Environmental DNA with Jonah Ventures: Knowledge in Sequence

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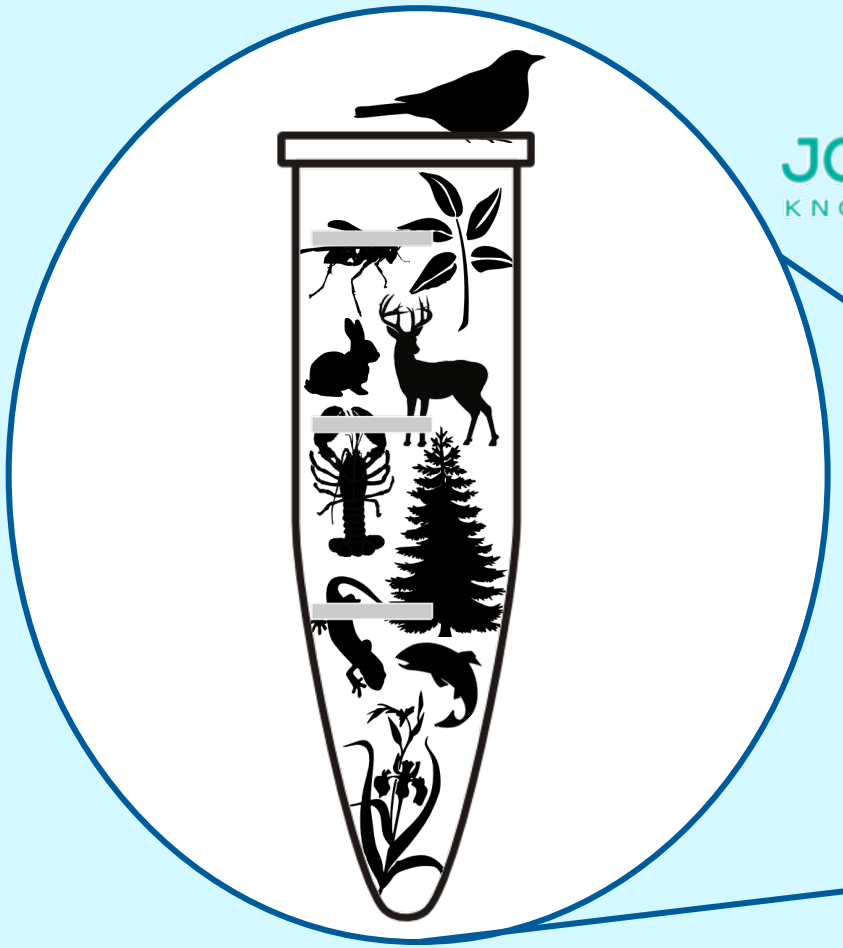
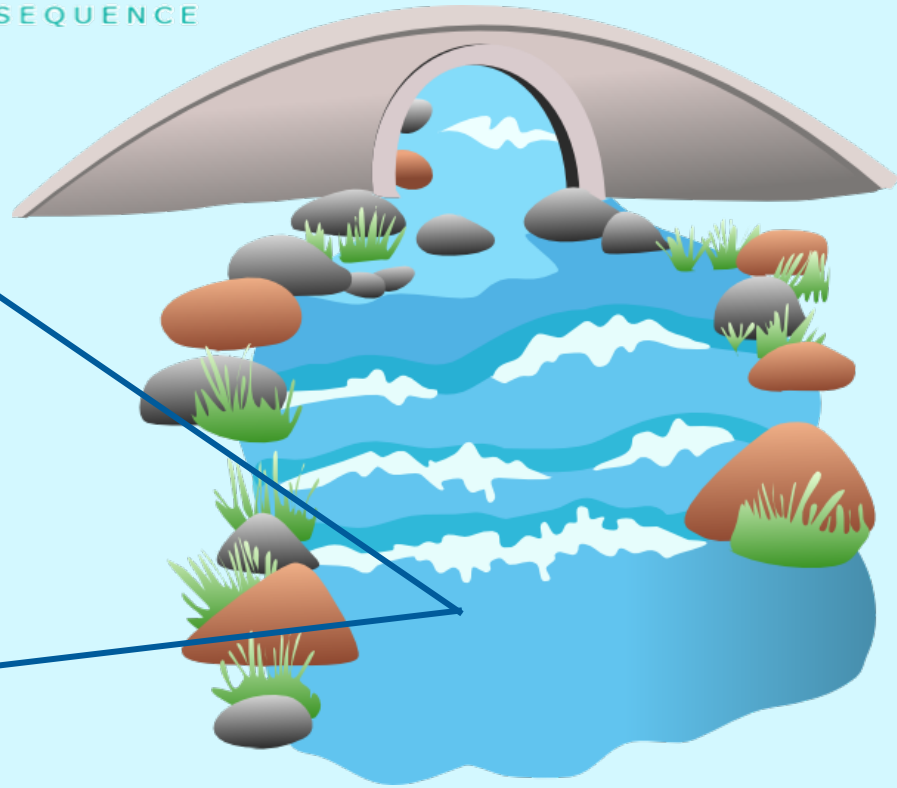
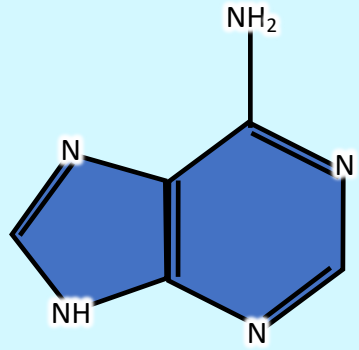


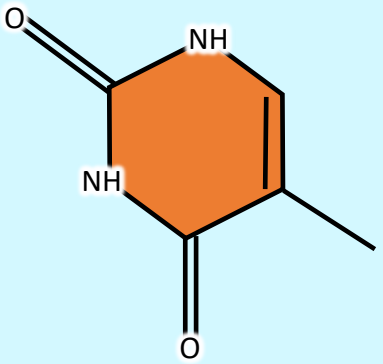


What is DNA (deoxyribonucleic acid)?

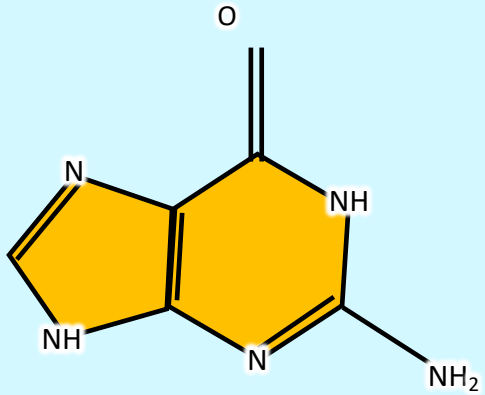
- The DNA of nearly every living thing is made up of four different nucleotides



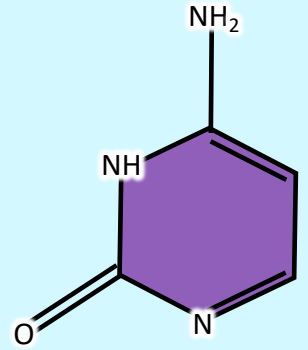
Adenine (A)



Thymine (T)



Guanine (G)



Cytosine (C)

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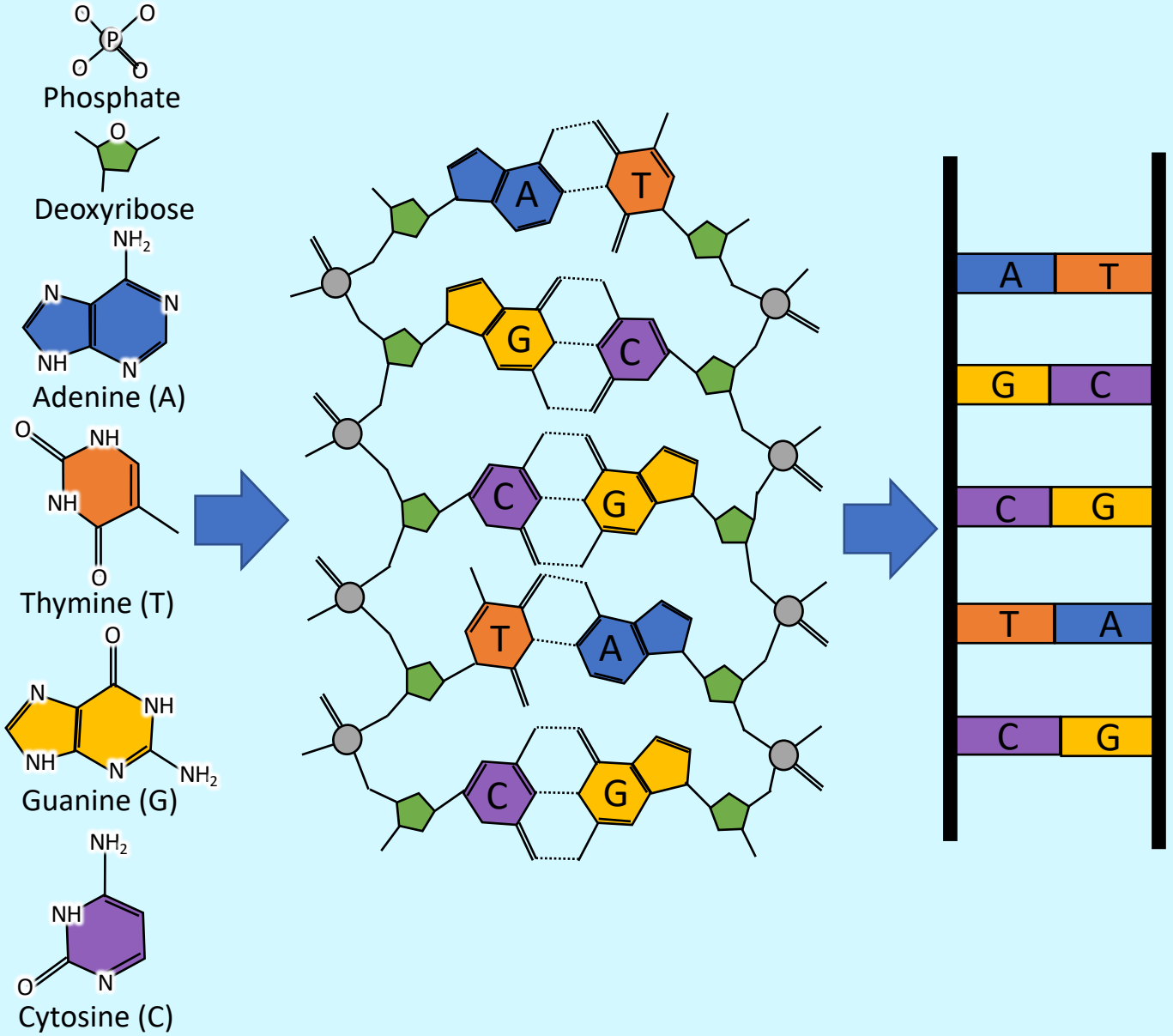
What is DNA?

- These nucleotides pair up into <u>base pairs</u>, A to T and G to C, and are held in place by a phosphate deoxyribose backbone
- The order of these base pairs determines the characteristics of nearly every organism on earth!



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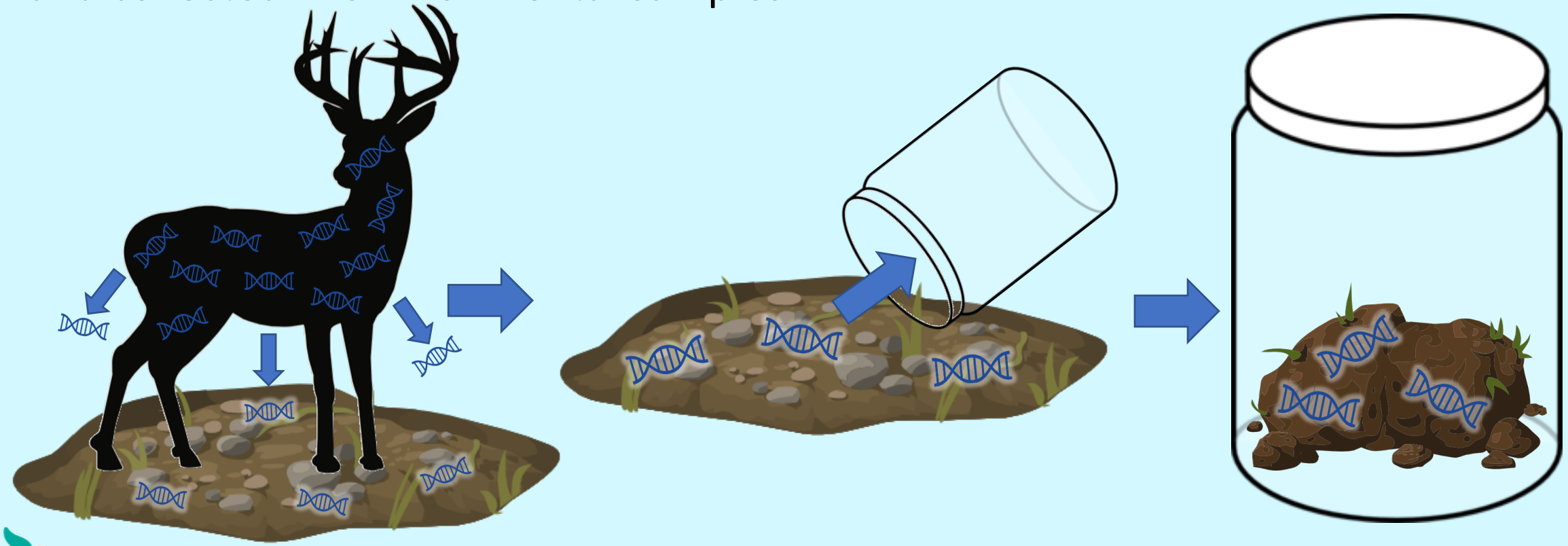
What is Environmental DNA?

- Environmental DNA (eDNA) is DNA that is shed by an organism into its environment and collected in environmental samples



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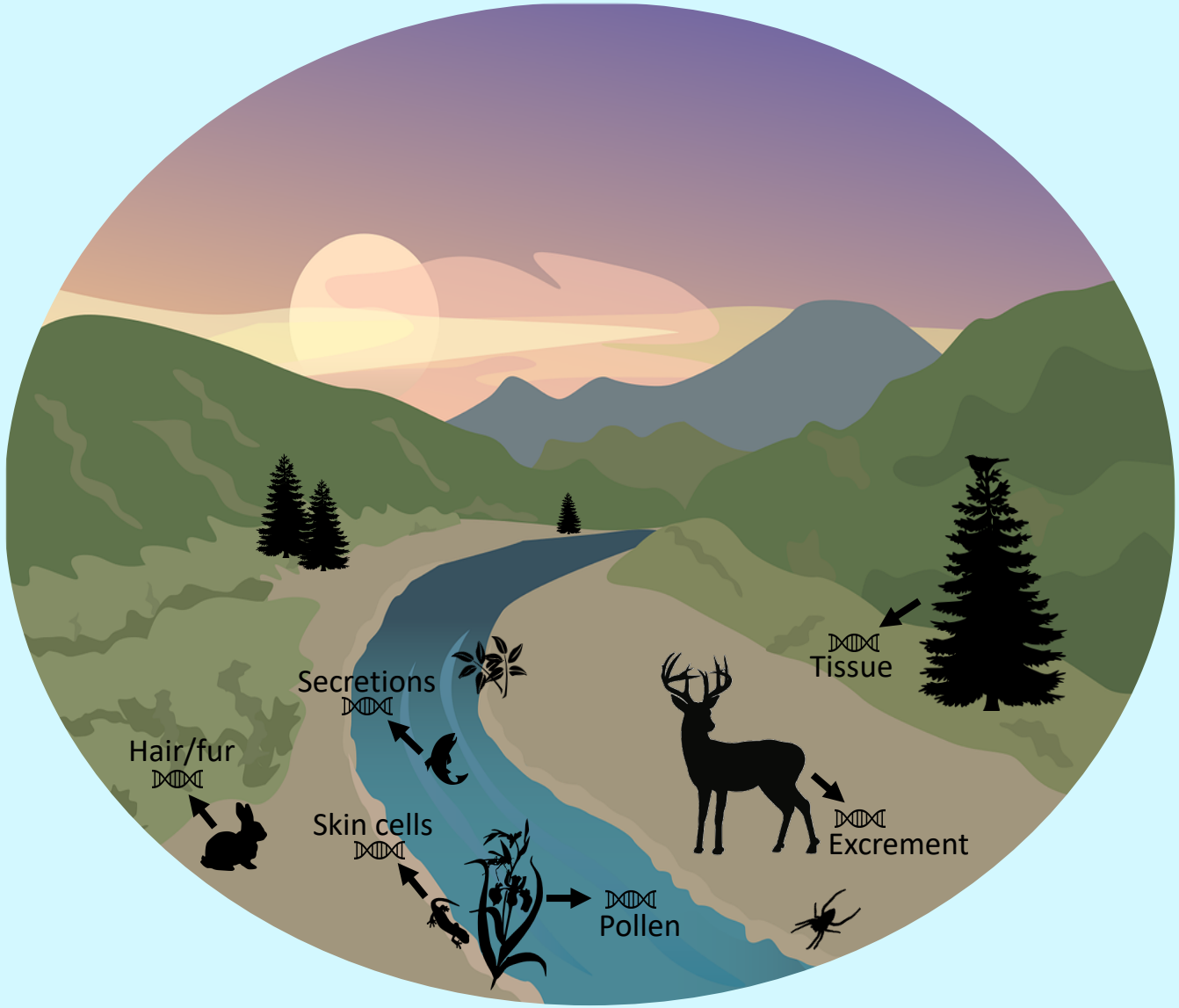
What is Environmental DNA?

- What do you think comes from an organism that could be considered environmental DNA?
  - Anything that comes from an organism can be environmental DNA
  - DNA is EVERYWHERE!



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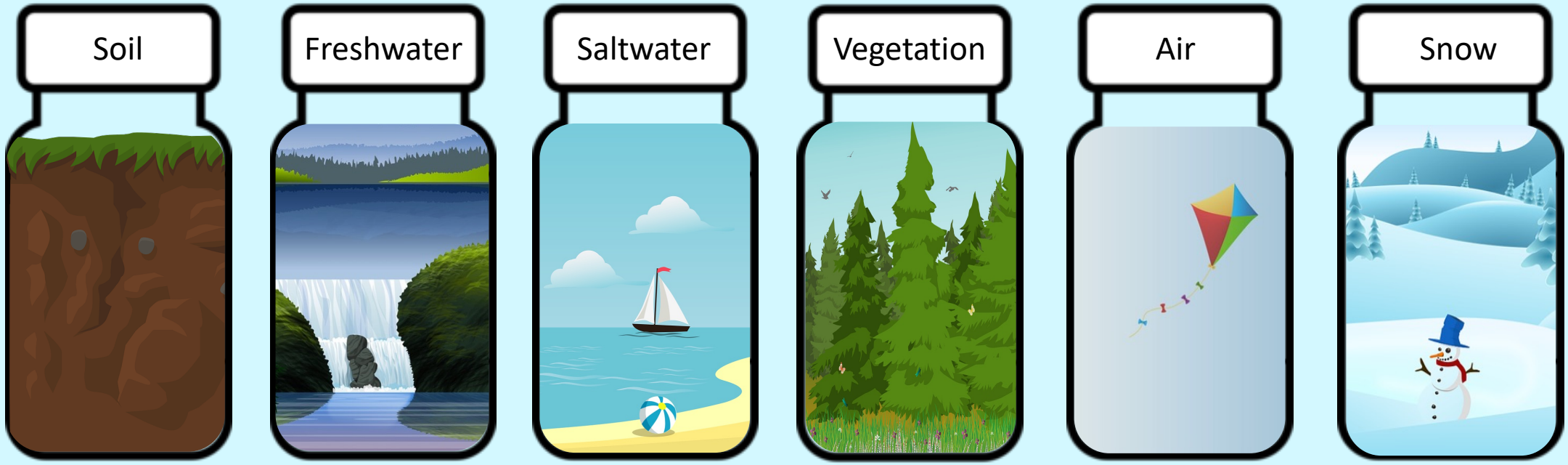
Environmental DNA Sampling

- This environmental DNA is collected by sampling substrate from the organisms environment
- Then the substrate samples are sent off to the lab



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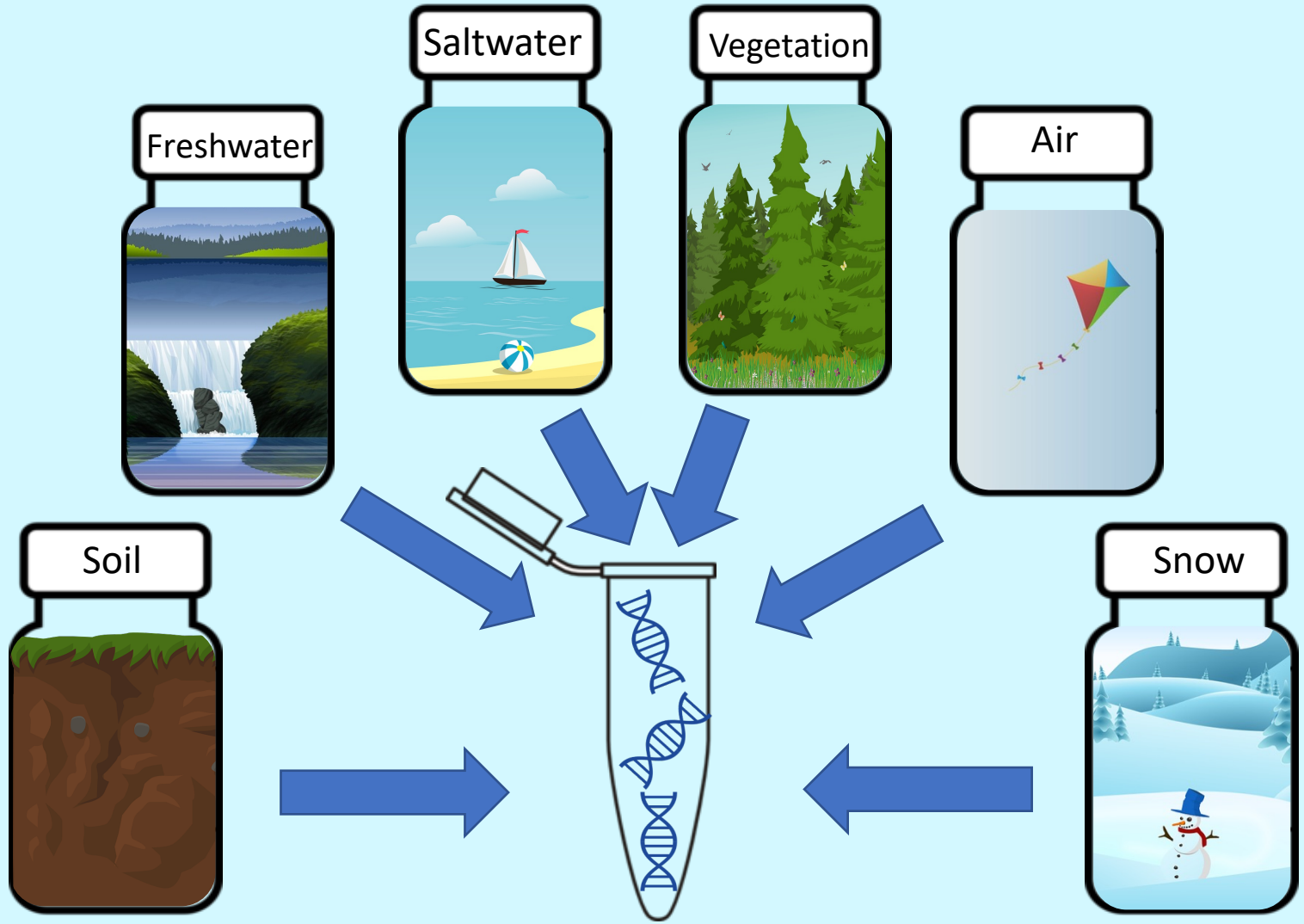
Extraction



