Modification Form for Permit BIO-UWO-0161

Permit Holder: Gilles Lajoie

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

Greg Vilk

Nicholas Carruthers

Additional Personnel
(Please list additional personnel here)

Chris Hughes

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	Candida albicans, Staphylococcus aureus (ATCC 25923)	Escherichia coli (DH5alpha)
Approved Primary and Established Cells		
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)		pCAGTetRnls plasmid is carried in the E. Coli
Approved Use of Animals	Balble	
Approved Biological Toxin(s)		

See also Modification #2

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

As the Principal Investigator, I have ensured 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.shs.uwo.ca/workplace/newposition.htm

Signature of Permit Holder: _____

Current Classification: 2 **Containment Level for Added Biohazards:** 1

Date of Last Biohazardous Agents Registry Form: Mar 12, 2010

Date of Last Modification (if applicable): May 20, 2010

BioSafety Officer(s): _____

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

We are ordering the plasmid pCAGTetRnls that is carried in the E. coli strain DH5 alpha in order to transfect HEK293 cells to produce a gene knockdown of our desired product. All work will be carried out in the lab of Dr. Lynne Marie Postovit by Christopher Hughes. The Lajoie lab is simply ordering the plasmid in the spirit of collaboration.

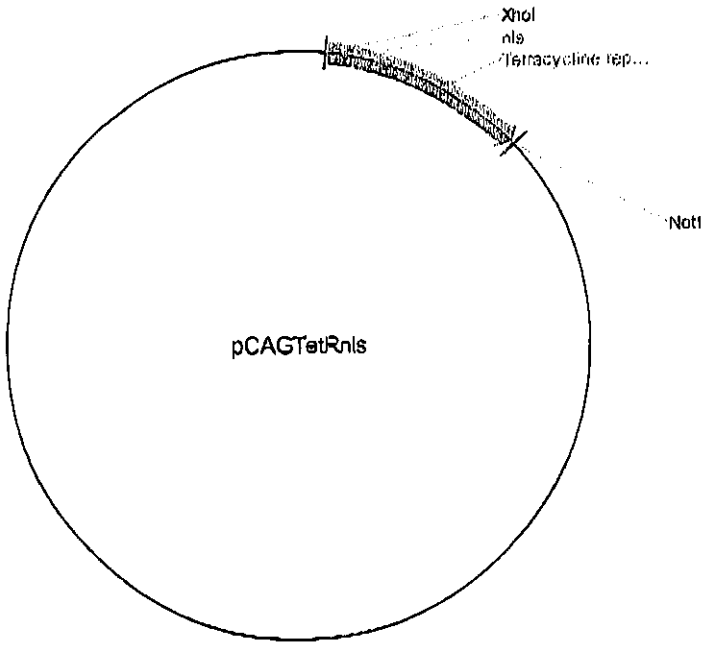
addgene
A better way to share plasmids

Browse > Peter Andrews > Zaharana et al > pCAGTetRnls

Plasmid 26599: pCAGTetRnls

Gene/insert name:	Tetracycline repressor
Alt name:	TetR
Insert size:	704
Species:	E.Coli
GenBank ID:	X00694
Entrez Gene:	TetR (IPF_209)
Fusion protein or tag:	nls
Terminal:	N terminal on insert
Vector backbone:	pCAG (Search Vector Database)
Vector type:	Mammalian Expression
Backbone size w/o insert:	6413
Cloning site 5':	XhoI
Site destroyed during cloning:	No
Cloning site 3':	NotI
Site destroyed during cloning:	No
5' sequencing primer:	n/a List of Sequencing Primers
3' sequencing primer:	n/a
Bacterial resistance:	Ampicillin
Growth strain:	DH5alpha
Growth temperature (°C):	37
High or low copy:	Low Copy
Selectable markers:	Puromycin
Sequence:	View sequences (2)
Map:	View map
Principal Investigator:	Peter Andrews
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Article: Specific knockdown of OCT4 in human embryonic stem cells by inducible short hairpin RNA interference. Zafarana et al (Stem Cells. 2009 Apr . 27(4):776-82. 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 26599" in your Materials and Methods section.

Modification Form for Permit BIO-UWO-0161

Permit Holder: Gilles Lajoie

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

Greg Vilk

Nicholas Carruthers

Additional Personnel
(Please list additional personnel here)

Modification #2

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	Candida albicans, Staphylococcus aureus (ATCC 25923)	Escherichia coli (DH5 alpha)
Approved Primary and Established Cells		
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)		pEGIP plasmid carried in the e. coli
Approved Use of Animals	Balble	
Approved Biological Toxin(s)		

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

As the Principal Investigator, I have ensured 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.shs.uwo.ca/workplace/newposition.htm

Signature of Permit Holder: _____

Current Classification: 2 **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Mar 12, 2010

Date of Last Modification (if applicable): May 20, 2010

BioSafety Officer(s): _____

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

We are ordering the plasmid pEGIP that is carried in the E. coli strain DH5 alpha in order to transfect HEK293 cells to produce a gene knockdown of our desired product. All work will be carried out in the lab of Dr. Lynne Marie Postovit by Christopher Hughes. The Lajoie lab is ordering the plasmid in the spirit of collaboration.

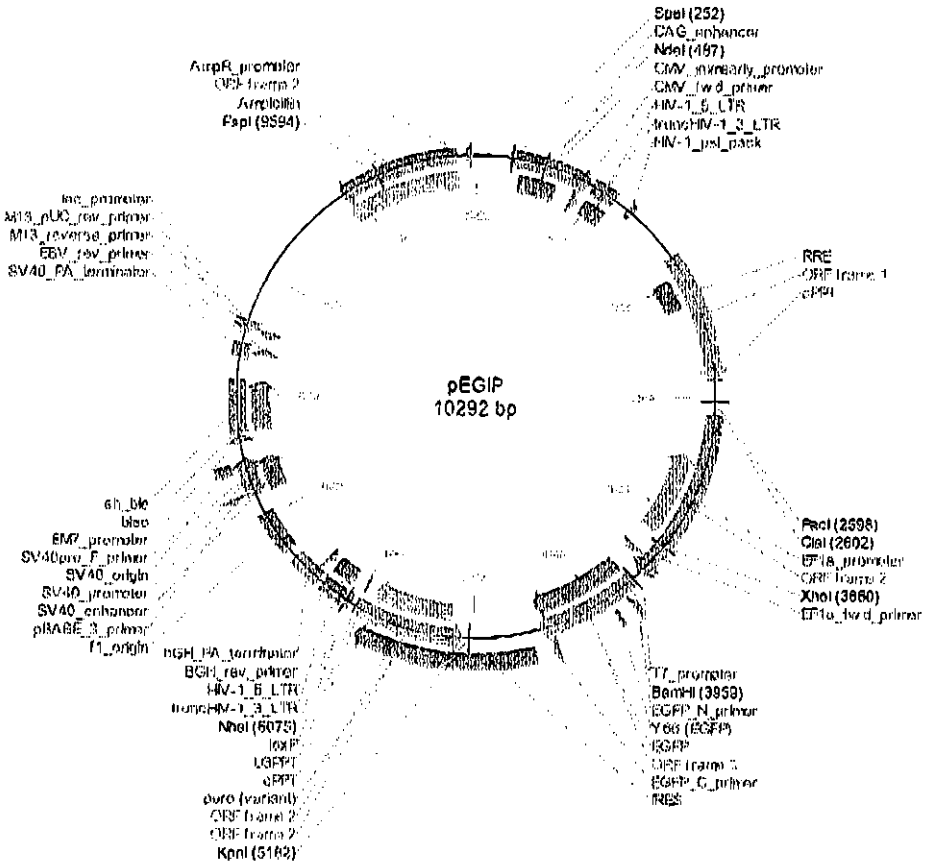
addgene
A better way to share plasmids

Browse > Linzhao Cheng > Zou et al > pEGIP

Plasmid 26777: pEGIP

Gene/insert name:	EF1a-GFP-IRES-Puro
Alt name:	GFP
Alt name:	Puromycin resistant gene
Alt name:	EF1alpha promoter
Insert size:	3229
Entrez Gene:	*gfp* (pCmGFP_001)
Vector backbone:	Duet011 (Search Vector Database)
Vector type:	Mammalian Expression, Lentiviral
Backbone size w/o insert:	7063
Cloning site 5':	PacI
Site destroyed during cloning:	No
Cloning site 3':	HpaI
Site destroyed during cloning:	Yes
5' sequencing primer:	n/a List of Sequencing Primers
Bacterial resistance:	Ampicillin
Growth strain:	Stbl3
Growth temperature (°C):	37
High or low copy:	High Copy
Selectable markers:	Puromycin
Selectable markers, other:	GFP
Sequence:	View sequences (2)
Principal Investigator:	Linzhao Cheng
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Feature Name	Start	End
CMV_immearly_promoter	239	815
CAG_enhancer	318	605
CMV_fwd_primer	772	792
HIV-1_5_LTR	835	1015
truncHIV-1_3_LTR	835	1015
HIV-1_psi_pack	1126	1170
RRE	1680	1913
cPPT	2444	2459
EF1a_promoter	2690	3673
EF1a_fwd_primer	3821	3841
T7_promoter	3890	3908
EGFP	3981	4694
EGFP_N_primer	4044	4023
Y66 (EGFP)	4161	4190
EGFP_C_primer	4631	4652
IRES	4748	5320
puro (variant)	5324	5923
U3PPT	5984	6005
cPPT	5984	5999
loxP	6039	6072
