IV. RESULTS

1. Preparation of genomic DNA

Genomic DNA was isolated from venous blood. The yield is approximately 280-950 μg/ml. The OD260/OD280 ratio was between 1.70-1.95 .

2. Restriction endonuclease digestion of genomic DNA

10 μg of genomic DNA was restricted with 40 units of *Eco* R I or *Bgl* II. The restriction pattern was shown in Figure 10 .

3. Preparation of the α2- and ζ-globin specific probes

3.1 The pEMBL α2 was completely restricted with *Pst* I, giving the 1.5 kb of α2-globin specific probe separated from the pEMBL vector as shown in figure 11,lane B.

3.2 The pEMBL ζ was completely restricted with *Pst* I and *Hin* d III, giving the 400 bp of ζ-globin specific probe separated from the 4.1 kb of pEMBL vector as shown in figure 11,lane D.

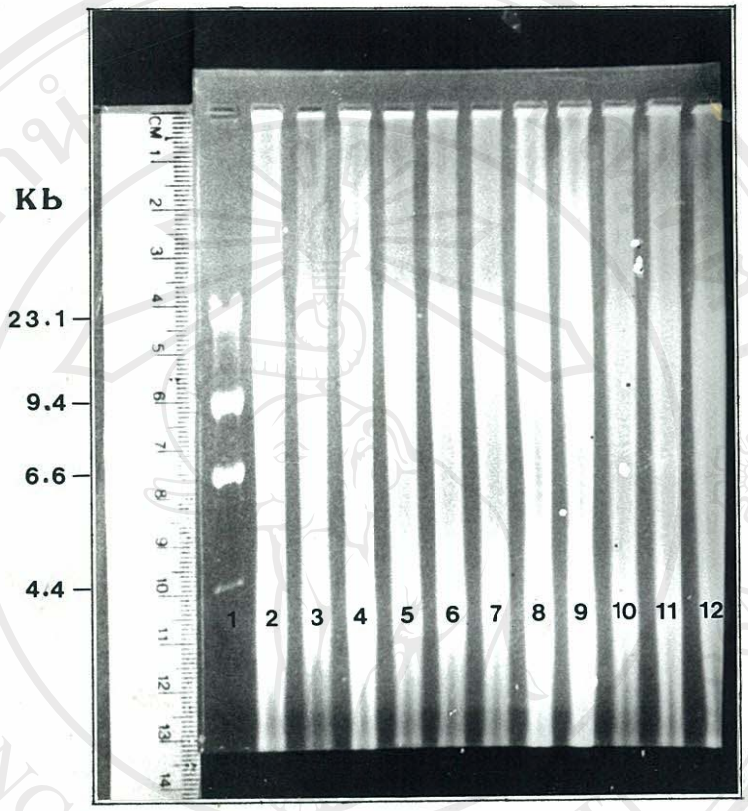

Figure 10. A pattern of genomic DNA digested with restriction endonuclease after running in 0.7 % agarose gel in 0.5xTBE and staining with EtBr solution (0.05 μg/ml)

lane 1 = λ/*Hin* d III (DNA marker)

lane 2 = Normal DNA

lane 3 - 12 = Hb H disease DNA

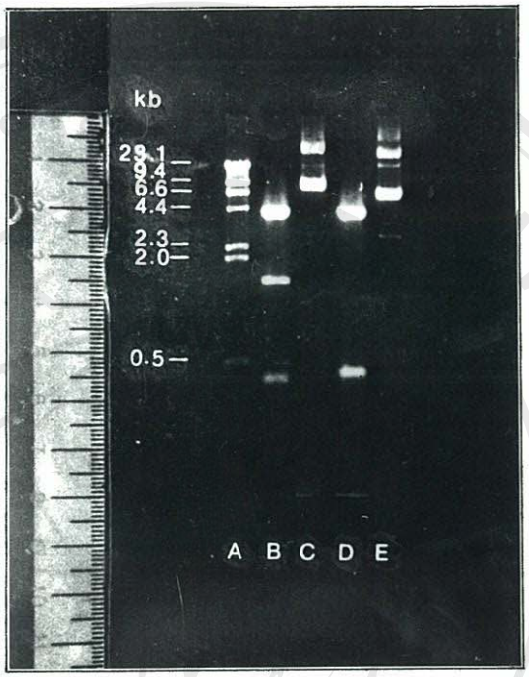

Figure 11. A pattern of the recombinant plasmids digested with restriction endonuclease after running in 0.65% agarose gel in 0.5xTBE and staining with EtBr solution (0.05 μg/ml)

