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Overview of genetic tools and techniques to study Notch signaling in mice

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Abstract

Aberrations of Notch signaling in humans cause both congenital and acquired defects and cancers. Genetically-engineered mice provide the most efficient and cost-effective models to study Notch signaling in a mammalian system. Here, we review the various types of genetic models, tools, and strategies to study Notch signaling in mice, and provide examples of their use. We also provide advice on breeding strategies for conditional mutant mice, and a protocol for tamoxifen administration to mouse strains expressing inducible Cre recombinase-estrogen receptor fusion proteins.

Keywords

Conditional mutations; Notch reporter lines; Notch receptor-Cre fusions; Fate mapping; Lineage analysis; Domain-swap mice; Tamoxifen administration

1. Introduction

The Notch signaling pathway is an evolutionarily ancient form of cell-cell communication. In vertebrates it plays a central role in the development and homeostasis of most major organ systems and has been implicated in many forms of hereditary and idiopathic diseases, including cancer. The many complexities of the Notch signaling pathway have been described extensively elsewhere (1–4). In this chapter, we describe how the laboratory mouse has been developed to study many aspects of Notch signaling in development and disease. In addition to the huge number of different genetically modified mouse lines that have been generated to cause loss or gain of function of different components of the Notch signaling pathway, recent years have seen the advent of new mouse lines to visualize Notch-responsive cells in the intact animal, to follow the fates of cells that have experienced Notch signaling, and to probe the structure-function relationships between different mammalian Notch receptors. In this chapter, we describe the current status of transgenic mouse

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technology that has addressed these questions, and provide some basic protocols for breeding and working with conditional mutants of the Notch pathway.

2. Loss and gain of function Notch pathway mutant mice

2.1. Constitutive loss of function mutant mice

The many different components of the Notch signaling pathway (2) has led to the generation of a huge number of genetically modified and mutagenesis-induced mouse lines that have loss of function in Notch signaling. As described in Section 6 below, there are comprehensive online databases that allow the investigator to search for a wide variety of different alleles of genes in the Notch pathway. Historically, these alleles were generated piecemeal in individual laboratories. More recently however, an international project has aimed to systematically produce targeted mutations in ES cells for every mouse gene (5, 6) (www.knockoutmouse.org/). Many of these ES cell lines have already been used to generate mouse lines, and an initial systematic analysis of these lines is ongoing (7). Individual investigators may order targeted ES cells from repositories derived from these projects, and in addition to generating mutant mice from such cell lines, it will be possible to re-engineer such lines for new purposes (for example, expression of fluorescent proteins, recombinases, optogenetic channels, calcium indicator proteins, or to add epitope tags) using technology such as recombination-mediated cassette exchange (8, 9).

2.2. Conditional loss of function mutant mice

The early embryonic lethality exhibited by many constitutive Notch pathway loss of function mutants, that is often a result of defects in formation of the embryonic vasculature (10), has emphasized the importance of generation of conditional and/or inducible Notch pathway mutants to facilitate study of Notch pathway function later in embryogenesis, and in the postnatal and adult mouse.

2.2.1: Generating conditional mutant mice with the Cre-Lox system—The ability of the P1 bacteriophage Cre recombinase to recombine palindromic LoxP sites at high efficiency (11) in mammalian cells (12), even when separated by large intervening DNA sequences, has made it the method of choice for creating spatially and temporally controlled gain and loss of function in mice (13–15). A huge armory of transgenic and knock-in lines expressing Cre recombinase under the control of different promoters and enhancers allows manipulation of genes in most tissues. Temporal control of Cre activity is also possible using fusions of the Cre protein with modified forms of the estrogen receptor, which allow nuclear localization and activation of Cre following administration of the estrogen analogue tamoxifen (16–20). Cre has also been fused with the progesterone receptor in a similar fashion to yield mice in which the recombinase activity can be activated by Mifepristone (RU486) (21), although this strategy is used far less than Cre-estrogen receptor fusions (22). Similarly, although the FLP-FRT recombination system has also been used to drive recombination in the mouse genome, its use has tended to be reserved for intersectional recombination strategies (23), or for the removal of selection cassettes following the construction of targeted alleles (24).

Many conditional alleles have been generated for components of the Notch signaling pathway. Conditional alleles are available for the *Notch1*, *Notch2*, and *Notch3* receptors, for the *Dll1*, *Dll4*, *Jag1* and *Jag2* ligands, as well as several non-canonical ligands such as *DNER*, *Contactin1* and *MAGP1* (25). Conditional alleles have also been generated for modifying and processing enzymes of Notch and its ligands, for nuclear co-activators and for direct downstream targets of Notch signaling. Section 6 gives advice for identifying and locating mouse mutants for various Notch signaling pathway components. More recently, the advent of large scale mouse targeting projects, such as the Knockout Mouse Project (KOMP) and the European Conditional Mouse Mutagenesis Program (EUCOMM), has generated a large number of new targeted mutations of genes in the Notch pathway. Many of these alleles have used the “knockout first” targeting strategy, in which a targeted null allele can be converted to a conditional allele by mating with ubiquitously-expressing FLP lines (26).

