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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 18654.12-2026

Replacing GB/T 18654.12-2008

Inspection of germplasm for fishes - Part 12: Test method for the karyotype

鱼类种质检验 第12部分：染色体组型分析

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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 12 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 18654.12-2026 is the Chinese national standard covering the chromosome complement of a fish - which identifies the species, detects hybrids, and confirms the triploids and gynogenetic lines that fish breeding now depends on. It replaces GB/T 18654.12-2008 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 18654.12-2008. 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 fish karyotype analysis. This document applies to karyotype analysis for fish germplasm testing.

4.Principles Stimulating fish lymphocyte division with cell division stimulants, or collecting gill filament epithelial cells and fin epithelial cells with vigorous cell division. Cells and embryonic cells were treated with colchicine to disrupt spindle fibers, thus arresting cell division at metaphase. After hypotonic treatment, fixation, slide preparation, and staining, the cells were then examined under a microscope. The cleavage phases were obtained by microscopic examination and karyotyping analysis was performed.

5 Reagents and Materials

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

5.1.1 Potassium permanganate (KMnO4).

5.1.2 Ethanol (CH3CH2OH), concentration (volume fraction) 95%.

5.1.3 Sodium chloride (NaCl).

5.1.4 Sodium bicarbonate (NaHCO3).

5.1.5 Fetal serum, in milliliters (mL).

5.1.6 Fetal bovine serum, in milliliters (mL).

5.1.7 Cell culture medium (commercial culture medium such as M-199, RPMI-1640 or EaglesMEM, or homemade culture medium).

5.1.8 Penicillin, in International Units (IU).

5.1.9 Streptomycin, in milligrams (mg).

5.1.10 Kanamycin, in International Units (IU).

5.1.11 Phytohemagglutinin (PHA).

5.1.12 Colchicine.

5.1.13 Anhydrous disodium hydrogen phosphate (Na2HPO4).

5.2 Solution Preparation

5.2.1 KMnO4 solution Weigh out KMnO4 (5.1.1) according to the volume of the disinfected water and prepare a 1mg/L~2mg/L KMnO4 solution. 5.2.2 75% ethanol solution Take 100 mL of 95% ethanol (5.1.2), add

26.67 mL of deionized water or distilled water, and mix well.

5.2.3 Physiological saline Prepare a 0.7%~0.9% NaCl solution by weighing 7.0g~9.0g NaCl (5.1.3), adding 1L of sterile water, mixing well, and incubating at 121°C. Sterilize at 103.4 kPa for 30 min in an autoclave (6.2). 5.2.4

0.1 mol/L NaHCO3 solution Weigh 8.4g NaHCO3 (5.1.4), add 1L of sterile water, mix well and set aside. Filter using a bacterial filter (5.3.6).

5.2.5 Culture medium A culture medium was prepared by mixing calf serum (5.1.5) or fetal bovine serum (5.1.6) with cell culture medium (5.1.7) at a volume ratio of 1.4.Each milliliter of culture medium... The solution contained penicillin (5.1.8), streptomycin (5.1.9), and kanamycin (5.1.10) at concentrations of 100 IU, 100 mg, and 50 IU, respectively, and was prepared using a

0.1 mol/L solution. Adjust the pH of the NaHCO3 solution (5.2.4) to 7.2.

5.2.6 PHA solution According to the fish size and the dosage used for injection or tissue culture, weigh PHA (5.1.11) and mix with sterile physiological saline (5.2.3) and deionized water. Prepare a PHA solution of appropriate concentration using water or distilled water. Filter the solution using a bacterial filter (5.3.6) during cell culture.

5.2.7 Colchicine solution Depending on the culture method, temperature, and fish size, weigh out colchicine (5.1.12) and prepare a solution with a mass concentration of 5 μg/mL. A 10 mg/mL colchicine solution. Filter the solution using a bacterial filter (5.3.6) during cell culture. 5.2.8

0.2 mol/L Na2HPO4 solution Weigh 28.4g of anhydrous Na2HPO4 (5.1.13), add 1L of

sterile water, mix well and set aside. 5.2.9

0.2 mol/L NaH2PO4 solution Weigh 24.0g of anhydrous NaH2PO4 (5.1.14), add 1L of sterile water, mix well and set aside.

5.2.11 Hypotonic solution Weigh out

5.59 g of KCl from 0.0375 mol/L,

0.05 mol/L, or

0.075 mol/L KCl solutions, respectively. (5.1.16) Add 1L of sterile water, mix well, and store at 4°C for later use.

5.2.12 Carnoy's fixative Methanol (5.1.17) or anhydrous ethanol (5.1.18) and glacial acetic acid (5.1.19) are mixed at a volume ratio of

3.1 and used immediately.

5.2.13 Chromic acid cleaning solution Mix potassium dichromate (5.1.20). water. concentrated sulfuric acid (5.1.21) in a ratio of 1 (g). 2 (mL). 18 (mL).

5.2.14 Giemsa stock solution 0.5g of Giemsa powder (5.1.22) and 33mL of glycerin (5.1.23) were mixed with a small amount of glycerin in a mortar (5.3.10) and ground. When there are no particles, pour in the remaining glycerin, keep warm at 56°C for 2 hours, add 33mL of methanol (5.1.13), and store away from light.

5.3 Materials

5.3.1 White porcelain plate.

5.3.2 Disinfect gauze or disinfectant paper towels.

5.3.3 Petri dish. 5.3.4 10mL beaker. 5.3.5 25mL culture flask.

5.3.6 Bacterial filter (membrane pore size 0.22μm).

5.3.7 Centrifuge tubes (10mL, 15mL).

5.3.8 Glass slides. Glass slides are immersed in chromic acid cleaning solution (5.2.13) for at least 12 hours, rinsed with purified water, and then soaked in anhydrous ethanol (5.1.18) for 1 hour. Store at -20°C until needed. Alternatively, purchase clean, ready-to-use glass slides.

5.3.9 Alcohol lamp.

5.3.10 Mortar and pestle.

5.3.11 Silk sieve [aperture 60 mesh (

0.250 mm) ~ 100 mesh (