Modification Form for Permit BIO-UWO-0035

Permit Holder: Gregory Gloor

Approved Personnel
(Please stroke out any personnel to be removed)

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E. Coli dh5 alpha, E.coli XL1-Blue S. S. cerevisae	
Approved Primary and Established Cells		
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	pUC18, pET11, standard yeast shuttle vectors	pPM28 (evoGFP CEN/ARS URA3) pPM47 (U2R-RFP CEN/ARS URA3)
Approved Use of Animals		
Approved Biological Toxin(s)		

** PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Current Classification: 1 **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Sep 16, 2009

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ **Date:** _____

Basically I am going to be inserting a histone acetylase gene into these vectors and use them to transform yeast. The reason I needed both RFP and GFP expressing vectors is that the yeast strains I transform will be assayed in competitive growth experiments, and their fluorescent protein expression will allow me to view frequencies of the cells under fluorescent activated cell sorting analysis.

Thanks,

Ramy

Plasmid(s)

addgene

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

Plasmid 20131: pPM28 (eroGFP CEN/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':	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
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)

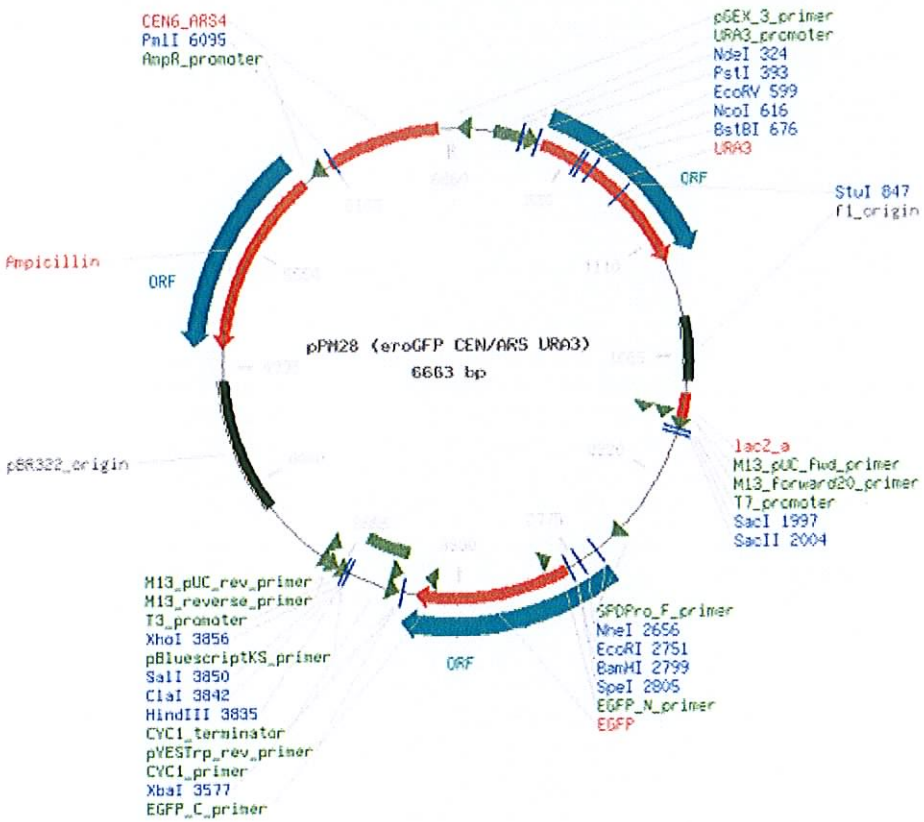
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

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.

addgene

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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addgene

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

Plasmid 20132: pPM47 (UPR-RFP CEN/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)

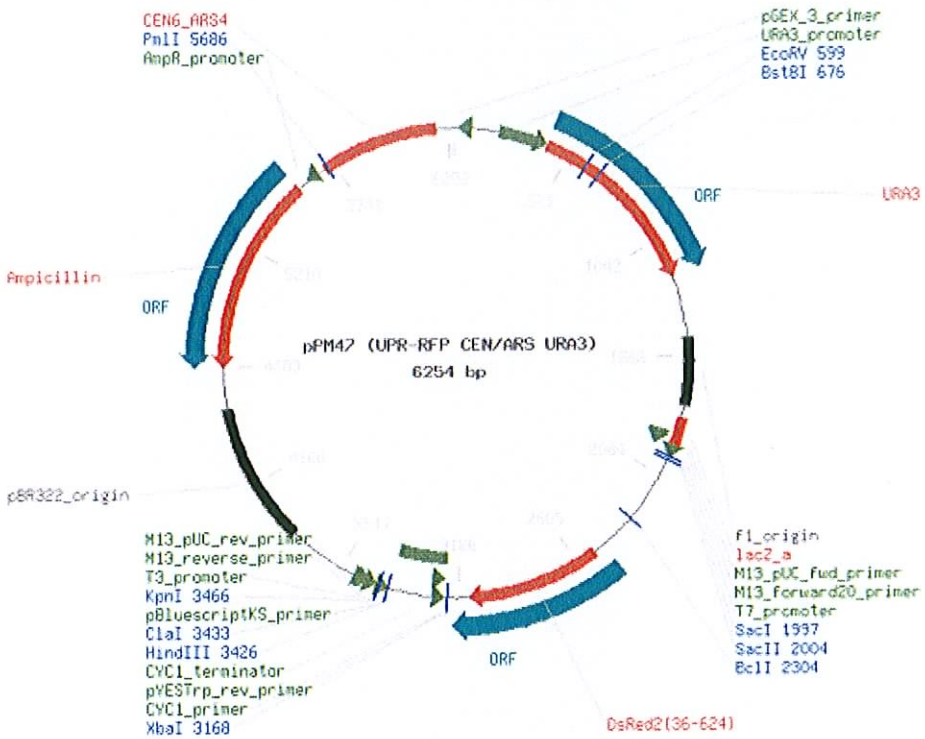
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

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.

addgene

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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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 ~~also~~ be updated at least every 3 years or when there are changes to the biohazards 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 Biohazard 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	GREG GLOOR
SIGNATURE	
DEPARTMENT	Biochem. Sty
ADDRESS	MBL C8
PHONE NUMBER	83526
EMERGENCY PHONE NUMBER(S)	519-645-8157
EMAIL	ggloor@uwo.ca

Location of experimental work to be carried out: Building(s) MBL Room(s) C7

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: NONE AT PRESENT
GRANT TITLE(S):

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

NONE AT PRESENT
ONLY PI

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? O YES ⊗NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

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: DH5 α	O Yes ⊗No	O Yes ⊗No	O Yes ⊗No	< 1	STANDARD LAB SUPPLY	⊗1 O2 O3
E. coli. XL1-Blue	O Yes ⊗ No	O Yes ⊗No	O Yes ⊗ No	< 1	"	⊗1 O2 O3
Saccharomyces cerevisiae.	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	< 1	"	⊗1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3

*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? O 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 in the table below

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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 O 1 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 ⊗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 Known to Be 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		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

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 O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. coli.	pUC18, pET11 Yeast shuttle vectors	Standard LAB Stocks	PGK	Ability to use Glucose.

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

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below ⊗NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O 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 using the viral vector in 4.0? O YES ⊗NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus 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 ⊗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 be used in live animals O YES, specify: _____ O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

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

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

8.3 What is the LD_{50} (specify species) of the toxin_____

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

8.5 How much of the toxin is stored*? _____

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊗NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ☒ NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

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

10.6 Do you do any modifications to the plant? O YES O 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? O YES O NO

10.9 Please 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_____
If no, please proceed to Section 10.0 ☒ NO

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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. Ø1 O2 O3

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

O YES, permit # if on-campus_____

O NO, please certify

ØNOT 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: July 6/09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 16 Sept. 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: Sept 14/09

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: BIO-UWO-035 Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

Introduction to Proteins: Proteins are made up of 20 chemically distinct amino acids. The sequence of amino acids determines how the protein folds into its characteristic 3-dimentional shape, and the function of that protein in the cell. About 20000 different proteins are found in humans and other mammals. Proteins compose the major building blocks of cells and they catalyze almost all the chemical reactions needed for life.

Protein Evolution in a nutshell: DNA is the cell's long-term information storage molecule. The information for each protein is contained in a single gene, which is encoded in the sequence of base pairs along the DNA molecule. A somewhat faithful copy of DNA is made every time the cell divides; on average there is one mistake per DNA molecule/cell division. Over millions of years (which translates into billions of cell divisions for single-celled organisms such as bacteria and yeast) every position in the sequence of a DNA molecule has sustained several changes. The cell dies if a change in a gene (which alters the amino acid sequence of the corresponding protein) is incompatible with proper structure or function of the protein; conversely, the cell survives if the change is compatible with protein structure or function. The net result is that over long periods of time many possible amino acid substitutions are attempted at each position in a given protein sequence, and only those compatible with life result in organisms that give progeny.

