VSV-G

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Instructions for culture of 293GPG clones

293GPG cells

Culture Media:

DMEM
10% IFS
2 mM L-Glutamine
50 u/ml Pen-Strep
1 µg/ml tetracycline (Sigma T-7660)
2 µg/ml puromycin (Sigma P-7255)
0.3 mg/ml G418

use puro for selecting stable Transfectants

10mg/ml 50mg/ml → make 20mg/ml stock in DMEM (x use at concentration)

1) Make up fresh media every 2 weeks
2) Change media on cells every 3 days
3) Passage cells with PBS⁻ and trypsin containing 1 µg/ml tetracycline (make up fresh every 14 days)
4) Prepare tetracycline as 1000x stock in 70% ethanol (store at -20 C)
5) Prepare puromycin as 500x stock in PBS⁻ (store at -20 C)
6) Split cells at maximum of 1:5; they are slow growing in selection
7) Freeze cells in 90%IFS/10%DMSO supplemented with 1 µg/ml tetracycline

GPGnlsLZ2 clone (aka GPG-nlsLZ2)

1) Supplement 293GPG media with zeocin 100 µg/ml (Invitrogen R250-01) 100mg/ml stock
2) After freeze downs of clone are obtained, cells can be passaged in 293GPG media alone.
3) Harvest of viral supernatants requires the following:
   a) grow in 10 cm dish to 95% confluence
   b) wash cells twice with 5 ml non-tetracycline-containing 293 media (DMEM/10%IFS/glutamine/Pen-Strep). **Take care to aspirate media completely between washes to ensure complete tetracycline de-repression.**
   c) harvest 24 hour supernatants serially at specified time points (see graph) by aspirating with 5cc syringe and 18g needle. Filter supernatant through a 0.45 µm low-protein-binding acrodisc (Gelman Sciences 4184).
   d) Add polybrene to supernatant to 8 µg/ml before freezing at -80 C (Prepare polybrene (Sigma H-9268) as 100x stock=800µg/ml in PBS⁻ and store at 4 C)
4) Note: Cells are loosely adherent. Be gentle during washes or addition of media.

Transient into 293GPG

Transfect at 90-95% confluence. Usually 1:2 split of confluent dish the morning before. It takes the cells along time to attach and flatten out--about 18-24 hours, when cells settle Add Suck off Media + Add Fresh Media to remove debris.

Protocol for a 10 cm dish.

Before transfection.
Wash off the regular media containing tet with PBS and replace with 4 cc of Optimem.

Prepare 10 ug of DNA in 500 ul Optimem
Prepare 62.5 ul of Lipofectamine in 500 ul Optimem
Mix and incubate at room temperature for 30'
Add another 1cc of Optimem and then add 2cc to cells (with the 4 cc of Optimem already on there).

At hours 8-10....add 4 cc of DME/10%IFS/with 25mM HEPES to the plate. Now the final volume is 10 cc.

At 24 hours post transfection, discard media and replace with 10 cc of fresh DME/10% IFS. Collect media every day for the next few days.

Titer rises to about 96 hours then falls off as the cells begin to fuse and die.