Journal of the American Association for Laboratory Animal Science
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 46, No 3
May 2007
Pages 37–40

A New Coat Color Mouse Line for Testing Germline Transmission of Embryonic Stem Cells while Retaining an Inbred Genetic Background

Ivana Barbaric,[1] Michelle Stewart,[2] Sara Wells[2] and T Neil Dear[1,2*]

Most gene-targeted mice are produced on a mixed genetic background of C57BL/6 and substrains of 129/Sv. Mating chimeric mice containing 129/Sv-derived embryonic stem cells that are wild type at the agouti locus (*A*) in a nonagouti (*a*) donor genetic background with inbred C57BL/6 mice that are homozygous for the nonagouti allele allows the use of coat color to detect germline transmission. Agouti pups from such a cross indicate germline transmission of embryonic stem cell-derived genetic material. However, 129/Sv substrains and C57BL/6 are genetically and phenotypically quite different and, consequently, differing genetic contributions of the 2 backgrounds may influence the phenotype under investigation. To avoid this problem yet maintain the usefulness of the coat color system in detecting germline transmission, we have generated a new strain of mouse by selectively introducing the nonagouti locus into a 129/Sv inbred background. This mouse strain contains 129/Sv-derived genetic material almost entirely except for a small region surrounding the nonagouti allele. Germline transmission can be detected in the usual manner, but the agouti offspring will be almost identical to 129/Sv inbred mice. Thus, the system allows the generation of gene-targeted mutations on a 129/Sv genetic background.

Abbreviations: ES cell, embryonic stem cell

Gene-targeted mice usually are produced on a mixed genetic background of C57BL/6 and a substrain of 129/Sv. This mixed background results because genetically modified 129-derived embryonic stem (ES) cells typically are injected into blastocysts derived from a strain with a different coat-color, such as C57BL/6. To detect germline transmission, the resultant chimeras typically are bred with wild-type inbred mice homozygous for the nonagouti coat color mutation (*a*), usually C57BL/6. Agouti offspring (genetically *A/a*) indicate inheritance of ES-cell derived genetic material. Such mice then are either intercrossed to generate homozygous mice, or heterozygotes for the targeted allele are again backcrossed to the wild-type strain.

A disadvantage to this approach is that mating chimeras to a nonagouti strain such as C57BL/6 generates a hybrid mouse that has genetic material from both the ES cells (129) and the wild-type strain background (C57BL/6). C57BL/6 and 129 strains of mice are genetically quite different,[38] and such a mixed background can profoundly affect phenotype. For example, 129/Sv substrains and C57BL/6 differ in behaviors such as grooming,[22] olfactory sensitivity,[24] learning ability,[17] anxiety levels,[30] activity,[12] and even 'wildness.'[36] They are differentially sensitive to drugs such as ethanol,[13,15] pentobarbital,[14] morphine,[5,27] and volatile anaesthetics.[18] They have different inflammatory responses,[20] cholesterol absorption kinetics,[21] and bone density.[4] In fact, the Mouse Phenome Database lists 272 phenotypes (among the 709 measured) at which 129S1/SvImJ and C57BL/6J differ by more than 1 standard deviation.[7] In addition, when compared with C57BL/6, 129/Sv substrains have a deletion in *Disc1*[11] as well as additional members of the *Ly49* gene family.[25]

Characterization of the effect of a genetic alteration on phenotype would be facilitated by the ability to generate an inbred genetically homogenous line containing the mutation of interest rather than breeding the mutation on an already heterogeneous background. One way of alleviating this problem would be to use C57BL/6 ES cells. Several such cell lines have been developed[3,10,23,32] but C57BL/6 ES cells are less efficient than 129 ES cells at generating germline-transmitting chimeras. For example, compared with 129 ES cells, C57BL/6 ES cells have a propensity to lose their ability to colonize the germline,[2] and their injection results in lower levels of chimerism.[34,37]

If a mutation is desired wholly on a 129 genetic background, chimeras generated from 129 ES cells to 129/Sv mice could be crossed to this end, but detection of germline transmission would require genotyping of every animal. This task would be particularly labor-intensive for chimeras with poor transmission efficiency. Therefore, to rapidly generate inbred genetically homogeneous mouse lines containing a targeted gene, yet still make use of the coat color system to identify germline transmitting chimeras, we have introduced the nonagouti allele into a 129/SvEv mouse background. When used with chimeras derived from ES cell lines such as CCE, intercrossing and selection of agouti offspring generates gene-targeted mice with a genetic background similar to that of 129/SvEv inbred mice.

Materials and Methods

Mouse breeding. Mice were bred at the Mary Lyon Centre (Medical Research Council, Harwell, UK) in a full-barrier facility consisting of individually ventilated cages with health monitoring according to guidelines from the Federation of European Laboratory Animal Science Associations.[28] Animal work was in accordance with both institutional[26] and UK Home Office regulations under the Animals (Scientific Procedures) Act.[19] All animals were used humanely, and the only invasive procedure involved removing a small tissue biopsy from the ear pinna for genotyping.

Received: 14 Sep 2006. Revision requested: 26 Oct 2006. Accepted: 30 Oct 2006.
[1]Mammalian Genetics of Disease Unit, School of Medicine and Biomedical Sciences, University of Sheffield, UK; [2]Mary Lyon Centre, Medical Research Council, Harwell, Didcot, UK.
[*]Corresponding author. Email: T.Dear@sheffield.ac.uk

Table 1. Details of markers used and genotypes of N_7 C57BL/6J × 129/SvEvH mice

Chromosome	Marker	Chromosome location (Mb)[a]	Genotyping marker details			
			C57BL/6J (size in bp)	129S6/SvEvTac (size in bp)	129/SvEvH (marker origin)[b]	N_7 B6 × 129 (marker origin)[b]
1	D1Mit231	21	266	273	129/129	129/129
	D1Mit217	122	171	189	129/129	129/129
	D1Mit362	191	117	156	129/129	129/129
2	D2Mit312	3	121	112	129/129	129/129
	D2Mit92	71	148	158	129/129	129/129
	D2Mit304	128	121	140	129/129	129/129
	D2Mit194	144	117	127	129/129	B6/129
	D2Mit285	153	139	158	129/129	B6/129
	Agouti	155	*a/a*	*A/A*	*A/A*	*A/a*
3	D3Mit151	31	148	169	129/129	129/129
	D3Mit230	53	150	168	129/129	129/129
	D3Mit212	83	123	116	129/129	129/129
	D3Mit45	148	145	133	129/129	129/129
4	D4Mit178	67	150	174	129/129	129/129
	D4Mit12	124	197	169	129/129	129/129
	D4Mit232	144	121	134	129/129	129/129
5	D5Mit259	89	137	118	129/129	129/129
	D5Mitl6l	127	121	138	129/129	129/129
6	D6Mit138	4	114	133	129/129	129/129
	D6Mit123	57	120	101	129/129	129/129
	D6Mit15	146	254	196	129/129	129/129
7	D7Mit246	30	135	152	129/129	129/129
	D7Mit250	79	126	132	129/129	129/129
	D7Mit44	129	186	166	129/129	129/129
8	DBMit141	13	236	211	129/129	129/129
	D8Mit249	84	149	195	129/129	129/129
	D8Mit211	106	154	167	129/129	129/129
9	D9Mit206	40	100	106	129/129	129/129
	D9Mit107	73	128	111	129/129	129/129
10	D10Mit186	75	131	108	129/129	129/129
	D10Mit35	122	229	242	129/129	129/129
11	D11Mit260	62	253	297	129/129	129/129
	D11Mit4	68	98	112	129/129	129/129
	D11Mit61	112	141	153	129/129	129/129
12	D12Mit182	11	131	148	129/129	129/129
	D12Mit172	47	196	181	129/129	129/129
13	D13MitB1	41	212	176	129/129	129/129
	D13Mit191	85	119	138	129/129	129/129
	D13Mit35	120	196	182	129/129	129/129
14	D14Mit141	46	139	126	129/129	129/129
	D14Mit203	65	153	172	129/129	129/129
	D14Mit165	105	137	126	129/129	129/129
15	D15Mit175	9	174	126	129/129	129/129
	D15Mit193	98	127	112	129/129	129/129
16	D16Mit131	7	145	183	129/129	129/129
	D16Mit4	36	131	148	129/129	129/129
	D16Mit139	66	150	175	129/129	129/129
17	D17Mit164	4	132	118	129/129	129/129
	Dl7Mit176	42	172	161	129/129	129/129
	D17Mit123	94	131	152	129/129	129/129
18	Dl8Mit149	45	134	115	129/129	129/129
	Dl8Mit103	70	118	94	129/129	129/129
	Dl8Mit4	84	210	179	129/129	129/129
19	D19Mit71	60	136	149	129/129	129/129

[a]According to Ensembl.[6]
[b]129 denotes an allele identical in size to that of 129/SvEvH; B6 denotes an allele identical in size to that of C57BL/6J.

Markers for genotyping. For the nonagouti locus, we designed primers that span part of the viral insertion site in the locus that generates the nonagouti (*a*) allele.[9] Primers for amplification were selected using the Primer 3 program.[31] These primers (forward, 5' CTC CTC CCT CCT TCT GCT TT 3'; reverse, 5' CTG TGT GCT TCC ATG TTG CT 3') generated a 359-basepair product specific for the *a* allele. To differentiate between 129/SvEvH and C57BL/6J genomes, we used the microsatellite marker panel reported by Bothe and colleagues[8] and available from the Taconic website (www.taconic.com/news-press/inbreds_for_transgenics.htm). We selected markers from this panel for each chromosome, primarily on the basis of a relatively large size difference between 129S6 and C57BL/6J to facilitate polymorphism discrimination. All selected markers from this panel differentiated between our 129/SvEvH strain and C57BL/6J.

DNA isolation. Tissue biopsies from the ear pinna were incubated overnight in 150 µl of a solution containing 50 mM Tris-HCl pH 8.5, 0.1 mM ethylenediaminetetraacetic acid, 0.5% (v/v) Tween 20, and 300 µg/ml proteinase K. After brief vortexing, the samples were heated at 100 °C for 12 min, followed by addition of 600 µl sterile water. Samples were stored at 4 °C and used directly in polymerase chain reaction (PCR) amplification without further purification.

PCR. Standard PCR conditions for all primer pairs comprised a 15-µl reaction volume containing 0.12 U *Taq*Gold polymerase (Applied Biosystems, Warrington, UK), 200 µM dNTPs, 1.5 mM $MgCl_2$, 1× reaction buffer, and 3 µl genomic DNA suspension. The PCR program consisted of 40 cycles of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. PCR products were separated by electrophoresis in 4% (w/v) agarose gels in 1× TBE buffer and 5 µg/ml ethidium bromide. Samples were run alongside a 100-basepair DNA ladder (New England Biolabs, Hitchin, UK).

Results

For the 129/Sv strain, we used a mouse line maintained at our institution and which had been designated as 129/SvEv; CCE ES cells were derived from this line.[29] Under the revised 129 nomenclature, 129/SvEv is similar to the 129S5 and 126S6 substrains.[16] Although the line has not gone through the required 20 generations of inbreeding to be designated a new substrain, we will refer to it as 129/SvEvH (H = Harwell) hereafter.

