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Biosafety assignment for lentiviral vectors

The use of lentivirus vector systems is becoming more prevalent in research, especially with the development of later generation systems that have reduced biosafety concerns. This document serves as general guidance on biosafety levels for *in vitro* and *in vivo* work with lentivirus.

Note that the IBC must review each individual project involving viral vectors to determine the appropriate biosafety level prior to initiating work with the vector. In all cases, additional biosafety precautions may be recommended.

System	Parameters of use	*Biosafety Level*	Special Requirements
In vitro **Lentivirus- 2nd or 3rd generation***	Non-oncogenic insert	BSL-2	
	Insert: -has oncogenic potential -expresses biological toxin[1]	BSL-2+	Special practices as outlined in the BSL-2+ template biosafety manual or as determined by a Biosafety Officer risk assessment.
	All inserts: Production scale	BSL-2+	- Any insert with a production scale > 500 ml of unconcentrated virus - Special practices as outlined in the BSL-2+ template biosafety manual or as determined by a Biosafety Officer risk assessment.
In vivo **Animal models other than non-human primates** **Lentivirus- 2nd or 3rd generation***	All inserts: Direct injection -or- Non-human cells infected *ex vivo*	ABSL-1	-Injection should take place using BSL-2 containment and practices, and needle protective devices should be used for injection procedures -After injection of animals, the injection site should be cleansed with 70% ethanol and then animals should be placed into a secondary container without bedding. Once injection site is dry, animals can be returned to original cage(s) and returned to the ABSL-1 animal facility. The secondary container should also be cleaned with 70% ethanol.
	All inserts: Human cells infected *ex vivo* –	ABSL-2	If lentiviral vectors are used to infect human cells which are subsequently injected into animals.
In vivo **Non-human primates**	All inserts: Lentivirus - 2nd generation	ABSL-2+	Must always have RCV[2]
	All inserts: Lentivirus - 3rd generation	ABSL-2	Must always have RCV[2]

*The IBC always recommends testing for replication competent virus (RCV) in the vector stock.

Guidance on use of 2nd or 3rd generation lentivirus vector systems[3]

The following are typical characteristics of 2nd and 3rd generation systems. Unique/novel systems will be reviewed by the IBC to determine the appropriate safety precautions.

2nd Generation Systems

- Uses two helper plasmids to separate the packaging and gene transfer functions in addition to the plasmid containing your gene of interest
    - Plasmid with Gag, Pol, Rev, and Tat and plasmid with envelope protein
- May either express the HIV envelope protein or may be pseudotyped with the vesicular stomatitis virus G (VSV-G) protein
- May or may not be self-inactivating

3rd Generation Systems

- The IBC recommends the use of 3rd Generation Systems for safety purposes
- Uses three or more plasmids to separate the packaging and gene transfer functions in addition to the plasmid containing your gene of interest
    - Plasmid with Gag and Pol, plasmid with Env (VSV-G), and plasmid with Rev
- Does not include Tat
- Typically pseudotyped to express VSV-G
- Is self-inactivating

Footnotes:

[1]link to biological toxin definition on IBC website: http://www.ohsu.edu/xd/research/about/integrity/ibc/#agentToxin

[2] Links to RCV testing protocols and references are available at: http://www.ohsu.edu/xd/research/about/integrity/ibc/protocols.cfm
There are labs at OHSU who routinely perform p24 ELISA tests. You may contact one of these labs for assistance:
- Dr. Ashlee Moses, mosesa@ohsu.edu
- ONPRC Virology Core - CoreyAyne Singleton, singletc@ohsu.edu or call 503-690-5568
or Greg Dissen, disseng@ohsu.edu or call 503-690-5382

[3]For additional information regarding the safety of Lentiviral vectors, please see:

- NIH guidance: http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html
- State-of-the-Art Lentiviral Vectors for Research Use: Risk Assessment and Biosafety Recommendations. 2009. Current Gene Therapy Vol 9(6): 459-474