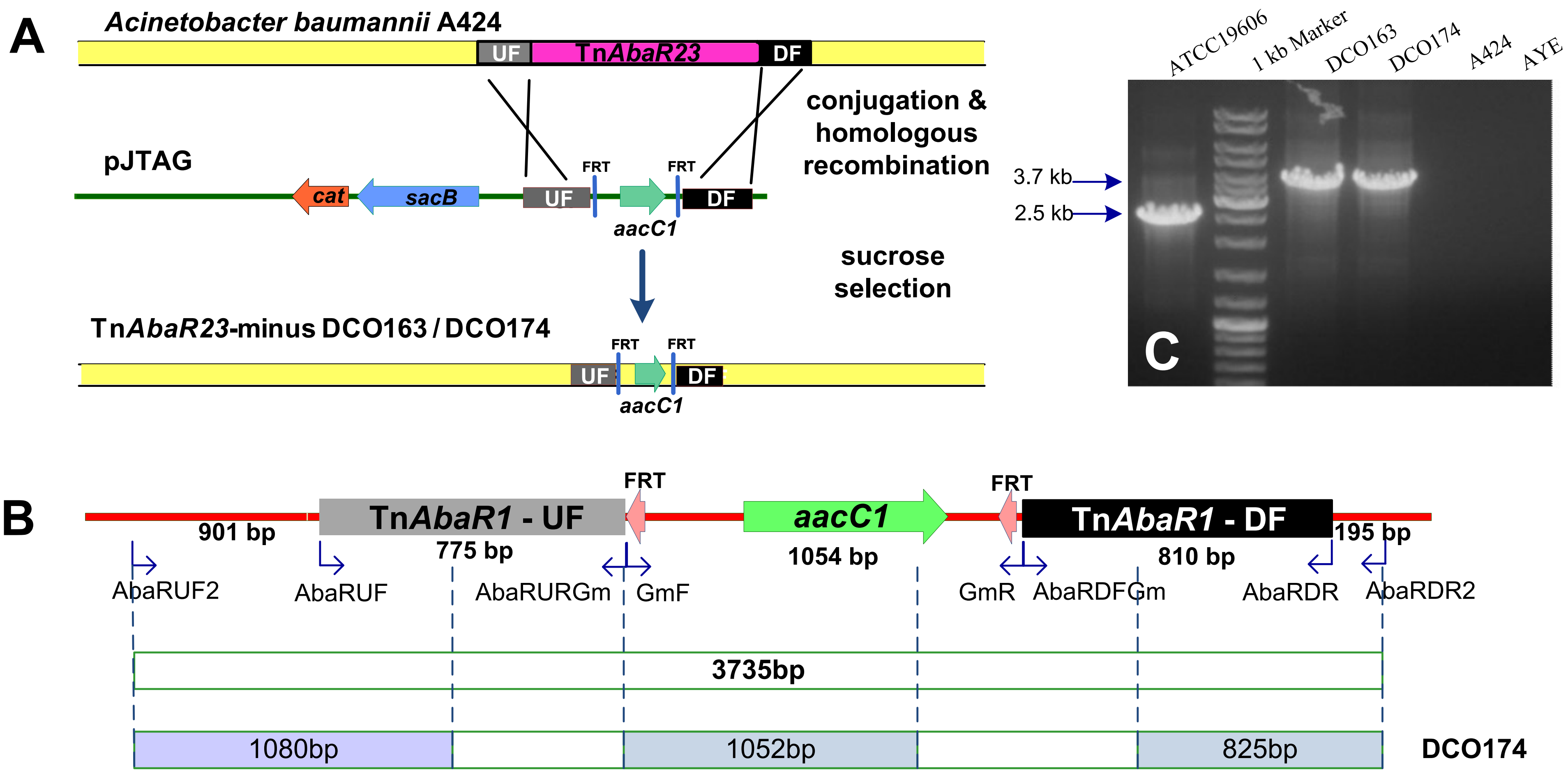

Fig. S1: Construction and analysis of the putative Tn*AbaR23*-minus mutants of *Acinetobacter baumannii* A424. **(A)** Schematic depiction of allelic exchange method used to construct Tn*AbaR23*-minus derivatives of A424. Mutant construction was mediated by conjugative-delivery of the pJTAG suicide plasmid into *A. baumannii* A424 from an *E. coli* λ *pir*-expressing donor. pJTAG was constructed by cloning an SOE-PCR fragment comprising Tn*AbaR1*-associated upstream and downstream flanks (UF & DF) amplified from strain AYE and an intervening FRT-flanked *aacC1* antibiotic resistance marker into the *Not*I site of pJTOOL-3 (32). Initial single cross-over mutants were recovered by gentamicin selection. Subsequent screening on sucrose medium was used to identify *sacB*-minus candidate double cross-over mutants. **(B)** Schematic representation of PCR- and sequencing-based validation of the Tn*AbaR23*-minus mutant DCO174. Blue angled arrows represent locations of primers, which were used for amplification of the different fragments. Blue boxes show the regions of the mutant that were confirmed by sequencing. Equivalent regions of both Tn*AbaR23*-minus mutants generated during this study were sequenced to confirm targeted deletion of Tn*AbaR23*. **(C)** PCR amplication across the targeted deleted locus in DCO163 and DCO174 using the primers AbaRUF2 and AbaRDR2 yields the expected ~3.7 kb product. Given the presence of the large intervening Tn*AbaR23* element, these same primers failed to produce an amplicon with wild-type A424 template DNA. The sequenced strains ATCC 19606 and AYE that lacked and harboured an island within the *comM* gene were used as positive (2.5 kb band expected) and negative PCR controls, respectively.

A

```
A424-ABAYE3778      TGCGCGATGCATTTACTCTATCAAGAGCAAAAAATTTCTCTTTTCCAAGATGATTCTGTT 60
DCO174-ABAYE3778    TGCGCGATGCATTTACTCTATCAAGAGCAAAAAATTTCTCTTTTCCAAGATGATTCTGTT 60
DCO163-ABAYE3778    TGCGCGATGCATTTACTCTATCAAGAGCAAAAAATTTCTCTTTTCCAAGATGATTCTGTT 60
                    ************************************************************

A424-ABAYE3778      GAACCGAATGAATGGATCATTACCATCTTTGTTACGATGGAACCAGAGTATTCAGATATT 120
DCO174-ABAYE3778    GAACCGAATGAATGGATCATTACCATCTTTGTTACGATGGAACCAGAGTATTCAGATATT 120
DCO163-ABAYE3778    GAACCGAATGAATGGATCATTACCATCTTTGTTACGATGGAACCAGAGTATTCAGATATT 120
                    ************************************************************

A424-ABAYE3778      GTTGATCTGGAGCAGTGGTTTTCGACAATCGCTGAGCAATTTAACGGCGAATATGATGGG 180
DCO174-ABAYE3778    GTTGATCTGGAGCAGTGGTTTTCGACAATCGCTGAGCAATTTAACGGCGAATATGATGGG 180
DCO163-ABAYE3778    GTTGATCTGGAGCAGTGGTTTTCGACAATCGCTGAGCAATTTAACGGCGAATATGATGGG 180
                    ************************************************************

A424-ABAYE3778      TGGGGCTGCATGGCTTATGTCTTCGACGAAGAAGAAGATGACATTTTACAATAAAGCCTA 240
DCO174-ABAYE3778    TGGGGCTGCATGGCTTATGTCTTCGACGAAGAAGAAGATGACATTTTACAATAAAGCCTA 240
DCO163-ABAYE3778    TGGGGCTGCATGGCTTATGTCTTCGACGAAGAAGAAGATGACATTTTACAATAAAGCCTA 240
                    ************************************************************

A424-ABAYE3778      AATATAGCCGGGAGGTTCTACAATGAGCTTATCTCAAAAAGAAATTGCGGTACGTTTTTT 300
DCO174-ABAYE3778    AATATAGCCGGGAGGTTCTACAATGAGCTTATCTCAAAAAGAAATTGCGGTACGTTTTTT 300
DCO163-ABAYE3778    AATATAGCCGGGAGGTTCTACAATGAGCTTATCTCAAAAAGAAATTGCGGTACGTTTTTT 300
                    ************************************************************

A424-ABAYE3778      AGAGCTTGCTGCTGCTGGCGAGGTCGATGAAGCCTACGGCAATTATACCGCTCCAAACTT 360
DCO174-ABAYE3778    AGAGCTTGCTGCTGCTGGCGAGGTCGATGAAGCCTACGGCAATTATACCGCTCCAAACTT 360
DCO163-ABAYE3778    AGAGCTTGCTGCTGCTGGCGAGGTCGATGAAGCCTACGGCAATTATACCGCTCCAAACTT 360
                    ************************************************************

A424-ABAYE3778      TAAGCATCACAATCCCTATTATGCTGGTGATAAGACTTCTTTGAAGGAAGGGATGCGTGA 420
DCO174-ABAYE3778    TAAGCATCACAATCCCTATTATGCTGGTGATAAGACTTCTTTGAAGGAAGGGATGCGTGA 420
DCO163-ABAYE3778    TAAGCATCACAATCCCTATTATGCTGGTGATAAGACTTCTTTGAAGGAAGGGATGCGTGA 420
                    ************************************************************

A424-ABAYE3778      AAGTGCAATTAAAACTCCAAACAAAGTTCTTGAAGTTCAACATGTCATTGAAGATGGCGC 480
DCO174-ABAYE3778    AAGTGCAATTAAAACTCCAAACAAAGTTCTTGAAGTTCAACATGTCATTGAAGATGGCGC 480
DCO163-ABAYE3778    AAGTGCAATTAAAACTCCAAACAAAGTTCTTGAAGTTCAACATGTCATTGAAGATGGCGC 480
                    ************************************************************

A424-ABAYE3778      TTTAGTTGCAGTACATTCGAAACTGGAAATGCAAATGAATAATAAGCTAACAATTTTAGC 540
DCO174-ABAYE3778    TTTAGTTGCAGTACATTCGAAACTGGAAATGCAAATGAATAATAAGCTAACAATTTTAGC 540
DCO163-ABAYE3778    TTTAGTTGCAGTACATTCGAAACTGGAAATGCAAATGAATAATAAGCTAACAATTTTAGC 540
                    ************************************************************

A424-ABAYE3778      TGTGGTTCATATATGCCGTTTTGAAAATGGAAAAATTGCGGAGTTTTGGGATATTGGGCA 600
DCO174-ABAYE3778    TGTGGTTCATATATGCCGTTTTGAAAATGGAAAAATTGCGGAGTTTTGGGATATTGGGCA 600
DCO163-ABAYE3778    TGTGGTTCATATATGCCGTTTTGAAAATGGAAAAATTGCGGAGTTTTGGGATATTGGGCA 600
                    ************************************************************

A424-ABAYE3778      AATACAGCCTGATCCACT----- 618
DCO174-ABAYE3778    AATACAGCCTGATCCACTCGTTA 623
DCO163-ABAYE3778    AATACAGCCTGATCCACTCGT-- 621
                    ******************
```

