# Student Checklist (1A)

**This form is required for ALL projects.**

1. a. Student/Team Leader: Randel Placino Grade: 12

   Email: randelplacino@gmail.com Phone: (347) 824-0942

   b. Team Member: David Durdaller c. Team Member: _____

2. Title of Project:

   Elevated temperature and DNA methylation affects Mago nashi expression and sexual developmen

3. School: G.W Hewlett High School School Phone: (516) 792-4001

   School Address: 60 Everit Ave

   Hewlett NY 11557

4. Adult Sponsor: Dr. Terrence Bissoondial Phone/Email: (516) 425-9991/bissoondialt@gmail.co

5. Does this project need SRC/IRB/IACUC or other pre-approval? ☐ Yes ☒ No Tentative start date: 02/04/2019

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:

   02/04/2019 (Actual Start Date: (mm/dd/yy)) 10/15/2019 (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.

**Elevated temperature and DNA methylation affects Mago nashi expression and sexual development in *Ceratopteris richardii***

**Student Names:** Randel Placino and David Durdaller

## A. Rationale:

Elevated temperature, a distinct characteristic of climate change, has previously been correlated with significant decreases in crop yield. Elevated temperatures has also been shown to reduce pollen production and fertility in various plants such as *Phaseolus vulgaris* (Gross and Kigel, 1994), *Brassica napus* (Young *et. al*, 2004), and *Lycopersicon esculentum* (Abdul-Baki and Stommel, 1995). With the world population predicted to grow to about 9 billion in 2050, it is crucial that a stable food supply is established.

Explanations for such crop reductions include increases in temperature that harm pollen production and development. Specifically, both microsporogenesis and microgametogenesis phases are extremely sensitive to heat (Mueller and Rieu, 2016). However, this mechanism of heat sensitivity is not well understood.

Mago nashi is a RNA-binding protein that is involved in germ cell differentiation. It is ubiquitous, being highly conserved throughout numerous species of plants and animals (Swidzinski *et. al*). Mago nashi has been found in plants such as *Oryza sativa*, *Physalis floridana*, *Marsilea vestita*, and *Arabidopsis thaliana* (Swidzinski *et al.*, 2001; He *et al.*, 2007; Weele *et al,* 2007). It affects the sex determination in the early development in plants, including spermatogenesis (He *et al.*, 2007), male fertility (Weele *et al*, 2007), and seed viability (Park *et al.*, 2009).

Mago nashi has been shown to partake in the export of mRNA from the nucleus to the cytoplasm in eukaryotic organisms. It has co-evolved with another mRNA binding protein, Y-14 (Gong, *et. al*, 2014), in the export of mRNA into the cytoplasm with export factors barentz (Btz) and eukaryotic initiation factor 4a-III (eIF4AIII) (Roignant and Treisman, 2010). These export factors, along with Mago nashi and Y-14, also play a key role in the function of the Exon Junction Complex (EJC). The knockdown of key EJC components such as Mago nashi and Y14 in *Oryza sativa* have resulted in much shorter plants compared to the wild type (Gong and He, 2014). This suggests the significance of EJC components in early plant development.

Further examination of Mago nashi in higher plants is challenging due to the complex nature of pollen and seeds (Bushart *et al.*, 2007). Mechanisms including Mago Nashi-mRNA binding is not well understood in plants. Studying these mechanisms in simpler plant models, such as ferns, may provide insight into gametophyte development in higher plants (Banks, 1999).

A 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 stage (Hickok *et al.*, 1995; Banks, 1999). The sexual expression

of gametophytes in *Ceratopteris* is determined by a chemical messenger, antheridiogen, which promotes the antheridia, the male sex organ (Warne and Hicock, 1988). Hermaphrodites produce antheridia as well as archegonia, the female sex organ. Two mutants characterized in *Ceratopteris* are the *him1* and *her1* (Banks, 1994; Banks, 1999). *him1* and *her1* display abnormalities in sexual development (Spiro *et al.*, 2008; Banks 1994). *him1* favors the development of males; *her1*, the development of hermaphrodites.

The focus of this study is to investigate the role of Mago nashi in sexual differentiation of males and hermaphrodites in response to temperature changes. We will examine the effects of high temperature and inhibition of DNA methylation on the expression of Mago nashi in *Ceratopteris richardii*. Investigation will shed light on the mechanisms of sexual differentiation and development in plants in response to the effects caused by climate change.

## B. Research Questions/Hypothesis/ Expected Outcome

*Problem:* How does elevated temperature due to climate change affect sexual differentiation of gametophytes in *Ceratopteris richardii*?

*Hypothesis:*

Elevated temperature (28 °C) and inhibition of DNA methylation by 5-Azacytidine (5-AC) will lead to a greater disparity between male and hermaphrodite gametophytes in *Ceratopteris richardii*. Resulting increases in the number of males will affect fertility and cause a subsequent decrease in quantity of sporophytes developed. Mago nashi will be expressed more in *Ceratopteris* spores grown at higher temperatures and spores treated with 5-AC than those at standard condition.

## 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 with the 25 μM 5-Azacytidine, or 5-AC (Sigma Aldrich; Product ID: A2385-100MG). Since this chemical is highly toxic, it will be prepared and handled by mentor. Personal protective devices include 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. The ratio of males to hermaphrodite will be assessed at 10 days. The number of sporophytes produced will be measured after 30 days.

**RNA Isolation/RT-PCR:**

Spores will be grown in the presence of 5 AC and elevated temperature as previously described. To determine Mago nashi expression, RT-PCR will be conducted. Gloves, goggles, and lab coats will be worn. Mentor will assist in handling and disposing of hazardous chemicals. RNA will be isolated using the Invitrogen RNAqueous-4PCR Total RNA Isolation Kit (Catalog # AM1914). 0.5-0.75 mg tissue samples will be used. Samples will be disrupted with Lysis/Binding Solution. An equal volume of 64% Ethanol will be added. The lysate/ethanol mixture will be drawn through a Filter Cartridge. It will then be washed with 700µl Wash Solution #1 as well as with the 2×500µl Wash Solution #2/3. RNA will be eluted with 40-60µl preheated Elution Solution. It will then be eluted with a second 10-60 µl aliquot of Elution Solution.

RNA will be reverse transcribed using Invitrogen SuperScript III One-Step RT-PCR System with Platinum *Taq* High Fidelity DNA Polymerase from Thermo Fisher Scientific (Catalog # 12574-030). These procedures will be followed according to manufacturer's instructions. The thermal cycler will be programmed so that cDNA synthesis is automatically followed with PCR amplification.

**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.

**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 |

**Risk and Safety:**

Material Safety Data Sheets from Carolina Biological, Sigma Aldrich, ThermoFisher Scientific were used to obtain the information for these chemicals:

**5-Azacytidine** - may cause genetic defects, cancer, and is 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/store/msds?partNumber=AC226620500&productDescription=5-AZACYTIDINE%2C+99%25+50MG&vendorId=VN00032119&countryCode=US&language=en

**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

**Invitrogen RNAqueous®-4PCR Total RNA Isolation 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://assets.fishersci.com/TFS-Assets/LSG/manuals/cms_059300.pdf

**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

**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 will 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 will 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 handles 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.

**Data Analysis:**
**Measurements of Germination:**
After collecting data on the emergence of rhizoids, the area of the prothalli, the number of cells in the gametophyte stage, and the percentage of ferns developing into the sporophyte stage, an average 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).

**Gene Identification of Mago nashi isolated from *Ceratopteris richardii*:**
To find an orthologue of Mago nashi in Ceratopteris, an Expressed Sequence Tag (EST) library will be screened. Since the Mago nashi sequence from *Arabidopsis thaliana* has already been sequenced, the sequence will be entered into Basic Local Alignment Search Tool (BLAST) to obtain protein sequences of orthologues. Selected genomes of plants will be found from www.phytozome.net. MEGA6 (http://www.megasoftware.net/) will be used to create a phylogenetic tree to compare the evolutionary relationship of the Mago nashi sequence from Ceratopteris to other organisms. Clustal omega will be used to compare known protein sequences of Mago nashi from Arabidopsis and other plants to the partial Mago nashi sequence from Ceratopteris.

## D. Bibliography


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ADDENDUM

No changes were made to the Research Plan