- DNA is extracted from the environmental samples in a laboratory

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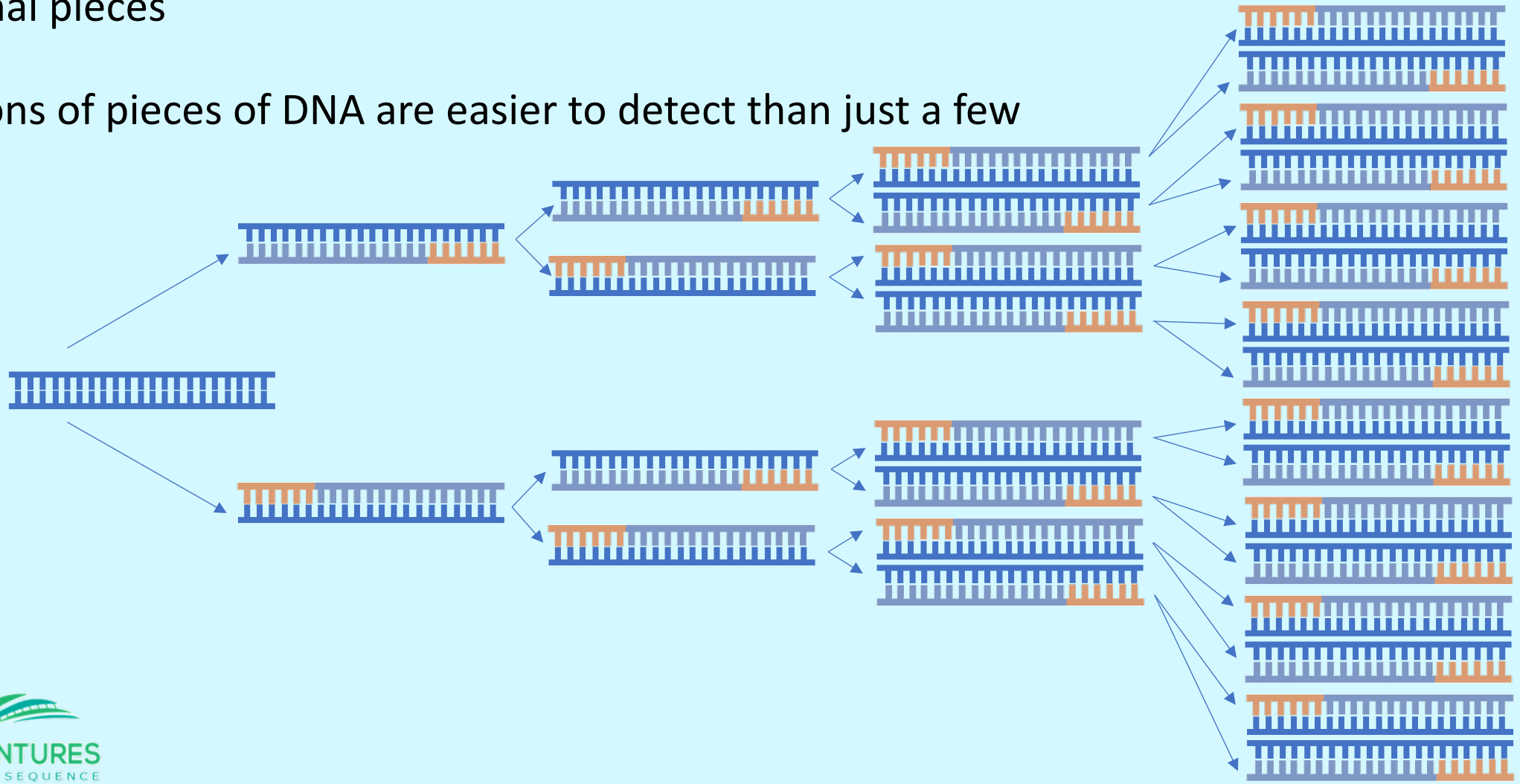
What is Polymerase Chain Reaction (PCR)?

- Polymerase chain reaction (PCR) is a technique used to make millions of copies of DNA from a few original pieces
- Millions of pieces of DNA are easier to detect than just a few



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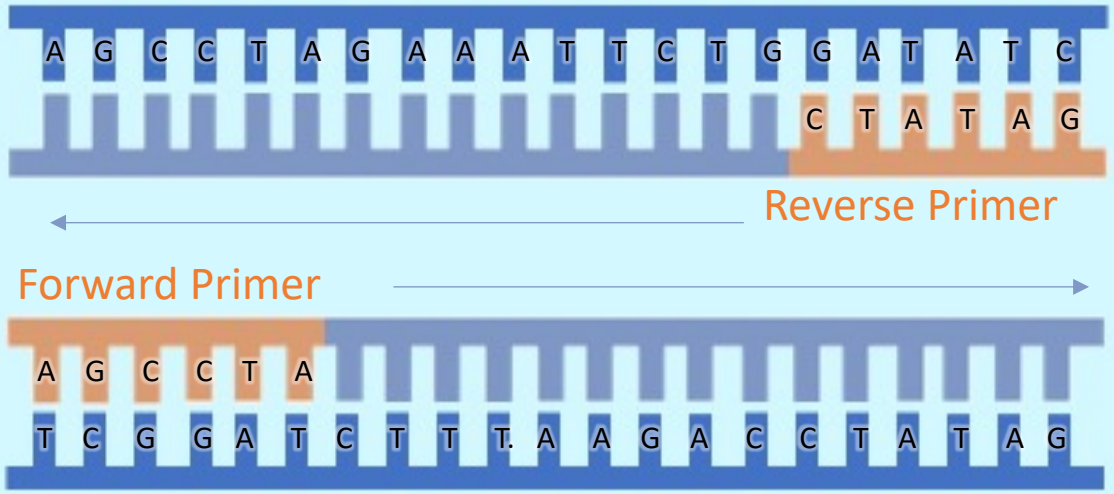
What is Polymerase Chain Reaction (PCR)?

- PCR involves using primers, which are specific nucleic acids that bind to the beginning and end of specific sequences (forward and reverse primers) of DNA and allow them to create copies
- The base pairs in the primers match to their complementary base pairs on the DNA (A to T, G to C)
- Different primers work for different groups of species



Invertebrates

Plants

Vertebrates

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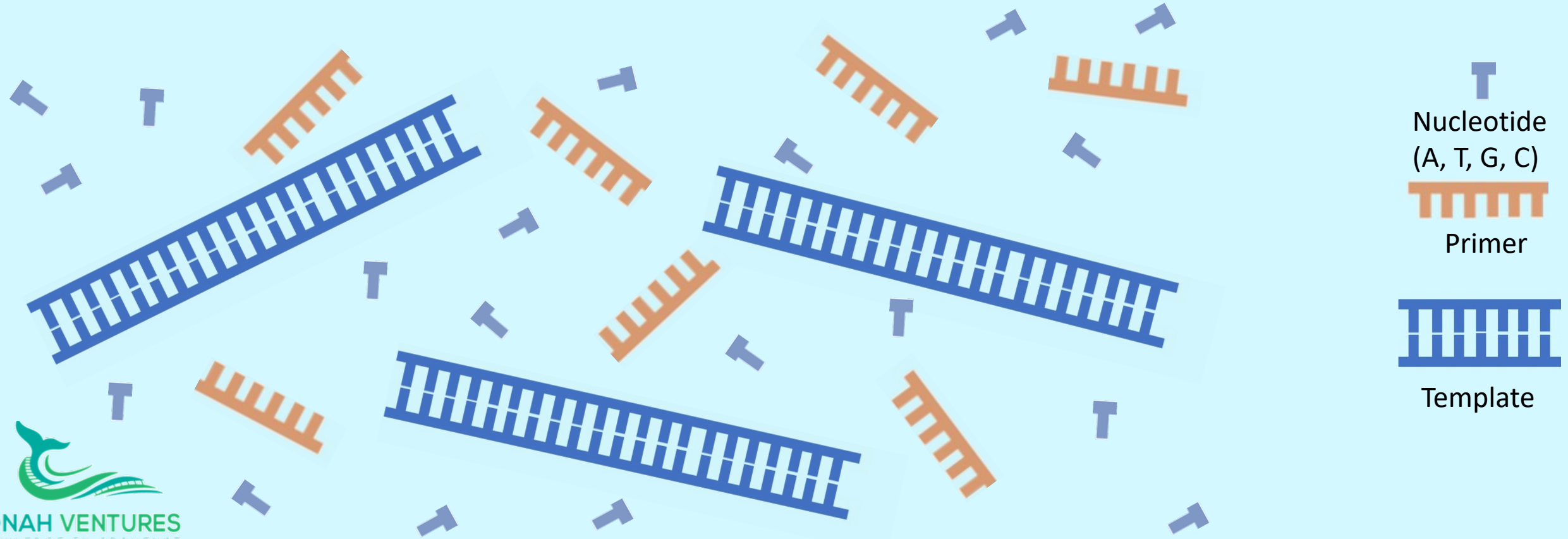
Polymerase Chain Reaction (PCR): The Process

- Template DNA is loaded into a reaction with specific primers and additional nucleotide
- At room temperatures, they all stay separate



