

The University of Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) 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/.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

| |
|--|
| PRINCIPAL INVESTIGATOR: Greg Gloor |
| DEPARTMENT: Biochemistry |
| ADDRESS: MBL room C8 |
| PHONE NUMBER: 83526 |
| EMERGENCY PHONE NUMBER(S): 519-280-1384 |
| EMAIL: ggloor@uwo.ca |

Location of experimental work to be carried out :

| | | | |
|------------|------------|----------|-----------|
| Building : | MBL | Room(s): | C7 |
| Building : | _____ | Room(s): | _____ |
| Building : | _____ | Room(s): | _____ |

***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 / CIHR**

GRANT TITLE(S): **The relationship between coevolution and protein structure/function (NSERC)
 MATERNAL-INFANT MICROBIOME AND IMMUNITY (MIMI) NETWORK (CIHR)
 The Canadian vaginal microbiome project (CIHR)**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): **None**

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

**Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.**

NSERC: Multiple sequence alignments are often used to identify conserved positions in protein families, and aid structure-function analysis by guiding decisions as to which residues to mutate. Mutagenesis of conserved positions usually results in disruption of the protein's structure or function. Most positions in a typical protein family are thought of as uninteresting because they are not conserved— but mutating non-conserved positions in protein families frequently reduces or ablates protein function. Interestingly, some positions show context-dependent or covariant, mutation phenotypes since a mutation at one site can be suppressed by a different mutation in the same protein. My overall objective is to determine how non-conserved yet covariant positions contribute to protein structure and function. I have three major goals: 1) To demonstrate the general importance of residue covariation in protein structure and function. 2) To determine which of the two competing models best explain how covariant positions arise^{5,9}. 3) To develop and implement validated computational tools that identify covarying positions in protein families.

CIHR: The CIHR vaginal microbiome grant, and the Maternal microbiome initiative grants have the same general focus: how does the composition and function of the bacteria that live in and on us (the human microbiome) affect health and well-being? In collaboration with Dr Gregor Reid locally, and researchers from various sites in Canada (UBC, U of T, U. Saskatchewan, NRC) human microbiome samples are collected, processed and sent for sequencing. My role in these projects are in the initial experimental design and in the downstream data analysis. I am not involved in the collection or processing of samples. The results of the sequencing are analyzed to determine microbiome composition patterns that correlate with health outcomes.

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:

| Full Scientific 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 |
|--|--|--|--|--|---------------------------|--------------------------------|
| <i>Escherichia coli DH5 alpha</i> | Yes No | Yes No | Yes No | less than 1 litre | common lab E. coli strain | <u>1</u> 2 2+ 3 |
| <i>Escherichia coli K12</i> | Yes No | Yes No | Yes No | less than 1 litre | common lab E. coli strain | <u>1</u> 2 2+ 3 |
| <i>Escherichia coli JM109</i> | Yes No | Yes No | Yes No | less than 1 litre | common lab E. coli strain | <u>1</u> 2 2+ 3 |
| <i>Escherichia coli XL1Blue</i> | Yes No | Yes No | Yes No | less than 1 litre | common lab E. coli strain | <u>1</u> 2 2+ 3 |
| <i>Saccharomyces cerevisiae</i> | Yes No | Yes No | Yes No | less than 1 litre | Dr Chris Brandl | <u>1</u> 2 2+ 3 |
| | Yes No | Yes No | Yes No | | | 1 2 2+ 3 |
| | Yes No | Yes No | Yes No | | | 1 2 2+ 3 |
| | Yes No | Yes No | Yes No | | | 1 2 2+ 3 |

**Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:*
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: _____

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 | Yes No | | Not applicable |
| Rodent | Yes No | | |
| Non-human primate | Yes No | | |
| Other (specify) | Yes No | | |

1.3 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 | Yes No | | | |
| Rodent | Yes No | | | |
| Non-human primate | Yes No | | | |
| Other (specify) | Yes No | | | |

