Transfection of Sf9 cells in suspension

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Version 1.0

Summary:

Figure 1 Vertiga Shaker manufactured by Thomson Instruments Co.

This protocol is used to generate baculoviruses containing GPCR genes of interest for expression in SF9 cells. This protocol is a slight modification of Expression Systems protocols for generating viruses. The following link to their protocols may be useful when searching for general ideas on how to proceed. For alternative reagents and protocols, please see or BD Boisciences web site or download the following file from Invitrogen. The JCIMPT protocol uses a specialized small-scale eukaryotic expression platform that was developed by the JCIMPT in collaboration with Thomson Instrument Company (Vertiga-IM;Figure 1) which is now commercially available. This shaker has enabled parallel micro-expression studies. The 12-Plate Position Incubator for Insect and Mammalian Cells has a platform that holds 12 plates, and a top shelf. The unit has a much milder 3D-orbit to help avoid any shearing of the delicate eukaryotic cells and temperature control within 0.1 °C.

Further improvements to the transfection and virus amplification protocols are currently under development and validation at the JCIMPT.

Materials:

1. Reagents:

Sapphire^{TM} Baculovirus DNA (Orbigen #BVD-10001)

Transfection Medium (Expression Systems #95-020)

FuGENE HD Transfection Reagent (Roche Applied Science #04709705001)

2.Equipment:

Vertiga Shaker (Thomson Instruments Co. Cat#381150)

Step by Step Methodology

1. Warm Transfection Medium to room temperature
2. Transfer 2.5mL of Sf9 at 1 x 10^6 cells/mL into each 24 deep well block.

3. Make the following solutions:
    a. Solution A:

pBac (plasmids)	pFastBac (bacmids)
5 uL plamids DNA at 200ng/uL 2.5 uL Sapphire baculovirus DNA 42.5 uL Transfection Medium	5 uL bacmid 50 uL Transfection Medium

    b. Solution B:

    50 uL Transfection medium

    Add 3 uL FuGENE HD directly to the Transfection Medium, avoid touching the side of the tube when adding FuGENE.

    Incubate at room temperature for 5 minutes.

    Note: one can make a master stock. So if you have 10 samples to transfect, then take 50*11=550 uL of Transfection medium + 3*11=33uL of Fugene HD. Mix, then add 52.5uL of mix to each DNA sample. To make the master stock, multiplication by 11 is done to have some extra left over in case the pipet is off.

4. Combine solutions A and B, mix gently, and incubate at room temperature for 30 minutes.
5. After incubation, add 400uL Transfection medium to each tubes from step 3, mix gently, and then add the cotransfection mix to the cells, drop-wise.
6. Cover and incubate with shaking for 4 days.
7. On day 4, transfer 170 uL of cell culture to a fresh 24 deep well plate (and generate P1 following the protocol for generating P1 stock.
8. Spin down the remaining cells and collect the supernatant. Label it P0.

Generation of P1 stock:

1. To the 24 deep well block containing 170uL of cells, add 4 mL Sf9 cells at 1 to 2 x 10^6 cells/mL.
2. Incubate the cells at 27°C with shaking for 4 days.
3. Remove cells by centrifugation at 2500 rpm for 10 minutes. Transfer the supernatant to fresh set of 15mL tubes and label it “P1”.

4. Determine the titer.
5. Archive some P1 stocks at -80°C for long term storage.

Generation of working P2 stock:

1. Seed a shake flask at desired volume with Sf9 cells at 10^6 cells/mL.
2. Infect the flask at an MOI of 0.1.
3. Incubate flasks at 27°C with shaking for 3-5 days.
4. Beginning 3 days after setting up the culture, determine the viability of the culture. Harvest the culture when the viability drops below 70%.
5. Remove cells by centrifugation at 2000 rpm for 10 minutes.
6. Filter virus stock through a 0.2 micron, low protein-binding filter.
7. Titer stock.
8. Store stock at 4°C for up to 6 months.

References

1. Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, Stevens RC., "The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist.",Science. (2008);**322**:1211-7
2. Hanson MA, Brooun A, Baker KA, Jaakola VP, Roth C, Chien EY, Alexandrov A, Velasquez J, Davis L, Griffith M, Moy K, Ganser-Pornillos BK, Hua Y, Kuhn P, Ellis S, Yeager M, Stevens RC., "Profiling of membrane protein variants in a baculovirus system by coupling cell-surface detection with small-scale parallel expression.", Protein Expr Purif. (2007) ;**56**(1):85-92.