# Mystery #2: Are these fish stocked or wild fish?

**Objective:** Students will determine if a fish is an offspring of a stocked or wild fish and why genetic diversity is important for population survival.

**Materials:**

- Chromatograms separated into 5 stacks per allele
- Data sheet

**Teacher set up:**

- Print Chromatogram pdf
- Cut the Fish chromatogram from the mother and father chromatograms
- Use a paperclip to keep Fish 1-5 and parent cards together for each Allele

**Background**:
What are some applications of DNA and genetic analysis that you know of? (medicine: vaccines and insulin, forensics: identify individuals, and agriculture: selective breeding dogs, modify food)

Genetic analysis is an important aspect of wildlife conservation and can give scientists a better idea of how species are doing in an area. Every plant and animal on earth has a unique genetic code stored in their DNA. DNA strands are made up of a series of four nucleotide bases; Adenine, Thymine, Guanine, and Cytosine. The DNA sequence of a species can be written like this: Periwinkle Snail (AGTCCAG) although in reality they are millions or billions of bases long. The bases pair up with each other to create two connected strands held together in the middle like a ladder. The complementary base pairs are A-T and G-C. Knowing the sequence of one strand automatically tells you the sequence of the other!

Biologists at South Carolina Department of Natural Resources use genetic analysis for multiple reasons. They use it for population health/genetic diversity, species distribution and abundance, genetic identification, and population structure. Here we are going to see how these tools can be used to identify stocked vs. wild fish of the same species.

Wild fish have never seen the walls of a concrete tank and have lived their whole lives in open water. Stocked fish are born and raised in a lab or culturing facility and are released into the environment when they reach a certain size. The goal of the SCDNR stocking program is to help maintain population levels and allow more people to fish, while also maintaining healthy, natural populations! We do not select broodstock (wild caught parent fish) for any genetic traits and rotate them periodically to produce genetically diverse stock. The parent fish used as broodstock are wild caught and temporarily held and then released. The four main MARINE species that we spawn and stock are red drum, spotted seatrout, cobia, and striped bass. Species of trout, bass, robust redhorse, and other freshwater fishes are grown in hatcheries by SCDNR biologists from around the state.

**Explanation of the analysis:** At SCDNR, we usually have 12-20 markers per species, so the chromatograms overlap exactly perfectly across 20 sites, or there are a few mismatches here and there. We have a cut-off of 2-3 mismatches is unknown parentage and >5 is wild fish. We have an algorithm that analyzes it. The important thing to remember would be that the offspring would match some combination of the parents *perfectly* because they SHOULD match to some degree (same species, same population) but the parent offspring pairs will match perfectly. Remember even as perfect matches they will only match HALF of the alleles of any given fish, half mother and half father.

**Answers for activity:**
Parents (N=5) have 5 alleles/parent ==25 total cards
Angler caught fish (N=5) have 5 alleles each ==25 total cards
2 fish are wild caught (match one set of parent alleles and no others)
1 fish is unknown (Matches 3 parent alleles but not all)
2 fish are cultured (matches all parent alleles perfectly)

**Extension:** Great video of interstate coordination of research and mariculture between SC and GA for a newly discovered freshwater species: https://video.gpb.org/video/mystery-fish-usak30/

Genetics Mystery #2
Stocked vs. Wild Fish
Answer Key for Data Sheet

Determine if the fish is wild, stocked or unknown:
**wild caught: one match**
**unknown: 3 matches**
**Cultured: all matches**

| Fish Sample | Parent Allele a | Parent Allele b | Parent Allele c | Parent Allele d | Parent Allele e | **The offspring is:** |
|---|---|---|---|---|---|---|
| Fish 1 | match | match | match | none | none | **unknown** |
| Fish 2 | match | none | none | none | none | **wild** |
| Fish 3 | match | match | match | match | match | **stocked** |
| Fish 4 | match | match | match | match | match | **stocked** |
| Fish 5 | none | none | none | none | match | **wild** |

# Student Worksheet

**Question:** What are some applications of DNA and genetic analysis that you know of?

**Pre-Activity Engagement:** Read this blog post as an introduction to SC Department of Natural Resources Stocking Program.
https://sccoastalresources.com/home/2017/4/19/stocking-south-carolinas-favorite-saltwater-fish?rq=genetics

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**Mystery 2**: You are one of the mariculture (saltwater aquaculture) biologist and it is important to know if the fish you are raising in the hatchery are growing, reproducing, and contributing to the natural population--in other words, is all your hard work paying off?! The state is threatening to cut off your funding if you cannot prove that this fish stocking program is paying off! It's your job to use genetic analysis to figure out if the fish caught by anglers are offspring of a stocked or wild fish.

1. Get one set of Allele Cards for each group. There are 5 sets total.
2. Lay out the parent cards and offspring fish cards on the table.

3. Match up the corresponding parent and offspring cards based on similar allele spikes on the chromatograms.
4. Fish offspring cards must have spikes that correspond to one from the mother and one from the father in order to have a match.
5. Fill in the data sheet one column at a time as you work through each allele. Write 'match' or 'no match' for Fish 1-5.
6. Finish matching up parents and offspring in their allele set, rotate allele cards to another group.
7. Every group should work through every allele to finish their data sheet. If low on time, groups can share their answers.

**Discussion:**

8. What are some advantages to stocking fish?

9. What can be some disadvantages?

10. What are other alternatives to managing a fish species without stocking?

Data Sheet

The offspring is:

- **Wild: one allele match**
- **Unknown: 3 allele matches**
- **Stocked: all 5 allele matches**

Determine if the fish has a match or no match to parents for each allele.

| Fish Sample | Parent Allele A | Parent Allele B | Parent Allele C | Parent Allele D | Parent Allele E | **The offspring is:** |
|---|---|---|---|---|---|---|
| Fish 1 | | | | | | |
| Fish 2 | | | | | | |
| Fish 3 | | | | | | |
| Fish 4 | | | | | | |
| Fish 5 | | | | | | |