

Table 1: Primers used in the ARMS-assays. Their position on the gene sequence was established according to the Gene Bank sequence accession number NG\_000006.1. Bases in bold and underscored at the 3' end of the primers were known mutations; those located upstream of the 3' ends were additional mismatches. In the primer for the mutation  $\alpha 2$  IVS-I -5 nt the symbol “/” indicate the 5 nt deletion. “Common” primers were chosen from divergent sequences of the  $\alpha 2$  and  $\alpha 1$ -globin genes; control primers from regions outside the genes. Experimental conditions relative to each primer were reported. Running names of the mutations are reported within brackets; the  $\alpha 2$  cod 26 GCG>ACG and  $\alpha 2$  cod 130 GCT>CCT were found to be associated *in cis* (see text). The asterisks mark mutations generating amplicons with the same length.

PRIMERS (P)							Experimental conditions			
Name	Length	Position	Direction (F/R) and 5'-3' sequence of the primers	p mol	“common” P (used with ) name p mol	Amplicons length (bp)	Control Primers name p mol	Annealing T (45'')	Synthesis duration (72°C)	
<b>A) Primers for multiplex-ARMS-assay for 9 mutations of the <math>\alpha 2</math>-globin gene</b>										
$\alpha 2$ ATG>ACG (Nco I)	21	33757-77	F-GACTCAGAGAGAACCCAGCAC	13		886 *				
$\alpha 2$ ATG>GTG (Nco I)	21	33756-76	F-AGACTCAGAGAGAACCCAGCG	11		887 *				
$\alpha 2$ IVS-I -5 nt (Hph I)	19	33854-71	F-GCGGAGGCCCTGGAGACG/C	6		789				
$\alpha 2$ cod 26 GCG>ACG (Hb Caserta)	20	33835-54	F-GCACGCTGGCGAGTATGCTA	10		808				
$\alpha 2$ cod 130 GCT>CCT (Hb Sun Prairie)	20	34406-25	F-CTCCCTGGACAAGTTCTGTG	10	$\alpha 2$ +904	237	P1 + P2	2.5+2.5	62°C	
$\alpha 2$ cod 142 TAA>AAA (Hb Icaria)	23	34439-61	F-CGTGCTGACCTCCAAATACGGTA	11		204 **			60''	
$\alpha 2$ cod 142 TAA>CAA (Hb Constant Spring)	23	34439-61	F-CGTGCTGACCTCCAAATACGGTC	9		204 **				
$\alpha 2$ AATAAA>AATAAG (Poly A Arabian)	24	34534-57	F-GGCCCTTCCTGGTCTTTGAATGTG	10		109 ***				
$\alpha 2$ AATAAA>AATGAA (Poly A Turkish)	22	34534-55	F-GGCCCTTCCTGGTCTTTGATTG	10		109 ***				
<b>B) Primers for ARMS-assay for normal sequences corresponding to reported <math>\alpha 2</math>-globin gene mutations</b>										
N-ATG>ACG	20	33758-77	F-ACTCAGAGAGAACCCAGCAT	21		885			60''	
N-Hb Icaria / N-Hb Constant Spring	20	34442-61	F-GCTGACCTCCAAATACGGTT	12	$\alpha 2$ +904	201		2.0+2.0	58°C	
N-Hb Caserta	20	33835-54	F-GCACGCTGGCGAGTATGCTG	10		808	P1 + P2	2.0+2.0	62°C	
N-Hb Sun Prairie	20	34406-25	F-CTCCCTGGACAAGTTCTGTG	8		237		3.0+3.0	61°C	
N-AATAAA	20	34576-57	R-GGCTGCCGCCACTCACT	9	$\alpha 2$ del 7bp	284		63°C	45''	
N-IVS-I -5 nt	20	33891-72	R-GAGCAGGGGAGGGAGCGTCA	13	$\alpha 2$ - 898	1.045		2.3+2.3	60°C	
<b>C) Primers for ARMS-assay for the mutations Hb J-Oxford (<math>\alpha 1</math>-globin gene) and -<math>\alpha 3.7</math> +35/36 -AC</b>										
$\alpha 1$ cod 15 GTA>ATA	20	33803-22	F-AACGTCAAGGCCGCCTCGGA	11	$\alpha 1$ +773	709	P3 + P2	3.0+3.0	62°C	
- $\alpha 3.7$ +35/36 -AC	20	33754-73	F-ACAGACTCAGAGAGAACGCC	8	$\alpha 1$ +916	901	P1 + P2	2.5+2.5	60°C	
<b>D) “Common” primers</b>										
$\alpha 2$ + 904	25	34642-18	R-GTCTGAGACAGGTAAACACCTCCAT							
$\alpha 1$ + 773	23	38315-293	R-GAGGCCCAAGGGCAAGAAGCAT							
$\alpha 1$ + 916	25	38458-34	R-TGTGTGTCAGCTGCTGTCCACGC							
$\alpha 2$ - 898	23	32840-62	F-CCCAGAGCCAGGTTTGTATTCT							
$\alpha 2$ del 7bp	23	34293-315	F-CGGCTGCGGGCCTGGGCCGCACT							
<b>E) “Control” Primers and control-amplicons length</b>										
$\alpha$ intergene 5' (P1)	23	35943-65	F-AGGCTGTGGCAGAGTCAGAAGA			714				
$\alpha$ intergene 3' (P2)	22	36656-35	R-CAATAGCTGGAACCGGCTGGAG							
5' $\alpha$ (P3)	21	36309-29	F-AAGTCCACCCCTTCTTCTC			348				
$\alpha$ intergene 3' (P2)	22	36656-35	R-CAATAGCTGGAACCGGCTGGAG							