Mos20 cells (Aedes aegyptii-Mosquito). A transfection protocol for stable transfection.

Mos20 cells (adherent) were seeded at a concentration of 5×10^5 /ml into 25cm^2 flasks and grown to 75% confluence at 27° C in 5 ml Medium 199 supplemented with FBS (10%), yeastolate (1µg/ml), lactalbumin hydrolysate (4µg/ml) and L-glutamine (2mM). Transfection mixtures were prepared by mixing 5µg of plasmid DNA with 30μ l INSECTOGENE in serum free medium 199 for 15 minutes at room temperature. Cells were washed twice in Hanks Buffered Saline and incubated with the transfection mixture at 27° C for 12 hours. Normal growth medium was restored and the cells allowed to recover for 48 hours at 27° C before selection. Ca. 50 surviving colonies became apparent after 2-4 weeks.

13 hygromycin resistant clones were established after intense selection.