
EPFL
ViTEST

The Origin of the Project

Maxime Guérinot
Professional winegrower

Flavescence Dorée

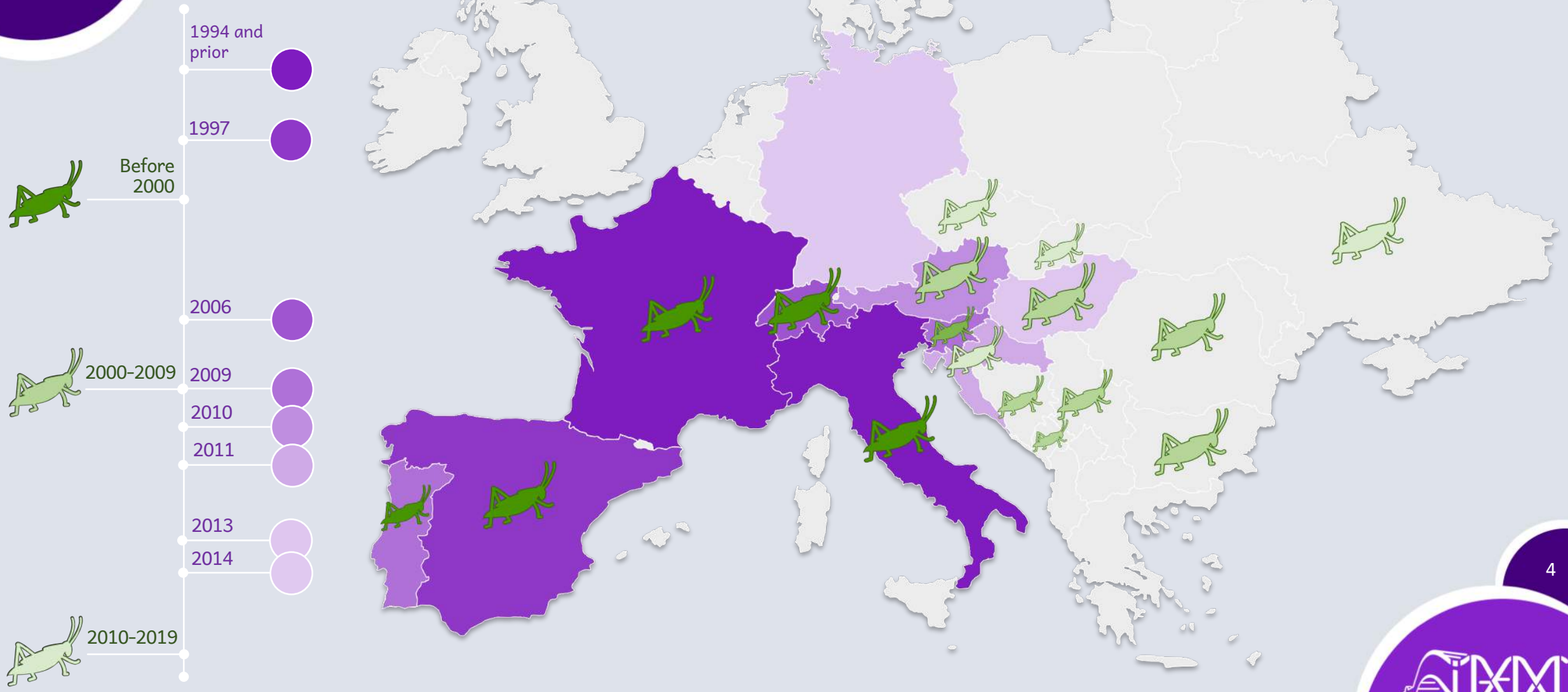
Flavescence Dorée symptoms on grapevine

QUARANTINE

- Deadly
- No cure
- Highly contagious

A growing problem in Europe

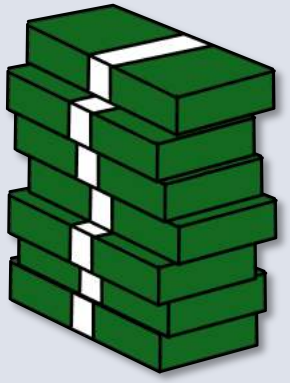
Source : EPPO reports



Timeline of 1ST reports of Flavescence Dorée and its vectors in Europe

An epidemic with far-reaching consequences

Economical



Ecological

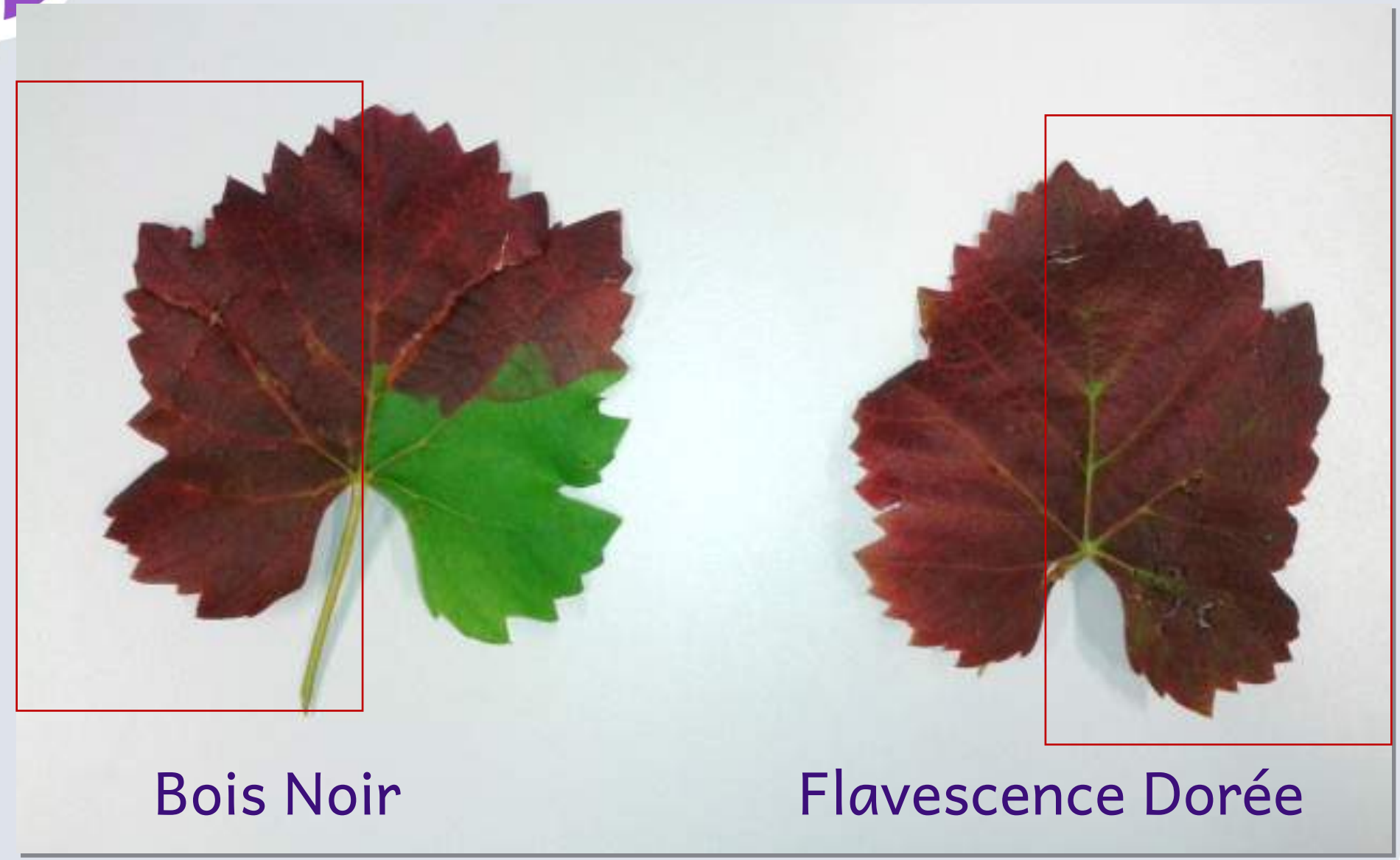
"Despite mandatory control in Europe for [flavescence dorée], it is still spreading and need permanent monitoring to detect new infected areas."

