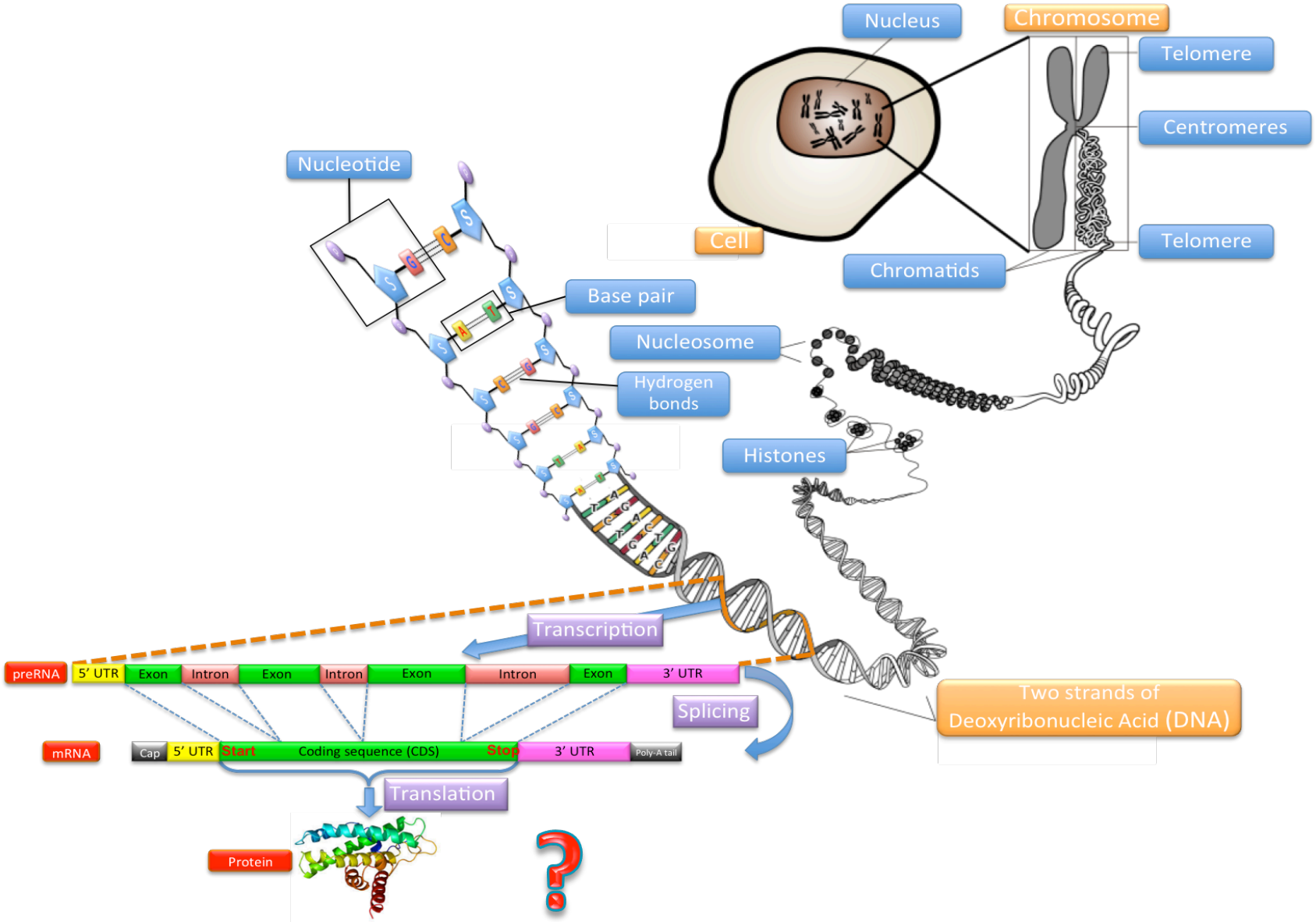
NBIS
NATIONAL BIOINFORMATICS INFRASTRUCTURE SWEDEN

SciLifeLab

Lucile Soler PhD

Functional annotation

Overview

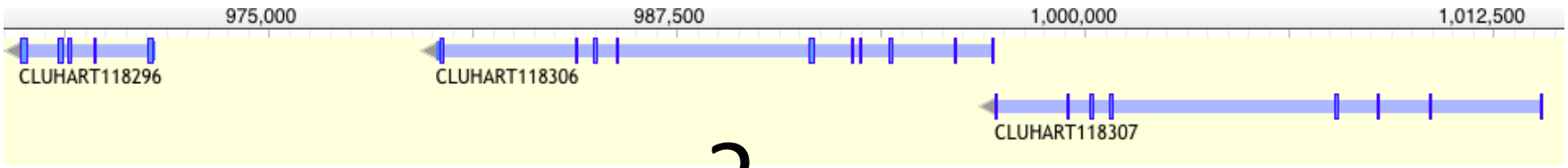

Nucleus
Chromosome
Telomere
Centromeres
Telomere
Cell
Chromatids
Nucleotide
Base pair
Nucleosome
Hydrogen bonds
Histones
Transcription
Splicing
Two strands of Deoxyribonucleic Acid (DNA)
preRNA
5' UTR
Exon
Intron
Exon
Intron
Exon
Intron
Exon
3' UTR
mRNA
Cap
5' UTR
Start
Coding sequence (CDS)
Stop
3' UTR
Poly-A tail
Translation
Protein

Functional annotation – Why?

Understanding the function of gene product is key to understanding how a limited number of interacting gene products can generate life, from simple unicellular organisms to the incredibly complex multi-cellular Homo sapiens.

Rison,S.C., Hodgman,T.C. and Thornton,J.M. (2000) Comparison of functional annotation schemes for genomes. Funct. Integr. Genomics, 1, 56–69.

Proteins vary in structure as well as function



?

Antibodies?

?

Energy?

?

Enzyme?

Contractile protein?

Transport Protein?

Hormone?

Storage Protein?

Structural Protein?

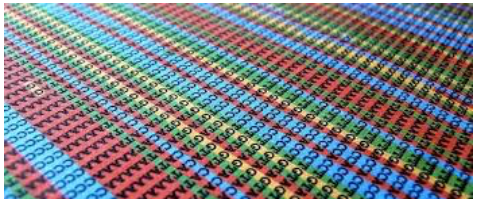
Functional annotation – HOW?

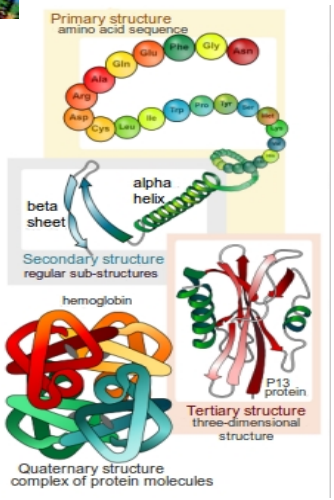
- Experimentally
  => Mutants, knockout, etc.

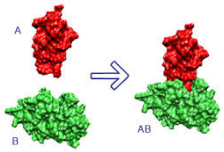
Accurate

Mice homozygous for the diabetes 3J spontaneous mutation

- Computationally
  - Sequence-based
  - Structure based
  - Protein-protein interaction data







limited accuracy

Methods - **Sequence-based**

- Based on similarity

  =>Best blast hit

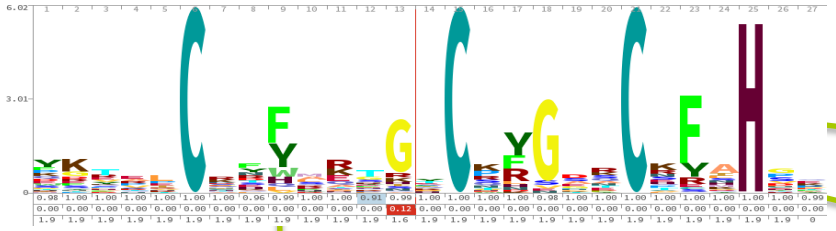
```
Q GLMDTAFEHIKATGGLTTESNYPYKGEDATCNS-K
  GLM+ AFE+IK +GG+TTES YPY+  + TC++ +
S GLMENAFEYIKHSGGITTESAYPYRAANGTCDAVR
```

- Based on Motif

  ⇒Proscan, MEME, QuasiMotiFinder

D-X-[KR]-P-{WYF}-X5

- Based on Profile (HMM or other statistical signature)



Whole sequence
e.g. Psi-BLAST*, PIRSF

domain
e.g. PFAM

Localization (e.g membrane, golgi, secreted)
e.g. SignalP, TMHMM

structural classification
e.g. SUPERFAMILY

...

