



INOCULUM DNA TEST REPORT

Customer Name

Customer Address

Customer E-mail

Customer Phone

Company Name

Company Address

Company E-mail

Company Phone

Date

All truffles that are used in LOT number inoculum shall be tested.

Protocol:

Take a sample from every truffle used in the inoculum of a specific Lot and extract the DNA using a Genotyping kit similar to:

Extract N-Amp PCR kit (Sigma – Aldrich).

Each DNA extract to be analyzed by end-point PCR, using the specific *Tuber melanosporum* (ITS4LNG – ITSML), *T. brumale* (ITS4LNG – ITSB) and *T. indicum* (IST4LNG – ITSCHCH) (Paolocci et al. 1999) primers and all PCR products have been separated by electrophoresis in 0.8% agarose gels. A negative control, using grade PCR water, has been included in the analysis to avoid having false positives. Three positive controls (one DNA extract of *T. melanosporum*, one of *T. brumale* and one of *T. indicum*) have also been included.

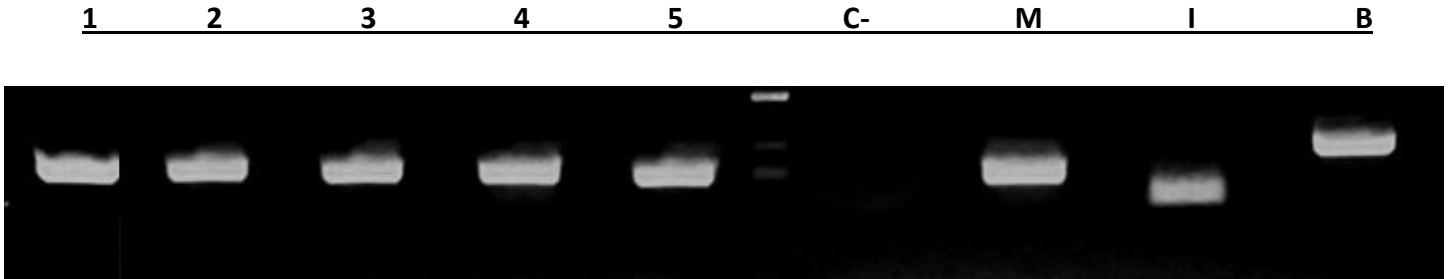
Paolocci F, Rubini A, Granetti B, Arcioni S. 1999. Rapid molecular approach for a reliable identification of Tuber spp. ectomycorrhizae. FEMS Microbiol. Ecol. 28:23–30

Example:

| LOT | Number of Truffles Tested | KG | Analysis Date | NUMBERS IN GEL | RESULTS |
|-----|---------------------------|----|---------------|----------------|---|
| 25 | 5 | 1 | 09/17/2020 | From 1 to 5 | All truffles are <i>T. melanosporum</i> |

The results should be presented with explanation as shown below in the agarose gel images.

All the samples amplified for *T. melanosporum*. No amplification was observed in the negative control (grade PCR water instead of DNA), as no band is expected. A band was observed for each of the positive controls, as expected.



Each number corresponds to a single truffle used in the inoculum.

The C- corresponds to the negative control grade PCR water instead of DNA; no band is expected.

The M corresponds to a positive control of *T. melanosporum*; a band is expected.

The I corresponds to a positive control of *T. indicum*; a band is expected

The B corresponds to a positive control *T. brumale*; a band is expected

All the truffles analyzed and used for the inoculum are *Tuber melanosporum*.

Date _____

Tested by: _____