

Genetic polymorphisms among C57BL/6 mouse inbred strains

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Abstract Mice from the inbred C57BL/6 strain have been commonly used for the generation and analysis of transgenic and knockout animal models. However, several C57BL/6 substrains exist, and these are genetically and phenotypically different. In addition, each of these substrains can be purchased from different animal providers and, in some cases, they have maintained their breeding stocks separated for a long time, allowing genetic differences to accumulate due to individual variability and genetic drift. With the aim of describing the differences in the genotype of several C57BL/6 substrains, we applied the Illumina[®] Mouse Medium Density Linkage Mapping panel, with 1,449 single nucleotide polymorphisms (SNPs), to individuals from ten C57BL/6-related strains: C57BL/6JArc, C57BL/6J

from The Jackson Lab, C57BL/6J from CrI, C57BL/6JRcHsd, C57BL/6JOlaHsd, C57BL/6JBomTac, B6(Cg)-Ty^{c-2j}/J, C57BL/6NCrI, C57BL/6NHsd and C57BL/6NTac. Twelve SNPs were found informative to discriminate among the mouse strains considered. Mice derived from the original C57BL/6J: C57BL/6JArc, C57BL/6J from The Jackson Lab and C57BL/6J from CrI, were indistinguishable. Similarly, all C57BL/6N substrains displayed the same genotype, whereas the additional substrains showed intermediate cases with substrain-specific polymorphisms. These results will be instrumental for the correct genetic monitoring and appropriate mouse colony handling of different transgenic and knockout mice produced in distinct C57BL/6 inbred substrains.

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Introduction

The inbred mouse strain C57BL/6 has been the preferred choice for the generation and analysis of transgenic and knockout mice. Genetically modified C57BL/6 mice appear repeatedly in many studies describing new animal models for human diseases (i.e. Al-Hasani et al. 2004; Tang et al. 2008). However, there are several C57BL/6 substrains that are related but not genetically identical and for which behavioral differences have been reported (Bothe et al. 2004; Bryant et al. 2008; Mulligan et al. 2008). Therefore, knowledge and recognition of the exact C57BL/6 strain that is used, or the C57BL/6 strain from which ES cells were originally derived for the generation of the corresponding mouse knock-out, are crucial for the correct design and interpretation of the corresponding experiment involving any of these mice.

There are two major substrains of C57BL/6 mice, known as C57BL/6J and C57BL/6N. The “original” Jax mouse strain is C57BL/6J (www.jax.org, The Jackson Laboratory, #000664), which was initially derived as C57BL in 1921 by Dr. CC Little, from the mating of female 57 with male 52 from Miss Abbie Lathrop’s stock. Substrains C57BL/6 and C57BL/10 were separated prior to 1937 (Festing 1996). C57BL/6N is a C57BL/6 subline originated at NIH that was separated from C57BL/6J in 1951. Further C57BL/6 substrains, J or N, were developed as breeding stock of either of these lines was distributed and propagated by various investigators and vendors. Because of genetic drift, each of these sublines is genetically distinct, although externally, they are very similar. Thus, though all C57BL/6 substrains are phenotypically black, associated with the *nonagouti* (*a*) mutant allele they all carry in homozygosity, mutations have been identified that arose within the various lineages and became fixed.

Most substrains derived from C57BL/6J mice carry a mutation in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene (Freeman et al. 2006), with the exception of C57BL/6JBomTac, suggesting that this mutation occurred in The Jackson Laboratory after 1971, when C57BL/6J mice were sent to

Hannover to create the C57BL/6JBom mouse stock. Likewise, the *Nnt* mutation is also not found in C57BL/6N mice (Mekada et al. 2009), since they had been separated from C57BL/6J mice since 1951. C57BL/6JolaHsd mice carry a mutation in the alpha-synuclein (*Snca*) gene, whereas, apparently, the rest of C57BL/6 substrains carry the wild-type allele for this locus (Specht and Schoepfer 2001).

C57BL/6J was the first inbred mouse strain whose genome was sequenced (Waterston et al. 2002) and has been used extensively to generate many transgenic and knock-out mouse lines. More recently, the International Knockout Mouse Consortium (www.knockoutmouse.org) agreed to use ES cells derived from C57BL/6N mice as the primary source for knocking out all mouse genes systematically (Pettitt et al. 2009). Although many investigators treat these different substrains as identical (and commonly refer to these as “C57” or “C57 black” or “B6”), it is important to distinguish the exact background when examining the genotype because of the possible effects on phenotype. Thus, knockout and transgenic mice produced in C57BL/6J and C57BL/6N inbred substrains might co-exist in a given animal house and, therefore great care should be taken to accurately monitor the genotypes of mouse colonies or when attempting to create new animal models involving individuals from both substrains. Nowadays, it is not uncommon for the generation of mouse models to involve the crossing of several independent transgenic and/or knock-out lines to obtain the necessary combination of alleles in which the biological questions can be addressed. Also, it is important to genetically differentiate any transgenic mouse model created with one of the various existing C57BL/6 substrains. With this aim we have prepared genomic DNA from ten C57BL/6-related substrains, both /J and /N, obtained from colleagues and/or different mouse providers distributed world-wide, and have explored the genetic polymorphisms (SNPs) that can be used to differentiate them.

Methods

Animals and SNP genotyping

Genomic DNA was prepared from five to ten individuals of all considered C57BL/6 substrains, both males

and females, according to standard protocols. A total of 93 DNA samples, plus three internal controls, were genotyped through the Illumina® Mouse Medium Density (MD) Linkage Mapping panel (1,449 single-nucleotide-polymorphisms, SNPs), using the GoldenGate® Assay, as recommended by the supplier. All the SNPs described in this manuscript have been submitted to the dbSNP at the NCBI (www.ncbi.nlm.nih.gov/projects/SNP/) and to the Mouse Phenome Database (MPD) site (www.jax.org/phenome) and will be freely available from the MPD collection of SNPs (phenome.jax.org/SNP/). The raw data used in this manuscript are also available to download from our laboratory home page (www.cnb.csic.es/~montoliu/C57/).

Hierarchical clustering of SNPs

Computational analysis of SNP data was performed with MATLAB version 7.5.0. (Natick, Massachusetts, USA: The MathWorks Inc., 2007, www.mathworks.com). The 12 SNPs presenting both, ‘AA’ and ‘BB’ alleles (polymorphic SNPs), according to Illumina® internal annotation, were used for the analysis. Each mouse was further represented as a binary vector using these polymorphic SNPs (0 coding for ‘AA’ and 1 for ‘BB’). Distances between mice were computed as the number of different positions in these vectors (number of SNPs in which they differ). Finally, Ward’s linkage method based on these distances was used to hierarchically cluster the mice and generate the final tree.

