**Supplementary file 1A. Strains used in this work.**

|  |  |  |
| --- | --- | --- |
| Strain | Genotype | Reference |
| MC4100 | F-Δ*lac*U169 *araD139 rpsL150 relA1 ptsF rbs flbB5301* | (56) |
| DADE | As MC4100, ∆*tatABCD* ∆*tatE* | (57) |
| MCDSSAC | As MC4100, *amiA*∆2-33, *amiC*∆2-32 | (26) |
| MCDSSACtat | As MCDSSAC, ∆*tatABC*::Apra | (22) |
| HS3018-A | F- Δ*lac*U169 *araD*139 *rpsL thi MalTc*-1 Δ*malE*444, AraR | (58) |
| HS3018-Atat | As HS3018-A, ∆*tatABCD* ∆*tatE* | (58) |
| DH5 | F- *endA1* *gln*V44 *thi*-1 *recA1* *relA1* *gyrA96* *deoR* nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, *hsdR17*(rK- mK+), λ– | Stratagene |

**Supplementary file 1B. Amino acid sequences of the fusion proteins used in this study.**

**RieskeTMD-Bla**

MSSQDIPEENLPAEQDRPHGAAARPADETNPFADPGLPPHEPRVQDVDERAAKRSERTVALLFTLSMLATIAFIAAFVAIDVDKSVYIFPLGHISALNFALGMTLGVALFAIGAGAVHWARTLMSDEEVADERHPIEASPEVRAKVHADFKQGAKESVIGRRKLIRNTMLGALTLVPLSGVVLLRSRHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**RieskeTMD-Bla 205 extended**

MSSQDIPEENLPAEQDRPHGAAARPADETNPFADPGLPPHEPRVQDVDERAAKRSERTVALLFTLSMLATIAFIAAFVAIDVDKSVYIFPLGHISALNFALGMTLGVALFAIGAGAVHWARTLMSDEEVADERHPIEASPEVRAKVHADFKQGAKESVIGRRKLIRNTMLGALTLVPLSGVVLLRDLGPLPGTKLRHTLWSKGKLSRHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**RieskeTMD-AmiA**

MSSQDIPEENLPAEQDRPHGAAARPADETNPFADPGLPPHEPRVQDVDERAAKRSERTVALLFTLSMLATIAFIAAFVAIDVDKSVYIFPLGHISALNFALGMTLGVALFAIGAGAVHWARTLMSDEEVADERHPIEASPEVRAKVHADFKQGAKESVIGRRKLIRNTMLGALTLVPLSGVVLLRSRAIAKDELLKTSNGHSKPKAKKSGGKRVVVLDPGHGGIDTGAIGRNGSKEKHVVLAIAKNVRSILRNHGIDARLTRSGDTFIPLYDRVEIAHKHGADLFMSIHADGFTNPKAAGASVFALSNRGASSAMAKYLSERENRADEVAGKKATDKDHLLQQVLFDLVQTDTIKNSLTLGSHILKKIKPVHKLHSRNTEQAAFVVLKSPSVPSVLVETSFITNPEEERLLGTAAFRQKIATAIAEGVISYFHWFDNQKAHSKKR

**MtbRieskeTMD-Bla (‘short fusion’)**

MSRADDDAVGVPPTCGGRSDEEERRIVPGPNPQDGAKDGAKATAVPREPDEAALAAMSNQELLALGGKLDGVRIAYKEPRWPVEGTKAEKRAERSVAVWLLLGGVFGLALLLIFLFWPWEFKAADGESDFIYSLTTPLYGLTFGLSILSIAIGAVLYQKRFIPEEISIQERHDGASREIDRKTVVANLTDAFEGSTIRRRKLIGLSFGVGMGAFGLGTLVAFAGGLISRHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**MtbRieskeTMD-Bla 243-extended (‘long fusion’)**

MSRADDDAVGVPPTCGGRSDEEERRIVPGPNPQDGAKDGAKATAVPREPDEAALAAMSNQELLALGGKLDGVRIAYKEPRWPVEGTKAEKRAERSVAVWLLLGGVFGLALLLIFLFWPWEFKAADGESDFIYSLTTPLYGLTFGLSILSIAIGAVLYQKRFIPEEISIQERHDGASREIDRKTVVANLTDAFEGSTIRRRKLIGLSFGVGMGAFGLGTLVAFAGGLIKNPWKPVVPTAEGKKASRHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**Sco3746TMD-AmiA**

MGHWPSGSGAPAYGRSMRDLASRLPSSPGFWRSPLRGPWLTSVLGTVLLVGITVLFVTGLLSYAAYNPDLAPVNDKTPDKGILGFYLFAWPTDPPWLYRLTQGVHVTLGLVLIPVLLAKLWSVVPRLFTLPPVRSLAHALERISLLLLVGGALFEFVTGVLNIQLDYLFPGSFYPLHFYGAWVFFAAFVAHAVLKTPIALRNLRAMREERDDLVSPRPAAPTVSRRGALWFVGGGSLLMFATNAGRSSRAIAKDELLKTSNGHSKPKAKKSGGKRVVVLDPGHGGIDTGAIGRNGSKEKHVVLAIAKNVRSILRNHGIDARLTRSGDTFIPLYDRVEIAHKHGADLFMSIHADGFTNPKAAGASVFALSNRGASSAMAKYLSERENRADEVAGKKATDKDHLLQQVLFDLVQTDTIKNSLTLGSHILKKIKPVHKLHSRNTEQAAFVVLKSPSVPSVLVETSFITNPEEERLLGTAAFRQKIATAIAEGVISYFHWFDNQKAHSKKR

**Sco3746TMD-MBP**

MGHWPSGSGAPAYGRSMRDLASRLPSSPGFWRSPLRGPWLTSVLGTVLLVGITVLFVTGLLSYAAYNPDLAPVNDKTPDKGILGFYLFAWPTDPPWLYRLTQGVHVTLGLVLIPVLLAKLWSVVPRLFTLPPVRSLAHALERISLLLLVGGALFEFVTGVLNIQLDYLFPGSFYPLHFYGAWVFFAAFVAHAVLKTPIALRNLRAMREERDDLVSPRPAAPTVSRRGALWFVGGGSLLMFATNAGRSSRKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTRITK

**Sco3746TMD-Bla (‘short fusion’)**

MGHWPSGSGAPAYGRSMRDLASRLPSSPGFWRSPLRGPWLTSVLGTVLLVGITVLFVTGLLSYAAYNPDLAPVNDKTPDKGILGFYLFAWPTDPPWLYRLTQGVHVTLGLVLIPVLLAKLWSVVPRLFTLPPVRSLAHALERISLLLLVGGALFEFVTGVLNIQLDYLFPGSFYPLHFYGAWVFFAAFVAHAVLKTPIALRNLRAMREERDDLVSPRPAAPTVSRRGALWFVGGGSLLMFATNAGRSFDSPLGTHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**Sco3746TMD-Bla extended (‘long fusion’)**

