High-throughput sequencing: de weg naar virusgetoetst en virusvrij uitgangsmateriaal in de containerteelt

VIRVRijUIT

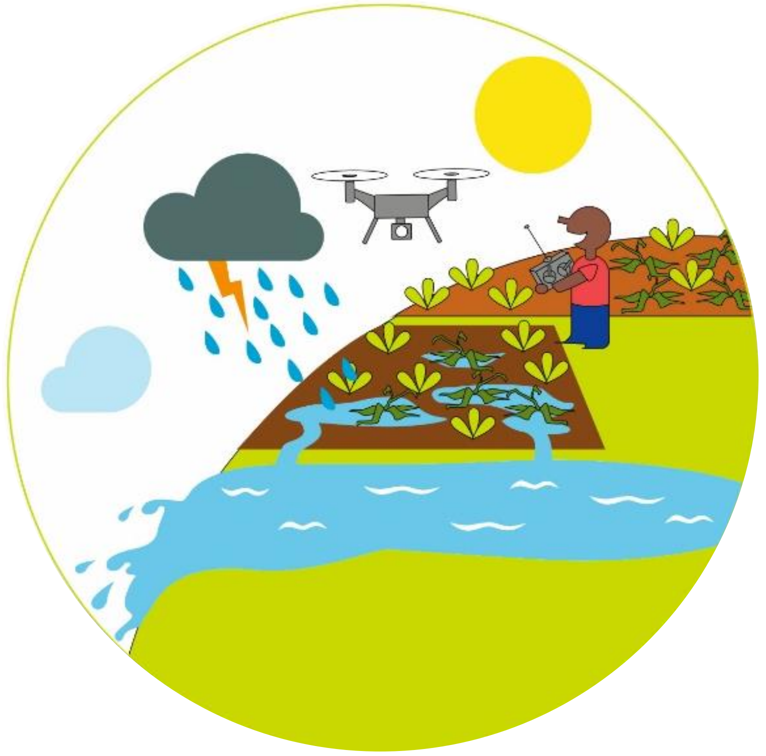
ILVO Institute for Agricultural and Fisheries Research — Ine Dewitte, Kris De Jonghe

UNIVERSITEIT GENT — Ine Dewitte, Stefaan Werbrouck

viaverda — Dominique Van Haecke

ILVO PLANT: ONDERZOEK VAN BODEM TOT PRODUCT

PLANTEN IN
EEN WIJZIGEND KLIMAAT



GEZONDE BODEM

PLANTEN VOOR DE
TOEKOMST

DUURZAME PLANT
PRODUCTIE SYSTEMEN

GEZONDE PLANTEN
TELEN

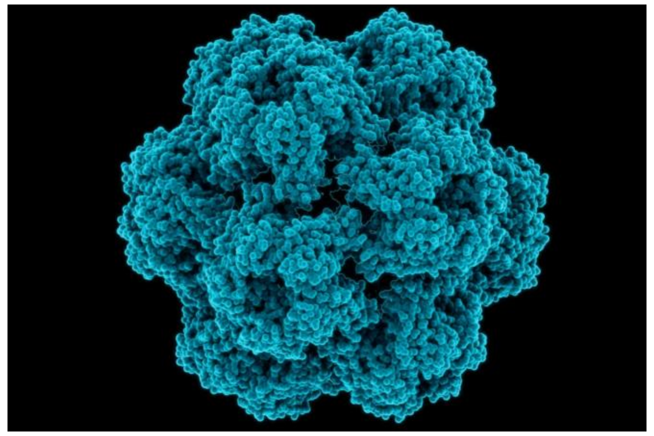
GEZONDE PLANTEN TELEN

Plantengezondheid

- Uitgangsmateriaal vegetatieve vermeerdering:
  - Stekken
  - Enten
  - Weefselteelt
  - …

(FarmProgress, 2017)

→ Vrij zijn van plantenziekten:
- Virussen – Virvrijuit
- Bacteriën
- Schimmels
- …



(Bailey, 2019)

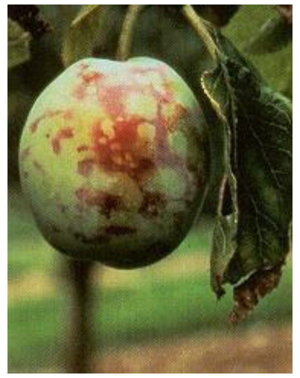
Plantenvirussen

- Mozaïek


- Mottling – vlekken

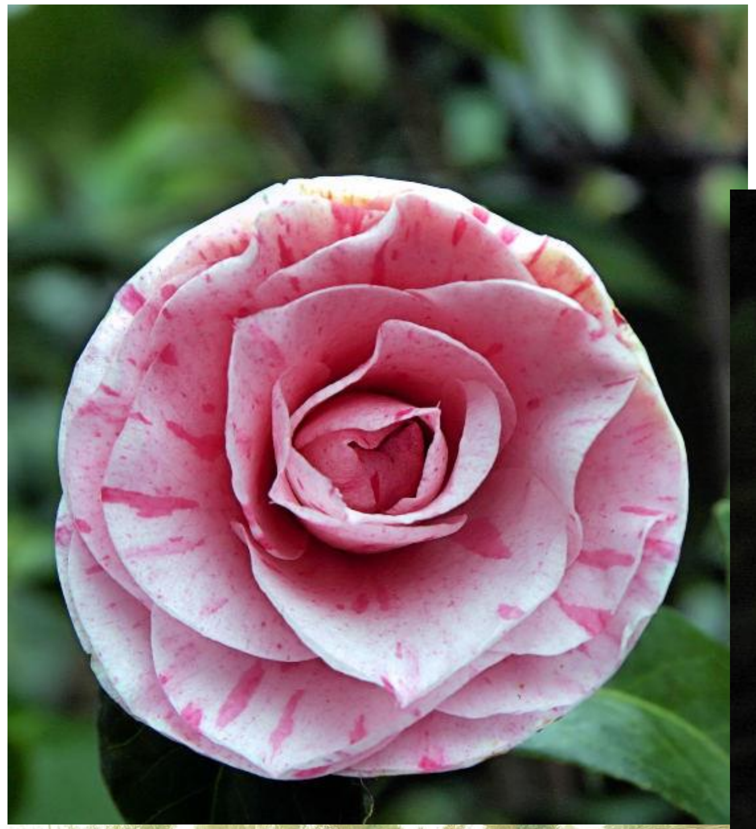
- Misvormingen en dwerggroei


- Kringvlekken



Plantenvirussen

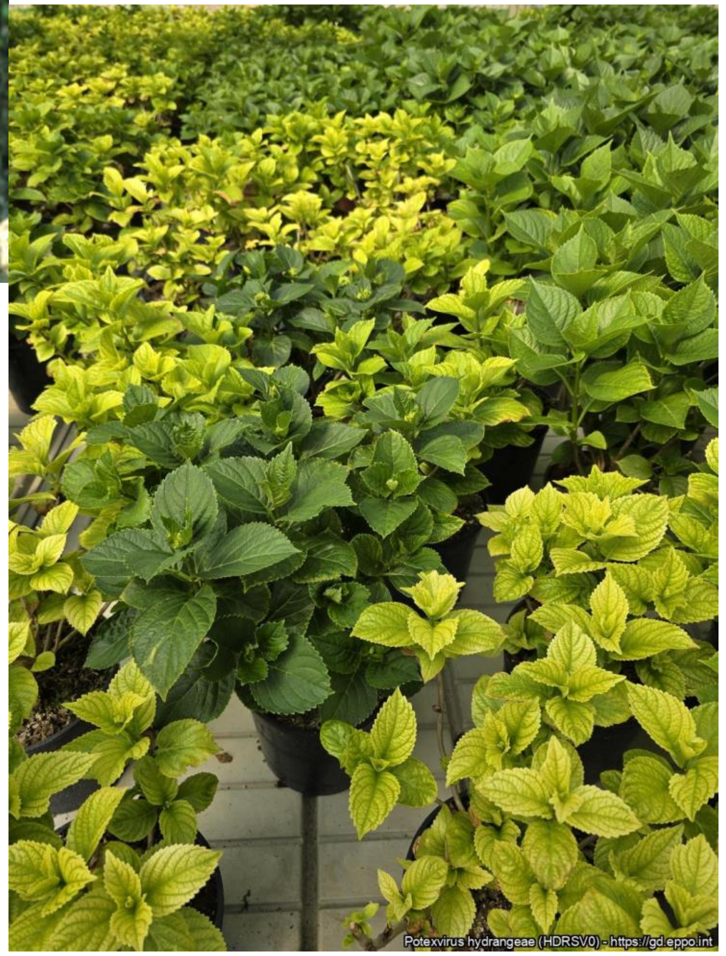
(Dr.Coffee, 2014.)



(450px-Tulip_With_Variegated_Colors-Tulip Breaking Virus, 2013)

(*Heavenly Bamboo Fire Power - Nandina Domestica Fire Power (BERBERIDACEAE)*, z.d.)

(Grower, 2001.)



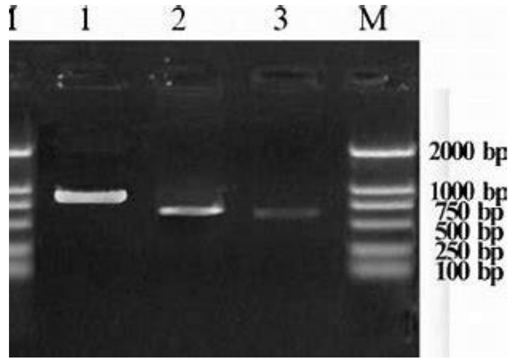
(*Potexvirus Hydrangeae (HDRSV0)[Photos]. EPPO Global Database*, z.d.)

