EUPHRESCO PHYLIB
Micro arrays for discrimination of phytoplasmas - proof of principle

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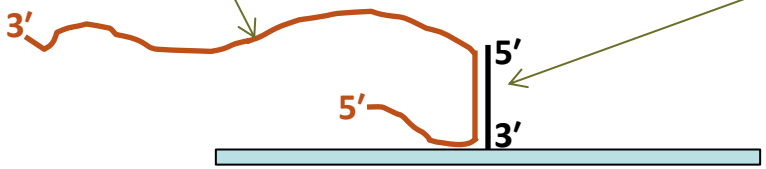
Development of the array

- Probe design
- Primer design
- Testing primers (real-time)
- Testing primer/probe combinations (array)
- Multi-plex PCR

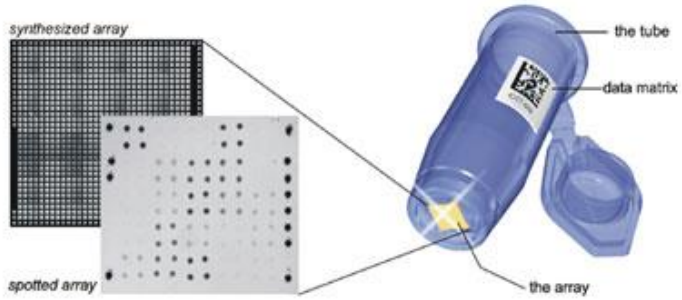
Probe design for the array

biotin-labelled target DNA
(PCR product)

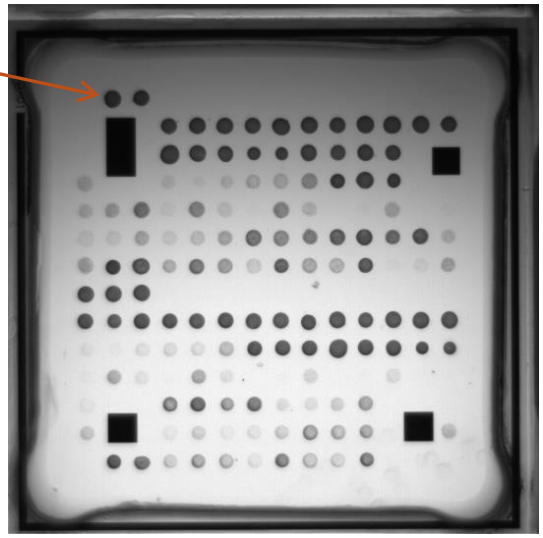
target-specific probe



array surface



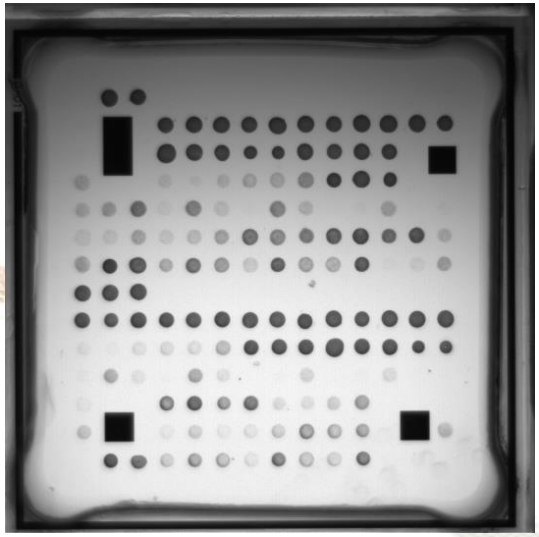
(Alere)



Each spot on the array =
a different probe

Probe design for the array

- (14 x 14) – 18 = 178 spots (89 probes: SecA, 16S, 23S)
- Multiple targets (genes) per species
- Multiple probes per target
- Redundancy = robustness

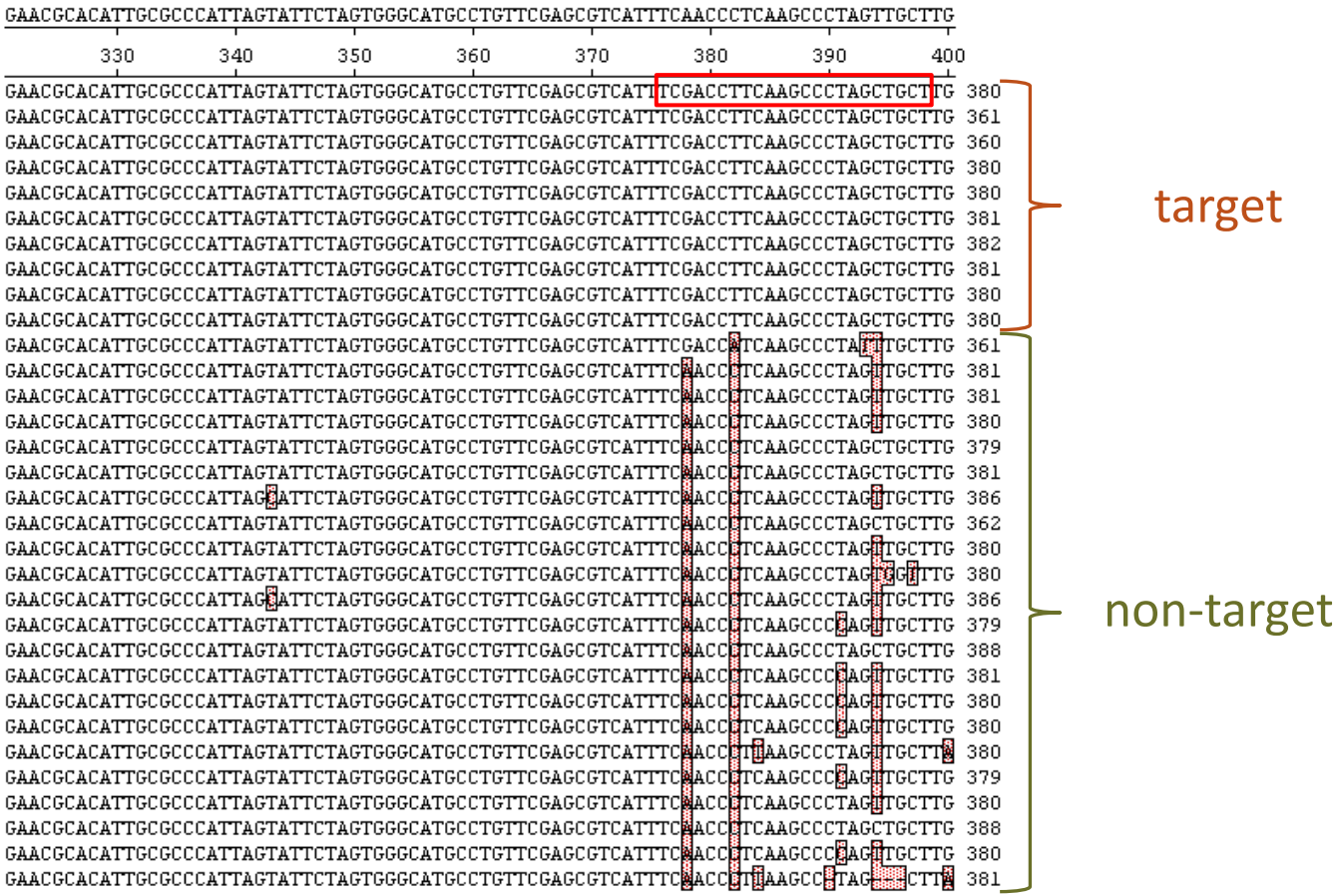


	74	75	76	77	78	79	80	81	82	83	84	85	
44			63	64	65	66	67	68	69	70	71	72	73
43			54	55	56	57	58	59	60	61	62		
42			45	46	47	48	49	50	51	52	53		
28	29	30	31	32	33	34	35	36	37	38	39	40	41
14	15	16	17	18	19	20	21	22	23	24	25	26	27
87	1	2	3	4	5	6	7	8	9	10	11	12	13
73	74	75	76	77	78	79	80	81	82	83	84	85	86
59	60	61	62	63	64	65	66	67	68	69	70	71	72
45	46	47	48	49	50	51	52	53	54	55	56	57	58
31	32	33	34	35	36	37	38	39	40	41	42	43	44
14			23	24	25	26	27	28	29	30			86
13			15	16	17	18	19	20	21	22			87
	1	2	3	4	5	6	7	8	9	10	11	12	

Probe design

Target organism	No. target genes	No. probes for each organism
Ca. L africanus	2	2
Ca. L americanus	2	3
Ca. L asiaticus	3	3
Ca. L crescens	2	2
Ca. L europaeus	1	1
Ca. L solanacearum	3	4
Group I	2	4
Group II	2	6
Group III	2	5
Group IV	2	5
Group V	2	4
Group VI	2	4
Group VII	2	4
Group IX	2	4
Group X	2	7
Group XI	2	5
Group XII	2	6
Group XIII	2	4
Group XXII	2	4
Group XXX	2	4

Probe design for the array



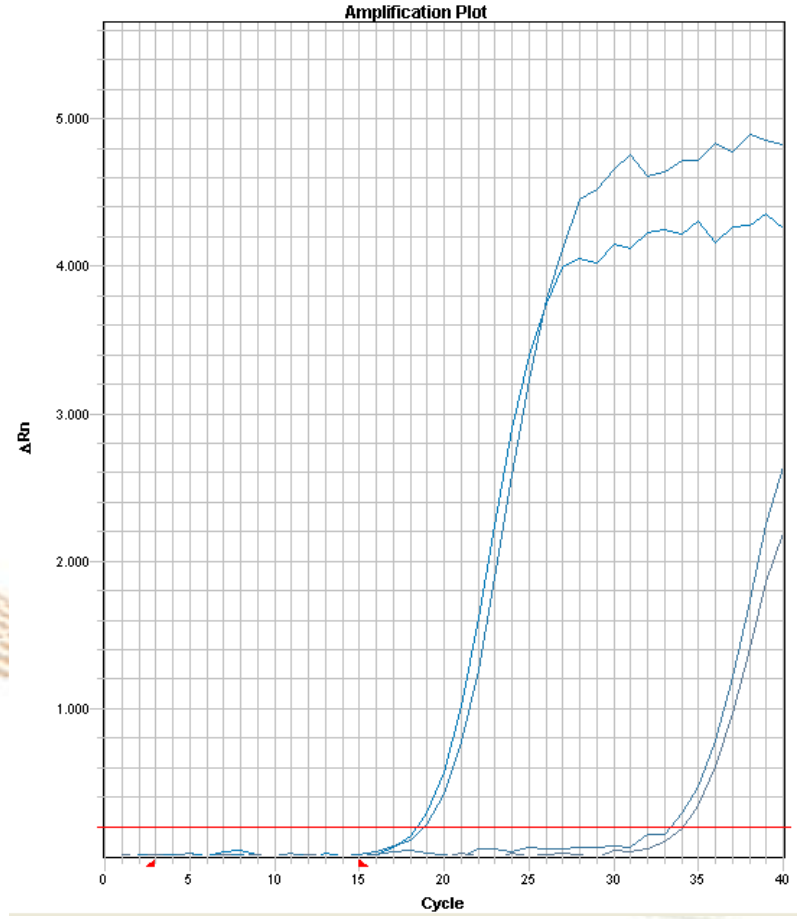
Primer design

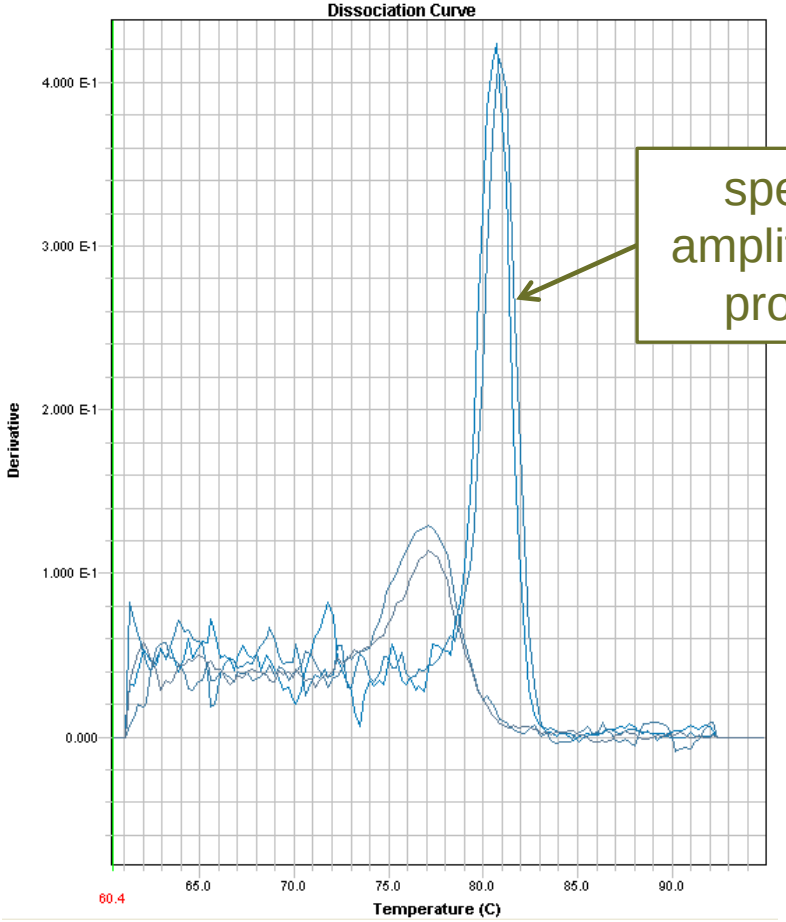
- Group-specific, species specific and universal probes and primer combinations (Phytoplasmas) and species-specific probes (Liberibacter)
- Some primers were designed for multiple target organisms to reduce the total number of primers required in the final multiplex
- Probe position can affect mismatch discrimination
- Primer ratio can affect mismatch discrimination

Testing the primers

- Primer combinations evaluated using real-time PCR with EvaGreen
- Looking to ensure primer pairs amplify well
- Using dissociation curves to look for non-specific amplification