MGHWPSGSGAPAYGRSMRDLASRLPSSPGFWRSPLRGPWLTSVLGTVLLVGITVLFVTGLLSYAAYNPDLAPVNDKTPDKGILGFYLFAWPTDPPWLYRLTQGVHVTLGLVLIPVLLAKLWSVVPRLFTLPPVRSLAHALERISLLLLVGGALFEFVTGVLNIQLDYLFPGSFYPLHFYGAWVFFAAFVAHAVLKTPIALRNLRAMREERDDLVSPRPAAPTVSRRGALWFVGGGSLLMFATNAGRSFDSPLRATAVLSPHGGPEPGGGPNGGTHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**PFDTMD-AmiA**

MNFSRLKSLRGWRLLSQWFFLLLFLFLFIETEGKGGDELGWPVKLFLDFNPLILLTTLLSAHTVPAIFLLALVVVALTLLLGRVFCGWICPFGTIHNLLSLASNRSRRLVGHPAWLRLKYLILIMMLAMAALGIQQAGLLDPISLLIRSLAVGIYPAFSYAISAFFDTIYLWQIGGVSAVAEWFYGILRDTVLPFQQPIFSQAGLIGGLFIAILALNFYERRFWCRYLCPLGALLGLLARWSLLSREVAEGCNHCGACGQHCPGGAEPHALPDFAGQHQTGAHQPTASPPWLRSECHACFNCDDICPQRLISFRWRLPGLGGKPRSAVSAPAVAGPDLGRRRLLGAAAAGLVVGPLLRVSNPPESRAIAKDELLKTSNGHSKPKAKKSGGKRVVVLDPGHGGIDTGAIGRNGSKEKHVVLAIAKNVRSILRNHGIDARLTRSGDTFIPLYDRVEIAHKHGADLFMSIHADGFTNPKAAGASVFALSNRGASSAMAKYLSERENRADEVAGKKATDKDHLLQQVLFDLVQTDTIKNSLTLGSHILKKIKPVHKLHSRNTEQAAFVVLKSPSVPSVLVETSFITNPEEERLLGTAAFRQKIATAIAEGVISYFHWFDNQKAHSKKR

**PFDTMD-MBP**

MNFSRLKSLRGWRLLSQWFFLLLFLFLFIETEGKGGDELGWPVKLFLDFNPLILLTTLLSAHTVPAIFLLALVVVALTLLLGRVFCGWICPFGTIHNLLSLASNRSRRLVGHPAWLRLKYLILIMMLAMAALGIQQAGLLDPISLLIRSLAVGIYPAFSYAISAFFDTIYLWQIGGVSAVAEWFYGILRDTVLPFQQPIFSQAGLIGGLFIAILALNFYERRFWCRYLCPLGALLGLLARWSLLSREVAEGCNHCGACGQHCPGGAEPHALPDFAGQHQTGAHQPTASPPWLRSECHACFNCDDICPQRLISFRWRLPGLGGKPRSAVSAPAVAGPDLGRRRLLGAAAAGLVVGPLLRVSNPPESRKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTRITK

**PFDTMD-Bla (‘short fusion’)**

MNFSRLKSLRGWRLLSQWFFLLLFLFLFIETEGKGGDELGWPVKLFLDFNPLILLTTLLSAHTVPAIFLLALVVVALTLLLGRVFCGWICPFGTIHNLLSLASNRSRRLVGHPAWLRLKYLILIMMLAMAALGIQQAGLLDPISLLIRSLAVGIYPAFSYAISAFFDTIYLWQIGGVSAVAEWFYGILRDTVLPFQQPIFSQAGLIGGLFIAILALNFYERRFWCRYLCPLGALLGLLARWSLLSREVAEGCNHCGACGQHCPGGAEPHALPDFAGQHQTGAHQPTASPPWLRSECHACFNCDDICPQRLISFRWRLPGLGGKPRSAVSAPAVAGPDLGRRRLLGAAAAGLVVGPLLRVSNPPEGRANPLLGTHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**PFDTMD-Bla extended (‘long fusion’)**

MNFSRLKSLRGWRLLSQWFFLLLFLFLFIETEGKGGDELGWPVKLFLDFNPLILLTTLLSAHTVPAIFLLALVVVALTLLLGRVFCGWICPFGTIHNLLSLASNRSRRLVGHPAWLRLKYLILIMMLAMAALGIQQAGLLDPISLLIRSLAVGIYPAFSYAISAFFDTIYLWQIGGVSAVAEWFYGILRDTVLPFQQPIFSQAGLIGGLFIAILALNFYERRFWCRYLCPLGALLGLLARWSLLSREVAEGCNHCGACGQHCPGGAEPHALPDFAGQHQTGAHQPTASPPWLRSECHACFNCDDICPQRLISFRWRLPGLGGKPRSAVSAPAVAGPDLGRRRLLGAAAAGLVVGPLLRVSNPPEGRANPLLIRPGTHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPELNEAIPNDERDTTMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

