

A genome-wide set of congenic mouse strains derived from CAST/Ei on a C57BL/6 background

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Abstract

We previously reported the construction of two sets of heterozygous congenic strains spanning the mouse genome. For both sets, C57BL/6J was employed as the background strain while DNA from either DBA/2 or CAST/Ei was introgressed to form the congenic region. We have subsequently bred most of these strains to produce homozygous breeding stocks. Here, we report the characterization of the strain set based on CAST/Ei. CAST/Ei is the most genetically distant strain within the *Mus mus* species and many trait variations relevant to common diseases have been identified in CAST/Ei mice. Despite breeding difficulties for some congenic regions, presumably due to incompatible allelic variations between CAST/Ei and C57BL/6, the resulting congenic strains cover about 80% of the autosomal chromosomes and will be useful as a resource for the further analysis of quantitative trait loci between the strains.

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For some time, researchers have used mouse genetic crosses to pursue identification of genes underlying complex traits [1–3]. These crosses take advantage of natural genetic variation between inbred mouse strains to perturb traits relevant to common disease in humans. Results from these studies identify multiple quantitative trait loci (QTL) that show genetic linkage to the trait of interest. However, to validate the effects of any single such locus and to investigate its metabolic impact, other approaches are required. One common approach is to construct a congenic strain that isolates the genetic locus from one of the parental strains on a common genetic background (for example, see Nadeau et al. [4] or Estrada-Smith et al. [5,6]). Comparison of the congenic strain directly with the background strain reveals the metabolic impacts of the congenic locus and provides a powerful tool to identify the affected pathways and

gene variants. For example, this approach recently led to the identification of a gene important in type 2 diabetes [7].

An important problem with congenic strains is that they are expensive and time-consuming to construct, requiring a minimum of four or five mouse generations, even with marker-assisted strategies [8]. To address this issue, we previously developed two libraries of congenic strains that comprehensively span the genome [9]. At this initial stage, most of the strains remained heterozygous for the congenic region. We have subsequently focused on producing homozygous breeding stocks and have recently described the congenic set carrying the DBA/2J genome introgressed onto C57BL/6J [10]. Strains from this set have already been used to validate and localize several behavioral QTL better [11,12], and other studies are in progress.

In the present paper, we describe the second library in which the CAST/EiJ genome is introgressed onto the same C57BL/6J genetic background. CAST/Ei is an inbred strain derived from the wild strain *Mus mus castaneus*, while the common lab-

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oratory mouse strains are hybrids of several wild strains, predominately *Mus mus domesticus*. Because *M. m. castaneus* diverged from *M. m. domesticus* about a million years ago [13], CAST/Ei carries a higher density of DNA polymorphisms than other laboratory strains. As a result, there is a high likelihood of natural variation in most biological pathways compared to standard laboratory strains such as C57BL/6, making *castaneus*-derived inbred strains attractive candidates for QTL mapping studies. However, while this natural variation increases chances of detecting QTLs, it also causes reduced fertility for some hybrid strains and has made it difficult to generate or maintain some of the congenic strains within the library. For example, we found that congenics carrying middle and distal regions of chromosome 2 from CAST/Ei were difficult or impossible to maintain by standard breeding, although, in independent experiments, we have produced such constructs by routinely employing foster mothers from high-fertility strains [5,6]. Despite these difficulties, we report here a set of congenic strains that cover about 80% of the autosomal chromosomes.

Results

We have used marker-assisted selective breeding to generate a genome-wide library of congenic strains carrying alleles from

CAST/EiJ introgressed onto a C57BL/6J background. For most of these strains, we have been able to establish homozygous breeding stocks, although some strains either did not achieve homozygosity or, after becoming homozygous, demonstrated such low fertility that it proved impractical to maintain the stock using routine breeding practices. The approximate boundaries of the set of strains are presented in Fig. 1. Our objective was to derive 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 strains carrying introgressed regions covering both proximal and middle regions (designated PM) or middle and distal regions (designated MD). And, for chromosomes 11 and 19, we created a near-consomic strain designated by C in the strain name.