In addition to conditional “floxed” alleles, some mouse lines have been developed to produce dominant-negative mutants of Notch pathway components. For example, *ROSA26 dnMAML1-GFP* mice express a Cre-inducible fusion protein of a truncated *Mastermind-like1* (*MAML1*) and GFP knocked into the *ROSA26* locus (27, 28). This fusion protein is capable of binding Rbpj/CSL, but cannot recruit transcriptional co-activators to this complex and thus acts as a dominant-negative protein. The use of a dominant loss-of-function mutation can make breeding strategies easier; however, it is possible that two mutant alleles may be required to give a strong loss of Notch signaling in some tissues.

2.2.2: Selection and verification of Cre driver lines—Several databases listing Cre driver lines have been developed (29). The Mouse Genome Informatics database maintains and curates an extensive and rapidly growing list of published Cre driver lines (www.informatics.jax.org/recombinase.shtml) that target many different tissues, cell populations and stages of development. The activity and recombination pattern of these lines can be monitored by crossing them with a variety of Cre reporter lines, in which the expression of a fluorescent or enzyme marker is activated after Cre recombination. Many of these lines are available from the Jackson Laboratory (cre.jax.org/crereporters.html), which also maintains a Cre driver line characterization pipeline (30). Additional Cre driver databases are maintained in Canada and Europe (31, 32).

It is *strongly recommended* that each investigator independently verifies the activity of a Cre driver line imported into their mouse colony before starting a conditional knockout experiment. It is common for Cre lines to produce patterns of recombination that are partially different from their first published descriptions. This can be due to differences in genetic background, or to differing sensitivities of the Cre reporter lines used. This advice is doubly true for studies using tamoxifen-inducible versions of Cre recombinase. Here, the degree and efficiency of recombination is exquisitely sensitive to the dose and route of administration of tamoxifen, and careful monitoring of recombination efficiency is essential for correct interpretation of results using such mice. If possible, it is recommended to include a Cre reporter allele alongside a conditional allele, so that recombination can be observed directly in the conditional mutant strain. A protocol for tamoxifen administration is given below (Section 2.2.4).

2.2.3: Breeding strategies for generation of conditional mouse mutants—A

typical breeding scheme to generate conditional loss-of-function mouse mutants using the Cre-LoxP system is shown in Figure 1. In brief, a Cre driver mouse is crossed with a mouse from a second line that is heterozygous for a null allele of the gene of interest. The offspring from this cross are screened for mice carrying both the Cre and null alleles. These mice are then crossed with mice carrying two alleles of the gene of interest that has all or part of its coding region flanked by loxP sites (“floxed” or “flox” alleles). Approximately 25% of the resulting offspring will inherit the Cre allele, a null allele and a floxed allele of the gene of interest. These mice will then display a loss of function of the gene of interest in cells in which the Cre recombinase is active. If a tamoxifen-inducible version of Cre recombinase is used, recombination will not occur until the mice are dosed with this drug.

Notes

1. Anecdotal evidence suggests that recombination is more efficient if a null allele is crossed on to the Cre driver line as opposed to a floxed allele. This is presumably due to the presence of fewer loxP sites in the target genome. A recent published study has verified these anecdotal findings (33). A null allele can easily be generated from a floxed allele by mating with a ubiquitously-expressed Cre line.
2. Since the maintenance of Cre driver; null allele mouse lines requires genotyping at each generation and does not yield 100% of the desired genotype, it is common to use male studs carrying these alleles, as a single stud can be mated repeatedly to multiple females. Since the propagation of a homozygous floxed line is far more efficient, homozygous floxed females are typically used in this mating scheme.
3. Before commencing a conditional breeding strategy, it is always advisable to check the chromosomal location of the Cre driver allele and the gene of interest to be used in the project, as well as any reporter alleles that may be included in the breeding scheme. Although the probability of two given loci occurring on the same chromosome in mice is low, a quick check may save months of wasted time.

2.2.4: Protocol: Administration of tamoxifen to mice by oral gavage

1. Introduction: As described above, it is strongly recommended that investigators perform preliminary experiments to verify the fidelity of their Cre lines, and to determine the speed and efficiency of Cre-mediated recombination after administration of tamoxifen. It is possible to observe the first evidence of recombination within 6–8 hours of tamoxifen administration (34), although this will obviously depend on the strength of the regulatory sequences in each Cre driver line. It should also be noted that recombination may continue for days, or in some cases weeks, after cessation of tamoxifen administration (35), so studies that make use of pulses of tamoxifen should be interpreted with appropriate caution.

2: Materials

1. Warm 5–6 ml corn oil (Sigma, C8267) in a 15 ml tube at 42°C for 30 minutes. Corn oil can be stored at room temperature, but replace every 3–6 months to prevent the oil from becoming rancid.

