

Biosafety of lentiviral vectors and particles

OriGene's lentiviral vectors (shRNA and overexpression lentiviral vectors) are 3rd generation lentiviral vectors with improved biosafety. These safety features are discussed below.

- The pLenti expression vector is replication deficient as it contains SIN (Self Inactivation), a deletion in the 3' LTR (Δ U3). This SIN deletion does not affect lentiviral packaging, yet results in "self-inactivation" after integration into the transduced cell. The integrated lentiviral genome is no longer capable of self-replication.
- The number of genes from HIV-1 that are used in the 3rd generation lenti system has been reduced to three (*i.e.* *gag*, *pol*, and *rev*). TAT, an essential gene for viral replication is eliminated.
- A constitutive promoter (CMV promoter) has been placed upstream of the 5' LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA.
- The packing plasmids (Gag, pol, rev and VSV-G) supporting lentiviral packaging are separated onto three plasmids; none of them contain LTRs or the Ψ packaging sequence. Only the lenti vector for gene overexpression or shRNA knockdown contains the packaging signal that can be packaged into viral particles.
- The VSV-G gene from Vesicular Stomatitis Virus is used as the pseudo-envelope, further reducing the chances of recombination with viral genome.
- The Woodchuck Hepatitis Virus (WHV) Posttranscriptional Regulatory Element (WPRE) is a regulatory element which is *transcribed, but not translated*. When WPRE is inserted in the 3' untranslated region (UTR) of a mammalian expression cassette, it can significantly increase mRNA stability and protein yield for genes delivered by viral vectors. Due to a lack of WPRE *protein expression*, the oncogenic potential is minimized/not present

Despite the safety features discussed, lentiviral particles produced with OriGene's lenti system can still pose some biohazard risks since the particles can transduce primary human cells. All lentiviral particles provided by OriGene are prepared from HEK293 cells under the **BSL-2** conditions using OriGene's 3rd generation packaging kit.

In order to control the risks with OriGene's lentiviral particles, **we highly recommend that you treat all lentiviral particles as Biosafety Level 2 (BSL-2) organisms and strictly follow the published BSL-2 guidelines with proper waste decontamination.** Furthermore, lentivirus carrying potential harmful or toxic genes (*e.g.* activated oncogenes) requires higher levels of protection such as BSL-2 enhanced containment.

For more information on biosafety levels, please read the NIH Biosafety Guidelines.