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	Dr. Mark A. Bernards
DEPARTMENT	Biology
ADDRESS	NCB 404/B&GS 2025E
PHONE NUMBER	x86477
EMERGENCY PHONE NUMBER(S)	519-200-3414
EMAIL	bernards@uwo.ca

Location of experimental work to be carried out: Building(s) NCB Room(s) 405/464

*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 Characterization of Plant Secondary Metabolism/Metabolites

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

Name	UWO E-mail Address	Date of Biosafety Training
Meg Haggit	meg.haggitt@uwo.ca	Aug 3, 2011
Jessica Koteles	jkotele@uwo.ca	Feb 14, 2011
Dimitre Ivanov	divanov2@uwo.ca	Pending (Nov 22/2011)
Pooja Sharma	psharm26@uwo.ca	Jan 13, 2011
Anica Bjelica	abjelica@uwo.ca	Pending (Nov. 22/2011)
Muna Basahi	mbasahi@uwo.ca	Pending

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.

We employ typical microorganisms used in the generation of experimental transgenic plants, the expression of recombinant proteins for biochemical characterization and the generation of "hairy roots" for studying root biology. These include E. coli (TOP10, DHalpha), Agrobacterium tumefaciens, Agrobacterium rhizogenes and yeast (WAT 11). We also use a number of fungal (Trichoderma hamatum) and oomycete (Pythium irregulare, Pythium ultimum, Phytophthora sojae) species to study plant-pathogen interactions. Routine culturing, preparation of freezer stocks and standard transformation protocols are used.

We employ a number of vectors/plasmids for this work, including commercially available Gateway vectors, as well as pGEMeasy, pCAMBIA-based vectors for expression analysis. For each of these, we clone-inthe gene/promoter constructs that we are interested in studying. We routinely prepare freezer stocks (-80c) of all vectors/plasmids at each stage of development. For this, we store untransformed and transformed bacterial and yeast cultures as glycerol stocks at -80°C. Single colonies of the strains are grown overnight in appropriate selectable liquid media; 0.5ml of overnight culture is mixed with 0.5 ml of 80% sterile glycerol and kept in -80°C.

All working cultures are grown under standard conditions (typically 37°C) in LB or the appropriate selectable medium and disposed of according to UWO policy (i.e., autoclaving with indicator cuultures).

Please include a one page research summary or teaching protocol.

Suberin is a biomacromolecule that plants use to reinforce the cell walls of specific cell types, including root epidermis and endodermis cells. The role of suberin in the resistance of plants to root diseases and tolerance to water stress has caught the interest of breeders and scientists, increasing the need to better understand this under-studied material. The long-term objectives of my research program are to understand the genetic and biochemical control of suberin biosynthesis. Ultimately, I would like to apply this knowledge to enhance suberin deposition and improve plant tolerance to pathogens and drought. In the short term, my objectives are to (i) identify critical genes of suberin biosynthesis, clone them and isolate their promoters, (ii) develop systems in which to manipulate the expression of these genes in tissue relevant to suberin deposition, and (iii) use the systems developed in (ii) to characterize the promoters of these genes, determine critical hormonal and genetic controllers of their expression, and determine their specific role(s) in suberin biosynthesis.

The hydroxylation and further oxidation of the terminal (omega) carbon of fatty acids is a critical step in the biosynthesis of aliphatic suberin monomers, since it introduces a second functional group and allows three-dimensional cross-linking. We have identified and cloned three fatty acid omega hydroxylases (FAwH) from potato and six from soybean. For potato, StFAwH1, has been shown to be tuber specific. For soybean, the expression of the six GmFAwH genes is more complex and we need to confirm which are expressed in roots during normal growth and development. For this we will use a "brute force" PCR approach involving gene specific primers. The recent (Fall 2010) preliminary release of the Solanum phureja genome (http://potatogenomics.plantbiology.msu.edu/index.html) has allowed us to clone 1.7 kb of sequence upstream of StFAwH1 and we will now analyse the regulation of expression of this gene (i.e., with GUS and GFP marker genes). Our preliminary data suggests that the first approx. 260 bp 5′ of the StFAwH1 transcriptional start site are sufficient for wound-induced GUS expression in transgenic potato hairy roots. We are now poised to explore the regulation of StFAwH1 gene expression in detail. However, this is hampered by the lack of a rapid and robust genetic transformation system in tissues relevant to suberin deposition. While Arabidopsis can be rapidly transformed and there are extensive genetic resources available for this species, it does not produce large amounts of suberin. Conversely, potato tubers produce an abundance of suberin; however, obtaining genetically modified potato tubers is laborious and time consuming. To overcome these obstacles, we propose to combine the in vitro propagation of potato microtubers with Agrobacterium-mediated genetic transformation to create a system in which genetically modified potato microtuber tissue can be generated on the same time scale as Arabidopsis transformants. Similarly, we are adapting a soybean hairy root system, in which A. rhizogenes-mediated gene transfer is used to create transgenic soybean roots, to the study of the soybean-Phytophthora sojae pathosystem. With this system we will be able to directly test whether altering suberin content of the root affects resistance or susceptibility to P. sojae. We will also be able to design experiments to help determine how suberin deposition is regulated in soybean roots, using GmFAwH genes as markers for suberin biosynthesis.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ⊘ YES O 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? O 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: (See also, attached table extension, pp 4b)

