Genomewide Search and Evaluation of Candidate genes for Detection and Identification of '*Candidatus* Liberibacter spp.'

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Acknowledgements

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Sequences Available before Genome

1. 16S rRNA and 16S/23S ITS

   Conventional (1996) and real-time PCR(2006)

2. β-operon

   Conventional (1999) and real-time PCR (2006)

3. DNA polymerase gene

   Conventional PCR (2008)

4. Outer membrane protein (omp) gene

   Conventional PCR (2008)

Species-signature 16S rDNA sequences

EU980389 – '*Candidatus* Liberibacter sp.'

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  61 gtcgagcgct tatttttaat aggagcggca gacgggtgag taacgcgtgg gaatctacct
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1441 cgactggggt gaagtcgtaa caaggtagcc gtaggggaac ctgtggctgg atcacctcct
1501 ttc
```

V1(domain) qPCR

V2

V3

V4

V5

V6

V7

V8(domain)

V9

OI1

OA1

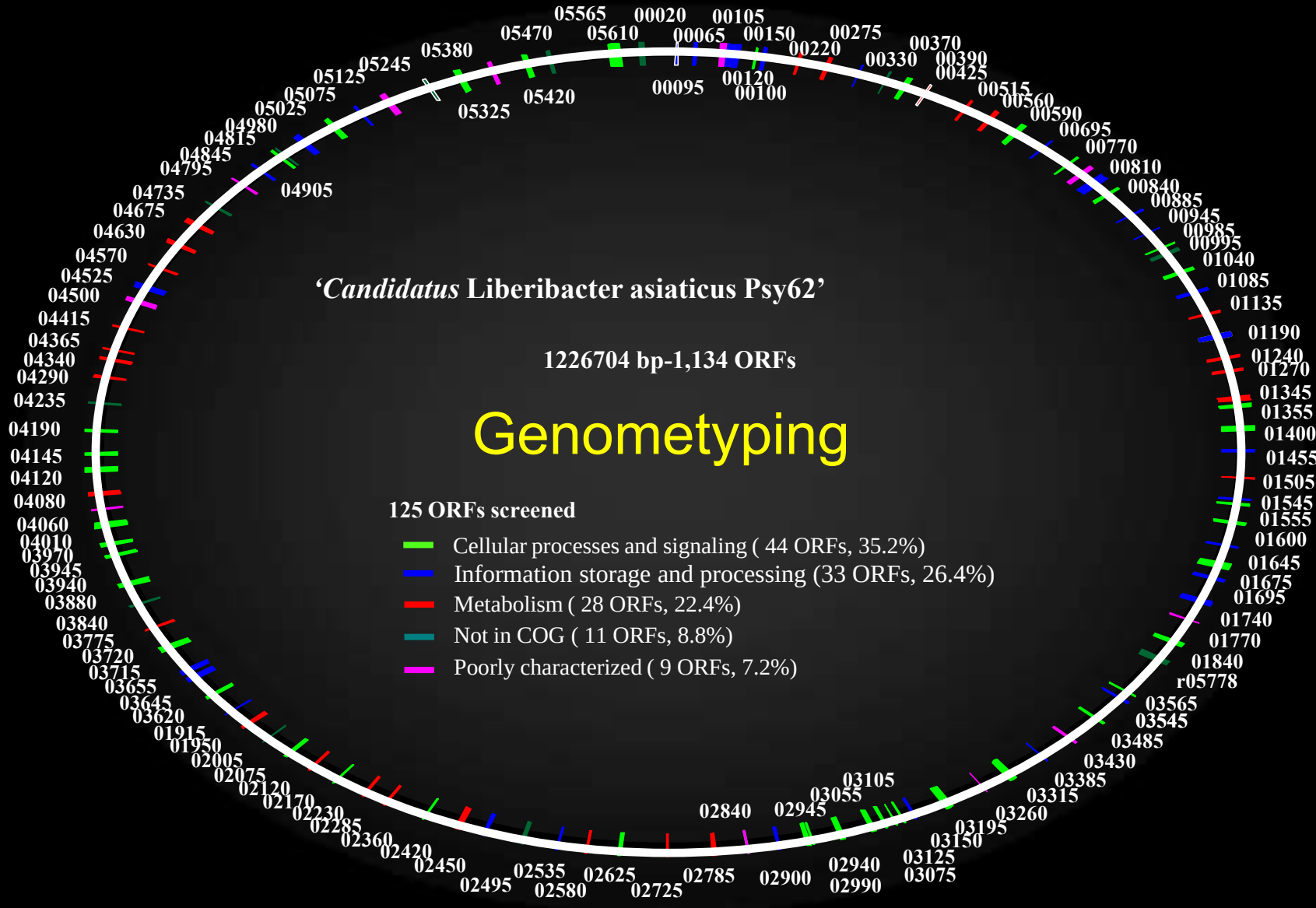
OA2

GB2

OI2c

GB5

Source: Chakravorty et al. J Microbiol Methods 69:330-339, 2007



'Candidatus Liberibacter asiaticus Psy62'

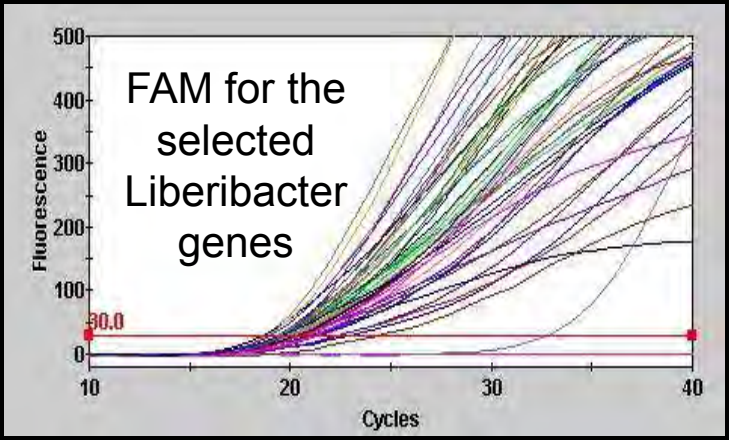
1226704 bp-1,134 ORFs

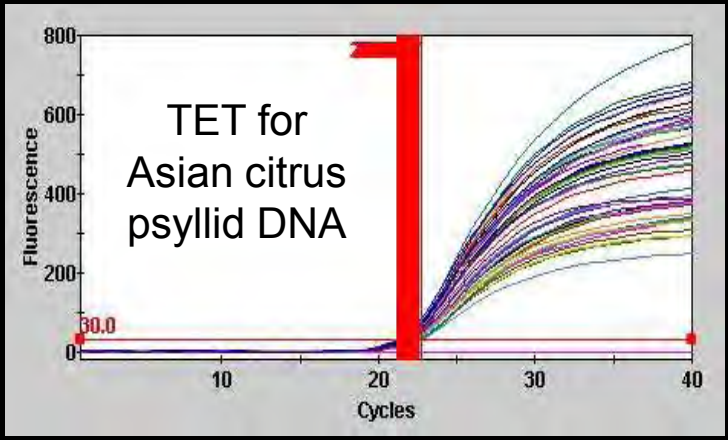
Genometyping

125 ORFs screened

- Cellular processes and signaling (44 ORFs, 35.2%)
- Information storage and processing (33 ORFs, 26.4%)
- Metabolism (28 ORFs, 22.4%)
- Not in COG (11 ORFs, 8.8%)
- Poorly characterized (9 ORFs, 7.2%)

Confirmation of the candidadte genes and evaluation of their TaqMan probes and primers

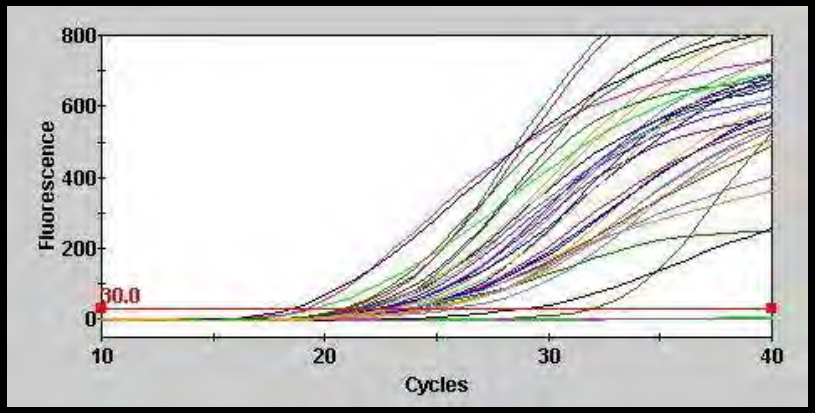