Introduction to Protein Families: In different organisms the protein that catalyzes the same chemical reaction will have the same overall shape, but will have somewhat different amino acid sequences because of protein evolution. These homologous proteins can be identified because they are more similar to each other than they are to other proteins. Groups of homologous protein can be identified from many different organisms, and the sequence of their amino acids can be aligned using bioinformatic methods to identify positions that are invariant, conserved or variable. Invariant positions in these multiple sequence alignments are generally required for the chemical activity of the protein and conserved positions are often seen to be substitutions of chemically similar amino acids for each other. Most positions in protein families are seen to vary substantially without affecting the structure or function of the protein. However, some positions can vary only if another position in the protein varies concurrently, or coevolves.

Coevolving positions: It has been difficult to identify positions in protein families that coevolve more than expected by chance, and many different methods have been developed. Analyses of variable positions in protein multiple sequence alignments have shown that some pairs or groups of positions share far higher levels of mutual information (MI) than most others. High MI is interpreted to mean that the optimal amino acid at one position is dependent on the amino acid(s) occupying the other position(s), so that the positions coevolve.

We have developed state-of-the-art methods that allows us to distinguish two classes of high MI positions, those that share high MI with one other position, which we call "isolated pairs", and those that are part of a group with high MI shared between many pairs, these are termed "group coevolving positions". When mapped onto protein structures, the isolated pairs are usually in contact, and often found far from the active site. The group coevolving positions are generally at or near chemically active sites or near protein-protein interaction surfaces, and are sometimes in contact but often interspersed

hypothesis 1 if mutations with high MI have more severe effects on yeast viability and growth than do matched positions with low MI.
Select revertant mutations to wild-type growth, of mutations (isolated in objective 1) that prevent or slow the growth of the yeast. Sequence and map the mutation to determine if the revertant arose because of a second-site suppression event. intragenic nature,

Experimental methods (each experiment would be an ideal honors biochemistry project):

Measuring the correspondence between coevolution score and mutability: *MAP1* genes carrying mutations for five single coevolving positions, five group coevolving positions and 10 corresponding control positions will be made using the Stratagene quick change system. Each of these 20 *MAP1* gene mutations will be transformed into a yeast strain that contains a wild-type *MAP1* gene that can be removed if the cells are grown on the drug, 5FOA. Therefore, if the transformed cells fail to grow, or grow more slowly, on 5FOA, this will tell us that the positions we mutated affect the function of the *MAP1* gene; by convention these defective genes are referred to in lower case, *map1*. We can carefully measure the growth of each transformed line to find the effect of each single mutation. We predict that mutations at positions with high coevolution scores will have stronger effects on Map1 activity than entropy-matched controls that are not coevolving. All *map1* mutations will be retained for use in the suppression analyses.

Direct test of second site suppression: We will mutate the identified co-evolving partner to the mutants that show no growth or slow growth on 5FOA media in the previous experiment. For example, the residue pair Y130 – C302 is in the yeast *MAP1* gene, but in other organisms if the first position is a K, the second position is generally an E or a T residue. So if the Y130K mutant in the yeast *map1* gene is not functional, we will attempt to suppress the mutation by making both the C302E and C302T mutants. Similar logic will be used to make presumed suppressors for each of the test mutants described above. If these suppress the original mutation, this will be an important first step towards verifying the coevolution hypothesis

Direct proof for coevolution of *map1* mutations: A more thorough, but slower, method to test the second site suppression hypothesis will be to identify natural revertants of the *map1* mutants. The slow-growing *map1* mutants will be grown in rich liquid media for long periods of time. This will select for outgrowth of spontaneous mutations that grow faster than the slow growing parents. We will identify individual colonies that grow more rapidly, characterize the change that led to the suppression of the slow growth phenotype. Isolated, suppressed *MAP1* genes will be sequenced and the site of the change that suppresses the original mutation idenfied by DNA sequencing. An alternative PCR-based strategy, termed unigenic evolution, can be used if we find that the outgrowth method is too slow (Dr Brandl is an expert in this second method).

We expect to recover three general classes of mutations in the *map1* gene that result in restoration of the protein to near-normal function. First, we expect to recover reversions of the initial mutation. These should be rare as we will design the original mutations so that reversion would require two independent changes to occur. Secondly, we expect to recover rare mutations that result in a residue with similar properties to the amino acid in the