

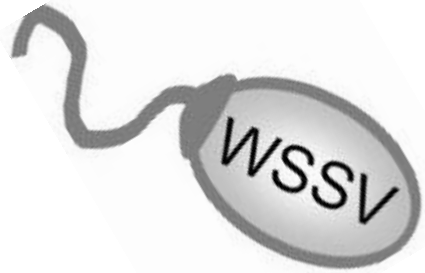
Highly sensitive and rapid detection kit for shrimp pathogens

Qingli Zhang

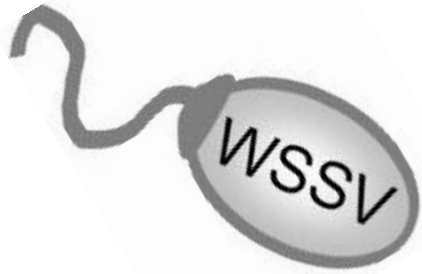
July 27 2016



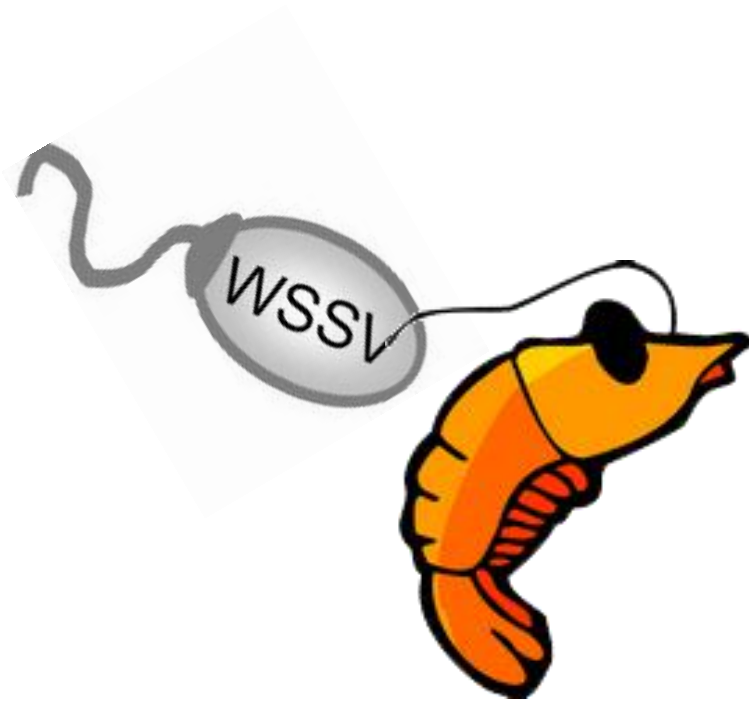
Healthy & Happy



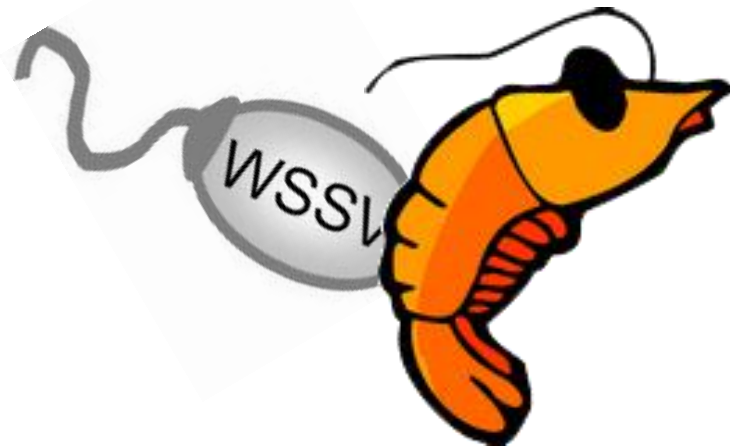
Wow, **virus** is coming.....



