CHAPTER II

LITERATURE REVIEW

Most of the reported genetic analyses were in African elephants *(Loxodonta africana*). Fewer studies in Asian elephant (*Elephas maximus*) were on their taxonomy, genetic variation and evolution (Fernando *et al*, 2001, 2003a, 2003b, Gunasekera *et al*, 2003, and Lertwatcharasarakul *et al*, 2003). In the previous studies, their used genetic marker loci in many regions of nuclear DNA and/or mitochondrial DNA (mtDNA). The most microsatellite loci in a nuclear DNA were specific for African elephant, and were applied to Asian elephant for some locus. However, the loci for Asian elephant were genotyped five of tri- and tetranucleotide microsatellite loci in 20 Asian elephants and reported that all loci were polymorphic (Fernando *et al*, 2001). The highly polymorphic loci were used and selected for elephant genetic studies. Beside the microsatellite loci, the mtDNA was used to identify a maternal inheritance and animal's evolution in many species included Proboscidean animals (Frankham *et al*., 2002). In Thailand, the studies were reported by Srikummool (1998), sequenced the 323 bp mtDNA D-loop of Asian elephant in Thailand and found that the sequence has the same nucleotides as the 280 bp D-loop registered in the gene bank. Second, Lertwatcharasarakul *et al* (2003) reported 8 haplotypes of 5′ end cytochrome *b* of mtDNA of Asian elephant in Thailand. The last, Siripunkaw (2003) used the cross-species primer of 8 microsatellite loci from African elephant to amplify these loci in 20 Asian elephants and suggested that the 4 highly polymorphic loci

could be used for individual identification. That loci can used for evaluate elephant pedigree. The microsatellite locus and mtDNA are now provides tools for genetic management of endangered species for such studies, especially individual identification and parentage test in animals, and forensic analysis (Ellegren 2004)

Microsatellite markers

Microsatellite loci are tandem repeats of very short nucleotide motifs, typically no more than six bases in length. The loci are presented in many thousands of loci in eukaryotic genomes, but also occur in prokaryotes at lower frequencies. Microsatellite loci can be classified on the basis of the repeat motif length, e.g. dinucleotide, trinucleotide, tetranucleotide, etc. For example, AC_{22} would be a shown of 22 repeat units of the dinucleotide repeat AC in genome. The microstaellite have a high mutation rates, the rate's range from 10^{-6} to 10^{-2} per generation (Schlötterer, 2000). According to the mechanism of slippage event in DNA synthesis, called "DNA (replication) slippage" (Schlötterer and Tautz, 1992, Schlötterer, 2000), it is a mechanism generating microsatellite variability. The mechanism is assumed that during DNA replication, when the nascent DNA separates and template strand out of register. If DNA synthesis continues on this molecule, the repeat number of the microsatellite is altered (Figure 1). Microsatellites are scattered along chromosomes and each locus has a unique flanking sequence, and their locus typically exhibits sequence length polymorphism involving variation in the number of times the basic motif is repeated. The illustrate as shown below (Figure 2), the picture shown the pattern on gel of microsatellite with three genotypes. Microsatellites have been widely

used for population-genetic studies, linkage mapping, paternity test, forensic analysis and as a tool for breeding strategies in various domestic and endangered species (Thitaram *et al*, 2006b).

Due to the mutation rate is high and inherited biparentally of the nuclear DNA or autosome. The individual genotype can be observed on an agarose or polyacrylamide gel with electrophoretic separation. The pattern shown the alleles with fragment length, a homologous individual have two alleles of equal length and a heterozygote have two alleles of different lengths. Therefore microsatellite has a highly polymorphic marker as following allele size on a gel. (Figure 3) Microsatellites are well-suited for studying the parentage and pedigree (Tautz, 1989), as well as individual identification in the forensic medicine (Randi *el al*, 2002). Although microsatellites are found to be highly polymorphic in elephants, we cannot use only single-locus to identify individual elephant or pedigree. Multiloci are therefore recommended, as Tsumagari *et al* (2003) reviewed that the parentage test could be done by using DNA fingerprints of multiloci microsatellite.

