

# Biological Materials and Recombinant DNA Protocol

Return completed form to:

**The Office of Research Compliance**  
**203 Foust Building**

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## Instructions for Completing Biological Materials and Recombinant DNA Protocol

The Office of Research Compliance requests submission of biosafety protocols for research activities involving:

- microbiological agents infectious to humans and/or animals; provide a copy of any required federal permit.
- exotic plants, animals, and microbes (e.g., nonindigenous plant or insect pathogen, or biological control agent); provide a copy of any required federal permit.
- carcinogens, mutagens, drugs, and toxins when administered *in vivo* or *in vitro* to induce a biological outcome.
- recombinant DNA molecules and recombinant DNA-containing organisms or cell cultures which are subject to the NIH *Guidelines for Research Involving Recombinant DNA Molecules* and USDA APHIS Guidelines.

This form provides the Institutional Biosafety Committee a detailed description of the research elements and their management, with emphasis on containment practices, and provides a basis for risk assessment. A single biosafety protocol may cover multiple grant submissions. Once registered and assigned a safety committee number (SC#), the protocol is valid for three years.

**Minor changes** to the biosafety protocol involve administrative information that does not alter the risk assessment. Examples of minor changes are the addition of grant applications that use the same materials and methods as the existing protocol and contact information for the principal investigator. Minor changes may be submitted by providing the first page of the protocol form.

**Major changes** are those that may alter the risk assessment. Examples of major changes include the addition or deletion of biological materials or methods, and a change in the location of the research facilities. This information may be submitted on the original protocol form.

Sections IV and V cover the use of recombinant DNA molecules and potentially biohazardous components of your research project. Skip sections that do not apply. In Section V, please provide an overview of the project and a detailed description of the practices employed in the management of biohazardous elements; discuss safety aspects of facility, containment equipment, personnel practices, and staff training that will ensure safe conduct of the investigation.

## References

Biosafety in Microbiological and Biomedical Laboratories. CDC/NIH. 4<sup>th</sup> edition, May 1999.  
<http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>

Laboratory Standard. 1990. Department of Labor, Occupational Safety and Health Administration. 29 CFR, Part 1910.1450. Federal Register Vol. 55, No. 21.

NIH *Guidelines for Research Involving Recombinant DNA Molecules*. Revised January 1 and subsequent amendments. National Institutes of Health. <http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

*Proposed Guidelines for Research Involving the Planned Introduction into the Environment of Organisms with Deliberately Modified Hereditary Traits*. 1991. USDA. Federal Register Vol. 56, No. 22.

# Biological Materials and Recombinant DNA Protocol

Institutional Biosafety Committee

## I. CORE REGISTRATION INFORMATION

Name of Principal Investigator (PI): S. Snape, M.D.  
 Job Title: Professor  
 Office Phone: \_\_\_\_\_ Lab Phone: \_\_\_\_\_ Fax: \_\_\_\_\_  
 Department: Pharmaceutical Sciences Box #: \_\_\_\_\_  
 Campus Address: Hogwart's Hall, Rm. B110  
 (Bldg, Rm #, Street)  
 Email Address: ssnape@uncg.edu  
 Name of Co-PI(s): n/a

Protocol Type	Applicable Registration Number & Review Date	
	Amendment or Renewal SC#	Review date
New, Amendment, Renewal, Training or Centers		
<u>New</u>		

General Protocol Title: Molecular mechanisms of NEWT signaling

Grant Title(s):	Granting Agency(s):	Grant #:
<u>EYE of NEWT: a Novel Enhancer of Wnt1 Transduction</u>	<u>NIH</u>	<u>Application pending</u>

Check this box if you have designated any portions of this protocol as confidential to protect proprietary or patentable information.

		<b>APPROVED:</b>	
Signature of Principal Investigator	Date	Chair, Institutional Biosafety Committee	Date

## II. RESEARCH FACILITIES

### Location:

Where are experiments performed? Is there anything unique about the location that allows the use of special precautionary measures such as an autoclave, containment facilities or biological safety cabinets? **[NOTE: The OFFICE OF RESEARCH COMPLIANCE requires prior notification, via written amendment to this protocol, regarding any change in location.]**

Building Name	Room number	Use of Room	Containment Equipment (autoclave, biosafety cabinet, fume hood, clean bench, other)
Hogwart's Hall	B106	General lab procedures	fume hood
" "	B111	Cell culture	biosafety cabinet, type IIA (Baker SG603). Last certified 03-21-06
" "	B114	Utility	autoclave
Other			

## III. LABORATORY/ADMINISTRATIVE PERSONNEL

List personnel involved with work covered under this research registration; include lab personnel: investigators, students, and research staff. Please mark (asterisk) the lab supervisor or administrative coordinator who should be contacted for information about this protocol.

