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| **Supplemental Table 1. Strains, Plasmids and Primers**  |
| ***E. coli* Strains used for clone screening and protein expression** |
| *Strain* | *Usage* |
| DH5α | Clone screening |
| BL21 (DE3) | Expression of the synthase domain of *Rt*MprF |
| C41 (DE3) | Expression of the full length *Rt*MprF, *Rt*MprF D234A/Y303A/R304A/E280Q/E280K/D753A mutant, *RtPa*MprF |
| **Plasmids used for protein expression**  |
| *Plasmid* | *Gene*  | *Restriction site* |
| pET21b | the synthase domain of *Rt*MprF*,* Full length *Rt*MprF, *Rt*MprF D234A/Y303A/R304A/E280Q/E280K/D753A mutant, *RtPa*MprF | Nde I, Hind III |
| **Primers used for plasmid construction** |
| *Construct* | *Primer Type* | *Primer Sequence* |
| *Rt*MprF | Forward primer | 5’-GATATGACCATATGTCGAGCCCAATCGATC-3’ |
| Reverse primer | 5'-ATGTTAAGCTTTTTCCTCACGACCCCCT- 3' |
| *Rt*MprF-Synthase | Forward primer | 5’-GGGAATTCCATATGGACGATGCGGTG-3’ |
| Reverse primer | 5’-CCCAAGCTTTCCGCCCCCGAT-3’ |
| *RtPa*MprF | Forward primer | 5’-CCGGCAACGAAGCGGCCGGAGCCTGTCAGCGCGGAAGAGCTG-3’ |
| Reverse primer | 5’-GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTGCGTTTCACCAA-3’ |
| *Rt*MprF-D234A | Forward primer | 5’-CACCGCATTCGCGATCGCGGCCTCG-3’ |
| Reverse primer | 5’-CGAGGCCGCGATCGCGAATGCGGTG-3’ |
| *Rt*MprF-Y303A | Forward primer | 5’-GTTCTCTGGTCCTCGCGCGCGTCATCTACAAC-3’ |
| Reverse primer | 5’-GTTGTAGATGACGCGCGCGAGGACCAGAGAAC-3’ |
| *Rt*MprF-R304A | Forward primer | 5’-CTGGTCCTCTATGCGGTCATCTACAAC-3’ |
| Reverse primer | 5’-GTTGTAGATGACCGCATAGAGGACCAG-3’ |
| *Rt*MprF-Y307A | Forward primer | 5’-CTATCGCGTCATCGCGAACGTCATTCC-3’ |
| Reverse primer | 5’-GGAATGACGTTCGCGATGACGCGATAG-3’ |
| *Rt*MprF-E280Q | Forward primer | 5’-CTTCGGCGTGTTCCAGACGATCATC-3’ |
| Reverse primer | 5’-GATGATCGTCTGGAACACGCCGAAG-3’ |
| *Rt*MprF-E280K | Forward primer | 5’-CTTCGGCGTGTTCAAAACGATCATCATC-3’ |
| Reverse primer | 5’-GATGATGATCGTTTTGAACACGCCGAAG-3’ |
| *Rt*MprF-D753A | Forward primer | 5’-AGGGATCCGTCGCGCTGATGCGTTTTT-3’ |
| Reverse primer | 5’-AAAAACGCATCAGCGCGACGGATCCCT-3’ |