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| --- | --- | --- | --- | --- |
| Mutation | Primer  | Sequence (altered base(s) underlined) | Generated Plasmid | Original Template |
| S131P/S263A | S131P: HDK1062 | CTCCGGTGGCCATCTCAACCCTGCCGTCACCGTTGGCAA | pHDK166 | pRPaGFP-AQP2 |
| S131P: HDK1063 | TTGCCAACGGTGACGGCAGGGTTGAGATGGCCACCGGAG |
| S263A: HDK1064 | TCTCCCCTTGCGATGAATCCCGCACTTGATTTCGGTCCCAGGG |
| S263A: HDK1065 | CCCTGGGACCGAAATCAAGTGCGGGATTCATCGCAAGGGGAGA |
| I110W | HDK607 | CTCGGTCTTACGATTGGTTGGGGTGTGGCTGTCACGATG | pHDK84 | pRPaGFP-AQP2 |
| HDK608 | CATCGTGACAGCCACACCCCAACCAATCGTAAGACCGAG |
| L264R | HDK609 | TCTCCCCTTGCGATGAATCCCTCACGTGATTTCGGTCCCAGGGTCTTC | pHDK167 | pRPaGFP-AQP2 |
| HDK610 | GAAGACCCTGGGACCGAAATCACGTGAGGGATTCATCGCAAGGGGAGA |
| I110W/L264R | L264R: HDK609 | TCTCCCCTTGCGATGAATCCCTCACGTGATTTCGGTCCCAGGGTCTTC | pHDK78 | pHDK84 |
| L264R: HDK610 | GAAGACCCTGGGACCGAAATCACGTGAGGGATTCATCGCAAGGGGAGA |
| L258Y | HDK1109 | CAACTTCGGCTTAGCGTCTCCCTATGCGATGAATCCCTCACTTGAT | pHDK168 | pRPaGFP-AQP2 |
| HDK1110 | ATCAAGTGAGGGATTCATCGCATAGGGAGACGCTAAGCCGAAGTTG |
| I190T | HDK1056 | GCCTTCGGTGAAAAGGGGACTGCGTGGGTGTTTGCCATG | pHDK163 | pRPaGFP-AQP2 |
| HDK1057 | CATGGCAAACACCCACGCAGTCCCCTTTTCACCGAAGGC |
| W192G | HDK1058 | CGGTGAAAAGGGGATTGCGGGGGTGTTTGCCATGTACCC | pHDK164 | pRPaGFP-AQP2 |
| HDK1059 | GGGTACATGGCAAACACCCCCGCAATCCCCTTTTCACCG |
| I190T/W192G | HDK1060 | GCCTTCGGTGAAAAGGGGACTGCGGGGGTGTTTGCCATGTACCC | pHDK165 | pHDK163 |
| HDK1061 | GGGTACATGGCAAACACCCCCGCAGTCCCCTTTTCACCGAAGGC |
| L84W | HDK1276 | AAACTTCGTCTGGATATATATCGCTAAGGG | pHDK210 | pRPaGFP-AQP2 |
| HDK1277 | CCCAGAAATTCAGCCACG |
| L118W | HDK1274 | CACCGCAGTGTGGCTGCTCTGTG | pHDK208 | pRPaGFP-AQP2 |
| HDK1275 | GAAATGAGTTCAGCAAAAATTG |
| L218W | HDK1272 | CACGATGGCTTGGTATGTTTCACTG | pHDK209 | pRPaGFP-AQP2 |
| HDK1273 | ACAGCCACACCAATACCA |
| L84W/L118W | HDK1276 | AAACTTCGTCTGGATATATATCGCTAAGGG | pHDK227 | pHDK208 |
| HDK1277 | CCCAGAAATTCAGCCACG |
| L84M | HDK1364 | AAACTTCGTCATGATATATATCGCTAAGG | pHDK234 | pHDK210 |
| HDK1367 | GAAATGAGTTCAGCAAAAATTGGATAAAATATAC |
| L118M | HDK1365 | CACGATGGCTATGTATGTTTCAC | pHDK235 | pHDK208 |
| HDK1367 | GAAATGAGTTCAGCAAAAATTGGATAAAATATAC |
| L218M | HDK1366 | CACCGCAGTGATGCTGCTCTGTG | pHDK236 | pHDK209 |
| HDK1367 | GAAATGAGTTCAGCAAAAATTGGATAAAATATAC |

**Supplementary File 4. List of primers used in the construction of mutations in TbAQP2.**