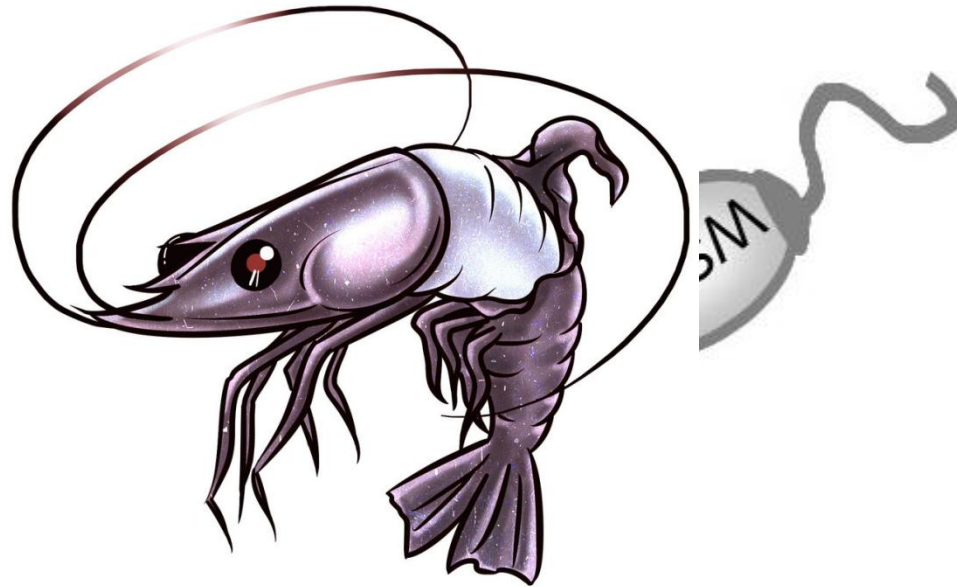
Run, run, run.....



Run, run, run.....



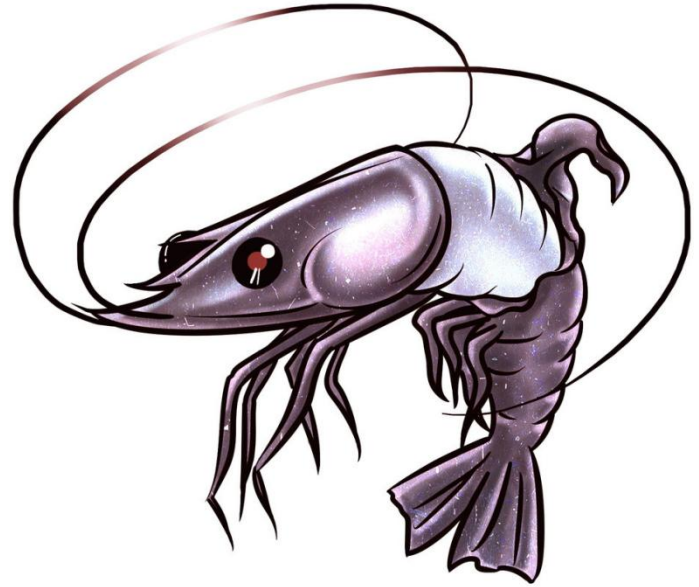
Oh, **my God**.....



