Figure S1: Comparison of the ARI-A region in *bla*_{NDM}-positive IncA/C plasmids. Numbers in parentheses indicate sequences with similar *bla*_{NDM} structures. Features are colour coded as follows: *bla*_{NDM}, red; other resistance genes, pink; IS/transposable elements, purple; DNA binding/modification, green; conjugation, blue; plasmid stability, yellow. Plasmids KU302801.1 and KU302802.1 were omitted as the right side of ARI-A has been deleted in these plasmids.
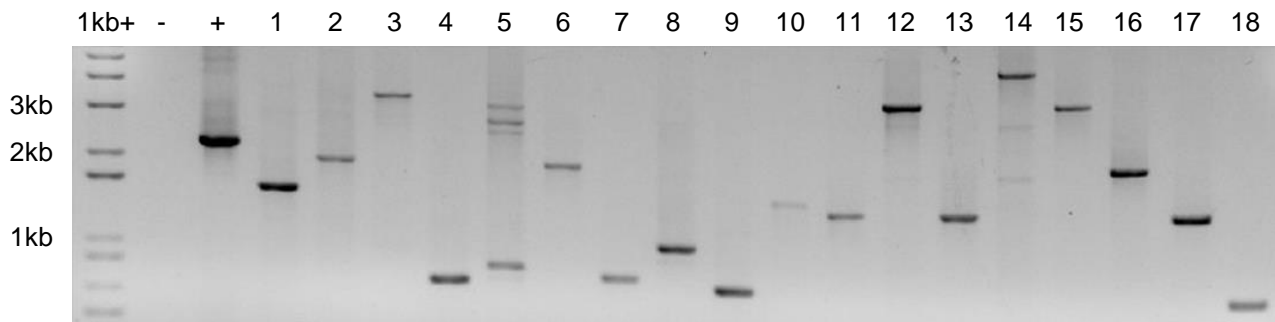
Figure S2: Randomness of *in vitro* mutagenesis of pMS6198A. Image of inverse PCR products from 18 individual mutants generated by *in vitro* transposon mutagenesis.
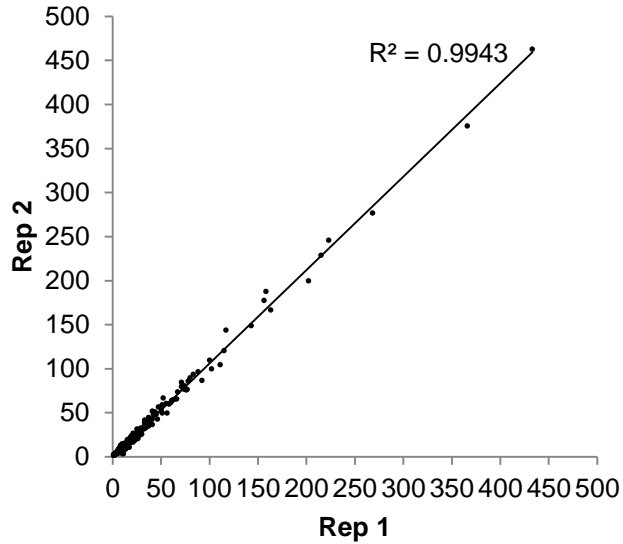
Figure S3: *In vitro* **mutagenesis and TraDIS is highly reproducible**. Correlation plot comparing the number of transposon insertion sites within each gene in replicate 1 compared to replicate 2.
