

**Additional file 2: Table S2** Primers employed for generating gene knockout strains.

| Primer                    | Sequence (5'→3')                            | Use                                                                                    |
|---------------------------|---------------------------------------------|----------------------------------------------------------------------------------------|
| DHFR-Gibson-Fw            | AAGCTTCGCCAGGCTGTAAATCC                     | Amplify the DHFR fragment to construct the pUPRT-DHFR-D plasmid                        |
| DHFR-Gibson-Rv            | GAATTCATCCTGCAAGTGCATAG                     | Amplify the DHFR fragment to construct the pUPRT-DHFR-D plasmid                        |
| pUC19-Gibson-Fw           | TGTGAAATTGTTATCCGCTC                        | Amplify the pUC19 fragment to construct the pUPRT-DHFR-D plasmid                       |
| pUC19-Gibson-Rv           | AACGTCGTGACTGGGAAAACC                       | Amplify the pUC19 fragment to construct the pUPRT-DHFR-D plasmid                       |
| sgRNA-KO-Rv               | AACTTGACATCCCCATTAC                         | Construct the CRISPR plasmid for deleting ROP genes (universal primer)                 |
| PCR3-DHFR-Rv              | GCCAAAGTAGAAAGGAATTAGCAT                    | Detect the insertion of 5' homologous fragment of ROP genes in PCR3 (universal primer) |
| PCR5-DHFR-Fw              | TGACGCAGATGTGCGTGTATCCAC                    | Detect the insertion of 3' homologous fragment of ROP genes in PCR5 (universal primer) |
| sgRNA-TGME49_312150-KO    | GCAGAAGGAATTCTCGGCCA                        | Designed SgRNA-KO sequence for TGME49_312150                                           |
| sgRNA-TGME49_312150-KO-Fw | GCAGAAGGAATTCTCGGCCAGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_312150                                |
| U5-TGME49_312150-Fw       | GGTTTTCCCAGTCACGACGTTGTGAAGCAAGTGCGATAGATG  | Amplify the 5' homologous arms of TGME49_312150 to construct the pUPRT-DHFR-D plasmid  |
| U5-TGME49_312150-Rv       | GGATTACAGCCTGGCGAAGCTTCCATTTTTCGTGCGTGCAAG  | Amplify the 5' homologous arms of TGME49_312150 to construct the pUPRT-DHFR-D plasmid  |
| U3-TGME49_312150-Fw       | CTATGCACTTGCAGGATGAATTCGTCGTTGTCACATCAGACTG | Amplify the 3' homologous arms of TGME49_312150 to construct the pUPRT-DHFR-D plasmid  |
| U3-TGME49_312150-Rv       | GAGCGGATAACAATTTACACAACATGCAGGTCAGTTGTC     | Amplify the 3' homologous arms of TGME49_312150 to construct the pUPRT-DHFR-D plasmid  |
| PCR3-TGME49_312150-Fw     | GTGTGACAAGTGCAACAGAG                        | Detect the insertion of 5' homologous fragment of TGME49_312150 in PCR3                |
| PCR4-TGME49_312150-Fw     | GTGACCATGCCATTCTCTTG                        | Detect the deletion of TGME49_312150 in PCR4                                           |
| PCR4-TGME49_312150-Rv     | CAGACGCATTCAACTTACTGT                       | Detect the deletion of TGME49_312150 in PCR4                                           |
| PCR5-TGME49_312150-Rv     | GCTGTGTGTCGAAACATCTC                        | Detect the insertion of 3' homologous fragment of TGME49_312150 in PCR5                |
| sgRNA-TGME49_246178-KO    | GCTACTGGATCCGCTTCCGG                        | Designed SgRNA-KO sequence for TGME49_246178                                           |

|                           |                                             |                                                                                       |
|---------------------------|---------------------------------------------|---------------------------------------------------------------------------------------|
| sgRNA-TGME49_246178-KO-Fw | GCTACTGGATCCGCTTCCGGGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_246178                               |
| U5-TGME49_246178-Fw       | GGTTTTCCCAGTCACGACGTTGAACCGTCCTTGTGATCGTC   | Amplify the 5' homologous arms of TGME49_246178 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_246178-Rv       | GGATTTACAGCCTGGCGAAGCTTGGACGAGACAGCTGATGATC | Amplify the 5' homologous arms of TGME49_246178 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_246178-Fw       | CTATGCACTTGCAGGATGAATTCGACGAAGACGTCGAACACG  | Amplify the 3' homologous arms of TGME49_246178 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_246178-Rv       | GAGCGGATAACAATTTACAGCCTCATGTGTGGTTTGCTG     | Amplify the 3' homologous arms of TGME49_246178 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_246178-Fw     | GCATCTCCTTTCGCAGCTTC                        | Detect the insertion of 5' homologous fragment of TGME49_246178 in PCR3               |
| PCR4-TGME49_246178-Fw     | GACTTGTGTATCTCGAGTGCG                       | Detect the deletion of TGME49_246178 in PCR4                                          |
| PCR4-TGME49_246178-Rv     | GCTTCGATTTGAGGAGCCAC                        | Detect the deletion of TGME49_246178 in PCR4                                          |
| PCR5-TGME49_246178-Rv     | GTACGAGGGAAGTCTGCAAG                        | Detect the insertion of 3' homologous fragment of TGME49_246178 in PCR5               |
| sgRNA-TGME49_254070-KO    | GCGCTCGGACAACGTCAACG                        | Designed SgRNA-KO sequence for TGME49_254070                                          |
| sgRNA-TGME49_254070-KO-Fw | GCGCTCGGACAACGTCAACGGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_254070                               |
| U5-TGME49_254070-Fw       | GGTTTTCCCAGTCACGACGTTGACTAGCGTAGCGGTTACTTC  | Amplify the 5' homologous arms of TGME49_254070 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_254070-Rv       | GGATTTACAGCCTGGCGAAGCTTGATGTAAACCACCGCTGCTC | Amplify the 5' homologous arms of TGME49_254070 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_254070-Fw       | CTATGCACTTGCAGGATGAATTCAGCGTGTAGCGTCGTAAAGC | Amplify the 3' homologous arms of TGME49_254070 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_254070-Rv       | GAGCGGATAACAATTTACAGTGTCAACAGGGCGATATG      | Amplify the 3' homologous arms of TGME49_254070 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_254070-Fw     | GTCAGTCCTGGACTCGAGAG                        | Detect the insertion of 5' homologous fragment of TGME49_254070 in PCR3               |
| PCR4-TGME49_254070-Fw     | GGCAGTAGACTAGATTGCGC                        | Detect the deletion of TGME49_254070 in PCR4                                          |
| PCR4-TGME49_254070-Rv     | CCATTGGTGAATTCAGTTGCC                       | Detect the deletion of TGME49_254070 in PCR4                                          |
| PCR5-TGME49_254070-Rv     | GAGAGGCAGCTACAAGTCAG                        | Detect the insertion of 3' homologous fragment of TGME49_254070 in PCR5               |
| sgRNA-TGME49_254880-KO    | GGACAGCATGAAGAAGATGG                        | Designed SgRNA-KO sequence for TGME49_254880                                          |

|                           |                                            |                                                                                       |
|---------------------------|--------------------------------------------|---------------------------------------------------------------------------------------|
| sgRNA-TGME49_254880-KO-Fw | GGACAGCATGAAGAAGATGGGTTTTAGAGCTAGAAATAGC   | Construct the CRISPR plasmid for deleting TGME49_254880                               |
| U5-TGME49_254880-Fw       | GGTTTTCCCAGTCACGACGTTGGTACAGCTAGCTGGTAGGAG | Amplify the 5' homologous arms of TGME49_254880 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_254880-Rv       | GGATTTACAGCCTGGCGAAGCTTCACGAACCGGCTCGAATG  | Amplify the 5' homologous arms of TGME49_254880 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_254880-Fw       | CTATGCACTTGCAGGATGAATTCGTGAACAGCCAGAGGCAAC | Amplify the 3' homologous arms of TGME49_254880 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_254880-Rv       | GAGCGGATAACAATTTACAGAAGAGAAGCGTCTCTCCTC    | Amplify the 3' homologous arms of TGME49_254880 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_254880-Fw     | GACGCTGCAGACTCAAGGTC                       | Detect the insertion of 5' homologous fragment of TGME49_254880 in PCR3               |
| PCR4-TGME49_254880-Fw     | GTTGCTGGCCTAGAACGTATC                      | Detect the deletion of TGME49_254880 in PCR4                                          |
| PCR4-TGME49_254880-Rv     | GACTAGCTGCTATGTAGGACG                      | Detect the deletion of TGME49_254880 in PCR4                                          |
| PCR5-TGME49_254880-Rv     | GTGGCTCGTCTACCGTTCAG                       | Detect the insertion of 3' homologous fragment of TGME49_254880 in PCR5               |
| sgRNA-TGME49_264600-KO    | GTTAATGCTTGTGTGAACCG                       | Designed SgRNA-KO sequence for TGME49_264600                                          |
| sgRNA-TGME49_264600-KO-Fw | GTTAATGCTTGTGTGAACCGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_264600                               |
| U5-TGME49_264600-Fw       | GGTTTTCCCAGTCACGACGTTGTAATTCTCGGAGCCTCTGC  | Amplify the 5' homologous arms of TGME49_264600 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_264600-Rv       | GGATTTACAGCCTGGCGAAGCTTGTTTTCTGCAGGATGTCCG | Amplify the 5' homologous arms of TGME49_264600 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_264600-Fw       | CTATGCACTTGCAGGATGAATTCGCTCATGAACGTGCTACAC | Amplify the 3' homologous arms of TGME49_264600 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_264600-Rv       | GAGCGGATAACAATTTACAGACTGTCGTTGATAGTGGCTG   | Amplify the 3' homologous arms of TGME49_264600 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_264600-Fw     | GTACCTGGAATGTCTCGTGC                       | Detect the insertion of 5' homologous fragment of TGME49_264600 in PCR3               |
| PCR4-TGME49_264600-Fw     | GTACATTCGCCTGGAGGTG                        | Detect the deletion of TGME49_264600 in PCR4                                          |
| PCR4-TGME49_264600-Rv     | GCGATGTTGCAGAGTCGTC                        | Detect the deletion of TGME49_264600 in PCR4                                          |
| PCR5-TGME49_264600-Rv     | GCTAGGTGTCACGGAACAAG                       | Detect the insertion of 3' homologous fragment of TGME49_264600 in PCR5               |
| sgRNA-TGME49_270200-KO    | GGAATCTGCGATTGAAGCGT                       | Designed SgRNA-KO sequence for TGME49_270200                                          |

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|---------------------------|---------------------------------------------|---------------------------------------------------------------------------------------|
| sgRNA-TGME49_270200-KO-Fw | GGAATCTGCGATTGAAGCGTGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_270200                               |
| U5-TGME49_270200-Fw       | GGTTTTCCCAGTCACGACGTTGCAAGTGTGAGAAGGCAAGC   | Amplify the 5' homologous arms of TGME49_270200 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_270200-Rv       | GGATTTACAGCCTGGCGAAGCTTGACTCGCGGAATATTGTCC  | Amplify the 5' homologous arms of TGME49_270200 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_270200-Fw       | CTATGCACTTGCAGGATGAATTCGGTTGGAGCAGATGCAGATC | Amplify the 3' homologous arms of TGME49_270200 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_270200-Rv       | GAGCGGATAACAATTTACAGGCAAGGTATCGCATATACG     | Amplify the 3' homologous arms of TGME49_270200 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_270200-Fw     | GCTGTCGTCATCGCATATACC                       | Detect the insertion of 5' homologous fragment of TGME49_270200 in PCR3               |
| PCR4-TGME49_270200-Fw     | GACTGGTCGTCGGTCTGTTC                        | Detect the deletion of TGME49_270200 in PCR4                                          |
| PCR4-TGME49_270200-Rv     | GAGATGAAGCGAGCACAGC                         | Detect the deletion of TGME49_270200 in PCR4                                          |
| PCR5-TGME49_270200-Rv     | GTGTCAGCGTTCTGACTGTG                        | Detect the insertion of 3' homologous fragment of TGME49_270200 in PCR5               |
| sgRNA-TGME49_271270-KO    | GCATGTCGACTCAACACAGG                        | Designed SgRNA-KO sequence for TGME49_271270                                          |
| sgRNA-TGME49_271270-KO-Fw | GCATGTCGACTCAACACAGGGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_271270                               |
