—Information for luciferase gene-introduced cell lines—

Origin of luciferase gene

The species of origin: firefly (*Photinus pyralis*) that is living in North America.

Gene: pGL3-luciferase (Promega)
Corresponding sequence will be Genbank ACC U47295, CDS 88..1740.
https://www.ncbi.nlm.nih.gov/nuccore/U47295

Vector used

One of following vector was used for the transfection of luciferase gene to host cells. Please check the each cell information in JCRB Cell Bank website.

・pMSCV-Luc
・pLVSIN-Luc
・pLL3.7-CMV-Luc

pMSCV-Luc (retrovirus vector)

pMSCV-luc was constructed by inserting pGL3 luciferase gene at XhoI-EcoRI site of pMSCVpuro. Selection marker is puromycin resistancy.
Please refer to the vector construction in page 3.

pLVSIN-Luc (lentivirus vector)

pLVSIN-luc was constructed by inserting pGL3 luciferase gene at XhoI-XbaI site of pLVSIN-CMV-pur or pLVSIN-EF1a-pur. Selection marker is puromycin resistancy.
Please refer to the vector construction in page 4.

pLL3.7/CMV-Luc (lentivirus vector)

pLL3.7/CMV-Luc was constructed by inserting pGL3 luciferase gene to pLL3.7 vector. EGFP gene that was included in pLL3.7 vector was eliminated in pLL3.7/CMV-Luc. This vector does not contain antibody selection marker.
Please refer to the vector construction in page 5.

Antibiotics selection

According to the developer, around 2.5-4 ug/mL puromycin was used if the cell line has puromycin-resistant marker. Please use several points of concentrations for the trial of selection.

These cell lines were established as stable transfectants, and therefore JCRB Cell Bank did not take place antibiotics selection for these cell lines. However, there is possibility that the population of luciferase-expressing cells may decrease such by silencing and by population sift. Therefore, we recommend to prepare the frozen stocks of cell lines at early opportunities when the cells grow well in order to avoid loss of specific characteristics of cell lines or accidental loss such as contamination by microorganisms.

