Food and Agriculture Organization
of the United Nations

# Pathology and diagnosis of TiLV

Kathy Tang-Nelson

**Project Inception Workshop of GCP/RAF/510/MUL:**
**Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture**
Southern Sun Myfair Hotel, 23-24 October 2018, Nairobi, Kenya

- **Discovery of TiLV in Israel in 2009,**
  **genome size: 10-kb,**
  **new species name: *Tilapia tilapinevirus***
- **TiLVD gross signs**
- **Susceptible species**

- **Diagnostic chart and reporting**

- **Diagnostic methods**
  1. **Histological examination**
  2. **Virus isolation and cell culture**
  3. **Conventional RT-PCR**
  4. **Real-time RT-PCR (RT-qPCR)**
  5. **In situ hybridization**

**Credited to Mr. Natan Wajsbrot**

Massive mortality (1,000s dead fish per day) of tilapia from TiLV outbreaks in the Valley of Bet She'an, Israel

**The occurrence of mass mortality due to TiLV in hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) cultured in Israel**.

**The TiLVD mortalities ranged 40-90%.**

**Credited to Mr. Natan Wajsbrot**

Birds were fed on the dead fish during the TiLV outbreaks in Israel

**Credited to Mr. Natan Wajsbrot**

Gross signs include erosions and ulcerations in the skin and unilateral or bilateral ocular alterations (cataracts).

**Credited to Dr. Win Surachetpong**

**The occurrence of mass mortality due to TiLV in red hybrid tilapia cultured in Thailand.**

**Credited to Dr. Win Surachetpong**


**Gross signs of infected red tilapia: distinct skin redness and erosion in skin and tail fin.**

**June, 6, 2017, Taiwan province**
**Mortality: 100's per day, for 2-weeks**
**clinical signs: abdominal swelling, hemorrhage, loss of scales**

**Farm operation: tilapia mix culture with other freshwater fishes (perches, grass carp, silver carp, black carp, marble eel), but other fishes were not affected.**

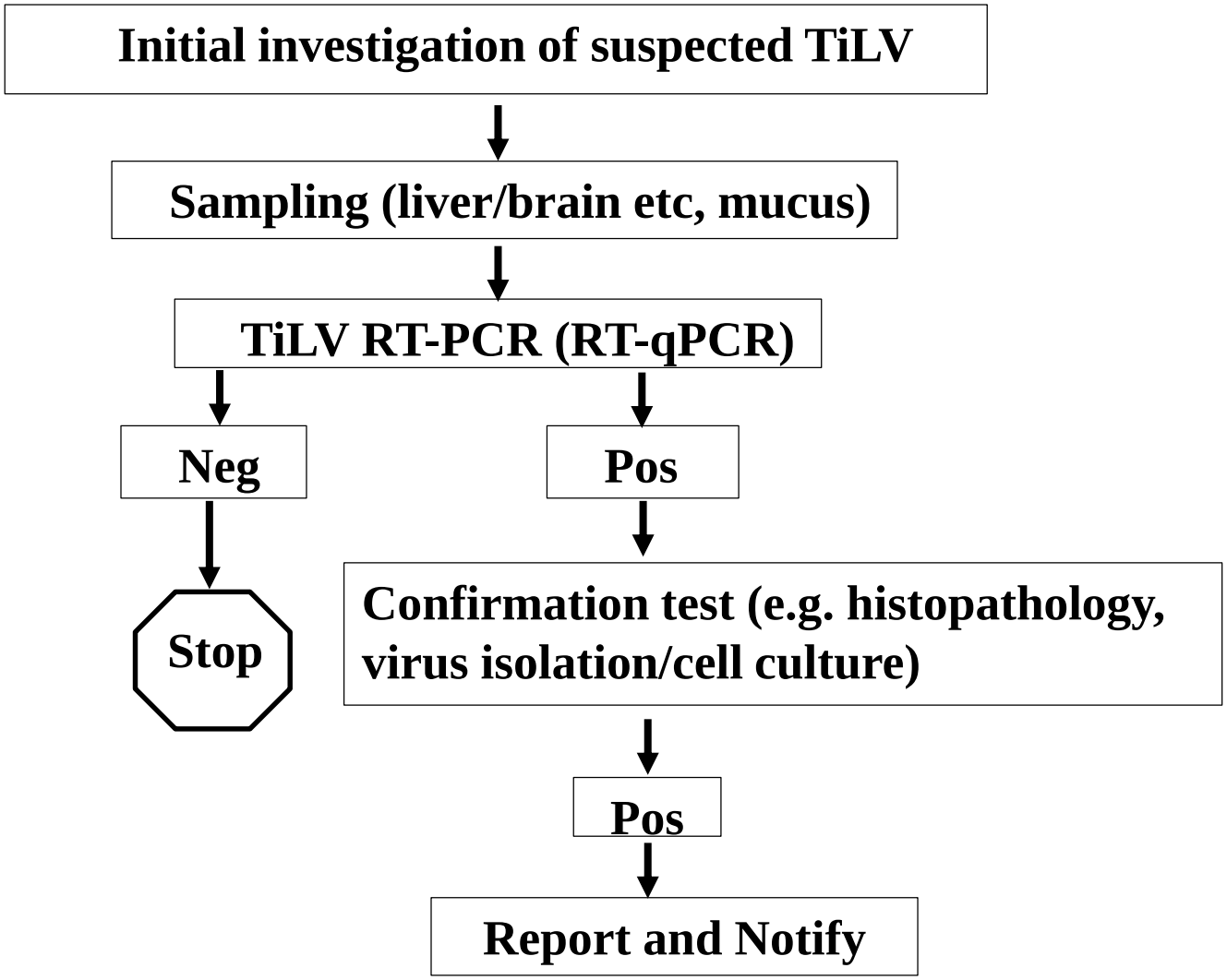
# Gross signs of TiLVD

**(Left photo) diseased Nile tilapia showed skin erosion, hemorrhage on various parts of body, loss of scales, abdominal swelling, and swelling of the eyeball (exophthalmos); (Right photo) diseased wild tilapia (*Sarotherodon galilaeus*) showed shrinkage of the eye and loss of ocular functioning.**

## Susceptible species

- **Nile tilapia (*O. niloticus*)**
- **blue tilapia (*Oreochromis niloticus x O. aureus* hybrids)**
- **red tilapia (*Oreochromis* sp.)**
- ***Tilapia zillii***
- ***Sarotherodon galilaeus* “St. Peter’s” fish**
- ***Oreochromis aureus***
- ***Tristamellasimonis intermedia***
- **River barb (*Barbonymus schwanenfeldii*)**
- **Giant gourami**

# Diagnostic flowchart



## Suspect and confirmation of TiLV infection

- **Suspect case**

Infection of TiLV is **suspected** if at least one of the following criteria is met:

