Cold Spring Harbor Laboratory
DNA Learning Center

DNALC Live

Barcoding Bioinformatics Part I

Jason Williams
Cold Spring Harbor Laboratory, DNA Learning Center
williams@cshl.edu
@JasonWilliamsNY

DNALC *Live*

This is an experiment, give us feedback on what you would like to see!

DNALC *Live*

- Provide genetics, molecular biology, and bioinformatics learning resources
- Laboratory and computer demos, short online courses for middle school, high school, and the general public
- Interviews with scientists, help for teachers
- At-home activities, social media contests, and more

DNALC Website and Social Media

dnalc.cshl.edu

dnalc.cshl.edu/dnalc-live

DNALC Website and Social Media

youtube.com/DNALearningCenter

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@dnalc

@dna_learning_center

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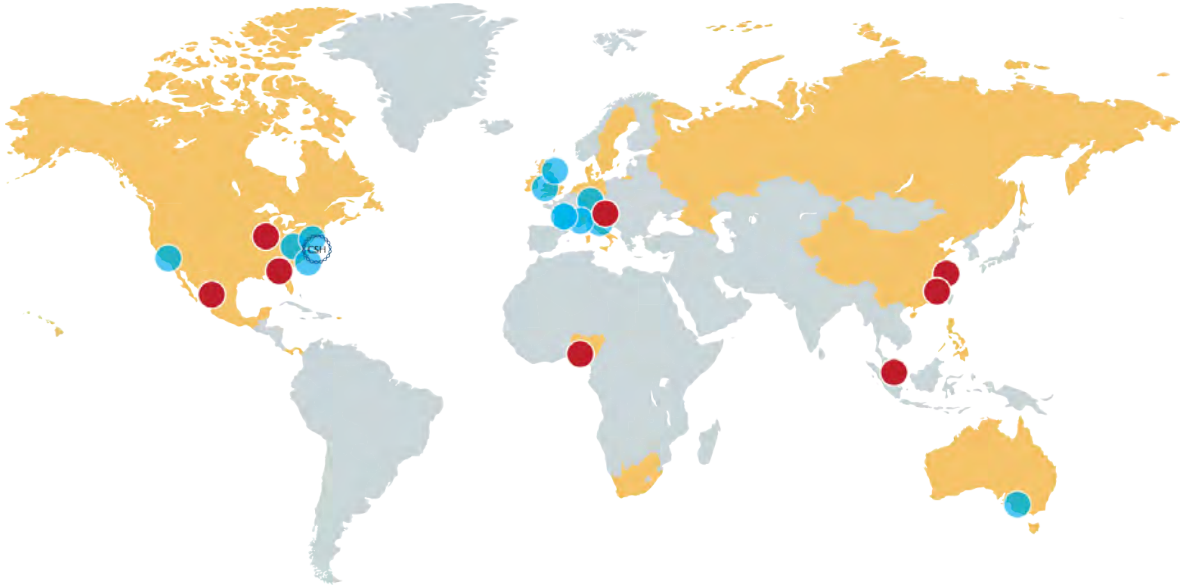


- CSHL
- Licensed Centers
- Programs Modeled on the DNALC
- Teacher Training Sites (US States and Countries)

Cold Spring Harbor Laboratory

Barcoding Bioinformatics Part I

Who is this course for?

- Audience(s): US AP Biology (high school grades 10-12) AND Intro undergraduate biology
- Format: 3 sessions (1 per week); ~ 45-60 minutes each
- Exercises: Follow along with our online bioinformatics tool DNA Subway
- Learning resources: Slides and packet available (teachers can also request the teacher edition)

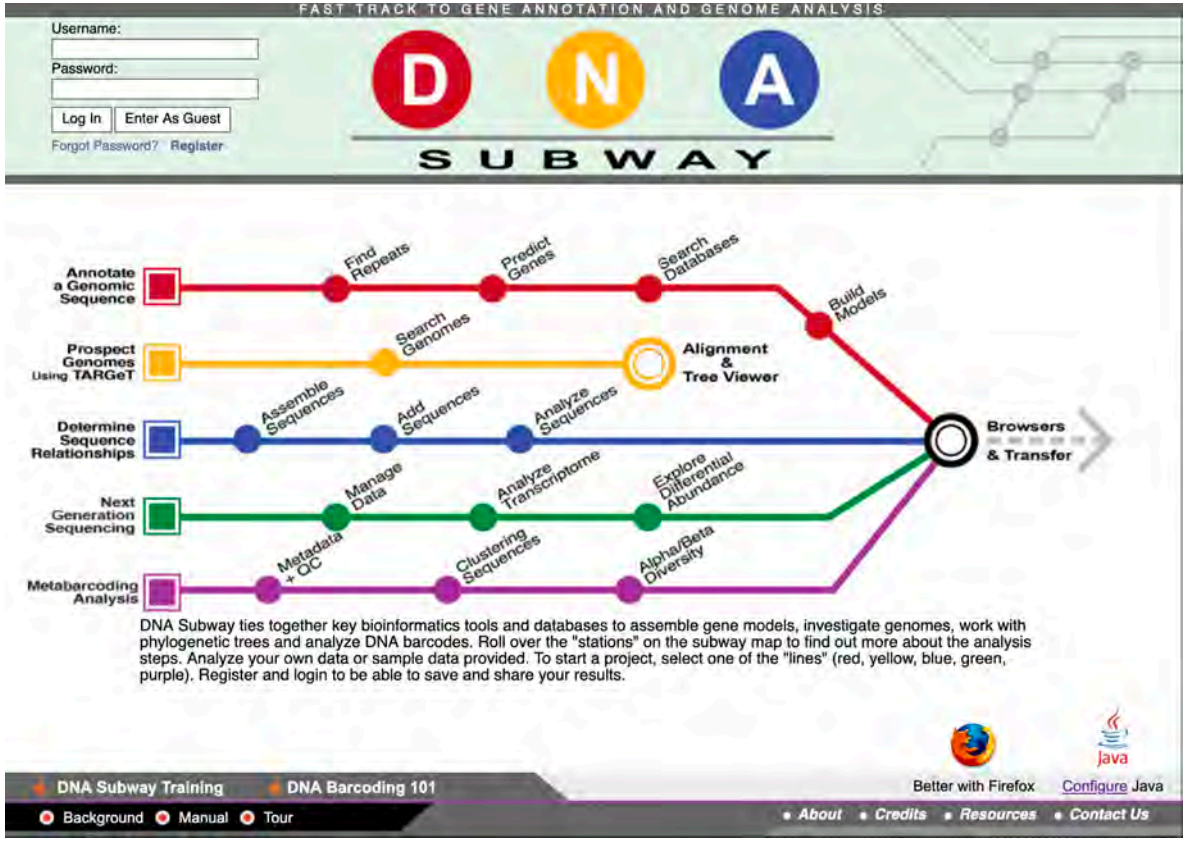
Course Learning Goals

- Learn how DNA can be used to identify unknown organisms
- Understand how we obtain DNA Sequence and access its quality
- Use BLAST* to compare an unknown DNA Sequence to known sequences
- Compare DNA Sequences using phylogenetics

*AP Bio (Lab 3 – Comparing DNA Sequences)

Lab Setup

- We will be using DNA Subway – You can get a free account at **cyverse.org** (optional)



Barcoding Bioinformatics Part I

(Background and Sequence Quality)

Steps for today's session

- Introduction to Bioinformatics
- Get background on DNA Barcoding
- Learn about our example experiment
- Start an experiment
- Examine DNA sequence quality

Introduction to Bioinformatics

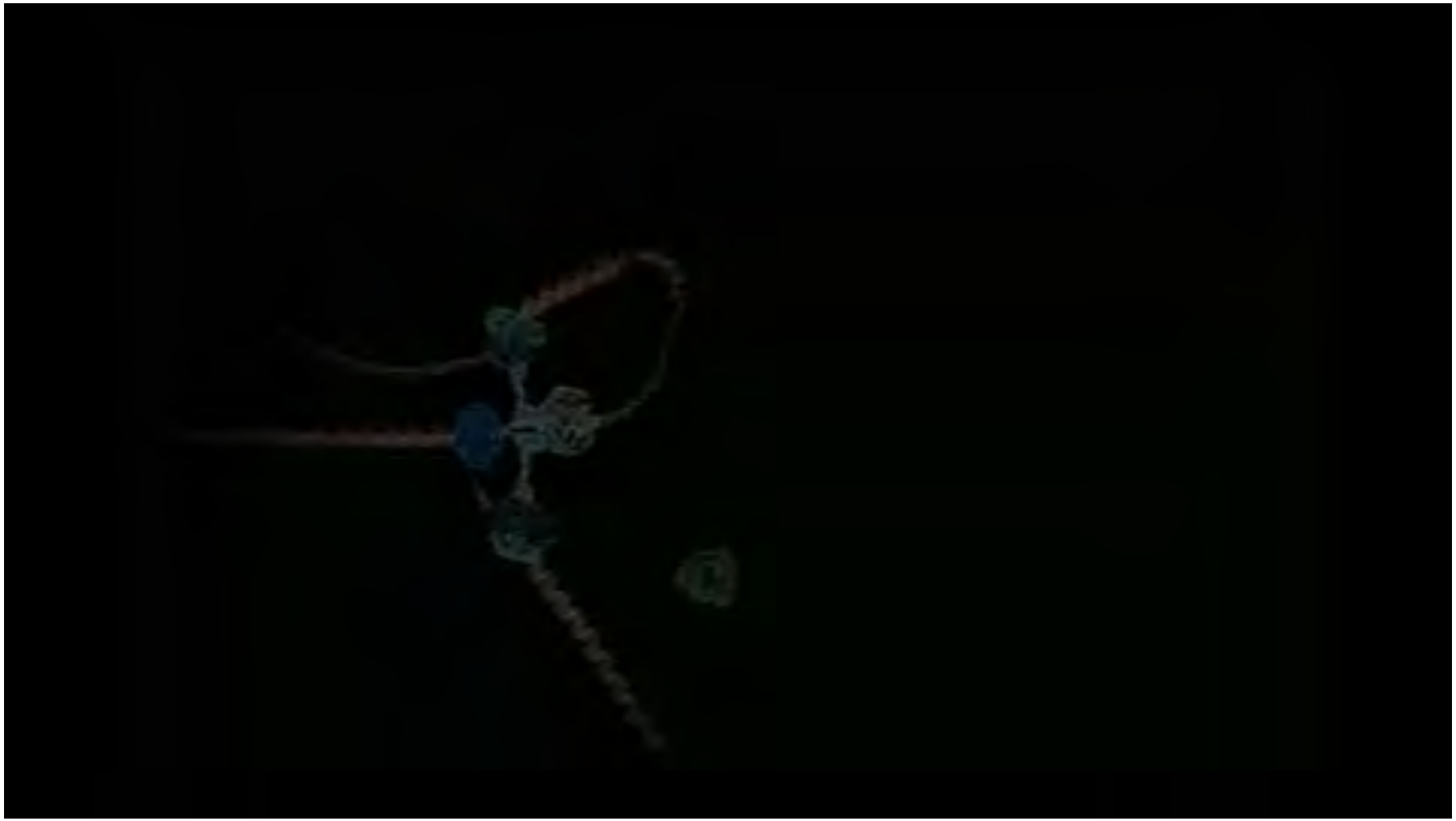
What is Bioinformatics?

