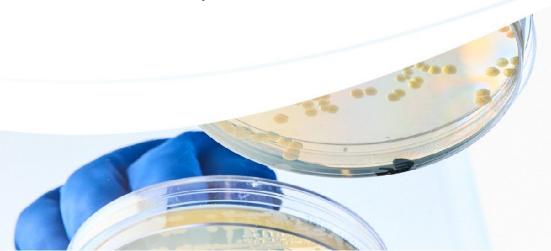


# Unlocking the Power of Authenticated Microbial Genomics with the ATCC Genome Portal

Jonathan Jacobs, PhD Senior Director, Bioinformatics & Genomics, BioNexus Principal Scientist, ATCC



# Introducing today's speaker





Jonathan Jacobs, PhD
Senior Director, Bioinformatics / BioNexus Foundation Principal Scientist, ATCC

Dr. Jonathan Jacobs is the Senior Director of Bioinformatics at ATCC, where he leads the Sequencing & Bioinformatics Center and oversees the development of the ATCC Genome Portal, an authenticated reference genome database for ATCC materials. With over 20 years of experience in molecular genetics, bioinformatics, and microbial genomics, Dr. Jacobs has consistently worked to solve problems and create solutions across academia, government, and industry. His own research has included functional genomics of viral pathogenicity and fungal multi-drug resistance mechanisms, cell line engineering for biopharma and industrial microbiology, and developing metagenomics tools for biosurveillance of emerging infectious diseases.

### **About ATCC**



Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD

World's premier biological materials resource and standards development organization

5,000+ cell lines

80,000 microorganisms

Genomic & synthetic nucleic acids

Media/reagents

ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions

Growing portfolio of products and services

Sales and distribution in 150 countries, 20 international distributors

Talented team of 600+ employees, over one-third with advanced degrees









# Agenda

1

### The discovery loop

2

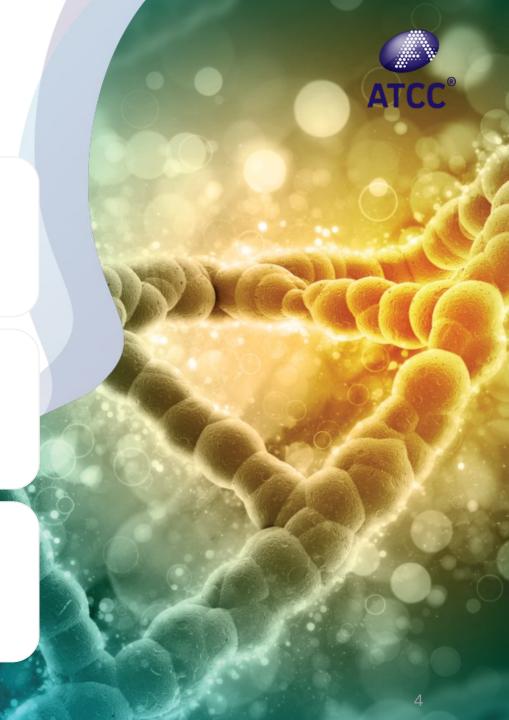
### How trustworthy is that data?

- Data provenance
- Examples of problems

3

### The ATCC® Genome Portal

- Overview
- How to access data
- Data exploration tools



### **ATCC® Genome Portal**



Download whole-genome sequences and annotations of ATCC® materials



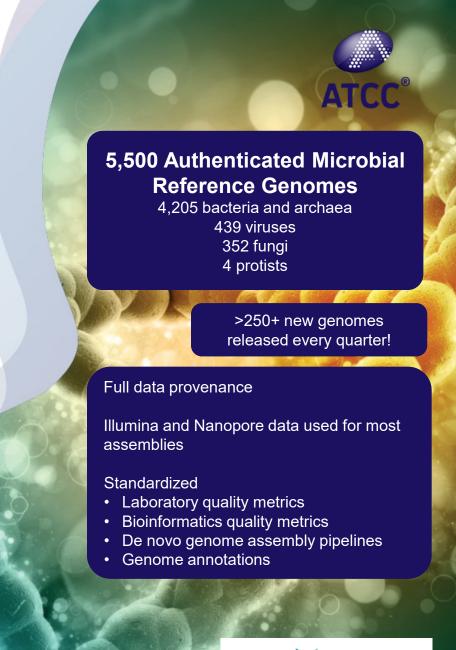
Search for nucleotide sequences or genes within genomes



View genome assembly metadata and quality metrics

Learn more about the Genome Portal at <a href="https://www.atcc.org/genomeportal">www.atcc.org/genomeportal</a>

Access the Genome Portal at genomes.atcc.org/





# The discovery loop

How we generally do everything

# The (improved) discovery loop



Have an idea



**Plan experiments** 



Find data & materials



Do experiment



**Analyze results** 



### A common scenario...





Microbiologist

"I need to design a new assay for the detection of antibiotic-resistant bacteria."



Plan your lab research and development

Find reference data (genomes, genes, etc.)

Design assay (bioinformatics)

Get materials, controls, strains, etc.







**Unexpected results** 

Why?

### A common scenario...





"I need to design a new assay for the detection of antibiotic-resistant bacteria."

Microbiologist

Overlooked assumptions



Plan your lab research and development

Find reference data (genomes, genes, etc.)

Design assay (bioinformatics)

Get materials, controls, strains, etc.







**Unexpected results** 

Why?

# The (improved) discovery loop



### Have an idea



Use trusted data resources to discover new materials

### **Plan experiments**



Use authenticated data to improve experimental planning

# Find data & materials



- Use authenticated materials & data
- Verify the source & history of external data
- Know the risks of using unverified data

### Do experiment



### **Analyze results**





# How trustworthy is that data?

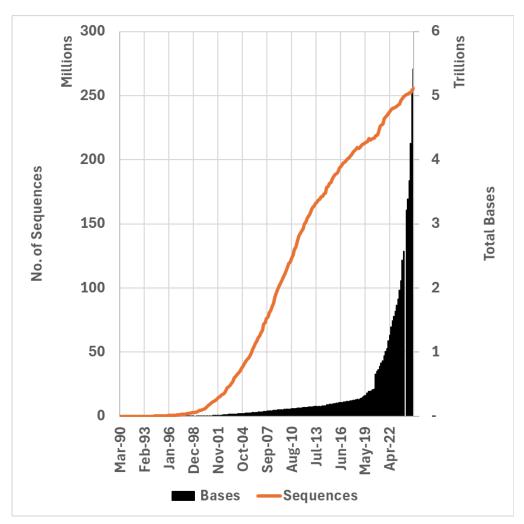
A few stories to remember...

# Authenticated, traceable, and reproducible?

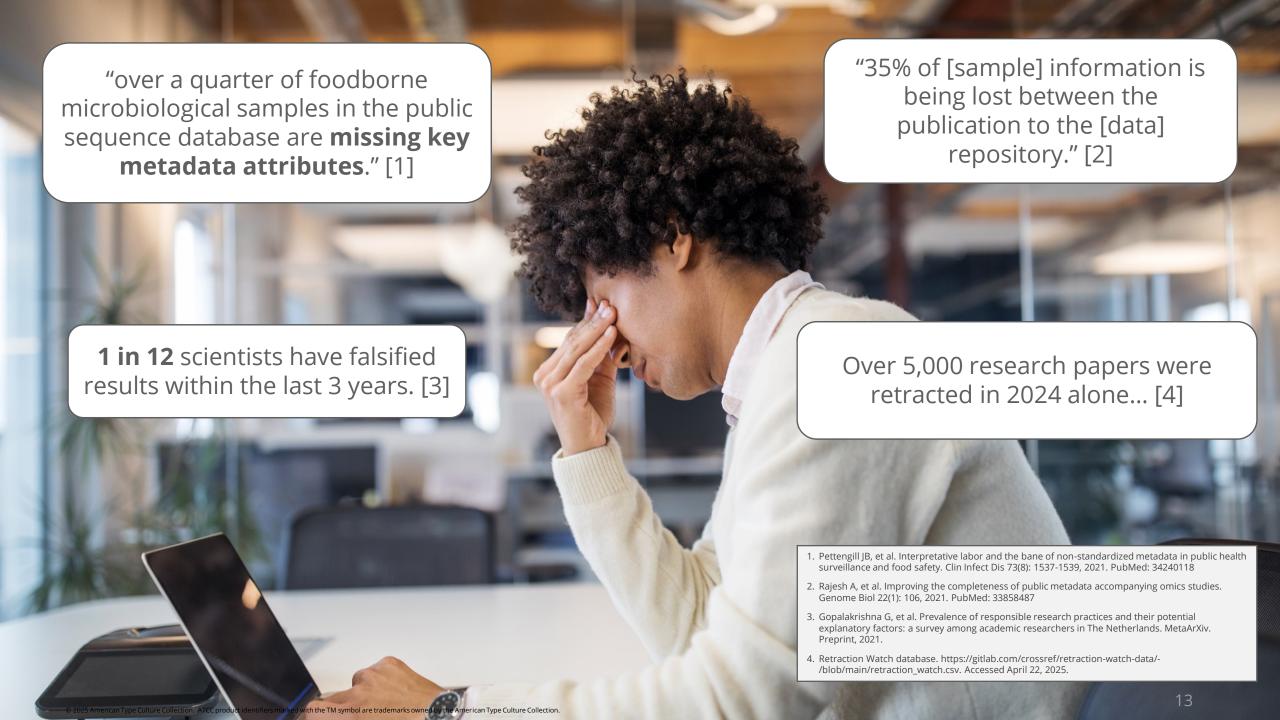


### Where do researchers turn to for "reference" genomes?

- NCBI the de facto standard
  - From 1982 to the present, the number of <u>bases</u> in GenBank has **doubled approximately every 18** months.
- Data submitted by thousands of labs, many with their own
  - laboratory protocols,
  - bioinformatics pipelines,
  - metadata curation preferences
- Very little human curation, mostly automated
- Highly variable quality
- Content is never retrospectively updated if methods or standards change
- NEVER authenticated by ATCC



