Food and Agriculture Organization of the United Nations

FAO/China Intensive Training Course on Tilapia Lake Virus (TiLV)

Sun Yat Sen University, Guangzhou, China

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Session 2

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In situ hybridization for TiLV

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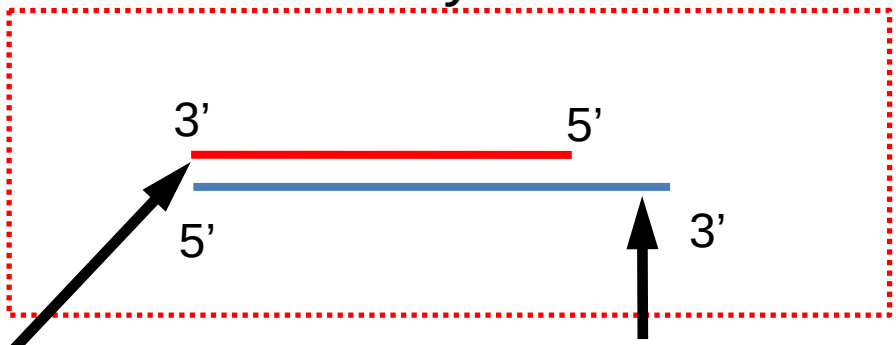
In - situ hybridization

inside site

"in position" or "in place"

a hybrid molecule



TiLV specific probe

TiLV RNA in the cell

"Basically it involves *formation of a hybrid molecule between an endogenous single-stranded RNA or DNA in the cell and a complementary single-stranded RNA or DNA probe*"

Gall (2016) Methods 98:4-9

In situ hybridization (ISH)

Purpose

- To detect and confirm the presence of TiLV (through its nucleic acid) in the tissues and histopathological lesions.
- To identify tissue tropisms of TiLV

Development of TiLV-specific ISH probe

TiLV-infected tilapia

↓

Extraction of total RNA

↓

RT-PCR amplification of 415 bp genomic segment-3

↓

clone a TiLV genomic fragment into a plasmid

↓

PCR labeling with digoxigenin-11-dUTP

↓

DIG-labeled TiLV probe

DNA probes preparation

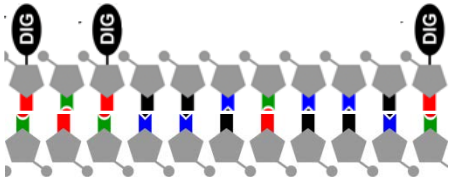
- Probes were prepared using DIG-labeling Mix (Roche, Germany)
- Plasmid pGEM-415 bp was used as a template in the labeling reaction
- 282-bp fragment derived from IMNV was employed as an unrelated negative probe

TATGCAGTACTTTCCCTGCCTGAGTTGTGCTTCTAGCAA
TCAACATCAAAAGCTCACGAGCAAGTGGGGCACTAGCTG
GTAGAGGCAATATCTTCTGTGTAGCAGGCTTATGAGAAG
CAACTGTATACCTTTGTATCCACCCTCCATTGCGGAACT
CAAATTCTC**TATCACGTGCGTACTCGTTCAGT**ATAAGCT
CTCTTGCCTCTTGGTCAAGACCACACTCCTCACCACAGG
CGAGGAACTTTGAGCACTCGAAGAACCCATATTGCCTCT
TTAGCTCAGCTGTCTCCTTGGATATGTCCGCGAGTCTGG
GTGGTGCCACCCACTCGATACGAGGCTTCGGGCCACTCT
TTGGATGTGGTAGTTCAAATAGCCGTTCCCTTAGCTCAG
CATCG**TAGGATGCCTTGTGCCCAAC**

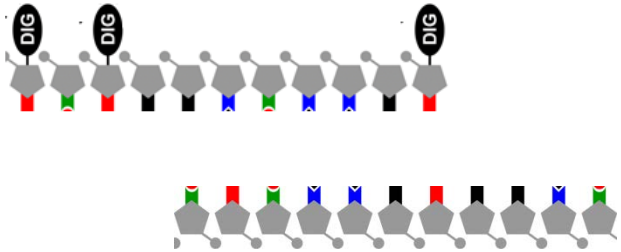
Sequence of a 415-bp derived from genome segment 3 of TiLV cloned in pGEM-T. (used primer Nested ext-1 & ME1)

Principle of *in situ* hybridization (ISH)

