**Table S2. sgRNA and primer sequences used for *hmx*2, *hmx3a* and *hmx2;hmx3a* CRISPR mutagenesis.**

| **SU Allele** | **Gene(s)** | **sgRNA(s) Sequence(s)** | **F0 Screening** | **Stable Mutant Screening** |
| --- | --- | --- | --- | --- |
| **Method(s)** | **Primer Sequences** | **Method(s)** | **Primer Sequences** |
| 35 | *hmx2* | GGTATGGATGGTGTCAAATG (E) | HRMA | FW: TGTTTGTGTGTCGCATTGGTTGRV: TGTCTTCAGAAACGGGAGACAT | Sequencing | FW: CAACAGTCGATCAAACAGTCAGAGRV: GTGGACTCGAGTTGATAAACCTG |
| 36 | *hmx2* | GGTATGGATGGTGTCAAATG (E) | HRMA | FW: TGTTTGTGTGTCGCATTGGTTGRV: TGTCTTCAGAAACGGGAGACAT | HRMA | FW: TGTTTGTGTGTCGCATTGGTTGRV: TGTCTTCAGAAACGGGAGACAT |
| 37 | *hmx2* | GGTATGGATGGTGTCAAATG (E) | HRMA | FW: TGTTTGTGTGTCGCATTGGTTGRV: TGTCTTCAGAAACGGGAGACAT | PCR | FW: CAACAGTCGATCAAACAGTCAGAGRV: GTGGACTCGAGTTGATAAACCTG |
| 38 | *hmx2* | 5’: GGTATGGATGGTGTCAAATG (E)3’: GGTTCCAGGATAATACAGTG (F) | PCR | FW: TCAACAGTCGATCAAACAGTCAGRV: GAACCGTGTTGCTTTTGTGTTTTAC | PCR | FW: TCAACAGTCGATCAAACAGTCAGRV: GAACCGTGTTGCTTTTGTGTTTTAC |
| 39 | *hmx2* | 5’: GGACCGGACTCCGTCGTTTG (C)3’: GGTTCCAGGATAATACAGTG (F) | Nested PCR | Nested 1FW: GCAAATGAGGGCACGTTTCACRV: GGACATATACAGAACCGTGTTGCNested 2FW: TGAATAATTCGGAGGACAGCGGAAGRV: CGTAGACTCGTTTAATTGCTGC | Nested PCR (for deletion) and WT amplicon\* | Nested 1FW: GCAAATGAGGGCACGTTTCACRV: GGACATATACAGAACCGTGTTGCNested 2FW: TGAATAATTCGGAGGACAGCGGAAGRV: CGTAGACTCGTTTAATTGCTGCWT AmpliconFW: CAACAGTCGATCAAACAGTCAGAGRV: GTGGACTCGAGTTGATAAACCTG |
| MENTHU | *hmx2* | TCGGAGGATGACTGCAGCGC (D) | T7 Endonuclease I | FW: GTCTATTCTGGGCACCTCAAACRV: GTACACCAGGCTCAGAGCAGTA | Sequencing | FW: GTCTATTCTGGGCACCTCAAACRV: GTACACCAGGCTCAGAGCAGTA |
| 42 | *hmx3a* | GGAATCTCGGTCGGTGCCTG (B) | HRMA | FW: CGAATGCTAATTTGGCCTCTATTACTRV: TTTTGTTGTCGTCTTCATCGTCC | PCR + restriction digest | FW: TGGCAAAGTGACACGACCAGRV: GAGAACACCGTGCGAGTTTTCRestriction enzyme: BanI |
| 3 | *hmx3a* | GGAATCTCGGTCGGTGCCTG (B) | HRMA | FW: CGAATGCTAATTTGGCCTCTATTACTRV: TTTTGTTGTCGTCTTCATCGTCC | PCR | FW: TGGCAAAGTGACACGACCAGRV: GAGAACACCGTGCGAGTTTTC |
| 43 | *hmx3a* | GGAATCTCGGTCGGTGCCTG (B) | Fluorescence and/or PCR | FW: GAGAACACCGTGCGAGTTTTCRV: AGTTTCCTGGCAGCATATCCAG | Fluorescence and/or PCR and WT amplicon\* | PCRFW: GAGAACACCGTGCGAGTTTTCRV: AGTTTCCTGGCAGCATATCCAGWT AmpliconFW: TGGCAAAGTGACACGACCAGRV: GAGAACACCGTGCGAGTTTTC |
| 44 | *hmx2;**hmx3a* | 5: GGGGCACGTACAATAGCAAA (A)3’: GGTTCCAGGATAATACAGTG (F) | Nested PCR | Nested 1:FW: GTTAGACTTTGCAATGCTCGTGRV: GGACATATACAGAACCGTGTTGCNested 2:FW: CCTTTTTGCTATTGCCTTCATCRV: CGTAGACTCGTTTAATTGCTGC | Nested PCR (for deletion) and WT amplicon\* | Nested 1:FW: GTTAGACTTTGCAATGCTCGTGRV: GGACATATACAGAACCGTGTTGCNested 2:FW: CCTTTTTGCTATTGCCTTCATCRV: CGTAGACTCGTTTAATTGCTGCWT AmpliconFW: TGGCAAAGTGACACGACCAGRV: GAGAACACCGTGCGAGTTTTC |
| 45 | *hmx2;**hmx3a* | 5: GGGGCACGTACAATAGCAAA (A)3’: GGTTCCAGGATAATACAGTG (F) | Nested PCR | Nested 1:FW: GTTAGACTTTGCAATGCTCGTGRV: GGACATATACAGAACCGTGTTGCNested 2:FW: CCTTTTTGCTATTGCCTTCATCRV: CGTAGACTCGTTTAATTGCTGC | Nested PCR (for deletion) and WT amplicon\* | Nested 1:FW: GTTAGACTTTGCAATGCTCGTGRV: GGACATATACAGAACCGTGTTGCNested 2:FW: CCTTTTTGCTATTGCCTTCATCRV: CGTAGACTCGTTTAATTGCTGCWT AmpliconFW: TGGCAAAGTGACACGACCAGRV: GAGAACACCGTGCGAGTTTTC |

Column 1 lists SU allele designation. Column 2 provides the name of the targeted gene or genes. Column 3 shows the sgRNA sequences used. The letter in brackets after each sgRNA sequence corresponds to the sgRNA locations depicted by the letters A-F in Figure 4. Columns 4 and 5 indicate the method and primer sequences used to screen founder fish (HRMA = high resolution melt analysis). Columns 6 and 7 indicate the method and primer sequences used to screen stable mutant fish. \*In the case of large deletion alleles (where the WT PCR product would be too large to detect by standard PCR conditions), a second PCR (a WT amplicon PCR) is performed. The genomic region amplified by this PCR is only present in WT and heterozygous animals. For experimental conditions, see materials and methods.