

**Table S4.** Genotype of 13 kDa prolamin-knockout lines (T<sub>2</sub> generation)

sgRNA	T <sub>2</sub> plants	Transgenic assay <sup>a</sup> <i>SpCas9/Hyg<sup>R</sup></i>	Edited gene	Frequency <sup>b</sup>	Type	Sequencing results
sgRNA-pro13a	WT	-/-			WT	TTGAAACGTAGCTGGTTGCCA...GAAGGGGGTT
	1a-8-1	-/-	<i>Pro13a.1</i>	99.50%	Ins (1 Nu)	*****A*****
			<i>Pro13a.2</i>	97.30%	Ins (1 Nu)	*****A*****
	2a-2-1	-/-	<i>Pro13a.1</i>	99.10%	Del (1 Nu)	*****-...*****
sgRNA-pro13b	WT	-/-			WT	GTTGAAACGCAGCTGATTGCA...AGAAGGTGCT
	4b-9-7	-/-	<i>Pro13b.1</i>	98.10%	Ins (1 Nu)	*****A*****
			<i>Pro13b.13</i>	99.80%	Ins (1 Nu)	*****A*****
	8b-3-9	-/-	<i>Pro13b.3</i>	97.80%	Del (4 Nu)	*****-----*****

<sup>a</sup>Symbol '+' and '-' indicate that *SpCas9/Hyg<sup>R</sup>* genes are present or absent, respectively

<sup>b</sup>Indel (Insertion & Deletion) frequency was calculated from Deep sequencing analysis

Red color dash and letter indicate the deletion and insertion of nucleotide, respectively

Green letters indicate the PAM sequence

Asterisks indicate the nucleotides with no change