CHAPTER IV

RESULTS

1. DNA Extraction

From total samples of 22 (15 blood samples and 7 hair follicle samples), all samples were successfully extracted. . DNA samples analyzed by spectrophotometer showed that the DNA extracting from blood had quite better quality than DNA extracting from hair follicle. According to a most extracted DNA samples from blood were lied between 1.80-2.00 ratios of $OD_{260/280}$ ratio, the ratio range of the best DNA quality, and all extracted DNA from hair follicles were upper to 2.00 ratio. The causes are might be from protein contamination from blood cells for ratio that lower to 1.80 ratio samples, and RNA is excess in an over 2.00 ratio samples. However DNA concentrations were similar. The amount of DNA extracting from blood range between 10.7-206.3 ng/µl, and from hair follicle range between 21.7-107.3 ng/µl. All data are shown in Appendix E.

2. DNA Amplification

One hundred percent of genomic DNA isolated from blood and hair follicle samples (n = 15 and 7 respectively) was successfully amplified at FH94, FH102 and LafMS03 microsatellite loci, as well as the partial D-loop and cytochrome *b* of mtDNA. The amplified products, analyzed by agarose gel electrophoresis, were shown in (Figure 6-8)

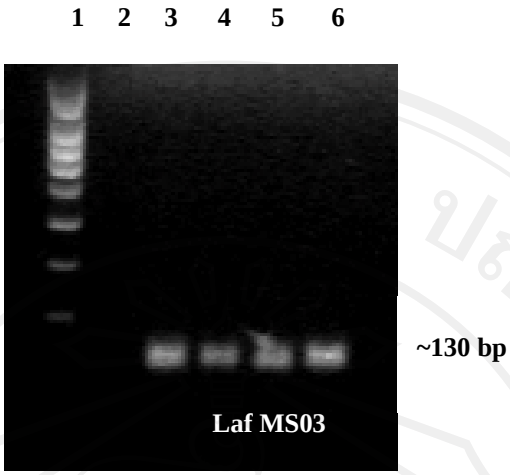


Figure 6 Agarose gel electrophoresis of genomic DNA from buffy coat samples amplified with micosatellite marker named LafMS03.: Lane 3-6; elephant DNA. Lane 1; 100 base-pair ladder size standard**,** and Lane 2; negative control.

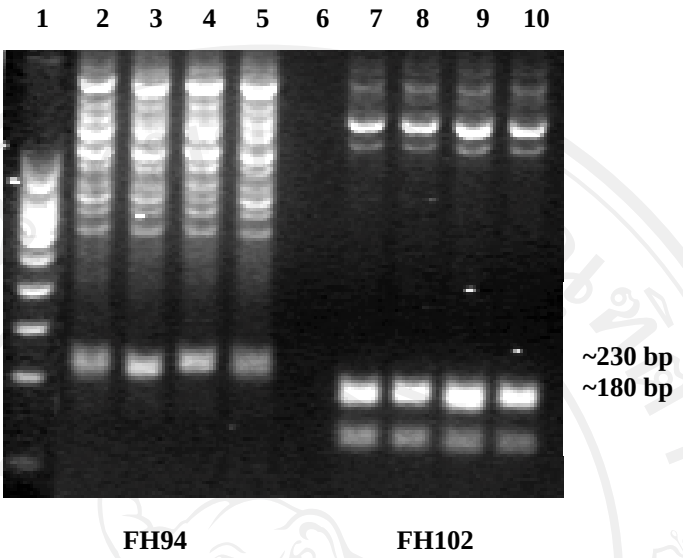


Figure 7 Agarose gel electrophoresis of amplified genomic DNA with micosatellite marker named FH94 and FH102.: Lane 1; 100 base-pair ladder size standard**,** Lanes 2-5 and 7-10; genomic DNA from buffy coat samples, and Lane 6; negative control.

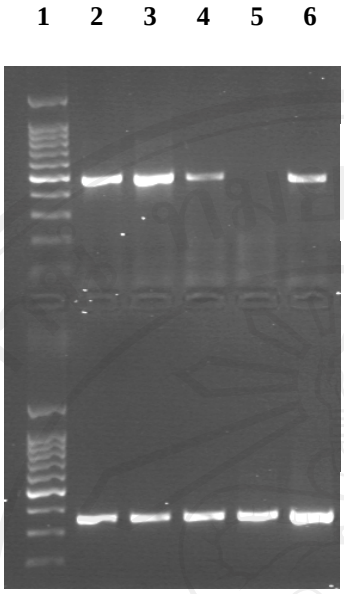
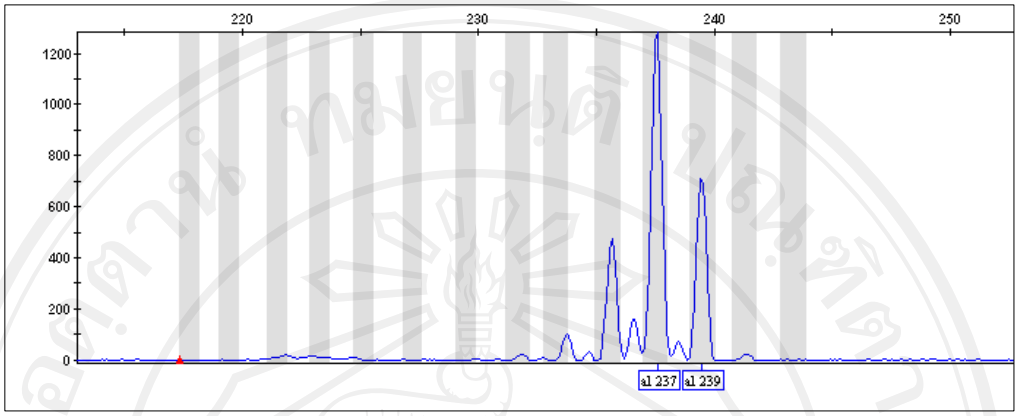


Figure 8 Agarose gel electrophoresis of mtDNA amplified with ELE and Mito-CB primer.: Lane 1; 100 base-pair ladder size standard**,** Lanes 2-5; template from buffy coat, and Lanes 6; template from hair follicle.

3. Fragment Size Determination

PCR products sizing or genotyping of the three microsatellite loci was obtained by using the automated ABI 3130XL DNA Analyzer (Applied Biosystem at the Ultrecht University, the Netherlands). The fragment sizes at three loci are illustrated in Figure 9-11. It should be noticed that in the electrophorograms there are smaller peaks located prior to the higher peaks or the true alleles determining by Genescan 4.0 software (Applied Biosystem). There are also the stray bands or non-specific amplification products, which might cause difficulty in the genotyping process. The discussion about this problem is in the next chapter.

(a)



(b)

