

## A genome-wide set of congenic mouse strains derived from DBA/2J on a C57BL/6J background

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### Abstract

In the analysis of complex traits, congenic strains are powerful tools because they allow characterization of a single locus in the absence of genetic variation throughout the remainder of the genome. Here, we report the construction and initial characterization of a genome-wide panel of congenic strains derived from the donor strain DBA/2J on the background strain C57BL/6J. For many strains, we have carried out high-density SNP genotyping to precisely map the congenic interval and to identify any contaminating regions. Certain strains exhibit striking variation in litter size and in the ratio of females to males. We illustrate the utility of the set by “Mendelizing” the complex trait of myocardial calcification. These 65 strains cover more than 95% of the autosomal genome and should facilitate the analysis of the many genetic trait differences that have been reported between these parental strains.

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### Introduction

Because of the extensive synteny between mouse and human genomes, the largely similar metabolism between mammalian species, and the large number of well-characterized inbred mouse strains, it is now clear that the mouse will play an important role in mapping and identifying genes in human disease and various complex traits [1,2]. Moreover, studies in mice provide a means of examining physiological,

genetic, and environmental interactions that will be extremely difficult to analyze directly in humans [3]. Efforts to make use of the rich naturally occurring variation in complex traits that exists among inbred strains of mice and rats have largely involved QTL mapping, an approach that attempts to statistically link inheritance of disease-related phenotypes with the parental origins of the DNA in segregating crosses. While QTL mapping has been remarkably successful at identifying broad genetic loci, attempts to more finely localize the underlying genes have proven difficult.

A very powerful resource in this effort is the congenic mouse. Congenic mice were first introduced by George Snell in the 1940s. He used them to isolate loci contributing to transplantation, in the process discovering the major histocompatibility complex, known as H2 in the mouse [4]. In essence, a congenic strain can “Mendelize” a complex trait, such that a single gene contributes to a phenotype between the

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background strain and the congenic strain. This allows a QTL to be confirmed and, more importantly, fine-mapped.

Unfortunately, congenic strains are costly and time consuming to construct, even using a marker-assisted protocol to accelerate the removal of contaminating donor regions [5,6]. It is much easier, though still time consuming, to construct recombinant inbred (RI) strains and recombinant inbred congenic (RIC) strains. For RI strains, pairs of progeny from an F2 cross are brother sister mated to produce new inbred strains each carrying approximately equal proportions of genes from both parental strains but randomly distributed over the chromosomes. Numerous sets of RI strains are available and can be very powerful gene-mapping tools as recently demonstrated by Chesler et al. [7] in their efforts to identify the genetic basis of brain morphology. Less commonly, investigators have constructed sets of “recombinant inbred congenic” strains by repetitive backcrossing of one parental strain to the other followed by inbreeding [8,9]. In the case of RICs, each strain carries about 10% of the genome from a donor parental strain with 90% derived from a recipient parental strain. Such RIC strains have been used to identify genes underlying a number of complex traits,

including cancer modifiers [8] and genes affecting susceptibility to malaria [10]. Nadeau and colleagues constructed a set of “consomic” strains in which an entire chromosome from one strain is substituted on the background of a second strain [11].

We have been developing two sets of congenic strains in which the congenic regions span the genome with the donor strains DBA/2J (DBA) or CAST/EiJ on a C57BL/6J (B6) background. We previously reported our progress in the first three generations of construction using microsatellite-assisted breeding [12]. We have now completed the construction of the homozygous B6.DBA panel and we report here the characteristics of this panel. The mice have been made freely available to interested researchers and have been deposited in The Jackson Laboratory strain repository.

## Results

We initiated the construction of a genome-wide set of congenic strains with strain DBA as donor strain and B6 as

