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Development of SNP markers for C57BL/6N-derived mouse inbred strains

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Abstract: C57BL/6N inbred mice are used as the genetic background for producing knockout mice in large-scale projects worldwide; however, the genetic divergence among C57BL/6N-derived substrains has not been verified. Here, we identified novel single nucleotide polymorphisms (SNPs) specific to the C57BL/6NJ strain and selected useful SNPs for the genetic monitoring of C57BL/6N-derived substrains. Informative SNPs were selected from the public SNP database at the Wellcome Trust Sanger Institute by comparing sequence data from C57BL/6NJ and C57BL/6J mice. A total of 1,361 candidate SNPs from the SNP database could distinguish the C57BL/6NJ strain from 12 other inbred strains. We confirmed 277 C57BL/6NJ-specific SNPs including 10 nonsynonymous SNPs by direct sequencing, and selected 100 useful SNPs that cover all of the chromosomes except Y. Genotyping of 11 C57BL/6N-derived substrains at these 100 SNP loci demonstrated genetic differences among the substrains. This information will be useful for accurate genetic monitoring of mouse strains with a C57BL/6N-derived background.

Key words: C57BL/6N, SNP, genetic background, inbred, substrain

Introduction

C57BL/6 is the best-known inbred mouse strain and has been used as the genetic background for spontaneous and induced mutations. To produce knockout mice, embryonic stem (ES) cells derived from 129 mouse substrains were used initially to manipulate the mouse genome [19]; however, these substrains were not suitable for most biomedical studies, especially in immunology, neurobiology, and physiology [4, 13, 28]. Backcrossing to C57BL/6 mice is carried out frequently to generate congenic strains to facilitate phenotypic analyses, but this procedure requires additional cost and time. In addition, the targeted locus from the original ES cell genome remains in the congenic mice and may confound the results of studies using these animals [6]. Therefore, ES cells with a pure C57BL/6 genetic background are more useful for the generation of knockout mice.

Recently, C57BL/6 mouse-derived ES cells were established in several laboratories. Importantly, ES cells derived from C57BL/6N mice maintained their pluripotency after homologous recombination [18, 25], and the methods used to generate germline-transmitting chimeric mice have been improved [5]. The International Knockout Mouse Consortium (IKMC) conducted large-scale mutagenesis to mutate all of the protein-coding genes in mice using gene trapping and targeting in ES cells [9, 22]. Since then, several mouse ES cells derived from the C57BL/6NTac strain have been used as standard ES cells for the production of mutant alleles [5, 18]. Moreover, the International Mouse Phenotyping Consortium has used the IKMC-targeted C57BL/6N ES cell clones to undertake the broad-based phenotyping of 20,000 mouse genes [2].

Since the 1950s, the C57BL/6 strain has diverged into several substrains, including two major groups, C57BL/6J and C57BL/6N. Currently, more than 20 inbred substrains derived from C57BL/6J and C57BL/6N

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Table 1. C57BL/6 substrains investigated in this study

Substrain		Source
C57BL/6N substrains	C57BL/6NJ	The Jackson Laboratory (Bar Harbor, MA, USA)
	C57BL/6NCrSim	Simonsen Laboratories, Inc. (Gilroy, CA, USA)
	C57BL/6NTac	Taconic Farm Inc. (New York, NY, USA)
	C57BL/6NJcl	CLEA Japan Inc. (Tokyo, Japan)
	C57BL/6NSeac	Kyudo Co. Ltd. (Tosu, Japan)
	C57BL/6NCrlCrlj	Charls River Laboratories Japan, Inc. (Yokohama, Japan)
	C57BL/6NCrl	Charls River Laboratories International, Inc. (Wilmington, MA, USA)
	C57BL/6NHsd	Harlan Laboratories, Inc. (Indianapolus, IN, USA)
	C57BL/6NCrSlc	Japan SLC, Inc. (Hamamatsu, Japan)
	C57BL/6By	The Jackson Laboratory (Bar Harbor, MA, USA)
	C57BL/6ByJ	The Jackson Laboratory (Bar Harbor, MA, USA)
C57BL/6J substrains	C57BL/6J	The Jackson Laboratory (Bar Harbor, MA, USA) via Charls River Laboratories Japan, Inc. (Yokohama, Japan)
	C57BL/6JJcl	CLEA Japan Inc. (Tokyo, Japan)
	C57BL/6JJmsSlc	Japan SLC, Inc. (Hamamatsu, Japan)
	C57BL/6JEiJ	The Jackson Laboratory (Bar Harbor, MA, USA)
	C57BL/6JOlaHsd	Harlan Laboratories, Inc. (Indianapolus, IN, USA)
	C57BL/6JRccHsd	Harlan Laboratories, Inc. (Indianapolus, IN, USA)
	C57BL/6JBomTac	Taconic Farm Inc. (New York, NY, USA)

Nomenclatured strain names of each C57BL/6 substrain were in accordance with JAX® NOTES [16].

mice have been established and distributed worldwide [1, 16]. The C57BL/6J strain has many specific single nucleotide polymorphisms (SNPs) that distinguish it from other inbred strains [17], and SNPs that can differentiate C57BL/6J substrains have also been identified [14, 32]. In addition, several phenotypic differences have been reported among C57BL/6J substrains [8, 20, 23, 24]. C57BL/6J-specific SNP information is useful for the genetic monitoring of mouse strains with a C57BL/6J-derived background and interpretation of phenotypic data.

At least, 11 C57BL/6N-derived substrains exist and are commercially available. However, genetic variation among C57BL/6N-derived substrains, including C57BL/6NTac, which was used to generate the IKMC ES cells has not yet been verified. Previously, C57BL/6J-specific SNPs detected by comparing the reference C57BL/6J sequence [15] with other inbred mouse strains have been reported [7, 17, 26, 27, 29, 31]; however, C57BL/6N was not included in these SNP data. Recently, the Wellcome Trust Sanger Institute (WTSI) published whole genome resequencing data of 17 key mouse inbred strains including C57BL/6NJ, which enabled us to identify C57BL/6NJ-specific SNPs through comparisons with other inbred strains [10, 30].

In this study, we searched for SNPs specific to the C57BL/6NJ strain using the resequence database of the WTSI. Moreover, in light of the branching history of C57BL/6N-derived substrains, we found variation in the number of accumulated C57BL/6NJ-specific SNPs among the C57BL/6N-derived substrains, which can be used to differentiate the substrains.

Materials and Methods

Animals

SNP genotyping was conducted in 11 C57BL/6N and 7 C57BL/6J-derived inbred substrains available from different breeders and holders around the world (Table 1). As for the C57BL/6NJ, C57BL/6By, C57BL/6ByJ, and C57BL/6JEiJ strains, genomic DNA from one animal of each strain was obtained from The Jackson Laboratory Mouse DNA Resources (stock #005304, #000663, # 001139, and #000924, respectively; Bar Harbor, ME). As for the other strains, live mice or frozen tissue from two animals of the C57BL/6N-derived substrain and one animal of the C57BL/6J-derived substrain were used, respectively. Genomic DNA was extracted from the tail tips or kidneys using an Autogen NA-2000 automatic nucleic acid isolation system (KURABO Industries Ltd., Osaka, Japan) and/or a DNeasy Blood & Tissue Kit (QIAGEN GmbH, Hilden, Germany). All animal experiments were conducted in accordance with the Regulations for Animal Experiments of RIKEN (October 1, 2003 Rule No. 129, last amendment on March 31, 2008 Rule No. 29). Our experimental protocols,

Table 2. Confirmation of C57BL/6NJ-specific SNPs by direct sequencing of PCR products

Chromosome	Number of SNPs selected	Number of SNPs sequenced	Direct sequencing succeeded	Confirmed	Data discrepancy
1	91	91	67	60	7
2	98	98	67	61	6
3	89	45	28	25	3
4	74	18	8	8	0
5	64	16	13	11	2
6	71	48	21	10	11
7	57	16	9	9	0
8	82	13	9	8	1
9	68	12	8	8	0
10	61	14	10	8	2
11	50	11	8	8	0
12	80	12	7	7	0
13	85	13	9	8	1
14	54	12	9	8	1
15	59	11	8	8	0
16	69	11	7	7	0
17	62	10	4	4	0
18	67	10	8	8	0
19	20	9	5	4	1
X	60	16	10	7	3
Total	1361	486	315	277	38

SNP: single nucleotide polymorphism.

including those involving animals (Exp10-002), were approved by the Animal Experiments Committee of the RIKEN Tsukuba Institute.

In silico selection of informative SNPs for C57BL/6NJ

Informative SNPs were selected from the public SNP database at the Mouse Genome Project, WTSI (http://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-1303), by comparing the sequence data of C57BL/6NJ with that of the C57BL/6J reference strain. SNPs marked with a "high confidence" call on the database were extracted, and then candidate C57BL/6NJ-specific SNPs were selected through a comparison with sequence data from the 12 other inbred mouse strains in the database (129P2/OlaHsd, 129S1/SvImJ, 129S5/SvEvBrd, A/J, AKR/J, BALB/cJ, C3H/HeJ, CBA/J, DBA/2J, LP/J, NOD/ShiLtJ, and NZO/HlLtJ).

Experimental confirmation of SNPs and genotyping

To confirm whether the *in silico*-selected C57BL/6NJ-specific candidate SNPs were present in mouse DNA samples, the SNP loci of the C57BL/6NJ and C57BL/6J strains were genotyped by PCR and direct sequencing. Target regions containing the candidate SNPs were amplified by PCR with flanking primers designed by using BatchPrimer3 v1.0 (probes.pw.usda.gov/batchprimer3/index.html). PCR was performed using a QIAGEN multiplex PCR Kit (QIAGEN GmbH) according to the manufacturer's protocol. The PCR products were electrophoresed and separated on an E-Gel CloneWell 0.8% SYBR Safe gel using an E-Gel iBase Power system (both from Life Technologies, Carlsbad, CA). Sequencing reactions were performed in a DNA Engine® and Dyad™ PTC-220 Peltier Thermal Cycler (Bio-Rad, Laboratories, Inc., Hercules, CA) using an ABI BigDye® Terminator v3.1 Cycle Sequencing Kit with AmpliTaq DNA polymerase (Life Technologies), following the protocols supplied by the manufacturers. Single-pass sequencing was performed on each template primer. The fluorescently labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and then subjected to electrophoresis in an ABI 3730 × l sequencer (Life Technologies). After the SNPs were confirmed in both strains, the other C57BL/6 substrains were genotyped by the same method. The flanking primers used for each SNP typing are listed in Table 4.

Results

From the SNP database, 1,361 informative SNPs on all of the chromosomes except Y were screened, and the

Table 3. Status of 10 nonsynonymous variant SNPs

dbSNP ID	Chromo some	Position (bp) (GRCm38)	Linked gene	C57BL/6J allele	C57BL/6NJ allele	Variant type of C57BL/6NJ allele
rs262569844	5	89,775,351	*Adamts3*	C/C	T/T	Missense (Val199Ile)
rs229712565	5	112,762,721	*Myo18b*	C/C	T/T	Missense (Arg1935His)
rs243575509	7	102,973,309	*Olfr577*	C/C	T/T	Missense (Val228Ile)
rs246274290	10	88,091,833	*Pmch*	T/T	C/C	Missense (Ile132Thr)
rs240617401	11	46,222,615	*Cyfip2*	G/G	A/A	Missense (Ser968Phe)
rs238893157	11	90,480,671	*Stxbp4*	C/C	T/T	Missense (Ala535Thr)
rs242991609	13	119,477,808	*4833420G17Rik*	C/C	A/A	Missense (Thr484Lys)
rs248157600	14	70,586,204	*Fam160b2*	G/G	T/T	Missense (Ser575Arg)
rs246033409	15	11,336,383	*Adamts12*	G/G	T/T	Missense (Cys1518Phe)
rs230596409	13	64,921,972	*Spata31*	C/C	T/T	Stop gained (Arg645Ter)

Information of the variant type was obtained from the Ensembl Mouse Genome Server.
SNP: single nucleotide polymorphism.

SNPs were able to distinguish C57BL/6NJ from the other 12 inbred strains *in silico* (Table 2). No informative SNPs on chromosome Y were found in the database. Among the informative SNPs, 486 candidate SNPs from chromosome 1–X were selected to include approximately 1 SNP locus per 10 Mb, and the genotypes of the selected SNP loci were examined by direct sequencing of C57BL/6J and C57BL/6NJ DNA samples. As a result, 315 SNP loci were sequenced successfully and 277 SNPs were confirmed to be specific to C57BL/6NJ. The genotypes of the remaining 38 SNP loci were not consistent with the data in the SNP database. According to the Ensembl Mouse Genome Server (www.ensembl.org/Mus_musculus/Info/Index), 10 of the 277 SNPs were nonsynonymous variant SNPs, such as missense or stop-gain variants, which were predicted to affect the amino acid sequence of the protein (Table 3).

Next, 100 SNPs for genetic monitoring of C57BL/6N-derived substrains were selected from the 277 SNPs to include 1 SNP locus per 10–40 Mb to cover all of the chromosomes except Y (Table 4), and these SNPs were genotyped in the other 10 C57BL/6N and 6 C57BL/6J-derived substrains. All C57BL/6N and C57BL/6J-derived substrains were homozygous for these 100 SNP loci. In addition, when two samples from the same strain were genotyped in the C57BL/6N-derived substrains, they were found to be completely identical. The genotyping results for the C57BL/6J-derived substrains were consistent with the C57BL/6J reference sequence in the database. SNP genotyping demonstrated variation in the number of C57BL/6NJ-specific SNPs among the C57BL/6N-derived substrains (Table 5). Fourteen SNPs at Locus Nos. 10, 13, 14, 22, 30, 42, 44, 45, 54, 58, 63, 68, 84, and 93 were shared commonly, while the remaining 86 SNPs were only shared partly among the 11 C57BL/6N-derived substrains.

Discussion

The relationship between the genealogy of the C57BL/6N-derived substrains used in this study and the number of the 100 C57BL/6NJ-specific SNPs in the substrains is summarized in Fig. 1. Milestones in the establishment of the strains were obtained from the product catalogs of the breeders and previous reports [14, 16]. The C57BL/6 strain was separated from the C57BL parental strain at the end of the 1940s and introduced to The Jackson Laboratory. A few years later, C57BL/6 mice were sent to the National Institutes of Health (NIH; Bethesda, MD) from The Jackson Laboratory, and the C57BL/6N mice were separated into the other C57BL/6N substrains, including the C57BL/6By strain, at different times. C57BL/6NCrSim mice from Simonsen Laboratories (Gilroy, CA) were derived from C57BL/6N mice at the NIH in 1995. C57BL/6NTac mice from Taconic Farms (New York, NY) were derived from the NIH Animal Genetic Resource at F151 in 1991. C57BL/6NJ mice were derived from embryos cryopreserved at the NIH in 1984. C57BL/6NJcl mice were introduced to the Central Institute for Experimental Animals (Kawasaki, Japan) from the NIH at F121 in 1978, and then transferred to CLEA Japan (Tokyo, Japan) at F146 in 1988. Charles River Laboratories (Wilmington, MA) obtained C57BL/6N mice from the NIH in 1974. The C57BL/6NCrl mice were further transferred to Charles River Laboratories Japan (Yokohama, Japan) at F101 in 1976, and since then, the mice have been distributed as C57BL/6NCrlCrlj. C57BL/6NSeac mice were intro-

Table 4. Position of the 100 selected SNP loci and their flanking primer sequence