Thirty-nine microsatellite loci were reported in elephants, mostly the African elephants (Table 1). Nyakaana and Arctander (1998) and Comstock *et al* (2000) isolated and characterized 5 and 12 microsatellite loci in African elephant respectively. Siripunkaw (2003) selected 4 of 5 and 4 of 12 from the above loci for genotyping the Asian elephants. She could amplify and genotype all these loci in Asian elephants. Fernanndo *et al.* (2001) described the isolation and characterization of 5 microsatellite makers in Asian elephants and used these markers to identify the unique elephant species in Borneo island (Fernando *et al*, 2003b). Eggert *et al* (2000) and Archie *et al* (2003) characterized 6 and 12 microsatellite loci in African elephants respectively.

These loci were further used for genetic evaluation in African elephants and then applied in Asian elephants.

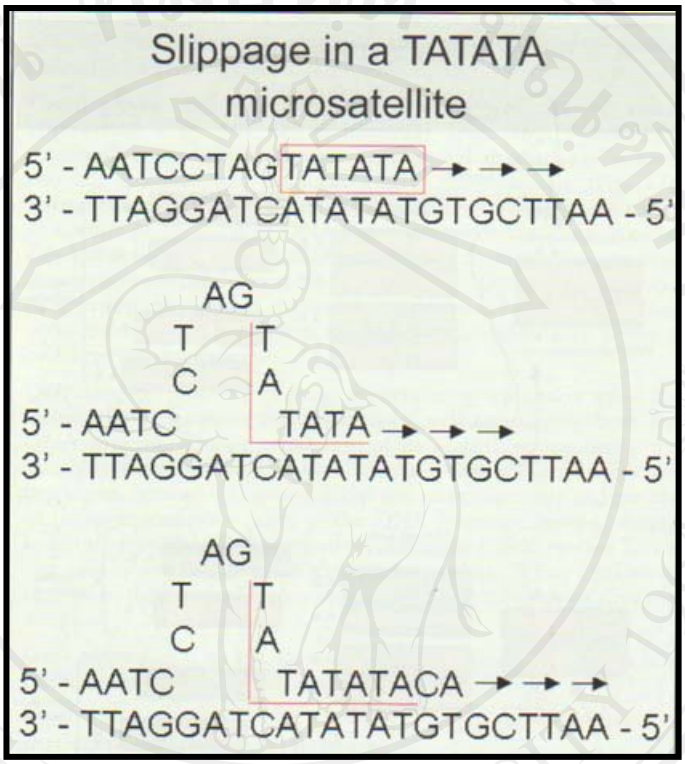

Figure 1 DNA slippage occurs during replication when the nascent DNA separates and reassociates itself temporary from the DNA template. If the replication continues, the nascent DNA is found to be longer or shorter than template with the repeat number of the microsatellite. (Randi *el al*, 2002)

