

SUPPLEMENTARY MATERIALS

to the article S.V. Gerasimova, A.M. Korotkova, C. Hertig, S. Hiekel, R. Hoffie, N. Budhagatapalli, I. Otto, G. Hensel, V.K. Shumny, A.V. Kochetov, J. Kumlehn, E.K. Khlestkina "Targeted genome modification in protoplasts of a highly regenerable Siberian barley cultivar using RNA-guided Cas9 endonuclease"

Supplementary material 1

List of oligonucleotides

Oligonucleotide	Structure	Application
Hv_Nud_1exF	5'-AGCGTAGGTGTATACGCATCCG-3'	<i>Nud</i> exon1 amplification
Hv_Nud_1exR	5'-GAGTCGTAGGCCGGAGAGTAG-3'	<i>Nud</i> exon1 amplification, NGS
Hv_Vrs1_F1	5'-CCGATCACCTTCACATCTCC-3'	<i>Vrs1</i> exon1 amplification
Hv_Vrs1_R1	5'-CAGAATCTCGTCTGCTCGT-3'	
Hv_Nud_F4	5'-ACTGCCTGCTGATCATTCT-3'	NGS
Hv_Vrs1_1exF	5'-CAGAACAACCTACCGTGTCTC-3'	
Hv_Vrs1_1exR	5'-GCACATACAAACACAGAATGGA-3'	
Nud_ex1-50-F	5'-TGGCGTCTCTGGGTCTCCGAGATC-3'	Cloning
Nud_ex1-50-R	5'-AAACGATCTCGGAGACCCAGGAG-3'	
Nud_ex1-45-F	5'-TGGCGGAGACCCAGGAGCCCCAG-3'	
Nud_ex1-45-R	5'-AAACCTGGGGCTCCTGGGTCTCC-3'	
Vrs_ex1-33-F	5'-TGGCGTGGACACGACTTTCTTCG-3'	
Vrs_ex1-33-R	5'-AAACCGAAGAAAGTCGTGTCCAC-3'	
OsU3-1_GibF	5'-AAGCTCACGTGACGGAATTAAGCTTAGTAATTCATCCAGGTCACCAAG-3'	
OsU3-1_GibR	5'-TTATGTCCACTGGGTGGGGTCTCCGCCACGGATCATCTGCAC-3'	
OsU3-2_GibF	5'-GTGCAGATGATCCGTGGCGGAGACCCCAACCCAGTGGACATAAG-3'	
OsU3-2_GibR	5'-ACTGCAGGAATTCGATATCAAGCTTAGCAGCAAGCAGTATCGATC-3'	

Supplementary material 2

Nud_Aley_1	ATGGTACAGT	CCAAGAAGAA	GTTTCGCGGC	GTCAGGCAGC	<u>GCCA</u> CTGGG	CICCTGGGTC	TCCGAGATCA	<u>GG</u> CATCCTCT	CCT	83
Morex	ATGGTACAGT	CCAAGAAGAA	GTTTCGCGGC	GTCAGGCAGC	<u>GCCA</u> CTGGG	CICCTGGGTC	TCCGAGATCA	<u>GG</u> CATCCTCT	CCT	
Barke	ATGGTACAGT	CCAAGAAGAA	GTTTCGCGGC	GTCAGGCAGC	<u>GCCA</u> CTGGG	CICCTGGGTC	TCCGAGATCA	<u>GG</u> CATCCTCT	CCT	
Bowman	ATGGTACAGT	CCAAGAAGAA	GTTTCGCGGC	GTCAGGCAGC	<u>GCCA</u> CTGGG	CICCTGGGTC	TCCGAGATCA	<u>GG</u> CATCCTCT	CCT	
Consensus	ATGGTACAGT	CCAAGAAGAA	GTTTCGCGGC	GTCAGGCAGC	<u>GCCA</u> CTGGG	CICCTGGGTC	TCCGAGATCA	<u>GG</u> CATCCTCT	CCT	

Vrs1_Aley_1	ATGGACAAGC	ATCAGCTCTT	TGATTCATCC	AA <u>CGTGGACA</u>	CGACTTTCTT	CG <u>GGCCAA</u> T	GSTACACAG	ACGCCGCGCG	CGCCCGGTCT	TTGGCGATGC	GATGATGCAG	C	111
Bowman	ATGGACAAGC	ATCAGCTCTT	TGATTCATCC	AA <u>CGTGGACA</u>	CGACTTTCTT	CG <u>GGCCAA</u> T	GSTACACAG	ACGCCGCGCG	CGCCCGGTCT	TTGGCGATGC	GATGATGCAG	C	
Barke	ATGGACAAGC	ATCAGCTCTT	TGATTCATCC	AA <u>CGTGGACA</u>	CGACTTTCTT	CG <u>GGCCAA</u> T	GSTACACAG	ACGCCGCGCG	CGCCCGGTCT	TTGGCGATGC	GATGATGCAG	C	
Morex	ATGGACAAGC	ATCAGCTCTT	TGATTCATCC	AA <u>CGTGGACA</u>	CGACTTTCTT	CG <u>GGCCAA</u> T	GSTACACAG	ACGCCGCGCG	CGCCCGGTCT	TTGGCGATGC	GATGATGCAG	C	
Consensus	ATGGACAAGC	ATCAGCTCTT	TGATTCATCC	AA <u>CGTGGACA</u>	CGACTTTCTT	CG <u>GGCCAA</u> T	GSTACACAG	ACGCCGCGCG	CGCCCGGTCT	TTGGCGATGC	GATGATGCAG	C	

Alignment of *Nud* and *Vrs1* target gene fragments (cv. Aley) to reference sequences. Bars correspond to target sites, PAM motifs are underlined.