Dec 31, 2019

Colony Formation Titering Assay for Lentivirus V.2

Addgene the nonprofit plasmid repository[1]

[1]Addgene

1 Works for me

dx.doi.org/10.17504/protocols.io.bawsifee

Addgene The Nonprofit Plasmid Repository
Addgene

ABSTRACT

This protocol is for running a colony formation titering assay for Lentivirus. To see the full abstract and additional resources, visit the Addgene protocol page.

Sample Data

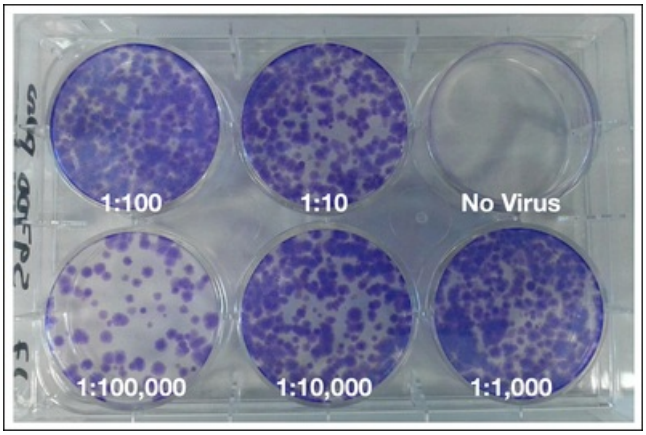

Figure 1: A549 cells were transduced with the indicated serial dilutions of the lentiviral vector pRosetta. The following day, cells were treated with puromycin. Colonies were allowed to grow in the presence of selective reagent for 12 days with media exchanges every 3-4 days. Colonies were then stained with 0.1% crystal violet and counted.

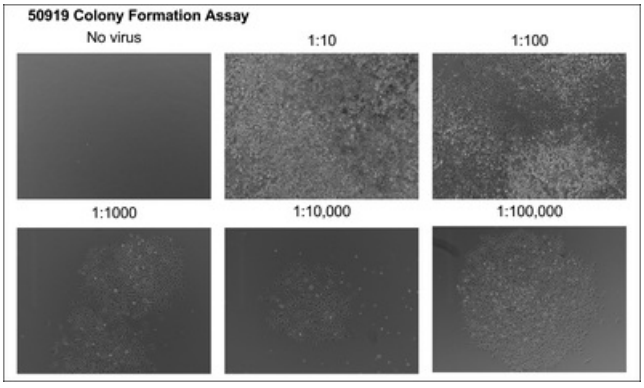

Figure 2: A549 cells were transduced with the indicated serial dilutions of the lentiviral vector pHAGE EF1α dCas9-KRAB. The following day, cells were treated with puromycin. Colonies were allowed to grow in the presence of selective reagent for 12 days with media exchanges every 3-4 days. After 12 days of selection, no cells have survived in the untransduced well, while lawns of cells are present in the lower dilutions, and single colonies are visible in the higher dilutions.

EXTERNAL LINK

https://www.addgene.org/protocols/colony-formation-titering-assay/

GUIDELINES

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Workflow Timeline

Day 0: Seed and Transduce Cells
Day 2: Replace media with fresh media containing selection reagent.
Days 3-14: Change media as needed
Days 14-18: Stain cells and count resistant colonies

MATERIALS TEXT

Equipment

- Biosafety cabinet
- Pipetman
- Pipettors
- Incubator

Reagents

- DMEM high glucose
- L-alanyl-L-glutamine (or alternative stable glutamine)
- Heat-inactivated FBS
- Polybrene
- PBS pH 7.4 without calcium or magnesium (cations can affect the attachment of adherent cells)
- Microcentrifuge tubes
- 6-well dishes
- Pipettes
- Pipette tips
- 0.1% Crystal Violet
- 0.22 µm polyethersulfone filter (for crystal violet solution)
- 0.45 µm polyethersulfone filter (for viral preps, if prep was not previously filtered)
- Syringe for filtering
- Lentivirus Preparation

Reagent Preparation

1. DMEM Complete: 10% v/v FBS and 4 mM L-alanyl-L-glutamine

- To a **500 ml** bottle of DMEM high glucose, add **55 ml** of heat inactivated FBS and **11 ml** of 200 mM L-alanyl-L-glutamine. Store at **4 °C** .

Pro-Tip
Different brands and lots of FBS can promote or inhibit transfection. Test a variety of brands and lots of FBS to find one suitable with your protocols. FBS can be purchased already head inactivated or it can be inactivated in the lab by heating to **56 °C** for **00:30:00** .

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

SAFETY WARNINGS

See SDS (Safety Data Sheet) for safety warnings and hazards.