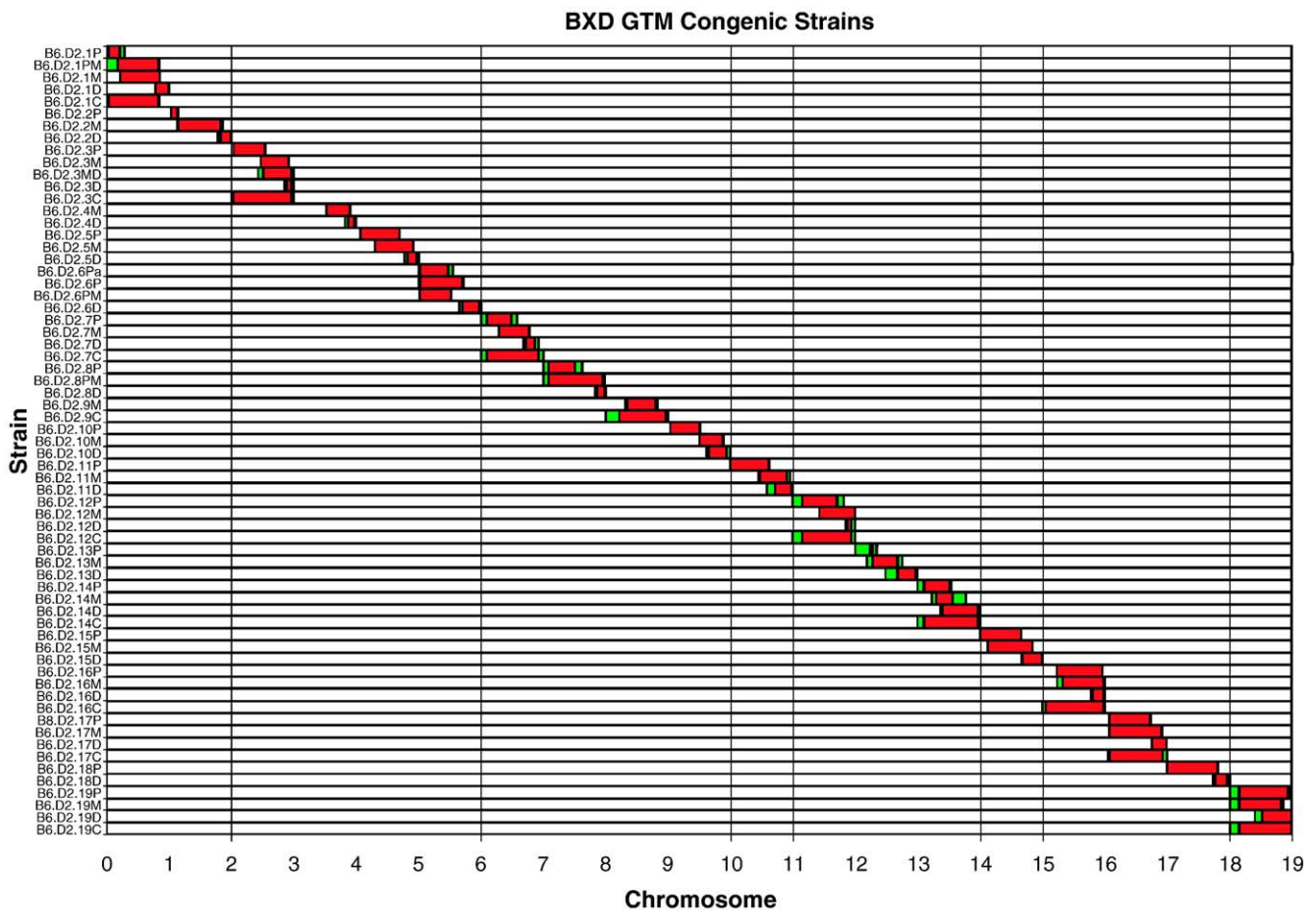


Fig. 1. Map positions (bp) of the introgressed segments in the congenic lines following 5 cM density genotyping. Most chromosomes are covered by proximal (P), middle (M), and distal (D) congenic strains. Some chromosomes are represented by additional strains carrying whole, or nearly whole, chromosomes of DBA. Such consomics are indicated by “C” in the strain name. Red indicates the chromosomal region known by mapping to be included in the congenic. Green indicates the distance to the nearest flanking marker.

the recipient strain a number of years ago, and we previously reported our progress for the initial stages of the construction [12]. We recently completed the marker-assisted backcrossing and the selection for homozygosity of the congenic strains and now report their completion and initial characterization. The set consists of 64 inbred strains with overlapping congenic regions covering nearly the entire genome with the exception of the sex chromosomes. These strains have been deposited with The Jackson Laboratory, and, thus, they are freely available to interested investigators. We maintain a website in which the updated characteristics of the congenic set will be available: <http://www.genetics.ucla.edu/GTM>.

The approximate boundaries of the set of strains are presented in Fig. 1. For most chromosomes, we have derived congenics that represent the proximal, middle, or distal regions of the chromosome, designated by P, M, and D, respectively, in the strain name. For some chromosomes, there are consomic or near-consomic strains designated by C in the strain name. For a few chromosomes, there are additional strains carrying introgressed regions covering both proximal and middle regions (designated PM) or middle and distal regions (designated MD).

We have now performed high-density SNP mapping of many of the congenic strains. For this, the parental strains and congenic strains were analyzed for ~12,000 common SNPs, allowing precise mapping of the congenic intervals (Fig. 2, Table 1). Fig. 2 shows the SNP genotype map for the congenic strain B6.D2.1M. Horizontal bars on the left of the figure show the positions of all STPs that were genotyped on Chr.1. Bars that extend to the middle of the figure represent those SNPs that were informative between B6 and DBA. Bars that extend to the right-hand side of the figure represent those informative SNPs that showed the DBA/2 allele in B6.D2.1M. Thus, the 1M congenic region begins at about 44 Mbp and extends to about 170 Mbp. The arrows show the location of the proximal (P) and distal (D) microsatellite markers used for selection of the introgressed region during construction of the congenic strain. SNP genotype maps for the other strains are presented online in supplementary Fig. 2. In Table 2, we show any residual regions of donor (DBA/2) DNA outside of the congenic regions detected by high-density SNP mapping. In approximately half the tested strains we identified small regions of DBA alleles outside of the congenic interval. Thus, the level of residual contamination is quite small and may be traced using the SNP marker data provided.

As shown in Fig. 2, there are regions up to 10 Mbp where no informative SNPs were identified between B6 and DBA. This result is consistent with the hypothesis that gaps in informative SNPs represent regions of shared ancestry between the B6 and the DBA strains. To verify that these gaps in informative SNPs represent regions of higher sequence conservation between B6 and DBA, we used the Celera sequence databases [13] to identify all SNPs between these two strains. For example, Fig. 3 shows

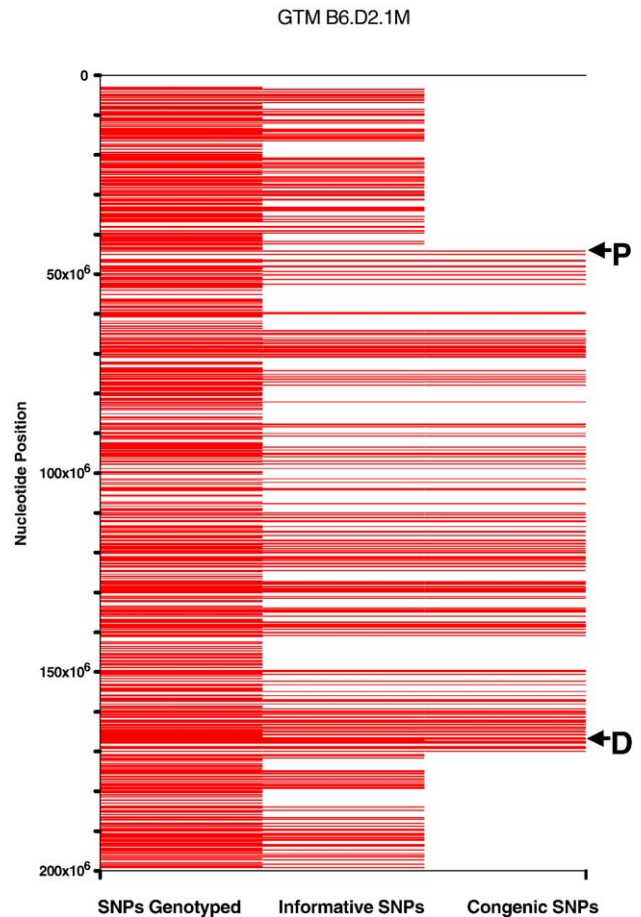


Fig. 2. SNP mapping of the introgressed segment in B6.D2.1M. Horizontal lines indicate the location, in bp, of SNPs along the chromosome (centromere at the top). The left-hand set of lines indicates the positions of all 924 SNPs tested on chromosome 1. Lines that extend to the center indicate those 376 SNPs that were informative between B6 and DBA. Lines that extend to the right-hand side indicate those SNPs showing the DBA/2 allele in the congenic. For maps of the other chromosomes, see Fig. 2, supplemental. Positions of the proximal (P) and distal (D) microsatellite markers used during construction of the congenic are indicated by arrows.

a comparison of resulting SNP density along chromosome 16 compared with the positions of informative SNPs genotyped for this report. It is clear that there is a very strong correspondence between regions of low overall SNP density and the gaps in the set of informative SNPs typed here. These regions were presumably inherited from a relatively recent common ancestor during the development of the strains nearly a century ago and, thus, are less likely to exhibit functional variation that may underlie quantitative trait loci (QTLs).

