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

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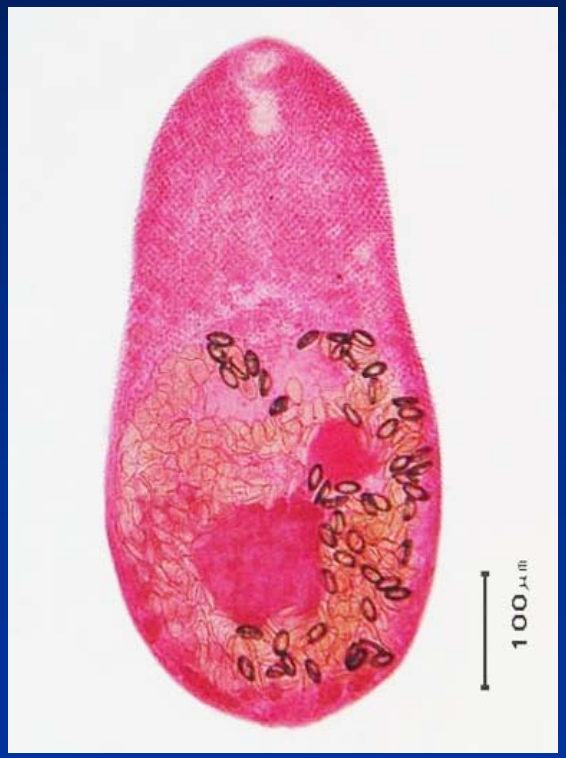
Haplorchis taichui



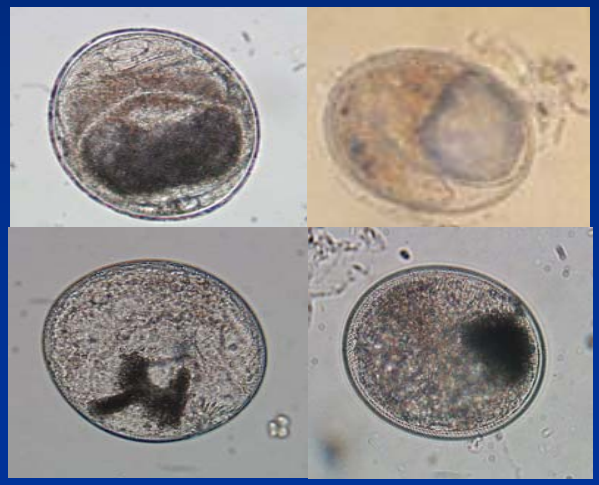
Endemic species in northern Thailand especially in Chiang Mai province.
(Wongsawad et al,2003;Kumchoo et al,2004)

-High prevalence and intensity

Henicorhynchus siamensis

Second Intermediate Host

Puntioplites proctozysron



metacercariae

Cyclocheilichthys repasson

Henicorhynchus siamensis

Barbodes schwanenfeldi

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

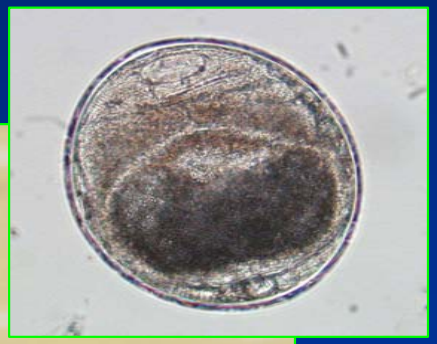
-Fisheries
-Aquaculture
-Travel, floating houses
-Personal interest,
wild plants / animals

-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

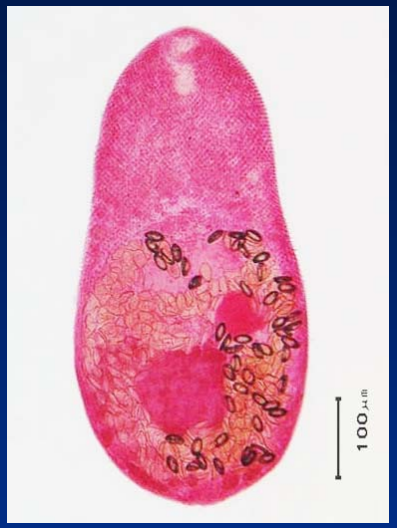
Target parasites

:For the development of specific detection

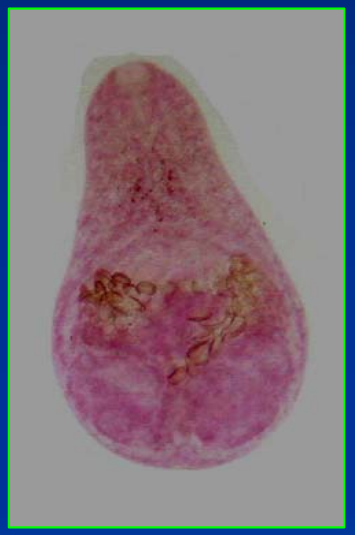
Intestinal fluke;

- *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)



H. taichui



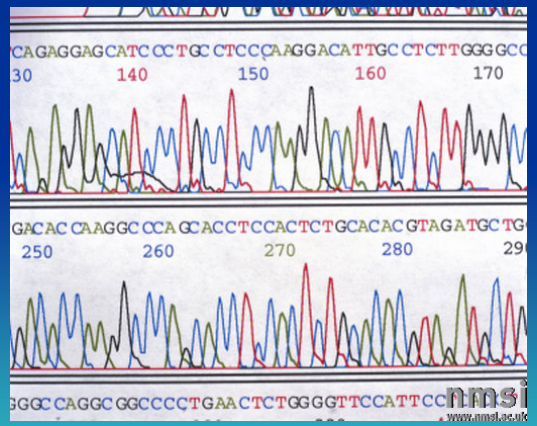
S. falcatus

O. viverrini

Samples of parasites

C. caninus

MATERIALS AND METHODS



Specimens preparation

Ground and homogenized

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

Passed through the Grading sieves

Rinsed with 0.85% NaCl

Examined for metacercariae

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.

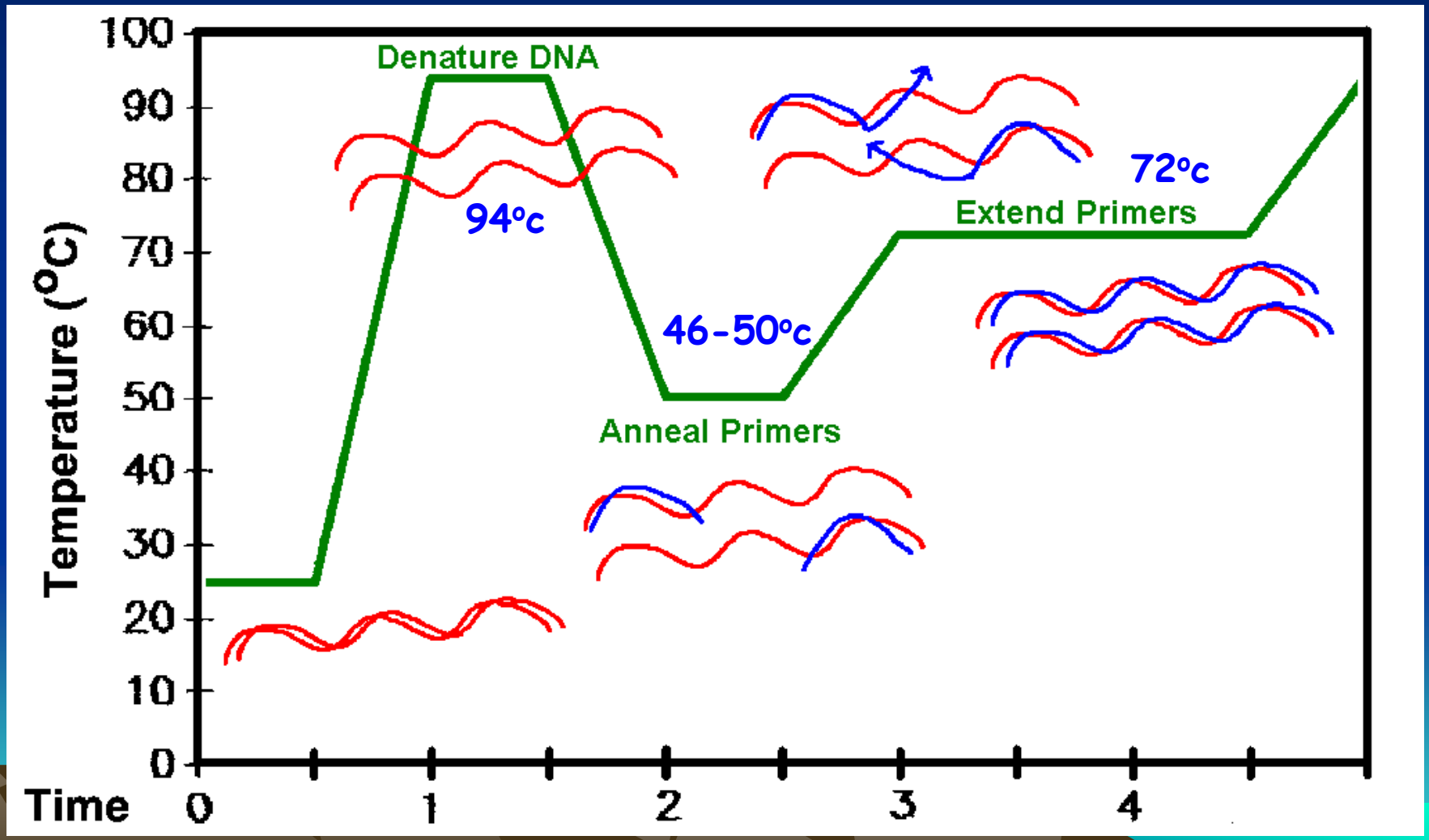
Methods

-DNA extraction

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

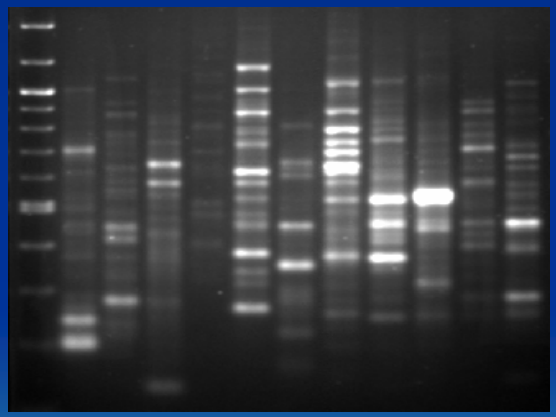
HAT-RAPD PCR

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

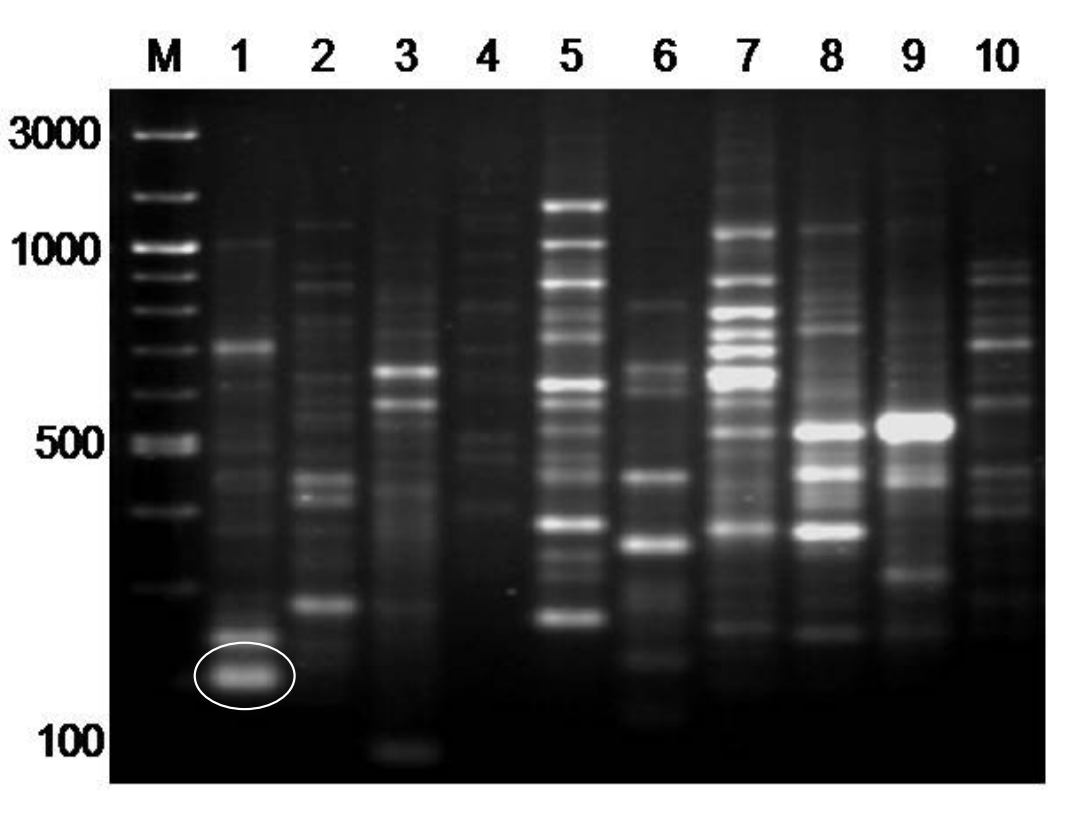


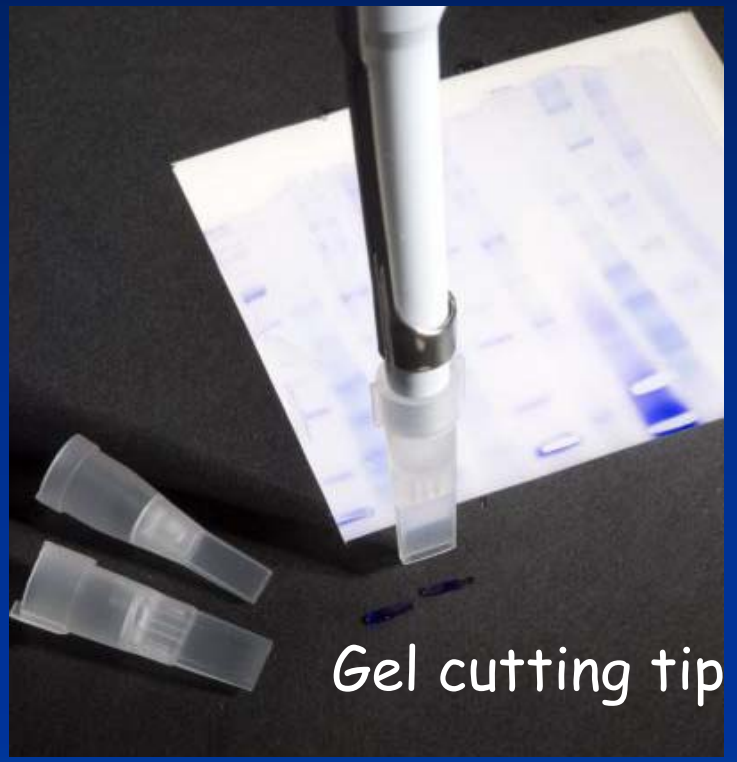
