Student Checklist (1A)

This form is required for ALL projects.

1. a. Student/Team Leader: Jude Tomas Grade: 11

   Email: eduj1samot@gmail.com Phone: 516-784-6552

   b. Team Member: Kelvin Cheng c. Team Member: _____

2. Title of Project:

   Regulation of a Temperature-Induced Lipocalin (TIL) by Cold Stress and Epibrassinolid

3. School: George W. Hewlett High School School Phone: 516-792-8004

   School Address: 60 Everit Avenue
   Hewlett, NY, 11557

4. Adult Sponsor: Terrence Y. Bissoondial Phone/Email: 516-425-9991

5. Does this project need SRC/IRB/IACUC or other pre-approval? ☐ Yes ☑ No Tentative start date: 6/06/19

6. Is this a continuation/progression from a previous year? ☐ Yes ☑ No
   If Yes:
   a. Attach the previous year's ☐ Abstract **and** ☐ Research Plan/Project Summary
   b. Explain how this project is new and different from previous years on
   ☐☐ Continuation/Research Progression Form (7)

7. This year's laboratory experiment/data collection:

   6/06/19 (Actual Start Date: (mm/dd/yy)) 10/18/19 (End Date: (mm/dd/yy))

8. Where will you conduct your experimentation? (check all that apply)
   ☐ Research Institution ☑ School ☐ Field ☐ Home ☐ Other: _____

9. List name and address of all non-home and non-school work site(s):

   Name: _____ _____

   Address: _____ _____

   _____ _____

   Phone/ email _____ _____

10. **Complete a Research Plan/Project Summary following the Research Plan/Project Summary instructions and attach to this form.**

11. **An abstract is required for all projects after experimentation.**

Research Plan/Project Summary Instructions

A complete Research Plan/Project Summary is required for ALL projects and must accompany Student Checklist (1A).

1. All projects must have a Research Plan/Project Summary
   a. Written prior to experimentation following the instructions below to detail the rationale, research question(s), methodology, and risk assessment of the proposed research.
   b. If changes are made during the research, such changes can be added to the original research plan as an addendum, recognizing that some changes may require returning to the IRB or SRC for appropriate review and approvals. If no additional approvals are required, this addendum serves as a project summary to explain research that was conducted.
   c. If no changes are made from the original research plan, no project summary is required.

2. Some studies, such as an engineering design or mathematics projects, will be less detailed in the initial project plan and will change through the course of research. If such changes occur, a project summary that explains what was done is required and can be appended to the original research plan.

3. The Research Plan/Project Summary should include the following:
   a. **RATIONALE:** Include a brief synopsis of the background that supports your research problem and explain why this research is important and if applicable, explain any societal impact of your research.
   b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), ENGINEERING GOAL(S), EXPECTED OUTCOMES:** How is this based on the rationale described above?
   c. Describe the following in detail:
      - **Procedures:** Detail all procedures and experimental design including methods for data collection. Describe only your project. Do not include work done by mentor or others.
      - **Risk and Safety:** Identify any potential risks and safety precautions needed.
      - **Data Analysis:** Describe the procedures you will use to analyze the data/results.
   d. **BIBLIOGRAPHY:** List major references (e.g. science journal articles, books, internet sites) from your literature review. If you plan to use vertebrate animals, one of these references must be an animal care reference.

Items 1–4 below are subject-specific guidelines for additional items to be included in your research plan/project summary as applicable.

1. **Human participants research:**
   a. **Participants:** Describe age range, gender, racial/ethnic composition of participants. Identify vulnerable populations (minors, pregnant women, prisoners, mentally disabled or economically disadvantaged).
   b. **Recruitment:** Where will you find your participants? How will they be invited to participate?
   c. **Methods:** What will participants be asked to do? Will you use any surveys, questionnaires or tests? If yes and not your own, how did you obtain? Did it require permissions? If so, explain. What is the frequency and length of time involved for each subject?
   d. **Risk Assessment:** What are the risks or potential discomforts (physical, psychological, time involved, social, legal, etc.) to participants? How will you minimize risks? List any benefits to society or participants.
   e. **Protection of Privacy:** Will identifiable information (e.g., names, telephone numbers, birth dates, email addresses) be collected? Will data be confidential/anonymous? If anonymous, describe how the data will be collected. If not anonymous, what procedures are in place for safeguarding confidentiality? Where will data be stored? Who will have access to the data? What will you do with the data after the study?
   f. **Informed Consent Process:** Describe how you will inform participants about the purpose of the study, what they will be asked to do, that their participation is voluntary and they have the right to stop at any time.

2. **Vertebrate animal research:**
   a. Discuss potential ALTERNATIVES to vertebrate animal use and present justification for use of vertebrates.
   b. Explain potential impact or contribution of this research.
   c. Detail all procedures to be used, including methods used to minimize potential discomfort, distress, pain and ~~injury~~ to the animals and detailed chemical concentrations and drug dosages.
   d. Detail animal numbers, species, strain, sex, age, source, etc., include justification of the numbers planned.
   e. Describe housing and oversight of daily care
   f. Discuss disposition of the animals at the termination of the study.

3. **Potentially hazardous biological agents research:**
   a. Give source of the organism and describe BSL assessment process and BSL determination.
   b. Detail safety precautions and discuss methods of disposal.

4. **Hazardous chemicals, activities & devices:**
   - Describe Risk Assessment process, supervision, safety precautions and methods of disposal.
   - Material Safety Data Sheets are not necessary to submit with paperwork.

Regulation of Temperature-Induced Lipocalin (TIL) by Cold stress and Epibrassinolide in the Gametophyte Stage of *Ceratopteris richardii*

Student names: Jude Tomas and Kelvin Cheng

A. Rationale:

While climate change is primarily known for its characteristic increases in temperature, it has also been shown to induce decreases in temperature as well, or extreme cold. The concern of extreme cold is that it can significantly alter important plant functions. Decreases in temperature leads to a reduction in metabolism rates of plants because enzymes, proteins, and other important cellular functions will not be in homeostasis (Pennisi *et. al*, 2017). With the world population predicted to grow to about 9 billion in 2050, it is crucial that a stable food supply is established.

Extreme cold is shown to correlate with a decrease in crop yield. Although it is known that extreme cold is detrimental to plant metabolism and functions, - specifically decreases in chlorophyll accumulation and sensitivity in chloroplasts for photosynthesis (Sanghera *et. al,* 2011) - the mechanism in which plants tolerate cold stress is poorly understood.

Temperature-induced lipocalin (TIL) is a transmembrane protein that belongs to a larger family of lipocalins well known for their protective roles in mitigating temperature stress, drought, and salinity stresses(Hernández-Gras, Francesc, et. al, 2015). They also have essential roles in the modulation of cell growth, induction of apoptosis and the regulation of immune response(Hernández-Gras, Francesc, et. al, 2015). For example, several studies in Arabidopsis have shown that TIL plays a role in basal and acquired thermotolerance salt stress as well as drought and high light stresses (Hernández-Gras, Francesc, et. al, 2015).

The Brassinosteroid (BR) pathway is known to regulate multiple aspects of physiological responses, such as male fertility (pollen development), senescence, cell elongation and division (Clouse, Steven D, 2011). Treatment with 24-epibrassinolide (Charron, Jean-Benoit Frenette, et al., 2002) increases heat and cold tolerance within plants. This enhanced resistance is attributed to membrane stability, and suggests that part of temperature response in plants involve brassinosteroids as signaling molecules to bring about the expression of steroid binding proteins such as lipocalins.

Further examination of the TIL gene and the Brassinosteroid pathway in higher plants is difficult because of the difficulty in attaining physiological data. The plant would have to be killed in order to attain gametophyte data from the plant's sexual organs such as the ovary.

A more viable plant model may be the aquatic fern, *Ceratopteris richardii* (Cooke *et al.*, 1995; Chasan, 1992). Gametophytes of *Ceratopteris* are either characterized as male or hermaphrodites. Since ferns have characteristics of a higher plant (sporophyte) and lower plant system (gametophyte), examination of *Ceratopteris* is highly advantageous as development can be examined separately in each developmental stage (Hickok *et al.*, 1995; Banks, 1999). Furthermore, *Ceratopteris* takes significantly less time to mature as a gametophyte, taking only 10-12 days (Hickock *et. al,* 1987), thus facilitating observation and analysis.

The focus of this study is to determine how Temperature-induced lipocalin is regulated by cold stress and the Brassinosteroid pathway, using Epibrassinolide (EBL) as a stimulant. We will test the effects of cold stress and treatment of EBL on the expression of TIL in *Ceratopteris richardii* spores, as well as physiological differences between room temperature and cold, control and treated. Investigation will bring forth more knowledge of the mechanism that

controls the regulation of the TIL gene, and hence how plants tolerate and acclimate to extreme cold.

B. Research Questions/Hypothesis/ Expected Outcome

Problem: How does cold stress due to climate change affect the expression of Temperature-Induced Lipocalin (TIL) in plants?

Hypothesis: The putative orthologue of TIL1 found in *Ceratopteris richardii* will be upregulated under cold stress conditions (4-10 °C) relative to the spores treated under room temperature conditions. Furthermore, TIL will be upregulated when the spores are treated with Epibrassinolide (EBL) relative to the control group.

C. Procedures:

Measuring Germination:

C-Fern® agar, Liquid C-Fern® media, and pre-sterilized dry spores of *Ceratopteris richardii* (Catalogue #156728) will be obtained from Carolina Biological. C-Fern® agar and Liquid C-Fern® media will be prepared according to manufacturer's procedures. Gloves, goggles, and labs coats will be worn when handling C-Fern® agar.

Spores will be incubated in the dark for 7 days before they are added to the agar plates. Agar plates will be prepared one day before the addition of spores. Personal protective devices includes goggles, gloves, lab coats and fume hood. After 7 days of incubation, spores will be plated onto C-fern media (Carolina Biological) and allowed to develop under continuous white light. This will be marked as Day 0. Starting on the third day, percent germination will be quantified.

Applying EBL and treating with cold stress:

On day 7, two of the agar plates will be treated with EBL, while two more agar plates will be marked as the control group. For 7 days, the two groups will be compared to look for physiological changes or differences. On day 16, both plates of the control and EBL will be flooded. Control will be flooded with 2 mL of distilled water and EBL groups will be flooded with 2 mL of EBL. On day 17, one control and one EBL will be separated with another control and another EBL to form one room temperature and one cold stress group. The cold stress group will be put under temperatures of 4-10 °C for 24 hrs. Room temperature group will be placed under temperatures of 22-25°C. After 24 hrs, the cold stress group will be placed under normal temperatures. Data will be taken for the inflation of gametophytes in the group that will be exposed to cold stress

Identification and Characterization of a putative TIL gene from Ceratopteris richardii.

Bioinformatics analysis will be performed to screen an Expressed Sequence Tag cDNA library of *Ceratopteris*. Any sequence identified will then be characterized using various bioinformatics programs. For example, the ORF finder, as well as the BLAST program on the National Center of Biotechnology Information (NCBI) will be utilized in order to find the putative orthologue of TIL in *Ceratopteris*, and to find orthologues in other plant species, respectively.

Isolation of RNA

RNA will be isolated from gametophytes tissue by mentor and provided to students

RT-PCR

RNA will be converted to cDNA using the Invitrogen SuperScript® III Platinum® One-Step RT-PCR Kit (Catalogue #A11732-020). The primers to amplify the CrTIL gene: CrTIL-forward: CGC CAT TAC TTT TAT and Cr-TIL-reverse: GTT GTG CAG TCA TGG TG. Gloves, goggles, and lab coats will be worn. Mentor will assist in handling and disposing of hazardous chemicals.

PCR Conditions and Agarose Gel Electrophoresis:

Step	Temperature (°C)	Time (Minutes)	Cycles
Initial Denaturation	94°C	3 minutes	1
Denaturation	94°C	0.5 minutes	30 -35
Annealing	45-68°C	0.5 minutes	
Extension	72°C	2 minutes	
Final Extension	72°C	10 minutes	1
Hold	4°C	Indefinitely	N/A

Agarose Gel Electrophoresis:
Agarose Gel Electrophoresis will be used to analyze the amplification of PCR products. Mentor will oversee the procedure. Gel tray will be assembled and 1% agarose gel in 1xTBE solution (89mM Tris Base, 89mM Boric Acid, 2mM EDTA) will be prepared. Agarose gel will be run in 1xTBE buffer. DNA will then be visualized using SYBR Safe DNA stain and UV light.

Risk and Safety:
Material Safety Data Sheets from US Research Nanomaterials, Cayman Chemicals, Sigma Aldrich, ThermoFisher Scientific, and Carolina will be used for the chemicals.

C-Fern Agar/Liquid C-Fern - more than minimal risk may cause eye, skin, and respiratory irritation and can cause gastrointestinal discomfort if swallowed. Gloves, goggles, lab coats will be worn when handling. https://www.carolina.com/teacher-resources/Document/msds-basic-c-fern-medium-prepared/tr-msds-basic-c-fern-medium-prepared.tr

Acetone- highly flammable liquid and vapor; more than minimal risk may cause serious eye irritation; toxic to aquatic life; potentially harmful if inhaled or absorbed through skin; potentially harmful if swallowed. Gloves, goggles, lab coats will be worn when handling. Will be disposed according to official regulations by the chemical safety officer of G.W. Hewlett High School.
https://www.fishersci.com/shop/msdsproxy?productName=AC177170010&productDescription=ACETONE

