Supplementary material 1

The CP-coding region is dispensable for RNA1-mediated S-RNAi suppression

Results

To examine whether the CP-coding region is required for RNA1-mediated S-RNAi suppression, we used pBICRC1-ΔCP (Supplementary figure 1A), in which the CP gene was precisely deleted from pBICRC1. Patches receiving pBICGFP + pBICRC1-ΔCP showed bright green fluorescence (Supplementary figure 1B). The accumulation of GFP mRNA was higher in patches receiving pBICGFP + pBICRC1-ΔCP than in those receiving pBICGFP + pBICRC1, and the accumulation of GFP-specific siRNAs in patches receiving pBICGFP + pBICRC1-ΔCP was similar to that in those receiving pBICGFP + pBICRC1 (Supplementary figure 1C). These results indicate that the CP-coding region is not essential for RNA1-mediated S-RNAi suppression.

Materials and methods

Construction of pBICRC1-ΔCP: A PCR fragment was amplified from pUCR1 using the primers CPAD+ and M4. The 0.1 kb BglII–SacII fragment of the amplified DNA was substituted for the small BglII–SacII fragment of pUCR1 to construct pUCR1-ΔCP. pUCR1-ΔCP was digested with XhoI, and then partially digested with KpnI to yield the 0.9 kb XhoI–KpnI fragment. The 0.9 kb fragment was inserted into pBICRC1 previously digested with XhoI and KpnI to create pBICRC1-ΔCP. Sequences of the
primers used are listed in supplementary table I.

Figure legends

Supplementary figure 1 The CP-coding region is not essential for S-RNAi suppression.

(A) Schematic representation of pBICRC1-ΔCP, in which the CP gene is precisely deleted from pBICRC1. (B) Leaves of line 16c were viewed under UV light at 4 dpi.

(C) Northern blot analysis of GFP mRNA (top) and GFP-specific siRNAs (middle) extracted from agro-infiltrated patches.
Takeda et al.,
EMBO J.
Supplementary figure 1

A

pBICRC1

\[
\begin{align*}
\text{P} & \quad \text{p27} \quad \text{p88} \\
\text{pBICRC1-\Delta CP} & \quad \text{p27} \quad \text{p88}
\end{align*}
\]

CP

B

GFP +

Vector  RC1  RC1-\Delta CP

C

GFP +

Vector  RC1  RC1-\Delta CP

GFP mRNA
GFP siRNAs
rRNA
Supplementary material 2

Materials and methods

Construction of the plasmids for agroinfiltration, in vitro transcription and transient expression in protoplasts.

The plasmids containing the prefix ‘pBIC’ were used for agroinfiltration. pBICP35 is the empty vector, which encodes transcription cassettes containing the 35S promoter and terminator of Cauliflower mosaic virus (Mori et al., 1993). pBICGFP has a GFP gene, which was used as an inducer of S-RNAi, and pBICNSs has a TSWV NSs gene, which suppresses S-RNAi (Takeda et al., 2002). pBICRC1 and pBICRC2 encode wild-type RNA1 and RNA2, respectively, of RCNMV Australian strain (Xiong and Lommel, 1991). pBICRC1, pBICRC2 and their derivatives have the ribozyme sequence of a satellite RNA of Tobacco ringspot virus (Kaido et al., 1997) to minimize extra nonviral nucleotides at the 3′ UTR of their transcripts. pBICp27, pBICp57, pBICp88 and pBICRCCP encode p27, p57, p88 and CP genes, respectively, of RCNMV. pBICp88-GVD encodes p88 with a mutation in the GDD motif. pBICRC1-p27fs has a frameshift mutation in the p27 gene of pBICRC1. pBICRC1-p27 has mutations leading to the loss of p88 expression, and pBICRC1-p88 has mutations leading to the loss of p27 expression without affecting its p88 expression (see Xiong et al., 1993). pBICRC2-MPfs has a frameshift mutation in the MP gene of pBICRC2. In pBICRC2-5D, the 5′ UTR was substituted with a nonviral 5′ leader (5′-GGGAAGGAGATATAACA-3′). In
pBICRC2-3D, a stem-loop structure required for RCNMV replication (Turner and Buck, 1999) was deleted. pBICRC2(−) encodes negative-strand RNA2 of RCNMV.

