CHAPTER III

RESULTS

3.1 GENOMIC DNA PREPARATION

The 114 genomic DNA samples were extracted from peripheral venous blood of patients with Hb H disease. The yield was approximately 20-80-ng/μl. the OD_{260}/OD_{280} ratio was between 0.9-1.1.

3.2 POLYMERASE CHAIN REACTION

3.2.1 PCR-based diagnosis of the ($--^{SEA}$) deletion

The Southeast Asian ($--^{SEA}$) α-thalassemia-1 deletion spans approximately 20-kb, removing both functional α-globin genes while leaving the ζ_2-globin gene intact. By using 3 primers (SEA-F, SEA-R, and α-R) that bind at the different sites inside and flanking the ($--^{SEA}$) deletion, one could easily determine the α-thalassemia-1 of this type by visualizing the amplified products generated from each of primer pair.

Representative results of DNA analysis in samples with normal α-globin genes, heterozygotes and homozygotes for α-thalassemia-1 ($--^{SEA}$) deletion are shown in Figure 18. The characteristic PCR products, a 314-bp band in normal samples, a deletion-specific 188-bp band in homozygotes for α-thalassemia-1, and both bands in heterozygotes were found clearly discernible.

3.2.2 PCR-based diagnosis of the ($--^{THAI}$) deletion

The Thai ($--^{THAI}$) α-thalassemia-1 deletion spans approximately 34-38-kb and removes the ζ_2-globin gene as well as both α-globin genes. By using 3 primers (THAI-F, THAI-R, and THAI-N) that bind at the different sites inside and flanking the ($--^{THAI}$)

deletion, one can easily determine the α-thalassemia-1 of this type by visualizing the amplified products generated from each of primer pair.

For ($--^{THAI}$) deletion, samples with the normal set of four α-globin genes gave a single 637-bp band. Heterozygotes for α-thalassemia-1 showed a deletion specific 480-bp band and the normal 637-bp band. Blood from fetuses homozygous for the α-thalassemia-1 haplotype gave only the 480-bp band (figure 19).

3.2.3 PCR-based diagnosis of the ($--^{FIL}$) deletion

The Filipino ($--^{FIL}$) α-thalassemia-1 deletion extends for 30-34-kb beyond the ζ_2-genes at the 5' end and beyond the α-genes at the 3' end. By using 3 primers (FIL-F, FIL-R, and FIL-N) that bind at the different sites inside and flanking the ($--^{FIL}$) deletion, one can easily determine the α-thalassemia-1 of this type by visualizing the amplified products generated from each of primer pair.

For ($--^{FIL}$) deletion, samples with the normal set of four α-globin genes gave a single 227-bp band. Heterozygotes for α-thalassemia-1 showed a deletion specific 597-bp band and the normal 227-bp band. Blood from fetuses homozygous for the α-thalassemia-1 haplotype gave only the 597-bp band (figure 20).

3.2.4 Optimization of the multiplex PCR

The concentrations of the primers used in the multiplex PCR, their locations and sequences in the α-globin gene cluster and expected amplicon sizes are shown in Table 4 and Figure 17. Using the optimal conditions for the amplification of individual DNA fragments as a starting point, we investigated the conditions required for the optimal amplification of all four fragments in a single reaction. Figure 21 illustrates the results obtained when four different amplification mixtures containing equal amounts (0.4-μM each) of different a single pair of primers were subjected to standard PCR with program A (PCR condition for $--^{SEA}$ deletion) and program B (PCR condition for $--^{FIL}$ deletion); the latter program had a higher annealing temperature (63°C) and longer denaturation and extension times, but shorter annealing time. In general, there was a

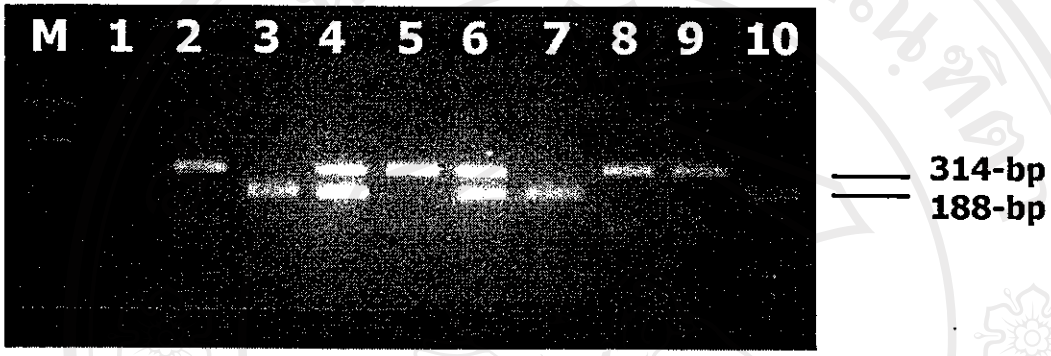

Figure 18. Detection of α-thalassemia-1 ($--^{SEA}$ deletion) by standard PCR with the SEA primer set.

The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Lane M, 100-bp DNA ladder; lane 1, negative control; lane 2 and 5, αα/αα; lane 3 and 7, $--^{SEA}/--^{SEA}$; lane 4 and 6, $--^{SEA}/\alpha\alpha$; lane 8, $--^{THAI}/\alpha\alpha$; lane 9, $--^{FIL}/\alpha\alpha$; lane 10, $--^{SEA}/--^{THAI}$. Specially sized PCR products were obtained for the normal (314-bp), and $--^{SEA}$ (188-bp) genotypes.

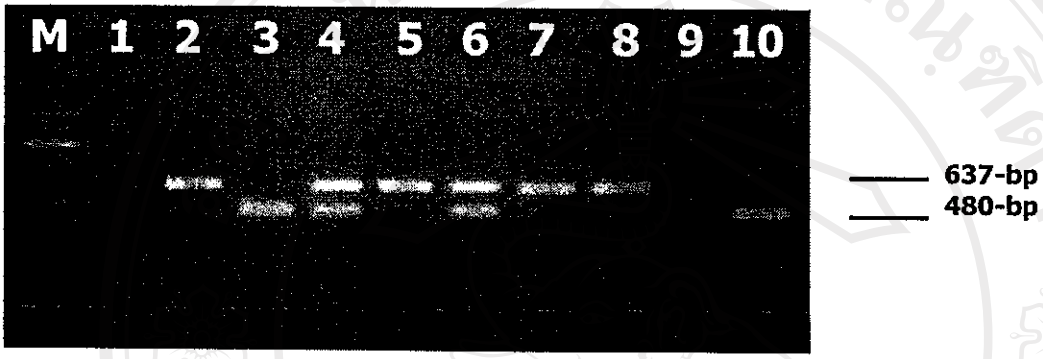

Figure 19. Detection of α-thalassemia-1 ($--^{THAI}$ deletion) by standard PCR with the THAI primer set.

