

- Seattle families, 1966–1979. *J. Infect. Dis.* 166, 646–649
- 7 Camm, A.J. and Gupta, S. (1999) *Chronic Infection, Chlamydia and Coronary Heart Disease*, Kluwer Academic Publishers
- 8 Kuo, C. *et al.* (1995) *Chlamydia pneumoniae* (TWAR). *Clin. Microbiol. Rev.* 8, 451–461
- 9 Barron, A.L. (1988) *Microbiology of Chlamydia*, CRC Press
- 10 Moulder, J.W. *et al.* (1981) Attachment defect in mouse fibroblasts (L cells) persistently infected with *Chlamydia psittaci*. *Infect. Immun.* 34, 285–291
- 11 Moulder, J.W. *et al.* (1982) Association between resistance to superinfection and patterns of surface protein labeling in mouse fibroblasts (L cells) persistently infected with *Chlamydia psittaci*. *Infect. Immun.* 35, 834–839
- 12 Byrne, G.I. and Faubion, C.L. (1982) Lymphokine-mediated microbistatic mechanisms restrict *Chlamydia psittaci* growth in macrophages. *J. Immunol.* 128, 469–474
- 13 de la Maza, L.M. *et al.* (1985) The anti-chlamydial and anti-proliferative activities of recombinant murine interferon- α are not dependent on tryptophan concentrations. *J. Immunol.* 135, 4198–4200
- 14 Koskiniemi, M. *et al.* (1996) *Chlamydia pneumoniae* associated with central nervous system infections. *Eur. Neurol.* 36, 160–163
- 15 Sriram, S. *et al.* (1999) *Chlamydia pneumoniae* infection of the central nervous system in multiple sclerosis. *Ann. Neurol.* 46, 6–14
- 16 Layh-Schmitt, G. *et al.* (2000) Evidence for infection with *Chlamydia pneumoniae* in a subgroup of patients with multiple sclerosis. *Ann. Neurol.* 47, 652–655
- 17 Boman, J. *et al.* (2000) Failure to detect *Chlamydia pneumoniae* in the central nervous system of patients with MS. *Neurology* 54, 265
- 18 Pucci, E. *et al.* (2000) Lack of *Chlamydia* infection of the central nervous system in multiple sclerosis. *Ann. Neurol.* 48, 399–400
- 19 Hammerschlag, M.R. *et al.* (2000) Is *Chlamydia pneumoniae* present in brain lesions of patients with multiple sclerosis? *J. Clin. Microbiol.* 38, 4274–4276
- 20 Morr , S.A. *et al.* (2000) Is *Chlamydia pneumoniae* present in the central nervous system of multiple sclerosis patients? *Ann. Neurol.* 48, 399
- 21 Meijer, A. *et al.* (1998) Detection of microorganisms in vessel wall specimens of the abdominal aorta: development of a PCR assay in the absence of a gold standard. *Res. Microbiol.* 149, 577–583
- 22 Tong, C.Y. and Sillis, M. (1993) Detection of *Chlamydia pneumoniae* and *Chlamydia psittaci* in sputum samples by PCR. *J. Clin. Pathol.* 46, 313–317
- 23 Gilden, D.H. *et al.* (1996) The search for virus in multiple sclerosis brain. *Multiple Sclerosis* 2, 179–183
- 24 Derfuss, T. *et al.* Intrathecal antibody production against *Chlamydia pneumoniae* in multiple sclerosis is part of a polyspecific immune response. *Brain* (in press)
- 25 Treib, J. *et al.* (2000) Multiple sclerosis and *Chlamydia pneumoniae*. *Ann. Neurol.* 47, 408

Jean C. Tsai

Dept of Microbiology and the Neuroscience Graduate Group, University of Pennsylvania School of Medicine, 3400 Spruce Street, Philadelphia, PA 19104, USA.

Donald H. Gilden*

Depts of Neurology and Microbiology, University of Colorado Health Sciences Center, 4200 E. 9th Avenue, Mail Stop B-182, Denver, CO 80262, USA.

*e-mail: don.gilden@uchsc.edu

Techniques & Applications

It's easy to build your own microarrayer!

Arthur Thompson, Sacha Lucchini and Jay C.D. Hinton

DNA microarrays are becoming the tool of choice for microbial gene-expression profiling and genotypic analysis. The construction of a gridding robot for the 'in-house' production of microarrays is a choice worth considering, and offers distinct advantages over other options in terms of cost effectiveness and scale. Having built our own robot, we want to dispel some of the myths that might be associated with such a project, as well as provide practical advice for potential builders in the UK and Europe.

