Industry allocated project number	PHI allocated project number

SATI	CFPA	SAAPPA/SASPA	DFTS	Winetech
tarryn@satgi.co.za Tel: 021 863-0366	inmaak@mweb.co.za Tel: 021 872-1501	theresa@hortgro.co.za Tel: 021 882-8470	dappies@dtd.co.za Tel: 021 870 2900	andraga@winetech.co.za Tel: 021 276 0499

Indicate (X) client(s) to whom this concept project proposal is submitted. Replace any of these with other relevant clients if required.

NB: The instructions in red, throughout the template, should be omitted from the final document.

FINAL REPORT
(2015)

1. PROGRAMME AND PROJECT LEADER INFORMATION

	Research Organisation Programme leader	Research Team Manager	Project leader
Title, initials, surname	Prof JT Burger	Prof JT Burger	Dr HJ Maree
Present position	Professor	Professor	Senior Researcher
Address	Department of Genetics, Stellenbosch University	Department of Genetics, Stellenbosch University	ARC Infruitec-Nietvoorbij c/o Dept Genetics, Stellenbosch University
Tel. / Cell no.	021 8085858	021 8085858	0835565567
Fax			
E-mail	jtb@sun.ac.za	jtb@sun.ac.za	mareeh@arc.agric.za
	Co-worker	**Student**	**Student**
Title, initials, surname		Mr N Molenaar	Mr K Oosthuizen
Present position		MSc Student	BSc (Hons) Student
Address		Department of Genetics, Stellenbosch University	Department of Genetics, Stellenbosch University
Tel. / Cell no.			
Fax			
E-mail			

2. PROJECT INFORMATION

Research Organisation Project number	Genus 11/3
Project title	Determination of the viral complexity of grapevine leafroll disease through deep sequencing
Short title	Deep sequencing of leafroll disease

Fruit kind(s)	Wine Grapes		
Start date (mm/yyyy)	01/01/2012	**End date** (mm/yyyy)	31/12/2014

Key words	Next-generation sequencing, GLRaV-3

Approved by Research Organisation Programme leader (tick box) ☐

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

3. EXECUTIVE SUMMARY

In this project we proposed to sequence typical viral diseased vines by next-generation sequencing (NGS) technology to determine the basal level of virus infection commonly associated with Grapevine leafroll disease (GLD).
Grapevine leafroll diseased vines were sampled during two seasons (2012/13) and dsRNA extracted for the construction of NGS libraries. The sequencing of the dsRNA in a metagenomic approach allowed for the construction of unbiased viral profiles.
Seventeen samples have been collected across the Western Cape representing red and white cultivars as well as rootstock plants. The samples have been sequenced through Illumina MiSeq (2012 samples) and Illumina HiSeq (2013 samples) sequencing platforms, both at the ARC Biotechnology Platform.
In excess of 190 million reads were generated through NGS. Read datasets were trimmed and filtered for quality and subjected to both read-mapping and *de novo* assembly. Contigs assembled *de novo* were analyzed with BLAST (Basic Local Alignment Search Tool) against the NCBI (National Centre for Biotechnology Information) database and it was determined that GLRaV-3 was the most common virus comprising 97.5% of the assembled contigs. *Grapevine virus F* (GVF) was detected through *de novo* assemblies and the complete genome sequence validated through direct Sanger sequencing. The complete GVF isolate V5 genome spans 7,539 nucleotides and shares 89.11% nucleotide identity to existing GVF genomes. No specific symptoms could be linked to GVF infection.
The data generated through this study will assist in further understanding the etiology of GLD, and currently supports the generally accepted belief that GLRaV-3 as the primary contributor to GLD.
This study also highlights the fact that grapevines accumulate viruses resulting in complex viral profiles that could potentially alter disease symptomology. The severe symptoms of GLD can potentially mask the symptoms of milder viruses and delay the detection of new viruses such as GVF.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

State the problem being addressed and the ultimate aim of the project.