(1) mortality and clinical signs consistent with TiLVD

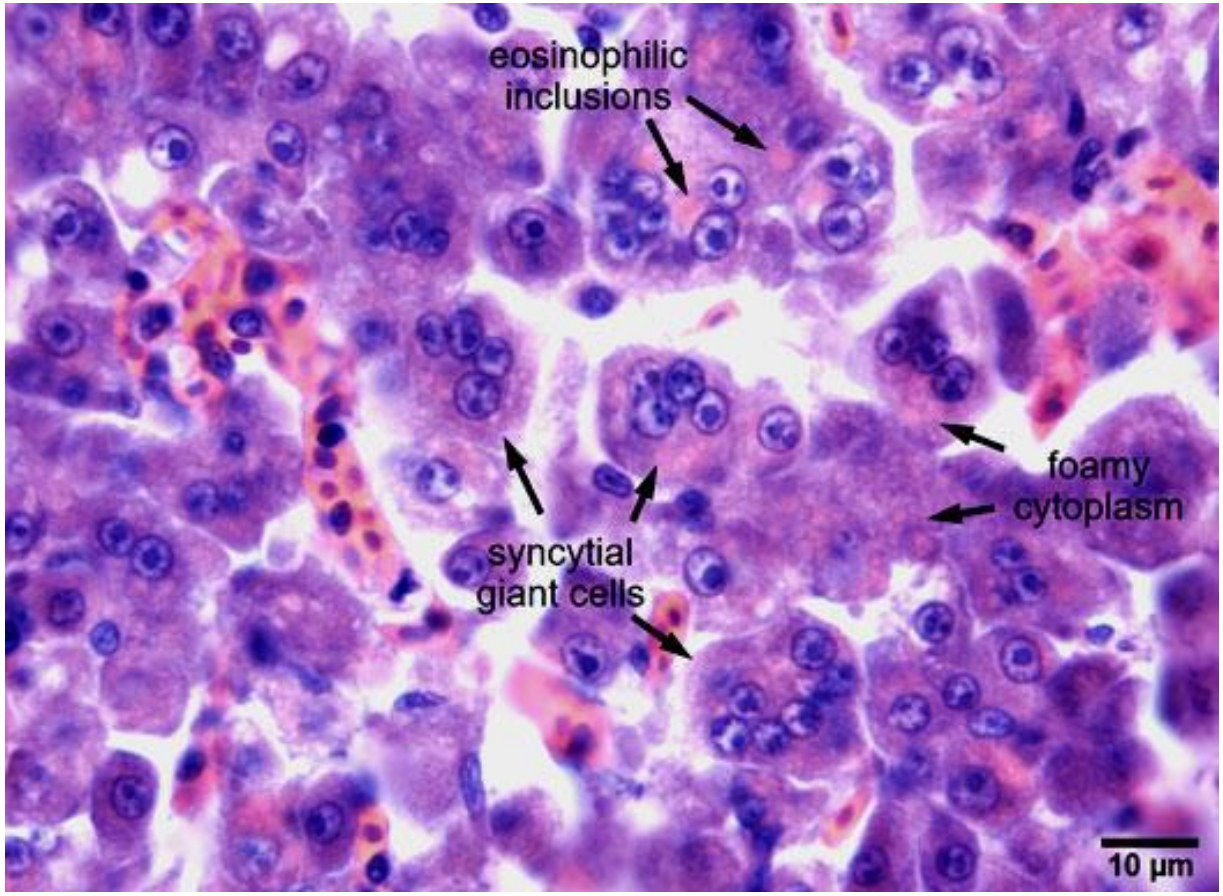
(2) histopathology consistent with disease

(3) detection of TiLV by RT-PCR (or RT-qPCR).

- Infection of TiLV is considered to be **confirmed** if two or more of the following criteria are met:

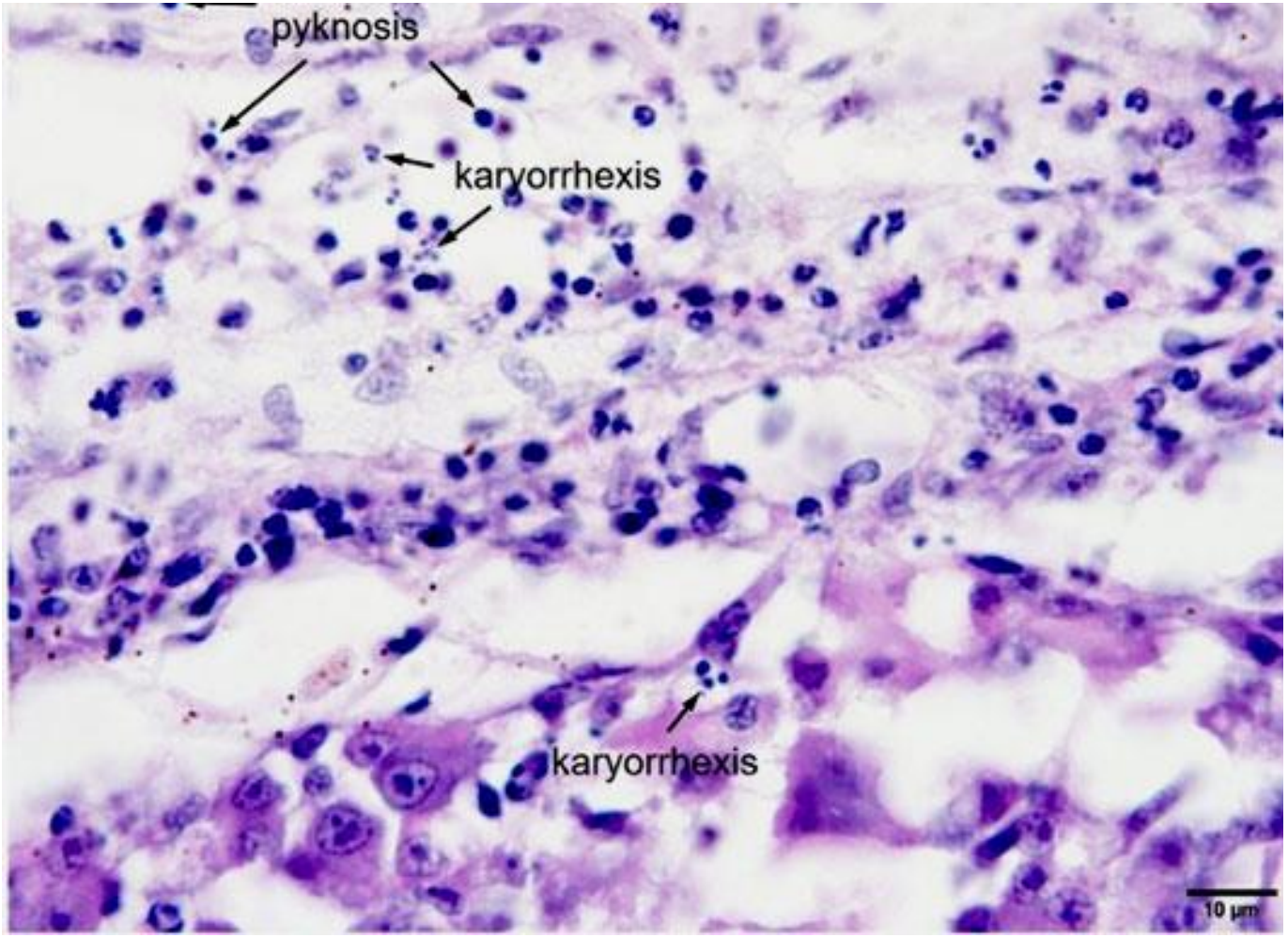
- histopathology consistent with disease
- detection of a TiLV by RT-PCR and amplicons' sequence analysis
- TiLV are isolated from infected fish, followed by performing the cell-culture or laboratory infection in conjunction with the diagnostic methods (histopathology, RT-PCR, sequencing, or RT-qPCR) for TiLV.

# A. Histopathology Credited to Dr. Dong Ha



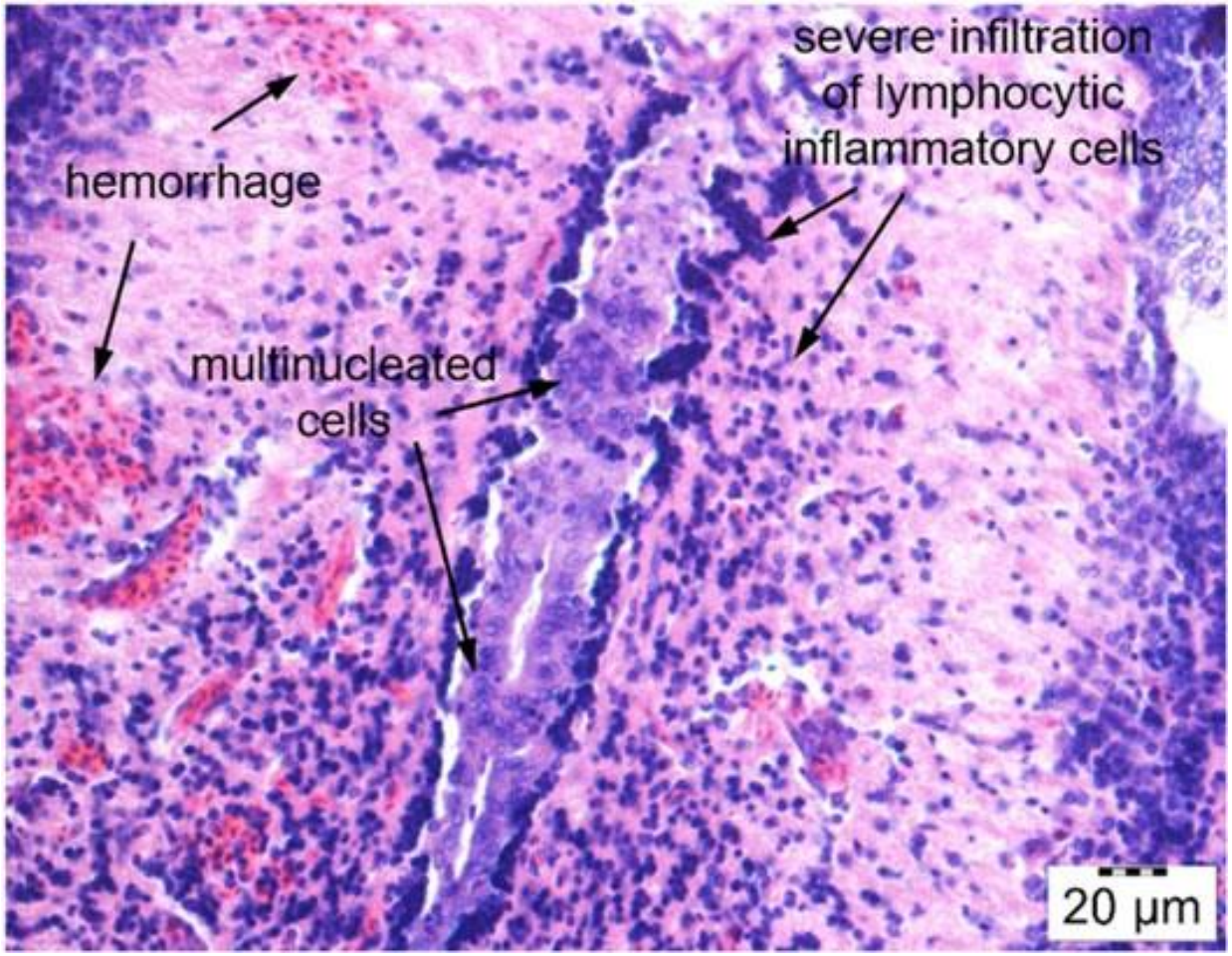

**H&E histopathology of liver in a TiLV-infected tilapia, it demonstrates the presence of syncytial cells as TiLVD was originally named as syncytial hepatitis of tilapia (SHT).**

**Credited to Dr. Dong Ha**




**H&E histopathology of liver in a TiLV-infected tilapia, it demonstrates the cellular necrosis, the appearance of pyknotic and karyorrhectic nuclei.**

**Credited to Dr. Dong Ha**



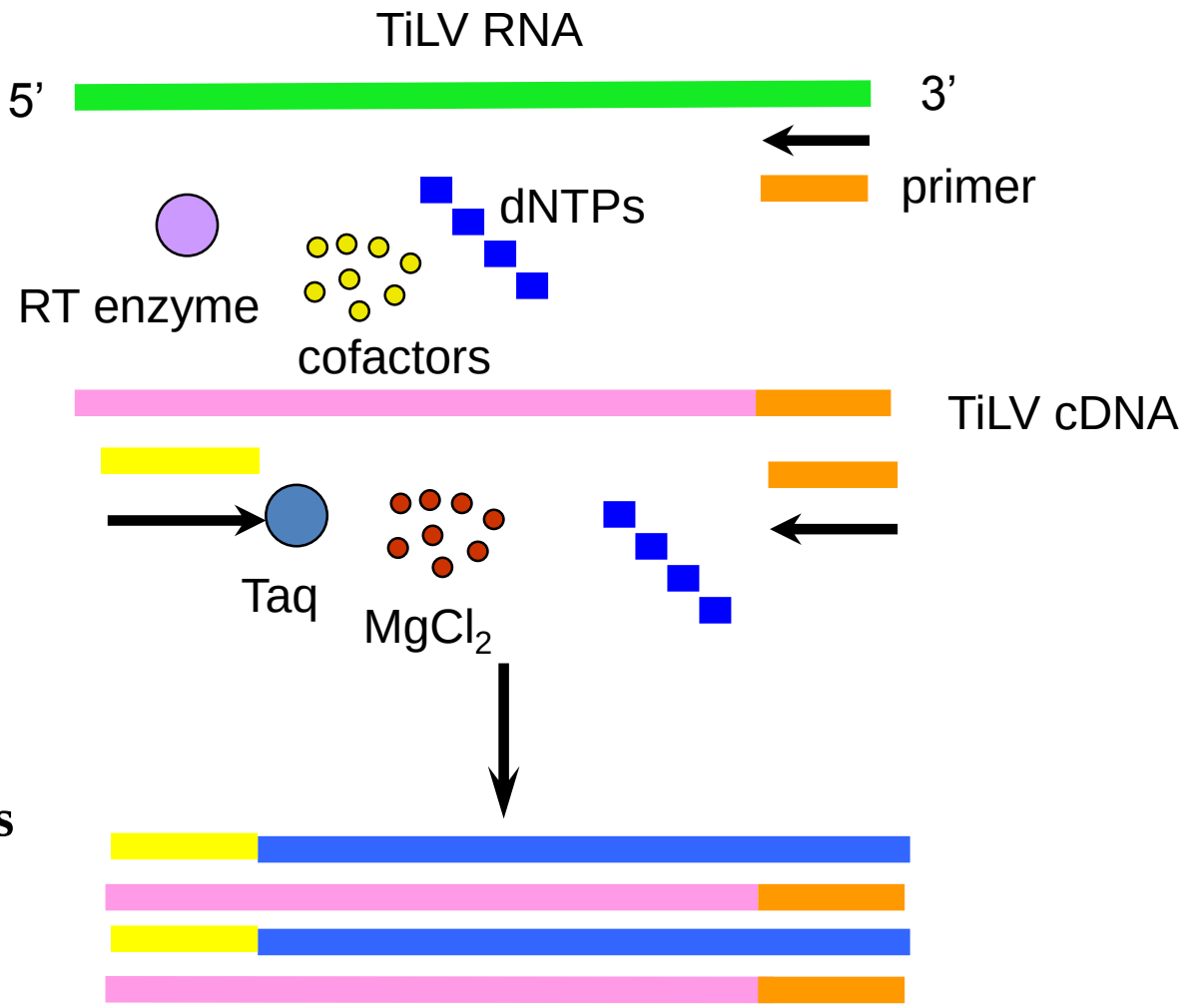

**H&E histology of  brain from a TiLV-infected tilapia**.

# TiLV isolation and cell culture

1. **Remove liver (or brain) from TiLV–infected fish**
2. **Homogenize the tissues in buffer (e.g. Hanks balanced salt solution), centrifuge to remove the cellular debris, keep the supernatant, filtrate through a 0.22 μm membrane.**
3. **The viral solution can be used in inoculating the cell lines, such as E-11, OmB, TmB, etc, and monitored for the appearance of CPE (cytopathic effect).**
4. **From the infected cell culture, the virus-containing supernatant can be prepared and stored at -80$^{o}$C as virus stock solution.**