2. Weigh out 100 mg tamoxifen (Sigma T5648) into a second 15 ml tube. Tamoxifen is light-sensitive, and should be stored in the dark at 4°C with a desiccant. Allow the tamoxifen to equilibrate to room temperature before weighing.
3. Add 5 ml of the warm corn oil to the tamoxifen, wrap the tube in foil to protect from light and incubate in a 37°C water bath to dissolve. The solution should be vortexed frequently to aid solubilization, which usually takes several hours. There should be no precipitate left in the solution.
4. The tamoxifen solution can be stored in the dark at 4°C wrapped in foil. Discard the solution after one month, or sooner if a precipitate starts to develop.

3: Methods

1. Weigh each mouse on a balance before administering tamoxifen. Accurate recording of weight is important to obtain consistent dosing.
2. Curved reusable metal gavage needles with a ball tip are available from a number of suppliers (for example, Fine Science Tools 18060-20) or from institutional veterinary staff. A 20 gauge needle with a tip of between 1–2 mm is suitable for adult mice. Alternatively, disposable needles are also available (for example, Fine Science Tools 18061-20).
3. Connect the needle to a syringe and fill with tamoxifen solution. Grasp the mouse by the scruff of the neck, and secure the tail in the manner one would use for an intraperitoneal injection. It is important that the mouse be securely restrained but relatively relaxed.
4. Introduce the needle tip into the side of the mouse's mouth. Pass the needle tip to the rear of the mouth along the roof. Push the mouse's head back and pass the needle fully down the esophagus into the stomach to deliver the tamoxifen dose. A minimum of force is required and the entire procedure should be performed as gently as possible to avoid distress. If the animal attempts to struggle, it is possible that the windpipe is obstructed – this should be avoided.

4: Notes

1. Some studies have administered tamoxifen by intraperitoneal injection. While this may require less training to administer, many anecdotal reports suggest pregnant females show signs of discomfort from the added burden of corn oil in their abdomens.
2. Adult mice can tolerate doses of tamoxifen as high as 0.3 mg/g body weight. Pregnant females and their litters are more sensitive to tamoxifen, so dosage should be determined empirically for each mouse strain. Females past the 10th day of pregnancy can typically tolerate as much as 0.12 – 0.13 mg/g body weight administered twice a day. Administration of tamoxifen at earlier stages can cause developmental problems or resorption of embryos, and the dose may need to be reduced appropriately (36).

3. As tamoxifen is an estrogen analogue, it can cause complications with pregnancy, especially in the final few days before delivery. To prevent late fetal abortion, progesterone (Sigma P3972) can be administered with the tamoxifen, typically at 50% of the dose of tamoxifen.
4. It is recommended that pregnant dams be disturbed as little as possible by laboratory and animal care staff for several days before and after delivery.
5. Tamoxifen can be administered to neonatal pups in several ways. Direct intraperitoneal injection of tamoxifen works effectively, although care must be exercised when handling the pups. Alternatively, tamoxifen is known to be transmitted in maternal milk (37), so continued administration of tamoxifen to the mother will provide dosing to neonates, albeit in a more variable fashion.
6. Tamoxifen is a carcinogen and appropriate caution should be taken when handling the powder and solution. Dispose of materials that have come into contact with tamoxifen in a manner consistent with local environmental safety regulations.
7. All handling and use of animals requires approval by local monitoring agencies and should adhere to local and national animal care guidelines. It is strongly recommended that users learning the gavage procedure be trained by a veterinarian or veterinary technician, ideally by first practicing on an animal that has been euthanized.

2.3. Conditional gain of function mice

Transgenic mis-expression of Notch pathway components can have very strong biological effects, such that constitutive gain of function transgenic or knock-in alleles are often lethal, or difficult to maintain. This has led a number of investigators to generate conditional gain of function mice, whose expression is activated by the Cre-Lox system. These alleles contain a transcriptional stop sequence, flanked on either side by a loxP site, located between the promoter and the coding sequences for the Notch pathway gene that will be expressed. These alleles have been generated either as transgenes or as knock-in alleles into the widely-expressed *ROSA26* locus. Commonly, the gene being mis-expressed is the transcriptionally-active Notch intracellular domain (NICD). For example, mice that conditionally express NICD1 (38) or NICD2 (39) have been reported; many additional publications using these or similar mice exist. Aifantis and colleagues recently reported generation of four knock-in strains, one for each NICD (1 through 4) (40). Each line used the same allele design, in which coding sequences for the NICD were inserted into the *ROSA26* locus behind a loxP-stop-loxP cassette. NICD coding sequence was followed by an internal ribosome entry site-yellow fluorescent protein (IRES-YFP) cassette to facilitate monitoring of expression. Interestingly, when activated by a human ubiquitin C promoter, only the NICD1 line was capable of causing T cell leukemia, despite comparable levels of expression of all four different NICDs, demonstrating sequence-specific differences among the four NICDs in vivo (40), similar to those previously described in vitro (41).