For homozygous stocks, we carried out high-density genome-wide SNP mapping to identify precisely endpoints of the congenic regions and to locate residual CAST/EiJ contamination on other chromosomes. Fig. 2 shows the SNP map for congenic strain B6.CAST.1M. Horizontal bars on the left of the figure represent the positions of SNPs informative between C57BL/6J and CAST/Ei. Bars that extend farther to the right side are those that show the CAST/Ei allele in the congenic strain. In this case, the congenic region extends from about 3.4 to 137 Mb on chromosome 1. The limiting SNPs for

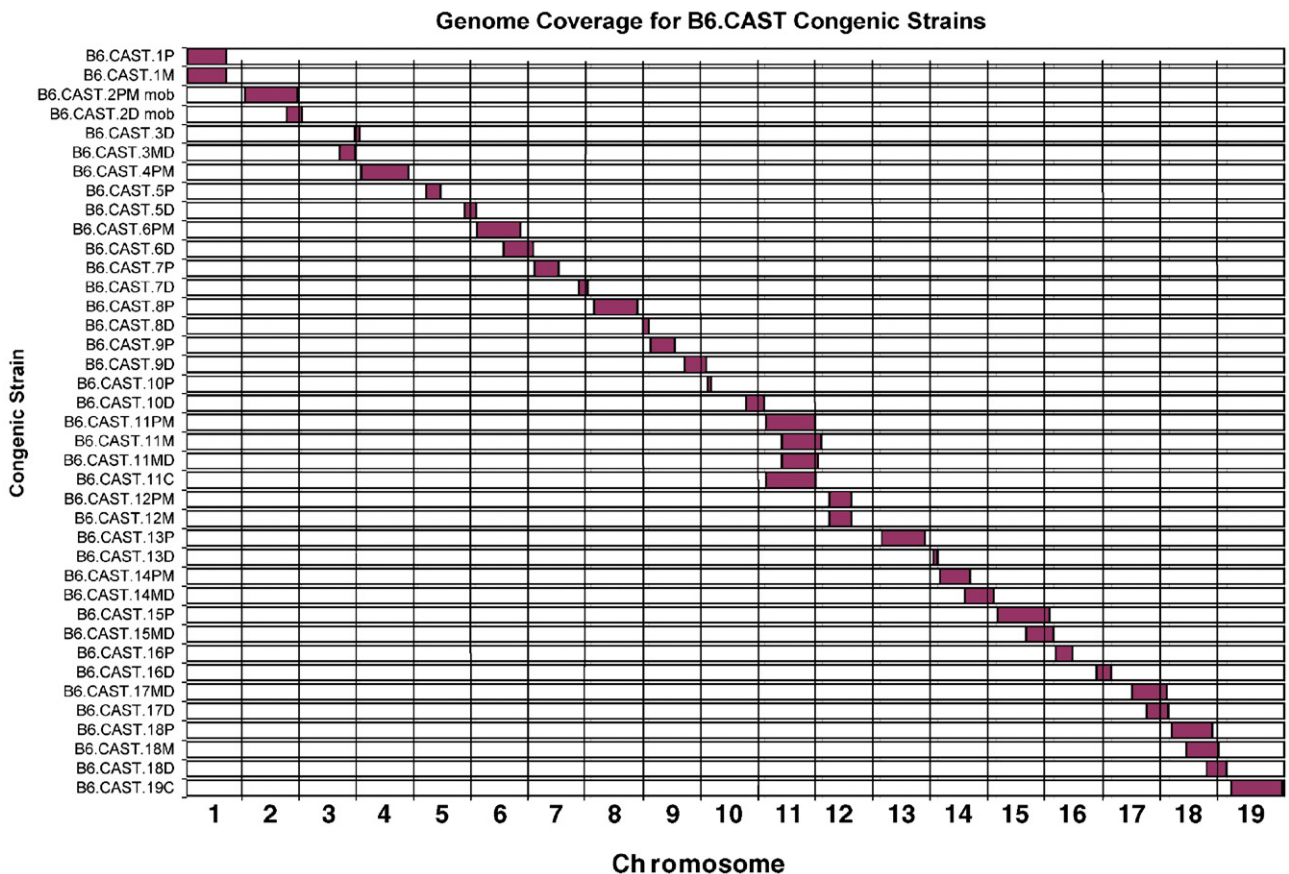


Fig. 1. Map positions of the introgressed segments for each congenic line. Most chromosomes are covered by proximal (P), middle (M), and distal (D) congenic strains. Chromosomes 11 and 19 are represented by additional strains carrying whole, or nearly whole, chromosomes of