Last name/First name	Job Title	Phone number
Snape, Severus	Principle Investigator	
Granger, Hermione	Graduate student	
Potter, Harry	Graduate student	
Weasley, Ginny	Undergraduate student	

## IV. RESEARCH ELEMENTS (Skip sections that do not apply)

**A. Recombinant DNA** subject to the NIH *Guidelines for Research Involving Recombinant DNA Molecules and / or USDA APHIS Guidelines.*

**Describe the rDNA constructs below in Section V. "Research Protocol Description".** Include a description, in molecular terms (e.g. promoter[s], ORFs, selectable markers), of the rDNA construct and provide a map if available. It is not necessary to provide details about every construct; categorical descriptions that are useful in assessing risks are acceptable. Describe the method of transfer or transfection. Describe measures taken to prevent or minimize expression of pathogenic/infectious sequences. Please avoid or explain acronyms. .

### A(i) Gene Source(s)

Gene Source(s) (Genus, species, strain)	Gene Name Explain acronyms (e.g. GFP - green fluorescent protein)	Nature of Insert or Protein expressed (Toxin, marker trait, virulence factor, DNA repair gene, oncogene, transcription factor, etc.)	Use of Construct Cloning for sequencing, PCR Expression in a microbe Expression in OTCC Expression assoc. w/ Organism
<i>Human herpes virus 6</i>	NEWT – novel enhancer of WNT1 transduction <sup>1</sup>	Signaling protein	Expression in cell lines
<i>Homo sapiens</i>	Axin <sup>2</sup>	Signaling protein	Expression in cell lines
<i>Homo sapiens</i>	Beta-catenin <sup>2</sup>	Signaling protein	Expression in cell lines
<i>Homo sapiens</i>	Promoter of c-myc binding protein (MBP)	Wnt—responsive gene	Expression in cell lines
<i>Aequorea victoria</i>	CFP and YFP (cyan and yellow fluorescent proteins)	Fluorescent proteins	Expression in cell lines
<i>Renilla reniformis</i>	luciferase	Luminescent protein	Expression in cell lines

<sup>1</sup> This gene was cloned by PCR from a non-infectious fragment of the HHV6 genome obtained from NIH and is in a eukaryotic expression plasmid (pSI vector, Promega)

<sup>2</sup> These genes will be obtained by RT-PCR from a human T-cell line and inserted in eukaryotic expression plasmids (see below)

### A(ii). Vector Description(s) Attach a construct map if a simple description is inadequate.

General description: The various genes of interest (NEWT, beta-catenin and Axin) will be cloned into either or both of the commercial fluorescent protein vectors listed below, designed to express the proteins as fluorescent conjugates in transfected cells. The NEWT coding region will be modified by site-directed mutagenesis for additional studies. The MBP promoter region will be cloned into a Promega Renilla luciferase vector for studies of Wnt-dependent gene regulation by NEWT, and its modulation by various small molecules of interest.

Gene Transfer Method (Conjugation; liposome; electroporation, viral infection, CaPO <sub>4</sub> polyplexes, naked DNA uptake, etc.)	Vector Backbone Source (Bacterial plasmid, cosmid, phage, virus, synthetic, YAC, BAC, transposon, etc.) Include genus and species of source if applicable	Vector Technical Name Include commercial vendor if applicable (e.g., pLXSN - Clontech)
Chemical (Lipofectamine 2000)	Bacterial plasmids	pECFP-C1, pEYFP-C1 (Clontech), pHRG-B (Promega) and other commercial cloning, sequencing and expression vectors as appropriate

### A(iii). Guidelines Assessment

**Assess the appropriate physical and biological containment.** State the appropriate biological safety level(s). Support your assessment **of any unusual potential hazards** by citing the relevant subsection(s) of the current NIH *Guidelines* and/or USDA APHIS *Guidelines*.

The only significant biohazard associated with this work is the usual risk associated with working with human cell lines, which is addressed by the use of BSL-2 precautions and a type 2 biosafety cabinet.



**B. Microbiological Agents** Identify agents and mark (y/n) in appropriate categories\*. Include microbes used to propagate recombinant plasmids and vectors or produce foreign proteins as described in section IV.A. above.

Microbe Source (Genus, species, strain)	Human Pathogen*	Animal Pathogen*	Plant Pathogen*	Toxin Production*	Large Scale Production >10 liters*	Recipient of rDNA construct*
E. coli BL21, DH5alpha, possibly other strains						Y

**Exposure Prophylaxis** For each microorganism, consider the consequences of an accidental exposure, i.e., mucosal splash, inhalation, or inoculation, which might occur during experimental handling. Consider that organisms normally not pathogenic for healthy humans may become so when the natural barriers to infection are circumvented. Prepare a response procedure. It could be a simple matter of washing the wound with soap and water, or it could involve reporting to a health service. Is a particular antibiotic preferred and readily available? The exposure response plan should be posted in the laboratory. In the event of an accident, inform the Office of Safety.

Microbe	Exposure Response
E. coli	Lab has posted SOPs for accidents etc. Lab strain bacterial exposure/spill requires washing of exposed area and disinfection of spills with bleach solution, iodine/povidone, or similar agent.

**C. Organ, Tissue or Cell Cultures (OTCC)** Identify species source, passage, and mark (y/n) in appropriate categories\*.