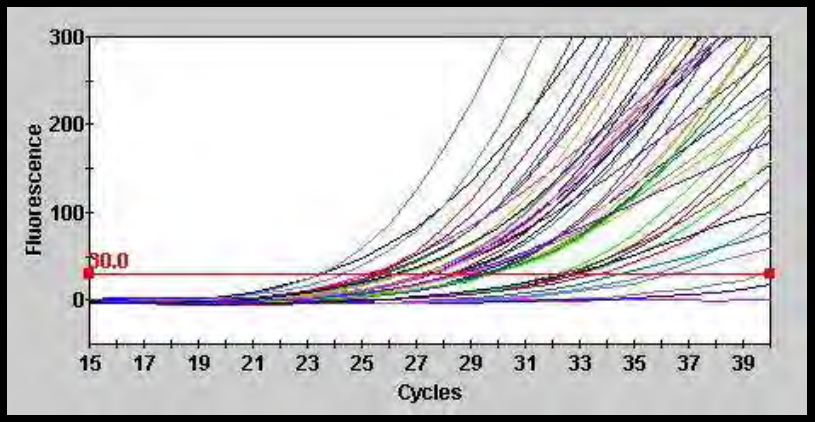


The presence of all the candidate genes in the genome was confirmed by their successful TaqMan real-time PCR amplification from '*Ca*. L. asiaticus'-infected Asian citrus psyllids collected in the field in Florida

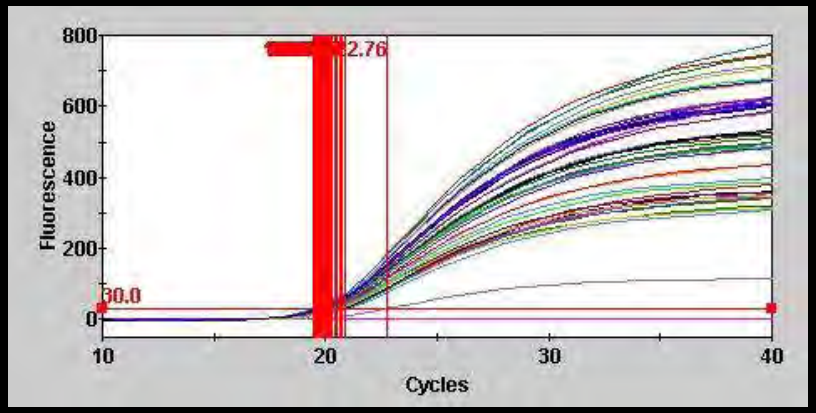
Presence of all the candidate genes in the Liberibacter from HLB-infected citrus trees in the field in Florida

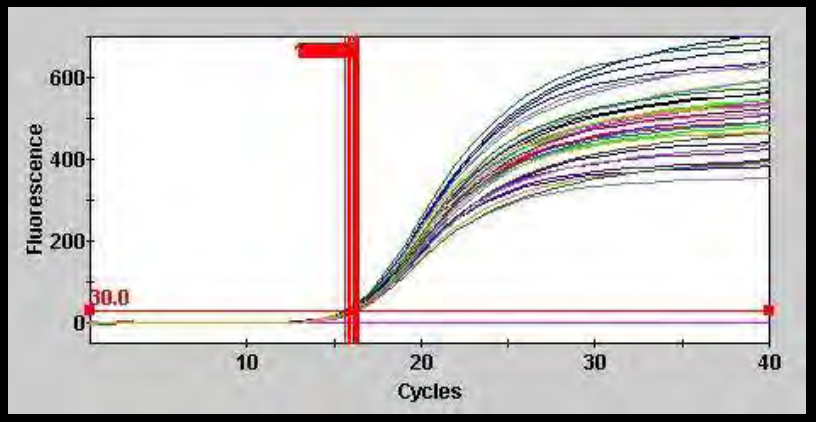
Liberibacter genometyping





(+) internal plant DNA

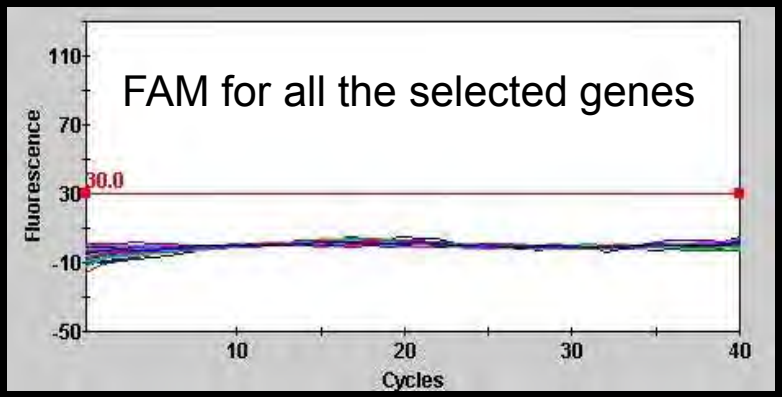


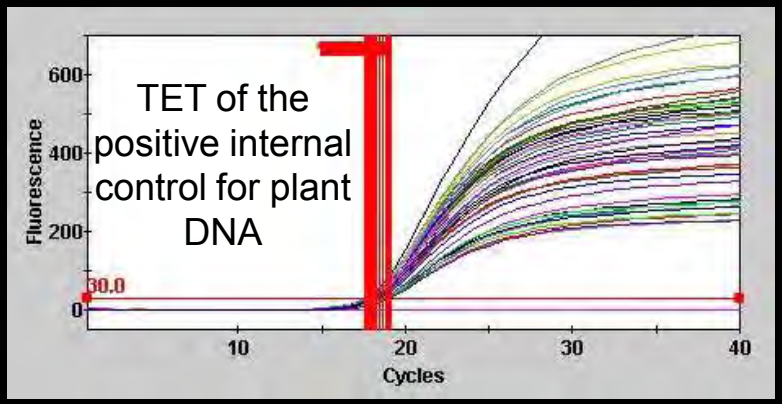


28 Liberibacter-free DNA extracts for specificity

- **10 international DNA extracts** from healthy trees in the field in 10 foreign nations (one DNA extract from each).
  China, Japan, India, Brazil, Mexico, Costa Rica, Saudi Arabia South Africa
- **10 domestic DNA extracts** from healthy trees in the field in 10 US States (one DNA from each).
  FL, CA, TX, GA, AL, LA, SC, HI, PR, GU
- **6 suspect DNA extracts** from HLB-like symptomatic but
- Liberibater-negative trees in the field
  Japan, Florida, Costa Rica, Porto Rico, Texas, Hawaii
- **2 DNA extracts** from healthy plants in greenhouses
  ARS green house and APHHIS greenhouse in Beltsville, MD

Specificity of the TaqMan probe/primer sets for the candidate genes



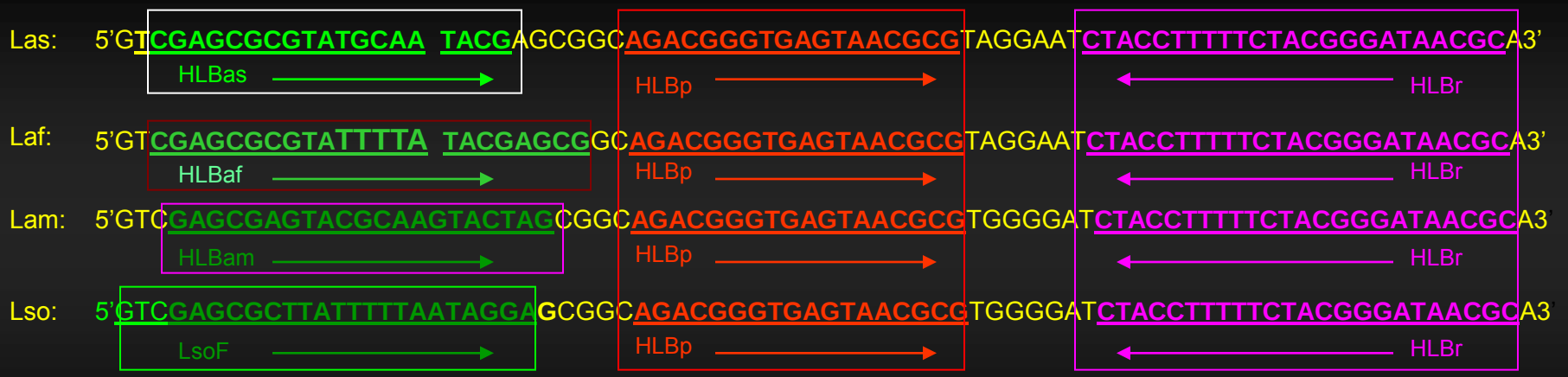


None of the TaqMan probe/primer sets designed for all the candidate genes caused any false positive reaction, in the multiplex real-time PCR, with any of the Liberibacter-free DNA extracts from HLB-free plants.

