

Safety and handling of shRNA^{mir} viral vectors

The Expression Arrest™ shRNA^{mir} viral vectors are **self inactivating (sin)** expression vectors. The viral vectors contain a specifically designed long terminal repeat (LTR) derived from the native virus. These LTR's differs from the native by several point mutations and a deletion, enhancing transcriptional activation and decreasing transcriptional suppression in embryonic stem and embryonal carcinoma cells.

Self inactivating vector are constructed by deleting the enhancer and/or the promoter in the U3 region of the 3' LTR. During reverse transcription, a circular intermediate is formed that transfers the deletion to the 5' LTR of the pro-viral DNA. The deletion abolishes any transcriptional activity driven by the LTR so that no full-length vector RNA is produced in transduced cells. Following a single round of replication, the changes are copied into both 5' and 3' LTRs resulting in inactive provirus. However, any promoter(s) internal to the LTRs in such vectors will still be active. This strategy has been employed to eliminate effects of the enhancers and promoters in the viral LTRs on transcription from internally placed genes.

The Expression Arrest™ shRNA^{mir} viral vectors are infectious **only** when packaged in a cell line with appropriate tropism but is not replication competent. Virus produced by both transient and stable transfections can infect target cells and transmit target genes; however, it cannot replicate within target cells because the viral structural genes are absent.

Viral packaging

To develop a packaging cell line, the viral gag, pol and env genes- necessary for particle formation and replication- are stably integrated or co-transfected into the genome of the packaging cell line. The separate introduction and integration of the structural genes minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation. Viral expression vectors provide the packaging signal, transcription and processing elements, and a target gene. Transfection of the retroviral vector into a packaging cell line produces high-titer, replication-incompetent virus.

The protocols for viral cell packaging require the producing, handling and storing of infectious retrovirus. An understanding of safe laboratory practices and potential retroviral hazards is necessary. Appropriate NIH, regional, and institutional guidelines apply, as well as specific guidelines for other countries. In the United States, NIH guidelines require that retroviral production and transduction be performed in a Biosafety Level 2 (BL2) facility. More information about BL2 guidelines is available at <http://bmbi.od.nih.gov/contents.htm>

Note: Viral supernatants produced using retroviral packaging cells, depending on your retroviral insert, may contain potentially hazardous recombinant virus. All users must exercise caution in the production, use and storage of recombinant virus, especially those with amphotropic or dualtropic host ranges.

References

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- (3) Morgenstern JP and Land H. (1990). Advanced mammalian gene transfer: High titer retroviral vectors with multiple drug selection markers and a complementary helper free packaging cell line. *Nucleic Acids Research.* 18:3587-3596