Pick your Poisson

Many scientists, particularly those using light to probe processes at the cellular or single-molecule level, count events and fit them to a distribution. These data are almost always best described by a Poisson distribution, but out of convenience, scientists invariably use a Gaussian distribution as an approximation. But the increasing importance of accurately fitting curves to photon-counting histograms, mostly for localizing individual fluorophores, has expanded interest in fitting these distributions as accurately as possible. Several articles in this issue of *Nature Methods* describe the use of the maximum-likelihood estimator (MLE) for fitting photon distributions and show how this outperforms the popular least-squares Gaussian estimator. New software tools implementing the MLE greatly reduce the practical hurdles that have impeded the use of this preferred fitting method.

Brief Communications p373, p377, Correspondence p338, News and Views p357

Adding to the reference genome

Sometimes an old-fashioned approach yields the best results. A case in point, in this issue, is Eichler’s analysis of fosmid clones that represent the genomes of nine human individuals by Sanger sequencing. The long Sanger reads can span repetitive regions in the genome and anchor clones that contain areas of high structural diversity in known sequence. The researchers added 1.6 megabases of sequence that are not currently contained in the human reference genome to hundreds of genomic loci. Not only will this be an interesting resource, it also outlines a strategy to analyze the more repeat-rich regions in the human genome.

Resource p365, The Author File p333

Super-SILAC

Stable-isotope labeling by amino acids in cell culture (SILAC) is a widely used method in quantitative proteomics. Proteins in one sample are metabolically labeled with ‘heavy’ versions of lysine and arginine, and another sample is left unlabeled; two samples can thus be analyzed together via mass spectrometry and quantitatively compared with high accuracy. However, SILAC has been limited to single-cell organisms and small animals, which can be metabolically labeled, precluding application to human tissues. Mann and colleagues now describe a twist to quantify proteins in human tumor tissues. Reasoning that a tumor proteome is likely to be fully represented by a mixture of immortalized cell lines, they combine several heavy isotope-labeled cancer cell lines in a ‘super-SILAC mix’. This serves as an internal quantitative standard for the unlabeled tissue specimen. Super-SILAC should lead to new insights in tumor biology.

Brief Communication p383, News and Views p361

Engineering temperature-sensitive expression

A classical system for obtaining conditional gene activity in model organisms is the use of temperature-sensitive alleles, but such alleles are not available for many genes. Chalfie and colleagues now report a way to engineer temperature-sensitive alleles in *Caenorhabditis elegans* for any gene for which a loss-of-function allele is available. Splicing, and therefore expression, of a gene of interest can be made dependent on the RNA processing factor MEC-8, and the use of temperature-sensitive *mec-8*, in turn, renders gene expression temperature dependent. The researchers apply this approach to engineer a temperature-sensitive allele of *mec-4* and to produce temperature-sensitive RNA interference in several tissues.

Article p407

Genotyping RNA molecules

Rare transcripts can have profound effects in cells, but population-based assays lack the sensitivity to detect them. Instead, one needs assays with single-cell, and preferentially even single-molecule, sensitivity. Several hybridization-based methods allow in situ detection of single mRNA molecules, but they cannot be used to distinguish between highly related transcripts. To accomplish this, Nilsson and colleagues used highly selective padlock probes—molecules that are converted into circular probes upon hybridization to the target—to detect cDNA molecules that are localized to the primary transcript. The researchers demonstrate detection of transcripts that differ in only a single base, and they determine the allelic ratio between a wild-type and mutant transcript.

Brief Communication p395