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敦品励学， 笃志尤能

Infectious Hematopoietic Necrosis Virus Detection System Established Based on MIRA Method

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Abstract

IHNV threatens salmonids. A MIRA method targeting IHNV N gene was established, yielding results in 25 min, 100% detection at 10^2 copies/μL RNA, showing high consistency with dPCR in testing isolates, supernatants and tissues, suitable for on-site detection.

Research Objective

To enable rapid on-site detection of IHNV in fish tissues, this study designs specific primer-probes for IHNV N gene's conserved region, establishes a MIRA method, validates its performance vs RNA standards and dPCR (8 isolates, 12 supernatants, 67 samples), and provides a new tech for IHN early diagnosis.

Introduction

- IHNV harms salmonids,causing high mortality and huge losses
- IHNV spreads via horizontal/vertical routes, with hard control due to variation
- Existing IHNV detection methods (eg, RT-PCR, dPCR) have limit ations.
- MIRA, a promising isothermal tech, is applied for IHNV

Methods

After DNAMAN V6-aligned IHNV N gene conserved regi on, 4 primer sets/2 probes (optimal F1+R1+P1) were desig ned. MIRA (50μL system, 42℃/20min, fluorescence read) a nd dPCR (30μL system, microdroplet amplification/detect io n) were used. MIRA's sensitivity (10-fold RNA dilution s, 32 repeats), specificity, reproducibility, coverage (8 IHN V g enotypes) were evaluated; 12 supernatants/67 samples were tested vs dPCR.

Results

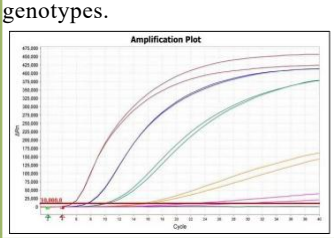
Result 1: Primer/Probe Screening: Optimal primer-prob e set (F 1+R1+P1) targeting the conserved region ofIHNV N gene was con firmed via screening.

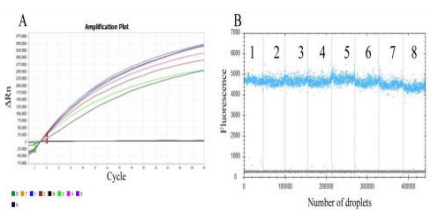
Result 2: 12 cell culture supernatants were positive by b oth metho ds. For 67 suspected tissue samples, MIRA detecte d 58 positives (86.6%), dPCR 59 (88.1%), with 98.3% consis tency (1 undetected sample by MIRA had 12.5 copies/μL, bel ow its limit).

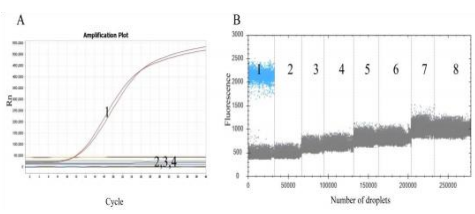
Results

Result 3: Sensitivity & Reproducibility: MIRA detected IHNV RNA at 10^2 copies/μL (100% detection rate in 4 experiments, 32 re peats); dPCR detected 10^1 copies/μL. MIRA showed stable results (no false positives/negatives) in 4 replicates for 10^1–10^5 co pies/μ L RNA.

Result 4:Both MIRA and dPCR were positive only for IHNV (no cross-reaction with 7 other fish viruses) and detected all 8 I HNV genotypes.







Conclusion

The MIRA-based IHNV detection system stably detects 10^2 copies/μL IHNV RNA without cross-reaction. It is faster (~25 minutes) than dPCR and only needs a temperature-controlled fluorescence detector. With high sensitivity, specificity, reproducibility, and coverage, it is ideal for on-site IHNV detection, providing technical support for cold-water aquaculture epidemic control.