Figure 1. Complete region of the *afpA* gene (615 bp) present in the UPEC-46 strain. In green, the oligonucleotide sequence of primers (4532-Fw and 4532-Rv) used for amplification of the internal fragment $afpA_1$ (307 bp), shown in red (reads 5' – 3').

> *afpA* (615 bp)

ATGAATATTTTTACAAAAAAAGTTTTTGATAAAAAAGCGCAGAAGC
GTAAAAGCTCCCTCCTTTTTCAGAAGGGCTTGTCATTAATCGAGG
CCTCTATGGTCCTCGCCCTCTCCGCCATCGTCGTCTCCGGCGTT
ATGTATTACTACCAGTCTGCTTCTGACAATAACAAGACTCAGAGC
ACCGTCTCCGAAGTCATGAGTATCGTCTCCGCTGTTAACGGTCT
CTACGTCGGTACTTCCGGTTATGAAGGGTTAAATGAATCCGTTAT
CCTCAAAACCTCTTCCGTCCCCGAAAATTATAAGTCCAAAGATGG
AAAAACCATTATGCATCCGTTCGGGGGGAATCTGGCTCTGGGGC
CAACACAAGGTTATACCGGCTATTATATTGAACTGACTAAGATTC
CTAAGAGTGCCTGTGTGAACCTCTCGTCAATGAACTTCGGGACC
TCACTGGGTGGTGTCGGGGTTAATGCGCCGAAGGGATCAGGGC
AAGATATTTCTACTGTTAACGGTCAGAACGGTAACAAAAACTACA
CCAAAAATGCTCTGACTCCAGCACTGGCTTCCACCGCTTGTAGC
CAGAACGAAAACACCATCACCTTCCTGCTGAAATAA

Figure 2. Amplification product of the afpA$_1$ using UPEC-46 as template. The arrow indicates the correct size of the afpA$_1$ fragment amplified (307 bp). M: 1 Kb plus DNA ladder (Invitrogem).

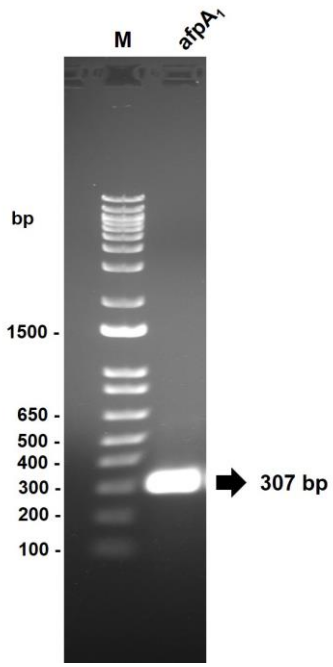

Figure 3. PCR for detection of the afpA1 fragment in selected transformants. All transformants showed positivity for the $afpA_1$ fragment and, consequently, for the plasmid pPAS1. M: 1 Kb plus DNA ladder (Invitrogem).

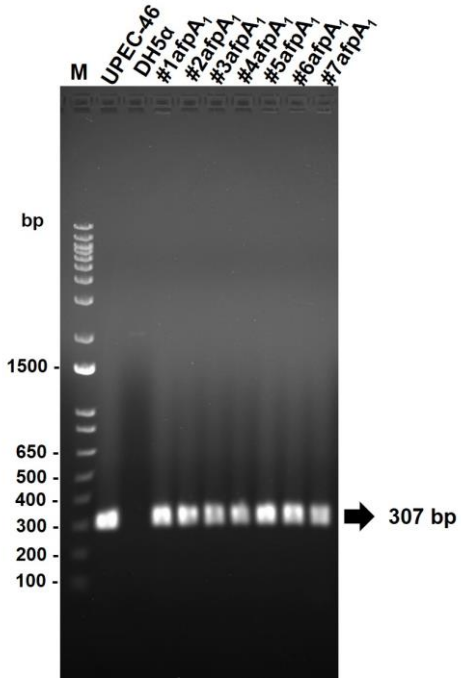

Figure 4. Restriction profile of plasmid pPAS1 extracted from DH5α(pPAS1) strain after digestion with EcoRI. M: 1 Kb plus DNA ladder (Invitrogem).

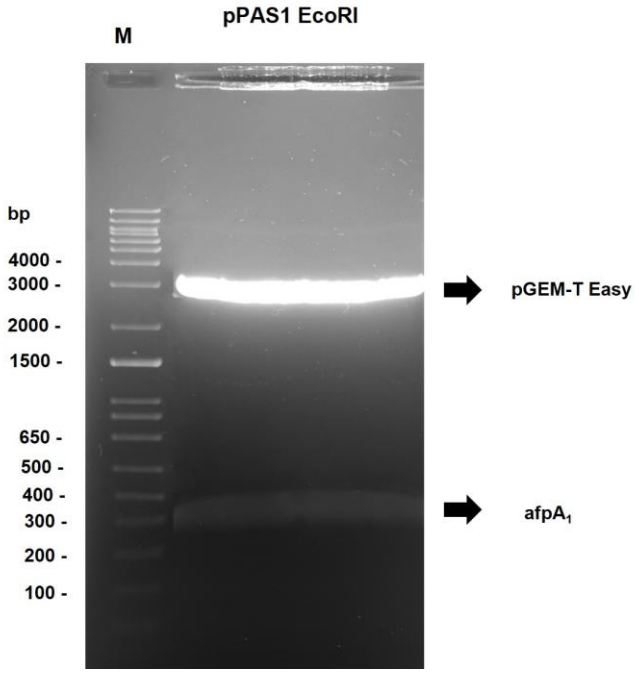

Figure 5. Confirmation of homologous recombination of transconjugant strains by PCR. Agarose gel electrophoresis for analysis of the *afpA* gene amplification product, using the primers FwAfpA and RvAfpA, and DNA of two transconjugants (Trans-1 and Trans-2) and UPEC-46 strain. In UPEC-46 there was amplification only of the *afpA* gene (~600 bp), while for the transconjugant strains (Trans-1 and Trans-2) the amplification product was higher (~4.1 kb), corresponding to the *afpA* gene recombined with the plasmid pPAS2. The amplification product was analyzed by Sanger's sequencing. M: 1 Kb plus DNA ladder (Invitrogem).