



Canadian Food  
Inspection Agency

Agence canadienne  
d'inspection des aliments

# EUPHRESKO P-172: Next Generation Sequencing Proficiency testing of virus infected grapevine and fruit trees.

NGS technologies workshop, Bari, 2017



# Background

- Kickoff meeting of participants of EUPHRESKO P-172, late 2016, Braunschweig.
- Decision was made that there was enough experience within the group working with viruses infecting grapevine and fruit trees to warrant undertaking a limited proficiency trial.
- 13-14 laboratories from 11 countries agreed to take part in the trial:

Adrian Fox  
Christina Varveri  
Denis Kutnjak  
Hano Maree  
Heiko Zeibell  
Jan Kreuze  
Kris De Jonghe  
Maher Al Rwahnih  
Marcel Westenberg  
Michael Rott  
Thierry Candresse  
Pascal Gentit  
Wilhelm Jelkmann

Fera  
BPI  
NIB  
U. Stellenbosch  
JKI  
CIP  
ILVO  
U. California  
NVWA  
CFIA  
INRA  
ANSES  
JKI

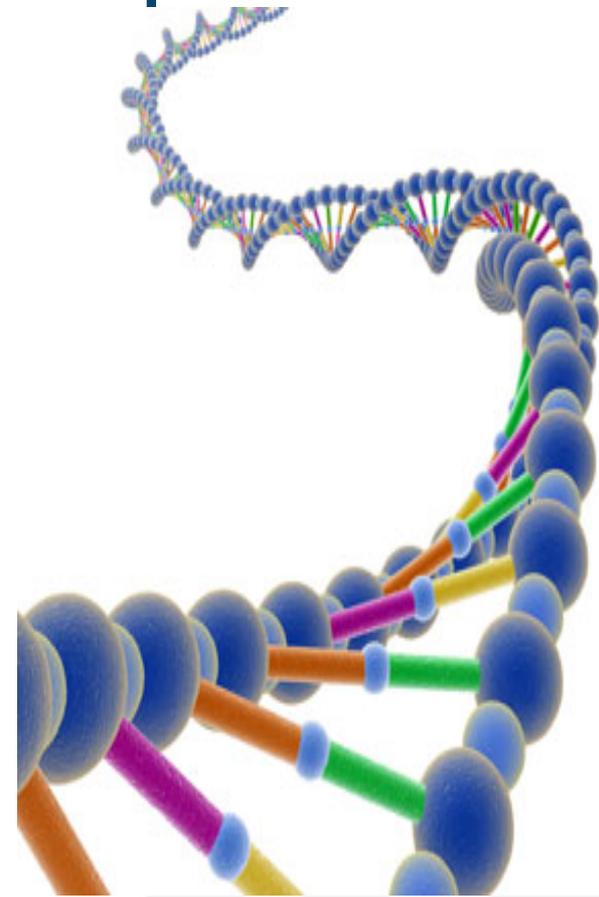
UK  
Greece  
Slovenia  
South Africa  
Germany  
Peru  
Belgium  
USA  
Netherlands  
Canada  
France  
France  
Germany

(Robert Hanner, BOL, Canada)

# NGS for the detection of plant viruses

- Detection of the plant virome
- Detection of plant viruses
- Detection on “novel” disease agents
- Detection of known viruses causing disease

wet lab vs dry lab



*Can NGS reliably detect known viruses in infected plant material?*



# Criteria

## 2) *Viral nucleic acid extraction:*

### a) total RNA extraction/ ribosomal depletion

- (mix of plant/ viral/microbial RNAs)

### b) Small RNAs

- siRNA

### c) Double stranded RNA

- viral and viral intermediate

## 3) *Sequencing:*

### a) Library construction

(preparation of viral nucleic acid for sequencing)