Oh, **I got disease.**



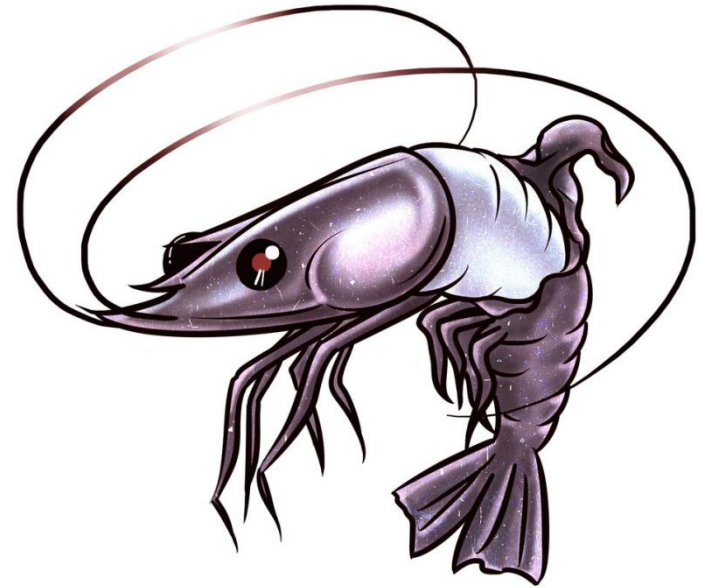
Healthy & Happy



Disease & Sad



Health & Happy



Disease & Sad



Virus is very very tiny. About 200 nanometers

Is it possible?

to determine whether the shrimp is carrying **100 virus copies in 60 minutes in the field.**

The answer is

YES.



Content

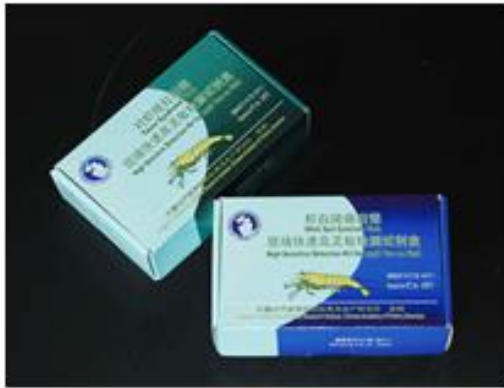
1. General information of the kits
2. Principle of the kit
3. How to use the kit
4. Demonstrations of virus detecting

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1. General information of the kits

Detection kit



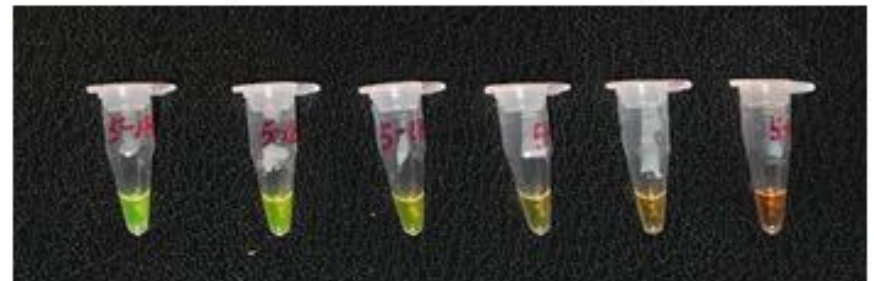
Detection process



OR



Detection result



Fast: Finish the detection in 60min;

Simple: Only need an incubator;

NO False-positive: Contamination-control measure integrated;

High specificity: No cross-reaction between viruses;

High sensitivity: 100-fold higher than PCR;

Easy reading: Read the result simply by color development.

Pathogens Detectable by the Kits

Application scope

kits for shrimp pathogens

WSSV

TSV

CMNV

ECZ

MrNV

IMNV

MBV

PvNV

YHV

BP

IHHNV

HPV

Vp_{AHPND}

Spiroplasma

kits for fish pathogens

NNV

SVCV

GCRV

CCV

TRBIV

Edwardsiella tarda

kits for mollusk viruses

OsHV-1

AVNV



One kit can be used for 4 samples detection.