Screening primers using real-time PCR

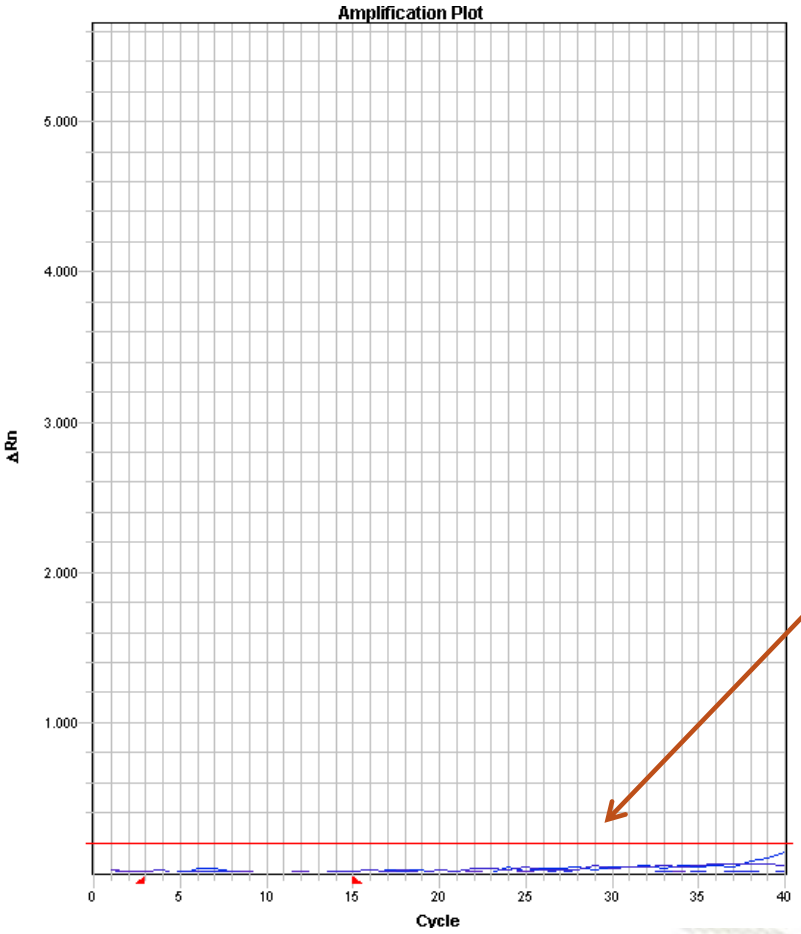


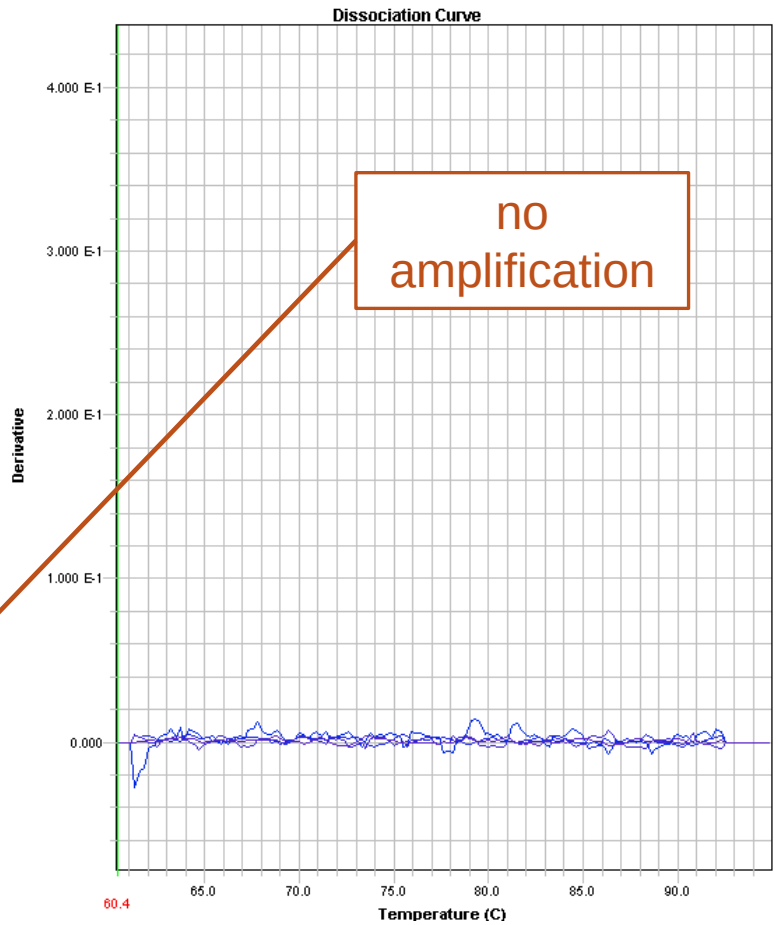


specific amplification product

✓

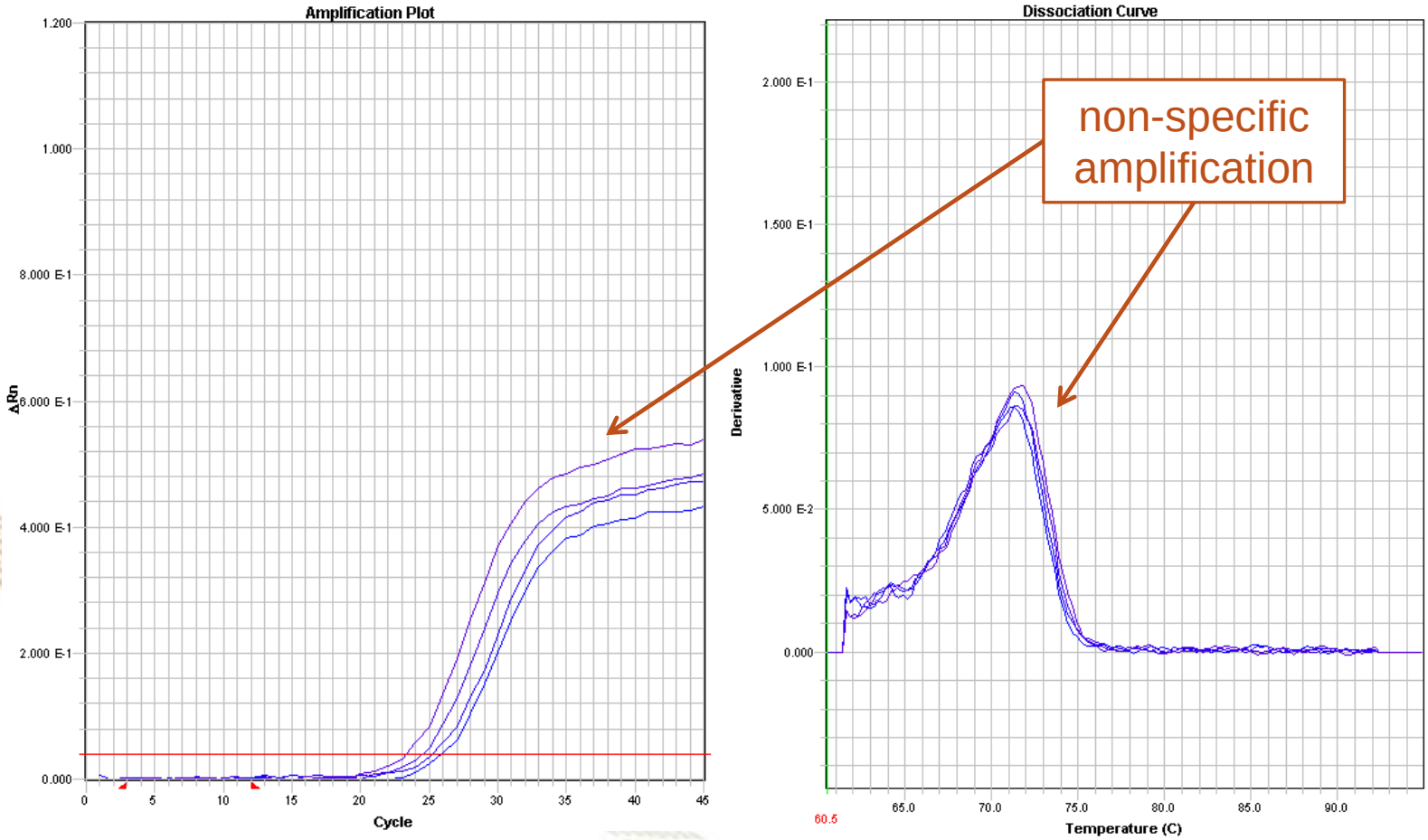
Screening primers using real-time PCR





no amplification

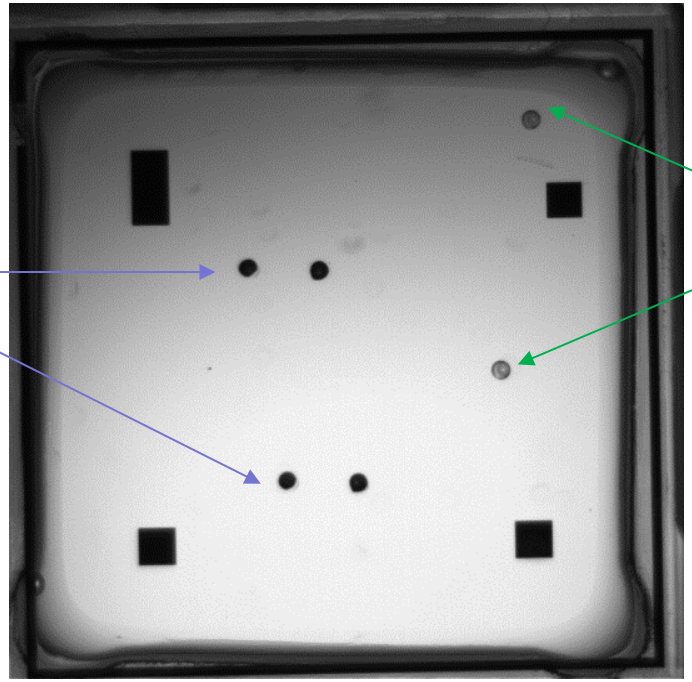
Screening primers using real-time PCR



Testing the probes on the array

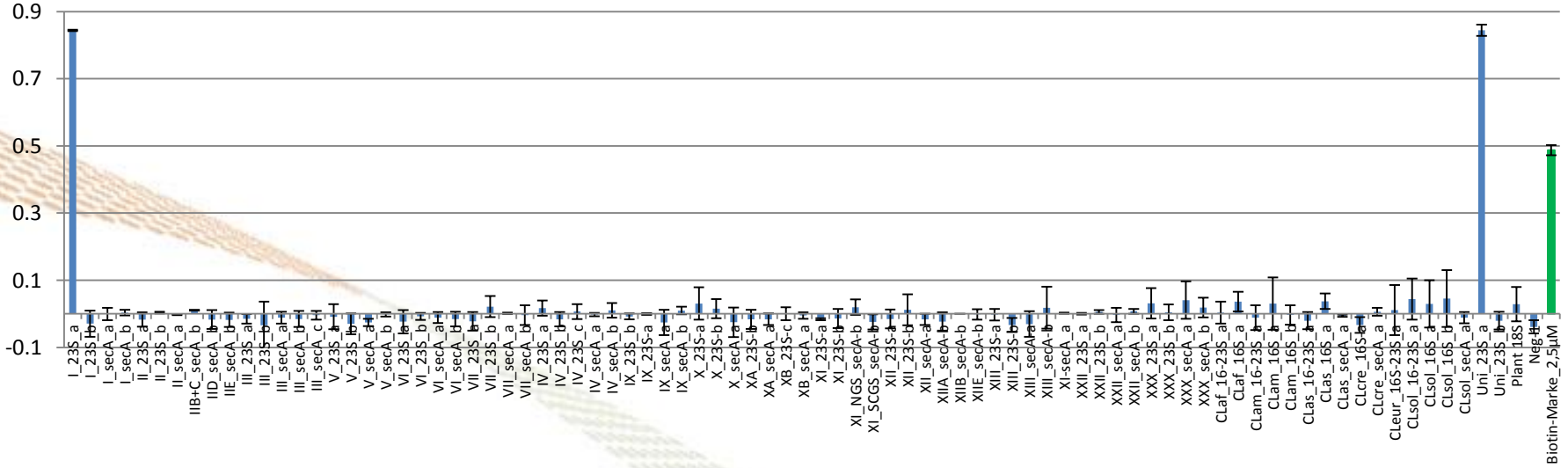
- Primer pair combinations were selected to take forward to test on the arrays
- Looking for a good signal and no cross reactions with other target probes

