Liquid-Handling Robotic Workstations for Functional Genomics

Matthias G. O. Lorenz
National Cancer Institute, Advanced Technology Center, Gaithersburg, MD

Keywords: automation, robots, robotics, workstation, genomics, functional genomics

More and more functional genomics laboratories are willing to invest in robotic workstations due to the higher throughput liquid-handling intensive nature of the work. In this report, the features of robotic workstations important for functional genomics are discussed. Workstations for functional genomics are useful for replication of clone sets, PCR and sequencing set-up and clean-up, hit picking, gel loading, and nucleic acid purification procedures. Workstations not only increase throughput, but also ensure that assay steps are performed consistently, human error- and learning curve-free from run to run. Workstations fit on a laboratory bench and may have several robotic arms in different configurations. Available configurations include grippers and single-, oligo-, or multi-probe heads, with uniform or independent spanning, and liquid-level detection or tracking options. Most hardware and software packages can be designed for integration with other automated devices, building towards a fully automated system. (JALA 2004;9:262–7)

With the completion of the human and mouse genomes, functional genomics laboratories emerged in fast pace. Functional genomics laboratories have to deal with typical molecular biological, liquid handling tasks in a higher throughput manner.

Justification for Automation

Robots have several advantages over manual sample processing in the laboratory[1–14]: Robotic workstations perform consistently and learning curve-free from run to run, 24 hours a day/7 days a week. Robots increase throughput, report each step to a log file, reduce labor costs and ensure a safe lab operation. Robotic workstations also allow principle investigators in the beginning of their careers and established investigators, if qualified personal is difficult to maintain or to attract, to maintain a fully operational laboratory. But despite the huge benefits of establishing an automated laboratory, the required budget and space, the limited training options for new workstation users and the challenges to organize the material and data flow should also be considered.

The Right Automation Strategy: Flexibility Versus Throughput

The optimal degree of laboratory automation depends on the laboratory setting. Flexibility is in general the priority in academia and throughput in industry. But there is a trade off between flexibility and throughput. If high flexibility is required, then throughput is generally low. In academia, a bench approach is mostly sufficient, since it allows higher flexibility. In genomics facilities or industry-like

Correspondence: Dr. Matthias Lorenz, NMRC, 503 Robert Grant Avenue, Silver Spring, MD 20190; E-mail: lorenzm@nmrc.navy.mil
1535-5535/$30.00
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doi:10.1016/j.jala.2004.03.010

laboratories, a workstation approach with medium flexibility and medium throughput is mostly appropriate. This semi-automated approach with lab technicians moving the plates from station to station combines the benefits for higher throughput and human-error free runs with a reasonable investment fitting to the laboratory's budget. If throughput requirements and laboratory budgets increase, robotic workstations can be upgraded and used to design fully automated robotic laboratories. Full lab automation ensures high throughput at lower flexibility, and is often desired in an industrial setting.

Considerations for Choosing Automations

Robotic workstations could be classified into liquid-handling "only," general molecular biological and process specific workstations. Liquid-handling "only" workstations move liquid from one deck location to another, from a reservoir to a plate, from one plate to another, column-by-column, row-by-row or entire plates. General molecular biological liquid-handling workstations (some vendors refer to as sample processors or assay processors) are able to do more than just liquid-handling tasks. They are able to clean up, filter, shake, incubate and move plates from one workstation to another. Some vendors also offer process-specific robots (e.g. plasmid purification robots), which are high throughput, but have limited flexibility. In general, liquid-handling "only" workstations are faster, but less flexible than general molecular biological workstations. But at the end, the availability of accessories decides, whether a liquid-handling workstation is liquid handling "only," or whether it can be extended to a general molecular biological workstation.

Workstations execute robotic tasks and robotic tasks can be more general or more genomics specific in nature. General robotic tasks include entering, identifying, de-capping or de-lidding, aliquotting, reformatting, replicating, normalizing, serial diluting and exiting of tubes, plates, or samples. These tasks are well accomplished by liquid handling 'only' workstations with certain features and accessories as outlined in Table 1. Genomics specific robotic tasks include nucleic acid extraction by solid phase, magnetic separation or precipitation, PCR and sequencing reactions setup and clean-up, gel loading, hit picking, RNA amplification or other enzymatic reactions. These tasks are well performed by molecular biological workstations, which are capable to accommodate special equipment such as vacuum manifolds, magnetic separators, shakers, incubators or indexed centrifuges on, beneath or under the work deck. Examples for genomics specific tasks reported in the literature include identification and picking of colonies,[15] purification of DNA from large samples,[16] automated sample-preparation technologies in genome sequencing projects,[17] high throughput probe production for DNA microarrays,[18] plasmid purification,[19] high throughput DNA extraction methods for PCR,[20] automated cycle sequencing,[21] purification of BigDye Terminator fluorescent DNA sequencing reaction,[22] purification of PCR products,[23] automated agarose gel electrophoresis,[24] high throughput RNA purification,[25,26] and printing of microarrays using liquid-handling robots.[27] However, some tasks are difficult to automate at all, such as initial sample (e.g. blood, serum) collection and initial bar coding of samples, tubes or plates.

There are literally dozens of companies out there (e.g. Apogent Discoveries, Autogen, Beckman Coulter, Colibri Robotics, Genemachines, Genovision AS, Gentra Systems, Gilson, Genetix, Hamilton, Korvis Automation, MacConnell Research, Matrix, MWG Biotech, Perkin Elmer, Qiagen, Roche Applied Science, Robbins Scientific, Sias, Tecan, Tepnel Life Science, Titertek Instruments, Tomtec, Zinsser Analytic, Zymark Corporation, ...), which are specialized in automation devices and are able to fulfill the requirements for genomics laboratories. Some vendors offer process specific robots others liquid handling "only" robots, others molecular biological liquid-handling workstations. In order to be able to narrow down the choices, one should look at the budget, the space available for the workstation, the desired flexibility, the throughput needs, the accepted frequency of user interactions and robotic break-downs, and the possible integration with other systems at both the software and hardware level before doing a purchase decision. Thus, typical questions are: How many tubes or plates do I process per day? How much space do I have available? How many different assays can I run within the same labware footprint or format? Do I process a large set of samples, that all have the basic same requirements? Do I need disposable or filtered tips to prevent cross-contamination or are washable probes sufficient? Does the robot uses universal or proprietary tips? What is the desired pipetting range of the probe or tip? What are my accepted accuracy and precision requirements for robotic pipetting? How much individual control over the probes do I need? What are my accuracy requirements for positioning? Which accessories of which capacity do I need (Table 1)? How much x-, y-, z-axes and rotation freedom should the gripper have? An additional plus is, if the vendor is willing to provide training, technical support, qualified service and maintenance, and is willing to design custom tools and adapters for 3rd party devices. In our experience, cost, space, throughput and flexibility are the most important parameters for the decision-making process. In general, good sources of information are personal sources (colleges), commercial sources (advertisement, sales people), public sources (scientific journals) and experiential sources (handling, examining, using the robot). Thus, good sources of robotics information are the material posted on the company's web site, flyers and sales representatives or product managers. In addition, trade shows offer a good opportunity to see robotic workstations in action and users or web-based discussion groups can be a good source for alternative information of robotic workstations.

Table 1. Examples of robotics tasks and its recommended accessories

Category	Robotic tasks	Recommended accessories
General	Entering and exiting of tubes, plates or samples	Robotic arms, conveyer belts or rail drive systems
	Identifying of tubes, plates or samples	1D or 2D barcode reader
	De-capping or de-lidding	Decapper, gripper
	Aliquotting, replicating, reformatting	Liquid-handling "only" workstation
	Normalizing	Single or oligo-probe capability with liquid level detection and individual probe control
	Serial diluting	Oligo-probe capability
	High throughput liquid handling	96 or 384 probe head
Genomics Specific	Nucleic acid extraction by solid phase, magnetic separation or precipitation	Vacuum filtration manifold, magnetic separator, indexing centrifuge or process specific workstation
	PCR and sequencing reaction setup	Liquid-handling "only" workstation
	PCR and sequencing reaction clean-up	Vacuum filtration manifold, magnetic separator or indexing centrifuge
	Gel loading	Oligo-probe capability, gel holder
	Hit picking	Single probe or oligo-probe capability with independent probe spanning
	RNA amplification or other enzymatic reactions	Sealer, Piercer, temperature controlled water system or Peltier element

Liquid Handling

Workstations are priced approximately from $50,000–$250,000 and are designed to fit on a laboratory bench (up to 80 in. length, 30 in. depth). Robotic workstations have pipetting heads, which have washable probes or allow attachment of disposable tips. Pipetting heads are available as 1, 4, 8, 12, 48, 96, or 384 probe heads. Pipetting heads can be mobile themselves or stationary with the deck or a part of the deck moving around. A 1 probe liquid handler is very flexible, but has only marginal throughput, vice versa 96 or 384 probe liquid handler provide high throughput with limited flexibility. Many vendors offer oligo-probe (4, 8, 12 probe heads) solutions to allow a compromise between flexibility and throughput. In most cases, the oligo-probe heads offer an appropriate functionality such as individual control of probes, uniform or independent spanning and individual y- and z-positioning. Independent probe spanning increases e.g. the throughput of hit picking. Also the use of a 384 probe head increases the throughput, but does not allow the access of 96 well microtiter plates. But some vendors offer 384 probe heads with 96 probe capability by supporting the appropriate attachment of 96 tips at 9 mm centers.

Most pipetting heads contain probes, channels, syringes and syringe drives. While probes are the parts that go into the sample, channels are the units of volumetric control. A probe is the steel probe itself, which is connected with a piece of tubing to a syringe. The syringe is on a syringe drive that is a precise worm gear, which withdraws the plunger with a stepper motor. One has control over the stepper motor by software. Individual control of a probe means that one probe is connected to one piece of tubing, which connects to one syringe and one syringe drive. More probes means higher throughput, more individual control over probes means more flexibility. Procedures such as sample normalization require individual probe control. But most 48, 96 or 384 probe heads have a reduced control over individual probes.

The standard spacing of probes to each other in a probe head is 9 mm centers, but 4.5 mm or 18 mm centers are also available. In functional genomics laboratories, most tasks are performed in a 96 or 384 well plate format to increase the throughput.[28] 1,536 well plates are less frequently used due to the associated evaporation problems. 96 well microtiter plates have 9 mm centers, while 384 well microtiter plates have 4.5 mm centers. If you have a probe head with 9 mm centers, most applications should work out for you. Depending on the manufacturer, the spacing of the probes within a probe head can also be customized.

The material of probes is mostly stainless steel and can be coated with Teflon or Ceramic. To choose the best probe material, one should ask, can the probe be cleaned or is it cheap enough to throw away? Does the probe contaminate the sample by leaching any contaminants? Does it adsorb any contaminants from the sample? Does it provide adequate mechanical and electrical properties required for proper operation of the workstation? But, whatever probe material you choose, it should be chemically compatible to the sample. Teflon-coated probes are cheaper than ceramic-coated probes and are more frequently used. Teflon is

non-sticky and it is inert to a variety of chemical conditions. Teflon coating prevents drops on the outside of the probe and allows for enhanced accuracy at microliter and submicroliter volumes. Probes also differ in volume range, accuracy and precision. Accuracy measures how close to a true or accepted value a measurement lies. Precision measures how closely the measurements can be duplicated. In general, accuracy and precision of the pipetting should be determined with aqueous and organic reagents, both under wet and dry dispense conditions. The smaller the pipetted volume is, the better the accuracy should be. The accuracy of fixed probes is in general better than the accuracy of probes with disposable tips. In general, the coefficient of variance for precision is better in wet dispense than in dry dispense and is better in an aqueous than in an organic solution. Probes may also vary in bore size. The inside diameter of the probe is in general between 0.01 and 0.02 in. But wider bore sizes are available for pipetting of viscous fluids.

Air displacement and positive displacement pipetting technologies are routinely used for liquid-handling robotic workstations. Both types of pipetting technologies have a piston that moves in a cylinder or capillary. In air displacement pipetting, a specified volume of air remains (air as ''system fluid'') between the piston and the liquid. In positive displacement pipetting, the piston is in direct contact with the liquid. Air displacement pipetting is used for standard pipetting applications and has a fair accuracy. However, conditions such as atmospheric pressure, the specific gravity and viscosity of the solution have an effect on the performance of air displacement pipetting. Air is very compressible, its density is temperature-dependent, it forms an interface with liquids, which have different surface tension and it mixes with liquids and changes its properties. If filtered tips are used, the system should also compensate for the presence of the filter in the tip, which reduces flow rates into and out of the air column. Air displacement pipettes are meant for general use with aqueous solutions. Positive displacement pipetting is used for applications requiring extreme accuracy. Positive displacement technologies use liquid as system fluid. But most liquids are slightly compressible and any air bubbles in the line compromises accuracy. There exist also a slight likelihood that the system fluid leaks and contaminates (and dilutes) the sample. Positive displacement pipettes are used for high viscosity and volatile liquids. Benefits of the positive displacement technology are the simple technology and the excellent accuracy and precision.

Liquid level detection (LLD) is important to prevent pipetting from an empty well and to reduce the risk of cross-contamination by ensuring minimal tip contact with liquid. Single probes or a row or column of probes or all probes within a multi-probe head can be capable of liquid level detection. Most liquid handler use an LLD method based on capacitance. In capacitive LLD, when the probe comes in contact with the liquid surface, a change in capacitance is measured indicating the location of the liquid surface. Accurate capacitive LLD is restricted to conductive and polar liquids in relatively large volumes. Capacitive LLD cannot detect non-conductive liquids such as DMSO, and can be fooled by foam. Capacitive LLD requires fixed probes or disposable conductive carbon tips to operate. Other available liquid level detection methods are contact LLD and pressure LLD. However, most 96- or 384 probe heads are not capable of liquid level detection, but of liquid level tracking. Liquid level tracking means, that the liquid level is calculated, not measured.

Contamination

For genomics applications, special considerations should also be given to prevent cross-contaminations. The methods favored to prevent carry-overs depend strongly on the individual investigator. But most researchers would agree, that fixed probes are sufficient for protocols using the same reagent, do not have downstream amplification steps (e.g. PCR, bacteria inoculation, ...) or do not contaminate the probe itself in such a way that it is difficult to clean it later on. All protocols with a potential of contamination are best performed by using disposable or disposable, filtered tips. But disposable tips add an additional expense to the protocol. In our experience, the use of disposable tips, selection of microtiter plates with sufficient well-to-well spacing and the prevention of involuntarily z-axes movements of the robotic gripper during plate transfer or manifold disassembly are the most important factors to prevent carry-overs. The success in fighting carry-overs can be observed by running the robot on microplates having ink/water (for simple liquid handling carry-over testing) or blank/sample (for complex biological sample carry-over testing, e.g., PCR) alternate filled wells.

Adding capability

The right choice of accessories for your robotic workstation can significantly increase the throughput or flexibility of your system, the walk away time or the life span of your reagents. Stackers, carousels and feeders are ideal for storing shallow plates, deep well plates and tip racks. In order to be able to allow enzymatic reactions or to increase the life span of your reagents, reagent reservoirs, deck positions and stackers can be temperature-controlled by water systems or peltier elements. For molecular biological applications such as nucleic acid preparations, vacuum and magnetic separation manifolds or indexing centrifuges could be necessary. Although internal grippers can move plates to all positions on the deck, robotic arms, conveyer belts or rail drive systems can move the plates from one robot to another or to peripheral devices. Grippers can be attached to a probe head or to a separate arm. If the gripper is attached to the probe head, the freedom of the gripper may be limited, but it saves the

installation of a separate robotic arm. In general, whenever you increase the complexity of the system with accessories, you also increase the chances for breakdowns.

Software

Most robotic software has a consistent and intuitive graphical user interface to control all movement and pipetting procedures of your workstation. The software should be easy to use and should not require extensive programming skills. Also, start-up screens or wizards to guide lab technicians through their daily routines are helpful. The software should allow the scheduling of the workflow and allow full integration with other software. The software should allow features such as tip touch, multi-target dispensing, timing procedures and complete control over accessories. Some software packages support FDA regulation requirements for multilevel user management, full audit trail, electronic records and electronic signatures. In addition, some vendors offer labware files and methods on their web sites.

Summary

Liquid-handling robotic workstations are valuable tools for genomics laboratories. Robots not only perform consistently and learning curve-free, they also increase throughput and cut labor costs. Liquid-handling robots differ in number and configuration of robotics arms, pipetting heads, functionality of the probes and the availability of accessories. Cost, space, throughput and flexibility are the most important parameters for the decision making process in purchasing a robotic workstation. You should contact multiple vendors and explore the available options to assemble your custom liquid-handling robot by keeping your desired automation needs in mind.

Acknowledgments

The author thanks Anton Burr (Tecan), Steve Cotterman (Beckman Coulter), John Duke (Sias), Jeff Drassler (Autogen), Chris Freezen (Perkin Elmer), Dane Herring (Haemilton), Dawna Houston (Beckman Coulter), Chuck Maw (Matrix), Doyle Stewart (Hamilton), and many others for helpful discussions. The author also thanks Dr. Lizette Cortes for critical reading of the manuscript. The author is not responsible for any incorrect information or any misrepresentation. The reader should contact the vendors directly to validate and update information.

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