

# Microbial sequence data

What's out there?

# Levels of sequence data



# Raw data

- **Amplicon**
  - PCR product, usually Sanger sequence (.ab1, .fasta)
- **Locus**
  - Multiple overlapping amplicons assembled (.fasta)
- **Genome**
  - Whole genome sequencing reads (.fastq.gz)
- **Transcriptome**
  - RNA (cDNA) sequencing reads (.fastq.gz)



# Derived data

- **Assembled genome (.fasta)**
  - Draft - multiple contigs
  - Complete - one contig per replicon
- **Annotated genome (.gbk or .gff)**
  - Genomic features labelled *e.g.* genes
- **Protein sequences**
  - Translated from predicted genes
  - Find in assembled transcripts



# Curated data

- “Curated” means
  - Assessed for quality
  - Usually some human contribution
- Orthologs
  - Protein families
- Sequence “profiles”
  - Alignments of orthologous sequences
  - DNA or Protein



# The INDSC



## International Nucleotide Sequence Database Collaboration

- The International Nucleotide Sequence Database Collaboration (INSDC) is a long-standing foundational initiative that operates between [DDBJ](#), [EMBL-EBI](#) and [NCBI](#). INSDC covers the spectrum of data raw reads, though alignments and assemblies to functional annotation, enriched with contextual information relating to samples and experimental configurations.

Data type	DDBJ	EMBL-EBI	NCBI
Next generation reads	<a href="#">Sequence Read Archive</a>	European Nucleotide Archive ( <a href="#">ENA</a> )	<a href="#">Sequence Read Archive</a>
Capillary reads	<a href="#">Trace Archive</a>		<a href="#">Trace Archive</a>
Annotated sequences	<a href="#">DDBJ</a>		<a href="#">GenBank</a>
Samples	<a href="#">BioSample</a>		<a href="#">BioSample</a>
Studies	<a href="#">BioProject</a>		<a href="#">BioProject</a>

NCBI Home

Resource List (A-Z)

- All Resources
- Chemicals & Bioassays
- Data & Software
- DNA & RNA
- Domains & Structures
- Genes & Expression
- Genetics & Medicine
- Genomes & Maps
- Homology
- Literature
- Proteins
- Sequence Analysis
- Taxonomy
- Training & Tutorials
- Variation

Welcome to NCBI

The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.

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Deposit data or manuscripts into NCBI databases



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Transfer NCBI data to your computer



Learn

Find help documents, attend a class or watch a tutorial



Develop

Use NCBI APIs and code libraries to build applications



Analyze

Identify an NCBI tool for your data analysis task



Research

Explore NCBI research and collaborative projects



Hosted at NCBI in Washington, USA





## European Nucleotide Archive

The European Nucleotide Archive (ENA) provides a comprehensive record of the world's nucleotide sequencing information, covering raw sequencing data, sequence assembly information and functional annotation. [More about ENA](#)

Access to ENA data is provided through the browser, through search tools, large scale file download and through the API.

### Text Search

Examples: [BN000065](#), [histone](#)

[Advanced search](#)

### Sequence Search

Enter or paste a nucleotide sequence or accession number

[Advanced search](#)

Hosted at EBI in  
Cambridge UK



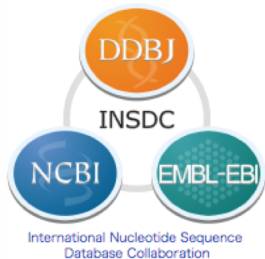
RSS



DDBJ Twitter



Mail Magazine



## DDBJ Service



Data Submission



Search / Analysis



Super Computer



ftp.ddbj.nig.ac.jp

## Hot Topics

 [News Archive](#)

[News](#) [Release](#) [PR](#) [Maintenance](#) [Operation](#) [All](#)

-  2016.12.07 [Suspension of the DDBJ activity during the New Year Holidays](#)
-  2016.11.28 [DDBJ center starts to provide ArrayExpress mirror ftp site](#)
-  2016.11.18 [Feature Table Definition revised](#)
-  2016.11.10 [UniProt 2016\\_10 released](#)
-  2016.11.09 [Updated tools related to Mass Submission System \(MSS\)](#)

Hosted at NIG in  
Mishima, Japan

# Sequence Read Archive (SRA)

# The FASTQ dumping ground



- NCBI → “SRA”  
Download “.sra” files  
Convert with “fastq-dump”
- ENA → “ENA”  
Download “.fastq.gz” files
- DDBJ → “DRA”  
???



**Sample 1**



**Sample 2**



# Study

**Sample 3**



**Sample 1**



**Sample 2**



**Experiment 2**



**Experiment 1**



**Study**

**Sample 3**



**Experiment 3**



**Sample 1**



**Experiment 1**



**Sample 2**



**Experiment 2**



**Study**

**Sample 3**



**Experiment 3**



**Sample 1**



**Experiment 1**



**Run 1**



**Sample 2**



**Experiment 2**



**Run 2**



**Sample 3**



**Experiment 3**



**Run 3**



**Study**



**Sample**



**Experiment**



**Experiment**



**Run**



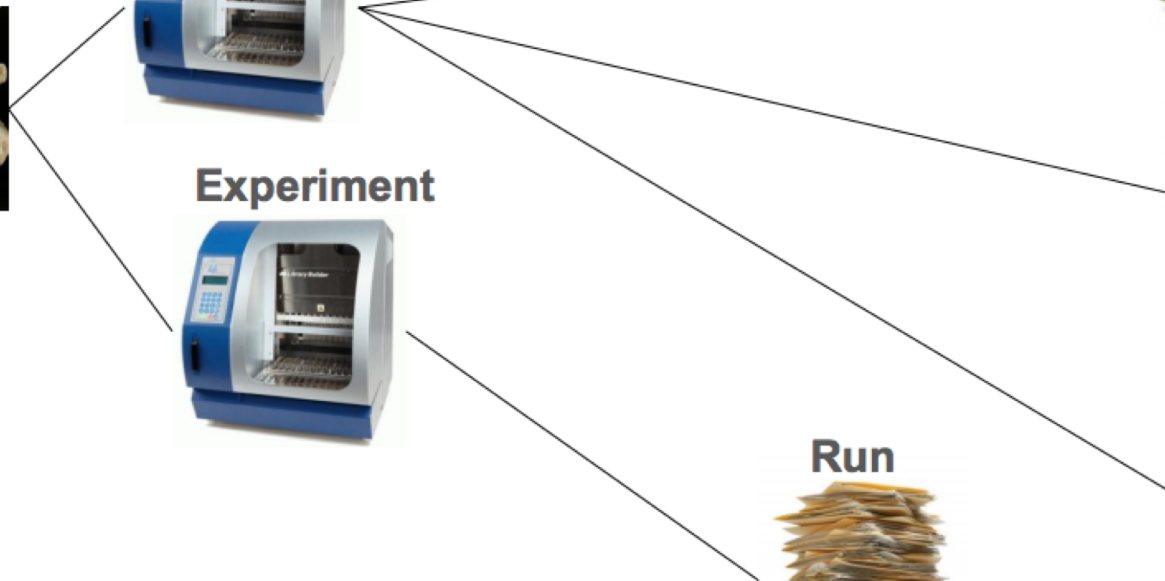
**Run**

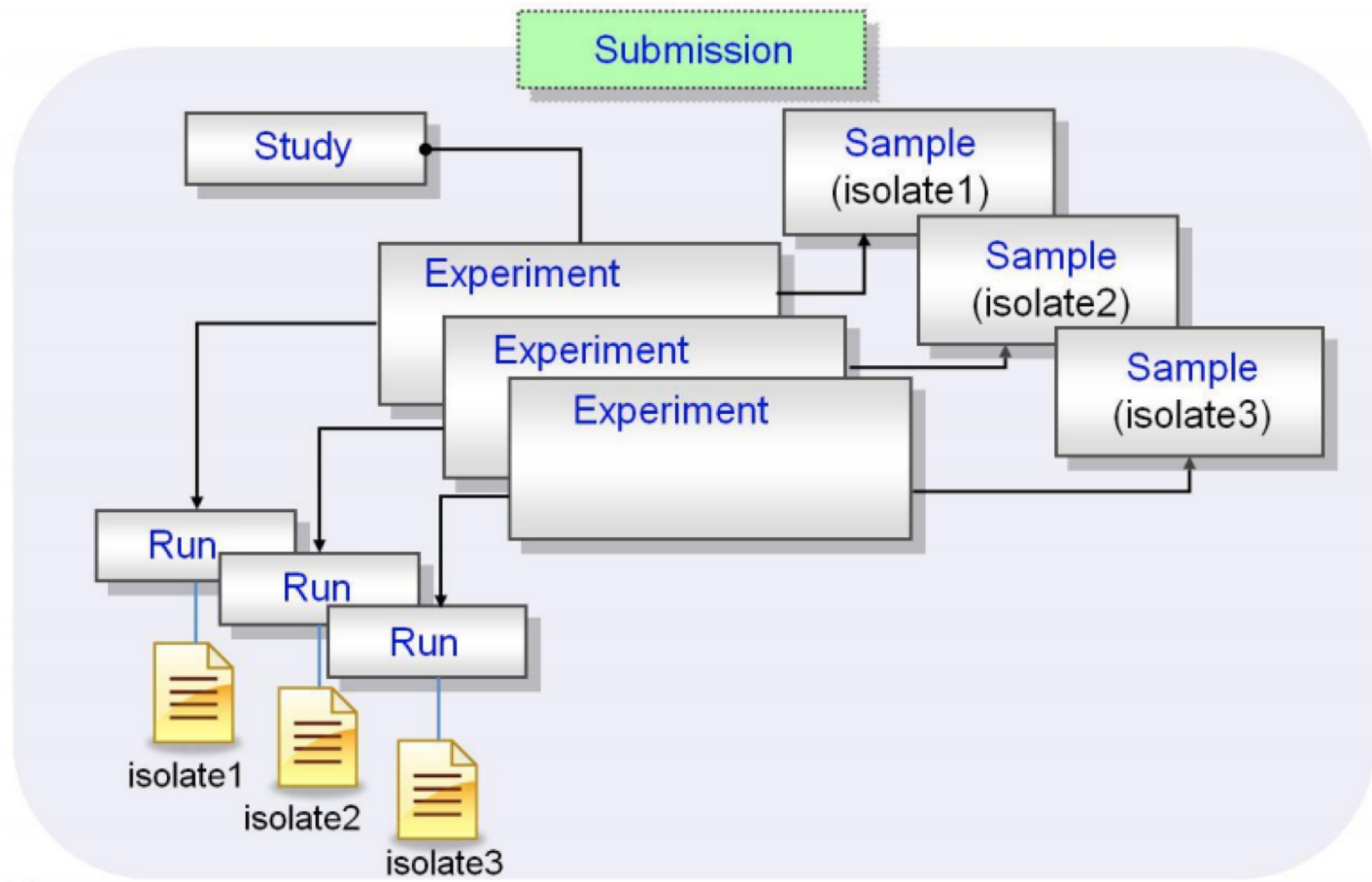


**Run**



**Run**





one

many



Sample



Experiment



Experiment



Run



Study



Experiment



# Reference genomes

# Reference genomes (ENA)

<http://www.ebi.ac.uk/genomes/bacteria.html>

When downloading use “*Sequence - Plain*”

List of available genomes (on 5-MAY-2015)