truncHIV-1_3_LTR	6097	6277
HIV-1_5_LTR	6097	6277
BGH_rev_primer	6320	6303
bGH_PA_terminator	6306	6533

f1_origin	6596	6902
pGABE_3_primer	7036	7076
SV40_enhancer	7237	7022
SV40_promoter	7034	7302
SV40_origin	7201	7278
SV40pro_F_primer	7263	7282
EM7_promoter	7396	7463
sh_ble	7464	7838
bleo	7464	7835
SV40_PA_terminator	7971	8090
EBV_rev_primer	8059	8078
M13_reverse_primer	8152	8134
M13_pUC_rev_primer	8173	8151
lac_promoter	8216	8187
Ampicillin	10159	9299
AmpR_promoter	10229	10201

ORF	Start	End
ORF frame 1	1558	2445
ORF frame 2	3613	2993
ORF frame 3	3978	4697
ORF frame 2	5974	5225
ORF frame 2	5312	5923
ORF frame 2	10159	9299

Enzyme Name	Cut
SpeI	252
NdeI	487
PacI	2598
ClaI	2602
XhoI	3660
BamHI	3959
KpnI	5182
NheI	6075
FspI	9594

Article: Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells, Zou et al (Cell Stem Cell. 2009 Jul 2. 5(1):97-110. 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 26777" in your Materials and Methods section.

Modification Form for Permit BIO-UWO-0161

Permit Holder: Gilles Lajoie

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

Nicholas Carruthers

Greg Vilk

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 appreviate.
Approved Microorganisms	Candida albicans Staphylococcus aureus. (ATCC 25923)	- level 2 - see attached MSDS. - storage in BSB Room 3004E.
Approved Primary and Established Cells		
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)		
Approved Use of Animals	Balble	
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: X _____

Current Classification: 2 **Containment Level for Added Biohazards:** 2

Date of Last Biohazardous Agents Registry Form: Mar 12, 2010

Date of Last Modification (if applicable): N/A

BioSafety Officer(s): Jennifer Stanley. _____ May 20/10

Chair, Biohazards Subcommittee: _____ **Date:** 20 May 2010

May 20/10 { Notes:
Volume - 5 mL
stored in fridge in DSB 3004 E

Subject: Re: Biohazardous Agents Registry Form - Lajoie
From: Greg Vilk <gvilk2@gmail.com>
Date: Thu, 20 May 2010 07:53:17 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

1. 5 ml of S. aureus culture

2. In fact, there is a fridge in DSB 3004E where I presently store my C. albicans. This is where I will store the S. aureus.

Greg

On Wed, May 19, 2010 at 4:37 PM, Jennifer Stanley <jstanle2@uwo.ca> wrote:

> Hello there
>
> Please clarify the following -
> 1. What volume of S. aureus will you will growing?
> 2. Please clarify the storage of cultures in DSB 3004E- there is no freezer or fridge...
>
> Regards
> Jennifer

--
Greg J A Vilk, PhD
Department of Biochemistry
Medical Sciences Building, Room M359 (lab)
Room M354 (office)
London, Ontario, CANADA
N6A 5C1
Phone: 1-519-661-2111 x86849 (lab)
Email: gvilk2@gmail.com

