

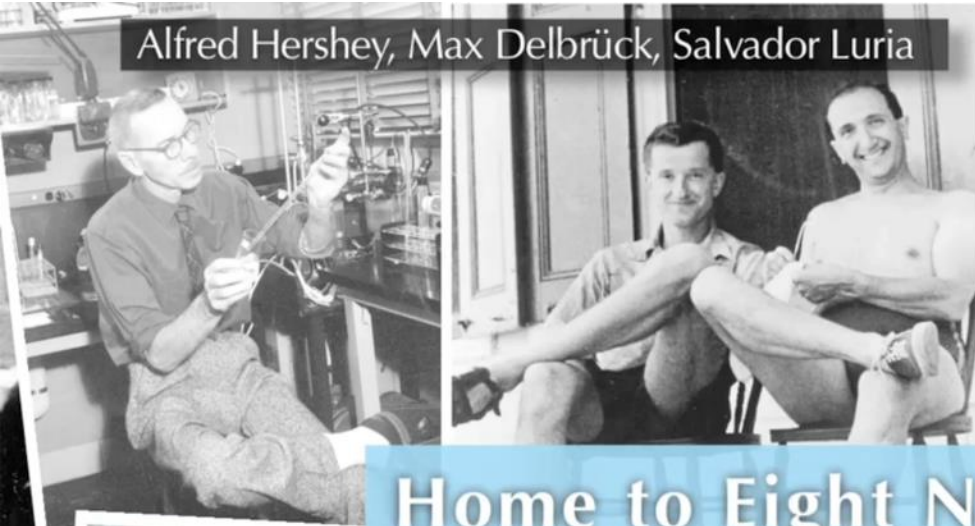
DNA Barcoding and DNA Subway: Infrastructure for Citizen Science

Dave Micklos
DNA Learning Center,
Cold Spring Harbor Laboratory
Banbury Nanopore Meeting, 9/23/24

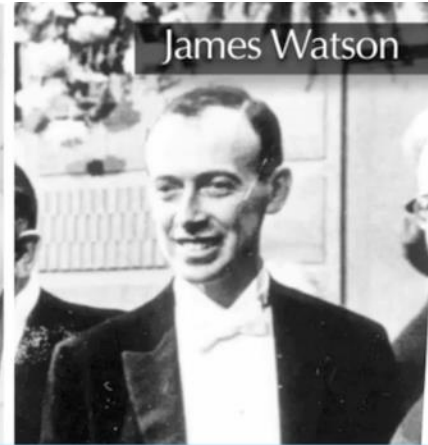


Cold Spring Harbor Laboratory

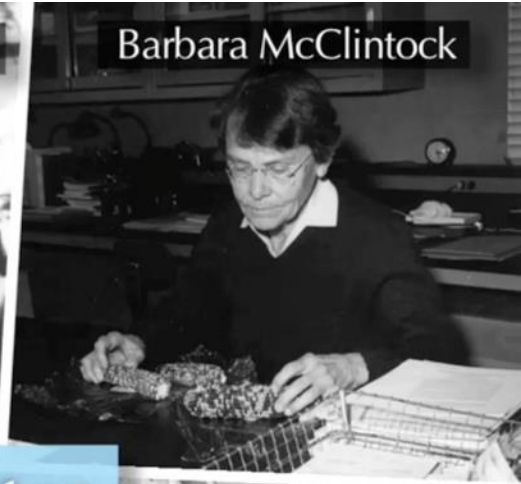
Alfred Hershey, Max Delbrück, Salvador Luria



