A Proposed Mouse Mutant Resequencing Initiative

A recommendation to augment the positional cloning of mouse mutations by resequencing of genetically-defined critical regions at Genome Centers.

The long-term goal of the human genome project (HGP) is to define and understand the functions of all human genes and their relationships to health and disease. The HGP has revolutionized genetic research, having yielded complete genome sequences, comprehensive polymorphic genetic marker sets, and other molecular reagents for humans, mice and many other experimental organisms. This has greatly simplified positional cloning of disease genes and genetic mapping of simple and complex traits. Concurrent efforts have identified thousands of expressed genes, and coupled with high-throughput gene expression and protein analysis technologies, we are in the powerful position of knowing the sequence identity of most genes, their expression patterns, the chromatin states around genes, and networks of interacting proteins.

While these data are valuable for deducing or suggesting the roles of genes in development and disease, the *in vivo* functions of most mammalian genes remain unknown. And their characterization remains a formidable challenge. With the advent of RNAi technologies, it has been possible to qualitatively assay large sets of genes for particular functions in simple animals such as *C. elegans*. In mammals, such technologies are being applied to cultured cells, but this approach will not address genes that function in developmental processes or differentiated tissues for which there is no *in vitro* model.

The task of elucidating the *in vivo* function of human genes in disease and development relies heavily upon utilization of the mouse, in which mutations can be created in various ways. By standard targeted mutagenesis in ES cells, mice containing null mutations in approximately 3500 mouse genes have been generated by independent laboratories. Gene trapping can be used to randomly mutate genes in ES cells, followed by the derivation of germline chimeras (1, 2). Successful gene trap alleles are either hypomorphs with reduced expression or null alleles. The International Gene Trap Consortium, consisting of several groups around the world, has generated an enormously useful resource for the mouse community. However, it has become clear that, due to various technical issues, only about 2/3 of genes will be effectively trapped (3). Germline transposon mutagenesis has more recently been developed in mice (4, 5), but it is not yet clear how effective this will be for mutagenizing a large fraction of the mouse transcriptome. The alleles generated by germline transposition will also be predominantly null alleles. Finally, the Knockout Mouse Project (KOMP; (6)) was initiated at NIH and funding has been awarded for high-throughput generation of gene-targeted ES cells (not mice). The ultimate goal is to have every mouse gene mutated in ES cells, and made available to investigators to convert into animal models. This complements similar international efforts in Canada and Europe.

These projects will be hugely important for our understanding of mammalian genetics and biology. However, as history has taught us, it is highly desirable to have not just null mutations in every gene, but an allelic series of mutations including missense mutations that vary in severity. It is also essential to generate information on the consequent phenotypes. Finally, it has recently become evident that large parts of the genome (of mammals and other organisms such as Drosophila) harbor transcription units for noncoding or unannotated RNAs that play unknown roles in genome regulation or function (7-10). These and other unknown elements will not be targeted by KOMP or other reverse genetic approaches. Notably, the first discovery of functional microRNAs in *C. elegans* came through a phenotypic genetic screen with positional cloning that yielded lin-4 (11, 12). Forward genetic methods that can uncover such functionally important genomic elements and associate them with specific phenotypes will continue to be

valuable. Clearly, a combination of phenotype-driven, gene-driven, and computational approaches will be required to approach a comprehensive understanding of the functions of all mammalian genes and other genome elements

In recent years, the NIH has supported large centers and individual labs in their efforts to generate ENU-induced mouse mutants. Most of these screens were phenotype-driven, and performed by groups with expertise in particular biological areas. These efforts have resulted in the isolation of hundreds of well-characterized mutants with defects in processes such as embryonic development, neurological function, reproduction, behavior, morphology and perception. Many of these ENU-induced mutants provide relevant human disease models. Overall, these funding initiatives have been highly successful in generating a rich resource of mutants. The discovery and analysis of spontaneous single-gene mutations with visible phenotypes also continues to augment the available collection of disease models. The diversity of biological process that has been informed by these studies is quite remarkable, as illustrated in the representative publications that are cited below (Appendix 1).

The collective experience of research teams involved in these forward genetic screens is that many investigators with interest in the biology of the mutants are reluctant to undertake the positional cloning task themselves. The majority of successfully cloned genes have been identified by the investigators that created the mutants. Only a fraction of the induced mutations have been positionally cloned to date, though many have been genetically mapped to varying degrees.

The primary value of these ENU mutants is the potential for associating the phenotype data with a specific genetic lesion. These lesions may be in genes or regulatory regions or non-coding RNAs or other DNA elements. An enormous amount of effort and resources has already been devoted to biological characterization, but the full value of this investment will not be realized until the mutations are identified. With the availability of high density genetic markers, it has become routine to map the mutants to a region of less than 5 Mb by generating and genotyping 100 to 200 F2 animals. The bottleneck for positional cloning is now the identification of point mutations within the nonrecombinant regions, which can take a substantial amount of time and money when carried out using traditional small-scale sequencing capabilities. We therefore propose that high-throughput resequencing of genetic intervals containing ENU and spontaneous mutations could significantly expedite positional cloning and the value of the collective mutant resource.

A practical solution would be to utilize the vast sequencing capacity of existing Genome Centers to sequence critical regions for mapped mutations. The cost of this resequencing is estimated at $5-6K per mutant, as discussed below, which compares favorably with the costs of the two alternative approaches to mouse gene function now in progress. The KOMP project will generate ES cell lines with mutations in particular genes, with no phenotypic information, at a cost of approximately $5,000 per line. Knockout mice with characterized phenotypes are being “repatriated” from the private sector at a cost of $45,000 per line. Since the ENU induced mutants are already phenotypically characterized, and can be assessed with respect to biomedical relevance, the investment of an additional $5-6K per line, is marginal compared with the previous investment and the inherent value of a mutant with known phenotype. Resequencing is thus a cost-effective approach to assigning functional data to this set of mutated genes, and provides an alternative to generating uncharacterized ES lines for the same genes. After the genes responsible for large numbers of ENU-induced mutations are identified, the genes being targeted by KOMP can be adjusted so as to be non-redundant, thereby increasing the number of mouse genes that will be mutated with available funding.

A key issue is the cost of identifying an ENU mutation by high-throughput resequencing. We suggest that eligible candidates mutations must first be mapped to a reasonable level of resolution, perhaps 5 Mb or less, which would then be followed by sequencing exons and flanking regions for all genes in the region. Positional cloning experience indicates that the great majority of ENU-induced mutations responsible for mutant phenotypes are located within or near coding sequence. Analysis of the most recent mouse genome build (courtesy of Peter Meric and Deanna Church, NHGRI) identified between 208,043 and 243,757 exons in the genome (based on gene models and transcripts, respectively), or 69 to 81 exons/ Mb on average. Less than 10% of these are > 800 bp in length. Thus, a 5 Mb nonrecombinant interval could be fully characterized by analysis of 350-400 amplicons. The cost of primers and sequencing for such a project would be approximately $5-6K and the process could be completed within 1-2 weeks in the context of a Genome Sequencing Center. The actual sequencing analysis required for even hundreds of ENU mutants would be a very small fraction of the capacity of a Genome Center.

Although most disease mutations lie within or near coding regions of genes, mutations located outside of annotated genes but within the genetically defined mutant region, although rare, are of particular interest for identification of novel functional elements of the genome. These cases represent valuable opportunities that are not achievable via the KOMP-style gene-by-gene approach. Because of the difficulty of finding such mutations, it remains unclear what proportion of mutants in the various ENU collections fall into this category. While there is only one proven case of an ENU-induced mutation of a regulatory sequence (13) there are numerous anecdotal reports in which mutations have not been found despite extensive exon-directed sequence analysis. For such cases, genomic resequencing of either conserved (presumptive regulatory) regions, or the entire nonrecombinant region is required. Even for these cases the value of identifying such mutants is high relative to the sequencing costs. Indeed, the identification of novel functional genomic elements is the raison d'etre of the ENCODE project.

It is important to emphasize that positional cloning of spontaneous mutants continues to be an important source of information regarding gene function and disease. These projects would also benefit greatly from access to resequencing of exons within 5 Mb nonrecombinant regions. In certain situations, QTL loci might also be eligible for the resequencing approach, i.e. when the parental strain is not included among the 15 strains being resequenced, and the locus of interest has been mapped with high confidence and resolution.

LOGISTICS:

A goal of this proposal is to minimize the impact on the Genome Center's normal workflow. To this end we are proposing an external review process for consideration of projects, as well as a Coordinating Center that will serve as an interface between investigators and the Sequencing Centers. The intent of the review is to insure that the minimal criteria for processing are met; specifically, **localization to a <5 Mb interval** and **agreement to submit the characterized mutant to a public repository prior to publication.** The aim of the Coordinating Center is to provide a consistent pipeline for handling DNA samples going to the Centers and the data being returned. This is especially desirable given that it would be inefficient to require the Centers to communicate with the large numbers of investigators who will utilize this program.

Coordination Center: For the interim, The Mouse Mutation and Developmental Analysis Program (MMDAP: UO1 HD43430, D. Beier, PI; J.Moran, Program Director) will serve as a coordinating center, and will be responsible for the following:

1) Receive requests from investigators by email.

2) Forward application to two reviewers from standing committee of six reviewers; monitor return of reviews.
3) Communicate outcome of review to investigators.
4) Receive DNA samples from investigators; verify concentration, quality, strain background (by SNP analysis).
5) Forward DNA samples to assigned genome center and monitor timely response.
6) Receive sequencing report from Genome Center and communicate results to Investigators.

Eligibility: In the first phase of the project, single-gene mutations of biomedical interest that have been mapped to a maximal recombinant interval of 5Mb. This will, on average, contain 50 genes and require sequencing of approximately 350-400 amplicons.

Application procedure: The investigator will provide the following information:
Phenotype class: e.g. immunity, deafness, eye
Phenotype description and biomedical justification for sequencing:
Phenotype assay:
Type of mutation: (ENU-induced, spontaneous mutation, strain difference)
Background strain:
Mapping strategy: (F2, backcross, congenic)
Chromosome position and mapping data:
    Proximal marker (bp location), # recombinants/total potential recombinants.
    Nonrecombinant markers tested (bp location)
    Distal marker (bp location), # recombinants/total potential recombinants
    # Refseq genes in nonrecombinant interval
Known mutant loci in nonrecombinant interval (can be obtained from Mouse Genome Database)
Publications:
Grant support, current or planned:
Plan for pre-publication submission to public repository.

Review Process:
Two independent reviewers will rate each application, using the following scale:

1. top priority for sequencing
2. defer for additional information
3. reject

Reviews will be "rolling", with a goal of 2-week turn-around for completion. The aim in this case is to review the data for genetic localization, not to make judgments regarding scientific significance. Upon acceptance, each mutant will be assigned an anonymous ID number for use in subsequent steps.

Submission of samples:
Four DNA samples (1 mg each) will be submitted, from 2 affected individuals and 2 controls. In the case of preimplantation lethal mutations, heterozygous carriers can be provided in place of homozygous affected samples. As previously noted, the Coordinating Center will confirm DNA concentrations and perform SNP analysis to document strain background.

Re-sequencing Report from Sequencing Center to Investigator:
A report of sequence variants (consistent differences between the 2 controls and 2 affected samples) will be returned to the Coordinating Center and forward to the Investigator. The data will be reviewed by the Coordinating Center to identify strain-specific polymorphisms (which will be submitted to dbSNP) and presumptive mutations (which will be reported to the Investigator).

The format of the report, and type of bioinformatic analysis, will be determined by the standards for Resequencing at each Genome Center.

Data and Resource sharing plan: First, all the DNA sequence traces will be submitted to the trace archive in accordance with the usual sequencing center procedures. Second, the P.I. will submit phenotype and strain data to the Mouse Genome Informatics website. Specifically, the P.I. will submit data about the strain and phenotype to MGI using this web-form: http://www.informatics.jax.org/mgihome/nomen/allmut_form.shtml. This form collects data on type and mode of inheritance of the mutation, genetic background, phenotype, publications, and contact details. This ensures that the information on the strain is readily available from a fully searchable database that is familiar to the scientific community. The data will go public upon acceptance of the project. Finally, the P.I. will fill out an MMRRC submission form, found at: http://www.mmrrc.org/submission/strain_submission_terms.html. Conditions of submission include agreements to provide genotyping data, sign the MTA, provide a health report, and ship the animals. The form also collects detailed information on the allele, background strain, phenotype, applicable research areas, breeding behavior, husbandry requirements, and relevant citations. The submission will be reviewed according to the standard procedures of the MMRRC steering committee. Furthermore, the members of the Coordinating Center will work closely with staff at the NHGRI to ensure full compliance with this resource sharing plan.

