**CLEVELAND STATE UNIVERSITY IBC APPLICATION**

**INSTRUCTIONS:**

* Please download the newest version of the IBC application forms before preparing any new submission, resubmission, continuing renewal, or amendment. The form may have been updated since your last application. A version number can be found in the lower-left corner of each form.
* Sections 1,2,3, and 5 must be filled out completely in all applications.
* The nature of the proposed research using recombinant or synthetic nucleic acids dictates which information in Section 4 is required. The questions in Section 1F may help one determine which parts of Section 4 must be completed.
* Certain research using recombinant or synthetic nucleic acids is exempt from IBC review. Summaries of some common exemptions can be found on the [CSU IBC webpage](https://www.csuohio.edu/sprs/ibc).
* An [example of a completed IBC application](https://www.csuohio.edu/sites/default/files/IBC%20application%20examples.docx) is available for download from the CSU webpage. It may help demonstrate the level of detail required for each section.
* *If you are unsure whether you need to complete a full application, you may complete Section 1 and send that to the IBC chair as a presubmission inquiry.*

In many versions of Microsoft Word, the Enter/Return key will not make a line break in form fields like those in this document. Instead, use Shift+Enter (Windows) or Shift+Return (Mac).

**SECTION 1: General information.**

**1A. Project Title**
Examples of answers for IBC applications

**1B. Principle Investigator**
Thanks to Colleen Karlo & the CWRU IBC for the example experimental details, which were only slightly modified before their use in section 3D of this document

**1C. Application Date**September 9, 2023

**1D. Is this a new submission, resubmission, continuing renewal, or amendment?** *New submissions are applications that have not been previously submitted. Resubmissions involve applications that have been previously rejected or returned for revision. Continuing renewals update the project details to allow the project to continue beyond the prior approval period. Amendments update the project when there are changes in personnel or experimental details before a renewal is required*Continuing Renewal

**If a resubmission, continuing renewal, or amendment, briefly summarize any changes since the last submission.***Example: “This amendment includes changes to the study team members, proposed risk group, viral vectors being used, and list of recombinant materials.”*This continuing renewal includes changes to the study title, the study team members, and the list of recombinant materials.

**If a continuing renewal or amendment, please note date and approval number for last submission.**9/1/2020 IBC-2020-99

**1E. What is the expected duration of the research project or grant-supported funding period following this submission?** *IBC approvals are for three years. Projects longer than three years will require a renewal prior to the start of the fourth year.*5 years

**1F. Please answer the following questions regarding areas of the research project *that involve recombinant materials/transgenic organisms.***

**Does the study involve human subjects (e.g., gene transfer/therapy, vaccine study, etc.)?** *If yes, provide IRB approval number.*No Click or tap here to enter approval number.

**Does the study involve live vertebrate animals?** *If yes, provide IACUC approval number.*Yes #####-XXX-AS

**Does the study involve known pathogens?** *If yes, section 4A is required.*No

**Does the study involve creation of an organism that harbors and/or expresses a known pathogenic agent, toxin, prion, oncogene, etc.?** *If yes, section 4A is required.*No

**Does the study involve using a virus/viral vector?** *If yes, section 4B is required.*Yes

**Does the study involve cell culture?** *If yes, sections 4C and 4D are required.*Yes

**Does the study involve bacterial strains other than *E. coli* K-12 derivatives (*e.g.*, BL21)?** *If yes, sections 4C and 4D are required.*No

**Does the study involve CRISPR mediated gene editing with gRNA and nuclease encoded on the same plasmid, vector, or delivery vehicle?** *If yes, section 4E is required.*Yes

**Does the study involve working with compacted DNA/nanoparticles?** *If yes, section 4F is required.*No

**Does the study involve non-pathogenic invertebrates or fungi?** *If yes, please contact the IBC before completing your application.*No

**Does the study involve transgenic plants?** *If yes, please contact the IBC before completing your application.*No

**SECTION 2: Personnel and Funding**

**2A: Study team information (include PI):** *If personnel have changed since the last approval, please list all members of the current study team.*

|  |  |  |
| --- | --- | --- |
| **NAME** | **ROLE** | **EMERGENCY CONTACT(Name and Phone Number)** |
| Nell Arthur | PI | Chester A Arthur 216-687-5555 |
| John Tyler | Postdoc | Julia Gardiner Tyler 555-555-5555 |
| Caroline Fillmore | Graduate Student | Millard Fillmore 123-456-7890 |
| Click here to enter name. | Click here to enter role. | Click here to enter contact info. |
| Click here to enter name. | Click here to enter role. | Click here to enter contact info. |

*Please note any additional team members on a separate page and indicate the number of continuation pages used in the field below.* [*A formatted continuation page can be downloaded from the SPRS webpage.*](https://www.csuohio.edu/sites/default/files/IBC%20application%20s2a%20continuation%20page.docx)0