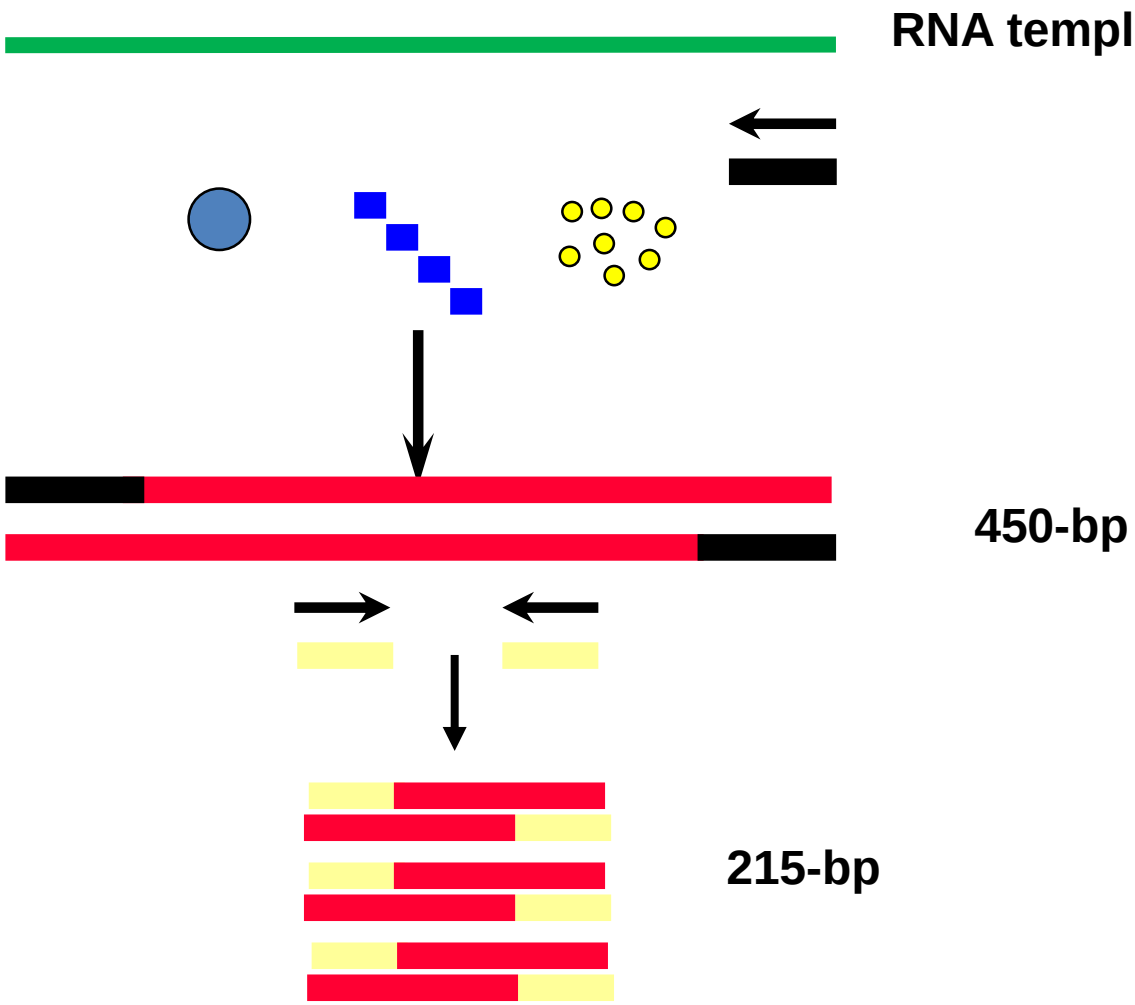
# Principle of RT-PCR

**Reverse transcription (RT)**

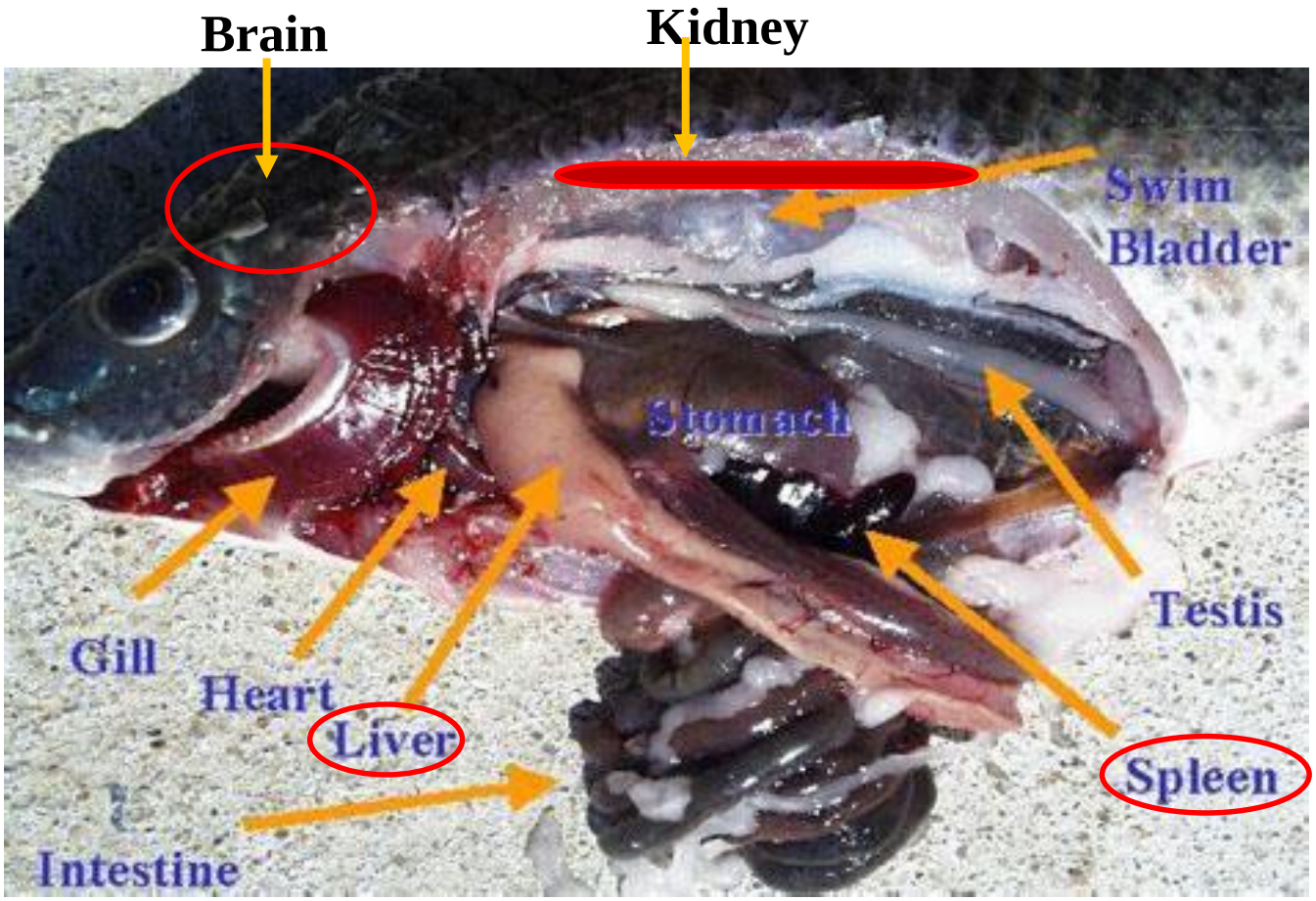



**PCR: Denature, anneal and extend for 25 - 40 cycles; Generate amplified products of a specific size**

# Principle of Nested RT-PCR



# Tilapia tissue sampling for RNA extraction



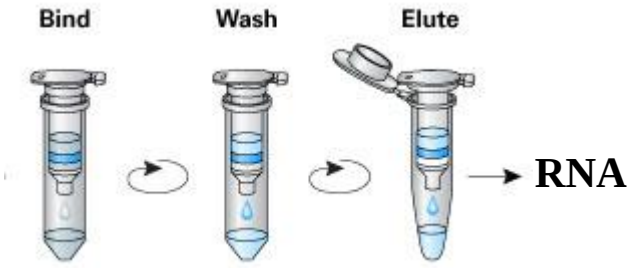

**Fresh, frozen, or preserve in ethanol (70-95%)**

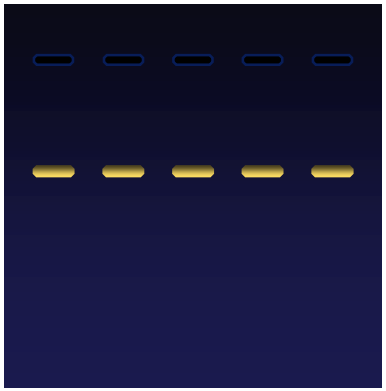
**Non-invasive: mucus**

# Conventional RT-PCR

**RNA extraction** → **RT-PCR** → **Endpoint Analysis**

**or**



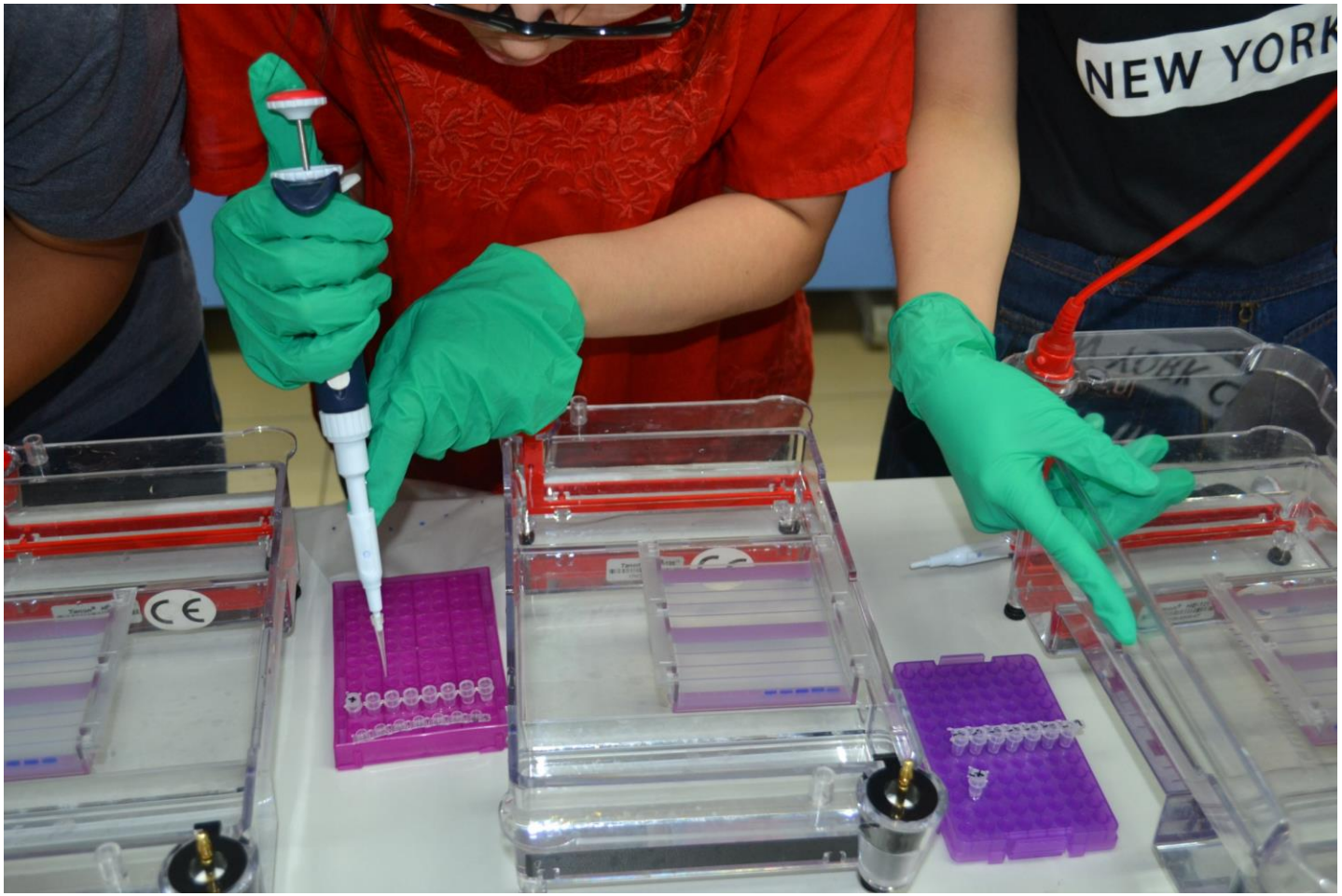



# TiLV RT-nPCR (semi-nested PCR)

| RT-PCR | Primers | Amplicon size | Sequence (5' to 3') | Reference |
|---|---|---|---|---|
| 1-step | Nested ext-1 | 415-bp | TATGCAGTACTTTCCCTGCC | Eyngor et al. 2014; Tsofack et al. 2016 |
| | ME1 | | GTTGGGCACAAGGCATCCTA | |
| 2-step | 7450/150R/ME2 | 250-bp | TATCACGTGCGTACTCGTTCAGT | |
| | ME1 | | GTTGGGCACAAGGCATCCTA | |

| Pathogen | Temperature (°C) | Time | Number of cycles |
|---|---|---|---|
| TiLV-1 | 50, 94 | 30 min, 2 min | 1 |
| | 94, 60, 72 | 30 s, 30 s, 30 s | 25 |
| | 72 | 5 min | 1 |

