Annotated NIAID SBIR Phase II Grant Application

Last updated on August 22, 2003.

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Introduction

We are indebted to Dr. John Trawick and Elitra Pharmaceuticals for permitting us to show his outstanding Phase I and Phase II SBIR applications. Dr. Trawick submitted his Phase I application as a new investigator in December 1999, and his Phase II application in April 2002.

To avoid confusing current applicants, we have updated his original SBIR Phase I form pages onto those of the PHS 398 which are required today. We have also made some minor changes in personnel names and deleted confidential salary information. Other than these minor changes, the applications are exactly as submitted.

We have also included the Summary Statement and Notice of Grant Award for each application. Further, we've added annotations to explain how these application reflect much of the advice we give in our "Advice on SBIR and STTR Grant Applications."

NIAID annotations are in yellow boxes, like this one.

If you have questions or comments, please contact:

Gregory Milman, Ph.D.
Director, Office for Innovation and Special Programs
Division of Extramural Activities
National Institute of Allergy and Infectious Diseases, NIH, DHHS
6700-B Rockledge Drive; Room 2140
Bethesda, MD 20892-7610 (US Mail)
Rockville, MD 20817-7610 (Delivery Services)
Tel (301) 496-8666 Fax (301) 402-0369
Email gmilman@niaid.nih.gov

SBIR Phase I and II applications must use PHS398 form pages

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed 56-character length restrictions, including spaces.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT
Target based antifungal drug discovery

Note that project title was changed for Phase II.

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☐ NO ☒ YES
(If "Yes," state number and title)
Number: PHS 2003-2 Title: Phase II SBIR

Indicates response to SBIR solicitation

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

New Investigator ☒ **No** ☐ **Yes**

3a. NAME *(Last, first, middle)*
Trawick, John, Douglas

3b. DEGREE(S)
PhD

3c. POSITION TITLE
Senior Scientist

3d. MAILING ADDRESS *(Street, city, state, zip code)*
3510 Dunhill Street
San Deigo, CA
92121

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT

3f. MAJOR SUBDIVISION

3g. TELEPHONE AND FAX *(Area code, number and extension)*
TEL: 858-410-3019 FAX: 858-410-3090

E-MAIL ADDRESS: jtrawick@elitra.com

4. HUMAN SUBJECTS RESEARCH
☒ No
☐ Yes

4a. Research Exempt ☐ No ☐ Yes
If "Yes," Exemption No.

4b. Human Subjects Assurance No.

4c. NIH-defined Phase III Clinical Trial ☒ No ☐ Yes

5. VERTEBRATE ANIMALS ☒ No ☐ Yes

5a. If "Yes," IACUC approval Date

5b. Animal welfare assurance no

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
12/1/2002	12/1/2004	420,019	580,602	533,163	1,194,765

9. APPLICANT ORGANIZATION
Name Elitra Pharmceuticals
Address 3510 Dunhill Street
San Diego, CA
92121

Institutional Profile File Number (if known)

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☐ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☒ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER

DUNS NO. *(if available)*

Congressional District 46

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name Gregory Tibbits
Title Chief Financial Officer
Address Elitra Pharmaceuticals
3510 Dunhill Street
San Deigo, CA
92121
Tel 858-410-3058 FAX 858-410-3810
E-Mail gtibbits@elitra.com

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name Gregory Tibbitts
Title Chief Financial Officer
Address Elitra Pharmaceuticals 3510 Dunhill Street
San Diego, CA
92121
Tel 858-410-3058 FAX 858-410-3810
E-Mail gtibbitts@elitra.com

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.	SIGNATURE OF PI/PD NAMED IN 3a. *(In ink. "Per" signature not acceptable.)*	DATE
15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.	SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)*	DATE

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

The incidence of serious fungal infections has increased markedly in the last two decades and effective treatment options are increasingly compromised by the emergence of drug-resistant strains. The goal of the proposed work is to develop novel anti-fungal drugs that are safer and more effective than those currently available. The work will focus primarily on the dimorphic yeast, Candida albicans, which is by far the leading cause of both life-threatening systemic fungal infections and more commonly occurring topical infections. A distinguishing feature of the drug-discovery strategy we are pursuing is that it is based on target discovery, target prioritization and screening, all conducted with the pathogen itself, rather than with a surrogate model system. This strategy has been enabled by gene-identification and screen-configuration technologies developed at Elitra Pharmaceuticals, including important technologies developed under Phase I funding for this grant. Under Phase I, an expression vector system was constructed that will allow screening for dominant-negative phenotypes. No such tools existed previously for C. albicans. Screens for dominant-negatives will identify new drug targets in C. albicans and help annotate essential physiological pathways in this important fungal pathogen. Critical functional features of the expression vector system have already been validated, a C. albicans cDNA library has been constructed in the vector, and pilot screening for dominant negatives is in progress. Under Phase II funding, we propose to implement the screen more broadly, characterize and prioritize the targets that are identified and conduct screening for drug leads. The dominant-negative phenotypes of the newly identified targets will be used to develop primary or secondary cell-based assays to screen chemical libraries for potential anti-fungal drugs and to facilitate the characterization of hits identified through other screening strategies. The proposed Phase II work will complement and enhance the value of internally funded target-identification and screening efforts at Elitra and will help promote the discovery of badly needed therapeutic agents in a medical area that generally receives insufficient attention from the pharmaceutical industry.

Describes Phase I success and logical extension of Phase I to Phase II.

PERFORMANCE SITE(S) *(organization, city, state)*

Elitra Pharmaceuticals
3510 Dunhill Street
San Diego, CA
92121

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
Trawick, John D.	Elitra Pharmaceuticals	Principal investigator, Program director
Bussey, Howard	McGill University, Montreal, Canada	Consultant
Carr, Grant	Elitra Pharmaceuticals	Collaborator, High throughput screening (HTS)
Corcos, Isabelle	Elitra Pharmaceuticals	Collaborator, High throughput screening
Foulkes, Gordon	Elitra Pharmaceuticals	Co-investigator, Head of Research
Kallel, Adam	Elitra Pharmaceuticals	Collaborator, Medicinal Chemistry, Chemi Informatics
Nouraini, Shahrzad	Elitra Pharmaceuticals	Co-investigator, Project leader

This page was added to list more key personnel than would fit on page 2.

Olson, Erica	Elitra Pharmaceuticals	Co-investigator, HTS support
Roemer, Terry	Elitra Pharmaceuticals	Consultant
Shangle, Starr	Elitra Pharmaceuticals	Co-investigator, HTS support
Youngman, Phillip	Elitra Pharmaceuticals	Co-investigator, Head of SBIR-directed research
Zamudio, Carlos	Elitra Pharmaceuticals	Collaborator, Bioinformatic

Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. **YesX** **No**

RESEARCH GRANT
TABLE OF CONTENTS

(Items A-D: not to exceed 25 pages)*

** SBIR/STTR Phase I: Items A-D limited to 15 pages.*

Appendix *(Five collated sets. No page numbering necessary for Appendix.)*

Check if Appendix is Included ☐

Appendices NOT PERMITTED for Phase I SBIR/STTR unless specifically solicited.

Number of publications and manuscripts accepted for publication *(not to exceed 10)* _____

Other items (list):

The detailed salary information on this page was deleted to protect privacy.

Principal Investigator/Program Director (Last, first, middle): Trawick, John, Douglas

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM | THROUGH

PERSONNEL *(Applicant organization only)* NAME	ROLE ON PROJECT	TYPE APPT. *(months)*	% EFFORT ON PROJ.	INST. BASE SALARY	DOLLAR AMOUNT REQUESTED *(omit cents)* SALARY REQUESTED	FRINGE BENEFITS	TOTAL
John Trawick	Principal Investigator		12.5				
Pill Youngman	Co-investigator		2.5				
Carlos Zamudio	Co-investigator		2.5				
Sherry Nouraini	Co-investigator		50				
Grant Carr	Co-investigator		2.5				
Isabelle Corcos	Co-investigator		25				
Adam Kallel	Co-investigator		5				
Gordon Foulkes	Co-investigator		2.5				
HTS RA	Co-investigator		50				
HTS RA	Co-investigator		22.5				
SUBTOTALS →							152936

Personnel should be listed in the same order as in the page 2 description. A detailed justification is necessary to explain to the review committee how an average of one or two hours per week (2.5% effort) will contribute to the project. "Co-investigator" is not a useful description of role on project.

These extra lines were added for more personnel. Although we can guess that "RA" means research associate, the applicant does not tell us what the meaning of HTS RA.

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		

Note absence of equipment in budget.

SUPPLIES *(Itemize by category)* Chemical compounds, enzymes, RNA purification reagents, cDNA synthesis reagents, DNA purification reagents, E. coli cells and media, PCR reagents, C. albicans media, electrophoration cuvettes, assay plates, assay blocks, aluminum seals, DMSO, seals, Robbin tips, p6v plates		85,250
TRAVEL		12,500
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$259,436
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)* →		$420,019
SBIR/STTR Only: FEE REQUESTED		

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		152,936	162,112			
CONSULTANT COSTS		8,750	8,750			
EQUIPMENT		0	0			
SUPPLIES		85,250	90,365			
TRAVEL		12,500	12,500			
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
SUBTOTAL DIRECT COSTS		259,436	273,727			
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT					
	F&A					
TOTAL DIRECT COSTS		259,436	273,727			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD *(Item 8a, Face Page)* —— **$** 533,163

SBIR/STTR Only Fee Requested					

SBIR/STTR Only: Total Fee Requested for Entire Proposed Project Period
(Add Total Fee amount to "Total direct costs for entire proposed project period" above and Total F&A/indirect costs from Checklist Form Page, and enter these as "Costs Requested for Proposed Period of Support on Face Page, Item 8b.) **$**

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

A separate page is used for Justification to provide enough room.

Budget Justification

The budget justification is the weakest part of this application. It would be better if the budget justification listed personnel in the same order as on the detailed budget (page 5) which should also be the same and in the same order as the list of key personnel in the description (page 2). An organizational chart, diagram, or flow chart would have been useful to show how personnel will work on the project.

Dr. John Trawick will be responsible for the overall supervision of the project.

Dr. Grant Carr will be responsible for the overall supervision of the high throughput screening efforts.

This section should have described how Dr. Carr will use his average one hour per week on this project. It seems unlikely that this effort will be sufficient.

Dr. Isabelle Corcos will be responsible for the design and implementation of the high throughput screening experiments. Starr Shangle and Erica Olson are Research Assistants that will be working under the direction of Dr. Corcos to perform the high throughput screening operations. Star Shangle also provide her expertise in microbiology preclinical development to characterize the spectrum of activity of confirmed hit compounds.

It seems unlikely that Dr. Corcos could supervise two research assistants in her average one hour per week on this project. Ms. Shangle's biosketch is not provided so there is no way of assessing her claimed expertise.

Dr. Shahrzad Nouraini will be responsible for performing the dominant-negative genetic screen, and characterization and prioritization of dominant-negative cDNAs. Dr. Nouraini will also be responsible for development of assays for high throughput screening. I addition, Dr. Nouraini will be in charge of coordinating the various aspect of the Phase II project.

Dr. Adam Kallel is our lead chemist who will be responsible for characterizing and prioritization of hit compounds.

This section should have described how Dr. Kallel will accomplish this in his average two hours per week on the project.

Dr. Phillip Youngman is the leader of SBIR-directed projects and will provide guidance and coordination with Elitra's gene identification projects.

This section should provide understandable information on Dr. Youngman's role.

Dr. Gordon Foulkes will be responsible for making final decisions with respect to prioritizing targets and compounds for further development.

This section should tell us how and when Dr. Foulkes is going to make decisions.

Drs. Howard Bussey and Terry Roemer have agreed to consult during Phase II. Both will be in close contact with Dr. Trawick and Dr. Nouraini throughout the development of the project.

There is no description of the percent effort for either Dr. Bussey or Dr. Roemer. There is no biosketch or letter confirming a consulting arrangement for Dr. Bussey.

In the second budget period, the 5.5% annual increase in personnel costs and supply costs has been included.

More detail would be better including estimates of time required for each part of proposed project. A justification of supplies and travel is also lacking.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
John Douglas Trawick	**Senior Research Scientist, Elitra Pharmaceuticals**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Gustavus Adolphus Coll., St.Peter, MN	B.A	1976	Biology
Northern Illinois University, Dekalb IL	M.S.	1979	Biological Sciences
University of Minnesota, Minneapolis, MN	Ph.D.	1984	Microbiology

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

1984-1985 Postdoctoral Fellow, Mayo Foundation, Rochester, MN. Research in actin gene expression in mammalian cells.

1985-1990 Postdoctoral Research Associate, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO. Expression and regulation of cytochrome oxidase genes in the yeast, *Saccharomyces cerevisiae*, nuclear-mitochondrial interactions in *S. cerevisiae*.

1990-1992 Assistant Professor, Department of Medicine, University of Colorado Health Sciences Center, Denver, CO. Research into the molecular biology and genetics of bile acid synthesis in rat hepatoma cells and assembly of lipoproteins.

1992-1996 Adjunct Assistant Professor, Department of Biology, San Diego State University. Research into the molecular biology and genetics of bile acid synthesis in rat hepatoma cells and assembly of lipoproteins.

1992-present Adjunct Assistant Professor, Department of Biology, San Diego State University.

1996-1997 Course instructor, Biology Departments San Diego State University and University of San Diego.

1997-present Senior Research Scientist, Drug Development Dept., Elitra Pharmaceuticals. Target evaluation and validation *in E. coli* and *Staphylococcus aureus*. Target discovery in *Salmonella typhimurium*, target discovery in *Candida albicans*, vector development and improvement, and new organism evaluation.

Patent applications

Genes identified as required for proliferation in Escherichia coli. 2000 Inventors: Zyskind, J. W., Ohlsen, K.L., **Trawick, J.D.,** Forsyth, R. A., Froelich, J. M., Carr, G. J., Yamamoto, R. T., Xu, H. **WO 00/44906**

Identification of essential genes in prokaryotes. 2001. Haselbeck, R., Ohlsen, K. L., Zyskind, J. W., Wall, D., **Trawick, J. D**., Carr, G. J., Yamamoto, R.T., Xu, H. H. **WO 01/70955**

B. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

Name in bold makes it easy to infer role on publication

Kline, B., Seelke, R. and **Trawick, J.** Replication and incompatibility functions in mini-F plasmids. *In* Levy, S.B., Clowes, R.L., and Koenig, E.L., eds., Molecular biology, pathogenicity, and ecology of bacterial plasmids, pp. 317-326. Proceedings of the International Plasmid Conference on Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids, January 5-9, 1981, Santo Domingo, Dominican Republic, Plenum Press, NY.

Kline, B.C., Seelke, R.W., **Trawick, J.D.,** Levy, S.B. and Hogan, J. Genetic studies on the maintenance of mini-F plasmids. *In* Proceedings of the Third Tokyo Symposium on Mechanisms of Antibiotic Resistance, October, 1981, Tokyo, Japan 1984.
Seelke, R.W., Kline, B.C., **Trawick, J.D.,** and Ritts, G.D. 1982. Genetic studies of F plasmid maintenance genes involved in copy number control, incompatibility and partitioning. *Plasmid* **7:** 163-179.
Kline, B.C. and **Trawick, J.** 1983. Identification and characterization of a second copy number control gene in mini-F plasmids. *Molec. Gen. Genet.* **192:** 408-415.
Trawick, J.D. and Kline, B.C. 1985. A two-stage molecular model for control of mini-F replication. *Plasmid* **13:** 59-69.
Wright, R.M., **Trawick, J.D.,** Trueblood, C.E., Patterson, T.E., and Poyton, R.O. Organization and expression of nuclear genes for yeast cytochrome c oxidase. *In*: Cytochrome systems: Molecular biology and bioenergetics, pp. 49-56. ed. S. Papa. 1987. Plenum Press, NY.
Trawick, J.D., Wright, R.M., and Poyton, R.O. 1989. Transcription of yeast COX6, the gene for subunit VI of the cytochrome c oxidase of S. cerevisiae, is dependent on heme and on the HAP2 gene. *J. Biol. Chem.* **264:** 7005-7008.
Trawick, J.D., Rogness, C.R., and Poyton, R.O. 1989. Identification of an upstream activation site and other cis-acting elements required for transcription of COX6 from Saccharomyces cerevisiae. *Mol. Cell. Biol.* **9:** 5350-5358.
Farrell, L.E., **Trawick, J.D.,** and Poyton, R.O. Mitochondrial-nuclear interactions: transcription of nuclear COX genes in yeast is reduced in cells that lack a mitochondrial genome. *In*: Structure, function, and biogenesis of energy transfer systems, pp.131-134, ed. E. Quaglieriello, S. Papa, F. Palmieri, and C. Saccone. 1990. Elsevier Press.
Trawick, J.D., Simon, F.R., Kraut, N.,and Poyton, R.O. 1992. Regulation of Yeast COX6 by the General Transcription Factor ABF1 and Separate HAP2 and Heme Responsive Elements. *Mol. Cell. Biol.* **12:** 2301-2314.
Leighton, J.K., Dueland, S., Straka, M.S., **Trawick, J.,** and Davis, R.A. 1991. Activation of the silent endogenous cholesterol-7-alpha-hydroxylase gene in rat hepatoma cells: A new complementation group having resistance to 25-hydroxycholesterol. *Mol. Cell. Biol.* **11:** 2049-2056.
Davis, R.A., Dueland, S. and **Trawick, J.** 1992. Bile Acid Synthesis and the Enterohepatic Circulation: Processes Regulating Total Body Cholesterol Homeostasis. *In* Molecular Genetics of Coronary Heart Disease and Stroke. Lusis, A., Rotter, J. and Sparkes, R.S., eds. Karger Press.
Thrift, R., Drisko, J. Dueland, S., **Trawick, J.D.**, and Davis, R.A.,. 1992. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in a nonhepatic cell line.*Proc. Natl. Acad. Sci. USA*. **89:**9161-9165.
Dueland, S., **Trawick, J.D.,** Nenseter, M.S., MacPhee, A.A., and Davis, R.A. 1992. Expression of 7alpha-hydroxylase in non-hepatic cells results in liver phenotypic resistance of the low density lipoprotein receptor to cholesterol repression. *J. Biol. Chem.* **267:** 22695-22698.
Trawick, J. D.,Lewis, K.D., Moore, G.L., Simon, F.R., and Davis, R.A. 1996. Rat hepatoma L35 cells, a liver-differentiated cell line, display resistance to bile acid repression of cholesterol 7 alpha-hydroxylase. *J. Lipid Res.* **37:** 588-599.
Moore, G. L., Drevon, C. A., Machleder, D., Lusis, A. J., **Trawick, J. D.**, Unson, M. A., McClelland, A., Roy, S., Lyons, R., Jambou, R., and Davis, R.A. 1997. Expression of human cholesterol 7 alpha-hydroxylase in atherosclerosis-susceptible mice via adenovirus infection. *Biochem. J.* **324:** 863-867.
Dueland, S., France, D., Wang, S.-L., **Trawick, J. D.,** and R. A. Davis. 1997. Cholesterol-7alpha-hydroxylase influences the expression of hepatic Apo AI in two inbred mouse strains displaying different susceptibilities to atherosclerosis and in hepatoma cells. *J. Lipid Res*. **38:** 1445-1453.
Trawick, J. D., Shui-Long Wang, David Bell, and R.A. Davis.1997. Transcriptional induction of 7 alpha-hydroxylase by dexamethasone in L35 hepatoma cells requires sulfhydryl reducing agents. *J. Biol. Chem*. **272:** 3099-3102.
R. A. Forsyth, R. J. Haselbeck, K. L. Ohlsen, R. T. Yamamoto, H. Xu, **J. D. Trawick**, D. Wall,L. Wang, V. Brown-Driver, J. M. Froelich, Kedar G. C., P. King, M. McCarthy, C. Malone, B. Misiner, D. Robbins, Z Tan, Z.-y. Zhu, G. Carr, D. A. Mosca, C. Zamudio, J. G. Foulkes & J. W. Zyskind. 2002. A genome-wide strategy for the identification of essential genes in *Staphylococcus aureus Molec. Microbiol*. **43 (6):** 1387-1400.

C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.

