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	Susanne Kohalmi
DEPARTMENT	Biology
ADDRESS	1151 Richmond Street North, London Ontario, N6A 5B7
PHONE NUMBER	519-661-2111 x86485
EMERGENCY PHONE NUMBER(S)	519-636-7717
EMAIL	skohalmi@uwo.ca

Location of experimental work to be carried out: Building(s) __WSC_____ Room(s)_319

*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:____N/A_____
GRANT TITLE(S): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Danielle Styranko	dstyrank@uwo.ca	Oct 10, 2008
Crystal Bross	cbross2@uwo.ca	Sept 17, 2010
Levent Karademir	lkaradem@uwo.ca	Will start in Sept 2011
Rene Boudreau	rboudre3@uwo.ca	Will start in Sept 2011
Tehmina Ahmad	tahmad3@uwo.ca	Sept 15, 2009

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 are using several microorganisms and plant species in the lab. The species are *Saccharomyces cerevisiae*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Arabidopsis thaliana* and *Nicotiana benthamiana*. All of the strains are research strains and none of the strains used have been associated with any health hazards. All microorganisms are stored at -80oC. Plants are stored in form of seeds at room temperature in glass vials. Microorganisms are grown in liquid cultures or on agar plates. They are used for plasmid propagation, expression studies, and protein-protein interaction assays. Plants are grown in soil or on agar plates in control conditions (incubators) and no field studies are conducted. Plants are grown for tissue harvesting or observation of fluorescence and phenotypic analysis. All biological material is autoclaved prior to disposal.

Please include a one page research summary or teaching protocol.

The work in my laboratory focuses on the analysis of plant genes and proteins from *Arabidopsis thaliana*. In particular we are interested in the molecular characterization of arogenate dehydratases, enzymes required for the synthesis of phenylalanine. We are interested in establishing RNA and protein expression profiles, protein-protein interaction capabilities *in vitro* and *in vivo*, protein complex formations, impact of allele variations on enzymatic function and interaction profiles, subcellular localization patterns and the genetic, phenotypic and functional analysis of mutants. To do so, numerous plant genes have been cloned into *E. coli* vectors to capture and modify gene sequences, or to express proteins for analysis. Gene sequences have also been cloned into yeast/*E. coli* shuttle vectors. In such cases the vectors are cloned and tested first in *E. coli* and then transformed into the yeast *Saccharomyces cerevisiae*. In yeast the cloned genes are expressed as proteins either to generate enough proteins for further biochemical analyses or to determine if they are able to form protein complexes (yeast two hybrid experiments). Numerous plant genes have been also cloned into *E. coli*/ *Agrobacterium*/ plant shuttle vectors. In these cases, *E. coli* is used for the cloning steps. The finished and tested plasmids are transformed into *Agrobacterium*. Transformed *Agrobacterium* strains are then used to introduce the inserted gene sequences into *Arabidopsis* or tobacco. Transformed plants are then used either for phenotypic analyses or for the localization and detection of proteins.

Lab members are trained in all procedures, protocols and assays, typically by initially following someone else who is trained, then performing the experiments on their own but with supervision, to gain independence and proficiency before pursuing procedures on their own. If expertise is not available in the lab itself training is achieved in collaboration with other laboratories. all lab members take part in the required safety training.

Notes to Biohazardous Agents Registry Form

1.2

Saccharomyces cerevisiae: several lab strains are in use. Strains have been in the lab since years. They are research strains and have been received from other researchers.

Agrobacterium tumefaciens: several lab strains are in use. Again the strains have been in use over years and as research strains have been supplied by other researchers.

Escherichia coli: standard lab strains are in use for cloning and transformations are in use: DH5alpha, DH10B and DB3.1. strains are classified as level one. Cells are purchased through companies like Invitrogen.

2.2

The only cell tissue cultures which are performed in the lab are plant based. They are used for the preparation of protoplasts for *Arabidopsis thaliana*.

4.2

All vectors listed below carry a multitude of different plant genes. The plant genes are predominantly genes encoding proteins for floral development and enzymes required for the synthesis of phenylalanine. Genes may have been cloned as full-length or partial sequences, they may have been cloned in several versions to accommodate different restriction enzymes for subcloning, or as fusions to GFP, YFP, GAL4 or antibody tags.

E. coli plasmids are present in either DH5alpha, DH10b DB3.1. *E.coli*/yeast shuttle plasmids are present in both host strains, and *E. coli*/*Agrobacterium*/plant shuttle plasmids are present in either of the host systems.

All plasmids with the exception of pGEM-T have been received from other researchers.

pGEM-T: standard *E. coli* cloning vector for the capture of PCR fragments.

pDONR 221: cloning vector.

pYES/NT, pEXP5-NT, pET-44 Ek/LIC: expression vectores for expression of proteins in yeast or *E. coli*.

pBI770, pBI771, pGBKT7, pGADT7: yeast/*E coli* shuttle vectors used for detecting protein interactions in yeast (yeast two hybrid system).

pBIN20, pEZT-NL, pEZS-NL, pRD420, pEarlyGate 201: *Arabidopsis*, *E. coli*, *Agrobacterium* shuttle vectors for the expression of genes in plants.

Base vector maps have been provided where ever possible.

See Notes - ie Sections 4 + 10

4.4 through 4.7

Sections have not been answered as I do not work with any viruses.

10.3

All plant lines are for research purposes only. Thye either have been obtained from other researchers over years or from ABRC (Arabidopsis Biological Research Center)

10.4

Plants are obtained and stored as seeds. For study they are grown from seeds, to seedlings to mature plants to collect seeds again.

10.5.

We use plants for research purposes only. Both plant species are standard model organisms. Plants are "stored" in form of seeds and then propagated under controlled conditions for tissue collection and/or for observation. Plants are not used as crop or for any commercial use and are not grown under field conditions. Individual seeds obviously are only grown once. However, seeds from the same batch (generation) or offspring from an individual seed can be grown more then once.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? x 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 x NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? __laboratory strains_____
Please describe the risk (if any) of escape and how this will be mitigated: _none of the microorganisms we are using have any associated effects on human health_____
_____
_____
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
Saccaromyces cerevisiae	O Yes x No	O Yes x No	O Yes x No	App 4 L		x 1 O 2 O 2+ O 3
Agrobacterium tumefaciens	O Yes x No	O Yes x No	O Yes x No	App. 2 L		x 1 O 2 O 2+ O 3
Escherichia coli	O Yes x No	O Yes x No	O Yes x No	App. 4 L		x 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			

*Please attach a Material Safety Data Sheet or equivaler

E. coli DH10B, DH5 alpha, DB3.1

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? x 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 x No		Not applicable
Rodent	O Yes x No		
Non-human primate	O Yes x No		
Other (specify)	x Yes O No	Arabidopsis thaliana	

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 x No			
Rodent	O Yes x No			
Non-human primate	O Yes x No			
Other (specify)	O Yes x 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 x 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 x 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? x YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? x 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 transfection
DH10B *DH5 alpha* *DB3.1* E. coli	*pGEM-T* *pDONR 221* *pYES/NT* *pEXP5-NT* *pET-44 Ek/LIC* *pBI770* *pBI771* *pGBKT7* *pGADT7* *pBIN20*			

See "Notes ... Form"

	pEZT-NL pEZS-NL pRD420 pEarlyGate 201			

pEarleyGate 202?
pEarleyGate 201

* Please attach a Material Data Sheet or equivalent if avai
** 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 x 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 _____ x NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ x NO
- SV 40 Large T antigen O YES x NO
- E1A oncogene O YES x NO
- Known oncogenes O YES, please specify _____ x NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ x NO

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES x 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 x 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 x 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 x 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 x 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

_____

_____

See "Notes ... Form"

10.0 Plants

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

10.2 If YES, please give the name of the species. Arabidopsis thaliana, Nicotiana benthamiana

10.3 What is the origin of the plant? _Research strains_____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? seed, seedlings, mature plants

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

10.6 Do you do any modifications to the plant? x YES O NO
If yes, please describe: __transient and stable transformations_____

_____

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

_____

_____

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

_____

_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ x 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 Susan Kohli

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. x 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
x 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:

_N/A_____

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 Susan Kohli Date: July 4, 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:

-------- 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

Please visit our website http://www.inspection.gc.ca/english/sci/bio/bioe.shtml / Veuillez visiter notre site internet http://www.inspection.gc.ca/francais/sci/bio/biof.shtml

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

Canada

MATERIAL SAFETY DATA SHEET

Revised 6/28/04
Replaces 6/28/04
Printed 6/28/04

LE DB3.1 COMPETENT CELLS
INVITROGEN CORPORATION
MSDS ID: 11782

1. PRODUCT AND COMPANY INFORMATION

INVITROGEN CORPORATION
1600 FARADAY AVE.
CARLSBAD, CA 92008
760/603-7200

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716/774-6700

INVITROGEN CORPORATION
3 FOUNTAIN DR.
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
44-141 814-6100

INVITROGEN CORPORATION
P.O. BOX 12-502
PENROSE
AUCKLAND 1135
NEW ZEALAND
64-9-579-3024

INVITROGEN CORPORATION
2270 INDUSTRIAL ST.
BURLINGTON, ONT
CANADA L7P 1A1
905/335-2255

INVITROGEN AUSTRALIA PTY LIMITED
2A/14 LIONEL ROAD
MOUNT WAVERLY VIC 3149
AUSTRALIA
1-800-331-627

EMERGENCY NUMBER (SPILLS, EXPOSURES): 301/431-8585 (24 HOUR)
800/451-8346 (24 HOUR)
NON-EMERGENCY INFORMATION: 800/955-6288

Product Name: LE DB3.1 COMP CELLS
Stock Number: 11782

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
These products are for laboratory research use only and are not intended for human or animal diagnostics, therapeutic, or other clinical uses, unless otherwise stated.

Synonyms:
Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

Component	CAS No.	Percent
No hazardous components		

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Occupational exposure presents little or no health hazard.
****

Potential Health Effects:
Eye:
May cause irritation of the eye.

Skin:
May cause skin irritation.

Inhalation:
No toxicity expected from inhalation.

Ingestion:
Mildly irritating to mouth, throat, and stomach. Can cause abdominal discomfort.

Chronic:
No data on cancer.

4. FIRST AID MEASURES

Eye:
Flush with water in an eyewash for at least 15 minutes, holding eyelids open. Remove contact lenses, clean before re-use. Obtain medical attention if symptoms develop (redness, itching, etc.).

Skin:
Wash with plenty of soap and water. Obtain medical attention if symptoms develop (redness, itching, etc.). Remove contaminated clothes, wash before re-use.

Inhalation:
Remove person to fresh air. Obtain medical attention unless effects are mild and temporary.

Ingestion:
Give plenty of water, if conscious. If vomiting occurs naturally, wash mouth out. Be prepared to induce vomiting upon a physician's advice. Obtain medical attention if symptoms develop.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:
Not available.

Upper Flammable Limit %:
Not available.

Lower Flammable Limit %:
Not available.

Autoignition Temperature Deg C:
Not available.

Extinguishing Media:
Use means appropriate for surrounding materials.

Firefighting Techniques/Equipment:
Standard turnout gear ("bunker gear"). Positive-pressure self-contained breathing apparatus.

Hazardous Combustion Products:
Includes carbon dioxide, carbon monoxide, dense smoke.

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

Spill Cleanup:
Absorb spill. Common absorbent materials should be effective. Deposit in appropriate containers for removal and disposal.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
Ambient

Handling Procedures:
Keep closed or covered when not in use.

Storage Procedures:
Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
No hazardous components		

Engineering Controls:
Area ventilation is generally adequate.

Personal Protective Equipment:

Eye:
Safety glasses should be the minimum eye protection.

Skin:
Gloves should be used as minimum hand protection.

Respiratory:
No respiratory protection will be needed under normal industrial operating conditions.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
Boiling Point (C):	100
Melting Point (C):	0
Solubility in water:	100%
pH:	7.0
Vapor Pressure:	0.0212 ATM AT 20°C
Vapor Density:	17.3 G/M3
Specific Gravity/Density:	1.00
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	0.3 (BUTYL ACETATE = 1.0)
Viscosity:	0.98 cp at 20°C

10. STABILITY AND REACTIVITY

Stability:
Stable under ordinary conditions of use and storage.

Conditions to Avoid:
Strong oxidizers.

Hazardous Decomposition Products:

10. STABILITY AND REACTIVITY (CONT.)

Carbon monoxide. Carbon dioxide.

Hazardous Polymerization:
Not expected to occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
Not determined.

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
Not determined.

Target Organs: No data found.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

13. DISPOSAL CONSIDERATIONS (CONT.)

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
Dispose of by incineration following Federal, State, Local, or Provincial regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not regulated.
Subsidiary Hazards:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.
Materials used in manufacturing processes which are subject to compliance with the Federal Food Drug and Cosmetic Act (FDA) are not subject to the Toxic Substance Control Act (TSCA). The TSCA research use only restriction does not apply to FDA regulated manufacturing processes nor the resulting product per 15 U.S.C. 2602(2).

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT WHMIS Classification
No hazardous components

EUROPEAN UNION:

PRODUCT RISK PHRASES:
None assigned.

PRODUCT SAFETY PHRASES:
Not applicable.

15. REGULATORY INFORMATION (CONT.)

PRODUCT CLASSIFICATION:
N/A

Component	EINECS Number
No hazardous components	

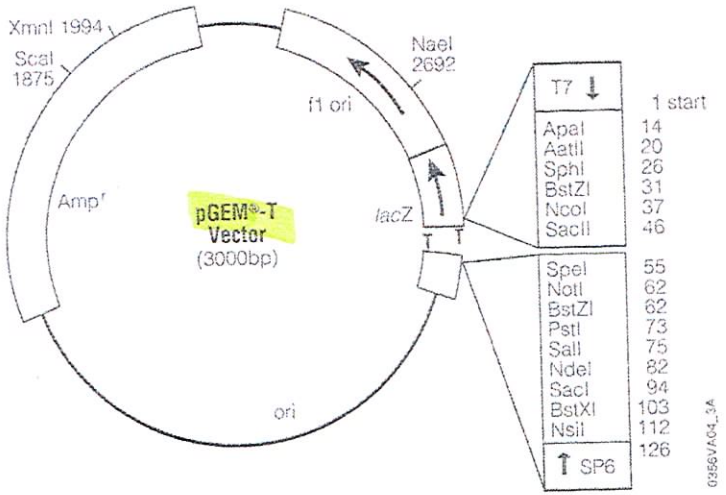
16. OTHER INFORMATION

HMIS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

Abbreviations
N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial Chemical Substances

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

5.B. pGEM®-T Vector Map and Sequence Reference Points



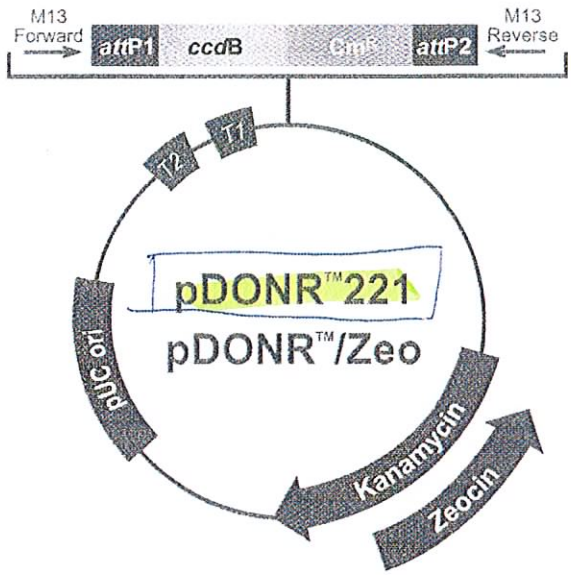
pGEM®-T Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–113
SP6 RNA polymerase promoter (–17 to +3)	124–143
SP6 RNA polymerase transcription initiation site	126
pUC/M13 Reverse Sequencing Primer binding site	161–177
*lac*Z start codon	165
lac operator	185–201
β-lactamase coding region	1322–2182
phage f1 region	2365–2820
lac operon sequences	2821–2981, 151–380
pUC/M13 Forward Sequencing Primer binding site	2941–2957
T7 RNA polymerase promoter (–17 to +3)	2984–3

Note: Inserts can be sequenced using the SP6 Promoter Primer (Cat.# Q5011), T7 Promoter Primer (Cat.# Q5021), pUC/M13 Forward Primer (Cat.# Q5601), or pUC/M13 Reverse Primer (Cat.# Q5421).

Note: A single digest with BstZI (Cat.# R6881) will release inserts cloned into the pGEM®-T Vector. Double digests can also be used to release inserts.

Info on Plasmid(s)



Comments for:	pDONR™221 4761 nucleotides	pDONR™/Zeo 4291 nucleotides
*rrn*B T2 transcription termination sequence (c):	268-295	268-295
rrnB T1 transcription termination sequence (c):	427-470	427-470
M13 Forward (-20) priming site:	537-552	537-552
*attP*1:	570-801	570-801
*ccd*B gene (c):	1197-1502	1197-1502
Chloramphenicol resistance gene (c):	1825-2505	1847-2506
*attP*2 (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

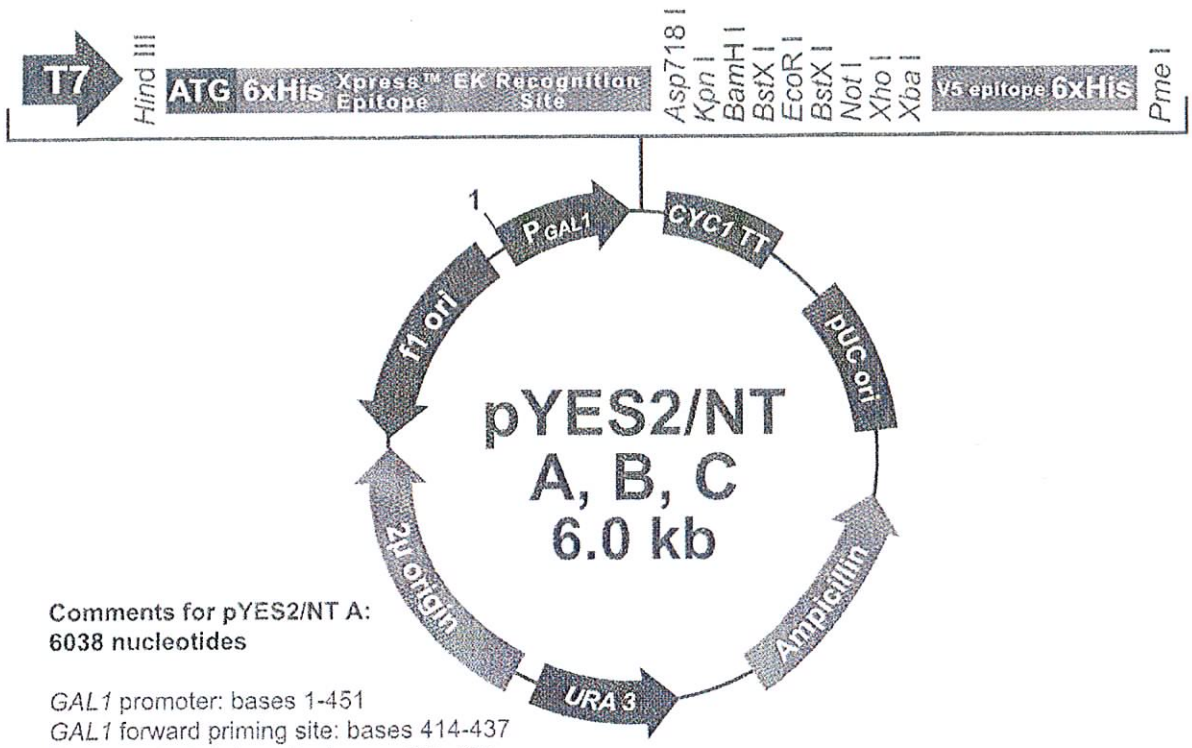
(c) = complementary strand

invitrogen™

pYES2/NT Vector

Map of pYES2/NT

The figure below summarizes the features of the pYES2/NT vectors. The complete sequences for pYES2/NT A, B, and C are available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 27).



Comments for pYES2/NT A:
6038 nucleotides

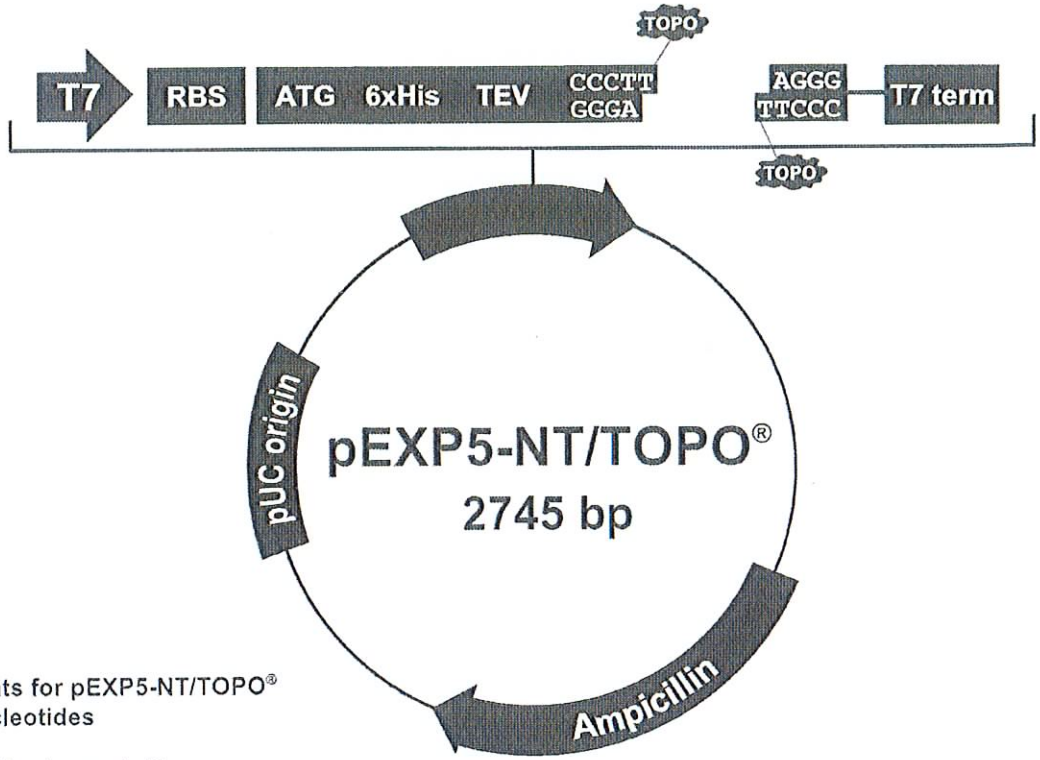
GAL1 promoter: bases 1-451
GAL1 forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
ATG initiation codon: bases 510-512
Polyhistidine (6xHis) region: bases 522-539
Xpress™ epitope: bases 579-602
Enterokinase (EK) recognition site: bases 588-602
Multiple cloning site: bases 602-669
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)

Continued on next page

Map and Features of pEXP5-NT/TOPO®

pEXP5-NT/TOPO® Map

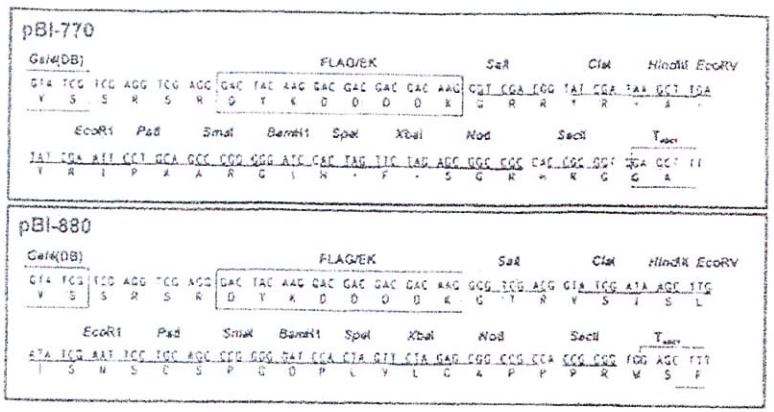
The figure below shows the features of the pEXP5-NT/TOPO® vector. The complete sequence of pEXP5-NT/TOPO® is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 36).



Comments for pEXP5-NT/TOPO®
2745 nucleotides

T7 promoter: bases 1-17
T7 forward priming site: bases 1-20
Ribosome binding site (RBS): bases 68-73
Initiation ATG: bases 80-82
Polyhistidine (6xHis) region: bases 92-109
HisG epitope: bases 92-112
TEV recognition site: bases 122-142
TOPO® recognition site 1: bases 141-145
TOPO® recognition site 2: bases 146-150
T7 reverse priming site: bases 198-217
T7 transcription terminator: bases 159-287
bla promoter: bases 399-497
Ampicillin resistance gene: bases 498-1358
pUC origin: 1503-2176

continued on next page



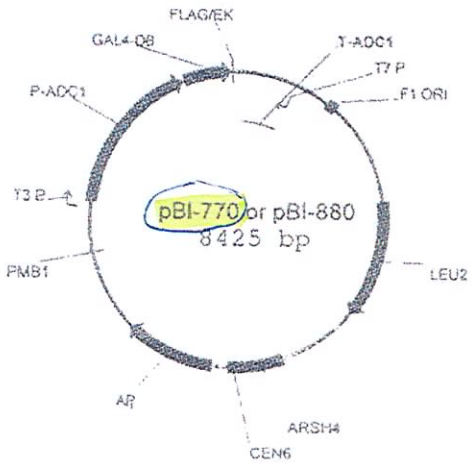
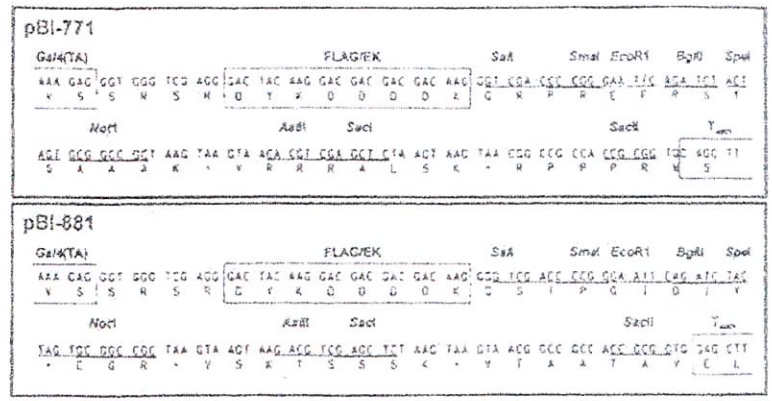


Fig. 1a



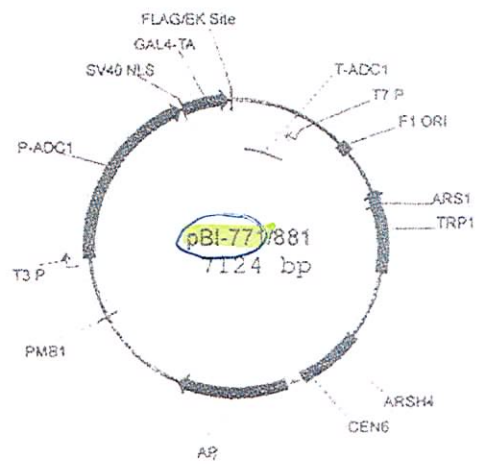
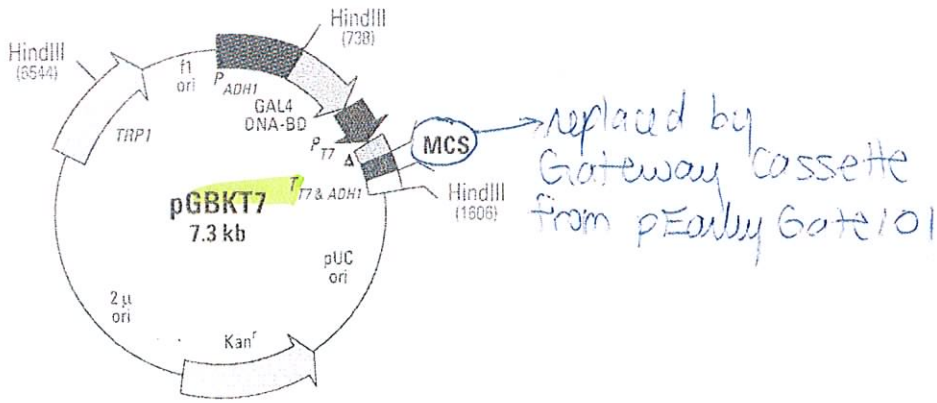
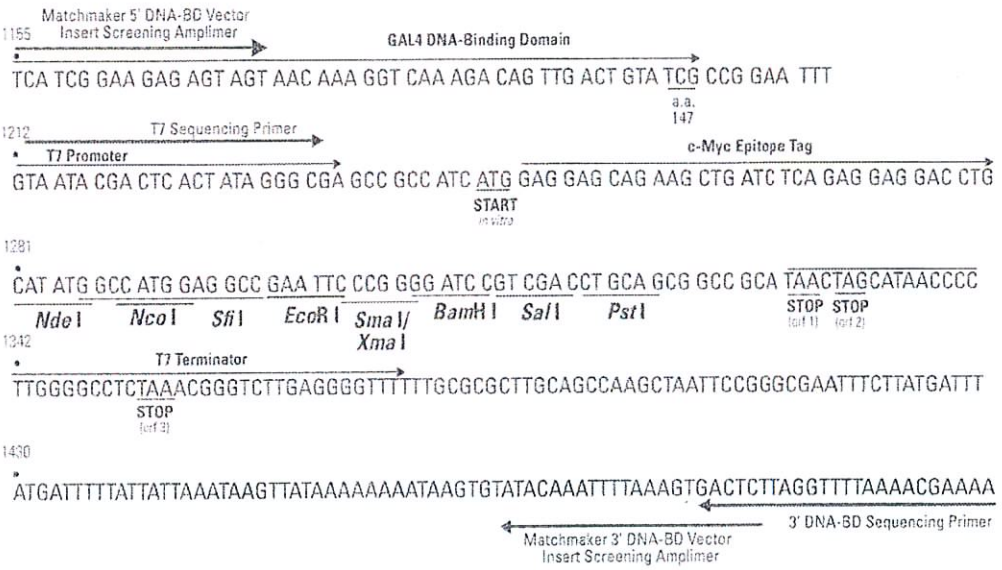


Fig. 1a,b. Sequence detail in the Multiple Cloning Site region, and overall maps of 2-hybrid 'bait' plasmids pBI-770-880 (Figure 1a) and 'prey' plasmids pBI-771/881 (Figure 1b).

...antibodies specific to GAL4 ... biological control. For screening putative



▲ c-Myc epitope tag



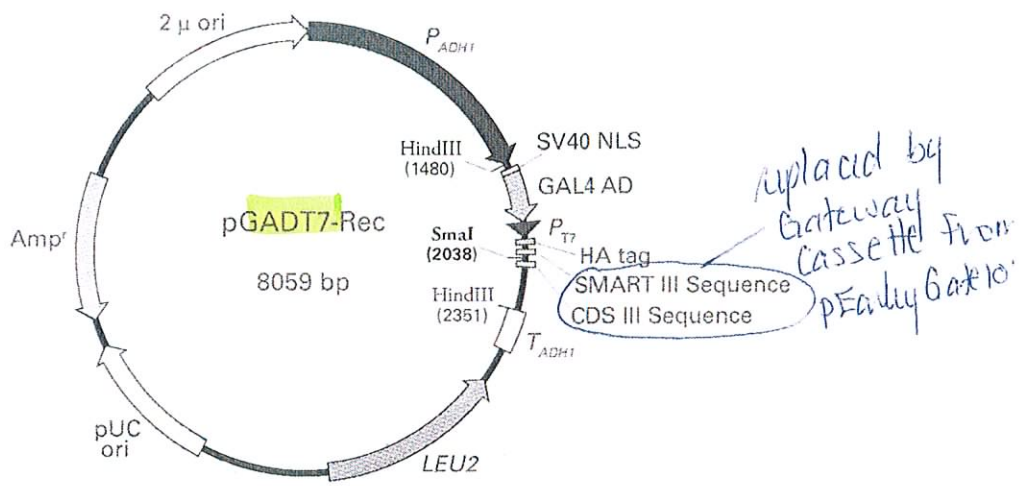
Restriction Map and Multiple Cloning Site (MCS) of pGBKT7. Unique restriction sites are in bold.

Description:

The pGBKT7 vector expresses proteins fused to amino acids 1–147 of the GAL4 DNA binding domain (DNA-BD). In yeast, fusion proteins are expressed at high levels from the constitutive *ADH1* promoter (P_{ADH1}); transcription is terminated by the T7 and *ADH1* transcription termination signals ($T_{T7\ \&\ ADH1}$). pGBKT7 also contains the T7 promoter, a c-Myc epitope tag, and a MCS. pGBKT7 replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 µ ori, respectively. The vector carries the Kan^r for selection in *E. coli* and the *TRP1* nutritional marker for selection in yeast. Yeast strains containing pGBKT7 exhibit a higher transformation efficiency than strains carrying other DNA-BD domain vectors (1).

(PR8Y2643; published 5 Dec 2008)

Sold as part of Cat. Nos. 630490 & 630491



SMART™ III terminus

MATCHMAKER 5′ AD LD-Insert Screening Amplimer → GAL4 Activation Domain (a.a. 881) — T7 Sequencing Primer / T7 Promoter

1858 CTA TTC GAT GAT GAA GAT ACC CCA CCA AAC CCA AAA AAA GAG ATC TTT AAT ACG ACT

START (*in vitro*) — HA Epitope Tag

1915 CAC TAT AGG GCG AGC GCC GCC ATG GAG TAC CCA TAC GAC GTA CCA GAT TAC GCT

SMART III Oligonucleotide Sequence

1969 CAT ATG GCC ATG GAG GCC AGT GAA TTC CAC CCA AGC AGT GGT ATC AAC GCA GAG TGG

2026 CCA TTA TGG CCC
Partial SmaI site

CDS III terminus

CDS III Primer Sequence

2038 GGG AAA AAA CAT GTC GGC CGC CTC GGC CTC TAG AGG GTG GGC ATC GAT ACG GGA TCC
Partial SmaI site

STOP

2095 ATC GAG CTC GAG CTG CAG ATG AAT CGT AGA TAC TGA AAAACCCCGCAAGTTCACTTC

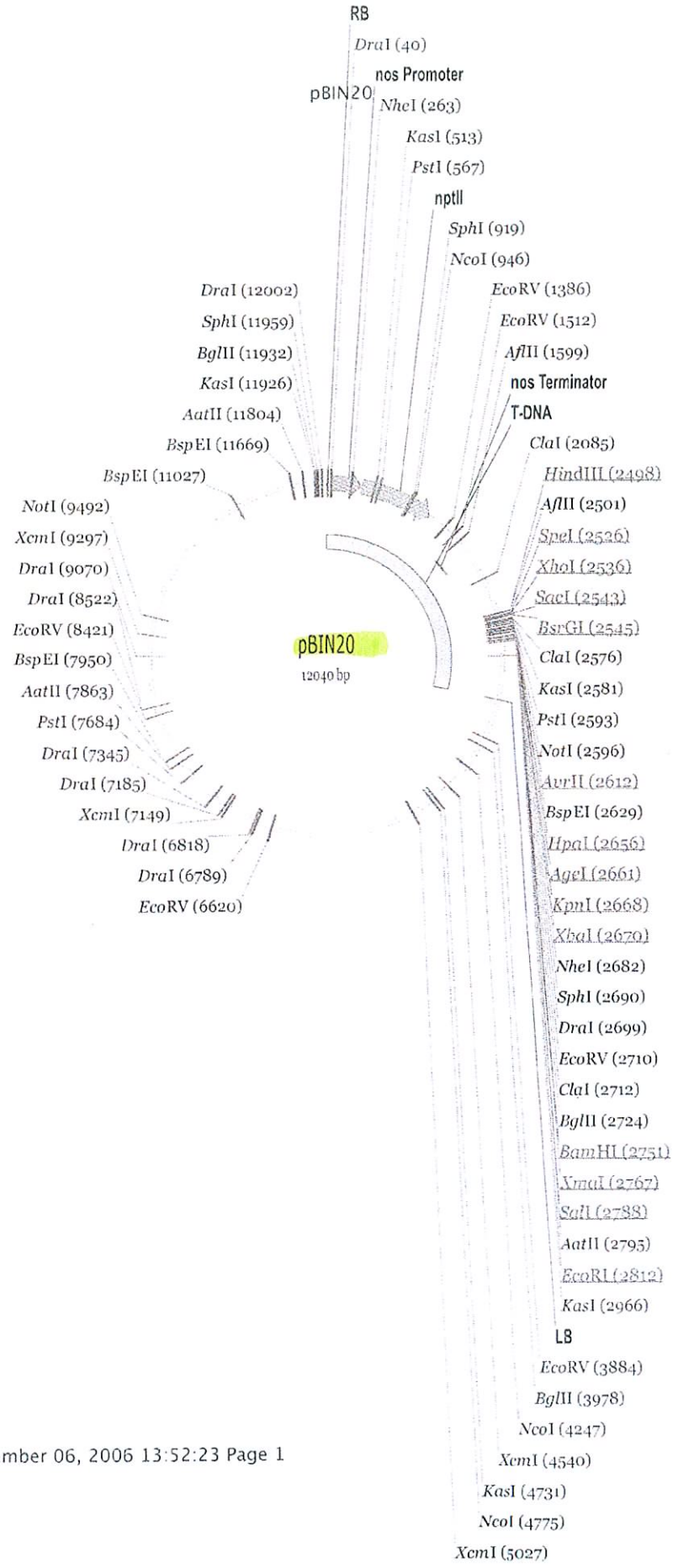
MATCHMAKER 3′ AD LD-Insert Screening Amplimer

2152 AACTGTGCATCGTGCACCATCT

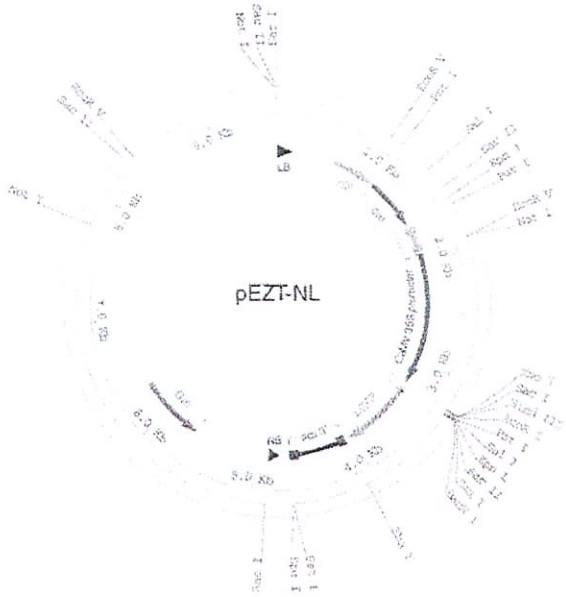
3′ AD Sequencing Primer

Figure 1. pGADT7-Rec Vector Map and Cloning Site. A unique restriction site (SmaI) is shown in bold. Both the Make Your Own "Mate & Plate" Library System and the Matchmaker™ Gold Yeast One-Hybrid System (Cat. Nos. 630490 and 630491, respectively) contain the SmaI-linearized form of this vector, the form used for recombination-mediated cloning in yeast.

(PR8Z2660 published 24 December 2008)


RB
DraI (40)
nos Promoter
pBIN20
NheI (263)
KasI (513)
PstI (567)
nptII
SphI (919)
NcoI (946)
EcoRV (1386)
EcoRV (1512)
AflII (1599)
nos Terminator
T-DNA
ClaI (2085)
HindIII (2498)
AflII (2501)
SpeI (2526)
XhoI (2536)
SacI (2543)
BsrGI (2545)
ClaI (2576)
KasI (2581)
PstI (2593)
NotI (2596)
AvrII (2612)
BspEI (2629)
HpaI (2656)
AgeI (2661)
KpnI (2668)
XbaI (2670)
NheI (2682)
SphI (2690)
DraI (2699)
EcoRV (2710)
ClaI (2712)
BglII (2724)
BamHI (2751)
XmaI (2767)
SalI (2788)
AatII (2795)
EcoRI (2812)
KasI (2966)
LB
EcoRV (3884)
BglII (3978)
NcoI (4247)
XcmI (4540)
KasI (4731)
NcoI (4775)
XcmI (5027)
DraI (12002)
SphI (11959)
BglII (11932)
KasI (11926)
AatII (11804)
BspEI (11669)
BspEI (11027)
NotI (9492)
XcmI (9297)
DraI (9070)
DraI (8522)
EcoRV (8421)
BspEI (7950)
AatII (7863)
PstI (7684)
DraI (7345)
DraI (7185)
XcmI (7149)
DraI (6818)
DraI (6789)
EcoRV (6620)
pBIN20
12040 bp

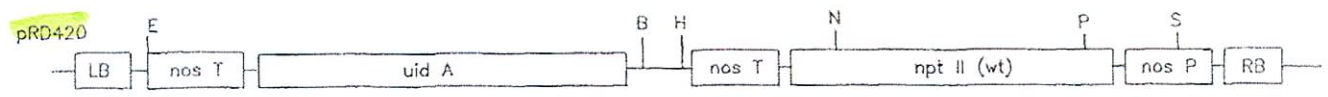
pEZT-NL map

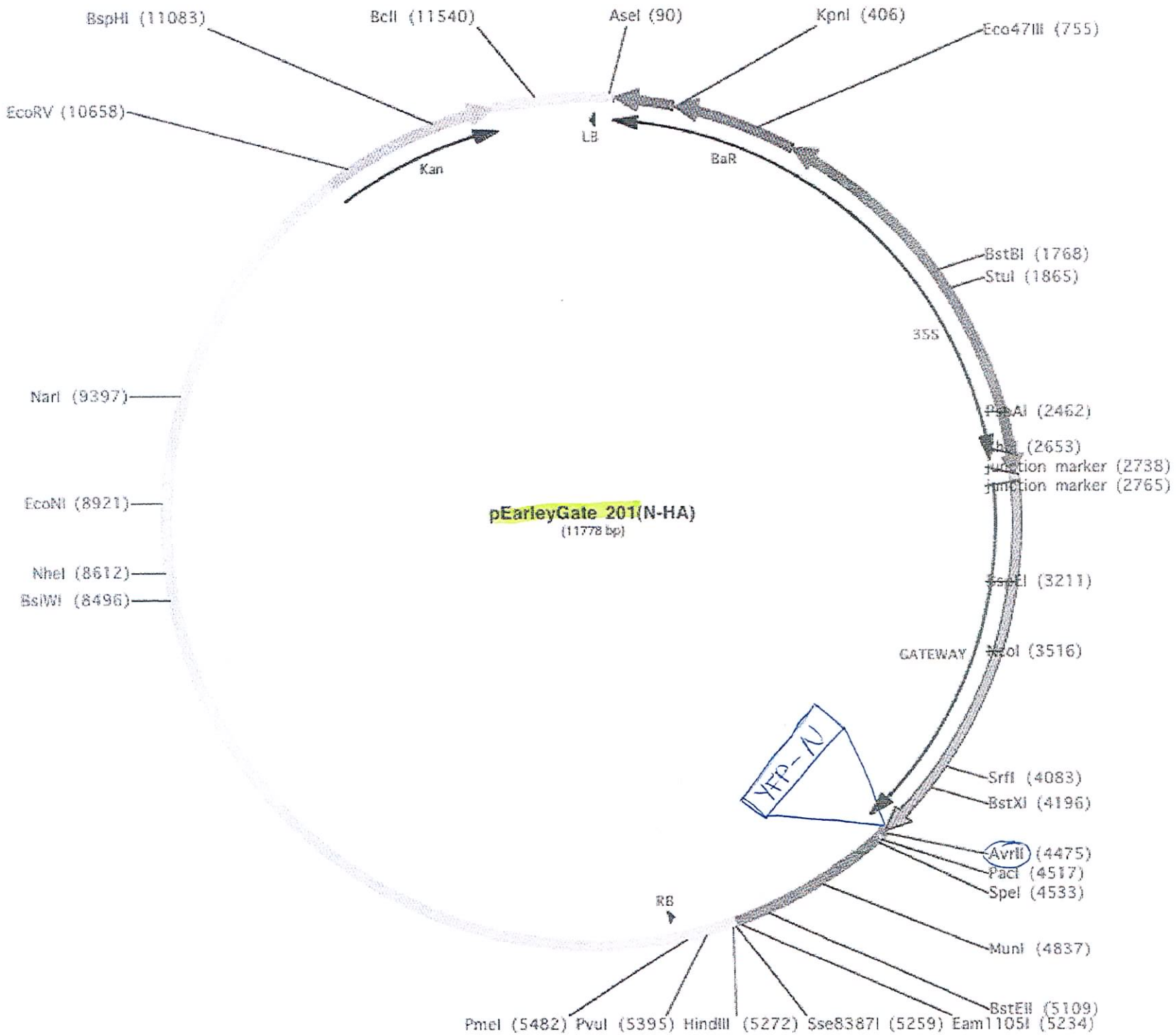


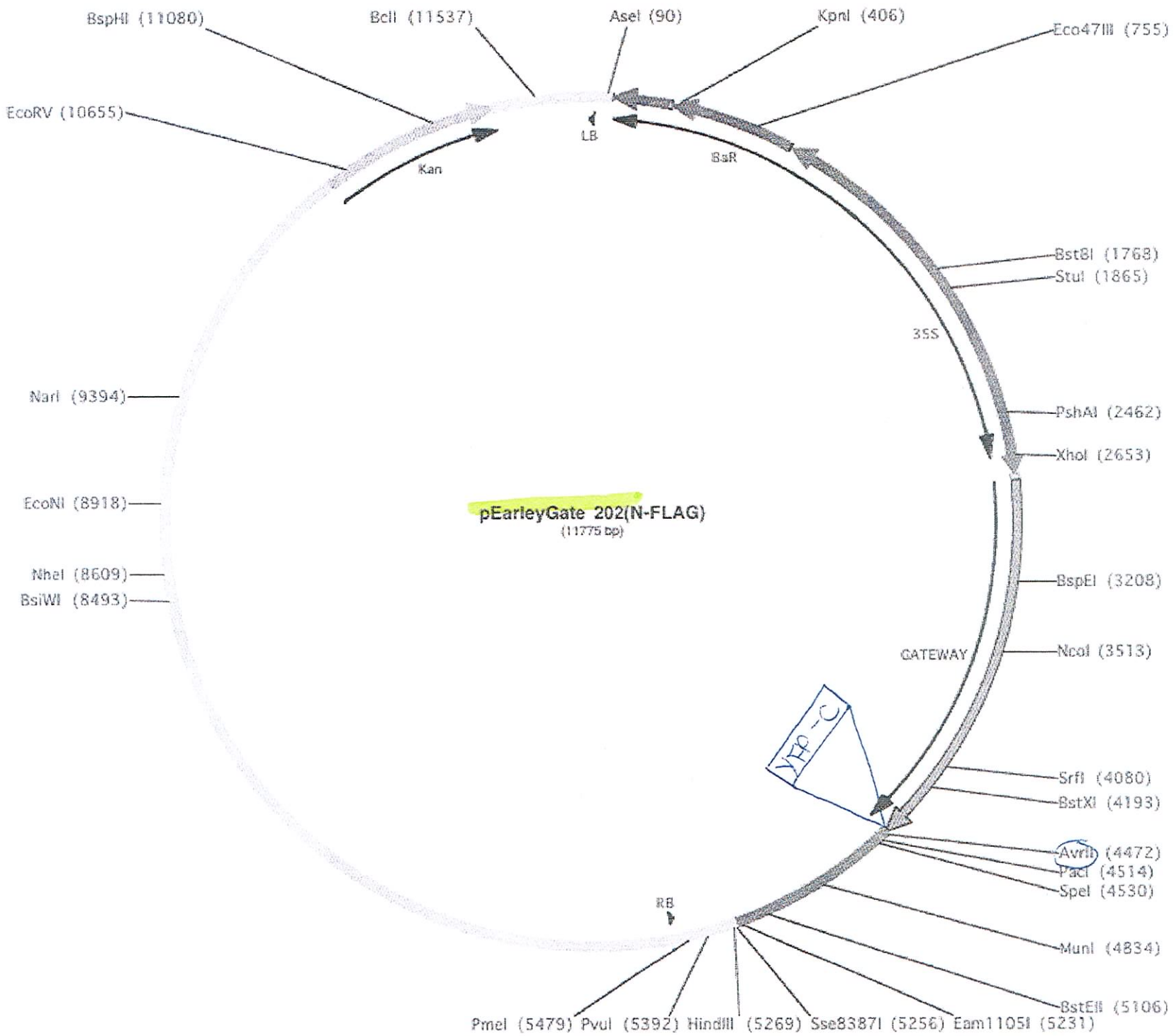
Unique sites are shown in red.

pEZS-NL map

Unique sites are shown in red.


pRD420
E
B
H
N
P
S
LB
nos T
uid A
nos T
npt II (wt)
nos P
RB


BspHI (11083)
BclI (11540)
AseI (90)
KpnI (406)
Eco47III (755)
EcoRV (10658)
LB
Kan
BaR
BstBI (1768)
StuI (1865)
35S
NarI (9397)
PstAI (2462)
(2653)
junction marker (2738)
junction marker (2765)
EcoNI (8921)
pEarleyGate 201(N-HA)
(11778 bp)
NheI (8612)
BslWI (8496)
SstI (3211)
GATEWAY
XcmI (3516)
YFP-N
SrfI (4083)
BstXI (4196)
AvrII (4475)
PacI (4517)
SpeI (4533)
RB
MunI (4837)
BstEII (5109)
PmeI (5482)
PvuI (5395)
HindIII (5272)
Sse8387I (5259)
Eam1105I (5234)


BspHI (11080)
BclI (11537)
AseI (90)
KpnI (406)
Eco47III (755)
EcoRV (10655)
LB
Kan
BsR
Bst8I (1768)
StuI (1865)
35S
NarI (9394)
PshAI (2462)
XhoI (2653)
pEarleyGate 202(N-FLAG)
(11775 bp)
EcoNI (8918)
BspEI (3208)
NheI (8609)
BsiWI (8493)
GATEWAY
NcoI (3513)
YFP-C
SrfI (4080)
BstXI (4193)
AvrII (4472)
PacI (4514)
SpeI (4530)
RB
MunI (4834)
BstEII (5106)
PmeI (5479)
PvuI (5392)
HindIII (5269)
Sse8387I (5256)
Eam1105I (5231)