**2B. Laboratory safety training**

\*All laboratory personnel should receive lab safety training either online or in person. Persons interested in the online training provided by Environmental Health and Safety can enroll at the following link:
<https://www.csuohio.edu/ehs/online-safety-training-sign>

\*Training for specialized techniques involving Recombinant DNA may be done in the lab.
**Please briefly summarize training that has been conducted for all lab members as well as any specialized training provided as needed:**
The PI and all personnel involved in the study have completed the online safety training provided by EH&S as well in-lab training in use and disposal of hazardous chemicals. All students involved in cell culture have in-lab training in proper cell culture technique and disposal. All personnel using lentivirus will have in-lab training with the Principle Investigator to ensure knowledge of appropriate SOPs for safe use and decontamination

**2C. Please provide the following information regarding project funding:**

**Agency or Source Name(s):**
Click or tap here to enter text.

**Agency or Source Grant/Contract/Protocol #(s):**
Click or tap here to enter text.

**SECTION 3. Study Description/Plan**

**3A. What is the nature of this recombinant experiment? (select all that apply):**[ ]  Creation of transgenic animals
[ ]  Purchase or transfer of transgenic animals
[x]  Viral infection and/or viral vectors
[ ]  Gene therapy
[x]  Use of modified cells in animals

**Will any of the following be introduced into a living cell? (select all that apply):**[x]  sh/si/dsRNA
[ ]  Toxin genes
[ ]  Oncogenes
[ ]  Vaccine
[ ]  Synthetic oligonucleotides or morpholinos
[ ]  Compacted DNA/nanoparticles
[ ]  Other

**\*\*If other, please explain.**Click or tap here to enter text.

**3B. Describe the purpose of the proposed experiments in 3 or 4 sentences, using language that can be understood by a non-scientist:**Oligodendrocytes are glial cells in the CNS that perform a critical role in assembly of the myelin sheath that insulates axons. Defects in oligodendrocyte function are implicated in cerebral palsy, schizophrenia, and bipolar disorder. To understand the mechanisms regulating the ability of oligodentrocytes to participate in myelinization, we will modulate the expression level of differentiation factors in an oligodendrocyte cell line and examine the behavior of modified oligodentrocytes when introduced into a mouse model.

**3C. Briefly summarize the broad goals of the study and how the proposed recombinant nucleic acid research fits in your overall project:**We will use CRISPR editing to generate missense mutations predicted to disrupt protein-protein interactions in transcription factors Olig1 and Olig2, both of which are required for oligodendrocyte differentiation. Lentiviral-mediated shRNA knockdown will be used to deplete cFLIP in oligodendrocyte HNK-1 cells to determine whether cFLIP-dependent protection of oligodendrocytes from inflammation is essential for efficient myelinization. Finally, we will use lentiviral transduction to overexpress TMEM10 and NDE1, both required for normal oligodendrocyte differentiation, in oligodendrocyte progenitor cells. Modified cells will be into mouse models to determine their ability to differentiate and incorporate into the CNS. These experiments will help elucidate the mechanisms that regulate oligodendrocyte number and differentiation.

**3D. Detail the recombinant and biosafety portions of your experiments in a step-by-step fashion. Indicate if you are generating or purchasing viral particles. Please detail safety measures used including Personal Protective Equipment (PPE), physical containment (biosafety cabinet), decontamination, and disposal of waste (If using bleach to decontaminate liquid media or surfaces, include bleach percentage and, if appropriate, minimum time between addition of bleach and disposal):**Overall Protocol:
1. design guide RNAs targeting genes of interest for CRISPR experiments
2. Clone guides or cDNA into lentiviral vectors (lentiCRISPRv2; lentiCRISPRv2\_GFP; pLV hU6-sgRNA hUbC-dCas9-KRAB-T2a-GFP; or related lentiviral CRISPR targeting vectors)
3. Package lentivirus using HEK293T cells cotransfected with lentiviral backbone using Lenti-X Single Shot 4th generation packaging system (Clontech)
4. Collect supernatant containing packaged lentivirus 72h after transfection and filter through a 0.45 um PVDF membrane
5. Transduce cells and analyze effects on formation of oligodendrocytes.

Gene editing studies:
CRISPR vectors will target Olig1 and Olig2 in murine oligodendrocyte progenitor cells. The planned mutations are amino acid changes thought to disrupt protein-protein interactions. After 6hrs to 3 days in culture, the cells will be assayed for protein expression.

Knockdown studies:
shRNA lentiviral particles will be purchased commercially from Dharmacon (4th generation) targeting cFLIP and used to transduce oligodendrocyte cell line HNK-1. After 3 days in culture, the cells will be collected for analysis of protein expression for cell markers of differentiation.

