Introducing Microbiome Bioinformatics

Part 11.

Introducing sequence databases.

Recap: Aims

- **Microbiome analysis**
  - with particular regard to **sequence informatics concepts**
- "Top down" – putting analysis tools and resources in context
- No highly detailed technicalities (yet)
  - No instructions on how to run particular programs
- Why you are using the bioinformatics approaches you use; pros, cons; alternatives

Series of talks

- 10 so far
- Open ended… as long there is demand
- Expected to be every 2 weeks
  - Notwithstanding some larger gaps for various reasons…
  - all dates will be confirmed in advance
  - *Please refer to:* ***Bite-size bioinformatics mailing list***
    - *Contact* ***Mark Fernandes,*** *or me*
- Informal and flexible
  - Please interrupt and ask questions
  - **Suggestions for topics for further focus**
- Previous talks will be repeated, starting this Autumn

Topics, top-down

Introduction

Types of environments studied

Variety of microbiomes, and domains of Life

Aims of studies:

"Communities"

Functions

Aims of DNA/ RNA sequencing approaches

"barcoding"- use of marker genes

"shotgun" metagenomics

metatranscriptomics

16S rRNA

Other markers

aims of whole-metagenome sequencing

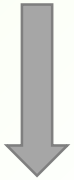
Dealing with the data: Informatics concepts

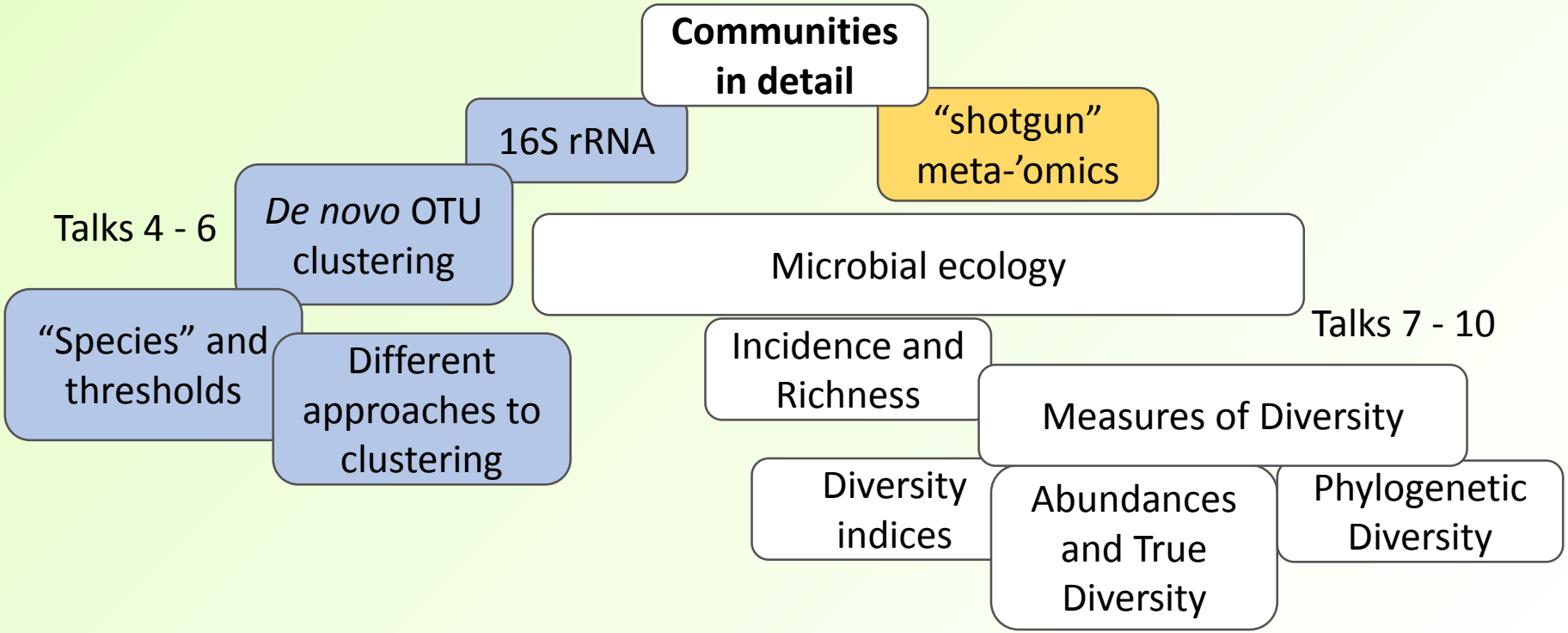
16S rRNA

"shotgun" meta-'omics

Communities in detail

Later: more technical details



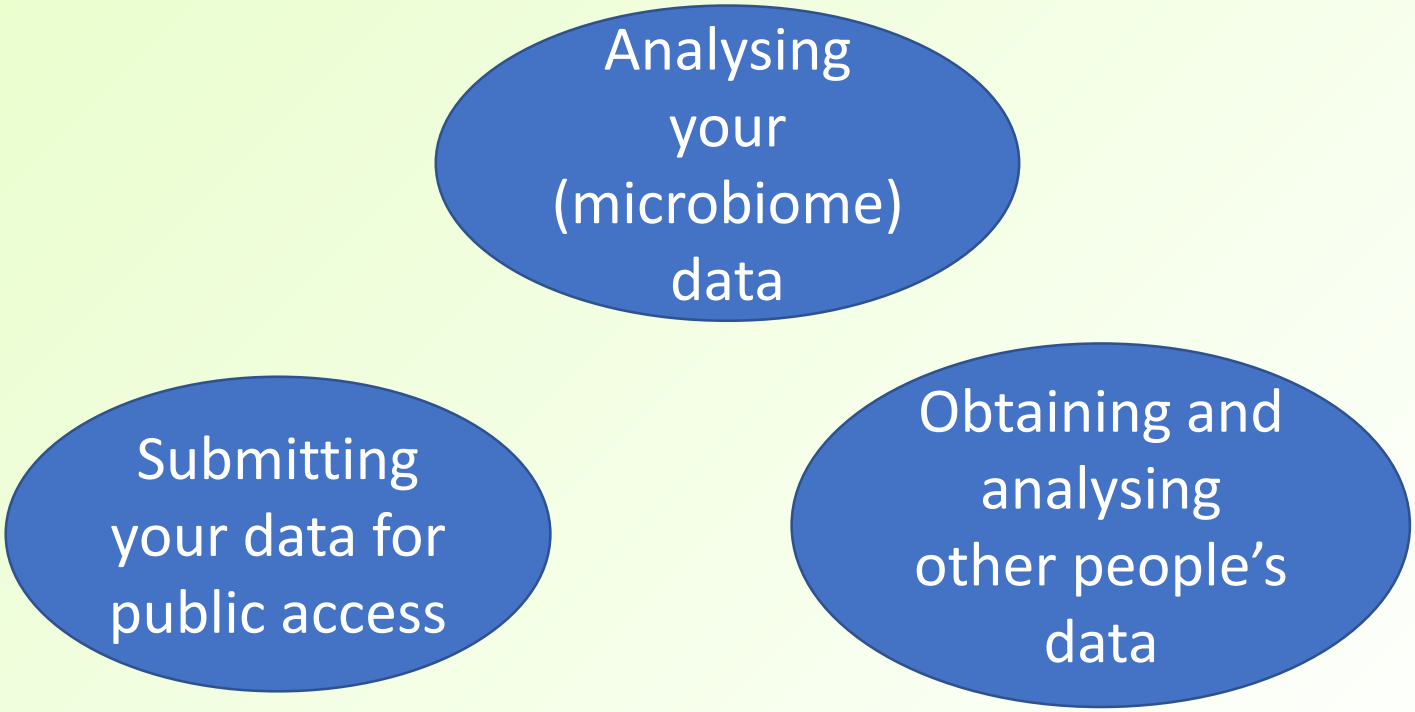

Communities in detail
16S rRNA
"shotgun" meta-'omics
Talks 4 - 6
De novo OTU clustering
Microbial ecology
"Species" and thresholds
Different approaches to clustering
Talks 7 - 10
Incidence and Richness
Measures of Diversity
Diversity indices
Abundances and True Diversity
Phylogenetic Diversity