| Pathogen | Temperature (°C) | Time | Number of cycles |
|---|---|---|---|
| TiLV-2 | 94 | 2 min | 1 |
| | 94, 60, 72 | 30 s, 30 s, 30 s | 25 |
| | 72 | 5 min | 1 |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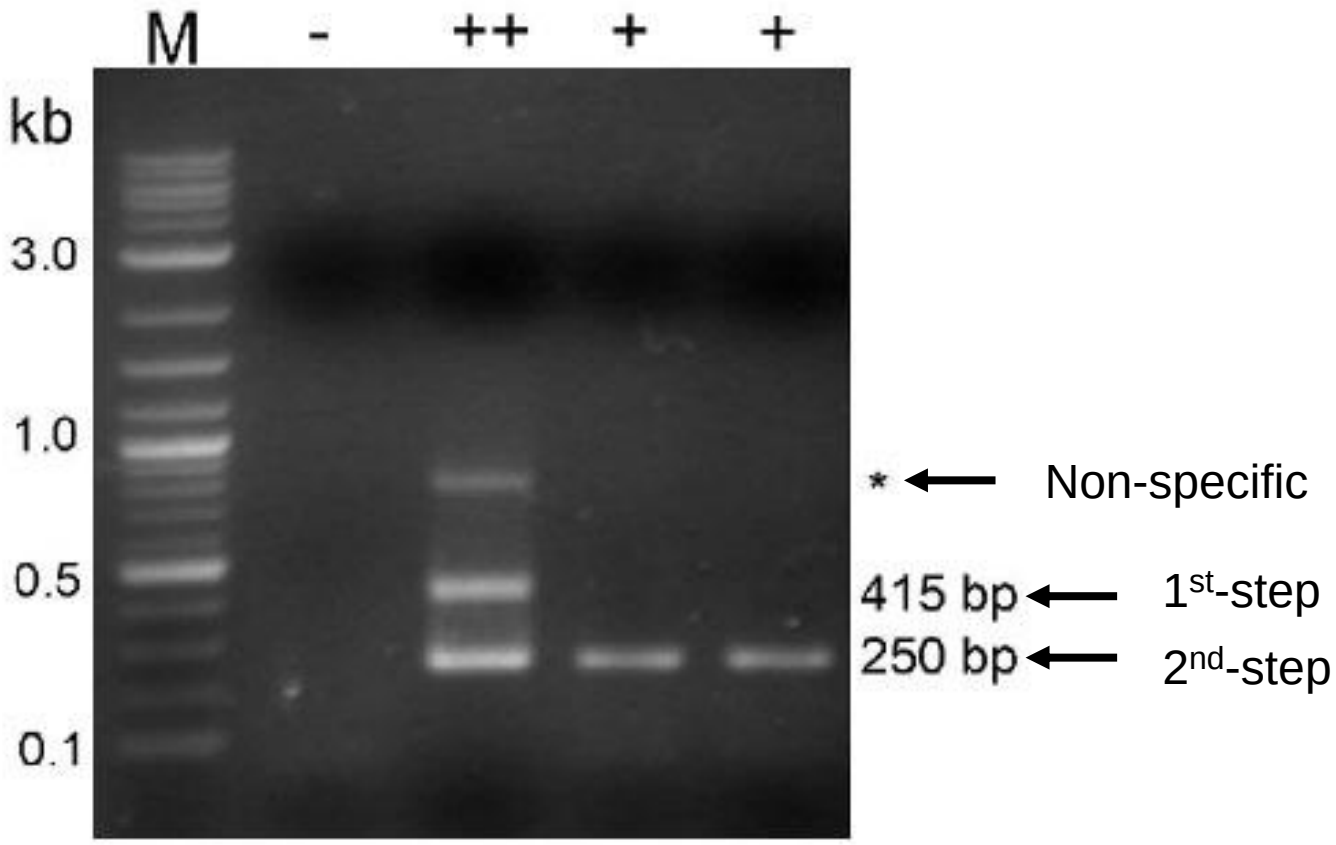
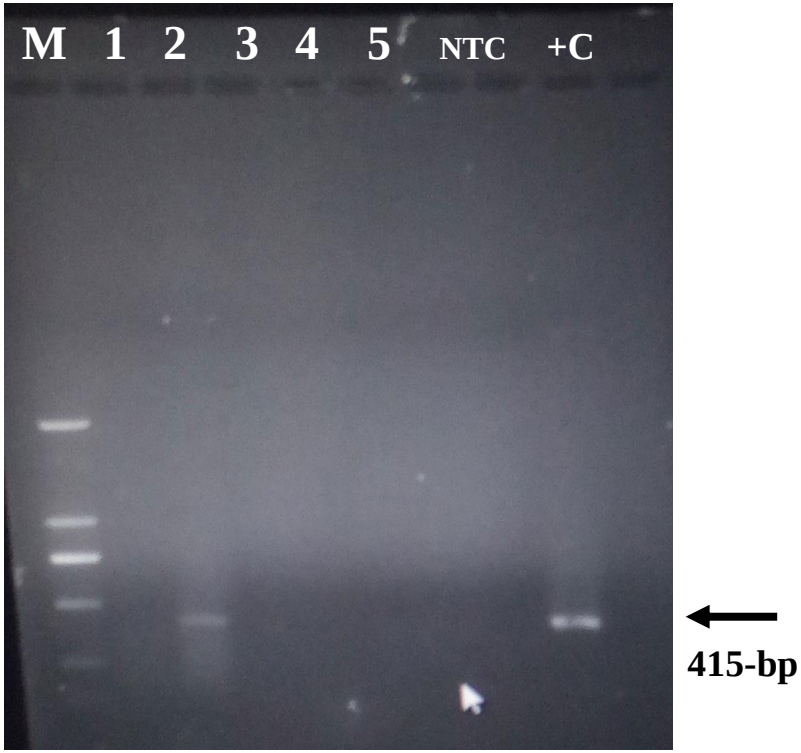


