IMBB

RESULTS

IMBB 2016

BecA-ILRI Hub, Nairobi

May 9 – 20, 2016

15th Anniversary biosciences eastern and central africa

NEPAD TRANSFORMING AFRICA

NC STATE UNIVERSITY

ILRI INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

Group1 a.k.a *Ndengu* Results

IMBB 2016

BecA-ILRI Hub, Nairobi

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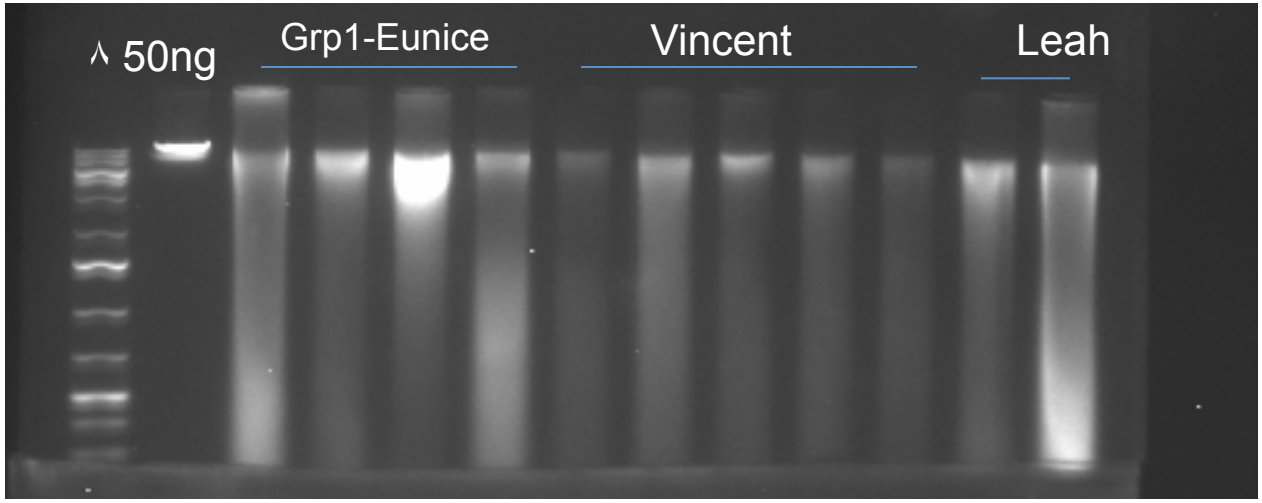
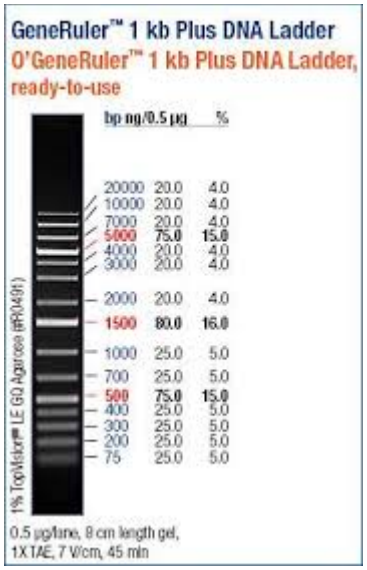
Eunice Machuka

Genomic DNA –Animal Tissue Extracted with Qiagen DNeasy Kit

0.8% Agarose Gel 11th May 2016



Sample ID	ng/ul	260/280	260/230
GIRIMA E1	332.36	2.01	1.95
YASSIR E1	111.4	1.97	1.58
JELALI E1	165.42	1.44	1.18
ESLAH E2	183.45	2.13	2.09

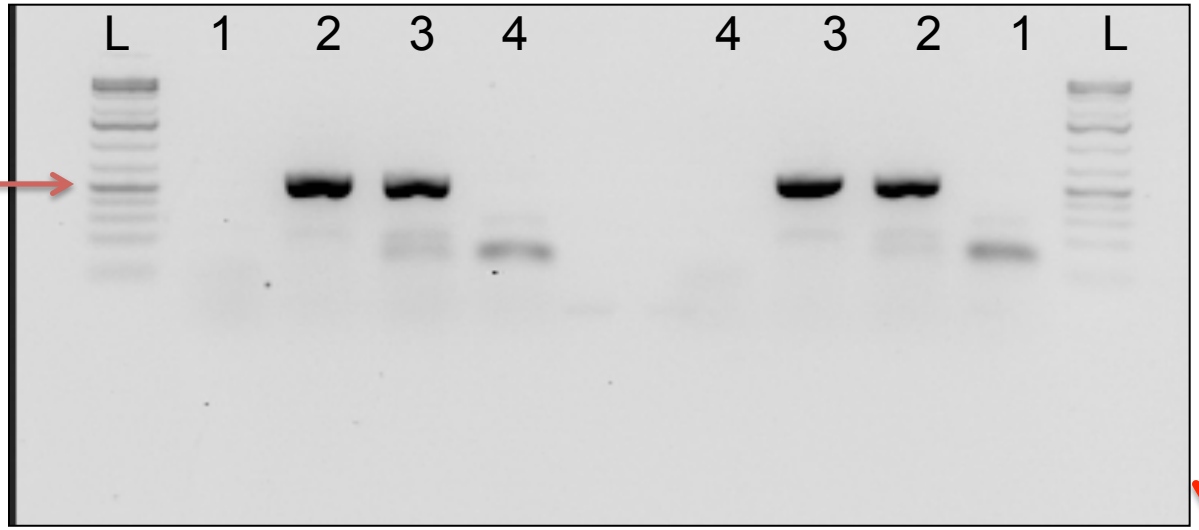


Phytophtora Diagnostics

1.5% Agarose Gel

11th May 2016

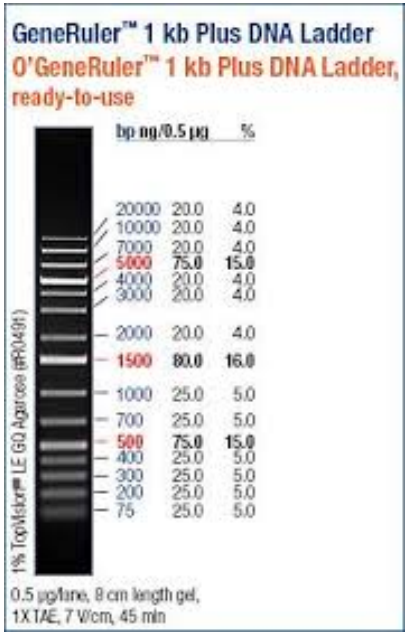
Onyango and Francine



KEY

L- 1kb ladder

1. Healthy leaf
2. Diseased leaf
3. Mycelia
4. NTC- H_20



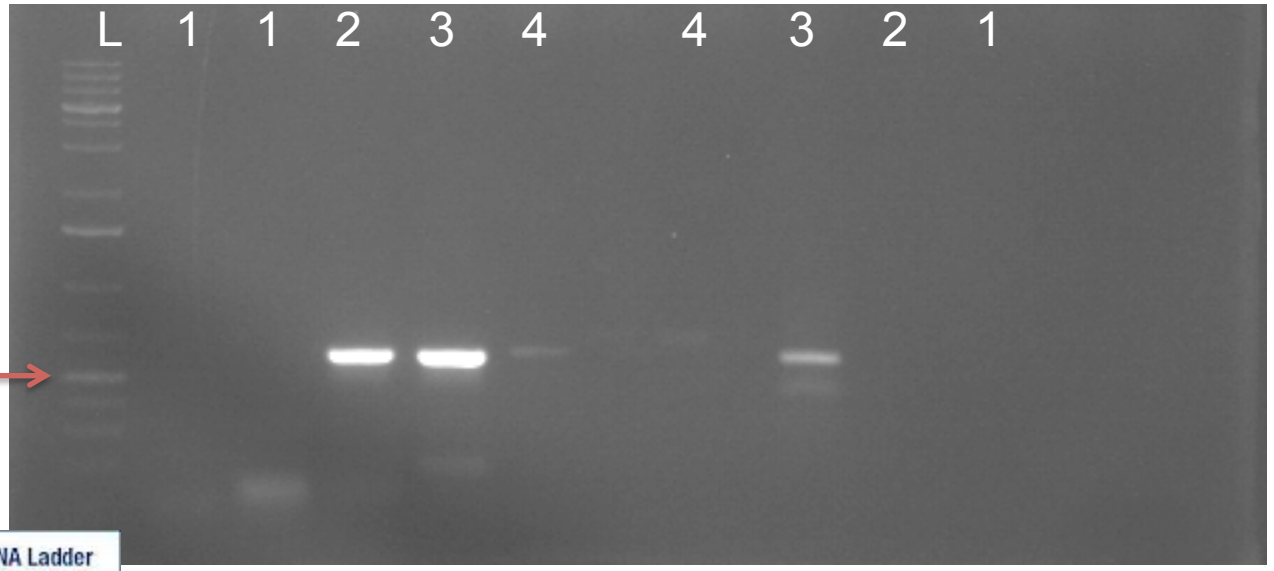
Phytophtora Diagnostics

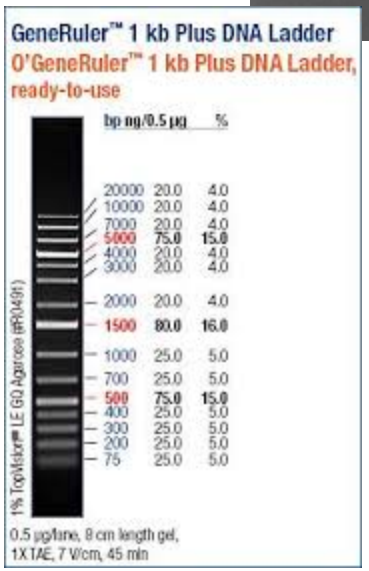
1.5% Agarose Gel

11th May 2016

Yassir

Jelalu





KEY

L- 1kb ladder

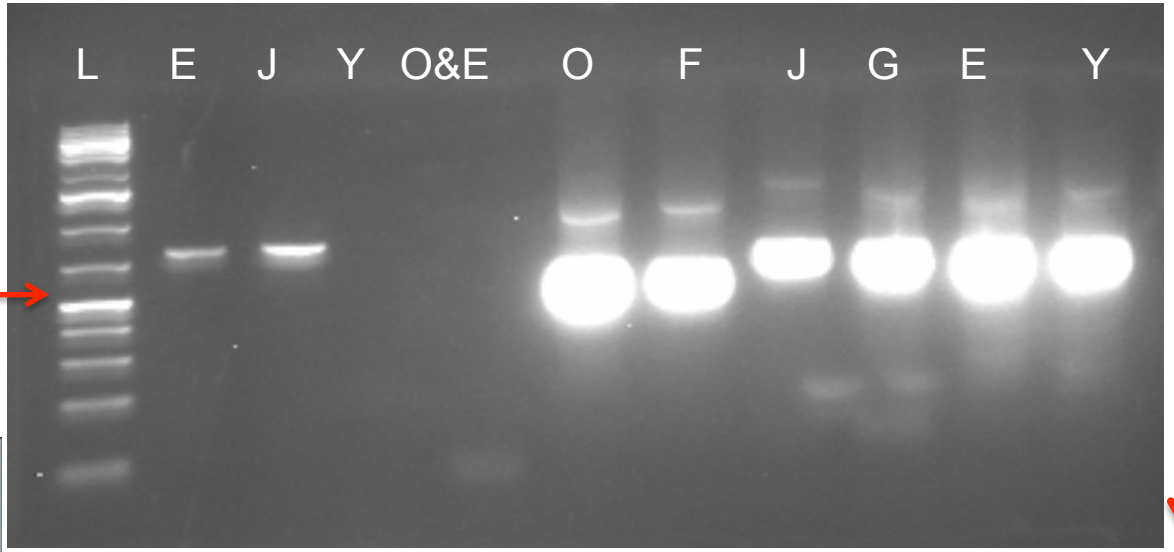
1. Healthy leaf
2. Diseased leaf
3. Mycelia
4. NTC- H_20

