charles river | research models

White Paper

The C57BL/6 Mouse

The Role of the C57BL/6N Mouse in the Creation of Future Genetically Engineered Models

Rosalba Sacca, PhD, Director, Scientific Development, Charles River
Bruce Elder, PhD, Director, Corporate Rodent Genetics, Charles River
Katherine Wasson, DVM, PhD, Associate Director, Mouse Biology Program, University of California, Davis

Introduction

Among animal species, the laboratory mouse has been instrumental in modeling human biology and disease states. The isolation of mouse embryonic stem (mES) cells in 1985 (Doetschman et al., 1985; Smithies, O. et al., 1985) and the subsequent ability to direct pre-planned mutagenesis of the murine genome by homologous recombination in the mES cells (Thomas et al., 1986; Kuehn et al., 1987), has allowed this seminal technology to address all aspects of mammalian biology. More recently, the availability of extensive information on mouse genetics, the use of inbred ES cell lines which significantly reduce the time to develop inbred knockout (KO) mice, and advances in molecular biology have made genetically engineered mouse models (GEMM) a valuable tool in understanding biological functions. In addition, KO mice have been used extensively in the drug discovery process.

Over the last 20 years, thousands of KO mice have been developed worldwide, providing drug discovery scientists with access to this critical resource to investigate the role of potential drug targets. KO mice have become an invaluable tool for determining target function, selectivity and potential toxicity liabilities (Sacca et al., 2009). For example, retrospective evaluation of KO phenotypes were shown to have a close correlation with the therapeutic effects on the targets of the 100 top-selling drugs (Zambrowicz et al., 2003), clearly demonstrating the value of applying KO mice in the validation of novel targets.

Recognizing the value of these mouse knockouts and their potential in facilitating the study of human disease, several international programs were launched in 2006 in the United States, Europe and Canada, with the intent to knockout every protein coding gene in the mouse genome (~21,000). These efforts have been conducted using a standardized C57BL/6N embryonic stem cell line, given the many advantages of using an inbred ES cell strain in GEMM production, as well as the marked differences in assay responses that can be observed when using different genetic backgrounds.

The intent of this paper is to provide a summary of the current efforts of these consortia as well as the best practices that have been put in place – such as the use of the C57BL/6N strain– to ensure the standardization of the mouse production and phenotyping data that will result from these programs over the next few years.

History and Role of the C57BL/6 Model

The C57BL/6 inbred strain was developed starting in 1921 by Clarence Little at the Jackson Labs. At that time, a “black subline” (C57BL) and a “brown subline” (C57BR) were established and bred independently. The C57BL subline was further separated into two sublines designated “subline 6” and “subline 10.” These sublines would eventually give rise to the C57BL/6 and C57BL/10 inbred stains we know today. At the time they were separated, the C57BL subline had been inbred to a high degree and it was thought that no other strains under development had contributed to this subline.

Over the next several decades, hundreds of other inbred strains were developed and selected for traits of interest. For example, the C57BL/6 strain shows an increased preference for narcotics and alcohol, so is preferentially used for genetic studies of substance abuse (Peirce et al., 1998). The real expansion of the mouse as a tool for genetic analysis came with the advent of recombinant DNA

askcharlesriver@crl.com
www.criver.com

techniques during the 1980s. Due to relatively robust breeding performance (when compared to many other inbred strains) and confidence in the strain origin, investigators used the C57BL/6 strain as the basis for large-scale biological resources such as genomic libraries.

Availability of resources such as this in turn drove the expansion of the use of the B6 strain for other endeavors. During the late 1980s, various groups led by Martin Evans, Mario Capecchi and Oliver Smithies created the first knockout mice. While the embryonic stem cells used to introduce the mutations were from the 129 inbred strain background, most lines were quickly crossed onto the B6 background for further expansion and analysis. By the mid 1990s, many engineered mutants had been generated by the scientific community, further expanding the B6 subline as the genetic origin for this type of biological resource.

In 1999, as part of the Human Genome Project, three major sequencing centers formed a consortium to undertake sequencing of the mouse genome. There was a good deal of debate in the scientific community regarding which mouse strain should be used to construct the bacterial artificial chromosome (BAC) library that would serve as the basis for this effort. It was ultimately decided that the C57BL/6 strain would be used, due in part to its extensive use in the development of genetically engineered models up to that point and its breeding characteristics and relatively well-documented history (Battey et al., 1999). In 2002, the first publicly available genomic sequence of the mouse was published (Waterston, 2002). This sequence was based on the J substrain of C57BL/6 and continues to serve as the index reference for other mouse sequences now available.