PI, SBIR phase I research. "Dominant expression for new targets in *Candida albicans***"** 2001. Phase I of present application. NIH grant #
Goal of project was to construct and validate vectors for dominant negative gene identification in *C. albicans*, construct cDNA libraries to carry out dominant negative gene identification and screen for *C. albicans* genes using this technology.
Task Force Leader of a task force in Elitra collaboration with Merck. 2001-present. Organize and carry out efforts to build proprietary Elitra genetic system for identification of cellular targets "hit" by active compounds in the human pathogen, *S. aureus*. Co-leader of task force, designed principle genetic tools in effort and have overseen efforts of several person team in implementing these genetic tools.
Task Force leader of target validation for *E.coli* and *S. aureus* genes. 2000-present. Organized and executed efforts to validate essential gene targets recognized in Elitra genetic screening. Responsible for Elitra validation of essential gene targets in both of these organisms.
Member of team, Collaboration between Elitra and LG Chem. 2000-present. Responsible for functional evaluation of targets presented to LG Chem (Republic of Korea) as part of Elitra collaboration. Has evolved into responsibility for functional (i.e., biological role) of potential antibacterial targets in Elitra collaborations with other pharmaceutical firms.
Leader of team for essential gene identification in *Salmonella enterica* Typhimurium. 1999. Led effort to apply Elitra genetic technology to *Salmonella enterica*. Led effort to improve genomic library construction and genetic screening.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Grant J. Carr	Director of Screening Operations

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Oxford University, England Imperial College, University of London, England	Ph. D. B. Sc.	1989	Cell & Molecular Biology Microbiology

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

D. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions and Employment

1989-1991 Postdoctoral Fellow, LEICESTER UNIVERSITY, Leicester, England
1991-1992 Scientist, OHMEDA PHARMACEUTICAL PRODUCTS DIVISION, THE BOC GROUP, Murray Hill, NJ
1992-1994 Senior Scientist, OHMEDA PHARMACEUTICAL PRODUCTS DIVISION, THE BOC GROUP, Murray Hill, NJ.
1994-1995 Lead Scientist, OHMEDA PHARMACEUTICAL PRODUCTS DIVISION, THE BOC GROUP, Murray Hill, NJ
6/95 - 6/96 Senior Scientist, AXYS PHARMACEUTICALS, South San Francisco, CA.
6/96 – 3/98 Group Leader, AXYS PHARMACEUTICALS, South San Francisco, CA.
4/98 - 9/99 Director, Biochemistry and High Throughput Screening, AXYS PHARMACEUTICALS, South San Francisco, CA.
6/95 - 9/99 Head of High Throughput Screening, AXYS PHARMACEUTICALS, South San Francisco, CA.
10/99 – Present Director of Screening Operations, ELITRA PHARMACEUTICALS, San Diego, CA

Note that the references in this CV have a different format than those in the previous one. Although there is no requirement that all CVs have the same reference format, an application looks more professional and coordinated when there is a unified format throughout.

E. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

1- Carr GJ, Ferguson SJ. The nitric oxide reductase of Paracoccus denitrificans. (1990) Biochem J. Jul 15;269(2):423-9.

2- Carr GJ, Ferguson SJ. Nitric oxide formed by nitrite reductase of Paracoccus denitrificans is sufficiently stable to inhibit cytochrome oxidase activity and is reduced by its reductase under aerobic conditions. (1990) Biochim Biophys ActaMay 15;1017(1):57-62.

3- Carr GJ, Page MD, Ferguson SJ. The energy-conserving nitric-oxide-reductase system in Paracoccus denitrificans. Distinction from the nitrite reductase that catalyses synthesis of nitric oxide and evidence from trapping experiments for nitric oxide as a free intermediate during denitrification. (1989) Eur J Biochem. Feb 15;179(3):683-92.

4- Page MD, Carr G, Bell LC, Ferguson SJ. Structure, control and assembly of a bacterial electron transport system as exemplified by Paracoccus denitrificans. (1989) Biochem Soc Trans. Dec;17(6):991-3.

5- Shrader WD, Young WB, Sprengeler PA, Sangalang JC, Elrod K, Carr G. Neutral inhibitors of the serine protease factor Xa. (2001) Bioorg Med Chem Lett Jul 23;11(14):1801-4

6- R. A. Forsyth, R. J. Haselbeck, K. L. Ohlsen, R. T. Yamamoto, H. Xu, J. D. Trawick, D. Wall,L. Wang, V. Brown-Driver, J. M. Froelich, Kedar G. C., P. King, M. McCarthy, C. Malone, B. Misiner, D. Robbins, Z Tan, Z.-y. Zhu, G. Carr, D. A. Mosca, C. Zamudio, J. G. Foulkes & J. W. Zyskind.. (2002) A genome-wide strategy for the identification of essential genes in Staphylococcus aureus Molec. Microbiol. 43 (6): 1387-1400.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
ISABEL ANNE CORCOS	Senior Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
The University of Michigan, Ann Arbor, Michigan	Ph. D.	1985-1992	Cell & Molecular Biology
Michigan State University.	B. Sc.	1985	Zoology

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

F. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions and Employment

1992 – 1994 Postdoctoral Fellow, The Scripps Research Institute, San Diego, CA.
1994 - 1998: Research Scientist, Trega Biosciences, Inc., San Diego, CA.
1998 - 1999: Senior Scientist, Trega Biosciences, Inc., San Diego, CA.
May 1999 to Feb. 2000: Principal Scientist, Aurora Biosciences Corp., San Diego, CA.
Feb. 2000 to Oct. 2000: Automated Screening Data Coordinator, Aurora Biosciences Corp. , San Diego, CA.
Oct. 2000 to Dec. 2001: Research Scientist, HTS, Elitra Pharmaceuticals, Inc., San Diego, CA.
2002-Present: Senior Scientist HTS, Elitra Pharmaceuticals, Inc., San Diego, CA.

G. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

1-C. Corcos, I. Corcos, B. Stockhoff. Double-take: A second look at cloning, law, and science fiction. *Louisiana Law Review* 59:1041-1099. Summer, 1999.

2-Corcos, I.A., E.U. Meese, R. Loch-Caruso. Human connexin43 gene locus, GJA1, sublocalized to 6q21-23.2. *Cytogenetics and Cell Genetics* 64:31-32; 1993.

3-Corcos, I.A., R.G. Lafreniere, C.R. Begy, R. Loch-Caruso, H.F. Willard, T.W. Glover. Refined localization of human connexin32 gene locus, GJB1, to Xq13.1. *Genomics* 13:479-480; 1992.

4-Loch-Caruso, R., I.A. Corcos, J.E. Trosko. Inhibition of metabolic coupling by metals. *J. Tox. Env. Hlth.* 32:33-48; 1991.

5-Loch-Caruso, R., D.R. Juberg, V. Caldwell, I.A. Corcos. Cultured myometrial cells establish communicating gap junctions. *Cell Biol. Int. Rep.* 14:905-916; 1990.

6-Loch-Caruso, R., J.E. Trosko, I.A. Corcos. Interruption of cell-cell communication in Chinese hamster V79 cells by various glycol ethers: Implications for teratogenicity. *Environ. Health Perspect.* 57:119-123; 1984.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
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NAME	POSITION TITLE
J. Gordon Foulkes	Executive Vice President, Research and Development

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Dundee, Scotland	Ph. D.	1979	Biochemistry
University College Cardiff, Wales	B. Sc.	1976	Biochemistry

A. Positions and Honors

Positions and employment

1980 – 1981 Postdoctoral fellow, University of Colorado, Denver, Colorado
1982 – 1984 Senior fellow, Massachusetts Institute of Technology, Massachusetts
1984 – 1987 Tenured Member of the Scientific Staff, The Medical Research Council (MRC), National Institute for Medical Research, London, U.K
1987 - 1990 Director of Therapeutics, Oncogene Science, Inc., New York, NY
1990 – 1992 Vice President and Director of Therapeutics, Oncogene Science, Inc., New York, NY
1992 – 1995 Vice President and Chief Scientific Officer, Oncogene Science, Inc., New York, NY
1994 – 1996 Appointed to the Office of the Chief Executive and the Board of Directors, Oncogene Science, Inc., New York, NY
1996 – 1998 Chief Technical Officer and Member of the Board of Directors, Aurora Biosciences Corporation, San Diego, CA
1999 – Present Executive Vice President, Research and Development, Elitra Pharmaceuticals, San Diego, CA

Publications:

Over 50 major publications and reviews prior to joining Oncogene Science in 1987. Examples:
Discovery and characterization of mammalian protein-tyrosine phosphatases: J. Biol. Chem. 258, 431-438; FEBS Lett. 130, 197-200.
Discovery in transformed cells of tyrosine phosphorylated nuclear proteins: Nature 325, 552-554.
Development of the first bacterial expression system for purification of a tyrosine kinase: J. Biol. Chem. 260, 8070-8077.
Identification of serine/tyrosine protein kinase cascade systems. Proc. Natl. Acad. Sci. U.S.A. 82, 272-276; EMBO J. 4, 3173-3178; Proc. Natl. Acad. Sci. U.S.A. 84, 4408-4412.
Identification of protein phosphatases in translational control. Proc. Natl. Acad. Sci. U.S.A. 82, 272-276; Proc. Natl. Acad. Sci. U.S.A. 79, 7091-7096; J. Biol. Chem. 258, 1439-1443.
Discovery of a new human oncogene. Nature 325, 635-637.
Cloning of TGF-β3. Proc. Natl. Acad. Sci. USA 85, 4715-4719.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
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NAME	POSITION TITLE
Edward Adam Kallel	Principle Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Los Angeles	Ph. D.	1991	Applied Quantum Mechanics
California State Polytechnic University, Pomona	B. Sc.	1987	Chemistry

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

H. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions and Employment

9/1/91-12/20/94 Computational Chemist, American Cyanamid Company ARD, Princeton NJ

1/3/95 - 4/1/97 Research Scientist, Medicinal Chemistry, Ligand Pharmaceuticals Inc., San Diego, CA

4/1/9 - 5/16/97 Senior Research Scientist, Medicinal Chemistry, Ligand Pharmaceuticals Inc., San Diego, CA

5/19/97–1/5/00 Staff Scientist Medicinal Chemistry, Chugai Biopharmaceuticals Inc., San Diego, CA

1/7/00–present Principle Scientist Drug Discovery Informatics, Elitra Pharmaceuticals Inc., San Diego, CA

Honors

Donald J. Cram Award For Research Excellence UCLA

Cal. Poly. Pomona, Alumnae Merit Scholarship

CRC Freshman Chemistry Award, Cal. Poly.

I. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

1) Zhi, L.; Tegley, C. M.; Kallel, E. A.; Marschke, K. B.; Mais, D. M.; Gottardis, M. M.; and Jones, T. K. (1998) *J. Med Chem*, *3*, 291.

2) Farmer, L. J.; Jeong, S.; Kallel, E. A.; Canan Koch, S. C.; Croston, G. E.; Flatten, K. S.; Heyman, R. A. and Nadzan, A. M. (1997) *Bioorganic and Medicinal Chemistry Letters*, *7* , 2393.

3) Farmer, L. J.; Jeong, S.; Kallel, E. A.; Canan Koch, S. C.; Croston, G. E.;

Flatten, K. S.; Heyman, R. A. and Nadzan, A. M. (1997) *Bioorganic and Medicinal Chemistry Letters*, *7*, 2747

4) Niwayama, S.; Kallel. E. A.; Spellmeyer, D. C.; Sheu, C.; Houk, K. N. (1996) *J. Org. Chem.*, *61*, 2813.

5) Niwayama, S.; Kallel, E. A.; Sheu, C.; Houk, K. N. (1996) *J. Org. Chem.*, *61*, 2517.

6) Gange, D.; Kallel, E. A. (1991) *J. Chem. Soc., Chem. Commun.*, , *11*, 824.

7) Kallel, E. A.; Wang Y.; Spellmeyer, D. C.; Houk, K. N. (1990) *J. Am. Chem. Soc. 112*, 6759.

8) Kallel E. A.; Houk, K. N. (1989) *J. Org. Chem.*, *54*, 6006.

9) Goldstein, E.; Kallel, E. A.; Beauchamp, P. S. (1987) *J. Mol. Structure (Theochem) 151*, 297.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
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NAME	POSITION TITLE
Shahrzad (Sherry) Nouraini	Post Doctoral Fellow

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Toronto, Toronto, Canada	Ph. D.	1997	Yeast Genetics
University of Toronto, Toronto, Canada	M. Sc.	1992	Yeast Genetics
York University, Toronto, Canada	B. Sc.	1989	Biology/Chemistry

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J. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions and Employment

1997-2001 Post doctoral fellow, Burnham Institute, San Diego, CA
2001-present Post doctoral fellow, Elitra Pharmaceuticals, San Diego, CA

Honors

1998-2000 University of California Tobacco Related Disease Research Program Fellowship
1992-95 National Cancer Institute of Canada Steve Fonyo Studentship
1990-92 Medical Research Council of Canada Studentship
1990 Ontario Graduate Scholarship
1989 University of Toronto Open Master's Fellowship
1989 Dean's Honor list
1988 Book prize award of Chemical Institute of Canada
1988 NSERC summer studentship
1986 NSERC summer studentship

K. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

1-The putative pore-forming domain of Bax regulates mitochondrial localization and interaction with Bcl-X_L. **Shahrzad Nouraini**, Emmanuelle Six, Shigemi Matsuyama, Stainslaw Krajewski, and John C. Reed (2000), Mol. Cell. Biol. 20: 1604-1615.

2-Yeast as a tool for apoptosis research. Shigemi Matsuyama, Shahrzad Nouraini, and John C. Reed (1999), Current Opinion in Microbiology 2: 618-623.

3-Genetic evidence for selective degradation of RNA polymerase subunits by the 20S proteasome in *Saccharomyces cerevisiae*. **Shahrzad Nouraini**, Deming Xu, Sue Nelson, Marcus Lee, and James D. Friesen (1997) Nucl. Acids. Res. 12: 3570-3579.

4-An RNA-dependent ATPase associated with U2/U6 snRNA in pre-mRNA splicing. Deming Xu, Shahrzad Nouraini, Deborah Field, Shou-Jiang Tang, and James D. Friesen (1996) Nature 381: 709-713.

5-Rpo26p, a subunit common to yeast RNA polymerases, is essential for the assembly of RNA polymerases I and II and for the stability of the largest subunits of these enzymes. **Shahrzad Nouraini**, Jacques Archambault, and James D. Friesen (1996), Mol. Cell. Biol. 16: 5985-5996.

6-Mutations in an Abf1p binding site in the promoter of yeast RPO26 shift the transcription start sites and reduce the level of RPO26 mRNA. **Shahrzad Nouraini**, Jim Hu, Linda D. B. McBroom, and James D. Friesen (1996) Yeast 12: 1339-1350.

C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and your role (e.g. PI, Co-Investigator, Consultant) in the research project. Do not list award amounts or percent effort in projects.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
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NAME	POSITION TITLE
Terry D. Roemer	Director of Fungal Genomics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Simon Fraser University, British Colombia, Canada	B.Sc.	1982-1987	Biology
McGill University, Montreal, Quebec	Ph.D.	1989-1994	Yeast Genetics

B. Positions and Honors

Positions and employment

1995-1997 Postdoctoral Fellow, Yale University
3/1/98-10/1/0 Co-founder and Director of Research and Development
Mycota Biosciences Inc., Montreal, Quebec
10/1/0-present Director of Fungal Genomics, Elitra Pharmaceuticals Inc.
San Deigo, CA

Honors

1995-1997	Medical Research Council of Canada (MRC) Postdoctoral Fellowship
1992-1994	Natural Sciences and Engineering Research Council of Canada (NSERC) Postgraduate Fellowship
1992-1994	Fonds pour la Formation de Chercheurs et L'Aide à la Recherche (FCAR) Postgraduate Fellowship (declined)
1988	National Research Council (NRC) Summer Student Program
1985-1987	NSERC Undergraduate Research Award recipient 4 times

B. Selected peer-reviewed publications

1-R.Haselbeck, D. Wall, B. Jiang, T. Ketela, J. Zyskind, H. Bussey, J.G. Foulkes, **T. Roemer**; Comprehensive Essential Gene Identification as a Platform for Novel Anti-infective Drug Discovery, Current Pharmaceutical Design, Volume 7, 2001 (in press).

2-P. Ross-Macdonald, Coelho, P., **Roemer, T**., Kumar, A., des Etages, S-S., Cheung, K., Sheehan, A., Symoniatis, D., Jansen, R., Umansky, L., Nelson, K., Agarwal, S., Hager, K., Kanada, D., Lugo, R., Miller, P., Roeder, G.S., and Michael Snyder (1999) Large-scale analysis of the yeast genome by transposon tagging and disruption. In press Nature.

3-**T. Roemer**, Vallier, L. G., Sheu, Y-L., and M. Snyder (1998). Sph1p localizes to sites of polarized growth and interacts with components of two MAP kinase modules. J. Cell Sci. 111: 479-494.

4-**T. Roemer**, Madden, K., Chang, J., and M. Snyder (1996). Selection of axial growth sites in yeast requires Axl2p, a novel plasma membrane glycoprotein. Genes & Dev. 10:777-793.

5-**T. Roemer**, Vallier, L. G., and M. Snyder (1996). Selection of polarized growth sites in yeast. Trends in Cell Biol. 6: 434-441.

6-**T. Roemer** and H. Bussey (1995). Yeast Kre1p is a cell surface O-glycoprotein. Mol. Gen. Genet. 249:209-216.

7-**T. Roemer**, Paravicini, G., Payton, M. A., and H. Bussey (1994). Characterization of the yeast (1-6)-β-Glucan biosynthetic components, Kre6p and Skn1p, and genetic interactions between the PKC pathway and extracellular matrix assembly. J. Cell Biol. 127:567-579.

8-**T. Roemer**, Fortin, N., and H. Bussey (1994). DNA Sequence analysis of a 10.4 kbp region on the right arm of yeast chromosome XVI positions GPH1 and SGV1 adjacent to KRE6, and identifies two novel tRNA genes. Yeast 10: 1527-1530.

9-**T. Roemer**, Delaney, S., and H. Bussey (1993). SKN1 and KRE6 define a pair of functional homologs encoding putative membrane proteins involved in β-Glucan synthesis. Mol. Cell. Biol. 13: 4039-4048.

10-**T. Roemer** and H. Bussey (1991). Yeast β-Glucan synthesis: KRE6 encodes a predicted type II membrane protein required for glucan synthesis in vivo and for glucan synthesis activity in vitro. Proc. Natl. Acad. Sci. USA 88:11295-11299.

BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Philip J. Youngman	Vice President, Discovery Biology Elitra Pharmaceuticals

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Harvard University, Cambridge MA	B.A.	1969-73	Biochemistry
MIT, Cambridge MA	Ph.D.	1974-79	Biology
Harvard University, Cambridge MA		1980-85	Postdoctoral

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L. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

1980-83 Postdoctor Fellow, Jane Coffin Childs Memorial Fund for Medical Research, Laboratory of Richard Losick, Harvard University
1983-85 Research Associate, Department of Cellular and Developmental Biology, Harvard University
1985-91 Assistant Professor, Associate Professor, Department of Microbiology, University of Pennsylvania School of Medicine
1991-96 Associate Professor, Department of Genetics, University of Georgia
1996- Adjunct Associate Professor, Department of Genetics, University of Georgia
1996-97 Director Molecular Biology, Antibacterial Project Team Leader, ChemGenics Pharmaceuticals
1997-01 Senior Director Bacterial Genetics, Millennium Pharmaceuticals
2001- Vice President Discovery Biology, Elitra Pharmaceuticals

M. Selected peer-reviewed publications (in chronological order). Do not include publications submitted or in preparation.

Perkins, J.B. and P. Youngman (1983). *Streptococcus* plasmid pAMα1 is a composite of two separable replicons, one of which is closely related to *Bacillus* plasmid pBC16. J. Bacteriol. 155:607-615.

Youngman, P., J.B. Perkins and R. Losick (1983). Genetic transposition and insertional mutagenesis in *Bacillus subtilis* with *Streptococcus faecalis* transposon Tn*917*. Proc. Natl. Acad. Sci. U.S.A. 80: 2305-2309.

Youngman, P., J.B. Perkins and R. Losick (1984). Construction of a cloning site near one end of Tn*917* into which foreign DNA may be inserted without affecting transposition in *Bacillus subtilis* or expression of the transposon-borne *erm* gene. Plasmid 12: 1-9.

Perkins, J.B. and P. Youngman (1984). A physical and functional analysis of Tn*917*, a *Streptococcus* transposon in the Tn*3* family that functions in *Bacillus*. Plasmid 12: 119-138.

Youngman, P., J.B. Perkins and R. Losick (1984). A novel method for the rapid cloning in *Escherichia coli* of *Bacillus subtilis* chromosomal DNA adjacent to Tn*917* insertions. Mol. Gen. Genet. 195: 424-433.

Youngman, P.J., P. Zuber, J.B. Perkins, K. Sandman, M. Igo and R. Losick (1985). New ways to study developmental genes in bacteria. Science 228: 285-291.

Perkins, J.B. and P. Youngman (1986). Construction and properties of Tn*917-lac*, a transposon derivative that mediates transcriptional gene fusions in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. 83: 140-144.

Sandman, K., R. Losick and P. Youngman (1987). Genetic analysis of *Bacillus subtilis spo* mutants generated by Tn*917*-mediated insertional mutagenesis.Genetics 117: 603-617.

Poth, H. and P. Youngman (1988). A new cloning system for *Bacillus subtilis* comprising elements of phage, plasmid and transposon vectors. Gene 73: 215-226.

Guzmán, P., J. Westpheling and P. Youngman (1988). Characterization of the promoter region of the *Bacillus subtilis spoIIE* operon. J. Bacteriol. 170:1598-1609.

Kunkel, B., L. Kroos, H. Poth, P. Youngman and R. Losick (1989). Temporal and spatial control of mother-cell regulatory gene *spoIIID* of *Bacillus subtilis*. Genes Dev. 3: 1735-1744.

Kenney, T., K. York, P. Youngman and C.P. Moran, Jr. (1989). Genetic evidence that RNA polymerase associated with σ^{A} uses a sporulation-specific promoter in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA 80: 9109-9113.

Olmedo, G., E. Ninfa, J. Stock and P. Youngman (1990). Novel mutations that enhance sporulation in *Bacillus subtilis*: evidence that phosphorylation of regulatory protein Spo0A controls the initiation of sporulation. J. Mol. Biol. 215: 359-372.

Camilli, A., D. Portnoy and P. Youngman (1990). Insertional mutagenesis of *Listeria monocytogenes* with a novel Tn*917* derivative that exhibits enhanced transposition frequency and allows direct cloning of DNA flanking transposon insertions. J. Bacteriol. 172: 3738-3744.