PCR Purification of COI and Rubisco PCR

1.5% Agarose Gel

12th May 2016

Phytophtora Rubisco/COI



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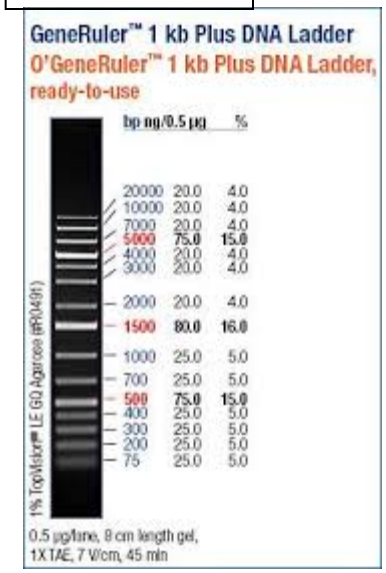
L- 1kb ladder



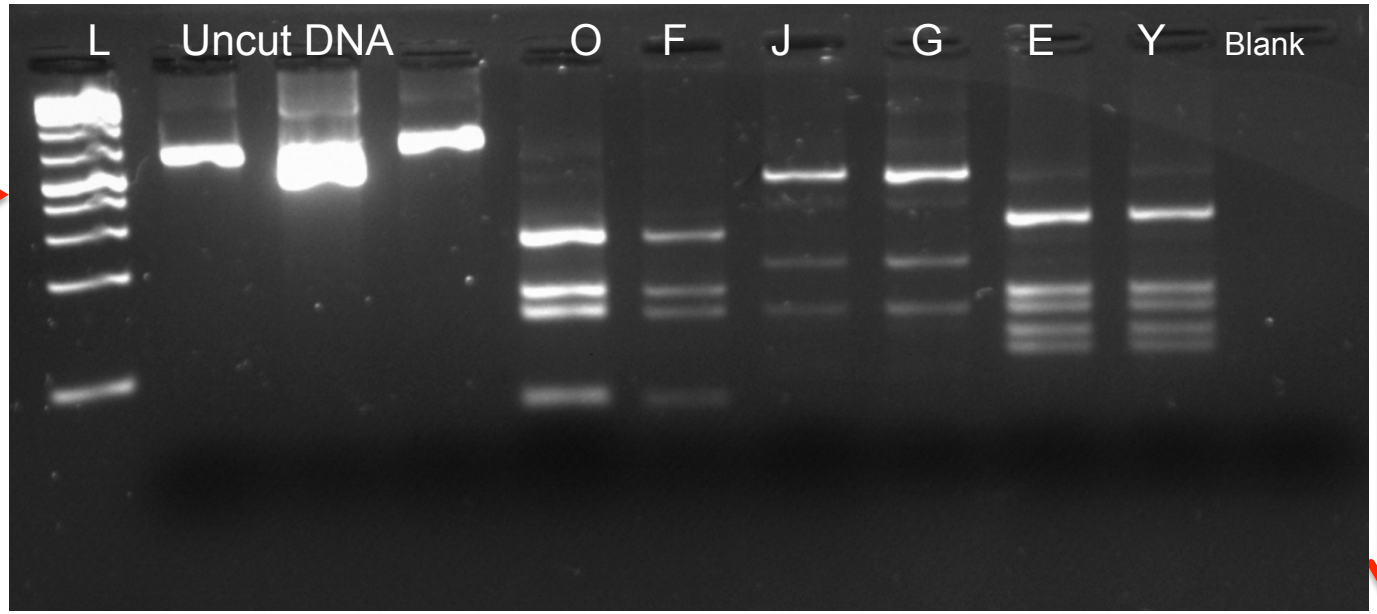
Phyto PCR	ng/ul	260/280	260/230
Eslah	24.98	0.91	0.15
Jalal	25.25	0.85	0.29
Yassir	29.37	0.93	0.24
OF	26.43	0.9	0.28

Rub/COI	ng/ul	260/280	260/230
Jelalu	72.78	1.29	0.59
Onyango	123.3	1.52	1.07
Eslah	109.62	1.42	1.09
Yassir	119.96	1.4	0.8
Frances	77.02	1.25	0.87
Girima	88.55	1.31	0.89

Restriction Digestion- Amplicons of COI and Rubisco PCR

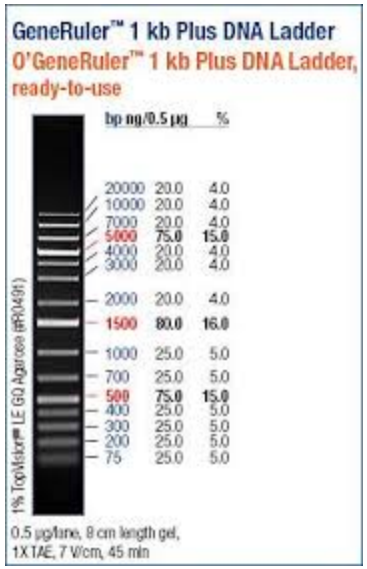
1.5% Agarose Gel 12th May 2016

AluI Digests



KEY

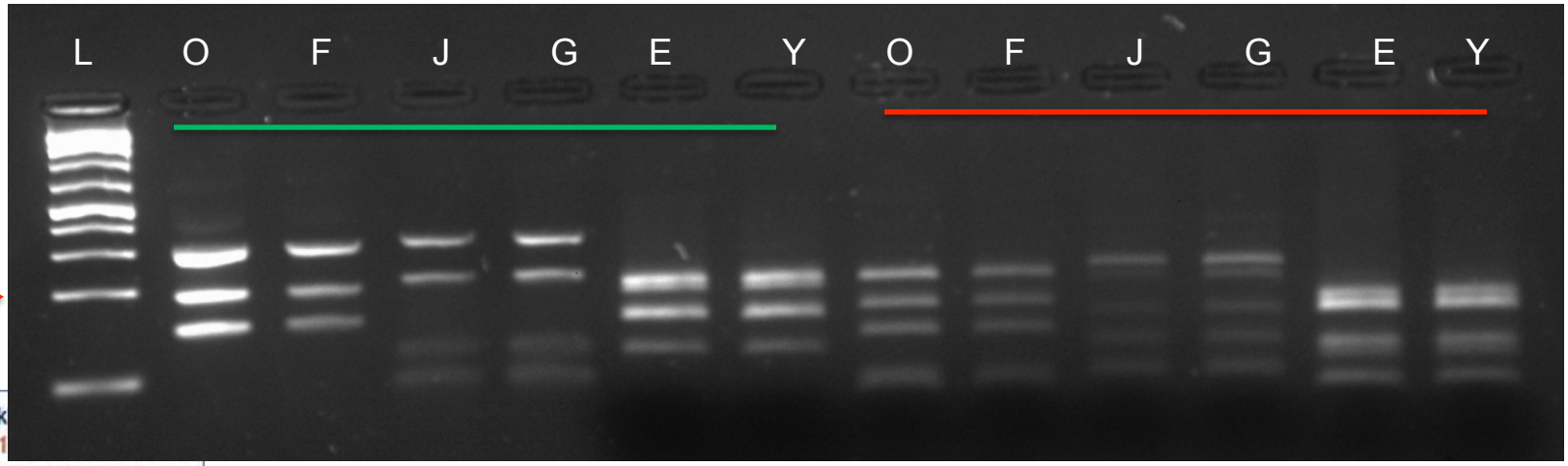
L- 1kb ladder



Restriction Digestion- Amplicons of COI and Rubisco PCR

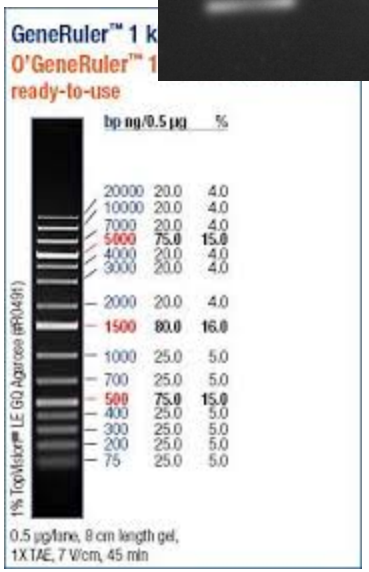
1.5% Agarose Gel 12th May 2016

Sau3I *AluI* and *Sau3ai*



100V
99min

500bp

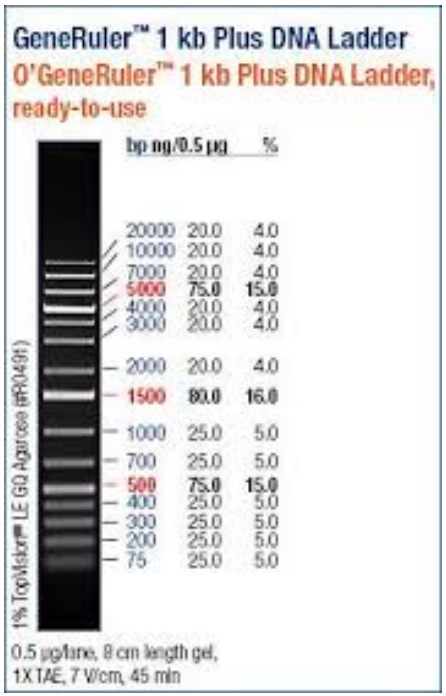


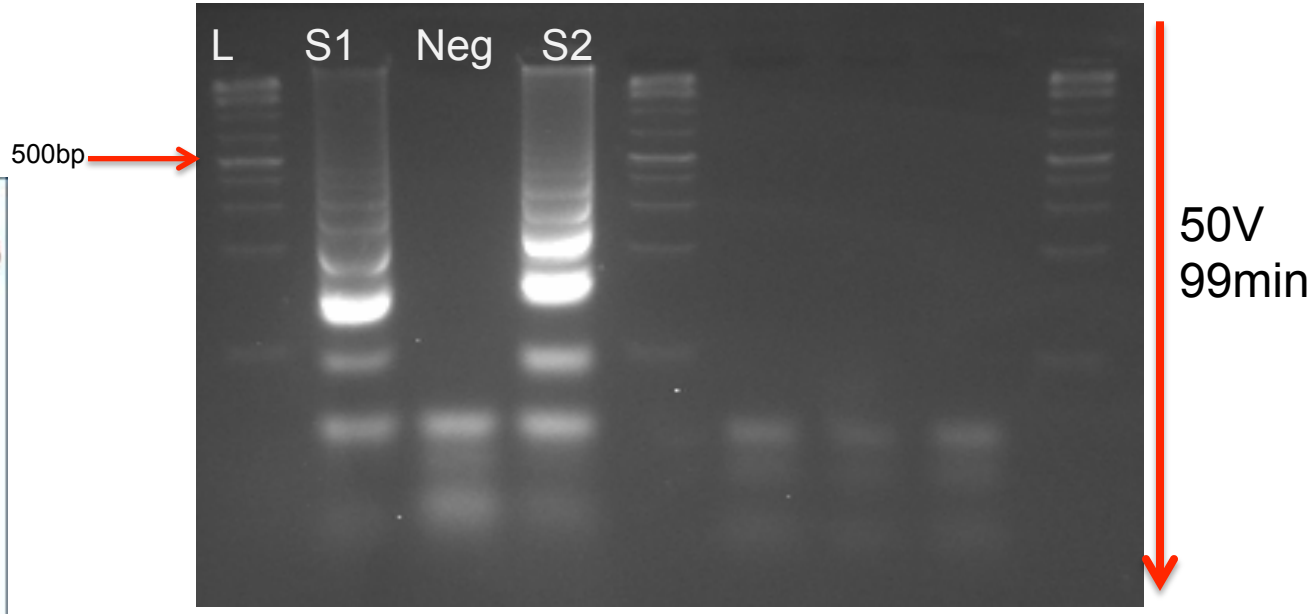
KEY

L- 1kb ladder

Phytophtora Diagnosis Using LAMP

1.5% Agarose Gel 12th May 2016





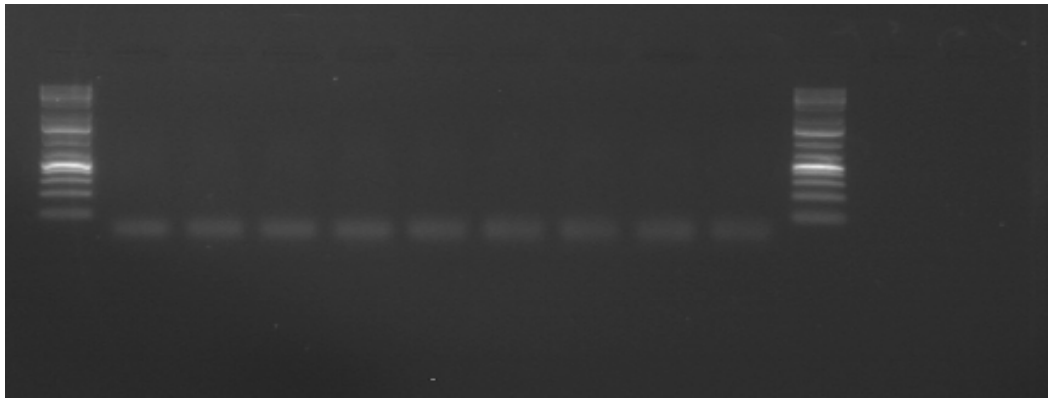
KEY

L- 1kb ladder

PCR Optimization for Mg Titration Rubisco PCR

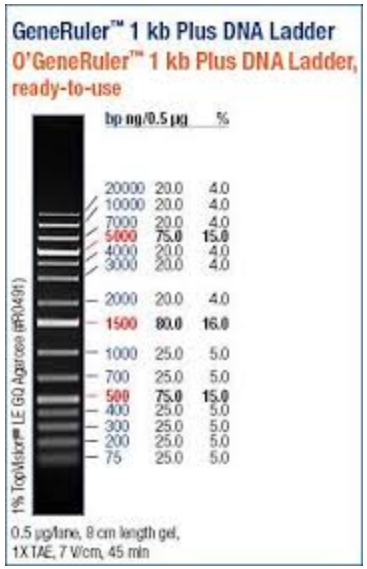
1.5% Agarose Gel 13th May 2016

L 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 Blank



100V
99min

KEY
L- 1kb ladder



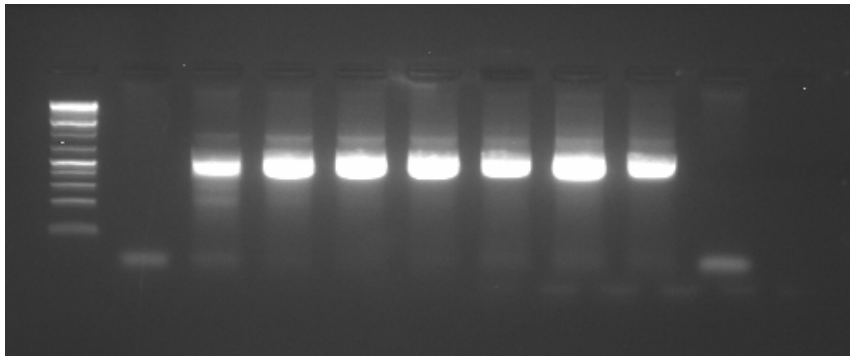
PCR Did not work, Why? DNA issues?
Plan: A repeat PCR set up with DNA of good concentration/quality

Repeat of PCR Optimization: Mg Concentration (mM) Titration Rubisco PCR

1.5% Agarose Gel

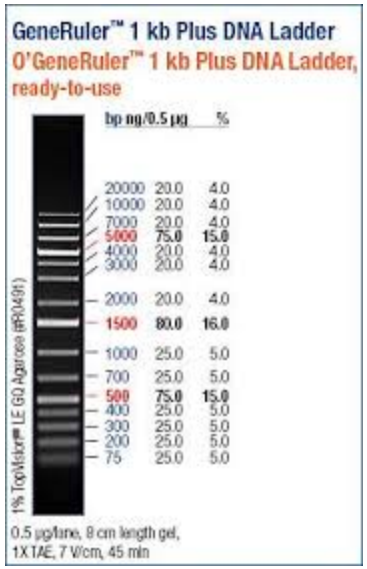
13th May 2016

L 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 Blank



100V
45 min

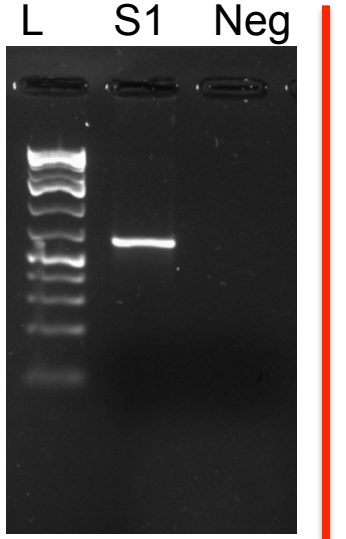
KEY
L- 1kb ladder



What is the effect of Magnesium here? Discuss optimal concentration based on product obtained.

Ethanol Precipitation and Purification

1.5% Agarose Gel 13th May 2016

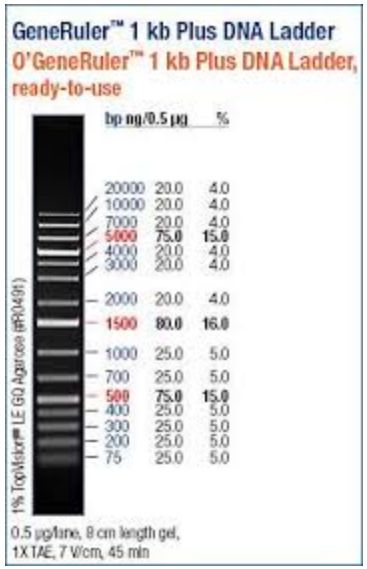


500bp

100V 45min

KEY

L- 1kb ladder



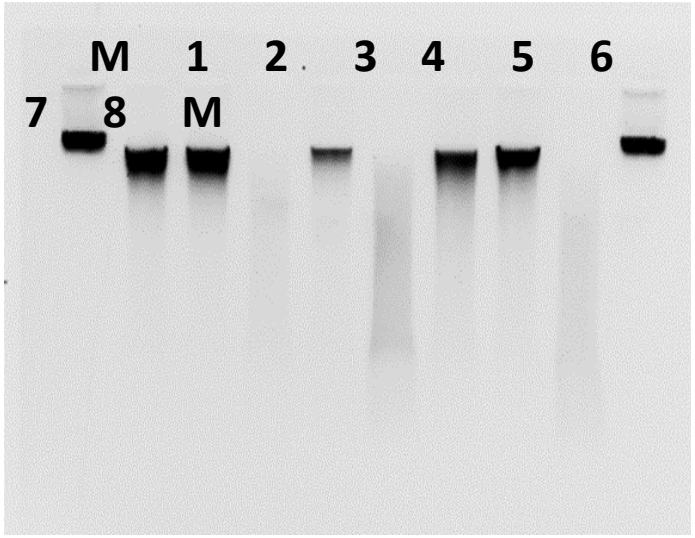
Sample ID	ng/ul	260/280	260/230
Pure PCR prod	38.68	1.69	2.45

GROUP 2

Mbuzi

Martina Kyalo

Genomic DNA



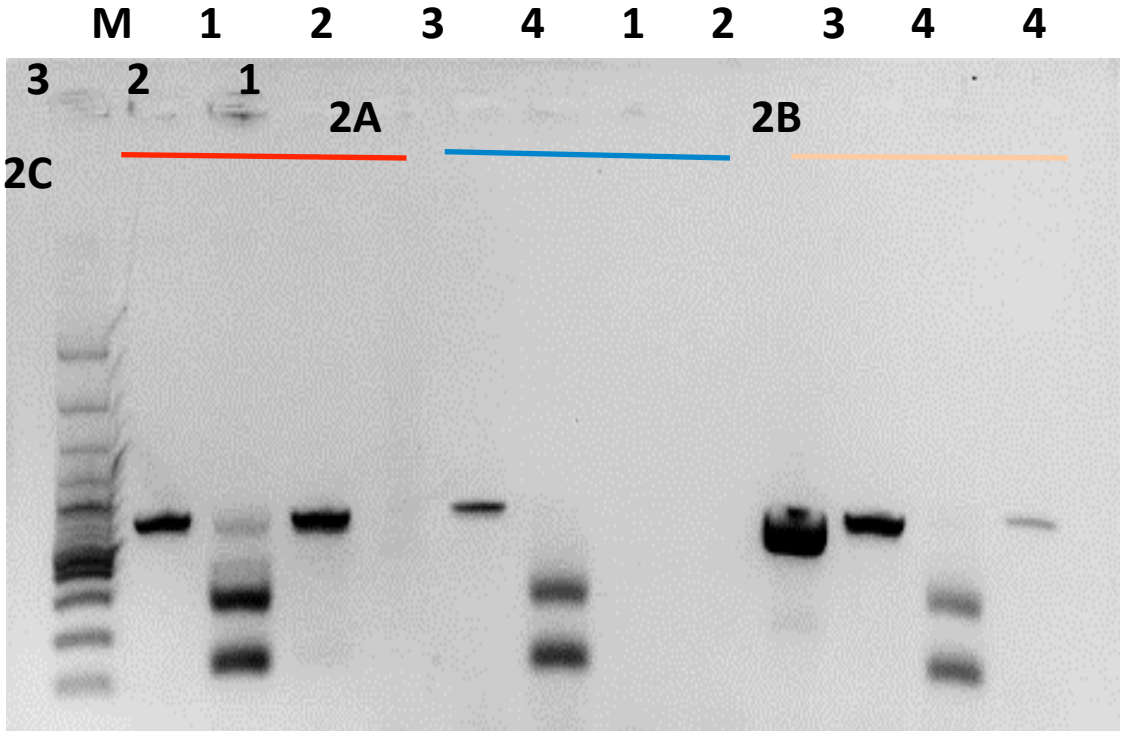
Sample ID	NA Conc.	Unit	260/280	260/230
Mulatu	84.2	ng/µl	1.79	0.49
Francine	139.3	ng/µl	1.83	1.66
Margarite	122.7	ng/µl	1.82	0.35
Elsie	56.2	ng/µl	1.47	0.48
Innocent	153.4	ng/µl	1.82	0.98
Tina-TK	376.8	ng/µl	1.84	1.91
Tina-P	98.8	ng/µl	1.81	0.6
Onyango	48.9	ng/µl	1.57	0.23

Key

1. **TK**
2. **P**
3. **MN**
4. **SF**
5. **ES**
6. **MU**
7. **IH**
8. **OA**

M λ DNA

Phytophthora diagnostic PCR

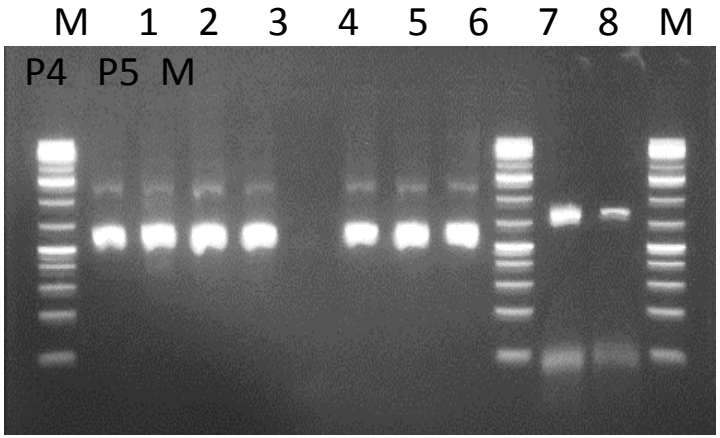


Key

1. Diseased
2. Healthy
3. Mycelia
4. NTC

M 100 bp DNA ruler

PCR PRODUCT PURIFICATION



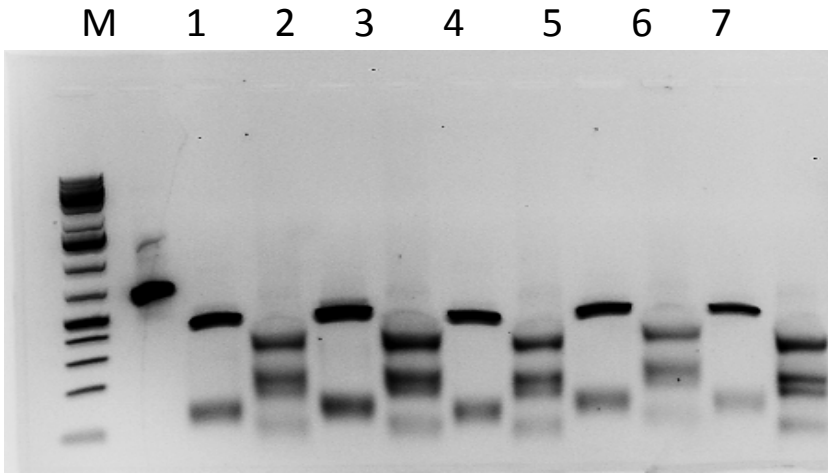
Key

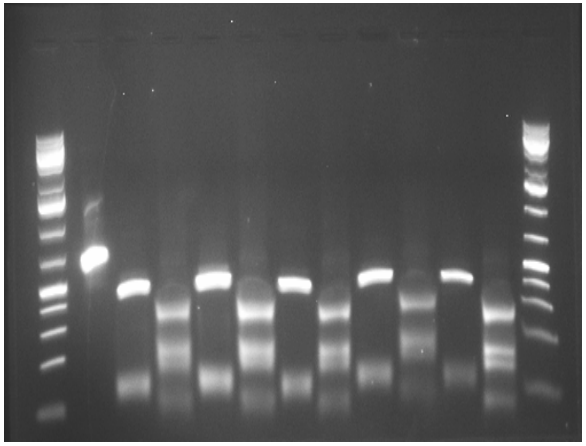
1. TK
2. P
3. MN
4. SF
5. ES
6. MU
7. IH
8. OA

M. 1Kb+ ruler

P4 & P5. Phytophthora PCR products

RESTRICTION DIGESTION OF RbCLa PCR PRODUCT





Key

1.	**Uncut RbCLa PCR fragment**
2, 4, 6, 8, 10	**MseI digestion**
3, 5, 7, 9, 11	**Sau3AI digestion**
M.	**1Kb+ ruler**

DNA purification

Sample ID	NA Conc.	Unit	260/280	260/230
IH_{CP}	153.4	ng/µl	1.82	0.98
IH_{EP}	145.3	ng/µl	1.79	2.34
TK_{CP}	376.8	ng/µl	1.84	1.91
TK_{EP}	551.6	ng/µl	1.82	2.19

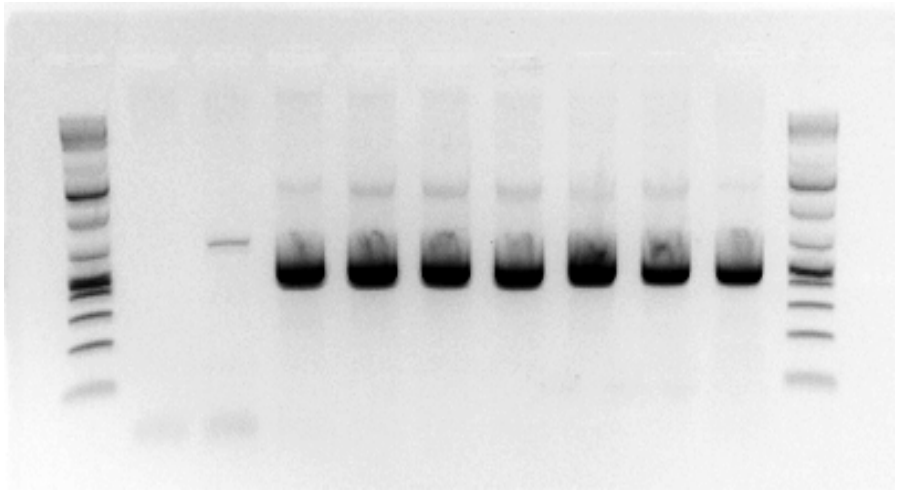
Key

CP column purification

EP ethanol purification

Magnesium chloride titration

M 1 2 3 4 5 6 7 8 9 M



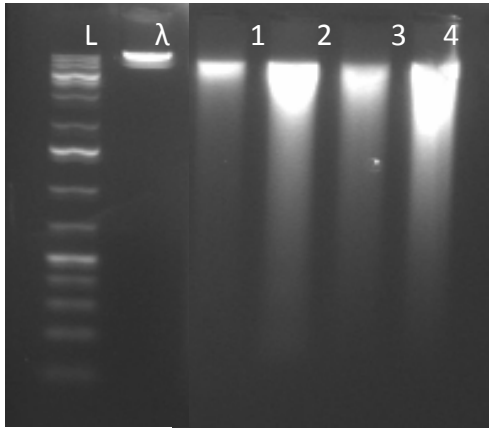
Key

1. 0 mM
2. 0.5 mM
3. 1 mM
4. 1.5 mM
5. 2 mM
6. 2.5 mM
7. 3 mM
8. 3.5 mM
9. 4 mM

GROUP 3
Simba

Pauline Asami

Genomic DNA

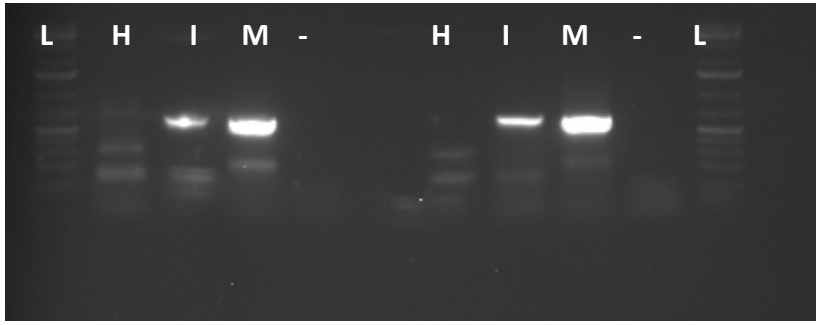


Sample ID	ng/ul	260/280	260/230
L1A	44.87	1.9	0.81
L1Ab	27.01	1.72	0.84
L1B	164.71	1.89	2.23
L1Bb	58.02	1.9	0.59
L2Aa	229.31	1.89	2.3
L2Ab	74.55	1.87	1.83
L2Ba	541.02	1.82	2.47
L2Bb	185.57	1.88	1.96

11th May 2016

L: 1Kb+ Ladder
Λ: Lambda DNA
1-4: DNA from leaf samples

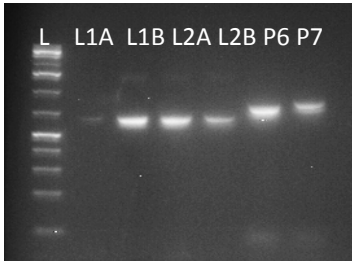
PCR from Quick Extraction

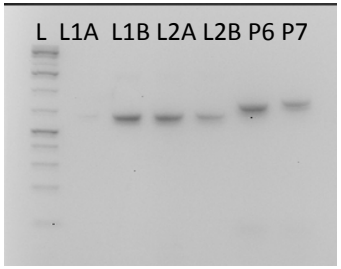


L: 1Kb+ Ladder
H: DNA from healthy leaf sample
I: DNA from infected leaf sample
M: DNA from Mycelia
-: Negative control (Water)

12th May 2016

Leaf and phytophthora PCR purification

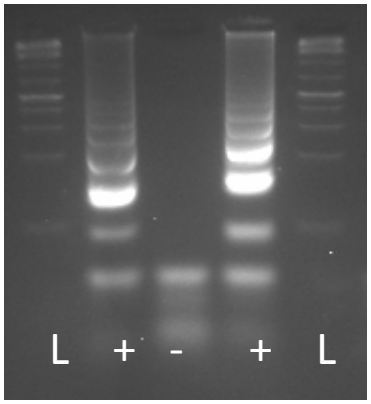




Sample ID	ng/ul	260/280	260/230
L1A	32.6	1.05	0.09
L1B	104.69	1.33	0.28
L2A	79.04	1.12	0.28
L2B	63.61	1.23	0.16

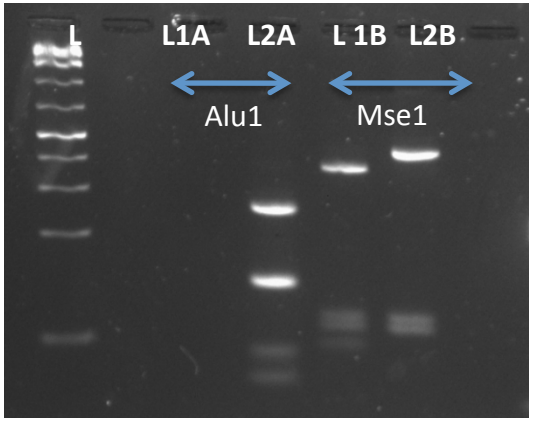
L1A-L2B: Purified PCR products (Leaf samples)
P6-P7: Purified Phytophthora sample 6,7

LAMP PCR Gel



L: 1Kb+ Ladder
+: Positive phytophthora sample 7
-: NWC

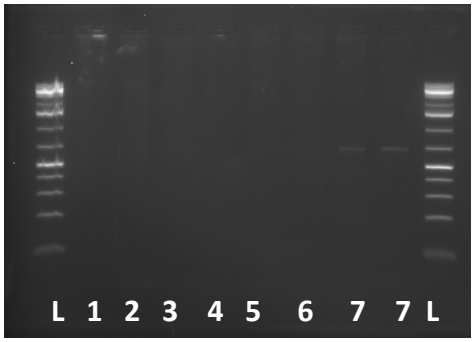
PCR-RFLP RUBISCO

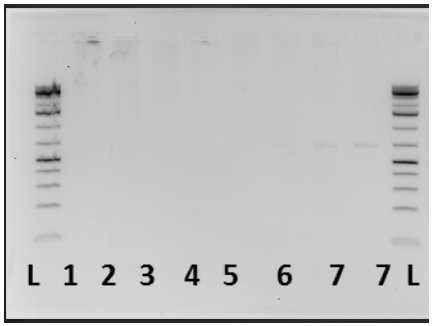


L: 100bp ladder
L1A-L2B: Purified PCR products

Ethanol precipitation of Mg titration results on 1.5% agarose gel

13th May, 2015



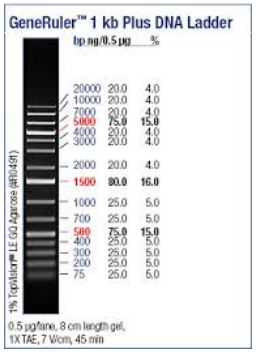


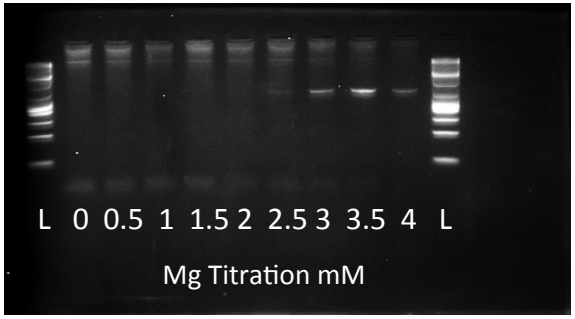
100volts
45 Mins

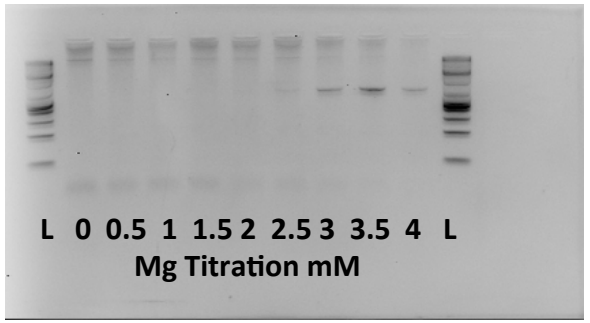
Sample ID	ng/ul	260/280	260/230
G3 C	66.46	1.63	2.38
G3 1	110.66	1.58	2.08
G3 2	45.47	1.63	1.99
G3 3	39.94	1.76	2.04
G3 4	24.75	1.65	2.11
G3 5	26.4	1.65	1.91
G3 6	29.72	1.72	2.05
G3 7	1.83	-1.32	-0.3
G3 7.	18.86	1.67	1.62

Mg^{2+} titration

13th May, 2016







Sample conc. of DNA used: 185.6ng/ul. The more the conc. of DNA sample the more Mg^{2+} required.

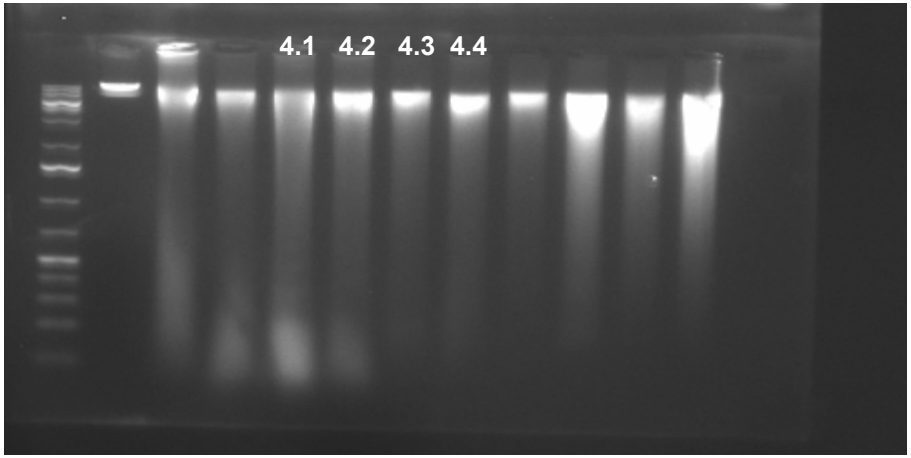
GROUP 4
NDOVU

Collins Mutai

Genomic DNA-Animal

First Elution

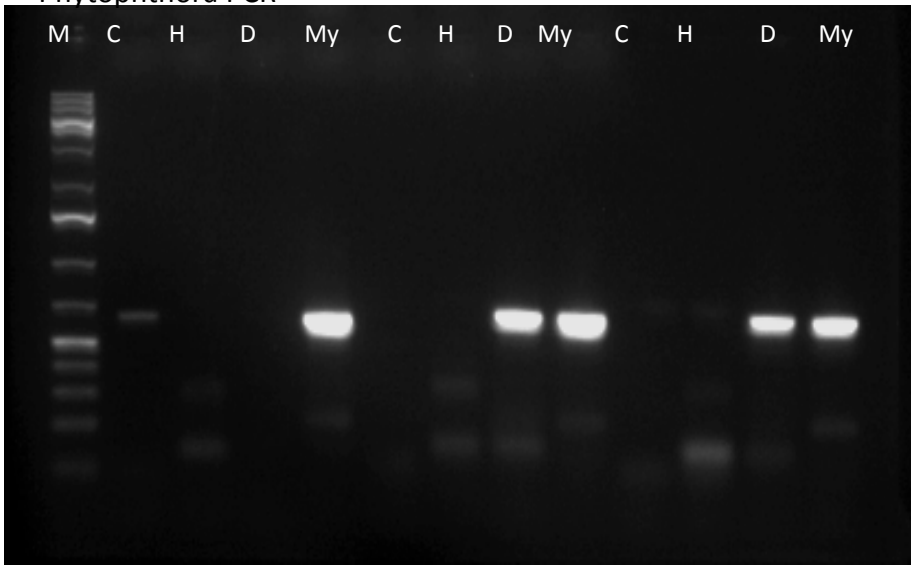
Samples	Concentration (ng/ul)	260/280	260/230
4.1	374.3	2.06	2.09
4.2	87.1	2.04	2.18
4.3	31.7	1.53	0.3
4.4	59.5	1.93	1.53



Second Elution

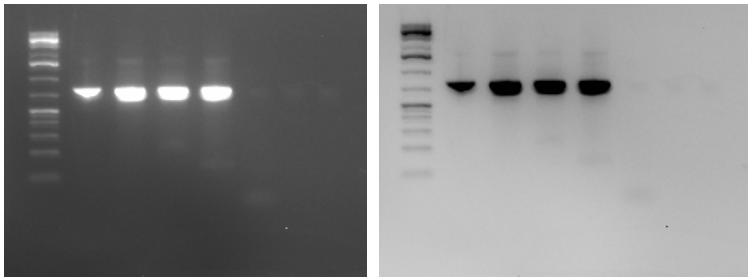
Samples	Concentration (ng/ul)	260/280	260/230
4.1.1	162.2	2.04	1.81
4.2.2	14.2	1.81	1.61
4.3.3	49.6	1.54	0.44
4.4.4	58.1	1.84	1.53

Phytophthora PCR

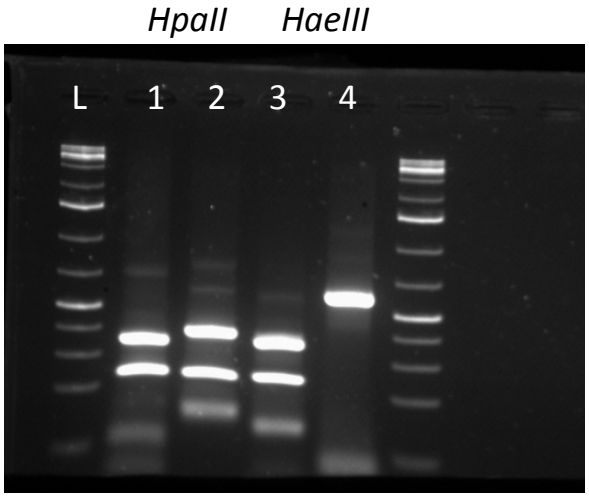


PCR purified CO1 products

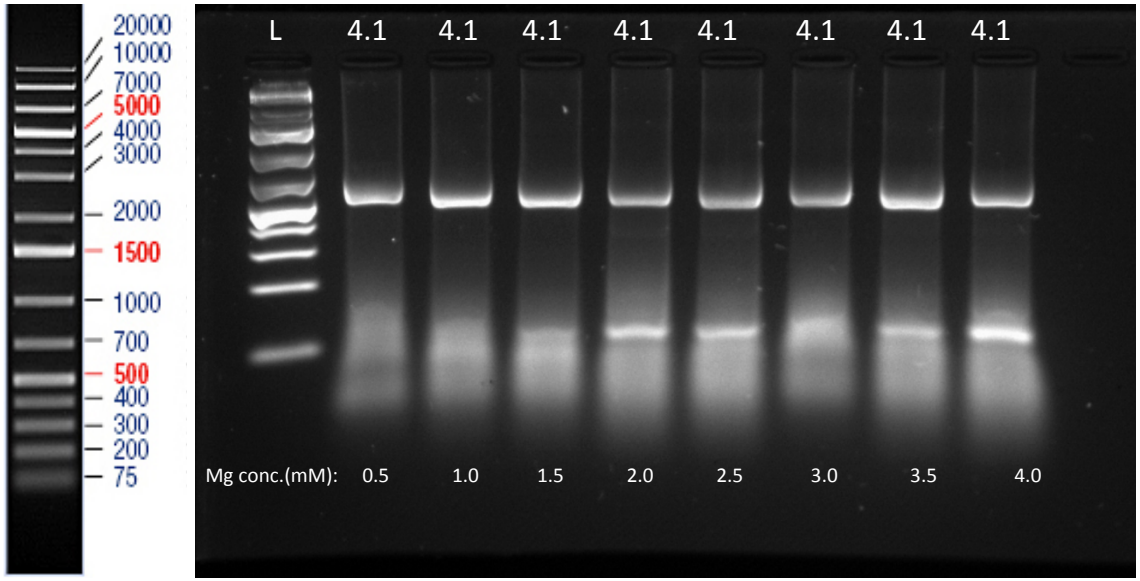
L 1 2 3 4



CO1 restriction digest



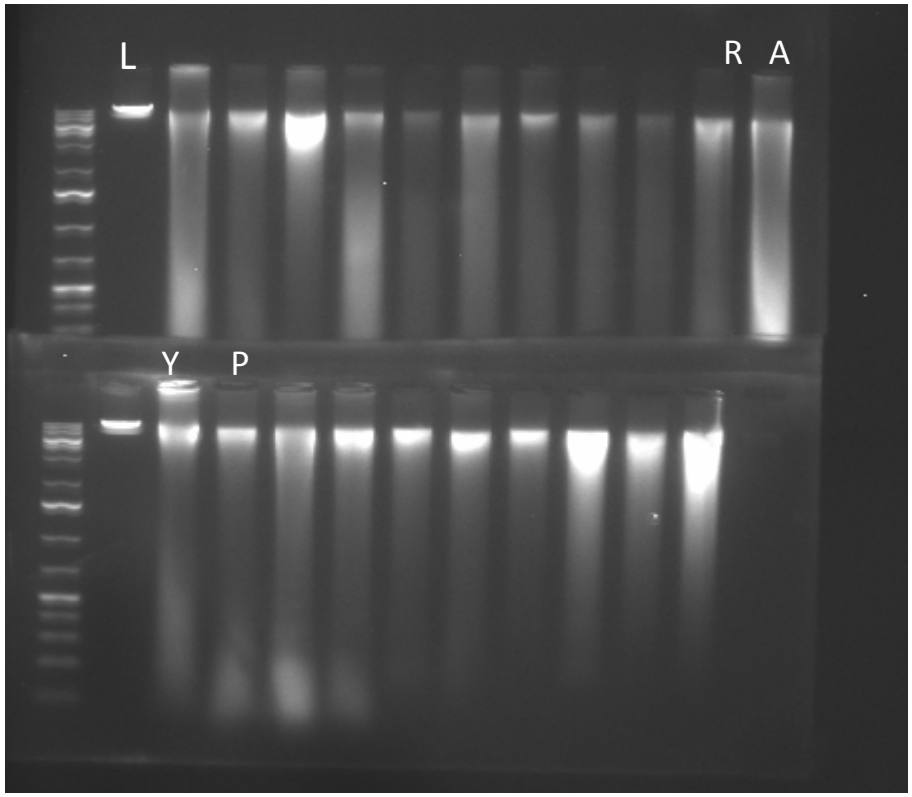
Magnesium Titration



GROUP 5
Mahindi

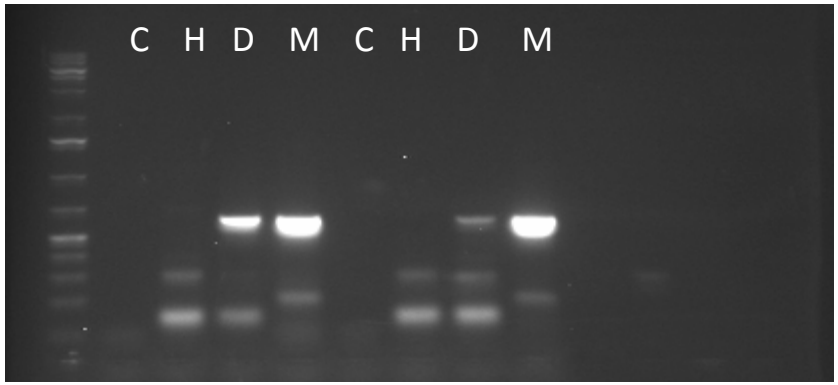
Leah Kago

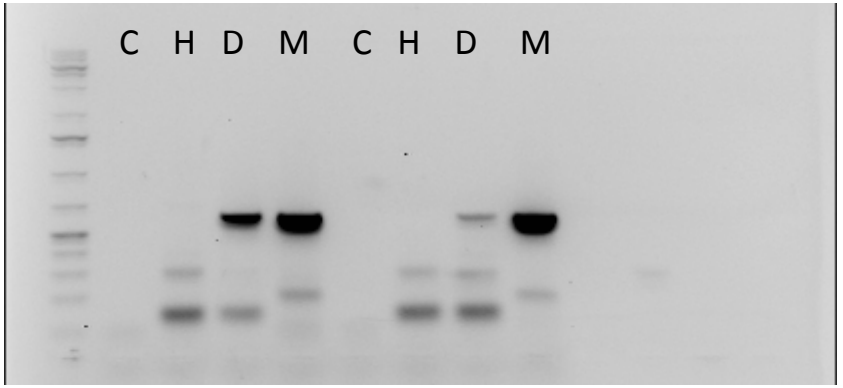
gDNA from animal tissue



Group 5	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
1Y	205.5	ng/µl	4.11	2.02	2.03	2.29
p1	249.4	ng/µl	4.99	2.422	2.06	2.1
5'2 A	532.2	ng/µl	10.6	5.247	2.03	1.99
5'7 R	99.9	ng/µl	2	1.019	1.96	2.28

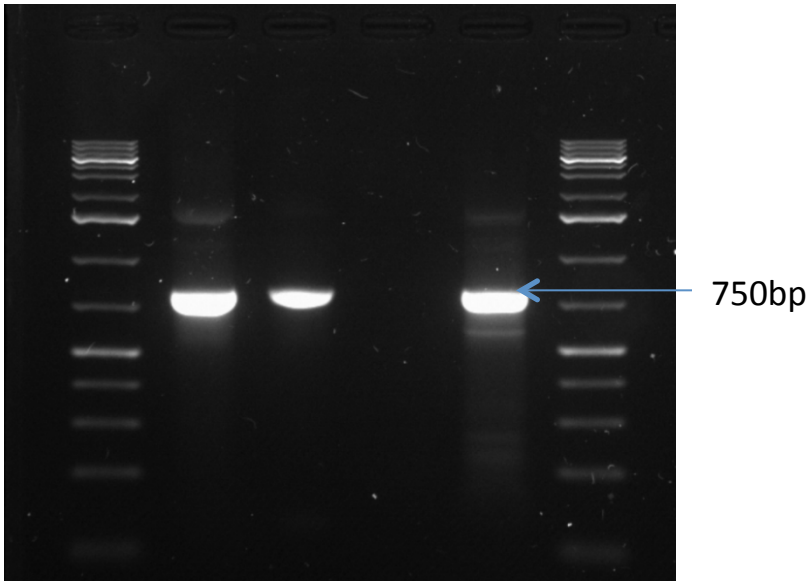
Quick PCR Phytophthora





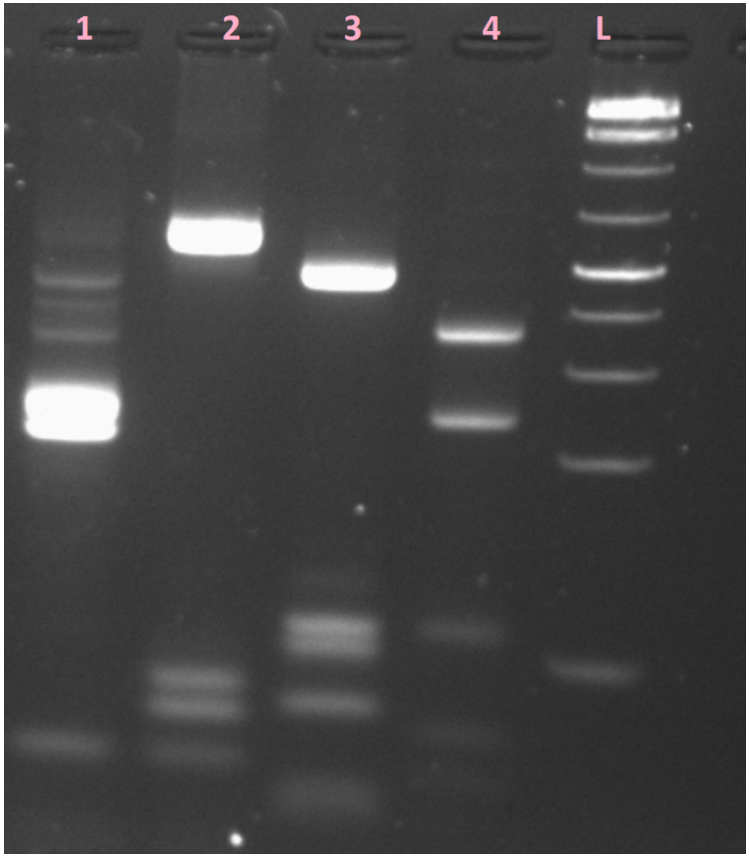
C=Control
H= Healthy
D=Diseased
M=Mycelium

Purified CO1 PCR products

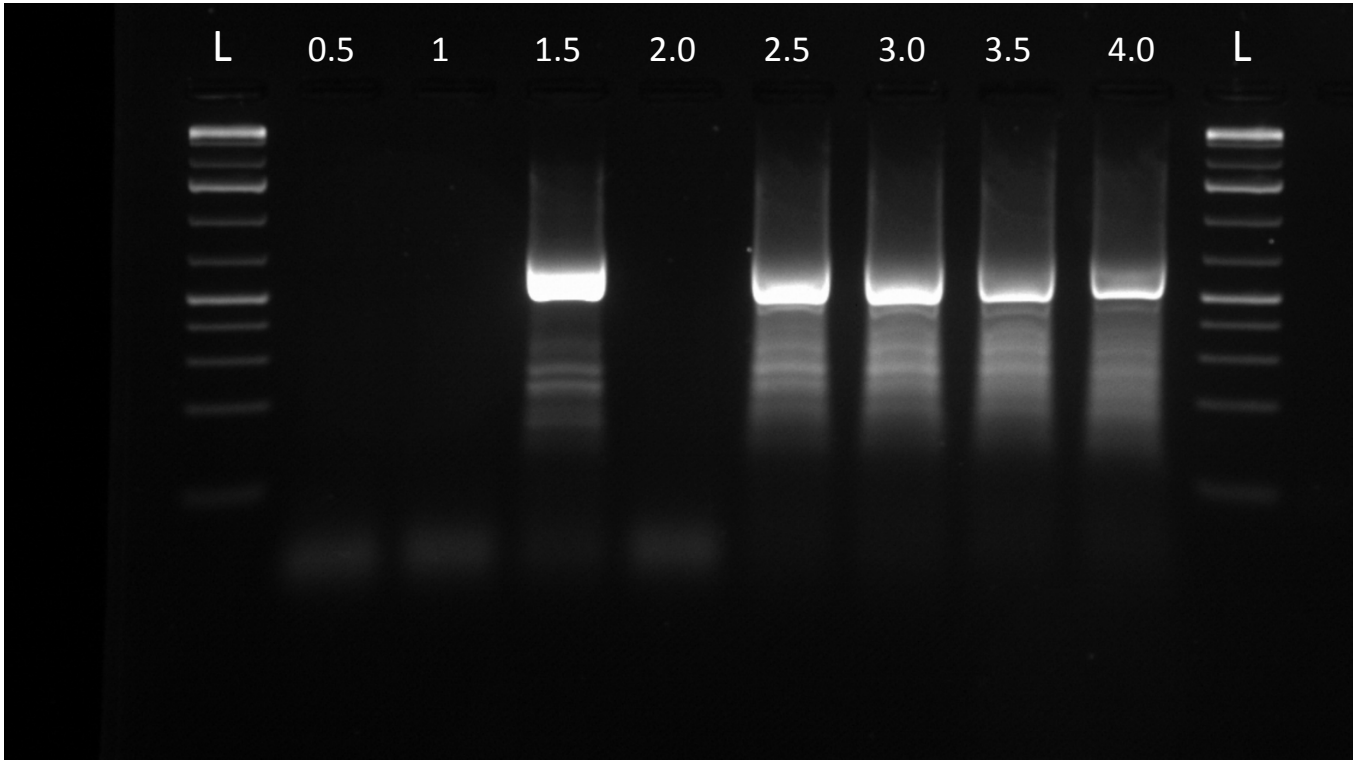


Group 5	Sample ID	Nucleic Acid Conc.	Unit	260/280	260/230
1	5'2	20.1	ng/µl	1.79	0.42
2	5'7	56.1	ng/µl	1.81	0.2
3	P1	86.7	ng/µl	1.76	0.81
4	Y1	62.7	ng/µl	1.78	0.31

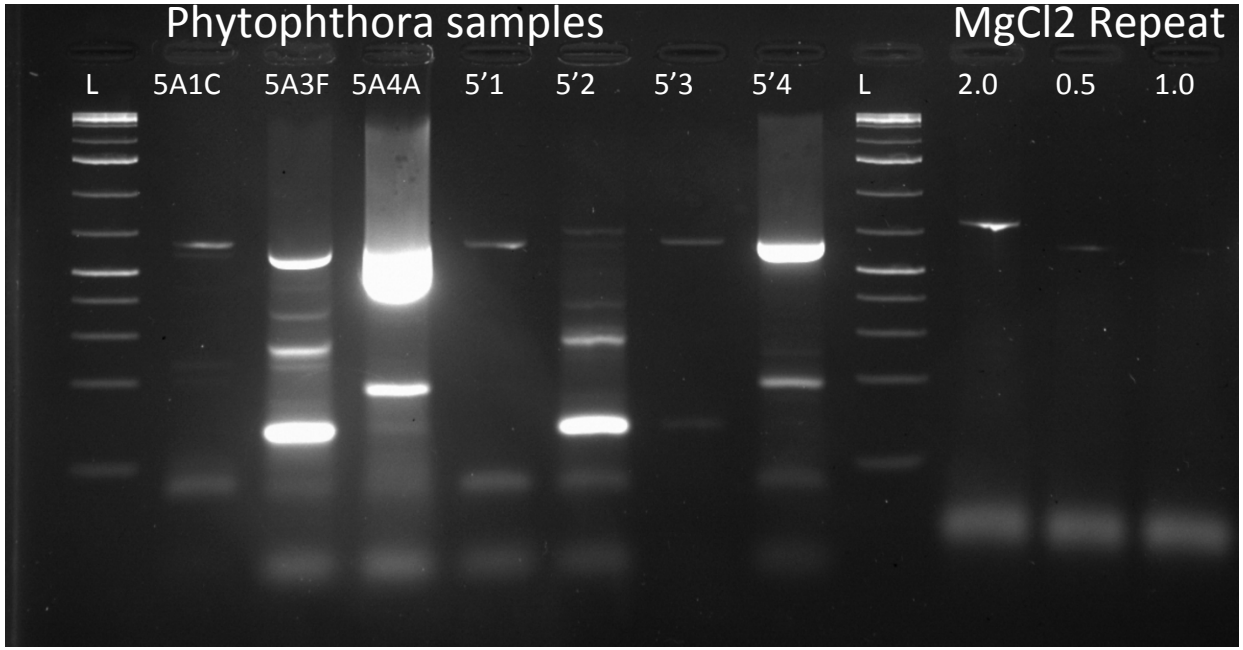
PCR RFLP CO1



$MgCl_2$ Titration



Phytophthora PCR products EtOH precipitation

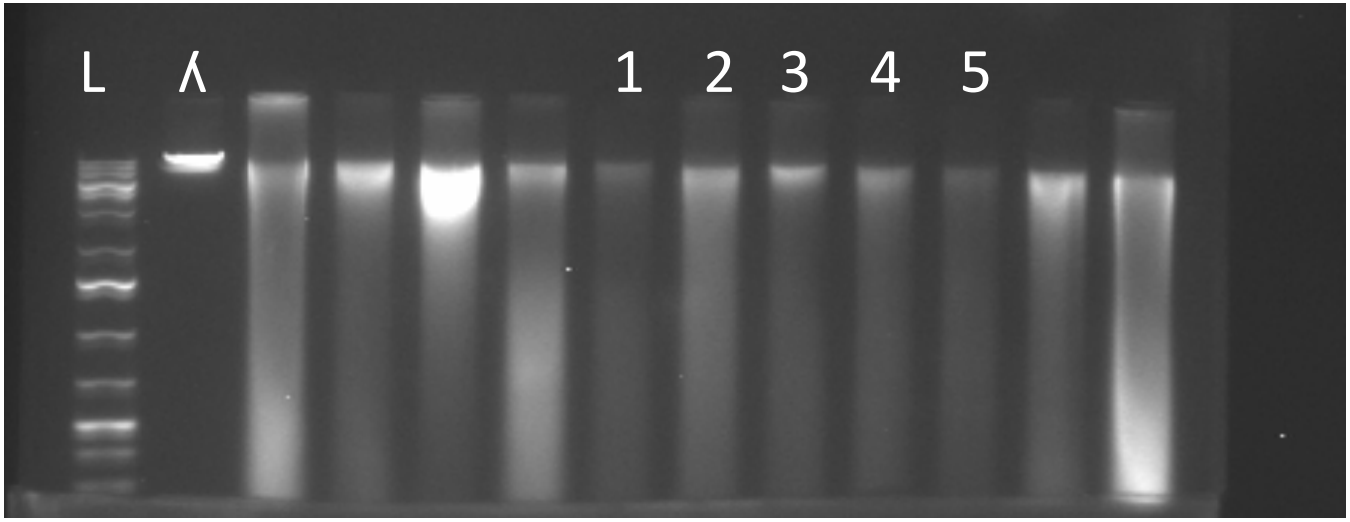


Note:Mgcl2 titration samples M1,M2 & M4 repeated for confirmation

#	Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
1	5A1C	6.8	ng/µl	0.14	0.11	1.19	0.21
2	5A3F	71.1	ng/µl	1.42	0.88	1.62	1.25
3	5A4A	138.3	ng/µl	2.77	1.66	1.67	1
4	5'1	16.6	ng/µl	0.33	0.2	1.66	0.95
5	5'2	16.8	ng/µl	0.34	0.2	1.7	1.38
6	5'3	0.9	ng/µl	0.02	0.01	1.61	0.44
7	5'4	52	ng/µl	1.04	0.62	1.69	1.74
8	5'3Repeat	0.8	ng/µl	0.02	0	15.18	0.58

GROUP 6
Maharagwe
Vincent Njunge

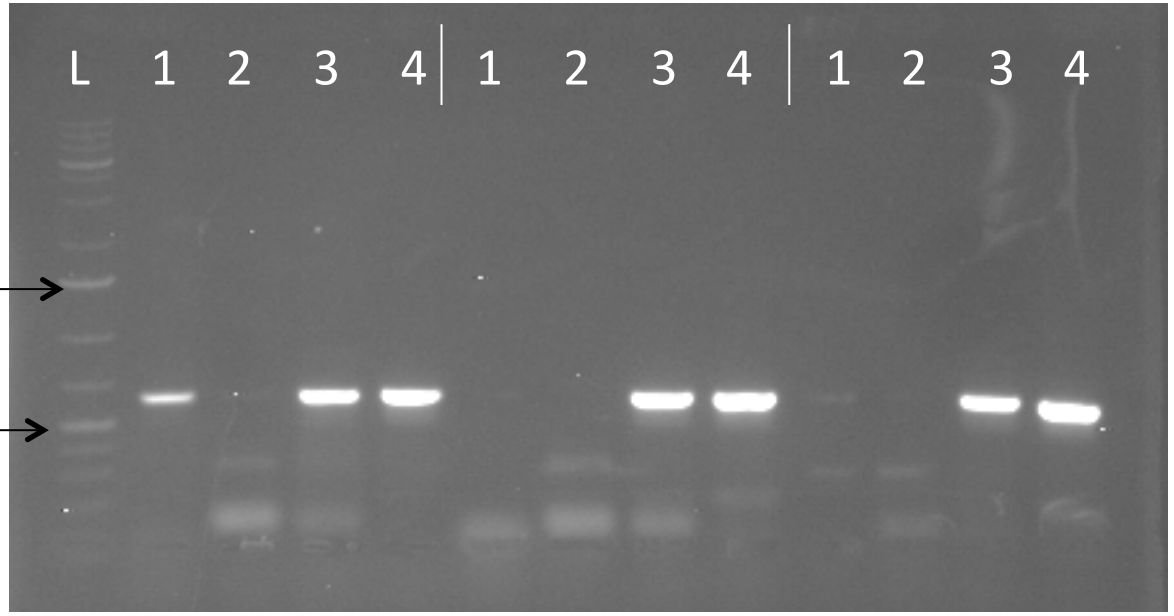
Genomic DNA



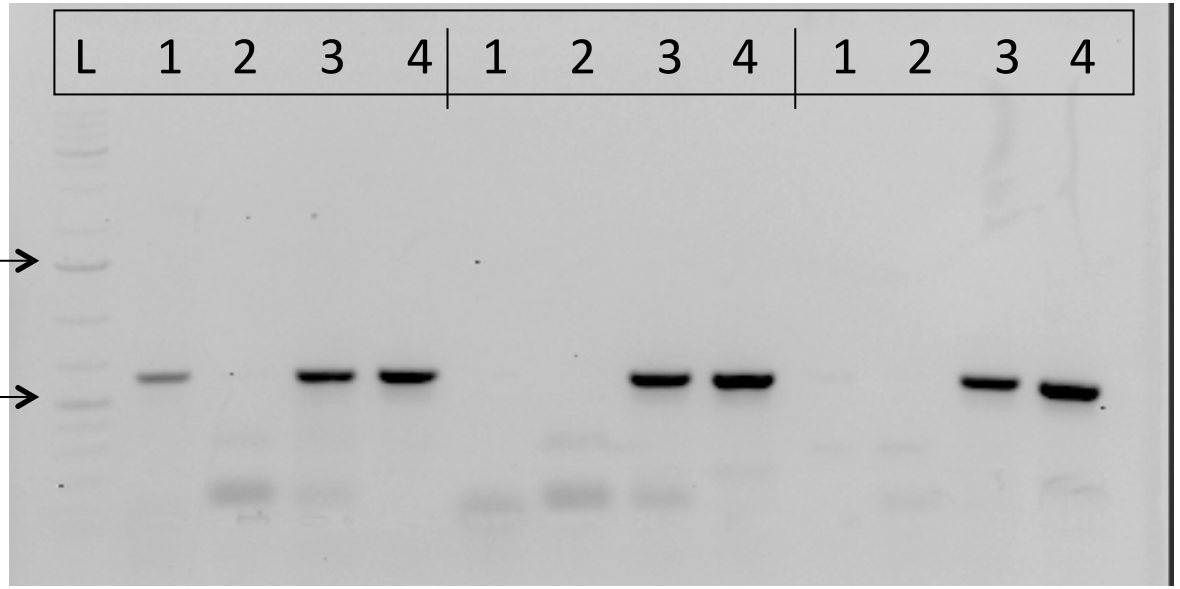
Sample No	Sample ID	Conc ng/ µl	260/280	260/230
1	Arsene_1	103.15	2.04	1.47
2	Urgesa_1	129.31	2.03	1.99
3	Abdi_1	50.22	2.02	1.65
4	Rose_1	71.28	2.03	1.61
5	Eunice_4	65.47	1.7	0.69

Quick PCR Phytophthora

Group 6 PCR Results

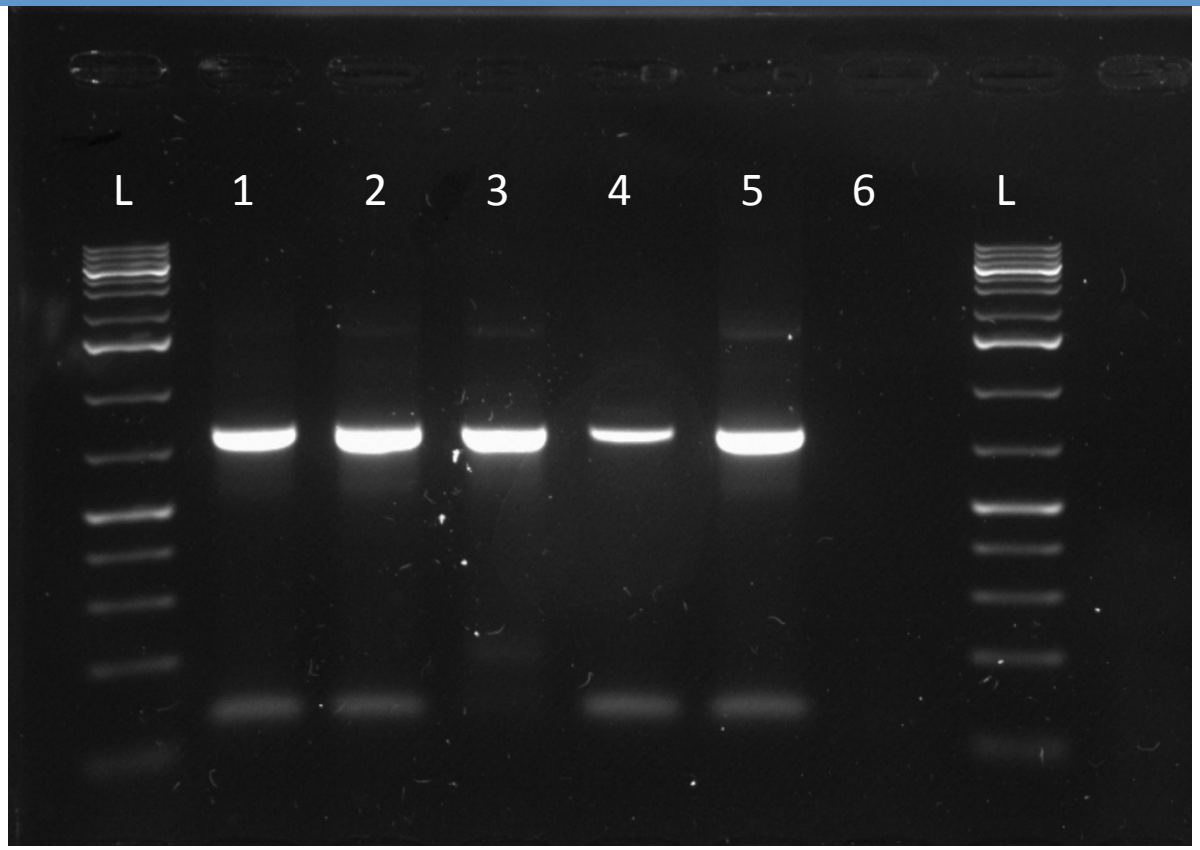


1. Healthy
2. Water
3. Disease
4. Cultured



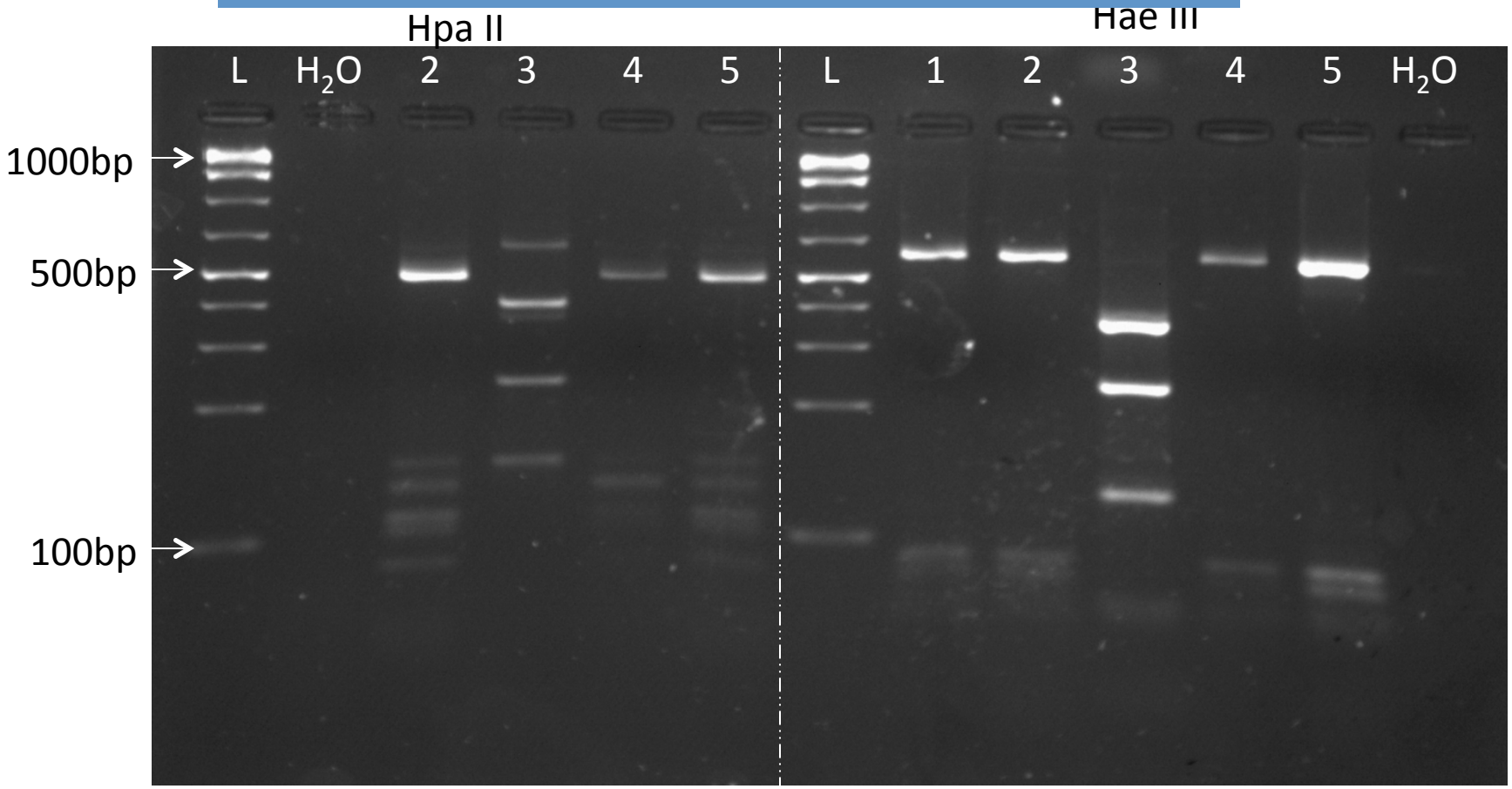
1. Healthy
2. Water
3. Disease
4. Cultured

Purified PCR products CO1

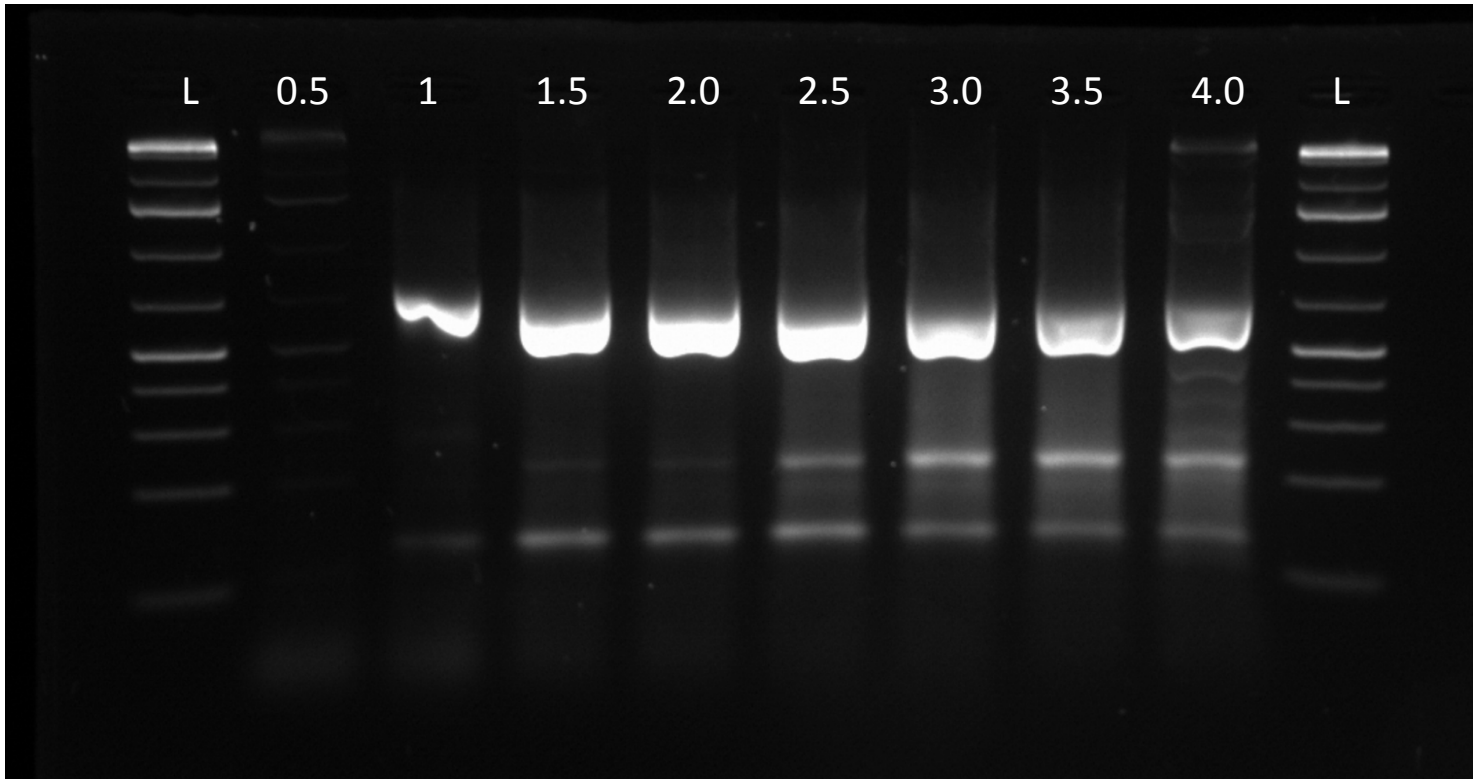


#	Sample ID	Nucleic Acid Conc.	260/280	260/230
1	Arsene	39.4	1.84	0.45
2	Urgesa	44.2	1.81	0.48
3	Eunice	38.5	1.79	1.22
4	Abdi	17.1	1.91	0.18
5	Rose	36.5	1.84	0.75
6	H_20	8.5	1.9	0.19

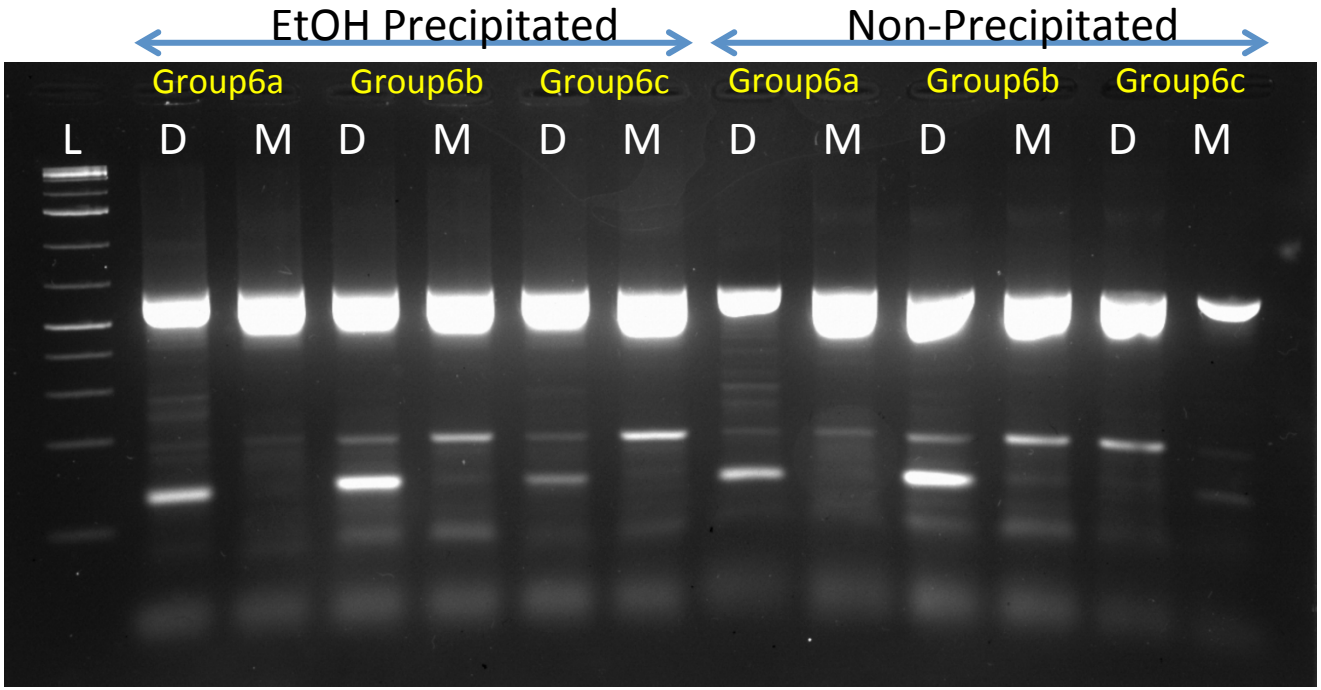
CO1 Products Restriction Digest



$MgCl_2$ Titration



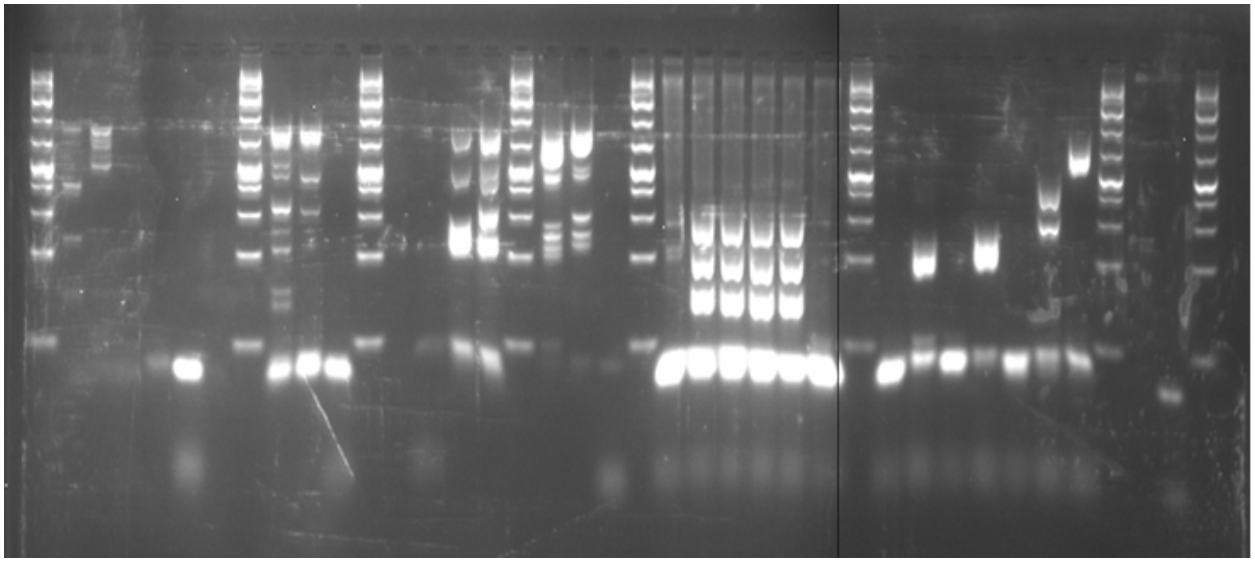
Ethanol Precipitation of Phytophthora PCR Products



D-Diseased
M-Mycelia

#	Sample ID	Nucleic Acid Conc.	260/280	260/230
1	**6a diseased**	481.4	1.73	2.01
2	**6a mycelia**	338.3	1.76	1.81
3	**6b diseased**	548.4	1.7	1.38
4	**6b Mycelia**	658.8	1.69	1.23
5	**6c diseased**	463.4	1.65	0.91
6	**6c Mycelia**	777.8	1.66	0.88
7	**Unprecpd 6a control (H20)**	337.6	1.74	1.55
8	**Unprecpd 6a diseased**	401.2	1.72	1.48
9	**Unprecpd 6b diseased**	408.1	1.72	1.5
10	**Unprecpd 6c diseased**	417.9	1.71	1.41
11	**Unprecpd 6c Mycelia**	394.7	1.72	1.46

PCR-RFLP Phytophthora for all samples



LAMP Gel Image Group 5 and 6