| U5-TGME49_271270-Fw       | GGTTTTCCCAGTCACGACGTTGAAGATATGCCAGCGACCAC   | Amplify the 5' homologous arms of TGME49_271270 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_271270-Rv       | GGATTTACAGCCTGGCGAAGCTTGCACTGATGCCACAGGTAG  | Amplify the 5' homologous arms of TGME49_271270 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_271270-Fw       | CTATGCACTTGCAGGATGAATTCGCTCGCTTGTCTGTGCATG  | Amplify the 3' homologous arms of TGME49_271270 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_271270-Rv       | GAGCGGATAACAATTTACAGCATCAGTGCATCTAGCTTG     | Amplify the 3' homologous arms of TGME49_271270 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_271270-Fw     | GCTCTTGGTCACTTGAAGGT                        | Detect the insertion of 5' homologous fragment of TGME49_271270 in PCR3               |
| PCR4-TGME49_271270-Fw     | GTCTACCTGCAGCGTATATGG                       | Detect the deletion of TGME49_271270 in PCR4                                          |
| PCR4-TGME49_271270-Rv     | GTGCGTGAAGGTAATGTGTC                        | Detect the deletion of TGME49_271270 in PCR4                                          |
| PCR5-TGME49_271270-Rv     | GAGTCTGGTGAGTCTCTGAG                        | Detect the insertion of 3' homologous fragment of TGME49_271270 in PCR5               |
| sgRNA-TGME49_273860-KO    | GATGAAGATTCGGCAGAAGG                        | Designed SgRNA-KO sequence for TGME49_273860                                          |

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|---------------------------|---------------------------------------------|---------------------------------------------------------------------------------------|
| sgRNA-TGME49_273860-KO-Fw | GATGAAGATTCGGCAGAAGGGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_273860                               |
| U5-TGME49_273860-Fw       | GGTTTTCCCAGTCACGACGTTGTGTGAGCAGAGTCACCATTG  | Amplify the 5' homologous arms of TGME49_273860 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_273860-Rv       | GGATTTACAGCCTGGCGAAGCTTGACGAAGAAACCTGGTTACG | Amplify the 5' homologous arms of TGME49_273860 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_273860-Fw       | CTATGCACTTGCAGGATGAATTCTACACGTGGAGAATCGAAGC | Amplify the 3' homologous arms of TGME49_273860 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_273860-Rv       | GAGCGGATAACAATTTACAGGTGTAGGCAGATGGATACAC    | Amplify the 3' homologous arms of TGME49_273860 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_273860-Fw     | GGACGTCAGGATCTATAACCAG                      | Detect the insertion of 5' homologous fragment of TGME49_273860 in PCR3               |
| PCR4-TGME49_273860-Fw     | GCCAGCAGAATGTACTATGTC                       | Detect the deletion of TGME49_273860 in PCR4                                          |
| PCR4-TGME49_273860-Rv     | GCACCTTGTATTTACCGCTAG                       | Detect the deletion of TGME49_273860 in PCR4                                          |
| PCR5-TGME49_273860-Rv     | GCATATCATACGCGTTCCG                         | Detect the insertion of 3' homologous fragment of TGME49_273860 in PCR5               |
| sgRNA-TGME49_279420-KO    | GCAGCTGAGGAGGCACGCGA                        | Designed SgRNA-KO sequence for TGME49_279420                                          |
| sgRNA-TGME49_279420-KO-Fw | GCAGCTGAGGAGGCACGCGAGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_279420                               |
| U5-TGME49_279420-Fw       | GGTTTTCCCAGTCACGACGTTGTGAGTGCTCTCCAACATCC   | Amplify the 5' homologous arms of TGME49_279420 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_279420-Rv       | GGATTTACAGCCTGGCGAAGCTTGAGCTGAAGCGAAGACATGC | Amplify the 5' homologous arms of TGME49_279420 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_279420-Fw       | CTATGCACTTGCAGGATGAATTCGGTAGCAGCACAACCAATAG | Amplify the 3' homologous arms of TGME49_279420 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_279420-Rv       | GAGCGGATAACAATTTACAGATACAGTAGCTCAGCACATG    | Amplify the 3' homologous arms of TGME49_279420 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_279420-Fw     | GTGGATGTCGGTCATGGAC                         | Detect the insertion of 5' homologous fragment of TGME49_279420 in PCR3               |
