NGS technology at CNR-IPSP: from *de novo* virus discovery to the sanitary status of plants for planting

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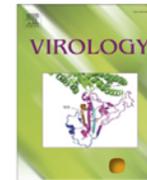
Naa! Is this now possible?

Rapid Communication

Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses

Jan F. Kreuze [a,d,*], Ana Perez [c], Milton Untiveros [a], Dora Quispe [a], Segundo Fuentes [c], Ian Barker [c], Reinhard Simon [b]

an unexplored territory

GOLD RUSH

Facility and sequencing at IPSP

Since 2011…

HiScanSQ apparatus
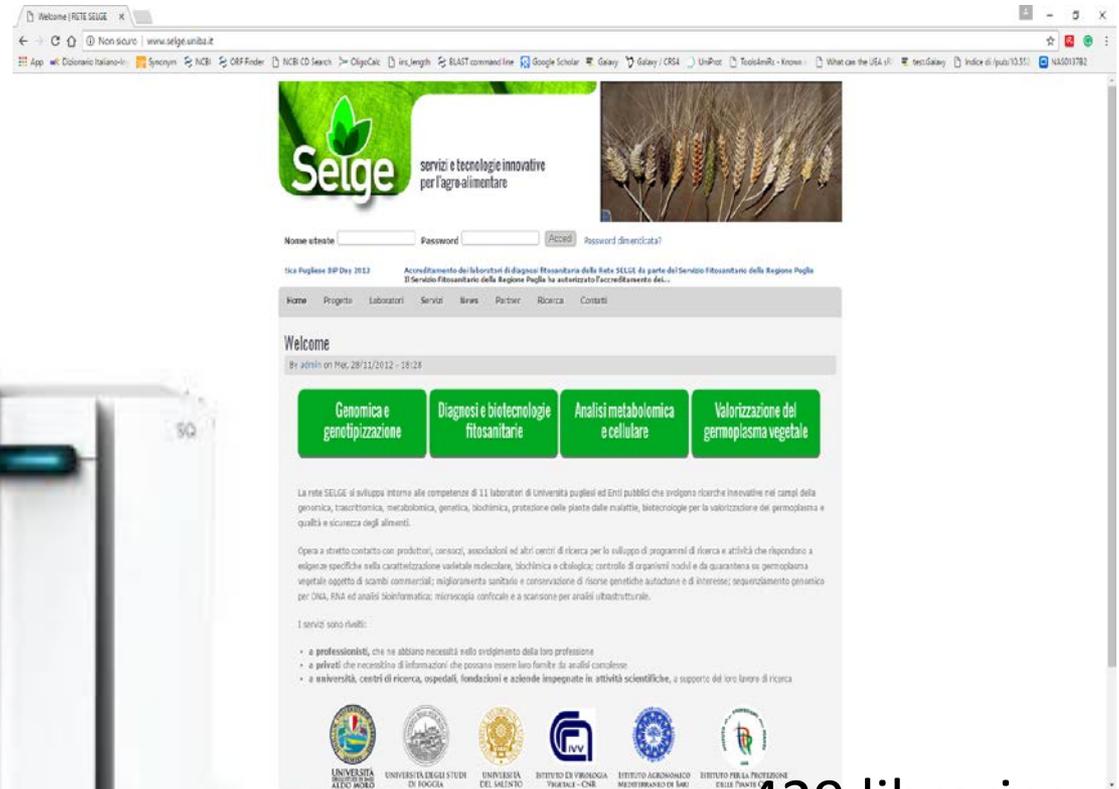
… 429 libraries

sequenced

In 10 runs

5,36 Average nr. of libraries/lane

Libraries and species

Among the 429 libraries

sRNA	206
mRNA	197
dsRNA	26

Genomic DNA (External service)