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

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

Additional Comments: _____

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 | | Yes Unknown | | 1 2 2+ 3 |
| Human Blood (fraction) or other Body Fluid | | Yes Unknown | | 1 2 2+ 3 |
| Human Organs or Tissues (unpreserved) | | Yes Unknown | | 1 2 2+ 3 |
| Human Organs or Tissues (preserved) | | Not Applicable | | Not Applicable |

Additional Comments: **Clinical swabs or faecal matter collected by collaborators under their permits and protocols in their labs. No material is ever sent to, or processed in my lab. Only the DNA or RNA sequence is received by my group**

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 Transformed or Transfected | Will there be a change due to transformation of the bacteria? | Will there be a change in the pathogenicity of the bacteria after the genetic modification? | What are the consequences due to the transformation of the bacteria? |
|---------------------------------|--|-------------------------------|--|---|---|--|
| E. coli DH5 alpha | ColE1 - replicon pUC (pProEXHTa I-Ltr, pCcdB/I-LtrI, LtrI_Litmus2 8i , pUC18, pPM28, pPM47) and pSC101 (pENdo/I-Ltr) based vectors and derivatives | Novagen and lab stocks | LAGLIDAG homing endonuclease, ccdB gyrase inhibitor | yes | No | Become resistant to ampicillin and/or chloramphenicol, overexpress proteins for biochemical studies |
| Saccharomyces cerevisiae | standard shuttle vectors (pYIplac128) | Dr Brandl | Leu2 | yes | no | ability to grow in the absence of leucine |

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

*** Please attach a plasmid map.*

****No Material Safety Data Sheet is required for the following strains of E. coli:*

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

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.*

4.3.1 Will virus be replication defective? YES NO

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

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

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? NO YES

Additional Comments:

6.0 Human Gene Therapy Trials

6.1 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 7.0

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

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

1.4 How will the biological agent be administered?

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

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

7.0 Animal Experiments

7.1 Will live animals be used? YES NO If NO, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

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

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

8.0 Use of Animal species with Zoonotic Hazards

8.1 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 9.0

8.2 Will live animals be used? YES NO

8.3 If YES, please specify the animal(s) used:

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

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

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? YES **NO** If **NO**, please proceed to Section 10.0

1.2 If YES, please name the toxin(s) or hormones(s)

Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

1.3 What is the LD₅₀ (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? YES NO

If **YES**, Please provide details:

*For information on biosecurity requirements, please see:

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? Initiate and maintain colony, give location:

"One-time" use, give location:

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

10.7 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:

11.0 Plants

1.1 Do you use plants? YES NO - If **NO**, please proceed to Section 12.0

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

1.3 What is the origin of the plant?

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

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

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

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

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

12.0 Import Requirements

12.1 Will any of the above agents be imported? YES, country of origin NO
If **NO**, please proceed to Section 13.0

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

12.3 Additional Comments: _____ Has
an

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

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

13.0 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.

An X in the check box indicates you agree with the above statement...

Enter Your Name X **Date:** _____

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

Special Conditions of Approval:



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. Shamiia Survery / Mr. Michael Decosimo
Cedariane 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 considered 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 | • DP50 | • 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

Plasmid 20131: pPM28 (eroGFP GEN/ARS URA3)

Gene/insert name: eroGFP
Alternative names: kar2ss-roGFP2-HDEL
KAR2
Insert size (bp): 1828
Gene/insert aliases: KAR2, GRP78
Species of gene(s): *S. cerevisiae* (budding yeast)
jellyfish
Vector backbone: pRS316
([Search Vector Database](#))
Type of vector: Yeast expression
Backbone size (bp): 4835
Cloning site 5': SacI
Site destroyed during cloning: No
Cloning site 3': HindIII
Site destroyed during cloning: No
5' Sequencing primer: n/a ([List of Sequencing Primers](#))
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard *E. coli* @ 37C: Yes
Selectable markers: URA3
If you did not originally clone this gene, from whom and where did you receive the plasmid used to derive this plasmid: S. James Remington University of Oregon
Sequence: Visit www.addgene.org/20131
Plasmid Provided In: DH5a
Principal Investigator: Feroz Papa