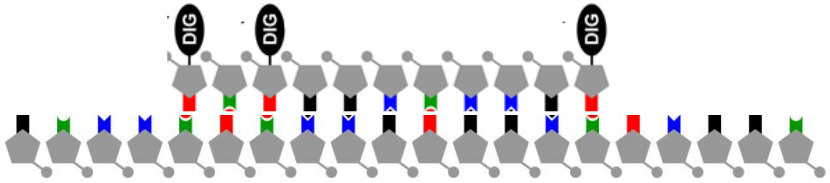
1. Labeling DNA probe



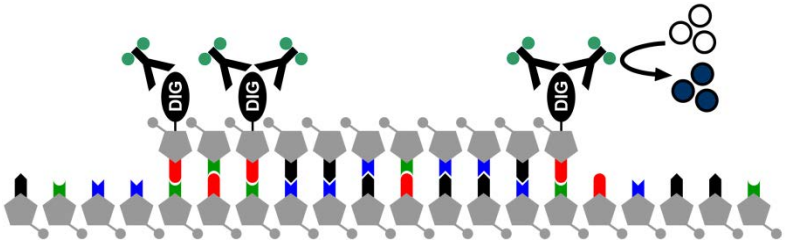
2. Denaturation of DNA probe



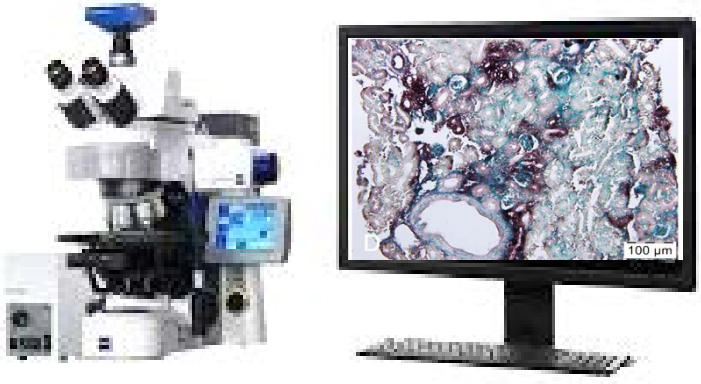
3. Hybridization