Source: http://www.winetwork-data.eu/intranet/libretti/0/libretto16625-01-1.pdf

Two diseases, same symptoms

Flavescence Dorée

Bois Noir

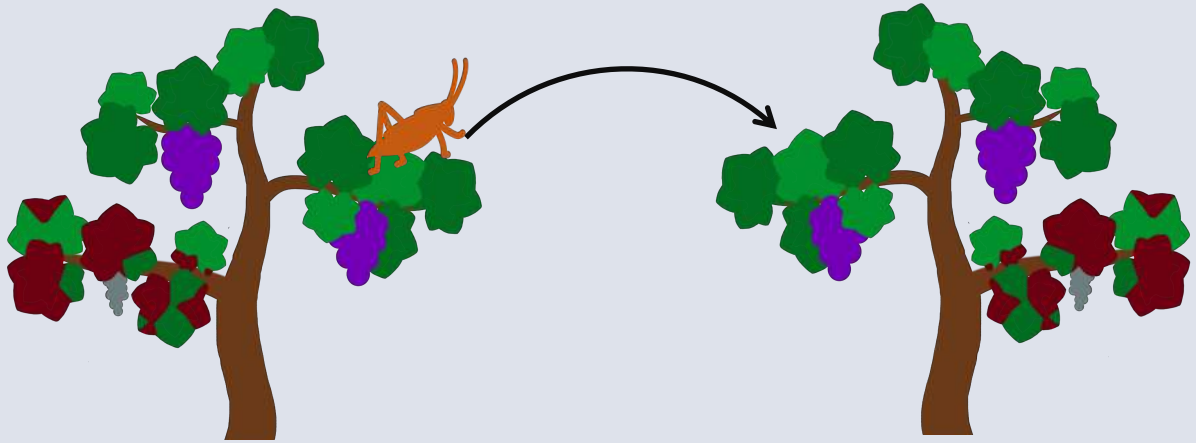


Bois Noir

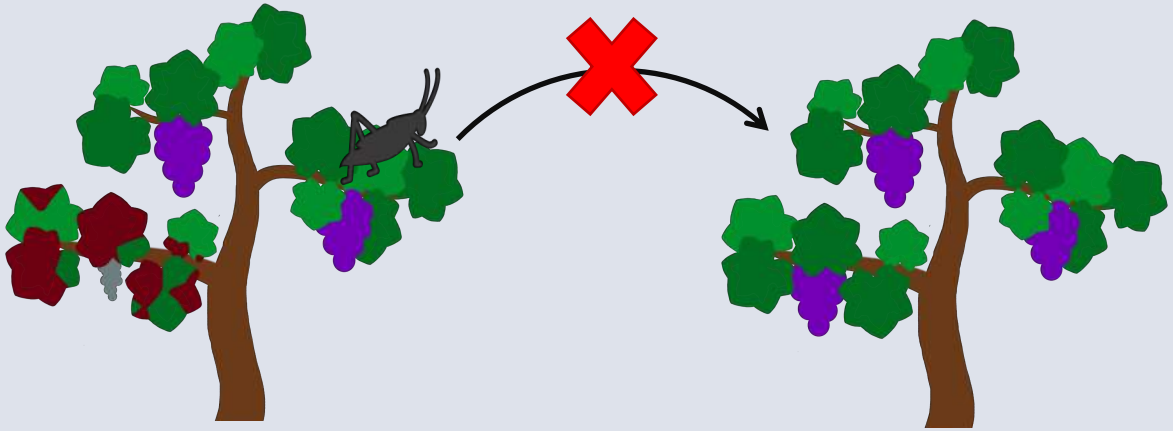
Flavescence Dorée

Disease transmission

Scaphoideus titanus



Hyalesthes obsoletus



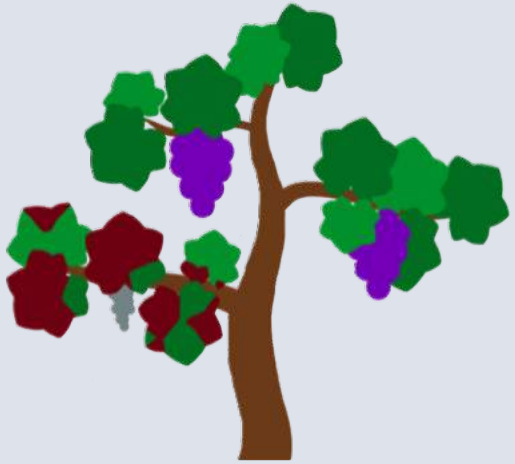
Interacting with local stakeholders

Winegrower

Phytosanitary police

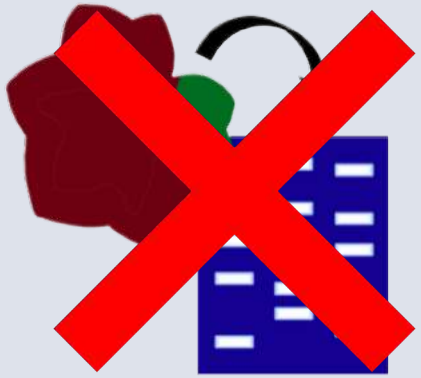
Today's diagnostic

1 WINEGROWER



2 PHYTOSANITARY POLICE

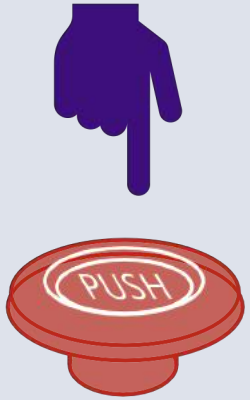
3 AGROSCOPE



4 WINEGROWER

2-5 weeks

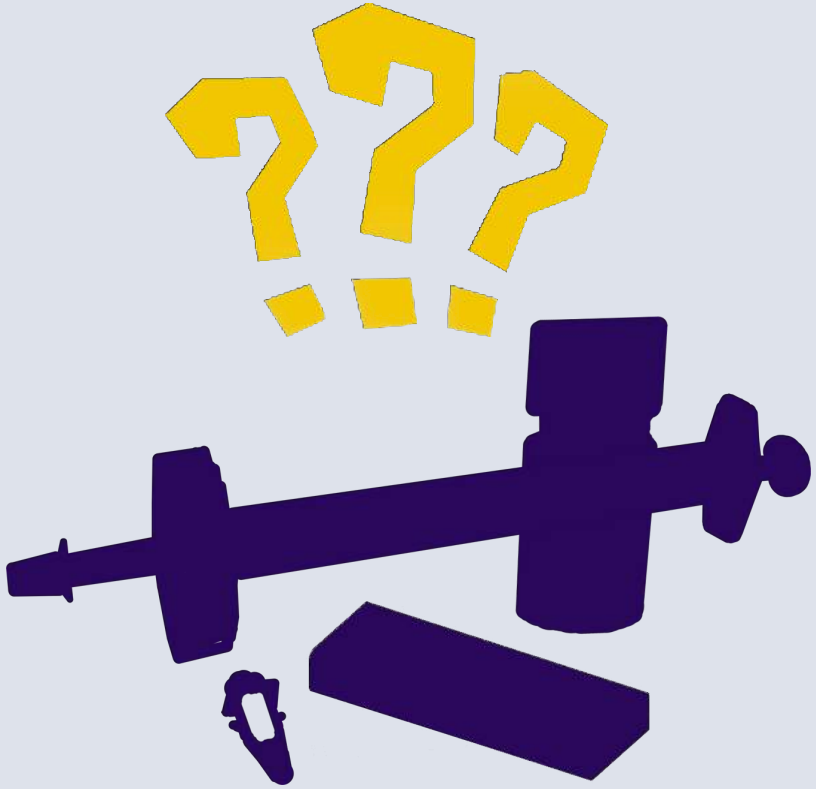
Our solution



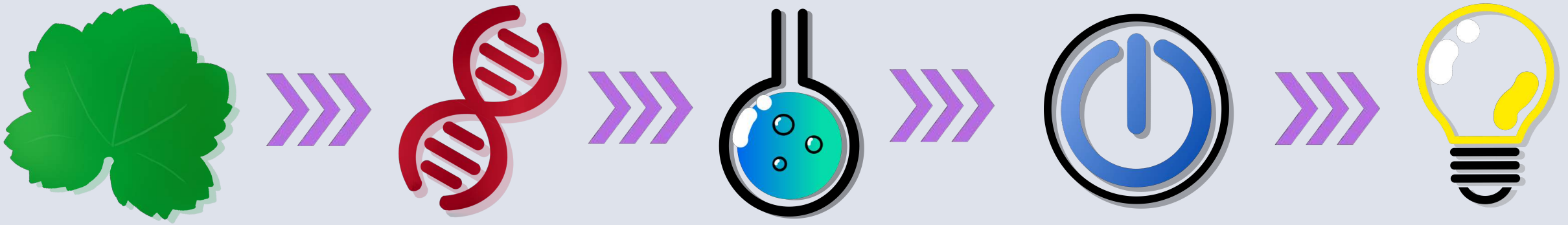
Easy-to-use

Fast

Field-based



Method



DNA Extraction

DNA Amplification

Expression System

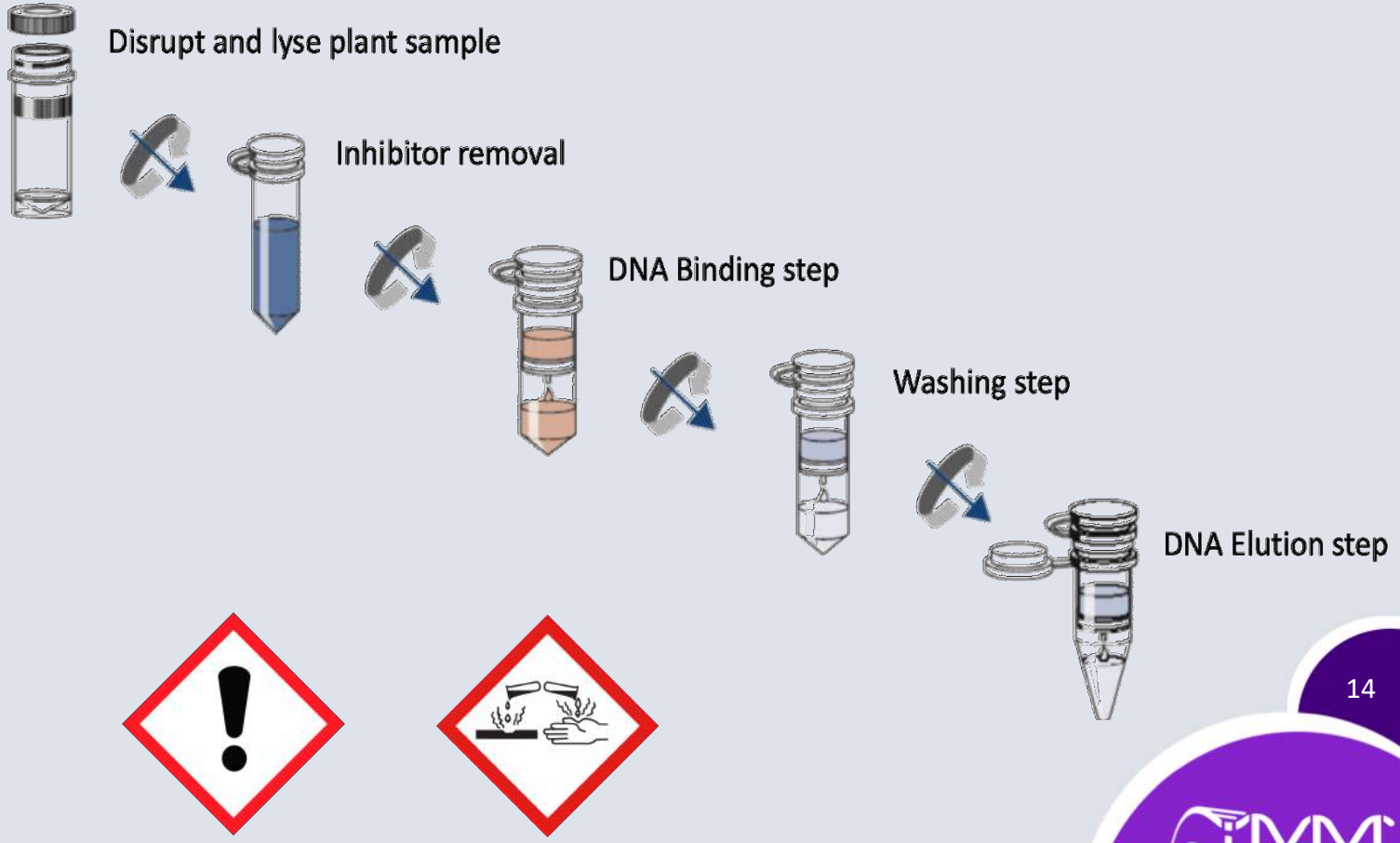
Toehold Switch

Signal Generation

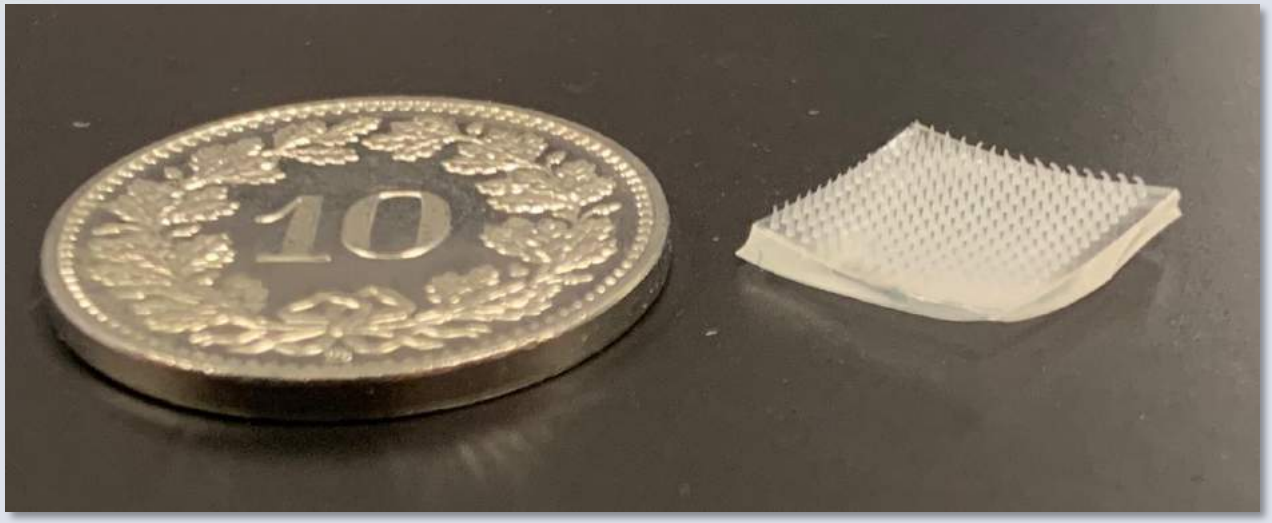
DNA extraction

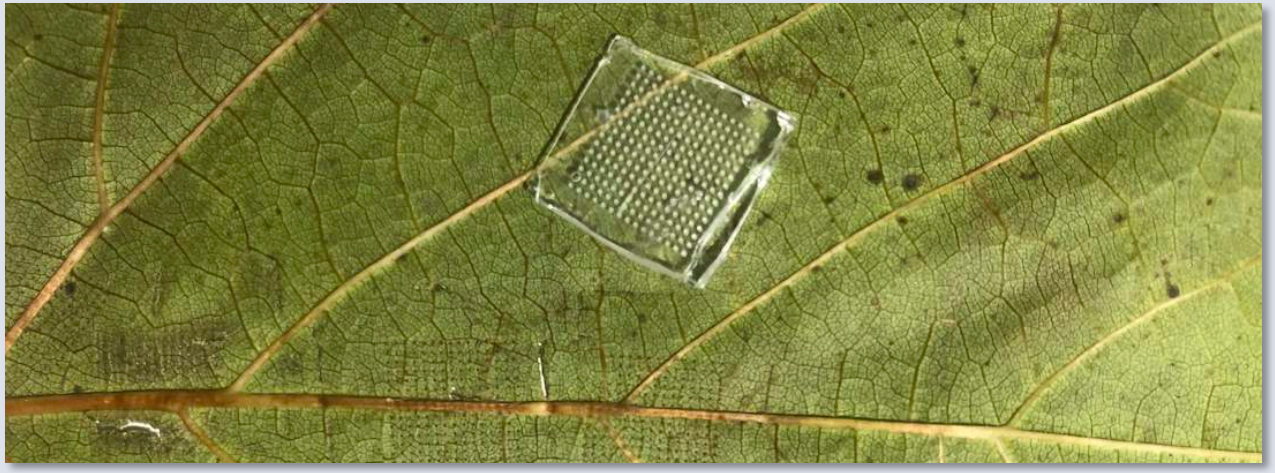
Conventional plant DNA extraction method