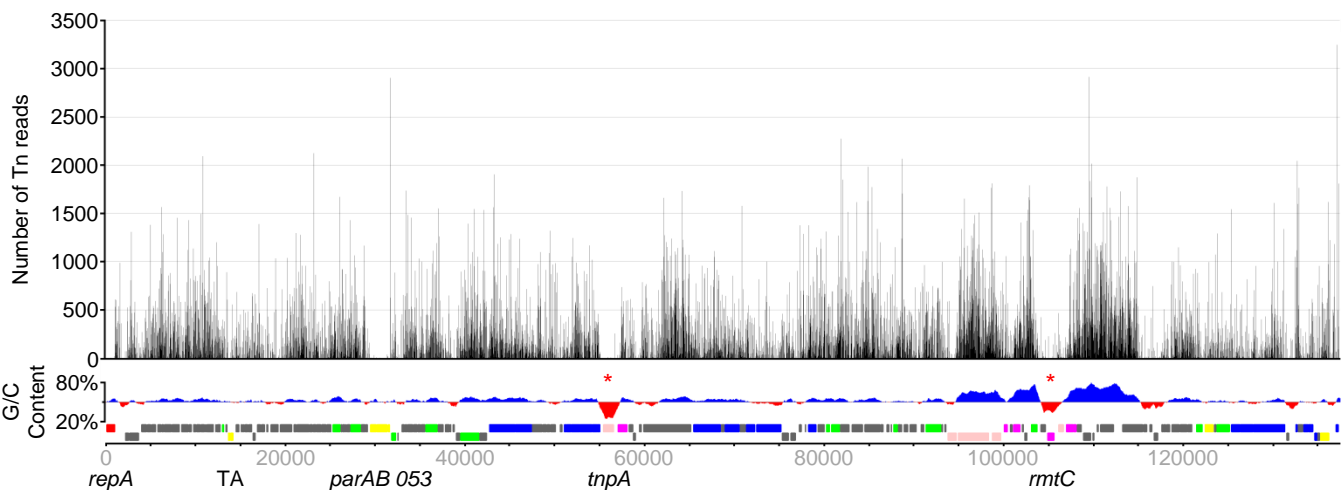
Figure S4: Comparison of Tn*5* insertion frequency and GC-content of pMS6198A. Tracks from top to bottom are; graph of the number of transposon specific reads mapped to each base; GC-content; CDS on reverse strand; CDS on forward strand. CDS are colour coded as per Figure 1. Regions of low GC content and low insertion frequency are indicated by an asterisk; these regions correspond to the false positive TraDIS hits *tnpA* and *rmtC*.
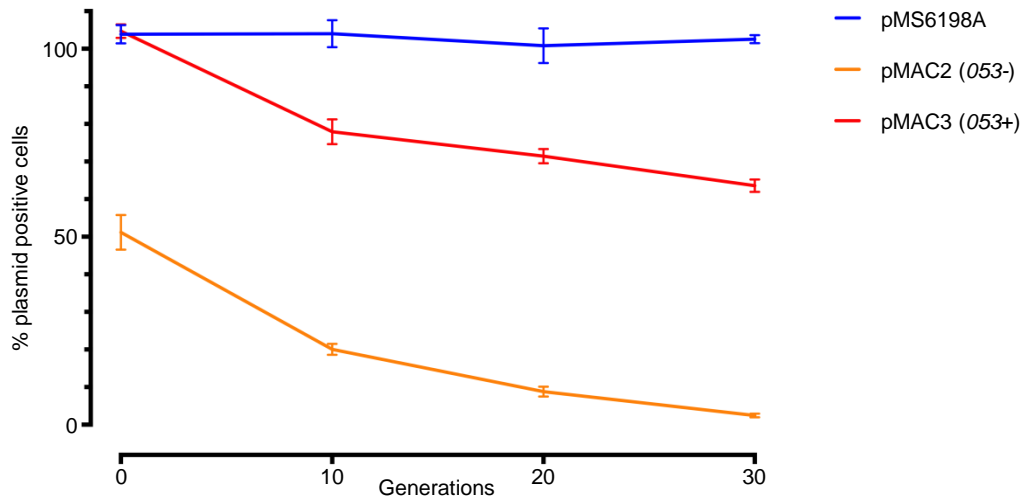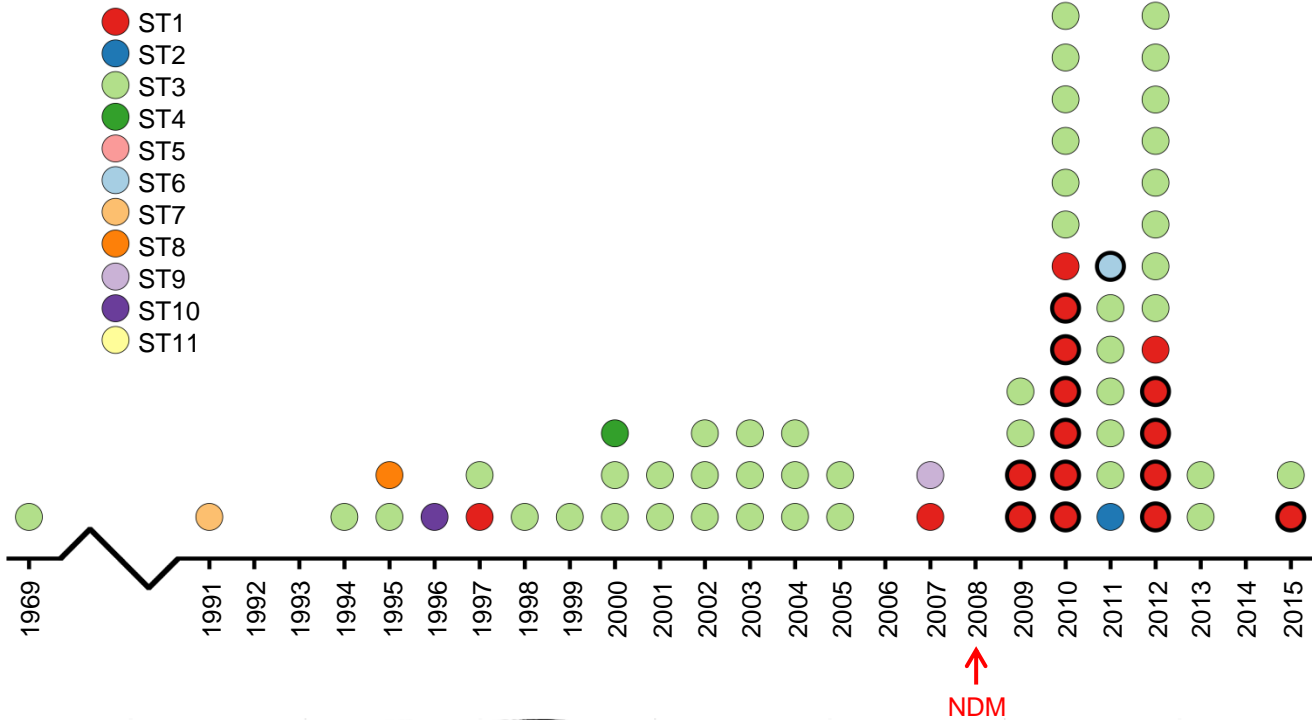
Figure S5: Time course stability assay in broth culture of pMS6198A and miniA/C plasmids, pMAC2 and pMAC3. The assays were performed in triplicate. Data points are mean ± standard deviation.

Figure S6: Minimal spanning tree showing the IncA/C core gene PMLST (cgPMLST). Branch lengths indicate the number of allele differences between cgSTs. The colour of each cgST indicates its respective ST as per the legend. Numbers inside each circle are the cgST subtypes. Unless otherwise shown in the circle n=1. Plasmids that contain a bla_{NDM} structure similar to that present in pMS6198A (Figure S1) are indicated by an asterisk.

Figure S7: Spatio-temporal distribution of fully-sequenced IncA/C plasmids from the NCBI database. A) Temporal distribution of currently available IncA/C plasmids by sequence type. Each circle represents a single plasmid colour coded according to its ST. Circles with thick borders indicate plasmids carrying bla_{NDM}; the red NDM arrow indicates the year of bla_{NDM} discovery. Data based on 71/82 plasmids with isolation date. B) Global distribution of currently available IncA/C plasmids by sequence type. Pie charts show the proportion of STs while the circle size indicates the number of plasmids from each country. Data based on 78/82 IncA/C plasmids with location information.