. The robot must be able to carry out these tasks according to a programmed sequence, often within tight geometric tolerances and scheduling constraints.

Robot Selection

Hewlett-Packard's Optimized Robot for Chemical Analysis (ORCA)[2,3] was found to be a good candidate for building an automated DNA plate replication system. The ORCA is a an anthropomorphic-like arm mounted on a rail that fits the rectilinear geometry of a typical laboratory workbench. The arm possesses six movable joints that provide six positional degrees of freedom: linear (x) motion along the rail, reach (y) and height (z) motions produced by combined shoulder and elbow rotation, wrist motion, and two finger motions—twist and grip. Robot fingers are interchangeable and may be designed for different tasks. Overall, the device exhibits a simple working geometry without external wiring that could become an obstacle during movement.

The ORCA design allows the robot to perform a variety of tasks with greater repeatability and accuracy than that obtained with an earlier generation of laboratory robot.* The rail can be located at either edge of the workbench, or in the middle when the robot is operating on both sides of rail (Fig. 2). The rectilinear configuration in combination with flexible geometry of motion and dexterity enables the ORCA to address a large and densely packed work surface and to access devices and manipulate objects in virtually any orientation. ORCA's positional accuracy is essential in developing replication systems capable of using the newer 384-well microtiter plate. Compared with the previously standard 96-well plate, the more densely packed 384-well plate increases plate replication throughput almost four times while decreasing freezer storage volume by 75%.

The ORCA software, Methods Development Software (MDS) 2.0, facilitates robot system implementation. MDS runs on PC-compatible computers under Microsoft® Windows, and uses industry standard interfaces to control and monitor external devices. MDS can be controlled by other Windows programs, enabling the robot to act as a server to client programs, and can also export data via Dynamic Data Exchange® for analysis and display by other programs such as Microsoft Excel®. MDS also has the ability to execute parallel command structures, enabling the robot to control external devices while simultaneously executing a motion control program. This feature is used in the current system to control a sterilizer at the same time the robot is fetching and storing microtiter plates.

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* In an earlier project, the author and other workers at the Lawrence Berkeley Laboratory (LBL) developed a prototype robotic system for automated colony picking and DNA library replication.[4] The robot employed utilized a circular geometry with a restricted motion envelope that limited its deployment to a relatively small production area and thus did not fit well with the rectangular geometry of the typical laboratory benchtop and instrument placement. More importantly, unlike the ORCA, this earlier generation robot was not sufficiently accurate to service the new 384-well plate—a major innovation for increasing DNA library replication throughput and storage efficiency.

ORCA movement is taught by using a joystick to physically step the robot through the positions required to carry out each basic task, such as removing the lid from a microtiter plate at a specific workstation on the table, or storing a plate in a stacker. These simple 'motions' are stored with names so that they may subsequently be executed from application programs that call them in the sequence required to accomplish specific tasks. When such a program is run, the sequence of positions in each referenced motion is transmitted to the robot's kinematics processor, which actually drives the robot. The kinematics processor ensures smooth and precise arrival at each specified Cartesian coordinate position by computing the complex joint motions necessary to reach the target along a straight-line trajectory.

Each taught motion is tied to a local coordinate system such as a plate workstation or stacker. A powerful feature of MDS is that it allows replicas of such 'frames' to be defined anywhere in the robot's work space, so that a motion taught at one location may be executed at another simply by specifying the corresponding execution frame for the appropriate motion in the application program. Similarly, MDS allows frames to be divided into evenly spaced sub-positions, such as vertical levels of a plate stacker (Fig. 2d), so that motion taught at one sub-position may be executed at any other. In addition, programmed offsets permit motions to be easily varied. For example, a motion for gripping a 96-well plate can be offset within a program to compensate for the dimensional differences of a 384-well plate. These features significantly reduce the burden of teaching and programming the machine.

System Design

Software

At its simplest level, replication of a DNA library is accomplished by inserting a multipin transfer tool into a source plate (Fig. 4). Cells adhering to the pins are transferred to copy plates, the wells of which have been filled with growth medium. The transferred cells of each clone then grow up into colonies. The procedure is repeated with each source plate until the required number of copies have been prepared. The transfer tool is sterilized and dried between source plates changes to prevent contamination (Fig. 5). The brevity of this description belies the considerable complexity in software required to carry out this process (Fig. 6.). The program must first ask the operator for relevant parameters describing plate size, number of source plates, presence of lids, number of copies desired, and quantity of growth medium per well to be dispensed; then execute the sequence of replication operations (including source plate access, filling new plates, and sterilizing the replication tool when necessary) so that all the input conditions are fulfilled. If the operator requests copying from 96- to 384-well plates (or vice versa), the program must provide for the 4:1 (or 1:4) source to copy plate ratio.

Hardware

In addition to the extensive programming requirements, a great deal of ORCA-compatible custom hardware had to be developed. These fixtures included special gripping fingers for the robot, multipin transfer tools, a sterilizing station, robot-accessible plate storage units, and workstations.[5] Some of this hardware is now being

supplied by a third-party developer.[6] Since major design goals were to maximize both the throughput and number of plates processed per run, fixtures were designed to conform to as dense a layout as possible, consistent with the robot's accuracy and maneuverability limits. Because of these tight spatial constraints, the design cycle for each item required several iterations before all the components were smoothly integrated.

Workstation plate fixtures were required to secure the microplate position within a narrow registration to ensure proper alignment of the pins of the transfer tool for entry into the small wells (3 mm across) of a 384-well plate. To compensate for small positional errors in teaching or executing the related robot motions, fixture edges were beveled so that plate entry did not produce collisions that could shut down the system. The same technique was used to enable proper docking of the transfer tools in the sterilizer (Figs. 4 and 5).

The transfer tools (Fig. 3) contain 96 or 384 stainless steel pins that slide vertically to ensure that all pins reach the bottom of the source wells—where cells may be clumped—without causing damage to the wells. The robot uses the same gripper for manipulating microplates and transfer tools, thus avoiding time-consuming exchanges of fingers. In order to compensate for differences in weight and centers of gravity between plate and tool, the grippers are designed with a second set of tool points which hold the heavier tool in a stable position directly below the axis of the robot hand.

The replicating tool sterilizer consists of an ethanol bath and an electrically heated dryer to evaporate the solvent. The robot places the tool into each unit in turn, and then uses the sterilization and drying intervals to fetch and store plates. A circulating pump linked to a feedback sensor maintains the ethanol bath at a constant level by replacing evaporated liquid from a reservoir. The bath is also equipped with an ultrasonic transducer that provides agitation to help remove cells from the pins. Tests of the sterilizer show 100% sterilization efficiency for *E. coli* cells with ten seconds of sonication in a mixture of 70/30 ethanol to water, followed by ten seconds of drying at 100° C. Programmed control of the sterilizer is by means of an off-the-shelf control unit and custom-designed relay box (Fig. 7).

The growth medium dispenser was obtained from a DNA library replicating system based on an earlier robot.[4] The robot moves each copy plate under a manifold that fills sixteen wells simultaneously. The manifold is supplied by a 10 mL syringe pump, also under program control.

Results and Conclusion

The automated plate replication system design is now sufficiently robust to evaluate in full-scale production trials. Test results indicate that the system can generate 180 new 96-well plates in a run time of approximately five hours. This more than doubles the number of plates per run of an earlier system with a lower throughput rate (80 new plates in 3.1 hours).[4] Moreover, throughput increases dramatically with the more densely packed 384-well plates. Here, the earlier system is no match since that robot is not sufficiently accurate to process 384-well plates.

Now that a working system has been achieved, additional improvements are contemplated. These include a stand-alone dispensing station instead of using the robot to dispense growth medium. Such a substitution will increase throughput by allowing the robot to be more productively employed during dispensing intervals. Also contemplated is a bar code reader for sample labeling and tracking. The reader will supply plate identifiers to a database of DNA source libraries and clones, making it easy to locate and retrieve colonies of interest. A comparable reader was successfully implemented on the earlier system.

The development of a competent robotics system for DNA library replication has generated interest in the robotic automation of other complex medical and biological applications such as gel loading of DNA for electrophoretic separation, collection, and manipulation of samples in clone pooling analysis. Like plate replication, these procedures are costly, time consuming, and make poor use of highly skilled personnel. Robotics-based systems of the type discussed in this article could be instrumental in reducing the cost and increasing both the efficiency and quality of applicable research.

Acknowledgment

This work was supported by the Director, Office of Energy Research, Office of Health and Environmental Research, Human Genome Program, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098. Windows, Dynamic Data Exchange, and Excel are registered trademarks of Microsoft Corporation. Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

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Figures

Figure 1 Preparation of DNA libraries. Source: DOE Human Genome 1991-92 Program Report, Primer on Molecular Genetics, United States Department of Energy, 18-19, April 1992.