- dependant of type of nucleic acid extract
- dependent on type of sequencer

### b) Sequencer?

### c) Reads

- number
- length



*To be determined by the individual lab: based on lab developed protocols*

# Criteria

## 4) *Data analysis:*

- Multiple strategies, algorithms and settings possible
  - Most groups have developed their own workflows
    - Compare sequence data from a sample with known virus genomes or virus motifs
    - Each group provided with the viral sequences present in each sample
- Compare results for the different workflows
  - Standard worksheet provide for metadata collection
- Standardize data from all participants using one of two workflows
  - Submission of raw sequence data
    - VirusDetect <http://virusdetect.feilab.net/cgi-bin/virusdetect/index.cgi>
    - Virtool [www.virtool.ca](http://www.virtool.ca)



# Samples

- Two samples sets consisting of 6 samples
  - Grapevine sample set
  - Tree fruit sample set
  - Wach sample previously characterized for viruses
  - Range of genome types
    - Single stranded positive sense RNA
    - Single stranded negative sense RNA
    - Double stranded RNA
    - ssDNA
- Participants could choose one or both sets

# Sample Preparation

- Leaf material collected June 2017
  - 20 accessions from the CFIA positive collection, previously characterized using NGS
  - Leaf material freeze-dried and aliquoted into 15ml falcon tubes,
    - 0.5g dried material/tube ca. 3-5g fresh weight/tube
  - dsRNA was extracted from one complete set of aliquots and analyzed by NGS
  - Total RNA was extracted from the same aliquots, selected extracts tested for specific viruses by PCR for CVA, ACLSV, GRSPaV, CV\_TF1, GV\_TF1, ARWV, GLRaV.
  - Total of 12 samples selected and shipped to participants in August.
  - Complete viral sequence sent to participants in September

# Accessions

Sample ID	Host	Viruses	Genomes	Sample set
VT1	Cherry	ACLSV, CGRMV, CNRMV, CTLaV, CVA, PDV, PNRSV	ss +RNA, ss - RNA, ssDNA Multiple genotypes monopartite, multipartite 14 virus species Trichvirus Ampelovirus Capillovirus Illarvirus Foveavirus Unclassified Bunyavirus Unclassified Geminivirus	Tree Fruit
VT2	Apple	ASPV, ASGV, ACLSV		
VT3	Apple	ACLSV, ASPV, ARWV-1, ARWV-2		
VT5	Apricot	CVA, GV_TF1		
VT6	Plum	PBNPaV		
VT18	Peach	PcMV		
VT8	Grape	GVB, GVA, GLRaV2, GRSPaV, GFkV, HSVd	ss +RNA, ssDNA, dsDNA Multiple genotypes mono and mutipartite 14 virus species Badnavirus Vitivirus Closterovirus Ampelovirus Foveavirus Macularvirus nepovirus Unclassified Geminivirus	Grapevine
VT9	Grape	GLRaV4, GRBaV, GLRaV1, GLRaV2, GRSPaV, HSVd, GYSVd		
VT11	Grape	RpRSP, GRSPaV, HSVd		
VT13	Grape	TBRV		
VT16	Grape	GVA, GVB, GVD, GVF, GLRaV3, HSVd, GYSVd		
VT17	Currant	GVBaV, CV-TF1		

## Preliminary results (CLCBio)

- dsRNA extract
- Illumina 75H single read

## Grapevine Samples

Sample	Virus	reads	%coverage
VT8 (7.6M reads)	GVB	3,2440	100
	GLRaV2	1,185,575	99.9
	GRSPaV	572,717	99.8
	HSVd	3,227	99.7
	GLRaV3	33,153	99.1
	GFkV	1,911	73.6
	GVA	11,992	99.7
VT9 (15M reads)	GLRaV4	1,798,071	100
	GLRaV1	15,440	99.9
	GYSVd	4,060	99.7
	HSVd	6,342	99.7
	GLRaV2	7,175	99.7
	GRSPaV	5,330	99.4
VT11 (32M reads)	GRSPaV	10,719,762	100
	HSVd	9,505	99.7
	RpRSV	1,6052	99.7
	ASGV	2,961	93.8
VT13 (11M reads)	TBRV	52,288	90.9
	GLRaV1	796	93.5
	GLRaV2	1,616	92.3
	GRSPaV	477	83.8
	GLRaV4	468	77.2
VT16 (6.1M reads)	GVB	8,353	99.9
	GYSVd	683	99.7
	HSVd	2,020	99.7
	GVF	4,952	98.1
	GVA	6,617	97.6
	GVD	2,691	88.9
	GLRaV3	7,492	97.1
VT17 (1.9M reads)	CV_TF1	73,735	99.5
	GVBaV	358	61.7

# Preliminary results: 2 lab results Tree fruit Samples

1) dsRNA extraction

2) Total RNA extraction ribosomal depletion

Illumina sequencing

CLCbio workflows

Sample	Virus	dsRNA extraction			Total RNA extraction		
		total reads (single 75)	reads	% coverage	total reads (paired 150)	reads	% coverage
VT1	CTLaV	32 M	6,633,670	100	6.6M	179,200	99
	CVA		12,695,237	100		851,151	100
	PNRSV		287,469	100		17,110	97
	PDV		1,150,260	99.9		151,445	99
	ACLSV		126,709	99.9		3,197	87
	CGRMV		922,884	99.6		30,631	
	CNRMV		205,795	99.6		20,994	99
VT2	ASGV	27M	6,021,067	100	12M	3,606	79
	ASPV		22,057	95.7		-	
	ACLSV		67,372	71.2		-	
VT3	ASPV	10M	308,084	100	14M	10,138	80
	ACLSV		306,398	99.9		8,851	93
	ARWV2		42,173	99.9		-	
	ARWV1		27,046	99.8		-	
VT5	CVA	8.6M	2,331,103	100	12M	31,104	100
	GV_TF1		7,717	97.2		-	
VT6	PBNSPV	2M	227,797	99.9	12M	-	
VT18	PcMV	23M	2,388,227	100	23M	308,801	100

# Ongoing

- Trial delayed
  - Sample preparation and shipping took longer than planned.
- All participating laboratories currently in various stages of sample processing/ data analysis
- Expect results and data over the next couple months
- Final data results/presentation/discussion by next EUPHRESKO P-172 meeting
- Publication 2018

Canada 

## Preliminary results (CLCBio)

- dsRNA extract
- Illumina 75H single read
- 24 samples/run (12 shown)

### Fruit Tree Samples

Sample	Virus	reads	%coverage
VT1 (32M reads)	CTLaV	6633670	100
	CVA	12695237	100
	PNRSV	287469	100
	PDV	1150260	99.9
	ACLSV	126709	99.9
	CGRMV	922884	99.6
VT2 (27M reads)	CNRMV	205795	99.6
	ASGV	6021067	100
	ASPV	22057	95.7
VT3 (10M reads)	ACLSV	67372	71.2
	ARWV1	308084	100
	ACLSV	306398	99.9
	ARWV2	42173	99.9
VT5 (8.6M reads)	ASPV	27046	99.8
	CVA	2331103	100
	GV_TF1	7717	97.2
VT6 (2.0M reads)	PBNSPV	227797	99.9
VT18 (23M reads)	PcMV	2388227	100

## Grapevine Samples

Sample	Virus	reads	%coverage
VT8 (7.6M reads)	GVB	32440	100
	GLRaV2	1185575	99.9
	GRSPaV	572717	99.8
	HSVd	3227	99.7
	GLRaV3	33153	99.1
	GFkV	1911	73.6
	GVA	11992	99.7
VT9 (15M reads)	GLRaV4	1798071	100
	GLRaV1	15440	99.9
	GYSVd	4060	99.7
	HSVd	6342	99.7
	GLRaV2	7175	99.7
	GRSPaV	5330	99.4
VT11 (32M reads)	GRSPaV	10719762	100
	HSVd	9505	99.7
	RpRSV	16052	99.7
VT13 (11M reads)	ASGV	2961	93.8
	TBRV	52288	90.9
	GLRaV1	796	93.5
	GLRaV2	1616	92.3
	GRSPaV	477	83.8
VT16 (6.1M reads)	GLRaV4	468	77.2
	GVB	8353	99.9
	GYSVd	683	99.7
	HSVd	2020	99.7
	GVF	4952	98.1
VT17 (1.9M reads)	GVA	6617	97.6
	GVD	2691	88.9
	GLRaV3	7492	97.1
	CV_TF1	73735	99.5
	GVBaV	358	61.7