Series of talks

Slideshows - http://ghfs1.quadram.ac.uk/ghfs/

- Part 1: 27/1/2017
  - "Biological and Experimental Stuff that a microbiome bioinformatician needs to know"
  - Overview of marker gene sequencing for community analysis
- Part 2: 10/2/2017
  - Overview of whole-metagenome sequencing
- Part 3: 24/2/2017
  - Focus on metatranscriptomics
- Part 4: 10/3/2017
  - Different bioinformatics approaches to processing 16S read data
- Part 5: 24/3/2017
  - *De novo* OTU clustering: sequence identities and how thresholds have been determined historically; relationships to taxonomic levels
- Part 6: 7/4/2017
  - The clustering problem: different approaches, and what can go wrong; the influence of amplification artefacts, sequencing errors and sequence lengths; computational OTUs versus species
- Part 7: 21/4/2017
  - Introducing microbial ecology: using observed abundances of OTUs (or species, or functions) to estimate the richness of the community (number of different OTUs, species etc)
- Part 8: 2/6/2017 – continuing microbial ecology: community diversity : diversity indices
- Part 9: 16/6/2017 – continuing microbial ecology: community diversity : true diversity
- Part 10: 28/7/2017 – concluding diversity (for now);
- Part 11: today – Introducing sequence databases

Sequence databases

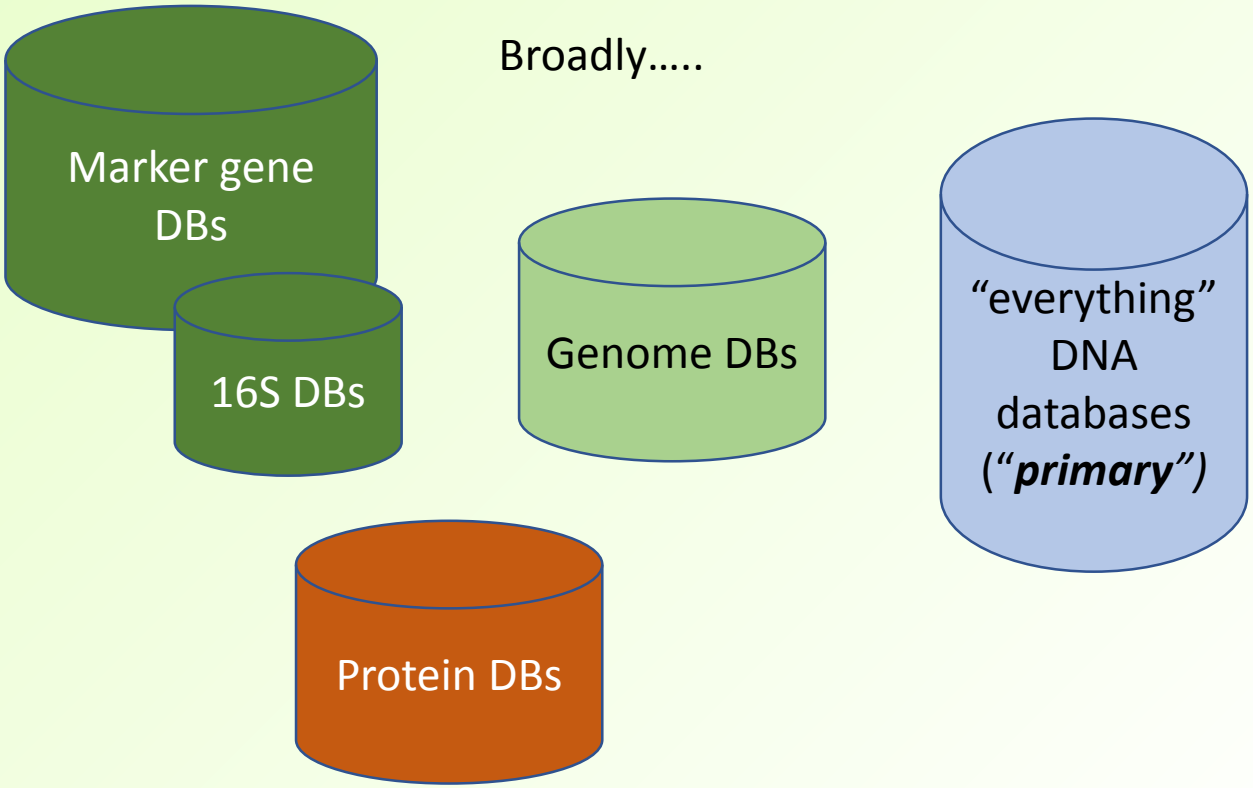


Sequence databases and microbiome analysis

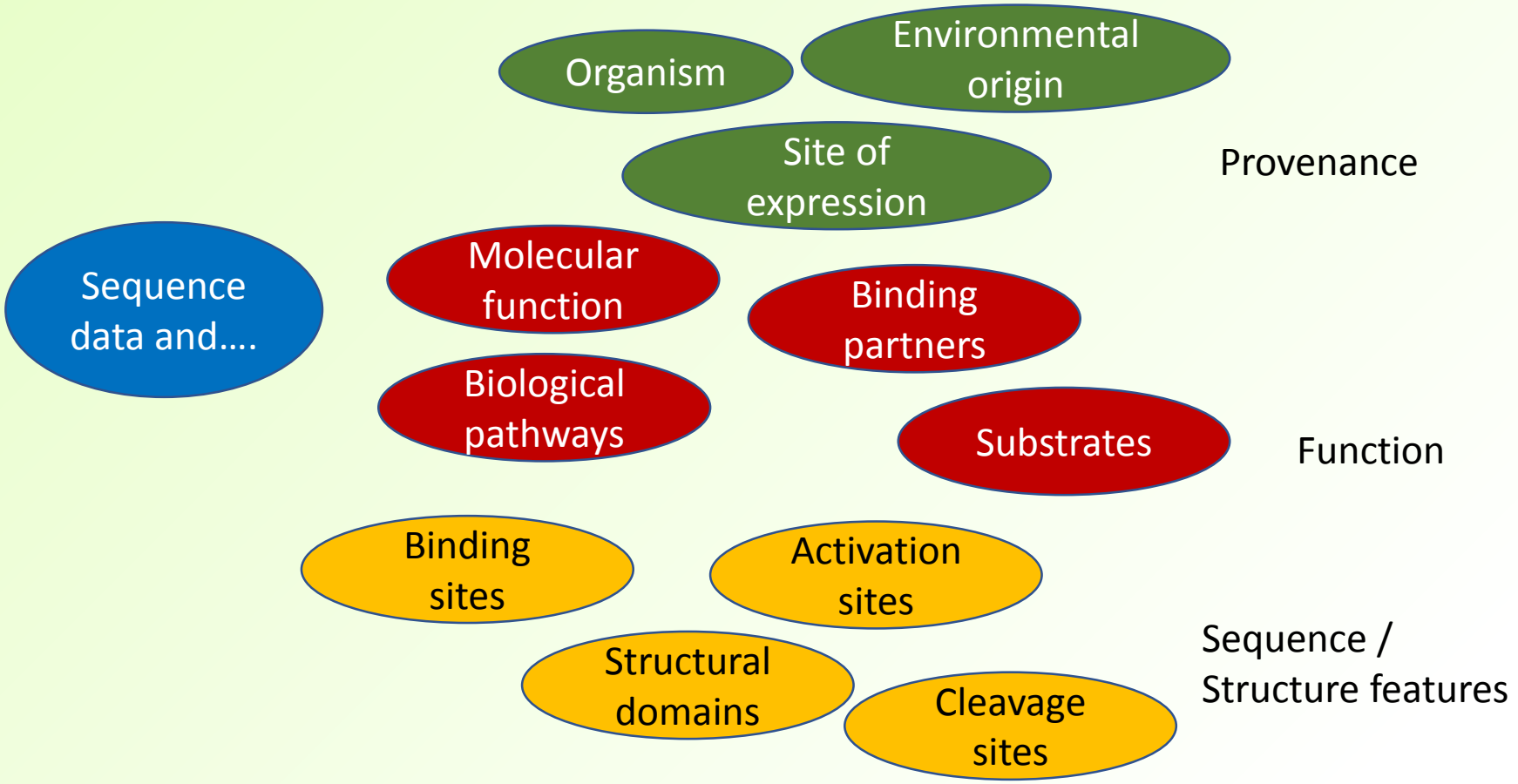
- Today – overview of potentially useful databases
  - Nucleic acid (nucleotide) sequence databases
  - Protein / peptide sequence databases
- Next time – principles of how these can be used to analyse 16S and whole-metagenome shotgun data

  (….part I)
  - In principle, you can usefully use any sequence database to find similar sequences to your sequence data
  - This direct approach is used in some methods of microbiome data analysis
  - But has pros/cons, and so various other methods can be used.There are also “indirect”methods. More next time.

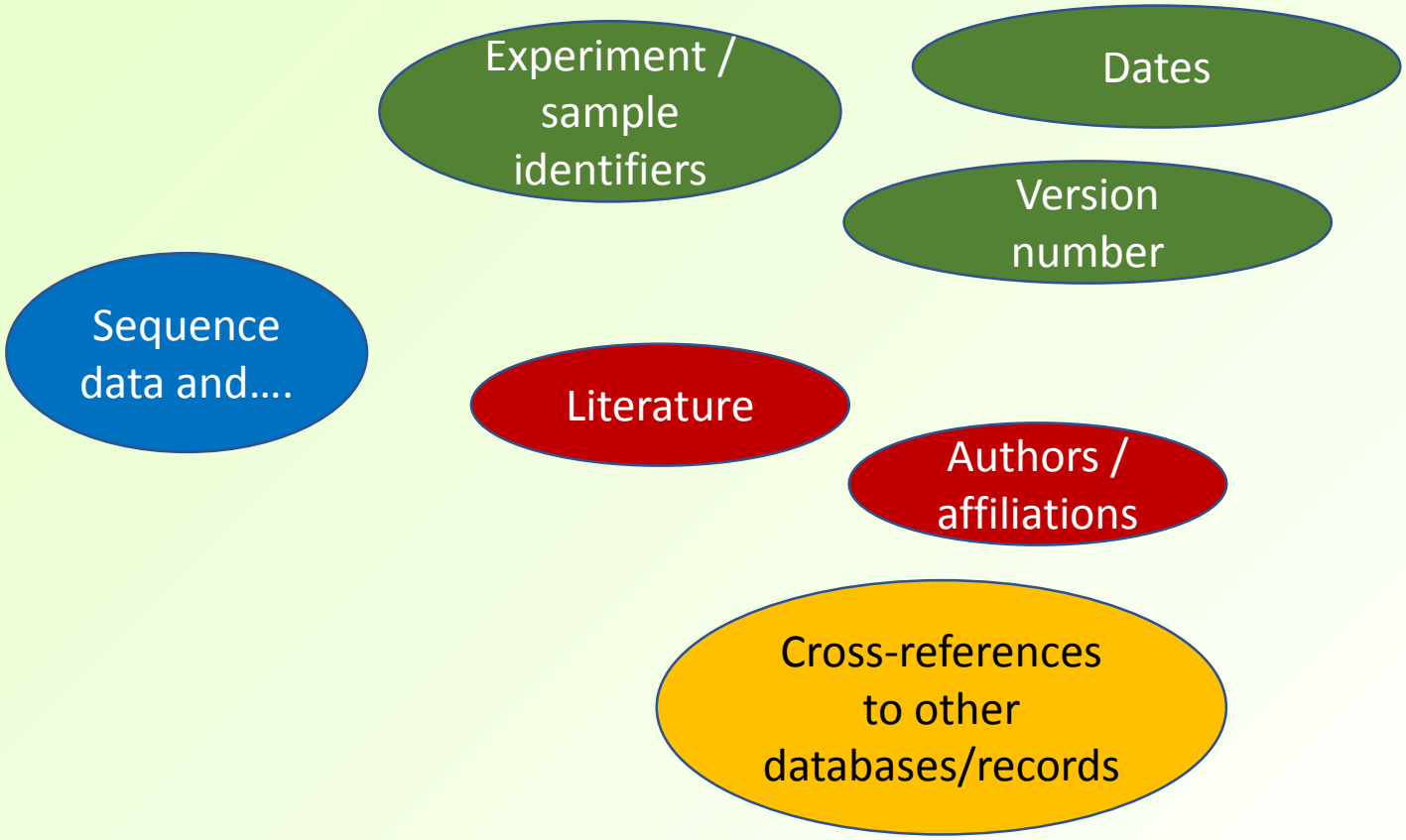
Sequence databases and microbiome analysis



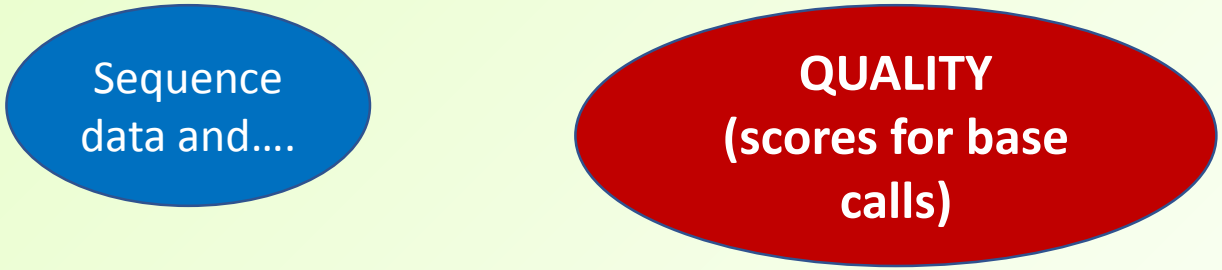
Annotations



“Metadata”


Experiment / sample identifiers
Dates
Version number
Sequence data and….
Literature
Authors / affiliations
Cross-references to other databases/records

Sequence **reads**



FASTQ format

General Database Concepts

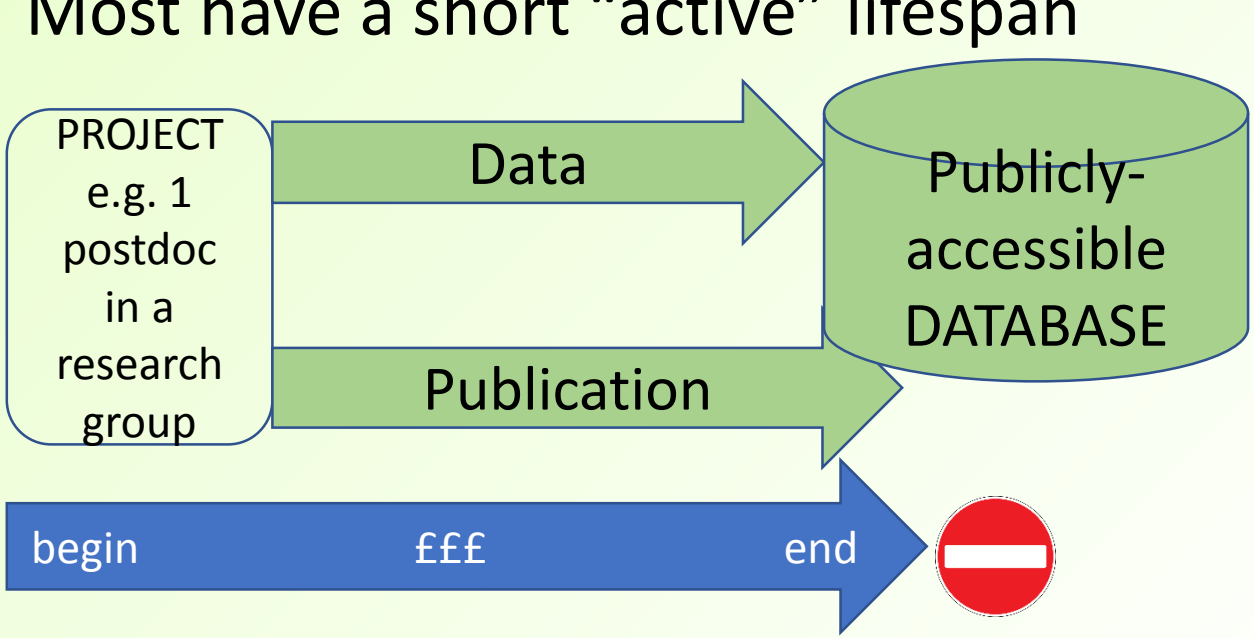
- Characteristics of any formalized database:
  - a collection of **records** (database '**entries**')
  - each record is uniquely identified by a **key** (a unique name/number)
    - in many databases, key is referred to as the "**Accession (number)**"
    - E.g. **'Q8X696**' (an example from the UniProt database)
    - a.k.a. "primary ID", "primary key"
  - a key is a special type (i.e. it is unique within the database) of **field**
  - other information about the record is held in other fields, e.g.
    - sequence
    - date
    - literature reference
    - functional annotation
    - etc

General Database Concepts

- Characteristics of any formalized database:
  - A given record may change over time:
    - be **updated** (same key, same record, different content in record)
    - be **retired** (deleted from the database)
  - Some records may be **merged into one** record (when the curators realize that two records represent the same thing)
  - Some databases will include **dates of records** (e.g. date of creation; most recent update; all updates)
  - Some databases make older versions of records available in an archive (including those now deleted)
- Some very large databases (e.g. ENA) consist of **regular full releases** (on a quarterly basis); the **Release-proper** is supplemented by an **Update** section, which changes daily and contains changes relative to the most recent Release.

Bioinformatics databases

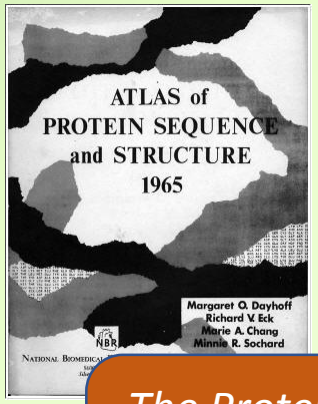
- Many thousands of them
- Many have a fairly short lifespan
- Most have a short “active” lifespan



NO LONGER MAINTAINED

- **Data not updated**
- **Problems not fixed**
- **“Zombie DB”**
- **or goes down forever**

Evolution of **Primary Protein Sequence Databases**



The Protein Sequence Database

Early 1960s
Dayhoff et al.
NBRF,
Washington D.C.

PIR, NBRF

MIPS collection, Munich

1990s

JIPID, Tokyo

The Protein Identification /Information Resource Protein Seq DB (PIR-PSD)

NBRF, 1984

Swiss-Prot

Bairoch et al.,
Univ. Geneva
1986

Swiss-Prot

TrEMBL

Bairoch, Apweiler
SIB Switzerland,
EBI, Hinxton

UNIPROT

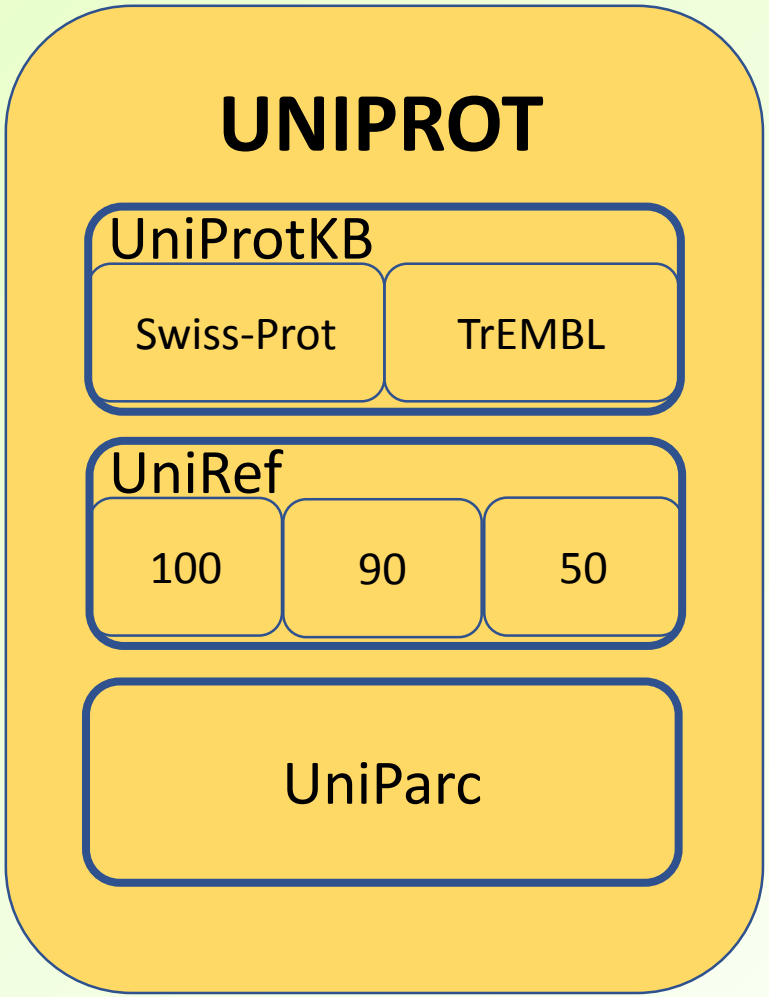
UniProtKB

UniRef

UniParc

2002

EBI / SIB / PIR collaboration



- Compartmentalised: different sections useful for different purposes
- Redundant and non-redundant sections
- Cross-references to other databases

Primary Sequence Databases

- Basically, role is to store all publicly-available DNA/RNA/protein sequences of all known life...
- "**Primary**" because they are the **principal** and **largest** databases; there aren't many of them
  - not the original sources of the data
- Include sequence data from many types of sources
- From many projects, large and small

Primary Sequence Databases

- Nucleic acid sequences:
- **ENA** (EMBL, Europe), **Genbank** (NCBI, USA), **DDBJ** (Japan)
  - Collaborate/duplicate (**INSDC**) – see http://www.insdc.org
- Protein sequences:
  - **UniProt**
    - Consortium: EMBL-EBI, SIB (Europe); PIR (USA)
  - **NCBI protein database** ('GenPept')
  - DDBJ Amino Acid Sequence DB (**DAD**)

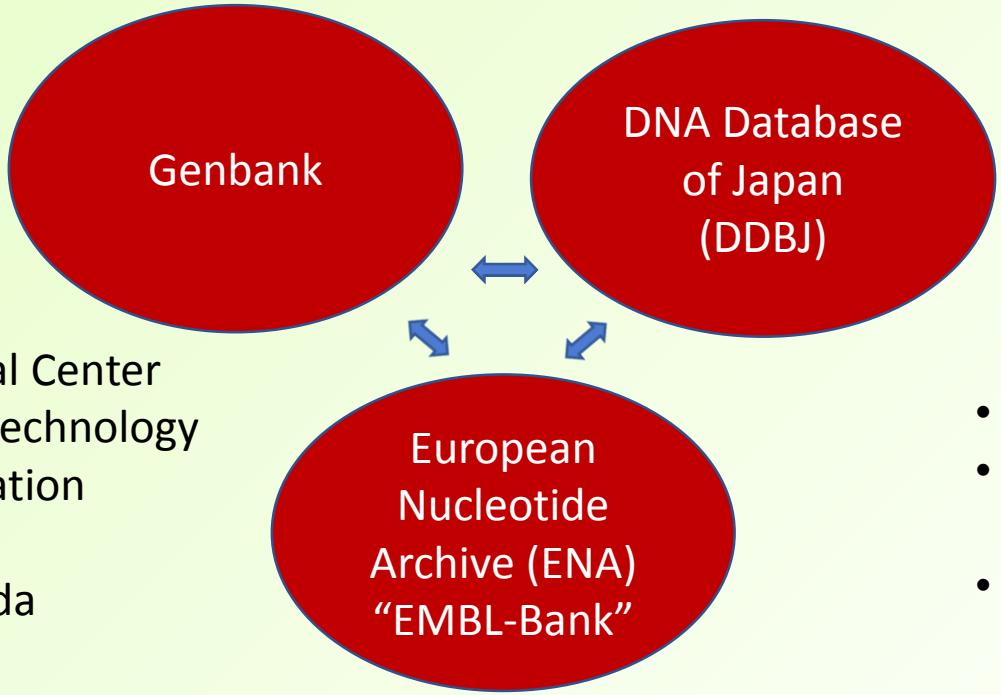
Primary Sequence Databases

- Include sequence data from many types of sources:
  - genome sequencing/resequencing
  - transcriptome sequencing
  - environmental samples (meta-'omes)
  - traditional low-throughput studies

Types of sequence in the DBs

- Proteins/peptides
- Whole chromosomes/pseudomolecules
- Plasmids
- Single genomic reads
- cDNA, ESTs
- various other types of sequence “tags”
- Constructs e.g. BACs, YACs etc
- Gene coding-region sequences
- Complete gene sequences
- Raw read data from all kinds of experiments
- genome-sequencing projects; marker-gene amplicons; metagenomes
- ...etc
- The primary databases consist of various DATA CLASSES
  - E.g. “**Standard**” sequences, i.e. assembled, annotated
  - See http://www.ebi.ac.uk/ena/about/formats

International Nucleotide Sequence Database Collaboration (INSDC)



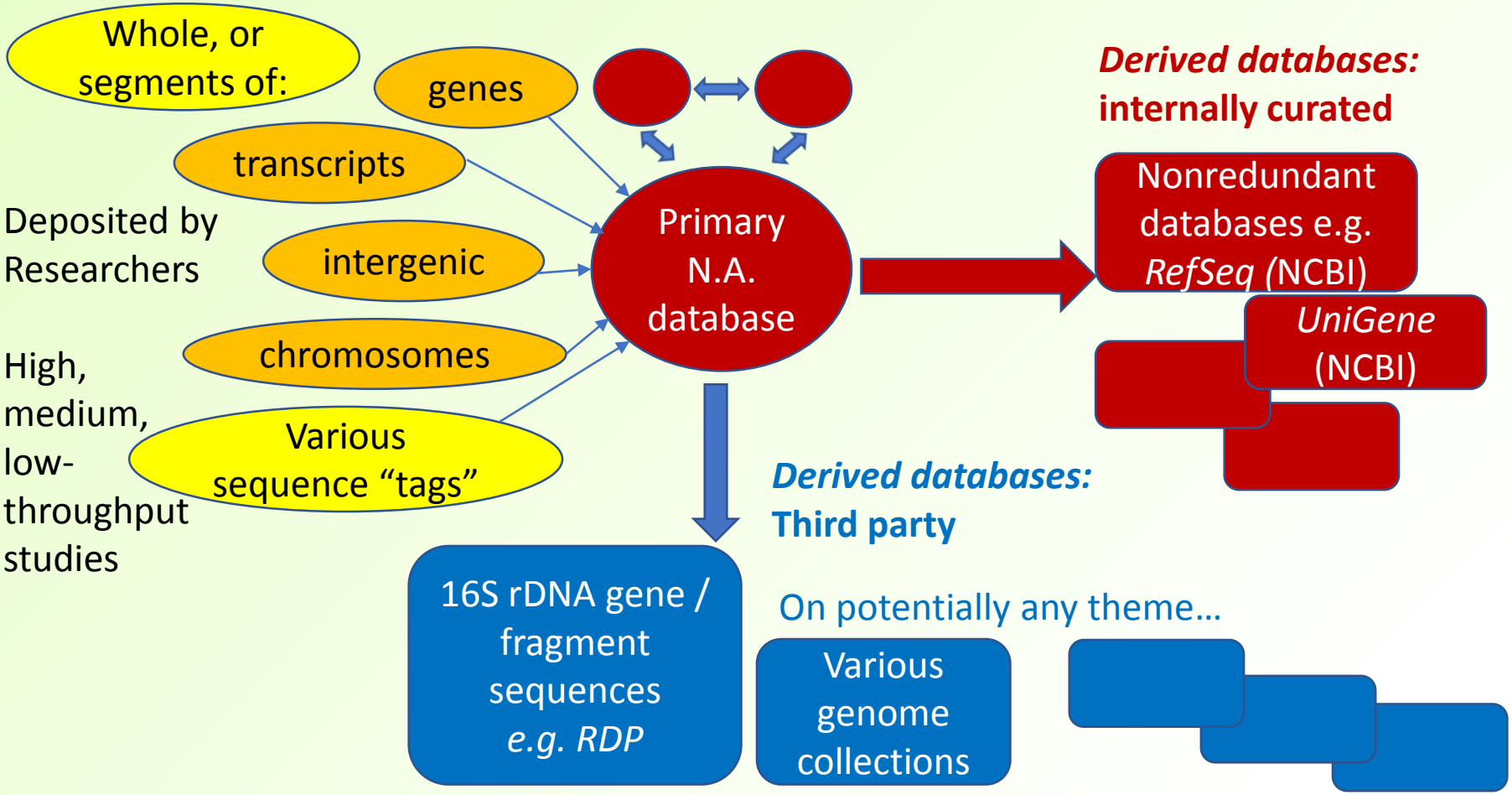
DDBJ Center, Natl. Inst. Genetics, Mishima

National Center for Biotechnology Information (NCBI), Bethesda

EMBL-European Bioinformatics Institute (EBI), Hinxton

- Same data
- synchronized daily
- Managed and presented in slightly different ways
- www.insdc.org

The ins and outs of primary sequence databases



What else is available from the primary database providers?

- Besides the annotated sequence records:
  - Sequence read data
    - **Sequence Read Archive (SRA)**: quality scores, in the form of FASTQ data
    - Trace Archive: equivalent for first-generation (Sanger) reads
  - Data describing **projects, experiments, samples**
    - BioProjects, BioSamples
- All the above are available at NCBI, DDBJ, EMBL-EBI
  - Again, presentation/modes of access at each site differ
  - Collectively, constitute ENA at EBI
  - (Organisation slightly different at NCBI, DDBJ)

Redundancy

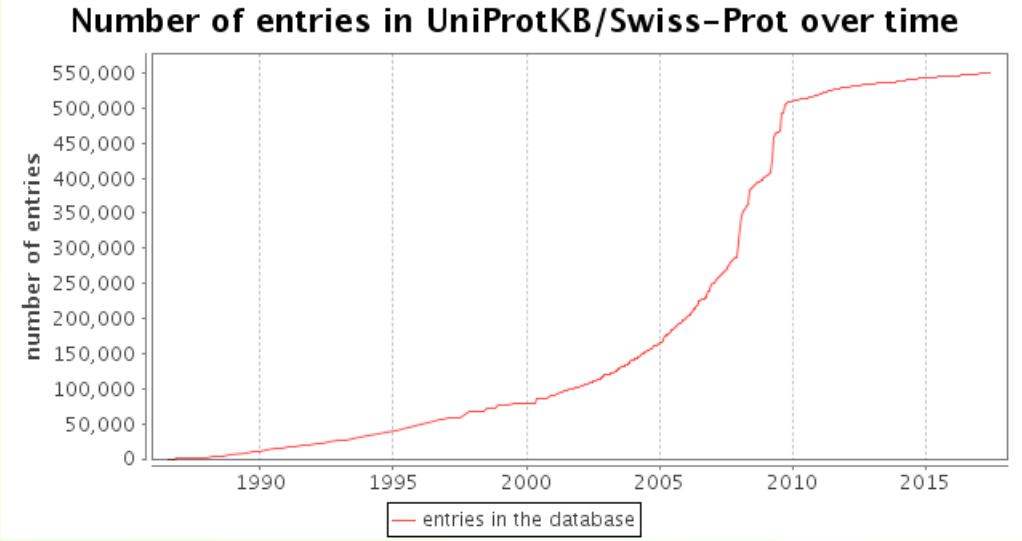
- Multiple research groups may sequence the same gene, chromosome etc
  - **and independently deposit the data in the same DB**
- Clearly this happens a lot with microbiome data
  - 16S sequences
  - Fragments of genomes from metagenomics reads
- Often it is important to be able to access **original data sets**, irrespective of redundancy
- For other purposes, it is essential to have access to **nonredundant** data

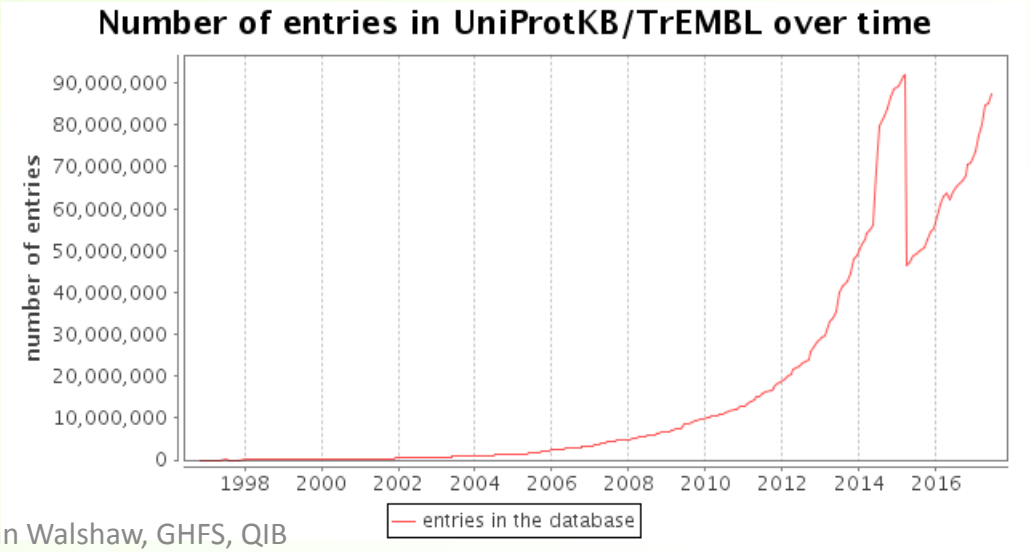
RefSeq : nonredundant sequences

- **RefSeq** – maintained by the NCBI
  - a database of sequences of:
    - Genomic DNA – including genes and whole chromosomes, and thus whole prokaryote genome sequences
    - Transcripts
    - Proteins
  - Non-redundant, i.e.: **Single standard reference sequence for each gene, chromosome, transcript, protein**
  - Cross-referenced
- Not to be confused with…
  - UniRef (nonredundant data sets from UniProt)
  - UniGene (NCBI) – which associates multiple fragments of **transcripts** with a single **gene sequence** (also achieves nonredundancy)

Primary databases are big

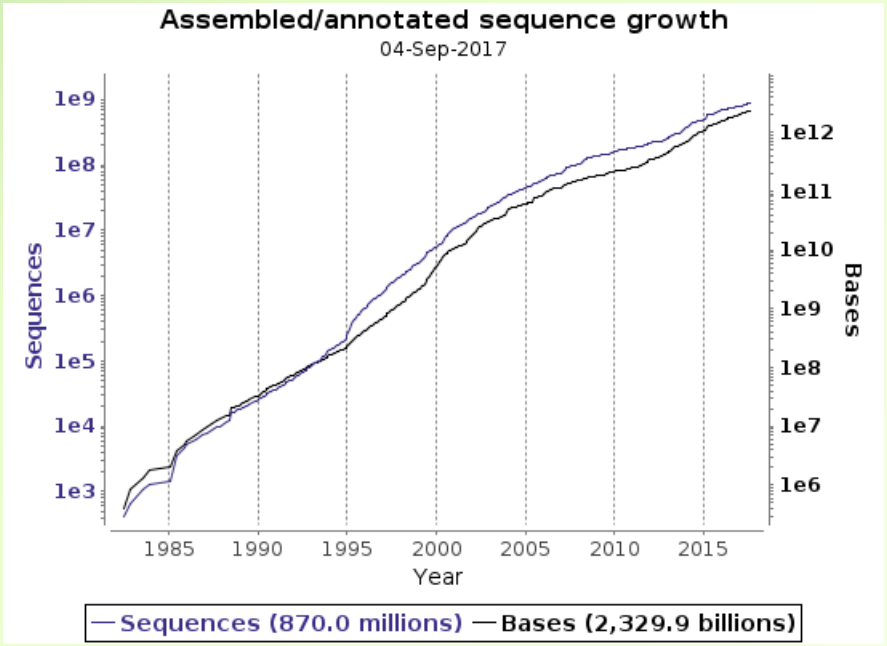
- UniProtKB - currently:
- 555,000 manually annotated/reviewed entries (Swiss-Prot)
- 89,000,000 auto-annotated/unreviewed entries (TrEMBL)
- www.uniprot.org/statistics/

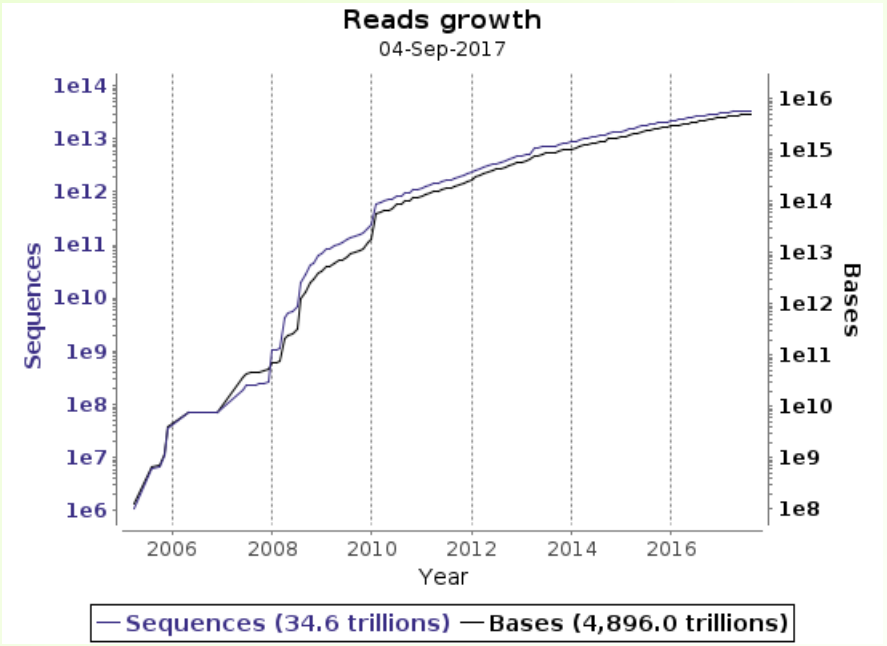


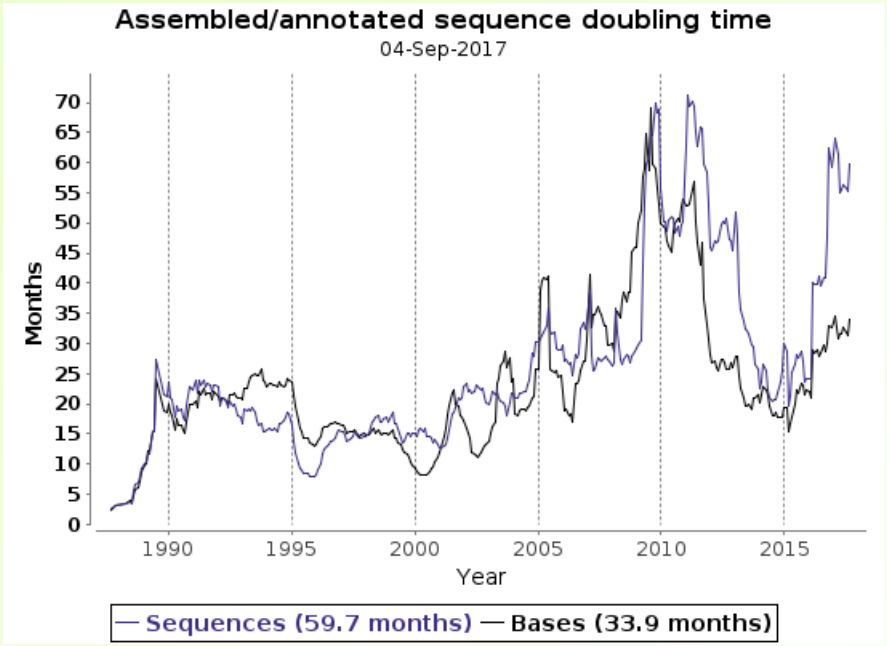


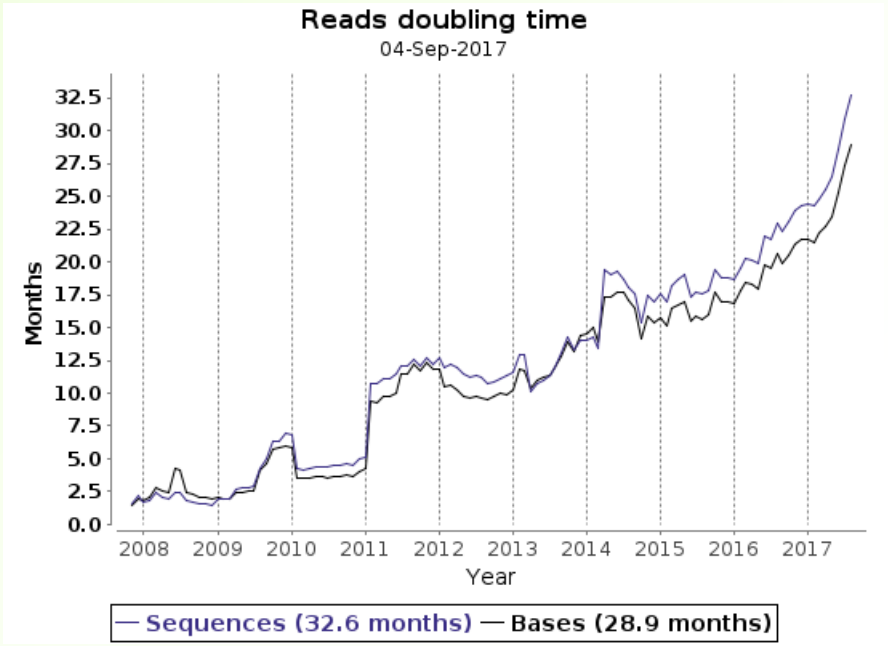
Primary nucleotide databases are VERY big

http://www.ebi.ac.uk/ena/about/statistics


Assembled/annotated sequence growth
04-Sep-2017
Sequences
Bases
Year
Sequences (870.0 millions)
Bases (2,329.9 billions)


Reads growth
04-Sep-2017
Sequences
Bases
Year
Sequences (34.6 trillions)
Bases (4,896.0 trillions)


Assembled/annotated sequence doubling time
04-Sep-2017
Months
Year
Sequences (59.7 months)
Bases (33.9 months)


Reads doubling time
04-Sep-2017
Months
Year
Sequences (32.6 months)
Bases (28.9 months)

Beyond primary DB providers

- Besides primary database maintainers-
  - numerous other providers of large datasets of many different kinds
  - Available for download, online interactive access and/or in conjunction with online analysis tools
- A few major examples (there are many others):
  - Kyoto University Bioinformatics Center
    - **Kyoto Encyclopedia of Genes and Genomes** (KEGG)
  - **Wellcome Trust Sanger Institute**, Hinxton
  - Swiss Institute of Bioinformatics, Lausanne
    - **ExPASy Bioinformatics Portal**
  - DOE-Joint Genome Institute (**JGI**), California
  - Human Microbiome Project Data Analysis and Coordination Centre (**HMP-DACC**)

Ribosomal RNA (gene) sequence databases

16S
Fungal 28S

Ribosomal Database Project (RDP)
Michigan State Univ.

16S/18S
23S/28S

SILVA
Max Planck Inst. for Marine Microbiology / Jacobs University

- Experts using their own systems for classification / curation
- Gives rise to **non-identical taxonomies**
- Which may also differ from other taxonomies (e.g. NCBI)

EzBioCloud 16S Database (formerly EzTaxon)
Chunlab, Inc.

Greengenes
Univ. Colorado

16S

- Associated with their own software, e.g.
- RDP Classifier
- SINA-Aligner (SILVA)

- Can be used in principle with various other software
- (may be some limitations)
- QIIME, MOTHUR, MEGAN etc

```
LOCUS       AY779786                 598 bp    DNA     linear   ENV 08-NOV-2004
DEFINITION  Uncultured archaeon clone sw3a52 small subunit ribosomal RNA gene,
            partial sequence.
ACCESSION   AY779786
VERSION     AY779786.1
KEYWORDS    ENV.
SOURCE      uncultured archaeon
  ORGANISM  uncultured archaeon
            Archaea; environmental samples.
REFERENCE   1  (bases 1 to 598)
  AUTHORS   Siering,P.L. and Wilson,M.S.
  TITLE     Geochemical and biological diversity in acidic hot springs in
            Lassen Volcanic National Park
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 598)
  AUTHORS   Siering,P.L. and Wilson,M.S.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-OCT-2004) Biological Sciences, Humboldt State
            University, 1 Harpst Street, Arcata, CA 95521, USA
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      121 ggggatactg ccgggctagg gggcgggaga ggccgggggt accccagggg taggggcgaa
      181 atccgataat ccctggggga ccaccagtgg cgaaggcgcc cggctggaac gcgcccgacg
      241 gtgaggggcg aaagccgggg gagcgaaccg gattagatac ccgggtagtc ccggctgtaa
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      481 gcgacagcag gatgacggcc aggctaacga ccttgcccga cgcgctgagg ggaggtgcat
      541 ggccgtcgcc agctcgtgct gtgaagtgtc ctgttaagtc aggcaacgag cgagaccc
//
```

GenBank record: AY779786.1

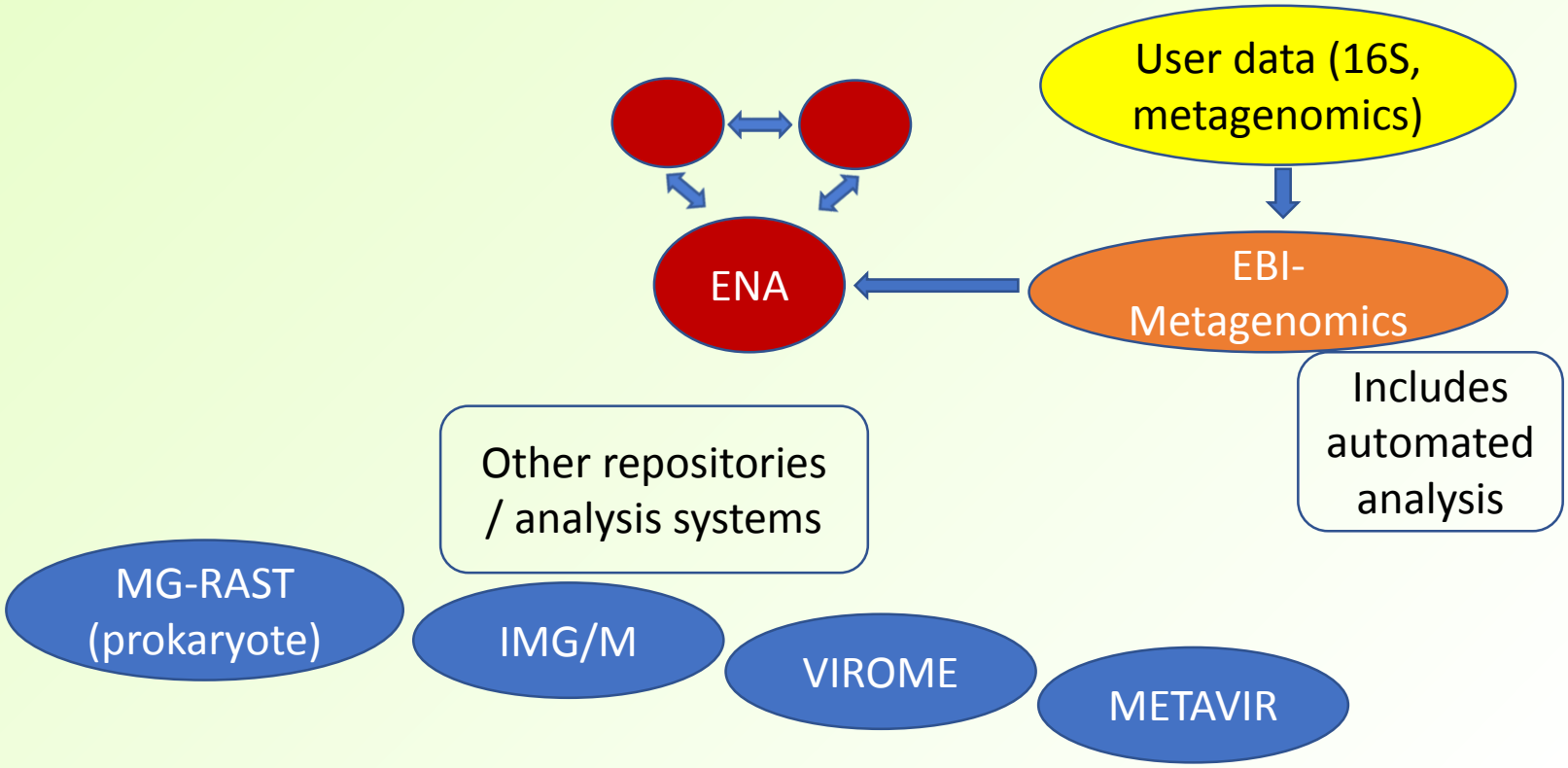
```
LOCUS       AY779786                 598 bp    DNA     linear   ENV 08-NOV-2004
DEFINITION  Uncultured archaeon clone sw3a52 small subunit ribosomal RNA gene,
            partial sequence.
ACCESSION   AY779786
VERSION     AY779786.1
KEYWORDS    ENV.
SOURCE      uncultured archaeon
  ORGANISM  uncultured archaeon
            Archaea; environmental samples.
```

GenBank record: AY779786.1

```
LOCUS       S000444337               598 bp    rRNA    linear   BCT 08-Nov-2004
DEFINITION  uncultured archaeon; sw3a52.
ACCESSION   AY779786 REGION: <1..>598
SOURCE      uncultured archaeon
  ORGANISM  uncultured archaeon
            Root; Archaea; "Crenarchaeota"; Thermoprotei; Acidilobales;
            Acidilobaceae; Acidilobus.
```

RDP record: S000444337

Microbiome-specific repositories



To be continued…

(Microbial) Genome Sequence Databases

References – Sequence Databases

Primary nucleotide sequence databases

- European Nucleotide Archive (ENA) http://www.ebi.ac.uk/ena
  - ("EMBL-Bank" is the ENA component equivalent to GenBank and DDBJ)
  - Leinonen, R. et al. (2011) The European Nucleotide Archive, *Nucleic Acids Res.* **39** (Database issue) D28-D31
- GenBank https://www.ncbi.nlm.nih.gov/genbank/
  - Benson *et al.* (2017) GenBank, *Nucleic Acids Res.* **45** (Database issue) D37-D42
  - Rindone, W.P. *et al.* (1983) GenBank™ - the Genetic Sequence Data-bank, *DNA-A Journal of Molecular & Cellular Biology* **2** (2) 173
  - Rindone, W.P. (1983) GenBank, *Trends in Pharmacological Sciences* **4** 326
- DNA Data Bank of Japan (DDBJ) http://www.ddbj.nig.ac.jp/
  - Mashima, J. *et al.* (2017) DNA Data Bank of Japan, *Nucleic Acids Res.* **45** (Database issue) D25-D31
  - Miyazawa, S. (1990) DNA Data-bank of Japan – Present Status and Future-plans, in *Computers and DNA*, pp. 47-61, eds G.I. Bell and T. Marr, Addison-Wesley, Reading, M.A.

References – Sequence Databases

Sequence databases associated with or derived from the primary nucleotide sequence databases – a few examples

- RefSeq https://www.ncbi.nlm.nih.gov/refseq/
  - O'Leary, N.A. (2016) Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation, *Nucleic Acids Res.* **44**(Database issue) D733-D745
- UniGene https://www.ncbi.nlm.nih.gov/unigene/
  - (see also NCBI Resource Coordinators (2016) Database resources of the National Center for Biotechnology Information, *Nucleic Acids Res.* **44**(Database issue) D7-D19

- Sequence Read Archive (sequences and quality data)
  - SRA at NCBI https://trace.ncbi.nlm.nih.gov/Traces/sra/
  - SRA at DDBJ (DRA) http://trace.ddbj.nig.ac.jp/dra/index_e.html
  - EBI SRA (sometimes referred to as "ERA") is integrated into ENA
  - Leinonen *et al.* (2011) The Sequence Read Archive, *Nucleic Acids Res.* **39** (Database issue) D19-D21

References – Sequence Databases

Primary protein sequence databases

- UniProt www.uniport.org
  - The UniProt Consortium (2017) UniProt: the universal protein knowledgebase, *Nucleic Acids Res.* **45** D158-D169

See also

- NCBI Protein https://www.ncbi.nlm.nih.gov/protein
- DDBJ Amino Acid Sequence Database (DAD) – see DDBJ, and ftp://ftp.ddbj.nig.ac.jp/ddbj_database/dad/

References – Sequence Databases

"Historical" primary protein sequence database references:

- Relating to the original Protein Sequence Database:
  - Dayhoff, M.O. (1965) Atlas of protein sequence and structure [Vol. 1], Silver Spring, MD, U.S.A. http://www.worldcat.org/title/atlas-of-protein-sequence-and-structure-vol-1-1965-margaret-o-dayhoff-et-al/oclc/605459794
  - Dayhoff, M.O. *et al.* (1975) Evolution of Sequences within Protein Superfamilies *Naturwissenschaften* **62** 154-161
  - **PIR-PSD** (see UniProt); *final release December 2004; now integrated into UniProt* http://pir.georgetown.edu/pirwww/dbinfo/pir_psd.shtml
    - Barker W.C. *et al.* (1991) The PIR sequence database, *Nucleic Acids Res.* **19** (Suppl) 2231-2236
    - Wu, C.H. *et al.* (2003) The Protein Information Resource, *Nucleic Acids Res.* **31** (1) 345-347
  - **MIPS** (formerly Martinsried Institute for Protein Sequences; latterly Munich Information Center for Protein Sequences, and the name of the database it maintained)
    - Mewes, H.W. *et al.* (1998) MIPS: a database for protein sequences and complete genomes , *Nucleic Acids Res.* **26** (1) 33-37
    - Mewes, H.W. *et al.* (2010) MIPS: curated databases and comprehensive secondary data resources in 2010, *Nucleic Acids Res.* **39** (Database issue) D220-D224
    - MIPS is now integrated into the Institute of Bioinformatics and Systems Biology (IBIS), German Research Center for Environmental Health https://www.helmholtz-muenchen.de/ibis
- Swiss-Prot/TrEMBL (now components of UniProt)
  - Bairoch, A. & Boeckmann, B. (1991) The SWISS-PROT protein sequence data bank, *Nucleic Acids Res.* **19** (Suppl) 2247–2249
  - Bairoch, A. & Apweiler, R. (1996) The SWISS-PROT Protein Sequence Data Bank and Its New Supplement TREMBL, *Nucleic Acids Res.* **24** (1) 21–25

References – Sequence Databases

Some mentioned example non-sequence databases associated with primary database providers:

- NCBI
  - BioProject
    https://www.ncbi.nlm.nih.gov/bioproject
  - Samples https://www.ncbi.nlm.nih.gov/biosample
  - Assembly
    https://www.ncbi.nlm.nih.gov/assembly

References – Sequence Databases

Institutes, consortia and miscellaneous projects

- International Nucleotide Sequence Database Collaboration (**INSDC**) http://www.insdc.org
- European Bioinformatics Institute (**EBI**) http://www.ebi.ac.uk
  - *is an outstation of*: European Molecular Biology Laboratory (**EMBL**) http://www.embl.de
- DNA Database of Japan **(DDBJ) Center** http://www.ddbj.nig.ac.jp
- National Center for Biotechnology Information (**NCBI**) http://www.ncbi.nlm.nih.gov
- Swiss Institute of Bioinformatics (**SIB**) http://www.isb-sib.ch
- Protein Information Resource (**PIR**) http://pir.georgetown.edu
- Joint Genome Institute (**JGI**) http://jgi.doe.gov
- Wellcome Trust Sanger Institute http://www.sanger.ac.uk
- Kyoto Encyclopedia of Genes and Genomes (**KEGG**)http://www.kegg.jp
- NIH Human Microbiome Project (**HMP**) https://commonfund.nih.gov/hmp
  - Data Analysis and Coordination Centre http://www.hmpdacc.org

References – Sequence Databases

Ribosomal RNA gene sequence databases

- RDP (Ribosomal Database Project) http://rdp.cme.msu.edu
  - Cole, J.R. *et al.* (2014) Ribosomal Database Project: data and tools for high throughput rRNA analysis, *Nucl. Acids Res.* **42**(Database issue) D633-D642
- SILVA http://www.arb-silva.de
  - Quast, C. *et al.* (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, *Nucl. Acids Res.* **41** (Database issue) D590-D596
- Greengenes http://greengenes.secondgenome.com
  - (previously http://greengenes.lbl.gov - data still available there)
  - McDonald, D. *et al.* (2011) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea, *ISME J.* **6**(3) 610-608
- EzBioCloud (formerly EzTaxon) http://www.ezbiocloud.net/taxonomy
  - Chun, J. *et al.* (2007) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species, *Int. J. Syst. Evol. Microbiol.* **57**(10) 2259-2261
  - Kim, O.S. *et al.* (2012) EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences, *Int. J. Syst. Evol. Microbiol.* **62**(3) 716-721

References – Sequence Databases

Some metagenome sequence repositories/annotation resources

- EBI Metagenomics https://www.ebi.ac.uk/metagenomics
  - Mitchell, A. *et al.* (2016) EBI metagenomics in 2016 - an expanding and evolving resource for the analysis and archiving of metagenomic data, *Nucleic Acids Res.* **44** (Database issue) D595-D603
- MG-RAST http://metagenomics.anl.gov
  - Meyer, F. *et al.* (2008) The Metagenomics RAST server — A public resource for the automatic phylogenetic and functional analysis of metagenomes, *BMC Bioinformatics* **9** 386
- IMG/M https://img.jgi.doe.gov
  - Chen, I.A. *et al.* (2017) IMG/M: integrated genome and metagenome comparative data analysis system, *Nucleic Acids Res.* **45** (Database issue) D507-D516
- VIROME http://virome.dbi.udel.edu
  - Wommack, K. E. *et al.* (2012) VIROME: a standard operating procedure for analysis of viral metagenome sequences, *Stand. Genomic Sci.* **6**(3) 427-439
- METAVIR / METAVIR2 http://metavir-meb.univ-bpclermont.fr
  - Roux, S. et al. (2011) Metavir: a web server dedicated to virome analysis, *Bioinformatics* **27** (21) 3074-3075
  - Roux, S. et al. (2014) Metavir 2: new tools for viral metagenome comparison and assembled virome analysis, *BMC Bioinformatics* **15** 76