ViTEST

Microneedle DNA Extraction 7/20-7/25

Microneedle Frabrication

EPFL iGEM

Saturday, 20 July 2019

Title: PVA solution

Aim: Make PVA solution

Participants: Hana, Stefania

Protocols: To make the poly(vinyl alcohol) (30– 70 kDa, 10 wt %) (PVA) solution, we add 28g of water and 4g of PVA powder and mix it at 110 degrees celsius for 20 minutes. We then leave it overnight.

Thursday, 25 July 2019

Title: Microneedle manufacturing

Aim: Make microneedles

Participants: Hana, Stefania

Protocols: To fabricate the microneedle patches, 1.5 mL of poly(vinyl alcohol) (30– 70 kDa, 10 wt %) solution was added to each silicone mold. After that, the molds are placed in a centrifuge for 20 min at 40 °C at 4000 rpm to draw the PVA solution into the cavities and achieve the desired viscosity. These molds were then kept overnight at 25 °C in a chemical hood vacuum. After being dried, the microneedle patches were carefully separated from the molds and stored at 25°C in a sealed Petri dish. The patches ought to be used within a month after their fabrication date.

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Microneedle DNA Extraction 8/10-8/20

Extraction method testing

EPFL iGEM

Saturday, 10 August 2019

Title: Amplifying different The CE using different amounts of TE buffer to extract the DNA extracted with the MN patch and using the Gblock as reference

Aim: Find the optimal concentration of EB for the extraction of DNA.

Participants: Hana, Stefania

Protocols: Gel electrophoresis, PCR

Notes:

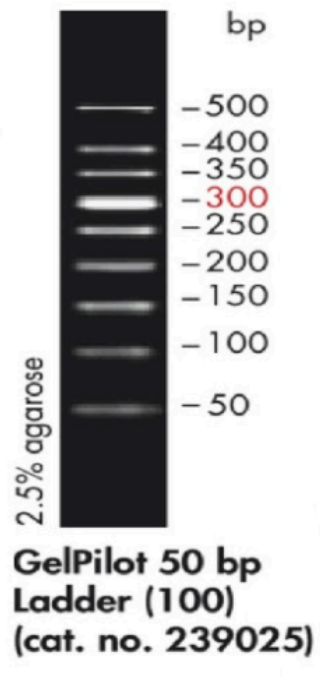
Gel electrophoresis

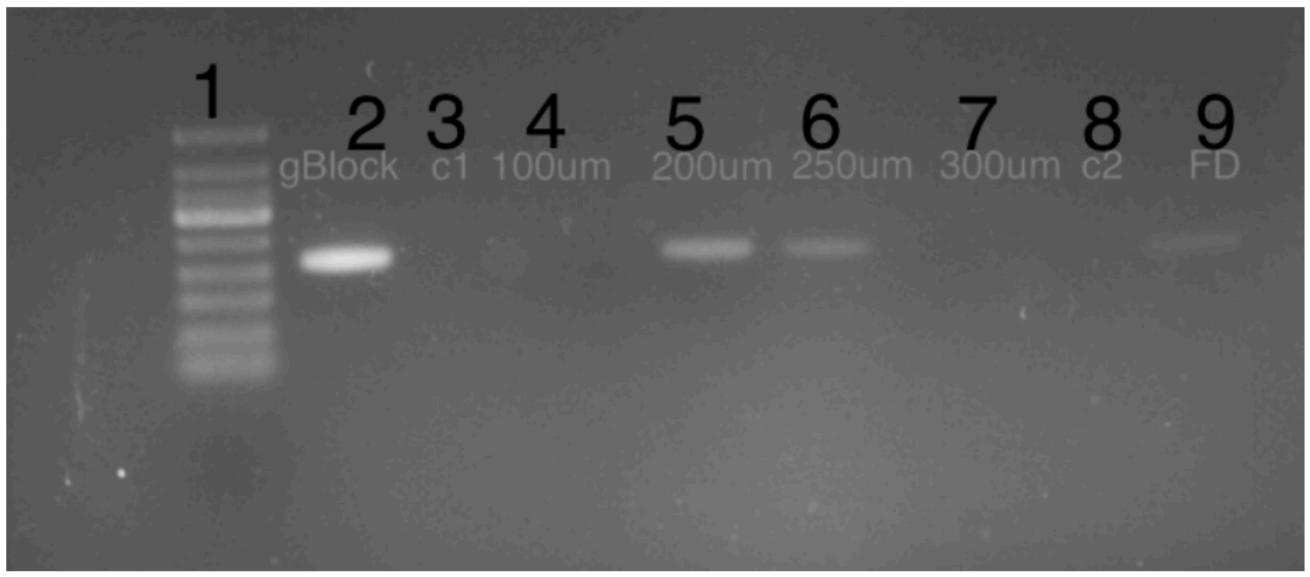
2% agarose gel, midori green gel stain and gel pilot 6X loading dye