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 (TOP10, DHa, BL21)	O Yes ⊘ No	O Yes ⊘ No	O Yes ⊘ No	0.1 - 0.5 L		⊘ 1 O 2 O 2+ O 3
Saccharomyces cerevisiae)	O Yes ⊘ No	O Yes ⊘ No	O Yes ⊘ No	0.1 - 0.5 L		⊘ 1 O 2 O 2+ O 3
Agrobacterium tumefaciens	O Yes ⊘ No	O Yes ⊘ No	O Yes ⊘ No	0.1-0.2 L		⊘ 1 O 2 O 2+ O 3
Agrobacterium rhizogenes	O Yes ⊘ No	O Yes ⊘ No	O Yes ⊘ No	0.1-0.2 L		⊘ 1 O 2 O 2+ O 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 O 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	O Yes ⊘ No		Not applicable
Rodent	O Yes ⊘ No		
Non-human primate	O Yes ⊘ No		
Other (specify)	⊘ Yes O No	potato leaves & tubers soybean cotyledons	

The following fungi and oomycetes were obtained from the Canadian Collection of Fungal Cultures. None are genetically modified or used for molecular biological work. They are routinely cultured on either potato dextrose agar, V-8 agar or Chepak-Dox minimal medium.

Genus	Species	Isolate No.	Host Species	Location
Trichoderma	*hamatum*	TH 215090	*Cucumis sativus*	British Columbia, Canada
Trichoderma	*hamatum*	TH 215955	*Cucumis sativus*	British Columbia, Canada
Pythium	*irregulare*	BR 574	*Picea mariana*	Ontario, Canada
Pythium	*irregulare*	BR 901	*Cucumis sativus*	Alberta, Canada
Pythium	*irregulare*	BR 486	*Phaseolus vulgaris*	Netherlands
Pythium	*irregulare*	BR 779	*Triticum aestivum*	Transvaal, South Africa
Pythium	*irregulare*	BR 962	Soil	Wairakei, New Zealand
Pythium	*irregulare*	BR 598	*Panax quinquefolius*	Ontario, Canada
Pythium	*irregulare*	BR 1040	*Phaseolus vulgaris*	British Columbia, Canada
Pythium	*irregulare*	BR 1068	*Panax quinquefolius*	Ontario, Canada
Pythium	*irregulare*	BR 426	*Rhododendron sp.*	Alberta, Canada
Pythium	*ultimum*	BR 638	*Pisum sativum*	Alberta, Canada

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	O Yes Ø No			
Rodent	O Yes Ø No			
Non-human primate	O Yes Ø No			
Other (specify)	O Yes Ø 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 Ø 1 O 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES Ø 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		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 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? Ø YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
Same as in Table 1.2.	See attached maps	See Supplemental Table on pp 5a	See Supplemental Table on pp 5a	See Supplemental Table on pp 5a

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O YES, complete table below Ø 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 O YES, please specify _____ Ø NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ Ø NO
- SV 40 Large T antigen O YES Ø NO
- E1A oncogene O YES Ø NO
- Known oncogenes O YES, please specify _____ Ø NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ Ø NO

4.5 Will virus be replication defective? O YES Ø NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES Ø 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES Ø 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 O YES, specify: _____ O NO

6.5 Will the agent(s) be shed by the animal: O YES O 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)? O YES ⊗ No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

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

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O 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? O 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 LD_{50} (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? O YES, Please provide details:_____ O 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? O YES ⊗ NO If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

_____
_____

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

10.0 Plants

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

10.2 If YES, please give the name of the species. potato, soybean, ginseng

10.3 What is the origin of the plant? potato tubers are purchased, soybean seed from Agriculture Canada, ginseng seed and roots from Agriculture Canada

10.4 What is the form of the plant (seed, seedling, plant, tree...)? seeds, tubers, roots

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

10.6 Do you do any modifications to the plant? ⊗ YES O NO
If yes, please describe: potato and soybean tissues are used for moelcular biology experiemtns; these are generated, used and maintained under sterile, restrictive conditions

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
The transgenic plants we create to study the role(s) of specific genes in suberin biosynthesis are readily re-generated, as long as we have the appropriate constructs in hand. These materials do not pose any threat to the environment.

10.8 Is the CFIA permit attached? O YES ⊗ NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
These tissues do not require CFIA permits

11.0 Import Requirements

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

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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. Ø 1 O 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, date of most recent biosafety inspection: _____

O NO, please certify

Ø NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

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.

N/A

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:

washing well with soapy water & doing accident report. Monitor employee to ensure there are no ill effects.

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines 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 _____ Date: November 2, 2011

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:

Table 1.2

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

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: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@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
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

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

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

Canada

-------- Original Message --------
Subject: Re: Containment Level Request (agrobacterium LBA4404)
Date: Thu, 05 May 2011 10:46:22 -0400
From: ImportZoopath <ImportZoopath@inspection.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Good Morning,

According to our database, Agrobacterium tumefaciens would be considered a containment level 1 animal pathogen. If you have further questions, do not hesitate to contact our office.

Thank you,
Steven Burns

Office of Biohazard Containment & Safety, CFIA | Bureau du confinement des biorisques et de la sécurité, ACIA
Government of Canada | Gouvernement du Canada
1400 Merivale, Ottawa ON K1A0Y9
Phone/Tél.: (613) 773-6520
Fax/ Téléc.: (613) 773-6521
ImportZoopath@inspection.gc.ca

------- Original Message --------
Subject: Re: Agrobacterium rhizogenes
Date: Wed, 13 Jul 2011 08:18:26 -0400
From: ImportZoopath <ImportZoopath@inspection.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Good Morning,

According to our database, Agrobacterium rhizogenes is a level 1 animal pathogen. If you have any further questions, do not hesitate to contact our office.

Thank you,

Lauren Baerg

Office of Biohazard Containment & Safety, CFIA | Bureau du confinement des biorisques et de la sécurité, ACIA
Government of Canada | Gouvernement du Canada
1400 Merivale, Ottawa ON K1A0Y9
Phone/Tél.: (613) 773-6520
Fax/ Téléc.: (613) 773-6521
ImportZoopath@inspection.gc.ca

Section 4.2

Supplemental Table (Section 4.2)

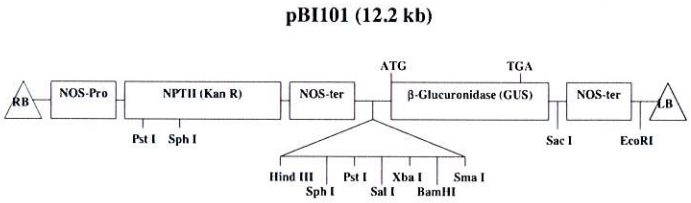
Bacteria Used for Cloning	**Plasmid(s)***	**Source**	**Gene Transfected**	**Describe the change that results from transformation**
E. coli DH5α	pGemTeasy	Promega	1.7 kb FAωHI coding region	The only change we are testing for and seeing regularly in the strains transformed with the plasmids (all the strains *E. coli*, *S. cerevisae* and *A. rhizogenes* listed) is their acquaintance of specific antibiotic resistance determined by plasmid sequence (selection marker for transformed strains). In addition, strains transformed with plasmids containing FAωHI coding region are expressing recombinant protein.
E. coli DH5α	pTrcHisA	Invitrogen	1.7 kb FAωHI coding region	
S. cerevisiae wat11	pYeDP60	Dr. Philip Urban, France	1.7 kb FAωHI coding region	
ccdB *E. coli*	pH2GW7,O	Invitrogen	none	
ccdB *E. coli*	pHGWFS7,0	Invitrogen	none	
E. coli DH5α	pHGWFS7,0	Invitrogen	upstream promoter region FAωHI; 300 bp	
A. rhizogenes	pHGWFS7,0	Invitrogen	upstream promoter region FAωHI; 300 bp	
E. coli DH105α	pCAMBIA1305.1	Invitrogen	None	
E. coli XL1Blue	pBI101:CML 37 promoter	Colleague	None	
E. coli XL1Blue	pBI101:CML 38 promoter	Colleague	None	

Abbreviations: FaωHI coding region of **F**atty **A**cid **ω** **H**ydroxylase from *Solanum tuberosum* (CYP86A homolog).

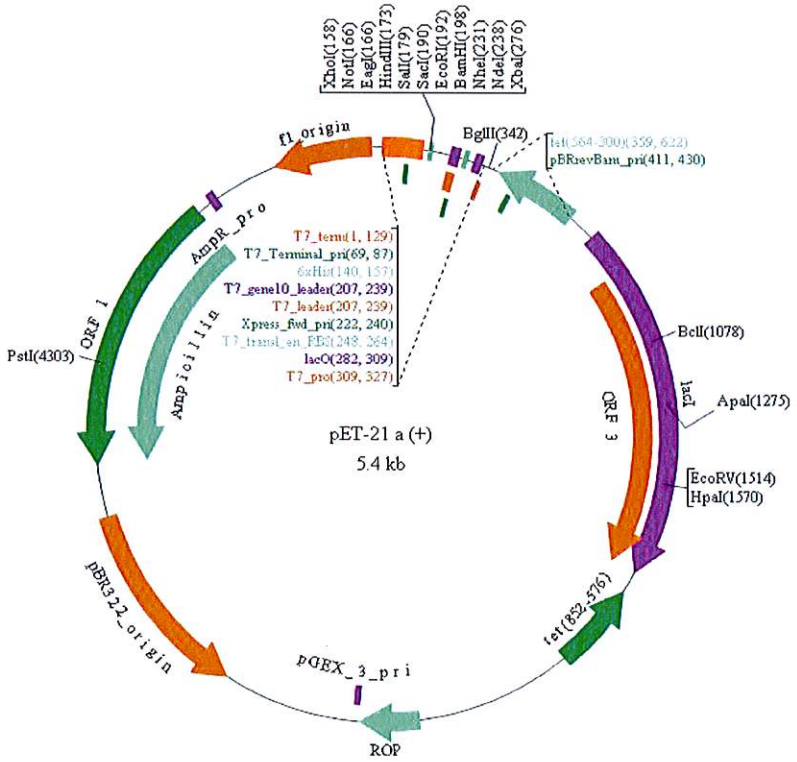
*plasmid maps attached

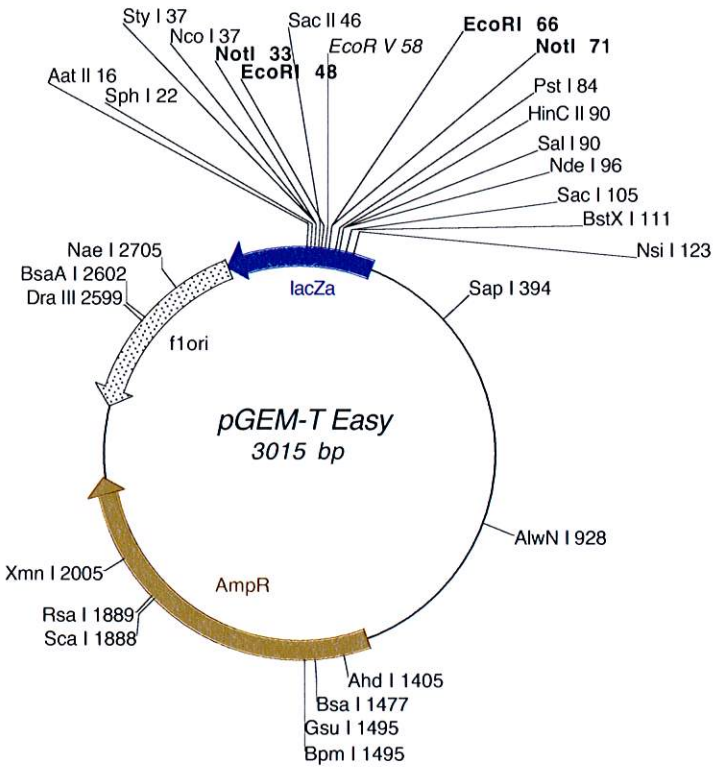
Vector Maps for vectors used in the Bernards lab.

1. Vectors used in cloning and bacterial expression of recombinant proteins.



AAG CTT GCA TGC CTG CAG GTC GAC TCT AGA GGA TCC CCG GGT GGT CAG TCC CTT ATG TTA
Hind III Sph I Pst I Sal I Xba I Bam HI Sma I



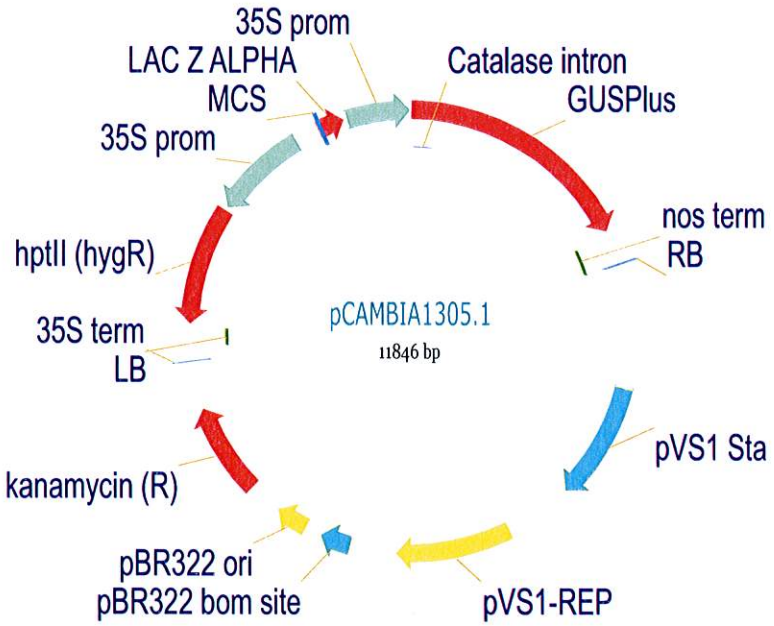


Plasmid name: pGEM-T Easy
Plasmid size: 3015 bp
Constructed by: Promega Corporation, Madison, WI.
Construction date:
Comments: T7 RNA Polymerase transcription initiation site 1
SP6 RNA Polymerase transcription initiation site 141
T7 RNA Polymerase promoter (-17 to +3) 2999-3
SP6 RNA Polymerase promoter (-17 to +3) 139-158
multiple cloning region 10-128
lacZ start codon 180
lac operon sequences 2836-2996, 166-395
lac operator 200-216
beta-lactamase coding region 1337-2197
phage f1 region 2380-2835
binding site of pUC/M13 Forward Sequencing Primer 2956-2972
binding site of pUC/M13 Reverse Sequencing Primer 176-192

The pGEM(R)-T Easy Vector has been linearized with EcoRV at Base 60 of this sequence (indicated by an asterisk *) and a T added to both 3' -ends.

2. Vectors used in plant expression

pCAMBIA1305.1



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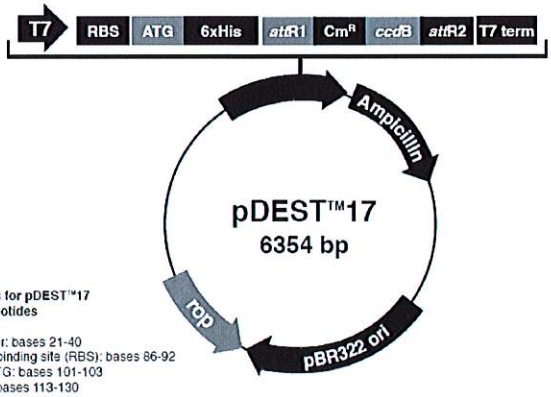
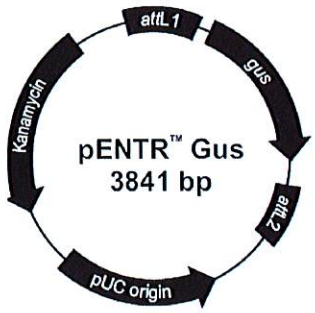
Gateway Vectors: used in plant expression systems



Comments for pDEST™17
6354 nucleotides

T7 promoter: bases 21-40
Ribosome binding site (RBS): bases 86-92
Initiation ATG: bases 101-103
6xHis tag: bases 113-130
*att*R1: bases 140-264
Chloramphenicol resistance gene (Cm^R): bases 373-1032
*ccd*B gene: bases 1374-1679
*att*R2: bases 1720-1844
T7 transcription termination region: bases 1855-1963
bla promoter: bases 2471-2569
Ampicillin (*bla*) resistance gene: bases 2570-3430
pBR322 origin: bases 3575-4248
ROP ORF: bases 4619-4810 (C)

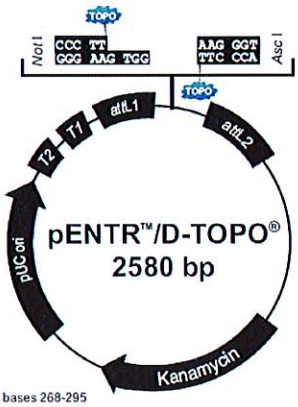
C=complementary strand



Comments for pENTR™ Gus
3841 nucleotides

*att*L1: bases 99-198 (complementary strand)
gus gene: bases 228-2039
*att*L2: bases 2041-2140
pUC origin: bases 2200-2873 (C)
Kanamycin resistance gene: bases 2990-3805 (C)

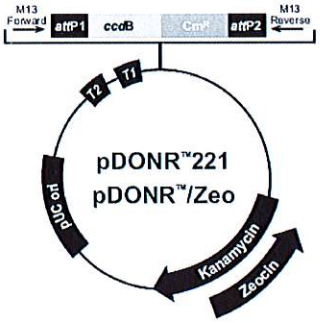
C = complementary strand



Comments for pENTR™/D-TOPO®
2580 nucleotides

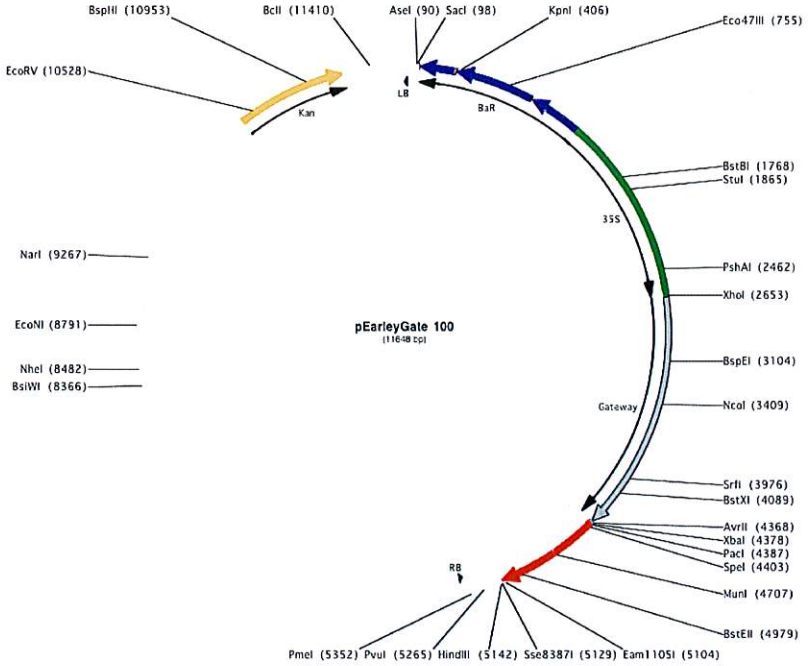
*rrn*B T2 transcription termination sequence: bases 268-295
*rrn*B T1 transcription termination sequence: bases 427-470
M13 forward (-20) priming site: bases 537-552
*att*L1: bases 569-668 (c)
TOPO® recognition site 1: bases 680-684
Overhang: bases 685-688
TOPO® recognition site 2: bases 689-693
*att*L2: bases 705-804
T7 Promoter/priming site: bases 821-840 (c)
M13 reverse priming site: bases 845-861
Kanamycin resistance gene: bases 974-1783
pUC origin: bases 1904-2577