Array testing

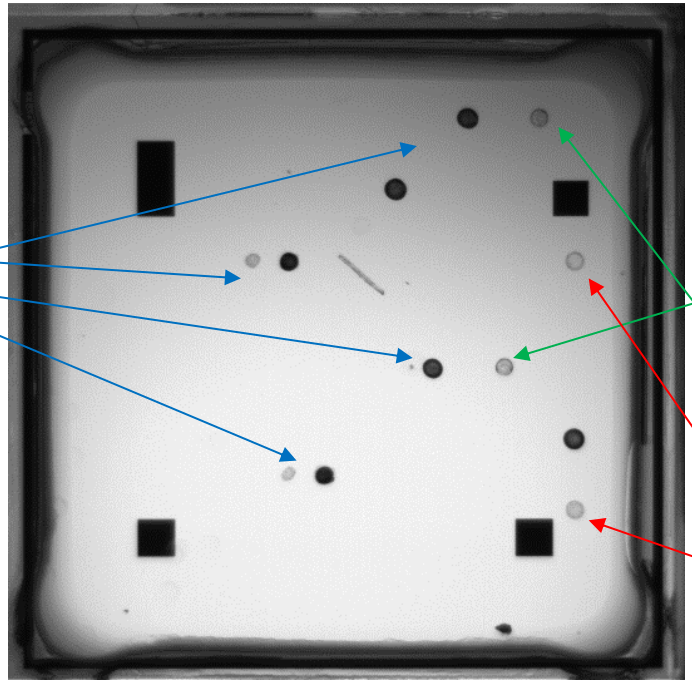


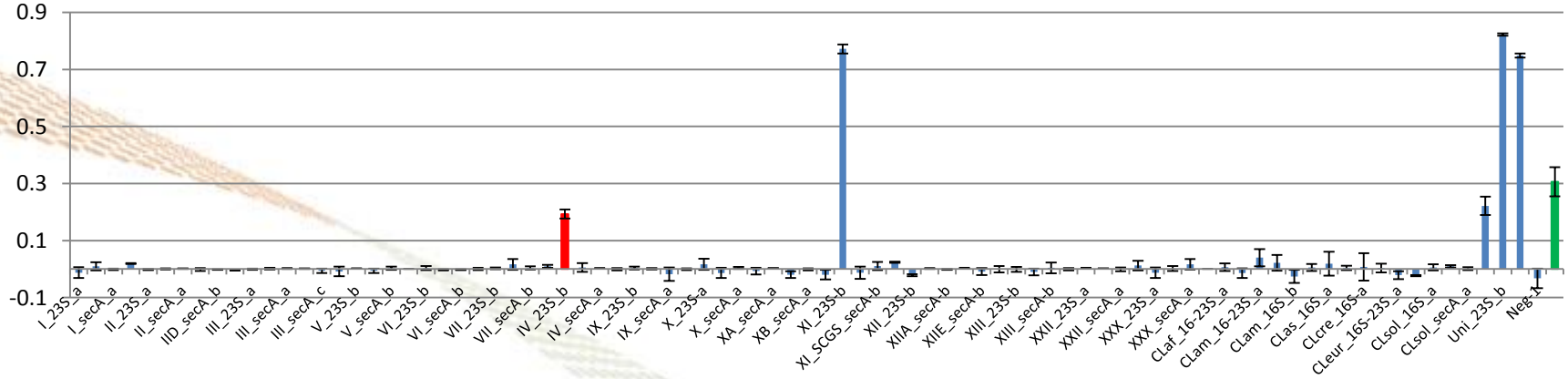
Group I and Universal 23S target probes

biotin control



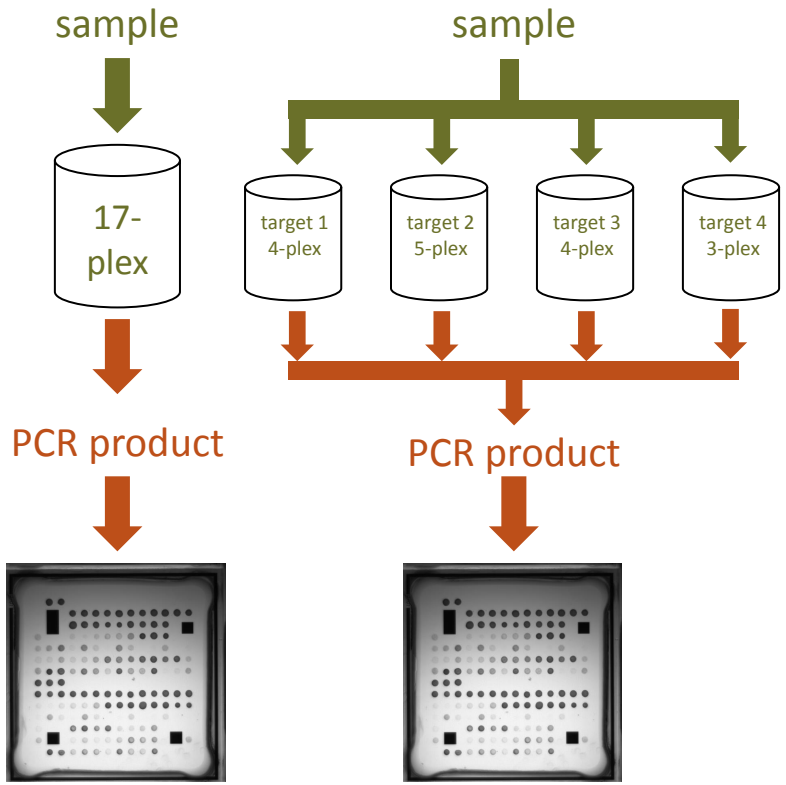
Array testing