In biology, **Bioinformatics** is an interdisciplinary field that develops and improves upon methods for storing, retrieving, organizing and analyzing **biological data**. A major activity in bioinformatics is to develop software tools to generate useful biological knowledge.

- http://en.wikipedia.org/wiki/Bioinformatics - retrieved April 23rd 2013

Bioinformatics is about data

Bioinformatics is about data



DNA Structure

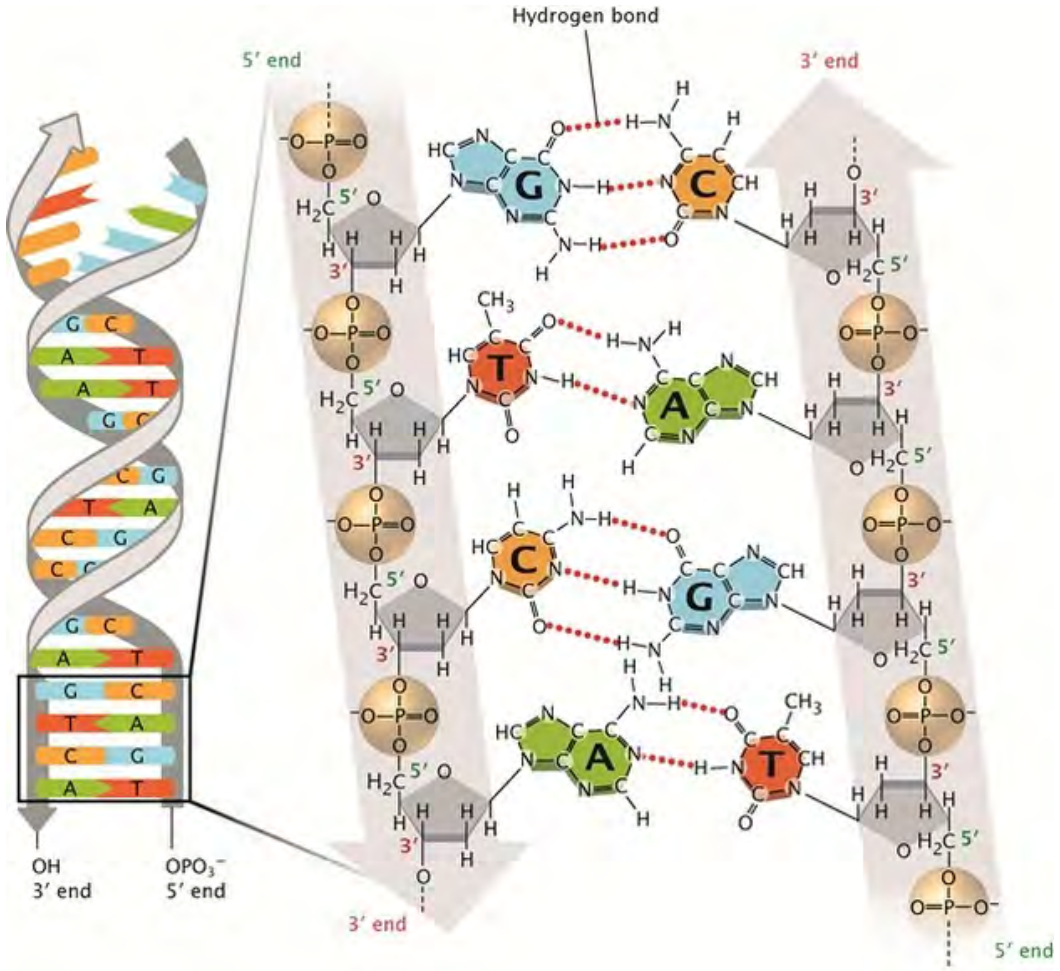


Image Credit: Discovery of DNA Structure and Function: Watson and Crick
By: Leslie A. Pray, Ph.D. © 2008 Nature Education
Citation: Pray, L. (2008) Discovery of DNA structure and function: Watson and Crick. Nature Education 1(1):100

Bioinformatics is about data

Often, we we are speaking about data in biology, we are talking about DNA Sequence

Bioinformatics is about data

Often, when we are speaking about data in biology, we are talking about DNA Sequence (there are lots of other data, we just won't be talking about that today)

DNA Sequence

National Center for Biotechnology Information
NCBI

Quick Tour

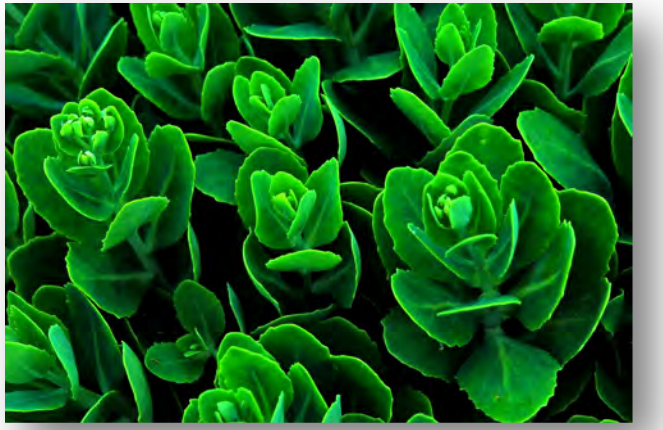
- NCBI Homepage https://www.ncbi.nlm.nih.gov/
- Human Genome: https://www.ncbi.nlm.nih.gov/projects/genome/guide/human/index.shtml
- Corona Virus Genome: https://mra.asm.org/content/9/11/e00169-20

Introduction to Barcoding

How can you identify an organism using just its DNA?

What is DNA Barcoding?