Figure 2 Automated DNA library replication system. System components: a) Polymer clad grippers for manipulating replicating tools, microtiter plates, and other objects. The grippers incorporate independent sets of tool points in order to manipulate both plates and transfer tools without requiring an exchange of robot fixtures (Also see Fig. 5). b) Clones are transferred from source to copy plates using 96- or 384-pin tools (See Figs. 3 and 4). During transfer, cells cling to the pins. When the tool is inserted in the wells of the copy plate, a mixing motion is imposed to facilitate release of adhering cells. Replication of a 384-well source plate into 96-well copy plates requires four sets of transfers—each with the 96-pin tool occupying one of four offset positions. Each offset results in the transfer of a unique set of 96 clones—384 in all. c) 96-well and 384-well plates (See Fig. 4 for a close-up of the latter). The 384-well plate increases replication throughput nearly four times while decreasing freezer storage space by a factor of four. d) The plate stacker provides sequential access and a dense plate storage configuration. The stacker cannot be used if source plates are sealed with sticky sealing tape which makes the lids impossible to remove. e) The "hotel" houses a less dense plate storage configuration which the ORCA can access in any order. Source plates which arrive sealed are stored here prior to use after lids and tape are removed manually. f) Workstations are platforms that hold microtiter plates or lids while colonies are transferred. g) The sterilization station employs ethanol as a bactericide and uses ultrasonics to physically dislodge contaminants, plus a heater for evaporating ethanol after sterilization is complete. Cycles are timed so that the robot can perform other tasks during sterilization and drying intervals (See Figure 6).

Figure 3 384-pin and 96-pin transfer tools custom designed at LBL. The aluminum heat shields keep the tool bases from overheating in the sterilizer dryer.

Figure 4 Transfer of clones to a copy plate containing growth medium.

Figure 5 Sterilization of transfer tool between source plate changes. The robot is moving the tool from the ethanol bath (center) to the electric heater (left). The heater controller is on the right.

Figure 6 DNA plate replication flow diagram

Notes:

1. If transfer is between 384-well source and copy plates, 384-pin replicating tool is used. Otherwise, 96-pin tool is used.
2. Transfer from 384-well source plate to 96-well copy plate. Replicating tool moves through a source plate offset program. Each offset delivers one unique 96-well transfer set so that one source plate generates four unique sets of N_c copy plates. Replicating tool is sterilized and dried between each unique 96-well transfer.
3. Transfer from 96-well source plate to 384-well copy plate. Replicating tool moves through a copy plate delivery offset program. Each offset delivers a 96-well transfer to 1/4 of the 384 wells of the copy plate so that transfers from four unique source plates produce one set of N_c copy plates.

Figure 7 Plate replication system command and control network. When the program is executed, commands are sent from the system controller (computer) to the robot's kinematics processor over an IEEE 488 bus. Computer control of other system functions is by means of a HP 44474A 16-bit digital I/O board in a HP 3488A interface control unit. The latter is also connected to the computer via the bus. The growth medium dispenser is linked directly to the computer using an RS232 interface.

About the Author: Donald Uber earned a B.S. in Electrical Engineering at Cornell University (1962), and a M.S. in Biomedical Engineering at Johns Hopkins University (1966). He then joined Lawrence Livermore Laboratory (Livermore, California) working on computer applications in the Laboratory's Biomedical Division. In 1978, Mr. Uber moved to Lawrence Berkeley Laboratory where he currently works on computer applications in the laboratory's Human Genome Program Instrumentation Group.