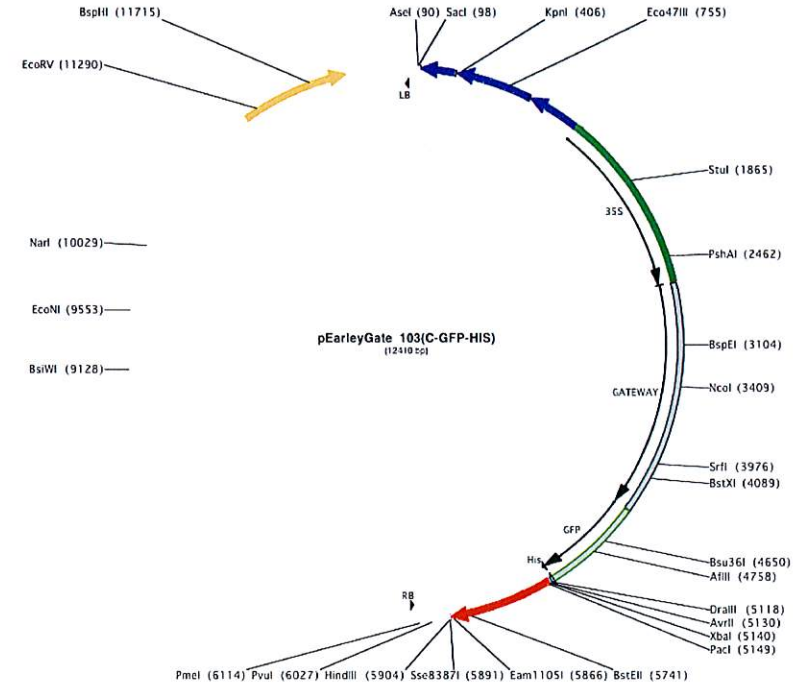
(c) = complementary sequence

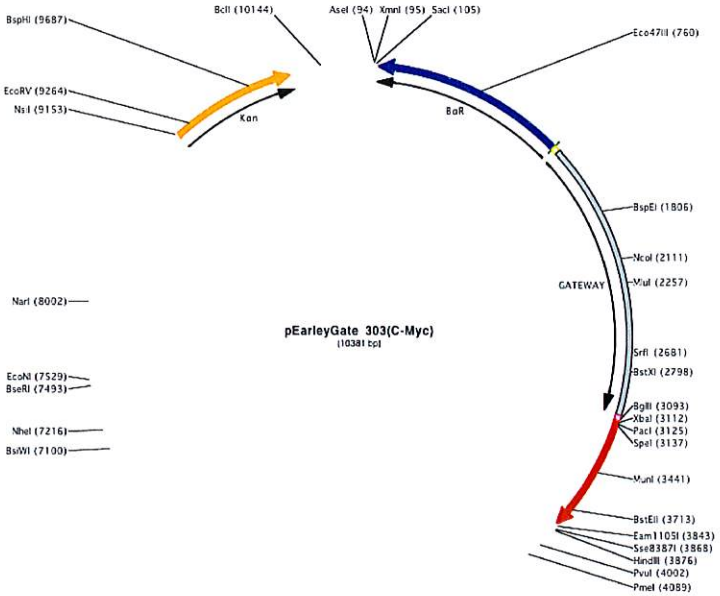


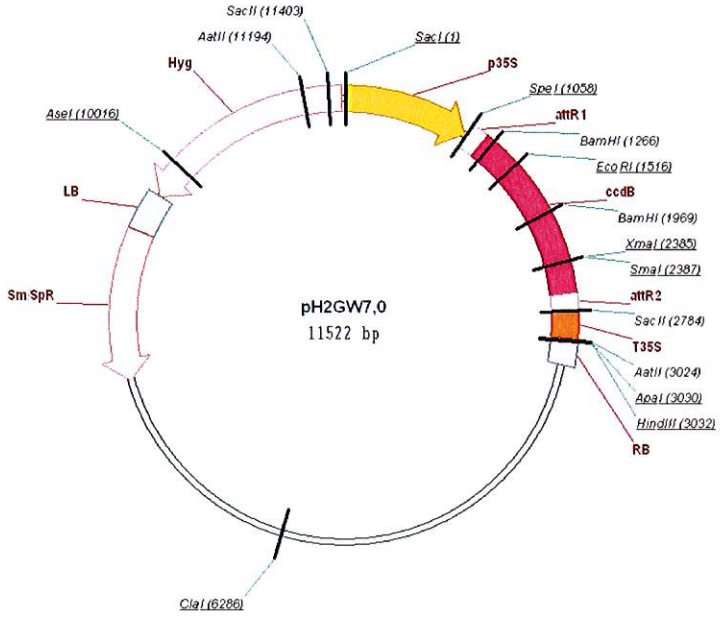
Comments for:	pDONR™221 4761 nucleotides	pDONR™/Zeo 4291 nucleotides
*rrn*B T2 transcription termination sequence (c):	268-295	268-295
*rrn*B T1 transcription termination sequence (c):	427-470	427-470
M13 Forward (-20) priming site:	537-552	537-552
*att*P1:	570-801	570-801
*ccd*B gene (c):	1197-1502	1197-1502
Chloramphenicol resistance gene (c):	1825-2505	1847-2506
*att*P2 (c):	2753-2984	2754-2985
M13 Reverse priming site:	3026-3042	3027-3043
Kanamycin resistance gene:	3155-3964	---
EM7 promoter (c):	---	3486-3552
Zeocin resistance gene (c):	---	3111-3485
pUC origin:	4085-4758	3615-4288

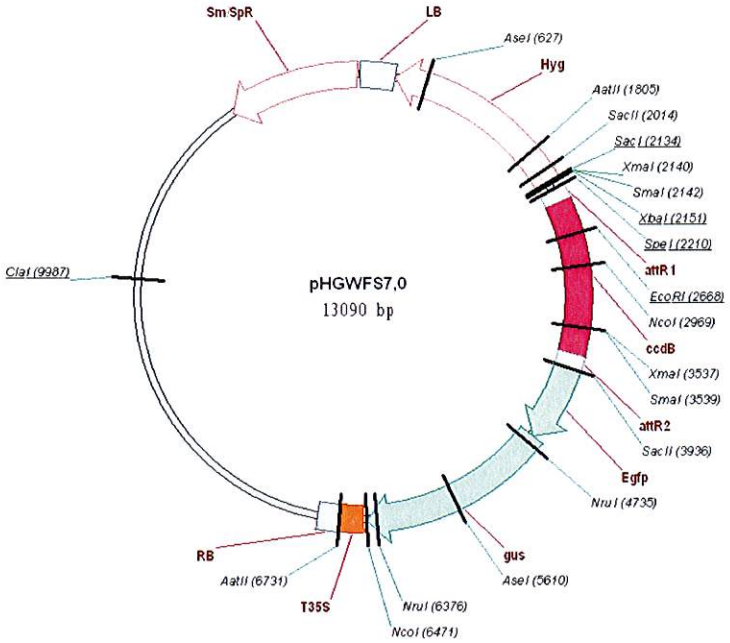
(c) = complementary strand


BspHI (10953)
BclI (11410)
AseI (90) SacI (98)
KpnI (406)
Eco47III (755)
EcoRV (10528)
LB
Kan
BsR
BstBI (1768)
StuI (1865)
35S
NarI (9267)
PshAI (2462)
XhoI (2653)
EcoNI (8791)
pEarleyGate 100
(11648 bp)
BspEI (3104)
NheI (8482)
BsiWI (8366)
Gateway
NcoI (3409)
SrfI (3976)
BstXI (4089)
AvrII (4368)
XbaI (4378)
PacI (4387)
SpeI (4403)
RB
MunI (4707)
BstEII (4979)
PmeI (5352) PvuI (5265) HindIII (5142) Sse8387I (5129) Eam1105I (5104)


BspHI (11715)
AseI (90) SacI (98)
KpnI (406)
Eco47III (755)
EcoRV (11290)
LB
StuI (1865)
35S
NarI (10029)
PshAI (2462)
EcoNI (9553)
pEarleyGate 103(C-GFP-HIS)
(12410 bp)
BspEI (3104)
BsiWI (9128)
GATEWAY
NcoI (3409)
SrfI (3976)
BstXI (4089)
GFP
His
Bsu36I (4650)
AflII (4758)
RB
DraIII (5118)
AvrII (5130)
XbaI (5140)
PacI (5149)
PmeI (6114) PvuI (6027) HindIII (5904) Sse8387I (5891) Eam1105I (5866) BstEII (5741)

