An NCBI Guide to Finding and Analyzing Metagenomic Data

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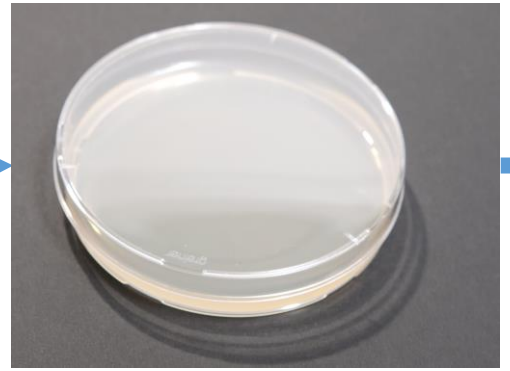
NIH National Library of Medicine
National Center for Biotechnology Information

Outline

- What is Metagenomics?
- How Does NCBI Support Metagenomics?
- Today's Case Study
  - Objective 1 – Find Metagenomic Reads in SRA
  - Objective 2 – Explore Taxonomic Composition of SRA reads using STAT
  - Objective 3 – Use MagicBLAST to align metagenomic reads against reference sequences
  - Objective 4 – Compare MagicBLAST and STAT output

Sequencing cultured organisms is not a perfect "science"

Environmental Community



Laboratory Isolation

DNA Sequencing

Bioinformatic Analysis

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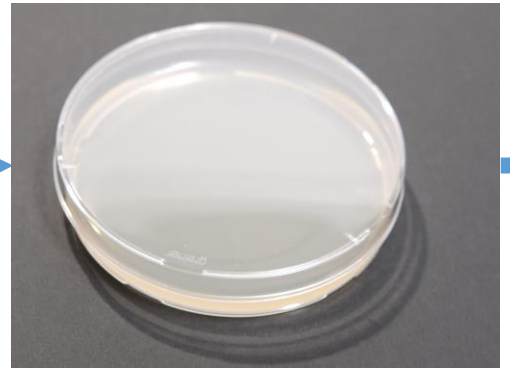

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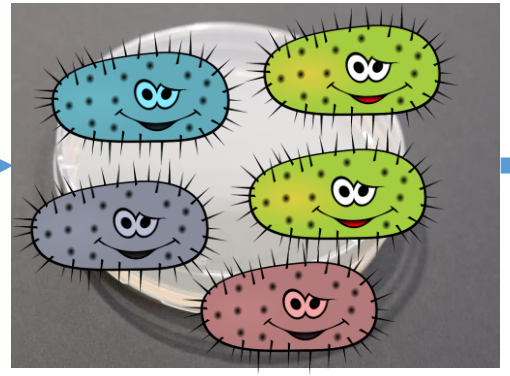

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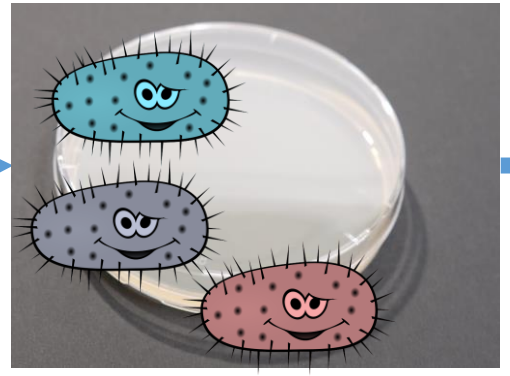

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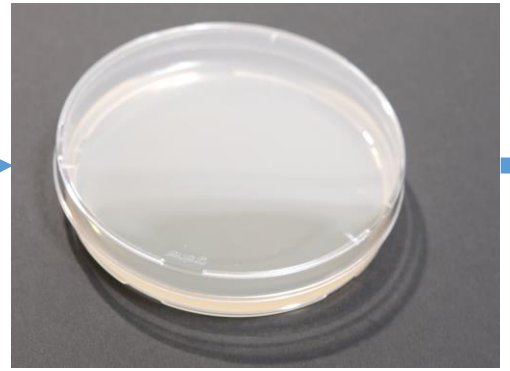

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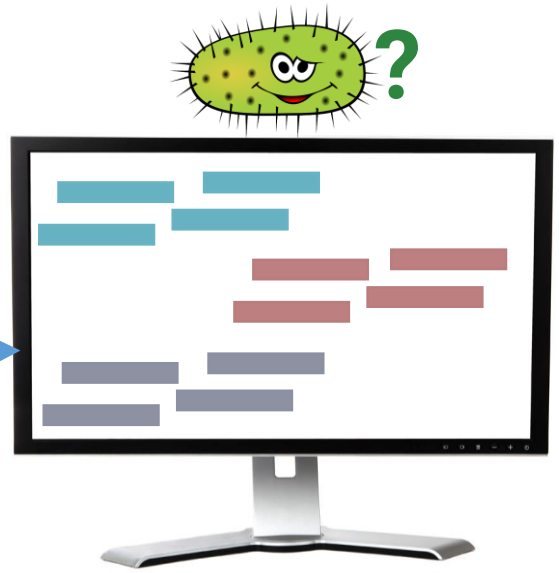