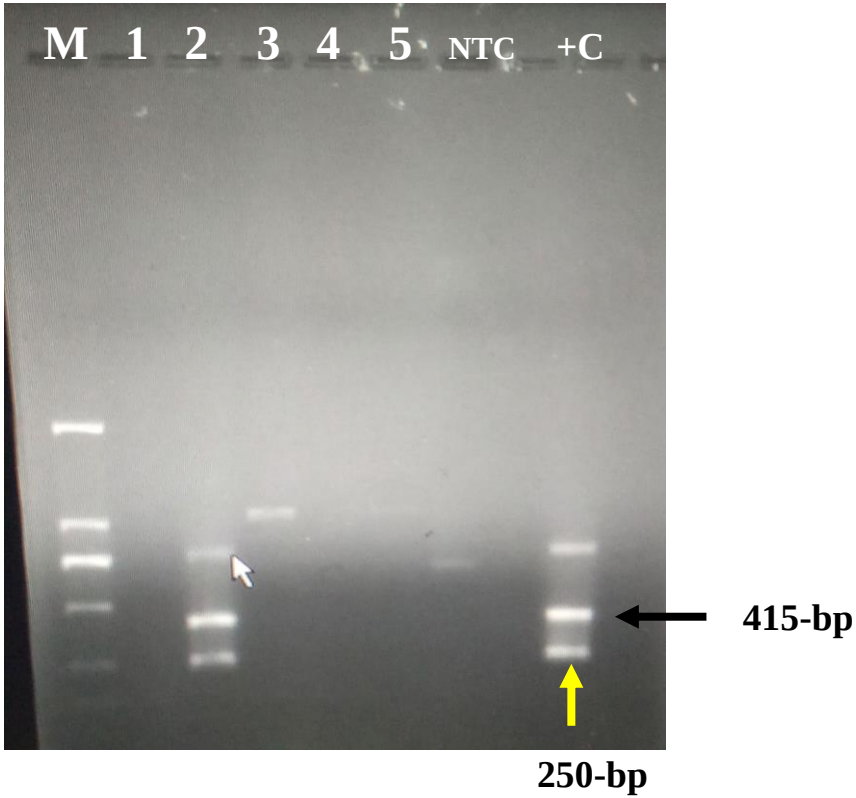

**TiLV RT-PCR of RNA extracted from tilapia samples. ++: heavy infection; +: light infection; -: not detected.**

## Group B

### 1st -step



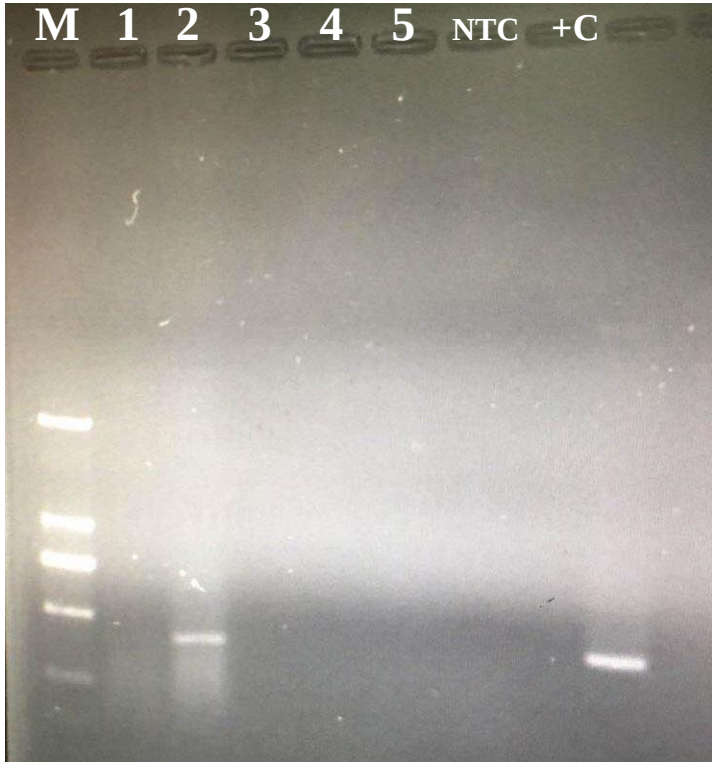

### 2nd -step



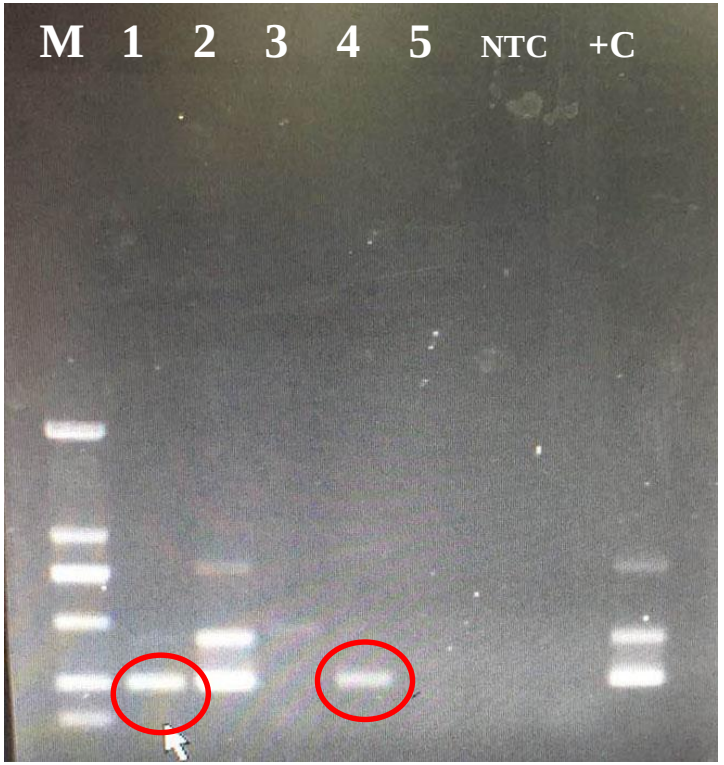

**#1: healthy tilapia;**
**#2 TiLV-infected tilapia;**
**#3-5: tilapia samples from countries 1-3;**
**NTC: none-template control;**
**+C: positive control (plasmid DNA)**

# Group E
# Contamination

## 1st -step




## 2nd -step




**#1: healthy tilapia;**
**#2 TiLV-infected tilapia;**
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**NTC: none-template control;**
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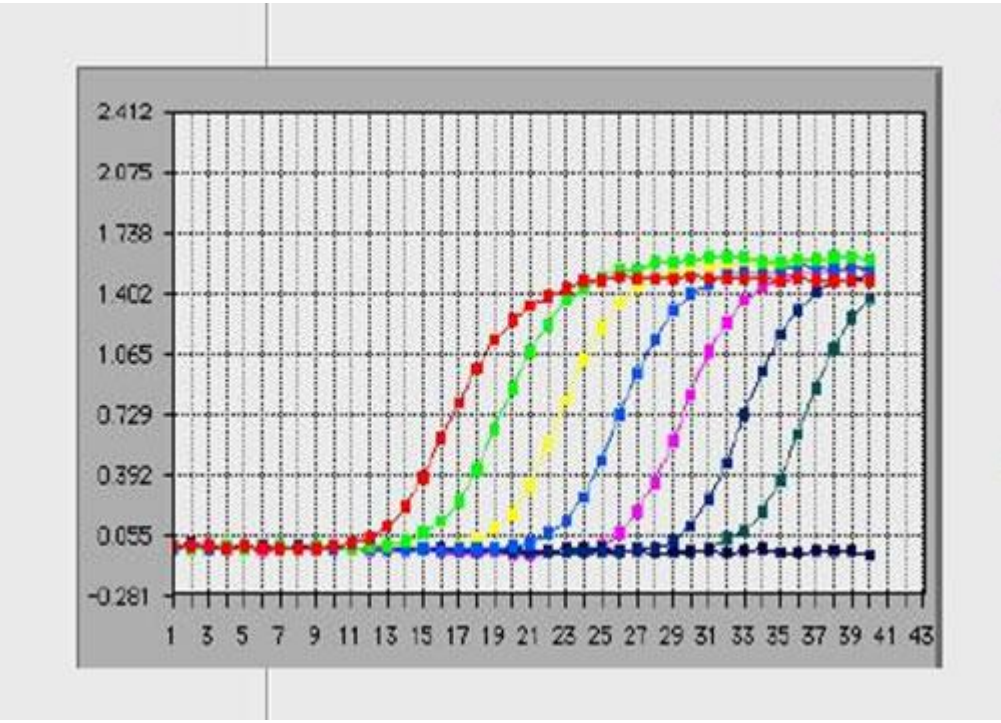
# Real-time PCR (qPCR)

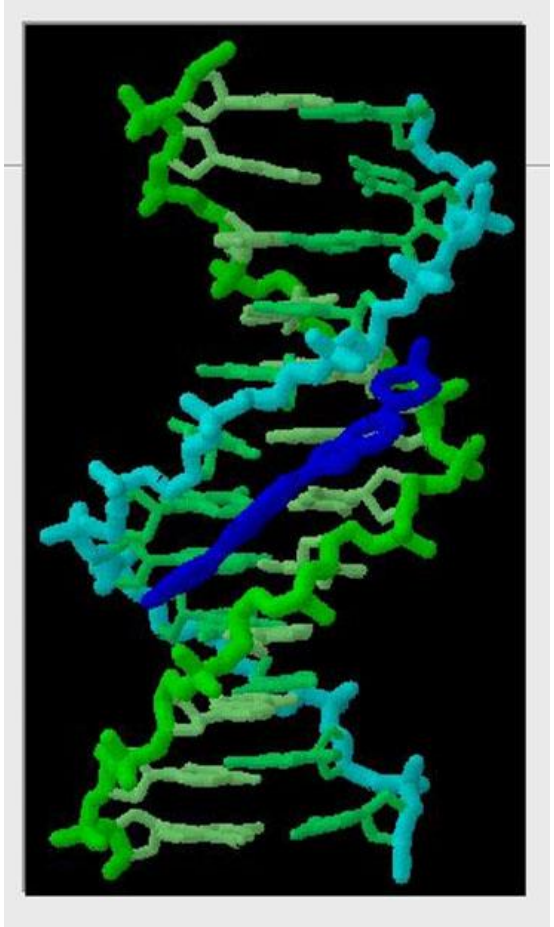



- **Data taken at each amplification cycle**
- **Fluorescent detection of PCR products**
- **Rapid assay, high sensitivity (< 100 copies)**
- **Low risk of carry-over contamination (closed-tube)**

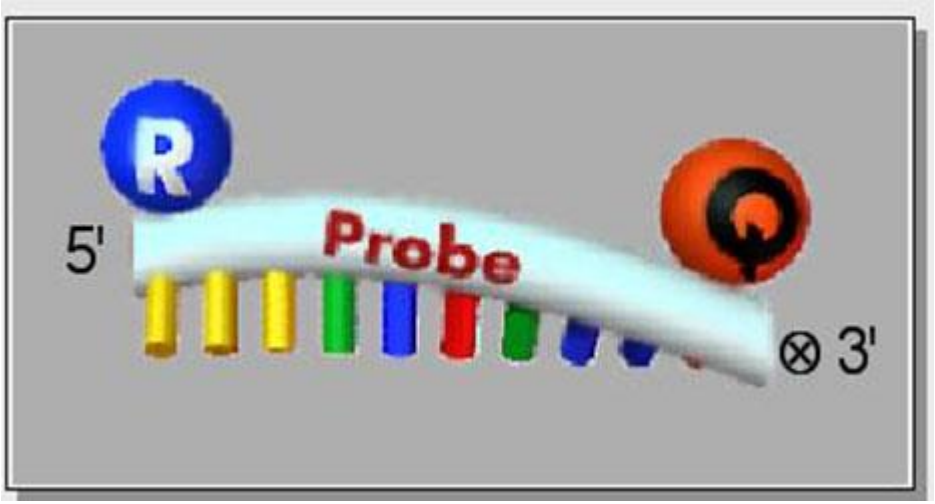
# Fluorescence detection chemistry in qPCR (RT-qPCR)