CAST/Ei. Such consomics are indicated by “C” in the strain name. The filled bar indicates the chromosomal region known by mapping to be included in the congenic strain.

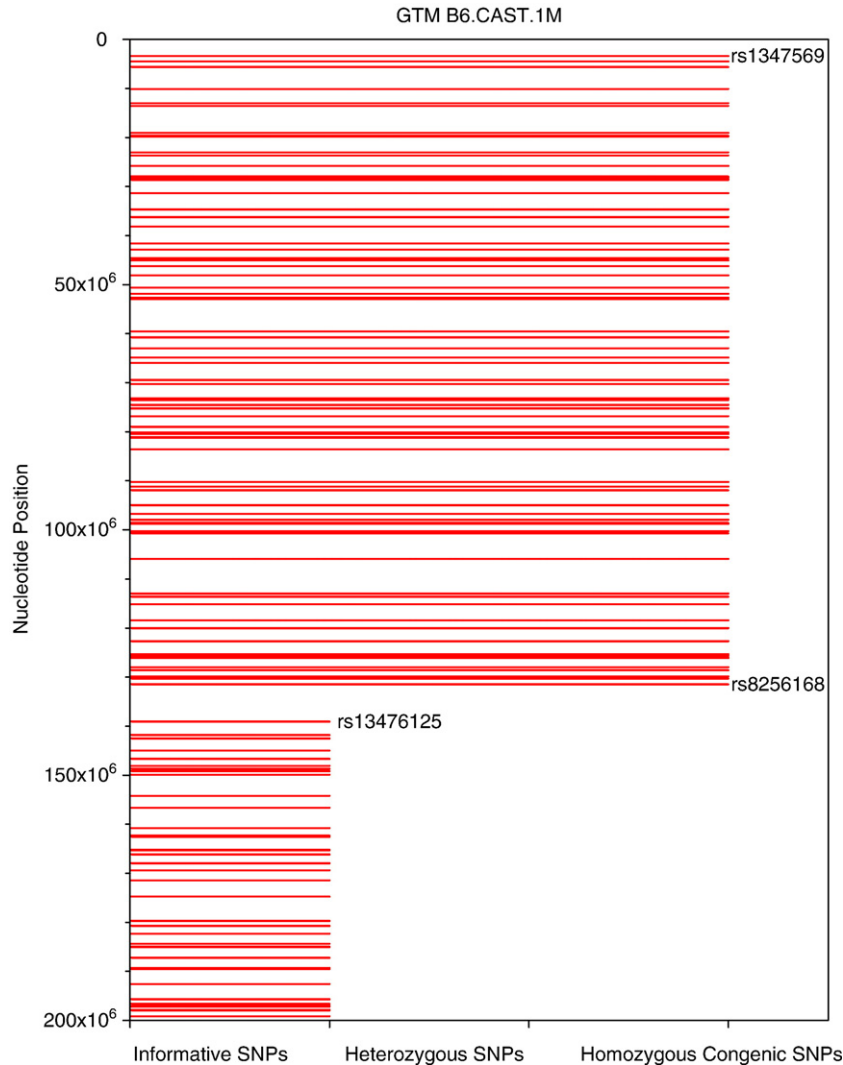


Fig. 2. SNP mapping of the introgressed segment in B6.CAST.1M. Horizontal lines indicate the locations, in base pairs, of SNPs along the chromosome (centromere at the top). The left-hand set of lines indicates the positions of SNPs that were informative between B6 and CAST/Ei on chromosome 1. Lines that extend to the right-hand side indicate those SNPs showing the CAST/Ei allele in the congenic. Lines that extend only to the center indicate heterozygous SNPs in the congenic. No SNPs were identified as heterozygous in this congenic. The limiting SNPs for each region are annotated by their public SNP identification number. For maps of the other chromosomes, see Supplemental Fig. 2.

