C57BL/6J-^{Aw-J}/J (B6 agouti) mouse embryonic stem cell lines

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Summary

Several embryonic stem (ES) cell lines were derived from an agouti coisogenic C57BL/6J mouse strain, C57BL/6J-^{Aw-J}/J. Standard C57BL/6J mice are non-agouti, or black. Because agouti is dominant to black, transmission of the ES cell genome through the germ line can be visualized by coat color while working within a coisogenic system. One line of XY sex chromosome constitution, called B6AW1, resulted in high frequency extreme chimerism when injected into blastocysts. It therefore has potential for genome modification experiments in mice.

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Introduction

C57BL/6J is the most popular strain of laboratory mouse in biological research. Standard C57BL/6J mice are non-agouti or black, a/a , while the coisogenic strain C57BL/6J- A^{w-J}/J is white-bellied agouti, A^w/A^w . ES cell lines derived from the latter strain would be useful because the dominant A^w allele marks the ES cell genome, that is, when C57BL/6J- $A^{w-J}/J \leftrightarrow a/a$ male chimeras, identified by coat color, are mated to standard C57BL/6J females, all germ line pups are C57BL/6J- a/A^w (agouti), while all non-germ line pups are black. This study describes the derivation of a strain C57BL/6J- A^{w-J}/J XY ES cell line that resulted in high frequency extreme chimerism when injected into blastocysts.

Materials & Methods

Mice

Strain C57BL/6J- A^{w-J}/J , stock no. 000051, The Jackson Laboratory, Bar Harbor, Maine, USA (JAX) originated from a spontaneous reversion from a to A^w in strain C57BL/6J (<https://www.jax.org/strain/000051>). The mechanism of reversion has been described (Bultman *et al.* 1994). For these experiments strain C57BL/6J- A^{w-J}/J was acquired from JAX in the mid-1990's at F107 or greater and therefore was a substrain of C57BL/6J (more than 20 generations of brother-sister mating). The ES cell lines described here were derived and tested in year 1997. Since then the strain at JAX has been backcrossed to standard C57BL/6J then brother-sister mated (N4F12 at 05-August-2014).

ES cell derivation

Blastocysts were plated, and disaggregated outgrowths were seeded (passage 0) onto mitomycin C-inactivated STO-*neo*-LIF transformed fibroblast feeder layers. Primary ES cell clones appearing at passage 0 were individually picked then seeded onto mitomycin C-inactivated primary mouse embryo fibroblast feeder layers (MEFLs), expanded on MEFLs and frozen at passage 4. No exogenous LIF (leukemia inhibitory factor) was added to the growth medium. The method is described in detail (Mann, 2001).

Results & Discussion

Seventeen lines were derived—designated with the prefix B6AW, and six of these were positive for Y chromosome sequences as assessed in Southern blots using the Y-specific probe pY353/B (Bishop *et al.* 1985). Four lines of putative XY sex chromosome constitution at passage 10 were tested for their potential to form chimeras by injection into C57BL/6J-*a/a* blastocysts: C57BL/6 blastocysts are a good host for C57BL/6 ES cells, for example (Schuster-Gossler *et al.* 2001; Pettitt *et al.* 2009). The degree of chimerism assessed by coat color was variable depending on the line. Line B6AW1 produced extreme chimeras at high frequency—from approximately 50 blastocysts injected, 16 pups were born all of which were male. The large majority of these were complete chimeras, that is, they were entirely agouti with no visible black fur. The remainder were near-complete chimeras with a small amount of black fur. All chimeras were overtly normal in appearance. Line B6AW17 produced a high frequency of high-level male chimeras while lines B6AW6 and B6AW8 produced only low-level chimeras.

The performance of line B6AW1 to date indicates utility for genome modification experiments in mice. Genetically modified clones derived from this line are capable of germ line transmission as shown in one experiment performed in year 1998, but the general frequency of germ line transmission at the clonal level is yet to be determined. The A^w/A^w genotype of this line would allow for coat color marking of the ES cell genome while working within strain C57BL/6J. By contrast, ES cell line JM8 is heterozygous for agouti (a/A) and is strain C57BL/6N (Pettitt *et al.* 2009). While exogenous LIF was not used in propagating line B6AW1, adding LIF does improve its clonal morphology and could be advantageous.

References

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