A knockout resource for mouse genetics

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An international consortium of researchers report today in *Nature* that they have knocked out almost 40 per cent of the genes in the mouse genome. The completed resource will power studies of gene activity in models of human disease.

The results are founded on a novel, efficient production line that is able to target each specific gene in turn. The consortium has cracked all the challenges of generating mutations of each gene in mouse embryonic stem cells, and has already knocked out 9,000 genes in the mouse genome as part of an international effort to knockout all 21,000. This developing resource will be essential in our understanding of the role of genes in all mammals - including humans.

The cells generated by this approach will allow researchers to ask and answer questions about the roles of genes at the scale of the whole mouse and human genome. The gold-standard method to uncover that role is to mutate a gene in mouse embryonic stem cells: the biochemical and developmental behaviour of the mutated cells can be studied in test tubes or in mice. Until this production system was developed, conducting gold-standard research on this scale was impossible.

The problem to be overcome was: how do you scale this approach to tackle the whole mouse genome?

"We have pioneered novel methods that enable us to deliver the most complex and accurate high-throughput functional genomics platform yet attempted," says Dr Bill Skarnes, Wellcome Trust Sanger Institute

researcher and lead author of the study. "We believe that our work raises the standards of achievement and expectation for genome-scale programmes.

"It is an investment for the future: the genome-engineering technologies developed here for the mouse will drive future model systems, including work on human stem cells."

Genomics was transformed in the 1990s from individual-based research to large-scale commodity resources: an equivalent success was needed for mouse mutagenesis - to provide resources efficiently and consistently and to release them freely. Previously attempted strategies to develop mouse models on a large scale suffered the twin disadvantages of not producing precise genetic changes and favouring only the genes that were active during the experiment, leaving the remainder unaltered.

The present work solves these problems. The team exploited a system called homologous recombination within mouse embryonic stem cells, which can deliver very precise alteration of any gene in the genome. It is founded on choosing the correct recombinant DNA molecules (vectors) to target genes efficiently.

However, some genes are essential to life of the cell or organism: disruption of these might cause the cell to die and so the mutation would be 'lost' from the project. Crucially, to ensure that all genes can be disrupted, the team developed DNA vectors that create a mutation only when required: gene targeted by the mutation can be identified, but the mutation activated only when it is to be studied.

But in the essential step to realize its ambitions of a comprehensive, freely available resource, the team designed and delivered a 'pipeline' that systematically designs and constructs the vectors, and efficiently introduces the engineered DNA molecules into the mouse embryonic

stem cell line developed specifically for these projects.

Finally, by employing a modular approach to the vector design, a number of other valuable resources are created en route to the generation of targeted ES cells: the paper reports that the consortium had produced vectors for more than half of the genes in the mouse genome. All of these outputs are being made available to the mouse research community through the consortium's web portal at http://www.knockoutmouse.org/

"We are producing mutations in embryonic stem cells with greater efficiency and speed than we predicted and at well above the historical average," says Allan Bradley, senior author of the study and Director Emeritus of the Wellcome Trust Sanger Institute. "We have taken careful steps to ensure we deliver quality resources of maximum utility that will stand the test of time. Indeed, we expect our systems will be increasingly adopted by researchers using human and other cells to seek advances in the understanding of disease."

The methods the team have developed will also accelerate studies on human stem cells - cells that have the potential to grow into many different types of adult tissue. Research into producing such induced pluripotent stem cells from adult tissues (forgoing the need for embryonic stem cells) is expected to be vital in understanding human disease and therapies. The systems developed for mouse stem cells are transferable to human cells and could drive research into mutation in the human genome and its biological and medical consequences.

"Biomedical research needs biological resources on a scale that match genomics resources," explains Colin Fletcher, Ph.D., Program Director of the Knock Out Mouse Program at the National Institutes of Health, a part of the international knockout effort. "Such knockout resources are the foundation for producing thousands of valuable mouse mutants for future large-scale international phenotyping programmes and will serve

the biological and biomedical research community worldwide."

More information: Skarnes WC et al. (20110 A conditional knockout resource for the genome-wide study of mouse gene function. Nature, published 16 June 2011 doi:10.1038/nature10163

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