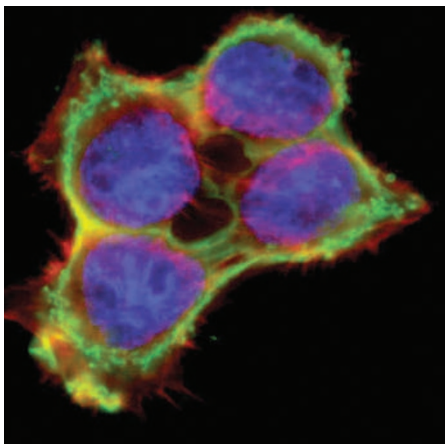
clones. The other two groups were dedicated to immunohistochemistry and the informatics infrastructure necessary to deal with the large volume of data.

To deal with image acquisition issues, the institute collaborated with Applied Imaging of San Jose, California, to develop an automated high-throughput image-analysis system suitable for tissue microarray applications. So far, the Sanger project has generated more than 4,000 monoclonal antibodies to 290 antigens, which are available to buy from Geneservice in Cambridge, UK.

Although the project is being discontinued, McCafferty says that much has been learned about the bottlenecks of high-throughput generation of antibodies and how these can be overcome. "Surprisingly, the generation of the antibodies was not the major issue," he says. "The bottlenecks were generating good quality protein product to do selection, and how to deal with the large amounts of image data a project such as this produces."

Finding affinity

Even as the Sanger project comes to a close, other initiatives are beginning to gather steam — although these have been hampered somewhat by a lack of funding. "There seems to be a reluctance from the funding agencies to put money into large-scale antibody initiatives," says Andrew Bradbury of the biosciences division at Los Alamos National Laboratory in New Mexico. The NCI's five-year, $104-million clinical proteomic technologies initiative



MILLIPORE

Cells stained with the 4G10 anti-phosphotyrosine antibody from Millipore.

that is now getting off the ground may be the start of a change.

In 2005, the NCI held a workshop to discuss affinity capture. It found that the scientific community wanted renewable resources that were well characterized for performance data, says Clark. The meeting also revealed that the community was concerned by the lack of characterization data for most available antibodies.

Following this lead, the NCI proteomics reagent core, one of the centres in the clinical proteomic technology initiative, is embarking on the production of affinity reagents. To focus its efforts, it has identified a list of protein targets: all cancer-related proteins for which no commercial antibodies are yet available. The core will develop monoclonal antibodies that will be characterized by Western blots, enzyme-linked immunosorbent assay (ELISA), immunohistochemistry and immunoprecipitation followed by mass spectrometry. "All the raw data on how the antibodies perform on a variety of assays will be provided and an investigator will be able to acquire these antibodies through a website organized by the NCI," says Clark.

In Europe, another group of investigators plans to generate affinity reagents against the human proteome. The group, called ProteomeBinders, consists of 26 European Union and two US institutional partners. "The goal or the hope is to get funding from the European Union to put a project together next year or the year after," says Bradbury, one the US participants.

Although the antibody remains the affinity reagent of choice, the exploration of alternative binders by large groups, such as ProteomeBinders, shows how far these non-traditional reagents have come in a relatively short time.

Gold standard

A quick glance through the catalogues from commercial vendors and researchers reveals thousands of antibodies not only to proteins, but also to specific protein changes such as post-translational modifications. Still other companies offer to produce antibodies to an investigator's antigen of interest.

Monoclonal antibodies produced by animal immunization remain the 'gold standard' of

ANTIBODIES IN THE FAST LANE

In making recombinant antibodies, the resulting antibody is only as good as the combinatorial library and the screening assay. The trick is to find the molecule of highest affinity and specificity for the target among a library of millions of clones. Traditionally, recombinant antibody libraries have been phage-based and the screening relied on enzyme-linked immunosorbent assay (ELISA), not a high-throughput method. Changes in both phage display and screening methods are now moving recombinant antibody production into a high-throughput world.

Flow cytometry has become the assay of choice for rapid screening of clones from recombinant antibody libraries. "We looked at different ways of screening. Flow cytometry was the only one that seemed to meet our throughput requirements," says Andrew Bradbury of Los Alamos National Laboratory in New Mexico. Bradbury's group has developed a flow-cytometry assay to screen its single-chain antibody-fragment phage libraries using a mixture of beads coated with specific and non-specific antigens. The method rapidly identifies antibodies that have good affinity for the protein of interest while discarding those that show low specificity.

Phage-display screening methods are also used to identify antibodies that target post-translational modifications (PTMs) such as phosphorylation or acetylation. As PTMs have a role in many processes — from gene regulation to apoptosis — they are of growing interest for the biological community. Companies have responded by developing antibodies targeting proteins in a specific state of modifications. "Antibodies to PTMs are gaining in importance with customers," says Kumar Bala, director of antibody technologies for Millipore in the company's lab in Temecula, California.

But obtaining antibodies directed against PTMs is not a trivial task. Rockland Immunochemicals of Gilbertsville, Pennsylvania, has put in a lot of effort to develop antibodies for looking at phosphorylated and non-phosphorylated forms of various proteins in a sequence independent context, says Daniel O'Shannessy, the company's vice-president of corporate development.

LANL

Andrew Bradbury develops recombinant single-chain antibodies.

Antibodies that recognize PTMs independently of the protein site on which the modification occurs are useful — particularly for enriching, for example, all phosphorylated proteins from a cell. But such antibodies are hard to make by animal immunization as the PTM itself is not immunogenic. So scientists are turning to recombinant molecules and *in vitro* screening such as phage display to isolate 'pan-PTM' affinity reagents.

Although making steps in the right direction, many more antibodies and further improvements in affinity reagent technology will be needed to understand and characterize the full range of PTMs found in nature. Still, Bala argues that the "best tools for purifying, identifying, differentiating and characterizing PTMs are antibodies". N.B.