**Supplementary file 1C. Plasmids used in this work.**

|  |  |  |
| --- | --- | --- |
| Plasmid | Relevant features | Reference |
| pSU-PROM | Expression vector with constitutive *E. coli* *tatA* promoter; KanR | (28) |
| pSU-PROM Sco2149TMD‑AmiA | pSU-PROM producing aa 1-185 of Sco2149 fused to aa 32-289 of *E. coli* AmiA | This work |
| pSU-PROM Sco2149TMD-Bla | pSU-PROM producing aa 1-185 of Sco2149 fused to aa 24-286 of β‑lactamase (from pBR322) | This work |
| Constructs based on pSU-PROM Sco2149TMD-Bla | | |
| Sco2149TMD RHHR-Bla | Substitution of aa 133-134 of Sco2149TMD from RH to HR | This work |
| Sco2149TMD RHKK-Bla | Substitution of aa 133-134 of Sco2149TMD from RH to KK | This work |
| Sco3149TMD A144 -Bla | Substitution of aa 144 of Sco2149TMD from A to P | This work |
| Sco3149TMD A148P-Bla | Substitution of aa 148 of Sco2149TMD from A to P | This work |
| Sco3149TMD A154P-Bla | Substitution of aa 154 of Sco2149TMD from A to P | This work |
| Sco3149TMD M124A-Bla | Substitution of aa 124 of Sco2149TMD from M to A | This work |
| Sco2149TMD S125A-Bla | Substitution of aa 125 of Sco2149TMD from S to A | This work |
| Sco2149TMD D126A-Bla | Substitution of aa 126 of Sco2149TMD from D to A | This work |
| Sco2149TMD E127A-Bla | Substitution of aa 127 of Sco2149TMD from E to A | This work |
| Sco2149TMD M124L-Bla | Substitution of aa 124 of Sco2149TMD from M to L | This work |
| Sco2149TMD S125L-Bla | Substitution of aa 125 of Sco2149TMD from S to L | This work |
| Sco2149TMD D126L-Bla | Substitution of aa 126 of Sco2149TMD from D to L | This work |
| Sco2149TMD E127L-Bla | Substitution of aa 127 of Sco2149TMD from E to L | This work |
| Sco2149TMD RRKK-Bla | Substitution of aa 161-162 of Sco2149TMD from RR to KK | This work |
| Sco2149TMD RRKQ-Bla | Substitution of aa 161-162 of Sco2149TMD from RR to KQ | This work |
| Sco2149TMD RRAA-Bla | Substitution of aa 161-162 of Sco2149TMD from RR to AA | This work |
| Sco2149TMD RRAD-Bla | Substitution of aa 161-162 of Sco2149TMD from RR to AD | This work |
| Sco2149TMD∆RR -Bla | Deletion of aa 161-162 of Sco2149TMD | This work |
| Sco2149TMD∆118-122-Bla | Deletion of aa 118-122 of Sco2149TMD | This work |
| Sco2149TMD∆123-127-Bla | Deletion of aa 123-127 of Sco2149TMD | This work |
| Sco2149TMD∆128-132-Bla | Deletion of aa 128-132 of Sco2149TMD | This work |
| Sco2149TMD∆133-137-Bla | Deletion of aa 133-137 of Sco2149TMD | This work |
| Sco2149TMD∆138-142-Bla | Deletion of aa 138-142 of Sco2149TMD | This work |
| Sco2149TMD∆143-147-Bla | Deletion of aa 143-147 of Sco2149TMD | This work |
| Sco2149TMD∆148-152-Bla | Deletion of aa 148-152 of Sco2149TMD | This work |
| Sco2149TMD∆153-157-Bla | Deletion of aa 153-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-127-Bla | Deletion of aa 118-127 of Sco2149TMD | This work |
| Sco2149TMD∆128-137-Bla | Deletion of aa 128-137 of Sco2149TMD | This work |
| Sco2149TMD∆138-147-Bla | Deletion of aa 138-147 of Sco2149TMD | This work |
| Sco2149TMD∆148-157-Bla | Deletion of aa 148-157 of Sco2149TMD | This work |
| Sco2149TMD∆138-152-Bla | Deletion of aa 138-152 of Sco2149TMD | This work |
| Sco2149TMD∆118-132-Bla | Deletion of aa 118-132 of Sco2149TMD | This work |
| Sco2149TMD∆123-137-Bla | Deletion of aa 123-137 of Sco2149TMD | This work |
| Sco2149TMD∆128-142-Bla | Deletion of aa 128-142 of Sco2149TMD | This work |
| Sco2149TMD∆133-147-Bla | Deletion of aa 133-147 of Sco2149TMD | This work |
| Sco2149TMD∆143-157-Bla | Deletion of aa 143-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-137-Bla | Deletion of aa 118-137 of Sco2149TMD | This work |
| Sco2149TMD∆138-157-Bla | Deletion of aa 138-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-142-Bla | Deletion of aa 118-142 of Sco2149TMD | This work |
| Sco2149TMD∆123-147-Bla | Deletion of aa 123-147 of Sco2149TMD | This work |
| Sco2149TMD∆128-152-Bla | Deletion of aa 128-152 of Sco2149TMD | This work |
| Sco2149TMD∆133-157-Bla | Deletion of aa 133-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-147-Bla | Deletion of aa 118-147 of Sco2149TMD | This work |
| Sco2149TMD∆123-152-Bla | Deletion of aa 123-152 of Sco2149TMD | This work |
| Sco2149TMD∆128-157-Bla | Deletion of aa 128-157 of Sco2149TMD | This work |
| Sco2149TMD∆123-157-Bla | Deletion of aa 123-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-152-Bla | Deletion of aa 118-152 of Sco2149TMD | This work |
| Sco2149TMD∆118-153-Bla | Deletion of aa 118-153 of Sco2149TMD | This work |
| Sco2149TMD∆118-154-Bla | Deletion of aa 118-154 of Sco2149TMD | This work |
| Sco2149TMD∆118-155-Bla | Deletion of aa 118-155 of Sco2149TMD | This work |
| Sco2149TMD∆118-156-Bla | Deletion of aa 118-156 of Sco2149TMD | This work |
| Sco2149TMD∆118-157-Bla | Deletion of aa 118-157 of Sco2149TMD | This work |
| Sco2149TMD∆126-127-Bla | Deletion of aa 126-127of Sco2149TMD | This work |
| Sco2149TMD∆127-128-Bla | Deletion of aa 127-128 of Sco2149TMD | This work |
| Sco2149TMD∆126-128-Bla | Deletion of aa 126-128 of Sco2149TMD | This work |
| Sco2149TMD∆131-132-Bla | Deletion of aa 131-132 of Sco2149TMD | This work |
| Sco2149TMD∆137Δ141-Bla | Deletion of aa 137 & 141of Sco2149TMD | This work |
| Sco2149TMD∆149Δ156-Bla | Deletion of aa 149 & 156 of Sco2149TMD | This work |
| Sco2149TMD∆131-132 Δ141-Bla | Deletion of aa 131 & 132 & 141 of Sco2149TMD | This work |