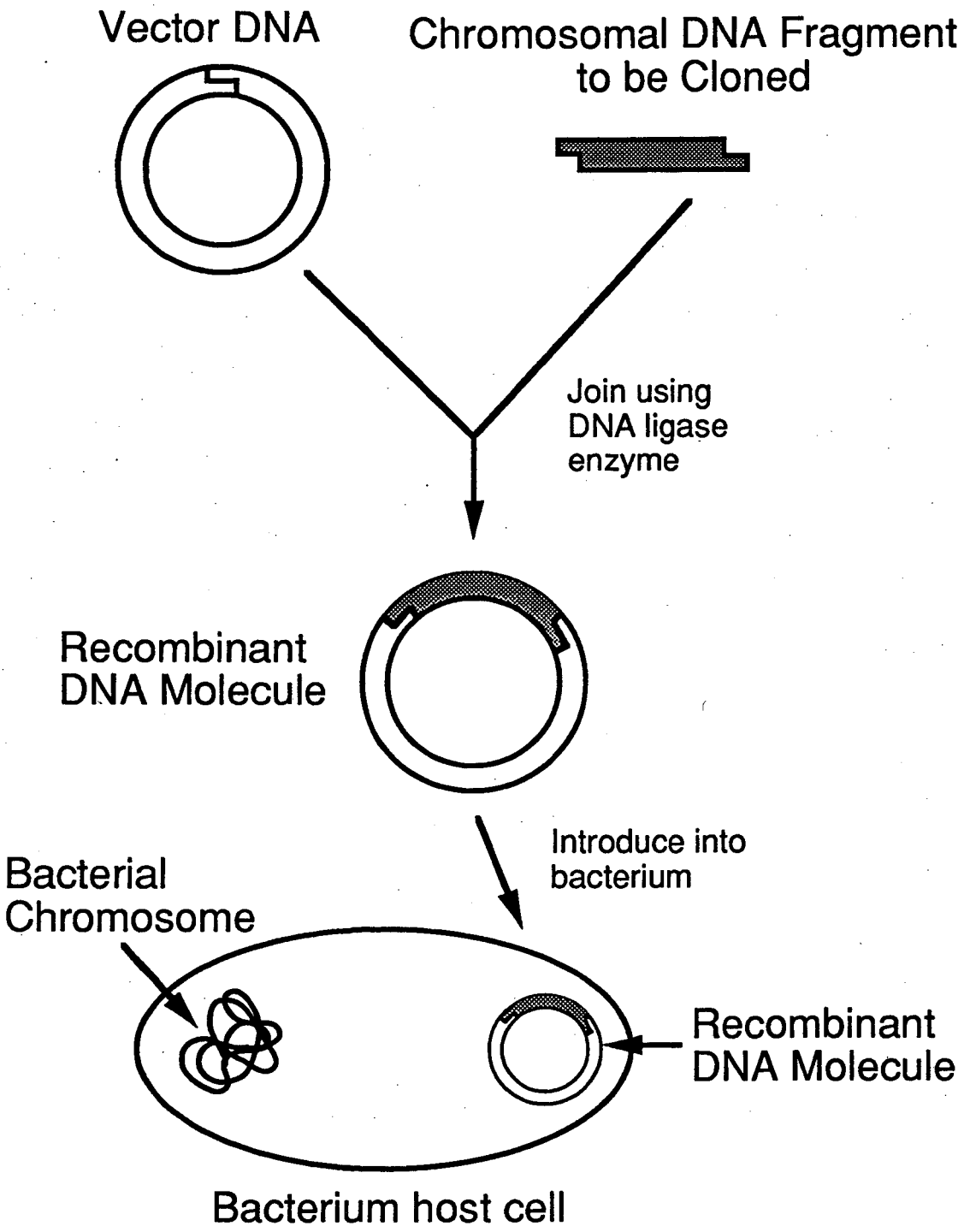
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FIGURE 1

