

Development of Specific DNA Marker for the Detection  
of Trematode, *Haplorchis taichui* using HAT-RAPD  
Derivation Method

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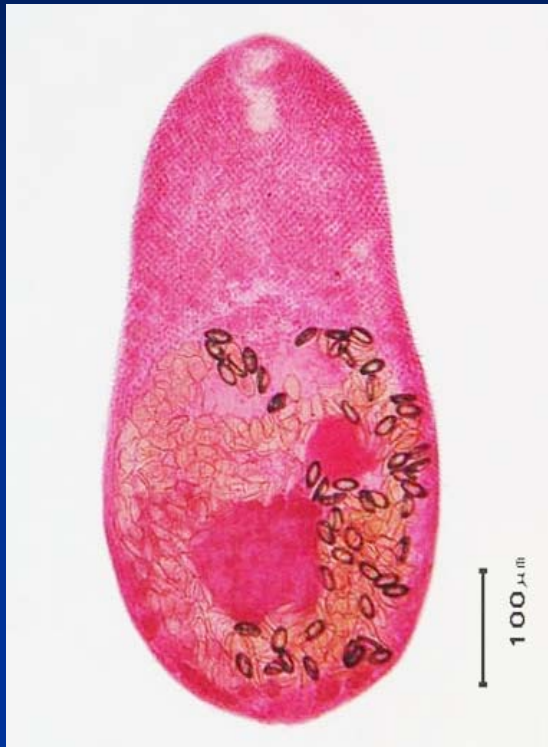
Thesis Advisor

Assoc.Prof.Dr. Chalobol Wongsawad

Prof. Dr. Jong Yil Chai, SNU, Korea

Assoc. Prof. Dr. Somboon Anuntalabhochai

# *Haplorchis taichui*



Endemic species in northern Thailand especially in Chiang Mai province.

(Wongsawad et al, 2003; Kumchoo et al, 2004)

-High prevalence and intensity



*Henicorhynchus siamensis*



# Second Intermediate Host



*Puntioplites proctozysron*



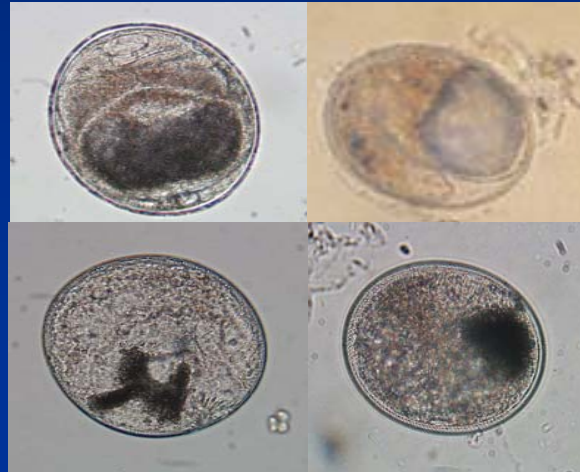
*Cyclocheilichthys repasson*



*Henicorhynchus siamensis*



*Barbodes schwanenfeldi*



metacercariae

# Mae-Ngad Somboon Chon Dam, Chiang Mai



Originated by His Majesty The King of Thailand.

Constructed for support irrigation areas in Mae Taeng district, Chiang Mai province.

Provides enough water for year-round agriculture.



# Human accession

- Fisheries
- Aquaculture
- Travel, floating houses
- Personal interest, wild plants / animals



- Accumulation of household-waste water
- Deposition of fecal material from floating houses
- High prevalence / intensity of *H. taichui* in fish



*Henicorhynchus siamensis*

# Infection

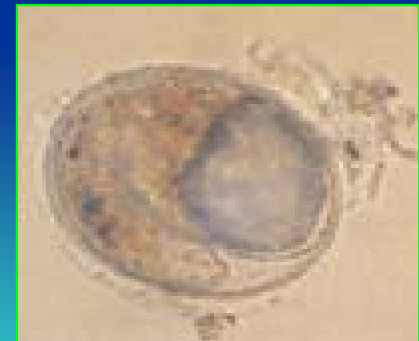
Human acquired these parasites by eating raw or undercooked fishes containing metacercaria.



*H. taichui*



*C. caninus*



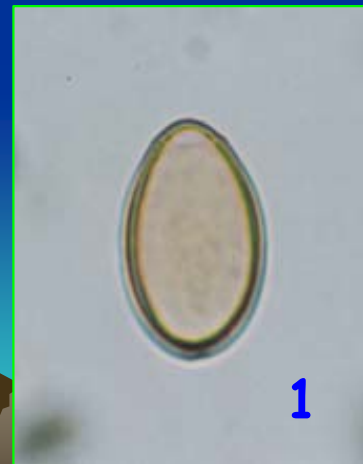
*O. viverrini*

# Identification and Detection

## Problems ;

- some parasites are monomorphology
- the similarity of larval stage and egg form which difficult to distinguish and indicate the epidemiology.
- One fish or snail host may be hold with several parasites

1 = Heterophyte's egg  
2 = *O. viverrini*'s egg



# Target parasites

:For the development of specific detection

Intestinal fluke;

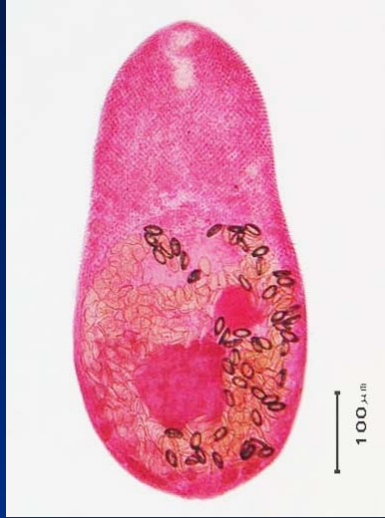
- *Haplorchis taichui*
- *H. pumilio*
- *Stellantchasmus falcatus*
- *Centrocestus caninus*
- *Haplorchoides* sp.