| Sco2149TMD∆126-128 Δ131-132-Bla | Deletion of aa 126-128 &131-132 of Sco2149TMD | This work |
| Sco2149TMD∆126-128 Δ137 Δ141-Bla | Deletion of aa 126-128 &137 &141 of Sco2149TMD | This work |
| Sco2149TMD∆131-132 Δ137 Δ141-Bla | Deletion of aa 131-132 & 137 &141 of Sco2149TMD | This work |
| Sco2149TMD-Bla ∆126-128 Δ131-132 Δ137 Δ141 | Deletion of aa 126-128 &131-132 &137 & 141 of Sco2149TMD | This work |
| Sco2149TMD-Bla ∆126-128 Δ131-132 Δ137 Δ141 Δ149 Δ156 | Deletion of aa 126-128 &131-132 &137 & 141 &149 & 156 of Sco2149TMD | This work |
| Sco2149TMD D131A E132A-Bla | Substitution of aa 131 & 132 of Sco2149TMD to A | This work |
| Sco2149TMD E137A E141A-Bla | Substitution of aa 137 & 141 of Sco2149TMD to A | This work |
| Sco2149TMD D126A E127A E128A -Bla | Substitution of aa 126 & 127 & 128 of Sco2149TMD to A | This work |
| Sco2149TMD D131K E132K-Bla | Substitution of aa 131 & 132 of Sco2149TMD to K | This work |
| Sco2149TMD E137K E141K-Bla | Substitution of aa 137 & 141 of Sco2149TMD to K | This work |
| Sco2149TMD D126K E127K E128K-Bla | Substitution of aa 126 & 127 & 128 of Sco2149TMD to K | This work |
| Sco2149TMDD129 E130 E131-Bla INS | Insertion of DEE at positions aa 129-131 of Sco2149TMD | This work |
| Sco2149TMDD126K E127K E128K E137K E141K-Bla | Substitution of aa 126-128 & 137 & 141 of Sco2149TMD to K | This work |
| Sco2149TMD Sco2149TMDD126K E127K E128K D131K E132K E137K E141K -Bla | Substitution of aa 126-128 & 131-132 & 137 & 141 of Sco2149TMD to K | This work |
| Sco2149TMDP177L S179L G180L-Bla | Substitution of aa 177 &179 & 180 of Sco2149TMD to L | This work |
| Sco2149TMDS179L G180L-Bla | Substitution of aa 179 & 180 of Sco2149TMD to L | This work |
| Sco2149TMDG180L-Bla | Substitution of aa 180 of Sco2149TMD to L | This work |
| Sco2149TMDS179L-Bla | Substitution of aa 179 of Sco2149TMD to L | This work |
| Sco2149TMDR185A-Bla | Substitution of aa 185 of Sco2149TMD to A | This work |
| Sco2149TMDV158K-Bla | Substitution of aa 158 of Sco2149TMD to K | This work |
| Sco2149TMD∆118-155 V158K -Bla | Deletion of aa 118-155 of Sco2149TMD & substitution of aa 158 of Sco2149TMD to K | This work |
| Sco2149TMD∆118-156 V158K -Bla | Deletion of aa 118-156 of Sco2149TMD & substitution of aa 158 of Sco2149TMD to K | This work |
| Sco2149TMD∆118-157 V158K -Bla | Deletion of aa 118-157 of Sco2149TMD & substitution of aa 158 of Sco2149TMD to K | This work |
| pSU-PROM Sco2149TMDextended-Bla | pSU-PROM Sco2149TMD-Bla extension of sequence from aa 185 to 205 | This work |
| Constructs based on pSU-PROM Sco2149TMD-AmiA | | |
| Sco2149TMD RHHR-AmiA | Substitution of aa 133-134 of Sco2149TMD from RH to HR | This work |
| Sco2149TMD RHKK-AmiA | Substitution of aa 133-134 of Sco2149TMD from RH to KK | This work |
| Sco2149TMD A144P-AmiA | Substitution of aa 144 of Sco2149TMD from A to P | This work |
| Sco2149TMD A148P-AmiA | Substitution of aa 148 of Sco2149TMD from A to P | This work |
| Sco2149TMD A154P-AmiA | Substitution of aa 154 of Sco2149TMD from A to P | This work |
| Sco2149TMD M124A-AmiA | Substitution of aa 124 of Sco2149TMD from M to A | This work |
| Sco2149TMD S125A-AmiA | Substitution of aa 125 of Sco2149TMD from S to A | This work |
| Sco2149TMD D126A-AmiA | Substitution of aa 126 of Sco2149TMD from D to A | This work |
| Sco2149TMD E127A-AmiA | Substitution of aa 127 of Sco2149TMD from E to A | This work |
| Sco2149TMD M124L-AmiA | Substitution of aa 124 of Sco2149TMD from M to L | This work |
| Sco2149TMD S125L-AmiA | Substitution of aa 125 of Sco2149TMD from S to L | This work |
| Sco2149TMD D126L-AmiA | Substitution of aa 126 of Sco2149TMD from D to L | This work |
| Sco2149TMD E127L-AmiA | Substitution of aa 127 of Sco2149TMD from E to L | This work |
| Sco2149TMD RRKK-AmiA | Substitution of aa 161-162 of Sco2149TMD from RR to KK | This work |
| Sco2149TMD RRKQ-AmiA | Substitution of aa 161-162 of Sco2149TMD from RR to KQ | This work |
| Sco2149TMD RRAA-AmiA | Substitution of aa 161-162 of Sco2149TMD from RR to AA | This work |
| Sco2149TMD RRAD-AmiA | Substitution of aa 161-162 of Sco2149TMD from RR to AD | This work |
| Sco2149TMD ∆RR-AmiA | Deletion of aa 161-162 of Sco2149TMD | This work |
| Sco2149TMD∆118-122-AmiA | Deletion of aa 118-122 of Sco2149TMD | This work |
| Sco2149TMD∆123-127-AmiA | Deletion of aa 123-127 of Sco2149TMD | This work |
| Sco2149TMD∆128-132-AmiA | Deletion of aa 128-132 of Sco2149TMD | This work |
| Sco2149TMD∆133-137-AmiA | Deletion of aa 133-137 of Sco2149TMD | This work |
| Sco2149TMD∆138-142-AmiA | Deletion of aa 138-142 of Sco2149TMD | This work |
| Sco2149TMD∆143-147-AmiA | Deletion of aa 143-147 of Sco2149TMD | This work |
| Sco2149TMD∆148-152-AmiA | Deletion of aa 148-152 of Sco2149TMD | This work |
| Sco2149TMD∆153-157-AmiA | Deletion of aa 153-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-127-AmiA | Deletion of aa 118-127 of Sco2149TMD | This work |
| Sco2149TMD∆128-137-AmiA | Deletion of aa 128-137 of Sco2149TMD | This work |
| Sco2149TMD∆138-147-AmiA | Deletion of aa 138-147 of Sco2149TMD | This work |
| Sco2149TMD∆148-157-AmiA | Deletion of aa 148-157 of Sco2149TMD | This work |
| Sco2149TMD∆138-152-AmiA | Deletion of aa 138-152 of Sco2149TMD | This work |
| Sco2149TMD∆118-132-AmiA | Deletion of aa 118-132 of Sco2149TMD | This work |
| Sco2149TMD-∆123-137AmiA | Deletion of aa 123-137 of Sco2149TMD | This work |
