**Supplementary file 2: All the PCR primers, qRT PCR primers and probes used in this study**

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| --- | --- | --- | --- | --- |
| **Sl.no** | **Primer name** | **Primer sequence 5’- 3’** | **Amplicon size** | **Source** |
| **Primers to confirm the gRNA clones**  |
| 1 | Sequencing F | gagggcctatttcccatgat | 539bp | This study |
| 2 | Sequencing R | tggatctctgctgtccctgt |   | This study |
| **Sanger sequencing primer for *HBG* promoter region** |
| 3 | HBF 1 F  | acaaaagaagtcctggtatc  | 490bp | This study |
| 4 | HBF 2 F | ttactgcgctgaaactgtgg | 772bp | This study |
| 5 | HBF 1 R | cttcccagggtttctcctcc |   | This study |
| **qRT PCR primers to amplify globin gene expression** |
| 6 | HBB E2E3 RT F | acctttgccacactgagtgag | 110bp | This study |
| 7 | HBB E2E3 RT R | tttgccaaagtgatgggcca |   | This study |
| 8 | HBA RT F | cgacaagaccaacgtcaagg | 99bp | This study |
| 9 | HBA RT R | gtggggaaggacaggaacat |   | This study |
| 10 | HBF E2E3 RT F | cttccttgggagatgccata | 136bp | This study |
| 11 | HBF E2E3 RT R | aaaacggtcaccagcacatt |   | This study |
| 12 | GAPDH E7E8 RT F | ctgcaccaccaactgcttag | 110bp | This study |
| 13 | GAPDH E7E8 RT R | gtcttctgggtggcagtgat |   | This study |
| **NGS primers for *HBG* promoter region** |
| 14 | NGS 2 F | gctcttccgatct tgaatcggaacaaggcaaagg | 325bp | This study |
| 15 | NGS 2 R | gctcttccgatct gtgaaatgacccatggcgtc |   | This study |
| 16 | NGS 3 F | gctcttccgatct cctggacctatgcctaaaaca | 318bp | This study |
| 17 | NGS 3 R | gctcttccgatct agtttagccagggaccgttt |   | This study |
| 18 | NGS 4 F | gctcttccgatct cggctgacaaaagaagtcct |   | This study |
| **Primers to differentiate *HBG1* and *HBG2*** |
| 19 | HBG1 F | ccacagtacctgccaaagaa | 940bp | This study |
| 20 | HBG2 F | ccatagtatctggtaaagagca | 940bp | This study |
| 21 | HBG1/2 R | ggcgtctggactaggag |   | This study |
| **Sanger sequencing primer for *AAVS1* locus** |
| 22 | AAVS1 seqF | gagatggctccaggaaatgg | 420bp | Matthew J.J et al., Sci Rep,2018. |
| 23 | AAVS1 seqR | acctctcactcctttcatttgg |   | Matthew J.J et al., Sci Rep,2018. |
| **ABE 8e lenti plasmid cloning primers** |
| 24 | ABE 8e F | acacaggtgtcgtgacgcgggatccgccaccatgaaacggacagccgacgg | 4872bp | This study |
| 25 | ABE 8e R | agttggtggcgccgctgccgctagcgactttcctcttcttcttgg |   | This study |
| **qRT PCR primers to validate Vector copy number** |
| 26 | Cas 9 var RT F | ccgaagaggtcgtgaagaag | 128bp | This study |
| 27 | Cas 9 var RT R | gccttatccagttcgctcag |   | This study |
| 28 | U6 RT F | agggcctatttcccatgatt | 151bp | This study |
| 29 | U6 RT R | aaactgcaaactacccaagaaa |   | This study |
| 30 | WREP RT F | caccacctgtcagctccttt | 135bp | This study |
| 31 | WREP RT R | acaacaccacggaattgtca |   | This study |
| 32 | HBB locus ctrl F | ttggacccagaggttctttg | 123bp | This study |
| 33 | HBB locus ctrl F | gagccaggccatcactaaag |   | This study |
| **qRT PCR primers to validate 4.9kb large deletion** |
| 34 | Del\_qPCR\_F | aggggctcaacgaagaaaaagtgt |   | Chang Li et al., Blood, 2018. |
| 35 | Del\_qPCR\_R | cacttcattgtagttaccgtggaaaga |   | Chang Li et al., Blood, 2018. |
| 36 | Del\_ctrl\_qPCR\_F | aaatgaatcagcagaggctcac |   | Chang Li et al., Blood, 2018. |
| 37 | Del\_ctrl\_qPCR\_R | atgcactaacatccaactatacaaaa |   | Chang Li et al., Blood, 2018. |
| **Probes used for EMSA** |
| 38 | HBG2 -110 to -132 Wild type (WT) | ggccagccttgccttgaccaata | Sense | This study |
| 39 | HBG2 -110 to -132 Wild type (WT) | tattggtcaaggcaaggctggcc | Antisense | This study |
| 40 | HBG2 -110 to -132 -123T>C/-124T>C | ggccagccccgccttgaccaata | Sense | This study |
| 41 | HBG2 -110 to -132 -123T>C/-124T>C | tattggtcaaggcggggctggcc | Antisense | This study |
| 42 | Hbbt1 CACCC (KLF1 +ve control) | tagagccacaccctggtaag | Sense | Merlin C et al., Mol Cell Biol, 1996. |
| 43 | *Hbbt1 CACCC (KLF1 +ve control)* | cttaccagggtgtggctcta | Antisense | Merlin C et al., Mol Cell Biol, 1996. |
| **qRT PCR primers used for ChIP analysis** |
| 44 | *γ-globin* promoter -73 to -179 F | caaatatctgtctgaaacggtccc | 106 bp | Gabriella E. M et al., Blood, 2019. |
| 45 | *γ-globin* promoter -73 to -179 R | actctaagactattggtcaagtttgc |   | Gabriella E. M et al., Blood, 2019. |
| 46 | *γ-globin* promoter -199 to -106 F | tcaatgcaaatatctgtctgaaacg | 93bp | Gabriella E. M et al., Blood, 2019. |
| 47 | *γ-globin* promoter -199 to -106 R | caaggctattggtcaaggcaa |   | Gabriella E. M et al., Blood, 2019. |
| 48 | *SP1* promoter F | acctctccgcccactagga |   | Beeke Wienert et al., Blood, 2017. |
| 49 | *SP1* promoter R | caacggccaaccagaatcc |   | Beeke Wienert et al., Blood, 2017. |
| 50 | *VegfA* F | ggtttgtatcctgcccttc |   | Beeke Wienert et al., Blood, 2017. |
| 51 | *VegfA* R | actgggtcttgctgttttcc |   | Beeke Wienert et al., Blood, 2017. |
| **Control gRNA for CD34+ cells editing** |
| 52 | AAVS1 | ggggccactagggacaggat |   | Matthew J.J et al., Sci Rep,2018. |
| **Primers to amplify DNA off-target** |
| 53 | G11 OT1 F | tacacgacgctcttccgatct ggcagaggggacacatcagt | 192bp | This study |
| 54 | G11 OT1 R | agacgtgtgctcttccgatct tgcgctatgatgttgggtatgtcc |   | This study |
| 55 | G11 OT2 F | tacacgacgctcttccgatct acttaatgctcacagattggttcaatcaggc | 190bp | This study |
| 56 | G11 OT2 R | agacgtgtgctcttccgatct agccaatcaggttcaagatggcag |   | This study |
| 57 | G11 OT3 F | tacacgacgctcttccgatct gtctactggaaagcccatttgcatagtaag | 200bp | This study |
| 58 | G11 OT3 R | agacgtgtgctcttccgatct gtgagagagagaggacttctgag |   | This study |
| 59 | G11 OT4 F | tacacgacgctcttccgatct tcctcagttccaagccttggg | 194bp | This study |
| 60 | G11 OT4 R | agacgtgtgctcttccgatct gtcatccccaatccacaaactcac |   | This study |
| 61 | G11 OT5 F | tacacgacgctcttccgatct cacacgtgtcttatctgtcacctc | 193bp | This study |
| 62 | G11 OT5 R | agacgtgtgctcttccgatct ctgaccactcttgcaactccatg |   | This study |
| 63 | G11 OT6 F | tacacgacgctcttccgatct atcagggaaaaacctgccatgtgc | 191bp | This study |
| 64 | G11 OT6 R | agacgtgtgctcttccgatct gcaattcctctgttcaagacaggatgtag |   | This study |
| 65 | G11 OT7 F | tacacgacgctcttccgatct gggcaaggggaaacttcatttgca | 192bp | This study |
| 66 | G11 OT7 R | agacgtgtgctcttccgatct gagaggcgacttctgagagg |   | This study |
| 67 | G11 OT8 F | tacacgacgctcttccgatct ttacaggcatgaaccaccacatcc | 194bp | This study |
| 68 | G11 OT8 R | agacgtgtgctcttccgatct gtcagagaactgagcctaatggag |   | This study |
| 69 | G11 OT9 F | tacacgacgctcttccgatct cacttgagatcaggatttggagaccag | 192bp | This study |
| 70 | G11 OT9 R | agacgtgtgctcttccgatct gcgcaatcttgggtcactgca |   | This study |
| 71 | G11 OT10 F | tacacgacgctcttccgatct ggcccacagattggttagatcag | 194bp | This study |
| 72 | G11 OT10 R | agacgtgtgctcttccgatct ctacttgggactaggcatgttcag |   | This study |
| 73 | G11 OT11 F | tacacgacgctcttccgatct gggggctactctaataaaaagactttaggaac | 200bp | This study |
| 74 | G11 OT11 R | agacgtgtgctcttccgatct gttccttatcagcttatgtagattttgggctgag |   | This study |