LAB TRAINING EUPHRESCO PHYTFIRE.

LISBON, 3-4 OCTOBER 2012.

Samples:

Set of samples for each of the following three bacterial pathogens:
***E. amylovora* (A), *E. pyrifoliae* (Py), *E. piriflorinigrans* (Pfn)**

Set of samples	Code used on tubes	
Bacterial suspensions of pure cultures in water	**S-A** **S-Py** **S-Pfn**	**Bacterial concentration (cfu/ml):** 10^6, 10^5, 10^4, 10^3, 10^2, 10
Plant material (pear leaves and shoots) extract spiked with bacterial suspensions	**P-A** **P-Py** **P-Pfn**	**Bacterial concentration (cfu/ml) in plant material:** 10^6, 10^5, 10^4, 10^3, 10^2, 10
DNA extractions of plant material (leaves and shoots) extract spiked with bacterial suspensions, according to Taylor *et al.* (2001)	**T-A** **T-Py** **T-Pfn**	**DNA extractions of samples spiked at bacterial concentration (cfu/ml):** 10^6, 10^5, 10^4, 10^3, 10^2, 10

Controls:

Positive and negative controls could be:

Negative controls	Positive controls
1) Water used for the suspensions 2) Water used for PCR mix 3) Healthy plant material extract 4) Taylor extraction buffer	1) 10^6 cfu/ml bacterial suspension 2) Plant material spiked with 10^6 cfu/ml bacterial suspension 3) DNA extract from target organism

REAL TIME PCR for *E. amylovora* (Pirc *et al.*, 2009)

Date _____

Components	**x 1**	
Ultrapure water	2,5 µl	
2X PCR Master Mix	12,5 µl	
Primer Ams 116F (10µM)	2,25 µl	
Primer Ams 189R (10µM)	2,25 µl	
Probe Ams 141T (10µM)	0,5 µl	
Aliquot to	**20 µl**	
Sample DNA	5 µl	

Samples:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

PCR Conditions:

95° C 10 min

95° C 15 sec
60° C 1 min
} 40 cycles

REAL TIME PCR for *E. amylovora* (Gottsberger *et al.*, 2010)

Date _____

Components	**x 1**	
Ultrapure water	7,95 µl	
2X PCR Master Mix	10 µl	
Primer hpEaF (10µM)	0,5 µl	
Primer hpEaR (10µM)	0,5 µl	
Probe hpEaP (10µM)	0,05 µl	
Aliquot to	**19 µl**	
Sample DNA	1 µl	

Samples:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

PCR Conditions:

95° C 10 min

95° C 15 sec
60° C 1 min
} 40 cycles

CONVENTIONAL PCR for *E. amylovora* (Taylor *et al.*, 2001)

Date _____

Components	**x 1**	
Ultrapure water	14,3 µl /14,1 µl	
10X Thermopol buffer	2,5 µl	
$MgCl_2$ (50 mM)	0,75 µl	
dNTPs (10 mM)	0,25 µl	
Primer G1-F (10µM)	1 µl	
Primer G2-R (10µM)	1 µl	
Taq polymerase (5U/µl)	0,2 µl /0,4 µl	
Aliquot to	**20 µl**	
Sample DNA	5 µl	

Samples:

1		11		21		31	
2		12		22		32	
3		13		23		33	
4		14		24		34	
5		15		25		35	
6		16		26		36	
7		17		27		37	
8		18		28		38	
9		19		29		39	
10		20		30		40	

PCR Conditions:

95° C	3 min	
94° C	30 sec	40 cycles
60° C	30 sec	
72° C	1 min	
72° C	5 min	
15 °C	∞	

Expected product size: 187 bp

Agarose gel (%): 2 %

LAMP PROTOCOL for *E. amylovora* (amsL gene)

Date _____

Components	**X1**	
10x Thermopol buffer	5 µl	
dNTPs (10 mM)	5 µl	
$MgSO_4$ (100mM)	2 µl	
BSA (10 mg/ml)	2 µl	
ALB FIP (100 µM)	1,2 µl	
ALB BIP (100 µM)	1,2 µl	
ALB F (10 µM)	1 µl	
ALB B (10 µM)	1 µl	
Bst DNA polymerase (8U/(100 µl)	2 µl	
Ultrapure water	24,6 µl	
Sample DNA	5 µl	

