Inducing Virus Production from stable cell lines harboring EBV (Looking for Producers)

modified from Wolfgang Hammerschmidt's lab's protocol
Jisook Lee May 2005

Virus Production

1. Seed 1.25x10^5 cells/well of a 6-well plate. Grow for 4 days or until they are 50% confluent. (I think it is important to transfect cells when they are not too confluent. 50% confluency or less works the best.)
2. Transfect cells to induce virus production:
    a. Mix 0.5ug (5ug) each of p509 (BZLF1) and p2811 (BARF 4) with 100ul (500ul) of Optimem (per well)
    b. Mix 7ul (25ul) of PEI with 100ul (500ul) Optimem (per well)
    c. Incubate DNA and PEI solutions for 20min at room temp.
3. During incubation, replace medium on cells with 2mls DMEM without FBS (optional)
4. Add DNA/PEI solution dropwise to cells
5. Incubate four hours and replace medium with 2mls (5mls) DMEM 10%FBS
6. Allow virus production to occur over three days. Harvest supernatant and filter through 0.8um filter. Store at 4 deg.

- When using 10cm plate

Titer Determination

Day 1 :
1. Using a 24-well plate for titering virus, add 500ul, 250ul and 125ul of virus containing supernatant to a well for each virus. Add media to 0.5ml.
2. Add 0.5ml of RPMI containing 1x10^5 Raji cells to each well.

Day 2 :
Change media by carefully removing old media while cells remain at the bottom of the well and replace with fresh RPMI 10%FBS

Day 3:
Add Sodium butyrate (final 3mM) and TPA (tumor promoting agent- final 20ng/ml) to the medium

Day 4
Check and count for GFP+ Raji cells
Determine GRU (Green Raji Units)
% green cells infected with 500ul * 2 * 10^5 = x GRU/ml
ex> 5% green in 500ul well would give you 10^4 GRU/ml virus stock

DNA in Producers

After finding a good producer cell line, extract total genomic DNA from the cells. Digest about 8-15ug of DNA with BamHI and run on an agarose gel. Also digest about 100ng of plasmid DNA as positive control. Using the linear DNA fragment from homologous recombination as a probe, perform Southern Blot analysis to ensure cells are maintaining the DNA.

Concentrating EBV
If the titer of your virus stock is too low, you could concentrate it by spinning at 17500rpm in the ultracentrifuge (Rotor : 50.Ti) at 4degree for 2hrs
Pellet contains your virus, resuspend in 1/100 of original volume and let the pellet dissolve for o/n. Then titer again.