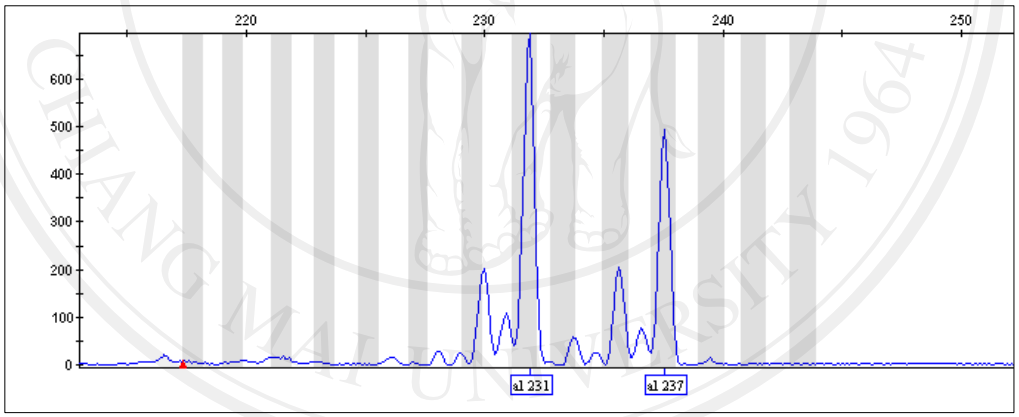
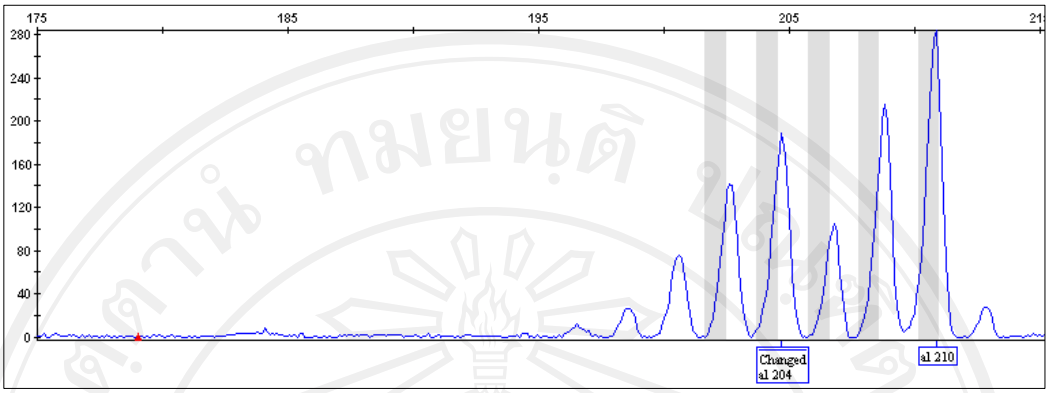


Figure 9 Fragment sizes of PCR products at FH94 locus are (a) 237/239 (LP1-C-PP) and (b) 231/237 (LP1-C-SK).

(a)



(b)

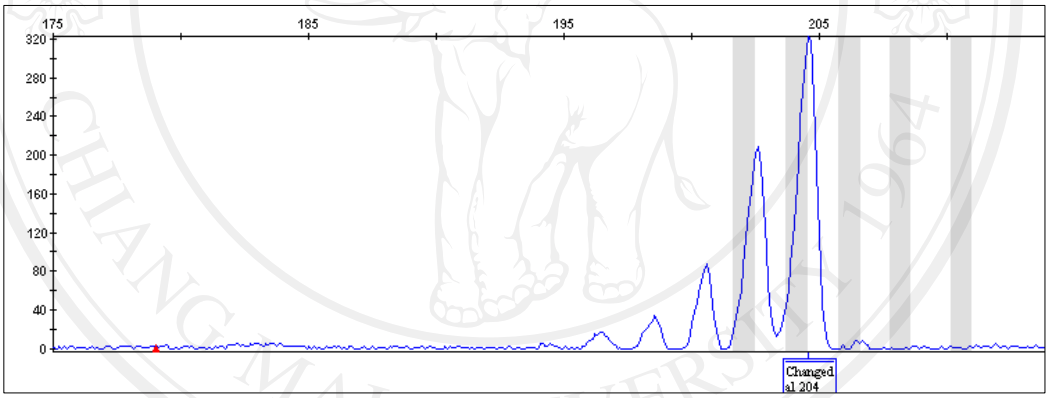
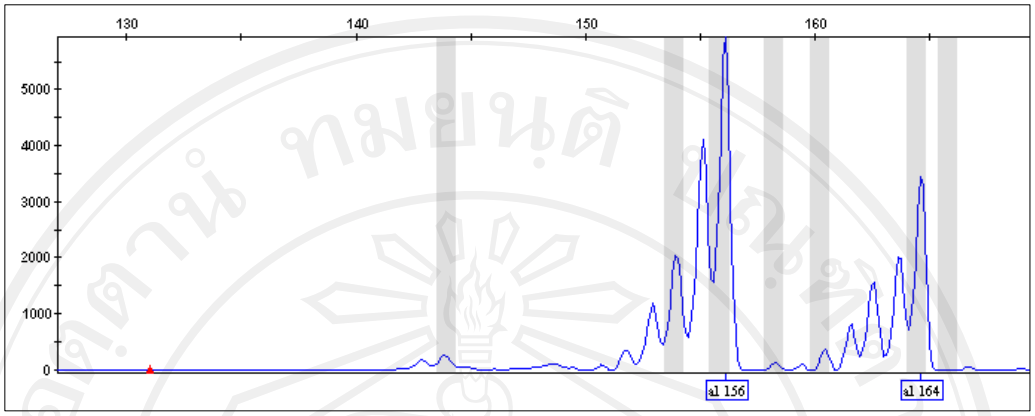


