Why Mouse?

Roger Reeves, Ph.D./ Tom Sussan, 5-6624
rreeves@jhmi.edu/ tsussan@jhmi.edu

Mouse as a Genetic Model System for Mammalian Genetics

1. Genetic structures in mice are not found in nature
   - Mapping in inbred strains
   - Recombinant inbred strains
   - Congenic strains/ speed congenics ("marker-assisted breeding ")
   - Haplotype mapping
   - Quantitative trait loci meet genomics
2. Make your own Genotype
   - Transgenesis
   - Gene targeting
   - Chromosome engineering
3. Make your own phenotype
   - Mutagenesis – insertion/ENU
   - Phenome project
4. Comparative genetics
   - Comparative maps/ sequence
   - Biology – comparative pheontypes
     - Functions of a genome
     - Evolution

1. **Special Genetic Structures in Mice: Mice can be inbred to homozygosity**

"A strain shall be regarded as inbred when it has been mated brother x sister (hereafter called bxs)
for twenty or more consecutive generations (F20), and can be traced to a single ancestral breeding
pair in the 20th or a subsequent generation. Parent offspring matings may be substituted for bxs matings provided that, in the case of consecutive parent x offspring matings, the mating in each case is to the younger of the two parents. Exceptionally, other breeding systems may be used, provided that the inbreeding coefficient achieved is at least equal to that at F20 (0.99)."

2. **Hundreds of inbred strains of mice capture many different types of genetic information**

nature genetics

Inbred strain categories
A) Swiss mice
B) Castle's mice
C) Strains derived from colonies from China and Japan
D) Other inbred strains
E) C57-related strains
F) Strains derived from wild mice
G) Mice derived from multiple inbred strains

Note: categories are intended to help the researchers find or define strains of interest and should not be regarded as fixed or mutually exclusive. Species and subspecies are as described in the relevant reference.

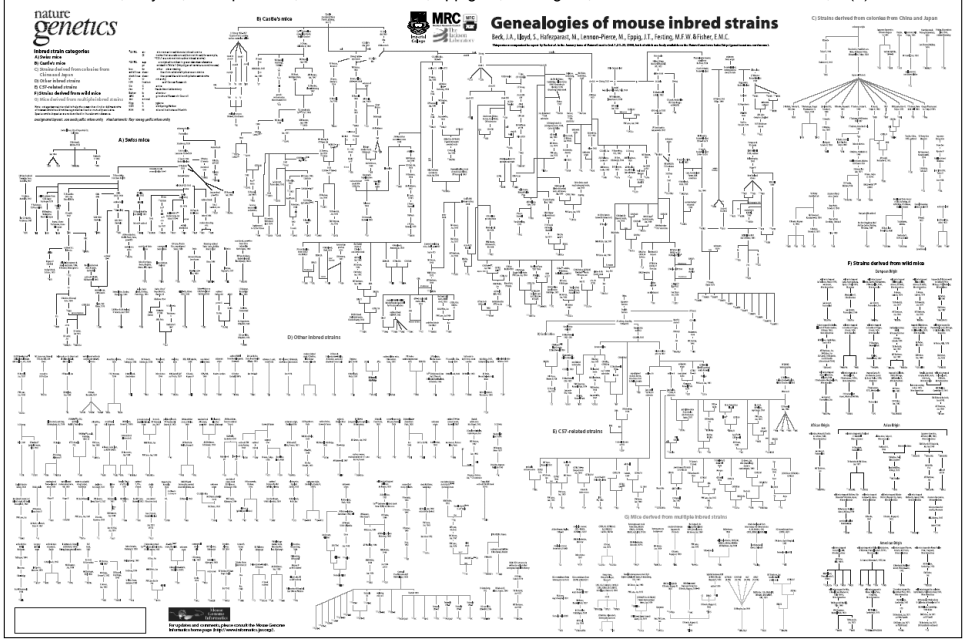
Design and layout: Jon Beck (MRC Prion Unit) Final artwork: Ray Young (MRC Prion Unit)

[B]C57BL	str	ain names in red denote inbred strains
	(S	trains that are referenced, but not in red, for example, [14]CD-1 are not maintained as inbred strains).
[D]C57BL	sup	erscripted number in green denotes reference
	pr	ovided in Table 1 (http://genetics.nature.com/mouse)
bxs	br	other sister mating
solid lines	descr	ibes firm relationship between strains
dash lines	descr	ibes possible relationship between strains
U	U	niversity
ICR	Institut	e of Cancer Research
Ctr	C	entre
Jax	T	he Jackson Laboratory
Behav	b	ehaviour
ARC	A	gricultural Research Council
Ani	animal	
Hyg	h	ygiene
CSH	C	old Spring Harbor
NIH	N	ational Institutes of Health

CBA

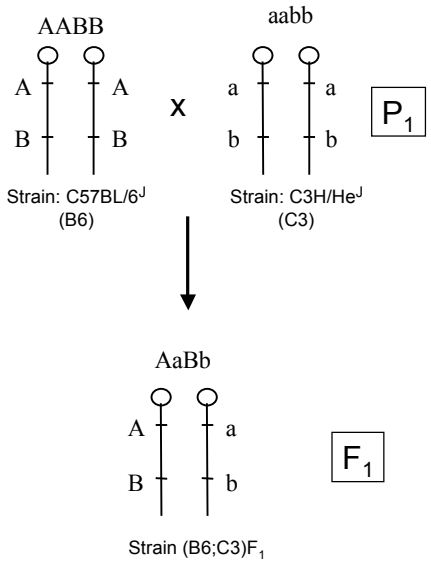
>450 inbred strains http://www.nature.com/ng/mouse/ng0100_treefinal.pdf

Beck JA, Lloyd S, Hafezparast M, Lennon-Pierce M, Eppig JT, Festing MF, Fisher EM. Nat Genet. 2000 Jan;24(1):23-5.



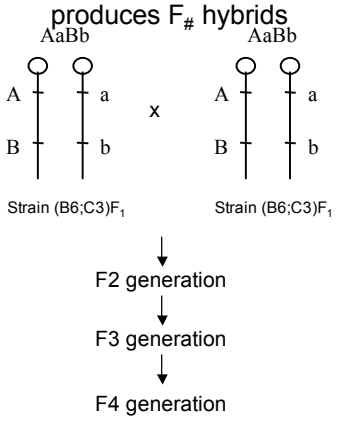
Nomenclature and genetic content

An **intercross** between two parental (P_1) inbred strains produces an F_1 hybrid

