**Easier FRET for protein-protein interaction studies**

Fluorescence resonance energy transfer (FRET) is a powerful method for detecting protein-protein interactions in living cells. Unfortunately, obtaining a strong and specific signal that does not interfere with the interaction under investigation requires more than simply fusing an appropriate donor fluorescent protein to a putative interactor and an acceptor fluorescent protein to its partner. Grünberg, Serrano and their colleagues describe two rational protein design strategies for incorporating weak helper interactions into fluorescent protein pairs. These interactions help ensure optimal FRET signals while minimizing undesirable side effects. One strategy in particular is simple and general enough for wide use, and the authors demonstrate its effectiveness by studying the interaction of two different protein pairs.  
**Article p1021**

**Single-gene mutant human cells**

Collections of single-gene knockout strains in model organisms such as yeast have proven invaluable for the study of gene function. Nijman, Brummelkamp, Superti-Furga, Bürckstümmer and colleagues report a collection of over 3,000 isogenic human cell lines, each with a mutation in a single gene. The Resource is based on the stable, almost-haploid KBM7 cell line and is generated using retroviral gene-trap insertional mutagenesis. The mutant alleles are barcoded and in most cases reversible. As demonstrated on several examples of disease-related genes, this growing collection will provide a rich resource for functional studies of biological processes in human cells.  
**Resource p965**

**Better microbial marker-gene sequencing and analysis**

Sequencing the gene encoding 16S ribosomal RNA is an established way to survey microbes in the environment. But amplifying this gene in preparation for sequencing introduces biases, sequence changes and artifacts that hurt the efficiency and accuracy of the approach. Dangl and colleagues offer improvements to 16S sequencing, which they demonstrate on microbial communities associated with plant tissues. They show that adding unique molecular barcodes to every copy of 16S before amplification limits quantification bias and flags erroneous sequences. The researchers also use peptide nucleic acids to enrich microbial reads by capturing and removing related sequences from mitochondria and chloroplasts of the host. Also in this issue, Edgar profiles sample diversity by clustering 16S sequence reads into operational taxonomic units (OTUs). His UPARSE-OTU pipeline filters out many artificial sequences, notably the chimeric reads that are often generated during amplification, to give a more accurate characterization of community composition than other current methods provide.  
**Brief Communications p996, p999**

**How to do an EWAS well**

To understand the impact of epigenetic variations on development and disease, one needs an unbiased, epigenome-wide approach. But epigenome-wide association studies (EWAS) are plagued by many confounding factors such as differences in DNA methylation between different tissues within a sample (and even between different cell types in the same tissue), population structure and high biological variability. Michels, together with other leading researchers in epigenetics, now propose recommendations for standardized EWAS design, including the selection of appropriate protocols, tissues and controls as well as data analysis with appropriate statistics and approaches for data interpretation. The authors stress the importance of verifying and validating results, and they caution that inferring causality is difficult because, unlike the genome, the epigenome of a cell can change over time.  
**Review p949**