Figure 10 Fragment sizes of PCR products at FH102 locus are (a) 204/210 (CM1-Cc-WP2) and (b) 204/204 (LP1-Cc-AN).

(a)



(b)

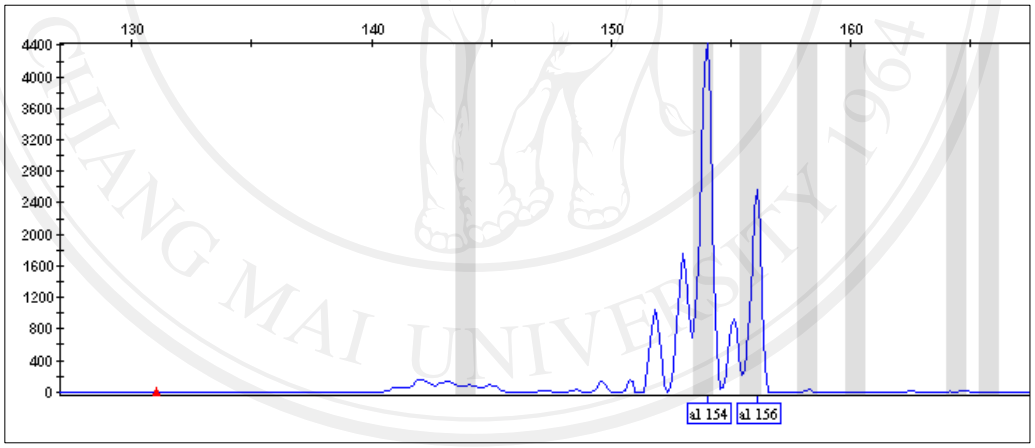


Figure 11 Fragment sizes of PCR products at LafMS03 locus are (a) 156/164 (CM1-C-NO2) and (b) 154/156 (CM1-C-ND)**.**

4. Genotyping and Parentage test

Genotypes of each elephant were determined and summarized in Table 4. The size range between 230-238 bp (4 alleles) at LafMS03, 197-223 bp (7 alleles) at FH94 and 154-172 bp (7 alleles) at FH102. Each locus showed variation in copy number. However the genotype of two elephants did not match with the parents' genotype. It's possible that the cause is the genotyping error, which is discussed in the next chapter.

The parentage test of five elephant families were performed by comparing the genotype between each elephant in the same family. Each family showed biparental inheritance, i.e., the calf inherited one allele from the father and another allele from the mother. The pedigrees of all families are described in Figure 12-16.

Table 4 Microsatellite genotyping in 5 elephant families.

Families	Codes	FH94		FH102		LafMS03		Remark
		A1	A2	A1	A2	A1	A2	
TECC-01	LP1-B-TD	230	236	203	207	153	163	
	LP1-C-SK	230	236	203	207	157	163	
	LP1-Cc-NU	230	236	207	207	153	163	
TECC-02	LP1-B-PM	236	238	205	207	155	165	
	LP1-C-PP	236	238	203	207	153	163	
	LP1-Cc-AN	236	238	203*	203*	163	165	
MSEC-01	CM1-B-BP	230	236	207	209	153	165	
	CM1-C-ND	234	236	201	203	153	155	
	CM1-Cc-DP	234	236	203	207	155	165	
MSEC-02	CM1-B-KH	234	236	207	207	153	169	
	CM1-C-NO2**	236	238	205	209	155	163	
	CM1-Cc-CT	236	238	205	207	153	155	
MSEC-03	CM1-B-YA	230	234	203	223	155	163	
	CM1-C-NO2**	236	238	205	209	155	163	mother 1
	CM1-Cc-WP2	234	238	203	209	163	163	
	CM1-C-MH	230	236	201	209	153	155	mother 2
	CM1-Cb-LC	230	230	201	203	153	155	
	CM1-Cb-TP	230	236	201	203	153	163	
	CM1-C-SY	236	236	197	197	153	155	mother 3
	CM1-Cb-SP	234	236	223*	223*	155	155	
	CM1-Cb-WP	230	236	197	223	155	163	
	CM1-C-SN	234	236	207	209	155	171	mother 4
	CM1-Cb-TT	234	234	209	223	155	171	