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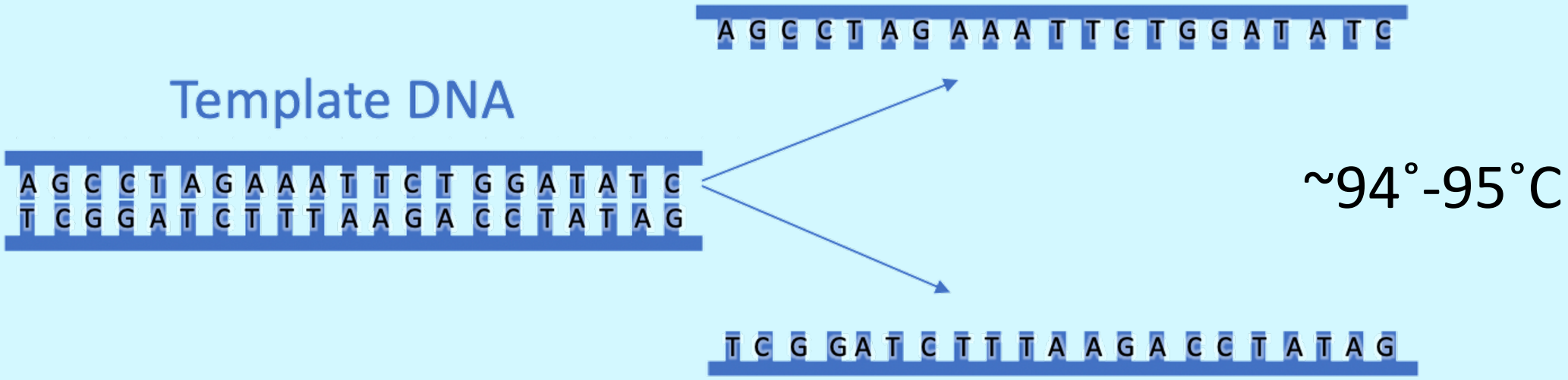
Polymerase Chain Reaction (PCR): The Process

- As the temperature is raised (denaturation) during PCR, the strands of DNA unwind and separate



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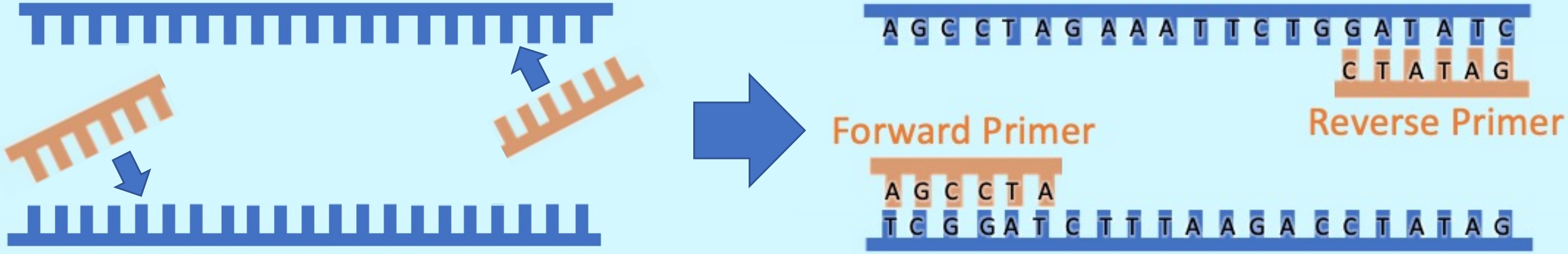
Polymerase Chain Reaction (PCR): The Process

- As the temperature is lowered (annealing) the primers match to their targeted sequences

~68˚C



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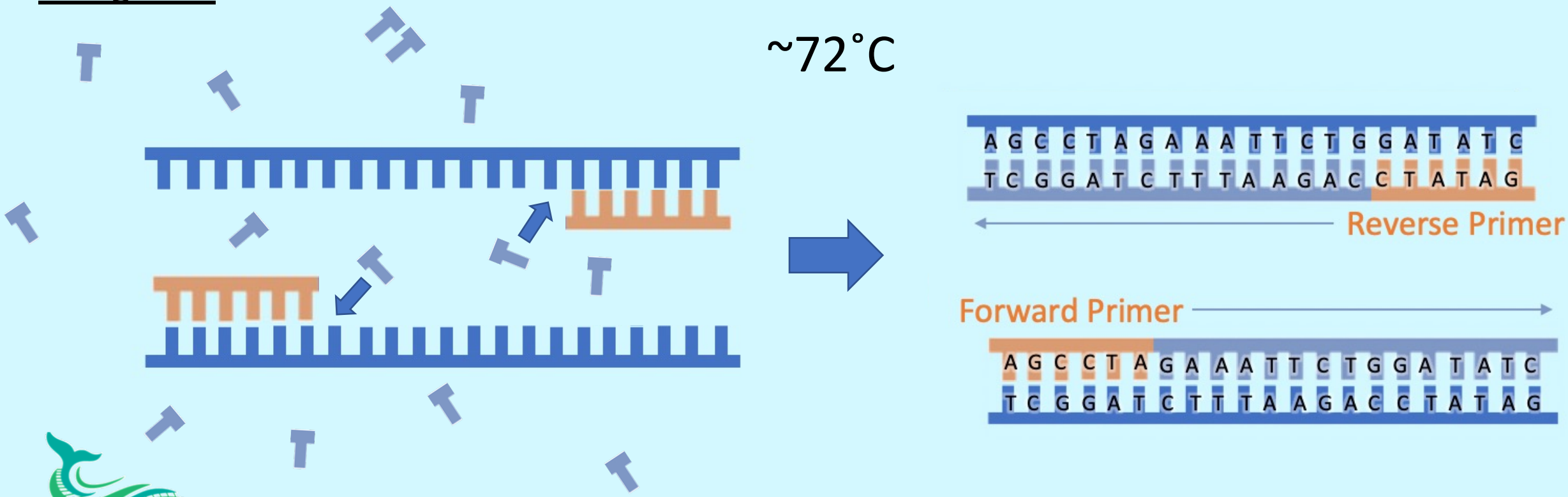
Polymerase Chain Reaction (PCR): The Process

- Next, the temperature is raised slightly and the spaces between the forward and reverse primers are filled in with complementary nucleotides, creating a new copy of DNA, called <u>elongation</u>



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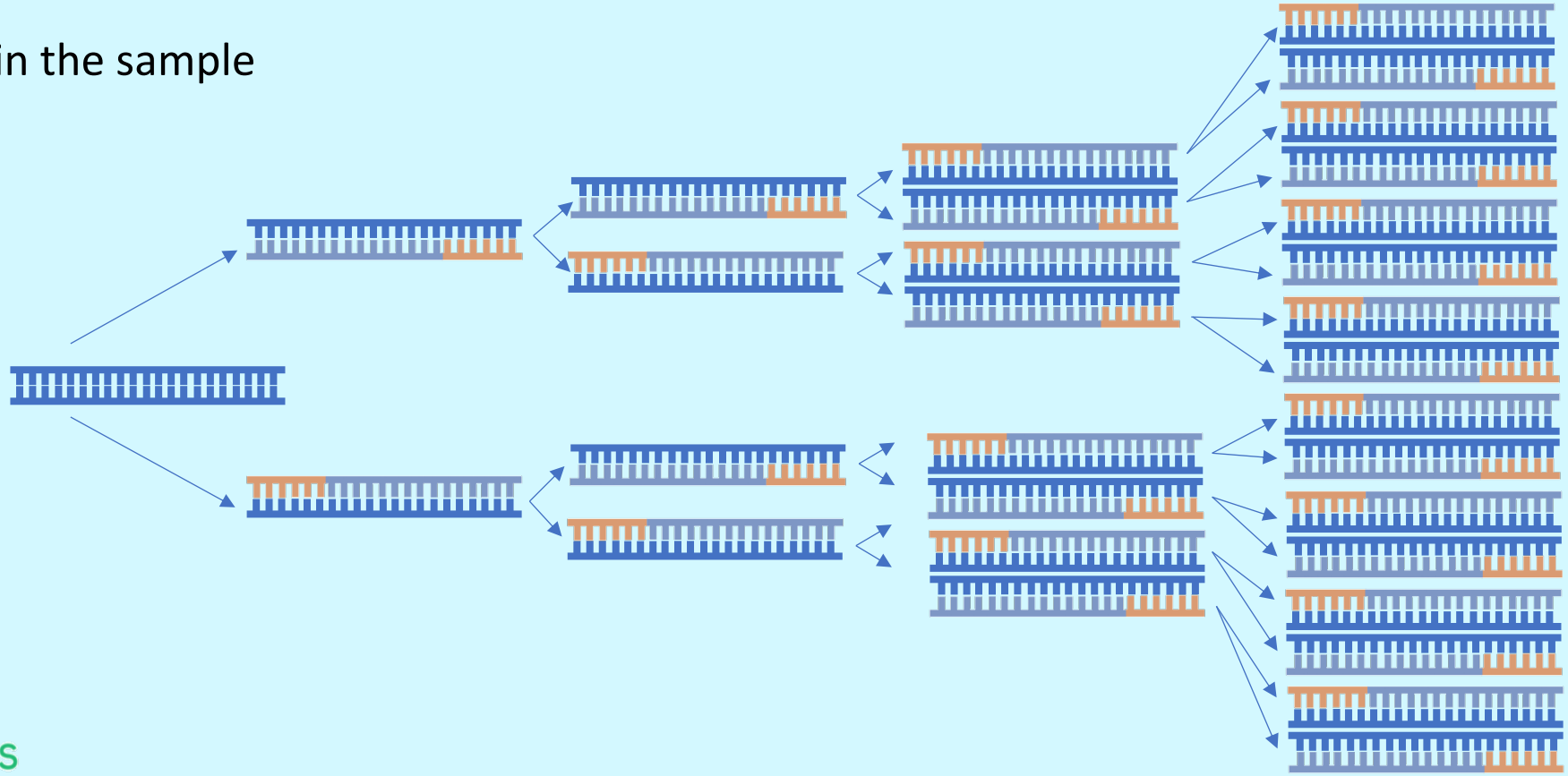
Polymerase Chain Reaction (PCR): The Process

- This process is repeated over and over again, doubling the amount of DNA in the reaction each time!
- These copied sequences flanked by forward and reverse primers are called amplicons
- Amplification is the exponential growth the DNA experiences and makes it easier for researchers to detect the DNA within the sample



