|  |  |
| --- | --- |
| **Name** | **Sequence (5’— 3’**) |
| pT25-seq | 5’ TCGGTGACCAGCGGC 3’ |
| pUT18C-seq | 5’ GAAACGGTGCCGGCG 3’ |
| pUT18c-sec-R | 5’ GGCTTAACTATGCGGC 3’ |
| pUT18-sec-F | 5’ TTCACACAGGAAACAGC 3’ |
| pUT18-sec-R | 5’ GTCGATGCGTTCGCG 3’ |
| pKT25-sec-R | 5’ TGGGTAACGCCAGGG 3’ |
| pKTN25-sec-R | 5’ ATGCCAGACTCCCGGTCG 3’ |
| GLNK-BYTH-1F | 5’ GAGGGATCCTATGAAGCTGGTG 3’ |
| GLNK-BYTH-1R | 5’ TGCCCCGGGTTACAGCGCCGC 3’ |
| pUT18-PipX-E4A-F | 5’ TCCCATGGCTTCCGCGAACTACCTCAA 3’ |
| pUT18-PipX-E4A-R | 5’ GATGGTTGAGGTAGTTCGCGGAAGCCA 3’ |
| CK1E4A-F | 5’ GAGTAATGGCTTCCGCGAACTACCTCAACCATCCC 3’ |
| CK1E4A-R | 5’GGGATGGTTGAGGTAGTTCGCGGAAGCCATTACTC 3’ |
| PipX-Y6A-F | 5’ CGCTTCCGAGAACGCCCTCAACCATCCCACC 3’ |
| PipX-Y6A-R | 5’ GGTGGGATGGTTGAGGGCGTTCTCGGAAGCG 3’ |
| PipX-H9A-1F | 5’ CTACCTCAACGCTCCCACCTTCG 3’ |
| PipX-H9A-1R | 5’ CGAAGGTGGGAGCGTTGAGGTAG 3’ |
| PipX-F12A-F | 5’ CCTCAACCATCCCACCGCCGGATTGCTCTACC 3’ |
| PipX-F12A-R | 5’ GGTAGAGCAATCCGGCGGTGGGATGGTTGAGG 3’ |
| PipX-Y16A-F | 5’ CCCACCTTCGGATTGCTCGCCCAAATCTGCAGC 3’ |
| PipX-Y16A-R | 5’ GCTGCAGATTTGGGCGAGCAATCCGAAGGTGGG 3’ |
| PipX-Y32A-1F | 5’ CGCCACTCTTGCTGCTCAGCGCC 3’ |
| PipX-Y32A-1R | 5’ GGCGCTGAGCAGCAAGAGTGGCG 3’ |
| PipX-R35A-F | 5’ GCCACTCTTTATGCTCAGGCCCTCTTTTTTCTCGTAGCC 3’ |
| PipX-R35A-R | 5’ GGCTACGAGAAAAAAGAGGGCCTGAGCATAAAGAGTGGC 3’ |
| PipX-F38A-F | 5’ GCTCAGCGCCTCTTTGCTCTCGTAGCCTTTGATGC 3’ |
| PipX-F38A-R | 5’ GCATCAAAGGCTACGAGAGCAAAGAGGCGCTGAGC 3’ |
| PipX-R54C-F | 5’ GCTTTGAGCCAATCGGTTGTAATGAAGCGCGGATGTTGG 3’ |
| PipX-R54C-R | 5’ CCAACATCCGCGCTTCATTACAACCGATTGGCTCAAAGC 3’ |
| PipX-L65Q-F | 5’ GGTCGACAACCGTCAGCGCCAGCTGCGCCGAGATGC 3’ |
| PipX-L65Q-R | 5’ GCATCTCGGCGCAGCTGGCGCTGACGGTTGTCGACC 3’ |
| PipX-R70A-F | 5’ CCGTCTGCGCCAGCTGCGCGCAGATGCCAGTCTGCAGGAATA 3’ |
| PipX-R70A-R | 5’ TATTCCTGCAGACTGGCATCTGCGCGCAGCTGGCGCAGACGG 3’ |
| PipX-L80Q-F | 5’ GCAGGAATACAACCAGCAGCAGCAAGTCTTCAAAC 3’ |
| PipX-L80Q-R | 5’ GTTTGAAGACTTGCTGCTGCTGGTTGTATTCCTGC 3’ |

**Table S1.** Oligonucleotides.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primer 1** | **Primer 2** | **Template** | **Resulting plasmid** | **Fusion protein expressed** |
| pUT18-PipX-E4A-F | pUT18-PipX-E4A-R | pUAGC934 | pUAGC1140 | PipXE4A-T18 |
| pUAGC1045 | pUAGC1166 | PipXE4A-T25 |
| CK1E4A-F | CK1E4A-R | pUAGC444 | pUAGC1032 | T18-PipXE4A |
| PipX-Y6A-F | PipX-Y6A-R | pUAGC934 | pUAGC1141 | PipXY6A-T18 |
| pUAGC444 | pUAGC1147 | T18-PipXY6A |
| pUAGC1045 | pUAGC1167 | PipXY6A-T25 |
| PipX-H9A-1F | PipX-H9A-1R | pUAGC934 | pUAGC1088 | PipXH9A-T18 |
| pUAGC444 | pUAGC800 | T18-PipXH9A |
| pUAGC1045 | pUAGC1168 | PipXH9A-T25 |
| PipX-F12A-F | PipX-F12A-R | pUAGC934 | pUAGC1089 | PipXF12A-T18 |
| pUAGC444 | pUAGC1034 | T18-PipXF12A |
| pUAGC1045 | pUAGC1169 | PipXF12A-T25 |
| PipX-Y16A-F | PipX-Y16A-R | pUAGC934 | pUAGC1142 | PipXY16A-T18 |
| pUAGC444 | pUAGC1148 | T18-PipXY16A |
| pUAGC1045 | pUAGC1170 | PipXY16A-T25 |
| PipX-Y32A-1F | PipX-Y32A-1R | pUAGC934 | pUAGC1090 | PipXY32A-T18 |
| pUAGC444 | pUAGC801 | T18-PipXY32A |
| pUAGC1045 | pUAGC1171 | PipXY32A-T25 |
| PipX-R35A-F | PipX-R35A-R | pUAGC934 | pUAGC1143 | PipXR35A-T18 |
| pUAGC444 | pUAGC1149 | T18-PipXR35A |
| pUAGC1045 | pUAGC1172 | PipXR35A-T25 |
| PipX-F38A-F | PipX-F38A-R | pUAGC934 | pUAGC1091 | PipXF38A-T18 |
| pUAGC444 | pUAGC1086 | T18-PipXF38A |
| pUAGC1045 | pUAGC1173 | PipXF38A-T25 |
| PipX-R54C-F | PipX-R54C-R | pUAGC934 | pUAGC1144 | PipXR54C-T18 |
| pUAGC444 | pUAGC806 | T18-PipXR54C |
| pUAGC1045 | pUAGC1175 | PipXR54C-T25 |
| PipX-L65Q-F | PipX-L65Q-R | pUAGC934 | pUAGC1145 | PipXL65Q-T18 |
| pUAGC444 | pUAGC807 | T18-PipXL65Q |
| pUAGC1045 | pUAGC1176 | PipXL65Q-T25 |
| PipX-R70A-F | PipX-R70A-R | pUAGC934 | pUAGC1151 | PipXR70A-T18 |
| pUAGC444 | pUAGC1152 | T18-PipXR70A |
| pUAGC1045 | pUAGC1177 | PipXR70A-T25 |
| PipX-L80Q-F | PipX-L80Q-R | pUAGC934 | pUAGC1146 | PipXL80Q-T18 |
| pUAGC444 | pUAGC1150 | T18-PipXL80Q |
| pUAGC1045 | pUAGC1178 | PipXL80Q-T25 |

**Table S2.** Quick-change mutagenesis of PipX derivatives for BACTH analysis.