Note: A1 = Allele 1 and A2 = Allele 2, ∗ = calf's allele does not fully match with the parents, ** = the elephant is duplicated

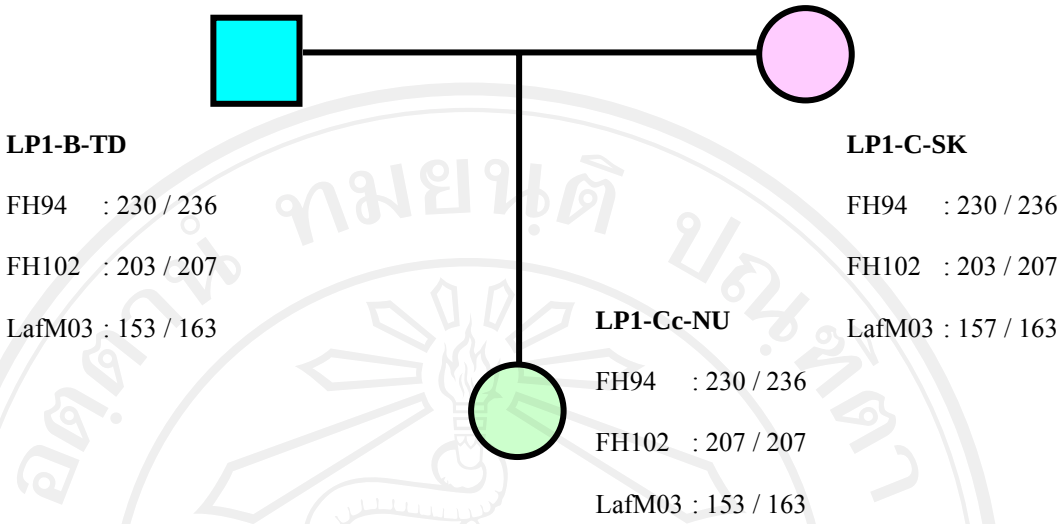


Figure 12 Pedigree of family TECC-01 shows detailed genotypes.

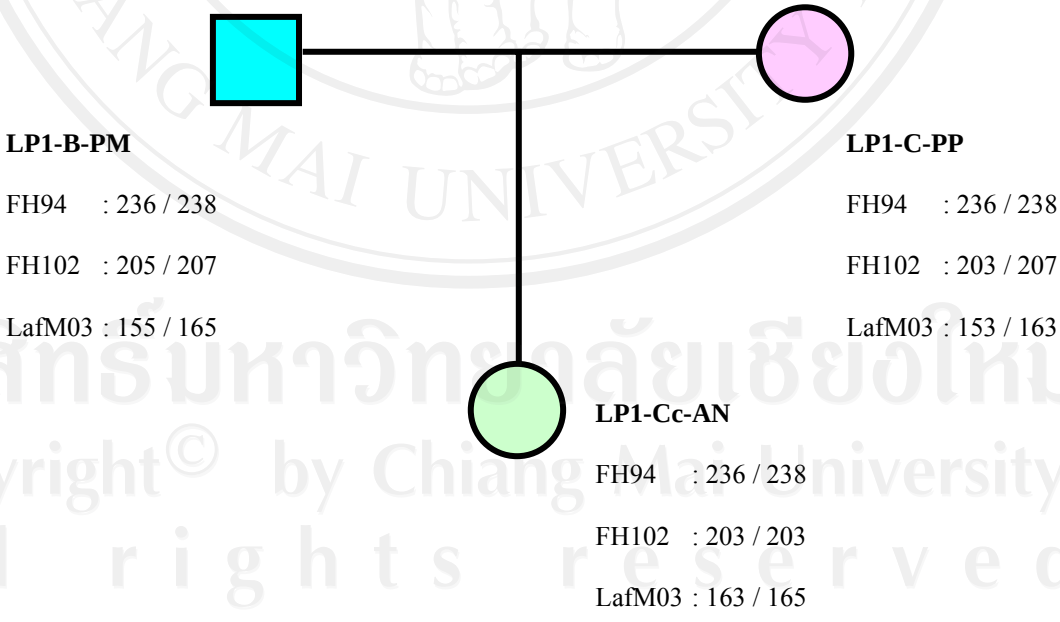


Figure 13 Pedigree of family TECC-02 shows detailed genotypes, * calf's allele does not fully match with the parents.

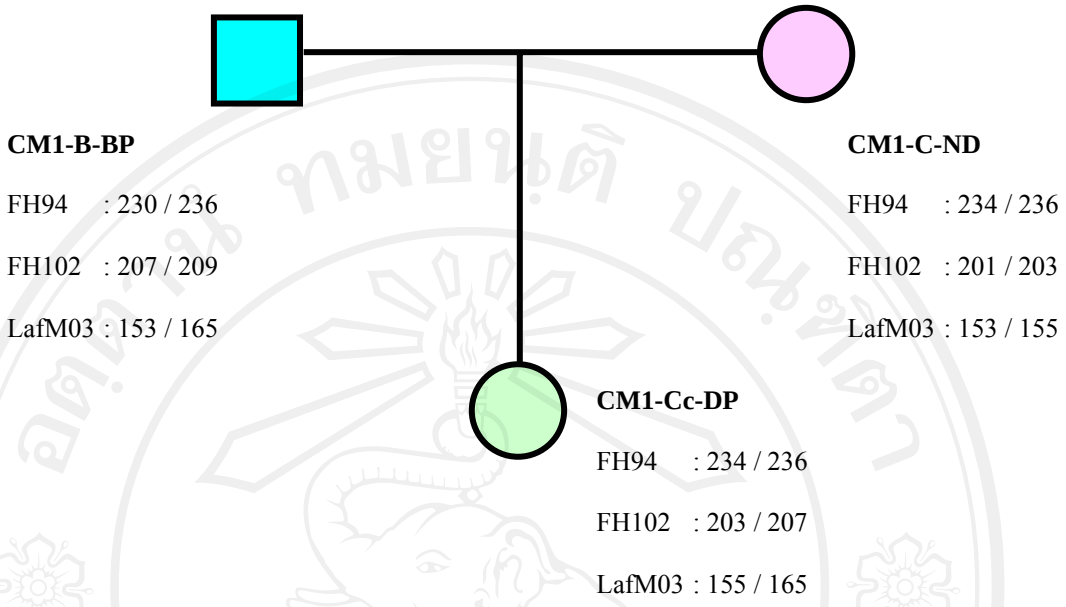


Figure 14 Pedigree of family MSEC-01 shows detailed genotypes.

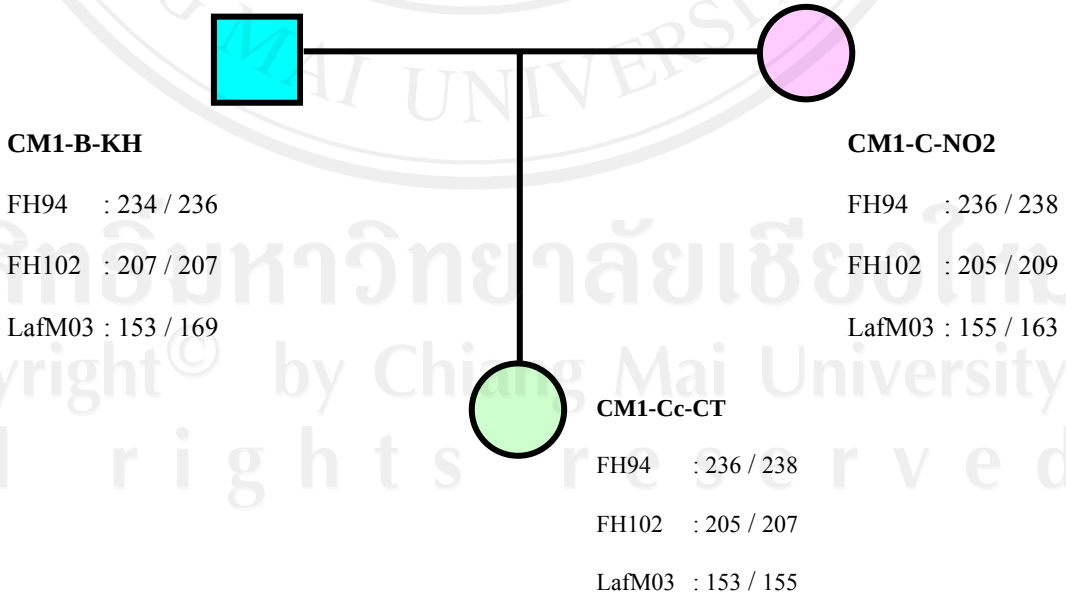


Figure 15 Pedigree of family MSEC-02 shows detailed genotypes.