BEFORE STARTING

- The health of the the target cell line is critical for obtaining accurate titers.
  - Check the cells for mycoplasma regularly
  - Do not over or under-grow your cells.
  - Thaw a new vial of cells after 20-30 passages.

- Do not add penicillin/streptomycin to the media.
- Titer will vary between cell lines.
- It is not recommended that lentiviral supernatants be subjected to multiple freeze-thaw cycles.
- This protocol outlines the seeding of the cells at the same time as the viral transduction. For some cell lines, transduction is optimized if the cells are seeded the day before viral transduction. Note that in that case, polybrene should only be added at the time of viral transduction, and not during the cell seeding step.

Procedure

1 Before beginning a colony formation assay, the dose of antibiotic required to kill your target cell line needs to be empirically determined. Treat the target cells with a range of doses of antibiotic. Determine the minimum concentration required to kill all of the cells. Use this dose for the colony formation assay.

2 Prepare a batch of DMEM complete containing 10 µg/mL polybrene by diluting **20 µl** of 10 mg/mL polybrene into **20 ml** media.

3 If using freshly collected virus, filter through a 0.45 µm polyethersulfone filter to remove cells and debris.

- Lentiviral titer can decrease during cycles of freeze-thaw. If you are freezing and aliquoting virus it is recommended that you titer from the frozen stock to account for any loss in titer associated with freeze-thaw.

4 If using frozen virus, rapidly thaw the lentiviral aliquot at **37 °C** by agitating in a warm water or bead bath.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

5 Prepare 10-fold serial dilutions of the lentivirus into DMEM complete containing 10 µg/mL polybrene:

Dilution	Volume of Lentivirus or Previous Dilution (µL)	Volume of DMEM Complete Containing 10 µg/mL Polybrene (µL)	Volume of Virus Added to Plate (µL)	Volume of Cells Added to Plate (µL)	Final Viral Dilution
1:10	100 of Stock Virus	900	150	1,350	1:100
1:100	100 of 1:10	900	150	1,350	1:1,000
1:1,000	100 of 1:100	900	150	1,350	1:10,000
1:10,000	100 of 1:1,000	900	150	1,350	1:100,000
1:100,000	100 of 1:10,000	900	150	1,350	1:1,000,000

Notes:
- Mix the dilutions well
- The 1:10 dilution can usually be omitted because this dilution typically produces a lawn of cells, which cannot be accurately quantified.

6 Add 150 µL of a viral dilution to each well (each well gets one dilution)

7 Seed 1,000 cells into each well of a 6-well dish.

- Prepare a batch of cells as follows: Dilute 7,000 cells into **9.45 ml** of DMEM complete containing 10 µg/mL polybrene.
- Mix well by pipetting or inverting.
- Aliquot **1.35 ml** of the cell suspension into each well of the 6-well dish directly on top of virus. Swirl gently to mix and place dish in incubator.

8 Incubate for **48:00:00** - **72:00:00** .

Pro-Tip
Different cells may be more sensitive to lentivirus or to polybrene, in which case this incubation step can be reduced to **08:00:00** and the media can be replaced with DMEM complete. However, antibiotic-containing media should not be added until **48:00:00** - **72:00:00** after transduction.

9 Gently aspirate the media from the cells.

10 Add **1.5 ml** of DMEM complete containing the appropriate antibiotic.

11 Incubate the cells for ~2 weeks. All of the cells in the untransduced (negative) control well should be killed and colonies should be visible.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

12 Every 3-4 days, gently aspirate the media and replace it with fresh DMEM complete containing the appropriate antibiotic.

13 When the untransduced (negative control) cells have died and colonies are visible in the transduced wells, aspirate the media from the wells.

14 Gently wash the cells with **1 ml** of PBS and aspirate wash.

15 Filter 0.1% crystal violet solution through a 0.22 µm filter to remove any precipitates.

16 Stain each well with **1 ml** of 0.1% crystal violet for **00:10:00** at room temperature.

17 Gently remove the stain.

18 Wash cells 3x with **1 ml** of PBS, being careful not to disturb the colonies.

19 Count the colonies for at least 2 of the dilutions.

Pro-Tip
The higher dilution wells tend to provide more accurate titers as the likelihood of multiple integration events decreases as the dilution increases.

20 Calculate the transduction units per mL (TU/mL) as follows:

- TU/mL = number of colonies/total volume in the well (mL) x dilution factor
- e.g., If the 1:100,000 well has 75 colonies, then there are 75 colonies / **1.5 ml** x 1,000,000 or 50,000,000 TU/mL

21 For a more accurate titer, take the average of multiple dilutions.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited