



# Detection of aquatic animal viruses by loop mediated isothermal amplification (LAMP)

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# Content

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1 . About the LAMP

2 . Detection of TRBIV by LAMP method

3. Detection of shrimp viruses by LAMP method

4. Detection of AVNV by LAMP method

5. Diagnosis kit of aquatic animal viruses



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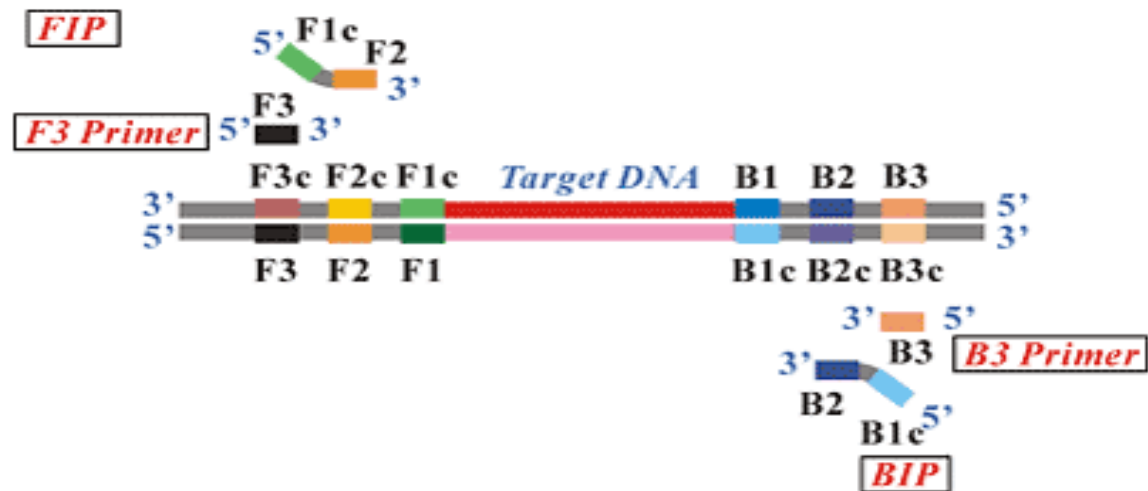
5. Diagnosis kit of aquatic animal viruses

## Introduction of the LAMP

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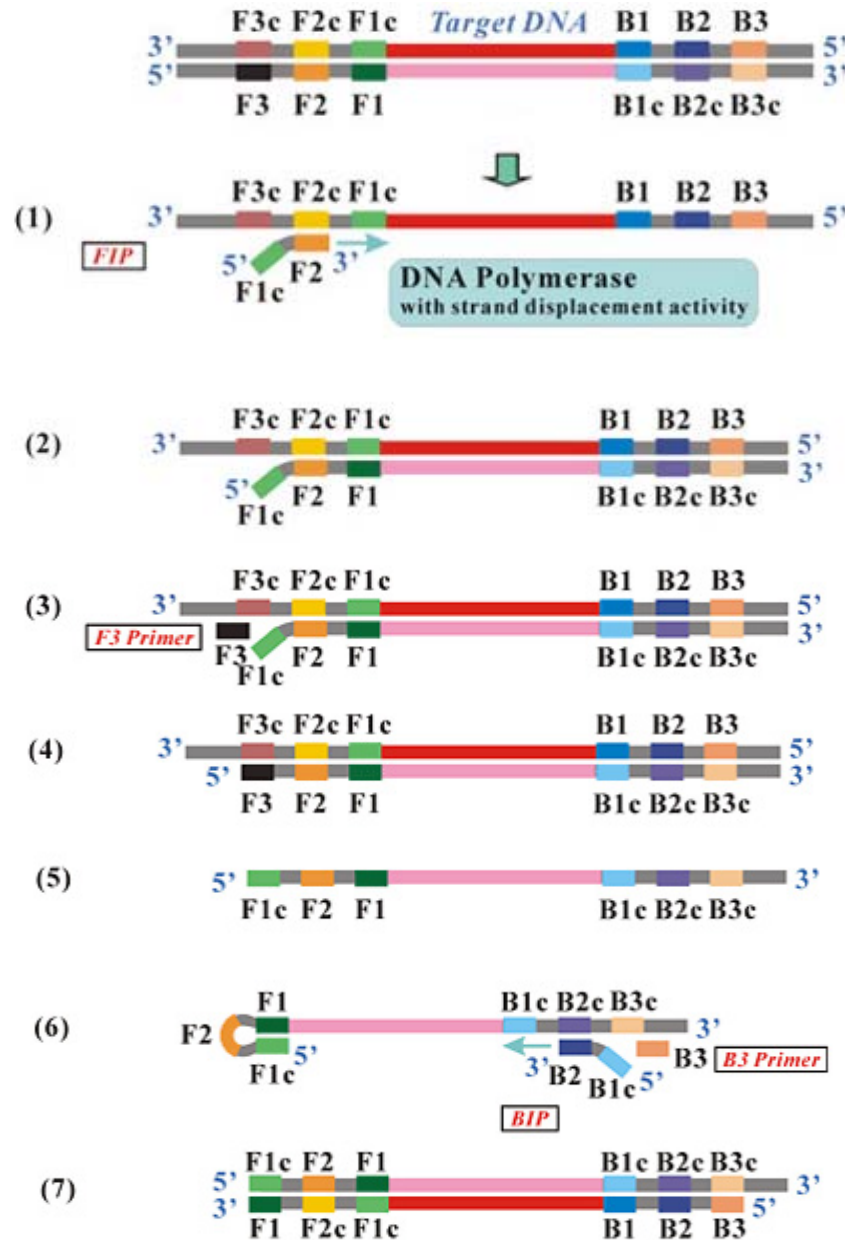
- LAMP: Loop-mediated Isothermal Amplification (Tsugunori Notomi et al, 2000)
- No need of thermal cycling (different from PCR) react at a constant temperature (usually 60~ 65° C)
- use 4 different primers ( recognize 6 distinct regions on the target gene)
- high amplification efficiency( target DNA can be amplified  $10^9$ - $10^{10}$  times in 60 minutes).
- a simple, rapid, specific and low-cost nucleic acid amplification method.