National Center for Biotechnology Information. GenBank Statistics. NCBI. Published April 22, 2025. Accessed April 22, 2025. https://www.ncbi.nlm.nih.gov/genbank/statistics/



## Falsified data was deposited in GenBank as early as 1995



Federal Register / Vol. 62, No. 135 / Tuesday, July 15, 1997 / Notices

37921

...graduate student "engaged in scientific misconduct by falsifying and fabricating research data in five published research papers, two published review articles, one submitted but unpublished paper, in his doctoral dissertation, and in a submission to the GenBank computer data base." – The Federal Register, v62, n135 (1997)

author of the application is identified and that person's role in the project is identified. 20 points

4. Organizational Experience. The application identifies the qualifying experience of the organization to demonstrate the applicant's ability to effectively and efficiently administer this project. The application specifically identifies the applicant as a nationallyrecognized organization, institution, or company with a record of study and analysis of rural and special transportation needs. Previous specific experience with work similar to the Tasks proposed is clearly and specifically described. The relationship between this project and other work planned, anticipated, or underway by the applicant is described, including a chart which lists all related Federal assistance received within the last five years. In the event a consortium of applicants is proposed, the project history of prior joint work should be provided. The previous Federal assistance is identified by project number, Federal agency, and grants or contracting officer. 25 points

#### Components of a Complete Application

A complete application consists of the following items in this order:

- 1. Application for Federal Assistance (Standard Form 424, REV 4–88);
- Budget Information—Nonconstruction Programs (Standard Form 424A, REV 4–88);
- Assurances—Non-construction Programs (Standard Form 424B, REV 4–88):
- 4 Table of Contents

Dated: July 9, 1997.

#### David F. Garrison,

Principal Deputy Assistant Secretary for Planning and Evaluation. [FR Doc. 97–18528 Filed 7–14–97; 8:45 am]

BILLING CODE 4151-04-M

### DEPARTMENT OF HEALTH AND HUMAN SERVICES

#### Office of the Secretary

#### Findings of Scientific Misconduct

**AGENCY:** Office of the Secretary, HHS. **ACTION:** Notice.

**SUMMARY:** Notice is hereby given that the Office of Research Integrity (ORI) has made a final finding of scientific misconduct in the following case:

Amitav Hajra, University of Michigan: Based upon a report from the University of Michigan, information obtained by the Office of Research Integrity (ORI) during its oversight review, and Mr. Hajra's own admission, ORI found that Mr. Hajra, former graduate student, University of Michigan, engaged in scientific misconduct by falsifying and fabricating research data in five published research papers, two published review articles, one submitted but unpublished paper, in his doctoral dissertation, and in a submission to the GenBank computer data base. Mr. Hajra's doctoral training and research was supported by two Public Health Service (PHS) grants, and his experiments were conducted at and submitted for publication from the

- Wijmenga, C., Gregory, P.E., Hajra, A., Schröck, E., Ried, T., Eils, R., Liu, P.P., and Collins, F.S. "Core binding factor β-smooth muscle myosin heavy chain chimeric protein involved in acute myeloid leukemia forms unusual nuclear rod-like structures in transformed NIH 3T3 cells." *Proc. Natl. Acad. Sci.* USA 93(4):1630–1635, 1996; and
- Liu, P.P., Wijmenga, C., Hajra, A., Blake, T.B., Kelley, C.A., Adelstein, R.S., Bagg, A., Rector, J., Cotelingham, J., Willman, C.L., and Collins, F.S. "Identification of the chimeric protein product of the CBFB-MYH11 fusion gene in inv(16) leukemia cells." *Genes, Chromosomes, and Cancer* 16:77–87, 1996 (Erratum in *Genes, Chromosomes, and Cancer* 18(1):71, 1997).

Mr. Hajra included fabricated and falsified data in the following review articles:

- Hajra, A., Liu, P.P., and Collins, F.S. "Transforming properties of the leukemic Inv(16) fusion gene CBFB–MYH11." In Molecular Aspects of Myeloid Stem Cell Development in Current Topics in Microbiology and Immunology (L. Wolff and A.S. Perkins, Eds.) 211:289–298, 1996 (Review). Berlin and New York: Springer-Verlag; and
- Liu, P.P., Hajra, A., Wijmenga, C., and Collins, F.S. "Molecular pathogenesis of the chromosome 16 inversion in the M4Eo subtype of acute myeloid leukemia." *Blood* 85:2289– 2302. 1995 (Review).

Mr. Hajra submitted a fabricated nucleotide sequence in computer data

Office of the Secretary, Department of Health and Human Services. Findings of Scientific Misconduct. Federal Register 62(135): 37921, 1997.

# 30 years later, it's still being cited...



37921

Received: 25 March 2021

Revised: 16 June 2021

Accepted: 13 July 2021

DOI: 10.100/20101110.2426

REVIEW

Human Mutatio

# Pathogenic noncoding variants in the neurofibron schwannomatosis predisposition genes

PEREZ-BECERRIL ET AL.

#### Cristina Perez-B

Division of Evolution and Gr Manchester Centre for Gen Mary's Hospital, Mancheste Health Science Centre, Schc Sciences, University of Man Manchester, UK

#### Correspondence

Miriam J. Smith, Division of Evolution and Genomic Science, Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester Academic Health Science Centre, School of Biological Sciences, University of Manchester, Manchester M13 9WL, UK. Email: miriam.smith@manchester.ac.uk

comparison of the full human and murine neurofibromin sequences revealed a high degree of similarity (>98%) and high conservation levels across 5'- and 3'-UTRs (Bernards et al., 19'3; Hajra et al., 1994). A subsequent in silico study compared the 5' upstream region and intron 1 of NF1 and homologous genes in human, mouse, rat, and puffer fish (Fugu rubripes). The authors found high homology segments throughout the region across all species, including two exact

have been identified in the SMARCB1 and LZTR1 genes, at the DGCR8 gene was recently reported to predispose to s the high detection rate for PVs in NF1 and NF2 (over 90% variants can be identified by routine genetic screening) up portion of clinical cases remain undetected. A higher

Perez-Becerril C, Evans DG, Smith MJ. Pathogenic noncoding variants in the neurofibromatosis and schwannomatosis predisposition genes. Hum Mutat 42(10):1187-1207, 2021. PubMed: 34273915

author of the application is identified and that person's role in the project is identified. 20 points

4. Organizational Experience. The application identifies the qualifying experience of the organization to demonstrate the applicant's ability to effectively and efficiently administer this project. The application specifically identifies the applicant as a nationallyrecognized organization, institution, or company with a record of study and analysis of rural and special transportation needs. Previous specific experience with work similar to the Tasks proposed is clearly and specifically described. The relationship between this project and other work planned, anticipated, or underway by he applicant is described, including a char which lists all related Federal assistance, occived within the last five years. In the evert a consortium of applicants is proposed, the project history of prior joint work should be provided. The previous Federal assistance is identified by project number, Federal agency, and grants or contracting officer. 25 points

#### Components of a Complete Application

A complete application consists of the following items in this order:

- 1. Application for Federal Assistance (Standard Form 424, REV 4–88);
- Budget Information—Nonconstruction Programs (Standard Form 424A, REV 4–88);
- Assurances—Non-construction Programs (Standard Form 424B, REV 4–88):
- 4 Table of Contenter

Dated: July 9, 1997.

#### David F. Garrison,

Principal Deputy Assistant Secretary for Planning and Evaluation.

Federal Register / Vol. 62, No. 135 / Tuesday, July 15, 1997 / Notices

[FR Doc. 97–18528 Filed 7–14–97; 8:45 am] BILLING CODE 4151–04–M

#### DEPARTMENT OF HEALTH AND HUMAN SERVICES

#### Office of the Secretary

#### Findings of Scientific Misconduct

**AGENCY:** Office of the Secretary, HHS. **ACTION:** Notice.

**SUMMARY:** Notice is hereby given that the Office of Research Integrity (ORI) has made a final finding of scientific misconduct in the following case:

Amitav Hajra, University of Michigan: Based upon a report from the University of Michigan, information obtained by the Office of Research Integrity (ORI) during its oversight review, and Mr. lajra's own admission, ORI found that Mr. Hajra, former graduate student, ity of Michigan, engaged in scientific misconduct by falsifying and fabricating research data in five published research papers, two published review articles, one submitted but unpublished paper, in his doctoral dissertation, and in a submission to the GenBank computer data base. Mr. Haya's doctoral training and research was supported by two Public Health Service (PHS) grants, and his experiments were conducted at and submitted for publication from the

- Wijmenga, C., Gregory, P.E., Hajra, A., Schröck, E., Ried, T., Eils, R., Liu, P.P., and Collins, F.S. "Core binding factor β-smooth muscle myosin heavy chain chimeric protein involved in acute myeloid leukemia forms unusual nuclear rod-like structures in transformed NIH 3T3 cells." Proc. Natl. Acad. Sci. USA 93(4):1630–1635, 1996; and
- Liu, P.P., Wijmenga, C., Hajra, A., Blake, T.B., Kelley, C.A., Adelstein, R.S., Bagg, A., Rector, J., Cotelingham, J., Willman, C.L., and Collins, F.S. "Identification of the chimeric protein product of the CBFB-MYH11 fusion gene in inv(16) leukemia cells." *Genes, Chromosomes, and Cancer* 16:77–87, 1996 (Erratum in *Genes, Chromosomes, and Cancer* 18(1):71, 1997).