Article: [Real-time redox measurements during endoplasmic reticulum stress reveal interlinked protein folding functions](#). Merksamer PI et al. (Cell. 2008 Nov 28. 135(5):933-47. [Pubmed](#))

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 20131" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

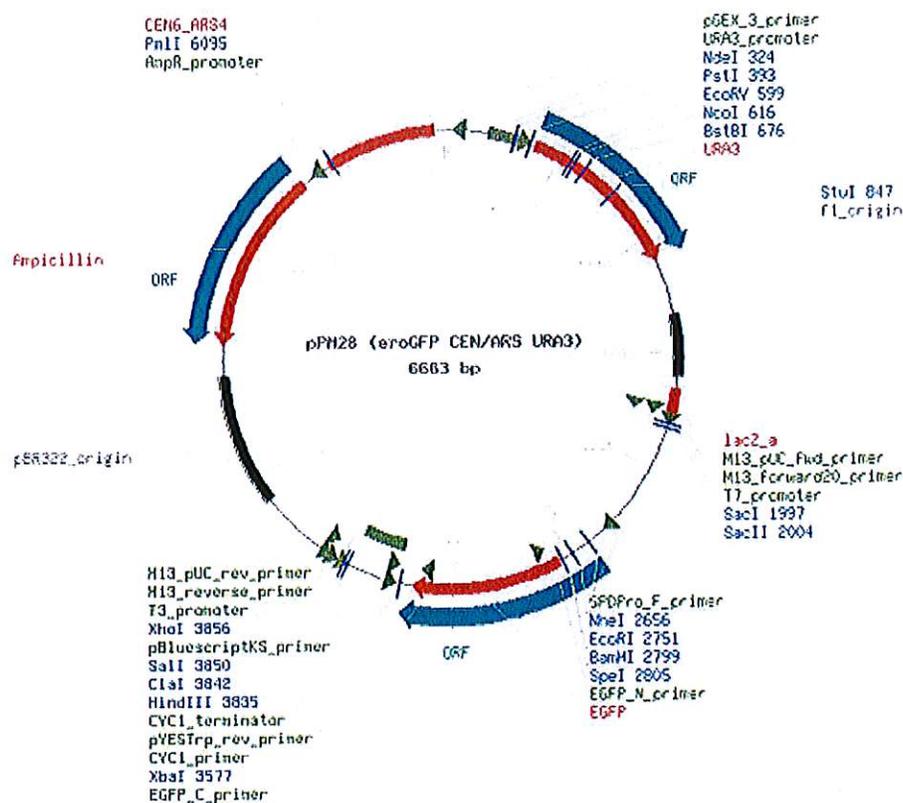
Please check www.addgene.org/20131 for updated plasmid information and related links.

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Information on this datasheet is provided pursuant to Addgene's Terms of Use at www.addgene.org.



Find this plasmid at: www.addgene.org
Enter "20131" in the search box



Please check www.addgene.org/20131 for updated plasmid information and related links.

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Find this plasmid at: www.addgene.org
Enter "20132" in the search box

Plasmid 20132: pPM47 (UPR-RFP GEN/ARS URA3)

Gene/insert name: UPR-RFP
 Alternative names: 4xUPRE-mCherry
 Insert size (bp): 1419
 Species of gene(s): *S. cerevisiae* (budding yeast)
 Other
 Vector backbone: pRS316
 ([Search Vector Database](#))
 Type of vector: Yeast expression
 Backbone size (bp): 4835
 Cloning site 5': SacII
 Site destroyed during cloning: No
 Cloning site 3': HindIII
 Site destroyed during cloning: No
 5' Sequencing primer: n/a ([List of Sequencing Primers](#))
 Bacteria resistance: Ampicillin
 High or low copy: High Copy
 Grow in standard *E. coli* @ 37C: Yes
 Selectable markers: URA3
 Sequence: Visit www.addgene.org/20132
 Plasmid Provided In: DH5a
 Principal Investigator: Feroz Papa

Comments: There is a 2 base gap between depositor's sequence and Addgene sequence; per depositor this gap is in the promoter region and shouldn't significantly affect the plasmid.

Article: [Real-time redox measurements during endoplasmic reticulum stress reveal interlinked protein folding functions.](#) Merksamer PI et al. (Cell. 2008 Nov 28. 135(5):933-47. [Pubmed](#))

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 20132" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

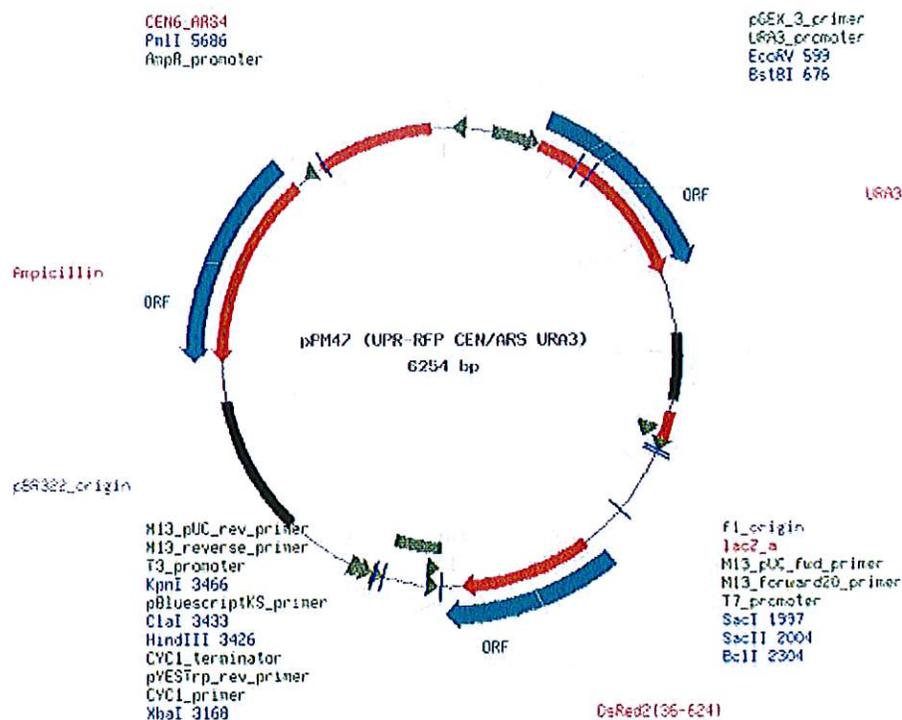
Please check www.addgene.org/20132 for updated plasmid information and related links.

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Find this plasmid at: www.addgene.org
Enter "20132" in the search box

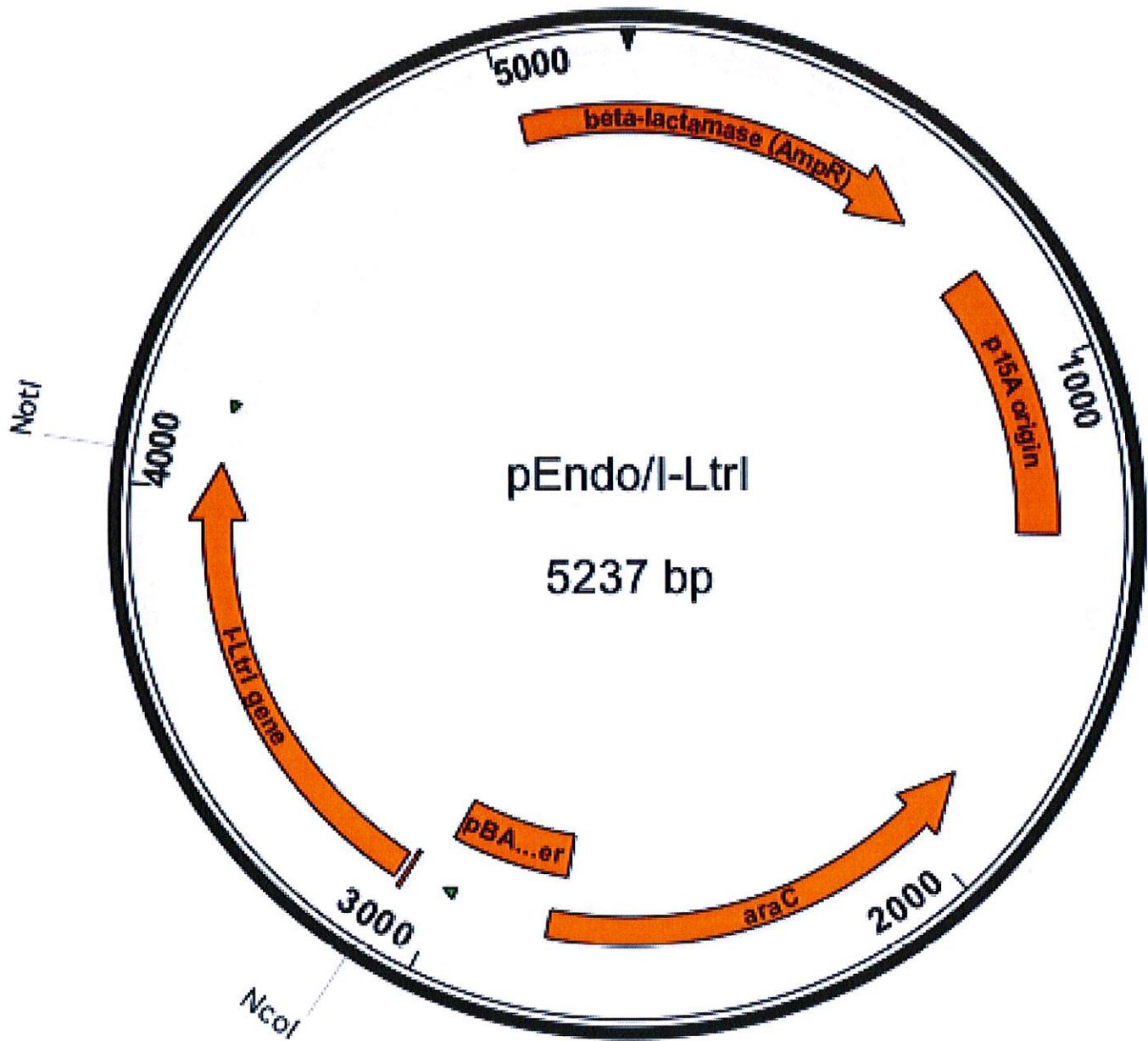


Please check www.addgene.org/20132 for updated plasmid information and related links.