The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Lane M, 100-bp DNA ladder; lane 1, negative control; lane 2 and 5, αα/αα; lane 3 and 10, $--^{SEA}/--^{THAI}$; lane 4 and 6, $--^{THAI}$/αα; lane 7, $--^{SEA}$/αα; lane 8, $--^{FIL}$/αα; lane 9, $--^{SEA}/--^{SEA}$. Specially sized PCR products were obtained for the normal (637-bp), and $--^{THAI}$ (480-bp) genotypes.

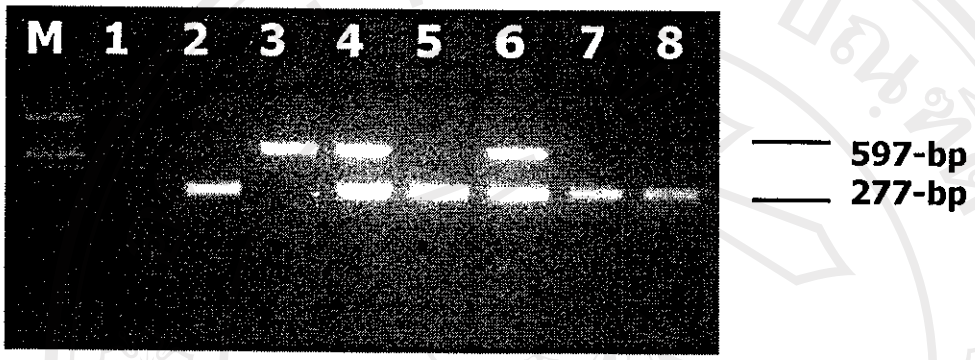

Figure 20. Detection of α-thalassemia-1 ($--^{FIL}$ deletion) by standard PCR with the FIL primer set.

The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Lane M, 100-bp DNA ladder; lane 1, negative control; lane 2 and 5, αα/αα; lane 3, $--^{FIL}$/αα (the PCR product of primer FIL-F and FIL-R); lane 4 and 6, $--^{FIL}$/αα (the PCR products of primer FIL-F, FIL-R, and FIL-N); lane 7, $--^{SEA}$/αα; lane 8, $--^{THAI}$/αα. Specially sized PCR products were obtained for the normal (277-bp), and $--^{THAI}$ (597-bp) genotypes.

visibly higher yield of PCR products for lane 7 and 8 with program B. In addition, with program A some unspecific products appear in lane 3 and 4. The results with program A were considered less desirable overall. Thus, we chose program B for using on next step of multiplex PCR optimization.

When the multiplex PCR is performed for the first time, it is useful to add the primers in equimolar amounts. The obtained results would be used to adjust the concentration and other parameters (Figure 21). Next, the optimization of multiplex PCR cycling conditions including the annealing temperature, the annealing time, the extension temperature, the extension time, and the number of PCR cycles were optimized to provide the best multiplex PCR cycling condition for the detection of three types of α-thalassemia-1 deletions. Then, the optimization of multiplex PCR reaction components including the concentrations of dNTP, PCR buffer (KCl), and $MgCl_2$, the amount of template DNA and Taq DNA polymerase and the usefulness of adjuvants: Q-solution, DMSO, and glycerol also were optimized.

In this experiment, the annealing temperatures were varied from 58°C to 64°C (58°C, 60°C, 62°C, and 64°C) as shown in Figure 22 that the best result was found at 60°C without non-specific bands. And the annealing times were varied from 10, 20, and 40 seconds (figure 23). The expected bands present in all of these times but the best had been observed in 20 seconds.

The extension temperatures were varied from 68°C, 72°C, and 75°C (Figure 24); the best result was found at 72°C. And the extension times were varied from 0.5, 1, and 2 minutes (figure 25). The expected bands present in all of these times but the optimal extension time was 1-2 minutes. The numbers of PCR cycles were varied from 30, 35, and 40 cycles (Figure 26), the best results was found at 40 cycles.

For the optimization of multiplex PCR reaction components, the PCR buffer (KCl) concentration were varied from 0.5x, 1x, and 1.5x concentration. Raising the buffer concentration to 1x improved the efficiency of the multiplex reaction. Generally, primer pairs with longer amplification products worked better at lower salt concentrations, whereas primer pairs with short amplification products worked better at higher salt

concentrations, where longer products become harder to denature (Figure 27). But the optimization of some parameters such as the titration of dNTPs, $MgCl_2$, and genomic DNA (Figure 30), result of the multiplex PCR system showed no significant differences.

No major differences due to the $MgCl_2$ concentration were seen when keeping dNTP concentration at 200-μM each and gradually increasing $MgCl_2$ from 1.5-3-mM (Figure 28). In this study, $MgCl_2$ concentration was kept constant (1.5-mM), while the dNTP concentration was increased stepwise from 100-400-μM each. At dNTP concentrations between 100 and 400-μM each, the results showed no significant difference (Figure 29).

And different concentrations of Taq DNA polymerase were tested using multiplex PCR (Figure 31). The most efficient enzyme concentration seemed to be around 0.5-2.0 unit per reaction. Too much enzyme, possibly because of the high glycerol concentration in the stock solution, resulted in an unbalanced amplification of PCR products and a slight increase in the nonspecific background products (Figure 31, as see at 2.0-U/reaction).

