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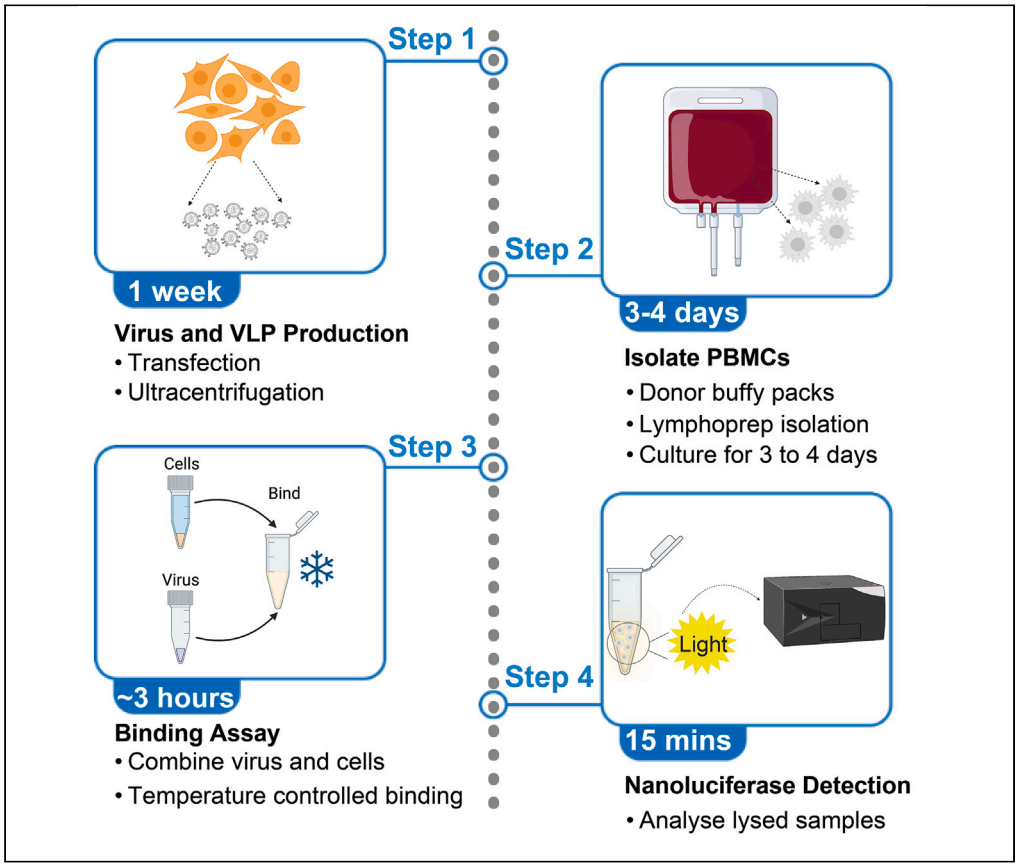

Prior to receptor engagement, a specific, non-electrostatic glycan-glycan interaction between viral particles and host cells may lengthen the dwell time of the virus at the cellular surface, thereby facilitating subsequent virus entry. Here, we present a protocol for quantifying the level of glycan-mediated binding between virus or virus-like-particles and human peripheral blood mononuclear cells (PBMCs) using a nanoluciferase reporter system. We describe steps for virus production, isolation of PBMCs, and performing a nanoluciferase binding assay.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Belinda L. Spillings, Johnson Mak

b.devilliers@griffith.edu.au (B.L.S.)
j.mak@griffith.edu.au (J.M.)

Highlights

Protocol for investigating glycan-mediated interactions

Population-level quantification of glycan binding using a nanoluciferase reporter

Steps for isolating human PBMCs and virus production

Visualizing glycan interactions between glycosylated substrates

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Protocol to quantify glycan-mediated binding between viral particles and human peripheral blood mononuclear cells using nanoluciferase

Belinda L. Spillings[1,2,*] and Johnson Mak[1,3,*]

[1]Institute for Glycomics, Griffith University, Gold Coast, QLD 4222, Australia

[2]Technical contact

[3]Lead contact

*Correspondence: b.devilliers@griffith.edu.au (B.L.S.), j.mak@griffith.edu.au (J.M.)
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SUMMARY