James Watson

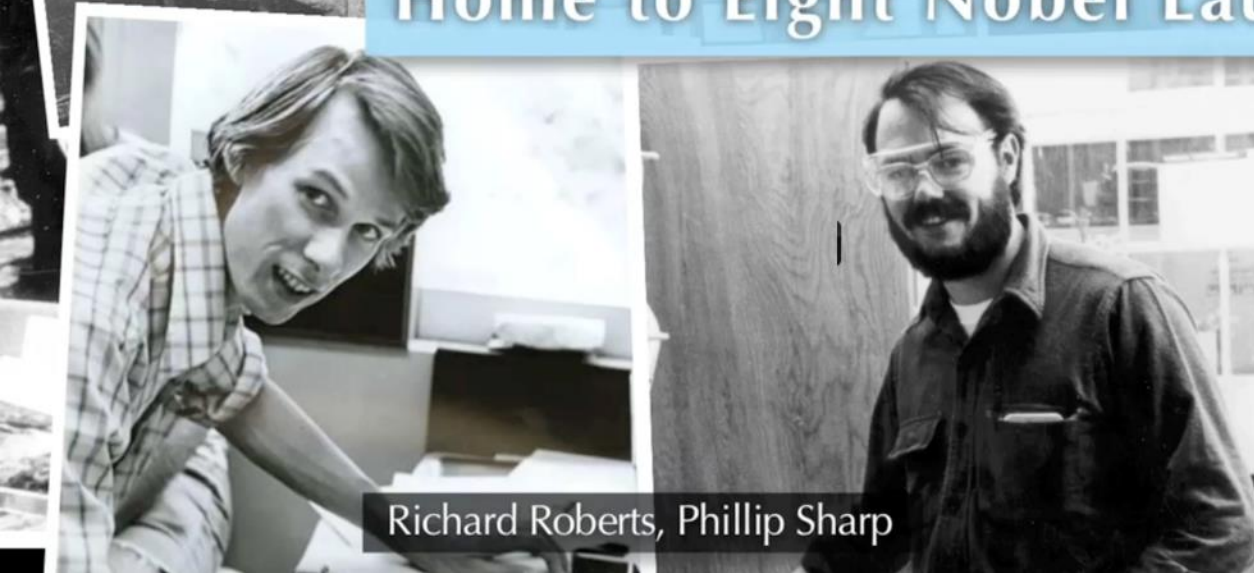


Barbara McClintock



Home to Eight Nobel Laureates

Richard Roberts, Phillip Sharp



Carol Greider



The Egalitarian Gene

Agarose Gel Electrophoresis, 1973



1958
Matt Meselson &
Ultracentrifuge, \$500,000



1973
Sharp, Sambrook, Sugden
Gel Electrophoresis
Chamber, \$250

Detection of Two Restriction Endonuclease Activities in *Haemophilus parainfluenzae* Using Analytical Agarose-Ethidium Bromide Electrophoresis†

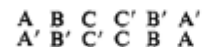
Phillip A. Sharp,* Bill Sugden, and Joe Sambrook

ABSTRACT: A rapid assay for restriction enzymes has been developed using electrophoresis of DNA through 1.4% agarose gels in the presence of 0.5 $\mu\text{g}/\text{ml}$ of ethidium bromide. The method eliminates lengthy staining and destaining procedures and resolves species of DNA which are less than 7×10^6 daltons. As little as 0.05 μg of DNA can easily be detected by direct examination of the gels in ultraviolet light. Using

activities have different chromatographic properties on phenylacrylamide-cellulose and Bio-Gel A-0.5m, DNA at different sites. One activity cleaves DNA at a single position situated 1.5 $\times 10^6$ length from the insertion point of the adenovirus SV40 hybrid Ad2⁺ND₁. This activity cleaves SV40 DNA at three sites within 13 of the 11 cleavage points attached to the DNA isolated from *H. influenzae* strain



lar to the axis of the DNA duplex; in other words, the DNA contains palindromic sequences of the type



Because different enzymes attack different palindromes, each enzyme generates a characteristic set of cleavage products when reacted with DNA. For any particular enzyme, the number of fragments obtained is a measure of the number of palindromic sites in the DNA specific to the enzyme. The size of the fragments reflects the distribution of the palindromes along the DNA.

The two principal methods which have been used to analyze the fragments of DNA produced by restriction enzymes are density gradient sedimentation and electrophoresis through agarose gels.

JOURNAL OF POLYMER SCIENCE: PART A: VOL. 11, NO. 1, 1973



The Egalitarian Genome

Third Generation Sequencing 2014

2001:

ABI 3730 Sequencer



Human Genome:
\$2.7 Billion, 13 Years,
1000s of people

2014

Oxford Nanopore
MinION



Human Genome:
\$900, 6 Hours, one person



DNA Barcoding Begins: CSHL Banbury Center Meetings 2003

Mark Stoeckle



Paul Hebert



Jesse Ausubel



The Inspiration

Mark and Kate Stoeckle

CNN.com /technology

DNA testing uncovers suspect sushi

- STORY HIGHLIGHT
- Two teenage girls
 - They used a genet
 - Samples were col
 - The results showe

Next Article in Techn

From Christina Chinnici
CNN

TEXT SIZE - +

NEW YORK (CNN) -- Two teenage girls used DNA bar coding to determine that some sushi on New York dinner plates was mislabeled with cheaper fish being passed off as a more expensive species.



Kate Stoeckle and Louisa Strauss were not science majors or even college students when they decided to take 60 samples of seafood and use a genetic fingerprinting technique to see whether the fish were labeled correctly.

The graduates of Manhattan's Trinity School in New York were inspired by Kate Stoeckle's father, Mark, a scientist and proponent of the use of DNA bar coding, a technique that greatly simplifies the process of identifying a species.

"Growing up, bar coding was dinner conversation, so I was familiar with it," Stoeckle said. "And then one night while out to dinner I asked could we

TIME Quotes of the Day



“ If you're paying for white tuna and you're eating tilapia, I think you'd want to know that. ”

KATE STOECKLE, 19, after she and Louisa Strauss, 18, freelanced a science project in which they checked 60 samples of seafood using genetic fingerprinting and found that one-fourth of the fish samples with identifiable DNA were mislabeled

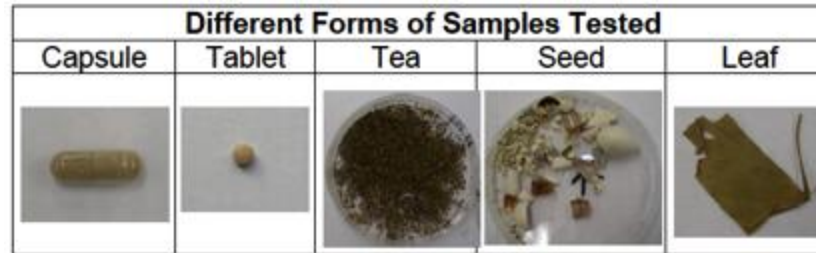
Friday, August 22, 2008

Ginkgo Product Fraud (2012)

Mary Acheampong, Bobby Glover, and Marisa VanBrakle
Mentor: Allison Granberry
Hostos-Lincoln Academy of Science, Bronx
2012 UBP Grand Prize Winners



Ginkgo Product Fraud (2012)