Finally, the usefulness some of the more popular of adjuvants to improve amplification efficiency (higher amount of product) and specificity (no unspecific products) of multiplex PCR. It was found that the Q-solution, in 1x concentration can be increased the efficiency of the multiplex PCR much more than either 5% DMSO or 7.5% glycerol. And many unspecific products appeared in the reaction mixture without adjuvant (Figure 32). Unexpected or inappropriate fragments were not observed in any of the sample tested, confirming the specificity of this assay. These results demonstrate the successful development of a multiplex PCR assay for α-thalassemia-1 that found in Thailand. As shown in Figure 33, the multiplex PCR results of various α-globin genotypes control.

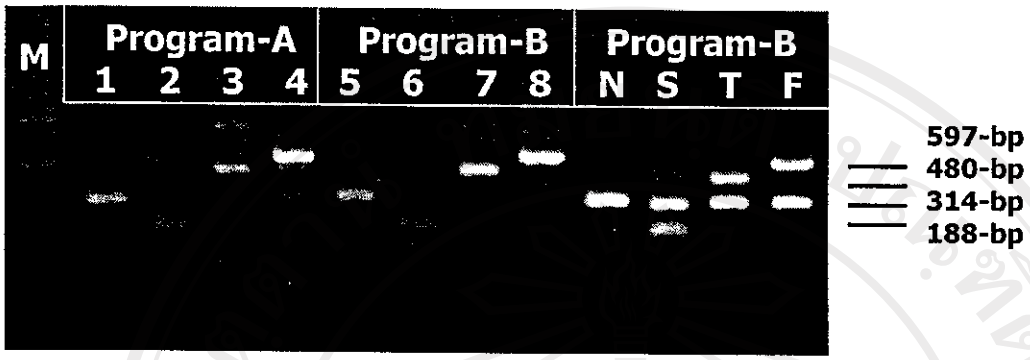

Figure 21. The effects of PCR program-A and PCR program-B on the detection of α-thalassemia-1 by multiplex PCR.

The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. The PCR products of primer SEA-F and α-R (amplified normal gene) are shown in lane 1 and 5 whereas in lane 2 and 6 are shown the PCR products of primer SEA-F and SEA-R (amplified $--^{SEA}$ deletion). The PCR products in lane 3 and 7 were obtained with primer THAI-F and THAI-R (amplified $--^{THAI}$ deletion) and lane 4 and 8 were obtained with primer FIL-F and FIL-R (amplified $--^{FIL}$ deletion).

Multiplex PCR was performed with the primers in equimolar amounts by using PCR program-B. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal (αα/αα)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

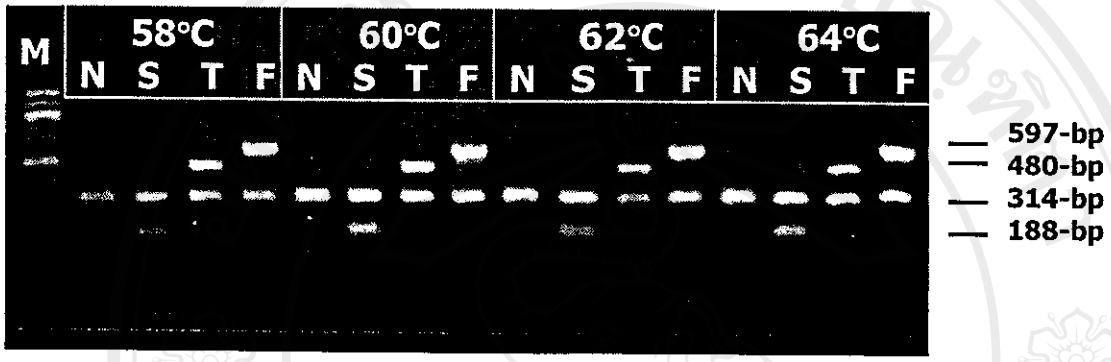

Figure 22. The optimization of the annealing temperature for detection of α-thalassemia-1 by multiplex PCR.

The annealing temperatures were adjusted to provide the best result. The temperatures were 58°C, 60°C, 62°C, and 64°C, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}/\alpha\alpha$

Lane T = $--^{THAI}/\alpha\alpha$

Lane F = $--^{FIL}/\alpha\alpha$

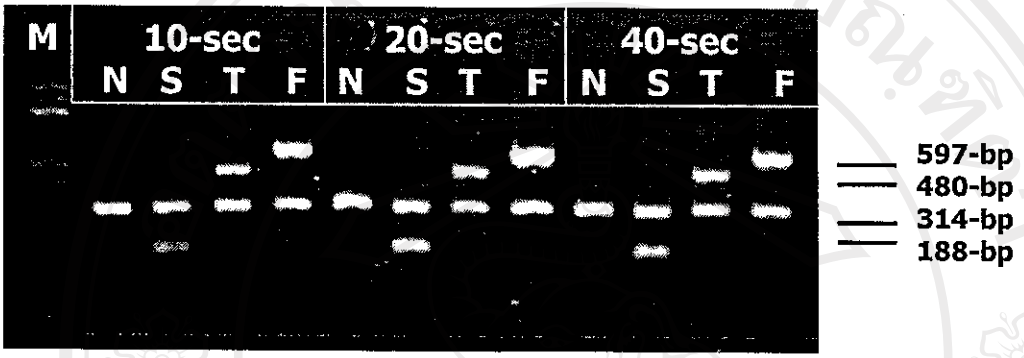

Figure 23. The optimization of the annealing time for detection of α-thalassemia-1 by multiplex PCR.

The annealing times were adjusted to provide the best result. The annealing times were 10-sec, 20-sec, and 40-sec, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}/\alpha\alpha$

Lane T = $--^{THAI}/\alpha\alpha$

Lane F = $--^{FIL}/\alpha\alpha$

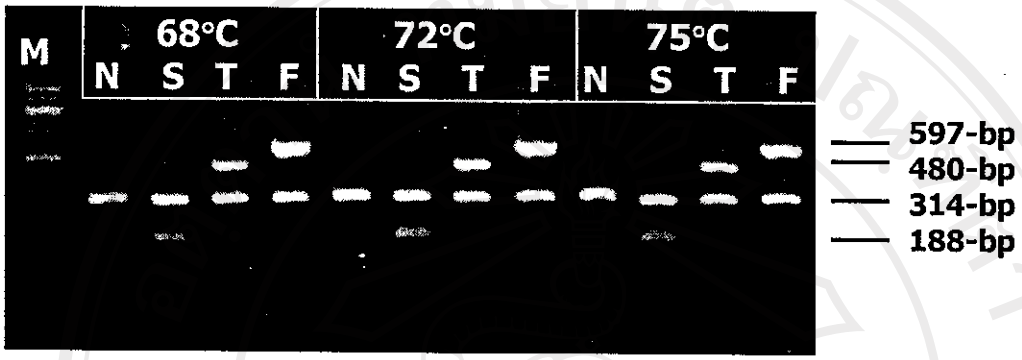

Figure 24. The optimization of the extension temperature for detection of α-thalassemia-1 by multiplex PCR.