Endemic species in northern Thailand especially  
In Chiang Mai province.

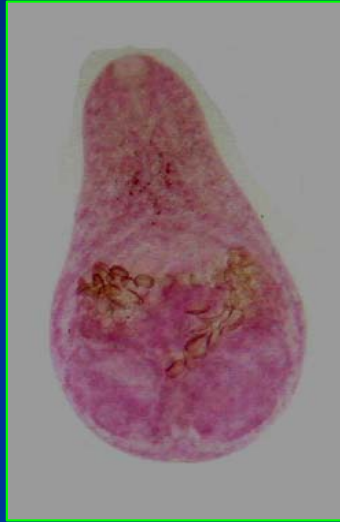
(Wongsawad et al,2003;Kumchoo et al,2004)



*O. viverrini*



*H. taichui*



*S. falcatus*

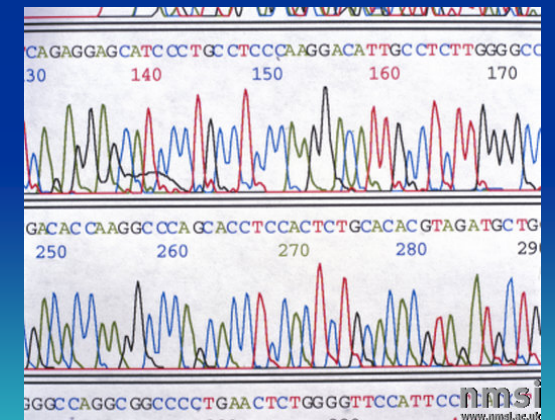
Samples of parasites

*C. caninus*



# MATERIALS AND

# METHODS

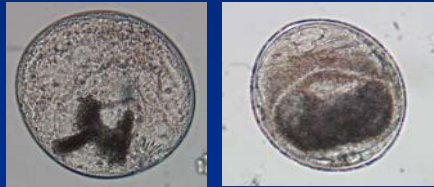


# Specimens preparation



Ground and homogenized

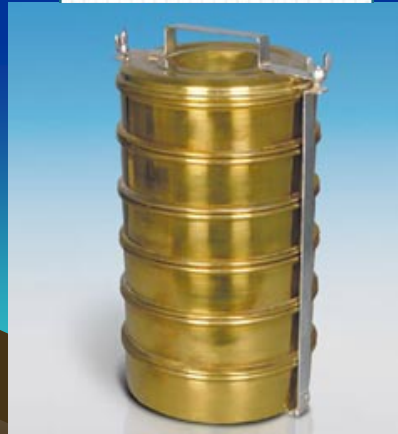
Examined for metacercariae



Digested with  
1% Pepsin solution  
Incubated 37°C  
2 hour

Passed through the  
Grading sieves

Rinsed with  
0.85% NaCl



## Obtaining of adult worms

➡ All collected metacercariae will be force-fed to definitive host such as chicks for heterophyid flukes and hamster for liver fluke.



➡ Adult worms will be obtained by dissecting of host intestine and liver.

## Other fluke specimens used

Three amphistome fluke from rumen  
of ruminant

1. *Paramphistomum epiclitum*
2. *Orthocoelium streptocoelium*
3. *Fischoederius elongatus*  
(Sripalwit et al., 2007)

*Ganeo tigrinus* from frog intestine

*Posthorchigenes* sp. from intestine  
of House lizard



# Investigation of molecular markers

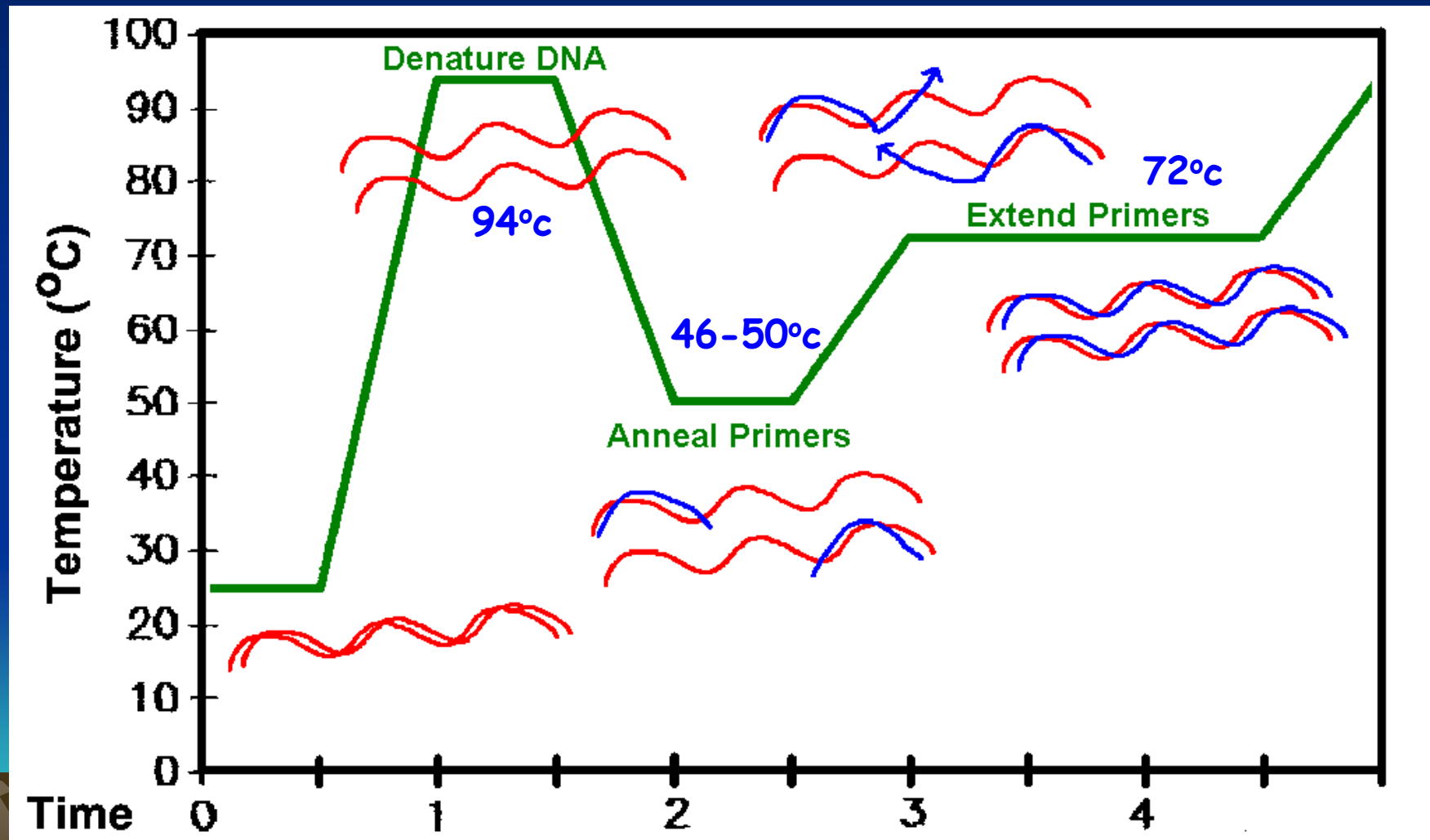
High annealing temperature-random amplified Polymorphic DNA (HAT-RAPD) analysis will be Performed in this study.

## Methods

- DNA extraction
- PCR (HAT-RAPD technique)  
(Anuntalabhochai et al., 2000)
- Agarose Gel Electrophoresis and visualizing
- Ligation, transformation and sequencing
- Design primer/synthesize and detection

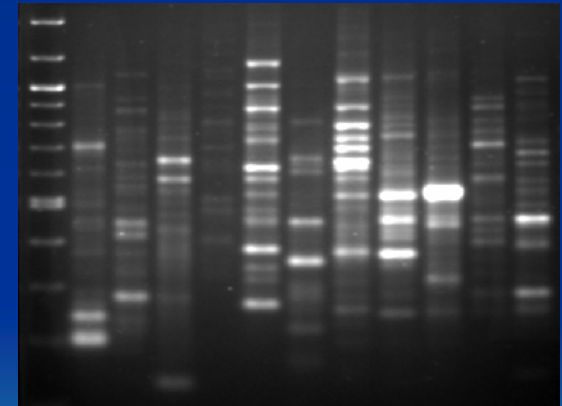
# HAT-RAPD PCR

HAT-RAPD markers will be generated with 20 random 10-mer arbitrary primers.

