Biosafety
Office of Research
UNIVERSITY OF GEORGIA

Tips for Successful Completion of Your IBC Protocol Form

The purpose of this document is to help you navigate the questions in this protocol that require more detail. Tips are not given for every question. If you have any questions while completing this form, please call 706.542.2697.

On Page 4: In **Key Personnel**, provide all personnel working on the project along with a description of their specific duties and degrees, number of years of training/experience, and a description of the experience associated with their duties. Be sure to include yourself in Key Personnel.

For example:

Add Row | Delete Row

Name and Degree	Specific Duties on Project	Number of Years Training and Description of Experience
[redacted], PhD	Oversight, molecular virology, virus rescue, phenotype assessment in vitro and in vivo, animal infection, sample collection	>20 yr microbiology & virology experience incl. culture, cloning, & animal infection studies. >10 yr BSL3 exp.
[redacted], BS	Molecular virology, virus rescue, phenotype assessment in vitro & in vivo, animal infection, sample collection, supervision, trainining	>20yr microbiology, >10yr virology exp incl. culture, cloning, & animal infection studies. >10 yr BSL3 exp.
[redacted], MS	Molecular virology, virus rescue, phenotype assessment in vitro & in vivo, animal infection, sample collection, supervision, trainining	>20 yr microbiology & virology experience incl. culture, cloning, & animal infection studies. >10 yr BSL3 exp.

Note:

1. A Proficiency for Standard and Special Microbiological Practices form must be filled-out for each person working in the laboratory, **whether they are listed on this protocol or not**. This is a one-time request for each person. This form should only be filled out and submitted once lab personnel have demonstrated proficiency in daily work practices in the laboratory. This form will need to be submitted for any personnel who join the lab later.

2. For PIs performing recombinant DNA work, you must complete the NIH Guidelines training before you can receive your IBC approval. The NIH requires PIs that conduct research involving rDNA to receive training on the NIH Guidelines. UGA NIH guidelines training can now be found on the UGA PEP. Login and go to **Browse Training**. In the search box, **search** for **'UGA NIH Guidelines for Recombinant or Synthetic Nucleic Acid Molecules"** and **select the title**. Next, select **Launch** and the PowerPoint will download in a new window. Read through the slides – there is no test. Once complete, close the PowerPoint, go to **Transcripts** and mark **Completed**. Print out the certificate for your records. This training only needs to be completed once, regardless of the number of active protocols involving rDNA you may have. **Please note this training is a component of the approval process and failure to respond to this requirement will delay the approval process.**

Page 4: In the **Technical Synopsis**, provide some detail of what the project entails. Provide enough information so the committee has a good representation of the work you are performing. Provide a detailed description of specific experiments that will be conducted, focusing on how infectious agents and toxins, rDNA materials, and human and/or non-human primate materials will be used/handled. Include the following information, as it applies:

- In vitro procedures used
- In vivo procedures used
- Maximum volumes and titers of cultures to be grown at any given time
- Method(s) for transportation of cultures/samples
- Identify any procedure that may create a splash or aerosol hazard
- If you are working with animals, provide an AUP number.

For example:

Apicomplexans are protozoan pathogens that cause morbidity, mortality and substantial economic loss. Notably, they contribute to more than half a million annual deaths due to malaria, and cause severe, debilitating infant diarrhea. Acute infection with the apicomplexan Toxoplasma gondii can cause fetal abnormalities, and severe complications or death in immunocompromised patients. Due to its genetic amenability, the Huet lab uses T. gondii as a model to study the unique and divergent aspects of apicomplexan biology.

Apicomplexans are able to survive in a wide variety of environments as they enter and exit host cells, and as they transfer between hosts. Their ability to propagate within a wide range of cell types demonstrates their metabolic plasticity and ability to access nutrients from diverse and changing environments. In apicomplexans, important metabolic pathways take place within two organelles: the mitochondrion and the apicoplast.

In the mitochondrion, an enzyme called the ATP synthase couples the proton-motive force, generated by respiration, to ATP synthesis. This crucial process is intricately regulated in eukaryotes. As such, expression and regulation of its subunits respond to the cellular demands that can drastically change depending on the environment. There is also increasing evidence that mitochondrial metabolites are able to regulate ATP synthase activity. In apicomplexans, phylum-specific subunits may regulate this enzyme. Metabolites of mitochondrial or apicoplast origin could also contribute to enzyme regulation, which is suggested by the metabolic interdependence of these two organelles. However, the metabolic cross-talk between the two organelles remains poorly understood. Studies have demonstrated that both organelles are physically associated, but the molecular basis of this interaction remains unknown. Our lab will focus on understanding how the ATP synthase is regulated during the parasite life cycle and how mitochondrion and apicoplast communicate. The highly divergent nature of the apicomplexan ATP synthase and organelles will provide evolutionary insights into this group of pathogens and opportunities to develop urgently needed anti-parasitic therapies.

To study T. gondii, our lab requires the propagation of the parasite in flasks containing 2.5 mL with approximately 1e7 parasites total, although larger volumes of parasites may be needed periodically. After isolation, the parasites can then be processed for routine molecular biology and biochemical techniques such as immunoprecipitations, western blotting and immunofluorescence microscopy. Additional techniques such as gene cloning and recombinant protein expression will also be performed routinely.
Regarding the recombinant work that will be performed, foreign DNA sequences will be introduced into the parasite using electroporation. As mentioned in part B of this form, DNA sequences to be inserted will be derived from the parasite genome and include genes identified as phylum-specific subunits of the ATP synthase.

