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| **Supplementary File 2.** Plasmids used in this study. | | | | |
| **Plasmids** | **Relevant genotypes or characteristics** | **Plasmid construction** | | **References** |
| **PCR templatea** | **PCR primersb** |
| pET22b(+) | P*T7lacO* inducible expression vector, contains N-terminal PelB signal peptide for periplasmic localization; AmpR | - | - | Novagen |
| pET23/42 | P*T7* inducible expression vector, contains multiple cloning site of pET42a(+) in pET23a(+) backbone, C-terminal His8-tag; AmpR | - | - | (Wu et al., 2006) |
| pET23/42(N) | P*T7* inducible expression vector, contains the first multiple cloning site of pETDuet-1 in pET23a(+) backbone, N-terminal His6-tag; AmpR | - | See Materials and Methods | This study |
| pET22/42 | P*T7lacO* inducible expression vector, contains multiple cloning site of pET42a(+) in pET22b(+) backbone; AmpR | - | - | (Chng et al., 2010b) |
| pCDF Duet-1 | P*T7lacO-*dependent dual expression vector; SmR, SpecR | - | - | Novagen |
| pET23/42*mlaF-His* | Encodes full length MlaF with C-terminal His8 tag; AmpR (p*mlaF-His*) | ch. DNA | MlaF-N-NdeI FWD MlaF-C-XhoI REV | This study |
| pET23/42*His-mlaE* | Encodes full length MlaE with N-terminal His6 tag; AmpR (p*His-mlaE*) | ch. DNA | MlaE-N-EcoRI FWD MlaE-C-AvrII REV | This study |
| pET23/42*mlaD-His* | Encodes full length MlaD with C-terminal His8 tag; AmpR (p*mlaD-His*) | ch. DNA | MlaD-N-NdeI FWD MlaD-C-XhoI REV | This study |
| pET23/42*His-mlaB* | Encodes full length MlaB with N-terminal His6 tag; AmpR (p*His-mlaB*) | ch. DNA | MlaB-N-EcoRI FWD MlaB-C-AvrII REV | This study |
| pET22/42*mlaF(His-E)DCB* | Encodes full length MlaF, MlaE, MlaD, MlaC and MlaB with N-terminal His6 tag before MlaE; AmpR | ch. DNA | See Materials and Methods | This study |
| **Plasmids** | **Relevant genotypes or characteristics** | **Plasmid construction** | | **References** |
| **PCR templatea** | **PCR primersb** |
| pET22/42*mlaF(His-E)D* | Encodes full length MlaF, MlaE and MlaD with N-terminal His6 tag before MlaE; AmpR | pET22/42*mlaF(His-E)DCB* | MlaF-N-NdeI FWD MlaD-C-AvrII REV | This study |
| pET22/42*mlaF(His-E)* | Encodes full length MlaF and MlaE with N-terminal His6 tag before MlaE; AmpR | pET22/42*mlaF(His-E)DCB* | MlaF-N-NdeI FWD MlaE-C-AvrII REV | This study |
| pET22/42*mlaFK46R(His-E)* | Encodes full length MlaFK46R and MlaE with N-terminal His6 tag before MlaE; AmpR | pET22/42*mlaF(His-E)* | MlaF-K46R-N FWD MlaF-K46R-C REV | This study |
| pET22/42*mlaF-His* | Encodes full length MlaF with C-terminal His8 tag; AmpR | ch. DNA | MlaF-N-NdeI FWD MlaF-C-XhoI REV | This study |
| pET22/42*mlaFK46R-His* | Encodes full length MlaFK46R with C-terminal His8 tag; AmpR | pET22/42*mlaF-His* | MlaF-K46R-N FWD MlaF-K46R-C REV | This study |
| pCDF*mlaB* | Encodes full length MlaB (replaces both cloning sites); SmR, SpecR | ch. DNA | MlaB-N-NcoI FWD MlaB-C-XhoI REV | This study |
| pCDF*mlaBT52A* | Encodes full length MlaBT52A (replaces both cloning sites); SmR, SpecR | pCDF*mlaB* | MlaB-T52A-N FWD MlaB-T52A-C REV | This study |
| pET22/42s*mlaD-His* | Encodes soluble domain of MlaD (a.a. 29-183) with C-terminal His8 tag; AmpR | ch. DNA | MlaD-SD-N-NdeI-FWD MlaD-SD-C-XhoI-REV | This study |
| pET22b*lolB-His* | Encodes LolB (a.a. 23-207) with a C-terminal His6 tag and the *pelB* signal peptide; AmpR | - | - | (Chng et al., 2010b) |

a ch. DNA = MC4100 chromosomal DNA

b primer sequences are listed in Supplementary File 3.