Methods - **Sequence-based**

- Based on evolutionary relationship (Orthology)
  - Clustering: KOG / COG
  - Based on synteny
    ⇒Whole genome alignment (lastZ)
    (NBIS) Satsuma + kraken + custom script
  - Based on phylogeny
    ⇒ Quite complicated at large scale

Methods - **Structure-based**

- Similarity to known structures.
  - Global structure-comparison
    - CATH and SCOP, the two most comprehensive structure-based family resources
  - localized regions
    - might be relevant to function: clefts, pockets and surfaces
  - active-site residues (catalytic clusters and ligand-binding sites)
    - active-site residues is often more conserved than the overall fold

    ⇒PDBSiteScan

no single method is always successful

Functional annotation – HOW?

It is actually kind of complex…

- Multi-dimensional problem :
  - e.g. A protein can have a molecular function, a cellular role, and be part of a functional complex or pathway
- Molecular function can be illustrated by multiple descriptive levels
  - (e.g. '**enzyme**' category versus a more specific '**protease**' assignment).

Functional annotation – HOW?

It is actually kind of complex…

- Similarities (structural or in sequence) VS function.
  - Similar sequence but different function (new domain => new combination => different function)
  - Different sequence may have same function (convergence) : Profiles helpful
  - Two proteins may have a similar fold but different functions

- Looks for conserved domains more reliable than whole sequence ?
  - How to go from conserved domains to assigning a function for your protein?

=> Importance to gathering as much information as possible

Functional annotation – HOW?

Sequence-based methods

- The most used (popular)
- Quick
- Easy to use
- Accurate (>70%)

Watson JD, Sanderson S, Ezersky A, Savchenko A, Edwards A, Orengo C, Joachimiak A, Laskowski RA, Thornton JM: Towards fully automated structure-based function prediction in structural genomics: a case study. J Mol Biol. 2007, 367: 1511-1522. 10.1016/j.jmb.2007.01.063.

- Many resources: even structural domains information
- Less computationally demanding

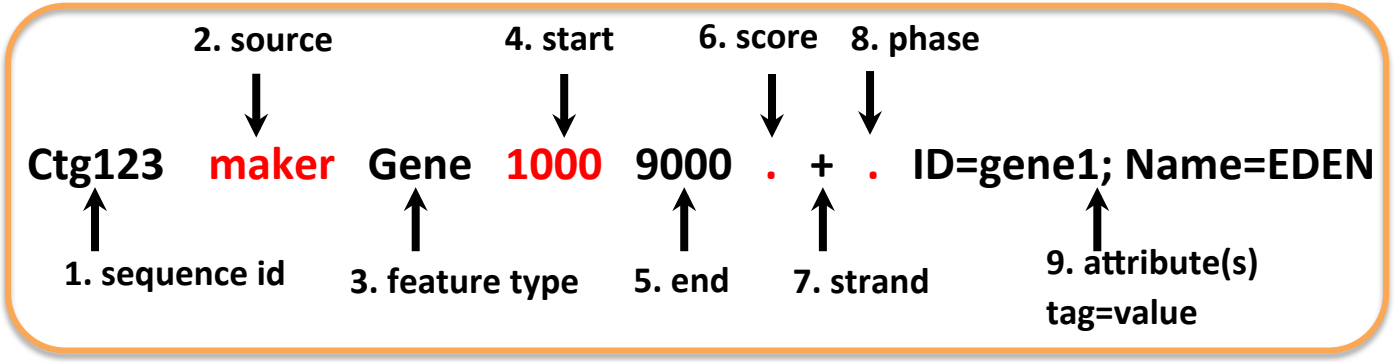
Functional annotation – HOW?

Get sequences

Functional annotation – HOW?

- Genome is in fasta format.

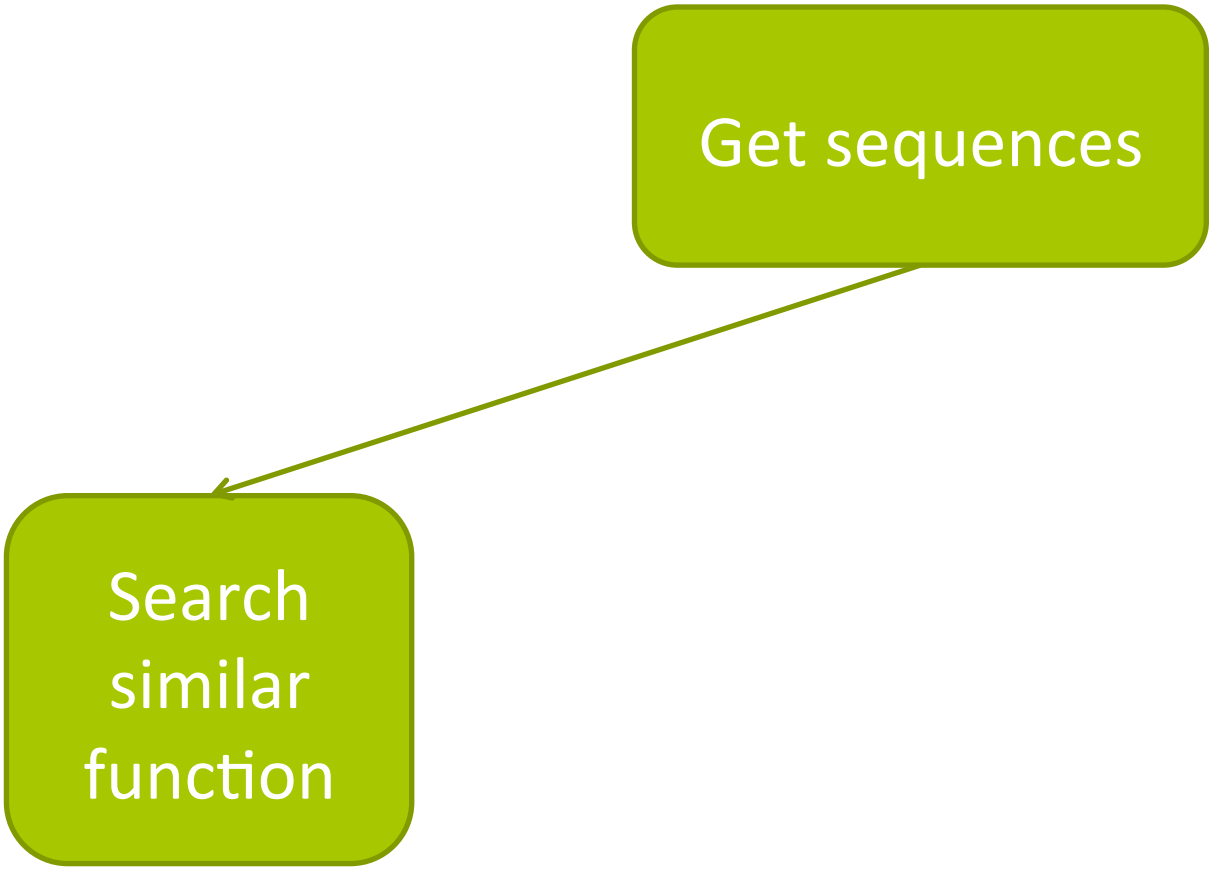
- Annotation is often in GFF-format. This format contains in general only coordinates, but sometimes it can include the sequence as well.



- You can use the GFF-file together with the genome-file to extract the gene sequences.

