Figure 4. Immunoblot analysis of SDS solubilised total bacterial protein probed with monoclonal anti-multiple banded antigen antibody. Of 26 individual transformation experiments only one strain (U6) showed altered mobility of the MBA following transposon mutagenesis (+Tn). Representative gel of several experiments is shown showing 3 strains of serovar (SV) 3 and 2 strains of serovar 6.

Figure 5. Growth kinetics for *U. parvum* parent strain (HPA5) compared to membrane protein disruption (UU390::mTn) or DEADbox RNA-helicase gene disruption (UU582::mTn) when incubated at 37°C (A.), 33°C (B.), or 25^{o}C (C.). Strains were titrated out in a 10-fold dilution series and growth measured at time points indicated by urease conversion of urea to ammonium ions. *Ureaplasma* growth is shown as colour (pH indicator) changing units per ml. Mean and standard deviation of dilutions performed in triplicate. Results were consistent through three repeated experiments.

Figure 6. Serum killing (A) and immunoblot analysis using human high titre seropositive serum (B) for parental *U. urealyticum* strain W11 and following successful transposon mutagenesis (+Tn). Serum killing of increases significantly following 1 h challenge with human serum containing anti-*Ureaplasma* antibodies and analysis of this serum shows the serum-sensitive transposon mutated strain has lost a 41 kDa band that was immunoreactive with the challenging serum. Bar graph shows mean +/- SEM of experiments performed in triplicate. Representative immunoblot from three repeat experiments shown.

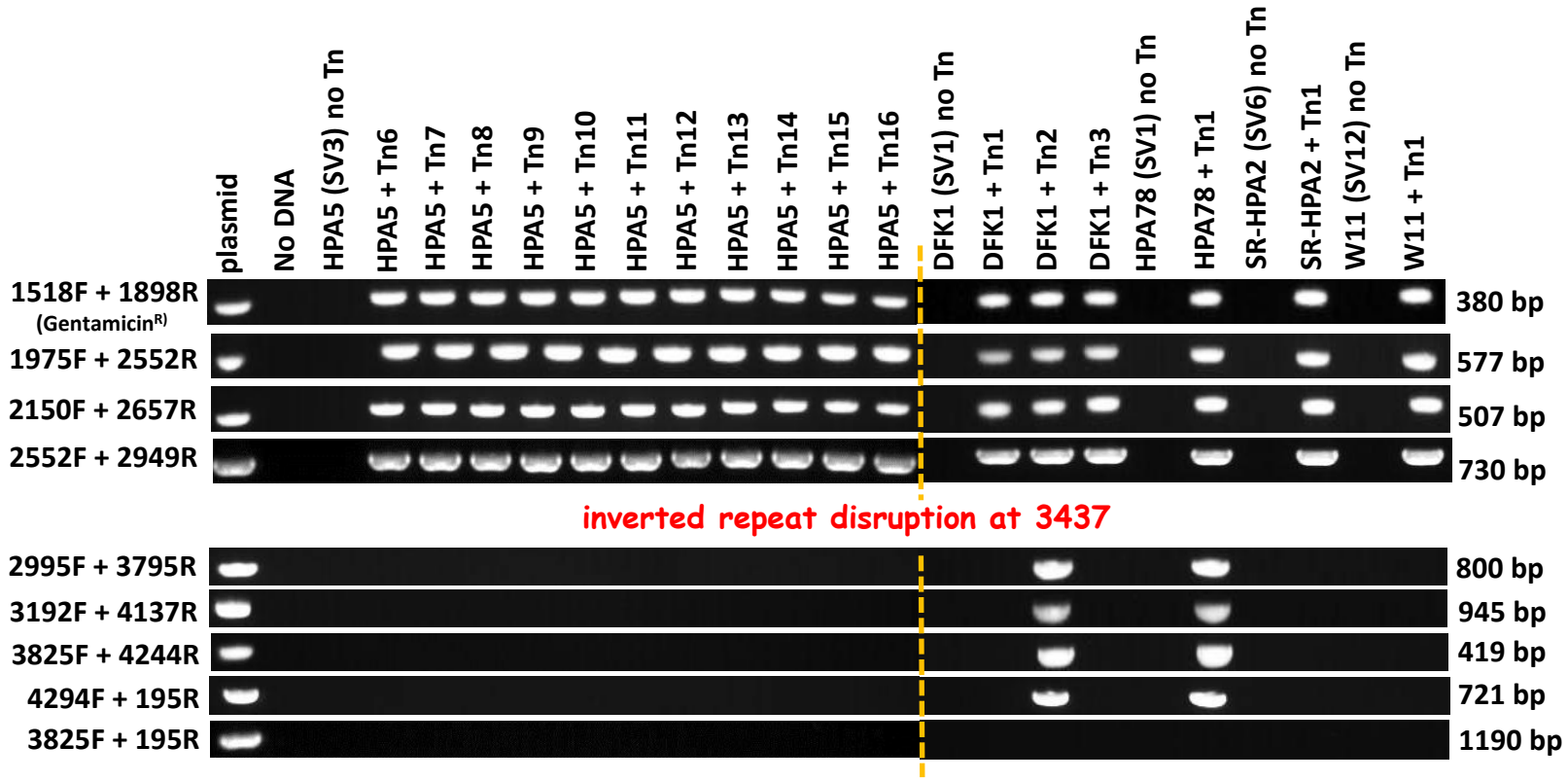
Supplementary Figure 1. Further PCR mapping of successful transposon-mutagenised strains *of Ureaplasma parvum* and W11 (*Ureaplasma urealyticum*) using the methods outlined for Figure 1. Presence of the gentamicin resistance gene (primers 1518F to 1898R) was only found in transposon mutated strains. PCR probing identified one of three DFK1 serovar 1 strains (three different experiments) and HPA78 to have some of the transposase (Tnase) gene integrated into the genome.. Expected amplicon size is indicated to the right of the figure.

Supplementary Figure 2. PCR amplification of *U. parvum* genes (as designated by ATCC strain 700970 nomenclature) UU390, UU450, UU520, and UU582 using primers designed within the coding region of these genes. An additional primer set amplifying the end of gene UU187 and the beginning of UU188 (including the 31 bp promoter region) was also included. Non-mutagenised HPA5 and U6 serovar 3 *U. parvum* are included as controls. Single clones with disrupted genes are shown for each primer set as well as 7 additional clones successfully transformed with the mini-transposon to show specificity.

Supplementary figure 3: Growth of HPA5 in triplicate at 25, 33, and 37^{o}C as measured by colour change of *Ureaplasma* selective medium from Mycoplasma Experience ltd. 180 μl were placed in each well and 20 μl of prototype laboratory *U. parvum* strain HPA5 was inoculated into the first well (in triplicate). Filter tips were changed between each dilution (transfer of 20 μl) across the plate. The plates were then incubated in separate incubators at the listed temperatures and the growth documented at various time points. These images were taken at the 48 hr time point for one of the repeats of the experiment.

Supplementary Figure 4. Sequence and detail of the pMT85 vector.

supplementary figure 1



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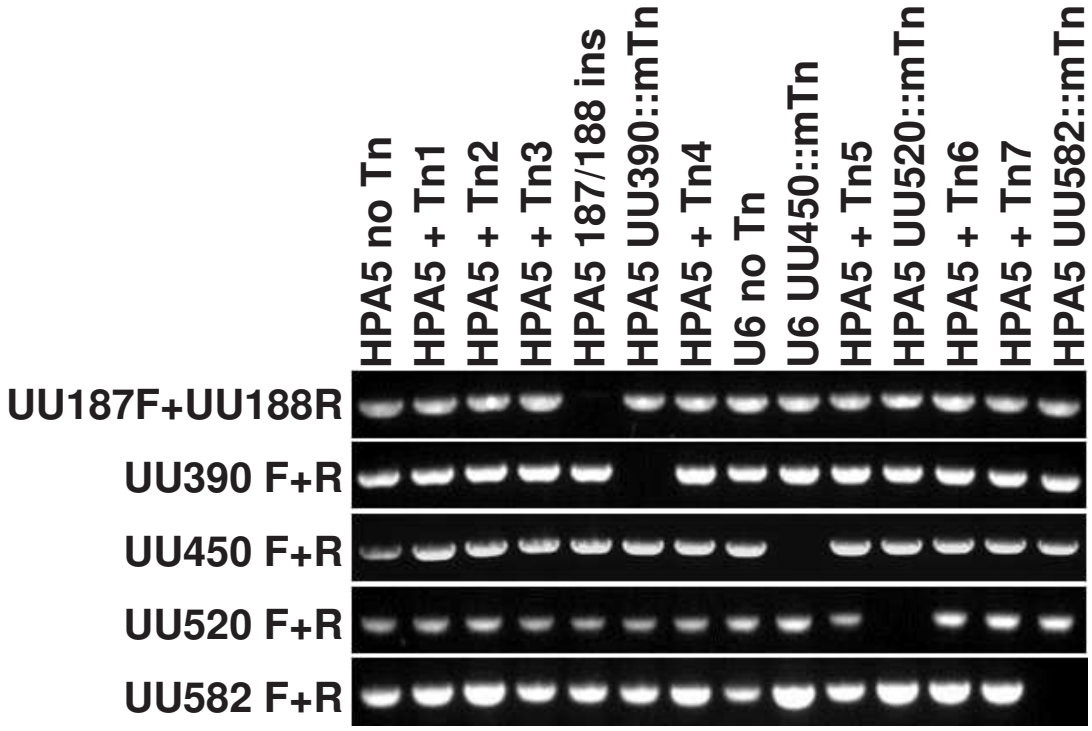

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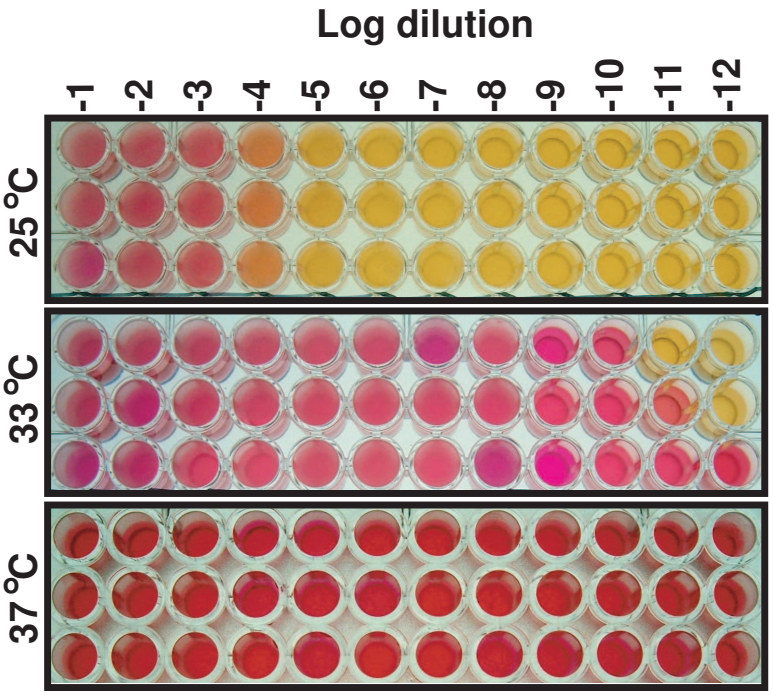
supplementary figure 3



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Legend:
Inverted repeat 1 : 1-26
Inverted repeat 2: 3412-3438

Gentamicin resistance: 1036 - 2475 479 a.a.
bifunctional AAC/APH (AAC(6'): 6'-aminoglycoside N-acetyltransferase and APH(2'): 2''-aminoglycoside phosphotransferase [synthetic Mycoplasma genitalium JCVI-1.0]
Sequence ID: gi|166079093|gb|ABY79711.1|Length: 495 (100% match)

transposase 3538-4710 390 a.a.
IS256, transposase [Enterococcus faecalis V583]
Sequence ID: gi|29374776|ref|NP_813928.1|Length: 390 (100% match)

>pMT85gen

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