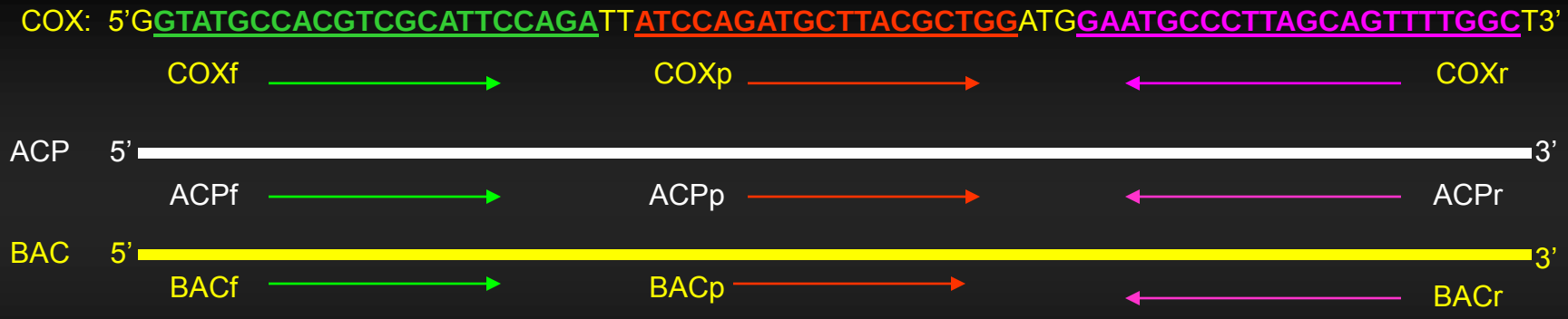
Table. Liberibacter-infected DNA isolates for genometyping.

Liberibacter species	DNA isolate origin	DNA isolate number	DNA isolates selected
'*Ca*. L. asiaticus'	USA	248	33
	Japan	16	16
	China	12	12
	India	7	7
	Brazil	5	5
	Mexico	3	0
	Jamaica	3	0
	Taiwan	3	2
	Belize	2	1
	Cambodia	1	1
	Indonesia	1	1
	Malaysia	1	1
	Saudi Arabia	1	0
	Thailand	1	1
	Vietnam	1	1
'*Ca*. L. americanus'	Brazil	9	6
'*Ca*. L. africanus'	South Africa	4	4
'*Ca*. L. solanacearum'	USA	28	10
Total		**346**	**101**

Multiplex real-time PCR for quantification of Liberibacters and host DNA



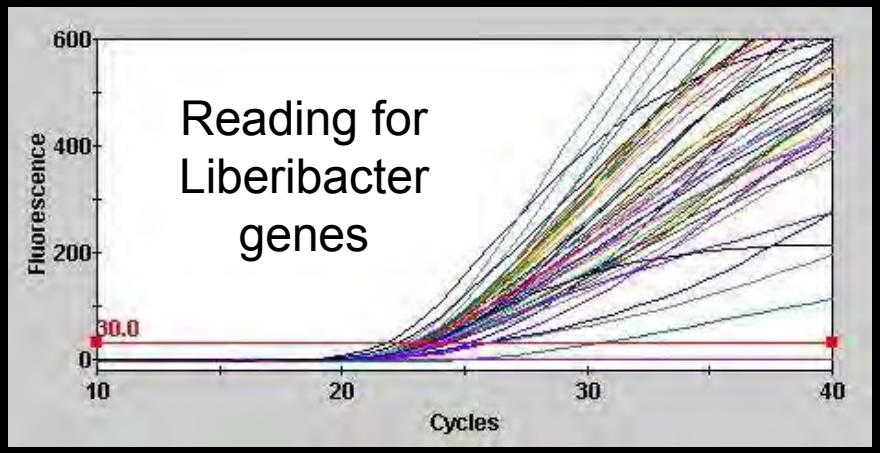
by 16S rDNA-based primer/probe sets specific to Liberibacters

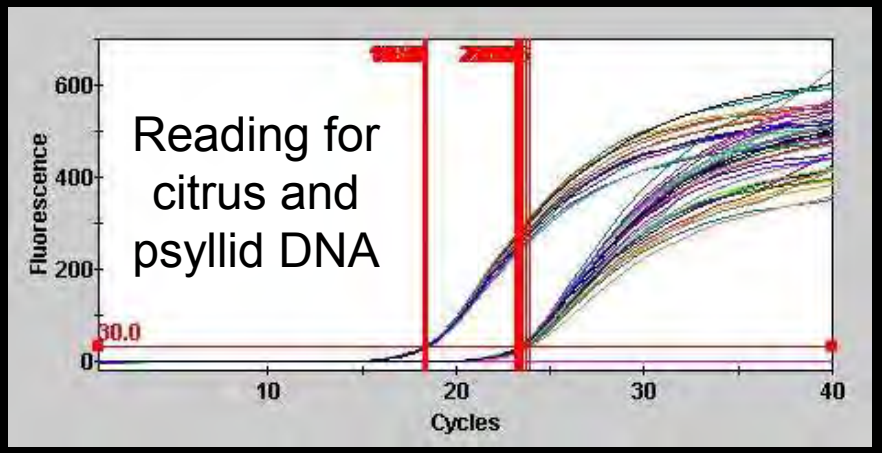


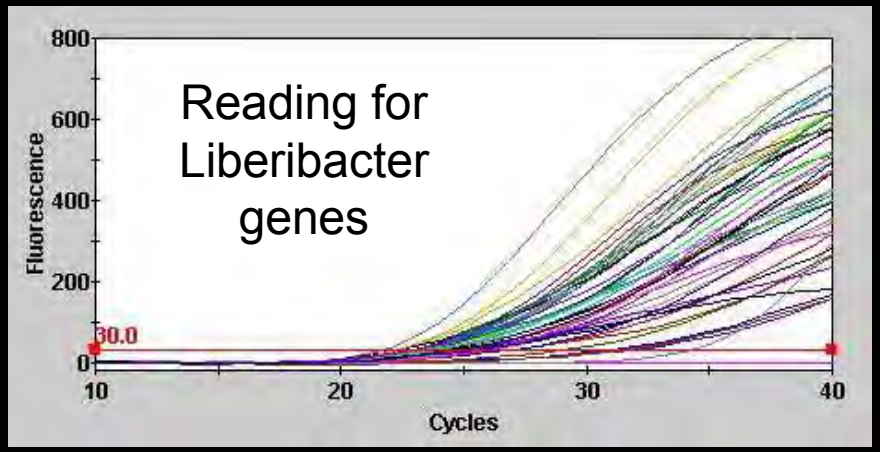
and host plant or psyllid vector-based positive internal control primer/probe sets

Source: Li et al.,2006, 2009 J. Microbiol Methods 66:104-115.

Figure. Scan of Liberibacter candidate genes against different isolates of the bacterium.







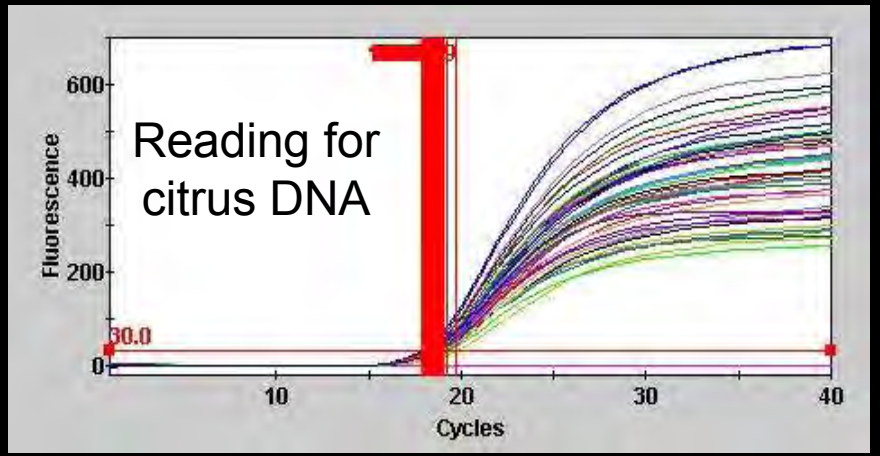


Table. Reliable positive internal control in multiplex qPCR* for all the candidate genes of Liberibacters.

Gene	L.bact	plant	Gene	L.bact	plant	Gene	L.bact	plant
16S	22.81	23.22	11	23.77	23.47	21	25.40	23.18
2	24.86	23.29	12	24.03	23.29	22	24.49	23.15
3	23.82	23.51	13	23.69	23.54	23	24.48	23.16
4	24.22	23.30	14	24.14	23.43	24	24.89	23.20
5	25.28	23.45	15	23.59	23.43	25	24.43	23.31
6	24.62	23.33	16	24.24	23.41	26	25.36	23.19
7	24.27	23.32	17	0.00	23.38	27	24.38	23.29
8	24.87	23.50	18	23.71	23.41	28	24.77	23.15
9	24.23	23.37	19	28.56	23.96	29	26.27	23.32
10	23.67	23.70	20	25.82	23.54	30	0.00	23.19

* Results for 30 of the selected genes, FAM for Liberibacter genes, TET of positive internal control for host plant DNA.

