III. RESULT

III.1 Genomic DNA Preparation

The 191 DNA samples extracted from cord blood had the yield around 100-500 mg. The OD260/OD280 ratio were 1.51-1.91.

III.2 Polymerase Chain Reaction

The PCR components and PCR condition had been titrated to bring about the best results, which provided a clear single band at the expected size of 276 bp, as shown in the "a"-lanes of Fig. 6, 7, and 8. The nucleic acid molecular weight markers (pUC18/HpaII) was shown in lane "M", which provided the marker sizes of 501, 489, 404, 348, 242, 190, 147, 110, 94, 67, 34, and 26 bp, respectively.

III.3 Restriction Enzyme Mse I Cut

Samples that could be cut with restriction enzymes Mse I generated the DNA fragments of 164 bp and 112 bp as expected, as shown in the "b"-lanes of Fig. 6, 7, and 8.

III.3.1 Results from Cord Blood Samples

Of the 191 cord blood samples, all except 5 were completely cut by restriction enzyme MseI. Therefore, 186 samples possessed the normal termination codon on their α_2-globin gene(s).

Approximately one half of the PCR product from sample no. 6, 163, 172, 187, and 218 was cut with restriction enzyme Mse I. The RFLP patterns of these samples were the same to RFLP pattern of sample no.163 (shown in the "b"-lane of Fig.6). These samples were interpreted as heterozygosity of normal and abnormal termination codon on α_2-globin gene.

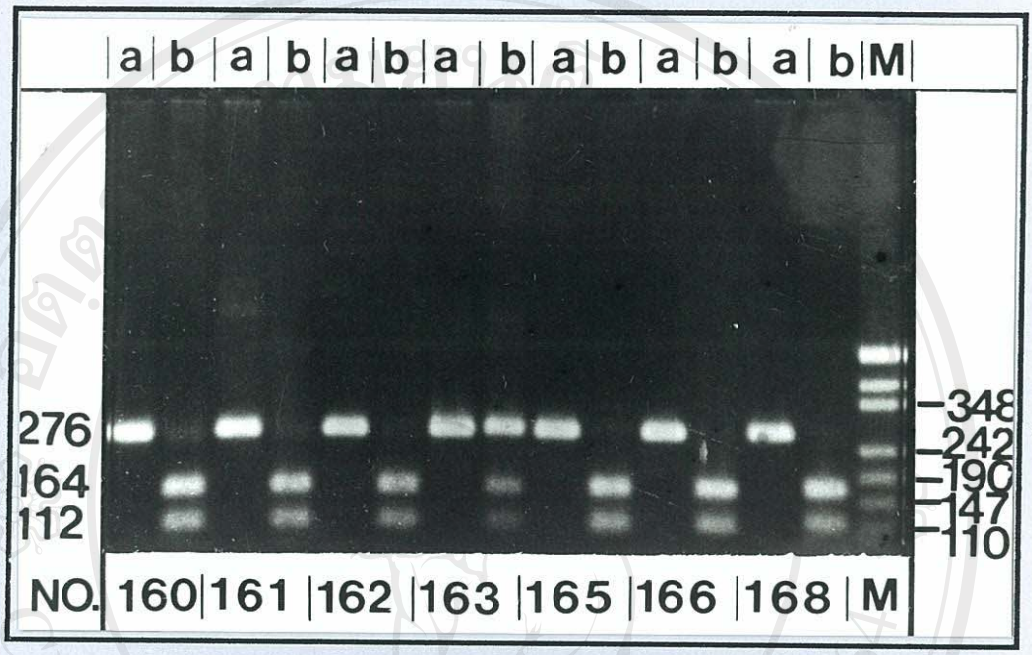

Figure 6 : RFLP Pattern from Cord Blood Samples Cut with Mse I

PCR products before cutting with Mse I are shown in lane "a" and after cutting are shown in lane "b". DNA molecular weight marker (pUC18/Hpa II) is shown in lane "M". The number of each sample is shown at the bottom line. They were run on 2.5% agarose gel in 0.5x TBE buffer.

