Supplemental Materials

Supplemental Section I: Construction of a Ribozyme Processing Cassette for Minireplicon Cloning and Analysis of Ribozyme Activity.

To evaluate ribozyme processing and provide a platform for cloning of minireplicon reporters, we engineered the *in vitro* transcription cassette $pSYNV_{HRz,\Delta Rz}$ (Note: See Fig. S1 for an overview of the steps used to construct $pSYNV_{HRz,\Delta Rz}$ and Table S1 for all primers used in Supplemental Section I). The $pSYNV_{HRz,\Delta Rz}$ cassette (Fig. S1B) has a bacteriophage T7 promoter (T7-Pro) inserted upstream of a hammerhead ribozyme (HRz) (1), followed by the SYNV leader (l) sequence (2), the 5' untranslated region (UTR) of the N gene (3), a multi cloning site, the 3' UTR of the SYNV L gene (4), the 162 nt trailer (t) sequence (5) and 89 nt from the ΔRz provided by Dr. Andrew Ball (6, 7).

To assess HRz processing, $pSYNV_{HRz,\Delta Rz}$ DNA (Fig. S2A) was digested with *Bsr*GI and transcribed *in vitro* with T7 polymerase to produce a 293 nt runoff transcript (T) containing the HRz. After 0.5, 1, 2, 3 and 4 hr transcription reactions in the presence of an RNase inhibitor (RNase OUT), aliquots were separated on sequencing gels. The primary runoff precursor transcript (T) was evident as a faint band at each of the transcription time points (Fig. S2B). The samples also contained a processed product RNA (HRz1) and a smaller RNA (HRz) that migrate close to the 235 nt (▷) and 56 nt (►) marker RNAs show in lanes P and M, respectively. Notably, both the P and HRz RNAs increased in intensity during reactions, whereas the 293 nt precursor transcript (T) remained relatively constant throughout the transcription reactions. These results suggest that the HRz processed the primary transcripts rapidly.

The 5' processing activities of HRz and the 3' processing of ΔRz were also analyzed by digesting $pSYNV_{HRz,\Delta Rz}$ DNA with *Bam*HI to facilitate runoff transcription of a 583 nt RNA (Rz2) containing both

ribozymes. Three high molecular weight RNAs were evident at the five transcription time periods (Fig. S2C). The larger RNA (Rz2) had a size corresponding to that expected of the unprocessed transcription product containing both ribozymes. A more rapidly migrating intermediate (Rz1) also appeared during the reactions that consisted of a faint but discernible doublet indicative of a mixture of transcripts containing either the HRz or the ΔRz ribozymes. The most rapidly migrating high molecular weight RNA (Rz0) corresponding closely in size to the 430 nt marker RNA (◀ in lane N) increased during reactions due to processing by both ribozymes. In addition, the intensity of two smaller RNAs corresponding to the expected 63 nt HRz fragment (15 nt *Stu*I and 47 nt HRz sequences) and the 89 nt ΔRz product increased throughout the reactions. In sum, the kinetics of the reactions confirmed that both ribozymes were active *in vitro*.

Time course experiments were carried out to characterize ribozyme activities by eluting the unprocessed Rz2 band containing both ribozymes from preparatory acrylamide gels (Fig. S2D). In these experiments, Rz2 recovered from the gels was incubated under Mg^{2+} concentrations suitable for ribozyme activity, and aliquots recovered after 2 to 200 min reactions were frozen on dry ice. Perusal of the sequencing gels revealed a decline in the intensity of the primary Rz2 transcript as the reactions proceeded, whereas the Rz1 and the Rz0 RNAs increased in intensity. More obvious increases in intensity of the ΔRz and HRz RNAs were also evident during the reactions. Taken together, these results indicate that the primary Rz2 transcript is processed at both the 5' and 3' termini to yield the Rz0 RNA, which is predicted to contain a single nucleotide preceding the 5' terminus of the ι sequence and the exact 3' terminus of the τ region.

Supplemental Section II: Construction of the SYNV pSYNV-MR$_{rsGFP-CAT}$ minireplicon.

The pSYNV-MR$_{rsGFP-CAT}$ derivative was engineered by substituting reporter genes between the *Nhe*I and *Bsr*GI sites of pSYNV$_{HRz,\Delta Rz}$ in manipulations that preserved the 5' and 3' UTRs and the gene junction sequences (GJ) separating the SYNV N, P and L genes (3, 8, 9). For construction of pSYNV-MR$_{rsGFP-CAT}$, a red-shifted green fluorescent protein (rsGFP) intermediate fragment was amplified from pGD-GFP (10) (See Fig. S3 and Table S2 for details of the cloning steps used for pSYNV-MR$_{rsGFP-CAT}$ construction). The gene junction (GJ) sequence separating the SYNV N and P genes (NPJ IF) was then inserted at the 3' end of GFP after amplification of RNA from SYNV-infected plants. Another fragment was engineered to introduce a second reporter, chloramphenicol acetyl transferase (CAT) from a *Barley stripe mosaic virus* (BSMV) gRNA-βbCAT construct described earlier (11) at a position corresponding to the P protein ORF. Then, the two fragments were joined by overlap PCR (12) and introduced between the *Nhe*I and *Bsr*GI sites of pSYNV$_{HRz,\Delta Rz}$ to generate pSYNV$_{rsGFP-CAT}$. (Note: In pSYNV$_{rsGFP-CAT}$, the CAT 3' UTR corresponds to the SYNV L gene 3' UTR). Next, the sequence flanked by the *Stu*I and *Sac*I sites of pSYNV$_{rsGFP-CAT}$ was cloned between the *Stu*I and *Sac*I sites of the pCass2 vector (13) to yield the derivative pSYNV-Cass$_{rsGFP-CAT}$. This manipulation eliminated the pSYNV$_{rsGFP-CAT}$ T7 promotor and positioned the GFP-CAT insert between the pCassRz2 CaMV 35S^2 promoter and the *A. tumefaciens* nopoline synthetase termination signal (*nos*) to facilitate RNA transcription and termination in agroinfiltrated plants. In the final step, a blunt end ligation reaction was implemented to insert the *Hae*II fragment from pSYNV-Cass$_{rsGFP-CAT}$ into the *Xmn*I and *Pme*I sites of pCAMBIA1300 to create the pSYNV-MR$_{rsGFP-CAT}$ reporter cassette (See Fig. S3 for cloning details). The complete sequence from the T-DNA right border to the left border of pSYNV-MR$_{rsGFP-CAT}$ has been submitted to GenBank and can be found under the accession number JNO38404.

