Step I: White Paper Application

Application Guidelines

1. *The application should be submitted electronically per NIAID's requirements (TBD). Include all attachments, if any, to the application.*
2. *GSC personnel at any of the three Centers can assist / guide you in preparing the white paper.*
3. *Investigators can expect to receive a response within 4-6 weeks of the review cycle.*
4. *Financial agreements would require appropriate justification and approval; payment arrangements need to be established in advance with the GSC.*
5. *Upon approval of the white paper concept, the NIAID Project Officer will assign the project to an NIAID GSC to develop a Management Plan in conjunction with the participating scientists.*

Please Note: This is a draft template. Users may have to adapt contents to a final template NIAID develops in the coming weeks and adhere to a process that will be established for submission and approval of projects; it shall be communicated once finalized.

White Paper Application

Project Title: *Cryptococcus gattii* Genomics and Transcriptomics

White Paper Submission Date (08/07/10):

Principal Investigator Contact:

Name	Vishnu Chaturvedi
Position	Director, Mycology Laboratory
Institution	Wadsworth Center, New York State Department of Health
Address	120 New Scotland Ave.
	Albany, NY 12208
Telephone	518-474 4177
Fax	518-486 7971
E-Mail	vishnu@wadsworth.org

Co-Principal Investigator Contact:

Name	K.J. Kwon-Chung
Position	Chief, Molecular Microbiology Section
Institution	Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases
Address	Bldg. 10, Room 11N234, NIH
	Bethesda, MD 20892
Telephone	301-496-1602
Fax	301-480-3240
E-Mail	June_Kwon-Chung@nih.gov

Co-Principal Investigator Contact:

Name	John Perfect
Position	Interim Chief, Division of Infectious Diseases
Institution	Department of Medicine, School of Medicine, Duke University
Address	DUMC 3353
	Durham, NC 27710
Telephone	919-684-8902
Fax	919-684-8902
E-Mail	perfe001@mc.duke.edu

Co-Principal Investigator Contact:

Name	Brian Wong
Position	Director, Division of Infectious Diseases
Institution	Oregon Health & Science University
Address	3181 SW Sam Jackson Park Road L457
	Portland OR 97239-3098
Telephone	503-494-7735
Fax	503-494-4264
E-Mail	wongbri@ohsu.edu

All white papers will be evaluated based on the following sections.

1. Executive Summary *(Please limit to 500 words.)*

Provide an executive summary of the proposal.

Cryptococcus gattii is an emerging global pathogen that was first recognized in 1970 from Central Africa. Earlier, *C. gattii* CNS, lung and skin infections were believed to be limited to healthy individuals in tropics and subtropics. Early investigations suggested that these infections were not seen in HIV-AIDS patients. These perspectives might have led to less attention being paid to *C. gattii* vis-à-vis *C. neoformans*, which has received maximum attention as an opportunistic pathogen in HIV-AIDS patients. However, many recent reports suggest that *C. gattii* cryptococcosis is more common in immunocompetent as well as HIV-AIDS patients globally; this is matched by isolations of fungus from more than 50 tree species in temperate and tropical areas. More recently, *C. gattii* infections and deaths in the Pacific Northwest and Vancouver, Canada have raised public health awareness in North America.

Cryptococcus gattii cryptococcosis has more pulmonary and cerebral nodules (cryptococcomas), increased neurological morbidity, and slower response to treatment vis-à-vis *C. neoformans* disease. Limited studies have indicated that outstanding questions regarding *C. gattii* cryptococcosis cannot be resolved by extrapolation from *C. neoformans* pathogenic factors. We do not know the reasons and significance of the disparity in the global distribution of *C. gattii* serotype B and C strains. The majority of isolates from Canada are serotype B, *MAT*α, VGIIa genotype and the emergence of a 'hypervirulent clone' was implied to be the reason for the outbreak. However, this is not entirely tenable since a similar strain *C. gattii* NIH444 (serotype B, VGIIa, *MAT*α) was isolated from sputum of a patient in 1970 from Seattle, WA. Beside, genotypes VGI, VGIII and VGIV and serotype C have been reported from patients from other areas. Thus, we have few clues as to what causes emergence or reemergence of highly pathogenic strains, why *C. gattii* split up from its pigeon guano dwelling sibling *C. neoformans*, and why it chose to climb into trees instead, and why immunocompetent individuals are vulnerable to this pathogen and what can be done to prevent the infection?

