New genetic resources for mammalian developmental biologists
David R Beier

Address: Genetics Division, Brigham and Women’s Hospital, Harvard Medical School New Research Building, 77 Avenue Louis Pasteur, Boston, MA 02115, USA
Email: beier@receptor.med.harvard.edu

F1000 Biology Reports 2010, 2:72 (doi:10.3410/B2-72)

Abstract
The utilization of homologous recombination in embryonic stem cells as a means to generate mice carrying pre-determined modifications of genomic sequences has revolutionized the study of developmental biology. Recognizing the potential efficiencies that can be obtained by high-throughput production at centralized technology centers, a number of large-scale efforts for generating mice with targeted mutations have been funded. These programs are reaching fruition, and a variety of libraries of embryonic stem cells with defined mutations are now available.

Introduction and context
There can be little doubt that the ability to use homologous recombination in embryonic stem (ES) cells as a method to generate mice with defined genomic modifications has made them the premier model system for the study of mammalian development and organogenesis. This has been further enhanced by recombinase-based methods that allow mutations to be induced in a tissue- or temporal-specific fashion. However, the task of generating mice with genome modifications remains time- and resource-intensive, and is particularly daunting for investigators without experience in this domain. This problem has been recently addressed by the initiation of large-scale targeted and gene-trap mutagenesis efforts, many of which have progressed to maturity, providing a vast resource for investigators wishing to use the mouse as a model system. The following summarizes the present status of several of these programs. For additional information, a thorough review of the history and methodologies of these and other programs has recently been published [1].

Major recent advances
In 2004, a proposal was made to pursue a large-scale project to generate a library of ES cells carrying mutations of all known or presumptive genes [2]. This effort has made remarkable progress, and very large numbers of targeted ES cells are now available to investigators at modest cost. The project is being undertaken by a consortium whose diverse responsibilities include the generation of targeting vectors, the selection and characterization of mutant ES cells, the distribution of reagents, and the presentation of data.

The largest components of this effort are represented in the KOMP (Knock-Out Mouse Project) and EUCOMM (European Conditional Mouse Mutagenesis) programs. KOMP was initiated in 2006 and is itself represented in two complementary efforts [3]. CSD is a collaborative team from the Children’s Hospital Oakland Research Institute (CHORI), the Wellcome Trust Sanger Institute, and the University of California at Davis School of Veterinary Medicine that has a goal of generating 5000 targeted ES cells. The CSD strategy uses a remarkable high-throughput method of recombineering developed at the Sanger Institute to generate targeting vectors that will generate conditional mutant alleles. This is paired with an automated ES cell colony-picking technology and allele characterization by long-range polymerase chain reaction (PCR). The other major component of KOMP is an effort by investigators at Regeneron to generate 3500 deletion alleles. This employs a method of bacterial artificial chromosome (BAC)-mediated homologous recombination and replacement that they have
developed and adapted for high throughput [4]. Successful targeting is assayed by using quantitative PCR to discriminate between wild-type and hemizygous cell lines for each mutant allele. The KOMP has been highly productive; as of May 2010 they have generated a total of 8092 targeting vectors, mutated ES cell lines for 5643 loci, and generated over 300 mutant mouse lines.

EUCOMM is a parallel effort with a goal of generating 8000 targeted conditional mutant alleles [5]. This program employs the same gene targeting strategy. The project was developed at the Wellcome Trust Sanger Institute, the Helmholtz Zentrum München German Research Center, the Medical Research Council (MRC) Mammalian Genetics Unit, Harwell, UK, the Institut Clinique de la Souris, Strasbourg, France, the Consiglio Nazionale delle Ricerche, Monterotondo, Italy, the University of Frankfurt, Germany, the Center for Cardiovascular Research at the Charité, Berlin, Germany, the University of Technology, Dresden, Germany, and the European Molecular Biology Laboratory (EMBL), Monterotondo, Italy. EUCOMM has produced 5980 vectors, 3473 mutated ES cells, and over 400 mutant mouse lines.

A third major project involving gene targeting by homologous recombination is being pursued as part of the NorCOMM (North American Conditional Mouse Mutagenesis) project [6]. The goal in this effort is up to 500 targeted loci, and the targeting protocol maximizes the utility of the modified locus for further manipulation (e.g., replacement with specific mutation [‘knock-in’], different reporters, recombinases, and so on).