| Sco2149TMD∆128-142-AmiA | Deletion of aa 128-142 of Sco2149TMD | This work |
| Sco2149TMD∆133-147-AmiA | Deletion of aa 133-147 of Sco2149TMD | This work |
| Sco2149TMD∆143-157-AmiA | Deletion of aa 143-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-137-AmiA | Deletion of aa 118-137 of Sco2149TMD | This work |
| Sco2149TMD∆138-157-AmiA | Deletion of aa 138-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-142-AmiA | Deletion of aa 118-142 of Sco2149TMD | This work |
| Sco2149TMD∆123-147-AmiA | Deletion of aa 123-147 of Sco2149TMD | This work |
| Sco2149TMD∆128-152-AmiA | Deletion of aa 128-152 of Sco2149TMD | This work |
| Sco2149TMD∆133-157-AmiA | Deletion of aa 133-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-147-AmiA | Deletion of aa 118-147 of Sco2149TMD | This work |
| Sco2149TMD∆123-152-AmiA | Deletion of aa 123-152 of Sco2149TMD | This work |
| Sco2149TMD-∆128-157AmiA | Deletion of aa 128-157 of Sco2149TMD | This work |
| Sco2149TMD∆123-157-AmiA | Deletion of aa 123-157 of Sco2149TMD | This work |
| Sco2149TMD∆118-152-AmiA | Deletion of aa 118-152 of Sco2149TMD | This work |
| Sco2149TMD∆118-153-AmiA | Deletion of aa 118-153 of Sco2149TMD | This work |
| Sco2149TMD∆118-154-AmiA | Deletion of aa 118-154 of Sco2149TMD | This work |
| Sco2149TMD∆118-155-AmiA | Deletion of aa 118-155 of Sco2149TMD | This work |
| Sco2149TMD∆118-156-AmiA | Deletion of aa 118-156 of Sco2149TMD | This work |
| Sco2149TMD∆118-157-AmiA | Deletion of aa 118-157 of Sco2149TMD | This work |
| Sco2149TMD∆124-157-AmiA | Deletion of aa 118-155 of Sco2149TMD | This work |
| Sco2149TMD∆125-157-AmiA | Deletion of aa 118-156 of Sco2149TMD | This work |
| pSU18 | Expression vector with a constitutive *E. coli* *lac* promoter. CmlR | (59) |
| pSU18 AmiA | pSU18 producing aa 1-289 of *E. coli* AmiA | (26) |
| pSU18 Sco2149TMD-AmiA (pSU-TM123-AmiA) | pSU18 producing aa 1-185 of Sco2149 fused to aa 32-289 of *E. coli* AmiA | (22) |
| pSU-PROM MtbRieskeTMD-Bla | pSU-PROM producing aa 1-227 of *M. tuberculosis* H37Rv *qcrA* codon optimised for *E. coli* fused to aa 24-286 of β‑lactamase (from pBR322) | This work |
| pSU-PROM MtbRieskeTMDextended -Bla | pSU-PROM MtbRiekeTMD-Bla extension of sequence from aa 227 to 243 | This work |
| pSU18-PROM Sco3746TMD-AmiA | pSU18 producing aa 1-247 of Sco3746 fused via an Ala-Ile-Ala linker to aa 32-289 of *E. coli* AmiA under control of constitutive *E. coli* *tat* promoter | This work |
| pSU18-PROM Sco3746TMD-RRKK-AmiA | pSU18-PROM Sco3746TMD-AmiA with substitution of aa 225-226 of Sco3746 from RR to KK | This work |
| pSU18-PROM Sco3746TMD-MBP | pSU18 producing aa 1-247 of Sco3746 fused to aa 29-396 of *E. coli* MBP under control of constitutive *E. coli* *tat* promoter | This work |
| pSU18-PROM Sco3746TMD RRKK-MBP | pSU18-PROM Sco3746TMD-MBP with substitution of aa 225-226 of Sco3746 from RR to KK | This work |
| pSU1-8PROM Sco3746TMD G14C-MBP | pSU18-PROM Sco3746TMD-MBP with substitution of aa G14 of Sco3746 to C | This work |
| pSU18-PROM Sco3746TMD G84C-MBP | pSU18-PROM Sco3746TMD-MBP with substitution of aa G84 of Sco3746 to C | This work |
| pSU18-PROM Sco3746TMD A137C-MBP | pSU18-PROM Sco3746TMD-MBP with substitution of aa A137 of Sco3746 to C | This work |
| pSU1-8PROM Sco3746TMD G171C-MBP | pSU18-PROM Sco3746TMD-MBP with substitution of aa G171 of Sco3746 to C | This work |
| pSU18-PROM Sco3746TMD A219C-MBP | pSU18-PROM Sco3746TMD-MBP with substitution of aa A219 of Sco3746 to C | This work |
| pSU18-PROM Sco3746TMD-Bla | pSU18 producing aa 1-252 of Sco3746 fused to aa 24-286 of β‑lactamase (from pBR322) under control of constitutive *E. coli* *tat* promoter. | This work |
| pSU18-PROM Sco3746TMDextended-Bla | As pSU18PROM Sco3746TMD-Bla but with extension of Sco3746TMD sequence from aa 252 to 272 | This work |
| pSU18PROM Sco3746TMD G234L S235L-Bla | pSU18PROM Sco3746TMD-Bla with substitution of aa 234-235 of Sco3746 to L | This work |
| pSU18PROM Sco3746TMD G234L S235L M239L F240L-Bla | pSU18PROM Sco3746TMD-Bla with substitution of aa 234-235 and 239-240 of Sco3746 to L | This work |
| pSU18 PFDTMD-AmiA | pSU18 producing aa 1-364 of PFD fused via an Ala-Ile-Ala linker to aa 32-289 of *E. coli* AmiA | This work |
| pSU18 PFDTMD RRKK-AmiA | pSU18 PFDTMD-AmiA with substitution of aa 340-341 of PFD from RR to KK | This work |
| pSU18 PFDTMD-MBP | pSU18 producing aa 1-364 of PFD fused to aa 29-396 of *E. coli* MBP | This work |
| pSU18 PFDTMD RRKK-MBP | pSU18 PFDTMD-MBP with substitution of aa 340-341 of PFD from RR to KK | This work |
| pSU18 PFDTMD-Bla | pSU18 producing aa 1-371 of PFD fused to aa 24-286 of β‑lactamase (from pBR322) under control of constitutive *E. coli* *tat* promoter. | This work |
| pSU18 PFDTMDextended-Bla | As pSU18 PFDTMD-Bla but with extension of PFDTMD sequence from aa 371 to 374 | This work |
| pSU18 PFDTMD G354L R358L-Bla | pSU18 PFDTMD-Bla with substitution of aa 354 and-358 of PFD to L | This work |
| pSU18 PFDTMD G354L P355L R358L-Bla | pSU18 PFDTMD-Bla with substitution of aa 354-355 and-358 of PFD to L | This work |
| pSU18 PFDTMDextended G354L R358L-Bla | pSU18 PFDTMDextended-Bla with substitution of aa 354 and-358 of PFD to L | This work |
| pSU18 PFDTMDextended G354L P355L R358L-Bla | pSU18 PFDTMDextended-Bla with substitution of aa 354-355 and-358 of PFD to L | This work |

