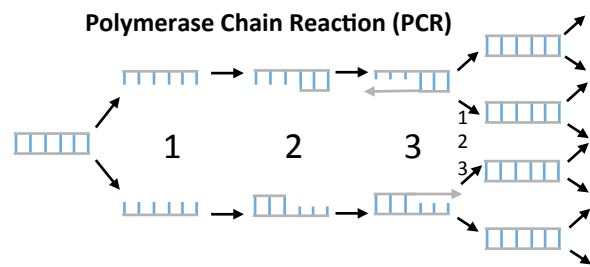


What is PCR?

Polymerase chain reaction (PCR) is a technology used to make many copies of a specific piece of DNA. PCR is a heating and cooling process that pulls apart the double stranded DNA (1), adheres small pieces of DNA to “prime” the creation of new DNA strands (2), and then builds a new strand of DNA that is the same as the original (3). This cycle repeats many times, doubling the amount of DNA each cycle. This increases the amount of DNA in a sample to a detectable level.



PCR detects the presence of DNA of the bacterium that causes HLB, but it does not distinguish between live or dead cells. However, the type of PCR that is being funded by the CRDF is a quantitative PCR (qPCR) that does estimate the amount of DNA present based on a Copy threshold (C_t) value.

What is a copy threshold?

Quantitative PCR will cycle 40 times. This type of PCR uses a signal that tells the PCR machine how much DNA is in the sample during each of the cycles. The machine will go through several cycles before it can even detect the target DNA, the cycle in which the DNA is finally detected by the machine is the cycle threshold or C_t . This is when enough DNA has been made to hit the threshold for detection. For example, if only one copy is in the sample, the machine may take 38 cycles to make enough copies until there is enough DNA for the machine to detect; in this case the C_t is 38. If there are 100 copies, the machine may take only 20 cycles and the C_t would be 20.

Do PCR results tell us how much bacteria is in the sample?

PCR labs produce C_t values which cannot be directly translated into bacterial concentrations. The C_t values will tell you if your tree is HLB positive or negative and to what extent the tree is infected compared to all of the samples in that sample batch. Extra steps are necessary to estimate the number of bacterial cells in a sample; this would limit the number of samples that could be processed and does not provide important decision-making information to growers.

My sample has a C_t value of 32, why does the testing lab consider this HLB free?

A number of falsely positive or falsely negative samples are expected when screening large numbers of samples from many sources; because of this a threshold is set by the PCR lab. The Southern Gardens threshold, for example, is a C_t value of 32, this value was selected after screening large numbers of known positive and negative samples. PCR results should be combined with a visual assessment of the tree to get the most accurate knowledge of tree health status.

If my sample has a C_t value of 40 does that mean it is HLB free?

No test is perfect. A C_t value that is above the “PCR negative” threshold may not mean the tree is disease-free; sampling methods including the type of tissue selected for sampling and the number of samples per tree, and the time of year effect the ability to get accurate results. Again, a visual assessment of the tree, combined with PCR results, will give a more accurate read on the health of the tree. A document describing methods to evaluate tree health titled “Field trial tree evaluation methods”, can be found on the CRDF website (citrusrdf.org).

What is the best tissue to submit to the PCR labs?

Leaf tissue that has shown symptoms of HLB such as blotchy mottle and yellow vein symptoms is the best tissue to select. New flush tissue should be avoided and only fully expanded leaves should be sampled. For more detailed instructions see this document: http://www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf.

What is the best time of year to sample?

During the summer when the weather is hot and the trees look their best is the time of year when HLB is the most difficult to detect by PCR. This may be because the tree is growing more rapidly than the bacteria is growing, the heat may suppress the bacteria, or the tree may be able to use defense mechanisms to suppress the bacterial growth. Southern Gardens recommends sampling from September through March for the most accurate results.

PCR Labs (these CRDF funded labs provide PCR analysis of plant and psyllid samples at no cost to growers):



SWFREC HLB Lab:
2685 State Road 29 North
Immokalee, Florida 34142
239-658-3431
www.imok.ufl.edu/programs/plant-path/extension-roberts/hlb-lab/

Southern Gardens Lab:
United States Sugar Corporation
Technical Operations
111 Ponce de Leon Ave.
Clewiston, FL 33440
863-902-2249
www.flcitrusmutual.com/content/docs/issues/canker/sg_samplingform.pdf