TITLE:

GUIDELINES FOR WORKING WITH REPLICATION INCOMPETENT ADENO-ASSOCIATED, LENTIVIRAL AND ADENO VIRAL VECTORS IN LAB AND ANIMAL RESEARCH

This document provides essential information for Research Investigators regarding work with human Adenovirus, Adenovirus vector systems, Adeno-Associated Virus (AAV) vector systems and Lentivirus in the lab and in animal models. Use of all viral vectors requires approval by Emory University's Institutional Biosafety Committee (IBC).

Adeno- Associated Viral Vectors:

Adeno-Associated viruses are in the Parvoviridae family of viruses and are considered to be non-pathogenic to humans even though the virus will integrate in the host genome.

Appendix B-I of the NIH Guidelines assesses Adeno-Associated Virus (AAV) and recombinant AAV (rAAV) as Risk Group 1 agents. RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include adeno-associated virus (AAV - all serotypes) and recombinant or synthetic AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the ***absence of a helper virus.***

Biosafety Level Required to work with AAV and rAAV:

To ensure the ongoing protection of our research staff with the increasing use of viral vectors we have adopted the following guidelines for work with AAV vectors.

ABSL1 requirements for the use of AAV and rAAV, ***only if all three requirements are met*:**

- Transgene does not express an oncogenic protein or toxin.
- AAV/rAAV is generated without using adenovirus or any other helper virus of human origin.
- AAV/rAAV is propagated in insect cell lines or is purified sufficiently before use. The method and assessment of purification needs to be documented.

BSL2/ABSL2 **must be** used when working with AAV and rAAV:

- Transgenes express an oncogenic protein or toxin.
- Helper virus of human origin is used to generate AAV/rAAV.
- AAV/rAAV is propagated in human cell lines without further purification before use.

Adeno and Lentiviral vectors:

Replication-incompetent lentiviral and adeno viral vectors should be used in at BSL2 conditions in laboratories. Due to their replication-incompetent status and inability to replicate (even in wild-type form) in rodents, long-term use in infected rodents within animal biosafety 1 (ABSL1) conditions is generally considered acceptable. However, the method by which animals are transferred from animal biosafety level 2 (ABSL2) following surgery, a time in which they may still have infectious virus on their wound or body secretions that could be transmitted to research staff, and when they can be 'stepped-down' to ABSL1 conditions requires clarification.

To ensure the ongoing protection of our research staff with the increasing use of viral vectors we have adopted the following guidelines for work with lentiviral and adeno viral vectors. These guidelines were based upon the NIH Recombinant DNA Advisory Committee (RAC) Guidance Document on Research with Lentiviral Vectors (document

can be viewed at the NIH lentivirus Guidance). Unless otherwise specifically approved by the Institutional Biosafety Committee (IBC) of Emory University, the use of lentiviral- and Adenoviral-based viral vectors for gene delivery in animal models requires the following procedures:

NOTE: Guidelines pertain only to replication-incompetent lentiviral vectors produced with established 3 and 4-plasmid systems or Adenoviral vectors produced without helper virus that pose minimal risk of replication-competent virus for the purpose of gene transfer into animals.

- The initial delivery of viral vector should be performed under laboratory BSL2 conditions, and animals should be housed in ABSL2 conditions in filter top cages and in designated containment areas.
- **Transfer of recipient animals from laboratory to the animal research facility containment site must be performed using filter top cages.**
- Once the period of potential infectivity is over, the containment level can be reduced to ABS1 provided this has been requested and approved by IBC and where the following procedures are involved:
    - Following surgical infection with the lentivirus or Adenoviral vectors, animals are housed in ABSL2 conditions for at least 72 hours following infection.
    - The ABSL2 conditions will be arranged with the director or designee of DAR and the veterinary staff at each research facility. These will typically be provided as a temporary, quarantine-type ABSL2 cubicle that the animals will be held in during the 72 hour period. In special cases in arrangement with vet staff, specified ABSL2 containment animal racks may be used within an otherwise ABSL1 designated vivarium room.
    - There will be specific signage/labeling on each ABSL2 cage stating ‘ABSL2 Biohazard Containment-Quarantine for Lentiviral (or Adenoviral) Vector Research’, and these cages will not be allowed out of the ABSL2 containment space.
    - On the fourth day following infection, animals can be transferred to ABSL1 standard conditions. The animals will be transferred to a clean cage, and the ABSL2 cage will stay in the ABSL2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL 1, they can be used within ABSL 1 behavioral facilities, etc. as with other ABSL1 animals.