Bacteria

ATCC® Number: **25923™** Order this Item Price: **$40.00**

Preceptrol® Culture

Organism:	*Staphylococcus aureus* subsp. *aureus* Rosenbach deposited as *Staphylococcus aureus* Rosenbach
Designations:	Seattle 1945
Isolation:	clinical isolate
Depositor:	FDA
History:	ATCC <<--FDA<<--F. Schoenknecht
Biosafety Level:	2
Shipped:	freeze-dried
Growth Conditions:	ATCC medium18: Trypticase soy agar **Temperature:** 37.0°C Duration: aerobic
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Cross References:	Nucleotide (GenBank) : AB047239 Staphylococcus aureus DNA, complete structure of cassette chromosome(SCC)-like element, strain:ATCC 25923. Nucleotide (GenBank) : AF053568 Staphylococcus aureus ATCC 25923 heat shock protein 60 gene, partial cds. Nucleotide (GenBank) : AX110511 Sequence 1244 from Patent WO0123604. Nucleotide (GenBank) : U02910 Staphylococcus aureus ATCC 25923 16S rRNA gene, partial sequence. Nucleotide (GenBank) : U39769 Staphylococcus aureus 16S-23S ribosomal RNA spacer region. Nucleotide (GenBank) : AX110995 Sequence 1728 from Patent WO0123604. Nucleotide (GenBank) : Z16422 S.aureus dfrB gene for dihydrofolate reductase.
Comments:	Incorrectly cited in previous editions of this catalog as identical to NCTC 6571.

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Products

Staphylococcus aureus - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Staphylococcus aureus***

SYNONYM OR CROSS REFERENCE: Staphylococcal diseases, impetigo, toxic shock syndrome, food poisoning, intoxication

CHARACTERISTICS: Gram positive cocci, usually in clusters; coagulase positive; non-spore forming; non-motile; many strains produce exotoxins including staphylococcal enterotoxins A,B,C,D,E, toxic shock syndrome toxin (TSST-1) and exfoliative toxins A, and B

SECTION II - HEALTH HAZARD

PATHOGENICITY: Opportunistic pathogen, normal flora; produces a variety of syndromes with a range of clinical manifestations; clinically different in general community, newborns, menstruating women, and hospitalized patients; food intoxication is characterized by abrupt/violent onset, severe nausea, cramps, vomiting, and diarrhea using lasting 1-2days; animal bites can result in localized infections; may cause surface or deep/system infections in both community and hospital settings; surface infections include impetigo, folliculitis, abscesses, boils, infected lacerations; deep infections include endocarditis, meningitis, septic arthritis, pneumonia, osteomyelitis; systemic infection may cause fever, headache malaise, myalgia; newborns are susceptible to scalded skin syndrome (SSS) caused by exfoliative toxins; my be colonized during delivery resulting in sepsis meningitis; toxic shock syndrome is an acute multi-system illness caused by TSST-1 a super antigen; characterized by sudden onset, high fever, vomiting, profuse watery diarrhea, myalgia, hypotension erythematous rash

EPIDEMIOLOGY: Occurs worldwide; particularly in areas where personal hygiene is suboptimal; in hospitals by development of antibiotic-resistant strains

HOST RANGE: Humans; to a lesser extent, warm-blooded animals

INFECTIOUS DOSE: Virulence of strains varies greatly

MODE OF TRANSMISSION: Contact with nasal carriers (30-40% of population); from draining lesions or purulent discharges; spread person-to-person; ingestion of food containing staphylococcal enterotoxin (food may be contaminated by food handlers hands); from mother to neonate during delivery

INCUBATION PERIOD: Variable and indefinite, commonly 4-10 days; disease may not occur until several months after colonization; interval between eating food and onset of symptoms is usually 2-4 hours (30 min to 8 hours)

COMMUNICABILITY: As long as purulent lesions continue to drain or carrier state persists; auto-infection may continue for the period of nasal colonization or duration of active lesions

SECTION III - DISSEMINATION

RESERVOIR: Human; patients with indwelling catheters or IVs act as reservoirs for nosocomial infections; food borne - occasionally cows with infected udders

ZOONOSIS: Yes - direct or indirect contact with infected animals

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Many strains are multi-resistant to antibiotics and are of increasing importance; methicillin resistant (MRSA) strains have caused major outbreaks world-wide; Vancomycin resistant (VRSA) are being increasingly isolated; sensitivity must be determined for each strain

