

## Supplementary material

# Multiplex CRISPR Mutagenesis of the Serine/Arginine-Rich (SR) Gene Family in Rice

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**Table S1.** Synthetic fragments used as sgRNAs for targeting single and multiple SR loci.

Target Loci	5'-Sequence-3'
<i>SR32</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAAAGATCC CTCCGAGGCCACC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SR33a</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAAAAATTC CTCCAAGGCCCTCC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SR33</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGAACCT CCCGGGCAGCATC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SR40</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAATAAGG ATATAGATCTTGC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SR32/SR33a/SR33/SR40</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAAAGATCC CTCCGAGGCCACC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAA AATTCCTCCAAGGCCCTCC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC AGTCCGGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGC CAGGAACCTCCCGGGCAGCATC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCAGTCCGGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGG TGGTGCAATAAGGATATAGATCTTGC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAA GTGGCACCAGTCCGGTGC TTTT TTTT
<i>RSZ21a</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGTGCAGC GCGCGGGAGCTCCG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>RSZ21</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGGATCCC CGAGTGACTCCG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>RSZ23</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGACCTG GATCCGCGGTGAG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>RSZ21a/RSZ21/RSZ23</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGTGCAGC GCGCGGGAGCTCCG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAG ATCCCGAGTGACTTCCG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC AGTCCGGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGC CAGAACCTGGATCCGGCCGTGAG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCAGTCCGGTGC TTTT TTTT
<i>SC25</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCACTCCGGC CGCTACGGCCAC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SC32</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAATGTCCG GCTTCGGCCGCTC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SC34</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCACTCCGGG AGTCCGGCCGCG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SC25/SC32/SC34</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCACTCCGGC CGCTACGGCCAC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAT GTCGGCTTCGGCCGCTC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC AGTCCGGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGC CACTCCGGAGTCCGGCCGCG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCAGTCCGGTGC TTTT TTTT
<i>SCL25</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGGACC TTCGTCGGCCATT GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SCL26</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGTCCCT GTGGCCGCTATGG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SCL28</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGGCGTA CCGGAGCCGAGC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC TTTT TTTT
<i>SCL25/SCL26/SCL28</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAAGGACC TTCGTCGGCCATT GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGTGC GGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGCAGT CCTCGTGGCGCTATGG GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC AGTCCGGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCCAGGCTGGTGC CACGGGTACCGGAGCCGAGC GTTTTAGAGCTAGAAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG CACCAGTCCGGTGC TTTT TTTT

Target Locus	5'-Sequence-3'
<i>SCL30a/SCL30</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGTCCCC CTAGGAGGGGATA GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GGTGC TTTT TTTT
<i>SCL57</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGGAGCCG CAGCCCCAGCAAG GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GGTGC TTTT TTTT
<i>SCL30a/SCL30/SCL57</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGTCCCC CTAGGAGGGGATA GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAG AGCCGACGCCACAGAG GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCG GAGTCGGTGC TTTT TTTT
<i>RS2Z36/RS2Z38</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGT TTTAA CTGTGGGATTGA GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GTGC TTTT TTTT
<i>RS2Z37</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAATTCCTCGC GCACCCGGACCC GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GTGC TTTT TTTT
<i>RS2Z39</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAACCCGGGA TGCAATGATGCG GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GGTGC TTTT TTTT
<i>RS2Z36/RS2Z37/RS2Z38/RS2Z39</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGT TTTAA CTGTGGGATTGA GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAATTC TCGCGCACCCGGACCC GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGA GTCCGTGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTG ACCCGGGATGCAATGATGCG GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGG ACCGAGTCGGTGC TTTT TTTT
<i>RS29</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGTATCCT TTTGCCCTGGG GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC TGC TTTT TTTT
<i>RS33</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGAGCCGC TCTTCAGCAAAATAGT TTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GTGC TTTT TTTT
<i>RS29/RS33</i>	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGTATCCT TTTGCCCTGGG GTTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC TGC AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTTCGATTCCC GGCTGGTGCAAGC GCCTTTCAGCAAAATAGT TTTAGAGCTAGAAATAGCAAGTAAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCG GTCCGTGC TTTT TTTT
U3 promoter	GACCATGATTACGCCAAGCTTAAGGAATCTTTAAACATACGAACAGATCACTTAAAGTTCTTCTGAAGCAACTTAAAG TTATCAGGCATGCATGGATCTTGAGGAATCAGATGTGCAGTCAGGGACCATAGCACAAAGACAGGCCTCTTACTG GTGCTACCAGCAAATGCTGGAAGCCGGGAACACTGGGTACGTTGGAACCACGTGATGGAAGAAAGTAAAGTAAACT GTAGGAGAAAAGCATTTCTAGTGGCCATGAAGCCTTTCAGGACATGATTGCGATGAGGCGGCCATTACGCA ATTGACGACAACAAAGACTAGTATTAGTACCACCTCGGCTATCCACATAGATCAAAGCTGATTTAAAGAGTTGTG CAGATGATCCGTGGCA

pre-tRNA: yellow background gRNA spacer: gray background Scaffold RNA: green background polIII terminator: red background