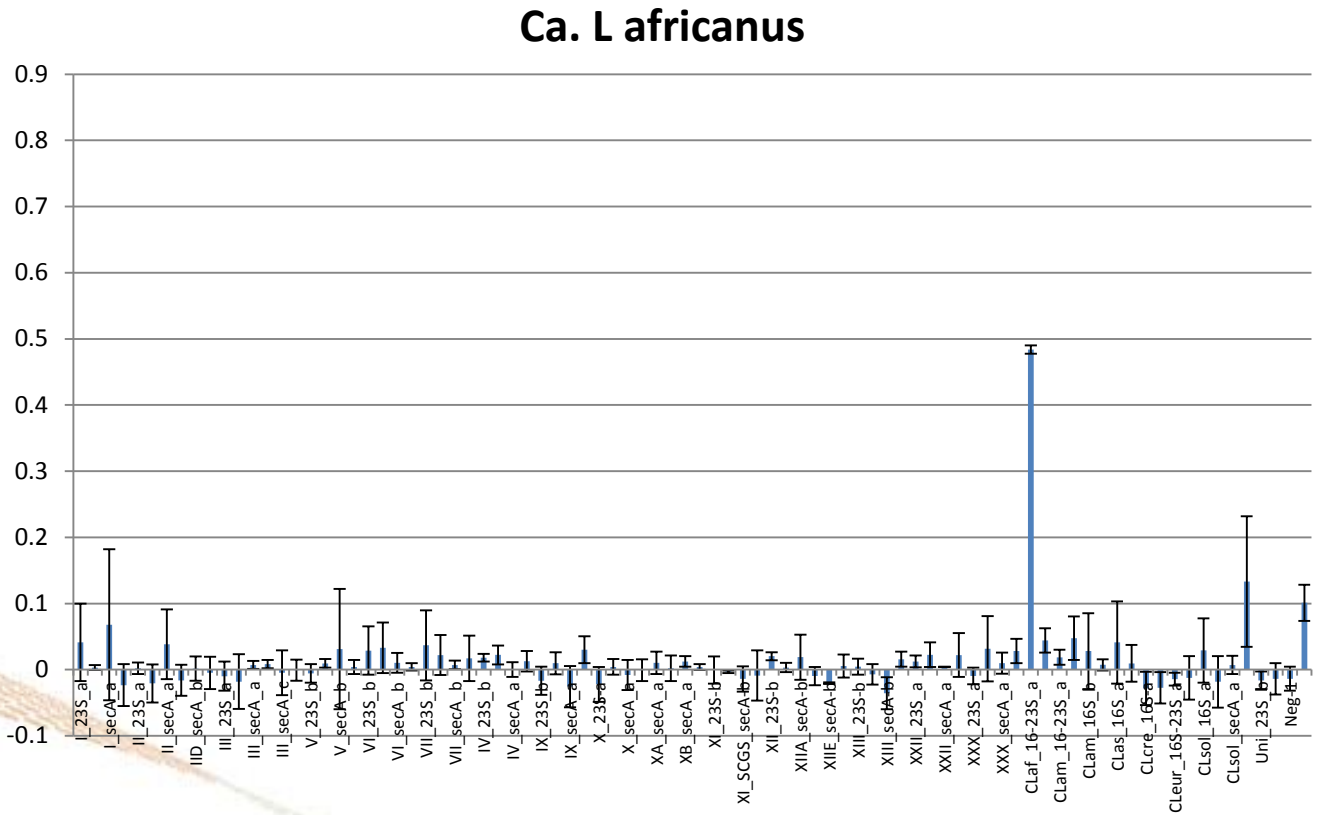


Multiplex PCR for the array

- Multiplex amplification – using specific primers
- Sensitivity limited in highly multiplexed PCR
- Can increase sensitivity by dividing amplification into smaller ‘mini-plexes’