To introduce 129/Sv X and Y chromosomes, we first backcrossed male C57BL/6J mice to female 129/SvEvH mice and chose F_1 male offspring, because the X chromosome would be derived from 129/SvEvH. We then crossed these male offspring to 129/SvEvH female mice and chose female N_2 offspring, because both X chromosomes in these animals would be derived from 129/SvEvH. We repeated this process several times during the backcrossing to ensure that sex chromosomes were wholly 129/SvEvH-derived. For introduction of 129/SvEvH autosomal genetic material, we used the standard congenic backcross approach.[35] We elected not to use a 'speed congenics' approach, because considerable amounts of donor material can still contaminate the recipient genetic background.[1] At each generation, we used PCR-based methods to genotype mice for presence of the nonagouti *a* allele; various mice containing this allele were then used for backcrossing with 129/SvEvH mice. This was repeated at each subsequent generation. At N_6 and N_7, the mice were genotyped by use of a panel of microsatellite markers that differentiated between 129/SvEvH and C57BL/6J and that spanned the genome. By N_6, all of the markers were derived from 129/SvEvH, with the exception of a region surrounding the nonagouti locus on chromosome 2 (Table 1). Intercrossing of N_7 *A/a* mice demonstrated that nonagouti *a/a* mice were recovered at a Mendelian frequency (Figure 1).

Figure 1. Photograph of littermates derived from intercross of N_7 C57BL/6J × 129/SvEvH *A/a* parents. The littermates are genetically similar and primarily contain 129/SvEvH genetic material, apart from the immediate region surrounding the nonagouti (*a*) locus, which is derived from C57BL/6J in the mice with a black coat color.

Discussion

This new mouse line, 129.B6-*a*, is particularly suitable for targeting experiments done using CCE cells, which originally were derived from a 129 strain background,[29] or W4/129S6 cells, which were derived from the 129S6 strain background and which clearly is genetically very similar based on our genetic analysis. In addition, even for ES cells derived from other 129 strains, the 129.B6-*a* strain is liable to provide a far more homogeneous background than would crossing to C57BL/6. One further possible use of the 129.B6-*a* line is as a blastocyst host for microinjection of 129 ES cells, because coisogenic host blastocysts may better support the development of injected ES cells.[2,33]

We are continuing to backcross the current mouse strain (129.B6-*a* [N_8]) to 129/SvEvH to obtain recombinants for mouse chromosome 2 that further reduce the C57BL/6J contribution to this chromosome, which currently is between 11 and 53 Mb (a maximum of approximately 1.9% of the total genome). The 129.B6-*a* strain will be made available to the scientific community, subject to a standard Material Transfer Agreement.

Acknowledgments

We thank Monica Neilan, Sarah Tull, Lucie Vizor, Charissa Warner, Gemma Law, and Michelle Hammett for technical assistance.

References

1. **Armstrong NJ, Brodnicki TC, Speed TP.** 2006. Mind the gap: analysis of marker-assisted breeding strategies for inbred mouse strains. Mamm Genome **17:**273–287.
2. **Auerbach W, Dunmore JH, Fairchild-Huntress V, Fang Q, Auerbach AB, Huszar D, Joyner AL.** 2000. Establishment and chimera analysis of 129/SvEv- and C57BL/6-derived mouse embryonic stem cell lines. Biotechniques **29:**1024–1028.
3. **Baharvand H, Matthaei KI.** 2004. Culture condition difference for establishment of new embryonic stem cell lines from the C57BL/6 and BALB/c mouse strains. In Vitro Cell Dev Biol Anim **40:**76–81.
4. **Beamer WG, Donahue LR, Rosen CJ, Baylink DJ.** 1996. Genetic variability in adult bone density among inbred strains of mice. Bone **18:**397–403.
5. **Belknap JK, Riggan J, Cross S, Young ER, Gallaher EJ, Crabbe JC.** 1998. Genetic determinants of morphine activity and thermal responses in 15 inbred mouse strains. Pharmacol Biochem Behav **59:**353–360.

