PCR protocol for amplification of MLST genes of Candidatus Liberibacter solanacearum

1) For each 50 µl PCR, use the following reaction components (multiply these volumes by the number of PCR reactions + 1 to make up a single master mix which can then be dispensed in 47 µl amounts into numbered, thin-walled PCR tubes):

Reagent	Volume	Notes
H_2O	30.5µl	
5x HF reaction buffer	10µl	
Forward primer	2.5µl	[10µM primer stocks = 0.5µM final conc.]
Reverse primer	2.5µl	
dNTP mix	1µl	[10mM stock]
Phusion polymerase	0.5µl	[2 units/µl stock]
Total	**47µl**	

2) Add 3 µl of the template DNA diluted appropriately (from 1/10 to 1/100) to each tube. Remember to set up a negative PCR control, which consists of reaction components and NO added template DNA. If you are optimising the PCR, using new template DNA or using a new set of primers, it is advisable to also set up a positive PCR control by including a reaction that contains template DNA which has been amplified reliably in previous PCRs.

3) Place the tubes into the thermal cycler and close the lid, ensuring that the heated plate inside the lid is in contact with the tops of the tubes.

4) PCR program:

Step	Temperature /^{0}C	Time
1	98	30 s
2	98	10 s
3	63	30 s
4	72	30 s
5	repeat steps 2, 3 and 4 a further 34 times	
6	72	10 min
7	4	hold

All the primers were designed to have an optimal annealing temperature near 63°C.

5) After cycling, check that amplification was successful by running 5 or 10 µl of each reaction on an agarose gel, with size standards.

6) Purification of PCR products: we recommend using a PCR purification kit, e.g. QIAquick PCR Purification kit (Qiagen) according to the manufacturer's instructions, and eluting the DNA with sterile H_2O. Measure the DNA concentration of each sample, for optimizing the sequencing reactions.

7) Store the samples at -20°C.