ncbi.nlm.nih.gov/cgr

# Revolutionizing Biological Research with the NIH Comparative Genomics Resource (CGR) Valerie Schneider, Ph.D. 10/26/23



In what ways does your work relate to CGR-related resources?

Where do you think CGR might have the greatest impact for your clients?

What types of CGR-engagement opportunities might be most valuable?



Why We Are Here

# Outline

- Intro to Comparative Genomics
- The Value of Research Organisms
- Problem
- CGR Solution
- CGR Impact Two use cases
- What's Next





# What is comparative genomics?





# Who does comparative genomics?















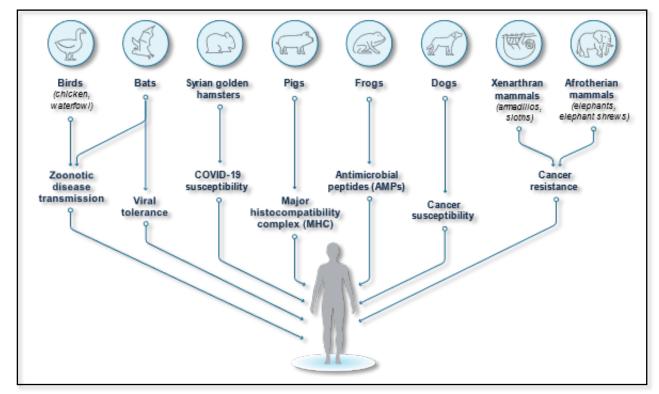
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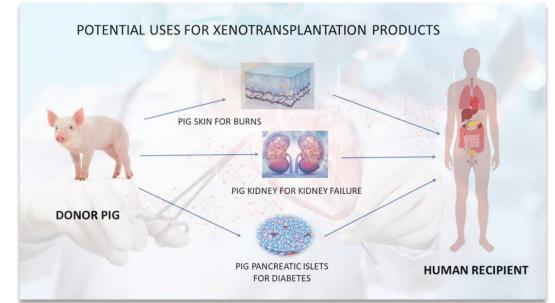
# The Value of Research Organisms Understand Basic Biological **Processes & Human Disease**

Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates

Joana Damas<sup>a,1</sup>©, Graham M. Hughes<sup>b,1</sup>©, Kathleen C. Keough<sup>c,d,1</sup>©, Corrie A. Painter<sup>e,1</sup>©, Nicole S. Persky<sup>f,1</sup>©, Marco Corbo<sup>a</sup><sup>(a)</sup>, Michael Hiller<sup>g,h,i</sup><sup>(b)</sup>, Klaus-Peter Koepfli<sup>(b)</sup>, Andreas R. Pfenning<sup>k</sup><sup>(b)</sup>, Huabin Zhao<sup>l,m</sup><sup>(b)</sup>, Diane P. Genereux<sup>n</sup><sup>(0)</sup>, Ross Swofford<sup>n</sup><sup>(0)</sup>, Katherine S. Pollard<sup>d,o,p</sup><sup>(0)</sup>, Oliver A. Ryder<sup>q,r</sup><sup>(0)</sup>, Martin T. Nweeia<sup>s,t,u</sup><sup>(0)</sup>, Kerstin Lindblad-Toh<sup>n,v</sup><sup>®</sup>, Emma C. Teeling<sup>b</sup><sup>®</sup>, Elinor K. Karlsson<sup>n,w,x</sup><sup>®</sup>, and Harris A. Lewin<sup>a,y,z,2</sup><sup>®</sup>

https://www.pnas.org/doi/10.1073/pnas.2010146117







https://www.fda.gov/vaccines-blood-biologics/xenotransplantation



# Problem

Comparative genomics research faces several limitations and challenges

- Exponential data growth; variable data quality
- Multiple different user interfaces
- Limited number of organisms supported
- Siloed data and applications
- Must download data to apply tools
- Limited scalability

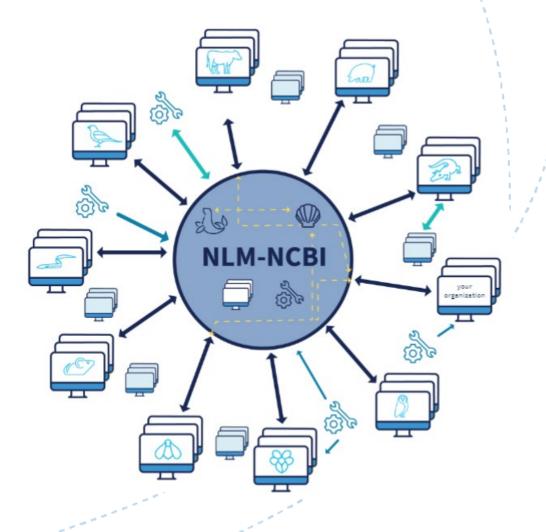
## Solution NIH Comparative Genomics Resource (CGR)

What: CGR maximizes the impact of eukaryotic research organisms and their genomic data to biomedical research.

**How**: CGR facilitates reliable comparative genomics analyses through **community collaboration** and an **NCBI genomics toolkit**. The toolkit includes highquality data, tools, and **interfaces** for connecting community-provided resources with NCBI.

**Outcome**: CGR provides you with information and examples about free tools and data so you can **confidently help educators, trainees, researchers, and bioinformaticians** working in comparative genomics.





# CGR Components

### **NCBI** Toolkit

- Interconnected databases
- Interoperable data and tools •

#### Data Resources

2

3

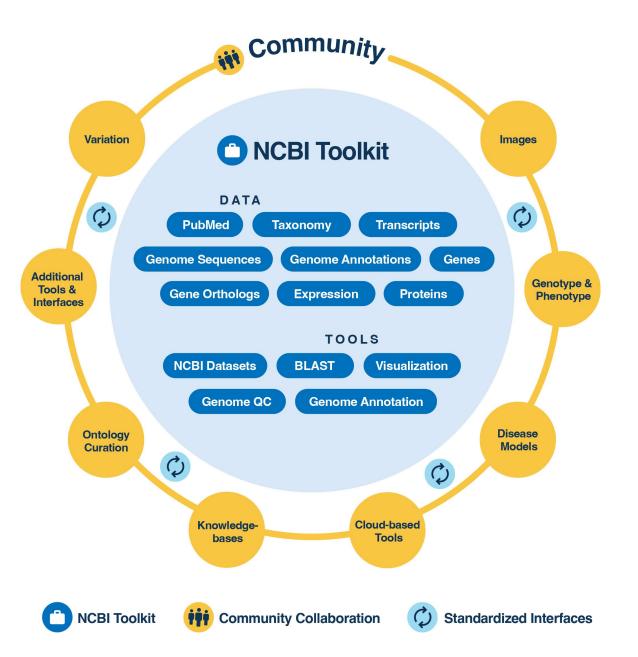
- NCBI Datasets Coenomes, Cenes, Proteins, Expression
- Gon netotogenome-related data and
- metadata with the NCBI toolkit

### And ty for most new developments and

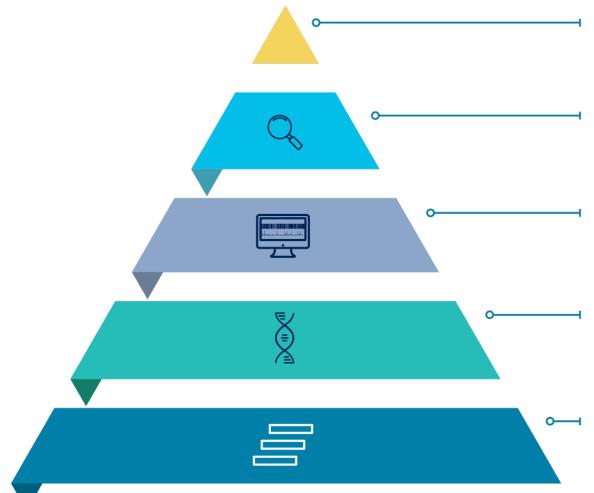
- Basic Local Alignment Search Tool (BLAST)
- Visualization tools .

- Stand Antipatetive Genome Viewer (CGV)
   Multiple Sequence Alignment (MSA) Viewer
   Congercise Gamme Wall & Genome With
- NCBI content via command line
- Data Quality Tools
   Foreign Contamination Screening (FCS) Tool
- Assembled genome QC .
- Eukaryotic Annotation Tool (EGAP) •





# **CGR** Impact



#### Scientific Advancement

Maximize impact of eukaryotic research organisms and their genomic data resources to biomedical research

#### **New Discovery Amplification**

Equal access to all eukaryotic organism data with better connections to community resources

#### **Scalable Analyses**

Support data growth with emerging big data approaches

#### New and Improved Comparative Genomics Tools

Promote high-quality data submission, exploration, analysis, and retrieval with seamless user experiences

#### High Quality Genomic Data

Serve standardized, uncontaminated, and consistently annotated eukaryotic genomic data from NCBI Archives



# **CGR Impact: Two Case Studies**





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# Finding genomic resources for organisms on NCBI

A public health biologist wants to find and contribute data for an invasive tick species

### They need to:

- Identify available genomic data on NCBI for tick species:
  - Datasets Taxonomy
  - Datasets Genome
  - Datasets Command Line
- Improve the quality of their own data:
  - Foreign Contamination Screen
  - Genome Annotation



Haemaphysalis longicornis Asian longhorned tick Recently found on the East Coast!

https://www.cdc.gov/ticks/longhorned-tick/index.html



### Taxonomy Browser

- Search by common name, species name, higher groups
- Returns table of matches with data availability
- Taxon names link to Taxonomy pages – one stop shop for links to available sequence NCBI data!



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Selected taxa Haemaphysalis longicornis (longhorned tick) 🐵 Enter one or more taxonomic names	
Taxonomic name	Genomes
✓ Eukaryota (eukaryotes)	34,077
<ul> <li>✓ Metazoa (animals)</li> </ul>	12,395
<ul> <li>Arthropoda (arthropods)</li> </ul>	4,797
<ul> <li>Arachnida (arachnids)</li> </ul>	150
<ul> <li>Ixodida (ticks)</li> </ul>	44
<ul> <li>Ixodidae (hardbacked ticks)</li> </ul>	30
✓ Haemaphysalis	4
Haemaphysalis longicornis (longhorned tick)	4

There are four available genome sequences for *H*. *longicornis* available on NCBI, and 44 for ticks in general.

https://www.ncbi.nlm.nih.gov/datasets/taxonomy/tree/

### Taxonomy Page

- Taxonomy-based portal to data: gene expres raw sequence, overarching project more!
- Direct link to designated  $\bullet$ reference genome
- Links to other CGR • resources – annotation table, visualization, BLAST

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s and	Datas
	Series

All nucleotide sequences	50,367	
Genomic sequences	2,577	
mRNA sequences	47,787	
GEO Datasets		
Datasets	C	
Series	3	
Samples	37	
Platforms	3	
PopSet		

**Phylogenetic studies** Population studies

**Database links** 

Nucleotide

### Summary of data available on NCBI for *H. longicornis*

29

9

Protein sequences	27,921
Conserved domains	1
3D structures	2
Sequence Read Archive (SF	RA)
All SRA experiments	268
DNA	192
RNA	76
	Genome
Projects and samples	Browse all 4 genomes
BioProject	D (
BioSample	Reference genome BIME_HaeL_1.3
	TIGMIC Group (2020). Isolate GenBank GCA_013339765.2



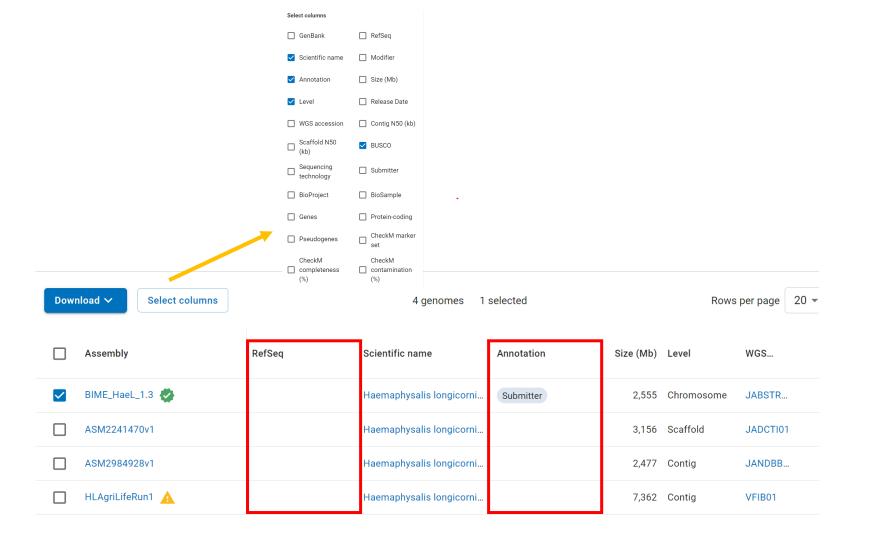


### NCBI Datasets Genome Table: *H. longicornis*

- Summary of available genome sequences, Includes metadata like genome size, quality and annotation availability
- Allows researchers to select most suitable assembly for their research
- Modify visible data using "Select Columns"



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None of the four *H. longicornis* genomes are curated RefSeq genomes or have RefSeq annotation...What about other species of tick?

### What genomic resources do we have for other ticks?

naphysalis longicornis (longhorned tick) 🛞 Enter one or more taxonomic names	
Taxonomic name	Genomes
Eukaryota (eukaryotes)	34,077
✓ Metazoa (animals)	12,395
<ul> <li>Arthropoda (arthropods)</li> </ul>	4,797
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✓ Haemaphysalis	4
Haemaphysalis longicornis (longhorned tick)	4



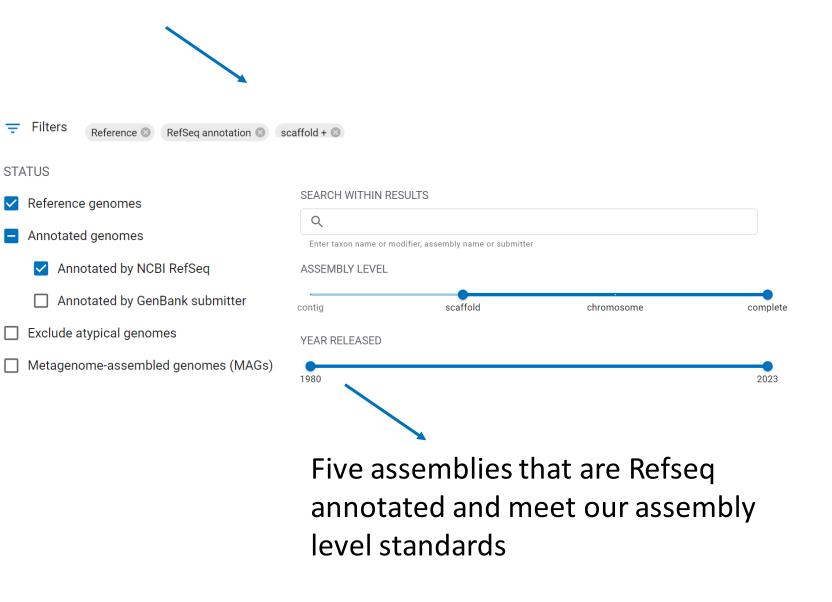
### NCBI Datasets Genome Table: Filtering

- Genome data from related species can be extremely useful to make inferences about our target species
- However, we likely can't analyze all 30 genomes in this family of ticks
- Apply filters for annotation availability and genome quality to select most informative assemblies to study furthers



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### 30 genomes assemblies for tick family Ixodidae

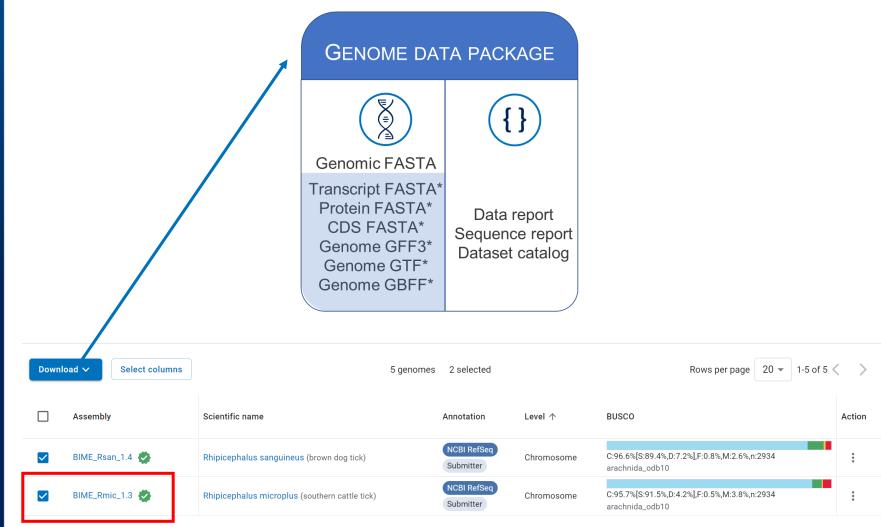


# Genome Data package

- Once you have identified relevant genome assemblies, download bulk data as efficient packages
- Get either actual sequencing and annotation data or metadata for further filtering
- Variety of industry-standard file formats for use in bioinformatics pipelines



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After filtering for genome quality and annotation availability, we select two species of related ticks to study further by downloading sequence data and associated metadata right from the Genome Table interface.

### NCBI Datasets: Genome Annotation Table

- Download gene, transcript and protein sequences, and metadata
- Tables are available for ~7500 eukaryotic annotated genomes
- Available for both RefSeq and GenBank submitted annotations
- Filter by gene type, gene name, and chromosome or location on the genome



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#### Southern Cattle tick – a better-studied relative!



### Genes

Genes annotated on *Rhipicephalus microplus* (southern cattle tick) BIME\_Rmic\_1.3 (GCF\_013339725.1)

Annotation Name: NCBI Annotation Release 100 (November 4, 2020)

₹	➡ Filters evasin ⊗					~
DOV	WNLOAD V Select columns	1 Gene		Rows per page 20	• ▼ 1-1 of 1 <	>
	Genomic location	Chromosome	Orientation	Name	Symbol	Action
	NC_051165.1:111928293-112022529	1	minus	evasin-1-like	LOC11915996	*

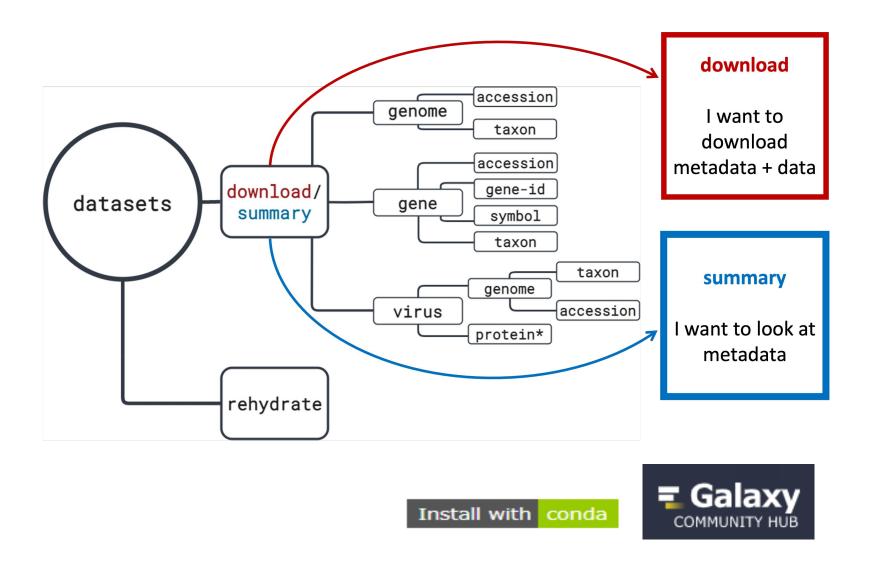
We get one result for searching the R. microplus annotation set for *evasin*, a protein in tick saliva that helps evade the host immune system. From here, download data or follow a link to the gene page to learn more!

### **NCBI** Datasets Command Line Interface

- Same information available in the web interfaces
- Look at metadata without downloading large files
- Available in bioinformatics ecosystems like Galaxy
- **NCBI** Datasets  $\bullet$ content also available via **RESTAPI**



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www.ncbi.nlm.nih.gov/datasets/docs/v2/download-and-install/

# Using the Comparative Genomics Resource we quickly...

- Found genomic related data for a focal species
- Found genomic related data for related species
- Sorted and filtered those assemblies using metadata
- Sorted and filtered annotated features (genes) using metadata

# But what if we want to improve or annotate our own genome assemblies?

- Foreign Contamination Screen
- Eukaryotic Genome Annotation Pipeline

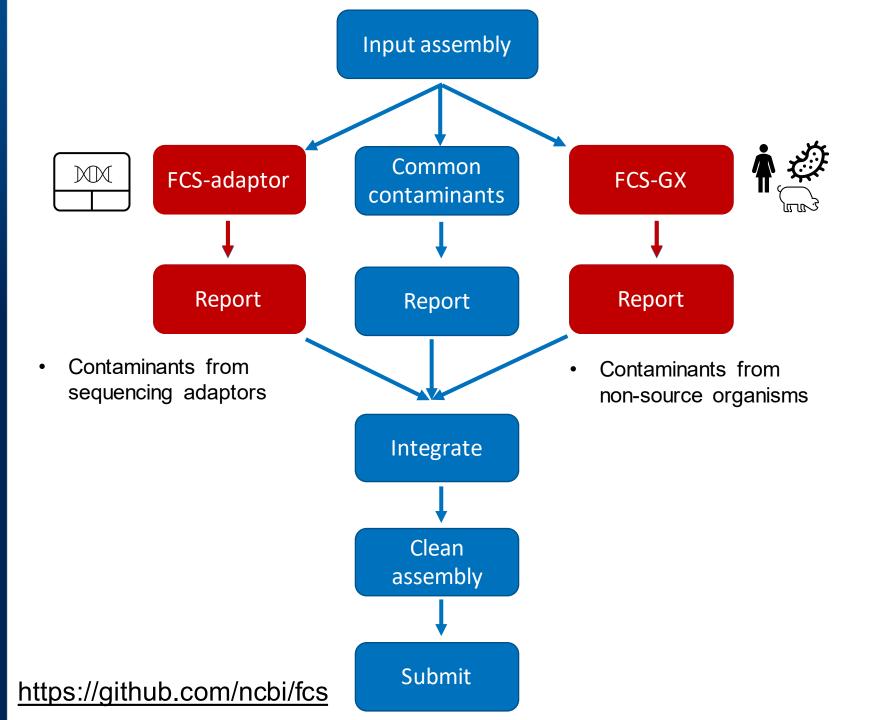
### Foreign Contaminant Screening (FCS)

- In 2022, 1 of every 3 eukaryotic genomes submitted to GenBank had detectable contamination
- Required User Inputs:
  - genome assembly
  - NCBI taxonomy identifier
- User gets:
  - contamination summary report

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- actions for cleaning genome
- cleaned genome, contaminants file



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# EGAP: Eukaryotic Genome Annotation Pipeline

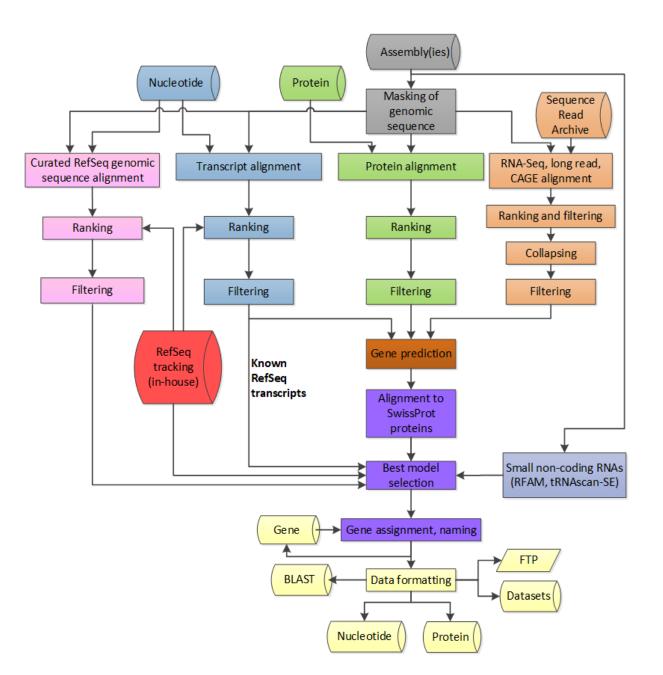
Used by NCBI to annotate >1000 species Evidence used for gene prediction:

- ✓ ESTs
- ✓ cDNAs
- Same and cross-species proteins
- ✓ RNA-Seq
- ✓ PacBio IsoSeq, ONT transcriptomes
- ✓ CAGE

#### **GIVE FEEDBACK!**

Cloud-compatible containerized EGAP for public use. Want to be an alpha tester?





# Case Study 1 Summary

Find and contribute genomic data for an invasive tick species

Find Data:

- Taxonomy Browser
- Improved Taxonomy Pages
- Datasets Genome Table
- Datasets Command Line
   Interface/API
- Datasets Annotation Table

Improve your data:

- Foreign Contamination Screen
- Eukaryotic Genome Annotation Pipeline



# Case Study 2

Making discoveries in cancers common to humans and dogs

Li-Fraumeni syndrome

Osteosarcoma Histiocytic sarcoma

### The following are some resources that can help in this research:

**TP53** 



NCBI Gene Multiple Sequence Alignment (MSA) Viewer



Comparative Genome Viewer (CGV) NCBI Orthologs



iCn3D





**Genome Data Viewer (GDV)** 

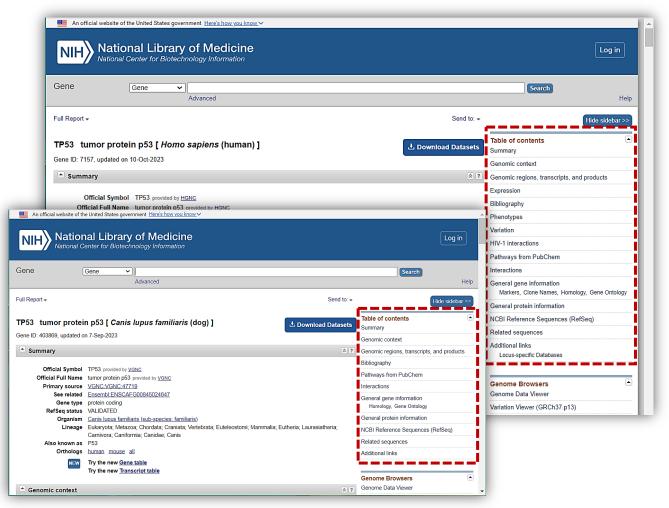


Summary records of an organism's gene-specific information, including sequences, expression data, published literature, functional domains, structures and homologs with access to more...

- Displayed information is aggregated from all relevant NCBI database records and tools
- Hyperlinks to related resources such as PubMed, GDV, NCBI Orthologs
- Data is accessible via the web, FTP, NCBI Datasets, Eutilities APIs, and the Edirect command-line tool



### www.ncbi.nlm.nih.gov/gene



The human TP53 gene record contains has much more information than displayed for the dog version. This data may help to fill in knowledge gaps for this lesser-studied gene.



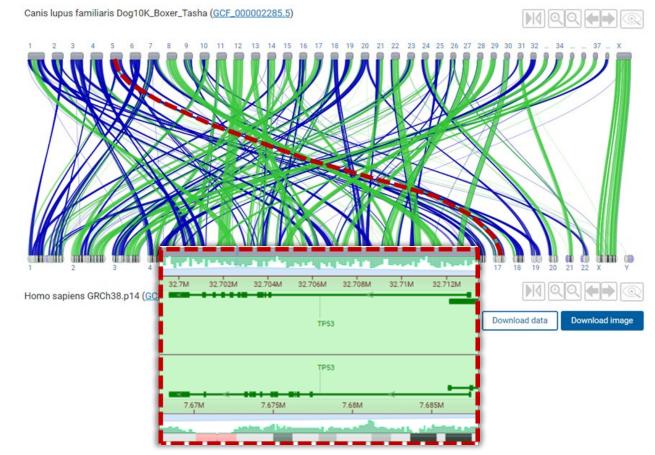
Compare two genomes and their gene annotations based on assembly-assembly alignments

- Zoom to multiple levels
- Search by gene symbol or name
- Compare gene
   annotations



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### www.ncbi.nlm.nih.gov/genome/cgv



The human and dog TP53 genomic regions appear largely similar but exhibit gene structural differences. This tool can be used as a gateway to do more detailed analysis by facilitating access to other key visualization resources.



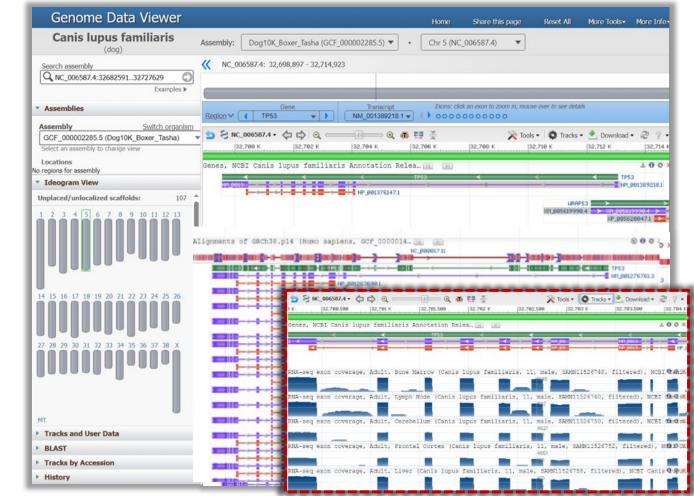
Explore and analyze genomic regions via annotations and alignments.

- The interactive display enables you to zoom in/out, search for annotations and features, customize the display and download an image or the underlying data.
- Explore available NCBI data tracks or upload Track Hub Registry tracks or your own data!
- This is a continually developing resource with new data tracks added as new data comes in.



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### www.ncbi.nlm.nih.gov/genome/gdv



The human and dog genomic alignments in the TP53 gene region enable direct comparison of differences in known transcript variants. In addition, the GDV browser enables comparisons with other track annotations, including RNA seq expression data for specific tissues.

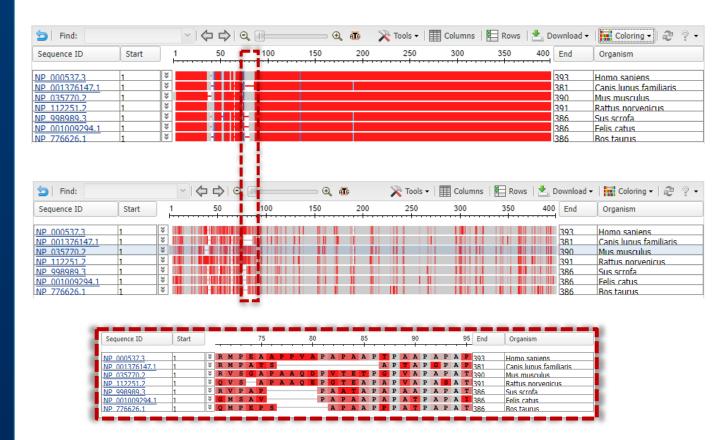
### Multiple Sequence Alignment (MSA) Viewer

Compare and examine nucleotide or protein sequence alignments.

- The interactive display enables you to zoom in/out, color residues based on several different schema, customize the display and download an image or the underlying data.
- This resource can be used to visualize your own sequence alignments or can be accessed from a BLAST search result or a Comparative Genome Viewer (CGV) alignment.
- The MSA Viewer is also available with an API call for embedding in your own webpage.



### www.ncbi.nlm.nih.gov/tools/msaviewer/



In comparison with the human TP53 protein sequence, the dog and other mammals have a region showing significant sequence diversity. Using MSA Viewer it is easy to zoom in and examine that region.



Find related genes in other organisms and perform quick sequence comparisons.

- Select transcript or protein sequences for download or alignment
- Refine your results with the taxonomy tree
- Drill down to species level information
- Access relevant PubMed citations for orthologs



#### Learn more: ncbiinsights.ncbi.nlm.nih.gov/2019/04/24/ searching-for-orthologous-genes-at-ncbi/

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Search NCBI	TP53			X Search		
esponds to diverse cellular changes in metabolism. Mut Alternative splicing of this g	uppressor protein contr stresses to regulate exp ations in this gene are a ene and the use of alter of alternate translation	pression of target genes, the ssociated with a variety of h nate promoters result in mu i nitiation codons from iden	ereby inducing cel suman cancers, ind Itiple transcript va	, and oligomerization domains. The l cycle arrest, apoptosis, senescen Luding hereditary cancers such as riants and isoforms. Additional iso riants (PMIDs: 12032546, 2093727	ce, DNA repai Li-Fraumeni s forms have al	r, or syndrome so been
🗮 0 items		421 genes for: jawed ver Add to cart	tebrates (Gnatho	stomata)		
SEARCH THE TAXONOMY T	REE					
Enter taxonomic name		6 selected		Download data	×	100
<ul> <li>jawed vertebrates</li> <li>birds</li> <li>turtles</li> <li>alligators and others</li> <li>lizards &amp; snakes</li> </ul>		Species Morno sapiens human	Gene TP53 tumor protein p53	File types RefSeq transcripts (FA	ASTA) 🖨	Nex
<ul> <li>mammals</li> <li>amphibians</li> <li>bony fishes</li> <li>cartilaginous fishes</li> </ul>		Mus musculus house mouse	Trp53 transformation related protein 53	RefSeq transcripts (F RefSeq proteins (FAS Tabular data (CSV)		
		Rattus norvegicus Norway rat	Tp53 tumor protein p53	Dow	nload	
		Danio rerio zebrafish	tp53 tumor protein p53		374 🗸	
		Canis lupus familiaris dog	TP53 tumor protein p53	:	381 🗸	
		Sus scrofa pig	TP53 tumor protein p53		386 🗸	
		Gallus gallus chicken	TP53 tumor protein p53		367 🗸	
		Bos taurus cattle	TP53 tumor protein p53	:	386 ~	
		Felis catus domestic cat	TP53 tumor protein	:	386 ~	

NCBI's genome annotation pipeline has identified TP53 orthologous sequences for over 400 organisms. In this resource, sets of selected sequences can be quickly aligned or downloaded.



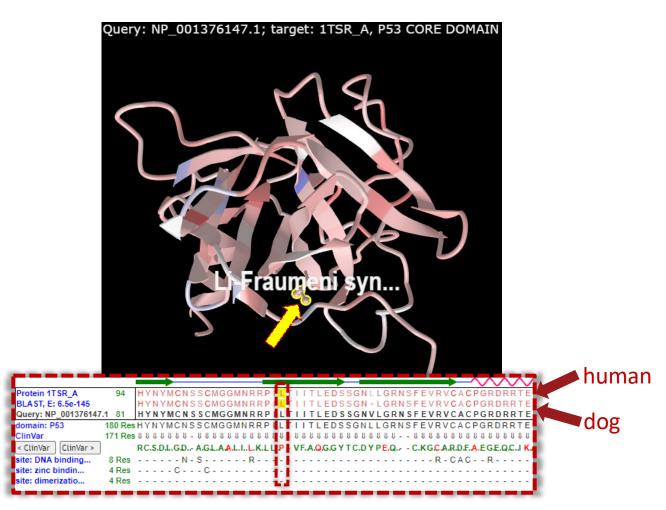
Visualize and map locations of a protein's key sequence residues to its 3D structure, along with NCBI annotations such as the positions of known clinical variants.

- Interactive display
- Customize and download image or the underlying data
- Align multiple structures
- Source code available on GitHub

### NIH

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#### www.ncbi.nlm.nih.gov/Structure/icn3d/icn3d.html



The core DNA binding domain for human TP53 has a sequence very similar to that of the dog. In mapping known ClinVar pathogenic variants to the structure, a similar genetic variant impact may also be predicted for the dog TP53.

# Case Study 2 Summary

Making discoveries in cancers common to humans and dogs

**NCBI Gene**: We learned what is known about the dog TP53 gene and what information we might infer from what is known about the well-studied human version.



**Comparative Genome Viewer (CGV)**: We were able to align and explore the synteny for the dog and human TP53 genomic regions.



**Genome Data Viewer (GDV)**: We examined the dog and human TP53 gene annotations alongside other annotation tracks including dog tissue-specific RNAseq expression data.



**Multiple Sequence Alignment (MSA) Viewer**: We were able to explore and directly compare the human, dog and other mammalian TP53 protein sequences.

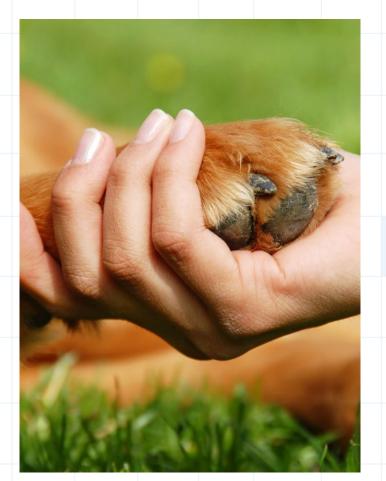


**NCBI Orthologs**: We quickly found TP53 orthologous sequences for over 400 organisms and were able to quickly download datasets for organisms we selected, such as human, dog, mouse, rat, and pig.



**iCn3D**: We were able to interactively visualize the human TP53 protein 3D structure and directly map aligned human and dog sequences and annotations such as known ClinVar pathological clinical variants.





# What's next for CGR?

- Ongoing resource improvements based on community feedback
- Making EGAPx publicly available and expanding its taxonomic scope
  - Alpha testers wanted!
- More data available in CGV



# How Do I Learn More and Get Involved?



Reach out to us at cgr@nlm.nih.gov

$\checkmark$

Sign up for our mailing list bit.ly/Subscribe CGR

#### Visit the CGR website ncbi.nlm.nih.gov/cgr

and click the yellow Feedback button on the bottom right of the page 0

Look out for future meetings, workshops, webinars, surveys, small group sessions, user testing, and interviews to inform the development process



NCBI will provide high-value data and assorted tools compatible with community-provided resources. Get CGR updates



reliable comparative genomics analyses for all eukaryotic organisms in collaboration with the genomics community. The project's vision is to maximize the biomedical impact of eukaryotic research organisms and their genomic data resources to meet emerging research needs for human health. To achieve this,



#### feedback.

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Data resources

across eukaryotic organisms

Learn how NIH is creating new opportunities for comparative genomics research and amplifying community resources.

Browse and download sequence and metadata

for genomes, genes, proteins and transcripts

Read the latest CGR news and find opportunities to engage-community input is essential to CGR's success

Explore and analyze biological sequences and

News and events



Help shape the future of comparative genomics

research. Email cgr@nlm.nih.gov to share your

thoughts, participate in a user feedback session or contribute data or tools

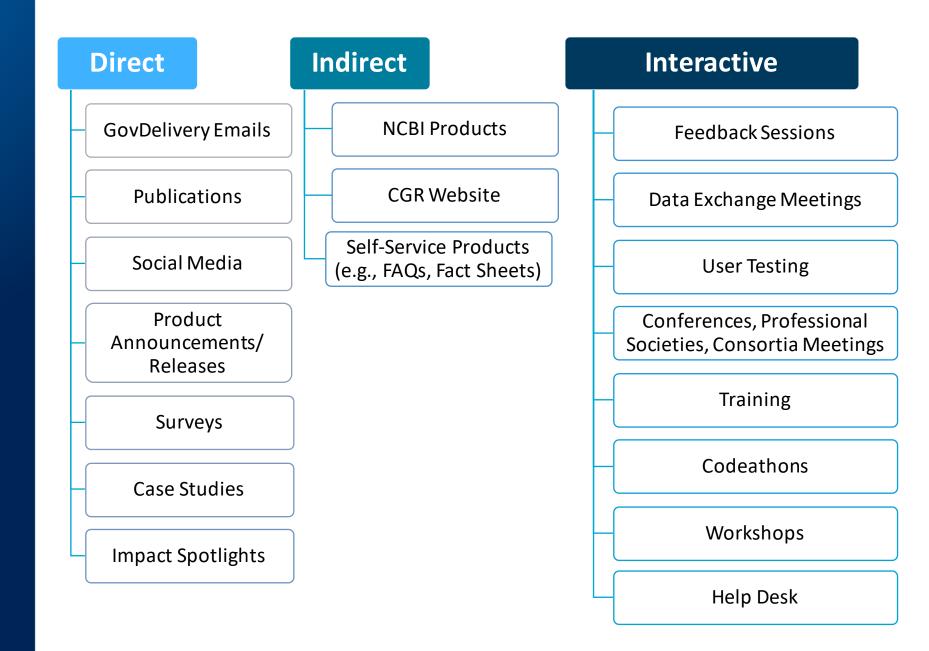
Data quality tools Improve your assembled genomes prior to

Data quality tool

submission.



# Engagement Opportunities



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# For you today:

### CGR Impact Spotlight

#### Research Summary

Publication: Redmond AK, Pettinello R, Bakke FK, Dooley H, Sharks Provide Evidence for a Highly Complex TNFSF Repertoire in the Jawed Vertebrate Ancestor, J. Immunol. 2022. Nov. 1;209(9):1713-1723. doi: 10.4049/ immunol. 2200300. Epub 2022 Sep 16. PMID: 36113883. n family evolution and orthology inference for a particular gene fam med a variety of bioinformatic analyses to survey tumor ne artilaginous fishes that occupy a critical phylogeneti rilies (TNFSFs), suggesting sharks cou vertebrates, due to retention of an and ions. Cytokines of the TNF superfamily are important ( upe function and implicated in many human diseases. Researchers of the cartilaginous fish immune system may be less primitive than predicted compared with mammalian system:

S

#### Potential CGR Impact on Research

BLAST<sup>®</sup> with ClusteredNR Database: Researchers could BLAST<sup>®</sup> the TNFSF query set against the new ClusteredNR database. This would make it easier to examine other would allow for rapid generation of a reference set of bony fish TNFSF sequences, as well as their rapid update as new es are sequenced and uploaded. NCBI cartilaginous fish sequences available at NCBI, create a create standardized and high-quality genom to gene predictions, reducing their need to

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Intended Users: Information Professionals Researchers

NIH Comparative Genomics Resource (CGR)

#### **NIH Comparative Genomics Resource**

Description: The NIH Comparative Genomics Resource (CGR) is a multiyear project intended to maximize the impact of

research on eukaryotic (non-bacterial, non-viral organisms such as animals, plants, and fungi) lifeforms and their genomic data. CGR facilitates reliable comparative genomics analyses, including the study of structure, function, evolution, and mapping of eukaryotic genomes. Researchers can compare characteristics of sequenced genomes across different species. Comparative genomics provides insight into evolution and how species change over time, how genes control biological functions, and how gene variants in a single species may contribute to disease. CGR facilitates this through community collaboration and an NCBI Toolkit of interconnected and interoperable data and tools. Its development is led by the National Center for Biotechnology Information.

#### Popular uses of this product:

Researchers
<ul> <li>Download comprehensive genomic data including gene, transcript, protein sequences, and metadata.</li> <li>Visualize and compare eukaryotic genomes assemblies and anotations.</li> <li>Use tools to improve the quality of your genome assemblies prior to GenBank submission.</li> <li>Request NCBI evaluation of your human, mouse, or rat genome assemblies for accuracy, completeness, and correctness.</li> <li>Share curated data with NCBI to expand and enhance genomic related content.</li> <li>Give feedback on the usability and usefulness of CGR (vellow feedback button).</li> </ul>
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# Outline

- Intro to Comparative Genomics
- The Value of Research Organisms
- Problem
- CGR Solution
- CGR Impact Two use cases
- What's Next





# Thank You



### **NLM-NCBI**

Steve Sherry

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Anne Ketter

Katya Sukharnikov

Wratko Hlavina

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Françoise Thibaud-Nissen

Nuala O'Leary

Sanjida Rangwala

Tom Madden

Aron Marchler-Bauer Rana Morris

Sally Chang

Patti Brennan Jodi Nurik Diane Tuncer NLM Board of Regents CGR Working Group

NLM

### **NIH Oversight**

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ncbi.nlm.nih.gov/cgr cgr@nlm.nih.gov