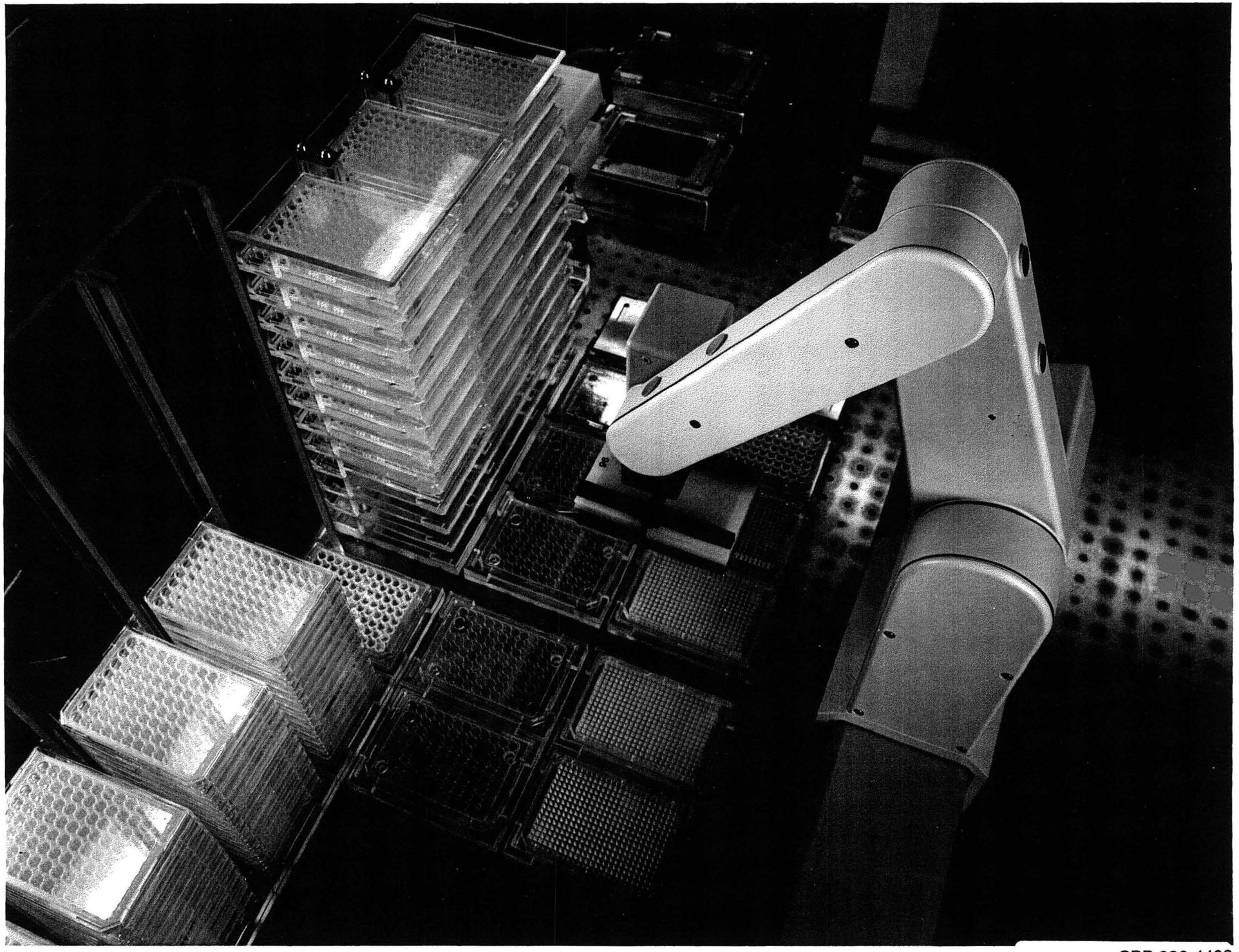

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FIGURE 2

FIGURE 3 CBB 931-373

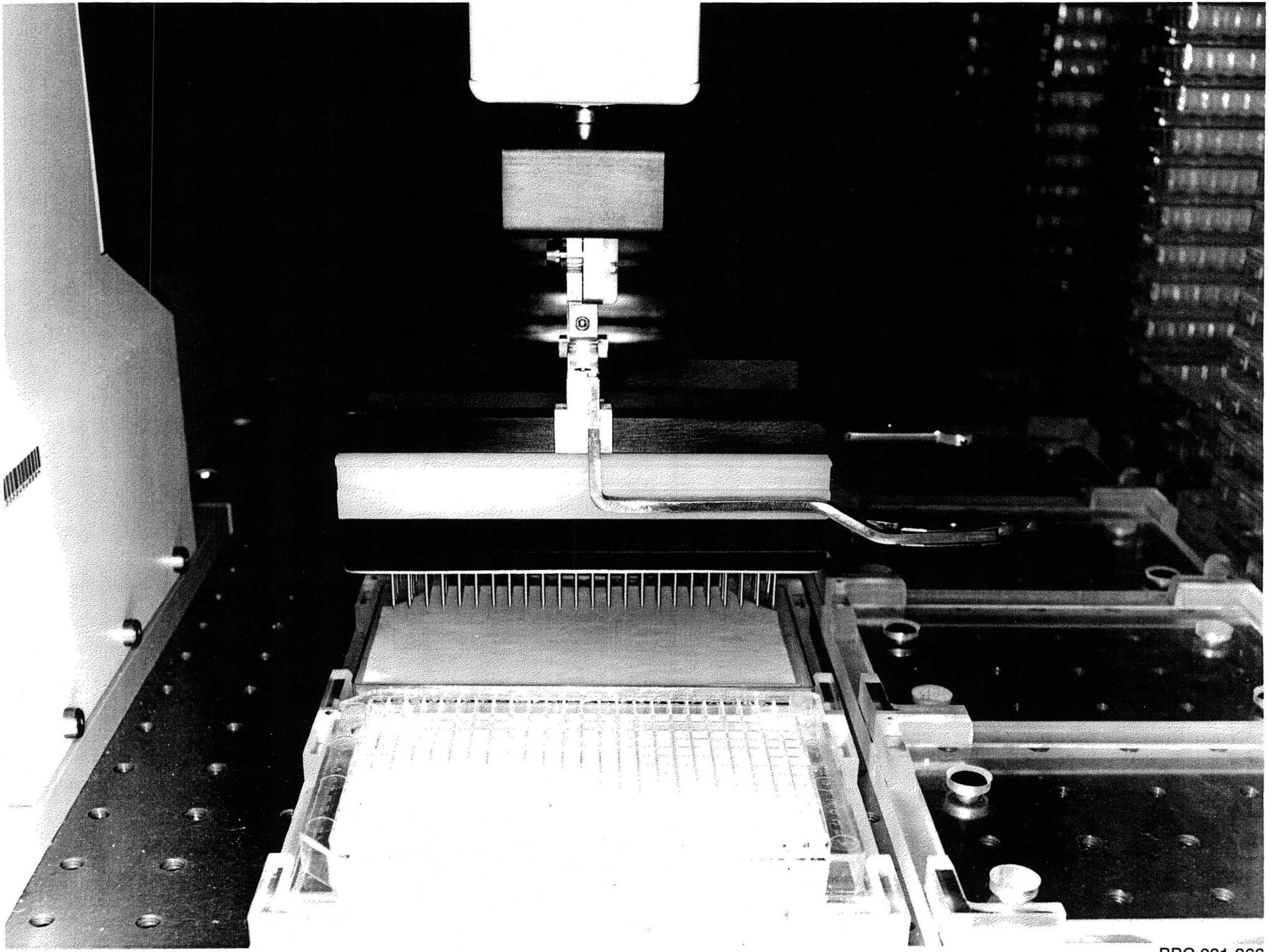

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FIGURE 4

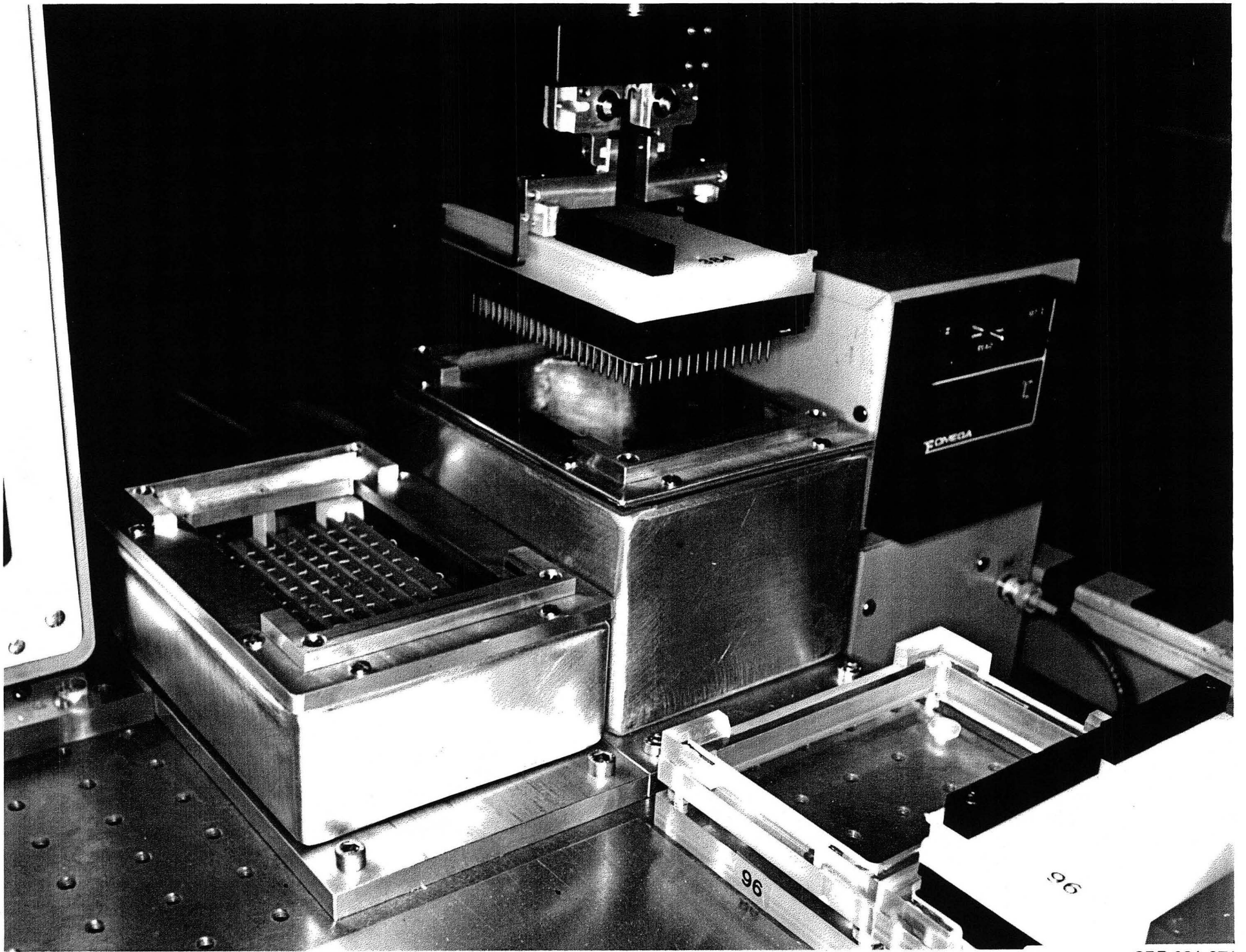

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FIGURE 5

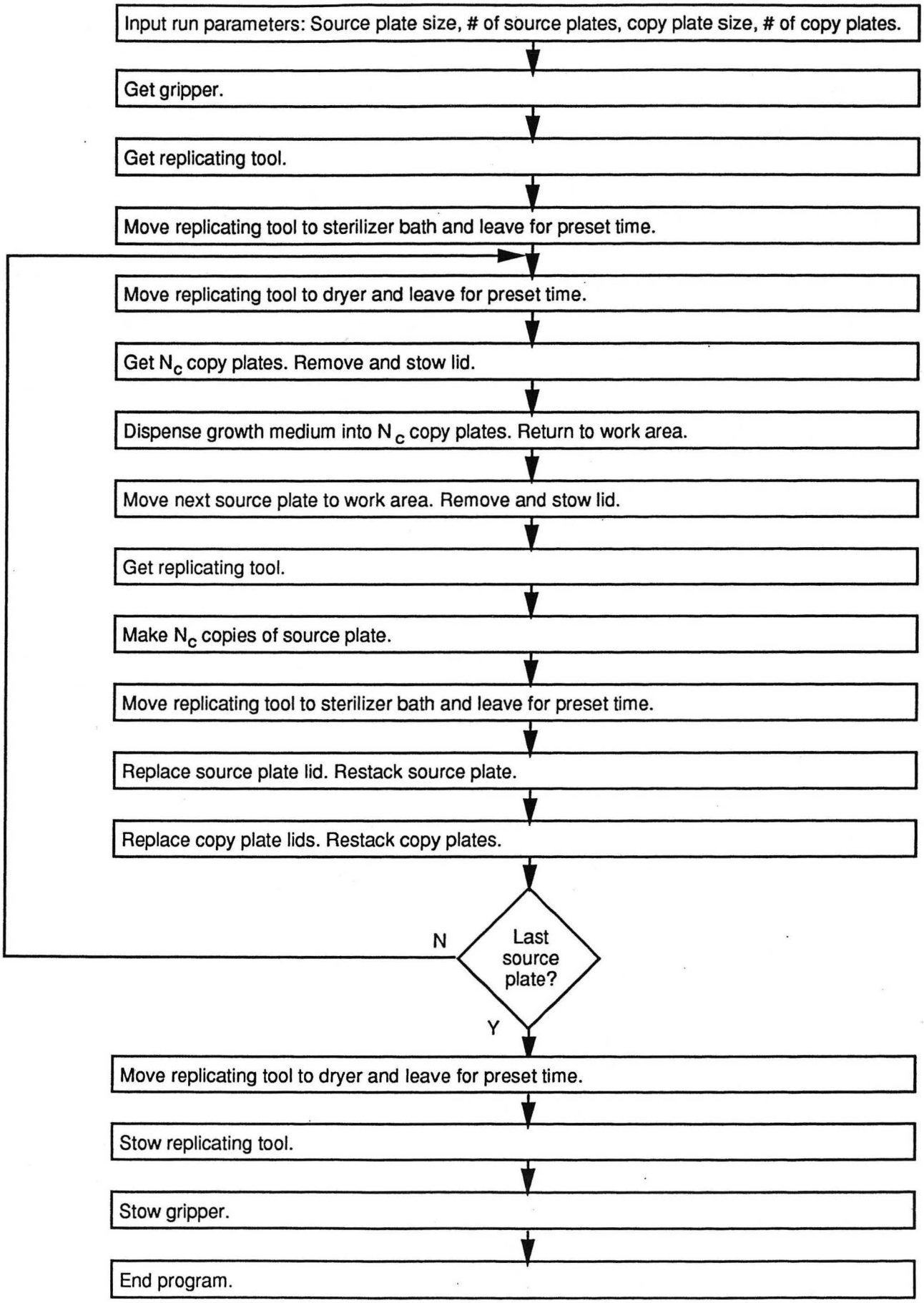