Mr. Hajra included fabricated and falsified data in the following review articles:

- Hajra, A., Liu, P.P., and Collins, F.S. "Transforming properties of the leukemic Inv(16) fusion gene CBFB–MYH11." In Molecular Aspects of Myeloid Stem Cell Development in Current Topics in Microbiology and Immunology (L. Wolff and A.S. Perkins, Eds.) 211:289–298, 1996 (Review). Berlin and New York: Springer-Verlag; and
- Liu, P.P., Hajra, A., Wijmenga, C., and Collins, F.S. "Molecular pathogenesis of the chromosome 16 inversion in the M4Eo subtype of acute myeloid leukemia." *Blood* 85:2289–2302. 1995 (Review).

Mr. Hajra submitted a fabricated nucleotide sequence in computer data

Office of the Secretary, Department of Health and Human Services. Findings of Scientific Misconduct. Federal Register 62(135): 37921, 1997.

## Falsified sequencing to support a false phylogeny





Biochemical Systematics and Ecology

Volume 96, June 2021, 104263



Scientific data laundering: Chimeric mitogenomes of a sparrowhawk and a nightjar covered-up by forged phylogenies

George Sangster <sup>a</sup>  $\stackrel{\triangle}{\sim}$   $\boxtimes$  , Jolanda A. Luksenburg <sup>b c</sup>  $\boxtimes$ 

Show more V

+ Add to Mendeley 🗬 Share 🗦 Cite

https://doi.org/10.1016/j.bse.2021.104263 >

Get rights and content 🗷

#### Highlights

- This manuscript presents evidence that a complete <u>mitochondrial</u> <u>genome</u> of a sparrowhawk published by Gang Liu and colleagues in a paper in *Biochemical Systematics and Ecology* in 2017 is not an authentic sequence of this species but represents a chimera of three different species (a sparrowhawk, a buzzard and a dove).
- The manuscript also presents evidence that the authors of the
  aforementioned paper have fabricated false phylogenies to cover-up
  this problematic genome, and that of a nightjar previously
  published by another team, which is also a chimera (of two owls). To
  our knowledge this is the first known case of scientific fraud in
  phylogenetics.



"The evidence indicates that Liu et al. (2017) published phylogenies that were not based on existing data **but were fabricated to reflect preconceived ideas** about phylogenetic relationships." – Sangster & Luksenburg (2021)

Sangster G, Luksenburg JA. Scientific data laundering: Chimeric mitogenomes of a sparrowhawk and a nightjar covered-up by forged phylogenies. Biochem Syst Ecol 96: 104263, 2021.

# Unfortunately, the data is still in GenBank...



### UNVERIFIED: Accipiter gularis mitochondrion sequence

GenBank: KX585864.1 FASTA Graphics

Go to: ✓

LOCUS KX585864 17918 bp DNA linear VRT 31-AUG-2021

DEFINITION UNVERIFIED: Accipiter gularis mitochondrion sequence.

ACCESSION KX585864 VERSION KX585864.1

KEYWORDS UNVERIFIED; UNVERIFIED\_ORGANISM.

SOURCE mitochondrion Accipiter gularis (Japanese sparrowhawk)

ORGANISM <u>Accipiter gularis</u>

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Archelosauria; Archosauria; Dinosauria; Saurischia; Theropoda; Coelurosauria; Aves; Neognathae; Accipitriformes; Accipitridae;

Accipitrinae; Accipiter.

REFERENCE 1 (bases 1 to 17918)

AUTHORS Liu, G.

TITLE The complete mtDNA of Accipiter gularis

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 17918)

AUTHORS Liu, G.

TITLE Direct Submission

JOURNAL Submitted (21-JUL-2016) School of life science, Anhui Medical

University, 81 Meishan Rd, Hefei, Anhui 230032, China

COMMENT GenBank staff is unable to verify source organism and sequence

and/or annotation provided by the submitter.

FEATURES Location/Qualifiers

source 1..17918

National Center for Biotechnology Information. Sequence: KX585864.1. NCBI. Published April 22, 2025. Accessed April 22, 2025. https://www.ncbi.nlm.nih.gov/nuccore/KX585864.1

- Labeled as "UNVERIFIED," but the sequence remains in GenBank
- And can be returned with a BLAST search
- GenBank record "comments" aren't visible directly in BLAST results

### Intentional falsification is rare... but... what about accidents?



### Over 2 million "accidents" might be something else...

Mukheriee et al. Standards in Genomic Sciences 2015. 10:18 http://www.standardsingenomics.com/content/10/1/18



COMMENTARY **Open Access** 

#### Large-scale contamination of microbial isolate genomes by Illumina PhiX control

Supratim Mukherjee1\*, Marcel Huntemann1, Natalia Ivanova1, Nikos C Kyrpides

With the rapid growth and development of sequencing technologies, genomes exploring solutions to some of the world's biggest challenges such as searching exploration of genomic dark matter. However, progress in sequencing has been that can occur during template or library preparation, sequencing, imaging or d screened over 18,000 publicly available microbial isolate genome sequences in t database and identified more than 1000 genomes that are contaminated with P during Illumina sequencing runs. Approximately 10% of these genomes have be contaminated genomes were sequenced under the Human Microbiome Project. contamination from various sources and are usually eliminated during downstre of PhIX contaminated genomes indicates a lapse in either the application or effimeasures. The presence of PhiX contamination in several publicly available isolated errors when such data are used in comparative genomics analyses. Such contain far-reaching consequences in the form of erroneous data interpretation and ana measures to proofread raw sequences before releasing them to the broader scie

Keywords: Next-generation sequencing, PhiX, Contamination, Comparative gen

#### Background

The ability to produce large numbers of high-quality, low-cost reads has revolutionized the field of microbiology [1-3]. Starting from a meager 1575 registered One such challenge projects in September 2005, there has been a steady in- used as a quality ar crease in the number of sequencing projects according runs. PhiX is an i to the Genomes OnLine Database [4]. As of November with a single-strand 17th 2014, there were 41,553 bacterial and archaeal 5386 nucleotides at isolate genome sequencing projects reported in GOLD [4,5]. This explosion of genome sequencing projects especially during the last 5 years has been largely catalyzed by the development of several next-generation sequencing platforms offering rapid and accurate genome using PhiX at a low information at a low cost. Among the different NGS raised up to 40% for technologies available commercially, the sequencing by on the concentration eventhoris technology [6] championed by Illumina [7] is the same lane along with the sample or used as a senar.

Despite its high platform does come need to be address sequenced by Fred defined genome se used as a control for majority of its librar Steinegger and Salzberg Genome Biology https://doi.org/10.1186/s13059-020-02023-1

Genome Biology

**Open Access** 

#### Terminating contamination: large-scale search identifies more than 2,000,000 contaminated entries in GenBank

Martin Steinegger<sup>1,2,3\*</sup> and Steven L. Salzberg<sup>2,4,5</sup>

#### \*Correspondence:

**METHOD** 

<sup>1</sup>School of Biological Sciences. Seoul National University, Seoul, 08826, South Korea <sup>2</sup>Center for Computational Biology. Whiting School of Engineering, Johns Hopkins University, 21218 Baltimore, Maryland, USA Full list of author information is available at the end of the article

#### Abstract

Genomic analyses are sensitive to contamination in public databases caused by incorrectly labeled reference sequences. Here, we describe Conterminator, an efficient method to detect and remove incorrectly labeled sequences by an exhaustive all-against-all sequence comparison. Our analysis reports contamination of 2,161,746, 114,035, and 14,148 sequences in the RefSeq, GenBank, and NR databases, respectively, spanning the whole range from draft to "complete" model organism genomes. Our method scales linearly with input size and can process 3.3 TB in 12 days on a 32-core computer. Conterminator can help ensure the quality of reference databases. Source code (GPLv3): https://github.com/martin-steinegger/conterminator

Keywords: Genomes, Contamination, Software, RefSeq, GenBank

Downloaded from genome.cshlp.org on October 20, 2021 - Published by Cold Spring Harbor Laboratory Press

#### Human contamination in bacterial genomes has created thousands of spurious proteins

Florian P. Breitwieser, Mihaela Pertea, 1,2 Aleksey V. Zimin, 1,3

thans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, er Science, Whiting School of Engineering, Johns Hopkins University, Baltimore, cal Engineering, Johns Hopkins University, Baltimore, Maryland 21218, USA; of Public Health, Johns Hopkins University, Baltimore, Maryland 21205, USA

lished genomes can cause numerous problems for downstream analyses, particiomics projects. Our large-scale scan of complete and draft bacterial and archaeal als that 2250 genomes are contaminated by human sequence. The contaminant thuman repeat regions, which themselves are not adequately represented in the 8. The absence of the sequences from the human assembly offers a likely explablies. In some cases, the contaminating contigs have been erroneously annotated iich over time have propagated to create spurious protein "families" across mul-. As a result, 3437 spurious protein entries are currently present in the widely e report here an extensive list of contaminant sequences in bacterial genome ashem. We found that nearly all contaminants occurred in small contigs in draft small contigs from draft genome assemblies may mitigate the issue of contamigenuine genomic sequences.

#### article.] dy available ge-

es to well over tal resources for ing microbiome mplex samples erence databases ), but for practile today are still tigs or scaffolds o chromosomes ete or "finished" ry chromosome the human gether animal geiffolds that conin sequence has itive regions are ng to problems cies vary widely ng thousands of assemblies in the NCBI and UCSC Genome Browser databases were contaminated with the primate-specific AluY repeats (Longo et al. 2011). Although validation pipelines have improved substantially since then (Tatusova et al. 2016; Haft et al. 2018), some contaminants still remain, as we describe below. Furthermore, when open reading frames (ORFs) in the contaminated contigs get annotated as protein-coding genes, their protein sequence may be added to other databases. Once in those databases, these spurious proteins may in turn be used in future annotation, leading to the so-called "transitive catastrophe" problem where errors are propagated widely (Karp 1998; Salzberg 2007; Danchin et al. 2018). Indeed, one study found that the percentage of misan notated entries in the NCBI nonredundant (nr) protein collection, which is used for thousands of BLAST searches every day, has been increasing over time (Schnoes et al. 2009).

