

Table S3: Primers used in the study

| Primers | Sequence (5' - 3') |
|--|---|
| Strain identification | |
| Tn7_F | AATGATACGGCGACCACCGAGATCTACACGTTTCAGAGTTCTACAG |
| Tn7_R ^a | CAAGCAGAAGACGGCATAACGAGATxxxxxxGTGACTGGAGTTCAGACGTGTG |
| KCGbTn7TTSv3F2 | AGCTTAGATCGGAAGAGCACACGTCTGAACTCCAGTCAACCCCGGGCTGCAGGAATTCCT |
| KCGbTn7TTSv3R1 | TGATCGTCGGACTGTAGAACTCTGAACGTGTAGAACTAGTAGTGCTCATGCATGA |
| Ancestor ID ^b | |
| PA14-1_F | ATCACA <u>AAAAGGACATGC</u> |
| PAO1-B11_F | ATCAGT <u>GTCGTGGG</u> TGC |
| B23-2_F | ATCAG <u>CCTATTGTGTGC</u> |
| CF18-1_F | ATCAG <u>TTACGTCAATGC</u> |
| MSH10-2_F | ATCA <u>TATCAGATTT</u> TGC |
| S54485-1_F | ATCA <u>TTAAACTAGG</u> TGC |
| AI07-08_R | GCTTATGTGCCACCAATCAACAG |
| <i>wspA</i> complementation ^c | |
| wt- <i>wspA</i> _A | taa ^a aacgacggccagtgccaagcttGCGCTGATGGTTCGAACTG |
| wt- <i>wspA</i> _B | gtgacctgcaCCGAAGAACGCTGGGCCT |
| wt- <i>wspA</i> _C | cgttcttcggtGCAGGTCACCACCTCGG |
| wt- <i>wspA</i> _D | cagctatgacctgattacgaattcACTCCGGCGGGAACAGCA |
| Generation of nucleotide barcodes | |
| KCGbTn7TagF1 | GTTCTACAGTCCGACGATCANNNNNNNNNNNTGCGCCGTAGTCCCAATGAAAAACCTATGG |
| KCGbTn7TagR1 | TTCACAGGGTTCAGATTCCTGATGCTACCCAAAACAAAGTCCATAGGTTTTTCATTGGGA |
| KCGbTn7TagF2 | AGGAATCTGAACCCTGTGAATGTGGGGTTCGCGCGCATAGACCTTTATCTCCGGTTCAAG |
| KCGbTn7TagR2 | AGTGTAGGTGTGACAACGTAGCATGCAGCCTCATGCCTAACTGTAACCGGAGATAAAGGT |
| KCGbTn7TagF3 | TACGTTGTACACCTACACTGCTCGAAGTAAATATGGGAAGCGCGCGCCTGGCCCGAGG |
| KCGbTn7TagR3 | CACCAATCAACAGTTAACGAACACGTGGCGGCGCGGAACGCCTCGGGCCAGGCCGCGCGC |
| KCGbTn7TagF4 | TCGTAACTGTTGATTGGTGGCACATAAGCAATACCGTAGTCGGCTAGGTCAAATAGAGT |
| KCGbTn7TagR4 | GTGCTCTCCGATCTAAGCTAGACATGCTGATATCAAAGCACTCTATTTGACCTAGCCGA |

^a xxxxxx indicates the strain-specific barcode sequence (Table 1).

^b underlined indicates the strain specific barcode sequence (Table 1).

^c Lower case is specific to pEX18Ap. Upper case is specific to *wspA*