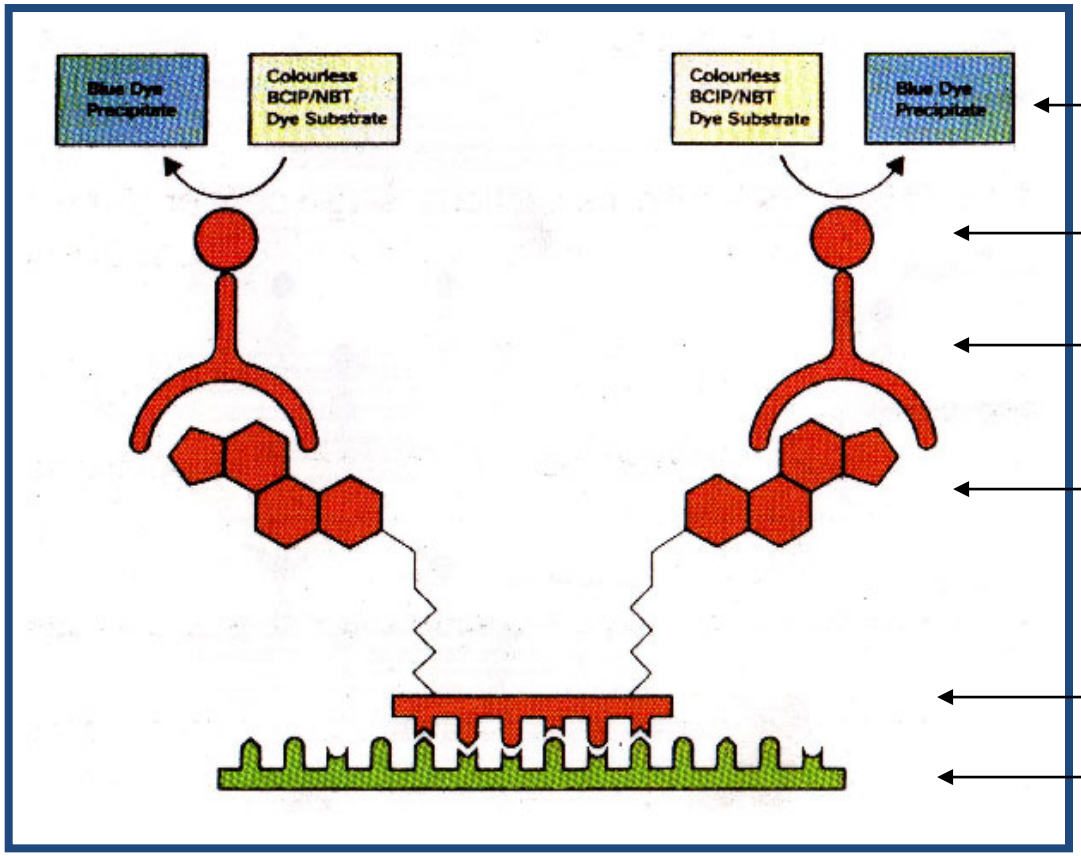
4. Color development



5. Result interpretation

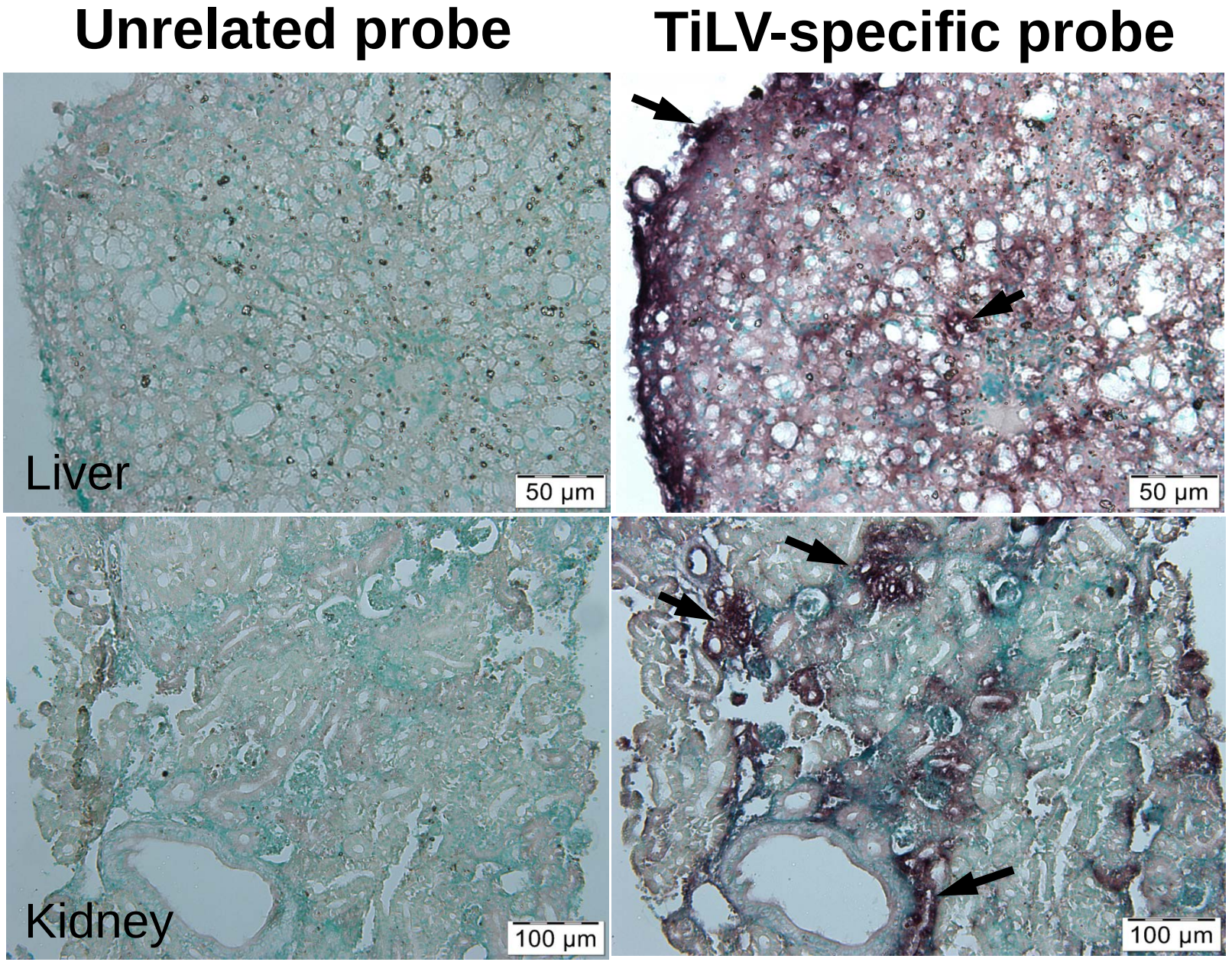


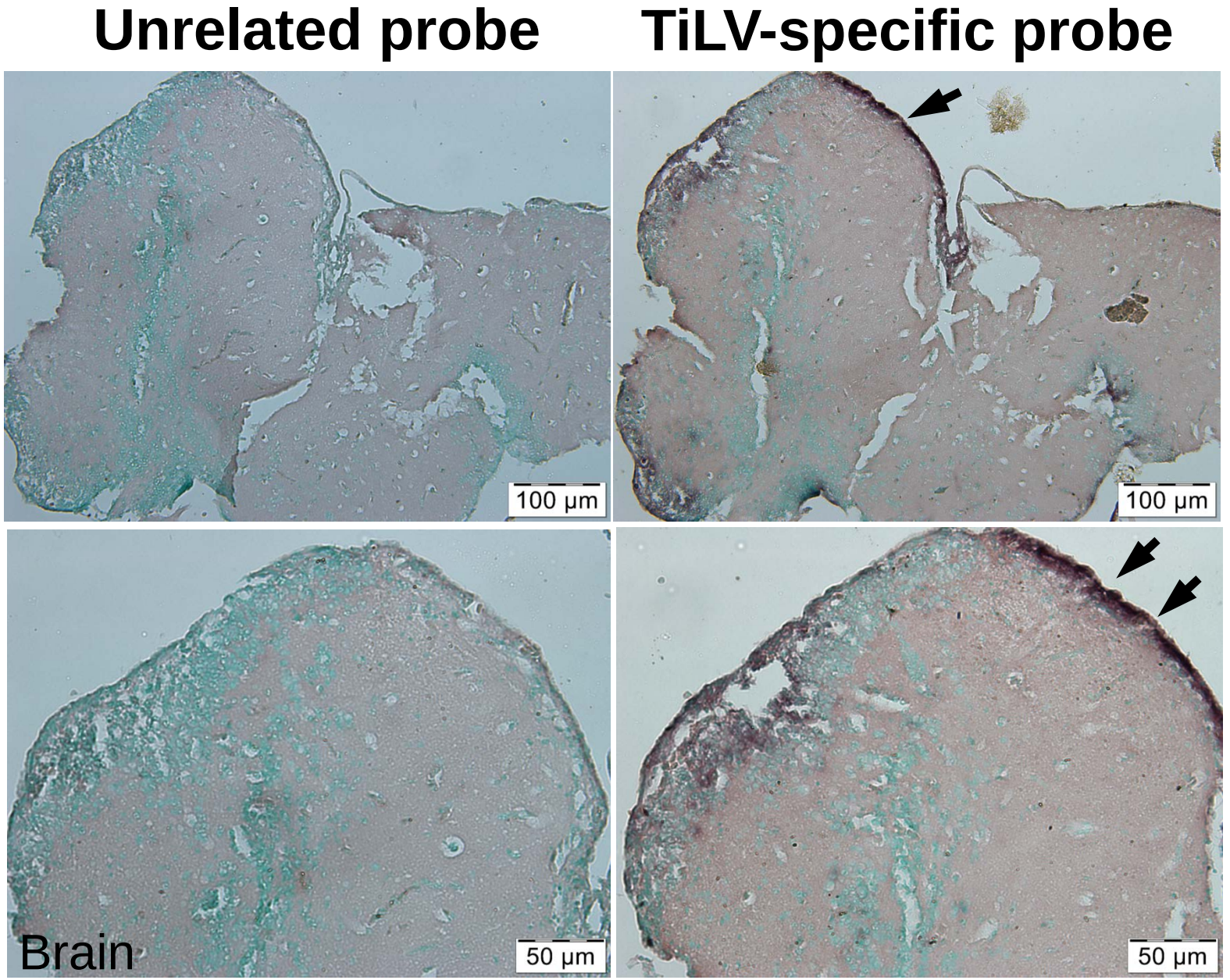
Non-Radioactive Detection Based on Digoxigenin-label

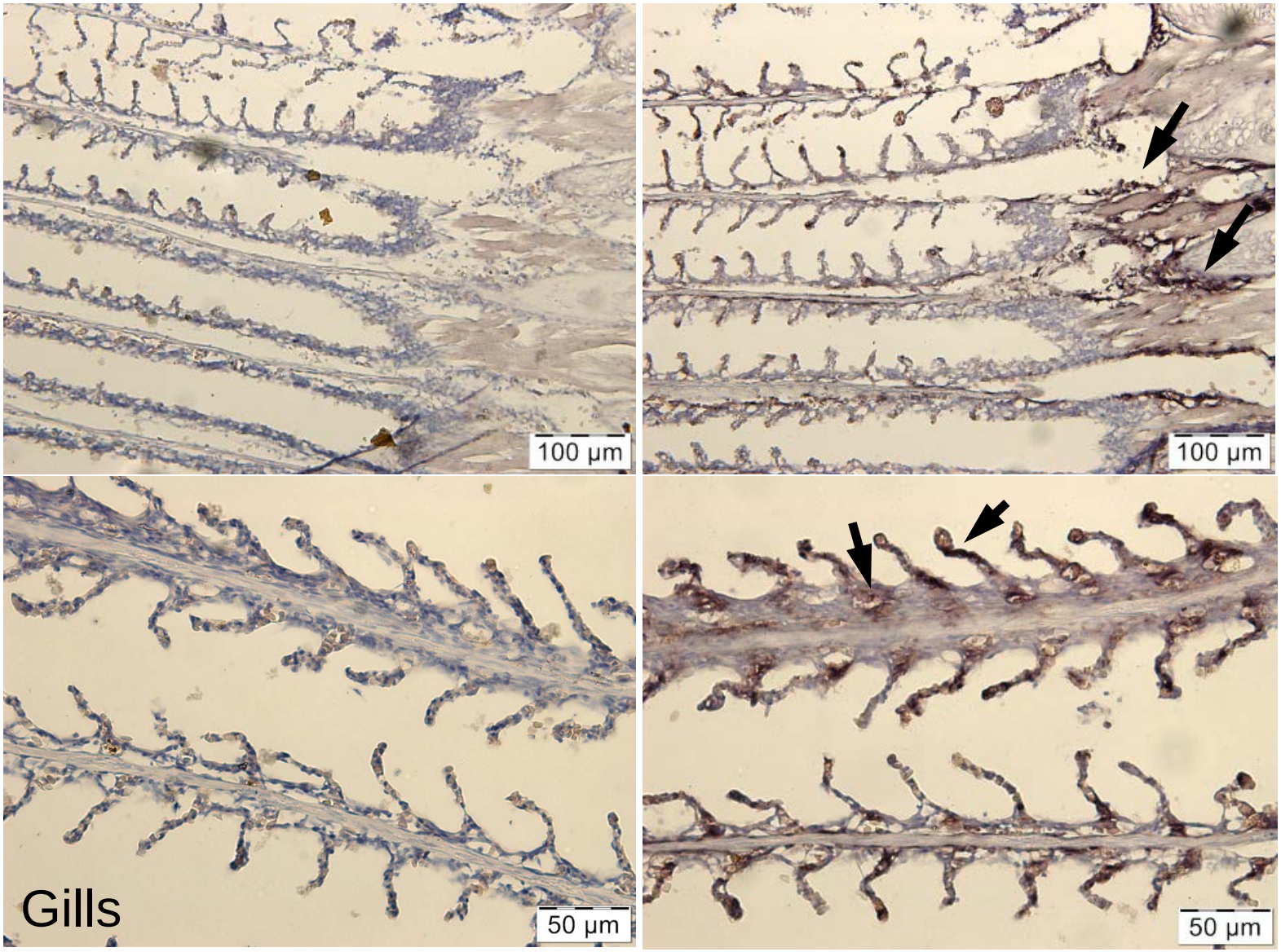


In Situ Hybridization Incubator

- Temperature control
- Trough for water to maintain humidity
- Rack to hold slides
- Cover to maintain temperature & humidity

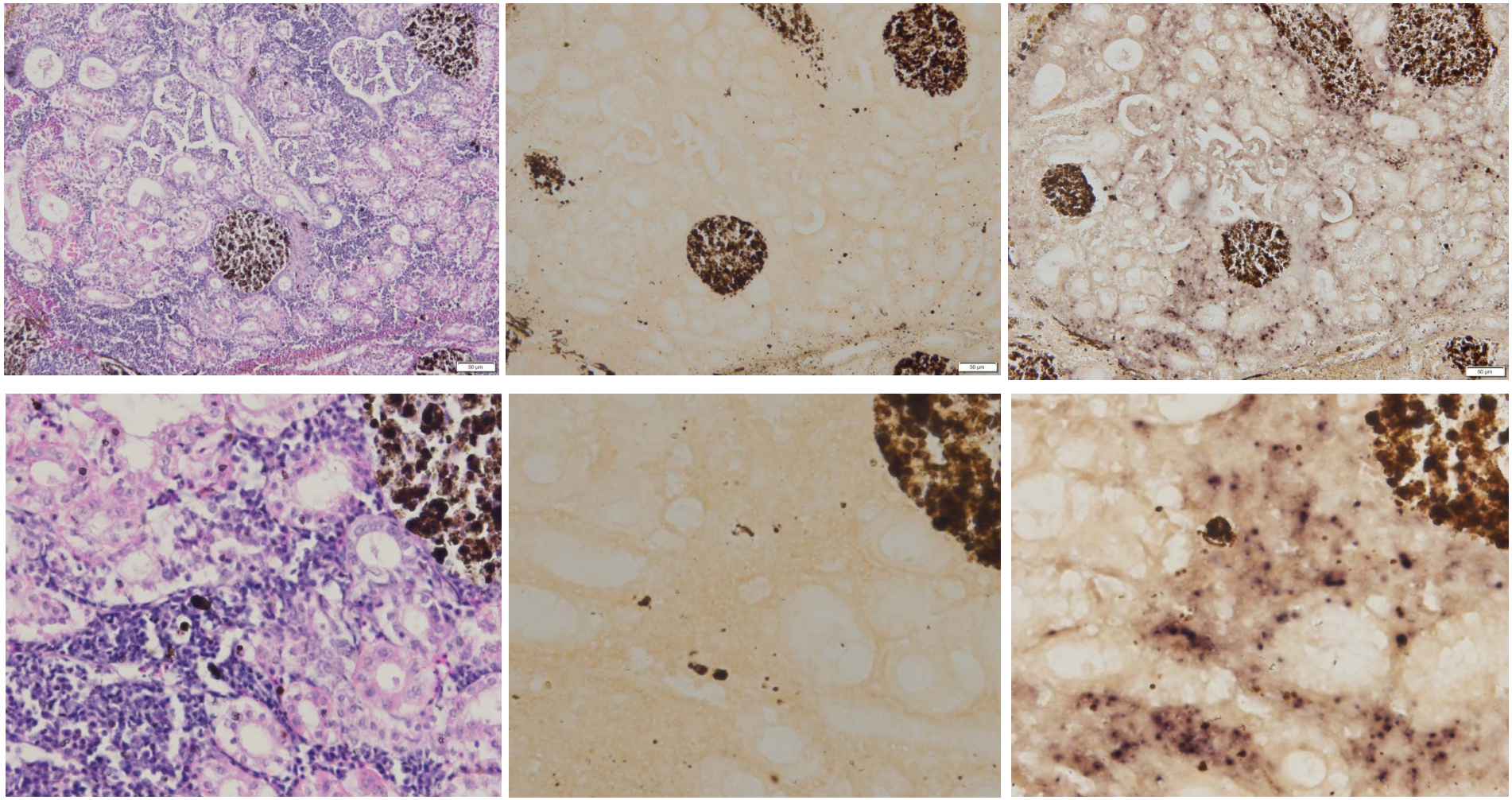

Unrelated probe
TiLV-specific probe
Liver
50 µm
50 µm
Kidney
100 µm
100 µm


Unrelated probe
TiLV-specific probe
100 µm
100 µm
Brain
50 µm
50 µm


Unrelated probe
TiLV-specific probe
100 µm
100 µm
Gills
50 µm
50 µm

Example of ISKNV (1)