6. **Birney E, Andrews D, Caccamo M, Chen Y, Clarke L, Coates G, Cox T, Cunningham F, Curwen V, Cutts T, Down T, Durbin R, Fernandez-Suarez XM, Flicek P, Graf S, Hammond M, Herrero J, Howe K, Iyer V, Jekosch K, Kahari A, Kasprzyk A, Keefe D, Kokocinski F, Kulesha E, London D, Longden I, Melsopp C, Meidl P, Overduin B, Parker A, Proctor G, Prlic A, Rae M, Rios D, Redmond S, Schuster M, Sealy I, Searle S, Severin J, Slater G, Smedley D, Smith J, Stabenau A, Stalker J, Trevanion S, Ureta-Vidal A, Vogel J, White S, Woodwark C, Hubbard TJ.** 2006. Ensembl 2006. Nucleic Acids Res **34:**D556–561.
7. **Bogue MA, Grubb SC, Maddatu TP, Bult CJ.** 2007. Mouse phenome database (MPD). Nucleic Acids Res. **35:**D643–649.
8. **Bothe GW, Bolivar VJ, Vedder MJ, Geistfeld JG.** 2004. Genetic and behavioral differences among five inbred mouse strains commonly used in the production of transgenic and knockout mice. Genes Brain Behav **3:**149–157.
9. **Bultman SJ, Klebig ML, Michaud EJ, Sweet HO, Davisson MT, Woychik RP.** 1994. Molecular analysis of reverse mutations from nonagouti (a) to black-and-tan (a(t)) and white-bellied agouti (Aw) reveals alternative forms of agouti transcripts. Genes Dev **8:**481–490.
10. **Cheng J, Dutra A, Takesono A, Garrett-Beal L, Schwartzberg PL.** 2004. Improved generation of C57BL/6J mouse embryonic stem cells in a defined serum-free media. Genesis **39:**100–104.
11. **Clapcote SJ, Roder JC.** 2006. Deletion polymorphism of disc1 is common to all 129 mouse substrains: implications for gene-targeting studies of brain function. Genetics **173:**2407–2410.
12. **Contet C, Rawlins JN, Deacon RM.** 2001. A comparison of 129S2/SvHsd and C57BL/6JOlaHsd mice on a test battery assessing sensorimotor, affective and cognitive behaviours: implications for the study of genetically modified mice. Behav Brain Res **124:**33–46.
13. **Crabbe JC, Metten P, Cameron AJ, Wahlsten D.** 2005. An analysis of the genetics of alcohol intoxication in inbred mice. Neurosci Biobehav Rev **28:**785–802.
14. **Crabbe JC, Metten P, Gallaher EJ, Belknap JK.** 2002. Genetic determinants of sensitivity to pentobarbital in inbred mice. Psychopharmacology (Berl) **161:**408–416.
15. **Crabbe JC, Metten P, Ponomarev I, Prescott CA, Wahlsten D.** 2006. Effects of genetic and procedural variation on measurement of alcohol sensitivity in mouse inbred strains. Behav Genet **36:**536–552.
16. **Festing MF, Simpson EM, Davisson MT, Mobraaten LE.** 1999. Revised nomenclature for strain 129 mice. Mamm Genome **10:**836.
17. **Gerlai R.** 1998. A new continuous alternation task in T-maze detects hippocampal dysfunction in mice. A strain comparison and lesion study. Behav Brain Res **95:**91–101.
18. **Homanics GE, Quinlan JJ, Firestone LL.** 1999. Pharmacologic and behavioral responses of inbred C57BL/6J and strain 129/SvJ mouse lines. Pharmacol Biochem Behav **63:**21–26.
19. **Home Office, UK** [Internet]**.** 2000. Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 [cited 29 Mar 2007]. Available at http://www.archive.official-documents.co.uk/document/hoc/321/321.htm.
20. **Ishola DA Jr, van der Giezen DM, Hahnel B, Goldschmeding R, Kriz W, Koomans HA, Joles JA.** 2006. In mice, proteinuria and renal inflammatory responses to albumin overload are strain-dependent. Nephrol Dial Transplant **21:**591–597.
21. **Jolley CD, Dietschy JM, Turley SD.** 1999. Genetic differences in cholesterol absorption in 129/Sv and C57BL/6 mice: effect on cholesterol responsiveness. Am J Physiol **276:**G1117–G1124.
22. **Kalueff AV, Tuohimaa P.** 2004. Contrasting grooming phenotypes in C57Bl/6 and 129S1/SvImJ mice. Brain Res **1028:**75–82.
23. **Kontgen F, Suss G, Stewart C, Steinmetz M, Bluethmann H.** 1993. Targeted disruption of the MHC class II *Aa* gene in C57BL/6 mice. Int Immunol **5:**957–964.
24. **Lee AW, Emsley JG, Brown RE, Hagg T.** 2003. Marked differences in olfactory sensitivity and apparent speed of forebrain neuroblast migration in three inbred strains of mice. Neuroscience **118:**263–270.
25. **Makrigiannis AP, Pau AT, Schwartzberg PL, McVicar DW, Beck TW, Anderson SK.** 2002. A BAC contig map of the Ly49 gene cluster in 129 mice reveals extensive differences in gene content relative to C57BL/6 mice. Genomics **79:**437–444.
26. **Medical Research Council** [Internet]**.** 1993. Responsibility in the use of animals in medical research [cited 29 Mar 2007]. Available at http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002463.
27. **Murphy NP, Lam HA, Maidment NT.** 2001. A comparison of morphine-induced locomotor activity and mesolimbic dopamine release in C57BL6, 129Sv, and DBA2 mice. J Neurochem **79:**626–635.
28. **Nicklas W, Baneux P, Boot R, Decelle T, Deeny AA, Fumanelli M, Illgen-Wilcke B; FELASA (Federation of European Laboratory Animal Science Associations Working Group on Health Monitoring of Rodent and Rabbit Colonies).** 2002. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. Lab Anim **36:**20–42
29. **Robertson E, Bradley A, Kuehn M.** 1986. Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector. Nature **323:**445–448.
30. **Rodgers RJ, Boullier E, Chatzimichalaki P, Cooper GD, Shorten A.** 2002. Contrasting phenotypes of C57BL/6JOlaHsd, 129S2/SvHsd and 129/SvEv mice in two exploration-based tests of anxiety-related behaviour. Physiol Behav **77:**301–310.
31. **Rozen S, Skaletsky HJ**. 2000. Primer3 on the WWW for general users and for biologist programmers, p 365–386. *In* Krawetz S, Misener S, editors. Bioinformatics methods and protocols: methods in molecular biology. Totowa (NJ): Humana Press.
32. **Schoonjans L, Kreemers V, Danloy S, Moreadith RW, Laroche Y, Collen D.** 2003. Improved generation of germline-competent embryonic stem cell lines from inbred mouse strains. Stem Cells **21:**90–97.
33. **Schuster-Gossler K, Lee AW, Lerner CP, Parker HJ, Dyer VW, Scott VE, Gossler A, Conover JC.** 2001. Use of coisogenic host blastocysts for efficient establishment of germline chimeras with C57BL/6J ES cell lines. Biotechniques **31:**1022–1024.
34. **Seong E, Saunders T, Stewart C, Burmeister M.** 2004. To knockout in 129 or in C57BL/6: that is the question. Trends Genet **20:**59–62.
35. **Silver LM.** 1995. Mouse genetics: concepts and applications. New York: Oxford University Press.
36. **Wahlsten D, Metten P, Crabbe JC.** 2003. A rating scale for wildness and ease of handling laboratory mice: results for 21 inbred strains tested in two laboratories. Genes Brain Behav **2:**71–79.
37. **Ware CB, Siverts LA, Nelson AM, Morton JF, Ladiges WC.** 2003. Utility of a C57BL/6 ES line versus 129 ES lines for targeted mutations in mice. Transgenic Res **12:**743–746.
38. **Wiltshire T, Pletcher MT, Batalov S, Barnes SW, Tarantino LM, Cooke MP, Wu H, Smylie K, Santrosyan A, Copeland NG, Jenkins NA, Kalush F, Mural RJ, Glynne RJ, Kay SA, Adams MD, Fletcher CF.** 2003. Genome-wide single-nucleotide polymorphism analysis defines haplotype patterns in mouse. Proc Natl Acad Sci U S A **100:**3380–3385.