- Chemical cell lysis
- 3 hours
- Lab bound



DNA extraction using Microneedle patches



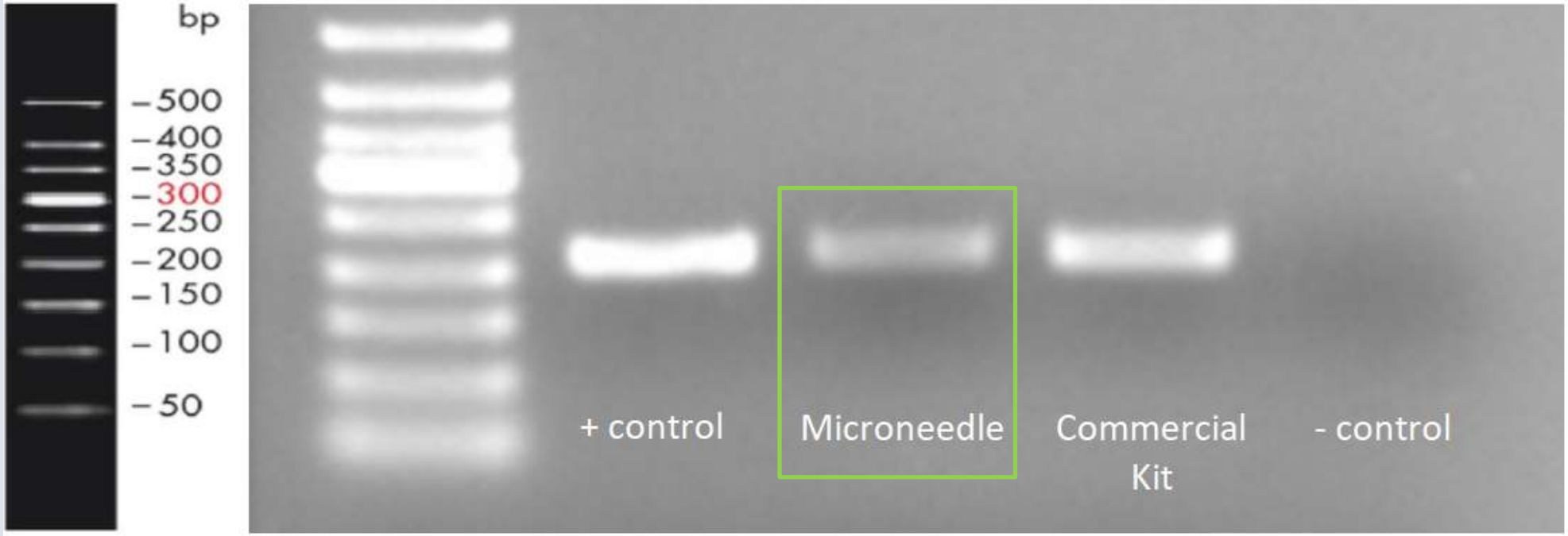


- No chemicals
- No DNA purification
- 1 minute

Prof. Zhen Gu

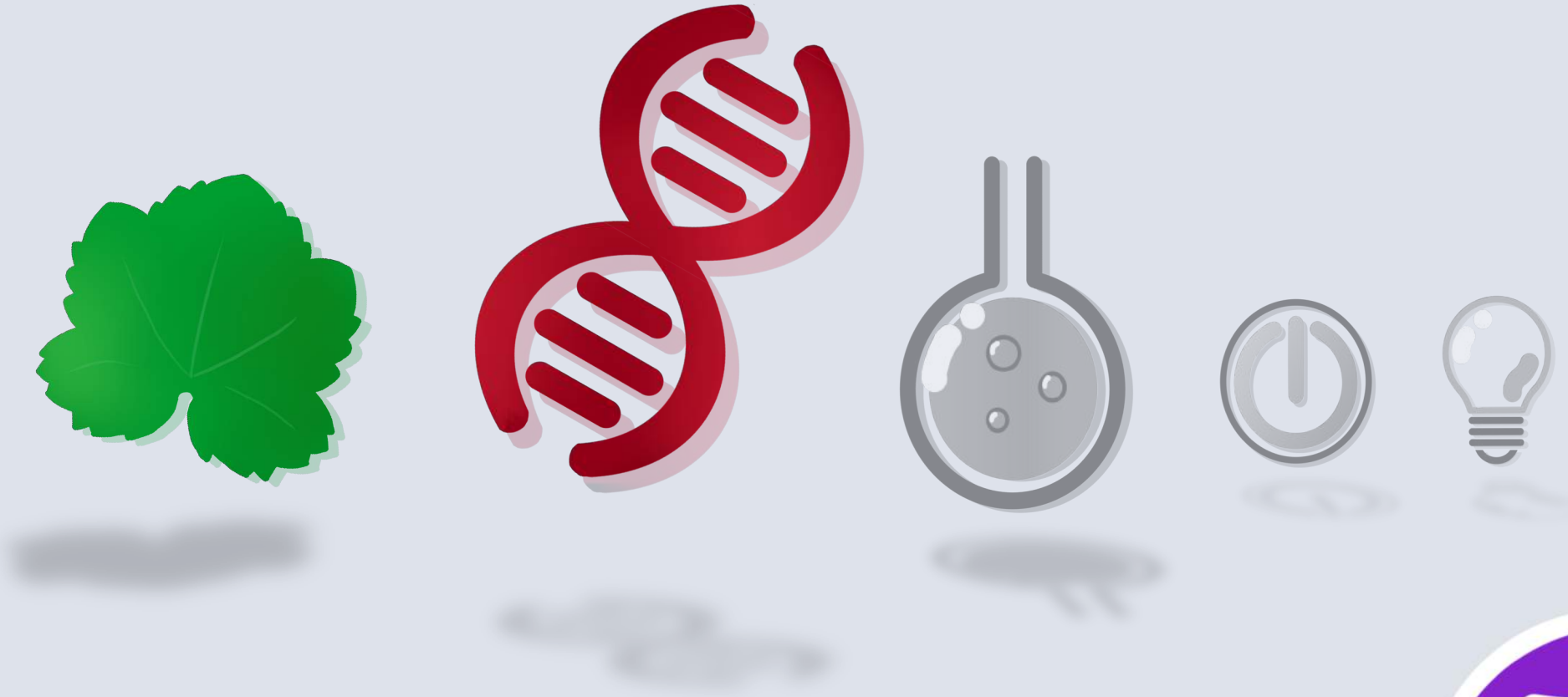
Rajesh Paul, et al, 2019.

Comparison of different DNA extraction techniques



Different extraction methods

DNA amplification

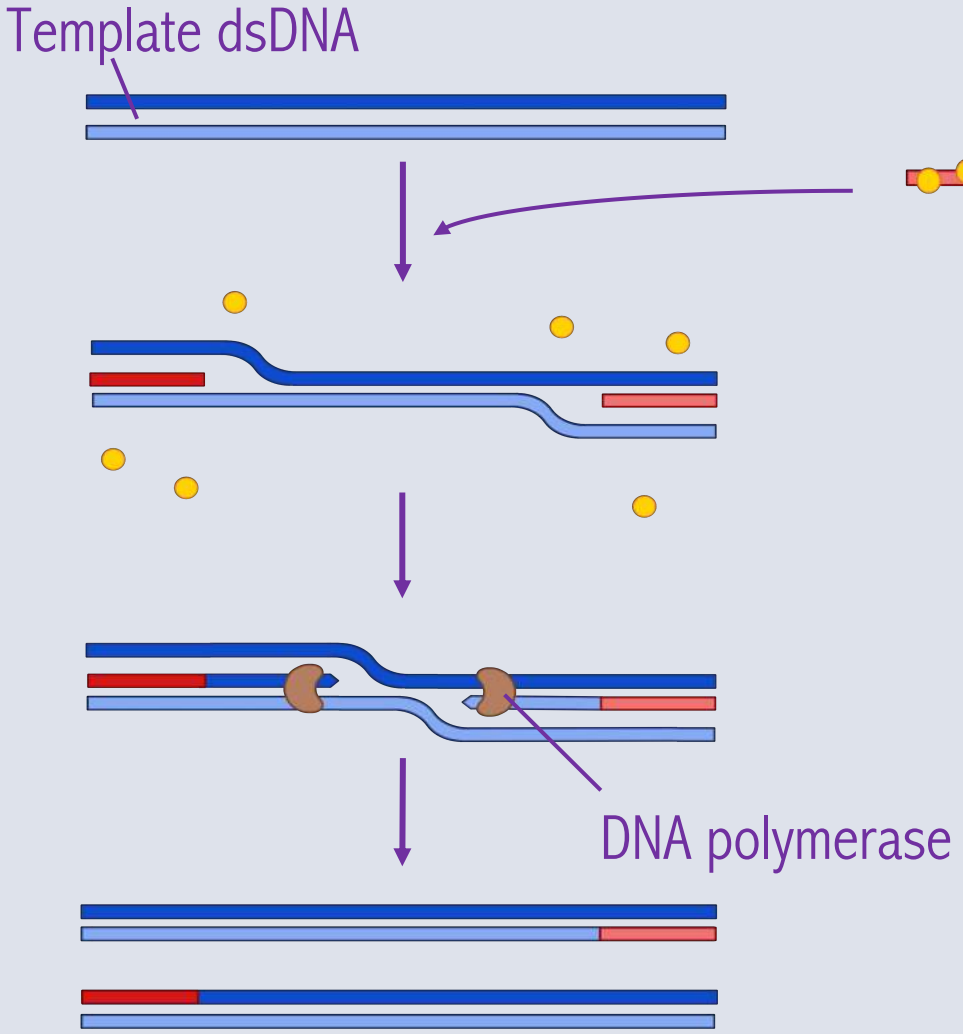


Choosing the Amplification Method

Loop Mediated Amplification (LAMP)