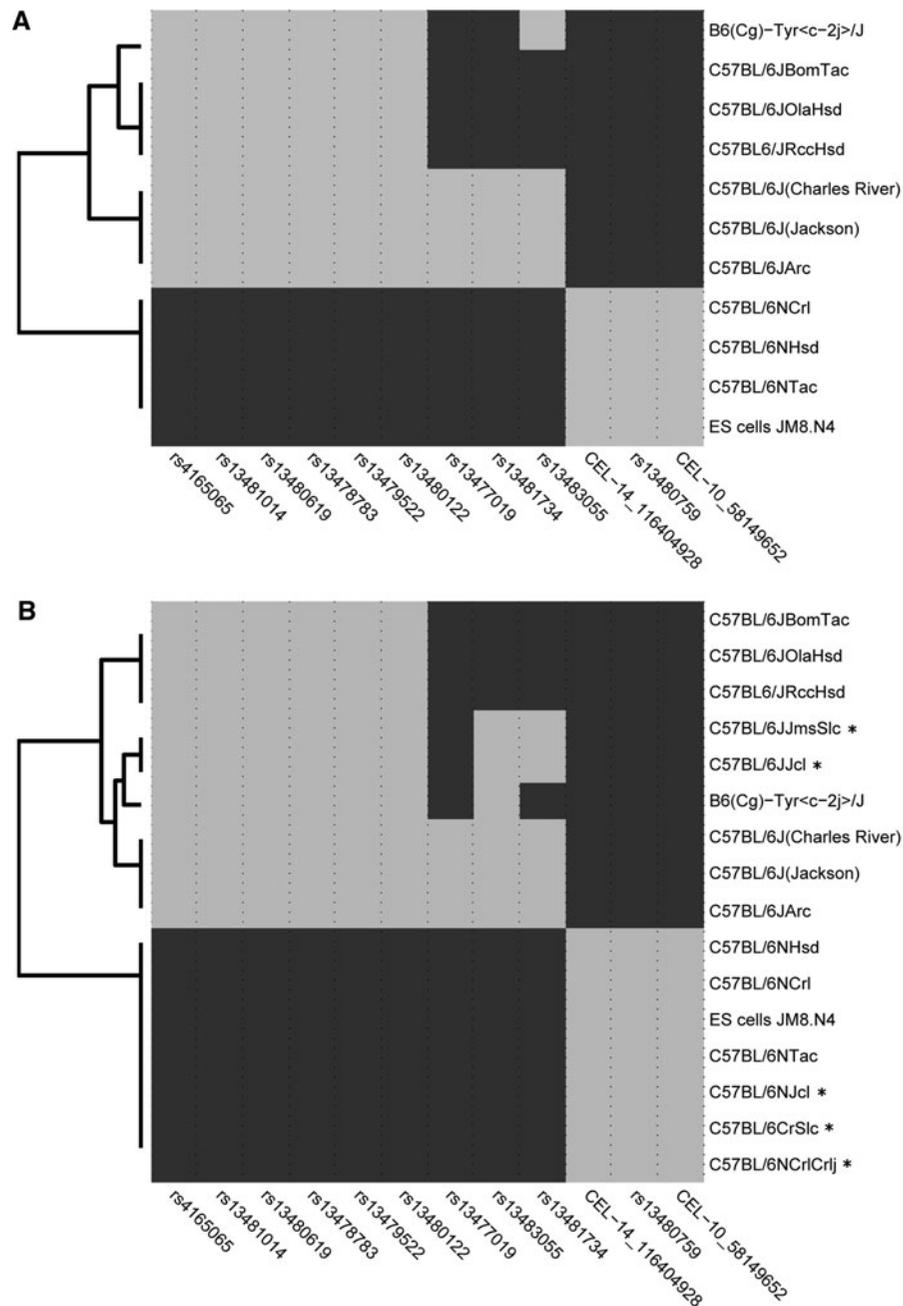
Results and discussion

The C57BL/6 substrains considered in this study, from European, North American and Australian sources, are: C57BL/6JArc (obtained from Animal Resources Centre, Arc, Perth, Australia, www.arc.wa.gov.au), C57BL/6J (original, www.jax.org, #000664, from The Jackson Laboratory, Bar Harbor, Maine, obtained from USA), C57BL/6J (www.crl.com; obtained from Charles River, Lyon, France, Europe), C57BL/6JRccHsd (www.harlan.com; Harlan), C57BL/6JOLA Hsd (Harlan), C57BL/6JBomTac (www.taconic.com; Taconic, Europe), C57BL/6NCr1 (Charles River, Europe), C57BL/6NHsd (Harlan, Europe) and C57BL/6NTac (Taconic, Europe and USA). In addition we included DNA from the coisogenic mouse line B6(Cg)-*Tyr^{c-2j}/J*

(“C57BL/6J albino”, The Jackson Laboratory, #000058) and from JM8.N4 mouse embryonic stem (ES) cells, used by the International Knockout Mouse Consortium (Pettitt et al. 2009) (www.eummc.org/products/wild_type_cells.php).

In order to investigate the genetic differences between the C57BL/6 substrains, we applied the Illumina® Mouse Medium Density (MD) Linkage Mapping panel, consisting of 1,449 single-nucleotide-polymorphisms (SNPs) that were selected from all 20 mouse chromosomes (approximately 3 SNPs per 5 Mb interval across the genome) and chosen to maximize genetic information across common inbred strains (www.illumina.com). Out of the 1,449 SNPs in the panel, only 12 SNPs were found to be polymorphic among the different C57BL/6 inbred substrains analyzed (Fig. 1a). These 12 SNPs are located on 10 different mouse chromosomes, and the positions of these SNPs, along with any associated gene, are shown in Tables 1 and 2. Most of these SNPs could not be directly associated to a known gene. However, one of these markers, rs13478783, maps about 140 kb 3’ of the *Snca* gene that is mutated in the C57BL/6JOLA Hsd strain. A detailed analysis of the resulting hierarchical clustering produced with the polymorphic SNPs offers a number of interesting conclusions (Fig. 1a). The three “original” C57BL/6J inbred strains analyzed, obtained directly from The Jackson Laboratory, Charles River (Europe) or ARC (Australia), are indistinguishable using these 1,449 SNPs. Therefore, even if purchased from different parts of the world, the genetic uniformity of the C57BL/6J “original” mice appears to be adequately monitored and preserved. This is not unexpected since all these animals are derived from the same foundation colony at The Jackson Laboratory.

Similarly, the three C57BL/6N substrains considered in this analysis, obtained independently from three animal providers (C57BL/6NCr1, C57BL/6NHsd and C57BL/6NTac) are also identical regarding these 1,449 SNPs, but are all different from C57BL/6J at 12 of the polymorphic SNPs. In particular, C57BL/6NTac mice, regardless of their origin, USA or Europe, are also identical regarding these 1,449 SNPs and, accordingly, are shown as C57BL/6NTac in Fig. 1a. As expected, the DNA from JM8.N4 ES cells, which are derived from C57BL/6N (Pettitt et al. 2009), follows the same pattern as any of the C57BL/6N mice. Thus, these 12 SNPs are useful to genetically differentiate C57BL/6J



mice from all C57BL/6N substrains, regardless of their origins, as described above. The additional C57BL/6J substrains C57BL/6JRccHsd, C57BL/6JOLA Hsd and C57BL/6JBomTac differ from the “original” C57BL/6J inbred strain at 3 of the 12 SNPs. Accordingly, only 9 of the 12 SNPs can be used to differentiate these additional C57BL/6J inbred substrains from C57BL/6N mice.

Finally, the coisogenic mouse strain B6(Cg)-Tyr^{c-2j}/J (“C57BL/6J albino mice”), commonly used to produce recipient blastocyst hosts for the identification of coat-colour chimeras after injection with C57BL/6-derived ES cell lines, shares 10 of 12 SNPs with the original C57BL/6J substrain, in agreement with its origin, in 1970, within a C57BL/6J congenic strain, carrying other mutations that were eliminated by

◀ **Fig. 1 a** Hierarchical clustering of the C57BL/6 mouse substrains reported in this study. Distances between strains are based on the allele distribution of SNPs. The two possible alleles for each SNPs are indicated in *dark/light gray*. The correspondence between a given allele and *dark/light gray* is arbitrary within each SNP (see Tables 1 and 2 for additional details on the SNP including the correspondence between alleles and nucleotides). Only the 12 SNPs (in which the clustering is based), that were found to be polymorphic among the 1,449 screened, are shown. All the individual mice of each of the C57BL/6 mouse substrains presented the same vector of polymorphism (produced the same genotype) and are hence collapsed into the same row. The tree shown is a graphical representation of the distances between strains based on the allele distribution of SNPs. **b** Hierarchical clustering of C57BL/6 mouse substrains that have been analysed with the Illumina Mouse Medium Density (MD) Linkage Mapping panel. The analysis and tree has been prepared as in A, using the ten C57BL/6 substrains reported in our study (plus the JM8.N4 ES cell line) and the five additional C57BL/6 substrains reported by Mekada et al. (2009): C57BL/6JJcl, C57BL/6JJmsSlc, C57BL/6NJcl, C57BL/6NCrCrIj and C57BL/6CrSlc, highlighted with an *asterisk*

further backcrossing to C57BL/6J. Although this strain has been backcrossed to C57BL/6J at least twice in its history, according to the information provided in the Jax web site, it is still clearly distinguishable from C57BL/6J using the identified 2 polymorphic markers on chromosomes 3 and 13 (Fig. 1a, Tables 1 and 2).

Of the 1,449 SNPs in the panel, only 12 of them were informative for differentiating between the C57BL/6 substrains we screened here (Fig. 1a). The 12 SNPs are located on 10 different mouse chromosomes, and their locations and any associated genes are shown in Table 1, and the sequence of each is shown in Table 2. Five of the 12 fall within the boundaries of known genes, and of the remaining seven, one, rs13478783, maps about 140 kb 3' of the *Snca* gene that is mutated in the C57BL/6JOlaHsd strain.

Recently, a very similar study was reported by Mekada et al. (2009). They also applied the Illumina® Mouse Medium Density (MD) Linkage Mapping panel to analyse the genetic differences between seven C57BL/6 mouse substrains commonly used in Japan (C57BL/6J, C57BL/6JJcl, C57BL/6JJmsSlc, C57BL/6NJcl, C57BL/6NCrCrIj, C57BL/6NTac and C57BL/6CrSlc), and they identified the same 12 SNPs reported here that distinguish their J and N sublines. Only two of these seven mouse lines (C57BL/6J and C57BL/6NTac) are in common with our study. Our results therefore both confirm and

extend the work of Mekada et al., and we have combined the five Japanese C57BL/6 substrains with our group of ten C57BL/6 substrains to build an enlarged dendrogram with the entire set of 15 C57BL/6 mouse strains (Fig. 1b). All N mouse substrains are identical and are accordingly grouped together, whereas the two additional J mouse substrains from Japan, C57BL/6JJcl and C57BL/6JJmsSlc, fall between C57BL/6J and the group of C57BL/6JRccHsd, C57BL/6JOlaHsd and C57BL/6JBomTac mice, according to the SNP variants they have retained.

When examining our results (Fig. 1a), several interesting observations become apparent. First, all the N substrains (C57BL/6NCrI, C57BL/6NHsd and C57BL/6NTac) as well as the ES cell line derived from C57BL/6N are identical. Secondly, all the N substrains differ from C57BL/6J and C57BL/6JArc at all 12 of the SNPs; by the same token, C57BL/6J obtained from both the Jackson Laboratory and Charles River as well as the Acc substrain are identical. Thirdly, the Ola, Rcc, Bom, Jcl and JmsSlc substrains of the J lineage are intermediate between the N substrains and “original” C57BL/6J, differing from N at 9 of the SNPs and from J at 3 SNPs. The C57BL/6J-derived B6(Cg)-*Tyr^{c-2j}/J* is slightly more “J-like”, differing from N at 10 SNPs and from J at 2 SNPs.

If we compare the dendrogram obtained depicting the distances between strains based on the allele distribution of these 12 polymorphic SNPs (Fig. 1a, b) to the known developmental lineage of the C57BL/6 lines (Fig. 2), this pattern becomes easy to explain. The N lineage was separated from the J lineage in 1951, and before these split into the various N substrains in 1971, 9 SNPs were acquired and fixed by one or the other of the N or J lineages: rs13481014, rs4165065, rs13480619, rs13478783, rs13479522, rs13480122, CEL-10_58149652 (rs29359333), rs13480759 and CEL-14_116404928 (rs31233932). Within the J lineage, two SNPs, rs13481734 and rs13483055, must have appeared and become fixed in the C57BL/6J line during the time between 1983, after C57BL/6JOla was separated, and 1989, when the divergence of C57BL/6JJcl occurred. The fixation of rs13477019 then must have occurred within the canonical C57BL/6J after this last split. The closer resemblance of B6(Cg)-*Tyr^{c-2j}/J* to C57BL/6J can be explained by the recent backcrossing to N3 of this strain to C57BL/6J. This