These three projects (and the Texas A&M Institute for Genomic Medicine, see below) are associated in the International Knockout Mouse Consortium (IKMC). The IKMC data coordination center provides unified access to information about vector design and status of ES cell line and mutant mouse generation [7]. A genome-view perspective of alleles that have targeted mutations can be obtained using the ‘IKMC genes’ ribbon on the University of California Santa Cruz Genome Browser [8] or the ‘KO alleles’ DAS (distributed annotation system) track on the ENSEMBL Genome Browser. A crucial aspect of any large-scale biological resource is facilitating utilization of genetically modified mice for developmental analysis, a number of unknowns exist. First and foremost is the robustness of the newly developed resource with respect to the generation of germline-competent mutant mice. Many variables affect this, including genetic background, genomic stability, culture conditions, and technical expertise. Both the Sanger Institute and Regeneron have excellent success in generating germline-competent mice in their own facilities. For example, targeted JM8 clones successfully colonized the germline in over 65% of experiments [10]. As multiple cell lines have been derived for each targeted locus, the likelihood of obtaining a germline-competent mouse for any specific gene of interest is high. This assumes such success can be obtained elsewhere.

**Future directions**

While we are potentially on the cusp of a new era in the utilization of genetically modified mice for developmental analysis, a number of unknowns exist. First and foremost is the robustness of the newly developed resource with respect to the generation of germline-competent mutant mice. Many variables affect this, including genetic background, genomic stability, culture conditions, and technical expertise. Both the Sanger Institute and Regeneron have excellent success in generating germline-competent mice in their own facilities. For example, targeted JM8 clones successfully colonized the germline in over 65% of experiments [10]. As multiple cell lines have been derived for each targeted locus, the likelihood of obtaining a germline-competent mouse for any specific gene of interest is high. This assumes such success can be obtained elsewhere.
Another issue is the fact that the resource has been created in a C57BL/6N-derived cell line. Although the J and N substrains are very closely related (only 102 of 139,561 genotyped single-nucleotide polymorphisms are discordant [10]), several phenotypic differences have been noted [13,14] and molecular differences, such as a deletion of the Nnt gene in C57BL/6J, have been found [15,16].

Finally, there is a spectrum of opinions regarding how such resources can best be utilized. The European Community and the Wellcome Trust Sanger Institute have developed a vigorous effort to support systematized and centralized phenotyping in a large-scale fashion [17]. This model has been less consistently embraced by the research community in the USA. While there are clear efficiencies and opportunities for discovery inherent by phenotypic screening in an unbiased fashion, there is also a case to be made for facilitating analysis by individual investigators who are invested in specific gene families, pathways, or phenotypes. As an example, relatively modest but targeted ENU (N-ethyl-N-nitrosourea) mutagenesis programs have been arguably as productive, with respect to mutation discovery and functional characterization, as much larger, broadly focused efforts [18-21].

In summary, an extraordinary resource of gene deletions, disruptions, and conditional mutant alleles is now available at relatively low cost to investigators worldwide. The substantial investment required to create these libraries is likely to yield substantial insight into the role of specific genes in mammalian biology.

**Abbreviations**

CHORI, Children’s Hospital Oakland Research Institute; CSD, CHORI/Sanger/UIC Davis; ES, embryonic stem; EUCOMM, European Conditional Mouse Mutagenesis; IKMC, International Knockout Mouse Consortium; KOMP, Knock-Out Mouse Project; PCR, polymerase chain reaction.

**Competing interests**

DRB is a member of the scientific advisory boards for the KOMP and EUCOMM programs.

**Acknowledgments**

This work was supported by grants from the National Institute of Child Health and Human Development (HD36404) and the National Institute of Neurological Disorders and Stroke (MH081187).