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38)	C57BL/6J allele	C57BL/6NJ allele	Flanking primer sequence (5' to 3')	
						Forward primer	Reverse primer
1	rs246236360	1	11,996,705	C/C	T/T	ACCCCCTGAACCTTCAATTC	TTTCCCATGGAATTCTGCTC
2	rs246490354	1	14,344,561	T/T	A/A	GGGGAAGAGTGGGATGACTA	TGGTCAGCATATTCCAGTGC
3	rs212521754	1	16,968,405	C/C	A/A	GCAACGAAGGAAATTGAAGC	TGTTGAGGCATGTCCCTTTT
4	rs227394849	1	19,544,960	A/A	T/T	GGGCAGAACTTCCTTTTCCT	TCTCACCTGAGTCCCTGGAT
5	rs232920323	1	21,639,642	C/C	G/G	GAAATAGCACAGGTCCATCAAA	CCCAGCAGACAAGAGACAAA
6	rs213024334	1	30,167,141	C/C	T/T	GGAATCTCAACCTAAGCAGCA	TGGAAAATTGAAGCAACCAG
7	rs244794780	1	40,107,883	T/T	A/A	TCTGTTGCTCTCCAGCATTG	CTACACCCTGGCCTGACACT
8	rs260670033	1	50,146,448	C/C	T/T	AGCAGAAATGCCAAAATGCT	TCAGACCCAAAAGGACATGC
9	rs249907793	1	61,950,812	C/C	A/A	CCAGTGGGTTAAGTGGGATT	ATCAAATGGGGTGGCATTTA
10	rs265151779	1	83,182,637	G/G	A/A	ATTCTGCTAACTGCGGAGGA	TTGTTCACCCTCTTCCCCTA
11	rs229124202	1	89,861,338	G/G	T/T	GGAAGGCAGATCACCAACTC	TCTATGGTGGCCCTAGGATG
12	rs237656339	1	99,547,673	G/G	A/A	TGGCTCCTGACATCTTTCCT	GCTCCTGGATCGGCATATTA
13	rs223540754	1	110,024,886	G/G	C/C	GACCAAATGCCTTGAAAATGA	CTCTCCCCATCCCTTTTCTT
14	rs259683638	1	119,116,297	C/C	T/T	AGGTCTTGGGCTCTTTAGGG	ACTTGCTGGCTGACTCCTTC
15	rs229911289	1	132,980,179	T/T	A/A	TTTTATTTCCTCCGCATTGG	ACTCGGGAACACACAAGCTC
16	rs215622703	1	142,008,378	C/C	A/A	TTTTTGTGTTGGCCAAGGAT	CCTTTCTCTTCAGAGGGGTTTT
17	rs239017398	1	154,474,620	C/C	T/T	CAGATCCCGGCTCAATTTTA	AGCTCATTAGCCTGGCATGT
18	rs214254072	1	161,859,644	C/C	T/T	GTTTGCTCCTCCCCCTACTC	TGTATGAAAATGTGCAGGTTCTG
19	rs255914894	1	172,611,934	G/G	A/A	ATGCCGGTGTACCTTCAGAG	CCCCAGTAACCATTCTCCTG
20	rs222303818	1	179,503,532	G/G	A/A	CTGCCCATACTCCTGTCCAT	AGGGCCTGGTACTGAGAACA
21	rs262282675	1	188,434,376	A/A	T/T	ACCCTTTGATGGTTCCCATT	AATTTTGCTAGGCCCATGAAT
22	rs251979693	2	11,214,185	C/C	T/T	CCCCACATTTGCTTATCCAG	GCCAATTGTGAGGAATGCTT
23	rs230600693	2	21,681,174	G/G	T/T	GGTCCAGCATTATTGGCATT	GTGATCCCATCTGCCATCTT
24	rs242780245	2	30,188,489	A/A	C/C	CCACTGTCACCAGCACATTC	CCACCACTCCTCTCCGAATA
25	rs228546410	2	41,205,764	T/T	A/A	ATGCCCACAATGCAAACATA	TAGCCCCTCTGACTGTCCAC
26	rs254996546	2	51,969,852	C/C	T/T	CCGTGACCAAGTATGCACAG	GCTGAGGGTTCAGTTGTGGT
27	rs256541267	2	70,251,451	C/C	T/T	CTGAAGAAAGGCCTGTTTGG	CGAATTCAATGCTGCCAATA
28	rs248280077	2	80,873,138	C/C	T/T	TGTGCCGATTCCTCTAGCTT	CTGCACCAATTAGCAGCAA
29	rs214356625	2	96,674,180	G/G	T/T	GTGTATGCCCCCAACCTTTA	CCAGTGATTGCATTTCACCTT
30	rs224344563	2	102,710,505	A/A	T/T	CAGGACAGGAGAGGGTCAAG	ATCCCAGGCCATAGGATTTT
31	rs251933504	2	112,966,408	T/T	C/C	GCTCGGTCTGAAAGGTCAAC	GGAAGCAAGAGCTTGGAAGA
32	rs258508221	2	122,708,738	T/T	A/A	ACTTTGTGCCTTTTGCAACC	GAGGGGGATCCAAGGATAAG
33	rs255014110	2	132,432,999	C/C	T/T	CAGCACAGATGGTTTCATGG	AGATGCACAAGTGGCTCTGA
34	rs242413924	2	140,793,056	T/T	G/G	AAATTTTCTTCCCCACAGCA	GTGAGCCAGTACAGGGGAGA
35	rs217443774	2	152,781,403	A/A	G/G	CTCTTCTTCCTGCCCTTCCT	AGCCATTGAGTGAGGTGCTT
36	rs253212197	2	164,813,748	C/C	T/T	CTGAACTGCAACCCTCATCA	AGTGTAGCCCTCCCTGTCCT
37	rs213376233	2	170,240,435	G/G	A/A	GCTAAGTGGTCTTGGGATGC	GCCACCACACACAGCTAATTT
38	rs264719247	2	180,149,012	A/A	G/G	TTCTGCTAGGCTTCCTGGTG	CTCCCTCACAACAGGCTCAT
39	rs221521392	2	181,868,891	C/C	T/T	TCTTTTTGCCTCTTGTTGGAA	CGTGCTTGTGAGCTCTCTGA
40	rs256520809	3	8,498,163	G/G	A/A	TGGCAGAAGTTTGTTTCAGG	CCATCTGGGGCTGAATACTT
41	rs214801792	3	32,773,111	G/G	C/C	TGCTGGGGTAGTTTTCCACT	GGAGGGAGTCAGGTGCAATA
42	rs222821429	3	66,305,330	C/C	T/T	CACCCAACACCCACAGAGTA	TGTCTTTTCAAAGGGCCAGA
43	rs243656799	3	72,616,062	G/G	A/A	CCCATTGGACACGAAAACTT	CACTGCTGCTCATTGGTTCA
44	rs262827930	3	109,597,274	A/A	T/T	GGCAGTTTGGCCTGTAGGTA	CTTTACTGGCTTGCCTCACC
45	rs254145219	3	147,657,255	C/C	T/T	CAGCAGGATATGCGTCCTCT	GCTTCCCCTCCCATAATTTC
46	rs219227155	4	19,328,298	T/T	C/C	ACACAAGAACTGGCACATGG	TTGGGACCTGTCAGCCTATC
47	rs235104023	4	56,463,984	C/C	T/T	AGCAGTTGGTGTGTTTGCTG	CCCCCATTGCTTTGTGTCTA
48	rs261879287	4	104,973,294	T/T	G/G	GACGAGGGAAAATGAGTGGA	CAAATGGCATGTTCGTTTGA
49	rs256724446	5	35,701,259	G/G	A/A	ATTCATTCCTGACCCATCCA	CTTCCTCAATTCCCCTCCAT
50	rs260260338	5	80,026,465	G/G	A/A	TGGGGAAAGAATGTGCCTAC	TTGGTCCAACATCAAACTACCTT
51	rs217297994	5	117,118,668	G/G	A/A	CCAAGGAGCAGCCCTACTAA	CAACTCCTGGTCAACGCTCT
52	rs221990668	5	150,224,989	G/G	T/T	CTTGTAGAACCCAGGCCATC	GTCCCCACCCATTACATCAG
53	rs257294810	6	39,971,164	G/G	A/A	GCATTCAGCTCTCCTTCCTG	GAGACCTGGGCACAATGACT
54	rs224069095	6	74,169,211	C/C	A/A	CTCATCATGACACAAGGAGCA	CATGTGTGGCCCCTAGTTCT
55	rs37540455	6	113,159,679	G/G	A/A	ATTCCTGGCCAGCCTTAGAT	TGTTGGTGAGAGTCCTTCCA
56	rs217544076	6	144,513,005	G/G	C/C	CACACATCCATCTGCCTCTG	GCAGCCGGAGTATTAGCAAG
57	rs212452109	7	16,595,985	A/A	T/T	GAGTTCAATCCCTGGGACAA	GTGTCACTGTGGCTGGCTTA
58	rs224103578	7	53,390,545	C/C	T/T	GGAGGGAATGTGTCAGTAACG	CCTGACCTCAGTGTGCAGAA
59	rs243575509	7	102,973,309	C/C	T/T	TCATCACAGGAGGGAAGAGG	GGCTATCTGTCGTCCTTTGC
60	rs229340185	7	140,821,590	A/A	T/T	CTTCAGGCCCTTCACGAGTA	GATTCCTATTGGCTGGCTTG
61	rs263791105	8	22,903,742	G/G	A/A	CGAATGTGCATTTGTCATCC	GCCCTTCCAACTACCTCACA
62	rs255341040	8	58,790,625	G/G	A/A	GGCACTGTTTATCTTGGGAAC	TGCCAAACAGCACTCAGAAG
63	rs239219835	8	79,117,401	G/G	A/A	TAAATGGCCCGAATTCACAT	TGTGCACCTTCCTTTGTTCA
64	rs256624163	8	94,046,068	G/G	A/A	TCAGAGCCCACAGAAAAAGG	CCATGGGTTTCACACATTCA
65	rs211750147	8	118,442,679	G/G	A/A	TCGGGGGCTTAATTTCTCTT	TGCCTAGACCTGGATTTGGT
66	rs52003732	9	10,125,248	T/T	C/C	TTCTCCCCTCTGTGAGCAAG	TTGCCACCACCTCAAAAACT
67	rs214490504	9	60,662,109	G/G	A/A	TCATCCCGGAACATAAATGG	AGTCTCGCCAATACGACTGC
68	rs243500146	9	116,160,235	C/C	T/T	CAGAAGGATCCTGGACTTGC	CTCTTATCTCCCCGCCAGA
69	rs51123066	10	11,070,460	G/G	T/T	CAAGGCCCCTGTAAATCCTT	CGAGGGCCTAGCATATTTCA
70	rs219489973	10	41,944,745	G/G	A/A	CCTCGGCTAACTTCAAGCAC	GCCTGTGCGCTTACTTTGAT
71	rs213583872	10	49,357,252	G/G	T/T	GTTGCACAGGCTGAGAATGA	CCCAAATGAATTGCAAAGGT
72	rs246274290	10	88,091,833	T/T	C/C	CACAGAACACAGGCTCCAAA	GCCAACATGGTCGGTAGACT
73	rs223857079	11	12,253,003	A/A	T/T	CTGGTTTGGAGGTGAGCATT	AAAAGCTCCGGAAGGTGAAT
74	rs240617401	11	46,222,615	G/G	A/A	TGACCCCCAATCACACATTA	GCCAGCTTATCCATCTGCAC
75	rs231656457	12	29,886,947	T/T	G/G	TCTTGGTTAAGGTGGCAAGG	ATTCACAAATGTCGGCATCA
76	rs217422777	12	70,772,479	C/C	T/T	CCGGGAAAAACATACACACC	ACCCTGCTCTCCTTGACAAA
77	rs221345442	12	97,702,669	A/A	T/T	TCCTCTCACCCGTATCTGCT	GCGTCTTCAGAGACCTTCTCA
78	rs226310424	13	41,494,375	G/G	C/C	CATCTCCATGGTGCTCGATA	TCCACAGTTCAGCCAAAGG
79	rs230596409	13	64,921,972	C/C	T/T	GGTGTTGACCATGAGCCTTC	CTGGGGTGAGCTTAGGTCTG
80	rs251507217	13	101,112,155	A/A	T/T	CCCTGTACCGTCCAATCATC	TTCTCCCCACCTCTGATGTC
81	rs242991609	13	119,477,808	C/C	A/A	TTTTGGCTGTGCAATTCTTG	CACACAGAGGTCGCCTATCA
82	rs265193270	14	39,164,780	C/C	T/T	GGCCATCTCATCAGTGCATA	AGGCTGACATGGTTTTGAGC
83	rs235428682	14	75,727,727	G/G	A/A	ACATCTCCAGCTTCCAGACC	GAGGCGGTGACTATGAAGGA
84	rs222607275	14	117,850,332	G/G	A/A	TTGTGGTTTCAGGAATGTCG	GGCAAACTTCTTGCCTCAGA
85	rs243245803	15	22,748,238	T/T	G/G	TGTTTCCTGAGAGGGTTTGC	TTTGGGAAAGACAAGGGAGA
86	rs243400512	15	55,816,925	C/C	T/T	TCAGAGGCTGAAGTGACAGC	GGGCAGTCTGTCTGTGGAAG
87	rs231321125	15	97,760,563	C/C	G/G	CTCTCACGAGGACATGAGCA	GGCTCCCCAGTAAAACATGA
88	rs230243864	16	20,458,800	C/C	T/T	TGGGGGCTTATCTTGTTCAC	ACTTAACCACAAGCCCAGGA
89	rs240948896	16	61,450,798	G/G	A/A	TGGAGCATGACAAGGAATCA	TTTGCAAAATCCATGATTGG
90	rs240067957	17	40,854,409	C/C	T/T	TGCTCATGGTAAATGCTGGA	TCAGCACTCAGGTGATTTCC
91	rs259144033	17	69,131,609	C/C	T/T	CATGCACACGGCAGTAGAAG	CAGAGGTGGAACCAGGAAGA
92	rs225963780	18	22,530,101	G/G	T/T	AGCGGTATGCTTGCTTTGAT	ACAAGGGCCAAATATTGCTG
93	rs214638331	18	41,344,993	T/T	C/C	CTGCCAGATAAGCCACCAAT	TCACCAATGACAGAGCCAAAAA
94	rs255789242	18	59,519,801	C/C	T/T	TTCCCCTAGCTTGGAAACCT	TCTTTCCTGGAGTTGCCCTA
95	rs263687961	18	90,448,757	A/A	G/G	TTCCCATTGTGGTCATTGAA	TGAGCTAAATTTGGAGCAAGC
96	rs232414357	19	23,329,888	C/C	T/T	CAGCCCTCCCCTTTATCTTC	GTATGCCCCTGTTGGGTCTA
97	rs230656170	19	40,364,531	G/G	A/A	AGCGCTCGCTTTGACATAAT	TGGGACAGGAGGAGGTTACA
98	rs246037535	X	84,805,631	C/C	T/T	CCCTAGGGCAACATGGTAAA	CATTCCGTGCAAATGAGATG
99	rs266019057	X	112,095,948	A/A	G/G	GGTGGCAGAGATGGAAACAT	CTGTCTTGCTTGGTCGCTAA
100	rs212226666	X	157,445,480	T/T	A/A	TGCACTTGCACATCCTACAG	GGGGTTTGGGTTTTCATTTT