Contamination of genomic sequences can be particularly problematic for metagenomic studies. For example, if a genome labeled as species X contains fragments of the human genome, then any sample containing human DNA might erroneously be identified as also containing species X. Since human DNA is virtually always present in the environment of sequencing laboratories, human contamination is very common in sequencing experiments of all types. Contamination of laboratory reagents with

Contamination of genome assemblies with sequences from

Mukherjee S, Huntemann M, Ivanova N, et al. Large-scale contamination of microbial isolate genomes by Illumina PhiX control, Stand Genomic Sci 10: 18. 2015. PubMed: 26203331

Steinegger M, Salzberg SL. Terminating contamination: large-scale search identifies more than 2,000,000 contaminated entries in GenBank. Genome Biol 21(1): 115, 2020. PubMed: 32398145

Breitwieser FP, Pertea M, Zimin AV, Salzberg SL. Human contamination in bacterial genomes has created thousands of spurious proteins. Genome Res 29(6): 954-960, 2019. PubMed: 31064768

### Poor quality genomes can result in misclassification



### Multiple papers have found widespread misclassification based on sequencing data (examples below)

Bioinformatics, 36(18), 2020, 4699-4705 doi: 10.1093/bioinformatics/btaa586 Advance Access Publication Date: 24 June 2020 Original Paper OXFORD

2020

Sequence analysis

#### Detecting and correcting misclassified sequences in the large-scale public databases

Hamid Bagheri @ 1,\*, Andrew J. Severin2 and Hridesh Rajan1

<sup>1</sup>Department of Computer Science and <sup>2</sup>Genome Informatics Facility, Iowa State University, Ames, IA 50011, USA

\*To whom correspondence should be addressed.

Associate Editor: Arne Elofsson

Received on April 2, 2020: revised on June 10, 2020; editorial decision on June 11, 2020; accepted on June 16, 2020

#### Abstract

Motivation: As the cost of sequ ata being deposited into public repositories is increasing rapidly. Public database a for each submission that is prone to user error. ~7.8% of Unfortunately, most publig rely on user input and do not have methods for identifying errors in the p error propagation. Previous research on a small subset of the NR databa genomes ce similarity. To the best of our knowledge, the amount of misclassifical ed. We propose a heuristic method to detect potentially misclassified t nnique and quality control misclassified to find the most probab tion from manually and ~4% at at the species Results: We found mor simulated data, we show teins. The proposed approthe genus level Availability and implement are available at https://github.

Contact: hbagheri@iastate.edu Supplementary information: Supple vailable at guency of each annota-NR database. Using misclassified pro-Docker container

level

#### 1 Introduction

Researchers use BLAST on the non-redundant (NR) database on a

are deposited. For example, if data for DNA sequences were deposited by a plant researcher studying soybeans obtained from a soy-

Bagheri H, Severin AJ, Rajan H. Detecting and correcting misclassified sequences in the large-scale public databases, Bioinformatics 36(18): 4699-4705, 2020, PubMed: 32579213

#### PLOS ONE

2021

RESEARCH ARTICLE

Large-scale k-mer-based analysis of the informational properties of genomes. comparative genomics and taxonomy

Yuval Bussi 61,2,3, Ruti Kapon 61, Ziv Reich1+

1 Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel, 2 Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot, Israel, 3 Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

\* ziv.reich@weizmann.ac.il

# Check for

#### OPEN ACCESS

Citation: Bussi Y, Kapon R, Reich Z (2021) Largescale k-mer-based analysis of the informational properties of genomes, comparative genomics and taxonomy. PLoS ONE 16 (10): e0258693. https:// doi.org/10.1371/journal.pone.0258693

Editor: Omri Finkel, University of North Carolina at Chapel Hill, UNITED STATES

Received: April 30, 2021

Accepted: October 2, 2021

Published: October 14, 2021

Copyright @ 2021 Bussi et al. This is an open

#### Abstract

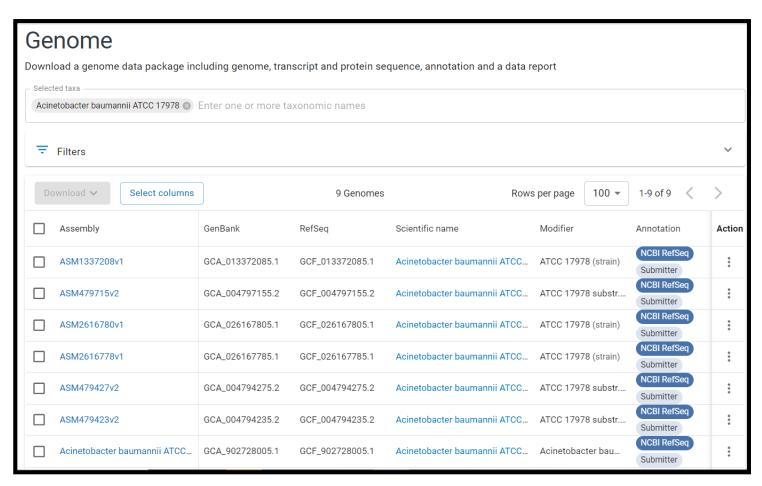
Information theoretic approach wide variety of bioinformatics applications. In comp ~7% of ds, based on short DNA words, or k-mers, are ity of varying k-mer lengths for genome comp verage of 5805 genomes genomes in the KEGG 0 four k-mer lengths misclassified spanning the relevant ra 34 genus-level rep resentative genomes us best recapitulated dom domains and a phylogenetic/taxonomi at genus or phylum). By analyzhigh subtree similarity for higher n-ancestor taxon leving ~14.2M prokaryotic ger els, we detected many potent database, further demonstrating the need for widetaxonomic classifications based on whole-genome similarity.

Bussi Y. Kapon R. Reich Z. Large-scale k-mer-based analysis of the informational properties of genomes, comparative genomics and taxonomy. PLoS One 16(10): e0258693, 2021. PubMed: 34648558

# Which reference? 9 and growing...







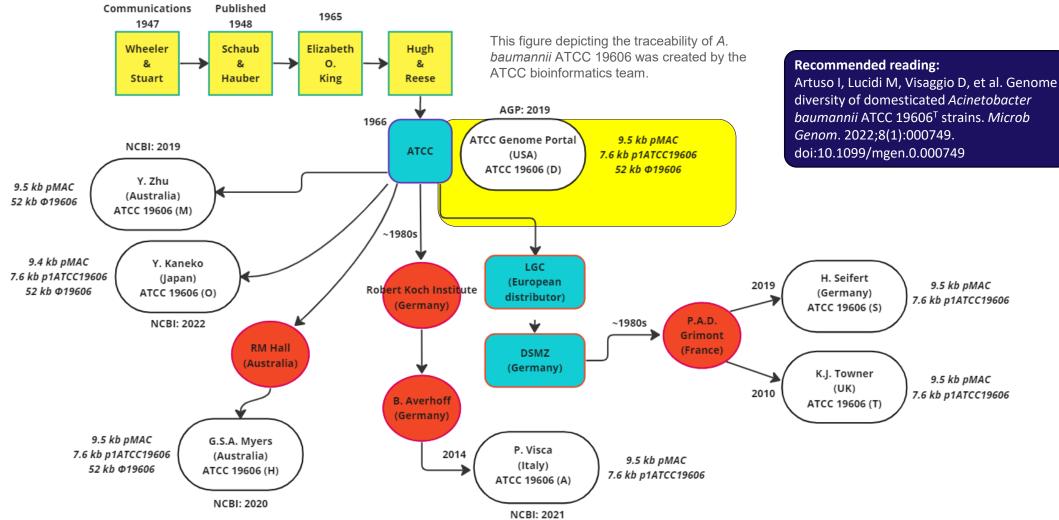
- Unverified chain of custody.
- Growth conditions?
- DNA extraction methods?
- DNA sequencing platforms?
- de novo assembly methods?

How do researchers \*know\* which data set to use for their research?

National Center for Biotechnology Information (NCBI). Genome Data Viewer [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [cited 2025 Apr 22]. Available from: https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=400667

# Which reference is the "right" reference?





The NCBI "reference genome"

Personal

## Comparison of ATCC® Genome Portal vs. RefSeq Assemblies





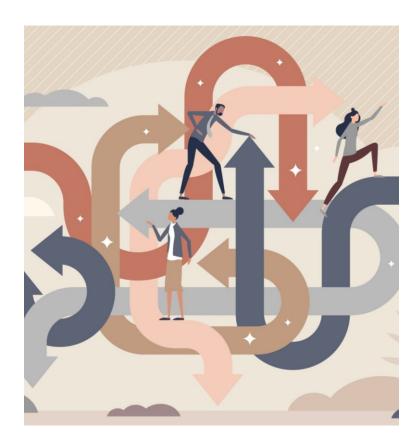
### refseq assembly

- 52kb region missing, which includes 74 genes
- 51 are not found anywhere else in the refseq assembly

# **Examples of other reliability issues**



- Misclassification of type strains used in FDA-approved probiotic foods.
- Misclassification of control strains used by clinical microbiology labs for a widely used AMR testing platform.
- Different phenotypes for the "same" strains.
- Unknown history or chain-of-custody of materials or data.
- There's no "track changes" with genome assemblies.
- Accidental mislabeling of files or rows in a table can lead to incorrect links between NGS data and metadata.





# The ATCC® Genome Portal

The *only* source for authenticated genome assemblies for ATCC® materials

### **Our solution**



### **Physical Repository**

### Authenticated Reference Data

- Strains
- Derivatives
- Standards
- Reference materials



- Sequencing data
- Assembled genomes
- Annotated genes

Drive scientific advancement by providing the scientific community with high-quality, annotated whole-genome sequence (WGS) information traceable to ATCC®'s authenticated biological materials.

# The arc of database quality in genomics





# **Expert Curated Data**

- Less risk
- Standardized metadata
  - Improved reproducibility
- Often commercial



### **Authenticated Reference Data**

- Lowest risk
- Standardized laboratory methods
- Standardized bioinformatics methods
- Quality assurance (ISO 9001)
- Traceable to materials in a biorepository
- Full data provenance
- Fully curated and authenticated
- Maximum reproducibility

ATCC® Genome Portal ATCC® Cell Line Land



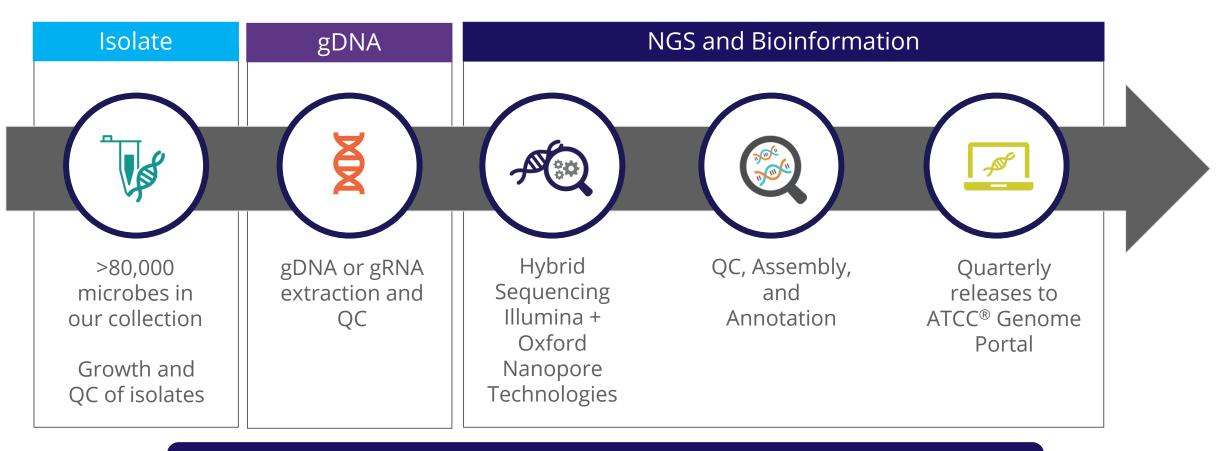
- Moderate risk
- Improved metadata
- More limited scope



- Moderate to high risk
- Unknown quality
- Missing / non-standard metadata
- Often no data provenance

# Authenticated physical material coupled with reference-quality genome sequences



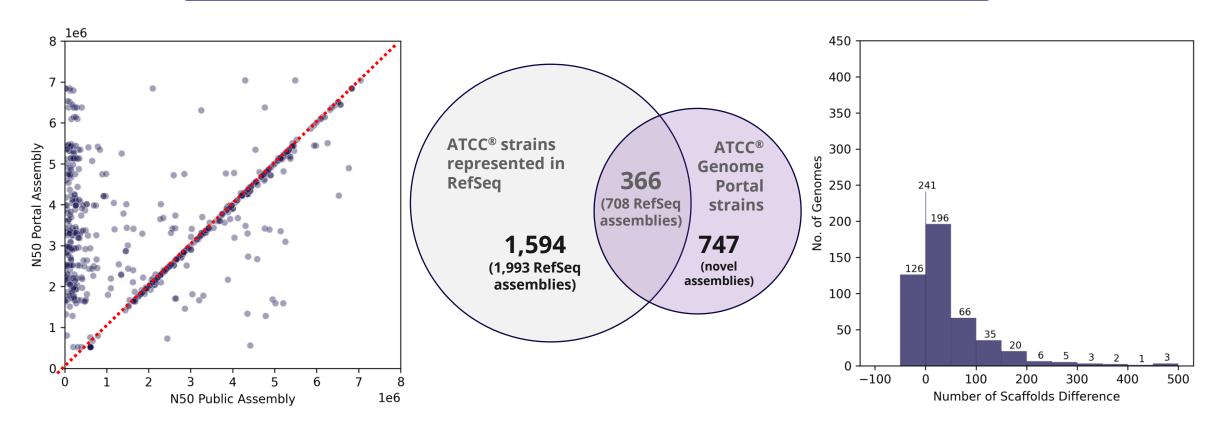


- Fully traceable and authenticated to ATCC® materials
- All genome assemblies produced in-house at ATCC® in an ISO-certified laboratory

# Comparison of ATCC® vs. NCBI RefSeq bacterial assemblies



>98% of our assemblies were more complete and of higher quality than RefSeq



Yarmosh DA, Lopera JG, Puthuveetil NP, et al. Comparative Analysis and Data Provenance for 1,113 Bacterial Genome Assemblies. mSphere 7(3): e0007722, 2022. PubMed: 35491842

# **Overview of our process**



### **ATCC Repository**



ATCC Repository
Retrieval from ATCC collection





**Bioproduction & QC** ATCC® Standards culturing and qc ■ VITEK 2 ■ 16S analysis





**Data Provenance** ISO-9001 batch number and metadata authentication of vials

### Sequencing & Bioinformatics Center (SBC)



**Nucleic Acid Extraction** Automated extraction and QC of HMW genomic DNA





**NGS Library Prep** Illumina and ONT specific WGS kits

dsDNA, dsRNA, ssRNA, ssDNA





**NextGen Sequencing** Illumina and ONT specific WGS



**Raw NGS Data** Run output QC and demultiplexing

- Illumina FASTQONT FASTQ/POD5



**Trimming / Filtering / QC**Contamination QC, read classification, fastp, NanoFilt



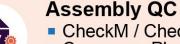
de novo Assembly Unicycler (Bacteria) SPAdes (Viruses) MaSuRCA (Fungi & Protists)



**Functional Annotation** 

PGAP, funannotate, VIGA





- CheckM / CheckV / Busco
- Coverage, Phred, and N Bases
- Length, contigs, circularization



#### **Technical Review**

- Taxonomic analysis consensusManual QC check

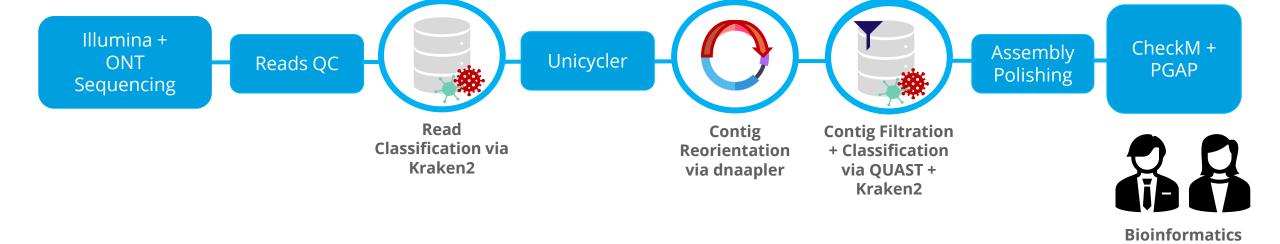
Refer the expanded list of references in the Appendix at the end of the presentation: 1.Simão FA, et al. Bioinformatics 2015:31:3210-3212. 2. Parks DH, et al. Genome Res 2015;25:1043-1055.

4.Palmer JM, Stajich J. Zenodo 2020. 5. Tatusova T, et al. Nucleic Acids Res 2016;44:6614-6624.

7. Zimin AV, et al. Bioinformatics 2013;29:2669-2677. 8. Wick RR, et al. PLoS Comput Biol 2017;13:e1005595. 9.Fu P, et al. Brief Bioinform 2023;25:bbad444.

# **Bacterial genome assembly**





- All bacterial strains are sequenced on both sequencing platforms.
- NGS reads are trimmed and filtered, assembled using a pipeline built around *Unicycler*, and finally polished.
- Assembly QC is based on CheckM.
- Annotation is based on NCBI's PGAP pipeline.

**Technical Review** 

# Virology genome assembly



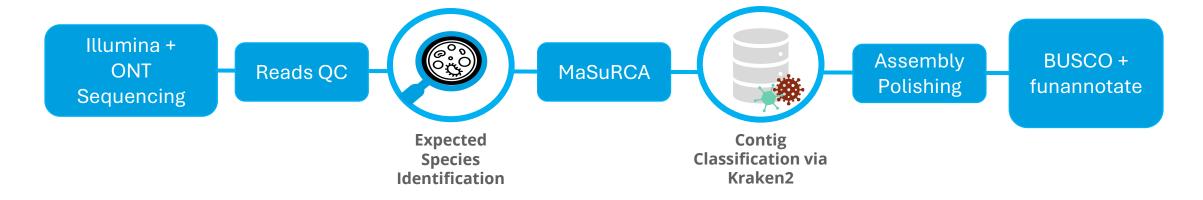


- All viral strains are sequenced on Illumina.
- (soon) DNA viruses are also sequenced on ONT.
- Samples go through an *in silico* de-hosting against each respective genomes for the host cell line used to produce the virus.
- De novo assembly is based around the SPAdes assembler.
- BLAST, VIGA, and CheckV are used for post-assembly QC.

# Microbial eukaryotes genome assembly



### **Fungi and Protists**



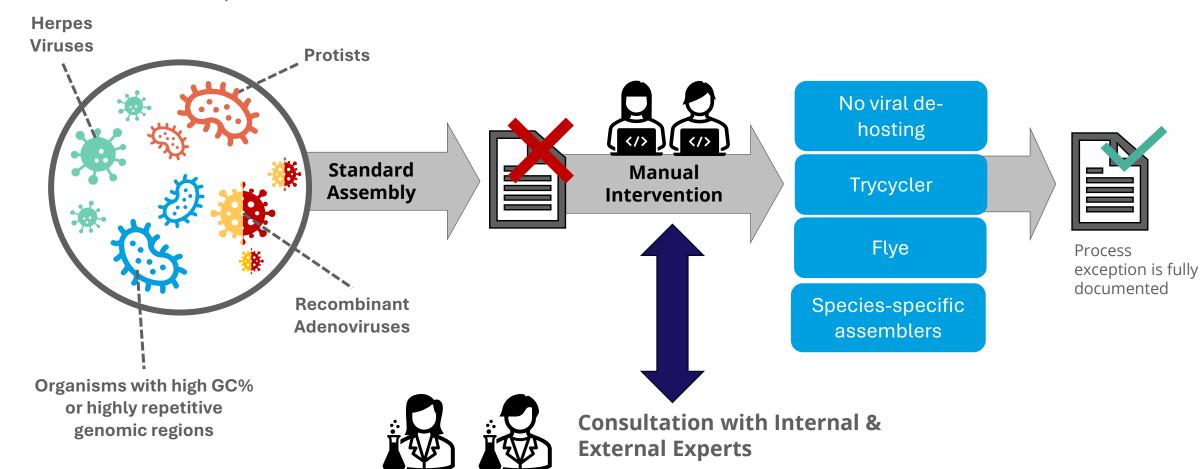
- All microbial eukaryotic samples are sequenced on both Illumina and Oxford Nanopore
- Assembly pipeline is built around the MaSuRCA assembler
- Annotation is done using BUSCO and FunAnnotate

# Our "white glove" genome assembly process



Sometimes, genome assemblies need some TLC, but for our group even this is documented and standardized.

### Some recent examples:







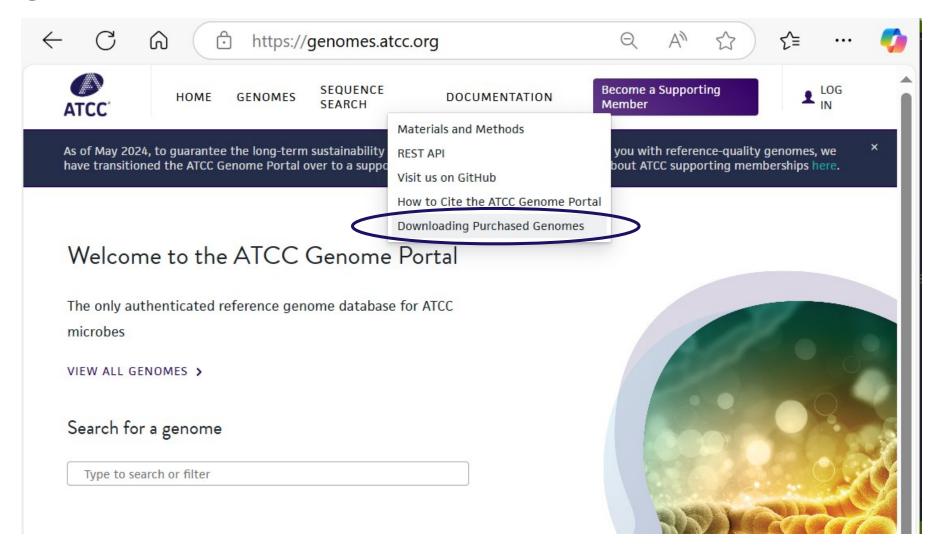
# The ATCC® Genome Portal

Accessing our data

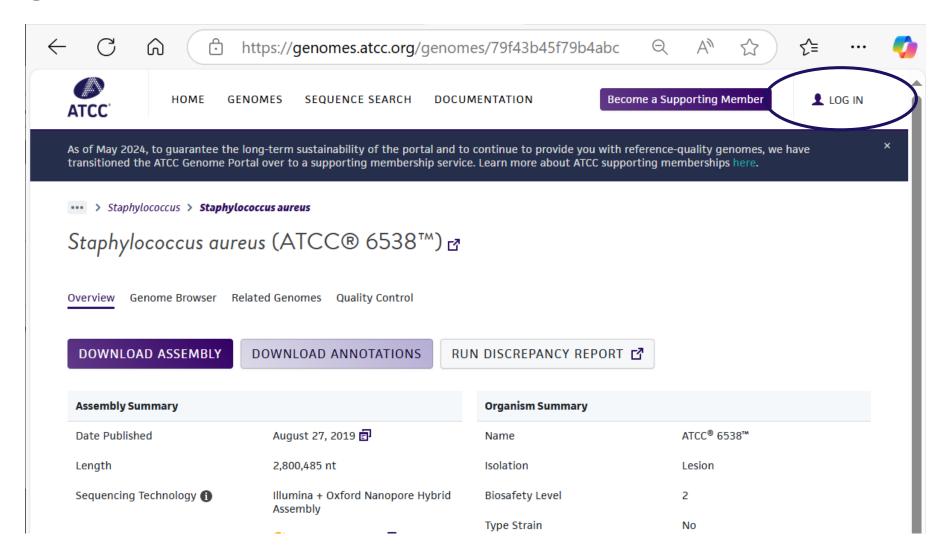
## If customers have purchased the physical product...



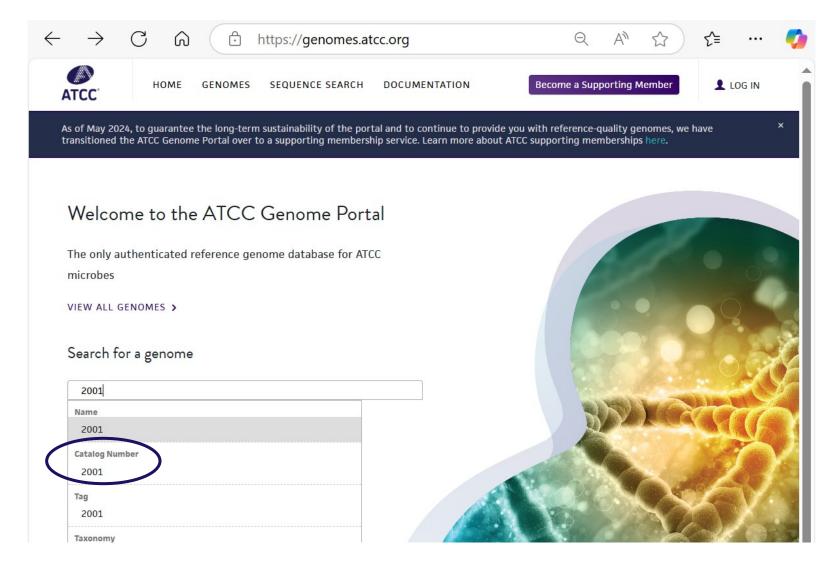
Download genome(s) with the lot number



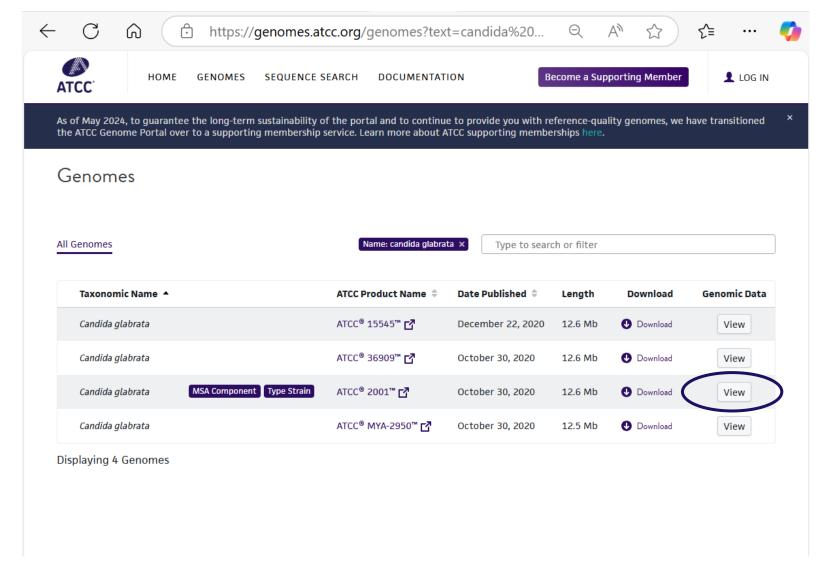




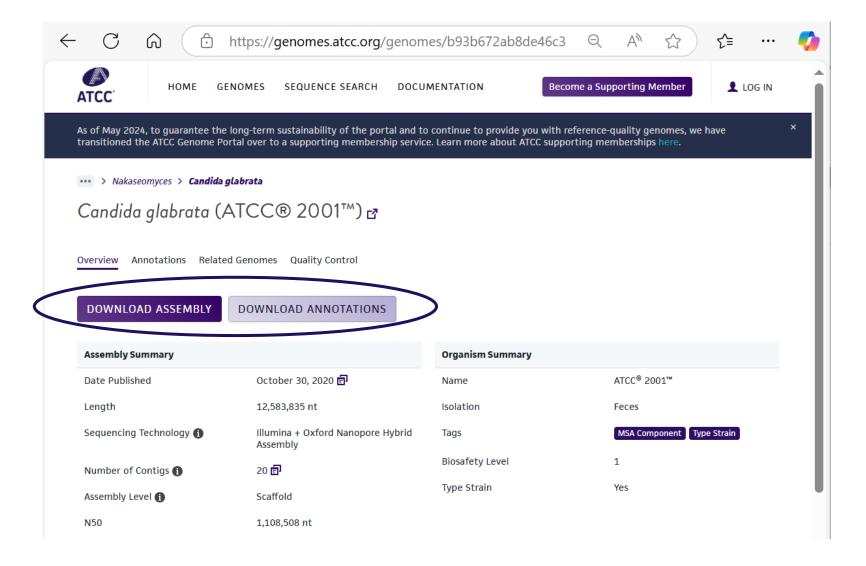




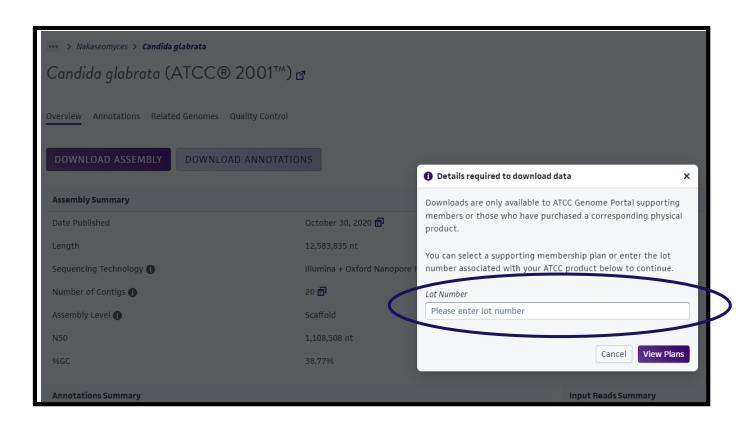














# Access to the entire database... .....purchase a Supporting Membership



## **Explore our annual Supporting Membership opportunities**

	Free	Individual	Research Group	Institution
View organism and genome metadata, assemblies, and annotations	✓	<b>✓</b>	✓	<b>√</b>
Search for genomes of interest	✓	<b>√</b>	✓	✓
Purchase the corresponding authenticated ATCC source materials	<b>✓</b>	<b>✓</b>	<b>✓</b>	<b>✓</b>
Download genome assemblies and annotations	Only for purchased products	All products	All products	All products
Access our secure REST-API	Not available	✓	✓	✓
Analyze isolates with Discrepancy Reports	Fee for each report	12 free reports per year	60 free reports per year	Inquire
Members with full access	0	1	5	Unlimited
		±600 / ±4 000	#2 400 / # <b>7</b> 200	

\$600 / \$1,800 \$2,400 / \$7,200

Inquire

## Access to the entire database...





HOME

GENOMES

SEQUENCE SEARCH

DOCUMENTATION

LOG IN

As of May 2024, to guarantee the long-term sustainability of the portal and to continue to provide you with reference-quality genomes, we have transitioned the ATCC Genome Portal over to a supporting membership service. Learn more about ATCC supporting memberships here.

## ATCC Genome Portal Pricing

ATCC has partnered with One Codex to provide access to premium ATCC Genome Portal content and easy to use analyses on One Codex. Learn more about the One Codex and ATCC partnership. If you wish to use a Purchase Order for your Supporting Membership, please contact nextgen@atcc.org.

#### Free Plan

Search for and view over 4,000 genomes from ATCC's catalog

#### Sign Up

- View organism and genome metadata, assemblies, and annotations
- Search for genomes of interest

#### Individual

View and download genomic data and access premium features

#### Log In To View Pricing

- View organism and genome metadata, assemblies, and annotations
- Search for genomes of interest
- Download genome assemblies and annotations
- Access the REST API
- Analyze isolates with 12
   Discrepancy Reports included
- 1 seat

### Research Group

Full access and premium features for up to 5 team members

#### Log In To View Pricing

- View organism and genome metadata, assemblies, and annotations
- Search for genomes of interest
- Download genome assemblies and annotations
- Access the REST API
- Analyze isolates with 60
   Discrepancy Reports included
- 5 seats

### Institutional

Full access and premium features for your entire institution

#### Log In To View Pricing

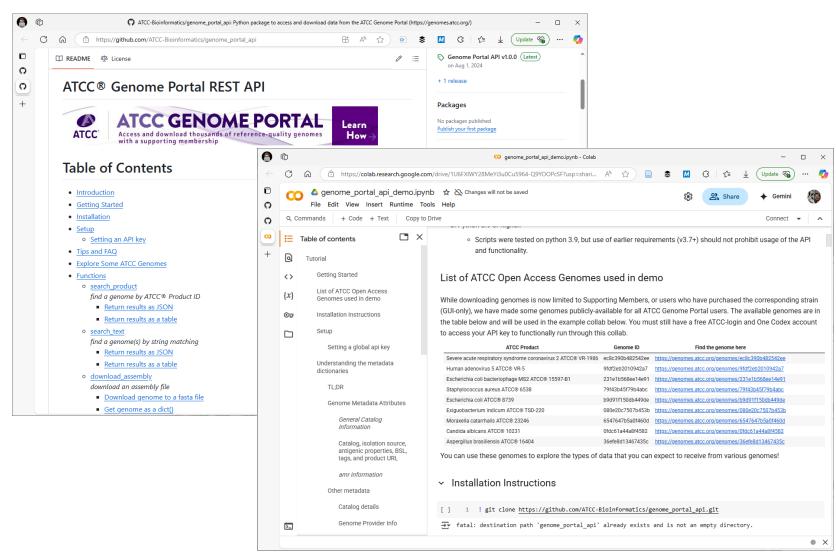
- View organism and genome metadata, assemblies, and annotations
- Search for genomes of interest
- Download genome assemblies and annotations
- Access the REST API
- Analyze isolates with Discrepency Reports (Inquire for details)
- Unlimited seats

# Programmatic access via our REST-API



## https://github.com/ATCC-Bioinformatics/genome\_portal\_api

- Documentation available on GitHub.
- Tutorials available on Google Colab. Openaccess assemblies available for testing. No Supporting Membership required.