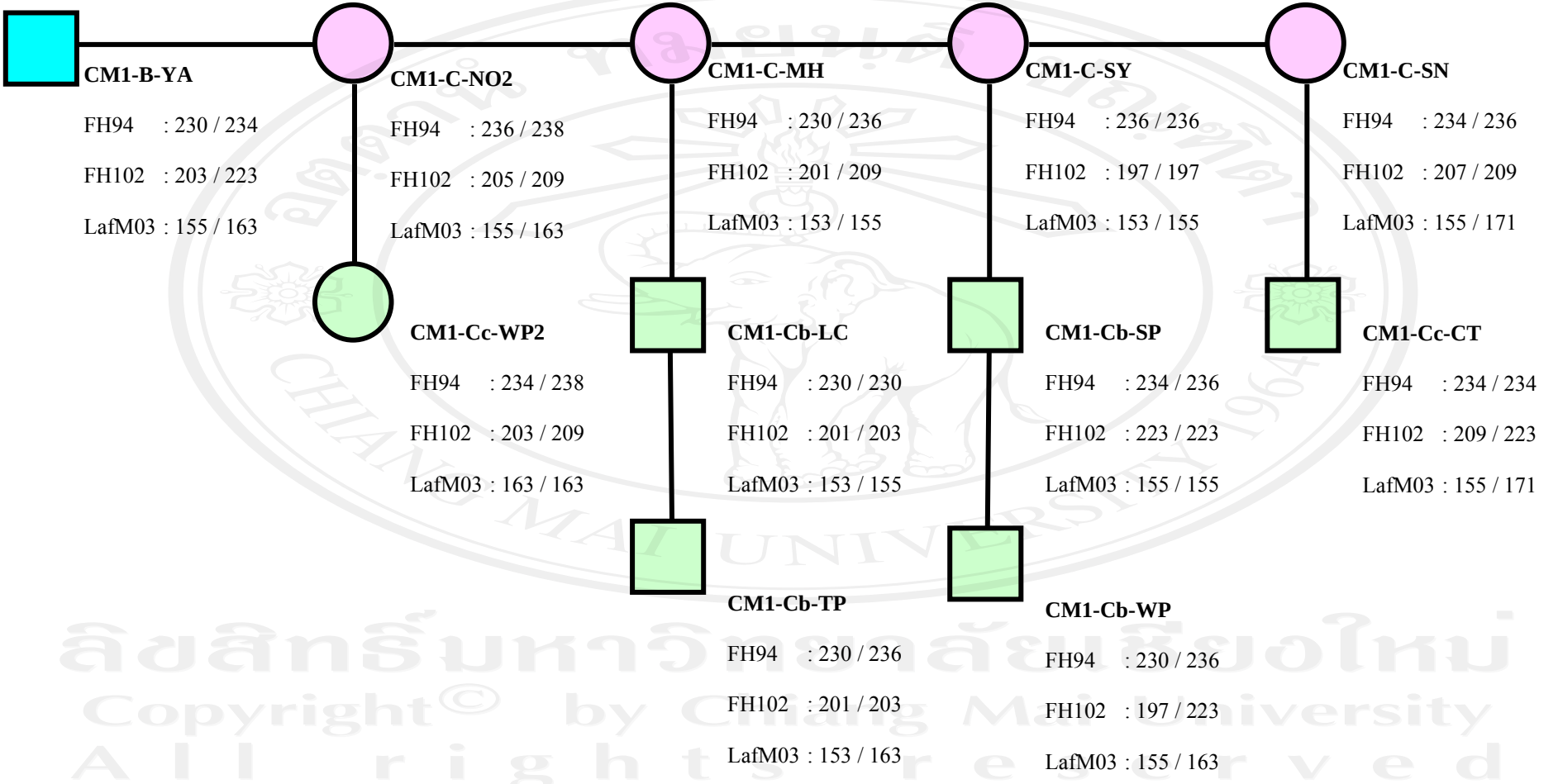


Figure 16 Pedigree of family MSEC-03 shows detailed genotypes, * calf's allele does not fully match with the parents

The frequency distribution of all alleles and genotypes at all loci were summarized in Table 5 and 6, respectively. The power of discrimination (PD) and power of exclusion (PE) were shown in the Table 7.

Table 5 Frequency distribution of all alleles at all loci.

Locus and alleles	Number of alleles	Frequency	Remark
FH94			
230	10	0.2273	
232	-	-	
234	9	0.2045	
236	19	0.4318	
238	6	0.1364	
FH102			
197	3	0.0682	
199	-	-	
201	4	0.0909	
203	11	0.2500	
205	3	0.0682	
207	12	0.0909	
209	6	0.1364	
--	-	--	
223	5	0.1136	
LafMS03			
153	11	0.2500	
155	14	0.3182	
157	1	0.0227	
159	-	-	
161	-	-	
163	11	0.2500	
165	4	0.0909	
167	-	-	
169	1	0.0227	
171	2	0.0455	

Table 6 Frequency distribution of genotype at all loci.

FH94		FH102		LafMS03	
phenotype	P_i	phenotype	P_i	phenotype	P_i
230/230	0.045	197/197	0.045	153/155	0.226
230/234	0.045	197/223	0.045	153/163	0.181
230/236	0.325	201/203	0.141	153/165	0.045
234/234	0.045	201/209	0.045	153/169	0.045
234/236	0.225	203/203	0.045	155/155	0.045
234/238	0.045	203/207	0.181	155/163	0.141
236/236	0.045	203/209	0.045	155/165	0.091
236/238	0.225	203/223	0.045	155/171	0.091
		205/207	0.091	157/163	0.045
		205/209	0.045	163/163	0.045
		207/207	0.091	163/165	0.045
		207/209	0.091		
		209/223	0.045		
		223/223	0.045		

Table 7 The power of discriminating (PD) and average power of exclusion (PE) of loci.

Loci	**FH94**	**FH102**	**LafMS03**
Discriminating power	0.7830	0.9043	0.8675
Combined PD	0.9972		
Power of exclusion	0.2789	0.4604	0.3654
Culmulative PE	0.7531		

5. Mitochondrial DNA sequencing

All mitochondrial DNA sequences of the female elephants and their calves were similar (Appendix F). The observed sequences were 269 bp at the nucleotide position 128-391 of the D-loop (Genbank, Referenced sequence AF132520) and the 377 bp at the nucleotide position 15361-15771 of the 5′ end cytochrome *b* (Genbank, Referenced sequence NC005129, a complete elephant mitochondrial DNA sequence).