

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR	<u>Dr. Anthony Percival-Smith</u>
DEPARTMENT	<u>Biology</u>
ADDRESS	<u>University of Western Ontario</u>
PHONE NUMBER	<u>519-661-4015</u>
EMERGENCY PHONE NUMBER(S)	<u>519-438-6571</u>
EMAIL	<u>aperciva@uwo.ca</u>

Location of experimental work to be carried out: Building(s) WSC Room(s) 361

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC
GRANT TITLE(S): Functional dissection of HOX proteins in Drosophila melanogaster

List all personnel working under Principal Investigators supervision in this location:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Lovesha Sivanantharajah</u>	<u>lsivanan@uwo.ca</u>	<u>2005</u>
<u>Elyse Burlingham</u>	<u>eburling@uwo.ca</u>	<u>2009</u>
<u>Melissa Bredow</u>	<u>mbredow@uwo.ca</u>	<u>2010</u>
<u>Tyler Luyben</u>	<u>tluyben@uwo.ca</u>	<u>2010</u>

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

E. coli DH5alpha: Stored on Petri dishes or in the freezer. Disposal: Autoclaved

Drosophila melanogaster: Stored in vials or bottles. Disposal: Ethanol morgue or Autoclaved

Please include a one page research summary or teaching protocol.

Nearly all animals exhibit some form of segmentation along the anterior-posterior (head to tail) axis. An example of segmentation of the human body plan is the vertebrae of the backbone; each vertebra represents an individual segment and each vertebra has a unique structure or morphology. I am interested in two general questions: first how the normal number of segments is established; and second how a segment is assigned a unique morphology? The insect body plan is divided into 14 segmental units (parasegments) along the anterior-posterior axis. The fruitfly (*Drosophila melanogaster*) fushi tarazu gene (*ftz*) encodes a protein FTZ required for determining the number of segments that form during development, and the proboscipedia (*pb*) and Sex combs reduced (*Scr*) genes encode proteins PB and SCR that determine the unique identities of 3 segments (maxillary, labial, and first thoracic). All three proteins share a DNA-binding protein domain called the homeodomain (HD). HD-containing proteins are transcription factors that regulate the transcription of genes, the process that converts information stored in DNA to RNA that can be read by the protein synthesis machinery. Even though many of the genes regulated by FTZ, PB and SCR have been identified and extensively characterized, there is a lack of knowledge about how these transcription factors FTZ, PB and SCR work to bring about changes in the pattern gene transcription. One key element lacking is a complete description the functional domain structure of these three proteins. These transcription factors have been analyzed for over twenty years, but the mapping of the functional domains has proven difficult. In recent years, we and others have found that HD-containing proteins are made up of small peptide motifs that make small contributions to overall activity. We are now identifying the small motifs and describing their requirements. This knowledge will greatly enhance our understanding of the mechanistic logic behind the segmentation of the insect body plan, and as HD-containing protein are also used during the segmentation of the human body plan, the work in flies is of general biological significance and application.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? YES NO

If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level
E. coli	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	2 litres		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
dHS alpha	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES NO
 If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input type="radio"/> Yes <input type="radio"/> No			
Rodent	<input type="radio"/> Yes <input type="radio"/> No			
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No			
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No			

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required 1 2 2+ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? YES, complete table below NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or transfection
<i>E. coli</i> DH5alpha	pBR322 based	Various	<i>Drosophila</i> genes	Developmental

* Please attach a Material Data Sheet or equivalent if available

** Please attach a plasmid map.

See E-mail

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made? YES, complete table below NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV YES, please specify _____ NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

4.6 Will virus be infectious to humans or animals? YES NO

4.7 Will this be expected to increase the containment level required? YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES NO
 (including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
 If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
 Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? YES NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____

5.5 Has human ethics approval been obtained? YES, number: _____ NO PENDING

6.0 Animal Experiments

6.1 Will live animals be used? YES NO If no, please proceed to section 7.0

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES No If no, please proceed to section 8.0

7.2 Will live animals be used? YES No

7.3 If yes, please specify the animal(s) used:

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES, please specify species _____ NO
- ◆ Non-human primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES, please specify species _____ NO
- ◆ Others (wild or domestic) YES, please specify _____ NO

7.4 If no live animals are used, please specify the source of the specimens:

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? YES NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) _____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD₅₀ (specify species) of the toxin _____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details: _____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. Drosophila melanogaster

9.3 What is the origin of the insect? Stock centers

9.4 What is the life stage of the insect? All stages

9.5 What is your intention? Initiate and maintain colony, give location: WSC 361/357
 "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated: No risk

9.7 Do you use insects that require a permit from the CFIA permit? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? YES NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES NO
If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

10.8 Is the CFIA permit attached? YES NO
If YES, Please attach the CFIA permit & describe any

See E-mail

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin__USA, Europe NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? YES NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO

11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, date of most recent biosafety inspection: _____
 NO, please certify
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-UWO-0046

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
Not applicable

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
Not applicable

See E-mail

14.3 As the Principal Investigator, I will ensure that this work complies with the Biosafety and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE [Signature] Date: Dec 13, 2010

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

----- Original Message -----

Subject:Re: Biological Agents Registry Form (Percival-Smith)

Date:Wed, 22 Dec 2010 16:16:35 -0800

From:Anthony Percival-Smith <aperciva@uwo.ca>

To:Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer,

14.2 Please outline what will be done if there is an exposure to the biological agents listed,
such as a needlestick injury or an accidental splash:

As none of the biological agents are pathogenic (E. coli DH5 alpha and Drosophila melanogaster), our procedure is to wash the area of the needlestick injury or accidental splash thoroughly. In case of a splash in the eyes, to wash the eyes thoroughly. After taking the appropriate measures, the worker reports the incident to the supervisor, and is instructed to monitor for changes. If there are changes to the exposed area, the worker is instructed to seek medical attention.

Tony



E-mail



Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: InroortZoopath@inspection.gc.ca

Bureau du confinement des biotiques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Téléc: (613) 228-6129
Courriel: InroortZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- | | | | | |
|---------------|--------------------|-----------|-------------------|----------------|
| • 5K | • CIE85 | • J52 | • MC4100 (MuLac) | • U5/41 |
| • 58 | • DH1 | • J53 | • MG1655 | • W208 |
| • 58-161 | • DH10 GOLD | • JC3272 | • MM294 | • W945 |
| • 679 | • DH10B | • JC7661 | • MS101 | • W1485 |
| • 1532 | • DH5 | • JC9387 | • NC-7 | • W3104 |
| • AB284 | • DH5-alpha | • JF1504 | • Nissle 1917 | • W3110 |
| • AB311 | • DP30 | • JF1508 | • One Shot STBL3 | • WA704 |
| • AB1157 | • DY145 | • JF1509 | • OP50 | • WP2 |
| • AB1206 | • DY380 | • JJ055 | • P678 | • X1854 |
| • AG1 | • E11 | • JM83 | • PA309 | • X2160T |
| • B | • EJ183 | • JM101 | • PK-5 | • X2541 |
| • BB4 | • EL250 | • JM109 | • PMC103 | • X2547T |
| • BD792 | • EMG2 | • K12 | • PR13 | • XL1-BLUE |
| • BL21 | • EPI 300 | • KC8 | • Rri | • XL1-BLUE-MRF |
| • BL21 (DE3) | • EZ10 | • KA802 | • RV308 | • XL0LR |
| • BM25.8 | • FDA Seattle 1946 | • KAM32 | • S17-1λ-PIR | • Y10 |
| • C | • Fusion-Blue | • KAM33 | • SCS1 | • Y1090 (1090) |
| • C-1a | • H1443 | • KAM43 | • SMR10 | • YN2980 |
| • C-3000 | • HF4714 | • LE450 | • SOLR | • W3110 |
| • C25 | • HB101 | • LE451 | • SuperchargeEZ10 | • WG1 |
| • C41 (DE3) | • HS(PFAMP)R | • LE452 | • SURE | • WG439 |
| • C43 (DE3) | • Hfr3000 | • MB408 | • TOP10 | • WG443 |
| • C600 | • Hfr3000 X74 | • MBX1928 | • TG1 | • WG445 |
| • Cavalli Hfr | • HMS174 | • MC1061 | | |

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Subject: Re: Biological Agents Registry Form (Percival-Smith)

From: Anthony Percival-Smith <aperciva@uwo.ca>

Date: Fri, 29 Apr 2011 10:28:01 -0400

To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer,

Attached is the Biohazards form with Sections 7, 8 10 completed.

Table 4.2

Bacteria used for cloning change	Plasmids	Source of Plasmid	Genes Transfected	Describe
DH5alpha change to bacteria	PUAST	Fly community	proboscipedia	No
plasmids used to carry genes; bacteria unaffected.	Pcasperhs	Fly community	Sex combs reduced fushi tarazu	the
			All above genes encode Drosophila HOX proteins	

Maps of PUAST and Pcasperhs are attached.

Section 11

Drosophila melanogaster requires no permit for importation through the Canadian Food Inspection Agency. See Canadian Food Inspection Agency Website for list of organisms that do not require a permit.

Insect containment

Containment is required for Plant pests. Drosophila melanogaster is not considered a plant pest and is harmless. No containment is required. However, using the Plant Pest containment scale our laboratory falls under Plant Pest Containment Level 1 (PPC-1). A summary of PPC-1 is attached.

Hope this addresses all outstanding issues.

Tony

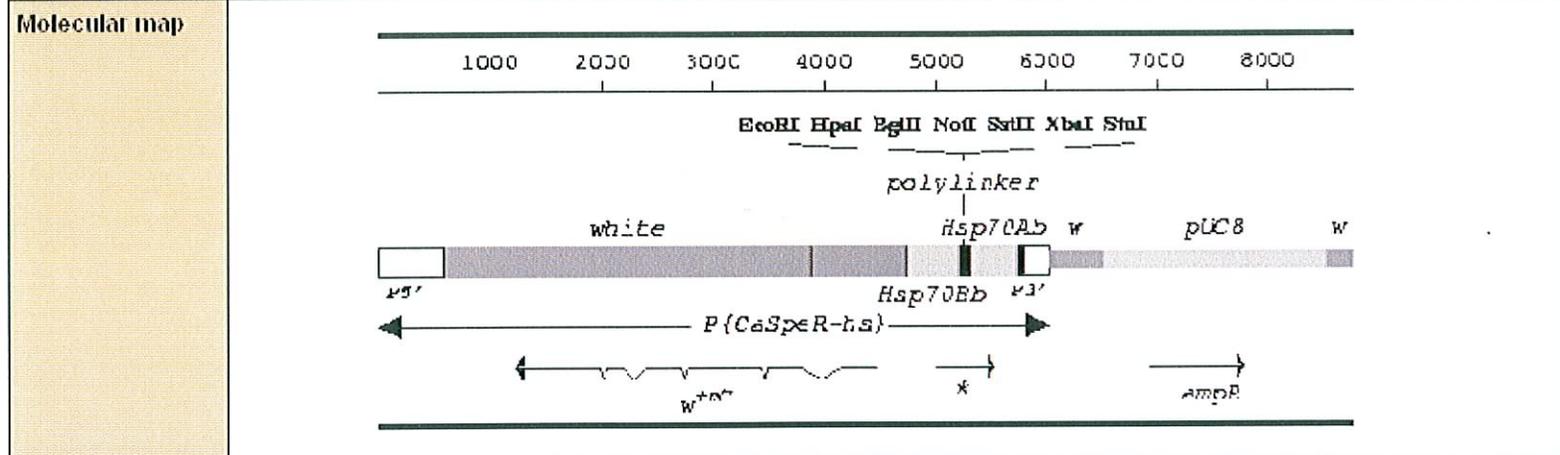


On 03/21/11, Jennifer Stanley <jstanle2@uwo.ca> wrote:

Hi there

Recombinant construct pP{CaSpeR-hs}

General Information			
Symbol	pP{CaSpeR-hs}	FlyBase ID	FBmc0000179
Feature type	engineered_construct		
Size		Expression data	
Associated insertions	0 available		