Microarrays are becoming the pre-eminent technology for the investigation of functional genomics. Many laboratories and research centres are deciding whether to invest in microarraying technology by setting up 'in-house' facilities or purchasing commercial pre-printed arrays, which can cost ~£500 each. One factor that can influence this decision is that a significant number of microarrays is required to obtain one set of publishable data. It is not possible to re-use microarrays for fluorescent DNA applications, and the results from several

microarray experiments are required to produce robust data¹. Microarrays are produced by specialized gridding robots², which are readily available from several US and European companies for upwards of £50 000. The machines differ in terms of the accessories, which include the type and number of print pins (4–48), the number of slides that can be printed in a single run, and the inclusion of cooled plate stackers, and humidity or temperature controls. An attractive alternative is the construction of your own microarraying robot. This is surprisingly easy, and offers a cheap and effective way to produce microarrays at a density of up to 40 000 spots per microscope slide. The total cost to build this robot is in the region of £18 000 plus the cost of at least 20 printing pins (£100 each). Plans for this robust and accurate basic machine, which was designed by Joe DeRisi and Pat Brown at Stanford University, are freely available at: <http://cmgm.stanford.edu/pbrown/mguide/index.html>. The site includes clear building instructions, and the details of suppliers of specific parts, as well as the software (free to academic institutions and available at a

small cost to industrial concerns) for programming the microarraying robot and the analysis of microarray data.

Inspiration

We are currently one of the two institutions within the UK to have built our own DNA microarrayer according to the Stanford design, the other location being at the MRC Toxicology Unit, University of Leicester (<http://www.le.ac.uk/cmht/twg1/array-fp.html>). The construction of our microarrayer was inspired by a Cold Spring Harbor course attended by A.T. from 20 October to 2 November 1999 entitled 'Making and Using DNA Microarrays', where a group of 16 participants constructed four microarrayers within two days³. This article is intended to address the reservations held by most molecular biologists about pursuing a DIY project of this type. We hope to dispel the impression that self-build is an intimidating undertaking. A 'state of the art' engineering workshop is certainly not required; all that is needed is a little patience, and some familiarity with the use of a screwdriver and wire stripper. As Joe DeRisi says 'a

14 year old could do it in a few hours'⁴¹. The truth behind this comment is that nearly all of the microarrayer components are modular and it is mostly a case of literally bolting them down onto a 'breadboard' once they arrive from the suppliers. The amount of expertise required with a wire stripper and screwdriver is no more than that required to wire up a 13 amp electric plug. Some rudimentary computing know-how is also useful for the downloading and installation of the freeware for controlling the arrayer. We found that the most time-consuming part of self-build stemmed from the shipping times of the components, particularly those sourced from the USA. We hope to relay some of the lessons we have learned from having constructed a machine in the UK and to describe the straightforward adaptation of the machine for use with UK and European mains voltage.

Motors and printing pins

The central components of the robot are the three custom-assembled linear actuators and motors, which provide 3-D movement to enable the printing process. The motors are computer controlled and connect to the 'linear actuators', which are built in the USA by Parker-Hannifen. The actuators convert rotary to linear motion and have a positional accuracy of $\pm 2.47 \mu\text{m}$. Briefly, microarray production involves the printing of DNA onto glass microscope slides that have been coated with a DNA-binding substrate and which are laid out onto a platter (Fig. 1). The platter carries up to 137 microscope slides, and is positioned by the X-axis actuator so that consecutive slides lie beneath the print head. The print head carries either 16 or 32 hardened steel pins, which are moved by two actuators (the Y and Z axes) to the washing and drying stations, before dipping into a 384-well microtitre plate containing the DNA solutions to be printed. The pins tap down and deposit sub-nanolitre amounts of DNA solution onto each consecutive slide. Delivery of the custom-built actuators takes around 2–3 months and these should be ordered first, along with the solid base (the breadboard), onto which the actuators and other components are bolted. It is important when ordering the breadboard to specify that it is laid out in imperial measurements. It is also worth remembering that the legs for the