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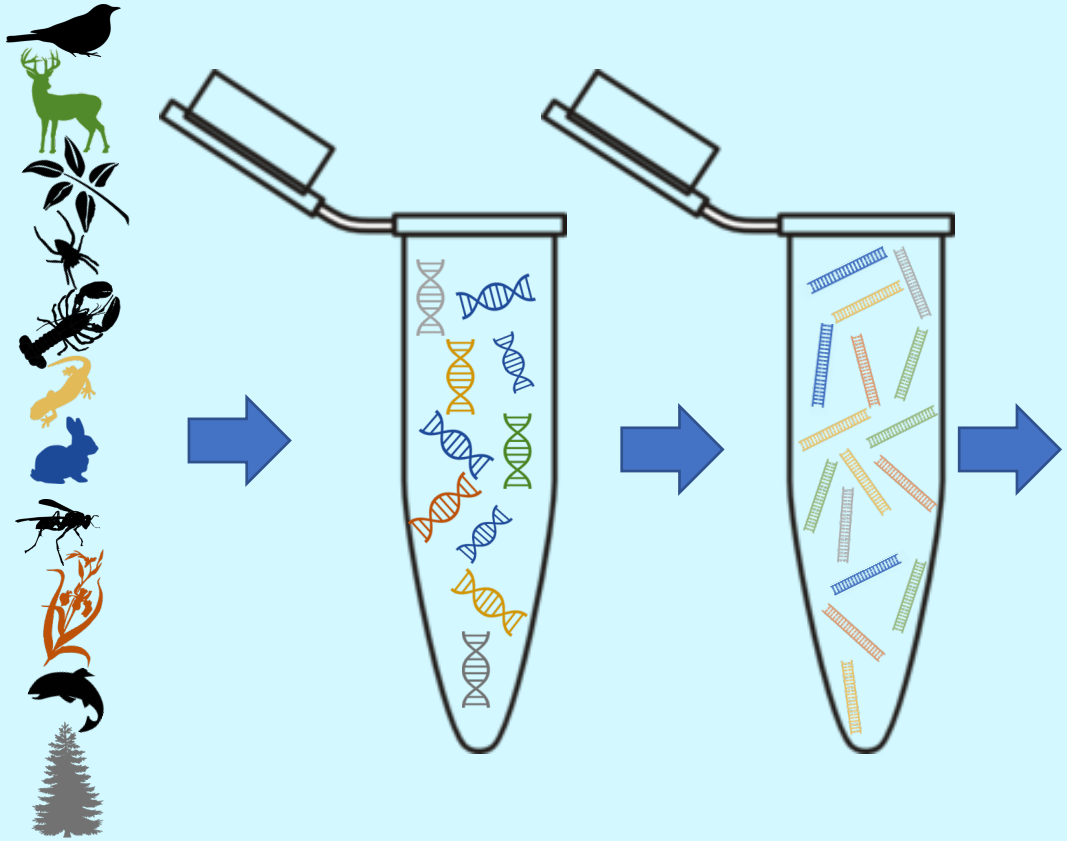
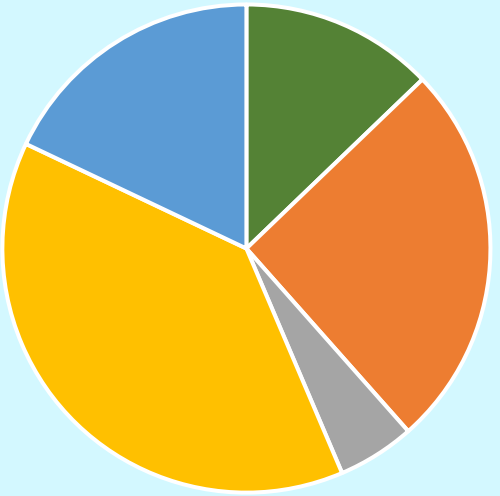
Next Generation Sequencing

- Next generation sequencing is where we determine the sequences of the DNA in our sample



TACGGCCTATAAGCTGAATCTTG
ACTTAGGACCCTAGGCCCTACCT
CTAATTCCGACAAGGTCAGTCGT
AGGTCAGTCGTCTAATTCCGACA
GCTGAATCTTGTACGGCCTATAAT



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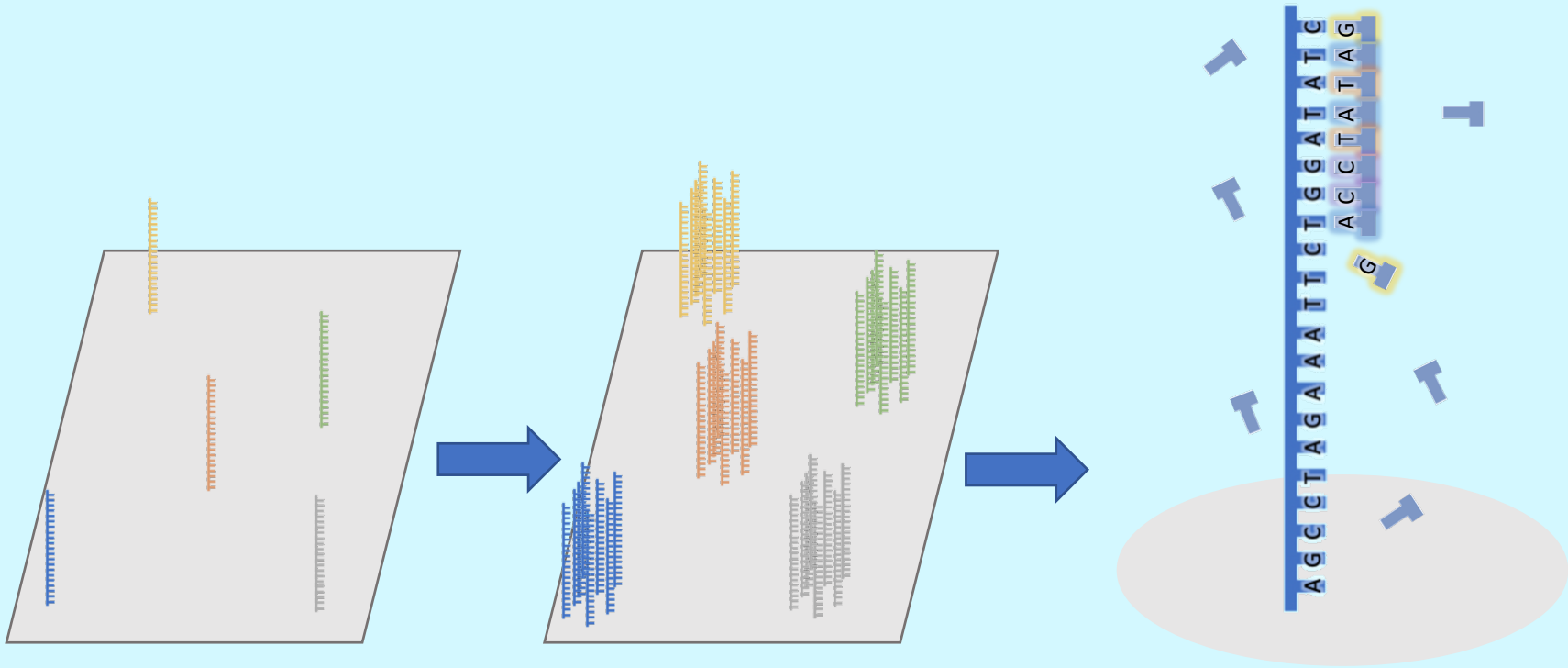
Next Generation Sequencing

- DNA is loaded into an Illumina MiSeq machine
- Single strands of DNA bind to the surface of a plate and replicate, forming clusters
- Nucleotides are added that flash a specific color as they match to their complements on the DNA



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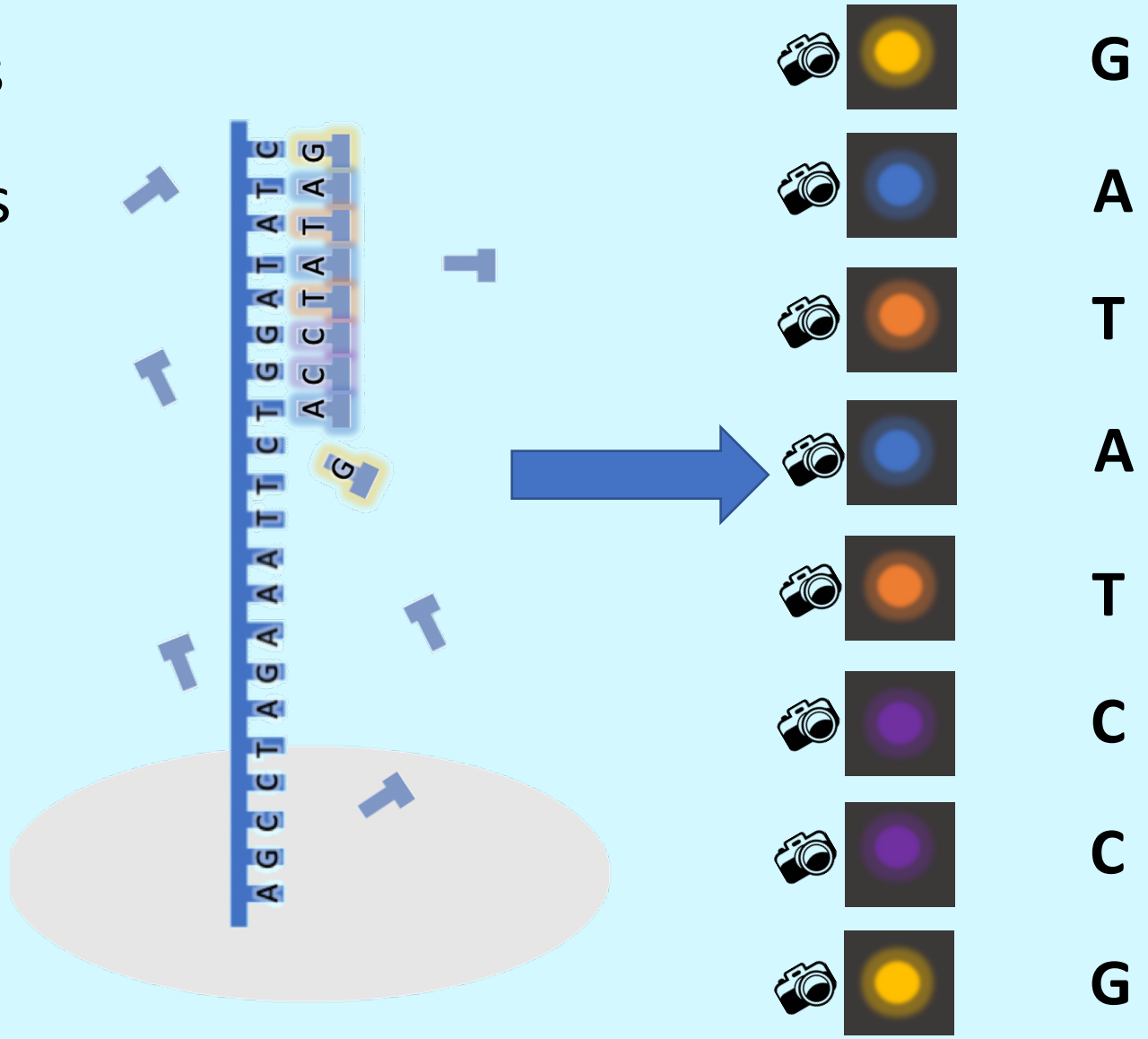
Next Generation Sequencing

- A camera captures the flashes of colors in order, and gives us a sequence for the DNA
- This happens thousands of times simultaneously for the DNA of all species present in the sample!