pBICdsGFP, from which a GFP hairpin dsRNA is expressed, was used as an IR-RNAi inducer. pBICp19 encodes a Tomato bushy stunt virus p19 gene, which suppresses IR-RNAi (Takeda et al., 2002). pBICmiR171prec encodes a precursor of miR171 (Parizotto et al., 2004). The plasmids containing the prefix ‘pUCR’ or ‘pRC’ were used as templates for in vitro transcription. pUCR1 and pRC2|G encode wild-type RNA1 and RNA2, respectively, of RCNMV Australian strain (Xiong and Lommel, 1991). pUCR1-p27 and pUCR1-p88 have mutations corresponding to those of pBICRC1-p27 and pBICRC1-p88, respectively. pRC2|G-MPfs (pRNA2fsMP in Tatsuta et al., 2005) has a frameshift mutation in the MP gene. The plasmids containing the prefix ‘pUB’ were used as inocula for transient gene expression in protoplasts. pUBR1-p27 and pUBR1-p88 have mutations corresponding to those of pBICRC1-p27 and pBICRC1-p88, respectively. pUBp27 and pUBp88 encode the p27 and p88 genes, respectively.

The regions derived from PCR, those yielded by partial digestion or those treated with T4 DNA polymerase were sequenced. Sequences of the primers used are listed in supplementary table I. pRC1|G and pRC2|G contain the full-length cDNAs of wild-type RNA1 and RNA2, respectively, of RCNMV Australian strain (Xiong and Lommel, 1991). The small SacI–SmaI fragment of pUC118 (Takara) was replaced with the 3.9 kb SacI–SmaI fragment of pRC1|G to create pUCR1. The small EcoRI–HindIII fragment of pBE2113-GUS (Mitsuhara et al., 1996) was replaced with the small EcoRI–HindIII fragment of pUC18 (Takara) to create pBIC18. pBICBPBR2R (Kaido et al., 1997) was
digested with *Pst*I, treated with T4 DNA polymerase, and then digested with *Eco*RI, yielding 0.06 kb *Eco*RI–*Pst*I fragment which contains the ribozyme sequence. pBICP35 (Mori *et al.*, 1993) was digested with *Kpn*I, treated with T4 DNA polymerase, and then digested with *Eco*RI, yielding 12.5 kb *Eco*RI–*Kpn*I fragment. Then the resulting 0.06 and 12.5 kb fragments were ligated to construct pBICP35R. pBluescript II SK(+) (Stratagene) was digested with *Sac*I and *Bam*HI, treated with T4 DNA polymerase and then self-ligated to construct pBS-ΔSB. The 0.6 kb *Hind*III–*Sall*I fragment of pBICP35 was cloned into a modified pUC19 (Takara), in which the cloning sites from *Eco*RI to *Bam*HI had been eliminated, to construct pUBP35.

**pBICRC1** A PCR fragment was amplified from pBICP35R using the primers Sa35+ and 35/RC1–, and another was amplified from pUCR1 using the primers 35/RC1+ and S/R1int3'P. Then a PCR fragment was amplified from a mixture of these two PCR fragments using the primers Sa35+ and S/R1int3'P, and the *Sac*I–*Eco*RI fragment of the amplified DNA was substituted for the small *Sac*I–*Eco*RI fragment of pUCR1/Neo (Mizumoto *et al.*, 2003) to construct pRCP35A1. A PCR fragment was amplified from pBICP35R using the primers RC1/Rt+ and RtSm+, and another was amplified from pUCR1 using the primers S/R1int5'P and RC1/Rt–. Then a PCR fragment was amplified from a mixture of these two PCR fragments using the primers S/R1int5'P and RtSm+, and the *Sac*II–*Sma*I fragment of the amplified DNA was substituted for the small *Sac*II–*Sma*I fragment of pRCP35A1 to construct pUBRC1. The 4.6 kb *Sac*I–*Sma*I fragment of pUBRC1 was inserted into pBIC18 previously digested with the same enzymes to create pBICRC1.
**pBICRC2** A PCR fragment was amplified from pBICP35R using the primers Ps35+ and 35/RC2−, and another was amplified from pRC2|G using the primers 35/RC2+ and S/R2int3’P. Then a PCR fragment was amplified from a mixture of these two fragments using the primers Ps35+ and S/R2int3’P, and the PstI–NheI fragment of the amplified DNA was substituted for the small PstI–NheI fragment of pRNA2fsMP (Tatsuta et al., 2005) to construct pRCP35A2. A PCR fragment was amplified from pBICP35R using the primers RC2/Rt+ and RtSm+, and another was amplified from pRC2|G using the primers Ps-Hi/Aus2-5’ and RC2/Rt−. Then a PCR fragment was amplified from a mixture of these two PCR fragments using the primers Ps-Hi/Aus2-5’ and RtSm+, and the NcoI–SmaI fragment of the amplified DNA was substituted for the small NcoI–SmaI fragment of pRCP35A2 to construct pUBRC2. The HindIII–SmaI fragment of pUBRC2 was inserted into pBIC18 previously digested with the same enzymes to create pBICRC2.

**pUBp27 and pBICp27** A PCR fragment was amplified from pUCR1 using the primers p27-5Sma and p27-3Kpn. The 0.7 kb SmaI–KpnI fragment of the amplified DNA was inserted into pUBP35 and pBICP35, which had been digested with SruI and KpnI, to create pUBp27 and pBICp27, respectively.

**pBICp57** A PCR fragment was amplified from pUCR1 using the primers p57-5Sma and p57-23BK. The 0.3 kb SmaI–KpnI fragment of the amplified DNA was inserted into pBS-ΔSB previously digested with the same enzymes to create pBSp57-PS. The 1.4 kb PstI–BglII fragment of pUCR1 was substituted for the small PstI–BglII fragment of pBSp57-PS to construct pBSp57. The 1.5 kb SmaI–KpnI fragment of
pBSp57 was inserted into pBICP35 previously digested with Stul and KpnI to create pBICp57.

*pUBp88 and pBICp88* Two PCR fragments were amplified from pUCR1 using two sets of primers, p27-5Sma plus Ausp88–, and Ausp88+ plus Aus137L. Then a PCR fragment was amplified from a mixture of these two PCR fragments using the primers p27-5Sma and Aus137L, and the 1.3 kb Smal–HindIII fragment of the amplified DNA was substituted for the small Smal–HindIII fragment of pBSp57 to construct pBSp88. The 2.4 kb Smal–KpnI fragment of pBSp88 was inserted into pUBP35 and pBICP35, which had been digested with Stul and KpnI, to create pUBp88 and pBICp88, respectively.

*pBICRCCP* A PCR fragment was amplified from pUCR1 using the primers RCCP-5Sma and RCCP-3Kpn. The 1.0 kb Smal–KpnI fragment of the amplified DNA was inserted into pBICP35 previously digested with Stul and KpnI to create pBICRCCP.

*pBICp88-GVD* Two PCR fragments were amplified from pUCR1 using two sets of primers, p88-156R plus p88-GVD-L, and p88-GVD-R plus p88-186L. Then a PCR fragment was amplified from a mixture of these two PCR fragments using the primers p88-156R and p88-186L, and the 0.2 kb Stul–Xhol fragment of the amplified DNA was substituted for the corresponding fragment of pBICp88 to construct pBICp88-GVD.

*pBICRC1-p27fs* pBICRC1 was digested with EcoRI, treated with T4 DNA polymerase, and then religated to construct pBICRC1-p27fs. The orientation of the religated fragments was checked by DNA sequencing.

*pUCR1-p27* Two PCR fragments were amplified from pUCR1 using two sets of
primers, 5'RS1T7 plus Ausp27–, and Ausp27+ plus AC1–2520. Then a PCR fragment
was amplified from a mixture of these two PCR fragments using the primers 5'RS1T7
and AC1–2520, and the 0.6 kb Th111I–BsiWI fragment of the amplified DNA was
substituted for the corresponding fragment of pUCR1 to construct pUCR1-p27.

pUCR1-p88 Two PCR fragments were amplified from pUCR1 using two sets of
primers, 5'RS1T7 plus Ausp88–, and Ausp88+ plus AC1–2520. Then a PCR fragment
was amplified from a mixture of these two PCR fragments using the primers 5'RS1T7
and AC1–2520, and the 0.6 kb Th111I–BsiWI fragment of the amplified DNA was
substituted for the corresponding fragment of pUCR1 to construct pUCR1-p88.

pBICRC1-p27 and pBICRC1-p88 The 1.0 kb SalI–HindIII fragments of pUCR1-p27 and pUCR1-p88 were ligated with the largest SalI–HindIII fragment of pBICRC1
to construct pBICRC1-p27-PS and pBICRC1-p88-PS, respectively. The 2.6 kb HindIII-
digested fragment of pBICRC1 was inserted into pBICRC1-p27-PS and pBICRC1-p88-
PS, which had been digested with HindIII, to create pBICRC1-p27 and pBICRC1-p88,
respectively. The orientation of the inserted fragments were checked by DNA
sequencing.