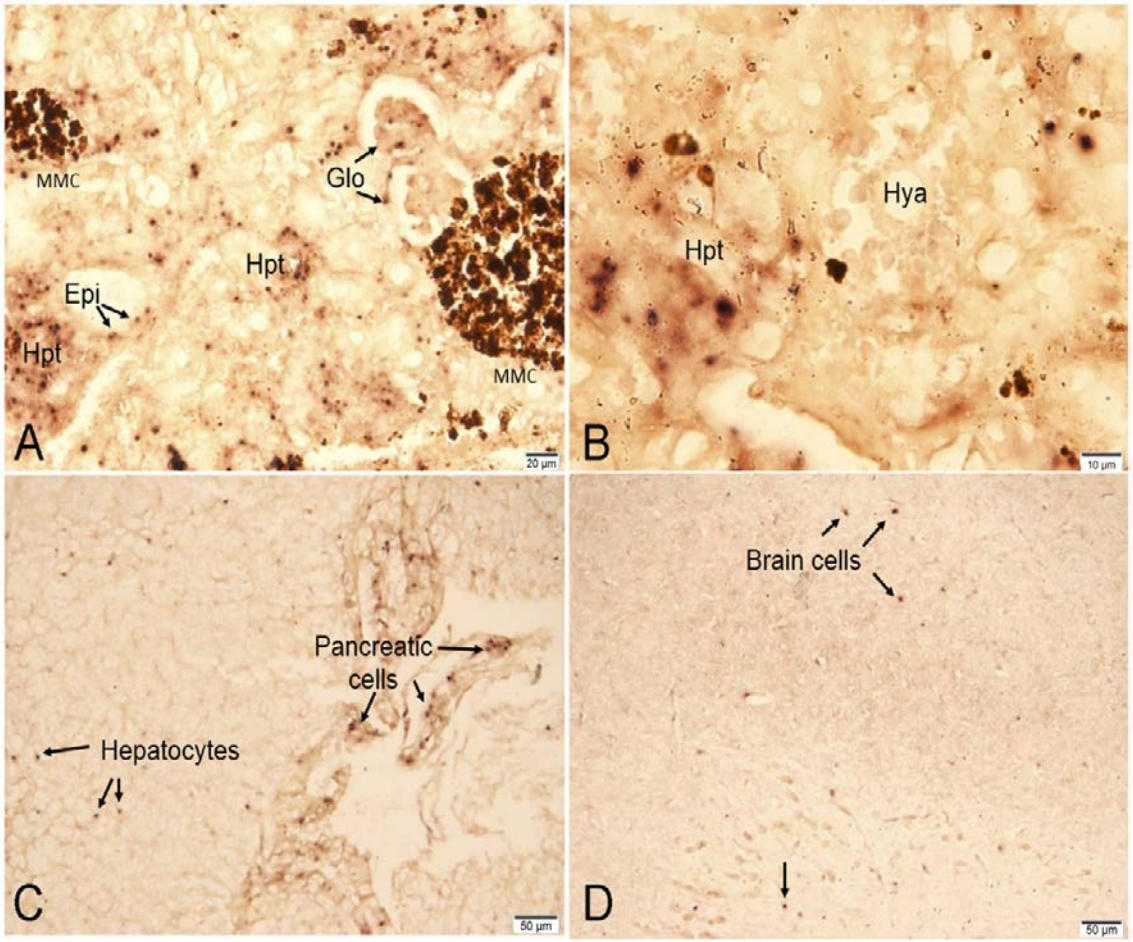
H&E | Unrelated probe | Specific-probe



Taken in conjunction with outbreaks described in Dong et al. 2017 Fish & Shellfish Immunology 68: 65-73

Example of ISKNV (2)

- ISKNV-specific probe (517 bp)
- Kidney (A, B), liver and pancreas (C) and brain (D).
- Strong signals were observed in the epithelial cells (Epi), of hematopoietic tissue (Hpt) and the glomerulus (Glo) of the kidney (A).



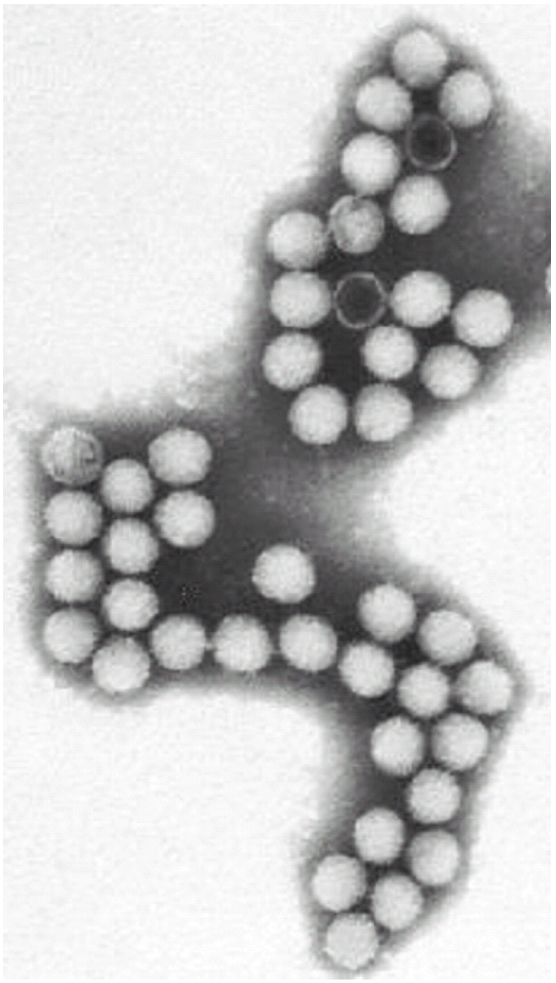
Dong et al. 2017 Fish & Shellfish Immunology 68: 65-73

Applications of ISH in the study of shrimp viruses

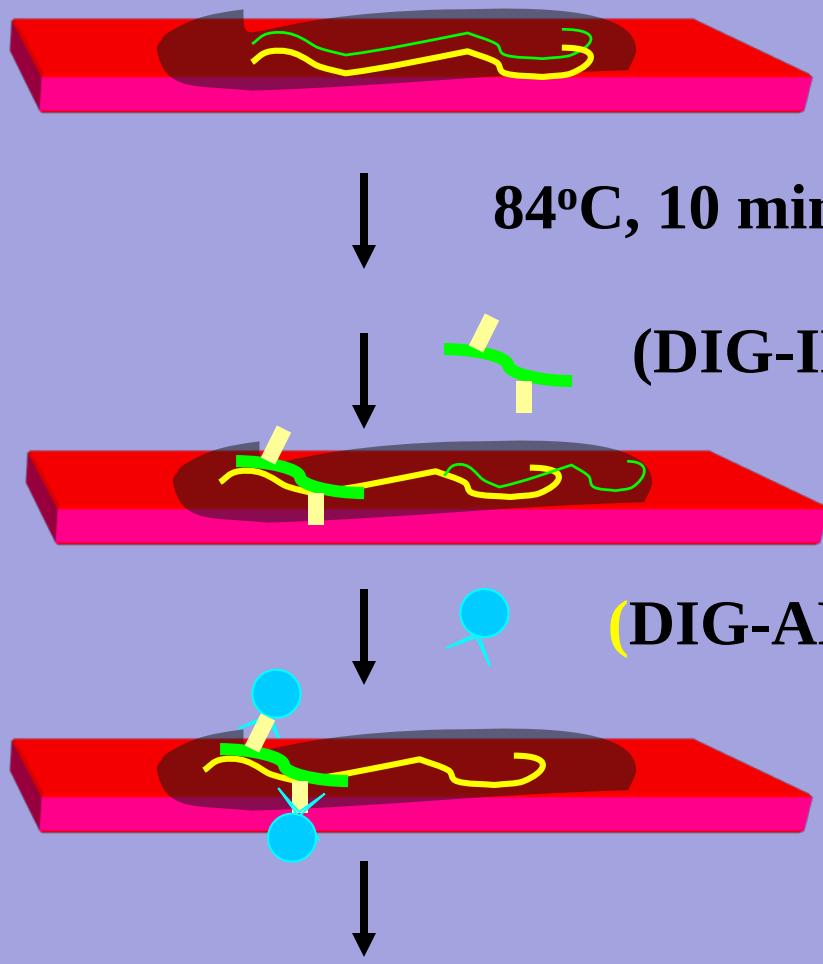
1. 3 major penaeid species, *P. vannmei*, *P. stylirostris*, *P. monodon*, are susceptible to IMNV infection