The information on the genome of *C. gattii* is inadequate and unrepresentative. Currently, draft genome sequences are available for two strains, R265 and WM276, which are *MAT*α, serotype B, genotype VGII/VGI from Canada and Australia, respectively. There is a gap in knowledge about the genomes of VGIII and VGIV strains, serotype C strains, and *MAT***a** strains. The geographical representation is inadequate in the absence of strains from California, South America, Asia and Africa. Additional obstacles are: a) complex molecular typing schemes, and b) lack of functional analyses (transcriptomics) that can foretell *C. gattii* response under pathogenic and non-pathogenic conditions.

We propose complete genome sequencing of 12 reference strains by next generation sequencing technology. Transcriptomes of 12 *C. gattii* strains will also be mapped by RNA-Seq technology. The selection of strains, sampling conditions, analytical strategies and data release plan were designed with input from the 3-Co-PIs, and additional researchers representing global *C. gattii* community. The project would provide: 1) insight into *C. gattii* genomes to anchor future research studies, 2) validation of single nucleotide

polymorphisms (SNPs) for molecular typing to improve epidemiology studies, 3) transcript analyses to fast forward the discovery of proteins for diagnostics, drug targets and vaccines. Overall, the project will fill-in important gaps in our knowledge about an important emerging pathogen, accelerate research efforts and translate laboratory findings into tangible public health benefits.

2. Justification

Provide a succinct justification for the sequencing or genotyping study by describing the significance of the problem and providing other relevant background information.

Public health significance.

C. gattii cryptococcosis is an emerging global problem: *C. gattii* is an emerging pathogen that has triggered serious public health concerns due to (i) its appearance in previously unknown geographic areas, (ii) its outbreaks among healthy humans, pets, and wildlife, (iii) the intractable nature of cryptococcal disease, and (iv) problem with routine diagnosis in clinical laboratories. (1, 2, 9, 16, 25, 28, 44, 45, 51, 54, 56). Initial studies suggested that HIV-AIDS patients rarely get infected with *C. gattii*, but more recent publications indicate a higher prevalence of *C. gattii* infections in both immunocompetent hosts and HIV-AIDS patients (9, 12, 23, 29, 40, 42, 67).

Global burden of *C. gattii* disease remains underestimated: There is an obvious disconnect between natural isolations of *C. gattii* and its finding in clinical materials in some areas while the converse is true in other areas (65). Reliable estimates for rates of cryptococcosis due to *C. gattii* are currently lacking, but one estimate suggests that one-third to one-tenth of cryptococcosis cases worldwide are caused by *C. gattii*(20, 27, 49, 55, 63).

C. gattii natural habitat(s) poses occupational and recreational risks: *C. gattii* natural habitat is believed to be on a variety of trees especially in tree-hollows (19, 38, 65). *C. gattii* poses travel-related risks to humans and animals traveling to the areas considered to be 'hot spots' for the natural occurrence of the fungus.(6, 7, 25, 39, 47). Environmental exposure due to occupational or recreational activities increases the risks for acquisition of infections especially among males who are over-represented among affected populations (18, 30, 31).

C. gattii cryptococcosis has many complications and is difficult to treat: *C. gattii* cryptococcosis has more complicated course than *C. neoformans* cryptococcosis. The complications include more pulmonary and cerebral nodules (cryptococcomas), persistent mental status abnormalities, increased neurological morbidity, surgical interventions and slower response to treatment vis-à-vis *C. neoformans* disease (15, 17, 24, 57-59, 62, 64).

C. gattii strains have higher level of heteroresistance to fluconazole: *C. gattii* strains exhibit relatively higher levels of heteroresistance to fluconazole (LHF) as compared to *C. neoformans* strains (61, 70). This phenomenon refers to variable intrinsic resistance exhibited by *C. gattii* strains to fluconazle, which is the most common drug used for the treatment of cryptococcosis. LHF could be an important determinant of outcome in *C. gattii* cryptococcosis. The mechanism of action of LHF remains undetermined since prior exposure to the drug is not a pre-requisite for the drug resistance.

Many diagnostic laboratories are unprepared to report *C. gattii* infections: The serotype variations in *C. neoformans* species complex and the existence of serotypes B and C, which would define future *C. gattii*, were known as early as 1950 (21). However, the wide-spread use of serotyping system became possible in diagnostic laboratories only after the reagents became commercially available (26). Alas, this reagent is no longer available and no serotyping is being carried out anymore. Similarly, selective isolation medium (nigerseed agar) and differentiation medium for *C. neoformans* and *C. gattii* are well described, but these require specialized reagents and therefore, they are also not readily available in most diagnostic laboratories (37, 66). Thus, there is a pressing need to find a facile method for routine identification of *C. gattii* in diagnostic laboratories.

Great heterogeneity in *C. gattii* isolates points to possible sub-species: The currently recognized serotypes (B, C) and genotypes (VGI-VGIV) do not fully represent the great heterogeneity in *C. gattii* strains isolated from clinical and environmental sources (52). The recent emergence of this infection in Pacific Northwest and Vancouver, Canada has revealed unique pathogenic attributes of sub-genotypes within VGII strains (5, 32). The information is also starting to emerge about similar association between other sub-genotypes and distinct virulence properties.

Host immune responses critical for *C. gattii* infection and recovery are not understood: A few surveys in high incidence areas have revealed many seropositive individuals, which suggest that the burden of exposure is much larger than can be estimated from the people with apparent disease (60). Thus, not all exposed individuals develop *C. gattii* infections and there are host factors like HLA types that are critical for the control and elimination of this pathogen (68). Conversely, there are yet undefined predisposition events that cause apparently healthy individuals to suffer from *C. gattii* infections (51). Preliminary data indicates a role for protective inflammation in controlling this disease (4, 11).

Apparently unique pathogenic attributes of *C. gattii* remain poorly defined: Both *C. gattii* and *C. neoformans* share obvious virulence characteristics such as capsule, thermal tolerance and laccase. However, *C. gattii* is suspected to prevent immune cells in the lungs from mounting effective defense, but we do not know about mechanisms behind such an exquisite strategy. Similarly, a number of proteins relevant to the virulence in *C. neoformans* are employed by *C. gattii* in apparently distinct manner and more in depth investigations in this area are needed.

Genomics tools are needed to find better targets for diagnostics, drugs and vaccine: A custom-designed *C. gattii* whole-genome tiling microarray was recently used to discover an important role for mitochondrial parasitism in fungal virulence (46). However, the poor annotation of existing *C. gattii* genomes prevented these investigators from full identification of other significant virulence genes in *C. gattii* R265. Due to better genome annotations, the transcriptomic analyses are far more advanced in *C. neoformans* and have revealed important insights into oxygen and CO2 sensing mechanisms, capsule synthesis, and cyclic AMP signaling (8, 13, 14, 33, 48). Clearly, whole genome profiling tools are needed for *C. gattii* for advancing our understanding of this enigmatic pathogen and for translating such knowledge for public health benefit.

3a. Rationale for Strain Selection

The strains proposed to be included in genome sequencing and/or transcriptomics are summarized in Table 1; accompanying details provide rationale for their selection.

Table 1. List of *C. gattii* strains to be sequenced.

No.	Strain	Source/Origin	Characteristics	Selection Criteria	Ref.
1	RV20186	CSF / Democratic Republic of Congo	VGI/ Serotype B	Type strain of *C. gattii*	(3, 69)
2	WM179	Human/Australia	VGI/ Serotype B/ *MAT*α	Pathogenic in a rat model of *C. gattii* cryptococcosis	(35, 50)
3	LA295	Human/ Argentina	VGIIa/ Serotype B/ *MAT*α	More pathogenic than R265- the major genotype in Vancouver outbreak	(53)
4	R272	Human/ Canada	VGIIb / Serotype B/ *MAT*α	'Minor'genotype in Vancouver outbreak; low pathogenic potential	(22)
5	EJB18	Human /Oregon	VGIIc /Serotype B/ *MAT*α	Novel genotype from Oregon outbreak; highly pathogenic in mice	(5)
6	WM198 (McBride)	Feline/Australia	VGIIb/Serotype B/ *MAT*α	Veterinary strain, reactivation disease, low virulence in mice, high virulence in rat	(34, 35)
7	NIH444	CSF /Washington	VGIIa /Serotype B/ *MAT*α	*MAT* $\boldsymbol{\alpha}$ Type strain of *F. bacillispora*; Host strain for pathogenesis studies	(10, 36)
8	VMGc3	Cactus plant/ Puerto Rico	VGII / Serotype B/ *MAT*$\boldsymbol{a}$	Environmental *MAT*$\boldsymbol{a}$ strain	(43)
9	NIH191	CSF/ California	VGIII (AFLP 5C)/ Serotype C/ *MAT*$\boldsymbol{a}$	*MAT* $\boldsymbol{a}$ Type strain of *Filobasidiella bacillispora* (Sexual form)	(36)
10	USC1499	CSF/ California	Genotype VGIII /Serotype B/ *MAT*α	Highly pathogenic strain from AIDS patient	(9)
11	Bt12	CSF/Botswana	VGIV/ Serotype C/ *MAT*α	Genetic identity to Serotype C strains from Botswana and Malawi	(41)
12	IND107-97	*Eucalyptus*/India	VGIV / Serotype B	Environmental strain /low fluconazole heteroresistance	(70)

Rationale for selection

***C. gattii* RV20186**

This is the type strain of *C. gattii* originally isolated from a seven-year old boy from the Democratic Republic of Congo who was successfully treated for cryptococcal meningitis. The CSF culture yielded two colony types – R and B, which were also reproduced when fungal cells were injected in mice. Although the case was diagnosed in 1966, this region is believed to have many similar infections in young men dating back to early 1950s and 60s. Thus, this strain could provide an invaluable window to the earliest known infections and a reference for tracking evolutional history and population biology of *C. gattii*.

***C. gattii* WM179**
This is a human strain from Australia, which represents relatively rare VGI genotype. It has been found to be pathogenic in a rat model of *C. gattii*. This will be a crucial for comparative pathogenic studies.

***C. gattii* LA295**
This human strain originated in Argentina. It belongs to VGIIa, the major hypervirulent genotype implicated in recent outbreak of *C. gattii* disease in Vancouver, British Columbia, Canada. Importantly, LA295 is more pathogenic than R265 – the type strain analyzed from the Canadian outbreak.

***C. gattii* R272**
This human strain originated from Canada. It is genotype VGIIb, the minor genotype in the Canadian outbreak and reportedly with low pathogenic potential.

***C. gattii* EJB18**
This human strain represents a novel genotype – VGIIc, which is predominant is Oregon outbreak. It is also highly pathogenic in mice models.

***C. gattii* WM198 (McBride)**
This veterinary strain is VGIIb genotype. It has low virulence in mice, but high virulence in rat model. Moreover, it is the only strain that has proven to be able to be reactivated in animal after full treatment more than 10 years.

***C. gattii* NIH444**
This VGIIa strain isolated from sputum of a patient from Seattle in 1970 is very similar to R265, the outbreak strain from Canada. It has been shown to be an optimal host strain for many experimental pathogenesis studies.

***C. gattii* VMGc3**
This is a rare VGII, *MAT**a*** environmental strain from Puerto Rico. This is one of the two *MAT**a*** strains included in this study to compare with remaining *MAT*α strains.

***C. gattii* NIH191**
This is the type strain of the sexual state of *C. gattii* termed *Filobasidiella bacillispora*. This is a clinical strain with three unusual characteristics- serotype C, *MAT**a*** and VGIII genotype. It presents unique opportunities for genomic and phenotypic comparisons.

