**vd-sRNA profiler: User-focused interface for viroid derived small RNA mapping and profiling**

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**Abstract**

Viroids are circular, highly structured, single-stranded non-coding RNA pathogens known to infect and cause disease in several plant species. Viroid is known to trigger RNA silencing machinery of the host plant. Detection of viroid-derived small RNA (vd-sRNA) in viroid infected host plant opened a new avenue in viroid-host pathogenicity. Since then, several viroid research groups studied vd-sRNA retrieved from different viroid-host combinations. Such studies require the segregation of 21- to -24-nucleotide long small RNAs (sRNA) from deep-sequencing databank, followed by separation of vd-sRNA from this sRNA groups that shows sequencing similarity with genomic and antigenomic strands of the viroid. For visualization, such mapped vd-sRNAs are then profiled on the viroid’s genomic and antigenomic strand. Although several commercial interfaces are available, they are all programmed for linear RNA molecules. Hence, viroid researchers must develop a computer program to accommodate the vd-sRNAs derived from the circular viroid. This is laborious and often a bottleneck for biologists. To overcome this constraint and help the viroid research community, a python-based pattern matching interface has been developed to profile and map vd-sRNA on a circular genome in the present study. Additionally, a feature of matching tolerance has been added to map the vd-sRNA derived from the quasi-species. The efficacy of the software is tested with previously reported deep-sequenced data obtained from two independent studies. Clearly this novel software should be a key tool to evaluate the production of vd-sRNA and profiling on their target viroid species.

**Introduction**

Viroids are the simplest and smallest known plant pathogens. They consist of a circular single-stranded non-coding RNA genome of 246-434 nucleotides (nt) in length [1]. Since viroid does not encode for any known peptides, they rely entirely on their sequence, structure, and host factors to replicate and induce disease symptoms in host plants. Upon infection, they induce a wide array of symptoms in host plants, such as leaf epinasty, leaf distortion, stunting, and flower distortion [2–4]. Due to their highly base-paired secondary structures and RNA-RNA mode of replication, viroids are both inducers and targets of RNA silencing [5].

RNA silencing (RNA interference [RNAi]) is a multilayer defence system, which protects plants from invading RNA pathogens such as viruses and viroids [6]. Silencing is triggered by the processing of double-stranded or highly structured RNA by the host’s RNase III-type ribonucleases (i.e., DICER or DICER-LIKE), resulting in the production of small interference RNAs (siRNAs) of 21–24 nt in length [7]. In 2001, two research groups independently reported the presence of viroid-derived small RNAs (vd-sRNA) in plants infected with PSTVd by RNA gel blot assays [8,9], suggesting that viroids are the targets of RNA silencing. The introduction of next-generation sequencing (NGS) technology allowed large-scale studies on the accumulation of such vd-sRNA in viroid-infected plants [10–13]. The role of vd-sRNA in RNAi-mediated down-regulation of the host transcripts and symptom induction has been explored using different host-viroid combinations [14–18]. This has been reviewed elsewhere [19].

Viroid researchers extensively use the NGS to study the viroid-host interaction emphasizing vd-sRNA production [20]. Profiling of vd-sRNAs on both the genomic on antigenomic strands of viroid gives several information such as but not limited to: (i) the proportion of vd-sRNA produced from genomic on antigenomic strands of the viroid; (ii) distribution of vd-sRNA on the genomic on antigenomic strands; and, (iii) regions of viroid that are susceptible, and resistance for host’s RNA silencing machinery. Such information could be used to understand host-viroid species relationships [11,13,21], viroid quasi-species [22,23], and to develop viroid resistance plants [24,25]. To get all this information, the first and foremost is mapping of vd-sRNA based on viroid sequence. The biggest hurdle for such studies is the lack of sRNA mapping software on the circular genome, such as viroids. Although several commercial platforms are available, they are all programmed for linear DNA or RNA molecules. Consequently, either viroid researchers develop their interface or depend on bioinformaticians to progress in their studies.

Mapping of vd-sRNA to a target viroid’s genomic and/or antigenomic strand sequence is innately tied to the classic example of determining the number of pattern matchings. In computer science, pattern matching is the process of checking a given sequence of characters (in the present scenario, nucleotides) that exists among the provided data. In other words, given two strings and , does string occur as a sub-string of? Knuth, Moris and Pratt first addressed the concept of pattern matching in 1970 [26]. Since then, many proposed solutions to this problem has came into existence that include but are not limited to industrial applications and supply chain performance. Pattern matching is extensively used in bioinformatics to determine the sequence similarity between the subject and query [27].

In the present study, a python language has been used to write the pattern matching program to provide a user-friendly and efficient bioinformatics tool to viroid researchers. Python is one of the computer languages that are easy to read and implement. Additionally, it is an open-source language and runs on different platforms such as Mac, Windows and Unix. Here we report the development of an interface that was initially tested on an illustrative genome and sRNAs and then applied to the previously reported NGS data.

**Materials and methods**

**Setting the setting for pattern matching**

The counting version of the standard pattern matching is a problem of enumerating all the occurrences of the string in the string [28,29]. Computational biology provides an archetypal context for counting problems of this sort [27,30,31], where for example, is considered an sRNA and as a viroid genome—computing the number of bindings of a vd-sRNA with the viroid genome. When an sRNA nucleotide sequence is represented by a string and the viroid genome by a string , computing the number is bindings of the sRNA sequence immediately admits the counting form of the standard pattern matching problem. Furthermore, we can safely assume that and are drawn from the alphabet . Before laying out the problem precisely, to solve several instances of the above counting problem, each corresponding to a different viroid RNA nucleotide . Hence, the problem that we would like to solve in this section is stated below.

*Definition 1: Viroid binding problem*

Given the following:

1. Set of N strings where each string has length is sampled from ,
2. Length m string sampled from the alphabet , and
3. Function from strings to to identify a matching. For two equal-length strings and , if and only if and match and otherwise.

Compute the size of the sets for each , where:

Equation 1:

The definition of is intentionally unspecified to accommodate various scenarios in the present work. That said, is used in four different ways, that can be divided into two categories, that is (i) matching of in forward and reverse direction with respect to , and (ii) linear or circular topology for .

(i) matching of in forward and reverse directions with respect to :

The first set of variations occurs from choosing an alignment of the viroid genome relative to the sRNA. While the natural alignment called the forward matching, setting coincides with the definition in “Definition 1”, its counterpart is referred to as the reverse matching set. The forward and reverse cases differ in their rule for deciding when two equal-length strings and, sampled from the same alphabet, are identical. The forward matching setting adopts the natural definition where if and only i , for all , where is the length of . On the contrary, the reverse matching setting places a non-standard requirement: if and only of , for all . Moving forward, only the case of forward matching is discussed as reverse matching adapts the same discussion of forward matching without affecting the conclusion.

Setting the topology is crucial to determining the boundaries for a string to differentiate the mapping of sRNA derived from a linear and a circular genome. The case of linear topology (used for sRNA derived from the linear genome), referred to as linear alignment, refers to a with a finite boundary, that is, a length string with following for and no character following . On the contrary, the case of circular topology (used for sRNA derived from the circular genome), referred to as circular alignment, is characterized by a gene with periodic boundary conditions, that is, a length string with the characterfollowing the character for . It is straightforward to note that the matching problem on a circular aligned gene trivially reduces to the linear alignment case. This can be realized by appending the gene string with the length substring of starting at position . So, we will not attribute any conceptual distinction to this setting and continue to analyze our solution for the linear alignment.

**Finding the occurrence of in**

This section presents the solution to forward matching with linear alignment for the pattern matching problem defined in *Definition 1*. First of all, let us introduce the following useful notations.

1. The *length* of a string is simply the number of characters in . We often use To denote the size of
2. The *Hamming weight* of a binary sequence is the number of ’s in the sequence. We often use to denote the Hamming weight of

A nsolution to the pattern matching problem entails a systematic search for a match between every sequence in to every substring of . In other words, makes calls to an elementary routine which compared two strings and to identify the number of times occurs in . Consequently, the time complexity of the naive solution is . We want to provide a solution that outperforms the naive one. This is achieved by mapping the pattern matching problem to a similar size matrix multiplication problem. The latter can be solved efficiently by vectorizing individual operations.

The first ingredient of this mapping is encoding each character in into a unique binary sequence of bits.

Equation 2:

The above encoding naturally implies an encoding for strings composed of the characters from , by simply concatenating the binary sequences corresponding to the individual alphabets in the string. Likewise, a nucleotide sequence of length is identified by a binary sequence of bits, denoted by .

It is straightforward to notice that distinct characters in Equation 2 correspond to orthogonal binary sequences. Therefore, two nucleotide strings and of length each, match exactly if and of only if the number of ones in the dot product: is equal to , i.e., . A similar observation can be made to account for mismatches wherein the the number of ones in should be at least . To summarize, two length $k$ strings and match approximately with at most mismatches if and only if the Hamming weight of is at most .