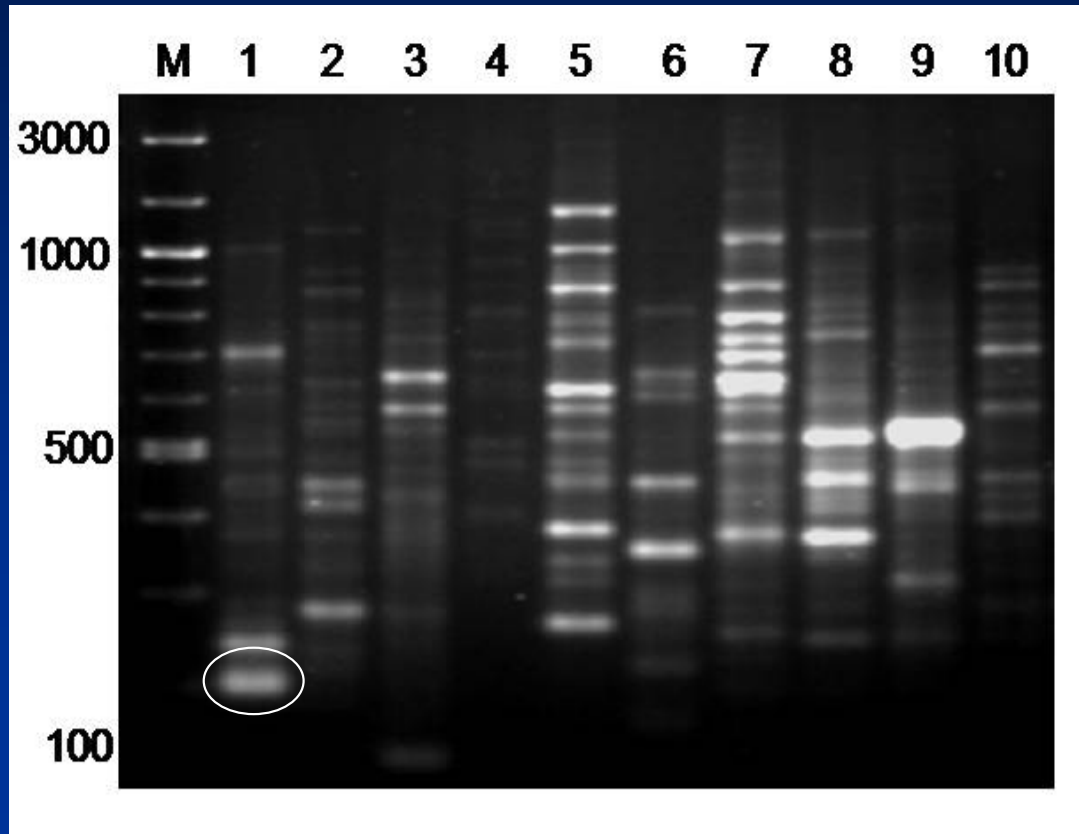


# Gel Electrophoresis

HAT-RAPD markers will be separated in 1.4% agarose gel, stained with Ethidium bromide and visualized by Kodak Gel Logic 100 digital camera.



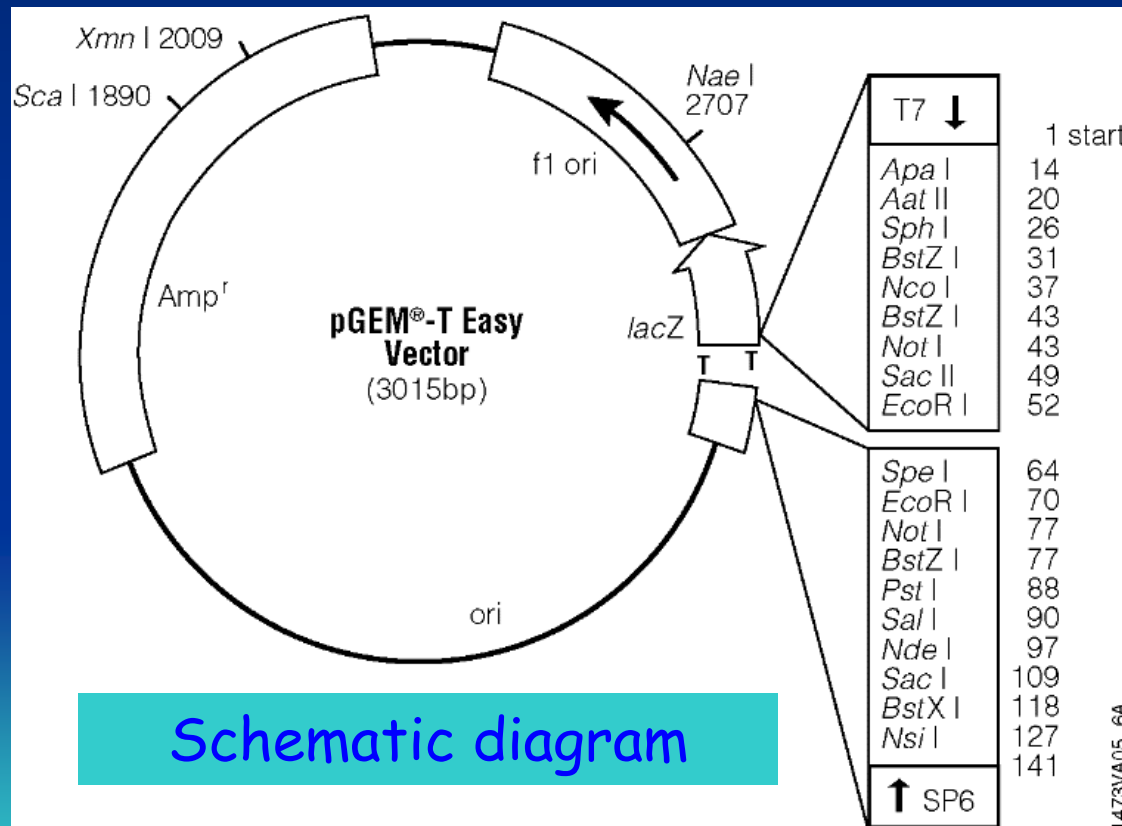
# Screening for species specific character



