Biotechnology Explorer™

Fish DNA Barcoding Kit

Planning Guide

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Catalog #166-5100EDU, Fish DNA Barcoding Kit
Catalog #166-5115EDU, DNA Barcoding Sequencing Module

Note: This document is for planning purposes only and may vary from the final product specifications.

For technical service, call your local Bio-Rad office or, in the U.S., call 1-800-424-6723.

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Overview

The intention of this planning guide is to provide an overview of the Fish DNA Barcoding kit, including details of timelines, materials included, and equipment requirements, so that instructors may prepare their curriculum and estimate budgetary requirements in advance. These are estimates based on current information available, and may change in the final version of the lab series.

The Fish DNA Barcoding kit (catalog #166-5100EDU) is made up of one wet lab module. There is an optional DNA sequencing module (catalog #166-5115EDU) available. **Sequencing is a required step** for the overall laboratory activity; Bio-Rad provides a prepaid sequencing service that can be purchased with the kit for your convenience. If you have access to a sequencing service, we can provide details on what sequencing requirements are needed to make sure your sequencing is done properly.

If you have further questions about the lab series, please contact biotechnology_explorer@bio-rad.com.

Fish DNA Barcoding Kit Part Numbers

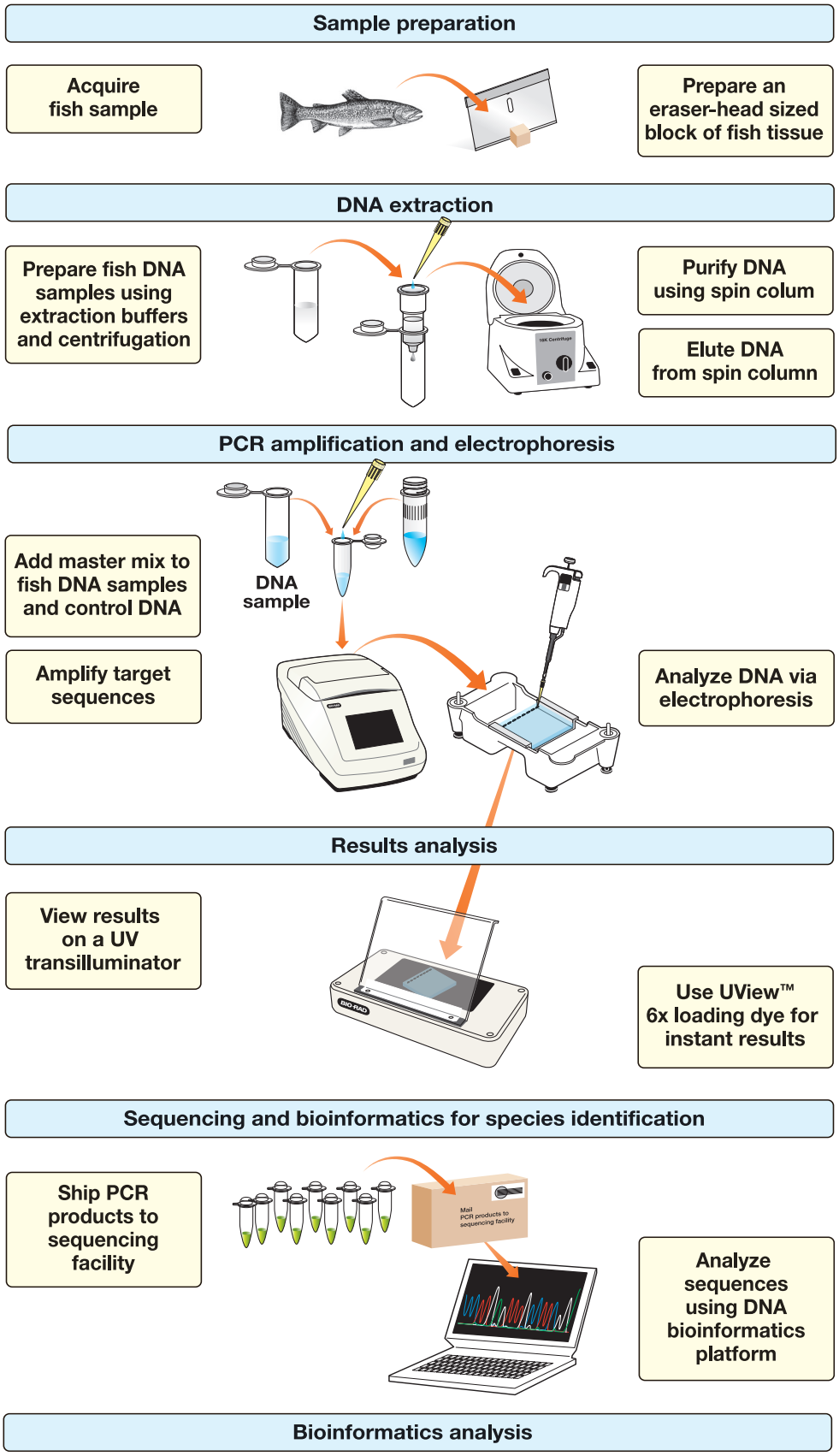
Catalog #166-5100EDU, Fish DNA Barcoding Kit, includes materials for 16 samples (eight student workstations):