	Description	Length (bp)	Sequence		Project
			Plain	HTML	
<u>Acaryochloris marina</u>					
1	<u>Acaryochloris marina MBIC11017</u>	6,503,724	<u>CP000828</u>	<u>CP000828</u>	<u>PRJNA12997</u>
<u>Acetobacter pasteurianus</u>					
2	<u>Acetobacter pasteurianus 386B</u>	2,818,679	<u>HF677570</u>	<u>HF677570</u>	<u>PRJEB1172</u>
3	<u>Acetobacter pasteurianus IFO 3283-01</u>	2,907,495	<u>AP011121</u>	<u>AP011121</u>	<u>PRJDA31129</u>
4	<u>Acetobacter pasteurianus IFO 3283-01-42C</u>	2,815,241	<u>AP011163</u>	<u>AP011163</u>	<u>PRJDA31141</u>

# Curated databases

## Available Databases

<p><b><i>Salmonella</i></b> Strains:186080</p> <p>Assembled</p> <ul style="list-style-type: none"><li>Legacy:7229</li><li>From NGS:178851</li><li>In Progress:5</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>rMLST:177783</li><li>Achtman 7 Gene MLST:184963</li><li>cgMLST V2:177394</li><li>wgMLST:174068</li><li>CBISPP:51158</li></ul> <p><a href="#">Database Home</a> </p>	<p><b><i>Escherichia/Shigella</i></b> Strains:92429</p> <p>Assembled</p> <ul style="list-style-type: none"><li>Legacy:9611</li><li>From NGS:82618</li><li>In Progress:2</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>wgMLST:81297</li><li>Achtman 7 Gene MLST:92123</li><li>rMLST:82304</li><li>cgMLST V1:82096</li></ul> <p><a href="#">Database Home</a> </p>	<p><b><i>Clostridioides</i></b> Strains:7215</p> <p>Assembled</p> <ul style="list-style-type: none"><li>From NGS:7215</li><li>In Progress:0</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>Griffiths 7 Gene:7208</li><li>cgMLST V1:7205</li><li>rMLST:7206</li><li>wgMLST:7202</li></ul> <p><a href="#">Database Home</a> </p>	<p><b><i>Vibrio</i></b> Strains:6680</p> <p>Assembled</p> <ul style="list-style-type: none"><li>From NGS:6680</li><li>In Progress:1</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>rMLST:6676</li></ul> <p><a href="#">Database Home</a> </p>
<p><b><i>Yersinia</i></b> Strains:3598</p> <p>Assembled</p> <ul style="list-style-type: none"><li>Legacy:1165</li><li>From NGS:2433</li><li>In Progress:1</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>Achtman 7 Gene:3229</li><li>McNally 7 Gene:2795</li><li>cgMLST V1:2433</li><li>rMLST:2430</li><li>wgMLST:2432</li></ul> <p><a href="#">Database Home</a> </p>	<p><b><i>Moraxella</i></b> Strains:557</p> <p>Assembled</p> <ul style="list-style-type: none"><li>Legacy:420</li><li>From NGS:137</li><li>In Progress:0</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>Achtman 7 Gene:509</li><li>rMLST:137</li></ul> <p><a href="#">Database Home</a> </p>	<p><b><i>Helicobacter</i></b> Strains:535</p> <p>Assembled</p> <ul style="list-style-type: none"><li>From NGS:535</li><li>In Progress:0</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>rMLST:531</li></ul> <p><a href="#">Database Home</a> </p>	<p><b><i>Dev. Sandbox</i></b> Strains:128</p> <p>Assembled</p> <ul style="list-style-type: none"><li>From NGS:128</li><li>In Progress:3</li></ul> <p>Schemes</p> <ul style="list-style-type: none"><li>Achtman 7 Gene:67</li><li>rMLST:64</li></ul> <p><a href="#">Database Home</a> </p>



## Search

Search our comprehensive database for:

- ▲ Genomes
- ▲ Genes & proteins
- ▲ Immune epitopes
- ▲ 3D protein structures
- ▲ Host Factor Data
- ▲ Antiviral Drugs

[Browse All Search Types](#)

## Analyze

Analyze data online:

- ▲ Sequence Alignment
- ▲ Phylogenetic Tree
- ▲ Sequence Variation (SNP)
- ▲ Metadata-driven Comparative Analysis
- ▲ BLAST

[Browse All Tools](#)

## Save to Workbench















Sign up for a workbench to:

- ▲ Store and share data
- ▲ Combine working sets
- ▲ Integrate your data with ViPR data
- ▲ Store and share analyses
- ▲ Custom search alert

[Sign In](#)

### ▲ Virus Families

Click on icon of family or species of interest. Click [here](#) to view all families and species in list format. Don't know family of species?

