High-Throughput Hybridoma Screening

EMBL Researchers Find that the Arrayjet Super-Marathon Inkjet Microarrayer Can Improve Process

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- **Introduction**

The maturation of proteomics as a separate experimental field from genomics has highlighted the importance of obtaining information concerning a given protein's activation state. This information can often be obtained by investigating the protein's post-translational modification status—in particular, its phosphorylation state. The difficulties in producing high specificity and sensitivity affinity reagents specific for phospho-epitopes in sufficient quantities is, however, limiting the scope of diagnostic and research assays, where a multitude of phosphorylation states need to be probed simultaneously.
To address this reagent shortage, a series of high-throughput methods were devised at the EMBL Monoclonal Antibody Core Facility in Montorotondo, Italy. These methods incorporated robotic liquid handlers to perform somatic cell fusions at the rate of eight reactions per week—one or two is the norm—on lymphocytes derived from antigen-primed mouse spleens to generate classical hybridomas.

These eight fusions generated a total of 15,360 different samples to be tested by an affinity capture assay such as ELISA.

Successful screening for specificity against a phospho-epitope requires probing of both the phosphorylated and unphosphorylated form of the peptide, and a third, unrelated target, which has the same phosphorylated amino acid present in a different primary sequence context. This strategy ensures that only cell lines specific for the relevant amino acids are isolated, but triples the number of capture assays required to 46,080.

Furthermore, these assays must be completed within 48 hours to enable isolation of antibody-generating cell lines before the growth medium is exhausted. These combined issues push throughput requirements beyond the capacity of a standard plate-based ELISA, as the screen would necessitate the 480 96-well plates be processed.

- **First Step to the Solution**

  The most parallel assay technology to emerge in recent years is the microarray. The potential to perform a single test and yet obtain tens or hundreds of thousands of data points simultaneously, made microarrays an attractive solution to effectively manage throughput.

  A novel antigen microarray assay was recently devised by EMBL in which a single peptide-coated glass microscope slide per test antigen (phosphorylated, unphosphorylated, and irrelevant peptide) would be printed with the cell culture supernatants from each fusion. At three slides per fusion, only 24 slides would be needed for eight fusions while maintaining the same level of screening complexity.

  Initial screens using microarrays produced on a contact microarrayer proved problematic. Unreliable contact deposition necessitated triplicate printing of each supernatant; as the maximum spot density per slide was 17,280, only three fusions could be spotted onto each slide in a single print run (5,760 spots were required per fusion). This restriction limited print-run length and significantly decreased unattended operation time, while increasing manual intervention.

- **The Complete Solution**

  A high-throughput noncontact microarrayer, the Super-Marathon Inkjet Microarrayer from Arrayjet was therefore employed. The Super-Marathon has the capacity to produce microarrays from up to 48 microtitre plates on up to 100 slides, without user intervention, handling up to 32 samples simultaneously. The instrument was installed within an enclosed, HEPA-filtered environment with control of temperature and humidity; maintaining ideal conditions for microarray production increased productivity and efficiency. Furthermore, due to the quality of the microarrays produced it was no longer necessary to print supernatants in triplicate.

  Finally, implementation of custom-designed Multiple Print Run capability within Arrayjet's Command Centre™ software enabled each print run to be divided into a number of sub-print runs, effectively making it unnecessary to print each supernatant on each slide, as in conventional microarray manufacture. Instead, one set of supernatants could be printed on one set of slides and another on a different set of slides.

  These factors significantly increased the number of fusions that could be printed in unattended operation, while simultaneously reducing printing time. The EMBL group could therefore perform a

single, high-speed print run on the Super-Marathon instead of what had previously required three low-speed print runs on their contact microarrayer.

- **Making and Screening mAbs**

  A hybridoma cell line was prepared by selecting and fusing splenocytes from one of two CD1 mice, each of which were immunized with a serine-phosphorylated synthetic peptide coupled to diphtheria toxoid as a carrier protein. The animal with the highest serum titre—determined by ELISA 10 days after each immunization—was selected for fusion eight weeks after immunization.