SNP: single nucleotide polymorphism.

Table 5. SNPs among C57BL/6 substrains

Chromosome	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2
Locus No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
C57BL/6NJ	T/T	A/A	A/A	T/T	G/G	T/T	A/A	T/T	A/A	A/A	T/T	A/A	C/C	T/T	A/A	A/A	T/T	T/T	A/A	A/A	T/T	T/T	T/T	C/C	A/A	T/T	T/T	T/T	T/T	T/T	C/C	A/A	T/T	G/G
C57BL/6NCrSim	T/T	A/A	A/A	T/T	G/G	T/T	T/T	C/C	A/A	A/A	T/T	A/A	C/C	T/T	A/A	A/A	T/T	T/T	A/A	A/A	T/T	T/T	T/T	C/C	T/T	T/T	C/C	T/T	T/T	T/T	C/C	A/A	T/T	T/T
C57BL/6NTac	T/T	A/A	A/A	T/T	G/G	T/T	T/T	C/C	A/A	A/A	T/T	A/A	C/C	T/T	A/A	A/A	T/T	T/T	A/A	A/A	T/T	T/T	T/T	C/C	T/T	T/T	C/C	T/T	T/T	T/T	C/C	A/A	T/T	T/T
C57BL/6NJcl	T/T	A/A	C/C	T/T	G/G	T/T	T/T	C/C	A/A	A/A	T/T	A/A	C/C	T/T	T/T	A/A	T/T	T/T	A/A	A/A	T/T	T/T	T/T	C/C	T/T	T/T	C/C	T/T	T/T	T/T	C/C	A/A	T/T	T/T
C57BL/6NSeac	T/T	A/A	C/C	T/T	G/G	T/T	T/T	C/C	A/A	A/A	T/T	A/A	C/C	T/T	T/T	A/A	T/T	T/T	A/A	G/G	T/T	T/T	T/T	C/C	T/T	T/T	C/C	T/T	T/T	T/T	C/C	T/T	T/T	T/T
C57BL/6NCrlCrlj	T/T	A/A	C/C	T/T	G/G	T/T	T/T	C/C	A/A	A/A	T/T	A/A	C/C	T/T	T/T	A/A	T/T	T/T	A/A	G/G	T/T	T/T	T/T	C/C	T/T	T/T	C/C	T/T	T/T	T/T	C/C	T/T	T/T	T/T
C57BL/6NCrl	T/T	A/A	C/C	T/T	G/G	T/T	T/T	C/C	A/A	A/A	T/T	A/A	C/C	T/T	T/T	A/A	T/T	T/T	A/A	G/G	T/T	T/T	T/T	C/C	T/T	T/T	C/C	T/T	T/T	T/T	C/C	A/A	T/T	T/T
C57BL/6NHsd	T/T	A/A	C/C	T/T	G/G	T/T	T/T	C/C	A/A	A/A	T/T	A/A	C/C	T/T	T/T	A/A	T/T	T/T	A/A	A/A	T/T	T/T	T/T	C/C	T/T	T/T	C/C	T/T	T/T	T/T	C/C	T/T	T/T	T/T
C57BL/6NCrSlc	T/T	T/T	C/C	A/A	C/C	T/T	T/T	C/C	A/A	A/A	T/T	G/G	C/C	T/T	T/T	C/C	C/C	C/C	G/G	G/G	T/T	T/T	G/G	A/A	T/T	T/T	C/C	C/C	T/T	T/T	T/T	T/T	C/C	T/T
C57BL/6By	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	A/A	G/G	G/G	C/C	T/T	T/T	C/C	C/C	C/C	G/G	G/G	A/A	T/T	G/G	A/A	T/T	C/C	C/C	C/C	G/G	T/T	T/T	T/T	C/C	T/T
C57BL/6ByJ	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	A/A	G/G	G/G	C/C	T/T	T/T	C/C	C/C	C/C	G/G	G/G	A/A	T/T	G/G	A/A	T/T	C/C	C/C	C/C	G/G	T/T	T/T	T/T	C/C	T/T
C57BL/6JBomTac	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	G/G	G/G	G/G	G/G	C/C	T/T	C/C	C/C	C/C	G/G	G/G	A/A	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	A/A	T/T	T/T	C/C	T/T
C57BL/6JRccHsd	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	G/G	G/G	G/G	G/G	C/C	T/T	C/C	C/C	C/C	G/G	G/G	A/A	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	A/A	T/T	T/T	C/C	T/T
C57BL/6JOlaHsd	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	G/G	G/G	G/G	G/G	C/C	T/T	C/C	C/C	C/C	G/G	G/G	A/A	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	A/A	T/T	T/T	C/C	T/T
C57BL/6JEiJ	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	G/G	G/G	G/G	G/G	C/C	T/T	C/C	C/C	C/C	G/G	G/G	A/A	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	A/A	T/T	T/T	C/C	T/T
C57BL/6JJmsSlc	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	G/G	G/G	G/G	G/G	C/C	T/T	C/C	C/C	C/C	G/G	G/G	A/A	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	A/A	T/T	T/T	C/C	T/T
C57BL/6JJcl	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	G/G	G/G	G/G	G/G	C/C	T/T	C/C	C/C	C/C	G/G	G/G	A/A	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	A/A	T/T	T/T	C/C	T/T
C57BL/6J	C/C	T/T	C/C	A/A	C/C	C/C	T/T	C/C	C/C	G/G	G/G	G/G	G/G	C/C	T/T	C/C	C/C	C/C	G/G	G/G	A/A	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	A/A	T/T	T/T	C/C	T/T