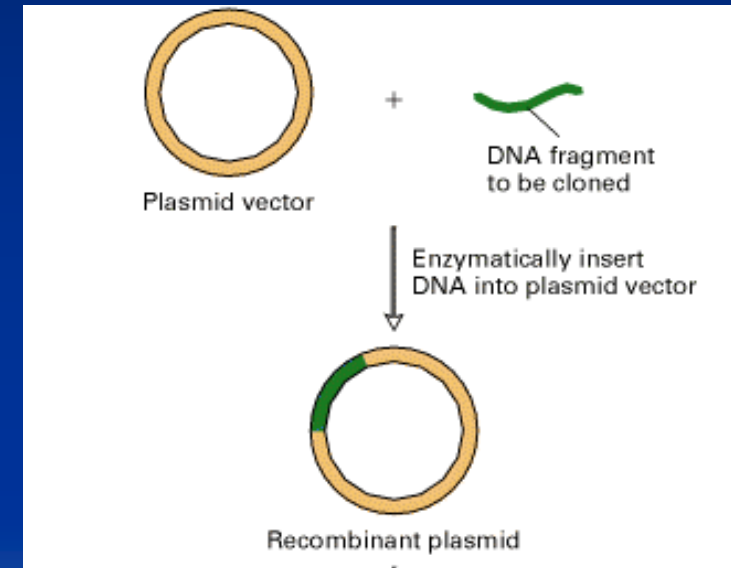
**For example:** HAT-RAPD markers generated by OPP-11 primer showing the expected species specific fragments (200 bp). M= molecular size markers , Ht= *H. taichui*

# Ligation to Plasmid vector

Selected amplicon will be purified from agarose gel and ligated to pGEM-T Easy Vector Using T4 ligation enzyme and incubated 4°C overnight.

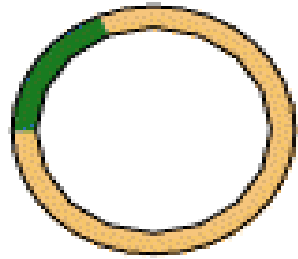


Schematic diagram

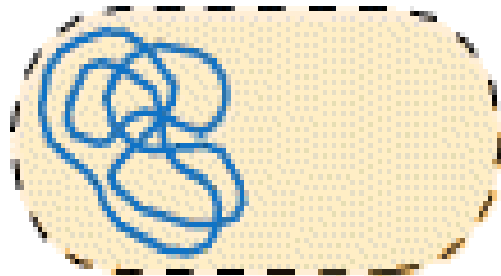


Plasmid recombination

# Transformation



Recombinant plasmid

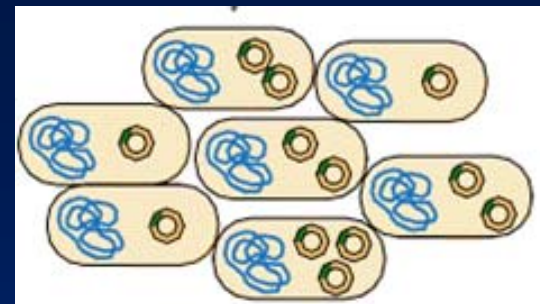


Ligated vector

Competent cells



electroporation



Colony of cells each containing copies of the same recombinant plasmid

Bacterial chromosome



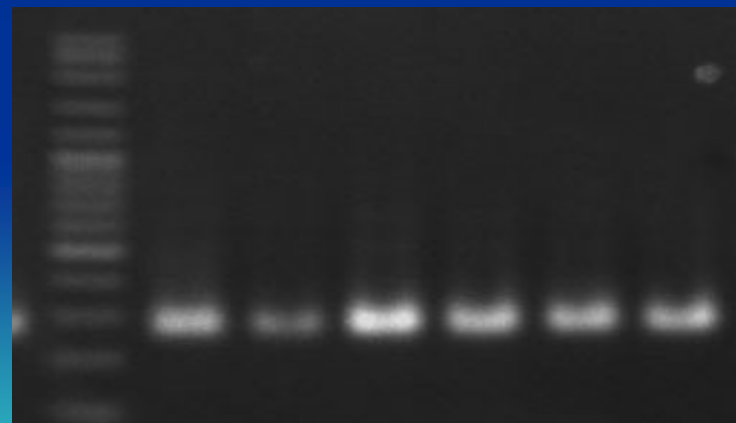
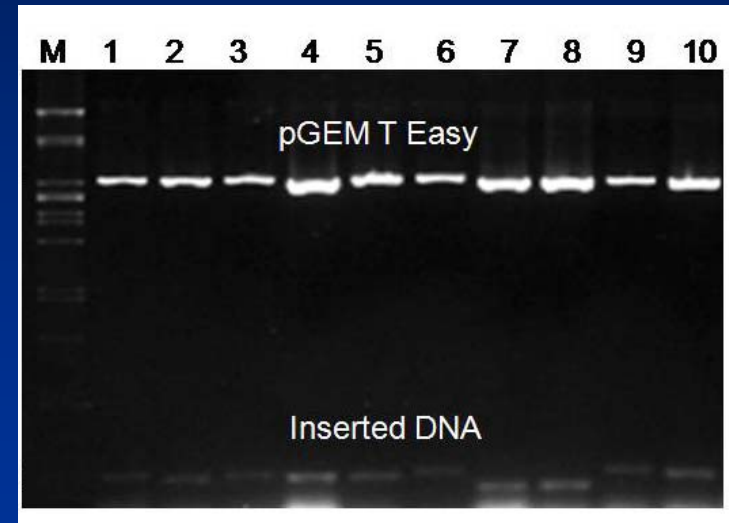
Transformed *E. coli* cell survives