Problem identification and objectives

The high incidence of GLRaV-3 in South African vineyards masks the contributions of other viruses to Grapevine leafroll disease (GLD) and influences the symptom expression in other diseases.
This study will generate sequence data that will give an unbiased profile of the viruses and their variants found in grapevines affected by leafroll disease. The data will be critical to develop accurate diagnostic tests, like RT-PCR and RT-qPCR, based on virus sequence, and may identify novel pathogenic and non-pathogenic viruses.

5. WORKPLAN (MATERIALS AND METHODS)

Amended workplan materials and methods

Approach and Techniques:

Sample collection will form the most critical part of this project in order to accurately calculate the viral complexity in our vineyards. Different groups of samples will be collected:

i) Leafroll diseased vines: Samples will be collected from old and new vineyards from different cultivars and species (red, white and rootstock). [MSc Project]

ii) "Healthy" vines: Samples will be collected from diseased vineyards that appear to be asymptomatic or atypical of that particular disease. Samples will also be collected from established vineyards (certified material) and motherblocks. [MSc Project and Post Doc]

Double stranded RNA is a clear indicator of virus infection. Samples will be subjected to dsRNA extraction and sequence data generated for dsRNA-containing samples by constructing indexed double stranded cDNA libraries. Bioinformatic analysis of the sequence data will identify viruses present in these samples and a viral profile for each sample can then be constructed. RT-PCR and Sanger sequencing will be used to validate new viruses and variants.

Materials and Methods:

1. Sampling:
    a. Collect samples from across the Western Cape.
       Different types of samples will be collected (approximate sample size indicated in brackets):
        I. Leafroll diseased vines: (±12 samples)
            i. Red cultivar
            ii. White cultivar
            iii. Rootstock
        II. "Healthy" vines in diseased vineyard: (±12 samples)
            i. Atypical leafroll symptoms, any cultivar
            ii. No symptoms, any cultivar [Mother blocks, certified material]
    b. Extract dsRNA from samples using cellulose affinity purification.
    c. Prepare dsRNA-containing samples for sequencing. [Nextera RNASeq or similar]
2. Sequencing (indexed run, ±1GB/sample) [Illumina MiSeq (2013) and HiSeq (2014), ARC Biotechnology Platform or similar]
3. Analyse data
    a. *De Novo* assemblies [Velvet, CLC Genomics workbench and other software]
    b. Re-assemblies to known viruses [MAQ, CLC Genomics workbench and other software]
4. Validate virus sequences detected especially novel viruses and variants. Develop diagnostic RT-PCR for new viruses.

6. RESULTS AND DISCUSSION

Leafroll diseased vines (17) were sampled across the Western Cape from 9 farms. Five red cultivars, six white cultivars and four rootstocks were sampled. Double stranded RNA has been extracted from these samples and has been sequenced.

Samples collected in the year 2012 were sequenced with an Illumina MiSeq platform in 2013, which generated less data when compared to that of the samples collected in 2013, which were subjected to an Illumina HiSeq sequencing platform in 2014. Commonly infecting grapevine viruses such as GLRaV-3, GVA, GVE and RSPaV were detected throughout the dataset as expected by read-mapping and *de novo* assembly. All variant groups of GLRaV-3 were detected throughout the sequenced samples. A new genetic variant of GLRaV-3 was identified in sample GH24 and confirmed with Sanger sequencing. The full genome of this new variant was sequenced (Sanger) and phylogenetic analysis and pairwise comparisons indicated that this variant is more diverse that any variant detected to date and after thorough phylogenetic analysis designated to the reference sequence of the new variant group VII. GLRaV-3 isolate GH24 was included in the screening process and found in 8/17 samples. Furthermore, a number of vitiviruses were detected among which was GVF, previously not detected in South African vineyards. GEEV, the mycovirus identified in project number GenUS10/1, was detected in 15 of the samples sequenced. A number of viroids were detected with high reference genome coverage.

Data clean up:
Raw NGS data was trimmed and filtered as follows:
Nucleotides on the 5'-ends of read datasets were trimmed of unevenly distributed nucleotides to remove potentially incorporated sequencing errors and improve the quality of reads for *de novo* assemblies. The 3' region of the read dataset sequences whose mean quality score was less than 28 were trimmed to improve the overall quality of read datasets to ensure more accurate read-mappings and *de novo* assemblies. Read datasets were then filtered for quality using the fastq_quality_filter command of FASTX_Toolkit (Hannon Lab); the minimum quality score to keep was set at Q20 (-q 20) and the minimum percent of bases with Q20 quality scores was set at 96% (-p 96) (Figure 4.3). Through filtering read datasets for Q20 Phred scores it was assured that 99% of bases were called correctly, further improving the quality of reads for subsequent analyses. Once the data had been trimmed of sequencing adaptors and reduced quality sequence sections as well as filtered for high quality reads, between 39.6% and 81.0% of the total reads, per sample, were discarded. The seventeen trimmed and filtered read datasets were imported as single reads into CLC Genomics Workbench 7 (CLC Bio) and additionally filtered for host contaminants using the 19 *Vitis vinifera* chromosomes as well as grapevine chloroplast and mitochondria genomes.

Read-mapping:
Cleaned datasets were mapped to 124 reference genomes, comprised of grapevine-infecting pathogens. Read-mapping results for each of the 17 read datasets were pooled and GLRaV-3 was found to be the most prevalent virus, contributing to 31.29% of the total virus population detected. GLRaV-3 variant groups 6 and 2 were the most commonly detected variants, contributing 22.16% and 8.23% of the total viral population, respectively. Members of the genus *Vitivirus* contributed to 0.55% of the total viral population, GVE being the best represented *Vitivirus* with 0.47% contribution to the combined viral load. Reads mapping to *Grapevine endophyte endornavirus* (GEEV) were detected in all samples and contributed towards 6.6% of the total viral load. Three families of viruses contributed towards greater than 1% of the total viral load each, they are: *Closteroviridae, Betaflexiviridae* and *Endornaviridae*, the remaining viruses were identified at lower percentages. A summarised table of the read mapping results can be seen in table 1.

Table 1: Average coverage and the fraction of the reference genome covered for each sample against viruses from the families *Closteroviridae, Betaflexiviridae, Pospiviridae, Endornaviridae* and *Narnaviridae,* generated through read-mappings.

Sample Number:	Description:	Cultivar:	Grapevine Infecting Pathogen and Corresponding Family																													
			Closteroviridae														*Betaflexiviridae*								*Pospiviridae*						*Endornaviridae*		*Narnaviridae*	
			GLRaV-2		GLRaV-3 (1)		GLRaV-3 (2)		GLRaV-3 (3)		GLRaV-3 (4)		GLRaV-3 (6)		GLRaV-3 (GH24)		GVA		GVE		GVF		GRSPaV		Hop Stunt Viroid		Yellow Speckled Viroid 1		Yellow Speckled Viroid 2		GEEV		GNV	
			Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.	Ave. Cov.	Frac. Ref. Cov.
1	1/49/1	Chardonnay	0,09	0,02	1,33	0,14	**16**	**0,19**	0,2	0,02			3,78	0,12					0,32	0,10	0,01	0,01	0,15	0,11							0,62	0,31		
2	27/16/74	Pinot Noir	0,03	0,01	1,4	0,11	9,6	0,18	0,18	0,02			2,12	0,11			0,01	0,01	0,26	0,10	0,02	0,02	0,05	0,04							0,91	0,40		
3	38/36/31	Carignan	**1165**	**1**	**225**	**0,33**	**27096**	**1**	74,79	0,08	0,51	0,08	**1E+06**	**1**	0,03	0,01	**11**	**0,66**	**198**	**0,97**	**113**	**0,54**	0,05	0,03	**117**	**1**	**1424**	**1**	0,18	0,18	0,23	0,16		
4	49STOK	Richter '99	0,04	0,02	**16411**	**1**	**29592**	**1**	**1780**	**0,11**	1,29	0,17	**7806**	**1**	0,31	0,02	**28**	**0,97**	**741**	**1**	0,27	0,18	0,15	0,11	**25**	**0,94**	1,34	0,50			**12950**	**1,00**	0,13	0,13
5	BJ3	Richter '99	0,07	0,01	4,6	0,25	**3249**	**0,81**	0,97	0,05	0,48	0,13	4,21	0,16					5,39	0,39			0,19	0,12			0,48	0,12			0,57	0,30		
6	BJ4	Richter '99	0,02	0,01	**937**	**0,32**	**66591**	**1**	**172**	**0,13**	**10**	**0,25**	1,08	0,12	0,09	0,01	**138**	**0,96**	0,05	0,05	0,01	0,01	**1889**	**1**	**12**	**0,81**	**47**	**1**	**19**	**1**	0,18	0,13		
7	GH23	Cab. Sav.					0,05	0,03					0,05	0,02	**21880**	**1**															0,07	0,03		
8	GH24	Cab. Sav.					0,07	0,03							**7507**	**1**									0,73	0,18					0,09	0,05		
9	H35	Semillion	0,02	0,01	**1712**	**0,90**	0,5	0,06	0,24	0,01			**565**	**0,92**			0,08	0,02	0,01	0,01							**21**	**0,96**			0,07	0,05		
10	H36	Shiraz	0,01	0,00	0,6	0,10	**43**	**0,14**					**18**	**0,13**					0,71	0,06							0,17	0,17			0,01	0,01		
11	H38	Chenin Blanc	0,01	0,01	**158**	**0,44**	**1209**	**0,60**	0,23	0,01			**378**	**0,61**			0,03	0,03	2,66	0,15							**11**	**0,86**			0,22	0,14		
12	V1	Chardonnay	0,23	0,04	0,4	0,08	7,5	0,19	0,03	0,01			**22644**	**1**			0,03	0,02	0,40	0,10	0,08	0,03	0,03	0,03	7,16	0,81	1,4	0,55	**13**	**0,71**	0,56	0,13	**11**	**0,7**
13	V3	Chardonnay	0,03	0,01	**9927**	**1**	**7494**	**1**	**252**	**0,11**	5,74	0,18	**3299**	**1**	0,01	0,01	**30**	**0,98**	**1398**	**1**	**94**	**0,71**	0,06	0,03	4,2	0,77	3,12	0,93			1,22	0,46	3,13	0,55
14	V4R	Ramsey	0,05	0,02	**1353**	**0,93**	**10114**	**0,93**	88	0,07	0,46	0,14	**7277**	**0,88**	0,02	0,00	0,24	0,18	0,18	0,07	**38**	**0,43**	0,19	0,09	0,45	0,45	1,99	0,44			0,76	0,33	0,52	0,28
15	V5	Chenin Blanc	0,05	0,02	**148**	**0,33**	**1E+05**	**1**	49	0,07	0,94	0,17	6,09	0,23	0,01	0,01	0,02	0,02	**2019**	**1**	**128**	**0,60**	0,03	0,03	3,81	0,79	8,9	0,96	**34**	**0,87**	0,21	0,13	4,05	0,76
16	V6	Merlot	0,03	0,02	0,9	0,12	6,9	0,16	0,06	0,02			1,73	0,12			0,03	0,03	0,11	0,08	0,01	0,01	0,04	0,04							0,31	0,23		
17	16/15/14	Shiraz	0,06	0,01	1,36	0,13	**10**	**0,14**	0,2	0,02			2,69	0,14			0,02	0,02	0,26	0,11	0,04	0,04	0,05	0,04			0,19	0,19			0,59	0,35		

De novo assembly:

Parameters for each of the *de novo* assemblies were: a default word size and bubble size of 20 and 50, respectively; a minimum contig length of 200; global alignment with reads mapping back to contigs using default settings (mismatch cost: 2, insertion cost: 3, deletion cost: 3, length fraction: 0.5 and similarity fraction: 0.8); and update contigs was selected. Contigs created through *de novo* assemblies were selected based on the criteria that they had an average coverage of greater than 50 X or were 1 kb in length and subjected to BLAST (Basic Local Alignment Search Tool) searches with both blastn (searches a nucleotide database with a nucleotide query) and tblastx (searches a translated nucleotide database using a translated nucleotide query) analysis against the NCBI non-redundant database with the use of Blast2GO (Conesa et al., 2005). To ensure that high confidence matches were selected the E-value cut-off was set at 1.0E-6. Contigs were classified according to the highest identity identified.

In figure 1 the relative percentages of viral contigs (weighted by reads) identified through BLAST can be seen.

GLRaV-3 was found to be the best-represented virus detected in de novo assemblies, comprising 97.5% of the total viral population detected (Figure 2). Of the GLRaV-3 variant groups, GLRaV-3 variant group I was detected at the highest frequency with 120 and 109 contigs matched through blastn and tblastx searches, respectively. The results, however, may be misleading due to conserved region matches across GLRaV-3 variant groups. Furthermore, the genome of GLRaV-3 isolate GH24 is not present in online databases. GLRaV-3 variant group II was detected with the second highest prevalence; however, was identified with the greatest average coverage across all variants.

The primary infectious agents of the pooled viral population across all samples was found to be viruses from the family Closteroviridae followed by members of the family Betaflexiviridae (Figure 2). Grapevine rupestris stem pitting-associated virus (GRSPaV) was identified as the most prevalent member of the family Betaflexiviridae followed by GVE and GVA. This finding supports the theory that GLRaV-3 is the primary causative agent of GLD and that the vitiviruses are a

common occurrence in GLD-affected grapevines. Grapevine virus F (GVF) was detected for the first time in South African vineyards through de novo assemblies performed on samples 13 and 15. GVF was represented by three contigs constructed from 13 675 reads.

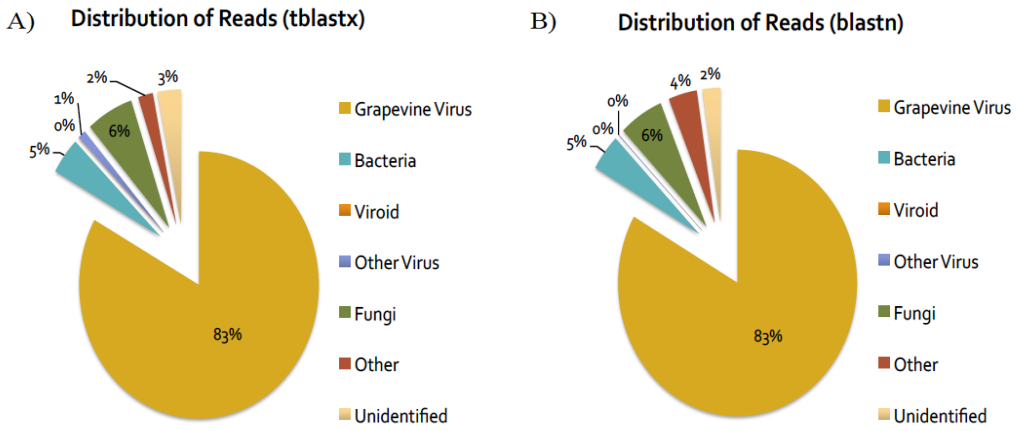

Figure 1: Graphical representations of the distribution of *de novo* assembly data to different categories of organisms, determined through Blast2GO. A) Distribution of *de novo* assembled reads using the tblastx function of Blast2GO. B) Distribution of *de novo* assembled reads using the blastn function of Blast2GO.