III.3.2 Results from Hb H Disease Patients

All 18 Hb H disease samples had been characterized by Southern blot hybridization as heterozygosity of α-thalassemia 1 and nondeletional α-thalassemia (Muangmoonchai, 1994).

There were 3 different RFLP patterns provided from these samples, after PCR product had been cut with restriction enzyme Mse I .

1. Sample No. H1-H4, H7, H8 (Fig. 7), H9-H13 (Fig. 8) and H5 (picture not included), the PCR product from these 12 samples were not cut with restriction enzyme Mse I.

2. Sample No. H6 (Fig. 7) was completely cut with restriction enzyme Mse I.

3. Sample No. H14-H18 (Fig. 8), the PCR product from these 5 samples were partially cut with restriction enzyme Mse I.

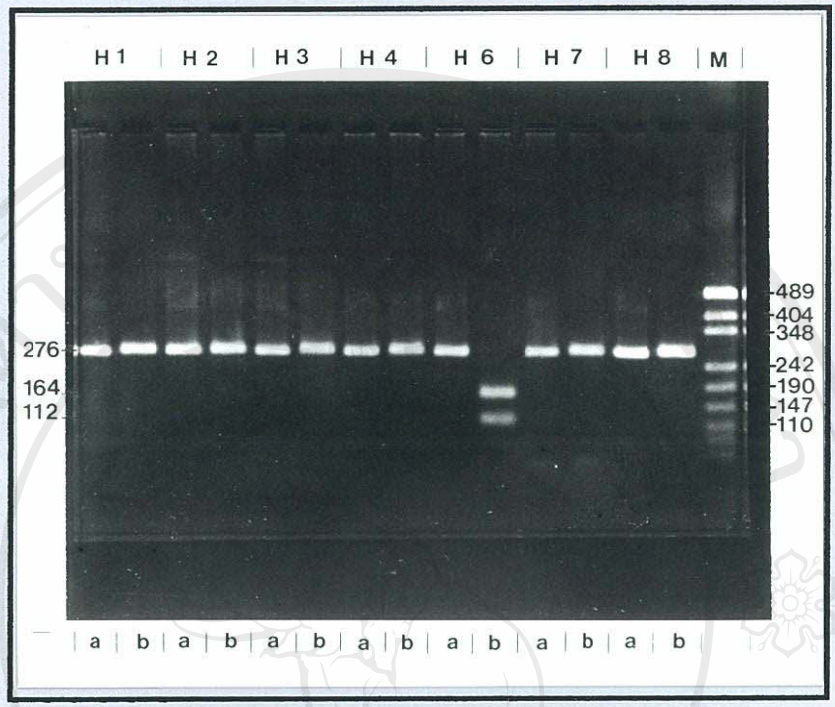

Figure 7 : RFLP Pattern from Hb H Disease Samples Cut with Mse I

PCR products before cutting with Mse I are shown in lane "a" and after cutting are shown in lane "b". DNA molecular weight marker (pUC18/Hpa II) is shown in lane "M". The number of each sample is shown at the above line. They were run on 2.5% agarose gel in 0.5x TBE buffer.