Application scope:

The kits can be applied for detection the pathogens of aquaculture animal:

selection of healthy broodstock,
disease early diagnosis,

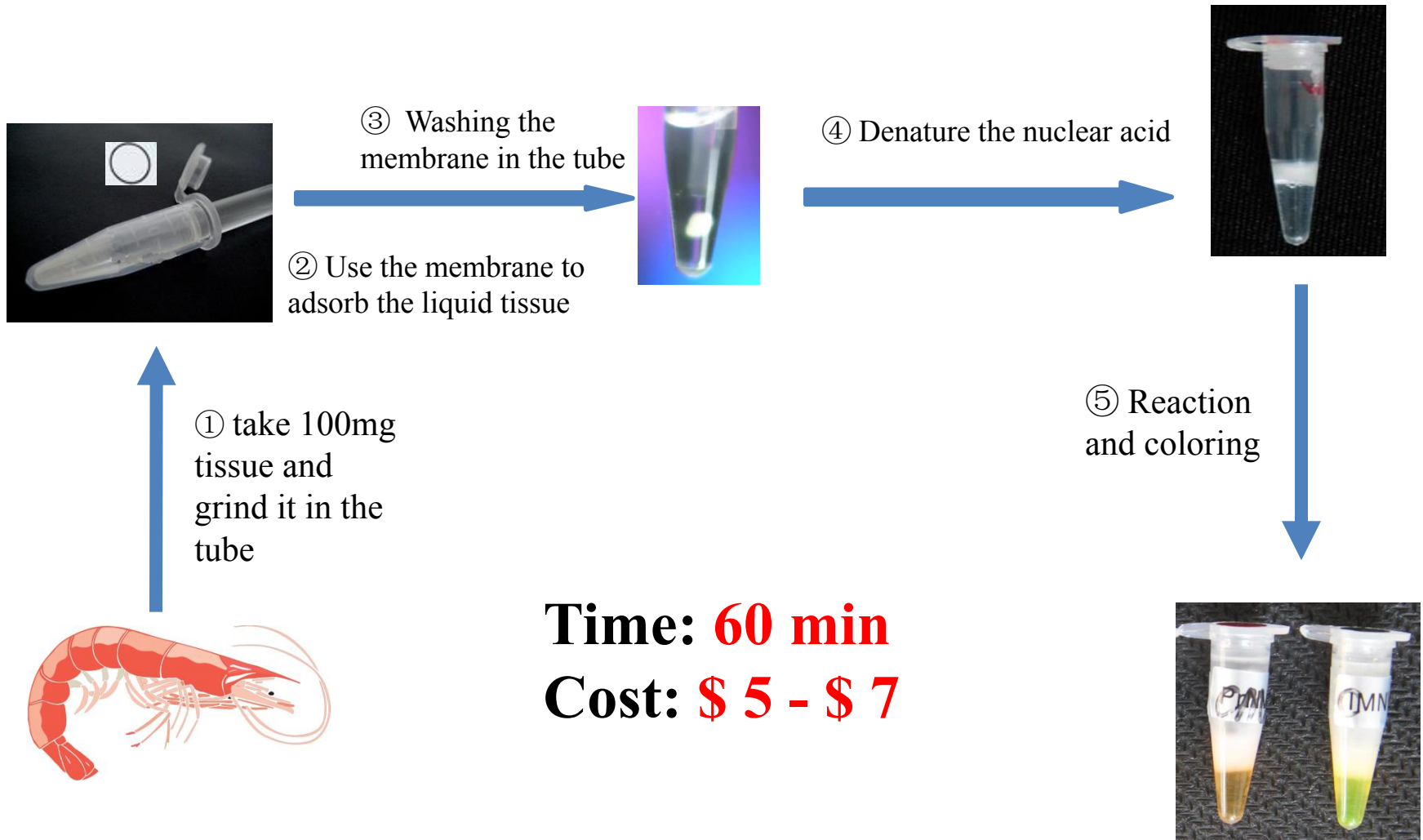
risk monitoring,

epidemiological surveillance.