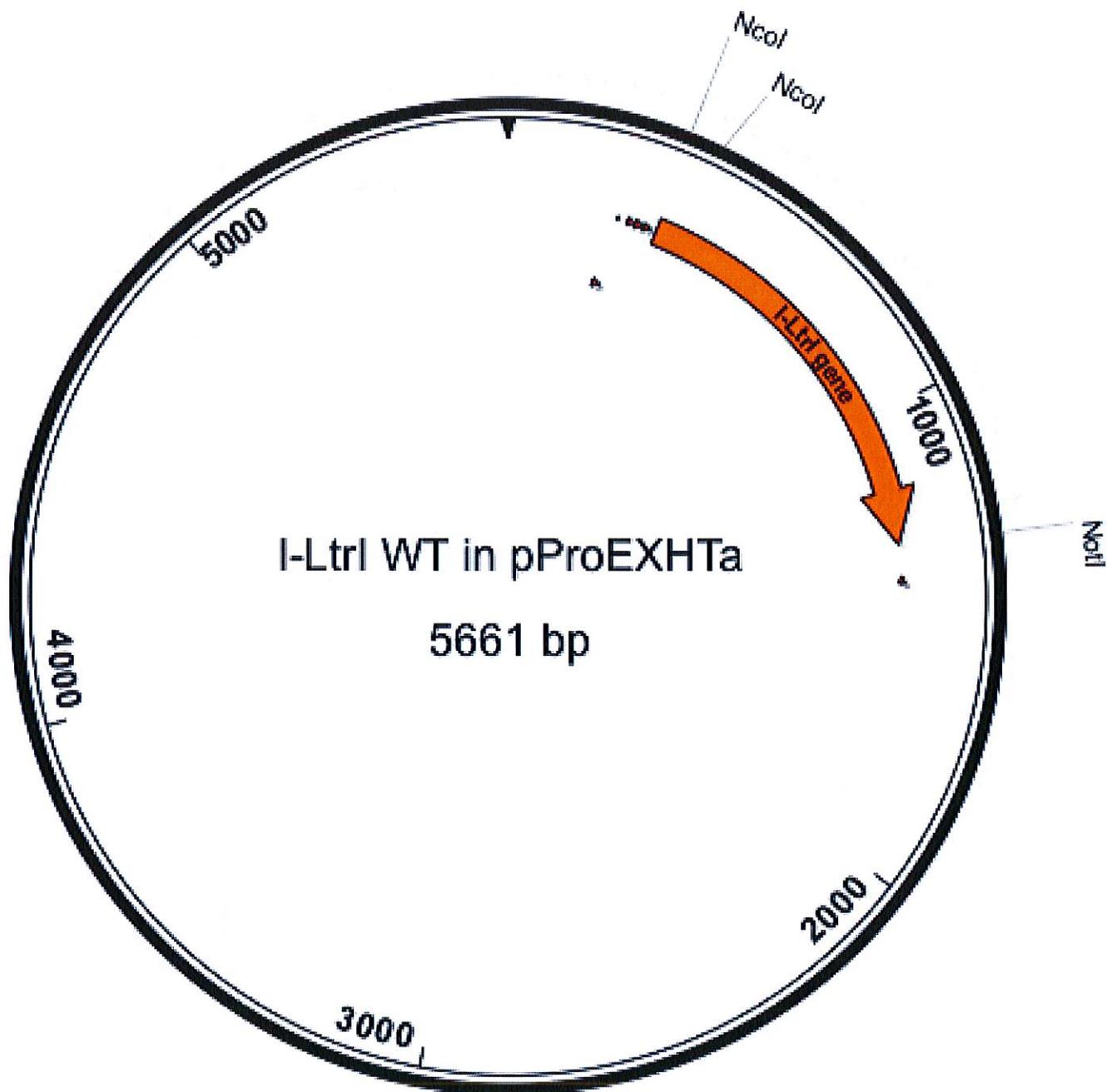
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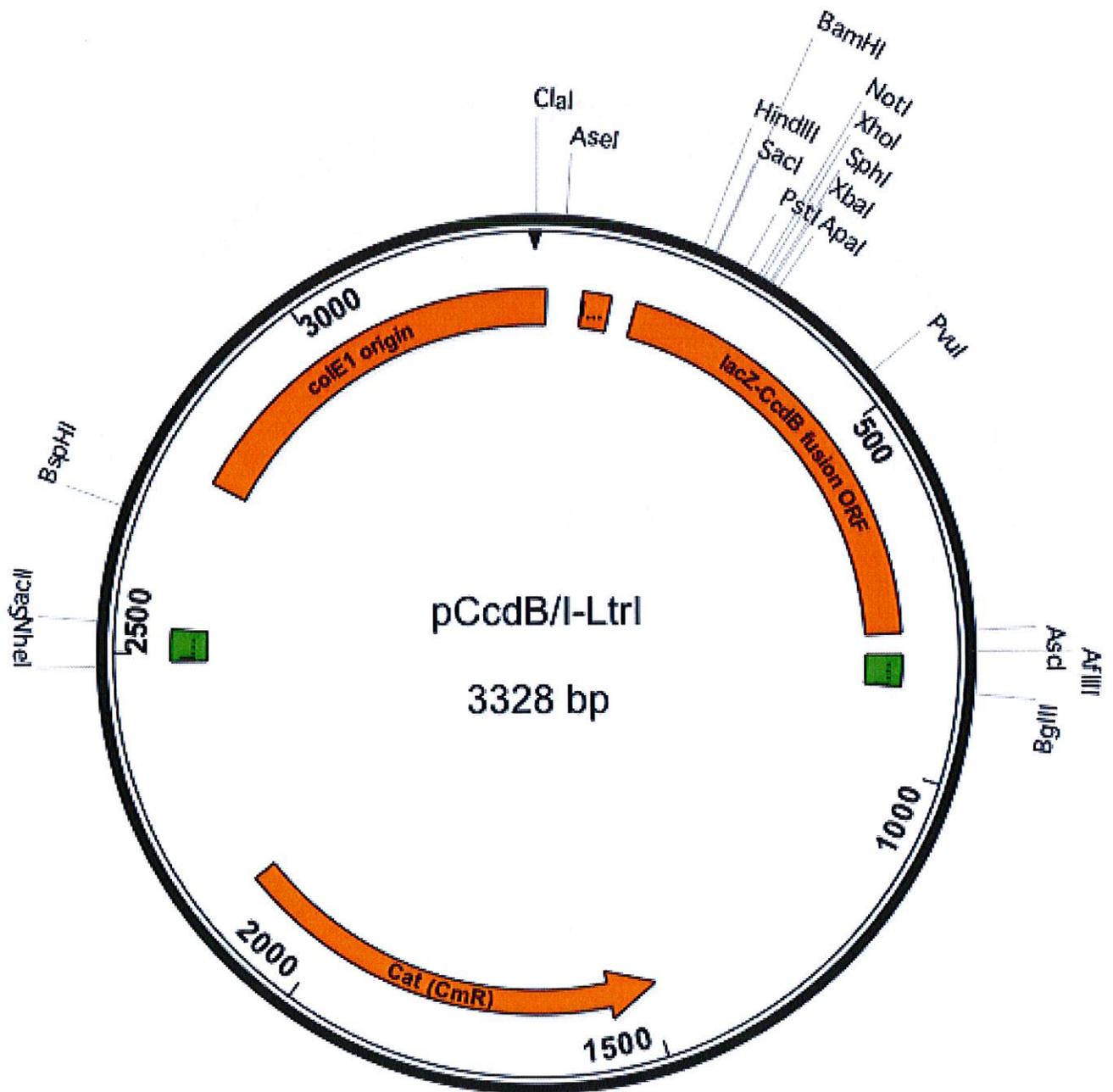
pEndo – used for protein expression within the genetic selection. LtrI and OnuI were directionally cloned into the vector using an n-terminal NcoI and c-terminal NotI. This vector (and ORFs within) were used as template for all quickchange.



pProExHta- protein expression vector used for inducing protein for purification. ORFs were cloned in using the n-terminal NcoI and c-terminal NotI. Note that the second NcoI site (within the LtrI ORF) was eliminated using a synonymous mutation.



pCcdB- the toxic plasmid used for the genetic selections. Two target sites for LtrI were cloned into this vector using NheI/SacII and AflIII/BglII. The same sites were also used to clone in the OnuI sites.



Litmus28i- used as substrate for the cleavage assays. LtrI/OnuI target sites were cloned into the vector using AflIII/BglII sites.

