

RAT AND OTHER GENOMES**ORAL PRESENTATION****MONDAY NOVEMBER, 13****1.30PM - 1.45PM****07****EURATOOLS: AN EU INTEGRATED PROJECT - EUROPEAN FUNCTIONAL GENOMIC TOOLS FOR THE RAT**X M Fernandez¹, N Hubner², J Mullins³, M Pravenec⁴, E Birney¹, A Dominiczak⁵, E Werner⁶, T Aitman⁶

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Determining the molecular basis of natural phenotypic variation, including inter-individual susceptibility to common diseases, is a central challenge of post-genome genetics. The laboratory rat (*Rattus norvegicus*) is the animal model of choice for physiology, pharmacology, toxicology and study of human diseases. Hundreds of quantitative trait loci (QTLs) containing genes that confer susceptibility to complex disease phenotypes have been localised. However, few genes underlying these genetically complex traits have as yet been identified.

The EURATools Consortium draws together leading researchers in rat genetics, pharmacology, toxicology, disease pathophysiology, genome biology and informatics. The project aims to develop integrated genome tools that will generate knowledge which can be translated into improvements in healthcare.

The EURATools aims will be achieved by integrating informatics with every aspect of the project's activities which include: high-throughput sequencing and genotyping for development of a dense single nucleotide polymorphism (SNP) and ultimately a haplotype map; intensive analysis of common disease phenotypes; investigation of gene sequence and gene expression in congenic strains to identify genes and regulatory pathways underlying complex rat disease phenotypes; optimising protocols for rat gene targeting and integration with new and existing data for rat cloning, and to develop new tools that will increase the utility and accessibility of rat genome databases. The Project is structured in six activity areas:

1. Genome Tools - SNP Detection and Map Construction.
2. Nuclear Transfer.
3. Biological Resources and Toxicogenetics.
4. Genome Informatics.
5. Gene Expression Analysis.
6. QTL Gene Discovery and Applications of the Rat Model to Human Disease.

**RAT AND OTHER GENOMES
ORAL PRESENTATION****MONDAY NOVEMBER, 13 1.45PM - 2.00PM****O8****THE PIRC RAT, A COMPLEMENTARY GENETIC MODEL FOR HUMAN COLON CANCER**

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The mouse has proven to be a strong genetic model to study the development of cancer; however, several limitations of the mouse, including size, lifespan, and genome organization hinder the mechanistic study of prolonged tumor development. As a complementary approach to modeling intestinal tumor development in the mouse we have turned to the rat model system. We have developed an isogenic knockout rat model of human colon tumor development by generating a nonsense mutation in the rat adenomatous polyposis coli (*Apc*) gene through ENU mutagenesis. Intriguingly, the rat differs from the mouse in several ways: the tumor distribution in the rat is shifted towards the colon, the tumor-bearing rat lives in excess of one year, and the colon tumors can grow to 1 cm in size. These differences, in addition to the rat's larger size, allow us to better follow tumor progression through both micro CT imaging and endoscopy. Quantitative allele specific analysis of tumors in the isogenic F344 line by pyrosequencing indicates that >90% of tumors show loss of heterozygosity of the wild-type allele. The metacentric rat karyotype, rather than the acrocentric mouse, has allowed for the full examination of the chromosomal mode of loss of the wildtype *Apc* allele. Analysis of [F344xWF] F1 *Pirc*/+ animals has revealed arm-specific loss of heterozygosity, strongly supporting an underlying, genomically conservative mechanism of somatic recombination. Thus we find the Pirc rat provides a model that is complementary to the Min mouse model in important physiological, genetic, and genomic ways.

RAT AND OTHER GENOMES**ORAL PRESENTATION****MONDAY NOVEMBER, 13****2.00PM - 2.15PM****O9****CONFIRMATION OF MAJOR QTL ON THE X CHROMOSOME INFLUENCING COPING BEHAVIOR IN WKY AND F344 RATS USING CONGENIC STRAINS**EE Redej, KL Debus, F Aird, N Ahmadiyah

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Wistar Kyoto (WKY) and Fisher 344 (F344) rats differ in anxiety/coping behavior in the defensive burying (DB) paradigm with WKY rats showing passive, and F344 active behavior. Previous genetic mapping studies identified *Stresp1* on the proximal part of chromosome X that explained 6.7 and 6.2 % of the genetic variance of latency to bury and duration of burying, respectively; phenotypes related to anxiety. We constructed a congenic strain, F344.WKY-DXRat64:DXRat21, by introgressing a 121.6 Mb region of the proximal WKY X chromosome onto a F344 background. Mapping confirmed and narrowed the *Stresp1* locus for duration and identified a new coping locus 33 Mb distal to *Stresp1*. This latter significant *duration* and suggestive *latency* locus overlapped at DXRat50. At DXWox17, within the *Stresp1* locus, males with WKY allele showed significantly shorter duration to bury ($F[1,35] = 10.24, p = 0.003$). At the new locus, DXRat50, WKY allele decreased the duration of burying as well ($F[1,39]=14.2, p<0.001$). In the elevated plus maze test of anxiety there were no effects of the WKY X chromosomal region, but grooming of congenic rats were significantly different from those of F344, but not from WKYs ($F[2,32]=36.58; p<0.001$). These latter results are intriguing, as we did not detect a grooming QTL on the X chromosome previously. Overall, these data confirm the existence of genes on the X chromosome at regions DXWox17 and DxRat50 that have strong and specific influence on the difference in duration in the DB between WKY and F344 rats.

**RAT AND OTHER GENOMES
ORAL PRESENTATION****MONDAY NOVEMBER, 13****2.15PM - 2.30PM****O10****RAT GENOME DATABASE DISEASE PORTALS: A PLATFORM FOR GENETIC AND GENOMIC RESEARCH**

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The Rat Genome Database Disease Portals provide a comprehensive research platform through the integration of heterogeneous datasets into the context of the genome using multiple ontologies and sophisticated data mining and visualization tools. The Disease Portals provide both the novice and experienced user with easy access to a comprehensive, integrated knowledgebase that can be tailored to the particular interests of the user. In addition, these initiatives define the focus and scope for data acquisition and curation projects. Current and proposed components of the Disease Portals include: 1) comprehensive rat, human and mouse gene sets associated with diseases, related phenotypes, pathways and biological processes; 2) all rat QTLs related to the disease area as well as associated mouse and human QTLs; 3) strains used as disease models; 4) phenotype data; 5) related references; 6) expression data; 7) genome-wide view of disease genes and QTLs via GViewer; 8) comparative maps of disease related regions, 10) customization of datasets and download options; 11) analysis and visualization of function and cellular localization makeup of gene sets. These portals are designed to highlight genetic and genomic data generated from rat research in diseases related to the cardiovascular, nervous, musculoskeletal, digestive, endocrine and immune systems as well as metabolic diseases and cancer.

**RAT AND OTHER GENOMES
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MONDAY NOVEMBER, 13

2.30PM - 2.45PM

O11

ADVANCES IN TRANSGENIC RAT PRODUCTION

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The rat is an important animal model for physiology, cancer, and pharmacological research. The use of transgenic technology to overexpress proteins in a cell specific fashion promises to further increase the value of rat models of human disease. We made three Sprague-Dawley CrI:(CD)SD and four Fischer 344 F344/NHsd transgenic rat models by DNA pronuclear microinjection. For example, transgenic F344 rats expressing the human diphtheria toxin receptor were developed to model human kidney disease (focal segmental glomerulosclerosis, Wharram et al., 2005. J Am Soc Nephrol. 16:2941).

Superovulation, microinjection, and pseudopregnant recipient preparation methods were evaluated to improve transgenic rat efficiency. Five superovulation treatments were compared. The most effective treatment was 30 IU PMSG followed by 20 IU HCG. Two microinjection needle geometries were compared. Needles longer and thinner than conventional needles produced the best egg survival rates. Four methods for preparing pseudopregnant recipients were compared. The preferred method combined LHRH agonist estrus synchronization and mating with vasectomized males. Compared to usual methods, our optimized procedures reduced the numbers of animals required for transgenic rat production.

Anecdotes suggest rat transgenesis is less efficient than mouse transgenesis. We observed that 1.9% of microinjected SD eggs and 1.4% of F344 eggs developed into transgenic founders. These rates compare favourably to C57BL/6J mice (1.0% of injected eggs developed into founders). We expect that transgenic units experienced in C57BL/6 transgenesis will find little difficulty in preparing transgenic rats. The combination of transgenic technology with extensively characterized rat genetic backgrounds will increase our ability to model human diseases.