

Providing information new vector

To add a new vector to a notification/permit, information about this vector must be available. The form below indicates which information must be submitted. Vector maps and any literature must be submitted separately. Relevant sections in the literature must be highlighted; if references are made to other documents, these must also be included.

On the following pages, a detailed explanation is provided for completing Form 2. Always consult this explanation when submitting information to ensure that the data is complete and accurate.

Send the completed form, together with any attachments, to the BSO ($\underline{v.vanleeuwen@maastrichtuniversity.nl}$) and $\underline{n.kisters@maastrichtuniversity.nl}$).

GMO NOTIFICATION/PERMIT INFORMATION	
IG number to which the vector	
must be added	
Number(s) of the section(s) to	
which the vector should be added	
VECTOR SPECIFICATIONS	
Name vector	
Addgene number	
Backbone vector	
Viral elements located on the	
vector	
Antibiotic resistance(s)	



Instructions regarding information to be provided on new vectors

To add new vectors to a notification or permit, detailed information about the vectors must be submitted. **Form 2** outlines the required information. These instructions provide a more detailed explanation of exactly what information needs to be included and why it is necessary.

GMO notification/permit information:

In this section of the form, the IG number of the notification/permit must be specified, along with the specific cell lines to which this vector should be added.

Specifications vector:

For each vector, it must be clearly indicated which elements are present. These elements may lead to a higher biosafety classification than ML-I, depending on their application. In general, the following sequences can increase the classification level of a given activity:

- Viral elements.
- Sequences encoding enzymatic functions involved in the transposition or integration of transposons or proviral sequences,
- Sequences that enable autonomous transfer of genetic material,
- Antibiotic resistance genes that do not naturally occur in the host species.

Examples for clarification:

Example 1 (SV40):

Some cell lines contain large fragments of the SV40 polyomavirus (e.g., COS7 cells). If these cells are transfected with a vector containing an SV40 origin of replication (SV40 ori), it can lead to the formation of a replicating genetically modified SV40 virus. Consequence: these activities must be performed at ML-II instead of ML-I.

Example 2 (lentiviral vectors):

For lentiviral vectors, the classification depends on:

- Whether the vector is SIN (self-inactivating, due to a deletion in the LTR), and
- Whether any accessory genes (*nef*, *vif*, *vpu*, *vpr*) are still present on the vector.

To correctly classify the activities, it is therefore essential that all elements on the vectors are fully specified (restriction sites and primer sequences are excluded).

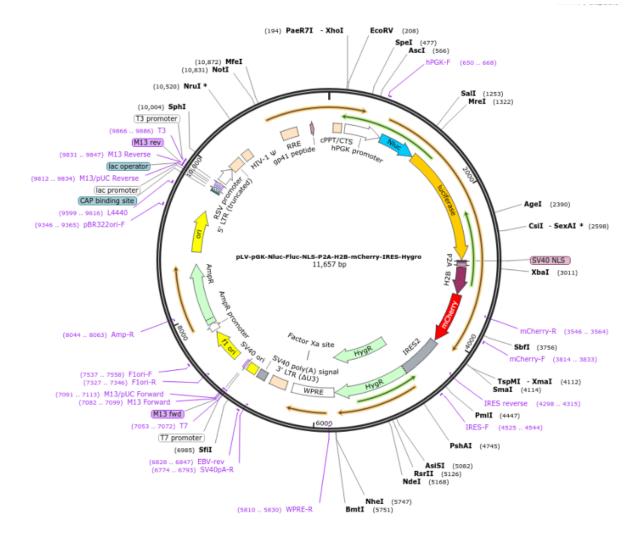
Researchers must specify on Form 2:

- Which viral sequences and antibiotic resistance genes are present,
- Provide a detailed vector map.

An Addgene number alone is not sufficient; a complete and detailed vector map must be supplied.

Below is an example of a detailed vector map.





For the vector described above, the following elements are important to specify:

- The truncated 5' LTR linked to the RSV promoter (one of the requirements for a third-generation system); the truncated U3 in the 3' LTR (which makes the vector SIN),
- HIV packaging signal, RRE, cPPT/CTS, as these are all components derived from the HIV, WPRE, P2A, SV40 NLS, SV40 poly A, and IRES, as these are viral elements,
- The SV40 ori since, together with a polyoma sequence in cells, it can lead to the formation of a replication-competent polyoma virus,
- The ampicillin and hygromycin antibiotic resistance markers.