# Test for the complete of insertion

Methodologies needed for verify that target DNA is completely inserted are;

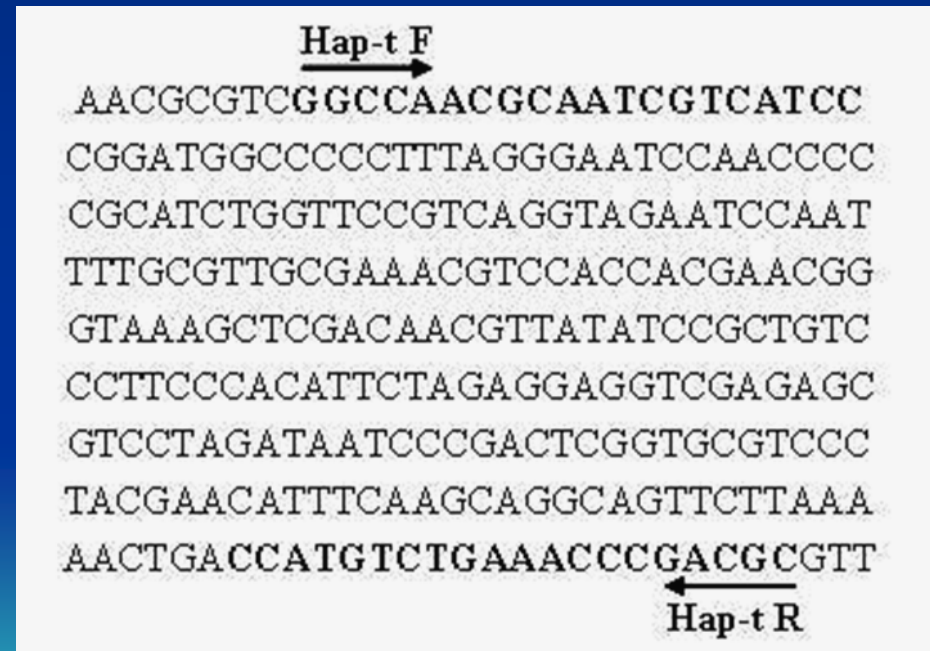
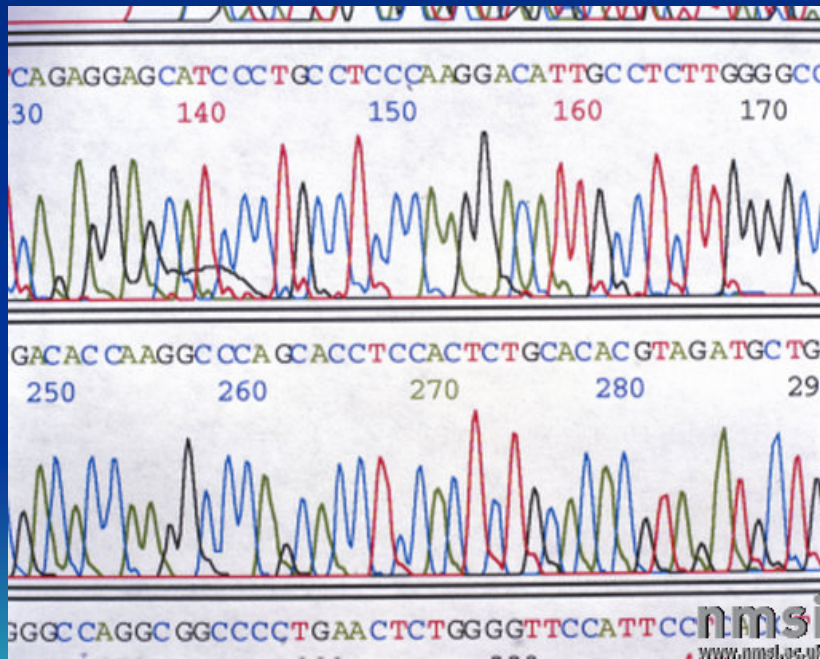
1. IPTG/X-gal plate
2. Colony PCR
3. Restriction enzyme



(white colonies will be selected)

# Sequencing

Colonies which possess complete insert, will be picked and extracted for plasmid DNA before subjected to sequencing at Genomes Institute, National Science Park, PathumThani.



Sequence chromatogram and data sheet

# Primer designing

Hapt\_F

```
AACGCGTCGGCCAACGCAATCGTCATCC  
CGGATGGCCCCCTTTAGGGAATCCAACCCC  
CGCATCTGGTTCCGTCAGGTAGAATCCAAT  
TTTGC GTTGCGAAACGTCCACCACGAACGG  
GTAAAGCTCGACAACGTTATATCCGCTGTC  
CCTTCCCACATTCTAGAGGAGGTCGAGAGC  
GTCCTAGATAATCCCGACTCGGTGCGTCCC
```

Hapt\_R

Forward primer ;

Hapt\_F : 5'-AACGCGTCGGCCAACGCAAT- 3'