2. Prove that yellow head virus has a positive sense RNA genome

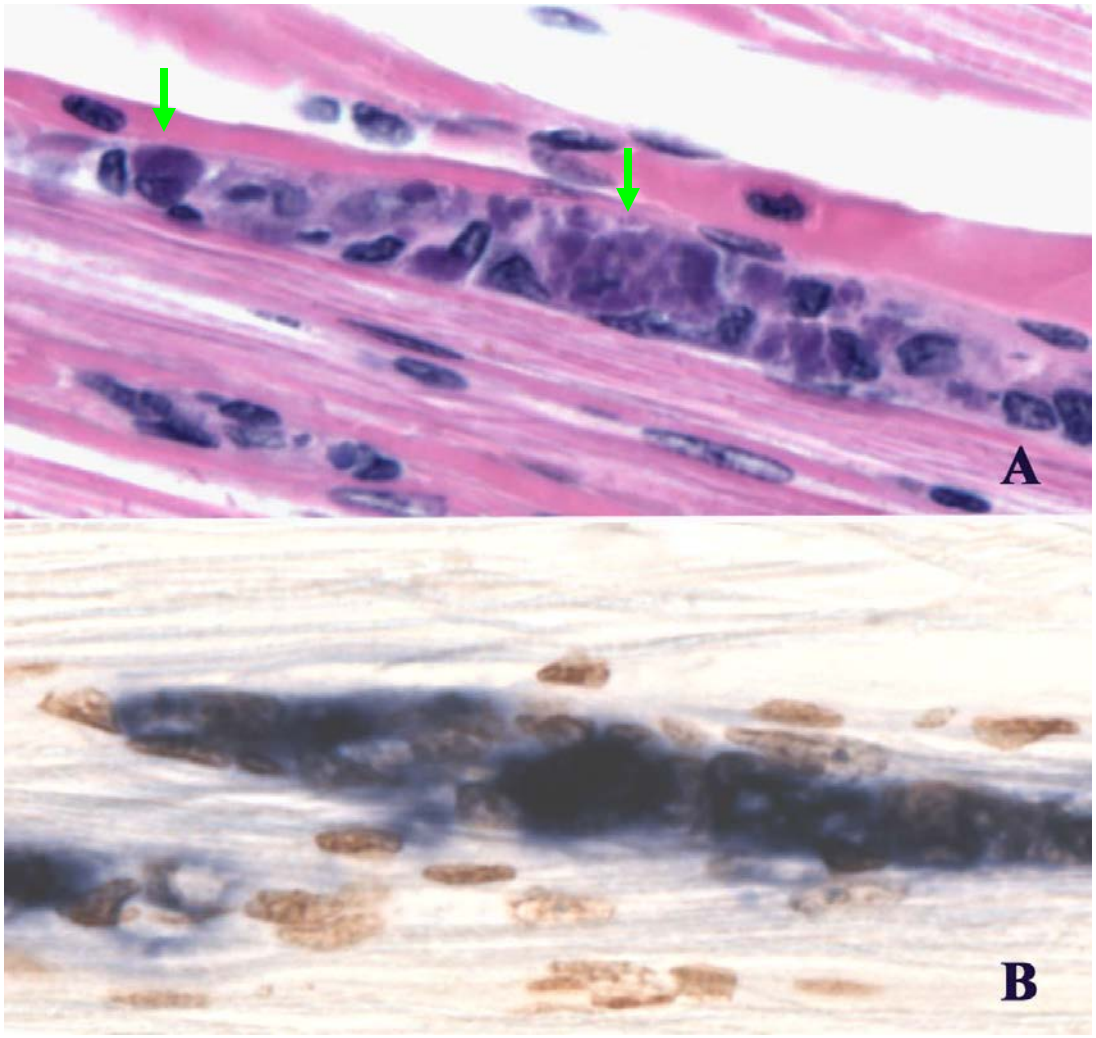
IMNV: non-enveloped ds RNA virus



In situ hybridization



H&E staining and ISH detection of IMNV in skeletal muscle of *L. vannamei*



H&E

ISH

Experimental infection

P. vannamei, P. stylirostris, P. monodon

↓

Injection with an IMNV inoculum

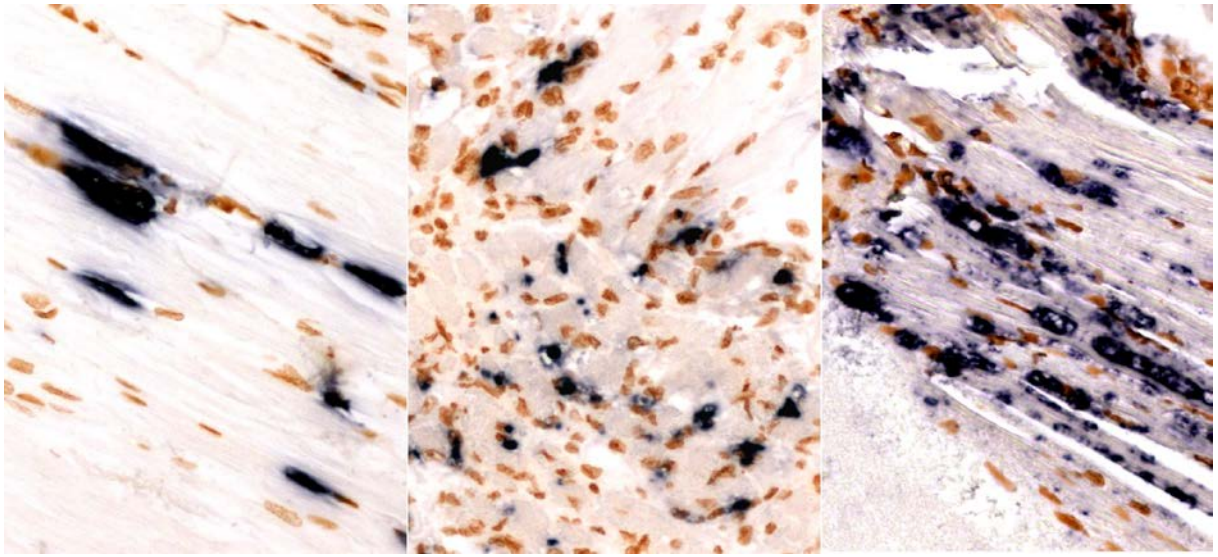
↓ **4 wks**

Appearance of clinical signs and mortality

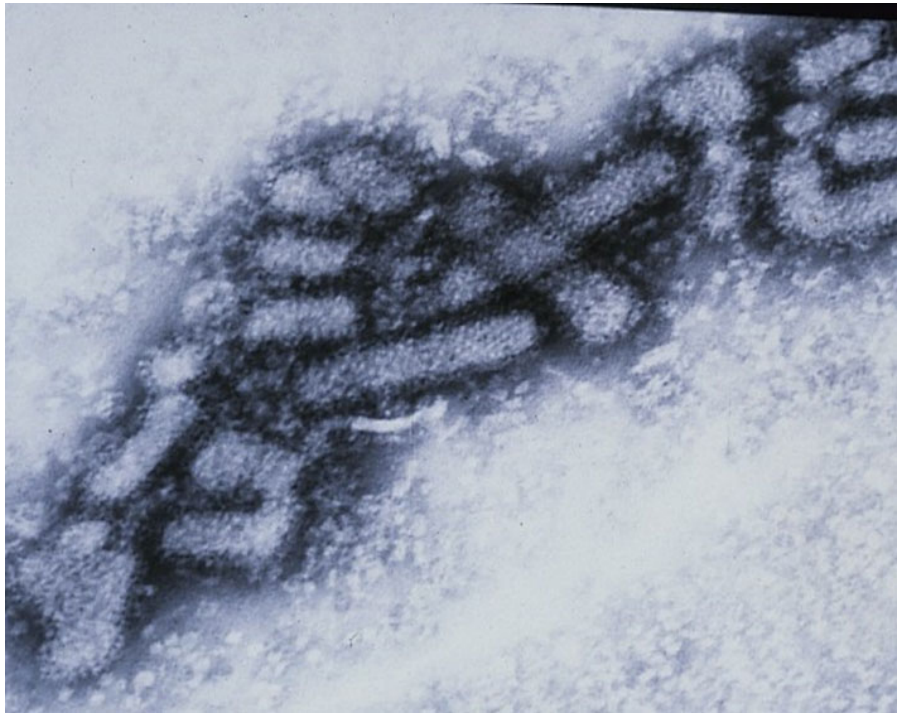
ISH: all positive for IMNV infection

Susceptibility of penaeid shrimp to IMNV infection

- **Laboratory infections to species of *P. vannamei, P. stylirostris, P. monodon***