As shown in Appendix 2, there is abundant community interest in the proposed program, and a large number of investigators prepared to utilize these resources as soon as they can be made available. An additional important consideration is that most of the eligible mutations are being characterized as part of existing NIH-supported research programs. The ”end game” in this characterization is notoriously tedious, time-consuming, labor-intensive, and costly for laboratories without access to robust informatic and sequencing resources. Thus, facilitating the use of a high throughput center for sequencing would ultimately result in the more efficient use of NIH funds. We urge the NIH to explore options to enable researchers with well-mapped ENU-induced mutations, spontaneous mutations, or QTLs, to partner with Genome Centers for re-sequencing of critical regions. This Mutant Resequencing Project would unlock the full value of the mouse mutant resources in which the NIH has invested over the past several years.

Respectfully submitted:

Miriam Meisler, Ph. D.
Professor of Human Genetics
University of Michigan

John Schimenti, Ph.D.
Professor of Genetics
Director, Center for Vertebrate Genomics
Cornell University

David R. Beier, M.D., Ph.D.
Professor of Medicine
Harvard Medical School
Brigham and Women’s Hospital

References:

1. Skarnes WC 1993 The identification of new genes: gene trapping in transgenic mice. Curr Opin Biotechnol 4:684-689

2. Mitchell KJ, Pinson KI, Kelly OG, Brennan J, Zupicich J, Scherz P, Leighton PA, Goodrich LV, Lu X, Avery BJ, Tate P, Dill K, Pangilinan E, Wakenight P, Tessier-Lavigne M, Skarnes WC 2001 Functional analysis of secreted and transmembrane proteins critical to mouse development. Nat Genet 28:241-249

3. Skarnes WC, von Melchner H, Wurst W, Hicks G, Nord AS, Cox T, Young SG, Ruiz P, Soriano P, Tessier-Lavigne M, Conklin BR, Stanford WL, Rossant J 2004 A public gene trap resource for mouse functional genomics. Nat Genet 36:543-544

4. Keng VW, Yae K, Hayakawa T, Mizuno S, Uno Y, Yusa K, Kokubu C, Kinoshita T, Akagi K, Jenkins NA, Copeland NG, Horie K, Takeda J 2005 Region-specific saturation germline mutagenesis in mice using the Sleeping Beauty transposon system. Nat Methods 2:763-769

5. Ding S, Wu X, Li G, Han M, Zhuang Y, Xu T 2005 Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. Cell 122:473-483

6. Austin CP, Battey JF, Bradley A, Bucan M, Capecchi M, Collins FS, Dove WF, Duyk G, Dymecki S, Eppig JT, Grieder FB, Heintz N, Hicks G, Insel TR, Joyner A, Koller BH, Lloyd KC, Magnuson T, Moore MW, Nagy A, Pollock JD, Roses AD, Sands AT, Seed B, Skarnes WC, Snoddy J, Soriano P, Stewart DJ, Stewart F, Stillman B, Varmus H, Varticovski L, Verma IM, Vogt TF, von Melchner H, Witkowski J, Woychik RP, Wurst W, Yancopoulos GD, Young SG, Zambrowicz B 2004 The knockout mouse project. Nat Genet 36:921-924

7. Furuno M, Pang KC, Ninomiya N, Fukuda S, Frith MC, Bult C, Kai C, Kawai J, Carninci P, Hayashizaki Y, Mattick JS, Suzuki H 2006 Clusters of internally primed transcripts reveal novel long noncoding RNAs. PLoS Genet 2:e37

8. Oliver B 2006 Tiling DNA microarrays for fly genome cartography. Nat Genet 38:1101-1102

9. Ravasi T, Suzuki H, Pang KC, Katayama S, Furuno M, Okunishi R, Fukuda S, Ru K, Frith MC, Gongora MM, Grimmond SM, Hume DA, Hayashizaki Y, Mattick JS 2006 Experimental validation of the regulated expression of large numbers of non-coding RNAs from the mouse genome. Genome Res 16:11-19

10. Bertone P, Stolc V, Royce TE, Rozowsky JS, Urban AE, Zhu X, Rinn JL, Tongprasit W, Samanta M, Weissman S, Gerstein M, Snyder M 2004 Global identification of human transcribed sequences with genome tiling arrays. Science 306:2242-2246