Chromosome	2	2	2	2	2	3	3	3	3	3	3	4	4	4	5	5	5	5	6	6	6	6	7	7	7	7	8	8	8	8	9	9	9
Locus No.	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
C57BL/6NJ	G/G	T/T	A/A	G/G	T/T	A/A	C/C	T/T	A/A	T/T	T/T	C/C	T/T	G/G	A/A	A/A	A/A	T/T	A/A	A/A	A/A	C/C	T/T	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	C/C	A/A	T/T
C57BL/6NCrSim	G/G	T/T	G/G	G/G	T/T	A/A	C/C	T/T	A/A	T/T	T/T	C/C	T/T	G/G	A/A	A/A	A/A	T/T	A/A	A/A	A/A	C/C	A/A	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	C/C	A/A	T/T
C57BL/6NTac	G/G	T/T	G/G	G/G	T/T	A/A	C/C	T/T	A/A	T/T	T/T	C/C	T/T	G/G	A/A	A/A	A/A	T/T	A/A	A/A	A/A	C/C	A/A	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	C/C	A/A	T/T
C57BL/6NJcl	G/G	T/T	G/G	G/G	C/C	A/A	C/C	T/T	A/A	T/T	T/T	C/C	T/T	G/G	G/G	A/A	G/G	T/T	A/A	A/A	A/A	C/C	A/A	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	C/C	A/A	T/T
C57BL/6NSeac	G/G	T/T	G/G	A/A	C/C	A/A	C/C	T/T	A/A	T/T	T/T	T/T	T/T	T/T	G/G	A/A	G/G	G/G	A/A	A/A	A/A	G/G	A/A	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	T/T	A/A	T/T
C57BL/6NCrlCrlj	G/G	T/T	G/G	A/A	C/C	A/A	C/C	T/T	A/A	T/T	T/T	T/T	T/T	T/T	G/G	A/A	G/G	G/G	A/A	A/A	A/A	G/G	A/A	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	T/T	A/A	T/T
C57BL/6NCrl	G/G	T/T	G/G	G/G	C/C	A/A	C/C	T/T	A/A	T/T	T/T	C/C	T/T	G/G	G/G	A/A	G/G	T/T	A/A	A/A	A/A	G/G	A/A	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	C/C	A/A	T/T
C57BL/6NHsd	G/G	T/T	G/G	G/G	C/C	A/A	C/C	T/T	A/A	T/T	T/T	C/C	T/T	G/G	G/G	A/A	G/G	T/T	A/A	A/A	A/A	G/G	A/A	T/T	T/T	T/T	A/A	A/A	A/A	A/A	A/A	C/C	A/A	T/T
C57BL/6NCrSlc	A/A	T/T	G/G	A/A	C/C	G/G	G/G	T/T	G/G	T/T	T/T	T/T	C/C	T/T	G/G	A/A	G/G	G/G	G/G	A/A	G/G	G/G	A/A	T/T	C/C	T/T	G/G	A/A	A/A	G/G	G/G	T/T	G/G	T/T
C57BL/6ByJ	A/A	C/C	G/G	A/A	C/C	G/G	G/G	T/T	G/G	T/T	T/T	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	A/A	G/G	G/G	A/A	T/T	C/C	A/A	G/G	G/G	A/A	G/G	G/G	T/T	G/G	T/T
C57BL/6By	A/A	C/C	G/G	A/A	C/C	G/G	G/G	T/T	G/G	T/T	T/T	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	A/A	G/G	G/G	A/A	T/T	C/C	A/A	G/G	G/G	A/A	G/G	G/G	T/T	G/G	T/T
C57BL/6JBomTac	A/A	C/C	G/G	A/A	C/C	G/G	G/G	C/C	G/G	A/A	C/C	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/A	C/C	C/C	A/A	G/G	G/G	G/G	G/G	G/G	T/T	G/G	C/C
C57BL/6JRccHsd	A/A	C/C	G/G	A/A	C/C	G/G	G/G	C/C	G/G	A/A	C/C	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/A	C/C	C/C	A/A	G/G	G/G	G/G	G/G	G/G	T/T	G/G	C/C
C57BL/6JOlaHsd	A/A	C/C	G/G	A/A	C/C	G/G	G/G	C/C	G/G	A/A	C/C	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/A	C/C	C/C	A/A	G/G	G/G	G/G	G/G	G/G	T/T	G/G	C/C
C57BL/6JEiJ	A/A	C/C	G/G	A/A	C/C	G/G	G/G	C/C	G/G	A/A	C/C	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/A	C/C	C/C	A/A	G/G	G/G	G/G	G/G	G/G	T/T	G/G	C/C
C57BL/6JJmsSlc	A/A	C/C	G/G	A/A	C/C	G/G	G/G	C/C	G/G	A/A	C/C	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/A	C/C	C/C	A/A	G/G	G/G	G/G	G/G	G/G	T/T	G/G	C/C
C57BL/6JJcl	A/A	C/C	G/G	A/A	C/C	G/G	G/G	C/C	G/G	A/A	C/C	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/A	C/C	C/C	A/A	G/G	G/G	G/G	G/G	G/G	T/T	G/G	C/C
C57BL/6J	A/A	C/C	G/G	A/A	C/C	G/G	G/G	C/C	G/G	A/A	C/C	T/T	C/C	T/T	G/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/A	C/C	C/C	A/A	G/G	G/G	G/G	G/G	G/G	T/T	G/G	C/C

