

An isoform-specific allele of the sax-7 locus

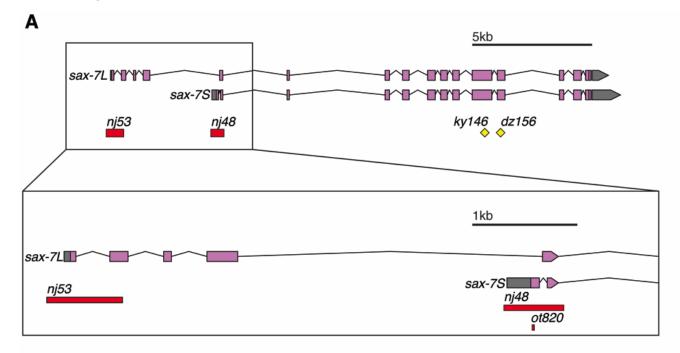
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В

sgRNA sequence PAM

ATG GGG TTA CGA GAG ACG ATG GGT CGT GGT GGA CCA CCG AAT ACC ACA ACC TTC ATA TTC Met Gly Leu Arg Glu Thr Met Gly Arg Gly Gly Pro Pro Asn Thr Thr Thr Phe Ile Phe

wt CTG CTA GGA TGT CTA CTG TTC CTT GTG gttagtttcatttcatttttctttttagggcacattgttctttcactatgttaatgaa Leu Leu Gly Cys Leu Leu Phe Leu Val

acggatttcaag TCG GAT CGC TAC TAC ACA TGT ACA GCC GAG AAC ATC GAA CTC AAG GAC TAC Ser Asp Arg Tyr Tyr Thr Cys Thr Ala Glu Asn Ile Glu Leu Lys Asp Try AAG TTT GGC AAT CAG TTT AGT ... Lys Phe Gly Asn Gin Phe Ser

ATG GGG TTA CGA GAG [------]TCG TGG TGG ACC ACC GAA TAC CAC AAC CTT CAT ATT Met Gly Leu Arg Glu Ser Trp Trp Thr Thr Glu Tyr His Asn Leu His Ile ot820 CCT GCT AGG ATG TCT ACT GTT CCT TGT G gttagtttcatttcattttctttttagggcacattgttctttcactatgttaat Pro Ala Ala Met Ser Thr Val Pro Cys gaaacggatttcaagTC GGA TCG CTA CTA CAC ATG TAC AGC CGA GAA CAT CGA ACT CAA GGA CTA Val Gly Ser Leu Leu His Met Tyr Ser Arg Glu His Arg Thr Gln Gly Leu CAA GTT TGG CAA TCA GTT TAG T ... Gln Val Trp Gln Ser Val *

Figure 1 : *ot820* is a novel isoform-specific allele of the *sax-7* locus. A. *sax-7* locus encodes two isoforms of an L1CAM homolog. Existing null alleles affect either the long isoform (*nj53*) or both isoforms (*nj48*, *ky146*, and *dz156*). *ot820* is a short deletion mutant that affects only the short isoform. B. sgRNA targets the beginning of the first exon of *sax-7S*, and *ot820* is a 8bp deletion 15bp from the start codon.



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Description

The immunoglobulin superfamily member *sax-7* produces a long and short isoform that appear to have distinct functions (Chen *et al.* 2001; Wang *et al.* 2005; Sasakura *et al.* 2005; Pocock *et al.* 2008). An isoform-specific allele for the long isoform, *sax-7L*, was previously reported (Sasakura *et al.* 2005), but an isoform-specific allele for the short isoform, *sax-7S*, has been lacking (Fig. 1A). We used CRISPR/Cas9 to generate such an allele, using the *unc-22* co-CRISPR method (Kim *et al.* 2014). The *ot820* allele was isolated using sgRNA targeted to the first exon of *sax-7S* (sgRNA sequece: 5' – TGGGGTTACGAGAGAGAGAT – 3'). Twitching progeny were screened by PCR and Sanger sequencing, and an 8bp deletion 15bp from the start codon was isolated (screening primers: 5' – GGTGCTTCTCTGGTGGTAGC – 3' and 5' – TGTTGGCAAACAAAATACACG – 3', Fig. 1B). While the *sax-7L* isoform is predicted to be entirely unaffected by this allele, the resultant frameshift is predicted to generate a 49 amino acid protein in which all but the first five amino acids of *sax-7S* are aberrant and has no predicted signal sequence, before terminating in a premature stop in the second exon (Fig. 1B). This allele therefore likely represents a null for the *sax-7S* isoform. After we generated this allele, a recent paper reported an additional *sax-7S*-specific allele, with somewhat similar, but not identical sequence properties (Chen et al., 2019). Consistent with previous evidence of distinct functions of *sax-7* isoforms, these authors showed differing axon fasciculation defects between these two alleles, which is further distinct from a total null allele. These new *sax-7S* alleles should help to reveal new insights into the role of L1CAM/SAX-7 isoforms in the nervous system.

Reagents

OH13830 *sax-7(ot820) IV; oyIs14 V*. Will be available at CGC.

References

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