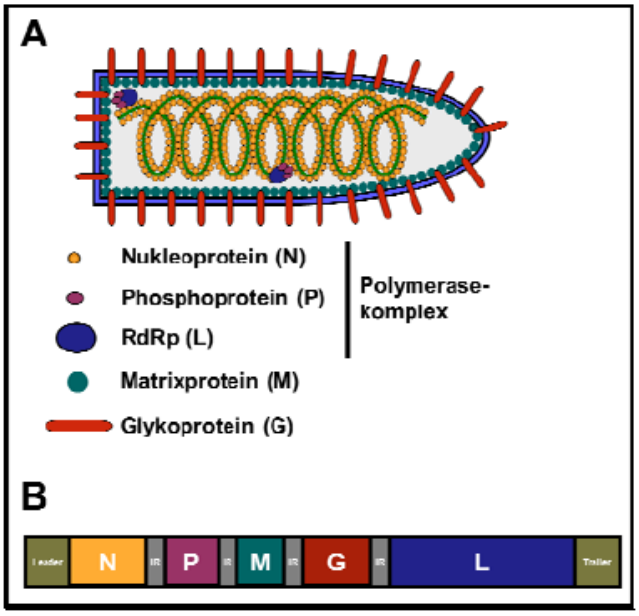
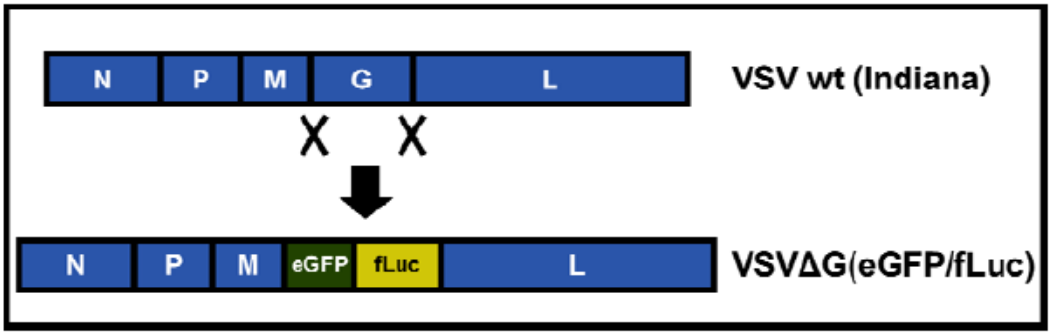
VSV*ΔG-Luc

General information:

This vector allows the assessment of viral glycoprotein-mediated entry of a huge panel of viruses. Vesicular stomatitis virus (VSV) is known to incorporate foreign viral glycoproteins into its envelope and this phenomenon can be used to study host cell entry of e.g. viruses for which no virus isolate is available or for high containment (BSL-3/4) viruses under BSL-2 settings (I think some people are even allowed to use it under BSL-1). Further, this system can also be used for serum neutralization assays.

