

## Oligonucleotides and conditions for *S-RNase* allele-specific PCR-digestion method

<i>S-RNase</i> allele	Primers	Sense/ antisense	Nucleotide sequence (5' → 3')	Annealing temp (°C)	Band sizes (bp)	Restriction enzyme	Band sizes (bp) after digestion	Reference
<i>SI+S20+S24</i>	Sf-sense 2 Sf-antisense 2	Sense Antisense	ATTAATCTGCCTCGCACTTG TTGGTGGGGCAGAAATTCC	56 or 62	1047 ( <i>S1</i> ) 1021 ( <i>S20</i> ) 1040 ( <i>S24</i> )	<i>RsaI</i>	337 <sup>z</sup> ( <i>S1</i> ) 122 <sup>z</sup> , 78 <sup>z</sup> ( <i>S20</i> ) 540 <sup>z</sup> ( <i>S24</i> )	Matsumoto et al., 1999b; Kitahara et al., 2000
<i>S2</i>	OWB122 OWB123	Sense Antisense	GTTCAAACGTGACTTATGCG GGTTTGGTCCTTACCATGG	64	449	<i>EcoRV</i>	349+100	Broothaerts et al., 1995
<i>S3</i>	OWB134 OWB145	Sense Antisense	ATGGATCGAATTCTTGACAAAYATTA TTCAATG TAGAATTCATTTGCTAGGGACATCGATC	56	375	<i>PstI</i>	226+149	Broothaerts et al., 1995
<i>S4+S16a</i> + <i>S16b+S16c</i>	FTC5 OWB249	Sense Antisense	ATGAATTCCCACAATACAGAACGAGA ATGAATTCAATCTATGAAATGTGCTCTG	58	278	<i>TaqI</i>	195+83 ( <i>S4</i> ) 241+37 ( <i>S16a, c</i> ) 197+44+37 ( <i>S16b</i> ) Nerum et al., 2001; Matsumoto and Furusawa, 2005	Verdoordt et al., 1998; Van
<i>S5+S33+S34</i>	S5-sense S5-antisense	Sense Antisense	TATCCTATCCAAGCCAATATCAAT CATCCTATATATGGATAATGGCAACCG	56	363 ( <i>S5</i> ) 319 ( <i>S33</i> ) 330 ( <i>S34</i> )	<i>EcoRI</i> <i>Sau3AI</i>	235+128 ( <i>S5</i> ) 235+84 ( <i>S33</i> ) 235+95 ( <i>S34</i> ) 363 ( <i>S5</i> ) 208+111 ( <i>S33</i> ) 330 ( <i>S34</i> )	Matsumoto et al., 1999a, 2010
<i>S6a+S17</i> ( <i>S28</i> )	FTQQYQ <sup>Y</sup> Anti- <sup>1</sup> /MWPNV <sup>Y</sup>	Sense Antisense	TTTACGCAGCAATATCAG ACGTTCGGCCAAT <sup>A/c</sup> ATT	48	367 369 ( <i>S28</i> )	<i>MluI</i>	367 ( <i>S6a</i> ) 251+116 ( <i>S17</i> ) 369 ( <i>S28</i> )	Morita et al., 2009 De Franceschi et al., 2018
<i>S7</i>	OWB126 OWB127	Sense Antisense	GCCTTCAGACTCGAATGGACA TGGCATTACAAATATCTACC	52	440	<i>AccI</i>	228+212	Janssens et al., 1995; Matsumoto et al., 1999a
<i>S9</i>	OWB154 OWB155	Sense Antisense	CAGCCGGCTGCTGCCACTT CGGTTCGATCGAGTACGTTG	62	343	<i>EcoRI</i>	212+131	Janssens et al., 1995;
<i>S10+S3</i>	Si-sense Si-antisense	Sense Antisense	AACAAATCTAACGCCAGC GGTTCTTATAGTCGATACTTG	60	282	<i>EheI</i>	185+97 ( <i>S10</i> )	Kitahara and Matsumoto, 2002a
<i>SI+S20+S24</i>	Sf-sense 3 Sf-antisense 2	Sense Antisense	ACGATCATGAAGGCTTCTGGCG TTGGTGGGGCAGAAATTCC	56	370	<i>SnaBI</i>	204+164 ( <i>S24</i> )	Kitahara et al., 2000
<i>S11+S21+S30</i>	FTQQYQ <sup>Y</sup> Anti- <sup>1</sup> /MWPNV <sup>Y</sup>	Sense Antisense	TTTACGCAGCAATATCAG ACGTTCGGCCAAT <sup>A/c</sup> ATT	48	373 ( <i>S11</i> ) 375 ( <i>S21</i> ) 378 ( <i>S30</i> )	<i>MluI</i>	373 ( <i>S11</i> ) 257+118 ( <i>S21</i> ) 378 ( <i>S30</i> )	Morita et al., 2009
<i>S20a+S20b</i>	FTQQYQ <sup>Y</sup> Anti- <sup>1</sup> /MWPNV <sup>Y</sup>	Sense Antisense	TTTACGCAGCAATATCAG ACGTTCGGCCAAT <sup>A/c</sup> ATT	48	512 ( <i>S20a</i> ) 514 ( <i>S20b</i> )	<i>NsiI</i> <i>SnaBI</i>	267+245 ( <i>S20a</i> ) 514 ( <i>S20b</i> ) 512 ( <i>S20a</i> ) 315+199 ( <i>S20b</i> )	Matsumoto et al., 2001
<i>S23</i>	S10-sense S10-antisense	Sense Antisense	CAAGGATCCTCCTGCCAAG CCAGAAGACCAAATGATTGG	60	327	<i>HaeIII</i>	246+42+39	Schneider et al., 2001
<i>S25</i>	Sz-sense Sz-antisense	Sense Antisense	TTGTCTCGTCCACTGTGGG GTAACATCCAAGGTTGTGTT	60	198	<i>BamHI</i>	96+92	Kitahara and Matsumoto, 2002b
<i>S26</i>	FTC14 FTC9	Sense Antisense	GAAGATGCCATACGCAATGG ATGAATTCTTAATACCGAATATTGGCG	55	193	<i>KpnI</i>	170+23	Verdoordt et al., 1998
<i>S28</i>	Se-sense Se-antisense	Sense Antisense	AAACGTCTCTGCATTCTCG ATCGTATCGCTTGTTGGTGGT	60	227	<i>KpnI</i>	136+91	Matsumoto and Kitahara, 2000
<i>S30+S21</i>	St-sense St-antisense	Sense Antisense	CAATAGATAACGAGAACAC CAATCTATGAAATGTTCTCC	48	259	<i>RsaI</i>	214+45 ( <i>S30, S21</i> )	Matsumoto et al., 2003
<i>S31</i>	AS31-SPF1 AS31-SPR1	Sense Antisense	ATGGGGACGGGGATGATATATG CAGTCTCCGGCTTTCTACC	60	757			Kim et al., 2008
<i>S32</i>	AS32-SPF1 AS32-SPR1	Sense Antisense	AACTTTTAGGACCTGACCCA TCTCTCCGTGTCCACTTTT	60	451			Kim et al., 2008