- DNA extraction reagents
- PCR reagents
- Microcentrifuge and PCR tubes
- Curriculum and instruction manual

Catalog #166-5115EDU, DNA Barcoding Sequencing Module, includes:

- Instructions for setting up your online sequencing order through our selected sequencing vendor
- Mailing kit for submitting PCR products for sequencing
- Serial number for claiming sequencing service for PCR cleanup and sequencing of nine samples

Laboratory activity flowchart



Kit Summary

The International Barcode of Life (iBOL) project is a global initiative to identify all species on Earth using a technology called DNA barcoding. DNA barcoding adds a genetic identification level to species classification. Traditionally, a taxonomist would make species determinations based on observations of physical and environmental characteristics of a specimen. In some cases, DNA barcoding can help make the distinction between species that look and behave so similarly that they would be considered the same species based on the traditional taxonomical criteria. Since the slight nuances found through DNA sequencing reveal the uniqueness of a species, these sequences are essentially barcodes that can be used to help identify previously unclassified species.

DNA barcoding is also being actively used by the U.S. Food and Drug Administration (US FDA) to monitor the labeling of fish in the marketplace. Once a fish has been processed for sale, it can be incredibly difficult for a consumer to tell the difference between the varieties available. Mislabeling might be done on purpose for profit, or accidentally, but the end result to consumers is the same — they do not get what they pay for.

This laboratory activity involves extraction of DNA from fish tissue, amplification of the DNA through PCR, running gel electrophoresis to verify PCR products, and sequencing of the cytochrome c oxidase 1 (CO1) gene. The CO1 gene is a mitochondrial gene found in fish, birds, butterflies, flies, and other animal groups, making it a useful benchmarking tool for comparison between species. Finally, bioinformatics analysis is performed to genetically identify the species using the sequence obtained. The unique sequence produced for a given fish sample is considered to be its DNA barcode. This exercise is an opportunity for students to perform an investigation into their local area to see if marketplace substitution is occurring, or to develop the skills to participate in the iBOL initiative and contribute scientific data to the Barcode of Life Database (BOLD).

The Fish DNA Barcoding kit (catalog #166-5100EDU) is designed to serve up to eight student teams, with two to four students per team. Each team can process two fish samples, so a single kit can prepare up to 16 samples for sequencing. The aim of this kit is to genetically identify fish species by generating a DNA barcode.

The DNA Barcoding sequencing module (catalog #166-5115EDU) may be purchased with a prepayment for sequencing services through Bio-Rad's validated sequencing vendor. This module entitles you to PCR cleanup and forward and reverse sequencing per sample, up to nine samples per module. The nine samples submitted should be eight fish samples and the positive control. This number is half the number of samples that can be prepared with the Fish DNA Barcoding kit. The configuration is modular so you can purchase sequencing services in multiples of nine as needed for your classroom.

Materials Required but Not Supplied

Chemical	Catalog Number	Qty/Amount
Molecular biology grade agarose	161-3116EDU	5 g
50x TAE	166-0742EDU	100 ml
Distilled water	–	5 ml
Ethanol	–	10 ml
Plastics and Consumables		
100–1,000 µl pipet tips, aerosol barrier	211-2021EDU	1 rack
20–200 µl pipet tips, aerosol barrier	211-2016EDU	8 racks
2–20 µl pipet tips, aerosol barrier	211-2006EDU	8 racks
Parafilm sealing film	–	–
Glassware		
Ice baths or beakers with ice	–	8
Bottle or Erlenmeyer flask, 1 L	–	1
Graduated cylinder (to measure ethanol)	–	1
Graduated cylinders, 3 L and 500 ml	–	1
Temperature Control Devices		
Water bath at 60°C (optional)	166-0504EDU	1
Water bath or dry bath set to 55°C	166-0504EDU,166-0562EDU	1
Other Equipment		
100–1,000 µl adjustable-volume micropipets	166-0508EDU	1
20–200 µl adjustable-volume micropipets	166-0507EDU	8
2–20 µl adjustable-volume micropipets	166-0506EDU	8
Horizontal electrophoresis chamber with gel combs and casting trays	166-4000EDU	8
Microcentrifuge, 16K	166-0602EDU	1–2
Microwave or magnetic hot plate and stir bar	–	1
Power supply	164-5050EDU	2–4
UV transilluminator	166-0531EDU	1
Computer with Internet access	–	8
Miscellaneous		
Fish samples (if not provided by students)	–	8–16
Lab tape (optional)	–	1 roll
Marking pens	–	8
Razor blades or plastic knives	–	16
Weigh boats	–	16
Gloves	–	Class set

Refills Available Separately

DNA Extraction Module, catalog #166-5105EDU

Fish DNA Barcoding Reagent Refill Pack, catalog #166-5110EDU

EZ Micro™ Test Tube, 2 ml, 500/box, catalog #223-9430EDU

UView™ 6x Loading Dye, 200 µl, catalog #166-5111EDU

UView 6x Loading Dye, 1 ml, catalog #166-5112EDU

Certified Molecular Biology Grade Agarose, 5 g, catalog #161-3116EDU

50x TAE, 100 ml, catalog #166-0742EDU

Course Objectives

The Fish DNA Barcoding kit aligns with a variety of courses and educational levels. Students will be most successful if they already have experience with micropipetting, gel electrophoresis, and PCR. This activity would also be suitable for students doing independent research.