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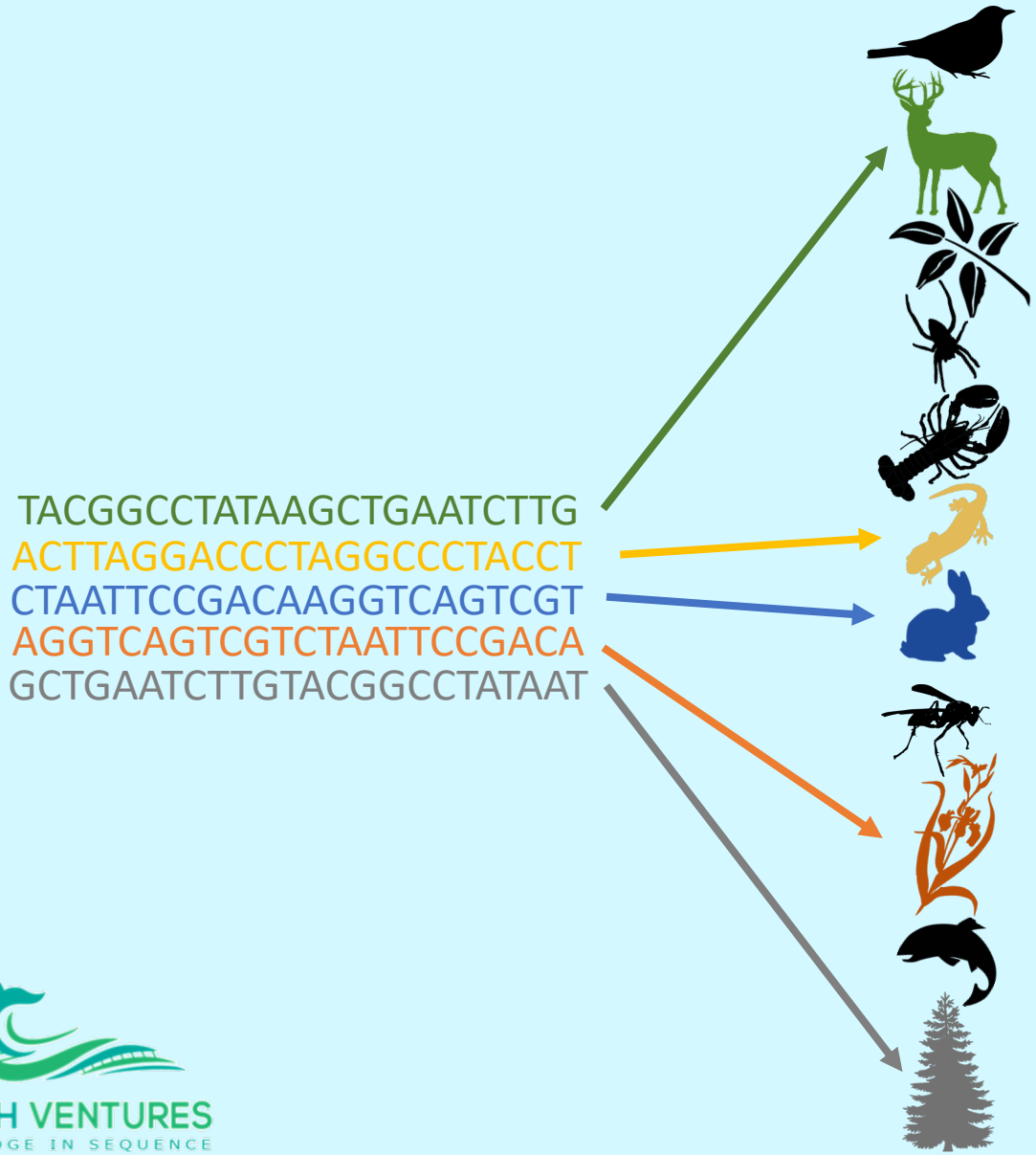
Next Generation Sequencing



- From here, all of the sequences are compared to a library of the sequences of thousands of different species and matched

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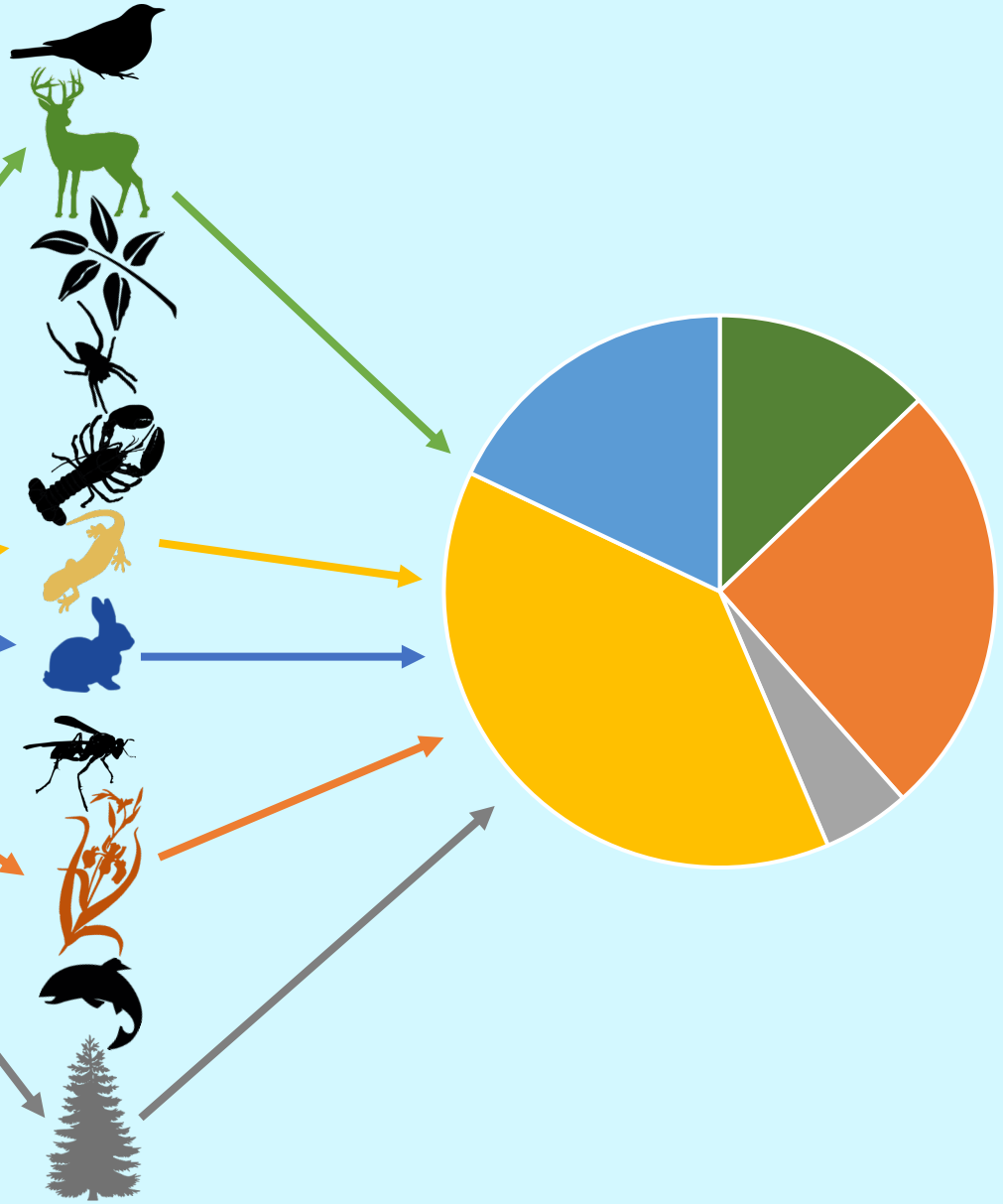
Analysis

- From this data, we can collect important information about the environment our sample came from

TACGGCCTATAAGCTGAATCTTG
ACTTAGGACCCTAGGCCCTACCT
CTAATTCCGACAAGGTCAGTCGT
AGGTCAGTCGTCTAATTCCGACA
GCTGAATCTTGTACGGCCTATAAT



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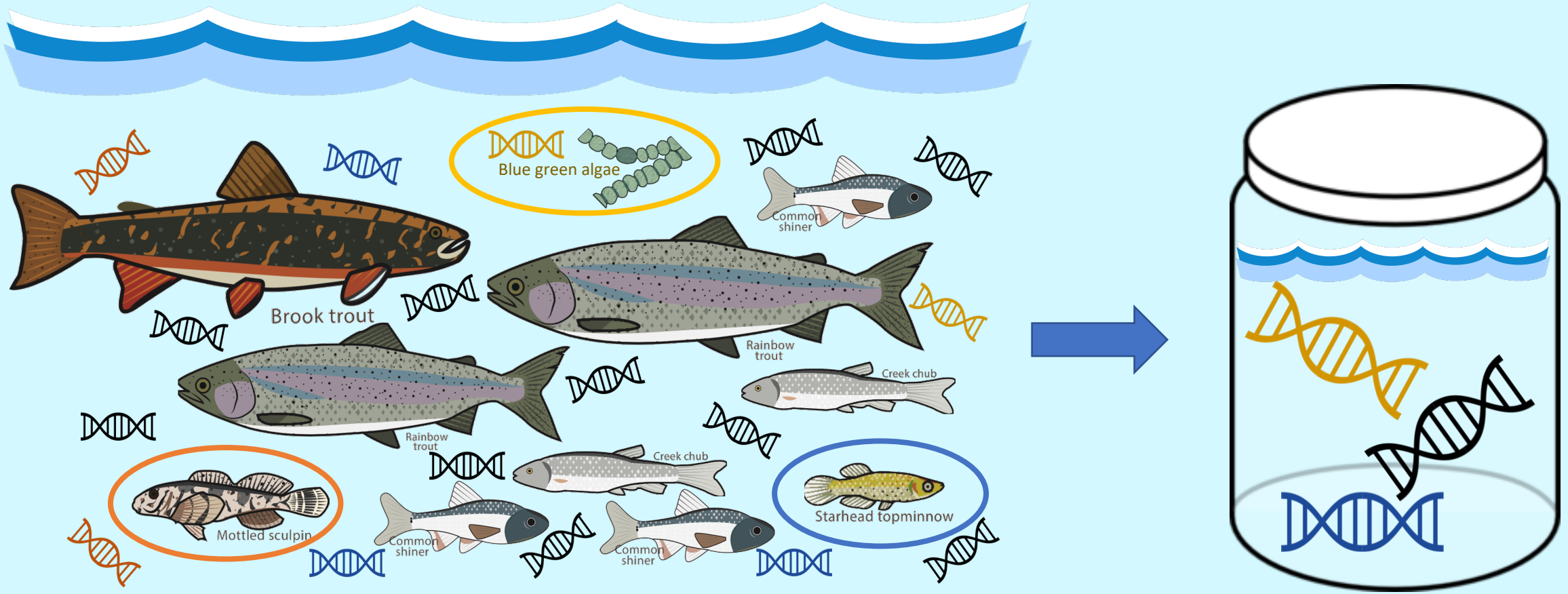
How is this information useful?