Note: <sup>z</sup>Specific band(s) are shown.

<sup>Y</sup>Other *S-RNase* alleles are amplified in different sizes.

## References

- Broothaert, M., Janssens, G.A., Proost, P., Broekaert, W.F., 1995. cDNA cloning and molecular analysis of two self-incompatibility alleles from apple. *Plant Mol Biol*, 27: 499–511.
- De Franceschi, P.D., Bianco, L., Cestaro, A., Dondini, L., Velasco, R., 2018. Characterization of 25 full-length S-RNase alleles, including flanking regions, from a pool of resequenced apple cultivars. *Plant Mol Biol*, <https://doi.org/10.1007/s11103-018-0741-x>
- Janssens, G.A., Goderis, I.J., Broekaert, W.F., Broothaerts, W., 1995. A molecular method for *S*-allele identification in apple based on allele-specific PCR. *Theor Appl Genet*, 91: 691–698.
- Kim, H., Park, J., Hirata, Y., Nou, I., 2008. Molecular characterization of new *S-RNases* ('S<sub>31</sub>' and 'S<sub>32</sub>') in apple (*Malus ×domestica* Borkh.). *J Plant Biol*, 51: 202–208.
- Kitahara, K., Soejima, J., Komatsu, H., Fukui, H., Matsumoto, S., 2000. Complete sequences of the *S*-genes, Sd-and Sh-RNase cDNA in apple. *HortScience*, 35: 712–715.
- Kitahara, K., Matsumoto, S., 2002a. Sequence of the *S<sub>10</sub>* cDNA from 'McIntosh' apple and a PCR-digestion identification method. *HortScience*, 37: 187–190.
- Kitahara, K., Matsumoto, S., 2002b. Cloning of the *S<sub>25</sub>* cDNA from 'McIntosh' apple and an *S<sub>25</sub>*-allele identification method. *J Hort Sci Biotech*, 77: 724–728.
- Matsumoto, S., Komori, S., Kitahara, K., Imazu, S., Soejima, J., 1999a. S-genotypes of 15 apple cultivars and self-compatibility of 'Megumi'. *J Japan Soc Hort Sci*, 68: 236–241.
- Matsumoto, S., Kitahara, K., Komori, S., Soejima, J., 1999b. A new *S*-allele in apple, 'Sg', and its similarity to the 'Sf' allele from 'Fuji'. *HortScience*, 34: 708–710.
- Matsumoto, S., Kitahara, K., 2000. Discovery of a new self-incompatibility allele in apple. *HortScience*, 35: 1329–1332.
- Matsumoto, S., Kitahara, K., Komatsu, H., Soejima, J., 2001. A functional *S*-allele, 'Sg', in the wild apple possessing a single amino acid, S-RNase 'Sg'-RNase', different from 'Sg-RNase' in *Malus ×domestica* cultivars. *J Hort Sci Biotech*, 76: 163–166.
- Matsumoto, S., Furusawa Y., Kitahara, K., Soejima, J., 2003. Partial genomic sequences of *S<sub>6</sub>*-, *S<sub>12</sub>*-, *S<sub>13</sub>*-, *S<sub>14</sub>*-, *S<sub>17</sub>*-, *S<sub>19</sub>*-, and *S<sub>27</sub>*-RNases of apple and their allele designations. *Plant Biotech*, 20: 323–329.
- Matsumoto, S., Furusawa, Y., 2005. Genomic DNA sequence of *S<sub>16c(=16)</sub>*-RNase in apple: re-numbering of *S<sub>16(=27a)</sub>*- and *S<sub>22(=27b)</sub>*-allele to *S<sub>16a</sub>* and *S<sub>16b</sub>*. *Sci Rep Fac Educ Gifu Univ (Nat Sci)*, 29: 7–12.
- Matsumoto, S., Yamada, K., Shiratake, K., Okada, K., Abe, K., 2010. Structural and functional analyses of two new *S-RNase* alleles, *S<sub>si5</sub>* and *S<sub>ad5</sub>*, in apple. *J Hort Sci Biotech*, 85: 131–136.
- Morita, J., Abe, K., Matsumoto, S., 2009. *S-RNase* genotypes of apple cultivars grown in Japan and development of a PCR-RFLP method to identify the *S<sub>6</sub>*- and *S<sub>27</sub>*-RNase alleles. *J Hort Sci Biotech*, 84: 29–34.
- Schneider, D., Stern, R.A., Eisikowitch, D., Goldway, M., 2001. Analysis of *S*-alleles by PCR for determination of compatibility in the 'Red Delicious' apple orchard. *J Hort Sci Biotech*, 76: 596–600.
- Van Nerum, I., Geerts, M., Van Haoute, A., Keulemans, J., Broothaerts, W., 2001. Re-examination of the self-incompatibility genotype of apple cultivars containing putative 'new' *S*-alleles. *Theor Appl Genet*, 103: 584–591.
- Verdoodt, L., Van Haute, A., Goderis, I.J., De Witte, K., Keulemans, J., Broothaerts, W., 1998. Use of the multi-allelic self-incompatibility gene in apple to assess homozygosity in shoots obtained through haploid induction. *Theor Appl Genet*, 96: 294–300.