pBICRC2-MPfs A PCR fragment was amplified from pUBRC2 using the primers
M4 and 35/RC2–, and another was amplified from pRNA2fsMP (Tatsuta et al, 2005)
using the primers 35/RC2+ and D3'H-1–. Then a PCR fragment was amplified from a
mixture of these two PCR fragments using the primers M4 and D3'H-1–, and the
EcoRV–NheI fragment of the amplified DNA was substituted for the corresponding
fragment of pUBRC2 to construct pRCP35A2RT-MPfs. The HindIII–SmaI fragment of
pUBRC2-MPs was inserted into pBIC18 previously digested with the same enzymes to create pBICRC2-MPs.

**pBICRC2-5D** pBICRC2 was digested with SacI, treated with T4 DNA polymerase, and then digested with XbaI, yielding 0.7 kb XbaI–SacI fragment. pBICRCMP was digested with HindIII, treated with T4 DNA polymerase, and then digested with XbaI, yielding 13.1 kb XbaI–HindIII fragment. Then the resulting 0.7 and 13.1 kb fragments were ligated to construct pBICRC2-5D.

**pBICRC2-3D** Two PCR fragments were amplified from pBICRC2 using two sets of primers, RCMP81R plus RC2-3D–, and RC2-3D+ plus BICEco. Then a PCR fragment was amplified from a mixture of these two fragments using the primers RCMP81R and BICEco, and the XbaI–SmaI fragment of the amplified DNA was substituted for the small XbaI–SmaI fragment of pBICRC2 to construct pBICRC2-3D.

**pBICmiR171prec** Genomic DNA was isolated from the leaf tissue of *A. thaliana* accession Col-0 by using DNeasy plant Mini Kit (QIAGEN) according to manufacture’s instruction. A PCR fragment was amplified from the prepared genomic DNA using the primers miR171-5SS and miR171-3Kpn. Then, SacI–KpnI fragment of the amplified DNA was inserted into pBluescript II SK(+) previously digested with the same enzymes to create pBSmiR171. A PCR fragment was amplified from pBSmiR171 using the primers miR171prec-5Stu and miR171prec-3Kpn. Then, Stul–KpnI fragment of the amplified DNA was inserted into pBICP35 previously digested with the same enzymes to create pBICmiR171prec.

**pBICRC2(−)** The 1.5 kb PstI–SmaI fragment of pRNA2fsMP (Tatsuta *et al*, 2005)
was treated with T4 DNA polymerase, and then inserted into pBICP35 previously
digested with Stul to construct pBICRC2(−). The orientation of the inserted fragment
was checked by DNA sequencing.

5  **S-RNAi suppression assay**

An S-RNAi suppression assay using *A. tumefaciens* GV3101(pMP90) and *N.
benthamiana* line 16c was performed as described previously (Takeda *et al.*, 2002). The
concentration of *Agrobacterium* in all infiltration experiments was normalized to 0.8
OD at 600 nm. In the S-RNAi suppression assay, the *Agrobacterium* with pBICGFP was
used in an equal concentration (0.4 OD at 600 nm). GFP fluorescence was detected as
described previously (Takeda *et al.*, 2002) using a Cyber-shot DSC-F828 digital camera
(Sony) to take photographs at 4 dpi. Isolation of total RNA from *Agrobacterium-
infiltrated leaves and northern blot analysis for GFP mRNA and GFP-specific siRNAs
were performed as described previously (Takeda *et al.*, 2002). Radioactive signals were
detected using a FUJIX BAS2500 phosphorimager.

17 **IR-RNAi suppression assay**

The IR-RNAi suppression assay was performed essentially as described
previously (Takeda *et al.*, 2002). In this assay, we used wild-type *N. benthamiana.*
*Agrobacterium* cultures were infiltrated in combinations at the following concentrations:
pBICGFP, pBICP35 or pBICp19, 0.32 OD at 600 nm; pBICdsGFP, pBICRC1 or
pBICRC2, 0.16 OD at 600 nm. RNA analysis was performed as described for the S-
RNAi suppression assay.