Parasite/host cell line cultures maintenance and harvesting will be performed in our Biosafety Cabinets. No use of live parasites will be performed on the open bench. The use of a centrifuge is often required and we have aerosol resistant o-ring containing caps for our centrifuges to prevent parasite release.

Page 5, Part A: In compliance with the NIH Guidelines, include transgenic insects in Part A. Insects to include (but not limited to) fruit flies, mosquitoes, bees, ants, butterflies.

Page 5, Part B: #5:

Most of the recombinant work at UGA falls into categories III-D, III-E, and III-F of the NIH Guidelines.

Use this rubric to help determine the category your research falls. Go to the NIH Guidelines for more information. If you need assistance, please call the Office of Biosafety at 706.542.2697.

Experiments using Risk Groups 2, 3, 4, or Restricted Agents as Host-Vector Systems	**Section III-D-1**
Experiments in which DNA form Risk Groups 2, 3, 4, or Restricted Agents is cloned into nonpathogenic E. coli or lower eukaryotic host-vector systems	**Section III-D-2**
Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA presence of helper virus in tissue culture systems	**Section III-D-3**
Experiments involving whole animals (including transgenic animals)	**Section III-D-4**
Experiments involving whole plants (including transgenic plants)	**Section III-D-5 and/or III-E-2**

Experiments involving more than 10 liters of culture	**Section III-D-6**
Experiments involving influenza viruses	**Section III-D-7**
Experiments involving the formation of recombinant or synthetic nucleic acid molecules containing no more than two-thirds the genome of any eukaryotic virus	**Section III-E-1**
Experiments involving transgenic rodents (generation)	**Section III-E-3**
Exempt Experiments **See the NIH Guidelines for examples. **Protocol submission is required by the UGA IBC	**Section III-F**

Page 6, Part B: #7: Provide some detail on the specific genes being cloned.
For example:

7. **Please describe the nature of the inserted DNA sequences,** including regulatory or coding region, entire genome, synthetic antisense sequences, etc.

Synthetic sense and anti-sense sequences. Almost all CRISPER will be removing genes or making amino acid substitutions to the native sequence. Some genes that will be targeted are ugt-1, ugt-49, ugt-23. In some cases we will be adding GFP, Green fluorescent protein. GFP and DPY-10 are both positive controls that have been reported in the literature. GFP is green fluorescent protein and DPY-10 is a well characterized C. elegans gene, whose defect causes worms to be fat and short. GFP is a 27 kDA protein consisitng of 238 amino acids deived from the crystal jelly fish, Aequorea victoria. The GFP used here will be purchased from a commercial vendor. Our other target genes are primarily in the family of glycosyl transferases. Information about specific genes will be communicated to OBS as it becomes available.

Page 6, Part B: #8: If you are using *E.coli* or a similar organism for plasmid expression make sure to add that here. Other types of recipients include plants, animals, cell culture (including human or non-human primate cell lines), etc.

Page 6, Part B: #9: Be sure to list the function of each vector system. Be sure to send in vector maps with the submission.

For example: *pET30a vector will be used for E. coil (DH5-alpha) replication, and insertion deletion mutagenesis in S. pneumoniae. Vector provides KanR2 gene for kanamycin resistance and allows genetic disruption of Inserted gene fragments.*

Page 7, #12: Include infectious vectors.

Page 11, #19: Waste Equipment and Decontamination/Disposal Method – when appropriate, include disposal of transgenic plants, soil, seeds, and transgenic insects.

Please refer to the following Biosafety Guidelines for solid and liquid waste:
Decontamination by autoclaving:
BSL-1/BSL-2 solid waste: Exposure time at least 30 minutes at 121oC, 15psi.
BSL-1/BSL-2 liquid waste: Exposure time at least 30 minutes at 121oC, 15psi.
Note: Take in account the volume of the waste and the ability of steam to penetrate the load. It may require more than 30 minutes exposure time.

Chemical treatment:
BSL-1/BSL-2 liquid waste: Add a chemical disinfectant such as Clorox brand bleach (5.25% sodium hypochlorite) for a final concentration of 10% bleach. Allow at least 20 minutes contact time. Dispose of according to Office of Research Safety recommendations.

For example:

Type of Waste	Decontamination/Disposal Method
liquid waste (cell culture or allantoic fluids)	bring to 10% final volume with household bleach, Virkon, or other approved disinfectant. Treat for >/= 20 minutes. Dispose down sanitary sewer.
Solid waste (plastics, PPE, etc)	Autoclave, decontamination cycle 121C for >/= 30 minutes OR package for disposal by Stericycle
Surface decontamination	1% Virkon S, 10% Microchem, 10% household bleach; prepared weekly; 10' contact time. 70% EtOH; indefinite shelf life in sealed container; 10' contact.

Page 11, #20: LIST ONLY FOR PPE TO BE USED IN THE LAB. Be sure to specify the type of face and respiratory protection used, if applicable.

Page 11, #21: As these apply, include this information in this section:

a. If an exposure control plan and/or reporting plan is in place.
b. If a protocol is in place for the safe handling and disposal of sharps.
c. If a strict hand washing policy is in place.
d. If a sharps and glass reduction plan is in place to eliminate potential laboratory hazards.
e. Indicate how biohazard waste is transported to the autoclave room.
f. If lab personnel are enrolled in the Research Occupational Health Program.
g. If agent specific training has been provided.
h. If the Board of Regent's On-line Blood-borne Pathogens Training has been completed and Hepatitis B vaccination series offered.
i. If air-purifying respirators (ex. N95) are required for work, are lab personnel enrolled in the Research Respiratory Protection Program.