- Detects presence of species (especially important with species that are rare, invasive, or dangerous!)
- It can also be used to estimate relative abundance of species and biodiversity (how many different species live in the ecosystem)

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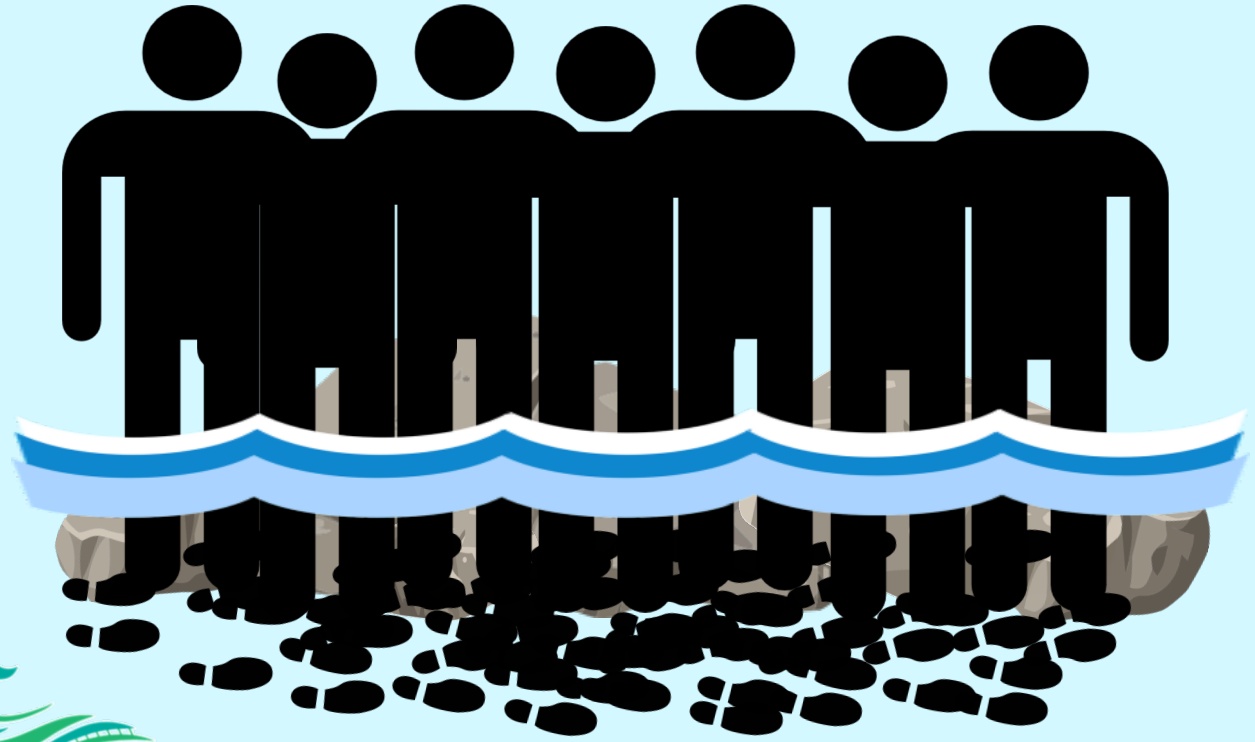
Advantages of eDNA

- Very little disturbance to a site

Traditional sampling for species usually means a team of people at a site



eDNA sampling only needs a single trained person!

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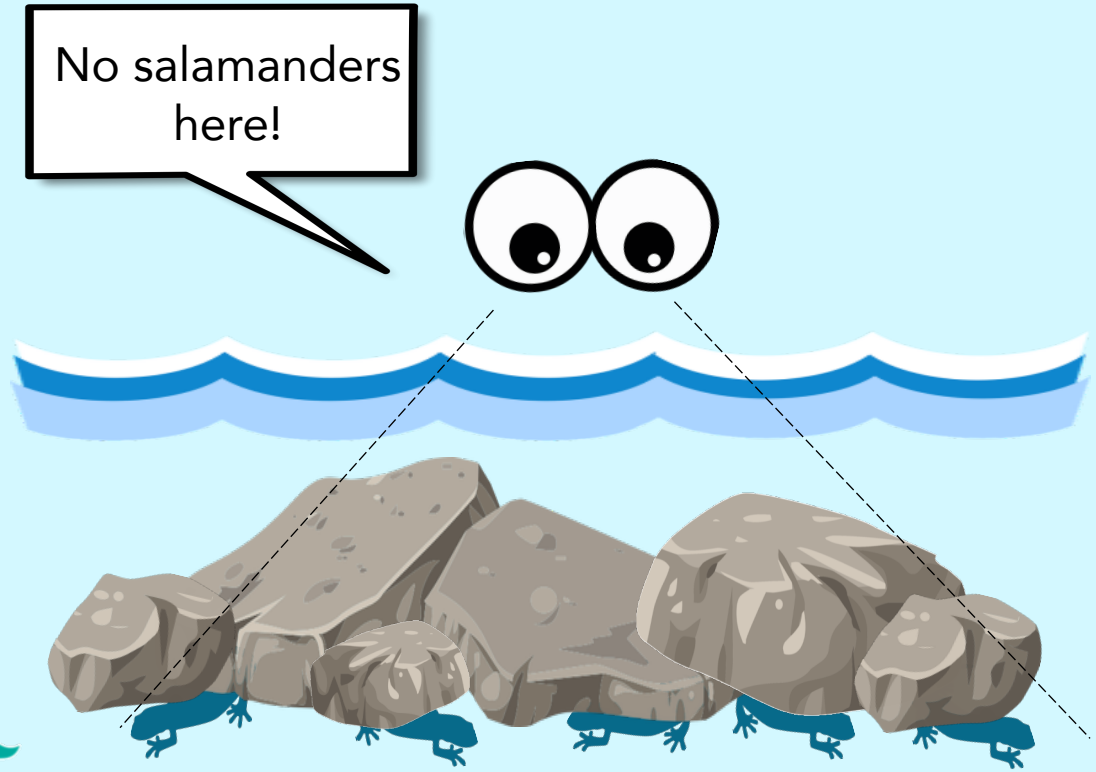
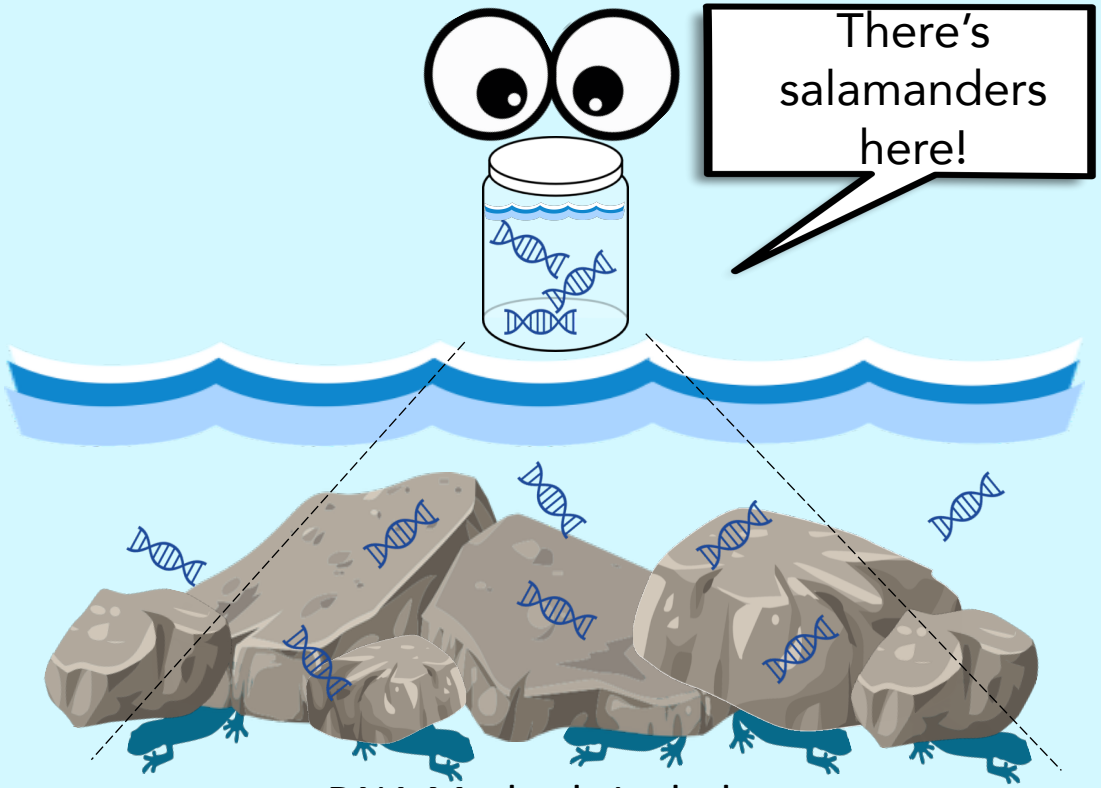
Advantages of eDNA

- Using eDNA can be more effective at finding species than traditional methods
  - An organism may be able to hide if you're looking for it, but it can't hide its DNA in the environment!



Traditional Methods Include:

- Visual Searches
- Aural Searches



eDNA Methods Include:

- Taking a substrate sample

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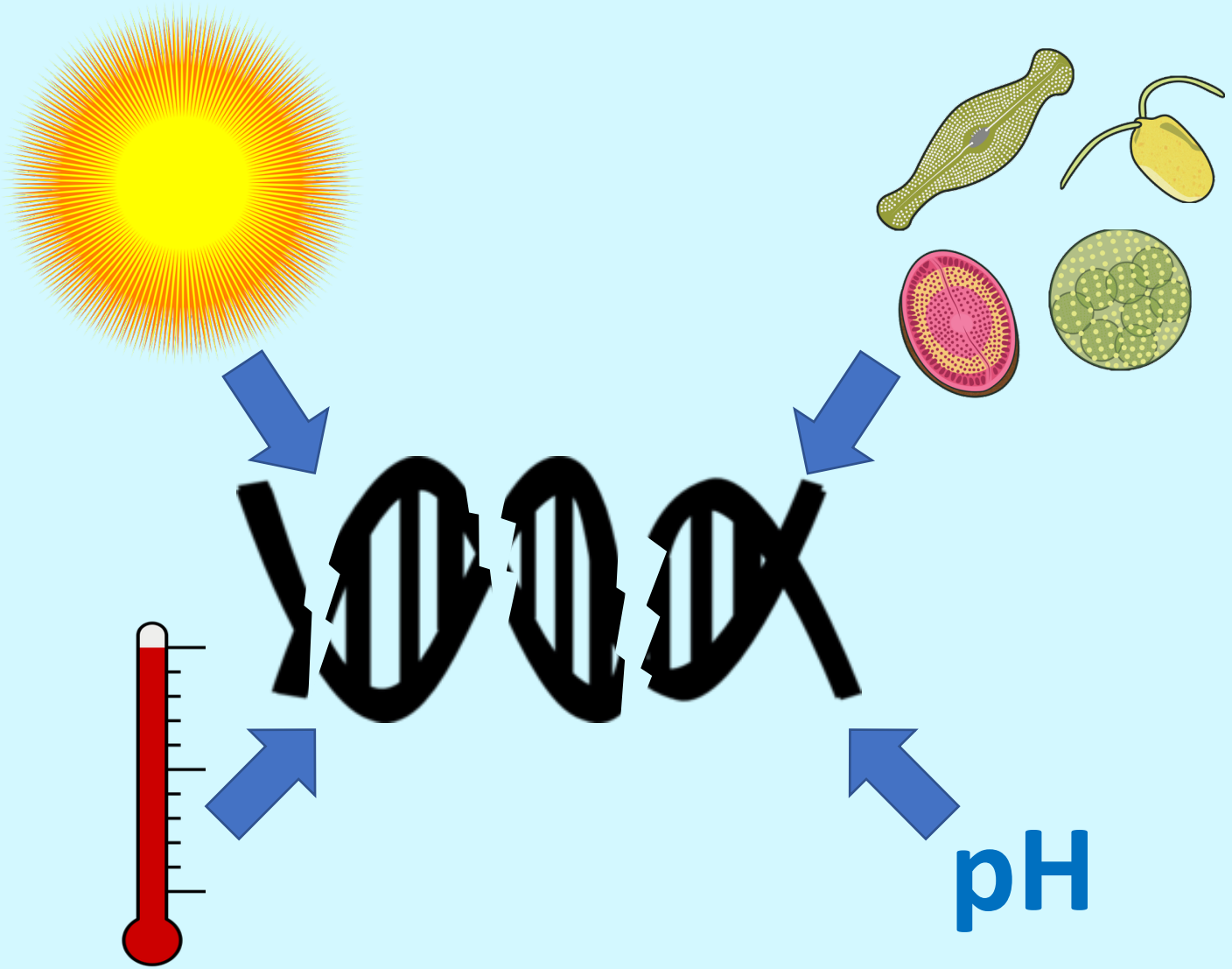
Limits of eDNA