each region are identified by public SNP identification numbers. As we previously discussed [10], gaps in the set of informative SNPs such as that seen from 53 to 59 Mb in Fig. 2 most likely represent regions where CAST/Ei and C57BL/6 are identical by descent, probably deriving from a *castaneus* component of the C57BL/6 hybrid. SNP mapping diagrams for the remaining congenic strains are shown in Supplemental Fig. 2.

Table 1 summarizes markers defining the endpoints of the congenic strains. Some strains carry regions of residual CAST/EiJ contamination in the remainder of the genome. These regions are summarized in Table 2.

As was observed with the B6.DBA congenic strain set [10], there were wide variations in the breeding characteristics of the various strains. These data are presented in Table 3. Notably, all the strains for chromosome 6 (B6.CAST.6PM, B6.CAST.6M, and B6.CAST.6D) showed a reduced production of male

pups. For instance, for B6.CAST.6PM, the observed male-to-female ratio was 0.71, compared with the expected ratio of 1.0 ($p < 0.05$). This suggests that a genetic variation on chromosome 6 in CAST/Ei strongly affects sex ratios in the offspring. Judging from the region of overlap between these strains, the relevant gene is likely located between 73 and 117 Mb (Table 1).

There was also wide variation in breeding productivity of the strains. To some extent this may reflect differences in the mean number of pups weaned per litter (Table 3). Overall, the mean weaned litter size was 5.16 pups (± 0.93 SD). Two strains (B6.CAST.5D and B6.CAST.10D) showed mean weaned litter sizes (2.27 and 7.80, respectively) that may be possible outliers as detected by outlier box-plot analysis. However, breeding productivity, measured as pups weaned per breeding pair per month, more closely reflects the difficulties encountered in developing a useful experimental strain. This measure takes

Table 1
B6.CAST congenic interval limits

Congenic strain	Flanking proximal marker		Proximal internal marker		Distal internal marker		Flanking distal marker	
	Marker ID	Position	Marker ID	Position	Marker ID	Position	Marker ID	Position
B6.CAST.1P	Terminus	0	rs13475697	3,396,817	rs13476119	137,096,558	rs13476125	139,040,156
B6.CAST.1M	Terminus	0	rs13475697	3,396,817	rs13476119	137,096,558	rs13476125	139,040,156
B6.CAST.2PM mob	Terminus	0	rs13476318	3,047,321	rs13476907	169,592,612	rs13476928	174,453,653
B6.CAST.2D mob	rs13476779	133,086,710	rs13476783	134,003,516	rs4223701	181,364,415	Terminus	181,572,269
B6.CAST.3D	rs13477462	147,530,207	rs13477466	148,405,960	rs13477526	163,858,174	Terminus	164,059,219
B6.CAST.3MD	rs13477294*	104,641,296	rs6339990	106,777,849	rs13477473	150,096,333	rs3022971*	150,653,009
B6.CAST.4PM	Terminus	0	rs13477534	4,046,388	rs6355453	129,755,364	rs13477985	130,446,260
B6.CAST.5P	rs3023036	20,960,382	rs13478145	22,798,348	rs13478279	59,532,947	rs13478280	59,860,058
B6.CAST.5D	D5Mit319	118,193,255	D5Mit242	121,515,329	D5Mit143	151,270,472	Terminus	152,003,063
B6.CAST.6PM	Terminus	0	rs6172481	4,362,933	rs4226214	117,571,843	rs13478999	120,465,626
B6.CAST.6D	rs4225987	72,847,262	rs13478816	73,426,012	rs13479094	149,822,665	Terminus	150,465,925
B6.CAST.7P	Terminus	0	rs8252589	3,778,826	rs13479311	60,328,845	rs13479314	60,962,142
B6.CAST.7D	rs13479450	99,210,233	rs6396580	108,464,052	rs6211135	137,457,344	Terminus	137,457,344
B6.CAST.8P	Terminus	0	D8Mit50	7,162,471	D8Mit211	105,606,100	D8Mit317	117,093,597
B6.CAST.8D	rs13479995	115,389,450	rs13480004	117,383,572	rs13480048	131,006,770	Terminus	131,006,770
B6.CAST.9P	Terminus	0	rs13477344	3,616,418	rs13480204	56,359,387	rs13480241	66,568,324
B6.CAST.9D	rs13480274	76,009,564	rs6161630	78,758,392	rs6376141	125,963,015	Terminus	126,414,413
B6.CAST.10P	Terminus	0	rs13480467	3,924,015	rs6208271	10,647,150	rs13480504	13,018,376
B6.CAST.10D	rs4228381	91,018,426	rs6182528	91,708,895	rs13480826	131,714,319	Terminus	132,810,849
B6.CAST.11PM	Terminus	0	rs13480835	3,232,122	rs13481211	106,069,315	rs13481216	107,075,376
B6.CAST.11M	rs13480944	31,490,879	rs13480968	36,095,047	rs4229215	119,162,793	Terminus	121,297,865
B6.CAST.11MD	rs13480944	31,490,879	rs13480968	36,095,047	rs13481235	111,949,691	rs3698585	113,509,261
B6.CAST.11C	Terminus	0	rs13480835	3,232,122	rs13481218	107,453,760	rs13481230	110,825,026
B6.CAST.12PM	rs13481304	12,818,628	rs6186506	13,938,008	rs13481491	57,244,405	rs3675722	60,068,847
B6.CAST.12M	rs6153111	40,400,408	rs13481435	42,830,106	rs3722988	114,701,743	Terminus	60,068,847
B6.CAST.13P	Terminus	0	rs13481666	3,715,647	rs13481946	91,668,560	rs3687604	96,198,202
B6.CAST.13D	rs13482007	108,172,879	rs13482011	109,427,087	rs6412462	117,826,177	Terminus	118,131,803
B6.CAST.14PM	Terminus	0	rs13482047	4,247,033	rs13482254	66,082,694	rs13459144	67,487,858
B6.CAST.14MD	rs13482206	50,489,812	rs6214828	55,085,849	rs13482398	113,081,474	rs13482403	114,480,623
B6.CAST.15P	Terminus	0	rs13459176	3,360,749	rs4230816	76,763,557	rs6236706	78,552,525
B6.CAST.15MD	rs13482572	51,179,216	rs13482588	55,680,041	rs13482750	104,749,350	Terminus	105,049,027
B6.CAST.16P	Terminus	0	rs4152838	4,518,524	rs4171059	32,714,426	rs4171440	33,213,186
B6.CAST.16D	rs4197745	68,340,892	rs4202793	74,403,922	rs4221365	99,198,206	Terminus	99,615,617
B6.CAST.17D	rs13482997	43,206,640	rs13483012	48,048,189	rs3023460	94,127,499	Terminus	95,583,272
B6.CAST.18P	Terminus	0	rs13483187	4,492,040	rs3675194	67,323,344	rs6238283	68,329,466
B6.CAST.18M	rs3705122	24,515,194	rs13483264	27,304,145	rs6298532	77,439,448	rs13483456	79,595,925
B6.CAST.18D	rs13483360	52,074,719	rs6338896	58,709,598	rs13483496	89,681,596	Terminus	89,681,596
B6.CAST.19C	rs13483499	3,217,016	rs13483505	4,868,758	rs13483693	58,187,377	Terminus	60,649,273

into account the fact that some strains only infrequently bring a litter to weaning age, either because of a failure to produce pups at all or because the pups die or are killed shortly after birth. This appears to be the case with B6.CAST.2M, which has normal weaned litter sizes but, overall, very low yield of pups per breeding pair per month (“Fecundity” in Table 3). In an independent set of B6.CAST congenics that we developed for this region [5,6], we found it mandatory to employ foster mothers from a more fecund strain to produce sufficient animals for meaningful experiments. Interestingly, of the strains in the B6.D2 congenic strain set previously described [10], the B6.D2.2M congenic had the lowest fecundity of all the strains in the set. This suggests that specific alleles in the middle region of chromosome 2 in C57BL/6 have an important impact on successful breeding in this strain and that substitution of alleles from another strain is disruptive to overall breeding success. The genes or mechanisms involved are unclear.

Discussion

We report the construction and breeding to homozygosity of a set of congenic strains in which subchromosomal regions derived from CAST/Ei have been introgressed on a C57BL/6 background. As discussed below, we anticipate that these strains will be useful for following up on QTL mapping studies by many investigators.

Congenic analysis is an important step in the positional cloning of genes affecting complex traits [1]. Quantitative traits are efficiently mapped to large chromosomal regions using F2 intercross and backcross populations. However, genetic analysis of these populations is limited by low genetic resolution and by the presence of a mixed genetic background. This complexity can make it difficult to discriminate between epistasis and other complex gene–gene interactions. The congenic model allows for isolation of strain-specific QTL alleles on an isogenic background. Thus, independent effects of the locus can be

Table 2
Gaps in the congenic region and additional CAST/Ei segments outside congenic region

Congenic strain	Type	Chromosome	Beginning SNP	Beginning Mb	Ending SNP	Ending Mb
B6.CAST.1P	Heterozygous gap	1	rs6405821	76.84	rs13475966	88.85
B6.CAST.2Dmob	Heterozygous flanking	2	rs13476739	121.64	rs13476778	132.74
	Heterozygous gap	2	rs13476872	159.92	rs13476907	169.59
B6.CAST.3MD	Heterozygous flanking	3	rs13477137	61.03	rs3159432	103.42
	Heterozygous flanking	3	rs3022971	150.65	rs13477526	163.86
	Heterozygous contamination	7	rs13479278	51.89	rs13479564	133.48
B6.CAST.4PM	Homozygous gap	5	rs6163246	20.78	rs4224426	25.24
B6.CAST.6PM	Heterozygous gap	6	rs13478707	34.96	rs13478834	77.91
	Heterozygous flanking	6	rs13478999	120.47	rs3024135	141.45
B6.CAST.6D	Heterozygous contamination	2	rs13476337	8.01	rs13478631	11.05
B6.CAST.7P	Heterozygous gap	7	rs4226520	23.31	rs6239325	31.42
B6.CAST.7D	Heterozygous contamination	6	rs13478818	74.15	rs13478976	114.02
	Heterozygous contamination	15	rs13482423	8.73	rs13482561	49.04
	Heterozygous contamination	18	rs13483221	15.40	rs13483237	19.61
B6.CAST.10P	Heterozygous flanking	10	rs13480504	13.02	rs13480619	59.97
B6.CAST.11PM	Heterozygous contamination	4	rs13477631	32.72	rs13477719	55.70
	Heterozygous contamination	10	rs13480722	100.18	rs3654717	109.70
	Heterozygous contamination	12	rs13481290	8.16	rs3665818	19.93
	Heterozygous contamination	13	rs13481867	67.66	rs3705446	78.80
	Heterozygous contamination	14	rs13482377	105.49	rs3711767	110.95
	Heterozygous contamination	16	rs3692015	59.25	rs4197458	67.48
B6.CAST.11M	Heterozygous contamination	11	rs3153215	104.00	rs13481218	107.45
B6.CAST.11MD	Heterozygous gap	11	rs3090849	41.71	rs3023311	58.57
	Homozygous gap	11	rs13481128	83.27	rs13481218	107.45
	Heterozygous gap	11	rs3698585	113.51	rs13481251	116.55
B6.CAST.11C	Heterozygous contamination	5	rs6163111	72.96	rs13478337	77.82
	Heterozygous gap	11	rs13481230	110.83	rs13481251	116.55
	Heterozygous contamination	18	rs13483221	15.40	rs13483296	35.37
B6.CAST.13P	Heterozygous contamination	11	rs13480835	3.23	rs6313528	18.94
	Heterozygous contamination	18	rs13483221	15.40	rs3023747	21.27
B6.CAST.15MD	Homozygous contamination	6	rs6172481	4.36	rs13478697	32.73
B6.CAST.18P	Heterozygous contamination	13	rs13481666	3.72	rs3691376	23.67
	Heterozygous gap	18	rs13483236	19.30	rs3705122	24.52
B6.CAST.18M	Heterozygous contamination	18	rs13483221	15.40	rs13483237	19.61
	Heterozygous chromosome	1	rs3022877	184.70	rs13476312	197.94
	Heterozygous chromosome	11	rs6163209	101.62	rs4229215	119.16
B6.CAST.18D	Homozygous contamination	10	rs13480467	3.92	rs13480490	9.24

