

# Sorting of virally transduced cells

## 3<sup>rd</sup> Generation lentivirus

1 Check your permit if host cells and vectors are included.

Location for sorting is the NIG building M379, Room number 4.148 (route number 475)

2 Perform risk analysis

$$(20^W * 200^I * 2^{2,4T}) / V \geq 100$$

W= # washing steps  
I= # trypsinisation steps  
T= # days after transduction  
V= virus titer =  $10^8$  (when unknown)

3 Make sure that cell lines used for transduction are negative for HIV, SIV and HTLV. This can be tested using PCR.

**When Risk analysis is >100 and cells are negative for HIV, SIV and HTLV, sort can be performed on MLI.**

Make sure to sign the logbook when the sort is performed.

## 2<sup>nd</sup> Generation lentivirus

1 Check your permit if host cells and vectors are included.

Location for sorting is the NIG building M379 Room number 4.148 (route number 475)

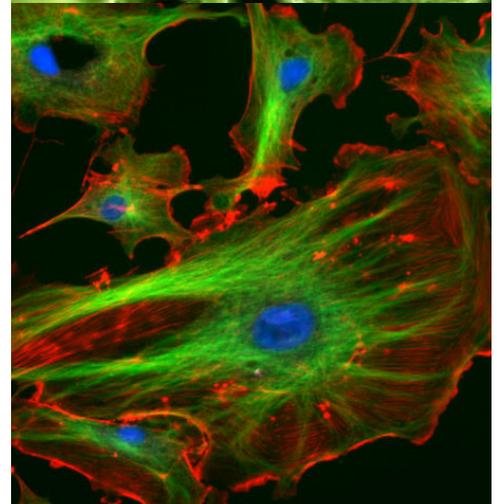
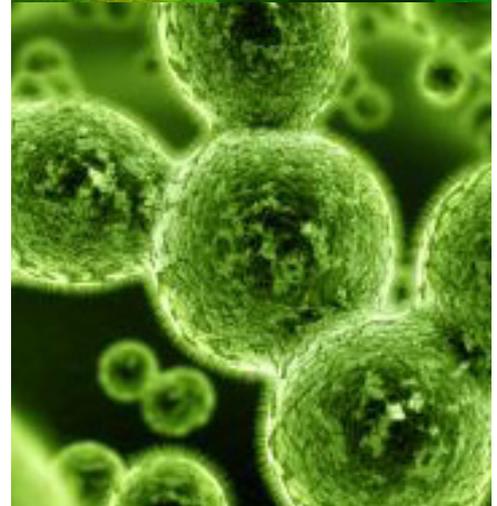
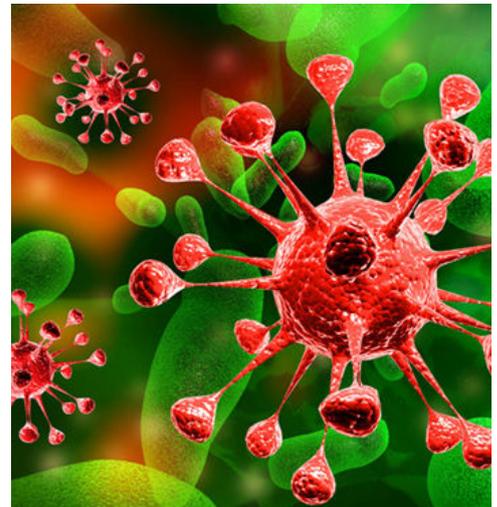
2 Perform risk analysis

$$(20^W * 200^I * 2^{2,4T}) / V \geq 100$$

W= # washing steps  
I= # trypsinisation steps  
T= # days after transduction  
V= virus titer =  $10^8$  (when unknown)

**Perform the sort on MLI.**

Make sure to sign the logbook when the sort is performed.



# Retrovirus

**1** Check your permit if host cells and vectors are included.

Location for sorting is the NIG building M379 Room number 4.148 (route number 475)

**2** Perform risk analysis

$$(20^W * 20^I * 2^{2,4T}) / V \geq 100$$

W= # washing steps

I= # trypsinisation steps

T= # days after transduction

V= virus titer =  $10^8$  (when unknown)

**3** Perform PERT assay and provide the document with the result.

**When Risk analysis is >100 and PERT assay is negative, sort can be performed on MLI. When PERT assay positive is or not performed, you have to sort on MLI.**

Make sure to sign the logbook when the sort is performed.

**Sort request by mail to [rob.woestenenk@radboudumc.nl](mailto:rob.woestenenk@radboudumc.nl) includes:**

Name:

Date of sort:

Host cells:

Vector:

Type of virus:

Days of culturing after transduction (T):

Washing steps (W):

Trypsinize steps (I):

Complete risk analysis:

**Sorting on MLI:**

Provide results PERT assay (retro), or prove that cell line is HIV, SIV and HTLV negative (3rd gen. lenti).