

1 Characterization of endogenous *Rubus* yellow net virus in raspberries

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13 Abstract

14 *Rubus* yellow net virus (RYNV) belongs to genus *Badnavirus*. Badnavirids are found in plants as
15 endogenous, inactive sequences, and/or in episomal (infectious and active) forms. To assess the state of
16 RYNV infections, we sequenced the genomes of various *Rubus* cultivars and mined eight additional
17 published whole genome sequencing datasets. Sequence analysis revealed the presence of a diverse
18 array of endogenous RYNV (endoRYNV) sequences that differ significantly in their structure; some
19 lineages have nearly complete, yet non-functional genomes whereas others have rudimentary, small
20 sequence fragments. We developed assays to genotype the six main endoRYNV lineages as well as the
21 only known episomal lineage in commercial *Rubus*. This study discloses the widespread presence of
22 endoRYNVs in commercial raspberries, likely because breeding programs have been using a limited pool
23 of germplasm that harbored endoRYNVs.

24 **Keywords:** *Rubus yellow net virus*, raspberry, *Rubus*, *Badnavirus*, virus diversity
25

26 1. Introduction

27 Raspberry is an economically important crop with global production in 2018 being over 820,000 tons
28 grown in 125,000 hectares in all continents except Antarctica ("Production of raspberries in 2018".
29 United Nations, Corporate Statistical Database (FAOSTAT) 2019, retrieved January 14, 2021).
30 Commercial breeding for red raspberry (*Rubus idaeus*) began about 200 years ago, and most of the
31 currently available cultivars share the same germplasm pedigree dating back to the late 1800s and early
32 1900s (Jennings, 2018).

33 More than 40 virus species are known to infect *Rubus*; yet *Rubus* yellow net virus (RYNV) is one of only
34 two badnaviruses known to infect the genus (Diaz-Lara et al., 2015; Shahid et al., 2017). The genus
35 *Badnavirus*, family *Caulimoviridae*, includes viruses that have an inactive, endogenous form and an
36 infectious, episomal form. *Banana streak OL virus* (BSOLV), *Banana streak GF virus* (BSGFV), and *Banana*
37 *streak IM virus* (BSIMV) integrants have been shown to reactivate from an inactive integrated
38 counterpart (Chabannes et al., 2013; Gayral et al., 2008; Ndowora et al., 1999), whereas no other
39 badnavirid is known to reactivate from integrated sequences (reviewed by Bhat et al., 2016).

40 RYNV is a component of raspberry mosaic, an important disease first described in the 1920s (Bennett,
41 1927; Stace-Smith, 1955). The virus infects all red raspberry and most blackberry and hybrid berry
42 cultivars in North America and Europe (Stace-Smith and Jones, 1987a) and can reduce yield from 30-75%
43 in the first year and up to 15% in subsequent years in mixed infections with black raspberry necrosis
44 virus (Stace-Smith and Jones, 1987b). Partial RYNV sequences were first obtained at the turn of the
45 century (GenBank accession number AF468454; Jones et al., 2002). The plant used in the Jones et al.
46 (2002) study had virus-like symptoms and bacilliform particles were observed under the electron
47 microscope. The first RYNV genome (RYNV-Ca, GenBank accession number KF241951), assembled from
48 two PCR amplicons, was obtained by Kalischuk et al. (2008). Another genome (RYNV-BS, KM078034,
49 Diaz-Lara et al., 2015) was sequenced from red raspberry 'Baumforth's Seedling A' using DNA from
50 rolling circle amplification. Since then, several RYNV sequences were published using small RNA
51 (Rajamäki et al., 2019) or whole genome sequencing (MN245240).

52 Diaz-Lara (2016) observed that red raspberry plants, supposedly free of RYNV based on aphid or graft
53 transmission onto *R. occidentalis* 'Munger' indicator, yielded positive results when indexed by PCR-
54 based assays. Moreover, those plants were tested positive for RYNV by PCR even after heat therapy and
55 meristem-tip culture for virus elimination. It was demonstrated that RYNV integrates into the red
56 raspberry genome (Diaz-Lara et al., 2020), but no further analysis was conducted for the reported
57 endogenous RYNV (endoRYNV) sequences. In this study, multiple cultivars were assayed to determine
58 the prevalence of endoRYNV and the lineages identified were validated and characterized in-depth.

59 **2. Materials and methods**

60 **2.1. Plant material**

61 Twenty-five raspberry cultivars maintained as tissue culture plantlets in Watsonville, California were
62 used in the study (Table 1). For 'Baumforth's Seedling A', an additional mature plant was obtained from
63 Corvallis, Oregon with the RYNV-BS (Diaz-Lara et al., 2015; Table 1) and used as a positive control for the
64 episomal form, hereafter referred to as epiRYNV-BS. In addition, the genome of 75 proprietary red
65 raspberry and 100 proprietary blackberry cultivars were sequenced and assayed for integration of RYNV-
66 BS and the episomal form of the virus but their identity is not provided to protect intellectual property
67 rights.

68 **2.2. DNA purification, sequencing, and virus discovery**

69 DNA was extracted using either the DNeasy(R) kit (Qiagen) or the method described by Poudel et al.
70 (2013). All DNA libraries were constructed using a TruSeq DNA HT Sample Prep(R) kit and sequenced
71 individually using paired-end (2 × 300 bp) Illumina HiSeq configuration by Novogene (Sacramento, CA).

72 Raw Illumina reads were subject to de novo assembly using Spades (Bankevich et al., 2012). BLASTn
73 search (Camacho et al., 2009) was performed on the output contigs with e-value=10 against published
74 RYNV nucleotide sequences (nt) downloaded from GenBank nt database (January 16, 2021). After RYNV
75 hits were filtered out, the remaining contigs were processed using BLASTx against a database containing
76 all RYNV protein sequences downloaded from GenBank nr (January 16, 2021). All Illumina datasets were
77 also submitted to VirFind (<http://virfind.org>, Ho and Tzanetakis, 2014) for virus detection and discovery.
78 Bowtie2 (Langmead and Salzberg, 2012) was used for mapping raw reads to RYNV contigs for visual
79 confirmation of the mapping assemblies with Tablet (Milne et al., 2013). BioEdit (Hall, 1999) was used to
80 calculate sequence identity matrix, and ClustalW (Thompson et al., 1994) of the MEGA X software
81 (Kumar et al., 2018) applied to align nucleotide and amino acid sequences. ExPasy
82 (<https://web.expasy.org/translate/>) was used to predict open reading frames (ORF). Conserved domain
83 search was done using the NCBI homonymous tool (Lu et al., 2020). Breaking points of the RYNV
84 lineages were identified by aligning raw Illumina reads with BLASTn against the assembled sequences
85 and partially aligned reads were manually analyzed for sequence identities.

86 **2.3. Electron microscopy**

87 Tissues were homogenized in 100 mM potassium phosphate pH 7.0, 2% polyvinylpyrrolidone
88 (MW:10,000) and 0.2% Na₂SO₃ at 1:20 (w:v). After a low-speed centrifugation at 10,000 g, the
89 supernatant was used for immunosorbent electron microscopy (ISEM) according to Lockhart (1986).
90 Briefly Formvar/carbon coated copper grids were floated on 10 µl of the capture antibodies of
91 sugarcane bacilliform virus (NanoDiagnostics, Arkansas) diluted 1:10 in 50 mM potassium phosphate
92 buffer pH7.0. After 30 min incubation at 37°C, grids were rinsed with 50 mM potassium phosphate
93 buffer pH7.0, and then floated on 30 µl of the sample preparations for 20-22 hrs at 4°C. The grids were
94 then rinsed with 50 mM potassium phosphate buffer pH7.0 containing 100 µg/ml bacitracin and stained
95 with 0.5% phosphotungstic acid pH7.0 containing 100 µg/ml bacitracin. The grids were examined using a
96 Hitachi H-7500 transmission electron microscope (Hitachi High-Tech Corporation, Fukuoka, Japan) with
97 an AMT Biosprint 12M-B CCD camera (Advanced Microscopy Techniques, Woburn, MA). Virus particles
98 were measured using the camera software.

99 **2.4. Data mining**

100 Published datasets were mined for RYNV sequences (Table 1) including raw Illumina data of red
101 raspberry cultivars 'Caroline', 'Cascade Bounty', 'Comox', 'Glen Cova', 'Meeker', and 'Willamette' from
102 the Diaz-Lara et al. (2020) study, 'Glen Moy' from Hackett et al. (2018), and the assembled 'Joan J'
103 genome, obtained using PacBio and Illumina sequencing (Wight et al. 2019). These eight datasets were
104 processed using the procedures described above.

105 **2.5. Development of RYNV lineage-specific primers and validation**

106 For each endo/epi RYNV lineage, 20 PCR primer sets were designed by processing the corresponding
107 sequences using PrimerQuest at default parameters for 'qPCR Intercalating Dyes' option (Integrated
108 DNA Technologies, IDT). The outputs were aligned with all RYNV sequences and 5-10 oligo pairs were
109 selected with each oligo, when possible, having at least 2nt mismatches to other RYNV lineages. SYBR
110 Green quantitative PCR was performed for each set against cultivars 1-25 (Table 1) using 20 ng plant
111 DNA, 5 µl Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, catalog number K0253), 1 µM

112 each of forward and reverse primers, and water to 10 μ l. Amplification was performed on QuantStudio 6
113 Flex instrument (Applied Biosystems) with the amplification program consisting of 95°C for 10 min,
114 followed by 40 cycles of 95°C, 53°C, and 72°C, for 20s each. The melting stage started at 53°C for 1m,
115 increased by 0.05°C/s and stopped after reaching 95°C for 15s. To investigate further the possibility of
116 integration of the epiRYNV-BS lineage, we used the most consistent assay against the epiRYNV-BS
117 lineage developed here against a panel of 271 public and proprietary genotypes bulked into 294 DNA
118 samples consisting of 876 plants (Supplemental table). Samples were considered positive for a lineage if
119 there was amplification with the correct melting point. The previously published assay of Diaz-Lara et al.
120 (2020) was also included in this validation for specificity comparison.

121 **3. Results**

122 ***3.1. A diverse array of endoRYNV is present in raspberry***

123 The presence of RYNV DNA in commercial raspberries was investigated by whole genome sequencing
124 and mining data of 25 and 8 cultivars, respectively (Table 1). Sequencing produced approximately 9 Gbp
125 for each cultivar, representing \sim 30X coverage of the predicted 300-Mbp raspberry genome (Wight et
126 al., 2019). BLASTn and BLASTx steps using contigs assembled from the raspberry genomes found no
127 evidence of RYNV DNA in cultivars ‘Korbfüller’, ‘Lloyd George’, ‘Malling Jewel’, ‘Octavia’, ‘Yellow
128 Antwerp’, or the black raspberry cultivar ‘Munger’. The remaining 27 cultivars showed a diverse array of
129 RYNV sequences, ranging from six partial genomic segments with duplicated and rearranged sequences,
130 to rudimentary fragments of a few hundred base pairs. None of the newly discovered lineages possesses
131 an intact genome. Bacilliform virus particles were consistently observed using electron microscope from
132 ‘Baumforth’s Seedling A’ from Corvallis OR, the only sample in the study with a verified episomal form of
133 the virus (Diaz-Lara et al., 2020) (Figure 1). The size of the virions is approximately 149 nm X 33 nm
134 ($n=102$). No additional badnavirid sequences were detected in this or any other sample using VirFind (Ho
135 and Tzanetakis, 2014) indicating that the observed particles belong to RYNV. Bacilliform particles were
136 not detected from any of the other samples, including the ‘Baumforth’s Seedling A’ from Watsonville CA
137 indicating that all other RYNV sequences are integrated in the raspberry genome.

138 ***3.2. Structure of main endoRYNV lineages***

139 RYNV-derived sequences more than 4 Kbp in length were named based on the oldest cultivars they were
140 identified in. RYNV has five conserved badnavirid domains including reverse transcriptase, ribonuclease
141 H (RNaseH), pepsin-like aspartate protease, a zinc knuckle which is a zinc binding motif from retroviral
142 gag proteins (nucleocapsid), and a ribosomal L25/TL5/CTC N-terminal 5S rRNA binding domain (Diaz-
143 Lara et al., 2015). The reverse transcriptase and RNaseH (RT_RNaseH) domains were concatenated and
144 used for sequence comparison against those of the three available genomes (GenBank accession
145 numbers KF241951, KM078034, and MN245240). The new lineages shared >78.5% in nucleotide
146 identities to each other and the epiRYNV-BS (Table 2).

147 **3.2.1. endoRYNV-CU1**

148 ‘Cuthbert’ (1865 release, New York), has three endoRYNVs (namely endoRYNV-CU1, -CU2, and -CU3)
149 and is the oldest cultivar in the study having an endoRYNV. endoRYNV-CU1 was discovered in 12
150 cultivars (Table 1). The 7268-nt segment has intact 5’ intergenic region (IG), ORF1, ORF2, but when
151 compared to epiRYNV-BS, ORF3 is missing 549 bp after nt1691, corresponding to 183 amino acids (aa),

152 at the site for the ribosomal L25/TL5/CTC N-terminal 5S rRNA binding domain, essential to the
153 badnavirus movement (Figure 2). The four other domains are similar to the epiRYNV-BS. ORFs 4 and 6
154 are embedded intact within ORF3.

155 endoRYNV-CU1 RT_RNaseH region shares 100% nt identity with that of RYNV-Ca (Kalischuk et al., 2013).
156 However, there is 12.9% nt diversity between the two lineages. endoRYNV-CU1 3' IG is intact but very
157 different from RYNV-Ca 3' IG, sharing only 29% nt identity (Figure 3). The RYNV-Ca has two inverted
158 repeats, at nt4325-4693 and nt7564-7932. The endoRYNV-CU1 integrant is highly fragmented and has
159 breaking points at genomic nt positions 12, 15, 767, 815, 1321, 1426, 2473, and 6527 (Table 3). One
160 plant-virus junction was detected at nt7268.

161 **3.2.2. endoRYNV-CU2**

162 The second 'Cuthbert' endoRYNV (CU-2) is present in 11 cultivars. The 7252-nt sequence starts with the
163 complete 5' IG, ORF1 and ORF2. When aligned against epiRYNV-BS, ORF3 is lacking a 141-nt stretch after
164 nt2455, corresponding to 47 aa. Similar to endoRYNV-CU1, it has four conserved domains similar to the
165 epiRYNV-BS but missing the ribosomal L25/TL5/CTC N-terminal 5S rRNA binding domain as well as about
166 428 nt of the 3' IG. Alike endoRYNV-CU1, its ORF4 and ORF6 are embedded intact within ORF3. The
167 endoRYNV-CU2 RT_RNaseH region shares 79% nt identity with that of epiRYNV-BS and the integrant is
168 fragmented at genomic nt positions 2217 and 5319, with plant-virus junctions detected at nt1, 188, and
169 5863.

170 **3.2.3. endoRYNV-CU3**

171 The third 'Cuthbert' endoRYNV (CU-3) is present in five cultivars and is heavily truncated. It is lacking 5'
172 IG, ORF1 and ORF2. Its sequence starts with a truncated ORF3 and together with the 3' IG accounting for
173 a 4550-bp stretch. Its RT_RNaseH region shares 99.6% to RYNV-Cu from Chile (MN245240). The
174 integrant is fragmented at nt4423 and 4550, and has plant-virus junctions at nt1 and 1492.

175 **3.2.4. endoRYNV-BS**

176 The endoRYNV-BS was first detected in 'Baumforth's Seedling A' (1880 release, UK) and is present in 17
177 cultivars. The lineage is 7602 bp, and has intact 5' IG, ORF1, and ORF2. When aligned against epiRYNV-
178 BS, ORF3 is missing a 132-nt stretch after nt2445 corresponding to 44 aa, and the 3' IG lacks 83 bp. This
179 lineage has all five conserved badnavirid domains. The lineage is present in the assembled genome of
180 'Joan J' as a single copy on chromosome 4. The integrant is 12,143 bp and composed of two fragments.
181 The first is in the forward orientation and contains complete 5' IG that forms a junction with the plant
182 DNA at its 5' end, followed by complete ORF1, ORF2, and part of ORF3 that is truncated at nt6317. The
183 second follows immediately after in the reverse orientation, with a truncated 3' IG at nt7602, then
184 continues with ORF3 but truncated at nt1777 fusing to the plant genome. No full-length ORF3 is present
185 in either of the fragments.

186 **3.2.5. endoRYNV-PH1**

187 First detected in Phoenix (1896 release, UK), endoRYNV-PH1 is present in nine cultivars. The 6631nt
188 sequence starts with the 5' IG, followed by the ORF1 of 177 nt and missing 380 nt after nt561 when
189 aligned against the epiRYNV-BS, before an intact ORF2. ORF3 is missing 675 nt after nt1307 as well as

190 the ribosomal L25/TL5/CTC N-terminal 5S rRNA binding domain. It has the four other conserved domains
191 similar to the epiRYNV-BS. The integrant is fragmented at genomic nt positions 335, 1373, 1814, and
192 5859, with nt1 and nt6631 connected to the plant DNA.

193 **3.2.6. endoRYNV-PH2**

194 The last substantial integrated RYNV sequence, endoRYNV-PH2, was only found in 'Phoenix'. The 7091-
195 nt fragment's 5' end has the intact 5' IG, ORF1 and ORF2. ORF3 misses 141 nt after nt2461
196 corresponding to 47 aa, and the fragment terminates at nt7091. This sequence has all conserved
197 domains found in the epiRYNV-BS. The integrant is fragmented at nt2201 and 2432, and has two plant-
198 virus junctions at nt3094 and 7091.

199 **3.3. Validation**

200 SYBR Green qPCR assays were able to differentiate between the epiRYNV-BS and each of the main
201 endoRYNVs in all cultivars used in this study, either in single or multiple integration events (Figure 4,
202 Table 1). Except the positive control 'Baumforth's Seedling A' OR, the epiRYNV-BS lineage was not
203 detected in any of the genetics used in this study (Figure 5). The epiBS-2604F/2715R assay did not have
204 off-target melting points and amplifications, compared to the Diaz-Lara et al. (2020) assay, presently
205 considered the better assay for RYNV detection, with off-target in 43 cases (Supplemental figure).

206 **4. Discussion**

207 We analyzed the genome sequence data of commercial cultivars from around the globe released as
208 early as 1802 and as recent as 2006. Integrated RYNV sequences were present in 27/33 cultivars (82%).
209 The endoRYNV population could be categorized into six main lineages and other short endogenous
210 fragments. The diversity of endoRYNV is complex with sometimes sequences having inversions,
211 duplications, or deletions.

212 *Rubus* domestication has resulted in a reduction of genetic diversity (Haskell, 1960; Jennings, 1988), and
213 modern cultivars are genetically similar to each other (Dale et al., 1993; Graham and McNicol, 1995).
214 This can be seen in the case of the cultivars analyzed in this study. All breeding programs share the same
215 endoRYNV lineages, which in turn were discovered in three cultivars commercialized in the 19th century:
216 'Cuthbert' (1865), 'Baumforth's Seedling A' (1880), and 'Phoenix' (1896). These endogenous sequences
217 presumably became widespread as the three aforementioned cultivars were used as parents, or are in
218 the lineages of most raspberry breeding programs worldwide.

219 endoRYNV-CU1 lineage is the closest isolate to the published RYNV-Ca sequence (KF241951) (Kalischuk
220 et al., 2013). Since RYNV-Ca has two inverted repeats, misses the true 3' IG, and hence likely is an
221 endogenous sequence, we consider epiRYNV-BS as the sole episomal RYNV lineage known to infect
222 *Rubus*. It is important to note that when aligned against the epiRYNV-BS sequence, all endoRYNV
223 lineages are truncated and missing genomic DNA stretches. From this data, we hypothesize that the
224 endoRYNVs are unable to reactivate and become episomal due to their incomplete genomes. In addition
225 to the raspberry cultivars of this study, we sequenced the whole genome of an additional 75 proprietary
226 red raspberry cultivars, and epiRYNV-BS was absent in all (data not shown), indicating that this lineage
227 may be unable to integrate in the raspberry genome. We also sequenced the genomes of 100

228 proprietary blackberry cultivars (data not shown) but did not find any evidence of endoRYNV, suggesting
229 that endoRYNV sequences may be limited to red raspberry.

230 Diagnostic tests for infectious agents are necessary so that phytosanitary agencies can protect a
231 country's natural resources and agriculture. However, the Rubus industry could be significantly
232 impacted if a diagnostic test was positive for RYNV but inadvertently a no-risk endoRYNV was detected.
233 Published PCR primers were designed to target either RYNV-Ca or epiRYNV-BS as they had been the only
234 known RYNV lineages (Diaz-Lara et al., 2020; Jones et al., 2002; Kalischuk et al., 2008). Diaz-Lara et al.
235 (2020) showed that primers currently used for RYNV detection could produce positive results in cultivars
236 only harboring endoRYNV DNA, indicating the urgency to have a good diagnostic test that can clearly
237 differentiate the two forms, similar to epiBS-2604F/2715R. This test should be developed and validated
238 for accuracy and sensitivity against a wide range of episomal isolates.

239 Theoretically, endoRYNV can be removed from the red raspberry by traditional breeding. However, the
240 effort required to remove endoRYNV DNA after multiple generations of backcrossing would be
241 considerable, especially when desired traits must be retained. CRISPR-Cas9 could be used to remove the
242 endoRYNVs, but for cultivars with multiple endoRYNV fragments, multiple gene-editing events will need
243 to be done. We believe that such actions are not necessary as endoRYNV fragments could not
244 reconstruct a full, infectious, genome.

245 **5. Conflict of interest**

246 T.H., J.C.B., J.P.B., W.O. are employees of Driscoll's Inc.

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251 **7. Data availability**

252 The assembled sequences of endoRYNV-CU1, endoRYNV-CU2, endoRYNV-CU3, endoRYNV-BS,
253 endoRYNV-PH1, and endoRYNV-PH2 were deposited on the NCBI's GenBank under accessions XXX-XXX,
254 and the raw Illumina reads of all cultivars sequenced in this study to the Sequence Read Archive (SRA)
255 under accessions XXX-XXX.

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- 353
354

355 **9. Tables**

356 Table 1. Description of the origin of the raspberry cultivars used as plants for Illumina sequencing and
 357 method development in this study, or mined from published literatures, sorted by year of release, and
 358 subsequent RYNV detection result* using analysis of whole genome sequencing data and validated by
 359 SYBR Green PCR detection. **

	Cultivar names	Year Release	Breeding Program	Endo RYNV -CU1	Endo RYNV -CU2	Endo RYNV -CU3	Endo RYNV -BS	Endo RYNV -PH1	Endo RYNV -PH2	Fragment	Epi RYNV -BS
1	Yellow Antwerp	1802	Belgium	No	No	No	No	No	No	No	No
2	Cuthbert	1865	New York	Yes	Yes	Yes	No	No	No	Yes	No
3	Baumforth's Seedling A	1880	UK	Yes	No	No	Yes	No	No	No	Yes/No***
4	Phoenix	1896	UK	No	Yes	No	Yes	Yes	Yes	Yes	No
5	Latham	1920	Minnesota	No	No	No	Yes	Yes	No	No	No
6	St. Regis	1920	New Jersey	No	Yes	No	Yes	Yes	No	Yes	No
7	Lloyd George	1923	UK	No	No	No	No	No	No	No	No
8	Malling Landmark	1943	UK	Yes	No	No	No	No	No	No	No
9	Korbüller	1945	Germany	No	No	No	No	No	No	No	No
10	September	1947	New York	No	Yes	No	Yes	Yes	No	Yes	No
11	Mandarin	1955	North Carolina	No	No	No	Yes	No	No	No	No
12	Chilcotin	1965	British Columbia	No	No	No	Yes	No	No	No	No
13	Southland	1968	North Carolina	No	Yes	Yes	Yes	Yes	No	No	No
14	Heritage	1969	New York	No	Yes	Yes	Yes	Yes	No	No	No
15	Malling Jewel	1980	UK	No	No	No	No	No	No	No	No
16	Titan	1982	New York	No	No	No	Yes	Yes	No	No	No
17	Autumn Bliss	1984	UK	No	Yes	No	Yes	Yes	No	Yes	No
18	Summit	1989	Oregon	No	No	No	Yes	Yes	No	No	No
19	Tulameen	1991	British Columbia	No	No	No	Yes	No	No	Yes	No
20	Qualicum	1995	British Columbia	Yes	No	No	No	No	No	Yes	No
21	Prelude	1998	New York	Yes	Yes	No	Yes	No	No	No	No
22	Polka	2001	Poland	No	No	Yes	No	No	No	No	No
23	Octavia	2002	UK	No	No	No	No	No	No	No	No
24	Chemainus	2006	British Columbia	No	No	No	Yes	No	No	Yes	No
25	Munger	1890	Ohio	No	No	No	No	No	No	No	No
26	Willamette	1943	Oregon	Yes	No	No	No	No	No	Yes	No
27	Meeker	1967	Washington State	Yes	No	No	No	No	No	No	No
28	Glen Cova	1969	UK	Yes	No	No	No	No	No	Yes	No
29	Comox	1978	British Columbia	Yes	No	No	No	No	No	Yes	No
30	Glen Moy	1986	UK	Yes	No	No	No	No	No	Yes	No
31	Caroline	1998	Maryland	Yes	Yes	Yes	No	No	No	No	No
32	Cascade Bounty	2005	Washington State	Yes	Yes	No	Yes	No	No	Yes	No
33	Joan J	2005	UK	No	Yes	No	Yes	No	No	No	No

360 *: Yes = positive; No = negative.

361 **: Samples 1-25 were plant samples in this study and validated using SYBR Green PCR detection, while
 362 26-33 were data mined from the published literature and not subject to SYBR Green PCR. Further
 363 descriptions are presented in sections 2.1 and 2.4.

364 ****: Yes for the plant housed in Corvallis OR that has the episomal RYNV-BS; No for the plant housed in*
365 *Watsonville CA.*
366

367 Table 2. Percent identity matrix of the reverse transcriptase and RNaseH region of the six Rubus yellow
 368 net virus (RYNV) lineages discovered in this study against the three published RYNV sequences,
 369 *Gooseberry vein banding virus* (GBVBV), *Grapevine vein clearing virus* (GVCV), and *Birch leafroll*
 370 *associated virus* (BLRaV).

	endoRYNV V -BS	endoRYNV V -CU1	endoRYNV V -CU2	endoRYNV V -CU3	endoRYNV V -PH1	endoRYNV V -PH2	epiRYNV -BS KM0780 34	RYNV -Cu MN2452 40	RYNV -Ca KF24195 1	GBVBV HQ8522 49.1	GVCV KT90747 8.1
endoRYNV- CU1	80.5										
endoRYNV- CU2	80	82.5									
endoRYNV- CU3	79.8	90.3	81.8								
endoRYNV- PH1	80.3	90.3	81.3	88.5							
endoRYNV- PH2	81.1	87.1	89.3	85.2	85.4						
epiRYNV-BS KM078034	78.5	81.2	79	82.1	84.4	81.3					
RYNV-Chile MN245240	79.7	90.1	81.7	99.6	88.3	85	82				
RYNV-Ca KF241951	80.5	100	82.5	90.3	90.3	87.1	81.2	90.1			
GBVBV HQ852249.1	71.1	71	71.5	71.1	72.1	71.8	71	70.9	71		
GVCV KT907478.1	67.7	68.1	66.7	67.5	68.4	66.9	68.5	67.4	68.1	68.2	
BLRaV MG6864191	66.9	66.4	65.4	66.4	67.3	65.7	65.7	66.3	66.4	67.3	69.8

371

372 Table 3. Break points in the genomic sequence, and plant-virus junctions of the endogenous RYNV
 373 lineages discovered in this study. Nucleotide positions relative to the corresponding RYNV genomic
 374 sequence are shown within parentheses. Vertical bars indicate break/junction points.

