A recent success of the phage-display technology is HUMIRA, the first fully human monoclonal antibody to be approved for therapy, developed by Cambridge Antibody Technology (CAT) in Cambridge, UK, and Abbott Laboratories in Abbott Park, Illinois. This antibody, which blocks the inflammatory cytokine TNF-α, was approved in the United States in 2002 and in Europe in September this year as a treatment for the symptoms of rheumatoid arthritis.

CAT's phage-display library holds single-chain variable fragments (scFv) which consist of the variable ends of a light and a heavy chain linked with a short peptide. The library now boasts more than 100 billion distinct antigen-binding specificities. And in the world of antibody discovery, most people think that size matters. The larger the library, the greater the chance that it contains an antibody for your given target. "It means you can get reasonably high-affinity antibodies out of your first collection," says Winter.

The library started as a collection of antibody genes corresponding to antibodies found in the blood of healthy people, but CAT has shuffled the DNA sequences that code for the variable region to give even more different types of antigen-binding site. Having started with human genes, CAT believes its antibodies are less likely to create immunogenic responses than libraries built with synthetic sequences.

BIOINVENT

Eskil Söderlind: 10 billion antibodies.

Antibody discovery and production company BioInvent in Lund, Sweden, has a similar library called n-CoDeR, which holds human scFv. Like CAT, BioInvent starts with natural human antibody genes as the source of the DNA sequences, which are then further diversified by making variants of the antigen-binding regions. "We have taken evolution beyond nature and the library now has more than 10^{10} members," says Eskil Söderlind, who invented the n-CoDeR technology with Carl Borrebaeck. BioInvent can screen up to 20,000 clones per day against a customer's target antigen on its automated system. The scFv fragment genes are then converted into complete antibody genes, and the antibody itself produced in larger quantities from genetically engineered mammalian cells.

The biopharmaceutical company Dyax in Cambridge, Massachusetts, has phage-display libraries holding Fab fragments — an entire single 'arm' of an antibody. Although the company holds many of the foundation patents for phage-display technology, Dyax is a relative newcomer in applying phage display to antibodies, having initially focused on peptide and protein display. The company has moved rapidly into the antibody arena by cross-licensing its intellectual property with other major players, such as Genentech, Xoma, Affimed, Biosite and CAT. Chief scientific officer Clive Wood believes that one of the company's strengths is the newness of its antibody libraries. "We are the new kid on the block, but because of that we have important advantages. We have been able to incorporate new features into our Fab library, such as having a mixture of natural and synthetic diversity that allows us to pull out antibodies with picomolar to nanomolar affinity." The largest Dyax library contains about 37 billion distinct human antibodies, and the company has two therapeutic proteins derived from phage display in three phase II trials.

Going for GOLD with MorphoSys.

The Human Combinatorial Antibody Library (HuCAL) of MorphoSys in Martinsried, Germany, offers 10 billion totally synthetic variable regions modelled on partial human variable regions and displayed in phage. MorphoSys has now introduced HuCAL GOLD, in which a disulphide bond

GOING INTO PRODUCTION

"Some of the therapeutic antibodies are required now in tens of thousands of grams per year," says John Birch, chief scientific officer of the Lonza Group, based in Basel, Switzerland, which is planning three new 20,000-litre mammalian cell-culture reactors in the United States for monoclonal antibody production, in addition to its present capacity. Antibody harvests from cultured mammalian cells are around a gram per litre of culture.

But the cost of scaling-up production using mammalian cells can be prohibitive, particularly for a clinical trial of an antibody that may not turn out to be worth it. Looking to fill a gap in the market are alternatives such as the transgenic goats of GTC Biotherapeutics in Framingham, Massachusetts, and the transgenic rabbits produced under a GTC Biotherapeutics licence by BioProtein Technologies of Paris, France. "If I need it, I breed it — the walls of my bioreactor are expandable," says Thomas Newberry, vice-president of GTC Biotherapeutics. He estimates that the cost of producing a founder goat is about US$10 million. Although pricey, this is a fraction of the estimated $200 million–500 million that is needed to build a large-scale mammalian cell-culture bioreactor. The company estimates that a single goat can produce up to a kilogram of pure antibody per year. Goat-produced antibodies are in clinical trials to determine whether they match the safety, efficacy and pharmacokinetics of more traditionally produced counterparts.

"There's a whole category of antibodies that have not been effectively explored for therapeutic or preventive use, and for facing down viruses in the mouth, genito-urinary and respiratory tract," says Elliott Fineman, president and chief executive of Planet Biotechnology in Hayward, California. These are the secretory IgA antibodies, which cannot be produced from mammalian cell culture in significant amounts. Instead, his company is using transgenic tobacco plants to produce CaroRx, a monoclonal secretory IgA against the bacterium *Streptococcus mutans*, a leading cause of tooth decay. Originally created as 'Guys 13' by Thomas Lehner and Julian Ma at Guy's Hospital in London, CaroRx has shown its ability to block colonization by *S. mutans* in phase II clinical trials, and Planet Biotechnology is hoping to apply shortly for a licence to market the antibody in Europe for topical application by dentists and patients following antiseptic cleansing of teeth. If the company succeeds, it will be the first 'plantibody' to reach the market.

Fineman estimates that building, equipping and validating a facility to produce 100 kilograms of antibody from plants would cost between $30 million and $50 million. Keeping cost down is particularly important for antibodies intended for non-therapeutic use against diseases that are not life-threatening. "Nobody's going to take a £2 hike in the cost of toothpaste," says Ma.

J.C.