VIRAL VECTORS FOR GENE TRANSFER

The table below lists the most common viral vectors used for gene transfer and the required biosafety levels. Technology for gene delivery is constantly changing. We welcome your feedback to help keep this information current.

Viral vector	Biosafety Level	Replication Competent Virus (RCV) Testing
Adeno-associated virus (adenovirus-free)	BSL-1	▪ Adeno-associated virus vector stocks generated with adenovirus-free packaging systems can be handled at a BSL-1 level, and no further testing is needed for studies in mice. ▪ Reference for adenovirus free packaging system: *Allen JM, Halbert CL, Miller AD. 2000. Improved adeno-associated virus vector production with transfection of a single helper adenovirus gene, E4orf6. Mol Ther 1:88-95.*
Adeno-associated virus (with adenovirus)	BSL-2	▪ Test for the presence of replication-competent adenovirus after heat-inactivation. ▪ Reference for a RCV assay: *Hehir KM, Armentano D, Cardoza LM, et a1. 1996. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. J Virol 70:8459-8467.*
Adenovirus	BSL-2	▪ First, the vector must contain less than 2/3 of the wild-type genome; currently this only includes what are known as "gutless" vectors. ▪ Second, the vector stocks must be tested for RCV by PCR for E1a prior to use. The vector stock should be tested at a limit of sensitivity of 1 in less than 10^6 virus particles compared to a known positive control and the results of the test must be available upon request. ▪ For murine studies, if vectors are not gutless but have tested negative for RCV, the vector can be administered at BSL-2 and the animals held for at least one hour prior to a cage change and return to standard ABSL-1 housing. ▪ Reference for E1a PCR assay: *Zhang WW, Kock PE, Roth JA. 1995. Detection of wild-type contamination in a recombinant adenoviral preparation by PCR. Biotechniques 18: 444-447*.
Foamy virus (replication competent)	BSL-2	Not applicable

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Foamy virus (replication defective)	BSL-1	▪ Foamy virus vector stocks generated with packaging systems shown to be free of RCV by a marker rescue assay can be used in mice and handled at a BSL-1 level without further testing. ▪ Results should be <1 infectious units/mL. ▪ Foamy virus vectors which are replication-competent must be handled at a BSL-2 level. ▪ Reference for a marker rescue assay: *Trobridge GD, Russell DW. 1998. Helper-free foamy virus vectors. Hum Gene Ther 1998 9:2517-2525.*
Gammaretrovirus (ecotropic pseudotype)	BSL-1	▪ Gamma retrovirus vectors pseudotyped with an ecotropic envelope (one which allows for the transduction of mouse cells but not human cells) and generated by direct plasmid transfection can be handled at a BSL-1 level. There is no requirement that the vector stocks and/or producer lines be tested prior to use in mice; however, a marker rescue assay for RCV is recommended as part of any experimental design. ▪ Ecotropic producer cells generated by transduction with non ecotropic pseudotyped producer cells must be handled at a BSL-2 level until demonstrated to be free of RCV by a marker rescue assay.
Gammaretrovirus (other pseudotype)	BSL-2	▪ Gammaretrovirus vectors with pseudotypes other than ecotropic must be tested for RCV by a marker rescue assay prior to being approved for use at BSL-1/ABSL-1. ▪ The vector stock or producer line should be tested at a limit of sensitivity of 1 infectious unit/mL and should include a known positive control. ▪ Reference for a marker rescue assay: *Miller AD, Buttimore C. 1986. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. Mol Cell Biol 6:2895-2902.*
Herpes virus amplicons	BSL-2	▪ Herpes virus vectors based on attenuated herpes virus must always be handled at a BSL-2 level. ▪ Herpes virus generated using amplicons must be tested for RCV by a plaque assay prior to being approved for use at BSL-1/ABSL-1. The vector stock should be tested at a limit of sensitivity of 1 infectious unit/mL and include a known positive control.

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Herpes virus amplicons *(continued)*		▪ Reference for a plaque assay: *Strathdee CA, McLeod MR. 2000. A modular set of helper-dependent herpes simplex virus expression vectors. Mol Ther 5:479-485.*
Lentivirus (non-HIV pseudotyped)	BSL-2	▪ Lentivirus vector stocks generated with packaging systems devoid of the HIV envelope gene must be tested for RCV by serial transfer in a cell line documented to be capable of supporting wildtype HIV and ELISA assay for p24 antigen prior to approval for use at BSL-1/ABSL-1. ▪ The vector stock should be tested at a limit of sensitivity of 1 infectious unit/mL. Test a volume of at least 1 mL (or equivalent if viral stock is concentrated). ▪ Lentiviral vector systems with HIV envelope sequences require BSL-2 with BSL-3 practices at a minimum. ▪ Strictly adhere to the published protocol: *Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. 1998. A third-generation lentivirus vector with a conditional packaging system. J Virol 72: 8463-8471.* ▪ See example lentiviral vector RCV protocol.
Vaccinia virus	BSL-2	▪ Not applicable

RCV Testing to Lower Containment

In general, vector stocks and/or producer lines must be tested for replication competent virus (RCV) prior to approval for use at a lower biocontainment level. The following steps are required to lower containment for viral vector use:

1. Contact EH&S at ehsbio@uw.edu or 206.221.7770 to determine if RCV testing to lower containment is possible for the desired work.
2. Complete the RCV test referenced above for vector type.
    a. Submit a complete Request for Change to Biological Use Authorization (BUA) with the testing results attached to EH&S ROS. Include the test protocol or reference to published protocol, vector name, batch number, and results for samples including controls
3. EH&S and the IBC chair will review and confirm that results that meet the testing requirements. A new BUA letter will be issued to reflect the change.

Questions?

Contact EH&S for assistance at ehsbio@uw.edu or 206.221.7770.