

Providing information new cell line

To add a new cell line to a notification/permit, information about this cell line must be available. The form below indicates which information must be submitted, 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 3. 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 cell line	
must be added	
Number(s) of the section(s) to	
which the cell line should be added	
CELL LINE SPECIFICATIONS	
Name cell line	
Synonym	
ATCC/DSMZ number	
Origin cell line	human mouse rat other:
Is the cell line genetically modified?	no
	yes, describe how:



Instructions regarding information to be provided on new cell lines

To add new cell lines to a notification or permit, detailed information about the cell lines must be submitted. Form 3 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 sections to which this cell line should be added.

Cell line specifications:

For each cell line, information must be provided describing how it was generated. In many cases, this involves a non-modified cell, but information must still be submitted for that cell line.

Always review ATCC or DSMZ documentation carefully. Often, a cell line turns out to be genetically modified despite assumptions to the contrary. In some cases, the documentation specifies that cells were (retro)virally transduced to immortalize them. If viral immortalization has taken place, it is essential to know how the virus used for immortalization was produced. You must provide:

- The producer cell line
- The vectors used, and
- The vector maps for those vectors (see Example 1)

In addition to cell lines obtained via DSMZ or ATCC, cells derived from genetically modified mice are frequently added to notifications/permits. In such cases, information must be provided on how the relevant mouse was generated. The submitted information must clearly describe all elements and vectors used.

A general description such as "via homologous recombination" is not sufficient (see Examples 2 and 3).

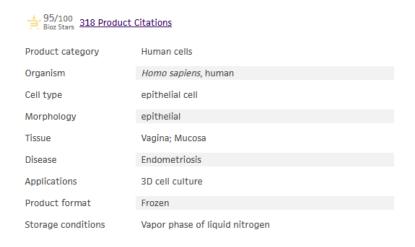
Special attention is required for iPS cells (iPSCs). For many of these cells, the manufacturer indicates that they are footprint-free, meaning that no genetic material remains after derivation. However, in the Netherlands, even these footprint-free iPSCs fall under GMO legislation and must be added to a notification or permit.

Example 1:

VK2/E6E7

CRL-2616 [™]

VK2/E6E7 is an epithelial cell line that was isolated in 1996 from the mucosa of a 32-year-old, female patient undergoing a hysterectomy for endometriosis. This cell line was deposited by D Anderson, RN Fichorova, and JG Rheinwald.





VK2/E6/7 cells are available from ATCC. When this cell line is looked up in the ATCC database, it appears at first glance to be a cell line isolated directly from a patient.

However, upon further review of the detailed information on the ATCC website, it becomes clear that this cell line was generated through transduction with a retroviral vector.

The VK2/E6E7 cell line was established in 1996 from the normal vaginal mucosal tissue taken from a premenopausal woman undergoing anterior-posterior vaginal repair surgery.

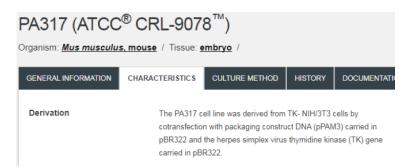
The ectocervical Ect1/E6E7 (ATCC CRL-2614) and endocervical End1/E6E7 (ATCC CRL-2615) cell lines were established in 1996 from normal epithelial tissue taken from a premenopausal woman undergoing hysterectomy for endometriosis.

Cells at passage 3 were immortalized by transduction with the retroviral vector LXSN-16E6E7 in the presence of polybrene. Clones were selected in medium containing G418.

To add this cell line to a notification or permit, the vector map of the LXCN-16E6E7 vector must be provided, and it must be determined which producer cell line was used for the production of this retrovirus. This information is often available in one of the references provided by ATCC. In this example, the relevant details were found in the first reference listed on the corresponding ATCC page.

The primary cell lines were immortalized by transduction with a retroviral vector (LXSN-16E6E7) packaged by the amphotropic fibroblast line PA317 (kindly provided by Denise Galloway, Fred Hutchinson Cancer Institute, Seattle, WA). Culture supernatants from the PA317 producer

It is then also necessary to obtain information about the PA317 cell line. This cell line is available from ATCC and was generated as follows:

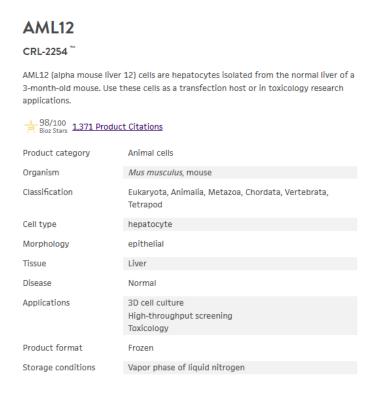


To complete the information, a vector map of the pBR322 vector must be provided, as well as information about the NIH/3T3 cell line.

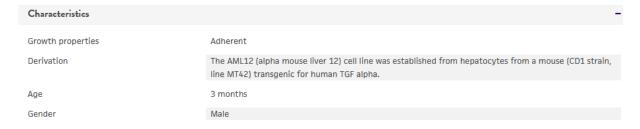


Example 2:

The AML12 cell line is a mouse cell line that is commercially available from ATCC. The initial information shown on the ATCC website does not indicate that this cell line is derived from a genetically modified mouse.



When the detailed information on the ATCC website is examined, it states that these cells are derived from a mouse transgenic for human TGF-alpha.



To be able to use this cell line, it must first be added to a notification or permit. For this purpose, it is necessary to determine how the mouse was generated. By reviewing the references cited in the first reference listed on the ATCC website, it can be determined that the CD1 mouse was generated as follows:

The CD1 strain was created by microinjection of the MT-TGFa fragment (770 bp of the mouse MT1 promoter, 917 bp of the human TGFa, and 625 bp of the 3' end of the human growth hormone gene) into the pronuclei of one-cell mouse embryos from the outbred strain CD1. The embryos were then implanted into CD1 pseudopregnant foster mothers.

The relevant references, together with this description, must be submitted to the BVF.



Example 3:

Through a collaboration, the mouse cell line HY15549 can be obtained. This cell line was isolated from a pancreatic ductal adenocarcinoma of a KPC mouse (p48-Cre+, Kras^{LSL-G12D/+}, Trp53^{lox/+}). This mouse was generated by crossing three mouse lines: Kras^{LSL-G12D}, p48-Cre, and a P53 conditional knockout mouse. To add this cell line to a notification or permit, the generation of all three mice must be described.

- The Kras mouse was generated by electroporation of the linearized pK-ras^{D12-LA} targeting construct into ES cells. C57BL/6 blastocyst-stage embryos were injected with K-ras^{LA} ES cells and subsequently transferred into pseudopregnant mice.
- The p48-Cre (Ptf1a-cre) mouse was generated by electroporation of the linearized Ptf1a-cre targeting construct into ES cells. These ES cells were injected into C57BL/6 blastocysts, which were then transferred into pseudopregnant ICR mice.
- To generate the P53 knockout mice, a Trp53 targeting construct was designed that encompassed Trp53 exons 1–11. This linearized targeting construct was electroporated into ES cells.