11. Wightman B, Ha I, Ruvkun G 1993 Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 75:855-862

12. Lee RC, Feinbaum RL, Ambros V 1993 The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843-854

13. Sagai T, Masuya H, Tamura M, Shimizu K, Yada Y, Wakana S, Gondo Y, Noda T, Shiroishi T, Phylogenetic conservation of a limb-specific, cis-acting regulator of Sonic hedgehog (Shh). 2004 Mamm Genome. 15:23-34

Appendix 1:

1. Ackerman, K.G., B.J. Herron, S.O. Vargas, H. Huang, S.G. Tevosian, L. Kochilas, C. Rao, B.R. Pober, R.P. Babiuk, J.A. Epstein, J.J. Greer, and D.R. Beier. 2005. Fog2 is required for normal diaphragm and lung development in mice and humans. PLoS Genet 1: 58-65.

2. Bannister, L.A., L.G. Reinholdt, R.J. Munroe, and J.C. Schimenti. 2004. Positional cloning and characterization of mouse mei8, a disrupted allelle of the meiotic cohesin Rec8. Genesis 40: 184-194.

3. Bialek, P., B. Kern, X. Yang, M. Schrock, D. Sosic, N. Hong, H. Wu, K. Yu, D.M. Ornitz, E.N. Olson, M.J. Justice, and G. Karsenty. 2004. A twist code determines the onset of osteoblast differentiation. Dev Cell 6: 423-435.

4. Bode, V.C., J.D. McDonald, J.L. Guenet, and D. Simon. 1988. hph-1: a mouse mutant with hereditary hyperphenylalaninemia induced by ethylnitrosourea mutagenesis. Genetics 118: 299-305.

5. Bosman, E.A., A.C. Penn, J.C. Ambrose, R. Kettleborough, D.L. Stemple, and K.P. Steel. 2005. Multiple mutations in mouse Chd7 provide models for CHARGE syndrome. Hum Mol Genet 14: 3463-3476.

6. Buchner, D.A., K.L. Seburn, W.N. Frankel, and M.H. Meisler. 2004. Three ENU-induced neurological mutations in the pore loop of sodium channel Scn8a (Na(v)1.6) and a genetically linked retinal mutation, rd13. Mamm Genome 15: 344-351.

7. Bultman, S.J., T.C. Gebuhr, and T. Magnuson. 2005. A Brg1 mutation that uncouples ATPase activity from chromatin remodeling reveals an essential role for SWI/SNF-related complexes in beta-globin expression and erythroid development. Genes Dev 19: 2849-2861.

8. Caspary, T., M.J. Garcia-Garcia, D. Huangfu, J.T. Eggenschwiler, M.R. Wyler, A.S. Rakeman, H.L. Alcorn, and K.V. Anderson. 2002. Mouse Dispatched homolog1 is required for long-range, but not juxtacrine, Hh signaling. Curr Biol 12: 1628-1632.

9. Curtin, J.A., E. Quint, V. Tsipouri, R.M. Arkell, B. Cattanach, A.J. Copp, D.J. Henderson, N. Spurr, P. Stanier, E.M. Fisher, P.M. Nolan, K.P. Steel, S.D. Brown, I.C. Gray, and J.N. Murdoch. 2003. Mutation of Celsr1 disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. Curr Biol 13: 1129-1133.

10. Eggenschwiler, J.T., E. Espinoza, and K.V. Anderson. 2001. Rab23 is an essential negative regulator of the mouse Sonic hedgehog signalling pathway. Nature 412: 194-198.

11. Flenniken, A.M., L.R. Osborne, N. Anderson, N. Ciliberti, C. Fleming, J.E. Gittens, X.Q. Gong, L.B. Kelsey, C. Lounsbury, L. Moreno, B.J. Nieman, K. Peterson, D. Qu, W. Roscoe, Q. Shao, D. Tong, G.I. Veitch, I. Voronina, I. Vukobradovic, G.A. Wood, Y. Zhu, R.A. Zirngibl, J.E. Aubin, D. Bai, B.G. Bruneau, M. Grynpas, J.E. Henderson, R.M. Henkelman, C. McKerlie, J.G. Sled, W.L. Stanford, D.W. Laird, G.M. Kidder, S.L. Adamson, and J. Rossant. 2005. A Gja1 missense mutation in a mouse model of oculodentodigital dysplasia. Development 132: 4375-4386.

12. Hentges, K.E., B. Sirry, A.C. Gingeras, D. Sarbassov, N. Sonenberg, D. Sabatini, and

A.S. Peterson. 2001. FRAP/mTOR is required for proliferation and patterning during embryonic development in the mouse. Proc Natl Acad Sci U S A 98: 13796-13801. Epub 12001 Nov 13713.

13. Huangfu, D., A. Liu, A.S. Rakeman, N.S. Murcia, L. Niswander, and K.V. Anderson. 2003. Hedgehog signalling in the mouse requires intraflagellar transport proteins. Nature 426: 83-87.

14. Jablonski, M.M., C. Dalke, X. Wang, L. Lu, K.F. Manly, W. Pretsch, J. Favor, M.T. Pardue, E.M. Rinchik, R.W. Williams, D. Goldowitz, and J. Graw. 2005. An ENU-induced mutation in Rs1h causes disruption of retinal structure and function. Mol Vis 11: 569-581.

15. Jiang, Z., P. Georgel, X. Du, L. Shamel, S. Sovath, S. Mudd, M. Huber, C. Kalis, S. Keck, C. Galanos, M. Freudenberg, and B. Beutler. 2005. CD14 is required for MyD88-independent LPS signaling. Nat Immunol 6: 565-570. Epub 2005 May 2015.

16. Jun, J.E., L.E. Wilson, C.G. Vinuesa, S. Lesage, M. Blery, L.A. Miosge, M.C. Cook, E.M. Kucharska, H. Hara, J.M. Penninger, H. Domashenz, N.A. Hong, R.J. Glynne, K.A. Nelms, and C.C. Goodnow. 2003. Identifying the MAGUK protein Carma-1 as a central regulator of humoral immune responses and atopy by genome-wide mouse mutagenesis. Immunity 18: 751-762.

17. Libby, B.J., L.G. Reinholdt, and J.C. Schimenti. 2003. Positional cloning and characterization of Mei1, a vertebrate-specific gene required for normal meiotic chromosome synapsis in mice. Proc Natl Acad Sci U S A 100: 15706-15711.

18. Liu, A., B. Wang, and L.A. Niswander. 2005. Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors. Development 132: 3103-3111. Epub 2005 Jun 3101.

19. Liu, H., X. Du, M. Wang, Q. Huang, L. Ding, H.W. McDonald, J.R. Yates, 3rd, B. Beutler, J. Horwitz, and X. Gong. 2005. Crystallin {gamma}B-I4F mutant protein binds to {alpha}-crystallin and affects lens transparency. J Biol Chem 280: 25071-25078. Epub 22005 May 25074.

20. May, S.R., N.J. Stewart, W. Chang, and A.S. Peterson. 2004. A Titin mutation defines roles for circulation in endothelial morphogenesis. Dev Biol 270: 31-46.

21. Pask, A.J., H. Kanasaki, U.B. Kaiser, P.M. Conn, J.A. Janovick, D.W. Stockton, D.L. Hess, M.J. Justice, and R.R. Behringer. 2005. A novel mouse model of hypogonadotrophic hypogonadism: N-ethyl-N-nitrosourea-induced gonadotropin-releasing hormone receptor gene mutation. Mol Endocrinol 19: 972-981. Epub 2004 Dec 2029.

22. Pinto, L.H., M.H. Vitaterna, K. Shimomura, S.M. Siepka, E.L. McDearmon, D. Fenner, S.L. Lumayag, C. Omura, A.W. Andrews, M. Baker, B.M. Invergo, M.A. Olvera, E. Heffron, R.F. Mullins, V.C. Sheffield, E.M. Stone, and J.S. Takahashi. 2005. Generation, characterization, and molecular cloning of the Noerg-1 mutation of rhodopsin in the mouse. Vis Neurosci 22: 619-629.

23. Rhodes, C.R., N. Parkinson, H. Tsai, D. Brooker, S. Mansell, N. Spurr, A.J. Hunter, K.P. Steel, and S.D. Brown. 2003. The homeobox gene Emx2 underlies middle ear and inner ear defects in the deaf mouse mutant pardon. J Neurocytol 32: 1143-1154.

24. Rutschmann, S., K. Hoebe, J. Zalevsky, X. Du, N. Mann, B.I. Dahiyat, P. Steed, and B.

Beutler. 2006. PanR1, a Dominant Negative Missense Allele of the Gene Encoding TNF-{alpha} (Tnf), Does Not Impair Lymphoid Development. J Immunol 176: 7525-7532.