One consequence of the popularity of the C57BL/6 strain has been the development of many substrains around the world. While all referred to as C57BL/6, substrains are distinguished from one another by the series of letters (called lab codes) following the C57BL/6 designation. For example, the primary B6 substrains raised by the major vendors (Charles River, Taconic, Harlan and Jackson Labs) are designated C57BL/6NCrl, C57BL/6NTac, C57BL/6NHsd and C57BL/6J, respectively. In the case of the Crl, Tac and Hsd substrains, the “N” indicates that these populations were originally raised at the National Institutes of Health (NIH). It is extremely important for researchers to note substrain designations, since populations raised in isolation will change genetically over time due to genetic drift. These changes can, and have, resulted in phenotypic differences between many of the C57BL/6 substrains available today (Bothe et al., 2004; Bryant et al., 2008; Mulligan et al., 2008; Mekada et al., 2009; Zurita et al., 2011).

While it is often not known if single nucleotide polymorphism (SNP) variation between substrains is associated with physiological or behavioral differences, there are some mutations known in genes for several of the substrains. Many C57BL/6J substrains carry a mutation at the nicotinamide nucleotide transhydrogenase (Nnt) locus, while N substrain mice are wild-type at that locus (Freeman et al., 2006; Mekeda et al., 2009). To date, C57BL/6JOlaHsd is the only substrain known to have a mutation in the alpha-synuclein (Snca) gene (Specht and Schoepfer, 2001), and all N substrain C57BL/6 mice tested thus far have a mutation called retinal degeneration 8 (rd8) in the crumbs homolog 1 (*Crb1*^{rd8}) gene (Mattapallil et al., 2012). Each of these mutations is associated with phenotypes that may or may not impact particular lines of research, and it is important for investigators to be aware of them.

Because of these known mutations, it is important for researchers to obtain accurate substrain information from collaborators for genetically engineered models with C57BL/6 backgrounds. Further, due to the unknown impact of substrain SNP variation, it is advisable for researchers to standardize any breeding using wild-type C57BL/6 mice to the appropriate substrain, and continue to supply their colonies with that line.

Model Creation Industry

With the impending publication of the complete mouse genome (Waterston, R.H. et al., 2002), members across multiple fields in the international scientific community gathered to discuss the opportunity to produce a consistent, uniform and publicly available collection of genome-wide knockout mice (Austin, Batty et al., 2004; Auwerx, Avner et al., 2004). Motivation for coordinated development of this resource included uniformity in the creation, availability and distribution of resources developed; decreased overall cost to the research community through economy of scale; reduction in the time to produce data through ready availability of experimental tools; and providing a baseline of tools and resources that would serve future scientific endeavors.

These scientists suggested that consideration should be given to technology and automation that allowed for high-throughput embryonic stem cell (ESC) production; reanimating a percentage of ESC into live, germline-tested mice for proof-of-concept and quality control/quality assurance; reporter tissue analysis to verify and discover sites of gene expression; and tier one phenotyping and transcriptome analysis to further prime the research pump and stimulate lines of hypothesis-driven questions regarding gene function. They also recommended these activities be coordinated at an international level to avoid duplication of effort and to maximize output across countries and organizations.

To address the latter suggestion, the International Knockout Mouse Consortium (IKMC) was established (Collins, FS and Rossant, J, 2007). Membership was comprised of the FP6 program of the European Commission, Genome Canada, the NIH and the Texas Institute for Genomic Medicine (TIGM). The IKMC supported the former suggestions of these scientists by providing funding and coordinated oversight to convert the published mouse genome into resources that are now becoming available to the scientific public. Within the United States, the National Institutes of Health (NIH) developed several request for applications (RFA) to support model creation opportunity. This included an RFA that resulted in the creation of an agouti C57BL/6N embryonic stem cell line that has been the basis for model creation within the IKMC (Pettitt, SJ, Liang, Q, et al., 2009). A second RFA launched what is now referred to as the Knockout Mouse Project (KOMP) Phase 1. This provided funding for the creation of a library of ~8500 C57BL/6N targeted deletion or targeted conditional ESC, and initiated production of the first 500 live germline-tested, PCR-verified knockout mice. A third RFA provided funding for the establishment of the KOMP Repository. This repository serves to collect and archive the mutant alleles generated by the KOMP and includes cryoarchived constructs, targeted ESC, embryos, sperm and live mice for distribution to the scientific community.

The IKMC website (www.knockoutmouse.org) tracks the production of vectors, ESC and live mutant mice produced by not only the NIH-funded KOMP initiatives, but also the European Commission's European Condition Mouse Mutagenesis Program (EUCOMM) and Genome Canada's North American Conditional Mouse Mutagenesis Program (NorCOMM). This website also provides additional information regarding the resources, publications and links to where and how resources can be obtained.