Transient miRNA assay

*Agrobacterium* cultures were infiltrated in combinations at the following concentrations: pBICmiR171prec, 0.68 OD at 600 nm; pBICRC1 or pBICRC2, 0.06 OD at 600 nm; pBICP35, variable. The concentration of *Agrobacterium* mixtures in the transient miRNA assay was normalized to 0.8 OD at 600 nm by varying the concentration of *Agrobacterium* containing pBICP35. Infiltrated patches were harvested at 2 dpi, total RNA was isolated from the patches, and 15 μg of total RNA was used for detection of miR171. To detect miR171, an oligonucleotide, miR171 probe (See Supplementary table I), which is complementary to miR171 was end-labeled with $^{32}$P and used as a probe.

Inoculation of protoplasts

Protoplast experiments using BY-2 suspension-cultured cells were performed as described elsewhere (Watanabe *et al*, 1987) with modifications as follows. In the incubation medium used to prepare BY-2 protoplasts, 20 mM MgCl$_2$ was added, and 0.1% pectolyase Y-23 (Seishin Pharmaceutical) was used instead of driserase. Disposable 4 mm gap cuvettes (BTX) and a Pulse Controller Plus (Bio-Rad) were used for electroporation. Cowpea protoplasts were prepared as described previously (Mizumoto *et al*, 2003) and were inoculated as described elsewhere (Matsuda *et al*, 2004) with minor changes; the washed protoplasts were resuspended in cold EB buffer.
at $1 \times 10^6$ cells/ml. Then 1.5 $\mu$g of \textit{in vitro} transcribed RNA, 10 $\mu$g each of pUBp27 and
pUBp88, and 2 $\mu$g of herring testes carrier DNA (BD Biosciences) were mixed with 0.3 ml of the protoplasts immediately before electroporation (950 $\mu$F capacitance, 90 V) using a pulse controller plus (Bio-Rad).