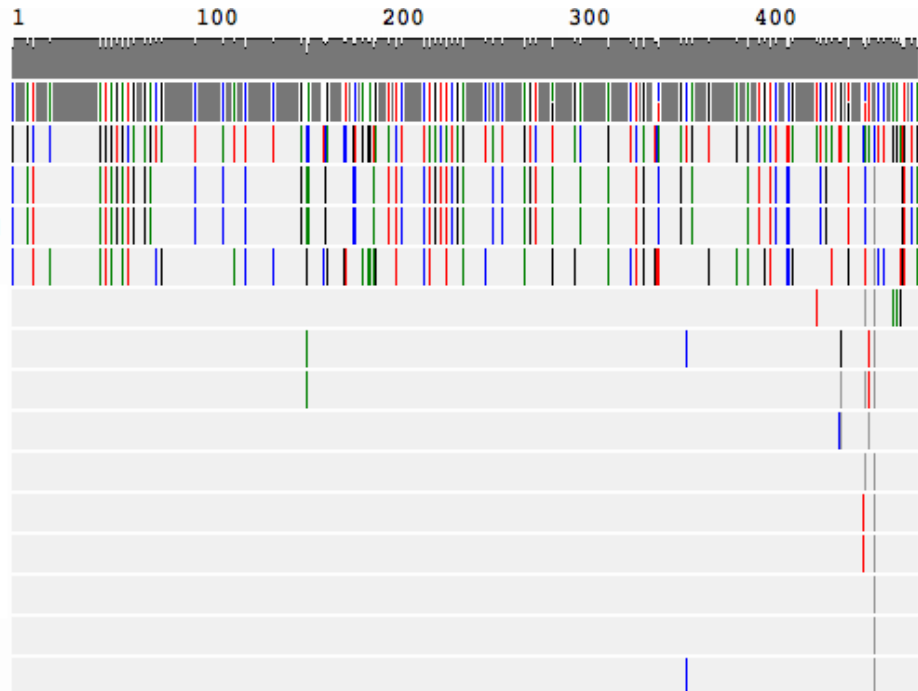
Sample Letter	Form	DNA Expected	DNA Results
A	Capsule	<i>Ginkgo biloba</i>	Rice: <i>Oryza rufipogon</i>
B	Capsule	<i>Ginkgo biloba</i>	Rice: <i>Oryza rufipogon</i>
C	Capsule	<i>Ginkgo biloba</i>	Rice: <i>Oryza rufipogon</i>
D	Tablet	<i>Ginkgo biloba</i>	No sequence available.
E	Capsule	<i>Ginkgo biloba</i>	Rice: <i>Oryza rufipogon</i>
F	Liquid	<i>Ginkgo biloba</i>	No sequence available
G	Capsule	<i>Ginkgo biloba</i>	No sequence available
H	Tea	<i>Ginkgo biloba</i>	Other rbcL DNA present but not <i>Mentha piperita</i>
L	Capsule	<i>Ginkgo</i>	Rice: <i>Oryza</i>

Sequence Conservation

Sequence Variation

Consensus

1. *Ginkgo-Ginkgoaceae*
2. *Ginkgo_biloba_Ginkgoaceae_DQ069500.1*
3. gi|315259862|gb|HQ619785.1|
4. Sample L
5. Sample A
6. Sample L
7. Sample CL
8. Sample E
9. Sample B2
10. Sample C
11. Sample B1
12. gi|57283873|emb|AJ746297.1|
13. *Rice-Poaceae*



Ginkgo Product Fraud Revisited

NY Attorney General Cease and Desist Order (2015)



STATE OF NEW YORK
OFFICE OF THE ATTORNEY GENERAL

ERIC T. SCHNEIDERMAN
ATTORNEY GENERAL

DIVISION OF REGIONAL AFFAIRS

February 2, 2015

Doug McMillon, President/CEO
Wal-Mart Stores, Inc.
702 SW 8th Street
Bentonville, Arkansas 72716

Certified—Return Receipt Requested

Re: **CEASE & DESIST NOTIFICATION**
Spring Valley—Walmart Distributed Herbal Dietary Supplements

Dear Mr. McMillon:

This letter constitutes a demand to cease and desist engaging in the sale of adulterated and/or mislabeled herbal dietary supplements, and in particular to immediately stop the sale of six “Spring Valley” dietary supplements as identified by lot number in the exhibit annexed hereto.

Walmart “Spring Valley” Ginkgo Biloba

Ginkgo Biloba. Negative. No ginkgo biloba DNA was identified. The only DNA identified was “oryza” (commonly known as rice) in 6 of the fifteen tests, with other tests identifying dracaena (a tropical houseplant), mustard, wheat, and radish. Four of the tests revealed no plant DNA whatsoever.