- eDNA can be negatively affected by sunlight, pH microbes, enzymes, and temperature, making it difficult to detect some species



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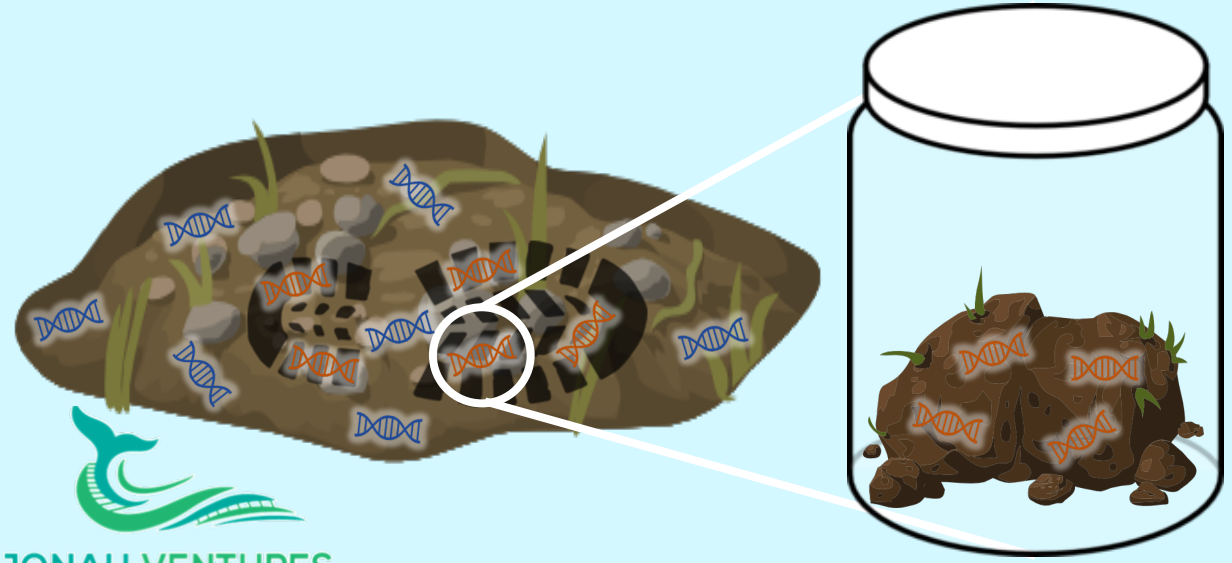
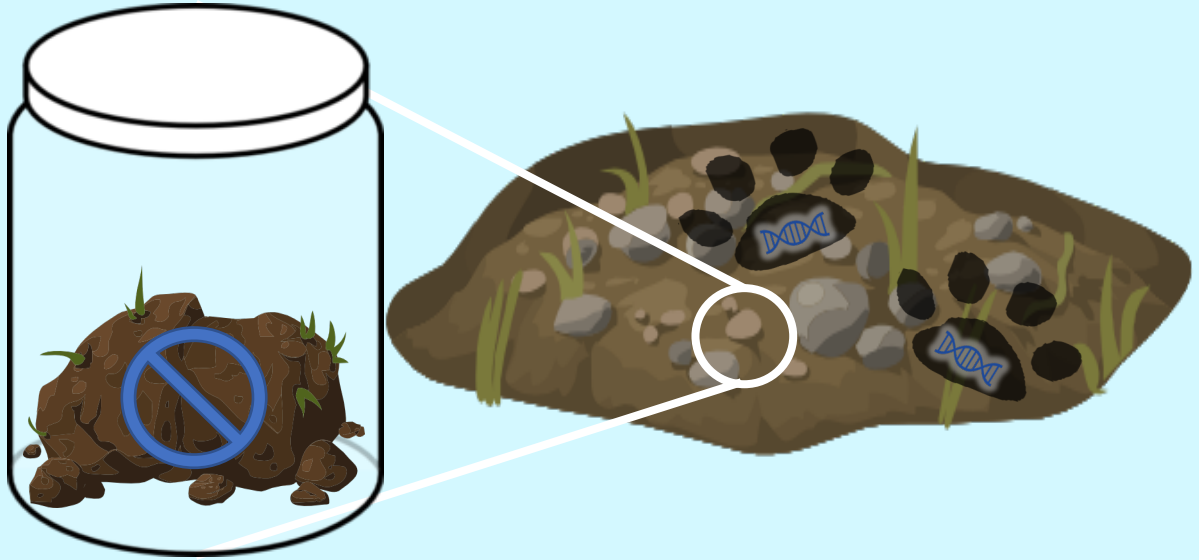
Limits of eDNA

- False positives: when a species is detected in a sample even though it does not occur there
  - Can be caused by DNA being brought to a site on equipment or other contamination



- False negatives: when a species is NOT detected in a sample even though it occurs there
  - Can be caused by collecting samples that are too small, not collecting in the correct area, DNA degradation, etc.



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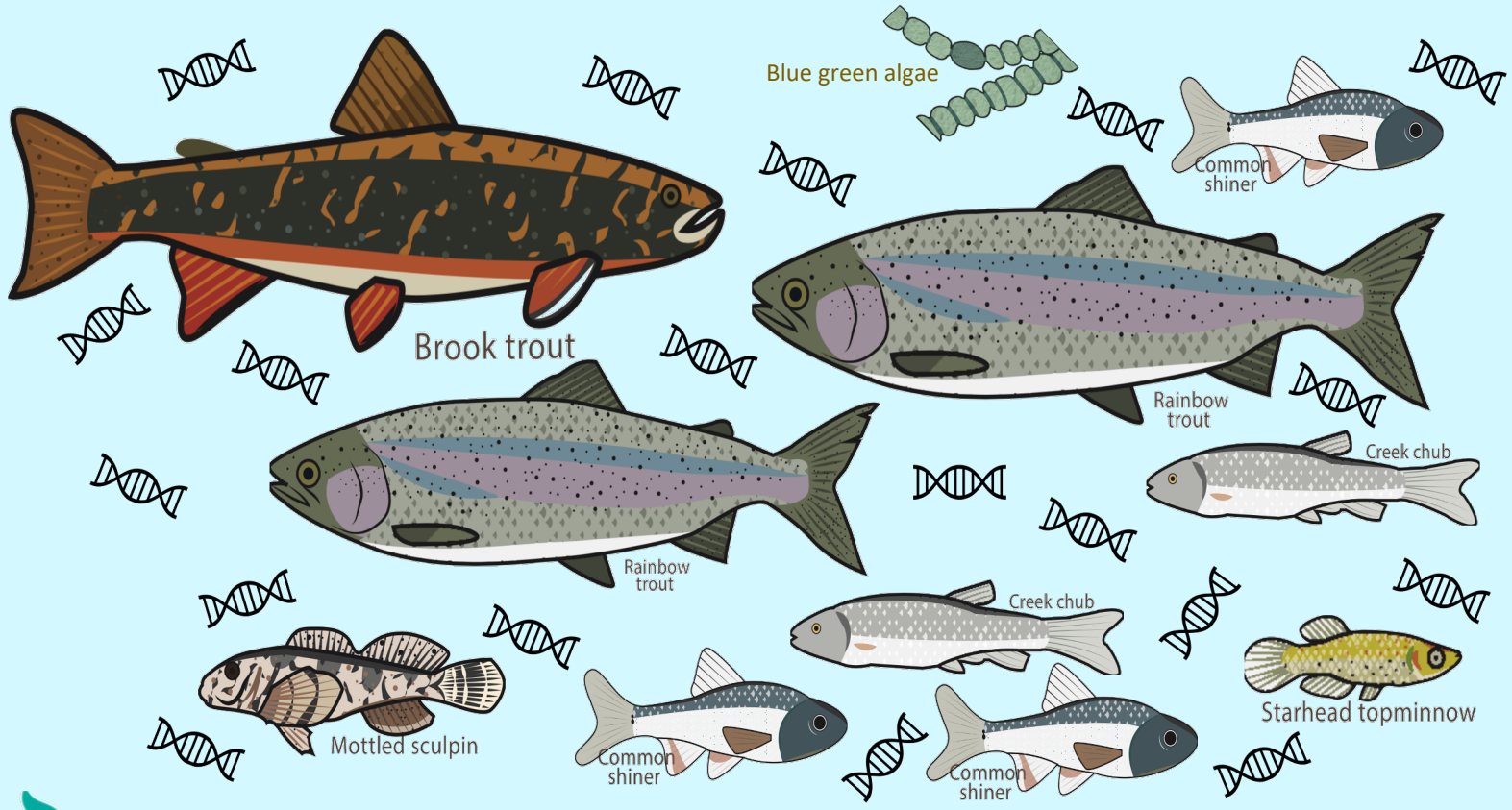
Conclusion



- Environmental DNA is a technology that can be used by anyone, anywhere to see what species live in the environment
- Environmental DNA is used to detect species in the environment without having to physically see them, including species that are invasive, rare, or dangerous to humans!

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Follow Up Questions

- What is environmental DNA?
  - DNA that is shed by an organism into its environment and collected in environmental samples
- What forms does eDNA come in and how is it collected?
  - Hair/fur, skin cells, secretions, excrement
  - Collected in environmental substrate such as snow, air, water, soil, sediment, vegetation
- Which nucleotides match up to form base pairs?
  - A to T, C to G
- What happens during the process of PCR?
  - Millions of copies of DNA are made from just a few fragments
- What is the process to find out what kind of DNA is in an environmental sample?
  - Sampling>Extraction>PCR>Next Generation Sequencing>Analysis
- What are some uses for eDNA?
  - Presence and absence of species, determine ecosystem biodiversity, estimate species abundance, and ecosystem health
- What are some advantages of eDNA?
  - Can be better at finding species than traditional methods, cost-effective, efficient, very little disturbance to a site
- What are some limits of eDNA?
  - It can be degraded by the sun, pH, microbes, enzymes, and temperature, it can give false positives/negatives

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Key Words

- DNA: Deoxyribonucleic acid, DNA is composed of paired nucleotides and determines the characteristics of nearly every organism on earth
- Nucleotides: Adenine, guanine, cytosine, and thymine, they are the primary components of DNA
- Base pairs: Pairs of nucleotides in DNA, A to T, G to C
- DNA Sequence: A precise order of base pairs
- Amplicon: A segment of DNA that is a product of amplification flanked by a forward and reverse primer
- Environmental DNA: DNA that is shed by an organism into its environment and collected in environmental samples
- Polymerase chain reaction: A technique used to make millions of copies of DNA from a few original pieces
- Primers: Specific nucleic acids that bind to the beginning and end of specific sequences of DNA and allow them to be amplified
- Denaturation: During PCR when the temperature is raised to a high temperature (94-96C) and strands of DNA unwind and separate
- Annealing: During PCR when the temperature is lowered (~68C) and the primers bind to their targeted sequences
- Elongation: During PCR when the temperature is raised slightly (~72C) and the spaces between the forward and reverse primers are filled in, creating a new copy of DNA
- Amplification: the exponential growth the DNA experiences during PCR
- Next Generation Sequencing: When the DNA in the sample is sequenced out and matched to sequences in a library of species
- Biodiversity: How many different species live in the ecosystem
- False positives: When a species is detected in a sample even though it does not occur at that site
- False negatives: When a species is not detected in a sample even though it occurs at the site

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