Seagrass Collection & Shipping Protocol

What to collect, where to collect it, and how to get it to the lab frozen — the field companion to the sequencing program.

Purpose

This document tells a collector three things: **what** species to prioritize, **where** the useful populations are, and **how** to handle and ship the tissue so it arrives frozen and sequence-ready. It pairs with the gDNA sampling steps (Section 5), which are unchanged from the field draft.

The sequencing goal is two data types per sample: a **whole genome** and a **methylome** (DNA methylation map). Both come from the same frozen leaf tissue, so a single clean collection serves both. Consistency of sampling matters more than volume — see the handling rules before going out.

Collection priorities

We are collecting where the public record is thin and the biology is most useful. Three tiers, in order:

Priority 1 — *Zostera marina*, paired hot and cold populations

Our primary target species. The scientific value is the **contrast**: collecting the same species from a cold site (Puget Sound) and a warm site (Southern California / N. Baja) gives us the naturally heat-adapted vs. cold-adapted methylomes that define which marks to switch on. Collect from **both ends of the temperature gradient**, ideally several plants per site. Proximity to the UC Berkeley / Decibel lab makes the California and Pacific Northwest sites the most practical to ship fresh-frozen.

Priority 2 — *Halodule wrightii*, the most heat-tolerant species

The single highest-value new genome. *Halodule* survives short-term exposure near 42 °C, yet **no public genome or methylome exists for it at all**. A clean collection here produces a genuinely first-of-its-kind resource and anchors the heat-tolerance work. Warmest accessible US populations are in Florida Bay / the Keys (~27 °C) and Tampa Bay; Texas (Laguna Madre) is a cooler-end contrast. *Thalassia testudinum* co-occurs at these sites and is worth collecting in parallel (Priority 2b — genome now public, but no methylome).

Priority 3 — *Cymodocea nodosa* & *Posidonia oceanica* (Mediterranean)

Comparative-reference species. Both already have public genomes, so the missing piece is the **methylome** — *Posidonia*'s naturally warm-adapted southern populations are the strongest natural warm-adaptation reference we can add. Lower urgency than Priorities 1–2 because they require international collection and shipping; collect opportunistically or through Mediterranean collaborators.

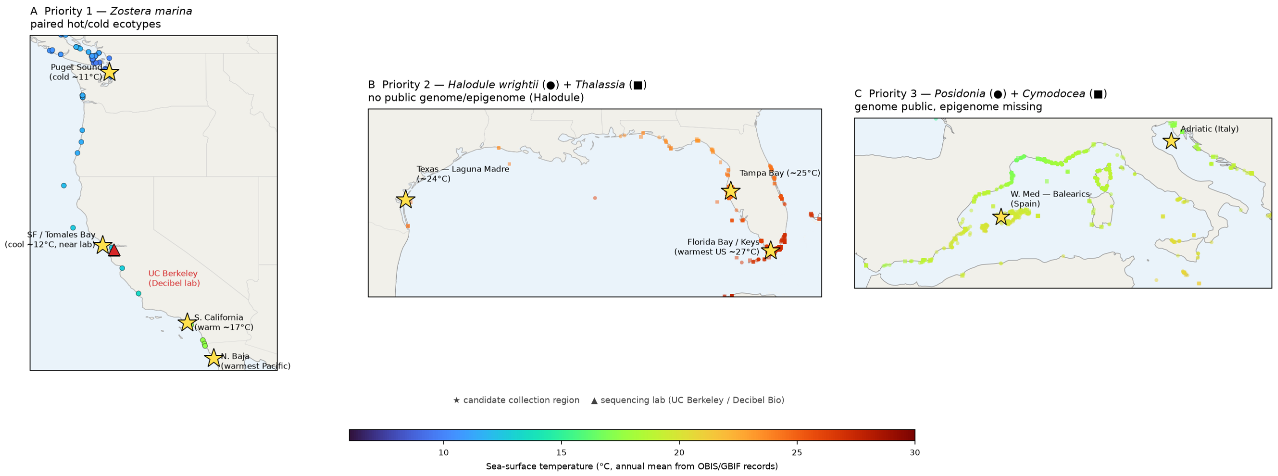

Figure 1. Collection priorities and candidate source regions. **(A)** *Zostera marina* across the NE Pacific thermal gradient — cold Puget Sound to warm Southern California / N. Baja — with UC Berkeley (Decibel lab) marked for shipping distance. **(B)** *Halodule wrightii* (circles) and *Thalassia testudinum* (squares) in the Gulf of Mexico and Florida, warmest US populations in Florida Bay / the Keys. **(C)** *Posidonia oceanica* (circles) and *Cymodocea nodosa* (squares) in the Mediterranean. Point colour is annual-mean sea-surface temperature from OBIS/GBIF occurrence records; stars mark candidate collection regions. Annual means understate summer maxima, so warm sites run hotter than shown in the growing season.

Candidate sites at a glance

PRIORITY	SPECIES	REGION	APPROX. SST	SHIPPING
1 (cold)	*Zostera marina*	Puget Sound, WA	~11 °C	Domestic
1 (cool)	*Zostera marina*	SF Bay / Tomales Bay, CA	~12 °C	Local to lab
1 (warm)	*Zostera marina*	S. California bays	~17 °C	Domestic
1 (warmest)	*Zostera marina*	N. Baja / San Quintín, MX	~17–18 °C	International
2	*Halodule wrightii*	Florida Bay / Keys, FL	~27 °C	Domestic
2	*Halodule wrightii*	Tampa Bay, FL	~25 °C	Domestic
2	*Halodule wrightii*	Laguna Madre, TX	~24 °C	Domestic
2b	*Thalassia testudinum*	Florida Bay / Keys, FL	~27 °C	Domestic
3	*Cymodocea nodosa*	W. Med (Balearics) / Adriatic	~18–20 °C	International
3	*Posidonia oceanica*	W. Med (Balearics) / Adriatic	~17–19 °C	International

Regions are drawn from OBIS/GBIF occurrence records and mark where the species is common, not specific permitted sites. Confirm access, collection permits, and any CITES/phytosanitary requirements for the specific location before sampling — this is especially important for international (Mexico, Mediterranean) collections.

How much to collect

- **Per site:** aim for **8–12 individual plants**, sampled far enough apart (meters) to avoid resampling the same clone. Seagrass spreads clonally, so nearby shoots may be genetically identical; spacing captures real genetic diversity.
- **Per plant:** one 2–3 inch leaf-tip segment is enough for both genome and methylome. A second segment in a separate tube is useful insurance if space and dry ice allow.
- **Label every tube** with site, plant number, date, and collector before freezing.

Specimen handling (gDNA sampling)

These steps are the field-tested protocol; follow them exactly. Consistency between plants is the single most important factor for clean methylome data.

1. **Before sampling, collect materials:** screw-cap tubes with labels **on the side**, forceps, scissors, a plastic freezer bag, a styrofoam box, and dry ice.
2. **Cut 2–3 inches of leaf tissue** with the scissors.

- Be **extremely consistent** across plants: sample roughly the same tissue age (don't mix an old damaged leaf from one plant with a young developing leaf from another) and the same part of the leaf (base vs. tip).
- Recommended: take the **tip of an undamaged leaf**.

1. **Fold the tissue** and use the forceps to stuff it into a screw-cap tube.
2. **Place the tube in the plastic freezer bag** — one bag holds all samples from a site; it makes the tubes easy to find in the lab.
3. **Cover the tube in dry ice as quickly as possible** for fast, even freezing. The faster tissue freezes, the better the DNA and methylation signal are preserved.
4. **Keep frozen until shipment.** Do not let samples thaw between collection and the lab.

Packaging & shipping

The rule is simple: **the samples must stay frozen from the field to the lab bench.** Dry ice sublimates (turns to gas, roughly 1 kg lost per 24 h from a typical cooler), so the whole process is built around keeping enough of it packed around the tubes and getting the box to the lab fast.

Safety first

Dry ice is **−78 °C** and turns directly into CO_2 gas. Two hazards to respect at every step:

- **Cold burns.** Always handle dry ice with **insulated gloves** and tongs — never bare skin. Wear eye protection when breaking up blocks.
- **Suffocation risk.** Subliming dry ice displaces oxygen. Work in a **ventilated space**, never in a sealed car cabin or small closed room, and never store the cooler in an airtight container.