**Supplementary file 1D. Oligonucleotides used in this work.**

|  |  |  |
| --- | --- | --- |
| Primer | Sequence | Use |
| BamHI AmiA | GCGCGGATCCATGAGCACTTTTAAACCACTAAAA | Construction of AmiA fusions |
| SU18.1 | CGTATGTTGTGTGGAATTGTGAGC | pSU18/pSU40 sequencing primer |
| SU18.2 | GGGTAACGCCAGGGTTTTCCC | pSU18/pSU40 sequencing primer |
| Uni-Rep1-Hind | GCGCAAGCTTTGTCGGTTGGCGCAAAACACGCTG | Amplification of *tat* promoter |
| Inside Bla reverse | GCTGCAGGCATCGTGGTGTCACGCTCGTC | *bla* sequencing primer |
| Inside AmiA reverse | TCAAACAGCACTTGTTGCAATAGGTGATC | *amiA* sequencing primer |
| MalE internal reverse | CCACGTCTTTAATGTCGTACTT | *malE* (MBP) sequencing primer |
| Sco2149TMD | GCGCGGATCCATGAGTAGCCAAGACATT | Construction of Sco2149TMD extended |
| Sco2149TMD extension | GCGCTCTAGAGAGCTTGCCCTTGGACCACAGGGTGTGGCGGAGCTTGGTCCCGGGCAGCGGACCGAGGTCGCGCAGCAGGACGACGCC | Construction of Sco2149TMD extended |
| MtbRieskeTMD | GCGCGGATCCATGAGCCGCGCGGATGAT | Construction of MtbRieskeTMD extended |
| MtbRieskeTMD extension | GCGCTCTAGACGCCTTCTTGCCTTCCGCGGTCGGCACCACCGGTTTCCACGGGTTTTTAATCAGGCCGCCCGCAAA | Construction of MtbRieskeTMD extended |
| Sco2149TMD RHHR | GTCGCCGACGAGCATCGCCCGATCGAGGCG | Sco2149TMD Quikchange |
| CGCCTCGATCGGGCGATGCTCGTCGGCGAC |
| Sco2149TMD RHKK | GTCGCCGACGAGAAAAAACCGATCGAGGCG | Sco2149TMD Quikchange |
| CGCCTCGATCGGTTTTTTCTCGTCGGCGAC |
| Sco2149TMD A144P | TCCCCCGAGGTCCGTCCCAAGGTCCACGCGGAC | Sco2149TMD Quikchange |
| GTCCGCGTGGACCTTGGGACGGACCTCGGGGGA |
| Sco2149TMD A148P | CGTGCCAAGGTCCACCCGGACTTCAAGCAGGGT | Sco2149TMD Quikchange |
| ACCCTGCTTGAAGTCCGGGTGGACCTTGGCACG |  |
| Sco2149TMD A154P | GACTTCAAGCAGGGTCCCAAGGAGTCCGTGATC | Sco2149TMD Quikchange |
| CAGCACGGACTCCTTGGGACCCTGCTTGAAGTC |  |
| Sco2149TMD M124A | TGGGCCCGCACCCTGGCCTCCGACGAGGAGGTC | Sco2149TMD Quikchange |
| GACCTCCTCGTCGGAGGCCAGGGTGCGGGCCCA |  |
| Sco2149TMD S125A | GCCCGCACCCTGATGGCCGACGAGGAGGTCGCC | Sco2149TMD Quikchange |
| GGCGACCTCCTCGTCGGCCATCAGGGTGCGGGC |  |
| Sco2149TMD D126A | CGCACCCTGATGTCCGCCGAGGAGGTCGCCGAC | Sco2149TMD Quikchange |
|  | GTCGGCGACCTCCTCGGCGGACATCAGGGTGCG |  |
| Sco2149TMD E127A | ACCCTGATGTCCGACGCCGAGGTCGCCGACGAG | Sco2149TMD Quikchange |
| CTCGTCGGCGACCTCGGCGTCGGACATCAGGGT |
| Sco2149TMD M124L | TGGGCCCGCACCCTGTTGTCCGACGAGGAGGTC | Sco2149TMD Quikchange |
| GACCTCCTCGTCGGACAACAGGGTGCGGGCCCA |
| Sco2149TMD S125L | GCCCGCACCCTGATGTTGGACGAGGAGGTCGCC | Sco2149TMD Quikchange |
| GGCGACCTCCTCGTCCAACATCAGGGTGCGGGC |
| Sco2149TMD D126L | CGCACCCTGATGTCCTTGGAGGAGGTCGCCGAC | Sco2149TMD Quikchange |
| GTCGGCGACCTCCTCCAAGGACATCAGGGTGCG |
| Sco2149TMD E127L | ACCCTGATGTCCGACTTGGAGGTCGCCGACGAG | Sco2149TMD Quikchange |
| CTCGTCGGCGACCTCCAAGTCGGACATCAGGGT |
| Sco2149TMD RRKK | GCCAAGGAGTCCGTGATCGGGAAGAAGAAGCTGATCCGCAACACG | Sco2149TMD Quikchange |
| CGTGTTGCGGATCAGCTTCTTCTTCCCGATCACGGACTCCTTGGC |
| Sco2149TMD RRKQ | GCCAAGGAGTCCGTGATCGGGAAGCAGAAGCTGATCCGCAACACG | Sco2149TMD Quikchange |
| CGTGTTGCGGATCAGCTTCTGCTTCCCGATCACGGACTCCTTGGC |
| Sco2149TMD RRAA | GCCAAGGAGTCCGTGATCGGGGCCGCCAAGCTGATCCGCAACACG | Sco2149TMD Quikchange |
| CGTGTTGCGGATCAGCTTGGCGGCCCCGATCACGGACTCCTTGGC |
| Sco2149TMD RRAD | GCCAAGGAGTCCGTGATCGGGGCCGATAAGCTGATCCGCAACACG | Sco2149TMD Quikchange |
| CGTGTTGCGGATCAGCTTATCGGCCCCGATCACGGACTCCTTGGC |
| Sco2149TMD ∆RR | TCCGTGATCGGGAAGCTGATCCGCAACACGATGCTG | Sco2149TMD Quikchange |
| CGCCTAGTCGAAGGGCTAGTGCCTGAGGAACCGTGG |
| Sco2149TMD ∆118-122 | GCGGGCGCGGTCCTGATGTCCGACGAGGAGGTCGCC | Sco2149TMD Modified Quikchange |
| GTCGGACATCAGGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆123-127 | TGGGCCCGCACCGAGGTCGCCGACGAGCGTCACCCG | Sco2149TMD Modified Quikchange |
| GTCGGCGACCTCGGTGCGGGCCCAGTGGACCGCGCC |
| Sco2149TMD ∆128-132 | ATGTCCGACGAGCGTCACCCGATCGAGGCGTCCCCC | Sco2149TMD Modified Quikchange |
| GATCGGGTGACGCTCGTCGGACATCAGGGTGCGGGC |
| Sco2149TMD ∆133-137 | GTCGCCGACGAGGCGTCCCCCGAGGTCCGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTCGGGGGACGCGTCGGCGACCTCCTCGTCGGACAT |
| Sco2149TMD ∆138-142 | CACCCGATCGAGCGTGCCAAGGTCCACGCGGACTTC | Sco2149TMD Modified Quikchange |
| GACCTTGGCACGCTCGATCGGGTGACGCTCGTCGGC |
| Sco2149TMD ∆143-147 | TCCCCCGAGGTCGCGGACTTCAAGCAGGGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTTGAAGTCCGCGACCTCGGGGGACGCCTCGATCGG |
| Sco2149TMD ∆148-152 | GCCAAGGTCCACGGTGCCAAGGAGTCCGTGATCGGG | Sco2149TMD Modified Quikchange |
| CTCCTTGGCACCGTGGACCTTGGCACGGACCTCGGG |
| Sco2149TMD ∆153-157 | GACTTCAAGCAGGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACCTGCTTGAAGTCCGCGTGGACCTT |
| Sco2149TMD ∆118-127 | GCGGGCGCGGTCGAGGTCGCCGACGAGCGTCACCCGATC | Sco2149TMD Modified Quikchange |
| GTCGGCGACCTCGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆128-137 | ATGTCCGACGAGGCGTCCCCCGAGGTCCGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTCGGGGGACGCCTCGTCGGACATCAGGGTGCGGGC |
