Recombining larger phage libraries

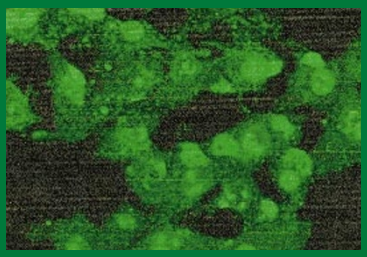
Isolation of the highest affinity antibodies from phage libraries is thought to require large complex libraries. On page 75, Bradbury and colleagues describe a simple way of making phage libraries larger and more diverse using Cre-mediated recombination. They have created a phagemid library in which heavy- and light-chain genes were separated by two *lox* sites, and then introduced the library into Cre-expressing bacteria at a high multiplicity of infection. This led—surprisingly—to entry and recombination of many phagemids into a single cell, and production of many new functional heavy- and light-chain combinations.

A paper on page 62 describes a transgenic zebra fish system for detection of mutagens in polluted water. Amanuma and colleagues have designed a shuttle plasmid containing the *Escherichia coli rpsL* gene, which confers streptomycin sensitivity, and used it to transform zebra fish. When the transgenic zebra fish are exposed to waterborne mutagens, *rpsL* accumulates mutations that confer streptomycin resistance. The plasmid is then rescued from the zebra fish chromosomes and transferred to *E. coli*, a procedure that allows appraisal of the mutation rate by the number of colonies that form under antibiotic selection (see also p. 21).

Peptides binding peptides

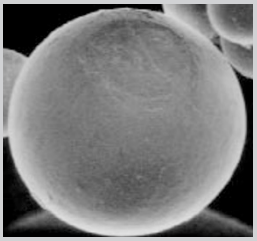
Recombinant antibodies have found numerous important applications as therapeutics and diagnostic reagents. However, they can be tedious and expensive to generate in very large quantities. In this issue, Kodadek and colleagues have studied the potential to generate peptide-binding peptides as alternative reagents to antibodies for diagnostic purposes (see p. 71). They first developed a genetic screen that allowed them to isolate peptides that could recognize their targets with high specificity. They then showed that the strengths of the peptide–peptide interactions, although much weaker than most antibody-antigen interactions, were sufficient to support immunoblot and affinity purification applications.

Alternative antisense



Antisense oligonucleotides are powerful tools to modify gene expression RNase H that exploit the cellular nuclease activity of RNase H. However, in some cases they have been shown to induce cleavage at sites other than the intended target. On page 58, Ma et al. have investigated the intracellular specificity of an alternative strategy that exploits Rnase P, an enzyme that normally cleaves the 5′ terminus of precursor tRNAs. A synthetic complementary oligonucleotide that mimics structural features of the precursor tRNA, termed the external guide sequence, was used to direct Rnase P to the PKC-α target gene. These EGSs were highly efficient in reducing expression of PKC-α protein and mRNA in cultured cells.

Buffering proteins against delivery problems



The ability to encapsulate drugs in biodegradable polymers has permitted the development of slow release systems for drug delivery in vivo. The extension of this delivery strategy to protein therapeutics involves additional considerations due to the instability of the fragile protein molecules in the polymer environment. In this issue, Schwendemann and colleagues examine factors leading to instability of proteins encapsulated in microspheres made from biocompatible polyesters made from lactic and glycolic acids (PLGA). They found that the acidic environment created by the gradual breakdown of the PLGA material was a prime factor leading to instability. Encapsulating a buffering agent along with the proteins was shown to reduce the acidic microclimate and enhanced the polymer stability of two therapeutic proteins (see pp. 24 and 52).

Technical Reports

Although matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) can readily detect mass differences of peptides, it is less sensitive for the detection of differences in base composition of DNA fragments. Garvin et al. have provided a way around this problem by first amplifying the DNA fragment to be analyzed with primers that contain sequences that allow subsequent transcription and translation of the DNA product into its peptide counterpart (see p. 95).

Reznikoff and colleagues describe an efficient in vitro method for inducing DNA transposition in bacteria and *Saccharomyces*. By mixing excised *Tn5* transposon with purified hyperactive transposase and electroporating the complexes into cells, they achieved high efficiency transposition while elimanting the need for in vivo expression of transposase and use of suicide vectors (see p. 97).

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An integrating adenoviral gene therapy vector.

A new chemistry for protease substrate libraries.

Stable transformation of an obligate fungal pathogen of barley.