lane A = λ/*Hin* d III (DNA marker)

lane B = 1.5 kb of the α2-globin specific probe was isolated from *Pst* I-digested pEMBL α2

lane D = 400 bp of the ζ-globin specific probe was isolated from the *Pst* I and *Hin* d III-digested pEMBL ζ

lane C,E = Undigested pEMBL α2 and pEMBL ζ, respectively.

4. α Globin genes analysis

4.1 Digestion of normal human genomic DNA by *Eco*R I produces a DNA fragment 23 kb long, carrying both α globin genes, the α globin gene and the ψζ1 globin gene on the same fragment. The ζ2 globin gene is located on a 5-kb *Eco* R I fragment which does not carry the α globin genes (Fig. 8). When *Eco*RI digested DNA fragments are hybridized with ζ-globin specific probe, normal DNA produces two ζ-globin gene carrying fragments, one 23.0 kb and the other 5.0 kb long (Fig. 8). In cases of 28 patients with Hb H disease , DNA from 10 cases had two shortened *Eco* R I ζ-specific fragments of 19.0 and 15.0 kb in addition to 5.0 kb, which is characteristic of a single α gene deletion; -α haplotype and of two α genes deletion; -- haplotype,respectively. The other 18 cases had only one shortened *Eco* R I ζ-specific fragment of 15.0 kb in addition to 23.0 kb and 5.0 kb, which indicated the presence of -- haplotype and a nondeletion mutation; $\alpha^{T}\alpha$ haplotype (Fig. 12-15).

4.2 To characterize haplotypes involving deletion of 3.7 kb and 4.2 kb, DNA was digested with *Bgl* II and hybridized with α-globin specific probe. Normal DNA generated *Bgl* II α-specific fragments of 12.6 kb and 7.4 kb. The $-\alpha^{3.7}$ and $-\alpha^{4.2}$ haplotypes were associated with 16.0 kb and 7.4 kb *Bgl* II α-specific fragments, respectively (Fig. 9, 16-18). Among 10 cases of patients who have --/-α genotype as above, 7 cases carrying a $-\alpha^{3.7}$haplotype and 3 cases carrying a $-\alpha^{4.2}$ haplotype were observed.