5 µL GelPilot 50 bp ladder (Qiagen)

All amplifications were performed using 1 µL of DNA , 25 µL of Q5 master mix and 2,5 µL of each primer in a 50 µL PCR reaction.

Results:





Lane	Fwd primer	Rev primer	DNA	Comment
1	X	X	X	Ladder
2	FD_T7_F1	FD_R1	gBlock	control
3	X	X	gBlock	control
4	FD_T7_F1	FD_R1	DNA + TE buffer standard volume (100um)	We must have messed something up here cause it the highest concentration and we don't have a signal.
5	FD_T7_F1	FD_R1	DNA + TE buffer larger volume (200um)	we can detect the CE using 200 µLof TE buffer
6	FD_T7_F1	FD_R1	DNA + TE buffer larger volume (250um)	We can still detect the CE using 200 µLof TE buffer though we have a lower signal
7	FD_T7_F1	FD_R1	DNA + TE buffer larger volume (300um)	We can no longer detect the CE using 300 µLof TE buffer though we have a lower signal
8	X	X	DNA 001 (diagnosed with FD)	control
9	FD_T7_F1	FD_R1	DNA 001 (Diagnosed with FD)	we can also detect CE from the FD affected sample that we have extracted from the field

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Conclusion:

We can keep a volume of up to 250 um of TE buffer during extraction.

We successfully extracted DNA from contaminated leaves.