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2. Principle of the kit



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Kit components

Number	Name in English	Quantity
1	Sampling membrane	4 pieces
2	Membrane for PC	1 piece
3	Membrane for NC	1 piece
4	Sample collection tube	4 pieces
5	Buffer A	0.8 mL
6	Washing tube	6 pieces
7	Nucleic acid denature tube	6 pieces
8	WSSV detection tube	6 pieces
9	Grinder (Pestle)	4 pieces
10	Toothpick	30 pieces
11	Dropper (Pipette)	1 piece
12	Manual	1 copy

Purification of Nuclear acid

[INSTRUCTIONS FOR USE]

1. Collect about 100 mg tissue from gills, appendages or the larvae body, put into the **sample collection tube** (采样管), quickly grind the samples into paste using the **grinder** (研磨棒). A total of four samples can be managed at one time.
2. Dip the toothpick in the sample paste and then daub the liquid adsorbed by the toothpick onto the **sampling membrane** (采样用膜片) until **it** is fully saturated. The different **sampling membranes** with number should be wetted by corresponding sample paste. Discard the toothpick. (The 0.2ml PCR tube containing the **sampling membrane** is kept in a plastic bag. The **sampling membranes** are assigned numbers (1-4) on the tube covers.)

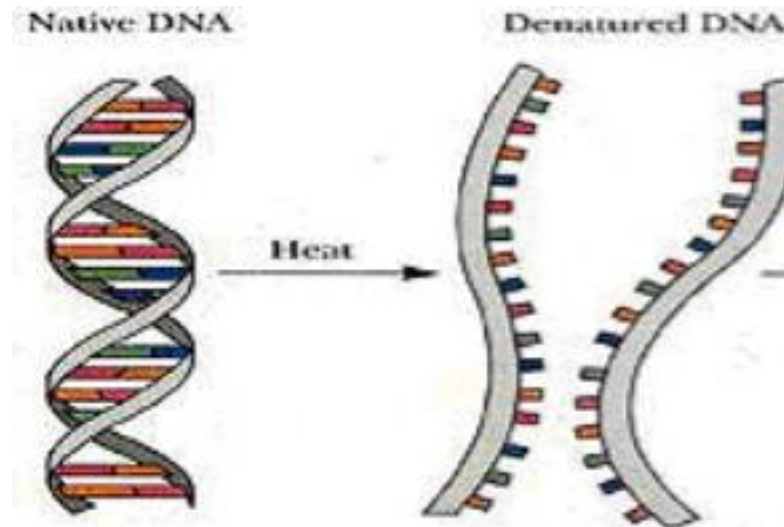
Washing the membranes

3. Add 2-3 drops of **Buffer A** onto the **sampling membrane** in the 0.2ml PCR tube, stir the membrane gently for 30 seconds with a new toothpick. Discard the toothpick.
4. Move the **sampling membrane** into the corresponding numbered **washing tube** (漂洗管) with a new **toothpick**, vortex the tube vigorously for 3-4 minutes. Discard the toothpick.

[Video: purification of nuclear acid and washing of the membrane](#)

Denature of nuclear acid

5. Move the **sampling membrane**, and the **positive and negative control membranes** into the corresponding **nucleic acid denature tube** (核酸变性管) with a new toothpick, incubate at 95°C for 4 minutes. Then put the tube into cold water or place at room temperature immediately for 2 minutes to cool it down.

