



Supplementary Figure 1. (Mekada & Yoshiki)

Supplementary Table 1. Major C57BL/6 mouse strains in Japan and worldwide, maintained by vendors and individual laboratories

Strain name	Source
C57BL/6J substrains	
C57BL/6J	The Jackson Laboratory (Bar Harbor, MA, USA)
C57BL/6JJcl	CLEA Japan, Inc. (Tokyo, Japan)
C57BL/6JJmsSlc	Japan SLC, Inc. (Hamamatsu, Japan)
C57BL/6JBomTac	Taconic Biosciences, Inc. (Rensselaer, NY, USA)
C57BL/6JOlaHsd	Envigo RMS, Inc. (Indianapolis, IN, USA)
C57BL/6JRccHsd	Envigo RMS, Inc. (Indianapolis, IN, USA)
C57BL/6JRj	Janvier Labs (Saint Berthevin, France)
C57BL/6JM5	National Institute of Genetics (Mishima, Japan)
C57BL/6JNrs	National Institutes for Quantum and Radiological Science and Technology (Chiba, Japan)
C57BL/6N substrains	
C57BL/6NJ	The Jackson Laboratory (Bar Harbor, MA, USA)
C57BL/6NCrl	Charles River Laboratories International, Inc. (Wilmington, MA, USA)
C57BL/6NCrlCrlj	Charles River Laboratories Japan, Inc. (Yokohama, Japan) (discontinued)
C57BL/6NJcl	CLEA Japan Inc. (Tokyo, Japan)
C57BL/6NCrSlc	Japan SLC, Inc. (Hamamatsu, Japan)
C57BL/6NSea	Kyudo Co. Ltd. (Tosu, Japan)
C57BL/6NTac	Taconic Biosciences, Inc. (Rensselaer, NY, USA)
C57BL/6NHsd	Envigo RMS, Inc. (Indianapolis, IN, USA)
C57BL/6NRj	Janvier Labs (Saint Berthevin, France)
C57BL/6NHla	Hilltop Lab Animals, Inc. (Scottsdale, PA, USA)
C57BL/6NCrSim	Simonsen Laboratories, Inc. (Gilroy, CA, USA)
C57BL/6ByJ	The Jackson Laboratory (Bar Harbor, MA, USA)

Supplementary Table 2. Profile of the 45 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6J substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6J allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
1	rs13475886	1	61,171,619		TF binding site variant	T/C	T/T	F: GCAGGCTTTTGTGTTTGT R: AGGGGTGGGATGTTATCTGT	503	T F: CAGGAAGAGAAAGTTGAGATTTT C R: GGGTAGAAGTAACCAGGGTCC	331 217
2	rs13476337	2	8,069,855		Intergenic variant	A/T	A/A	F: GCTGATTGGACAACACAGAC R: TCCAAAGAGTGTGCAATCA	556	A R: GGAGAGAAATCCTTTGGAAGATAT T R: GGAGAGAAATCCTTTGGAAGAAAA	286 286
3	rs13476359	2	16,685,831	<i>Pbx2</i>	Intron variant	G/A	G/G	F: CATTGTGGAGATGAAAATGC R: GAAACAATGCCCTTCACAAT	498	G F: CAGGCAATCTGAAACTTAGAAATG A F: CCAGGCAATCTGAAACTTAGAAATA	416 417
4	rs33142586	2	23,887,704		Intergenic variant	A/C	A/A	F: CCCATCCTCCATTATCTTT R: AAATCCTCCAGGAGAACTGG	498	A F: CCCATTTCTCCTCTCTGCTA C F: CCATTCTCCTCTCTGCTC	309 308
5	rs13476956	3	5,370,727	<i>Zfx4</i>	Intron variant	C/T	C/C	F: AAGAAAGCAATGTGCCAAGC R: TGCATAAGGCTTAAACAGATGAA	500	C R: TTAGCAGAGGTTGACTACGCG T R: TTAGCAGAGGTTGACTACGCA	274 277
6	rs13477019	3	23,824,920	<i>Naaladl2</i>	Intron variant	T/A	T/T	F: TCCAGAAATCACTCTGCTTCC R: CTGGGTTCTCTCTGAAACCA	534	T F: TGTGCATGCAGTTCTAAACACTT A F: TGTGCATGCAGTTCTAAACACTA	310 310
7	rs13477132	3	58,109,942	<i>Gm40056</i>	Intergenic variant	G/C	G/G	F: CCCAGCGATCTGTAGAGTTG R: GAACTGAGGGTTC AAGACCA	493	G F: TGCAGGCATGGTATAAATTGG C R: AGGCCTTCCAGGGGG	295 232
8	rs13477622	6	28,322,410		Intergenic variant	T/C	T/T	F: TTGCAGTTTGGGATGTGAAA R: TCCATGACTGGTAGATGGA	499	T F: CATGGCAGGGCTAGTGTTTT C F: CATGGCAGGGCTAGTGTTC	316 316
9	rs13477746	7	65,944,235	<i>Astm2</i>	Intron variant	T/C	T/T	F: CCCTGCTGTTCCTGCTAC R: GATTCAAGCCACTTTGGAA	516	T F: GAAGGAAGCACTGGTTAAGGT C F: AAGGAAGCACTGGTTAAGGC	248 247
10	rs13478320	5	70,742,061		Intergenic variant	C/A	C/C	F: GCCCTTAAACCACACTGCTG R: TGTCTTGCCATATGTTCCA	574	C F: GAAACCACACTGGAACAGATTC A F: GGAACCACACTGGAACAGATTA	317 318
11	rs3662161	5	117,459,347	<i>Ksr2</i>	Intron variant	A/G	A/A	F: ACTCTGCTTCTCAGGCTTGG R: AACTAAGCCACCGACTGCAC	487	A No allele-specific primers found G No allele-specific primers found	
12	rs33208334	5	149,626,021	<i>Hsph1</i>	Intron variant	T/C	T/T	F: TGAAGCTCACATCAACACCTG R: TGGTTTTGTGCTTGCACTGT	485	T R: CCAGAACAGGACTTCTCAAACA C R: CCAGAACAGGACTTCTCAAACG	317 318
13	rs30764547	6	28,715,963	<i>Snd1/Lrrc4</i>	Intron variant	T/A	T/T	F: CAAGCATGAAGCAGACAAGC R: ATGCAAAAGCAGGAGGAATG	495	T No allele-specific primers found A No allele-specific primers found	
14	rs13478783	6	60,591,379	<i>Gm35386</i>	Downstream gene variant	A/G	A/A	F: CTGGCCCAAAGCAAGGTAT R: TATTGAGCGTGTGATGG	396	A R: CCCAGGCCTACCTTTCTTATTT G R: CCCAGGCCTACCTTTCTTAGTC	318 317
15	rs6157367	6	67,287,180	<i>Serbp1</i>	3 prime UTR variant	T/A	T/T	F: TTTGGCTGATTTGAAATGCT R: TGCAAAACACAAGCCAAGTAA	491	T R: GACTATGGATAAATGGCTACCTCAA A R: GACTATGGATAAATGGCTACCTGAT	216 216
16	rs13479522	7	129,035,694		Intergenic variant	A/G	A/A	F: AACGATGGAATGGAGACTGA R: CTCCTTCCAGCACTGTGT	494	A R: CTCAGCATTGACGAGAACA G No allele-specific primers found	257
17	rs13479540	7	133,996,942	<i>Adam12</i>	Intron variant	A/G	A/A	F: CTACGAACATTGGCTCTCTGT R: CCACACAAAACACTGAAATTGC	504	A F: CATCAGCTTACTTCTTTTCCA G F: TCAGCTTCATTTCTTTCCG	275 273
18	rs3709624	8	15,191,287	<i>rs3709624</i>	Intron variant	T/C	T/T	F: CACAATGATTTCAAGGGTCCA R: CAAAGGCAAGTCAGGGGTAG	500	T F: TGTAGTTCATTATCAACGAGAATCT C F: TGTAGTTCATTATCAACGAGAATCC	315 315
19	rs13480100	9	21,450,142	<i>Dnm2</i>	Intron variant	T/C	T/T	F: GGCTGGCTTAGTGGCTATCA R: GCCCTGCCAGATTTCTTTT	559	T No allele-specific primers found C No allele-specific primers found	
20	rs13480109	9	25,889,972		Intergenic variant	A/G	A/A	F: CCTGGATATTTGGGGGTTT R: AAGGGACAAAATAGCCTCAAAA	541	A No allele-specific primers found G No allele-specific primers found	
21	rs13480122	9	31,156,626	<i>Aplp2</i>	Intron variant	T/C	T/T	F: TGCTGAGATACCATAAACAA R: GAATCCACGCTGACAGTTCA	564	T R: CGGTGAGGTTGTGGGGTA C R: CGGTGAGGTTGTGGGGTTG	315 315
22	rs13480575	10	33,653,023	<i>Gm15939</i>	Intron variant	T/C	T/T	F: TATCTCGCCTGGTTTGTG R: TGATGAGGGAGTGTGGA AAA	490	T R: GACAAAGGGAGTAGAGATGAAGAAA C No allele-specific primers found	325
23	rs13480619	10	57,752,462		Intergenic variant	T/C	T/T	F: TGTCACGGAGAATTTAGAGCA R: AGATGGGCCCTCTCTTTGTT	558	T F: GCTTCCTACTCTTTGTTTGT C F: GCTTCCTACTCTTTGTTTGT	296 296

Supplementary Table 2. Profile of the 45 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6J substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6J allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
24	rs29359333	10	58,401,504	<i>Lims1</i>	Intron variant	G/A	G/G	F: GCTTCTGTTCACAGCGGATAC R: TGGGTCTAACCTGTACACAT	485	G No allele-specific primers found A F: TGGACTACAGTCACAACAGATTTA	226
25	rs13480628	10	67,238,174	<i>Jmjd1c</i>	Missense variant	T/C	T/T	F: TTCTTGCTGCCTCGATCAAT R: GGGAGGAGGAACACATGAAT	491	T R: GCCAGGGATAATCTGAGGCA C R: CCAGGGATAATCTGAGGCC	302 301
26	rs13459122	10	80,795,365	<i>Dot1l</i>	3 prime UTR variant	A/C	T/T	F: AAACAGGCAAGCTTTGACAC R: CTCAGTCATCCTTGCCACTC	521	T F: GGAAGAGAACAATGCACCCT A F: GGAAGAGAACAATGCACCCA	241 241
27	rs13480759	10	109,378,627		Intergenic variant	C/T	C/C	F: TGACTAAGTGTGGCCATTCATC R: TTGAAAATTAGATCGCTCCCTA	581	C F: GCTTTGTCTTCTGTAATGTTTCAC T F: GCCTTTGTCTTCTGTAATGTTTCAT	322 323
28	rs3659787	11	4,508,730	<i>Mtmr3</i>	Intron variant	G/A	G/G	F: AGCTCAGGTCAAACATGCAAA R: TTCTTGCTGTCTGAACATCA	589	G R: CCTGCTCAGTGGGTTTGC A R: CCTGCTCAGTGGGTTTGT	307 307
29	rs13481014	11	48,117,382		Intergenic variant	T/C	T/T	F: TGAAAAATTTCACCATTCAAAGG R: GGTGCACCCTTGAACAAAAC	512	T F: CCAGTGCATAATGTAGGGTGTGT C F: CCAGTGCATAATGTAGGTGTGTC	266 266
30	rs13481117	11	79,252,230	<i>Wsb1</i>	Intron variant	G/T	G/G	F: TAGTGGCACACGCCTTAAT R: TGTTGCTCCCTTTGAAGAA	479	G F: CCCACCCACCTATTGAACG T F: CCCACCCACCTATTGAAC	300 300
31	rs13481403	12	39,488,993	<i>Gm34923</i>	Non-coding transcript exon variant	G/A	G/G	F: TAGATCCCTTCCCTCAGTC R: CCTGGGCCAAACATATACAA	282	G F: AATGTTATTCAAGGTTGTGGTCAG A F: AATGTTATTCAAGGTTGTGGTCAA	200 200
32	rs13481439	12	48,965,551		Intergenic variant	A/G	A/A	F: AGCTGTGGTTGCAGGATAG R: AGACATGGGATTTGTTTTCG	488	A R: CTATTAGATAGCCAGGAAACAGTAT G No allele-specific primers found	391
33	rs223935946	12	56,480,365		Intergenic variant	T/A	T/T	F: GAGGTCATGGAGAGGTGCTA R: GTGCTCTCCACTGCTGTCT	499	T R: GGCTCACTGTTAAGAGAGACTGGTA A F: GCTCACTGTTAAGAGAGACTGCTTT	271 271
34	rs13481569	12	85,645,337		Intergenic variant	G/A	G/G	F: GGCTCTCCTTTCACCTCTTC R: CCTTCTCATCTCTGGAT	399	G R: TGGCTTGGTTTTGTGTATGC A R: GAATGGCTTGGTTTTGTGTATGT	162 165
35	rs13481634	12	106,833,655	<i>1700013N06Rik</i>	Upstream gene variant	A/C	A/A	F: GATGAGCTGACAGGATGGTC R: ACAGCTTTGTTCTCATTGC	300	A F: TTCTCACACGGGCTCATACA C F: TCTCACACGGGCTCATACC	158 157
36	rs13481676	13	6,304,055		Intergenic variant	A/G	A/A	F: TTCTCCCTTTCACATCCTTG R: TTGGAGGGACAAAGAAAGAGA	497	A R: ATGCCACATGCAGCTCCT G R: GCCACATGCAGCTCCC	265 263
37	rs13481734	13	27,037,150	<i>Gm47888</i>	Intron variant	A/G	A/A	F: GATGAGGGAGGGAGACCAAT R: GCAAAGGACACTGCTGATAGG	529	A R: AAAGAATTGATCCCAGAACTGAAT G R: AAAGAATTGATCCCAGAACTGAAC	300 300
38	rs3722313	13	41,442,786	<i>Nedd9</i>	Intron variant	T/C	T/T	F: ATGCTGCAGAAAGGAAAGGA R: CTGAGGTTTCTGCCATAA	522	T F: AAAGAATTGATCCCAGAACTGAAC C F: TGCCTTCTCAGCTCCTTACT	246 247
39	rs31233932	14	124,108,797	<i>Fgf14</i>	Intron variant	C/T	C/C	F: CTTGTCTCAGTGGCTCCTGT R: GCCAGAAAACCAAGATGAAA	507	C R: AATGAAGAAATCGACAAAATTACCTG T R: GATAATGAAGAAATCGACAAAATTACCTA	248 251
40	rs3702158	15	57,160,486		Intergenic variant	A/G	A/A	F: TGGTTTCTATACATATGCATTATCAA R: AGGTGTTGAGGAGATACATCCA	521	A F: GATACTGAGAAAAGGCCAGTTTA G F: ACTGAGAAAAGGCCAGTTTG	292 289
41	rs4165065	16	17,412,079	<i>Snap29</i>	Intron variant	T/C	T/T	F: AGGACTTCTGAGAACAGGTTGC R: TGTCACGGGAGAAAATGAGA	536	T No allele-specific primers found C No allele-specific primers found	
42	rs13483055	17	60,319,945		Intergenic variant	T/C	T/T	F: GGCTTAAATGACAGCCCTGA R: GCAGCCTACAGTATGGGTGAA	536	T R: TCCTTCTGATAAAGGTCA C R: TCCTTCTGATAAAGGTGCG	292 292
43	rs13483237	18	19,289,435		Intergenic variant	G/C	G/G	F: CAAACTCAAGAACATCTGGGACT R: TCTTAACTCCTGGCTTGGAA	473	G F: TTAAGGAAATGGTTGAATCTTGG C F: TTAAGGAAATGGTTGAATCTTGG	253 253
44	rs3724876	19	49,976,154		Intergenic variant	G/T	G/G	F: TGACTTTACTGCCCATTTGG R: CCATGTGTGAACCAGCTCCT	598	G F: TCAGGAGATCACAGAGCG T F: GCAGGAGATCACAGAGCT	319 319
45	rs33867485	X	146,165,950		Intergenic variant	A/T	A/A	F: TCAATTTTCCTTTTGGCAGTG R: CCAATGTTCTCAGCATTTCTT	447	A No allele-specific primers found T No allele-specific primers found	

Supplementary Table 2. Profile of the 45 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6J substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6J allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
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Information about the chromosomal position, linked genes, and SNP type (sequence variant type) was obtained from the Ensembl Mouse Genome Server (ensembl.org/Mus_musculus/Info/Index). SNPs were amplified by PCR with allele flanking primers. PCR was performed using a QIAGEN Multiplex PCR kit (QIAGEN GmbH, Hilden, Germany) 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, USA). Sequencing reactions were performed in a DNA Engine® and Dyad® PTC-220 Peltier Thermal Cycler (Bio-Rad, Laboratories, Inc., Hercules, CA, USA) 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. The samples were resuspended in distilled water and then subjected to electrophoresis in an ABI 3730xl sequencer (Life Technologies). SNP detection was also performed using the allele-specific PCR method. Two PCR reactions were run in parallel, combining a common forward or reverse allele flanking primer and one of the two reverse or forward allele-specific primers. The PCR products were visualized with agarose gel electrophoresis. All PCR primers were designed using BatchPrimer3 v1.0 (robes.pw.usda.gov/batchprimer3/index.html).

Supplementary Table 3. Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6NJ allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
1	rs246236360	1	11,996,705		Intergenic variant	C/T	T/T	F: ACCCCCTGAACCTTCAATTC R: TTTCCTCATGGAATTCGTCTC	493	T No allele-specific primers found. C No allele-specific primers found.	
2	rs246490354	1	14,344,561		Intergenic variant	T/A	A/A	F: GGGGAAGAGTGGGATGACTA R: TGGTCAGCATATTCAGTGC	506	A No allele-specific primers found. T No allele-specific primers found.	