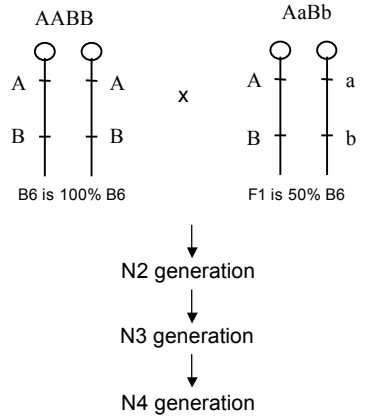


Nomenclature and genetic content

An **advanced intercross** produces $F_{\#}$ hybrids

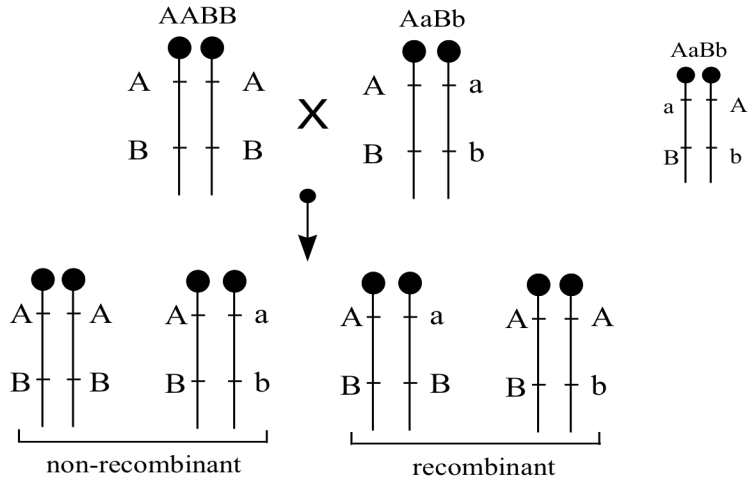


A **backcross** produces $N_{\#}$ generations



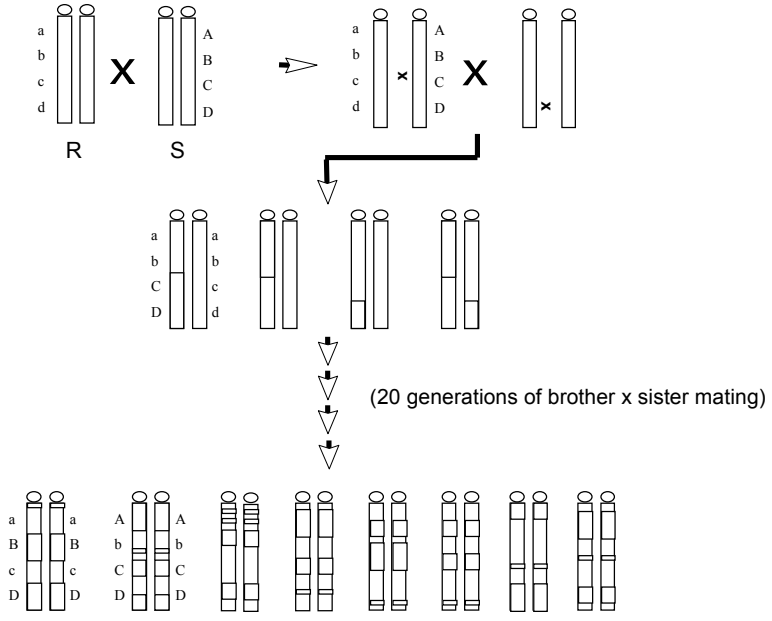
Mapping in inbred mice

For any polymorphic marker, crosses are completely phase-known

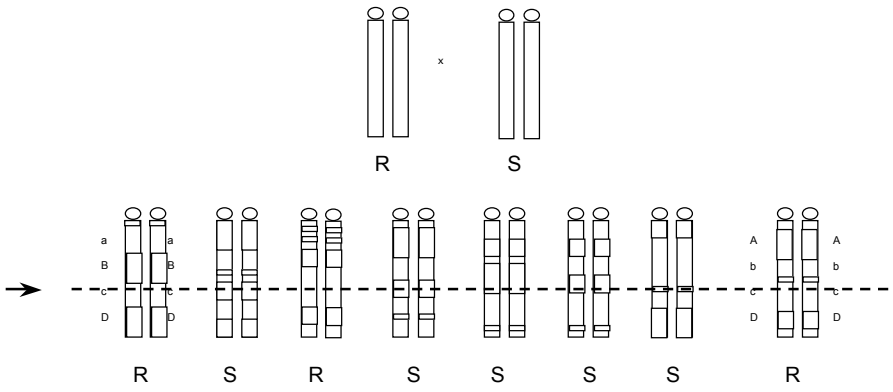


Recombinant inbred mouse strains

resource for genotype and phenotype



Mapping in Recombinant inbred (RI) strains



QTX: *software for complex trait analysis*
by Jane M. Meer, Robert H. Cudmore, Jr., and Kenneth F. Manly

http://mapmgr.roswellpark.org/mmQTX.html

Strain distribution patterns for Chr 6 in the AKXD RI strain set.

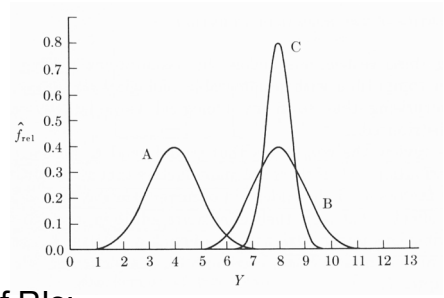
Chromosome: 6

AKXD	1	2	3	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28	Experiment (Reference)
Calu	A	D	A	A	D	A	D	A	D	A	D	A	A	A	.	A	D	A	A	A	A	A	D	D	D	Yabe D (J:41711)
Mtv23	D	A	A	D	D	A	A	D	D	D	D	D	D	D	A	A	D	D	D	A	D	.	A	A	D	Lee BK (J:10675)
D6Mit33	D	A	A	D	D	A	A	D	D	D	A	D	D	A	.	A	A	D	D	D	D	D	D	A	D	Taylor BA (J:11923)
Igk	D	A	D	A	D	A	A	.	.	A	A	D	D	A	.	A	D	A	D	D	A	D	D	A	.	Elliott RW (J:10507)
Igk-J	D	A	D	A	D	A	A	D	D	A	A	D	D	A	A	A	D	A	D	D	A	D	D	A	.	Boyd RT (J:8431)
Igk-V28	D	A	D	A	D	A	A	D	D	A	A	D	D	A	A	A	D	A	D	D	A	D	D	A	D	Taylor BA (J:8097)
Odc-rs5	D	A	D	A	D	A	A	D	D	A	A	D	D	A	A	A	D	A	D	D	A	D	D	A	D	Richards-Smith BA
Rn7s6	D	A	D	A	D	A	A	D	D	A	A	D	D	A	A	A	D	A	D	D	A	D	D	A	D	Taylor BA (J:8097)
D6Nds3	D	A	A	A	D	A	A	A	D	D	D	D	D	D	.	A	D	D	D	A	D	D	A	A	D	Cornall RJ (J:3227)
Cd8b	D	A	D	A	D	A	A	D	D	A	A	D	D	A	A	A	D	A	D	D	A	D	D	A	D	Taylor BA (J:8097)
Tgfa	D	A	D	A	D	A	A	D	D	A	A	D	D	A	.	D	D	A	D	D	A	D	A	A	A	Fowler KJ (J:12769)
D6Nds2	D	A	D	D	D	A	A	D	D	A	A	D	D	A	.	D	D	A	A	D	A	D	D	A	A	Cornall RJ (J:3227)
Rho	D	A	A	A	D	A	D	D	D	A	A	D	D	A	.	D	D	D	A	D	A	D	D	A	A	Elliott RW (J:10507)
Raf1	D	A	A	.	D	A	D	D	D	A	A	.	D	A	.	D	D	A	A	D	A	D	D	.	A	Elliott RW (J:10507)
D6Mit15	D	D	D	A	A	D	D	D	A	A	A	D	D	A	A	.	A	D	D	A	D	A	A	A	D	A	Taylor BA (J:11923)
Xmmv54	D	D	D	D	A	D	D	A	A	D	A	D	A	A	A	A	D	D	A	D	A	A	A	D	A	Wejman JC (J:7348)

Mouse Genome Informatics:
http://www.informatics.jax.org/searches/riset_form.shtml

Benefits of RI strains:

- "Pre-genome scan" – cumulative information
- Reassay the "same" individual many times (find true mean and deviation for variable traits)
- Highly beneficial for quantitative traits
- Stock of genetic variation (recombinant congenic mice)



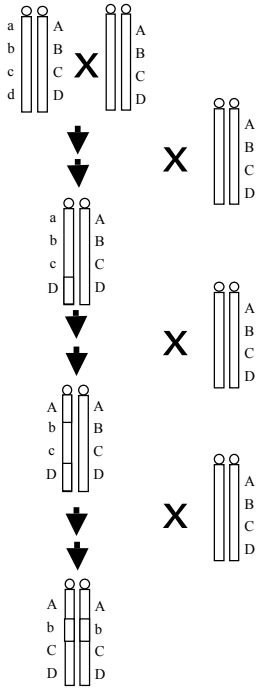
Limitations of RIs:

- Small strain sets have limited statistical power.
- Mapping is relatively low resolution on the first pass.