B

```
DCO163-filE     -TGTAGTCGATCAACTTTAGATCAGCTTTTCAATCGACAAACTCAAGACCAAGACTCTAC 59
DCO174-filE     ----AGTCGATCAACTTTAGATCAGCTTTTCAATCGACAAACTCAAGACCAAGACTCTAC 56
A424-filE       GTGTAGTCGATCAACTTTAGATCAGCTTTTCAATCGACAAACTCAAGACCAAGACTCTAC 60
                    ********************************************************

DCO163-filE     TGTTGTTTTAGCGCCGACGTATATGCGTTTGTCTTCACAAGAGCTAGAAGCAGCTTTTGA 119
DCO174-filE     TGTTGTTTTAGCGCCGACGTATATGCGTTTGTCTTCACAAGAGCTAGAAGCAGCTTTTGA 116
A424-filE       TGTTGTTTTAGCGCCGACGTATATGCGTTTGTCTTCACAAGAGCTAGAAGCAGCTTTTGA 120
                ************************************************************

DCO163-filE     ACAAGATAAGTGCTTTATTAAAGATTATAAAAAATCAATTAAAACGCTTAGCACCAATAA 179
DCO174-filE     ACAAGATAAGTGCTTTATTAAAGATTATAAAAAATCAATTAAAACGCTTAGCACCAATAA 176
A424-filE       ACAAGATAAGTGCTTTATTAAAGATTATAAAAAATCAATTAAAACGCTTAGCACCAATAA 180
                ************************************************************

DCO163-filE     GGAAGTAAACTTATGGCCACGGAAGCCATTGAAAGAAAAATTTGAATATGAGTTGGTACA 239
DCO174-filE     GGAAGTAAACTTATGGCCACGGAAGCCATTGAAAGAAAAATTTGAATATGAGTTGGTACA 236
A424-filE       GGAAGTAAACTTATGGCCACGGAAGCCATTGAAAGAAAAATTTGAATATGAGTTGGTACA 240
                ************************************************************

DCO163-filE     GTTAAATCCTTCTGTTCAGCATTTAAAAGTTATGTCTTTTGCTTCTAGTAATGAGAAGCC 299
DCO174-filE     GTTAAATCCTTCTGTTCAGCATTTAAAAGTTATGTCTTTTGCTTCTAGTAATGAGAAGCC 296
A424-filE       GTTAAATCCTTCTGTTCAGCATTTAAAAGTTATGTCTTTTGCTTCTAGTAATGAGAAGCC 300
                ************************************************************

DCO163-filE     TCTATATTATTGGCCACTCGTTGTATTTTTGGATGATAAAGGCTGTATTCTGGAAGGAGT 359
DCO174-filE     TCTATATTATTGGCCACTCGTTGTATTTTTGGATGATAAAGGCTGTATTCTGGAAGGAGT 356
A424-filE       TCTATATTATTGGCCACTCGTTGTATTTTTGGATGATAAAGGCTGTATTCTGGAAGGAGT 360
                ************************************************************

DCO163-filE     GAGTGGATTTAAAAGCAAATCTTATCCTTCGACTATGTTACAGCATGCATCGATTCAAGG 419
DCO174-filE     GAGTGGATTTAAAAGCAAATCTTATCCTTCGACTATGTTACAGCATGCATCGATTCAAGG 416
A424-filE       GAGTGGATTTAAAAGCAAATCTTATCCTTCGACTATGTTACAGCATGCATCGATTCAAGG 420
                ************************************************************

DCO163-filE     AGTTTTAAAAGTACCGCCTTCAGCACATTATATTATGATGACGCCTTTATCTTCTGCCGT 479
DCO174-filE     AGTTTTAAAAGTACCGCCTTCAGCACATTATATTATGATGACGCCTTTATCTTCTGCCGT 476
A424-filE       AGTTTTAAAAGTACCGCCTTCAGCACATTATATTATGATGACGCCTTTATCTTCTGCCGT 480
                ************************************************************

DCO163-filE     GGATGTTACAGAAAAAGAGTTAACAAATCAGGGACAAATT- 519
DCO174-filE     GGATGTTACAGAAAAAGAGTTAACAAATCAGGGACAAATTC 517
A424-filE       GGATGTTACAGAAAAAGAGTTAACAAATCAGGGACAAATT- 520
                ****************************************
```

C

```
A424-ompA       TCGGTAGATAAATGATCAGCAGGAATATTCTTACTAATTAAATAATTCTTAACTGCCAAA 60
DCO163-ompA     TCGGTAGATAAATGATCAGCAGGAATATTCTTACTAATTAAATAATTCTTAACTGCCAAA 60
DCO174-ompA     -CGGTAGATAAATGATCAGCAGGAATATTCTTACTAATTAAATAATTCTTAACTGCCAAA 59
                 ***********************************************************

A424-ompA       GCTCGATCTTGGCTGAGTTTTAAGTTTTTGGTCGCATCACCAGAACTATCGGTATGCCCC 120
DCO163-ompA     GCTCGATCTTGGCTGAGTTTTAAGTTTTTGGTCGCATCACCAGAACTATCGGTATGCCCC 120
DCO174-ompA     GCTCGATCTTGGCTGAGTTTTAAGTTTTTGGTCGCATCACCAGAACTATCGGTATGCCCC 119
                ************************************************************

A424-ompA       ACAATTTTGACTTTTTTCCCGCCCACTTTATTTAAAGCAACAGCCATTTCATCAAGAATT 180
DCO163-ompA     ACAATTTTGACTTTTTTCCCGCCCACTTTATTTAAAGCAACAGCCATTTCATCAAGAATT 180
DCO174-ompA     ACAATTTTGACTTTTTTCCCGCCCACTTTATTTAAAGCAACAGCCATTTCATCAAGAATT 179
                ************************************************************

A424-ompA       TTTTGCCCAGCTTCAGTTAATACTGCACTACCAGATTCAAATTCAATAATTCGATTTTTT 240
DCO163-ompA     TTTTGCCCAGCTTCAGTTAATACTGCACTACCAGATTCAAATTCAATAATTCGATTTTTT 240
DCO174-ompA     TTTTGCCCAGCTTCAGTTAATACTGCACTACCAGATTCAAATTCAATAATTCGATTTTTT 239
                ************************************************************

A424-ompA       AAAGCATCATCAATAATCTTCTGCTCAGCCTGATTTACAGAAAGCTGGGAAAATAATTGA 300
DCO163-ompA     AAAGCATCATCAATAATCTTCTGCTCAGCCTGATTTACAGAAAGCTGGGAAAATAATTGA 300
DCO174-ompA     AAAGCATCATCAATAATCTTCTGCTCAGCCTGATTTACAGAAAGCTGGGAAAATAATTGA 299
                ************************************************************

A424-ompA       TATGGAGGCTGTACCAAACTCTGAAAAGACTGAATGGTTGGCTGTATTTCTGTCGGATTT 360
DCO163-ompA     TATGGAGGCTGTACCAAACTCTGAAAAGACTGAATGGTTGGCTGTATTTCTGTCGGATTT 360
DCO174-ompA     TATGGAGGCTGTACCAAACTCTGAAAAGACTGAATGGTTGGCTGTATTTCTGTCGGATTT 359
                ************************************************************

A424-ompA       AGCATTTTCCCGGTCAGTTCAAATCGAGTTCCATTAACACTTAACTTACCCTGAGAAACT 420
DCO163-ompA     AGCATTTTCCCGGTCAGTTCAAATCGAGTTCCATTAACACTTAACTTACCCTGAGAAACT 420
DCO174-ompA     AGCATTTTCCCGGTCAGTTCAAATCGAGTTCCATTAACACTTAACTTACCCTGAGAAACT 419
                ************************************************************

A424-ompA       TTTTTAAGATCAGGTGTAATCACTCGTGTGACAGAGTCACTCCATCCATTTGGAGCGGCA 480
DCO163-ompA     TTTTTAAGATCAGGTGTAATCACTCGTGTGACAGAGTCACTCCATCCATTTGGAGCGGCA 480
DCO174-ompA     TTTTTAAGATCAGGTGTAATCACTCGTGTGACAGAGTCACTCCATCCATTTGGAGCGGCA 479
                ************************************************************

A424-ompA       ACTGGTCGAACTTGTATTTTATCGACAACTTGATCTGCACCATAAACAGACTGCATTTTC 540
DCO163-ompA     ACTGGTCGAACTTGTATTTTATCGACAACTTGATCTGCACCATAAACAGACTGCATTTTC 540
DCO174-ompA     ACTGGTCGAACTTGTATTTTATCGACAACTTGATCTGCACCATAAACAGACTGCATTTTC 539
                ************************************************************

A424-ompA       ATTAAAATAGCCTGTTTACTTGCTTCATTTGGCACTACACCTTCTACAACTATAGGCTGT 600
DCO163-ompA     ATTAAAATAGCCTGTTTACTTGCTTCATTTGGCACTACACCTTCTACAACTATAGGCTGT 600
DCO174-ompA     ATTAAAATAGCCTGTTTACTTGCTTCATTTGGCACTACACCTTCTACAACTATAGGCTGT 599
                ************************************************************

A424-ompA       GCTAAAGCATAATTCGC--- 617
DCO163-ompA     GCTAAAGCATAATTCGC--- 617
DCO174-ompA     GCTAAAGCATAATTCGCAAT 619
                *****************
```

D

```
DCO163-lldD     TGTGTTACGTGACCGTGGTTTTATGCGTAATGCTTTGGAACGTGCAAAAGCAGCAGGTTG 60
DCO174-lldD     TGTGTTACGTGACCGTGGTTTTATGCGTAATGCTTTGGAACGTGCAAAAGCAGCAGGTTG 60
A424-lldD       TGTGTTACGTGACCGTGGTTTTATGCGTAATGCTTTGGAACGTGCAAAAGCAGCAGGTTG 60
                ************************************************************

DCO163-lldD     TTCAACACTGGTATTTACCGTAGATATGCCTGTACCAGGTGCACGTTATCGTGATGCCCA 120
DCO174-lldD     TTCAACACTGGTATTTACCGTAGATATGCCTGTACCAGGTGCACGTTATCGTGATGCCCA 120
A424-lldD       TTCAACACTGGTATTTACCGTAGATATGCCTGTACCAGGTGCACGTTATCGTGATGCCCA 120
                ************************************************************

DCO163-lldD     TTCGGGTATGAGTGGGCCAAATGCTGCGATGCGTCGTTATATGCAGTCAGTGTTTCATCC 180
DCO174-lldD     TTCGGGTATGAGTGGGCCAAATGCTGCGATGCGTCGTTATATGCAGTCAGTGTTTCATCC 180
A424-lldD       TTCGGGTATGAGTGGGCCAAATGCTGCGATGCGTCGTTATATGCAGTCAGTGTTTCATCC 180
                ************************************************************

DCO163-lldD     GCATTGGTCATGGAATGTCGGTTTAATGGGGCGTCCGCACGACTTGGGAAATATCTCTAA 240
DCO174-lldD     GCATTGGTCATGGAATGTCGGTTTAATGGGGCGTCCGCACGACTTGGGAAATATCTCTAA 240
A424-lldD       GCATTGGTCATGGAATGTCGGTTTAATGGGGCGTCCGCACGACTTGGGAAATATCTCTAA 240
                ************************************************************

DCO163-lldD     ATATTTAGGTAAGCCGACTGGGCTTGAAGATTATATTGGTTGGTTAGGCTCGAACTTTGA 300
DCO174-lldD     ATATTTAGGTAAGCCGACTGGGCTTGAAGATTATATTGGTTGGTTAGGCTCGAACTTTGA 300
A424-lldD       ATATTTAGGTAAGCCGACTGGGCTTGAAGATTATATTGGTTGGTTAGGCTCGAACTTTGA 300
                ************************************************************

DCO163-lldD     CCCATCCATTTCATGGAAAGACCTTGAGTGGATACGTGAGTTTTGGGATGGCCCAATGGT 360
DCO174-lldD     CCCATCCATTTCATGGAAAGACCTTGAGTGGATACGTGAGTTTTGGGATGGCCCAATGGT 360
A424-lldD       CCCATCCATTTCATGGAAAGACCTTGAGTGGATACGTGAGTTTTGGGATGGCCCAATGGT 360
                ************************************************************

DCO163-lldD     GATTAAGGGCATTTTAGATCCTGAAGATGCCAAAGATGCTGTGCGTTTTGGTGCTGACGG 420
DCO174-lldD     GATTAAGGGCATTTTAGATCCTGAAGATGCCAAAGATGCTGTGCGTTTTGGTGCTGACGG 420
A424-lldD       GATTAAGGGCATTTTAGATCCTGAAGATGCCAAAGATGCTGTGCGTTTTGGTGCTGACGG 420
                ************************************************************

DCO163-lldD     GATTGTGGTATCAAATCATGGCGGTCGTCAGTTAGATGGCGTGATGTCATCTGCTCGTGC 480
DCO174-lldD     GATTGTGGTATCAAATCATGGCGGTCGTCAGTTAGATGGCGTGATGTCATCTGCTCGTGC 480
A424-lldD       GATTGTGGTATCAAATCATGGCGGTCGTCAGTTAGATGGCGTGATGTCATCTGCTCGTGC 480
                ************************************************************

DCO163-lldD     ATTGCCTGCAATTGCCGATGCGGTGAAAGGTGACTTAGCGATTTTGGCTGACTCAGGCAT 540
DCO174-lldD     ATTGCCTGCAATTGCCGATGCGGTGAAAGGTGACTTAGCGATTTTGGCTGACTCAGGCAT 540
A424-lldD       ATTGCCTGCAATTGCCGATGCGGTGAAAGGTGACTTAGCGATTTTGGCTGACTCAGGCAT 540
                ************************************************************

DCO163-lldD     TCGTAATGGTTTAGATGTGGTGCGTATGTTGGCGCTCGGTGCGGATACGGTTTTACTTGG 600
DCO174-lldD     TCGTAATGGTTTAGATGTGGTGCGTATGTTGGCGCTCGGTGCGGATACGGTTTTACTTGG 600
A424-lldD       TCGTAATGGTTTAGATGTGGTGCGTATGTTGGCGCTCGGTGCGGATACGGTTTTACTTGG 600
                ************************************************************

DCO163-lldD     CCGTGCGTTTGTTTATGCCTTGGCAGCAGCAGGTGGACAAGGTGTGTCGAACTTACTTGA 660
DCO174-lldD     CCGTGCGTTTGTTTATGCCTTGGCAGCAGCAGGTGGACAAGGTGTGTCGAACTTACTTGA 660
A424-lldD       CCGTGCGTTTGTTTATGCCTTGGCAGCAGCAGGTGGACAAGGTGTGTCGAACTTACTTGA 660
                ************************************************************

DCO163-lldD     TTTAATTGATAAGGAAATGCGTGTTGCGATGACGCTCACGGGTGCTAAGTCGATTTCGGA 720
DCO174-lldD     TTTAATTGATAAGGAAATGCGTGTTGCGATGACGCTCACGGGTGCTAAGTCGATTTCGGA 720
A424-lldD       TTTAATTGATAAGGAAATGCGTGTTGCGATGACGCTCACGGGTGCTAAGTCGATTTCGGA 720
                ************************************************************

DCO163-lldD     TATTAATACGGATTGTTTAGTACAGGCGATTAAC 754
DCO174-lldD     TATTAATACGGATTGTTTAGTACAGGCGATT--- 751
A424-lldD       TATTAATACGGATTGTTTAGTACAGGCGATTAAC 754
                *******************************
```

