# Preliminary Detection of SSR Markers in Mound Building Termite, *Macrotermes gilvus* (Hagen) (Termitidae: Macrotermitinae)

by

G. Veera Singham, Ahmad Sofiman Othman and Chow Yang-Lee

Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

## Abstract

Five potential SSR markers were successfully detected in pooled DNA sample tissue of *Macrotermes gilvus* (Hagen) worker caste. Genomic DNA was enzyme-restricted (*Rsal*) followed by enrichment using Hybond N+ membrane bound with oligonucleotides. A total of 96 positive clones were selected and sequenced. Locus specific primers were developed and optimized for PCR reaction. The success rate of finding SSR motifs in pooled DNA samples was significantly higher (16.8%) than that of DNA sampled from single individual (2.1%). Cross amplification was successful for two out of the four tested primer sets.

**Key words:** SSR marker, *Macrotermes gilvus*, enrichment procedure, cross amplification

## Introduction

*Macrotermes gilvus* (Hagen) is a common mound building termite that is indigenous to South East Asia from Myanmar, Thailand, Vietnam, and Peninsular Malaysia to Indonesia, Borneo and Philippines (Snyder 1949, Roonwal 1970, Thapa 1981, Tho 1992). *M. gilvus* is regarded as a pest of economic importance as it may cause damages to wooden structures besides being responsible for secondary reinfestation in premises previously infested by other termite species (Roonwal 1970, Lee 2002, Lee et al. 2007). Despite the fact that it is widely distributed in this region, few studies have been made on this species. Most of the previous studies focused on biological and ecological aspects of this species (Inoue et al. 1997, Acda 2004, Neoh and Lee 2009, Neoh et al. 2010). There has been a serious lack of information on the population aspects of this species. The use of genetic and molecular markers will aid and enable more accurate determination of population dynamics and colony structure. This preliminary study is dedicated on detecting sensitive molecular marker using enrichment procedure. Simple Sequence Repeat (SSR) or microsatellite marker has been proven as a sensitve molecular tool found widely in the genome of prokaryotes and eukaryotes (Field and Wills 1998, Schlotterer 2000). It has been repeatedly used in many fields of genetics as in genetic diversity, population genetic structure, genomic mapping and phylogeography (McCouch et al. 1997, Steinberg et al. 2002, Menke et al. 2010). The outcome of this study is vital in providing essential molecular tool to elucidate the population genetic structure of *M. gilvus*.

## Methods and materials

***Sample collection.*** Termites were collected from five colonies of *Macrotermes gilvus* within main campus of Universiti Sains Malaysia (USM) (5º 21' N, 100º 18' E). Sampling was made by breaking open the termite mound using a pick and both minor and major worker termites were directly collected using a fine forcep and were immediately stored in absolute ethanol for molecular analysis. Soldier termites were also collected and stored in 70% ethanol for species identification based on morphometric features (Roonwal 1970).

***Genomic DNA extraction*.** DNA from pooled sample tissue of five individual worker termites (representing five different colonies) and another single worker termite were extracted using CTB Tissue Extraction Kit (Intron, Seongnam-Si, Gyeonggi-do, Korea) after being pulverized in liquid nitrogen. DNA extraction was carried out according to manufacturer's standard procedure. Extracted DNA was quantified for concentration and tested for purity based on optical density (OD) values obtained from UV spectrophotometer. DNA with $OD_{260/280}$ value ranging from 1.8-2.0 is chosen for the isolation of SSR markers.

***Isolation of SSR markers.*** The pooled genomic DNA was digested with *Rsal* and followed by ligation with M1uI annealed adaptor. The ligation was enriched with microsatellite repeats by hybridizing on Hybond N+ membrane with bound oligonucleotides comprising of di-, tri- and tetra-nucleotide motifs (GATA, AAAT, GATG, GACA, AAG, CAA, AAT, CA, GT, and CT).Fragments with SSR repeats (enriched fragments) were then incorporated into a cloning vector pGEM-T Easy vector (Promega, Madison, Wisconsin, USA) before being transformed into JM109 *E. coli* competent cells (Promega) to obtain a clone library. Blue-white screening was performed to select out positive clones (i.e colonies with vector incorporated with insert).95 positive clones were selected and sequenced.

***Primer design and Optimization*.** Sequence results were traced for SSR repeats and any overlapping sequences were eliminated. Primer sets were designed using a web-based Primer 3 plus program (www.bioinformatics.nl/cgi-bin/primer3plus.cgi). Designed primers were optimized via Polymerase Chain Reaction (PCR). PCR amplification was performed in a standard 50 µl reaction volume with 4 varying $MgCl_2$ concentrations (i.e. 1.5 mM, 2.0 mM, 2.5mM and 3.0 mM), 2mM dNTPs, 5U/µl *Taq* DNA polymerase, 1 pmol of each forward and reverse primer and 4µl of total genomic DNA. All PCR amplification was performed on a MJ Research PTC-200, Peltier Thermol Cycle (Waltham, USA), with an initial denaturation step at 94 ℃ for 2min, followed by 35 cycles of three step PCR ; denaturation step at 94 ℃ for 30 sec, annealing step was optimized on a gradient of temperatures based on the melting point of the designed primers for 30 sec and extension step at 72 ℃ for 1 min followed by a post cycle extension at 72 ℃ for 10 min. PCR products were electrophoresed on 2% agarose gel and post stained with Ethidium Bromide before being viewed under a UV illuminator.

**Cross amplification.** Four sets of microsatellite primers previously developed for *Macrotermes michaelseni* (Kaib et al. 2000) were tested against *Macrotermes gilvus* for detecting any cross amplification across the two species.

## Results and discussion

Sequence analysis of DNA sampled from a single individual termite resulted in two sets of sequence data comprising short SSR motifs of which both failed to be developed into locus specific primers. Pooled DNA samples, however, showed an enhanced sequence results with 16 sequence data comprising relatively long SSR motifs. 12 sets of locus specific primers (forward and reverse primers) were successfully designed out of the total 16 sequences after elimination of overlapping sequences. Five of the 12 developed primer sets successfully PCR amplify with a single distinct band being observed on 2% agarose gel electrophoresis. The rest of the primer sets were either non-amplifying or amplifying with multiple unspecific bands. The optimum conditions and amplification success of all the primer sets are as shown in Table 1.

Table 1. Developed primer sets (Primer 3 plus), amplification success and optimum conditions.

| Locus | Repeat motif | Product size | Primer sequence (5'-3') Forward (top); Reverse (bottom) | Amplify success | Optimum temperature (°C)/ [$MgCl_2$] mM |
|---|---|---|---|---|---|
| B6-V | $(GA)_{20}$ | 195 bp | GGAACTAGAACAGGCCATGAA<br>TCTTGCTTACGCGTGGACTA | Multiple bands | n.a |
| B9-V | $(TG)_{21}$ | 245 bp | GCGTGGACTATGTGCTCTTG<br>ACACAACCGGTGGTTAGTCC | Multiple bands | n.a |
| B12-V | $(CAGA)_5$ | 176 bp | TCCCCAGATTTGTTAGTCCA<br>ACAGCTGGCCATTTAACCAA | ✓ | 55.0/2.0 |
| E1-V | $(CA)_{30}$ | 241 bp | CATTTGTCAGCCTGTCTCCA<br>AGCATTCAGACAACGTGCAG | Multiple bands | n.a |
| E8-V | $(TG)_{15}$ | 213 bp | TTGCGTGAAGTAATCGCTGA<br>TTAGAAATATGGCGGCAACG | ✓ | 57.0/2.0 |
| E10-V | $(AC)_{34}$ | 150 bp | GGCTCTGATCTGAAGCGAAC<br>CAATCCTGCATTGAAACCAC | Multiple bands | n.a |
| F3-V | $(CA)_{13}$ | 225 bp | ACAGCAGGAACCAGAAAGGA<br>TCTGCACCGAGTGGTAACAA | ✓ | 59.0/2.5 |
| G1-V | $(TG)_{17}$ | 155 bp | AAGGCTGTTTTTCGCTTGAA<br>AGCAAGAGCACAACTTTCACC | ✓ | 55.0/1.5 |
| G3-V | $(AG)_{27}$ | 226 bp | GTTAACCGAGCAGGAAAACG<br>TAATCATTGTTGCCGTGGAA | Multiple bands | n.a |
| G9-V | $(CA)_{12}$ | 160 bp | AAGCTAACTCGCGCTTTTTG<br>AAGGAACATTCTCCCGAAGTC | Multiple bands | n.a |
| D10-V | $(TG)_{10}$ | 150 bp | AAATCCAGCAACAACACGAA<br>TGTGGGCACGTTTTATTTTG | ✓ | 55.0/2.5 |
| H12-V | $(GT)_{10}$ | 156 bp | TCACGGTCATGTGGCAGTAT<br>TCCATCATCAGGAGCAACTTT | X | n.a |

* x – no amplification; ✓- amplify with single distinct band ; *bp – base pair ; n.a. – not applicable

The success rate of finding SSR motifs in the sequence data is 2.1% for DNA sampled from single individual whilst 16.8 % for pooled DNA samples. This result indicates that the success rate of isolating SSR marker is relatively higher in pooled DNA sample than that of single individual termite. The amplifying primers will be fluorescently labeled and tested for polymorphism for future applications in population genetics.

Only two sets of primers were successfully PCR amplify in cross amplification using primers previously developed for *Macrotermes michaelseni*. This shows that some regions in the genome of *Macrotermes gilvus* are relatively similar to that of its sibling species *M. michaelseni* leading to development of cross amplifying primers. This condition is essentially useful for inter population studies between sibling species.

## Conclusion

In conclusion, this study provides an insight in detecting essential molecular tool for revealing the elusive world of a common mound building termite, *Macrotermes gilvus*.

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