



Microbial genome

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Louis Pasteur (1822-1885)

Phylogenic tree of life



 Through variation in rDNA sequences we can distinguish organisms to the species level and trace evolutionary relationships

Three separate
"Domains" of life;
Eukaryotes, Bacteria, and
Archaea

 It is now possible to sequence and analyze ribosomal DNAs without culturing the microbes

Microbial genetics

- Microbes are among our planet's most ubiquitous organisms. They are present in every biosphere, including some of the most extreme locations on Earth.
- Microbes, in general, possess genomes much smaller in size compared to plants and animals, which makes them ideal for genetic and physiological studies.
- Microbial genomics is largely the identification and characterization of their genetic compositions.

Classification

| Characteristic | Viruses | Bacteria | Fungi | Protozoa and Helminths |
|----------------------------------|--|--|---------------------------------|---------------------------|
| Cells | No | Yes | Yes | Yes |
| Approximate diameter $(\mu m)^1$ | 0.02-0.2 | 1–5 | 3–10 (yeasts) | 15-25 (trophozoites) |
| Nucleic acid | Either DNA or RNA | Both DNA and RNA | Both DNA and RNA | Both DNA and RNA |
| Type of nucleus | None | Prokaryotic | Eukaryotic | Eukaryotic |
| Ribosomes | Absent | 70S | 80S | 80S |
| Mitochondria | Absent | Absent | Present | Present |
| Nature of outer surface | Protein capsid and lipoprotein envelope | Rigid wall containing peptidoglycan | Rigid wall containing chitin | Flexible membrane |
| Motility | None | Some | None | Most |
| Method of replication | Not binary fission | Binary fission | Budding or mitosis ² | Mitosis ³ |

Virus

Spike protein

Envelope

Lipid envelope

Bacterial cell



 $^1 \text{For comparison, a human red blood cell has a diameter of 7 <math display="inline">\mu\text{m}.$

²Yeasts divide by budding, whereas molds divide by mitosis.

³Helminth cells divide by mitosis, but the organism reproduces itself by complex, sexual life cycles.

Genomic data-Viruses



| An offic | cial website of the United States government Here's how you know. | | | |
|---|---|------------|---|--------|
| NIH | National Library of Medicine National Center for Biotechnology Information | | | Log in |
| Nucleotid | e Nucleotide Advanced | | Search | Help |
| GenBank 🗸 | | Send to: 🕶 | Change region shown | • |
| Pseudomonas putida strain C54, complete sequence GenBank: MZ361366.1 FASTA Graphics | | | Customize view | |
| <u>Go to:</u> ⊘ | | | Analyze this sequence Run BLAST | |
| LOCUS DEFINITION | MZ361366 22782 bp DNA linear BCT 06-SEP-2021 Pseudomonas putida strain C54, complete sequence. | | Pick Primers Highlight Sequence Features | |
| VERSION KEYWORDS | MZ361366.1 | | Find in this Sequence | |
| SOURCE ORGANISM | Pseudomonas putida <u>Pseudomonas putida</u> Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae: Pseudomonas | | Related information | |
| REFERENCE AUTHORS | ERENCE 1 (bases 1 to 22782) JTHORS Rajabal,V., Taner,F., Sanlidag,T., Suer,K., Guler,E., Sayan,M. and Petrovski,S. ITLE Genetic characterisation of antibiotic resistance transposons Tn6608 and Tn6609 isolated from clinical Pseudomonas strains in Cyprus | | Protein | |
| TITLE | | | Taxonomy | |
| | | | PubMed (Weighted) | |
| JOURNAL PUBMED | J Glob Antimicrob Resist (2021) In press 34363995 | | | |



Microbial Gene Expression

•Gene expression analysis - the study of the way genes are transcribed to synthesize functional gene products

•Provides insights into normal cellular processes

 Transcription- the process of creating a complementary RNA copy of a DNA sequence, can be regulated in a variety of ways

•DNA microarrays — an array of oligonucleotide probes bound to a chip surface enables gene expression profiling of many genes in response to a condition.

•REAL TIME PCR— steady-state levels of mRNA are quantitated by reverse transcription of the RNA to cDNA followed by quantitative PCR (qPCR) on the cDNA

Identification of an Open Reading Frame



Structure of a gene and primers



Operons and Transcriptional Regulation in Bacteria



mRNA

mRNA

What if we want to study gene function?

Approach to take:

- Transposon Mutagenesis
- Assess if there is phenotypic change
- Locate transposon Tn insertion in genome, using primers specific to Tn
- Clone Tn-gDNA junction fragment and sequence Growth a
- Identify gene similarity in database (NCBI)

Growth at permissive T + kanamycin e Growth at restrictive T + kanamycin

Cloning of entire gene



Blue/White screening-

Detection of recombinant bacteria in vector-based molecular cloning experiments



Restriction enzymes



Cloning of gene into expression vector for protein purification