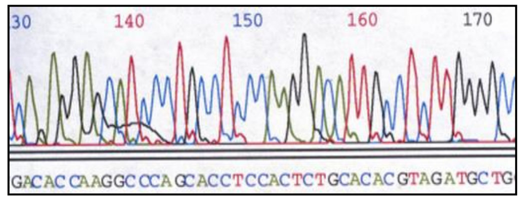
ILVO Institute for Agricultural and Fisheries Research

UNIVERSITEIT GENT

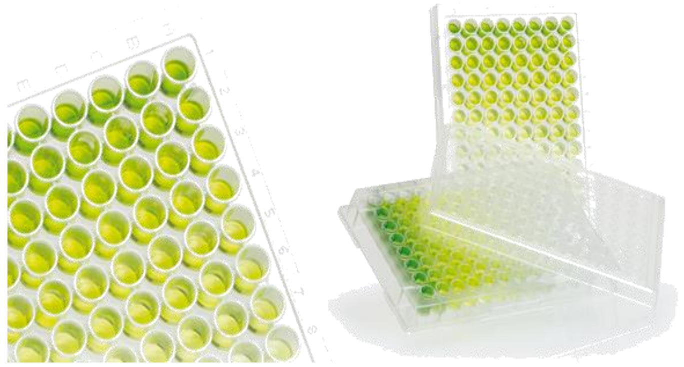
viaverda

Huidige virusdetectie





- ELISA
- PCR
- Plant testen (indicator planten)



→ Specifiek
→ Beperkt aantal virussen
→ Tijdsrovend

Innovatie

- **High-throughput sequencing (HTS)**

→ 1 test tegen alle gekende virussen

→ Ontdekking van nieuwe virussen

→ Minder arbeidsintensief

→ Meerdere stalen poolen

→ Kostenbesparend en sneller voor grote screenings

Waar nuttig?

- Virusvrij uitgangsmateriaal/certificering/import-export
- Voor niet doelgerichte, brede testen
- Ongewone symptomen

(Paddock, 2017)


(450px-Tulip_With_Variegated_Colors- Tulip Breaking Virus, 2013)

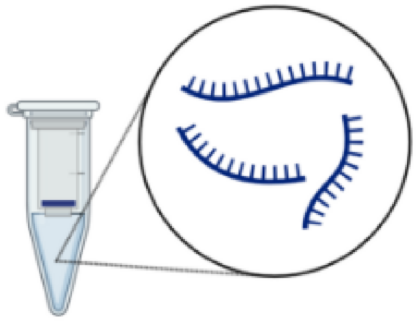
HIGH-THROUGHPUT SEQUENCING

Workflow

Staalname:

- Blad
- Wortel
- Nerven

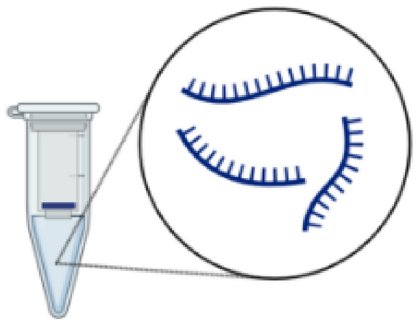
Workflow

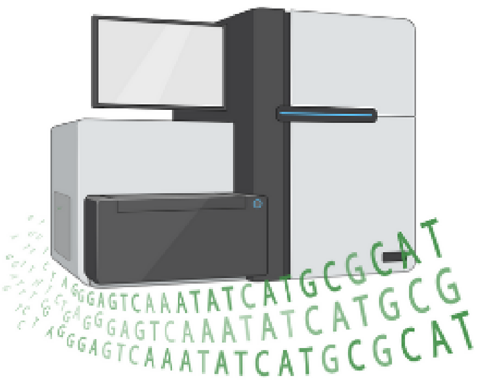


Staalname:
- Blad
- Wortel
- Nerven

RNA extractie

Workflow





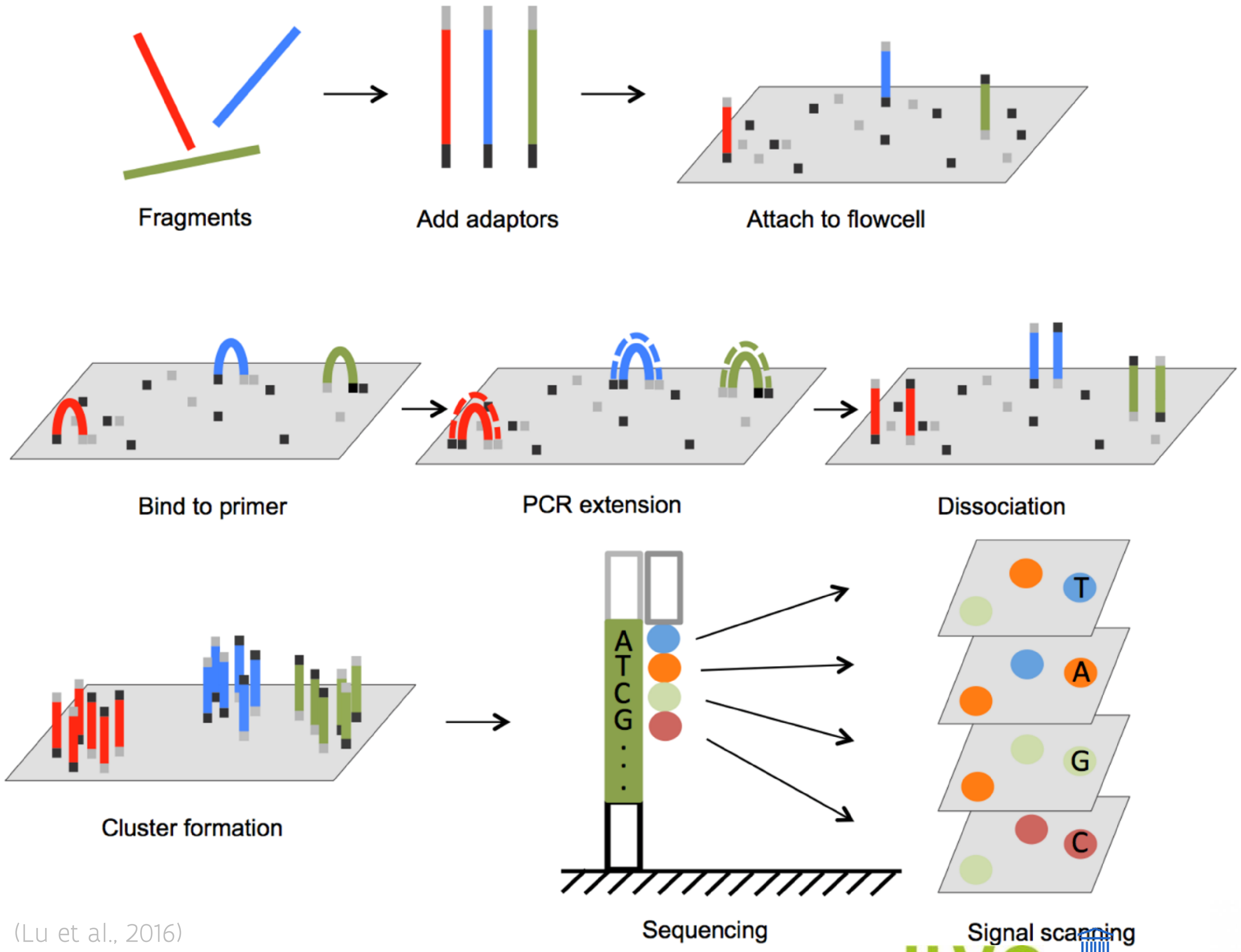
Staalname:
- Blad
- Wortel
- Nerven

RNA extractie

RNA sequencing

Illumina sequencing

1. Fragmentatie
2. Amplificatie
3. Sequencing

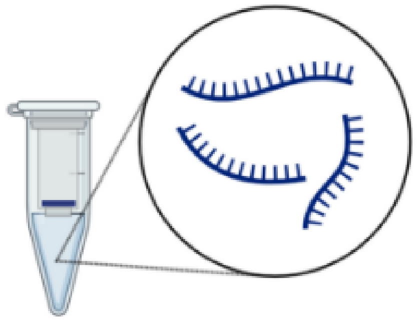


(Lu et al., 2016)

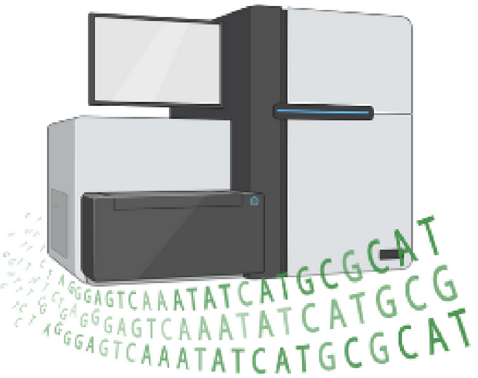
ILVO Institute for Agricultural and Fisheries Research
UNIVERSITEIT GENT
viaverda