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, iodine/alcohol solutions, glutaraldehyde, formaldehyde

PHYSICAL INACTIVATION: Organisms are destroyed by heat (moist heat - 121° C for at least 15 min, dry heat - 160-170° C for at least 1 hour; enterotoxins are heat resistant, stable at boiling temperature

SURVIVAL OUTSIDE HOST: Carcass and organs - up to 42 days; floor - less than 7 days; glass - 46 hours; sunlight - 17 hours; UV - 7 hours; meat products - 60 days; coins - up to 7 days; skin from 30 min to 38 days

SECTION V - MEDICAL

SURVEILLANCE: Monitor for skin inflammation if wounded by a sharp instrument; isolation of organism from wound or blood, CSF, urine; isolation of> 10^5 organisms or enterotoxin from suspected food

FIRST AID/TREATMENT: Fluid replacement for food poisoning; in localized skin infections, drain abscesses; antibiotic therapy for severe infections

IMMUNIZATION: None

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 29 reported cases up to 1973 with 1 death

SOURCES/SPECIMENS: Clinical specimens - blood, abcesses, lesion exudates, CSF, respiratory specimens, feces, urine

PRIMARY HAZARDS: Injuries from contaminated sharp instruments; ingestion; aerosols

SPECIAL HAZARDS: Direct contact with open cuts and lesions of skin

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities with cultures or potentially infectious clinical materials

PROTECTIVE CLOTHING: Laboratory coat: gloves when skin contact is unavoidable

OTHER PRECAUTIONS: Thorough handwashing before leaving the laboratory and after handling infectious materials

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wear protective clothing; gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: March, 2001

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2001-04-23

BIO-UWO-0161

Animal Project 2010-036 Description:

Background: Fungal and bacterial infections are a rising health risk as evidenced by the emergence of 9 million patients afflicted with these infections in the seven major markets worldwide. Patients at risk include those undergoing surgery, leukemic and HIV/AIDS patients and those who are immuno-compromised due to cancer, surgery or organ transplantation. This is partially as a result of the develoment of antibiotic-resistance in micro-organisms. As a result, there is a growing unmet need in connection with anti-microbial agents, and in particular, anti-fungal and anti-bacterial agents, which represents a market opportunity to develop non-toxic anti-fungal agents with high potency in humans. One such naturally-occurring component found in human saliva is Histatin. This peptide has been shown to possess anti-microbial action on various fungal and bacterial organisms. However, its mode of action is still controversal and its potency is lacking for use as a marketable compound for clinical use. We therefore developed a cyclical analogue of Histatin that has been shown to be very stable and more effective than its naturally-occuring counter-part (Brewer and Lajoie, 2002, Biochem.). This cyclical analogue, termed DB2121, is effective against various fungal (eg. C. albicans) and microbial organisms (eg. S. aureus) and its mode of action has been implied by recent research by the authors (PCT Patent No. WO/2008/134882) (2) in which it targets key mitochondrial proteins in C. albicans. To investigate whether this compound would be effective as a dermatological agent, we would now like to test DB2121 in a BALB/c mouse model that has been previously-infected with C. albicans (SC5314) (2, 4, 5) or **S. aureus** (ATCC 25923) (3) on the skin.

Hypothesis: The optimal concentration of C. albicans when applied to a skin defect created on BALB/c mice will produce an overt and measurable infection.

Objectives: Confirm model system prior to efficacy testing of DB2121 by introducing various concentrations of C. albicans (SC5314) or **S. aureus** (ATCC 25923) to skin defect created on BALB/c mice.

Experimental Procedure (PILOT): PURPOSE IS TO CONFIRM MODEL SYSTEM. Six 25 g BALB/c mice will be prepped by shaving an area on the dorsal, thoracic surface. It will be cleaned with chlorhexidine soap and alcohol. Surgery will consist of TWO skin defects over the dorsal, thoracic area made with a six mm circular biopsy punch. Then, 10 ul of 1X10E7, 1X10E8, and 1X10E9 logarithmically-growing C. albicans (SC5314) cells/ml in sterile PBS or 2 X10E5, 2X10E6, 2X10E7 CFUs of S. aureus in PBS will be applied to the wound site of each of the test mice. One control mouse will be included in which PBS only will be applied to the skin defects. Mice and the wound site will be monitored for a period of 4 days after which the animals will be euthanized. The mice will be monitored for physical signs that will infer the degree of infection; decreased food and water consumption, weight loss, self-imposed isolation/hiding, rapid breathing, opened-mouth breathing, increased/decreased movement, abnormal posture/positioning, dehydration, twitching, trembling, and tremor. The wound will also be monitored for degree of infection and its inability to heal due to advanced infection. If

these signs are clearly apparent PRIOR to the four-day experimental end-period, the observations will be noted and the animals will then be euthanized immediately.

BIOHAZARD LEVEL 2 REQUIREMENT

S. aureus will be grown in DSB Room 3004E prior to each experiment and will be applied to Balb/c mice in ACVs's level 2 containment facility.

DISPOSAL: All animals that are exposed to the agent will be incinerated at the end of the experiment. Procedures specific to a level 2 facility will be adhered. Safety glasses, gloves, lab coat, and biosafety hood will be used. Bleach will be used for sterilization.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 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 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: Dr. Gilles Lajoie.
SIGNATURE:
DEPARTMENT: Biochemistry.
ADDRESS: SBRI, Room G31
PHONE NUMBER: x83054
EMERGENCY PHONE NUMBER(S):
EMAIL: glajoie@uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 3004 E

*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: CA CIHR GRANT #: R3095A16
GRANT TITLE(S): Efficacy of Histatin Analogue, DB2121, in Candida-infected Mouse Models.

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:
Dr. Greg J.A. Vilk (gvilka@gmail.com)

* 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 biological agents? ⊗ YES O NO
(including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ⊗ NO
If YES, please give the name of the species. Candida albicans (SC5314)
What is the origin of the microorganism(s)? Obtained from Dr. Raymond (IRCM), Montreal, Quebec. - Institute of Clinical Research.
Please describe the risk (if any) of escape and how this will be mitigated:
10% Bleach &/or Ethanol sterilization of contaminated area.

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
C. albicans (SC5314)	⊗ Yes O No	⊗ Yes O No	⊗ Yes ⊗ No	0.1 L	MSDS Attached.	O 1 ⊗ 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? 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:

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:

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 Ø NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/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 Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES Ø NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	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 _____ ☒ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ☒ NO
- SV 40 Large T antigen O YES ☒ NO
- E1A oncogene O YES ☒ NO
- Known oncogenes O YES, please specify _____ ☒ NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ☒ NO

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

6.1 Will live animals be used? ☒ YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used Balb/c (female)

6.3 AUS protocol # In progress for approval

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ ☒ NO

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

_____

_____

* 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*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide detais:_____ O NO

*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 life stage of the insect? _____

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

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

_____
_____
_____

9.7 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
If NO, please forward the permit to he Biosafety Officer when available.

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 12.0 ⊗ 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. O 1 Ø 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
Ø YES, permit # if on-campus BIO-UWO-0161
O NO, please certify
O 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: March 2 2010

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

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:
Standard procedures outlined for such incidents with Level 2 bioorganisms

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 15 March 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: J Stanley
Date: Mar 12, 2010

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

Approval Number: BIO-UWO-0161 Expiry Date (3 years from Approval): March 11, 2013

Special Conditions of Approval:

- Preparation of C. albicans must be done in Level 2 room/lab. Injections to be done in biological safety cabinet in Level 2 animal room.

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

Subject: Re: Lajoie BARF at today's meeting
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Mon, 15 Mar 2010 10:28:36 -0400
To: Williams <Fred.Williams@schulich.uwo.ca>
CC: skoval@uwo.ca, "avpres@uwo.ca" <avpres@uwo.ca>

Hi Fred
I doublechecked Dr. Lajoie's recent application to use Candida albicans. He does state DSB 3004E as the room he is intending to use for this work.
There should be a biosafety sign posted for Dr. Lajoie's work (with Candida albicans listed) - I think there is a sign already there but I will mail one to you just in case.
If there is a problem with him using the room, please let me know.
Jennifer

On 3/12/2010 4:32 PM, Susan Koval wrote:

> I checked with Fred Williams (Teaching and Research Coordinator in our department) about use of the departmental Biosarety Level 2 facility. His response is as follows:
> "Several years ago when Greg Vilk was working with Lajoie we allowed them access to the facility. They purchased and installed the bench top incubator. As far as I know they have done essentially no work in our room up to now."
>
> It was news to Fred today that Vilk and Lajoie now planned to use the Level 2 facility. He will ask Greg about this. We do acknowledge that the facility has been able to use the incubator they provided. However, it would be wise for all parties concerned to know that these researchers plan to grow Candida there.
> Regards,
> Susan

Candida albicans - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Candida albicans***

SYNONYM OR CROSS REFERENCE: Candidiasis, Thrush, Moniliasis

CHARACTERISTICS: Oval, budding yeast, produces pseudohyphae in culture and in tissues and exudates

SECTION II - HEALTH HAZARD

PATHOGENICITY: Mycosis of superficial layers of skin or mucous membranes (oral thrush, vulvovaginitis, paronychia, onychomycosis, intertrigo); ulcers or pseudomembranes in esophagus, gastrointestinal tract or bladder; hematogenous dissemination may produce lesions in kidney, spleen, lung, liver, prosthetic cardiac valve, eye, meninges, brain

EPIDEMIOLOGY: Worldwide

HOST RANGE: Humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Endogenous spread (part of normal human flora); by contact with excretions of mouth, skin, and feces from patients or carriers; from mother to infant during childbirth; disseminated candidiasis may originate from mucosal lesions, unsterile narcotic injections, catheters

INCUBATION PERIOD: Variable

COMMUNICABILITY: Communicable for duration of lesions

SECTION III - DISSEMINATION

RESERVOIR: Humans (normal human flora)

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Sensitive to nystatin, clotrimazole, ketoconazole, fluconazole, amphotericin B for invasive candidiasis

DRUG RESISTANCE: Resistant strains have been described for all the above antifungal drugs

SUSCEPTIBILITY TO DISINFECTANTS: Sensitive to 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde; only moderately sensitive to 70% ethanol (phenolic may be substituted)

PHYSICAL INACTIVATION: Inactivated by moist heat (121°C for at least 15 min)

SURVIVAL OUTSIDE HOST: Survives outside of host, especially in moist, dark areas

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; microscopic demonstration of pseudohyphae and/or yeast cells in infected tissue or fluid; confirmation by culture

FIRST AID/TREATMENT: Administer antibiotic therapy as required

IMMUNIZATION: None

PROPHYLAXIS: None

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 2 reported laboratory-acquired infections with *Candida*

SOURCES/SPECIMENS: Sputum, bronchial washings, stool, urine, mucosal surfaces, skin or wound exudates, CSF, blood

PRIMARY HAZARDS: Accidental parenteral inoculation, exposure of mucous membranes to droplets and aerosols, ingestion

SPECIAL HAZARDS: None

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for the manipulation of this organism

PROTECTIVE CLOTHING: Laboratory coat; gloves when contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with absorbent paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal; steam sterilization, chemical disinfection, incineration

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: November 1999

Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2001-01-23

Lajoie

Infectious/Opportunistic diseases represent a significant health concern due to their increasing prevalence in society and the occurance of drug-resistent forms. Thus one requires to develop novel compounds to combat these diseases. We have developed a novel compound that can effectively eliminate fungal diseases in the laboratory. Before clinical testing, we now would like to test this compound in mouse models to determine its effectiveness alone or in combination with other known anti-fungal drugs. This study would therefore allow us to prepare a superior treatment for infectious disease control than the ones that are currently provided.

Background: Fungal and bacterial infections are a rising health risk as evidenced by the emergence of 9 million patients afflicted with these infections in the seven major markets worldwide. Patients at risk include those undergoing surgery, leukemic and HIV/AIDS patients and those who are immuno-compromised due to cancer, surgery or organ transplantation. This is partially as a result of the develoment of antibiotic-resistance in micro-organisms. As a result, there is a growing unmet need in connection with anti-microbial agents, and in particular, anti-fungal and anti-bacterial agents, which represents a market opportunity to develop non-toxic anti-fungal agents with high potency in humans. One such naturally-occurring component found in human saliva is Histatin. This peptide has been shown to possess anti-microbial action on various fungal and bacterial organisms. However, its mode of action is still controversal and its potency is lacking for use as a marketable compound for clinical use. We therefore developed a cyclical analogue of Histatin that has been shown to be very stable and more effective than its naturally-occuring counter-part (Brewer and Lajoie, 2002, Biochem.). This cyclical analogue, termed DB2121, is effective against various fungal (eg. C. albicans) and microbial organisms (eg. S. aureus) and its mode of action has been implied by recent research by the authors (PCT Patent No. WO/2008/134882) in which it targets key mitochondrial proteins in C. albicans. To investigate whether this compound would be effective as a dermatological agent, we would now like to test DB2121 in a BALB/c mouse model that has been previously-infected with C. albicans on the skin (SC5314).