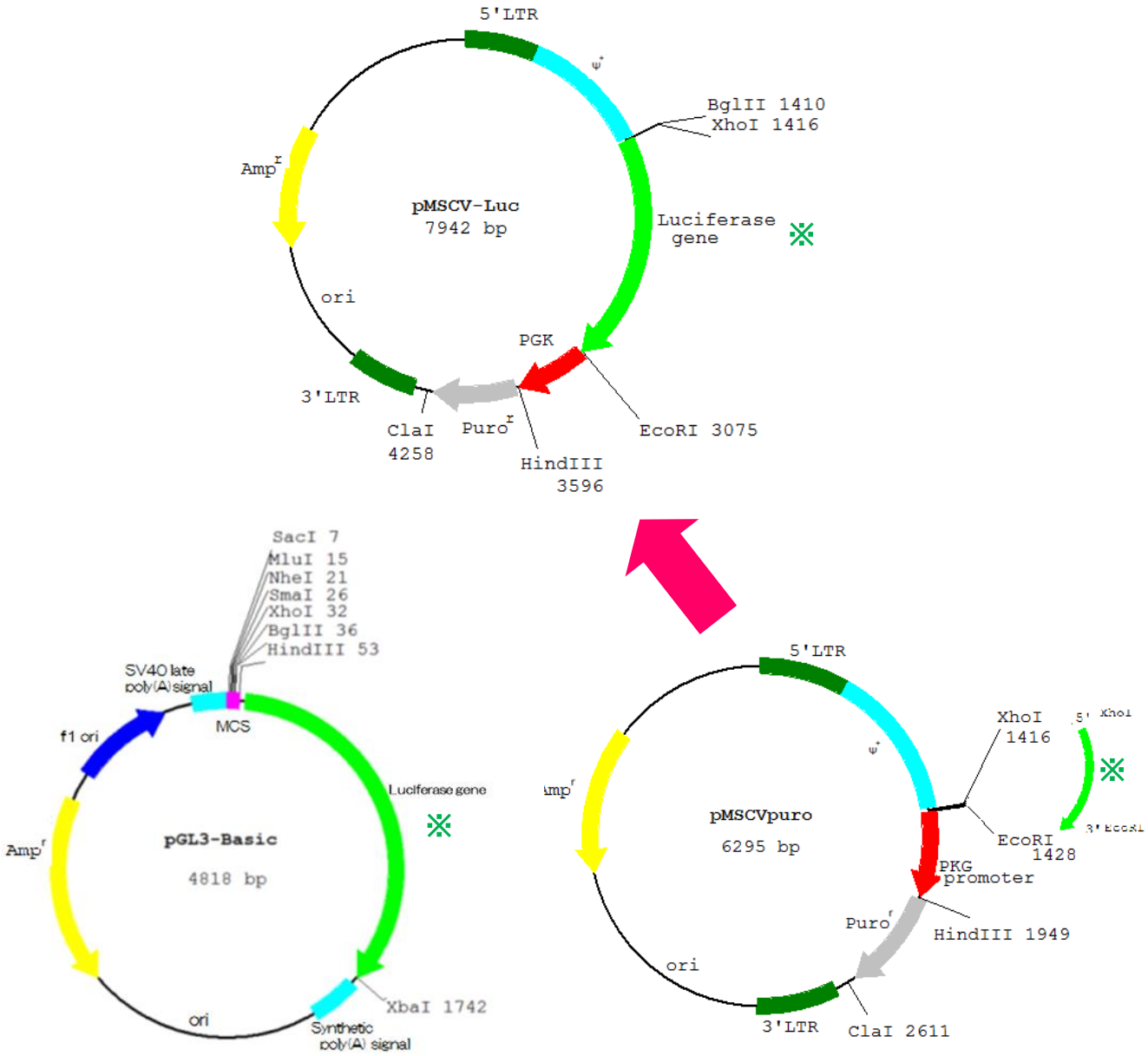
Safety

The retrovirus or lentivirus vectors used are replication incompetent, and therefor the recombinant virus is not produced by the host cells after the transfection.

Also, we judged that no infectious virus is remained in the cells due to the denature and dilution of transfected recombinant virus by long serial cultures.

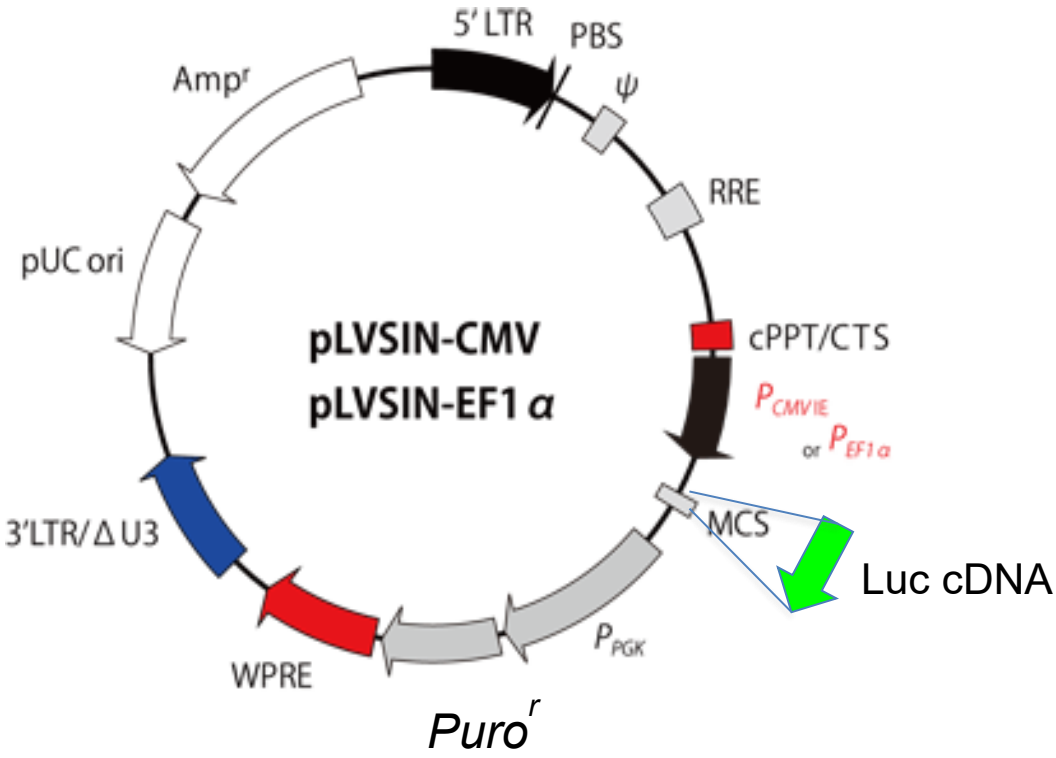
However, we recommend to handle these cell lines as potentially biohazadous materials. In most cases for human cell lines, the handling as biosafety level 2 is recommended. This not means that the cell line produces BSL-2 pathogens, but is needed to avoid potential risk.

pMSCV-Luc



※The insert was amplified from pGL3-Luciferase using PCR

pLVSIN-Luc


5′ LTR
PBS
Ψ
RRE
cPPT/CTS
P_{CMVIE}
or $P_{EF1\alpha}$
MCS
Luc cDNA
P_{PGK}
Puro^r
WPRE
3′LTR/ΔU3
pUC ori
Amp^r
pLVSIN-CMV
pLVSIN-EF1α

pLL3.7-CMV-Luc