Divergence and inheritance of neocortical heterotopia in inbred and genetically-engineered mice

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Abstract

Cortical function emerges from the intrinsic properties of neocortical neurons and their synaptic connections within and across lamina. Neurodevelopmental disorders affecting migration and lamination of the neocortex result in cognitive delay/disability and epilepsy. Molecular layer heterotopia (MLH), a dysplasia characterized by over-migration of neurons into layer I, are associated with cognitive deficits and neuronal hyperexcitability in humans and mice. The breadth of different inbred mouse strains that exhibit MLH and inheritance patterns of heterotopia remain unknown. A neuroanatomical survey of numerous different inbred mouse strains, 2 first filial generation (F1) hybrids, and one consomic strain (C57BL/6J-Chr 1$^{A/J}$/NaJ) revealed MLH only in C57BL/6 mice and the consomic strain. Heterotopia were observed in numerous genetically-engineered mouse lines on a congenic C57BL/6 background. These data indicate that heterotopia formation is a weakly penetrant trait requiring homozygosity of one or more C57BL/6 alleles outside of chromosome 1. These data are relevant toward understanding neocortical development and disorders affecting neocortical lamination.

Keywords

neocortex; malformation; heterotopia; C57BL/6

Introduction

The neocortex is highly vulnerable to neurodevelopmental malformations caused by a neuronal migration defect. Malformations characterized by incomplete migration, such as

periventricular nodular heterotopia (PVH) [1–3] and subcortical band heterotopia (SBH) [4–7], have been extensively studied. In contrast, much less is known about neocortical molecular layer heterotopia (MLH), which are characterized by over-migration of neurons into the molecular layer (layer I) and present in disorders such as dyslexia [8–10] and epilepsy [11,12]. Greater understanding of MLH has implications for various disorders with diverse clinical presentations.

MLH are present in several knockout (KO) lines [13–22] and inbred mouse strains [23,24], including C57BL/6 and C57BL/10 mice [25–27]. The cytoarchitecture of MLH in mice is indistinguishable from human heterotopia, suggesting similar causal mechanisms. Moreover, mice with heterotopia exhibit impaired learning of spatial and non-spatial memory tasks [28–32] and deficits in sensory discrimination tasks [33–36], consistent with cognitive deficits associated with MLH. Furthermore, mice with MLH have lower seizure thresholds and shorter latency to seizures following chemi-convulsant treatment [37,38], which mimic brain excitability changes observed in epileptics with MLH. Thus, behavioral changes in mice with MLH closely resemble those observed in humans with MLH, demonstrating the utility of mice as a model of human MLH.

The prevalence of MLH and underlying mechanisms of heterotopia formation remain unclear. In this study, we performed a neuroanatomical survey for the presence of MLH using 6 widely-used inbred mouse strains, including strains commonly used to produce genetically-engineered (GE) mice. We investigated the basic inheritance patterns of MLH by examining 2 first filial generation (F1) hybrids, one consomic strain, and GE mice on a congenic C57BL/6 background. Our results indicate MLH formation is a weakly penetrant trait requiring homozygosity of one or more C57BL/6 alleles outside of chromosome 1. These data are relevant for understanding neocortical development and the mechanisms of cortical lamination. Since C57BL/6 is the most widely used inbred strain in neuroscience research, our results have broad implications for their use in diverse studies and the creation of GE mice.

Materials and methods

This study was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Institutional Animal Care and Use Committee of New York Institute of Technology and Wadsworth Center. Description of the housing and care of mice has been described previously [25,26,39,40]. Five week-old, A/J, DBA/2J, and B6D2F1/J male mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Five week-old, 129S6/SvEvTac and B6129SF1/Tac male mice were obtained from Taconic Farms (Germantown, NY). Breeding pairs of 129S1/SvImJ, FVB/NJ, BALB/cJ inbred mice, and C57BL/6J-Chr 1[A/J]/NaJ (which carry chromosome 1 from strain A/J on a C57BL/6J background) were obtained from The Jackson Laboratory to generate mice used in this study. Brains from male and female retired breeders (6 months-old) or male and female offspring from these strains (3 weeks-old) were used. Since MLH are visible as early as P2 and the presence/absence of heterotopia does not change with age [25], we compared heterotopia prevalence between groups of mice because all mice were 3 weeks-old at time of sacrifice. Data from male and female mice

were combined since MLH prevalence does not differ between sexes [25,26]. Finally, no differences in heterotopia prevalence were previously observed between mice obtained directly from commercial vendors and mice bred in an academic vivarium from commercially-obtained breeders [25,26].

Histological and analytical methods were previously described by our lab [25,26,39]. We also used digital histological data from the Allen Brain Atlas (ABA; www.brain-map.org) and Mouse Brain Architecture Project (MBAP; http://mouse.brainarchitecture.org) to document heterotopia as previously described [25,27,39,41,42]. Data from the ABA *Mouse Strains* dataset contains high-resolution sagittal and coronal sections from the following strains (The Jackson Laboratory): 129S1/SvlmJ, C57BL/6J, Cast/EiJ, DBA/2J, PWD/PhJ, SPRET/EiJ, WSB/EiJ. Methods used in the creation of the *Mouse Strains* dataset were previously reported [43]. Data from the ABA *Transgenic Characterization* dataset (http://connectivity.brain-map.org/transgenic) contains histology from 221 Cre-driver lines that have been crossed with one of 38 "reporter" mouse lines (loxP, etc.). Methods and mice used in the creation of the *Transgenic Characterization* dataset were previously described [44,45]. Data from the MBAP *Transgenic Cell Counts* dataset (http://mouse.brainarchitecture.org/cellcounts/) contains histology from 79 F1 hybrid mice generated by crosses between Cre-driver lines and reporter lines (loxP, etc.). Methods and mice used in the creation of the *Transgenic Cell Counts* dataset were found on the MBAP website.

Results

Prevalence of MLH in common inbred strains

The prevalence of MLH in C57BL/6J and C57BL/10J strains is well established [25–27], but only a small number of brains from a few other inbred mouse strains have been examined [25]. Consequently, we used well-established histological methods [25–27] to examine brains from numerous inbred mice including: 129S6/SvEvTac (n=20), 129S1/SvImJ (n=17), A/J (n=17), BALB/cJ (n=16), DBA/2J (n=20), FVB/NJ (n=18) strains. We used methods previously described [25,27,39,41,42] to examine high-resolution digital histological material from the *Mouse Strains* dataset from the ABA, including 129S1/SvImJ (n=117 cases), C57BL/6J (n=314), Cast/EiJ (n=117 cases), DBA/2J (n=117 cases), PWD/PhJ (n=93 cases), SPRET/EiJ (n=117 cases), WSB/EiJ (n=93 cases) strains. Remarkably, we did not observe MLH in any inbred strain except C57BL/6J brains in the ABA *Mouse Strains* dataset. Additionally, heterotopia were not observed in DBA/2J or 129S1/SvImJ mice in the ABA or in the cohort examined using primary histological data. This suggests that causal alleles for heterotopia formation are present in C57BL/6 linage.

Inheritance of MLH in the C57BL/6 linage

Since first filial generation (F1) hybrid mice are heterozygous at all loci where the parental strains differ in allelic composition, we examined F1 hybrid mice to determine if MLH formation is a recessive trait. After establishing that DBA/2J mice and 129S6/SvEvTac mice do not exhibit MLH, we tested whether F1 hybrid crosses of these inbred strains with C57BL/6 mice would exhibit heterotopia. We did not observe MLH in 20 male B6D2F1/J or 20 male B6129SF1/Tac mice, which were generated by crossing a female C57BL/6 mouse

with a male DBA/2J mouse or male 129S6/SvEvTac mouse, respectively. This indicates that homozygosity, at one or more loci, is required for heterotopia formation.

Since consomic strains are characterized by the substitution of a homologous chromosome of a donor inbred strain backcrossed into a host inbred strain, they can be used to determine the chromosome associated with a given trait [46,47]. We examined brains from C57BL/6J-Chr 1$^{A/J}$/NaJ mice, which have introgressed chromosome 1 from A/J on an inbred C57BL/6J background. We observed MLH in 6 out of 22 (27.27%) consomic mice. Fisher's Exact Test of the prevalence of MLH in C57BL/6J-Chr 1$^{A/J}$/NaJ mice compared to measures previously determined for C57BL/6J mice (35.71%) [26] revealed no significant difference. The absence of MLH in A/J mice indicates that the locus responsible for heterotopia formation is outside chromosome 1 of the C57BL/6 background.