Alignment

High School	Community College/ Technical College	University
AP Biology, Biotechnology, AP Environmental Science, Marine Biology, Ecology	Biotechnology, Molecular Biology, Genetics, Laboratory Methods	Biotechnology, Molecular Biology, Genetics, Biochemistry, Cell Biology

The actual laboratory procedures are routine, safe, and relatively inexpensive, provided basic laboratory equipment is available. Measures have been taken to ensure the safety of the reagents used. While proper laboratory safety techniques must always be used, the reagents provided are safe to use in the classroom. In order to complete the laboratory project in six to eight sessions, it is assumed that students meet at least once per week in a one-hour laboratory session.

Specific Objectives Met by This Project

1. Students will experience a wide range of laboratory techniques. Some of the techniques implemented in this DNA-based project are: DNA extraction, PCR, gel electrophoresis, DNA sequencing, and analysis.
2. Students will see that these individual techniques are just steps in a longer investigatory process. Few researchers can complete an entire research project in one- or two-hour laboratory sessions (the timeframe of most commercially available kits), so this multi-period project more accurately reflects what goes on in a contemporary molecular biology laboratory.
3. Students will be active participants in the process. There are opportunities during the project for students to troubleshoot or have general discussions about their results, or to make judgments about what to do next.

This exercise does not take a simple “cookbook” approach, but rather engages students in true scientific practices. Students will employ critical thinking as they carry out their investigations and use data to construct explanations for their scientific results.

DNA Barcoding Timeline

The timeline will depend greatly on the level of the students, the length of class periods, and whether other techniques and analyses are performed in addition to the basic protocol. To assist in planning for the laboratory, the following pages provide a workflow and timeline.

50-Minute Lessons

- ☐ Lesson 0: Read instruction manual and inventory (as needed)
- ☐ Lesson 1: DNA extraction (50 min)
- ☐ Lesson 2: Set up PCR reactions and pour gels (50 min)
- ☐ Run PCR reactions (4 hrs, typically overnight)
- ☐ Lesson 3: Gel electrophoresis (50 min)
- ☐ Lesson 4: DNA sequencing
- ☐ Wait for sequencing reaction results (3–5 days)
- ☐ Lesson 5: Bioinformatics

Lesson	Lab Session	Task	Step Performed By	Estimated Duration
0. Advanced preparation		Read instruction manual	Instructor	2 hr
		Label tubes and aliquot DNA extraction reagents	Instructor	1 hr
		Acquire fish samples	Instructor	As needed
		Inventory required reagents, equipment, and accessories	Instructor	1 hr
		Remove tops from 2 ml microcentrifuge tubes	Instructor	30 min
		Set up student workstations	Instructor	30 min
		Read background materials/pre-lab materials	Student	As needed
1. DNA extraction	Sample preparation	Cut samples and place in resuspension buffer	Student	>5 min
		Add lysis solution to sample, mix and incubate	Student	>10 min
		Add neutralization solution, mix and centrifuge	Student	>15 min
		Prepare spin columns, load supernatant of fish sample, prepare and add matrix, centrifuge	Student	>10 min
		Wash matrix via centrifugation 2x	Student	>5 min
		Dry out columns via centrifugation	Student	2 min
		Elute DNA via centrifugation	Student	1 min

Lesson	Lab Session	Task	Step Performed By	Estimated Duration
2. Set up PCR reactions and prep PCR product for electrophoresis and sequencing	PCR amplification	Label tubes and aliquot PCR reagents	Instructor	1 hr
		Set up student workstations	Instructor	30 min
		Add master mix to DNA samples	Student	15 min
		Run PCR reaction	Instructor	2.5 hr to overnight
		Label tubes and transfer aliquot of PCR products to tubes — one set for electrophoresis, one set for sequencing	Student	15 min
3. Gel electrophoresis	DNA electrophoresis	Label tubes and aliquot electrophoresis reagents	Instructor	1 hr
		Pour 1% gels	Instructor	2 hr
		Set up student workstations	Instructor	30 min
		Add fluorescent loading dye to electrophoresis samples	Student	10 min
		Set up electrophoresis equipment and load gel	Student	10 min
		Run the gel and immediately visualize	Student	25 min
4. DNA sequencing	Sample sequencing	Send sequencing samples to vendors	Instructor	3–4 days
5. Bioinformatics	Bioinformatics snalysis	Use bioinformatics tool to identify samples	Instructor/ Student	As needed

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