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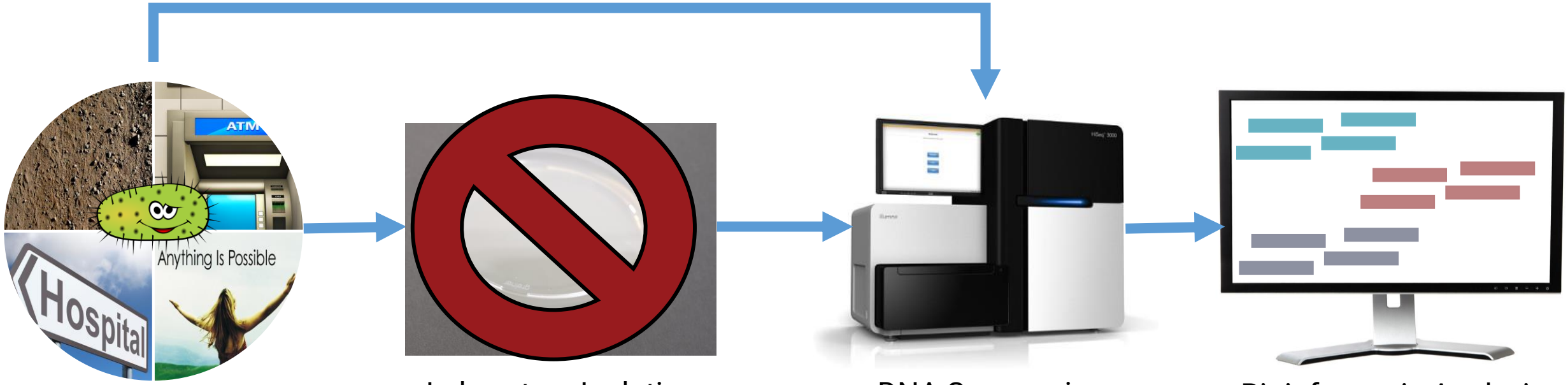

Metagenomics is sequencing without culturing

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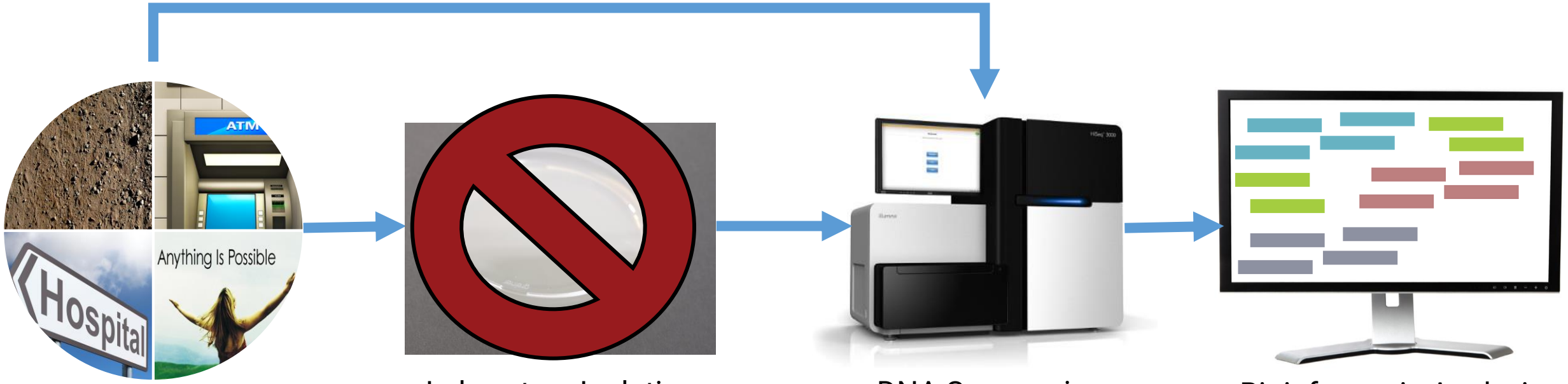
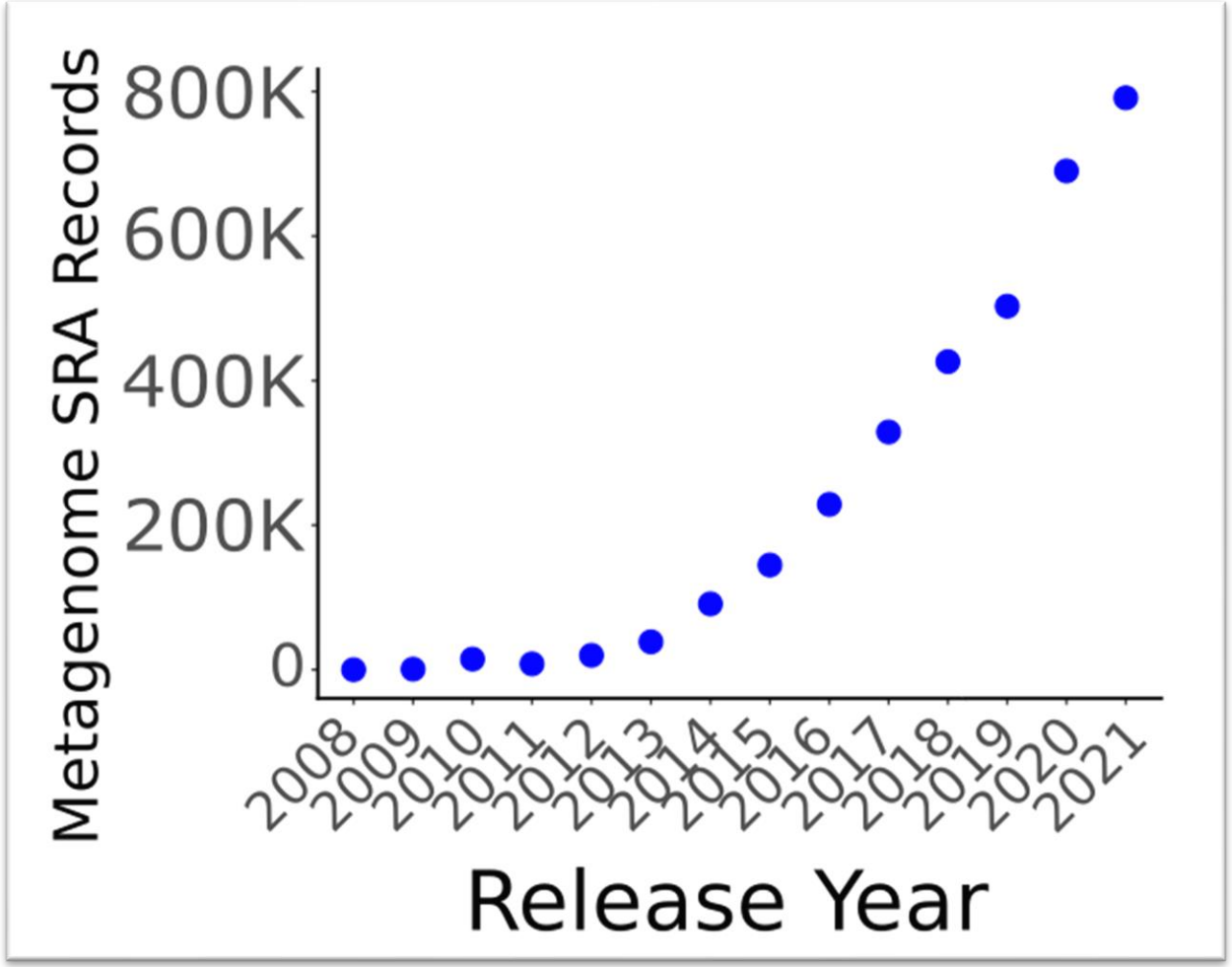


Metagenomics is sequencing without culturing

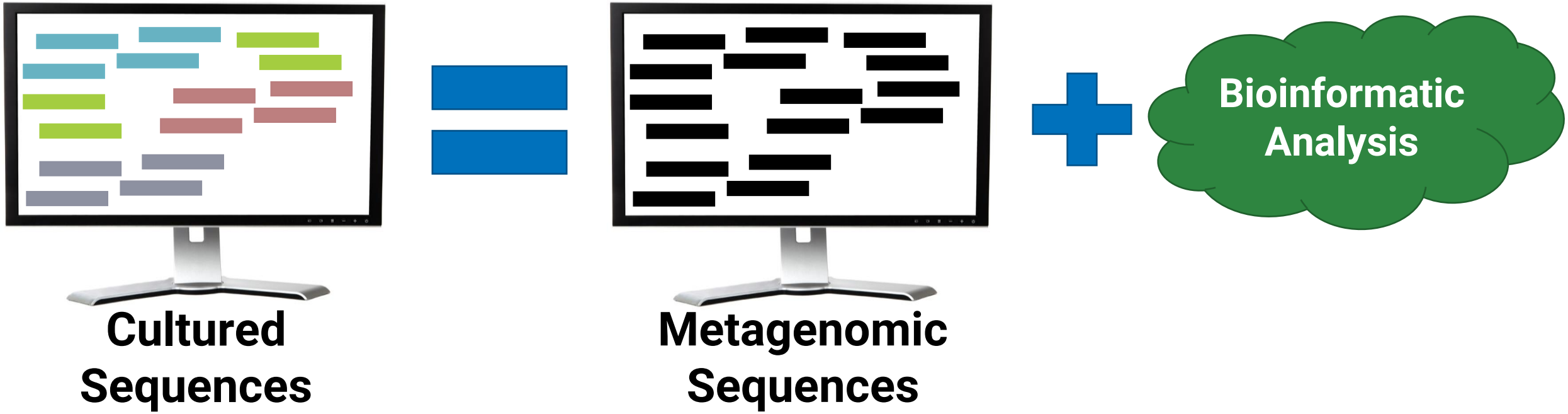
Current trends of metagenomic data production

- Over 3 million metagenomic records in NCBI
  - ~20% of SRA database
- >900 Terabytes of read data
- Annual Rate of new data growing exponentially



Source: SRA AWS metadata tables queried on 09/05/2021

There’s still a catch…



NCBI Metagenomic Resources

Data Storage

Sequence Read Archive

NCBI

Genbank & RefSeq

metagenome[ORGN]

Taxonomy Keyword

Data Analysis

SRA Taxonomy Analysis Tool

MagicBLAST

NCBI Metagenomic Resources

Data Storage

SRA - Now available on the cloud

Sequence Read Archive (SRA) data, available through multiple clou
available repository of high throughput sequencing data. The archive
metagenomic and environmental surveys. SRA stores raw sequenci
and facilitate new discoveries through data analysis.

