Hellbender eDNA Citizen Science Protocol - Updated 25 Aug 2015

Site Selection

- **Before sampling, volunteers MUST complete a training/refresher course OR accompany a trained volunteer to one site.**
- Select sites from google map. https://www.google.com/maps/d/edit?hl=en&authuser=0&mid=zd-DXattx7T8.krigUx69ypPE Change marker color from red to gray after adding your name to the site.
- Red markers indicate available sites. Change the marker to black after you sample it.
- Email Kim if you have ideas for other sites. DO NOT sample other sites without discussing with her first.
- *Before* sampling, confirm that your site has suitable habitat and get permission for access (if needed).
- Suitable habitat = flowing water, large rocks (>1.5 ft across) and cobble. Avoid bedrock, sand, and silt.
- Samples should be collected when the river is clear, with **low or average flows**. Check USGS data before visiting sites http://waterwatch.usgs.gov/?m=real&r=va
- Collect samples **150 ft downstream** of suitable habitat.
- Collect samples during the hellbender breeding season (Aug 15 – Oct 15).
- *Do not disturb rocks or animals.*

Disinfection

- **Disinfect the sampling kit when you receive it, BEFORE collecting your samples.**
- Disinfect collection bottles, flasks, rubber stopper, blue adaptor, tubing, and cooler with 10% bleach for 30 minutes. Rinse with tap water and air dry.
- Disinfect tweezers with 50% bleach for 30 min, rinse with distilled water, air dry on clean paper towel.
- Wrap tweezers in a clean paper towel until use. Do NOT put them back into a dirty container.
- Rinse collection bottles an additional 3 times in stream water immediately before samples are collected.

Water Collection

- Label collection bottles (1A, 1B, 2A, 2B, 3A, 3B) with tape and a sharpie.
- Be careful not to touch the inside of the bottles or bottle caps.
- Target pools or areas of low stream flow, as DNA tends to accumulate in these places.
- Enter downstream from where samples will be collected, taking care not to kick up sediment.
- When collecting samples, face upstream with the bottle in front of your body to avoid contamination. Rinse each collection bottle 3 times with stream water.
- Collect 3 samples (**2 bottles each**) at the site, for a total of 6 bottles. Collect one sample in the center of the stream and the other 2 samples from each bank. If river volume restricts sampling to one bank, collect all 3 samples from this bank.
- Cap bottles immediately. Filter on site OR dry the outside of each bottle, place it in a plastic bag in a cooler with ice packs, and filter within 24 hours.
- Be sure you have a negative control to filter at the same time as the sample (see below).
- Record stream data (see data sheet) immediately after sample collection.

FILTRATION

IMPORTANT NOTES:
Try to collect your sample when the river is at its clearest. Sediment makes it very difficult to filter.

Electric Pump: Do not allow the filter cup to run dry, as this could eventually burn out the pump motor.

Hand Pump: The hand pump will hold a vacuum if the sample has high sediment, so you might not need to pump continuously. It's ok to let the filter cup run dry.

If it takes >1 hour to filter one bottle, you can filter less than two bottles per sample. MAKE SURE you note how much water was filtered. Keep in mind that I can't use the sample if you filter less than 1 bottle/sample.

SET UP

- Assemble the filtering apparatus
    - Put on a new pair of gloves
    - Attach hose to the side arm of the filtering flask. Attach the other end of the hose to the vacuum pump.
    - Place the rubber stopper (with plastic adaptor and hose) on top of the filtering flask.
    - Attach a new filter cup to the adaptor. Make sure everything fits tightly.

Filter the negative control BEFORE filtering the sample water.

1. Fill two clean collection bottles with negative control (i.e., distilled) water.
2. Carefully remove the filter lid and pour some of the water into the filter cup.
3. Start pumping! Continue to pour water into the cup as you pump.
4. After filtering the first bottle, remove the stopper and empty the flask.
5. After filtering the second bottle, twist off the top of the filter cup.
6. Put on a new pair of gloves.
7. Using disinfected tweezers, carefully fold the filter paper in half several times.
8. Pick up the filter paper with the tweezers and place it in the sample tube.
9. Label the top and side of the sample tube with the collection date (format: 10 Aug 2015) and site ID.
10. Empty the flask and attach a new filter.
11. Repeat steps 2 – 10 for the 3 stream samples.
12. You do not need to disinfect gear in between samples from the same site. However, you ***must*** filter the negative control first. If you forget to do this, you will need to disinfect everything before filtering the negative control.
13. Place the negative control tube in a clean sample bag and label it "NEG CON" with the date and site ID.
14. Place the stream sample tubes in a clean sample bag and label it with the date and site ID.
15. Please the sample bags in a freezer until ready to ship.

Make sure that nothing touches the filter paper, the tips of the tweezers, or the inside of the sample tube. In your datasheet, make a note of any mistakes or potential contamination.