25. Shima, N., R.J. Munroe, and J.C. Schimenti. 2004. The mouse genomic instability mutation chaos1 is an allele of Polq that exhibits genetic interaction with Atm. Mol Cell Biol 24: 10381-10389.

26. Smyth, I., X. Du, M.S. Taylor, M.J. Justice, B. Beutler, and I.J. Jackson. 2004. The extracellular matrix gene Frem1 is essential for the normal adhesion of the embryonic epidermis. Proc Natl Acad Sci U S A 101: 13560-13565.

27. Su, L.K., K.W. Kinzler, B. Vogelstein, A.C. Preisinger, A.R. Moser, C. Luongo, K.A. Gould, and W.F. Dove. 1992. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science 256: 668-670.

28. Tabeta, K., K. Hoebe, E.M. Janssen, X. Du, P. Georgel, K. Crozat, S. Mudd, N. Mann, S. Sovath, J. Goode, L. Shamel, A.A. Herskovits, D.A. Portnoy, M. Cooke, L.M. Tarantino, T. Wiltshire, B.E. Steinberg, S. Grinstein, and B. Beutler. 2006. The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. Nat Immunol 7: 156-164.

29. Van Raamsdonk, C.D., K.R. Fitch, H. Fuchs, M.H. de Angelis, and G.S. Barsh. 2004. Effects of G-protein mutations on skin color. Nat Genet 36: 961-968. Epub 2004 Aug 2022.

30. Vitaterna, M.H., D.P. King, A.M. Chang, J.M. Kornhauser, P.L. Lowrey, J.D. McDonald, W.F. Dove, L.H. Pinto, F.W. Turek, and J.S. Takahashi. 1994. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science 264: 719-725.

31. Zohn, I.E., Y. Li, E.Y. Skolnik, K.V. Anderson, J. Han, and L. Niswander. 2006. p38 and a p38-Interacting Protein Are Critical for Downregulation of E-Cadherin during Mouse Gastrulation. Cell 125: 957-969.

32. Zoltewicz, J.S., N.J. Stewart, R. Leung, and A.S. Peterson. 2004. Atrophin 2 recruits histone deacetylase and is required for the function of multiple signaling centers during mouse embryogenesis. Development 131: 3-14.

Appendix 2:

An informal and incomplete tabulation of positional cloning projects (Table 1 below) reveals an extraordinary potential interest in the utilization of high-throughput mouse mutant resequencing. There are presently nearly 60 projects ready for such an analysis (i.e., mapped to a recombinant interval <5 Mb) and 35 nearly so (i.e., mapped to a recombinant interval of between 5-10 Mb).

Even this large number of projects will not tax the capacity of the Genome Center resequencing program. For example, according to Dr. John McPherson, the capacity of the Baylor Center effort is presently 400,000 PCR *per month*. Conservatively estimating each mouse project as requiring sequence analysis of 400 wild-type and mutant amplicons, the execution of 60 projects over one year would utilize about 2% of a single Genome Center's capacity. Assuming an approximate cost of $5500/ project, for a total of $330K/yr, this would be a highly cost-effective means to maximize the biological informativeness of phenotype-driven mutational analysis. Given our experience that community interest in phenotype-characterized mouse mutants is greatest for those in which the mutated gene is known, this would be an extremely productive use of NIH resources.

Table 1:

		<2 Mb	2-5 Mb	5-10 Mb	10-20 Mb	>20 Mb
Anderson, Kathryn	Sloan-Kettering	5	6	11	10	
Barsh, Greg	Stanford	2	10	0	0	
Beier, David	BWH/HMS	0	0	1	1	4
Beutler, Bruce	Scripps	1	3	2	0	4
Bucan, Maja	U Penn	0	0	8	0	0
Carlson, George	McLaughlin	1	0	2	3	6
Handel,Mary Ann	Cornell, Jax	0	2	3	9	9
Justice, Monica	Baylor	7	4	6	21	
Nishima, Patsy	Jax	4	1	3	0	0
Pavan, Bill	NHGRI	0	1	0	1	0
Schimenti, John	Cornell	12	4	3	0	4
Takahashi, Joe	Northwestern	0	2	2	10	2
Total		**32**	**33**	**41**	**55**	**29**