**Primers and proteins constructs**

Primer sequences used for the cloning and mutagenesis of Doc, Phd and EF-Tu constructs.a

|  |  |  |
| --- | --- | --- |
| **Cloning primer** | **Target vector** | **Primer sequence** |
| Doc\_NcoI\_fw | pET28 | GGTAATCCATGGGCATGACCCTACAACTTATCTCAGC |
| Doc\_XhoI\_rv | pET28 | GGTAATCTCGAGGGCACGTAAGCGAACG |
| Phd\_NheI\_fw | pET-FLAG | GGTGGTGCTAGCATGTTTATGCGTACGG |
| Phd\_BamHI\_rv | pET-FLAG | GGTAATGGATCCTCATTTATCCGCCAGCTCC |
| EF-Tu\_SacI\_fw | pBAD33 | GGTGGTGAGCTCATGTCTAAAGAAAAGTTTGAACG |
| EF-Tu\_HindIII\_rv | pBAD33 | CCACCAAAGCTTTTAGCCGAGAACTTTAGC |
| EF-Tu\_NdeI\_fw | pET24a | GGTGGTCATATGTCTAAAGAAAAGTTTGAACG |
| EF-Tu\_Xho1\_rv | pET24a | CCATTACTCGAGGCCGAGAACTTTAGCAACA |
| pBAD\_RBS\_fw | pBAD33 | pCTAGCAGGAGGAAGAGCTb |
| pBAD\_RBS\_rv | pBAD33 | pCTTCCTCCTGb |
| **Mutagenesis primer** | **Target vector** | **Primer sequence** |
| EF-Tu\_T383A\_HindIII\_rv | pBAD33 | CCTATTAAGCTTTTAGCCGAGAACTTTAGCAACAACGCCCGCGCCAACGGCACGGCC |
| EF-Tu\_T383A\_XhoI\_rv | pET24a | CCTATTCTCGAGGCCGAGAACTTTAGCAACAACGCCCGCGCCAACGGCACGGCC |
| EF-Tu\_T383V\_fw | pBAD33/pET24a | GGCGGCCGTGTAGTTGGCGCG |
| EF-Tu\_T383V\_rv | pBAD33/pET24a | TTCACGGATTGCGAAACGCAGACCGTCG |

afw: forward primer, rv: reverse primer, b5' phosphorylated primer

Full amino acid sequences of the proteins purified in this work. The desired protein sequence is underlined, mutated residues are highlighted in red, tags are highlighted in purple and cleavage sites are indicated with a slash in the middle of the sequence.

|  |  |
| --- | --- |
| **Protein** | **Sequence** |
| Doc1-122–His6 | MGMTLQLISAEEIIQFHDRLLRVTPGVTGMPDPGRAEALMYRVLKQIEYEGVTDVWLLAAMHLLAISRGHIFNDGNKRTALFITLLFLKRNGISLAANPDFVDMTVDAAAGRLTLEQIAVRLRALEHHHHHH |
| His6 / FLAG–Phd1-73 | MGSSHHHHHHSSGLVPR**/**GSHMDYKDDDDKASMFMRTVNYSEARQNLAEVLESAVTGGPVTITRRGHKSAVIISAEEFERYQTARMDDEFAAIMAVHGNELRELADK |
| EF-Tu1-394–His6 (pET24a) | MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFDQIDNAPEEKARGITINTSHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTREHILLGRQVGVPYIIVFLNKCDMVDDEELLELVEMEVRELLSQYDFPGDDTPIVRGSALKALEGDAEWEAKIIELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGIIKVGEEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRGIKREEIERGQVLAKPGTIKPHTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTIELPEGVEMVMPGDNIKMVVTLIHPIAMDDGLRFAIREGGRTVGAGVVAKVLGLEHHHHHH |
| EF-Tu1-394 T383A–His6 (pET24a) | MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFDQIDNAPEEKARGITINTSHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTREHILLGRQVGVPYIIVFLNKCDMVDDEELLELVEMEVRELLSQYDFPGDDTPIVRGSALKALEGDAEWEAKIIELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGIIKVGEEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRGIKREEIERGQVLAKPGTIKPHTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTIELPEGVEMVMPGDNIKMVVTLIHPIAMDDGLRFAIREGGR**A**VGAGVVAKVLGLEHHHHHH |
| EF-Tu1-394 T383V–His6 (pET24a) | MSKEKFERTKPHVNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFDQIDNAPEEKARGITINTSHVEYDTPTRHYAHVDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTREHILLGRQVGVPYIIVFLNKCDMVDDEELLELVEMEVRELLSQYDFPGDDTPIVRGSALKALEGDAEWEAKIIELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGRVERGIIKVGEEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRGIKREEIERGQVLAKPGTIKPHTKFESEVYILSKDEGGRHTPFFKGYRPQFYFRTTDVTGTIELPEGVEMVMPGDNIKMVVTLIHPIAMDDGLRFAIREGGR**V**VGAGVVAKVLGLEHHHHHH |