Table. Three groups of the candidate genes for Liberibacter detection and identification

Gene group	Gene	Liberibacter isolate					
		1	2	3	4	5	6
H. keeping	16S	22.81	22.64	23.02	22.12	22.78	23.12
Group I	1	24.22	24.02	24.50	23.52	24.32	24.60
	2	24.38	24.08	25.62	23.60	24.41	26.65
Group II [a]	1	24.52	32.18	24.26	24.03	34.89	25.12
	2	28.89	25.12	34.67	28.09	24.98	34.67
Group III [b]	1	0.00	24.67	21.08	37.78	24.63	0.00
	2	20.15	38.87	0.00	23.65	0.00	24.35

[a] β-operon belongs to Gene Group II; [b] the bacteriophage-type DNA polymerase gene belongs to Gene Group III.

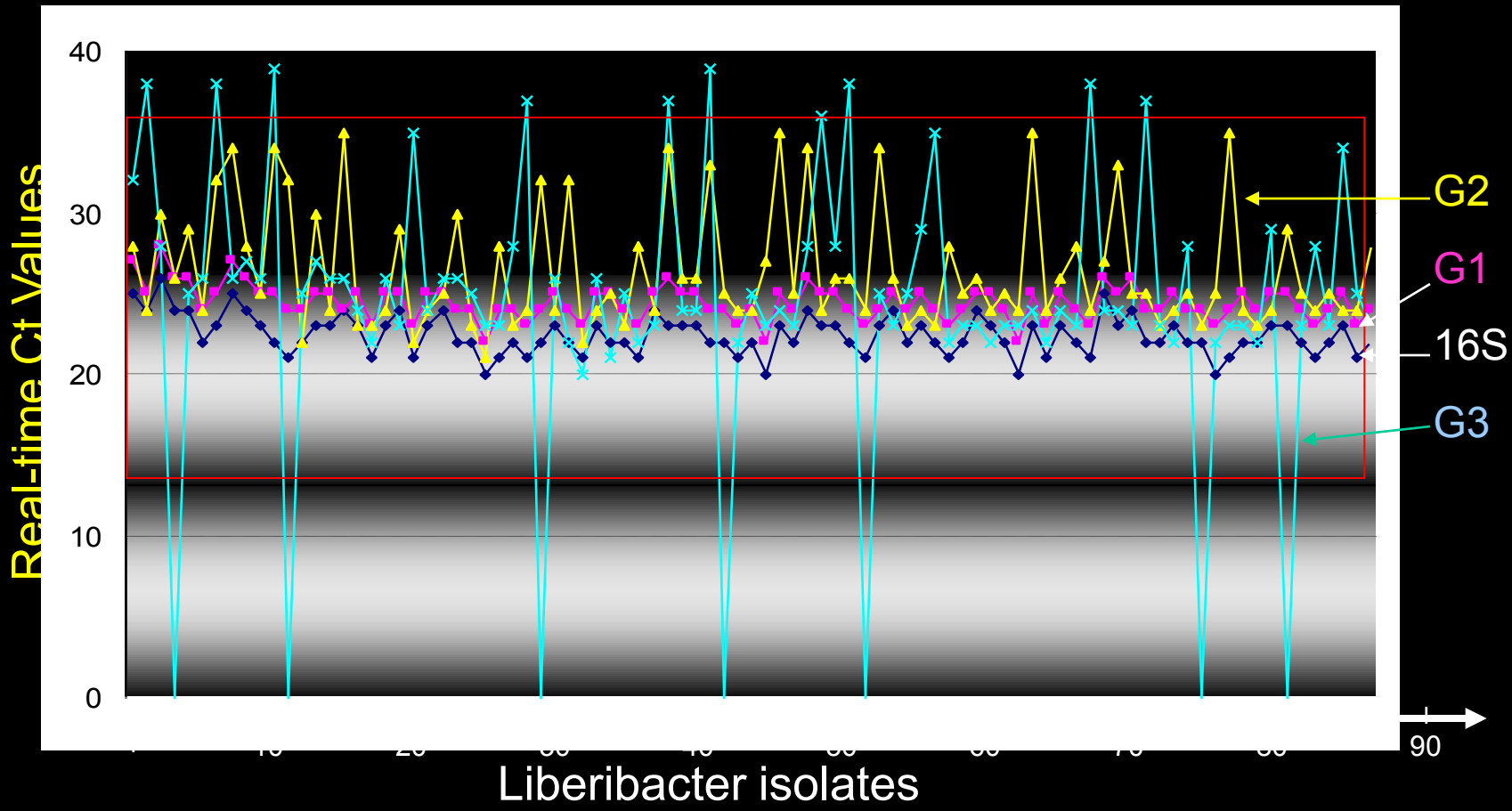
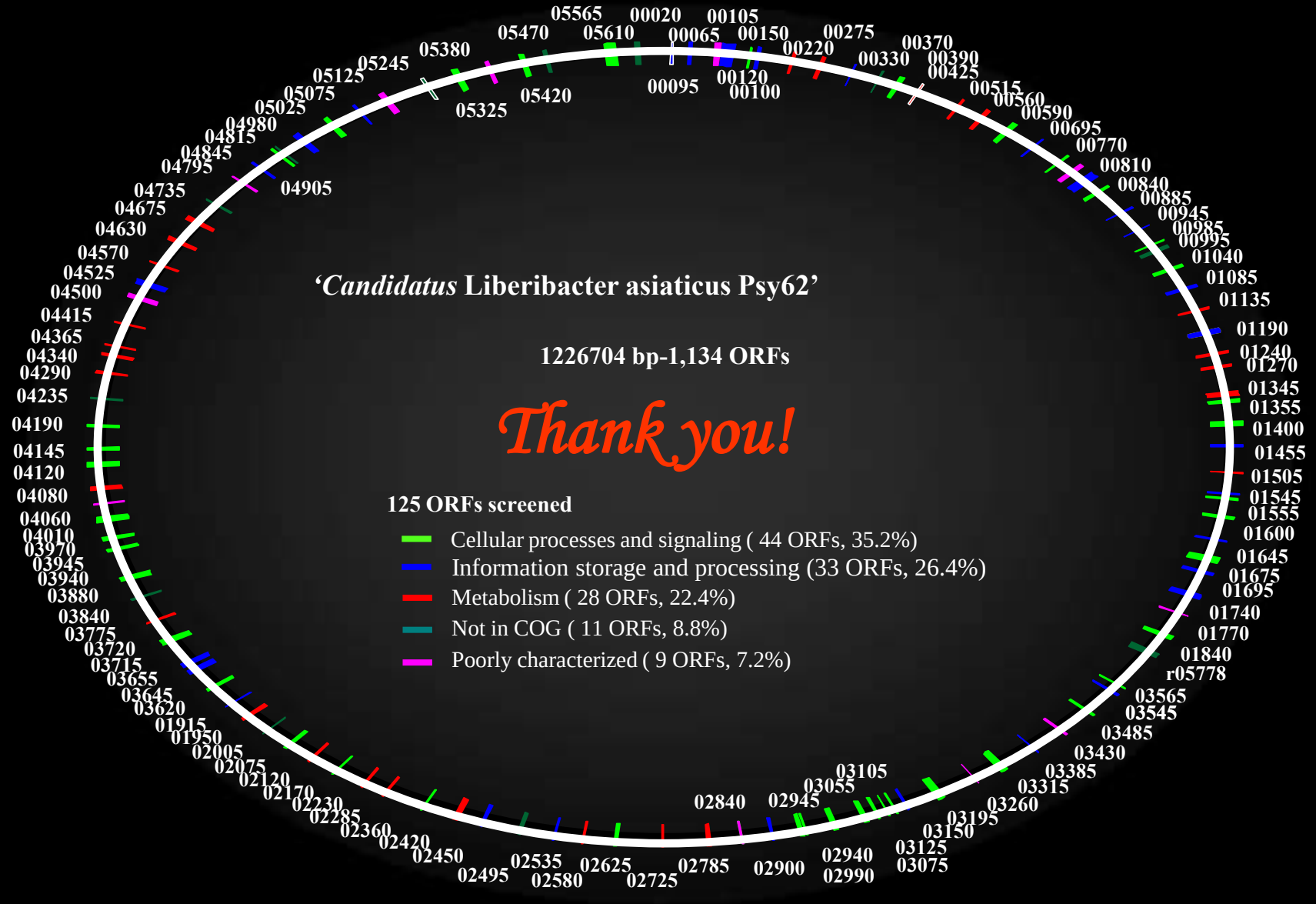


Figure. Gene groups for Liberibacter diagnosis



A system available for genometyping one Liberibacter isolate in 2 hrs.