***C. gattii* USC1499**
This is a clinical strain from HIV-AIDS patient from California. It has a VGIII genotype and high virulence for mice similar to a hypervirulent VGIIa strain involved in recent outbreak from Vancouver Island, British Columbia. It has been characterized extensively for its phenotypes and genotypes. It could provide better understanding of the pathogenesis of yet to be investigated VGIII strains. The choice is also relevant from the perspective of the earliest known geographical distribution of *C. gattii* in Southern California.

***C. gattii* Bt12**
This strain has been isolated from a patient in Southern Africa. The two rare characteristics are serotype C and VGIV genotype. It has been used in genetic analyses that revealed a lack of genetic diversity in clinical strains from Botswana and Malawi; these two countries have high incidence of HIV-AIDS.

***C. gattii* IND107-97**
This is an environmental strain, rare VGIV genotype and geographically diverse (India). Another interesting feature is low level of fluconazole heteroresistance.

Environmental, Human and Veterinary Strains
VMGc3, IND107-97, WM179 LA295, R272, EJB18, WM198, NIH444 provide widest possible diversity in clinical and environmental sources of *C. gattii* strains. These strains have been the subject of preliminary studies and this set would be optimal in transcriptomics to unravel changes in global expression in strains encountered in nature and in disease.

Strains for pathogenesis studies
NIH444, USC1499, WM179, WM198 are strains that would be very useful for molecular pathogenesis studies as they have stable differences in virulence, measurable putative virulence factors that could be manipulated by gene knockouts and good animal models. Transcriptomics on these strains would facilitate a correlation of virulence with genome-wide expression of known and yet to be recognized virulence traits.

Strains for fluconazole heteroresistance analysis
C. gattii exhibits high level of fluconazole heteroresistance (LHF) with direct correlation with high virulence. By implication, fluconazole heteroresistance could be an important factor behind the poor therapeutic responses seen in patients treated with standard regimen of antifungal drugs. Two high–low heteroresistance strains (NIH444 and IND107-97) would provide an ideal set for transcriptomic analysis to understand the mechanisms behind the observed resistance.

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3c. Nature, Availability & Source of Reagents/Samples:

Genomic DNAs from reference strains for high quality sequencing.
The Mycology Laboratory at the Wadsworth Center, Albany, NY has extensive experience with genes and genomics of *C. gattii* including sequencing of large contigs (*Biochemical and Biophysical Research Communications*. 2004: 326, 233–241). Many of the reference strains to be sequenced are already in this laboratory and stored under liquid nitrogen. Remaining strains are being procured from collaborating institutions. High quality genomic DNA will be made in Albany and quality checked before sequencing at the J. Craig Venter Institute.

***C. gattii* nucleic acids and other reagents source**
The interactive group of principal investigators represents US, Europe, Australia, Africa and South America. The interests and strengths of these laboratories are in clinical mycology, ecology, molecular pathogenesis, drug resistance and genomics. The

investigators are:
Vishnu Chaturvedi, New York State Department of Health, Albany, NY
K.J. Kwon-Chung, National Institute of Allergy and Infectious Diseases, Bethesda, MD
Brian Wong, Oregon Health Sciences Center, Portland, OR
John Perfect, Duke University Medical Center, Durham, NC
Joe Heitman, Duke University Medical Center, Durham, NC
Teun Boekhout, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands
Thomas Harrison, St. George's University of London, UK
Wieland Meyer, University of Sydney, Sydney Australia
Tania Sorell, University of Sydney, Australia
M.H. Veinstein, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
H.F. Vismer, PROMEC Unit, Medical Research Council, Tygerberg, South Africa

***C. gattii* RNAs will be provided by the following collaborators on a schedule to be developed.**
Vishnu Chaturvedi, New York State Department of Health, Albany, NY
K.J. Kwon-Chung, National Institute of Allergy and Infectious Diseases, Bethesda, MD
Brian Wong, Oregon Health Sciences Center, Portland, OR
John Perfect, Duke University Medical Center, Durham, NC
Joe Heitman, Duke University Medical Center, Durham, NC
Wieland Meyer, University of Sydney, Sydney Australia
M.H. Veinstein, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

3d. Proposed Methods and Protocols to Prepare Reagents:

Genomic DNAs from reference strains.
Standard methods for high quality genomic DNA preparations of *C. gattii* are available and used routinely in the laboratories of the participating PIs.

