

Establishing stable 293/maxiEBV clones

1. Seeded 2×10^6 cells on a 10cm plate
2. After 2 days, transfected 10ug 3227/2089(maxiEBV) using lipofectamine (Invitrogen)
 - (1) Mix 10ug DNA with 500ul Optimem
 - (2) Mix 15ul lipofectamine with 500ul Optimem
 - (3) Wait for 5min
 - (4) Mix (1)+ (2) incubate for 20min
 - (5) Add (4) to cells
3. 24 hrs after transfection, check for GFP expression
(My transfection efficiency was around 20%.)
4. Trypsinized cells and plated 1×10^6 cells ($\approx 2 \times 10^5$ green cells) on a 15cm plate
 - 1×10^5 cells ($\approx 2 \times 10^4$ green cells)
 - 1×10^4 cells ($\approx 2 \times 10^3$ green cells)

Added 200ug/ml Hygromycin for selection
5. Waited 2-3 weeks for clones to grow.
My cloning efficiency was around 0.1% ($1/10^3$ green cells gave rise to clones).