**Supplementary Materials and Methods**

**1. Identification of HBB gene mutations using PCR followed by DNA sequencing**

The primer sequences for PCR and sequencing of HBB gene are listed in the Supplementary Table 1. The total volume of PCR reaction was 25 μl, and it was contained 20-50 ng genomic DNA, 2.5μl of 10×LA Taq buffer (Takara, Dalian, China), 10 pmol of each primer, 2.5 μl of 2.5mM dNTPs, 1 Unit of LA Taq DNA polymerase. The PCR conditions were as follows: 94°C for 4 min for initial denaturation, followed by 35 cycles of 94°C for 45 sec, 63°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 10 min. The purified PCR product of a 1973 bp fragment was sequenced by using the respective sequencing primer and BigDye Teminator V3.1 cycle sequencing Kit (Life Technologies Corporation, Carlsbad, USA).

**Supplementary** **Table 1** The primers used in PCR-DNA sequencing of HBB gene and the tetra-primer ARMS PCR for detecting allelic variant of Hb Rush

|  |  |  |  |
| --- | --- | --- | --- |
| Primer | Description | Sequence (5'-3') | Product (bp) |
| HBB-F | PCR and sequencing | TGGTATGGGGCCAAGAGATA | 1973 |
| HBB-R | PCR and sequencing | TTTGCAGCCTCACCTTCTTT |
| HBB-R2 | Internal sequencing | TAAGTATAATAGTAAAAATTGCGG |  |
| HBB-F2 | Internal sequencing | AAGTCTCAGGATCGTTTTAGT |  |

**2. Tetra primer** **ARMS PCR for Hb Rush (G>C) variant detection**

The primer set was designed according to HBB gene sequence (NCBI accession # NG\_000007). The optimized primers used for the tetra-primer ARMS PCR for Hb Rush G>C variant detection were presented in Supplementary Table 2. The schematic illustration of the ARMS PCR assay was shown in Supplementary Figure 1. The total volume of PCR reaction was 20 μl, containing 20-50 ng of genomic DNA, 200nM of each outer primers and 100nM of each inner primers, 1.5 mM MgCl2, 20 μl of 2.5Mm dNTPs, 0.5 Unit of PlatinumTM Taq DNA polymerase and 1×PCR buffer(life Technologies, Carlsbad, CA, USA ).The PCR conditions were as follows: initial denaturation at 95°C for 2 min, followed by 32 cycles of 94°C for 45 sec, 57°C for 35 sec , 72°C for 45 sec, and a final extension step at 72°C for 10 min. For result visualization, 10μl of PCR product was mixed with loading dye and electrophoresed on 2% agarose stained with ethidium bromide, against DL500 DNA marker 50-500 bp (Takara, Dalian, China) on a UV transilluminator.

**Supplementary Table 2** PCRprimers used in the tetra-primer ARMS PCR for detecting Hb Rush variant

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer | Sequence (5'-3') | Allele | Tm（℃） | Product size |
| Outer F | GAAGACTCTTGGGTTTCTGATAGG |  | 62.0 | 438 bp |
| Outer R | CTAAAACGATCCTGAGACTTCCAC |  | 62.8 |
| Inner F | ACAAGCTGCACGTGGATCCTC | Mutant C | 56.4 | 181 bp |
| Inner R | CGTCCCATAGACTCACCCTGAAGTTCTC | Wilde G | 57.9 | 305 bp |



**Supplementary Figure 1**. An Illustration of the tera-primer ARMS PCR for detecting Hb Rush G>C variant using the primer set in supplementary Table 2. The outer primers produce a 438 bp of control fragment. Wilde type G allele specific primer Inner R pairing Outer F primer produces a 305 bp band representing G allele. Likewise, mutant C allele specific primer Inner F pairing Outer R primer produces a 181 bp band representing the C mutant allele.