Heterozygous gap, segments within the congenic region remaining heterozygous; Homozygous gap, segments within the congenic region remaining homozygous for B6 alleles; Heterozygous flanking, segments adjacent to the congenic region remaining heterozygous; Heterozygous contamination, regions on other chromosomes remaining heterozygous; Homozygous contamination, regions on other chromosomes carrying homozygous CAST alleles.

confirmed and quantified. The utility of a genome-wide set of congenics is in the ability to evaluate QTL-relevant strains for a variety of phenotypes in a relatively short period of time.

Because of its genetic distance from common laboratory strains, use of CAST/Ei in QTL mapping studies maximizes the chances for observing multiple natural genetic variations impacting the traits concerned. Thus, CAST/Ei has been used in crosses to identify loci affecting a diverse set of phenotypes. These include traits associated with growth and obesity [6,14–16], gallstones [17], plasma lipids and insulin resistance [6,18–22], eating behavior [23], B-lymphocyte deficiency [24], neuron number control [25] (with further mapping shown at <http://www.nervenet.org/papers/Strom99/Thesis.html> and <http://www.nervenet.org/papers/Strom99/Chapter5.html>), bone mineral density [26–29], soft tissue regeneration [30], cytokine-regulated growth [31], neuromuscular degeneration [32], hearing loss [33], and spherocytosis [34]. Further, from phenotypic data, it is clear the CAST/Ei strain is an attractive strain

for pursuing genes underlying a variety of other traits. For instance, in the Mouse Phenome Database (<http://phenome.jax.org/pub/cgi/phenome/>), CAST/Ei is listed among the outlier strains for activity and motor function, kidney weight, plasma cholesterol, response to atherogenic diet, body weight, blood pressure, ventricle weight, cranial–facial measures (mandible), drinking preference for NH₄Cl, food and water intake, sandy gallstones on an atherogenic diet, hearing, red cell distribution, total and HDL phospholipids, metabolism (oxygen consumption), plasma triglycerides, and wildness. In summary, we believe that the B6.CAST congenic library presented here will be invaluable in the investigation of a broad range of complex phenotypes. Moreover, because of the high-density SNP mapping carried out on most of these strains, investigators will have the additional advantage of knowing the precise end-points of the congenic intervals as well as comprehensive knowledge of residual CAST contamination present on other chromosomes.

Table 3
Breeding status and characteristics of the B6.CAST congenic strains

Congenic strain		Total pups	Litters	Mean	Standard error	Males	Females	M:F ratio	M:F <i>p</i> value	Fecundity ^a
B6.CAST	1M	172	29	5.93	0.32	79	89	0.89		2.6
B6.CAST	1P	119	20	5.95	0.41	66	53	1.25		2.1
B6.CAST	2D	97	23	4.22	0.5	43	53	0.8		1.1
B6.CAST	2M	37	7	5.29	1.15	23	14	1.64		1.0
B6.CAST	2MD	Not homozygous								
B6.CAST	2P	279	42	6.64	0.32	146	134	1.1		3.2
B6.CAST	3D	251	39	6.44	0.44	123	121	1.02		3.8
B6.CAST	3MD	187	36	5.19	0.38	95	92	1.03		3.0
B6.CAST	4PM	41	8	5.13	0.55	18	20	0.86		0.8
B6.CAST	5D	25	11	2.27	0.51	11	14	0.79		0.4
B6.CAST	5P	229	51	4.49	0.29	107	116	0.89		2.4
B6.CAST	6D	25	4	6.25	1.25	7	18	0.39	0.046	1.1
B6.CAST	6M	10	2	5	2	4	6	0.67		0.6
B6.CAST	6PM	60	14	4.29	0.67	25	35	0.71		2.0
B6.CAST	7D	184	41	4.49	0.36	94	90	1.04		1.9
B6.CAST	7P	70	14	5	0.58	37	33	1.12		1.0
B6.CAST	8D	152	35	4.34	0.36	70	82	0.85		1.4
B6.CAST	8P	135	31	4.35	0.3	74	61	1.21		1.7
B6.CAST	9D	193	34	5.68	0.34	85	108	0.79		1.8
B6.CAST	9P	87	21	4.14	0.42	34	53	0.64		0.9
B6.CAST	10D	39	5	7.8	1.24	18	21	0.86		2.1
B6.CAST	10P	107	26	4.12	0.46	56	51	1.1		1.3
B6.CAST	11C	144	27	5.33	0.41	73	71	1.03		2.1
B6.CAST	11M	181	32	5.66	0.4	111	70	1.59	0.003	3.4
B6.CAST	11MD	Not homozygous								
B6.CAST	11PM	124	24	5.17	0.34	57	67	0.85		2.6
B6.CAST	12M	51	10	5.1	0.64	21	22	0.96		1.3
B6.CAST	12MD	Not homozygous								
B6.CAST	12PM	220	36	6.11	0.32	103	117	0.88		3.0
B6.CAST	13D	207	34	6.09	0.35	97	110	0.88		2.7
B6.CAST	13P	83	16	5.19	0.43	33	50	0.66		1.9
B6.CAST	14MD	183	45	4.07	0.24	85	98	0.87		1.8
B6.CAST	14P	87	21	4.14	0.34	41	46	0.89		1.6
B6.CAST	14PM	57	9	6.33	1.04	27	30	0.9		2.7
B6.CAST	15D	84	19	4.42	0.41	42	42	1		1.1
B6.CAST	15MD	151	30	5.03	0.41	80	71	1.13		1.8
B6.CAST	15P	176	37	4.76	0.37	98	77	1.29		2.4
B6.CAST	16D	162	29	5.59	0.38	85	77	1.1		2.1
B6.CAST	16P	168	30	5.6	0.39	70	96	0.71		2.3
B6.CAST	16PM	Not homozygous								
B6.CAST	17D	56	11	5.09	0.55	25	31	0.81		1.3
B6.CAST	18D	212	41	5.17	0.34	105	107	0.98		2.4
B6.CAST	18M	99	18	5.5	0.57	50	49	1.02		2.4
B6.CAST	18MD	Not homozygous								
B6.CAST	18P	94	20	4.7	0.45	44	50	0.88		2.0
B6.CAST	19C	71	13	5.46	0.62	33	38	0.87		2.2
B6.CAST	19P	97	22	4.41	0.45	51	46	1.11		2.0

^a Pups weaned per month per breeding pair.

Currently, most strains are maintained at UCLA, although some have been transferred to The Jackson Laboratory and we are working with The Jackson Laboratory to establish cryo-preserved stocks for a core comprehensive panel of B6.CAST congenic strains. Investigators interested in using these strains should contact Customer Service at The Jackson Laboratory.

Methods and materials

Construction of congenic strains

We previously reported the initial stage of the construction of the CAST × B6 whole-genome congenic library [9]. Briefly, (B6 × CAST)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 [9]. 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 same chromosome carried by different congenics, (4) coverage of the entire genome by sets of congenic strains at each generation of development. Mice at the N6 generation or later were intercrossed and homozygous lines selected for propagation.

SNP mapping

Most homozygous congenic strains were typed using a panel of 5000 SNPs to map the congenic boundaries precisely and test for contaminating regions. SNP genotyping was performed at Affymetrix using the mouse MegAllele Genotyping Mouse 5K SNP panel [35].

Statistical analyses

Statistical significance was based upon a pairwise analysis of variance between C57BL/6J and each congenic strain.

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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.2007.05.009.

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