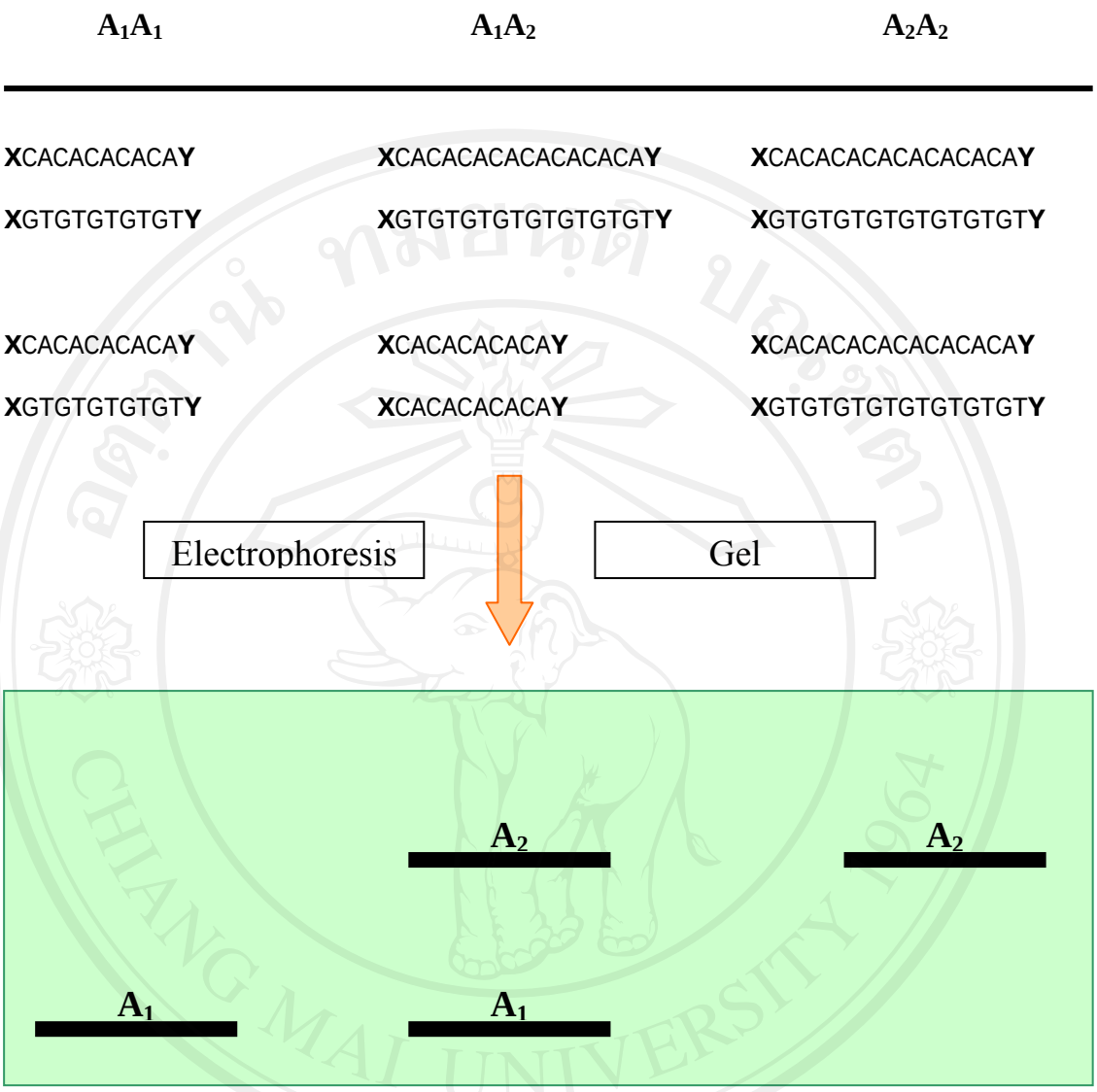

Figure 2 Microsatellite's patterns on gel of microsatellite with three genotypes, two different homozygotes and a heterozygote. The X and Y are invariant DNA sequence flanking the microsatellite repeat. (Frankham *el al*, 2002)

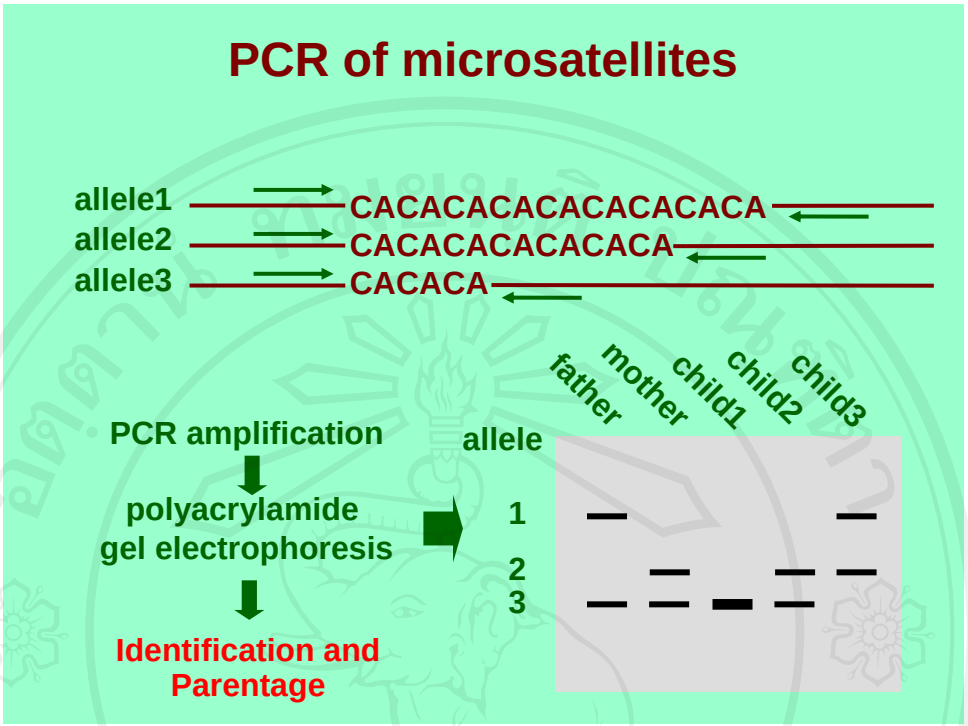

Figure 3 The microsatellites are used for individual identification and parentage test. The child 1-3 are received an allele from father and mother, a same allele is made one fragment length of heterozygote in child 1. The multiloci are necessary for used in the identification and parentagetesting (Thitaram, 2006a).

Table 1 Microsatellite markers are reported in the previous studies.

No.	Locus	Repeat motif	Primer	Sequenc of 5' to 3'	length[b]	Tm[c]	alleles	Size range	Reference
1	LafMS01	$(CA)_{13}(CA)_{16}$	MS01F	CGT-CGC-CCG-AGC-ACA-GTC-GCT	21	72	4 E	187-205	Siripunkaw (2003)
			MS01R	ACC-TGA-TTC-AGG-GAG-CAC-GG	20	64		189	Nyakaana (1998)
2	LafMS02	$(AC)_{16}$	MS02F	GAA-ACC-ACA-ACT-TGA-AGG-G	19	56	3 E	126-130	Siripunkaw (2003)
			MS02R	TCG-CTT-GTA-GAA-GGC-GTG	18	58		149	Nyakaana (1998)
3[a]	LafMS03	$(TG)_{15}$	MS03F	CAT-ATG-AAC-ATA-CCG-GAA-C	19	54	7 E	132-150	Siripunkaw (2003)
			MS03R	GAA-ACT-CCT-CGA-GTA-GTA-GAA	20	60		142	Nyakaana (1998)
4	LafMS04	$(TG)_{14}$	MS04F	GGG-ACA-CAT-GTG-TGC-ATA-A	19	56	3 E	138-142	Siripunkaw (2003)
			MS04R	ATG-TCT-GCA-TAG-ACA-GGT-TGG	22	70		155	Nyakaana r (1998)
5	FS48	$(CA)_{22}$	FH48F	GAG-TCT-CCA-TAA-TCA-AGA-GCG	21	62	3 E	156-168	Siripunkaw (2003)
			FH48R	CCT-CCC-TGG-AAT-CTG-TAC-AG	20	62	4 E	178	Comstock (2000)
							6 L		
6	FS60	$(CA)_{13}$	FH60F	CAA-GAA-GCT-TTG-GGA-TTG-GG	20	60	5 E	144-156	Siripunkaw (2003)
			FH60R	CCT-GCA-GCT-CAG-AAC-ACC-TG	20	64	6 E	148	Comstock (2000)
							5 L		
7[a]	FH94	$(CA)_{16}$	FH94F	TTC-CTC-CCA-CAG-AGC-AGC	18	58	6 E	213-225	Siripunkaw (2003)
			FH94R	ATT-GGT-TAA-TTT-GCC-AGT-CCC	21	90	6 E	229	Comstock (2000)
							4 L		
8[a]	FH102	$(CT)_{11}(CA)_{14}$	FH102F	TTC-ATT-ACT-GAC-CTA-AAC-GAG	22	58	9 E	179-217	Siripunkaw (2003)
			FH102R	GGA-CAG-GGC-TGG-AGA-AAT-ATG	21	64	6 E	179	Comstock (2000)
							3 L		

Table 1 Microsatellite markers are reported in the previous studies (continued).

No.	Locus	Repeat motif	Primer	Sequenc of 5' to 3'	length[b]	Tm[c]	alleles	Size range	Reference
9	EMX-1	$(GTT)_{14}$	EMX-1F	AGG-ACT-TAT-TTG-CTT-AGA-TGG	22	64	4 E	137-152	Fernando (2001)
			EMX-1R	AGG-CAA-TGT-TTC-GTT-CTG-T	20				
10	EMX-2	$(GTT)_5$	EMX-2F	CCC-ATG-AGT-CGG-AAT-CCA-CTT	22	70	2 E	217-223	Fernando (2001)
			EMX-2R	CCA-TAG-GGT-TGC-CAA-GGA-ATG	22				
11	EMX-3	$(GGAA)_3...(GAA)_5...(GGAA)_4$	EMX-3F	CAT-GGT-TAA-CTC-ATT-GCT-TGC	22	64	2 E	238-254	Fernando (2001)
			EMX-3R	GTG-TTC-CCT-CCC-TCT-CAT-CAT	22				
12	EMX-4	$(GGAA)_3A(GA)_3A(GGAA)_3$	EMX-4F	AGT-TCG-TGT-CTC-GGT-GCT-GTA	22	61	3 E	351-387	Fernando (2001)
			EMX-4R	GTA-TGC-TGA-TGG-AAA-TGT-CTA	22				
13	EMX-5	$(GGAA)_3...(GGAAGGGA)_4....$	EMX-5F	AAA-TAG-GAA-AAG-TCT-GAG-GTT	22	59	3 E	248-263	Fernando (2001)
		$..(GGAA)_3...(AGGG)_3$	EMX-5R	CCC-CTG-GAT-TTT-CTT-CAC-CTG	22				
14	LafMS05	$(AC)_{11}$	MS05F	CCT-TAG-GCT-GGG-TTG-TAT	18	49	3 E	160	Nyakaana (1998)
			MS05R	AAT-GGA-CTT-GGG-ACT-TGC-CAA-AAT-GT	26				
15	LA1	$(CA)_{10}(TA)_5$	LA1F	TGG-GTT-GTT-CCA-CCC-TCT-AC	20	53	not amplified	non data	Eggert (2000)
			LA1R	GTA-ACC-GGG-CAA-GTG-TGT-G	19		6 L	139-149	
16	LA2	$((CA)_6(CGTA))_2(CA)_6$	LA2F	CTT-GGT-GGG-AGT-CAT-GAC-CT	20	58	4 E	226-234	Eggert (2000)
			LA2R	GGA-GAA-ATG-ACT-GCC-CGA-TA	20		3 L		
17	LA3	$(CA)_{10}$	LA3F	TAC-TCT-GCT-CCT-CTG-CCT-ATC-C	22	55	3 E	166-172	Eggert (2000)
			LA3R	GCA-GAA-TTT-TGG-TCT-TGG-AGG	21		3 L		
18	LA4	$(CA)_{12}(CGTA)_4(CA)_7$	LA4F	GCT-ACA-GAG-GAC-ATT-ACC-CAG-C	22	54	4 E	111-117	Eggert (2000)
			LA4R	TTT-CCT-CAG-GGA-TTG-GGA-G	19		11 L		

Table 1 Microsatellite markers are reported in the previous studies (continued).