Gel Electrophoresis

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

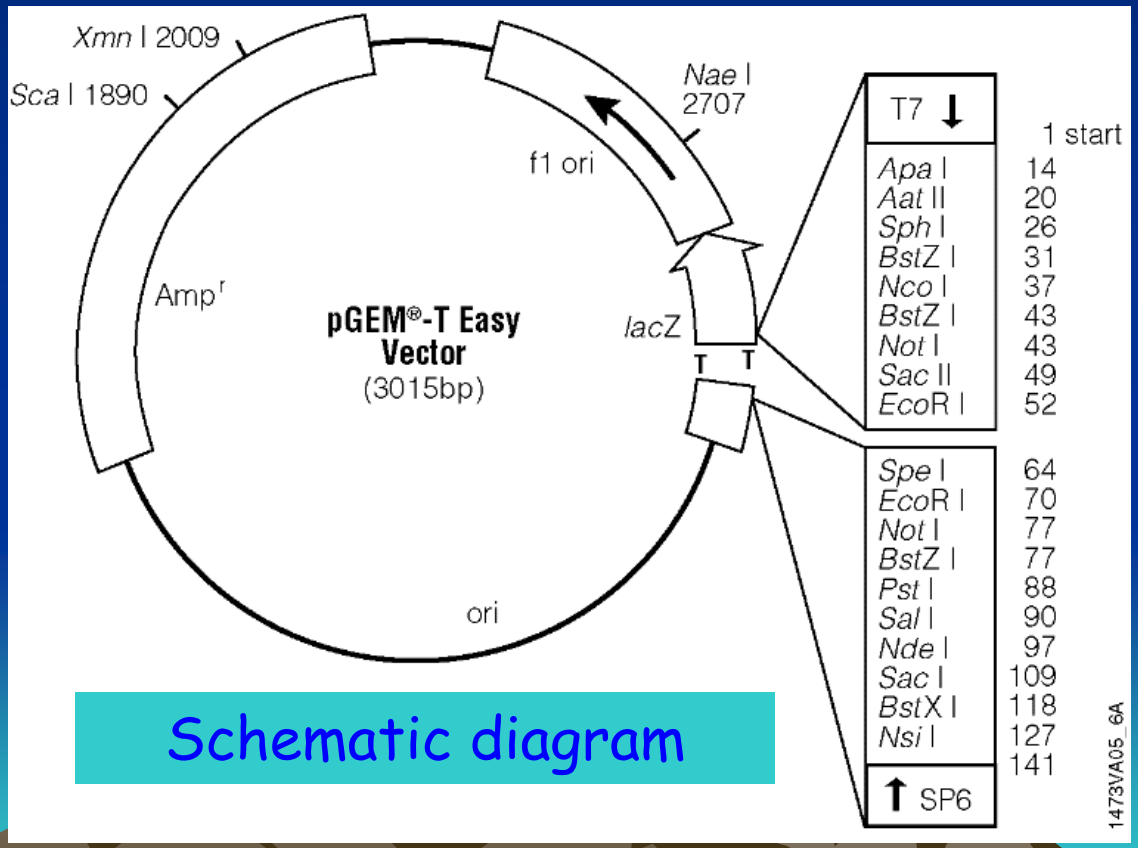




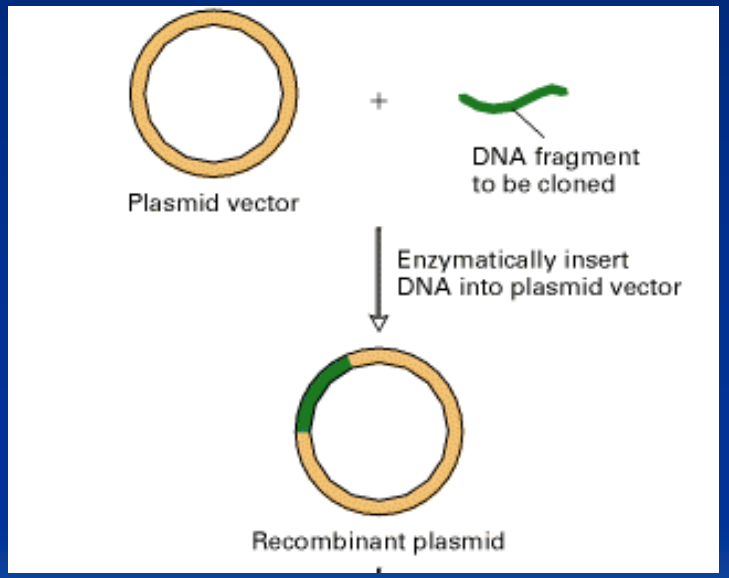
For example: HAT-RAPD markers generated by OPP-11 primer showing the expected species specific fragments (200 bp). M= molecular size markers , Ht= *H. taichui*