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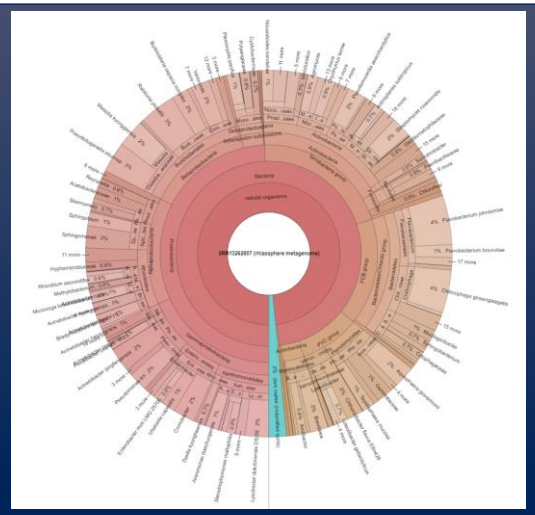
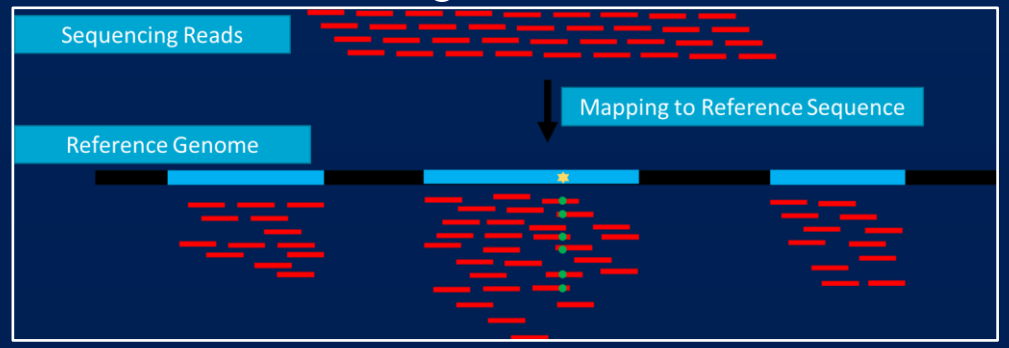


SRA Taxonomy Analysis Tool

MagicBLAST



Today's Case Study – Microbial Keratitis

Microbial Keratitis is a bacterial infection of the cornea (clear dome covering colored part of the eye)

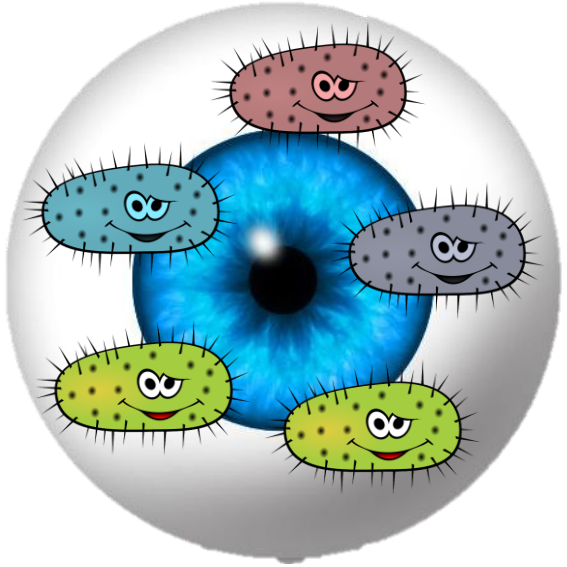
- Leading cause of preventable blindness worldwide
- Typically caused by *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus sp.*

Diagnosis is typically done via sampling and culturing of corneal samples

- Unreliable (~40% of cases are culture-negative)
- Time-consuming (~48hr turnaround + antibiotic resistance testing)

Source - https://peerj.com/articles/10778/

Today's Case Study – Methods



Corneal Scraping Community

16s rRNA Sequencing

Bioinformatic Analysis

Patient B has one infected eye and one healthy eye.

A) Is the taxonomic distribution of each "cornea microbiome" different between eyes?

B) Do the taxonomic distributions of the eyes match our expectations for healthy and infected eyes?

Source - https://peerj.com/articles/10778/

Today's Case Study – Our objectives

Objective 1 – Find Metagenomic Reads in SRA

Objective 2 – Explore Taxonomic Composition of SRA reads using STAT

Objective 3 – Use MagicBLAST to align metagenomic reads against reference sequences

Objective 4 – Compare MagicBLAST and STAT output

Objective 1 - Find Metagenomic Reads in SRA and Explore Taxonomic Composition using STAT

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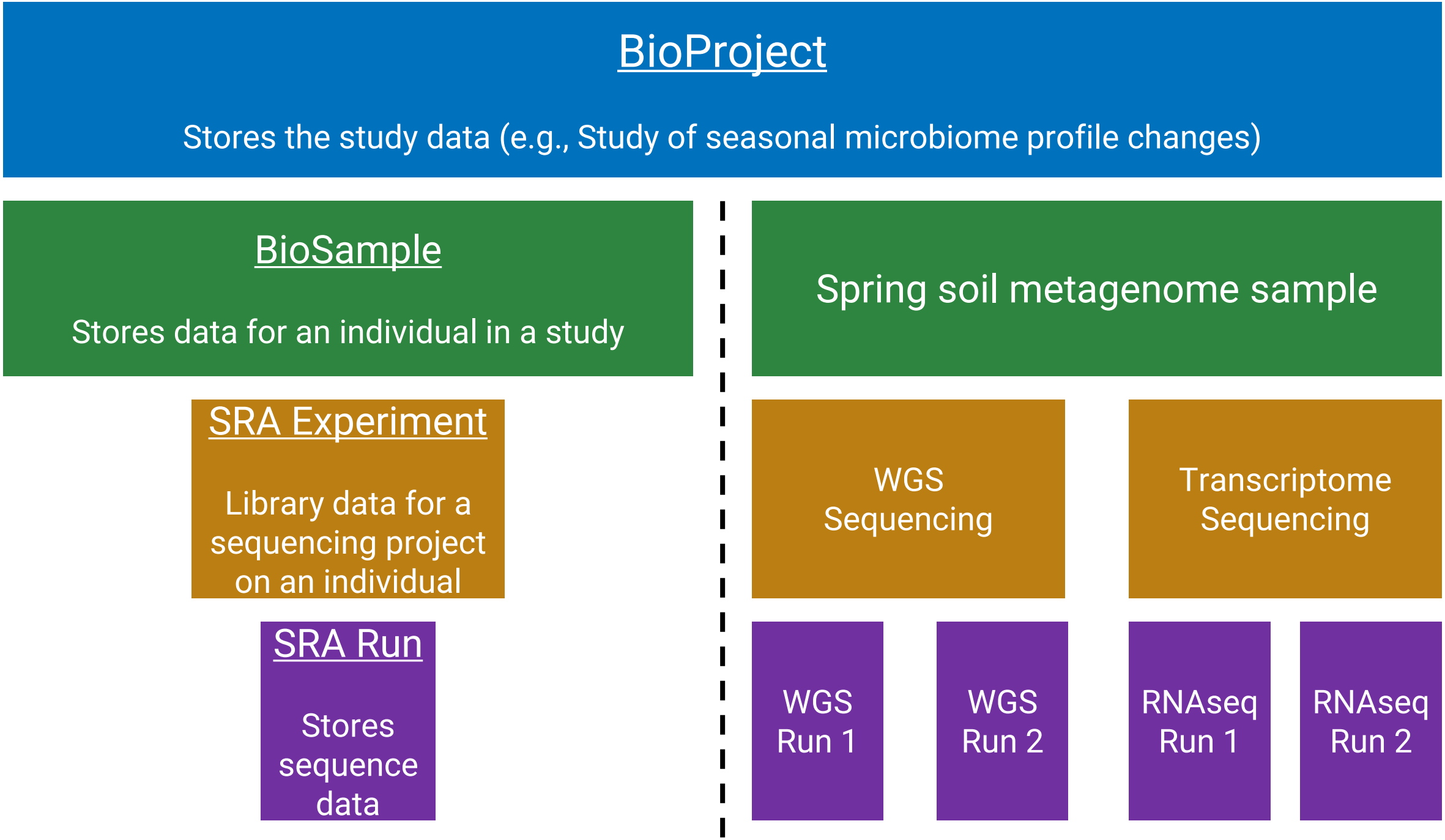
What is the Sequence Read Archive

https://www.ncbi.nlm.nih.gov/sra

- Collection of user-submitted nucleotide sequencing reads, most of which are publicly available to download
  - Current size = >23 petabytes
- You can search the data online using the URL above, or by exploring their metadata in the cloud


BioProject
Stores the study data (e.g., Study of seasonal microbiome profile changes)
BioSample
Stores data for an individual in a study
Spring soil metagenome sample
SRA Experiment
Library data for a sequencing project on an individual
WGS Sequencing
Transcriptome Sequencing
SRA Run
Stores sequence data
WGS Run 1
WGS Run 2
RNAseq Run 1
RNAseq Run 2

Finding the case study accessions

*Letter depends on original collection group:

S = SRA (NCBI)
E = ERA (ENA)
D = DRA (DDBJ)

Evaluation of full-length nanopore 16S sequencing for detection of pathogens in microbial keratitis

Data Availability

The following information was supplied regarding data availability:

Bioinformatics scripts and the DNA sequencing (FASTQ) files are available at European Nucleotide Archive (PRJEB37709): SAMEA7573840, SAMEA7573841, SAMEA7573842, SAMEA7573843, SAMEA7573844, SAMEA7573845, SAMEA7573846, SAMEA7573847, SAMEA7573848, SAMEA7573849, SAMEA7573850, SAMEA7573851, SAMEA7573852, ERX4706745, ERX4706746, ERX4706747, ERX4706748, ERX4706749, ERX4706750, ERX4706751, ERX4706752, ERX4706753, ERX4706754, ERX4706755, ERX4706756, ERR4836967, ERR4836968, ERR4836969, ERR4836970, ERR4836971, ERR4836972, ERR4836973, ERR4836974, ERR4836975, ERR4836976, ERR4836977, ERR4836978, SAMEA7573853, ERX4706757, ERR4836979, SAMEA7573854, ERX4706758, ERR4836980, SAMEA7556110, ERX4692670, ERR4822680.

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BioSample "SAM*"

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BioProject "PRJ*"

SRA Experiment "*RX"

BioSample "SAM*"

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BioProject "PRJ*"

SRA Experiment "*RX"

BioSample "SAM*"

SRA Run "*RR"

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Objective 2 - Explore Taxonomic Composition of SRA reads using STAT

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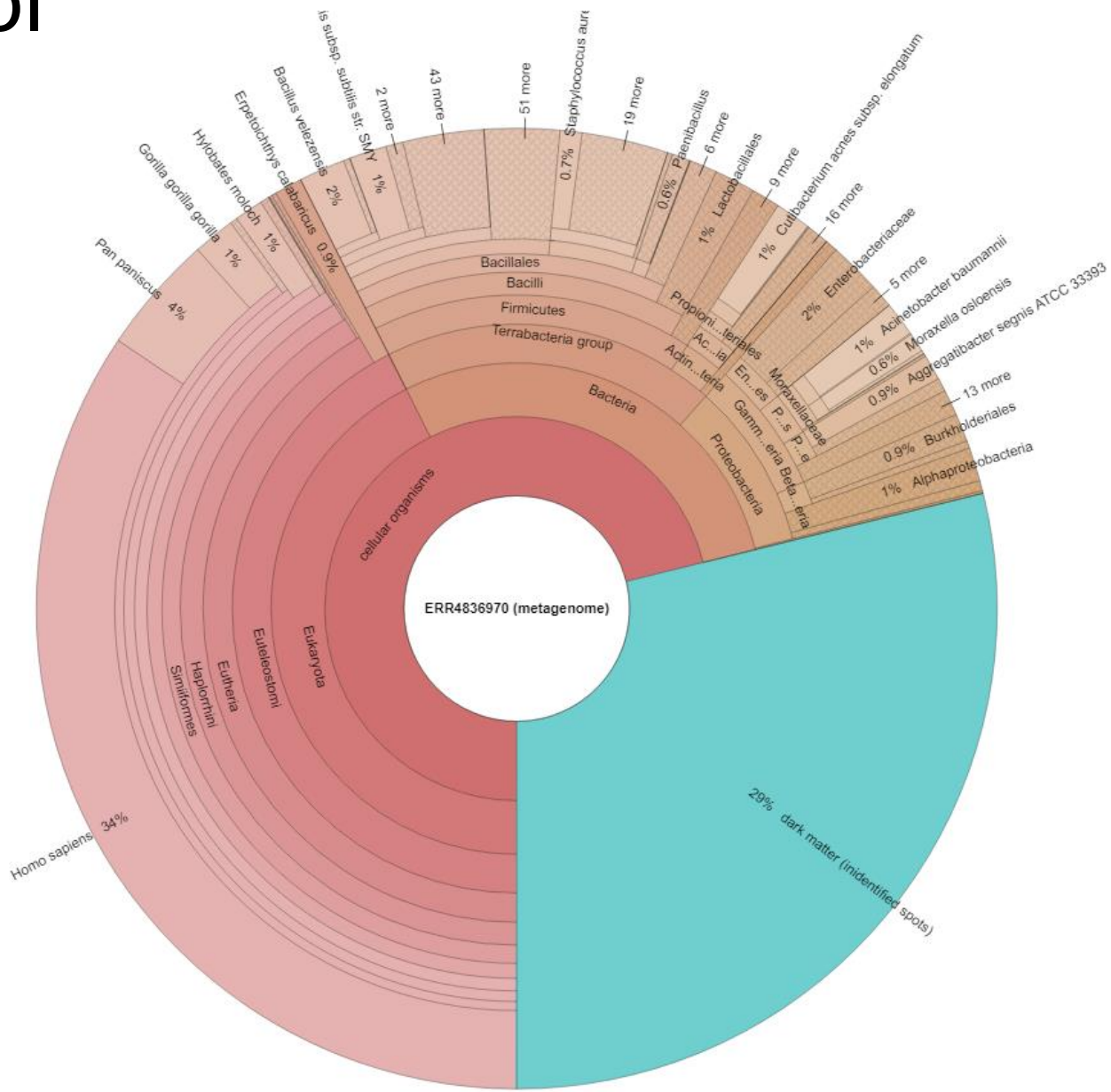
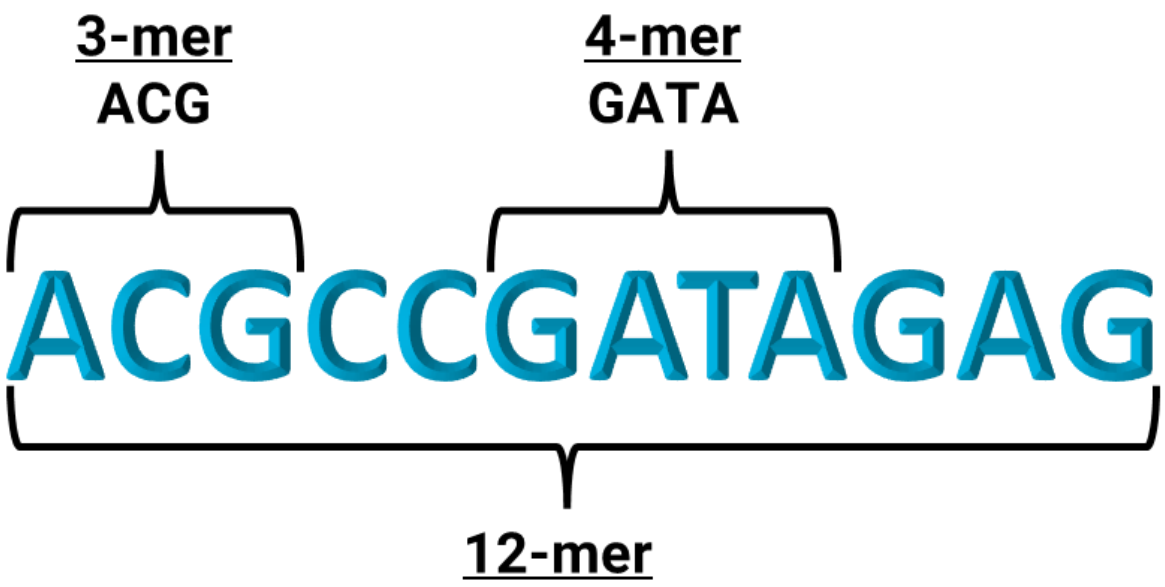
SRA Taxonomy Analysis Tool

- Characterizes taxonomic distribution of reads in *every* SRA submission
  - Measured as a % of reads within the run
- Reads may be mapped to multiple taxa. If so, read is assigned to lowest common taxonomic group
  - e.g., two species share a genus, so the read is assigned to genus
- Uses a Kmer hit approach to predict taxonomy of individual reads



A “Kmer” is fancy-talk for a “K-length sequence”



- Some Kmers, of sufficient length, can be unique to a taxonomic group
- STAT compiles 32-mers of NCBI’s RefSeq database for taxonomy predictions
- Under equal conditions, larger genomes naturally generate more reads
  - This should be considered when viewing results

Visit **Objective 2** of the Jupyter Notebook to get started!

Watch the chat box for the login link

Username: Email name (before the @), all lowercase

Password: <whatever you want, 7 chars min>

Objective 3 - Use MagicBLAST to Align Metagenomic Reads Against Reference Sequences

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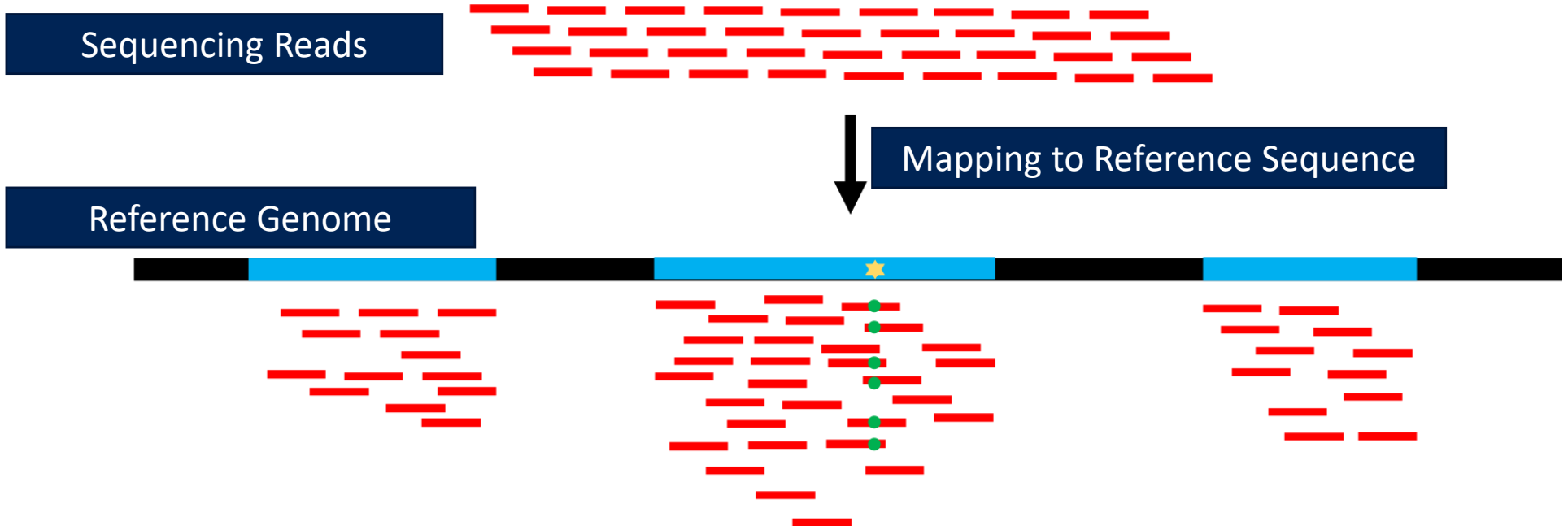
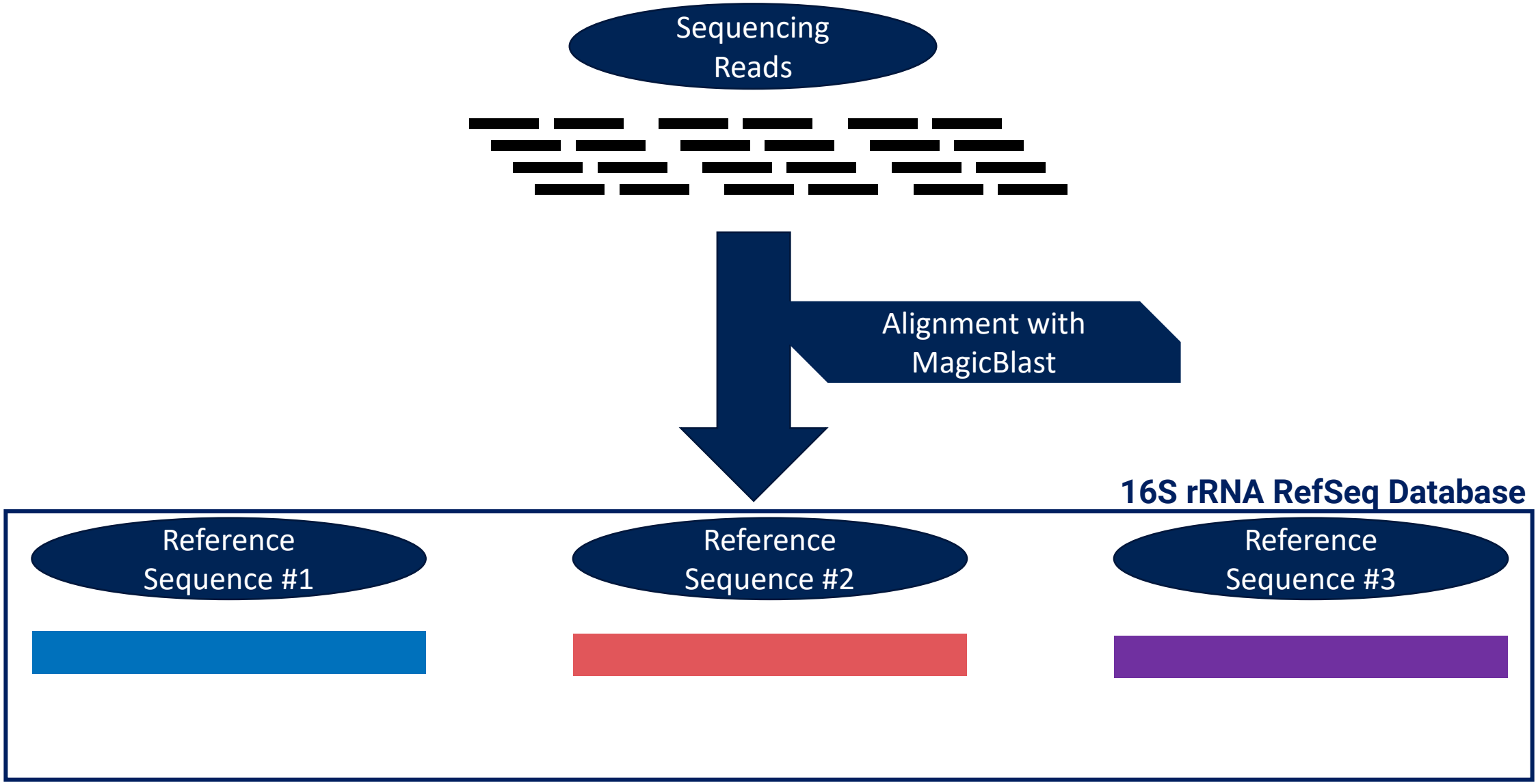
MagicBLAST

- A “flavor” of BLAST which aligns next-generation RNA or DNA sequencing reads against BLAST databases
  - Can use user-created custom databases OR NCBI maintained ones



Documentation - https://ncbi.github.io/magicblast/

Metagenomes align against a collection of sequences



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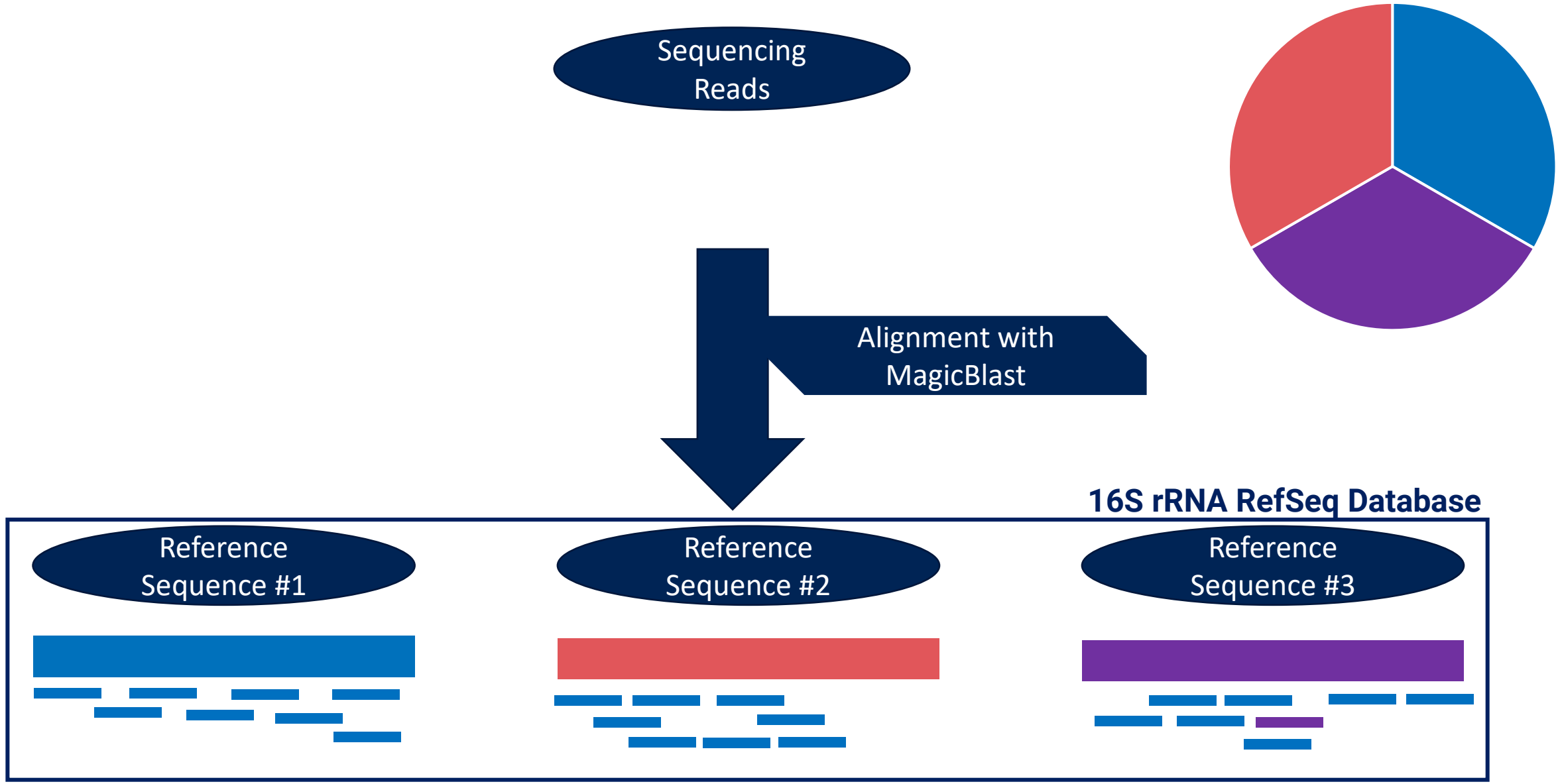

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Advanced Metagenomics With NCBI

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  - Functional profiling
  - Higher accuracy taxonomic characterization
  - *Coming Soon: Clustered BLAST dbs for faster read mapping*

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  - Explore in-depth STAT metadata in the cloud!

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  - Explore in-depth STAT metadata in the cloud!
- Submit your sequences to SRA!
  - No excuse to provide little metadata!

Thank you!