DNALC's Three High School DNA Barcoding Projects



Students	1834	2042
Teams	671	702
Mentors	134	241
Schools	91	68
Academic Institutions	32	
DNA Samples	>10,600	>10,800
DNA Sequences	>16,000	>8,600
	111 million NextGen sequencing reads	~22 billion bp and 75 mil reads from microbiome projects
Sequences in GenBank	67 novel 15 with polymorphisms 115 GPS data	55 novel 275 with polymorphisms 966 GPS data
Years Active	UBP 2011- UBRP 2014-	2014-



Barcode Long Island Symposium Cold Spring Harbor Laboratory




RESEARCH ARTICLE

DNA barcoding Brooklyn (New York): A first assessment of biodiversity in Marine Park by citizen scientists

Christine Marizzi^{1e*}, Antonia Florio^{2e}, Melissa Lee¹, Mohammed Khalfan³, Cornel Ghiban¹, Bruce Nash¹, Jenna Dorey^{1,4}, Sean McKenzie⁵, Christine Mazza⁶, Fabiana Cellini⁶, Carlo Baria⁷, Ron Bepat⁸, Lena Cosentino⁷, Alexander Dvorak⁹, Amina Gacevic¹⁰, Cristina Guzman-Moumtzis¹¹, Francesca Heller¹², Nicholas Alexander Holt^{8†}, Jeffrey Horenstein¹³, Vincent Joralemon¹¹, Manveer Kaur¹⁰, Tanveer Kaur¹⁰, Armani Khan⁸, Jessica Kuppan⁸, Scott Laverty⁷, Camila Lock¹⁴, Marianne Pena¹⁰, Ilona Petrychyn¹⁴, Indu Puthenkalam¹⁴, Daval Ram⁸, Arlene Ramos¹⁰, Noelle Scoca¹⁵, Rachel Sin¹², Izabel Gonzalez¹⁰, Akansha Thakur¹⁴, Husan Usmanov¹², Karen Han⁸, Andy Wu¹², Tiger Zhu¹³, David Andrew Micklos¹



 OPEN ACCESS

Citation: Marizzi C, Florio A, Lee M, Khalfan M, Ghiban C, Nash B, et al. (2018) DNA barcoding Brooklyn (New York): A first assessment of biodiversity in Marine Park by citizen scientists. *PLoS ONE* 13(7): e0199015. <https://doi.org/10.1371/journal.pone.0199015>

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© These authors contributed equally to this work.

† Deceased.

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DNA Barcoding Program Outcomes

NCBI **2,393**
Total GenBank
Publications

NCBI **220**
First GenBank
Barcodes

NCBI **579**
New Sequence
Variants

NCBI **1,623**
Unique GenBank
Authors

1,075
Unique Taxa
Identified

[Learn More](#)

Student Research Programs



Learn about metro New York (*Barcode Long Island*, *Urban Barcode Project*, and *Urban Barcode Research Program*), and China-based (*Barcode Suzhou* and *Barcode Beijing*) student research programs.

Citizen Science



Explore citizen science programs (*Barcoding US Ants* and *Citizen DNA Barcode Network*) that ignite community interest in biodiversity and science while contributing to our knowledge of species through DNA barcoding.

Laboratory & Resources



Protocol and resources supporting DNA barcoding to identify plants or animals—or products made from them. Online tools, animations, videos, presentations, and references that support students, teachers, or citizen scientists.

Walk?



...ride!