Prior to receptor engagement, a specific, non-electrostatic glycan-glycan interaction between viral particles and host cells may lengthen the dwell time of the virus at the cellular surface, thereby facilitating subsequent virus entry. Here, we present a protocol for quantifying the level of glycan-mediated binding between virus or virus-like-particles and human peripheral blood mononuclear cells (PBMCs) using a nanoluciferase reporter system. We describe steps for virus production, isolation of PBMCs, and performing a nanoluciferase binding assay. For complete details on the use and execution of this protocol, please refer to Spillings et al.[1]

BEFORE YOU BEGIN

Introduction

Glycans are increasingly being recognized for their role as surface attachment factors which enable pathogens to bind to and enter into target host cells and tissues. Early glycan studies focused on the proteinaceous-glycan interactions between glycans and cellular lectins.[2–4] More recently direct glycan-glycan interactions between bacteria[5–7] and viruses[1] and their target host cells have been described.

Sophisticated biophysical analyses using glycan arrays, isothermal calorimetry, surface plasmon resonance and mass spectrometry enable researchers to determine the structure and affinity of glycans within a closed, non-biological system. However, the interactions of glycan structures occur within the context of biological complexes. The ability to survey their broad responses to the presence of inhibitors, competimers, glycosidase enzymes, etc. within a biologically "whole system" would be advantageous.

Herein we describe a whole-cell system technique for quantifying the relative binding affinity of viral glycans for cellular glycans, using highly sensitive nanoluciferase as a readout.

Institutional permissions (if applicable)

We have used these glycan binding assays to determine the relative binding of both infectious HIV virus and non-infectious VLPs to peripheral blood mononuclear cells (PBMCs).

All infectious virus work should be undertaken in a laboratory which has the appropriate approvals for your state and country. All infectious HIV work undertaken for this work was conducted within a

certified PC3 laboratory (pursuant to section 90 of the Australian Gene Technology Act, 2000, and in compliance with the relevant Australian/New Zealand standards).

Handling of untested, deidentified donor blood products (sourced from Australian Red Cross Lifeblood, Brisbane) was performed using universal precautions, assuming all components may be infective. When processing blood products, ensure that the procedures are carried out in accordance with your local health and safety guidelines.

Virus and VLP production

Timing: 1 week

Prior to starting the assay, ensure that viral or VLP stocks are sufficient for the experiment. If necessary, prepare fresh stocks by transfection according to the protocol described in by Spillings et al.[8] with the following modifications.

1. Each transfection requires a total of 12 μg transfection-grade plasmid DNA per T175 flask.
2. Either the HIV proviral plasmid pNL4-3 (NIH AIDS Reagent program) or pNL4-3 ΔRTΔINΔVifΔVpr plasmid (as described in Spillings et al.[1]) is co-transfected with a plasmid encoding the nanoluciferase reporter in an 8:1 ratio.
   a. This equates to 10.67 μg pNL4-3 plasmid (or pNL4-3 ΔRTΔINΔVifΔVpr) and 1.33 μg nanoluciferase reporter plasmid per T175 flask.

Note: Clarification of the transfection supernatant through a syringe filter can be performed once without any detrimental effect on the subsequent nanoluciferase binding assay.

Note: The nanoluciferase-Vpr chimeric protein expression vector was constructed by replacing the *β-lactamase* gene of the β-lactamase-Vpr plasmid (NIH AIDS Reagent program) with the *nanoluciferase* gene.[1] The restriction sites BamHI and NheI were used for this cloning strategy. NEBuilder HiFi Master Mix was used to insert the nanoluciferase gene (gBlock, IDT) into the digested vector. SnapGene software was used to design the cloning strategy.

Note: The additional OptiPrep™ gradient steps from Spillings et al.[8] are not necessary for this nanoluciferase based binding assay and can be omitted.

3. Aliquot resuspended virus or VLPs in screw-cap tubes and store at −80°C until needed.
4. Determine the quantity and concentration of virus and VLP produced using a $p24^{CA}$ enzyme-linked immunosorbent assay (ELISA), following manufacturer's recommendations (Xpress Bio).

Isolation of peripheral blood mononuclear cells (PBMCs) from donor buffy coats

Timing: 3–4 days

We have only used freshly isolated PBMCs for the glycan binding assays. If frozen PBMCs are to be used, culture conditions may need to be optimized to ensure the cells are healthy. PBMCs can be isolated according to the protocol detailed in Spillings et al.[8], with the following modifications.

5. After step 19 of the referred protocol, use a hemocytometer to count the cells and seed them into complete RPMI media containing 10% FBS, 2 mM L-glutamine and 100 U/mL penicillin/streptomycin antibiotic, in a culture flask, at the cell density of 2×10^6 cells/mL.
6. Maintain the PBMCs in complete RPMI supplemented with iL-2 (50 U/mL) and PHA (10 μg/mL).
7. On the day of the binding experiment, collect the PBMCs into a 50 mL screw-cap centrifugation tube. Gently pellet the cells at 150–300 *g*, for 10 min at room temperature (20°C–25°C).

8. Gently resuspend the pelleted cells in fresh complete RPMI at 1 × 10^6 cells/mL.

Note: Within our laboratory we have noticed variations in the efficiency of PBMC isolation, based on the length of time between blood draw and buffy pack processing. Where possible we limit the time between blood draw to the start of PBMC processing to 18–24 hours. Buffy packs older than 24 hours old are avoided.

Note: All reagents, plasticware and equipment used should be sterile. DPBS and PBS used for PBMC related work is always Mg^{2+} and Ca^{2+} free.

⚠ CRITICAL: When working with blood products, universal precautions should be followed to ensure the health and safety of personnel.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Buffy coat	Australian Red Cross Lifeblood	N/A
Chemicals, peptides, and recombinant proteins		
RPMI	Thermo Fisher Scientific	Cat # 21870092
FBS, qualified, Australian origin	Thermo Fisher Scientific	Cat # 10099141
L-glutamine	Thermo Fisher Scientific	Cat # 25030081
Penicillin Streptomycin (10 000U/mL)	Thermo Fisher Scientific	Cat # 15140122
HBSS	Thermo Fisher Scientific	Cat # 14025134
Phytohemagglutinin (PHA)	Thermo Fisher Scientific	Cat #R30852701
0.25% (w/v) Trypsin without EDTA	Thermo Fisher Scientific	Cat # 15050065
Interleukin-2 (iL-2)	Sigma-Aldrich	Cat # 11147528001
NEBuilder HiFi DNA Assembly Master Mix	New England Biosciences	Cat #E2621S
NP40 IGEPAL CA-630	Sigma-Aldrich	Cat # 56741
Triton X-100	Sigma-Aldrich	Cat #T8787
NaCl	Sigma-Aldrich	Cat #S9888
Tris-Cl	Sigma-Aldrich	Cat #T3253
0.5M EDTA	Thermo Fisher Scientific	Cat # AM9261
PNGase F, recombinant	New England Biosciences	Cat #P0708L
Critical commercial assays		
$p24^{CA}$ ELISA	Xpress Bio	Cat # XB-1010
Nano-Glo Luciferase Assay System	Promega	Cat #N1110
Experimental models: Cell lines		
HEK293T: human, female, embryonic kidney derived cell line that is epithelial-like in morphology, hypotriploid and contains the SV40 T-antigen.	ATCC	Cat # CRL-3216
Recombinant DNA		
Human Immunodeficiency Virus 1 (HIV-1), Strain NL4-3 Infectious Molecular Clone (pNL4-3), ARP-2852, contributed by Dr. M. Martin	NIH AIDS Reagent Program	https://www.hivreagentprogram.org/Catalog/Clones/ARP-114.aspx
pNL4-3 ΔRTΔINΔVifΔVpr	Mak Laboratory	Spillings et al.[1]
NanoLuciferase-Vpr	Mak Laboratory	Spillings et al.[1]
Human Immunodeficiency Virus 1 (HIV-1) YU2 Vpr b-lactamase Expression Vector (pMM310), ARP-11444 contributed by Dr. Michael Miller (Merck Research Laboratories)	NIH AIDS Reagent Program	https://www.hivreagentprogram.org/Catalog/HRPPlasmidVectors/ARP-11444.aspx

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
SnapGene software	N/A	Insightful Science: https://www.snapgene.com
Other		
Falcon Tissue culture flasks, T175, vented	Corning	Cat # 353112
Costar 96 Flat Bottom, White, Polystyrene Plate	Corning	Cat # 3917
Microtube, 1.5 mL, screw cap with internal o-ring	SSI	Cat # SSIB2231-S0S
Plate Reader capable of Luminescence Detection e.g. Tecan MP200 Pro	Tecan Trading, AG	https://lifesciences.tecan.com/microplate-readers

MATERIALS AND EQUIPMENT

10 x Cell Lysis Buffer		
Reagent	Final concentration	Amount
NP40 (IGEPAL CA-630)	10%	10 mL
Triton X-100	10%	10 mL
5 M NaCl	1 M	20 mL
1 M Tris-Cl, pH 7.5	100 mM	10 mL
0.5 M EDTA	10 mM	2 mL
ddH_2O	N/A	48 mL
Total		**100 mL**
Prepare fresh 1 x Cell Lysis buffer from this stock solution as needed, by diluting 1 in 10 with sterile ddH_2O.		

Alternatives: As an alternative to the lysis buffer described above, we have compared the nanoluciferase activity using the homemade 1 x cell lysis buffer versus the 5 x Reporter Lysis buffer (RLB, diluted to working stock of 1 x RLB at assay) supplied by Promega. In our hands, there is no significant difference in nanoluciferase signal.

⚠ CRITICAL: For the binding assay, to ensure that virus and VLPs do not fuse with and enter cells, entry non-permissive temperatures should be used for all reagents and handling steps. Chill all buffers to 4°C ahead of the experiment and where possible, perform all steps on ice. Pre-cool the microfuge to 4°C and ensure that all wash steps are carried out as close to 4°C as possible.

STEP-BY-STEP METHOD DETAILS

Nanoluciferase binding assay

Timing: >3 hours, depending on pre-treatments that may need to be performed

Virus and VLPs bearing nanoluciferase-tagged-vpr are bound to target host cells at entry non-permissive temperatures and the resulting nanoluciferase signal read on a plate reader. For HIV, temperatures below 22°C do not support efficient fusion and cell entry. Thus, virus that is bound and attached to the cell surface can be quantified. This protocol is an updated, optimized version of the viral binding assay described by Janas and Wu (2009).[9]

Note: Prior to performing a binding assay, the sensitivity of the plate reader as well as the dynamic range of the nanoluciferase containing particles should be assessed. This is detailed within the technical manual for the Promega Nano-Glo Luciferase Assay System.

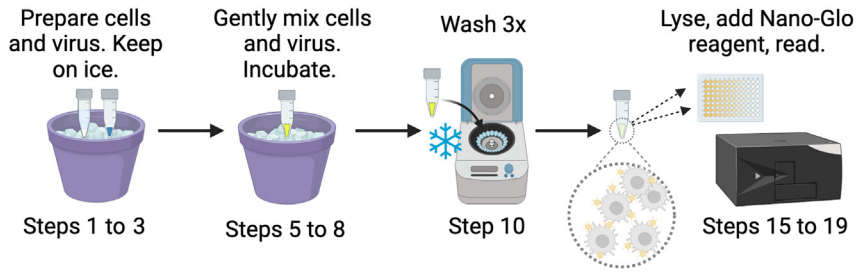

Figure 1. Cartoon schematic of the nanoluciferase binding assay

1. Where necessary, pretreat the cells or virus/VLP mixes with the chosen drug/competimer/ compound etc prior to starting this assay.
   a. Include all necessary controls, such as a mock (cells only, with no virus or VLP) and an untreated control (untreated cells with untreated virus or VLP).
   b. Where possible include a trypsin control (untreated cells with untreated virus or VLP that are treated with trypsin after binding).
   c. When investigating glycan mediated binding responses, a suitable negative control involves PNGase F treatment of virus or VLPs.

   Note: The trypsin control above will allow an estimation of the proportion of virus or VLPs that are either internalised into the cell during the binding assay or those that are protected from proteolytic activity. A high nanoluciferase signal obtained for these samples would suggest that either the cells are internalising bound virus/VLPs or the trypsin treatment has not been effective and the length of treatment needs to be optimised.

Troubleshooting: To reduce high trypsin control signals, review the handling steps to ensure that entry non-permissive temperatures are always maintained. Alternatively, the trypsin treatment has not been effective in removing the bound particles. Refer to the troubleshooting section below.

   Note: Recombinant PNGase F is an amidase able to cleave *N*-linked glycans. New England Biosciences PNGase F (P0708) is guaranteed to be endoglycosidase F1-, F2- and F3-free. When using this treatment as a control in these assays we have used the manufacturer's supplied glycobuffer2 that maintains native conditions.

2. For our PBMC based assays we use 1×10^6 PBMCs per treatment.
   a. Prepare the cells by determining the cell density and resuspend the cells at 1×10^6 cells/mL in fresh, complete RPMI.
   b. Aliquot 1 mL of cells into the appropriate number of screw-cap tubes needed for the experiment.
   c. Just before the experiment is started, centrifuge the cells at 200 *g* for 5 min at room temperature (20°C–25°C).
   d. Carefully remove 900 μL of media from each tube, ensuring that the cell pellet remains undisturbed.
   e. Gently resuspend the cells by tapping the tubes or by gentle, brief vortexing.
   f. Stand the tubes at 4°C for 10–15 min. Ensure that the rack allows sufficient cooling and does not insulate the tubes.
3. Ensure that the virus or VLP stocks being used for the assay are cool and on ice (Figure 1). Where possible, cool all consumables, including pipette tips in the refrigerator.
4. From this point forward, work on ice and maintain all buffers at 4°C.
5. Mix the virus or VLPs into their respective cell containing tubes.
6. Pipette the cell and virus/VLP mixes at least five times up and down to mix. Do not vortex.
7. Place the tubes immediately back on ice, in the refrigerator.

8. Incubate for 20 min (Figure 1).

Note: For our binding assays using infectious NL4-3 virus a $p24^{CA}$ equivalent of 50 ng results in a strong nanoluciferase signal. For our bindings assays using pNL4-3 ΔRTΔINΔVifΔVpr VLPs a $p24^{CA}$ equivalent of 100 ng was suitable. These values were used based on the dynamic range of the nanoluciferase on our Tecan MP200 Pro reader.

9. Add 1 mL ice-cold HBSS to each tube followed by centrifugation at 100 *g*, 4°C, 3–5 min.
10. Wash the cell and virus/VLP mixes at least three times using ice-cold HBSS buffer (Figure 1). Wash with 1 mL of HBSS per tube, per wash. Take care not to disturb the cell pellet when removing the HBSS wash.

Note: When washing the cell pellets do not attempt to remove all of the HBSS wash buffer between washes. Allow approximately 50 μL to remain in the tube. PBMC cell pellets tend to be loose and cells can be inadvertently removed when trying to clear all of the HBSS. Where a researcher is concerned that their cells have not been adequately washed, they can increase the number of washes to five.

11. If a trypsin control is being included, add 50–100 μL 0.25% trypsin (EDTA free) to this sample after the initial HBSS washes.
12. Incubate this tube at 37°C for 4 min.
13. Add 800 μL cold, complete RPMI to dilute and inactivate the trypsin.
14. Immediately wash the cells with ice-cold HBSS at least three times, as detailed above.

Note: The length of time required for the trypsin treatment may need to be optimised for the assay conditions in use. See troubleshooting points for potential solutions.

15. After the final wash, resuspend the cell pellets in 100 μL 1 x cell lysis buffer containing 1% Triton X-100 and 1% NP40 (Figure 1).

Note: 1 x Cell lysis buffer can be prepared ahead of the experiment and stored indefinitely at room temperature (20°C–25°C) until either contaminating microbial growth is seen or precipitation of the constituents occurs. Stock preparations of 10x cell lysis buffer should be stored 4°C, indefinitely until either precipitation or contamination becomes apparent.

Pause point: After the cells have been washed and resuspended in lysis buffer, the screw cap tubes can be directly frozen at -80°C. We have frozen lysates for up to one week, but have not tested the long-term stability past this point. For infectious virus work, this step is preferred as it is a failsafe to ensure that cells are fully lysed prior to removal from the PC3 laboratory for analysis. Allow all tubes to warm to room temperature (20°C–25°C) prior to continuing with the nanoluciferase assay. Pulse vortex prior to distributing thawed samples into the 96-well plate below.

16. Prepare Nano-Glo luciferase reagents as per manufacturer's instructions in the technical guide (Promega).
    a. Calculate the number of assays that need to be performed and prepare reagent with 5–10% excess to accommodate pipetting error.
17. Transfer 25 μL of each lysed sample to a 96-well plate suitable for luminescence reading for the plate reader.
18. To each sample well pipette 25 μL Nano-Glo reagent.
19. Pipette to mix and then allow to stand at room temperature (20°C–25°C) for at least 3 min before reading the plate in the plate reader (Figure 1).

Table 1. Example of raw nanoluciferase data measured on a plate reader

Plate Reader Data:
Application: Tecan i-control
Tecan i-control , 1.11.1.0
Device: infinite 200Pro
Serial number: 1501003715
Firmware: V_3.37_07/12_Infinite (Jul 20 2012/13.56.47)
MAI, V_3.37_07/12_Infinite (Jul 20 2012/13.56.47)Date: XX/YY/2023
Time: 4:32:29 PMSystem: DESKTOP-G*#@$K
User: Joe Bloggs
Plate: Costar 96 Flat Bottom White Polystyrene Cat No.: 3600/3362/3917/3912/3922/3596 [COS96fw.pdfx]Shaking (Orbital) Duration: 5s
Shaking (Orbital) Amplitude: 1mmLabel: Label1
Mode: Luminescence
Attenuation: Automatic:
Integration Time: 1000 ms
Settle Time: 10 ms
Part of Plate: A1 to B9
Start Time: 4:32:29 PM
End Time: 4:33:34 PMTemperature: 24°C

	1	2	3	4	5	6	7	8	9
	Cells: Donor 1			Cells: Donor 2			Cells: Donor 3		
	Untreated	PNGaseF	Trypsin	Untreated	PNGaseF	Trypsin	Untreated	PNGaseF	Trypsin
A mock	43	60	46	53	37	25	36	30	28
B	1131856	125601	6571	493377	87668	1038	177977	64807	938

EXPECTED OUTCOMES

Assessing nanoluciferase activity by the measurement of total relative light units (RLU) produced is possible once researchers analyze their data, taking into consideration their background controls (e.g., mock and trypsin-treated control). Analysis will be dependent on the experimental design. For the nanoluciferase binding assays described in Spillings et al.,[1] the relative nanoluciferase activity was determined by normalizing test samples for background (by subtracting mock RLU, Table 1) and then calculating the ratio of the total RLU of the test over the total RLU for the untreated, infected control.

For the above data set, the average mock RLU for each donor would be calculated as the mean of the luminescence values. For donor 1 this would equate to mean (A1:A3). This is the background luminescence generated from the cells only.

All of the RLU values for the samples would be adjusted for by subtracting their respective mock background RLU to result in a background adjusted RLU.

The ratio of the test RLU relative to the untreated, infected control sample would then be calculated. For example for donor 1:

$$\textit{Mean background RLU} = \frac{\textit{Sum of mock RLUs}}{\textit{number of wells}}$$

$$\textit{Mean background RLU} = \frac{\textit{Sum}\,(A1:A3)}{3} = \frac{43+60+46}{3} = 49.66$$

$$\textit{Relative Nanoluciferase}_{\textit{PNGaseF Treated Cells}} = \frac{\textit{Experimental RLU} - \textit{Mean background RLU}}{\textit{Untreated RLU} - \textit{Mean background RLU}} * 100$$

$$= \frac{(125601 - 49.66)}{(1131856 - 49.66)} * 100$$

$$= 11.09\ \%$$

LIMITATIONS

This binding assay should be optimized for each laboratory and cell/virus system for which it is being used. An important consideration is the need to determine the linear dynamic range of the plate reader/luminometer. The nanoluciferase assay system is sensitive to very high levels of nanoluciferase and should the levels be too high, the assay may need to be optimized to bring the levels within the linear dynamic range.

TROUBLESHOOTING

Problem 1

Cell pellets disappear during centrifugation steps (Nanoluciferase Binding Assay, Steps 6 and 7).

Potential solution

If you are working with PBMCs or other suspension cells do not try to remove all of the HBSS buffer from above the cell pellet. Allow approximately 50 μL to remain in the tube. In our hands we have noticed that PBMC cell pellets tend to be loose. The cell pellets can be easily dislodged and cells can be inadvertently removed during the pipetting steps to remove the HBSS. Where a researcher is concerned that their cells have not been adequately washed, they can increase the number of washes to five.