Steps to DNA Barcoding



Organism is sampled

DNA is extracted

“Barcode” amplified

ACGAGTCGGTAGCTGCCCTCTGACTGCATCGAA
TTGCTCCCCTACTACGTGCTATATGCGCTTACGAT
CGTACGAAGATTTATAGAATGCTGCTACTGCTCC
CTTATTCGATAACTAGCTCGATTATAGCTACGATG

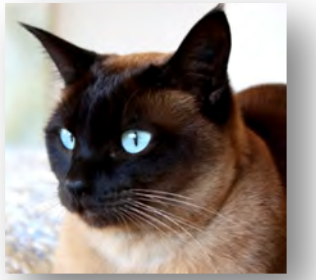
Sequenced DNA is compared with DNA in a barcode database

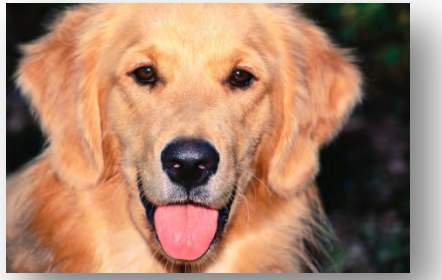
The Start of Barcoding

How many species can you name?

How many Animals did you name?
How many mammals?
How many plants?
How many insects?

"Dog"
Canis lupus familiaris





"Cat"
Felis catus

"Oak Tree"
Quercus alba

"Shark"
Ginglymostoma cirratum

"Beetle"
Popillia japonica

Problem 1: No one know how many species there are.

How many species are there?

Vertebrates	Species
Mammals	5,490
Birds	9,998
Reptiles	9,084
Amphibians	6,433
Fishes	31,300
Total	62,305

Invertebrates	Species
Insects	1,000,000
Mollusks	85,00
Crustaceans	47,000
Corals	2,175
Arachnids	102,248
Total (+others)	1,305,250

Plants	Species
Angiosperms	281,821
Gymnosperms	1,021
Ferns and Allies	12,000
Mosses	16,236
Green and Red Algae	10,134
Total	321,212

- **There are currently between 1.5 and 2 million described species**
- **It is estimated that this number may represent as little as half of the true number of species**
- **Perhaps more than 1/3 of all species are threatened**

(IUCN Red list version 2010.1)

Problem 2: Even though there are millions of species, there is also a lack of agreement on what a “species” means.

What is a species?

Canis lupus

Canis lupus (familiaris)

Anas platyrhynchos

Defining what species are is a complex task

Dependent on many factors

- Interbreeding capabilities
- Morphological variation
- Ecological context
- Genetic similarities

Problem 3: Current taxonomic methods may be inadequate (or at least too slow) to capture vanishing biodiversity

Traditional taxonomies

Leaves alternate proximally, opposite and ultimately decussate distally, 6–16 × 4–13 cm; petiole ca. as long as blade, winged, base clasping, basal lobes stipulate, growing as extensions of wings, less than 1 mm wide; blade 5–7-veined, ovate, glabrous, base typically sagittate, margins entire, apex acute to acuminate. Staminate inflorescences axillary, 1–2 per axil, paniculate, fasciculate; panicles bearing flowers singly,bracteolate, in a zigzag pattern along rachis, internodes less than 2 mm; rachis to 25 cm, secondary axes 1–3(–6), fasciculate, less than 3 cm, each subtended by deltate-ovate bracteole shorter than 1 mm. *Pistillate* inflorescences solitary, 4–8(–20)-flowered, 6–35 cm, internodes ca. 1 cm

The body form ranges from hemispherical (e.g., *Cleidostethus*) to elongate oval (e.g., *Clypastraea*) to latridiid-like (e.g., *Foadia*). Corylophids are typically dull brown, but some species have contrasting yellowish-brown patches on the pronotum or elytra. The integument is often densely punctured and may be glabrous or bear short, fine recumbent setae. Most corylophid adults can be diagnosed using the following morphological features: Maxilla with single apical lobe; Mesotrochanter short and strongly oblique; Head usually covered by pronotum; Frontoclypeal suture absent; Antennae elongate with 3-segmented club; Procoxal cavities closed externally; Tarsal formula 4-4-4; Pygidium exposed

Sequence is less complex

Leaves alternate proximally, opposite and ultimately decussate distally, 6–16 × 4–13 cm; petiole ca. as long as blade, winged, base clasping, basal lobes stipulate, growing as extensions of wings, less than 1 mm wide; blade 5–7-veined, ovate, glabrous, base typically sagittate, margins entire, apex acute to acuminate. Staminate inflorescences axillary, 1–2 per axil, paniculate, fasciculate; panicles bearing flowers singly,bracteolate, in a zigzag pattern along rachis, internodes less than 2 mm; rachis to 25 cm, secondary axes 1–3(–6), fasciculate, less than 3 cm, each subtended by deltate-ovate bracteole shorter than 1 mm. *Pistillate* inflorescences solitary, 4–8(–20)-flowered, 6–35 cm, internodes ca. 1 cm