3. Notch signaling reporter lines

Signaling pathway-specific transgenic reporter lines are very useful in analyzing pathway activation in both wildtype and mutant genetic backgrounds. These lines generally contain a gene encoding a reporter protein, such as β -galactosidase or a fluorescent protein, under the control of a promoter that is transcriptionally regulated by the specific pathway being analyzed (e.g., the Notch pathway). The two most commonly used transgenic Notch reporters are the Transgenic Notch Reporter (*TNR*) EGFP reporter line (42), and the Notch Activity Sensor (*NAS*) nuclear lacZ reporter (43). Neither of these Notch reporter lines, however, has gained the extremely wide utilization of the Wnt pathway transgenic reporter lines *TopGal* (44) and *BatGal* (45). The *TNR* line utilizes a synthetic promoter consisting of four Rbpj binding sites linked to the basal SV40 promoter to drive EGFP expression, while the *NAS* reporter uses 12 multimerized Rbpj binding sites upstream of a minimal promoter from the beta globin gene. Other Notch reporters use endogenous Notch target genes containing Rbpj binding sites to drive reporter gene expression. Transgenic or knock-in reporters utilizing the *Hes1* (46) and *Hes5* (46–48) promoters to drive β -galactosidase or EGFP expression have been described.

Novel transgenic Notch pathway reporters continue to be developed. Two groups have developed new reporters that utilize improved, brighter fluorescent proteins in order to better enable in vivo or ex vivo imaging at the single cell level, as well as flow cytometry analyses. The *Hes1-emGFP* line is a knock-in allele that places the emerald variant of the GFP protein under the transcriptional control of the endogenous *Hes1* promoter (49). *Hes1-emGFP* mice have been used in the analysis of Notch signaling in intestinal stem cells (49), during mammary gland development (including the sorting by flow cytometry of emGFP-expressing cells from the mammary gland) (50), and in the analysis of Notch pathway activation in specific hematopoietic cell lineages during both normal and stress hematopoiesis (40). The *CBF:H2B-Venus* transgenic line (*Cbf1* is an alternative symbol for the Rbpj gene) expresses a fusion protein comprising human histone H2B (to provide nuclear localization) sequences linked to the yellow fluorescent protein Venus (51), and uses the same synthetic Notch target promoter as the *TNR* reporter line (42). The sensitivity of the *CBF:H2B-Venus* reporter facilitated identification of new sites of Notch signaling in embryos, particularly within the epiblast of the early postimplantation embryo, and could be used for live cell imaging in ES cells, primary mouse embryonic fibroblasts, and in cultured postimplantation embryos (51).

4. Notch receptor-Cre fusions for fate mapping and functional analyses

One criticism of Notch reporter lines such as those described in the preceding section, which utilize promoters containing synthetic arrangements of Rbpj binding sites or natural Notch target promoters containing Rbpj binding sites, is that they do not distinguish signal reception mediated by the different Notch receptor proteins. Several groups have generated knock-in or transgenic lines expressing fusions of Cre recombinase or the transcriptional activator with Gal4VP16 with various Notch receptors. These lines can be used for both fate mapping of Notch receptor-expressing cell lineages, or for functional analyses of these lineages.

Two groups have developed bi-transgenic Notch1 reporter systems that are dependent on gamma secretase-mediated proteolysis of a modified *Notch1* gene to identify cells that have undergone active (i.e., proteolysis-mediated) Notch1 signaling. Kopan and colleagues used homologous recombination to generate a *Notch1* knock-in allele in which sequence encoding the Notch1 (N1) intracellular domain downstream of the gamma secretase cleavage site were replaced with sequence encoding Cre recombinase (52). The line, currently named *N1::Cre^{LO}* (but referred to in the original publication as *N₁IP-Cre*, for *Notch1 Intramembrane Proteolysis-Cre*), when crossed with a Cre reporter such as the *ROSA26R* strain (53), permits in vivo mapping of Notch1 activation by gamma secretase-mediated proteolytic cleavage. While not a Notch receptor-Cre fusion, Radtke and colleagues have developed a conceptually similar bi-transgenic Notch1 reporter system (54). They constructed a bacterial artificial chromosome (BAC) transgenic line (*N1-Gal4VP16*) containing a modified *Notch1* cDNA, in which the intracellular domain of the Notch1 receptor was replaced with sequence encoding the Gal4VP16 transcriptional activator. When crossed to a transgenic Gal4 reporter line, such as *UAS-lacZ* mice (55), the *N1-Gal4VP16* line permits the identification in vivo of cells undergoing active Notch1 signaling (54).

Kopan and colleagues have generated several additional Notch receptor-Cre fusion knock-in lines, including *N1::Cre^{HI}* (which, not surprisingly, expresses higher levels of Cre recombinase than the *N1::Cre^{LO}* line) (56), *N1::CreERT2* (57), and *N2::Cre^{LO}* (57, 58). Like the *N1::Cre^{LO}* line, all of these lines have Cre coding sequences replacing the Notch receptor intracellular domain, and must be activated by gamma secretase-mediated proteolytic cleavage in order to generate Cre recombinase activity. In addition to fate mapping studies (52, 58–60), some of these lines have been used in functional assays. Since the Cre coding sequences replace the Notch receptor intracellular domain in these alleles, they are functional null alleles that must be analyzed as heterozygotes. In a clever utilization of this system, trans-heterozygous *N1::Cre^{LO}/N1^{flox}* mice were generated. The low efficiency of the *N1::Cre^{LO}* allele created sporadic clones of cells with *Notch1* loss of heterozygosity (LOH) in these mice, which subsequently experienced widespread vascular tumors and lethality secondary to massive hemorrhage (56).