Workflow

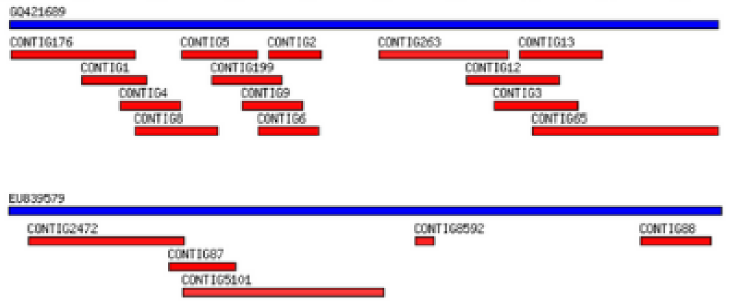
Staalname:
- Blad
- Wortel
- Nerven



RNA extractie



RNA sequencing

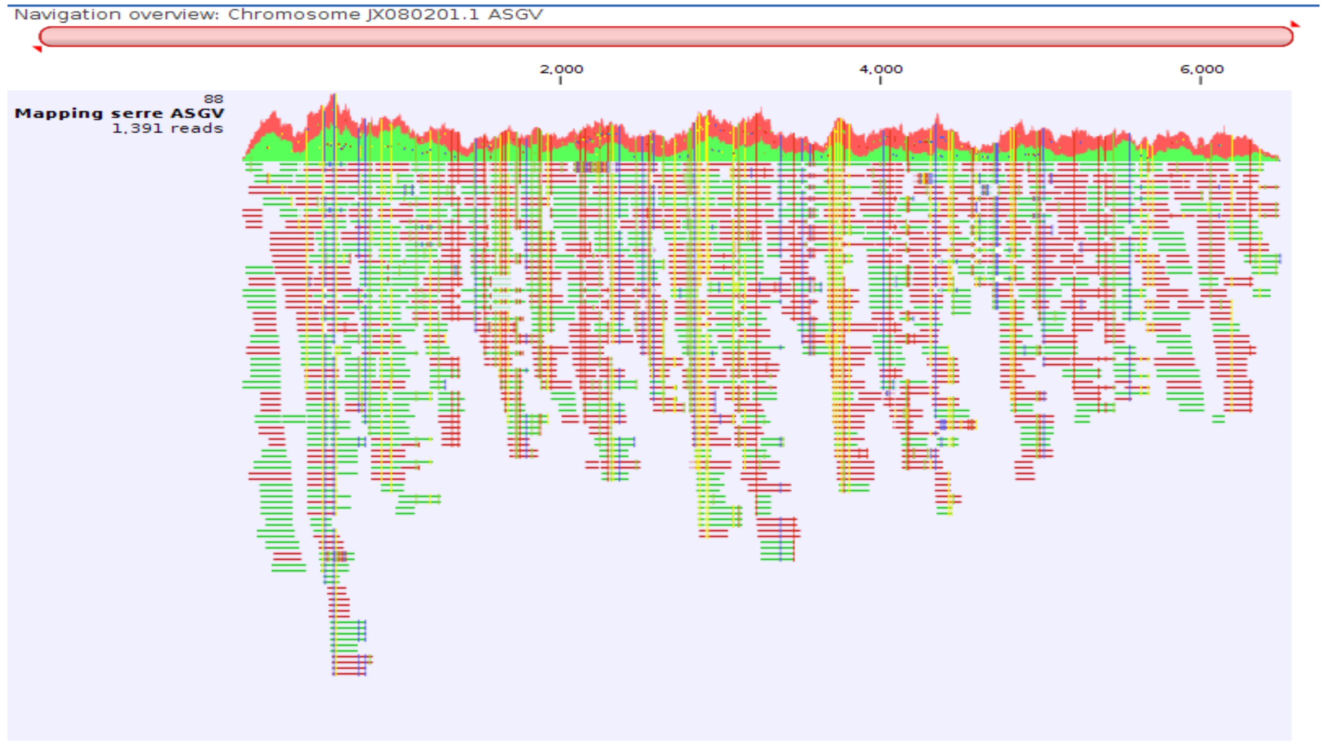


Data analyse

Data analyse

- Fragmenten vergelijken met database virussen

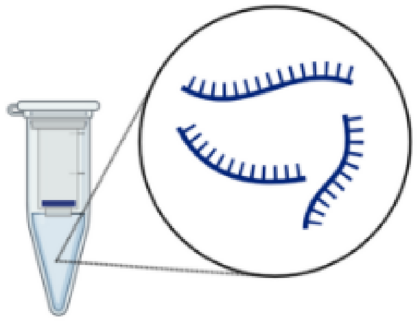
Apple stem grooving virus



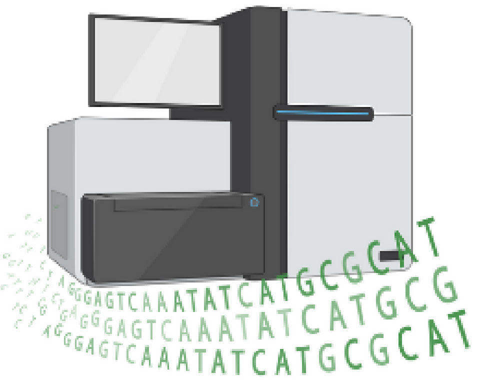
Dahlia common mosaic virus

Workflow

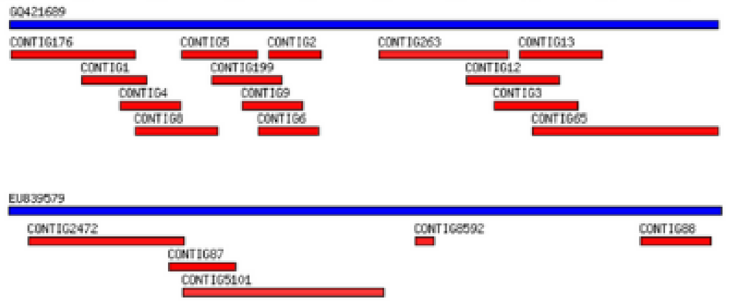
Staalname:
- Blad
- Wortel
- Nerven



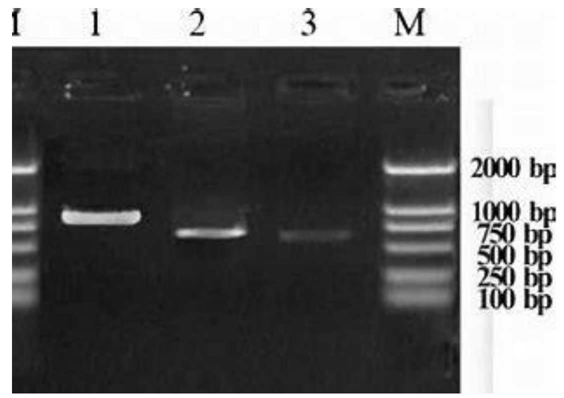
RNA extractie



RNA sequencing



Data analyse



PCR/ELISA

Blauwe bes

RNA sequencing

Locatie 1

- Blueberry latent virus (symptoomloos)
- Blueberry mosaic associated virus (chloroses & mozaïek) (RNQP)
- Blueberry virus A (brons/roodverkleuring & bladkrulling)

(Wikipedia, 2024.)

(Martin et al., 2012.)

Reference	Length	Coverage (%)	#contig	Depth	Depth (Norm)	%Identity	%Iden Max	%Iden Min	Genus	Description
AB608991	3434	3217 (93.7)	2	5.7	0.3	99.91	99.96	99.51	amalgavirus	Blueberry latent virus genomic RNA, complete genome, Japanese isolate.
LC066299	7944	7521 (94.7)	3	6.1	0.3	99.71	99.83	99.60	ophiovirus	Blueberry mosaic associated virus viral cRNA, segment RNA1, complete sequence, strain: JAT8 isolate.
KJ704366	7963	7195 (90.4)	16	7.0	0.4	99.57	100	99.18	ophiovirus	Blueberry mosaic associated virus segment RNA1, complete sequence.
LC066301	1570	1540 (98.1)	4	31.0	1.8	99.03	99.90	94.85	ophiovirus	Blueberry mosaic associated virus viral cRNA, segment RNA3, complete sequence, strain: JAT8 isolate.
KJ849173	1200	1200 (100)	5	20.5	1.2	96.75	100	94.75	ophiovirus	Blueberry mosaic associated virus isolate Oregon15 nucleocapsid protein gene, partial cds.
KJ849092	1200	1199 (99.9)	2	12.2	0.7	96.47	100	95.33	ophiovirus	Blueberry mosaic associated virus isolate Michigan1 58kDa Movement protein gene, partial cds.

ILVO Institute for Agricultural and Fisheries Research
UNIVERSITEIT GENT
viaverda

Blauwe bes

RNA sequencing

Locatie 2

- Blueberry scorch virus (verwelking) (RNQP)

(Blueberry (Vaccinium Corymbosum)-Scorch, 2024)

Reference	Length	Coverage (%)	#contig	Depth	Depth (Norm)	%Identity	%Iden Max	%Iden Min	Genus	Description
AY941199	8525	8437 (99)	5	617.8	38.6	91.46	96.77	89.65	carlavirus	Blueberry scorch virus isolate BC-2, complete genome.

ILVO Institute for Agricultural and Fisheries Research
UNIVERSITEIT GENT
viaverda

Hydrangea

RNA sequencing

- Geen virussen

(Stadhouder, 2024)

Prunus

RNA sequencing

- Cherry virus A (symptoomloos)
- Prunus necrotic ringspot virus (kringvlekken, chloroses, mozaïek…)
- Prunus virus F (bladvervormingen)

‹ Run6_Admera

Illumina mNGS Pipeline v8.3 | NCBI Index Date: 2024-02-06 | processed 4 months ago | SAMPLE DETAILS

Prunus_trimmed

Share | Download

Metagenomic

Taxon name | Name Type: Scientific | Background: None | Categories: 2 | Threshold filters: 1 | Read Specificity: All | Annotation | Pathogen Flags

NT rPM >= 10 ✕ | Viruses ✕ | Phage ✕

Report values are computed from 3.503.056 reads (2.000.000 unique reads) subsampled randomly from the reads that passed host and quality filters.
6 rows passing the above filters, out of 9119 total rows CLEAR FILTERS

Taxon	Score	Z Score	rPM	r	con...	con...	%id	L	E v...	NT NR
Capillovirus (*1 viral species*)	-	-	**13,569.2** 13,569.2	**51,917** 51,917	**25** 24	**51,271** 51,246	**99.2** 99.3	**4,104.4** 1,298.1	**10^{-291}** 10^{-249}	
Cherry virus A	-	-	**13,569.2** 13,569.2	**51,917** 51,917	**25** 24	**51,271** 51,246	**99.2** 99.3	**4,104.4** 1,298.1	**10^{-291}** 10^{-249}	
Ilarvirus (*1 viral species*)	-	-	**4,609.1** 4,540.9	**17,635** 17,374	**10** 9	**17,626** 17,366	**98.6** 98.7	**1,885.1** 388.9	**10^{-304}** 10^{-305}	
Prunus necrotic ringspot virus	-	-	**4,609.1** 4,540.9	**17,635** 17,374	**10** 9	**17,626** 17,366	**98.6** 98.7	**1,885.1** 388.9	**10^{-304}** 10^{-305}	
Fabavirus (*1 viral species*)	-	-	**582.1** 582.1	**2,227** 2,227	**4** 4	**2,227** 2,227	**96.5** 95.2	**3,259.1** 1,015.2	**10^{-308}** 10^{-304}	
Fabavirus pruni	-	-	**582.1** 582.1	**2,227** 2,227	**4** 4	**2,227** 2,227	**96.5** 95.2	**3,259.1** 1,015.2	**10^{-308}** 10^{-304}	

ILVO Institute for Agricultural and Fisheries Research

UNIVERSITEIT GENT

viaverda

VIRUSELIMINATIE

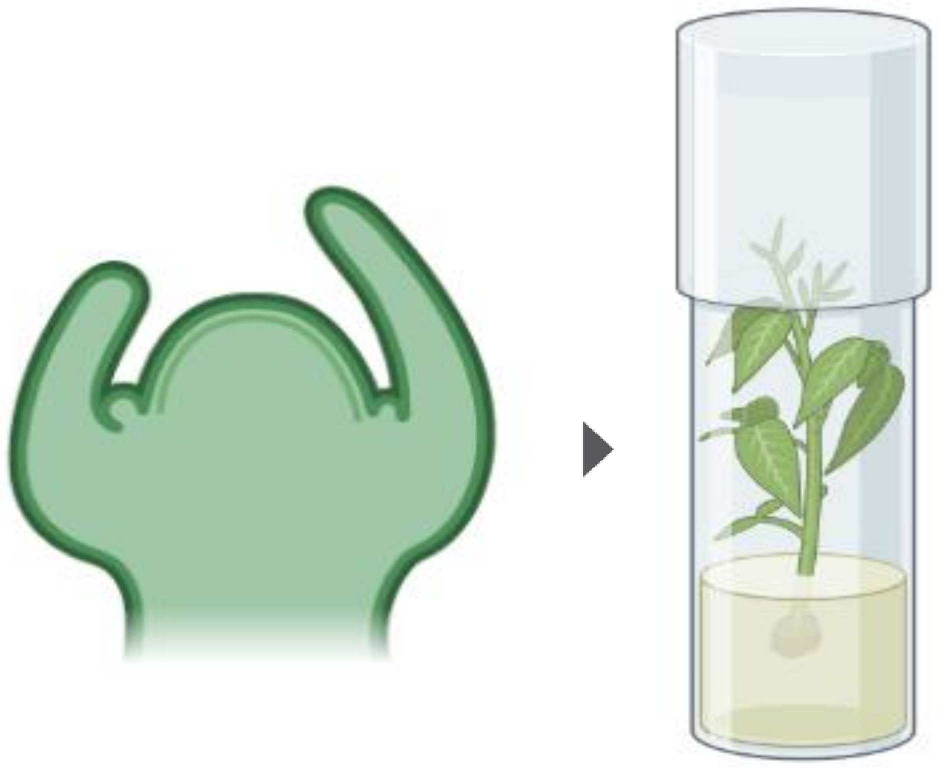
(Rosslee, 2024)

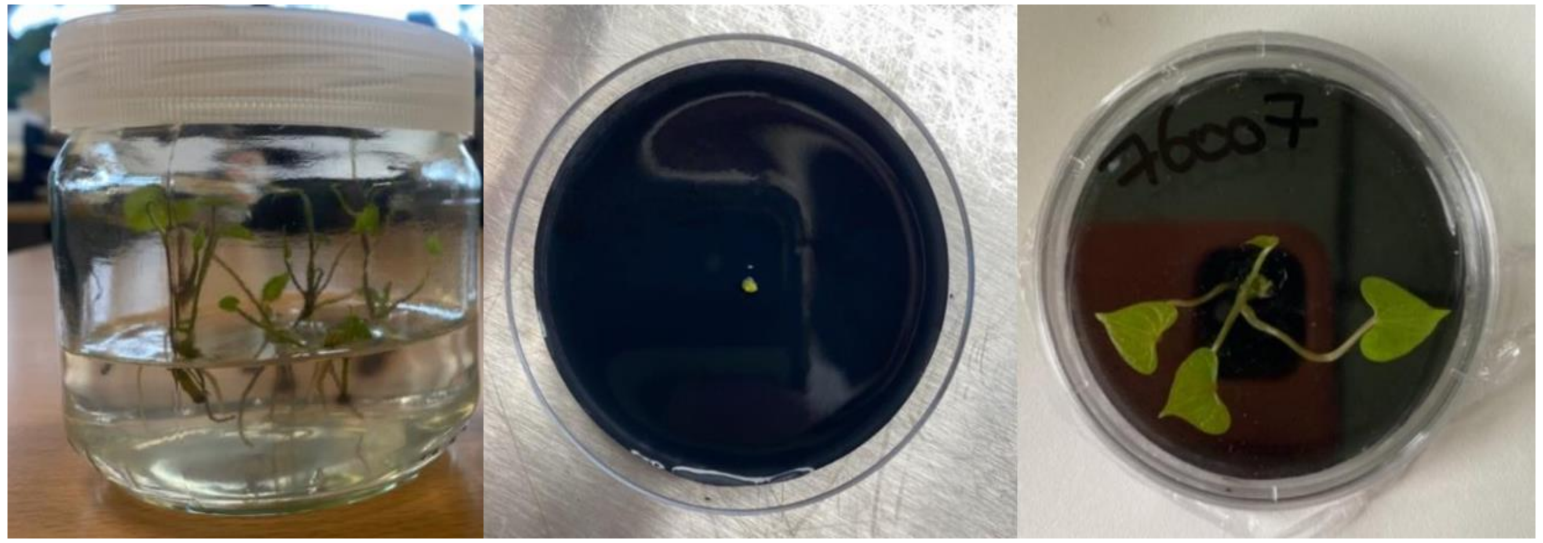
In vitro therapieën

- Meristeemcultuur
  - Afzonderen meristeemtopjes
  - Virus in lage concentratie aanwezig/ niet aanwezig

- Warmtetherapie
  - Met hitte
- Cryotherapie
  - Met koude
- Chemoherapie
  - Met antiviraal middel: ribavirine

→ stress

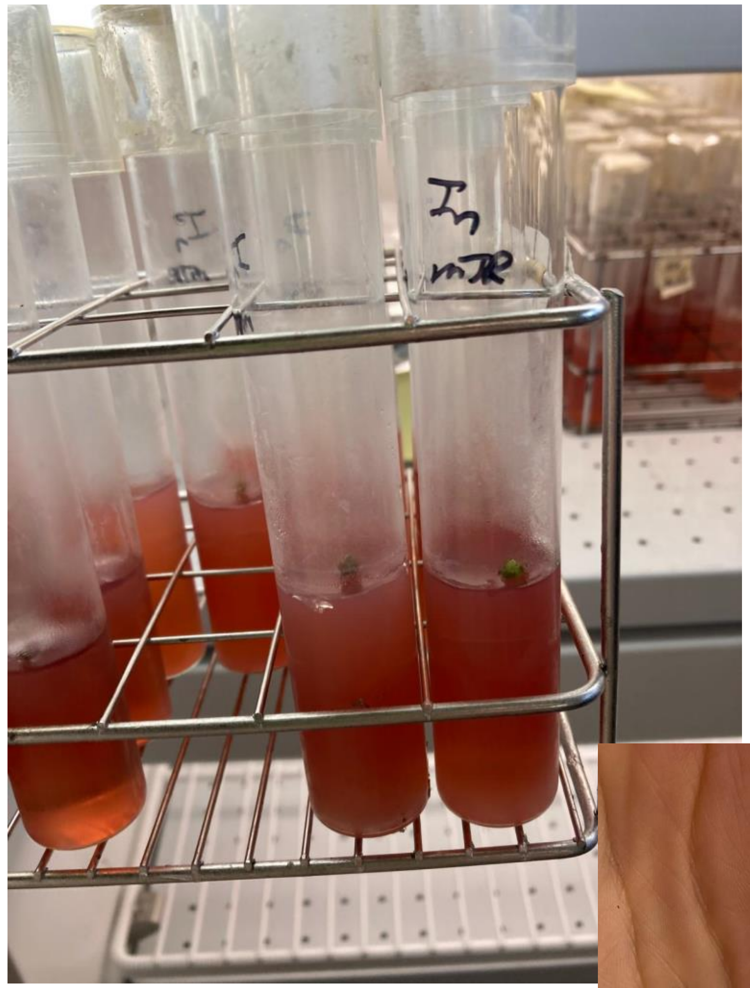


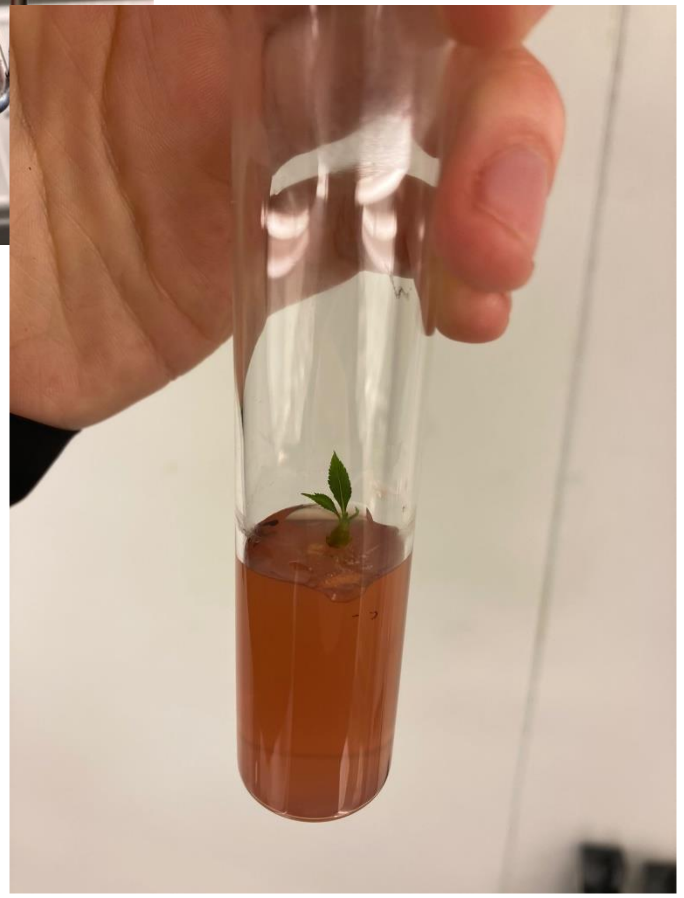


Prunus

- HTS detecteerde:
  - Cherry virus A
  - Prunus necrotic ringspot virus
  - Prunus virus F

- Viruseliminatie (Review (Szabó et al., 2023))
  - Thermotherapie
  - Chemotherapie (ribavirine)
  - Shoot tip culture





Chrysant

- Chrysanthemum stunt viroïde

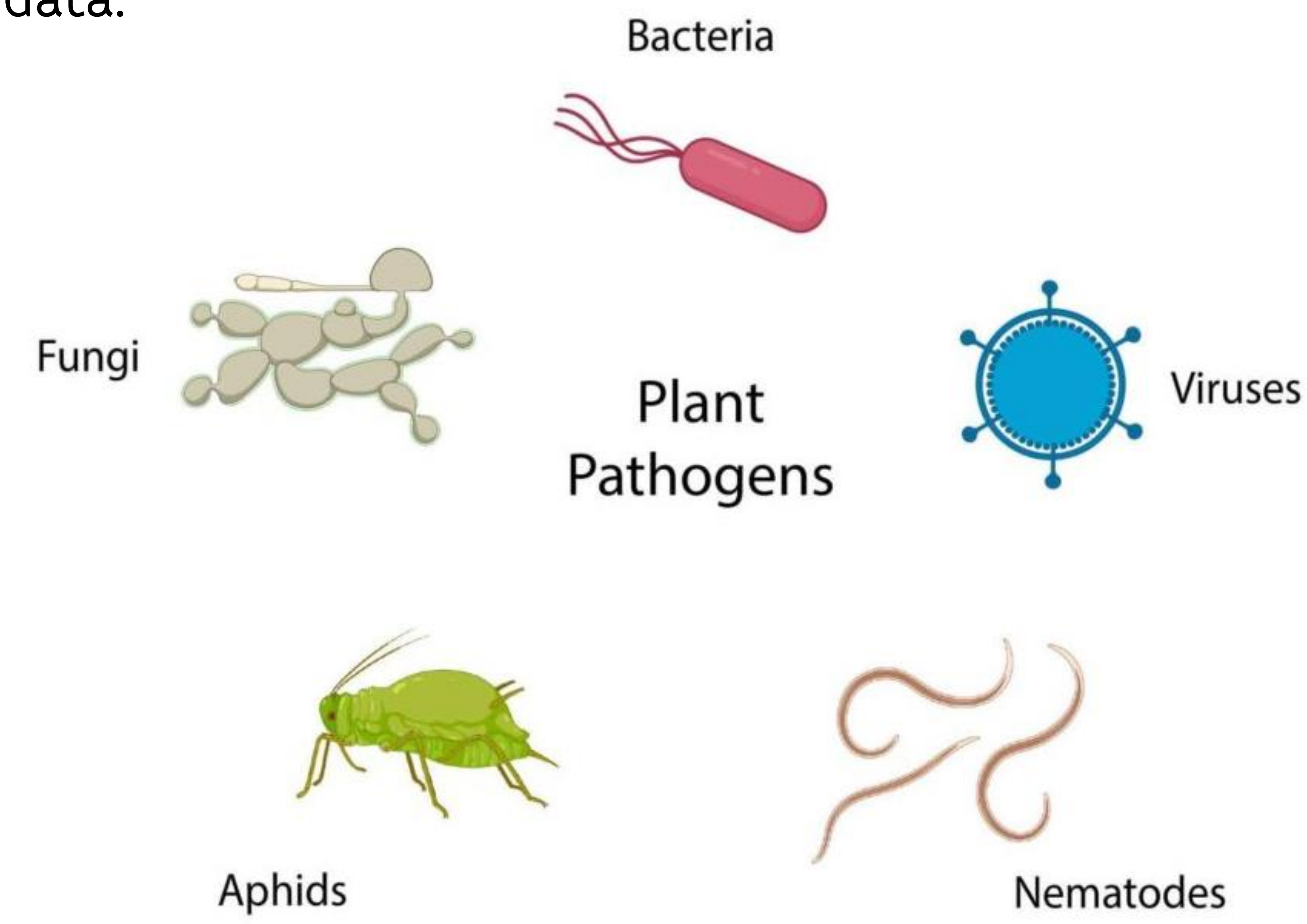
- Viruseliminatie:
  - Meristeemcultuur
  - Chemotherapie
  - 30 mg/L ribavirine voor 4 weken
  - 40 mg/L ribavirine voor 4 weken
  - 40 mg/L ribavirine voor 4 weken

- CSVd verwijderd

DETECTIE VAN ANDERE PATHOGENEN

Andere pathogenen

RNA sequencing data:



(*Plants Get Sick Too*, z.d.-b)