3	rs212521754	1	16,968,405		Intergenic variant	C/A	A/A	F: GCAACGGAAGAAATTGAAGC R: TGTTGAGGCATGTCCCTTTT	500	A No allele-specific primers found. C No allele-specific primers found.	
4	rs227394849	1	19,544,960		Intergenic variant	A/T	T/T	F: GGGCAGAACTTCCTTTTCCT R: TCTCACCTGAGTCCCTGGAT	505	T No allele-specific primers found. A No allele-specific primers found.	
5	rs232920323	1	21,639,642	<i>Kenq5</i>	Intron variant	C/G	G/G	F: GAAATAGCACAGGTCCATCAAA R: CCCAGCAGACAAGAGACAAA	501	G F: AACCCCTCCATTTGTATTTTGTTC C F: AACCCCTCCATTTGTATTTTGTTC	196 196
6	rs213024334	1	30,167,141		Intergenic variant	C/T	T/T	F: GGAATCTCAACCTAAGCAGCA R: TGGAAAATTGAAGCAACCAG	512	T No allele-specific primers designed. C No allele-specific primers designed.	
7	rs244794780	1	40,107,883	<i>Illr2</i>	Intron variant	T/A	A/A	F: TCTGTTGCTCTCCAGCATTG R: CTACACCCTGGCCTGACACT	500	A No allele-specific primers designed. T No allele-specific primers designed.	
8	rs260670033	1	50,146,448		Intergenic variant	C/T	T/T	F: AGCAGAAATGCCAAAATGCT R: TCAGACCCAAAAGGACATGC	489	T No allele-specific primers designed. C No allele-specific primers designed.	
9	rs249907793	1	61,950,812	<i>Pard3b</i>	Intron variant	C/A	A/A	F: CCAGTGGGTTAAGTGGGATT R: ATCAAATGGGGTGGCATTTA	494	A R: TTAATAATGGAGACATGTGGAGGT C R: AAATGGAGACATGTGGAGGG	122 119
10	rs265151779	1	83,182,637	<i>Wdr69</i>	Intron variant	G/A	A/A	F: ATTCGTACTACTGCGGAGGA R: TTGTTACCCCTTCCCCCTA	496	A No allele-specific primers designed. G No allele-specific primers designed.	
11	rs229124202	1	89,861,338	<i>Agap1</i>	Intron variant	G/T	T/T	F: GGAAGGCAGATCACCAACTC R: TCTATGGTGGCCCTTAGGATG	496	T No allele-specific primers designed. G No allele-specific primers designed.	
12	rs237656339	1	99,547,673		Intergenic variant	G/A	A/A	F: TGGCTCCTGACATCTTTCTC R: GCTCCTGGATCGGCATATTA	516	A F: TTTTCTGGTGTGTATCTAGGATGAA G F: TTTTCTGGTGTGTATCTAGGATGAG	184 184
13	rs223540754	1	110,024,886	<i>Cdh7</i>	Intron variant	G/C	C/C	F: GACCAAATGCCTGAAAATGA R: CTCTCCCATCCCTTTCTT	500	C No allele-specific primers designed. G No allele-specific primers designed.	
14	rs259683638	1	119,116,297		Intergenic variant	C/T	T/T	F: AGGTCTTGGGCTCTTTAGGG R: ACTTGCTGGCTGACTCCTTC	501	T No allele-specific primers designed. C No allele-specific primers designed.	
15	rs229911289	1	132,980,179		Intergenic variant	T/A	A/A	F: TTTTATTTCTCCGACTTGG R: ACTCGGGAACACACAAGCTC	499	A F: CTAGAGACCAGAGCTAGGTTTTGATA T F: CTAGAGACCAGAGCTAGGTTTTGATT	394 394
16	rs215622703	1	142,008,378		Intergenic variant	C/A	A/A	F: TTTTGTGTGGCCAAGGAT R: CCTTCTCTCAGAGGGGTTTT	521	A No allele-specific primers designed. C No allele-specific primers designed.	
17	rs239017398	1	154,474,620	<i>Caena1e</i>	Intron variant	C/T	T/T	F: CAGATCCCGGCTCAATTTTA R: AGCTCATTAGCCTGGCATGT	501	T No allele-specific primers designed. C No allele-specific primers designed.	
18	rs214254072	1	161,859,644	<i>A1848100</i>	Intron variant	C/T	T/T	F: GTTTGCTCCTCCCTACTC R: TGATGAAAATGTGCAGGTTCTG	494	T No allele-specific primers designed. C No allele-specific primers designed.	
19	rs255914894	1	172,611,934		Intergenic variant	G/A	A/A	F: ATGCCGGTGTACCTCAGAG R: CCCAGTAAACCATCTCCTG	503	A F: GGATTGGACAGAAAGGTTGG G F: GGGATTGGACAGAAAGGTTGA	166 167
20	rs222303818	1	179,503,532	<i>Smyd3</i>	Intron variant	G/A	A/A	F: CTGCCATACTCCTGTCCAT R: AGGGCCTGGTACTGAGAACA	501	A No allele-specific primers designed. G No allele-specific primers designed.	
21	rs262282675	1	188,434,376	<i>Ush2a</i>	Intron variant	A/T	T/T	F: ACCCTTTGATGTTCCCAT R: AATTTTGTAGGCCCATGAAT	496	T No allele-specific primers designed. A No allele-specific primers designed.	
22	rs251979693	2	11,214,185	<i>Prkcq</i>	Intron variant	C/T	T/T	F: CCCACATTGCTTATCCAG R: GCCAATTGTGAGGAATGCTT	493	T No allele-specific primers designed. C No allele-specific primers designed.	
23	rs230600693	2	21,681,174	<i>Gpr158</i>	Intron variant	G/T	T/T	F: GGTCCAGCATTATGGCATT R: GTGATCCCATCTGCCATCTT	498	T No allele-specific primers designed. G No allele-specific primers designed.	