Supplemental Section III: Construction of additional SYNV minireplicon derivatives.

Four additional minireplicons were generated on the basis of the pSYNV-MR$_{rsGFP-CAT}$ scaffold sequence to provide different reporter substitutions in the SYNV N and P ORFs (See Fig. S4 for an overview). The enhanced GFP (eGFP) reporter gene was amplified from pEGFP-N1 (Clontech, PaloAlto, CA), and DsRed (14), was amplified from pGD-DsRed (10). The expression cassettes were divided into 5 fragments, **1)** the leader sequence plus the N gene 5′ UTR sequence, **2)** the ORF1 gene, **3)** the N/P junction including the P protein gene 5' UTR, and **4)** the ORF2 gene and **5)** the L gene 3′UTR with the trailer sequence. Each of these fragments were amplified by PCR with the primers listed in Table S3. To join two fragments together, two contiguous primers were phosphorylated with T4 DNA polynucleotide kinase (T4 PNK; New England Biochemical (NEB), Beverly, MA) before PCR fusion of the fragments. The resulting PCR products were ligated by T4 DNA ligase (NEB) and subjected to a second PCR amplification with two flanking primers. After four rounds of PCR, the full-length cassettes were digested with *Xma*I and *Sac*I, and substituted for the rsGFP-CAT sequence in pSYNV-MR$_{rsGFP-CAT}$ to produce the pSYNV-MR$_{eGFP-CAT}$, pSYNV-MR$_{CAT-eGFP}$, pSYNV-MR$_{eGFP-DsRed}$ and pSYNV-MR $_{DsRed-eGFP}$ minireplicons (Fig. S4).

Supplemental Section IV: Mutant Minireplicon Derivatives.

To destroy rHRz activity, a 16 nt deletion preceding the normal HRz l sequence cleavage site was constructed in the 5' HRz of pSYNV-MR$_{eGFP-DsRed}$ by amplification with the forward GNPRHindIIIF and reverse Sma1095R primers (See Table S4 for primers used in Section IV). The amplified product was inserted into the *Hin*dIII/*Xma*I sites of pSYNV-MR$_{eGFP-DsRed}$ to assess biological activity of the HRz deficient derivative. To evaluate the importance of the 5' l region, an 18 nt deletion in the 144 nt l

sequence was created by amplifying pSYNV-MR$_{eGFP-DsRed}$ with the ι18F and Sac3143R primers and the mutant fragment was substituted into the *Xma*I/*Sac*I sites of pSYNV-MR$_{eGFP-DsRed}$. An 18 nt deletion of the τ sequence was also incorporated into pSYNV-MR$_{eGFP-DsRed}$ as described above by amplification with the NPJF and τ18R primer pair and the τ18-r and LBAscIR primer pair, to produce the intermediate fragments NP-$\tau\Delta$18IF and $\tau\Delta$18-LBIF respectively. These two fragments were joined by overlap PCR using the forward NPJF and reverse LBAscIR primers to yield the final NP-LB product with an 18 nt deletion in the τ sequence. This fragment was digested with *Apa*I/*Sac*I restriction enzymes and cloned into the *Apa*I/*Sac*I sites of pSYNV-MR$_{eGFP-DsRed}$.

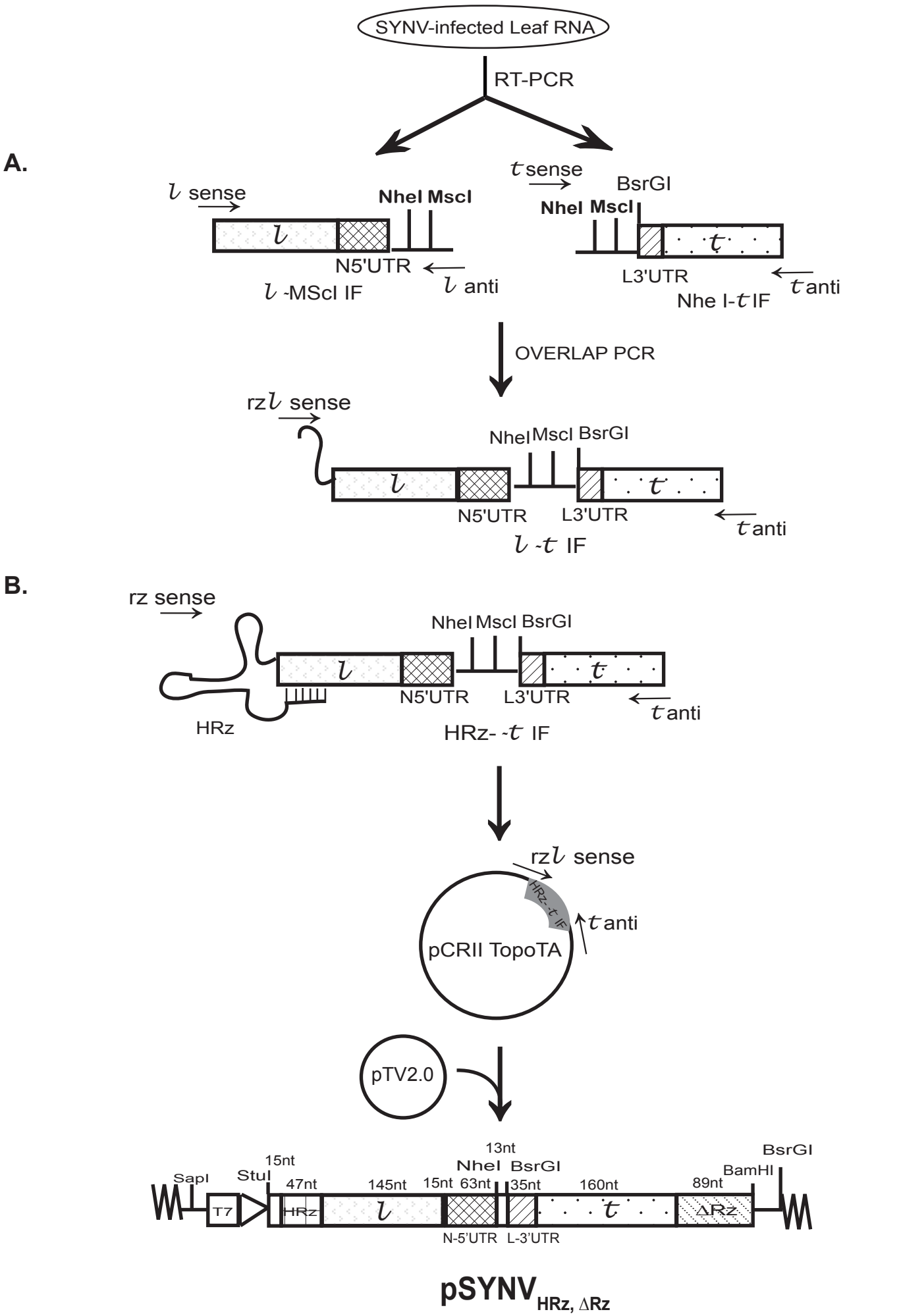
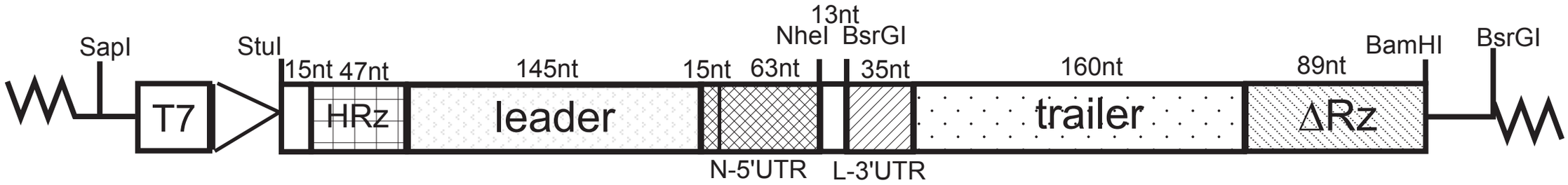

A.
SYNV-infected Leaf RNA
RT-PCR
l sense
NheI MscI
N5'UTR
l anti
l -MScI IF
t sense
BsrGI
NheI MscI
L3'UTR
t anti
Nhe I-t IF
OVERLAP PCR
rzl sense
NheI MscI BsrGI
N5'UTR
L3'UTR
t anti
l -t IF
B.
rz sense
NheI MscI BsrGI
N5'UTR
L3'UTR
t anti
HRz
HRz- -t IF
rzl sense
HRz-t IF
t anti
pCRII TopoTA
pTV2.0
SapI
StuI
15nt
47nt
145nt
13nt
NheI BsrGI
15nt 63nt
35nt
160nt
89nt
BamHI
BsrGI
T7
HRz
ΔRz
N-5'UTR
L-3'UTR
pSYNV HRz, ΔRz

Figure S1. Construction of pSYNV$_{HRz,\Delta Rz}$ to evaluate hammerhead (HRz) and Hepatitis Delta (ΔRz) ribozyme activity.

A) The SYNV leader (l) sequence through the N protein 5' untranslated region (UTR) was amplified from SYNV infected leaf RNA in an RT-PCR reaction containing the forward $l$sense and reverse $l$anti primers (See Table S1 for all primers used in pSYNV$_{HRz,\Delta Rz}$ cloning) to produce the l-MscI intermediate fragment (l-MscI IF) which contained the *Nhe*I and *Msc*I sites. A second intermediate (NheI-tIF) was amplified from SYNV RNA with the forward $t$sense and reverse $t$anti primers to produce a fragment containing the *Nhe*I, *Msc*I and *Bsr*GI sites fused to a portion of the L protein gene 3' UTR and the SYNV trailer (t) sequence. The two fragments were then fused in an overlap PCR reaction using the rz$l$ sense and $t$anti primers to generate the l-$t$intermediate fragment (l-tIF), which contains a portion of the hammerhead ribozyme (HRz) and the internal multicloning site.

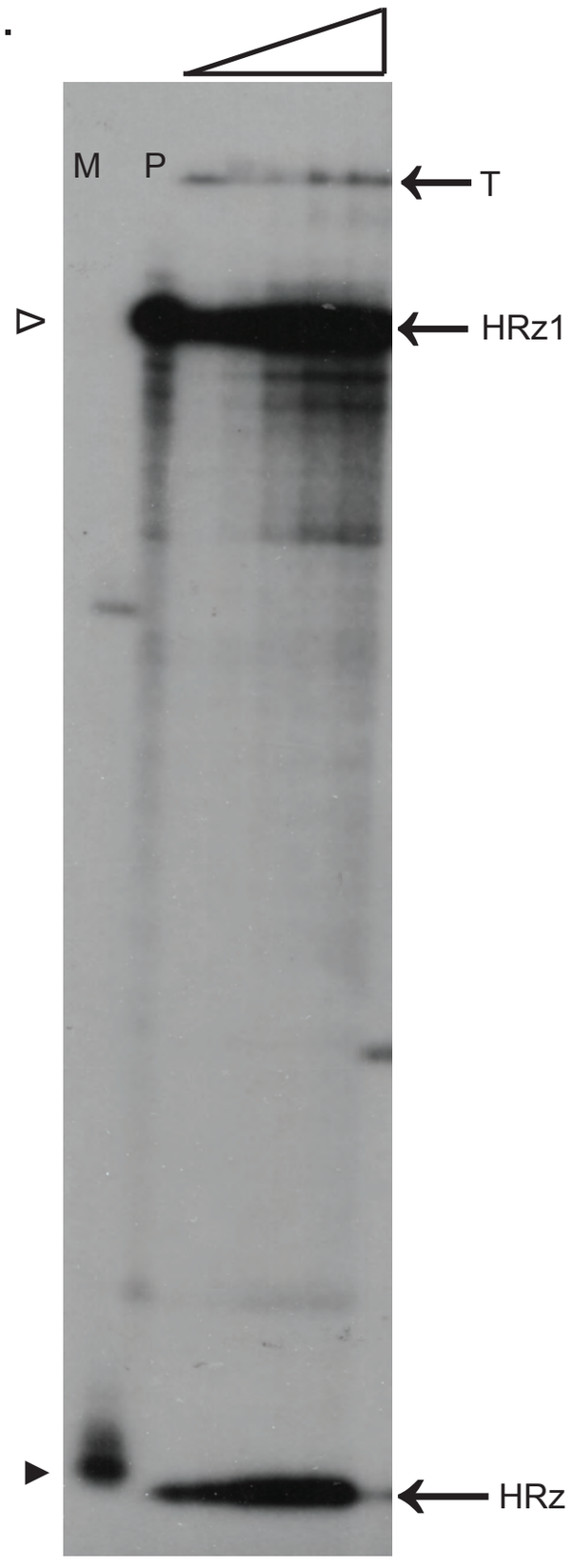
B) The 5' HRz was completed in a PCR reaction containing the rz sense and $t$anti primers to generate the intermediate fragment HRz-t, which was introduced into pCRII TopoTA to generate an intermediate plasmid pSM15-15 for use in other experiments. To place HRz-t under the control of the bacteriophage T7 promoter and introduce the 3' ΔRz, a second amplification was carried out with the rz$l$ sense and t anti primers for insertion into the *Stu*I site of the plasmid pTV2.0 to generate the transcription cassette, pSYNV$_{HRz,\ \Delta Rz.}$ (Note: The cartoons in Figs S1 to 4 are not drawn to scale).

A.

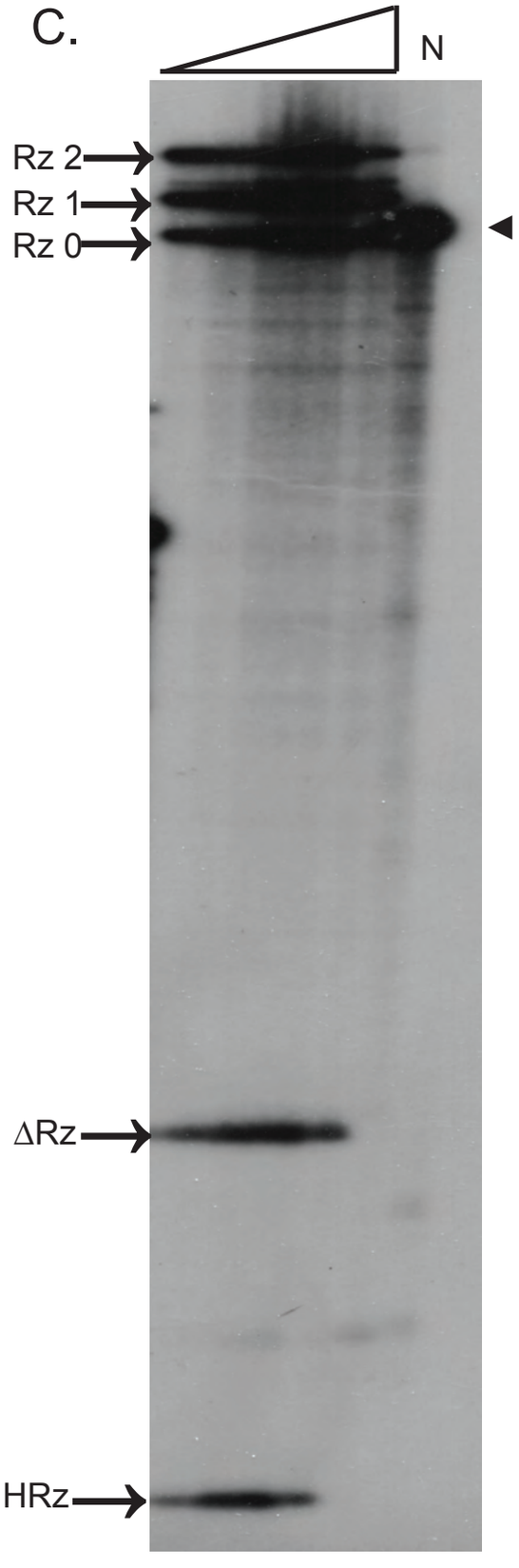


pSYNV$_{HRz,\ \Delta Rz}$

B.



C.



D.

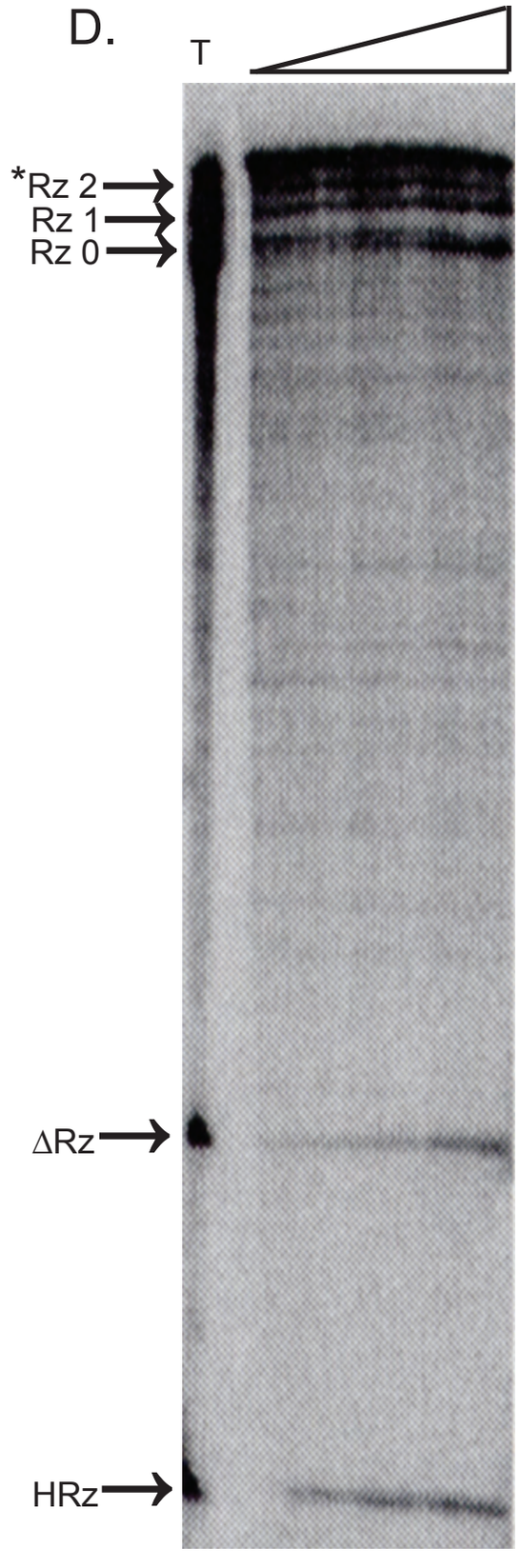


Figure S2. Hammer head and Hepatitis Delta Ribozyme transcript processing.

A) The pSYNV$_{HRz,\Delta Rz}$ cassette. The pSYNV$_{HRz,\ \Delta Rz}$ transcription cassette was used to facilitate *in vitro* transcription of SYNV antigenomic (ag) RNAs for evaluation of HRz and ΔRz processing. The sequence downstream of the T7 promoter (T7), consists of a 15 nt *Stu*I restriction site fused to a 47 nt HRz designed to cleave the agRNA transcript one nucleotide (nt) before the 5' terminus of the 145 nt leader (l) sequence. The l sequence is followed by a conserved 15 nt gene junction sequence (Vertical line) corresponding to that present in SYNV agRNAs and the 63 nt untranslated region (N-5' UTR) of the N gene mRNA. The N-5' UTR region is located adjacent to a 13 nt multiple cloning site (MCS) containing three unique restriction enzymes, including *Bsr*GI. The MCS is followed by a sequence consisting of the 35 nt 3' untranslated region of the L gene (L-3' UTR), the 160 nt SYNV trailer (t) sequence and an 89 nt ΔRz positioned to process transcripts at the immediate 3' terminus of the t sequence. A *Bam*HI restriction site flanks the 3' end of the ΔRz sequence. The 5' *Stu*I and 3' *Bam*HI sites, respectively, were designed to facilitate minireplicon cloning and transfer of the cassette into the other plasmid backgrounds.

B) Analysis of HRz processing *in vitro*. The sequencing gel shows the processing of a 293 nt precursor transcript (T) containing only the HRz that was produced by T7 transcription of pSYNV$_{HRz,\Delta Rz}$ plasmid DNA that had been digested with *Bsr*GI in the MCS. Transcription reactions were carried out for two hours at 37°C in the presence of ^{32}P-UTP in reactions containing 11 mM $MgCl_2$. The reactants were then incubated at 25 °C for time periods ranging from 0.5 to 4 hr, frozen on dry ice and separated in 5% polyacrylamide denaturing gels. **Lane M**: 56 nt marker RNA (►); **Lane P:** ~235 nt marker RNA (▷). The triangle above the lanes shows the 0.5, 1, 2, 3 and 4 hr reaction products. T = migration of the 293 nt HRz precursor transcript from the *Bsr*GI digested plasmid. HRz1 = migration of the 231 nt HRz product.

C) HRz and ΔRz *in vitro* processing. Processing of a transcript derived from *Bam*HI digested DNA that contains both the HRz and the ΔRz. **Lane N**: 430 nt Marker RNA lacking both the HRz

and ΔRz (◀); ΔRz and HRz = residual ribozyme products; Rz2 = 583 nt primary transcript that contains both HRz and ΔRz. Rz1 = processed product mixture forming a doublet bands, the upper of which contains the ΔRz and the lower containing the HRz; Rz0 = Transcript produced by both ΔRz and HRz cleavage. The triangle above the lane in as is panel B.

D) HRz and ΔRz processing kinetics. Analysis of ribozyme processing of the 583 nt RNA transcription product from *Bam*HI digested $pSYNV_{HRz,\Delta Rz}$ DNA. The RNA was separated in preparatory acrylamide gels in buffer containing EDTA and a ribonuclease inhibitor (RNase Out) and eluted before ribozyme reactions. The triangle represents lanes loaded with aliquots recovered after 2, 5, 10, 20, 40, 60, 120, 180, and 200 min reactions at 37 °C in a $MgCl_2$ containing buffer. **Lane T** = Marker reaction products before preparatory acrylamide gel separation. Designations are as indicated in Fig. S1B and C.

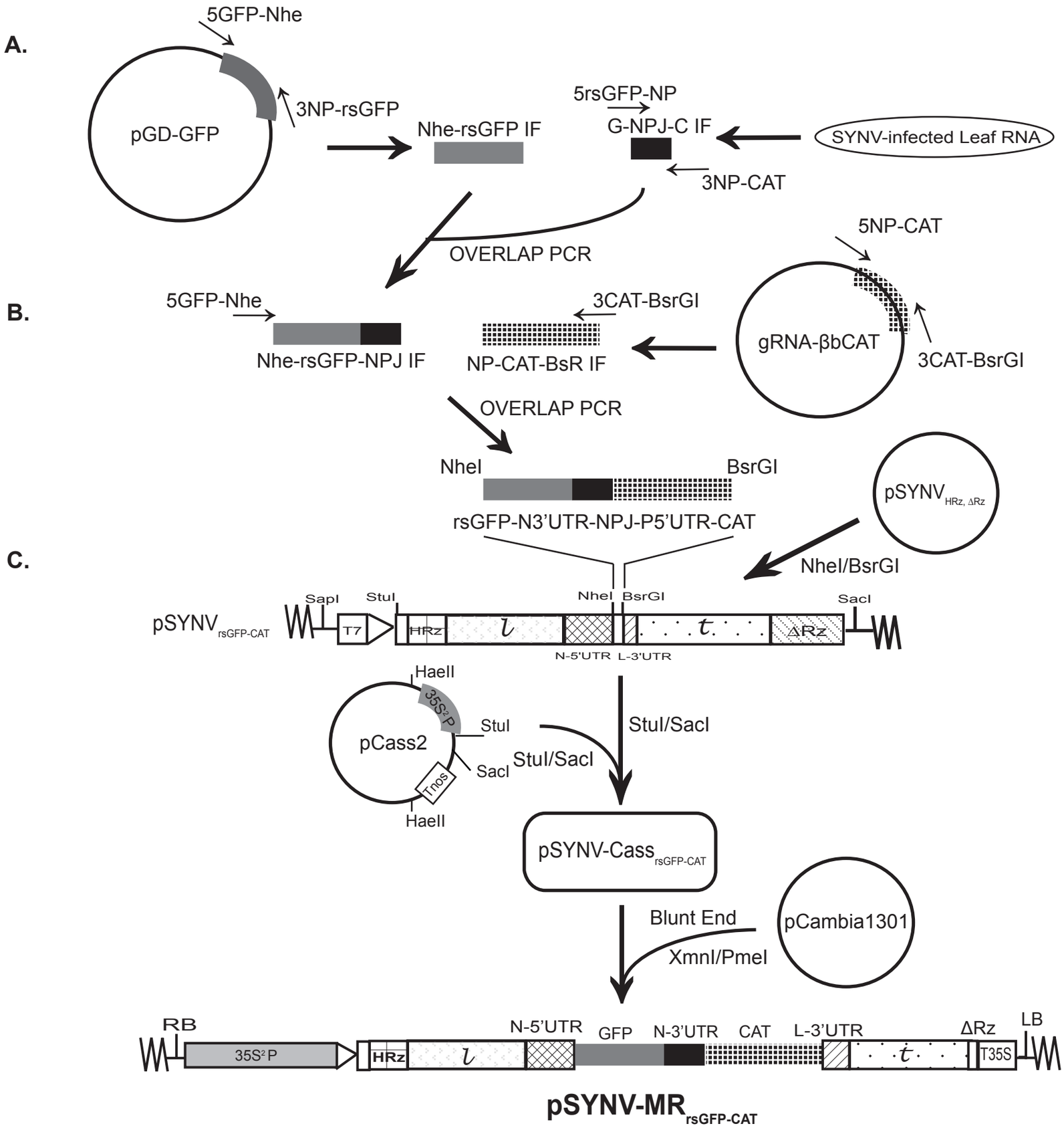

A.
5GFP-Nhe
pGD-GFP
3NP-rsGFP
Nhe-rsGFP IF
5rsGFP-NP
G-NPJ-C IF
SYNV-infected Leaf RNA
3NP-CAT
OVERLAP PCR
B.
5NP-CAT
5GFP-Nhe
3CAT-BsrGI
gRNA-βbCAT
3CAT-BsrGI
Nhe-rsGFP-NPJ IF
NP-CAT-BsR IF
OVERLAP PCR
NheI
BsrGI
rsGFP-N3'UTR-NPJ-P5'UTR-CAT
pSYNV HRz, ΔRz
NheI/BsrGI
C.
pSYNV rsGFP-CAT
SapI
StuI
NheI
BsrGI
SacI
T7
HRz
ΔRz
N-5'UTR
L-3'UTR
HaeII
35S2 P
pCass2
StuI
SacI
Tnos
HaeII
StuI/SacI
StuI/SacI
pSYNV-Cass rsGFP-CAT
Blunt End
XmnI/PmeI
pCambia1301
RB
35S2 P
HRz
N-5'UTR
GFP
N-3'UTR
CAT
L-3'UTR
ΔRz
T35S
LB
pSYNV-MR rsGFP-CAT

Figure S3. Engineering the pSYNV-MR$_{rsGFP-CAT}$ plasmid.

A) The Nhe-rsGFP intermediate fragment (IF) was constructed by using the forward 5GFP-Nhe and reverse 3NP-rsGFP primers (See Table S2) to amplify rsGFP from pGD-GFP (10). In another reaction, the N protein gene junction sequence was amplified from SYNV-infected plant RNA in a reverse transcription reaction using the primers 5rsGFP-NP and 3NP-CAT to produce the G-NPJ-C IF. Then, the rsGFP and G-NPJ-C IF derivatives were fused in an overlap PCR reaction containing the 5GFP-Nhe and 3NP-CAT primers to create Nhe-rsGFP-NPJ IF.

B) The *Barley stripe mosaic virus* plasmid, gRNA-βbCAT (11) was used as a source for PCR amplification of NP-CAT-BsR IF with the primers 5NP-CAT and 3CAT-BsrGI. This PCR product was then fused with Nhe-rsGFP-NPJ IF in an overlap PCR reaction with the 5GFP-Nhe and 3CAT-BsrGI primers to produce rsGFP-N3'UTR-NPJ-P5'UTR-CAT.

C) The rsGFP-N3'UTR-NPJ-P5'UTR-CAT fragment was inserted between the *Nhe*I and *Bsr*GI sites of pSYNV$_{HRZ-\Delta RZ}$ to generate pSYNV$_{rsGFP-CAT}$. Then, pSYNV$_{rsGFP-CAT}$ was digested with *Stu*I and *Sac*I and inserted between the *Stu*I and *Sac*I sites of the pCass2 vector to introduce the CaMV 35S[2] promoter and a 3' flanking *A. tumefasciens nos* termination signal to create pSYNV-Cass$_{rsGFP-CAT}$. As a final step, pSYNV-Cass$_{rsGFP-CAT}$ was digested with *Hae*II and the resulting fragment was inserted by a blunt end ligation between the *Xmn*I and *Pme*I sites of pCambria1301 to create the pSYNV-MR$_{rsGFP-CAT}$ reporter cassette for use in agroinfiltration. Note: For pSYNV-MR$_{rsGFP-CAT}$ and other plasmids in Fig. S4 the CAT 3' UTR corresponds to the SYNV L gene 3' UTR rather than the P gene 3' UTR.