Chromosome	10	10	10	10	11	11	12	12	12	13	13	13	13	14	14	14	15	15	15	16	16	17	17	18	18	18	19	19	X	X	X
Locus No.	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
C57BL/6NJ	T/T	A/A	T/T	C/C	T/T	A/A	G/G	T/T	T/T	C/C	T/T	T/T	A/A	T/T	A/A	A/A	G/G	T/T	G/G	T/T	A/A	T/T	T/T	T/T	C/C	T/T	G/G	T/T	A/A	T/T	G/G	A/A
C57BL/6NCrSim	T/T	A/A	T/T	C/C	T/T	A/A	G/G	T/T	T/T	G/G	T/T	T/T	A/A	T/T	A/A	A/A	G/G	T/T	C/C	T/T	A/A	C/C	T/T	T/T	C/C	T/T	G/G	T/T	A/A	C/C	G/G	A/A
C57BL/6NTac	T/T	A/A	T/T	C/C	T/T	A/A	G/G	T/T	T/T	G/G	T/T	T/T	A/A	T/T	A/A	A/A	G/G	T/T	C/C	T/T	A/A	C/C	T/T	T/T	C/C	T/T	G/G	T/T	A/A	C/C	G/G	A/A
C57BL/6NJcl	T/T	A/A	T/T	T/T	T/T	A/A	G/G	T/T	T/T	G/G	T/T	A/A	A/A	C/C	A/A	A/A	G/G	T/T	C/C	T/T	A/A	C/C	T/T	T/T	C/C	C/C	G/G	T/T	A/A	C/C	G/G	T/T
C57BL/6NSeac	T/T	G/G	T/T	T/T	T/T	A/A	G/G	T/T	T/T	G/G	T/T	A/A	A/A	C/C	A/A	A/A	G/G	T/T	C/C	T/T	A/A	C/C	T/T	T/T	C/C	C/C	G/G	T/T	A/A	C/C	G/G	T/T
C57BL/6NCrlCrlj	T/T	G/G	T/T	T/T	T/T	A/A	G/G	T/T	T/T	G/G	T/T	A/A	A/A	C/C	A/A	A/A	G/G	T/T	C/C	T/T	A/A	C/C	T/T	T/T	C/C	C/C	G/G	T/T	A/A	C/C	G/G	T/T
C57BL/6NCrl	T/T	A/A	T/T	C/C	T/T	A/A	G/G	T/T	T/T	G/G	T/T	A/A	A/A	C/C	A/A	A/A	G/G	T/T	C/C	T/T	A/A	C/C	T/T	T/T	C/C	C/C	G/G	T/T	A/A	C/C	G/G	T/T
C57BL/6NHsd	T/T	G/G	T/T	T/T	T/T	A/A	G/G	T/T	T/T	G/G	T/T	A/A	A/A	C/C	A/A	A/A	G/G	T/T	C/C	T/T	A/A	C/C	T/T	T/T	C/C	C/C	G/G	T/T	A/A	C/C	G/G	T/T
C57BL/6NCrSlc	G/G	G/G	G/G	T/T	A/A	A/A	T/T	T/T	A/A	G/G	C/C	A/A	C/C	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	C/C	C/C	G/G	C/C	A/A	C/C	A/A	T/T
C57BL/6ByJ	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	C/C	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6By	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	A/A	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	C/C	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6JBomTac	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	G/G	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	T/T	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6JRccHsd	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	G/G	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	T/T	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6JOlaHsd	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	G/G	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	T/T	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6JEiJ	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	G/G	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	T/T	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6JJmsSlc	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	G/G	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	T/T	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6JJcl	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	G/G	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	T/T	C/C	A/A	C/C	G/G	C/C	A/A	T/T
C57BL/6J	G/G	G/G	G/G	T/T	A/A	G/G	T/T	C/C	A/A	G/G	C/C	A/A	C/C	C/C	G/G	G/G	T/T	C/C	C/C	C/C	G/G	C/C	C/C	G/G	T/T	C/C	A/A	C/C	G/G	C/C	A/A	T/T

Locus No. corresponds "Locus No." in Table 4. SNP: single nucleotide polymorphism.

duced to Kyudo (Tosu, Japan) from the Charles River Laboratory Japan in 1981. C57BL/6NHsd mice were derived from a nucleus colony at the NIH in 1974. C57BL/6NCrSlc mice were introduced to the Institute of Medical Science, The University of Tokyo (Tokyo, Japan) in 1972 by Mr. Samuel M. Poiey, and then the mice were transferred to Japan SLC (Hamamatsu, Japan) in 1975. C57BL/6By (C57BL/6ByJ) mice were derived from the breeding stocks of Dr. Donald Bailey at the NIH in 1961.

The number of C57BL/6NJ-specific SNPs in each C57BL/6N-derived substrain was well correlated with

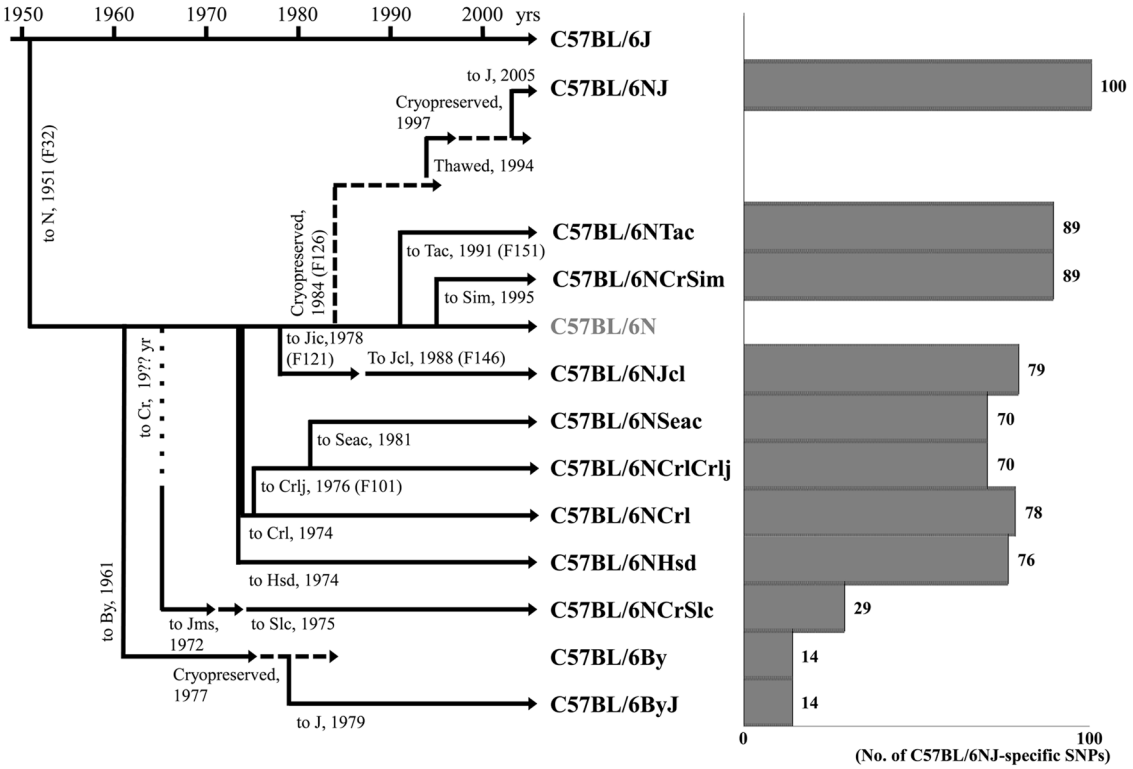

Fig. 1. Genealogy of C57BL/6N-derived substrains (left) and a bar graph for the number of C57BL/6NJ-specific SNPs (single nucleotide polymorphisms) (right). Abbreviations in the genealogy: N: National Institutes of Health; By: Dr. Donald Bailey; J: The Jackson Laboratory; Cr: National Cancer Institute; Jms: Institute of Medical Science, The University of Tokyo; Slc: Japan SLC; Crl: Charles River Laboratories; Crlj: Charles River Laboratories Japan; Seac: Kyudo; Hsd: Harlan Laboratories; Jic: Central Institute for Experimental Animals; Jcl: CLEA Japan; Sim: Simonsen Laboratories; Tac: Taconic Farm. Doted line: the year of introduction was unknown. Broken line: the strain was stored as frozen embryos. The C57BL/6NHsd and C57BL/6NCrl strains were derived independently from the NIH on unknown dates in 1974. On the right (bar graph), the number of C57BL/6NJ-specific SNPs of each C57BL/6N-derived substrain is indicated as a solid bar. The number of SNPs is also indicated at the right side of each bar.

its branching date from the original C57BL/6N strain (Fig. 1). Each C57BL/6N-derived substrain, except C57BL/6NTac and C57BL/6NCrSim, could only share C57BL/6NJ-specific SNPs that had arisen before its branching from the original C57BL/6N strain. The oldest C57BL/6By and C57BL/6ByJ strains may well preserve the genotype of C57BL/6N in approximately 1961 and only share 14 SNPs with other later substrains. As for the C57BL/6NTac and C57BL/6NCrSim strains, they only shared C57BL/6NJ-specific SNPs that had arisen before the C57BL/6NJ mice branched from the original C57BL/6N strain in 1984. Eleven SNPs that were detected only in the C57BL/6NJ strain were assumed to have arisen in C57BL/6NJ after it branched from the C57BL/6N strain in 1984. In other words, we could determine retrospectively when the C57BL/6NJ-specific SNPs had arisen by comparing the branching history and accumulated C57BL/6NJ-specific SNPs in different C57BL/6N-derived substrains. Similar observations have been reported previously in C57BL/6J-derived substrains [14, 32].

The C57BL/6NCrlCrlj and C57BL/6NSeac substrains that branched directly from C57BL/6NCrl and C57BL/6NCrlCrlj, respectively, but not directly from the original C57BL/6N, had only 70 C57BL/6NJ-specific SNPs, which is less than that of C57BL/6NCrl (Fig. 1). This corresponds to the number of discontinuous distribution patterns of SNPs seen in Table 5. One possible explanation for the discontinuous distribution of SNPs is that the 9 SNP loci, Nos. 20, 32, 38, 46, 48, 52, 66, 70, and 72 (Table 5), were heterozygous just before the branching of the C57BL/6NHsd and C57BL/6NCrl strains in 1974. Then, the SNPs were independently fixed to either C57BL/6NJ type or C57BL/6J type in the sub-

sequent inbreeding of each substrain. Finally, 6 SNP loci (Nos. 20, 38, 46, 48, 52 and 66) of C57BL/6NHsd and 8 SNP loci (Nos. 32, 38, 46, 48, 52, 66, 70 and 72) of C57BL/6NCrl were thought to be fixed to C57BL/6NJ type, while the 9 SNP loci of C57BL/6NCrlCrlj and C57BL/6NSeac were fixed to C57BL/6J type. Although there was no significant difference in the pattern of the 100 selected SNPs between C57BL/6By and C57BL/6ByJ, and C57BL/6NTac and C57BL/6NCrSim, informative SNPs that can be used to discriminate these substrains can be found by genotyping additional candidate SNPs in the future.

In the selected 277 SNPs, 10 nonsynonymous SNPs were identified that were predicted to affect the amino acid sequence of the protein (Table 3). According to the Ensembl Mouse Genome Server, 4 of these 10 SNP variants, which occurred in the *Myo18b*, *Olfr577*, *Cyfip2,* and *Adamts12* genes, were predicted to affect adversely each protein's function. Notably, the Ser968Phe variant of *Cyfip2* in C57BL/6N mice was reported recently by Kumar *et al.* [11]; this mutation destabilizes CYFIP2 protein and leads to acute and sensitized cocaine-response phenotypes. Although the C57BL/6N-derived substrains are closely related strains, such genetic differences would undoubtedly affect the phenotypes in various studies and interact with targeted mutations. Other phenotypic differences in behavior have been also reported among several C57BL/6N-derived substrains [3, 12]. As for the C57BL/6J and C57BL/6N strains, a relationship between genome variation and phenotypic changes has also been demonstrated [21]. In the future, advanced phenotypic analyses among the C57BL/6N-derived substrains will reveal novel functions of the genome variations detected among these substrains.

All of the SNPs detected in this report, including several nonsynonymous SNPs, will also be instrumental in the accurate identification of the C57BL/6N-derived substrain status of mutant mice and avoid the incorrect interpretation of data due to background effects. Furthermore, the selected SNP markers will be useful for quickly producing congenic strains to move a targeted mutation from one C57BL/6N-derived substrain to another substrain. Hence, our findings will serve as useful markers for the accurate and sophisticated genetic monitoring of C57BL/6N-derived congenic strains.

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