Supplementary Table 3. Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6NJ allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
24	rs242780245	2	30,188,488	<i>Ccbl1</i>	Intron variant	A/C	C/C	F: CCACTGTCACCAGCACATTC R: CCACCCTCTCTCCGAATA	494	C F: CTAGCAGCCTGGCCATAGA A R: AGCTCTGGGTCTGGCG	294 234
25	rs228546410	2	41,205,764	<i>Lrp1b</i>	Intron variant	T/A	A/A	F: ATGCCACAATGCAAACATA R: TAGCCCTCTGACTGTCCAC	504	A No allele-specific primers designed. T No allele-specific primers designed.	
26	rs254996546	2	51,969,852	<i>Nmi</i>	Intron variant	C/T	T/T	F: CCGTGACCAAGTATGCACAG R: GCTGAGGGTTCAGTTGTGGT	509	T No allele-specific primers designed. C No allele-specific primers designed.	
27	rs256541267	2	70,251,451	<i>Myo3b</i>	Intron variant	C/T	T/T	F: CTGAAGAAAGCCCTGTTTGG R: CGAATTCATGCTGCCAATA	512	T R: GCTGGCTAGAACTCCCATCA C R: CTGGCTAGAACTCCCATCG	185 184
28	rs248280077	2	80,873,138		Intergenic variant	C/T	T/T	F: TGTGCCGATTCCTCTAGCTT R: CTGCACCAATTAGCAGCAA	462	T No allele-specific primers designed. C No allele-specific primers designed.	
29	rs214356625	2	96,674,180	<i>Lrrc4c</i>	Intron variant	G/T	T/T	F: GTGTATGCCCAACCTTTA R: CCAGTGATTGCATTTACCTT	469	T No allele-specific primers designed. G No allele-specific primers designed.	
30	rs224344563	2	102,710,505	<i>Slc1a2</i>	Intron variant	A/T	T/T	F: CAGGACAGGAGAGGGTCAAG R: ATCCAGGCCATAGGATTTT	502	T F: CACTTAACATACTCCGAAGCCT A F: ACTTAACATACTCCGAAGCCA	159 158
31	rs251933504	2	112,966,408	<i>Ryr3</i>	3' UTR variant	T/C	C/C	F: GCTCGGTCTGAAAGGTCAAC R: GGAAGCAAGAGCTTGAAGA	502	C No allele-specific primers designed. T No allele-specific primers designed.	
32	rs258508221	2	122,708,738	<i>4930417H01Rik</i>	Intron variant	T/A	A/A	F: ACTTTGGCTTTTGCACAC R: GAGGGGATCCAAGGATAAG	499	A No allele-specific primers designed. T No allele-specific primers designed.	
33	rs255014110	2	132,432,999	<i>4921508D12Rik</i>	Intron variant	C/T	T/T	F: CAGCACAGATGGTTTCATGG R: AGATGCACAAGTGGCTCTGA	505	T No allele-specific primers designed. C No allele-specific primers designed.	
34	rs242413924	2	140,793,056	<i>MacroD2</i>	Intron variant	T/G	G/G	F: AAATTTTCTCCACACAGCA R: GTGAGCCAGTACAGGGGAGA	499	G R: GTAGAGGATCAGGGAATAGCAGAC T R: GTAGAGGATCAGGGAATAGCAGAA	398 398
35	rs217443774	2	152,781,403	<i>Bcl2l1</i>	3' UTR variant	A/G	G/G	F: CTCTTCTTCTGCCCTTCTCT R: AGCCATTGAGTGAGGTGCTT	498	G No allele-specific primers designed. A No allele-specific primers designed.	
36	rs253212197	2	164,813,748		Intergenic variant	C/T	T/T	F: CTGAACTGCAACCCATCA R: AGTGATGCCCTCCCTGTCT	501	T No allele-specific primers designed. C No allele-specific primers designed.	
37	rs213376233	2	170,240,434		Intergenic variant	G/A	A/A	F: GCTAAGTGGTCTTGGGATGC R: GCCACCACACACAGCTAATTT	502	A No allele-specific primers designed. G No allele-specific primers designed.	
38	rs264719247	2	180,149,012	<i>Osbpl2</i>	Intron variant	A/G	G/G	F: TTCTGTAGGCTTCTGAGTG R: CTCCTCACAACAGGCTCAT	502	G R: GCATCAGCAAGCAGGGAC A R: GCATCAGCAAGCAGGGAT	285 285
39	rs221521392	2	181,868,891	<i>Polr3k</i>	3' UTR variant	C/T	T/T	F: TCTTTTGGCTCTGTGGAA R: CGTGCTGTGAGCTCTCTGA	502	T No allele-specific primers found. C No allele-specific primers found.	
40	rs256520809	3	8,498,163		Intergenic variant	G/A	A/A	F: TGCCAGAAGTTTGTTCAGG R: CCATCTGGGGCTGAATACTT	505	A No allele-specific primers designed. G No allele-specific primers designed.	
41	rs214801792	3	32773111		Intergenic variant	G/C	C/C	F: TGCTGGGGTAGTTTTCACT R: GGAGGGAGTCAAGTGAATA	502	C F: GACAATTCTCCATAAAAAGGGTCC G F: ACAATTCTCCATAAAAAGGGTCCG	402 401
42	rs222821429	3	66,305,330		Intergenic variant	C/T	T/T	F: CACCAACACCCACAGAGTA R: TGTCTTTCAAAGGGCCAGA	499	T F: AGCCATTAACACTGCACAT C F: AGCCATTAACACTGCACAC	219 219
43	rs243656799	3	72,616,062		Intergenic variant	G/A	A/A	F: CCCATTGGACACGAAAACCT R: CACTGCTGCTCATTGGTTCA	488	A No allele-specific primers designed. G No allele-specific primers designed.	
44	rs262827930	3	109,597,274	<i>Vav3</i>	Intron variant	A/T	T/T	F: GGCAGTTTGGCCTGTAGGTA R: CTTTACTGGCTTGCCTCACC	482	T F: GCAGTGCCTTCTAGTTCGT A R: AGTCTTACTGCACACCTATCATCTTATT	395 136
45	rs254145219	3	147,657,254		Intergenic variant	C/T	T/T	F: CAGCAGGATATGCGTCTCT R: GCTTCCCCTCCATAATTTT	487	T No allele-specific primers found. C R: GAGACAGTGAATGCTTTGTAGGG	236
46	rs219227155	4	19,328,298	<i>Cngb3</i>	Intron variant	T/C	C/C	F: ACACAAGAAGTGGCACATGG R: TTGGGACCTGTACGCCTATC	501	C No allele-specific primers found. T No allele-specific primers found.	

