



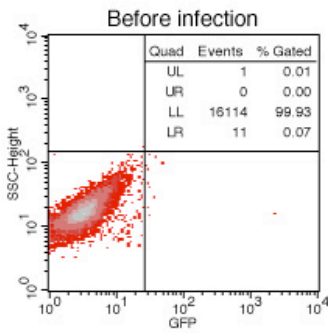
# Lentivirus Replication Safety Assay

Lentiviruses are powerful vectors for gene transfection, and may infect nondividing cells, including human tissue. Most lentiviruses that are used in labs have been modified to provide a safer virus version in which the viral replication genes have been removed.

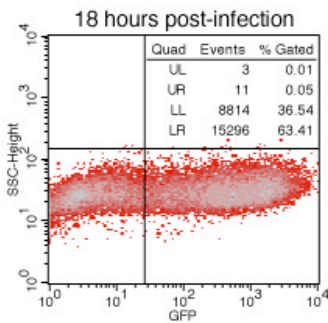
During infection, there is a slight possibility that the lentivirus may convert to a replication competent state. Although this scenario is highly unlikely, it is encouraged to monitor for such a possibility, since such a conversion could compromise laboratory safety. The below protocol was designed for monitoring the conversion of replication deficient virus to replication competent virus in a cell culture assay. It is suggested to perform this protocol for newly created vector backbones.

You should also be sure that your laboratory and safety policies are set up to work with lentiviruses.

## GFP Lentiviral Replication Safety Check

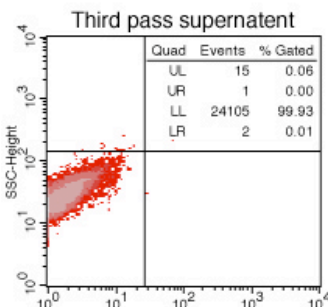


Day 1  
infect (1) well in a six-well in a plate of ~60% confluent Human 293 cells with 4 ml supernatant-containing virus.



Day 2 (~18 hours post infection)  
analyze infection rate by harvesting and reseeding cells by 1:4 dilution.

-Pass cells when confluency reaches ~100%. Repeat for a total of three passages.



~Day 6  
remove 4 ml supernatant from third passage cells and add to (1) well in a six-well in a plate of ~60% confluent Human 293 cells. Analyze GFP fluorescence on the following day (~18 hours post addition).