THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 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	Stanley D. Dunn
DEPARTMENT	Biochemistry
ADDRESS	M324 Medical Sciences Building
PHONE NUMBER	83055
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Location of experimental work to be carried out: Building(s) _Medical Sciences Building__ Room(s)_M323__

*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:____CIHR_____
GRANT TITLE(S): _____Energy Coupling in ATP Synthase_____

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

Name	UWO E-mail Address	Date of Biosafety Training
Yumin Bi*	ybi@uwo.ca	15-Sep-2010 (registered)*
Ardeshir Goliaei	agoliaei@uwo.ca	31-Aug-2010
David Goodwin	dgoodwi3@uwo.ca	23-Sep-2009
*Yumin took Biosafety training in 1998 when she started working for me; this will be a refresher.		

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.

The goals of my research program are to elucidate structural and mechanistic features of the bioenergetic enzyme ATP synthase, which produces ATP in oxidative phosphorylation. We use the enzyme from *Escherichia coli* as a mode system for these studies. The strains we use are all standard, nonpathogenic laboratory strains.

Our use of the bacteria could be divided into 3 general areas:

1. Study of the growth of the bacteria under various conditions, as a test of the functionality of their biioenergetics.

2. Study of the bioenergetic functionality of enzymes or membrane vesicles prepared from the bacteria, which would usually be grown in 1-liter cultures, though occasionally larger cultures are made to provide larger amounts of materials.

3. Engineering of ATP synthase or of its constituent pypeptides or fragments thereof. In these studies the genes are carried on plasmids such as pUC8, pACYCDuet, or pACYC184. Subcloning DNA fragments and introduction of mutations are carried out by standard recombinant DNA methods. Plasmids are prepared by the classical Quick-Prep or newer QIA-Prep methods. Most mutations are introduced by PCR and ligation of PCR products into appropriate plasmid vectors. Some related ATP synthase polypeptides from the nonpathogenic soil bacterium Rhodobacter capsulatus are also currently under study. We do not culture or study this organism, rather we have the cloned genes originally provided by another investigator, and these are expressed in E. coli.

General Procedures regarding storage, use and disposal

Strains of E. coli, usually expressing various polypeptides and proteins from plasmids, are used in the types of studies listed under 1 and 2.

Strains are stored as permanent frozen cultures in screw-cap tubes at -80 C. Cells are grown in standard laboratory incubators or fermentors and stored frozen at -80 C. Bacteria are disrupted using a French Pressure Cell and their contents fractionated by centrifugation and column chromatography.

Culture plates, samples from recombinant DNA experiments, and small cultures are disinfected by autoclaving. Larger amounts of used growth medium or unwanted cell fractions and materials, and glassware used in handling these materials, are disinfected by addition of chlorine bleach.

Please include a one page research summary or teaching protocol.

Summary of CIHR grant (2008-2013):

ATP synthase couples the translocation of protons across a membrane to the synthesis of ATP in the process of oxidative phosphorylation. Coupling occurs through a remarkable rotational mechanism, leading to the enzyme being called the "world's smallest motor". Basic principles of how the motor functions are best studied in the relatively simple enzyme of the common bacterium, *Escherichia coli*. Our interest is focussed on determining how the two connections, or stalks, between the membrane-integral F0 sector and the membrane-peripheral F1 sector ensure that ion-driven turning of the rotor is linked to the production of ATP. The central γε stalk is part of the rotor along with the *c* subunit ring, while the peripheral stator stalk is formed by a pair of *b* subunits that interact through a novel asymmetric right-handed coiled coil in the dimerization domain. We denote one *b* subunit *b*N and the other *b*C, based on an offset of their helices by approximately five and one half residues. Some species have homodimeric *b*2 stalks, while others have heteromeric *bb'* stalks composed of nonidentical *b*-type polypeptides. Recent work in this laboratory has demonstrated how mutation or alteration to either the ε or *b* subunits can lead to loss of coupling efficiency. The goal of this project is to determine how the structures, conformational changes, and subunit interactions of these two subunits are involved in energy coupling in ATP synthase.

The **central hypotheses** are: 1) that the two *b*-type subunits in the heterodimers of some species each preferentially occupies one of the two offset positions, 2) that the offset of the helices of the two *b* subunits occurs in intact ATP synthase, with the *b* subunit in each position making different interactions with other subunits and that together they provide elastic resistance to distortion from the torque imparted by turning of the rotor, and 3) that the C-domain of the ε subunit is involved in energy coupling because it relates rotational events to specific steps in ATP hydrolysis or synthesis, particularly the binding and release of phosphate.

The **objectives** of the proposal and the **approaches** to be adopted are as follows:

1. Express the dimerization domains of the heterodimeric *bb'* systems seen in some species, determine their interhelical relationship by analysis of disulfide bond formation between positions central to the helix-helix interface, and extend the analysis using FRET between positions peripheral to the interface. Investigate the potential for high resolution structural determination of the heterodimeric systems.
2. Extend our studies of the different roles and positions of the two *b* subunits, previously conducted in an *in vitro* *b*2-F1 system, to intact ATP synthase in cells and membrane vesicles, through ongoing development and characterization of a system using a pair of heterochimeric *b* subunits, each consisting largely of *E. coli b* sequence but incorporating regions essential for dimerization from the two *b*-type subunits of a *bb'* system. This will make it possible to independently label or manipulate the two polypeptides. Confirm the positional occupancy of each polypeptide, then use the system to study conformational or positional effects of the addition of substrates or the imposition of protonmotive force, utilizing a FRET approach. Analyze how the *b* dimer determines the relationship between the *a* subunit of F0 and the δ subunit of F1, which it links, in the presence and absence of a protonmotive force. Determine how these relationships change in uncoupled *b* mutants, and in functional *b* mutants carrying insertions or deletions, making them longer or shorter. Extend our analysis of uncoupling mutations in *b* subunits by construction of a series of glycine substitution mutants, designed to reduce the torsional stability of the coiled coil without affecting the subunit relationship in the C-terminal F1-binding domain.
3. Test the role of ε in promoting appropriate substrate binding by relating the effects of deleting or mutationally altering the C-terminal domain to the ability to promote ADP- and phosphate-dependent high-efficiency coupling. Identify positions of the C-domain that contribute to function through an alanine-scanning approach; relate effects to structural and coevolutionary data. Undertake collaborative single-molecule rotational studies to determine effects of uncoupling mutations on rotational steps in the catalytic mechanism.