The breeding characteristics of the congenic strains are presented in Table 3. Very notable and consistent breeding differences were observed among the congenic strains, and several could be bred with only considerable difficulty. This is reflected in significant differences in the size of litters and the sex ratios of the progeny between the strains.

Some basic physiologic characteristics of the congenic strains have been determined and are shown in the

Table 1  
Markers defining introgressed congenic regions

Congenic	Flanking prox.	FP bp	Prox. marker	Proximal bp	Distal marker	Distal bp	Flanking Dist.	FD bp
B6.D2.1P		0	D1mit295	8,270,642	D1mit213	43,659,838	D1mit324	58,882,946
B6.D2.1PM		0	D1mit278	36,376,211	D1mit57	167,372,496	D1mit145	167,321,928
B6.D2.1M	rs4222315	43,809,590	rs3662842	44,143,758	rs6412182	169,881,502	rs4222785	169,911,486
B6.D2.1D	rs3688785	155,962,218	rs6267646	155,979,491	mCV24145570	199,245,316	mCV24145570	199,245,316
B6.D2.1C		0	D1mit295	8,270,642	D1Mit57	167,372,496	D1Mit145	167,321,928
B6.D2.2P	rs3678168	5,576,815	mCV25103560	5,888,354	rs3681655	26,634,960	mCV23574676	27,146,909
B6.D2.2M	D2mit32	25,611,096	D2mit151	26,757,884	D2mit405	150,385,381	D2mit286	156,358,310
B6.D2.2D	D2mit422	143,062,840	D2mit405	150,385,381	D2mit457	180,673,957		181,655,546
B6.D2.3P	rs6247681	6,002,977	mCV23626472	6,523,929	rs3726528	89,735,357	rs4224044	90,038,587
B6.D2.3M	rs6322772	78,327,397	rs3653769	78,507,668	rs3022971	150,653,009	rs6250754	150,832,665
B6.D2.3MD	D3mit22	70,581,096	D3mit97	84,218,446	D3mit163	157,273,873		164,265,201
B6.D2.3D	D3mit194	139,404,629	D3mit259	147,005,845	D3mit163	157,273,873		164,265,201
B6.D2.3C		0	D3mit60	6,737,582	D3mit163	157,273,873		164,265,201
B6.D2.4M	rs6309721	81,856,180	rs3674783	82,471,285	rs6250787	140,808,075	rs4224893	140,981,824
B6.D2.4D	D4mit203	127,663,411	D4mit251	134,989,216	D4mit209	151,064,577		155,096,104
B6.D2.5P	rs3659713	10,609,330	rs3714258	10,945,199	rs6218435	104,171,620	rs3722596	104,385,813
B6.D2.5M	rs3694939	44,878,782	rs6315432	45,458,047	rs3701629	137,805,717	rs4225541	137,809,460
B6.D2.5D	D5mit431	117,293,634	D5mit370	124,156,134	D5mit223	144,668,511		150,139,705
B6.D2.6Pa		0	D6mit86	4,167,068	D6mit16	71,526,812	D6mit69	84,109,548
B6.D2.6P	rs4225651	3,156,699	rs3661828	3,165,742	rs3663024	78,580,432	rs6412632	78,689,920
B6.D2.6D	D6mit230	98,883,927	D6mit149	106,666,147	D6mit15	146,783,077		150,465,925
B6.D2.7P		0	D7mit265	14,404,862	D7mit62	71,778,812	D7mit321	85,129,145
B6.D2.7M	rs3023125	42,034,947	rs3680765	42,107,515	rs6322316	112,745,946	rs6258892	113,728,223
B6.D2.7D	D7mit328	98,538,811	D7mit253	103,834,006	D7mit291	125,499,057	D7mit259	133,810,804
B6.D2.7C		0	D7mit265	14,404,862	D7mit259	133,810,804		144,519,782
B6.D2.8P		0	D8mit256	11,756,348	D8mit29	67,134,871	D8mit106	82,445,598
B6.D2.8PM		0	D8mit256	11,756,348	D8mit42	126,174,392	D8mit56	128,673,808
B6.D2.8D	D8mit113	108,302,379	D8mit318	113,407,519	D8mit56	128,673,808		130,865,253
B6.D2.9M	D9mit206	40,465,133	D9mit329	43,463,185	D9mit115	101,650,063	D9mit77	104,501,941
B6.D2.9D	D9mit301	57,105,731	D9mit259	70,050,580	D9mit151	121,482,582		126,510,050
B6.D2.9C		0	D9mit64	28,444,701	D9mit151	121,482,582		126,510,050
B6.D2.10P	rs8244291	4,595,881	rs6185923	4,856,264	mCV22766787	68,268,511	rs6263039	68,359,010
B6.D2.10M	rs6172306	67,671,065	mCV22766787	68,268,511	rs3706484	118,064,029	rs4228488	118,337,950
B6.D2.10D	D10Mit42	82,236,495	D10Mit209	88,065,150	D10Mit145	125,141,573		133,230,318
B6.D2.11P	rs4228736	740	rs3721534	1,792	rs8241202	76,539,840	rs8247972	76,540,491
B6.D2.11M	D11Mit272	56,424,677	D11Mit208	59,231,252	D11Mit291	112,450,205	D11Mit103	118,040,201
B6.D2.11D	D11Mit279	72,360,343	D11Mit39	88,577,147	D11Mit104	120,186,501		123,310,986
B6.D2.12P		0	D12Mit269	16,780,275	D12Mit76	81,897,398	D12Mit30	94,595,517
B6.D2.12M	rs6370023	49,757,120	rs3689063	50,305,248	rs3679276	115,981,123	rs3679276	115,981,123
B6.D2.12D	D12Mit289	97,026,496	D12Mit232	101,158,439	D12Mit134	108,830,560		115,981,123
B6.D2.12C		0	D12Mit269	16,780,275	D12Mit134	108,830,560		115,981,123
B6.D2.13P		0	D13Mit79	29,524,010	D13Mit198	34,369,884	D13Mit63	42,085,609
B6.D2.13M	D13Mit217	22,665,651	D13Mit198	34,369,884	D13Mit314	82,205,288	D13Mit258	91,236,948
B6.D2.13D	D13Mit186	58,841,231	D13Mit314	82,205,288	D13Mit35	116,310,890		121,083,641
B6.D2.14P		0	D14Mit244	11,725,873	D14Mit39	59,213,108	D14Mit68	62,973,703
B6.D2.14M	D14Mit174	26,373,058	D14Mit212	34,733,669	D14Mit239	65,340,508	D14Mit228	90,399,852
B6.D2.14D	D14Mit257	42,633,369	D14Mit84	46,872,245	D14Mit136	112,147,361		116,386,191
B6.D2.14C		0	D14Mit244	11,725,873	D14Mit136	112,147,361		116,386,191
B6.D2.15P	rs3724697	9,211	rs6348185	11,461	rs6363050	69,945,774	rs4138576	69,961,179
B6.D2.15M	rs3693764	12,768,882	rs3681808	13,071,095	rs3710055	89,700,473	rs3694472	89,700,804
B6.D2.15D	rs3089472	71,049,770	mCV24506549	71,554,115	rs3686133	104,469,414	rs6248280	104,767,106
B6.D2.16P	rs4165301	22,437,844	rs4174828	22,594,901	rs6233949	94,939,922	rs6375622	95,488,249
B6.D2.16M	D16Mit146	23,489,111	D16Mit103	31,769,535	D16Mit71	97,709,102		99,700,954
B6.D2.16D	D16Mit217	77,720,366	D16Mit178	80,867,778	D16Mit71	97,709,102		99,700,954
B6.D2.16C		0	D16Mit55	5,826,147	D16Mit71	97,709,102		99,700,954
B8.D2.17P	rs3724616	6,274,955	rs3694629	6,371,892	rs3657117	69,443,195	rs6192583	70,188,347
B6.D2.17M	rs3724616	6,274,955	rs3694629	6,371,892	rs3689581	87,849,282	rs6371637	88,154,034
B6.D2.17D	rs3654545	72,504,749	rs6322076	72,664,271	rs3706382	95,281,464	rs6391399	95,466,092
B6.D2.17C	D17Mit19	4,750,579	D17Mit143	7,751,108	D17Mit221	88,796,812		96,171,066
B6.D2.18PM	rs3696933	2,381	rs3696933	2,381	rs3672215	72,787,137	rs6335278	73,345,400
B6.D2.18D	D18Mit161	66,094,003	D18Mit33	70,088,095	D18Mit144	85,959,208		90,108,150
B6.D2.19P		0	D19Mit42	9,182,086	D19Mit105	56,909,195	D19Mit71	59,402,815

Table 1 (continued)

Congenic	Flanking prox.	FP bp	Prox. marker	Proximal bp	Distal marker	Distal bp	Flanking Dist.	FD bp
B6.D2.19M		0	D19Mit42	9,182,086	D19Mit70	50,400,732	D19Mit103	53,134,639
B6.D2.19D	D19Mit40	24,662,668	D19Mit13	32,052,137	D19Mit6	60,848,712		61,092,598

The proximal and distal markers for each congenic strain define the known limits of the introgressed region. The flanking-proximal and flanking-distal markers are the nearest markers tested external to the congenic region. In cases where the markers are microsatellites, such as B6.D2.1P, these represent the markers used during construction of the congenic strain. For strains where high-density SNP genotyping was carried out, the limiting SNP markers are given using the “rs” (RefSNP) prefix for SNP accession numbers from the public database and the “mCV” (mouse Celera Variant) prefix for SNPs developed by Celera. When no flanking marker is identified, the end of the chromosome is given for the flanking marker position. All marker locations given are from NCBI Build 32 of the mouse genome database. Typically, the SNP mapping defined the limits of the introgressed segment within a few hundred thousand basepairs.

supplementary data (supplemental data Table 1 and supplemental data Table 2). These include plasma lipids (total cholesterol, HDL cholesterol, unesterified cholesterol, free fatty acids), plasma glucose, and plasma insulin levels (supplemental data Table 1) as well as determinations of lean body mass and fat body mass using nuclear magnetic resonance (supplemental data Table 2). With the exception of parameters such as weight, most of these measurements were performed on a relatively small number of mice of each strain (due to budgetary restrictions). This makes it difficult to identify subtle variations that segregate between the strains. As a demonstration of the utility of the mice for the mapping and fine mapping of QTL, we have expanded certain strains that have been reported to harbor QTL.

For example, we previously mapped the complex trait of dystrophic calcification (*Dyscalc*) to proximal mouse chromosome 7 in crosses between C3H/HeJ and B6 and DBA/2J and B6 [14,15]. The trait results in the necrosis and calcification of cardiac myocytes in strain DBA but not B6 nor (DBA × B6)F1 mice. Proximal chromosome 7 exhibited the strongest evidence of linkage to the trait but other chromosomes also exhibit evidence of linkage. Fig. 4 shows that congenic strain 7P exhibit myocardial calcification in contrast to mice of the 7M strain, confirming the QTL identified between B6 and DBA and demonstrating

that the presence of proximal chromosome 7 alone can cause the presence of the trait. These congenics should enable fine mapping of the underlying gene.

## Discussion

We report the creation of a complete autosomal panel of congenic strains between the extensively studied strains B6 (background) and DBA (donor). These strains were chosen because they differ for a large number of traits, including various disease models and behavioral traits as shown in Table 4. We recently used some of these congenic strains to confirm QTLs for complex behavioral traits [16,17]. The creation of subcongenic strains will allow yet finer localization of such QTLs [2]. Once the QTL has been finely localized, one general approach to identifying the underlying gene is to examine genes in the congenic interval that show differential expression between the congenic and the background strains. Genes that show such differential expression become candidate genes for the phenotype and may be tested by a variety of strategies including the creation of the corresponding transgenic or knockout strains. In fact, for this particular set of congenic strains, a broad comparison of gene expression in the congenic with the background may not be necessary. This is because a large set of *cis*-acting and *trans*-

Table 2  
Residual contaminants in GTM congenics

Congenic strain	Contaminated chromosome	Proximal marker	Proximal location	Distal marker	Distal location	Length, bp	Length, SNPs	Comments
B6.D2.1M	3	rs3708442	161,348	rs3708508	161,371	23	2	
	12	rs3688676	10,414,102	rs3655651	19,220,541	8,806,439	21	Heterozygous region
	12	rs3666132	21,503,520	rs3724198	29,476,925	7,973,405	25	Homozygous region
B6.D2.4M	4	rs3655655	2,631	rs3666852	3,211	580	2	
	4	rs3663744	31,042,378	rs6389474	33,199,587	2,157,209	9	>50 Mbp from congenic
B6.D2.5P	11	rs3659787	4,406,639	rs3716790	18,003,054	13,596,415	16	Heterozygous
B6.D2.5M	5	rs3705659	903	rs3694939	44,878,782	44,877,879	14	Het. extension of congenic
B6.D2.6PM	16	rs4199265	68,523,205	rs4214683	89,039,848	20,516,643	63	Heterozygous
B6.D2.7M	2	rs3672719	58,423,194	mCV23778822	68,953,410	10,530,216	21	
B6.D2.10P	13	rs6316705	114,805,579	rs3676640	116,665,914	1,860,335	7	
B6.D2.17M	11	rs4137882	102,249,410	rs3688691	116,134,690	13,885,280	27	
B6.D2.17M	14	rs3691209	92,755,476	rs6179144	94,064,405	1,308,929	6	

Residual DBA donor DNA identified by high-density SNP genotyping. The proximal and distal markers for each congenic strain define the known limits of the contaminating region. Except where noted, the contaminating regions are homozygous. SNP markers are given using the “rs” (RefSNP) prefix for SNP accession numbers from the public database and the “mCV” (mouse Celera Variant) prefix for SNPs developed by Celera.

### Chromosome 16 SNP Density

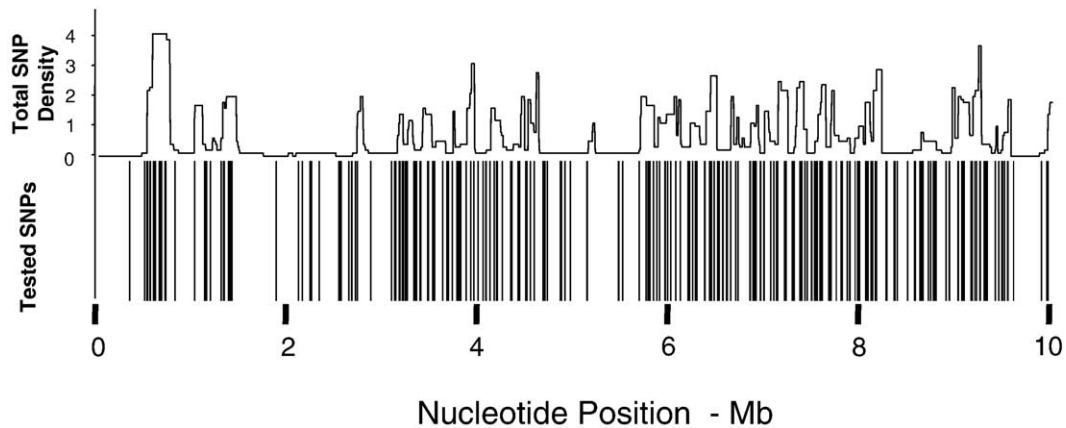


Fig. 3. Regions showing a low density of informative SNPs are identical by descent. The graphs compare distribution of informative SNPs tested here with total SNP density distribution between the known sequences of B6 and DBA for chromosome 16 [13]. Vertical bars at the bottom represent the position of the 354 SNPs that were informative between B6 and DBA. Above this, is plotted the density of known sequence differences between these two strains. The plot shows a moving average of SNP counts along the chromosome. The average at any position is calculated as the median SNP count in 13 overlapping 50-kb intervals centered at this position and spaced at 10-kb increments. As seen in these plots, gaps in the set of informative SNPs (bottom) correspond to regions of very low total SNP density (top), suggesting that regions inherited in B6 and DBA are from a recent common ancestral strain.

acting expression QTL (eQTL) have already been identified in a cross between strains DBA/2J and C57BL/6J [18,19] and in RI strains derived from the same parental strains [7]. In fact, colocalization of such a *cis*-acting eQTL with the trait

QTL provides evidence for functional variations in the positional candidate gene. Furthermore, the availability of the congenic strain provides an ideal tool for validating such positional candidate genes. Finally, the complete sequences

Table 3  
Breeding status and characteristics of the B6 × DBA congenic strains

Congenic strain		Total litters	Litter size		M:F	P value	Pups weaned/month/breeding pair
			Mean	STD. ER.			
B6.D2.	1P	29	4.07	0.41	1.11	0.0630	2.2
B6.D2.	1PM	25	5.80	0.47	0.93	0.0607	2.4
B6.D2.	1M	59	5.20	0.30	1.12	0.0483	2.2
B6.D2.	1D	42	5.48	0.35	0.91	0.0507	2.5
B6.D2.	1C	13	6.38	0.84	1.13	0.0752	3.5
B6.D2.	2P	45	6.27	0.35	1.01	0.0611	3.7
B6.D2.	2M	8	4.50	0.83	1.00	0.1321	1.0
B6.D2.	2D	20	6.85	0.44	<b>1.25</b>	<b>0.0301</b>	2.5
B6.D2.	3P	58	5.33	0.27	1.06	0.0583	2.1
B6.D2.	3M	77	4.84	0.25	1.12	0.0457	2.0
B6.D2.	3MD	27	5.56	0.63	1.00	0.0650	3.0
B6.D2.	3D	68	4.54	0.24	1.18	0.0344	2.4
B6.D2.	3C	5	5.00	0.94	1.08	0.1550	2.4
B6.D2.	4M	Not homozygous					
B6.D2.	5P	50	5.68	0.32	0.91	0.0507	2.9
B6.D2.	5M	49	6.04	0.41	0.91	0.0483	2.1
B6.D2.	5D	52	5.50	0.39	0.93	0.0531	3.1
B6.D2.	6PA	41	5.68	0.36	1.04	0.0583	3.1
B6.D2.	6P	33	6.27	0.35	0.85	0.0344	2.8
B6.D2.	6D	45	5.71	0.29	1.11	0.0507	2.4
B6.D2.	7P	1	2.00		1.00	0.5000	1.4
B6.D2.	7M	44	4.11	0.28	1.15	0.0401	1.5
B6.D2.	7D	46	5.70	0.32	<b>0.66</b>	<b>0.0020</b>	2.2
B6.D2.	7C	Not homozygous					
B6.D2.	8P	24	<b>3.67</b>	0.39	0.87	0.0693	1.5
B6.D2.	8PM	58	5.16	0.29	0.98	0.0604	2.7
B6.D2.	8D	46	5.15	0.27	1.10	0.0509	2.7