Tuesday, 20 August 2019

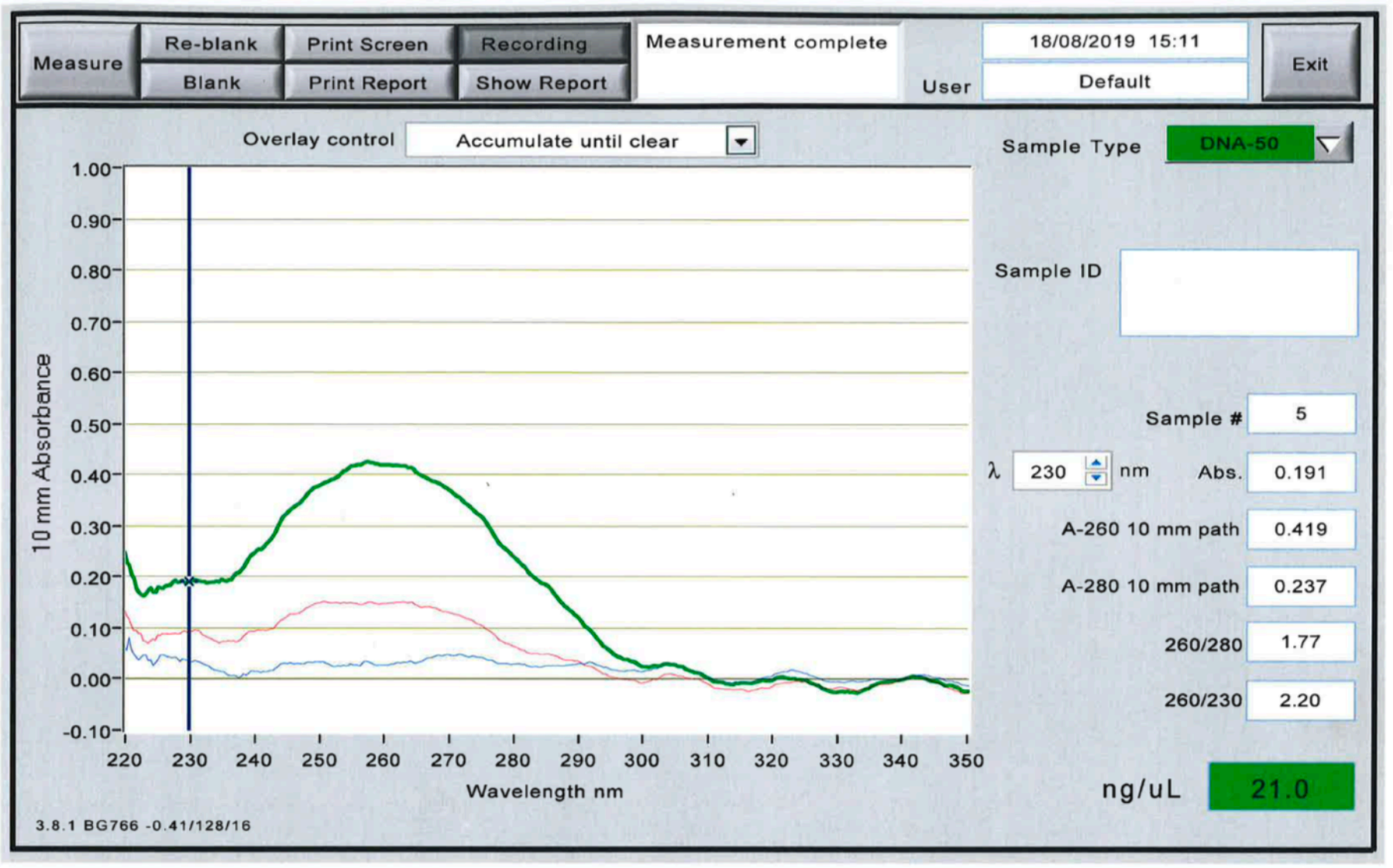
Title: Nanodrop

Aim: Verify correct amplification of DNA using microneedles

Participants: Hana, Stefania

Protocols: Nanodrop UV absorption spectra, with gBlock amplificatiom, MN patch amplification, and control

Notes: gBlock: green,
MN: red,
control: blue



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Microneedle DNA Extraction 10/18

Endogenous Control gene screening

EPFL iGEM

Friday, 18 October 2019

Title: EC screening, Amplicons: EC =214 , PHYTOPLASMA = 1850bp , FD = 1100bp

Aim: amplifying EC from the DNA extracted with the MN patch, kit estandard extraction and the Gblock of the EC.

Participants: Stefania

Protocols: Gel electrophoresis, PCR

Notes:

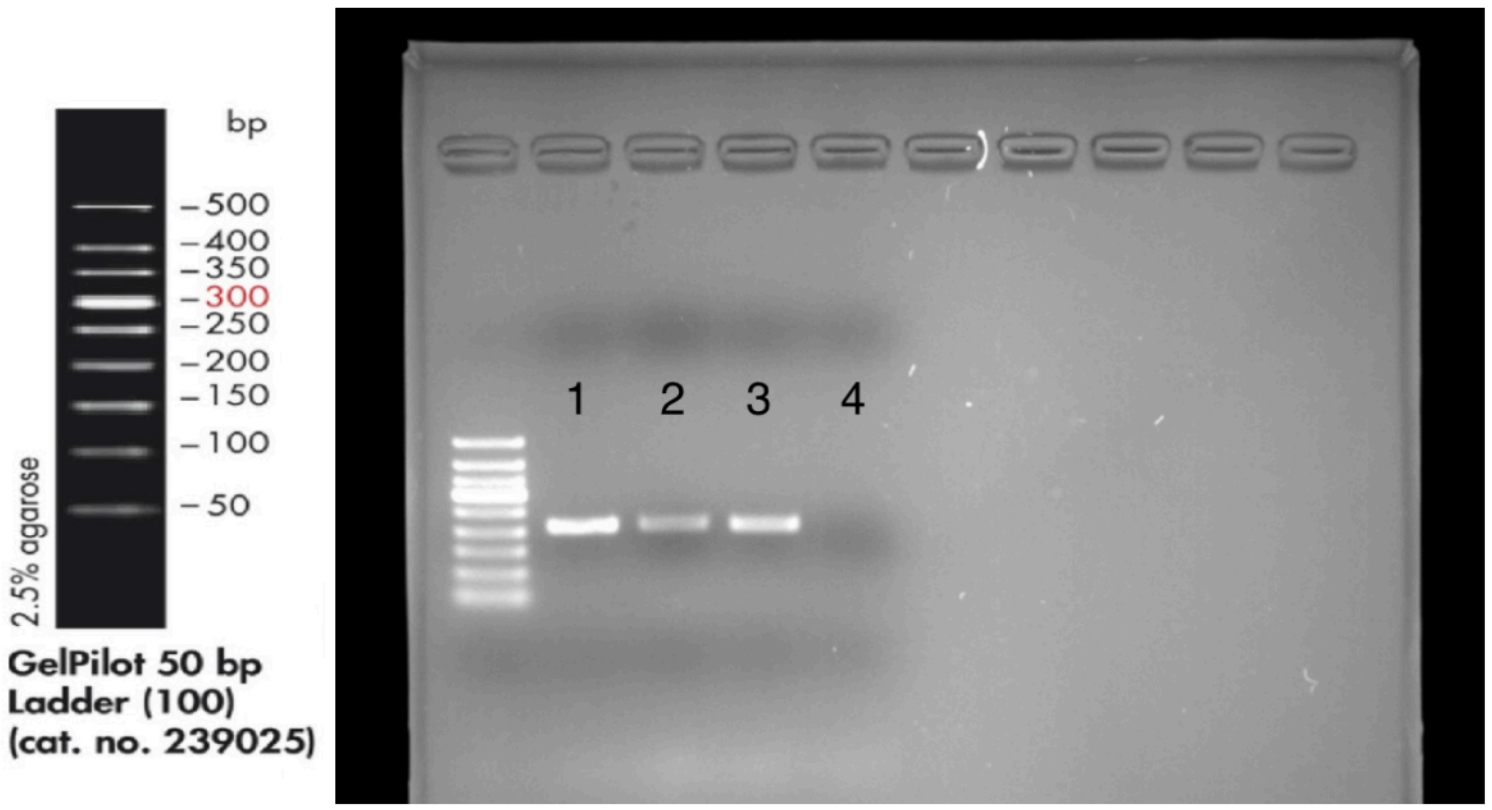
Gel electrophoresis

2% agarose gel, midori green gel stain and gel pilot 6X loading dye

5 µL GelPilot 50 bp ladder (Qiagen)

All amplifications were performed using 1 µL of DNA , 25 µL of Q5 master mix and 2,5 µL of each primer in a 50 µL PCR reaction.

Results:



Lane	Fwd primer	Rev primer	DNA	Comment
0	X	X	X	Ladder
1	X	X	gBlock	control
2	FD_T7_F1	FD_R1	MN DNA	
3	FD_T7_F1	FD_R1	kit - extracted DNA	

Analysis:

We have successful amplification of the MN extracted DNA

Conclusion:

Since we have managed to detect the vine DNA with the help of the microneedle patch we can now proceed with our test regarding the range of concentration and extraction of the phytoplasma and specifically FD .