

3. Restriction fragment length polymorphism analysis

The ITS1 PCR products of each species were further digested with several restriction endonucleases; *Acc* I, *Ase* I or *Hinf* I. *Ase* I or *Rsa* I were used for the ITS2 PCR products. There was no intraspecific variation in ITS restriction patterns within each filarial nematodes species tested.

Digestion of ITS1 PCR products with *Acc* I could differentiate among *W. bancrofti*, *Brugia* sp. (both *B. malayi* and *B. pahangi*) and *D. immitis* (figure 7). Besides *W. bancrofti* and *D. immitis*, digestion of ITS1 PCR products with *Ase* I could also differentiate *B. malayi* and *B. pahangi* (figure 8). The RFLP patterns of ITS1 PCR products using *Hinf* I could not differentiate among all filarial species tested.

Digestion of ITS2 PCR products with *Ase* I could differentiate among *W. bancrofti*, *Brugia* sp. (both *B. malayi* and *B. pahangi*) and *D. immitis* (figure 9), while RFLP patterns using *Rsa* I could not be used to differentiate all filarial parasite species tested.

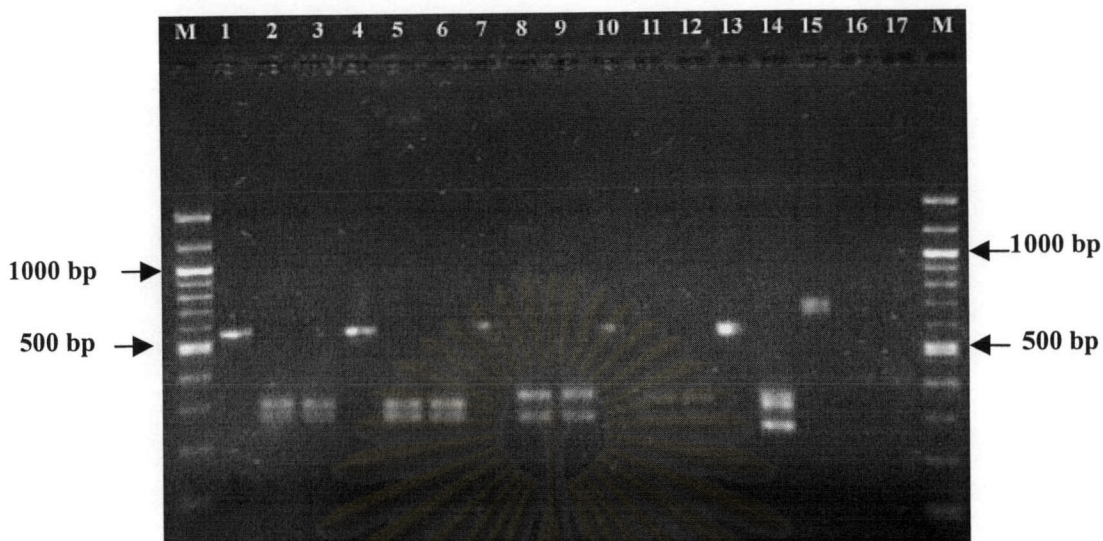


Figure 7 PCR-RFLP analysis of filarial ITS1 PCR products digested by *Acc* I. Lane M, 100 bp ladder; lane1, undigested Thai-Karen *W. bancrofti* (~570 bp); lanes 2-3, digested Thai-Karen *W. bancrofti* (~310/260 bp); lane 4, undigested Myanmar *W. bancrofti* (~570 bp); lanes 5-6, digested Myanmar *W. bancrofti* (~310/260 bp); lane 7, undigested human *B. malayi* (~580 bp); lanes 8-9, digested human *B. malayi* (~320/260 bp); lane10, undigested cat *B. malayi* (~580 bp); lanes 11-12, digested cat *B. malayi* (~320/260 bp); lane 13, undigested cat *B. pahangi* (~590 bp); lane 14, digested cat *B. pahangi* (~330/260 bp); lane 15, undigested dog *D. immitis* (667 bp); lanes 16-17, digested dog *D. immitis* (413/254 bp).

จุฬาลงกรณ์มหาวิทยาลัย



Figure 8 PCR-RFLP analysis of filarial ITS1 PCR products digested by *Ase* I. Lane M, 100 bp ladder; lane1, undigested Thai-Karen *W. bancrofti* (~570 bp); lanes 2-3, digested Thai-Karen *W. bancrofti* (~210/170/110/80 bp); lane 4, undigested Myanmar *W. bancrofti* (~570 bp); lanes 5-6, digested Myanmar *W. bancrofti* (~210/~170/110/80 bp); lane 7, undigested human *B. malayi* (~580 bp); lanes 8-9, digested human *B. malayi* (~290/150/140 bp); lane10, undigested cat *B. malayi* (~580 bp); lanes 11-12, digested cat *B. malayi* (~290/150/140 bp); lane 13, undigested cat *B. pahangi* (~590 bp); lane 14, digested cat *B. pahangi* (~300/290 bp); lane 15, undigested dog *D. immitis* (667 bp); lanes 16-17, digested dog *D. immitis* (455/212 bp).

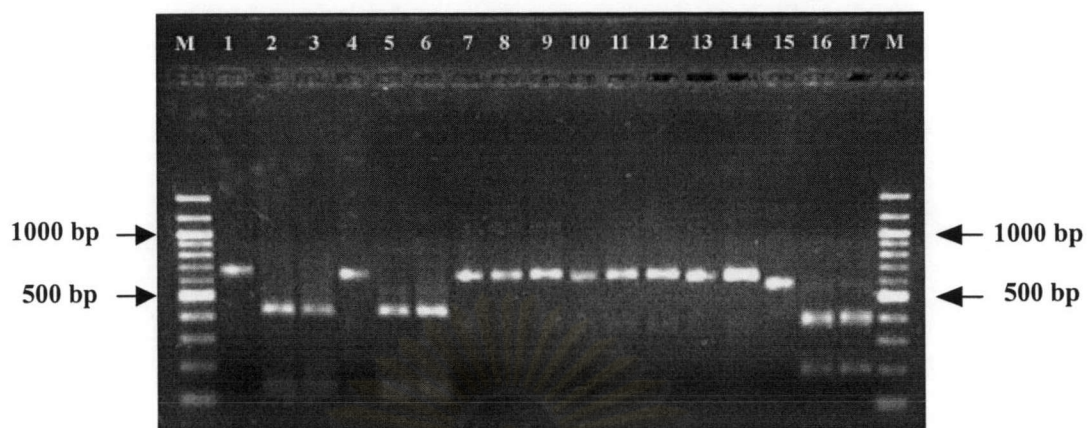


Figure 9 PCR-RFLP analysis of filarial ITS2 PCR products digested by *Ase* I. Lane M, 100 bp ladder; lane1, undigested Thai-Karen *W. bancrofti* (~660 bp); lanes 2-3, digested Thai-Karen *W. bancrofti* (~430/120/110 bp); lane 4, undigested Myanmar *W. bancrofti* (~660 bp); lanes 5-6, digested Myanmar *W. bancrofti* (~430/120/110 bp); lane 7, undigested human *B. malayi* (~650 bp); lanes 8-9, digested human *B. malayi* (undigested); lane10, undigested cat *B. malayi* (~650 bp); lanes 11-12, digested cat *B. malayi* (undigested); lane 13, undigested cat *B. pahangi* (~620 bp); lane 14, digested cat *B. pahangi* (undigested); lane 15, undigested dog *D. immitis* (570 bp); lanes 16-17, digested dog *D. immitis* (197/373 bp).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย