



Environmental Analysis Program

Pomona College
Biogeochemistry Laboratory

SOP-L700

DNA Extraction, Quantification, and Purity Assessment

Standard Operating Procedure

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1. Purpose

This procedure describes the extraction of genomic DNA from environmental samples (soil, sediment, water filters, and plant tissue), followed by quantification of DNA concentration and assessment of DNA purity using spectrophotometric and fluorometric methods. The protocol is based on commercial bead-beating extraction kits and is suitable for downstream applications including PCR amplification, qPCR, and amplicon sequencing (Qiagen, 2024a, 2024b).

2. Scope

- **Applies to:** Genomic DNA extraction from soil, sediment, water filter membranes, and plant tissue using the DNeasy PowerSoil Pro Kit (Qiagen) or equivalent bead-beating kit; DNA quantification using a NanoDrop spectrophotometer and Qubit fluorometer
- **Does not apply to:** RNA extraction, plasmid isolation, ancient DNA, or clinical/diagnostic samples
- **Location:** Molecular biology bench, Pomona College Biogeochemistry Lab

3. Responsibilities

Role	Responsibility
Lab PI	Approve SOP, ensure training compliance
Trained Personnel	Follow this SOP, report deviations
New Students	Complete training before independent use

4. Definitions

- **A_{260}/A_{280} ratio:** The ratio of absorbance at 260 nm to 280 nm; pure dsDNA yields ~1.8; values below 1.7 indicate protein or phenol contamination that may inhibit PCR amplification.
- **A_{260}/A_{230} ratio:** The ratio of absorbance at 260 nm to 230 nm; values of 2.0–2.2 indicate pure nucleic acids; low values indicate contamination with chaotropic salts, humic acids, or carbohydrates.
- **Bead beating:** Mechanical cell lysis in which sample is agitated at high speed with ceramic or glass beads in a tube; disrupts cell walls and membranes including the tough walls of fungal spores and gram-positive bacteria.
- **Chaotropic salt:** A salt (e.g., guanidinium isothiocyanate, guanidinium hydrochloride) that disrupts hydrogen bonds and hydrophobic interactions; used in DNA extraction to denature proteins and facilitate binding of DNA to silica membranes.
- **Environmental DNA (eDNA):** Genetic material shed into the environment by organisms through mucus, feces, skin cells, and gametes; collected from soil, water, or air without organism capture.
- **Inhibitor:** A substance co-extracted with DNA that reduces PCR amplification efficiency; common inhibitors in soil and water eDNA include humic acids, tannins, heavy metals, and residual ethanol.

- **Silica column (spin column):** A miniaturized chromatography column containing a silica membrane that selectively binds DNA in the presence of chaotropic salts; DNA is washed and then eluted with low-salt buffer or water.

5. Safety and Hazards

Hazards

- **Chemical:** Lysis buffer contains guanidine salts, which are harmful if swallowed or inhaled and can cause skin and eye irritation. Solution C4 contains ethanol. Review the Safety Data Sheets (SDS) for all kit reagents before use.
- **Physical:** Bead-beating generates aerosols; keep tubes sealed during vortexing. Centrifuge rotor imbalance can cause equipment damage.
- **Biological:** Environmental samples may contain unknown pathogens. Treat all samples as potentially infectious (Centers for Disease Control and Prevention & National Institutes of Health, 2020).

5.1. Required PPE

- ☐ Safety glasses or goggles
- ☐ Lab coat
- ☐ Nitrile gloves (change frequently to avoid cross-contamination)
- ☐ Face shield (if working with large volumes of lysis buffer)

5.2. Emergency Procedures

In case of chemical splash or skin/eye contact, flush affected area with water for at least 15 minutes and consult the SDS. For spills of lysis buffer, absorb with paper towels, clean with water, and dispose as chemical waste. Refer to [SOP-L003 Hazardous Materials Handling](#) for general chemical spill response.

6. Equipment and Materials

6.1. Equipment

Item	Location	Notes
Vortex adapter for bead tubes	Mol bio bench	Holds 24 tubes horizontally
Vortex-Genie 2	Mol bio bench	Set to maximum speed
Microcentrifuge	Mol bio bench	Must reach 10,000 x g minimum
NanoDrop One spectrophotometer	Instrument bench	UV-Vis micro-volume
Qubit 4 fluorometer	Instrument bench	For dsDNA quantification
Micropipettes (10, 100, 1000 uL)	Mol bio bench	Calibrated annually

Item	Location	Notes
–80 C freezer	Cold room	For long-term DNA storage
–20 C freezer	Lab	For short-term DNA storage

6.2. Reagents and Consumables

Item	Catalog No.	Storage
DNeasy PowerSoil Pro Kit (Qiagen)	47014 (50 prep)	Room temperature
Qubit dsDNA BR Assay Kit	Q32850	Room temperature
Qubit dsDNA HS Assay Kit	Q32851	Room temperature
Qubit assay tubes (0.5 mL)	Q32856	Room temperature
Nuclease-free water	Various	Room temperature
Molecular-grade ethanol (100%)	Various	Flammables cabinet
1.5 mL microcentrifuge tubes (DNase-free)	Various	Room temperature
Filter pipette tips (10, 100, 1000 uL)	Various	Room temperature

7. Procedure

7.1. Sample Preparation

1. Record sample identity, collection date, and mass/volume in the lab notebook and in the [Freezer Inventory Google Sheet](#)
2. For soil/sediment: weigh 0.20–0.25 g of sample into the PowerBead Pro tube. Record exact mass.
3. For water filters: use sterile forceps to transfer the membrane filter into the PowerBead Pro tube. Cut the filter into strips if needed to fit inside the tube.
4. For plant tissue: weigh 0.02–0.10 g of tissue (fresh or flash-frozen) into the PowerBead Pro tube. Use less tissue for highly mucilaginous samples (Doyle & Doyle, 1987).

! Important

Keep samples on ice during preparation to minimize DNA degradation by endogenous nucleases. Process all samples within a single batch if possible to reduce extraction variability (Frosteg aard et al., 1999).

7.2. Cell Lysis and DNA Extraction

Follow the DNeasy PowerSoil Pro Kit protocol (Qiagen, 2024b):

1. Add 800 uL of Solution CD1 to the PowerBead Pro tube
2. Secure tubes horizontally on the vortex adapter and vortex at maximum speed for 10 minutes
3. Centrifuge at 15,000 x g for 1 minute

4. Transfer the supernatant to a clean 2 mL collection tube
5. Add 200 uL of Solution CD2, vortex briefly (5 seconds), and centrifuge at 15,000 x g for 1 minute
6. Avoiding the pellet, transfer the supernatant to a clean 2 mL collection tube
7. Add 600 uL of Solution CD3, vortex briefly (5 seconds)
8. Load 650 uL onto an MB Spin Column seated in a 2 mL collection tube. Centrifuge at 15,000 x g for 1 minute. Discard flow-through. Repeat to load remaining lysate.
9. Add 500 uL of Solution EA. Centrifuge at 15,000 x g for 1 minute. Discard flow-through.
10. Add 500 uL of Solution C5 (wash buffer). Centrifuge at 15,000 x g for 1 minute. Discard flow-through.
11. Centrifuge again at 16,000 x g for 2 minutes to remove residual wash buffer
12. Transfer the MB Spin Column to a clean 1.5 mL microcentrifuge tube
13. Add 50–100 uL of Solution C6 (elution buffer) or nuclease-free water to the center of the membrane. Allow to stand for 1 minute at room temperature, then centrifuge at 15,000 x g for 1 minute.

 Tip

Use a smaller elution volume (50 uL) when higher DNA concentration is needed for downstream applications. A second elution with an additional 50 uL can recover 10–20% more DNA but at lower concentration (Qiagen, 2024b).

7.3. DNA Quantification – NanoDrop Spectrophotometry

The NanoDrop measures DNA concentration and provides absorbance ratios used to assess purity (Desjardins & Bhatt, 2010):

1. Clean the pedestal with 2 uL of nuclease-free water; wipe with a lint-free tissue
2. Blank the instrument with 2 uL of the elution buffer (Solution C6 or nuclease-free water, matching your elution)
3. Load 2 uL of DNA extract onto the pedestal
4. Record the concentration (ng/uL), A_{260}/A_{280} ratio, and A_{260}/A_{230} ratio for each sample

7.4. DNA Quantification – Qubit Fluorometry

The Qubit fluorometer uses fluorescent dyes that bind selectively to dsDNA, providing more accurate quantification than spectrophotometry, especially at low concentrations or in the presence of contaminants (Thermo Fisher Scientific, 2024):

1. Prepare the Qubit working solution: dilute the Qubit dsDNA BR (or HS) reagent 1:200 in Qubit buffer. Prepare enough for all samples plus two standards (e.g., 200 uL per assay tube).
2. Prepare standards: add 190 uL of working solution to each of two Qubit assay tubes. Add 10 uL of Standard 1 to one tube and 10 uL of Standard 2 to the other. Vortex briefly.
3. Prepare samples: add 198 uL of working solution to a Qubit assay tube. Add 2 uL of DNA extract. Vortex briefly.

4. Incubate all tubes at room temperature for 2 minutes (but not longer than 30 minutes)
5. Calibrate the Qubit with the two standards, then read each sample
6. Record the concentration (ng/uL, corrected for dilution by the instrument)

7.5. DNA Purity Assessment

Evaluate DNA purity using the NanoDrop absorbance ratios (Desjardins & Bhatt, 2010; Wilfinger et al., 1997):

Ratio	Ideal Range	Interpretation
A_{260}/A_{280}	1.8–2.0	Values below 1.8 suggest protein or phenol contamination
A_{260}/A_{230}	2.0–2.2	Values below 2.0 suggest contamination with humic acids, carbohydrates, salts, or residual guanidine from extraction buffers

Note

For environmental DNA extracts, especially from humic-rich soils, A_{260}/A_{230} ratios are frequently below 2.0. If downstream applications (e.g., PCR) fail or show inhibition, consider additional cleanup using a OneStep PCR Inhibitor Removal Kit or gel purification (Young et al., 2014).