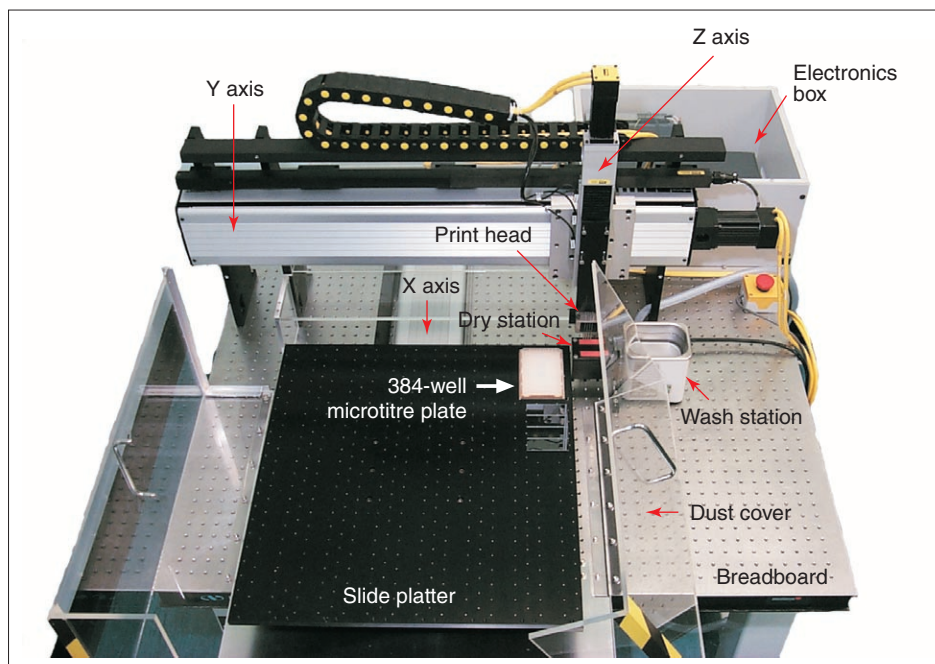


Fig. 1. The self-built 'Stanford-design' microarrayer at the Institute of Food Research, Norwich.

breadboard are packed on top of what looked to us like a pallet bottom, but which in fact contains the breadboard itself! A British company Micromech, will carry out the actuator construction (for the Z axis at least) and should be a more rapid supplier than Parker-Hannifen for those building in Europe. Alternately, the actuators can be supplied already correctly set up on a breadboard from Western Technology in the USA; this option will again take a few months to

process. On the Cold Spring Harbor course, the actuators and breadboard were supplied separately.

Printing pins are the most crucial variable in the generation of high-density microarrays. The printing pins on the Stanford arrayer are what is known as the 'quill' type, and have a thousandth of an inch width slit at the tapered tip of the pin, which wicks up the DNA solution. Because the pins tap repeatedly onto the surface of the

Table 1. Sources of key components required for DIY construction of a microarraying robot

Item (catalogue no.)	Supplier	Web or e-mail address
Scientific grade breadboard (SG-44-2) & workstation frame (VH3648W-OPT-NN-01)	Newport Ltd	http://www.newport.com
Linear actuators	Micromech	http://www.micromech.co.uk
Transformers step-down 230V AC input, 115V AC output (347-6652)	RS components	http://rswww.com
Encapsulated auto step-up/step-down (293-2678)		
Plastic enclosures (1796434); AC connectors (1749250); 3-conductor power supply cord (1289019); Belden 10-conductor cable (1284277); snap-in inlets (1407200)	Farnell Scientific (for Newark Electronics)	http://www.farnell.com/uk
Slide platter	Die-Tech	e-mail: DIE-TECH@ix.netcom.com
Wash station (Compact ultrasonic cleaner — part # 32695 K21)	McMaster-Carr	http://www.mcmaster.com
Printing pin head and printing pins	Majer Precision Die-Tech	http://www.majerprecision.com e-mail: DIE-TECH@ix.netcom.com
Vacuum Pump (Model # 71R655-V10-C222TX)	Gast Mfg	http://www.gastltd.com

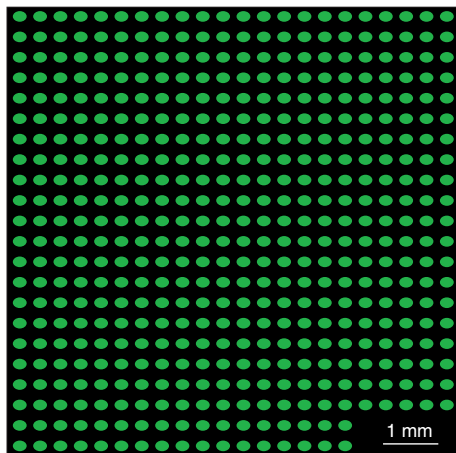


Fig. 2. A subgrid of 484 features printed out sequentially from a single pin on the microarraying robot. The spotting solution contained 3 X SSC, 0.01% Sarkosyl, 100 ng μl^{-1} salmon sperm DNA and 5 pmol of Cy3 dCTP (Amersham Pharmacia Biotech.).

slides they need to be robust and to be kept free of blockages. We have found the pins produced by Majer Precision to be particularly robust (<http://www.majerprecision.com>). Alternately, Die-Tech also manufacture suitable pins (DIE-TECH@ix.netcom.com).