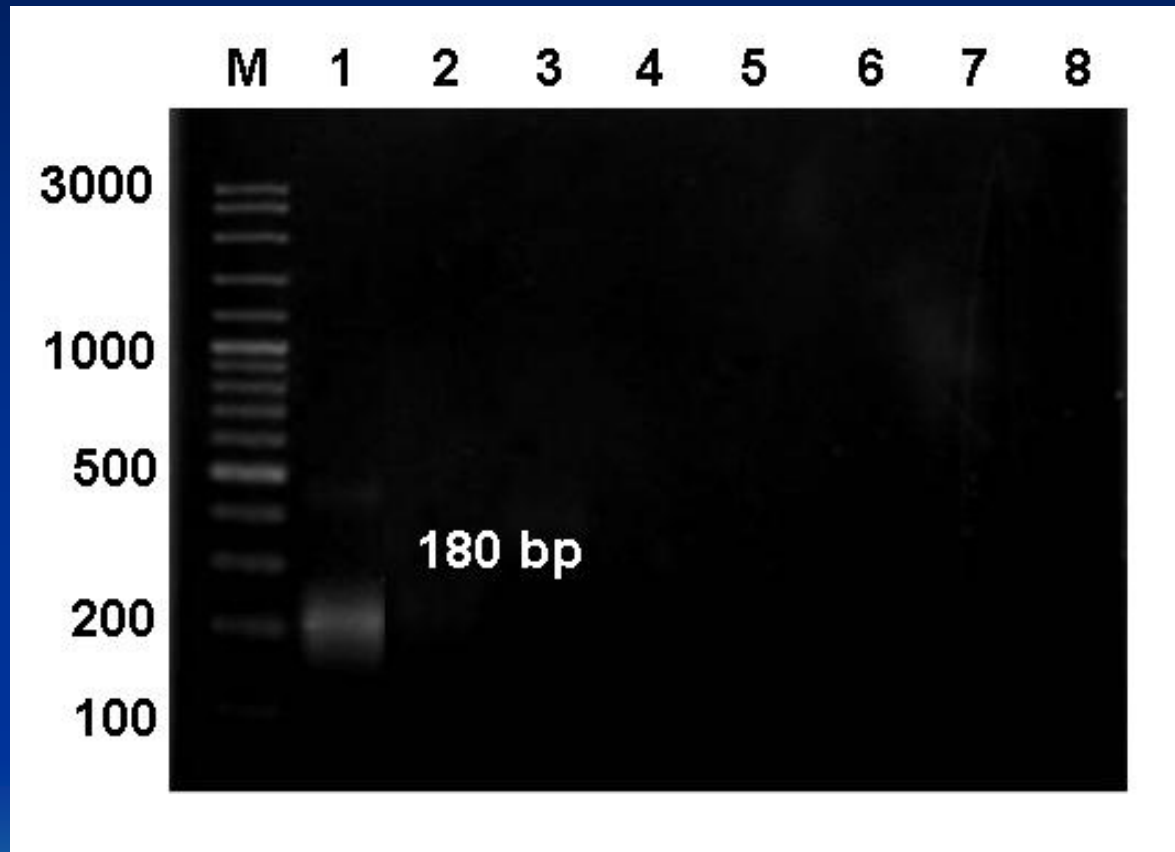
Reverse primer

Hapt\_R : 5'- GCTCTCGACCTCCTCTAGAA- 3'

Completely designed primer will be subjected to synthesize at Genomes Institute.

Example Sequence;  
These primers yield a 256 bp PCR product

# Specificity testing

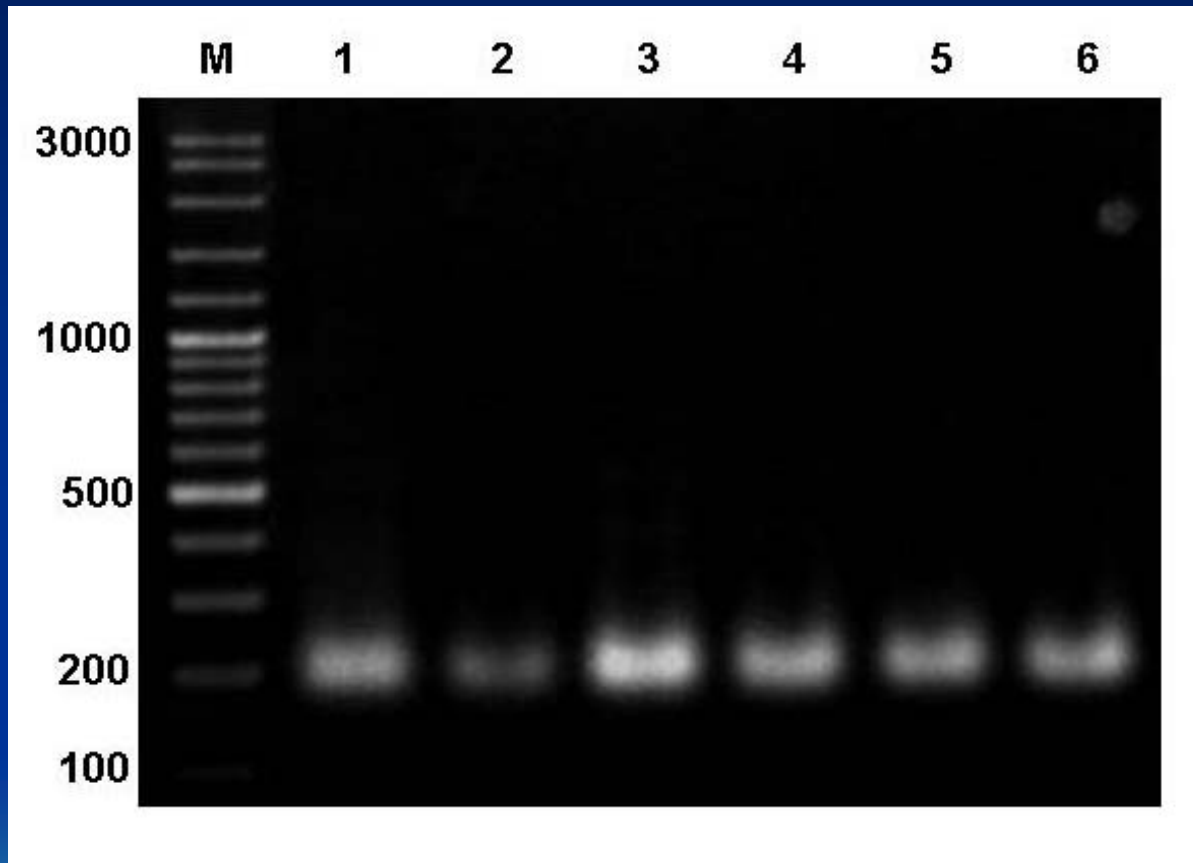


Result ;

Specific amplicon  
Was observed  
In only *H. taichui*  
Sample, No cross  
reaction whith other  
Testing parasites.

M = molecular size markers, 1 = *H. taichui*, 2-8 = other testing parasites

# Independent reaction



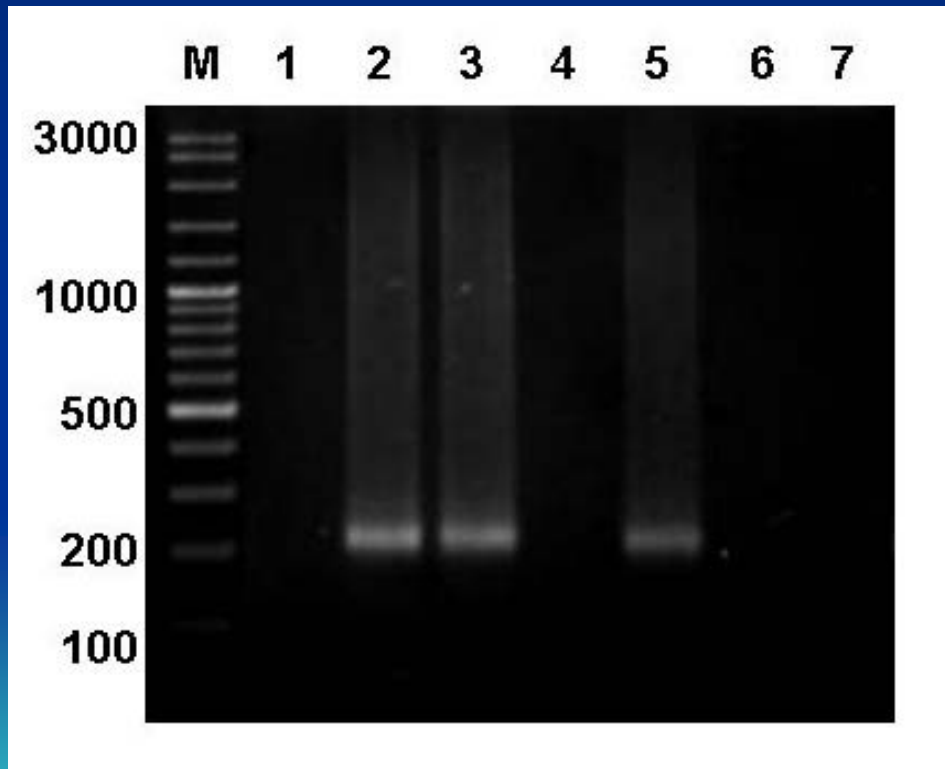
Result ;

Specific amplicon  
Was observed  
in *H. taichui* DNA  
Sample, even they  
were individually  
extracted and  
obtained from  
Different places.

Lane 1-3: metacercarial stage, lane 4-6: adult worm

# Detection in field-collected snails

Specific primers designed in this study were attempted to amplify in field-collected snails and found that 180bp amplicon was generated in the sample of *Pleurolophocercous* cercariae obtained from *Tarebia granifera* snail



Preference habitat for  
*T. granifera* snail



Lane 3, 5: *Pleurolophocercous* cercariae

1: negative, 2: positive, 3-7: different cercarial types

# Conclusion

The 200 bp amplicon generated from OPP-11, was successfully developed to found to *H. taichui*-specific DNA marker which yielding a product size of 180 bp.

Availability / validated HAT-RAPD method for development of specific DNA marker

Pleurolophocercous cercaria obtained from *T. granifera* snail will develop to be *H. taichui*.

The *H. taichui*-specific primers with successfully developed in this study can be use in several applications base on epidemiological monitoring and detection in snail intermediate hosts

Serve as usefulness diagnostic tool for prevention, management and epidemiological control program.

# THANK YOU



Parasitology Research  
Laboratory, Biol., Science, CMU.

Commission on Higher Education;  
CHE-PhD-SW 2549

Molecular Biology Laboratory, Biol.,  
Science, CMU

Economic Plant Genomes Research  
and Service Center



Applied Technology for  
Biodiversity Study, IST, CMU.