Overexpression studies:
Lentiviral particles expressing cDNA for TMEM10 and NDE1 will be used to transduce oligodendrocyte progenitor cells. Cells will be cultured for 1-7 days, and analyzed for RNA/protein expression to evaluate signaling pathways downstream of these genes.

Animal Studies:
Transduced cells expressing edited Olig1/2 or overexpressing TMEM10 may undergo selection with puromycin for 2 weeks, after which the cells will be introduced into athymic mice. The animals will be analyzed 1 week to 2 months following injection to evaluate differentiation and proliferation, as well as their electrophysiological properties.

Statement of safety procedures in place:
This CRISPR design does package the gRNA and CAS9 enzyme within the same viral particle. In addition to safety protocols designed to prevent exposure (described below), the risk to researchers is mitigated by the exclusive targeting of mouse genes within our mouse oligodendrocyte progenitor cells. Although the targeted genes are conserved between mouse and human, the guides we have chosen show low complementarity to the human homolog sequence (and other human genomic sequences) as indicated by BLAST alignment.

Numerous administrative controls, engineering controls, and use of proper personal protective equipment support the safety of these proposed experiments.

General: The lentiviral packaging constructs are not replication competent. All users of lentiviral techniques will have personal training with the Principal Investigator to ensure that each user is well informed about the SOP. All lentiviral work will be done during normal working hours to decrease the risk of serious adverse events.

Biosafety Cabinet: All work is conducted in a certified Class II Biosafety Cabinet. Before use, the Biosafety Cabinet will be run for 5 min to allow for a fully efficacious air shield. The cabinet and its contents will be wiped with bleach at the conclusion of experiments, followed by a water rinse to minimize corrosion of the hood surfaces. All items to be removed from the hood will be similarly decontaminated with bleach prior to removal from the biosafety cabinet.

Culture Technique: Cells are cultured within a dedicated ‘virus’ incubator to minimize unanticipated contamination of uninfected cell cultures. All centrifugation of virus will be done in centrifuges containing o-rings to decrease the risk of aerosol generation. All flasks, plates, and containers will be explicitly labeled with markers indicating the presence of virus. We will use plastic aspiration tips instead of glass ones to decrease the risk of virus transfer to the personnel via needle-sticks. Vortexing will only be performed within the confines of the biosafety cabinet.

PPE: Personal protective equipment, including nitrile disposable gloves and standard lab coats, further protect researchers from unwanted exposure. Additional protective equipment including: gloves, face shield, and surgical masks, are available for use.

Waste Procedure: To prevent virus from contaminating surfaces outside the biosafety cabinet, all serological pipettes, culture flasks, and pipette tips are bleached inside the cabinet for at least 30 minutes prior to disposal in the appropriate biohazardous waste container (burn box for pipettes; standard red bag for non-sharp items). A spill kit will be readily on hand for use in the event of a spill. Spills greater than 25 mls will be notified to the Principal Investigator. Aspirated waste will be aspirated into a solution which will contain a final concentration of at least 10% bleach. The aspiration line will also be bleached at the end of each session.

**3E. Indicate the laboratory facilities (building and room number) where work will be conducted.**Click or tap here to enter text.

**3F. What is the proposed Risk Group/Biosafety Level (BSL)/Animal Biosafety Level (ABSL) for this project:**Risk Group 3 Biosafety Level 2 Animal Biosafety Level 1

For more info on Risk Groups/BSLs/ABSLs please refer to the [CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL)](https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf) pages 12, 68, 71, and 147.

More information can also be found in the [NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)](https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf)

Reference Appendix B for an extensive list of Risk Groups for various agents.

**3G. Will BSL/ABSL levels increase or decrease during the course of research?** *For example, do you propose to move animals from ABSL 2 to ABSL 1 after testing for viral shedding?*Yes

**If YES, please describe which components of the proposed research will be affected and whether any testing will be done before changing biosafety levels:**
For most experiments involving lentivirus, transduced cells will be maintained in culture for over 72 hours prior to injection into mice. In some overexpression experiments, cells will be introduced into animals less than 72 hours after transduction. In these cases, animals will be maintained at ABSL2 until 72 hours post transduction, then transferred to ABSL1

**SECTION 4. Recombinant Nucleic Acid/Recombinant Material Details.**

**4A. If Recombinant experiments involve working with a pathogen, please list the names. Note that Baculovirus experiments that involve a pathogen are NOT exempt from the NIH Guidelines for Research Involving Recombinant DNA Molecules.**
Click or tap here to enter text.

