

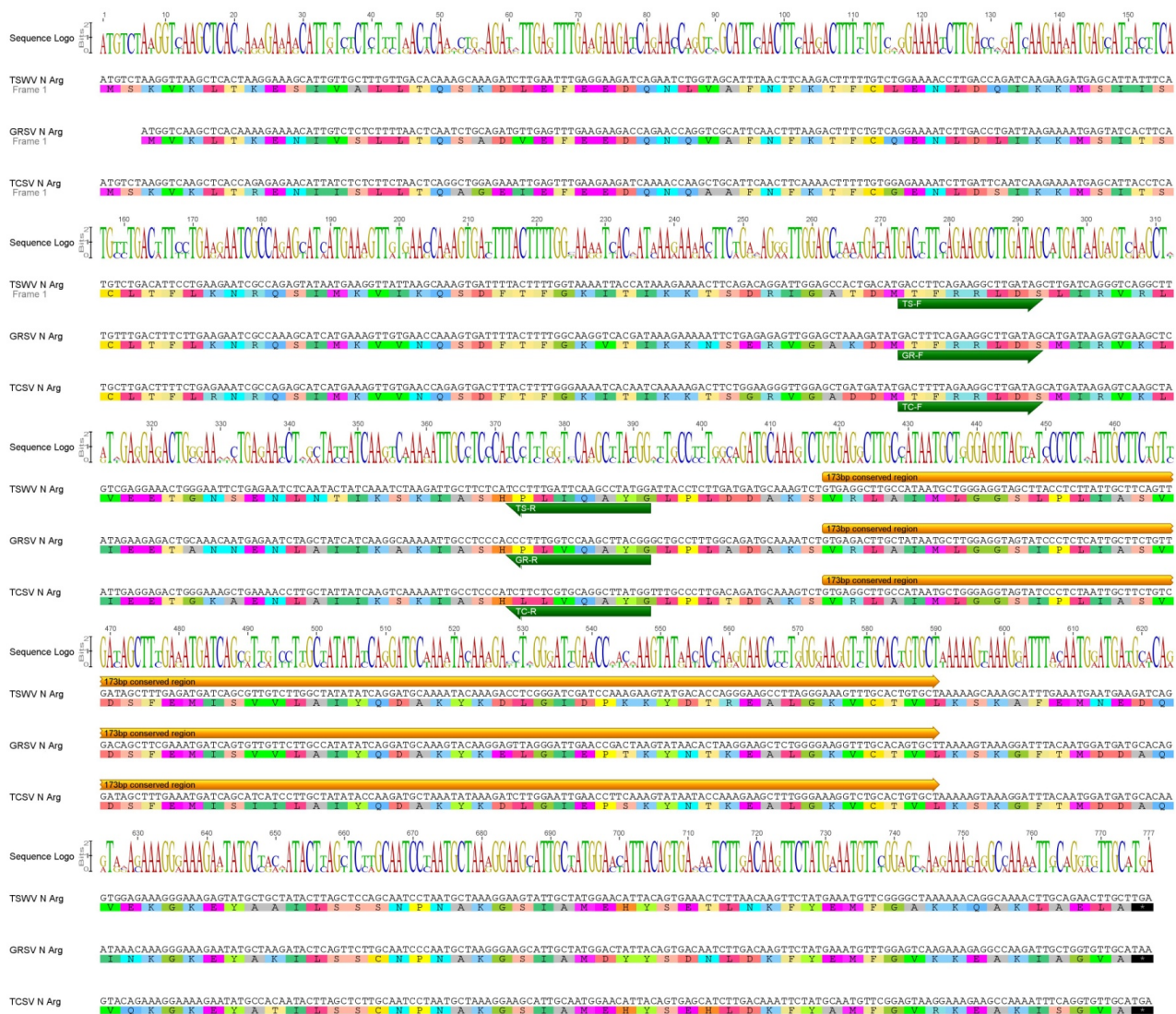


Fig. 1 Suppl. The sequence alignment of the *N* gene of TSWV, GRSV, and TCSV. Yellow annotations denote the 173 bp conserved sequence. Green annotations highlight qPCR primer binding sites. A global *Geneious* alignment with a cost matrix of a 65 % similarity (5.0/-4.0), gap open penalty: 12, gap extension penalty: 3, refinement iterations: 2. Software: *Geneious* 5.6, Biomatters Ltd, Auckland, New Zealand.

The Off-Target *in silico* determination of the conserved 173bp selected region of the TSWV *N* gene:

SUMMARY OF QUERY

Number of sequences submitted = 1

siRNA length = 21 nt

The 5' end of antisense strand must be A or U

The first seven bases of antisense strand (5' - 3') have at least 5 A or U bases

The 5' end of sense strand must be G or C

Percentage GC content: 30% to 70%

Mismatch allowed = 2

Target dataset = Nicotiana benthamiana DFCI Gene Index (NBGI) version 4 release on 4/23/2010

Hit Summary

Query: query\_seq. Select IDs for analysis of their functional domains and associated GO terms

| Target ID                                                                                  | Functional Annotation                                                                                                                                                                                                                       |
|--------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  EH369565 | similar to UniRef100_A7PNC0 Cluster: Chromosome chr1 scaffold_22, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr1 scaffold_22, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (44%) |

Report of query sequence fragments that have hits in target dataset

|                        |                       |
|------------------------|-----------------------|
| query_seq (98 - 118)   | CAACGCTGATCATCTCAAAGC |
| siRNA AS (3'-5')       | GUUGCGACUAGUAGAGUUUCG |
| EH369565 (270 - 290) + | caacactgctcatctcaaagc |
| query_seq (98 - 118)   | CAACGCTGATCATCTCAAAGC |
| siRNA AS (3'-5')       | GUUGCGACUAGUAGAGUUUCG |
| EH369565 (270 - 290) + | caacactgctcatctcaaagc |

The Off-Target *in silico* determination of the complete *N* gene sequence of TSWV:

SUMMARY OF QUERY

Number of sequences submitted = 1

siRNA length = 21 nt

The 5' end of antisense strand must be A or U

The first seven bases of antisense strand (5' - 3') have at least 5 A or U bases

The 5' end of sense strand must be G or C

Percentage GC content: 30% to 70%

Mismatch allowed = 2

Target dataset = Nicotiana benthamiana DFCI Gene Index (NBGI) version 4 release on 4/23/2010

Hit Summary

Query: query\_seq. Select IDs for analysis of their functional domains and associated GO terms

| Target ID                                                                                    | Functional Annotation                                                                                                                                                                                                                        |
|----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  CK285234 | similar to UniRef100_A7PFA3 Cluster: Chromosome chr11 scaffold_13, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr11 scaffold_13, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (4%) |

|                                   |                                                                                                                                                                                                                                                                                           |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> CK286012 | similar to UniRef100_Q8MVZ4 Cluster: Acetylcholinesterase; n=3; <i>Bactrocera oleae</i>  Rep: Acetylcholinesterase - <i>Bactrocera oleae</i> (Olive fruit fly), partial (3%)                                                                                                              |
| <input type="checkbox"/> CK291288 | similar to UniRef100_Q4J827 Cluster: Conserved protein; n=1; <i>Sulfolobus acidocaldarius</i>  Rep: Conserved protein - <i>Sulfolobus acidocaldarius</i> , partial (5%)                                                                                                                   |
| <input type="checkbox"/> CK294202 | similar to UniRef100_A7Q060 Cluster: Chromosome chr8 scaffold_41, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr8 scaffold_41, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (25%)                                               |
| <input type="checkbox"/> CN655446 | similar to UniRef100_A7NZ17 Cluster: Chromosome chr6 scaffold_3, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr6 scaffold_3, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (16%)                                                 |
| <input type="checkbox"/> EH365072 | Annotation not available.                                                                                                                                                                                                                                                                 |
| <input type="checkbox"/> EH369565 | similar to UniRef100_A7PNC0 Cluster: Chromosome chr1 scaffold_22, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr1 scaffold_22, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (44%)                                               |
| <input type="checkbox"/> EH369631 | Annotation not available.                                                                                                                                                                                                                                                                 |
| <input type="checkbox"/> EH370639 | homologue to UniRef100_Q40161 Cluster: Polygalacturonase-1 non-catalytic subunit beta precursor; n=1; <i>Solanum lycopersicum</i>  Rep: Polygalacturonase-1 non-catalytic subunit beta precursor - <i>Solanum lycopersicum</i> (Tomato) ( <i>Lycopersicon esculentum</i> ), partial (4%)  |
| <input type="checkbox"/> EH370783 | similar to UniRef100_A7P3I6 Cluster: Chromosome chr1 scaffold_5, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr1 scaffold_5, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (26%)                                                 |
| <input type="checkbox"/> GO604767 | similar to UniRef100_A7PU45 Cluster: Chromosome chr7 scaffold_31, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr7 scaffold_31, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (17%)                                               |
| <input type="checkbox"/> GO605380 | Annotation not available.                                                                                                                                                                                                                                                                 |
| <input type="checkbox"/> GO607687 | similar to UniRef100_A7P3I6 Cluster: Chromosome chr1 scaffold_5, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr1 scaffold_5, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (26%)                                                 |
| <input type="checkbox"/> GO607940 | Annotation not available.                                                                                                                                                                                                                                                                 |
| <input type="checkbox"/> TC16872  | similar to UniRef100_A5X5I9 Cluster: Pectin methylesterase inhibitor protein 1; n=1; <i>Capsicum annuum</i>  Rep: Pectin methylesterase inhibitor protein 1 - <i>Capsicum annuum</i> (Bell pepper), partial (98%)                                                                         |
| <input type="checkbox"/> TC17961  | similar to UniRef100_A7QF34 Cluster: Chromosome chr16 scaffold_86, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr16 scaffold_86, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (40%)                                             |
| <input type="checkbox"/> TC20344  | similar to UniRef100_Q6LWQ7 Cluster: Probable amino acid ABC transporter, periplasmic component precursor; n=1; <i>Methanococcus maripaludis</i>  Rep: Probable amino acid ABC transporter, periplasmic component precursor - <i>Methanococcus maripaludis</i> , partial (8%)             |
| <input type="checkbox"/> TC20649  | Annotation not available.                                                                                                                                                                                                                                                                 |
| <input type="checkbox"/> TC22330  | homologue to UniRef100_Q40161 Cluster: Polygalacturonase-1 non-catalytic subunit beta precursor; n=1; <i>Solanum lycopersicum</i>  Rep: Polygalacturonase-1 non-catalytic subunit beta precursor - <i>Solanum lycopersicum</i> (Tomato) ( <i>Lycopersicon esculentum</i> ), partial (20%) |
| <input type="checkbox"/> TC22644  | weakly similar to UniRef100_Q8S944 Cluster: Dynamin-related protein 3A; n=1; <i>Arabidopsis thaliana</i>  Rep: Dynamin-related protein 3A - <i>Arabidopsis thaliana</i> (Mouse-ear cress), partial (11%)                                                                                  |

Report of query sequence fragments that have hits in target dataset

query\_seq (26 - 46)  
siRNA AS (3'-5')  
EH369631 (224 - 244) +  
query\_seq (55 - 75)  
siRNA AS (3'-5')  
GO607687 (203 - 223) +  
query\_seq (58 - 78)  
siRNA AS (3'-5')  
GO605380 (253 - 273) +  
query\_seq (84 - 104)  
siRNA AS (3'-5')  
CN655446 (185 - 205) +  
query\_seq (148 - 168)  
siRNA AS (3'-5')  
GO604767 (437 - 457) +  
query\_seq (189 - 209)  
siRNA AS (3'-5')  
GO607940 (598 - 618) +  
query\_seq (326 - 346)  
siRNA AS (3'-5')  
CK286012 (624 - 644) +  
query\_seq (594 - 614)  
siRNA AS (3'-5')  
EH370639 (85 - 105) +  
TC22330 (618 - 638) +  
query\_seq (622 - 641)  
siRNA AS (3'-5')  
CK285234 (56 - 75) +  
query\_seq (687 - 707)  
siRNA AS (3'-5')  
TC17961 (619 - 639) +  
query\_seq (50 - 70)  
siRNA SS (5'-3')  
CK294202 (355 - 335) -  
query\_seq (54 - 74)  
siRNA SS (5'-3')  
EH370783 (622 - 602) -  
query\_seq (56 - 75)  
siRNA SS (5'-3')  
TC22644 (476 - 457) -  
query\_seq (196 - 216)  
siRNA SS (5'-3')  
CK291288 (892 - 872) -  
query\_seq (286 - 306)  
siRNA SS (5'-3')  
TC20649 (578 - 558) -  
query\_seq (472 - 492)

AAAACATTGTTGCTTTGTTGA  
UUUUGUAACAACGAAACAACU  
aaaacatggtcgtttgtga  
AAAGATCTTGAATTTGAGGAA  
UUUCUAGAACUAAAACUCCUU  
aaaattcttgaattgaggaa  
GATCTTGAATTTGAGGAAGAT  
CUAGAACUAAAACUCCUUCUA  
gatcttgaattcagaagat  
TCTGGTAGCATTCAACTTCAA  
AGACCAUCGUAAGUUGAAGUU  
tctgtagcatccaatttcaa  
ATTATTTTCATGTCTGACATTC  
UAAUAAAAGUACAGACUGUAAG  
attatctcatggtgacattc  
AATGAAGGTTATTAAGCAAAG  
UUACUUCCAAUAAUUCGUUUC  
aatgagggttatgaagcaaag  
GGAATTCTGAGAATCTCAATA  
CCUUAAGACUCUUAGAGUUAU  
ggaattctgagaatttcaata  
AAGCAAAGCATTGAAATGAA  
UUCGUUUCGUAACUUUACUU  
aagcaaaggatttgaattgaa  
aagcaaaggatttgaattgaa  
CAGGTGAAGAAAGGGAAAGA  
GUCCACUUCUUUCCCUUUCU  
caggtaagaaagggaaga  
TATTGCTATGGAACATTACAG  
AUAACGAUACCUUGUAAUGUC  
tatggctatggaacattacaa  
AAAGCAAAGATCTTGAATTTG  
AAAGCAAAGAUCUUGAAUUUG  
aaagaaagatcgtgaatttg  
CAAAGATCTTGAATTTGAGGA  
CAAAGAUCUUGAAUUUGAGGA  
caaaattcttgaatttgagga  
AAGATCTTGAATTTGAGGAA  
AAGAUCUUGAAUUUGAGGAA  
aagatcttgaatttgagtaa  
GTTATTAAGCAAAGTGATTTT  
GUUAUUAAGCAAAGUGAUUUU  
gtaattaagcaaagagatttt  
CTTGATAGCTTGATCAGGGTC  
CUUGAUAGCUUGAUCAGGGUC  
cttgatagcttttcagggtc  
AGCTTTGAGATGATCAGTGTT

|                                        |                                |
|----------------------------------------|--------------------------------|
| siRNA SS (5'-3')                       | AGCUUUGAGAUGAUCAGUGUU          |
| <a href="#">EH369565</a> (291 - 271) - | agctttgagatga <b>gc</b> agtgtt |
| <a href="#">query_seq</a> (607 - 627)  | GAAATGAATGAAGATCAGGTG          |
| siRNA SS (5'-3')                       | GAAAUGAAUGAAGAUCAGGUG          |
| <a href="#">EH365072</a> (92 - 72) -   | gaaa <b>aca</b> atgaagatcaggtg |
| <a href="#">query_seq</a> (610 - 630)  | ATGAATGAAGATCAGGTGAAG          |
| siRNA SS (5'-3')                       | AUGAAUGAAGAUCAGGUGAAG          |
| <a href="#">TC16872</a> (232 - 212) -  | atgaatgaaga <b>aca</b> agtgaag |
| <a href="#">query_seq</a> (621 - 641)  | TCAGGTGAAGAAAGGGAAAGA          |
| siRNA SS (5'-3')                       | UCAGGUGAAGAAAGGGAAAGA          |
| <a href="#">TC20344</a> (116 - 96) -   | tcagctgaagaa <b>at</b> ggaaaga |

The Off-Target *in silico* determination of the selected sequences corresponding to TSWV, GRSV and TCSV in Bucher *et al.* (2006):

#### SUMMARY OF QUERY

Number of sequences submitted = 1

siRNA length = 21 nt

The 5' end of antisense strand must be A or U

The first seven bases of antisense strand (5' - 3') have at least five A or U bases

The 5' end of sense strand must be G or C

Percentage GC content: 30% to 70%

Mismatch allowed = 2

Target dataset = Nicotiana benthamiana DFCI Gene Index (NBGI) version 4 release on 4/23/2010

#### Hit Summary

Query: . Select IDs for analysis of their functional domains and associated GO terms

| Target ID                         | Functional Annotation                                                                                                                                                                                                                                                                    |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> CK292196 | similar to UniRef100_A7PYV2 Cluster: Chromosome chr12 scaffold_38, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr12 scaffold_38, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (17%)                                            |
| <input type="checkbox"/> CN742562 | homologue to UniRef100_Q3HRW1 Cluster: 60S ribosomal protein L13a-like protein; n=1; <i>Solanum tuberosum</i>  Rep: 60S ribosomal protein L13a-like protein - <i>Solanum tuberosum</i> (Potato), partial (78%)                                                                           |
| <input type="checkbox"/> EH369565 | similar to UniRef100_A7PNC0 Cluster: Chromosome chr1 scaffold_22, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr1 scaffold_22, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (44%)                                              |
| <input type="checkbox"/> EH370639 | homologue to UniRef100_Q40161 Cluster: Polygalacturonase-1 non-catalytic subunit beta precursor; n=1; <i>Solanum lycopersicum</i>  Rep: Polygalacturonase-1 non-catalytic subunit beta precursor - <i>Solanum lycopersicum</i> (Tomato) ( <i>Lycopersicon esculentum</i> ), partial (4%) |
| <input type="checkbox"/> GO604194 | similar to UniRef100_A7NTE7 Cluster: Chromosome chr18 scaffold_1, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr18 scaffold_1, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (15%)                                              |
| <input type="checkbox"/> GO609975 | homologue to UniRef100_Q3HRW1 Cluster: 60S ribosomal protein L13a-like protein; n=1; <i>Solanum tuberosum</i>  Rep: 60S ribosomal protein L13a-like protein - <i>Solanum tuberosum</i> (Potato), partial (46%)                                                                           |

|                                   |                                                                                                                                                                                                                                                                                           |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> GO612005 | homologue to UniRef100_Q5NT78 Cluster: Potassium channel TORK1; n=1; <i>Nicotiana tabacum</i>  Rep: Potassium channel TORK1 - <i>Nicotiana tabacum</i> (Common tobacco), partial (10%)                                                                                                    |
| <input type="checkbox"/> TC17566  | similar to UniRef100_A7PYV2 Cluster: Chromosome chr12 scaffold_38, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr12 scaffold_38, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (73%)                                             |
| <input type="checkbox"/> TC19001  | similar to UniRef100_A7NUN5 Cluster: Chromosome chr18 scaffold_1, whole genome shotgun sequence; n=1; <i>Vitis vinifera</i>  Rep: Chromosome chr18 scaffold_1, whole genome shotgun sequence - <i>Vitis vinifera</i> (Grape), partial (33%)                                               |
| <input type="checkbox"/> TC20986  | Annotation not available.                                                                                                                                                                                                                                                                 |
| <input type="checkbox"/> TC22302  | homologue to UniRef100_Q3HRW1 Cluster: 60S ribosomal protein L13a-like protein; n=1; <i>Solanum tuberosum</i>  Rep: 60S ribosomal protein L13a-like protein - <i>Solanum tuberosum</i> (Potato), partial (54%)                                                                            |
| <input type="checkbox"/> TC22330  | homologue to UniRef100_Q40161 Cluster: Polygalacturonase-1 non-catalytic subunit beta precursor; n=1; <i>Solanum lycopersicum</i>  Rep: Polygalacturonase-1 non-catalytic subunit beta precursor - <i>Solanum lycopersicum</i> (Tomato) ( <i>Lycopersicon esculentum</i> ), partial (20%) |

Report of query sequence fragments that have hits in target dataset

|                         |                        |
|-------------------------|------------------------|
| 5 (- 25)                | AACTCTTATCATACTATCAAG  |
| siRNA AS (3'-5')        | UUGAGAAUAGUAUGAUAGUUC  |
| GO609975 (125 - 145) +  | aactattatgatactatcaag  |
| TC22302 (194 - 214) +   | aactattatgatactatcaag  |
| 76 (- 96)               | TTGTGATTTCCCAAAGGTAA   |
| siRNA AS (3'-5')        | AACACUAAAAGGGUUUCCAUI  |
| TC19001 (69 - 89) +     | ttgtattcttccaaagtaa    |
| 80 (- 100)              | GATTTTCCCAAAGGTAAATC   |
| siRNA AS (3'-5')        | CUAAAAGGGUUUCCAUIUUUAG |
| GO604194 (463 - 483) +  | gattatcctaaagtaaatac   |
| 289 (- 309)             | ATCCCTCTCATTGCTTCAGTT  |
| siRNA AS (3'-5')        | UAGGGAGAGUAAACGAAGUCA  |
| TC17566 (1400 - 1420) + | atcactgtcattgcttcagtt  |
| 290 (- 310)             | TCCCTCTCATTGCTTCAGTTG  |
| siRNA AS (3'-5')        | AGGGAGAGUAAACGAAGUCA   |
| TC17566 (1401 - 1421) + | tcactgtcattgcttcagttg  |
| 435 (- 455)             | AAGCAAAGCATTGAAATGAA   |
| siRNA AS (3'-5')        | UUCGUUUCGUAACUUUACUU   |
| EH370639 (85 - 105) +   | aagcaaaggattgaattgaa   |
| TC22330 (618 - 638) +   | aagcaaaggattgaattgaa   |
| 436 (- 456)             | AGCAAAGCATTGAAATGAAT   |
| siRNA AS (3'-5')        | UCGUUUCGUAACUUUACUU    |
| EH370639 (86 - 106) +   | agcaaaggattgaattgaat   |
| TC22330 (619 - 639) +   | agcaaaggattgaattgaat   |
| 5 (- 25)                | AACTCTTATCATACTATCAAG  |
| siRNA SS (5'-3')        | AACUCUUAUCAUACUAUCAAG  |
| CN742562 (294 - 274) -  | aactattatgatactatcaag  |
| 67 (- 87)               | TCTTTTTGATTGTGATTTCC   |
| siRNA SS (5'-3')        | UCUUUUUGAUUGUGAUUUUCC  |
| TC20986 (228 - 208) -   | tcttatgttgattttcc      |
| 237 (- 257)             | GCCTCTGGCAGATGCAAAATC  |



|                        |                       |
|------------------------|-----------------------|
| siRNA SS (5'-3')       | GCCUCUGGCAGAUGCAAAAUC |
| GO612005 (22 - 2) -    | gcttctggaagatgcaaaatc |
| 289 (- 309)            | ATCCCTCTCATTGCTTCAGTT |
| siRNA SS (5'-3')       | AUCCUCUCAUUGCUUCAGUU  |
| CK292196 (629 - 609) - | atcactgtcattgcttcagtt |
| 290 (- 310)            | TCCCTCTCATTGCTTCAGTTG |
| siRNA SS (5'-3')       | UCCUCUCAUUGCUUCAGUUG  |
| CK292196 (628 - 608) - | tcactgtcattgcttcagttg |
| 313 (- 333)            | AGCTTTGAGATGATCAGTGTT |
| siRNA SS (5'-3')       | AGCUUUGAGAUGAUCAGUGUU |
| EH369565 (291 - 271) - | agctttgagatgagcagtgtt |
| 314 (- 334)            | GCTTTGAGATGATCAGTGTTG |
| siRNA SS (5'-3')       | GCUUUGAGAUGAUCAGUGUUG |
| EH369565 (290 - 270) - | gctttgagatgagcagtgttg |

Fig. 2 Suppl. The *in silico* off-target determination of a 173 bp conserved *N* gene region, the complete TSWV *N* gene coding sequence or the TSWV, GRSV and TCSV fragments of the Bucher *et al.* (2006) construct. The siRNA scan tool (Xu *et al.* 2006) determined 20 and 12 putative off-targets using the *N. benthamiana* DFCI Gene Index as target for the complete TSWV *N* gene and the Bucher *et al.* (2006) construct, respectively. When the tool was applied to the 173 bp conserved *N* gene region, the number of putative off-targets was strongly reduced to one.

Xu, P., Zhang, Y., Kang, L., Roossinck, M.J., Mysore, K.S.: Computational estimation and experimental verification of off-target silencing during posttranscriptional gene silencing in plants. - Plant Physiol. **142**: 429-440, 2006.