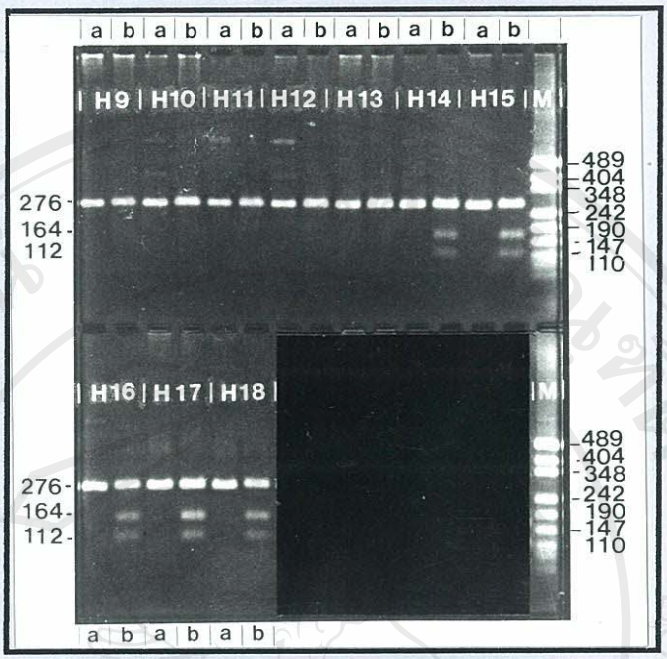

Figure 8 : RFLP Pattern from Hb H Disease Samples Cut with Mse I

PCR products before cutting with Mse I are shown in lane "a" and after cutting are shown in lane "b". DNA molecular weight marker (pUC18/Hpa II) are shown in lane "M". The number of each sample is shown at the above line, H represents Hb H disease patients. There were two sets of samples loaded on the same gel, which can be seen from the slots. They were run on 2.5% agarose gel in 0.5x TBE buffer.

III.4 Semi-Nested PCR and the Cut with Tth 111I

All the samples in which their α_2-specific PCR product were not completely cut with restriction enzyme Mse I were subjected to semi-nested PCR. The objective of semi-nested-PCR was to create restriction site of restriction enzyme Tth 111I for the sample that had the Hb CS gene, in order to characterize whether the abnormal termination codon of these samples was due to Hb CS mutation. The samples in which their semi-nested PCR could be cut with restriction enzyme Tth 111I were considered to possess α^{CS} gene.

Semi-nested PCR product from all the samples tested are shown in the "a"-lanes of Fig. 9, 10, 11 and 12. The size of the product was around 131 bp compared to the DNA molecular marker (ϕX174 DNA/Hae III, lane M), as expected from the model of study. ϕX174 DNA/Hae III generates 11 fragments of 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 bp, respectively.

Approximately one half of semi-nested PCR product from cord blood samples No. 6, 163 (Fig. 9),172, 187 and 218 (Fig. 10) was found to be cut with restriction enzyme Tth 111I (see lane "b"). This confirms previous results that these samples are heterozygous for normal α_2-termination codon and the Hb CS gene.

From 17 Hb H disease samples, 12 samples (H1-H5, and H7-H13) had their semi-nested PCR products almost completely cut with restriction enzyme Tth 111I (in "b"-lanes of Fig. 9, 10, and 11). This confirms previous results that these samples have the Hb CS gene on their only α_2-globin gene.

Another 5 samples from Hb H disease patients (H14-H18) had their semi-nested PCR product partially cut with restriction enzyme Tth 111I (in b-lanes of Fig. 11 and 12). This also confirms with their PCR product cut with Mse I, suggesting that there are two species of DNA templates. One generates the normal stop codon and one generates the Hb CS gene.

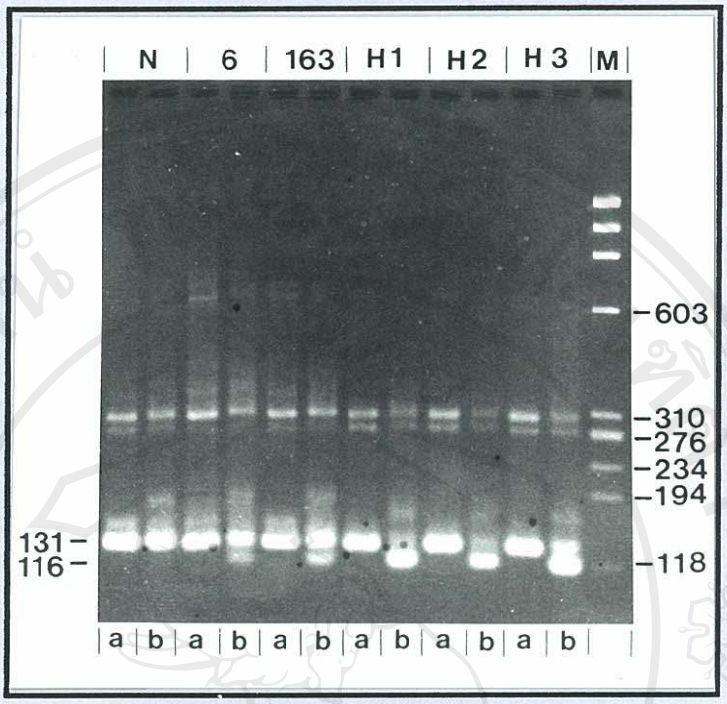

Figure 9 : RFLP Pattern of Semi-Nested PCR Product Cut with Tth 111I

Semi-nested PCR products before cutting with Tth 111I are shown in lane "a" and after cutting are shown in lane "b". DNA molecular weight marker (ϕX174 DNA/Hae III) is shown in lane "M". The number of each sample is shown at the above line, N represents normal, H represents Hb H disease samples, and number alone are the samples from cord blood. They were run on 3.0% agarose gel in 0.5x TBE buffer.

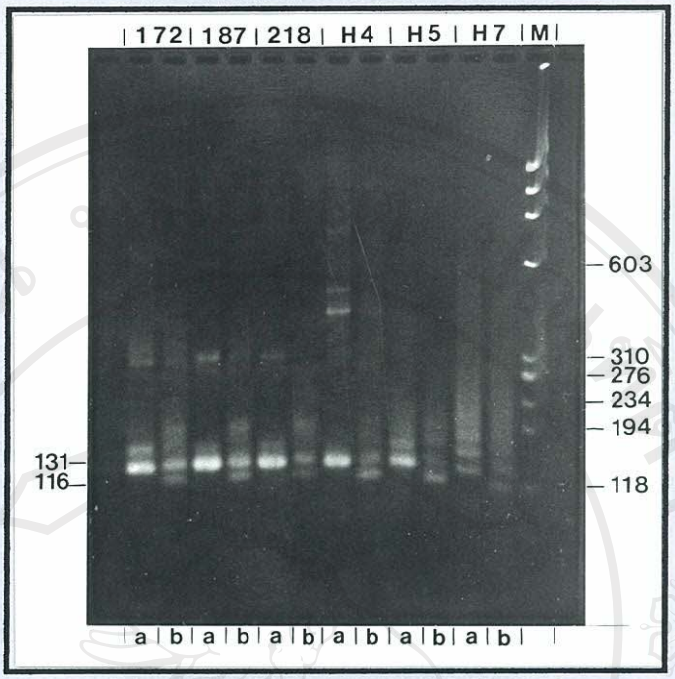

Figure 10 : RFLP Pattern of Semi-Nested PCR Product Cut with Tth 111I

Semi-nested PCR products before cutting with Tth 111I are shown in lane "a" and after cutting are shown in lane "b". DNA molecular weight marker (ϕX174 DNA/Hae III) is shown in lane "M". The number of each sample is shown at the above line, H represents Hb H disease samples, and number alone are the samples from cord blood. They were run on 3.0% agarose gel in 0.5x TBE buffer.

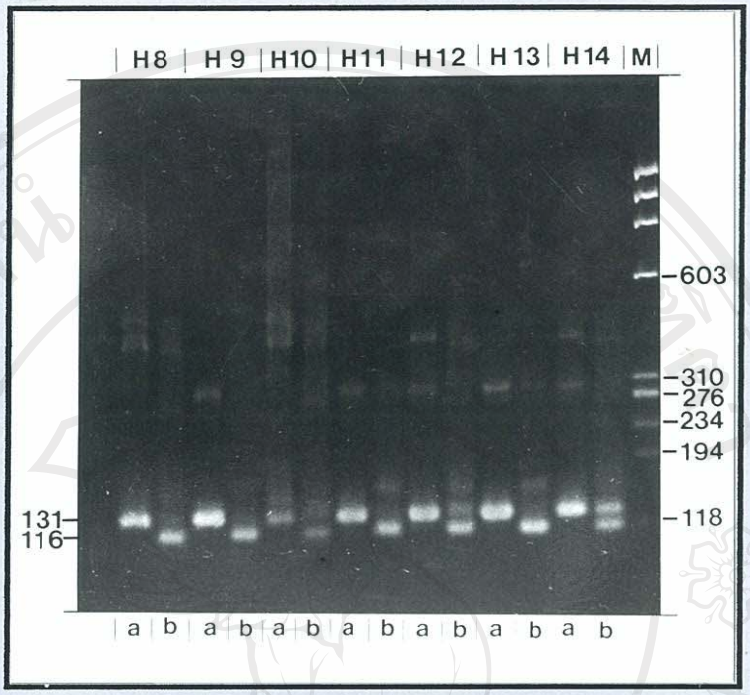

Figure 11 : RFLP Pattern of Semi-Nested PCR Product Cut with Tth 111I

Semi-nested PCR products before cutting with Tth 111I are shown in lane "a" and after cutting are shown in lane "b". DNA molecular weight marker (ϕX174 DNA/Hae III) is shown in lane "M". The number of each sample is shown at the above line, H represents Hb H disease samples. They were run on 3.0% agarose gel in 0.5x TBE buffer.

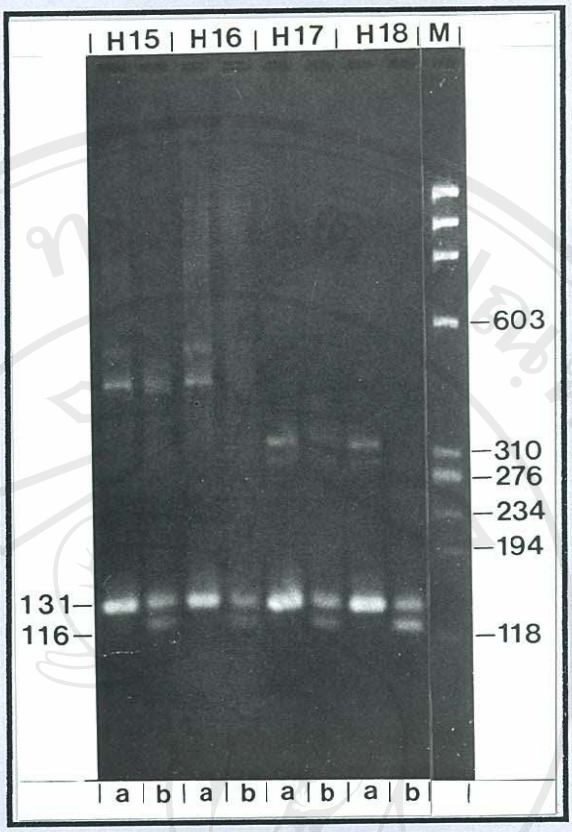

Figure 12 : RFLP Pattern of Semi-Nested PCR Product Cut with Tth 111I

Semi-nested PCR products before cutting with Tth 111I are shown in lane "a" and after cutting are shown in lane "b". DNA molecular weight marker (ϕX174 DNA/Hae III) is shown in lane "M". The number of each sample is shown at the above line, H represents Hb H disease samples. They were run on 3.0% agarose gel in 0.5x TBE buffer.

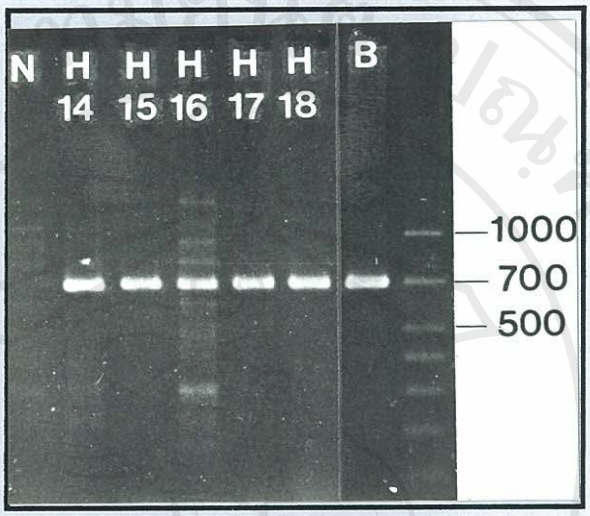

Figure 13 : The Detection of α-Thalassemia 1 (--^{SEA}) by PCR

N represents normal control, H represents Hb H disease patients, and B represent Hb Bart's (--/--). DNA molecular weight marker (Gel Marker^{TM}, Biolabs) on the last lane generated the DNA fragments of 1000, 700, 500, 400, 300, 200, 100, and 50 bp. They were run on 2% agarose gel in 0.5X TBE buffer.