Complex and Somewhat objective

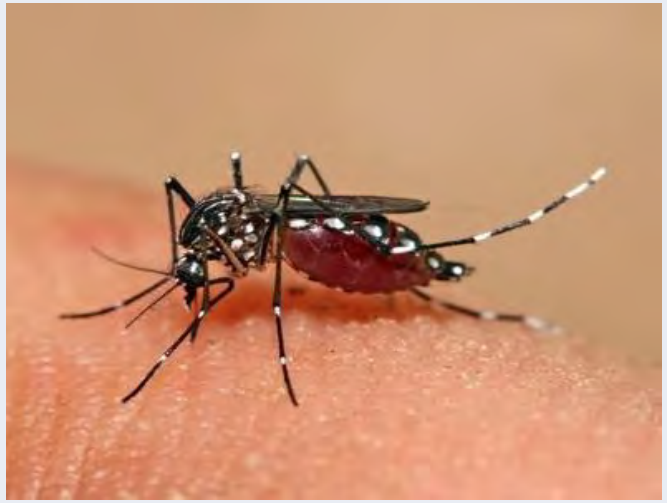
>Dioscorea alata (matK) gene, partial

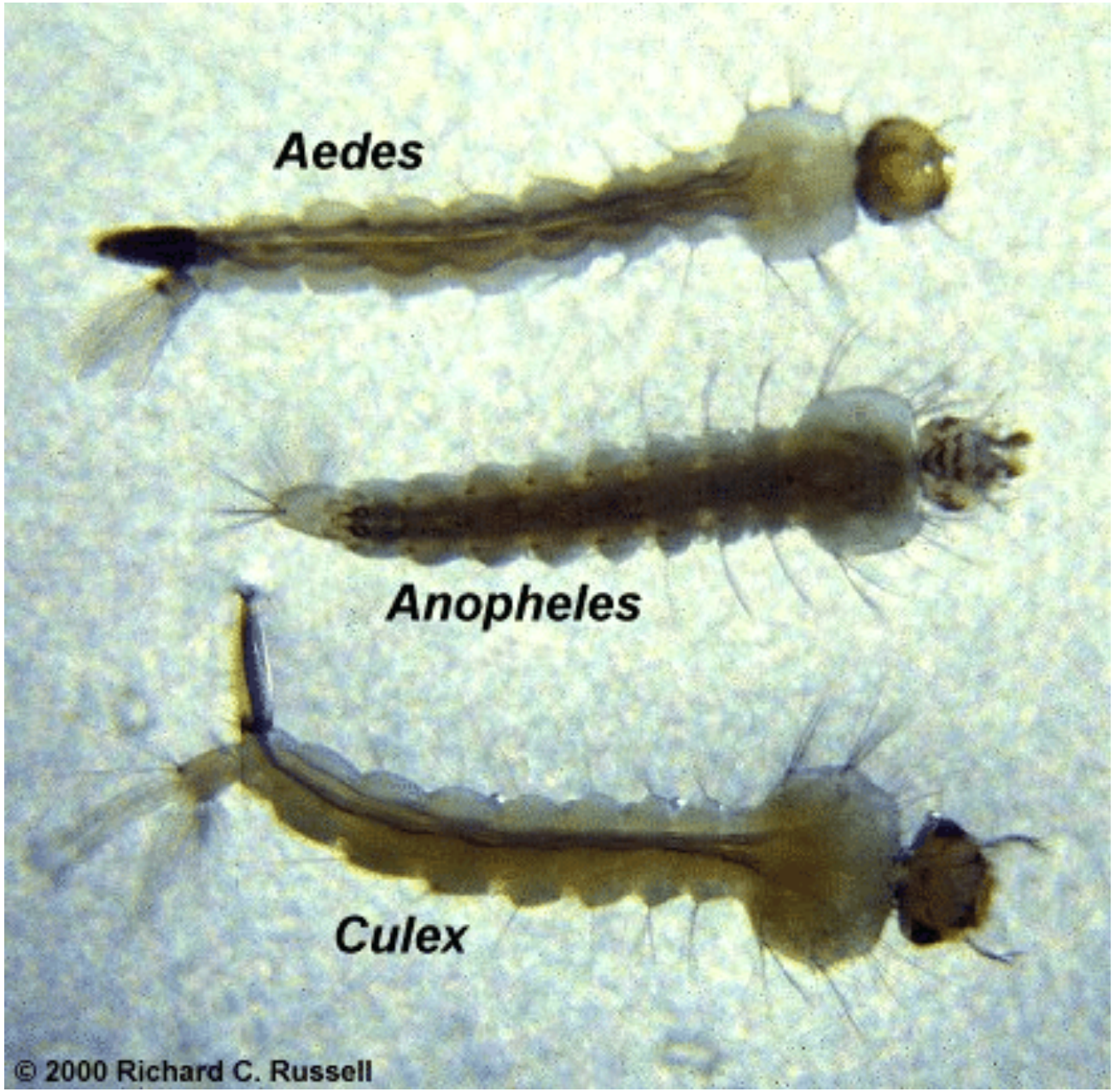
ATTTAAATTATGTGTCAGATATATTAATACCCCATCCCATCCATCTGGAAATCCTGGTTCAAATACTTCAATGCTGGACTCAAGATGTTTCCTCTT
TGCATTTATTGCGATTCTTTCTCCACGAATATCATAATTCGAAT AGTTTCATTACTCCGAAAAAACCTATTTACGTGATTTCAATTTCAAAAGAAA
ATAAAAGATTTTTTCGAT TCCTATATAATTCTTATGTATTTGAATGTGAATTTGTATTAGTTTTTTTTCATAAGCAATCCTCTTATTT ACGATCAA
GGTCCTCTGGAGTCTTTCTTGAGCGAACACATTTCTATGGAAAAATGGGGCATTTTTTAGTAGTGTGTTGTAATTATTTTCAGAAGACCCAATG
GTTCTTCAAAGATCCTTTTCTGCATTATGTTCGATATC AAGGAAAAGCAATTCTGGTGTCAAAGGGAACTCGTCTTTTGATGAGGAAATGGAGA
TCTTACCTTGTCCATTTTTGGCAATATTATTTTCAATTTTGGTCTCATCCGCATAGGATTCATATAAACCAATTATCAAATTATTCCTTCTGTTTTC
TGGGTTATCTTTCAAATGTACTAATAAATTTTTCCGTGGTAAGGAGTCAAATGTTAGAAAATTCATTTGTAATAGATACTCTTACTAAGAAATT
TGATACCAGAGTTTCAGTTATTGCTCTTATTCG ATCATTGTCTAAAGCGAAATTTTGTACCGTATCCGGGCATCCTATTAGTAAGTCAATATGGA
CAAATTTC TCAGATTTGGATATTATTCATCGATTTGGTTGGATATGTAGAA

Simple (A,T,G, or C) and objective

Lab: Mosquito Identification

Can we tell the difference between larvae that look (nearly) identical?

Aedes adult	Anopheles adult	Culex adult
 By Muhammad Mahdi Karim - Own work, GFDL 1.2, https://commons.wikimedia.org/w/index.php?curid=11185617	 By Jim Gathany - (PHIL), ID #5814. https://commons.wikimedia.org/w/index.php?curid=799284	 By Muhammad Mahdi Karim - Own work, GFDL 1.2, https://commons.wikimedia.org/w/index.php?curid=7673048
Aedes larva	**Anopheles larva**	**Culex larva**
 Photograph by Michele M. Cutwa, University of Florida.		 Photograph by Michelle Cutwa-Francis, University of Florida.


Aedes
Anopheles
Culex
© 2000 Richard C. Russell

Why does this matter?

***Aedes*:**

- Chikungunya
- Dengue fever
- Lymphatic filariasis
- Rift Valley fever
- Yellow fever
- Zika

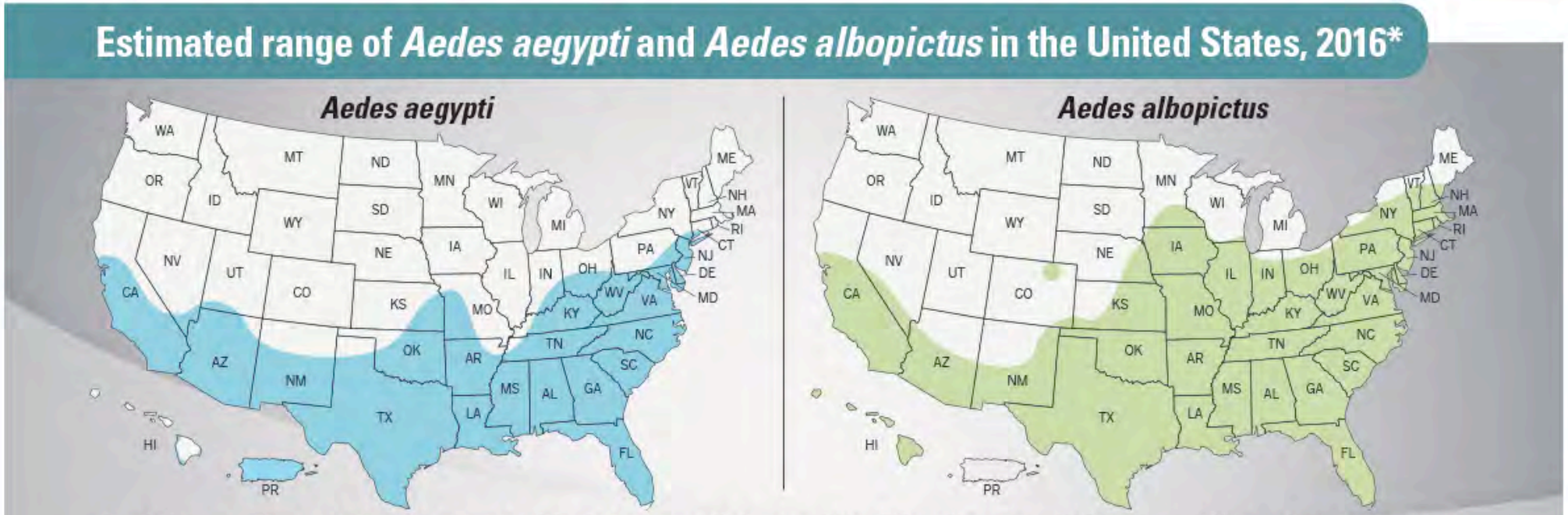
***Anopheles*:**

- Malaria
- Lymphatic filariasis

***Culex*:**

- Japanese encephalitis
- Lymphatic filariasis
- West Nile fever

Why does this matter?



***Aedes aegypti* mosquitoes are more likely to spread viruses like Zika, dengue, chikungunya than other types of mosquitoes such as *Aedes albopictus* mosquitoes.**

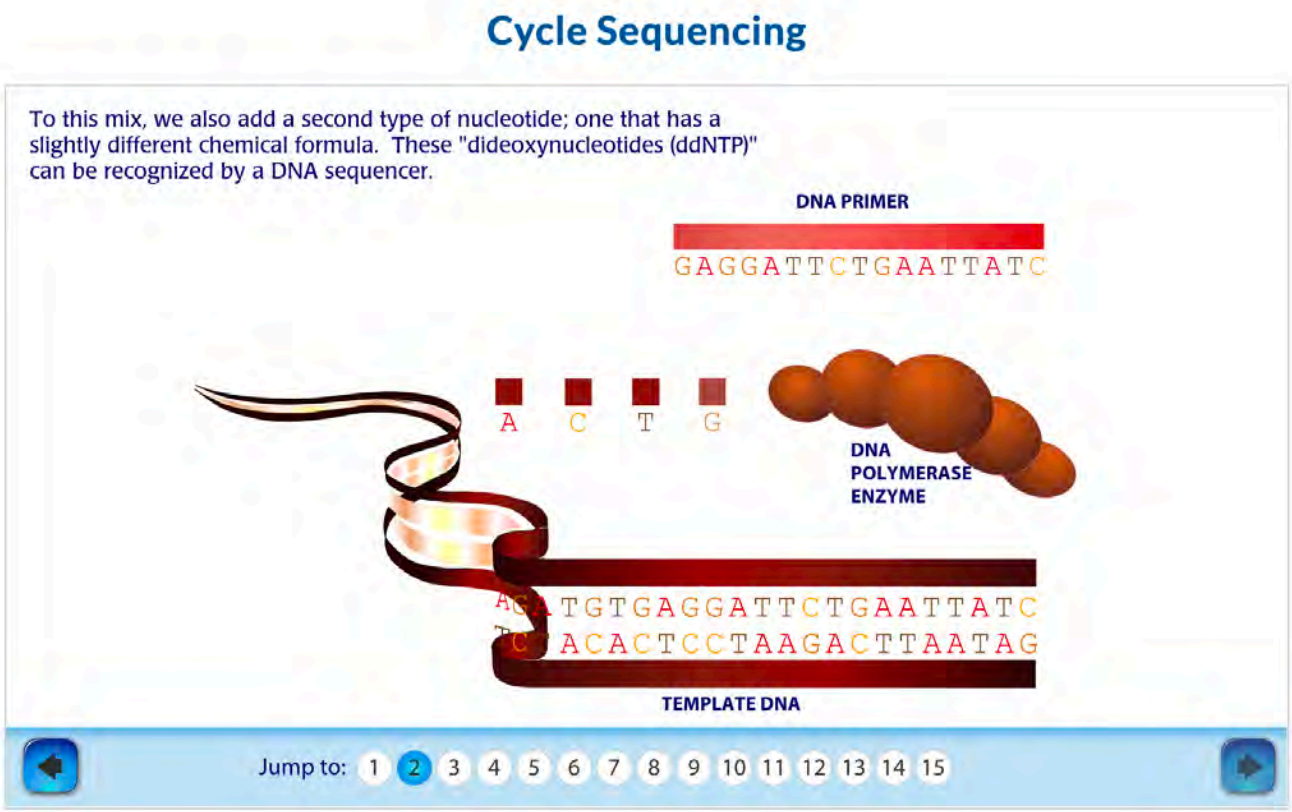
- These maps show CDC's best estimate of the potential range of *Aedes aegypti* and *Aedes albopictus* in the United States.
- These maps include areas where mosquitoes are or have been previously found.
- Shaded areas on the maps do not necessarily mean that there are infected mosquitoes in that area.

**Maps have been updated from a variety of sources. These maps represent CDC's best estimate of the potential range of Aedes aegypti and Aedes albopictus in the United States. Maps are not meant to represent risk for spread of disease.*

SOURCE: Zika: Vector Surveillance and Control. www.cdc.gov/zika/vector/index.html

Lab: DNA Sequencing Background

DNA Sequencing



https://dnalc.cshl.edu/resources/animations/cycseq.html

Some Anopheles DNA…

Anopheles gambiae isolate 10016 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MK592083.1

