



Alligare Aquatic Technical Services  
*Genetic Profile Analysis (GPA)*  
Hydrilla Fluridone Susceptibility Determination

## Process and Procedures for Hydrilla Collection

**Note: 15 eight-inch hydrilla stems with whorls = 1 Composite Sample**

1. Contact an Alligare Aquatic Specialist with questions pertaining to the number of composite samples to be collected per lake and cost. Generally, 1 Composite Sample for lakes under 40 acres is acceptable.
2. Collect Hydrilla plant stems from key locations and from multiple sites to best represent the hydrilla distribution. Samples from areas of special interest should be placed in a separate ZipLock bag and labeled differently. *NOTE: Collect clean hydrilla apical stems 8 in. long with some leaf whorls. Stems should be healthy; however, if plants have been recently treated with fluridone and have not yet begun to degrade or turn mushy the DNA will be useable.*



3. Rinse stems and remove silt, algae and sediment.
4. Wrap stems in a moist paper towel - two layers of wrap
5. Label a gallon sized Ziploc bag with collector's name, lake name or personal ID code, sample number and date. Use a simple sample numbering. For example, start with 1 and end with the number of samples provided.

6. Place wrapped stems in a gallon Ziploc bag and **drain** out water – DO NOT ADD WATER
7. Complete the GPA -Hydrilla test Chain of Custody form and place in a separate Ziploc bag. Forms can be downloaded from [alligare.com](http://alligare.com).
8. **Prior to shipping please call or text Dr. Ryan Thum at 406-994-4039 with shipping date.**
9. **Place the samples, USDA transportation permit and completed Chain of Custody form** in a cooler with cool packs and ship overnight via express mail (FedEx recommended). Leaky coolers will not be accepted. **Please do not use U.S. Mail for shipping and do not ship on Friday.**

Ship to: Dr. Ryan Thum  
Montana State Univ.  
Dept. of Plant Sciences and Plant Pathology Lab  
313 Plant Biosciences Building  
Bozeman, MT 59717-3150

*NOTE: If possible, ship the stems the date of collection or if shipping is delayed store in a cooler with cool packs. Freezers or refrigerators are too cold for sample storage.*

10. The cost per composite sample, \$595, covers MSU/Alligare expenses and is provided as a pass-through service to our customers. Please complete the Alligare Payment Information form and send via mail, fax or email to:

Alligare, LLC.  
ATTN: GPA Test Payment Information  
1565 5<sup>th</sup> Avenue  
Opelika, AL 36801  
Fax: 334-741-9395  
[hdaniell@alligare.com](mailto:hdaniell@alligare.com) (Hope Daniell in Accounts Receivable)

***Should you have any questions or need additional information, please contact Denise Haynes (334-364-0325 or [dhaynes@alligare.com](mailto:dhaynes@alligare.com) ) or Alligare Customer Service (334-741-9393 or [customerservice@alligare.com](mailto:customerservice@alligare.com)).***

Proc031519

P.O. Box 967, Opelika, AL 36803 · 1565 5<sup>th</sup> Avenue, Opelika, AL 36801  
888-ALLIGARE (255-4427) · 334-741-9393 · FAX 334-741-9395 · [www.alligare.com](http://www.alligare.com)



**Alligare Aquatic Technical Services**  
***Genetic Profile Analysis (Hydrilla Test)***  
**Chain of Custody Form**

Customer Name \_\_\_\_\_ Date \_\_\_\_\_

Plants Collected By: \_\_\_\_\_

Plants Shipped By: \_\_\_\_\_ Time/Date \_\_\_\_\_

Phone Number: \_\_\_\_\_ Email \_\_\_\_\_

Alligare Aquatic Specialist Contacted: \_\_\_\_\_

**Use one Chain of Custody Form per pond or lake**

Pond/Lake Name or Private ID Code \_\_\_\_\_

Waterbody Avg. Depth: \_\_\_\_\_ Surface Acres \_\_\_\_\_

Number of Composite Samples (**15 eight-inch stems with whorls = 1 Composite Sample**):

\_\_\_\_ *Hydrilla* (monoecious)      \_\_\_\_ *Hydrilla* (dioecious)      \_\_\_\_ Eurasian watermilfoil

Outside ZipLock Bag: Label Composite Sample Number

Composite Sample Number (Draw or attach map, provide GPS coordinates and sample location)

- 1.
- 2.
- 3.