While we have just described a method to check for a match between two strings of equal length, this can be readily extended to the strings of unequal lengths, i.e., when and for . To realize this, note that can be found in if and only if there are consecutive characters in that match with . In other words, for some . Furthermore, there are exactly substrings of that can be formed by taking consecutive characters, described by the set . Each of these substrings can be encoded into a binary sequence having bits. The substrings that match with will can be identified by checking if is at least .

Lastly, by combining the tests for matchings between each of for and into a single condition as follows. Let be a matrix whose columns are , respectively. It turns out that the dot product is a vector of size whose th component satisfies if and only if the substring matches with at all but at most locations. In other words, the number of occurrences of in is given by the number of entries in whose value is at least . This inference forms the backbone of our solution for the underlying pattern matching problem.

Let us now summarize our solution to the forward matching problem with linear alignment, in definition 1. Recall that the problem instance is described by a set of sRNA nucleotides and a gene . The encoding in equation 1, leads to two key observations:

1. is mapped to a binary matrix , whose th row denotes the binary encoding of the the nucleotide in the sRNA pool.
2. is mapped to a binary matrix whose th column denotes the binary encoding of the length substring of starting at position .

These observations imply that the dot product yields an ( binary matrix, where is equal to if and only if the th sRNA nucleotide binds to the gene, at position . Finally, taking the sum of all the rows of , yields a vector of size , where It is easy to realize that is nothing but the number of sRNA nucleotides in which binds to the gene at location . In other words, , from Equation 2. This completely specifies our solution to the pattern matching problem. Casting the pattern matching problem as matrix multiplication is instrumental [32] to its low runtimes for large number of sRNA nucleotides. Table 1 shows the runtimes for computing the solutions to the pattern matching problem in definition 1, for a few different sizes of the sRNA pool.

**Profiling of vd-sRNA on viroid genomic and antigenomic strands**

The key inputs to the software are the genome sequence, specified in a text file without line-breaks, and the pool of vd-sRNA, specified in a text file wherein each new line identifies a nucleotide sequence composed of “A”, “T”, “G” and “C” characters. If there is any nucleotide sequences with “N”, that sequence is ignored. To accommodate various formats, software accepts a whole number which specifies the number of lines to skip before reading every nucleotide sequence in the pool file. Additional settings for the matching problem include: (i) tolerance: the maximum number of mismatches allowed to accommodate the vd-sRNA derived from the quasispecie;, (ii) topology of the gene, i.e., a Boolean that takes the value 0 for linear matching and 1 for circular matching; and, (iii) the number of cores to be used by the software. For example, “gene.txt pool.txt 2 1 1 1” is a complete input specification, indicating that the problem of computing bindings, in the circular topology while allowing for at most one mismatch, between the sequence in pool.txt with those in the pool.txt. All such instances of the matching problem can be gathered in a text file, placed in vbind/data/input. The output format for the solution of the matching problem is a matrix, each of whose rows correspond to a unique length of vd-sRNA sequences in the pool. Each row is of length equal to the gene sequence, where its i-th column specifies the number of matchings amongst sequences of a given length in the pool, with the sequence in the gene starting at position 1.

**Viroid small RNA data**

Previously published two sRNA data obtained from tomato plants infected with variants of PSTVd is used in the present study for the analysis. Specifically, the total sRNA sequence obtained from the tomato plants infected with PSTVd-I (GenBank Acc. No.: AY937179) and PSTVd-RG1 (GenBank Acc. No.: U23058) having the GEO Acc. No.: GSM1695657 and GSM1717894, respectively [16,33]

**Software accessibility and instructions**

The script is available at <https://github.com/paviudes/vbind>. Example input and running instructions as shown in data/input/example.txt

**Results**

**Overview of sRNA mapping and profiling**

To verify the working of the currently developed tool, a 30-nt long DNA string (5’- GCT TCA GGG ATC CCC GGG GAA ACC TGG TCG-3’) used as “genome,” an equivalent to the viroid genomic strand (Fig. 1A). From this DNA string, a pool of sRNAs was prepared randomly such that the pool has at least one non-matching sRNA, genomic sRNA and antigenomic sRNA of 5- and 6-nt in length. Additionally, a couple of sRNAs were taken between positions 27 and 5 of the DNA string to imitate the sRNA derived from circular genomic strand (Table 1). This pool is equivalent to NGS data of sRNA. Running the patterning matching to find the matching of sRNAs in the pool with genome strand summarizes the results in the terminal as shown in figure 1 (panels A and B). Summary of obtained data includes the name of the gene used, sRNA pool, topology, tolerance level, cores used for mapping, and a table summarizing total number of sRNA of different lengths, number of sRNA matching in the forward direction, number of sRNA matching in the reverse direction, the total number of sRNA matching and % of sRNA mapped for the given sRNA species. Details of each sRNA sequence occurred in the pool is presented in Fig. S1 as generated by vd-sRNA profiler.