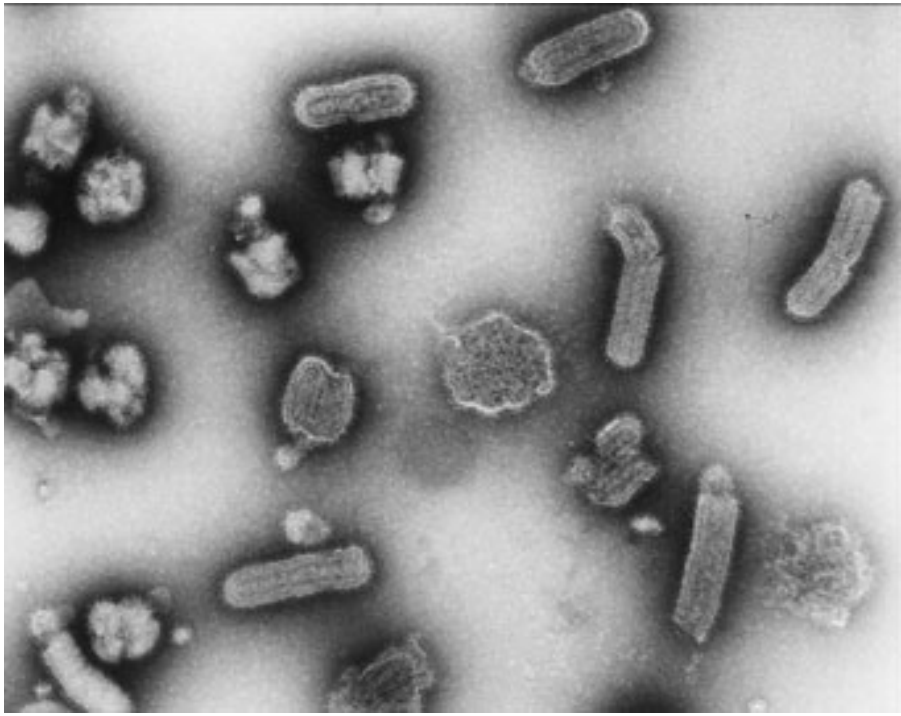


P. vannamei ***P. stylirostris*** ***P. monodon***



Yellow head virus
44 nm x 173 nm

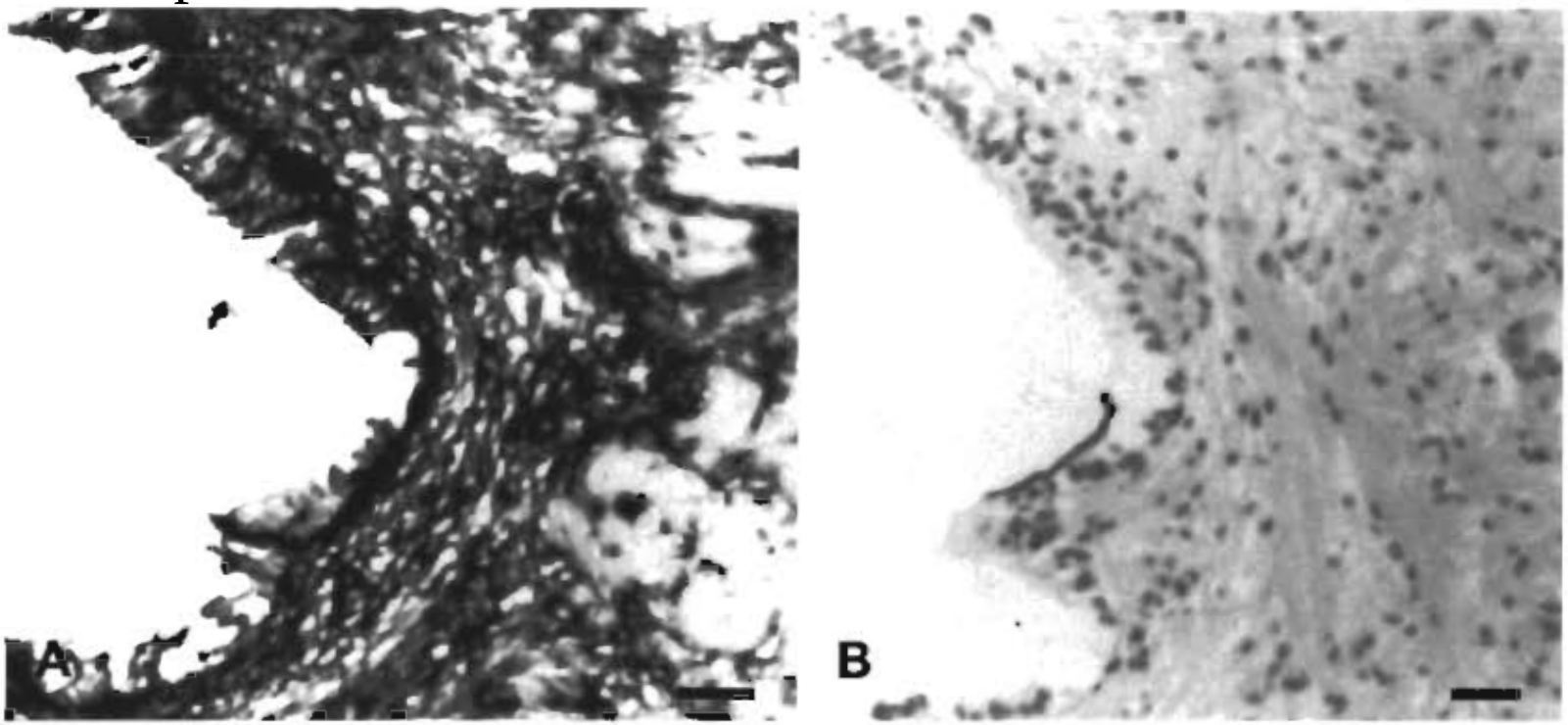
Was suspected to be a negative-sense RNA virus?



Rhabdovirus
70 nm x 180 nm

With a single, negative-sense, RNA genome

