

Characterization of endogenous Rubus yellow net virus in raspberries

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Abstract

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

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

1. Introduction

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

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

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

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

2. Materials and methods

2.1. Plant material

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

2.2. DNA purification, sequencing, and virus discovery

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

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

2.3. Electron microscopy

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

2.4. Data mining

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

2.5. Development of RYNV lineage-specific primers and validation

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

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

3. Results

3.1. A diverse array of endoRYNV is present in raspberry

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

3.2. Structure of main endoRYNV lineages

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

3.2.1. endoRYNV-CU1

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

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

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

3.2.2. endoRYNV-CU2

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

3.2.3. endoRYNV-CU3

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

3.2.4. endoRYNV-BS

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

3.2.5. endoRYNV-PH1

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

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

3.2.6. endoRYNV-PH2

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

3.3. Validation

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

4. Discussion

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

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

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

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

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

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

5. Conflict of interest

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

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7. Data availability

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

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353
354

9. Tables

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

*: Yes = positive; No = negative.

**: Samples 1-25 were plant samples in this study and validated using SYBR Green PCR detection, while 26-33 were data mined from the published literature and not subject to SYBR Green PCR. Further 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

Table 2. Percent identity matrix of the reverse transcriptase and RNaseH region of the six Rubus yellow net virus (RYNV) lineages discovered in this study against the three published RYNV sequences, *Gooseberry vein banding virus* (GBVBV), *Grapevine vein clearing virus* (GVCV), and *Birch leafroll 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 MG686419.1	66.9	66.4	65.4	66.4	67.3	65.7	65.7	66.3	66.4	67.3	69.8

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

Lineage	Break point	Nucleotide sequence
endoRYNV -CU1	6527 12	(6390)GGCTTCCCAAGGAACTAGCGGAGCTCACGGCCGAAGTGTCAAAGGAAGGGA CGAAGCCCTGGTGAACAA GGAGGTACAGAGGAACATCTCATGTTTCTCGAGACTGCCCTCTCCAAGCGGAGAAATCCGTGACTA(6527) TAC(12)GCTCTGATACCA(1)(7268)GAAAAATGGCCCGAAAAACATGGATCAAACTTCTTAAGTGCGCTCTTGAACA CTTAGAAGACATAAAATTCAGAGACAATGCGGAAGATAGAACTGAATAAACTCTTTATTTCTCAAGAAAGTTAATA CAATACAAGAGAAGGTT(7123)
	15 815	(132)TTTGACACAGACACTCACTCCTCTTCCCTCCGAAAAAGAGAGCAAACCTCAAGGTTCTGGTCTGAAACTTGGGT TTCTCGACAAGAAGGAAAGTGTTTAAAGCTACCATGGTGAGAGCTA(15)TG (815)AAGGCCACACAGTCAGCCCTGAAGCTAGGCTTCGCACAACTGCAGGA GGCAGTTCTAGTCTATCACAAGG GAAAAATGATCCCAACCAATCGAAGCAGCTACTGCACAAAGTAGCCGAA CAGCTGAGGAAGCAGCTTATTGAGGTC AAGTCCGTCTCGAGGAGACCAAGAAAGATCGC(993)
	767 1426	(685)CTCCAGACTCACCCAGTACCTGACAACAAAGGTTGGTTCCTACCAACAATCCCGGAGGATTACCCCTCCTG GACCAAGCCA(767) (1426)CAATACAATGAGCAGAAGCCAAACAAGGCTCCAGGCTCCGCCGCAACTGAAAGGG CAACAAGCAGCTCG GAATCAGGCACCCCACTTGGAGGACCAGATCCGAGGATACAGGCGCTCGCAAGGTTACGACACAGGC(1566)
	2473 1321	(2367)ACGAGGCAGAGGACGAGGAGGATCAGGAAGTACAGGTGATAGGAGCTACCACATTGAGGAGCCAGA GA TGGAGTACCAACTAGGCTCGAAGAAGTTATGGGCAAG(2473) (1321)CTCACTAAGGAGTTCGGAAGGTCAACTTAGGGAAAAGGAGGATAGAAGGAGCAGTCTCATCCAGAG ACAAGAACTTCTACGTCTGGAAGAACCCCTTCAATCAATACAATGAGCAGAAGCCAAACAAGGCTCCAGGCTCCGC CCGCAACTGAAAGGGCAACAAGCAGCTCGGAATCAGGCACCCCACT(1513)
	7268 plant	(7103)ATCGAGTAAGCTCAATAGCCAACCTTCTTGTATTGTATTAACCTTCTTGAGAAATAAAGAGTTTATTCAGTT CTATCTCCGATTGTCTCTGAATTTTATGTTCTTCTAAGTGTTCAAGAGCGCACTTAAGAAGTTTGTATCCATGTTTT CGGGCCATTTTTC(7268) (plant)TGGTAACATCGACTAAATATTGTTGAAATAATCTCCACCTTTTAACAAAGATCTGTACTTTTATTTCTGTCA TGGATTGATATGCAACAAAACCTTCGATTGACATTTTTTTCTTTTGATTCTTCATTT(plant)
endoRYNV -CU2	2217 5319	(2087)TGGGCAGGTGGAGACAGCAATATGATCATCACCAGATCTCTGGTGGGACGATTGACTAACACCAGCATGA C CAACCTTGAGTACCGGATAGAACAAGTACAGACTACTTGGCGAGCAATGGAGTCGATG(2217) (5319)ACTACTCAGAAGAGGAGTAAGCAGCTGAGAAACCAAGCTGCTACAAGAACTGAAAGAGCAGGGATA CATCGGAGAAGAGCCCATGAAGCACTGGGCAAGAATGGGA(5429)
	plant 1	(plant)GAATTTAATTAGAGAAACAATTTAAACCATTCAAATTTTATTACAATGCGTGTATATGTGAAAATAGGAAAA CTAGCAATAATTGGCACAAGGGGAGTTCCATAATTA(plant) (1)TGGTAGATTGTACTTTAGCTCTATTCAACATGTTAGCTTAACTTTTCTTTCTTGTGAGAAACCAAGTTTCAGA CTAGAACCTTGAGTTCTCTCTCTTTTTCAGGGAGGAGAGAGTAGTGAGCATTGCAAACTTCTGCAACTTTCAAACC CCCATGAAAGCTTTCC(279)
	plant 188	(plant)TAACATCCATCCTTGTTAAAAATACTTGTTATTCCTTGCCTGATATTTCTGTATTTGAACTGTACAATCTGCT GTCCTGTTATTTGCCTAGCCAC(plant) (188)TTCTGATTTTCACTAGTTCAAACCTACTGCTTAACTGCAGGCTTAGGCGTCGAAGCGAAGTACCCTTGAGCC GTTAGCTGGAGTGCGTTAGGCGTTGATTGGGGAACGACGTAAAGAAGCAGCAGCAACTAGCAAGAA AACT(335)
	5863 plant	(5738)GGCAACGCCAAAATCTTCAGCAAGTTCGACTTGAAGTCTGGTTTTCCACAGGTAATGGATGAAGAGTCC ATCCCATGGACGGCGTTTGTACACCTGTAGGCTTCTACGAGTGAAGGTGATG(5863) (plant)TCAGAAAATTGGCCGAACATAGGATCAAAAACCTTCTAAGTGCGCTCTTGAACACTCAGAACTCATAAAT CAGAGAAAATTGCGGAAGATAAGATGAACAAAAGCTCTTATTTCTCAAGATGTTAATACAATTCTAGAGAAGGTT GGCTATTGAGCTTACTCGATGCCTA(plant)

endoRYNV -CU3	4550 4423	(4434)AGATTGTTCTTCAGTTTTCCGCAATATCTCTCAGTTTTATGAGTTCCTAAGTGTCAAGAGCGCATTTAAGAA TTTTTGATATTGCGGAAAACTGAAGAACAACTCTTATTTCTCT(4550) (4423)TGAACGTTAATACAATACAGGAGAAGGTTGGCTATTGAGATTGCTCGATGCCTAAGCCTTCAGACTACACTC CCTTATATAGGAAGTAGGCTAAGCAAAACGACAGCAACTACTAGCTCGGGTGCCTTTCTGGGCCCCATCGACAG CTAATAAGTTCTGCTTTACAGCTTTGCTAAAGCT(4242)
	plant 1	(plant)TGCTAGCGTGCCAATATGATGAAGTGAAGCTATGAACCATTTGCTAGTTTGACTGAATTGTGGTACTTGA TTTGGTTGAGGTTGTATTAGGATGGGTATGCCAGGTAGGGGAACTCTGCAGTGAATAGCTACAGCAAGAGGA AGTTGCAGCAAAATGCAAGACTCTGCAGTGAATAGCTACAGCAAGAGGAAGTTGCAGCAAAATGCAAGAGGACC TCTTGT(plant) (1)ACAACGGCTGTATGCTAGTCTACCGCAGGACTTCACCTTAGTCCCTGACGTGATCAACA(60)
	plant 1492	(plant)AAACAAAAACAAAATGTATGGTCCATTAGCCGAAATCAATTCTGCTGTAGGCATCATTTTGCAGTGTGGGA TCTGACTGATCTTGTCAAGGTCCTCTTGTGATAGCTCCAGTCAAACAC(plant) (1492)TCCGATTTGGAACAACGATTCCACCGATCACATCCAAAACCTTGAAAATGTAGCAAGGATGATCGAACAA GGAAGGAAACCCCGAGGTAAATCATCAAAGAAACAGCTGAGAGCAGCAGCAACACCATTTGGAGCCCTCTAGCA AAGAAGGAATAGAGGAGCTAGCCGAGCTGTA(1670)
endoRYNV -BS	7602 6317	(7445)CCAAGCGCACCCGGGCTAATTTCTCTTGTCTTTAGCATAAGCCCCCTTCTATATAAGGAAGCTAAGTTAG AAGGCTTAGGCATCGAGCAACCTCAATAGCCAACCTTCTCTTGAATATCAGTATTCAGAAATTAGTATTCAAGAA ATTAGTTG(7602) (6317)CTATCCCATGTTCACTTGTCTTGTCTATACAGAGGCCAAGCAAGGTTCCGCATTTGGGAATGTAGTTTCTGG CGTAGTTGAGGACTCCTAGCCAGCTCCTAATCCTTTGAGGGTC(6199)
	plant 1	(plant)TCAAATTTTGTGAAAAATTTGTAGAAGTGATCTACTCATGAATATTTAATACTGAACAGTTGATTTGTGAG ATGTAATCGAAAAATAGATATCACAATATTAATCAAATAATACTCATTTAAGTGAATTTTTC(plant) (1)TGGTATCAGAGCTTTAGCTCTTCTTATTATGTCAGCTTAAACACCTTTTTCTGTGTGAGAAACCAAGTTTCGGA TCTGAACCTTGAGTTCTCTCTTTTTCAGGAGGGTAGTGAGTAAGCCAGCTTTGCAACTTTCAAACCCCGAGGAA ACTTTCCC(161)
	plant 1777	(plant)AATTAAGTGATCGATTATCAGACCGAGAATTAATCTATTCATGGTTCAATAATCAGTCCGATAATATATAT ATCAATATATGCACTGATCAACAATTAA(plant) (1777)TTCAGGTACATTGAAGAATCCTCTTACCAAAGGCTAGCAGCAGAGAAGGCATGCAGTTTATACATGTAGGCATG GCCATGGTAAGAATACAGATGCTGCACAGGACTGACGCA(1887)
endoRYNV -PH1	335 5859	(452)AGTCGCCCCGTTAGATCTTTCCAGGAGCTACTCTCTTCTCGAAGGCAGAGATTTCGTGTGTTGCATCAAAA CCACCAAGATCTAGCCGCTTACTAGCTTTCCGGCCGGTGATCTG(335) (5859)TCACCTGGTCAAATGGCTTCCAGAAGAACTGAAGGATCTCGCGCCGAGCTAGCCAAGAAAGAAGGCAA GACCTCCCTGAAGGGAGAGGTGCAGGAGGAGATCTCCTGTTTTCTCAGAACTGCC(5984)
	1373 1814	(1215)GGGACTTCAACGGCAACTAGATCCGGATGCCAGCTCTCTCTCAGCAGAAGGGGAAGAGCAAACTGTGA CCAGCAGAGGTAACACACTGAAAGAAGAAGGACCATCCGAGCCAGAAGGAAGGCCAGAAGGAGAGGACGA GAGCACACATTATG(1373) (1814)ATCCCCGAAGAAGAAGAAGTCTACCTCAAATATGAGGCAGAAGACGAAGAGGAGGATCAGGAGCTTC AAGTGATTGGAGCCACCACCATAGAAGAGCCAGAAATGGAATACCCAACAGGCTCAGGAAGTTATGGGC(1954)
	plant 1	(plant)AAGGACGAGCTGATATTCAAGTGAATAAAGATTGTTTCATTTTTCTCCGCATTTTCTCTGAGTTTCATGA GTTCTTAAGTGTTCCAGAGCGCACTTTCGAATATTAGATCCATGTTTTTCGGACCCCATTC(plant) (1)TGGTATCAGAGCTTTAGCTCTACCATGGTAGCTTAAACACCTTTTTCTGTGAGAAACCAAGTTTCAGATCTG AACCTTGAGCTTCTCTCT(95)
	6631 plant	(6542)GCTTAGCCTACTTCTATATAAGGGAGTATAGTCTGAAGGCTTAGGCACAGAGCAATCTTTAGCCAACCTT CTCTTGAGTTGTATTAA(6631) (plant)TATTCAACCTTCCCTAAACCTAAACCTAAACCTAAACCTAAGCACACCCATGGTGAGAGCTAAAGTCT GATACCAAGTGGGGTCCGAAAAACATGGATCTAATATTGAAAGTGCCTCTGGAACACTTAAGAACTCATGAAA CTCAGAGAAAATGCGGAAGAAAATGATGAACAATCTTTATTTACCAAGTGAATCTGTTT(plant)
endoRYNV -PH2	2201 2432	(1990)ACAATTGGTATACATAATACCAAACGCAATGATGTCAATACAGATTTTACAATTGTATACAGGTGAGCGTG CAAACCCGAGGCTACGGGACAGGATGGGCTGGAGGAGACAGCAGTATGATCATCACTAGATCACTGGTGGGCGT CTCACCAACACCAGCATGACAACTTCGAGTACCGGATAGATCAAGTACAGACTATCTAGCAA(2201)

		(2432)AGCACGCACTACGTGCTCGTCTTCAGACATAACTCCTATGAGACCAACCTCAGAGGAGAAAGGAGGCCAAGGCAGAACGAACCTTC(2517)
	7091 plant	(6970)GCAGAGATGGACGAGTCTAGAAGACTGGCCAAACAACGCCGAGACAAAGTCTTCGACGACG CAGG GCAAAACATCTGCGACACGGTCTACATCACCGGTGTCGACCTCGCCGCCGCCAAGGC(7091) (plant)ACGCGGCCACATTTGCAGCCTTGGTGGTGATAGTTTGAAGTGTGCTTGCAATGCTCCACTTGCTAAGTTGCAACATAAGTTTCAGAGTAAGCTTGTGGTGTCACAAGATGAAAAAGGCTCAAGGCTAGTTCTGAAGCTCTTGAAACA TGAAAGTATTAAATTATATTCATGGGAA(plant)
	3094 plant	(2924)AGTGGCATGGATAGCGAGTCCAGCATCACAGGTGGAGGATTATACCACCAAGCCAGTACCAGGAGCGCAGGGATACCCACCTGCCACAGGAGCGTCTGCTCAACCATAGGGCCAGCAGATCTGCAAGGATGGGGAGGACGAT TGCCAAGGAGCAGATCGCCGATAGGA(3094) (plant)AAATGGCCCAACATATAGATAGGAAAATGGCCCAACATATAGATCGAAAAATGTCTAAGTGCGCGCTTGAA CACTTAGAAACTCATAAAAACTCAGAGAATATGCGGAAGATAAGATGAATAAACTCTT(plant)

375

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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: CACAACTCAGGGACGTCTATC endoCU3-2575R: ATCCCTGGCAAGGTGTATTG	126	79.15
endoRYNV-BS	endoBS-4668F: CCTCCAGAACATCGAGAATGTC endoBS-4767R: GCTAGGCATTTCTGTCACCTATTA	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

10. Figures

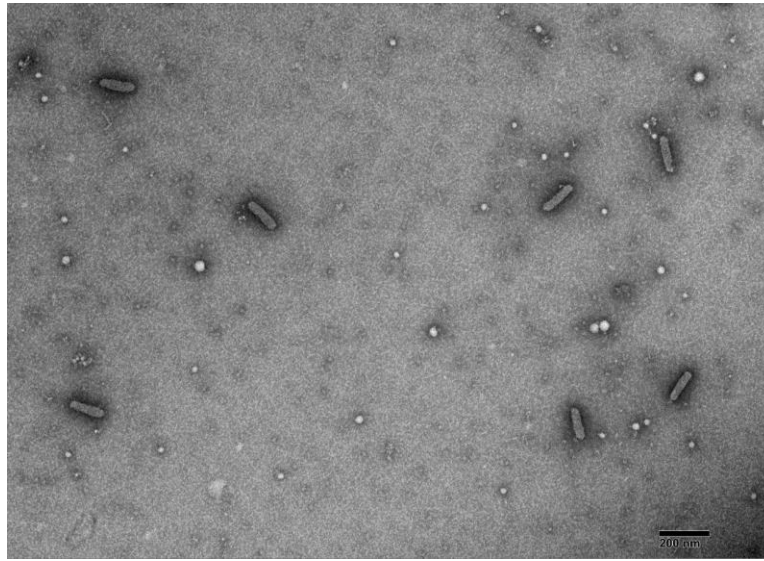


Figure 1. Bacilliform particles of rubus yellow net virus from 'Baumforth's Seedling A' (epiRYNV-BS) captured using antibody against sugarcane bacilliform virus and stained in phosphotungstic acid.

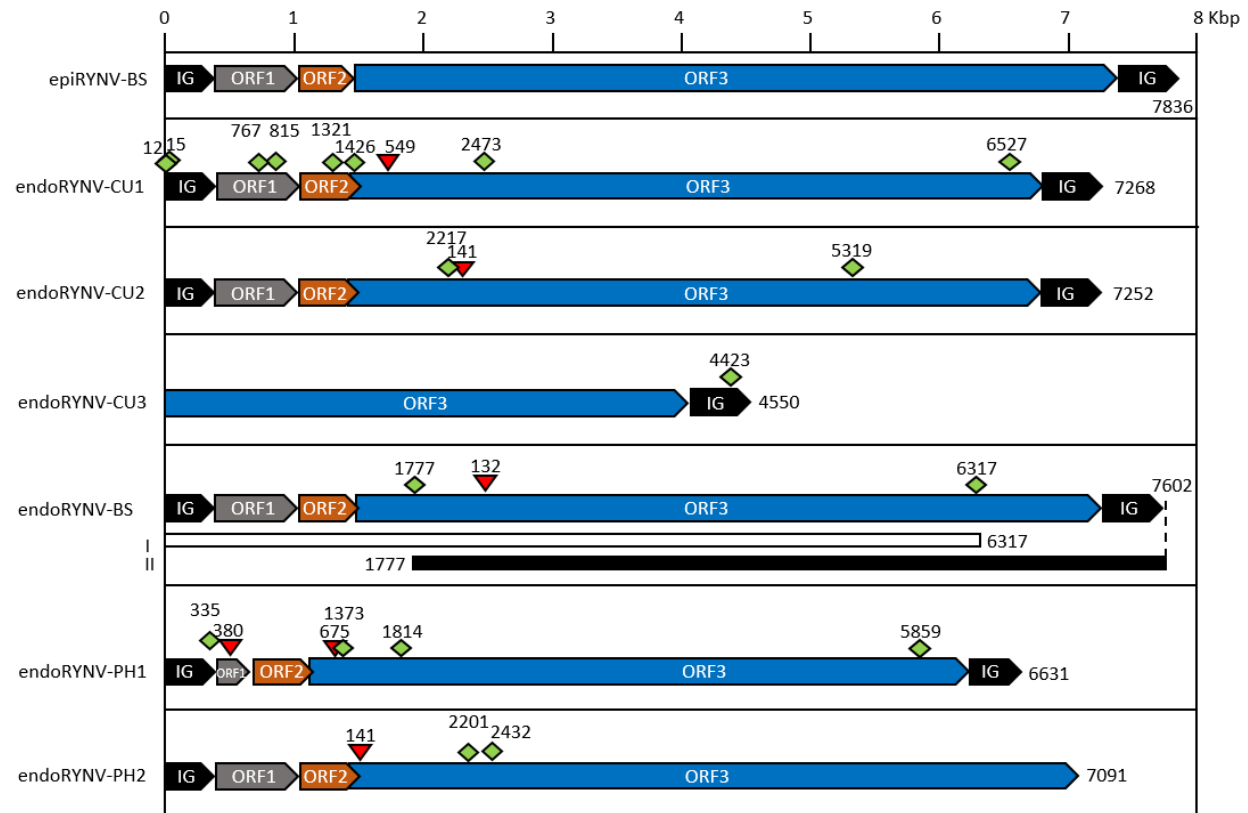


Figure 2. Overview of endogenous rubus yellow net virus (endoRYNV) lineage sequence structures in red raspberry. The sequences are represented with intergenic region (IG) in black; grey, orange, and blue boxes indicating ORF1, ORF2 and ORF3 of the virus, respectively. For simplicity, ORF4 and ORF6 embedded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

```

endoRYNV_CU1      ACGTCTAGTGAAGTGACGCAATGAATGACTTCACAATTGCCAATGTCGTCACCTGCTTACG
RYNVCaKF241951    ACGTCTAGTGAAGTGACGCAAGGAATGACTTCACAATTGCCAATGTCGTCACCTGCTTACG
*****

endoRYNV_CU1      ACTTGGAACCTTATCGTTTTGTGTGCGGAGCATCTCTTAGCTGTGCTTTGTGTGTAAGTGC
RYNVCaKF241951    ACTTGGAACCTTATCCTTTAGTGTGCGGAGCATCTCTTAGCTGTGCTAAGTGTGTAAGTGC
*****

endoRYNV_CU1      GCCGGTAGTGCGCTGTGTGTCAGGATAAGGAATCTTATCTCCTTATCTTTTTCG--TTTGT
RYNVCaKF241951    GCCAGTAGTGCGCTGTGTGTCAGGATAAGGAATCTTATCTCCTTATCTTCTTCCCTTTGT
*** *****

endoRYNV_CU1      -AAAGCTA--GCTGTAAAGCAGATTCTCTTAGCTGTGATGGGGCCAGAAAGCGCACCC
RYNVCaKF241951    TAAAGGTAAAGCTGTAAAGCAGGACTAATTAGCTGCAGGTCA--TCAGGTTTGGCGTTGT
**** * *****

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

endoRYNV_CU1      TAGAAGGCTTAGGCATCGAGTAAGCTCAATAGCCAACCTTCTCTGTATTGTAT--TAA
RYNVCaKF241951    CATAGAGATGTCGTATAAGCTCATCTTTGTTGTGGTATTGCCACTTAGCTGCGTCCTTGG
* * * * *

endoRYNV_CU1      CTTTCTTGAGAAATAAAGAGTTTATTTCAGT--TCTATCT-TCCGCATTGTCT-CTGAATT
RYNVCaKF241951    TGTCCCTTTGCGGCTATAAGCTTGATCCCATGATCCATGTATGCGCAAAGGGGACAGAGT
* *** ** * * * * * * * * * * * * * * * * * * * * * * * * *

endoRYNV_CU1      TTATGTTCTTCTAAGTGTTCAAGAG---CGCACTTAAGAAGTTTGTAT-CCATGTTTTC
RYNVCaKF241951    TGAGGTTGCAGGTGGTGCAGGTGACTCTTCGCCATGAGGCGTTTCGTCGCTGCATATGC
* * * * * * * * * * * * * * * * * * * * * * * * * * * * *

endoRYNV_CU1      GGGCCATTTTC-----
RYNVCaKF241951    TGCATATCCTCCCTTCGATCGGCACCTCTTGTGTATCACTCCAGGCGTGAGTGCAATTCTT
* ** * *

endoRYNV_CU1      -----
RYNVCaKF241951    GCTGTGCCTTTGGTATCTCCTTCCTTCTTCCAGGAAGGTTTTTC

```

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

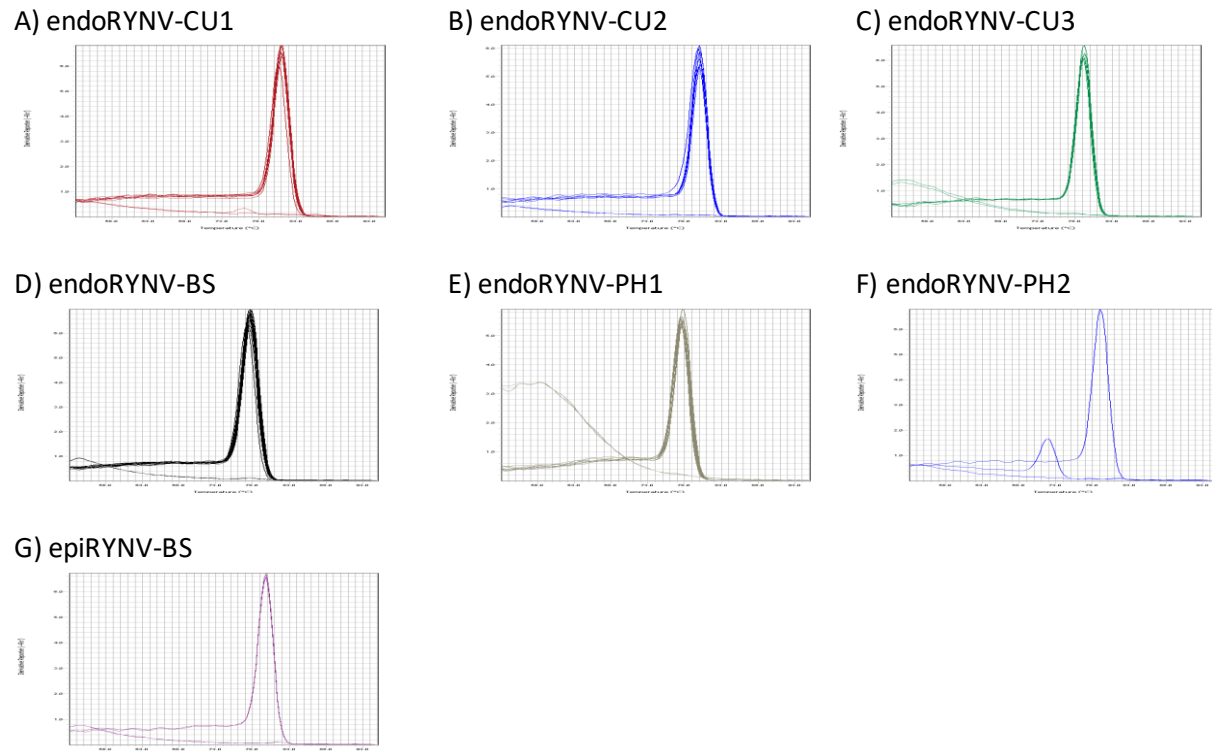
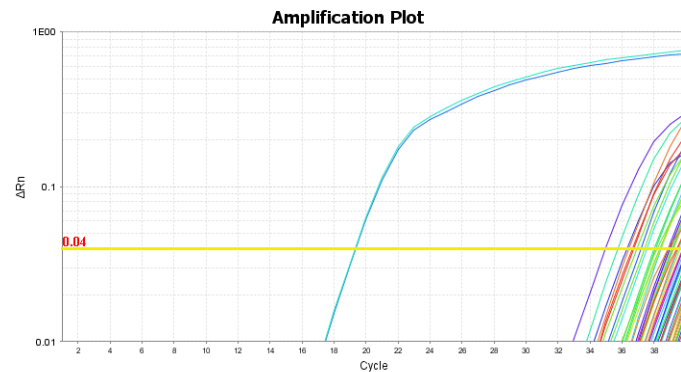