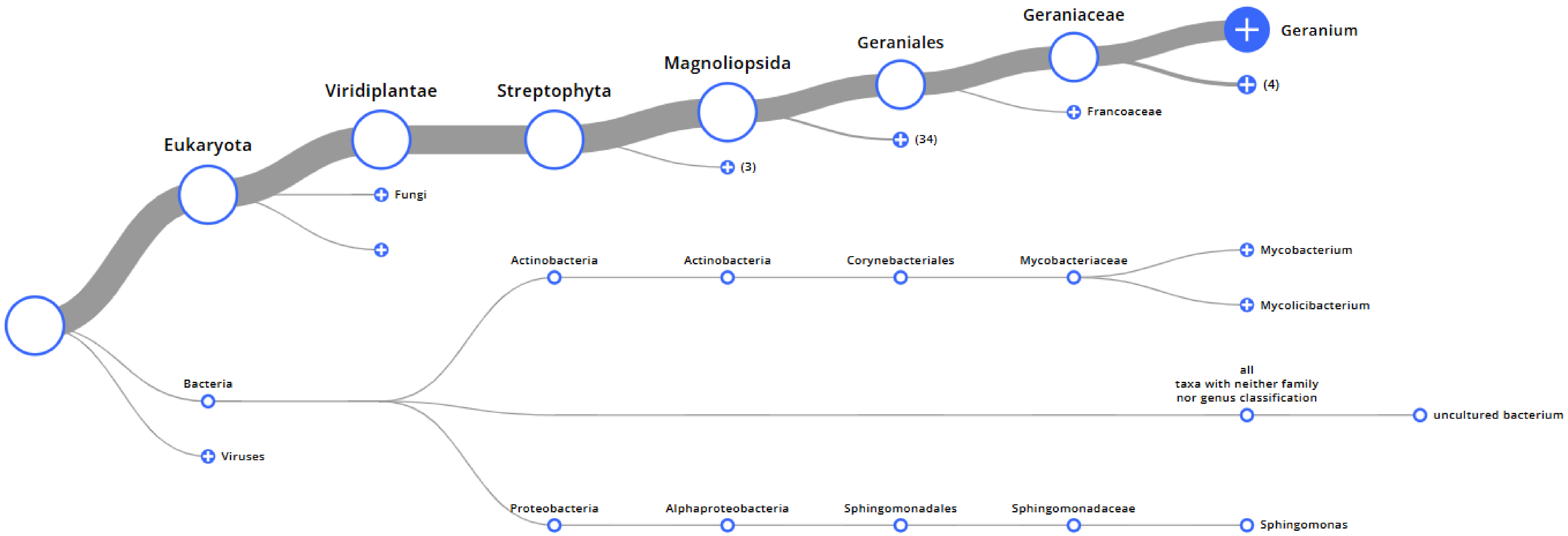
Geranium

Bacteria

NT rPM >= 10 ✕

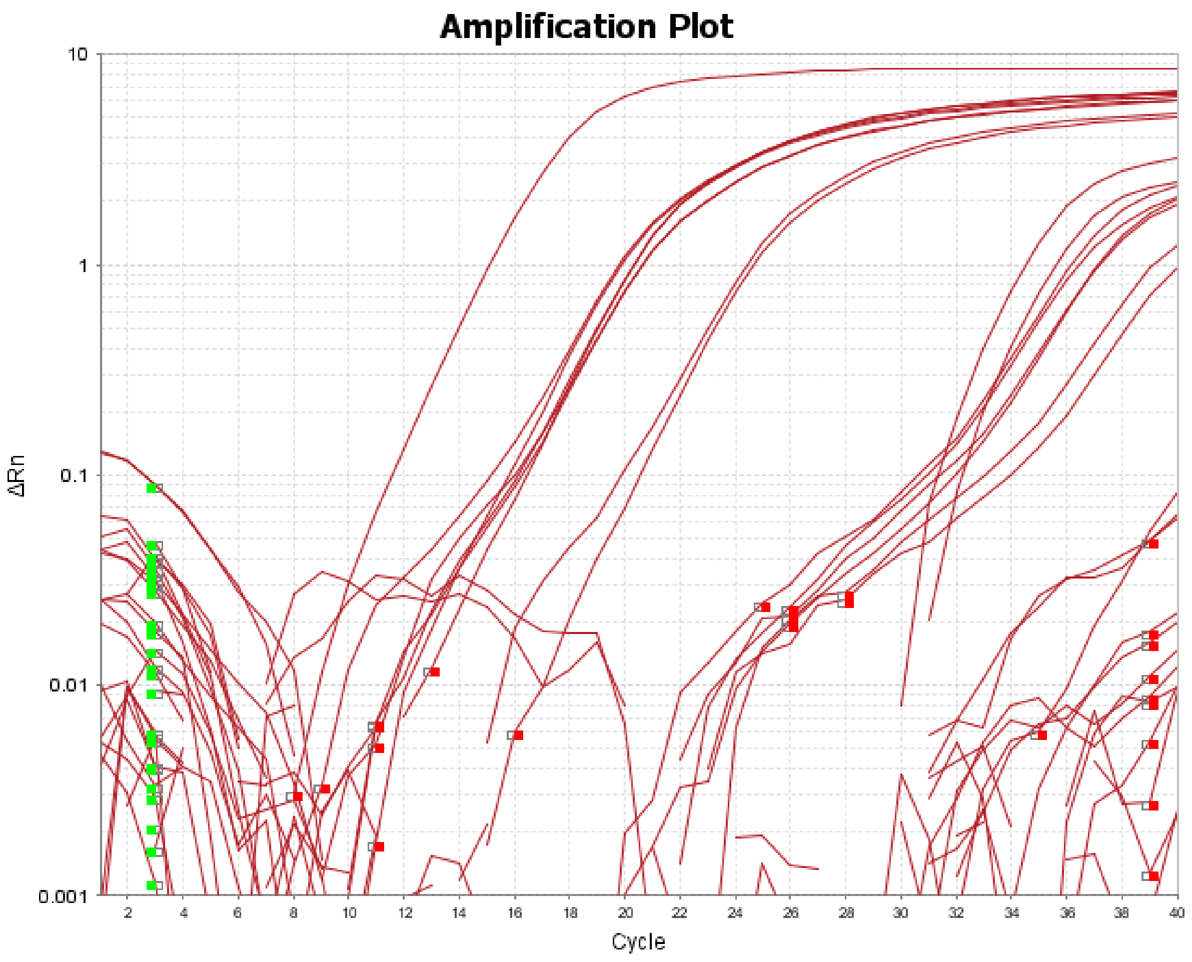
Report values are computed from 5.584.020 reads (2.000.000 unique reads) subsampled randomly from the reads that passed host and quality filters.

436 rows passing the above filters, out of 3809 total rows **CLEAR FILTERS**

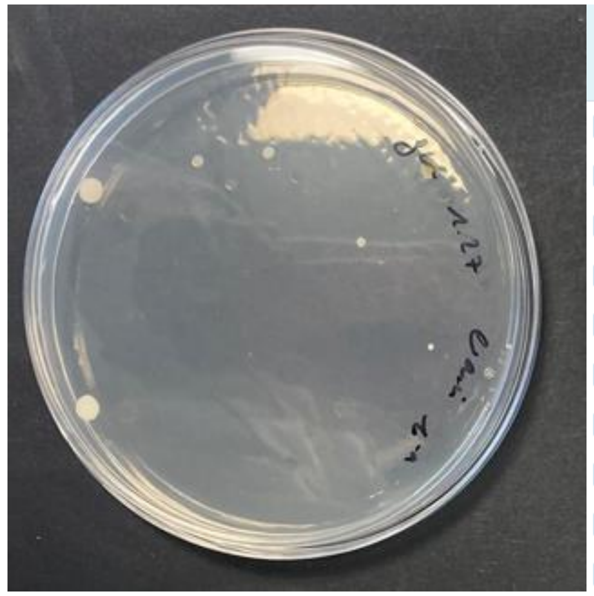


Geranium

Bacteria



- Bevestigd:
  - *Sphingomonas*
  - *Mycobacterium*



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
☑	Uncultured alpha proteobacterium gene for 16S ribosomal RNA, partial sequence, clone: B24	uncultured Alphaproteobacte...	686	686	22%	0.0	99.47%
☑	Uncultured bacterium clone A3 16S ribosomal RNA gene, partial sequence	uncultured bacterium	675	675	22%	0.0	98.44%
☑	Uncultured Sphingomonas sp. clone 9759 16S ribosomal RNA gene, partial sequence	uncultured Sphingomonas sp.	630	630	22%	3e-175	96.13%
☑	Sphingomonas pokkalii strain MMS22-ER13 16S ribosomal RNA gene, partial sequence	Sphingomonas pokkalii	627	627	22%	4e-174	96.58%
☑	Sphingomonas pokkalii strain L3B27 16S ribosomal RNA, partial sequence	Sphingomonas pokkalii	625	625	22%	2e-173	96.09%
☑	Sphingomonas pokkalii strain L1A4 16S ribosomal RNA gene, partial sequence	Sphingomonas pokkalii	625	625	22%	2e-173	96.09%
☑	Sphingomonas pokkalii strain 3CNBAF 16S ribosomal RNA gene, partial sequence	Sphingomonas pokkalii	625	625	22%	2e-173	96.09%
☑	Sphingomonas sp. WG 16S ribosomal RNA gene, partial sequence	Sphingomonas sp. WG	625	625	22%	2e-173	96.09%
☑	Sphingomonas cannabina strain DM2-R-LB4 chromosome, complete genome	Sphingomonas cannabina	625	625	22%	2e-173	96.09%
☑	Sphingomonas cannabina 16S ribosomal RNA gene, partial sequence	Sphingomonas cannabina	625	625	22%	2e-173	96.09%

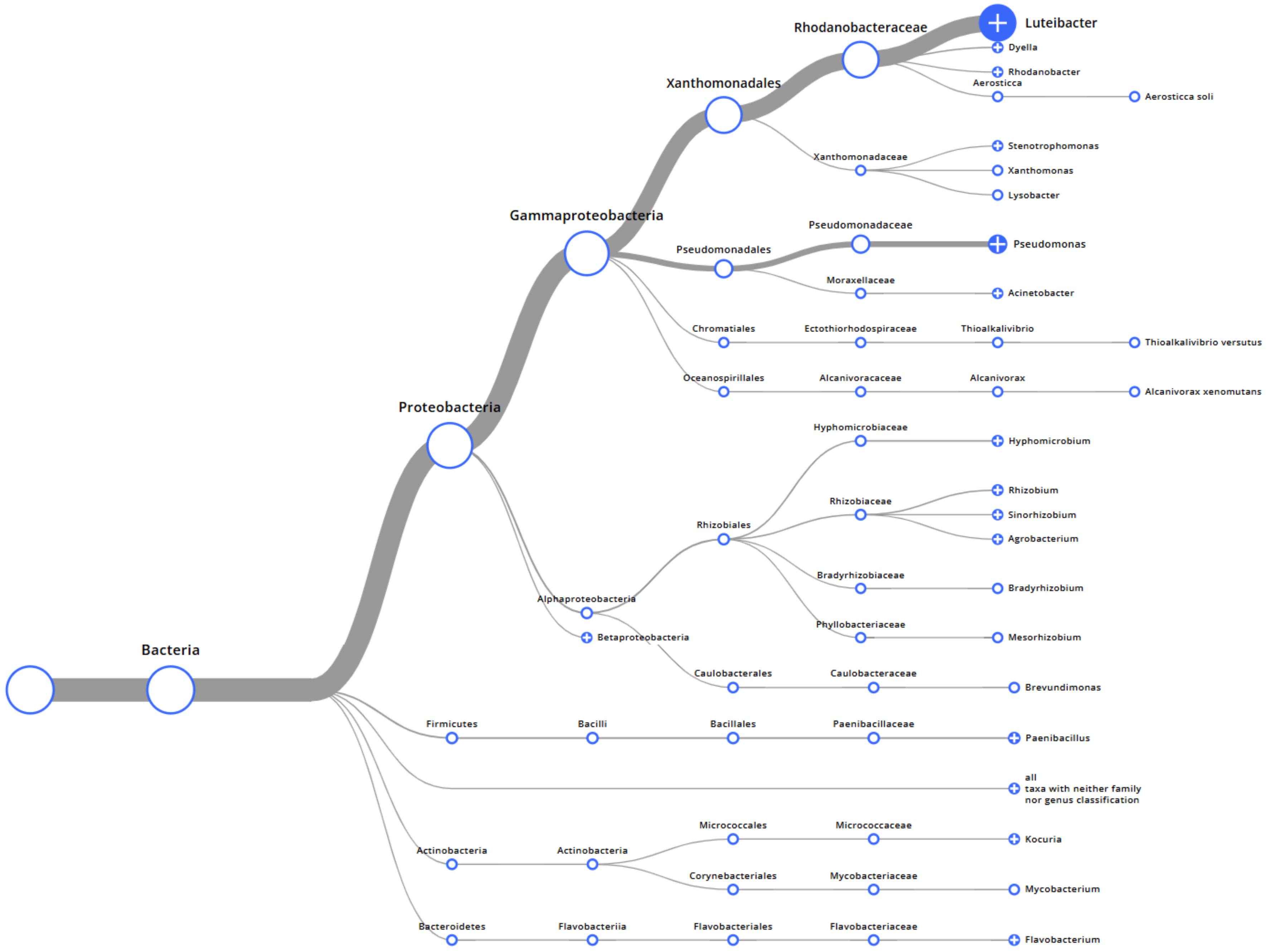
ILVO Institute for Agricultural and Fisheries Research
UNIVERSITEIT GENT
viaverda

Helleborus

Bacteria

NT rPM >= 10 ✕ Bacteria ✕

Report values are computed from 3.976.454 reads (2.000.000 unique reads) subsampled randomly from the reads that passed host and quality filters.
145 rows passing the above filters, out of 6830 total rows **CLEAR FILTERS**

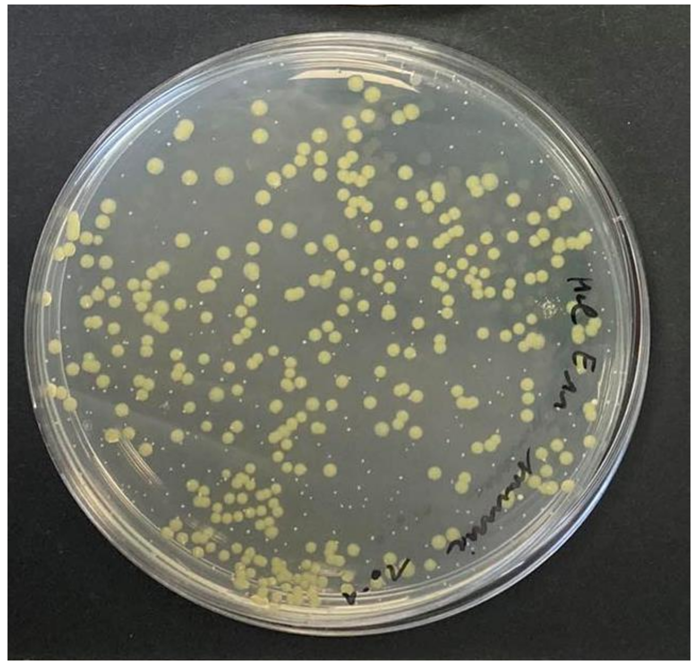


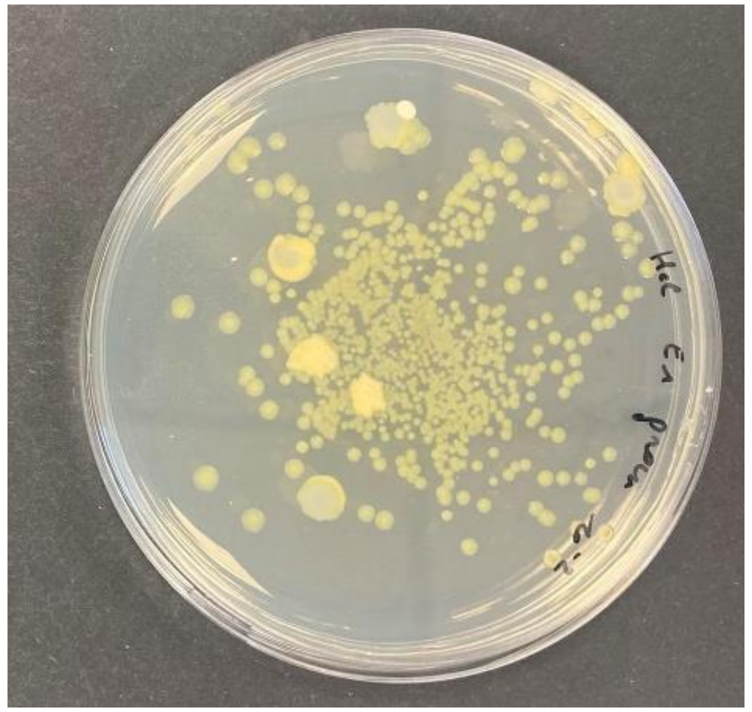
ILVO Institute for Agricultural and Fisheries Research
UNIVERSITEIT GENT
viaverda

Helleborus

Bacteria

- Bevestigd:
  - *Luteibacter*
  - *Paenibacillus*
  - *Pseudomonas*
  - *Kocuria*

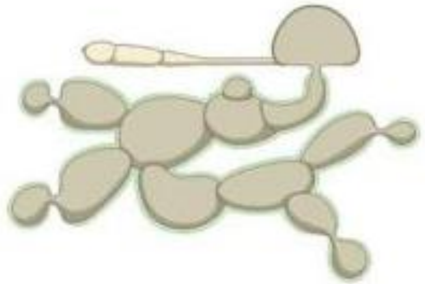




ILVO Institute for Agricultural and Fisheries Research
UNIVERSITEIT GENT
viaverda

Malus

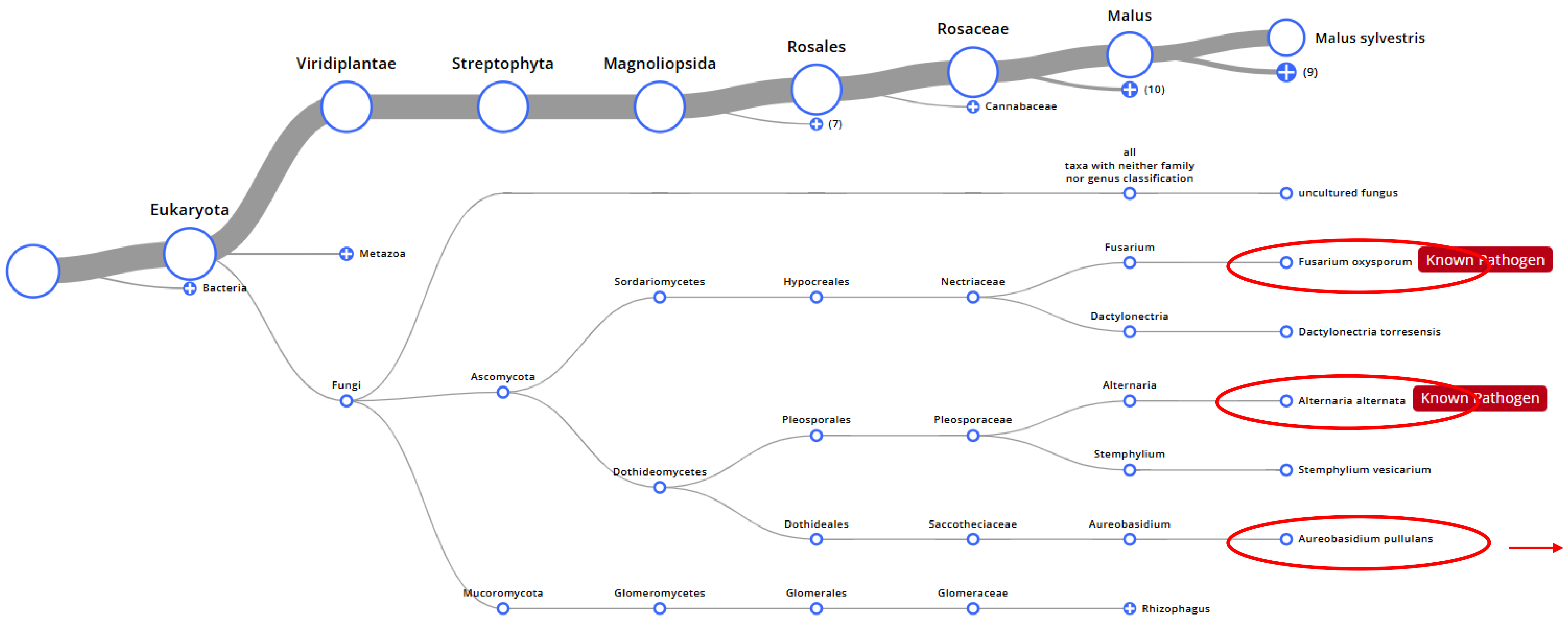
Fungi



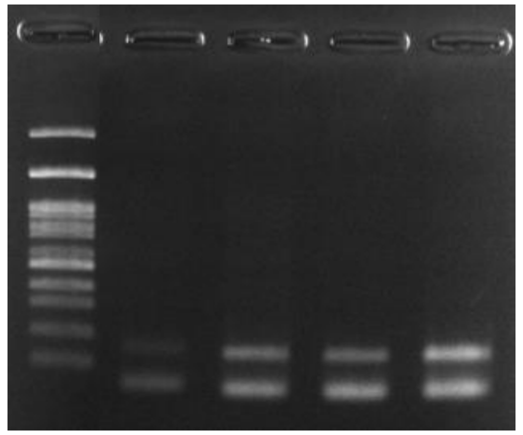
NT rPM >= 100 ✕

Report values are computed from 1.000.000 unique reads subsampled randomly from the 13.424.100 reads passing host and quality filters.