ISH to YHV-infected shrimp using single-stranded DIG-RNA probes

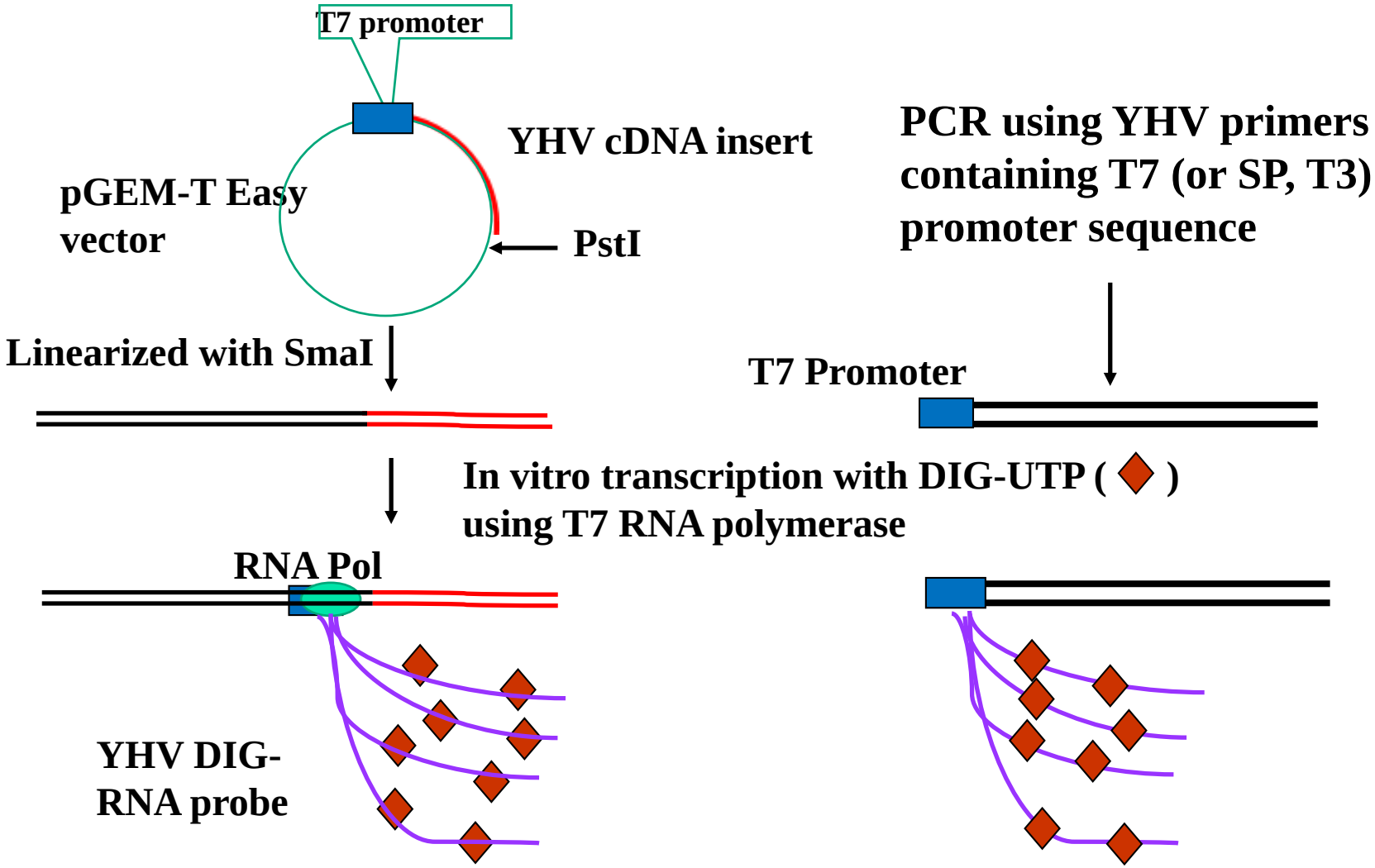


ISH with a anti-sense DIG-RNA probe: Positive reaction

Probe with a DIG-sense RNA: Negative reaction

The result indicated that YHV has a positive-sense RNA genome

In vitro transcribed DIG-RNA probe



Thank you for your attention!