PacBio	1
PE150	5
PE100	5
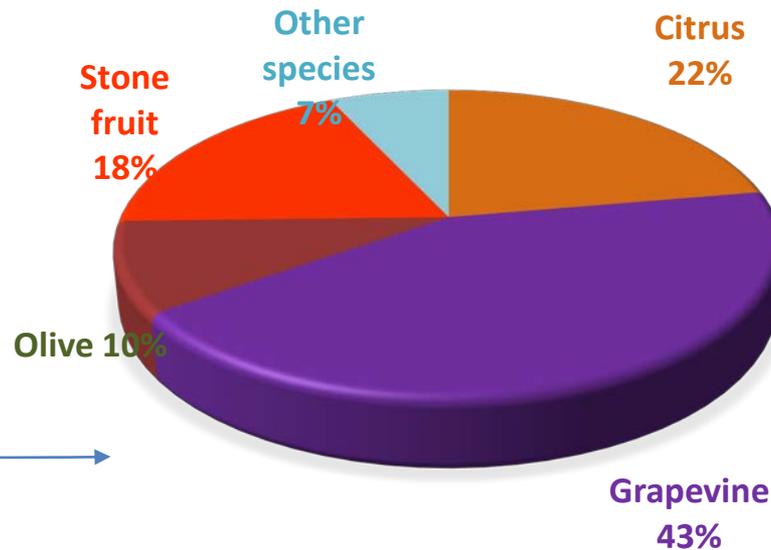
Technique of sRNA library synthesis – Illumina and HD adapters (Sorefan et al., 2012)

Bioinformatic pipeline

Quality Control

•Fastx toolkit.

The FASTX-Toolkit is a collection of command line tools for Short-Reads FASTA/FASTQ files preprocessing. Preprocess the FASTA/FASTQ files before mapping the sequences to the genome - manipulating the sequences to produce better mapping results.

De novo assembly

•Velvet 0.7.55;
•sRNAs: Kmer 15 and 17;
•dsRNAs: kmer 21, 23, 25.

Velvet is an algorithm package that has been designed to deal with *de novo* genome assembly. This is achieved through the manipulation of de Bruijn graphs for genomic sequence assembly via the removal of errors and the simplification of repeated regions.

NCBI Blast

•Blastn and Blastx;
•Local and/or web.

BLAST is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of proteins or the nucleotides of DNA sequences.

Analysis of Blast results

•E-value thresholds (10^-6 Blastn, 10^-4 Blastx);
•Grouping of results into family/genus clusters.

Mapping to reference

•SOAP Aligner;
•Patman;
•Variable nr of mismatches.

New viruses

unknown organism in symptomatic plant material

Grapevine Pinot gris virus

genus *Trichovirus;*   *Colomerus vitis*

New viruses
## *unknown organism in symptomatic plant material*

Grapevine roditis leaf discoloration associated virus
Maliogka et al. 2015, Chiumenti et al. 2016

# New viruses

unknown organism in symptomatic plant material
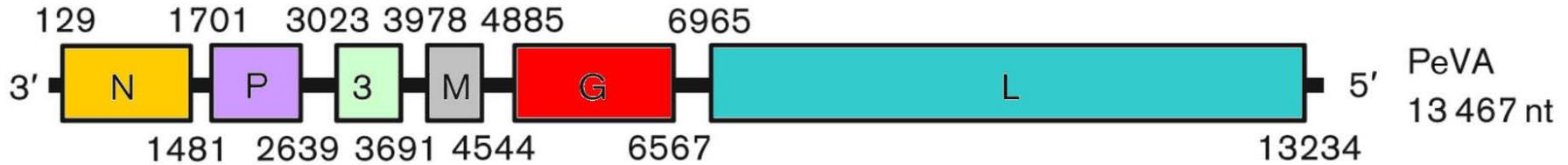
Persimmon virus A (PeVA)
Genus *Cytorhabdovirus*

Known viruses

known organism in symptomatic plant material

REPLICASE TGB CP

5' M A O P H R m C H

Apple green crinkle associated virus (AGCaV)
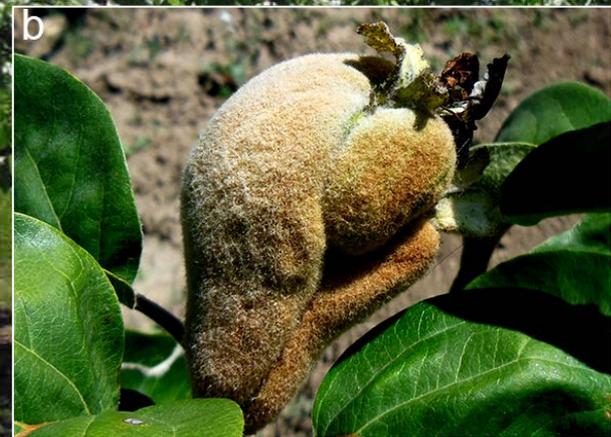
A viral disease on Quince *(Cydonia oblonga)*

New viruses

unknown organism in symptomatic plant material

Mulberry badnavirus 1 (MBV-1)
genus *Badnavirus*

a viral disease of mulberry (*Morus alba*)

New viruses

unknown organism in symptomatic plant material

A new Geminivirus associated with citrus chlorotic dwarf

VIRUS –LIKE DISEASES		
Chlorotic dwarf	Unknown causal agent	Diagnosis: Biological indexing on host range

Templates	Technology
siRNA	Single reads 50bp
DNA library	Pair-end 100-200bp

Initiation site (TAATATTAC conserved across geminiviruses)

3462   3510

C1 (Rep)

C2 (Rep)

V2 (MP)

V1 (CP)

V3 (MP)

P3   P1

P2

T1

T3 T2

3000

2000

1000

seq 3.640nt

New viruses

unknown organism in symptomatic plant material

Citrus vein clearing virus

VIRAL DISEASE		
Vein clearing	Citrus yellow vein clearing virus	Filamentous virus (?)

Templates	Technology
siRNA	Single reads 50bp
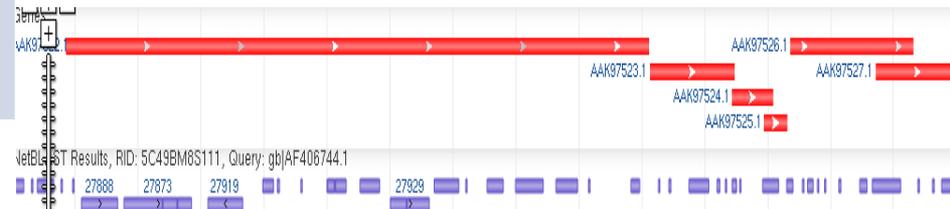
Contigs mapped on Indian citrus ringspot virus (genus mandarivirus) genome sequence identity ranged from 72 to 75%
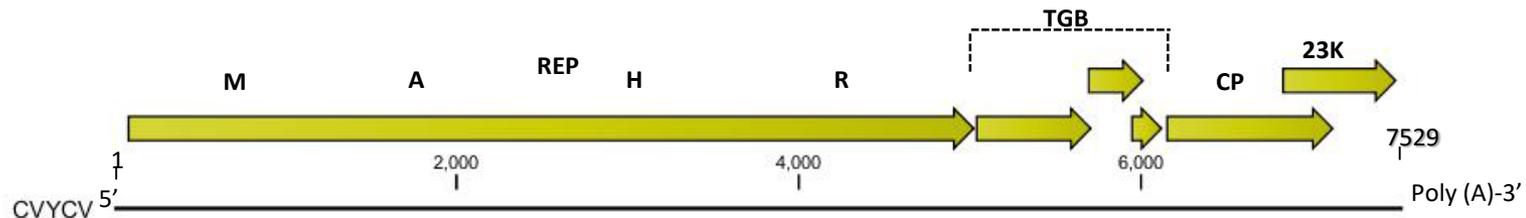
NGS contigs + conventional sequencing + RACE-PCR

New host for a viroid

uknown organism in symptomatic plant material

Apple dimple fruit viroid in Fig

healthyplants.wsu.edu
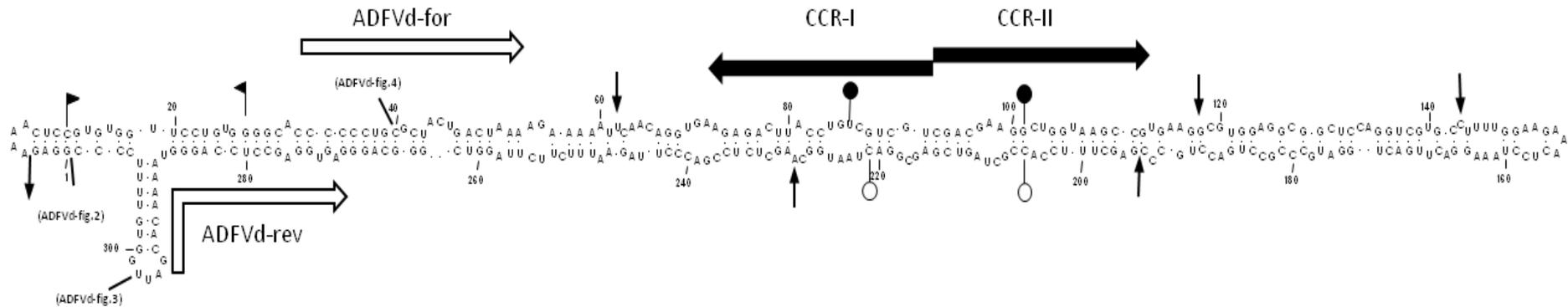
A dimeric infectious clone has been inoculated on seedlings of FIG and APPLE !!

# NGS assessing of the sanitary status of certified clones and rootstocks

G1 stock

Biological indexing

Laboratory assays
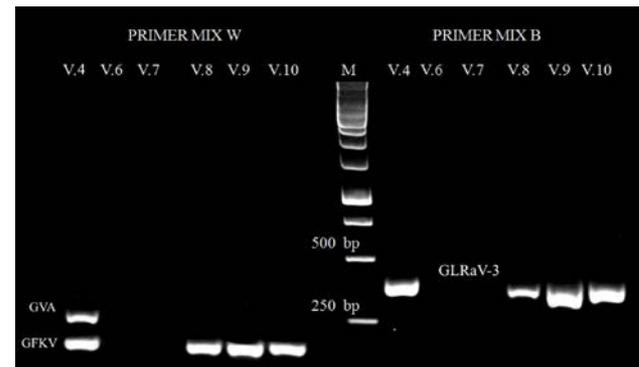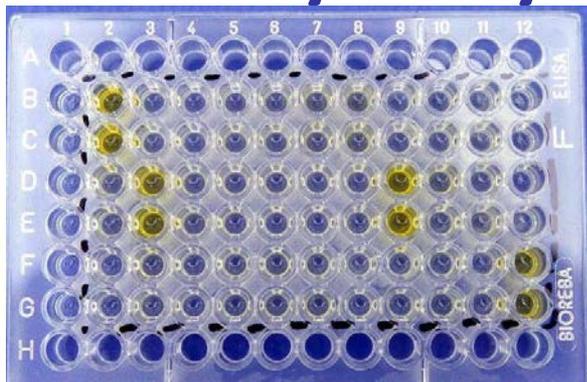
Woody indexing

dependent on climatic conditions

reliability affected by success in bud take

long time

place to place protocols (no standardization)

Constable et al. (2013) Aus Grape, Wine Res 19:74-80

Laboratory assays

specific tests

needs previous info on pathogens

Both tests can fail in identifying new and unknown viruses

Next generation sequencing

unbiased approach to study plant virome

| Cultivar/Rootstocks	Clone code	Redundant reads
Uva di Troia	UBA 49M	16.400.133
Malvasia Nera	UBA 69E	3.472.517
Bombino Nero	CRSA Reg. Puglia D205	22.872.057
Aglianico	CRSA Reg. Puglia D382	23.426.017
Baresana Rossa	CRSA 203	8.276.729
Italia	CRSA 121	23.332.063
Vittoria	CRSA 41	22.691.173
Regina dei Vigneti	CRSA 76	21.841.038
Lattuario Nero	CRSA 277	3.302.822
Verdeca	UBA 47	12.128.430
Susumaniello	CRSA Reg. Puglia D382	8.607.208
Bombino Bianco	CRSA Reg. Puglia D382	11.011.780
Negramaro	CRSA Reg. Puglia D382	19.435.282
Regina Bianca	CRSA 11	6.066.427
Michele Palieri	CRSA 229	6.078.556
Kober 5BB	UBA 01	15.016.373
1103 Paulsen	UBA 08	21.296.816
140 Ruggeri	UBA 05	19.737.418
420 A Mill.de Gr.	UBA 08	14.990.088
110 Richter	UBA 05	13.619.296

libraries from phloem and leaf tissues

GRLDaV discovered

GFLV ArMV, GFkV GLRaV-1,-2,-3 GVA, GVB free

A vine virome passport

Thanks to