113 rows passing the above filters, out of 10801 total rows CLEAR FILTERS



PCR *A. pullulans*



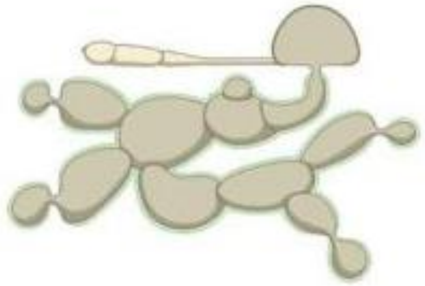
ILVO Institute for Agricultural and Fisheries Research

UNIVERSITEIT GENT

viaverda

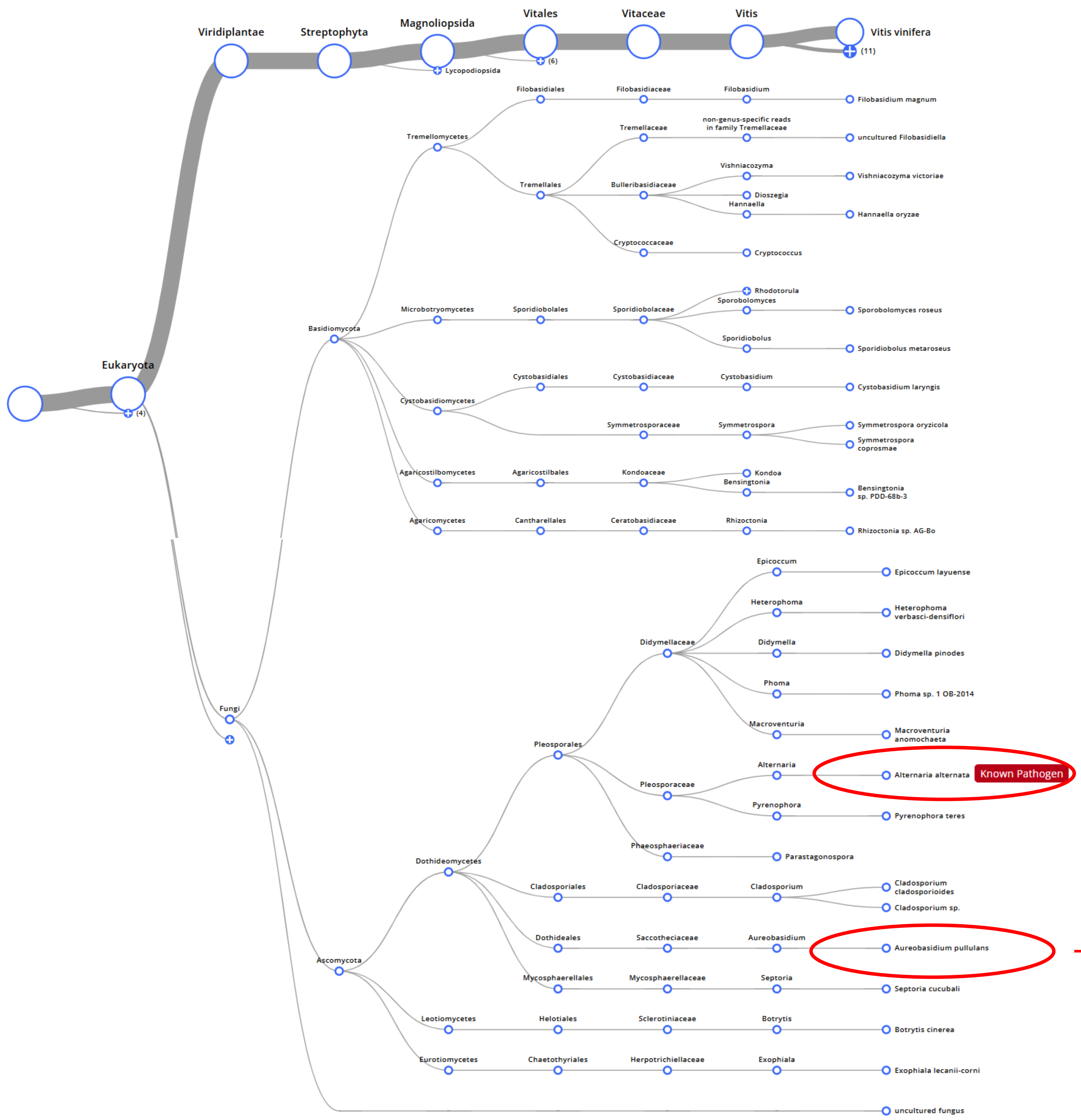
Vitis

Fungi

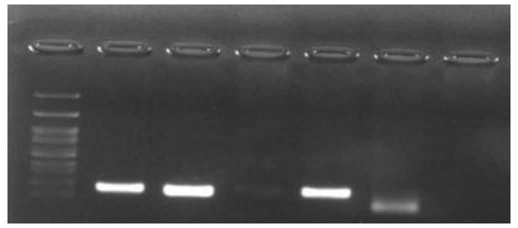


NT rPM >= 100 ✕

Report values are computed from 1.000.000 unique reads subsampled randomly from the 14.441.988 reads passing host and quality filters.
113 rows passing the above filters, out of 9904 total rows CLEAR FILTERS



PCR *A. pullulans*



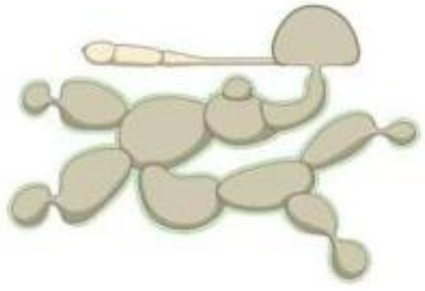
ILVO Institute for Agricultural and Fisheries Research

UNIVERSITEIT GENT

viaverda

Bamboe

Fungi

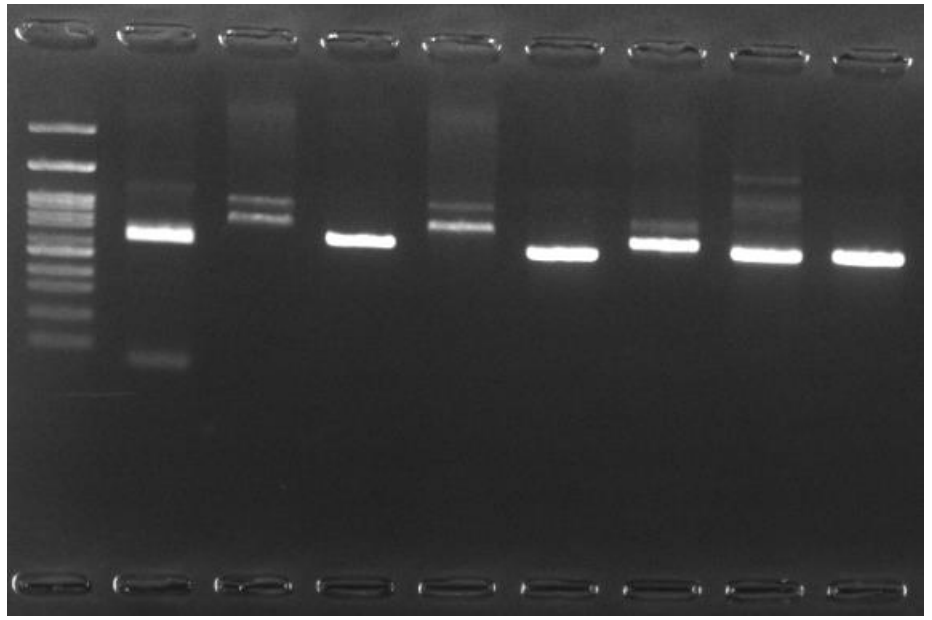


NT rPM >= 1000 ✕ Eukaryota ✕

Report values are computed from 6.413.034 reads (2.000.000 unique reads) subsampled randomly from the reads that passed host and quality filters.

90 rows passing the above filters, out of 8678 total rows CLEAR FILTERS

PCR *Sarocladium spp.* – ITS1F/ITS4



ILVO Institute for Agricultural and Fisheries Research
UNIVERSITEIT GENT
viaverda

Bedankt!

VRAGEN?

CONTACT:
Ine Dewitte – ine.dewitte@ilvo.vlaanderen.be

Kris De Jonghe – kris.dejonghe@ilvo.vlaanderen.be

Stefaan Werbrouck – stefaan.werbrouck@ugent.be

Dominique Van Haecke - dominique.vanhaecke@viaverda.be

Institute for Agricultural
and Fisheries Research (ILVO)
Burg. Van Gansberghelaan 96
9820 Merelbeke – Belgium
T + 32 (0)9 272 24 00

plant@ilvo.vlaanderen.be
www.ilvo.vlaanderen.be