No.	Locus	Repeat motif	Primer	Sequenc of 5' to 3'	length[b]	Tm[c]	alleles	Size range	Reference
19	LA5	$(CA)_{13}$	LA5F	GGG-CAG-CCT-CCT-TGT-TTT	18	52	2 E	142-144	Eggert (2000)
			LA5R	CTG-CTT-CTT-TCA-TGC-CAA-TG	20		7 L		
20	LA6	$(CA)_{13}$	LA6F	AAA-ATT-GAC-CCA-ACG-GCT-C	19	57	3 E	155-159	Eggert (2000)
			LA6R	TCA-CGT-AAC-CAC-TGC-GCT-AC	20		7 L		
21	LaT05	$(CCAT)_2CCAC(CCAT)_{14}CAT$	LaT05F	CAC-CAC-CCA-TCC-ATC-TGT	18	56	12 L	255-307	Archie (2003)
		$(CCAT)_{17}$	LaT05R	TGG-CTT-CTG-TGA-GTT-CAC-C	19				
22	LaT06	$(CCAT)_{13}$	LaT06F	AGC-CAG-GCA-CAT-TAA-GTG-T	19	52	6 L	281-366	Archie (2003)
			LaT06R	TCT-CCT-AGA-AAA-GGT-TAC-CAC-A	22				
23	LaT07	$(ATCT)_{19}$ … $(ATCT)_{16}AT$	LaT07F	CCT-GAG-CCA-TTT-TCT-TGA-G	19	56	15 L	340-398	Archie (2003)
		$(CATC)_{15}CATT(CATC)_4$	LaT07R	GAT-GGA-GAG-ACA-GAT-TTG-CTA-G	22				
24	LaT08	$(TAGA)_{16}$	LaT08F	ATG-GAC-AGG-CAG-AAA-GAT-TT	20	56	10 L	166-234	Archie (2003)
			LaT08R	TCC-CAA-TAA-CAG-GAT-AGC-ATT	21				
25	LaT13	$(CATC)_{21}$	LaT13F	TGA-GCT-TCT-GTA-GGC-TCT-GA	20	56	8 L	234-262	Archie (2003)
			LaT13R	GCA-CTC-GAT-AAA-CAG-TGT-TGA	21				
26	LaT16	$(GGAT)_3GGCG(GGAT)_{18}$	LaT16F	TGG-ATG-AAT-GGC-AAA-TGG	18	52	8 L	295-327	Archie (2003)
			LaT16R	GCA-CAA-CAC-CTG-CCT-GTC-A	19				
27	LaT17	$(GGAT)_{15}$ … $(GGAT)_{10}$	LaT17F	TTC-ACT-GAG-ACC-TAT-GCA-GGG	21	56	8 L	323-355	Archie (2003)
			LaT17R	AAA-ATA-CCA-GCC-TGA-GTG-TGC	21				
28	LaT18	$(CCAT)_{22}$	LaT18F	AAT-CCA-AGA-TTG-GGC-AAC-AC	20	56	8 L	286-318	Archie (2003)
			LaT18R	GCT-CAG-ATA-ACA-AAA-TGA-ATG-G	22				

Table 1 Microsatellite markers are reported in the previous studies (continued).

No.	Locus	Repeat motif	Primer	Sequenc of 5' to 3'	length[b]	Tm[c]	alleles	Size range	Reference
29	LaT24	$(GGAT)_{22}$	LaT24F	AAG-TTG-AGA-GAT-CAG-CAA-AGC-A	22	56	6 L	211-231	Archie (2003)
			LaT24R	GAT-GTT-CAG-TCC-TTC-CTT-AGC-A	22				
30	LaT25	$(CCAT)_{15}$	LaT25F	TGA-GAC-CGT-CTT-CAT-GAG-ATG	21	52	6 L	298-318	Archie (2003)
			LaT25R	ATG-CAA-GCT-TAC-AAT-GGC-AG	20				
31	LaT26	$(GGAT)_3G(GGAT)_5(GGGAT)_7$	LaT26F	AAC-CCA-GGC-TAA-AGC-ACC-AA	20	52	11 L	352-392	Archie (2003)
		$GGAT(GGGAT)_3CGAT(GGGAT)_4$	LaT26R	TTT-CCT-GCT-TGA-GAG-CCA-AA	20				
32	FH1	$(CA)_{12}$	FH1F	GAT-CAG-ACC-ATG-GCA-TGA-G	19	55	1 E	81	Comstock (2000)
			FH1R	ACA-GTC-TCC-CTT-GGG-AAG-AC	20		3 L		
33	FH65	$(CA)_{19}$	FH65F	GGC-TGT-AGC-ATT-TTA-CAC-TCC-C	22	60	3 E	241	Comstock (2000)
			FH65R	CAT-GAA-TAA-ACC-CAG-CCT-CTG	21		4 L		
34	FH71	$(CA)_{14}$	FH71F	GGG-ATT-GGC-TAA-AAT-AG	17	58	6 E	69	Comstock (2000)
			FH71R	CTA-AGC-ACA-TCA-GGG-AC	17		4 L		
35	FH40	$(CA)_{17}$	FH40F	GGC-TTT-CTA-GCC-ACC-TCC-TTC	21	60	2 E	243	Comstock (2000)
			FH40R	GCT-CAC-ATT-CAC-TTG-CTG-ACC	21		5 L		
36	FH39	$(CA)_{18}$	FH39F	GTA-TTC-CTG-GGC-ATT-CCA-TG	20	60	1 E	242	Comstock (2000)
			FH39R	CTT-GGA-ATA-TGA-CCC-TGT-TTG	21		7 L		
37	FH103	$(CA)_{13}$	FH103F	TGT-GCT-GCC-ACT-TCC-TAC-AC	20	58	3 E	154	Comstock (2000)
			FH103R	GAT-GTT-GAG-ACA-GTT-CTG-TAA-G	22		5 L		
38	FH67	$(CA)_{15}$	FH67F	GCT-TCT-CTA-GAA-ATG-TGT-ATG-C	22	58	2 E	97	Comstock (2000)
			FH67R	GGC-GTA-TAG-GAT-AGT-TCC-AC	20		6 L		