To profile the sRNA on both the genomic and antigenomic strands of the genome, above matched sRNAs were run on the genome strand. As presented in figure 1C, the X-axis indicates the length of viroid RNA, whereas Y-axis shows the number of matching sRNAs. By testing the above example, it is clear that this tool can segregate and output the total sRNA count, the sRNA matching genomic and antigenomic strands, thus addressing the fundamental questions of mapping the sRNA from the NGS data on a given genome sequence. Further, profiling of mapped sRNAs on the illustration DNA strand explains that the vd-sRNA profiler developed in this work could be applied to analyse vd-sRNA obtained from viroid infected plants.

**Mapping of sRNA obtained PSTVd-I infected plants**

To map and profile the sRNA on both the genomic and antigenomic strands of viroid RNA, an sRNA pool of 15- to 37-nt obtained from PSTVd-I infected tomato plant was retrieved from GEO. The data set was processed with the 359-nt long PSTVd-I genome sequence with zero tolerance to segregate genomic and antigenomic matching vd-sRNA.

As vd-sRNAs of 21- to 24-nt are the one researcher interested in, the matching obtained for these sizes are presented in Table 2. Out of 4,316,543 sRNA of 21- to 24-nt, a total of 488,176 vd-sRNAs showed 100% sequence similarity with PSTVd-I sequence accounting for 11.3% of total recovered sRNAs. Specifically, 380,731 (8.8%) and 107,445 (2.5%) vd-sRNAs of 21- to 24-nt derived for the genomic and antigenomic strands of PSTVd-I, respectively. In order to have a better picture of the expression level of individual-sized vd-sRNA, obtained vd-sRNAs were normalized per million reads. Detailed analysis showed that 22-nt long sRNA had a maximum number of vd-sRNA (34.3 %) whereas, the least expressed vd-sRNA is 24-nt (1.2 %). Interestingly, the vd-sRNA based on individual sizes revealed that the genomic strand produced more 22-nucleotide sRNAs (29.3%), while the antigenomic strand produced more 21-nucleotide sRNAs (7.9%). Overall, more genomic strand-derived sRNAs were covered when compared to the antigenomic strand of the PSTVd-I. In other words, (+) vd-sRNA expressed 3.5 times more than (-) vd-sRNA. This can be attributed to the higher recovery of the genomic strand of viroid in infected plants as described elsewhere [34]. These data are in agreement with the previous report where the same sRNA pool was used for the analysis [16], confirming the reproducibility of the new software.

**Profiling mapped sRNA on PSTVd-I**

In order to examine the regions of the PSTVd, that produced more vd-sRNA, the above-mapped vd-sRNA was plotted on the genomic and antigenomic strands of the PSTVd-I. Hence, each 21- to 24-nt long vd-sRNA and cumulative 21- to 24-nt long vd-sRNAs are profiled on the PSTVd-I (Fig. 2). The data presented here clearly show the regions that produce more vd-sRNA on both the genomic and antigenomic strand. This is in agreement with the previous report [16], thus confirming the reproducibility of the here developed profiling tool.

**Accommodating vd-sRNA of PSTVd-I quasi-species**

Viroids are known to form quasi-species in host plant [22,23,35–37]. That said, a single sequence type could give rise to several hundreds or thousands of sequence variants in host plant. In order to accommodate the vd-sRNA derived from the PSTVd-I sequence variants, in the present analysis, vd-sRNA mapping against the genomic and antigenomic strand of the PSTVd-I was decreased by allowing 1 mismatch. This decrease of the stringency increased overall matching by 0.4% compared with the matching at 0 mismatch (also called 0 tolerance), specifically from 11.3 % to 11.7 % as presented in Table 3. In other words, 113,094 and 117,011 vd-sRNAs of 21- to 24-nt long are present per million sRNA with zero tolerance and 1 mismatch, respectively. Allowing one mismatch increased the percentage of matching as low as 0.1 % (for 24-nt long vd-sRNA) to much as 1.1 % (for 22-nt long vd-sRNA) compared to zero mismatches. However, overall distribution of (+) vd-sRNA to (-) vd-sRNA remained almost the same as with that of zero mismatches. Further, as with zero tolerance mapping, 1 mismatch too had highest genomic derived vd-sRNAs for 22-nt long sRNAs whereas antigenomic derived vd-sRNAs of 21-nt long. To evaluate the regions of the PSTVd, that produced more vd-sRNA, the above mapped vd-sRNA with 1 mismatch is plotted on the genomic and antigenomic strands of the PSTVd-I as described before (Fig. 3).