Samples:

Samples	**Results**

LAMP Conditions:

65° C 60 min

CONVENTIONAL PCR for *E. pyrifoliae* (Wensing *et al.*, 2011)

Date _____

Components	**x 1**	
Ultrapure water	36,3 µl /36,1 µl	
10X Thermopol buffer	5 µl	
$MgCl_2$ (50 mM)	1,5 µl	
dNTPs (10 mM)	1 µl	
Primer EPPSGL1646 (10µM)	0,5 µl	
Primer EPPSGL2698c (10µM)	0,5 µl	
Taq polymerase (5U/µl)	0,2 µl /0,4 µl	
Aliquot to	**45 µl**	
Sample DNA	5 µl	

Samples:

1		11		21		31	
2		12		22		32	
3		13		23		33	
4		14		24		34	
5		15		25		35	
6		16		26		36	
7		17		27		37	
8		18		28		38	
9		19		29		39	
10		20		30		40	

PCR Conditions:

95° C 3 min

94° C 30 sec
57° C 30 sec
72° C 1 min
} 40 cycles

72° C 5 min

15° C ∞

Expected product size: 1052 bp

Agarose gel (%): 2 %

CONVENTIONAL PCR for *E. pyrifoliae* (Kim *et al.*, 2001)

Date _____

Components	**x 1**
Ultrapure water	35,3 µl /35,1 µl
10X Thermopol buffer	5 µl
$MgCl_2$ (50 mM)	1,5 µl
dNTPs (10 mM)	1 µl
Primer CPS1 (10µM)	1 µl
Primer CPS2 (10µM)	1 µl
Taq polymerase (5U/µl)	0,2 µl /0,4 µl
Aliquot to	**45 µl**
Sample DNA	5 µl

Samples:

1		11		21		31	
2		12		22		32	
3		13		23		33	
4		14		24		34	
5		15		25		35	
6		16		26		36	
7		17		27		37	
8		18		28		38	
9		19		29		39	
10		20		30		40	

PCR Conditions:

95° C	3 min	
94° C	30 sec	40 cycles
57° C	30 sec	
72° C	1 min	
72° C	5 min	
15° C	∞	

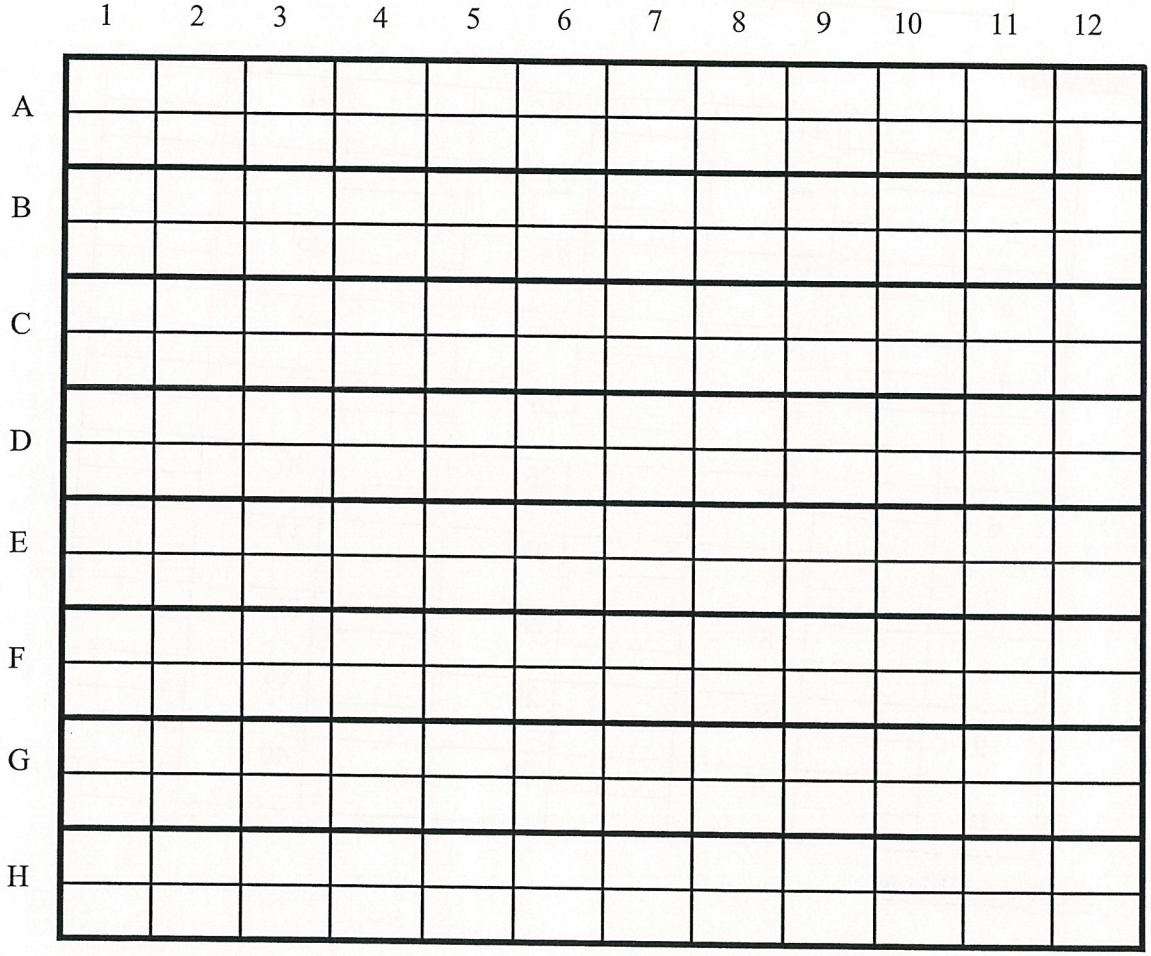
Expected product size: 1200 bp
Agarose gel (%): 2 %

REAL TIME PCR for *E. pyrifoliae* (based on Wensing *et al.*, 2011)

Date _____

Components	**x 1**	
Ultrapure water	7,25 µl	
2X PCR Master Mix	12,5 µl	
Primer EPPSGS1089Q (10µM)	1 µl	
Primer EPPSGS1228Qc (10µM)	1 µl	
Probe EPPSGS (10µM)	0,25 µl	
Aliquot to	**22 µl**	
Sample DNA	3 µl	

Samples:



PCR Conditions:

95° C 10 min

95° C 15 sec
60° C 1 min
} 45 cycles

CONVENTIONAL PCR for *E. piriflorinigrans* (Barbé *et al.*, 2012)

Date _____

Components	**x 1**	
Ultrapure water	34,4 µl /34,2 µl	
10X Thermopol buffer	5 µl	
$MgCl_2$ (50 mM)	3 µl	
dNTPs (10 mM)	0,4 µl	
Primer P37F (10µM)	1 µl	
Primer P37R (10µM)	1 µl	
Taq polymerase (5U/µl)	0,2 µl /0,4 µl	
Aliquot to	**45 µl**	
Sample DNA	5 µl	

Samples:

1		11		21		31	
2		12		22		32	
3		13		23		33	
4		14		24		34	
5		15		25		35	
6		16		26		36	
7		17		27		37	
8		18		28		38	
9		19		29		39	
10		20		30		40	

PCR Conditions:

94° C 3 min

94° C 30 sec
56° C 30 sec } 40 cycles
72° C 45 sec

72° C 10 min

8 ° C ∞

Expected product size: 255 bp

Agarose gel (%): 2 %

REAL TIME PCR for *E. piriflorinigrans* (Barbé *et al.*, 2012)

Date _____

Components	**x 1**	
Ultrapure water	1,2 µl /3,12 µl	
2X PCR Master Mix	6 µl /12,5 µl	
Primer p37F (10µM)	0,8 µl /1,95 µl	
Primer p37R (10µM)	0,8 µl /1,95 µl	
Probe p37 (10µM)	0,2 µl /0,48 µl	
Aliquot to	**9 µl /20 µl**	
Sample DNA	3 µl/5 µl	

Samples:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

PCR Conditions:

95° C 10 min

95° C 15 sec
70° C 1 min
} 40 cycles