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**This portion to be completed by Montana State University Lab staff:**

Date plant samples received \_\_\_\_\_ Number of Composite Samples \_\_\_\_\_

Received by: \_\_\_\_\_

Container Description: \_\_\_\_\_ Overnight \_\_\_\_\_ Mail \_\_\_\_\_ Cooler \_\_\_\_\_ Box

Composite Plant Sample Condition: \_\_\_\_\_ Excellent \_\_\_\_\_ Average \_\_\_\_\_ Poor \_\_\_\_\_ Unsuitable

Lab Technician: \_\_\_\_\_ Date Analyzed: \_\_\_\_\_

Date Results Emailed to Customer and Alligare: \_\_\_\_\_

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Alligare Aquatic Technical Services  
*Genetic Profile Analysis (GPA)*  
Payment Information Form

*Important – On the day of shipping the plant samples to the independent lab at Montana State University, this form must be completed and sent to Alligare @ 1565 5<sup>th</sup> Avenue, Opelika, AL 36801 or Fax (334-741-9395) or email ([hdaniell@alligare.com](mailto:hdaniell@alligare.com)).*

Customer or Company Name: \_\_\_\_\_

Primary Contact: \_\_\_\_\_ Phone Number: \_\_\_\_\_

Email Address \_\_\_\_\_

Billing Address \_\_\_\_\_

Alligare Aquatic Specialist Contacted: \_\_\_\_\_

Number of Composite Samples for the GPA (Genetic Profile Analysis) Test: \_\_\_\_\_

*(Note: 15 eight-inch stems with whorls = 1 Composite Sample; Each composite sample = \$595 + applicable sales or use tax).* Total Cost of GPA Test(s): \_\_\_\_\_

Select the Payment Options:  Check  VISA  Master Card  P.O. #

Credit Card Holder's Name: \_\_\_\_\_

Card No. \_\_\_\_\_ Exp. Date \_\_\_\_\_ Security Code \_\_\_\_\_

Call Alligare Customer Service with billing questions\*: 888-255-4427

Send this payment form to: Alligare LLC - GPA Hydrilla Test @ 1565 5<sup>th</sup> Avenue, Opelika, AL 36801, or Fax 334-741-9395 or Email [hdaniell@alligare.com](mailto:hdaniell@alligare.com). You will not be charged for this technical service until the *Hydrilla* GPA Test results have been emailed to you. As always, we greatly appreciate your business!

\*Contact your Alligare Aquatic Specialist directly for questions on sampling, pricing and results.

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**FROM:**

**TO:** Dr. Ryan Thum  
MSU Dept of Plant Sciences Lab  
313 Plant Biosciences Building  
Bozeman, MT 59717-3150



**FROM:**

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MSU Dept of Plant Sciences Lab  
313 Plant Biosciences Building  
Bozeman, MT 59717-3150

United States Department of Agriculture  
Animal and Plant Health Inspection Service  
Plant Protection & Quarantine  
4700 River Road  
Riverdale, MD 20737

**Permit to Move Live Plant Pests, Noxious Weeds, and Soil**  
Interstate Movement  
**Regulated by 7 CFR 360**

This permit was generated electronically via the ePermits system


<b>PERMITTEE NAME:</b>	Dr. Ryan Thum	<b>PERMIT NUMBER:</b>	P526P-18-03205
<b>ORGANIZATION:</b>	Montana State University	<b>APPLICATION NUMBER:</b>	P526-180307-015
<b>ADDRESS:</b>	313 Plant Biosciences Building Bozeman, MT 59717	<b>FACILITY NUMBER:</b>	4805
<b>MAILING ADDRESS:</b>	313 Plant Biosciences Building Bozeman, MT 59717	<b>HAND CARRY:</b>	No
<b>PHONE:</b>	406-994-4039	<b>DATE ISSUED:</b>	08/27/2018
<b>FAX:</b>		<b>EXPIRES:</b>	<b>08/27/2021</b>
<b>DESTINATION:</b>	Montana State University Dept. of Plant Sciences , 313 Plant Biosciences Building, Bozeman, MT 59717		
<b>RELEASE:</b>	No		

Under the conditions specified, this permit authorizes the following:					
<b><u>Regulated Article</u></b>	<b><u>Life Stage(s)</u></b>	<b><u>Intended Use</u></b>	<b><u>Shipment Origins</u></b>	<b><u>Originally Collected</u></b>	<b><u>Culture Designation</u></b>
Hydrilla verticillata (Hydrocharitaceae)	Plant Parts	Research - Lab FL, NC		Originally Collected from Within the Continental U.S.	