**Evaluating the mapping tool on vd-sRNA obtained from PSTVd-RG1 infected plants**

To increase the confidece of the programing tool, one more sRNA data set that is publicly available was analyzed. Specifically, the sRNA sequence obtained from tomato plants infected with PSTVd-RG1 variant was used for the analysis. Both PSTVd-I and PSTVd-RG1 are 359-nt long in their genome size. PSTVd-I and PSTVd-RG1 have 3 mismatches in their genome and they induce intermediate and lethal disease symptoms in tomato cultivar Rutgers (*Lycopersicum esculantum* cv Rutgers) [18]. As described earlier, a total of 730,499 sRNAs of 21- to 24-nt are mapped on the PSTVd-RG1 in both forward and reverse direction to segregate genomic (+) and antigenomic (-) vd-sRNA at 0 and 1 mismatch tolerance. Obtained data is normalized per million reads (Table 4). Results showed that total of 102,555 vd-sRNA are recovered per million reads which contained 88,493 (8.8 %) and 14,062 (1.4%) of genomic and antigenomic strands derived vd-sRNA that showed 100% sequence similarity with PSTVd-RG1. This accounted for 10.3% of total sequenced 21- to 24-nt long sRNA. Out of all 21- to 24-nt long vd-sRNAs, 21-nt long vd-sRNA species is the highly expressed (25.8%) whereas 24-nt long vd-sRNA is the least expressed (1.2%) for both genomic and anti-genomics strand of PSTVd-RG1.

To allow the vd-sRNA derived from sequence variants of PSTVd-RG1, one mismatch was allowed in the mapping. Decreasing the stringency increased the number for vd-sRNA by 3.3 % (from 102,555 per million reads with zero mismatches to 135,654 per million reads with one mismatch) (Table 5). This attributed to a 2.9% and 0.4% increase in genomic and antigenomic strand vd-sRNA compared to zero tolerance mapping. Analyzing the vd-sRNA derived from genomic and antigenomic strands revealed that genomic strand-derived sRNAs expressed at least 6.4 times higher than antigenomic strand derived sRNAs.

To visualize the regions of the PSTVd-RG1 that produced vd-sRNA in both genomic and antigenomic strands, the mapped vd-sRNA with zero and 1 mismatch is plotted on the genomic and antigenomic strand of the PSTVd-RG1 (Figure 4). The profiles obtained for 21-, 22-, 23-, 24-nt long vd-sRNA and the cumulative 21- to 24-nt long vd-sRNAs are presented in figure 4, panels A and B, for zero and 1 mismatch, respectively.

**Discussion**

Though viroids are single-stranded and circular, due to (i) sequence complementarity, they form highly base-paired secondary structures, and (ii) replication through asymmetric rolling circle mechanism, viroid act as both the inducers of RNA and targets of the host’s RNA silencing machinery (reviewed in [38]). That said, upon infection, all the viroids trigger RNA silencing and that in-turn cleaves viroid RNA into sRNAs of 21- to 24-nt. The accumulation of such [vd-sRNAs](javascript:;) has been extensively studied in different viroid-host combinations [10,11,13]. Since, viroids are non-coding pathogens, recent works were directed towards understanding the role of such vd-sRNA in its pathogenicity and symptom induction [14–16,21,39–42]. This works including predicted the vd-sRNA:target mRNA duplex formation and then, looking for the number of vd-sRNA in the viroid infected plant that potentially binds predicted target mRNA. For latter studies, it is important to map the vd-sRNA from NGS data obtained from viroid infected plants. Profiling obtained vd-sRNA on both the genomic and antigenomic strands of the viroid helps to understand that regions of viroid that produces more vd-sRNA and the region that produces less vd-sRNA. That indirectly suggests which region of the viroid is more susceptible and resistance to host RNA silencing. Such information helps in developing RNAi mediated viroid resistance transgenic plant development [43]. That said, mapping of vd-sRNA and profiling on viroid is very important in understanding host-viroid interaction.

Viroids being circular in nature, a specific computer software is required to map the sRNAs derived from the junction of first and last nucleotide of the viroid. This feature is not available in commercially available software. Here we developed vd-sRNA profiler, a python-based software tool that is tailormade to address this issue along with the detection of both genomic and antigenomic strand derived sRNAs. Besides its capabilities for detecting mappings, the tool is attractive for its efficiency (Table 6). For instance, the sRNA pool of 5.8 million reads can be solved with 30 minutes on a desktop computer with a single core whereas below 1 million reads needs approximately 2 minutes. This eliminates the need for high-end hardware requirements which are often the case for commercial software. Moreover, the fact that vd-sRNA profiler is wrote in python permits to customize for various studies of both circular and linear RNAs.

The solution to the instance of a viroid binding problem, computed by vd-sRNA profiler is represented by two matrices with whole number entries; that is: (i) solution for forward matchings, and, (ii) solution for reverse matchings. In each of the two cases, the matchings are specified by a matrix – for the forward matchings and for the reverse matchings – of size where is the number of distinct nucleotide lengths in the pool, and is the length of the gene. Each entry or, is the number of sRNA sequences in the pool, of length indexed by that have a forward or reverse matching, respectively with a substring of the gene starting at position . In addition to solving the viroid bindings problem, the vd-sRNA profiler is capable of a visual representation of the output, by profiling the vd-sRNA on the genomic and antigenomic strand of the viroid strand (Fig. 2; Fig.3, and Fig. 4). Here, the X-axis shows the positions on the viroid sequence. While the forward matching solutions are represented on the positive Y-axis, and the reverse matchings are on the negative Y-axis, respectively. For each index on the x-axis, the absolute value on the Y-axis for a fixed length is the maximum number of sequences in the pool that match with the substring of the gene starting at position . The area under the curve is filled for a visual appeal. It is important to note that the message conveyed in figures 2 and 4 is identical to the previous results [33,44], albeit the later adopting a slightly different visual representation for the plot. In previous studies [33,44], a visual representation was used wherein the height of the curve at a given position on the X-axis denotes the number of vd-sRNA nucleotides that match with a substring containing the character of the gene at position . This can be derived from the matrices of the vd-sRNA profiler followed by a simple postprocessing routine.

To provide the user friendly and the efficient bioinformatics tool to viroid researchers, vd-sRNA profiler was developed by pattern matching using python language. This software seamlessly allows researchers to map and plot vd-sRNAs on their parent viroid molecule. Choosing the different matching tolerance allows the user to consider and visualize the vd-sRNA derived from viroid quasi-species. This software will help viroid researchers to use in their studies to evaluate the production of vd-sRNA and its profiling on their target viroid species.

**Supplementary information**

**Fig. S1**. **Data generated from vd-sRNA profiler showing details of sRNA sequences and its occurrence in the pool.** Note that if a single sRNA shows matching in more than one place of the genome sequence, then it is counted as number of times it showed matching with the genome sequence.

**Author Contributions:**

Conceptualization: C.R.A.-P., P.S.I., T.S., and J.-P.P.; writing—original draft preparation: C.R.A.-P., and PI writing—review and editing: C.R.A.-P., T.S., and J.-P.P.; supervision: J.-P.P.; funding acquisition: J.-P.P. All authors have read and agreed to the published version of the manuscript.

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**Tables**

**Table 1. Summary of sRNA sequences used test run of vd-sRNA profiler.**

| sRNA length | Tolerance: 0 | | Tolerance: 1 | | Non-matching sRNA |
| --- | --- | --- | --- | --- | --- |
| Forward matching | Reverse matching | Forward matching | Reverse matching |
| 5-nt | GGGAT | ACCAC | ACCCG | AGATT | AGGGA |
|  | ATCCC | CCACG | GGATC | CACGA | CAAGT |
|  | AAACC | CCGTG\* | TTGAC\* | CCCTG\* | GGGGC |
| 6-nt | TTCAGG | AGTTCC | ACAAAA | CAGAGG | ACGCAG |
|  | CGGGGA | GTGAAC\* | GGCTCA | GTGGAC\* | GTAGAT |
|  | CGGCTT\* | CCGTGA\* | CACCGA\* | CCGCGA\* | CGGAAA |

\*sRNA derived between the nucleotide positions 28 and 5, representing the sRNA of circular genome.