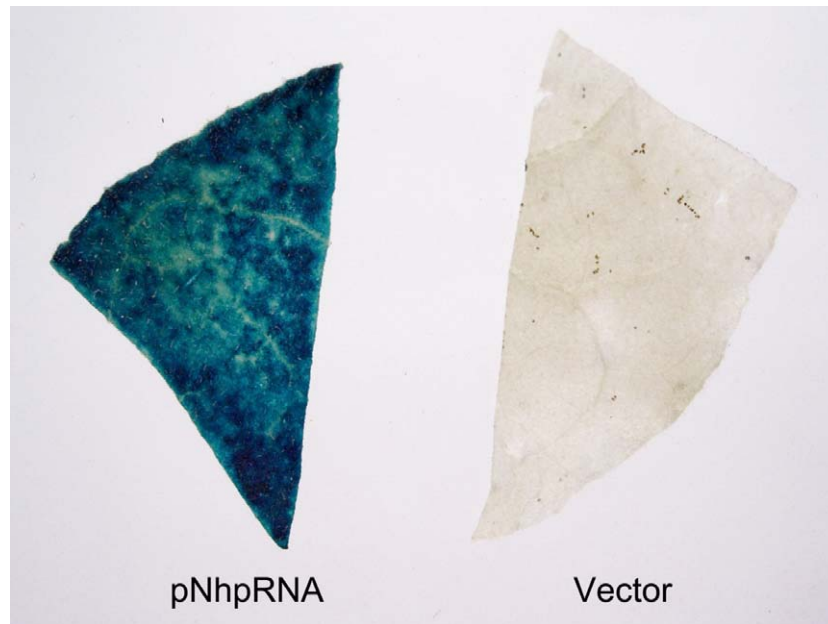


Fig. 3 Suppl. A representative agroinfiltrated leaf tissue (*N. benthamiana*) showing strong uniform GUS staining (pNhpRNA) or no GUS staining (vector). Histochemical staining GUS activity with X-gluc was performed as described by Jefferson (1987).

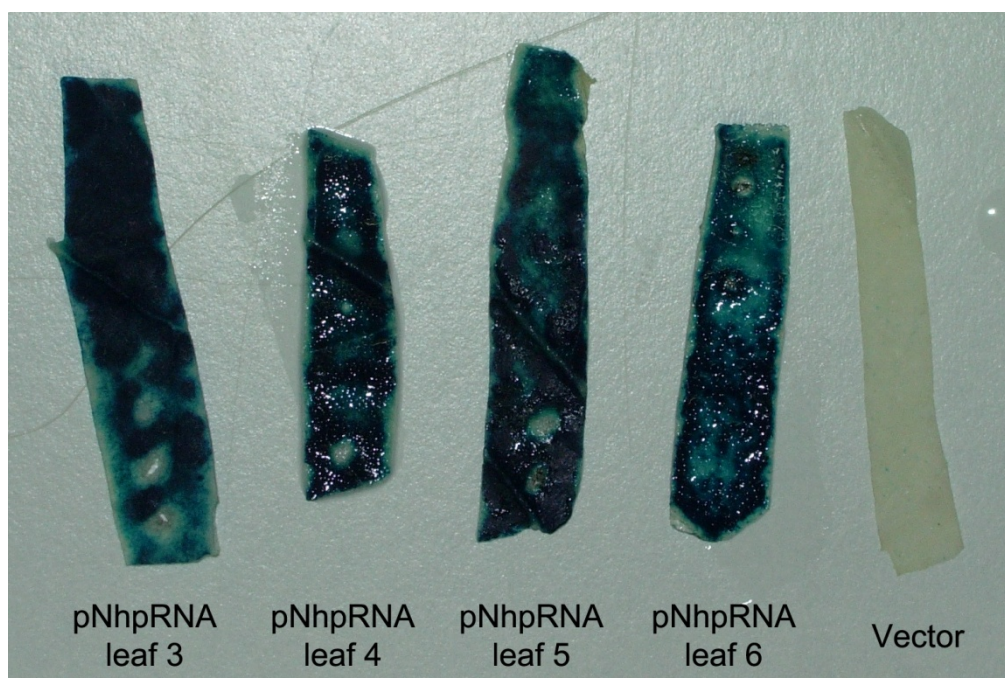


Fig. 4 Suppl. Representative leaf tissues of different leaves of an intact plant (*N. benthamiana*). Shoots were agroinfiltrated with pNhpRNA showing strong uniform GUS staining in every leaf. Histochemical staining GUS activity with X-gluc was performed as described by Jefferson (1987).

Jefferson, R.A.: Assaying chimeric genes in plants: The GUS gene fusion system. - Plant Mol. Biol. Rep. 5: 387-405, 1987.