The extension temperatures were adjusted to provide the best result. The temperatures were 68°C, 72°C, and 75°C, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder
Lane N = normal ($\alpha\alpha/\alpha\alpha$)
Lane S = $--^{SEA}/\alpha\alpha$
Lane T = $--^{THAI}/\alpha\alpha$
Lane F = $--^{FIL}/\alpha\alpha$

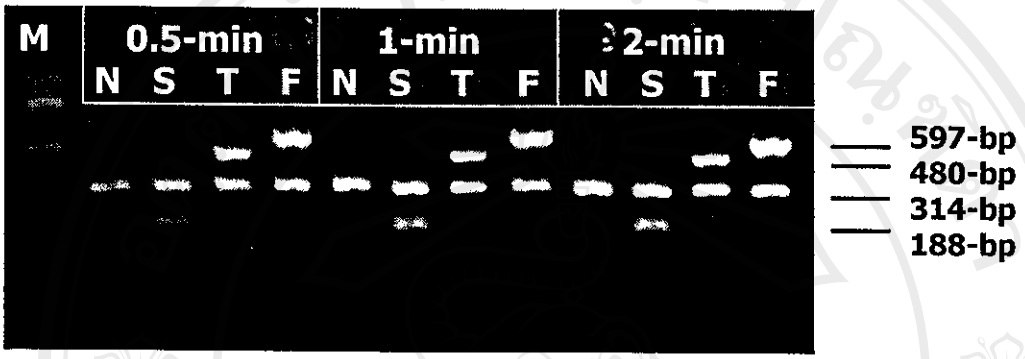

Figure 25. The optimization of the extension time for detection of α-thalassemia-1 by multiplex PCR.

The extension times were adjusted to provide the best result. The extension times were 0.5-min, 1-min, and 2-min, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}/\alpha\alpha$

Lane T = $--^{THAI}/\alpha\alpha$

Lane F = $--^{FIL}/\alpha\alpha$

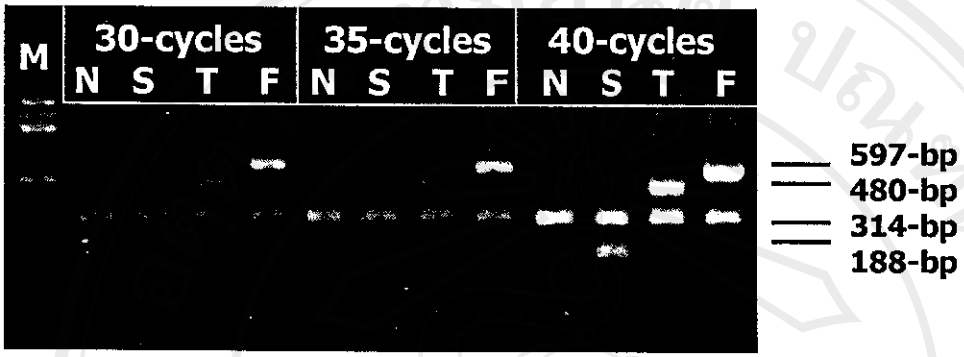

Figure 26. The optimization of the number of PCR cycles for detection of α-thalassemia-1 by multiplex PCR.

The numbers of PCR cycles were adjusted to provide the best result. The numbers of cycles were 30-cycles, 35-cycles, and 40-cycles, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

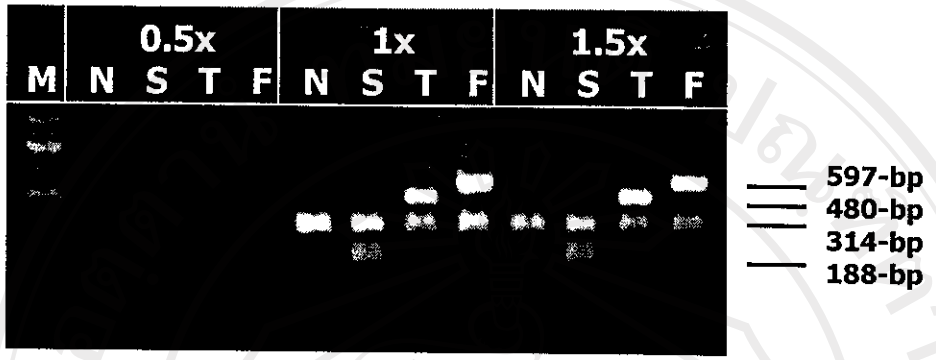

Figure 27. The optimization of the PCR buffer concentration for detection of α-thalassemia-1 by multiplex PCR.

The PCR buffer concentrations were adjusted to provide the best result. The concentrations were 0.5x, 1x, and 1.5x PCR buffer, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal (αα/αα)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

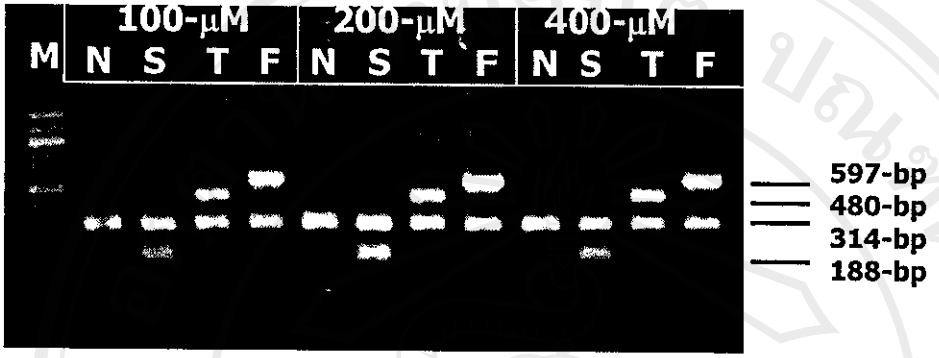

Figure 28. The optimization of the deoxynucleoside 5'-triphosphates concentration for detection of α-thalassemia-1 by multiplex PCR.

