



DEVELOPING A HIGH-THROUGHPUT GENETIC METHOD TO ELUCIDATE THE DIVERSITY AND ABUNDANCE OF ZOOPLANKTON FROM SOUTHERN- OCEAN CONTINUOUS PLANKTON RECORDER (CPR) COLLECTIONS

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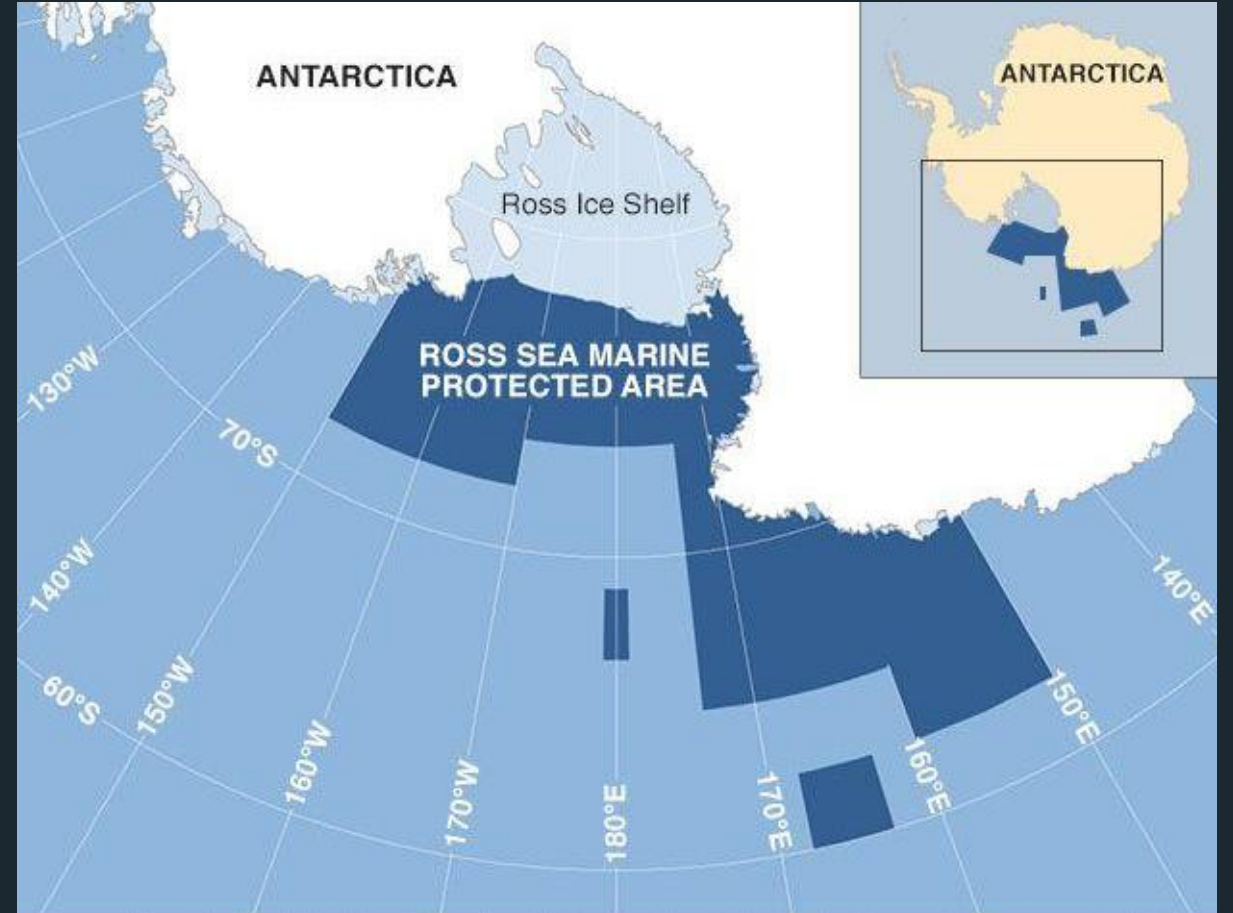
01

INTRODUCTION



ZOOPLANKTON IN THE SOUTHERN OCEAN

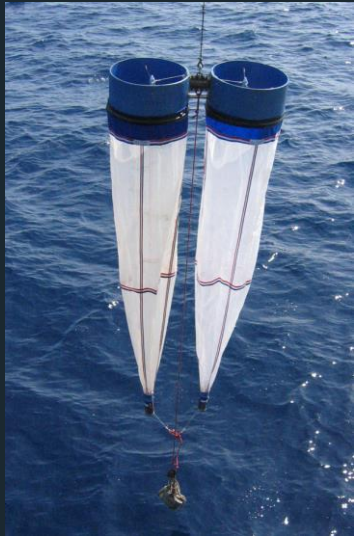
- Zooplankton are important to Southern Ocean ecosystems and carbon flux
- Climate Change Predictions:
 - Increased temperature*
 - Acidification*
 - Shifts in sea ice coverage*
 - Increased upwelling*
- Marine Protected Area
 - Climate change vs fishing pressure*



Credit: BBC

CURRENT METHODOLOGIES

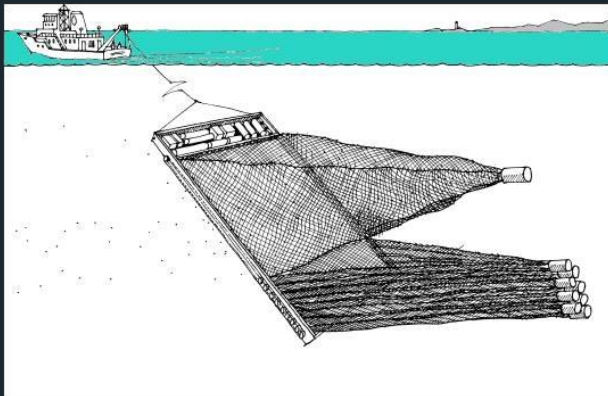
Sample Collection



Credit: NOAA



Credit: Mary Sewell



Credit: NOAA

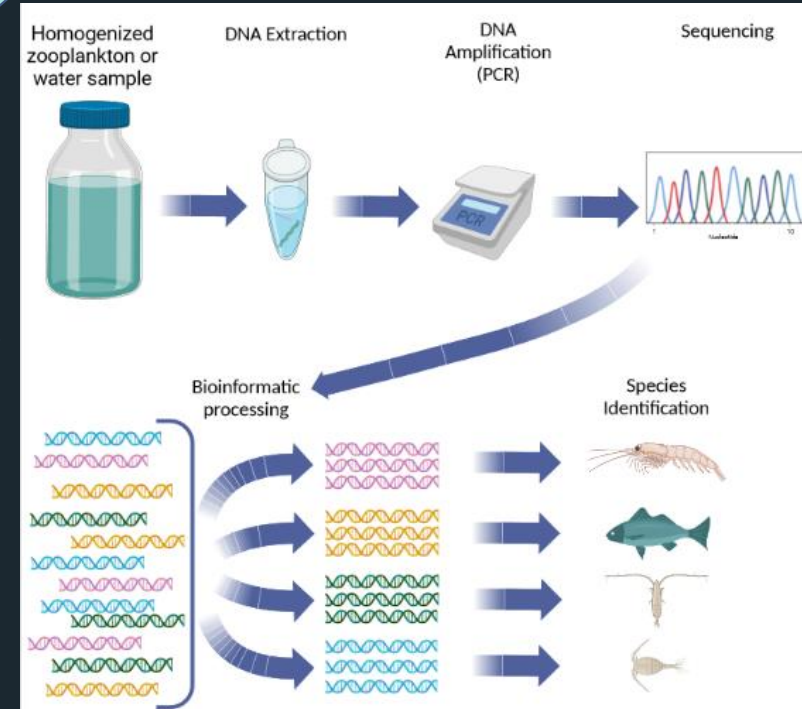
Processing



Analysis



Morphological Identification



Metabarcoding

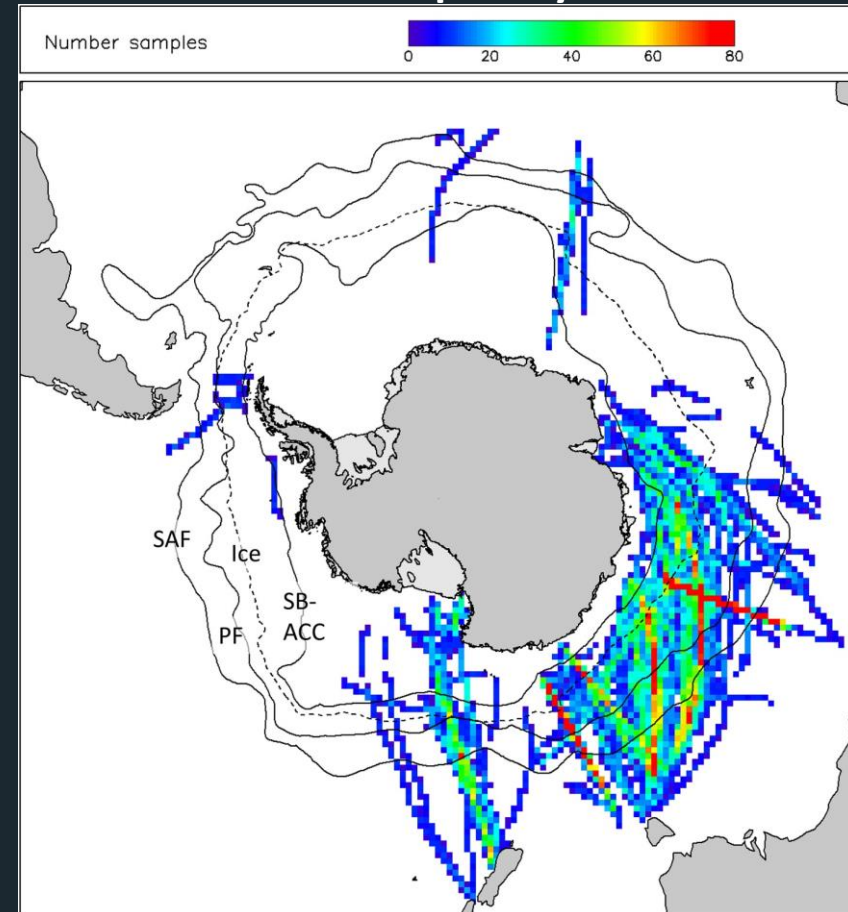
CURRENT METHODOLOGIES - COMPARISON

Method	Advantages	Disadvantages	Best Uses
Morphological Identification	<ul style="list-style-type: none"> Information about individuals <ul style="list-style-type: none"> Life stage Species abundance 	<ul style="list-style-type: none"> Large proportion unidentifiable Specialized knowledge required Expensive Small sample sizes 	<ul style="list-style-type: none"> Species abundance Developmental stages
Metabarcoding	<ul style="list-style-type: none"> Identification irrespective of appearance Low cost High throughput 	<ul style="list-style-type: none"> No information about individuals <ul style="list-style-type: none"> Not reliable for abundance Biases can be introduced throughout process Databases <ul style="list-style-type: none"> Prior identification Misidentification Unknown unknowns 	<ul style="list-style-type: none"> Species presence/absence

GOALS

- Current research limitations
 - *Methods of analysis*
 - Resource intensive
 - Introduce biases
 - *Sample processing backlog*
- A new methodology is needed
 - *Accurate*
 - *High throughput*
 - *Universal*

Number of CPR samples by area



Pinkerton et al, 2020

A large, white iceberg floats in the center of a dark, calm sea. The iceberg has a jagged, irregular shape with a prominent peak on the left. In the background, a range of dark, rugged mountains is covered in patches of snow and ice. The sky is a uniform, overcast grey. The foreground is filled with numerous smaller, broken pieces of ice floating on the water's surface.

02 METHOD DEVELOPMENT

METHOD DEVELOPMENT OVERVIEW

Universal Procedure Across Zooplankton Taxa

Sample
Collection

- Duration
- Preservation

DNA
Extraction

- Reagents
- Duration
- Treatments

Amplification

- Reagents
- Primers
- Protocols

Amplicon
Analysis

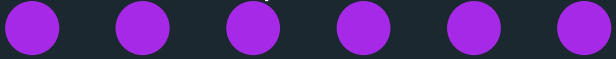
- Sequencing
- Sequence-independent Analyses

SAMPLE COLLECTION / IDENTIFICATION



Credit:
Mary
Sewell

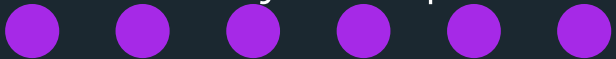
CPR Samples



Bongo Samples



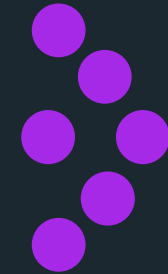
Underway Samples



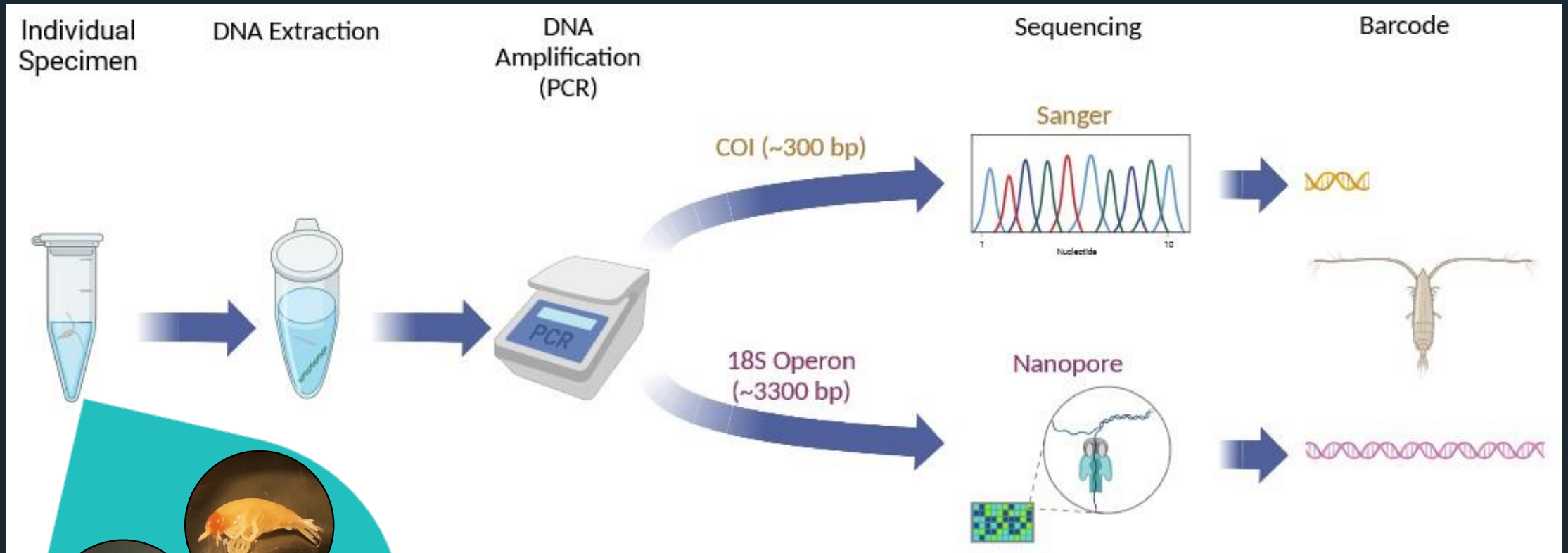
Morphological
Identification



Voucher
Specimens
for
Reference
Database



SPECIMEN PROCESSING



SEQUENCE ANALYSIS



Quality Control
Check sequence quality and BLAST search sequences to confirm ID

Finalize reference sequences
Trimming

Generate sequence alignments

Southern Ocean sequence database
Barcodes and sample data



03 RESULTS

RESULTS OVERVIEW

Amplicon	COI	18S Operon
# Taxa Sequenced	38	48

Phyla	COI Sequenced?	18S Operon Sequenced?
Annelida	Inconclusive	Yes
Arthropoda	Yes	Yes
Brachiopoda	No	No
Bryozoa	Yes	Yes
Chaetognatha	Yes	Yes
Chordata	Yes	Yes
Cnidaria	Inconclusive	Yes
Echinodermata	No	Yes
Foraminifera	Inconclusive	Yes
Hemichordata	No	No
Mollusca	Yes	Yes
Porifera	No	No
Radiozoa	No	Yes

SEQUENCE ALIGNMENT – 18S OPERON

Alignment from 38 sequences, representing 32 taxa

5'

3'



18S (SSU)

ITS

5.8S

ITS

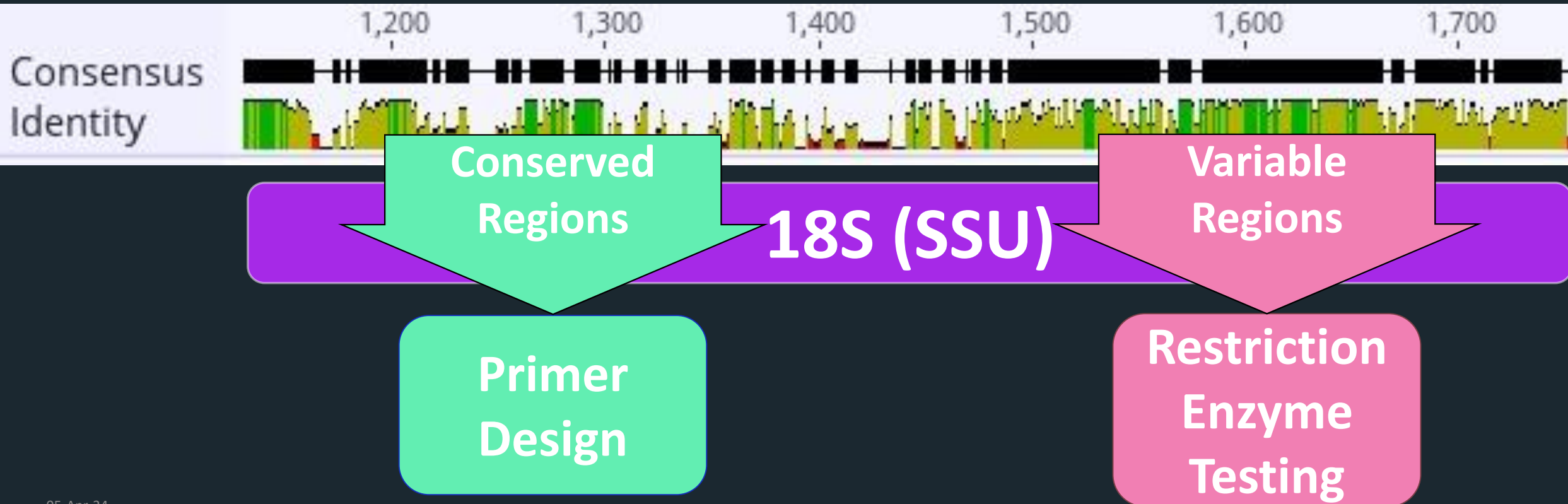
28S (LSU)

SEQUENCE ALIGNMENT – 18S OPERON



18S (SSU)

SEQUENCE ALIGNMENT – 18S OPERON



PRIMER DESIGN – 18S OPERON

5'

3'



18S (SSU)

ITS

5.8S

ITS

28S (LSU)

Amplicon
~1500-2500 bp

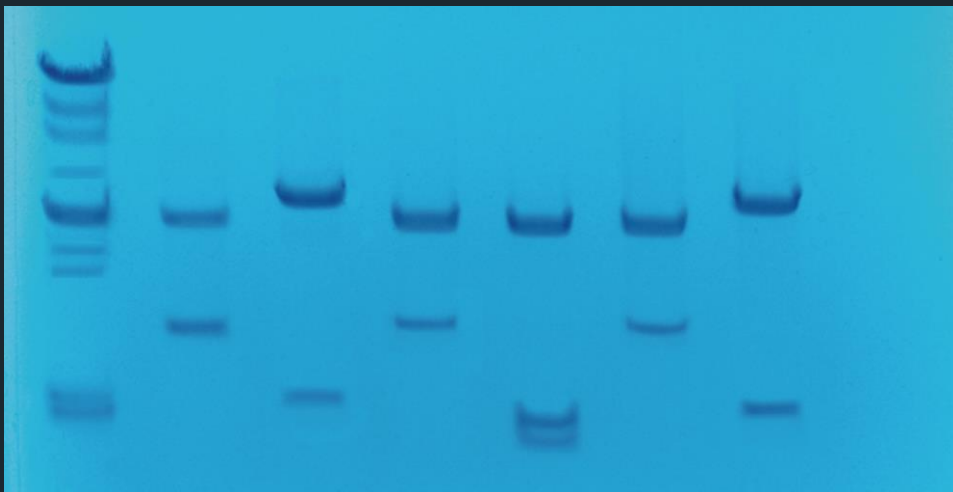
RESTRICTION ENZYME TESTING

GOALS

- Find 1 or more restriction enzymes that would differentially digest amplicons from different species to produce unique fingerprints

PROBLEMS

- Co-amplification
- Unknown sequences

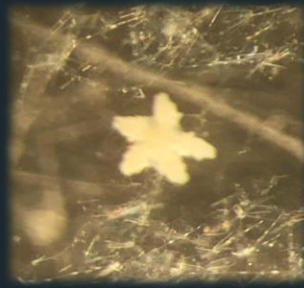


Example fingerprinting gel

Decision: proceed with sequencing method



04 FUTURE WORK



FUTURE WORK



1. Final determination of which primer set to use for this method
 1. COI or 18S operon
 2. Considerations:
 1. Amplification success across phyla
 2. Taxonomic resolution
2. Validation of the method versus morphological ID and metabarcoding
3. High-throughput processing of historical and recent ethanol samples

IN MEMORIAM: PROF. CRAIG CARY

May 13, 1954-Feb 29, 2024

An exceptional scientist and
mentor without whom this project
would not have been possible.



THANK YOU

Special thanks to Karen Robinson and Dr. Svenja Halfter for providing samples



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