Serving up unnatural proteins

One method for incorporating unnatural amino acids into recombinant proteins involves evolving new aminoacyl-tRNA synthetases (aaRS) that pair tRNA normally assigned to a stop codon with unnatural amino acids. These aaRS-tRNA pairs are orthogonal to all others in the cell and thus allow the incorporation of unnatural amino acids into the growing protein chain with high fidelity and efficiency. Schultz and colleagues previously demonstrated the utility of the system in bacteria and yeast, and in this issue now report the extension of the method to mammalian systems. This development should open up a new range of basic research experiments requiring protein labeling in mammalian cells, and could also have a substantial impact on industry and medicine. Article p239, News & Views p205

SLIC cloning

Cloning no longer needs to be confined to the insertion of one or two pieces of DNA into a vector; a new method by Li and Elledge facilitates the correct assembly of up to 10 fragments simultaneously. This sequence and ligation–independent cloning (SLIC) uses an exonuclease to generate single-stranded DNA overhangs in inserts and vector and relies on in vitro homologous recombination for the assembly of large intermediate molecules that efficiently transform

Target-decoy searching explained

The really difficult step in a proteomics study, peptide identification, begins after MS/MS data collection. Researchers use databases to compare their observed data to predicted peptide sequence patterns (called peptide-spectral matching) and must make inferences about the quality of the ‘match’. But this is far from straightforward for many peptides, and the matches are often ambiguous. Though there are several strategies available to help researchers gain confidence in their peptide identifications, one particularly powerful approach is the target-decoy search strategy. This approach allows researchers to estimate the false positive rate of peptide-spectral match identification, using a composite ‘target’ database appropriate to the proteomic sample, and a ‘decoy’ database containing the reversed target sequences. Elias and Gygi clarify their preferred methodology for target-decoy searching and make recommendations for tailoring the strategy for various types of experiments. Perspective p207