| PCR4-TGME49_279420-Fw     | CATGAGCATGTCTTCGCTTC                        | Detect the deletion of TGME49_279420 in PCR4                                          |
| PCR4-TGME49_279420-Rv     | GGATTCAATCTGGTTGCTGC                        | Detect the deletion of TGME49_279420 in PCR4                                          |
| PCR5-TGME49_279420-Rv     | GCGACAATGAACACACCAC                         | Detect the insertion of 3' homologous fragment of TGME49_279420 in PCR5               |
| sgRNA-TGME49_305270-KO    | GTTCCGCCTGAATCCACCGA                        | Designed SgRNA-KO sequence for TGME49_305270                                          |

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|---------------------------|---------------------------------------------|---------------------------------------------------------------------------------------|
| sgRNA-TGME49_305270-KO-Fw | GTTCCGCCTGAATCCACCGAGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_305270                               |
| U5-TGME49_305270-Fw       | GGTTTTCCCAGTCACGACGTTGTTCCCTTCGTGTGTCTTTGC  | Amplify the 5' homologous arms of TGME49_305270 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_305270-Rv       | GGATTTACAGCCTGGCGAAGCTTGGAACAAGGCAAGCAAGAG  | Amplify the 5' homologous arms of TGME49_305270 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_305270-Fw       | CTATGCACTTGCAGGATGAATTCCATCGTTGAGAATCCTCCAG | Amplify the 3' homologous arms of TGME49_305270 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_305270-Rv       | GAGCGGATAACAATTTACACAGTCGATTCCGATAACGATC    | Amplify the 3' homologous arms of TGME49_305270 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_305270-Fw     | CGCACATCAGTGA CTTATCTC                      | Detect the insertion of 5' homologous fragment of TGME49_305270 in PCR3               |
| PCR4-TGME49_305270-Fw     | GGTCGGGCGTAAGTTTCA                          | Detect the deletion of TGME49_305270 in PCR4                                          |
| PCR4-TGME49_305270-Rv     | TGTTGTCCAGCGGTGTTT                          | Detect the deletion of TGME49_305270 in PCR4                                          |
| PCR5-TGME49_305270-Rv     | GGACAACACTCAAGTCACGAG                       | Detect the insertion of 3' homologous fragment of TGME49_305270 in PCR5               |
| sgRNA-TGME49_306895-KO    | GTCAACCACCTCATTGCTGG                        | Designed SgRNA-KO sequence for TGME49_306895                                          |
| sgRNA-TGME49_306895-KO-Fw | GTCAACCACCTCATTGCTGGGTTTTAGAGCTAGAAATAGC    | Construct the CRISPR plasmid for deleting TGME49_306895                               |
| U5-TGME49_306895-Fw       | GGTTTTCCCAGTCACGACGTTGACGTA ACTGGTGGACCTCG  | Amplify the 5' homologous arms of TGME49_306895 to construct the pUPRT-DHFR-D plasmid |
| U5-TGME49_306895-Rv       | GGATTTACAGCCTGGCGAAGCTTGTGTTGATTGAGCAGTGTCC | Amplify the 5' homologous arms of TGME49_306895 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_306895-Fw       | CTATGCACTTGCAGGATGAATTCGCACTGCACATCTTCCTCTC | Amplify the 3' homologous arms of TGME49_306895 to construct the pUPRT-DHFR-D plasmid |
| U3-TGME49_306895-Rv       | GAGCGGATAACAATTTACAGGCACTCTAGTGTACTCTACG    | Amplify the 3' homologous arms of TGME49_306895 to construct the pUPRT-DHFR-D plasmid |
| PCR3-TGME49_306895-Fw     | CAGACAGTAGTCAGCAAGCG                        | Detect the insertion of 5' homologous fragment of TGME49_306895 in PCR3               |
| PCR4-TGME49_306895-Fw     | GAGTTAGCTGTGCACTGTCA                        | Detect the deletion of TGME49_306895 in PCR4                                          |
| PCR4-TGME49_306895-Rv     | GCTAGTGGAGGCTCGATCAG                        | Detect the deletion of TGME49_306895 in PCR4                                          |
| PCR5-TGME49_306895-Rv     | GAGCACATCGTCACATGC                          | Detect the insertion of 3' homologous fragment of TGME49_306895 in PCR5               |