Lineage	Break point	Nucleotide sequence
endoRYNV -CU1	6527 12	(6390)GGCTTCCCAAGGAACTAGCGGAGCTCACGGCCGAACTGTCAAAGGAAGGGA CGAAGCCCTGGTGAACAA GGAGGTACAGAGGAACATCTCATGTTTCTCGAGACTGCCCTCTCCAAGCGGAGAAATCCGTGACTA(6527) TAC(12)GCTCTGATACCA(1)(7268)GAAAAATGGCCGAAAACATGGATCAAACCTTCTAAGTGCCTCTTGAACA CTTAGAAGACATAAAATTCAGAGACAATGCGGAAGATAGAACTGAATAAACTCTTTATTTCTCAAGAAAGTTAATA CAATACAAGAGAAGGTT(7123)
	15 815	(132)TTTGACACAGACACTCACTCTTCCCTCCGAAAAAGAGAGCAAACCTCAAGGTTCTGGTCTGAAACTTGGGT TTCTCGACAAGAAGGAAAGTGTTTAAAGCTACCATGGTGAGAGCTA(15)TG (815)AAGGCCACACAGTCAGCCCTGAAGCTAGGCTTCGCACAACTGCAGGA GGCAGTTTCAGCTGATCATCAAGG GAAAACGATCCCAAACCAATCGAAGCAGCTACTGCACAAGTAGCCGAA CAGCTGAGGAAGCAGCTTATTGAGGTC AAGTCCGTCCTCGAGGAGACCAAGAAGATCGC(993)
	767 1426	(685)CTCCAGACTCACCCAGTACCTGACAACAAAGGTTGGTTCCTACCAACAATCCCGGAGGATTACCCCTCCTG GACCAAGCCA(767) (1426)CAATACAATGAGCAGAAGCCAAACAAGGCTCCAGGCTCCGCCGCAACTGAAAGGG CAACAAGCAGCTCG GAATCAGGCACCCCACTTGGAGGACCAGATCCGAGGATACAGGCGCTCCGCAAGGTTACGACACCAGGC(1566)
	2473 1321	(2367)ACGAGGCAGAGGACGAGGAGGATCAGGAAAGTACAGGTGATAGGAGCTACCAACCATTGAGGAGCCAGA GA TGGAGTACCAACTAGGCTCGAAGAAGTTATGGGCAAG(2473) (1321)CTCACTAAGGATTCGGAAGGTTCAACTTAGGGAAAAGGAAAGGGGATAGAAGGAGCAGTCTCATCCAGAG ACAAGAACTTCTACGTCTGGAAGAACCCCTTCAATCAATACAATGAGCAGAAGCCAAACAAGGCTCCAGGCTCCGC CCGCAACTGAAAGGGCAAACAGCAGCTCGAATCAGGCACCCCACT(1513)
	7268 plant	(7103)ATCGAGTAAGCTCAATAGCCAACCTTCTTGTATTGATTAACCTTCTTGAGAAATAAAGAGTTTATTTCAGTT CTATCTCCGATTGTCTCTGAATTTTATGTTCTTCTAAGTGTTCAAGAGCGCACTAAGAAGTTTTGATCCATGTTTT CCGGCCATTTTT(7268) (plant)TGGTAACATCGACTAAATATTGTTGAAATAATCTCCACCTTTTAAACAAGATCTGTACTTTTATTCTGTCA TGGATTTGATGCAACAAAACCTTCGATTGACATTTTTTTCTTTTGATTCTTCATTT(plant)
endoRYNV -CU2	2217 5319	(2087)TGGGCAGGTGGAGACAGCAATATGATCATCACCAGATCTCTGGTGGGACGATTGACTAACACCAGCATGA C CAACTTTGAGTACCGGATAGAACAAGTACAGACTACTGGCGAGCAATGGAGTCGCATG(2217) (5319)ACTACTCAGAAGGAGGATGAGCAGCTGAGAAACCAAGCTGCTACAAGAACTGAAAGAGCAGGGATA CATCGGAGAAGAGCCCATGAAGCACTGGGCAAGAATGGGA(5429)
	plant 1	(plant)GAATTTAATTAGAGAAACAATTTAAACCATTCAAATTTTCATTACAATGCGTGTATATGTGAAAATAGGAAAA CTAGCAATAATTGGCACAAGGGGAGTTCCATAATTA(plant) (1)TGGTAGATTGTACTTTAGCTCTATTCAACATGTTAGCTTAAACTTTTCATTTCTTGTGCGAAAACCAAGTTTCAGA CTAGAACCCTTGAGTTCTCTCTTTTTCAGGGAGGAGAGAGTAGTGAGCATTGCAAAAACCTTCTGCAACTTTCAAACC CCCATGAAAGCTTTCC(279)
	plant 188	(plant)TAACATCCATCCTTGTAAAAATACTTGTATTCTTGCCTGATATTTTCTGATTTGAACTGTACAATCTGCT GTCCTGTTATTTGCCTAGCCAC(plant) (188)TTCTGATTTTCACTAGTTCAAACCTACTGCTTAAACTGCAGGCTTAGGCGTCGAAGCGAAGTACCTTGTAGCC GTTAGCTGGAGTGCGTTAGGCGTTGATTGGGAAAACGACGTAAGAAGCAGCAGCAACTAGCAAGAAAACCT(335)
	5863 plant	(5738)GGCAACGCCAAAATCTTCAGCAAGTTCGACTTGAAGTCTGGTTTTCCACAGGTAATGGATGAAGAGTCC ATCCATGGACGGCGTTTGTACACCTGTAGGCTTCTACGAGTGGAAAGGTGATG(5863) (plant)TCAGAAAATTGGCCGAACATAGGATCAAAAACCTTCTAAGTGCCTCTTGAACACTCAGAACTCATAAATT CAGAGAAAATTGGGAAGATAAGATGAACAAAAGCTCTTATTTCTCAAGATGTTAATAACAATTCAGAGAAGGTT GGCTATTGAGCTTACTCGATGCCTA(plant)

endoRYNV -CU3	4550 4423	(4434)AGATTGTTCTTCAGTTTTCCGCAATATCTCTCAGTTTTATGAGTTCCTAAGTGTCAAGAGCGCATTTAAGAA TTTTTGATATTGCGGAAACTGAAGAACAACTTTATTTCTCT(4550) (4423)TGAAACGTTAATACAATACAGGAGAAGGTTGGCTATTGAGATTGCTCGATGCCTAAGCCTTCAGACTACACTC CCTTATATAGGAAGTAGGCTAAGCAAAACGACAGACAACACTAGCTCGGGTGCCTTTCTGGGCCCCATC GACAG CTAATAAGTTCTGCTTTACAGCTTTGCTAAAGCT(4242)
	plant 1	(plant)TGCTAGCGTGCCAATATGATGAACTGAAGCTATGAACCTTTGCTAGTTGTGACTGAATTGTGGTACTTGA TTTGGTTGAGGTTGTATTAGGATGGGTATGCCAGGTAGGGGAACTCTTGCAGTGAATAGCTACAGCAAGAGGA AGTTGCAGCAAAATGCAAGACTCTTGCAGTGAATAGCTACAGCAAGAGGAAGTTGCAGCAAAATGCAAGAGGACC TCTTGT(plant) (1)ACAACGGTGTATGCTAGTCTACCGCAGGACTTCACCTTAGTCCCTGACGTGATCAACA(60)
	plant 1492	(plant)AAACAAAAAATAATGTATGGTCCATTAGCCGAAATCAATTCTGCTGTAGGCATCATTTTGCCTGTGGGA TCTGACTGATCTTGTCAAGTCTCTTGTGATAGCTCCAGTCAAACAC(plant) (1492)TCCGATTTGGAACAACGATTCCACCGATCACATCCAAAACCTTGAAAATGTAGCAAGGATGATCGAACAA GGAAGGAAACCCCAAGGTAATCATCAAAGAAACAGCTGAGAGCAGCAGCAACACCATTGGAGCCCTTAGCA G AAGAAGGAATAGAGGAGCTAGCCGAGCTGTA(1670)
endoRYNV -BS	7602 6317	(7445)CCAAGCGCACCCGGGCTAATTTCTCTTGTCTTTAGCATAAGCCCCCTTCTATATAAGGAAGCTAAGTTAG AAGGCTTAGGCATCGAGCAACCTCAATAGCCAACCTTCTCTTGAATATCAGTATTCAAGAAATTAGTATTCAAGAA ATTAGTTG(7602) (6317)CTATCCCCTGTTCACTTGTCTTGTCTATACAGAGGCCAAGCAAGGTTCCGCATTTGGGAATGTAGTTTCTGG CGTAGTTGAGGACTCTAGCCAGCTCTAATCCTTTGAGGGTCT(6199)
	plant 1	(plant)TCAAATTTTGTGAAAAATTTGTAGAAGTGATCTACTCATGAATATTTAATACTGAACAGTTGATTTGTGCGAG ATGTAATCGAAAAATAGATATCACAACCTAATCAAATAAATACTCATTTAAGTGAATTTTTT(plant) (1)TGGTATCAGAGCTTTAGCTCTTATTATGTCAGCTTAAACCTTTTTTCTGTCGAGAAACCAAGTTTCGGA TCTGAACCTTGAGTTCTCTCTTTTTAGGAGGGTAGTGAGTAAGCCAGCTTTGCAAATTTCAAACCCCAAGGAAA ACTTTCCC(161)
	plant 1777	(plant)AATTAAGTGATCGATTATCAGACCGAGAATTAATCTATTCATGGTTCAATAATCAGTCCGATAATATATATAT ATCAATATATGCACTGATCAAACAATTA(plant) (1777)TTCAGGTACATTGAAGAATCCTCTTACCAAAGGCTAGCAGGAGAAGGCATGCAGTTTATACATGTAGGCATG GCCATGGTAAGAATACAGATGCTGCACAGGACTGACGCA(1887)
endoRYNV -PH1	335 5859	(452)AGTCGCCGTTTCAAGTCTTTCCAGGAGCTACTCTTCTTCTGAAAGGCAGAGATTCGTGTGTTGCATCAAAA CCACCAAGTCTAGCCGCTTACTAGCTTTCCGGCCGGTGTCTG(335) (5859)TCACCTGGTCAAATGGCTTCCAGAAAGAACTGAAGGATCTCGCGCCGAGCTAGCCAAGAAAGAGGCAA GACCTCCCTGAAGGGAGAGGTGACAGGAGGATCTCTGTTTTCTCAGAACTGCC(5984)
	1373 1814	(1215)GGGACTTCAACGGCAACTAGATCCGGATGCCGAGCTCTCTCAGCAGAAAGGGGAAAGCAACCTGTGA CCAGCAGAGGTAACACACTGAAAGAAAGGACCATCCGAGCCAGAAGGAAGGCCAGAAGGAGAGGACGA GAGCACACATTATG(1373) (1814)ATCCCCGAAAGAAAGAAAGAAAGTCTACCTCAAATATGAGGCAGAAAGCAGAAAGGAGGATCAGGAGCTT AAGTATTGGAGCCACCACATAGAAGAGCCAGAAATGGAATACCAAAGGCTCAGGAAGTTATGGGC(1954)
	plant 1	(plant)AAGGACGAGCTGATTTCAAGTGAATAAAGATTGTTTCATTTTTCTCCGCATTTTCTCTGAGTTTCATGA GTTCTTAAGTGTCCAGAGCGCACTTTCGAATATTAGATCCATGTTTTTCGGACCCATT(plant) (1)TGGTATCAGAGCTTTAGCTCTACCATGTTAGCTTAAACCTTTTTCTGTCGAGAAACCAAGTTTCAGATCTG AACCTTGAGCTTTCTCT(95)
	6631 plant	(6542)GCTTAGCCTACTTCTATATAAGGGAGTATAGTCTGAAGGCTTAGGCACAGAGCAATCTTTAGCCAACCTT CTCTTGAGTTGTATTA(6631) (plant)TATTCAACCTTCCCTAAACCTAAACCTAAACCTAAACCTAAAGCAGCAGGATGAGAGCTAAAGCTCT GATACCAAGTGGGGTCCGAAAAACATGGATCTAATATTGAAAGTGCCTCTGGAACACTTAAGAACTCATGAAA CTCAGAGAAAATGCGGAAGAAAATGATGAACAATCTTTATTTACCAAGTGAATCTGTTT(plant)
endoRYNV -PH2	2201 2432	(1990)ACAATTGGTATACATAATACAAACGCAATGATGTCAATACAGATTTTTACAATTGTATACAGGTCAGCGTG CAAACCGAGGCTACGGGACAGGATGGGCTGGAGGAGACAGCAGTATGATCATCACTAGATCACTGGTGGGCGT CTCACCAACACCAGCATGACAAACTTCGAGTACCGGATAGATCAAGTACAGACTATCTAGCAA(2201)

		(2432)AGCACGCACTACGTGCTCGTCTTCAGACATAACTCCTATGAGACCAACCTCAGAGGAGAAAGGAGGCCAAGGCAGAACGAACTTTC(2517)
	7091 plant	(6970)GCAGAGATGGACGAGTCTAGAAGACTGGCCAAACAACGCCGAGACAAAGTCTTCGACGACG CAGG GCAAAACATCTGCGACACGGTCTACATCACCGGTGTCGACCTCGCCGCCGCCAAGGC(7091) (plant)ACGCGGCCACATTTGCAGCCTTGGTGGTGATAGTTTTGACTGTTGCTTGCAATGCTCCACTTGCTAAGTTGCAACATAAGTTTCAGAGTAAAGCTTGTGGTGTCACAAGATGAAAAAGGCTCAAGGCTAGTTCTGAAGCTCTTGTAACA TGAAAGTATTTAAATTATATTCATGGGAA(plant)
	3094 plant	(2924)AGTGGCATGGATAGCGAGTCCAGCATCACAGGTGGAGGATTCATACCACCAAGCCAGTACCAGGAGCGCAGGGATACCCACCTGCCACAGGAGCGTCTGCTTCAACCATAGGGCCAGCAGATCTGCAAGGATGGGGAGGACGAT TGCCAAGGAGCAGATCGCCGATAGGA(3094) (plant)AAATGGCCCAACATATAGATAGGAAAATGGCCCAACATATAGATCGAAAAATGTCTAAGTGCGCGCTTGAA CACTTAGAAACTCATAAAAACCTCAGAGAATATGCGGAAGATAAGATGAATAAACTCTT(plant)

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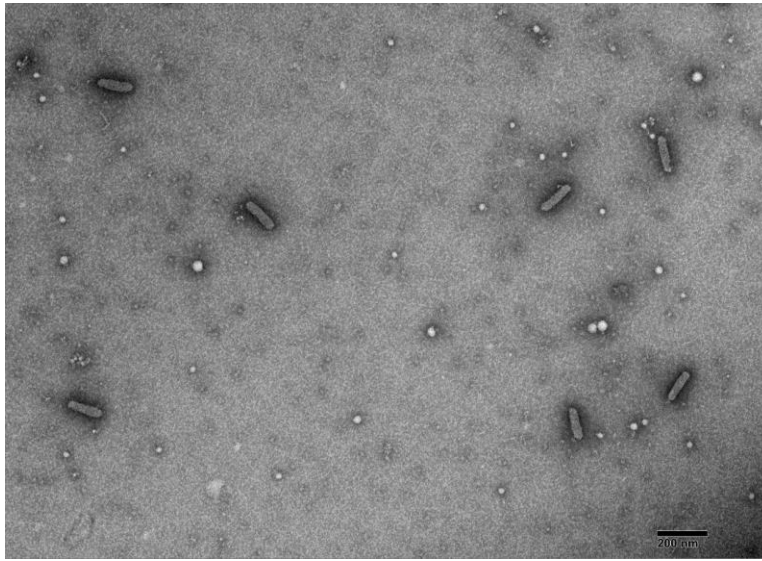
377 Table 4. Primer pairs used for Rubus yellow net virus lineage-specific SYBR Green PCR validation.

Lineage	Primer name and sequence	Amplicon size (bp)	Melting temperature (°C)
endoRYNV-CU1	endoCU1-6376F: CACTGTAGTAAGATGGCTTCCC endoCU1-6475R: GTTCCTCTGTACCTCCTTGTTT	100	80.99
endoRYNV-CU2	endoCU2-2415F: CGGAAGAAGAAAGCACACATTAC endoCU2-2527R: CTCTGGCGTGAACCTCTGAAA	113	79.94
endoRYNV-CU3	endoCU3-2450F: CACAAACTCAGGGACGTCTATC endoCU3-2575R: ATCCCTGGCAAGGTGTATTG	126	79.15
endoRYNV-BS	endoBS-4668F: CCTCCAGAACATCGAGAATGTC endoBS-4767R: GCTAGGCATTTTCGTACCTATTA	100	77.57
endoRYNV-PH1	endoPH1-2633F: GAGCTGTCGGAGAAGCTATTT endoPH1-2725R: TATCGTCAACCCTGGGTATCT	93	77.83
endoRYNV-PH2	endoPH2-3892F: GTGCTACCTTTGCGGAATAGA endoPH2-3989R: CTAAGCCCATGCCATTGAAATAC	98	79.02
epiRYNV-BS	epiBS-2604F: TCCTACGAGGTAAGCCTAAGAG epiBS-2715R: CCGAGTTTCGAGAGTTGGTTTAG	112	79.81

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379

380 **10. Figures**

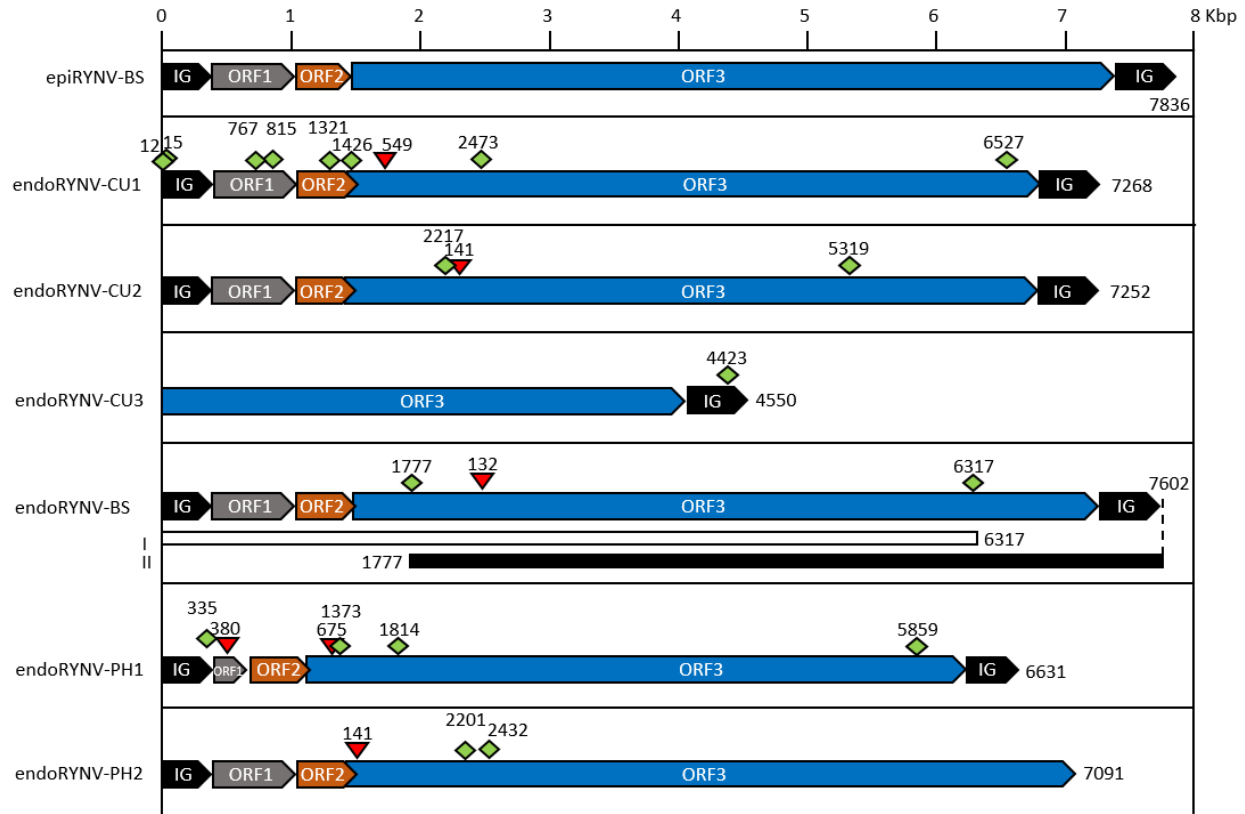


381

382 Figure 1. Bacilliform particles of rubus yellow net virus from 'Baumforth's Seedling A' (epiRYNV-BS)
383 captured using antibody against sugarcane bacilliform virus and stained in phosphotungstic acid.

384

385



386

387 Figure 2. Overview of endogenous rubus yellow net virus (endoRYNV) lineage sequence structures in red
 388 raspberry. The sequences are represented with intergenic region (IG) in black; grey, orange, and blue
 389 boxes indicating ORF1, ORF2 and ORF3 of the virus, respectively. For simplicity, ORF4 and ORF6
 390 embedded within ORF3 are not illustrated. Break points and deletions in the genomic sequence are
 391 represented in green diamond and red triangle shapes, respectively, with nucleotide location on top.
 392 The two fragments of endoRYNV-BS that were found in high-quality 'Joan J' genome are shown in high
 393 and black bars.

394

395

```

endoRYNV_CU1      ACGTCTAGTGAAGTGACGCAATGAATGACTTCACAATTGCCAATGTCGTCACCTGCTTACG
RYNVCaKF241951   ACGTCTAGTGAAGTGACGCAAGGAATGACTTCACAATTGCCAATGTCGTCACCTGCTTACG
*****

endoRYNV_CU1      ACTTGGAACCTTATCGTTTTGTGTGCGGCAGCATCTCTTAGCTGTCAATTTGTGTGTAAGTGC
RYNVCaKF241951   ACTTGGAACCTTATCCTTTAGTGTGCGGCAGCATCTCTTAGCTGTCAATAAGTGTGTAAGTGC
*****

endoRYNV_CU1      GCGGCTAGTGCCTGTGTCAAGATAAGGAATCTTATCTCCTTATCTTTTGC--TTTGT
RYNVCaKF241951   GCGGCTAGTGCCTGTGTCAAGATAAGGAATCTTATCTCCTTATCTTTTGC--TTTGT
*** ***** ** *

endoRYNV_CU1      -AAAGCTA--GCTGTAAAGCAGATTCTCTTAGCTGTGATGGGGCCAGAAAGCGCACCC
RYNVCaKF241951   TAAAGTTAAAGCTGTAAAGCAGGACTAATTAGCTGCAGGTCA--TCAGGTTTGGCGTTGT
*** * ***** * * * *

endoRYNV_CU1      GAG--CTAGTGGTCATCTGTCTTTTGTCTTAGCCTCTCCCTATATAAGGGAGCTCAGT
RYNVCaKF241951   GGAACCTCTGCAGCTGACTGGTGAGCTCTTCGACTTTTCTAGTGAGGAAAGCGTTGTGCT
* * * * *

endoRYNV_CU1      TAGAAGGCTTAGGCATCGAGTAAGCTCAATAGCCACCTTCTCTGTATGTAT--TAA
RYNVCaKF241951   CATAGAGATGTCGTATAAGCTCATCTTTGTTGTGGTATTGCCACTTAGCTGCGTCCTTGG
* * * * *

endoRYNV_CU1      CTTTCTTGAGAAATAAAGAGTTTATTCAGT--TCTATCT-TCCGCATTGTCT-CTGAATT
RYNVCaKF241951   TGTCTTTGCGGCTATAAGCTTGATCCCATGATCCATGTATGCGCAAAGGGGACAGAGT
* *** ** * * * * * * * * * * * * * * * * * * * * * * * * *

endoRYNV_CU1      TTATGTTCTTCTAAGTGTCAAGAG---CGCACTAAGAAGTTTGTAT-CCATGTTTTC
RYNVCaKF241951   TGAGGTTGCAGGTGGTGCAGGTGACTCTTCGCCCATGAGGCGTTTCGTCGCTGCATATGC
* * * * * * * * * * * * * * * * * * * * * * * * * * * * *

endoRYNV_CU1      GGGCCATTTTTC-----
RYNVCaKF241951   TGCATATCCTCCCTTCGATCGGCACCTTCTTGTGTATCACTCCAGGCGTGAGTGCATTCCT
* * * *

endoRYNV_CU1      -----
RYNVCaKF241951   GCTGTGCCTTTGGTATCTCCTTCTTCTCCAGGAAGGTTTTC

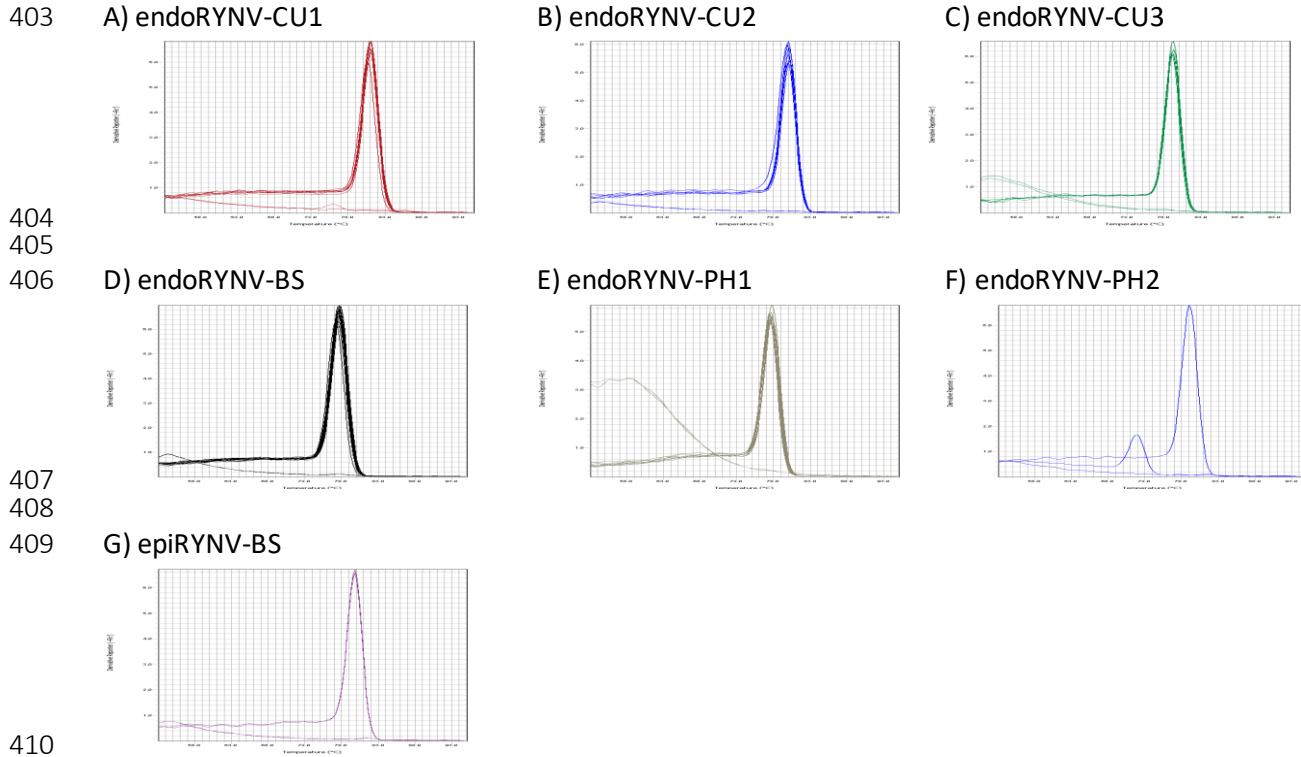
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397 Figure 3. ClustalW alignment of the 3' intergenic regions of the endogenous rubus yellow net virus
 398 (endoRYNV) lineages Cuthbert and Canada (endoRYNV-CU1 and RYNV-Ca respectively. Although the two
 399 lineages share 100% nucleotide (nt) identity at the reverse transcriptase and RNaseH domain, their 3'
 400 IGs share 29% nt identity.

401

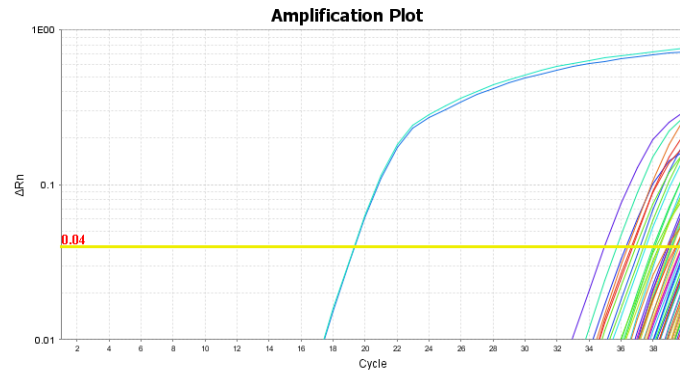
402



411 Figure 4. Melting curves for the lineage-specific SYBR Green PCR validation assays detecting the
412 endogenous and episomal rubus yellow net virus (endoRYNV and epiRYNV respectively) lineages from
413 the raspberry cultivars with corresponding endo/epiRYNVs. A) endoRYNV-CU1; B) endoRYNV-CU2; C)
414 endoRYNV-CU3; D) endoRYNV-BS; E) endoRYNV-PH1; F) endoRYNV-PH2; G) epiRYNV-BS.

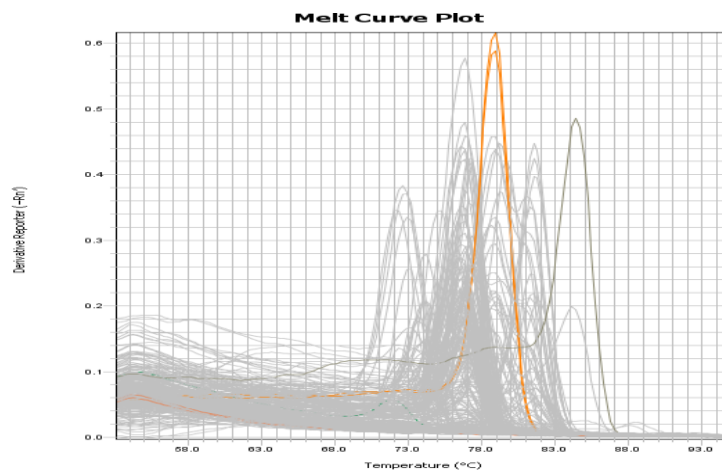
415
416

417 (A)



418

419 (B)



420

421 Figure 5. Detection of the rubus yellow net virus from 'Baumforth's Seedling A' (epiRYNV-BS) lineage
422 using the epiBS-2604F/2715R assay against a panel of 271 public and proprietary genetics. The plants
423 were as described in 2.5. A) Amplification plot (the epiRYNV-BS positive control from Corvallis, Oregon,
424 with two replicates were labeled in blue and light blue); B) Melting plot (the two positive control
425 replicates were labeled in orange with unique melting point compared to all other non-specific
426 amplifications, labeled in grey). epiRYNV-BS was detected at Ct=19, whereas non-specific amplifications
427 initiated after Ct=35 but their melting points were different from that of the positive control.

428

429

430 11. Supplemental data

431 *>endoRYNV-CU1*

432 TGGTATCAGAGCTTTAGCTCTACCATGGTAGCTTAAACACTTTCCTTCTGTGCGAGAAACCAAGTTTCAGACCAGAACCTTGA
433 GTTTGTCTCTTTTTTCGGAGGGAAGAGGAGTGAGTGTCTGTGTCAAAACCTTCAAAGATCAAAACCCCATGAAAACCTTTCCTCA
434 CGGTACCATAAGTTTTCTATCCTTCACTAGTTTGAACCTACTGCTCAAACCTGCAGGCTTAGGCGTCGAAGCGAAGTACCCTTGT
435 GCCGTTAGCAGGAGGCGTTAGGCGTTGATTGGGGAAAACCTGACGTAAGAAGCAGCAGCAACTAGGCAAGAAAACCTGACGG
436 GTAGATCACCGGCCGGAAGCCAGTAAGCGGCTAGATCTGGGCAGTTTTGATGCAACCTCACGAAATCTCAGCCTCGAAGAA
437 GAAAGCAGCTCTGGGAAAGTCTGAACGGGCGTATCGACAAGACTTTTTATTAGAAAATCTCAGAAATCTCAGAAATCCACGTTGGGA
438 GGCAATCAGAAAACACCTCTCTAGACTTTCCTTGTACCACTTCAACAACAACCGGACCAACAGTCCACCGCACTCTCTGC
439 AGACAAGAGAACAGTAAGGATTTACATTTCTGGTAAACACCTGTTGATCTCAACATCAACCGAGATCCACAATCAGGCGATT
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441 CTGGACCAAGCCACAATATCCTTAGATCTTCAAGCCCTCAAGGAGATCTGAAAGAAATCAAGGCCACACAGTACGACCTGAA
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443 GCACAAGTAGCCGAACAGCTGAGGAAGCAGCTTATTGAGGTCAAGTCCGCTCTCGAGGAGACCAAGAAGATCGCGAGATCTC
444 TGTCCCCGACGGATGAACCTAGGTGGCAGGATACTGCAACCAAGGAAACCTACCTCAAAGCCAACAAGCTACCTCATCTCT
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466 CCTCATCAACAAGATGATGTTTATTCCCTACAGGACAAGGCGGACTACGTAGAAAACCTCCTTGGAGAAAAGAAAAGGAG
467 ACATGGATGACATGGAGAATGAGTACGAGGAAGAGTACAGGCAACTCCTACCATGAGCGGAGACGTAAGGAACCTTACTG
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520 **>endoRYNV-CU2**

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607 CAGGGACAACCTGGTATGGCGACCTCGTCCACTCTTGGAGAA GAGAGCAGCTGCATGGAAGCTCCTCGCTGCACACGCA
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830 TCAGGCCATCATCACCTTCTATGAGAAGCTGAACGCTAAGAAA CTTACGGGTAAGGTGGTTAGCTTTTTGCGACTATATAAC
831 AAATCAGGGGTGAAGATGAAGTTGCAACACATCAAAGGCAAAGACAATCAGCTTGCCGATAACCTCAGCCGATTGACCCAA
832 CTCATCACCTGGTCAAATGGCTTCCAGAA GAAGTGAAGGATCTCGCGGCCGAGCTAGCCAAGAAAAGAAAGGCAAGACCTCCCT
833 GAAGGGAGAGGTGCAGGAGGAGATCTCCTGTTTTCTCAGAACTGCCCTCCGCCGAGCAGAGGAATCCGCGACTACTCGCCCA
834 TCAGAGCCGCGCCATGTA CTATGGCAGAGATGGA TGAATCCCGAAGACTGGCCTTACAACGAAGAAA CAAGTCTTCGATGA
835 CATCGCACAGAA CATACGCGATGCGGTCTACATCA CCGGATCGACCTCGCAGCGGCAAAAGCAAGAGCTACCAGGGACAAC
836 TGGTACAATGACGTCACACCCGACTGGAGCAAAGAGCA GCTGCAGCATGGAGACTCATGGCAGCCTACTCAGACTTTGCCAC
837 GTGGAAGGACGTAACGCTAGTGAA GTGACGCAAGGGATGACTTCACAATTGCCAATGTCGTCTATTGCTTACGACTTGGAAC
838 TTATCAGTTTGTGTCGGCAGCATCTATTAGCTGCATACTTTATGTAAGTGCGCCAGTAGTGCCTGAGTCATAAGGATAAGGA
839 ATCTTAGCTCCTTATCGTCTTTAGCTTTAGCAAAGCTGTA AAGATGAA TTTATTAGCTGTGATGGGGCCCA GAAAGCGCAC
840 TGAGCTGATATTTCTATCTTTTGCTTAGCCTACTTCTATATAAGGGAGTATAGTCTGAAGGCTTAGGCACAGAGCAATCTCT
841 TTAGCCAACCTTCTTGGAGTTGATTA

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843 TGATACCAGAAACAAAGAATTAACCATGGTCGCTTAAATTTTCTTTTCTTGTCGAGAAACCCAAGTTTCAGACCAGAACCTTGAG
844 TTGTCTCTCCTTTTTACAGGGGAGGAGGGAGTA CTGAGTGTTGCTAAAATTCTGCAAATTTCAAACCCCATGAAAACTTCTCT
845 CACGGTATTATAAGTTTTCTGACCCTACTAGTTCAAACCTACTGCTGAAACTGTAGACTTAGGCGTCGAAGCGAAGTACCTT
846 GTAGCCGTTAGCAGAGTGGCGTTAGGCGTTGATTGGGGAAAACTGACGTAAAGGAACAGCTGCAACTAGGCAA GAAAACTG
847 AAGGTACAGATCACCGGCCGGAGAGCTAGTACGCGGCTAGATCTGGGCAGATCTCTTGATGCAACCTCACGAAATCTCATCTTT
848 TGAAGAAGAGAGCAACTCTGGGAAAGGTCTGAACGGGCGTATCGACAAGACTTCTTATTAGGAATCTAAGATCTTACCTC
849 GTTACGAAGCAAACAGAAAGTCACTTCTGTGATTTCCCTTGCTATCACTTCAAACAACACCGGACCACCAGTCCACCGCA
850 CTCTCTGCAGACAGAAGAACAGCGAAGATTTACCTTCTGGTAAATACTCTGTTGATCTTAGTATACCAAGATTCACAACC
851 AAGCGGTCTTAGACGATAAGATCTCAAGACTCACCCAGTACTTGGTGGCAAAGTCCGGCACTTACCGACAATCCCGGAGGAA
852 TCACCCCTCTGGACGACAGCCATCTCTAGATCTTCAAGCCCTCAAGGCAGATCTAAAAAGATCAAGGCTACCCAGTCA
853 TTCTTGAAGCTAGGCTTTCACAGCTCCAAGAAGCGGTACAGCTGATCATCAAGGGAGAACGATCCAAACCGATTGAGGC
854 AGCTACAGCTCAGGTGGCTGAACAACCTGAGGAAGCAGCTTATCGAGGTTAAAGCCGTCTAGAGGAAACAAA GAAGATCGCG
855 AGATCTCTGTCCCCGACGGATGAACCCAGGTGGCAAGACACCGCCGCAAAGAAAACCTACATAAAAGCTATCCAGGCTACC
856 TCATCTTTACATCCAACAACACCGGGCTAGGGTTTATTGAACCTCACACCTATACCGAGGCAACTGTCCACAAGCCTAGCA
857 AAACAAAACAACACGCTCATACAGCTGTTGGTTAGGTGCTAGAGAAATCTCGACCTTGAACAGGCCATCGCGAACCTGTC
858 CGCACAAGGTACGAGACTTGAGAAGACCGTCCGACAGAGAAAGACACGGTCAAACCTCCAGAAAAGTGTCTCAACGACCTCACT
859 AAGGAGTTCGGAAAAGTCAACCTTGGGAAAAGGAAAGGGGCTAGAAGGAGCAGTCTCATCCAGAGACAAAACTTCTACGTTT
860 GAAAGAATCCCTCAATCAATACAATGAGCAGAAGCCAAACAAGGCTCCAGGCTCCGCCGCAACTGAAAAGGCAACAAGCA
861 GCTCGGACTCAGGCACCCCACTCTGGAGGACCAGATCCGAGGATACAGGCGCTCTGCAAGGTTACGACACCAAGCACAGAG
862 AGCGGTACGAAGGACCTCAGCAGGGACTTCAGGAACACCATA GAAAGGCAGCTAGATCCGGACGCCGAGTTATCACTCAGC
863 AGAAGGAGAAGAGCAAACCTAGTACCAGCAGAGGTA CTCTACGCTCAACCGTTCAGAACCTGTGAATCGTGTGTATGAGC
864 ACTATAGTGAGCTCGGGGCTCATATAGTAGACAGGCAACAAGACTTCCGATATATCGAGGAAGCATCTACAGAGACTAGTC
865 AGAGAAGGCATGCAATTCATACATGTCCGCATGGCAATGGTTAGGATCCAAA TGTGCA CAGGACAGATGCAGGTATATCTG
866 CGTTGGTGGTGTTCAGAGACACTAGTGGAGCGATGACAGGCAGGTCATCGGCAGCATGTCCGTA GACATGACCAAGGGGAG
867 CACAATTGGTATACATAATACCAAACGCAATGATGTCAA TACACGATTTTACAATCGTATACAGGTCAGCGTGC AAAACCCGAG
868 GCTACGGGACAGGATGGGCTGGAGGAGACAGCAATATGATCATCACTAGATCACTGGTGGGGCGTCTACCAACACAGCAT
869 GACAACTTCGAGTACCGGATAGATCAAGTACAGACTATCTAGCAAGCAACGGAGTCGCTTGATACCCGGACAGAAGTGG
870 GATGTGGCCAAACAGTCCGGCGAATGGGAGCTACAGCCAGCAGGATTGTTGCACCACTCATGACACCAACAGAGGGCGAGAC
871 TCAGCCAGAACAGGAGTGGCAGTATAAGTCTCAGATTCACCGACTTCCGAGATCAGAGGATCGTGGAAAGAAAGGCCAGTCCGA
872 GGATGAGGGCAGGCCAGAAGGTGAAGAGGAGAGACGCACTACGTGCTCGTCTTACAGACATAACTCCTATGAGACCAACCTC
873 AGAGGAGAAAGGAGGCCAAGGCAGAACGAACTTTCAGAGTTCACACCAGAGACAGATTTGGTGAGCCAATGGCTAAGCCAG
874 CTATCCGCATCAGCACACAACAGTGGAGTGTCAAGCTCAGAAGAAGAACCGCTAGGTTGATGAAACTGATGAAGACAGTG
875 ATGGCACAATAATGAGAAAACCTGGCAGAAGGAAGACCAAGGAAAGGAGAAATCTGGAGCTGCAAGGATGGAAAGAGACCT
876 GCAGACCAGGCATATACGAACTGATCCAGAGGAAGAAAGGAAATCTACCTCAGATACGAGGCAAGACGAGGATCAGG
877 AGGTACAGGTACTAGGGGCAACAACCATGGAGGAACCA GAAATGGAGTACCCACCAGACTGGAAGAGGTTATGGGCAAGC
878 TAAAGAATGTCAGCATGGAGAAACTTTTCCAGTAAAGTGGCATGGATA GCGAGTCCAGCATCACAGGTGGAGGATTCATACCA
879 CCAAGCCAGTACCAAGGAGCGCAGGGATACCCACCTGCCACAGGAGCGTCTGCTTCAACCATAGGGCCAGCAGATCTGCAAG
880 GATGGGGAGGACGATTGCAAGGAGCAGATCGCCGATAGGATATGGCCGCCACAGCAGCCATGGTCTTACCATCAGCCCA
881 GTCTGACAACGGCTGTATGCTAGTCTACTCAGGATTTACCTTAGTTCCCGATGTGATCAACCGATGGGAGTCTATTA CTGT
882 CAACCTCATCAACAAATGATGTTTATTCCCTACAGGATAAGGCGGACTATGTAGAAAATCTCTTGGGAGAAA GAGAAAAGG
883 AGACATGGATGACTTGGAGGATGCAGTACGAAAGAAGATAAAGCAACTCCTACCATGAGCGGAGACGTGAGGAATCTCAC
884 TGCCGCACTCAAAGGGTCTTTGGCGTACATGACCCTCATACTGGATCAGTCCACATACAGAACCAGGCGTATGCAGAACTGG
885 AGAGACTGTACTGTAAGCGGACAGACGACGTGATCCCTTCTCTACGACTACTACCAGCTAGCAGCTAAGTCTGGAAGGATG
886 TGGCTCGGACCAGAGCTATCAGAAAACTTTT CAGAAAGCTTCCACTGAGATAGGGCCAATATTGAACAGGCTACAAAAGA
887 CAGGTACCCAGGTCTCACCATTGGAGTCTTGGCAAGGGCCAATTCATCCTGGAATACCTACAAAACGCTCTGCAAGCAAGCAG
888 CGTTACAGAGTCCCTGAAGAGCCTCAGTTTTTGCAGAAACATGCCGGTGCCTGGATACTATGAGAAAGAACAGTATGGCATC
889 AGGAAAGCAAACCTTACAAGGGAAAGCCTACCCAACGCACGTA AAGGTGATCAAAAACAAGTACAAGCACACCTCCGGGA
890 AGAAGTGCAAGTGTACTTTGCGGAATAGAAGGCCATTACGCCAGGGAATGTCCAAA GAAGGTGGTAAAA CCACAAAAGGC
891 GGCATTTTCAATGGCATGGGCTTAGATGACAACCTGGGATGTTGTATCCGTA GAGCCAGGAGAGTCAAGTACGACGAAATC
892 TGCAGCATCTCCGAAGGAGAGAACGCTGGGGGAATGCATGAACTCATGGCATTCAAAACTCAACTCCCGTACCCAGTCAAGTA
893 CGAAGCCAGCACATCAACAGTTCTGCCATGGATA CAGGTAAGTGTGAGAAAAGTGATAAGCCCTCTTGGAGGAGAAGA

894 AGAGATATCCACAGGCCAAAAGGACTGCGCTCACACTTGGAGCGACACACAGGAAGTGCCGATAGAAGGGAGGATATGC
895 AGCATTTGCAGTGATGAAACGCCTCATGGAAGGAGAGTCACTGCACGACCTGCAGCCTAACCTGTGTCCAATTTGCGCATA
896 TATGGATCATGGAATTAAGCTGATCGCCGCAAGGGACACCAGAGACGCAGCAAAGTGCCAGTACCACAACAAAGATGAACTC
897 ATCAGGCCACTCTATGAACA CAATGCCTTCTAACCGAAAGTGGAGGAACTGACGACCCAGCTGCAAGAATCCAAAACCG
898 CAAGCCTGAAGACCTAATCAGCTTGGCGGATGATATGGAGGACGTGTCCATTCTGGA CAACGCCTCAAAA GGGGAA GGAG
899 AAGGAATCTTTCAAATTCGGAA CGACGATTCCCA TCGATCACATCCAGAATCTTGAAAC GTAGCAAGAATGATCGAA CAATG
900 GAAGGAGACCCCCAGAGTCACTATCAAGGAAA CAGCAGAAAGCAGCAACA CAATAGGAGCCCTCTA GCA GAAGAAGGGAT
901 TGAAGAGCTAGCTGCAGCTGTTGATACGGCATA CACAGAAATGCCAAAAGGAGGTCTGAACAACTA TACAACACCA TTGTTG
902 AGTTTGTAAATCCTCAGGAAAAGGGGCACCCACAAGATTCAGGGTTAGAGCAGTCATA GACA CAGGATGCACCTGCACATG
903 CATCAACAGCAAGAAGTCCCTAAGGAAGCCCTGGAAGAA GCAAAGTATCAGATGAACTTTGCAGGAGTAAATTCCTACTGGA
904 GAAACCAAGCTGAAAATGAAGATGGCAAGATGATAGTATCAGGAA GTGATTTCTACACGCCATATA TTGCA GCTTTCCCAAT
905 GGAATTAACAGATGTGGACATGCTCATTGGCTGCAACTTCTTGGAGCCATGAAAGGAGGAGTCAAGCTGGAAGGAAC GGAG
906 GTAACCATCTACAAGAGGTTACA ACTATCCA GACGACTCTAGAACACAGAAGATCTCTACTCCGTGCAGAAGCTGAAGC
907 AGGAGAAGAAATCGAGAGACTTTACTACGCCAACGACTACTCTGAAGAAGGAGTAAACAGGCTGAGAAA CCATAA GCTGCTA
908 CAAGAGCTCAAGGAGCAGGGTTACATTGGGGAAGAACCAATGAAGCACTGGGCGAAGAACGGAATCAAGTGAAGCTTGAT
909 ATCAAGAACCAGATATAGTCATCAGCAGCAAACCTCCGGATGCAGTCTCAAAA GAGACGAAGGCCAGTACCAGAGACACA
910 TCGATGCTCTATTGAAGATCAAGGTAATCCAA CCCAGCAAGAGCAAGCATCGAACTGCCACCTTCA TCAAAA CTGCGGTACA
911 ACCATAGACCCGATA ACAAAGAA GGAGATCCGAGGAAAAGAGAGGATGGTCTTTGATTACAGA GCCTCAACGATAACACCC
912 ACAAGGATCAGTACACCTTGCCTGGGATTAATACCA TCA TCTCCGCAATTGGCAACGCAAAAATCTTCAGCAAATTTGACTTGA
913 AGTCTGGCTTCCACCAAGTACTTA TGATGAGGAGTCCATCCCTTGGACCGCTTTGTACACACAGTAGTTTCTACGAATGGA
914 AGGTAATGCCTTTCCGACTCGCGAACGCTCCTGCA GTCTTCCAGAGGAAGATGGACCAGTGTTCGAGGAACTCTGAGTTC
915 ATAGCCGTCTACATCGACGACATTCTGGTCTTCAGTAAGACGCTGAAGGAACATGAGAAGCATCTCAGCATCATGCTGGGAAT
916 ATGTCGAGATAACGGCTGGTACTGTACCCAGCAAAATGAAGCTCGCCGCAACAGAGATAGATTTCTTGGGAGCAACCATTG
917 GTGACGGAAAAATCAAGCTCCAGCCTCACATCATCAAGAAGATTGCCGAGGTGGACGATGAATCCCTAAAGACCCCTCAAAGG
918 GCTGAGAAGCTGGTTGGGAGTTCTGAACTA TGCCAGGAACTACATCCCAA GTGTGGGACACTCCTTGGCCCACTCTACAGCA
919 AGACGAGTGAGCACGGGGACAGAA GATGGCATGCATCGGATTGGGCCTTAGTCAAGAAAA TCAAAGCCTGGTCCAAAACCT
920 CCCAGACCTCAA ACTGCCACTGAAGAGGCCTATATGATCATCGAGACAGATGGTTGTATGGAAGGATGGGGCGGAGTCTGT
921 AAATGGAAGCCCAACAAGGCAGACTCAGCAGGAAAGGAAGAAATCTGCGCATATGCAAGTGGGAAATCCCAA CGGTCAAAT
922 CAACTATTGACGCAGAAATCTTCGCGGTATGGAATCCTTGGAAAAATCAAGA TATTTTACATGAACAAGGATGAGATCACA
923 TCAGGACCGACTGCCACGCCATCATAACCTTTTACGAGAAGTTAAACGCCAAGAAGCCTTACGCGTAAGGTGGTTAGCTTTTT
924 GCGACTATA TAA CAACTCCGGGGT GAGGATGAAGTTCGAACATATCAAAGGCCAAGATAATCAATTAGCTGATAA TCTCAGT
925 CGCCTAACCCAACTCATCA CCGCAGTAAGATGGCTTCCAGAGGAAATGGCAGGAATCGCGGCAGAACTAGTCAAA GACAGGG
926 AAGAGTCCCCCGGATGCGAAGGTACAGGAGAGCCTCTCAGGCTTTCTCAAAGCTGCCCTCCTCAA GTCGAGAAATCCTCG
927 ACTACAGCCCATCAGAGCCGCACCATGCTCTTGGCAGAGATGGACGAGTCTAGAAGACTGGCCAAA CAACGCCGAGACAA
928 AGTCTTCGACGACGACGAGGGCAAACATCTGCGACACGGTCTACATCACCGGTGTCGACCTCGCCGCCGCAAGGC

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930 TGGTATCAGAGCTTTAGCTTTACCATGGTAGCTTAAACCCCCCATCTTGTGCGAGAAACCCAA GTTTCAGATCCGAACCTTGA
931 GTTTGCTCTTTTTCGAAGAGGAGAGGGAGTGTGACCTTACTCAAACCTTTT GCGGATCAAACCCCA CGAAAACCTCCTTAC
932 GGTATCATAAGTTTTCTGTCTTCACTAGTCCAACCTACTGCTCAAATCGCAGGTCTAGGCGTCAAGCGAAGTACCCTTGTA
933 GCCGTTAGCAGAGTGGCGTTAGCGTTGATGGGGAAA ACTGACGTAAGA AAGCGACAGCAA CTAGGCGAGGAAA CTGACG
934 GACAAATACCCGGCCGGAAGCTAGTAAGCGGCTAGATCTGTGTGGTTTTGATGCAACCACACGAAA TCTCCGAATTCGAAGC
935 AGAGAGCAGCTCTTGGGAAAGATCTGAACGGGCGTATCGACAAGACTTCTCATT CAGGAA TCTCAGAACCTATCCACGTTGGG
936 AATCAAACCAGAGGACACCCTCTTTGAA TTTCCGTGCTACCA TTACAACACAACA ACTGGACCTCCAGTCCACCGTACTCTCT
937 CAGACAAGGTGACGGCAAGGATTTACCATACTTAGTAAACACCTTGTTCGATCTCAATATCACC GAGATCCA CAATCAGGCGAT
938 CCTTGACGATAAGATCTCAAGGCTCACCGA GTACCTGACAA CAAAAGTGGTTCCCTACCAGCATCCCGGAGGATTCGCCCT
939 CCTGGACCAAATAGCCTTTTCTTAGATCTACAGGCCCTCAAGGCAGATCTGAAGGAAATCAAGGCCACACAGTCAAGCAAAA
940 AGCTAGCCTTCTACA ACTGCAGGAGGCA GTCCAGCTTTTTATCGCTCGGGAGAACGATCCCAAGCCGATCGAGGACGACTACT
941 GCACAAGTAGCCGAACAGCTGAGGAAAGCA ACTCATCGAGGTCAAGACAGTCTCGAAGAGACCAAGAAGATCGCCAGGTCTCT
942 TATCCCCGACGGATGAACCTAGGTGGCAGGAACTGCAGCCAA GGAACCTATCAAAGCGATCCAAGCAACCGCTTCCC
943 TCACCTCAAACGGCACCGGCCAAGGCTTATTGAGCCCCACACTACCCGGAGGACAGTTATCTACCAATCTAGCCAAGCAAA
944 ACAACACCATAATCGAATTA TTGGTGCAAGTGCTAGAAA GAATCTGACCTTGAACGGGGCGTAGCCAACCTCACAGGTCAA

945 GTC ACTAGGCTCGAAAAAGCCGTA GCAGACAAAGAAGCAGTCAAGCTTCCGGAAAAAGTCTA GAAGACCTAACCAAGGAAT
946 TTGGAAAGGTTAACTTAGGCAAAGGAAAGGGGAAAAAAGGAAAGTCTCAAGCCGAGACAGAACTTCTACGTGTGGAAAAC
947 CCCTACACTCAATACAA TGAGCAGAAGCCACACAAGGCTCCAA GCGCCCCCGCAACTGAAAGGGCCA CAAGCA GCTCGGATT
948 CAGGGACACCAACCTGGAAGACCAGATCCGAGGATACAGGAGATCTGCACGCA TGAGGCATCAAGCGCAGCAGAGACTAC
949 GGAGGACCTTCGGAAGGGACTTCAGAAAACGATCGAGAGACAACTCGATCCTGATGCAGAGCTCTCCCTCAGTCGAAGGAG
950 AAGAGCCAACCTTGGTACCGGCGGAAGTGCTCTACGCACACAACGGACAAGAACC GGTC AACCGAGTATACGAGCACTACAGT
951 GAGCTACGCGCTCATGTGGTAGACAGGCA GCAGGACTTCCGATTTATAGAGGAAGCGTCCTATCAAAGGCTGACCA GAGAAG
952 GAATGCAGTTCATCCACGTA GGTATGGCGATGGTCAGGATA CAAATGCTGCACAGGACAGATGCGGGTATATCCGCACTAGT
953 GGTGTTCCGAGACACCCGATGGA GCGATGACAGGCAGGTCATCGGTAGTATGTACGTCGATATGACACGAGGAGCGCAGTTG
954 GTATACATCATACCAACGCCATGATGTGATTCTACTTCTACAA CCGGATTCAGGTCAGCATACAGACCAGAGGATACGG
955 CACAGGCTGGGAAGGAGGCGACAGCAACATGATTATCACAAGATCA TTAGTCGGCCGACTCAAAACACCAGCATCACAAGC
956 TTCGAGTACAGGATAGACAACTGACAGACTACCTAGCCA GCAACGGCGTAGCCTGCATTCCCGGACAGAAAGTGGTCCGTGG
957 CAAAACAGGTCTGGAGAATGGAACTCCAGCCAAGCAGGATAGCAGCACCACTTGCAGTCCCCACAGATGCCAGGCTAAGACA
958 GAACCCAAACGGCAACATCAGCCTGAGGTTACGGATTTCCGCGA CCAAAGGATCGTGGAAGAAGGAGAGACATCCGAGCCA
959 GAAGGAAGACCGGAGACGAAGGAGGATGAGAGCAGCACTACGTCTTATGTTCAAACACTCCAGCCCTAGGTGGGATACG
960 CTCGGA CAGCCCAGCGTAAAACGACTACATGTTCCGATATGACGCACCAAGACCGACCATGGCCAACAA CAAACAGAG
961 GATGGGACGATGACCTCCTAAGCCA CCAAGCCCAAAGGATCCTACGAGGTAAGCCTAAGAGGCGAAAAGAAGCTGAAAAG
962 AAAAGGAACTCGCAGAGTTACCCCAGAGACAGATCTGGTCAGCCA GTGGCTAA ACCAACTCTCGAACTCGGCACACAACAGT
963 GGAGCTTCAAGCTCAGACGATGAGCCAAAGTTGATGAGGCAGACGACGAGGACGACGTCTACAATCAGAAAACCTGGGAG
964 AAGGAAGACCAGGAAAAGAGGGAGCTGGAACCTCAAAGGTGGAA ACCCACAGGGAGGCCAGGCCTCTATGAAATGATCCCT
965 GAGCAA GAAGAAGAAGTCTACCTCAGGTACGAGGCAGAGGACGAAGAGGAGGATCAGGAGTTGCAGGTCATAGGAGCCGC
966 AACAAATGGATGAGCCAGAGATGGAATACCCAA CAGGCTGAAAAA GTAATGGGCAAACTCAAAAA CGTAAGCATGGAGAA
967 GCTGTTCCAGTGAGCGGGATGGATAGCGAATCCAGCATAACAGGAGGTGGTGGAGGTTTTATACCA CCAAGCCAGTACCG
968 GGAGCA CAAGGATACCCCCAGCAACAACTCCACTATGTCCACCATTGGAC CAGCAGACATGCAGGGATGGGGAGGG AGA
969 GTGCCAGAAGTAGGTCACCATTAGGGTATGGCAGACCACAACAGCCATGGTCACTACCA TCAGCAGTCAGACAACGGCT
970 GCATGCTAGTCTTCCACAGGACTTACCCTAATACCGGATGTCA TCAACCGATGGGAATCCATAA CAGTCAACCTCATCAACA
971 AAATGATGTTTGACTCCCTGCAGGACAAGGCCGACTACGTGAGAACCTTCTGGAGAACGAGAAAAGGAGACGTGGATGAC
972 ATGGCGGATGCAATATGAA GAGGAGTATAACA ACTCCTTACGATGAGCGGAGATGTGAGGAATATCACAGCCGCA GTCAAG
973 CGGGTCTTCGGAGTACACGATCCGCATACAGGATCAGTCCACA TCCAGAATCAAGCATATGCAGAGCTCGAAAGGCTCTACTG
974 CAAACGGACGGACGACGTGATCCCTTCTATACGACTACTATCAGCTAGCAGCAAATCGGGAAGGATGTGGCTCGGACCC
975 GAGCTATCAGAGAAGCTGTT CAGAAA GCTTCCACCTGAGATAGGACCTACCATCGAGCAGGCCTATAAGGATCGATATCCAGG
976 GCTGACCA TTGGATTTTGGCCAGAGCAAACCTTATCCTGGAATATCTACAGAA CGTGTGCAAGCAGGCA GCACTGCAGCGTT
977 CGCTAAAAGCCTGAGCTTCTGCAGGAATA TGCCGGTCCCCGGA TACTATGAGAAGAAGCAATATGGTATCAGAAAGGCTAA
978 AACCTCAAAGGTAAGCCTCATCCGACCCACGTTAAGGTGATAAAAAACAAGTA CAAGCATACGAGGGGAAGAAATGCAAG
979 TGCTACTTGTGTGGGATCGAAGGTCACTATGCCCGA GAGTGCCCAAAGAAAGGTGGTCAAA CCACAACGAGCGGCCTATTCAA
980 TGGCATGGGACTAGACGTA AACTGGGATGTAGTCTCGGTGCAACCAGGAGAAGAA GACGACGACGAGATCTGCAGCATCTCA
981 GAAGGAGAAAACACTGGCGGAATGCACGAACTTATGGCATTCAA AACACA ACTCCCTTATCCAGTGGAGTATGAAGCCAGCA
982 CACCA CAGTTCCTGATGCCATGGA CACAGGTACCTGTGGAAAAGAGCGACAAAACCTTCCCTGGAGAAGACGGAAAGGATATCTC
983 ACAAGTCCAGAAGGACTGCACACA CACTGGAGTGACACCCAGGAAGTGCCTATCAGCGACAGGGTTTGCAGCATCTGTAGT
984 GACGAAAACCTCA CGGTAGAA GAGTCACTGCATACATGCAACATCAACCTCTGTCCGATATGTGCAAGGATGGACTATGG
985 GATCATGCTGATAGCAGCAAAA GACCAAGAGCGCAGCA CATTGGCAGTACCAAAA CAAGGATGAGCTCATACAGCACTG
986 TACGAA CACAACGCTTTTCTCACCAGGAAA GTAGCAGAGCTACTAGCCAGCTGCAGGAATTCCACAACCGCAGGCCTGAAGA
987 CCTGATCAGCCTAGCGGATGACCTGGAGGACGTGTCCATCCTGGACAACGCCTCAAACAGGGGGAAGGAGGAGAA GGAATT
988 GTTCCAA TTTGGA ACTACAATTC CATCGACCACATA CAAAACCTTGAAAATGTGGCCAAGATCATA GAAAAGTGGAAAGATA
989 CCCCAGGGTCTGTAATCAAGGA AACACCA GAAAGCAGTACCA GTAACACCA TCGGAGCACTTCTAGCTGAGGAAGGAATCGA
990 AGAACTGGCTGCAGCGTTGACACCGCCTATACCGAGATGCCAAAAGGAGGCCTCAACAA ACTTTACAACACAATTGTGGAGT
991 TCGTCA TACCTCAGGAAAAGGGAGCA CCCACGAAGTTCAGAGTTCGTGCTGTGATAGATACTGGATGCACCTGCACGTGCATT
992 AACAGTAAAAAGTTCCAAAGGAGGCACTCGAAGAA GCGAAGTACCAGATGAACTTCGAGGGGTTAACTCAACAGGGGAG
993 ACCAAGCTGAAAATGAAGAACGGGAAGATGATCGTGTGTCAGGCAGCGACTTCTACACCCGATATATAGCAGCTTCCCAATGGA
994 GTTGCCAGACGTAGACATGCTGATTGGCTGCAACTTCTTGCAGCCATGAAGGGAGGCGTAAGACTCGAAGGAACGGAAGTG
995 ACTATCTACAAGAAAGTCAACCAA TCCAAA CAACCTAGAGCCACA GAAGATATCCCTCCTCGAGCAGAGGCTGAAGTCGG
996 AGAAGAACTAGAGCGCATGTA CTAAGCCAATGACTATTCCGAGGAAGGAATAAGTCGGCTGAAGAACCACAGGCTGCTGCAG
997 GAACTCAGAGAACAAGGGTACATTGGTGAAGAGCCAATGAGACACTGGGCAAA GAACGGCATCAAATGCAAGCTGGATATC

998 AAGAATCCAGACATAGTCATCAGCAGCAAACCGCCTGACTCTGTATCAAAAGAGACGAAAGCCCAATACCAAAGGCATATAGA
999 TGCCCTGCTCAAATCGGAGTAA TCCAGCCCAGCAAGAGCAAACACAGGACGGCGGCTTTTATCACACTCGGGTACGTCAA
1000 TTGACCCGATTACCAAGAAAGAA GTCAGAGGGAAAGAACGGATGGTATTGACTACCGAAGTCTCAACGACAATACCCACAA
1001 AGATCAGTACACTGCCGGGTATCAATACCATCATATCCGCGATTGGCAACGCTAAAATATTTAGCAAGTTGATCTAAAGTC
1002 CGGATTCCATCAGGTGCTCATGGACGAAGAATCCATACCATGGACGGCTTTGTAACGCCAGTCGGATTCTATGAATGGAAGG
1003 TCATGCCCTTTGGCCTTGCCAATGCTCCAGTGTCTTCCAAGGAAGATGGACCAATGCTTCGCTGGAACTTCGGAATTCATCG
1004 CAGTCTACATCGATGACATCCTGGTGTTAGCAAAAACCTAAAGGAGCAGAGAAA CACCTTAGCATCATGCTAGGGATATGC
1005 CGTGATAACGGTTTGGTTTTATCGCCAGCAAATGAAGTTGGCCGCCACA GAGATAGACTTCCTTGGCGCCACCATA GGCGA
1006 TGGAAGGATCAAGCTCCAGCTCACATCAAAA GAAGATAGCCGAGGTGGATGACGAATCCCTGAAAACCTCAAAGGGTTA
1007 CGAAGCTGGTTGGGAGTGCTCAACTACGCGCGCAACTACATCCAAA GTGTGGCACACTGTTAGGCCACTATA CAGCAAGAC
1008 CAGCGAGCATGGTGATCGTAGGTGGCACGCTGTATTGGCCTTAGTCAAAGAATTAAGGGCTGGTCCAAAACCTCCCA
1009 GACCTAAAACCTCCCACGGAAGAGGCATACATGATCATTGAGACTGATGGATGCATGGAGGGCTGGGGAGGAGTCTGCAAT
1010 GGAAGCCCATGAAGGCAGACTCAGCAAGCAAGGAA GAAATCTGCGCTTACGCCAGTGGTAAATTCCCCACGGTAAAAATCAAC
1011 AATAGACGCAGAAATCTTCGAGTTATGGAGTCTTGGAAAATTCAAGATTTTTTACATGAACAAGGACGAGGTCACTATCA
1012 GGACTGATTGTCAAGCAATAATCACCTTCTACGAGAAGCTGAATGCAAAA GAAACCTTCGAGGGTAAGTGGCTTGCCTTTTGC
1013 GATTATATAACGAACTCCGGGGTAA GAATGAAGTTCGAACATA TAAAAGGTAAGACAAC CAGCTCGCAGATAACCTCAGCC
1014 GTCTCACAACTGATTACATTTGTGAAATGGCTTCCAACCGAGCTCAAGGACCTCGCGGCA GAACTAAC CAGGAAAGACGAC
1015 GGGACGCCCCGGAAGAA GGAAGTGAGGAGGAAATCTCTGTTTTCTCGAAGCTGCCCTCCGCCGAGCCAAGAGATCCGTGA
1016 CTACTACCAATCCGAGCCACGCCATGTACTATGGCAGAAATGGCAGAATCCAGAAGGCTGGCTCTACTGCGACGAGAGGAG
1017 ATCTTCAACAGCCTTGCCCAACACATCAGCGACACGGTCTTCA TACC GGAGTCGACCTTGC GGCAGCAAAA GGCCAGAGCAAC
1018 CAGGGACAACCTGGTATGCTGACATCACCAACACTGGAACGACGAGCCAC CGCAGCATGGAAGCTCATGGCCGTTACGAG
1019 GAATTCGCCACGTGTAA GGATGTGAACGTTTAGTGAA GCGACGTCAGCAATGACTTCA CAATCGCCCAAGTGCCTACTGCTT
1020 ACGCTTGGGAACTTATCTTTTAGTGTGCGGTAGCATCTTCTAGCTGCCATACTTTATTGTAAGTGC GCGGATAGTGCCTGAGTC
1021 ATAGTGATAAGGAATCTTATCACCTTATCGTCTTTCTTAGCTTTAGTAGCTGTAAAGACGAACTTATTAGCTGTGATGGGGCC
1022 CAGAAAGCGCACCCGAGCTGATTTTTCTCTTTCTGCTAAGCCCTCCCCATATAAGGGAGAAGAGTTTGAAGGCTTAGGC
1023 ACAGAGCAATCTCTAGCCAACCTTTCTCTTGAGTTGTATTA AAACATTCAAGTGAAATAAAGACTTGTTCATCTTTTCCGCAT
1024 ATCTCTGAGTTTTTATGAGTTCTTAAGTGTTCGAAAGCGCACTTTTGAATTAGATCCA TGTTTTTCGGACCCCATTC
1025

1026
1027

Supplemental table. 271 public and proprietary genetics were bulked into 294 samples and used in validation of epiRYNV-BS detection assay.

Bulked sample number	Genetic/cultivar
1	Selection 1, Selection 2
2	Selection 2, Selection 3
3	Selection 4, Selection 5
4	Selection 5, Selection 6
5	Selection 7
6	Selection 7
7	Selection 8, Selection 9
8	Selection 9
9	Selection 10, Selection 9
10	Selection 10, Selection 11
11	Selection 12, Selection 13
12	Selection 13, Selection 14
13	Selection 15, Selection 16
14	Selection 16, Selection 17
15	Selection 18, Selection 19
16	Selection 19, Selection 20
17	Selection 20
18	Selection 20, Selection 21
19	Selection 22, Selection 23
20	Selection 23, Selection 24
21	Selection 25, Selection 26
22	Selection 26
23	Selection 27
24	Selection 27, Selection 28
25	Selection 28, Selection 29
26	Selection 29
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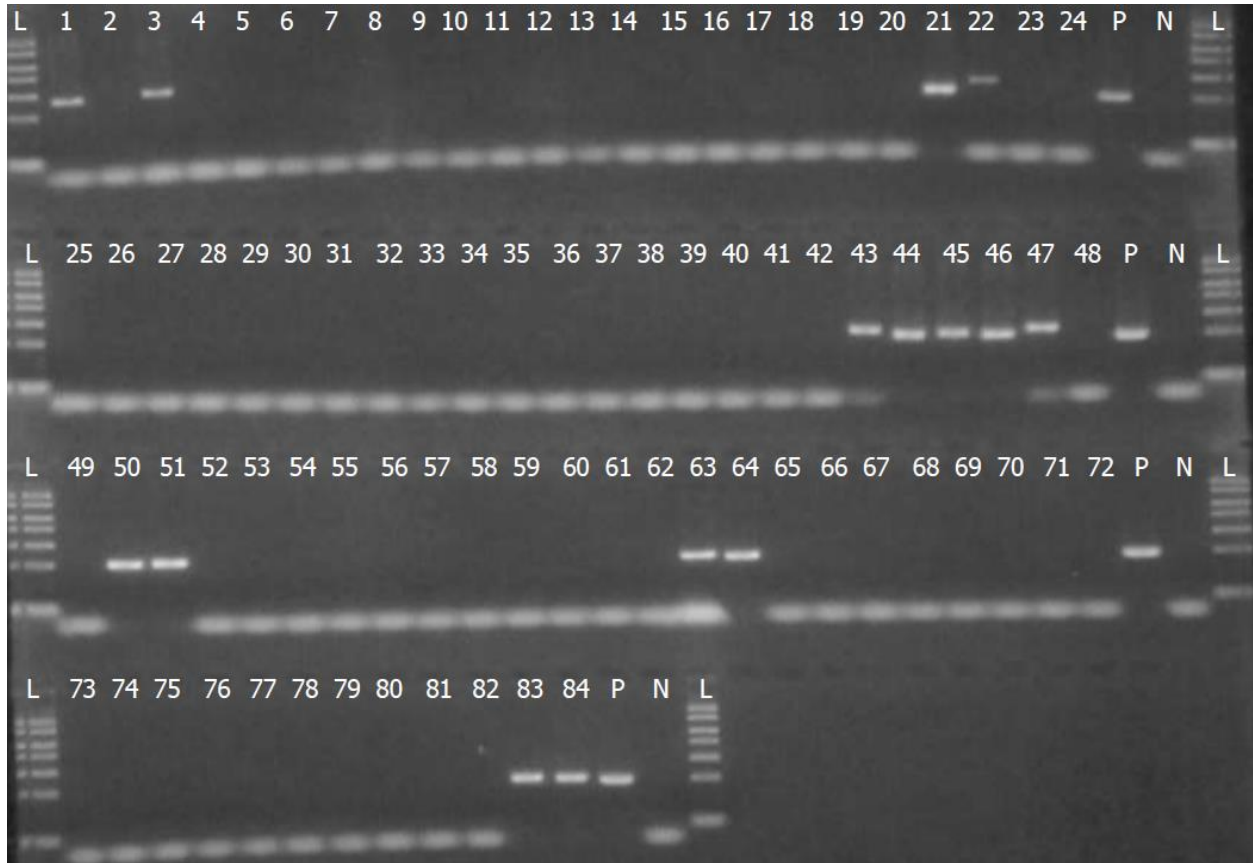
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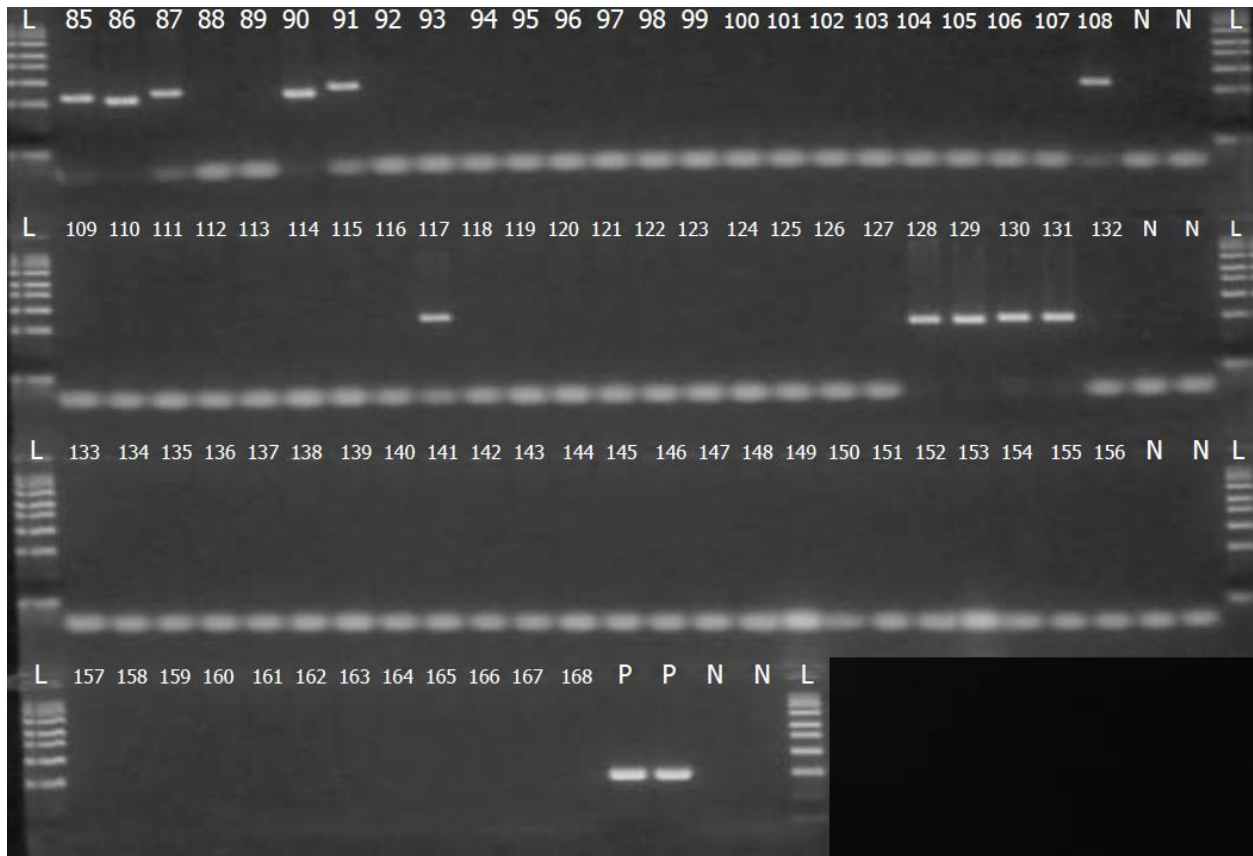
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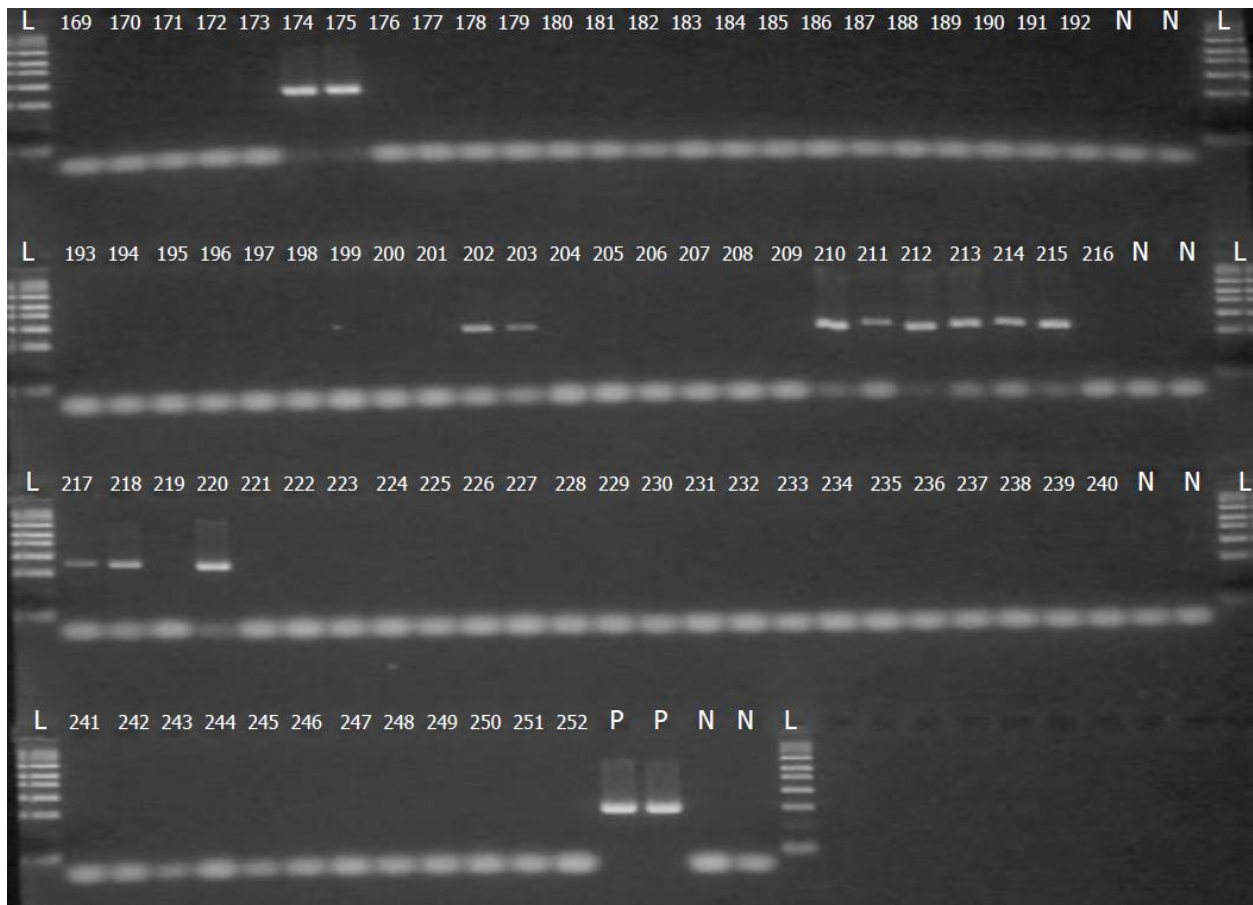
1030 **Supplemental figure. epiRYNV-BS detection using RYNV6-F/R PCR assay. 271 public and proprietary genetics**
1031 **were bulked into 294 samples. Off-target was observed in 43 cases.**



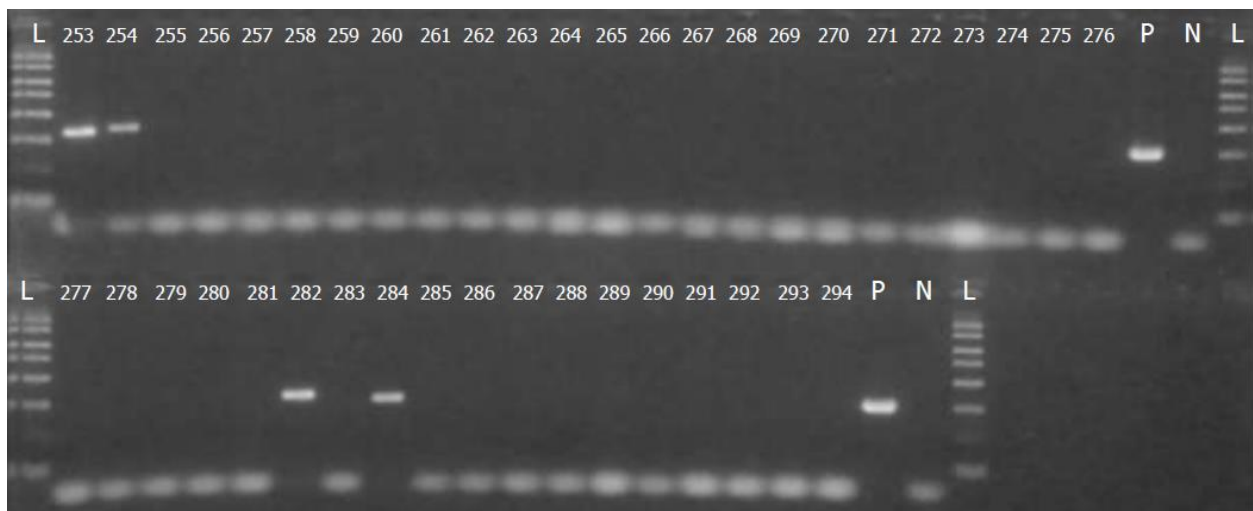
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