Notch receptor fusion lines such as *N1::Cre^{LO}* and *N1-Gal4VP16* identify cell lineages that have undergone proteolysis-mediated Notch1 signaling. Artavanis-Tsakonas and colleagues have developed four CreERT2-expressing lines that, when mated to Cre reporter lines, identify cell lineages expressing the *Notch1* through *Notch4* genes without requiring their activation by proteolytic cleavage (49). These lines have been used recently to identify hematopoietic cell lineages that express each of the Notch receptors during hematopoietic differentiation (40). By crossing each of the four *Notch-CreERT2* lines to the *ROSA26^{lsRFP}* reporter line (61), administering tamoxifen and waiting for various chase periods, the authors found that only *Notch1-CreERT2* and *Notch2-CreERT2* were expressed in bone marrow. *Notch1-CreERT2* primarily labeled cells with lymphoid potential, while *Notch2-CreERT2*-labeled cells were found mostly in nonlymphoid progenitors (40). The *Notch2-CreERT2* line also was used to identify, by mating to conditional β -galactosidase and Tomato red fluorescent protein Cre reporter lines, two previously unrecognized mammary epithelial cell lineages that are distinct from classical luminal and myoepithelial cells (50).

The *Notch2-CreERT2* line was used to functionally assess the role of these lineages during mammary gland development in virgin and lactating females, by mating the line to a conditional diphtheria toxin receptor line (62) to perform cell ablation of *Notch2-CreERT2*-expressing cell lineages. These experiments demonstrated that the *Notch2-CreERT2*-expressing cell lineages were critical for the formation of tertiary branches of mammary gland ductal trees (50).

5. Domain-swap mice

In vivo structure-function studies are a powerful tool to dissect protein function in mice. Kraman and McCright generated a knock-in allele (*Notch2^{N1in}*) in which sequences encoding the carboxy-terminal 426 amino acids of the Notch2 protein were replaced with the equivalent region of the Notch1 protein (63). Despite the fact that the amino acid sequences of the replaced region are only 37% identical, no aberrant phenotypes were detected in mice homozygous for the *Notch2^{N1in}* allele, indicating that the carboxy-terminal region of the Notch1 intracellular domain is able to functionally replace that of the Notch2 protein in vivo.

In a genetic engineering tour de force, Kopan and colleagues recently reported much more extensive, and reciprocal, intracellular domain swaps of the Notch1 and Notch2 proteins (58). They described construction of two knock-in lines in which the genomic regions encoding the intracellular domains (ICD) of the Notch1 and Notch2 proteins were interchanged. The *N12* allele encodes the extracellular domain (ECD) of Notch1 with the ICD of Notch2, while the *N21* allele encodes the ECD of Notch2 with the ICD of Notch1. The swapped regions extended from exon 28, encoding the transmembrane domain, to the stop codon in exon 34. In order to retain transcript-specific regulation of mRNA stability and translation (by microRNAs, for example), the 3' untranslated regions of the two genes were not interchanged. Mice homozygous for either allele (*N12/N12* or *N21/N21*) were viable. The initial publication was confined to an analysis of kidney development in these mice (58). The authors found that just a single copy of Notch1-ICD could fully rescue the loss of Notch2-ICD, if it was expressed from the *Notch2* locus. These results demonstrated that, at least for kidney development, the Notch1-ICD and Notch2-ICD were fully interchangeable. They further demonstrated that the Notch2-ECD increased Notch receptor localization to the cell surface during kidney development, and was cleaved more efficiently than the Notch1-ECD upon ligand binding (58).

6. Locating mouse genetic resources for in vivo analyses of Notch signaling

One of the greatest factors contributing to how widely a mouse line is utilized by the research community is whether the line is available from a public strain repository (64). Many of the lines summarized in this overview are available from such repositories. Researchers can search the web site of the International Mouse Strain Resource (IMSR; www.findmice.org) to locate mouse lines available from strain repositories around the world. The search options on the IMSR site permit one to filter the results in various ways, and it can be useful to exclude the ES cell category (which can contain hundreds of gene

trap alleles that have never been made into mice) from the search (Fig. 2). Information on different alleles of particular genes of interest is also available on the Mouse Genome Informatics site (www.informatics.jax.org). Table 1 provides examples of Notch signaling reporter and Notch receptor-Cre lines available (or soon to be available) from public mouse strain repositories. We have not attempted to summarize individual Notch pathway mutants deposited in mouse repositories, as the number of these strains is too numerous, but encourage investigators to utilize the search procedures described here, and elsewhere (5, 6, 29, 64).

Strains not deposited in repositories may be available directly from the investigator who produced them. Recovering cryopreserved strains from repositories generally costs several thousand dollars, or its equivalent in other currencies. It is not unusual to see requests from investigators on email list servers such as MGI-list (hosted by the Mouse Genome Informatics site), inquiring whether anyone willing to share breeder stock has a certain strain live on the shelf. It may also be useful to contact laboratories that have published recently using the desired strains.

7. Concluding remarks

The number and variety of genetic reagents and tools to study Notch signaling in mice has increased dramatically in recent years, and is sure to continue. The creation of large scale international programs to generate and distribute mouse genetic resources (5, 6), the ability to re-engineer these alleles (8, 9), and the development of novel techniques for genome engineering and generation of genetically-engineered mice (65, 66), all promise to keep mouse biologists interested in studying the Notch pathway busy for many years to come.

References

1. Guruharsha KG, Kankel MW, Artavanis-Tsakonas S. The Notch signalling system: recent insights into the complexity of a conserved pathway. *Nat Rev Genet.* 2012; 13:654–66. [PubMed: 22868267]
2. Ilagan MX, Kopan R. SnapShot: Notch signaling pathway. *Cell.* 2007; 128:1246. [PubMed: 17382890]
3. Kopan R. Notch signaling. *Cold Spring Harb Perspect Biol.* 2012; 4
4. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. *Development.* 2011; 138:3593–612. [PubMed: 21828089]
5. Bradley A, et al. The mammalian gene function resource: the International Knockout Mouse Consortium. *Mamm Genome.* 2012; 23:580–6. [PubMed: 22968824]
6. Bucan M, Eppig JT, Brown S. Mouse genomics programs and resources. *Mamm Genome.* 2012; 23:479–89. [PubMed: 22968825]
7. White JK, et al. Genome-wide generation and systematic phenotyping of knockout mice reveals new roles for many genes. *Cell.* 2013; 154:452–64. [PubMed: 23870131]
8. Osterwalder M, et al. Dual RMCE for efficient re-engineering of mouse mutant alleles. *Nat Methods.* 2010; 7:893–5. [PubMed: 20953177]
9. Schnutgen F, et al. Resources for proteomics in mouse embryonic stem cells. *Nat Methods.* 2011; 8:103–4. [PubMed: 21278719]
10. Gridley T. Notch signaling in the vasculature. *Curr Top Dev Biol.* 2010; 92:277–309. [PubMed: 20816399]

11. Sternberg N, Hamilton D. Bacteriophage P1 site-specific recombination. I. Recombination between loxP sites. *J Mol Biol.* 1981; 150:467–86. [PubMed: 6276557]
12. Sauer B, Henderson N. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proc Natl Acad Sci U S A.* 1988; 85:5166–70. [PubMed: 2839833]
13. Gu H, et al. Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. *Science.* 1994; 265:103–6. [PubMed: 8016642]
14. Kwan KM. Conditional alleles in mice: practical considerations for tissue-specific knockouts. *Genesis.* 2002; 32:49–62. [PubMed: 11857777]
15. Orban PC, Chui D, Marth JD. Tissue- and site-specific DNA recombination in transgenic mice. *Proc Natl Acad Sci U S A.* 1992; 89:6861–5. [PubMed: 1495975]
16. Danielian PS, et al. Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. *Curr Biol.* 1998; 8:1323–6. [PubMed: 9843687]
17. Feil R, et al. Ligand-activated site-specific recombination in mice. *Proc Natl Acad Sci U S A.* 1996; 93:10887–90. [PubMed: 8855277]
18. Feil R, et al. Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. *Biochem Biophys Res Commun.* 1997; 237:752–7. [PubMed: 9299439]
19. Feil S, Valtcheva N, Feil R. Inducible Cre mice. *Methods Mol Biol.* 2009; 530:343–63. [PubMed: 19266339]
20. Hayashi S, McMahon AP. Efficient recombination in diverse tissues by a tamoxifen-inducible form of Cre: a tool for temporally regulated gene activation/inactivation in the mouse. *Dev Biol.* 2002; 244:305–18. [PubMed: 11944939]
21. Kellendonk C, et al. Regulation of Cre recombinase activity by the synthetic steroid RU 486. *Nucleic Acids Res.* 1996; 24:1404–11. [PubMed: 8628671]
22. Rose MF, et al. Excitatory neurons of the proprioceptive, interoceptive, and arousal hindbrain networks share a developmental requirement for Math1. *Proc Natl Acad Sci U S A.* 2009; 106:22462–7. [PubMed: 20080794]
23. Dymecki SM, Ray RS, Kim JC. Mapping cell fate and function using recombinase-based intersectional strategies. *Methods Enzymol.* 2010; 477:183–213. [PubMed: 20699143]
24. Meyers EN, Lewandoski M, Martin GR. An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. *Nat Genet.* 1998; 18:136–41. [PubMed: 9462741]
25. D'Souza B, Meloty-Kapella L, Weinmaster G. Canonical and non-canonical Notch ligands. *Curr Top Dev Biol.* 2010; 92:73–129. [PubMed: 20816393]
26. Skarnes WC, et al. A conditional knockout resource for the genome-wide study of mouse gene function. *Nature.* 2011; 474:337–42. [PubMed: 21677750]
27. Tu L, et al. Notch signaling is an important regulator of type 2 immunity. *J Exp Med.* 2005; 202:1037–42. [PubMed: 16230473]
28. Weng AP, et al. Growth suppression of pre-T acute lymphoblastic leukemia cells by inhibition of notch signaling. *Mol Cell Biol.* 2003; 23:655–64. [PubMed: 12509463]
29. Murray SA, et al. Beyond knockouts: cre resources for conditional mutagenesis. *Mamm Genome.* 2012; 23:587–99. [PubMed: 22926223]
30. Heffner CS, et al. Supporting conditional mouse mutagenesis with a comprehensive cre characterization resource. *Nat Commun.* 2012; 3:1218. [PubMed: 23169059]
31. Nagy A, Mar L, Watts G. Creation and use of a cre recombinase transgenic database. *Methods Mol Biol.* 2009; 530:365–78. [PubMed: 19266338]
32. Chandras C, et al. CreZOO--the European virtual repository of Cre and other targeted conditional driver strains. *Database.* 2012; 2012:bas029. [PubMed: 22730454]
33. Bao J, et al. Incomplete cre-mediated excision leads to phenotypic differences between Stra8-iCre; Mov10l1(lox/lox) and Stra8-iCre; Mov10l1(lox/Delta) mice. *Genesis.* 2013; 51:481–90. [PubMed: 23554062]
34. Cai T, et al. Conditional deletion of Atoh1 reveals distinct critical periods for survival and function of hair cells in the organ of Corti. *J Neurosci.* 2013; 33:10110–22. [PubMed: 23761906]

35. Reinert RB, et al. Tamoxifen-Induced Cre-loxP Recombination Is Prolonged in Pancreatic Islets of Adult Mice. *PLoS One*. 2012; 7:e33529. [PubMed: 22470452]
36. Park EJ, et al. System for tamoxifen-inducible expression of cre-recombinase from the *Foxa2* locus in mice. *Dev Dyn*. 2008; 237:447–53. [PubMed: 18161057]
37. Leone DP, et al. Tamoxifen-inducible glia-specific Cre mice for somatic mutagenesis in oligodendrocytes and Schwann cells. *Mol Cell Neurosci*. 2003; 22:430–40. [PubMed: 12727441]
38. Murtaugh LC, et al. Notch signaling controls multiple steps of pancreatic differentiation. *Proc Natl Acad Sci USA*. 2003; 100:14920–5. [PubMed: 14657333]
39. Varadkar PA, Kraman M, McCright B. Generation of mice that conditionally express the activation domain of Notch2. *Genesis*. 2009; 47:573–8. [PubMed: 19530136]
40. Oh P, et al. In vivo mapping of Notch pathway activity in normal and stress hematopoiesis. *Cell Stem Cell*. 2013; 13:190–204. [PubMed: 23791481]
41. Ong CT, et al. Target selectivity of vertebrate notch proteins. Collaboration between discrete domains and CSL-binding site architecture determines activation probability. *J Biol Chem*. 2006; 281:5106–19. [PubMed: 16365048]
42. Mizutani K, et al. Differential Notch signalling distinguishes neural stem cells from intermediate progenitors. *Nature*. 2007; 449:351–5. [PubMed: 17721509]
43. Souilhol C, et al. Nas transgenic mouse line allows visualization of Notch pathway activity in vivo. *Genesis*. 2006; 44:277–86. [PubMed: 16708386]
44. DasGupta R, Fuchs E. Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development*. 1999; 126:4557–68. [PubMed: 10498690]
45. Maretto S, et al. Mapping Wnt/beta-catenin signaling during mouse development and in colorectal tumors. *Proc Natl Acad Sci USA*. 2003; 100:3299–304. [PubMed: 12626757]
46. Ohtsuka T, et al. Visualization of embryonic neural stem cells using *Hes* promoters in transgenic mice. *Mol Cell Neurosci*. 2006; 31:109–22. [PubMed: 16214363]
47. Basak O, Taylor V. Identification of self-replicating multipotent progenitors in the embryonic nervous system by high Notch activity and *Hes5* expression. *Eur J Neurosci*. 2007; 25:1006–22. [PubMed: 17331197]
48. Imayoshi I, et al. Essential roles of Notch signaling in maintenance of neural stem cells in developing and adult brains. *J Neurosci*. 2010; 30:3489–98. [PubMed: 20203209]
49. Fre S, et al. Notch lineages and activity in intestinal stem cells determined by a new set of knock-in mice. *PLoS One*. 2011; 6:e25785. [PubMed: 21991352]
50. Sale S, Lafkas D, Artavanis-Tsakonas S. Notch2 genetic fate mapping reveals two previously unrecognized mammary epithelial lineages. *Nat Cell Biol*. 2013; 15:451–60. [PubMed: 23604318]
51. Nowotschin S, et al. A bright single-cell resolution live imaging reporter of Notch signaling in the mouse. *BMC Dev Biol*. 2013; 13:15. [PubMed: 23617465]
52. Vooijs M, et al. Mapping the consequence of Notch1 proteolysis in vivo with NIP-CRE. *Development*. 2007; 134:535–44. [PubMed: 17215306]
53. Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet*. 1999; 21:70–1. [PubMed: 9916792]
54. Smith E, et al. Generation and characterization of a Notch1 signaling-specific reporter mouse line. *Genesis*. 2012; 50:700–10. [PubMed: 22488965]
55. Govindarajan V, et al. Intracorneal positioning of the lens in Pax6-GAL4/VP16 transgenic mice. *Mol Vis*. 2005; 11:876–86. [PubMed: 16270027]
56. Liu Z, et al. Notch1 loss of heterozygosity causes vascular tumors and lethal hemorrhage in mice. *J Clin Invest*. 2011; 121:800–8. [PubMed: 21266774]
57. Liu Z, et al. Rapid identification of homologous recombinants and determination of gene copy number with reference/query pyrosequencing (RQPS). *Genome Res*. 2009; 19:2081–9. [PubMed: 19797679]
58. Liu Z, et al. The extracellular domain of Notch2 Increases Its cell-surface abundance and ligand responsiveness during kidney development. *Dev Cell*. 2013; 25:585–98.

59. Morimoto M, et al. Canonical Notch signaling in the developing lung is required for determination of arterial smooth muscle cells and selection of Clara versus ciliated cell fate. *J Cell Sci.* 2010; 123:213–24. [PubMed: 20048339]
60. Liu Z, et al. In vivo visualization of Notch1 proteolysis reveals the heterogeneity of Notch1 signaling activity in the mouse cochlea. *PLoS One.* 2013; 8:e64903. [PubMed: 23741415]
61. Luche H, et al. Faithful activation of an extra-bright red fluorescent protein in “knock-in” Cre-reporter mice ideally suited for lineage tracing studies. *Eur J Immunol.* 2007; 37:43–53. [PubMed: 17171761]
62. Saito M, et al. Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice. *Nat Biotechnol.* 2001; 19:746–50. [PubMed: 11479567]
63. Kraman M, McCright B. Functional conservation of Notch1 and Notch2 intracellular domains. *Faseb J.* 2005; 19:1311–3. [PubMed: 15897231]
64. Donahue LR, et al. Centralized mouse repositories. *Mamm Genome.* 2012; 23:559–71. [PubMed: 22945696]
65. Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.* 2013; 31:397–405. [PubMed: 23664777]
66. Wang H, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell.* 2013; 153:910–8. [PubMed: 23643243]

Fig. 1.

A sample conditional breeding strategy to generate conditional loss of function mutations in Notch pathway genes. GOI = Gene of interest, for which a null allele (GOI^{null}) conditional, “floxed” allele (GOI^{flox}) are available. The Cre driver allele is referred to as Driver^{Cre}. This mating scheme will produce approximately 25% offspring that carry a conditional loss-of-function mutation.

You searched for:

Query: notch1

12 strains(s) match your unfiltered search.

Filters:

Remove All Filters

State: embryo

State: Mutation: targeted mutation

8 item(s) match after applying filters

Export:
Filter by: State Type Provider Mutation
N	Strain Name	Synonyms	States	Repository	Mutation Types	Alleles	Genes
+	B6.129X1-Notch1 ^{tm2Rko} /GridJ		live	JAX Order	targeted mutation	Notch1 ^{tm2Rko} targeted mutation 2, Raphael Kopan	Notch1 notch 1
+	STOCK Gt(Rosa)26Sor ^{tm1(Notch1)Dam} /J		live	JAX Order	targeted mutation targeted mutation targeted mutation	Notch1 notch 1 Gt(Rosa)26Sor ^{tm1(Notch1)Dam} targeted mutation 1, Douglas A Melton Gt(Rosa)26Sor ^{tm1(Notch1)Dam} targeted mutation 1, Douglas A Melton	Notch1 notch 1 Gt(Rosa)26Sor gene trap ROSA 26, Philippe Soriano Notch1 notch 1
?	B6129S5-Notch1 ^{tm1Lex}		sperm	TAC Order	targeted mutation	Notch1 Notch gene homolog 1 (Drosophila)	Notch1 Notch gene homolog 1 (Drosophila)
?	ICR.B6CB- Mesp2 ^{tm5(Notch1)Ysa} /YsaRbrc		embryo	RBRC Order	targeted mutation	Mesp2 ^{tm5(Notch1)Ysa} targeted mutation 5 (Notch1), Yumiko Saga	Mesp2 mesoderm posterior 2

Fig. 2. Results of a search on the IMSR web site (www.findmice.org) for *Notch1*-related targeted mutations. Not all of the returned results are shown. The results show examples of live mice and cryopreserved sperm or embryos, available from the Jackson Laboratory (JAX), Taconic (TAC), and the RIKEN BioResource Center (RBRC).

Table 1

Notch reporter and Notch-Cre fusion lines available from strain repositories

Name ^a	Reporter/Cre	Repository	Stock Number	Reference
TNR	EGFP	JAX	18322	(41)
NAS	β-galactosidase	EMMA	2412	(42)
CBF:H2B-Venus	Venus-YFP	JAX	20942	(50)
N1::Cre^{LO}	Cre	JAX	6953	(51)

^aThis is the name used in the author's publications, and in this review. The repositories may have these strains listed under different names. We recommend searching repository stock lists with the stock number. JAX: Jackson Laboratory; EMMA: European Mouse Mutant Archive.