- Isothermal
- 60 min
- Not Multiplexable

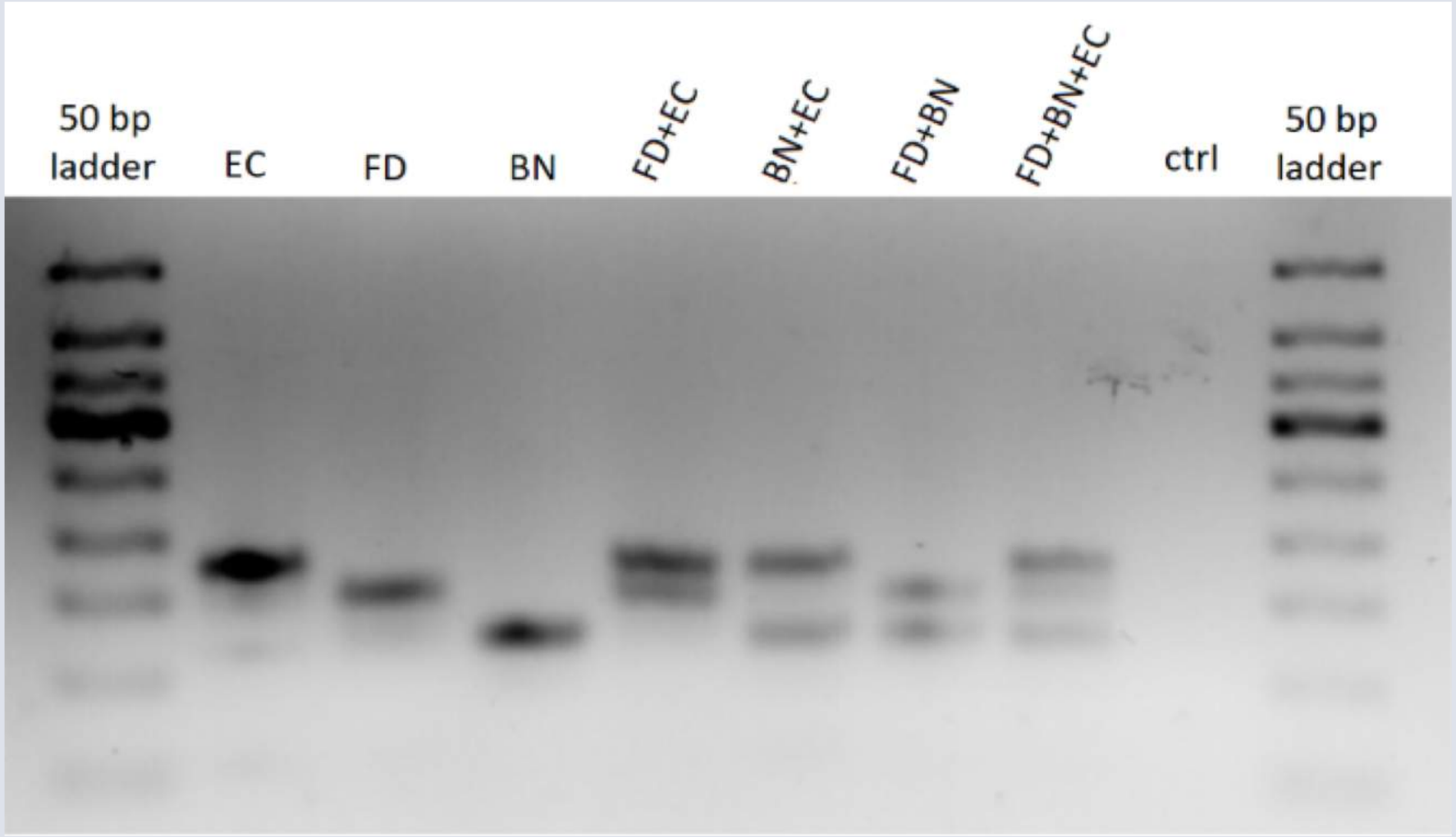
Recombinase Polymerase Amplification (RPA)



Adapted from Piepenburg, et al, 2006.

- Isothermal: 37°C
- Fast: 20 min
- Multiplexable

Simultaneous amplification of 3 DNA targets using RPA

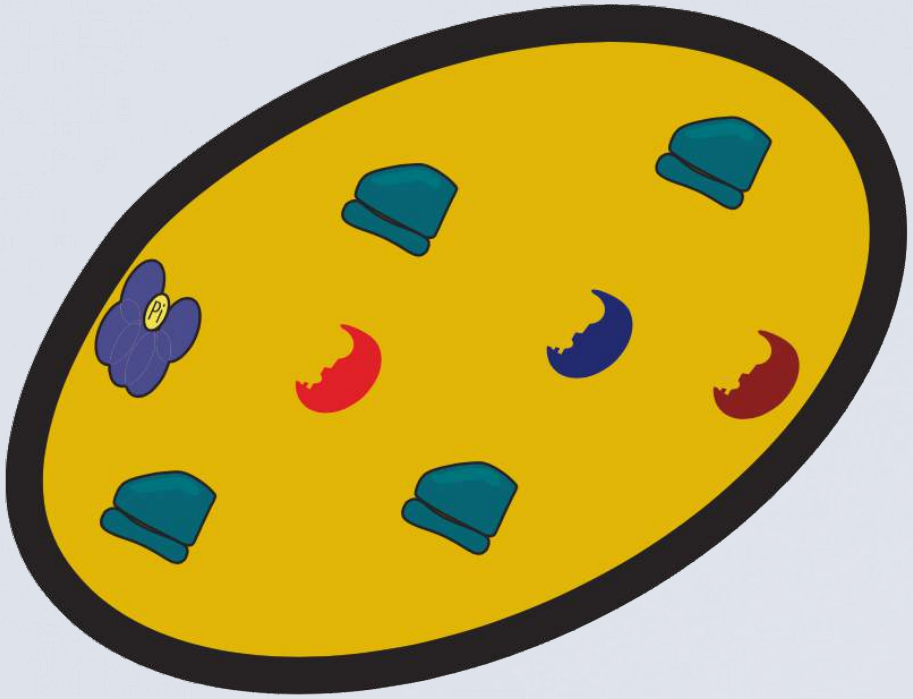


Agarose gel electrophoresis of multiplexed RPA reactions.

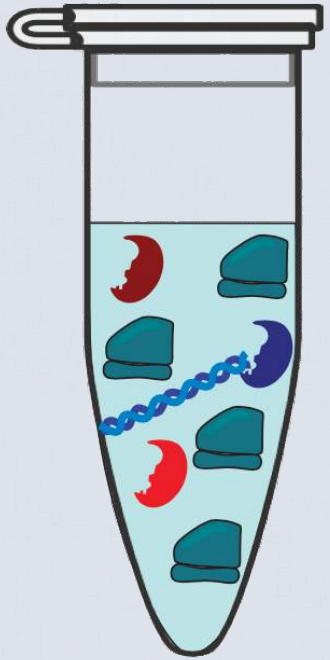
Expression system

Transcription-Translation System

Bacteria



Cell-Free system



Schemes from Lavickova Barbora

Transcription-Translation System

Bacteria

- Time consuming
- Complex
- Strict regulations

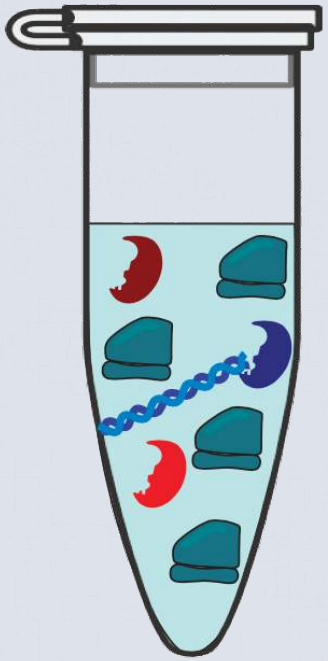
- $

Cell-Free system

- Quick
- Easy to use
- Field deployable

- $$$ (eg:PURExpress®)

In-house "OnePot" PURE



36 purified Proteins

Ribosomes

Energy solution

One co-culture

↓

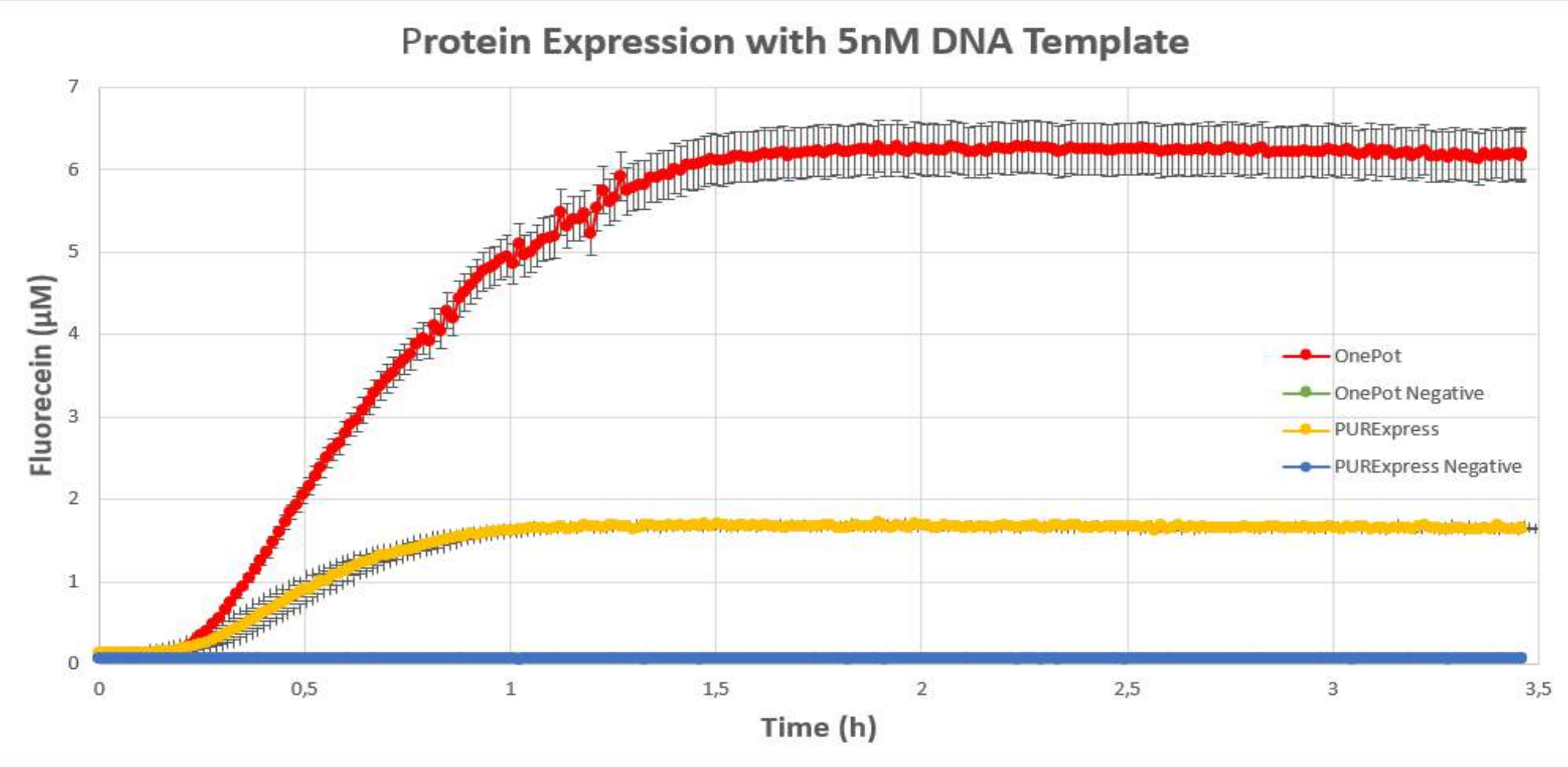
One Purification

↓

14x cheaper

Lavickova Barbora, and Sebastian J. Maerkl. A., 2018

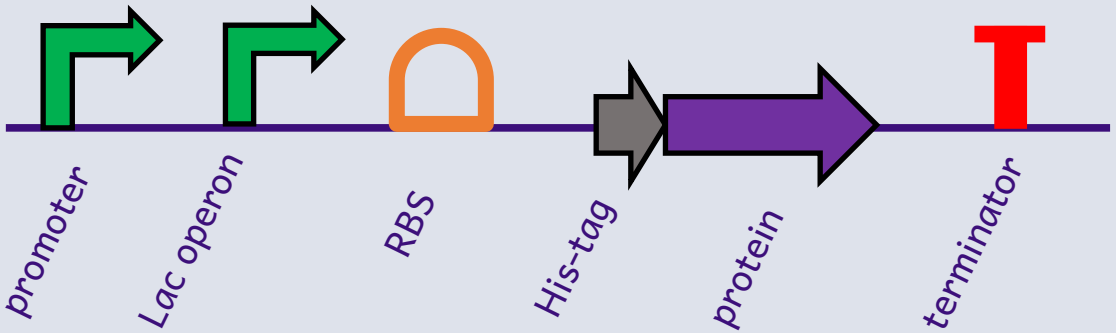
Characterization of "OnePot" PURE



sfGFP Gene Expression (in fluorescein con.)

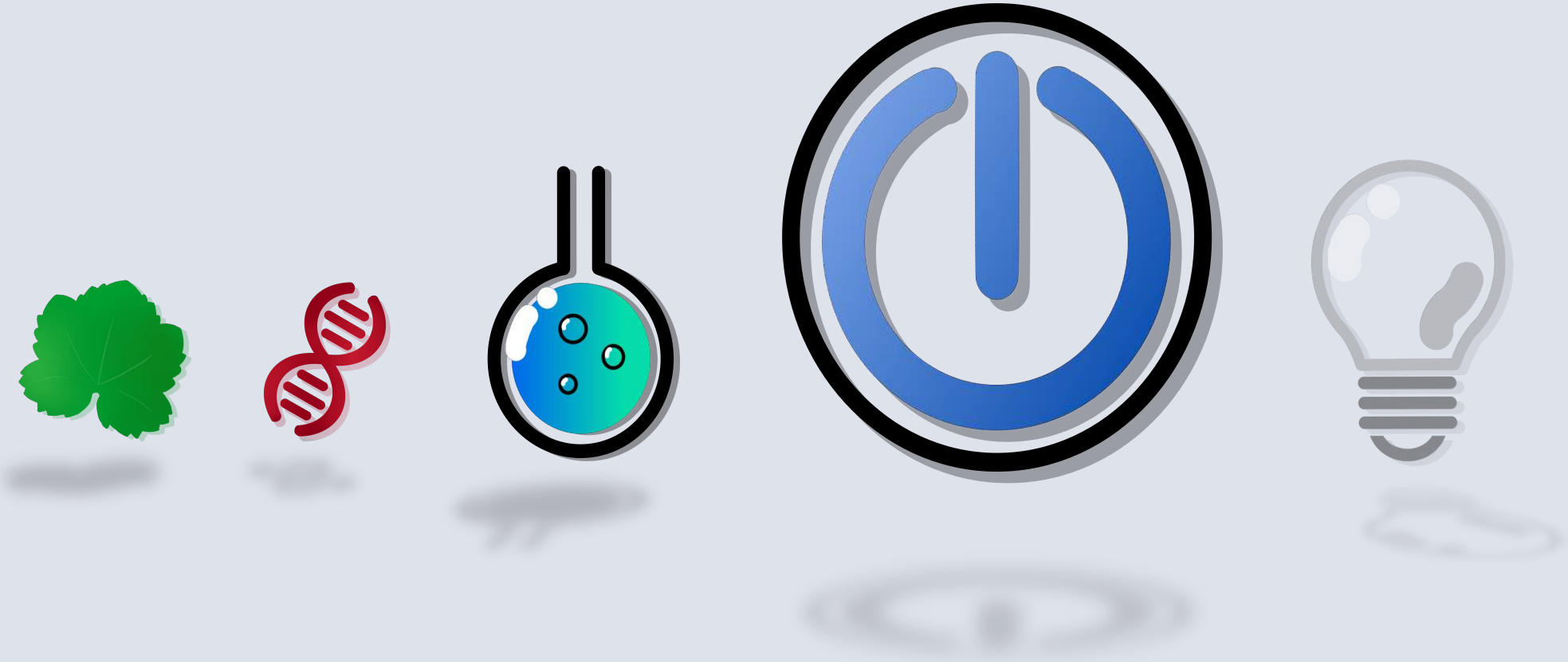
Part collection

Proteins:

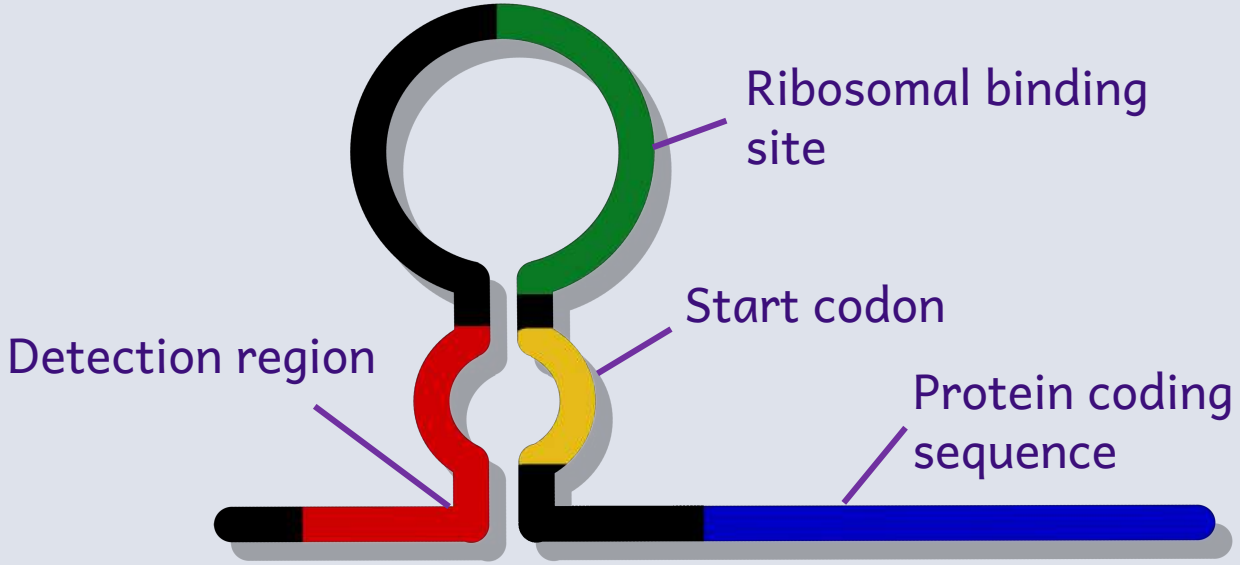


1. Registry(BBa_K2916000 to 49)
2. Protocol
3. Video tutorial (Search: “OnePot iGEM EPFL”)

Toehold switch

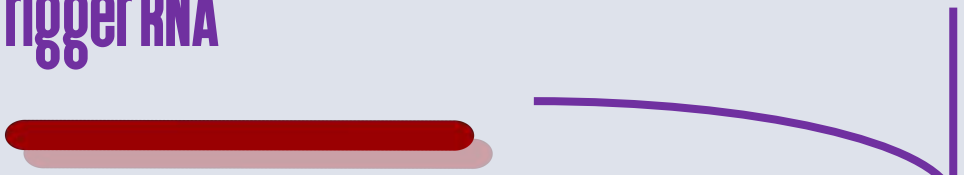


How Toehold works :



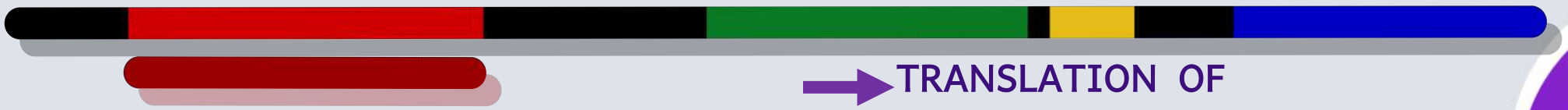
CLOSED (HAIRPIN) TOEHOLD

Trigger RNA



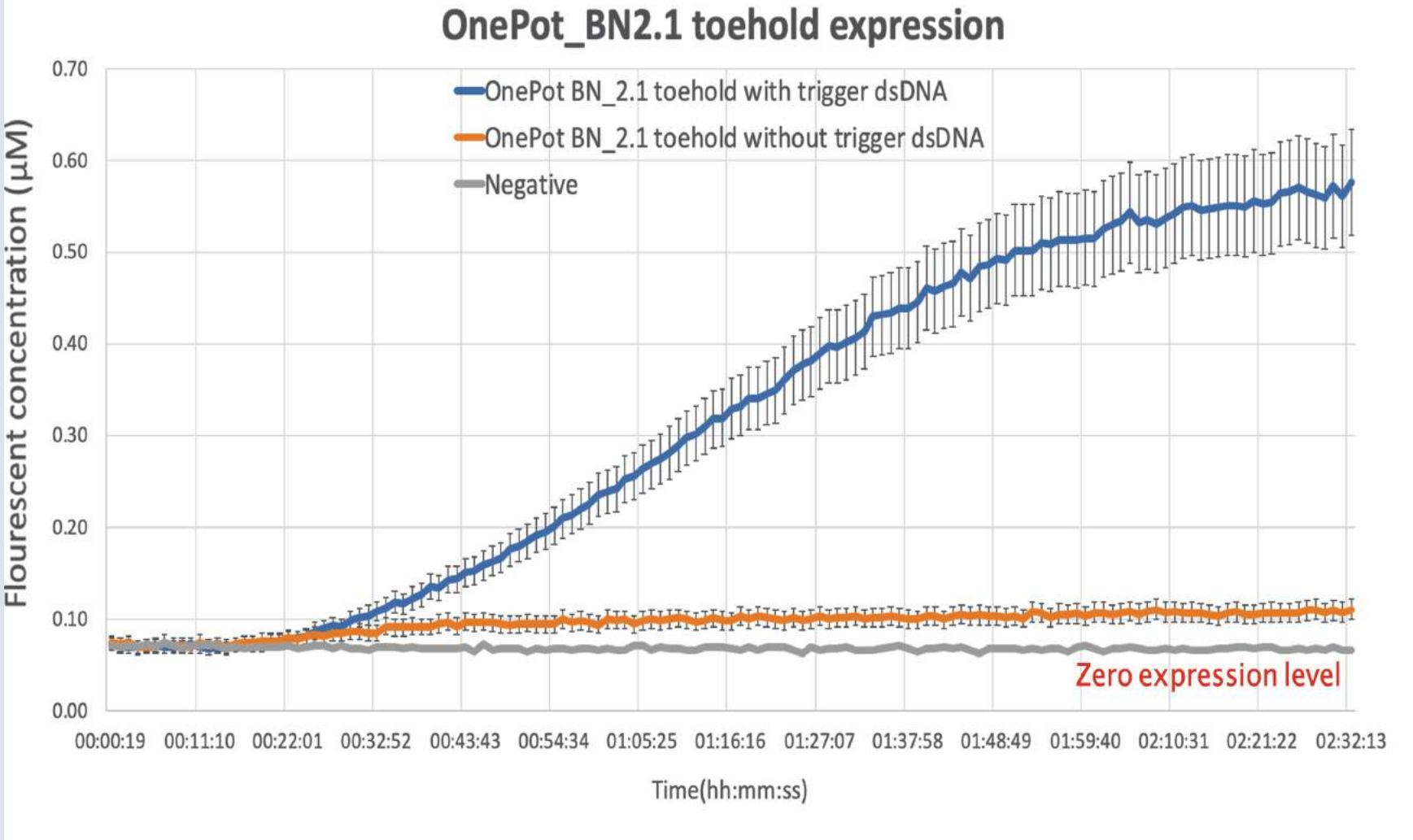
Ribosome

LINEARIZED TOEHOLD



TRANSLATION OF PROTEIN SEQUENCE

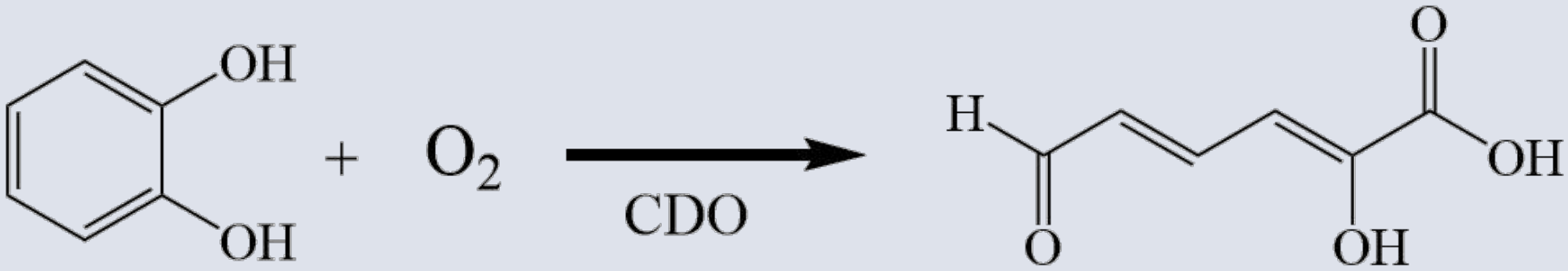
Toehold Regulation of GFP expression



Toehold functionality test
(Bois Noir 2.1 toehold v.s. commercially available toehold)

Signal generation

Signal Generation with CDO (catechol 2,3-dioxygenase)

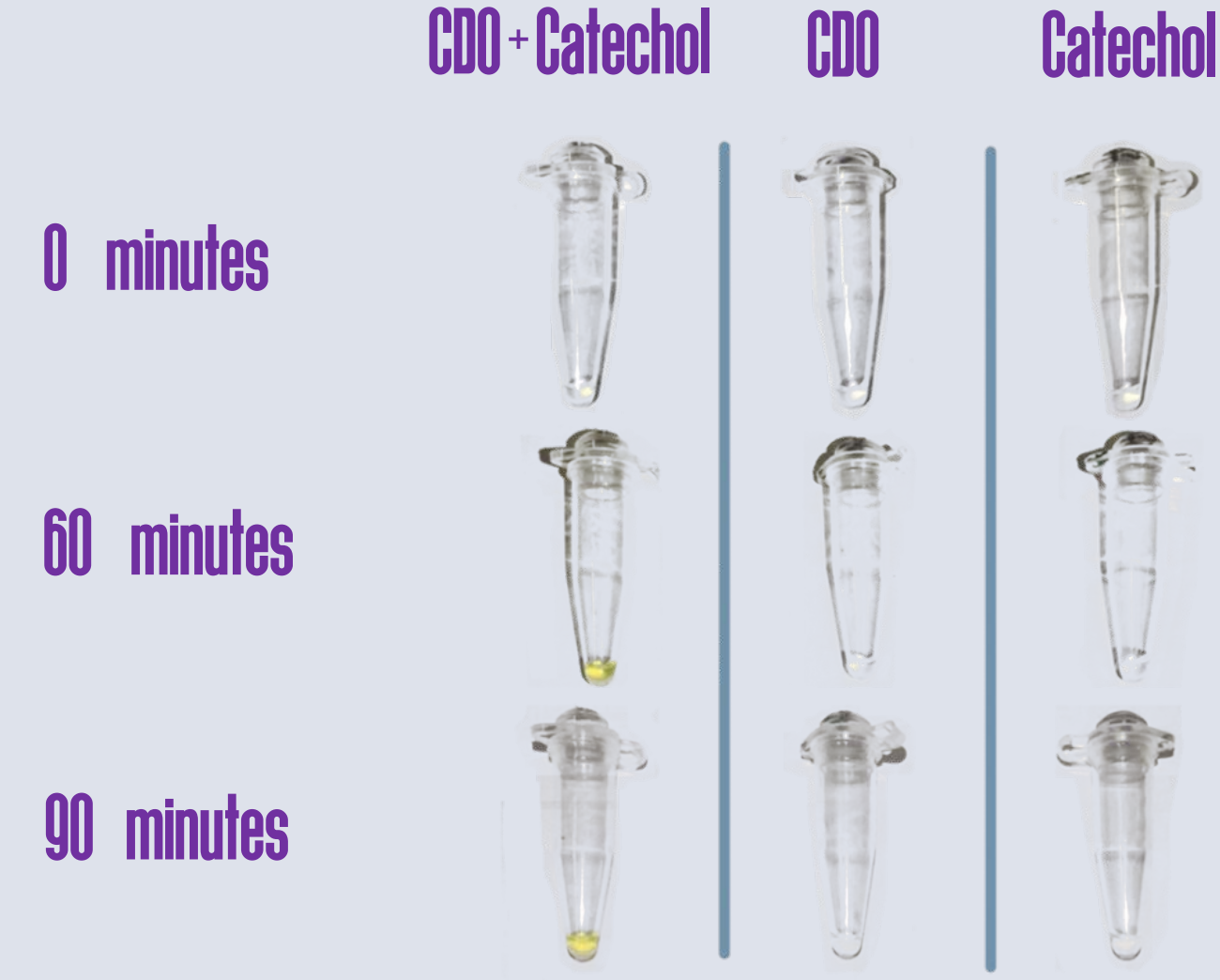


Catechol

2-Hydroxymuconic semialdehyde

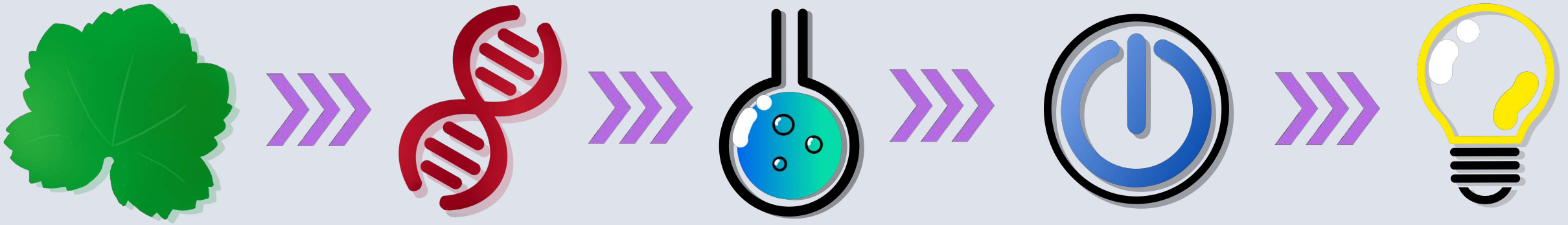
Verosloff, M., et al.,2019

Colorimetric Signal in OnePot PURE



Colorimetric signal preservation

Summary



Extraction

Polymerase Amplification

Toehold switch

signal

Winegrowers

- 2 Similar Diseases
- How it affects them
- Feedback

Phytosanitary Police

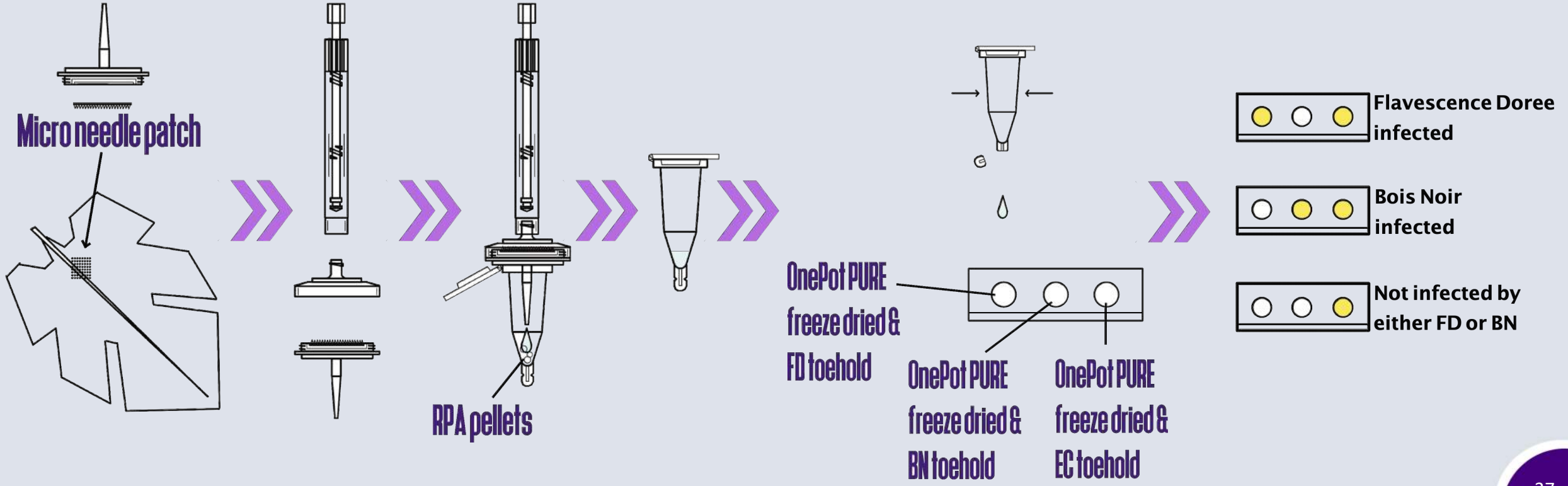
- Symptoms Identification
- Diagnostic Process
- Collect Infected Samples

Agroscope

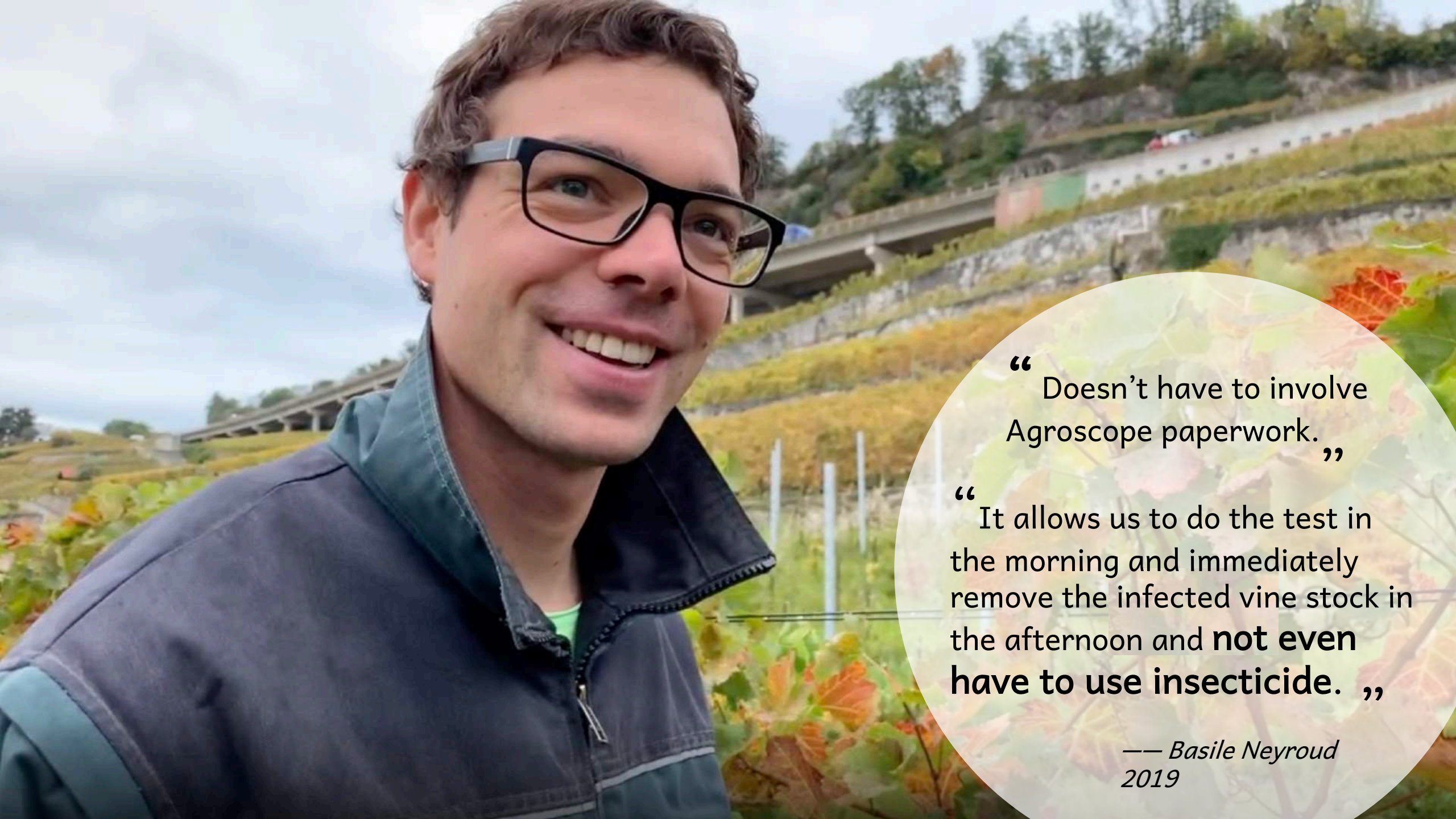
- Underlying biology of phytoplasma
- Technical Guidance
- Test Requirements

Agroscope

Hardware design



Hardware demonstration

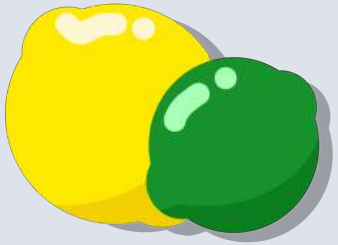


“Doesn’t have to involve Agroscope paperwork.”

“It allows us to do the test in the morning and immediately remove the infected vine stock in the afternoon and **not even have to use insecticide.** ”

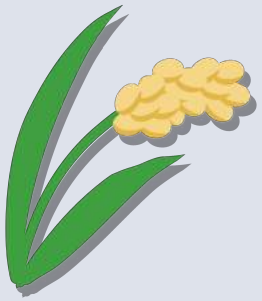
—— Basile Neyroud 2019

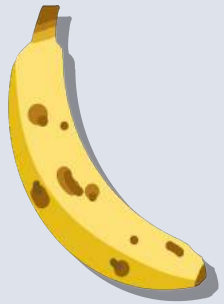
A local solution to a global problem



10% decline in citrus population in Iran

€100 million loss in apple production in Italy and **€25 million in Germany.**





Sources : https://gd.eppo.int/reporting/article-6205
https://link.springer.com/article/10.1007/s10327-014-0512-8

Thank You!

Supervisor

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TWIST BIOSCIENCE

Microsynth THE SWISS DNA COMPANY

Nikon

NEW ENGLAND BioLabs Inc. enabling technologies in the life sciences

Agroscope

robopooly

JEAN-FRANÇOIS

NEYROUD FONJALLAZ VIGNERON-ENCAVEUR CHARDONNE

iGEM EPFL iGEM


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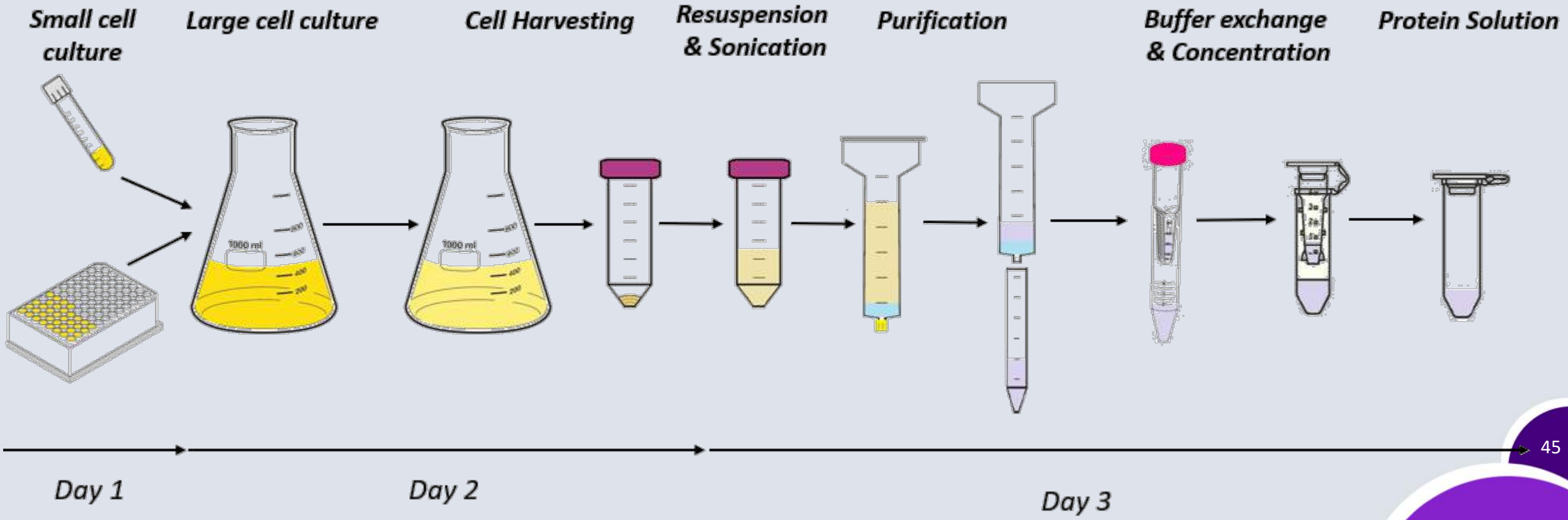
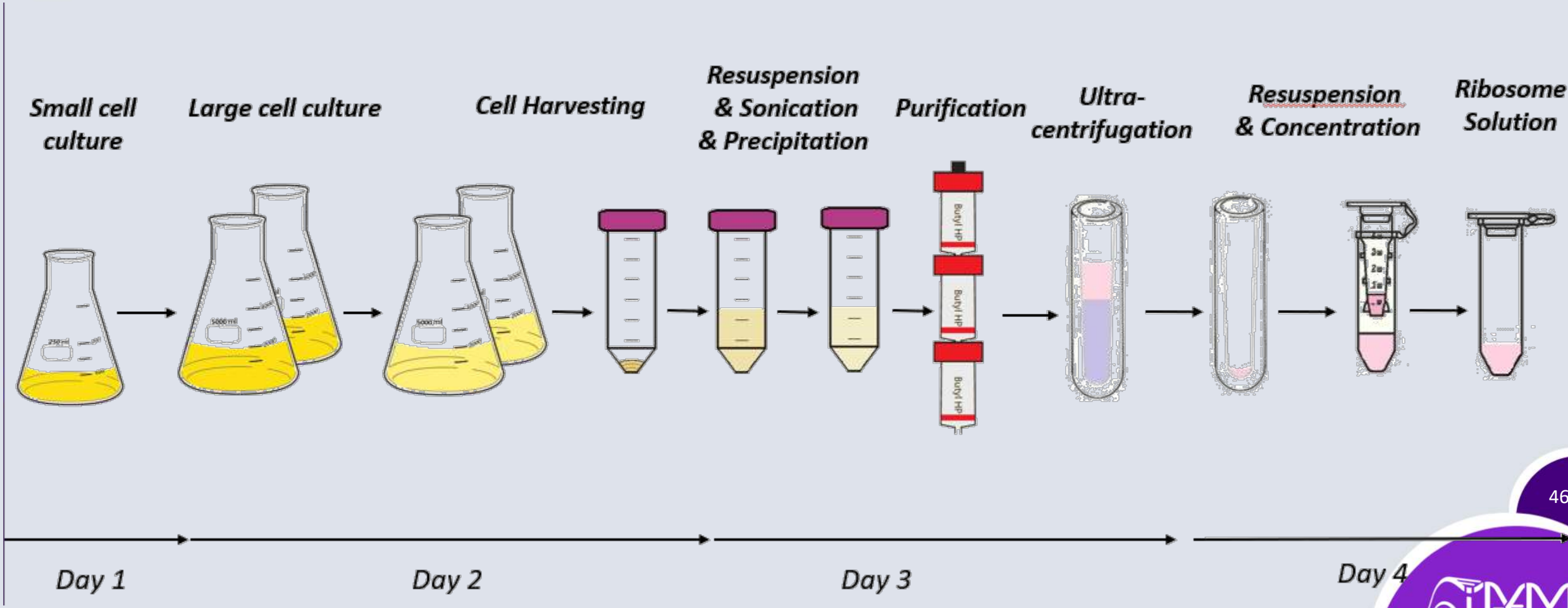
Appendix

Protein Purification



Ribosome Purification



36 Proteins

Transcription

- *T7 RNAP*

Translation

Initiation

- *IF1*
- *IF2*
- *IF3*

Elongation

- *EF-G*
- *EF-Tu*
- *EF-Ts*
- *AlaRS ArgRS*
- *AsnRS AspRS*
- *CysRS GlnRS*
- *GluRS GlyRS*
- *HisRS IleRS*
- *LeuRS LysRS*
- *MetRS PheRS*
- *ProRS SerRS*
- *ThrRS TrpRS*
- *TyrRS ValRS*

