CHAPTER V

CONCLUSION

1. In the study of molecular background for silent α-thalassemia, it was concluded that HbCS is one of the genetic factor producing the silent form of α-thalassemia. Therefore, in the area where HbCS is common, determination of Hb Bart's level could be helpful in detection of silent α-thalassemia caused by this defect. Other genetic factors involved in this phenomenon still exist and need further elucidation

2. Multiplex allele-specific PCR for detection double heterozygote of α/β-thalassemia and α-thalassemia/HbE using the Chelex™-extracted DNA target was successfully developed. The optimal condition for PCR system included the use of 5 µl of genomic target DNA (Chelex™ extracted) corresponding to approximately 100 – 200 ng/µl, 140 µM of dNTPs, 0.5 units i-Taq DNA polymerase (Intron), 0.25 ng (0.380 µM) for "Beta-common-multiplex" primer and 0.125 ng (0.018 µM) for the rest of beta primers, 1.0 ng (0.150 µM) of "SEA-1-multiplex" primers, 0.25 ng (0.035 µM) "SEA-2-multiplex" primers and 0.5 ng (0.075 µM) of "SEA-3-multiplex" primers in 10 mM Tris-HCl (pH 8.3), 50 mM HCl, 2.0 % DMSO and 2.0 $MgCl_2$. A total of 37 themal cycles was carried out in thermal cycler with each cycle comprising denaturation at 95^{o}C for 1 min, primer annealing at 62^{o}C for 30 sec and primers extension

at 72^{o}C for 30 sec. Initial denaturation was extended to 5 min and final extension was prolonged to 7 min for PCR. The optimal condition for sample to be used in the direct Chelex™ DNA extraction was a 200-µl volume of whole EDTA blood or 40-µl volume of buffycoat.