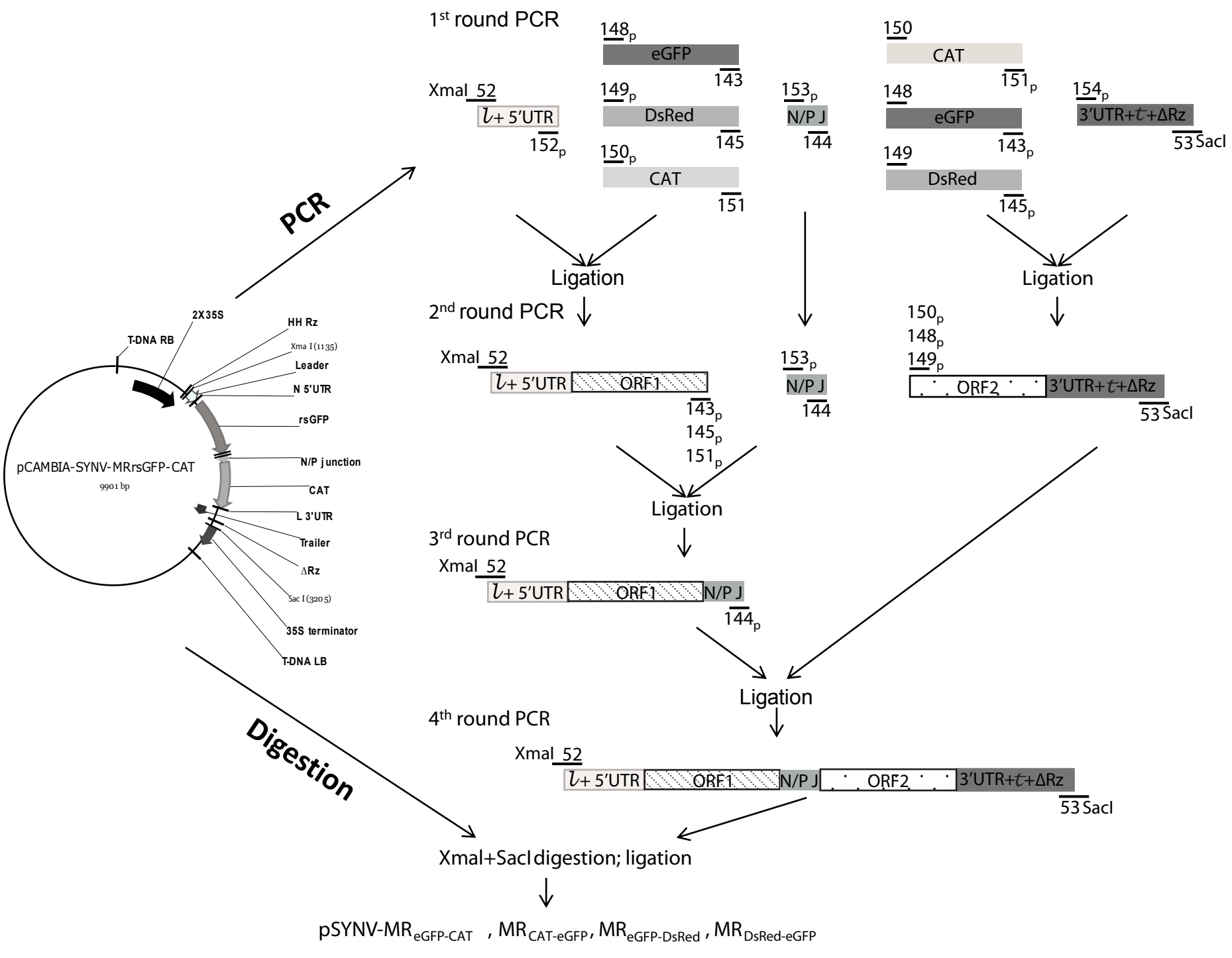

1st round PCR
148p
eGFP
143
Xmal 52
149p
DsRed
145
153p
N/P J
144
150
CAT
151p
148
eGFP
143p
154p
3'UTR+t+ΔRz
53 SacI
149
DsRed
145p
150p
CAT
151
PCR
Ligation
Ligation
2nd round PCR
Xmal 52
153p
N/P J
144
150p
148p
149p
ORF2
3'UTR+t+ΔRz
53 SacI
143p
145p
151p
Ligation
3rd round PCR
Xmal 52
ORF1
N/P J
144p
Ligation
4th round PCR
Xmal 52
ORF1
N/P J
ORF2
3'UTR+t+ΔRz
53 SacI
Digestion
XmaI+SacI digestion; ligation
pSYNV-MReGFP-CAT , MRCAT-eGFP, MReGFP-DsRed , MRDsRed-eGFP
T-DNA RB
2X35S
HH Rz
Xma I(1135)
Leader
N 5'UTR
rsGFP
N/P junction
CAT
L 3'UTR
Trailer
ΔRz
Sac I(3205)
35S terminator
T-DNA LB
pCAMBIA-SYNV-MRrsGFP-CAT
9901 bp

Figure S4. Construction of the pSYNV-MR$_{eGFP-CAT}$, pSYNV-MR$_{CAT-eGFP}$, pSYNV-MR$_{eGFP-DsRed}$ and pSYNV-MR$_{DsRed-eGFP}$ plasmids.

In a series of first round PCR reactions, reporter genes and regulatory sequences from pSYNV-MR$_{rsGFP-CAT}$ were amplified by PCR with primers shown on top of each fragment. The contiguous primers (as indicated by subscript $_p$) were phosphorylated with T4 PNK to facilitate ligation. The joined PCR products were amplified with their flanking primers (See Table S3). In a final series of substitution reactions, the reporter expression cassettes and pSYNV-MR$_{rsGFP-CAT}$ were digested with *Xma*I and *Sac*I, and then ligated to create the pSYNV-MR$_{eGFP-CAT,}$ pSYNV-MR$_{CAT-eGFP}$, pSYNV-MR$_{eGFP-DsRed}$ and pSYNV-MR$_{DsRed-eGFP}$ plasmids.

Table S1. Primers for creation of the pSYNV$_{HRz, \Delta Rz}$ transcription cassette and SYNV L gene cloning.

Clone Name		Primer Name	Primer sequence	Purpose
pSYNV$_{HRz, Rz}$				
l -MScI IF	F	$l$sense	AGAGACAGAAACTCAGAAAATACAATCACCG	To amplify the SYNV leader sequence through the N5'UTR sequence.
	R	$l$anti	GTATTTTAATTGCAGGTAATCGCTAGCTGGCCA	
Nhe I-tIF	F	$t$sense	ATCGCTAGCTGGCCATGTACATCTATATCTCTTGAA	To amplify sequence from the *Bsr*GI site at the 3' end of the SYNV L gene through the end of the genomic RNA.
	R	$t$anti	GGTATAGGGATTGTTCTGAGCTTTTGTCTCT	
l-t IF	F	rz$l$sense	CCCGGTATCCCGGGTTCAGAGACAGAAACTCAGAA	To generate a product extending from 8 bases before the 5' side of the *Sma*I site in the Hammerhead ribozyme to the end of the SYNV genomic RNA.
	R	$t$anti	GGTATAGGGATTGTTCTGAGCTTTTGTCTCT	
HRz-t IF	F	rzsense	GTCTCTCTGATGAGGCCGAAAGGCCGAAAACCCGGTATCCCGGGTTC	To attach the remainder of the hammerhead ribozyme to the transcription cassette.
	R	$t$anti	GGTATAGGGATTGTTCTGAGCTTTTGTCTCT	
L gene cloning				
	F	L-sense	GTCGACATGGAAGGGATGGATCACTGG	To amplify the SYNV L gene.
	R	L-anti	GTCGACTTATACTTCAAGAGATATAGATG	

Table S2. Primers used for cloning SYNV minireplicon pSYNV-MR$_{rsGFP-CAT}$

Clone Name		Primer Name	Primer sequence	Purpose
Nhe-rsGFP IF	F	5GFP-Nhe	GCTAGCATGGGTAAAGGAGAAGAAC	To introduce rsGFP into pSYNV$_{HRz,Rz}$.
	R	3NP-rsGFP	GTAGAGTTGTGGCTGTAGTTTATCTAGATCCGGTGGAT	
G-NPJ-C IF	F	5rsGFP-NP	ATCCACCGGATCTAGATAAACTACAGCCACAACTCTACC	To amplify the NPJ sequence having an overlap sequence with rsGFP at 5'end and CAT gene at the 3' end.
	R	3NP-CAT	TCCAGTGATTTTTTTCTCCATGGCCTAGACAAATAATACAAACAG	
Nhe-rsGFP-NPJ IF	F	5GFP-Nhe	GCTAGCATGGGTAAAGGAGAAGAAC	To introduce NPJ at the 3' end of the rsGFP gene.
	R	3NP-CAT	TCCAGTGATTTTTTTCTCCATGGCCTAGACAAATAATACAAACAG	
NP-CAT-BsR IF	F	5NP-CAT	CTGTTTGTATTATTTGTCTAGGCCATGGAGAAAAAAATCACTGGA	To fuse NPJ to the 5' end of the CAT gene.
	R	3CAT-BsrGI	TGTACATTACGCCCCGCCTGCCA	
rsGFP-N3'UTR-NPJ-P5'UTR-CAT	F	5GFP-NheI	GCTAGCATGGGTAAAGGAGAAGAAC	To introduce the CAT gene into pSYNV$_{HRz,Rz}$.
	R	CAT-BsrGI	TGTACATTACGCCCCGCCTGCCA	

Table S3. Primers used for cloning, the pSYNV-MR$_{eGFP-CAT}$, MR $_{CAT-eGFP}$, MR$_{DsRed-eGFP}$ and MR$_{CAT-eGFP}$ minireplicons.

No.	Sense	Target Annealed	Primer Sequence
52	F	SYNV l	CAGCGGATCCAGGCCTGGATCTTCCCGTCTC
152	F	SYNV 5′ UTR	GCTAGCGATTACCTGCAATTA
53	R	HDV_{rz}	GACGCTCGAGGAGCTCTCCCTTAGCCATCCGA
153	F	SYNV NP junction	TAAACTACAGCCACAACTCTACCT
144	R	SYNV NP junction	GGCCTAGACAAATAATACAAACAGACAAAT
146	F	SYNV L 3'UTR	GAAAAACCTTCCGTTATACACAAGCAG
154	R	SYNV 3'UTR	TGTACATCTATATCTCTTGAAG
148	F	eGFP	ATGGTGAGCAAGGGCGAGGA
143	R	eGFP	TTACTTGTACAGCTCGTCCATGCCGA
149	F	DsRed	ATGGCCTCCTCCGAGAACGT
145	R	DsRed	TTATAGGAACAGGTGGTGGCGGCCCT
150	F	CAT	ATGGAGAAAAAAATCACTGGAT
151	R	CAT	TTACGCCCCGCCCTGCCACTCATCGCAGTACT

Table S4. List of primers designed to introduce cis-acting mutations into the SYNV minireplicon.

Clone Name		Primer Name	Primer sequence	Purpose
ΔHRz	F	GNPRHindIIIF	CCATGATTACGCCAAGCTTGCATGCC	To inactivate the hammerhead ribozyme from the minireplicons.
	R	Sma1095R	GACCCCGGGTACCAAGATCCAGGCCTCTCCAAATGAAATG	
l Δ18	F	l18F	GAAAACCCGGTATCCCGGGTTCAATACAATCACCGTAGATTAACAG	To delete 18 nt from the leader sequence.
	R	Sac3143R	TTCGAGCTCTCCCTTAGCCATCCG	
NP-tΔ18 IF	F	NPJF	ACTACAGCCACAACTCTACC	To introduce an 18 nt deletion in the trailer region and create a 5'overlap with NPJ.
	R	t18R	GGAGGTGGAGATGCCATGCCGACCCCAATCCCTATACCCCATTTATCAT	
tΔ18 -LB IF	F	t18R-r	GATGATAAATGGGGTATAGGGATTGGGGTCGGCATGGCATCTCCACCTCC	To introduce an 18 nt deletion in the trailer region and create a 3' overlap with the left border of the MR.
	R	LBAscIR	GGGGCGCGCCTGGCAGGATATATTGTGGTGTAAACAAATTGACG	
NP-LB	F	NPJF	ACTACAGCCACAACTCTACC	To join the NP-tΔ18 IF and tΔ18-LB IF by overlap PCR
	R	LBAscIR	GGGGCGCGCCTGGCAGGATATATTGTGGTGTAAACAAATTGACG	

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