Supplementary Table 3. Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6NJ allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
47	rs235104023	4	56,463,984		Intergenic variant	C/T	T/T	F: AGCAGTTGGTGTGTTTGCTG R: CCCCATTGCTTTGTGCTA	501	T No allele-specific primers found. C F: CAGAGAGATCAATCGGTGTCC	412
48	rs261879287	4	104,973,294	<i>1700024P16Rik</i>	Intergenic variant	T/G	G/G	F: GACGAGGAAAAATGAGTGGA R: CAAATGGCATGTTTCGTTTGA	499	G R: TGCTGGCTCTAGAGGTGAAAAC T R: GCTGGCTCTAGAGGTGAAAA	267 266
49	rs256724446	5	35,701,259	<i>Sh3tc1</i>	Intron variant	A/G	A/A	F: ATTCATTCCCTGACCCATCCA R: CTTCTCAATCCCTCCAT	498	A R: GGAGCCAGGGAAGGTCT G R: GAGCCAGGGAAGGTCC	274 273
50	rs260260338	5	80,026,465		Intergenic variant	A/G	A/A	F: TGGGAAAGAATGTGCCTAC R: TTGGTCCAACATCAAACCTACCT	493	A R: CACAGGCTTTCAGCTCCTTACT G R: ACAGGCTTTCAGCTCCTTACC	153 152
51	rs217297994	5	117,118,668	<i>9530046B11Rik</i>	Non-transcript variant	A/G	G/G	F: CCAAGGAGCAGCCCTACTAA R: CAACTCCTGGTCAACGCTCT	499	A F: GGGGAAATACCAGAGAGAGATCA G F: GGGAAATACCAGAGAGATCG	279 278
52	rs221990668	5	150,224,989	<i>Fry</i>	Intron variant	G/T	T/T	F: CTTGTAGAACCAGCCATC R: GTCCCCACCCATTACATCAG	501	T F: CATAGTAAGCCTTCATTCTTTCTGAT G F: AGTAAGCCTTCATTTCTTTCTGAG	316 313
53	rs257294810	6	39971164		Intergenic variant	G/A	A/A	F: GCATTCAGCTCTCCTTCCTG R: GAGACCTGGGCACAATGACT	505	A F: AGAAGCTCCAGGAGTCACGA G F: GAAGCTCCAGGAGTCACGG	146 145
54	rs224069095	6	74169211		Intergenic variant	C/A	A/A	F: CTCATCATGACACAAGGAGCA R: CATGTGTGGCCCTAGTTCT	509	A F: AAAAAAAAAATCCTTATCACTTTACAAGA C R: TGCTAAAACCTTTCTTCAGGGG	405 157
55	rs37540455	6	113159679		Intergenic variant	G/A	A/A	F: ATTCCTGGCAGCCCTAGAT R: TGTTGGTGAGAGTCCTCCA	502	A F: AGTACCAGGTGATTCAGGCA G No allele-specific primers found.	223
56	rs217544076	6	144513005	<i>Sox5</i>	Intron variant	G/C	C/C	F: CACACATCCATCTGCCTCTG R: GCAGCCGGAGTATTAGCAAG	495	C R: TGTGCTTCTGATCCAGCG G R: ACTGTGCTTCTGATCCAGCC	365 367
57	rs212452109	7	16,595,985	<i>Grhl1</i>	Intron variant	A/T	T/T	F: GAGTTCAAATCCCTGGGACAA R: GTGTCACGTGGCTGGCTTA	501	T F: CAACAAGAAGGTTTCAGGTGCTT A F: CAACAAGAAGGTTTCAGGTGCTA	396 396
58	rs224103578	7	53390545		Intergenic variant	C/T	T/T	F: GGAGGGAATGTGTCAAGTAAACG R: CCTGACCTCAGTGTGCAGAA	510	T R: CCCAGATCCCAATAAATTTCA C F: AAATGAGATGATAAATTCGCTCAC	197 358
59	rs243575509	7	102,973,309	<i>Olf-577</i>	Missense variant	C/T	T/T	F: TCATCACAGGAGGGAAGAGG R: GGCTATCTGTCTCCTTTGC	491	T No allele-specific primers found. C No allele-specific primers found.	