We prepare new pins by doing thousands of preliminary taps on a few slides. There is a special 'test-array' program included with the Stanford software that permits this before full-scale printing. In our experience, some of the pins that do not work initially will start to print, and others will stop printing. We think this is caused by bending of the prongs on either side of the slit, which seems to affect deposition and might be the result of a very slightly off-centred slit position. Bending of the prongs too far inwards or outwards seems to prevent spot deposition. Occasionally, pins can become blocked with salt deposits or other contaminants and can be cleaned by gently wiping the tips with ethanol or water. Failing this, pins can be inspected under a low-power microscope and carefully unblocked by inserting a 0.001 inch feeler stock (RS components cat. no. 572-139) into the slit. Once a set of reliable pins is obtained after a few hours test printing, it should go on to print many thousands of spots consistently. One advantage of using the sturdy Majer Precision pins is that they can be repeatedly sonicated during the wash stage without apparent damage. We recommend ordering twice as many pins as you plan to use for arraying to allow for pins that do not print.

Wiring

Once the motors and linear actuators have been bolted down (a matter of an hour), the wiring of the motors to their respective amplifiers and the 'breakout box' can begin. The breakout box is the interface between the computer card and the amplifiers. This is pretty straightforward work consisting of wire stripping and using a small screwdriver to screw the wires into their respective terminals. The 'Mguide' (Pat Brown's self-build guide on the web) gives careful instruction concerning which coloured wires to put where. The motors themselves are controlled via a DMC-1739 controller card and it is important to ensure that the computer contains an ISA slot for this. The one and only encounter with a soldering iron will be the construction of a small relay box that coordinates the switching on and off of the sonicator and dry stations. A member of our laboratory (S.L.) who had no previous experience of soldering managed this task in just 10 min. The wash station sonicating water bath was ordered from the USA; however, the Gast vacuum pump connected to the dry station was of UK origin. Both items are controlled via the relay box, which is connected via a multi-board adapter to a 110V step-down transformer, purchased from RS components. Thus, it was necessary for us to use a step-up transformer to reconvert the 110V output from the relay box to the vacuum pump to 240V (UK voltage). Alternatively, a 240V sonicating water bath of suitable size could be used, and the whole set-up run at 240V to circumvent the need for a step-up transformer. The suppliers and distributors we used in the UK for the electronic and other components are listed in Table 1.

Custom-built covers

The entire building process took around a week, working part-time. To finish off the robot, we had a dust cover made in perspex to fit over the front and back of the arrayer and also a simple cover for the amplifiers and breakout box (Fig. 1), the dimensions of which are available from us on request. We house the machine in an air conditioned, HepA-filtered room, to minimize dust deposition during microarray printing, and typically maintain the ambient temperature at 18°C.

Conclusion

Overall, we are very pleased with the results of our efforts and the reproducibility of the printed spots is superb, typically between 100–150 μm in diameter. Figure 2 shows that 484 spots, or features, can be printed from a single pin loaded with DNA just once. The main advantages of the Stanford-designed microarrayer lie in its modest cost, robustness, accuracy, simplicity and its ability to print a maximum of 137 slides in one run. The one disadvantage compared with many commercial microarrayers is that it does not have a plate stacker, making it necessary to return to the machine every 30 min or so to change microtitre plates; however, this removes the necessity to have humidity or temperature controls, which are required to prevent evaporation from uncovered plates. So far, our machine has been used to array the *Campylobacter jejuni* genome, which took around four hours to produce 137 microarrays, each containing 1600 genes. It will shortly be used to array the *Escherichia coli* K12 and *Salmonella typhimurium* LT2 genomes. We hope that this article will encourage potential self-builders, and will be happy to advise on specific issues.

Acknowledgements

We are grateful for support from the BBSRC to set up the IFR Microarray Facility, for assistance from Bruce Pearson with construction and to Joe DeRisi, Pat Brown, Tim Gant and David Judah for their encouragement and advice. We thank David Jones and Barry Murrell for building the dust cover and electronics box.

References

- 1 Lucchini, S. *et al.* Microarrays for microbiologists. *Microbiology* (in press)
- 2 Shalon, D. *et al.* (1995) A DNA microarray system for analysing complex DNA samples using two-color fluorescent probe hybridisation. *Genome Res.* 6, 639–645
- 3 Fitcher, B. (1999) Blast ahead. *Nat. Genet.* 23, 377–378
- 4 Dalton, R. (2000) DIY microarrayers promise DNA chips with everything. *Nature UK Product Review* March, 5

Arthur Thompson*

Sacha Lucchini

Jay C.D. Hinton

Molecular Microbiology, Institute of Food Research, Colney Lane, Colney, Norwich, UK NR4 7UA.

*e-mail: Arthur.Thompson@bbsrc.ac.uk