Table 1 Microsatellite markers are reported in the previous studies (continued).

No.	Locus	Repeat motif	Primer	Sequenc of 5' to 3'	length[b]	Tm[c]	alleles	Size range	Reference
39	FH19	$(CA)_{15}$	FH19F	GAA-GCT-CAT-GGT-CAA-GGT-CAC	21	60	1 E	185	Comstock (2000)
			FH19R	CTG-CAT-ACT-CAT-CGA-AGT-CAC-C	22		7 L		

Remark : Tm (^{o}c) = [(A+T) x 2] + [(G+C) x 4]

E ; *Elephas*

L ; *Loxodonta*

a ; the locus with high polymorphism that used in this study

b ; length of primer (base)

c ; unit (^{o}c)

Mitochondrial DNA (mtDNA)

The mtDNA is a circular DNA, has a high mutation rate and inherited maternally (Figure 4-5), especially in displacement loop (D-loop) and cytochrome *b* (Phupat, 1994, Srikummool, 1998, Noro *et al*, 1998 and Lertwatcharasarakul *et al*, 2003). The D-loop, cytochrome *b* and 12S rRNA locus (partial or complete) were widely used for genetic analysis in many animal. It can be used to particularly trace the female line (Frankham *et al*, 2002). The DNA is located in the interior of the mitochondrion, the region called matrix, and encoding protein for proteins essential for mitochondial function. The mtDNA is inherited exclusively in a uniparental fashion through the female parent (ovum). Because of the offspring is derived the mitochondia which located in ovum cytoplasm only, not the sperm. However, some living organism has a biparental inheritance, e.g. a mating with fusion of haploid yeast cells. In mammals, some time the small part of mtDNA is inherited from the male parent, the sperm contributes little cytoplasm to the zygote, and virtually all the mitochondria in the embryo are derived from those. In Thailand, Srikummool (1998) used mtDNA D-loop and Lertwatcharasarakul *et al* (2003) used the cytochrome *b* for analyzing Asian elephant haplotypes. The regions is selected to use in this study for confirm an elephant mother and calf with DNA sequencing.

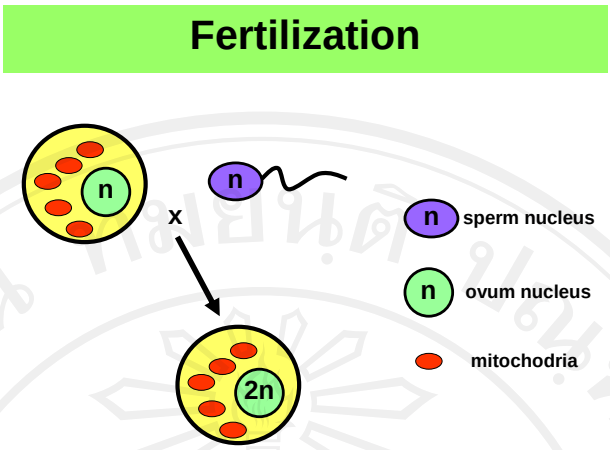

Figure 4 In the fertilization, egg cell is fertilized by a sperm. The zygote nucleus is formed the diploid chromosome from haploid chromosome of egg and sperm, and derived mitochondria from ovum cytoplasm.

