The making of ‘knock-out’ rats by target-selected mutagenesis

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The rat is currently the most widely studied experimental animal in biomedical research. Despite the wealth of knowledge that is now available on rat physiology, pharmacology, toxicology and behaviour, there is a wide gap in the understanding of genetics of nervous system function and regulation of behaviour. Although the rat genome has now been sequenced and some technologies are available for manipulating the rat genome, the possibility to generate so-called ‘knock-out’ animals using embryonic stem cells has not been successful for the rat.
We aim to generate knock-out rats by target-selected mutagenesis as a reverse genetics approach. ENU-mutagenesis conditions were optimized for the rat and male animals treated with ENU (3 x 35, 40 mg/kg, once a week) were crossed, when fertile, with wild-type females to generate a cohort of F1 animals that are expected to be heterozygous for many independent random point mutations. The mutation detection technology involves high-throughput amplification of target genes, followed by endonuclease (CEL-I)-mediated heteroduplex DNA cleavage, and subsequent re-amplification and resequencing of the target genes from genomic DNA to confirm potential mutations (for details see http://www.niob.knaw.nl/researchpages/cuppen/protocols.html). Mendelian inheritance of possible germ-line mutations is established by outcrossing. After the mutants have been bred to homozygosity they will be phenotyped in extensive behavioural screens to establish as to whether the mutations have functional consequences.
Currently, the mutant F1 animals are kept as a living stock and are used in a systematic forward genetic screen for behavioural phenotypes (see poster of Olivier et al.), but we ultimately want to create a permanent resource. To this end, we are implementing the rat sperm cryopreservation method of Yasushi Okuda (Azabu University Sagamihara, Japan). By means of intra-uterine insemination we are able to rederive live offspring from fresh sperm and are currently optimizing conditions to rederive live offspring from thawed sperm.

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