Bielecki, J., P. Youngman, P. Connelly and D. Portnoy (1990). *Bacillus subtilis* expressing a haemolysin gene from *Listeria monocytogenes* can grow in mammalian cells. Nature 345: 175-176.

Freitag, N.E., P. Youngman and D. Portnoy (1991). Transcriptional activation of the *Listeria monocytogenes* hemolysin gene in *Bacillus subtilis*. J. Bacteriol. 174: 1293-1298.

Smith, K. and P. Youngman (1992). Use of a new integrational vector to investigate compartment-specific expression of the *Bacillus subtilis spoIIM* gene. Biochimie 74: 705-711.

York, K., T.J. Kenney, S. Satola, C.P. Moran, Jr., H. Poth and P. Youngman (1992). Spo0A controls the σ^{A}-dependent activation of *Bacillus subtilis* sporulation-specific transcription unit *spoIIE* . J. Bacteriol. 174: 2648-2658.

Smith, K., M.E. Bayer and P. Youngman (1993). Physical and functional characterization of the *Bacillus subtilis* spoIIM gene. J. Bacteriol. 175: 3607-3617.

Smith, K. and P. Youngman (1993). Evidence that the spoIIM gene of *Bacillus subtilis* is transcribed by RNA polymerase associated with σ^{E} . J. Bacteriol. 175: 3618-3627.

Brown, D.P., L. Ganova-Raeva, B.D. Green, S.R. Wilkinson, M. Young and P. Youngman (1994). Characterization of *spo0A* homologues in diverse *Bacillus* and *Clostridium* species identifies a probable DNA-binding domain. Molec. Microbiol. 14:411-426.

Baldus, J.M., B.D. Green, P. Youngman and C.P. Moran Jr. (1994). Phosphorylation of *Bacillus subtilis* transcription factor SpoOA stimulates transcription from the *spoIIG* promoter by enhancing binding to weak OA boxes. J. Bacteriol. 176: 296- 306.

Bramucci, M.G., B.D. Green, N. Ambros and P. Youngman (1995). Identification of a *Bacillus subtilis spo0H* allele that is necessary for suppression of the sporulation-defective phenotype of a *spo0A* mutation. J. Bacteriol. 177:1630-1633.

Barák, I. and P. Youngman (1996). SpoIIE mutant of *Bacillus subtilis* comprise two distinct phenotypic classes consistent with a dual functional role for the SpoIIE protein. J. Bacteriol. 178:4984-4989.

Gutierrez, J.A., P.J. Crowley, D.P. Brown, J.D. Hillman, P. Youngman, A.S. Bleiweis (1996). Insertional mutagenesis and recovery of interrupted genes of *Streptococcus mutans* using transposon Tn917: Preliminary characterization of mutants displaying acid sensitivity and nutritional requirements. J. Bacteriol. 178:4166-4175.

Barák, I., J. Behari, G. Olmedo, P. Guzmán, D.P. Brown, E. Castro, D. Walker, J. Westpheling and P. Youngman (1996). Structure and function of the *Bacillus* SpoIIE protein and its localization to sites of sporulation septum assembly. Molec. Microbiol. 19:1047-1060.

Framson, P.E., A. Nittayajarn, J. Merry, P. Youngman and C.E. Rubens (1997). New genetic techniques for Group B streptococci: high-efficiency transformation, maintenance of temperature-sensitive pWV01 plasmids, and mutagenesis with Tn917. Appl. Environ. Microbiol. 63: 3539-3547.

Milenbachs, A.A., D.P. Brown, M. Moors and P. Youngman (1997). Carbon-source regulation of virulence gene expression in *Listeria monocytogenes*. Mol. Microbiol. 23: 1075-1085.

Kasman, L.M., A.A. Lukowiak, S.F. Garczynski, R.J. McNall, P. Youngman, and M.J. Adang (1998). Phage display of a biologically active *Bacillus thuringiensis* toxin. Appl. Envion. Microbiol. 64: 2995-3003.

Hatt, J. and P. Youngman (1998). SpoOA mutants of *Bacillus subtilis* with sigma factor-specific defects in transcription activation. J. Bacteriol. 180: 3584-91.

Fawcett, P., A. Melnikov and P. Youngman (1998). The *Bacillus* SpoIIGA protein is targeted to sites of spore septum formation in a SpoIIE-independent manner. Mol. Microbiol. 28: 931-43.

Melnikov, A. and P.J. Youngman (1999). Random mutagenesis by recombinational capture of PCR products in *Bacillus subtilis* and *Acinetobacter calcoaceticus*. Nucleic Acids Res. 27: 1056-1062.

Hatt, J.K. and P. Youngman (2000). Mutational analysis of conserved residues in the putative DNA-binding domain of the response regulator Spo0A of *Bacillus subtilis*. J Bacteriol 182:6975-82.

Ravagnani, A., K.C. Jennert, E. Steiner, R. Grunberg, J.R. Jefferies, S.R. Wilkinson, D.I. Young, E.C. Tidswell, D.P. Brown, P. Youngman, J.G. Morris, M. Young (2000). Spo0A directly controls the switch from acid to solvent production in solvent-forming clostridia. Mol Microbiol. 37:1172-85.

Fawcett, P., P. Eichenberger, R. Losick and P. Youngman (2000). The transcriptional profile of early to middle sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA 97: 8063-8068.

Price, C.W., P. Fawcett, H. Ceremonie, N. Su, C.K. Murphy, P. Youngman (2001). Genome-wide analysis of the general stress response in *Bacillus subtilis*. Mol Microbiol. 41: 757-774.

C. Research Support. List selected ongoing or completed (during the last three years) research projects (federal and non-federal support). Begin with the projects that are most relevant to the research proposed in this application. Briefly indicate the overall goals of the projects and responsibilities of principal investigator identified above.

No sponsored research during the last three years.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
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NAME	POSITION TITLE
Carlos Zamudio	Vice president, Drug Discovery Informatics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Los Angeles	B. Sc.	1979	Computer Science and Mathematics

A. Positions and Honors

Positions and Employment

1979-1984 Technical Staff, Defense Systems Group, TRW, Los Angeles, CA
1984-1986 Technical Staff, Jet Propulsion Laboratories, Pasadena, CA.
1986-1988 Product Development Manager, Technology Systems Organization, TRW, Los Angeles, CA
1988-1993 Group Leader, DNA Technology Cosnortium, Applied Biosystems Inc./Perkin Elmer, Foster City, CA
1993-1997 Director, Bioinformatics and Engineering, Sequana Therapeutics, La Jolla, CA.
1997-1999 Vice President, Bioinformatics, Axiom Biotechnologies Inc., San Diego, CA
1/99-10/99 President, Drug Discovery Informatics Consulting, San Diego, CA
1999-2002 Director of Drug Discovery Informatics, Elitra Pharmaceuticals, San Diego, CA
2002-Present Vice President, Drug Discovery Informatics, Elitra Pharmaceuticals, San Diego, CA

B. Selected peer-reviewed publications

N/A

C. Research Support

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:
Elitra Pharmaceuticals' laboratories are located in La Jolla, CA, where many biotech companies, UCSD, the Salk Institute, and Scripps Research Institute are also located. The Elitra facilities occupy around 32,000 square feet. The laboratories contain all necessary standard equipment standard for molecular biology and biochemistry research including incubators, cold room, warm room, centrifuges, freezers, chemical hood, a biosafety containment level 2 facility, electroporators, phase contrast microscope, spectrophotometer, autoclaves, 9 PCR machines, ice machine, ABI 377 and 3700 DNA sequencers, ABI Taqman system for RNA quantitation, radiation room and hybridization equipment, Molecular Dynamics Typhoon system for blot/gel imaging and quantitation, chemical balances, etc. Also included is equipment for automated picking and replica gridding of bacterial and fungal colonies. We have added an automated chemical screening system that was in place and in use since October of 1999. A fully equiped mammalian cell culture facility including laminar flow hoods and CO2 incubators is in operation for toxicity studies. Informatics support includes an internal TCP/IP network connected to the Internet through a high-bandwidth connection, and both multi-processor Windows 2000 and Sun Microsystems' Solaris computers serving as computer and file servers.

Impressive company laboratory facilities are described.

Clinical:
N/A

Animal:
Animal work is not part of this grant application. However, Elitra does rent an animal facility and has local and state approval for experiments involving animals. Future work beyond the scope of this application will require proof of efficacy of antifungal candidate compounds in animals. Approval of that work will be sought when necessary.

Indicates animal studies can be accomplished later if work progresses sufficiently. No need to include approval with this application.

Computer:
Elitra computer resources include servers providing company-wide network access to proprietary Elitra PathoSeq database and other licensed informatic resources including MycoPathDB and LifeTools. Elitra also has separate systems with RS3 and other HTS screening applications to monitor and analyze chemical screening and inventory compounds.

Office:
Adminstrative personel sufficient for all accounting, ordering, inventory, and other day to day management tasks are in place. The finance department has administered 3 SBIR Phase I grants in addition to the approximately $50 million raised during Elitra's history.

Highlights administrative experience with NIH grant management requirements.

Other:
Tele-video conference capability to communicate with Elitra Canada division in Montreal, Canada.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

ABI 377 and 3700 DNA Sequencers

2 Colony pickers that each pick bacterial colonies at a rate of 1800/hour

2 Gridders that can move bacterial cells from microtiter plates containing liquid or solid media into liquid or solid media

Biomek robots that carry out a variety of tasks including serial dilutions, plasmid minipreps, setting up of PCR reactions for sequencing, and PCR product cleanup.

Cell culture: laminar flow hoods, CO2 incubators, etc for culturing human cell lines in toxicity studies.

High-throughput screening systems. A comprehensive chemical screening laboratory has been set up for HTS cell-based assays and usable for biochemical assays as well. In clean rooms, automated systems are capable of handling and performing 25,000 cell-based assays per day in 384 well plates. Goal in 2002 is to increase capacity to 100,000 cell-based assays per day in 1536 well plates. These capacities are excellent by industry standards.

Chemical libraries: current libraries number ~250,000 compounds. Efforts are to increase in 2002 to ~500,000 compounds including natural products.

Describes equipment that is available for proposed studies.

RESEARCH PLAN

SPECIFIC AIMS

Phase I funding for this project supported the successful development of genetic tools that will enable new approaches to target-identification and cell-based screening in *Candida albicans*, the most important fungal pathogen of humans. The ultimate goal of the proposed Phase II project is to identify novel anti-fungal compounds that target newly identified *C. albicans* essential genes.

Describes how they accomplished Phase I goals.

The approach we are taking for identification of *C. albicans* essential genes is to utilize the ability of some essential genes to induce a dominant-negative phenotype upon over-expression (21). In phase I we constructed, tested, and validated a set of vectors and promoters suitable for over-expression of *C. albicans* cDNAs. Demonstrating a dominant negative phenotype upon over-expression of a known essential gene validated our expression system. We constructed a cDNA library in our validated expression vector and are now in the process of performing a pilot screen for dominant-negative *C. albicans* cDNAs.

Under Phase II support, we will implement more broadly the screening strategy developed in Phase I with the goal of identifying novel *C. albicans* essential genes. The expression vector tools developed in Phase I will also provide a method to use these genes as targets in high-throughput cell-based screening to discover new anti-fungal compounds.

Specific aims:

The three specific aims are logical and progress from one to the next.

1-Perform dominant negative screening to identify essential genes. A pilot screen currently on the way will be followed by a more comprehensive screen during Phase II of the project with the goal of identifying >100 essential genes.

2-Prioritize targets for screening. The essentiality of dominant-negative *C. albicans* genes will be verified by an independent method that involves sequential deletion of the two alleles of the gene. With the goal of development of broad-spectrum fungal-specific compounds, genes confirmed to be essential will be further prioritized by bioinformatic means based on presence of orthologues in other fungal pathogens, and absence of such functionally homologous genes in humans.

3-Use prioritized targets in a drug screening effort to find compounds acting specifically on the targeted gene product. Depending on the nature of the target gene, either cell-based or biochemical assays will be developed to screen chemical libraries for potential anti-fungal drugs. In cell-based assays, target-specific compounds will be identified based on their toxicity to cells only when the expression of the target gene is reduced. Using a reciprocal target-based screening method, the target-specificity of compounds will be validated based on their ability to alleviate the dominant-negative phenotype conferred by over-expression of our targets. Targets that have a known biochemical function will be purified and used to screen for compounds that inhibit their activity.

A phase III follow up work will include testing the in vivo efficacy of the potential lead compounds using in vivo models of candidiasis and moving qualified Candidates to clinical evaluation.

Background and significance

Candida albicans, importance in human health

There is an urgent need for novel and efficacious drugs to treat severe fungal infections (39)(18). Over the past decade, these infections have risen dramatically in immunocompromised and critically ill patients. Fungi now represent the fourth most common cause of bloodstream infection in U.S. hospital patients. Although the incidence of mortality caused by severe fungal infections is difficult to estimate, well over 250,000 patients are treated for severe fungal infections annually in the U.S. alone. Many of these patients are usually very ill already; therefore, fungal infections add significantly to overall health costs by prolonging their hospitalization.

Describes why research and product are important to human health and the mission of NIAID.

From an epidemiological standpoint, *Candida* comprises the most medically significant genus of human fungal pathogens, having caused approximately 173,000 cases, or nearly 70% of severe fungal infections in the U.S. in 1999 (26). *C. albicans* is the primary fungal pathogen within this genus, being responsible for over 72,000 (or 42%) of reported cases of invasive candidiasis. In particular, *C. albicans* causes oral and systemic candidiasis in immunocompromised patients and vulvovaginal candidiasis in women. *C. albicans* is also closely related to other clinically relevant non-albicans pathogens, including *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei*- currently estimated to contribute to nearly 50% (or over 86,000 cases) of *Candida* infections. Taken together, this information demonstrates the significant medical and economic importance of *C. albicans* pathogenesis.

There is an increasing need for safer and more effective antifungal agents. Some of the more effective antifungal agents including amphotericin B and the azoles (e.g. fluconazole, itraconazole) have toxicity problems because their cellular targets have homologues in mammalian cells. The azoles inhibit lanosterol 14alpha-demethylase, a cytochrome P450 enzyme critical for sterol synthesis in fungi and mammals; the azoles are also effective inhibitors of many cytochrome P450 reactions and because of this are useful tools in mammalian cell biology (22). In addition, cytochrome P450 inhibitors such as fluconazole are inherently prone to drug-drug interactions. Amphotericin B targets plasma membrane sterols and is nephrotoxic (13). Additionally, *C. albicans* strains resistant to the azoles have been on the increase in recent years (13).

C. albicans exhibits a complex life cycle dependent upon in vitro and in vivo growth conditions. Normally, *C. albicans* grows as a yeast cell or blastospore at 30^{o} C and with glucose as a carbon source. However, when cultured in the presence of serum, with carbon sources such as N acetyl-glucosamine, at elevated (37 ^{o} C) temperatures, or at altered (higher) pH, *C. albicans* switches to a predominantly hyphal form (32). The transitions between the yeast and hyphal forms appear to be essential for virulence. Non-hyphal *C. albicans* strains are avirulent (9) as are obligately hyphal *C. albicans* strains.

Despite the importance of *C. albicans* to human disease, work on this organism has often been hindered due to its largely asexual diploid nature and variant CTG codon (in *C. albicans* CTG encodes serine instead of leucine; (23),(35). The more facile organism for molecular biology, *S. cerevisiae,* is able to transition between haploid and diploid forms, and is suited to mutagenic analysis of gene function using knockouts. Recently, mating has been demonstrated in *C. albicans* (41), (25), but the normal, in vivo, role for this process in *C. albicans* is uncertain. Mated *C. albicans* cells become tetraploid but fail to undergo meiosis (41); production of haploids is still viewed as impossible. Due to these and other factors, much work has focused on

Describes roadblocks to previous research that will be overcome by proposed approach.

using *Saccharomyces cerevisiae* as a surrogate model for *C. albicans* genetics and biology. However, the many significant genetic, developmental, and pathogenic differences between the two organisms show that much more can be learned by developing methods to study *C. albicans* directly.

Finding new targets using dominant negative screening

An anti-fungal drug that can effectively eradicate the fungal pathogen from a site of infection would target a gene that is essential for the viability of the invading pathogen. Thus, various methods have been developed for *C. albicans* to decipher the essentiality of fungal genes, which include, 1) sequential deleting or knocking out the chromosomal copies of the gene(11;28), and 2) disruption of one allele and promoter replacement of the other allele to achieve conditional expression (7),(5),(16). If a gene is readily deleted from the chromosome it is deemed non-essential, thus assignment of essentiality to a gene relies on a negative answer. Conditional expression of genes often involves expression of the gene of interest from a conditional promoter, allowing examination of the consequence of shutting off gene expression on cell viability. Although this can be a very powerful method for identification of essential genes, it is not suitable for identification of essential genes that are required in very low amounts for cell survival. An alternative methodology would be induction of growth inhibition (a dominant negative phenotype) upon over-expression of an essential gene. This method of essential gene identification would most probably identify genes whose function can be perturbed by small alterations. Examples of such essential genes include: 1) ribosomal subunits whose synthesis and assembly into a ribosome are under tight regulation, 2) components of cytoskeleton, such as actin and tubulin, whose over-expression is known to confer a dominant-negative phenotype in yeast (6;24), 3) cell cycle regulatory components or other genes involved in the regulation of processes in which timing and concentration are critical (10), and 4) components of cell wall synthesis whose inhibition can propagate into large effects (10). An added advantage of the dominant negative expression as a means of essential gene identification is that it mimics the effects of a drug perturbing the function of an essential gene. Although essential genes can be identified by various methods, those identified due to their dominant-negative over-expression phenotype might make better drug targets because they are more sensitive to small perturbations. In fact, the examples provided above for this method of gene identification are known targets of widely used drugs (ribosome/spectinomycin, tubulin/nocodazole, and bacterial cell wall synthesis/beta-lactams). The only other large-scale functional gene identification efforts with *C. albicans* have been shotgun methods such as restriction enzyme mediated integration (REMI) (5) and antisense RNA expression (8). The REMI screen was not designed to identify essential genes while the antisense approach identified about 80 potential essential genes.

In *S. cerevisiae* results of several large-scale screens for dominant negative mutants have been published (24), (34),(10),(41), and have identified genes involved in growth (1),(33), mating type regulation (44) and other processes. Dominant negative gene analysis works in *C. albicans*. Both a directed dominant negative (5) and identification of a filamentation-causing dominant gene from a library screen (4) have been reported. A dominant negative allele of SEC4 was successfully used to demonstrate the essential role of SEC4 in cell growth and protein secretion (26). Some genes in *C. albicans* appear to be uniquely sensitive to minor copy number alterations (19),(31), thus their over-expression can lead to a dominant phenotype Regulation of the sorbose utilization gene, SOU1, appears to be through a regulated shift in copy number of *C. albicans* chromosome 5, since monosomic strains assimilated sorbose while non-assimilating disomic strains did not (19). Selection for fluconazole resistant strains of *C. albicans* also resulted in a shift in chromosomal copy number (31). Results such as these suggest that there is a

distinct possibility that many *C. albicans* genes are regulated through dosage effects and would likely be susceptible to dominant screens involving overexpression.

The goals of this project are to optimize a *C. albicans* expression vector and to exploit the unique characteristics of this vector in dominant negative phenotype screening of *C. albicans* genes involved in growth control and viability of the organism.

Elitra's fungal genomics program

Recently the *C. albicans* genome has been sequenced (http://www-sequence.stanford.edu/group/*Candida*/), identifying 8 chromosomes totaling approximately 16Mb and estimated to encode greater than 6000 genes. To obtain functional information from *C. albicans* on a genomic scale Elitra Canada developed a method that allows the systematic

Goals of project.

construction of conditional mutants(T. Roemer, B. Jiang, C. Boone & H. Bussey. Gene Disruption Methodologies for Drug Target Discovery. International patent application Publication No. WO 01/60975,(16).. This procedure, termed GRACE (Gene Replacement And Conditional Expression) employs two successive steps, both of which involve a PCR-based methodology that permits precise construction of mutant alleles by homologous recombination (2),(45). In the first step, a precise gene disruption of one allelic copy of the diploid pair is made, creating a heterozygous mutant strain. In a second step, controllable expression of the remaining second allelic copy is engineered by replacing the native promoter with the tightly regulatable tetracycline (Tet) promoter (30). Thus, one copy of an essential gene is deleted and the other is placed under the control of a regulatable promoter. Tetracycline-dependent repression of conditionally regulated genes is achieved by supplementing growth media with tetracycline.

For large-scale GRACE analysis of *C. albicans* genes, putative *C. albicans* orthologs to the *S. cerevisiae* essential gene set were used as a starting point. Of the estimated 5651 *S. cerevisiae* genes, 1026 are essential for life on rich medium at 30^{o}C. *C. albicans* genes which showed limited matches (BLAST p<1.0 e-10) to *S. cerevisiae* essential genes were subjected to the GRACE procedure. Of these, 650 showed a substantial growth defect deemed to represent gene essentiality. Analysis of other genes in the *C. albicans* genome chosen randomly or by other bioinformatic prioritization suggest that up to ~1400 (21%) *C. albicans* genes are important for growth on rich agar-medium at 30C, a proportion quite similar to 18% essential genes found for *S. cerevisiae*. Thus, a significant number of essential genes remain to be identified.

Determining the requirement of all *C. albicans* genes for cell survival will require a comprehensive genome-wide program, which remains a major task quite separate from the needs of drug screening. Shotgun methods for selecting essential phenotypes have an advantage in that the remaining *C. albicans* essential genes, a set not apparent from the *S. cerevisiae* data, can be found by selection in the organism without resorting to knocking out 2 copies each of about 5,000 genes. This is very important from both the standpoint of the resources required for knocking out about 10,000 loci, of which most will be non-essential, and the limitations in any gene knockout method. The GRACE technology, for instance, utilizes conditional expression of genes to elucidate essentiality, a method that fails to assign essentiality to genes that are required in small amounts for cell survival. Dominant negative screening may allow selection for some of these missed genes and for selection of more novel genes.

Target-based drug screening

Describes significance of Phase II.

Screening efforts initiated during the phase I SBIR work and continued during phase II, in combination with our bioinformatic target prioritization will deliver a number of highly

valuable targets for anti-fungal drug screening. In addition, using the GRACE methodology, we have identified and prioritized 650 essential genes. The full complement of our essential genes, identified either by GRACE or dominant-negative screening, will be used in drug screening campaigns with the goal of identifying target-specific drugs. Using a target-specific screening strategy, not only anti-fungal hit compounds are identified, clues as to the mechanism of their actions will also be obtained. Conceivably, some prioritized targets will have a known and assayable biochemical activity. Target-based drug identification for these targets will be based on the ability of a compound to inhibit their *in vitro* biochemical activity. For targets that are not amenable to high-throughput biochemical assays, a cell-based assay will be used to identify target-specific drugs. Such a cell-based assay will be designed based on our previous observation that reducing the expression of a drug-target leads to hypersensitivity of cells to the drug. More specifically, we have shown that reducing the expression of Erg11 (target of flucanozole) or His3 (target of 3-amino triazole) in a GRACE strain setting, hypersensitizes cells to flucanozole or 3-amino triazole, respectively (see Figure 1). Cell-based assays enable the screening of novel targets of unknown function or non-assayable targets with well-characterized functions. Thus, for our target-based drug screening effort, we will screen for compounds that are toxic to cells only when the expression of our targets is reduced. The compounds identified as such, will be validated by a reciprocal methodology that relies on over-expression of the target gene. For essential genes identified by the GRACE methodology, a compound/target connection will be validated if over-expression of the target alleviates toxicity of the compound. There is precedence (20) for such validation strategy which strengthens the legitimacy of this approach; overexpression of the NADP-cytochrome oxidoreductase of *S. cerevisiae* produces resistance to azoles (43). Azoles target the cytochrome P450 enzyme, lanosterol demethylase in the ergosterol biosynthesis pathway; NADP-cytochrome oxidoreductase is a rate-limiting component of lanosterol demethylase activity. Conversely, it is conceivable that the dominant negative phenotype conferred by our cDNA clones could be alleviated by treatment with compounds that block the functions of these cDNAs. Therefore, for compound/target validation in the case of dominant-negative targets, we will rely on the ability of the compound to inhibit the dominant-negative phenotype caused by over-expression of the target. The combination of biochemical and cell-based assays should deliver a series of target-specific compounds that will bring us closer to development of a new generation of anti-fungal compounds.

PHASE I FINAL REPORT

Beginning date: 8/1/01
Ending date:2/15/02

Key Personnel	Title	Date of Service	Percentage Effort
Sherry Nouraini	Post Doctoral Fellow	8/1/01-2/15/02	100%
John Trawick	Principal Investigator	8/1/01-2/15/02	4.14%
Gordon Foulkes	Co-investigator	8/1/01-2/15/02	5.0%
Carlos Zamudio	Co-investigator	8/1/01-2/15/02	5.0%

Detailed Final Report describes Phase I accomplishments.

The primary objective of the Phase I study was to develop tools for a dominant negative screen of *Candida* albicans genes, as a means of identifying antifungal drug targets.

The Phase I specific aims were:

1-Construction and optimization of a *C. albicans* site-specific integration expression vector. An integrative expression vector should contain the following components: 1) a regulatable promoter, 2) sequences to target for site-specific recombination, and 3) A selectable marker. For regulated expression we used as template the tetracycline inducible promoter system developed in house for the GRACE methodology described above. In this promoter, four copies of a Tet-responsive element are placed upstream of basal promoter sequences for the *Saccharomyces cerevisiae* Cyc1 gene. To achieve maximum level of transcription, we constructed variations of the promoter template in which the basal Cyc1 promoter elements (15) were replaced with promoter sequences of strongly expressed genes. The alternative promoters were *Candida albicans* PGK1 promoter (in house expression studies), *Saccharomyces cerevisiae* GPD promoter (29) and *C. albicans* Met3 promoter (7). The latter promoter also has the added advantage of being repressible in the presence of Cysteine and Methionine (7).

To achieve transcription activation, DNA-binding sequences from the bacterial Tet-repressor have been fused to the transcriptional activation domain of the *Saccharomyces cerevisiae* GAL4 transcription activator. This hybrid transcriptional activator (TetGal) binds to Tet-responsive elements in the absence of tetracycline, and activates transcription. When tetracycline is present, it inhibits transcriptional activation by binding to the DNA-binding domain of the hybrid transcriptional activator. The gene encoding TetGal is expressed from the *C. albicans* ACT1 promoter in a *C. albicans* strains called Cass1 (for *Candida* starting strain). In addition to expressing the TetGal4 activator, in the Cass1 strain both copies of the His3 gene have been replaced with HisG sequences from (name of the organism). The HisG sequences have been used extensively in *C. albicans* as a target sequence for chromosomal integration of plasmids by homologous recombination (11). Thus, we included a copy of HisG sequence in our

expression vector to facilitate integration into the chromosome. Targeted integration into the HisG locus is mediated by digestion of the expression vector with the restriction enzyme EagI to free the hisG arms. This restriction enzyme was chosen due to the paucity of its recognition sequence in the AT-rich eukaryotic genomes with an estimated one site for every 27,000 bp in *S. cerevisiae* (NEB catalogue, 2000, page 231). Detection of chromosomal integration requires the presence of a selectable marker on the vector. We chose the bacterial SAT1 gene that confers resistance to the drug nourseothricin (27). This marker has been developed and used by Elitra Canada for construction of the panel of Grace strains discussed above.

In the phase I application, we described construction and testing of a vector with URA3 as a selectable marker, and with sequences recognized by SceI meganuclease mediating chromosomal integration. However, we modified the design of our vector to contain components that are in widespread use for *C. albicans* strain construction and are also compatible with our system of GRACE methodology. The current vector design allowed us to use the Cass1 strain for strain construction and it will allow investigation of functional interaction between dominant negative clones and our Grace strains.

Describes modifications improving initial Phase I design.

2-Construction of a cDNA library in the optimized vector. This has been accomplished by contracting the construction of the cDNA library to ACGT Inc.

3-Perform a dominant negative screen with the library. A pilot dominant-negative screen is currently underway.

Should say here that pilot will be described in the Materials and Methods section below.

MATERIALS AND METHODS (PHASE1)

It is important to include detail in the Phase I report even though only the primary reviewers are expected to look carefully at this section.

Construction of a site-specific integration, expression vector. Construction of the site-specific integration, expression vectors is depicted in Figure 2. The various components of these

Reviewers probably would have appreciated the inclusion of the figures and tables in the body of the text instead of at the end as in this application.

vectors were assembled in the multi-cloning site of pBluescript SK vector. The components of the vector include: 1) HisG sequence directing site-specific integration, 2) The SAT1 gene, driven by the *Candida* ACT1 promoter, which confers resistance to the drug Nourseothricin, 3) transcription termination sequences from *Saccharomyces cerevisiae* ADH1 gene, 4) Tet operator sequences, 5) Promoter, 6) Transcription termination sequences from *Candida albicans MAL2* (3'-UTR). *MAL2* 3'-UTR was PCR amplified from the pAU22 plasmid (42) (a gift of A. Johnson) using primers MAL2UTR Up (5'- CGGCTCGAGTAAATATACACTAGATGC-3') and MAL2UTR DN (5'-GGGGTACCAGACATACGCTTTGCAGG-3'). HisG sequences were PCR amplified from chromosomal DNA prepared from C. albicans Cass1 strain with primers HisG upper (5'-GGCGAGCTCTTAGATCTTCCAGTGG-3') and HisG lower (5'-TCCCCGCGGAGATCCGGCCAGATCCGG-3'). Tet operator sequences were PCR amplified, either with or without the downstream CYC1 promoter sequences from our GRACE promoter

replacement vector pSATTET. The primer TetO4fwdnew (5'-TCCCCCGGGTGATCCGCTAGG-3') was used with primers TetO4rev (5'-GGGGATCCGAGCTCGACTTTC-3') or SATTETCYC1BamHIrev (5'-CGGGATCCCGAATTGATCCGG-3') to amplify TetO4 alone, or TetO4-Cyc1, respectively. The Met3 promoter was PCR amplified from pCaDIS (a gift of P. Sudbery) (7) with primers Met3BamHIfwd (5'-CGGGATCCAATTGTCTATTCCAAGCC-3') and Met3BamHIrev (5'-CGGGATCCGGGGAGGGTATTTAC-3'). The PGK1 promoter was PCR amplified from Cass1 chromosomal DNA using primers PGK1BHIfwd (5'-CCGGATCCAACGGAACTACACTAG-3') and PGK1BHIrev (5'-CCGGATCCTTTGATAGTTATTCTTC-3'). The GPD promoter was isolated from p426GPD (from ATCC) on a SacI/XhoI fragment. This fragment was treated with T4 DNA polymerase to generate a blunt ended fragment. To construct TetO4 promoter fusions, the TetO4 sequences were first cloned into SmaI/ BamHI digested pBluescriptSKII plasmid to generate pBSKTEtO4, followed by insertion of PGK1 or Met3 promoters into the BamHI site. Cloning of the GPD promoter was done similarly, except pBSKTetO4 was digested with BamHI and blunt-ended with T4 DNA polymerase and ligated to blunt ended GPD promoter fragment. The final constructs were called pLIB3MET3, pLIB3CYC1, pLIB3PGK, and pLIB3GPD that contained the promoters MET3, CYC1, PGK and GPD, respectively, fused to the Tet operator sequences. To make the pLIB3MET3 vector compatible for use with the SMART cDNA synthesis strategy, two complementary oligonucleotides were synthesized which when hybridized, contained sFiI sites for cloning of cDNAs. These oligos, SfiHAUp (5'-AATTCGGCCATTACGGCCTACCCATACGACGTCCCAG-3') and SfiHADn (5'-TCGAGGCCGAGGCGGCCAGCGTAGTCTGGGACGTCG-3') were hybridized and cloned into the EcoRI/XhoI sites of pLIB3Met3 to construct pTETMet3, which was used for library construction.

Transfomration of *C. albicans*. Cells were transformed by a traditional LiAcetate-based method (14),(5) or by using a yeast transformation kit (#T2001) from Zymogen Research Inc. (Orange, CA). To obtain SAT^r colonies, at the end of the transformation protocol cells were plated on YPD media without Noursethricin and incubated at 30°C overnight. The next day, cells were replica plated onto solid media containing 400 μg/ml Nourseothricin (NST) and incubated at 30 °C for two days.

Functional testing of alternative promoters by ACT1 over-expression. The *C. albicans* ACT1 gene was PCR amplified from Cass1 chromosomal DNA using primers CaACT1rev (5'-TTTCTCGAGGGCCGTCGACATTTTATG-3') and CaACT1fwd (5'-CGGAATTCATGGACGGTGGTATG-3'). The ACT1 gene was cloned as an EcoRI/XhoI digested fragment into pLIB3Met3, pLIB3CYC, pLIB3PGK and pLIB3GPD vectors. The resulting ACT1 constructs were digested with EagI, transformed into Cass1 strain and transformants were selected on YPD+NST plates. To monitor the growth phenotype induced by ACT1 over-expression, cell suspensions of NST-resistant colonies in sterile water were subjected to a 10-fold serial dilution and spot plated on the following solid media: 1) YNB+100μg/ml Histidine+400 μg/ml NST, 2) YNB+100μg/ml Histidine+400 μg/ml NST+ 50μg/ml Tetracycline, 3) YNB+100μg/ml Histidine+400 μg/ml NST+ 50μg/ml Tetracycline+ 0.5mM Cysteine+0.5mM Methionine, and 4) YNB+100μg/ml Histidine+400 μg/ml NST+0.5mM Cysteine+0.5mM Methionine.

Construction of cDNA library. To construct the cDNA library, total RNA was prepared from an equal mixture of CAI4 cells grown in YPD and harvested at early (OD600 of 0.5) and late (OD600 of 3.0) log phase. RNA was prepared using the RNeasy kit from Qiagen. The

construction of cDNA library was contracted to ACGT Inc, who used the SMART cDNA library kit from Invitrogen for library construction.

RESULTS AND DISCUSSION (Phase 1)

Construction and validation of integrative expression vectors. Four different integrative expression vectors were constructed as depicted in Figure 2. The four expression vectors were functionally tested to determine the ideal vector for our dominant-negative screen. The *C. albicans* ACT1 gene was PCR amplified from the chromosome and cloned downstream of each promoter. Overexpression of ACT1 leads to a dominant-negative phenotype in *S. cerevisiae* (24). The various ACT1 constructs were digested with the restriction enzyme EagI and transformed into the Cass1 strain. NST-resistant transformants were tested for a growth defect in the presence and absence of tetracycline. Although presence or absence of tetracycline was inconsequential to the growth rate of cells, repression of the Met3 promoter by inclusion of cysteine and methionine in the growth medium relieved the Met3-ACT1 mediated growth defect (Figure 3).

To confirm that the constructs have integrated into the HisG locus, genomic DNA was prepared from transformants and analyzed by PCR using an upstream primer hybridizing to genomic DNA and a downstream primer hybridizing to SAT1 sequence (Figure 4A). Correct integration of the ACT1 constructs is predicted to lead to appearance of a 3.1 kb PCR generated fragment. A control PCR reaction was performed with genomic DNA from Cass1 that should fail to support production of the 3.1 kb diagnostic band. As shown in Figure 4B, the ACT1 constructs have correctly integrated into the HisG locus, with Cass1 lacking such an integrant. To test whether the absence of a diagnostic band with the Cass1 sample is due to inhibition of PCR reaction with contaminants in the genomic DNA prep, a control PCR reaction was set up to amplify the ACT1 gene. As shown in Figure 4B, all genomic DNA samples supported amplification of the ACT1 gene (2.1 kb band), confirming that the absence of a 3.1kb diagnostic fragment in the Cass1 sample represents lack of a plasmid integrant.

Construction of a *C. albicans* cDNA library. Construction of the cDNA library was contracted to ACGT Inc. To capture cDNA from genes expressed at various stages of cell growth, total RNA was prepared from an equal number of cells grown to early (O.D.600=0.5) and late (O.D.600=3.0) log phase. The cDNA library constructed by ACGT Inc. has a total of 340,000 individual members with ~88% members containing an insert.

Optimization of transformation conditions: The standard transformation method used at Elitra for construction of strains is based on the traditional LiAcetate protocol (14). The efficiency of transformation obtained by this protocol is not suitable for library screening purposes. We attempted to increase transformation efficiency by using a yeast transformation kit available from Zymogen Research Inc. Cass1 cells were transformed with EagI-digested MET3-ACT1 constructs by three different protocols: 1) by the LiAcetate method, 2) using the Zymogen kit, and 3) the Zymogen kit along with 100μg Salmon Sperm (SS) DNA as carrier. The transformation efficiencies obtained from each protocol are outlined below:

Protocol	transformation efficiency (#SAT^r colonies/μg DNA)	Fold increase in efficiency
LiAc	0.06	1
Zymogen	0.16	2.7
Zymogen +SSDNA	32.4	540

Thus, for the purpose of library screening, we will be using the Zymogen yeast transformation protocol along with SS Carrier DNA.

PHASE II EXPERIMENTAL DESIGN AND METHODS

Identification of dominant negative genes in *C. albicans*

Screening for dominant-negative overexpression *C. albicans* cDNAs has already been initiated in Phase I on a pilot scale. This work will be extended to perform a large-scale screen to identify additional dominant negative clones present in our cDNA library. The *C. albicans* host strain, CASS-1 will be transformed with 1000μg of the library digested with EagI to obtain approximately 40,000 transformants which represents ~5 fold coverage of C. albicans genome. Selection for NST-resistance will be performed essentially as described above in "Phase I Progress Report".

Transformant colonies resistant to NST will be picked and transferred to liquid repressing medium (YNB medium plus, cysteine and methionine) in 384 well plates with a robotic colony picker (GeneMachines, Inc, San Carlos, CA). This colony picking and inoculating device can aseptically identify and pick colonies from transformant plates at the rate of 2,000 colonies per hour; colonies are transferred to recipient plates containing liquid growth medium. The colonies will be screened for dominant-negative inhibition as described in phase I report**.** Briefly, individual colonies will be robotically replica plated (BioGrid replica gridding device from BioRobotics, Ltd., Cambridge, U.K.) onto both repressing (+0.5 mM methionine/cysteine) and inducing (0 mM methionine/cysteine) plates Robotic colony picking and replica plating enable the screening of 10,000 to 50,000 colonies per day at Elitra. Dominant negative clones will be identified based on a reduction in growth rate or inability to grow under transcription induction conditions (0.5mM cys and met). Screening for a growth phenotype by gridding is subject to artifacts that may arise by uneven delivery of liquid from different wells of a growth plate. Serial dilutions of all putative dominant negative clones will be performed as described above to rule out gridding artifacts.

The approach outlined above depends on ectopic expression or overexpression of genes resulting in a dominant negative phenotype. There is considerable precedent for this phenomenon (24). However, some dominant phenotypes require point mutations altering an enzymatic activity (26) while deletion mutations may favor other dominant phenotypes (17), (37),(38). To this end, the cDNA library will be subjected to an in vitro mutagen. One such mutagen is hydroxylamine that hydroxylates cytosine residues and leads to a transition mutation after replication. The mutagenized library will then be purified from the mutagens and used to screen for dominant-negative clones, as described above.

A dominant negative phenotype might arise due to illegitimate insertion of the library plasmid outside of the HisG sequences, causing an artifactual dominant-negative phenotype. Furthermore, it is known from studies in *Saccharomyces cerevisiae* that transformation protocols are intrinsically mutagenic (14). To rule out these possible artifacts, two experiments will be performed. First, proper integration of the library plasmid will be tested by PCR as described in the phase I report. Second, the cDNA insert from dominant negative clone will be PCR amplified and cloned into freshly prepared pTETMET3. The newly constructed cDNA clone will be transformed into Cass1, and the dominant-negative phenotype will be assessed as described above. cDNA clones that show a reproducible dominant-negative phenotype will be further processed for target prioritization as described below.

Bioinformatics and target prioritization

The Pathoseq database and its tools will facilitate characterization of dominant negative clones; Pathoseq is a proprietary, Elitra, database of microbial genomes. DNA sequences of the dominant-negative *C. albicans* cDNAs will be included in the Pathoseq database. In addition to the Pathoseq database, we have licensed the MycoPathPD™ database of Proteome™ and integrated their data into our relational database. The MycoPathPD™ database archives all literature relating to published work spanning 17 different human fungal pathogens including multiple species of *Candida* (nine) and *Aspergillus* (three), as well as *Cryptococcus neoformans*, *Coccidiodes immitis*, *Pneumocystis carinii*, *Blastomyces dermititidis* and *Histoplasma capsulatum*.

Describes company's unique resource, proprietary Pathoseq database technology, that will be used for this project.

It is critical that the targets we select for drug screening are both present and essential in the second major human fungal pathogen, *Aspergillus fumigatus*. *A. fumigatus* is a fungal pathogen whose importance has increased significantly in the last 15 years as more aggressive medical interventions, including bone marrow and solid organ transplants have become common (18),(3). These organisms infect only profoundly immunodeficient patients. The Aspergilli are by far the most common human pathogens amongst the filamentous ascomycetes, at least in North America. Through an exclusive collaboration with Celera Genomics, we have recently obtained 10 X sequence coverage of the 29 Mb genome of *A. fumigatus*. Our internal annotated of the *A. fumigatus* genome identifies over 10, 000 genes (data not shown). Thus, internal bioinformatic analyses based on both the *A. fumigatus* and *C. albicans* genomic sequences enables for the first time rapid confirmation and prioritization of candidate broad-spectrum drug targets identified through our dominant negative screen.

An additional valuable resource available at Elitra is the database of *C. albicans* essential genes identified by the GRACE methodology at Elitra Canada. Dominant-negative cDNAs derived from genes found essential by the GRACE methodology are perhaps the most ideal targets for anti-fungal drugs, both due to their necessity for cell survival and their sensitivity to small perturbations. It is conceivable that some dominant-negative genes would be found non-essential for *C. albicans*. Since assignment of essentiality by the GRACE methodology depends on repression of a conditional promoter, essential genes that can survive at very low levels might be classified as non-essential. Furthermore, we have not determined the essentiality of all the >6,000 genes of *C. albicans*. To investigate the essentiality of dominant-negative genes with uncertain essentiality, the two chromosomal copies of the genes will be sequentially knocked out using the URA-blaster methodology. A hisG-URA3-hisG cassette flanked by gene-specific sequences is transformed into *C. albicans*. Gene disruption is achieved by homologous recombination between the gene specific regions on the cassette and a homologous copy of the gene on the chromosome. Then, intrachromosomal homologous recombination between hisG sequences leads to Ura^- revertants that can be selected by the drug 5-floroorotic acid (5-FOA). We will prepare genomic DNA from 5-FOA resistant colonies and use PCR and Southern blotting to confirm deletion of one chromosomal copy of the target gene. The second chromosomal copy of the target gene can then be deleted using the same disruption cassette. Failure to obtain Ura^+ colonies in the second round of transformation is an indication of essentiality of the target gene.

Describes company's unique resource, GRACE methodology, that will be used for this project.

Assay development for target-based drug screening

Describes two approaches, biochemical assays and cell based assays, for drug screening; first, basic principles of each assay, then detailed description. This provides information for secondary reviewers to learn about the approach without wading through all the details.

Biochemical assays: Prioritized cDNAs that encode known and measurable biochemical activities will be cloned and expressed in *E. coli* for the purpose of protein purification. When necessary, to alleviate problems arising from the variant CTG codon (in *C. albicans* CTG encodes serine instead of leucine,(23),(36),(35), the CTG codons will be modified by site-directed mutagenesis to a serine-encoding codon in *E. coli*. Protein expression and purification will be based on the QIAexpress protein purification system from Qiagen that is designed for one-step purification by metal-chelate affinity chromatography of 6 x Histidine-tagged proteins. Target-specific compounds will be identified based on their ability to inhibit the biochemical activity of the protein. The "readouts" of enzymatic assays can vary, as will the criteria for selecting inhibitory compounds. Therefore, if a biochemical assay is employed in screening, assay conditions will need to be configured for that specific reaction. Elitra has personnel in HTS and other research areas with considerable experience in developing specific biochemical assays as well as cell-based hypersensitive assays.

Cell-based assay for drug screening: As mentioned above, target-specific compounds will be identified based on their toxicity only when the target is under-expressed. Assay development efforts will be modeled based on our previous work on optimizing and developing cell-based assays for our bacterial antisense technology. Elitra has been able to identify a large number of essential genes based on the growth inhibition observed upon over-expression of bacterial genes in the antisense orientation (12),(16). In addition, the antisense growth inhibition phenotype has been used to screen for compounds that are toxic to cells only upon over-expression of the antisense fragment, thus sensitizing cells to a drug when the expression of its target is reduced. The inducer used for induction of the antisense insert is titratable, thus allowing optimizing growth inhibitory conditions for development of these assays. Repression of transcription from the Met3 promoter is also titratable between 0.01mM and 0.5mM range of cysteine and/or methionine concentrations, which will allow us to find optimized conditions for assay development. We will use the well-known EGR11/flucanozole combination to identify the ideal Cys/Met concentration (a desirable level of protein expression) for the assay. A *C. albicans* strain (hereafter called Met3-ERG11) will be created in which one chromosomal copy of ERG11 is deleted, and the Met3 promoter replaces the promoter of the other allele. Similar strains conferring Met3 promoter-dependence on essential genes are in widespread use in the *C. albicans* community (Met3 paper). The Met3-ERG11 strain will be grown in YNB medium overnight, and subsequently diluted 1000 fold into YNB media supplemented with a range of Cys/Met concentrations between 0.0 and 0.5mM. Cells will be grown into stationary phase and monitoring turbidity of cultures at 600nm will generate a growth profile. Modeled based on our Fluconazole hypersensitivity experiments described in Figure 1, the ideal Cys/Met concentration will induce a 90% growth inhibition as compared to the absence of Cys/Met. Similar criteria are used in Elitra's bacterial antisense-based assays described above.

Cell-based assay for validation: As mentioned above, the dominant-negative over-expression phenotype will be used to validate drug/target connections. Thus, the dominant-negative phenotype of each target will be evaluated to find suitable conditions for a cell-based assay. Cells containing dominant-negative clones will be grown in YNB medium supplemented with

0.5mM Cysteine and Methionine. Overnight cultures will be diluted 1000 fold into YNB without Cysteine and Methionine and with a range of Cysteine and Methionine concentrations of 0.01mM to 0.5mM. The growth profile of cells will be determined by measuring cell turbidity at 600nm (O.D.600) until all cultures reach stationary phase. A suitable Cysteine and Methionine concentration will be deduced based on reduction of growth rate by 90% (IC90) as compared to conditions with 0.5mM Cysteine and Methionine. In another words, the ideal conditions will results in an O.D.600 measurement that is 10% of the measurement at 0.5mM Cysteine and Methionine at a given point during log phase.

Assay conditions developed at Elitra for compound screening against *C. albicans* rely on monitoring number of viable cells in a culture by colorimetric measurement of cellular respiration. This is achieved by inclusion of a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4—sulfophenyl)-2H-tetrazolium) or MTS, which is reduced by cells into formazan. The quantity of formazan product is measured by absorbance at 490nm and is directly proportional to the number of living cells. Thus, we will adapt our optimized assay conditions to the above methodology which is the preferred method for monitoring growth rate in the 384 well plates used in high throughput screening of compounds.

This is the beginning of the detailed assay description. The application would have been better organized if the same order of assays was used in the detailed description as in the general description above, i.e., biochemical assays before cell based assays.

CELL BASED SCREENING

a. Preliminary drug screen:

The various steps involved in our proposed drug screening efforts are shown as a flow chart in Figure 5. First, *C. albicans* strains (hereafter called MRT for Met3 regulated target) will be constructed for each target in which one allelic copy of the target gene is deleted and the promoter of the remaining copy is replaced with the Met3 promoter. MRT strains will be grown overnight in YNB medium (transcription repression conditions) to log phase. Cells will be diluted 1000 fold into YNB supplemented with appropriate concentrations of Cysteine and Methionine in the presence of 333ug/ml MTS and 25uM PMS that is an electron coupling reagent and increases the efficiency of MTS reduction. Using our Multidrop robot, cells will be aliquoted into 384 well plates (45 μl/well) already containing compounds at 11uM final concentration, and solvent only wells which will be inoculated with cells diluted 1000 fold into YNB. Plates will be gently mixed and sealed with plastic plate sealer and then equilibrated at 30^{o} C for 5-20 min to reduce condensation prior to starting the assay. Absorbance at 490nm will be measured at the beginning and at the endpoint of the assay that will be determined during assay development. The primary screen will be performed twice, and compounds that show a reproducible decrease in absorbance at 490nm as compared to solvent-only wells will be chosen as primary hit compounds (PHC). The minimal fold shift in sensitivity to compounds is determined as the value that is greater than or equal to 2 standard deviations above the mean fold shift (usually close to 1) for the entire chemical library. The Met3-ERG11 strain and a range of flucanozole concentrations will also be tested as positive control.

b. Characterization of PHCs

A dose-response curve of PHCs will be performed against the parent wild-type yeast strain (Cass1) to determine IC50s and IC20s of the compounds. Then, PHCs will be retested against the MRT strains strain at their IC20 concentrations determined for the parent strain. Compounds

that continue to confer sensitivity to the MRT strains under these conditions are chosen and named confirmed hit compounds (CHC).

c. Prioritization of CHCs

At this point, a number of tests will be performed to prioritize CHCs for further development. First, a dose-response of CHCs will be done in the MRT strains to confirm the hypersensitivity phenotype. Second, a dose-response of CHCs will be performed in the dominant-negative assay system for each corresponding target to test ability of compounds to confer resistance to the growth inhibition due to over-expression of the target. Third, the structure of compounds will be reviewed and predictions are made as to the suitability of compounds for further development. Fourth, the activity of compounds against human cells will be measured to determine potential toxicity. The mammalian cell line used at Elitra for this purpose is the hepatoma line, HepG2. While these do not exactly mimic the human body, they are an adequate measure of gross toxicity. A therapeutic index (TI, [human IC50]/ [fungal IC50]) will be calculated as part of this analysis. A high value of the TI indicates low toxicity. Altogether, compounds that have a favorable structure, show reproducible target-specificity in both the MRT and dominant-negative strains, have an IC50 of less than or equal to 16μg/ml against the wild-type parent strain, and show a TI value of higher than 10 will be carried further into clinical microbiology.

BIOCHEMICAL SCREENING

Compounds, tested at ~11uM concentration, will be identified based on their ability to inhibit the in vitro activity of the target. Dose response curves for each active compound will be determined to calculate an IC50. Next, compounds that significantly reduce enzymatic activity will be retested to determine the kinetics of that activity. Active compounds will be further characterized for specificity to the target enzyme by investigating the ability of the compounds to inhibit other enzymes with related biochemical activity. Compounds shown to be specific inhibitors of the target enzymatic activity will be tested for selectivity to fungal- versus human-originated protein. Compounds with specificity and selectivity for the fungal target will be chosen to test for anti-fungal activity in standard preclinical microbiological assays established at Elitra for *C. albicans*. Compounds with anti-fungal activity and with an MIC of less than 16 μg/ml will be prioritized based on criteria used for prioritization of CHCs identified through cell-based screening, as described above.

d. Determine spectrum of activity (MIC) and kill kinetics to identify potential lead series (PLS)

Clinical microbiology tests will use wild-type *C. albicans* to determine Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) to further characterize the mode of action of all the candidate compounds. In addition, the compounds will be tested against other fungal pathogens and known drug-resistant C. albicans strains to determine spectrum of anti-fungal activity. The panels of fungal pathogens will include, *C. albicans* clinical isolates, *C. albicans* azole-resistant clinical isolates, *C. glabrata*, *C. parapsilosis*, *C. krusei*, and *Aspergillus fumigatus*. Panels of the appropriate organisms and SOPs for MIC determinations are already present in house at Elitra. Compounds with a broad-spectrum of activity will be chosen as Potential Lead Series (PLS).

The summary provides a "take home" message to all reviewers that this project will be successful.

Summary

The goal of the proposed project is to discover novel anti-fungal drugs that target essential genes identified by screening a cDNA expression library for dominant-negative phenotypes. The proposed work builds on the foundation of tools constructed and validated under Phase I funding. The strategy of identifying essential gene targets by dominant-negative screening complements another target-discovery technology developed at Elitra independent of the Phase I funding referred to as GRACE. Together, these target-discovery and cell-based screening technologies enable an important new initiative in the area of antifungal drug discovery. Elitra has systematically assembled and developed the bioinformatic capabilities, molecular genetic tools, functional genomics technologies and high throughput-screening infrastructure required to identify antifungal lead compounds and develop them into clinical candidates. Given these resources and its commitment to the therapeutic area of antimicrobials, Elitra is uniquely positioned to make effective use of the Phase II funding to discover novel anti-*Candida* drugs.

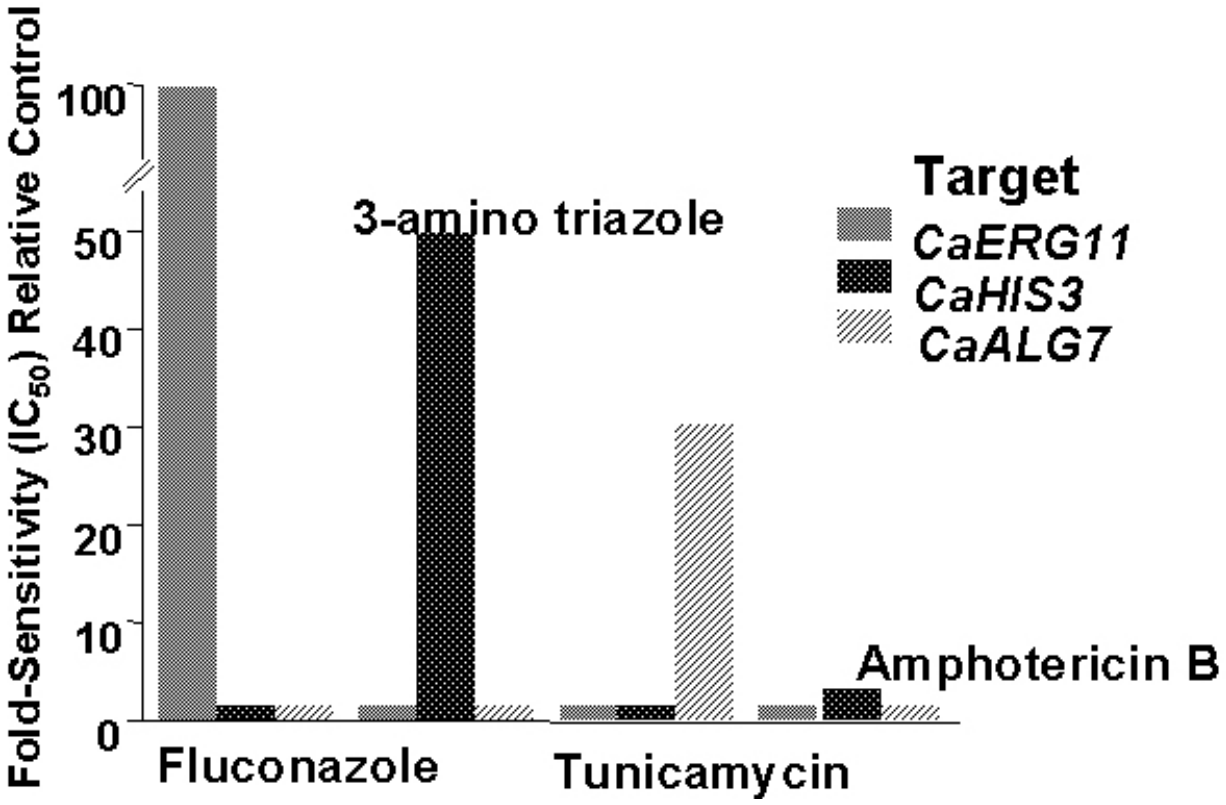

Figure 1. *C. albicans* Sensitized Whole Cell Assays. GRACE strains conditionally regulating the known drug targets, C*aERG11 CaHIS3,* and *CaALG7* were constructed and IC50 values were determined against a matrix of antifungal compounds including their cognate inhibitor. All strains were assayed in a suitable tetracycline concentration to underexpress the drug target to such an extent where growth rate reduced ~90% (24). As indicated, sensitized cells display a range between 30 fold and 100 fold lower IC50 values that are specifically detected between the drug target and its known inhibitor. IC50 determination to Amphotericin B (whose mechanism of action involves disrupting the plasma membrane) revealed no elevated drug sensitivity amongst any of the sensitized cells.

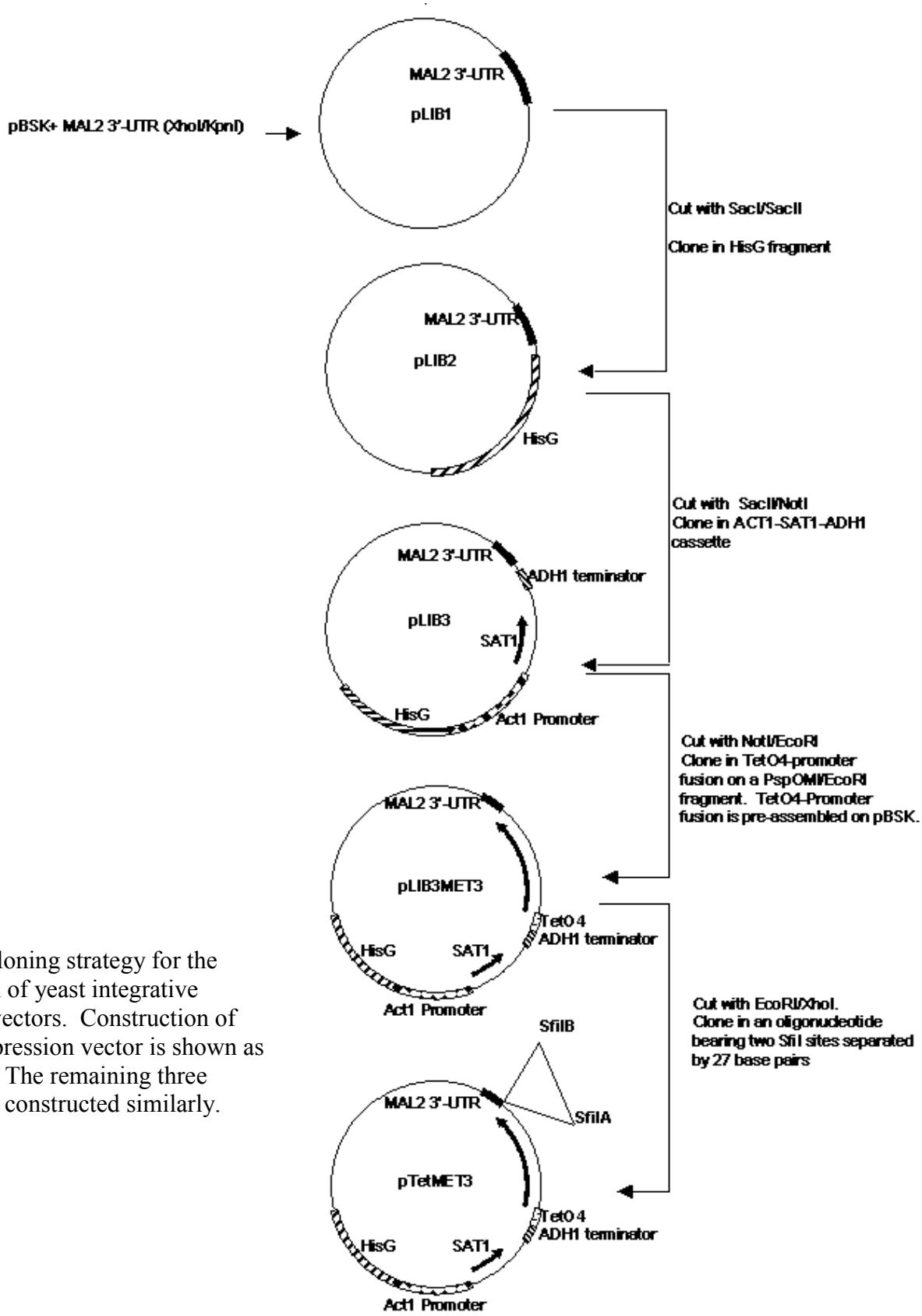

Figure 2. Cloning strategy for the Construction of yeast integrative Expression vectors. Construction of the Met3 expression vector is shown as an example. The remaining three vectors were constructed similarly.

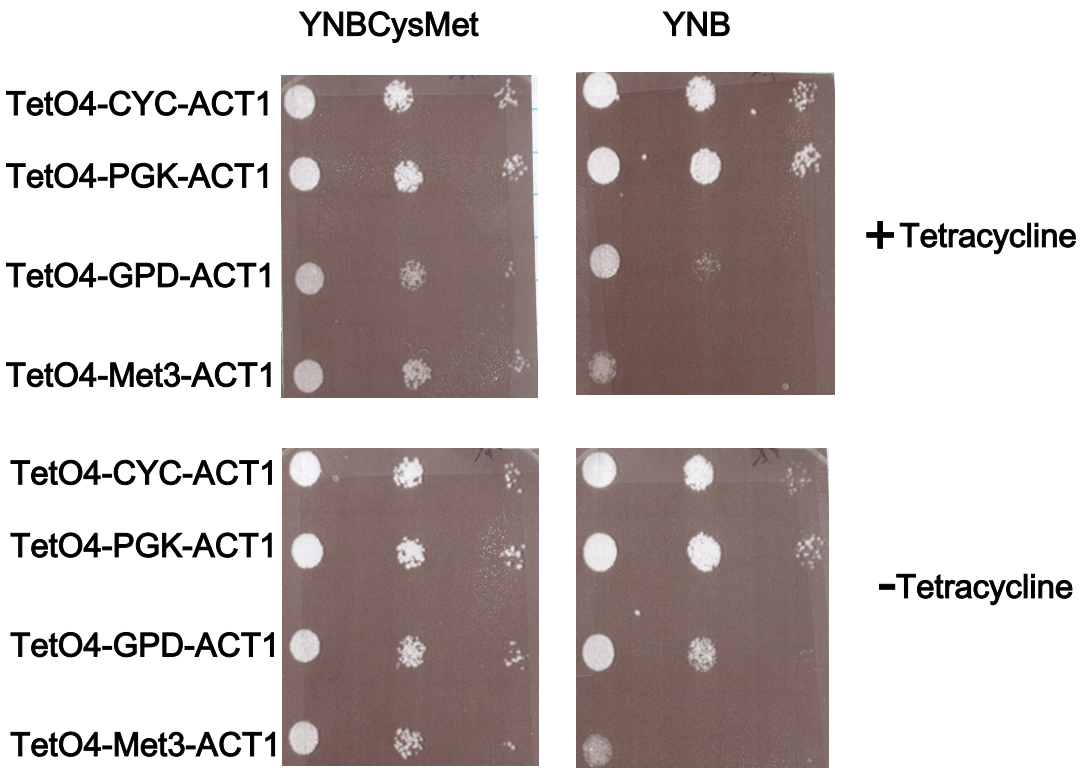

Figure 3- Effect of expression of ACT1 on growth of Candida albicans. Serial dilutions of cells transformed with various vectors driving ACT1 expession were spotted on YNB+NST, YNB+NST+0.5mM Cys and Met in the absence or presence of Tetracylcine. The Met3 promoter is repressed in the presence of Cys and Met.

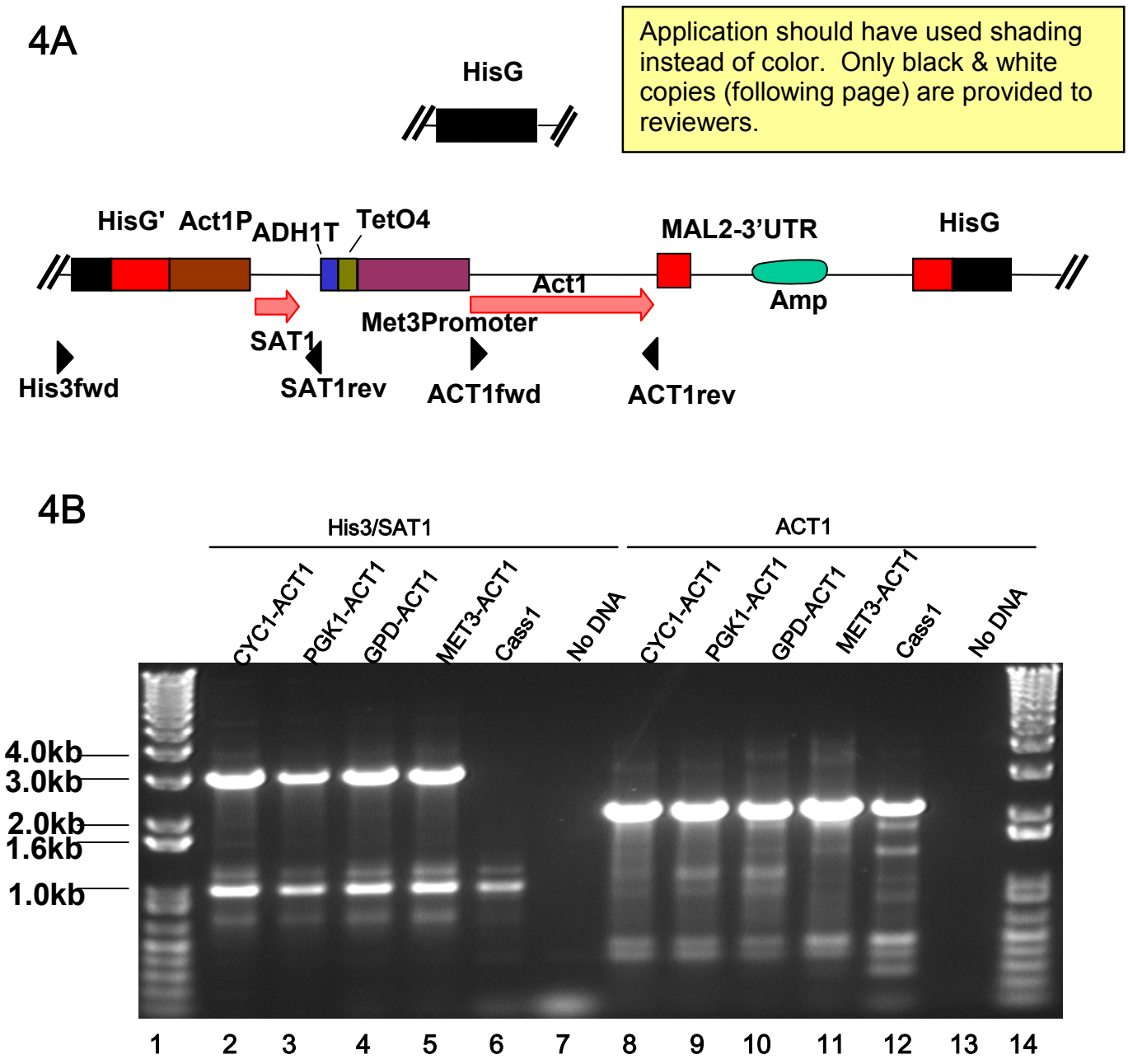

Figure 4- Chromosomal Integration of expression vectors by homologous recombination at the HisG sequence within the His3 locus. 3A) Schematic representation of the His3 locus before (Top) and after (bottom) plasmid integration. Digestion of the vector with *Eag*I, derives integration at HisG sequences. Homologus integrative recombination leads to duplication of the HisG sequence. The arrowheads represent primers used for PCR amplification of chromosomal DNA as a means of testing correct integration of the vector.
B) PCR amplification of chromosomal DNA from Cass1 transformants, and Cass1 as control. Lanes 2 through 7 show amplification with His3 and SAT1 primers and lanes 8 to 13 are results of amplification with ACT1 primers.

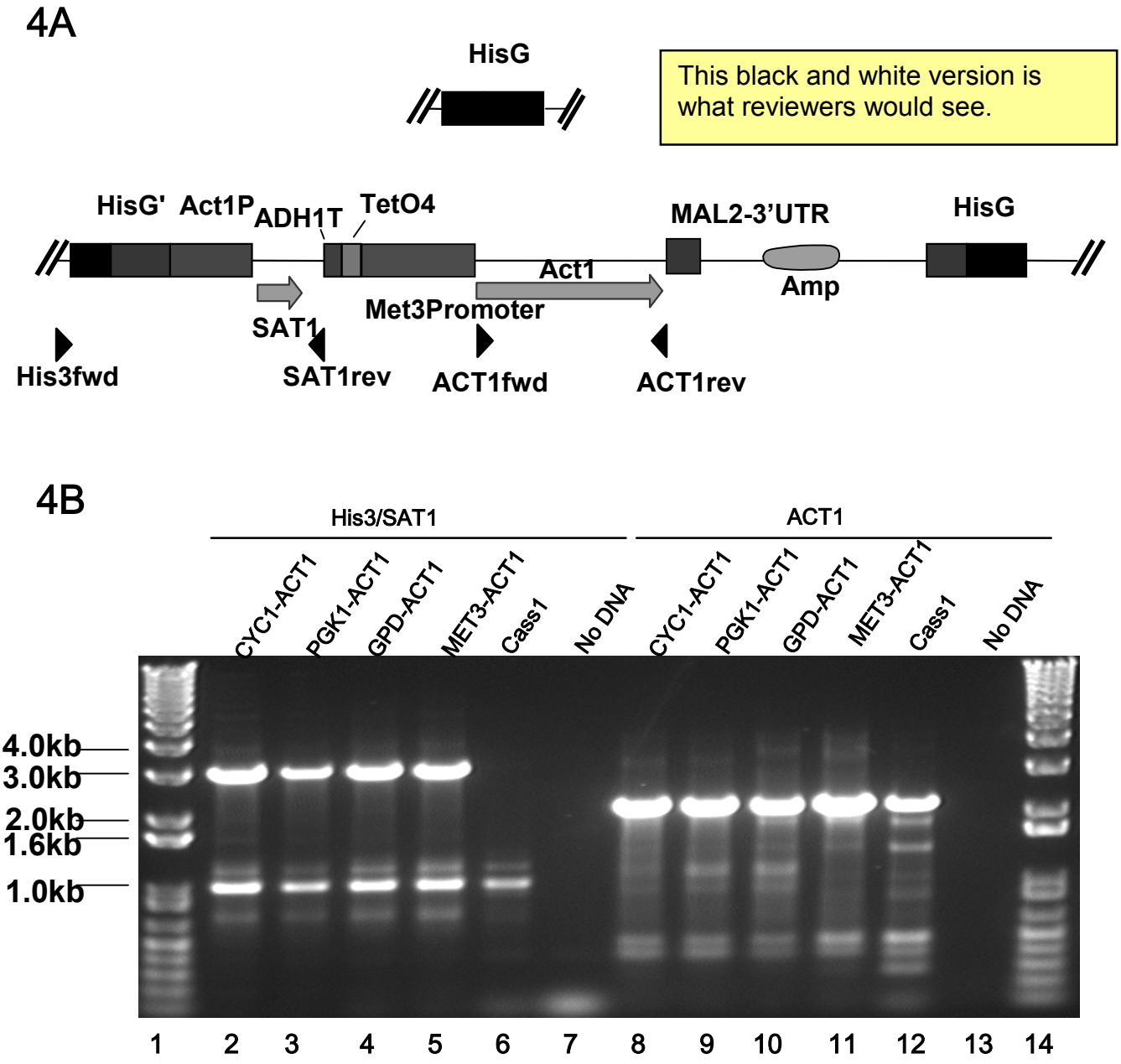

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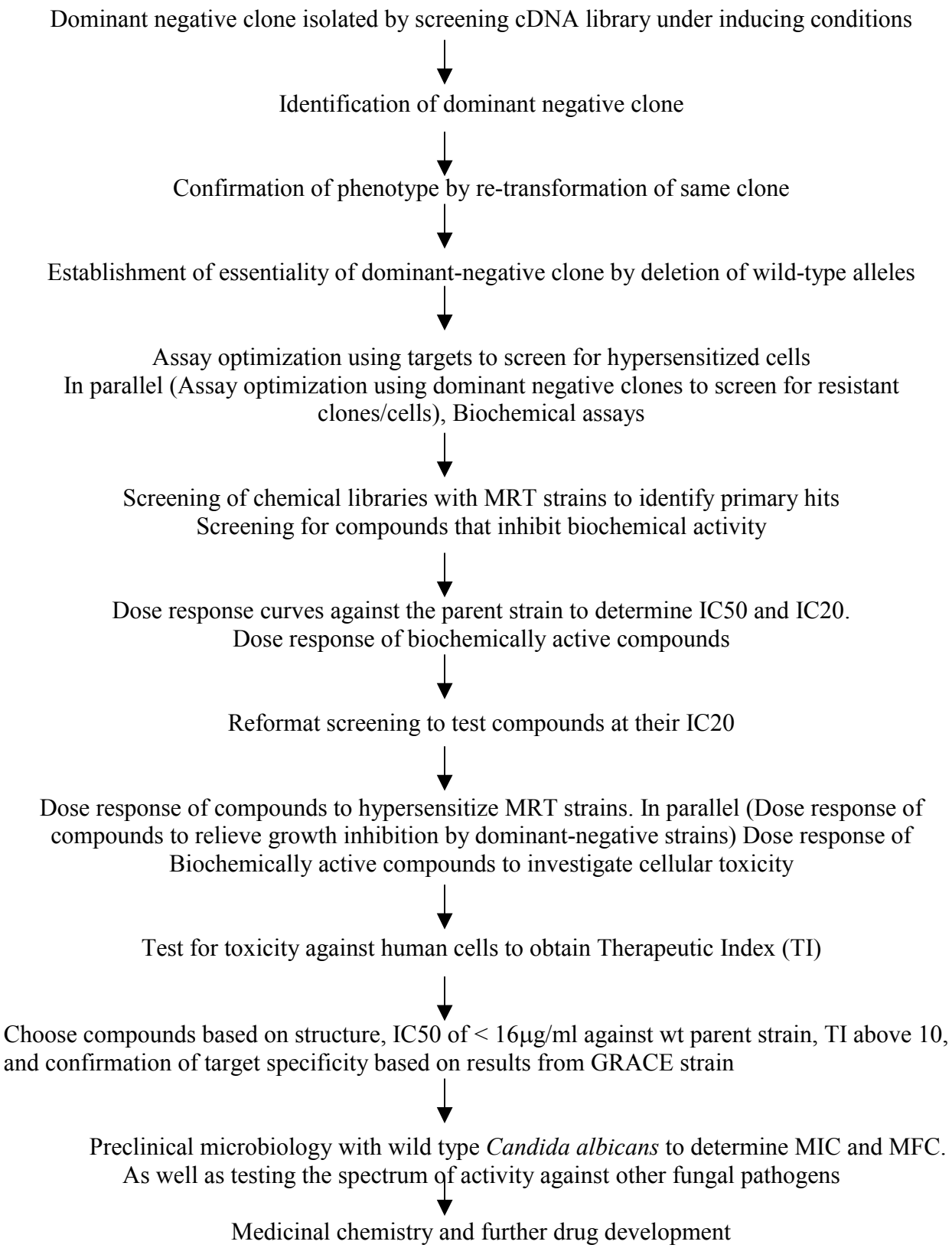
Figure 5-Flow Chart of the Phase II proposed project



Reference List

1. **Akada, R., J. Yamamoto, and I. Yamashita**. 1997. Screening and identification of yeast sequences that cause growth inhibition when overexpressed. Mol.Gen.Genet. **254**:267-274.
2. **Baudin, A**, et al. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. Nucleic Acids Res 21, 3329-3330.
3. **Brookman, J. L. and D. W. Denning**. 2000. Molecular genetics in *Aspergillus fumigatus*. Curr.Opin.Microbiol. **3**:468-474.
4. **Brown, D. H., Jr., A. D. Giusani, X. Chen, and C. A. Kumamoto**. 1999. Filamentous growth of *Candida albicans* in response to physical environmental cues and its regulation by the unique CZF1 gene. Mol.Microbiol. **34**:651-662.
5. **Brown, D. H., Jr., I. V. Slobodkin, and C. A. Kumamoto**. 1996. Stable transformation and regulated expression of an inducible reporter construct in Candida albicans using restriction enzyme-mediated integration Mol.Gen.Genet. **251**:75-80.
6. **Burke, D., P. Gasdaska, and L. Hartwell**. 1989. Dominant effects of tubulin overexpression in *Saccharomyces cerevisiae*. Mol.Cell Biol. **9**:1049-1059.
7. **Care, R. S., J. Trevethick, K. M. Binley, and P. E. Sudbery**. 1999. The MET3 promoter: a new tool for *Candida albicans* molecular genetics. Mol.Microbiol. **34**:792-798.
8. **De Backer, M. D., B. Nelissen, M. Logghe, J. Viaene, I. Loonen, S. Vandoninck, R. de Hoogt, S. Dewaele, F. A. Simons, P. Verhasselt, G. Vanhoof, R. Contreras, and W. H. Luyten**. 2001. An antisense-based functional genomics approach for identification of genes critical for growth of *Candida albicans*. Nat.Biotechnol. **19**:235-241.
9. **Diez-Orejas, R., G. Molero, I. Rios-Serrano, A. Vazquez, C. Gil, C. Nombela, and M. Sanchez-Perez**. 1999. Low virulence of a morphological *Candida albicans* mutant. FEMS Microbiol.Lett. **176**:311-319.
10. **Espinet, C., M. A. de la Torre, M. Aldea, and E. Herrero**. 1995. An efficient method to isolate yeast genes causing overexpression- mediated growth arrest. Yeast **11**:25-32.
11. **Fonzi, W. A. and M. Y. Irwin**. 1993. Isogenic strain construction and gene mapping in *Candida albicans*.Genetics **134**:717-728.
12. **Forsyth, R. A. et. al.** 2002. A genome-wide strategy for the identification of essential genes in *Staphylococcus aureus*. Molecular Microbiology **43**:1387-1400.
13. **Ghannoum, M. A. and L. B. Rice** . 1999. Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin.Microbiol.Rev. **12**:501-517.
14. **Gietz, R. D. and R. A. Woods**. 2001. Genetic transformation of yeast. Biotechniques **30**:816-6, 828.
15. **Guarente, L. and M. Ptashne**. 1981. Fusion of *Escherichia coli* lacZ to the cytochrome c gene of *Saccharomyces cerevisiae*. Proc.Natl.Acad.Sci.U.S.A **78**:2199-2203.
16. **Haselbeck, R. et al.** 2002. Comprehensive Essential Gene Identification as a Platform for Novel Anti-infective Drug Discovery, p. 99-1101. Current Pharmaceutical Design, vol. 8.
17. **Herskowitz, I.** 1987. Functional inactivation of genes by dominant negative mutations. Nature **329**:219-222.
18. Infectious disease study #50. Strategic overview of severe fungal infections. 2000. Infectious disease (A Pharmacor Serive) Decision Resource, Inc. Bay Colony Corporate Center-1100 Winter Street Waltham, Massachuesetts 02451.
    Ref Type: Report
19. **Janbon, G., F. Sherman, and E. Rustchenko**. 1998. Monosomy of a specific chromosome determines L-sorbose utilization: a novel regulatory mechanism in *Candida albicans*. Proc.Natl.Acad.Sci.U.S.A **95**:5150-5155.
20. **Kontoyiannis, D. P., N. Sagar, and K. D. Hirschi**. 1999. Overexpression of Erg11p by the regulatable GAL1 promoter confers fluconazole resistance in *Saccharomyces cerevisiae*. Antimicrob.Agents Chemother. **43**:2798-2800.
21. **Lee, S. A., Y. Mao, Z. Zhang, and B. Wong**. 2001. Overexpression of a dominant-negative allele of YPT1 inhibits growth and aspartyl protease secretion in *Candida albicans*.. Microbiology **147**:1961-1970.
22. **Leighton, J. K., S. Dueland, M. S. Straka, J. Trawick, and R. A. Davis**. 1991. Activation of the silent endogenous cholesterol-7-alpha-hydroxylase gene in rat hepatoma cells: a new complementation group having resistance to 25-hydroxycholesterol. Mol.Cell Biol. **11**:2049-2056.
23. **Leuker, C. E. and J. F. Ernst**. 1994. Toxicity of a heterologous leucyl-tRNA (anticodon CAG) in the pathogen *Candida albicans*: in vivo evidence for non-standard decoding of CUG codons. Mol.Gen.Genet. **245**:212-217.
24. **Liu, H., J. Krizek, and A. Bretscher**. 1992. Construction of a GAL1-regulated yeast cDNA expression library and its application to the identification of genes whose overexpression causes lethality in yeast. Genetics **132**:665-673.

25. **Magee, B. B. and P. T. Magee**. 2000. Induction of mating in *Candida albicans* by construction of MTLa and MTLalpha strains. Science **289**:310-313.
26. **Mao, Y., V. F. Kalb, and B. Wong**. 1999. Overexpression of a dominant-negative allele of SEC4 inhibits growth and protein secretion in *Candida albicans*. J.Bacteriol. **181**:7235-7242.
27. **McDade, H. C. and G. M. Cox**. 2001. A new dominant selectable marker for use in *Cryptococcus neoformans*. Med.Mycol. **39**:151-154.
28. **Morschhauser, J., S. Michel, and P. Staib**. 1999. Sequential gene disruption in *Candida albicans* by FLP-mediated site- specific recombination. Mol.Microbiol. **32**:547-556.
29. **Mumberg, D., R. Muller, and M. Funk**. 1995. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene **156**:119-122.
30. **Nakayama, H., T. Mio, S. Nagahashi, M. Kokado, M. Arisawa, and Y. Aoki**. 2000. Tetracycline-regulatable system to tightly control gene expression in the pathogenic fungus *Candida albicans*. Infect.Immun. **68**:6712-6719.
31. **Perepnikhatka, V., F. J. Fischer, M. Niimi, R. A. Baker, R. D. Cannon, Y. K. Wang, F. Sherman, and E. Rustchenko**. 1999. Specific chromosome alterations in fluconazole-resistant mutants of *Candida albicans*. J.Bacteriol. **181**:4041-4049.
32. **Pla, J., R. M. Perez-Diaz, F. Navarro-Garcia, M. Sanchez, and C. Nombela**. 1995. Cloning of the Candida albicans HIS1 gene by direct complementation of a *C. albicans* histidine auxotroph using an improved double-ARS shuttle vector. Gene **165**:115-120.
33. **Powers, S., K. O'Neill, and M. Wigler**. 1989. Dominant yeast and mammalian RAS mutants that interfere with the CDC25- dependent activation of wild-type RAS in *Saccharomyces cerevisiae* Mol.Cell Biol. **9**:390-395.
34. **Ramer, S. W., S. J. Elledge, and R. W. Davis**. 1992. Dominant genetics using a yeast genomic library under the control of a strong inducible promoter. Proc.Natl.Acad.Sci.U.S.A **89**:11589-11593.
35. **Santos, M. A., G. Keith, and M. F. Tuite**. 1993. Non-standard translational events in *Candida albicans* mediated by an unusual seryl-tRNA with a 5'-CAG-3' (leucine) anticodon. EMBO J. **12**:607-616.
36. **Santos, M. A. and M. F. Tuite**. 1995. The CUG codon is decoded in vivo as serine and not leucine in *Candida albicans*. Nucleic Acids Res. **23**:1481-1486.
37. **Shamah, S. M. and C. D. Stiles** . 1995. Transdominant negative mutations. Methods Enzymol. **254**:565-576.
38. **Sheppard, D.** 1994. Dominant negative mutants: tools for the study of protein function in vitro and in vivo. Am.J.Respir.Cell Mol.Biol. **11**:1-6.
39. **Sobel, J. D.** 2000. Practice guidelines for the treatment of fungal infections. Clinical and Infectious Diseases **30**:652.
40. **Stevenson, L. F., B. K. Kennedy, and E. Harlow**. 2001. A large-scale overexpression screen in *Saccharomyces cerevisiae* identifies previously uncharacterized cell cycle genes. Proc.Natl.Acad.Sci.U.S.A **98**:3946-3951.
41. **Tzung, K. W., R. M. Williams, S. Scherer, N. Federspiel, T. Jones, N. Hansen, V. Bivolarevic, L. Huizar, C. Komp, R. Surzycki, R. Tamse, R. W. Davis, and N. Agabian**. 2001. Genomic evidence for a complete sexual cycle in *Candida albicans*. Proc.Natl.Acad.Sci.U.S.A **98**:3249-3253.
42. **Uhl, M. A. and A. D. Johnson**. 2001. Development of *Streptococcus thermophilus* lacZ as a reporter gene for *Candida albicans*. Microbiology **147**:1189-1195.
43. **Venkateswarlu, K., D. E. Kelly, N. J. Manning, and S. L. Kelly**. 1998. NADPH cytochrome P-450 oxidoreductase and susceptibility to ketoconazole. Antimicrob.Agents Chemother. **42**:1756-1761.
44. **Whiteway, M., D. Dignard, and D. Y. Thomas**. 1992. Dominant negative selection of heterologous genes: isolation of Candida albicans genes that interfere with *Saccharomyces cerevisiae* mating factor-induced cell cycle arrest. Proc.Natl.Acad.Sci.U.S.A **89**:9410-9414.
45. **Wilson, R. B., D. Davis, and A. P. Mitchell**. 1999. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. J.Bacteriol. **181**:1868-1874.

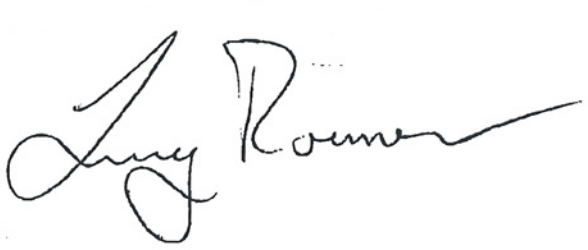
Dr. John Trawick
Principal Investigator
Elitra Pharmaceuticals
3510 Dunhill Street
San Diego, CA 92121

Dear John,

I certainly welcome the invitation and agree to serve as a consultant for your phase II SBIR grant, proposal to apply a dominant negative genetic screen towards identifying novel drug targets in *Candida albicans*. As you are well aware, such an initiative strongly complements the approach we have taken in identifying essential genes at Elitra Canada. Our approach relies on conditional expression using a method referred to as GRACE (gene replacement and conditional expression). Consequently, depending on the basal level of expression in our system, many essential genes are not discovered since the basal level of expression is sufficient for cell growth. However, controllable over expression of a cDNA library and screening for, dominant negative clones is a wonderfully elegant strategy to overcome the limitation of the GRACE method to identifying essential genes previously missed. Importantly, your proposal serves to discover novel antifungal targets displaying strong phenotypes that are highly sensitive to small perturbations in gene activity. Moreover as in any genetic screen, your approach selects for the very genes you are interested in without the biases of a systematic strategy we have implemented. Finally, the proposal outlines target-based biochemical and whole cell screens that focus directly to the commitment of drug discovery.

To my knowledge, an over expression-based genetic screening in *C. albicans* is entirely novel and will provide a number of important uses in addition to gene discovery. The development of secondary whole cell assays as you have described, as well as over expression-based suppressor screens are clear extensions of the molecular reagents you have constructed. You obviously have the tools and people to succeed in this important endeavor; I wish you the best of luck in your application and I believe it deserves strong support.

Sincerely,



Teary Roemer.

Director of Fungal Genomics
Elitra Canada
225 President Kennedy West,
Montreal, Quebec H2X 3Y8

Product Development Plan

This "Product Development Plan", now called the "Commercialization Plan" was prepared before the NIH suggested outline for current commercialization plans. It contains information about the company, previous funding history, regulatory experience, value of SBIR project, milestones and estimated achievement times, and brief market analysis. Missing are plans for intellectual property and potential exit strategies (e.g., licensing, merger or acquisition, or product production and sales).

Company Information

The mission of Elitra Pharmaceuticals Inc. is to discover, develop and commercialize new antimicrobial drugs against novel targets to be used to fight infectious diseases in humans. Elitra was founded in San Diego 1997 and has facilities in San Diego, California (where the SBIR work will take place) and Montreal, Canada. Elitra currently has 77 employees, over 50 of who are involved in research efforts. Current facilities are over 32,000 sq feet and include all of the equipment essential for target discovery, validation, and drug screening as well as initial preclinical studies. Facilities include HTS labs, molecular biology labs, preclinical microbiology labs, animal vivarium, and administrative facilities in 26,000 sq feet in San Diego.

Elitra has nearly completed the identification of essential genes in 9 target bacterial pathogens. Current antibacterial efforts are focused on validation and prioritization of targets for screening as well as high throughput screening of multiple targets in order to identify antibacterial drugs against *Staphylococcus aureus* and other pathogenic bacteria. Initial screening campaigns have succeeded to the point where lead optimization efforts are being initiated. Medicinal chemistry resources are available to Elitra through a unique risk sharing collaboration with MediChem Research Inc. and through pharmaceutical partnerships with Merck & Co. and LG Chem (Taejon, Korea). Additional partnerships in the antibacterial field are actively being sought.

Antifungal efforts at Elitra include identifying essential genes in the two most important fungal pathogens, *C. albicans*, and *A. fumigatus*, and development of high throughput screening assays. The proposed funding will support these efforts and accelerate the process of identifying potential antifungal leads. When such leads are identified, lead optimization chemistry resources will likely be available through MediChem and, possibly, through future commercial partnerships. Elitra also plans to build its own chemistry department within the next two to three years. Novel “Lead-to-Gene” technologies that allow for the rapid identification of the target(s) for a drug of unknown mechanism and monitoring target specificity are being developed at Elitra Canada and will augment Elitra’s antifungal lead discovery and lead optimization efforts.

As a start-up company, Elitra has no current products. However, Elitra’s senior management team has significant pharmaceutical company experience in developing and commercializing new drugs (see below) and remains committed to the commercialization of new antimicrobial drugs through a combination of pharmaceutical partnerships and retained commercial rights. For example, Elitra’s commercial collaboration with LG Chem (Taejon, Korea) is a 5-year collaboration to jointly discover and develop new antibacterial drugs for which Elitra retains commercial rights in North America and shared commercial rights in Western Europe. Additional commercial collaborations are ongoing with Merck, MediChem, and Incyte Genomics. These collaborative efforts are intended to achieve the following: 1) development execution of screens against antibacterial targets, 2) identification of chemicals active against these targets, 3) optimization and development of leads through medicinal chemistry efforts, 4) identification of mechanisms of action of antimicrobial compounds, 5)

establishment of genomics and drug discovery informatics databases of pathogenic microorganisms and Elitra compound libraries and 6) generally, the discovery and development of antimicrobial drugs. These companies have sufficient belief in the capabilities of Elitra's technologies to enhance and accelerate their own drug discovery efforts that three of them (Merck, LG Chem and Incyte) have invested in Elitra through equity participation.

Funding, Federal and non-federal

Federal funding:

1-Phase I SBIR grant (Grant #1 R43 AI47516-01)
Title: Identification of New Antibiotic Targets using BACs
Amount: $100,134
Period of support: 5/15/00 – 11/14/01
Principal investigator: Robert Haselbeck

2-Phase I SBIR grant (Grant #1 R43 Ai48345-01)
Title: Dominant Expression of New Targets in *C. albicans*
Amount: $100,114
Period of support: 8/15/00 – 2/14/02
Principal investigator: John Trawick

3-Phase I SBIR grant (Grant #1 R43 AI49694-01)
Title:Screen for essential genes in *Aspergillus fumigates*
Amount: $100,764
Period of support: 3/15/01 – 9/14/02
Principal Investigator: Daniel Tishkoff

Non-federal funding:

Elitra is a privately held, privately financed firm funded by venture capital investors. Funds raised to date total nearly $50 MM and include equity participation by Merck, LG Chem and Incyte.

Regulatory experience and subsequent commercialization

Elitra management has extensive experience in the pharmaceutical and biotechnology industries including direct experience in drug discovery, development and commercialization. Harry Hixson, CEO, was formerly President and Chief Operating Officer of Amgen during the launches of Epogen® and Neupogen®, its two blockbuster drug products (Thousand Oaks, CA) and held prior positions at Abbott. Gordon Foulkes, Executive Vice President of Research and Development, has many year of experience in drug screening efforts at Oncogene Sciences Inc. (OSI) and Aurora BioSciences and is thoroughly familiar with the drug development process. Deborah Mosca, Vice President of Drug Development was formerly involved in the development and commercialization of antimicrobial drugs at American Home Products (Lederle) and Intrabiotics. Ed Baracchini, Vice President of Business Development held former positions at Warner-Lambert, Agouron and Isis Pharmaceuticals. Others include: Iraj Beheshti, General Manager of Elitra Canada, (Abbott & Nymox); Phil Youngman, Vice President of Discovery Biology, (Millennium Pharmaceuticals); Carlos Zamudio, Vice President of Drug Discovery Informatics (Sequana & Perkin Elmer); Grant Carr, Director of Screening Operations (Axys) Elitra's advisors include experts in drug discovery and development including Marty Rosenberg,

former Executive VP of Infectious Diseases at GlaxoSmithKline, who is both an advisor to Elitra and a Board member.
Drug development is a complex process often taking 10 years or longer. Elitra plans to access drug development support initially through consultants, CROs and other contract organizations and also through access to the capabilities of its commercial partners. However, Elitra is dedicated to ultimately building an in-house drug development team including regulatory experts. Elitra will seek specific clinical indications and market opportunities dependent on the attributes of lead compounds discovered and will seek to accelerate the process of getting these important drugs to the market place where patients will have access to them through their doctors.

Value of SBIR project

Antimicrobial drugs target infectious diseases caused by pathogens such as bacteria and fungi. Worldwide sales of antimicrobial drugs totaled over $30 billion in 2000 (IMS Health). The majority of infections are acquired in the general community, resulting in approximately 145 million courses of antibiotics prescribed annually in the United States alone. The increasing use of such antimicrobials worldwide has led to an increasing emergence of drug resistance in microbial pathogens that is reaching catastrophic proportions. This provides a compelling reason to identify new antimicrobial drugs that work through novel drug targets. The inadequacies of current drugs (especially antifungal drugs) provide another and include side effects, toxicity and poor efficacy against some organisms. Yet current drugs target less than 30 microbial gene products. When considered by sales, the vast majority of antibacterials target just three or four pathways and the mechanisms of action of essentially all systemic antifungal drugs relate to just a single pathway – ergosterol biosynthesis and function.

Historically, researchers discovered most antibiotics and antifungals by exposing live bacteria or fungi to large random collections of chemicals to determine which chemicals killed them or prevented their proliferation. This whole-cell screening technique led to the identification of many of the antimicrobials still in use today. Examples include the antibiotics penicillin, erythromycin and tetracycline, and the antifungal polyenes and azoles. However, this has lead to the identification of compounds that address a limited number of potential drug targets and does not adequately distinguish between specific agents that kill bacteria or fungal pathogens and non-specific toxic compounds that can also have adverse effects in humans.

In order to discover new antimicrobial drugs that work through novel mechanisms, researchers developed new assays that enabled direct access to specified target gene products. By the mid-1980s, biochemical assays, in which scientists first purify and study the protein target in isolation outside of the cell, became a primary method of screening chemical libraries to identify potential drugs. While this approach has worked well for the identification of drugs acting on human drug targets in human cells, it has worked poorly for identifying new drugs acting through novel targets in microbial organisms. Many companies were successful in generating compounds that inhibited the function of isolated bacterial or fungal proteins only to find that these compounds failed to be effective against live, intact cells of the pathogen, resulting in wasted development time and resources.
By the mid-1990s, advances in both molecular biology and automation allowed researchers to develop a new generation of assays that use a variety of techniques to modify living cells. These functional assays allow researchers to assess more directly a compound's ability to inhibit a protein of interest in the protein's native environment. Although functional assays provide some advantages in the screening process, the widespread use of this type of assay can be limited by: 1) required knowledge of the function of the protein to develop an assay, 2) long assay development timelines, which can take six to nine months and 3) insufficient assay sensitivity to detect potential drugs.

Although there have been general advances in the areas of genomic information, molecular biology tools and new screening technologies, these advances have produced few new antimicrobial drugs. Researchers have introduced only two new classes of antimicrobial drugs in over 20 years (the antibacterial oxazolidinones and the antifungal echinocandins). Historically, the failure of new technologies to address the issue of novel mechanism led to the continuing practice among pharmaceutical chemists of altering the chemical structure of established antimicrobials in successive iterations in order to stay one step ahead of developing resistance in pathogen populations. However, it has become increasingly difficult to create new drugs that are as effective as current drugs yet are less toxic and also effective against resistant organisms and maintain their effectiveness over time. Thus, there is a critical need to develop new and effective antimicrobial drugs. For instance, existing antifungal agents target a limited number of cellular functions (Trends Microbiol 1998 6(3):117-24 Emerging targets for the development of novel antifungal therapeutics. Groll AH, De Lucca AJ, Walsh TJ.). This number is further reduced if only the major selling drugs for systemic fungal infections are considered. Together, the top 7 antifungal drugs for systemic infections account for $2.8 billion in worldwide sales (IMS Health; commissioned MIDAS report) but target either the fungal sterol, ergosterol, (polyenes like Amphotericin B) or one of 2 committed steps of ergosterol biosynthesis: azole/triazole antifungals such as fluconazole target lanosterol demethylase and allylamines target squalene epoxidase. All of these drugs have serious problems in spectrum of action, acquired resistance, and toxicity that can seriously handicap their use (Trends Microbiol 1998 6(3):117-24 Emerging targets for the development of novel antifungal therapeutics. Groll AH, De Lucca AJ, Walsh TJ.).). For example, the most popular antifungal agent, fluconazole, is not effective against some fungal species such as *C. glabrata* (Hitchcock CA, Pye GW, Troke PF, Johnson EM, Warnock DW. Fluconazole resistance in Candida glabrata**.** Antimicrob Agents Chemother. 1993 37(9):1962-5.) and *C. krusei* . Infections by these other species of *Candida* are on the increase in immunocompromised patients. Furthermore, resistance to fluconazole can arise during the chronic treatment of *C. albicans* infections thereby increasing the risk of mortality or long-term morbidity. Resistance to azoles may occur by one of several mechanisms and is likely to increase in incidence.

Other antifungal drugs such as Amphotericin B have more serious problems in use against systemic infections. This drug and its various formulations are associated with significant risk of nephrotoxicity (Curr Opin Crit Care 2001 7(6):379-83
Can we decrease amphotericin nephrotoxicity? Costa S, Nucci M.). Though emergence of resistance to polyenes such as Amphotericin B isn't a significant problem, the other challenges presented by this class of drug are daunting.

Taken together, existing antifungal therapies represent an extremely limited range of targeted cellular processes. Each of these drugs has a significant risk of toxicity because they target cellular processes that are highly conserved between fungi and mammals - sterol biosynthesis and sterol action in cell membranes. Finally, the target pathogens acquire resistance to the most commonly used and least toxic class, the azoles and triazoles.

The inherent deficiencies of existing antimicrobial drugs have motivated a number of pharmaceutical and biotech firms to attempt target-based screening in order to identify novel antifungals. To date, little has come of this approach. The only new and promising class of antifungal drugs, the lipopeptide antifungals including echinocandins, were first identified over a quarter of a century ago through classic whole cell screening (Besson F, Peypoux F, Michel G, Delcambe L.; Characterization of iturin A in antibiotics from various strains of *Bacillus subtilis* J Antibiot (Tokyo). 1976 Oct;29(10):1043-9). These lipopeptides have been aggressively pursued over the last 15 years but have proven very difficult to develop due to lack of systemic bioavailability. Only one, Caspofungin, has been approved to date and only for a very limited indication. Eli Lilly, one of the earliest companies to pursue development of echinocandins and

the related class of lipopeptide antibacterial compounds (e.g. Daptomycin) has apparently abandoned development and outlicensed all promising compounds. Indeed, Lilly appears to have abandoned R&D in antimicrobial drugs entirely. Other pharmaceutical companies that have apparently abandoned or severely curtailed R&D related to antifungal drugs include some of the prior leaders in the field: GlaxoSmithKline, Janssen / Johnson & Johnson, Aventis. Numerous others are notably absent: American Home Products, Bayer, Bristol Meyers Squibb, Eisai, Otsuka, Proctor and Gamble, Roche, Takeda, Tanabe, Yamanouchi. In fact, only four major US companies and three major Japanese companies appear to have significant R&D activities related to antifungal discovery and development. In short, at the height of the battle against fungal infections, the key armies have abandoned the field – presumably to focus on drugs that have the potential to achieve >$1 billion in peak sales.

This shift in the antifungal landscape has created an opportunity for smaller innovative companies such as Elitra. There is now both a compelling application for new antifungal technologies such as those being developed by Elitra and a compelling, unmet patient need for their results.

Commercialization Plan

The specific aims of this SBIR Phase II proposal are limited to target identification and compound screening. The term and amount of funding for Phase II applications are such that no clinical trials or commercial compounds will be developed as part of the work proposed here. Those goals will have to be met in subsequent research and development. However, the ultimate goal of Elitra's antifungal research is to develop and commercialize viable antifungal drugs as indicated above.

As envisioned in the specific aims, it should be possible during the term of PhaseII to identify "hit" compounds that are hyperpotent against a specific *C. albicans* target identified during the first part of PhaseII. Elitra will investigate Structure-Activity-Relationships for synthesized compound analogs of such hits and attempt to identify and optimize leads with the goal of producing broad-spectrum antifungal agents effective against systemic and topical infections. The actual indications and actual market will be determined by many factors including the specific properties of the lead compound(s) and evolving patient/clinical needs, which cannot be accurately predicted at this time. However, Elitra continues to monitor the antifungal market and emerging clinical needs and this information, in conjunction with the scientific data achieved during Phase II, will be applied to the processes of prioritizing potential targets for screening and selecting drug leads for development.

Time and milestones for antifungal drug development

NOTE: Only the beginning of the project will be funded by this Phase II application. This provides an opportunity to have additional parts of the project funded by future SBIR grants.

First two years (scope of this application): Target identification, validation, and primary screening with compounds. This will lead to identification of preliminary hits to be confirmed and validated. Elitra experience in antibacterial screening suggests that ~2,000 preliminary hits can be obtained in hyperpotent screening of >250,000 compounds.

Compounds with confirmed growth inhibition, excellent therapeutic indices, etc are likely be considerably fewer; projections are 30 to 50 potential lead compounds at the end of two years of screening.

Third and fourth years The potential lead compounds will be scored for *in vitro* sensitivity, preliminary *in vivo* validation in animal models to select series for medicinal chemistry. Elitra projects that a couple lead series of potential antifungal drugs will result from these efforts. By the end of the third year or into the fourth year, a successful drug screening and medicinal chemistry campaign should result in antifungal compounds with improved potency and pharmacodynamic properties that can serve as potential clinical leads. Activity in the fourth or fifth year is likely focus on preclinical development, selection of a proposed clinical lead and preparation of the IND package to allow filing of an IND (Investigational New Drug) with the US FDA.

Fifth year and beyond. The clinical lead will be entered into Phase I clinical trials for monitoring compound safety in normal human volunteers. A clinical lead that is efficacious *in vitro* and in animals, has an acceptable toxicity profile in animals and is safe in humans will be moved into Phase II and Phase III clinical trials. The goal is to establish appropriate dosing and demonstrate efficacy in the treatment of major systemic fungal infections. Ultimately the goal is have an NDA (New Drug Application) approved by the US FDA so that the drug can be launched in the market and reach the patients who need it.

As discussed above, these goals are beyond the actual scope of this application and are presented to indicate the commercial goals of antifungal research at Elitra. These are projections and it is likely that some deviation from this plan will occur. The drugs resulting from these efforts could either fit our goal of broad-spectrum drugs capable of treating even systemic mycoses or could be developed as products with more limited applications. The nature of the current antifungal market is such that there is competitive potential for almost any drug that is more efficacious and/or has a better toxicity profile than currently marketed drugs.

Market analysis and market size.

Worldwide sales of antifungal drugs exceeded $4 billion in 2000 (IMS Health; commissioned report). Of significant interest, sales of antifungal drugs in Japan and Korea have increased more than 50% from 1998 to 2000 (IMS Health; commissioned report). Worldwide sales data for antifungal drugs are presented in the table below and are from Med Ad News “Top 500 Drugs” (Med Ad News, 2001 20 (5)76-77).

Each of the top-selling antifungal agents exhibits significant toxicity in humans. One possible explanation for this toxicity is that these drugs target essential steps in sterol biosynthesis or cell membrane sterols directly. These targets all exist in human cells and share reasonably high amino acid sequence identity or structural similarity. The most toxic antifugal agents are the polyenes, specifically, Amphoterocin B and its various formulations. These compounds account for approximately 10% of the total market (ca. $280 million) and their continued use reflects a critical need for effective drugs to treat life threatening systemic mycoses. The top three drugs consist of two triazoles and one allylamine derivative. All three target enzymes in the sterol (and therefore ergosterol) biosynthesis pathway. They account for over 80% of the market. The most common clinical indications for these drugs according to IMS Health are Dermatophytosis, Candidiasis, Other Superficial Mycosis, Unspecified Mycosis and Other Inflammatory Indications – Vag./Vulva. Shortcomings of these drugs include liver toxicity, especially with chronic use, and fungistatic activity rather than true fungicidal activity resulting commonly in re-emergence of the infection post-treatment.

Thus, the market for systemic antifungals is large and current drugs have significant shortcomings. An effective antifungal with a good safety profile should be very competitive in this market. Elitra believes the way to discover such drugs is to focus on screening for molecules that target the products of essential fungal genes that are broadly conserved in fungi but have diverged or do not exist in mammals and which act within pathways unrelated to sterol biosynthesis or action.

Disease/Brand	Class of Drug	Pharmaceutical Co.	Y2000 Sales
			$ millions
Antifungal			**$2,849.90**
Diflucan	Triazole	Pfizer	$1,014.00
Lamisil	Allylamine derivative	Novartis	$756.00
Sporanox	Synthetic triazole	Johnson & Johnson	$604.00
Lotrisone	Antifungal agent	Schering-Plough & Galen Holdings	$192.00
AmBisome	Polyene antifungal	Gilead Sciences	$141.10
AmBisome	Polyene antifungal	Fujisawa Pharmaceutical	$77.80
Abelcet	Polyene antifungal	Elan	$64.00

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ SBIR Phase I ☒ SBIR Phase II: SBIR Phase I Grant No. _R43 Ai48345_____ ☐ SBIR Fast Track

☐ STTR Phase I ☐ STTR Phase II: STTR Phase I Grant No. _ _____ ☐ STTR Fast Track

☐ REVISION of application number: _____

(This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)

☐ COMPETING CONTINUATION of grant number: _____

(This application is to extend a funded grant beyond its current project period.)

INVENTIONS AND PATENTS *(Competing continuation appl. and Phase II only)*

☐ No ☐ Previously reported

☐ Yes. If "Yes," ☐ Not previously reported

☐ SUPPLEMENT to grant number: _____

(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.

Name of former principal investigator/program director: _____

☐ FOREIGN application or significant foreign component.

1. PROGRAM INCOME ***(See instructions.)***

All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)
	0	

2. ASSURANCES/CERTIFICATIONS ***(See instruction***

The following assurances/certifications are made and ve
signature of the Official Signing for Applicant Organizatio
Page of the application. Descriptions of individual assura
certifications are provided in Section III. If unable to cert
where applicable, provide an explanation and place it aft

•Human Subjects; •Research Using Human Embryonic S
•Research on Transplantation of Human Fetal Tissue •V
Minority Inclusion Policy •Inclusion of Children Policy• Ve Participation.

At the time of the application, the applicant did not have an F&A rate. The company will receive temporary a 10% F&A rate until an actual rate is established. The negotiated rate may be less than the one proposed which would decrease the amount of the total award.

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☐ DHHS Agreement dated: _____ ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with _____ Regional Office.

☒ No DHHS Agreement, but rate established with _____ Date _____

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	152,936	x Rate applied	105.00	% = F&A costs	$	160,583
b. 02 year	Amount of base $	162,112	x Rate applied	105.00	% = F&A costs	$	170,218
c. 03 year	Amount of base $		x Rate applied		% = F&A costs	$	
d. 04 year	Amount of base $		x Rate applied		% = F&A costs	$	
e. 05 year	Amount of base $		x Rate applied		% = F&A costs	$	
					TOTAL F&A Costs	$	330,801

*Check appropriate box(es):

☒ Salary and wages base ☐ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

4. SMOKE-FREE WORKPLACE ☒ Yes ☐ No *(The response to this question has no impact on the review or funding of this application.)*

SUMMARY STATEMENT

Dennis Dixon (DMID) Ph.D.
301-496-7728
dd24a@nih.gov

(Privileged Communication)

Release Date: **07/25/2002**

Application Number: **2 R44 AI048345-02**

TRAWICK, JOHN D PHD
ELITRA PHARMACEUTICALS, INC.
3510 DUNHILL ST
SAN DIEGO, CA 92121

Review Group: **ZRG1 SSS-K (10)**
Center for Scientific Review Special Emphasis Panel

Meeting Date: **07/10/2002**
Council: **OCT 2002**
Requested Start: **12/01/2002**

PCC: **M31**
Dual PCC: **88BP**
Dual IC(s): **CA, DK**

Project Title: **Target-based antifungal drug discovery**

SRG Action: **Priority Score: 141**
Human Subjects: **10-No human subjects involved for competing applications**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
2	259,436	581,370
3	273,727	613,395
TOTAL	533,163	1,194,765

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

R44 A1048345-02 Trawick, J.D.

BIOHAZARD NOTE

The summary is prepared by the Scientific Review Administrator.

RESUME AND SUMMARY OF DISCUSSION: This is an outstanding application to further develop new drug targets in *Candida albicans* and elucidate essential physiological pathways in this important fungal pathogen. Building on the success of their innovative and imaginative Phase I program, Elitra Pharmaceuticals promises to fill an unmet medical need with respect to development of candidates for antifungal therapy. This Phase II application has high commercial potential. The Principal Investigator, co-investigators, and consultants have impressive credentials and constitute a highly competent team. The environment at Elitra Pharmaceuticals is a significant strength and was strengthened by the acquisition of Mycota Biosciences Inc. last year. Although the level of support requested seems appropriate and was recommended in full, there was some concern regarding the small percentage effort proposed for some investigators and the level of bioinformatics expertise indicated. Potential personnel adjustments by the applicants were viewed primarily as an administrative matter. The reviewers noted that the application does not address the biohazard precautions that will be employed for personnel working with *C. albicans,* an issue that should have been addressed more thoroughly.

DESCRIPTION (provided by applicant): The incidence of serious fungal infections has increased markedly in the last two decades and effective treatment options are increasingly compromised by the emergence of drug-resistant strains. The goal of the proposed work is to develop novel antifungal drugs that are safer and more effective than those currently available. The work will focus primarily on the dimorphic yeast, *C. albicans,* which is by far the leading cause of both life-threatening systemic fungal infections and more commonly occurring topical infections. A distinguishing feature of the drug-discovery strategy the applicants are pursuing is that it is based on target discovery, target prioritization and screening, all conducted with the pathogen itself, rather than with a surrogate model system. This strategy has been enabled by gene-identification and screen-configuration technologies developed at Elitra Pharmaceuticals, including important technologies developed under Phase I funding for this program. Under Phase I, an expression vector system was constructed that will allow screening for dominant-negative phenotypes. No such tools existed previously for *C. albicans.* Screens for dominant-negatives will identify new drug targets in *C. albicans* and help annotate essential physiological pathways in this important fungal pathogen. Critical functional features of the expression vector system have already been validated, a *C. albicans* complementary deoxyribonucleic acid (cDNA) library has been constructed in the vector, and pilot screening for dominant-negatives is in progress. Under Phase II funding, the investigators propose to implement the screen more broadly, characterize and prioritize the targets that are identified and conduct screening for drug leads. The dominant-negative phenotypes of the newly identified targets will be used to develop primary or secondary cell-based assays to screen chemical libraries for potential antifungal drugs and to facilitate the characterization of hits identified through other screening strategies. The proposed Phase II work will complement and enhance the value of internally funded target-identification and screening efforts at Elitra and will help promote the discovery of badly needed therapeutic agents in a medical area that generally receives insufficient attention from the pharmaceutical industry.

CRITIQUE 1:

SIGNIFICANCE: The discovery tools for researchers intent on the identification of targets, inhibitors and development candidates for antifungal agents lags far behind antibacterial research primarily due to the rudimentary nature of the fungal genetics. Antifungal therapy remains an unmet medical need with all but one agent, amphotericin B, either having holes in pathogen coverage issues with resistance emergence, and/or adverse side effects. This Phase II application, as a continuation of the Phase I program, has high commercial potential and intends to fill this shortcoming.

This application has two primary reviewers whose critiques are reproduced verbatim.

APPROACH: The research plan detailed in this application intends to continue the work from a Phase I Small Business Innovation Research (SBIR) grant (which is updated within this application), and take the best of the validated antifungal targets into Phase II with the intention of identifying novel compounds as antifungal agents. Phase I technology achievements in *S. cereviseae will* be adapted to the relevant host, *C. albicans,* using molecular technologies devised in the Phase I SBIR project. In short, Elitra Pharmaceuticals will screen for inhibitors of essential genes by screening a cDNA expression library for dominant-negative phenotypes. Together with their proprietary GRACE (gene replacement and conditional expression) technology, Elitra Pharmaceuticals will screen validated essential antifungal genes to identify inhibitors. A critical path of 'hit' confirmation and progression is provided, and demonstrated a maturity and expertise not always observed in these SBIR grant applications. The commercial plan to take compounds beyond Phase II is sound.

INNOVATION: The overexpression-based genetic screening in C. *albicans* represents great novelty and innovation. It complements the down-regulation system (GRACE) previously used by Elitra Pharmaceuticals to identify/screen essential antifungal genes.

INVESTIGATORS: Elitra Pharmaceuticals sports an all-star cast of talented individuals with extensive mammalian and/or antifungal biology and genetics. The involvement of Drs. Roemer and Bussey is considered a plus. Dr. Trawick is well qualified to serve as the Principal Investigator.

Although a biosketch was not provided for either consultant, the reviewers recognize their names and expertise.

ENVIRONMENT: Elitra Pharmaceuticals, with the acquisition of Mycota Biosciences Inc. offers an excellent environment for this research effort. The multi-disciplinary research team will provide an environment of creativity.

OVERALL EVALUATION: Building on the successful completion of Phase I SBIR grant goals (updated/deleted within the application), the research team at Elitra Pharmaceuticals proposes to apply the tools (i.e., dominant-negative screening) to identify essential genes in *C. albicans.* Once identified, these essential genes will be screened for inhibitors by a combination of whole-cell and/or biochemical assays, with the intention of identifying chemical starting points for candidate optimization.Building on the identification of essential genes in S. cereviseae, candidate genes in *C. albicans will* be validated as essential in *C. albicans* using their dominant-negative screening. Targets shown to be essential in both *S. cereviseae* and *C. albicans will* be prioritized based on informatics analyses. Anti-sense downregulation-based whole cell screens will provide screening platforms (similar to those undertaken with anti-bacterials). The proposed program is sound, bears high commercial potential, and has a excellent chance of technology

success. The identification of a 'lead' novel compound may be overly optimistic, but does not detract from this proposal.

CRITIQUE 2:

SIGNIFICANCE: The incidence of serious fungal infections is on the increase and the number of treatment options is decreasing as the fungi develop drug resistance. In addition, the number of pharmaceutical companies engaged in antifungal work is dropping. Therefore there is both the need and the opportunity for biotechnology companies to fill the void.

By far the most prevalent human fungal pathogen is *C. albicans.* In this application the investigators propose to identify novel antifungal compounds that target newly-identified *C. albicans* essential genes. A distinguishing feature of the research is that the work will be conducted in the pathogenic fungus itself rather than in a heterologous model system (like *S. cerevisiae.)*

In addition to the obvious need for more and better antifungal drugs, the project has the benefit of potentially providing interesting scientific insights into the biology of *C. albicans.*

APPROACH: The investigators have clearly outlined their goals for Phase I I of the work. The goals are ambitious, but they are measurable and well-prioritized. The level of experimental detail included is ample, so as to instill confidence in the investigators' abilities and their level of preparation for the ensuing work.

The success of the project is dependent on the quality of the cDNA library employed, that it be representative of the whole genome. The cDNA library to be used was contracted out for production, and no mention was made of the quality control tests given to it. Therefore, it is advised that the cDNA library be adequately tested for its completeness prior to use.

Reviewers may provide useful advice.

INNOVATION: The investigation is innovative in its adaptation and application of molecular biological techniques for use in the pathogenic fungus of interest, *C. albicans,* rather than using the traditional model system intermediary. It does make the experimentation more difficult and potentially more hazardous to the experimenters, but it has the distinct benefit of providing immediacy to their results. The project can also provide a wealth of information about the fundamental biology of *C. albicans, i.e.,* how many of its genes are essential, and, on a more basic level, serve as a confirmation of the existence of genes identified by the DNA sequence analysis.

INVESTIGATORS: A major weakness of the program is the number of investigators named to the project who will be devoting very small percentage efforts to it. Of the 10 positions enumerated, five will contribute less than ten percent effort; four will contribute only two and one-half percent effort (One wonders how that level of effort can even be measured.). It seems as if the Principal Investigator, will need to expend a considerable amount of his 12.5 percent effort to keeping track of the others.

This reviewer focuses on the large number of listed personnel with low levels of effort. Fortunately, the reviewer considers this an administrative, not a fatal, flaw.

From the project description there seems to be a substantial bioinformatic component, ranging from running and interpreting the experiments, assessing and prioritizing the targets, and then choosing the candidate compounds to be tested. From the list of personnel and description of their responsibilities, it isn't obvious that an adequate level of bioinformatic support has been requested.

A major strength of the proposal is that the Principal Investigator and the major personnel involved have excellent credentials. Also, having Drs. Bussey and Roemer, both renown fungal experts, as consultants on the program is a definite plus.

ENVIRONMENT: The environment at Elitra Pharmaceuticals is a significant strength. The organization has all the necessary equipment and facilities to conduct molecular biological and biochemical research on microbes. It has the facilities and expertise to run high-throughput screening (HTS) experiments in drug discovery research.

Since the Phase I award of this project, Elitra Pharmaceuticals has acquired Mycota Biosciences Inc., which substantially increases the materials, resources and expertise available to this project. The company also has extensive bioinformatic resources that are important for the success of the project.

Furthermore, and equally important for success, Elitra Pharmaceuticals has developed productive business relationships with other biotechnology companies, like Incyte Genomics, and pharmaceutical companies, like Merck & Co., Inc. To actually succeed in a drug discovery project requires the involvement of a large pharmaceutical company, and the earlier such relationships are developed the better.

EVALUATION OF PHASE I PERFORMANCE: The goals set for Phase I of this program were ambitious and were met by interesting and innovative means. For example, the goal of designing an integrative vector was accomplished by amending the original procedure to incorporate an inducible promoter system that had been developed at Mycota Biosciences Inc. In addition, the investigators now have access to over 600 essential genes of *C. albicans* that were identified using Mycota's proprietary GRACE technology. These will augment the genes to be identified by the dominant negative technology, still in development from Phase I of the project. Construction of the cDNA library, is a difficult and exacting task. Yet it is this resource upon which the success of the subsequent steps are dependent. This task was contracted out to AGCT, Inc., a company that specializes in providing molecular biological services to other companies. These actions have freed up in-house resources to focus on developing the dominant-negative technology, which is the most unique part of the proposal, and designing the high-throughput screens. These examples suggest that Elitra Pharmaceuticals has both the necessary scientific and business acumen and flexibility to take advantage of opportunities as they arise, all of which are necessary components for success.

PRODUCT DEVELOPMENT PLANS: The investigators have put together a credible product development plan which adequately addresses the four key points.

OVERALL EVALUATION: Infections by fungal pathogens are a serious health problem that is on the rise, and is not being adequately addressed by the major pharmaceutical companies. Elitra Pharmaceuticals has put together a highly detailed, credible and innovative plan for identifying fungal drug targets and screening compounds for treatment. The applicant has assembled an impressive set of personnel and resources for the effort, and has also made

important relationships with other biotechnolopgy and pharmaceutical companies that will be essential for actually developing new drugs. All drug development is inherently fraught with risk, but Elitra Pharmaceuticals has made a good start. Therefore, this reviewer is highly enthusiastic about this project.

Biohazard: Much of the work will involve the use of live *C. albicans* cells. However, the proposal does not specifically mention the type of biohazard precautions that will be employed.

Budget: The assignment of personnel to the project is a concern. There is a significant number of investigators that are named to the project who will be devoting very minor amounts of their effort to it. Therefore personnel assignments should be reconsidered.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

BIOHAZARD NOTE: Much of the work will involve the use of live *C. albicans* cells; however, the application does not specifically mention the type of biohazard precautions that will be employed, personnel who may be affected, or the training of these personnel. The reviewers felt that these issues should have been addressed more thoroughly.

Biohazard considerations should have been addressed in the application. This note will require NIAID program staff to review biohazard plans prior to award.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

The application requests direct costs of $533,163 over two years and total costs of $1,194,765. The reviewers recommend support for the full time and amounts of the request.

MEETING ROSTER

Center for Scientific Review Special Emphasis Panel
CENTER FOR SCIENTIFIC REVIEW
ZRG1 SSS-K (10) B
July 10, 2002 - July 11, 2002

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

********** NOTICE OF GRANT AWARD **********
SMALL BUSINESS INNOVATION RESEARCH PROG Issue Date:06/03/2003
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
****

Grant Number: 2 R44 AI048345-02 (Revised)
Principal Investigator: TRAWICK, JOHN D PHD
Project Title: Target-based antifungal drug discovery

TIBBITS, GREGORY
CHIEF FINANCIAL OFFICER
ELITRA PHARMACEUTICALS, INC.
3510 DUNHILL ST
SAN DIEGO, CA 92121
UNITED STATES

This is a revised Notice of Award deleting funds initially provided in a prior Notice of Award because excessive salary was requested. The award amount can be revised by NIH for cause.

Budget Period: 02/01/2003 - 01/31/2004
Project Period: 08/15/2000 - 01/31/2005

Dear Business Official:

The National Institutes of Health hereby revises this award to reflect a decrease in the amount of $9,742 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to ELITRA PHARMACEUTICALS, INC. in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR PART 52 15 USC 638 and is subject to terms and conditions referenced below.

Acceptance of this award including the Terms and Conditions is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Award recipients are responsible for reporting inventions derived or reduced to practice in the performance of work under this grant. Rights to inventions vest with the grantee organization provided certain requirements are met and there is acknowledgement of NIH support. In addition, recipients must ensure that patent and license activities are consistent with their responsibility to make unique research resources developed under this award available to the scientific community, in accordance with NIH policy. For additional information, please visit http://www.iedison.gov.

If you have any questions about this award, please contact the individual(s) referenced in the information below.

Sincerely yours,

Jane W. Unsworth
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

See additional information below

SECTION I - AWARD DATA - 2 R44 AI048345-02 (Revised)

AWARD CALCULATION (U.S. Dollars):

Salaries and Wages	$123,486
Fringe Benefits	$24,698
Personnel Costs	$148,184
Consultant Services	$8,750
Supplies	$85,250
Travel Costs	$12,500
Federal Direct Costs	$254,684
Federal F&A Costs	$155,593
APPROVED BUDGET	$410,277
TOTAL FEDERAL AWARD AMOUNT	$410,277

AMOUNT OF THIS ACTION (FEDERAL SHARE) -$9,742

The personnel costs were decreased by $9,742 because the salary for one or more of the staff exceeded that allowed on federal grants ($171,900)

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project, is as follows.
03 $421,632

FISCAL INFORMATION:
CFDA Number: 93.856
EIN: 1330779254A1
Document Number: RAI048345B

IC/ CAN / FY2003 / FY2004
AI/8425730/ 410,277/ 421,632

NIH ADMINISTRATIVE DATA:
PCC: M31 / OC: 41.4B /Processed: JUNSWORTH 030601 0331

SECTION II - PAYMENT/HOTLINE INFORMATION - 2 R44 AI048345-02 (Revised)

For Payment and HHS Office of Inspector General Hotline Information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III - TERMS AND CONDITIONS - 2 R44 AI048345-02 (Revised)

This award is based on the application submitted to, and as approved by, the NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

- The grant program legislation and program regulation cited in this Notice of Grant Award.
- The restrictions on the expenditure of federal funds in appropriations acts, to the extent those restrictions are pertinent to the award.
- 45 CFR Part 74 or 45 CFR Part 92 as applicable.
- The NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
- This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(see NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

By accepting an award recipients agree to these conditions. Review this information carefully.

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This applies to funds carried over from one year to the next year of an award. An extension must be requested to carry over funds beyond the last year of an award.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

For instructions, see http://grants1.nih.gov/grants/funding/2590/phs2590.pdf

Treatment of Program Income:
Additional Costs

This REVISED Notice of Grant Award is issued to reflect downward adjusts in year -02 and future year due to salary in excess of the cap of $171,900. This supersedes Notice of Grant Award issued on 01/31/2003. None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of Executive Level I of the Federal Executive Pay Scale. The application for this project proposed a salary at a rate greater than Executive Level I per year for Drs. Youngman, Zamudio and Foulkes.

Pending the establishment of a negotiated facilities and administrative (F&A) cost rate, this award provides an allowance of F&A costs of 10% salaries and wages. Therefore, $12,349 facilities and administrative costs are available, and $143,244 of year 02 funds are restricted and may not be drawn down until a rate is negotiated and a revised Notice of Grant Award is issued to release those funds. Please contact Ms. Ruth Bishop, OFM/NIH/DHHS at (301) 496-2444 for assistance.

The grantee was awarded a temporary 10% F&A rate until an actual rate is negotiated. The grantee will only receive the negotiated rate which may be less (but not more) than that proposed in the application.

To meet institute program objectives the costs in the future budget period is escalated by 3%.

Intellectual property rights: Normally, the awardee organization retains the principal worldwide patent rights to any invention developed with United States Government support. Under Title 37 Code of Federal Regulations Part 401, the Government receives a royalty-free license for its use, reserves the right to require the patent holder to license others in certain circumstances, and requires that anyone exclusively licensed to sell the invention in the United States must normally manufacture it substantially in the United States.

Any product resulting from this award should be manufactured in the United States.

Rights and obligations related to inventions created or reduced to practice as a result of this award are detailed in 35 U.S.C. 205 and 37 CFR Part 401. These inventions must be reported to the Extramural Invention Reporting and Technology Resources Branch, OPERA, NIH, 6701 Rockledge Drive, MSC 7750, Bethesda, MD 20892-7750, (301) 435-1986. For additional information, access the NIH link on the Interagency Edison web site (www.iedison.gov)which includes an electronic invention reporting system, reference information and the text to 37 CFR 401.

The grantee is required to report inventions.

To the extent authorized by 35 U.S.C., Section 205, the Government will not make public any information disclosing an NIH-supported invention for a 4-year period to allow the awardee organization a reasonable time to file a patent application, nor will the Government release any information that is part of that patent application

When purchasing equipment or products under this SBIR award, the grantee shall use only American-made items, whenever possible.

If equipment is purchased, it should be American made.

Allowable costs conducted by for-profit organizations will be determined by applying the cost principles of Contracts with Commercial Organizations set forth in 48 CFR, Subpart 31.2.

Rory A. Duncan, Program Official
Phone: 301-402-8613
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SPREADSHEET
GRANT NUMBER: 2 R44 AI048345-02 (Revised)

P.I.: TRAWICK, JOHN D
INSTITUTION: ELITRA PHARMACEUTICALS, INC.

	YEAR 02	YEAR 03
Salaries and Wages	123,486	126,803
Fringe Benefits	24,698	25,361
Personnel Costs	148,184	152,164
Consultant Services	8,750	9,013
Supplies	85,250	87,808
Travel Costs	12,500	12,875
TOTAL FEDERAL DC	254,684	261,860
TOTAL FEDERAL F&A	155,593	159,772
TOTAL COST	410,277	421,632

	YEAR 02	YEAR 03
F&A Cost Rate 1	105%	105%
F&A Cost Base 1	148,184	152,164
F&A Costs 1	155,593	159,772