A new virus discovered by immunocapture of double-stranded RNA, a rapid method for virus enrichment in metagenomic studies

Abstract

Next-generation sequencing technologies enable the rapid identification of viral infection of diseased organisms. However, despite a consistent decrease in sequencing costs, it is difficult to justify their use in large-scale surveys without a virus sequence enrichment technique. As the majority of plant viruses have an RNA genome, a common approach is to extract the double-stranded RNA (dsRNA) replicative form, to enrich the replicating virus genetic material over the host background. The traditional dsRNA extraction is time-consuming and labour-intensive. We present an alternative method to enrich dsRNA from plant extracts using anti-dsRNA monoclonal antibodies in a pull-down assay. The extracted dsRNA can be amplified by reverse transcriptase–polymerase chain reaction and sequenced by next-generation sequencing. In our study, we have selected three distinct plant hosts: Maori potato (Solanum tuberosum), rengarenga (Arthropodium cirratum) and broadleaved dock (Rumex obtusifolius) representing a cultivated crop, a New Zealand-native ornamental plant and a weed, respectively. Of the sequence data obtained, 31–74% of the reads were of viral origin, and we identified five viruses including Potato virus Y and Potato virus S in potato; Turnip mosaic virus in rengarenga (a new host record); and in the dock sample Cherry leaf roll virus and a novel virus belonging to the genus Macluravirus. We believe that this new assay represents a significant opportunity to upscale virus ecology studies from environmental, primary industry and/or medical samples.

Keywords: antibodies, dsRNA, metagenomics, NGS, plant virus

Introduction

For rapid and efficient screening of large numbers of environmental, primary industry and medical samples for the presence of viruses, a virus nucleic acid enrichment step is required prior to sequencing. Because nucleic acids are difficult to isolate from some plant tissue samples, the development of an effective virus detection assay would be an important contribution to the effectiveness of such screening. Many new viruses have been identified with the advance in sequencing technologies. To detect and sequence plant viruses using next-generation sequencing (NGS) platforms, four strategies have been adopted based on the replicative cycle of a virus. The first strategy involves the sequencing of total RNA and then using bioinformatics to distinguish the viral RNAs from the plant sequence (Verbeek et al. 2014; Seo et al. 2015). The second strategy is to sequence RNA extracted from virions purified from the sample (Thapa et al. 2012, 2015; Tatineni et al. 2014), while the third method is to sequence the short, small interfering RNAs, some of which are derived from the plant antiviral defence mechanism (Giampetruzzi et al. 2012; Loconsole et al. 2012). Finally, the fourth method is to sequence the double-stranded RNA (dsRNA) produced during the replication of RNA viruses (Roossinck et al. 2010; Marais et al. 2015; Thapa et al. 2015). Each of these methods has its advantages and limitations, as reviewed by Massart et al. (2014).

Three-quarters of the plant viruses described to date have a positive-sense, single-stranded RNA genome (Hull 2014). Most RNA viruses produce a dsRNA intermediate replicative form, and as viruses are the dominant source of long dsRNA molecules in plants, this is commonly exploited for enrichment of viral nucleic acid.
A dsRNA extraction method described by Morris & Dodds (1979) has since been adapted and used for plant virus discovery. Recently, this traditional dsRNA enrichment method has been used for virus nucleic acid enrichment prior to NGS to diagnose viral infection in a plant (Al Rwahnih et al. 2012; Marais et al. 2014) but also in ecological studies (Coetzee et al. 2010; Roossinck et al. 2010; Thapa et al. 2015). However, the traditional dsRNA extraction method based on chromatography on cellulose is a bottleneck for streamlined, large-scale analyses of viral populations as it is time-consuming and requires large amounts of sample tissue. A rapid and efficient method for dsRNA enrichment would enable very high throughput of samples and identification of virus sequences from environmental, primary industry and/or medical samples.

Moffitt & Lister (1975) made the first antibodies against dsRNA and developed a ‘simple and sensitive’ serological test for dsRNA mycovirus after a phenol nucleic acid extraction from fungi. This was followed by several other publications of anti-dsRNA monoclonal antibodies (mAbs) being used in enzyme-linked immunosorbent assay (ELISA) (Garcia-Luque et al. 1986; Aramburu et al. 1991; Powell 1991; Schonborn et al. 1991; Aramburu & Moreno 1994). Although Powell was ‘in some way disappointed’ by the mAb that they had developed for use in ELISA, due to the high background it gave (Powell 1991), the same mAb was found to be sensitive when used in an immunocapture reverse transcriptase–polymerase chain reaction (IC-RT–PCR) by Nolasco et al. (1993). This was the first time the broad-spectrum dsRNA binding method was combined with the sensitivity of PCR, enabling confirmation of the presence of eight viruses and one viroid from a range of plant species (Nolasco et al. 1993). The restrictions of this method for virus discovery were the specificity of the PCR primers and the prerequisite for knowing the viral target sequence prior to amplification. To our knowledge, only the mAb described by Schonborn et al. (1991) (J2) is available commercially and has been used in research, mostly to localize dsRNA in infected cells (Fontana et al. 2008; Triantafilou et al. 2012).

Recently, O’Brien et al. (2015) described two anti-dsRNA IgM mAbs (3G1 and 2G4) raised against virus purification products from an infected mosquito cell culture and subsequently referred to as Monoclonal Antibodies against Viral RNA Intermediates in Cells (MAVRIC). In the characterization of the mAbs for ELISA and immunofluorescence, they showed the utility of these mAbs to detect arthropodborne viruses from a diverse range of families.

In this study, we have assessed a new protocol for the isolation of dsRNA in a pull-down experiment with the anti-dsRNA mAb 2G4 described by O’Brien et al. (2015). This protocol enables rapid dsRNA enrichment. Combined with a sensitive and sequence-independent PCR approach prior to NGS the utility of this protocol was confirmed by the detection of five viruses in three plant samples, including one virus which represents a new species of the Macluravirus genus.

Materials and methods

mAb concentration

Two mAbs 2G4 and 3G1 were tested in the first assay. The specificity of 2G4 and 3G1 to long dsRNA (>30 nucleotides) and not to ssRNA or RNA:DNA hybrids was demonstrated (O’Brien et al. 2015). The optimal conditions for immunoprecipitation were established by determining the quantity of hybridoma supernatant required to saturate 10 μg of Protein L beads (Thermo Scientific PierceTM).

Preparation of synthetic dsRNA

A synthetic dsRNA molecule was generated from the sequence of the virus Actinidia virus X (AVX accession KC568202). The RNA from AVX-infected plant tissue was reverse-transcribed and amplified by PCR using the primers T7 AVX 3048F (GAATTAATACGACTCACTATAGGGAGA CTGGTGATAGCCGTCAGTCC) and T7 AVX 5508R (GAATTAATACGACTCACTATAGGGA GATGGAA GTGACGACCCGA) using SuperScript® III One-Step RT–PCR System with Platinum® Taq DNA Polymerase (Thermo Fisher Scientific) following the manufacturer’s protocol. From the amiplicon, RNA was synthesized in both directions using a T7 RNA polymerase (Epicentre, Illumina) following the manufacturer’s protocol and then treated with DNase I (Thermo Fisher Scientific) and RNase H (Thermo Fisher Scientific) following the manufacturer’s protocol. Quantification was performed with a NanoDrop spectrophotometer. The concentration of the 2460-bp molecule was measured at 175.8 ng/μL by NanoDrop, equivalent to about 6.78 × 10^10 copies per μL as calculated by Endmemo.com (http://www.endmemo.com/bio/dncopynum.php).

Pull-down assay for the recovery of synthetic dsRNA

Protein L Magnetic beads (Thermo Scientific PierceTM) were washed with TBST as per the manufacturer’s protocol. Anti-dsRNA mAbs 2G4, 3G1 and negative IgM isotype control mAb 3D6 as hybridoma supernatant (Hobson-Peters et al. 2013; O’Brien et al. 2015) were coated onto the beads at saturating conditions as previously determined with 12.5 μg of beads used per sample. These were placed on a rotary tube suspension mixer for...
was transferred to a microcentrifuge tube and spun at 17 000 g in a benchtop centrifuge for 5 min to clarify the extract.

