GONNA: a Gene Ontology Nearest Neighbor Approach for the functional prediction of *P. falciparum* orphan genes

The database of the predictions

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Méthodes
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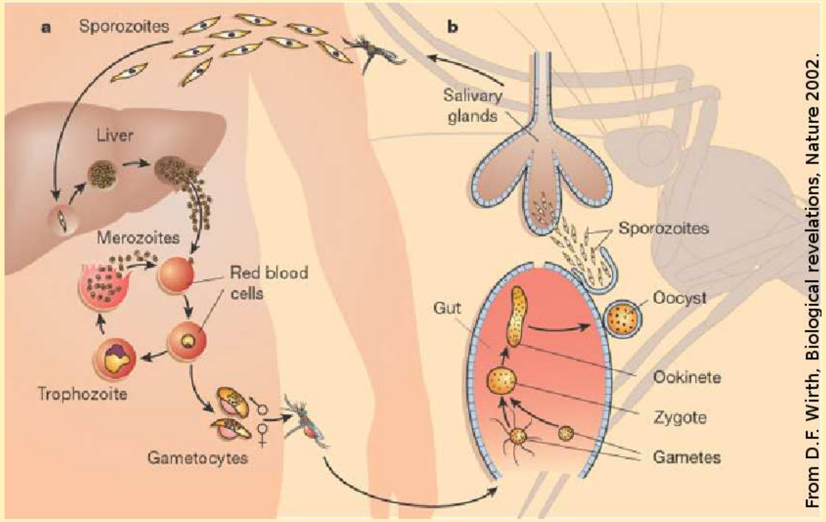
Plasmodium falciparum



From D.F. Wirth, Biological revelations, Nature 2002.

An atypical genome [Gardner et al., 2002]

- above 80% of A/T,
- only $\sim 40\%$ of the $5,300$ predicted genes can be annotated by sequence homology
  - because no homologous genes have already been characterized in other genomes
  - because standard tools fail to detect homology (sequence divergence is too large)

Non-homology based methods are needed to better characterize the $\sim 60\%$ of orphan genes

Guilt By Association (GBA) methods

Works in an intra-species way: the genes already characterized in the genome, *e.g.* by wet experiments or using sequence homology, help for the annotation of the other genes (the guilt by association principle)

Different postgenomic data can be used

- Transcriptomic data: genes with similar transcriptomic profiles are likely to share common functional roles [Eisen et al., 1998, Lockhart and Winzeler, 2000]
- Protein interaction data: proteins that share common interactors likely share common functions [Brun et al., 2003, Vazquez et al., 2003, Chen and Xu, 2004]
- Proteomic data, etc.

Two frameworks

- Unsupervised methods: unsupervised classification algorithms (clustering) + statistical test to search for over-represented functions
- Supervised methods: supervised classification algorithms to learn a gene function predictor

The Gene Ontology (GO)

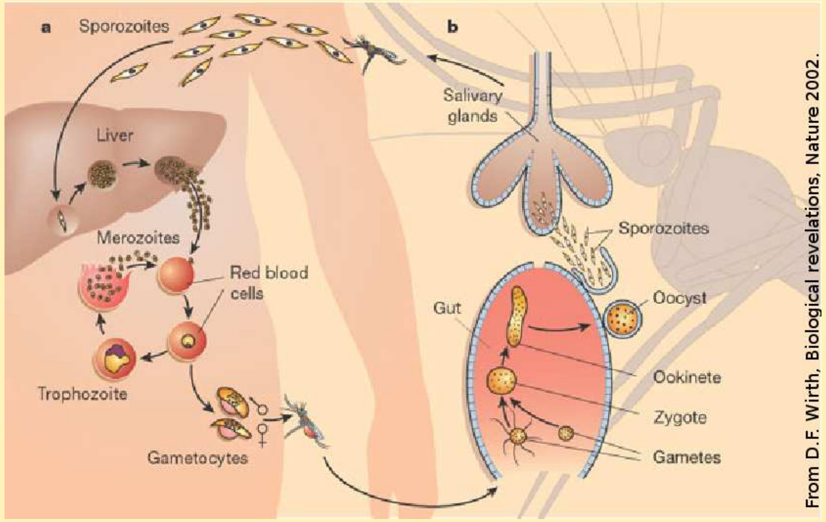
http://www.geneontology.org

- A systematic and standardized nomenclature to annotate genes in various organisms
- Three main ontologies:
  - Molecular Function
  - Biological Process
  - Cellular Component

- GO:0008150 : biological process
  - GO:0050789 : regulation of biological process
  - GO:0007582 : physiological process
    - GO:0008152 : metabolism
      - GO:0009058 : biosynthesis
        - GO:0044249 : cellular biosynthesis
          - GO:0009165 : nucleotide biosynthesis
          - GO:0016053 : organic acid biosynthesis
    - GO:0050875 : cellular physiological process
      - GO:0044237 : cellular metabolism
        - GO:0044249 : cellular biosynthesis

- Describes generalization relationships between hundreds of terms
- A gene may be annotated with several GO terms
- If a gene is annotated with a term t, then it is also annotated with all the terms that generalize t

P. falciparum: several postgenomic datasets available



From D.F. Wirth, Biological revelations, Nature 2002.

- **6 transcriptomic datasets:**
  - [Le Roch et al., 2003] 9 stages of the entire cycle of strain 3D7
    Measurements for $\sim 5,100$ genes
  - [Bozdech et al., 2003, Llinas et al., 2006] 48h intraerythrocytic developmental cycle for 3 strains: HB3, 3D7 and Dd2
    Measurements for $\sim 4,200$ genes
  - [Young et al., 2005] sexual developmental cycle (gametocytes) for 2 strains: 3D7 and NF54
    Measurements for $\sim 5,100$ genes
- **1 proteomic dataset:**
  [Florens et al., 2002, Le Roch et al., 2004] 7 stages of the entire cycle of strain 3D7
  Measurements for $\sim 2,900$ genes
- **1 protein interaction dataset:**
  [LaCount et al., 2005]
  Measurements for $\sim 1,300$ genes

GONNA - 1

Parameters

- For each postgenomic dataset d, compute a function $\mathcal{D}^d$ measuring the level of similarity $\mathcal{D}^d(g,h)$ of every pair of genes (g,h)
  - transcriptomic/proteomic data: Pearson correlation coefficient
    $\longrightarrow$ genes with correlated transcriptomic/proteomic profile have high similarity
  - protein interaction data: Czekanovski-Dice metric [Dice, 1945]
    $\longrightarrow$ genes that share many interactors have high similarity
- K and $K' \leq K$, two integers

Principle

Let g be an orphan gene

1. use the function $\mathcal{D}^d$ and the already characterized genes to search for the K nearest neighbors of g
2. for each GO term t, if at least K' of the K nearest neighbors are annotated with t, predict g to be annotated with t

GONNA - 2

Advantages

- predictions can be explained
- can be used with any present and future postgenomic dataset, as long as we have a relevant similarity measure
- consistent with the structure of the ontology
- low computing time: the confidence of the predictions can be assessed by cross-validation

Critical choices

- the similarity measure
- K: neither too large (neighbors are not similar) nor too small (sample is not representative)
- K':
  - high (close to K)
    - proportion of good predictions is high
    - few predictions on the most specific terms of the ontology
  - low
    - proportion of good predictions is lower
    - more predictions on the the most specific terms of the ontology

Assessing the confidence of the predictions made with a dataset

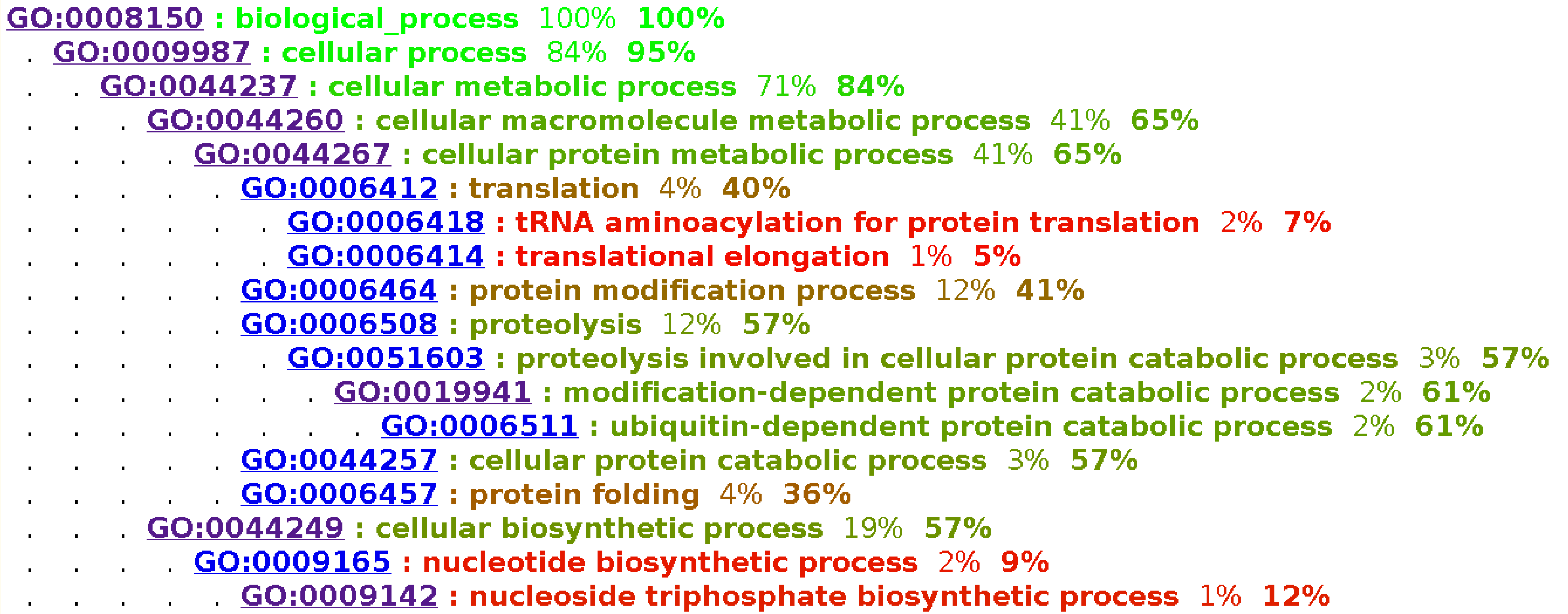
Leave-one-out Cross-validation (CV) [Hastie et al., 2001]

1. run GONNA on each characterized gene as it was an orphan gene
2. for each GO term t, compute the proportion of times GONNA is right when predicting that a gene has annotation t:
   True Discovery Rate (TDR) associated with t

Features

- confidence of the predictions can be estimated for each GO term
- highlights the parts of the ontology that are more suitable to apply a GBA approach with the considered dataset

An extract achieved with [Le Roch et al., 2003]



```
GO:0008150 : biological_process  100%  100%
 . GO:0009987 : cellular process  84%  95%
 .   . GO:0044237 : cellular metabolic process  71%  84%
 .   .   . GO:0044260 : cellular macromolecule metabolic process  41%  65%
 .   .   .   . GO:0044267 : cellular protein metabolic process  41%  65%
 .   .   .   .   . GO:0006412 : translation  4%  40%
 .   .   .   .   .   . GO:0006418 : tRNA aminoacylation for protein translation  2%  7%
 .   .   .   .   .   . GO:0006414 : translational elongation  1%  5%
 .   .   .   .   . GO:0006464 : protein modification process  12%  41%
 .   .   .   .   . GO:0006508 : proteolysis  12%  57%
 .   .   .   .   .   . GO:0051603 : proteolysis involved in cellular protein catabolic process  3%  57%
 .   .   .   .   .   .   . GO:0019941 : modification-dependent protein catabolic process  2%  61%
 .   .   .   .   .   .   .   . GO:0006511 : ubiquitin-dependent protein catabolic process  2%  61%
 .   .   .   .   . GO:0044257 : cellular protein catabolic process  3%  57%
 .   .   .   .   . GO:0006457 : protein folding  4%  36%
 .   .   . GO:0044249 : cellular biosynthetic process  19%  57%
 .   .   .   . GO:0009165 : nucleotide biosynthetic process  2%  9%
 .   .   .   .   . GO:0009142 : nucleoside triphosphate biosynthetic process  1%  12%
```