Table 3 (continued)

Congenic strain		Total litters	Litter size		M:F	P value	Pups weaned/month/breeding pair
			Mean	STD. ER.			
B6.D2.	9M	42	4.67	0.30	1.09	0.0531	2.1
B6.D2.	9C	15	6.80	0.74	<b>0.76</b>	<b>0.0303</b>	2.9
B6.D2.	10P	56	5.82	0.34	1.12	0.0507	3.1
B6.D2.	10M	22	6.00	0.37	1.03	0.0683	2.0
B6.D2.	10D	19	5.58	0.62	<b>1.41</b>	<b>0.0169</b>	2.6
B6.D2.	11P	27	6.74	0.51	<b>1.22</b>	<b>0.0262</b>	3.1
B6.D2.	11M	24	6.67	0.45	0.98	0.0622	3.1
B6.D2.	11D	24	5.88	0.64	<b>1.27</b>	<b>0.0335</b>	2.7
B6.D2.	12P	35	4.97	0.31	0.81	0.0262	1.3
B6.D2.	12M	31	5.68	0.50	1.17	0.0401	3.2
B6.D2.	12D	11	6.00	0.60	0.94	0.0950	1.6
B6.D2.	12C	30	5.90	0.47	0.86	0.0401	3.0
B6.D2.	13P	29	4.97	0.37	<b>1.30</b>	<b>0.0193</b>	2.3
B6.D2.	13M	36	4.61	0.38	1.18	0.0344	2.1
B6.D2.	13D	13	5.54	0.62	<b>1.40</b>	<b>0.0348</b>	2.9
B6.D2.	14P	14	<b>7.93</b>	0.48	0.98	0.0752	4.3
B6.D2.	14M	44	5.45	0.35	1.09	0.0006	2.6
B6.D2.	14D	23	<b>7.57</b>	0.53	0.98	0.0597	4.1
B6.D2.	14C	38	5.76	0.37	0.96	0.0569	3.7
B6.D2.	15P	27	5.19	0.37	<b>0.77</b>	<b>0.0213</b>	2.0
B6.D2.	15M	27	5.96	0.44	0.99	0.0626	3.1
B6.D2.	15D	30	6.50	0.44	0.95	0.0607	4.2
B6.D2.	16P	42	4.62	0.30	1.02	0.0611	1.7
B6.D2.	16M	10	5.70	0.89	<b>1.59</b>	<b>0.0242</b>	1.7
B6.D2.	16D	28	5.25	0.43	0.86	0.0501	2.7
B6.D2.	16C	Not homozygous					*
B6.D2.	17P	42	5.00	0.33	0.88	0.0401	2.4
B6.D2.	17M	47	4.77	0.30	0.91	0.0550	2.2
B6.D2.	17D	53	4.94	0.36	0.98	0.0615	2.6
B6.D2.	17C	19	4.79	0.49	1.17	0.0639	2.2
B6.D2.	18PM	46	5.52	0.31	1.23	0.0237	2.4
B6.D2.	18D	25	6.28	0.65	1.34	0.0118	3.6
B6.D2.	19P	29	4.45	0.40	0.84	0.0289	1.5
B6.D2.	19M	29	4.83	0.39	0.89	0.0536	2.8
B6.D2.	19D	12	5.58	0.69	<b>0.49</b>	<b>0.0018</b>	4.1
B6.D2.	19C	27	6.19	0.54	<b>0.80</b>	<b>0.0210</b>	3.9

Breeding characteristics of the congenic strains suggest gene interactions between the introgressed segment and the background strain. Several of the strains showed significantly lower or higher litter size than average (shown in bold). Also the M:F sex ratio in litters of some strains suggests gene variation affecting development. The *P* value for the sex ratio being significantly different than 1.0 was calculated using the binomial distribution. For strains where the M:F ratio is less than 0.8 or greater than 1.2 with a *P* value < 0.05, the M:F ratio is shown in bold. As indicators of breeding productivity, we show mean litter size (weaned animals) as well as average number pups weaned per breeding pair per month. In cases where this latter value is low such as B6.D2.2M, litters were infrequent or, if produced, failed to survive to weaning.

of both DBA and B6 have recently been determined and will be particularly useful in this regard (<http://bacpac.chori.org/mouse27.htm>).

Recombinant inbred congenic strains are based on a similar concept [8,9]. The usual RIC strains carry only about 12% of the donor genome with the remainder of the genome coming from the recipient strain. However, they differ from congenic strains in that each strain within the set carries a random mixture of parental alleles scattered over multiple chromosomes. This is in contrast to the congenic strains we describe here that typically contain less than 5% of the donor genome in a single contiguous block. Thus, when comparing a single RIC strain with its background parental strain, it is difficult to assign a unique genetic locus to any phenotypic difference. By contrast, with congenic strains, there is near certainty that the genes

underlying all phenotypic differences between the congenic and the background strains lie within the introgressed congenic segment.

Nadeau and colleagues [11,20] have also constructed a set of chromosome substitution strains (CSS) between strains A/J and B6. These strains (essentially whole-chromosome congenics and also called consomics) have the advantage that relatively small number of strains (one for each chromosome and another for mitochondria) are required to cover the entire genome. Although for complex traits, there may well be multiple QTLs on a single chromosome, possibly with opposite impact on the trait. The CSS strains have been phenotyped for a variety of traits including plasma sterols, diet-induced obesity, anxiety, and serum levels of amino acids. These congenic strains will also provide valuable assistance in identifying genes underlying complex traits.

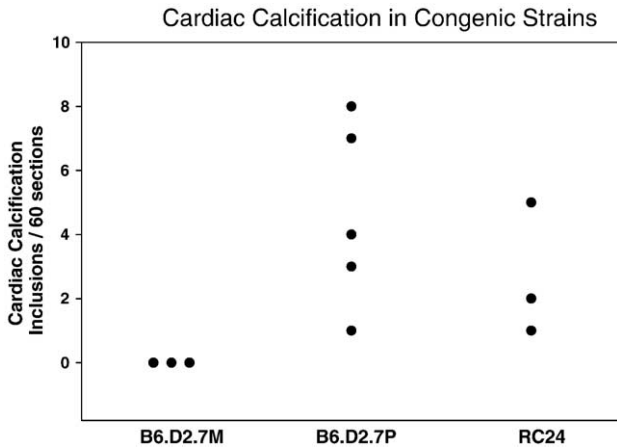


Fig. 4. Confirmation and localization of a QTL for myocardial calcification. DBA/2 mice are susceptible to myocardial calcification while C56BL/6 are not and, we have previously mapped the responsible locus, *Dyscalc*, to proximal chromosome 7 [15]. Of the two congenic strains B6.D2.7P and B6.D2.7M, only B6.D2.7P overlaps the *Dyscalc* locus. Aortic sections from B6.D2.7P and B6.D2.7M were compared with the recombinant inbred strain RC24, a susceptible strain previously shown to carry DBA alleles at the *Dyscalc* locus. None of 3 B6.D2.7M mice exhibited calcification whereas calcification was observed in 5 of 5 B6.D2.7P mice and 3 of 3 RC24 mice.

In all of these cases (RIC, CSS, and now the genome-wide congenic set presented here) it is possible to map genetic variation underlying complex phenotypes. This mapping is carried out by surveying the complete strain set for variation in the phenotype and looking for association between the phenotype and the parental genotype throughout the genome. While each of the congenic strains we report here carry about one-third of a chromosome, the mapping resolution can be substantially improved by examining the various overlapping congenics for an individual chromosome. Thus, for instance, we were able to localize a learning and memory trait within 8.8 cM on chromosome 3 [16].

Thus far, the only traits that have been examined in detail in the entire B6 × DBA congenic panel relate to breeding performance and sex ratios. In contrast to the consomic set between A/J and B6, the breeding performance of the B6 × DBA congenics is highly variable, likely due to gene–gene interactions. As shown in Table 3, several of the strains were very poor breeders, with infrequent litters. For instance, B6.D2.2M has produced an average of only 1 pup weaned per month per breeding pair. For such troublesome strains, greatly increased numbers of cages are required for colony maintenance. There were also some significant differences in the average litter size, ranging from 7.9 mice per litter (B6.D2.14M) to 3.7 mice per litter (B6.D2.8P). There was a poor relationship between the frequency of litters and the size of litters; for example, strain 2M, the poorest breeder, had only slightly reduced litter sizes. These differences are not unexpected since there are clearly striking differences in breeding performance and litter sizes among inbred strains [4]. The ratio of males to females in the B6 × DBA congenic strain litters varied from 1.41 (B6.D2.10D) to 0.49 (B6.D2.19D). The number of loci contributing to sex ratios

is large since, about 25% of the strains exhibited M:F sex ratios below 0.8 or above 1.2 ( $P < 0.05$ ), many more than would be expected based on the number of strains tested (~10%). There are rare monogenic effects in both humans and mice that distort sex ratios in the offspring (see, for example, de la Casa-Esperon et al. [21]). There is also evidence that sex ratios vary with gestation time [22], parental sex hormone levels [23], and metabolic response to dietary fat [24], all likely to be influenced by common multigenic variations.

The B6 × DBA congenic strains will be powerful tools for identifying such variations and characterizing the responsible pathways. Previous experience with consomic strain sets [11] showed very high sensitivity in detecting loci for multigenic traits, with 150 such loci identified in a set of consomic strains derived from A/J on a C57BL/6J background. Much of this sensitivity derives from the fact that multiple animals of a given strain may be measured for any given phenotype, thus ensuring the ability to achieve statistical significance for differences between strains. Moreover, congenic and consomic strains allow investigation of traits for developmental differences or differences in response to environment or drug treatment. Such traits cannot be followed in a genetic cross if measurement of the phenotype requires euthanasia of the animal.

Because congenic strains represent fixed genetic constructs, phenotype data for these strains are cumulative. Thus, phenotype differences between strains such as behavioral comparisons or alterations in bone morphology may be correlated with RNA expression patterns or with genome-wide proteomics data previously gathered by others. The power of this cumulative data to illuminate relationships among multifactorial genetic and environmental influences has been beautifully demonstrated for recombinant inbred strains in the recent work of Chesler et al. [7] and Cheverud et al. [25]. As these authors show, RI strain sets are convenient and powerful tools for gene mapping particularly for complex traits such as behavior and obesity where overall heritability of the trait is low and the influence of any one locus is modest. Conversely, however, because each strain carries roughly half of its genome from each parental strain, and because these parental alleles are widely scattered over all chromosomes, it is almost impossible, using a single RI strain, to study the phenotypic effects of a locus in isolation. In fact, isolation of loci mapped in RI strains using DBA/2 on a C56BL/6 background is most suitably carried out in the congenic strains presented here. And, while data for these strains is cumulative, for many traits, different laboratories may obtain different data for the same phenotype [26,27]. Thus, to obtain the full power of these resources it is increasingly recognized that standards of data collection will need to be established [28].

The B6 × DBA congenic strains are available to interested investigators from The Jackson Laboratory, and we maintain a website ([www.genetics.ucla.edu/GTM](http://www.genetics.ucla.edu/GTM)) that contains updated information on the characteristics of the strains.



## Methods and materials

### Construction of congenic strain

We previously reported the initial stage of the construction of the DBA × B6 whole genome congenic library [12]. Briefly, (B6 × DBA)F1 females were mated with B6 males to produce the N2 generation. Thus, the Y chromosome in all our congenic lines is derived from strain C57BL/6J. At subsequent generations, the male progeny were backcrossed

to C57BL/6J females. Sires for each generation were selected based on microsatellite markers polymorphic between B6 and DBA using DNAs isolated in a 24-well format. Genome-wide sets of marker panels at ~20 cM density were used in the N2 and N3 generations, and 5 cM density sets in subsequent generations, as previously described [12]. The selection of animals at each generation was based on: (1) the presence of the desired introgressed segments; (2) lower overall heterozygosity; (3) overlap between neighboring single introgressed segments from the

Table 4  
Phenotypic differences with identified QTLs between C57BL/6 × DBA/2

Trait	QTL location (chromosome)	Reference
Aging	2, 4, 7, 8, 9, 10, 11, 12, X	(Gerhard, Kaufmann, Wang, Erikson, Abraham, Grundy, Beard, et al., 2002)[34] (Hsu, Zhang, Li, Yi, Yang, Wu, Zhou, Sun, Xu, Yang et al. 2003)[35] (Hsu, Li, Zhang, and Mountz 2005)[36] (Henckaerts, Langer, and Snoeck 2004)[37]
Angiogenesis	2, 4, 11, 13, 15, 18	(Rogers, Rohan, Birsner, and D'Amato 2003)[38] (Rogers, Rohan, Birsner, and D'Amato 2004)[39]
Alcohol preference	1, 2, 3, 4, 6, 9, 10, 11	(Peirce, Derr, Shendure, Kolata, and Silver 1998)[40] (Tarantino, McClearn, Rodriguez, and Plomin 1998)[41] (Whatley, Johnson, and Erwin 1999)[42]
Arthritis	6, 7, 8, 10	(Yang, Jirholt, Svensson, Sundvall, Jansson, Pettersson, and Holmdahl 1999)[43]
Atherosclerosis/ vascular calcification	7, 8, 10	(Colinayo, Qiao, Demant, Krass, Lusic, and Drake 2002)[15] (Colinayo, Qiao, Wang, Krass, Schadt, Lusic, and Drake 2003)[44]
Behavioral	1, 3, 4, 10, 12	(Liu, Singh, Khan, Bhavsar, Lusic, Davis, and Smith 2003)[16] (Liu, Singh, Khan, Lusic, Davis, and Smith 2004)[17] (Steinberger, Reynolds, Ferris, Lincoln, Datta, Stanley, Paterson et al., 2003)[45]
Bone density	1, 2, 3, 5, 6, 7, 8, 10, 13, 15, X	(Drake, Schadt, Hannani, Kabo, Krass, Colinayo, Goldin et al., 2001)[46] (Klein, Turner, Skinner, Vartanian, Serang, Carlos, Shea, Belknap et al. 2002)[47]
Brain/CNS phenotypes	1, 2, 7, 8, 10, 11, 14, 17, 19	(Belknap, Phillips, and O'Toole 1992)[48] (Williams, Strom, and Goldowitz 1998)[49] (Airey, Lu, and Williams 2001)[50] (Krass, Colinayo, Ghazalpour, Vinters, Lusic, and Drake 2003)[51]
Cancer	4, 10	(Ferraro, Golden, Smith, Martin, Lohoff, Gieringer, Zamboni et al. 2004)[52] (Naito, Chenicek, Naito, and DiGiovanni 1988)[53] (DiGiovanni, Imamoto, Naito, Walker, Beltran, Chenicek, and Skow 1992)[54] (Lee, Bennett, Carabeo, and Drinkwater 1995)[55] (Schwarz, Davis, Vick, and Russell 2001)[56]
Cholesterol absorption	–	(Patel and Hitzemann 1999)[57]
Drug response/substance abuse	1, 2, 5, 9, 10, 11, 18	(Bergeson, Helms, O'Toole, Jarvis, Hain, Mogil, and Belknap 2001)[58] (Hood, Belknap, Crabbe, and Buck 2001)[59] (Ferraro, Golden, Smith, Martin, Schwebel, Doyle, Buono, and Berrettini 2004)[60] (Mueller, Ellenberger, Vaughn, Belknap, and Quock 2004)[61] (Chang, Smith, Hawes, Anderson, Zabaleta, Savinova, Roderick et al. 1999)[62] (Azua and Pereira 2000)[63] (Hasegawa, Baldwin, Metcalf, and Foote 2000)[64] (Roberts, Hasegawa, Metcalf, and Foote 2000)[65] (Myrick, DiGuisto, DeWolfe, Bowen, Kappler, Marrack, and Wakeland 2002)[66]
Glaucoma	4, 6	(Delano and Brownstein 1995)[67]
Immune system	1, 2, 6, 11, 13, 15	(Fierer, Walls, Wright, and Kirkland 1999)[68] (Mitsos, Cardon, Fortin, Ryan, LaCourse, North, and Gros 2000)[69] (Hardy, Lu, Nguyen, Woodland, Williams, and Blackman 2001)[70]
Infectious diseases	1, 3, 4, 6, 7, 17	(Drake, Schadt, Hannani, Kabo, Krass, Colinayo, Greaser Iii, Goldin et al. 2001)[46] (Colinayo, Qiao, Wang, Krass, Schadt, Lusic, and Drake 2003)[44] (Keightley, Morris, Ishikawa, Falconer, and Oliver 1998)[71] (Drake, Schadt, Hannani, Kabo, Krass, Colinayo, Greaser Iii, Goldin et al. 2001)[46] (Schadt, Monks, Drake, Lusic, Che, Colinayo, Ruff, Milligan, Lamb et al. 2003)[18]
Lipids	2, 3, 4, 5, 6, 7, 11, 17	(Ferraro, Golden, Smith, Schork, St Jean, Ballas, Choi, and Berrettini 1997)[72] (Ferraro, Golden, Smith, St Jean, Schork, Mulholland, Ballas, Schill et al. 1999)[73] (Libert, Wielockx, Hammond, Brouckaert, Fiers, and Elliott 1999)[74] (Dalton, Miller, Wu, Menon, Cianciolo, McKinnon, Smith, Robinson et al. 2000)[75] (Risinger, Quick, and Belknap 2000)[76]
Obesity/diabetes	2, 4, 6, 13, 15, 19	
Toxicity	1, 3, 4, 5, 6, 7, 9, 11, 12, 15, 18	

same chromosome carried by different congenics; (4) coverage of the entire genome by sets of congeneric strains at each generation of development. Mice at the N6 generation or later were intercrossed and homozygous lines selected for propagation. A subset of the congeneric strains was typed using a high-density SNP map to precisely map the congeneric boundaries and test for contaminating regions. SNP genotyping was performed at Illumina, Inc., San Diego, California, as described [19,29]. Most of the strains have been cryopreserved at The Jackson Laboratory (TJL) with sperm or small numbers of embryos for each strain. TJL will make these strains available in a limited offering in the future depending on the cryopreserved material available for each strain. Investigators interested in using these strains should contact Customer Service at The Jackson Laboratory.

### Physiological characterization

The congeneric strains were assessed for breeding characteristics, body weight, growth rate, fat depots, and a number of basic physiologic parameters [30]. Plasma lipid measurements, including total triglyceride and cholesterol levels, high-density lipoprotein (HDL) cholesterol levels, very low-density and low-density lipoprotein levels (VLDL and LDL), esterified and unesterified cholesterol levels, and free fatty acid levels were determined as indicated [31]. Glucose, leptin, and insulin levels were determined as described [32]. Unless otherwise indicated, all measurements were performed on mice that had been fasted overnight. Blood was collected under isoflurane anesthesia from the retro-orbital sinus. Lean body mass and fat body mass were determined by nuclear magnetic resonance [33] using the Bruker Minispec adapted for measurement of body composition by Echo Medical Systems (Houston, TX).

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ygeno.2005.05.010](https://doi.org/10.1016/j.ygeno.2005.05.010).

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