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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 5198-2020

**Technical specifications for the quarantine of Bumblebee
Brachyhymenium**

熊蜂短膜虫检疫技术规范

(English Translation)

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Table of Contents

Foreword
1 Scope
2 Normative references
3 Terms, definitions and abbreviations
4 Equipment, materials and reagents
4.1 Equipment
4.2 Reagents
4.3 PCR primers
4.4 Real-time PCR primers and probes
4.5 Quality control substances
5 Sampling
5.1 Sampling ratio
5.2 Sampling method
6 PCR detection
6.1 DNA extraction
6.2 PCR amplification
6.3 Agarose gel electrophoresis
6.4 Gel imaging analysis and sequencing

Foreword

This document is drafted in accordance with GB/T 1.1-2020.

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Drafting organizations of this document. Fuzhou Customs of the People's Republic of China, Haikou Customs of the People's Republic of China, Beijing of the People's Republic of China Customs, Fujian Agriculture and Forestry University, Xiamen Ridley Calibration and Testing Technology Co., Ltd.

1 Scope

This document specifies the polymerase chain reaction and real-time fluorescent polymerase chain reaction detection technologies for the quarantine of Bumblebee

Brachyhymenium.

This document applies to the quarantine of Bumblebee Brachyhymenium.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated references Documents, only the version corresponding to the date is applicable to this document; undated reference documents, the latest version (including all amendments) Applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms, definitions and abbreviations

There are no terms and definitions that need to be defined in this document. The following abbreviations apply to this document.

Ct cycle threshold (cycle threshold) DNA deoxyribonucleic acid (deoxyribonucleic acid) dNTP deoxynucleoside triphosphate EB Ethidium bromide (ethidium bromide) ITS internal transcribed space PCR polymerase chain reaction (polymerase chain reaction) TBE trihydroxymethyl aminomethane-boric acid-ethylene diamine tetraacetic acid) TE TE buffer solution (Tris-EDTA buffer solution)

4.1 Equipment

4.1.1

Autoclave.

4.1.2

Gradient PCR amplification instrument.

4.1.3

Fluorescent PCR machine.

4.1.4

High-speed refrigerated centrifuge (rotating speed 12 000 r/min).

4.1.5

Micro pipette.

4.1.6

Biological safety cabinet.

4.1.7

DNA relative molecular quality standards (100 bp ~1 000 bp).

4.1.8

Horizontal electrophoresis apparatus.

4.1.9

Gel imaging system.

4.1.10

Electronic balance (with a sense of 0.01 g).

4.1.11

Refrigerator (2 °C~8 °C and -20 °C).

4.2 Reagents

4.2.1

Water. Meet the first grade water specification in GB/T 6682.

4.2.2

Liquid nitrogen.

4.2.3

Anhydrous ether.

4.2.4

Absolute ethanol.

4.2.5 0.01 mol/L phosphate (PBS) buffer, the preparation method is shown in Appendix A, A.1.

4.2.6 10% SDS solution, the preparation method is shown in Appendix A, A.2.

4.2.7

Proteinase K.

4.2.8 5 mol/L NaCl solution, the preparation method is shown in Appendix A, A.3.

4.2.9

CTAB/NaCl solution, the preparation method is shown in Appendix A, A.4.

4.2.10

Trichloromethane/isoamyl alcohol mixture, the preparation method is shown in Appendix A, A.5.

4.2.11

Phenol/chloroform/isoamyl alcohol mixture, the preparation method is shown in Appendix A, A.6.

4.2.12

Isopropanol.

4.2.13

TE buffer.

4.2.14 10xPCR buffer.

4.2.15

dNTPs (including dCTP, dGTP, dATP, dTTP).

4.2.16

Taq DNA polymerase.

4.2.17

Agarose.

4.2.18 0.5xTBE buffer, the preparation method is shown in Appendix A, A.7.

4.2.19

Loading buffer, the preparation method is shown in Appendix A, A.8.

4.2.20

EB (10 mg/mL). For the preparation method, see A.9 in Appendix A.

4.2.21

DNA relative molecular mass standard Marker.

4.2.22

DNA purification and recovery kit.

4.3 PCR primers

Designed according to the nucleic acid sequence of the ITS gene of Bumblebee Bremenworm, the amplified length is 447 bp (see Appendix B in B.1), and it is dissolved in water to 10 After $\mu\text{mol/L}$, store at $-20\text{ }^{\circ}\text{C}$ for later use.

4.5 Quality control substances

Use the bumblebee's abdominal tissue or DNA containing the target fragment infected with *Bombus brachymemma* as a positive control, and use the uninfected *Bombus brachymembranaceus* the healthy bumblebee abdomen tissue of the insect was used as a