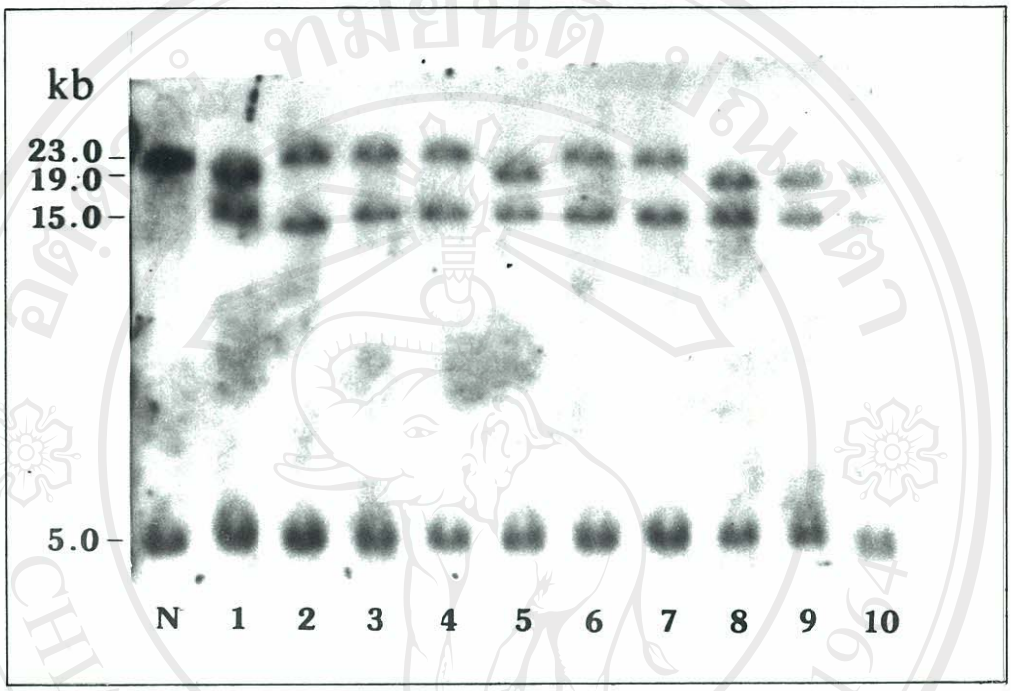

Figure 12. Autoradiogram of genomic DNA digested with *Eco*R I and hybridized with the ζ-globin specific probe.

N = Normal

1 and 8 = --/-$\alpha^{4.2}$

5,9,and 10 = --/-$\alpha^{3.7}$

2,3,4,6,and 7 = --/$\alpha^{T}\alpha$

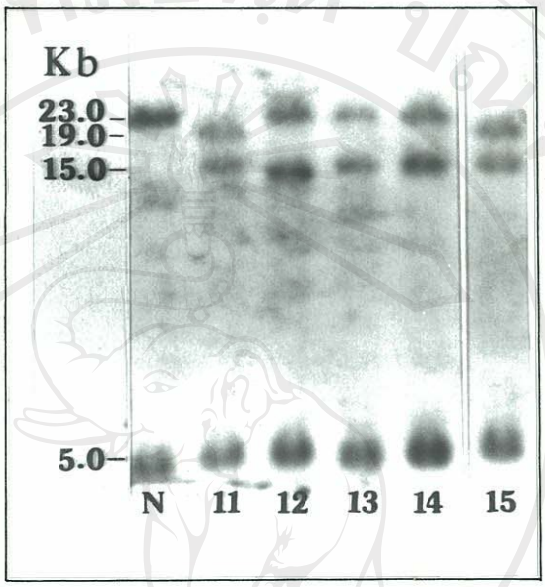

Figure13. Autoradiogram of genomic DNA digested with *Eco* R I and hybridized with the ζ-globin specific probe.

N = Normal

11 = --/-$\alpha^{4.2}$

12,13,and 14 = --/$\alpha^{T}\alpha$

15 = --/-$\alpha^{3.7}$

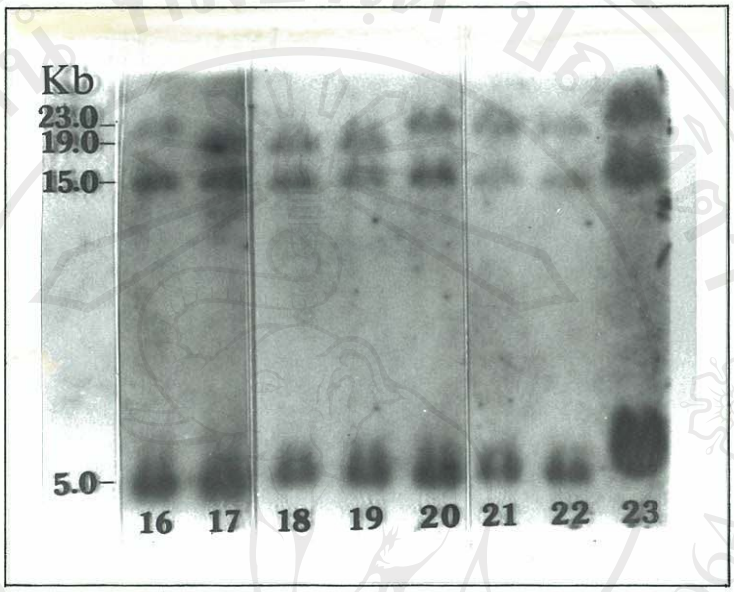

Figure14. Autoradiogram of genomic DNA digested with *Eco* R I and hybridized with the ζ-globin specific probe.

16,20,21,22,and 23 = --/$\alpha^T\alpha$

17,18,and 19 = --/-$\alpha^{3.7}$

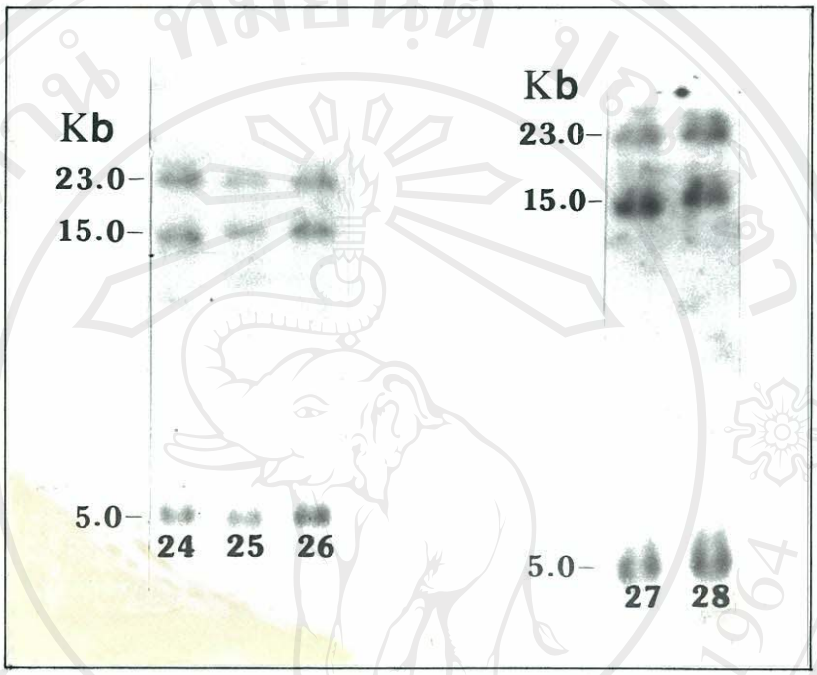

Figure15. Autoradiogram of genomic DNA digested with *Eco* R I and hybridized with the ζ-globin specific probe.

24,25,26,27,and 28 = --/$\alpha^T\alpha$

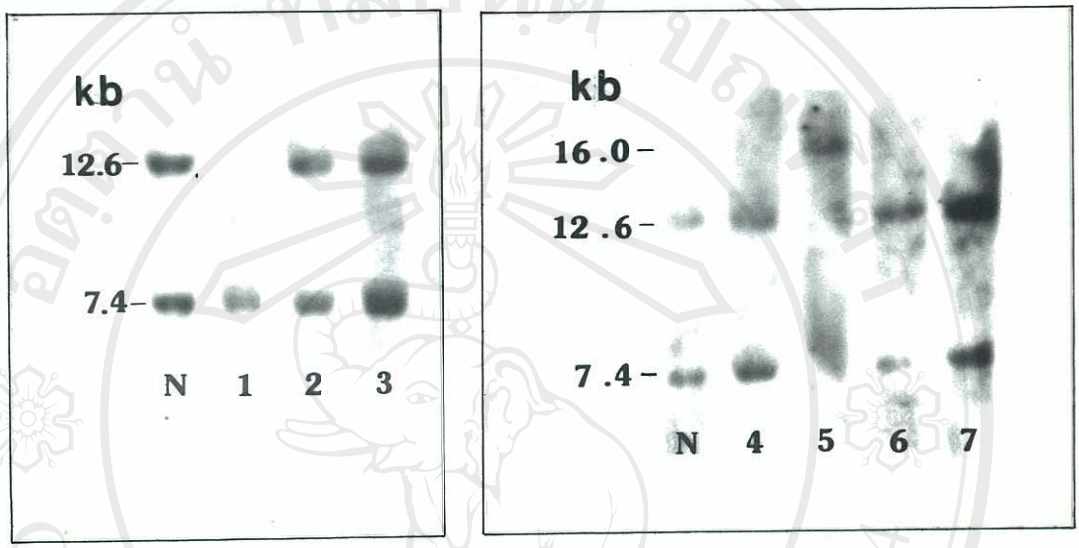

Figure 16. Autoradiogram of genomic DNA digested with *Bgl* II and hybridized with the α-globin specific probe.