# LAMP primers

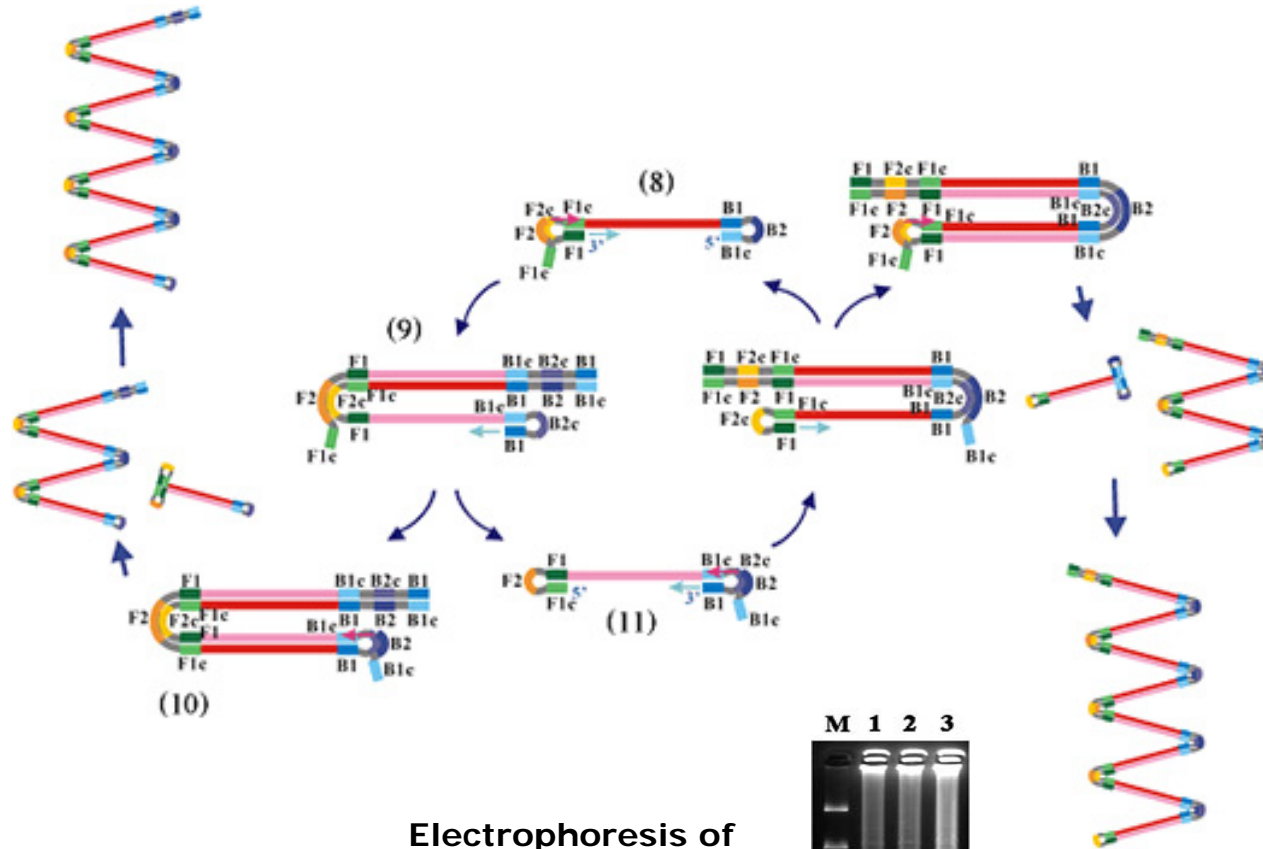


- FIP** : Forward Inner Primer (FIP) consists of the F2 region (at the 3' end) that is complementary to the F2c region, and the same sequence as the F1c region at the 5' end.
- F3 Primer** : Forward Outer Primer consists of the F3 region that is complementary to the F3c region.
- BIP** : Backward Inner Primer (BIP) consists of the B2 region (at the 3' end) that is complementary to the B2c region, and the same sequence as the B1c region at the 5' end.
- B3 Primer** : Backward Outer Primer consists of the B3 region that is complementary to the B3c region.

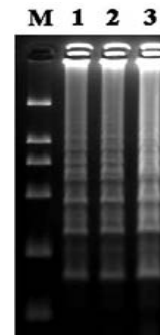
# Basic principle of LAMP



# LAMP basic principle



Electrophoresis of LAMP products





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# TRBIV

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- Turbot ( *Scophthalmus maximus* )

  - one of the most valuable species in coastal areas of northern China

- viral pathogen

  - Turbot Reddish Body Iridovirus (TRBIV)

# Design of primers for LAMP

Fig.1

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TRBIV 1 CATATGTGGTGCCTCAACAGGGCCGTGGTGGCG---TACTATCGCCAGGTGAGGTAGAAAATCTAACCTACGACGTGCCC
ISKNV 1 CNTATGTGGTGCCTCAACAGGACCGTGGTGGCAATATACCTCGCCAGGTGAGGTGGAATACTAACCTATGACGTGCCC
REIV 1 ATTACAGAGGGTCGTGAAAGGGCCGT---TGGCGAC---CTGTCCGCCGACGGGTGGAAAACGTAACATATGACGTACCC
OSGIV 1 ATTACAGAGGGTCGTGAAAGGGCCGT---TGGCGAC---CTGTCCGCCGACGGGTGGAAAACGTAACATATGACGTACCC
LYCIV 1 ATTACAGAGGGTCGTGAAAGGGCCGT---TGGCGAC---GTGTCACCCGACGGGTGGAAAACGTAACATATGACGTACCC

TRBIV 78 ACTCCCAGGAGGACCA---CGCAAC-----CTTGAACAGG---GTGGCATG---TCACCGATGA
ISKNV 81 ACTCCCAGGAGGACCA---TATGTGGT-----CTTGAACAGGACCGTGGTAAATATACCTCACCAGGATGA
REIV 76 ACTCCCAGGAGGACCAACAGCATATGTGGTGGCATAACCTCGAACAGG---GTGGCGCAATGTACCATCACCAGGATGA
OSGIV 76 ACTCCCAGGAGGACCAACAGCATATGTGGTGGCATAACCTCGAACAGG---GTGGCGCAATGTACCATCACCAGGATGA
LYCIV 76 ACTCCCAGGAGGACCA---TATGTGGTGGCATAACCTTGAACAGG---GTGGCGCAATGTACCATCACCAGGATGA

TRBIV 131 CATTGAAAATCTAACGTATGACGTGGCCACCCCGGACGATCGTACACACACCGTATGTGTGCCAAGCGATATCATGC
ISKNV 149 GGTGAAAATCTAACCTATGACGTGGCCACCCCGGACGATCGTACACACACCGTATGTGTGCCAAGCGATATCATGC
REIV 153 GGTGAAAATCTAACGTATGATGTGCCACCCCGGACGATCGTACACGACACCATATGTGTTGCCAAGCGATATCATGC
OSGIV 153 GGTGAAAATCTAACGTATGATGTGCCACCCCGGACGATCGTACACGACACCATATGTGTTGCCAAGCGATATCATGC
LYCIV 150 GGTGAAAATCTAACGTATGACGTGGCCACCCCGGACGATCGTACACGACACCATATGTGTTGCCAAGCGATATCATGC

TRBIV 211 C-ATATGCAGTGTCACTCCATTGCGTGCA
ISKNV 229 C-ATATGCAGTGTCACTCCATTGCGTGCA
REIV 233 C-ATATTCAGTGTCACTCCATTGCGTGCA
OSGIV 233 CCATATTCAGTGTCACTCCATTGCGTGCA
LYCIV 230 C-ATATTCAGTGTCACTCCATTGCGATCA
    
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A

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1 5'-GGGCCGTGGTGGCGTA<u>CTATCGCCAGGTGAGGTAGA</u>AAATC<u>TAACCTACGACGTGCCAC</u>
3'-CCCGGCACCACCGCATGATAGCGGTCCACTCCATCTTTTAGATTGGATGCTGCACGGGTG

61 5'-TCCCACGAGGACGCCACGCAACCTTGAACAGGGTGGCATGTCACCCGATGACATTGAAAA
3'-AGGGTGCTCCTGCGGTGCGTTGGAACTTG<u>TCCCACCGTACAGTGGGCTA</u>CTGTAACCTTTT

121 5'-T<u>CTAACGTACGACGTGGCCACC</u>C<u>CGGCAGCATCGTACACAACACCGTATGTGCTGCCAAG</u>
3'-AGATTGCATGCTGCACCCGGTGGG<u>GCCGTCGTAGCATGTGTTGTG</u><u>GCATACAGCAGGTTTC</u>

181 5'-CGATATCATGCCATATGCAATGTCATCTCCATTGCGTGCAGGCGATGCATATACGCCGTG
3'-<u>GC</u>TATAGTA<u>CGGTATACGTTACAGTAG</u>AGGTAACGCACGTCCGCTACGTATATGCGGCAC
    
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B

GenBank accession  
number is AY994122 .

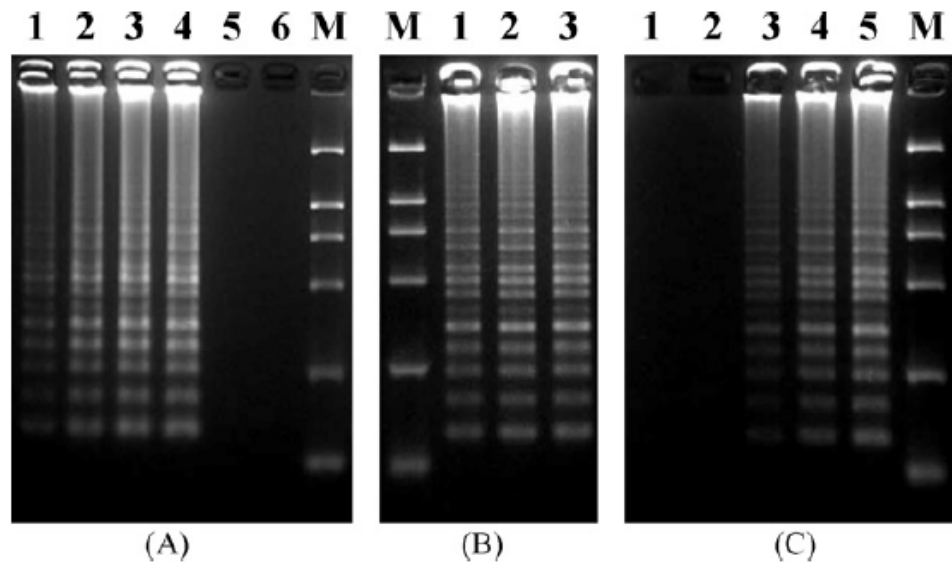
# Optimization of LAMP reactions

## Three factors of the reaction

(A). Magnesium chloride concentration.

(B). Reaction temperature.

(C). Reaction time.

