# METHODS IN BRIEF

GENETICS

## Multiplex mouse genetic engineering with CRISPR

The bacterial clustered, regularly interspaced, short palindromic repeats (CRISPR)/Cas9 system can be engineered to achieve site-specific DNA cleavage in several experimental systems. Wang *et al.* now demonstrate its use for multiplex gene modification in the mouse, performing the simultaneous mutagenesis of eight alleles of five genes (two of the genes being on the Y chromosome) in mouse embryonic stem cells. They further showed that the CRISPR/Cas9 system can be used via direct injection into mouse zygotes to rapidly generate animals with biallelic mutations in two genes. Finally, by supplying donor sequence with a single-stranded oligonucleotide, they demonstrated the precise biallelic insertion of short sequences in two genes via homologous recombination. The CRISPR/Cas9 system should greatly improve the efficiency of genetic engineering in the mouse.

Wang, H. *et al*. *Cell* **153**, 910–918 (2013).

CELL BIOLOGY

## Tissue molding in the dish

Tissue engineering of complex organs such as liver, kidney and heart remains challenging owing to the hierarchical arrangement of tissue compartments. To construct complex engineered tissues in the dish, and particularly for potential clinical applications, one needs technologies that enable multicellular patterning and that are scalable. Stevens *et al.* report a method called Intaglio-Void/Embed-Relief Topographic (InVERT) molding that is compatible with several hydrogel systems and can be used to pattern different types of cells into specific compartments and build large, complex engineered tissues. They used InVERT to organize primary hepatocytes (including human induced pluripotent stem cell–derived hepatocytes), fibroblasts and endothelial cells and to reproduce the microstructure and multicellular composition of liver tissue. The engineered hepatic structures displayed native function *in vitro* and good viability after *in vivo* transplantation in rodents.

Stevens, K.R. *et al. Nat. Commun.* **4**, 1847 (2013).

GENOMICS

## Profiling transcriptional heterogeneity

Full appreciation of a genome's functional repertoire requires looking at all the isoforms transcribed from each gene—a challenging endeavor. Pelechano *et al.* reasoned that to profile isoform diversity, the 5′ and 3′ ends of every transcript needed to be retained on the same sequence read. To accomplish this, they developed transcript isoform sequencing (TIF-seq). They attached a biotin tag to the 5′ end of full-length cDNA from *Saccharomyces cerevisiae*, circularized each molecule before fragmenting and then isolated the joint 5′-3′ end via the biotin tag. Deep sequencing revealed surprising isoform diversity: for every protein-encoding gene, they saw evidence of extensive alternative splicing, often affecting post-transcriptional regulation. And for the majority of genes, they detected variations in transcript boundaries.

Pelechano, V. *et al*. *Nature* **497**, 127–131 (2013).

STEM CELLS

## Functional thymic progenitors from stem cells

Human pluripotent stem cells can in principle differentiate into any somatic cell type, but mature functional cells have been generated in only a few instances. Thymic epithelial cells (TECs) are of substantial interest for many applications: they are required for T-cell development and are therefore important both for immune function and for establishing self-tolerance. Parent *et. al* achieved directed differentiation of human embryonic stem cells into thymic epithelial progenitors by mimicking *in vitro* the signals operative during *in vivo* thymus development. Upon implantation under the kidney capsule of athymic nude mice, these cells matured further into TECs and supported the development of functional mouse T cells.

Parent, A.V. *et al. Cell Stem Cell* doi:10.1016/j.stem.2013.04.004 (16 May 2013).