CHAPTER 5
CONCLUSIONS

1. The in-house multiplex allele-specific PCR protocol for α-thalassemia and hemoglobinopathy was successfully developed with the following condition;

1.1 Reaction volume : 50 µl,

1.2 Ingredients : 0.4 µM each of SEA1, SEA3, CS2 and C3 primers, 0.025 µM αG-17 primer, 1.2 µM each of Alpha3.7A and Alpha3.7B primers in 10 mM Tris pH 8.5, 50 mM KCl, 1 mM $MgCl_2$, 140 µM dNTPs, 2% DMSO, 2M betaine, 0.1 unit DNA polymerase (iTaq; iNtRON Biotechnology, Inc.) and 5 µl DNA.

1.3 Thermal cycles : 29 rounds comprising denaturation at 94^{o}C for 6 minutes 30 seconds in the first cycle and for 1 minute 30 seconds in subsequent 28 cycles, primer annealing at 60^{o}C for 1 minute 30 seconds and extension at 72^{o}C for 2 minute 20 seconds in the first 28 cycles and for 7 minutes during the last cycle.

2. The in-house multiplex allele-specific PCR protocol for β-thalassemia and hemoglobinopathies was successfully developed with the following condition;

2.1 Reaction volume : 25 µl

2.2 Ingredients : 0.025 µM αG-17, 0.4 µM C3, 0.032 µM "beta-common multiplex", 0.054 µM "beta 41/42 multiplex", 0.054 µM "beta17 multiplex", 0.018 µM "beta E multiplex" and 0.14 µM M-28M1 primer in 10 mM Tris pH 8.5, 50 mM KCl, 4 mM $MgCl_2$, 140 µM dNTPs, 2% DMSO, 0.1 unit DNA polymerase (iTaq; iNtRON Biotechnology, Inc.) and 5 µl DNA.

2.3 Thermal cycles : 27 rounds comprising denaturation at 95^{o}C for 5 minutes in the first cycle and for 1 minute in subsequent 26 cycles, primer annealing at 62^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds in the first 26 cycles and for 7 minutes during the last cycle.

3. The in-house whole blood PCR was successfully developed utilizing 9% (w/v) betaine as PCR facilitator, 2 µl blood sample in 50 µl PCR reaction combined with 3 repeating heat-cool steps for aged blood lysate and 10 repeating heat-cool steps for fresh whole blood (repeating heat-cool steps consisted of 3 minute at 94°C, then cooled for 3 minute at 55°C).

4. The in-house whole blood PCR protocol for SEA-α thalassemia 1 was successfully developed with the following condition;

4.1 Reaction volume : 25 µl

4.2 Ingredients : 0.10 µM SEA1, 0.07 µM SEA2 and 0.04 µM SEA3 primers, 1x iProof GC buffer, 1.5 mM $MgCl_2$, 140 µM dNTPs, 2% DMSO, 9% (w/v)

betaine, 0.02 units iProof DNA polymerase (iProof; Bio-Rad, Hercules, CA) and 2 µl fresh whole blood.

4.3 Thermal cycles : A 10 repeating heat-cool steps preceded the typical 27-round thermal cycles of 5-minute denaturation at 95°C in the first cycle, followed by 25 cycles of 95°C denaturation for 1 minute, 62°C annealing for 30 seconds and 72°C extension for 30 seconds. A final 5-minute extension at 72°C completed the reaction.

5. The in-house whole blood PCR protocol for HbCS was successfully developed with the following condition;

5.1 Reaction volume : 25 µl

5.2 Ingredients : 0.1 µM CS-2 primer, 0.04 µM αG-17 primer and 0.4 µM C3 primer, 10 mM Tris pH 8.5, 50 mM KCl, 2.5 mM $MgCl_2$, 100 µM dNTPs, 2% DMSO, 9% (w/v) betaine, 0.1 units DNA polymerase (iTaq; iNtRON Biotechnology, Inc.) and 2 µl fresh whole blood.

5.3 Thermal cycles : A 10 repeating heat-cool steps preceded the typical 32-round thermal cycles of 5-minute denaturation at 95°C in the first cycle, followed by 30 cycles of 95°C denaturation for 1 minute, 60°C annealing for 30 seconds, and 72°C extension for 1 minute. A final 5- minute extension at 72°C completed the reaction.

6. The in-house whole blood PCR protocol for β-thalassemia/hemoglobinopathies was successfully developed with the following condition;

6.1 Reaction volume : 25 µl

6.2 Ingredients : 0.032 µM "beta common multiplex", 0.054 µM 'beta 41/42 multiplex", 0.054 µM "beta17 multiplex", 0.14 µM M-28M1, 0.025 µM αG-17 and 0.4 µM C3 and 0.0108 µM "beta E multiplex" primers in 10 mM Tris pH 8.5, 50 mM KCl, 4 mM $MgCl_2$, 140µM dNTPs, 2% DMSO, 9% (w/v) betaine, 0.1 unit DNA polymerase (iTaq; iNtRON Biotechnology, Inc.) and 2 µl fresh whole blood.

6.3 Thermal cycles: A 10 repeating heat-cool steps preceded the typical 26-round thermal cycles of initial denaturation at 95^{o}C for 5 minutes and for 1 minute in subsequent 24 cycles, primer annealing at 62^{o}C for 30 seconds and extension at 72^{o}C for 30 seconds and 7 minutes at last cycle.