Table 1 Polymorphic SNPs described in this work

Polymorphic SNPs	Chromosome and position# associated to gene ^{\$}	C57BL/6JArc (Australia)	C57BL/6J (Jackson)	C57BL/6J (Charles River)	C57BL/6JRecHsd (Harlan)	C57BL/6JOlalHsd (Harlan)	C57BL/6JBomTac (Taconic)	B6(Cg)-Tyrc ^{-2j} /J ("B6 albino"/Jackson)	C57BL/6NCl (Charles River)	C57BL/6NHsd (Harlan)	ES cells JM8.N4	C57BL/6NTac (Taconic)
rs13477019	3 : 23723842 <i>Nactadl2</i>	T	T	T	A	A	A	A	A	A	A	A
CEL-14_116404928 (rs31233932)	14 : 124508019 <i>Fgf14</i>	C	C	C	C	C	C	C	T	T	T	T
rs13480759	10 : 108815683	C	C	C	C	C	C	C	T	T	T	T
CEL-10_58149652 (rs29359333)	10 : 57864252 <i>Lims1</i>	G	G	G	G	G	G	G	A	A	A	A
rs13483055	17 : 60459368	T	T	T	C	C	C	T	C	C	C	C
rs13481734	13 : 27129019	A	A	A	G	G	G	G	G	G	G	G
rs13480122	9 : 30964211 <i>Aplp2</i>	T	T	T	T	T	T	T	C	C	C	C
rs13479522	7 : 136179208	A	A	A	A	A	A	A	G	G	G	G
rs13478783	6 : 60541373	A	A	A	A	A	A	A	G	G	G	G
rs13480619	10 : 57472268	T	T	T	T	T	T	T	C	C	C	C
rs4165065	16 : 17412172 <i>Snrap29</i>	T	T	T	T	T	T	T	C	C	C	C
rs13481014	11 : 47930884	T	T	T	T	T	T	T	C	C	C	C

Nucleotide positions, in bp, of SNPs refer to mouse genome build NCBI 37.1, as reported in the SNPs collection of the Mouse Phenome Database (phenome.jax.org/SNP/)

\$ In case this is available/known, the gene associated with the SNP is indicated

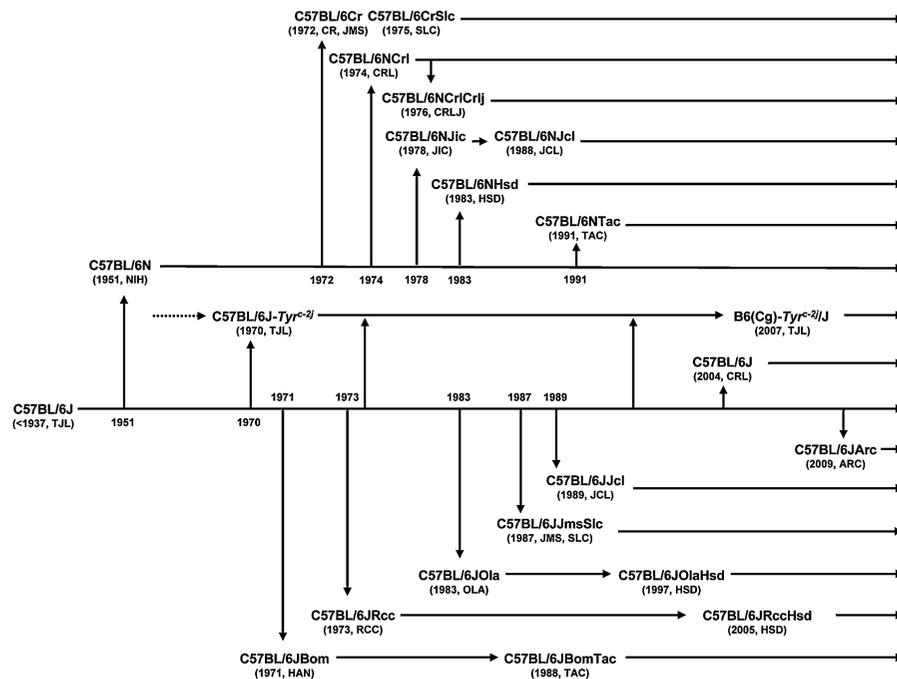


Fig. 2 Lineage relationships between C57BL/6 mouse substrains. The milestones in the establishment of all C57BL/6 substrains reported in this study plus the five additional C57BL/6 Japanese substrains (Mekada et al. 2009) are shown. Depicted dates, relationships and information have been obtained and summarized from the respective web sites at The Jackson Laboratory (TJL: www.jax.org), Charles River (CRL: www.crl.com), Taconic (TAC: www.taconic.com), Harlan (HSD: www.harlan.com) and the Animal Resources Centre (ARC, Perth, Australia: www.arc.wa.gov.au). Japanese C57BL/6 substrain lineage data have been obtained from Mekada et al. (2009). CRL and TJL began sharing the same

introduced one of the recent C57B6/J-specific SNPs while retaining two of the more ancestral SNPs. The agreement of the SNP data (Fig. 1a, b) with the developmental lineage of the various C57BL/6 substrains (Fig. 2) suggests strongly that the SNPs represent real mutation and fixation events during the development of the strains rather than de novo SNP generation or genetic contamination between the strains.

These results thus confirm that, at least at the level of sensitivity detectable here, C57BL/6J is identical whether it is supplied directly from The Jackson Laboratory or via Charles River. This is of course not surprising since the same foundation colony supplies both distribution colonies. C57BL/6JArc is also identical to C57BL/6J since it was re-imported from The Jackson Laboratory in 2009. It also confirms the

foundation colony in 2004, therefore C57BL/6J purchased from TJL and CRL are genetically identical and are not substrains. Likewise, ARC acquired its last foundation stock from TJL in 2009, therefore these are also indistinguishable from C57BL/6J. The two additional arrows connecting C57BL/6J and the B6(Cg)-Tyr^{c-2j}/J line represent the two known backcrosses to C57BL/6J that have occurred in the development of the coisogenic line. A similar lineage graphic has been reported for the additional five C57BL/6 substrains (C57BL/6Jcl; C57BL/6JmsSlc; C57BL/6NJcl; C57BL/6NCrICrlJ and C57BL/6CrSlc) reported by Mekada et al. (2009). Not drawn to time scale

recent nomenclature change of the co-isogenic “B6 albino” strain B6(Cg)-Tyr^{c-2j}/J from C57BL/6-Tyr^{c-2j}/J. Blastocysts from these mice are often used as recipients for C57BL/6 ES cells in order to produce coat color chimeras in gene targeting experiments, and an ES cell line was produced from these “B6 albino” mice and have been used for gene targeting by some with the understanding that these were actually on a C57BL/6J background. However, as indicated by the nomenclature change and confirmed by our data, B6(Cg)-Tyr^{c-2j}/J is not identical to C57BL/6J.

Previous work (Pettitt et al. 2009) described 19 SNPs that could genetically differentiate C57BL/6J and C57BL/6N inbred mice. Two of these overlapped with the set of 12 polymorphic SNPs reported in this study and by Mekada et al. (2009), rs13481734 and

rs4165065. Thus, these studies now provide a merged list of 29 SNPs that can be applied to differentiate these strains. These and additional SNPs for some of these mouse C57BL/6 substrains can be found at the SNPs collection of the Mouse Genome Database (MPD) (phenome.jax.org/SNP/).

The collection of SNPs described in this and other similar reports should be useful for genetically monitoring and handling C57BL/6 mouse colonies of diverse origin appropriately, and will be useful for such applications as producing speed congenics to move targeted and transgenic mutations to a particular background or for detecting genetic contamination between strains in a facility.

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