pCF1 construction

A: Plasmid extraction (miniprep), revealing the plasmid obtained after sat 930bp amplicon cloned in pGEM-Teasy (pCF1)

B: Cloning confirmation by PCR. The sat fragment present in pCF1 amplification resulted in a 930 bp amplicon.
A: pCF1 EcoRI digestion. A *sat* fragment flanked by EcoRI restriction sites was obtained.

B: pJP5603 EcoRI digestion.

**pCF2 construction**

A: pCF1 EcoRI digestion. A *sat* fragment flanked by EcoRI restriction sites was obtained.

B: pJP5603 EcoRI digestion.
pCF2 construction

A: Plasmid extraction (miniprep), revealing the plasmid obtained after a sat fragment flanked by EcoRI restriction sites was cloned in pJP5603 (pCF2)

B: Cloning confirmation by PCR. The sat fragment present in pCF2 amplification resulted in a 930 bp amplicon.
PCR confirmation of pCF2 insertion on EC071 sat gene.

Different combinations of sat and M13 primers were used.

The highlighted amplicons were sequenced by Sanguer’s method.
pCF3 construction

A and B: EC071 complete sat gene and pettac vector amplified using primers with NdeI and XhoI restriction sites.

C: Plasmid extraction (miniprep), revealing the plasmid obtained after EC071 sat into pettac vector (pCF3)
pCF4 construction

A: Plasmid extraction (miniprep), revealing the plasmid obtained after pCF3 complete amplification using primers with specific point mutations in target nucleotides of pCF3 sat serinoprotease active site (pCF4).

B: Amplicon obtention for sequencing confirmation of the site-directed mutagenesis.