FAST TRACK TO GENE ANNOTATION AND GENOME ANALYSIS

LOG OUT *David Micklos*

My Projects

Public Projects

D N A

SUBWAY

Annotate a Genomic Sequence

Find Repeats

Predict Genes

Search Databases

Build Models

Prospect Genomes Using TARGET

Search Genomes

Alignment & Tree Viewer

Determine Sequence Relationships

Assemble Sequences

Add Sequences

Analyze Sequences

Next Generation Sequencing

Manage Data

Analyze Transcriptome

Explore Differential Abundance

Metabarcoding Analysis

Metadata + QC

Clustering Sequences

Alpha/Beta Diversity

Browsers & Transfer

DNA Subway ties together key bioinformatics tools and databases to assemble gene models, investigate genomes, work with phylogenetic trees and analyze DNA barcodes. Roll over the "stations" on the subway map to find out more about the analysis steps. Analyze your own data or sample data provided. To start a project, select one of the "lines" (red, yellow, blue, green, purple). Register and login to be able to save and share your results.

▲ DNA Barcoding 101

● Background ● Manual ● Tour

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CYVERSE

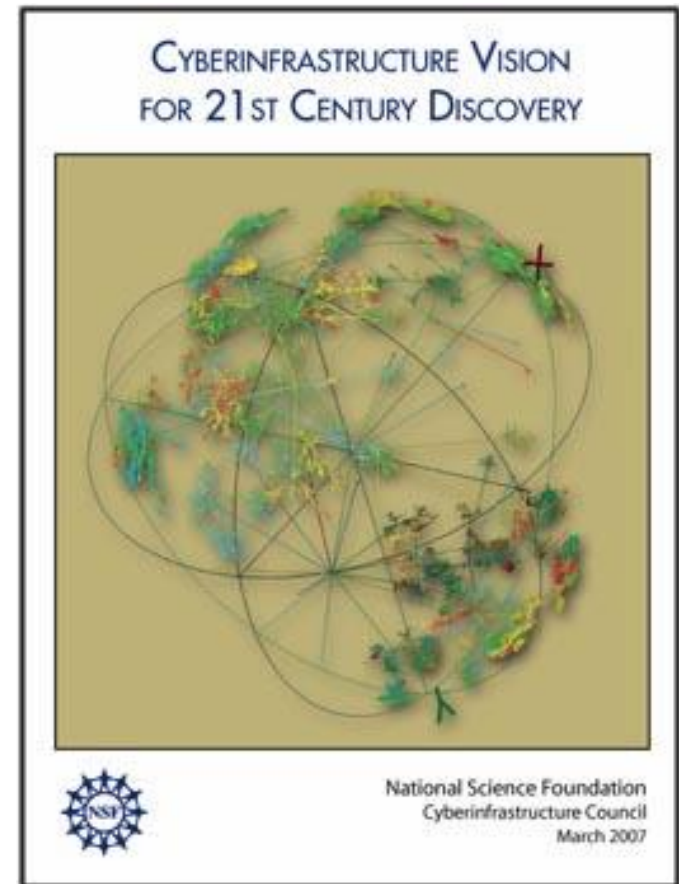
CSH Cold Spring Harbor Laboratory



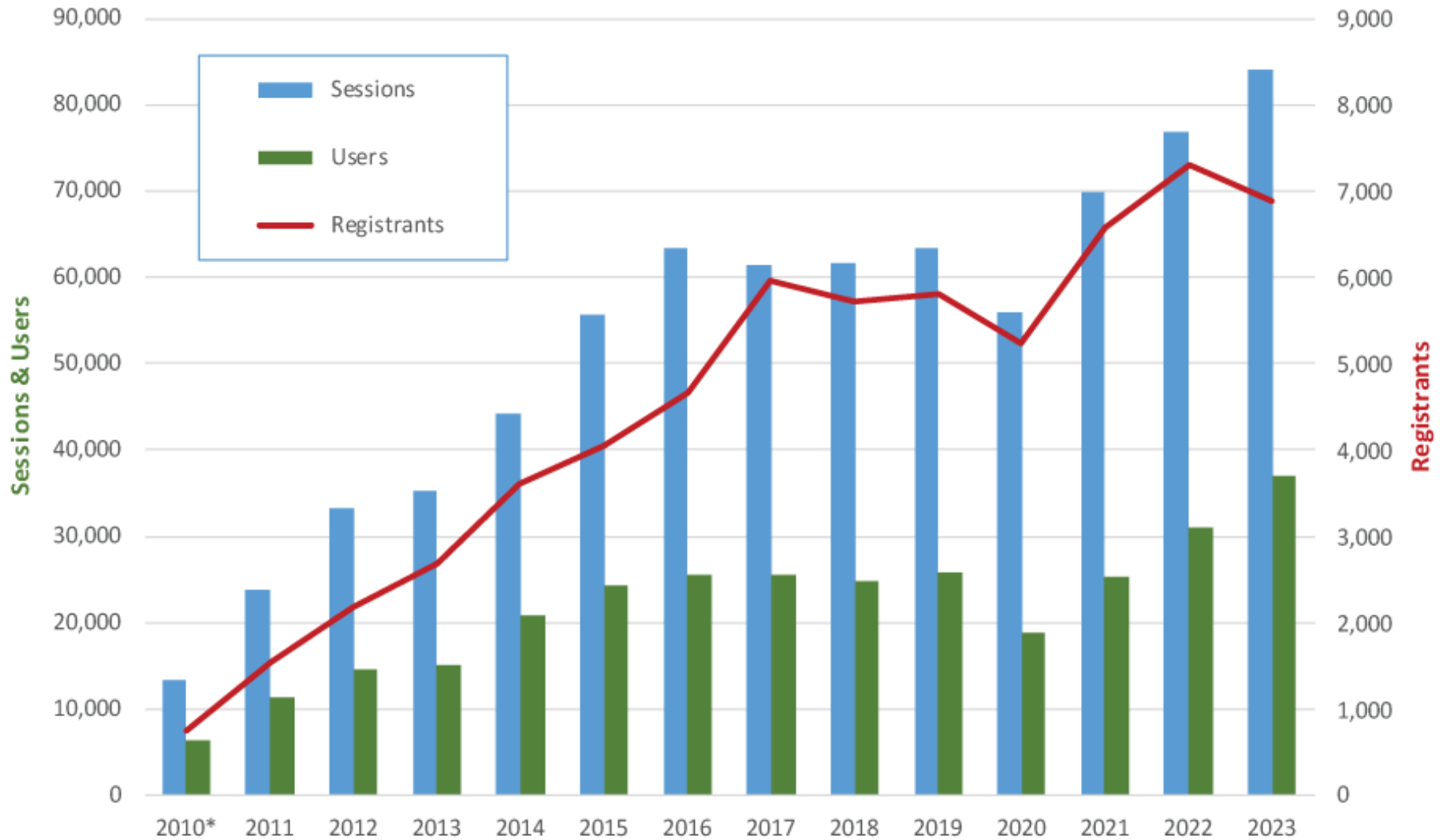
DNA Subway

an educational *Discovery Environment*

- Developed as educational outreach for iPlant (Cyverse), a 10 year NSF project to develop a computer infrastructure to apply computational thinking to solve big-data biological
- Students “ride” on any of five different lines to access simplified workflows for gene discovery, annotation, and comparison
- 25 collaborators at 11 institutions
- Launched in March 2010



DNA Subway Visitation to Date



DNA Subway Visitation to Date

- 66,652 registered users
- 329,653 total users (including guests)
- 799,058 user sessions, averaging 17 minutes (25.8 person years)
- Blue line analyses account for 71% of traffic
- 277,534 DNA sequences uploaded from GENEWIZ (mainly DNA barcodes)

Pop-up Survey Results (n=5,000)

- 73% said *DNA Subway* is easy to use
- 76% were likely to use in again
- 73% K-16 students, 27% teachers
- Students and faculty were: 75% four-year, 10% CC, 15% precollege
- 41% of non-K-16 students were researchers or graduate students