- The functional annotation tools want sequences in amino acid format, so when you extract the sequences you also need to convert the nucleotides to amino acids.

Functional annotation – HOW?



Blast-based approach

Blast-based approach

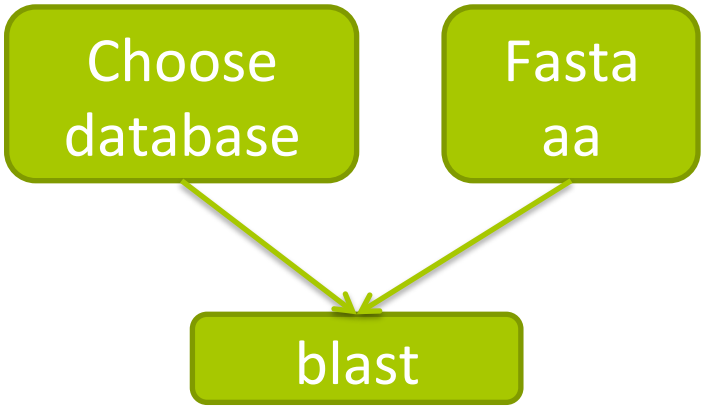
Annotate the sequences functionally using Blast

Choose database

Uniprot	Swissprot
exhaustive	reliable

Blast-based approach

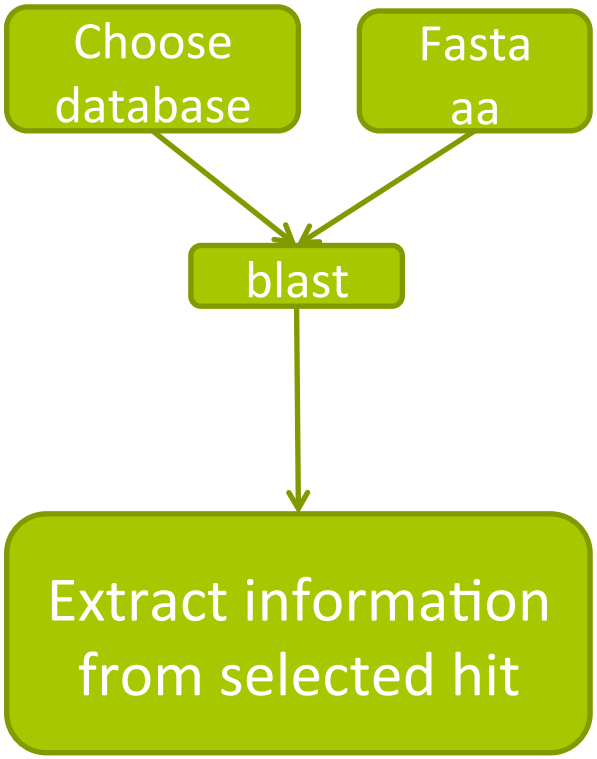
Annotate the sequences functionally using Blast



Minimum Threshold

Blast-based approach

Annotate the sequences functionally using Blast

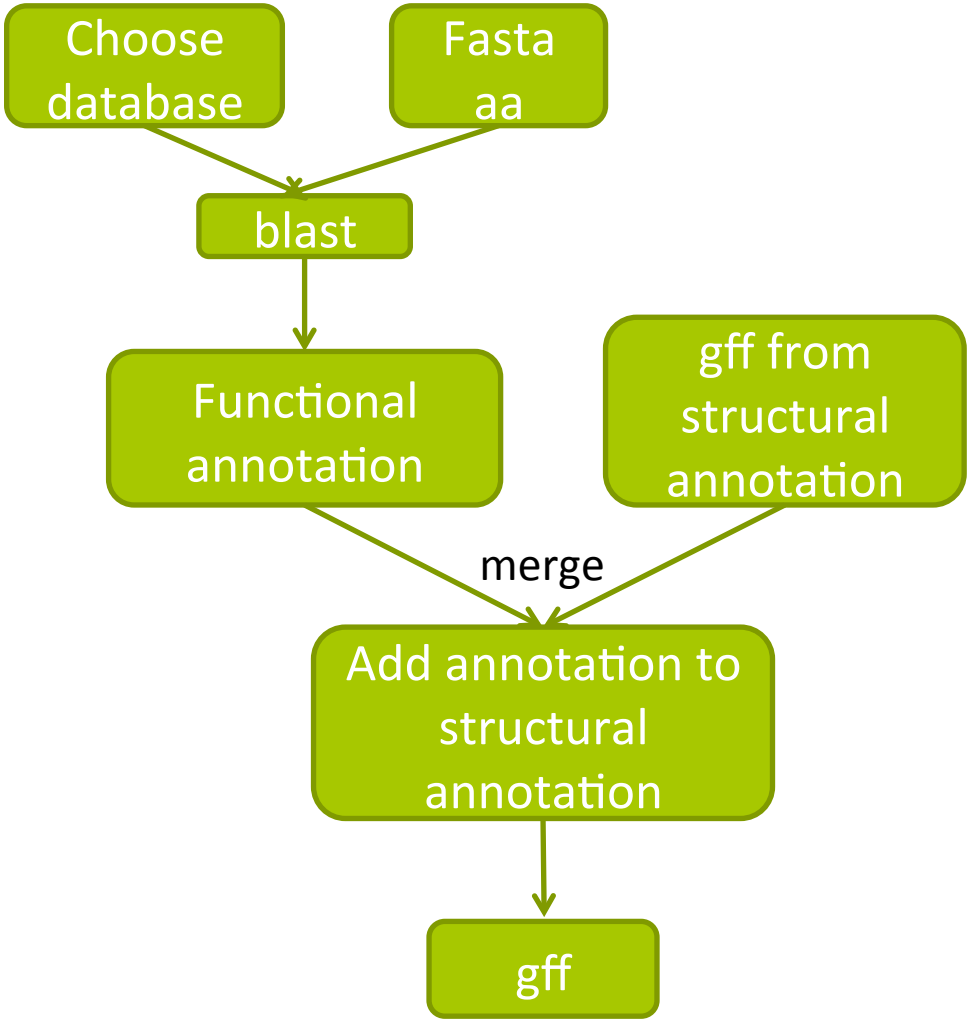


How to filter ?

- Minimum e-value
- Best blast hit
- You could prioritize by species

Blast-based approach

Annotate the sequences functionally using Blast



Blast-based approach

Strengths

- Fairly fast and easy
- Allow gene naming (e.g. plip)
- Overall function (e.g. Phosphatidylglycerophosphatase and protein-tyrosine phosphatase 1)

Limits

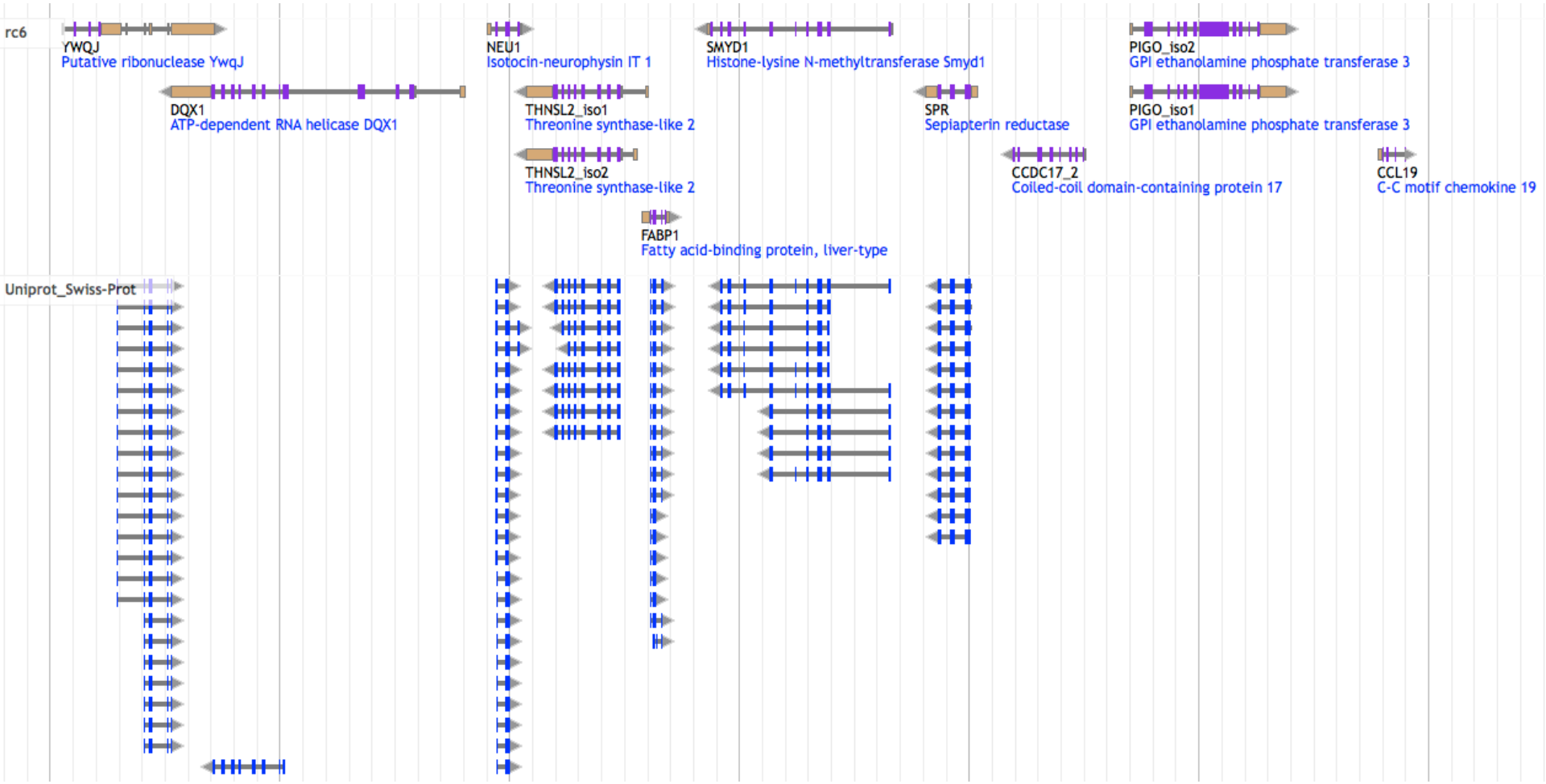
- Orthology not certain - best blast-hit does not equal orthologous!
- Bias due to well conserved domains
- Best Hit (use as template) is not necessary the best annotated sequence to use => Could apply a prioritization rule (Human first, then mouse, etc).

Blast-based approach

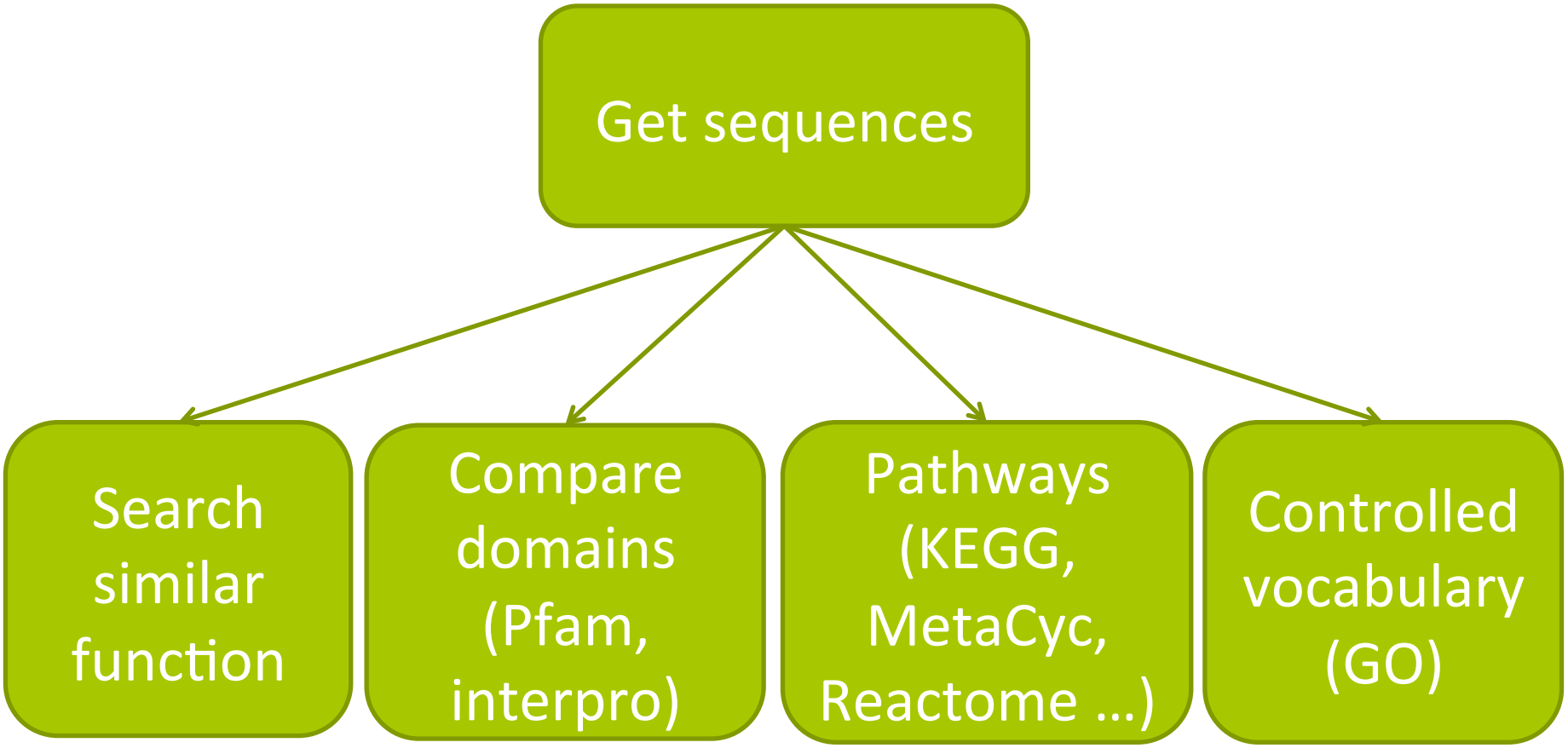
Blast-based annotation are tightly dependent to the quality of the structural annotation

- Gene Fusion
- Gene split
- Gene Partial (Well conserved domain)
- Over prediction
- Wrong ORF

Blast-based approach : results



Functional annotation – HOW?



Databases

Database	Information	Comment
KEGG	Pathway	Kyoto Encyclopedia of Genes and Genomes
MetaCyc	Pathway	Curated database of experimentally elucidated metabolic pathways from all domains of life (NIH)
Reactome	Pathway	Curated and peer reviewed pathway database
UniPathway	Pathway	Manually curated resource of enzyme-catalyzed and spontaneous chemical reactions.
GO	Gene Ontology	Three structured, controlled vocabularies (ontologies) : biological processes, cellular components and molecular functions
Pfam	Protein families	Multiple sequence alignments and hidden Markov models
Interpro	Protein families, domains and functional sites	Run separate search applications, and create a signature to search against Interpro.
Have a look on the Interpro web page: All the database they search into are listed. It gives a nice overview of different types of databases available.		

Gene Ontology

Gene Ontology: the framework for the model of biology.
The GO defines concepts/classes used to describe gene function, and relationships between these concepts. It classifies functions along three aspects:

More than 60 000 terms

GO term prediction

Biological Process

- GO:0006631 fatty acid metabolic process
- GO:0006635 fatty acid beta-oxidation
- GO:0008152 metabolic process
- GO:0055114 oxidation-reduction process

pathways and larger processes made up of the activities of multiple gene products.

Molecular Function

- GO:0003824 catalytic activity
- GO:0003857 3-hydroxyacyl-CoA dehydrogenase activity
- GO:0004300 enoyl-CoA hydratase activity
- GO:0016491 oxidoreductase activity
- GO:0016616 oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor
- GO:0050662 coenzyme binding

molecular activities of gene products

Cellular Component

- GO:0005739 mitochondrion
- GO:0016507 mitochondrial fatty acid beta-oxidation multienzyme complex

where gene products are active

Gene Ontology

http://www.geneontology.org/

Tools

Tool	Approach	Comment
Trinotate	Best blast hit + protein domain identification (HMMER/ PFAM) + protein signal peptide and transmembrane domain prediction (signalP/tmHMM), and leveraging various annotation databases (eggNOG/GO/Kegg databases).	Partially automated
Annocript	Best blast hit	Collects the best-hit and related annotations (proteins, domains, GO terms, Enzymes, pathways, short)
Annot8r	Best blast hits	A tool for Gene Ontology, KEGG biochemical pathways and Enzyme Commission EC number annotation of nucleotide and peptide sequences.
Sma3s	Best blast hit + Best reciprocal blast hit + clusterisation	3 annotation levels
afterParty	BLAST, InterProScan	web application
Interproscan	Run separate search applications HMMs, fingerprints, patterns => InterPro	Created to unite secondary databases
Blast2Go	Best* blast hits	Commercial !

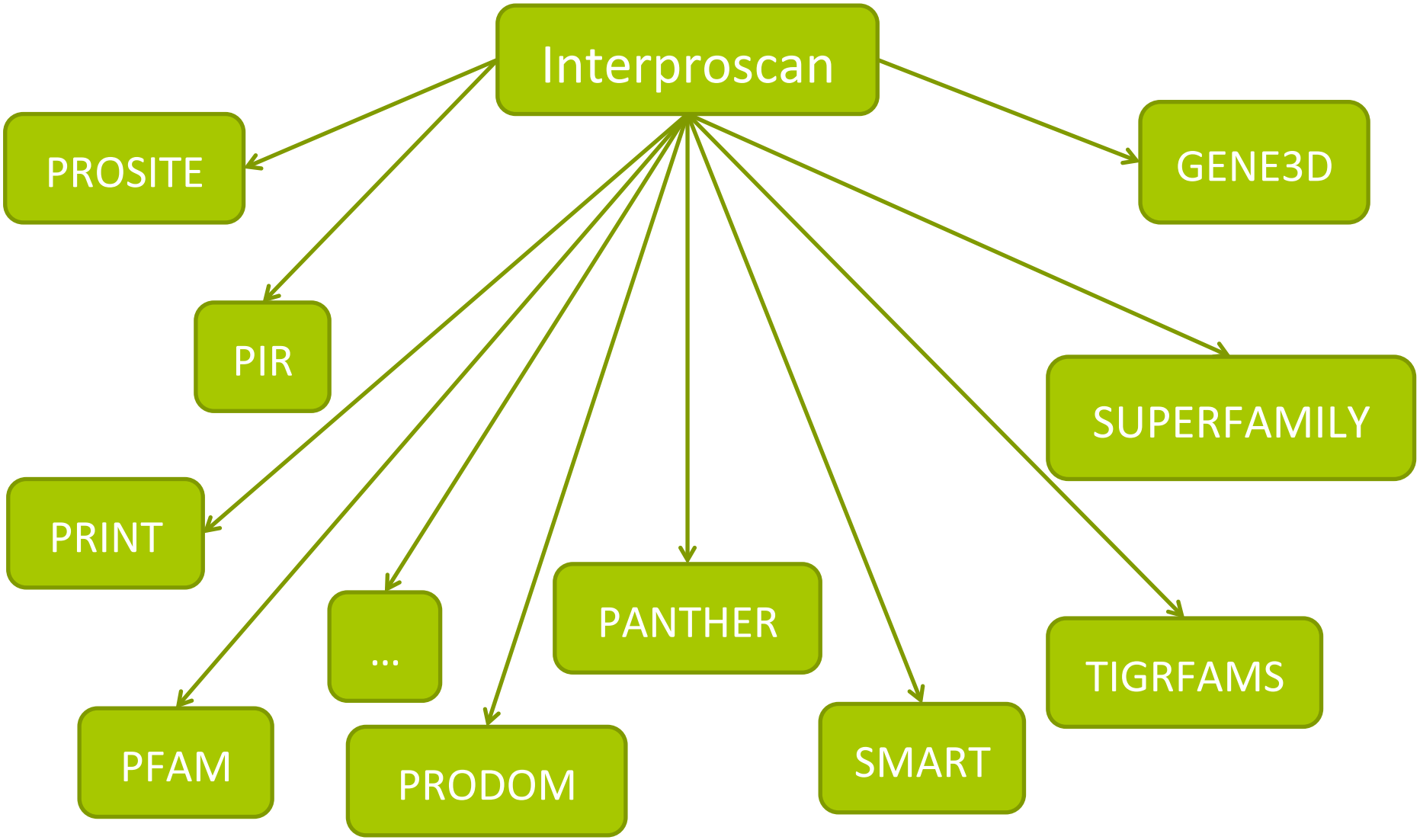
Interproscan

"InterPro is a resource that provides functional analysis of protein sequences by classifying them into families and predicting the presence of domains and important sites.

To classify proteins in this way, InterPro uses predictive models, known as signatures,
provided by several different databases (referred to as member databases) that make up the InterPro consortium."

https://www.ebi.ac.uk/interpro/about.html

Interproscan



Interproscan

- Annotate the sequences functionally using Interproscan : http://www.ebi.ac.uk/interpro/

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InterPro

Protein sequence analysis & classification

Search InterPro...

Examples: IPR020405, kinase, P51587, PF02932, GO:0007165

Home | Search | Release notes | Download | About InterPro | Help | Contact | InterPro BETA

InterPro: protein sequence analysis & classification

InterPro provides functional analysis of proteins by classifying them into families and predicting domains and important sites. We combine protein signatures from a number of member databases into a single searchable resource, capitalising on their individual strengths to produce a powerful integrated database and diagnostic tool. Read more about InterPro

Analyse your protein sequence

Submit | Clear | Example protein sequence

Documentation

About InterPro: core concepts, update frequency, how to cite, team and consortium members.

FAQs: what are entry types and why are they important, interpreting results, downloading InterPro?

Web services documentation

Protein focus

What's ape

The genus *Homo*, to which all human beings belong, is believed to have evolved from *Australopithecus* around 2–3 million years ago. Lucy, the *Australopithecus afarensis* ape, whose skeleton was pieced together from several hundred pieces of bone fossils, is the best known example of

Publications

InterPro in 2019: improving coverage, classification and access to protein sequence annotations

Our latest paper describing new developments on the InterPro website (*Nucleic Acids Research*, Jan 2019).

HTML | PDF (5.7Mb) | All publications

v.74 InterPro 74.0
9th May 2019

Features include:

- The addition of 156 InterPro entries.
- Integration of 174 new methods from the CATH-Gene3D (100), CDD (28) and PANTHER (46) databases.
- Removal of the ProDom (2006.1) database.

Download | Read more

Tweets by @InterProDB

InterPro
@InterProDB
Replying to @InterProDB
InterProScan 5 (version 5.34-73.0) is now available! For more details please visit: github.com/ebi-pf-team/in…

ebi-pf-team/interproscan
Contribute to ebi-pf-team/i…
github.com

Mar 28, 2019

InterPro

Interproscan

Contents and coverage of InterPro 74.0

InterPro protein matches are now calculated for all UniProtKB and UniParc proteins. The following statistics are for all UniProtKB proteins.

InterPro release 74.0 contains 36713 entries (last entry: IPR042311), representing:

- H Homologous superfamily (3078)
- F Family (21769)
- D Domain (10637)
- R Repeat (316)
- S Sites
  - Active site (132)
  - Binding site (76)
  - Conserved site (688)
  - PTM (17)

InterPro cites 58657 publications in PubM

→ Structural domains

Member database information

Signature database	Version	Signatures*	Integrated signatures**
CATH-Gene3D	4.2.0	6119	2369
CDD	3.16	12805	3284
HAMAP	2019_01	2274	2245
PANTHER	14.1	123151	9043
Pfam	32.0	17929	17421
PIRSF	3.02	3285	3217
PRINTS	42.0	2106	1953
PROSITE patterns	2019_01	1310	1287
PROSITE profiles	2019_01	1232	1173
SFLD	4	303	147
SMART	7.1	1312	1264
SUPERFAMILY	1.75	2019	1601
TIGRFAMs	15.0	4488	4435

* Some signatures may not have matches to UniProtKB proteins.

** Not all signatures of a member database may be integrated at the time of an InterPro release

Other sequence features

☑ Coils ☑ Phobius ☑ SignalP ☑ TMHMM

Interproscan

Sequence database	Version	Count	Count of proteins matching	
			any signature	integrated signatures
UniProtKB	2019_04	156637804	130888307 (83.6%)	126806860 (81.0%)
UniProtKB/TrEMBL	2019_04	156077686	130343729 (83.5%)	126265196 (80.9%)
UniProtKB/Swiss-Prot	2019_04	560118	544578 (97.2%)	541664 (96.7%)

InterPro2GO

Total number of GO terms mapped to InterPro entries - 34141

Not integrated signatures = signature not yet curated or do not reach InterPro's standards for integration

pathway information available as well:

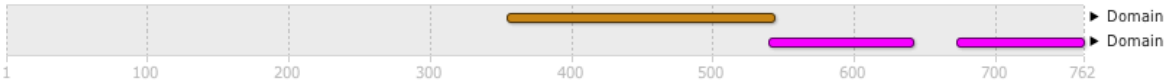
- KEGG
- MetaCyc
- Reactome
- UniPathway

Interproscan results

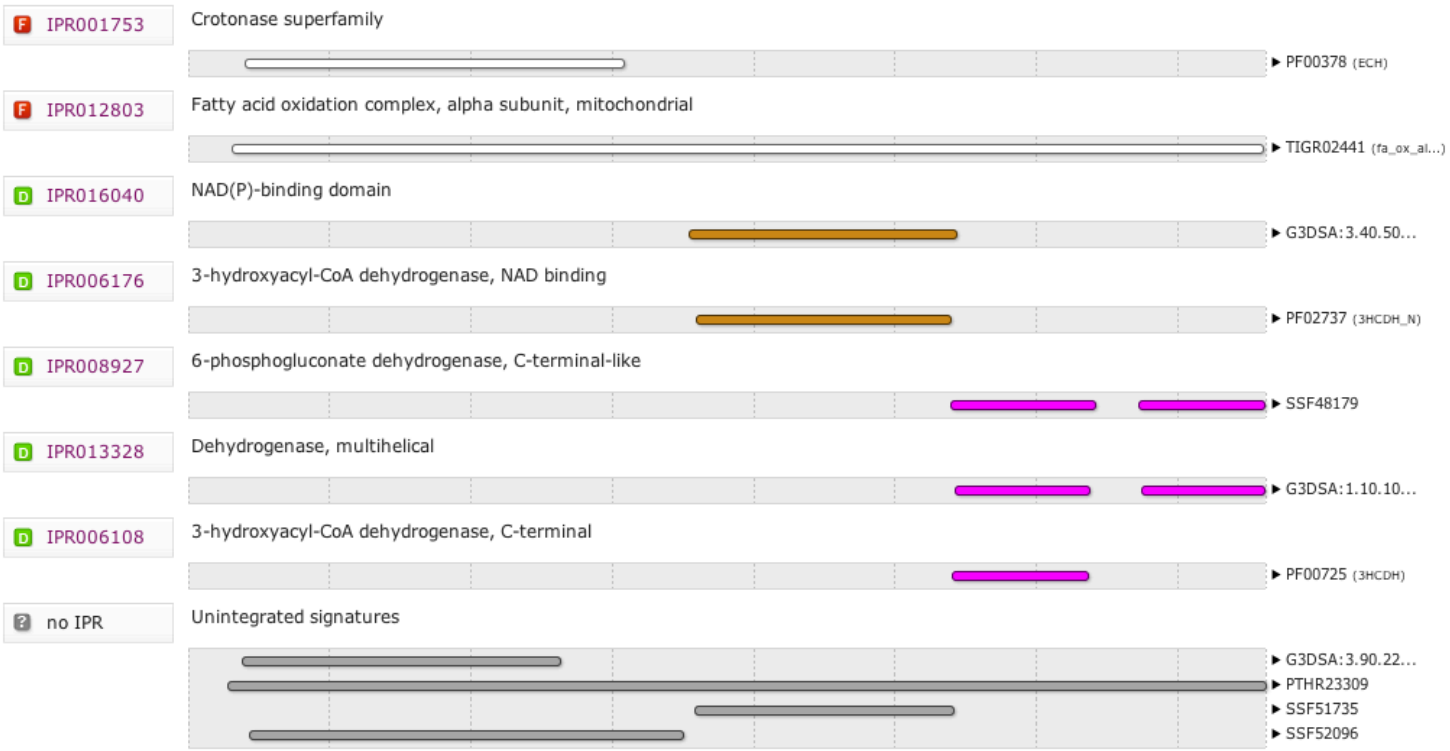
Protein family membership

- Crotonase superfamily (IPR001753)
  - Fatty acid oxidation complex, alpha subunit, mitochondrial (IPR012803)

Domains and repeats



Detailed signature matches



Interproscan results

Ouput: TSV, XML, SVG, etc

gene-2.44-mRNA-1 a9deba5837e2614a850c7849c85c8e9c 447 Pfam PF02458 Transferase family 98 425 1.4E-15 T 31-10-2015 IPR003480 Transferase GO:0016747

gene-0.13-mRNA-1 61882f1a46b15c8497ed9584a0eb1a35 459 Pfam PF01490 Transmembrane amino acid transporter protein 49 439 2.0E-39 T 31-10-2015 IPR013057 Amino acid transporter, transmembrane

gene-1.4-mRNA-1 b867bbb377084bba6ea84dcda9f27f4e 511 SUPERFAMILY SSF103473 42 481 4.19E-50 T 31-10-2015 IPR016196 Major facilitator superfamily domain, general substrate transporter

gene-1.4-mRNA-1 b867bbb377084bba6ea84dcda9f27f4e 511 Pfam PF07690 Major Facilitator Superfamily 67 447 3.5E-30 T 31-10-2015 IPR011701 Major facilitator superfamily GO:0016021|GO:0055085

Scripts exist to merge the interproscan-results to the structural annotation gff file

Blast2GO

Another way : use the (mostly) commercial alternative

- Combines a blast-based search with a search for functional domains
- Blast at NCBI -> picks out GO terms based on blast hits and uniprot -> statistical significance test -> done!
- Blast2Go relies entirely on sequence similarity … but InterProScan searches can also be launched within blast2go
- Command line tool or Plugin for Geneious or CLC bio Workbench (commercial tools for downstream analyses)

=> Contain nice downstream analysis/visualization components

Blast2GO

/Users/hobbe/Documents/Artemis_files_current/blast2go_20101001_0816.dat - Blast2GO V.2.4.4

File Blast Mapping Annotation Analysis Statistics Select Tools View Info

GO:0007067,GO:0016021 | transport;binding;apoptos | SPO_2518,DDX18_HUMAN

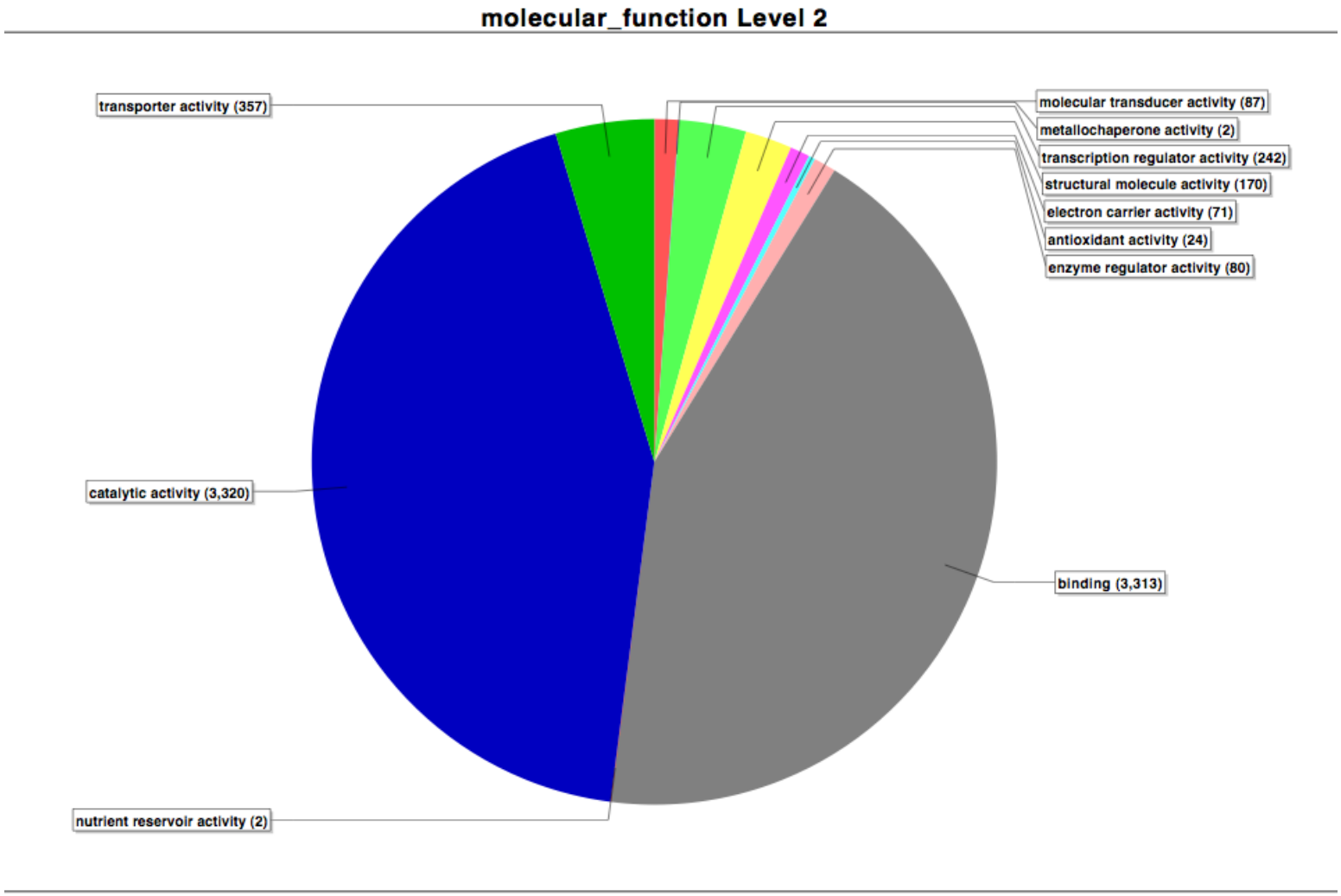
nr	sequence name	seq description	length	#...	min. eValue	sim mean	#G...	GO IDs	Enzyme	InterPro
3884	gene_3884|GeneMar...	c6 transcription	977	20	1.0E-171	59.85%	7	F:transcription factor activity; F:zinc ion binding; P:regulation of transcription, DNA-dependent; C:transcription factor complex; F:transporter activity; C:membrane; P:transmembrane transport		IPR005829; IPR007219
3885	gene_3885|GeneMar...	hypothetical protein NFIA_039100 [Neosartorya fischeri NRRL 181]	312	20	1.0E-39	63.15%	1	C:viral capsid	-	no IPS match
3886	gene_3886|GeneMar...	sin3 complex subunit	870	20	0.0	73.2%	0		-	-
3887	gene_3887|GeneMar...	mitochondrial intermembrane space translocase subunit	87	20	1.0E-40	88.55%	5	F:metal ion binding; P:protein import into mitochondrial inner membrane; C:mitochondrial inner membrane; C:mitochondrial intermembrane space protein transporter complex; P:transmembrane transport		IPR004217; PTHR11038 (PANTHER), PTHR11038:SF8 (PANTHER)
3888	gene_3888|GeneMar...	lysyl-trna synthetase	592	20	0.0	73.55%	7	C:cytoplasm; P:auxin biosynthetic process; F:nucleic acid binding; F:lysine-tRNA ligase activity; P:lysyl-tRNA aminoacylation; F:ATP binding; P:lysine biosynthetic process	EC:6.1.1.6	IPR004364; IPR004365; IPR006195; IPR012340; IPR016027; IPR018149; IPR018150; G3DSA:3.30.930.1((GENE3D), SSF5568 (SUPERFAMILY)
3889	gene_3889|GeneMar...	transcription factor	1569	20	0.0	70.9%	0		-	-
3890	gene_3890|GeneMar...	conserved hypothetical protein [Aspergillus clavatus NRRL 1]	240	20	1.0E-51	56.25%	0		-	-
		udp-glc gal endoplasmic						C:integral to membrane; C:endoplasmic reticulum membrane; P:transmembrane transport; P:carbohydrate		IPR013657; PTHR10778

GO Graphs | Application Messages | Blast/IPS Results | Statistics | Kegg Maps

```
17:59   InterProScan for gene_8871|GeneMark.hmm|286_aa done.
17:59   --------------------
17:59   InterProScan Result:
17:59   InterProId: IPR001715
17:59   InterProName: Calponin-like actin-binding
17:59   InterProType: Domain
17:59           DB-Name: GENE3D - G3DSA:1.10.418.10
17:59   InterProId: IPR016146
17:59   InterProName: Calponin-homology
17:59   InterProType: Domain
17:59           DB-Name: SUPERFAMILY - SSF47576
17:59   InterProId: noIPR
17:59   InterProName: unintegrated
17:59   InterProType: unintegrated
17:59           DB-Name: PANTHER - PTHR19961
17:59           DB-Name: PANTHER - PTHR19961:SF9
```

Annotation already running

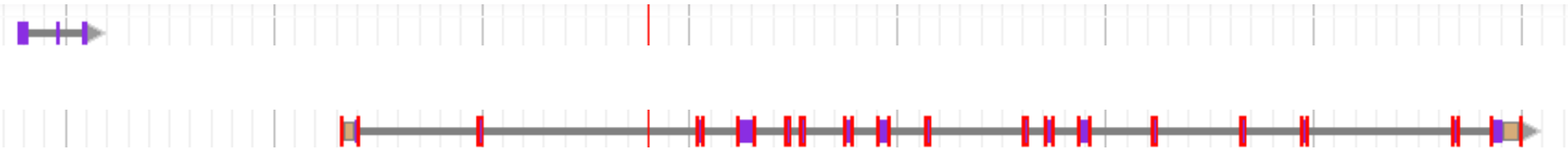
Blast2GO


molecular_function Level 2
transporter activity (357)
molecular transducer activity (87)
metallochaperone activity (2)
transcription regulator activity (242)
structural molecule activity (170)
electron carrier activity (71)
antioxidant activity (24)
enzyme regulator activity (80)
catalytic activity (3,320)
binding (3,313)
nutrient reservoir activity (2)

Quick view of synteny-based method

Liftovers are very useful for orthology determination

- Align two genomes (Satsuma) (http://satsuma.sourceforge.net/)
- Transfer annotations between aligned regions (Kraken)(https://github.com/nedaz/kraken)
- Transfer functional annotations between lifted genes that overlap



One word about network

Categorizations of gene function (e.g GO) in a hierarchy of categories is helpful

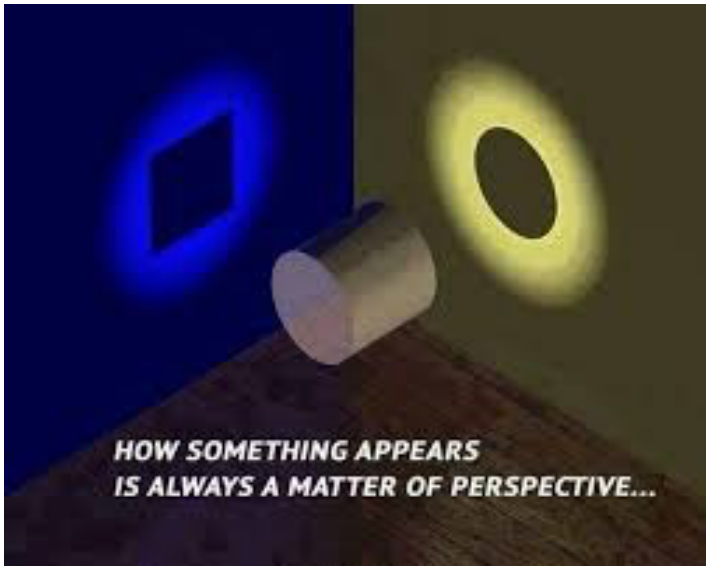
BUT

gene has no function alone

⇒ Pathways / regulatory networks explain how genes interact so what they are doing!

E.g. databases for pathway :

- KEGG
- MetaCyc
- Reactome
- UniPathway



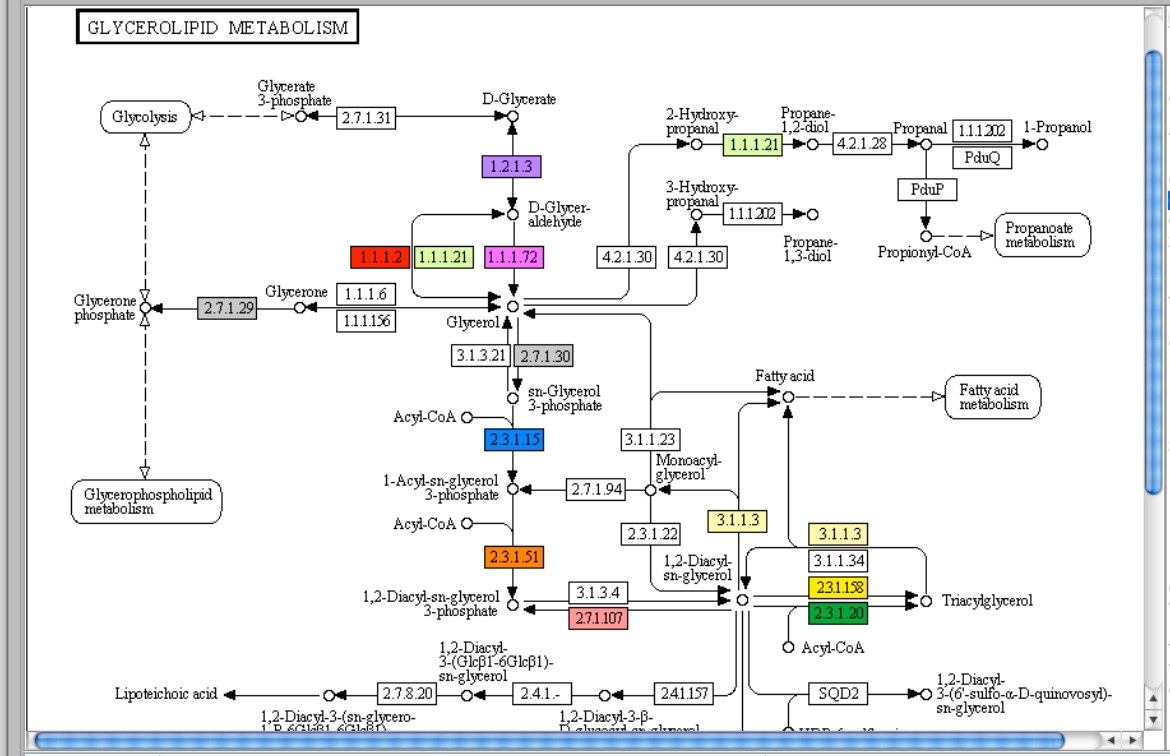
KEGG-mapping

File Blast Mapping Annotation Analysis Statistics Select Tools View Info

GO:0007067,GO:0016021 | transport;binding;apoptos | SPO_2518,DDX18_HUMAN

nr	sequence name	seq description	length	#...	min. eValue	sim mean	#G...	GO IDs	Enzyme	InterPro
		succinyl- synthetase subunit						F:ATP binding; F:succinate-CoA ligase (GDP-forming) activity; P:tricarboxylic acid cycle; C:succinate-CoA ligase		IPR003781; IPR005810;

GO Graphs | Application Messages | Blast/IPS Results | Statistics | Kegg Maps



Pathways
- Pentose phosphate pathway
- Fructose and mannose metabolism
- Butanoate metabolism
- Carbon fixation in photosynthetic organisms
- Lysine degradation
- Tyrosine metabolism
- Methane metabolism
- Glyoxylate and dicarboxylate metabolism
- Glycerolipid metabolism
- Glutathione metabolism
- Selenoamino acid metabolism
- Phenylalanine metabolism
- Benzoate degradation via CoA ligation
- Valine, leucine and isoleucine biosynthesis
- Reductive carboxylate cycle (CO2 fixation)
- Galactose metabolism
- Phenylalanine, tyrosine and tryptophan biosynthesis
- N-Glycan biosynthesis
- Photosynthesis
- Drug metabolism - other enzymes
- Sulfur metabolism
- Fatty acid biosynthesis
- Inositol phosphate metabolism
- beta-Alanine metabolism
- Drug metabolism - cytochrome P450
- Pantothenate and CoA biosynthesis
- Biosynthesis of unsaturated fatty acids
- Cyanoamino acid metabolism
- Terpenoid backbone biosynthesis
- Histidine metabolism
- T cell receptor signaling pathway
- Tropane, piperidine and pyridine alkaloid biosynthesis
- One carbon pool by folate
- Pentose and glucuronate interconversions
- Phosphatidylinositol signaling system

Color	Enzyme	Sequences
red	ec:1.1.1.2 - alcohol dehydrogenase (NADP+)	gene_674|GeneMark.hmm|333_aa, gene_5801|GeneMark.hmm|312_aa
yellow	ec:2.3.1.158 - phospholipid:diacylglycerol acyltransferase	gene_2604|GeneMark.hmm|188_aa, gene_6532|GeneMark.hmm|505_aa
orange	ec:2.3.1.51 - 1-acylglycerol-3-phosphate O-acyltransferase	gene_176|GeneMark.hmm|429_aa, gene_6693|GeneMark.hmm|292_aa
green	ec:2.3.1.20 - diacylglycerol O-acyltransferase	gene_176|GeneMark.hmm|429_aa, gene_7213|GeneMark.hmm|521_aa, gene_8170|GeneMark.hmm|470_aa
blue	ec:2.3.1.15 - glycerol-3-phosphate O-acyltransferase	gene_886|GeneMark.hmm|748_aa, gene_2640|GeneMark.hmm|823_aa
pink	ec:1.1.1.72 - glycerol dehydrogenase (NADP+)	gene_3376|GeneMark.hmm|325_aa, gene_4577|GeneMark.hmm|326_aa
violet	ec:1.2.1.3 - aldehyde dehydrogenase (NAD+)	gene_2201|GeneMark.hmm|497_aa, gene_5247|GeneMark.hmm|502_aa, gene_5611|GeneMark.hmm|471_aa
light-red	ec:2.7.1.107 - diacylglycerol kinase	gene_5292|GeneMark.hmm|409_aa

Annotation already running

Conclusion

- Functional annotation found

  /!\ Transmission of error from databases !

  Experimental check is good !

- Hypothetical protein / Uncharacterized protein

  => depends largely on conventional experiments.

Knowing the function is not enough: Chimp and human => 98% similarity

=> Knowledge of other parameters useful (pathway, positional and temporal regulation of genes)