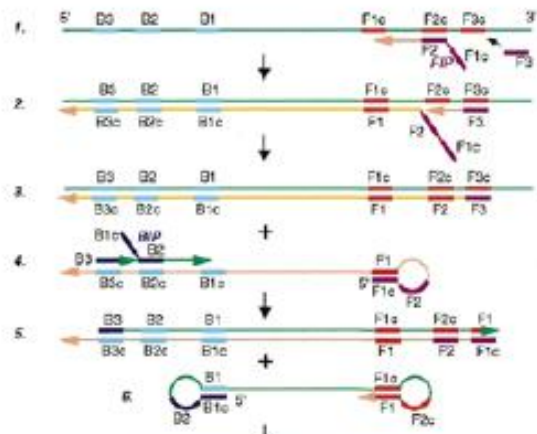


Amplification of the target nuclear acid

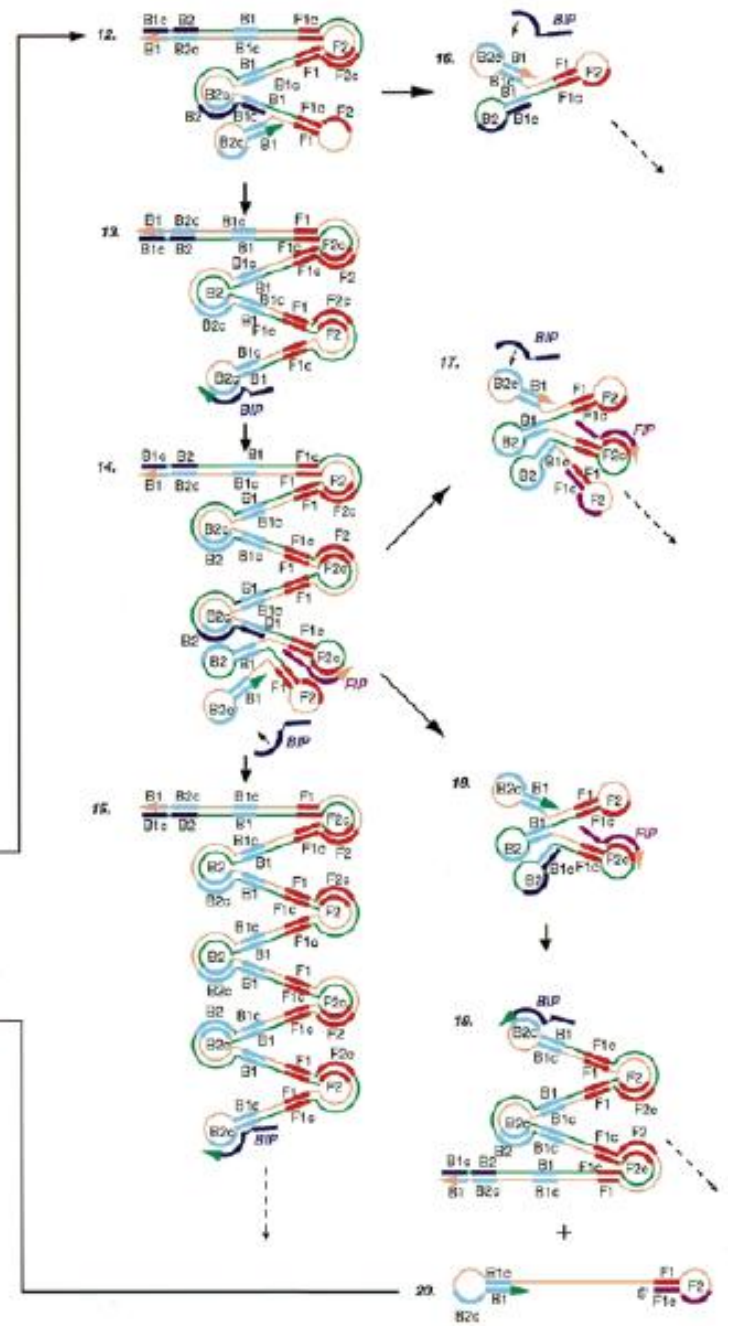
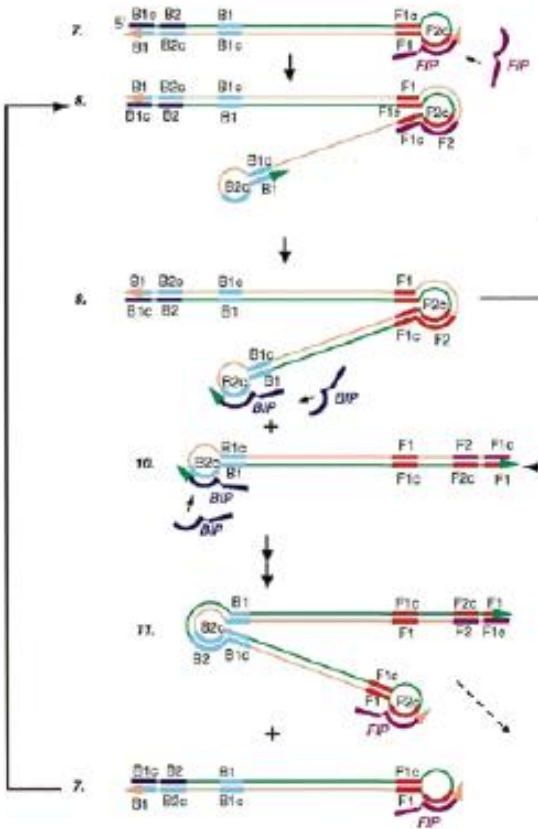
6. Move the membranes into corresponding **WSSV detection tube** (WSSV 検
測管) with a new toothpick, incubate at 57-60 °C for 50 minutes. (Water bath, metal bath or PCR machine. **Note:** if PCR machine is used, the lid-heating program must be turned off.)

View of amplification

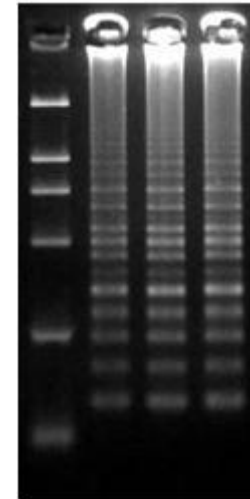
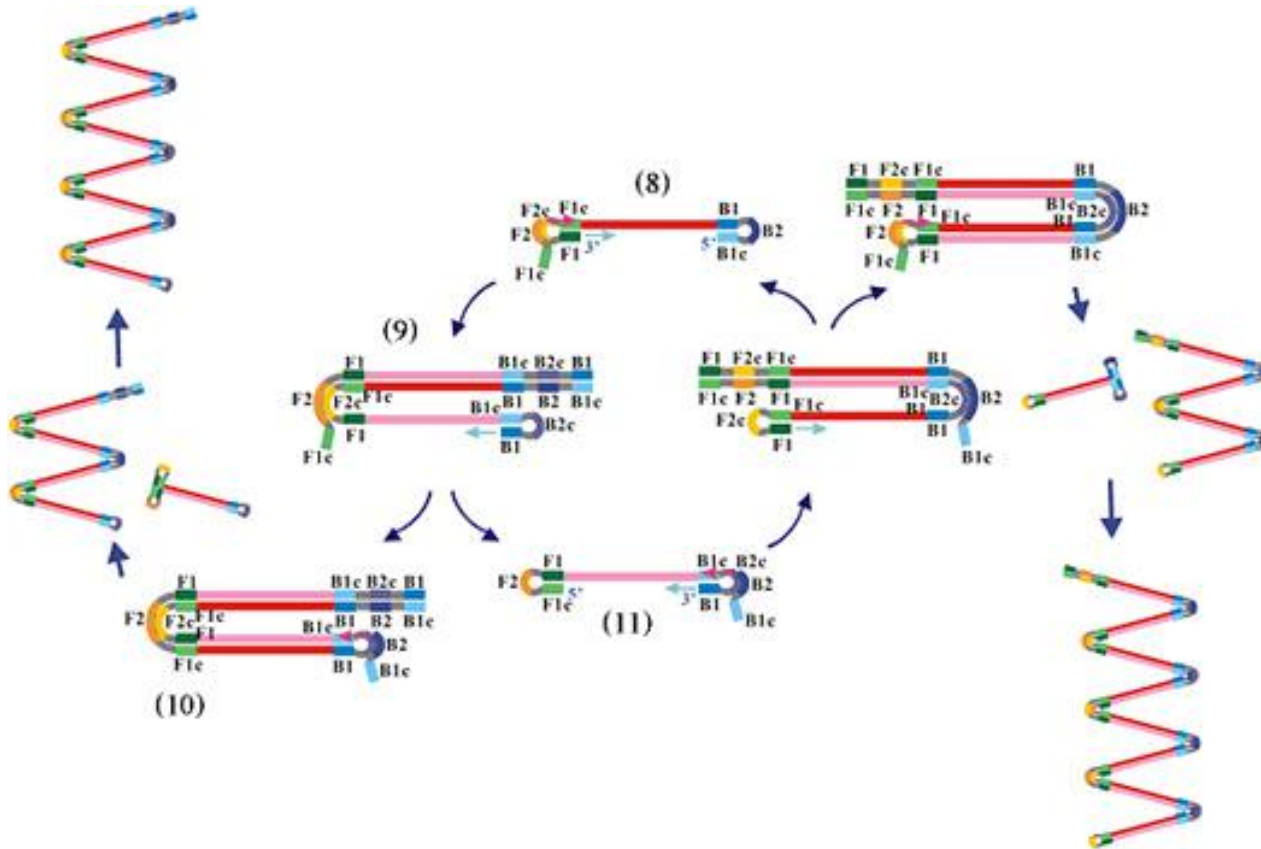
A. I. Starting material producing step