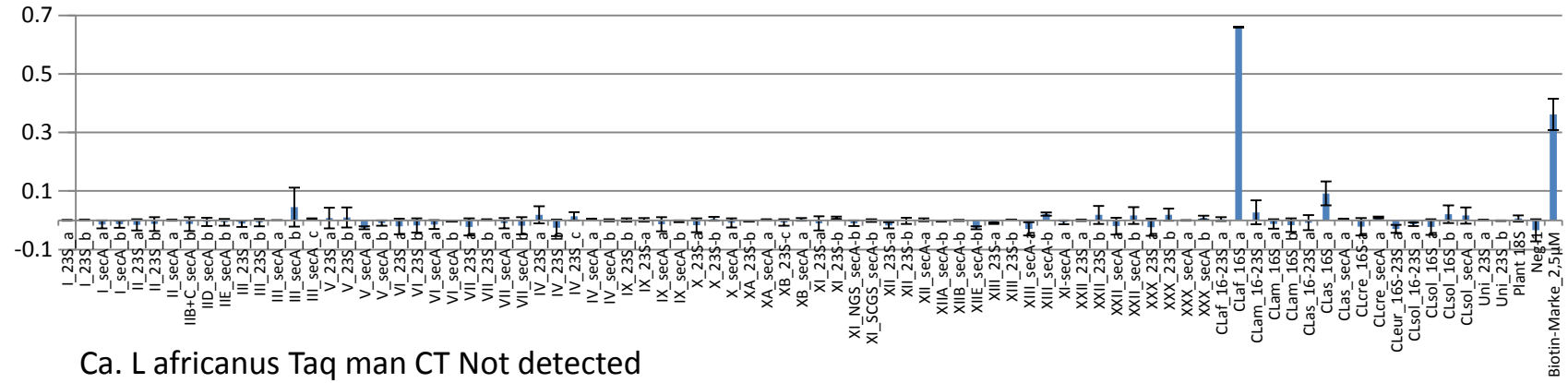
Multiplex PCR: example results



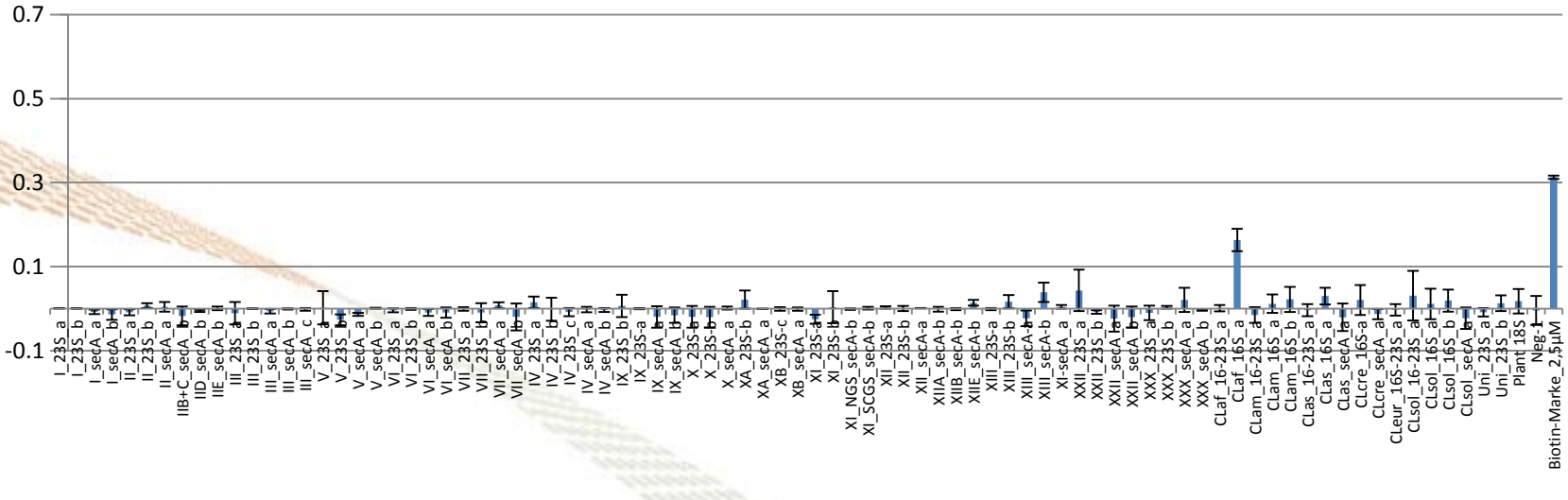
Signal reduced from 0.82 to 0.48

Sensitivity: example results

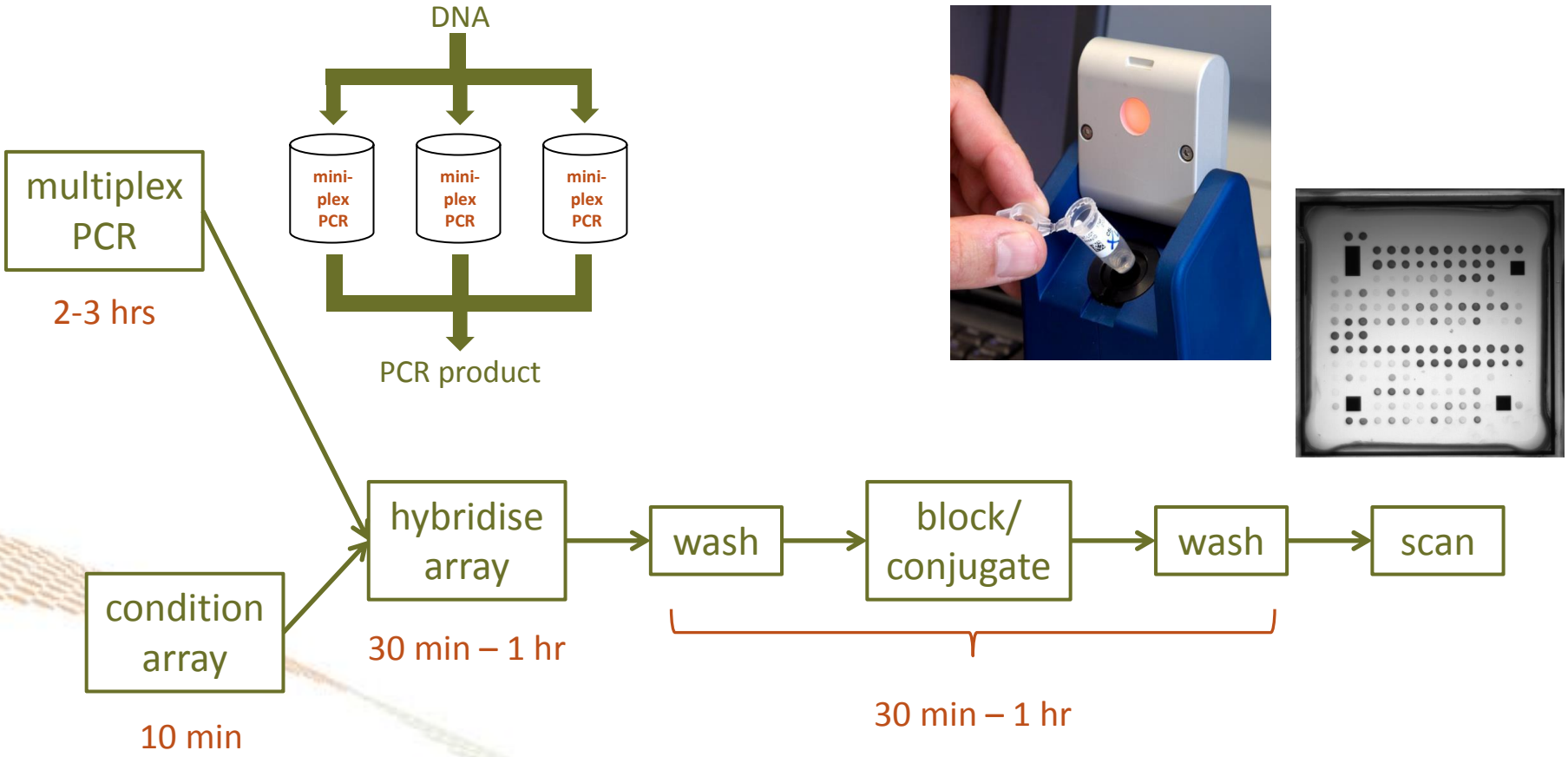
Ca. L africanus Taq man CT 36



Ca. L africanus Taq man CT Not detected



Summary of the array method



Future work: next steps

- Array detection of Phytoplasma and Liberibacter is feasible
- Optimisation of the multiplex
- Second iteration of design (probes and/or primers)
- Validation