  Fusions were automated using a suite of cell culture robotics and dispensed into 20 96-well tissue culture plates, following standard protocols. After two weeks of incubation at 37°C, 40 µL of culture supernatant from each well was transferred by robotic liquid handler from the 96-well plates into the 384-well plates intended as source plates for the Super-Marathon. An additional seven fusions were simultaneously carried out in an identical fashion, using different protein and peptide targets.

  Glass microscope slides were coated with an aminosilane to enable the peptide antigens to bind to the slide surface. Three aminosilane-coated chips were then coated with a monolayer of each of the three peptides: one phosphorylated, one unphosphorylated, and one an irrelevant peptide containing a phospho-serine moiety. Protein and peptide chips from each of the additional seven fusions were similarly prepared. The peptide and protein chips were subsequently placed on the Super-Marathon and 40 x 384-well plates, with lids, placed in the Microplate Stacker.

  A Multiple Print Run definition enabled the designated supernatants to be printed on the designated slides in a single, unattended overnight print run. Each slide was washed and incubated with a fluorophore-labeled antimouse antibody, washed again, and visualized using a fluorescence microarray scanner. The phospho-peptide, nonphospho-peptide, and irrelevant-phospho-peptide chips were analyzed using standard software and compared using a custom-made database to look for phospho-specific signals.

Click Image To Enlarge +

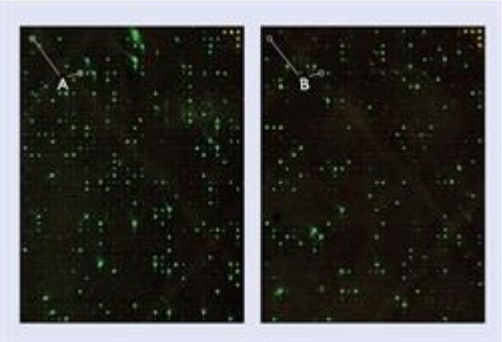

Figure 1. A comparison of a chip coated with a peptide containing a phosphor-serine (left), with a chip coated with an unphosphorylated version of the same peptide. Spots labeled A are specific to the phosphorylated form and are therefore not present at point B.

- **Results**

  A comparison of images of the three slides in the phospho-specificity test fusion enabled isolation of hybridomas that produced antibodies specific for the phospho-peptide (*Figure 1*). In addition, a comparison of the microarray images produced by the contact microarrayer with those produced on the Super-Marathon demonstrated the superior spot morphology of the microarrays produced by the Super-Marathon.

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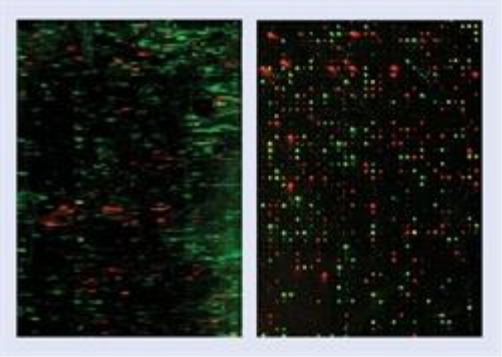

Figure 2. A comparison of a typical protein chip spotted with 32 fixed pins (left) with the typical spotting pattern of the Arrayjet Super-Marathon

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The improved microarray quality simplified and accelerated the analysis (*Figure 2*), while enabling rapid and simultaneous screening of the supernatants from the seven other fusions. Clone selection and isolation were completed within 36 hours of commencing the microarray assay (data not shown).

- **Conclusion**

  Robust systems for high-throughput hybridoma production and screening are absolutely necessary in order to produce high-quality antibodies in the most efficient manner possible. The Super-Marathon Inkjet Microarrayer has been shown to improve the speed and robustness of manufacture of the screening assay due to its flexibility, reliability, and the quality of the microarrays it produces.

  In addition, the quality and accessibility of the microarrays produced on the Super-Marathon enabled more rapid data analysis and interpretation. The capacity and flexibility of the Super-Marathon could enable the introduction of new methods, such as epitope-mapping of fusions at the primary screen, particularly useful when attempting to raise matching pairs of monoclonals for sandwich assays such as ELISA, proximity ligation assays, multiplex bead assays, and for protein quantitation.