**Pull-down of dsRNA from plant specimens**

Protein L Magnetic beads (Thermo Scientific PierceTM) were coated as described for the pull-down assay with the following modifications: 50 μg of beads saturated with 2G4 hybridoma supernatant per sample. These were placed on a rotary tube suspension mixer for 1 h at 37 °C. The beads were then washed with TBST buffer as per the manufacturer’s recommendation. The beads were resuspended in TBST, transferred into the plant extract and incubated for 1 h at 37 °C on a rotary tube suspension mixer. The beads were recovered and washed as previously and resuspended in 30 μL of sterile deionized water.

**Amplification and barcoding**

The RT reaction from dsRNA template was performed following the method described by Roossinck et al. (2010) using 6 μL of the resuspended beads–dsRNA, heated at 95 °C for 2 min with 3 μL random primer (5′ CCTTCCGATCCTCC N6,12 3′) at a concentration of 20 μM (equimolar mix of N6, N8 and N12) and 9 μL of water and placed on ice for 1 min. The RT mix was then added as per the manufacturer’s recommendation comprising 300 U of SuperScript III (Thermo Fisher Scientific), 0.5 mM dNTPs, 6 μL of 5× buffer and 3 μL of DTT. Samples were incubated on ice for 15 min and then at 50 °C for 1 h. After the incubation, 0.75 μL of ribonuclease A was then added (20 mg/mL Sigma), and the samples were incubated at room temperature for 15 min and then at 85 °C for 2 min. Primers were removed using the Qiagen PCR purification column following the manufacturer’s protocol. The samples were eluted in 30 μL 1:10 dilution elution buffer provided.

The PCR amplification protocol followed the method described by Roossinck et al. (2010). Samples were amplified individually, using 17.5 μL of the RT product in a 50 μL reaction using Takara Prime script reagents (Clonetech) following the manufacturer’s protocol for PCR (25 μL 2× buffer, 2.5 μL of 50 mM MgCl2) with 5 μL of a single primer (10 μM, barcode CCTTCGGA TCCTCC). The barcodes used were CAGCG, CAGCG and CAGCG for the potato, rengarenga and dock, respectively. The first cycle was 94 °C for 5 min, 65 °C for 30 s, 72 °C for 5 s and 1 min and then 40 cycles consisting of 94 °C for 15 s, 45 °C for 15 s and 72 °C for 1 min. The PCR product was excised and purified using ZymocleanTM Gel

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DNA Recovery Kit following the manufacturer’s protocol.

Each sample was quantified using Qubit fluorometric quantification (Thermo Fisher Scientific) and normalized to 5 ng per sample. Library preparation on the combined samples (TruSeq Nano DNA Library Prep Kit, Illumina) and sequencing was performed by Macrogen Korea on HiSeq 2000 (Illumina) using a partial run with 100-bp paired end.

Bioinformatics

All the bioinformatics analyses were performed in the Galaxy web-based platform running on a Plant & Food Research Limited internal server. The sequence data were demultiplexed using barcode splitter (https://toolshed.g2.bx.psu.edu/view/devteam/fastx_barcode_splitter) with no mismatches or deletions allowed. The adaptors were removed and poor quality sequence was removed using the Fastq-mcf tool with the following parameters: C = 1000000; % = 0.0001; 1 = 50; q = 25; and x = 5. For each pool of data, a de novo assembly was performed by Trinity on Galaxy (https://toolshed.g2.bx.psu.edu/view/bhas/trinityrnaseq) using the two directions of sequence with no strand-specific library type, 1500 group pairs distance and 75 path reinforcement distance. The contigs obtained were compared online by BLAST to identify the virus present. BOWTIE2 (version 2.2.4) was used to align each read-pair set separately (single-end mode), with alignment mode ‘end to end’ and the preset option ‘very sensitive’ (Langmead & Salzberg 2012). The preset option minimum alignment score to be considered mapped is −49.8 with the 82 nucleotides reads. The mapped reads were counted with Samtools Flagstat in Galaxy, and the numbers of reads mapped by the forward and reverse direction were added.

Alignment and tree construction

The virus tentatively named as Rumex virus Y was compared with members of Potyviridae on a partial coat protein fragment (most conserved region) at the amino acid level. The samples were trimmed to the Potyviridae coat protein motif (pfam00767) to a size ranging from 195 to 244 amino acids. The sequences were aligned using CLUSTALW (BLOSUM matrix, 10 gap opening, 0.1 gap extension). A maximum-likelihood tree was built using PHYML v3 with the proportion of invariant sites estimated, empirical amino acid frequencies, variation in rates across sites modelled as a gamma distribution with four categories and 100 bootstrap replicates using the BLOSUM62 scoring matrix (Guindon & Gascuel 2003; Guindon et al. 2010).

Results

The binding of dsRNA to mAb 2G4 in a pull-down assay is not affected by the concentration of dsRNA

Initial studies were performed to investigate the adaptation of the anti-dsRNA mAbs to a pull-down assay platform. The saturation of the beads with the mAbs was equivalent to approximately 7 μg of purified antibody per 10 μg of beads (Fig. S1, Supporting information). At this saturating concentration, more mAb 2G4 could be eluted from the beads than mAb 3G1 indicating that more of the former mAb was actually bound to the beads when saturated. To assess the binding capability of the mAb-saturated beads to dsRNA, and its direct detection by RT–PCR, dsRNA obtained by transcription of a 2460-nucleotide PCR product originating from the ORF1 of the virus Actinidia virus X (AVX; GenBank KC568202) was recovered by mAb 2G4 and 3G1 in a pull-down assay using magnetic Protein L beads as assessed by reverse transcriptase–quantitative PCR (RT–qPCR) (Fig. 1). The non-dsRNA-binding mAb 3D6 (isotype-matched control) was used as a negative control and had only one very late Ct value (>38) out of three biological replicates at the highest concentration confirming the dsRNA recovery observed with 2G4 and 3G1 treatments was due to the binding to the antibodies and not to non-specific binding to the beads. Both mAbs showed similar binding performance to the dsRNA solution.

NGS results from plant extracts

The efficiency of the viral enrichment with the anti-dsRNA mAb-saturated beads from virus-infected plants was measured by sequencing the recovered dsRNA. After the read deconvolution, the three plant host samples resulted in a similar number of reads: 127 898 for the potato sample, 198 597 for the rengarenga sample and 154 027 reads for the dock sample. From the total sequencing data, 95% of bases had the minimum quality score of 20 (Q20), and 87% of Q30.

From the Māori potato sample, 125 426 reads passed the quality control (98%). De novo assembly with Trinity yielded 96 contigs longer than 200 nucleotides. The two longest contigs were 8469 and 5015 nucleotides and both matched the virus Potato virus S (PVS) by nucleotide BLAST; however, the two sequences only shared 76% nucleotide identity across their common region (ORF1). To compare the two contigs, we examined the common 5015 nucleotides region in the ORF1. The first contig matched PVS isolate WaDef-US (GenBank FJ813512) and isolate 09.369 (GenBank LN851191) equally with 98% identity both for the nucleotide and amino acids. When the isolate 09.369 was used as a reference for mapping
using BOWTIE2, 52,216 reads assembled (42% of the total number of reads after QC) forming an 8452-nucleotide consensus sequence named NZ-O (GenBank KU058656) that is 98% identical to PVS isolates WaDef-US and 09.369. When compared with the isolate WaDef-US, NZ-O covers 99.6% of the genome with only the extremities not sequenced (33 nucleotides in total across both the 5’ UTR and the 3’ UTR). When using NZ-O as a reference, it mapped to 53,909 reads (43% of the total number of reads after QC; Table 1).

The second contig matched the PVS isolate BB-AND (GenBank JQ647830) with 96% nucleotide and amino acid identity. When used as a reference, the isolate BB-AND mapped 29,576 reads (24% of the total number of reads after QC) forming a consensus sequence called NZ-A of 8390 nucleotides (GenBank KU058657). The isolate BB-AND was the closest match to NZ-A with 96% identical nucleotides and 97% identical amino acids. When compared with the isolate BB-AND genome, only the extremities were not sequenced representing a total of 129 nucleotides across both the 3’ and 5’ UTRs. When used as a reference, NZ-A was mapped by 35,983 reads (29% of the total number of reads after QC; Table 1).

When compared, NZ-O and NZ-A shared only 80% nucleotide identity, confirming that they are different strains of PVS.

In addition to PVS, 15 contigs were found to match Potato virus Y (PVY) with length varying between 208 and 1491 nucleotides that match PVY isolate N (GenBank D00441). When PVY isolate N was used as a reference, 3405 reads mapped (3% of the total number of reads after QC; Table 1).

From the rengarenga sample, 193,322 reads passed the quality control (97%). The de novo assembly yielded 113 contigs longer than 200 nucleotides. The longest contig (6241 nucleotides) as well as four additional contigs shared 99% nucleotide identity with the NZ290 isolate of Turnip mosaic virus (TuMV; GenBank AB093612). When this TuMV accession was used as a reference, it mapped 56,738 reads (29% of the total number of reads after QC). The consensus sequence obtained (GenBank KU053508) was at 9787 nucleotides long and shared 99.5% nucleotide identity with TuMV isolate NZ290.

From the dock sample, 149,708 reads passed the quality control (97%). In total, 127 contigs larger than 200 nucleotides were obtained from de novo assembly. The longest contig (6496 nucleotides) did not match any known sequence in the GenBank, but its translated sequence shared 52% amino acid identity with the polyprotein of the virus Chinese yam necrosis mosaic virus (CYNMC GenBank NC_018455). Another translated contig of 1727 nucleotides also matched the same virus and these two contigs overlapped by 65 nucleotides (with three mismatches). When using the assembled contig as a reference, 58,305 reads mapped (39% of the total

| Table 1 | Number of viral reads mapped to a reference sequence using BOWTIE2 |
|---------|---------------------------------------------------------------|
| Host    | Total read number after QC | Virus       | Viral reads | Viral percentage |
| Potato  | 125426                        | PVS<sup>Q</sup> | 53909   | 43.0          |
|         |                               | PVS<sup>A</sup> | 35983   | 28.7          |
|         |                               | PVY          | 3405    | 2.7           |
| Rengarenga | 193322                        | TuMV         | 60738   | 31.4          |
| Dock    | 149708                        | Rumex virus Y | 58305   | 39.0          |
|         |                               | CLRV         | 370     | 0.2           |

PVS<sup>Q</sup> [KU058656]; PVS<sup>A</sup> [KU058657]; PVY [D00441]; TuMV [KU053508]; Rumex virus Y [KU053507]; CLRV [KC937025 and KC937030].

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The consensus sequence formed is 8174 nucleotides with 11 ambiguities. This CYNMC-like virus is further characterized below as a novel Rumex virus. Of the remaining contigs, seven matched CLRV sequences with the longest 685 nucleotides long. When mapped on the CLRV genome 441, 126 reads mapped the RNA1 (GenBank KC937025) and 244 mapped the RNA2 (GenBank KC937030).

The viral sequence detected in dock represents a new Macluravirus species

The novel Rumex virus is a monopartite RNA virus of 8174 nucleotides extending from the 5’ UTR (154 nucleotides) to the 3’ UTR (208 nucleotides) that encodes one single polyprotein of 2603 amino acids. The closest relative to this virus is CYNMC with only 52% amino acid identity over the coding region. The new virus genome encodes the Potyviridae motifs listed in the NCBI’s conserved motif database (Marchler-Bauer et al. 2015); a peptidase C4; a helicase (GSGKSX3P and DEXH); an RNA dependant RNA polymerase (RdRp) motif; and the Potyviridae coat protein (CP) motifs. It also has the conserved motif GAx responsible for a polymerase slippage resulting in an additional ORF (namely PIPO) and the production of the P3N-PIPO protein (Chung et al. 2008; Olspert et al. 2015; Rodamilans et al. 2015). A phylogenetic tree based on the CP encoded by members of the Potyviridae family shows that the putatively named Rumex virus Y (RVY) is in the Macluravirus genus (Fig. 2). A phylogenetic tree estimated using the full-length polyprotein indicates similar clustering (Fig. S2, Supporting information).

Discussion

The use of mAbs raised against dsRNA to enrich for viral sequences shows potential to increase both speed and cost efficiency for identification of virus genomes including those from difficult to process tissues. Purification of dsRNA on cellulose is a common enrichment step for plant virus, but it is performed over 2 days. In this study, we have demonstrated a cost- and time-efficient method to permit enrichment of viral dsRNA from plant tissues that can be completed in less than 3 h. Using this method, we successfully identified five viruses from

![Fig. 2 The relationship of a new Macluravirus detected in dock with representative members of the family Potyviridae in an amino acid conserved region of the coat protein (pfam00767). Alignment performed with CLUSTALW and maximum-likelihood tree obtained with PHYML v3.](image-url)
three plants, and we were able to distinguish the different strains of a virus infecting a single plant as well as to retrieve the almost full genome of a previously uncharacterized virus.

Our method retrieved a high proportion (31–74%) of reads of viral origin and substantial portions of viral genomes. This level of viral recovery is comparable with the one obtained from cellulose chromatography by Minutillo et al. (2015) with 48–70%, but much higher than the results shown by Roossinck et al. (2010) with values ranging from 1% to 5%. However, the host plants and virus types are highly likely to also contribute to the differences in dsRNA recovery between those studies rather than simply the dsRNA enrichment methods.

Using the dsRNA pull-down assay coupled with NGS, we were able to detect these five different viruses representing three families (\textit{Betatenuiviridae}, \textit{Potyviridae} and \textit{Picornaviridae}) from three different hosts representing a commercial crop, a New Zealand-native ornamental and a weed. From potato, two common viruses were identified: PVS that comprised 72% of the reads, and PVY with 3% of the reads. The PVS reads formed two distinct isolates matching the highly divergent PVS\(^A\) and PVS\(^O\) clades (Cox & Jones 2010). It is the first time these two variant strains were detected in the same plant in New Zealand.

The potyvirus TuMV was recovered from rengarenga for the first time. It is now common to find this ubiquitous potyvirus within New Zealand-native plants (Fletcher et al. 2009, 2010; Veerakone et al. 2015).

From the dock composite sample we identified almost the full sequence of a novel Macluravirus. As this sample represents eight dock plants showing diverse symptoms (mild mottle-to-general necrosis), and another virus was also detected (CLR), it is not known which symptom is caused by the novel Macluravirus, and therefore, it was tentatively named Rumex virus Y (RVY), the Y reflecting the \textit{Potyviridae} family. The impact of this virus and its host range are unknown.

The mAb-based enrichment protocol was performed in microfuge tubes from only a small amount of freeze-dried samples (100 mg) demonstrating its convenient use in low-tech laboratories that might be associated with processing of high numbers of ‘field’ samples for an ecological or medical study. In 2010, Roossinck presented the result of a large viral metagenomics study from 384 environmental samples that alongside the publications by Thapa et al. (2012, 2015) represent the largest plant virus metagenomics studies published to date. The Roossinck protocol used 5 g of starting material followed by a phenol: chloroform extraction and chromatography using cellulose CF11 (Morris & Dodds 1979). We believe that the anti-dsRNA antibodies used in the immunocapture protocol presented in this current report are a major advance over the traditional dsRNA enrichment due to their simplicity and rapidity that will enable similar studies at a reduced cost; our protocol used a crude extract, eliminating the need for phenol: chloroform extraction. In future, this protocol could be streamlined for large-scale studies as long as the sequencing depth remains sufficient to detect multiple virus infections as the number of reads recovered was very variable between the coinfecting viruses of potato and the Rumex, perhaps reflecting the different rates of replication of the viruses at the time of sampling. In addition, multiplexing large number of samples may be restricted by the normalization process and the tag jumps where a small number of sequences are not reported to the correct tag (Schnell et al. 2015). In the case of an ecological study, a ‘lawn-mower’ metagenomics approach is possible where no individual barcoding is required because the emphasis is on a comparison between different environments (Roossinck 2012).

