The latest generation of molecular tools for cranberry: long-range PCR and long-read sequencing to detect fungicide resistance

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apcq Association des Producteurs de Canneberges du Québec

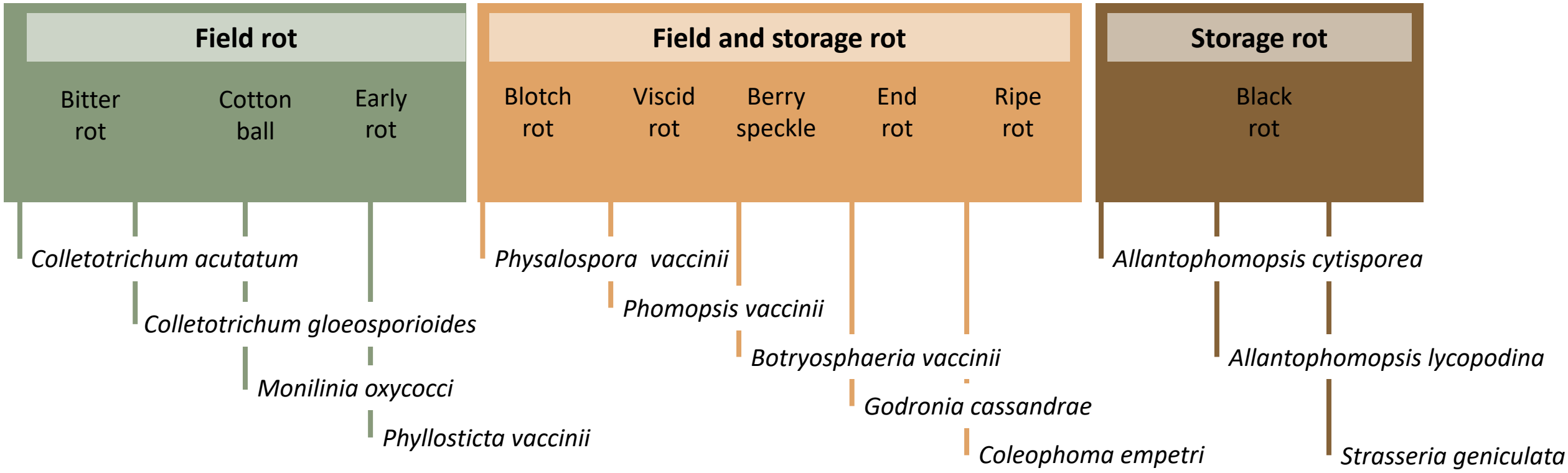
UNIVERSITÉ LAVAL

CRIV

Introduction

Cranberry fruit rot

Species and diseases in field and storage rot[1]



[1] Polashock et *al.*, 2017

Fungicides used in the cranberry industry

Strobilurins = Quinone outside Inhibitors (QoIs)[1, 2]

(Fungicide Resistance Action Committee (FRAC) group 11)

Azoxystrobin
*(**Quadris,** Abound, Aframe, Satori, Azoxy, Azoshy)*

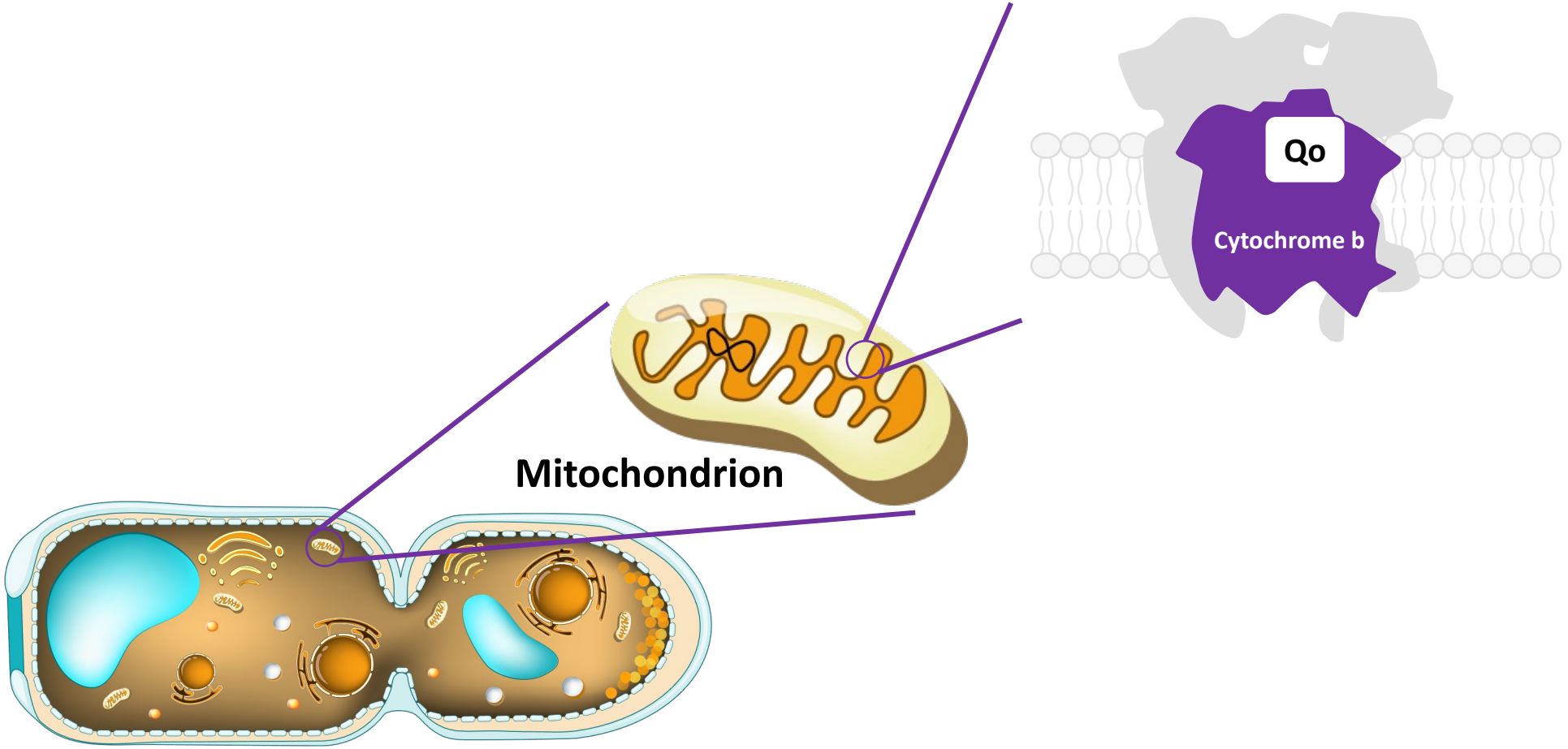
Fluoxastrobin
(Evito, Aftershock)

[1] FRAC, 2022

[2] Wells et al., 2014

Genetic mechanisms of fungicide resistance

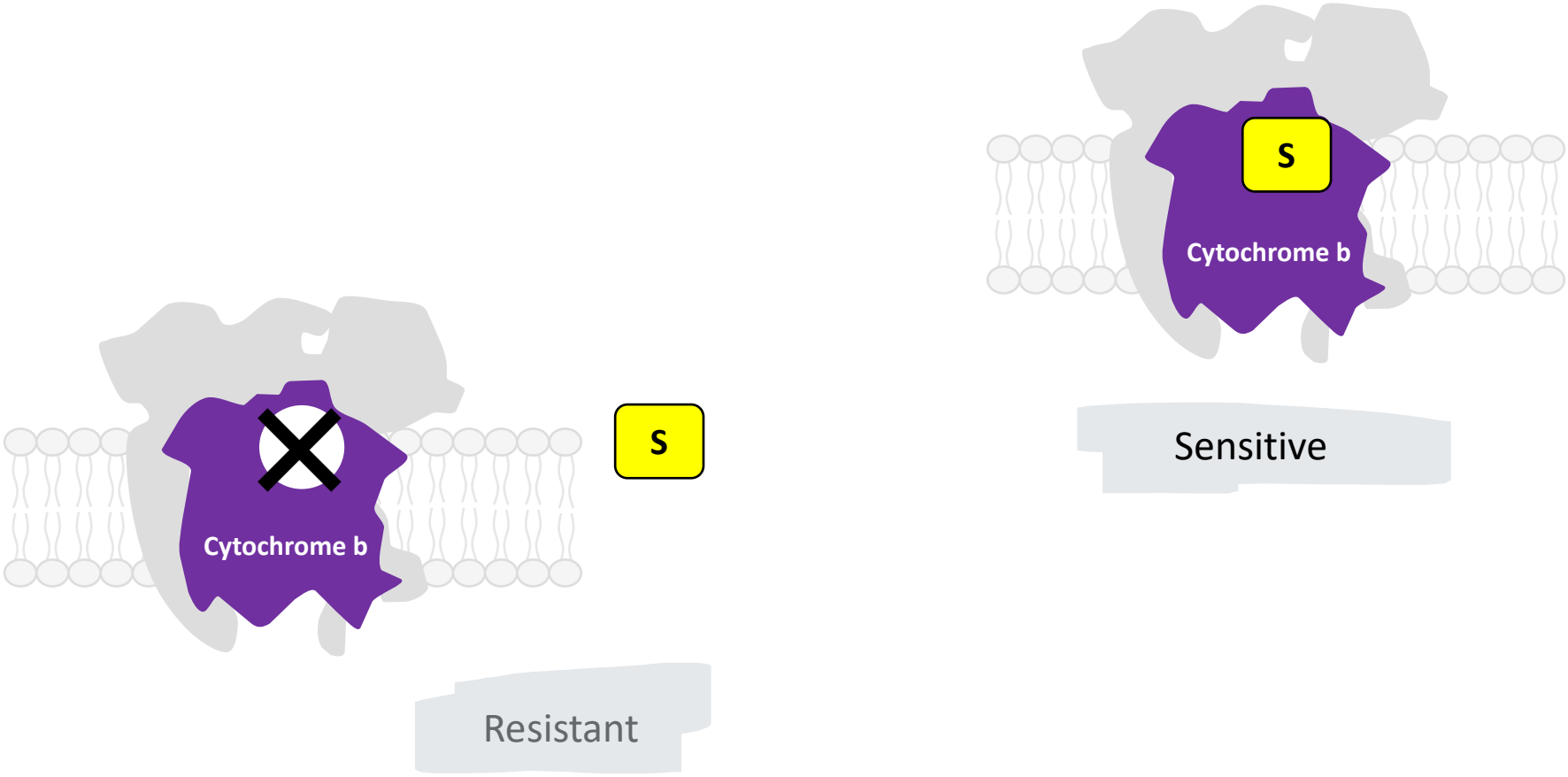
Mode of action[1]: **S** Strobilurins



[1] FRAC, 2022

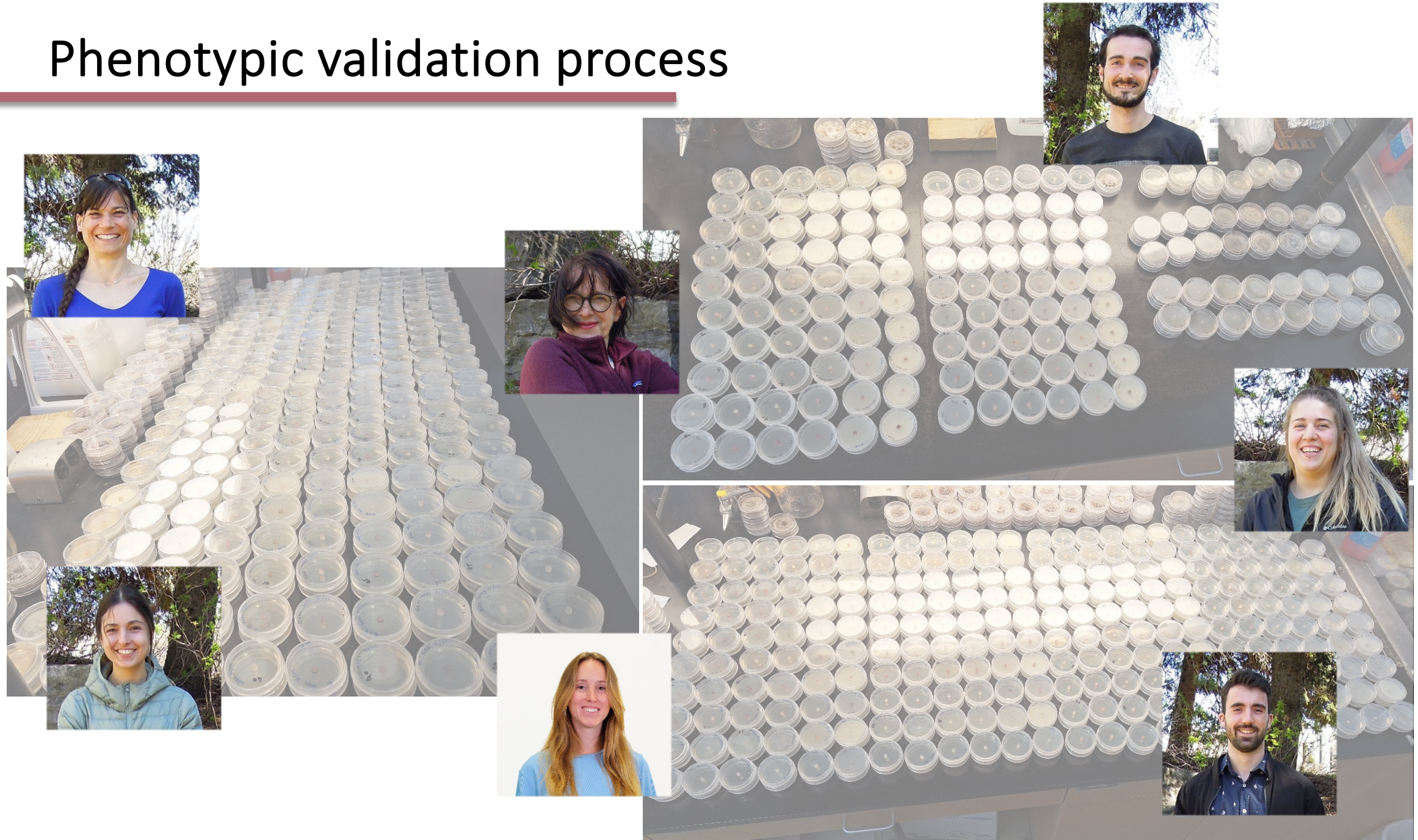
Genetic mechanisms of fungicide resistance

Mode of action[1]:

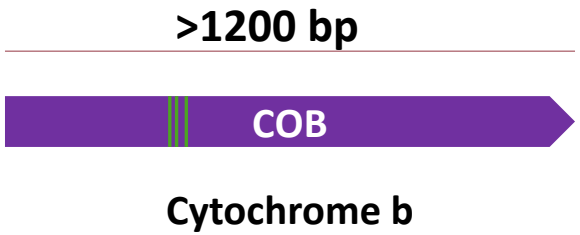


[1] FRAC, 2022

Phenotypic validation process



Genetic mechanisms of fungicide resistance



QoI resistance

In fungi

The main mutation involved[1]:

G143A

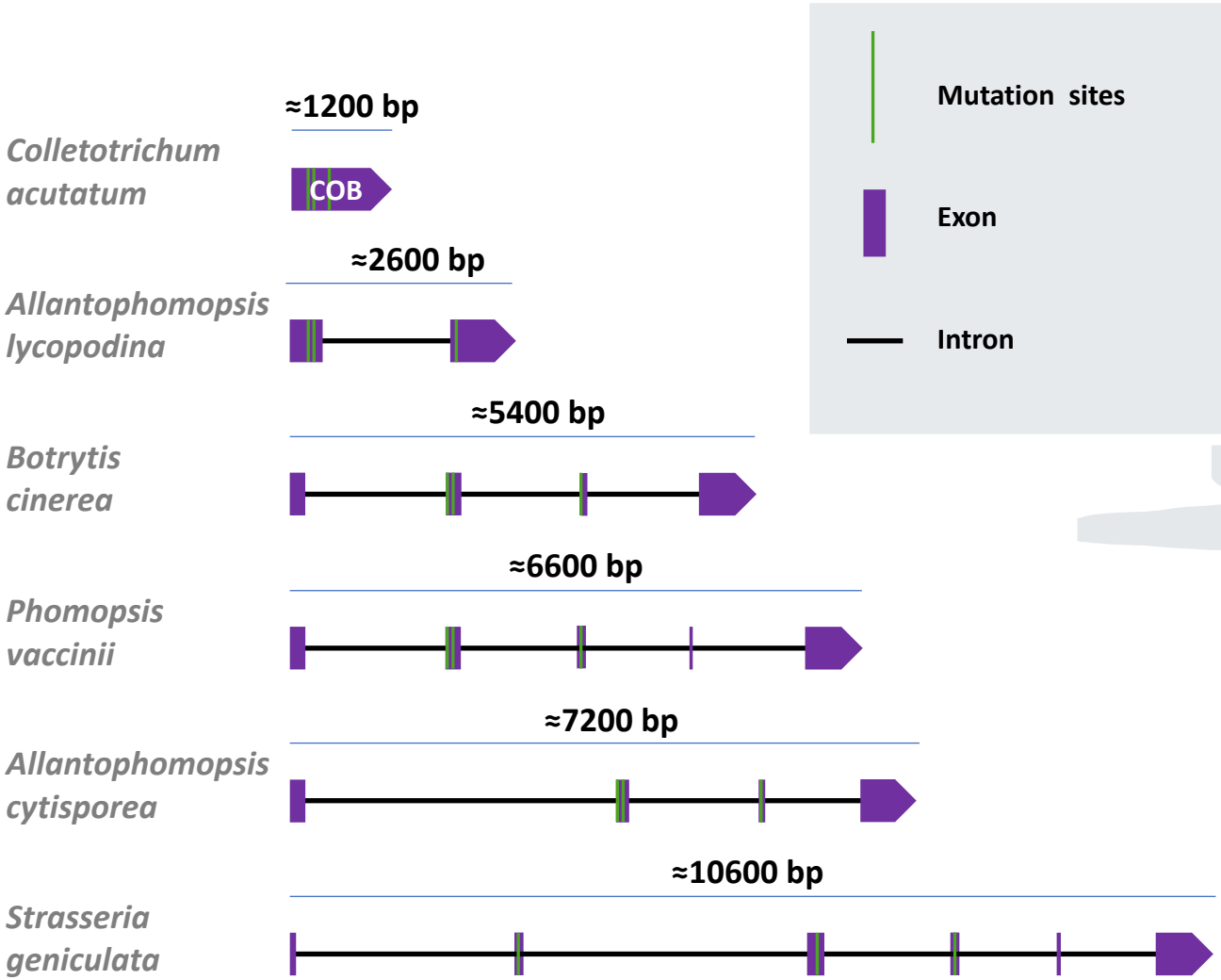
2 other mutations involved[1]:

F129L

G137R

[1] Fisher *et al.*, 2021

Genetic mechanisms of fungicide resistance



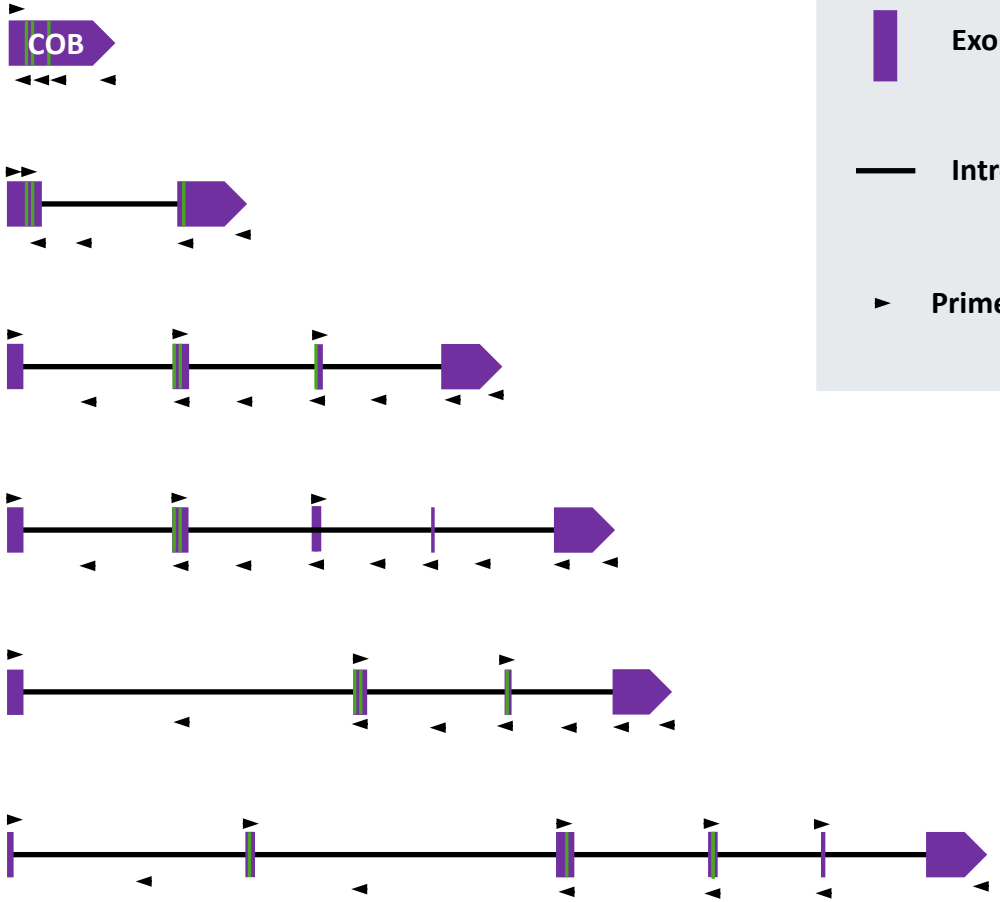
High intron variability in COB gene

- Impacts the development of resistant mutations
- Requires the use of many primer pairs

Cinget and Bélanger, 2020

Current challenge

Current method to capture fungicide resistance mutations

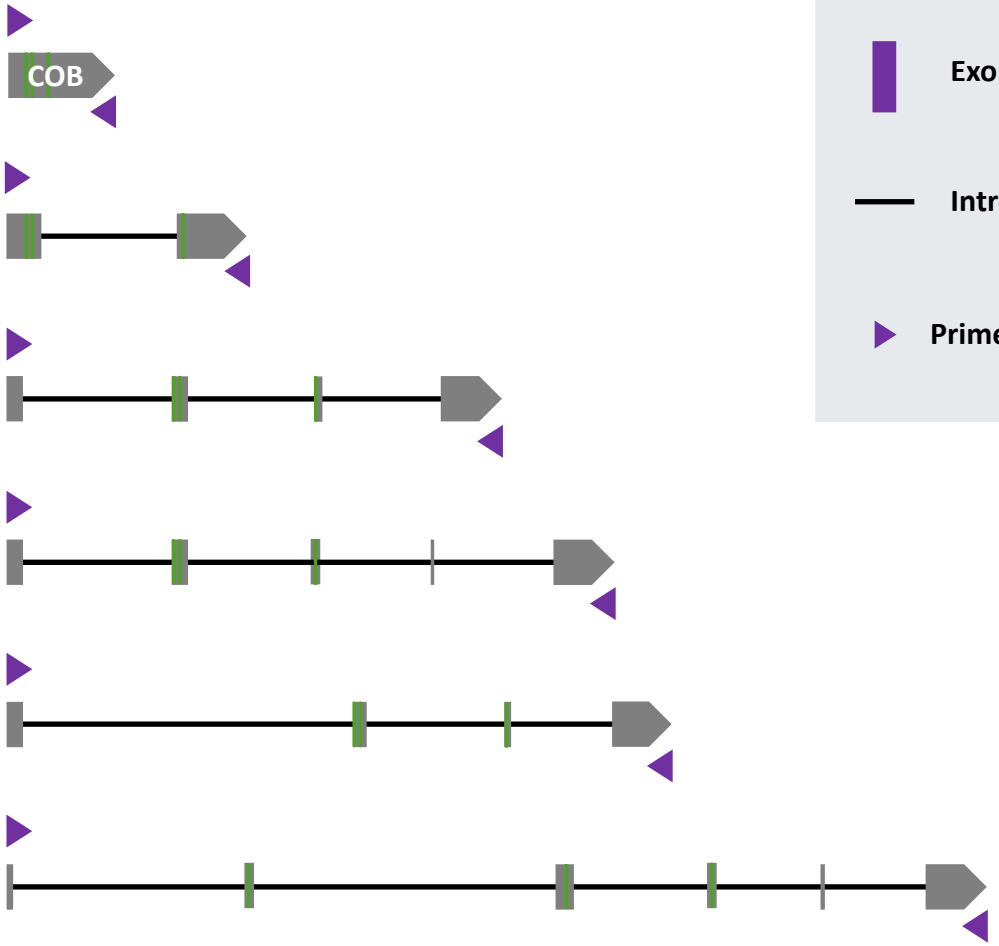


2 primers per known mutation
x (3 COB mutations)
X 12 species

=

> 72 primers to be designed
+
36 PCR reactions per sample

New approach



Mutation sites

Exon

Intron

Primer

2 gene-specific primers **BUT** universal for all 12 species

Advantages:

- ✓ Allows to obtain the gene sequence and its structure
- ✓ Captures diversity within each species

Hypothesis & Objectives

Hypothesis

Target gene sequencing allows to identify and predict fungicide resistance of the 12 main fungal species involved in cranberry fruit rot, simultaneously.

Hypothesis & Objectives

- **Objective 1**: Design universal primers for resistance gene.
- **Objective 2**: Build gene sequence reference bank for resistance gene.
- **Objective 3**: Develop a bioinformatic pipeline for processing sequenced data.

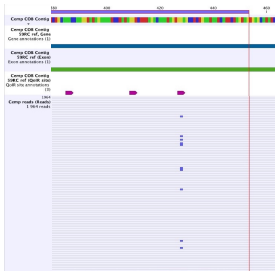
Fungicide resistance diagnosis

1. Fungal samples sourced

2. DNA extraction

3. Resistance gene targeting via PCR

4. Resistance gene sequencing



5. Data analysis

6. Sequencing report

Fungal species identification
Fungicide resistance profile

Fungicide resistance diagnostic workflow

Results

Photo: J. Moreau

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Fungicide resistance diagnostic workflow

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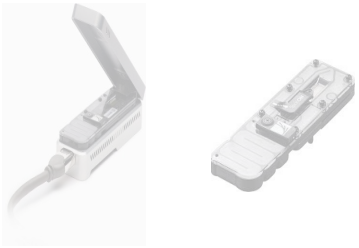

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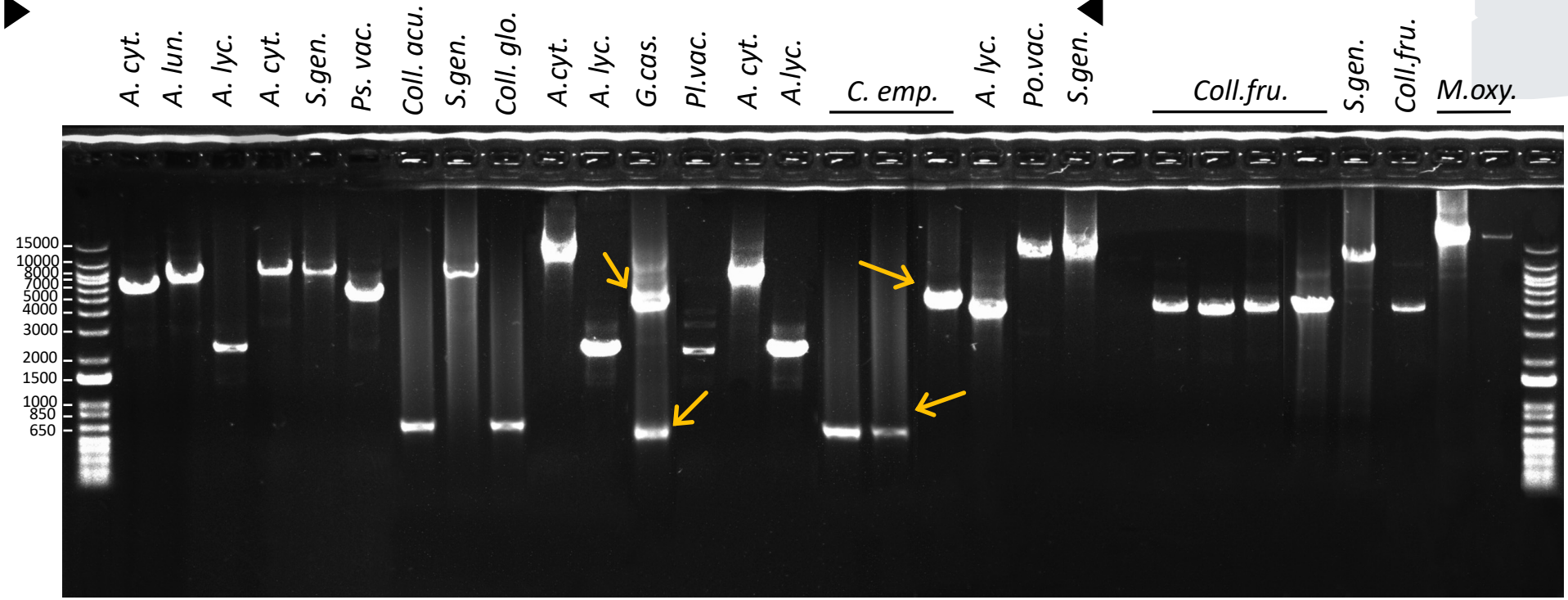
Design of gene specific primers : COB (Cytochrome b)

- **Primers**

- ✓ 2 primers only
- ✓ 13 species
- ✓ 9 genera



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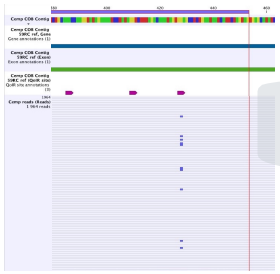

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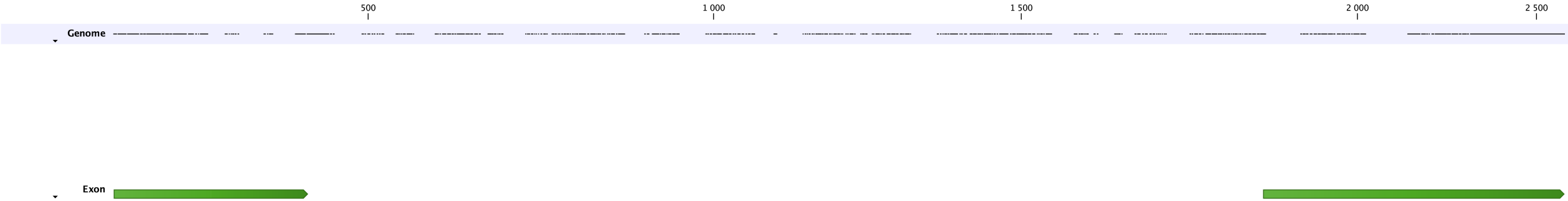

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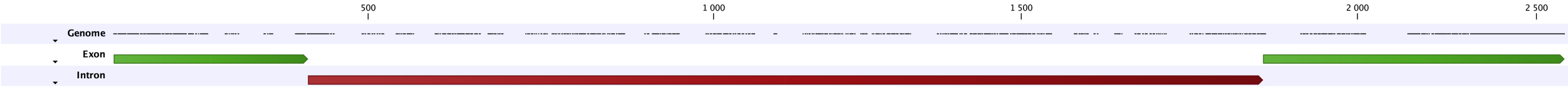
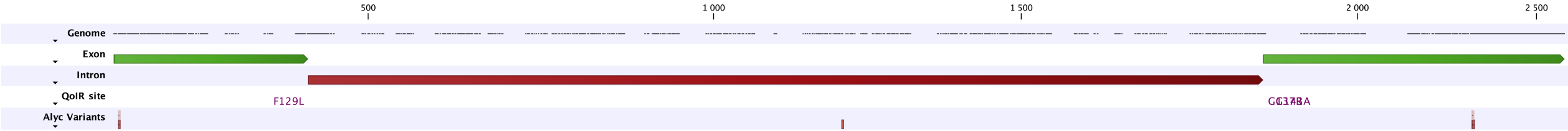


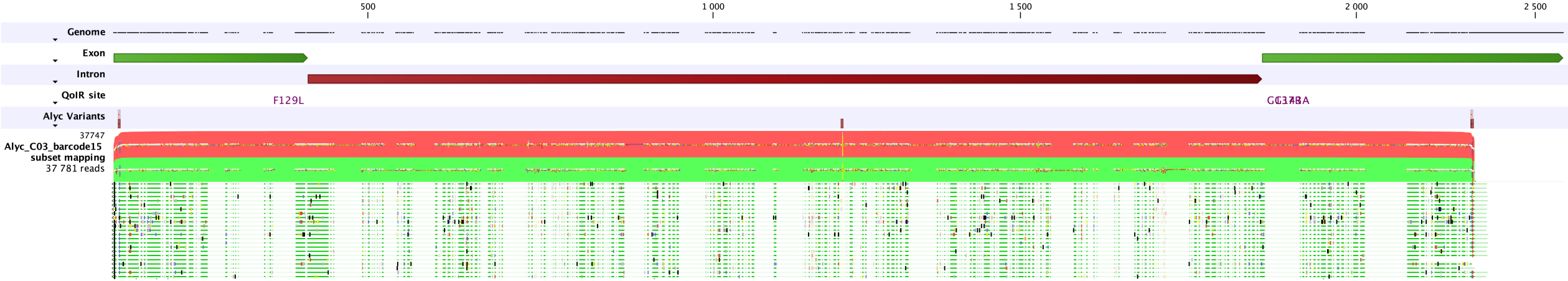
Results


500
1 000
1 500
2 000
2 500
Genome
Exon
Intron
QoIR site
F129L
G137A

Results

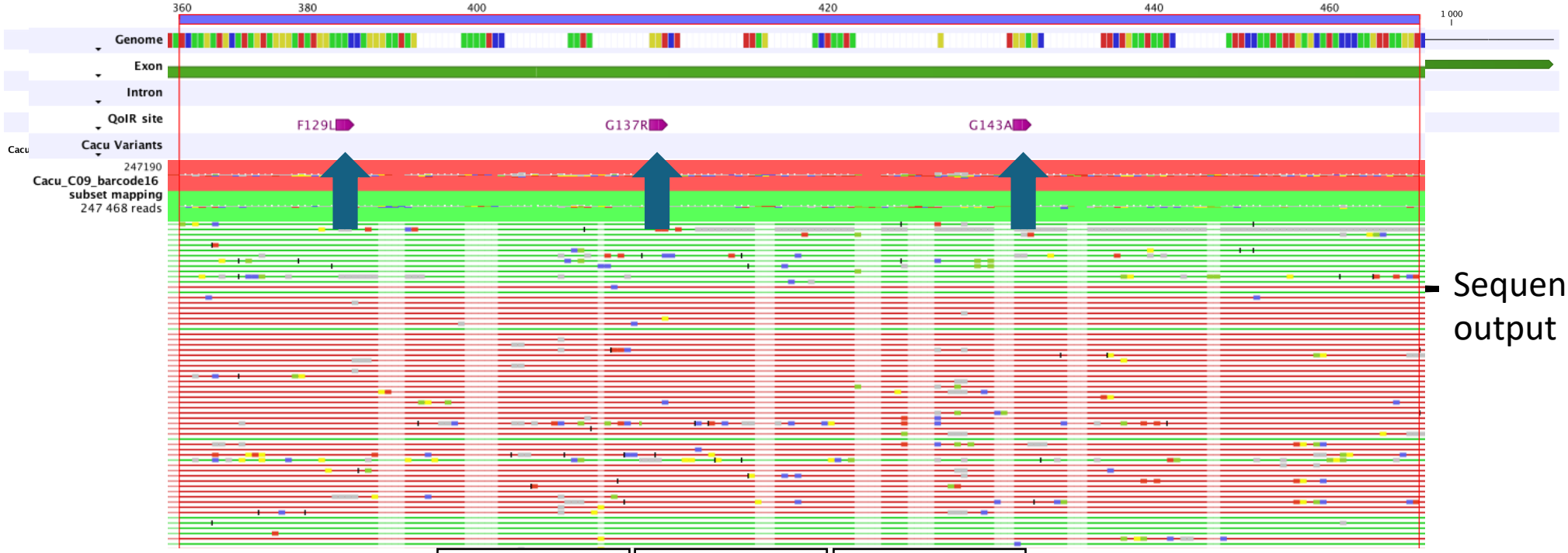

500
1 000
1 500
2 000
2 500
Genome
Exon
Intron
QoIR site
F129L
G137A
Alyc Variants

Results


500
1 000
1 500
2 000
2 500
Genome
Exon
Intron
QoIR site
F129L
G137A
Alyc Variants
37747
Alyc_C03_barcode15
subset mapping
37 781 reads

Results

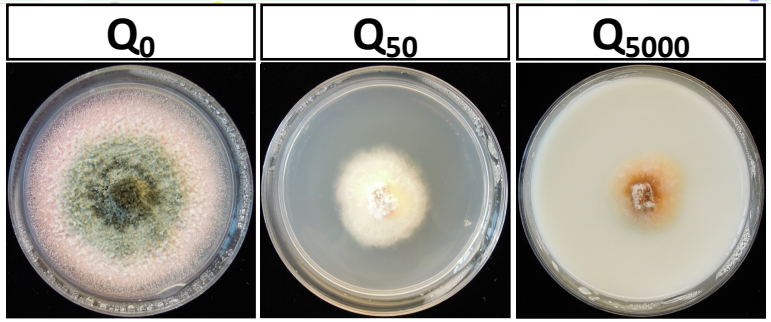
Colletotrichum acutatum (DAOM:214992)



Reference sequence

Sequencing output

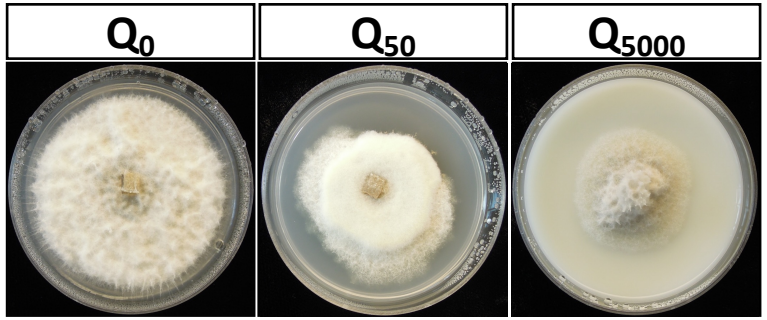
Growing time = 11 days



Results

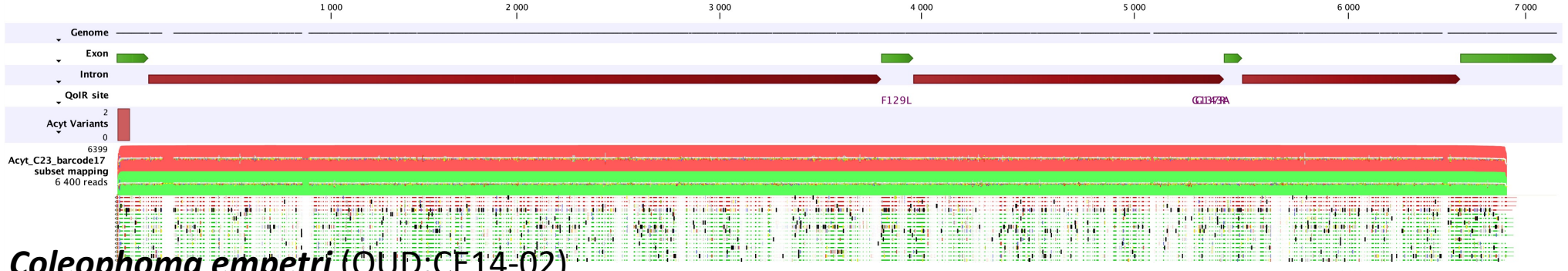
Colletotrichum fructivorum (OUD:GL20-02)

Growing time = 11 days

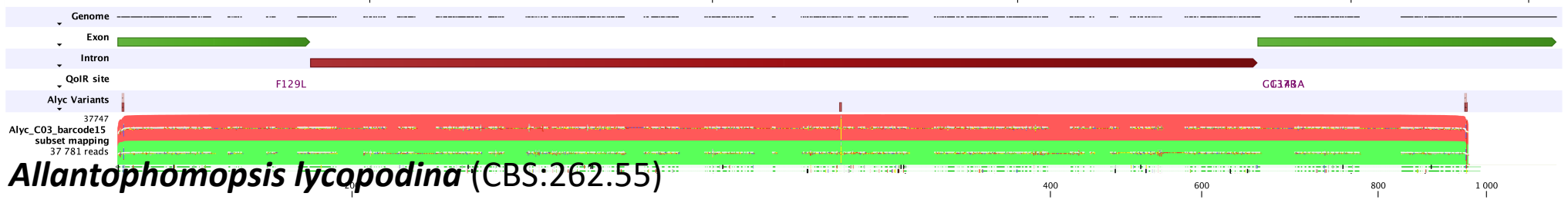


Results

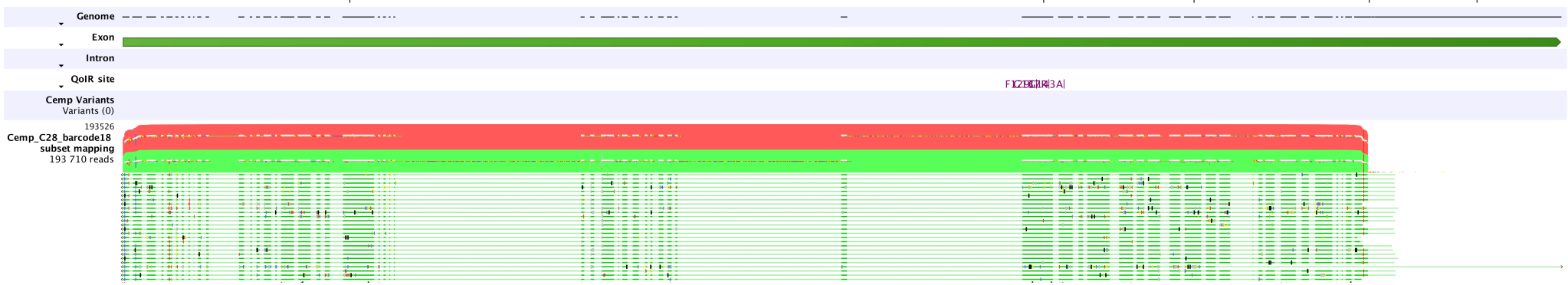
Allantophomopsis cytisporea (ATCC:66955)



Coleophoma empetri (QUD:CE14-02)



Allantophomopsis lycopodina (CBS:262.55)



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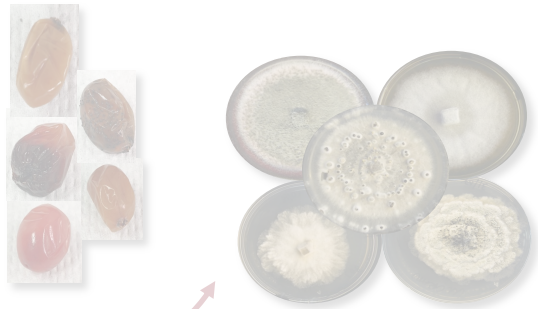

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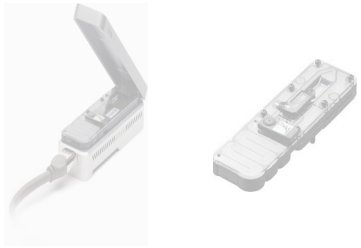

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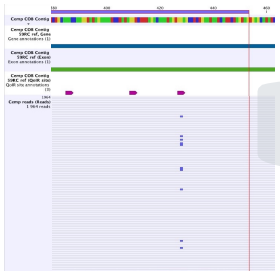

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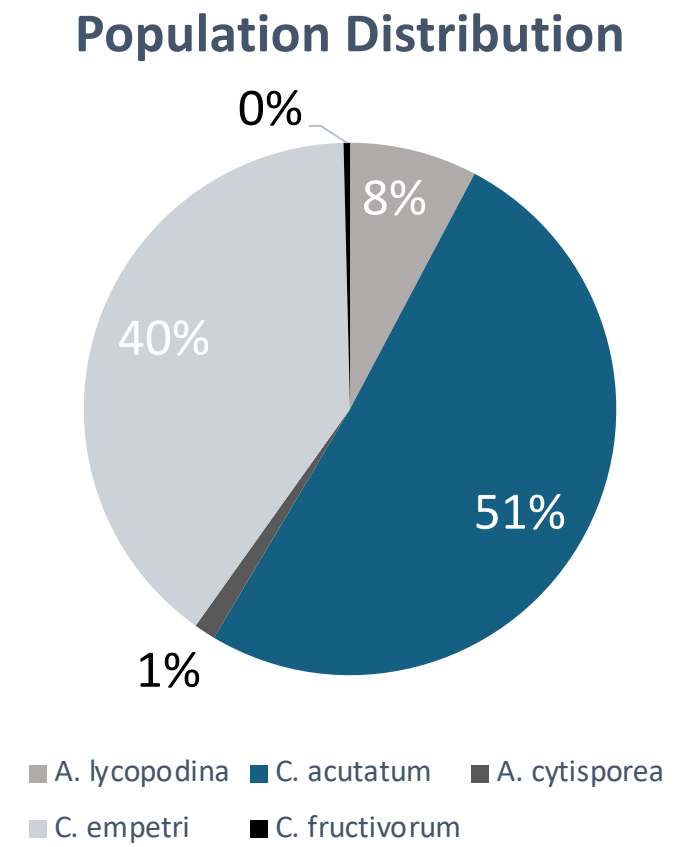
Application

Species Identified	Number of reads	Correctly Mapped Reads	Wildtype reads	COB mutated reads	COB mutation Rate (%)
Allantophomopsis lycopodina	38 541	37 781	37 037	744	1.97
Colletotrichum acutatum	260 946	247 468	247 394	74	0.03
Allantophomopsis cytisporea	6402	6400	6069	331	5.17
Coleophoma empetri	206 792	193 710	181 448	12 262	6.33
Colletotrichum fructivorum	1917	1907	8	1 899	99.58
Unknown Species	27 330				
Total	**514 598**				



Conclusion

→ Target gene sequencing allowed to identify and predict fungicide resistance of the 12 main fungal species involved in cranberry fruit rot.

→ Application is universal, no matter the gene structure

→ ↓ Labour, ↓ cost, ↑ time effectiveness

→ Possible expansion: fungi, fungicide resistance genes

→ Better management strategies

Thank You

Richard Bélanger

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Yannick Asselin
Vanessa D'amour
Camille Gagnon