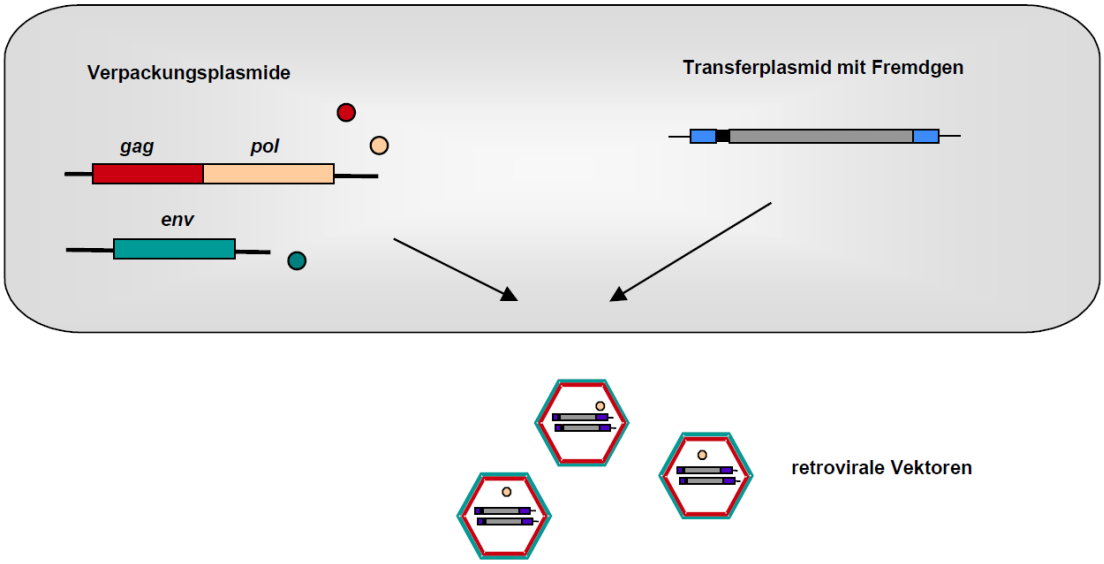
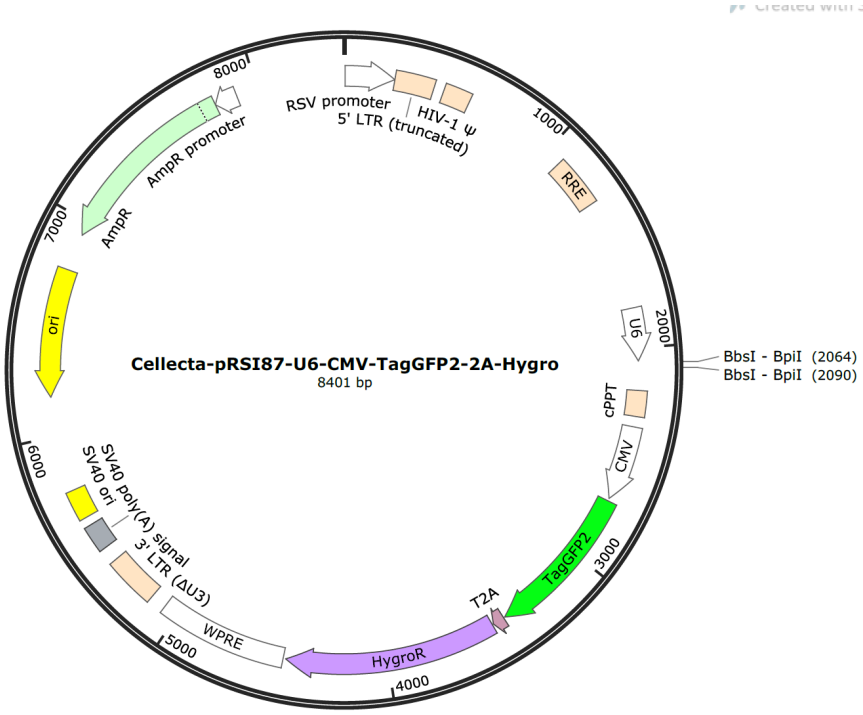
The company produced **replication-incompetent lentiviral vectors.** The genes for the viral proteins are distributed on **3 plasmids**: **Two packaging plasmids and a transfer plasmid** (with GFP gene). They are used to produce high amounts of the complete lentiviral vectors in HEK293T cells (see scheme below) which are then used to transduce the HUVECs. After transduction, the remaining vectors are washed away (as described in their mail).



Regarding the envelope-expressing plasmid (see below), they used another virus (VSV-g) than that which is contained in the transfer plasmid (HIV-1). This is called **pseudotyping**.

From their packaging plasmids (see below) we can also deduct that they are using **2nd or 3rd generation plasmids.**

Lentiviral Transfer plasmide:



Packaging plasmids

- **psPAX2**
  - 2nd generation packaging plasmid
  - contains Gag, Pol, Rev, and Tat
  - can be used with 2nd and 3rd generation transfer plasmids and envelope expressing plasmid
- **pMD2.G**
  - VSV-G envelope expressing plasmid

With this information, we can now classify our cells according to the **ZKBS guideline** step by step:

„Generation of lentiviral vectors:

4.14. If a transfer plasmid and at least two packaging plasmids of the 2nd, 3rd or newer generation, which are based on a lentivirus, are transfected into a cell line of risk group 1, the genetically modified organisms are to be assigned to risk group 2. The gene of the coat protein and the gag / pol gene must be present on separate packaging plasmids. Genetic engineering work with genetically modified organisms that meet the criteria mentioned can be compared with one another and assigned to security level 2.

4.15. Lentiviral vectors, which are delivered from the cell lines described under 4.14. are assigned to risk group 2. A recombination between the plasmids to replication-competent lentiviruses cannot be assumed when using packaging systems of the 2nd, 3rd or newer generation. Genetic engineering work with these vectors, including the transduction of further cells from risk group 1 and the inoculation of animals, can be compared with one another and is assigned to safety level 2.“

Together with
„Retroviral vectors with modified envelopes:

4.22. If the under 4.5., 4.6., 4.10., **4.14.** or 4.16. described generation of retroviral vectors, the retroviral env gene is replaced by the possibly modified gene of a coat protein of any virus (except unmodified proteins of an ecotropic murine γ-retrovirus) or if this gene is additionally expressed, the genetically modified organisms are to be assigned to risk group 2. Genetic engineering work with genetically modified organisms that meet the criteria mentioned can be compared with one another and assigned to security level 2.

4.23. Pseudotyped retroviral vectors with possibly modified envelope proteins of foreign viruses, which are delivered from those cell lines described under 4.22. are assigned to risk group 2, provided that the coat proteins are not exclusively unmodified proteins of ecotropic murine γ-retroviruses. Genetic engineering work with these vectors, including the transduction of further cells from risk group 1 and the inoculation of animals, can be compared with one another and is assigned to safety level 2.”

Together with
“Infection of cells with retroviral vectors:

4.27. **Cells of risk group 1, which were transduced or infected with the retroviral vectors described under** 4.9., 4.10., 4.12., **4.15.,** 4.16., 4.18., **4.23**. or 4.25., in which contamination with replication-competent retroviruses is not to be expected, **are to be assigned to risk group 1**, provided that the cells do not complement the replication defect and the cells no longer adhere to infectious retroviral vectors. Genetic engineering work with genetically modified organisms that meet the criteria mentioned can be compared with one another and assigned to security level 1.”