Hypothesis: The cyclic analogue, DB2121, can eliminate a C. albicans skin infection in a BALB/c mouse model.

Objectives: 1. Confirm model system prior to DB2121 testing for efficacy by introducing various concentrations of C. albicans (SC5314) to wound site of BALB/c mice (PILOT). 2. Test the efficacy of DB2121 in vivo to eliminate a C. albicans infection that has been applied to a wound site on female BALB/c mice. 3. Determine if a combination therapy with ketoconazole is more effective than using DB2121 alone.

Experimental Procedure (PILOT): PURPOSE IS TO CONFIRM MODEL SYSTEM. Three 20 g BALB/c mice will be prepped by shaving an area on the dorsal, thoracic surface. It will be cleaned with chlorhexidine soap and alcohol. Surgery will consist of one skin defect over the dorsal, thoracic area made with a six mm circular biopsy punch. Then, 100 ul of 1X10E5, 1X10E6, and 1X10E7 logarithmically-growing C. albicans (SC5314) cells/ml in sterile PBS will be applied to the wound site of each of the three test mice. Mice and the wound site will be monitored for a period of 2 weeks after which the animals will be euthanized. The mice will be monitored for physical signs that will infer the degree of infection; decreased food and water consumption, weight loss, self-imposed isolation/hiding, rapid breathing, opened-mouth breathing, increased/decreased movement, abnormal posture/positioning, dehydration, twitching, trembling, and tremor. The wound will also be monitored for degree of infection and its inability to heal due to advanced infection. If these signs are apparent PRIOR to the two-week experimental period, the observations will be noted and the animals will then be euthanized immediately.

Experimental Procedure (FULL PROJECT): Once the Pilot study has confirmed the model system, we then would like to test the cyclic analogue, DB2121, on the C. albicans-infected BALB/c mice. Six (6) groups of 20g female BALB/c mice (n=5 for PBS CONTROL and n=6 for test articles = TOTAL 35 mice) will be used for the study. BALB/c mice will be prepped by shaving an area on the dorsal, thoracic surface. It will be cleaned with chlorhexidine soap and alcohol. Surgery will consist of one skin defect over the dorsal, thoracic area made with a six mm circular biopsy punch. A concentration (ie previously-determined in the PILOT study) of logarithmically-growing C. albicans (SC5314) cells in sterile PBS will be premixed with either (A) 100 ul PBS CONTROL, (B) 0.1 mg/kg DB2121 in distilled water, (C) 1 mg/kg DB2121 in distilled water, (D) 10 mg/kg DB2121 in distilled water, (E) 0.1 mg/kg DB2121 and 1 mg/kg ketoconazole in 0.01% DMSO/distilled water, or (F) 1 mg/kg ketoconazole in 0.01% DMSO/distilled water and applied to wound site. At 1, 2, 3, 5, 7, 9, 11, and 14 days post application of infectious organism and test article, the wound site will be swabbed with a sterile Q-tip applicator and placed in YPD and SDA culture media that is optimized for growth of yeast and fungal organisms. The cultures will then be serially-diluted and grown in Room 3004E of the Dental Sciences Building (Level 2 Biosafety approved) overnight on Sabouraud dextrose agar (SDA) or yeast peptone dextrose (YPD) agar plates located in a 30oC incubator. The plates will then be examined after 24 hours of growth and the efficacy of DB2121 will then be determined. At 14 days post-application, the study will be terminated in which the BALB/c mice will be sanguinated and the wound site of infection, liver, kidney, and heart will be fixed in formalin for later histological analysis.

REFERENCES:

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8. Tsai, H. (1998) Human Salivary Histatins: Promising Anti-Fungal Therapeutic Agents. Crit Rev in Oral Biol & Med. 9(4):480-497
9. Zumbuehl, et al. (2007) Antifungal Hydrogels. PNAS 104 (32): 12994-12998

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