Congenic mice

are made by repeated backcrossing while selecting for a specific locus of parental type

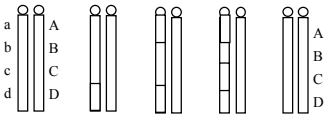
10-12 generations of backcrossing/selection



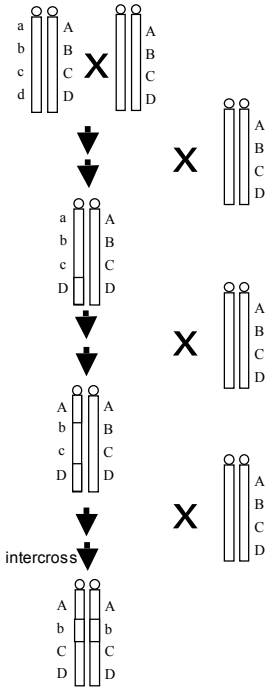
“Speed” congenic mice

are made by repeated backcrossing while selecting for a specific locus of parental type AND screening large sets of progeny with a genome scan to identify those mice which are most inbred

4-5 generations using marker assisted selection to choose the progenitors of the next generation

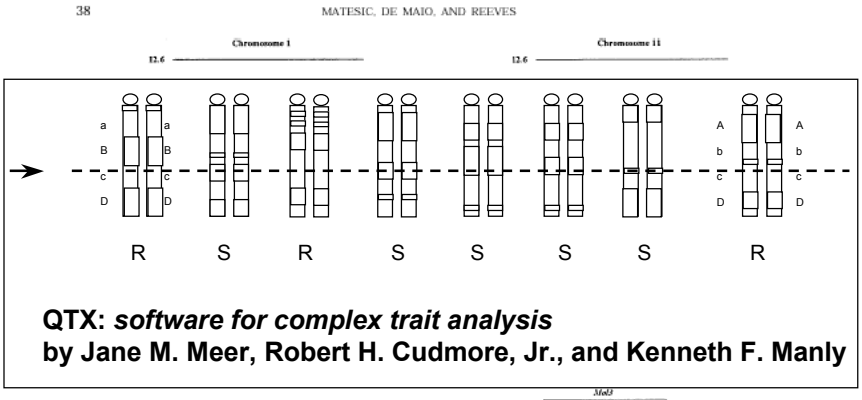


Chromosome pairs from five different F3 individuals assessed by a genome scan – which should be selected for the next generation?

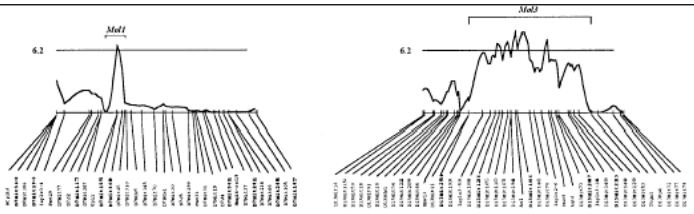


Markel et al., Nat Genet. 1997 Nov;17(3):280-4.

Overcoming limited statistical power of RI strains



QTX: *software for complex trait analysis*
by Jane M. Meer, Robert H. Cudmore, Jr., and Kenneth F. Manly

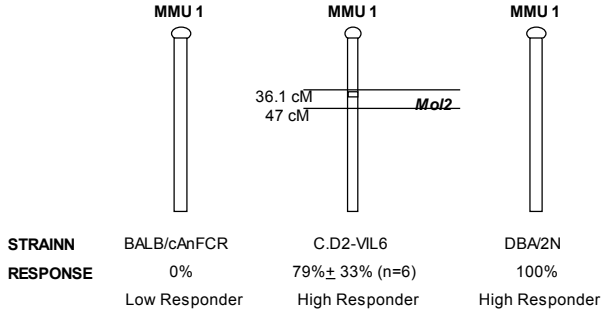


Matesic et al., Genomics 62:34-41.

Congenic mice can be used to confirm and refine localizations made with other approaches.

Further, they demonstrate sufficiency of an individual locus to a cause a phenotype (e.g. in QTL studies).

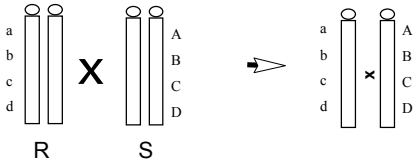
Independent confirmation of *Mol2* on Chr1



Matesic, L.E., A. De Maio, R.H. Reeves. 1999. Mapping LPS Response Loci in Mice Using Recombinant Inbred and Congenic Strains. Genomics 62:34-41.

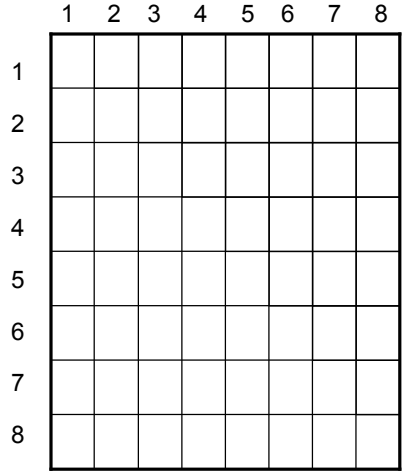
Increased resolution from RI mapping

1. RIX - Threadgill



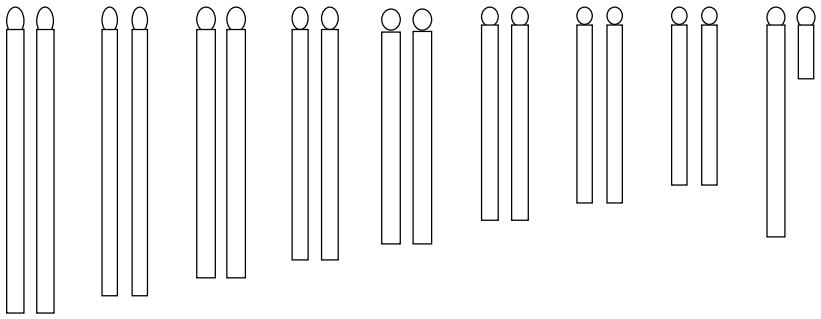
2. Complex Trait Consortium

- 8-way cross
- 2048 RI strains (256 on the shelf)
- www.complextrait.org/Workshop1.pdf



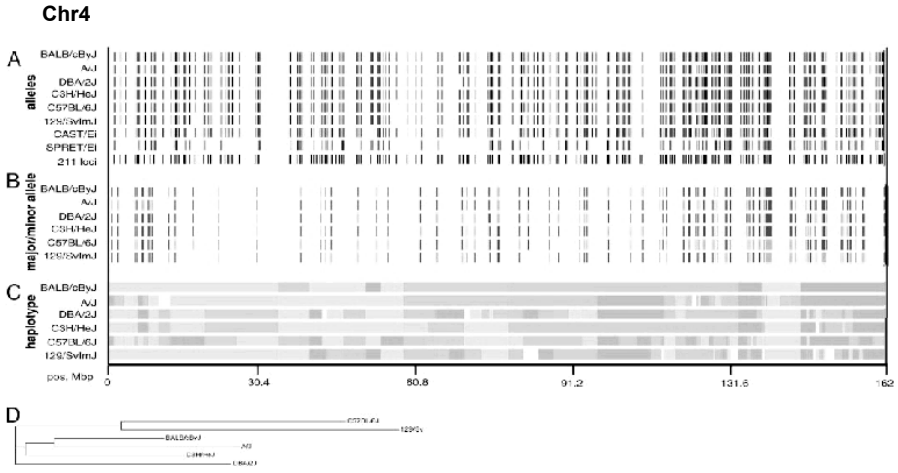
Consomic mice

are made by repeated backcrossing to place one chromosome pair from strain "A" onto the genetic background of strain "B"



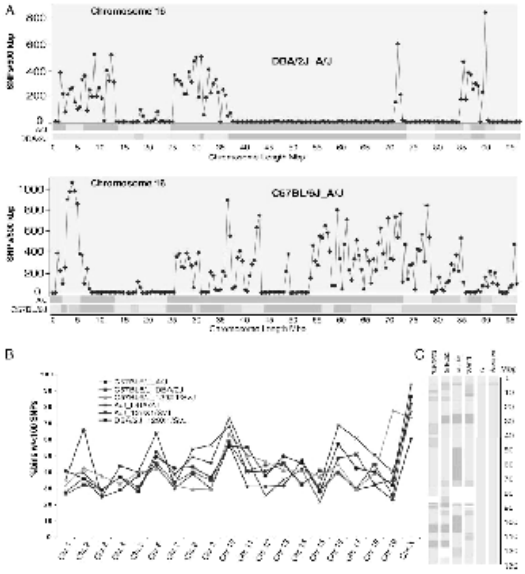
Nadeau et al., Nat Genet. 2000 Mar;24(3):221-5

Haplotype structure in the world of inbred mice



Wiltshire et al., PNAS 100:3380-3385 (2003); www.gnf.org/SNP/

Large haplotype blocks correspond to regions of low SNP density



SNP density on Chr16

Wiltshire et al., PNAS 100:3380-3385 (2003); www.gnf.org/SNP/

Mapping with haplotypes: Tyr

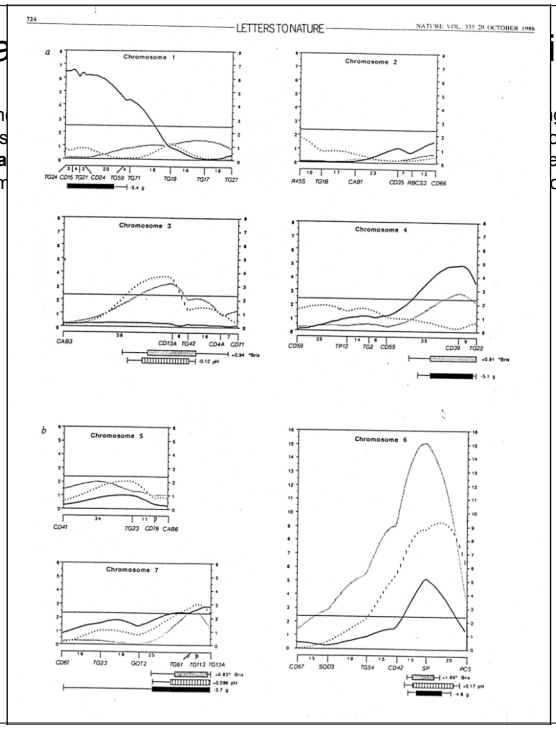
	−1,000	−500	−400	−100	−50	0	50	200	350	1,000 kb
129/SvimJ	AT	A	CTG DEL A	CA	CA	A	A	CC	CGG	C INS TT
DBA/2J	AT	A	CTG DEL A	CA	CA	A	A	CC	CGG	C INS TT
C3H/HeJ	AT	A	CTG DEL A	CA	CA	A	A	CC	CGG	C INS TT
NZB/BINJ	AT	A	—	CA	CA	A	A	CC	CGG	C INS TT
C57BL/6J	GA	T	TAA INS T	TG	AG	A	A	CC	AAA G	DEL CC
C58/J	GA	T	—	TG	AG	A	A	CC	AAA G	DEL CC
A/J	GA	T	TAA INS T	TA	CA	C	C	TA	CGG	C INS TT
AKR/J	GA	T	TAA INS T	TA	CA	C	C	TA	CGG	C INS TT
BALB/cByJ	GA	T	TAA INS T	TA	CA	C	C	TA	CGG	C INS TT
NOD/LtJ	GA	T	TAA INS T	TA	CA	C	C	TA	CGG	C INS TT
SJL/J	GA	T	TAA INS T	TA	CA	C	C	TA	CGG	C INS TT
FVB/NJ	GA	T	TAA INS T	TA	CA	C	C	TA	CGG	C INS TT

Figure 5 Association between a single haplotype and the albinism phenotype caused by a mutation at the tyrosinase locus[20]. Columns show SNPs discovered in ten 500-bp assays with positions (kb) relative to the centre of the genomic segment containing the gene (GenBank accession GI:12852585). The causal mutation (Cys103Ser) is located at +32.6 kb. The association between phenotype and ancestral haplotype for 12 strains would be sufficient to identify a haplotype background and 'critical region' of ~500 kb (including the assays from −100 kb before Tyr to 200 kb after Tyr) likely to contain the albinism mutation.

Wade et al., Nature 420:574-578 (2002)

Complex tra ic modifiers

Many traits are influen g standard pedigree analysis. Experiments oblem that can be used to map such **qua** e entire genomes of experimental organism omato.



mass
pH
brix

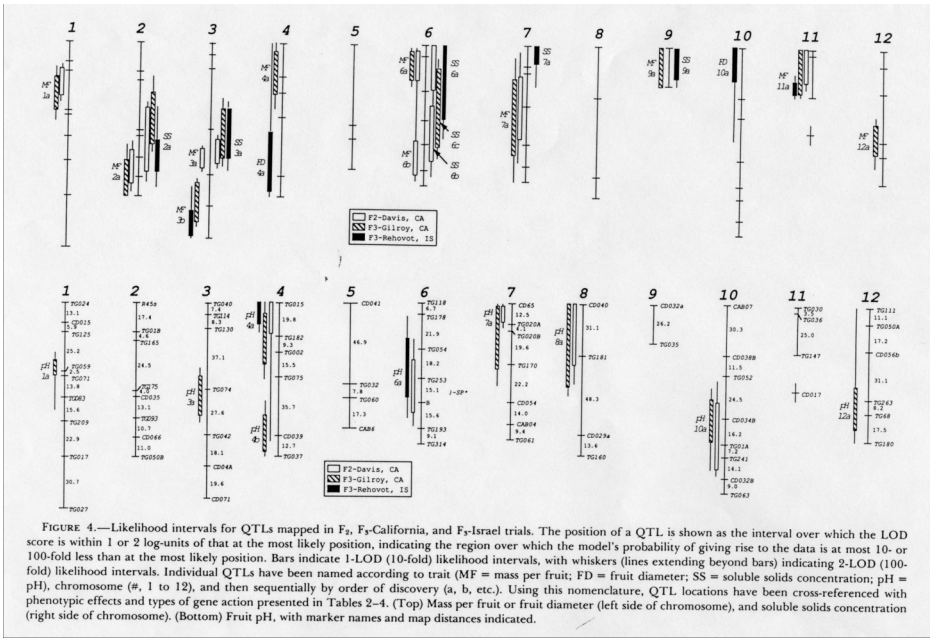
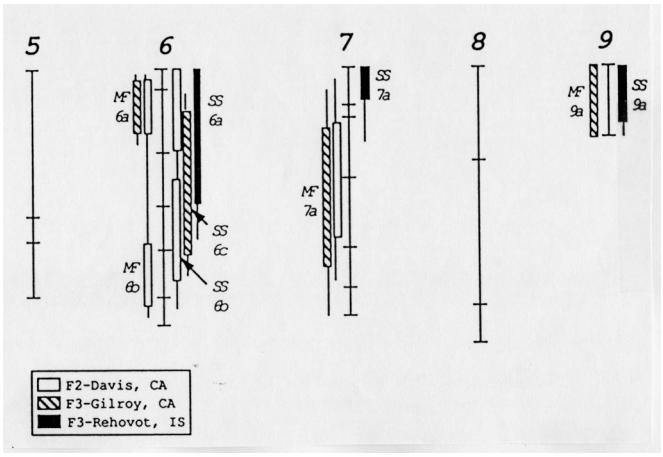


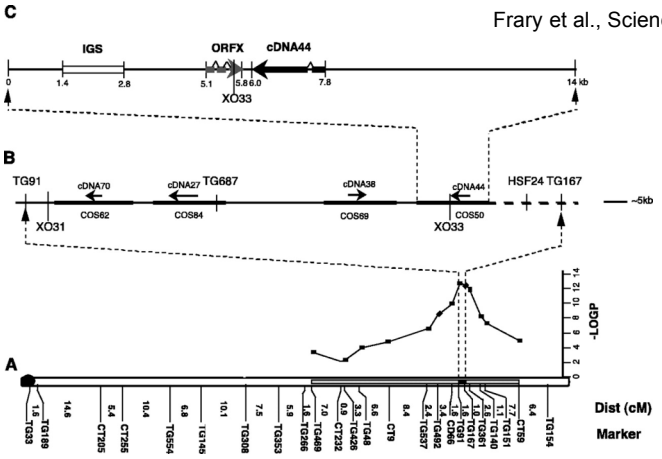
FIGURE 4.—Likelihood intervals for QTLs mapped in F_2, F_3-California, and F_3-Israel trials. The position of a QTL is shown as the interval over which the LOD score is within 1 or 2 log-units of that at the most likely position, indicating the region over which the model's probability of giving rise to the data is at most 10- or 100-fold less than at the most likely position. Bars indicate 1-LOD (10-fold) likelihood intervals, with whiskers (lines extending beyond bars) indicating 2-LOD (100-fold) likelihood intervals. Individual QTLs have been named according to trait (MF = mass per fruit; FD = fruit diameter; SS = soluble solids concentration; pH = pH), chromosome (#, 1 to 12), and then sequentially by order of discovery (a, b, etc.). Using this nomenclature, QTL locations have been cross-referenced with phenotypic effects and types of gene action presented in Tables 2–4. (Top) Mass per fruit or fruit diameter (left side of chromosome), and soluble solids concentration (right side of chromosome). (Bottom) Fruit pH, with marker names and map distances indicated.