E

```
A424WT-ABAYE3780    ATTTGAAGATGACGATTTAATTGTGGTTGATAAACCTGCGGGTTTGTTGTCGGTCATGGG 60
DCO174-ABAYE3780    ATTTGAAGATGACGATTTAATTGTGGTTGATAAACCTGCGGGTTTGTTGTCGGTCATGGG 60
DCO163-ABAYE3780    ATTTGAAGATGACGATTTAATTGTGGTTGATAAACCTGCGGGTTTGTTGTCGGTCATGGG 60
                    ************************************************************

A424WT-ABAYE3780    CCGGTTACCTGAACATCATGACAGTGCGTATTTAAGGGTATTGGAAAAGTTCCCTTTAGC 120
DCO174-ABAYE3780    CCGGTTACCTGAACATCATGACAGTGCGTATTTAAGGGTATTGGAAAAGTTCCCTTTAGC 120
DCO163-ABAYE3780    CCGGTTACCTGAACATCATGACAGTGCGTATTTAAGGGTATTGGAAAAGTTCCCTTTAGC 120
                    ************************************************************

A424WT-ABAYE3780    AAAGGTTACGCACCGTTTAGATATGGCAACCTCAGGTTTACTTATGTTTGCCAAGCATCG 180
DCO174-ABAYE3780    AAAGGTTACGCACCGTTTAGATATGGCAACCTCAGGTTTACTTATGTTTGCCAAGCATCG 180
DCO163-ABAYE3780    AAAGGTTACGCACCGTTTAGATATGGCAACCTCAGGTTTACTTATGTTTGCCAAGCATCG 180
                    ************************************************************

A424WT-ABAYE3780    CGATGCAGAAGTTGCGGTAAGTAAAATGTTTCAGGCCAGAACAGTCAAAAAACATTATAT 240
DCO174-ABAYE3780    CGATGCAGAAGTTGCGGTAAGTAAAATGTTTCAGGCCAGAACAGTCAAAAAACATTATAT 240
DCO163-ABAYE3780    CGATGCAGAAGTTGCGGTAAGTAAAATGTTTCAGGCCAGAACAGTCAAAAAACATTATAT 240
                    ************************************************************

A424WT-ABAYE3780    TGCTTTGGTGCAAGGCCAAGTAAAACAAGAGGGCAGTGTTGAAGTTCCACTCATTACTGA 300
DCO174-ABAYE3780    TGCTTTGGTGCAAGGCCAAGTAAAACAAGAGGGCAGTGTTGAAGTTCCACTCATTACTGA 300
DCO163-ABAYE3780    TGCTTTGGTGCAAGGCCAAGTAAAACAAGAGGGCAGTGTTGAAGTTCCACTCATTACTGA 300
                    ************************************************************

A424WT-ABAYE3780    CTGGGAAAATCGCCCAAGACAAATTGTACATTTTGAATTAGGTAAACATGCTAAAACCTT 360
DCO174-ABAYE3780    CTGGGAAAATCGCCCAAGACAAATTGTACATTTTGAATTAGGTAAACATGCTAAAACCTT 360
DCO163-ABAYE3780    CTGGGAAAATCGCCCAAGACAAATTGTACATTTTGAATTAGGTAAACATGCTAAAACCTT 360
                    ************************************************************

A424WT-ABAYE3780    GTTTCAACCATTGGTTTATGATGCAACAACAGATCAAAGTCGAGTGTTACTTGAGCCTGT 420
DCO174-ABAYE3780    GTTTCAACCATTGGTTTATGATGCAACAACAGATCAAAGTCGAGTGTTACTTGAGCCTGT 420
DCO163-ABAYE3780    GTTTCAACCATTGGTTTATGATGCAACAACAGATCAAAGTCGAGTGTTACTTGAGCCTGT 420
                    ************************************************************

A424WT-ABAYE3780    GACAGGGCGTTCGCACCAGTTACGTGTACATATGATGCATATTGGGCATCCGATTATGGG 480
DCO174-ABAYE3780    GACAGGGCGTTCGCACCAGTTACGTGTACATATGATGCATATTGGGCATCCGATTATGGG 480
DCO163-ABAYE3780    GACAGGGCGTTCGCACCAGTTACGTGTACATATGATGCATATTGGGCATCCGATTATGGG 480
                    ************************************************************

A424WT-ABAYE3780    GGACAAGTTATATCATCCTGAACCTAAACGGTTTCATTTGAATCGTATGGCTTTACACGC 540
DCO174-ABAYE3780    GGACAAGTTATATCATCCTGAACCTAAACGGTTTCATTTGAATCGTATGGCTTTACACGC 540
DCO163-ABAYE3780    GGACAAGTTATATCATCCTGAACCTAAACGGTTTCATTTGAATCGTATGGCTTTACACGC 540
                    ************************************************************

A424WT-ABAYE3780    TGCATATCTCGCTTTTCAACATCCTTTGAAGGGAACAGACGTGGTGATTGA 591
DCO174-ABAYE3780    TGCATATCTCGCTTTTCAACATCCTTTGAAGGGAACAGACGTGGTGATTGA 591
DCO163-ABAYE3780    TGCATATCTCGCTTTTCAACATCCTTTGAAGGGAACAGACGTGGTGATTGA 591
                    ***************************************************
```

F

```
DCO163-filB     AACTCGCCGCATCGTAGAAGCCTTATTTGCTAAACGGTCATATTTATATGTGGGATATTG 60
DCO174-filB     AACTCGCCGCATCGTAGAAGCCTTATTTGCTAAACGGTCATATTTATATGTGGGATATTG 60
A424-filB       AACTCGCCGCATCGTAGAAGCCTTATTTGCTAAACGGTCATATTTATATGTGGGATATTG 60
                ************************************************************

DCO163-filB     CACATCAACAAAAGCATATCTATTGACTGTTGCATCCTCTACATGACGCATATCCGAATC 120
DCO174-filB     CACATCAACAAAAGCATATCTATTGACTGTTGCATCCTCTACATGACGCATATCCGAATC 120
A424-filB       CACATCAACAAAAGCATATCTATTGACTGTTGCATCCTCTACATGACGCATATCCGAATC 120
                ************************************************************

DCO163-filB     TTTTAATGCTAATAAGTCTTTACGCTGAGATTCAGGAACTGAAATTACAAATAACGTATT 180
DCO174-filB     TTTTAATGCTAATAAGTCTTTACGCTGAGATTCAGGAACTGAAATTACAAATAACGTATT 180
A424-filB       TTTTAATGCTAATAAGTCTTTACGCTGAGATTCAGGAACTGAAATTACAAATAACGTATT 180
                ************************************************************

DCO163-filB     ATTATCCCAAATTTCCTTAAAACGATTTTCATCAAAGCTGATATTCCCTAAAGCTGGATC 240
DCO174-filB     ATTATCCCAAATTTCCTTAAAACGATTTTCATCAAAGCTGATATTCCCTAAAGCTGGATC 240
A424-filB       ATTATCCCAAATTTCCTTAAAACGATTTTCATCAAAGCTGATATTCCCTAAAGCTGGATC 240
                ************************************************************

DCO163-filB     AGCTACATATACTCGTCCATCTTTATAAGCTTTATATACGACAAAATGTTTAAAACCAGC 300
DCO174-filB     AGCTACATATACTCGTCCATCTTTATAAGCTTTATATACGACAAAATGTTTAAAACCAGC 300
A424-filB       AGCTACATATACTCGTCCATCTTTATAAGCTTTATATACGACAAAATGTTTAAAACCAGC 300
                ************************************************************

DCO163-filB     ATATGAAATCGGAACAATTGCAGGTTGACCGAGTTTAACTAAATCAGAAAACTCTCCTCG 360
DCO174-filB     ATATGAAATCGGAACAATTGCAGGTTGACCGAGTTTAACTAAATCAGAAAACTCTCCTCG 360
A424-filB       ATATGAAATCGGAACAATTGCAGGTTGACCGAGTTTAACTAAATCAGAAAACTCTCCTCG 360
                ************************************************************

DCO163-filB     GTAACCACCACTCTCTAAACCAATTGCTGTGACAAAGCGTTTCATATCAAGGAGCGAAAA 420
DCO174-filB     GTAACCACCACTCTCTAAACCAATTGCTGTGACAAAGCGTTTCATATCAAGGAGCGAAAA 420
A424-filB       GTAACCACCACTCTCTAAACCAATTGCTGTGACAAAGCGTTTCATATCAAGGAGCGAAAA 420
                ************************************************************

DCO163-filB     GCTTCGGCGTTCAATAATACGTTGATACTCACCGTACTGTAGCAAACCACTCATTGTTTG 480
DCO174-filB     GCTTCGGCGTTCAATAATACGTTGATACTCACCGTACTGTAGCAAACCACTCATTGTTTG 480
A424-filB       GCTTCGGCGTTCAATAATACGTTGATACTCACCGTACTGTAGCAAACCACTCATTGTTTG 480
                ************************************************************

DCO163-filB     CTGTTCCGTTAAACTCGTACCAACATAACCATTTAATAAAGTCGTAAGGGCAGCAGACCC 540
DCO174-filB     CTGTTCCGTTAAACTCGTACCAACATAACCATTTAATAAAGTCGTAAGGGCAGCAGACCC 540
A424-filB       CTGTTCCGTTAAACTCGTACCAACATAACCATTTAATAAAGTCGTAAGGGCAGCAGACCC 540
                ************************************************************

DCO163-filB     ACAACTATAATCGTAGGCTTGTCGAACAATTCCACGAAACTGGTCTTCAACAGCTGGTTT 600
DCO174-filB     ACAACTATAATCGTAGGCTTGTCGAACAATTCCACGAAACTGGTCTTCAACAGCTGGTTT 600
A424-filB       ACAACTATAATCGTAGGCTTGTCGAACAATTCCACGAAACTGGTCTTCAACAGCTGGTTT 600
                ************************************************************

DCO163-filB     TATAGTAACTGGTTCAATATGATTTCGATGAAAAGATAAATTACGTGAATCGGCAGTCTC 660
DCO174-filB     TATAGTAACTGGTTCAATATGATTTCGATGAAAAGATAAATTACGTGAATCGGCAGTCTC 660
A424-filB       TATAGTAACTGGTTCAATATGATTTCGATGAAAAGATAAATTACGTGAATCGGCAGTCTC 660
                ************************************************************

DCO163-filB     TGTATAATAAACAGTTCCCGGCGGTTTTTTTTCTATTTCGAAGGCTTCTGTCGCANAGTA 720
DCO174-filB     TGTATAATAAACAGTTCCCGGCGGTTTTTTTTCTATTTCGAAGGCTTCTGTCGCANAGTA 720
A424-filB       TGTATAATAAACAGTTCCCGGCGGTTTTTTTTCTATTTCGAAGGCTTCTGTCGCANAGTA 720
                ************************************************************

DCO163-filB     ATATATCAATGCTGAGCCTAAAGCAATCTCTAACATAAGCACTTGGTCTAAATGGTAGCC 780
DCO174-filB     ATATATCAATGCTGAGCCTAAAGCAATCTCTAACATAAGCACTTGGTCTAAATGGTAGCC 780
A424-filB       ATATATCAATGCTGAGCCTAAAGCAATCTCTAACATAAGCACTTGGTCTAAATGGTAGCC 780
                ************************************************************

DCO163-filB     TAACTGGCCTTATTCA 796
DCO174-filB     TAACTGGCCTTATTCA 796
A424-filB       TAACTGGCCTTATTCA 796
                ****************
```

G

```
DCO163-gyrA       -TGTCGTTGGGGACGTAATCGGTAAATATCACCCGCATGGTGACTTAGCTGTTTATGAAA 59
DCO174-gyrA       GTGTCGTTGGGGACGTAATCGGTAAATATCACCCGCATGGTGACTTAGCTGTTTATGAAA 60
A424-gyrA         ------------ACGTAATCGGTAAATATCACCCGCATGGTGACTTAGCTGTTTATGAAA 48
                              ************************************************

DCO163-gyrA       CCATTGTTCGTATGGCTCAAGACTTTAGCTTACGTTATTTATTGGTTGATGGTCAGGGTA 119
DCO 174-gyrA      CCATTGTTCGTATGGCTCAAGACTTTAGCTTACGTTATTTATTGGTTGATGGTCAGGGTA 120
A424-gyrA         CCATTGTTCGTATGGCTCAAGACTTTAGCTTACGTTATTTATTGGTTGATGGTCAGGGTA 108
                  ************************************************************

DCO163-gyrA       ACTTCGGTTCGATCGATGGTGATAGCGCTGCGGCAATGCGTTATACCGAAGTCCGTATGA 179
DCO174-gyrA       ACTTCGGTTCGATCGATGGTGATAGCGCTGCGGCAATGCGTTATACCGAAGTCCGTATGA 180
A424-gyrA         ACTTCGGTTCGATCGATGGTGATAGCGCTGCGGCAATGCGTTATACCGAAGTCCGTATGA 168
                  ************************************************************

DCO163-gyrA       CTAAGCTGGCACATGAGCTTCTTGCAGATTTAGAAAAAGACACAGTTGACTGGGAAGATA 239
DCO174-gyrA       CTAAGCTGGCACATGAGCTTCTTGCAGATTTAGAAAAAGACACAGTTGACTGGGAAGATA 240
A424-gyrA         CTAAGCTGGCACATGAGCTTCTTGCAGATTTAGAAAAAGACACAGTTGACTGGGAAGATA 228
                  ************************************************************

DCO163-gyrA       ACTACGACGGTTCGGAACGTATCCCTGAAGTACTTCCGACACGTGTTCCAAACTTATTAA 299
DCO174-gyrA       ACTACGACGGTTCGGAACGTATCCCTGAAGTACTTCCGACACGTGTTCCAAACTTATTAA 300
A424-gyrA         ACTACGACGGTTCGGAACGTATCCCTGAAGTACTTCCGACACGTGTTCCAAACTTATTAA 288
                  ************************************************************

DCO163-gyrA       T------------------- 300
DCO174-gyrA       TTAACGGTGCTGCTGGTATC 320
A424-gyrA         TTAACGGTGCTG-------- 300
                  *
```

H

```
A424-parC         -TAAACCTGTTCAGCGCCGCATTGTCTATGCCATGAGCGAGCTAGGCTTAAAAAGCAGTG 59
DCO174-parC       TTAAACCTGTTCAGCGCCGCATTGTCTATGCCATGAGCGAGCTAGGCTTAAAAAGCAGTG 60
DCO163-parC       ---AACCTGTTCAGCGCCGCATTGTCTATGCCATGAGCGAGCTAGGCTTAAAAAGCAGTG 57
                     *********************************************************

A424-parC         GCAAGCCAAAAAAATCAGCGCGTACAGTGGGTGATGTACTTGGTAAATACCACCCACATG 119
DCO174-parC       GCAAGCCAAAAAAATCAGCGCGTACAGTGGGTGATGTACTTGGTAAATACCACCCACATG 120
DCO163-parC       GCAAGCCAAAAAAATCAGCGCGTACAGTGGGTGATGTACTTGGTAAATACCACCCACATG 117
                  ************************************************************

A424-parC         GTGACTCGGCATGTTATGAAGCCATGGTACTCATGGCTCAGCCATTTAGTTACCGCTATC 179
DCO174-parC       GTGACTCGGCATGTTATGAAGCCATGGTACTCATGGCTCAGCCATTTAGTTACCGCTATC 180
DCO163-parC       GTGACTCGGCATGTTATGAAGCCATGGTACTCATGGCTCAGCCATTTAGTTACCGCTATC 177
                  ************************************************************

A424-parC         CTTTAATCGAAGGTCAGGGGAACTGGGGCTCACCTGATGATCCTAAGTCTTTTGCTGCGA 239
DCO174-parC       CTTTAATCGAAGGTCAGGGGAACTGGGGCTCACCTGATGATCCTAAGTCTTTTGCTGCGA 240
DCO163-parC       CTTTAATCGAAGGTCAGGGGAACTGGGGCTCACCTGATGATCCTAAGTCTTTTGCTGCGA 237
                  ************************************************************

A424-parC         TGCGTTATACCGAAGCAAAACTCTCGGCTTATAGTGAATTATTGCTGAGCGAATTAGGTC 299
DCO174-parC       TGCGTTATACCGAAGCAAAACTCTCGGCTTATAGTGAATTATTGCTGAGCGAATTAGGTC 300
DCO163-parC       TGCGTTATACCGAAGCAAAACTCTCGGCTTATAGTGAATTATTGCTGAGCGAATTAGGTC 297
                  ************************************************************

A424-parC         AGGGCACTAGTGAATGGCAAGACAACTTT--- 328
DCO174-parC       AGGGCACTAGTGAATGGCAAGACAACTTTATA 332
DCO163-parC       AGGGCACTAGTGAATGGCAAGACAACTTTA-- 327
                  *****************************
```

Fig. S2: ClustalW-based alignment of PCR-derived sequences mapping to selected target genes in *Acinetobacter baumannii* A424 and its two Tn*AbaR23*-minus mutants. Gene-specific primers were used to amplify and subsequently sequence internal segments of the following genes: **(A)** *ABAYE3778*, **(B)** *filE*, **(C)** *ompA*, **(D)** *lldD*, **(E)** *ABAYE3780,* **(F)** *filB*, **(G)** *gyrA*, and **(H)** *parC*. For the *gyrA* and *parC* alignments, residues shown in green indicate codons that are commonly implicated in ciprofloxacin resistance in *Acinetobacter* but that are 'unmutated' in these strains (GyrA, Gly81 and Ala84; ParC, Ser80 and Glu84), whilst residues shown in red highlight the GyrA Ser83Leu ciprofloxacin resistance-associated substitution common to all three strains. Numbers shown to the right correspond to sequencing coordinates.

Table S1. Details of oligonucleotide primers used in this study

Number	Name	Sequence 5' -> 3'	Reference
PR121	5'-CS	GGCATCCAAGCAGCAAG	This study
PR122	3'-CS	AAGCAGACTTGACCTGA	This study
PR126	int1R	CCCGAGGCATAGACTGTA	This study
PR131	1F	CTTAATTGCCTCTGGTCAAC	S12
PR135	4F	TATCAGCAGCAAAACGATGG	S12
PR136	2R	TTGGGGATTCTGTCCGTAAG	S12
PR138	3R	CTGCTACGGCTGAAACATCC	S12
PR164	trxBR	AAGTGTCCGTCCATTTTTGC	This study
PR165	tnpAF	GACGAAATGCACTTCCAGGT	This study
PR167	arsHR	TGCACCACCTGCTAATGGTA	This study
PR168	arsRF	CACAACCCTGAATGTGGTACA	This study
PR301	arsRR	GCAGACATTTGCTCAAACTCA	This study
PR302	tnpAR	ACCTGGAAGTGCATTTCGTC	This study
PR303	orf5F	TCGCAGAACGCCTAGAACTC	This study
PR304	pbrR-R	GTTCGTAGAAGCGGATCGTC	This study
PR320	oxa51a	CTAATAATTGATCTACTCAAG	This study
PR321	oxa51b	CCAGTGGATGGATGGATAGATTATC	This study
PR351	Gm-F	CGAATTAGCTTCAAAAGCGCTCTGA	S2
PR352	Gm-R	CGAATTGGGGATCTTGAAGTTCCT	S2
PR356	AbaRURGm	TCAGAGCGCTTTTGAAGCTAATTCGGTAAATGACAGCGGTTCAC	This study
PR357	AbaRDFGm	AGGAACTTCAAGATCCCCAATTCGTATTTGTGGAACTGACAACC	This study
PR533	trkAF*	aaacgcaaaagaaaaTGCCGGCACTTTAAAAATACTTTAATAACCC	This study
PR544	2F	TCCATTTTACCGCCACTTTC	S12
PR545	4R	AATCGATGCGGTCGAGTAAC	S12
PR572	AbaRUF	ATAAGAATGCGGCCGCTACAAAAAAGCAGGCTTATTCGCTTTGGTTTTGCAG	This study

PR573	AbaRDR	ATAAGAATGCGGCCGCTACAAGAAAGCTGGGTGCCTCTCATTGAGGTTGAGG	This study
PR720	AbaRDR2	CGTTTAGAAGCGCTACAA	This study
PR721	3664R^	ggcgttaattaaCCACTTGGGTCACAGTAAC	This study
PR722	3663R^	ggcgttaattaaGGTTGCATCCATGACAGTC	This study
PR723	3662R^	ggcgttaattaaCGTATCAACCACGATGAGA	This study
PR768	aadA1R	GTTCCATAGCGTTAAGGTTTCATT	This study
PR804	AbaRUF2	CGCGGTATGGCTGAACTA	This study
PR806	sacB-R-Gm	AGGAACTTCAAGATCCCCAATTCGTCTTTAGGCCCGTAGTCTGC	This study
PR807	sacB-F	CGGCATTTTCTTTTGCGTTT	This study
PR871	sul1R	ATGATTCATATCGTAAAGTG	This study
PR872	3662F	CTCCAGCAAAAGCGAATGTT	This study
PR873	tniAintF	ATGAAGCTTGGCAGATTGCT	This study
PR874	tniAintR	CCATTTAACGATTGGGGATG	This study
PR875	merAF	AGATGTTCGGGCTACTGGTG	This study
PR876	merAR	TCAAGGAAGCCTTGGAGAAA	This study
PR881	tetR-F	CGTCTGTTTCCAACTCGTGA	This study
PR901	tetR-R	GAGCTGTTGAACGAGGTTGG	This study
PR902	aphA1-F	AGAAAACTCACCGAGGCAGT	This study
PR903	aphA1R	AAACGTCTTGCTCGAGGC	This study
PR904	lspAF	GGCAAACATCTGTCACCGTA	This study
PR905	lspAR	AAGTAAGGCACCGAGGACAA	This study
PR906	aadA1F	GAAGCCACACAGTGATATTGATTT	This study
PR907	RH377-R	GACACAACGCAGGTCACATTGAT	S11
PR908	RH212-R	GCATATCGCGACCTGAAAGC	S11
PR909	VEB-F	TGTGTATTCAAATGCTCAAACTGAC	This study
PR910	VEB-R	AATTCCACGTTATTTTTGCAATGT	This study
PR911	TEM-F	CCGTGTCGCCCTTATTCC	S7
PR912	TEM-R	AGGCACCTATCTCAGCGA	S7

PR913	oxa23F	GATGTGTCATAGTATTCGTCGT	This study
PR914	oxa23R	TCACAACAACTAAAAGCACTGT	This study
PR915	IMP-F	CATGGTTTGGTGGTTCTTGT	This study
PR916	IMP-R	ATAATTTGGCGGACTTTGGC	This study
PR917	VIM-F	ATTGGTCTATTTGACCGCGTC	This study
PR918	VIM-R	TGCTACTCAACGACTGAGCG	This study
PR919	aac6'R	CGCATTTTTCATAACAGGCAATAG	This study
PR920	cadRF	CAGCGGTACAGGACTGATGA	This study
PR921	cadAR	CCTCCCGATGTTTGGATATG	This study
PR940	pecMF	CAGCGTCTGATCCAAGAACA	This study
PR941	pecMR	TACCTGCCGAACTTCTCACC	This study
PR942	IS26F	AGCGTGACATCATTCTGTGG	This study
PR943	IS26R	CGTAAGCCGTCTTCATGGAT	This study
PR944	oxa66F	AATCCAAATCACAGCGCTTC	This study
PR945	oxa66R	GCTGAACAACCCATCCAGTT	This study
PR946	folAF	CTTGCTGTTCGCTTGCAGTT	This study
PR947	folAR	CGCAATGGACAAAAATCACT	This study
PR948	ISAba1-F	TGAGAGTCGTCCATGCTTCTT	This study
PR949	ISAba1-R	CGCTCATCATGCTTGTGATT	This study
PR953	aac6'F	TGCGTTACAGAAAAGCGATG	This study
PR954	adeAF	ATCGCTAACAAAGGCTTGAA	This study
PR955	adeAR	CGCCCCCTCAGCTATAGAA	This study
PR956	oxa10F	AATTATCGCGTGTCTTTCGAGTA	This study
PR957	oxa10R	CTCACTTTCCATGATTTTGGTG	This study
PR958	SHV-F	ATGCGTTATATTCGCCTGTG	This study
PR959	SHV-R	TTAGCGTTGCCAGTGCTC	This study
PR960	arr2F	CAGCCAAATCCCAACAATTAAGG	This study
PR961	arr2R	CCTCCCAGTCTTCAACAACG	This study
PR962	folPF	GACGGTGTTCGGCATTCT	This study

PR963	folPR	AGGGTTTCCGAGAAGGTGAT	This study
PR964	lysRF	CGAGTGCCAATGATGACCT	This study
PR965	lysRR	AAGCATGGCTGCTGACAAC	This study
PR966	tetA(A)	GCTACATCCTGCTTGCCTTC	S8
PR967	tetA(A)	CATAGATCGCCGTGAAGAGG	S8
PR968	RH513	GTACTGTTGTAATTCATTAAGCAT	S11
PR969	RH514	TCCCAATGGCATCGTAAAGAACA	S11
PR970	ybjAF	CGCTGCGGCAATAACATTAC	This study
PR971	ybjAR	CCTCCCAGAGCCTGATAAAA	This study
PR975	clmA5F	TACGACAGCGAGCACAATTC	This study
PR975	clmA5R	AAGACAGACCGAGCACGACT	This study
PR976	aphEF	ATGATGTCTAACAGCAAACTG	This study
PR977	ahpER	TCAACCCCAAGTAAGAGG	This study
PR978	ebrF	GCGAAGTAATCGCAACATCC	This study
PR979	ebrR	GATCGGGCGAGCAAAAAG	This study
PR980	topAF	GCCGACCGTATTTTGCTG	This study
PR981	topAR	AGGTCTTGCACTCGGGATAG	This study
PR1001	orf5F2	GAGGTTGTGCGGCTGATG	This study
PR1002	orf5R2	TTCTGCGATGAAGGTTGGAT	This study
PR1003	oxa69F	GCTGAACAACCCATCCAGTT	This study
PR1004	oxa69R	CCATCAAGGTCAAACTCAACAA	This study
PR1008	tnpAWF	AGGACAAGAACCGTGAAG	This study
PR1009	ArmAf	TGCATCAAATATGGGGGTCT	S1
PR1010	ArmAr	GGATTGAAGCCACAACCAAA	S1
PR1011	gyrA1	AAATCTGCCCGTGTCGTTGGT	S14
PR1012	gyrA2	GCCATACCTACGGCGATACC	S14
PR1013	parC1	AAACCTGTTCAGCGCCGCATT	S12
PR1014	parC2	AAAGTTGTCTTGCCATTCACT	S12
PR1023	CTX-M2F	TTTGCGATGTGCAGTACCAGTAA	This study

PR1024	CTX-M2R	CGATATCGTTGGTGGTGCCATA	This study
PR1025	qnrAf	ATTTCTCACGCCAGGATTTG	S1
PR1026	qnrAr	GATCGGCAAAGGTTAGGTCA	S1
PR1073	pecMF2	TCGCACCAATCCACCTGC	This study
PR1074	topAF2	CTATCCCGAGTGCAAGACCT	This study
PR1108	topAF3	GCAACCTGACCGATGGAA	This study
PR1109	pecMR2	TGTTCTTGGATCAGACGCTG	This study
PR1111	pecMF3	GCCACTTCACCCACTTGA	This study
PR1212	sul1C	GTATTGCGCCGCTCTTAGAC	This study
PR1213	sul1D	CCGACTTCAGCTTTTGAAGG	This study
PR1247	adeBF	CTTGCATTTACGTGTGGTGT	This study
PR1248	adeBR	GCTTTTCTACTGCACCCAAA	This study
PR1249	adeJF	GGTCATTAATATCTTTGGC	This study
PR1250	adeJR	GGTACGAATACCGCTGTCA	This study
PR1758	ModA-F	TTAAAGACTAAGCAAAAGAA	This study
PR1759	ModC-R	GCAAAGTCAGTATCTCACA	This study
PR1760	ModF-F	TGTGCCACGGCGTCATTGCT	This study
PR1761	ModG-R	GCTCAATCATCGACAGTGGCG	This study
PR1762	ModG-F	ATCAAGCAGGGCACCGTCAC	This study
PR1763	ModI-R	CAAATTGCAGTTCGCGCTTAGC	This study
PR1764	ModI-F	CCGGCCCTTTGTTTCATCCT	This study
PR1765	ModE-R	TATCCCTAGTTCCTTCGGCGGCAAGT	This study
PR1766	ModC-F	CATCCGCGATCATTTGAATCAGTTC	This study
PR1767	ModK-R	CGTAGCAGCCGTTGATAAAGTTGTC	This study
PR1776	OXA-51-likeF	TAATGCTTTGATCGGCCTTG	S15
PR1777	OXA-51-likeR	TGGATTGCACTTCATCTTGG	S15
PR1778	OXA-23-likeF	GATCGGATTGGAGAACCAGA	S15
PR1779	OXA-23-likeR	ATTTCTGACCGCATTTCCAT	S15

PR1780	OXA-24-likeF	GGTTAGTTGGCCCCCTTAAA	S15
PR1781	OXA-24-likeR	AGTTGAGCGAAAAGGGGATT	S15
PR1801	OXA-58-likeF	AAGTATTGGGGCTTGTGCTG	S15
PR1802	OXA-58-likeR	CCCCTCTGCGCTCTACATAC	S15
PR1803	OXA-143-F	TGGCACTTTCAGCAGTTCCT	S3
PR1804	OXA-143-R	TAATCTTGAGGGGGCCAACC	S3
PR1805	GIM-F1	TCAATTAGCTCTTGGGCTGAC	S6
PR1806	GIM-R1	CGGAACGACCATTTGAATGG	S6
PR1807	SPM-F1	CTAAATCGAGAGCCCTGCTTG	S6
PR1808	SPM-R1	CCTTTTCCGCGACCTTGATC	S6
PR1809	Int1F	CAGTGGACATAAGCCTGTTC	S4
PR1810	Int1R	CCCGAGGCATAGACTGTA	S4
PR1811	RH935	GCAGTCGCCCTAAAACAAAG	S9
PR1812	RH936	CCCGTATGCCCAACTTTGTA	S9
PR1813	NDM1-F	GCGCAACACAGCCTGACTTT	S5
PR1814	NDM1-R	CAGCCACCAAAAGCGATGTC	S5
PR1832	CTXM9-F	GTGACAAAGAGAGTGCAACGG	S10
PR1833	CTXM9-R	ATGATTCTCGCCGCTGAAGCC	S10

Footnotes:

* Nucleotides complementary to the *sacB* cassette are shown in lower case

^ Nucleotides carrying an introduced *Pac*I restriction site are shown in lower case

Table S2. Tn*AbaR* and antibiotic resistance-associated gene- and/or locus-specific PCR survey

Target gene(s)	NCBI	Tn*AbaR*	Primer combination	*In silico* prediction (bp)‡	AYE	A424	DCO163	DCO174
intI1	ABAYE3633	Tn*AbaR1*	PR1809/PR1810	160 (AYE)	+	-	±	±
lspA	ABAYE3651	Tn*AbaR1*	PR904/PR905	435 (AYE)	+	+	-	-
ebr	ABAYE3569	Tn*AbaR1*	PR978/PR979	271 (AYE)	+	+	+ (~1.8 Kb)	+ (~1.8 Kb)
adeB	ABAYE1822	NA	PR1247/PR1248	169 (AYE)	+	+	+	+
ybjA	ABAYE3588	Tn*AbaR1*	PR970/PR971	242 (AYE)	+	+	-	-
tetA(A)	ABAYE3597	Tn*AbaR1*	PR966/PR967	210 (AYE)	+	+	-	-
cadAR	ABAYE3559, ABAYE3560	Tn*AbaR1*	PR920/PR921	1,045 (AYE)	+	+	-	-
merAD	ABAYE3605, ABAYE3606	Tn*AbaR1*	PR875/PR876	1,143 (AYE)	+	+	-	-
pecM	ABAYE3596	Tn*AbaR1*	PR940/PR941	696 (AYE)	+	+	-	-
adeB-like	ABAYE0747	NA	PR1249/PR1250	222 (AYE)	+	+	+	+
tniA	ABAYE3666	Tn*AbaR1*	PR873/PR874	1,055 (AYE)	+	+	-	-
$\mathit{orf5}_{\mathit{In2}\text{-type}}$	ABAYE3567	Tn*AbaR1*	PR1001/PR1002	426 (AYE)	+	+	+ (~1.7 kb)	+ (~1.7 kb)
aadA1	ABAYE3570	Tn*AbaR1*	PR906/PR768	397 (AYE)	+	+	-	-
aacC1	ABAYE3578	Tn*AbaR1*	PR1811/PR1812	456 (AYE)	+	-	+	+
catA	ABAYE3587	Tn*AbaR1*	PR968/PR969	594 (AYE)	+	-	-	-
folA	ABAYE3315	NA	PR946/PR947	425 (AYE)	+	+	+	+
$\mathit{bla}_{\text{OXA-69}}$*	ABAYE2122	NA	PR1003/PR1004	545 (AYE)	+	+	+	+
trkA-trxB	ABAYE3661, ABAYE3662	Tn*AbaR1*	PR533/PR164	1,891 (AYE)	+	+	-	-
arsCRCBH	ABAYE3656-ABAYE3660	Tn*AbaR1*	PR167/PR168	2,688 (AYE)	+	+	-	-
topA	AB57_0262	Tn*AbaR3*	PR980/PR981	1,555 (AB0057)	-	+	-	-
orf4-sup	ABAYE3552, ABAYE3553	Tn*AbaR1*	PR138/PR871	2,982 (AYE)	+	+	-	-
adeA	ABAYE1821	NA	PR954/PR955	159 (AYE)	+	+	+	+
$\mathit{bla}_{\text{OXA-66}}$*	ACICU_01560	NA	PR944/PR945	637 (ACICU)	+	+	+	+
qnrA	GI:66474467	NA	PR1025/PR1026	657 (AY931017.1)	-	-	-	-
$\mathit{tnpA}_{\text{IS}\mathit{26}}$	ABAYE3576	Tn*AbaR1*	PR942/PR943	561 (AYE)	+	+	+	+

IS*AbaI*	ABAYE0193	NA	PR948/PR949	877 (AYE)	+	+	+	+
lysR	ABAYE3636	Tn*AbaR1*	PR964/PR965	660 (AYE)	+	-	-	-
sul1	ABAYE3612	Tn*AbaR1*	PR1212/PR1213	408 (AYE)	+	+	-	-
aphE	ABAYE3648	Tn*AbaR1*	PR976/PR977	837 (AYE)	+	-	-	-
aphA1	ABAYE3578	Tn*AbaR1*	PR902/PR903	701 (AYE)	+	-	-	-
aadB	ABAYE3622	Tn*AbaR1*	PR907/PR908	524 (AYE)	+	-	-	-
aac6'	ABAYE3630	Tn*AbaR1*	PR953/PR919	416 (AYE)	+	-	-	-
armA	GI:156105450	NA	PR1009/PR1010	214 (EUO14811.1)	-	-	-	-
tetR(G)	ABAYE3639	Tn*AbaR1*	PR881/PR901	505 (AYE)	+	-	-	-
clmA	ABAYE3620	Tn*AbaR1*	PR974/PR975	1034 (AYE)	+	-	-	-
$bla_{\text{OXA-58-like}}$	ACICU_p0019	NA	PR1801/PR1802	599 (ACICU)	-	-	-	-
bla_{IMP}	GI:4210822	NA	PR915/PR916	448 (AJ223604)	-	-	-	-
bla_{VIM}	GI:14133992	NA	PR917/PR918	780 (AF369871.1)	-	-	-	-
bla_{TEM}	AB57_0283	Tn*AbaR3*	PR911/PR912	824 (AB0057)	-	-	-	-
bla_{SHV}	GI:18656796	NA	PR958/PR959	861 (AAL77983.1)	-	-	-	-
$bla_{\text{VEB-1}}$	ABAYE3623	Tn*AbaR1*	PR909/PR910	830 (AYE)	+	-	-	-
$bla_{\text{CTX-M-9}}$	GI:16357504	NA	PR1832/PR1833	857 (pMSP071)	-	-	-	-
$bla_{\text{CTX-M-2}}$	GI:74055071	NA	PR1023/PR1024	544 (AAZ95872.1)	-	-	-	-
arr-2	ABAYE3621	Tn*AbaR1*	PR960/PR961	401 (AYE)	+	-	-	-
$bla_{\text{OXA-51-like}}$	ABAYE2122	NA	PR1776/PR1777	353 (AYE)	+	+	+	+
$bla_{\text{OXA-23-like}}$	AB57_0551	Tn*AbaR4*	PR1778/PR1779	501 (AB0057)	-	-	-	-
$bla_{\text{OXA-24-like}}$	GI: 258445192	NA	PR1780/PR1781	249 (GQ377752.1)	-	-	-	-
$bla_{\text{OXA-143}}$	GI:261348124	NA	PR1803/PR1804	150 (GQ861437.1)	-	-	-	-
bla_{GIM}	GI:42516777	NA	PR1805/PR1806	72 (AJ620678.1)	-	-	-	-
bla_{SPM}	EMBL: AJ492820	NA	PR1807/PR1808	798 (AJ492820)	-	-	-	-
bla_{NDM1}	GI:310892440	NA	PR1813/PR1814	155 (HQ256747.1)	-	-	-	-
$bla_{\text{OXA-10-like}}$	ABAYE3619	Tn*AbaR1*	PR956/PR957	760 (AYE)	+	-	-	-

Footnote:

* primers used may have resulted in amplification of other *bla* genes

[‡] Predicted sizes of amplicons with templates as indicated in brackets (strain names for genome sequences or GenBank accession numbers)

±, faint bands as compared to AYE

Table S3. PCR mapping of Tn*AbaR23*

Primer combination	*In silico* prediction (bp)	AYE	A424	DCO163	DCO174
PR545/PR723	4,114 (AYE)	+	+	-	-
PR545/PR721	7,194 (AYE)	+	+	±	±
PR872/PR168	8,464 (AYE)	+	+	-	-
PR167/PR302	5,566 (AYE)	+	+	-	-
PR872/PR905	11,166 (AYE)	+	+	-	-
PR167/PR921	4,755 (AYE)	+	+	-	-
PR872/PR131	83,727 (AYE)	-	-	-	-
PR920/PR131	7,710 (AYE)	+	+	-	-
PR138/PR131	4,110 (AYE)	+	+	-	-
PR301/PR131	75,241 (AYE)	-	-	-	-
PR165/PR131	5,874 (AYE)	+	+	-	-
PR1008/PR131	4,841 (AYE)	+	+	-	-
PR545/PR874	2,465 (AYE)	+	+	-	-
PR873/PR723	2,704 (AYE)	+	+	-	-
PR872/PR168	8,464 (AYE)	+	+	-	-
PR167/PR921	4,755 (AYE)	+	+	-	-
PR301/PR905	2,680 (AYE)	+	+	-	-
PR904/PR126	4,949 (AYE)	+	-	-	-
PR121/PR921	8,338 (AYE)	+	-	-	-
PR303/PR921	3,844 (AYE)	+	+	-	-
PR962/PR876	5,499 (AYE)	+	+	-	-
PR966/PR971	3,904 (AYE)	+	+	-	-
PR904/PR871	5,337 (AYE)	+	+	-	-
PR970/PR902	11,462 (AYE)	+	-	-	-
PR121/PR122	1,242 & 3,080 & 7,148 (AYE)	+^	-	-	-
PR1758/PR1759	2,580 (AYE)	+	+	-	-
PR1766/PR1765	5,148 (AB0057)	-	+	-	-
PR1760/PR1761	5,887 (AB0057)	-	+	-	-
PR1762/PR1763	17,203 (AYE)	-	+ (3.9 kb)*	-	-
PR1764/PR1767	4,195 (AYE)	+	+	-	-

Footnotes:

±, faint bands as compared to AYE and A424

*, 3.9 kb amplicon size predicted from A424 genome sequence data

Orange shading, PCR assays performed following availability of A424 whole genome sequence data

^, all three expected bands were detected

Table S4. CDS identified in Tn*AbaR1* in *A. baumannii* AYE (NC_010410) [a]

Locus tag in AYE (NC_010410) / TnAbaR1 (CT025832)	Coordinates in AYE		Locus tag in TnAbR1	Orientation	Module	Description
	start	end				
ABAYE3668	3696928	3696245		-		C-terminal ComM, Mg^{2+} chelatase
ABAYE3667	3696168	3695410	TnAbaR1_01	+	A	TniA transposition protein A
ABAYE3666	3695409	3693499	TnAbaR1_02	+	A	TniB transposition protein B
ABAYE3665	3693494	3692574	TnAbaR1_03	+	A	TniC transposition helper protein C
ABAYE3664	3692544	3691429	TnAbaR1_04	+	A	Orf 2 hypothetical protein
ABAYE3663	3691451	3689988	TnAbaR1_05	+	A	Orf 3 hypothetical protein
ABAYE3662	3689884	3688763	TnAbaR1_06	-	B	TrkA putative monooxygenase
ABAYE3661	3688715	3687762	TnAbaR1_07	-	B	TrxB thioredoxin reductase
ABAYE3660	3687744	3687040	TnAbaR1_08	-	C	ArsH arsenic resistance protein
ABAYE3659	3687034	3685991	TnAbaR1_09	-	C	ArsB transmembrane protein of arsenate pump
ABAYE3658	3685983	3685510	TnAbaR1_10	-	C	ArsC arsenate reductase
ABAYE3657	3685503	3685171	TnAbaR1_11	-	C	ArsR arsenite inducible repressor
ABAYE3656	3685125	3684625	TnAbaR1_12	-	C	ArsC arsenate reductase
ABAYE3655	3684488	3683961	TnAbaR1_13	-	C	Truncated N-terminal end of UspA universal stress protein
ABAYE3654	3683868	3683446	TnAbaR1_14	-	D	Cad R transcriptional regulator, MerR family
ABAYE3653	3683365	3682469	TnAbaR1_15	+	D	Cad A heavy metal detoxification protein
ABAYE3651	3682465	3681953	TnAbaR1_16	+	D	LspA lipoprotein signal peptide
ABAYE3650	3681931	3680642	TnAbaR1_17	+	D	Transposase IS*204*/IS*1001*/IS*1096*/IS*1165* family
ABAYE3649	3680620	3680489	TnAbaR1_18	+	E	Truncated Sat, streptomycin 3"-adenylyltransferase
ABAYE3648	3680492	3679656	TnAbaR1_19	+	E	StrA streptomycin 3"-kinase
ABAYE3647	3679665	3678820	TnAbaR1_20	+	E	StrB streptomycin 3"-kinase
1_780	3678848	3678498	TnAbaR1_21	-	E	TnpM transposase

ABAYE3646	3678559	3677546	TnAbaR1_22	-	E	Int integrase/recombinase
ABAYE3645	3677616	3677386	TnAbaR1_23	+	E	Truncated Sat, streptomycin acetyl-transferase
ABAYE3644	3677389	3676916	TnAbaR1_24	+	E	DrfI dihydrofolate reductase type I
New annotation	3676822	3676343	TnAbaR1_25	+	E	Hypothetical protein
ABAYE3642	3676212	3675865	TnAbaR1_26	+	E	QacEdelta1
ABAYE3641	3675958	3674975	TnAbaR1_27	+	E	Truncated Sul1 dihydropteroate synthase type-1
ABAYE3640	3674758	3673544	TnAbaR1_28	+	E	CmlA chloramphenicol and florfenicol resistance protein
ABAYE3639	3673337	3672711	TnAbaR1_29	-	E	TetR tetracycline repressor protein, class G
ABAYE3637	3672607	3671432	TnAbaR1_30	+	E	TetA tetracycline repressor protein, class G
ABAYE3636	3671411	3670686	TnAbaR1_31	+	E	LysR transcription regulator
1_732	3670594	3669062	TnAbaR1_32	-	E	IS*CR3C* transposase
ABAYE3633	3668835	3668182	TnAbaR1_33	-	E	GroEL-integrase fusion protein
ABAYE3631	3668252	3667998	TnAbaR1_34	+	E	Truncated Sat, streptothricin acetyl-transferase
ABAYE3630	3668004	3667408	TnAbaR1_35	+	E	Aac6' aminoglycoside 6-acetyltransferase
ABAYE3629	3667456	3665606	TnAbaR1_36	-	E	RecG ATP-dependent DNA helicase
1_710	3665481	3663937	TnAbaR1_37	-	E	IS*CR3C* transposase
ABAYE3627	3663798	3663145	TnAbaR1_38	-	E	GroEL-integrase fusion protein
ABAYE3625	3663215	3663030	TnAbaR1_39	+	E	Truncated aminoglycoside adenylyltransferase
ABAYE3624	3663020	3661770	TnAbaR1_40	-	E	Transposase of IS*10A*, IS4 family
ABAYE3623	3661636	3660737	TnAbaR1_41	+	E	bla(VEB-1) β-lactamase
ABAYE3622	3660592	3660059	TnAbaR1_42	+	E	AadB aminoglycoside adenylyltransferase
ABAYE3621	3660013	3659522	TnAbaR1_43	+	E	Arr-2 rifampin ADP-ribosylating transferase
ABAYE3620	3659255	3657942	TnAbaR1_44	+	E	CmlA chloramphenicol resistance protein
ABAYE3619	3657677	3656877	TnAbaR1_45	+	E	bla(OXA-10) β-lactamase
ABAYE3618	3656899	3656069	TnAbaR1_46	+	E	AadA1 aminoglycoside adenylyltransferase
ABAYE3617	3655905	3655558	TnAbaR1_47	+	E	QacEdelta1

ABAYE3616	3655651	3654725	TnAbaR1_48	+	E	Sul1 dihydropteroate synthase type-1
ABAYE3615	3654320	3652779	TnAbaR1_49	+	E	ORF513 transposase
ABAYE3614	3652397	3651834	TnAbaR1_50	+	E	DfrA10 dihydrofolate reductase type A10
ABAYE3613	3651797	3651450	TnAbaR1_51	+	E	QacEdelta1
ABAYE3612	3651543	3650617	TnAbaR1_52	+	E	Sul1 dihydropteroate synthase type-1
ABAYE3611	3650489	3659989	TnAbaR1_53	+	E	GCN5-like N-acetyltransferase
1_213	3649518	3648718	TnAbaR1_54	+	G	TnpA transposase for insertion sequence IS*6100*
1_627	3648480	3648268	TnAbaR1_55	-	G	Urf2Y
ABAYE3607	3648202	3647966	TnAbaR1_56	-	G	MerE mercuric resistance protein
ABAYE3606	3647969	3647571	TnAbaR1_57	-	G	MerD HTH-type transcriptional regulator
ABAYE3605	3647586	3645841	TnAbaR1_58	-	G	MerA mercuric ion reductase (Hg(II) reductase)
ABAYE3604	3645862	3645437	TnAbaR1_59	-	G	MerC mercury resistance inner membrane protein
1_612	3645409	3645134	TnAbaR1_60	-	G	MerP mercuric transporter protein, periplasmic binding protein
1_609	3645118	3644753	TnAbaR1_61	-	G	MerT mercuric ion transport protein
ABAYE3601	3644681	3644226	TnAbaR1_62	+	G	MerR mercury resistance operon regulatory protein
ABAYE3599	3644088	3643846	TnAbaR1_63	+	G	Putative relaxase/helicase
ABAYE3598	3643814	3643137	TnAbaR1_64	-	G	TetR tetracycline repressor protein, Class A
ABAYE3597	3643133	3641859	TnAbaR1_65	+	G	TetA tetracycline resistance protein, Class A
ABAYE3596	3641827	3640943	TnAbaR1_66	-	G	PecM-like putative membrane protein
ABAYE3593	3640805	3640398	TnAbaR1_67	-	G	Putative isochorismatase hydrolase
1_275	3640478	3638658	TnAbaR1_68	+	G	TnpA transposase Tn*1721*
ABAYE3588	3638594	3638214	TnAbaR1_69	+	G	Putative GCN5-related N-acetyltransferase
ABAYE3587	3638013	3637354	TnAbaR1_70	-	G	CatA chloramphenicol acetyltransferase
1_285	3637075	3636800	TnAbaR1_71	+	G	InsA IS*1* transposase
1_287	3636881	3636363	TnAbaR1_72	+	G	InsB IS*1* transposase
ABAYE3584	3636366	3633817	TnAbaR1_73	-	G	Transposase of Tn*1*

ABAYE3583	3633566	3632844	TnAbaR1_74	-	I	TnpA transposase of IS*26*, IS*6* family
ABAYE3582	3632885	3629949	TnAbaR1_75	+	I	Transposase
ABAYE3581	3629838	3629179	TnAbaR1_76	+	I	TnpR resolvase
ABAYE3580	3629179	3628463	TnAbaR1_77	+	I	Transposase of IS*15DI*, IS*6* family
ABAYE3578	3627863	3627048	TnAbaR1_78	-	I	AphA1 aminoglycoside 3'-phosphotransferase
ABAYE3576	3626801	3626614	TnAbaR1_79	+	I	TnpA transposase of IS*26*, IS6 family
1_516	3625452	3626066	TnAbaR1_80	-	I	TnpM transposase
ABAYE3575	3625513	3624500	TnAbaR1_81	-	I	Int integrase/recombinase
ABAYE3574	3624570	3624352	TnAbaR1_82	+	I	Truncated Sat, streptothricin acetyl-transferase
ABAYE3573	3624398	3623865	TnAbaR1_83	+	I	AacC1 gentamicin 3'-acetyltransferase
ABAYE3572	3623767	3623234	TnAbaR1_84	+	I	OrfX putative GCN5-related N-acetyltransferase
ABAYE3571	3623208	3622696	TnAbaR1_85	+	I	OrfX putative GCN5-related N-acetyltransferase
1_344	3622680	3622375	TnAbaR1_86	+	I	Hypothetical protein
ABAYE3570	3622283	3621492	TnAbaR1_87	+	I	AadA1 aminoglycoside 3'-(9)-O-adenylyltransferase
ABAYE3569	3621328	3620981	TnAbaR1_88	+	I	QacEdelta1
ABAYE3568	3621074	3620148	TnAbaR1_89	+	I	Sul1 dihydropteroate synthase type-1
1_360	3620020	3619520	TnAbaR1_90	+	I	Orf5
New annotation	3619496	3619209	TnAbaR1_91	+	I	Hypothetical protein
1_365	3618853	3618368	TnAbaR1_92	+	I	Resolvase N-term
1_366	3618614	3618225	TnAbaR1_93	+	I	Resolvase C-term
ABAYE3562	3617997	3617779	TnAbaR1_94	+	I	Hypothetical protein
ABAYE3561	3617722	3617459	TnAbaR1_95	-	I	TrbI fragment of conjugal transfer protein
ABAYE3560	3617337	3616915	TnAbaR1_96	-	J	CadR transcriptional regulator, MerR family
ABAYE3559	3616834	3615938	TnAbaR1_97	+	J	CadA heavy metal transport/detoxification protein
ABAYE3558	3615934	3615422	TnAbaR1_98	+	J	IspA prolipoprotein signal peptidase (SPase II)
ABAYE3556	3615400	3614111	TnAbaR1_99	+	J	Transposase

ABAYE3554	3613725	3614050	TnAbaR1_100	-	K	Truncated N-terminal end of UspA universal stress protein
ABAYE3553	3614075	3612223	TnAbaR1_101	-	K	Sup sulphate transporter, MFS superfamily
ABAYE3552	3613710	3610132	TnAbaR1_102	-	K	Orf4 hypothetical protein
ABAYE3551	3611928	3609192	N/A	-	N/A	N-terminal ComM, Mg^{2+} chelatase
Total number of genes	102					
Total number of resistance genes	48					

[a] Putative resistance genes are highlighted in blue. Changes or additions to original annotations are highlight in red.

Table S5: Protein coding sequence identified in Tn*AbaR23* [a]

Locus_tag in Tn*AbaR23*	**Coordinates**		**Orientation**	**Module**	**Homolog in TnAbaR1 or other *A. baumannii* genome**	**Matching locus tag in Tn*AbaR1*/ other genomes**	**% identity [b]**
	start	**end**					
N/A	2931	2248	-	N/A	C-terminal ComM, Mg^{2+} chelatase	N/A	100
TnAbaR23_01	3008	3765	+	A	TniA transposition protein A	TnAbaR1_01	100
TnAbaR23_02	3767	5677	+	A	TniB transposition protein B	TnAbaR1_02	100
TnAbaR23_03	5682	6602	+	A	TniC transposition helper protein C	TnAbaR1_03	100
TnAbaR23_04	6632	7747	+	A	Orf 2 hypothetical protein	TnAbaR1_04	100
TnAbaR23_05	7725	9188	+	A	Orf 3 hypothetical protein	TnAbaR1_05	100
TnAbaR23_06	10386	9292	-	A	TrkA putative monooxygenase	TnAbaR1_06	100
TnAbaR23_07	11318	10461	-	B	TrxB thioredoxin reductase	TnAbaR1_07	100
TnAbaR23_08	12136	11432	-	B	ArsH arsenic resistance protein	TnAbaR1_08	100
TnAbaR23_09	13185	12142	-	C	ArsB transmembrane protein of arsenate pump	TnAbaR1_09	100
TnAbaR23_10	13666	13193	-	C	ArsC arsenate reductase	TnAbaR1_10	100
TnAbaR23_11	14005	13673	-	C	ArsR arsenite inducible repressor	TnAbaR1_11	100
TnAbaR23_12	14551	14051	-	C	ArsC arsenate reductase	TnAbaR1_12	100
TnAbaR23_13	15215	14688	-	C	Truncated N-terminal end of UspA universal stress protein	TnAbaR1_13	100
TnAbaR23_14	15730	15309	-	D	Cad R transcriptional regulator, MerR family	TnAbaR1_14	100
TnAbaR23_15	16707	15811	+	D	Cad A heavy metal detoxification protein	TnAbaR1_15	100
TnAbaR23_16	17223	15811	+	D	LspA lipoprotein signal peptide	TnAbaR1_16	99
TnAbaR23_17	17223	15811	+	D	Transposase IS*204*/IS*1001*/IS*1096*/IS*1165* family	TnAbaR1_17	100
TnAbaR23_18	19120	18687	-	F	Truncated hypothetical protein	AB57_0261	99
TnAbaR23_19	19342	21360	+	F	TopA DNA topoisomerase I	AB57_0262	100
TnAbaR23_20	21387	21740	+	F	Ssb protein	AbauAB059_010100015377	100
TnAbaR23_21	21768	22100	+	F	Hypothetical protein	AbauAB0_10281	100
TnAbaR23_22	22863	22432	-	F	Hypothetical protein	AB57_0264	100

TnAbaR23_23	22971	23912	+	F	Sul1 dihydropteroate synthase	AB57_0265	100
TnAbaR23_24	24040	24540	+	F	Orf 5 acetyltransferase, GNAT family	AB57_0266	100
TnAbaR23_25	25011	25811	+	G	TnpA transposase for insertion sequence IS*6100*	TnAbaR1_54	100
TnAbaR23_26	26261	26049	-	G	Urf2Y	TnAbaR1_55	100
TnAbaR23_27	26563	26327	-	G	MerE mercuric resistance protein	TnAbaR1_56	100
TnAbaR23_28	26946	26563	-	G	MerD HTH-type transcriptional regulator	TnAbaR1_57	100
TnAbaR23_29	28628	26943	-	G	MerA mercuric ion reductase (Hg(II) reductase)	TnAbaR1_58	100
TnAbaR23_30	29092	28667	-	G	MerC mercury resistance inner membrane protein	TnAbaR1_59	100
TnAbaR23_31	29395	29122	-	G	MerP mercuric transporter protein, periplasmic binding protein	TnAbaR1_60	100
TnAbaR23_32	29776	29411	-	G	MerT mercuric ion transport protein	TnAbaR1_61	100
TnAbaR23_33	29848	30303	+	G	MerR mercury resistance operon regulatory protein	TnAbaR1_62	100
TnAbaR23_34	30441	30683	+	G	Putative relaxase/helicase	TnAbaR1_63	100
TnAbaR23_35	31392	30715	-	G	TetR tetracycline repressor protein, Class A	TnAbaR1_64	100
TnAbaR23_36	31396	32670	+	G	TetA tetracycline resistance protein, Class A	TnAbaR1_65	100
TnAbaR23_37	33586	32702	-	G	PecM-like putative membrane protein	TnAbaR1_66	100
TnAbaR23_38	34131	33724	-	G	Putative isochorismatase hydrolase	TnAbaR1_67	100
TnAbaR23_39	34075	35871	+	G	TnpA transposase Tn*1721*	TnAbaR1_68	100
TnAbaR23_40	35935	36315	+	G	Putative GCN5-related N-acetyltransferase	TnAbaR1_69	100
TnAbaR23_41	36716	36641	-	G	CatA chloramphenicol acetyltransferase	TnAbaR1_70	100
TnAbaR23_42	37234	36623	-	I	Transposase of IS*15DI*, IS*6* family	TnAbaR1_77	98
TnAbaR23_43	37466	37951	+	I	Truncated AacC1 gentamicin 3'-acetyltransferase	TnAbaR1_83	99
TnAbaR23_44	38070	38582	+	I	OrfX putative GCN5-related N-acetyltransferase	TnAbaR1_85	100
TnAbaR23_45	38598	38903	+	I	Hypothetical protein	TnAbaR1_86	100
TnAbaR23_46	38995	39786	+	I	AadA1 aminoglycoside 3'-(9)-O-adenylyltransferase	TnAbaR1_87	100
TnAbaR23_47	39950	40297	+	I	QacEdelta1	TnAbaR1_88	100
TnAbaR23_48	40204	41130	+	I	Sul1 dihydropteroate synthase type-1	TnAbaR1_89	100

TnAbaR23_49	41258	41758	+	I	Orf5	TnAbaR1_90	99
TnAbaR23_50	41782	42069	+	I	Hypothetical protein	TnAbaR1_91	100
TnAbaR23_51	42425	42910	+	I	Resolvase N-term	TnAbaR1_92	100
TnAbaR23_52	42664	43053	+	I	Resolvase C-term	TnAbaR1_93	100
TnAbaR23_53	43281	43499	-	I	Hypothetical protein	TnAbaR1_94	100
TnAbaR23_54	43819	43556	-	I	TrbI fragment of conjugal transfer protein	TnAbaR1_95	100
TnAbaR23_55	44363	43941	-	J	CadR transcriptional regulator, MerR family	TnAbaR1_96	100
TnAbaR23_56	44444	45340	+	J	CadA heavy metal transport/detoxification protein	TnAbaR1_97	100
TnAbaR23_57	45344	45856	+	J	IspA prolipoprotein signal peptidase (SPase II)	TnAbaR1_98	100
TnAbaR23_58	45878	47167	+	J	Transposase	TnAbaR1_99	100
TnAbaR23_59	47553	47226	-	K	Truncated N-terminal end of UspA universal stress protein	TnAbaR1_100	100
TnAbaR23_60	49055	47568	-	K	Sup sulphate transporter, MFS superfamily	TnAbaR1_101	100
TnAbaR23_61	51146	49350	-	K	Orf4 hypothetical protein	TnAbaR1_102	100
N/A	52086	51229	-	N/A	N-terminal ComM, Mg^{2+} chelatase	N/A	
Total number of genes	61						
Total number of resistance genes	27						

[a] Putative resistance genes are highlighted in blue. Changes or additions to original annotations are highlight in red.

[b] Percentage identity between the predicted amino acid sequences of Tn*AbaR23* gene and its matching locus.

Table S6. Annotation details of non-Tn*AbaR1* modules identified to date within the Tn*AbaR* family of transposons [a]

Locus_tag	Coordinates		Orientation	Module	Description
	start	end			
AB57_0261	279360	279794	-	F	Hypothetical protein
AB57_0262	280016	282034	+	F	TopA DNA topoisomerase I
AB57_0263	282061	282414	+	F	Ssb protein
New annotation	282442	282774	+	F	Hypothetical protein
AB57_0264	283106	283537	-	F	Hypothetical protein
AB57_0265	283659	284585	+	F	Sul1 dihydropteroate synthase
AB57_0266	284713	285231	+	F	Orf 5 acetyltransferase, GNAT family
AB57_0281	298836	301841	-	H	TnpA Transposase Tn*3*
AB57_0282	302004	302561	+	H	TnpR Transposon Tn*3* resolvase
AB57_0283	302744	303604	+	H	Bla(TEM) β-lactamase
AB57_0549	584648	585082	-	L	Transposase
AB57_0550	585156	585738	-	L	Transposase 1
AB57_0551	585844	586665	+	L	Bla(OXA-23) β-lactamase
AB57_0552	586770	587078	-	L	AAA ATPase superfamily protein
AB57_0553	587330	587884	-	L	Hypothetical protein
AB57_0554	587892	588224	-	L	YeeA Type II restriction enzyme
AB57_0555	588326	588895	+	L	Transposase 1
AB57_0556	588982	589416	+	L	Transposase subunit
ACICU_00223	250782	251414	+	M	Aac(6)-1b Aminoglycoside 6'-N-acetyl transferase type Ib
ACICU_00225	251553	251876	+	M	Hypothetical protein
ACICU_00226	251962	252762	+	M	bla(OXA-20) β-lactamase
A1S_0208	230959	231921	-	N	Is*Aba11*
AB57_0558	590899	591747	-	O	Universal stress protein
AB57_0559	592187	592558	-	O	Hypothetical protein
AB57_0560	592667	592786	-	O	UspA

[a] Putative resistance genes are highlighted in blue. Changes or additions to original annotations are highlight in red.

Supplementary References

S1. **Adams-Haduch, J. M., D. L. Paterson, H. E. Sidjabat, A. W. Pasculle, B. A. Potoski, C. A. Muto, L. H. Harrison, and Y. Doi.** 2008. Genetic basis of multidrug resistance in *Acinetobacter baumannii* clinical isolates at a tertiary medical center in Pennsylvania. Antimicrob. Agents Chemother. **52:**3837-3843. doi: 10.1128/AAC.00570-08.

S2. **Choi, K. H., and H. P. Schweizer.** 2005. An improved method for rapid generation of unmarked *Pseudomonas aeruginosa* deletion mutants. BMC Microbiol. **5:**30. doi: 10.1186/1471-2180-5-30.

S3. **Higgins, P. G., M. Lehmann, and H. Seifert.** 2010. Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents. **35:**305. doi: 10.1016/j.ijantimicag.2009.10.014.

S4. **Koeleman, J. G., J. Stoof, M. W. Van Der Bijl, C. M. Vandenbroucke-Grauls, and P. H. Savelkoul.** 2001. Identification of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR. J. Clin. Microbiol. **39:**8-13. doi: 10.1128/JCM.39.1.8-13.2001.

S5. **Kusradze, I., S. M. Diene, M. Goderdzishvili, and J. M. Rolain.** 2011. Molecular detection of OXA carbapenemase genes in multidrug-resistant *Acinetobacter baumannii* isolates from Iraq and Georgia. Int. J. Antimicrob. Agents. **38:**164-168. doi: 10.1016/j.ijantimicag.2011.03.021.

S6. **Mendes, R. E., K. A. Kiyota, J. Monteiro, M. Castanheira, S. S. Andrade, A. C. Gales, A. C. Pignatari, and S. Tufik.** 2007. Rapid detection and identification of metallo-β-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. J. Clin. Microbiol. **45:**544-547. doi: 10.1128/JCM.01728-06.

S7. **Nagano, N., Y. Nagano, C. Cordevant, N. Shibata, and Y. Arakawa.** 2004. Nosocomial transmission of CTX-M-2 β-lactamase-producing *Acinetobacter baumannii* in a neurosurgery ward. J. Clin. Microbiol. **42:**3978-3984. doi: 10.1128/JCM.42.9.3978-3984.2004.

S8. **Ng, L. K., I. Martin, M. Alfa, and M. Mulvey.** 2001. Multiplex PCR for the detection of tetracycline resistant genes. Mol. Cell. Probes. **15:**209-215. doi: 10.1006/mcpr.2001.0363.

S9. **Nigro, S. J., V. Post, and R. M. Hall.** 2011. Aminoglycoside resistance in multiply antibiotic-resistant *Acinetobacter baumannii* belonging to global clone 2 from Australian hospitals. J. Antimicrob. Chemother. **66:**1504-1509. doi: 10.1093/jac/dkr163.

S10. **Novais, A., R. Cantón, A. Valverde, E. Machado, J. C. Galán, L. Peixe, A. Carattoli, F. Baquero, and T. M. Coque.** 2006. Dissemination and persistence of *bla*$_{\text{CTX-M-9}}$ are linked to class 1 integrons containing CR1 associated with defective transposon derivatives from Tn*402* located in early antibiotic resistance plasmids of IncHI2, IncP1-alpha, and IncFI groups. Antimicrob. Agents Chemother. **50:**2741-2750. doi: 10.1128/AAC.00274-06.

S11. **Post, V., and R. M. Hall.** 2009. AbaR5, a large multiple-antibiotic resistance region found in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **53:**2667-2671. doi: 10.1128/AAC.01407-08.

S12. **Shaikh, F., R. P. Spence, K. Levi, H. Y. Ou, Z. Deng, K. J. Towner, and K. Rajakumar.** 2009. ATPase genes of diverse multidrug-resistant *Acinetobacter baumannii* isolates frequently harbour integrated DNA. J. Antimicrob. Chemother. **63:**260-264. doi: 10.1093/jac/dkn481.

S13. **Vila, J., J. Ruiz, P. Goñi, and T. Jimenez de Anta.** 1997. Quinolone-resistance mutations in the topoisomerase IV *parC* gene of *Acinetobacter baumannii*. J. Antimicrob. Chemother. **39:**757-762.

S14. **Vila, J., J. Ruiz, P. Goñi, A. Marcos, and T. Jimenez de Anta.** 1995. Mutation in the *gyrA* gene of quinolone-resistant clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **39:**1201-1203.

S15. **Woodford, N., M. J. Ellington, J. M. Coelho, J. F. Turton, M. E. Ward, S. Brown, S. G. Amyes, and D. M. Livermore.** 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents. **27:**351-353. doi: 10.1016/j.ijantimicag.2006.01.004.