60	rs229340185	7	140,821,590	<i>Zfp941</i>	Intron variant	A/T	T/T	F: CTTCAGGCCCTTACAGAGTA R: GATTCCTATTGGCTGGCTTG	500	T R: CGGCCAAATTTTCCACTATTTA A R: CGGCCAAATTTTCCACTATTTT	378 378
61	rs263791105	8	22,903,742	<i>Myst3</i>	Intron variant	G/A	A/A	F: CGAATGTGCAATTTGCATCC R: GCCCTTCAACTACCTCACA	529	A F: ACTACAGCCAAGGTCAGAAAA G F: ACTACAGCCAAGGTCAGAAAAG	410 410
62	rs255341040	8	58,790,625	<i>Galnt6</i>	Intron variant	G/A	A/A	F: GGCAGTGTATCTTGGGAAC R: TGCCAAACAGCACTCAGAAG	500	A No allele-specific primers found. G R: ATTGATTTCTGAAAAATTATACCATTC	232
63	rs239219835	8	79,117,401	<i>Zfp827</i>	Intron variant	G/A	A/A	F: TAAATGGCCGAATTCACAT R: TGTGCACCTTCCTTTGTCA	523	A R: CCCAGCTGCTATTTCCATT G R: CCCAGCTGCTATTTCCATC	155 155
64	rs256624163	8	94,046,068	<i>Ogfod1</i>	Intron variant	G/A	A/A	F: TCAGAGCCCAAGAAAAAGG R: CCATGGGTTTACACATTTCA	480	A F: GGAAGAATGAAGCCTGGGA G F: GGAAGAATGAAGCCTGGGG	406 406
65	rs211750147	8	118,442,679	<i>Cdh13</i>	Intron variant	G/A	A/A	F: TCGGGGCTTAATTTCTCTT R: TGCCTAGACCTGGATTGGT	511	A F: AGCTACAGAGTGCTCCATGACA G F: GCTACAGAGTGCTCCATGACG	221 220
66	rs52003732	9	10,125,214	<i>Cnm5</i>	Intron variant	T/C	C/C	F: TTCTCCCTCTGTGAGCAAG R: TTGCCACCACCTCAAAAACCT	464	C R: CCCTTCATTTGTTCTTATACCG T R: ACCCTTCATTTGTTCTTATACCA	178 179
67	rs214490504	9	60,662,109	<i>Lrrc49</i>	Intron variant	G/A	A/A	F: TCATCCCGAACATAAATGG R: AGTCTCGCAATACGACTGC	499	A R: ACTGAGAAATGATCTCCTCCAGATGT G F: GCTACCTGGTGTGCCCG	375 166
68	rs243500146	9	116,160,235	<i>Tgfb2</i>	Intron variant	C/T	T/T	F: CAGAAGGATCTGGACTTGC R: CTCTTATCTCCCGCCAGA	489	T F: GTATGCAGCCAACCTCAACTGTAAT C F: GTATGCAGCCAACCTCAACTGTAAC	119 119
69	rs51123066	10	11,070,460	<i>Grm1</i>	Intron variant	G/T	T/T	F: CAAGGCCCTGTAAATCCTT R: CGAGGGCTTAGCATATTTCA	493	T No allele-specific primers found. G F: CCTGTTCTTATCACTAGAGTCG	

Supplementary Table 3. Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6NJ allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
70	rs219489973	10	41,944,745	<i>Armc2</i>	Intron variant	G/A	A/A	F: CCTCGGCTAACTTCAAGCAC R: GCCTGTGCGCTTACTTTGAT	492	A F: CCTCCTTGGGCACATCA G F: CCTCCTTGGGCACATCG	365 3665
71	rs213583872	10	49,357,252	<i>Grik2</i>	Intron variant	G/T	T/T	F: GTTGACACAGGCTGAGAATGA R: CCCAAATGAATTGCAAAGGT	540	T R: GCAGTACAGCAATAACACGA G R: GCAGTACAGCAATAACACGC	217 217
72	rs246274290	10	88,091,833	<i>Pmch</i>	Missense variant	T/C	C/C	F: CACAGAACACAGGCTCCAAA R: GCCAACATGGTCGGTAGACT	499	C F: TCCACACAGGAAAAGAGAGACAC T F: TCCACACAGGAAAAGAGAGACAT	371 371
73	rs223857079	11	12,253,003	<i>Cobl</i>	Intron variant	A/T	T/T	F: CTGGTTTGGAGGTGAGCATT R: AAAAGCTCCGGAAGGTGAAT	498	T F: CTGTCAGCTCTGCCTTTCTT A F: ACTTGCAGCTCTGCCTTTCTA	119 120
74	rs240617401	11	46,222,615	<i>Cyfp2</i>	Missense variant	G/A	A/A	F: TGACCCCAATCACACATTA R: GCCAGCTTATCCATCTGCAC	505	A F: TCCATCCACCCACCTGTAA G F: CATCCACCCACCTGGAG	137 135
75	rs231656457	12	29886947	<i>Myt1l</i>	Intron variant	T/G	G/G	F: TCTTGGTTAAGGTGGCAAGG R: ATTCACAAATGTCGGCATCA	500	G F: CAGTTCCTTGTATGACTCCTTGAG T F: CAGTTCCTTGTATGACTCCTTGAT	353 353
76	rs217422777	12	70772479		Intergenic variant	C/T	T/T	F: CCGGAAAAACATAACACACC R: ACCCTGCTCTCCTTGACAAA	497	T F: GAGGCTGCATCCCAGATTT C F: GAGGCTGCATCCCAGATTC	366 366
77	rs221345442	12	97702669		Intergenic variant	A/T	T/T	F: TCCTCTCACCCGTATCTGCT R: GCGTCTTCAGAGACCTTCTCA	489	T R: TTGTCTGTGTTTGTGTGGAA A R: TTTGTCTGTGTTTGTGTGGAT	334 335
78	rs226310424	13	41494375		Intergenic variant	G/C	C/C	F: CATCTCCATGGTCTCGATA R: TCCACAGTTCAGCCAAAGG	500	C F: CAACATCCCATGTGCC G F: CAACATCCCATGTCCCG	219 219
79	rs230596409	13	64921972	<i>Spata31</i>	Stop gained	C/T	T/T	F: GGTGTGACCATGAGCCTTC R: CTGGGGTGAGCTTAGGCTCG	511	T R: GGCAGTAAAACGACTTCGCA C No allele-specific primers found.	152
80	rs251507217	13	101112155		Intergenic variant	A/T	T/T	F: CCCTGTACCCTCAATCATC R: TTCTCCCCACCTCTGATGTC	500	T R: ACTTTCTGGGATGAAATAAGAGAGTTA A R: CTTTCTGGGATGAAATAAGAGAGTTT	212 211
81	rs242991609	13	119477808	<i>4833420G17Rik</i>	Missense variant	C/A	A/A	F: TTTTGGCTGTGCAATCTTIG R: CACACAGAGGTCGCCTATCA	517	A F: TCTTTTTGGATGGCGTCAA C F: TGTCTTTTTGGATGGCGTTAC	247 249
82	rs265193270	14	39164780	<i>Nrg3</i>	Intron variant	C/T	T/T	F: GGCCATCTCATCAGTGACATA R: AGGCTGACATGGTTTTGAGC	502	T F: CCACCTCTAATGTGGTGTGT C No allele-specific primers found.	345
83	rs235428682	14	75727727	<i>Cog3</i>	Synonymous variant	G/A	A/A	F: ACATCTCCAGCTTCCAGACC R: GAGGCGGTGACTATGAAGGA	492	A F: CATAACATACCAGACTTTCTTAAACCAATA G F: AACATACCAGACTTTCTTAAACCAATG	252 249
84	rs222607275	14	117850332	<i>Gpc6</i>	Intron variant	G/A	A/A	F: TTGTGGTTTCAGGAATGTGC R: GGCAAACCTTCTGCCTCAGA	497	A F: GGAGCCAGCACTCGGA G R: TCACAATAGCACTGCCTCAATC	385 149
85	rs243245803	15	22748238	<i>Cdh18</i>	Intron variant	T/G	G/G	F: TGTTCCTGAGAGGGTTTGC R: TTTGGGAAAAGACAAGGAGA	492	G No allele-specific primers designed. T No allele-specific primers designed.	