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 xNO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____

_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____

_____

1.2 Please complete the table below:

Name of Biological agent(s)*	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 strains K12, MM294, JM109, BL21(DE3)	Yes x No	Yes x No	Yes x No	100	Laboratory stocks of many years	x 1 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.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES x 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		

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)*	Supplier / Source
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)

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES 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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		Yes Unknown		O 1 O 2 2+ O 3
Human Blood (fraction) or other Body Fluid		Yes Unknown		O 1 O 2 2+ O 3
Human Organs or Tissues (unpreserved)		Yes Unknown		O 1 O 2 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 NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
E. coli strains K12, MM294, JM109, BL21(DE3)	*pACYCDuet and derivatives, e.g. pSD451; pUC8 and derivatives, e.g. pSD51; pACYC184 And derivatives, e.g. pACWU1.2*	*Novagen, laboratory stocks, other investigators (e.g. Robert Nakamoto of the University of Virginia provided pACWU1.2)*	*Subunits of ATP synthase*	*Nonpathogenic, harmless proteins are expressed*

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

4.3 Will genetic modification(s) involving viral vectors be made? 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 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 YES x NO
- E1A oncogene YES x NO
- Known oncogenes YES, please specify _____ x NO
- Other human or animal pathogen and or their toxins YES, please specify _____ x NO

4.5 Will virus be replication defective? NA YES NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? 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? YES NO

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

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

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? 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? YES, Please provide details:_____ NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES 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? 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? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

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

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

10.3 What is the origin of the plant? _____

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

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

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

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

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

11.0 Import Requirements

11.1 Will any of the above agents be imported? 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? YES NO

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

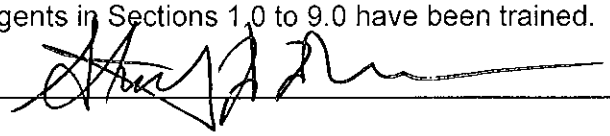
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE 

13.0 Containment Levels

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

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

x YES, permit # if on-campus__BIO-UWO-0088_____

NO, please certify

NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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: Sept 2, 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

___NA_____

_____

_____

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

__We would encourage bleeding from the wound, wash the area with soap and water, apply bandage, identify the potential biological agent (noting antibiotic resistance provided by plasmid that may have been carried by bacterium), notify the UWO Biosafety Officer, seek medical attention, file an accident report, ensure that the affected individual is monitored for any effects or evidence of infection over several days/weeks._

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:

NEW ENGLAND BioLabs Inc.
New England Biolabs
240 County Road
Ipswich, MA 01938

MATERIAL SAFETY DATA SHEET (MSDS)

Telephone: (978) 927-5054
Toll free: (800) 632-5227
Fax: (978) 921-1350
Email: info@neb.com
Revision Date: 9/10

NEB #C2527

SECTION 1–CHEMICAL INFORMATION

Product Name: BL2(DE3) Competent *E. coli* Cas.# None

SECTION 2–COMPOSITION/INFORMATION ON INGREDIENT

1. Glycerol 1–10% Cas.# 56-81-5
2. Dimethyl Sulfoxide 1–10% Cas.# 67-68-5

The ingredients listed in this section include only those items that have more than 1% of a component classified as hazardous and 0.1% of a component classified as carcinogenic. If you have any questions, please contact info@neb.com.

SECTION 3–HAZARDOUS IDENTIFICATION

Emergency Overview: Warning: May cause irritation to skin, eyes, and respiratory tract, may affect kidneys, blood and liver.

HMIS and NFPA Ratings: 0 – Minimal or None, 1 – Slight, 2 – Moderate, 3 – Serious, and 4 – Severe

Health: 1
Flammability: 1
Reactivity: 0

SECTION 4–FIRST AID MEASURES

Eyes: Flush eyes with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating eyelids. Call a physician.

Skin: Wash skin with soap and copious amount of water.

Ingestion: If the person is conscious, wash out mouth with water. Call a physician.

Inhalation: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

SECTION 5–FIRE FIGHTING MEASURES

Extinguishing Media: Water spray. Carbon dioxide, dry chemical powder or appropriate foam.

Special Fire Fighting Procedures: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

Fire and Explosion Hazards: Combustible liquid. Emits toxic fumes under fire conditions.

SECTION 6–ACCIDENTAL RELEASE MEASURES

Personal Precautions: Avoid breathing or contact with vapors, mist or gas.

Procedure of Personal Precaution: Wear self-contained breathing apparatus, rubber boots and heavy rubber gloves and chemical safety goggles. Use non-sparking tools and equipment. Ventilate and evacuate area of leak or spill.

Environmental Precautions: Do not let product enter drains.

Methods For Cleaning Up: Cover with dry lime, sand, or soda ash. Sweep up and shovel. Place in covered container for disposal.

SECTION 7–HANDLING AND STORAGE

Handling: Provide appropriate exhaust ventilation.

User Exposure: Avoid inhalation. Avoid contact with DMSO solutions containing toxic materials or material with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

Storage: Keep tightly closed in a dry and well ventilated place. Store at -20°C .

SECTION 8–EXPOSURE CONTROLS/PPE

Engineering Controls: Safety shower and eye wash. Mechanical exhaust.

Personal Protective Equipment

Eye Protection: Safety goggles.

Hand Protection: Compatible resistant gloves.

Respiratory Protection: Government approved respirator.

Hygiene Measure: General practice, wash (hands and skin) thoroughly after handling. Remove and wash contaminated clothing.

SECTION 9–PHYSICAL AND CHEMICAL PROPERTIES

Physical State: Form: Liquid Color: Clear or colorless Odor: No Data Available

Property	Value	Temperature or Pressure	
Boiling Point Range:		>189°C	
Melting Point Range:		>18.4°C	
Flash Point:		>87°C	Method: Closed cup
Auto Ignition Temp:		>215°C	
Vapor Pressure:	.42 mmHg	20°C	
Vapor Density:	2.7 g/l		
Specific Gravity:	1.1		
Solubility in Water:	Soluble		

SECTION 10–STABILITY AND REACTIVITY

Stability: Stable under recommended storage conditions.

Conditions to Avoid: Moisture

Materials to Avoid: Acid chlorides, Phosphorus halides, strong oxidizing agents, strong acids, strong reducing agents.

Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide, Sulfur dioxides.

Hazardous Polymerization: Will not occur.

Hazardous Exothermic Reactions: Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermicreaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phorphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver flioride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan and sulfur dioxide.

SECTION 11–TOXICOLOGICAL INFORMATION

Acute and Chronic Affects Based On Routes Of Exposure

Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).
Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus).

Specific Developmental Abnormalities: Musculoskeletal System

Eye Contact: May cause irritation.

Skin Contact: May cause irritation.

Ingestion: May cause nausea, coughing, headache or diarrhea.

Inhalation: Unlikely at room temperature, inhalation of mist may cause irritation of respiratory tract.

Chronic Exposure

Target Organ(s): May cause kidney and liver damage.

Aggravation of Pre-existing Conditions: Persons with pre-existing skin disorder or eye problems or impaired liver or kidneys may be more susceptible to the effects of the material.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen.

IARC: No component of this product present at levels greater than or equal to 0.1 % is identified as probable, possible or confirmed human carcinogen.

ACGIH: No component of this product present at levels greater than or equal to 0.1 % is identified as a known or suspected human carcinogen or confirmed animal with unknown relevance humans.

Route of Exposure
Skin Absorption: May be harmful if absorbed.
Contact: May cause skin irritation.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.
Conditions Aggravated By Exposure: Avoid contact with DMSO solutions containing toxic materials or material with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

Target Organ (s) or System (s): Eyes and Skin

Toxicity Data

Inhalation
Rat
40,250 ppm
LC50

Oral
Rat
3,300 mg/kg
LD50

Oral
Rat
14,500 mg/kg
LD50

Remarks: Sense Organs and Special Senses (Nose, Eye, Ear and Taste): Eye: Hemorrhage. Sense Organs and Special Senses (Nose, Eye, Ear and Taste): Eye: Conjunctive irritation.

Skin
Rat
40,000 mg/kg
LD50

Intraperitoneal
Rat
8,200 mg/kg
LD50

Subcutaneous
Rat
12 gg/kg
LD50

Remarks: Behavorial: Change in motor activity (specific assay), Lungs, Thorax, or Respiration: Dyspnea.

Intravenous
Rat
5,360 mg /kg
LD50

Remarks: Behavorial: Tremor, Muscle weakness. Lungs, Thorax or Respiration: Dyspnea.

Chronic Exposure - Carcinogen
Species: Rat
Route of Application: Oral
Dose: 59 gm/kg
Exposure Time: 81W
Frequency: I

Result: Tumorigenic: Equivocal tumorigenic agent by RTECS criteria, Skin and Appendages: Other: Tumors.

Species: Rat
Route of Application: Subcutaneous
Dose: 220 gm/kg
Exposure Time: 82W
Frequency I

Result: Tumorigenic: Equivocal tumorigenic agent by RTECS criteria, Skin and Appendages: Other: Tumors.

Chronic Exposure - Mutagen
Species: Rat
Route: Intraperitoneal
Dose: 25 gm/kg
Exposure Time: 5D
Mutation Test: Cytogenetic analysis.

Chronic Exposure - Reproductive Hazard
Species: Rat
Dose: 56 gm/kg
Route of Application: Intraperitoneal
Exposure Time: (6–12D PREG)
Result: Effects on Fertility: Abortion

Species: Rat
Dose: 6,600 mg/kg
Route of Application: Intraperitoneal
Exposure Time: (7–15D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species : Rat
Dose: 30,750 mg/kg
Route of Application: Subcutaneous
Exposure Time: (8–10D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Effects on Fertility: Litter size (e.g.; # fetuses per litter; measured before birth).

SECTION 12–ECOLOGICAL INFORMATION

Elimination Information (persistence and degradability): No data available.

Ecotoxicity Effects

Toxicity to fish	LC50-Pimephales promelas (fathead minnow) - 34,000 mg/l - 96 h
	LC50-Oncorhynycus mykiss (rainbow trout) - 35,000 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	EC50-Daphnia pulex (water flea) - 27,500 mg/l
Toxicity to algae	EC50 - Lepomis macrochirus (Blue Gill) - > 400,000 mg/l - 96 h

Further Information On Ecology: No data available.

SECTION 13–DISPOSAL CONSIDERATIONS

Dispose of container, unused contents and contaminated packaging in accordance with federal, state and local requirement. Contract with a licensed Chemical Waste Disposal Service.

SECTION 14–TRANSPORT INFORMATION

This product is not dangerous and no special precautions are needed according to DOT, ADR/RID (cross border), IMDG and IATA/ICAO.

SECTION 15–REGULATORY INFORMATION

OSHA Hazards: None known.

US Classification and Label Test

US Statements: Combustible. Readily absorbed through skin. Target Organ (s): Eyes, skin, liver and kidneys. Caution. Avoid contact and inhalation.

United States Regulatory Information:

Sara Listed: No

TSCA Inventory Item: Yes

Canada Regulatory Information

WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR and the MSDS contains all the information required by the CPR.

DSL: Yes

NDSL: No

EU Additional Classification

S: 23 24/25

Safety Statements: Do not breath vapor. Avoid contact with skin and eyes.

SECTION 16–OTHER INFORMATION

DISCLAIMER

The information provided on the MSDS is furnished in good faith and based on our present knowledge. However, this MSDS shall not constitute a guarantee of any kind. Personnel handling this material must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of this material and the safety and health of employees and customers. NEB assumes no additional liability or responsibility resulting from the use of, or reliance on this information. This product is for R&D use only. Not for drug, household or other uses.

Questions about the information found on this MSDS should be directed to info@neb.com.

MATERIAL SAFETY DATA SHEET

New England Biolabs
32 Tozer Road
Beverly MA 01915

EMERGENCY TELEPHONE NO. 1-800-632-5227
OTHER INFORMATION CALLS 1-978-927-5054
FAX: 1-978-921-1350
INTERNET e-mail: info@neb.com

Strain
#E4107S

SECTION 1 - PRODUCT

Product Name: *E. coli* K12 JM109

SECTION 2–COMPOSITION/ INFORMATION ON INGREDIENT

Strains supplied by NEB are all derivatives of *E. coli* K12, *E. coli* B or hybrids of these two strains. *E. coli* K12 and B are nonpathogenic isolates. K12 is the standard nonpathogenic host, exempt from the NIH Recombinant DNA Advisory Committee (RAC) guidelines (1).

E. coli B has also been shown to lack common pathogenicity-related sequences (2).

References:

1. Federal Register, (1986) Vol. V1: 88, 6952–16985.
2. Kuhnert, P., Hacker, I. Muldorfer, A. P. Burnens, J. Nicolet, and J. Frey (1997). Detection system for Escherichia coli-specific virulence genes.: absence of virulence determinants in B and C strains. Appl. Environ. Microbiol. 63(2): 703– 709.

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FAX: 1-978-921-1350
INTERNET e-mail: info@neb.com

Strain

#E4102S

SECTION 1 - PRODUCT

Product Name: *E. coli* K12 ER1821

SECTION 2–COMPOSITION/ INFORMATION ON INGREDIENT

Strains supplied by NEB are all derivatives of *E. coli* K12, *E. coli* B or hybrids of these two strains. *E. coli* K12 and B are nonpathogenic isolates. K12 is the standard nonpathogenic host, exempt from the NIH Recombinant DNA Advisory Committee (RAC) guidelines (1).

E. coli B has also been shown to lack common pathogenicity-related sequences (2).

References:

1. Federal Register, (1986) Vol. V1: 88, 6952–16985.
2. Kuhnert, P., Hacker, I. Muldorfer, A. P. Burnens, J. Nicolet, and J. Frey (1997). Detection system for Escherichia coli-specific virulence genes.: absence of virulence determinants in B and C strains. Appl. Environ. Microbiol. 63(2): 703– 709.

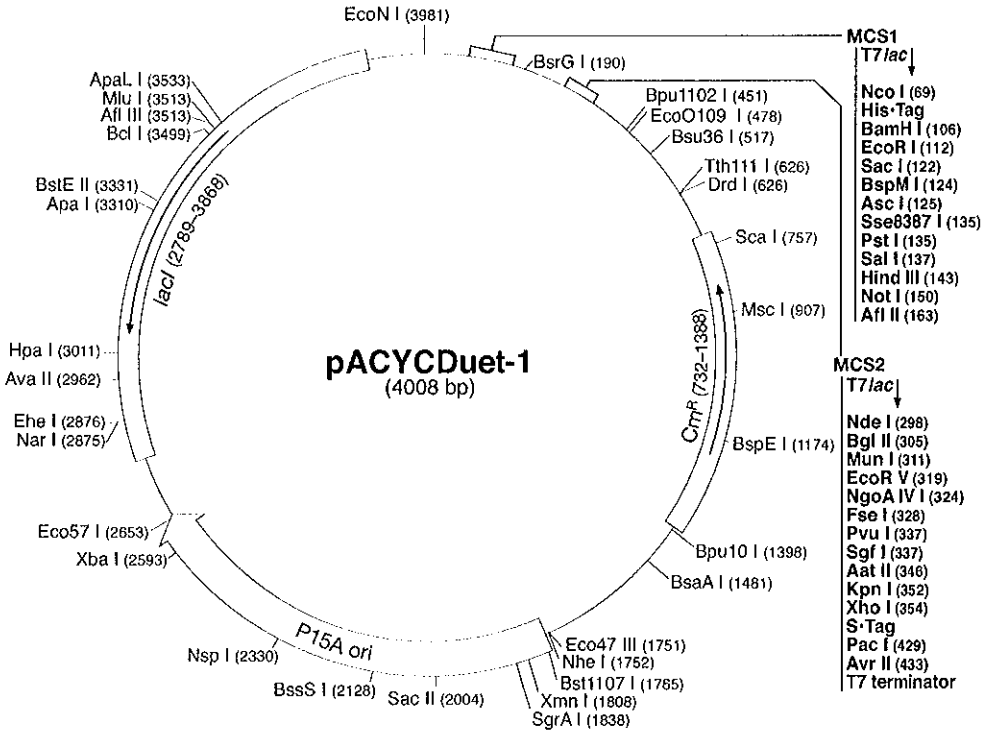
Novagen®

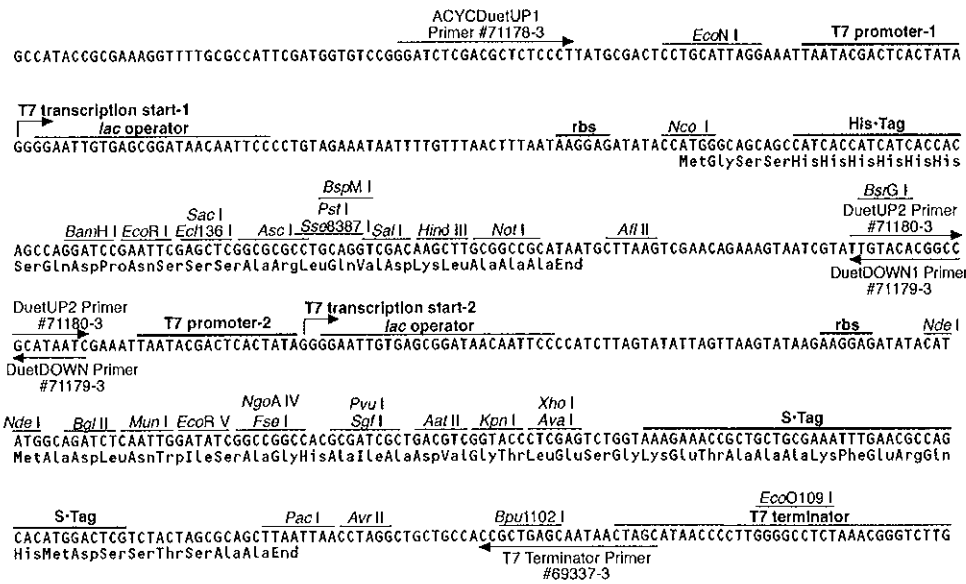
pACYCDuet-1 Vector

TB336 10/02

	Cat. No.
pACYCDuet-1 DNA	71147-3
pACYCDuet-1 sequence landmarks	
T7 promoter-1	3992–4008
T7 transcription start-1	1
His•Tag® coding sequence	83–100
Multiple cloning sites-1 (*Nco* I–*Afl* II)	69–168
T7 promoter-2	214–230
T7 transcription start-2	231
Multiple cloning sites-2 (*Nde* I–*Avr* II)	297–438
S•Tag™ coding sequence	366–410
T7 terminator	462–509
P15A origin	1750–2662
cat (Cm^R) coding sequence	732–1388
lacI coding sequence	2789–3868

pACYCDuet™-1 is designed for the coexpression of two target genes. The vector contains two multiple cloning sites (MCS), each of which is preceded by a T7 promoter/*lac* operator and ribosome binding site (rbs). The vector also carries the P15A replicon, *lacI* gene and chloramphenicol resistance gene. This vector can be used in combination with pETDuet™-1 (Cat. No. 71146-3) in an appropriate host strain for the coexpression of up to 4 target genes. Genes inserted into MCS1 can be sequenced using the ACYCDuetUP1 Primer (Cat. No. 71178-3) and DuetDOWN1 Primer (Cat. No. 71179-3). Genes inserted into MCS2 can be sequenced using the DuetUP2 Primer (Cat. No. 71180-3) and T7 Terminator Primer (Cat. No. 69337-3).





pACYCDuet-1 cloning/expression regions

Novagen

pACYCDuet-1 Restriction Sites

TB336 10/02

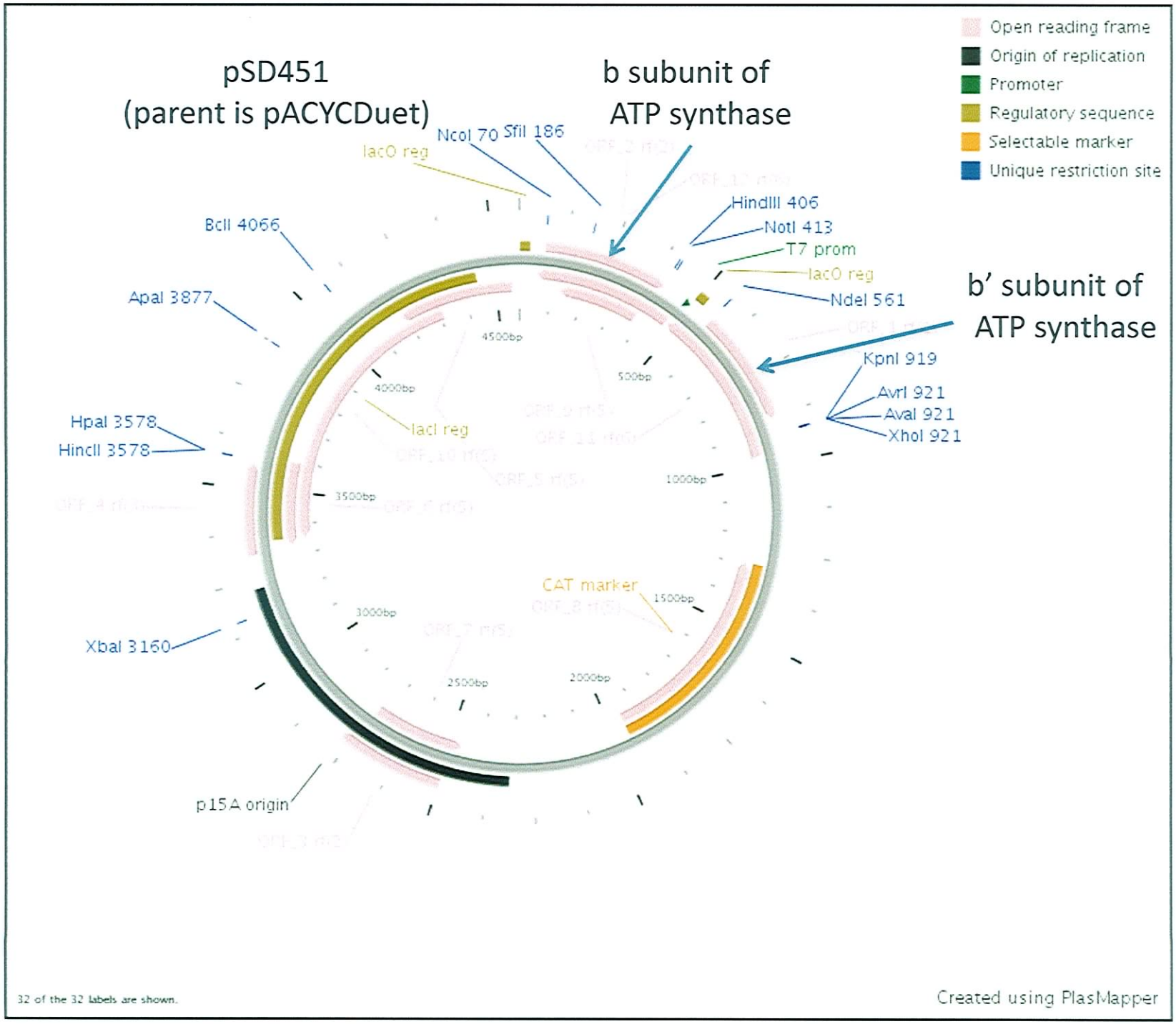
Enzyme	# Sites	Locations				
AatII	1	346				
AccI	3	138	411	1764		
AciI	49					
AflII	1	163				
AflIII	1	3513				
AluI	18					
Alw26I	7	946	1499	2198	2898	3285
		3411	3816			
AlwI	4	101	114	2732	3957	
AlwNI	2	1706	2354			
ApaI	1	3310				
ApaLI	1	3533				
ApoI	5	112	384	632	644	3238
AscI	1	125				
AvaI	1	354				
AvaII	1	2962				
AvrII	1	433				
BamHI	1	106				
BanI	5	348	704	2744	2874	3593
BanII	2	122	3310			
BbsI	2	3028	3367			
BbvI	16					
BcgI	2	162	3193			
BclI	1	3499				
BfaI	5	415	434	462	1753	2594
BglII	1	305				
BpmI	4	1054	1647	3192	3681	
Bpu10I	1	1398				
Bpu1102I	1	451				
BsaAI	1	1481				
BsaHI	3	343	2875	3558		
BsaJI	11					
BsaWI	8	551	566	1174	1838	2161
		2291	2691	3194		
BseRI	2	2385	2428			
BsgI	3	1819	3468	3668		
BsiEI	8	153	199	325	337	625
		1909	2278	2734		
BsiHKAI	3	122	1663	3537		
BsiI	11					
BsmBI	3	946	1499	2898		
BsmFI	2	1554	1674			
BsmI	2	776	1183			
Bsp1286I	5	122	707	1663	3310	3537
BspEI	1	1174				
BspMI	1	124				
BsrBI	3	13	243	1926		
BsrDI	3	1157	3106	3472		
BsrFI	6	324	566	1838	2161	2394
		3827				
BsrGI	1	190				
BsrI	16					
BssHII	2	125	3102			
BssSI	1	2128				
Bst1107I	1	1765				
BstEII	1	3331				
BstXI	3	3467	3590	3719		
BstYI	4	106	305	2737	3949	
Bsu36I	1	517				
Cac8I	22					
CviJI	61					
DdeI	8	262	451	517	950	1398
		2213	2476	2942		
DpnI	13					
DraI	2	915	1254			
DrdI	1	626				
DsaI	2	69	2001			
EaeI	7	150	196	322	326	905
		2182	2839			
EagI	3	150	196	322		
EarI	2	2621	3896			
Ecl136II	1	120				