**References**

1. Guan C, Ye C, Yang X, Gao J: A review of current large-scale mouse knockout efforts. *Genes* 2010, 48:73-85.
2. Austin CP, Battey JF, Bradley A, Buca M, Capecci M, Collins FS, Dove WF, Duky G, Dynecki S, Eppling JT, Grieder FB, Heintz N, Hicks G, Insel TR, Joyner A, Koller BH, Lloyd KC, Magunson T, Moore MW, Nagy A, Pollock JD, Rosas AD, Sands AT, Seed B, Skarnes WC, Snoddy J, Soriano P, Stewart DJ, Stewart F, Stillman B, et al.: The knockout mouse project. *Nat Genet* 2004, 36:921-4.
3. Jones EA, Baron MH, Fraser SE, Dickinson ME: Measuring hemodynamic changes during mammalian development. *Am J Physiol Heart Circ Physiol* 2004, 287:H1561-9.
4. Valenzuela DM, Murphy AJ, Frendewey D, Gale NW, Economides AN, Auerbach YV, Poueymire WT, Adams NC, Rojas J, Yasenchak J, Chernenmorsky R, Boucher M, Elsasser AL, Esau L, Zheng J, Griffiths JA, Wang X, Su H, Xue Y, Dominguez MG, Noguera I, Torres R, Macdonald LE, Stewart AF, DeChiara TM, Yancopoulos GD: High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. *Nat Biotechnol* 2003, 21:652-9.
5. Jones EA, Baron MH, Fraser SE, Dickinson ME: Dynamic in vivo imaging of mammalian hematovascular development using whole embryo culture. *Methods Mol Med* 2005, 105:381-94.
6. Pettet SJ, Lian Q, Rairdan KY, Moran JL, Prosser HM, Beier DR, Lloyd KC, Bradley A, Skarnes WC: Agouti C57BL/6N embryonic stem cells for mouse genetic resources. *Nat Methods* 2006, 3:493-5.
7. Nord AS, Chang PJ, Conklin BR, Cox AV, Harper CA, Hicks GG, Huang CC, Johns SJ, Kawai M, Liu S, Meng EC, Morris JH, Rossant J, Ruiz P, Skarnes WC, Soriano P, Stanford WL, Stryke D, von Melchner H, Wurst W, Yamamura K, Young SG, Babbitt PC, Ferrin TE: The International Gene Trap Consortium Website: a portal to all publicly available gene trap cell lines in mouse. *Nucleic Acids Res* 2006, 34:D642-8.
8. Hansen GM, Marksich DC, Burnett MB, Zhu Q, Dionne KM, Richter LJ, Finnell RH, Sands AT, Zambrowicz BP, Abuin A: Large-scale gene trapping in C57BL/6N mouse embryonic stem cells. *Genome Res* 2008, 18:1670-9.
9. Stiedl O, Radulovic J, Loehmann R, Birkenfeld K, Palve M, Kummermeier J, Sananbenesi F, Spiess J: Strain and substrain differences in context- and tone-dependent fear conditioning of inbred mice. *Behav Brain Res* 1999, 104:1-12.
10. Khisti RT, Wolstenholme J, Shelton KL, Miles MF: Characterization of the ethanol-deprivation effect in substrains of C57BL/6 mice. *Alcohol* 2006, 40:119-26.
11. Huang TT, Naeemuddin M, Elchuri S, Yamaguchi M, Kozy HM, Carlson EJ, Epstein CJ: Genetic modifiers of the phenotype of mice deficient in mitochondrial superoxide dismutase. *Hum Mol Genet* 2006, 15:1187-94.
trait locus accounting for glucose intolerance in C57BL/6J mice. Diabetes 2006, 55:2153-6.

17. Brown SD, Wurst W, Kuhn R, Hancock JM: The functional annotation of mammalian genomes: the challenge of phenotyping. Annu Rev Genet 2009, 43:305-33.

18. Shimano, Hartford SA, Duffy T, Wilson LA, Schimenti KJ, Schimenti JC: Phenotype-based identification of mouse chromosome instability mutants. Genetics 2003, 163:1031-40.

19. Huangfu D, Anderson KV: Cilia and Hedgehog responsiveness in the mouse. Proc Natl Acad Sci U S A 2005, 102:11325-30.

20. Georgel P, Du X, Hoebe K, Beutler B: ENU mutagenesis in mice. Methods Mol Biol 2008, 415:1-16.

21. Boles MK, Wilkinson BM, Wilming LG, Liu B, Probst FJ, Harrow J, Graffham D, Henges KE, Woodward LP, Maxwell A, Mitchell K, Risley MD, Johnson R, Hirschi K, Lupski JR, Funato Y, Miki H, Marin-Garcia P, Matthews L, Coffey AJ, Parker A, Hubbard TJ, Rogers J, Bradley A, Adams DJ, Justice MJ: Discovery of candidate disease genes in ENU-induced mouse mutants by large-scale sequencing, including a splice-site mutation in nucleoredoxin. PloS Genet 2009, 5: e1000759.