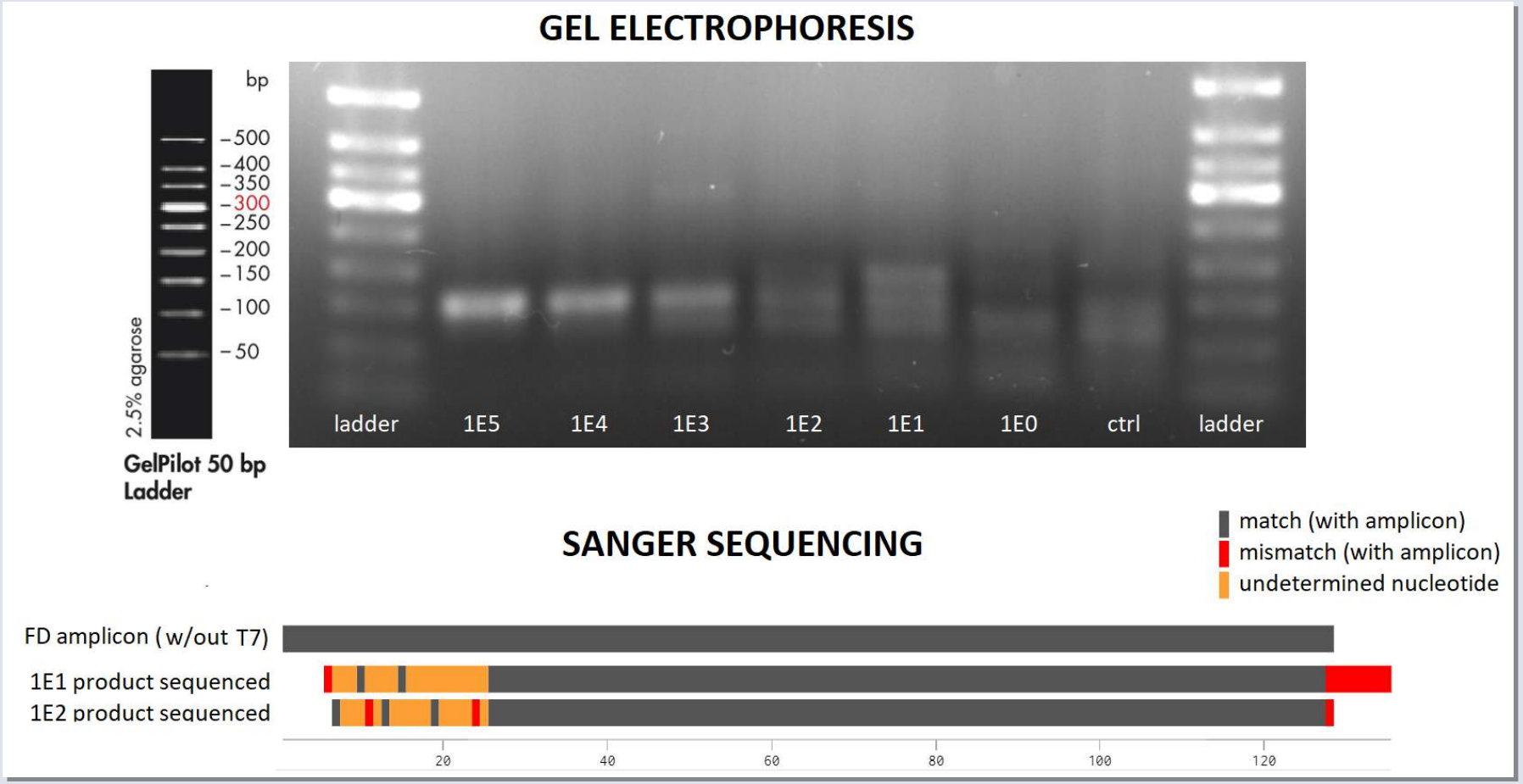
Termination

- *RF1*
- *RF2*
- *RF3*
- *RRF*

Catalyzing

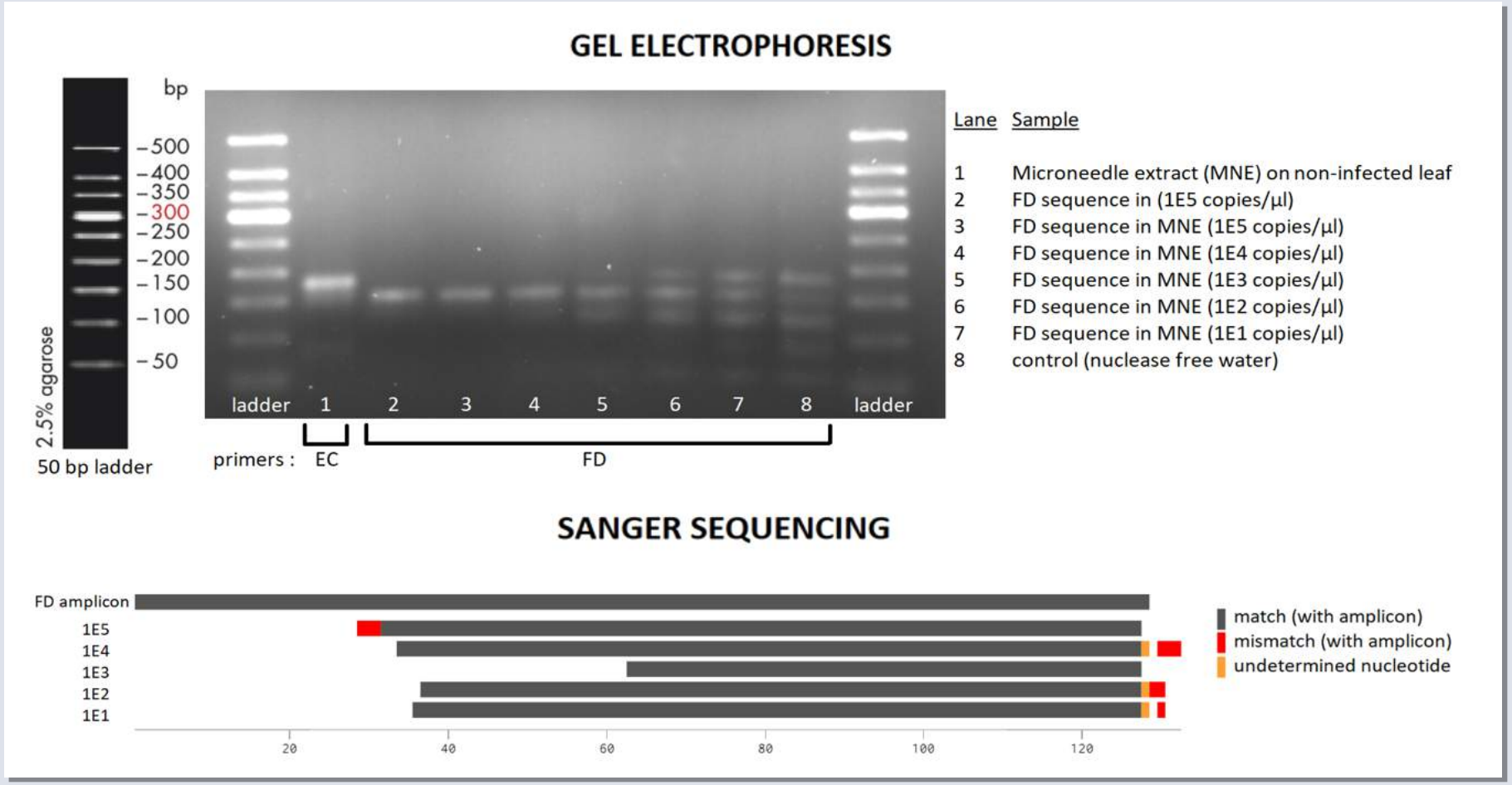
- *MTF*
- *CK*
- *MK*
- *NDK*
- *PPiase*

Limit of detection



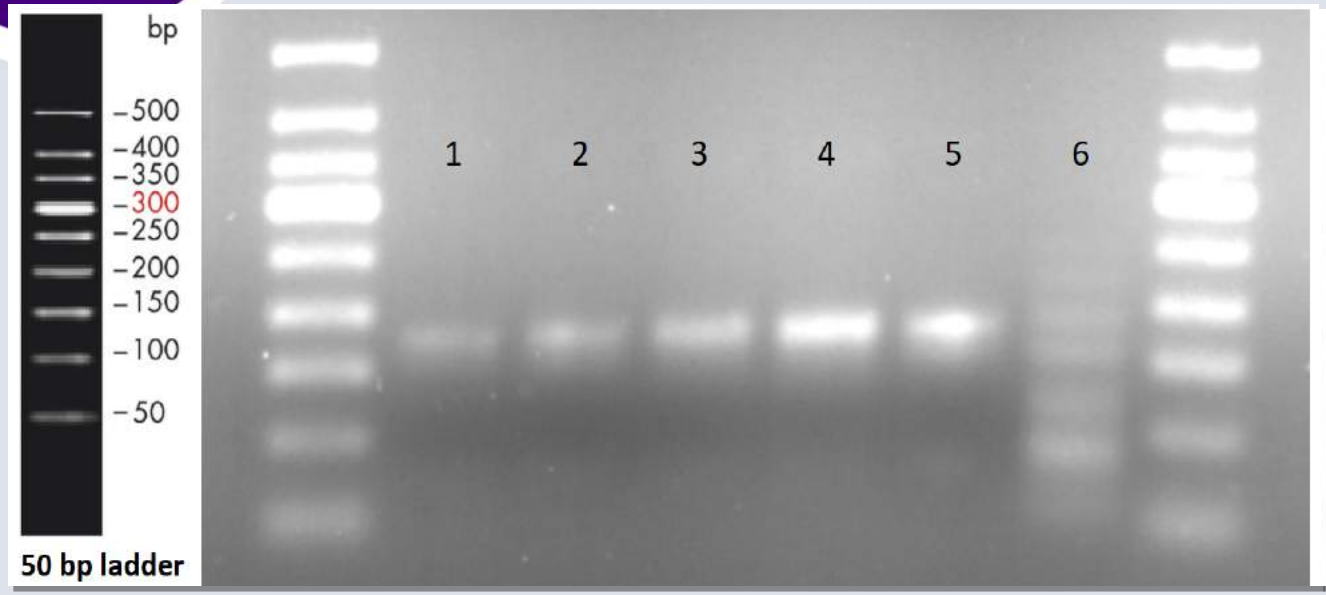
Agarose gel electrophoresis and sequence alignments of limit of detection of FD synthetic DNA

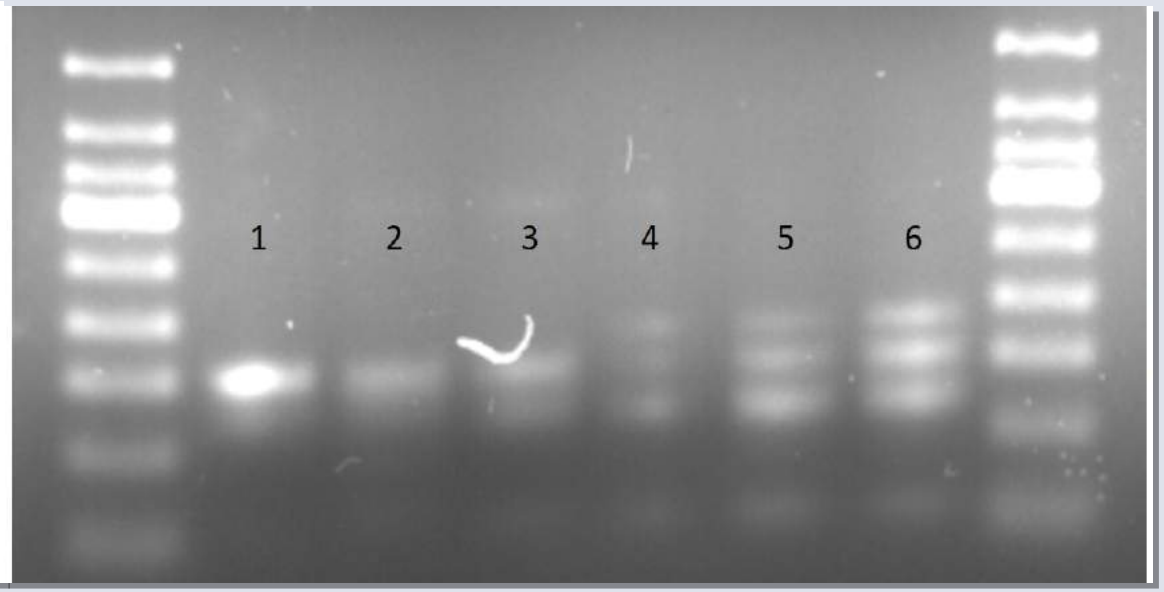
Limit of detection in microneedle extract



Agarose gel electrophoresis and sequence alignments of limit of detection of FD synthetic DNA in presence of plant extract

Testing on grapevine





Lane	Template
1	Respective synthetic sequence
2	Microneedle extraction (MNE) on non-infected plant
3	MNE on FD-infected plant
4	MNE on BN-infected plant
5	Prototype extraction on non-infected plant
6	Water

Summary

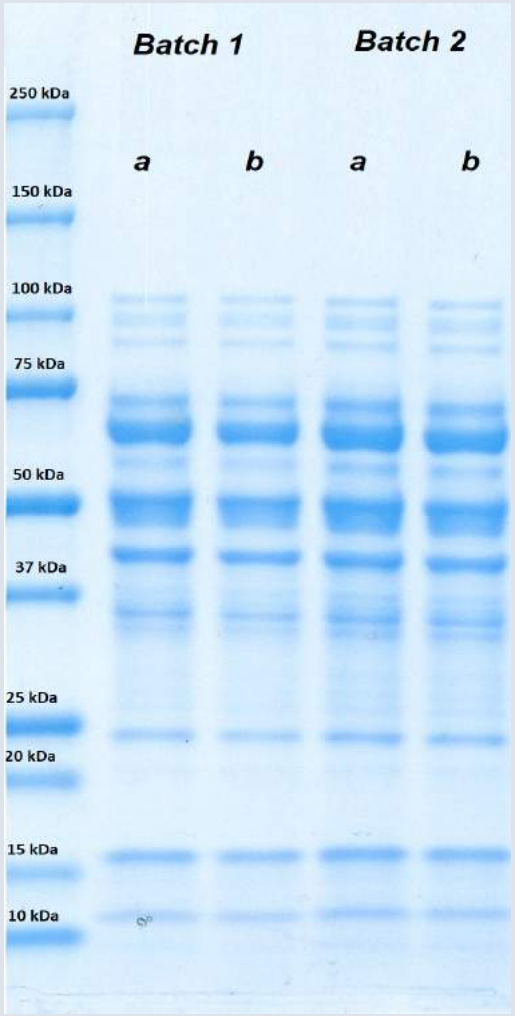
- Detection of phytoplasma plant diseases in 4 steps including DNA extraction with microneedle patch, isothermal amplification (RPA), toehold regulation, signal generation by using our in-house OnePot PURE system.

- An easy-to-use field-based diagnostic hardware was developed to allow us to detect the infectious grapevine diseases within 3 hours.

Frabrication cost:

ViTest Mk I	Amount:	Fabrication cost ($):
BD 1ml Syringe	1	0.34
Micro-needle patch	1	2.50
ER Buffer	0.3ml	0.10
3D printing	1	0.85
TwistAmp® Basic	1rxn	3.16
Test discs	3	0.61
Plastic dropper 0.5ml	1	0.02
Total:		**7.58**

OnePot protein



Reproducibility of OnePot protein