| Sco2149TMD ∆138-147 | CACCCGATCGAGGCGGACTTCAAGCAGGGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTTGAAGTCCGCCTCGATCGGGTGACGCTCGTCGGC |
| Sco2149TMD ∆148-157 | GCCAAGGTCCACGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACGTGGACCTTGGCACGGACCTCGGG |
| Sco2149TMD ∆131-132 | GAGGAGGTCGCCCGTCACCCGATCGAGGCGTCCCCC | Sco2149TMD Modified Quikchange |
| GATCGGGTGACGGGCGACCTCCTCGTCGGACATCAG |
| Sco2149TMD ∆126-128 | ACCCTGATGTCCGTCGCCGACGAGCGTCACCCGATC | Sco2149TMD Modified Quikchange |
| CTCGTCGGCGACGGACATCAGGGTGCGGGCCCAGTG |
| Sco2149TMD ∆137 ∆141 | CACCCGATCGCGTCCCCCGTCCGTGCCAAGGTCCACGCG | Sco2149TMD Modified Quikchange |
| GGCACGGACGGGGGACGCGATCGGGTGACGCTCGTCGGC |
| Sco2149TMD ∆149 ∆156 | AAGGTCCACGCGTTCAAGCAGGGTGCCAAGTCCGTGATCGGG | Sco2149TMD Modified Quikchange |
| CCCGATCACGGACTTGGCACCCTGCTTGAACGCGTGGACCTT |
| Sco2149TMD E137A E141A | CGTCACCCGATCGCGGCGTCCCCCGCGGTCCGTGCCAAG | Sco2149TMD Quikchange |
| CTTGGCACGGACCGCGGGGGACGCCGCGATCGGGTGACG |
| Sco2149TMD D131A E132A | GACGAGGAGGTCGCCGCGGCGCGTCACCCGATCGAG | Sco2149TMD Quikchange |
| CTCGATCGGGTGACGCGCCGCGGCGACCTCCTCGTC |
| Sco2149TMD D126A E127A E128A | GCCCGCACCCTGATGTCCGCGGCGGCGGTCGCCGACGAGCGTCAC | Sco2149TMD Quikchange |
| GTGACGCTCGTCGGCGACCGCCGCCGCGGACATCAGGGTGCGGGC |
| Sco2149TMD Ins D129 E130 E131 | TCCGACGAGGAGGACGAGGAGGTCGCCGACGAGCGTCACCCGATC | Sco2149TMD Modified Quikchange |
| CTCGTCGGCGACCTCCTCGTCCTCCTCGTCGGACATCAGGGTGCG |
| Sco2149TMD ∆126-8 ∆131-2 | ATGTCCGTCGCCCGTCACCCGATCGAGGCGTCCCCC | Sco2149TMD Modified Quikchange |
| GATCGGGTGACGGGCGACGGACATCAGGGTGCGGGC |
| Sco2149TMD ∆126-7 | ACCCTGATGTCCGAGGTCGCCGACGAGCGTCACCCG | Sco2149TMD Modified Quikchange |
| GTCGGCGACCTCGGACATCAGGGTGCGGGCCCAGTG |
| Sco2149TMD ∆127-8 | CTGATGTCCGACGTCGCCGACGAGCGTCACCCGATC | Sco2149TMD Modified Quikchange |
| CTCGTCGGCGACGTCGGACATCAGGGTGCGGGCCCA |
| Sco2149TMD ∆118-132 | GCGGGCGCGGTCCGTCACCCGATCGAGGCGTCCCCC | Sco2149TMD Modified Quikchange |
| GATCGGGTGACGGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆123-137 | TGGGCCCGCACCGCGTCCCCCGAGGTCCGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTCGGGGGACGCGGTGCGGGCCCAGTGGACCGCGCC |
| Sco2149TMD ∆128-142 | ATGTCCGACGAGCGTGCCAAGGTCCACGCGGACTTC | Sco2149TMD Modified Quikchange |
| GACCTTGGCACGCTCGTCGGACATCAGGGTGCGGGC |
| Sco2149TMD ∆133-147 | GTCGCCGACGAGGCGGACTTCAAGCAGGGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTTGAAGTCCGCCTCGTCGGCGACCTCCTCGTCGGA |
| Sco2149TMD ∆138-152 | CACCCGATCGAGGGTGCCAAGGAGTCCGTGATCGGG | Sco2149TMD Modified Quikchange |
| CTCCTTGGCACCCTCGATCGGGTGACGCTCGTCGGC |
| Sco2149TMD ∆143-157 | TCCCCCGAGGTCGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACGACCTCGGGGGACGCCTCGATCGG |
| Sco2149TMD ∆118-137 | GCGGGCGCGGTCGCGTCCCCCGAGGTCCGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTCGGGGGACGCGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆138-157 | CACCCGATCGAGGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
|  | GCGCCCGATCACCTCGATCGGGTGACGCTCGTCGGC |  |
| Sco2149TMD ∆131-2 | GCGCATGTCCGTCGCCCGTCACCCGATCGCGTCCCCCGTC | Sco2149TMD Modified Quikchange |
| GCGCGATCGGGTGACGGGCGACGGACATCAGGGTGCGGGC |
| Sco2149TMD D126K E127K E128K | GCGCACCCTGATGTCCAAGAAGAAGGTCGCCGACGAGCGTCACCCG | Sco2149TMD Quikchange |
| GCGCCTCGTCGGCGACCTTCTTCTTGGACATCAGGGTGCGGGCCCA |
| Sco2149TMD D131K E132K | GAGGAGGTCGCCAAGAAGCGTCACCCGATCGAGGCGTCC | Sco2149TMD Quikchange |
| GATCGGGTGACGCTTCTTGGCGACCTCCTCGTCGGACAT |
| Sco2149TMD E137K E141K | CGTCACCCGATCAAGGCGTCCCCCAAGGTCCGTGCCAAGGTCCACGCG | Sco2149TMD Quikchange |
| CTTGGCACGGACCTTGGGGGACGCCTTGATCGGGTGACGCTCGTCGGC |
| Sco2149TMD ∆118-142 | GCGGGCGCGGTCCGTGCCAAGGTCCACGCGGACTTC | Sco2149TMD Modified Quikchange |
| GACCTTGGCACGGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆123-147 | TGGGCCCGCACCGCGGACTTCAAGCAGGGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTTGAAGTCCGCGGTGCGGGCCCAGTGGACCGCGCC |  |
| Sco2149TMD ∆128-152 | ATGTCCGACGAGGGTGCCAAGGAGTCCGTGATCGGG | Sco2149TMD Modified Quikchange |
| CTCCTTGGCACCCTCGTCGGACATCAGGGTGCGGGC |  |
| Sco2149TMD ∆133-157 | GTCGCCGACGAGGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACCTCGTCGGCGACCTCCTCGTCGGA |
| Sco2149TMD ∆118-147 | GCGGGCGCGGTCGCGGACTTCAAGCAGGGTGCCAAG | Sco2149TMD Modified Quikchange |
| CTTGAAGTCCGCGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆123-152 | TGGGCCCGCACCGGTGCCAAGGAGTCCGTGATCGGG | Sco2149TMD Modified Quikchange |
| CTCCTTGGCACCGGTGCGGGCCCAGTGGACCGCGCC |
| Sco2149TMD ∆128-157 | ATGTCCGACGAGGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACCTCGTCGGACATCAGGGTGCGGGC |
| Sco2149TMD D131KE132K | AAGAAGGTCGCCAAGAAGCGTCACCCGATCAAGGCGTCC | Sco2149TMD Quikchange |
| GATCGGGTGACGCTTCTTGGCGACCTTCTTCTTGGACAT |
| Sco2149TMD ∆118-152 | GCGGGCGCGGTCGGTGCCAAGGAGTCCGTGATCGGG | Sco2149TMD Modified Quikchange |
| CTCCTTGGCACCGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆123-157 | TGGGCCCGCACCGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACGGTGCGGGCCCAGTGGACCGCGCC |
| Sco2149TMD ∆118-157 | GCGGGCGCGGTCGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆118-153 | GCGGGCGCGGTCGCCAAGGAGTCCGTGATCGGGCGC | Sco2149TMD Modified Quikchange |
| GGACTCCTTGGCGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆118-154 | GCGGGCGCGGTCAAGGAGTCCGTGATCGGGCGCCGC | Sco2149TMD Modified Quikchange |
| CACGGACTCCTTGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD ∆118-155 | GCGGGCGCGGTCGAGTCCGTGATCGGGCGCCGCAAG | Sco2149TMD Modified Quikchange |
|  | GATCACGGACTCGACCGCGCCCGCGCCGATGGCGAA |  |
| Sco2149TMD ∆118-156 | GCGGGCGCGGTCTCCGTGATCGGGCGCCGCAAGCTG | Sco2149TMD Modified Quikchange |
| CCCGATCACGGAGACCGCGCCCGCGCCGATGGCGAA |
| Sco2149TMD Δ124-157 | GCCCGCACCCTGGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACCAGGGTGCGGGCCCAGTGGACCGC |
| Sco2149TMD Δ125-157 | CGCACCCTGATGGTGATCGGGCGCCGCAAGCTGATC | Sco2149TMD Modified Quikchange |
| GCGCCCGATCACCATCAGGGTGCGGGCCCAGTGGAC |
| Sco2149TMD V158K | GGTGCCAAGGAGTCCAAGATCGGGCGCCGCAAG | Sco2149TMD Quikchange |
| CTTGCGGCGCCCGATCTTGGACTCCTTGGCACC |
| CTTGCGGCGCCCGATCTTGGACTCCTTGACCGC |
| Sco2149TMD ∆118-155 V158K | GGCGCGGTCGAGTCCAAGATCGGGCGCCGCAAG | Sco2149TMD Quikchange |
| CTTGCGGCGCCCGATCTTGGACTCGACCGCGCC |
| Sco2149TMD ∆118-156 V158K | GCGGGCGCGGTCTCCAAGATCGGGCGCCGCAAG | Sco2149TMD Quikchange |
| CTTGCGGCGCCCGATCTTGGAGACCGCGCCCGC |
| Sco2149TMD ∆118-157 V158K | GGCGCGGGCGCGGTCAAGATCGGGCGCCGCAAG | Sco2149TMD Quikchange |
| CTTGCGGCGCCCGATCTTGACCGCGCCCGCGCC |
| Sco2149TMD S179L | ACCCTGGTGCCGCTCCTGGGCGTCGTCCTGCTGCGC | Sco2149TMD Quikchange |
| GCGCAGCAGGACGACGCCCAGGAGCGGCACCAGGGT |
| Sco2149TMD G180L | ACCCTGGTGCCGCTCTCCCTGGTCGTCCTGCTGCGC | Sco2149TMD Quikchange |
| GCGCAGCAGGACGACCAGGGAGAGCGGCACCAGGGT |
| Sco2149TMD S179LG180L | ACCCTGGTGCCGCTCCTGCTGGTCGTCCTGCTGCGC | Sco2149TMD Quikchange |
| GCGCAGCAGGACGACCAGCAGGAGCGGCACCAGGGT |
| Sco2149TMD P177L S179L G180L | GCGCTCACCCTGGTGCTGCTCCTGCTGGTCGTCCTGCTGCGC | Sco2149TMD Quikchange |
| GCGCAGCAGGACGACCAGCAGGAGCAGCACCAGGGTGAGCGC |
| Sco2149TMD R185A | TCCGGCGTCGTCCTGCTGGCGTCTAGACACCCAGAAACG | Sco2149TMD Quikchange |
| CGTTTCTGGGTGTCTAGACGCCAGCAGGACGACGCCGGA |
| Sco3746For | GCGCAGATCTATGGGTCACTGGCCCTCTGGGAGTGGGGCCCCG | Sco3746TMD amplification |
| Sco3746Rev | GCGCTCTAGAGCTGCGCCCCGCGTTCGTGGCGCCAATCAGC |
| Sco3746(252)rev | GCGCTCTAGACAGCGGACTGTCGAAGCTGCGCCCCGCGTT | Construction of Sco3746TMDBla |
| Sco3746TMDBla For | GCGCACGAACGCGGGGCGCAGCGGTACCCACCCAGAAACGCTGGTG | Quickchange of *Xba*I site out of Sco3746TMDBla (replaced with *Kpn*I) |
| Sco3746TMDBla Rev | CACCAGCGTTTCTGGGTGGGTACCGCTGCGCCCCGCGTTCGTGCGC |
| Sco3746TMDextension | CGCGCCATGGTGGCAAGCCTGGCGGCGGGCCAAGCCCGGGCGGCACGCCCCTCTCGTGCCGCCACCGGGCGTCGCCTGACAGCTTCGACGCGGG | Construction of Sco3746TMD extended |
| Sco3746TMD RRKK | CCGGCCGCGCCCACCGTCTCGAAGAAGGGGGCCCTGTGGTTCGTCGGG | Sco3746TMD Quikchange |
| CCCGACGAACCACAGGGCCCCCTTCTTCGAGACGGTGGGCGCGGCCGG |
| Sco3746TMD G14C | GCGCGAGTGGGGCCCCGGCCTACTGTCGGTCCATGCGCGACC | Sco3746TMD Quikchange |
| GGTCGCGCATGGACCGACAGTAGGCCGGGGCCCCACTCGCGC |
| Sco3746TMD G84C | GCGCcccggacaaggggatcctctgcttctacctcttcgcctGG | Sco3746TMD Quikchange |
| CCAGGCGAAGAGGTAGAAGCAGAGGATCCCCTTGTCCGGGGCGC |
| Sco3746TMD A137C | GCGCccgccggtccgctcgctctgccacgcgctggagcggatct | Sco3746TMD Quikchange |
| AGATCCGCTCCAGCGCGTGGCAGAGCGAGCGGACCGGCGGGCGC |
| Sco3746TMD G171C | GCGCagctggactacctgtttccctgttccttctacccgctgca | Sco3746TMD Quikchange |
| TGCAGCGGGTAGAAGGAACAGGGAAACAGGTAGTCCAGCTGCGC |
| Sco3746TMD A219C | GCGCcgacctggtctccccgcgcccgtgcgcgcccaccgtctcgcgg | Sco3746TMD Quikchange |
| CCGCGAGACGGTGGGCGCGCACGGGCGCGGGGAGACCAGGTCGGCGC |
| Sco3746TMD G234L S235L | TTCGTCGGGGGCCTGCTGCTGCTGATGTTC | Sco3746TMD Quikchange |
| GAACATCAGCAGCAGCAGGCCCCCGACGAA |
| Sco3746TMD G234L S235L M239L F240L | TTCGTCGGGGGCCTGCTGCTGCTGCTGCTGGCCACGAACGCG | Sco3746TMD Quikchange |
| CGCGTTCGTGGCCAGCAGCAGCAGCAGCAGGCCCCCGACGAA |
| PFDTMDBlaRev | GCGCGGTACCAAGCAGTGGGTTAGCTCTACCTTCCGGTGGGTTGGATACGCG | Construction of PFDTMDBla |
| PFDTMD extension | GCGCGGTACCCGGACGAATAAGCAGTGGGTTAGCTCTACCTTCCGGTGGGTTGGATACGCG | Construction of PFDTMD extended |
| PFDTMD RRKK | GTGGCCGGTCCGGATCTGGGCAAGAAGCGCCTCCTGGGTGCCGCGGCA | PFDTMD Quikchange |
| TGCCGCGGCACCCAGGAGGCGCTTCTTGCCCAGATCCGGACCGGCCAC |
| PFDTMD G354L R358L | GGCCTGGTAGTCCTGCCGCTCCTGCTGGTATCCAACCCA | PFDTMD Quikchange |
| TGGGTTGGATACCAGCAGGAGCGGCAGGACTACCAGGCC |
| PFDTMD G354L P355L R358L | GGCCTGGTAGTCCTGCTGCTCCTGCTGGTATCCAACCCA | PFDTMD Quikchange |
| TGGGTTGGATACCAGCAGGAGCAGCAGGACTACCAGGCC |

For all Quikchange primers, the forward primer sequence is given first and the reverse second. Restriction enzymes sites are underlined