Presence of MLH in genetically-engineered C57BL/6 congenic mice

These results suggest that GE mice produced from C57BL/6 ES cells and maintained on this background would exhibit MLH. Furthermore, GE mice produced from ES cells of another inbred strain but backcrossed to a C57BL/6 background would also exhibit MLH. We tested this prediction by examining over 1500 histological cases of F1 hybrid mice generated by crossing one of 221 Cre-driver lines with one of 38 reporter lines in the ABA *Transgenic Characterization* dataset. A total of 57 cases from this database exhibited MLH. Quantitative analyses of MLH prevalence were not performed because not every histological section is available for examination for each case. Thus, errors of omission (i.e. cases erroneously reporting absence of MLH) may explain the small number of cases of MLH in this dataset.

We identified 39 unique combinations of Cre-driver and reporter line hybrids with MLH as summarized in Figure 1 and Table 1. MLH were observed in F1 hybrids produced by crossing Pvalb-IRES-Cre mice with either Ai14 or Ai39 reporter mice and in F1 hybrids produced by crossing Emx1-IRES-Cre with either Ai27 or Ai32 reporter mice. All driver and reporter mice used to generate the F1 hybrids in Table 1 were identified as being on a congenic C57BL/6 background per the ABA, The Jackson Laboratory (jax.org), or the Mutant Mouse Resource and Research Center (mmrrc.org) websites. These data indicate a causal allele for heterotopia formation can be present in the background of diverse GE mice and inherited in F1 crosses of GE lines.

MLH were also observed in the MBAP *Transgenic Cell Counts* database (Figure 2). Only 4 of 77 cases exhibited MLH which may be due to the mixed background in these congenic mice or fewer histological sections from which to evaluate. Nonetheless, heterotopia were identified in F1 hybrids crossed with the same Cre-driver mice shown to have MLH in the ABA database (e.g. Emx1-Cre and Pvalb-Cre mice). These data, from two different databases, indicate that driver-reporter F1 crosses can exhibit heterotopia.

Discussion

Heterotopia in the C57BL/6 linage require homozygosity at one or more alleles outside chromosome 1 and the sex chromosomes

We provide novel data on the prevalence and inheritance of spontaneous neurodevelopmental malformations of the neocortex using a combination of inbred and consomic strains as well as F1 hybrid GE mice. Our results indicate MLH may be unique to the C57BL linage as heterotopia were not observed in any other inbred strains examined. This suggests that other strains within the C57BL linage may also exhibit heterotopia, which is consistent with our previous observations of MLH in C57BL/10 mice. Since the natural history and genetic evolution/drift of this linage is well understood, documenting which C57BL stains exhibit MLH is instrumental toward a mechanistic understanding of heterotopia formation.

Using two different F1 hybrid mice produced from parental strains, including C57BL/6 and a strain with 0% prevalence for MLH (DBA/2J or 129S6/SvEvTac), our results indicate that homozygosity for one or more C57BL/6 alleles is required for heterotopia formation. This genetic model for heterotopia formation is consistent with observations of MLH in recombinant inbred mice, which by definition are homozygous at all alleles for some combination of each parental genotype [6,26,28–30,32]. Consequently, quantitative trait loci studies using a panel of recombinant inbred mice is a potential avenue to determine the genetic locus/loci responsible for MLH formation.

We used consomic mice to demonstrate that heterotopia formation requires one or more alleles outside of chromosome 1. Thus, future neuroanatomical analyses from an entire panel of consomic mice would reveal one or more strains that never exhibit heterotopia, thereby revealing the chromosome(s) where casual alleles are found. Since the prevalence of MLH in male and female C57BL/6 mice is the same [39] and F1 mice of both sexes do not display these malformations, these data argue that alleles on the sex chromosomes or chromosome 1 are not required for heterotopia formation.

Neocortical MLH in genetically-engineered mice

We document neocortical MLH in F1 hybrids of Cre-driver and reporter mouse lines even though most of these lines are on a mixed/congenic C57BL/6 background. The finding that MLH may be exclusive to the C57BL/6 linage suggests that heterotopia formation in these GE mice results from the inheritance of one or more causal C57BL/6 alleles during crossing. Consequently, GE mice created with C57BL/6 ES cells will exhibit some prevalence of heterotopia. Likewise, GE mice created with ES cells from another inbred strain but backcrossed to the C57BL/6 background will also exhibit heterotopia. Thus, results from studies using GE mice that exhibit heterotopia should be carefully evaluated so as not to attribute heterotopia formation to a genetic manipulation that was experimentally produced.

Implications of MLH on the use of C57BL/6J mice in neuroscience research

Our findings have broad implications on the use of C57BL/6 mice or GE mice on this background in neuroscience research. Firstly, results from neuroanatomical studies of

neocortical development in C57BL/6 mice using experimental agents/perturbations to disrupt growth or neuronal migration should be carefully evaluated as some percentage of control and experimentally-treated mice are expected to exhibit heterotopia solely due to the genetic background. For example, in a study where C57BL/6 dams were treated with sodium acetazolamide, 34% of acetazolamide-exposed pups had heterotopia which was not significantly different than the 28% of water-treated control pups with heterotopia [48]. Secondly, results from behavioral studies using C57BL/6 mice or GE mice on this background should be carefully evaluated. Heterotopia in control and experimentally-treated mice may affect performance on the task(s) tested or interact with treatment regimes. This assertion is supported by previous findings that mice with MLH exhibit deficits in learning, memory [28–30,32], and sensory discrimination tasks [33,34,36] as well as neocortical hyperexcitability based on response to chemi-convulsant treatment [38,49]. Finally, the extent to which gene/protein expression changes are observed in neurons and glia in heterotopia remains unknown; these changes could affect neocortical expression studies, particularly when the presence/absence of heterotopia is not known prior to tissue harvesting. Thus, until the potential effects of neocortical MLH are elucidated, results from studies using C57BL/6 mice should be carefully evaluated. Histological confirmation of the presence/absence of heterotopia in mice used in a study would be critical to interpretation of experimental results.

Acknowledgments

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Highlights

- Neocortical MLH is a weakly penetrant, divergent trait among inbred mice.

- Homozygosity, at one or more loci, is required for the formation of heterotopia.

- MLH formation requires one or more C57BL/6 alleles outside of chromosome 1.

- MLH are present in diverse genetically-engineered lines on a C57BL/6 background.
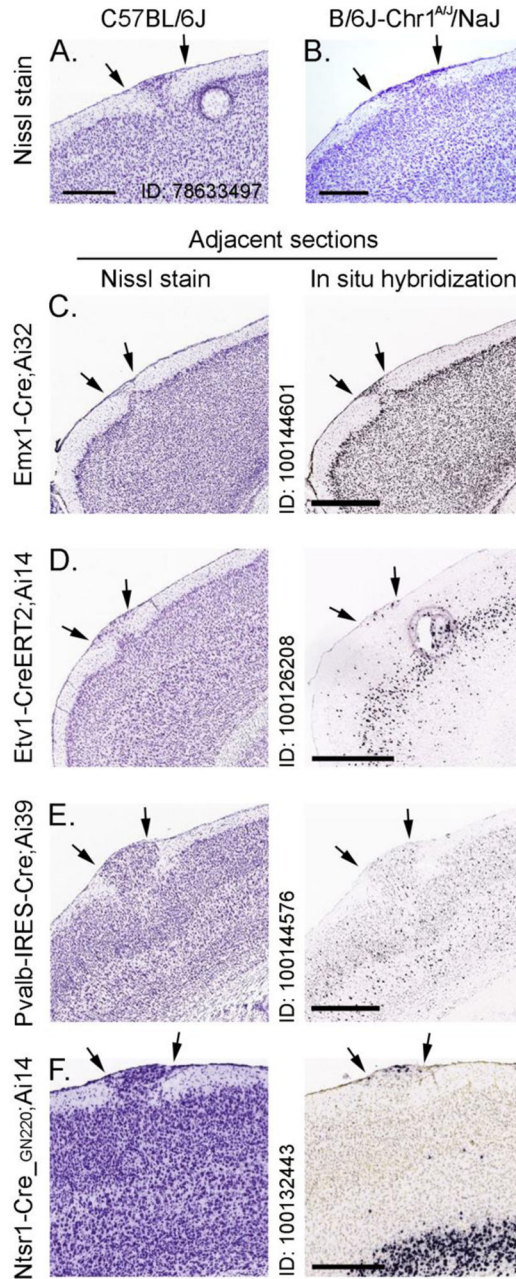
Figure 1.
A–B, Representative photomicrographs of Nissl-stained sections with heterotopia (arrows) from C57BL/6J (from the ABA *Mouse Strains* dataset) and C57BL/6J-Chr 1$^{A/J}$/NaJ mice (abbreviated B/6J-Chr11$^{A/J}$/NaJ), respectively. C–F, Representative photomicrographs of adjacent sections of F1 hybrid lines taken from the *Transgenic Characterization* dataset of the ABA containing heterotopia (arrows). Nissl-stained and hybridized sections in left and right panels, respectively. Hybridized gene listed next to each photomicrograph taken from the ABA. Scalebars (in microns): A = 420, B = 320, C = 798, D = 960, E = 632, F = 444.
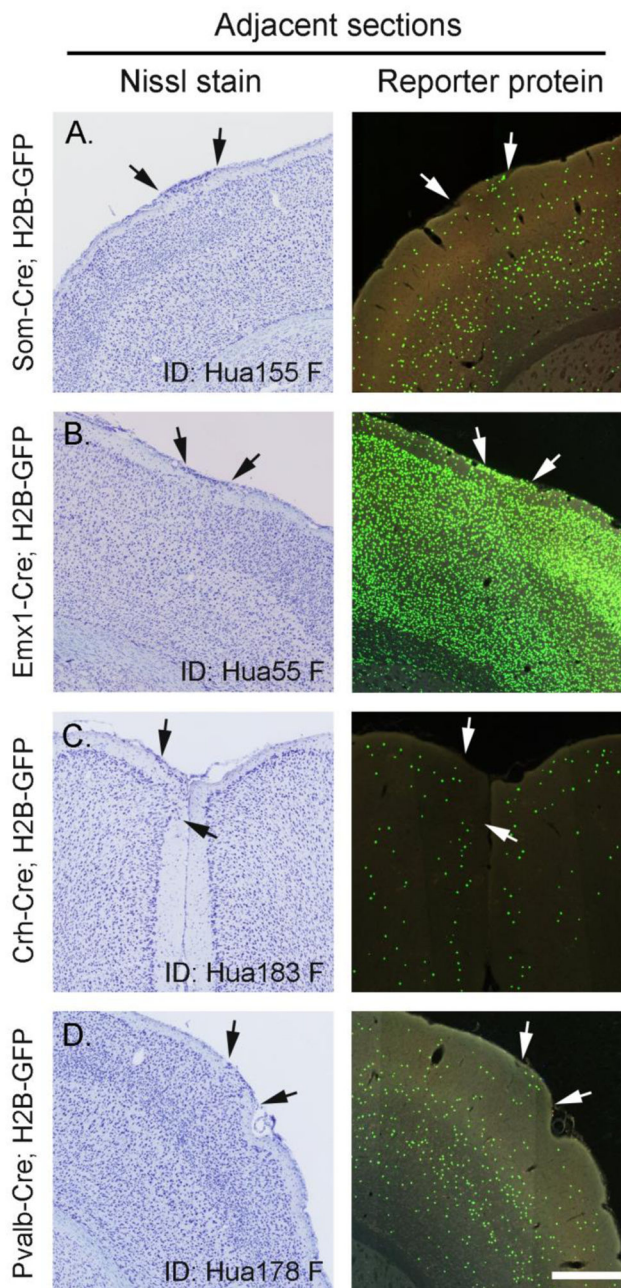
Figure 2.
A–D, Representative photomicrographs taken from F1 hybrid lines from the MBAP *Transgenic Cell Counts* database containing heterotopia (arrows). Nissl-stained and reporter protein expression shown in the left and right panels, respectively. Scalebar (in microns): A–B = 500; C–D = 600 microns.

Table 1

List of all unique driver-reporter F1 hybrid lines with MLH found in the ABA *Transgenic Characterization* dataset including experiment identification number of a representative example of each mouse line.

Experiment	F1 Hybrid Line Name	Driver line	Reporter line
293795159	Avp-IRES2-Cre;Ai14	Avp-IRES2-Cre	Ai14
175707182	Camk2a-tTA;Ai14	Camk2a-tTA	Ai14
268328793	Chrnb4-Cre_OL57;Ai14	Chrnb4-Cre_OL57	Ai14
100132436	Crh-IRES-Cre (BL);Ai14	Crh-IRES-Cre (BL)	Ai14
146455849	Crh-IRES-Cre (ZJH);Ai14	Crh-IRES-Cre (ZJH)	Ai14
80203010	Cyp39a1-Tg1-Cre;R26-stop-EYFP	Cyp39a1-Tg1-Cre	R26-stop-EYFP
100141756	Dbh-Cre_KH212;Ai14	Dbh-Cre_KH212	Ai14
100141754	Drd1a-Cre;Ai14	Drd1a-Cre	Ai14
175194677	EE313-lacZ-CreERT2-Tg2;Ai14	EE313-lacZ-CreERT2-Tg2	Ai14
167245573	EE342-lacZ-CreERT2-Tg3;Ai14	EE342-lacZ-CreERT2-Tg3	Ai14
167242744	EE609-lacZ-CreERT2-Tg2;Ai14	EE609-lacZ-CreERT2-Tg2	Ai14
167246779	EE921-lacZ-CreERT2-Tg2;Ai14	EE921-lacZ-CreERT2-Tg2	Ai14
100144601	Emx1-IRES-Cre;Ai32	Emx1-IRES-Cre	Ai32
100095125	Emx1-IRES-Cre;Ai27	Emx1-IRES-Cre	Ai27
100132637	Et(cre/ERT2)7089Rdav;Ai14	Et(cre/ERT2)7089Rdav	Ai14
196126585	Et(EGFP/cre)16059Rdav;Ai14	Et(EGFP/cre)16059Rdav	Ai14
100144041	Et(EGFP/cre)16102Rdav;Ai14	Et(EGFP/cre)16102Rdav	Ai14
100141712	Et(EGFP/cre)16261Rdav;Ai14	Et(EGFP/cre)16261Rdav	Ai14
156350546	Et(icre)21468Rdav;Ai14	Et(icre)21468Rdav	Ai14
114374599	Et(icre/ERT2)14163Rdav;Ai14	Et(icre/ERT2)14163Rdav	Ai14
100126208	Etv1-CreERT2;Ai14	Etv1-CreERT2	Ai14
157078989	Gabrr3-Cre_KC112;Ai14	Gabrr3-Cre_KC112	Ai14
100130925	Lepr-IRES-Cre;Ai14	Lepr-IRES-Cre	Ai14
100132443	Ntsr1-Cre_GN220;Ai14	Ntsr1-Cre_GN220	Ai14
112608505	Otof-Cre;Ai14	Otof-Cre	Ai14
159119080	Otof-CreERT2;Ai14	Otof-CreERT2	Ai14
100130512	Oxt-IRES-Cre;Ai14	Oxt-IRES-Cre	Ai14
100144576	Pvalb-IRES-Cre;Ai39	Pvalb-IRES-Cre	Ai39
100117968	Pvalb-IRES-Cre;Ai14	Pvalb-IRES-Cre	Ai14
146454705	Rbp4-Cre_KL100;Ai14	Rbp4-Cre_KL100	Ai14
281575505	Slc17a7-IRES2-Cre;Ai14	Slc17a7-IRES2-Cre	Ai14
100141516	Slc17a8-iCre;Ai14	Slc17a8-iCre	Ai14
100104685	Slc6a3-Cre;Ai3	Slc6a3-Cre	Ai3
112200098	Slc6a4-CreERT2_EZ13;Ai14	Slc6a4-CreERT2_EZ13	Ai14
181446571	Syt17-Cre_NO14;Ai14	Syt17-Cre_NO14	Ai14
146452634	Tac1-IRES2-Cre;Ai14	Tac1-IRES2-Cre	Ai14
156348437	Trib2-2A-CreERT2;Ai14	Trib2-2A-CreERT2	Ai14
100141745	Ucn3-Cre_KF43;Ai14	Ucn3-Cre_KF43	Ai14

Experiment	F1 Hybrid Line Name	Driver line	Reporter line
148054441	Vipr2-Cre_KE2;Ai14	Vipr2-Cre_KE2	Ai14