APC0266 ABL Data Report – Coastal Seining Collection

## Objectives – As Per the Work Plan Agreement

1. Extract and quantify 64 eDNA samples.
2. Develop metabarcoding libraries for COI-1 and 12S for a total of 2 libraries.
3. Sequence COI and 12S libraries on the MiSeq.
4. Provide raw sequencing data and corresponding sample index information.
5. Provide ABL Report upon completion of the project.

## 1. Sample Extraction & Quantification

A total of 64 eDNA samples were received, provided as glass fiber filters preserved in ethanol. These consisted of 48 Samples and 16 Field Blanks, collected from six different stations and, with the exception of Keji Seaside, at three different times from May to October of 2024 (Table 1).

All samples were extracted using the Qiagen DNeasy Blood and Tissue Kit (240) for the QIAcube Connect according to ABL’s modified eDNA extraction protocol. For example, sample filters were cut in half and digested in the Qiagen Lyse and Spin Baskets. Each set of extractions consisted of 11 samples and one Extraction Negative (ENEG), producing a total of six ENEGs. All extracted samples, Field Blanks, and ENEGs were quantified using the dsDNA High Sensitivity Assay Kit (ThermoFisher) for the Qubit 4 Fluorometer.

In general, eDNA concentrations were much higher than those from the Perley Offshore collection. The highest eDNA concentrations were observed in samples from the Keji Seaside, while the lowest concentrations were observed in the samples collected from Goldboro in May (Table 1). All ENEGs had DNA concentrations that were below quantifiable limits (BQL) for the Qubit Fluorometer, and considered to be 0 ng/µL. However, all of the Field Blanks had measurable DNA concentrations, some of which were as high as sample concentrations (Table 1). This may have implications for downstream analyses of the metabarcoding libraries.

**Table 1:** The number of eDNA filter samples and Field Blanks processed from each station, the collection dates, and average eDNA concentration of samples by station.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Station | Date Collected | No. of Replicates | No. of Field Blanks | Aver. eDNA Sample Conc. (ng/µL) | Field Blank Conc. (ng/µL) |
| Conrod Beach | 16-May-24 | 3 | 1 | 26.53 | 0.894 |
| 02-Jul-24 | 3 | 1 | 35.07 | 5.91 |
| 29-Oct-24 | 3 | 1 | 24.27 | 0.739 |
| Goldboro | 22-May-24 | 3 | 1 | 5.95 | 0.829 |
| 17-Jul-24 | 3 | 1 | 35.40 | 2.63 |
| 29-Oct-24 | 3 | 1 | 22.60 | 0.590 |
| Keji Seaside | 19-Jul-24 | 3 | 1 | 44.67 | 0.636 |
| Little Harbour | 16-May-24 | 3 | 1 | 33.60 | 1.67 |
| 03-Jul-24 | 3 | 1 | 31.43 | 13.0 |
| 29-Oct-24 | 3 | 1 | 24.37 | 0.804 |
| Moosehead | 21-May-24 | 3 | 1 | 23.60 | 2.82 |
| 16-Jul-24 | 3 | 1 | 21.33 | 5.47 |
| 29-Oct-24 | 3 | 1 | 22.53 | 0.454 |
| TaylorHead | 17-May-24 | 3 | 1 | 21.17 | 1.28 |
| 04-Jul-24 | 3 | 1 | 15.10 | 11.10 |
| 29-Oct-24 | 3 | 1 | 30.13 | 0.607 |

## 2. Develop metabarcoding libraries for COI and 12S

The loci used for metabarcoding were:

* COI-1:
  + mICOIintF / jgHCO2198 (Lacoursière‐Roussel *et al*. (2018))
    - mlCOIlintF (Leray *et al*. (2013))
      * Sequence: GGWACWGGWTGAACWGTWTAYCCYCC
    - jgHCO2198 (Geller *et al*. (2013))
      * Sequence: ACTTTCGTTCTTGATYRA
* 12S:
  + 12S\_248F\_RADS\_For / Mifish\_UR\_Miya (He *et al*. (2023))
    - 12S\_248F\_RADS\_For (He *et al*. (2022))
      * Sequence: CGTGCCAGCCACCGCGGTT
    - Mifish\_UR\_Miya (Miya *et al*. (2015))
      * Sequence: CATAGTGGGGTATCTAATCCCAGTTTG

The eDNA samples were randomized prior to the PCR reactions to avoid bias and plated to a 96-well LoBind plate according to the randomized sample number. Each library started with 70 samples. PCR reactions were performed with three replicates per sample. The PCR replicates were pooled and screened on the TapeStation 4200 (Agilent Technologies) to confirm amplification in the samples and to ensure there was no amplification in any associated PCR negatives (PNEG). If no amplification was observed in a sample or if a PNEG showed amplification the sample was redone. Three samples would not amplify for COI-1 (MCRG24\_31, MCRG24\_35, and MCRG24\_47) and one for 12S (MCRG24\_47), after three rounds of PCRs. Therefore, these samples were excluded from their respective libraries.

Once verified, the PCR product was purified using the AMPure XP Reagent (Beckman Coulter), followed by quantification using the AccuClear Ultra High Sensitivity dsDNA Quantitation kit (Biotium). Purified PCR products were multiplexed with equal amounts per sample when possible, with a target input of 50 ng/sample in (10 µL) for COI and 12S libraries. For samples with very low DNA (such as Field Blanks and Extraction Negatives), a maximum of 10µL was added to void diluting the final library concentration.

The multiplexed libraries were purified using AMPure XP Reagent, then quantified using the NEBNext Library Quant kit for Illumina (New England Biolabs) on the QuantStudio 7 Flex (Applied Biosystems). Two libraries were developed, one library for each target, with 67 and 69 randomly assigned samples and controls for COI-1 and 12S respectively.

## 3. COI and 12S Library Sequencing on the MiSeq

All libraries were sequenced using the MiSeq platform (Illumina). The MiSeq Reagent Kit v3 (600-cycle) was used for the COI-1 library, while the v2 kit (500-cycle) was used for the 12S library.

The COI-1 library was prepared to a final concentration of 13pM, with a 15% PhiX spike-in (13pM). This was decreased from the 15pM library/PhiX concentrations from APC0264 because there was evidence of over-clustering. For this project, there was a small improvement in the clusters passing filter (83.55% ± 3.33); however, the % >Q30 slightly decreased (Table 2.). The COI-1 library run metrics looked good for the first read; the Intensity, Clusters PF, and % Aligned looked really good and were fairly consistent across surfaces of the flow cell.

**Table 2.** Summary of sequencing run parameters and data output quality metrics.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Library/Run | MiSeq Reagent Kit/Chemistry | Library Input (pM) | PhiX Spike-in (%) | % > Q30 | Cluster Density (K/mm2) | Clusters PF (%) | Data Yield (Gb) |
| APC0266 COI-1 R1 | v3 (600-cycle)  PE | 13 | 15 | 76.20 | 1092 ± 21 | 83.55 ± 3.33 | 14.51 |
| APC0264 COI-1 A R1 | v3 (600-cycle)  PE | 15 | 15 | 80.05 | 1118 ± 18 | 79.4 ± 13.0 | 14.0 |
| APC0266 12S R1 | v2 (500-cycle)  PE | 7 | 20 | 76.96 | 774 ± 43 | 91.95 ± 1.78 | 8.20 |

PE = paired end

The 12S (ribosomal RNA) library was prepared to a final concentration of 7pM with a 20% PhiX spike-in (7pM). The sequencing run metrics looked good, with output quality metrics being close to or within optimal ranges for the kit chemistry (Table 2). The indexing looked good as well, with the % reads being fairly even across samples. The Field Blanks and ENEGs had the lowest % reads, as expected. However, samples MCRG24\_031 and MCRG24\_35 had reads as low as the blanks. This is not surprising considering the difficulties getting these samples to amplify (and which did not amplify for COI).

## 4. Provide Raw Sequencing Data

The sequence data for each of the COI-1 and 12S library sequencing runs were retrieved from the MiSeq. The sequence data (.fasta) and metadata were uploaded to the shared Teams project page “APC0266 Coastal Seining Collection”.

## Conclusions

##### Extract & quantify 140 eDNA

* 64 samples (SmithRoot filters preserved in ethanol) from six different stations (Conrod Beach, Goldboro, Keji Seaside, Little Harbour, Moosehead, and TaylorHead) were provided to the ABL.
* All samples were extracted from half-filter using Qiagen’s DNeasy Blood and Tissue kit for the QIAcube, with 6 Extraction Negatives being generated. The DNA was quantified using the Qubit dsDNA HS Assay for the Qubit v4.

##### Develop libraries for COI-1 and 12S and sequence on the MiSeq

* One library was produced for COI-1 and 12S (with 67 and 69 randomized samples respectively), for a total of 2 libraries.
* The COI-1 library was successfully sequenced on the MiSeq.
* The 12S library was also successfully sequenced on the MiSeq. There were no obvious issues with the runs; the sequencing output values were within optimal ranges.
* The raw sequencing data for the COI-1 and 12S libraries were uploaded to the Teams project page, “APC0266 Coastal Seining Collection”.

## References

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