Development of Specific DNA Marker for the Detection of Trematode, \textit{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

Henicorhynchus siamensis

Cyclocheilichthys repasson

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.
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)
Samples of parasites

- H. taichui
- S. falcatus
- O. viverrini
- C. caninus
MATERIALS AND METHODS
Specimens preparation

Digested with 1% Pepsin solution
Incubated 37°C 2 hour
Passed through the Grading sieves
Rinsed with 0.85% NaCl
Ground and homogenized
Examined for metacercariae
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
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.
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

Ligated vector $\rightarrow$ Competent cells $\rightarrow$ electroporation

Colony of cells each containing copies of the same recombinant plasmid

Bacterial chromosome

Transformed E. coli cell survives

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

Forward primer:
Hapt_F: 5'-AACGCGTCGGCCAACGCAAT- 3'

Reverse primer:
Hapt_R: 5'-GCTCTCGACCTCCTCTTAGAA- 3'

Example Sequence:
These primers yield a 256 bp PCR product.

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

Result:
Specific amplicon was observed in only *H. taichui* Sample, no cross reaction with other testing parasites.

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

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