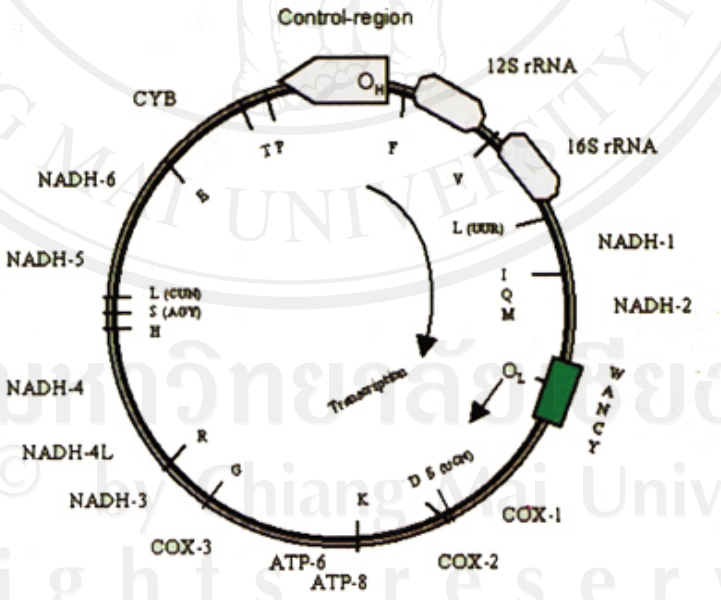

Figure 5 The mitochondrial DNA, a circular double helix made up of 15,000-20,000 nucleotide. (Randi *el al*, 2002)

Parentage/Kinship relation

In animal genetic management, the parentage test plays an important role on investigating the accuracy of pedigree, inbreeding and effective population size (Frankham *et al*, 2002). The parentage test is often used in endanger species with small population size for breeding management and conservation. For the examples, the studied animals were chimpanzee, (Ely *et al,* 1998), harbor seal (Coltman *et al,* 1999), loggerhead turtle (Moore, 2000), langur (Launhardt *et al,* 2001), Humpback whale (Nielsen *et al,* 2001), dollar sunfish (Mackiewicz *et al,* 2002), savannah baboon (Buchan *et al,* 2003), and snow geese (Frankham *et al*, 2002). In elephants, the parentage test was reported in African elephant at Addo elephant national park in South Africa by Whitehouse and Herley (2001) and African elephant population by Hollister-Smith *et al* (2004).

The parentage test is common used DNA fingerprint or fragment analysis with various method, such as Nuclear and Mitochondrial RFLPs (restriction fragment length polymorphisms), multi-locus minisatellite or DNA fingerprints, single-locus minisatellite or VNTRs (variable number tandem repeats) and DNA Sequencing (Phupat, 1994 and Randi *el al*, 2002 and Frankham *et al*, 2002). All method is accepted from CITES, especially genotyping and electrophorogram.

The parentage test and kinship relation can check and confirm with an observed peaks in eletrophograms. The elephant calf should have their genome similar to their father and/or mother with microsatellite data. About the statistic analysis, normally there use a discriminating power (PD) and the power of exclusion (PE) for evaluate an efficiency of loci that used in a parentage test (Phupat, 1994).

The locus is strongly for used to discriminate the individual animal from another when PD is nearly to 1 or 100 percentage. Similar to PD, a PE can indicate the high probability of the elephant bull or cow is a father or mother of elephant calf. The mtDNA is helpful for indicating mother and calf (Phupat, 1994, Rojanasunan, 2000 and Randi *el al*, 2002), but not father and calf, because of the relation between mother and calf is normally found in elephant herds or camps. Multiloci microsatellites can be supplemented for more genetic information. In this study, individual and pedigree identification of domesticated Asian elephants in Thailand, using both genetic markers, is performed.