

### Abstract

Over 65 viruses and viroids have been recorded until now in grapevine. Reliable and accurate individual detection of all those agents via serological and/or PCR based methods in each sample is rather difficult. The Next Generation Sequencing (NGS) technologies allow the simultaneous recovery of hundreds of thousand sequences from total RNA of infected plants that can derive from a multiplicity of viruses and/or viroids present in grapevine sample. NGS platform was here used to identify viruses and viroids from a grapevine variety Trakya İlkeren showing severe mosaic symptoms collected in Bursa province of Turkey in 2015. Total RNA was extracted and rRNA depletion was performed by Ribo-Zero rRNA Plant Removal Kit. NEBNext® Ultra™ RNA Library Prep Kit was used for library preparation. Deep sequencing was performed using Illumina HiSeq2000 RNASeq technology with 2x150 read length and 40 million depths for each read. In total, deep sequencing analysis yielded around 150 million sequences. For bioinformatic analyses, Geneious R10 software was used. After de novo assembly, 28,863 contigs were obtained with Tadpole assembler and the obtained contigs were analyzed by blastn against NCBI viral RefSeq database to detect known viruses and viroids. Afterwards, genome mapping was performed against reference sequences of each detected virus/viroid using all the reads. At the end of the genome mapping analyses, reference genomes for *Grapevine yellow speckle viroid-1* (GYSVd-1) (100 %), *Grapevine deformation virus* (GDefV) RNA 2 segment (29 %), *Grapevine Syrah virus-1* (GSYV-1) (20.2 %), *Grapevine fanleaf virus* (GFLV) RNA 1 segment (4.4 %), GFLV RNA 2 segment (3.9 %), GDefV RNA 1 segment (3.8 %), *Arabis mosaic virus* (ArMV) RNA 2 segment (2.7 %), *Grapevine asteroid mosaic associated virus* (GAMaV) (1.9 %), *Grapevine rupestris stem pitting associated virus* (GRSPaV) (1.6 %) and *Grapevine leafroll-associated virus-1* (GLRaV-1) (0.7 %) were recovered with indicated percentages.

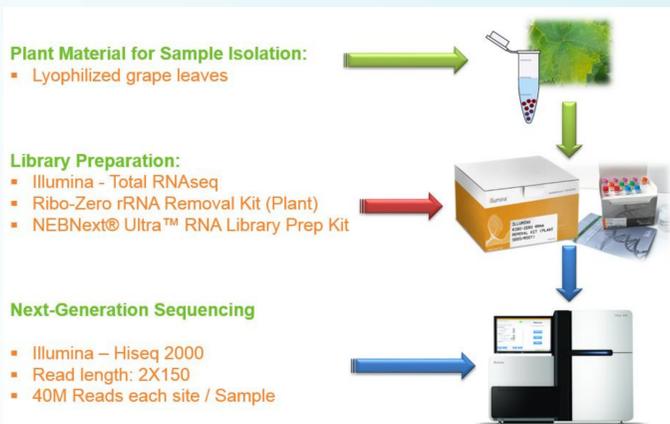
These results showed that recovering full genome for all of detected viruses and viroids with RNASeq is still very complicated and achieving a complete genome sequence may require the highest depth sequencing approach. Noticeably, to our best of knowledge GAMaV is the first report for Turkey grapevine production areas, which still needs to be confirmed by other identification tools.

### Grapevine Sample Information

Sample Code.....: S10  
 Grape Variety.....: Trakya İlkeren  
 Location.....: Bursa / TR  
 Collection Date.....: June 2015

Observed Symptoms:  
 Severe mosaic symptoms on the leaf area.

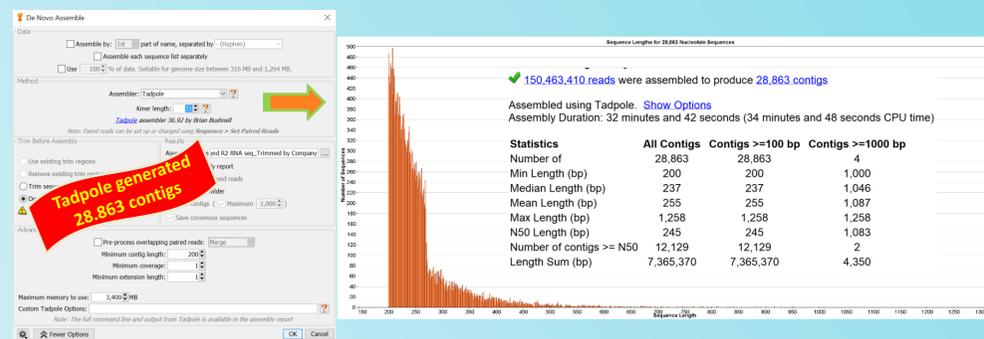
### NGS Laboratory Analyses



### Derived NGS Data Information



### De novo Assemble with Tadpole



### NCBI Nucleotide BLAST Results against Tadpole Contigs

Virus/Viroid Name	RefSeq Accession No	Range of the Matched Sequence Length (bp)	% Pairwise Identity Range of Sequences
GYSVd-1	NC_001920	199	89.4
GDefV RNA 2	NC_017938	223 to 560	70.2 to 93.0
GSYV-1	NC_012484	25 to 383	75.3 to 97.7
GFLV RNA 1	NC_003615	29 to 397	70.6 to 90.4
GFLV RNA 2	NC_003623	202 to 299	81.7 to 91.8
GDefV RNA 1	NC_017939	223 to 560	70.2 to 93.0
ArMV RNA 2	NC_006056	230 to 336	84.5 to 88.7
GAMaV	NC_031692	28 to 217	89.3 to 92.6
GRSPaV	NC_001948	33 to 218	84.8 to 90.4
GLRaV-1	NC_016509	25 to 240	72.5 to 92.0
GRGV	NC_030693	25 to 106	68.9 to 92.0
GVA	NC_003604	29 to 213	73.4 to 89.7

### Genome Mapping with Geneious for RNA Seq

Virus/Viroid Name	RefSeq Length (bp)	Coverage of RefSeq (bp)	% Coverage of RefSeq
GYSVd-1	366	366	100
GDefV-RNA 2	3753	1090	29
GSYV-1	6506	1313	20.2
GFLV RNA 1	7342	325	4.4
GFLV RNA 2	3774	149	3.9
GDefV RNA 1	7386	277	3.8
ArMV RNA 2	3820	104	2.7
GAMaV	6719	125	1.9
GRSPaV	8774	143	1.6
GLRaV-1	18659	128	0.7
GRGV	6863	---	---
GVA	7351	---	---

### Acknowledgement

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