Contents

1 Protocol Template – PCR

1.1 PROTOCOL INFORMATION

1.1.1 Minimum Information about an Omics Protocol (MIOP)

- MIOP terms are listed in the YAML frontmatter of this page.
- See MIOP_definition.md for list and definitions.

1.1.2 Making eDNA FAIR (FAIRe)

- FAIRe terms are listed in the YAML frontmatter of this page.
- See https://fair-edna.github.io/download.html for the FAIRe checklist and more information.
- See https://fair-edna.github.io/guidelines.html#missing-values for guidelines on missing values that can be used for missing FAIRe or MIOP terms.

1.1.3 Authors

- All authors known to have contributed to the preparation of this protocol, including those who filled in the template.
- Visit https://orcid.org/ to register for an ORCID.
- Date is the date the author first worked on the protocol.

PREPARED BY	AFFILIATION	ORCID	DATE
Content Cell	Content Cell	Content Cell	yyyy-mm-dd

PREPARED BY	AFFILIATION	ORCID	DATE
Content Cell	Content Cell	Content Cell	yyyy-mm-dd

1.1.4 Related Protocols

- This section contains protocols that should be known to users of this protocol.
- Include the link to each protocol.
- Include the version number and release date (if available).
- Internal/External: "Internal" are derivative or altered protocols, or other protocols in this workflow. "External" are protcols from manufacturers or other groups.

PROTOCOL NAME	LINK	VERSION	RELEASE DATE	INTERNAL/EXTERNAL
Content Cell	Content Cell	Content Cell	yyyy-mm-dd	Content Cell
Content Cell	Content Cell	Content Cell	yyyy-mm-dd	Content Cell

1.1.5 Protocol Revision Record

- Version numbers start at 1.0.0 when the protocol is first completed and will increase when changes that impact the outcome of the procedure are made (patches: 1.0.1; minor changes: 1.1.0; major changes: 2.0.0).
- Release date is the date when a given protocol version was finalised.
- Description of revisions includes a brief description of what was changed relative to the previous version.

VERSION	RELEASE DATE	DESCRIPTION OF REVISIONS
1.0.0	yyyy-mm-dd	Initial release

1.1.6 Acronyms and Abbreviations

ACRONYM / ABBREVIATION	DEFINITION
Content Cell	Content Cell
Content Cell	Content Cell

1.1.7 Glossary

SPECIALISED TERM	DEFINITION
Content Cell	Content Cell
Content Cell	Content Cell

1.2 BACKGROUND

This document describes the required protocol to conduct [insert name of the method/protocol].

1.2.1 Summary

Insert a short description of the background for the method/protocol (e.g. why and for which purpose do you perform water sampling). Please provide a brief summary of your method including, as appropriate, a brief description of what techniques your best practice is about, which ocean environments or regions it

targets, the primary sensors covered, what type of data/measurements/observing platform it covers, limits to its applicability.

1.2.2 Method Description and Rationale

Insert a short description of the functioning principal of the methodology used in the protocol (i.e. how does the method work?). Please note that this is different from the step-by-step description of the protocol procedure. Insert a short statement explaining why the specific methodology used in the protocol has been selected (e.g. it is highly reproducible, highly accurate, procedures are easy to execute etc….).

1.2.3 Spatial Coverage and Environment(s) of Relevance

If applicable, please specify the region where the protocol is applied. For regional term guidance see the GAZ ontology. If applicable, please indicate here the environment(s) of relevance for the protocol, e.g. Abyssal plain. Select from the ENVO ontology.

1.3 PERSONNEL REQUIRED

Insert the number of technicians, data managers, and scientists required for the good execution of the procedure

1.3.1 Safety

Identify hazards associated with the procedure and specify protective equipment and safety training required to safely execute the procedure

1.3.2 Training Requirements

Specify technical training required for the good execution of the procedure.

1.3.3 Time Needed to Execute the Procedure

Specify how much time is necessary to execute the procedure.

1.4 EQUIPMENT

- Opentrons Consumables: If using Opentrons OT-2 Robot for KF Plate Prep.
- Description: E.g., “filter”.
- Product Name and Model: Provide the official name of the product.
- Manufacturer: Provide the name of the manufacturer of the product.
- Quantity: Provide quantities necessary for one application of the standard operating procedure (e.g., number of filters).
- Remark: For example, some of the consumable may need to be sterilized, some commercial solution may need to be diluted or shielded from light during the operating procedure.

DESCRIPTION	PRODUCT NAME AND MODEL	MANUFACTURER	QUANTITY	REMARK
Durable equipment				
Content Cell	Content Cell	Content Cell	Content Cell	Content Cell
Content Cell	Content Cell	Content Cell	Content Cell	Content Cell
Consumable equipment				
Content Cell	Content Cell	Content Cell	Content Cell	Content Cell
Content Cell	Content Cell	Content Cell	Content Cell	Content Cell
Chemicals				
Content Cell	Content Cell	Content Cell	Content Cell	Content Cell
Content Cell	Content Cell	Content Cell	Content Cell	Content Cell

1.5 STANDARD OPERATING PROCEDURE

In the following SOP, please use the exact names of equipment as noted in the table above.

Provide a step-by-step description of the protocol. The identification of difficult steps in the protocol and the provision of recommendations for the execution of those steps are encouraged.

1.5.1 Preparation

Please specify the preparatory actions you took before you collected the samples and note what equipment was needed to do so (e.g. disinfection of work surfaces, preparations to the equipment you intend to use later on).

1. [Step 1]
2. [Step 2]

1.5.2 PCR

Please specify the actions you took to amplify the previously extracted DNA and the equipment and primers you used (ingredients for the PCR reaction, number of triplicates, PCR cycle parameter)

Primers: PCR primer sequences

PCR Primer Name	Direction	Sequence (5' -> 3')
content	forward	content
content	reverse	content

Reaction Mixture: PCR reagents, volumes, initial and final concentrations

Reagent	Volume	Initial Concentration	final concentration
content	content	content	content
content	content	content	content

PCR Cycling Program:

PCR Step	Temperature	Duration	Repetition
content	content	content	content
content	content	content	content

1. [Step 1]
2. [Step 2]

1.5.3 Quality Control

Please specify the actions you took to confirm the quality of the PCR output, to clean up the PCR output and the equipment you used (e.g. agarose gel to confirm quality, purification of PCR products).

1. [Step 1]
2. [Step 2]

1.5.3.1 Positive Control Please include information about any positive controls, used in every PCR run to verify success of the PCR reaction. This should include a description of the sequence(s), the concentration and volume added, and the reference sequence(s).

1.5.3.2 Negative Control Please include information about any negative controls, such as PCR-grade water used as a no template control (NTC) when setting up each PCR plate.

1.5.4 PCR Clean-up

Please specify the actions you took to clean up the PCR output and which equipment you used for this (e.g. agarose gel to confirm quality, purification of PCR products).

1. [Step 1]
2. [Step 2]

1.5.5 Basic Troubleshooting Guide

- Identify known issues associated with the procedure, if any.
- Provide troubleshooting guidelines when available.

1.6 REFERENCES

- Insert all references cited in the document.
- Please insert full DOI address when available, e.g. http://doi.dx.org/10.1007/s11258-014-0404-1.

1.7 APPENDIX A: DATASHEETS

Link templates (e.g. preformatted spreadsheets) used to record measurements and report on the quality of the data as well as any documents such as manufacturer specifications, images, etc that support this protocol. Please include a short note describing the document's relevance.