***Admp$^{ioz20/+}$; im:7138239$^{ioz11/+}$* (AB) (CZRC catalog ID: CZ1245)**

Nature of mutation

This is a double mutant line, induced by the CRISPR-cas9 in the AB genetic background. Admp gene is located near the im:7138239 gene in the reverse strand of chromosome 2. 132-142 bp of the wildtype admp coding sequence is deleted. The mutated admp codes for a truncated protein containing 72 aa, 43 aa of which is identical to wildtype admp. -27-22 bp of the wildtype im:7138239 coding sequence is deleted. The transcription start site of the im:7138239 gene has been deleted.

Genotyping assay

Genotyping of this double mutant line can be performed via allele-specific PCR using allele-specific primers.

Primers 1:

Ioz20_forward: 5’ TCATGTTGTATGCAATGTTC 3’

Ioz20_reverse: 5’ GTGACTCCGTCGACATCAGC 3’

PCR program:

94℃ 4min

94℃ 30 sec

52℃ 30 sec } 30 Cycles

72℃ 30 sec

72℃ 5min

4℃ hold

Product size: 250 bp

Primers 2:

Ioz11_forward: 5’ GCTCTTTGATCTGCAGCCAT 3’

Ioz11_reverse: 5’ GTAGAATATGCAGTACTCAC 3’

PCR program:

94℃ 4min

94℃ 30 sec

52℃ 30 sec } 30 Cycles

72℃ 30 sec

72℃ 5min

4℃ hold

Product size: 201 bp