86	rs243400512	15	55816925	<i>Sntb1</i>	Intron variant	C/T	T/T	F: TCAGAGGCTGAAGTGACAGC R: GGGCAGTCTGTCTGTGGAAG	512	T No allele-specific primers designed. C No allele-specific primers designed.	
87	rs231321125	15	97760563	<i>Rapgef3</i>	Intron variant	C/G	G/G	F: CTCTCACGAGGACATGAGCA R: GGCTCCCCAGTAAAACATGA	507	G No allele-specific primers designed. C No allele-specific primers designed.	
88	rs230243864	16	20462996		Intergenic variant	C/T	T/T	F: TGGGGGCTTATCTTTTCAC R: ACTTAACCAAGCCAGGA	500	T No allele-specific primers designed. C No allele-specific primers designed.	
89	rs240948896	16	61450798		Intergenic variant	G/A	A/A	F: TGGAGCATGACAAGGAATCA R: TTTGCAAAATCCATGATTGG	491	A No allele-specific primers designed. G No allele-specific primers designed.	
90	rs240067957	17	40854409		Intergenic variant	C/T	T/T	F: TGCTCATGGTAAATGCTGGA R: TCAGCACTCAGGTGATTTC	515	T No allele-specific primers designed. C No allele-specific primers designed.	
91	rs259144033	17	69131609		Intergenic variant	C/T	T/T	F: CATGCACACGGCAGTAGAAG R: CAGAGGTGAAACCAGGAAGA	506	T No allele-specific primers designed. C No allele-specific primers designed.	
92	rs225963780	18	22530101	<i>Asxl3</i>	3' UTR variant	G/T	T/T	F: AGCGGTATGCTTGTCTTGTAT R: ACAAGGCCAAATATTGCTG	485	T No allele-specific primers designed. G No allele-specific primers designed.	

Supplementary Table 3. Profile of the 100 selected single nucleotide polymorphism (SNP) markers developed for the C57BL/6N substrains

Locus No.	dbSNP ID	Chromosome	Position (bp) (GRCm38.p6)	Linked gene	SNP type	SNP allele	C57BL/6NJ allele	Allele flanking primer sequence (5' to 3')	Product size (bp)	Allele-specific primer sequence (5' to 3') SNP	Product size (bp)
93	rs214638331	18	41344993		Intergenic variant	T/C	C/C	F: CTGCCAGATAAGCCACCAAT R: TCACCAATGACAGAGCAAAAA	495	C No allele-specific primers designed. T No allele-specific primers designed.	
94	rs255789242	18	59519801		Intergenic variant	C/T	T/T	F: TTCCTAGCTTGGAAAACCT R: TCTTCCTGGAGTTGCCCTA	481	T No allele-specific primers designed. C No allele-specific primers designed.	
95	rs263687961	18	90448757		Intergenic variant	A/G	G/G	F: TTCCTATGTGGTCATTGAA R: TGAGCTAAATTTGGAGCAAGC	507	G No allele-specific primers designed. A No allele-specific primers designed.	
96	rs232414357	19	23329888	<i>Mamdc2</i>	Intron variant	C/T	T/T	F: CAGCCCTCCCTTTATCTTC R: GTATGCCCTGTGGGTCTA	492	T No allele-specific primers designed. C No allele-specific primers designed.	
97	rs230656170	19	40364531	<i>Sorbs1</i>	Intron variant	G/A	A/A	F: AGCGCTCGCTTGGACATAAT R: TGGGACAGGAGGAGTTACA	500	A No allele-specific primers designed. G No allele-specific primers designed.	
98	rs246037535	X	84805631	<i>Dmd</i>	Intron variant	C/T	T/T	F: CCCTAGGGCAACATGGTAAA R: CATTCCGTGCAAAATGAGATG	493	T No allele-specific primers designed. C No allele-specific primers designed.	
99	rs266019057	X	112095948		Intron variant	A/G	G/G	F: GGTGGCAGAGATGGAACAT R: CTGTCTTGCTTGGTCGCTAA	559	G No allele-specific primers designed. A No allele-specific primers designed.	
100	rs212226666	X	157445480	<i>Sms</i>	3' UTR variant	T/A	A/A	F: TGCACCTGCACATCCTACAG R: GGGGTTGGGTTTCATTT	500	A No allele-specific primers designed. T No allele-specific primers designed.	

Information about the chromosomal position, linked genes, and SNP type (sequence variant type) was obtained from the Ensembl Mouse Genome Server (ensembl.org/Mus_musculus/Info/Index). SNPs were amplified by PCR with allele flanking primers. PCR was performed using a QIAGEN Multiplex PCR kit (QIAGEN GmbH, Hilden, Germany) 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, USA). Sequencing reactions were performed in a DNA Engine® and Dyad® PTC-220 Peltier Thermal Cycler (Bio-Rad, Laboratories, Inc., Hercules, CA, USA) 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. The samples were resuspended in distilled water and then subjected to electrophoresis in an ABI 3730xl sequencer (Life Technologies). SNP detection was also performed using the allele-specific PCR method. Two PCR reactions were run in parallel, combining a common forward or reverse allele flanking primer and one of the two reverse or forward allele-specific primers. The PCR products were visualized with agarose gel electrophoresis. All PCR primers were designed using BatchPrimer3 v1.0 (robes.pw.usda.gov/batchprimer3/index.html).