**Table 2. Summary of sRNAs Identified by NGS of PSTVd-I inoculated tomato plants cv. Rutgers at 0 mismatch with the PSTVd-I genome.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **sRNA length** | **Total sRNA in the pool** | **Tolerance: 0** | | | **Normalized reads (per million reads)** | | | | | | | **(+):(-) vd-sRNA** |
| **(+) vd-sRNA** | **(-) vd-sRNA** | **Total vd-sRNA** | **total sRNA** | **(+) vd-sRNA** | | **(-) vd-sRNA** | | **total vd-sRNA** | |
| **No. of reads** | **%** | **No. of reads** | **%** | **No. of reads** | **%** |
| 21-nt | 700,131 | 105,294 | 55,304 | 160,598 | 162,197 | 24,393 | 15.0 | 12,812 | 7.9 | 37,205 | 22.9 | 1.9 |
| 22-nt | 839,033 | 245,515 | 42,665 | 288,180 | 194,376 | 56,878 | 29.3 | 9,884 | 5.1 | 66,762 | 34.3 | 5.8 |
| 23-nt | 622,373 | 10,064 | 3,154 | 13,218 | 144,183 | 2,331 | 1.6 | 731 | 0.5 | 3,062 | 2.1 | 3.2 |
| 24-nt | 2,155,006 | 19,858 | 6,322 | 26,180 | 499,243 | 4,600 | 0.9 | 1,465 | 0.3 | 6,065 | 1.2 | 3.1 |
| 21-24-nt | 4,316,543 | 380,731 | 107,445 | 488,176 | 1,000,000 | 88,203 | 8.8 | 24,891 | 2.5 | 113,094 | 11.3 | 3.5 |

**Table 3. Summary of sRNAs Identified by NGS of PSTVd-I inoculated tomato plants cv. Rutgers at 1 mismatch to the PSTVd-I genome.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **sRNA length** | **Total sRNA in the pool** | **Tolerance: 1** | | | **Normalized reads (per million reads)** | | | | | | | **(+):(-)**  **vd-sRNA** |
| **(+) vd-sRNA** | **(-) vd-sRNA** | **Total vd-sRNA** | **total sRNA** | **(+) vd-sRNA** | | **(-) vd-sRNA** | | **total vd-sRNA** | |
| **No. of reads** | **%** | **No. of reads** | **%** | **No. of reads** | **%** |
| 21-nt | 700,131 | 109,114 | 57,707 | 166,821 | 162,197 | 25,278 | 15.6 | 13,369 | 8.2 | 38,647 | 23.8 | 1.9 |
| 22-nt | 839,033 | 251,128 | 45,522 | 296,650 | 194,376 | 58,178 | 29.9 | 10,546 | 5.4 | 68,724 | 35.4 | 5.5 |
| 23-nt | 622,373 | 10,984 | 3,511 | 14,495 | 144,183 | 2,545 | 1.8 | 813 | 0.6 | 3,358 | 2.3 | 3.1 |
| 24-nt | 2,155,006 | 20,556 | 6,561 | 27,117 | 499,243 | 4,762 | 1.0 | 1,520 | 0.3 | 6,282 | 1.3 | 3.1 |
| 21-24-nt | 4,316,543 | 391,782 | 113,301 | 505,083 | 1,000,000 | 90,763 | 9.1 | 26,248 | 2.6 | 117,011 | 11.7 | 3.5 |

**Table 4. Summary of sRNAs Identified by NGS of PSTVd-RG1 inoculated tomato plants cv. Rutgers at 0 mismatch to the PSTVd-RG1 genome.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **sRNA length** | **Total sRNA in the pool** | **Tolerance: 0** | | | **Normalized reads (per million reads)** | | | | | | | **(+):(-)**  **vd-sRNA** |
| **(+) vd-sRNA** | **(-) vd-sRNA** | **Total vd-sRNA** | **total sRNA** | **(+) vd-sRNA** | | **(-) vd-sRNA** | | **total vd-sRNA** | |
| **No. of reads** | **%** | **No. of reads** | **%** | **No. of reads** | **%** |
| 21-nt | 160,262 | 35,533 | 5,778 | 41,311 | 219,387 | 48,642 | 22.2 | 7,910 | 3.6 | 56,552 | 25.8 | 6.1 |
| 22-nt | 145,487 | 24,528 | 3,913 | 28,441 | 199,161 | 33,577 | 16.9 | 5,357 | 2.7 | 38,934 | 19.5 | 6.3 |
| 23-nt | 124,625 | 1,448 | 197 | 1,645 | 170,603 | 1,982 | 1.2 | 270 | 0.2 | 2,252 | 1.3 | 7.4 |
| 24-nt | 300,125 | 3,135 | 374 | 3,509 | 410,849 | 4,292 | 1.0 | 512 | 0.1 | 4,804 | 1.2 | 8.4 |
| 21-24-nt | 730,499 | 64,644 | 10,272 | 74,906 | 1,000,000 | 88,493 | 8.8 | 14,062 | 1.4 | 102,555 | 10.3 | 6.3 |

**Table 5. Summary of sRNAs Identified by NGS of PSTVd-RG1 inoculated tomato plants cv. Rutgers at 1 mismatch to the PSTVd-RG1 genome.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **sRNA length** | **Total sRNA in the pool** | **Tolerance: 1** | | | **Normalized reads (per million reads)** | | | | | | | **(+):(-)**  **vd-sRNA** |
| **(+) vd-sRNA** | **(-) vd-sRNA** | **Total vd-sRNA** | **total sRNA** | **(+) vd-sRNA** | | **(-) vd-sRNA** | | **total vd-sRNA** | |
| **No. of reads** | **%** | **No. of reads** | **%** | **No. of reads** | **%** |
| 21-nt | 160,262 | 46,752 | 7,461 | 54,213 | 219,387 | 64,000 | 29.2 | 10,214 | 4.7 | 74,214 | 33.8 | 6.3 |
| 22-nt | 145,487 | 32,779 | 5,121 | 37,900 | 199,161 | 44,872 | 22.5 | 7,010 | 3.5 | 51,882 | 26.1 | 6.4 |
| 23-nt | 124,625 | 2,049 | 280 | 2,329 | 170,603 | 2,805 | 1.6 | 383 | 0.2 | 3,188 | 1.9 | 7.3 |
| 24-nt | 300,125 | 4,162 | 491 | 4,653 | 410,849 | 5,697 | 1.4 | 672 | 0.2 | 6,370 | 1.6 | 8.5 |
| 21-24-nt | 730,499 | 85,742 | 13,353 | 99,095 | 1,000,000 | 117,375 | 11.7 | 18,279 | 1.8 | 135,654 | 13.6 | 6.4 |

**Table 6. The runtime of vd-RNA profiler on a single core of a 3.2 GHz**

|  |  |  |  |
| --- | --- | --- | --- |
| **Size of the sRNA pool** | **Size of the viroid genome** | **Tolerance** | **Runtime in seconds** |
| 5875050 | 359 | 0 | 1450 |
| 5875050 | 359 | 1 | 1800 |
| 730499 | 359 | 0 | 128 |
| 730499 | 359 | 1 | 131 |

**Figure legends**

**Figure 1. vd-sRNA profiler data output for test sample.** (**A**) Summary of mapping data obtained from “0” tolerance and (**B**) “1” tolerance. (C) Profiling of mapped sRNAs on the 30-nt long circular test genome at 0” and “1” tolerance for 5-nt and 6-nt long sRNAs.

**Figure 2. Profiling of the vd-sRNAs recovered from PSTVd-I infected tomato plants at zero mismatches.** Sequence profiles of the (+) and the (−) PSTVd-sRNA populations recovered from the leaf tissues of infected tomato plants. Panels (A), (B), (C), (D) and (E) represent the profiles of the (+) and the (−) PSTVd-I derived sRNAs of sizes 21-, 22-, 23-, 24 and cumulative of 21- to 24-nt, respectively. Data were normalized to reads per million.

**Figure 3**. **Profiling of the vd-sRNAs recovered from PSTVd-I infected tomato plants at 1 mismatch.** Sequence profiles of the (+) and the (−) PSTVd-sRNA populations recovered from the leaf tissues of infected tomato plants. Panels (A), (B), (C), (D) and (E) represent the profiles of the (+) and the (−) PSTVd-I derived sRNAs of sizes 21-, 22-, 23-, 24 and cumulative of 21- to 24-nt, respectively. Data were normalized to reads per million.

**Figure 4**. **Profiling of the vd-sRNAs recovered from PSTVd-I infected tomato plants at zero and 1 mismatches.** Sequence profiles of the (+) and the (−) PSTVd-sRNA populations recovered from the leaf tissues of infected tomato plants. Panels (A), (B), (C), (D) and (E) represent the profiles of the (+) and the (−) PSTVd-I derived sRNAs of 0 mismatches of sizes 21-, 22-, 23-, 24 and cumulative of 21- to 24-nt while, Panels (F), (G), (H), (I) and (J) represent the profiles of the (+) and the (−) PSTVd-I derived sRNAs of of 1 mismatches of sizes 21-, 22-, 23-, 24 and cumulative of 21- to 24-nt, respectively. Data were normalized to reads per million.