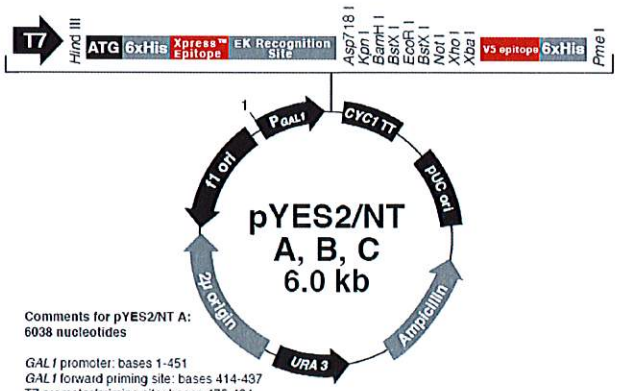

BclI (10144)
AseI (94) XmnI (95) SacI (105)
Eco47III (760)
BspHI (9687)
EcoRV (9264)
NsiI (9153)
Kan
BoR
BspEI (1806)
NcoI (2111)
MluI (2257)
GATEWAY
NarI (8002)
pEarleyGate 303(C-Myc)
10381 bp
SrfI (2681)
BstXI (2798)
EcoNI (7529)
BseRI (7493)
BglII (3093)
XbaI (3112)
PacI (3125)
SpeI (3137)
NheI (7216)
BsiWI (7100)
MunI (3441)
BstEII (3713)
Eam1105I (3843)
Sse8387I (3868)
HindIII (3876)
PvuI (4002)
PmeI (4089)


SacII (11403)
AatII (11194)
SacI (1)
Hyg
p35S
SpeI (1058)
AseI (10016)
attR1
BamHI (1266)
EcoRI (1516)
LB
ccdB
BamHI (1969)
XmaI (2385)
SmaI (2387)
Sm/SpR
attR2
pH2GW7,0
11522 bp
SacII (2784)
T35S
AatII (3024)
ApaI (3030)
HindIII (3032)
RB
ClaI (6286)


Sm/SpR
LB
AseI (627)
Hyg
AatII (1805)
SacII (2014)
SacI (2134)
XmaI (2140)
SmaI (2142)
XbaI (2151)
SpeI (2210)
attR1
EcoRI (2668)
NcoI (2969)
ccdB
XmaI (3537)
SmaI (3539)
attR2
SacII (3936)
Egfp
NruI (4735)
gus
AseI (5610)
NruI (6376)
NcoI (6471)
T35S
RB
AatII (6731)
ClaI (9987)
pHGWFS7,0
13090 bp

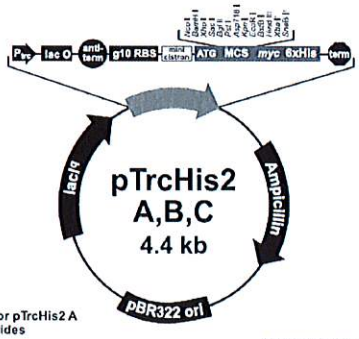
pp 5h

3. Vectors used for recombinant protein expression in yeast



Comments for pYES2/NT A:
6038 nucleotides

GAL1 promoter: bases 1-451
GAL1 forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
ATG initiation codon: bases 510-512
Polyhistidine (6xHis) region: bases 522-539
Xpress™ epitope: bases 579-602
Enterokinase (EK) recognition site: bases 588-602
Multiple cloning site: bases 602-669
V5 epitope: bases 682-723
Polyhistidine (6xHis) region: bases 733-750
CYC1 transcription termination signal: 783-1036
CYC1 reverse priming site: bases 800-818
pUC origin: bases 1220-1893
Ampicillin resistance gene: bases 2038-2898 (complementary strand)
URA3 gene: bases 2916-4023 (complementary strand)
2µ origin: bases 4027-5498
f1 origin: bases 5566-6021 (complementary strand)



Comments for pTrcHis2 A
4406 nucleotides

trc promotor region: bases 190-382
-35 region: bases 193-198
-10 region: bases 216-221
lac operator (*lac*O): bases 228-248
*rrn*B antitermination signal: bases 264-333
gene 10 region: bases 346-354
Ribosome binding site: bases 369-373
pTrcHis forward priming site: bases 370-390
Minicistron ORF: bases 383-409
Reinitiation RBS: bases 398-403
Expression ATG: bases 413-415
Multiple cloning site: bases 411-464
myc epitope: bases 471-503
Polyhistidine tag: bases 516-533
*myc*His reverse priming site: bases 508-527
*rrn*B T1 and T2 transcriptional terminators: bases 639-796
Ampicillin resistance ORF: bases 1076-1936
pBR322 origin: bases 2081-2754
Lac Repressor (*lacI*^{q}) ORF: bases 3408-4367

* *Xba* I is only found in pTrcHis2 B

† *Sna*B I is only found in pTrcHis2 C