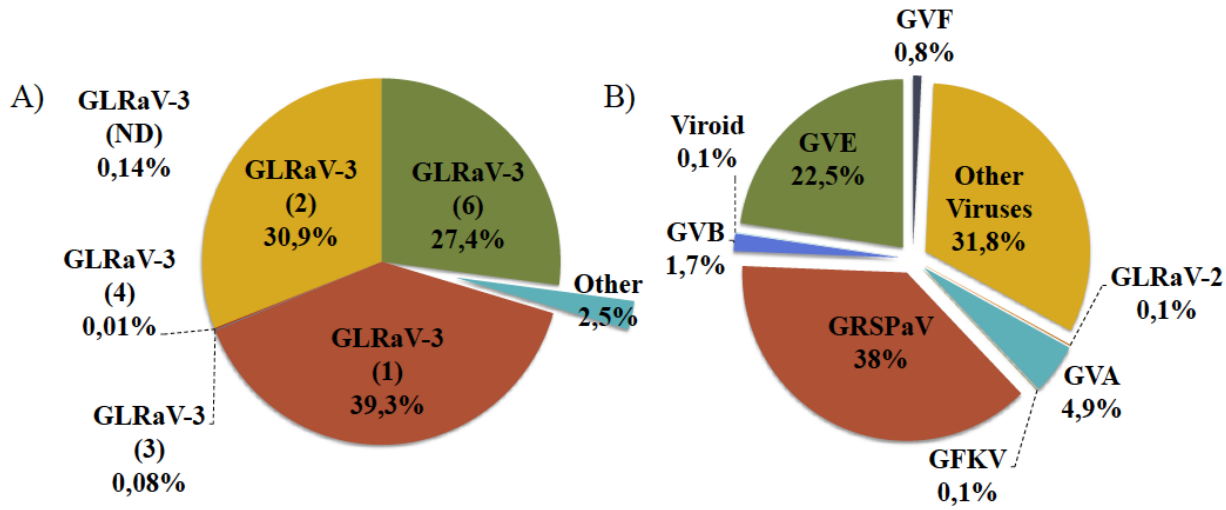

Figure 2: Chart representations of the pooled viral population within sampled grapevines, established through de novo assemblies. A) Percentage distribution of GLRaV-3 variant groups (denoted in brackets), as the primary causative agents of GLD. B) Viruses detected at lower frequencies whose percentage distribution comprises “Other” (1A).

7. COMPLETE THE FOLLOWING TABLE

Milestone	Target Date	Extension Date	Date completed	Achievement
1. Sampling 2012	July 2012		July 2012	Completed
2. Sampling 2013	July 2013		July 2013	Completed
3. Sequencing (two seasons)	March 2013	Dec 2013	Dec 2013	Completed
4 Data analysis	June 2013	Dec 2014	Dec 2014	Completed
5. Validation and Diagnostics	December 2013	Dec 2014	Dec 2014	Completed
5. Journal publication(s) – final milestone	Dec 2013	Dec 2014	Dec 2014	Completed

8. CONCLUSIONS

In this study, the viromes of 17 GLD symptomatic grapevines were investigated. It was determined that there is an average of four different viruses per sample, with rootstock and white-fruited cultivars hosting more diverse populations of viruses (Table 1). The most abundant virus was GLRaV-3, supporting the hypothesis that GLRaV-3 is the primary causative agent of GLD in South Africa (Pietersen, 2004). Through read mapping it was determined that viruses from the family *Closteroviridae* are the most prevalent in GLD-affected vines, comprising up to 31% of the mapped reads, while 0.6% of the total mapped reads originated viruses of the genus *Vitivirus*. In the de novo data GLRaV-3 accounted for 97.5% of the data. Although 76% of the raw data generated was lost through quality trimming and filtering, NGS functioned effectively in identifying grapevine infecting viruses and was capable of identifying a new variant of GLRaV-3 as well as a new isolate of GVF. Further research, with focus on viral populations and associations between virus species, is needed to broaden the understanding of GLD etiology and assist in reducing the negative effects of this disease.

For more detail on project GenUS11/3 please refer to Chapters 4 and 5 of Mr N Molenaar's MSc thesis. (http://scholar.sun.ac.za/handle/10019.1/97012).

9. ACCUMULATED OUTPUTS

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

Two RT-PCR diagnostic assays were developed that could specifically detect the new variant of GLRaV-3 (Isolate GH24) and GVF. An assay to detect the newly discovered GEEV was also developed.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

The influence of newly discovered viruses on plant health needs to be evaluated to ensure that they do not have a negative impact.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Graduation date	Total cost to industry throughout the project
Honours students					
Kristin Oosthuizen	RSA	BSc Hons	Honours	2013	0
Masters Students					
Nicholas Molenaar	RSA	MSc	Masters	2015	0
PhD students					
None					
Postdocs					
Hano Maree	RSA	PD		2013	0
Support Personnel					
Rachelle Bester	RSA	PhD	PhD		0

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

1. Molenaar, N., Burger, J.T. and Maree, H.J. (2015) Detection of a divergent variant of Grapevine virus F with next-generation sequencing. Archives of Virology. DOI: 10.1007/s00705-015-2466-3
2. Naidu, R.A., Maree, H.J., and Burger, J.T. (2015) Grapevine Leafroll Disease and Associated Viruses – A 'Unique' Pathosystem. Annual Review of Phytopathology. 53, 613-34. DOI: 10.1146/annurev-phyto-102313-045946
3. Burger, J.T. and Maree, H.J. (2015) Deep sequencing analysis of viruses infecting grapevines. Plant Pathology - Techniques and Protocols, Methods in Molecular Biology. Ed. Christophe Lacomme. Springer Science and Business Media, New York. Volume 1302, 315-330. DOI: 10.1007/978-1-4939-2620-6_23
4. Maree, H.J., Pirie, M.D., Bester, R., Oosthuizen, K., and Burger, J.T. (2015) Phylogenomic analysis reveals deep divergence and recombination in an economically important grapevine virus. PLoS ONE. 10(5): e0126819. DOI:10.1371/journal.pone.0126819
5. Jooste, A.E.C., Molenaar, N., Maree, H.J., Bester, R., De Koker, W.C. and Burger, J.T. (2015) Identification and distribution of multiple virus infections in grapevine leafroll diseased vineyards. European Journal of Plant Pathology. 142, 363–375. DOI: 10.1007/s10658-015-0620-0.
6. Bester, R., Pepler, T., Burger, J.T., Maree, H.J. (2014) Relative quantitation goes viral: RT-qPCR assay for a grapevine virus. Journal of Virological Methods. 210, 67–75. DOI: 10.1016/j.jviromet.2014.09.022.
7. Maree, H.J., Almeida, R.P.P., Bester, R., Chooi, K.M., Cohen, D., Dolja, V.V., Fuchs, M.F., Golino, D.A., Jooste, A.E.C., Martelli, G.P., Rayapati, N., Rohwani, A., Saldarelli, P. and Burger, J.T. (2013) Review: Grapevine leafroll-associated virus 3. Frontiers in Microbiology - Virology. 4, 82.

e) PRESENTATIONS/PAPERS DELIVERED

International

1. Maree, H.J., Bester, R., Pirie, M.D., Pepler, P.T., Oosthuizen, K. and Burger, J.T. (2015) GLRaV-3: diversity, detection and quantitation. Presentation at the 18th meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG), Ankara, Turkey. 7-11 September 2015.
2. Molenaar, N., Maree, H.J. and Burger, J.T. (2014) Determination of the efficiency of next-generation sequencing as a diagnostic tool for plant viral diseases. Presented at the Agricultural Biotechnology International Conference (ABIC). Saskatoon, Canada. 5-8 October 2014.

Local

1. Molenaar, N., Maree, H.J., Burger, J.T. (2013) Determination of the Viral Diversity Associated with Grapevine Leafroll Disease making use of Next Generation Sequencing. Presented at the 35th Conference of the South African Society for Enology and Viticulture, Somerset West, South Africa. 13-15 November 2013.
2. Maree, H.J. et al., (2014) Presentation: Final reports project GenUS 11/3 at Winetech virus workshop XII, Stellenbosch, South Africa. 3 Oct 2014.
3. Maree, H.J. et al., (2012) Presentation: Progress report GenUS11/3 at Winetech virus workshop XI, Stellenbosch, South Africa. 29 May 2012.

10. BUDGET

a) TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
1 (2012)					80 000	40 000		120000
2 (2013)					80 000	**18400***		98400
3 (2014)					0	0		0

*** THRIP Paid only 46% of allocation.**

b) FINAL BUDGET/FINANCIALS OF PROJECT

Please ensure that the budget is sufficiently detailed and add notes to explain all significant variations from the budget – you may submit this in an EXCEL document. Please report on the budget for the entire duration of the project. Add additional rows if required.

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
TOTAL INCOME				
Industry Funding				

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
PHI Funding				
Other Funding				
TOTAL EXPENDITURE				
Running Expenses				
General operating costs (printing, communication, etc.)				
Local Travel				
Publication costs				
Lab Analysis				
Lab Consumables				
Other				
Running expenses SUB-TOTAL				
HR Administration and Project Management				
HR Technical				
HR Research				
Student Bursaries				
HR SUB-TOTAL				
OTHER EXPENSES				
SURPLUS / DEFICIT				