Packing the box — step by step

1. **Confirm samples are already frozen.** Every tube should have been snap-frozen in dry ice at collection (handling step 5). Do not pack tubes that have warmed or thawed.
2. **Line the styrofoam cooler.** Use a hard-walled styrofoam box (or a styrofoam insert inside a cardboard carton). Put a **base layer of dry-ice pellets** (2–3 cm) across the bottom.
3. **Add the bagged tubes.** Place the freezer bag(s) of labeled tubes on the base layer. Keep the tubes together so they're easy to find on receipt.

4. **Bury the tubes in dry ice. Surround and cover the tubes completely** with dry-ice pellets — tubes in the middle of the ice, never sitting on top. Pellets pack more densely around small tubes than block ice; if only block ice is available, break it small. The goal is even, all-around cold.
5. **Fill void space.** Top up with dry ice and/or crumpled packing paper so the tubes **cannot shift** during transit. A partly empty box lets samples slide into a warm corner.
6. **Do not seal airtight.** Subliming CO_2 **must be able to vent** — leave the inner bag open or loosely closed, and use a styrofoam lid (not a vacuum seal). A sealed container will build pressure and can rupture.
7. **Close and tape the outer carton**, leaving the styrofoam vented. The outer cardboard can be taped normally; the venting happens through the styrofoam seams.

Amount and timing

- **How much dry ice:** carriers cap dry ice at **2.5 kg (5.5 lb) per package** for shipments on passenger aircraft, which is what most overnight air uses. Stay at or under that limit — **about 2 kg in a small styrofoam shipper is enough** to keep a dozen tubes frozen through a 24-hour overnight transit. There's no need to overpack: a small, well-insulated box with ~2 kg beats a large box with more.
- **Top off at the last moment.** Add a fresh layer of dry ice **right before carrier pickup**, not the night before.
- **Ship early in the week (Mon–Wed)** and **overnight** whenever possible, so a package never sits in a facility over a weekend. A 2-day ground shipment will usually arrive thawed.

Labeling and regulations

Dry ice is a regulated shipping material — **UN1845, IATA/DOT Class 9 (miscellaneous dangerous goods)**:

- The outer box needs a **Class 9 dry-ice hazard label** and the **UN1845 marking**, with the **net weight of dry ice in kg** written on it.
- The airway bill / shipping manifest must **declare dry ice and its weight** (see the per-package limit above).
- Most carriers (FedEx, UPS) handle dry-ice shipments routinely — **tell them it contains dry ice when you book**, and they'll apply the correct service and paperwork.
- **Frozen plant tissue** itself needs no special permit for **domestic US** shipment. **International shipments (Mexico, Mediterranean) additionally require phytosanitary / import clearance** — arrange this *before* collecting, since it can take time.

Ship to

Send frozen samples to Decibel Bio's lab, topped off with dry ice, overnight where possible:

> ***Decibel Bio***
> *c/o Brandon Pfannenstiel*
> *2625 Durant Avenue*
> *Berkeley, CA 94720*

Before shipping, email Brandon (brandon@decibel.bio) the expected **carrier, tracking number, and delivery date**, plus a quick manifest (species, sites, number of tubes) so the lab can receive and re-freeze the samples immediately on arrival. Avoid shipments that would arrive on a weekend unless arranged in advance.

Quick field checklist

- [ ] Permits / site access confirmed (and phytosanitary clearance for international sites)
- [ ] Materials: labeled screw-cap tubes, forceps, scissors, freezer bags, styrofoam shipper, **dry ice (~2 kg; ≤2.5 kg per package)**, insulated gloves

- [ ] 8–12 spaced plants per site; one labeled leaf-tip tube per plant
- [ ] Consistent tissue age and leaf position across all plants
- [ ] Tubes frozen in dry ice immediately, kept frozen
- [ ] Box packed with tubes buried in dry ice, vented, Class 9 / UN1845 labeled
- [ ] Overnight shipment booked; tracking + manifest emailed to Decibel before pickup

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Good Ancestor Foundation

Seagrass Climate Resilience Project — goodancestor.com

Internal field protocol. Genomic DNA (gDNA) sampling for whole-genome and methylome sequencing.