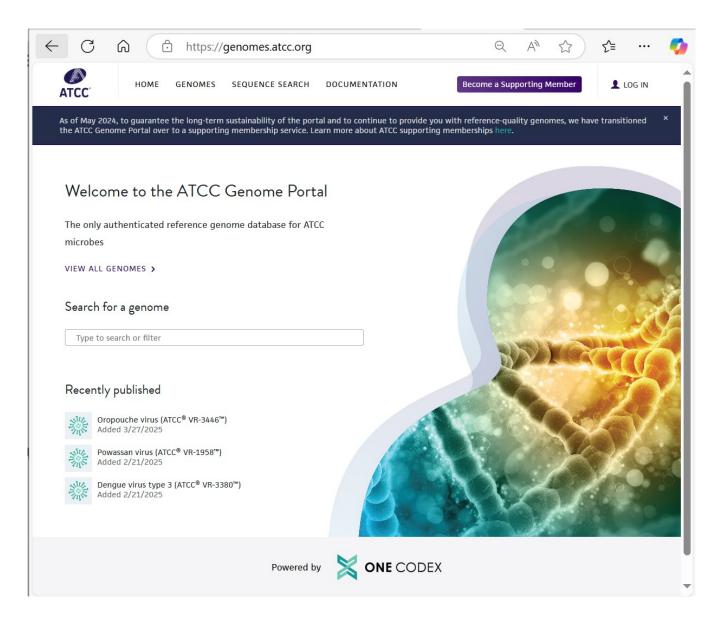


# The ATCC® Genome Portal

Search, exploration, and analysis tools

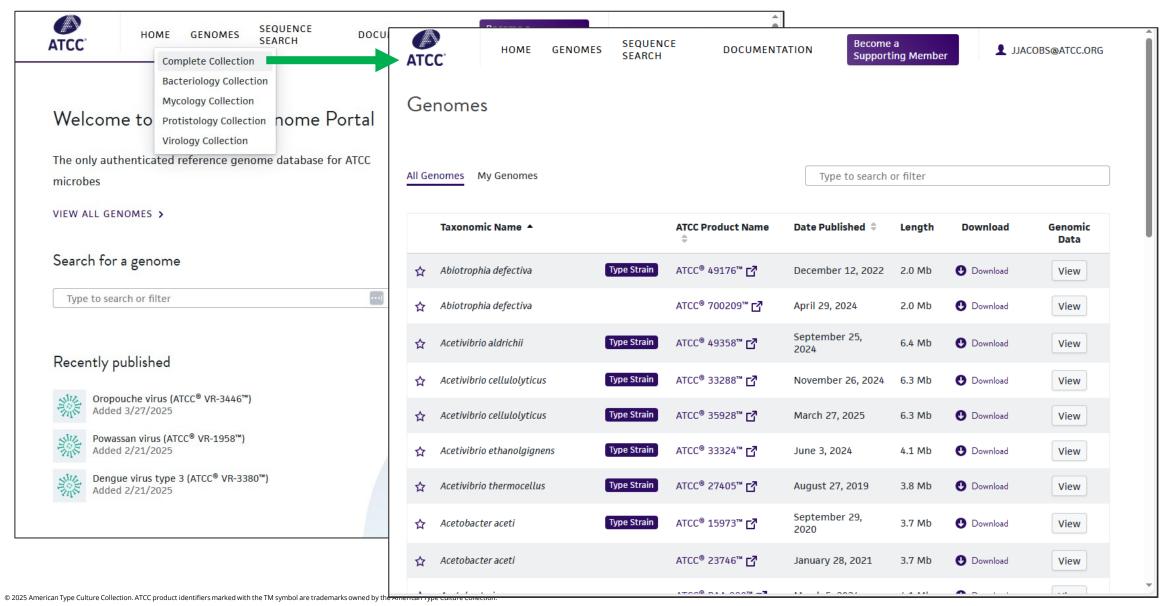
## The ATCC® Genome Portal





# **Browse for your data**

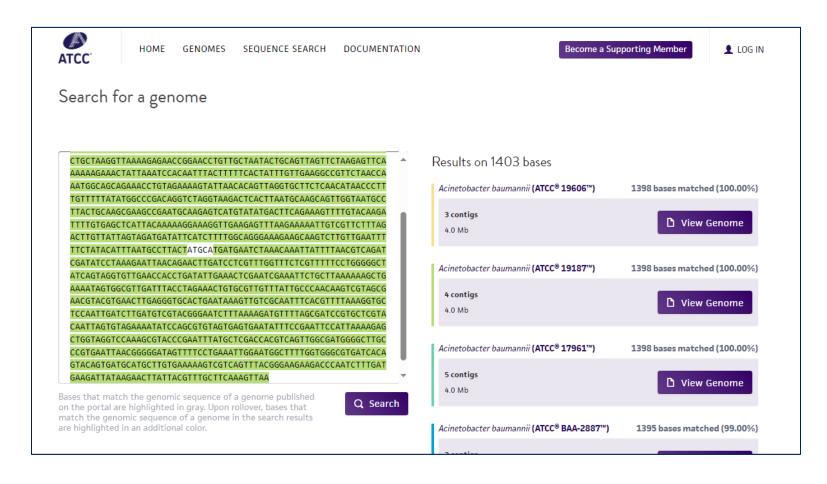




# Fast sequencing search

## https://genomes.atcc.org/sequence-search





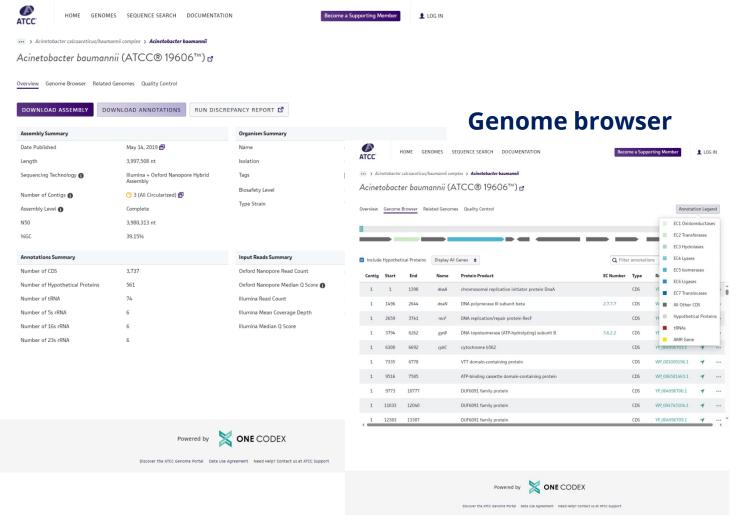
Search results are almost instantaneous!

## **ATCC®** Genome Portal: Reference Genome Details

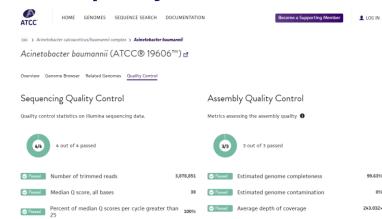


## **Example:** *Acinetobacter baumannii* (ATCC® 19606™)

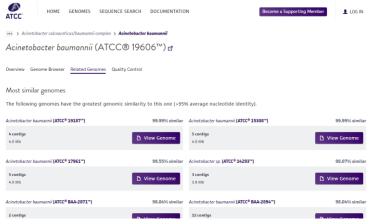
## **Overview page**



## View quality control data



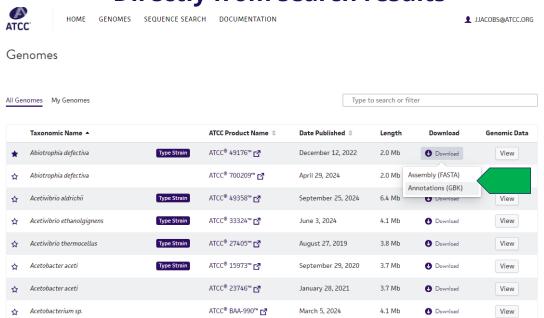
## Find related genomes



# Download genome references



## **Directly from search results**



Two file formats for download:

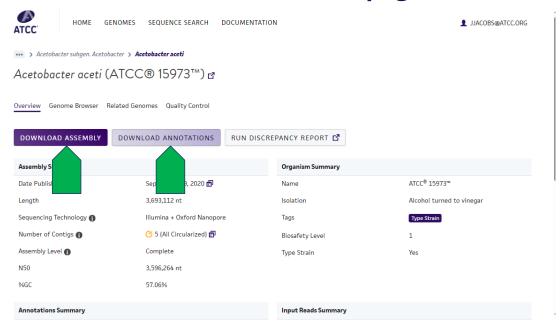
#### FASTA files

• **.fasta** files are smaller and include only basic information and unannotated DNA sequence for entire genome.

#### 2. GenBank files

• **.gbk** files are larger but include annotations for all known genes and rich metadata for the organism.

## **Download from details page**

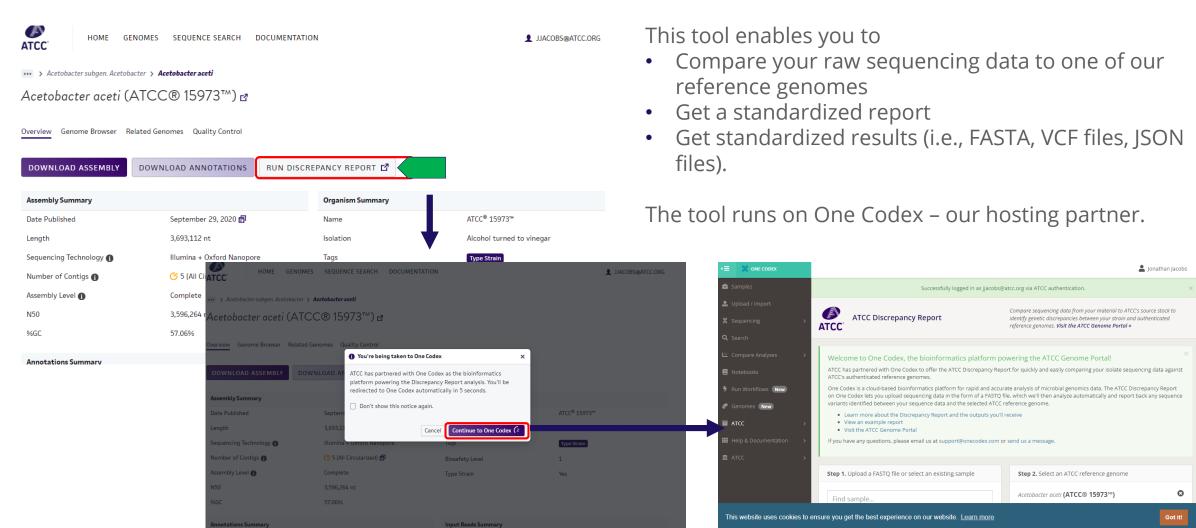


While both formats can be opened with a plain-text editor (i.e., Notepad), these files will often be thousands of lines long. They are intended to be imported into 3<sup>rd</sup> party bioinformatics software for data visualization.

We also have a REST-API for programmatic access to the ATCC® Genome Portal, including downloads. This is often the preferred approach by data scientists and bioinformaticians.

# Run a Discrepancy Report







# Summary

# Recent publications ....





Benton B, et al. **The ATCC® Genome Portal: Microbial Genome Reference Standards with Data Provenance.** Microbiology Resource Announcements 10(47): e00818-21, 2021



Yarmosh DA, et al. Comparative Analysis and Data Provenance for 1,113 Bacterial Genome Assemblies. mSphere 7(3): e0007722, 2022.



Nguyen SV, et al. **The ATCC**<sup>®</sup> **Genome Portal: 3,938 authenticated microbial reference genomes.** Microbiology Resource Announcements Epub ahead of print e0104523, 2024.

# Thank you!

## □ nextgen@atcc.org



## **Sequencing & Bioinformatics Center**



### Jonathan Jacobs, PhD

Senior Director, Bioinformatics BioNexus Principal Investigator

## **Genomics Lab**

### **Briana Benton, PMP**

Ana Fernandes
Ajeet Singh, PhD
Stephen King, MSc
James Duncan, MSc
Robert Marlow
Corina Tabron, MSc
Jade Kirkland
Noah Wax, MSc
Rula Khairi
Hannah McConnell
Kaitlyn Gaffney

## **Bioinformatics Lab**

## John Bagnoli

David Yarmosh, MSc. Nikhita Putheveetil, MSc. Joseph Petron, PhD. Amy Reese, MSc

Scott V Nguyen, PhD
Senior Biocuration Scientist

### **Our Partner**





# Questions



# Appendix

# **Open-source tools referenced on slide 29**



- 1.Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212.
- 2.Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055.
- 3. Nayfach S, Camargo AP, Schulz F, Eloe-Fadrosh E, Roux S, Kyrpides NC. 2021. CheckV assesses the quality and completeness of metagenome-assembled viral genomes. Nat Biotechnol 39:578–585.
- 4. Palmer JM, Stajich J. 2020. Funannotate v1.8.1: Eukaryotic genome annotation (v1.8.1). Zenodo.
- 5.Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624.
- 6.Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology 19:455–477.
- 7.Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. Bioinformatics 29:2669–2677. 8.Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595.
- 9.Fu P, Wu Y, Zhang Z, Qiu Y, Wang Y, Peng Y. 2023. VIGA: a one-stop tool for eukaryotic virus identification and genome assembly from next-generation-sequencing data. Briefings in Bioinformatics 25:bbad444.