GenBank Graphics

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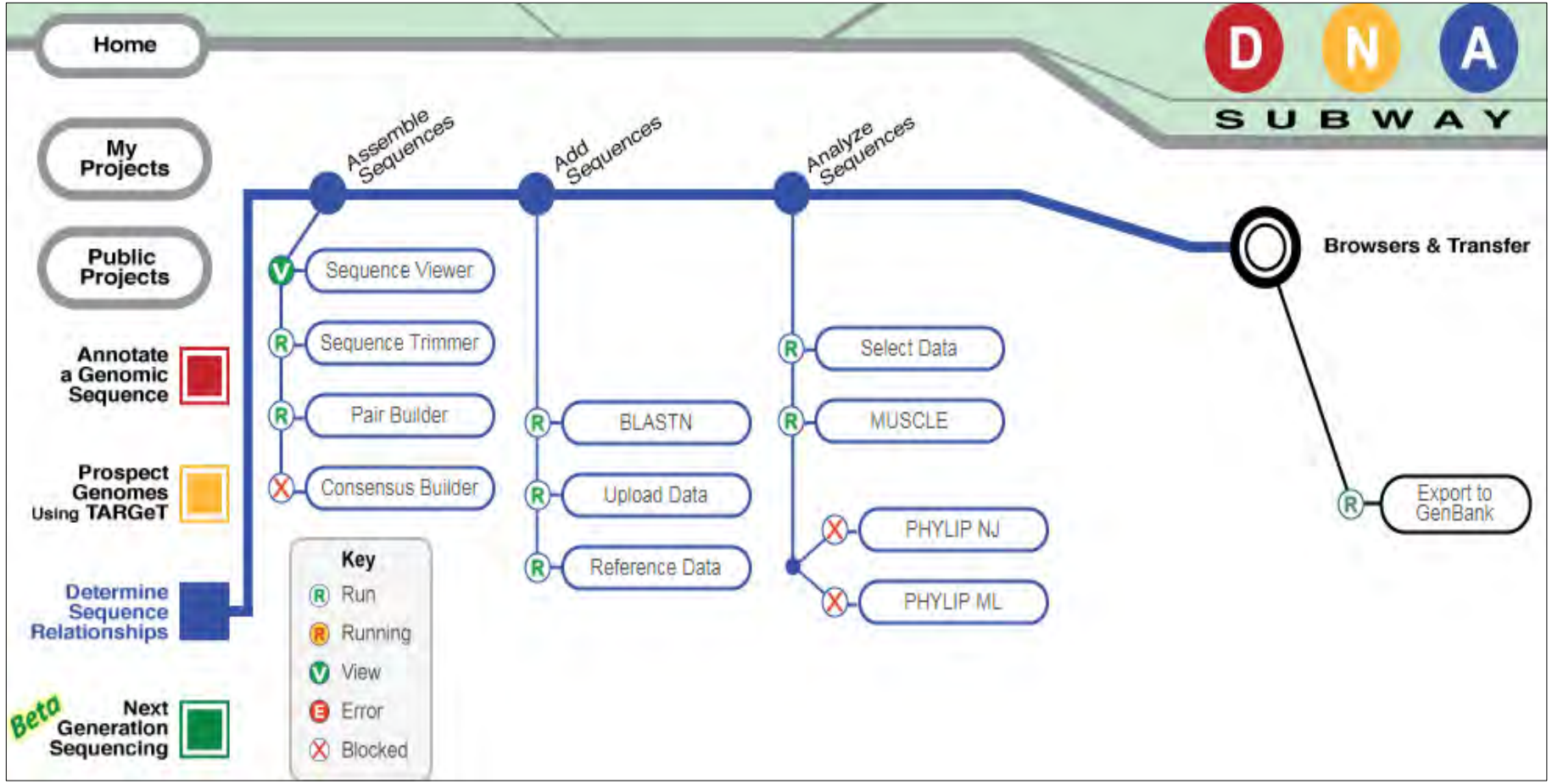
>MK592083.1 Anopheles gambiae isolate 10016 5.8S ribosomal RNA gene, partial
sequence; internal transcribed spacer 2, complete sequence; and large subunit
ribosomal RNA gene, partial sequence
ACACATGAACATTGATAAGTTGAACGCATATGGCGCATCGGACGTTTAATCCCGACCGATGCACACATTC
TTGAGTGCCTACTAATTACCAAAGTCTCATTTAGTTAACTACAGTGGCCGTCCGCGAAGGTGCCCGGGTC
ATCCGACGCACTGGGCGGTCGCTGTGCATAATGACGTGCTTGGTCCCCGTCTGCGGGTCCTCGGGCGTTG
AAAGTGGACACTCTCGAGCGTATGTTGGATGCGTTTCGTGTTGGTGGTGTTTGATGCGTAGGGCTTGTGG
TGTGTGTCAAGCCGCATGGTTCGAACTAATGCTACGTCGTTCCCGATGGCCACCGGCAGTCTACTCTCCA
GGCTAAAGTCGGCTCGTCTAGGGATTCGGAAAGCTAAGTCGCTGTAACTCATGTGGGCCCATACACGGCG
TTGCGCTACCACGCTAAGTTAGCCCTACATACACAAGCATCAACCCACGGCACGGGCGTAGCTGTAATAC
TTACGTCTCGGTTATACCACGTAGGCCTCAAGTGATGTGTGACTACCCCC
```

Lab: Creating a DNA Subway Project

(follow along in the packet)

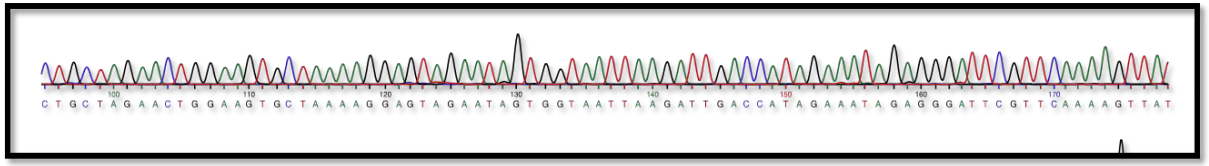
Working on DNA Subway Blue Line



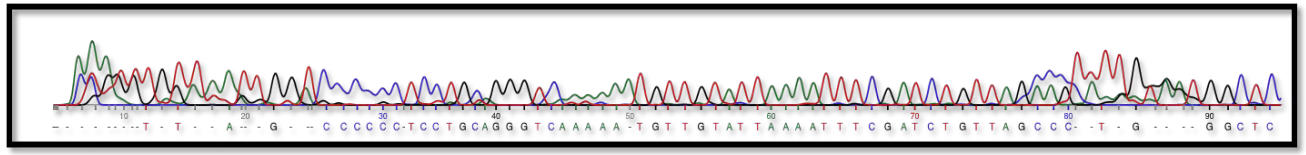
Key Concept: Data Quality

Some sequence examples…

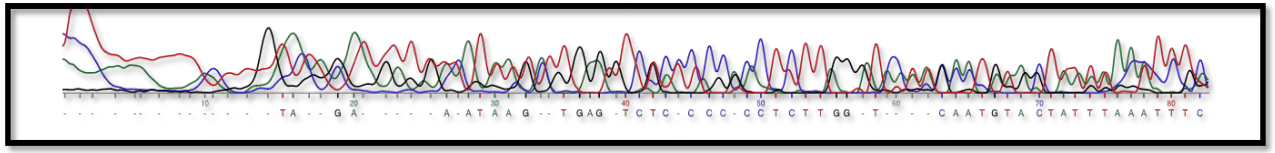
High Quality Sequence



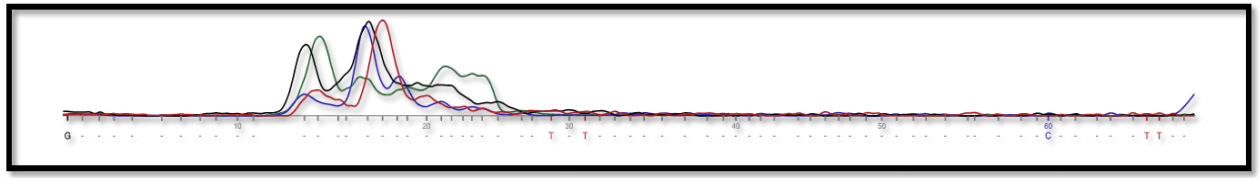
Acceptable Quality Sequence



Low Quality Sequence (multiple base calls per position)



Low Quality Sequence (no base calls)



Phred scores…

Phred Score	Error (bases miscalled)	Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1,000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%

Next time:
Comparing sequences with BLAST

DNALC Website and Social Media

dnalc.cshl.edu

dnalc.cshl.edu/dnalc-live