Problem 2

PNGaseF is a suitable negative control for gross removals of N-glycans but not for O-glycans or removal of specific glycan components (Nanoluciferase Binding Assay, Step 1).

Potential solution

When investigating glycan-based interactions, a suitable negative control should be chosen. For our study we specifically investigated glycan-glycan-based interactions between cells and virus. In order to remove a majority of the surface glycan structures, we chose to utilize the glycosidase enzyme PNGase F. This enzyme cleaves *N*-linked glycans between the innermost GlcNAc and asparagine residues on high mannose, hybrid and complex oligosaccharides. We have successfully used this enzyme to treat both PBMCs as well as VLPs and virus. Other glycosidase enzymes include neuraminidases (for the removal of sialic acid), galactosidases (for the removal of galactosidase), fucosidases (for the removal of fucose) and mannosidases (for the removal of mannose). These alternative glycosidases could be applied to investigate specific glycan-based interactions. For example, studies investigating proteinaceous lectin and complex *N*-linked glycan interactions may elect to utilize sialidase enzyme treatments as their negative control.

Problem 3

Nanoluciferase signal too low or too high (Nanoluciferase Binding Assay, Step 10).

Potential solution

Prior to performing a nanoluciferase based binding assay, the sensitivity of the plate reader should be assessed relative to your VLP or virus particles. This is detailed within the technical manual for the Promega Nano-Glo Luciferase Assay System. The linear dynamic range of the luminometer or spectrophotometer should be assessed by determining the luminescence or relative light units (RLU) produced across a concentration range of VLP or virus concentrations prepared in lysis buffer. A quantity of VLP or virus within the linear dynamic range should be used for the binding experiments. If, however, the signal across a dilution series does not permit accurate

quantification of small changes in nanoluciferase across the dilution range due to high signal intensities, the assay can be modified by using a luciferase-based assay. In this instance the nanoluciferase-Vpr reporter plasmid would be replaced with an appropriate luciferase-Vpr chimeric protein expression vector.

Problem 4

Nanoluciferase signal is the sum of binding at a population level rather than the occurrence at single cell level within a population (Nanoluciferase Binding Assay, Step 10).

Potential solution

The binding assay can be modified by using a fluorescent protein-based assay. In this instance the nanoluciferase-Vpr reporter plasmid would be replaced with an appropriate fluorescent protein-Vpr chimeric protein expression vector. It is understood that fluorescent protein conjugated with viral proteins (such as HIV Vpr) will not yield highly sensitive signals as nanoluciferase-Vpr chimera, but the relationship between viral particles and target cell will be revealed at the single cell level, including the spatial distribution of the surface binding with a resolution of 200 nm in accordance to the diffraction limit of visible light.

Problem 5

High RLU values for trypsin treated controls (Nanoluciferase Binding Assay, Step 7).

Potential solution

The length of time required for the trypsin treatment may need to be optimized for the assay conditions in use. The trypsin control should give an indication of the background levels of nanoluciferase carry over in the system, including the quantity of particles that may have been endocytosed. Surface bound virus or VLPs should be cleaved due to the tryptic activity.

To reduce high trypsin control signals, review the handling steps to ensure that temperatures are more stringently controlled and that entry non-permissive temperatures are always maintained.

Alternatively, the trypsin treatment itself has not been effective in removing the bound particles. If using thawed aliquots of trypsin, ensure that the aliquots are at room temperature (20°C–25°C) before use. The length of time for the trypsin treatment should be optimized prior to initiating a large experiment. Trypsinisation is optimal at 37°C and the length of treatment will also depend on how sensitive your target cells are to trypsin.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Johnson Mak (j.mak@griffith.edu.au).

Materials availability

Plasmids generated in this study will be provided, upon request to the lead contact, and may require fulfillment of an MTA.

Data and code availability

This study did not generate/analyze datasets or code.

ACKNOWLEDGMENTS

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nanoluciferase-vpr plasmid construct used in the binding assays. Figures were created using BioRender.com.

AUTHOR CONTRIBUTIONS

Conceptualization, B.LS., J.M.; Methodology and Investigation, B.L.S.; Writing – Original Draft, B.L.S.; Writing – Review and Editing, B.L.S., J.M.; Supervision, J.M.; Funding Acquisition, J.M. All authors have reviewed the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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