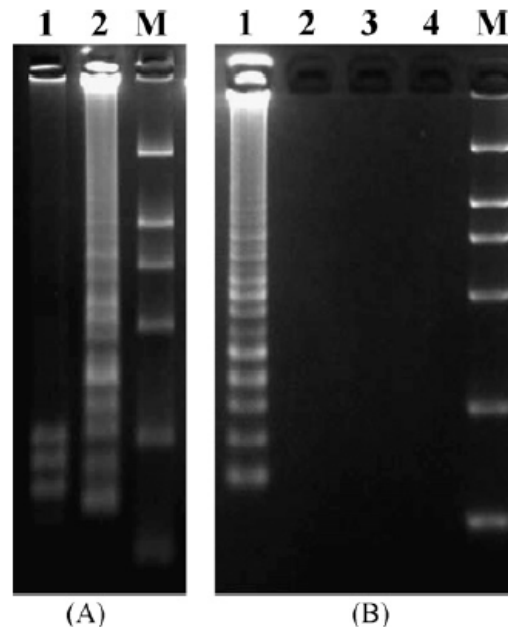


**Fig. 2.** Optimization of the LAMP reaction's detection of the *Msp* I restriction fragment DNA from the TRBIV genome. (A) Effect of  $MgCl_2$  concentrations on the LAMP reaction at  $63^\circ C$ . Lanes 1–6: 12, 10, 8, 6, 4, and 2 mM  $MgCl_2$ , respectively. (B) Effect of temperature on the LAMP reaction using 6 mM  $MgCl_2$ . Lanes 1–3:  $60^\circ C$ ,  $63^\circ C$ , and  $65^\circ C$ , respectively. (C) Effect of reaction time on the LAMP reaction using 6 mM  $MgCl_2$  at  $60^\circ C$ . Lanes 1–5: 15, 30, 45, 60, and 75 min, respectively. M: DNA marker DL2000 with 2000, 1000, 750, 500, 250, and 100 bp.

## Specificity of the TRBIV LAMP assay

(A). Digest LAMP product by *Msp* I .

(B). Amplify different irridoviruses by TRBIV primers.

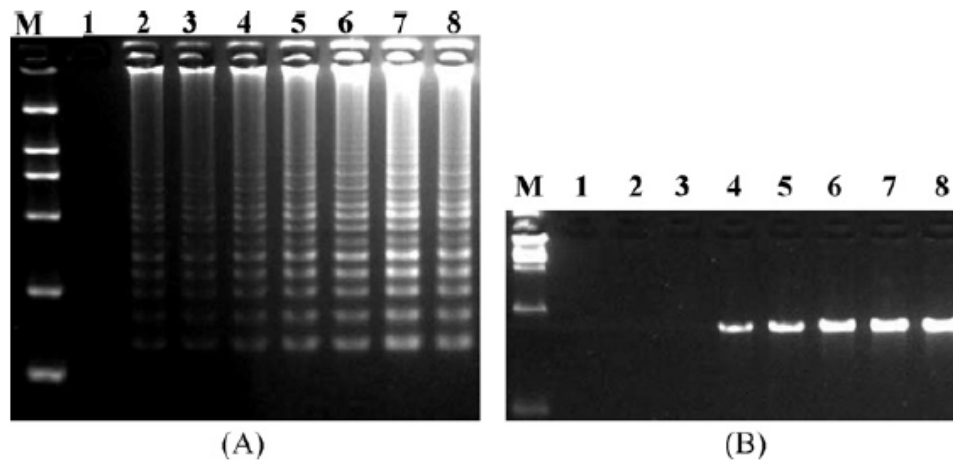


**Fig. 3.** Agarose gel illustrating the specificity of the TRBIV-LAMP assay for four *Megalocytivirus* species. A. Electrophoretic pattern of the LAMP and *Msp* I digested product. (1) *Msp* I digested product; (2) LAMP product; M, DNA marker DL2000 with 2000, 1000, 750, 500, 250, and 100 bp. (B). Specificity of LAMP for the different species. (1) TRBIV; (2) ISKNV; (3) RSIV; (4) LYCIV; M, DNA marker DL2000.

# Detection limit of TRBIV by LAMP and PCR

(A). Detection limit of TRBIV by LAMP.

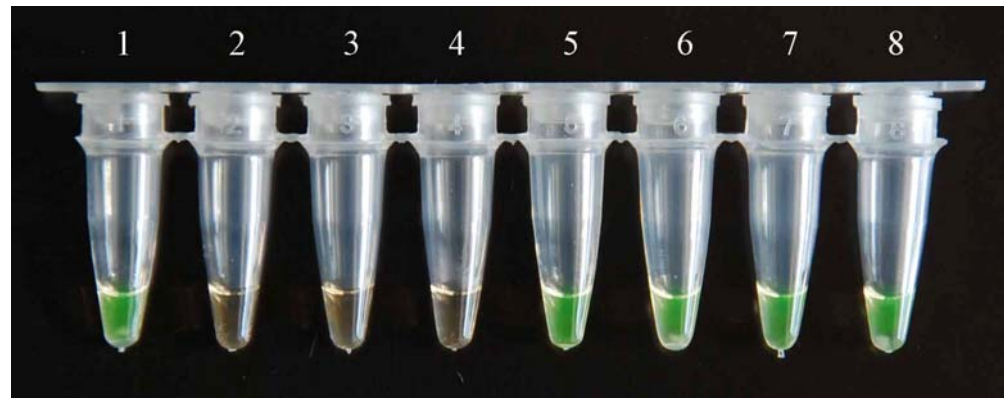
(B). Detection limit of TRBIV by PCR.



**Fig. 4.** Sensitivities of LAMP and conventional PCR detection methods for TRBIV. Lanes 1–8: reaction conducted using 10-fold serial dilutions of the plasmid (pMD18-T-TRBIV) DNA:  $7 \times 0$ , 100,  $10^1$ ,  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ , and  $10^7$  copies, respectively. (A) Sensitivities of LAMP detection of the pMD18-T-TRBIV plasmid containing the *Msp* I restriction DNA fragment of the TRBIV genome (M: DNA marker DL2000 with 2000, 1000, 750, 500, 250, and 100 bp). (B) Sensitivities of conventional PCR detection of the pMD18-T-TRBIV plasmid (M: DNA marker DL15000 with 15000, 10000, 7500, 5000, 2500, 1000, and 250 bp).

## Detection of LAMP products with fluorescent dyes

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- **Fig. 5.** Visual inspection of the product of TRBIV-LAMP. Positive reaction showed green while negative reaction showed orange in daylight with black background. Lane 1: the pMD18-T –TRBIV plasmid (positive control); Lane 2: Water (negative control); Lane 3 and 4: healthy turbot; Lanes 5-8: TRBIV-infected turbot. All the products in the reaction tubes were dyed by diluted 50 times GeneFinder™.



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## Detection of shrimp viruses by LAMP

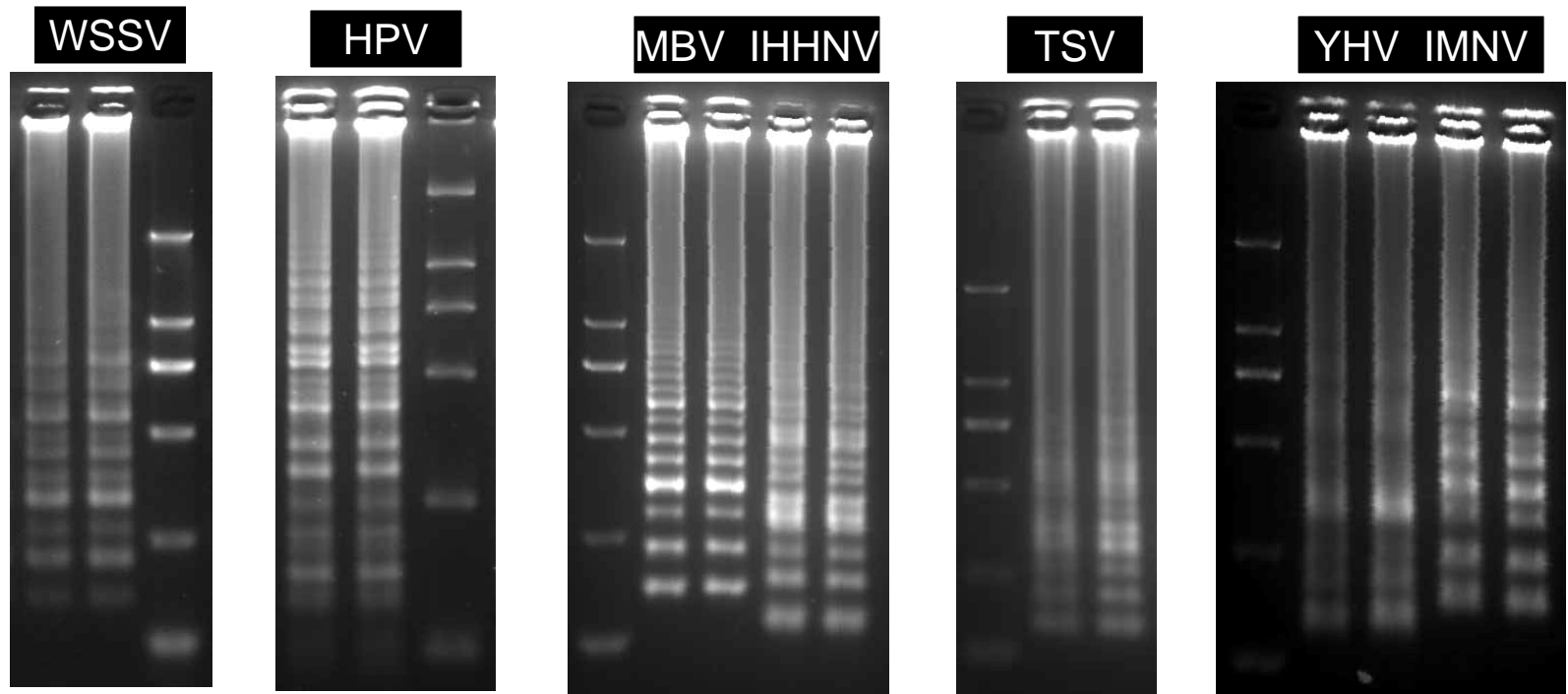
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**Seven shrimp viruses have been developed LAMP detection method.**

<b>Virus</b>	<b>Abbreviation</b>	<b>Genome type</b>
Whit spot syndrome virus	WSSV	Double strand DNA
Taura syndrome virus	TSV	Single strand RNA
Yellow head disease	YHV	Single strand RNA
Hepatopancreatic parvovirus	HPV	Single strand DNA
Infectious hypodermal and hematopoietic necrosis virus	IHHNV	Single strand DNA
Infectious myonecrosis virus	IMNV	Double strand RNA
Monodon baculovirus disease	MBV	Double strand DNA

# Detection of shrimp viruses by LAMP

Electrophoresis of LAMP products of seven shrimp viruses.





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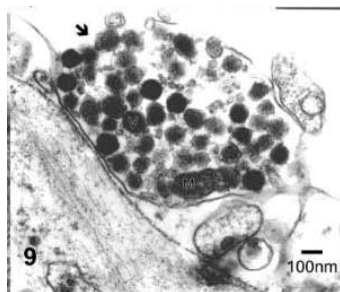
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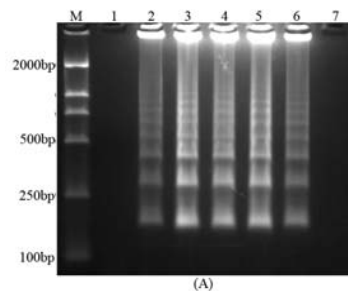
5. Diagnosis kit of aquatic animal viruses

# Detection of AVNV by LAMP

□  
□  
□



Wang et al, 2007



Wang et al, 2009

**Acute Viral Necrosis Virus (AVNV)**  
**scallop virus**

Detection of AVNV by LAMP  
was finished by **Professor Wang**  
**Congming** of our lab.



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# Detection Kit for Rapid Use on Field

Detection kits of the above viruses were developed .



# Usage of the Detection Kit

Detection Kit



Simple Instrument



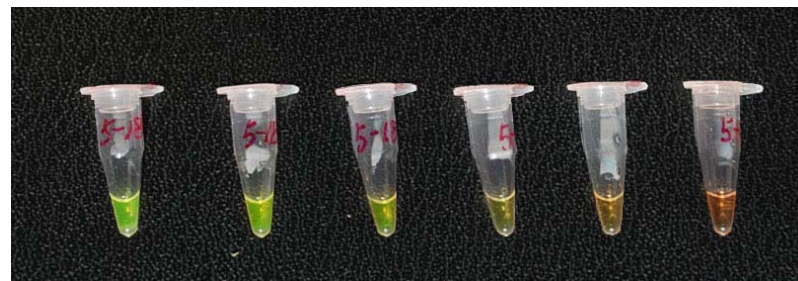
Whatman FTA card (We modified)

Extract of nucleic acid: **5 min**

Denature: 94 °C, **3 min**

Reaction: **60 min**

Detection Result





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**Thanks for your  
attention!**