The dNTPs concentrations were adjusted to provide the best result. The concentrations were 100-μM, 200-μM, and 400-μM, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal (αα/αα)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

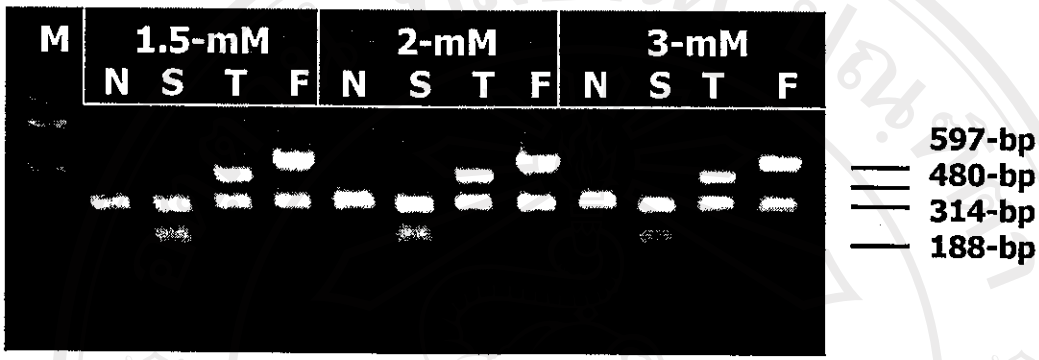

Figure 29. The optimization of the magnesium chloride concentration for detection of α-thalassemia-1 by multiplex PCR.

The $MgCl_2$ concentrations were adjusted to provide the best result. The concentrations were 1.5-mM, 2.0-mM, and 3.0-mM, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

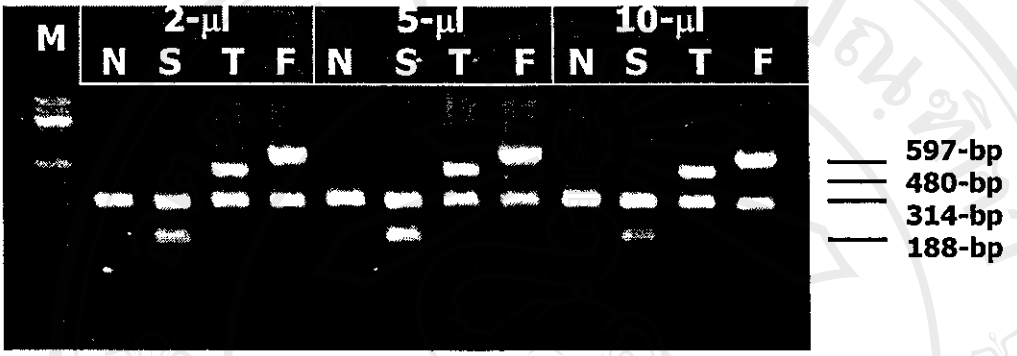

Figure 30. The optimization of the volume of genomic DNA samples for detection of α-thalassemia-1 by multiplex PCR.

The volumes of genomic DNA were adjusted to provide the best result. The volumes were 2-µl, 5-µl, and 10-µl, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

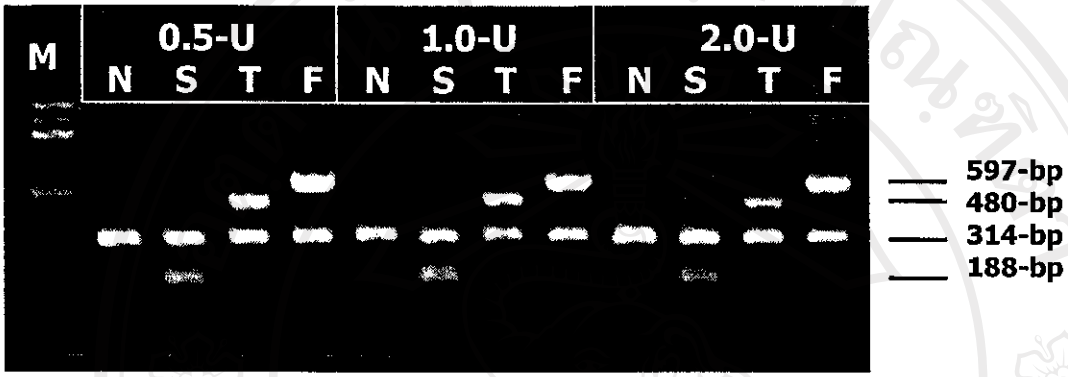

Figure 31. The optimization of the amount of *Taq* DNA polymerase for detection of α-thalassemia-1 by multiplex PCR.

The amounts of Taq DNA polymerase were adjusted to provide the best result. The concentrations were 0.5-U/reaction, 1.0-U/reaction, and 2.0-U/reaction, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

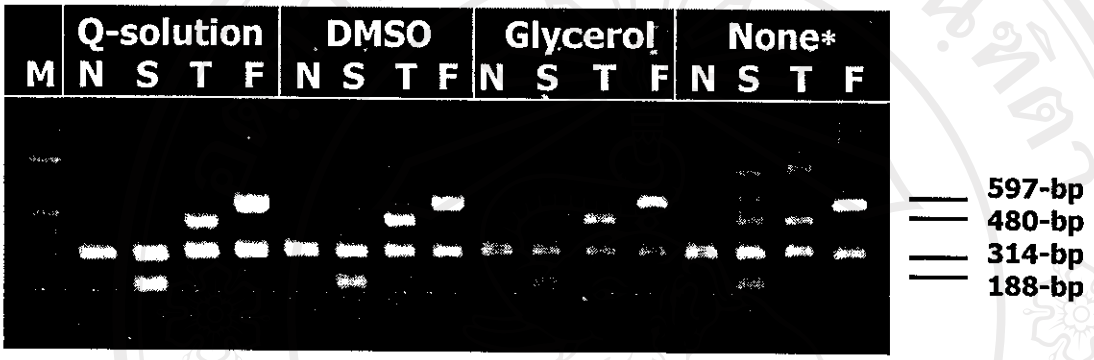

Figure 32. The effects of various adjuvants on the modified multiplex PCR.