**PERMIT GUIDANCE**

- 1) This permit does not authorize importation or interstate movement of strains of genetically engineered regulated organisms (created by the use of recombinant DNA technology) or their use outside of a confined laboratory setting.
- 2) If an animal pathogen is identified in your shipment, to ensure appropriate safeguarding, please refer to [http://www.aphis.usda.gov/import\\_export/animals/animal\\_import/animal\\_imports\\_an\\_products.shtml](http://www.aphis.usda.gov/import_export/animals/animal_import/animal_imports_an_products.shtml)
- 3) If a human pathogen is identified, please refer to the CDC Etiologic Agent Import Permit Program at <http://www.cdc.gov/od/eaipp/>
- 4) This permit does not fulfill the requirements of other federal or state regulatory authorities. Please contact the appropriate agencies, such as the U.S. Environmental Protection Agency, the U.S. Fish and Wildlife Service, the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, the APHIS Veterinary Services unit, the APHIS Biotechnology Regulatory Services, or your State's Department of Agriculture to ensure proper permitting.

Permit Number P526P-18-03205

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
  <b>Gregg Goodman</b>	<b>08/27/2018</b>

WARNING: Any alteration, forgery or unauthorized use of this Federal Form is subject to civil penalties of up to \$250,000 (7 U.S.C.s 7734(b)) or punishable by a fine of not more than \$10,000, or imprisonment of not more than 5 years, or both (18 U.S.C.s 1001)

5) If you are considering renewal of this permit, an application should be submitted at least 90 days prior to the expiration date of this permit to ensure continued coverage. Permits requiring containment facilities may take a longer period of time to process.


### PERMIT CONDITIONS

USDA-APHIS issues this permit to Ryan Thum with MT State Univ. in Bozeman, MT. This permit authorizes the interstate movement of the listed regulated materials/organisms from the listed countries/states and/or territories.

This permit authorizes Ryan Thum in MT to use the regulated materials/organisms for lab research in the APHIS approved facility # 4805.

1.
  - This permit is issued by the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). It conveys APHIS regulations and requirements for the material(s) listed on this permit. It does not reduce or eliminate your legal duty and responsibility to comply with all other applicable Federal and State regulatory requirements.
  - The permit number or a copy of the permit must accompany the shipment.
  - You must be an individual at least 18 years old, or legal entity such as partnership, corporation, association, or joint venture.
  - You are legally responsible for complying with all permit requirements and permit conditions.
  - If you violate any applicable laws associated with this permit, you may face substantial civil or criminal penalties. We may cancel all current permits and deny future permit applications.
  - Without prior notice and during reasonable hours, authorized Federal and State Regulators must be allowed to inspect the conditions associated with the regulated materials/organisms authorized under this permit.
  
2. The permit holder must:
  - maintain a valid PPQ526 permit so long as the regulated materials/organisms are alive or viable,
  - not assign or transfer this permit to other persons without APHIS PPQ authorization,
  - maintain an official permanent work assignment, residence, or affiliation at the address on this permit,

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- notify the Pest Permit Staff as soon as possible of any change in the permit holder's work assignment, residence, or affiliation,
- notify the Pest Permit Staff of the receipt of unauthorized and/or misdirected shipments of regulated materials/organisms,
- adequately mitigate environmental impacts resulting from unauthorized release of regulated materials/organisms and notify the Pest Permit staff immediately if one occurs,
- notify the Pest Permit Staff if the facility is damaged/destroyed or if you wish to decommission the facility,
- destroy all regulated materials/organisms prior to departure from the organization unless other arrangements are confirmed by the Pest Permit Staff.


Notifications to the Pest Permit Staff must be made via 866-524-5421 or pest.permits@aphis.usda.gov within one business day of the event triggering a notification.

3. All persons working with the listed regulated materials/organisms must be informed of these permit conditions. Anyone working with these materials/organisms must agree to and sign/initial these conditions before beginning work. These signed conditions do not need to be submitted to USDA/APHIS but must be readily accessible and made available to Federal and State regulators upon request.