***C. gattii* RNAs**
Total RNA preparations and enrichment for mRNA will be carried out with Qiagen RNA extraction kits or similar commercial products. All samples will be quality checked for Illumina RNA-seq application. The source of RNA samples will be different *in vitro, in vivo, ex vivo* and *ex planta* conditions used in participating laboratories to study various facets of *C. gattii* in its natural habitat and in human and animal infections.

4. Approach to Data Production:

Reference strain genome sequencing.
The proposed sequencing and annotation of 12 *C. gattii* strains would fill-in gaps in knowledge from the ongoing efforts on genome sequencing of two strains. The end product would ensure coverage of all *C. gattii* serotypes, genotypes, mating types, and clinical and environmental samples. High resolution genome coverage will be provided by using multiple runs of short-read next generation sequencing technology. The availability of reference *C. gattii* genomes would facilitate fast and accurate assembly and annotation of the new genomes.

RNA-seq
Seven collaborating laboratories will provide total RNA from 8 *C. gattii* strains selected for functions studies. The conditions of fungal growth will mimic natural habitat, growth under stress and during various stages of infection in the animal models. The respective laboratories will use whole genome sequences to analyze expression profiles generated under various conditions. Eventually,

5. Community Support and Collaborator Roles:

***Cryptococcus gattii* community**. The *C. gattii* community is currently small and also includes researchers who study cryptococcosis caused by *C. neoformans*. However, clinical and research interest in *C. gattii* is growing rapidly as exemplified by the coverage of relevant topics in peer-reviewed and popular press. A specialized international meeting (*Cryptococcus* and cryptococcosis) is organized every four years to bring together interested parties. This meeting attracts from 200 -600 participants hailing from all parts of the world including researchers who work exclusively with *C. gattii*. Nevertheless, it is accepted that much more needs to be done to expand the resources and the researchers currently working on *C. gattii* cryptococcosis vis-à-vis *C. neoformans* cryptococcosis. The White Paper draft process has allowed a number of investigators to work together to develop consensus on the existing gaps and future needs in this area.

6. Compliance Requirements:
6a. Review NIAID's Reagent, Data & Software Release Policy:
http://www3.niaid.nih.gov/research/resources/mscs/data.htm
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-013.html

Accept X Decline ☐

6b. Public Access to Data and Materials:

Strains.
Most of the *C. gattii* strains used in this project are available from a number of recognized culture collections. Similarly, the remaining few strains will be deposited at the NIAID sponsored BEI at ATCC.

Annotated reference genomes.
C. gattii genomes sequenced in this project, their annotations including the RNA-seq and sRNA-seq data will be deposited to GenBank at NCBI.

Expression profiling data.
All information pertaining to mRNA and small RNA expression profiling including descriptions of the biological samples, processed data files, and transcription levels for each gene will be uploaded to the NCBI's GEO database.

Sequence reads.
All original short read sequence reads will be deposited to the NCBI's Short Read Archive sequence database.

6c. Internal Review Board (IRB) / IACUE Yes ☐ No X

NIAID approval of IRB documentation is required prior to commencement of work.

No human subjects review is required as all C. gattii strains were obtained from strain collections and are devoid of any patient identifiers.

Investigator Signature: [electronically signed]

Vishnu Chaturvedi

Investigator Name: Vishnu Chaturvedi **Date:** August 7, 2010

Step II: Development of the Management Plan

(This section is to be completed upon approval of the white paper and should be done with the assigned GSC project leader in conjunction with the participating/collaborating scientists.

Rules of Engagement on Project Approval

1) *All sample acquisition will require a MTA and IBC approval.*
2) *Samples derived from human subjects and / or animals require IRB or IACUC approvals respectively prior to initiating work.*
3) *Samples acquired from outside the United States will have to adhere to import/export regulations.*
4) *Participants are required to comply with the NIAID data & reagent sharing and dissemination policy. <provide url>*
5) *Abstracts, Posters and Manuscripts in preparation should be submitted to NIAID for review prior to submission. Publications should acknowledge and cite the NIAID contract: "This project has been funded in whole or part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under contract number HHSN272200900007C."*

Please Note: This is a draft template. Users may have to adapt contents to a final template NIAID develops in the coming weeks and adhere to a process that will be established for submission and approval of projects; it shall be communicated once finalized.

Management Plan

Project Title:

Date (*MM/DD/YY*):

GSC Project Leader

Name	
Position	
Institution	
Address	
State	
ZIP Code	
Telephone	
Fax	
E-Mail	

Project Approach:

Outline the experimental design / strategy. This needs to be within the scope as well as service offerings / capabilities available at the GSC.

Deliverables:

Describe the deliverables to be generated from the project, including reagents, data and / or software. Researchers are required to be aware of the Data release/sharing and IP policy of the Center.

Project Timeline:

Estimate the duration of the project and list the milestones and deliverables with due dates.

Research Compliance Documentation Requirements:
(If required please attach completed/ approval documentation)

1. Material Transfer Agreement **Yes ☐ No ☐**
Please attach the completed UBMTA or the participating institutions MTA.
http://www.autm.net/Content/NavigationMenu/TechTransfer/TechnologyTransferResources/UBMTA.htm

2. **Internal Review Board (IRB) / IACUE** **Yes ☐ No ☐**
Please attach the IRB/IACUE application submitted at the institution where the samples were collected and prepared, the protocol description, consent form along with the approval letter from the participating institution(s).

3. Institutional Biosafety Committee **Yes ☐ No ☐**
IBC approval is a requirement to commence the project. <Provide contact information for Institutional IBC/ Environmental health and Safety officer's contact information >

4. Other permit requirements based on the project / samples under study
To be procured as relevant by the GSC. <Provide contact person>

a. CDC permit **Yes ☐ No ☐**
b. USDA permit **Yes ☐ No ☐**
c. Import/Export Authorization **Yes ☐ No ☐**

Detailed Budget for Proposed Project in US Dollars

(Include all costs to be incurred by the Center including sample preparation and shipping as well as acquisition of permits, if applicable. Note: The project may incur additional expenses at other participating institutions which are not included here.)

Budget Item	Cost
Total Estimated Costs	**US $**

NIAID Project Officer Approval:

Maria Y. Giovanni, Ph.D.
Assistant Director for Microbial Genomics & Advanced Technology
Division of Microbiology and Infectious Diseases
NIAID/NIH/DHHS
6610 Rockledge Dr. MSC 6603; Room 6007
Bethesda, MD 20892-6603
301-496-1884 Phone
301-480-4528 FAX
mgiovanni@niaid.nih.gov

NIAID Contract Officer Approval:

Robert Singman
MID Research Contracts Branch A
Office of Acquisitions, DEA, NIAID, NIH, DHHS
6700B Rockledge Drive
Room 3153, MSC 7612
Bethesda, MD 20892-7612 (FedEx use 20817)
301-451-2607 Phone
301-480-4675 FAX

Participating Institution Business/Administrative Contact:

(Required if there are financial transactions between the participating institutions)

Name	
Position	
Institution	
Address	
State	
ZIP Code	
Telephone	
Fax	
E-Mail	

Participating Institution Business/Administrative Approval**:**

GSC Principal Investigator's Acceptance and Approval:

Name	
Position	
Institution	
Address	
State	
ZIP Code	
Telephone	
Fax	
E-Mail	

Signature:

GSC Research Administrative Acceptance and Approval

Name	
Position	
Institution	
Address	
State	
ZIP Code	
Telephone	
Fax	
E-Mail	

Signature:

GSC Project Manager's Contact:

Name	
Position	
Institution	
Address	
State	
ZIP Code	
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