24-Epibrassinolide - not a hazardous substance or mixture. Nevertheless, safety equipment will be used when administering the substance to the organism. Will be handled with gloves; avoiding skin contact in mind, and safety goggles will be used. Will be disposed of according to manufacturer's instructions and according to official regulations by the chemical safety officer of G.W. Hewlett High School.
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=E1641&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fsearch%3Fterm%3Depibrassinolide%26interface%3DAll%26N%3D0%26mode%3Dmatch%2520partialmax%26lang%3Den%26region%3DUS%26focus%3Dproduct

Invitrogen SuperScript® III Platinum® One-Step RT-PCR Kit- more than minimal risk can potentially harmful if inhaled, absorbed through skin, or when in contact with the eyes, causing irritation; potentially harmful if swallowed. Gloves, goggles, lab coats will be worn when handling. Will be disposed of according to manufacturer's instructions and according to official regulations by the chemical safety officer of G.W. Hewlett High School.
https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/LSG/manuals/superscript_onestep_qrtpcr_man.pdf&title=Product%20Info%20Sheet:%20SuperScript%20III%20Platinum%20One-Step%20qRT-PCR%20Kit

SYBR Safe® DNA Stain- nonhazardous; nonetheless, gloves, goggles, lab coats will be worn when handling.
https://www.thermofisher.com/content/dam/LifeTech/Documents/PDFs/PG1290-PJ8342-CO128458-SYBRsafe-Green-Fact-Sheets-Corp-FHR.pdf

1xTBE has no health effects and is not flammable. Gloves, goggles, lab coats will be worn when handling.
https://www.fishersci.com/shop/msdsproxy?productName=BP24304&productDescription=1X+TRIS-BORATE-EDTA

Ethanol - more than minimal risk of eye, skin, gastrointestinal, and respiratory irritation, ignition, reaction with oxidizers, and formation of carbon monoxide and carbon dioxide via decomposition. Will be disposed according to official regulations by the chemical safety officer of G.W. Hewlett High School.
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=E7023&brand=SIGALD&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigald%2Fe7023%3Flang%3Den

Agarose gel - more than minimal risk of skin, eye, respiratory, and gastric irritation. Gloves, goggles, lab coats will be worn when handling.
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=P5472&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fp5472%3Flang%3Den

Lysis/Binding Buffer – more than minimal risk of damage to eyes and respiratory tract, neuro- and nephrotoxic effects. Will be handled and disposed by mentor.

Wash Solution #1 - more than minimal risk of skin, eye, respiratory, and gastrointestinal irritation, instability caused by excess heat and moisture, and reaction with oxidizing agents. Gloves, goggles, lab coats will be worn when handling. Will be disposed of according to manufacturer's instruction.
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=W0263&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fw0263%3Flang%3Den

Wash Solution #2/3 - more than minimal risk of eye and gastric irritation. Gloves, goggles, lab coats will be worn when handling. Will be disposed of according to manufacturer's instructions.
https://www.sigmaaldrich.com/MSDS/MSDS/PleaseWaitMSDSPage.do?language=&country=US&brand=SIGMA&productNumber=W4639&PageToGoToURL=https://www.sigmaaldrich.com/catalog/product/sigma/w4639?lang=en&region=US

Elution Buffer- more than minimal risk of eye and gastric irritation. Gloves, goggles, lab coats will be worn when handling.
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=C0241&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fc0241%3Flang%3Den

Activities and Devices:

Agarose Gel Electrophoresis- more than minimal risk of electric shock. Mentor will oversee use and assist with using device.

PCR Thermo-cycler- more than minimal risk of skin irritation and slight burns minor risk of burns to hand/fingers. Mentor will oversee use and assist with using device

Describe the Safety precautions and procedures that will be used to reduce the risks.

We will wear gloves, goggles, lab coats, and use the fume hood when necessary. Face masks will be used when handling chemicals that are hazardous with inhalation. Mentor will handle safety precaution training, hazardous chemicals, and will assist with the use of potentially harmful equipment.

Describe the disposal procedures that will be used.

Hazardous and organic chemicals will be stored in hazardous and organic chemical waste containers to be properly disposed by the Chemical safety officer. Most other chemicals are safe to dispose of down the drain diluted with water according to manufacturer's instructions.

List sources of Safety Information

All information for hazardous chemicals will be obtained from Safety Data Sheets obtained from:

- Carolina Biological
- Life Technologies
- Flinn Scientific
- Sigma Aldrich

Data Analysis:

Measurements of Germination:

After collecting data on the percentage of gametophytes with inflations and the number of inflations per gametophyte for the groups exposed to cold stress, the averages will be taken. Then, an ANOVA test will be conducted between the experimental data sets and the control data set of each data type to determine statistical significance (p-values lower than 0.05 indicate statistical significance between two sets of data).

D. Bibliography

Charron, Jean-Benoit Frenette, et al. "Identification, Expression, and Evolutionary Analyses of Plant Lipocalins." *Plant Physiology*, vol. 139, no. 4, 2005, pp. 2017–2028., doi:10.1104/pp.105.070466.

Charron, Jean-Benoit Frenette, et al. "Molecular and Structural Analyses of a Novel Temperature Stress-Induced Lipocalin from Wheat And Arabidopsis." *FEBS Letters*, vol. 517, no. 1-3, 2002, pp. 129–132., doi:10.1016/s0014-5793(02)02606-6.

Chasan, R. "Ceratopteris: A Model Plant for the 90s." The Plant Cell, 1992, pp. 113–115., doi:10.1105/tpc.4.2.113.

Clouse, Steven D. "Brassinosteroid Signal Transduction: From Receptor Kinase Activation to Transcriptional Networks Regulating Plant Development." *The Plant Cell*, vol. 23, no. 4, 2011, pp. 1219–1230., doi:10.1105/tpc.111.084475.

Cooke, Todd J., et al. "The Fern Ceratopteris Richardii as a Lower Plant Model System for Studying the Genetic Regulation of Plant Photomorphogenesis." International Journal of Plant Sciences, vol. 156, no. 3, 1995, pp. 367–373., doi:10.1086/297258.

Hernández-Gras, Francesc, and Albert Boronat. "A Hydrophobic Proline-Rich Motif Is Involved in the Intracellular Targeting of Temperature-Induced Lipocalin." *Plant Molecular Biology*, vol. 88, no. 3, 2015, pp. 301–311., doi:10.1007/s11103-015-0326-x.

Hickok, Leslie G. et al. (1987, August). "CERATOPTERIS RICHARDII: APPLICATIONS FOR EXPERIMENTAL PLANT BIOLOGY." *American Journal of Botany, Vol. 74 No. 8.* Pp. 1304-1316. Retrieved from http://www.jstor.org/stable/2444165.

Hickok, Leslie G., et al. "The Biology of the Fern Ceratopteris and Its Use as a Model System." International Journal of Plant Sciences, vol. 156, no. 3, 1995, pp. 332–345., doi:10.1086/297255.

Ihara-Ohori, Yuri, et al. "Cell Death Suppressor Arabidopsis Bax Inhibitor-1 Is Associated with Calmodulin Binding and Ion Homeostasis." *Plant Physiology*, vol. 143, no. 2, 2006, pp. 650–660., doi:10.1104/pp.106.090878.

Li, Boqiang, et al. "Brassinolide Enhances Cold Stress Tolerance of Fruit by Regulating Plasma Membrane Proteins and Lipids." *Amino Acids*, vol. 43, no. 6, 2012, pp. 2469–2480., doi:10.1007/s00726-012-1327-6.

Pennisi, Bodie, et. al "Effects of Low Temperature on Plants." *Department of Horiculture*, UGA Cooperative Extension B167.

Sanghera, Gulzar S., et al. "Engineering Cold Stress Tolerance in Crop Plants." Current Genomics, vol. 12, no. 1, 2011, pp. 30–43., doi:10.2174/138920211794520178.

Siegel, Ethan. "This Is Why Global Warming Is Responsible For Freezing Temperatures Across

The U.S." *Forbes*, Forbes Magazine, 30 Jan. 2019, www.forbes.com/sites/startswithabang/2019/01/30/this -is-why-global-warming-is-responsible-for-freezing-temperatures-across-the-usa/#aa6a1bfd8cf8.

Staff, Science X, and Chinese Science Academy. "Scientists Reveal Spring Cold Spells That Reduce Crop Yields." *Phys.org*, Phys.org, 7 Nov. 2018, phys.org/news/2018-11-scientists-reveal-cold-crop-yields.html.

Vogel, Elisabeth, et al. "The Effects of Climate Extremes on Global Agricultural Yields." *Environmental Research Letters*, vol. 14, no. 5, 2019, p. 054010., doi:10.1088/1748-9326/ab154b.

Wang, Zhi-Yong, et al. "The Brassinosteroid Signal Transduction Pathway." *Cell Research*, vol. 16, no. 5, 2006, pp. 427–434., doi:10.1038/sj.cr.7310054.

ADDENDUM

No changes were made to the Research Plan