TECHNOLOGY

Kcnma1fl-tdTomato Conditional Knockout Mice

OVERVIEW

Kcnma1fl-tdTomato conditional mutant mice possess loxP sites flanking exon 2 and a tdTomato reporter (that only expresses upon cre-recombination) in the Kcnma1 (potassium large conductance calcium-activated channel, subfamily M, alpha member 1) gene and may be useful in generating conditional mutations to study the function of BK currents.

Kcnma1 (potassium large conductance calcium-activated channel, subfamily M, alpha member 1) encodes the pore-forming alpha subunit of the BK voltage and calcium-sensitive potassium channel (KCa1.1). BK channels regulate membrane potential and repolarization of action potentials and are widely expressed in neurons, smooth muscle, neuroendocrine, and non-excitable cells such as bone and kidney. Mutations in Kcnma1 are associated with paroxysmal non-kinesigenic dyskinesia (PKND3) and epileptic seizures, as well as neurodevelopmental disorder.

These conditional Kcnma1fl-tdTomato mice possess loxP sites flanking exon 2 and a 2A-tdTomato reporter in reverse orientation. Mice that are homozygous for this floxed allele are viable and fertile. When bred to mice that express tissue-specific Cre recombinase, resulting offspring will have exon 2 inverted and the 2A-tdTomato reporter brought in frame (and expressed) in cre-expressing tissues.

For example, when bred to mice with Cre recombinase expression in neurons of the central and peripheral nervous systems (for example, Tg(Nes-cre)1Kln and SM222a-Cre), homozygous knockout offspring were verified to lack BK currents in neurons of the suprachiasmatic nucleus and mesenteric artery smooth muscle cells.

The targeting construct was designed to insert a lox71 site upstream of exon 2, and an FRT-flanked neomycin resistance cassette in reverse orientation followed by 2A-tdTomato-polyA sequence also in reverse orientation and a lox66 site into the Kcnma1 gene on chromosome 14. The construct was electroporated into hybrid (C57BL/6 x 129S/SvEv)F1-derived BA1 embryonic stem (ES) cells. Correctly targeted ES cells were injected into blastocysts. The resulting chimeric males were crossed to B6.SJL-Tg(ACTFLPe)9205Dym/J to remove the neomycin cassette. Progeny were backcrossed to C57BL/6J for 11 generations and the FLP allele bred out. Upon arrival, mice were bred to C57BL/6J for at least 1 generation to establish the colony.

LICENSING POTENTIAL

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LICENSE STATUS

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CATEGORIES

- Research Tools, Antibodies, & Reagents

INVESTIGATOR(S)

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EXTERNAL RESOURCES

- Generation of Kcnma1fl-tdTomato, a conditional deletion of the BK channel ? subunit in mouse

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