N = Normal
1 = --/-$\alpha^{4.2}$
5 = --/-$\alpha^{3.7}$
2,3,4,6,and 7 = --/$\alpha^{T}\alpha$

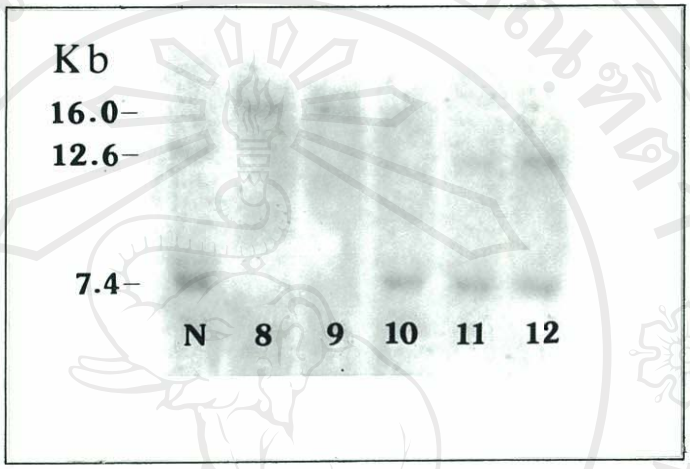

Figure17. Autoradiogram of genomic DNA digested with *Bgl* II and hybridized with the α-globin specific probe.

N	=	Normal
8 and 9	=	$--/-\alpha^{3.7}$
10	=	$--/-\alpha^{4.2}$
11 and 12	=	$--/\alpha^{T}\alpha$

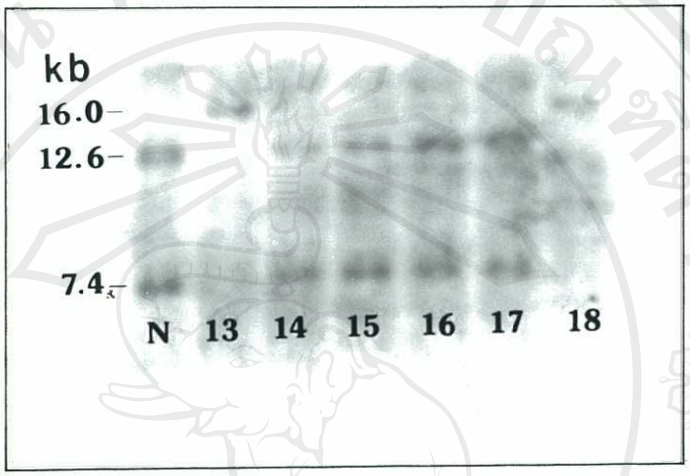

Figure18. Autoradiogram of genomic DNA digested with *Bgl* II and hybridized with the α-globin specific probe.

N = Normal
13 and 18 = --/-$\alpha^{3.7}$
14,15,16,and 17 = --/ $\alpha^{T}\alpha$

Table 3. Restriction fragment length(kb) detected with the α- and ζ-globin specific probes in various conditions.

Probe	Enzyme	αα/αα	--/-α	--/$\alpha^{T}\alpha$
ζ	*Eco* R I	23.0	19.0*	23.0
		5.0	15.0*	15.0*
			5.0	5.0
α	*Bgl* II	12.6	16.0*a	12.6
		7.4	7.4*b	7.4

a observed in $-\alpha^{3.7}$ haplotype

b observed in $-\alpha^{4.2}$ haplotype

* abnormal band

Table 4. Freguency of different genotypes observed in patients with Hb H disease.

No.(n=28)	*Eco* R I/ ζ	*Bgl* II/α	Genotype	%
7	19.0,15.0,5.0	16.0	--/-$\alpha^{3.7}$	25
3	19.0,15.0,5.0	7.4	--/-$\alpha^{4.2}$	11
18	23.0,15.0,5.0	12.6,7.4	--/$\alpha^{T}\alpha$	64