Standard Operating Procedure (SOP) for Eelgrass DNA Extraction

About this resource:
This resource is a detailed laboratory procedure for isolating high-quality DNA from *Zostera marina* (eelgrass) tissue using a modified CTAB (cetyltrimethylammonium bromide) method. The protocol outlines reagent preparation, tissue processing, incubation steps, organic extractions, and DNA precipitation using isopropanol. It includes guidance on troubleshooting issues related to polysaccharides, optimizing spin conditions, and maximizing DNA yield and purity. The document also offers practical advice on resuspension, storage, and safe disposal of hazardous waste.

This protocol is intended for researchers conducting genetic or genomic analyses on seagrasses or other marine plants. It is designed for users with basic to intermediate laboratory experience in molecular biology, particularly those conducting genetic or genomic studies requiring high-quality DNA. The protocol is also useful for restoration scientists and graduate students involved in population genetics, conservation genomics, or ecological monitoring programs where DNA extraction from plant tissues is a foundational step.

Citation: Kamel, S.. (2025). *Standard Operating Procedure (SOP) for Eelgrass DNA Extraction*. NERRS Science Collaborative.

About the project:
This resource was developed through a 2022-2025 Collaborative Research project titled *Evaluating and Enhancing Eelgrass Resiliency and Restoration Potential in a Changing Climate*.

In the lower Chesapeake Bay, Virginia, warmer water temperatures in recent years have resulted in large scale diebacks of eelgrass meadows (Zostera marina). In contrast, many eelgrass populations in Back Sound, North Carolina appear to be more resilient to warming water temperatures. Understanding the drivers of these regional differences in eelgrass resilience could help more effectively restore eelgrass meadows in a changing climate.

With a network of the intended users from reserves, state agencies, and Chesapeake Bay nonprofits, this project compared resiliency traits of eelgrass populations in Virginia and North Carolina by conducting reciprocal restoration trials and genomic sequencing. The project results indicate the importance of seed sources in potential future eelgrass restoration, in addition to site selection.

This webpage provides more information about the project.

3x CTAB DNA Extraction Protocol for *Zostera marina*

3x CTAB recipe
10 ml 1M Tris HCl pH 8.0
35ml 4M NaCl
4ml 0.5M EDTA pH 8.0
3 gm CTAB
Nanopure water to 100ml

Autoclave

If you plan on using all the buffer very quickly, add **200 ul of 2-mercaptoethanol** to the 100ml volume. If you will be using the buffer over the course of 2-4 weeks, you can add 2-mercaptoethanol to each portion of the buffer as you are using it.

Add mercaptoethanol at the rate of 2ul/1 ml buffer.

This buffer has a limited shelf life. If the buffer is more than a few weeks old, you should probably consider making up a new batch.

General notes:
Volumes may be adjusted according to tissue sample sizes. Volumes of chloroform and chloroform:isopropanol should each be **1:1** to the volume of 3x CTAB buffer used.

Standard extraction protocol:

1. Use between 0.02 and 0.04g of ground eelgrass tissue.
2. Isolate tissue into 2.0ml tubes and add 800ul 3x CTAB buffer, and 4µl Proteinase K to each tube. To save time you can calculate how much buffer and Proteinase K you need for all your samples and combine them in a 15 or 50ml Falcon tube before aliquoting to each tube.
3. Incubate all samples at 55˚C and 500rpm in the Thermomixer for one hour.
4. Transfer to incubator and incubate overnight at 42˚C.

5. When tissue is completely dissolved, add 800µl of ice cold chloroform:isoamyl alcohol (24:1) to each tube and mix gently and thoroughly. Briefly pop open the caps to release the gas.

6. Spin for 12 minutes at 15,000rpm.

7. Carefully transfer the upper layer to a new 2ml tube and add 2ul of RNAase A

8. Incubate at 37°C for one hour.

9. Add 800µl of ice cold chloroform:isoamyl alcohol (24:1) to each tube and mix gently and thoroughly. Briefly pop open the caps to release the gas.

10. Spin for 12 minutes at 15,000rpm.

11. Carefully transfer the clean upper layer to a new tube and add 800ul of 100% ice cold isopropanol. Gently invert the tube several times to mix the layers. Placing the tubes in the freezer will increase the DNA yields. Samples can be placed in the freezer for a few minutes to overnight.

12. Pellet the DNA by spinning for 20 minutes at 4°C.

13. Gently tip off liquid being careful not to lose the pellet. If the pellet is slippery, you can also use a pipette tip to draw off liquid. Wash pellet in 700ul 70% EtOH. If salt is a problem in your samples, allow the 70% EtOH to sit in the tube at room temperature for a few minutes before spinning at room temperature for 5 minutes at 11,000rpm.

14. Repeat this wash-spin step.

15. Let samples dry. You can dry them by leaving them covered loosely on the counter several hours to overnight.

16. Re-suspend in 100µl of buffer. Make sure the samples are thoroughly re-suspended and mixed before quantifying.

NOTES AND SAFETY PRECAUTIONS:

5M NaCl Addition: High salt concentrations in the extraction buffer inhibit the co-precipitation of polysaccharides and DNA. If polysaccharides are a problem (many plants and marine organisms), the addition of 50µl of 5M NaCL per each 800µl sample can help. If polysaccharides are left behind, then the DNA recovered may be subject to degradation over time.

SPIN TEMPERATURES: These spins can be done at 4C or at room temp. Room temp keeps the salts in solution and helps get the chloroform completely out of the extraction. Spinning in the fridge helps precipitate DNA but also precipitates the salts.

ISOPROPANOL vs EtOH for DNA precipitation:
DNA precipitation is mediated by a combination of high salt concentrations and alcohol. Isopropanol precipitation requires 0.6-1 volume of alcohol and can be performed at room temperature which minimizes salt co-precipitation. Since isopropanol is typically cheaper and you don't need as much, this is a more common method. The DNA yields tend to be less than with EtOH precipitation. EtOH precipitation requires 2-3 volumes of alcohol and is typically carried out in the freezer. While cold will increase the co-precipitation of salts, the use of EtOH generally recovers more DNA.

RESUSPENDING DNA PELLETS:
A good volume to start with is 50-100 ul of buffer. Once you have done a few extractions and quantified your samples you will get a better idea of the correct volume for re-suspension. Pre-warming the buffer can help speed up the process. Samples can be left on the counter or placed in the refrigerator for several hours. After adding the buffer you should flick the tubes to dislodge the pellet to get it floating in the buffer, this allows for quicker resuspension.

DNA Storage: DNA is usually best stored in the freezer. Caution should be taken to minimize freeze-thaw cycles. If you do not have problems with degradation and your samples are high quality, storing them for a few days in the refrigerator may be okay. If you anticipate needing to access your samples frequently, your extraction protocol is long or tricky, your DNA is subject to degrading , or your DNA samples can not be replaced, you might want to consider making a working solution of your DNA and keeping the original stock DNA solution in a separate freezer for long term storage. The best places for storing DNA are freezers that tend to build up ice (non-frost free). They hold their temperature better and samples tend to last longer.

WASTE DISPOSAL:

Phenol and Chloroform solutions should only be used in the fume hood. They are both considered hazardous and need to be disposed of only in the labeled waste container in the fume hood. Please use the funnel to make sure waste goes in the bottle and is not spilled on the outside of the container. The label should have a date of when waste was first put in container, and it should be picked up by EH&S within 9 months of that date. Only put waste in the bottle if it is listed on the label. If you are not sure, ask.

Waste tips and tubes should be placed in the fume hood trash bin for proper disposal.