

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

SN/T 5106-2019

Dengue vector mosquito DNA barcode identification method

登革热媒介蚊类DNA条形码鉴定方法

(English Translation)

Issued on: September 3, 2019

Implemented on: March 1, 2020

Issued by: General Administration of Customs

Table of Contents

Foreword
1 Scope
2 Normative references
3 Method principle
4 Identification procedure
4.1 Preparation
4.2 Specimen processing
4.3 DNA Preparation
4.4 PCR amplification
4.5 Detection of PCR products
4.6 Sequence determination
4.7 Sequence analysis
5 Result judgment

Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard is proposed and managed by the General Administration of Customs of the People's Republic of China.

This standard was drafted. Fuzhou Customs, People's Republic of China.

Drafters of this standard. Fang Yiliang, Zhang Jianqing, Lin Xingzhong, Wang Yuping, Huang Enjiong, Fang Changtian, Wu Rongquan.

SN/T 5106-201919 ?????, ????, ????, ??

Identification method of dengue vector mosquito DNA barcode

1 Scope

This standard specifies DNA extraction, gene amplification, and sequence alignment of dengue fever vector mosquito DNA barcode identification methods.

Analysis, results judgment, etc.

DNA barcode identification of specimens such as stubble and incomplete limbs.

2 Normative references

The following documents are essential for the application of this document. For dated references, only the dated version applies to this document.

file. For undated references, the latest version (including all amendments) applies to this document.

GB 1849 General requirements for laboratory biosafety SN/T 1876 Procedures for the collection, production and preservation of medical vector biological specimens SN/T 275.4 Self-protection code for health and quarantine personnel Part 4. Laboratory personnel SN/T 4278 Code of Practice for Identification of Medical Vector Insects DNA Bar Codes at Frontier Ports Application of polymerase chain reaction (PCR) in clinical diagnosis of WS/T 230

3 Method principle

Use universal primers to amplify specific fragments of the COI gene in mitochondrial DNA, clone the PCR product or directly sequence Then, an effective sequence with a length of not less than 500 bp is obtained, and compared with the standard sequence of this standard, so as to achieve rapid and accurate identification of species purpose.

4.1 Preparation

See Appendix A for equipment and reagents required by the laboratory. Laboratory biosafety complies with the regulations of GB 1849 and SN/T 275.4 the anti-pollution measures of the PC comply with the requirements of WS/T 230.

Configuration of electrophoresis solution TAE (1 x TAE). Tris base 242g, glacial acetic acid 57.1mL, 0.5mol/LEDA (pH8.0) 100mL, make up to 1L, and dilute 50 times with double distilled water.

4.2.1 Sampling

Specimens are frozen or anesthetized with ether. After death, a single specimen of the same sample is taken in batches. Rare single specimens are taken on the symmetrical side of the tissue.

(Such as unilateral mosquito feet) Keep it in a sterilized clean 1.5mL centrifuge tube. If it is not used for a long time, the volume fraction must be more than 90% ethanol.

Store at -20 ° C. Other specimens of the same species or part of the needle insert are used as voucher specimens.

SN/T 5106-201919 ?????, ?????, ??

4.2.2 Voucher specimen production

The sampled specimens are made into voucher specimens, and the specimens are prepared in accordance with the requirements of Sn/T 1876.

4.3 DNA Preparation

Place a single sample (adult, larva, pupae) in a sterilized clean 1.5mL centrifuge tube and grind it with a grinding rod, using commercial insects DNA extraction kit for mosquito genome extraction (sample is a single mosquito, after removing the head (if you need blood to remove the abdomen) Grinding); the sample is a small amount of symmetrical one-sided tissue (unilateral mosquito foot) placed in a sterilized clean 1.5mL centrifuge tube and ground with a grinding rod, using Micro tissue DNA extraction method (see Appendix B) for mosquito genome preparation, and prepared DNA for concentration and purity determination After that, store it in -20 °C refrigerator for future use.

4.4.1 Primers

LCO1490. 5'-GGTCACACAAAATCATATAGAGTAT-3G ' HCO21198.
5'-TAAAACTTTCAGGGTGTAGACCAAAAAAATCA-3 '

4.4.2 Amplification system

A 50μL system is recommended, and a commercial PCRIx is available. To verify whether the PCR system is polluted, a blank control and a negative control can be set at the same time; to verify that the PCR reaction is normal, a positive control can be set.

TaQPCMasterix 25μL forward and reverse primers (10μM) 4μL each template (50 ~ 100μg/μL) 3μL ddH₂O 14μL In order to improve the effect of the reaction, the volume of the template can be appropriately increased according to the concentration of the template, and the volume of ddH₂O should be reduced accordingly the volume is unchanged.

4.4.3 Amplification conditions

Step 1. 94 ° C for 5 min;

The second step. 95 ° C for 30s, 46 ° C for 30s, 72 ° C for 40s, 35 cycles;

Step 3. 72 min at 72 ° C.

4.5.1 Configuration of agarose gel

Weigh 1.5g of agar powder and pour it into a flask containing 100mL1 x TAE, put it in a microwave oven and heat it to fully dissolve until clear Just fine.

4.5.2 Electrophoresis

5 µL PCR amplification product (containing dye) was spotted on an agarose gel (containing ethidium bromide) with a mass concentration of 1.5%, the running buffer was 1 x TAE, and electrophoresis was performed at constant voltage 80V for 30 min. The gel imaging system images and shoots.

4.6 Sequence determination

The electrophoresis band of the COI sequence amplification product is around 750 bp (see Appendix C).

SN/T 5106-201919 ?????, ????, ????, ??

Ask a professional sequencing company to perform a two-way assay (sequencing primers and amplification primers), and send samples according to the requirements of the selected company. Conditional the laboratory can choose to clone and sequence, and the cloning process is performed according to the provisions of Sn/T 4278.

4.7 Sequence analysis

Sequencing results were edited using bioinformatics software to compare peak plots and forward and reverse sequencing results for errors. Remove primers at both ends.

Partial sequence. Compare with the standard sequence of *Aedes albopictus* in Appendix D, and the standard sequence of *Aedes aegypti* in Appendix E.

Record F.

5 Result judgment

The result of the comparison is *Aedes albopictus* and the similarity is greater than or equal to 98%.

Aedes aegypti and its similarity greater than or equal to 98% are *Aedes aegypti* (see Appendix F).

SN/T 5106-201919 ?????, ????, ????, ??

Appendix A

(Informative appendix) Main equipment and reagents A. 1 Instruments and appliances A. 2 Consumables 1.5ml centrifuge tube, 0.2ml PCR tube, pipette TIPS of each range, powder-free disposable gloves.

A. 3 Reagents and materials Reagents required for PCR. 75% ethanol by volume, DNA extraction kit for animal tissue genomic DNA, TaQPCRMastirix (Containing dye), PCR product purification kit, agarose, DNA standard molecular weight standard, TAE