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Determination for Trace Gene Residues of Microorganisms - Microdroplet Digital PCR

微生物痕量基因残留测定

微滴数字 PCR 法

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Determination for Trace Gene Residues of Microorganisms - Microdroplet Digital PCR

1 Scope

This document specifies the microdroplet digital PCR method for the determination of trace gene residues (below 100 pg/mL) of microorganisms.

This document is applicable to the determination of trace characteristic gene residues (below 100 pg/mL) of Saccharomyces cerevisiae and Lactobacillus plantarum in processed products.

2 Normative References

The contents of the following documents constitute indispensable clauses of this document through the normative references in this text. In terms of references with a specified date, only versions with a specified date are applicable to this document. In terms of references without a specified date, the latest version (including all the modifications) is applicable to this document.

GB/T 6682 Water for Analytical Laboratory Use - Specification and Test Methods

3 Terms and Definitions

The following terms and definitions are applicable to this document.

3.1 Trace Gene Residue

Trace gene residue refers to trace (below 100 pg/mL) nucleic acid that is difficult to remove in the subsequent separation and purification of microbial processed products.

4 Principle

Adopt the droplet microfluidic technology to divide the target nucleic acid PCR amplification system remaining in the biological sample to be tested into dozens to tens of thousands of microliter water-in-oil microdroplets containing one copy of the nucleic acid molecule. In accordance with the conventional PCR system, amplify it; read out the signal through the florescent probe. The number of the generated microdroplet positive signals represents the copy number of target nucleic acid molecules in the original sample, through which, the number of target nucleic acid molecules can be directly counted, and the trace gene residues in the initial sample can be quantified.

5.8 Lactobacillus plantarum CGMCC 1.2437 pure culture: pick a single colony of lactobacillus plantarum CGMCC 1.2437 in the activated plate; inoculate it into 25 mL of MRS liquid medium. In a constant-temperature shaker, at 30 °C ± 1 °C and 180 r/min, culture it overnight to obtain the seed solution. Take 0.5 mL of the seed solution and transfer it to 10 mL of MRS liquid medium; in a constant-temperature shaker, at 30 °C ± 1 °C and 180 r/min, culture it, until OD_{600} is 0.3 ~ 0.4 (about 2 h ~ 3 h) to obtain a pure culture of lactobacillus plantarum CGMCC 1.2437.

6 Instruments and Equipment

6.1 Microdroplet digital PCR instrument.

6.2 Nucleic acid quantometer.

6.3 Thermal cycler.

6.4 Electronic balance: with an accuracy of 0.01 g.

6.5 Pipette: with a measuring range of 0.1 μL ~ 2.5 μL, 2 μL ~ 20 μL, 10 μL ~ 100 μL and 100 μL ~ 1,000 μL.

6.6 Constant-temperature shaker: the temperature can achieve (28 ± 1) °C and (37 ± 1) °C; the speed is at 125 r/min ~ 250 r/min.

6.7 pH meter: with an accuracy of 0.1.

6.8 UV-visible spectrophotometer: the detectable wavelength includes (600 ± 20) nm; equipped with a 1 cm cuvette.

7 Preparation of Reaction System

7.1 Preparation of Saccharomyces Cerevisiae Residue Gene Detection Reaction System

7.1.1 DNA template preparation for test sample

Take 1.00 g of the test sample. In accordance with the instructions of the nucleic acid extraction kit, carry out DNA extraction. Finally, use 50 μL of sterile water for DNA elution. The extracted DNA sample is tested for nucleic acid concentration and purity with a nucleic acid quantometer.

7.1.2 DNA template preparation for positive control

Take 100 μL of the lactobacillus plantarum pure culture (5.8); use sterile water to dilute it, until OD_{600} is 0.2 (at this moment, the bacterial concentration is about 2×10^8 CFU/mL). Adopt the method of gradient dilution; continue to use sterile water to dilute it, until the bacterial concentration is 1×10^3 CFU/mL. Take 100 μL of the diluted lactobacillus plantarum pure culture; add 1.00 g of the test sample as a positive control. Use a DNA extraction kit to perform

number of effective microdroplets shall not be lower than the number of microdroplets specified by the microdroplet digital PCR equipment, otherwise, the test sample need to be re-tested.

In accordance with the operating instructions of the microdroplet digital PCR instrument, within 30 min, perform PCR amplification, or place it in a refrigerator at 4 °C for PCR amplification within 4 h. The amplification can be performed on the digital PCR or a thermal cycler, and the heating and cooling rate is ≤ 2.5 °C/s; see the amplification procedure in Table 3. After the amplification is completed, read the fluorescence signals of all microdroplets for the subsequent data analysis.

9 Result Analysis and Expression

9.1 Threshold Setting

In accordance with the end-point fluorescence value of the negative amplification system in the system, set the threshold value of florescence. The threshold value needs to clearly distinguish between negative and positive microdroplets in the amplification system.

9.2 Detection Result Analysis of Control Group

There was no amplification phenomenon in the negative control group and the blank control, and obvious amplification phenomenon appeared in the positive control group, which indicates that this detection with the microdroplet digital PCR method is normal and reliable, and there is no need to repeat the detection.

9.3 Specimen Detection Result Analysis

The result of specimen detection is analyzed in accordance with the following principles:

a) Both the positive control and the test sample manifested obvious amplification

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