All the viruses recovered in the present study are positive-sense single-stranded RNA viruses, by far the most common plant virus described to date (Hull 2014) and the detection of replication intermediates from positive-sense ssRNA viruses is also widely reported (Marais et al. 2015; Minutillo et al. 2015). Whether our approach can be further extended to detect negative-sense single-stranded RNA viruses and DNA plant viruses should be investigated in future studies. While previous reports (Weber et al. 2006; O’Brien et al. 2015) suggest that mAbs to dsRNA cannot be used for the detection of negative-sense single-stranded RNA viruses, we successfully detected a putative member of the genus \textit{Enaraviruses} (negative-sense RNA virus) from a symptomatic plant using the pull-down assay described herein in a parallel study (data not shown). The detection of dsRNA from DNA viruses has been reported before (Roossinck et al. 2010; Al Rwahnih et al. 2013; Thapa et al. 2015) and is explained by the overlapping of convergent transcription. The sequencing of dsRNA is therefore a very powerful method capable of detecting all virus types; however, the routine use of dsRNA sequencing for negative-sense RNA and DNA viruses warrants further validation. Another area that needs to be explored is whether our protocol can be used to detect replicating virus in recalcitrant plants such as strawberry, banana or yam. Such plants can accumulate significant quantity of tannins, phenolics and polysaccharides that may interfere with the nucleic acids and prevent their binding to the anti-dsRNA mAbs. However, in this case, we believe that the pull-down experiment can be performed on diluted extract to reduce the concentration of these interfering compounds, or alternatively it could be used after total RNA extraction.

An alternative approach to dsRNA extraction was developed by Kobayashi et al. (2009) using the
recombinant plant dsRNA-binding protein GST-DRB4*, derived from Arabidopsis thaliana. Similar to the method presented here, the DRB4* protein fused to a GST-tag was recovered with a Sepharose bead enabling a fast extraction. One aspect their publication does not cover is the plant weight required to detect the infected virus by RT–PCR following the extraction. In addition, their work was exclusively based on herbaceous indicator plants (Chenopodium quinoa and Nicotiana benthamiana) infected with a single virus. The GST-DRB4* protein was used for an analysis of the dsRNA present in aquatic microbial communities (Decker & Parker 2014). The dsRNA was extracted from total microbial RNA isolated from water and sequenced by NGS (Ion torrent). The method was able to detect unique dsRNA reads (about 30% of the total reads) including some that could be assembled into new RNA virus-like elements.

Conclusion
The results presented demonstrate enrichment for viral nucleic acids from soft plant tissues that is comparable with that achieved using the traditional dsRNA extraction method by cellulose chromatography, but with substantial gains in time and ease including the ability to translate to high throughput formats.

In conclusion, Son et al. (2015) suggest that dsRNA antibodies have a role in animal virus discovery using NGS, and here, we present that this dsRNA enrichment method is efficient for virus detection and discovery in plants. In addition, recent publications suggest that dsRNA is a good target for virus identification in environmental samples (Decker & Parker 2014), invertebrate samples (O’Brien et al. 2015) and vertebrate samples (Son et al. 2015), and dsRNA enrichment with mAbs represents an appropriate tool for large-scale viral population genetic studies across different environments.

Acknowledgements
We would like to thank John Fletcher for the recommendation and collection of the South Island samples. We thank Mahmoud Khalifa and Simon Bulman for their useful comments on the manuscripts as well as Karmun Chooi and Dan Cohen for the constructive discussions during the research period. Finally, we acknowledge Plant & Food Research for the funding and New Zealand Winegrowers for the financial support through the Rod Bonfiglioli Memorial Scholarship.

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A.G.B. contributed to the study design, conducted the laboratory work, analysed the data and wrote the manuscript. H.A.R. contributed to the phylogenetic analysis. C.A.O. performed the bead saturation experiment and provided critical review of the manuscript. J.H.P. contributed the mAbs and critical review of the manuscript. B.W. implemented the Galaxy support. R.M.M. contributed in the study design and overview of the research and manuscript. All authors reviewed and commented on the manuscript.

Data accessibility

Virus sequences: GenBank accessions KU053507–KU053508–KU058656–KU058657; DRYAD entry doi:10.5061/dryad.0bg04.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Bead saturation experiment: Dilutions of purified mAbs (2.5–100 μg) or hybridoma supernatant (25–500 μL) were in TBST. A total of 500 μL of each dilution was added to 7.5–10 μL magnetic Protein L beads and incubated for 1 h at room temperature with mixing.

Fig. S2 The relationship of a new Macluravirus detected in dock with representative members of the family Potyviridae in the full polyprotein using CLUSTALW alignment (BLOSUM matrix, 10 gap opening, 0.1 gap extension).