"PURE" positionally cloned QTL in *lypersicum*

Fig. 2. High-resolution mapping of the fw2.2 QTL. (A) The location of fw2.2 on tomato chromosome 2 in a cross between L. esculentum and a NIL containing a small introgression (gray area) from L. pennellii [from (8)]. (B) Contig of the fw2.2 candidate region, delimited by recombination events at XO31 and XO33 [from (8)]. Arrows represent the four original candidate cDNAs (70, 27, 38, and 44), and heavy horizontal bars are the four cosmids (cos62, 84, 69, and 50) isolated with these cDNAs as probes. The vertical lines are positions of restriction fragment length polymorphism or cleaved amplified polymorphism (CAPs) markers. (C) Sequence analysis of cos50, including the positions of cDNA44, ORFX, the A-T-rich repeat region, and the "rightmost" recombination event, XO33.

Frary et al., Science 289:85 (2000).



From QTL to gene: the harvest begins

In the past decade, quantitative trait locus (QTL) mapping has identified hundreds of chromosomal regions containing genes affecting asthma, atherosclerosis, diabetes, hypertension, obesity and other complex phenotypes. The ultimate goal of QTL mapping is to identify the genes underlying these polygenic traits and to gain a better understanding of their physiology and biochemistry. But identifying the QTL genes has been slow and difficult. A commentary in *Nature Genetics* questioned the value of QTL mapping and proposed that mutagenesis strategies offer more promise for identifying the genes determining complex traits[1]. That pessimistic opinion was premature; we herein report that 29 QTL genes have been identified, almost half of them in 2001. We suggest that QTL mapping of complex traits is a promising technique and that the harvest of QTL genes is just beginning.

The 29 genes identified from mammalian QTL through the end of 2001 are from humans, mice, rats and pigs (Table 1; Fig. 1). We counted the genes identified for common complex diseases, whether the study in question used a quantitative measure (for example, bronchial hyperresponsiveness) or a qualitative evaluate (for example, clinically diagnosed asthma) to measure the trait. We counted any trait for which linkage was first found by QTL analysis or by human linkage studies showing several genes, even though the next stage of analysis involved reducing the trait to a mendelian pattern of inheritance by constructing a congenic strain (animal studies) or selecting only those families linked to a particular region (Alzheimer disease and breast cancer in human studies). These genes are related to many phenotypes, from cancer, asthma, obesity, diabetes and Crohn disease to hearing loss and meat quality. Some were identified because they were obvious candidates in a QTL region, whereas others required positional cloning (Table 1).

A total of only 8 genes were identified from 1991–1998, but 4 were identified in 1999, 7 in 2000 and 11 in 2001. This acceleration reflects the increasing availability of simple sequence length polymorphisms (SSLPs), which facilitated mapping; publication of the human and mouse genomic sequences, which eliminated the need to build contigs experimentally; and comparative genomics, which facilitated the identification of genes. The availability of microarray technology will probably accelerate QTL gene identification; already microarrays have been used to identify *CD36* and *C5* (*Hc*), genes underlying insulin resistance and asthma, respectively[2,3].

The analysis of QTL has also been made easier by recently developed statistical tools[4] for detecting gene–gene interactions. For example, gene association studies yielded conflicting results as to whether the vitamin D receptor affects bone density;

Table 1 • Genes identified from QTL studies

Polygenic trait	Year	Ref.	Gene	Species	pos	tg	ko	fu
Alzheimer disease	1991	9	*APP*	human				X
Alzheimer disease	1993	10	*APOE*	human				
Ovarian and breast cancer	1994	11	*BRCA1*	human	X			X
Breast cancer	1995	12	*BRCA2*	human	X			X
Insulin resistance	1995	13	*FABP2*	human				
HDL-cholesterol levels	1997	14	*LIPC*	human				
Intestinal cancer	1997	15	*Pla2g2a*	mouse	X	X		
Blood pressure	1998	16	*Atp1a1 / ATP1A1*	rat/human		X		X
Leptin levels	1999	17,18	*POMC*	human			X[a]	X
Asthma	1999	19	*Il4*	mouse	X	X		
Asthma	1999	19	*Il13*	mouse	X	X		
Insulin-mediated glucose uptake	1999	2	*Cd36*	rat		X		
Obesity	2000	20	*Ptpn1/PTPN1*	mouse/human			X[b]	X
Alzheimer disease	2000	21	*PSEN1*	human	X			
Diabetes	2000	22	*Il2*	mouse	X		X[b]	X
Gallstones	2000	23	*Abcc2*	mouse	X			X
Asthma	2000	3	*Hc*	mouse				
Muscle glycogen content	2000	24	*Prkag3*	pig	X		X[c]	X
Crohn disease	2001	25,26	*NOD2*	human	X		X[a]	X
Blood pressure	2001	27	*SCNN1A1*	human			X[a]	
Blood pressure	2001	28	*SCNN1G*	human			X[a]	
Blood pressure	2001	29	*Slc12a1*	rat				
Blood pressure	2001	30	*Cyp17b1*	rat				X
Bone density	2001	5	*COL1A*	human				
Left ventricular mass	2001	31	*Nppa*	rat			X[b]	X
Modifier of tubby hearing	2001	32	*Mtap1a*	mouse	X	X		X
Taste, saccharin response	2001	33	*Tas1r3*	mouse	X	X		X
Tumor susceptibility	2001	34	*Cdkn2a*	mouse	X		X[b]	X
Diabetes	2001	35	*B2m*	mouse		X	X	

pos, found by positional cloning; tg, transgenic insertion of normal gene changes phenotype to normal (for example, transgenic rescue); ko, knockout provides additional evidence (human monogenic syndrome, [a]deletion of gene by homologous recombination produces a mouse with the phenotype typical of the disease, [b]knockout in yeast); fu, functional difference in candidate gene. *APP*, amyloid precursor protein; *APOE*, apolipoprotein E; *BRCA*, breast cancer gene; *FABP2*, fatty acid binding protein 2; *LIPC*, hepatic lipase; *ATP1A1*, α_1Na,K-ATPase; *POMC*, pre-pro-opiomelanocortin; *Il*, interleukin; *Cd36*, fatty acid translocase; *PTP1B*, protein tyrosine phosphatase-1B; *PSEN*, presenilin 1; *Abcc2*, ATP-binding cassette, subfamily C2; *Hc*, hemolytic complement (C5); *Prkag3*, protein kinase, AMP-activated, γ3; *NOD2*, caspase recruitment domain-containing protein 15 (*CARD15*); *SCNN*, sodium channel, non-voltage gated; *Slc12a1*, $Na,K,2Cl$-cotransporter; *Cyp17b1*, 17β-hydroxylase; *COL1A*, collagen-1A; *Nppa*, natriuretic peptide precursor A; *Mtap1a*, microtubule-associated protein 1a; *Tas1r3*, taste receptor-3; *Cdkn2a*, cyclin-dependent kinase inhibitor 2a; *B2m*, β2-microglobulin.

Mapped QTLs

R Korstanje & B Paigen
Nature Genetics **31**, 235 - 236 (2002).

Intercross analysis shows that Hpi1 is epistatic to Hpi2.

Genotype at *Hpi2*, Chromosome 5

Genotype at *Hpi1*, Chromosome 13	A/A		A/B		B/B		Totals	
A/A	33.5 (9)	± 4.6	35.6 (12)	± 4.8	35.6 (8)	± 6.9	35.0	± 3.0
A/B	28.9 (11)	± 5.0	35.7 (40)	± 3.0	37.8 (11)	± 4.8	34.9	± 2.3
B/B[b]	42.5 (2)	± 4.1	44.7 (14)	± 5.3	69.9[c] (11)	± 5.5	54.8	± 4.3
Totals	32.0	± 3.2	37.6	± 2.3	49.0	± 4.3	39.5	± 1.9

[a] Avg. number of PMN per h.p.f. ± s.e. are given for (n) animals of each genotype class.[b] Mice with a B/B genotype at *Hpi1* showed significantly higher PMN infiltration values than other *Hpi1* genotypes (p=1.22 X 10-4, t-test assuming unequal variance)[c] Mice with a B/B genotype at both Hpi1 and Hpi2 showed significantly higher PMN infiltration than other genotype classes (p=7.83X10-5, t-test assuming unequal variance)

Matesic, LE, EL Niemitz, A De Maio, and RH Reeves. 2000. Quantitative trait loci modulate neutrophil infiltration in the liver during LPS-induced inflammation. Matesic et al., FASEB Journal 14:2247-54.

Mouse phenome project

MPD Priority Set of Strains

GROUP A	GROUP B	GROUP C	GROUP D
B17 129S1/SvImJ	B85 AKR/J	D41 BUB/BnJ	none BTBR T+ tf/tf
B68 A/J	E4 C57L/J	E9 C57BL/10J	E5 C57BR/cdJ
B78 BALB/cByJ	E2 C58/J	E23 C57BLKS/J	D110 CE/J
B47 C3H/HeJ	F25 MOLF/Ei	B55 CBA/J	B29 I/LnJ
E12 C57BL/6J	A14 NOD/LtJ	F13 CZECHII/Ei	F34 JF1/Ms
F22 CAST/Ei	B112 NZB/BINJ	C29 KK/HlJ	E10 MA/MyJ
B42 DBA/2J	F48 PERA/Ei	B7 LP/J	A13 NON/LtJ
A25 FVB/NJ	D75 PL/J	F30 MSM/Ms	B114 NZW/LacJ
A29 SJL/J	B37 SM/J	D107 RIIIS/J	F10 PWK/Ph
F14 SPRET/Ei	A33 SWR/J	F38 WSB/Ei	B35 SEA/GnJ

- See explanation of color coding.
- BALB/cJ may be used at investigator's discretion.
- 129S1/SvImJ was renamed in Feb '01. Previous name was 129S3/SvImJ.
- BTBR T+ tf/tf was renamed in May '02. Previous name was BTBR+Ttf/tf.

www.jax.org/pheno

Transgenesis/ gene-targeting/ chromosome engineering

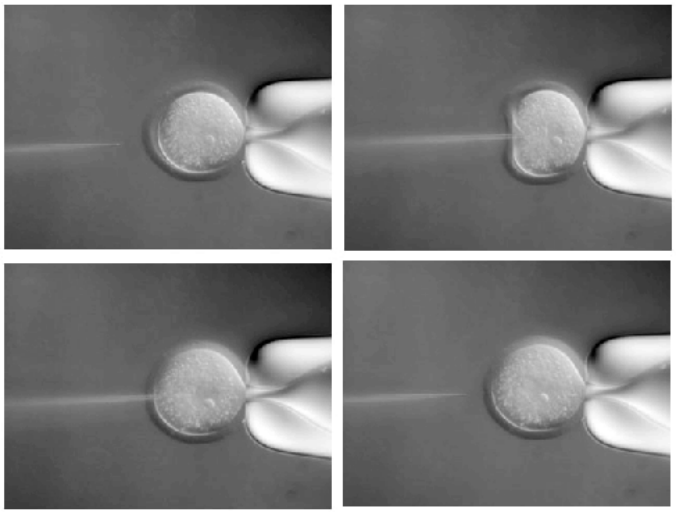
1. Transgenic mice (pronuclear injection) Hogan B, Beddington R, Constantini F, Lacy E. Manipulating the Mouse embryo: A laboratory manual. 1994. Cold Spring Harbor Laboratory Press.

2. "Knock out" (null alleles) and "Knock-in" mice (mutations, reporters), tissue-targeted and conditional mutations
   Shin MK, Levorse JM, Ingram RS, Tilghman SM. The temporal requirement for endothelin receptor-B signalling during neural crest development. Nature. 1999 Dec 2;402(6761):496-501.

3. Chromosome engineering
   Ramirez-Solis R, Liu P, Bradley A. Chromosome engineering in mice. Nature. 1995 378(6558):720-4.

4. Whole genome gene targeting
   Zheng B, Mills AA, Bradley A. A system for rapid generation of coat color-tagged knockouts and defined chromosomal rearrangements in mice. Nucleic Acids Res. 1999 27(11):2354-60.
   Zambrowicz BP, Friedrich GA, Buxton EC, Lilleberg SL, Person C, Sands AT. Disruption and sequence identification of 2,000 genes in mouse embryonic stem cells. Nature. 1998 392(6676):608-11.

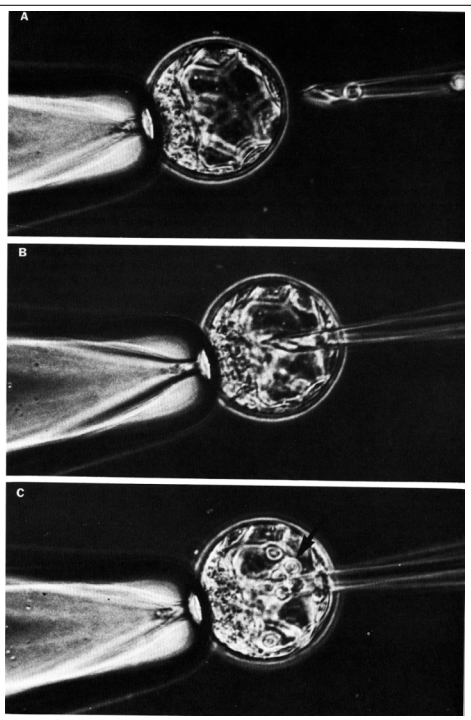
Micro-injection of DNA into a male pronucleus to create a transgenic mouse.



http://www.med.ic.ac.uk/db/dbbm/tgunit.htm

Blastocyst injection

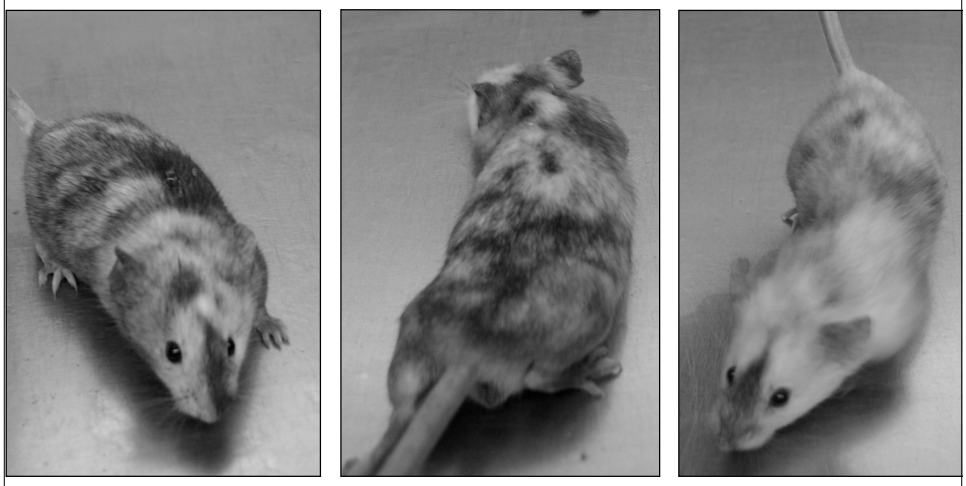
Embryonic stem cells can be manipulated in culture, then injected into mouse blastocysts to form part of the inner cell mass (ICM). The ICM will form the embryo proper while the surrounding trophectoderm cells contribute to extra-embryonic structures (placenta).



Hogan et al., Manipulating the Mouse Embryo, CSHL Press

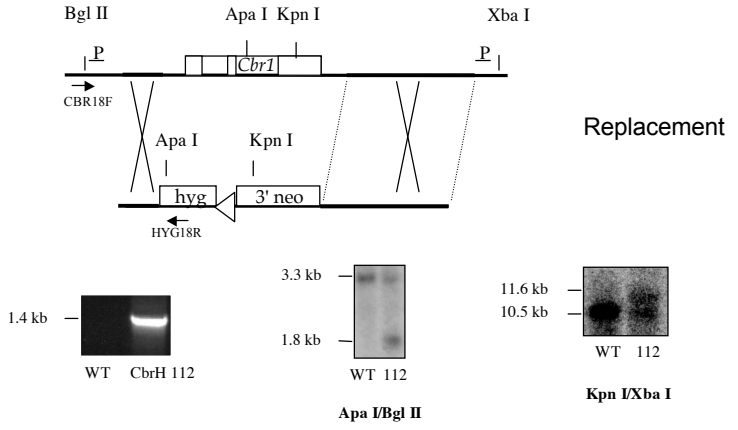
Chimeras formed from ES cells and host cells.

A chimeras has four parents, but individual cells have genetic information from only one of the two pairs.



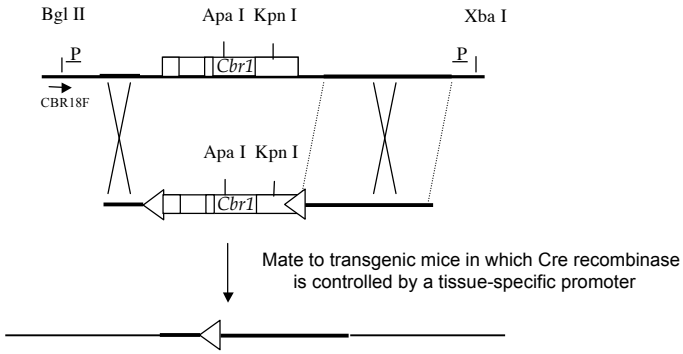
Manipulating the germ-line in embryonic stem cells

"Knock out" mice create null alleles to study loss of function.



Manipulating the germ-line in embryonic stem cells

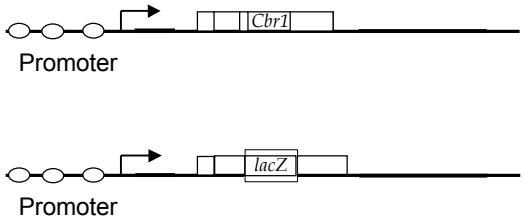
Conditional knock-outs allow a much greater range of studies for similar investment of effort.



A null allele of *Cbr1* can now be created in a specific tissue type.

Manipulating the germ-line in embryonic stem cells

"Knock-in" mice (mutations, reporters), allow precise temporal and spatial regulation of gene expression.



“Two stage” strategies control gene expression in space and time

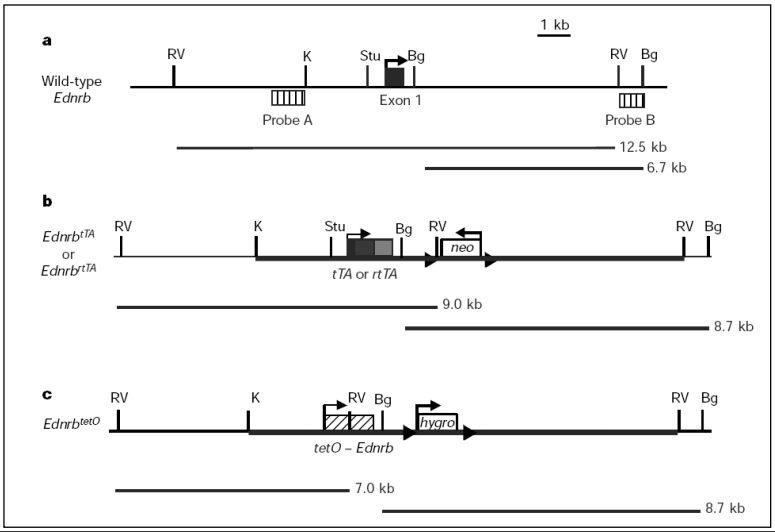
a. Cre-recombinase with a cell type-specific promoter plus marker gene

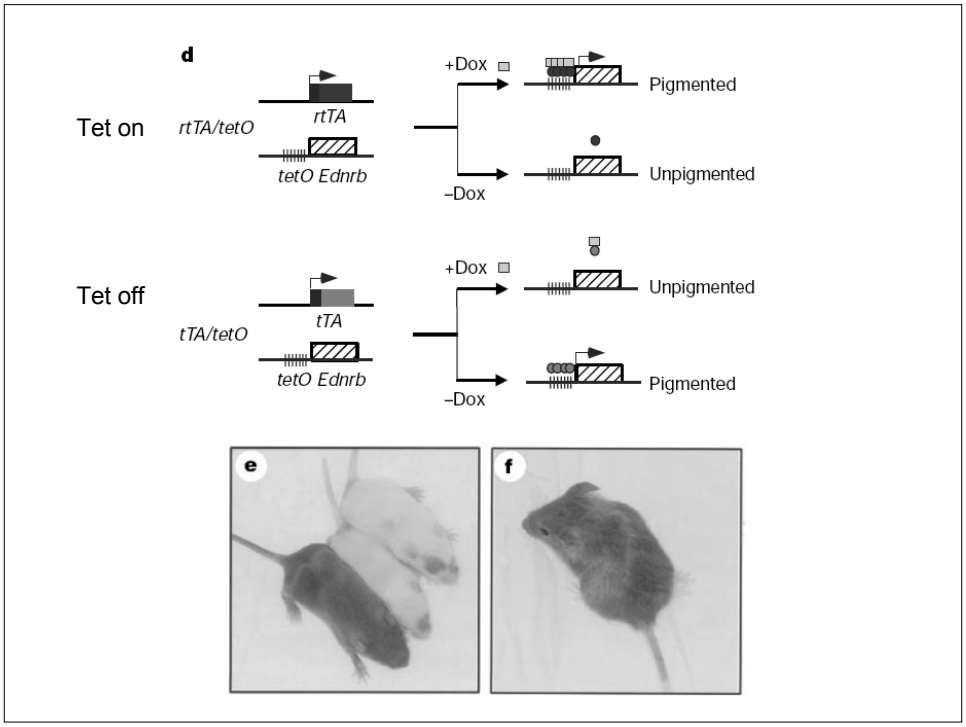
b. Transcriptional activators under pharmaceutical control

articles

The temporal requirement for endothelin receptor-B signalling during neural crest development

Shin et al., Nature 402:496, 1999





Chromosome Engineering

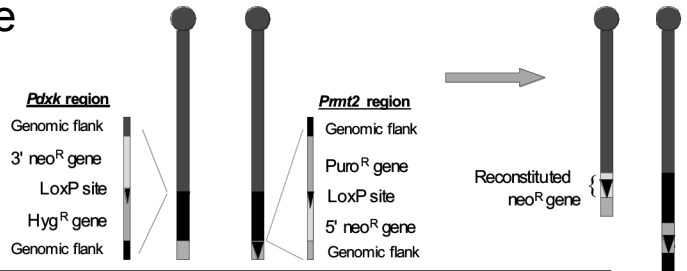
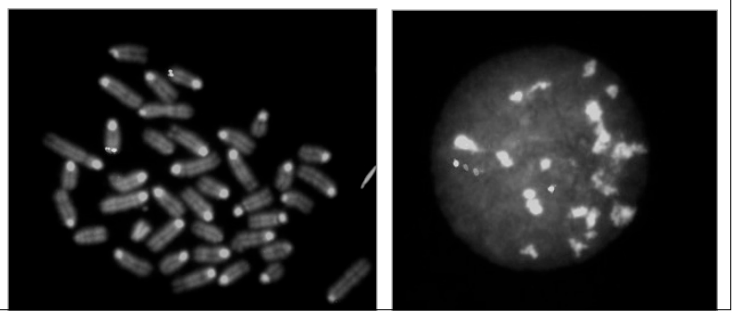


Fig. 8. Targeting lox sites asymmetrically by homologous recombination in ES cells provides target sites to create a reciprocal translocation by expressing Cre recombinase.

1) Olson et al., Science 306:687, 2004. 2) Adams et al., Nat Genet. 2004 Aug;36(8):867, 2004



Mapping Genome Function: Creating Phenotypes using Mutagenesis

Mutagenesis provides a means of generating new phenotypes in mouse.

1. Justice, M. J. 2000. Capitalizing on large-scale mouse mutagenesis screens. Nat Rev Genet 1: 109-15. Review.
2. MJ Justice *in* IJ Jackson and CM Abbott, Mouse Genetics and Transgenics: A Practical Approach. 2000. Oxford University Press, 299 pp.

Mutagenesis provides a means of generating new phenotypes in mouse.

1. Sources of mutations
   - Spontaneous, frequency is 10-5/locus/generation, all types of mutations;
   - Radiation, frequency is dose dependent, primarily chromosomal rearrangement;
   - Chemical, ENU gives point mutations at 1/600 gametes per locus at some loci

2. Targets/ mutation types
   - Visible single gene dom. or recessive
   - Allelic series
   - Biochemical pathway
   - Sensitization (Shedlovsky A, McDonald JD, Symula D, Dove WF. Mouse models of human phenylketonuria. Genetics. 1993. 134:1205-10).

Mutagenesis provides a means of generating new phenotypes in mouse.

3. Screens
   - •Specific locus test
   - •MutaMouse/ Big Blue
   - •SHIRPA
   - •Special targeted screens
   - •Dominant vs. recessive (1st vs. 3rd generation)
   - •Mutagenesis in combination with deletion (recessives in first generation)

4. Breeding schemes
   - Recessive over deletion;
   - Modifier (dominant mutation modifies another mutation)
   - Sensitization (recessive mutations in genes that interact in a pathway/ allelic

Large mutagenesis centers, see Trans-NIH Mouse Initiative

http://www.nih.gov/science/models/mouse/index.html

- Mouse Genome Center, ENU Mutagenesis Programme, Harwell, http://www.mgu.har.mrc.ac.uk/mutabase/
- German ENU Mutagenesis Center, http://www.gsf.de/isg/groups/enu-mouse.html

Enhancer, gene and promoter trapping

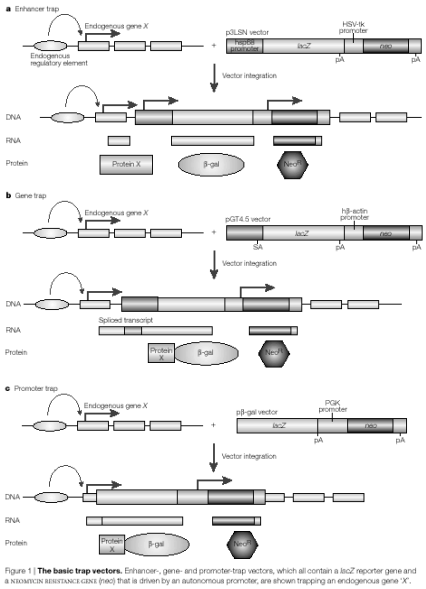
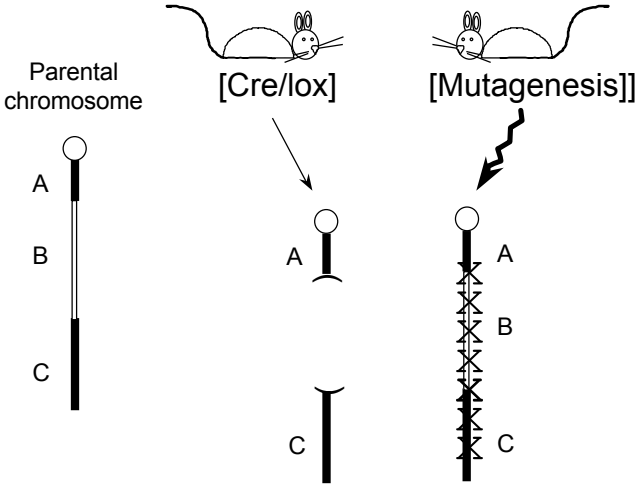


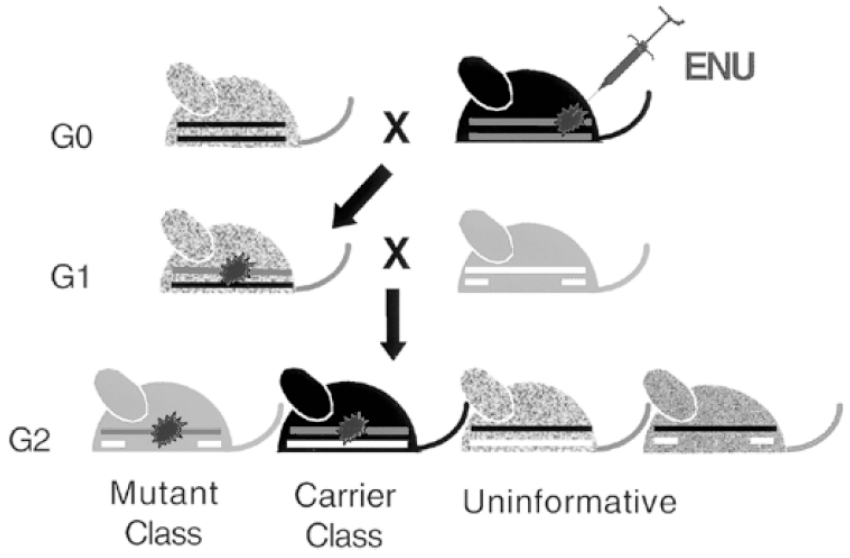
Figure 1 | **The basic trap vectors.** Enhancer-, gene- and promoter-trap vectors, which all contain a *lacZ* reporter gene and a NEOMYCIN RESISTANCE GENE (neo) that is driven by an autonomous promoter, are shown trapping an endogenous gene 'X'.

Stanford et al., Nature Reviews Genetics 2, 756-768 (2001)

Uncovering recessive mutations with a deletion/ ENU mutagenesis screen

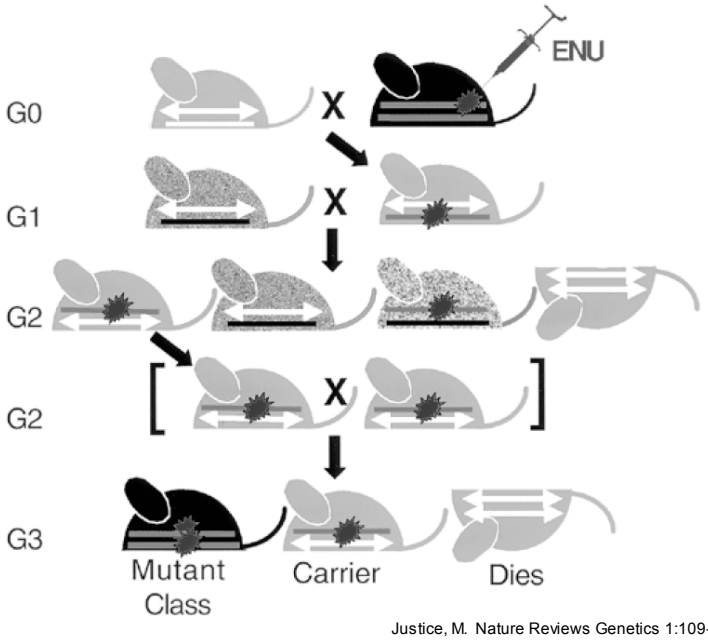


ENU mutagenesis in balancer strains

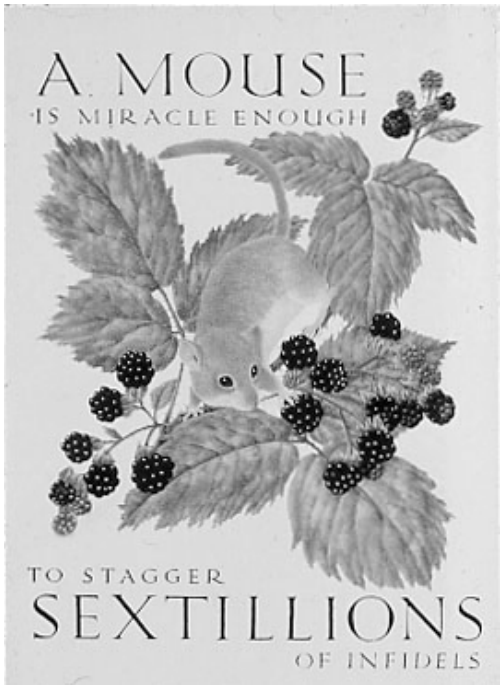


Justice, M. Nature Reviews Genetics 1:109-116 (2000)

ENU mutagenesis in balancer strains



Justice, M. Nature Reviews Genetics 1:109-116 (2000)



Walt Whitman