II. Cycling amplification step



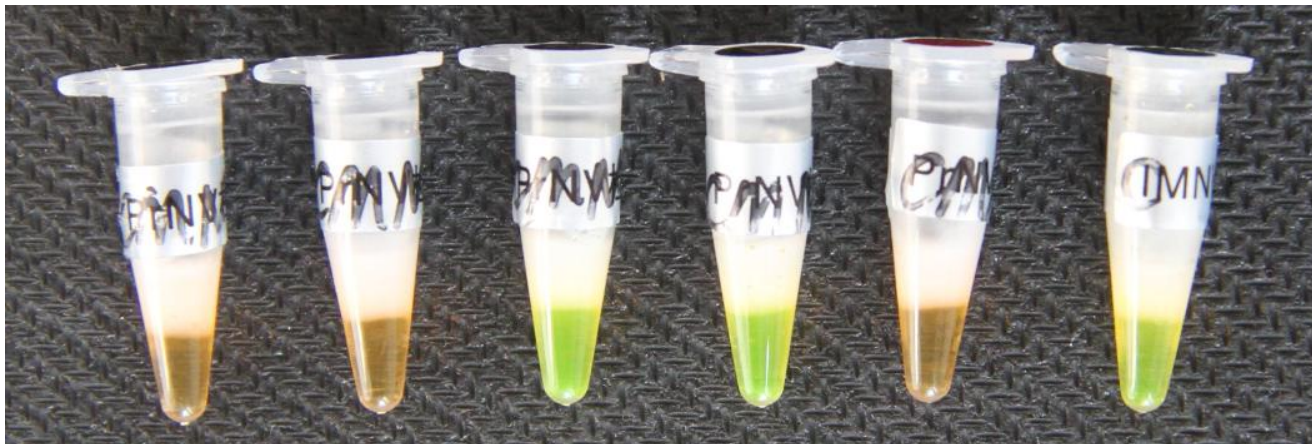
Principle of LAMP



Electrophoresis of LAMP

Get detecting result

7. Incubate the tube at 90~95 °C for 4 minutes, then shake the **WSSV detection tube** immediately (5-6 times within 3 seconds), to make the dye drop from the lid of the tube and blend with the reaction buffer. ↵
8. Swing the **WSSV detection tube** downwards **immediately**, to collect all buffer down to the bottom. Observe the color after 1-2 minutes by naked eye.↵



Thank you.

Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences