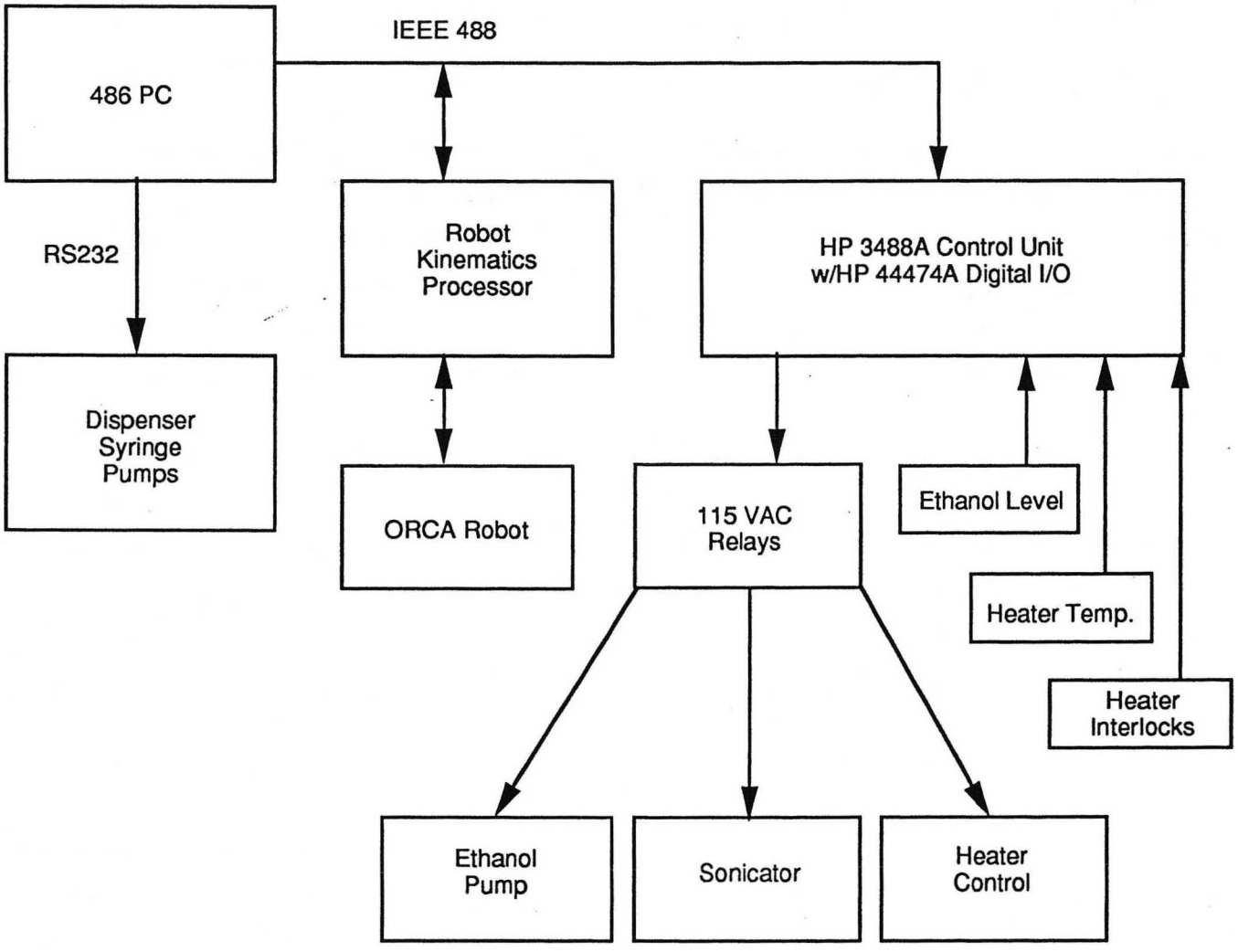

Input run parameters: Source plate size, # of source plates, copy plate size, # of copy plates.
Get gripper.
Get replicating tool.
Move replicating tool to sterilizer bath and leave for preset time.
Move replicating tool to dryer and leave for preset time.
Get N_c copy plates. Remove and stow lid.
Dispense growth medium into N_c copy plates. Return to work area.
Move next source plate to work area. Remove and stow lid.
Get replicating tool.
Make N_c copies of source plate.
Move replicating tool to sterilizer bath and leave for preset time.
Replace source plate lid. Restack source plate.
Replace copy plate lids. Restack copy plates.
Last source plate?
N
Y
Move replicating tool to dryer and leave for preset time.
Stow replicating tool.
Stow gripper.
End program.

FIGURE 6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RS232
Robot
Kinematics
Processor
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Dispenser
Syringe
Pumps
ORCA Robot
115 VAC
Relays
Ethanol Level
Heater Temp.
Heater
Interlocks
Ethanol
Pump
Sonicator
Heater
Control

FIGURE 7

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