References


Takeda et al.,
EMBO J.
Supplementary material 2


<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>35/RC1+</td>
<td>GGAGAGGACAAACGTTTTTACCGGGTTTG</td>
</tr>
<tr>
<td>35/RC1–</td>
<td>CTTTTGCTCCTTCCAAATGGAATGAC</td>
</tr>
<tr>
<td>35/RC2+</td>
<td>GGAGAGGACAAACGCTCCTTCTAAACAG</td>
</tr>
<tr>
<td>35/RC2–</td>
<td>CGAGGTGGTCTCCTCCTCCTAAATGGAATGAC</td>
</tr>
<tr>
<td>5’RS1T7</td>
<td>GGAGGTGCTCTTAAGAGCTCAGTACAGAAAGCGTTTTACCGGGTTTG</td>
</tr>
<tr>
<td>AC1–2520</td>
<td>ATGATTGTCCTTGCG</td>
</tr>
<tr>
<td>Aus13L</td>
<td>CATTATACTGAGATATCAG</td>
</tr>
<tr>
<td>Ausp27+</td>
<td>GAGATTTTTAGTTAGGCTAGGCCACCTC</td>
</tr>
<tr>
<td>Ausp27–</td>
<td>GAGTGGGGCCTTACCTACTAAAACTCTC</td>
</tr>
<tr>
<td>Ausp88+</td>
<td>CAAATCCCTTGGAGACTCATTAGGCCGCCC</td>
</tr>
<tr>
<td>Ausp88–</td>
<td>GGGCCGCCTAGAGTCTCAGGAGTTTTG</td>
</tr>
<tr>
<td>BICEco</td>
<td>TGGCACAAGTTGCCCTCTCAATC</td>
</tr>
<tr>
<td>CPAD+</td>
<td>CCACAAAGATCTCTACAAAGCCCGATATAATTGTCTTTTTAAGTGAGC</td>
</tr>
<tr>
<td>D3’H–1–</td>
<td>CCATCCTGCTGGGCAAACTCCCAATACG</td>
</tr>
<tr>
<td>M4</td>
<td>GTTTTCCAGTCAGC</td>
</tr>
<tr>
<td>miR171-probe</td>
<td>GATATTTGCGCGCTCTAATCA</td>
</tr>
<tr>
<td>miR171-3Kpn</td>
<td>GGTGGTGTTACCTCTGCTTTTCTCCCTGGTTTG</td>
</tr>
<tr>
<td>miR171-5SS</td>
<td>GTTGGTACGGCTACGCTATGATTTGATTGCATTCAATAC</td>
</tr>
<tr>
<td>miR171prec-3Kpn</td>
<td>GGTGGTACCCAGAGAGTACGGAGAAGCTA</td>
</tr>
<tr>
<td>miR171prec-5Stu</td>
<td>GTTGGTACCTAGAGGAGTACGGAGAAGCTA</td>
</tr>
<tr>
<td>p27-3Bam</td>
<td>ACAGATCCAAATCTCTCAAGATTTG</td>
</tr>
<tr>
<td>p27-3Kpn</td>
<td>GGTGGTGTTAGGCTACCTAAATGATAGAGGAT</td>
</tr>
<tr>
<td>p27-5Sm</td>
<td>GGTGGGGCCTTGGAGAGATATCCAAATGGAATTGCTTTTTCCTTGCTCTTC</td>
</tr>
<tr>
<td>p57-23BK</td>
<td>GGTGGTTACCTATGATCTTCTCTCTCTCCTCCTGTGGAGA</td>
</tr>
<tr>
<td>p57-5Sm</td>
<td>GGTGGTGGCTGGAGAGATATCAATGAAAGTGAGGAGGCAGTCG</td>
</tr>
<tr>
<td>p88-156R</td>
<td>TTACACAGAGGAGAAGCTA</td>
</tr>
<tr>
<td>p88-186L</td>
<td>ATGCACTTTAGCTAGAGAAGCTA</td>
</tr>
<tr>
<td>p88-GVD-L</td>
<td>CTACAGAGAGAGAAGCTGAGACACACCCCTGGGCAGAGCC</td>
</tr>
<tr>
<td>p88-GVD-R</td>
<td>GGTGGCCTAAGATGTTACGGTCTCCTCTCTCTGAGAGCT</td>
</tr>
<tr>
<td>Ps-Hi/Aus2-5’</td>
<td>AAACGTCGATCAGTCAAACTCCAGCTCATAC</td>
</tr>
<tr>
<td>Ps35+</td>
<td>AACTGACGAATATGTTGAGCGACGACAGC</td>
</tr>
<tr>
<td>RC1/Rt+</td>
<td>GTATCCGCTGAGCAGATGCTTTTCC</td>
</tr>
<tr>
<td>RC1/Rt–</td>
<td>CCGTACGGGGTACCTCAAGGCTTACATAC</td>
</tr>
<tr>
<td>RC2-3D+</td>
<td>GAGAAAATCTAATACCCGTCACGGATGT</td>
</tr>
<tr>
<td>RC2-3D–</td>
<td>CGGTACGGGGTATTAGTTTTTCTCCCTGCTG</td>
</tr>
<tr>
<td>RC2/Rt+</td>
<td>GGCAGGCCGGCCAGGTGTCTTTTTCCTGG</td>
</tr>
<tr>
<td>RC2/Rt–</td>
<td>CCGTACGGGGTACGCTCCTAGCCTGCTG</td>
</tr>
<tr>
<td>RCCP-3Kpn</td>
<td>GGTGGTGTGTAACCAACAGGTATGAAAGTGT</td>
</tr>
<tr>
<td>RCCP-5Sm</td>
<td>GGTGGTGGCTGAGAGGCAGATATAAACAGGTCTTGCAAAAGCTCCC</td>
</tr>
<tr>
<td>RCMP-27XK</td>
<td>GGTGGTGTTACCTCTTGAGTCTCCTCCCTCCCTGGTTTG</td>
</tr>
<tr>
<td>RCMP-5Sm</td>
<td>GGTGGTGGCTGAGAGGATATAACATGGCTTCTCAGTGAGA</td>
</tr>
<tr>
<td>RCMP81R</td>
<td>TTCTCCGGTCTCCAGTTC</td>
</tr>
<tr>
<td>RtSm+</td>
<td>TCCCCGGGTATAGGGACTTTAGTGACT</td>
</tr>
<tr>
<td>S/R1int3’P</td>
<td>TTGCGCTGCACTATGCAAAACCG</td>
</tr>
<tr>
<td>S/R1int5’P</td>
<td>TGACGACATAAACCAGCAATC</td>
</tr>
<tr>
<td>S/R2int3’P</td>
<td>TCTCCATCTGCTTGTGTC</td>
</tr>
<tr>
<td>Sa35+</td>
<td>GCGAGCTCAACATGGTAGGAGCAGCACACGC</td>
</tr>
</tbody>
</table>