The adjuvants were added to provide the best result. The adjuvants were added in the PCR reaction mixture includes; 1x Q-solution, 5% DMSO, and 7.5% glycerol, respectively. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5x TBE buffer. The best result was found at 1x PCR buffer.

Lane M = 100-bp DNA ladder

Lane N = normal ($\alpha\alpha/\alpha\alpha$)

Lane S = $--^{SEA}$ genotype

Lane T = $--^{THAI}$ genotype

Lane F = $--^{FIL}$ genotype

None* = no adjuvant in PCR reaction mixtures

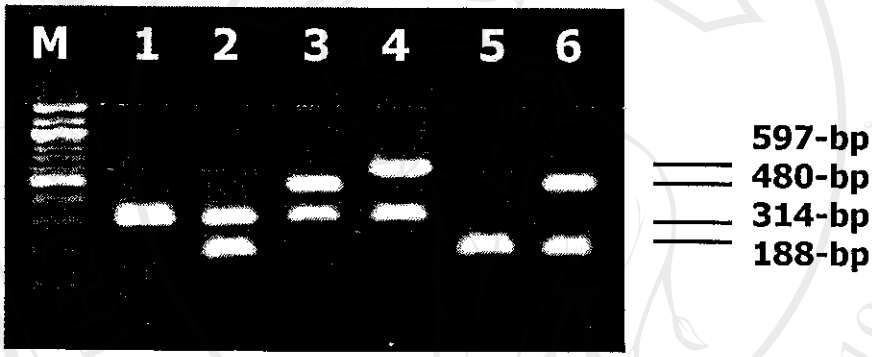

Figure 33. Multiplex PCR results for various α-globin genotypes. Specially sized PCR products were obtained for the normal (314-bp), $--^{SEA}$ (188-bp), $--^{THAI}$ (480-bp), and $--^{FIL}$ (597-bp) genotypes. Lane M, 100-bp DNA ladder (New England Biolabs), lane 1, αα/αα; lane 2, $--^{SEA}$/αα; lane 3, $--^{THAI}$/αα; lane 4, $--^{FIL}$/αα; lane 5, $--^{SEA}$/$--^{SEA}$; lane 6, $--^{SEA}$/$--^{THAI}$.

3.2.5 Detection of α-thalassemia-1: ($--^{SEA}$), ($--^{THAI}$) and ($--^{FIL}$) deletion types in-patients with Hb H disease and infants with Hb Bart's hydrops fetalis by multiplex PCR

In this study, the multiplex PCR assay was carried out on 114 genomic DNA samples from patients with Hb H disease. Out of these 114 cases, 113 cases were heterozygous for $--^{SEA}$ deletion and only one case was heterozygous for $--^{THAI}$ deletion. None of them has the $--^{FIL}$ deletion. Furthermore, we analyzed DNA of 33 Thai infants with the Hb Bart's hydrops fetalis (--/--) from Maharaj Nakorn Chiang Mai hospital. Only two out of 33 cases were of compound heterozygotes for the $--^{THAI}$ and $--^{SEA}$ mutants ($--^{THAI}/--^{SEA}$). The remaining 31 cases were homozygous for the common $--^{SEA}$ mutants ($--^{SEA}/--^{SEA}$). None of them has the compound heterozygotes for the $--^{THAI}$ or $--^{SEA}$ and $--^{FIL}$ mutants. The results were summarized in Table 7.

Table 7. The results from the detection of α-thalassemia-1 deletion in 114 cases of Hb H disease and 33 cases of Hb Bart's hydrops fetalis by multiplex PCR

Genotype	Cases
$--^{SEA}/-\alpha$ or $\alpha^T\alpha$	113
$--^{THAI}/-\alpha$ or $\alpha^T\alpha$	1
$--^{SEA}/--^{SEA}$	31
$--^{SEA}/--^{THAI}$	2
Total	147

3.3 DNA SEQUENCING

To confirm that the deletion breakpoints of each α-thalassemia-1 deletion types and the PCR products were the result of the binding of the primers to the intended region, we determined the DNA sequence of each amplicon by using automated DNA sequencing.

3.3.1 Confirmation of the deletion breakpoints of ($--^{SEA}$) deletion by using DNA sequencing technique

Chang *et al.* (1991) were identified the ($--^{SEA}$) deletion breakpoint by using direct DNA sequencing. The 5' breakpoint lies between 26259 and 26260 of GenBank accession number Z84721, and the 3' breakpoint lies between 2608 and 2609 of Z69706. A total of 19,304 nucleotides were deleted.

Z84721: 26236 5'-TTGGGGGGCGCCTTGGGGAGGTTC↓TAGCCCCTGAGCACCGGAGCT

$--^{SEA}$ deletion 5'-TTGGGGGGCGCCTTGGGGAGGTTC↓↑ACTTGGAGGCTGGGGCAGGAG

Z69706: 2584 5'-GGTAGTGCACACCTATGTCCCAGTT↑ACTTGGAGGCTGGGGCAGGAG

Note: Comparison of the 5' normal (top), 3' normal (bottom), and the $-^{SEA}$ deletion sequences (middle). The arrows indicate the deletion breakpoints of $--^{SEA}$ deletion.

3.3.2 Confirmation of the deletion breakpoints of ($--^{THAI}$) deletion by using DNA sequencing technique

The identity of the ($--^{THAI}$) junction bands was confirmed by DNA sequencing. The ($--^{THAI}$) deletion breakpoint was characterized during the course of developing the multiplex PCR. The 5' breakpoint lies between 10725 and 10726 of GenBank accession number Z84721, and the 3' breakpoint lies between 1220 and 1221 of Z69706 (Figure 39). A total of 33,450 nucleotides were deleted.

3.3.3 Confirmation of the deletion breakpoints of ($--^{FIL}$) deletion by using DNA sequencing technique

The identity of the ($--^{FIL}$) junction bands was confirmed by DNA sequencing. The identity of the ($--^{FIL}$) junction bands was confirmed by DNA sequencing. The ($--^{FIL}$) deletion breakpoint located within the completely homologous 21-bp segment. The 5' breakpoint lies between 12483 to 12503 of GenBank accession number Z84721, and the 3' breakpoint lies between 204 and 224 of Z69706 (Figure 40). A total of 30,656 nucleotides were deleted.

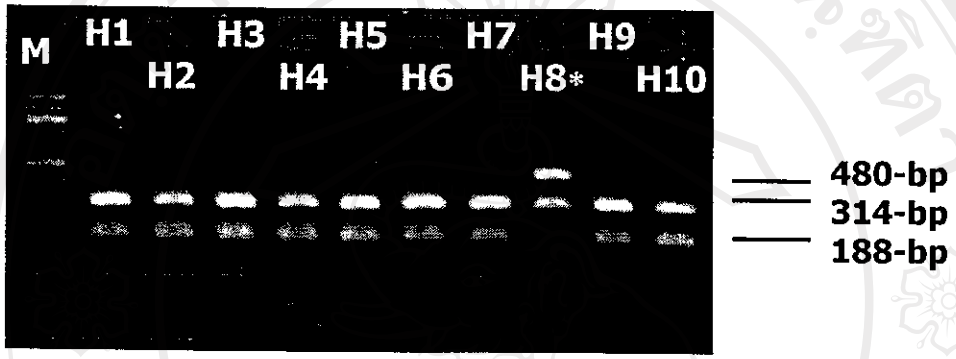

Figure 34. Detection of α-thalassemia-1 in genomic DNA samples by multiplex PCR. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5X TBE buffer.

Lane M = 100-bp DNA ladder

Lane H1, H2, H3, H4, H5, H6, H7, H9, and H10 = genomic DNA samples ($--^{SEA}$ genotype)

H8* = genomic DNA samples ($--^{THAI}$ genotype)

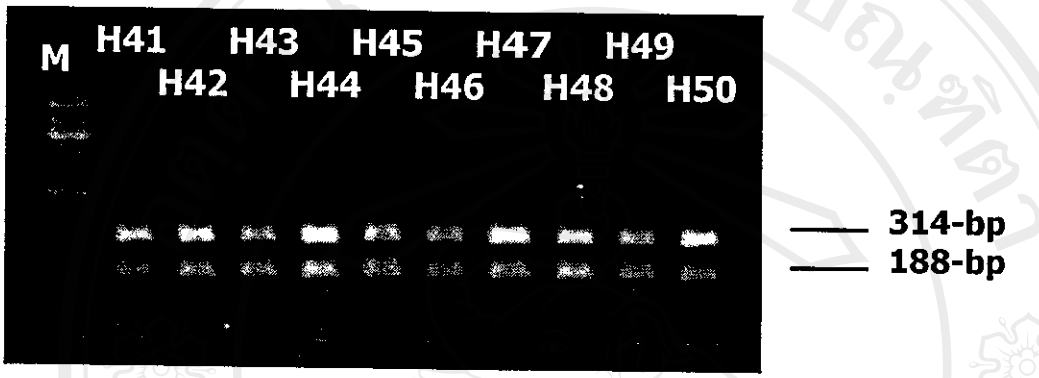

Figure 35. Detection of α-thalassemia-1 in genomic DNA samples by multiplex PCR. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5X TBE buffer.

Lane M = 100-bp DNA ladder

Lane H41, H42, H43, H44, H45, H46, H47, H48, H49, and H50 = genomic DNA samples ($--^{SEA}$ genotype)

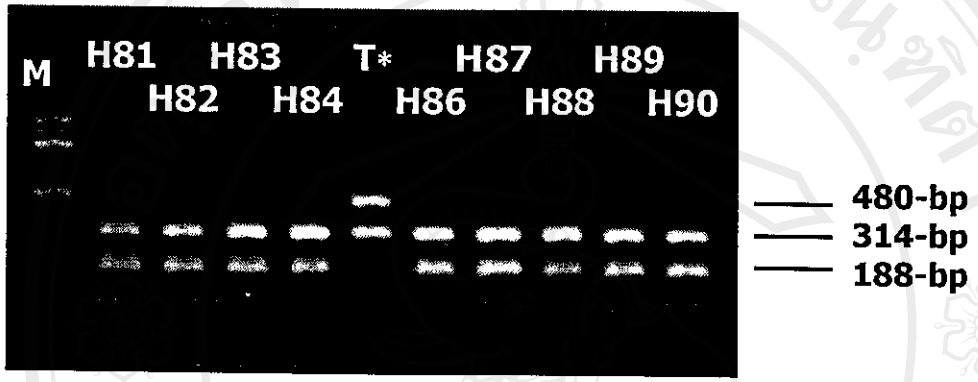

Figure 36. Detection of α-thalassemia-1 in genomic DNA samples by multiplex PCR. The PCR products were subjected to electrophoresis on 2% agarose gel in 0.5X TBE buffer.

Lane M = 100-bp DNA ladder

Lane H81, H82, H83, H84, H86, H87, H88, H89, and H90 = genomic DNA samples ($--^{SEA}$ genotype)

T* = genomic DNA samples ($--^{THAI}$ genotype)

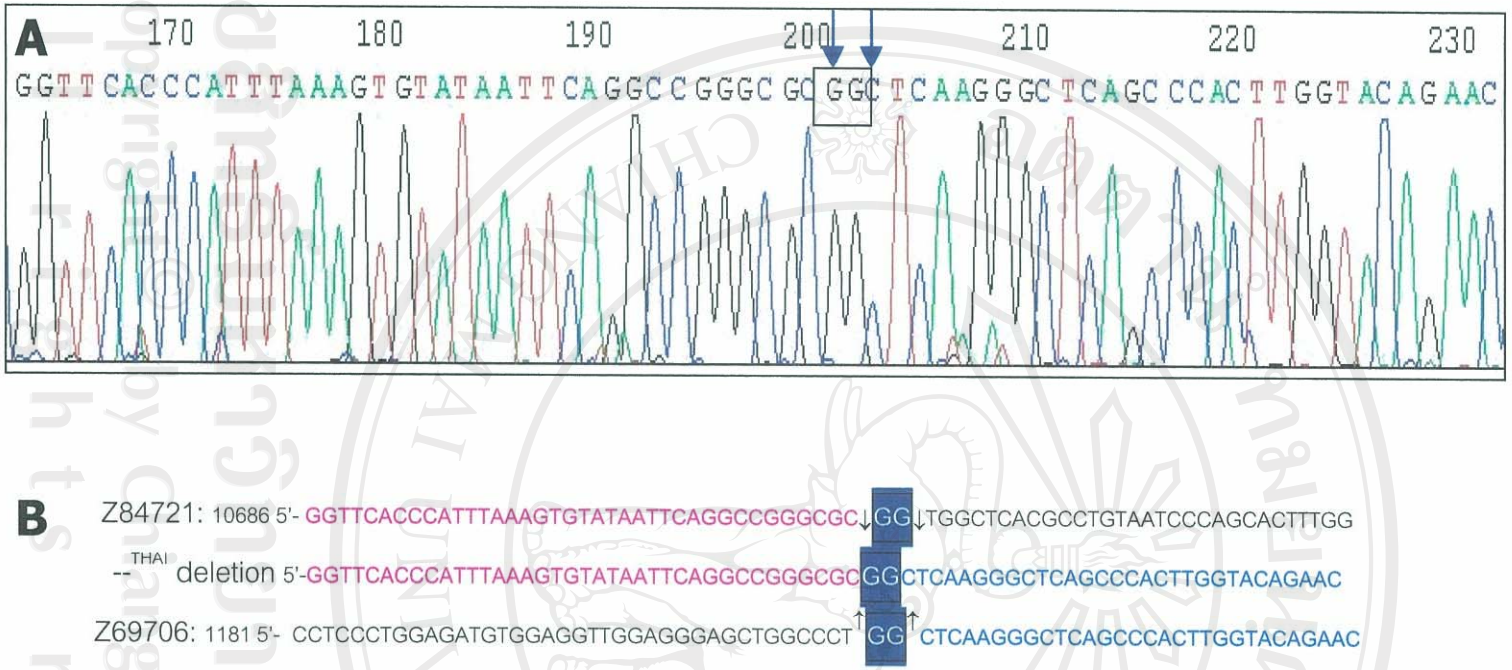

Figure 37. (A) DNA sequencing across the –^{THAI} breakpoints. (B) Comparison of the 5' normal (top), 3' normal (bottom), and the --^{THAI} deletion sequences (middle). The arrows indicate the deletion breakpoints of --^{THAI} deletion.

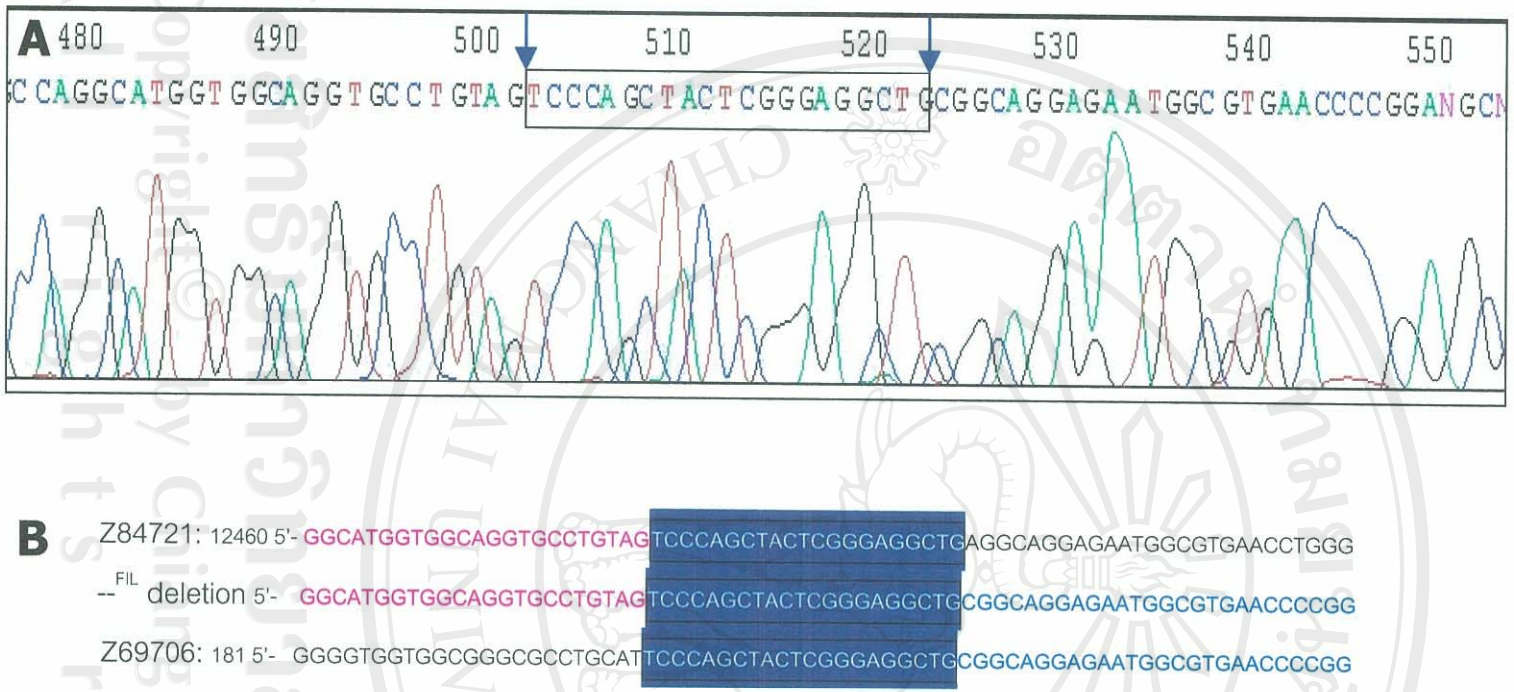

Figure 38. (A) DNA sequencing of the crossover regions between parent sequences, 5' to the ζ_2 and 3' to the θ_1 genes (B) Comparison of the 5' normal (top), 3' normal (bottom), and the --^{FIL} deletion sequences (middle). The breakpoints is assigned within completely homologous 21-bp sequence (square boxes).