

## Short description

LAMP (Loop-mediated isothermal Amplification) is a simple technique for identification based on the amplification of DNA at a constant temperature using pre-selected sets of primers. The primers are considered unique for a particular taxon or taxon group. The amplification product is then detected through photometry, usually by a fluorescence technique.

Within the framework of the project FF-IPM, different sets of LAMP primers are developed, targeting different representatives of two genera: *Ceratitis* and *Bactrocera*.

For *Ceratitis*, in addition to the target species *Ceratitis capitata*, the emphasis is on related species that are also considered of economic significance (as agricultural pests): *Ceratitis* FARQ complex (i.e. *fasciventris*, *anonae*, *rosa*, *quilicii*), and *Ceratitis cosyra*. To effectively differentiate between these *Ceratitis* species, based on mitochondrial *cox1* and *cob* genes, specific *Ceratitis* LAMP primer sets were designed and screened. 986 homologous sequences of 58 species of *Ceratitis* from GenBank and BOLDSYSTEM were served as negative controls to guarantee the high specificity of the primers. The experimental conditions optimization results showed that F3/B3:FIP/BIP=1:8 was the optimal primer concentration ratio, and 63°C was the optimal reaction temperature. A similar approach is under development for representatives of the genus *Bactrocera*, where the focus is on differentiating, primarily, between *B. dorsalis*, *B. zonata*, *B. latifrons* and *B. oleae*.

## Efficacy

LAMP allows identification of material that is morphologically not or difficult to identify (e.g. parts of whole body, immature stages, etc). Although it is a molecular approach it does not require a fully equipped molecular laboratory. The infrastructure is limited (mainly a hot water bath) and can be easily installed in-situ.

The identification flow can be completed within one hour including samples processing. As such, integrating fluorescent dye (for visual detection) and the rough DNA extraction method, a rapid and efficient visualization and identification process is established. It allows identification at points of entry, but is not limited to port quarantine. It can also take into account the field application scenarios, providing a new way of thinking for more agricultural pest workers.

An additional advantage is that LAMP primers can show higher sensitivity in the detection process than conventional PCR techniques.