Ligasion to Plasmid vector

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

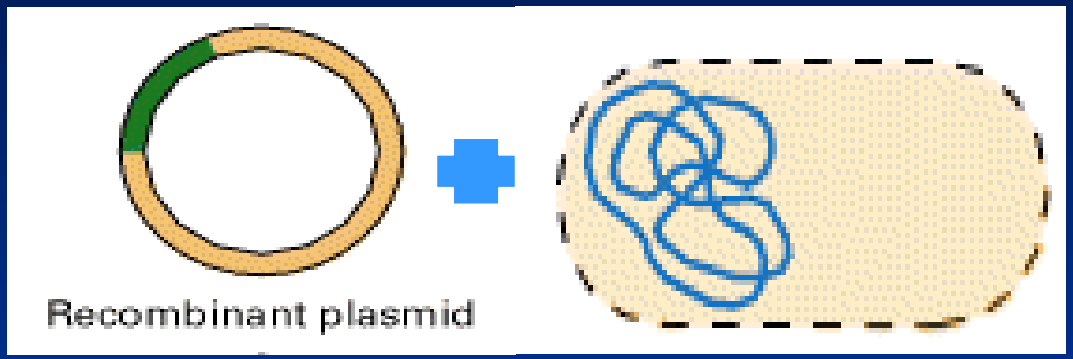


Schematic diagram



Plasmid recombination

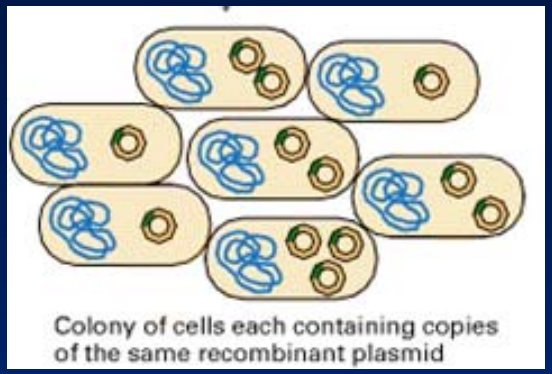
Transformation

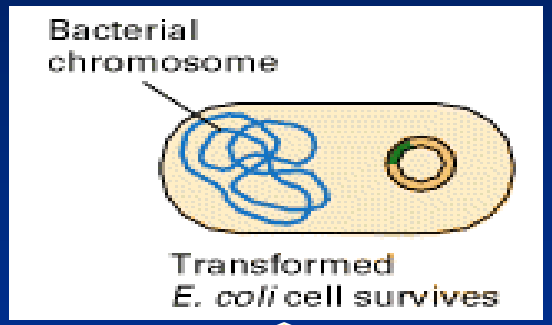


Ligated vector

Competent cells

electroporation

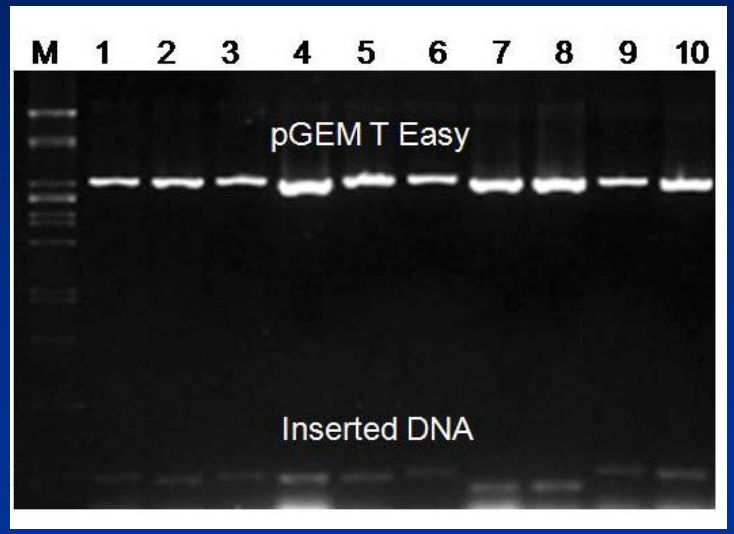


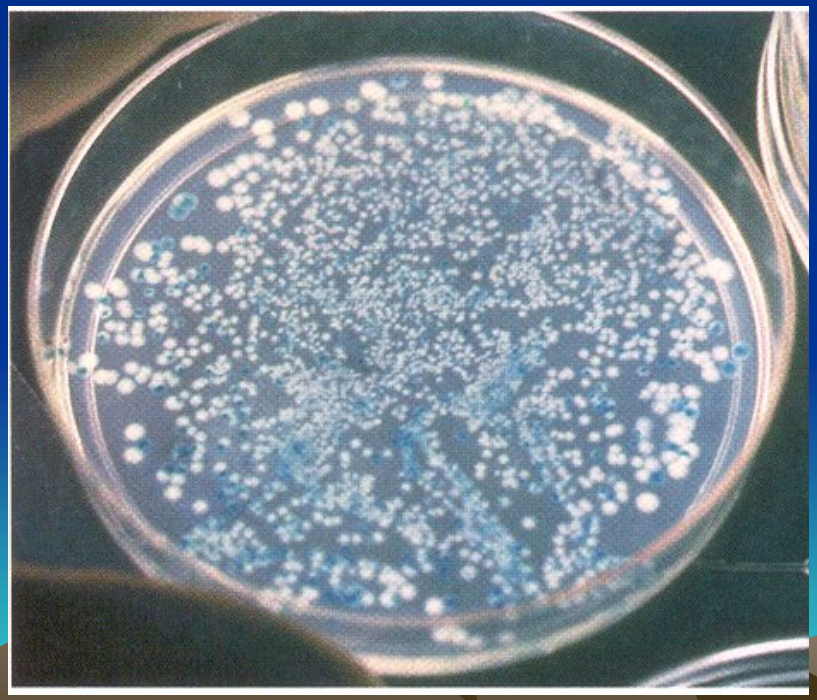


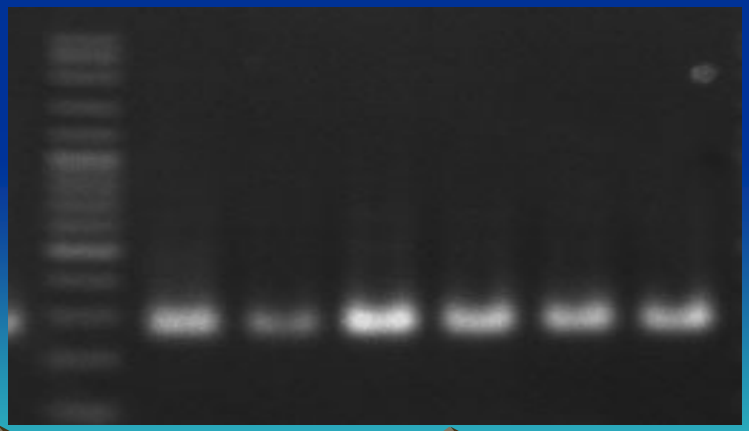
Test for the complete of insertion

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

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



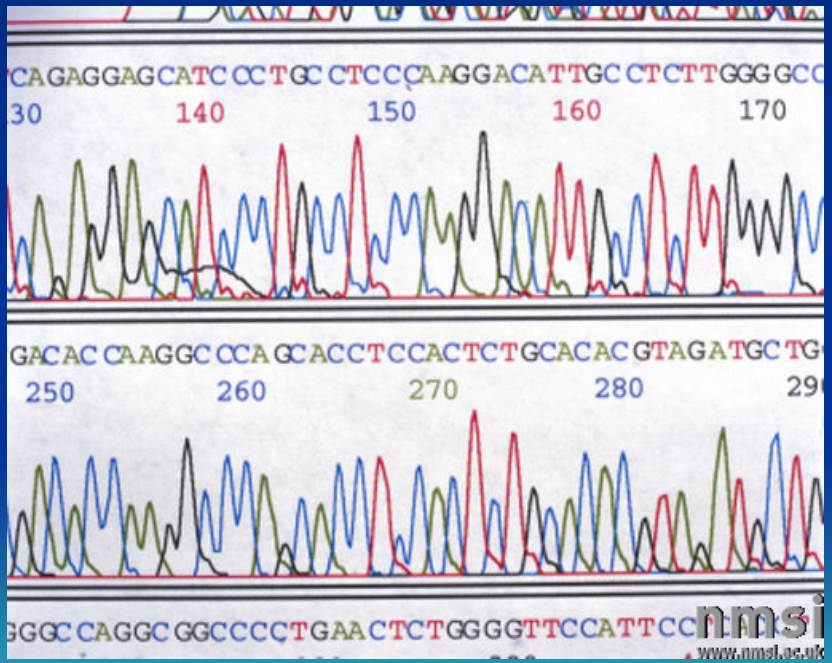


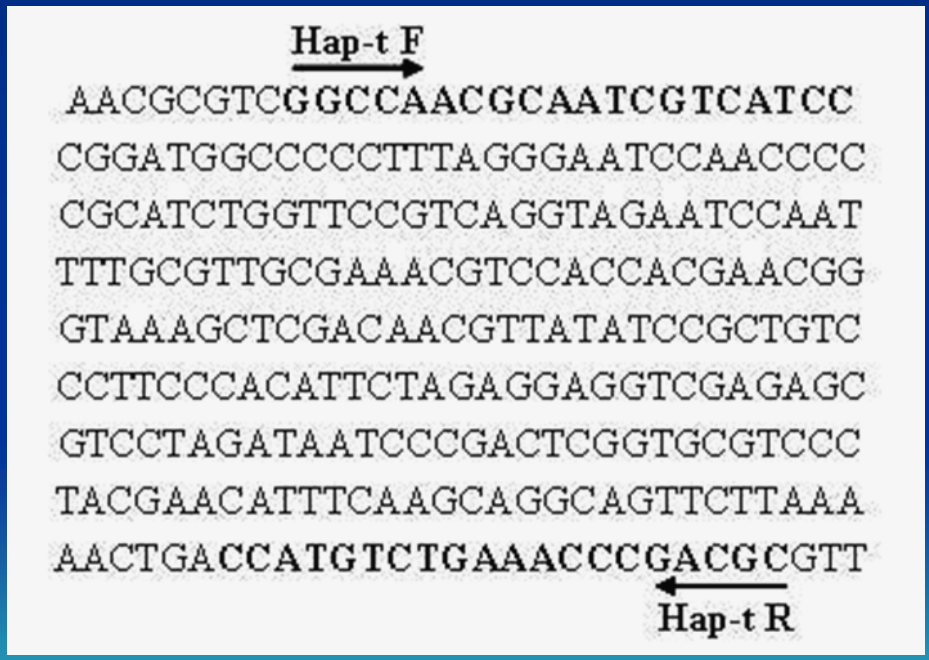


(white colonies will be selected)

Sequencing

Colonies which posses 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
TTTGCGTTGCGAAACGTCCACCACGAACGG
GTAAAGCTCGACAACGTTATATCCGCTGTC
CCTTCCCACATTCTAGAGGAGGTCGAGAGC
GTCCTAGATAATCCCGACTCGGTGCGTCCC

Hapt_R

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

Example Sequence; These primers yield a 256 bp PCR product

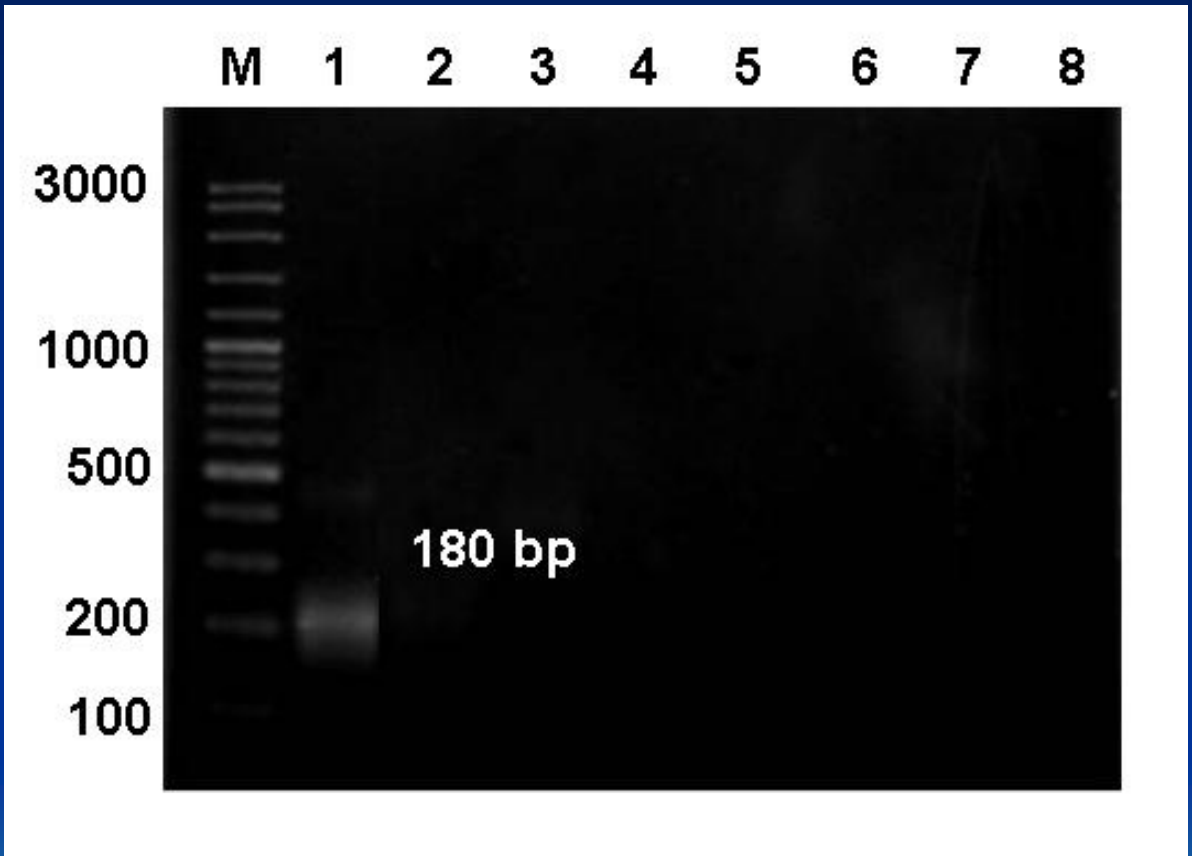
Forward primer ;

Hapt_F : $5'$-AACGCGTCGGCCAACGCAAT- $3'$

Reverse primer

Hapt_R : $5'$- GCTCTCGACCTCCTCTAGAA- $3'$

Specificity testing

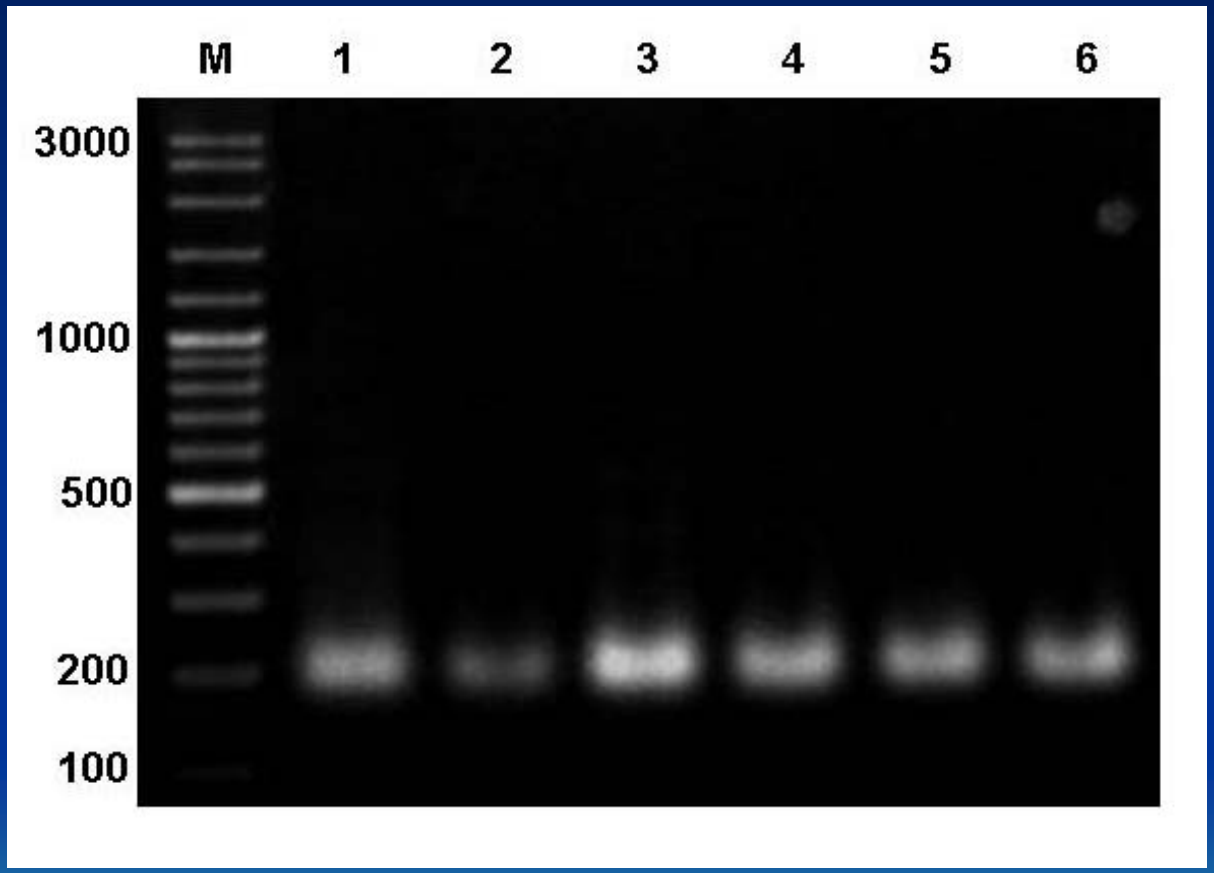


Result ;

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



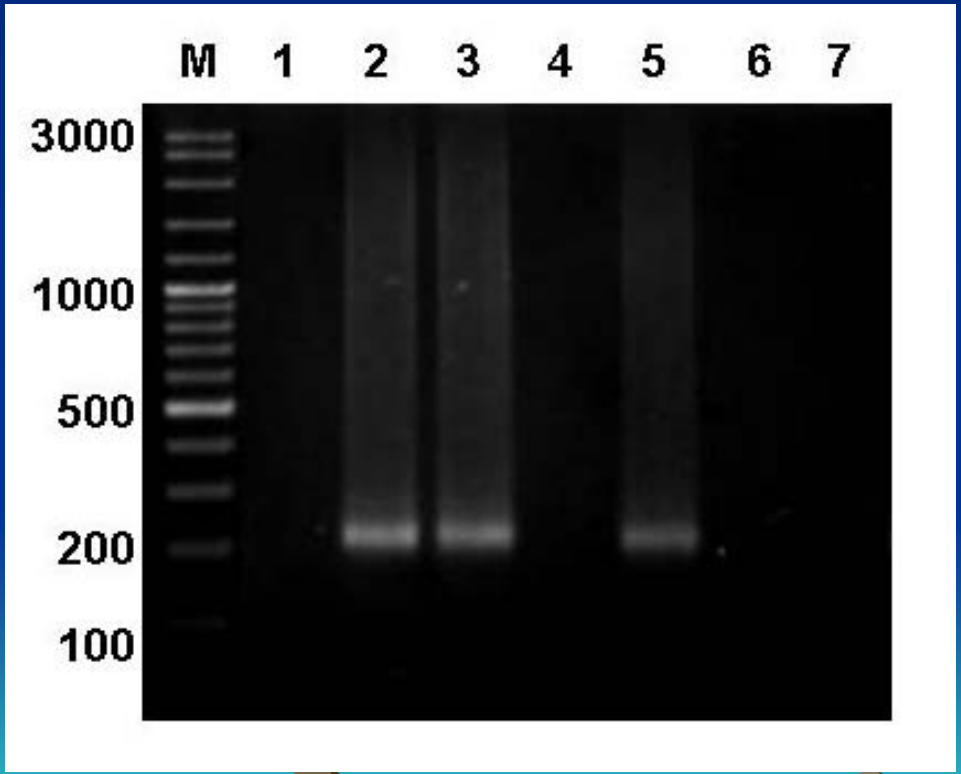
Result ;

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
Department of Biology
Faculty of Science
Chiang Mai University

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.