

## View Agent Approval Form

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PI	[REDACTED]
Department	[REDACTED]
Investigator	(none)
Investigator	(none)
Investigator	(none)
Status	<b>Approved</b>
Date of original submission	2010-02-02
Date of initial approval	2010-02-10
Date of most recent approval	2010-02-10
Date of expiration	2011-02-10
Agent	<b>Adenovirus</b>
Strains	subtype 5 deletion mutant (Ad5dl309) and AdEasy vector
Human risk category	2
Vaccine available for people?	N
Vaccine used for personnel	N
Pathogenic for animals?	Y
Pathogenic for plants?	N
Antibiotic resistant?	N
CDC or USDA permit required?	N
Select agent?	N
Largest unconcentrated volume > 10 liters?	N
Will organism be radiolabeled	N
Planned release of the agent into the environment?	N
Methods of inactivation	Autoclave and chemical (Coverage plus viricidal)

### Overview of how agent will be used

Normal morphological development of tissues during embryogenesis requires precise coordination of multiple cellular events such as cell migration and cell differentiation. Aberrant patterning of the nervous system results in severe congenital human diseases such as spina bifida. Prevention or treatment of these diseases requires an understanding of the machinery that controls neural patterning. The actin cytoskeleton is a fundamental cellular scaffold which dictates cell morphology and multiple important dynamic cell processes.

Cofilin is a member of a family of important actin assembly-regulatory proteins. Inactivation of cofilin-1 in mice results in neural tube closure defects, impaired neural crest migration and abnormal neuronal differentiation. The molecular and cellular basis of these developmental defects remains to be determined. We hypothesize that Wnt PCP signaling is coupled to cofilin regulation during vertebrate neural crest development. In this proposal we aim to identify means by which Wnt signaling and cofilin govern neural crest patterning. We will use the chick as a model system to determine what Wnt activity controls neural crest migration in this vertebrate. Neural crest migration will be directly assessed in situ and in vitro by time lapse microscopy following specific inhibition of non canonical Wnt signaling through expression of dominant negative signaling intermediates or pharmacological inhibition. We will differentiate roles for canonical Wnt signaling versus non

canonical Wnt PCP and Wnt Calcium signaling pathways in neural crest patterning. We will identify Wnt ligands that stimulate neural crest migration and establish their cellular and molecular mechanisms. Cofilin activity in neural crest cells will be evaluated in response to treatments that either disrupt or stimulate Wnt PCP signaling. The role of cofilin and Wnt signaling on neural crest cell migration will be evaluated through 4D fluorescence confocal microscopy live cell imaging. We will analyze migration in whole chick embryos and neural crest explant cultures.

### In vitro uses

In vitro BSL 2

**Buildings for in vitro use** [REDACTED]

**Rooms for in vitro use** [REDACTED]

**Procedures used to protect personnel from exposure to agent during in vitro studies**

BL-2: [REDACTED] has been approved as a BL-2 facility for generating and working with the recombinant bacterial plasmids and adenovirus in cell culture. The room is so labeled. It contains appropriate biological safety cabinet, centrifuge for viral work only, incubators for growing the cells, appropriate waste disposal facilities, and lab coats, gloves and eye protection that are worn when any viral work is performed. Adenovirus will be used

only in tissue culture. All personnel receive appropriate training on potential hazards and the necessary precautions. Eating, drinking, and other activities, which place individuals at risk, are not allowed in any laboratories in the building, including the BL-2 labs. Gloves, protective eyewear, and lab coats will be worn. All pipetting is done using mechanical pipettors. Solid waste of all types will be autoclaved prior to disposal. Liquid wastes are treated with 3% Coverage Plus or bleach to inactivate the virus prior to sewage disposal.

Cell culture work is done in a certified biological safety cabinet (service and certification is done annually). As is currently done for all work with human cell lines, solid waste of all types is autoclaved prior to disposal. Liquid wastes are processed with 3% Coverage Plus or 10% bleach prior to sewage disposal. No sharp objects of any kind are allowed in the biological safety cabinet under normal working conditions to prevent contamination by

puncture wounds. In the event of an accidental spill, the area will be immediately decontaminated with Coverage Plus (3%). All contaminated materials will be autoclaved. Prior to autoclaving contaminated materials, they are kept in a leakproof container in autoclavable bags. Both the container and bags are

clearly labeled as containing biohazardous materials. Janitorial staff will be notified of any changes in container location or type to avoid any accidental removal of materials [REDACTED] is labelled as BL-2 with indication that adenovirus is an infectious agent. This room has controlled access and only authorized workers who have been informed of the biohazard use this room.

**In vivo uses**

None

**Original IBC Review**

The CSU Biosafety Committee has reviewed your agent application for:

- Adenovirus
- In vitro at BSL 2

This application was APPROVED AS SUBMITTED.

The IBC greatly appreciated the level of detail provided in this Approval Request and would like to save it as an example for future applicants.

If you have any questions regarding this approval please contact Christine Johnson at 491-8690 or Chrstine.Johnson@Colostate.Edu.

Thank you,

Christy

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