**4B. If Recombinant experiments involve working with viruses, select all that apply.** *A map for each vector is required. This may be submitted as a separate file (e.g., graphic map cut and pasted into a Word document) or a web link or vendor AND product number that leads directly to information/maps for the vector/system used. The IBC may reject applications lacking documentation of the viral elements present in the vectors used.*

[ ]  Adenovirus
[ ]  Baculovirus
[ ]  Lentivirus 3 plasmids
[x]  Lentivirus 4 or more plasmids
[ ]  Retrovirus
[ ]  AAV
[ ]  Other

**If other, please specify**
Click or tap here to enter text.

**Will more than 2/3 of a viral genome be used?**Yes

**Will a helper virus be used? If using a helper plasmid, please answer no.**Yes

**Will a virus be used as a vector to introduce a foreign gene?**Yes

**If yes, please list the vector(s) name(s):**
pMDLg/pRRE (3rd gen packaging vector, encodes GAG and POL) <https://www.addgene.org/12251/>
pRSV-Rev (3rd gen packaging vector, encodes REV) <https://www.addgene.org/12253/>
pMD2.G (VSV G envelope protein) <https://www.addgene.org/12259/>
pRRLSIN.cPPT.PGK-GFP.WPRE (3rd generation transfer vector) <https://www.addgene.org/12252/>

Note: psPAX2 (second gen/3 plasmid packaging vector compatible with 3rd gen/4 plasmid systems, encodes GAG,POL,TAT and REV) <https://www.addgene.org/12260/> is often used instead of pMDLg/pRRE & pRSV-Rev or equivalent, this is a 2nd gen Lentivirus/3 plasmid system, even if a 3rd gen transfer vector is used, and this should be indicated in the checkboxes above

**4C. If Recombinant experiments involve cell lines or bacterial strains other than *E. coli K-12*, please provide cell information, if applicable. Include packaging cell lines in this list.**

|  |  |  |
| --- | --- | --- |
| **Type of cell/cell line (including species):** | **Origin/Source of cells (including name of supplier):** | **IRB approval number (if necessary):** |
| HNK-1 mouse oligodendrocyte progenitor | gift of XXX YYY lab, University of Make Believe | Click here to enter IRB #. |
| HEK293T Human Kidney | ATCC | Click here to enter IRB #. |
| Click here to enter text. | Click here to enter text. | Click here to enter IRB #. |
| Click here to enter text. | Click here to enter text. | Click here to enter IRB #. |
| Click here to enter text. | Click here to enter text. | Click here to enter IRB #. |

*Please note any additional cell lines on a separate page and indicate the number of continuation pages used in the field below.* [*A formatted continuation page can be downloaded from the SPRS webpage.*](https://www.csuohio.edu/sites/default/files/IBC%20application%20s4c%20continuation%20page.docx)0

**4D. Will the research project involve more than 10 Liters of culture at any time?**No

**4E. For CRISPR experiments, please answer the following:**

**If gRNA and nuclease are encoded on the same plasmid, vector, or delivery vehicle, can this vector transfect or infect a human cell?**Yes

**Does this research involve the creation of a gene drive experiment?** *If yes, please explain.*No
Click or tap here to enter text.

[*More information on gene drive experiments*](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6069294/)*.*

**Will the gene editing technology be used to target embryos or germ line cells?** *If yes, please explain.*No
Click or tap here to enter text.

**4F. If Recombinant experiments involve working with compacted DNA/nanoparticles, please specify the type of nanoparticle being used and safety procedures to minimize risk of exposure:**
Click or tap here to enter text.

**SECTION 5: Sources and nature of recombinant materials:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name of gene/protein** | **Species of origin** | **Will a foreign gene be expressed?** | **Activity/function of gene (tissue inhibitor, marker gene)** |
| Cas9 | *S. pyogenes* | Yes | nuclease |
| sgRNAs to Olig1 & 2 | mouse/*S. pyogenes* chimeras | Yes | single guide RNAs |
| shRNA to cFLIP | shRNA with complementarity to mouse | Yes | knock down mouse cFLIP regulator of apoptosis |
| TMEM10 | mouse | No | transmembrane protein, promotes oligodendrocyte differentiation |
| NDE1 | mouse | No | Centrosomal protein, promotes oligodendrocyte differentiation |

*Please note any additional recombinant materials on a separate page and indicate the number of continuation pages used in the field below.* [*A formatted continuation page can be downloaded from the SPRS webpage.*](https://www.csuohio.edu/sites/default/files/IBC%20application%20s5%20continuation%20page.docx)– NOTE: I should actually use a continuation page to include GFP since one of the viral vectors listed in section 4B (pRRLSIN.cPPT.PGK-GFP.WPRE) includes sequences that will lead to GFP expression in transduced mammalian cells…

**Resources:**

[CSU IBC forms, instructions, and biosafety requirements & exemptions on the SPRS website](https://www.csuohio.edu/sprs/ibc)

[NIH Guidelines April 2019](https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf)
[Biosafety in Microbiological and Biomedical Labs 6th Ed.](https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf)