Meta-omics

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16S

Using the shape of the handle to explain the function of the oven

Who what why… in summary

16S	Meta-Genomics	Meta-Transcriptomics
To understand who is there up to genus level Understand the diversity $	To understand what is possible Mostly high abundant players $$	To understand why things are happening Mostly high abundant players $$

Generating the data

Sequencing techniques

- Current generation
  - **Illumina (Many short reads, good quality)**
  - **Pacbio (Few long reads, medium quality)**
- Next generation (which is already here)
  - **Oxford nanopore (Few very long reads, medium quality)**

illumina

- Suitable for meta- samples
- Generates relatively short reads
- Assembly will result in many short contigs
- Cheap

16S

Genomics

Transcriptomics

PacBio

- Suitable for meta- samples (when not alone)
- Generates relatively long reads ~15 kb
- Assembly will be difficult due to low coverage
- New development on whole rna sequencing
- Expensive

16S

Genomics

Transcriptomics

Oxford

- Suitable for meta- samples (when not alone)
- Yield average per cell 2.3 Gb
- Needs less DNA than PacBio (±20x) (rumour)
- Generates long reads
- Assembly will be difficult due to low coverage
- New development on whole rna sequencing
- Expensive
- Still under heavy development and improvements

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Genomics

Transcriptomics

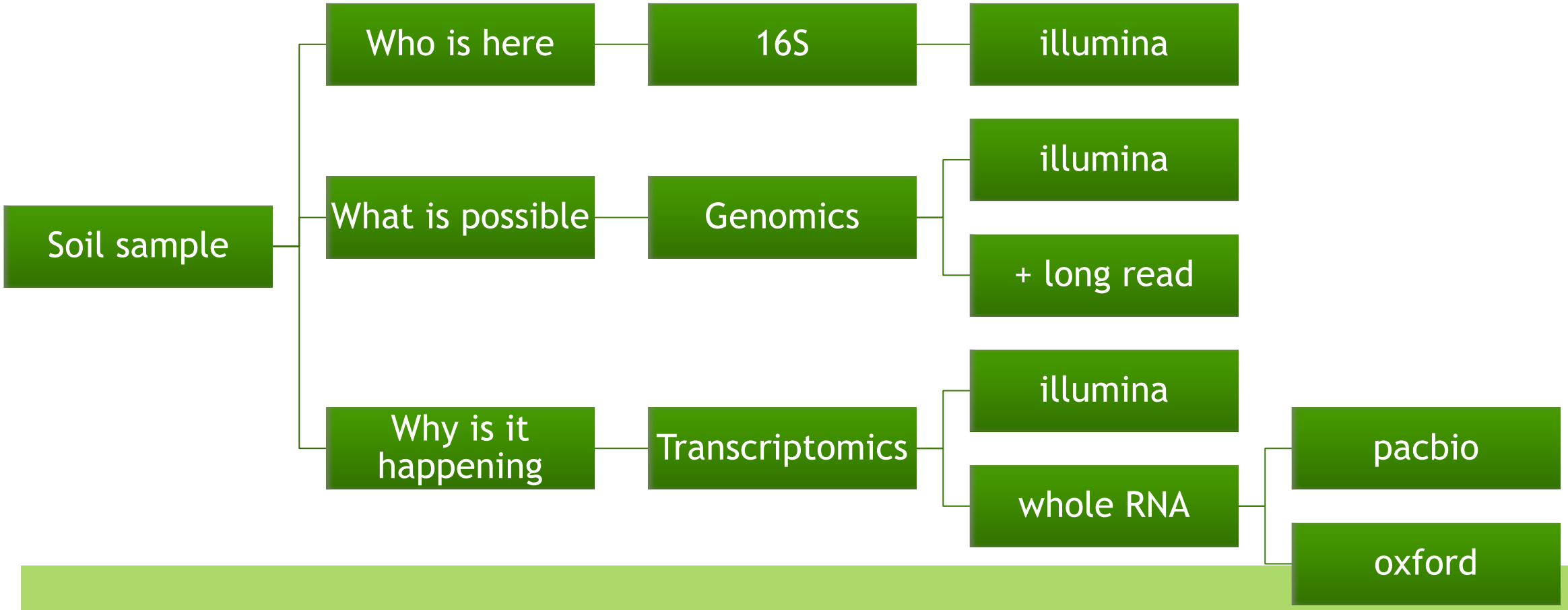
Congratulations!

The first >1 Mb DNA read, achieved with nanopore sequencing

Martin Smith & collaborators, Kinghorn Centre for Clinical Genomics, Garvan Institute for Medical Research, Australia

December 2017

Sequencing a meta-sample



What do I want to achieve?

- Have a general understanding who is here
- Extract specific complete genomes from a microbiome sample
- Identify pathways that are expressed under certain conditions
- Is it important to know who is there?

When is illumina good enough?

- When you want to know what is happening
- When you do not want to extract complete genomes
- To perform expression analysis
  - You do **not** need metagenomics for this

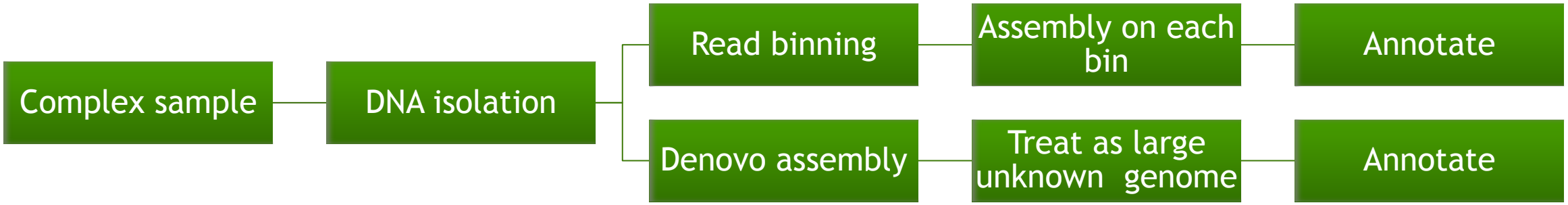
When are long reads good enough?

- When you want to extract complete genomes
- For the extraction of complete genomes but its not that easy
  - Use information obtained from short read sequencing
    - Antibiotic resistance, specific growth media, etc to isolate a strain of interest

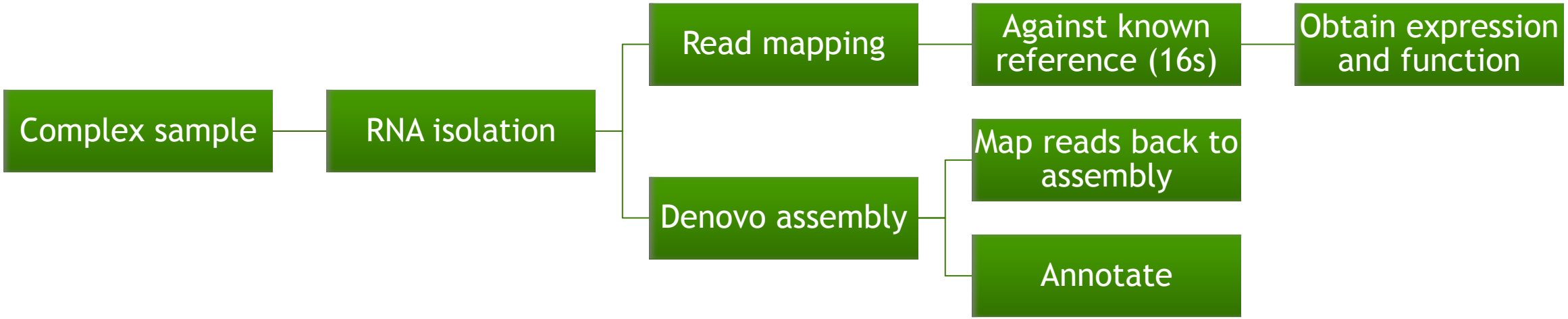
Workflow

- Meta-genomics workflow
- Meta-transcriptomics workflow

Meta-genomics workflow



Meta-transcriptomics workflow



What to expect?

- 16s
  - **Low abundant species will be picked up**
- Metagenomics
  - **Only high abundant groups are detected (2-4000x coverage required for the same resolution as 16s)**
    - **1 Gene vs ±4000 genes**
  - **Many contigs (fewer when long reads are used)**
  - **After annotation**
    - **You know what the theoretical possibilities are within your sample**
    - **Identify the landscape variation between different samples**
- Transcriptomics
- **Only high abundant groups are detected**
  - **Using a reference (difficult in an unknown or variable community)**
    - **Map reads, differential expression**
    - **De-novo assembly, annotate each transcript, …**
    - **Obtain the genes of interest, identify the function,**
      - **Hopefully understand why this is happening**

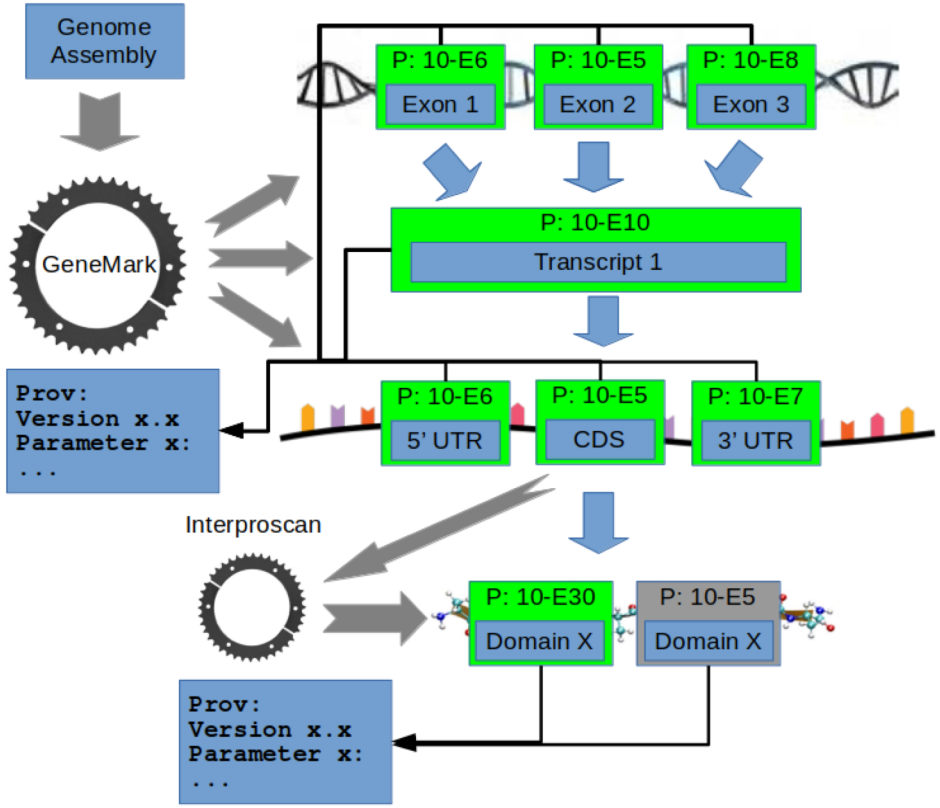
Putting the reads together

Annotation information storage

Example questions e.g:

- What (functions) distinguish “environmental” samples?
- Which enzymes are there that can catalyze reaction X (maybe with different cofactors?

Requires a resource of consistently annotated samples that can be easily mined



Requirements for genome mining

- A semantic annotation platform that incorporates common tools and stores the results in “proper” format. **SAPP**
- A graph database that can be mined: **SAGERP**
- A definition of the “proper format”: definitions of biological terms and their relationships: **GBOL ontology**
- Interface to use the ontology: **GBOL stack**
- Tools to develop all of these:
  - **Empusa**: code generator

- SAPP is the only thing a user would need to use to annotate a genome
- Sager-P is the only thing a user would need to mine the data

A “proper” format

```
0  ##gff-version 3.2.1
1  ##sequence-region ctg123 1 1497228
2  ctg123 . gene            1000  9000  .  +  .  ID=gene00001;Name=EDEN
3  ctg123 . TF_binding_site 1000  1012  .  +  .  ID=tfbs00001;Parent=ge
4  ctg123 . mRNA            1050  9000  .  +  .  ID=mRNA00001;Parent=ge
5  ctg123 . mRNA            1050  9000  .  +  .  ID=mRNA00002;Parent=ge
6  ctg123 . mRNA            1300  9000  .  +  .  ID=mRNA00003;Parent=ge
7  ctg123 . exon            1300  1500  .  +  .  ID=exon00001;Parent=mRNA00003
8  ctg123 . exon            1050  1500  .  +  .  ID=exon00002;Parent=mRNA00001,mRNA00002
9  ctg123 . exon            3000  3902  .  +  .  ID=exon00003;Parent=mRNA00001,mRNA00003
10 ctg123 . exon            5000  5500  .  +  .  ID=exon00004;Parent=mRNA00001,mRNA00002,mRNA00003
11 ctg123 . exon            7000  9000  .  +  .  ID=exon00005;Parent=mRNA00001,mRNA00002,mRNA00003
12 ctg123 . CDS             1201  1500  .  +  0  ID=cds00001;Parent=mRNA00001;Name=edenprotein.1
13 ctg123 . CDS             3000  3902  .  +  0  ID=cds00001;Parent=mRNA00001;Name=edenprotein.1
14 ctg123 . CDS             5000  5500  .  +  0  ID=cds00001;Parent=mRNA00001;Name=edenprotein.1
15 ctg
16 ctg
17 ctg
18 ctg
19 ctg
20 ctg
21 ctg
22 ctg
23 ctg
24 ctg
```

```
LOCUS       SCU49845     5028 bp    DNA             PLN       21-JUN-1999
DEFINITION  Saccharomyces cerevisiae TCP1-beta gene, partial cds, and Axl2p
            (AXL2) and Rev7p (REV7) genes, complete cds.
ACCESSION   U49845
VERSION     U49845.1  GI:1293613
KEYWORDS    .
SOURCE      Saccharomyces cerevisiae (baker's yeast)
  ORGANISM  Saccharomyces cerevisiae
            Eukaryota; Fungi; Ascomycota; Saccharomycotina; Saccharomycetes;
            Saccharomycetales; Saccharomycetaceae; Saccharomyces.
REFERENCE   1  (bases 1 to 5028)
  AUTHORS   Torpey,L.E., Gibbs,P.E., Nelson,J. and Lawrence,C.W.
  TITLE     Cloning and sequence of REV7, a gene whose function is required for
            DNA damage-induced mutagenesis in Saccharomyces cerevisiae
  JOURNAL   Yeast 10 (11), 1503-1509 (1994)
  PUBMED    7871890
REFERENCE   2  (bases 1 to 5028)
  AUTHORS   Roemer,T., Madden,K., Chang,J. and Snyder,M.
  TITLE     Selection of axial growth sites in yeast requires Axl2p, a novel
            plasma membrane glycoprotein
  JOURNAL   Genes Dev. 10 (7), 777-793 (1996)
  PUBMED    8846915
REFERENCE   3  (bases 1 to 5028)
  AUTHORS   Roemer,T.
  TITLE     Direct Submission
  JOURNAL   Submitted (22-FEB-1996) Terry Roemer, Biology, Yale University, New
            Haven, CT, USA
FEATURES             Location/Qualifiers
     source          1..5028
                     /organism="Saccharomyces cerevisiae"
                     /db_xref="taxon:4932"
                     /chromosome="IX"
                     /map="9"
     CDS             <1..206
                     /codon_start=3
                     /product="TCP1-beta"
                     /protein_id="AAA98665.1"
                     /db_xref="GI:1293614"
                     /translation="SSIYNGISTSGLDLNNGTIADMRQLGIVESYKLKRAVVSSASEA
                     AEVLLRVDNIIRARPRTANRQHM"
```

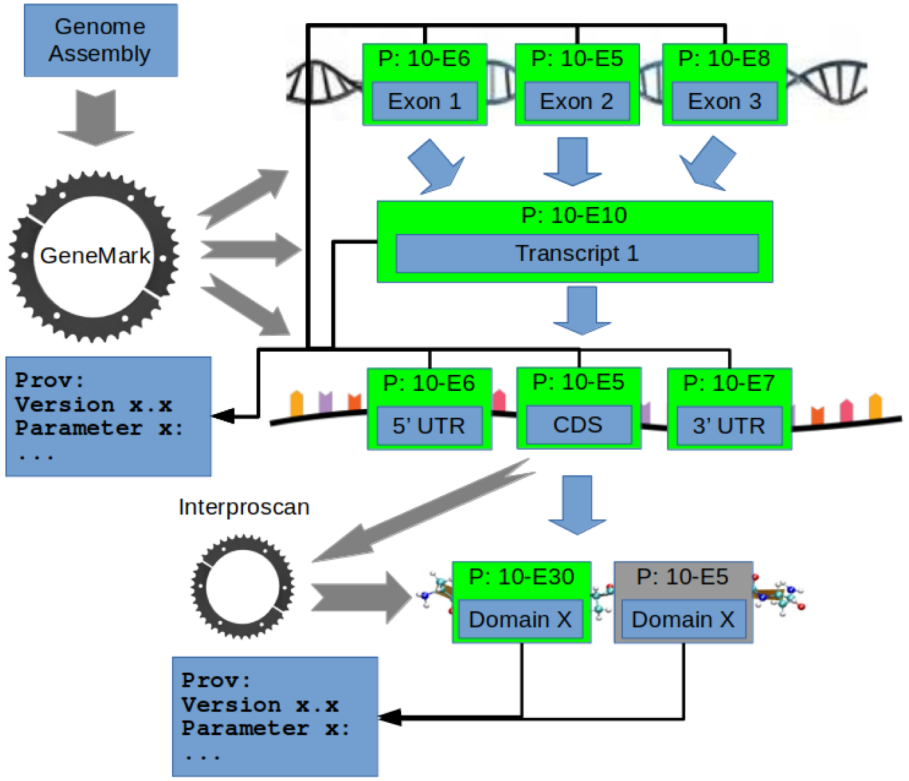
- Dataset-wise and element-wise provenance
- Mining enabled
- Query enabled

SAPP: Annotation information storage

- Wrapper to commonly used annotation tools (prokaryotes and eukaryotes) that generates FAIR data
- Current usage:
  - Uniform annotation of over 10 000 bacterial species.
  - Uniform annotation of salmonoids (fish)
  - Uniform annotation of plant genomes
  - Uniform annotation of environmental samples

Koehorst et al Bioinformatics 2017
https://gitlab.com/sapp

Documentation:
https://sapp.gitlab.io

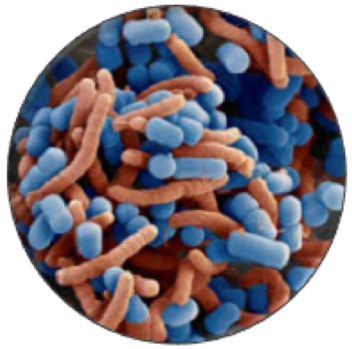
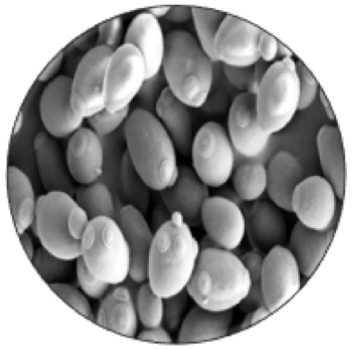


Modular design

Conversion types
- EMBL / GenBank
- FASTA
- GFF
- QTL
- VCF
- …

Genetic elements
- Gene prediction
- tRNA/rRNA
- Crispr
- …

Functional annotation
- BLAST
- Enzyme predictions
- Domain annotation
- Signal peptides
- Transmembrane
- Localization
- …

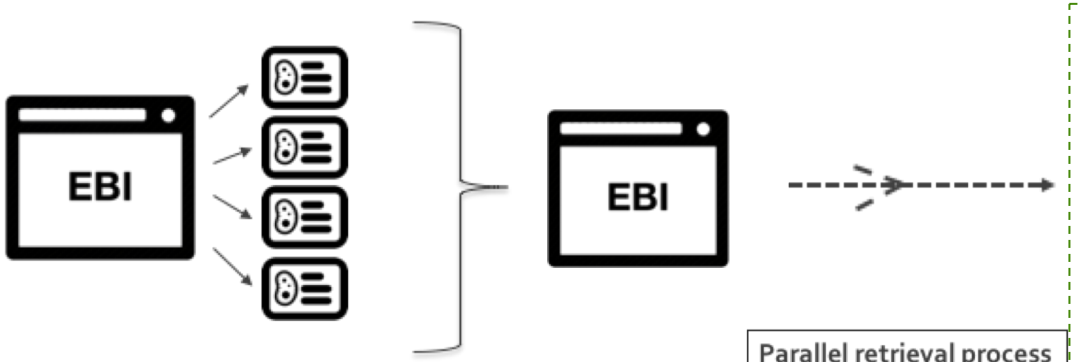
Use cases:

Computational genomics

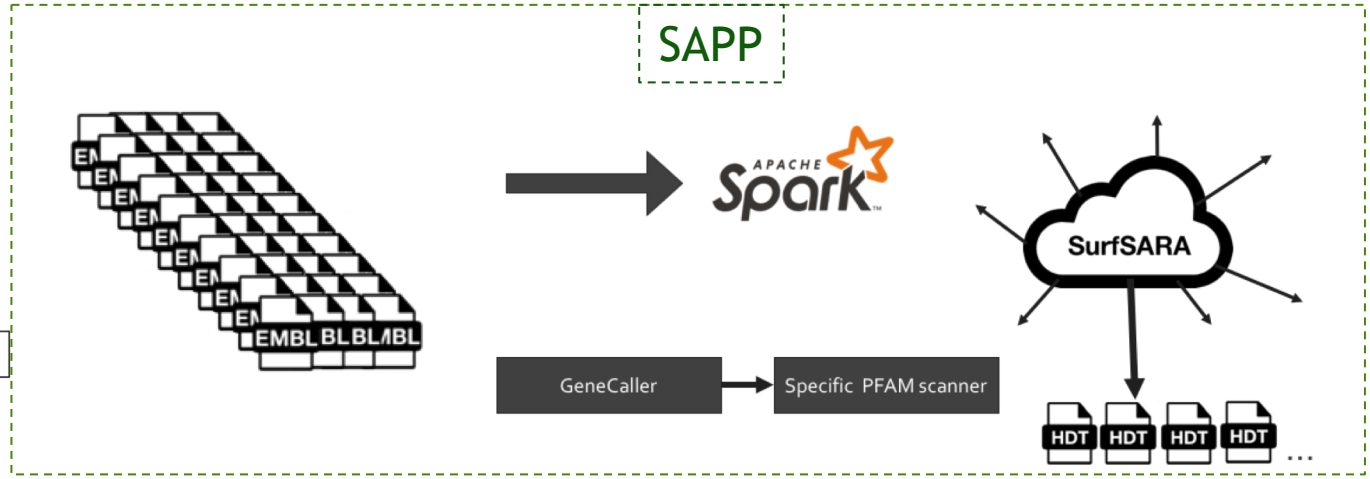
&

Environmental samples

High Throughput annotation



Parallel retrieval process



Dynamic search and filtering

Bacteria

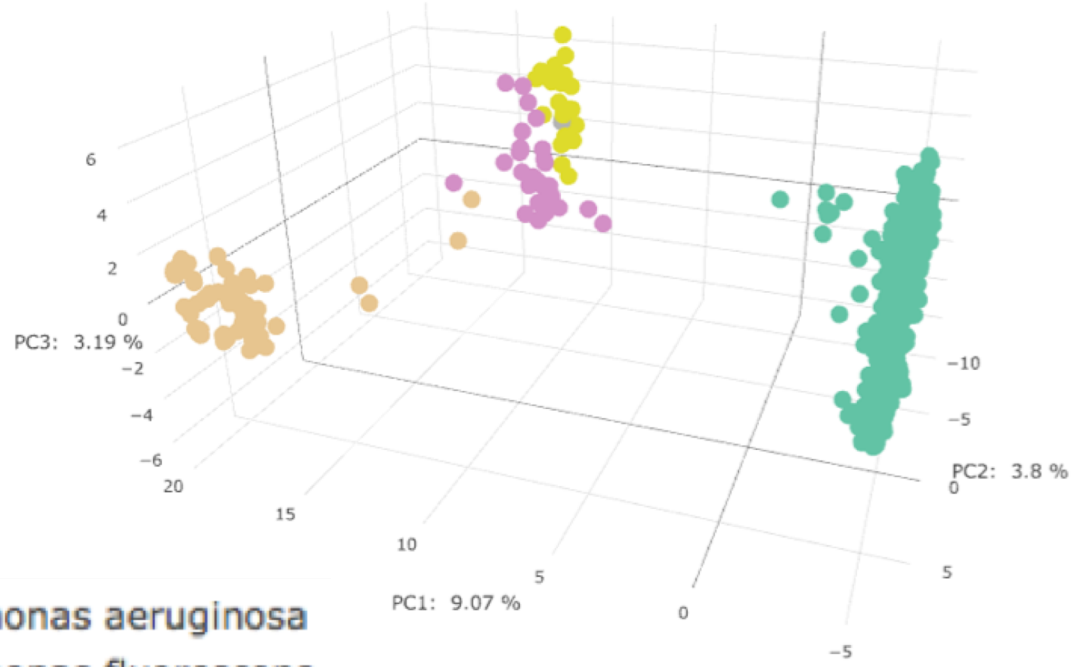
Functional domains retrieval

```
PREFIX gbol: <http://gbol.life/0.1/>
SELECT ?sample ?accession ?db
WHERE {
    ?sample a gbol:Sample .
    ?dnaobject gbol:sample ?sample .
    ?dnaobject gbol:feature ?gene .
    ?gene gbol:transcript ?transcript .
    ?transcript gbol:feature ?cds .
    ?cds gbol:protein ?protein .
    ?protein gbol:feature ?domain .
    ?domain gbol:xref ?xref .
    ?xref gbol:db <http://gbol.life/0.1/db/pfam> .
    ?xref gbol:accession ?accession .
} LIMIT 10
```

SAGERP

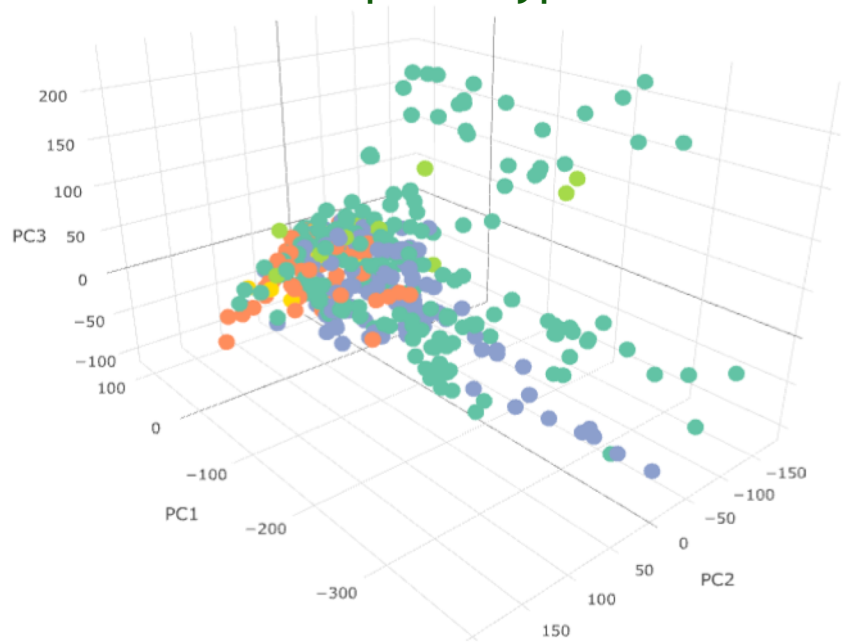
Functional variation

Phylogeny and phenotype relationships with the functional landscape



- Pseudomonas aeruginosa
- Pseudomonas fluorescens
- Pseudomonas putida
- Pseudomonas syringae

Scored phenotypes



- Aerobe
- Anaerobe
- Facultative
- Microaerophilic
- Obligate aerobe
- Obligate anaerobe

BacDive
The Bacterial Diversity Metadatabase

Pfam

Koehorst, Jasper J., et al. *Scientific reports* 6 (2016):

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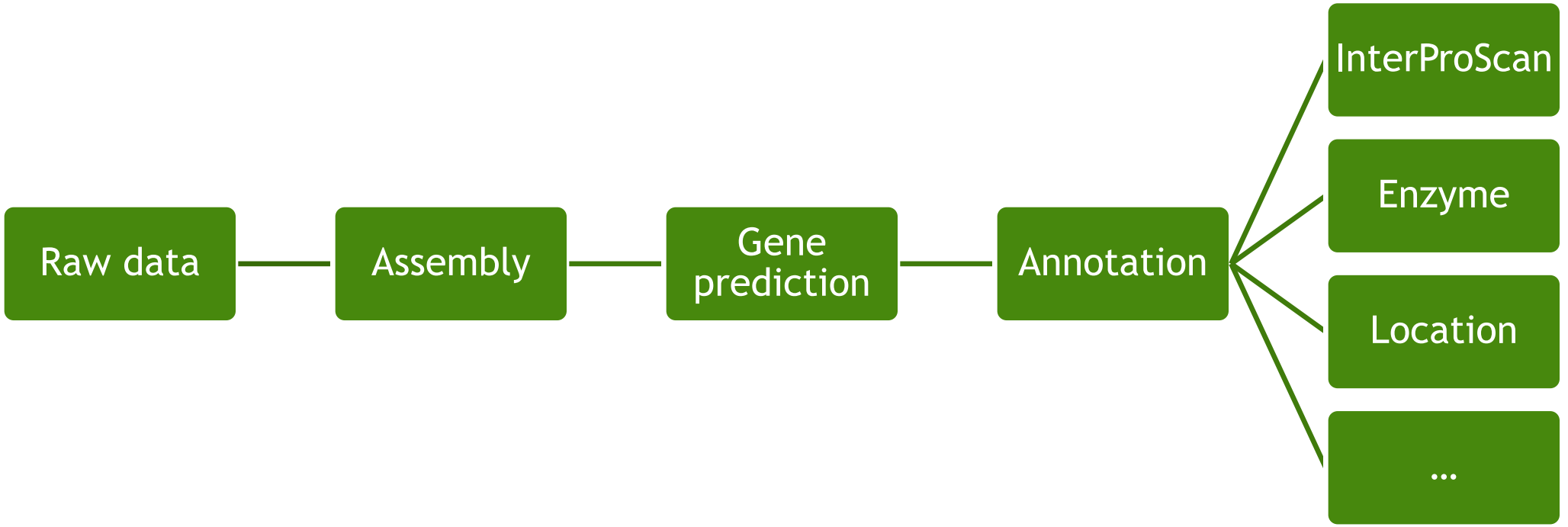
100years

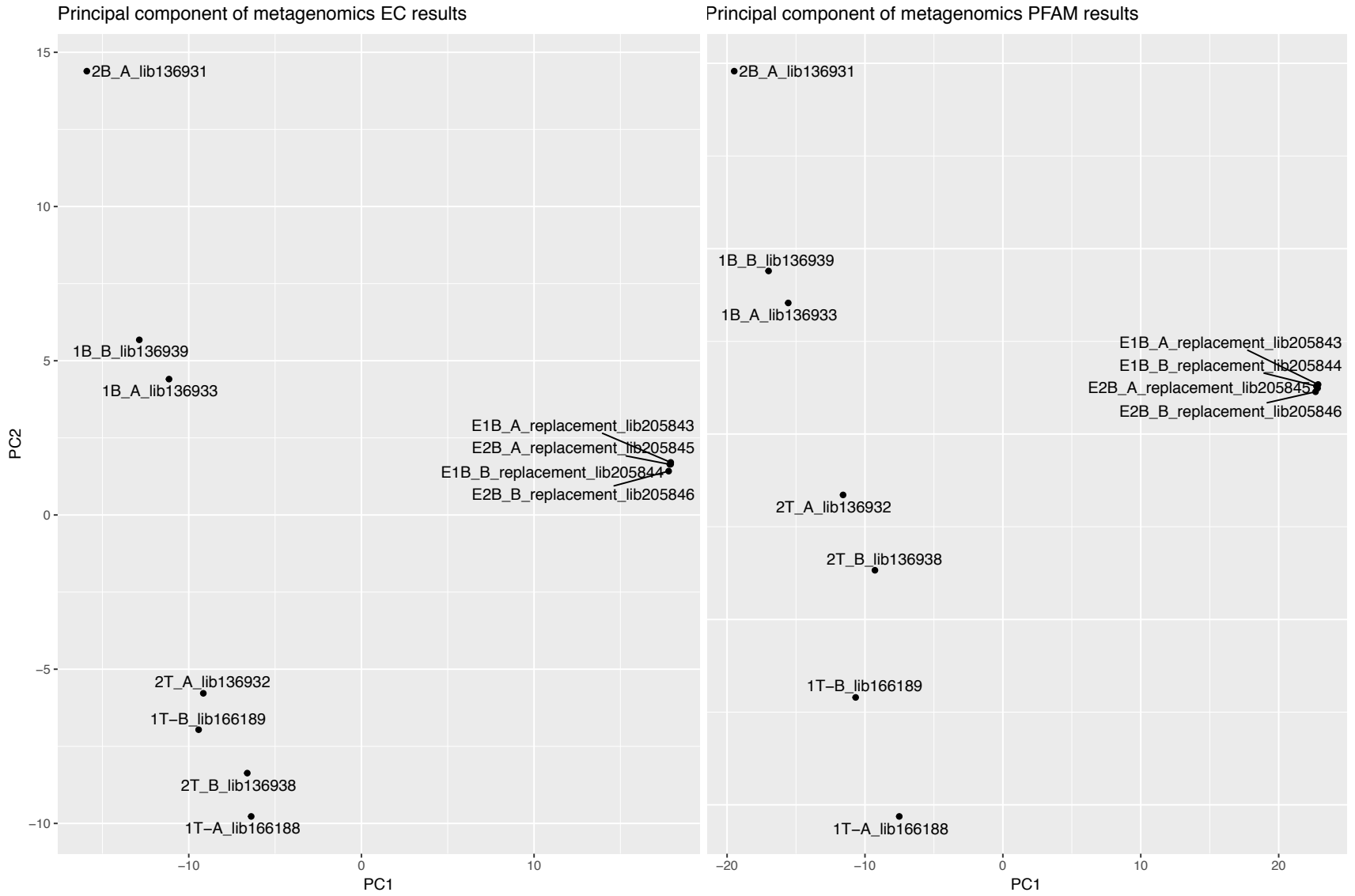
Environmental samples

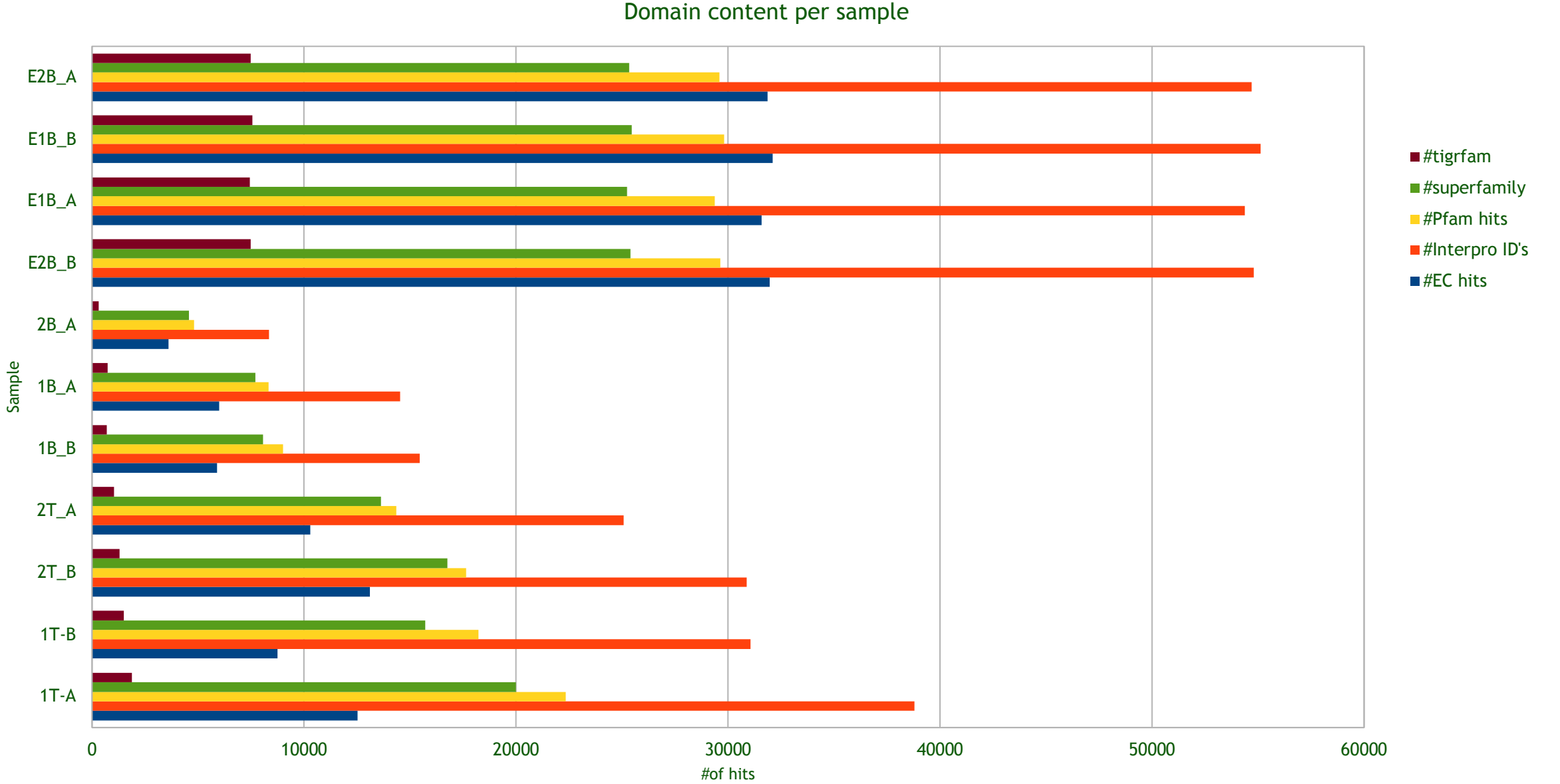
(current study)



Functional landscape

518.613 proteins in total




Domain content per sample
E2B_A
E1B_B
E1B_A
E2B_B
2B_A
1B_A
1B_B
2T_A
2T_B
1T-B
1T-A
Sample
0
10000
20000
30000
40000
50000
60000
#of hits
#tigrfam
#superfamily
#Pfam hits
#Interpro ID's
#EC hits

Current publications using SAPP

1. Comparative genomics highlights symbiotic capacities and high metabolic flexibility of the marine genus Pseudovibrio
   D Versluis, B Nijsse, MA Naim, JJ Koehorst, J Wiese, JF Imhoff, ... **Genome biology and evolution** 10 (1), 125-142

2. Concurrent haloalkanoate degradation and chlorate reduction by Pseudomonas chloritidismutans AW-1T
   P Peng, Y Zheng, JJ Koehorst, PJ Schaap, AJM Stams, H Smidt, ... **Applied and environmental microbiology** 83 (12), e00325-17

3. Persistence of Functional Protein Domains in Mycoplasma Species and their Role in Host Specificity and Synthetic Minimal Life
   T Kamminga, JJ Koehorst, P Vermeij, SJ Slagman, ... **Frontiers in cellular and infection microbiology** 7, 31

4. Complete Genome Sequence of Akkermansia glycaniphila Strain PytT, a Mucin-Degrading Specialist of the Reticulated Python Gut
   JP Ouwerkerk, JJ Koehorst, PJ Schaap, J Ritari, L Paulin, C Belzer, ... **Genome announcements** 5 (1), e01098-16

5. Interoperable genome annotation with GBOL, an extendable infrastructure for functional data mining
   JCJ van Dam, JJ Koehorst, JO Vik, PJ Schaap, M Suarez-Diez **bioRxiv**, 184747

6. Reverse methanogenesis and respiration in methanotrophic archaea
   PHA Timmers, CU Welte, JJ Koehorst, CM Plugge, MSM Jetten, ... **Archaea** 2017

7. Comparison of 432 Pseudomonas strains through integration of genomic, functional, metabolic and expression data
   JJ Koehorst, JCJ Van Dam, RGA Van Heck, E Saccenti, ... **Scientific reports** 6, 38699

8. Complete genome sequence of thermophilic Bacillus smithii type strain DSM 4216 T
   EF Bosma, JJ Koehorst, SAFT van Hijum, B Renckens, B Vriesendorp, ... **Standards in genomic sciences** 11 (1), 52

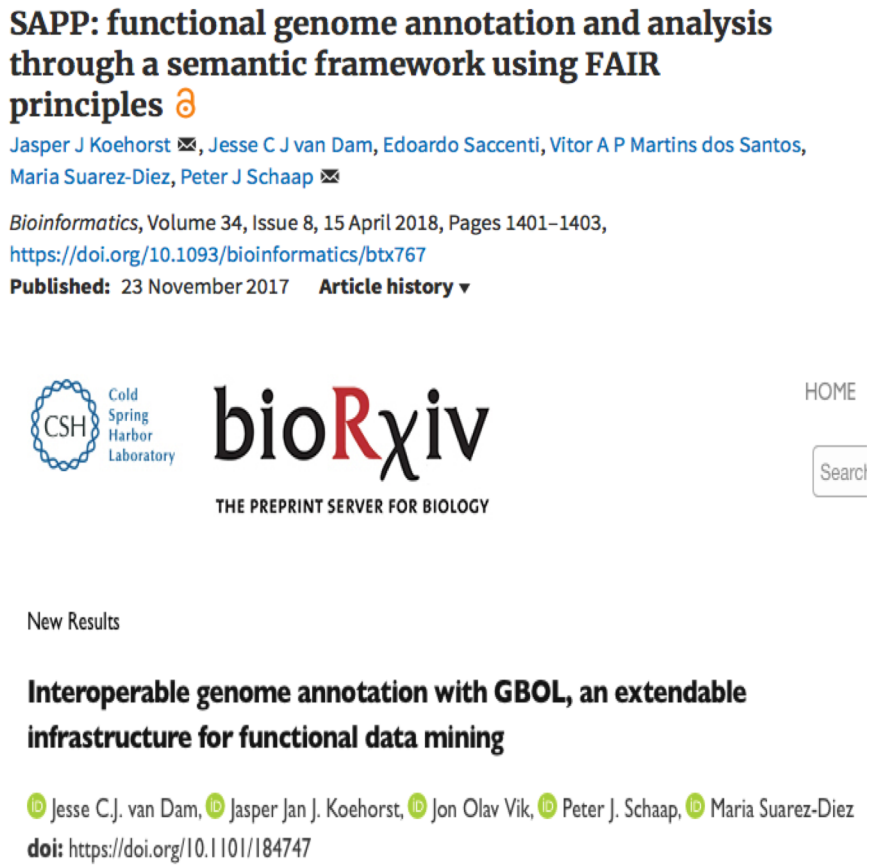
9. Protein domain architectures provide a fast, efficient and scalable alternative to sequence-based methods for comparative functional genomics
   JJ Koehorst, E Saccenti, PJ Schaap, VAPM dos Santos, M Suarez-Diez **F1000Research**

10. Assessing the metabolic diversity of streptococcus from a protein domain point of view
    E Saccenti, D Nieuwenhuijse, JJ Koehorst, VAPM dos Santos, PJ Schaap **PloS one** 10 (9), e0137908

11. A genomic view on syntrophic versus non-syntrophic lifestyle in anaerobic fatty acid degrading communities
    P Worm, JJ Koehorst, M Visser, VT Sedano-Núñez, PJ Schaap, ... Biochimica et Biophysica Acta **(BBA)-Bioenergetics** 1837 (12), 2004-2016

Availability

- **SAPP** Koehorst et al Bioinformatics 2017 https://sapp.gitlab.io
- **Empusa**: https://gitlab.com/Empusa
- **GBOL:** Documentation & namespace: http://gbol.life/0.1/



SAPP: functional genome annotation and analysis through a semantic framework using FAIR principles

Jasper J Koehorst, Jesse C J van Dam, Edoardo Saccenti, Vitor A P Martins dos Santos, Maria Suarez-Diez, Peter J Schaap

Bioinformatics, Volume 34, Issue 8, 15 April 2018, Pages 1401–1403, https://doi.org/10.1093/bioinformatics/btx767

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Interoperable genome annotation with GBOL, an extendable infrastructure for functional data mining

Jesse C.J. van Dam, Jasper Jan J. Koehorst, Jon Olav Vik, Peter J. Schaap, Maria Suarez-Diez

doi: https://doi.org/10.1101/184747

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100years


Poor Data Quality
Garbage In
Any method
Garbage Out
Poor Data Quality