**Table S2:** List of oligos used for genotyping

Oligo Name	5'-Sequence-3'
SR32_F	GGGAAATAAGTAAGGCATGGC
SR32_R	GTAACCAGAACTGTGTGAAGTG
SR33a_F	CACTTGGAGAGATTCCACCAG
SR33a_R	CACCTCGCTACCTCCTATTC
SR33_F	GTGGTCCAGTTTGGGGTTCAG
SR33_R	GTAGCTGCTAGAAGCATAACAG
SR40_F	GTACTAAAGGATGGACCACTTG
SR40_R	GAATAGTTAGGTACCATGCG
RSZ21a_F	CTCTCGCTCCCCCTCTTAC
RSZ21a_R	GCCTGGATGCCATAGCTGGG
RSZ21_F	CAGCTTCAGATTTCTGACACC
RSZ21_R	CTTATAGTACAAGGGGTACC
RSZ23_F	GTACTCTCCAGATCCCCCGC
RSZ23_R	CCCAATCCCACCAGTAGAAC
SC25_F	GCCGCGTCATCTTCTCTTC
SC25_R	GAACGCGAACCCTCTCGAATC
SC32_F	GTGGCCACCCTCACCTAC
SC32_R	CAGCCGACCTAATCAGCTCCC
SC34_F	CTCCACTGCACACACTCGCTG
SC34_R	GAATCCCCTCGAGTCCCCAGTC
SCL25_F	CTTAAGCCGGTTATAGCGGCC
SCL25_R	CCCTTCTCTTCAACAGACTTG
SCL26_F	GCTTGTTTTATTCCCTACCAG
SCL26_R	CCTTACAGTTACGAACTTCTG
SCL28_F	ACCTCTCCTCGCCCAAGCC
SCL28_R	GTAAGCCCTAGATCAAATGGGC
SCL30a_F	GTACTTGAAGTTACCTATGGG
SCL30a_R	GGAACTCTAAGATCCTCCGC
SCL30_F	GTTGGCCCAACATATTTTCTG
SCL30_R	CTGCATAACATCAGAACAGC
SCL57_F	CTGCCTAGCAAGTAGAATACC
SCL57_R	CCAGACATGACAGAAGTTAGAC
RS2Z36_F	CCTTGATGGCAGGGATGTTG
RS2Z36_R	GACCGGCTGATATAAAGAATG
RS2Z37_F	CCCTAGCTTCTCTGTAGATC
RS2Z37_R	CAGCATGCTGCTGGCCTAGAC
RS2Z38_F	GGGATGTTGATGGAAGCCGC
RS2Z38_R	CTGGACCTAGATCGGCTGATAG
RS2Z39_F	CACAGACTAGTGGATGTTCCC
RS2Z39_R	CCCTCTCTCCACAGCCATAG
RS29_F	GCAGGTGCAGTACTCTTCTG
RS29_R	GTGGCCTCTTCTGTGTTTC
RS33_F	CCTAGCATTGGCTATGGTGG
RS33_R	CAAATGGAGTACCACTCGC

**Table S3:** Summary for genotyping of SR mutants

Target gene(s)	No. of plants genotyped	No. of plants mutated
<i>SR32</i>	20	1
<i>SR33a</i>	5	3
<i>SR33</i>	5	4
<i>SR40</i>	5	3
<i>SR32/SR33a/SR33/SR40</i>	10	None of the plant is homozygous mutant for all loci
<i>RSZ21a</i>	5	1
<i>RSZ21</i>	5	4
<i>RSZ23</i>	5	2
<i>RSZ21a/RSZ21/RSZ23</i>	10	None of the plant is homozygous mutant for all loci
<i>SC25</i>	5	4
<i>SC32</i>	5	5
<i>SC34</i>	5	3
<i>SC25/SC32/SC34</i>	10	None of the plant is homozygous mutant for all loci
<i>SCL25</i>	8	4
<i>SCL26</i>	5	4
<i>SCL28</i>	4	1
<i>SCL25/SCL26/SCL28</i>	10	None of the plant is homozygous mutant for all loci
<i>SCL30a/SCL30</i>	5	None of the plant is homozygous mutant for all loci
<i>SCL57</i>	5	5
<i>SCL30a/SCL30/SCL57</i>	5	None of the plant is homozygous mutant for all loci
<i>RS2Z36/RS2Z38</i>	5	1 double mutant
<i>RS2Z37</i>	5	5
<i>RS2Z39</i>	5	5
<i>RS2Z36/RS2Z37/RS2Z38/RS2Z39</i>	10	None of the plant is homozygous mutant for all loci
<i>RS29</i>	5	2
<i>RS33</i>	5	3
<i>RS29/RS33</i>	10	1 double mutant