OTCC Source (Genus, species, strain)	Technical Name of OTCC (e.g. 3T3NIH, HepG2)	Passage (primary, established, immortal)	Comment (transforming, oncogenic, helper)	Recipient of rDNA* (transient/stable)	Recipient of Microbe*
Homo sapiens	HEK 293T*	immortal		Transient & stable	
Homo sapiens	Jurkat, Clone E6-1	immortal	Source of human gene by PCR		

\* Other standard human cell lines, or possibly non-human mammalian cell lines such as MDCK or CHO cells may be used for this research if found necessary or preferable for attaining our research objectives. This will not alter the risk level of the work.

**D. Research Organisms - Vertebrates, Invertebrates, or Plants** Identify organisms and mark (y/n) in appropriate categories\*.

Organism (Genus, species, strain)	Recipient of rDNA construct* (germ line or somatic transformation)	Recipient of Microbe*	Recipient of OTCC*
N/a			

### E. Chemicals Administered to Vertebrates, Invertebrates, Plants or OTCC

Identify chemicals and mark (y/n) in appropriate categories\*.

Nature of Chemical (carcinogens, mutagens, drugs, pesticide or toxins, etc).	Chemical Name	Route of Admin. (IV, IP, etc.)	Highest Concentration Administered	Administered to Microbe*	Administered to OTCC*	Administered to Organism*
Antioxidants, hormones	e.g. lipoic acid, dexamethasone, others, all of low toxicity	In cell culture	10 uM	N	Y	N

### F. Disposal

Describe the method of disposal of hazardous substances (e.g., incineration, autoclaving, chemical disinfection). If chemical disinfectant is used, state kind and concentration. Is autoclave monitored with biological indicator (e.g., spore strips)?

Disposal Substance	Disposal method/procedure
Used mammalian cells and cell culture media	Liquid bleach at 1:9 dilution added, disposed of in sink after a minimum of 30 mins.
Used culture plates, plasticware, pipettes, etc.	Autoclaving; autoclave is monitored periodically, not every cycle

**Chemical Hygiene Plan** Does your laboratory have a Chemical Hygiene Plan?  
If **no**, please contact the Office of Safety.

YES:   X   NO:           

## V. RESEARCH PROTOCOL DESCRIPTION

### A. Design and objectives

Briefly describe the experimental design and research objectives, tying together the research materials described above.

Wnt ligands are highly expressed in tumors, and the Wnt pathway may be exploited by some viruses. Wnt stabilizes cytoplasmic beta-catenin and activates beta-catenin/LEF-1 (lymphoid enhancer factor)-dependent gene transcription.

In the genome of human herpes virus 6 (HHV6), we previously identified a new virally-encoded modulator of the Wnt pathway, NEWT (Novel Enhancer of Wnt Transduction), a small 12 kDa protein. NEWT is a positive regulator of beta-catenin in the Wnt pathway. We hypothesize that NEWT mimics Wnt by stabilizing beta-catenin, thereby increasing expression of beta-catenin-dependent genes. Alternatively, NEWT may form a complex with Axin, a downstream component of the Wnt pathway.

The aim of these studies is to identify the functional link between upstream signals and the intracellular complexes that regulate beta-catenin.

We will clone and co-express fluorescent protein conjugates of NEWT, beta-catenin and Axin to assess their putative interactions in transfected cells using confocal microscopy and fluorescence resonance energy transfer (FRET), which will be assessed in HEK 293T cells (Human Embryonic Kidney cell line).

In order to study the effects of NEWT on downstream LEF-mediated gene expression, we will use the promoter of the c-myc binding protein (MBP) gene, a direct target of the beta-catenin/LEF-1 pathway. We will engineer the MBP promoter region (obtained by PCR) into the Promega luciferase reporter vector phRG-B, and create a stably transfected 293T cell line of that construct. Various small molecules, e.g. glucocorticoids and antioxidants, will be examined for their ability to up- or down-regulate the expected activation of LEF-dependent promoter activity mediated by NEWT.

Using site-directed mutagenesis (megaprimer method), we will also test whether a conserved Glu-Tyr-Glu (EYE) sequence motif of NEWT ("EYE of NEWT") is essential for its ability to enhance Wnt signaling and/or binding to beta-catenin or Axin.

## B. Potential environmental impact

Please describe aspects of the protocol which have potential environmental impact. If you plan to conduct a field trial, include the location and size of the environmental release.

There will be no environmental release.

## C. Risk assessment safety precautions

Describe methods for handling rDNA materials and/or hazardous substances. If you will be employing exotic organisms, or Risk Group 2 or 3 pathogenic microorganisms, give particular attention to the following: Adequacy of facility design and containment equipment, decontamination and disposal, investigator experience, personnel practices such as use of personal protective equipment, staff training, and Laboratory Standard considerations.

This project will not involve the use of infectious virus, only an isolated viral gene. Thus no exceptional protections will be required other than those used for the handling of any recombinant DNA molecules, lab strain E. coli bacteria and cell cultures. Work with the latter will be conducted in biosafety cabinets, with gloves and eye protection.

Nonetheless, all biological materials will be treated as potentially infectious, and disposed of as biohazard waste as described under section F above.

