Detect Plant Diseases In 4 Steps

ViTEST

EPFL iGEM

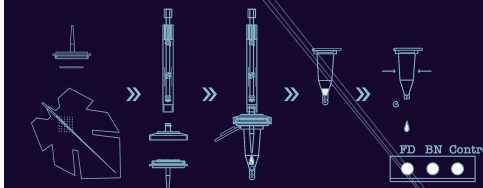
ViTEST is a rapid, field-based diagnostic tool to detect infectious grapevine diseases within 3 hours. It requires no lab equipment or special training, and saves on crop loss and pesticide use.

Background

We chose to focus on a quarantined grapevine disease, ***Flavescence Dorée (FD)***, which shares the same symptoms as the non-contagious grapevine disease ***Bois Noir (BN)***. Both are caused by Phytoplasma (a type of bacteria) and have no cure. A reliable diagnostic tool is needed to distinguish between them in order to take the heavy measures necessary to contain Flavescence Dorée.

However, current diagnostic methods tend to be either very expensive (restrictive), slow (can take months), imprecise (high false positive rates), or a combination of all three, making it hard for winegrowers to react in time. This leads to colossal crop loss and excessive amounts of pesticides are applied.

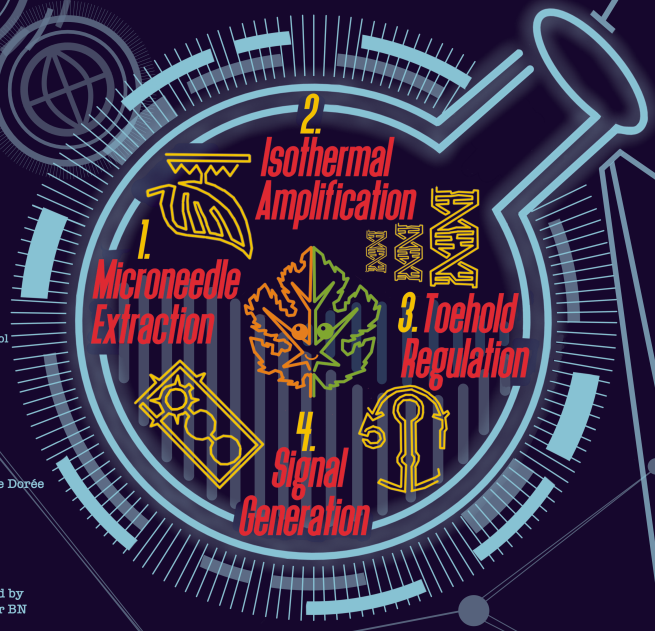
Hardware



Flavescence Dorée infected

Bois Noir infected

Not infected by either FD or BN



#1 Microneedle Extraction

fig 1.1 Microneedle patch

1. Synthetic endogenous control sequence
2. MN extraction on non infected leaf
3. Conventional extraction on non infected leaf
4. Nuclease free water

fig 1.2 Comparison of different extraction techniques

Extracting and isolating pathogenic DNA from plants is currently limited by cumbersome multi-step protocols that mostly require a laboratory environment.

To address this, a **disposable polymeric microneedle (MN) patch** was employed to extract **amplification-ready** DNA from a plant leaf within **1 minute.** The extracted DNA is **ready-to-use** for **field-based** molecular diagnosis of grapevine diseases.

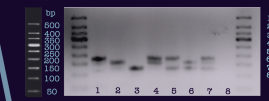
#2 Isothermal Amplification

Our preliminary research showed that detecting phytoplasma DNA directly from contaminated plant extract was almost impossible, as the amounts present were too low. For amplification, we chose **RPA(Recombinase Polymerase Amplification)** for the following reasons:

1.Isothermal - We wanted to bring our test into the vineyard. **A simple battery powered heat-block** would allow an isothermal method to function **anywhere.**

2.Multiplexable - It allows us to amplify Flavescence Dorée, Bois Noir and Endogenous Control in **one reaction.** (see fig 2.1)

3.Fast - It only takes ***20 min*** at its ideal temperature(37°C).



1. Endogenous Control (EC) amplicons
2. Flavescence Dorée (FD) amplicons
3. Bois Noir (BN) amplicons
4. EC + FD amplicons
5. EC + BN amplicons
6. FD+ BN1 amplicons
7. EC + FD + BN amplicons
8. Negative control (Nuclease free water)

fig 2.1 Electrophoresis gel of multiplexed RPA

#3 Toehold Regulation

fig 3.1 Detailed Toehold design schema *Green et al.,2014*

In order to eliminate unwanted interactions in the complex synthetic network, we use **low crosstalk toehold switches** to regulate the expression of a reporter gene, which is composed of four main parts as above. The hairpin structure **sequesters** the **Ribosome Binding Site (RBS) and start codon**, making it impossible for the ribosome to bind to the mRNA and translate the gene. When the **trigger RNA is present**, it will linearize the hairpin structure, **exposing the RBS** and allowing the gene to be expressed.

fig 3.2 OnePot BIN2.1 toehold expression

fig 3.3 ON/OFF ratio of toehold BIN 2.1 activation

#4 Signal Generation

To be field-deployable, we are using a **colorimetric signal, catechol 2,3-dioxygenase (CDO),** instead of a fluorescent one (GFP), because it **can be seen by human eyes** without the aid of any other equipment.

Also since CDO is a **small protein** of about 300 amino acids, it requires **less energy** from the cell-free transcription and translation system, which increases its efficiency.

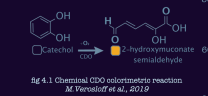


fig 4.1 Chemical CDO colorimetric reaction *M. Veraldi et al., 2019*

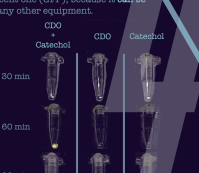


fig 4.2 CDO colorimetric reaction in OnePot PURE system

Conclusion

Our novel plant diagnostic system is a proof-of-concept to detect phytoplasma plant diseases in 4 steps including microneedle extraction, isothermal amplification (RPA), toehold regulation, signal generation by using our *in house* Onepot PURE system. We also developed an easy-to-use, field-based diagnostic hardware that allows us to detect infectious grapevine diseases within 3 hours.

OnePot PURE System

A main part of our project is the **expression of proteins** that will produce an observable signal. In order to have a **field-based** diagnostic test, we opted to use a **cell-free expression system**. However, the commercially available PURE systems **are quite costly**, especially for small research teams. To cope with this problem, we thus decided to produce our own PURE system based on a new method that was developed at **LBNC, EPFL** (*Lavickova et al., 2019*). Here, instead of **expressing the 36 proteins** in different flasks and then performing as many purifications, we **co-culture** them in **one flask**, hence the name **"OnePot"**, so only one purification step is required. We achieved a **14-fold decrease in cost** and we still get high yields on the produced proteins.

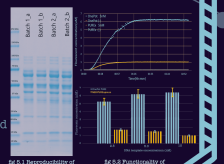


fig 5.1 Reproducibility of OnePot PURE

fig 5.2 Functionality of OnePot PURE

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Attributions

LBNC, EPFL, Switzerland
Administration Suisse, Agroscope
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