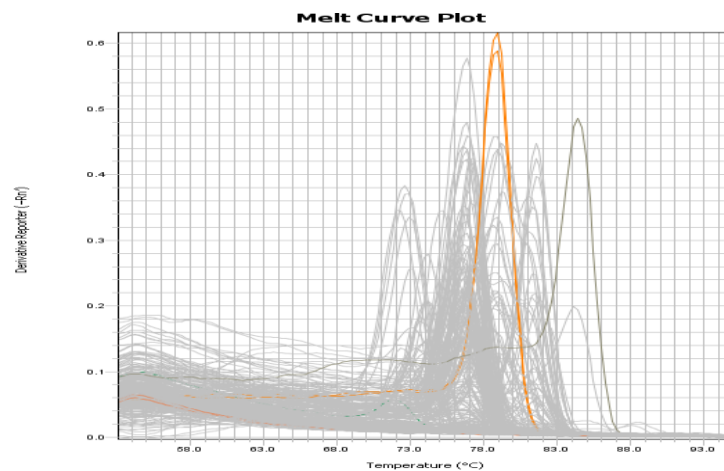
Figure 4. Melting curves for the lineage-specific SYBR Green PCR validation assays detecting the endogenous and episomal rubus yellow net virus (endoRYNV and epiRYNV respectively) lineages from the raspberry cultivars with corresponding endo/epiRYNVs. A) endoRYNV-CU1; B) endoRYNV-CU2; C) endoRYNV-CU3;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

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

11. Supplemental data

>endoRYNV-CU1

TGGTATCAGAGCTTTAGCTCTACCATGGTAGCTTAAACACTTTCCTTCTGTGCGAGAAACCCAAGTTTCAGACCAGAACCTTGA
GTTTGCTCTCTTTTCGGAGGGAAGAGGAGTGAGTGTCTGTGTCAAAACCTTGAAAGATCAAAACCCCATGAAACTTTCCTCA
CGGTACCATAAGTTTCTATCCTTCACTAGTTTGAACCTACTGCTCAAACCTGCAGGCTTAGGCGTCGAAGCGAAGTACCCTTGTA
GCCGTTAGCAGGAGGCGTTAGGCGTTGATTGGGGAAAACCTGACGTAAAGAAGCAGCAGCAACTAGGCAAGAAACCTGACGG
GTAGATCACCGGCCGGAAGCCAGTAAGCGGCTAGATCTGGGCGAGTTTGTATGCAACCTCACGAAATCTCAGCCTTCAAGAA
GAAAGCAGCTCTGGGAAAGGTCTGAACGGGCGTATCGACAAGACTTTTATTAGAAAATCTCAGAACTATCCACGTTGGGA
GGCAATCAGAAAACACCTCTCTAGACTTTCCTTGTACCACTTCAACAACAACCGGACCAACAGTCCACCGCACTCTCTGC
AGACAAGAGAAGCAGTAAGGATTTACATTTCTGGTAAACACCTGTTGATCTCAACATCACCGAGATCCACAATCAGGCGATT
CTGGACGATAAGATCTCAGACTACCCAGTACCTGACAACAAAGGTTGGTTCACTACCAACAATCCCGGAGGATTACCCCTC
CTGGACCAAGCCACAATATCCTTAGATCTTCAAGCCCTCAAGGCAGATCTGAAGGAAATCAAGGCCACACAGTCAGCCCTGAA
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855 AGATCTCTGTCCCCGACGGATGAACCCAGGTGGCAAGACACCGCCGCAAAAGAAACCTACATAAAAGCTATCCAGGCTACC
856 TCATCTCTTACATCCAACAACACGGGGCTAGGGTTCATTGAACCTCACACCTATACCGGAGGACAACTGTCCACAAGCCTAGCA
857 AAACAAAACAACAGCTCATACAGCTGTTGGTTCAGGTGCTAGAGAAAGTCTCGACCTTGAACAGGCCATCGCGAACCTGTC
858 CGCACAGGTCACGAGACTTGAGAAGACCGTCGCAGAGAAAGACACGGTCAAACCTCCAGAAAAGTGTCTCAACGACCTCACT
859 AAGGAGTTCGGAAAAGGTCAACCTTGGGAAAGGAAAGGGGCTAGAAGGAGCAGTCTCATCCAGAGACAAAACTTCTACGTTT
860 GAAAGAATCCCTTCAATCAATACAATGAGCAGAAGCCAAACAAGGCTCCAGGCTCCGCCGCAACTGAAAGGGCAACAAGCA
861 GCTCGGACTCAGGCACCCCACTCTGGAGGACCAGATCCGAGGATACAGGCGCTCTGCAAGGTTACGACACCAAGCACAGAG
862 AGCGGTACGAAGGACCTTCAGCAGGGACTTCAGGAACACCATAGAAAGGCAGCTAGATCCGGACGCCGAGTTATCACTCAGC
863 AGAAGGAGAAGAGCAAACCTAGTACCAGCAGAGGTACTCTACGCTCAACCGTTCAGAACCTGTGAATCGTGTGTATGAGC
864 ACTATAGTGAGCTCGGGGCTCATATAGTAGACAGGCAACAAGACTTCCGATATATCGAGGAAGCATCTACAGAGACTAGTC
865 AGAGAAGGCATGCAATTCATACATGTCGGCATGGCAATGGTTAGGATCCAAATGTTGCAAGGACAGATGCAGGTATATCTG
866 CGTTGGTGGTGTTTCAGAGACACTAGTGGAGCGATGACAGGCAGGTCATCGGCAGCATGTCCGTAGACATGACCAGGGGAG
867 CACAATTGGTATACATAATACCAAACGCAATGATGTCAATACACGATTTTACAATCGTATACAGGTCAGCGTGCAAAACCCGAG
868 GCTACGGGACAGGATGGGCTGGAGGAGACAGCAATATGATCATCACTAGATCACTGGTGGGGCGTCTACCAACACCAGCAT
869 GACAACTTCGAGTACCGGATAGATCAAGTCACAGACTATCTAGCAAGCAACGGAGTCGCTTGATACCCGGACAGAAGTGG
870 GATGTGGCCAAACAGGTCCGGCGAATGGGAGCTACAGCCAGCAGGATTGTTGCACCACTCATGACCAACAGAGGCGAGAC
871 TCAGCCAGAACAGGAGTGGCAGTATAAGTCTCAGATTACCGACTTCCGAGATCAGAGGATCGTGGAAAGAAAGGCCAGTCGA
872 GGATGAGGGCAGGCCAGAAGGTGAAGAGGAGAGCAGCACTACGTGCTCGTCTTCAGACATAACTCCTATGAGACCAACCTC
873 AGAGGAGAAAGGAGGCCAAGGCAGAACGAACTTTCAGAGTTCACACCAGAGACAGATTTGGTGAGCCAATGGCTAAGCCAG
874 CTATCCGCATCAGCACACAACAGTGGAGTGTCAAGCTCAGAAGAAGAACCGCTAGGTTGATGAAACTGATGAAGACAGTG
875 ATGGCACAATACATGAGAAAACCTGGCAGAAGGAAGACAGGAAGGAGAAATCTGGAGCTGCAAGGATGGAAGAAGACCT
876 GCAGACCAGGCATATACGAACTGATCCAGAGGAAGAAAGAGGAAATCTACCTCAGATACGAGGCAGAAAGACGAGGATCAGG
877 AGGTACAGGTACTAGGGGCAACAACCATGGAGGAACCAAGAAATGGAGTACCCACCAGACTGGAAGAGGTTATGGGCAAGC
878 TAAAGAATGTCAGCATGGAGAAACTTTTCCAGTAAGTGGCATGGATAGCGAGTCCAGCATCACAGGTGGAGGATTATACCA
879 CCAAGCCAGTACCAAGAGCGCAGGGATACCCACCTGCCACAGGAGCGTCTGCTTCAACCATAGGGCCAGCAGATCTGCAAG
880 GATGGGGAGGACGATTGCAAGGAGCAGATCGCCGATAGGATATGGCCGCCACAGCAGCCATGGTCTTACCATCAGCCCA
881 GTCTGACAACGGCTGTATGCTAGTCTACCTCAGGATTTACCTTAGTTCCCGATGTGATCAACCGATGGGAGTCTATTACTGT
882 CAACCTCATCAACAAATGATGTTTGATTCCCTACAGGATAAGGCGGACTATGTAGAAAATCTCTTGGGAGAAAAGAGAAAAGG
883 AGACATGGATGACTTGGAGGATGCAGTACGAAGAAGAATATAAGCAACTCCTACCATGAGCGGAGACGTGAGGAATCTCAC
884 TGCCGCAGTCAAAAGGGTCTTTGGCGTACATGACCCTCATACTGGATCAGTCCACATACAGAACCAGGCGTATGCAGAACTGG
885 AGAGACTGTACTGTAAGCGGACAGACGACGTGATCCCTTCTCTACGACTACTACCAGCTAGCAGCTAAGTCTGGAAGGATG
886 TGGCTCGGACCAGAGCTATCAGAAAACTTTTCAAGAACTTCACTGAGATAGGGCCAACCTATTGAACAGGCCTACAAAGA
887 CAGGTACCCAGGTCTCACCATTGGAGTCTTGGCAAGGGCCAACCTCATCTGGAATACCTACAAAACGTCTGCAAGCAAGCAG
888 CGTTACAGAGGTCCCTGAAGAGCCTCAGTTTTTGCAGAAACATGCCGGTGCCTGGATACTATGAGAAGAAACAGTATGGCATC
889 AGGAAGGCAAAAACCTTACAAGGGAAAGCCTACCCAACGCACGTAAGGTGATCAAAAACAAGTACAAGCACACCTCCGGGA
890 AGAAGTGCAAGTGCTACCTTTGCGGAATAGAAGGCCATTACGCCAGGGAATGTCCAAAGAAGGTGGTAAAAACCAAAAGGC
891 GGCATTTTCAATGGCATGGGCTTAGATGACAACTGGGATGTTGTATCCGTAGAGCCAGGAGAGTCAGATGACGACGAAATC
892 TGCAGCATCTCCGAAGGAGAGAACGCTGGGGGAATGCATGAACCTCATGGCATTCAAACTCACTCCCGTACCCAGTCGAGTA
893 CGAAGCCAGCACATCACAACAGTTCCTGCCATGGATACAGGTAAGTGTGAGAAAAGTGATAAGCCCTCTGGAGGAGAAGA

894 AGAGATATCCACAGGCACAAAAGGACTGCGCTCACACTTGGAGCGACACACAGGAAGTGCCGATAGAAGGGAGGATATGC
895 AGCATTTGCAGTGATGAAACGCCTCATGGAAGGAGAGTCACTGCACGACCTGCAGCCTAAACCTGTGTCCAATTTGCGCATA
896 TATGGATCATGGAATTAAGCTGATCGCCGAAGGGACACCAGAGACGCAGCAAAGTGGCAGTACCACAACAAAGATGAACTC
897 ATCAGGCACCTCTATGAACACAATGCCTTCTAACCAGGAAAGTGGAGGAACTGACGACCCAGCTGCAAGAATTTCAAAAACCG
898 CAAGCCTGAAGACCTAATCAGCTTGGCGGATGATATGGAGGACGTGTCCATTCTGGACAACGCCTCAAAAAGGGGAAAGGAG
899 AAGGAATCTTTCCAATTCGGAACGACGATTCCCATCGATCACATCCAGAATCTTGAAAACGTAGCAAGAATGATCGAACAATG
900 GAAGGAGACCCCCAGAGTCACTATCAAGGAAACAGCAGAAAGCAGCAACAATAGGAGCCCTCTAGCAGAAGAAGGGAT
901 TGAAGAGCTAGCTGCAGCTGTTGATACGGCATAACAGAAATGCCAAAAGGAGGTCTGAACAACTATACAACACCAATTGTTG
902 AGTTTGTAACTCAGGAAAAGGGGCACCCACAAGATTCAGGGTTAGAGCAGTCATAGACAGGATGCACCTGCACATG
903 CATCAACAGCAAGAAGTCCCTAAGGAAGCCCTGGAAGAAACAAAGTATCAGATGAACCTTGACAGGAGTAAATTCACCTGGA
904 GAAACCAAGCTGAAAATGAAGATGGCAAGATGATAGTATCAGGAAGTGATTTCTACACGCCATATATTGCACTTTCCCAAT
905 GGAATTACCAGATGTGGACATGCTCATTGGCTGCAACTCTTGCGAGCCATGAAAGGAGGAGTCAAGCTGGAAGGAACGGAG
906 GTAACCATCTACAAGAAGGTTACAACATCCAGACGACTCTAGAACCACAGAAGATCTCTACTCCGTGCAGAAGCTGAAGC
907 AGGAGAAGAAATCGAGAGACTTTACTACGCCAACGACTACTCTGAAGAAGGAGTAAACAGGCTGAGAAAACATAAGCTGCTA
908 CAAGAGCTCAAGGAGCAGGGTTACATTGGGGAAGAACCAATGAAGCACTGGGCGAAGAACGGAATCAAGTGAAGCTTGAT
909 ATCAAGAACCAGATATAGTCATCAGCAGCAAACTCCGGATGCAGTCTCAAAAGAGACGAAGGCACAGTACCAGAGACACA
910 TCGATGCTCTATTGAAGATCAAGGTAATCCAAACCAGCAAGAGCAAGCATCGAAGTCCACCTTCATCAAACTCGGGTACA
911 ACCATAGACCCGATAACAAAGAAGGAGATCCGAGGAAAAGAGAGGATGGTCTTTGATTACAGAAGCCTCAACGATAACACCC
912 ACAAGGATCAGTACACCTTGCTGGGATTAATACCATCATCTCCGCAATTGGCAACGCAAAAATCTTCAGCAAATTTGACTTGA
913 AGTCTGGCTTCCACCAAGTACTTATGGATGAGGAGTCCATCCCTTGACCGCTTTGTACACCAAGTAGGTTTCTACGAATGGA
914 AGGTAATGCCTTTTCGACTCGCGAACGCTCCTGCACTTCCAGAGGAAGATGGACCAAGTCTTCGAGGAACCTCTGAGTTC
915 ATAGCCGTCTACATCGACGACATTCTGGTCTTCAGTAAGACGCTGAAGGAACATGAGAAGCATCTCAGCATCATGCTGGGAAT
916 ATGTCGAGATAACGGCTGGTACTGTACCCAGCAAAATGAAGCTCGCCGCAACAGAGATAGATTTCTTGGGAGCAACCATTG
917 GTGACGGAAGAAATCAAGCTCCAGCCTCACATCATCAAGAAGATTGCCGAGGTGGACGATGAATCCCTAAAGACCTCAAAGG
918 GCTGAGAAGCTGGTTGGGAGTTCTGAACATGCCAGGAACTACATCCCCAAGTGTGGGACACTCCTTGCCCACTCTACAGCA
919 AGACGAGTGAGCACGGGGACAGAAAGATGGCATGCATCGGATTGGGCCTTAGTCAAGAAAATCAAAAGCCTGGTCCAAAACCT
920 CCCAGACCTCAAACCTGCCACTGAAGAGGCCTATATGATCATCGAGACAGATGGTTGTATGGAAGGATGGGGCGGAGTCTGT
921 AAATGGAAGCCCAACAAAGGCAGACTCAGCAGGAAAGGAAGAAATCTGCGCATATGCAAGTGGGAAATTTCCAAACGGTCAAAT
922 CAACTATTGACGCAGAAATCTTCGCGGTATGGAATCCTTGGAAGAAATCAAGATATTTTACATGAACAAGGATGAGATCACC
923 TCAGGACCGACTGCCACGCCATCATAACCTTTACGAGAAGTTAAACGCCAAGAAGCCTTACGCGTAAGGTGGTTAGCTTTT
924 GCGACTATATAACAACTCCGGGGTGAGGATGAAGTTCGAACATATCAAAGGCCAAGATAATCAATTAGCTGATAATCTCAGT
925 CGCCTAACCCAACTCATACCGCAGTAAGATGGCTTCCAGAGGAAATGGCAGGAATCGCGGCAGAACTAGTCAAAAGACAGGG
926 AAGAGTCCCCCGGATGCAAGAAGGTACAGGAGAGCCTCTCAGGCTTTCTCAAAGCTGCCCTCCTCAAAGTCGAGAAATCCTCG
927 ACTACAGCCCATCAGAGCCGCACCATGCTCTCTGGCAGAGATGGACGAGTCTAGAAGACTGGCCAAACACGCCGAGACAA
928 AGTCTTCGACGACGACGAGGGCAAAACATCTGCGACACGGTCTACATCACCGGTGTGACCTCGCCGCCGCAAGGC

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930 TGGTATCAGAGCTTTAGCTTTACCATGGTAGCTTAAACCCCCCATCTTGTGAGAAACCCAAAGTTTCAGATCCGAACCTTGA
931 GTTTGCTCTCTTTTCGAAGAGGAGAGGGAGTGTGACCTTACTCAAAACTTTTTCGCGATCAAACCCCCACGAAAACCTCCTTAC
932 GGTATCATAAGTTTTCTGTCTTCACTAGTCCAAACCTACTGCTCAAATCGCAGGTCTAGGCGTCAAGCGAAGTACCCTTGTA
933 GCCGTTAGCAGAGTGGCGTTAGGCGTTGATTGGGGAAGAACTGACGTAAGAAAGCGACAGCAACTAGGCGAGGAACTGACG
934 GACAAATCACCGGCCGGGAAGCTAGTAAGCGGCTAGATCTGTGTGGTTTGTGCAACCACACGAAATCTCCGAATTCGAAGC
935 AGAGAGCAGCTCTTGGGAAGATCTGAACGGGCGTATCGACAAGACTTCTCATTAGGAATCTCAGAACCTATCCACGTTGGG
936 AATCAAACAGAGGACACCTCTCTTGAATTTCCGTGCTACATTACAACACAACAACCTGGACCTCCAGTCCACCGTACTCTCT
937 CAGACAAGGTGACGGCAAGGATTTACCATACTTAGTAAACACCTTGTTTCGATCTCAATATCACCAGATCCACAATCAGGCGAT
938 CCTTGACGATAAGATCTCAAGGCTACCCAGTACCTGACAAACAAAGTCGGTTCCCTACCGACGATCCCGGAGGATTGCCCCCT
939 CCTGGACCAAAATAGCCCTTTCCCTAGATCTACAGGCCCTCAAGGCAGATCTGAAGGAAATCAAGGCCACACAGTCAGCACAAA
940 AGCTAGCCTTCTCAACTGCAGGAGGCAGTCCAGCTTTTATCGCTCGGGAGAACGATCCCAAGCCGATCGAGGACGCTACT
941 GCACAAGTAGCCGAACAGCTGAGGAAGCAACTCATCGAGGTCAAGACAGTCTCGAAGAGACCAAGAAGATCGCCAGGTCCT
942 TATCCCCGACGGATGAACCTAGGTGGCAGGAACTGCAGCCAAAGGAACCTATCAAAGCGATCCAAGCAACCGCTTCCC
943 TCACCTCCAACGGCACCGGCCAAGGCTTCATTGAGCCCCACCTACACCGGAGGACAGTTATCTACCAATCTAGCCAAGCAAA
944 ACAACACCATAATCGAATTAATGGTGCAAGTGCTAGAAAAGAACTCTGACCTTGAAACGGGGCGGTAGCCAACCTCACAGGTCAA

945 GTCCTAGGCTCGAAAAAGCCGTAGCAGACAAAGAAGCAGTCAAGCTTCCGAAAAAGTCCTAGAAGACCTAACCAAGGAAT
946 TTGGAAAGGTTAACTTAGGCAAAGGAAAGGGGAAAAAAGGAAAGTCTCAAGCCGAGACAGAACTTCTACGTGTGGAAAAC
947 CCCTACACTCAATACAATGAGCAGAAGCCACACAAGGCTCCAAAGCGCCCCCGCAACTGAAAGGGCCACAAGCAGCTCGGATT
948 CAGGGACACCAACCTGGAAGACCAGATCCGAGGATACAGGAGATCTGCACGCTAGAGGCATCAAGCGCAGCAGAGACTAC
949 GGAGGACCTTCGGAAGGGACTTCAGAAAACGATCGAGAGACAACTCGATCCTGATGCAGAGCTCTCCCTCAGTCGAAGGAG
950 AAGAGCCAACCTTGGTACCGGCGGAAGTGCTCTACGCACACAACGGACAAGAACCAGGTCAACCGAGTATACGAGCACTACAGT
951 GAGCTCAGCGCTCATGTGGTAGACAGGCAAGGACTTCCGATTTATAGAGGAAGCGTCCTATCAAAGGCTGACCAAGAGAAG
952 GAATGCAGTTCATCCACGTAGGTATGGCGATGGTCAGGATACAAATGCTGCACAGGACAGATGCGGGTATATCCGCACTAGT
953 GGTGTTCCGAGACACCCGATGGAAGCATGACAGGACAGGTATCGGTAGTATGTCAGTCGATATGACACGAGGAGCGCAGTTG
954 GTATACATCATACCAACGCCATGATGTCGATTCTGACTTCTACAAACCGGATTCAGGTCAGCATACAGACCAGAGGATACGG
955 CACAGGCTGGGAAGGAGGCGACAGCAACATGATTATCACAAGATCATTAGTCGGCCGACTCAAAACACCAGCATCACAAGC
956 TTCGAGTACAGGATAGACAACGTGACAGACTACCTAGCCAGCAACGGCGTAGCTGCATTCCCGGACAGAAAGTGGTCCGTGG
957 CAAACAGGTCTGGAGAATGGGAATCCAGCCAAGCAGGATAGCAGCACCATTGCACTCCACAGATGCCAGGCTAAGACA
958 GAACCCAAACGGCAACATCAGCCTGAGGTTACGGATTTCCGCGACCAAGGATCGTGGAAGAAGGAGAGACATCCGAGCCA
959 GAAGGAAGACCGGAGACGAAGGAGGATGAGAGCAGCACTACGTGCTTATGTTCAAACACTCCAGCCCTAGGTGGGATACG
960 CTCGACAGCCAGCGGTAAATACGACTACATGTCGATATGACGCACCAAGACGACATGGCCAACAAACAGAG
961 GATGGGACGATGACCTCCTAAGCCACCAAGCCAAAGGATCCTACGAGGTAAGCCTAAGAGGCGAAAAGAAGCTGAAAG
962 AAAAGGAACCTCGCAGAGTTACCCAGAGACAGATCTGGTCAGCCAGTGGCTAAACCACTCTCGAACTCGGCACACAACAGT
963 GGAGCTTCAAGCTCAGACGATGAGCCAAAGTTCGATGAGGCAGACGACGAGGACGACGTCTACAATCAGAAAACCTGGGAG
964 AAGGAAGACAGGAAAGAGGGAGCTGGAATCCAAAGGTGGAAACCCACAGGGAGGCCAGGCCTCTATGAAATGATCCCT
965 GAGCAAGAAGAAGTCTACCTCAGGTACGAGGACAGGACGAAGAGGAGGATCAGGAGTTGACGGTCATAGGAGCCGC
966 AACATGGATGAGCCAGAGATGGAATACCCAAACAGGCTCGAAAAAGTAATGGGCAACTCAAAAAAGTAAGCATGGAGAA
967 GCTGTTCCAGTGAGCGGGATGGATAGCGAATCCAGCATAACAGGAGGTGGTGGAGGTTTTATACCAACAGCCAGTACCG
968 GGAGCACAAGGATACCCCCAGCAACAATCCACTATGTCCACATTGGACAGCAGACATGCAGGGATGGGGAGGGAGA
969 GTGCCAGAAAGTAGGTACCATTAGGTATGGCAGACCACAACAGCCATGGTCACTACCATCAGCAGTCAGACAACGGCT
970 GCATGCTAGTCTTCCACAGGACTTACCCTAATACCGGATGTCTCAACCGATGGGAATCCATAACAGTCAACCTCATCAACA
971 AAATGATGTTTGACTCCCTGCAGGACAAGGCCGACTACGTCGAGAACCTTCTTGAGAACGAGAAAAAGGAGACGTGGATGAC
972 ATGGCGGATGCAATATGAAGAGGAGTATAACAACCTTACGATGAGCGGAGATGTGAGGAATATCACAGCCGAGTCAAG
973 CGGGTCTTCGGAGTACACGATCCGCATACAGGATCAGTCCACATCCAGAATCAAGCATATGCAGAGCTCGAAAGGCTCTACTG
974 CAAACGGACGGACGACGTGATCCCTTCTATACGACTACTATCAGCTAGCAGCAAAATCGGGAAGGATGTGGCTCGGACCC
975 GAGCTATCAGAGAAGCTGTTTCAAGAAAGCTTCCACCTGAGATAGGACCTACCATCGAGCAGGCCTATAAGGATCGATATCCAGG
976 GCTGACCATGGAGTTTTGGCCAGAGCAAACTTATCTGGAATATCTACAGAACGTGTGCAAGCAGGCAGCACTGCAGCGTT
977 CGCTAAAAGCCTGAGCTTCTGCAGGAATATGCCGGTCCCCGGA TACTATGAGAAGAAGCAATATGGTATCAGAAAGGCTAA
978 AACCTACAAAGGTAAGCCTCATCCGACCCACGTTAAGGTGATAAAAAACAAGTACAAGCATACGAGGGGAAGAAATGCAAG
979 TGCTACTTGTGTGGGATCGAAGGTCACTATGCCCGAGAGTGCCCAAGAAAGGTGGTCAAAACCAACGAGCGGCCTATTCAA
980 TGGCATGGGACTAGACGATAACTGGGATGTAGTCTCGGTGCAACCAGGAGAAGAAAGACGACGAGAGATCTGCAGCATCTCA
981 GAAGGAGAAAAACTGGCGGAATGCACGAACCTATGGCATTCAAAACACAACCTCCCTTATCCAGTGGAGTATGAAGCCAGCA
982 CACCACAGTTCCTGATGCCATGGACACAGGTACCTGTGGAAAAAGGCGACAAAACCTTCTGGAGAAGACGGAAGGATATCTC
983 ACAAGTCCAGAAGGACTGCACACACCTGGAGTGACACCCAGGAAGTGCCTATCAGCGACAGGGTTTGCAGCATCTGTAGT
984 GACGAAACACCTACGGTAGAAGAGTCACTGCACTACATGCAACATCAACCTCTGTCCGATATGTGCAAGGATGGACTATGG
985 GATCATGCTGATAGCAGCAAAAGACCAAGAGCGCAGCAATTGGCAGTACCAAAACAAGGATGAGCTCATACAGCACTG
986 TACGAACACAACGCTTTTCTACCAGGAAAAGTAGCAGAGCTACTAGCCAGCTGCAGGAATTCCACAACCGCAGGCCTGAAGA
987 CCTGATCAGCCTAGCGGATGACCTGGAGGACGTGTCCATCCTGGACAACGCCTCAAACAGGGGGAAGGAGGAGAAAGGAATT
988 GTTCCAATTTGGAATAAATCCCATCGACCACATAAAAACCTTGAAAATGTGGCCAAGATCATAGAAAAGTGGAAAGATA
989 CCCCAGGGTCTGTAATCAAGGAACACCAAGAGCAGTACCAAGTAACACCATCGGAGCACTTCTAGCTGAGGAAGGAATCGA
990 AGAACTGGCTGCAGCGTTGACACCGCCTATACCGAGATGCCAAAAGGAGGCCTCAACAACTTTACAACACAATTGTGGAGT
991 TCGTCATACCTCAGGAAAAGGGAGCAACACGAAGTTCAGAGTTCGTGCTGTGATAGATACTGGATGCACCTGCACGTGCATT
992 AACAGTAAAAAGTTCCCAAGGAGGCACTCGAAGAAGCGAAGTACCAGATGAATTCGAGGGGTTAACTCAACAGGGGAG
993 ACCAAGCTGAAAATGAAGAACGGGAAGATGATCGTGTGAGGACGCACTTCTACACCCGTATATAGCAGCTTTCCCAATGGA
994 GTTGCCAGACGTAGACATGCTGATTGGCTGCAACTTCTGCGAGCCATGAAGGGAGGCGTAAGACTCGAAGGAACGGAAGTG
995 ACTATCTACAAGAAAGTCAACACAATCCAAACAACCTAGAGCCACAGAAGATATCCCTCCTCGAGCAGAGGCTGAAGTCGG
996 AGAAGAACTAGAGCGCATGTACTACGCAATGACTATTCCGAGGAAGGAATAAGTCGGCTGAAGAACCACAGGCTGCTGCAG
997 GAACTCAGAGAACAAGGTACATTGGTGAAGAGCCAATGAGACACTGGGCAAGAAGCGGCATCAATGCAAGCTGGATATC

998 AAGAATCCAGACATAGTCATCAGCAGCAAACCGCCTGACTCTGTATCAAAAGAGACGAAAGCCCAATACCAAAGGCATATAGA
 999 TGCCCTGCTCAAAATCGGAGTAATCCAGCCCAGCAAGAGCAAACACAGGACGGCGGCTTTTATCACACTCGGGTACGTCAA
 1000 TTGACCCGATTACCAAGAAAGAAATCAGAGGGAAAGAACGGATGGTATTGACTACCGAAGTCTCAACGACAATACCCACAA
 1001 AGATCAGTACACTGCCGGGTATCAATACCATCATATCCGCGATTGGCAACGCTAAAATATTTAGCAAGTTGATCTAAAGTC
 1002 CGGATTCCATCAGGTGCTCATGGACGAAGAATCCATACCATGGACGGCTTTGTAACGCCAGTCGGATTCTATGAATGGAAGG
 1003 TCATGCCCTTTGGCCTTGCCAATGCTCCAGCTGTCTTCCAAAGGAAGATGGACCAATGCTTCGCTGGAACCTCGGAATTCATCG
 1004 CAGTCTACATCGATGACATCCTGGTGTTTCAGCAAAACCTAAAGGAGCAGAGAAACACCTTAGCATCATGCTAGGGATATGC
 1005 CGTGATAACGGTTTGGTTTTATCGCCAGCAAAATGAAGTTGGCCGCCACAGAGATAGACTTCCTTGGCGCCACCATAGGCGA
 1006 TGGAAGGATCAAGCTCCAGCTCACATCATAAAGAAGATAGCCGAGGTGGATGACGAATCCCTGAAAACCTCAAAGGGTTA
 1007 CGAAGCTGGTTGGGAGTGCTCAACTACGCGCGCAACTACATCCCAAAGTGTGGCACACTGTTAGGCCACTATACAGCAAGAC
 1008 CAGCGAGCATGGTGATCGTAGGTGGCACGCTGTATTGGCCTTAGTCAAAAGAATTAAGGGCTGGTCCAAAACCTCCCA
 1009 GACCTAAAACCTCCCCACGGAAGAGGCATACATGATCATTGAGACTGATGGATGCATGGAGGGCTGGGGAGGAGTCTGCAAT
 1010 GGAAGCCCATGAAGGCAGACTCAGCAAGCAAGGAAGAAATCTGCGCTTACGCCAGTGGTAAATTCCCCACGGTAAAAATCAAC
 1011 AATAGACGCAGAAATCTTCGAGTTATGGAGTCTTGGAAAAATTCAAGATTTTTTACATGAACAAGGACGAGGTCACTATCA
 1012 GGACTGATTGTCAAGCAATAATCACCTTCTACGAGAAGCTGAATGCAAAAGAAACCTTCGAGGGTAAGGTGGCTTGCCTTTTGC
 1013 GATTATATAACGAACTCCGGGGTAAGAATGAAGTTCAACATATAAAAGGTAAAGACAACAGCTCGCAGATAACCTCAGCC
 1014 GTCTCACACAACTGATTACATTTGTGAAATGGCTTCCAACCGAGCTCAAGGACCTCGCGGCAGAACTAACAGGAAAGACGAC
 1015 GGGACGCCCCGGAAGAAAGGAAGTGCAGGAGGAATCTCCTGTTTTCTGAAGCTGCCCTCCGCCGAGCCAAGAGATCCGTGA
 1016 CTACTACCAATCCGAGCCACGCCATGTACTATGGCAGAAATGGCAGAATCCAGAAGGCTGGCTCTACTGCGACGAGAGGAG
 1017 ATCTTCAACAGCCTTGCCCAACACATCAGCGACACGGTCTTCATCACCGGAGTCGACCTTGCGGCAGCAAAAGCCAGAGCAAC
 1018 CAGGGACAACCTGGTATGCTGACATCACCAACACTGGAACGACGAGCCACCGCAGCATGGAAGCTCATGGCCGCTTACGAG
 1019 GAATTCGCCACGTGTAAAGGATGTGAACGTTTGTGAAAGCGACGTGAGCAATGACTTCACAATCGCCCAAGTGCCTACTGCTT
 1020 ACGCTTGGGAACCTTATCTTTTGTGCTCGGTAGCATCTTCTAGCTGCCATACTTTATTGTAAGTGCGCCGATAGTGCGCTGAGTC
 1021 ATAGTGATAAGGAATCTTATCACCTTATCGTCCTTTCTTAGCTTTAGTAGCTGTAAAGACGAACCTTATAGCTGTGATGGGGCC
 1022 CAGAAAGCGCACCCGAGCTGATTTTTCTCTTTCTGCTAAGCCCTCCCCCTATATAAGGGAGAAGAGTTTGAAGGCTTAGGC
 1023 ACAGAGCAATCTCTAGCCAACCTTTCTCTTGAGTTGTATTAACATTCAGTGAAATAAAGACTTGTTTCATCTTTTTCCGCAT
 1024 ATCTCTGAGTTTTTATGAGTTCTTAAGTGTTGAAAGCGCACTTTTGAATTAGATCCA TGTTTTTCGGACCCCATTC
 1025

1026 **Supplemental table. 271 public and proprietary genetics were bulked into 294 samples and used in validation of**
 1027 **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
27	Selection 30
28	Selection 30, Selection 31
29	Selection 31, Selection 32
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33	Selection 33, Selection 34
34	Selection 35, Selection 36
35	Selection 36, Selection 37

36	Selection 38, Selection 39
37	Selection 39, Selection 40
38	Selection 41, Selection 42
39	Selection 42
40	Selection 42, Selection 43
41	Selection 43, Selection 44
42	Selection 45, Selection 46
43	Selection 46, Selection 47
44	Selection 47, Selection 48
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104	Selection 111, Selection 94
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110	Selection 101, Selection 102
111	Selection 102

112	Selection 103, Selection 111
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121	Selection 119, Selection 120
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124	Selection 123, Selection 124
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132	Selection 130
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143	Selection 140, Selection 141
144	Selection 141, Selection 142
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146	Selection 143
147	Selection 143
148	Selection 144
149	Selection 144, Selection 145

150	Selection 145, Selection 146
151	Selection 146
152	Selection 147, Selection 148
153	Selection 148, Selection 149
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203	QUALICUM, YELLOW ANTWERP
204	CHILCOTIN, LATHAM
205	CHILCOTIN, KORBUELLER
206	SOUTHLAND, SUMMIT
207	MALLING JEWEL, SUMMIT
208	MANDARIN, Selection 188
209	PHOENIX, Selection 188
210	BAUMFORTH SD.A, HERITAGE
211	SEPTEMBER, BAUMFORTH SD.A
212	Selection 189, ST. REGIS
213	CUTHBERT, ST. REGIS
214	MALLING LANDMARK, Selection 190
215	MALLING LANDMARK, TITAN
216	AUTUMN BLISS, CHEMAINUS
217	Selection 248, CHEMAINUS
218	Selection 191, Selection 192
219	Selection 192, TULAMEEN
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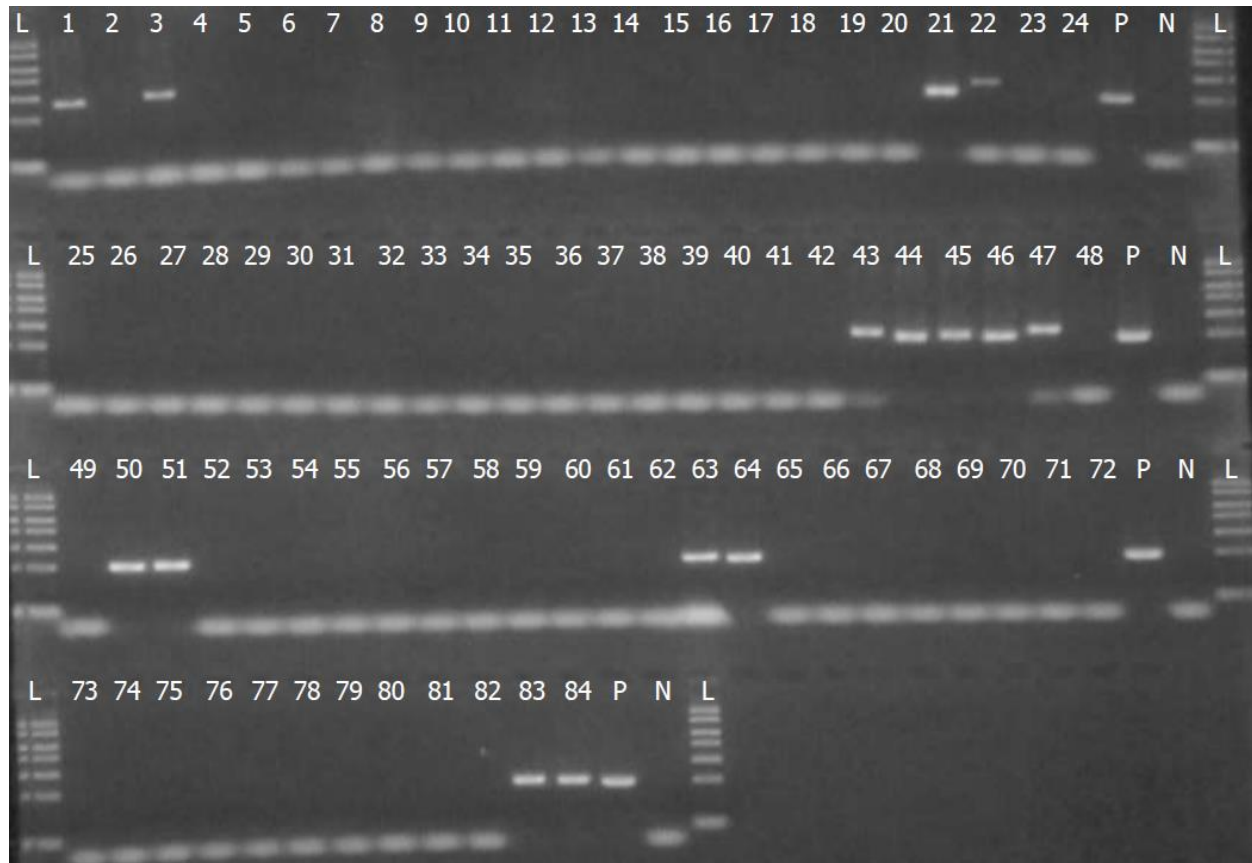
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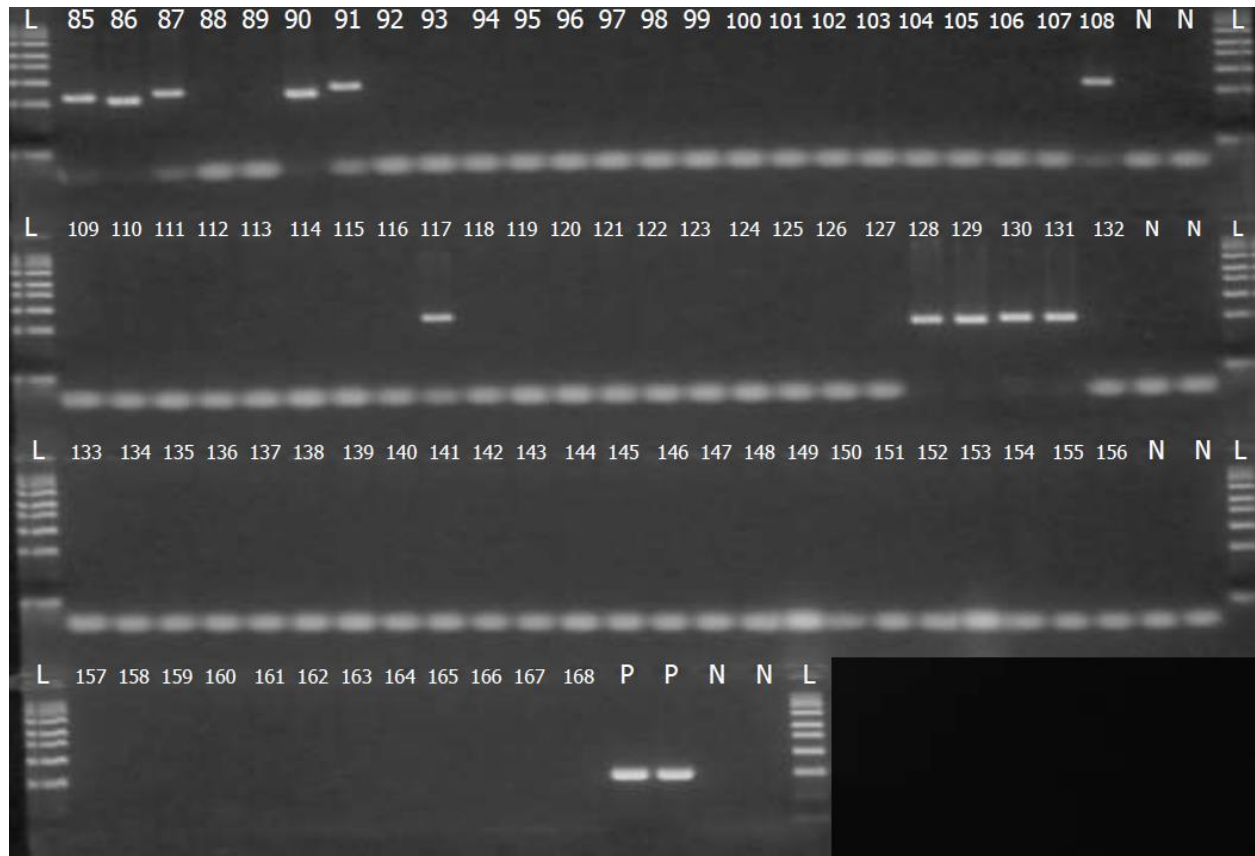
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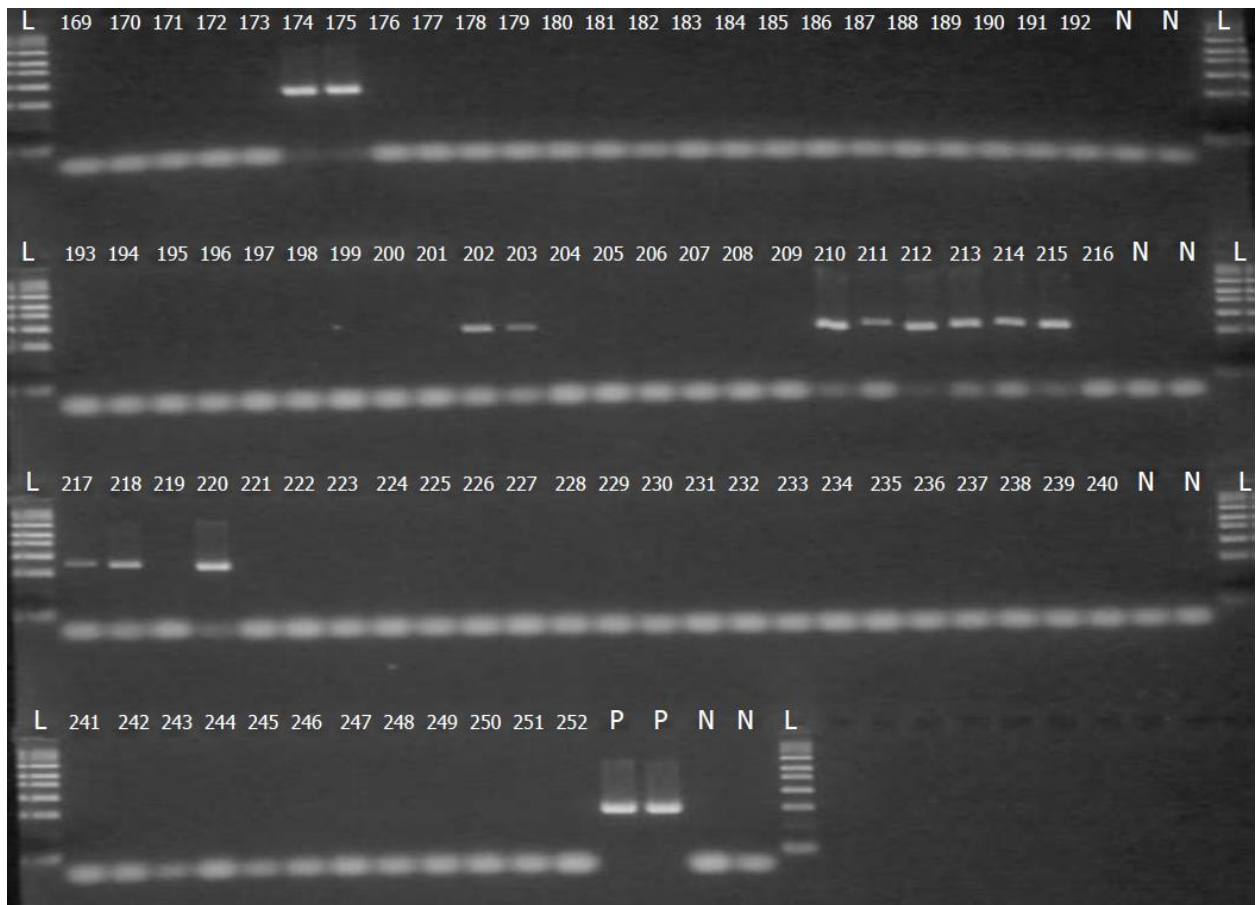
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Supplemental figure. epiRYNV-BS detection using RYNV6-F/R PCR assay. 271 public and proprietary genetics were bulked into 294 samples. Off-target was observed in 43 cases.





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