Streamlining the selection of recombinant antibodies

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Antibodies make good drug candidates because their natural role in the body's immune system means that they are potent, specific, well tolerated and can be readily raised to a variety of different targets. The most established method of generating monoclonal antibodies (MAbs) is to create hybridomas. This is achieved by innoculating a mouse with a target antigen thereby stimulating the production of a population of antibody secreting B-cells. These can be fused with cancer cells to produce hybrid cell lines — a sustainable source of the original antibodies. Although these MAbs are effective as drugs, successive treatments can induce a severe allergic response known as the HAMA (human anti-mouse antibody) reaction in some patients.

> Automation can eliminate a substantial part of the unpopular task of plating out and counting antibody clones.

To overcome immunogenicity problems, efforts have focused on making antibodies appear more human by using 'humanising' antibody peptide sequences. An alternative technique that uses entirely human antibody repertoires is phage display technology. Using a bacterial virus or 'phage' to display antibody fragments on its surface (*Figure 1*), the human antibody genes are cloned and introduced into phage particles to produce huge libraries of diverse antibodies. An advantage of this approach is that the antibody phenotype is linked to genotype thereby allowing the antibody to be propagated and its structure manipulated in order to optimise its properties.

A number of companies use phage display to generate human monoclonal antibody libraries. **Cambridge Antibody Technology** (CAT), for instance, uses libraries containing more than 100 billion different antibodies. To date, the company has produced seven antibody drug candidates from these libraries that are seen to be progressing well in clinical trials.

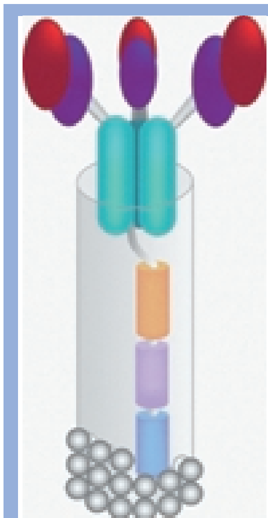

Figure 1 A filamentous phage displaying an antibody protein at its tip.

CAT has invested considerable efforts in trying to resolve procedural bottlenecks, efforts that have focused largely on the introduction of automation. In the past, several stages in the production process would be consumed by the tedious plating out and counting of antibody clones. Automation has now eliminated a substantial part of this unpopular task, resulting in considerable time savings and increased productivity.

Manual plating and counting method

The expression of recombinant antibodies in phage begins with cloning the heavy and light chain variable regions of human derived antibodies together with an antibiotic resistance gene into a specialised phagemid vector. The vector is then used to infect *E. coli* TGI cells. The transfected TGI cells are serially diluted in growth media to produce five or six dilutions. Precise volumes are plated out onto the selective antibiotic-containing agar, which kills the non-transfected host bacteria. However, those cells infected with the phagemid have resistance to the antibiotic carried by the vector and will therefore continue to grow. Each colony growing on any of the serial dilution plates will contain one phagemid clone and therefore one recombinant antibody. The number of colony forming units per mL is more than a simple measure of phage concentration; it can be both a powerful indicator of selection progress and a guide for post-isolation strategies used to refine the antibody's functional properties. It is an important component of quality control and efficient decision making.

It is usual to count the colonies manually using a light box and pen. With 30 technical and scientific staff performing colony counts on a daily basis, laboratories regularly use between 500 and 700 plates each day. Preparation and enumeration processes take an estimated 30 hours of collective research time to complete.

Streamlining plate preparation and colony counting

Researchers at CAT decided to automate the plating and counting processes to enable them to spend longer working on the more technically demanding parts of the process. To this end, the company evaluated a WASP Spiral Plater (**Don Whitley**, Shipley, UK). Spiral plating can cut the number of plates used in each selection round down from five or six to just two, which serves to reduce plate preparation time and media costs, as well as producing smaller amounts of hazardous waste. In conjunction with the assessment of the spiral plating method, CAT installed two automated colony counters: a ProtoCOL RGB and an åCOLyte system (**Synbiosis**, Cambridge, UK [*Figures 2* and *3*]), both of which integrate easily with the WASP to automatically count the number of recombinants on spiral plates. Two different systems were chosen because two separate groups perform the colony counting and the enumeration needs of each project are very different.

Figure 2 A ProtoCOL RGB automated colony counter.

The SuperCount version of åCOLyte is used as a simple, cost-effective method of automating total counts. Since the system can count up to 10^3 colonies in less than two seconds while automatically correcting for background variations and different media types, researchers can make instant decisions on experimental success and subsequent strategy.

Figure 3 The åCOLyte automated colony counter.

The ProtoCOL RGB was installed by a second research group at CAT because it uses high-resolution colour information to differentiate, analyse and count both coloured and white colonies. Although the ProtoCOL RGB is currently being used for total colony counting tasks in the same way as the åCOLyte system, there are plans to use it for the counting of recombinants produced using LacZ-based selection vectors. The system can do this by simultaneously differentiating and automatically counting blue (non-recombinant) and white (recombinant) colonies or plaques that are plated out on the same plate.

Both the åCOLyte and ProtoCOL RGB produce live, colour on-screen images that can be saved with a time and date stamp. This provides GLP compliance and allows images to be easily retrieved and printed out for reports or presentation material. This feature is important to companies such as CAT because it provides secure records that are compliant with the information required by external regulatory auditors.

Automating the counting of colonies has been so successful that CAT is now in the process of automating further plating out processes in order to improve productivity and reduce costs. Future plans involve adding a robotic arm to the system, so that plates can be moved directly from the spiral plater to an incubator and then to the ProtoCOL RGB without human intervention. This will fully automate the whole front end of the plating out and counting process.

Conclusion

The use of automated colony counting has reduced the time it takes each CAT researcher to make decisions from one hour to just five minutes (a 12-fold improvement). Further automation of the plating process will also help to cut the cost of materials and plate preparation labour by around 60%, ensuring that the initial expense of installing the equipment will be rapidly recovered.

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