- Recent Updates
- Description & Uses
- Sequence Data

Sequence (FB) FlyBase-compiled

Associated Sequence Data

DDBJ / EMBL / ... GenBank	DNA sequence	Extent
	U59056	(bases 1-8780)

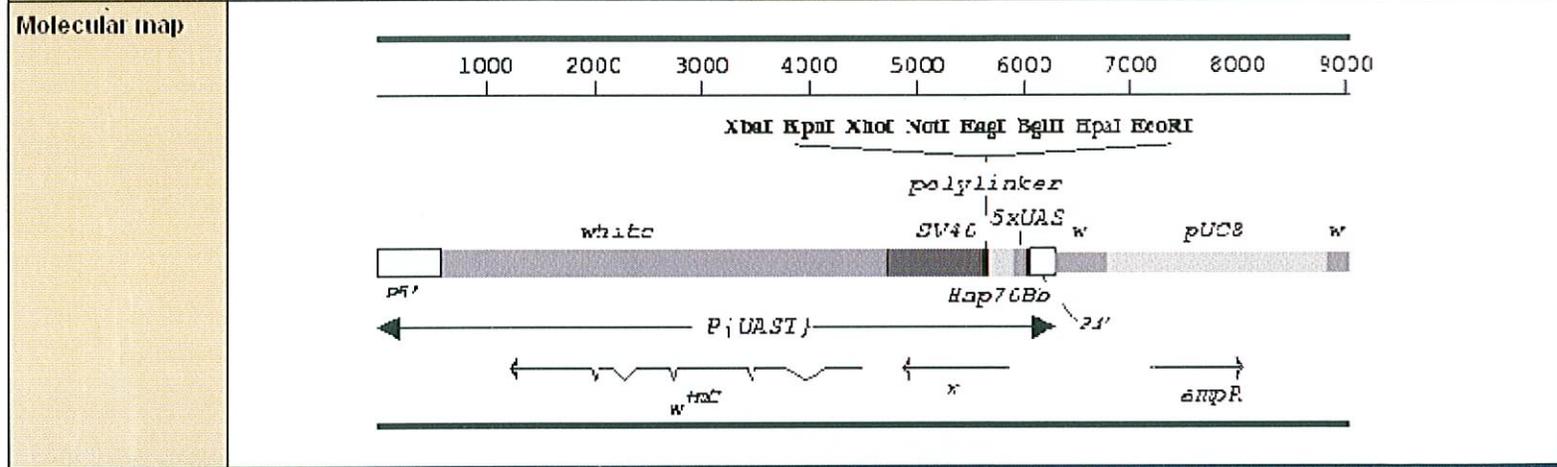
- Segments & Size
- Features
- Component Alleles

- Expression Data
- Progenitors & Descendants
- Comments
- Stocks Listed in FlyBase (0)



General Information

Symbol	pP{UAST}	FlyBase ID	FBmc0000383
Feature type	engineered_construct		
Size		Expression data	
Associated insertions	0 available		



- Recent Updates
- Description & Uses
- Sequence Data

Sequence (FB) FlyBase-compiled

Associated Sequence Data

DDBJ / EMBL / GenBank	DNA sequence	Extent

- Segments & Size
- Features
- Component Alleles

- Expression Data
- Progenitors & Descendants
- Comments
- Stocks Listed in FlyBase (0)

2.3.2 Plant Pest Containment Level 1 (PPC-1)

PPC-1 containment is the next highest containment level for plant pests. Facilities include permanent structures such as laboratories, greenhouses and screenhouses. Windows that can be opened must be fitted with appropriate screens, and greenhouses must be fully screened and caulked to both contain and exclude arthropods. An autoclave must be available to treat waste and waste water must be treated to kill pests where appropriate.

The following are examples of the types of work that could be appropriately conducted (with or without supplemental conditions) in PPC-1 containment:

- inoculating host plants with isolates of plum pox or other plant viruses in the absence of the vectors of those viruses;
- importing low-risk tropical insects into butterfly houses for study, display or rearing; or
- studying and rearing nematodes of quarantine concern in Canada that have low spread potential (e.g. *Globodera rostochiensis* and *Ditylenchus destructor*).