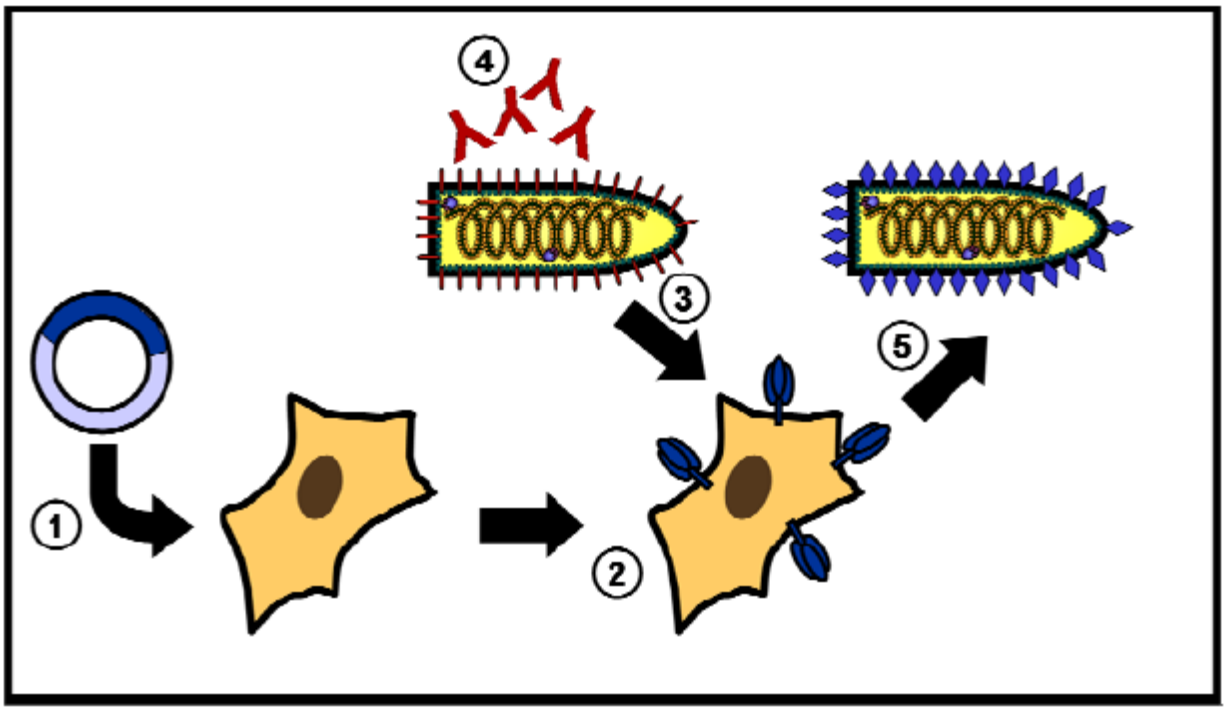


VSV particle and genome structure



Genome of wildtype VSV and VSV*ΔG-Luc

(eGFP and firefly luciferase genes are separated by introduction of an additional intergenic regions (compared to wildtype VSV, VSV*ΔG-Luc has one additional open reading frame).



Production of VSVpp (schematic overview)

1) Transfection of HEK-293T or BHK-21 cells with expression plasmids for viral glycoproteins

2) Cells express viral glycoproteins on their surface

3) Inoculation with VSV*ΔG-Luc

4) Addition of anti-VSV G antibodies for neutralization of unbound input virus (NOT used for VSVpp(VSV G))

5) Harvest of VSVpp-containing culture supernatant

Production of VSV*ΔG-Luc(VSV G) stocks (T-75 flask)

Info:

The BHK(G43) is stably transfected with an inducible vector for VSV G expression (pGENE-C, GeneSwitch system). Following addition of mifepristone to the medium, VSV G expression is immediately started.

For prolonged passaging of this cell line zeocin (0.5 mg/ml) and hygromycin B (0.25 mg/ml) should be added every fourth passage.

Day 1:

- Seeding of BHK(G43) cells (cells should be around 90 % the next day), medium: MEM or DMEM supplemented with 5 % FCS and 1 % Pen/Strep

Day 2:

- Replace culture medium by medium supplemented with 10^{-8} M mifepristone (from 10^{-5} M stock)
- Incubate for 6 h at 37 °C & 5 % CO_2
- Remove culture supernatant
- Add 10 ml medium (without supplements) containing 10^5 to 10^6 ffu/ml of VSV*ΔG-Luc(VSV G) → usually a 1:100 to 1: 1,000 dilution of the stock
- Incubate for 6 h at 37 °C & 5 % CO_2
- Remove inoculum
- Wash 1x with PBS
- Add 20 ml of medium (without supplements) containing 10^{-8} M mifepristone

Day 3:

- Check cells microscopically (If possible check eGFP expression via fluorescence microscopy)
- If the cytopathic effect (rounded and detached cells) is >90 % collect the culture supernatant in a 50 ml falcon tube
- Centrifuge for 10 min at 4,000 rpm and 4 °C to remove cell debris
- Transfer clarified supernatant into fresh tube
- Prepare aliquots of 0.5 or 1 ml and snap-freeze them using liquid nitrogen
- Store aliquots at -80 °C

Quantification of VSV*ΔG-Luc(VSV G) stock titers (96well plate)

Info:

Quantification of VSV*ΔG-Luc(VSV G) stock titers should be performed using the cell line chosen for production of VSV pseudotypes (BHK-21 or HEK-293T).

Day 1:

- Seeding of cells in 96well plates

Day 2:

- Thaw aliquot of VSV*ΔG-Luc(VSV G) stock (water bath)
- Prepare 10-fold serial dilution of the stock (up to 10^{-9})
- Remove culture supernatant
- Add 50 µl of each dilution (triplicates)
- Incubate for 1 h at 37 °C & 5 % CO_2
- Remove inoculum
- Wash 3x with PBS
- Add 100 µl culture medium
- Incubate for 16-20 h at 37 °C & 5 % CO_2
- Count GFP-positive cells and calculate stock titer

VSVpp preparation (6well format)

Day 1:

- Seeding of HEK-293T or BHK-21 cells

Day 2:

- Transfection with empty vector (ne. control), VSV G (pos. control) or expression plasmids for glycoprotein(s) of interest

Day 3:

- Prepare VSV*ΔG-Luc(VSV G) solution (in DMEM, w/o supplements) for inoculation (usually 1:10 to 1:100, depending on stock)
- Remove culture medium and add 1 ml of VSV*ΔG-Luc(VSV G) solution
- Incubate for 1 h at 37 °C & 5 % CO_2
- Prepare anti-VSV G antibody (I1) in DMEM (no supplements) for neutralization of residual input virus (1:1,000)
- Remove inoculum
- Wash 2-3x with PBS
- Add 2 ml of anti-VSV G antibody solution (!!! Not to wells transfected with VSV G, here add only DMEM)

Day 4:

- Transfer culture supernatant into 2 ml reaction tube
- Centrifuge for 10 min at 4,000 rpm and 4 °C to remove cell debris
- Transfer clarified supernatant into fresh tube
- VSVpp can be used directly for transduction, stored at 4 °C (up to two weeks) or at -80 °C (long term storage)

Quantification of VSVpp entry (96well plate)

Info:

VSVpp entry relies on the glycoprotein incorporated into VSV*ΔG-Luc particles. For each target cell line include VSVpp(empty vectors) and VSVpp(VSV G) as negative and positive transduction controls, respectively. Since VSV G permits entry into a very broad set of cell lines from various target species with high efficiency, it should be included to see the general susceptibility for the target cell line.

Day 1:

- Seeding of target cells in 96well plates

Day 2:

- Remove culture supernatant
- Add 50 µl of undiluted VSVpp in triplicates (also different dilutions could be used)
- Incubate for 1 h at 37 °C & 5 % CO_2
- Remove inoculum
- Wash 1x with PBS
- Add 100 µl culture medium
- Incubate for 16-20 h at 37 °C & 5 % CO_2
- Count GFP-positive cells or measure firefly luciferase activity* in the cell lysate
    - To quantify luciferase activity in the cell lysates remove culture supernatant
    - Wash cells 1x with PBS
    - Add 100 µl of lysis reagent (can be scaled down to 50 µl)
    - Incubate cells for 30 min at room temperature
    - Transfer lysates into white, opaque-walled microtiter plates
    - Add luciferase substrate and measure luminescence in a plate luminometer (optimal incubation time between substrate addition and measurement should be determined, for me it is 1 min)

*: We have good experiences with the kits from Promega (Luciferase Assay System) and PJK (Beetle Juice). For lysing of the cells we have bought the “Cell Culture Lysis Reagent” (5x concentrate from Promega. Nowadays, I prepare the lysis buffer by myself (I found the recipe for the Promega lysis reagent, see below).

Lysis buffer “Promega”

125 mM Tris-HCl pH 7.8

10 mM DTT

10 mM 1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid

50% glycerol

5% Triton X-100