- **SYBR Green I**
- **TaqMan probe**

# Fluorescence detection chemistry in qPCR (RT-qPCR)

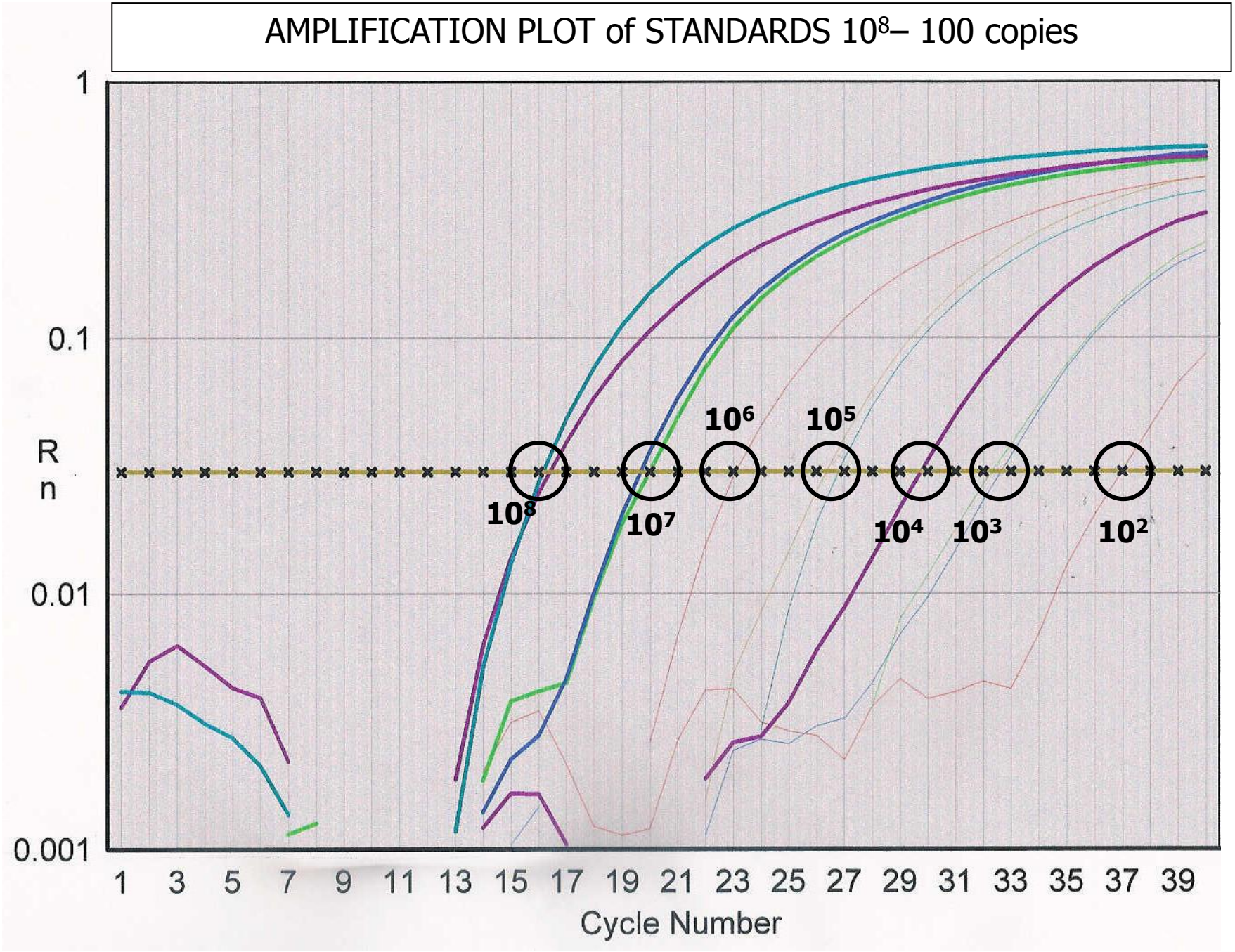


SYBR-Green, DNA-binding dye




TaqMan probe

# AMPLIFICATION PLOT of STANDARDS $10^8$– 100 copies



Credited to Dr. Dong Ha

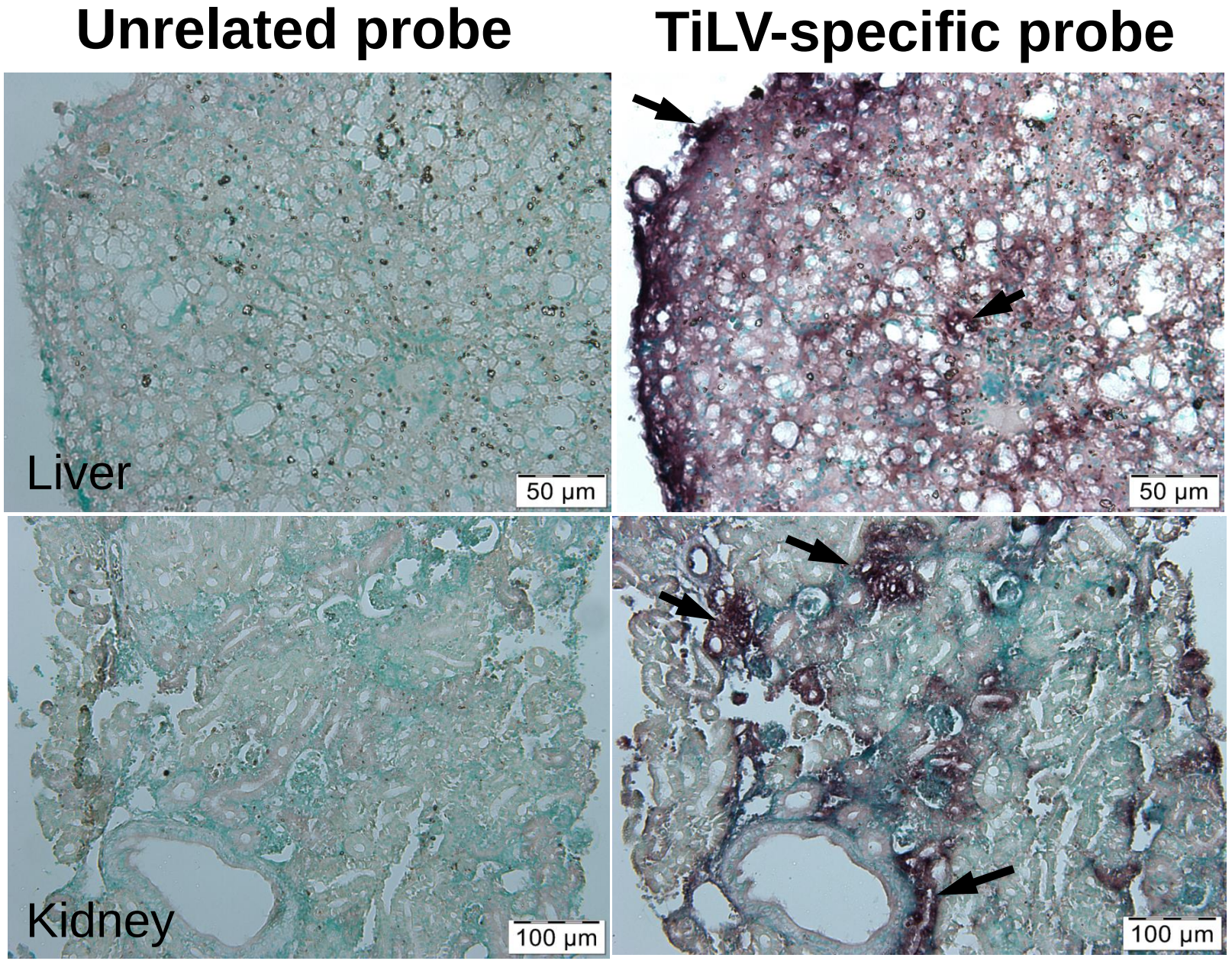
# In situ hybridization (ISH)

## Purpose

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

# 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

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# Thank you for your attention

ktangnelson@gmail.com

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