Single-Stranded Positive-Sense RNA	Single-Stranded Negative-Sense RNA	Double-Stranded RNA	Double-Stranded DNA
 <b>Caliciviridae</b>  <b>Hepeviridae</b>	 <b>Arenaviridae</b>  <b>Paramyxoviridae</b>	 <b>Reoviridae</b>	 <b>Herpesviridae</b>
 <b>Coronaviridae</b>  <b>Picornaviridae</b>	 <b>Bunyaviridae</b>  <b>Rhabdoviridae</b>		 <b>Poxviridae</b>
 <b>Flaviviridae</b>  <b>Togaviridae</b>	 <b>Filoviridae</b>		



## Data Summary

## News and Tweets

- 13 October 2016 PlasmoDB 29 Released
- 30 March 2016 PlasmoDB 28 Released
- 19 February 2016 PlasmoDB 27 Released

[All PlasmoDB News >>>](#)

## Tweets by @eupathdb



**EuPathDB**  
@eupathdb

Our paper is out in @NAR\_Open #EuPathDB: the eukaryotic pathogen genomics database resource nar.oxfordjournals.org/content/early/

## Community Resources

expand for 50 new items

## Education and Tutorials

expand for 8 new items

## About PlasmoDB

## Search for Genes

[expand all](#) | [collapse all](#)



 

- ▶ Text
- ▶ Gene models
- ▶ Annotation, curation and identifiers
- ▶ Genomic Location
- ▶ Taxonomy
- ▶ Orthology and synteny
- ▶ Phenotype
- ▶ Genetic variation
- ▶ Epigenomics
- ▶ Transcriptomics
- ▶ Sequence analysis
- ▶ Structure analysis
- ▶ Protein properties
- ▶ Protein targeting and localization
- ▶ Function prediction
- ▶ Pathways and interactions
- ▶ Proteomics
- ▶ Immunology

[expand all](#) | [collapse all](#)

## Search for Other Data Types

[expand all](#) | [collapse all](#)

- ▶ Popset Isolate Sequences
- ▶ Genomic Sequences
- ▶ Genomic Segments
- ▶ SNPs
- ▶ SNPs (from Array)
- ▶ ESTs
- ▶ ORFs
- ▶ Metabolic Pathways
- ▶ Compounds

[expand all](#) | [collapse all](#)

## Tools

### BLAST

Identify Sequence Similarities

### Results Analysis

Analyze Your Strategy Results

### Sequence Retrieval

Retrieve Specific Sequences using IDs and coordinates

### Companion

Annotate your sequence and determine orthology, phylogeny & synteny

### EuPaGDT

Eukaryotic Pathogen CRISPR guide RNA/DNA Design Tool

### PubMed and Entrez

View the Latest Pubmed and Entrez Results

### Genome Browser

View Sequences and Features in the genome browser

*For additional tools, use the **Tools** menu in the gray toolbar above.....*

# Conclusions

# Conclusions

- **Be skeptical!**
  - ENA + Genbank accept anything
  - Garbage in, Garbage out
- **Curated data**
  - Refseq vs GenBank
  - Specialised sites (better QC)
  - Be wary of draft assemblies
  - Go back to primary reads





**I'D TURN BACK  
IF I WERE YOU!**

## Further reading

[https://www.ncbi.nlm.nih.gov/core/assets/sra/files/Factsheet\\_SRA.pdf](https://www.ncbi.nlm.nih.gov/core/assets/sra/files/Factsheet_SRA.pdf)

<https://www.ddbj.nig.ac.jp/dra/index-e.html>

<https://p.ddbj.nig.ac.jp/pipeline/Login.do>