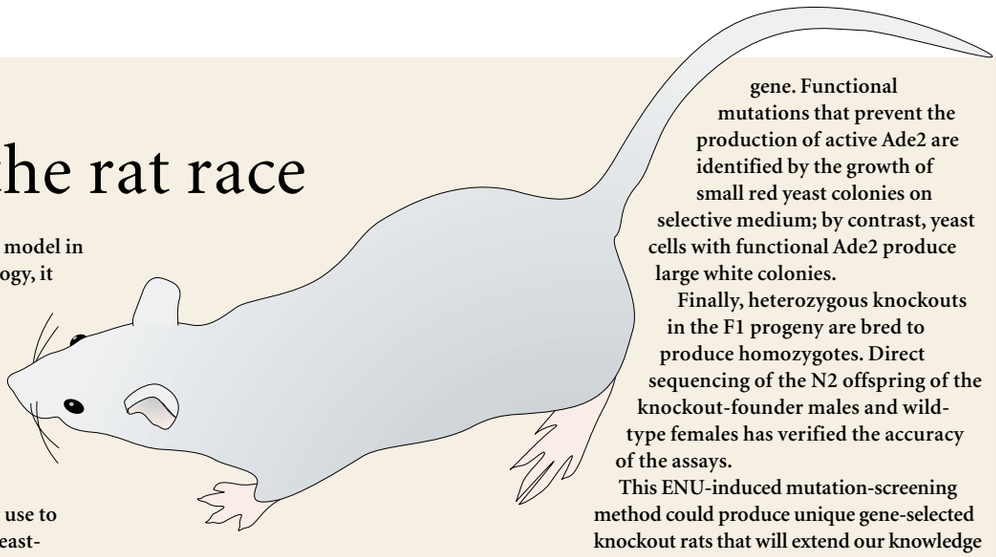
TECHNOLOGY

Winning the rat race



Although the rat is an important model in studies of behaviour and physiology, it lags behind the mouse in the genetic popularity stakes, as rat embryonic stem cells cannot be used to produce gene-disrupted knockout lines.

However, this is about to change, as Zan *et al.*, in *Nature Biotechnology*, describe an alternative approach, which they use to produce rat knockouts for the breast-cancer supressor genes *Brca1* and *Brca2*. Their innovative technique combines rat germline mutagenesis using *N-ethyl-N*-nitrosourea (ENU) with yeast truncation assays that screen for functional mutations in selected genes.

Initially, male rats are mutagenized and bred with wild-type females. The F1 pups are then screened for functional mutations in the gene of interest, using two related yeast truncation assays — one based on genomic DNA and the other on RNA that has been reverse transcribed to cDNA.

Whole-gene sequences or large fragments from the target gene are cloned into gap-repair vectors that are then transformed into competent *Saccharomyces cerevisiae* yIG397 cells. Once incorporated, the gene fragment is cloned behind the yeast *Adh1* promoter and in front of the *Ade2* reporter gene. Functional mutations that prevent the production of active Ade2 are identified by the growth of small red yeast colonies on selective medium; by contrast, yeast cells with functional Ade2 produce large white colonies.

Finally, heterozygous knockouts in the F1 progeny are bred to produce homozygotes. Direct sequencing of the N2 offspring of the knockout-founder males and wild-type females has verified the accuracy of the assays.

This ENU-induced mutation-screening method could produce unique gene-selected knockout rats that will extend our knowledge of the genetics that underlie human diseases and aid in drug development. So, in the future, rat models might give knockout mice a good run for their money.

Victoria Kitchener

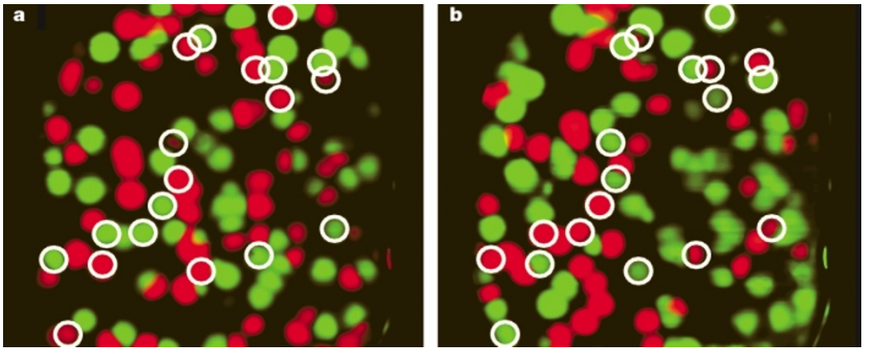
References and links

ORIGINAL RESEARCH PAPER Zan, Y. *et al.* Production of knockout rats using ENU mutagenesis and a yeast-based screening assay. *Nature Biotechnol.* **21**, 645–651 (2003)

FURTHER READING Jacob, H. J. & Kwitek, A. E. Rat genetics: attaching physiology and pharmacology to the genome. *Nature Rev. Genet.* **3**, 33–42 (2002)

TECHNOLOGY

Spot the SNPs



Genotyping of two SNPs in a single DNA sample. In the reaction for the first SNP (**a**), green signals indicate thymine residues and red signals indicate adenine residues; for the second SNP (**b**), green indicates thymine and red indicates cytosine. Haplotypes can be determined when the signal from two genotyping reactions overlaps (white circles). Image courtesy of R. Mitra, Washington University School of Medicine.

There is increasing evidence that, when located *in cis*, specific combinations of single nucleotide polymorphisms (SNPs) affect an individual's susceptibility to disease or treatment response. Methods that are suitable for haplotyping in the clinic are needed for the benefits of these results to reach patients. Computational methods for determining haplotypes are prone to errors, whereas direct haplotyping procedures are time consuming and expensive, or cannot be applied to distant SNPs. Now, Mitra *et al.* present an elegant new haplotyping method that overcomes these limitations. Indeed, they show that it can be used to successfully determine the genotype and phase of SNPs that are separated by as much as 45 kb.

The authors immobilized a dilute sample of patient DNA on a microscope slide in a thin acrylamide gel, which contains all the reagents necessary for a polymerase chain reaction (PCR). Two primer pairs incorporated in the gel allowed two SNPs of interest to be amplified. The PCR products — referred to as polonies — are immobile because the primers are modified such that one strand of the amplified DNA is covalently linked to the acrylamide matrix. So, DNA amplified by different primer pairs, but from a single DNA molecule, co-localizes. The SNPs are genotyped, one at a time, by a single base-pair extension method that uses fluorescently-labelled oligonucleotides. After each genotyping reaction the slide is scanned and the genotype of each polony is recorded digitally. The images from different reactions are merged and a computer algorithm identifies haplotypes based on the genotypes of overlapping polonies.

This is a promising method for SNP haplotyping. It has the potential to be used in the clinic, as a buccal swab provides sufficient DNA to make one slide, which can be reused for multiple genotyping reactions. This tool will also be useful for researchers interested in characterizing genetic variation, as it can be used to determine allele and haplotype frequencies in a population from pooled DNA samples.

Catherine Baxter

References and links

ORIGINAL RESEARCH PAPER Mitra, R. D. *et al.* Digital genotyping and haplotyping with polymerase colonies. *Proc. Natl Acad. Sci. USA* **100**, 5926–5931 (2003)

WEB SITE

Harvard-Lipper Center for Computational Genetics: http://twod.med.harvard.edu