7.6. Storage and Freezer Inventory

1. Label each tube with: sample ID, extraction date, elution volume, and concentration (in ng/uL)
2. For short-term storage (less than 1 month): store DNA extracts at -20°C
3. For long-term storage: store DNA extracts at -80°C in the designated molecular biology section of the freezer
4. Record the storage location (freezer, rack, box, and position) in the [Freezer Inventory Google Sheet](#)
5. Update the Google Sheet immediately upon placing samples in the freezer or removing them

-80°C Freezer Protocol

- Minimize the time the freezer door is open. Retrieve the correct box before opening the freezer.
- Place DNA extracts in designated cryoboxes labeled “DNA Extracts” with the project name and date range.
- Do not store DNA extracts in the same box as tissue samples or reagents.
- Avoid repeated freeze-thaw cycles. If multiple downstream analyses are planned, prepare aliquots at the time of extraction (Shao et al., 2012).

8. Quality Control and Acceptance Criteria

- Include one extraction blank (empty PowerBead Pro tube processed through the full protocol) per extraction batch to detect contamination (Salter et al., 2014)
- Run Qubit standards with each new batch of working solution
- Acceptable DNA yield from 0.25 g of soil: typically 1–20 ng/uL in a 100 uL elution, depending on soil type (Dineen et al., 2010)
- Acceptable A_{260}/A_{280} : 1.7–2.0. Samples outside this range should be noted and may require re-extraction.
- Acceptable A_{260}/A_{230} : greater than 1.5 for most downstream applications. Values below 1.0 indicate significant contamination and the extract should be purified or re-extracted.
- If NanoDrop and Qubit concentrations disagree by more than a factor of two, the NanoDrop reading is likely inflated by contaminants; use the Qubit value for planning downstream reactions (Nakayama et al., 2016).

9. Waste Disposal

Waste Type	Container	Disposal Method
Used PowerBead Pro tubes	Biohazard bag	Autoclave, then standard trash
Flow-through from spin columns	Chemical waste carboy	EH&S hazardous waste pickup (contains guanidine salts)
Used spin columns	Chemical waste bag	EH&S hazardous waste pickup
Pipette tips and tubes	Biohazard sharps/trash	Standard biohazard disposal
Empty reagent bottles	Rinse and recycle	Check SDS for specific instructions

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11. Training Record

Before performing this procedure independently, personnel must:

1. Read this SOP and [SOP-L003 Hazardous Materials Handling](#) in full
2. Review the SDS for all kit components (especially Solutions CD1, CD2, and CD3)
3. Complete the Qiagen DNeasy PowerSoil Pro Kit quick-start guide
4. Demonstrate competence with micropipetting, the NanoDrop, and the Qubit fluorometer
5. Perform a full extraction under supervision, including quantification and freezer logging
6. Be signed off by the PI or lab manager

12. Appendix A: Troubleshooting

Symptom	Probable Cause	Corrective Action
Very low DNA yield	Insufficient lysis; too little starting material	Increase vortexing time to 15 min; verify sample mass
Low A_{260}/A_{280} (below 1.6)	Protein carryover	Re-extract with fresh kit reagents; ensure full removal of CD2 pellet

Symptom	Probable Cause	Corrective Action
Low A_{260}/A_{230} (below 1.0)	Humic acid or guanidine carryover	Add a second C5 wash step; use inhibitor removal kit
NanoDrop reads much higher than Qubit	Co-extracted contaminants inflating UV absorbance	Trust Qubit value; consider cleanup before downstream use
PCR inhibition despite good yield	Co-extracted humic acids or polysaccharides	Dilute template 1:10; use inhibitor-tolerant polymerase; clean up with OneStep kit (Young et al., 2014)
Extraction blank shows amplification	Reagent or cross-contamination	Discard batch; use fresh reagents; clean workspace with 10% bleach then ethanol

13. Appendix B: DNA Concentration Calculation Worksheet

```
# Calculate total DNA yield from extraction
elution_vol_uL <- 100      # Volume of elution buffer used (uL)
qubit_conc_ng_uL <- 8.5    # Qubit reading (ng/uL)
sample_mass_g <- 0.25      # Starting sample mass (g)

total_dna_ng <- qubit_conc_ng_uL * elution_vol_uL
dna_per_g <- total_dna_ng / sample_mass_g

cat(sprintf("Total DNA yield: %.1f ng\n", total_dna_ng))
```

Total DNA yield: 850.0 ng

```
cat(sprintf("DNA yield per gram of sample: %.1f ng/g\n", dna_per_g))
```

DNA yield per gram of sample: 3400.0 ng/g