Mutant microdrops

Isolating mutant yeast strains can be complicated by their comparatively slow growth, leading to out-competition and overgrowth by wildtype strains. By double-encapsulating single yeast cells in agarose microdrops, researchers, led by James Weaver at the Massachusetts Institute of Technology, are able to isolate slow-growing mutant cells from a mixed population using fluorescent-activated cell sorting (see p.884). By using two fluorescent signals—green fluorescence as an indicator of the biomass and, therefore, microcolony size, and far-red fluorescence signals to eliminate signals from empty microdrops—they have been able to separate slow growers from free cells and noise.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

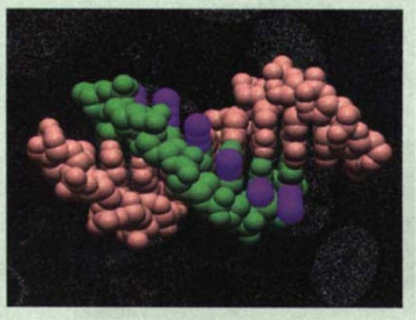
Designer primers

A surprisingly small number of statistically designed primers are theoretically able to identify the majority of protein coding regions from large genomic subsets. By using twenty statistically designed G-protein coupled receptor (GCPR) primer pairs, López-Nieto and Nigam, at Harvard Medical School, are able to detect 122 out of the total of 157 GPCRs (see p.828 and p.857). They have also identified, using computer-simulated PCR analysis and a set of 30 octanucleotides, over 70% of the known human coding regions. Using a consensus primer-set generated from 1000 human mRNAs, López-Nieto and Nigam have simulated PCR reactions in the 1000 human mRNAs, producing a PCR product in 76% of them.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

In this issue of *Nature Biotechnology*, Pech's group at the École Nationale Supérieure Agronomique (Toulouse, FR) demonstrate that an antisense ACC oxidase gene expressed in melons inhibits ethylene accumulation delaying fruit ripening for up to 60 days (see p.828 and p.862).

Statistical considerations have prompted the routine use of 12 to 25 nucleotide-long oligonucleotides (ONs) in antisense experiments. However, Wagner et al. at Gilead Sciences (Foster City, CA) have shown that exceptionally small 7mer ONs specifically inhibit expression of a target gene (see p.824 and p.840). Presumably, this unexpected specificity is due to the flanking sequences of the target RNA, which affect the structure, thus determining the ON's accessibility.



Bacterial hydrogen evolution

The potential of hydrogen as a fuel has led to the development of its in vitro synthesis by Woodward and his colleagues at the Oak Ridge National Laboratory. By coupling glucose dehydrogenase (GDH) from *Thermoplasma acidophilum* with hydrogenase from *Pyroccocus furiosus,* sustained evolution of molecular hydrogen from glucose was achieved (see p.872). GDH oxidizes glucose, which is subsequently hydrolyzed to gluconic acid; both enzymatic steps use the electron donor NADPH, which is continuously regenerated and recycled. Woodward et al. anticipate that this system could be used to derive hydrogen from abundant and renewable carbohydrate sources such as cellulose.

Fibrinogen bioreactor

The low yields of recombinant human fibrinogen production in mammalian cell culture have inspired Prunkard and her coworkers at Zymogenetics (Seattle, WA) and PPL Therapeutics (Edinburgh, UK) to develop mammalian bioreactors as an alternative (see p.867). Prunkard's team coinjected equal amounts of three expression cassettes encoding α, β, and γ fibrinogen chains, under the control of lactation-specific promoters, into mice embryos in order to integrate them in a single colinear conformation. They have obtained triply transgenic offspring in over 80% of cases that express levels of up to 100% of fully assembled, active fibrinogen.

Blechl and Anderson at the US Department of Agriculture (Albany, CA) have altered the glutenin content of wheatgerm, demonstrating the potential to improve the bread-making qualities of flour (see p.875).

Cytotoxic trigger

A combinatorial library has been screened to identify inhibitory peptide sequences to generate a mutant α-hemolysin, whose pore-forming toxic activity is specifically initiated by the site-directed cleavage of metastatic tumor cell proteases (see p.826 and p.852). Thus the group, led by Hagan Bayley at the Worcester Foundation for Biomedical Research (Shrewsbury, MA), envisions that these pore-forming proteins will be specifically toxic to tumor cells, either alone or when combined with cell-impermeable cytotoxic drugs, upon the tumor cells' release of the normally intracellular site-specific protease cathepsin B.

"Human" hybridomas

A group from GenPharm (Mountain View, CA) have created a new strain of transgenic mice for hybridoma production, as demonstrated by the isolation of human antiCD4 Mabs (see p.826 and p.845). By inserting a germline configuration minilocus transgene and a human κ light chain transgene, and preventing expression of mouse IgM and Igκ via targeted deletions, Fishwild's team has produced mouse hybridomas that secrete high-avidity human monoclonal antibodies to CD4, and do not secrete murine heavy or light chains.

Acidifying ion transport

Coupling genetic engineering with the powerful technology of microphysiometry has allowed a research team at Bristol-Myers Squibb (Princeton, NJ) to develop a prototype drug screen for the analysis of effectors of ion transport (see p.880). By engineering channels into potassium transporter-defective yeast cells, Stephen Kurtz's group has shown that extracellular acidification can be used to measure transmembrane ion and proton flux, thus developing the rationale for a high-throughput screen.