BROAD INSTITUTE | GENETIC PERTURBATION PLATFORM

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Protocol: Introducing Cas9 lentivirus into cell lines and determining infection efficiency

This protocol describes lentiviral infection of pLX_311Cas9v2 (also known as pXPR_111) and selection with blasticidin. At the end of the experiment, you will be able to determine the infection efficiency of pLX_311Cas9v2. You will also have on hand Cas9-expressing cells for subsequent experiments.

Should you need to test multiple Cas9-expressing viruses, you will need to scale up accordingly.

Prior to this protocol, cells should be tested for polybrene sensitivity and a blasticidin kill curve should be performed.

Material required:
2.5 ml of pLX_311Cas9v2 lentivirus
polybrene
blasticidin
6-well plates
12-well plates
10-cm dishes or T-75 flasks

DAY 1

1. Trypsinize and count cells. Resuspend 3 million cells in a final volume of 6 mL.
2. Add 18 µL of 1000x polybrene (optimal concentration previously determined).
3. Add 1 mL of cell suspension to 5 wells of a 12-well plate. Add 0, 200, 400, 800, 1000 µL of Cas9 virus per well. Make up the volume with media.

4. Spin plate for 2 hours at 1,000 x g at 30˚C; return plate to incubator.
5. 4 - 6 hours after the spin is complete, transfer the cells from each well to a 10cm dish or T-75 flask.

DAY 3

1. Trypsinize the non-infected cells or Cas9-infected cells and count.
2. For each volume of the Cas9 virus-infected cells, seed 50,000 cells into 2 wells of a 6-well plate. Add blasticidin at the appropriate final concentration to 1 well, and no blasticidin to the other. Seed the remainder of the cells into a 10cm dish with blasticidin.
3. For the non-infected cells, seed 50,000 cells into 2 wells of the 6-well plate. Add blasticidin at the appropriate final concentration to 1 well, and no blasticidin to the other. There is no need to continue to expand the remainder.

DAYS 5 – 10

1. Passage the Cas9-infected cells in the 10cm dish as needed, maintaining them in blasticidin. When splitting, it is not necessary to keep all the cells; passage them at the usual splitting schedule and density.
2. For the 6-well dish, when the non-infected well with no selection reaches 100% confluence, split all 4 wells 1-to-5 into a new 6-well plate. It is critical to split each well equally *by volume* not by cell number in order to determine infection efficiency.
3. After the split in step (2), when the uninfected well *with* selection is completely dead and the uninfected well *without* selection again reaches 100% confluency, trypsinize and count the cells.[1] The infection efficiency is the number of cells in the with-infection/with-selection well divided by the number of cells in the with-infection/no-selection well. By definition, for the uninfected cells, this should be close to 0%.
4. Once step (3) is complete and a reasonable infection efficiency has been confirmed (10-50%), the cells in the 10cm dish are ready for experimental use. They can also be frozen for future use. As a next step, we recommend determining Cas9 activity.

[1] If the no-selection well of the uninfected well reaches 100% confluence before the with-selection well is completely dead, split again. It is also possible that you are not using the right concentration of blasticidin, and this should be re-optimized.