More recently, in September 2011 a new international effort to systematically record and disseminate the phenotype of 20,000 knockout mice was launched. The consortium consists of 22 research institutes across four continents with funding provided by the NIH, European national governments and the partner institutions. This initiative is projected to take 10 years (until 2021), and will focus on analyzing the homozygous mutant mice generated on the isogenic C57BL/6 background previously generated by IKMC. The phenotyping strategy, called IMPReSS, is focused on revealing insights into human disease and will include neuromuscular, sensory, cardiovascular, metabolic, respiratory, hematological and neurological parameters collected for each mouse line. The phenotypic data is recorded in a freely accessible, fully searchable database (www.mousephenotype.org).

Conclusion

Mice are by far the most widely used animal model in present day research. Recent breakthroughs in mouse genetics and technological advances in transgenic methods have made mice a powerful system by which to better understand disease pathways and pharmacological intervention. The choice of mouse strains and substrains suitable for these studies is a critical one, in that it may influence the phenotype observed in a particular model. Therefore, one must understand the potential research implications that come with certain substrains. The use of C57BL/6N in the International Mouse Knockout program has led to the availability of reagents, all of which were created in a specific substrain. Maintaining the genetic integrity of these mice will be critical to ensuring the accuracy of the data obtained from these models.

References

Austin, CP, Batty, J. et al. The Knockout Mouse Project. *Nat. Genet.,* **36**:921-924 (2004).

Auwerx J, Avner P. et al. The European dimension for the mouse genome mutagenesis program. *Nat. Genet.*, **36**:925-927 (2004).

Batty J, et al. An action plan for mouse genomics. *Nat. Genet.*, **21**:73-75 (1999).

Bothe, G.W. et al. Genetic and behavioral differences among five inbred mouse strains commonly used in the production of transgenic and knockout mice. *Genes Brain Behav.*, **3**:149-157 (2004).

Bryant, C.D. et al., Behavioral differences among C57Bl/6 substrains: implications for transgenic and knockout studies. *J Neurogenet.*, **22**:315-331 (2008).

Collins, F.S., Rossant J. A Mouse for All Reasons. *Cell,* **128**:9-13 (2007).

Doetschman, T.C. et al. The *in vitro* development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J. Embryol. Exp. Morphol.,* **87**:27-45 (1985).

Freeman, H.C. et al. Deletion of nicotinamide nucleotide transhydrogenase: a new quantitative trait locus accounting for glucose intolerance in C57Bl/6J mice. *Diabetes*, **55**:2153-2156 (2006).

Kuehn, M.R. et al. A potential animal model for Lesch-Nyhan syndrome through introduction of HPRT mutations into mice. *Nature,* **326**(6110):295-298 (1987).

Mattapallil, M.J. et al. The Rd8 mutation of the Crb1 gene is present in vendor lines of C57Bl/6N mice and embryonic stem cells, and confounds ocular induced mutant phenotypes. *Invest. Ophthalmol. Vis. Sci.*, **53**(6):2921-2927 (2012).

Mekada, K. et al. Genetic differences among C57Bl/6 substrains. *Exp. Anim.*, **58**:141-149 (2009).

Mulligan, M.K. et al. Alcohol trait and transcriptional genomic analysis of C57Bl/6 substrains. *Genes Brain Behav*, **7**:677-689 (2008).

Peirce, J.L. et al. A major influence of sex-specific loci on alcohol preference in C57Bl/6 and DBA/2 inbred mice. *Mamm. Genome*, **9**:942-948 (1998).

Pettitt, S.J., Liang, Q. et al. Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nat. Methods*, **6**:493-495 (2009).

Sacca, R., et al. Genetically Engineered Mouse Models in Drug Discovery Research. In: Mouse Models for Drug Discovery. *Methods in Molecular Biology*, 602, G. Proetzel, M.V. Wiles, eds. Humana Press pp. 37-54 (2010).

Smithies, O. et al. Insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. *Nature*, **317**(6034):230-234 (1985).

Specht, C.G., Schoepfer, R. Deletion of the alpha-synuclein locus in a subpopulation of C57Bl/6J inbred mice. *BMC Neurosci.*, **2**:11 (2001).

Thomas, K.R., Capecchi, M.R. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell,* **51**:503-12 (1987).

Waterston, R.H. et al. Initial sequencing and comparative analysis of the mouse genome. *Nature*, **420**:520-562 (2002).

Zambrowicz, B. P., & Sands, A.T. Knockouts model the 100 best-selling drugs—will they model the next 100? *Nat. Rev. Drug Discov.,* **2**(1):38-51 (2003).

Zurita E. et al. Genetic polymorphisms among C57Bl/6 mouse inbred strains. *Transgenic Res.*, **20**(3):481-489 (2011).