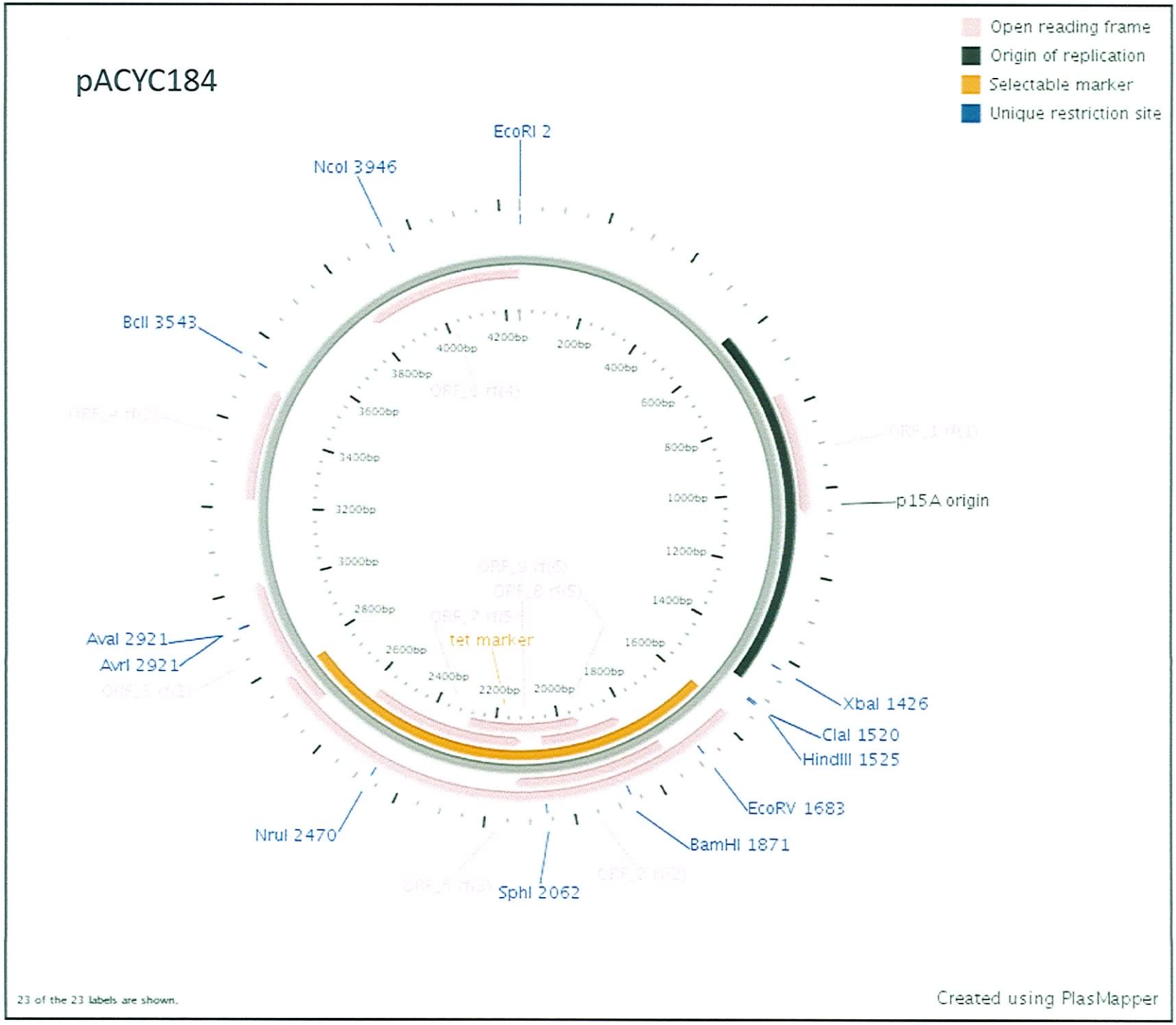
Enzyme	# Sites	Locations				
Eco47III	1	1751				
Eco57I	1	2653				
EcoNI	1	3981				
Eco0109I	1	478				
EcoRI	1	112				
EcoRII	14					
EcoRV	1	319				
EheI	1	2876				
FauI	10	743	1187	2690	2800	2842
		3009	3317	3704	3771	3796
Fnu4HI	30					
FokI	4	644	1190	3458	3467	
FseI	1	328				
HaeII	4	1753	2878	3121	3902	
HaeIII	17					
HgaI	8	1595	1833	2067	3286	3292
		3521	3566	3965		
HhaI	26					
HincII	2	139	3011			
HindIII	1	143				
HinfI	11					
HpaI	1	3011				
HphI	16					
KpnI	1	352				
MaeIII	10	539	1007	1112	1578	1721
		2294	2427	2449	3331	3854
MboII	10	900	1565	1974	1985	2574
		2608	3028	3367	3538	3883
MluI	1	3513				
MnlI	18					
MscI	1	907				
MseI	23					
MslI	4	1458	3147	3177	3465	
MspA1I	11					
MspI	24					
MunI	1	311				
MwoI	24					
NarI	1	2875				
NciI	9	626	1436	1528	2221	2318
		2742	3087	3896	3947	
NcoI	1	69				
NdeI	1	298				
NgoAIV	1	324				
NheI	1	1752				
NlaIII	15					
NlaIV	11					
NotI	1	150				
NspI	1	2330				
NspV	2	642	2488			
PacI	1	429				
PflMI	4	401	945	1512	3938	
PinAI	3	566	1838	2161		
PleI	9	214	365	399	1887	2317
		3084	3880	3967	3992	
Psp1406I	2	1085	3853			
PstI	1	135				
PvuI	1	337				
PvuII	4	1274	1686	2824	2917	
RsaI	5	192	350	757	1295	3370
SacI	1	122				
SacII	1	2004				
SalI	1	137				
Sau3AI	13					
Sau96I	8	478	1515	1998	2938	2962
		3306	3307	3652		
ScaI	1	757				
ScrFI	23					
SfaNI	10	842	1327	1605	1955	2053
		2736	3148	3151	3339	3480
SfcI	4	29	131	226	4004	
SgfI	1	337				
SgrAI	1	1838				

Enzyme	# Sites	Locations				
Sse8387I	1	135				
SspI	2	862	2589			
StyI	3	69	433	473		
TaiI	7	346	913	1088	1483	1495
		3783	3856			
TaqI	15					
TfiI	2	821	2835			
ThaI	22					
TseI	16					
Tsp45I	4	539	1578	2427	3331	
Tsp509I	23					
TspRI	14					
Tth111I	1	626				
VspI	5	213	2575	2771	2830	3991
XbaI	1	2593				
XcmI	3	3128	3146	3662		
XhoI	1	354				
XmnI	1	1808				

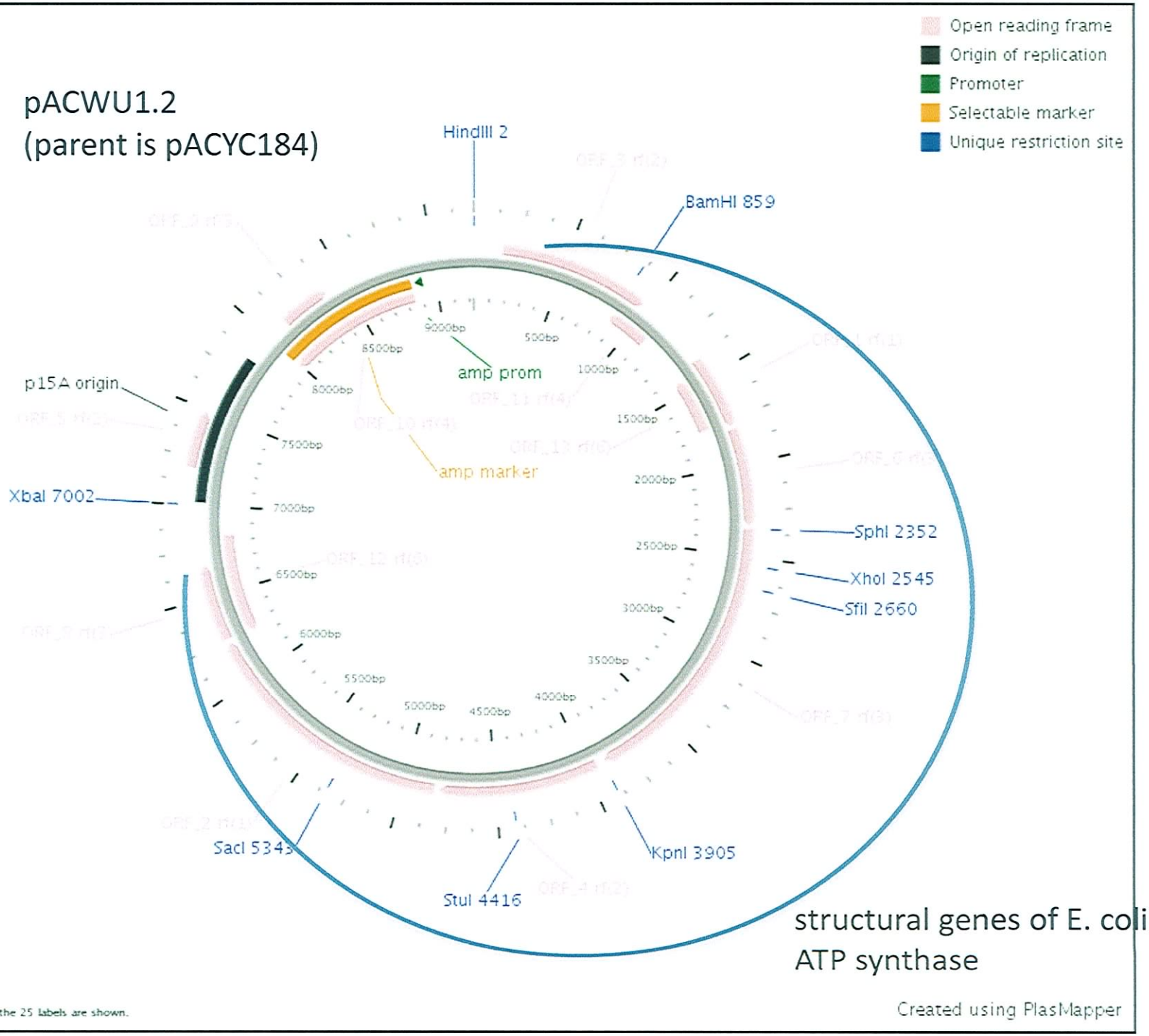
Enzymes that do not cut pACYCDuet-1:

AhdI	BglI	BsaBI	BsaI	BspLU11I	ClaI
DraIII	FspI	NruI	NsiI	PmeI	PmlI
PshAI	Psp5II	RcaI	RsrII	SanDI	SapI
SexAI	SfiI	SmaI	SnaBI	SpeI	SphI
SrfI	StuI	SunI	SwaI		

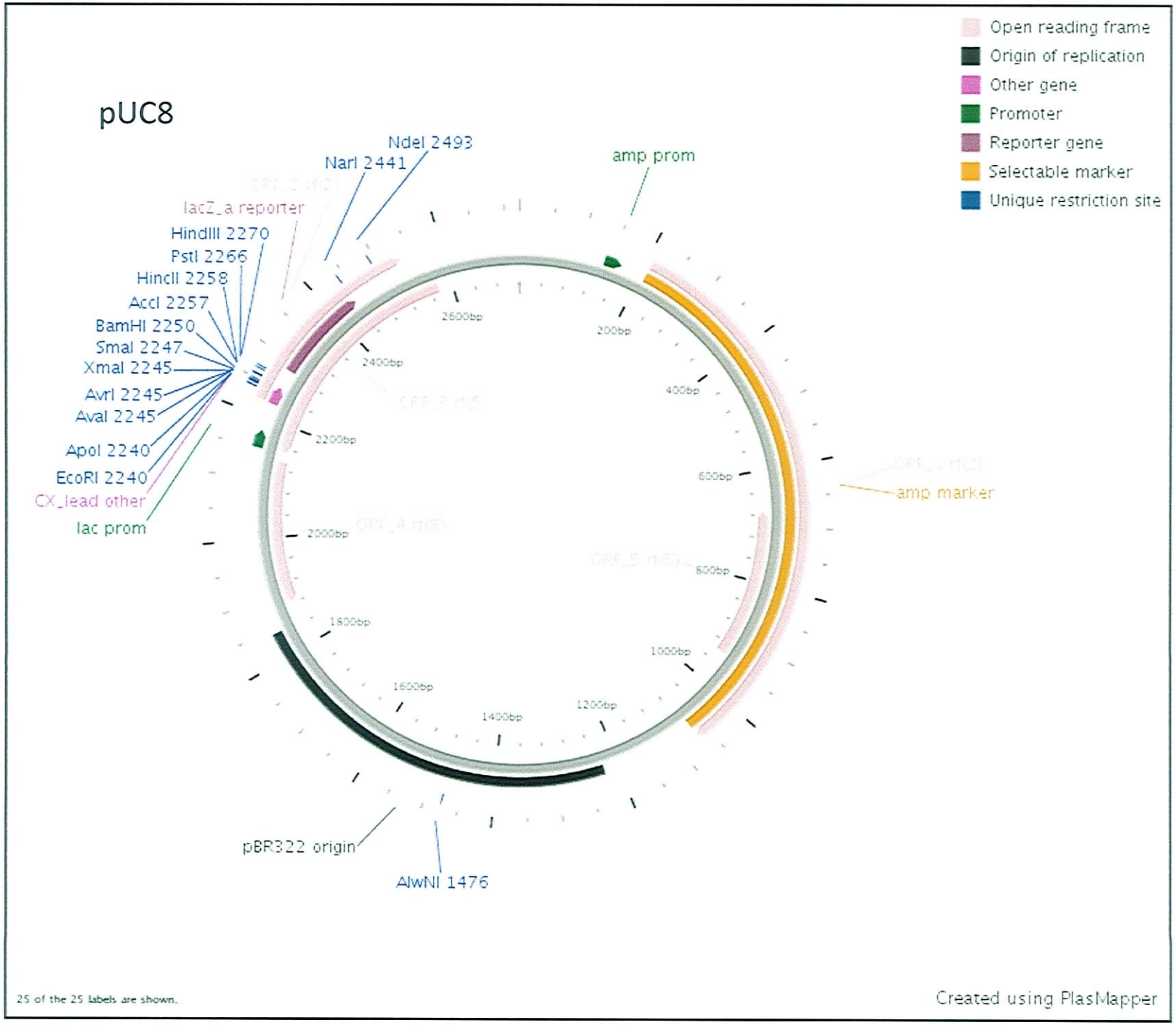

pSD451
(parent is pACYCDuet)
b subunit of
ATP synthase
b' subunit of
ATP synthase
Open reading frame
Origin of replication
Promoter
Regulatory sequence
Selectable marker
Unique restriction site
NcoI 70
SfiI 186
lacO reg
HindIII 406
NotI 413
T7 prom
lacO reg
NdeI 561
KpnI 919
AvrI 921
AvaI 921
XhoI 921
BclI 4066
ApaI 3877
HpaI 3578
HincII 3578
XbaI 3160
p15A origin
lacI reg
CAT marker
500bp
1000bp
1500bp
2000bp
2500bp
3000bp
3500bp
4000bp
4500bp
32 of the 32 labels are shown.
Created using PlasMapper


pACYC184
Open reading frame
Origin of replication
Selectable marker
Unique restriction site
EcoRI 2
NcoI 3946
BclI 3543
p15A origin
XbaI 1426
ClaI 1520
HindIII 1525
EcoRV 1683
BamHI 1871
SphI 2062
NruI 2470
AvaI 2921
AvrI 2921
tet marker
200bp
400bp
600bp
800bp
1000bp
1200bp
1400bp
1600bp
1800bp
2000bp
2200bp
2400bp
2600bp
2800bp
3000bp
3200bp
3400bp
3600bp
3800bp
4000bp
4200bp
23 of the 23 labels are shown.
Created using PlasMapper

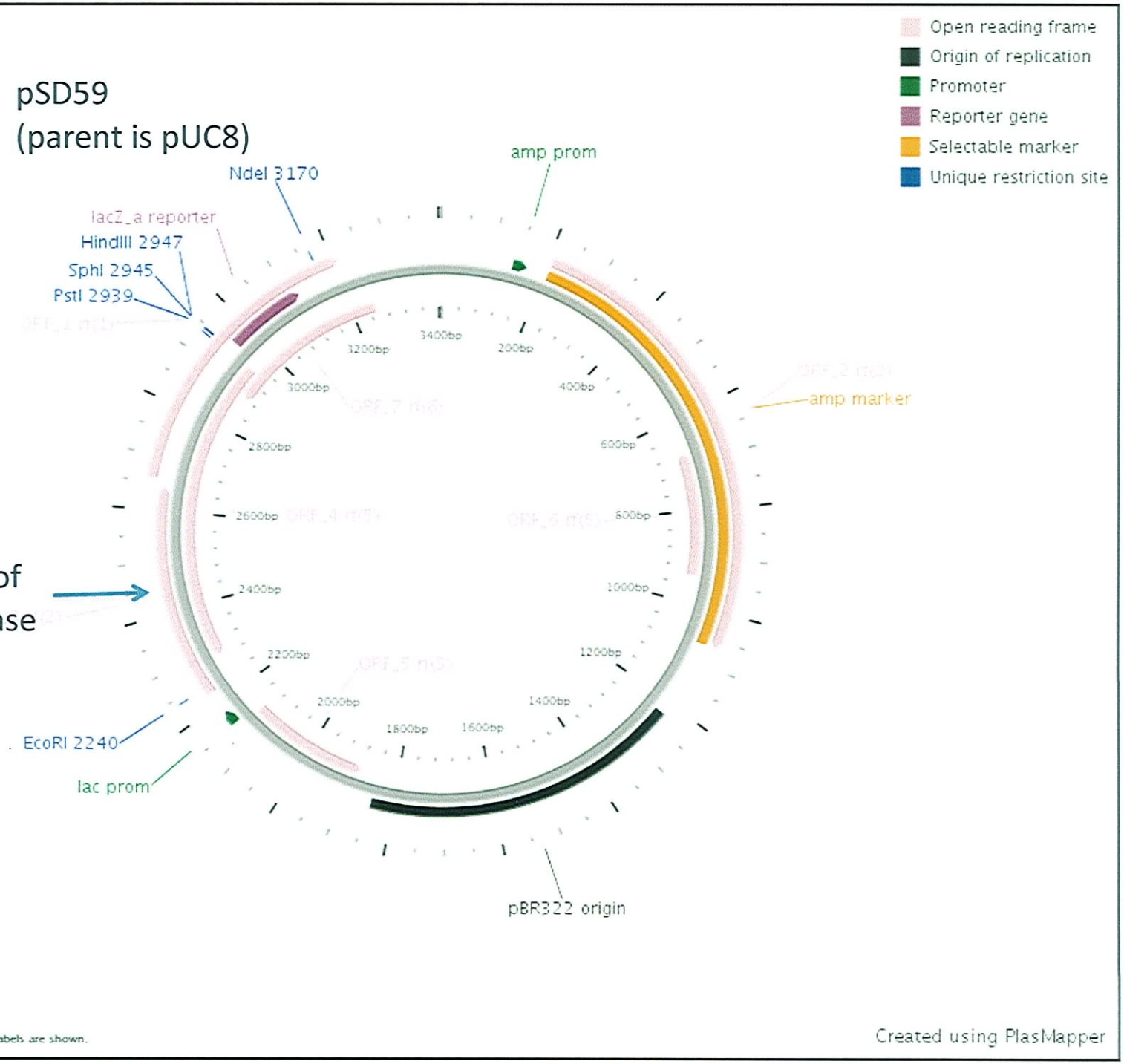
pACWU1.2
(parent is pACYC184)



structural genes of E. coli
ATP synthase


pUC8
Open reading frame
Origin of replication
Other gene
Promoter
Reporter gene
Selectable marker
Unique restriction site
NdeI 2493
NarI 2441
amp prom
lacZ_a reporter
HindIII 2270
PstI 2266
HincII 2258
AccI 2257
BamHI 2250
SmaI 2247
XmaI 2245
AvrI 2245
AvaI 2245
ApoI 2240
EcoRI 2240
CX_lead other
lac prom
amp marker
pBR322 origin
AlwNI 1476
1
200bp
400bp
600bp
800bp
1000bp
1200bp
1400bp
1600bp
1800bp
2000bp
2200bp
2400bp
2600bp
25 of the 25 labels are shown.
Created using PlasMapper

pSD59
(parent is pUC8)



b subunit of
ATP synthase