YCCEH Protocols

VIRUS INFECTION

Contributing Lab: Jun Lu

Date of Revision: August 16th, 2021

Reagents

Polybrene: 10mg/ml American Bioanalytical Cat#AB01643, or Millipore TR-1003-G (10mg/ml is 1250x)
Retronectin: Takara Bio #T100A.
Culture medium for the cells of interest: regular culture medium, including any necessary growth factors

Viral Infection

Overview

This protocol can be used for retrovirus or lentivirus infection. Infection with retrovirus or lentivirus is a useful technique to transduce genetic materials stably into hematopoietic cells.

Compared to contemporary lentivirus vectors, commonly used retroviral vectors tend to be smaller in size, and tend to produce virus with higher titer compared with lenti-vectors. Thus, retrovirus could be sometimes advantageous when the genetic payload is large. However, while lentivirus can enter the nucleus and integrate into the genomes of non-dividing cells, retrovirus integrates preferentially into dividing cells.

The half-lives of retrovirus or lentivirus at 37C or room temperature are relatively short. So avoid exposing virus to high temperatures whenever possible.

Polybrene is used to enhance infection efficiency, presumably through neutralizing charge interaction between cells and virus.

CAUTION: Proper Biological Safety precaution should be followed. Bleach pipets, tips and plates that have been in contact with virus.

Retroviral infection of attaching cells

Attaching cells can be infected when grown on a plate or through spin-infection.

Infecting attached cells

This protocol is for 6 well plates. It can be adapted for 10cm dish or other container size if needed. (Note: In the case of 10 cm dish, as low as 2.5 ml viral mixture can be used. Tilt plate to mix every 15 to 30 mins for a total of 2 hours before adding 10ml of growth medium).

We routinely use up to 1 ml of virus. Less virus can be used if titer is high.

Combine virus and culture medium, if any (medium for the cells to be infected) to a total of 1ml. Add 0.8 ul of 1250x polybrene. If growth factor is needed, supplement with the virus-medium mixture with 1x growth factor (1x growth factor means the same concentration of growth factor used for regular culture).

Aspirate out the medium of the well to be infected. Add the viral mixture into the well. Tilt plate several times to mix well. Incubate in TC incubator for 2 hours. Tilt once every 30 mins to mix.

Add 2 ml of growth medium to the well and culture overnight.

Change medium the second day.

Spin-infection

Trypsinize the cells to be infected and inactivate trypsin by adding medium with FBS. Count cells.

Prepare cells to be infected in 0.25 ml of culture medium. Add 1 ul 1250x polybrene. If growth factors are needed, add growth factors to 5x the concentration for culture (5x is relative to 0.25ml medium, so that after combining with 1ml virus below, the final concentration will be 1x).

Add up to 1 ml of virus to a well on a 6-well plate (add medium if <1ml of virus is used). Add 250ul of cell/poplybrene mixture. Pipet up and down to mix.

Spin in a centrifuge with a plate adaptor at 500 g or 600 g for 30mins at room temperature. Return plate to incubator after spinning.

Return to incubator. Add 2ml of fresh culture medium after 2 hours.

Change medium the next day.

Retroviral infection of floating cells

We use retronectin to enhance infection efficiency of hematopoietic cells.

Dissolve retronectin powder with 1XPBS, to a final concentration of 500ug/ml.
Store at -20C or short term at 4 degrees.

Before use, dilute to 50 to 100 μg/ml with PBS. Coat 6 well plates by pipetting and wetting the well and removing the liquid. 1ml can coat 12 to 18 6-well plate wells.
Let dry in air for >30 mins with cover lid on. Left-over diluted retronectin can be stored in 4C and re-used several times if needed.

Count cells to be infected, use 0.1 to 0.5 million cells per infection, depending on cell type. Resuspend cells at 0.25ml per infection. Add 1ul of 1250x polybrene. If growth factors are needed, add growth factors to 5x concentration (5x is relative to 0.25ml medium, so that after combining with 1ml virus below, the final concentration will be 1x).

Use up to 1ml of virus per infection. Supplement volume with culture medium if <1ml of virus is used. Add 1ml of virus to a retronectin-coated well. Add 0.25ml cells with polybrene and growth factors. Pipet up and down once to mix.

Spin in a centrifuge with a plate adaptor at 500 g or 600 g for 30mins at room temperature. Return plate to incubator after spinning.

Add 2ml of fresh growth medium with growth factor after 2 hours.

Drug selection

If your viral vector contains a drug resistance gene, drug selection can be applied 24 to 48 hours post infection. For cell lines, we normally apply selection 24 hours after infection. For primary hematopoietic cells, we normally apply selection 48 hours after infection. Here are some commonly used concentrations. Note: different cells may have different dose response. For a new cell type, optimize the concentration first before use.

Puromycin: 1-2 ug/ml (2ug/ml is the most common). Puromycin selects reasonably fast. In most cases, non-infected cells are all killed off within 2 to 3 days.

Blasticidin: 5 to 15ug/ml for most cell lines (10 or 15 ug/ml is often used for hematopoietic cells). Blasticidin selects slightly slower than puromycin. In most cases, non-infected cells are all killed off within 3 to 5 days.

Neomycin (geneticin): 0.3 to 1 mg/ ml (1mg/ml is often used in hematopoietic cells). Neomycin selects slowly. It can take up to 2 weeks for completing selection.

Hygromycin: 300ug/ml. Hygromycin selects faster than neomycin but slower than blasticidin. Often takes 7 to 10 days for selection.

Zeocin: 100-750ug/ml