

## How to Complete a Risk Assessment of Lentiviral Experiments in Animals and Cell Culture

1. **Organism or Agent:** LENTIVIRUS VECTORS
2. **Synonym:** Retroviruses
3. **Characteristics:** single stranded RNA virus, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase
4. **Containment Requirements:** Depends on agent: Consider biosafety level 2 practices, containment equipment and facilities for all activities involving the manipulation of the virus; primary containment devices and biological safety cabinets are recommended. Centrifuge safety precautions, secondary containers for transport between incubator and BSC. Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective.
5. **Manipulations of Lentivirus:** Depending on the vector, work may need to be performed within a biosafety cabinet, and the use of sharps including needles, blades and glassware should be minimized.
6. **Spills:** Allow aerosols to settle; wear protective clothing including an N95 respirator, gently cover spill with paper towel and apply disinfectant, starting at perimeter and working towards the center; allow sufficient contact time before clean-up (30 min).
7. **Biohazardous Waste:** Collect in double red bags and transport in a rigid container.
8. **Approved Disinfectants:**
  - a. 0.05% Sodium Hypochlorite (1:10 bleach/water) allow 10 minutes of contact time.
  - b. Stabilized prediluted bleach (preferred to lab prepared bleach)
  - c. Other HIV-approved hospital disinfectants
  - d. Alcohols are not acceptable disinfectants.
9. **Disposal:** Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.
10. **Storage:** Store in sealed containers appropriately labeled with a biohazard label, description and contact information.
11. **Pathogenicity:** Hazards depend on multiple factors: whether the vector is capable of infecting human cells, whether the vector is replication competent, how many viral genes are contained in the vector, and the specific transgenes present in the vector. On an emergent basis, the PI should be the best source of information regarding potential health hazards.
12. **Modes of Transmission:** Virus may be transmitted in the following ways: 1) a skin puncture or injection, 2) ingestion, 3) contact with mucous membranes (eyes, nose, or mouth), 4) contact with non-intact skin, and 5) low risk exposures include bites from an animal inoculated with lentivirus, percutaneous contact with body fluids from an animal inoculated with lentivirus and aerosols.
13. **Length of gene expression:** Variable, may be months to years

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14. **Communicability:** Replication incompetent vectors: Not communicable.
15. **Medical surveillance and clinical treatment procedure:**  
A clinical operating procedure for Lentivirus exposure is required.
16. **Safety Generation of Lentivirus Vector:** State which generation (usually 3<sup>rd</sup> or 4<sup>th</sup>) vector is being used. Information can be found in manufacture's documentation. First or second generations vectors are not permitted.
17. **Stability in Environment:** Drying in environment causes rapid (within several hours) 90-99% reduction in virus concentration. Half-life at 37°C is approx. 24 hours.
18. **Vector concentration, dosage per experiment:** State your stock vector concentration, and the amount used per experiment or kg.
19. **Vector shedding from animals:** Animals dosed intravenously may shed virus for up to 72 hours; animals dosed intracranially may shed for up to 24 hours.
20. **Transgene Information:** Discuss effects of transgene on animal or cell line. A good source for understanding the transgene being silenced or over-expressed is GENE CARDS (<http://www.genecards.org/>). A snapshot of a sample gene card is shown below:

The screenshot shows the GeneCards website interface. At the top, there is a navigation bar with links for Home, GeneCards Guide, Suite, Terms and Conditions, About Us, User Feedback, and Mirror sites. Below the navigation bar, there is a search bar and a section for 'Set Analysis' with buttons for 'GeneCards' and 'TumorCards'. The main content area displays the gene entry for TNFRSF10B, including its protein-coding status, GIIS score of 73, and its classification as a member of the Tumor Necrosis Factor Receptor Superfamily. A list of related genes is provided, such as TRICK2BP, TRICK2P, TRICK2L1, TRICK2L2, TRICK2L3, TRICK2L4, TRICK2L5, TRICK2L6, TRICK2L7, TRICK2L8, TRICK2L9, TRICK2L10, TRICK2L11, TRICK2L12, TRICK2L13, TRICK2L14, TRICK2L15, TRICK2L16, TRICK2L17, TRICK2L18, TRICK2L19, TRICK2L20, TRICK2L21, TRICK2L22, TRICK2L23, TRICK2L24, TRICK2L25, TRICK2L26, TRICK2L27, TRICK2L28, TRICK2L29, TRICK2L30, TRICK2L31, TRICK2L32, TRICK2L33, TRICK2L34, TRICK2L35, TRICK2L36, TRICK2L37, TRICK2L38, TRICK2L39, TRICK2L40, TRICK2L41, TRICK2L42, TRICK2L43, TRICK2L44, TRICK2L45, TRICK2L46, TRICK2L47, TRICK2L48, TRICK2L49, TRICK2L50, TRICK2L51, TRICK2L52, TRICK2L53, TRICK2L54, TRICK2L55, TRICK2L56, TRICK2L57, TRICK2L58, TRICK2L59, TRICK2L60, TRICK2L61, TRICK2L62, TRICK2L63, TRICK2L64, TRICK2L65, TRICK2L66, TRICK2L67, TRICK2L68, TRICK2L69, TRICK2L70, TRICK2L71, TRICK2L72, TRICK2L73, TRICK2L74, TRICK2L75, TRICK2L76, TRICK2L77, TRICK2L78, TRICK2L79, TRICK2L80, TRICK2L81, TRICK2L82, TRICK2L83, TRICK2L84, TRICK2L85, TRICK2L86, TRICK2L87, TRICK2L88, TRICK2L89, TRICK2L90, TRICK2L91, TRICK2L92, TRICK2L93, TRICK2L94, TRICK2L95, TRICK2L96, TRICK2L97, TRICK2L98, TRICK2L99, TRICK2L100. The page also includes external IDs, aliases, and a list of database identifiers.

To better understand potential human outcomes from accidental silencing, you can see if information exists in the Mouse Genome Informatics (<http://informatics.jax.org/>)

You must discuss the potential effects due to accidental worker exposure. If unknown, state that. Is the gene sequence or siRNA specific to an animal, humans or could it affect both.

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## How to Complete a Risk Assessment of Lentiviral Experiments in Animals and Cell Culture

### **Derogation of Transfected Cells to Lower Biosafety Classification:**

Lentivirus post-transduced cells may be removed for downstream assays in BSL 1 laboratories provided the cell line meets the following criteria (Dutch COGEM advisory CGM/090331-03 “Grading of Laboratory Work with Lentiviral Vectors.”):

$$\text{Reduction ratio} = (20W \times 200I \times 2^{2.4T})/C_i$$

In this formula *W* is the number of wash steps, (*I*) the number of loss-of-function wash steps with trypsin and *T* stands for the growth time in days after transduction. The factor 2.4 is based on the half-life of a lentivirus vector at a temperature of 37°C. The *C<sub>i</sub>* is the original number of virus particles in the inoculum. For example, a virus transfection experiment using 10<sup>6</sup> virus particles which requires a single wash and trypsin resuspension step occurring over 7 days at 37°C. The virus reduction ratio, compared to the starting virus concentration is:

$$20(1) \times 200(1) \times 2^{2.4(7)} / 1E6 = 456\text{-fold}$$

The reduction is 456 times less than the starting concentration. The cell line may be handled at RG-1. This risk group reduction methodology is only available for lentivirus vectors.

**Animal Biosafety and Containment:** Following transfection, animals are held at ABSL-2 for 72 hours. Following a cage change (performed at BSL-2), the animals may be housed at ABSL-1.