Note: these conditions may be copied and stored electronically for electronic signature and initialing provided that the permit number, authorized materials/organisms and life stages, release locations if applicable, and authorization statement all appear on the document with the permit number. Signing these conditions only indicates that the person working under this permit has read them; the permit holder is the sole responsible party under this permit.

4. The regulated material must be visually free of diseases, insects, or other plant pests and/or contaminants.
5. Field-collected samples of regulated material must be, "bare-root" or washed free of soil.
6. All packages for transport must minimally consist of both inner/primary and outer/secondary packages securely sealed so that both are effective barriers to escape or unauthorized dissemination of the listed materials/organisms. The inner/primary package(s) will contain all regulated materials/organisms and must be cushioned and sealed in such a way that it remains sealed during shock, impact, and pressure changes that may occur. The outer/secondary shipping container must

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be rigid and strong enough to withstand typical shipping conditions (dropping, stacking, impact from other freight, etc.) without opening.


7. Regulated materials must not be allowed to flower and set seed. Regulated materials must be inspected daily for the production of flowers and if any flowers are found, they must be removed from the material immediately and destroyed.
8. All research areas where work with the regulated material is undertaken must display a sign that reads, "Quarantined material - to be used only in accordance with current USDA Noxious Weed permit," or similar language.
9. All packets/containers of regulated material must be labeled with, "Quarantined material - to be used only in accordance with current USDA Noxious Weed permit," or similar language.
10. Regulated seeds must be in a sealed container during transport within the APHIS approved containment facility identified above.
11. When not working with the regulated materials/organisms, you must store and/or maintain them within the APHIS approved containment facility identified above. All stored regulated materials/organisms must be kept in a locked area with access limited to authorized personnel.
12. Modifications to the containment facility or any changes that affect the containment of the regulated materials/organisms must be approved by APHIS prior to making changes. Please contact the Pest Permit Staff (email: pest.permits@aphis.usda.gov; phone: 866-524-5421; address: 4700 River Rd., Unit 133, Riverdale, MD 20737; Fax: 301-734-8700).
13. All persons working with the regulated material/organism must be trained on the importance of APHIS approved containment practices as described in the permit conditions and approved SOPs associated with the APHIS approved containment facility listed above.
14. Standard Operating Procedures (SOPs) must be filed with, and approved by, the APHIS PPQ Pest Permit Staff at: email: pest.permits@aphis.usda.gov; phone: 866-524-5421; fax: 301-734-8700; address: 4700 River Road, Unit 133, Riverdale, MD 20737. All contact information must be kept current and the SOPs must be dated. If requirements in the permit conditions are more restrictive than the SOPs, permit conditions take precedence. APHIS PPQ must approve any changes to the SOPs before implementation.

A list of all persons with access to the containment facility must be maintained and available upon request by Federal or State Regulatory Officials.

15. DEVITALIZATION AND WASTE DISPOSAL

All regulated materials, including un-used and leftover material, and all packaging directly in contact with the regulated material must be devitalized/treated via one or more of the following methods: 1) autoclaved (see protocol below), 2) disposed of off-site by a facility holding a valid PPQ compliance agreement (contaminated waste must be stored in sealed containers prior to pick up by this company), or 3) Incineration.

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Treated waste will be double bagged prior to disposal.


Other sterilization methods are only allowed with prior written agreement from the USDA/APHIS PPQ Pest Permit Staff.

If using an autoclave the following protocol must be used:

- a. Waste must be autoclaved at 121 Celsius (250 Fahrenheit) for a minimum of 30 minutes at 15 psi.
  - b. Autoclave tape or other indicators must be placed on each load prior to treatment. The autoclave tape or other indicator on each container must be checked to verify color change before disposal.
  - c. The autoclave must be calibrated according to the manufacturer's instructions annually and a written record must be maintained.
  - d. Every 3 months, you must use a commercially available biological indicator kit that uses bacterial spores of *Geobacillus stearothermophilus* that are rendered unviable at 121 Celsius (250 Fahrenheit). You must follow the manufacturer's instructions. If any growth is observed, you must have the autoclave serviced and retested.
16. There is to be no further movement or distribution of the listed regulated materials/organisms within the United States and its territories unless the recipient holds, or is named as a responsible party on a valid PPQ526 permit for receipt of such materials/organisms.

**END OF PERMIT CONDITIONS**

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