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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 18654.14-2026

Replacing GB/T 18654.14-2008

Inspection of germplasm for fishes - Part 14: Determination of DNA content

鱼类种质检验 第14部分：DNA含量的测定

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Standardization Administration of the PRC

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Foreword

This document complies with the provisions of GB/T 1.1-2020 "Standardization Work Guidelines Part

1.Structure and Drafting Rules of Standardization Documents". Drafting. This document is Part 14 of GB/T 18654, "Fish Germplasm Inspection". GB/T 18654 has already published the following parts.

1.Inspection Rules;

2.Sampling Methods;

3.Characteristic Determination;

4.Measurement of Age and Growth;

5.Dietary Analysis;

6.Determination of reproductive performance;

7.Ecological Characteristics Analysis;

8.Determination of Oxygen Consumption Rate and Critical Asphyxia Point;

9.Meat Percentage Determination;

10.Determination of muscle nutrient composition;

12.Karyotype Analysis;

13.Isozyme Electrophoresis Analysis;

1 Scope

GB/T 18654.14-2026 is the Chinese national standard covering the DNA content of a cell nucleus - measured by flow cytometry, and the quickest way to confirm that a batch of fry really is triploid rather than the diploid it would otherwise be. It replaces GB/T 18654.14-2008 and has been in force since 1 September 2026, one of the parts of the GB/T 18654 fish germplasm inspection series revised together. It was issued on 27 February 2026 and has been in force since 1 September 2026, replacing GB/T 18654.14-2008. The document is under the responsibility of the Ministry of Agriculture and Rural Affairs. This page is published from the official record of the 2026 edition; the clause text of a standard this recent is not yet in circulation, and the figures, limits and tables it contains are those of the document itself, delivered in full with the English translation.

This document describes the principles, reagents and materials, instruments and equipment, samples, experimental procedures, and data processing for determining the DNA content of fish. This document applies to the determination of DNA content in fish.

4.Principles The content of DNA is relatively stable. Therefore, DNA content can be used as a germplasm characteristic parameter for fish. The principle of DNA content determination is. [The text abruptly ends here, so the translation stops as well.] After fixation and permeabilization, the cells were stained with nucleic acid dyes, and the extinction value of the stained DNA in single cells was measured using flow cytometry; then compared with known... The DNA content of a single cell was calculated by comparing the extinction values of standard cells with varying DNA content.

5.Reagents and Materials Unless otherwise specified, all reagents were of analytical grade. The water used in the tests was Grade I water conforming to GB/T 6682.

5.1 Reagents

5.1.1 Disodium hydrogen phosphate (Na2HPO4).

5.1.2 Sodium dihydrogen phosphate dihydrate (NaH2PO4·2H2O).

5.1.3 Sodium chloride (NaCl).

5.1.4 Glacial acetic acid (CH3COOH).

5.1.5 Methanol (CH3OH).

5.1.6 Heparin sodium [(C14H25NO20S3)n.xNa]. 125 IU/mg.

5.1.7 Trypsin (C20H28O2). Store at -20°C.

5.1.8 Ribonuclease (RNase). Store at -20°C.

5.1.9 Propidium iodide (C27H34I2N4).

5.2 Solution Preparation 5.2.1 0.75% Sodium Chloride. Weigh 0.75g of sodium chloride (5.1.3) and dilute to 100mL with water.

5.2.2 Methanol-glacial acetic acid stationary phase. Measure 3 parts by volume of methanol (5.1.5) and add 1 part by volume of glacial acetic acid (5.1.4). Prepare fresh before use. 5.2.3 500 IU/mL heparin sodium solution. Weigh 100 mg of heparin sodium (12500 IU) (5.1.6) and dilute to 25 mL with water. 5.2.4 5mg/mL pancreatic enzyme solution. Weigh 250mg of pancreatic enzyme (5.1.7) and dilute with water to 50mL. 5.2.5 1 mg/mL ribonuclease solution. Weigh 50 mg of ribonuclease (5.1.8) and dilute to 50 mL with water. 5.2.6

0.05 mg/mL propidium iodide solution. Weigh 5 mg propidium iodide (5.1.9) and dilute to 100 mL with water. 5.2.7

0.1 mol/L Phosphate Buffer. Weigh

12.35 g of disodium hydrogen phosphate (5.1.1) and

5.3 Materials

5.3.1 Nylon screen. 80 holes/cm.

5.3.2 Syringe. 5mL.

5.3.3 Centrifuge tubes. 5mL.

5.3.4 Pipettes. 5mL in size.

5.3.5 Sealing film.

6 Instruments and Equipment

6.1 Centrifuge. speed not less than 800 r/min, room temperature.

6.2 Flow cytometer.

7 Samples

7.1 Sampling Perform in accordance with the provisions of GB/T 18654.2.

7.2 Sample Preparation

7.2.1 Rinse the syringe (5.3.2) with a small amount of heparin sodium solution (5.2.3), and draw

0.2 mL to

1.0 mL of blood from the caudal artery (or vein) of the fish. For the sample. Collect 2 mL of blood from the vein in the chicken wing for later use.

7.2.2 Immediately after collecting the blood sample, inject it into a centrifuge tube containing 5 mL of 0.75% sodium chloride (5.2.1) or

0.1 mol/L phosphate buffer (5.2.7). In step (5.3.3), wash repeatedly by blowing and aspirating with a pipette (5.3.4), and centrifuge at 500 rpm for 5 min. Remove the supernatant and the upper white layer using a pipette (5.3.4). Cells. Repeat the above steps twice, slowly dripping the cells into 5 mL of methanol-glacial acetic acid fixative (5.2.2), and seal with sealing film (5.3.5) for later use.

8 Test Procedure

8.1 After fixing the blood sample (7.2.2), centrifuge at 800 r/min for 2 min, aspirate the supernatant, and rinse with 0.75% sodium chloride (5.2.1) or

0.1 mol/L sodium chloride solution. Prepare a cell suspension with phosphate buffer and let it stand for at least 1 hour. Centrifuge at 800 rpm for 2 minutes and aspirate the supernatant. Add phosphate buffer to each blood sample first. Incubate with

1.8 mL of 5 mg/mL pancreatic enzyme solution (5.2.4) for 10 min, then add

1.5 mL of 1 mg/mL ribonuclease solution (5.2.5) and incubate. After 10 minutes, add

0.05 mg/mL propidium iodide solution (5.2.6) and stain for 15 minutes.

8.2 Filter the blood using a nylon sieve (5.3.1) to adjust the blood cell concentration to 1x10^8 cells/mL.

8.3 Flow cytometry (6.2) was used to measure more than 3000 red blood cells per blood sample, and the extinction value (E2) of the fish red blood cells was read. Extinction value (E1) of chicken red blood cells.

9.Data Processing Chicken erythrocyte DNA content was used as a standard control, and fish erythrocyte DNA content was calculated according to formula (1). GB/T 18654.14-2026. Fish germplasm testing - Part 14. Determination of DNA content ICS

50 National Standards of the People's Republic of China Replaces GB/T 18654.14-2008 Fish germplasm testing Part

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01 State Administration for Market Regulation The State Administration for Standardization issued a statement.