The database of the predictions

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http://atgc.lirmm.fr/plasmo_draft/
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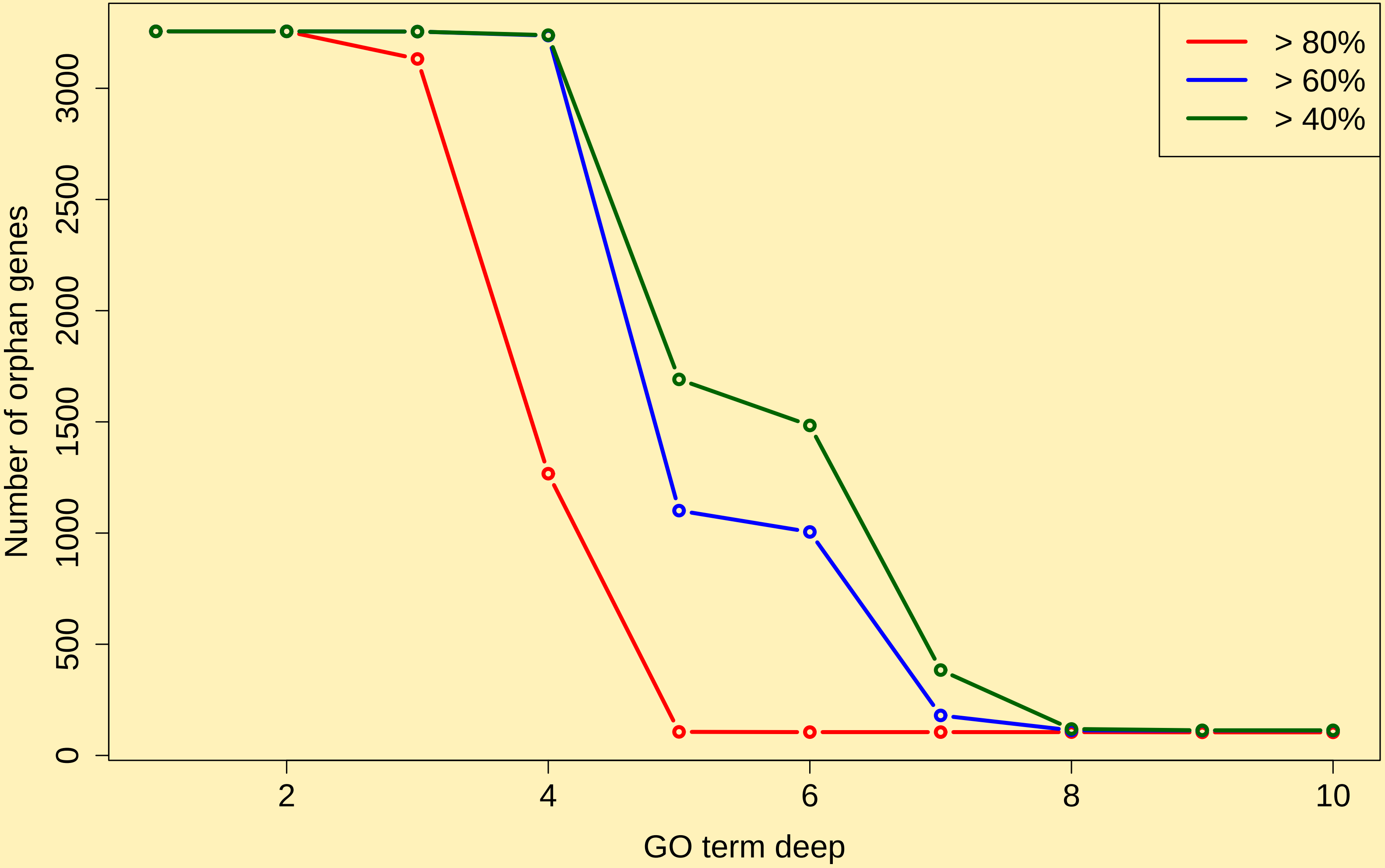
- Run GONNA on all available datasets using two sets of parameters (K, K'):
  - a stringent set $(K = 6, K' = 4)$ to achieve high TDRs in the most suitable GO terms
  - a non-stringent set $(K = 6, K' = 2)$ to allow predictions in the more “difficult” GO terms
- Pool all the predictions made with the different postgenomic datasets
- The database can be
  - browsed through the Gene Ontology
  - queried by GO terms and genes

Assessing the global performances

Achieved with the transcriptomic dataset of [Le Roch et al., 2003]



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