Supplementary data

Materials and methods

Cloning cDNA.Briefly, the purified cDNAs were mixed with: 50 ng of the cloning vector pGEM-T Easy (Fig.1), T4 DNA ligase, 2X Rapid Ligation Buffer (Tris-HCl 60 mM, pH 7.8, $MgCl_2$ 20 mM, DTT 20 mM, ATP 2 mM, 10% polyethylene glycol), and sterile water, until a volume of 10 µl. The ligation reaction was performed at 4 °C for 16 hours

Extraction of plasmid DNA: Plasmid DNA was extracted using the Miniprep DNA Purification System (Promega).The grown bacterial cells were placed in 1.5 ml tubes and centrifuged at 13,000 rpm for 5 min.; the supernatant was discarded and 250 µl of Cell Resuspension Solution were added,pipetting to re-suspend the pellet. Cell Lysis Solution (250 µl of) was added and the tubes mixed by inversion (4 times) and left for 5 min at room temperature. Ten µl of alkaline proteases were then added, and the tubes inverted 4 times and left for 5 min at room temperature, subsequently 350 µl of Neutralization Solution were added and samples were centrifuged at 14,000 rpm for 10 min. Clarified supernatants weretrans ferred to columns provided by the kit, and centrifuged at 14,000 rpm for 1 min., then 750 µl of Wash Solution were added and samples were centrifuged at 14,000 rpm for 1 min.The washing was repeated with 250 µl of Wash Solution and samples were centrifuged at 14,000 rpm for 2 min. A centrifugation in vacuum was performed to remove all ethanol; then the columns were moved in new tubes,adding70-100 µl of water "nuclease-free" for each sample, following placing at room temperature for 1 min. and then centrifuging at 14,000 rpm for 1 min. The DNA was stored at -20 °C.

Fig. 1 supp. Map of vector. Ori: origin of bacterial replication, lacZ: codifying region of the α-peptide β-galactosidase, lacO: lac operator, Sp6: promoter and codon of transcription start of Sp6 polymerase. In the MCS multiple cloning site T7: promoter and start codon of the transcription polymerase T7, f1: origin of replication of f1 phage, Amp: ampicillin resistance site. .