10 - Usage of Gene Ontology

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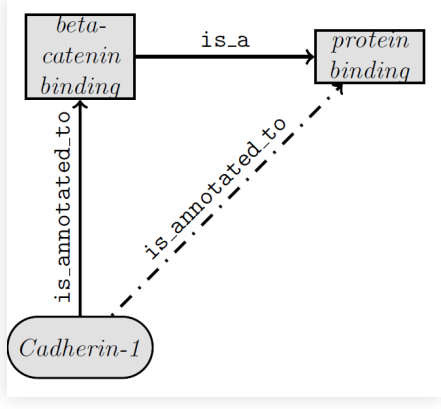
Inference in the Gene Ontology

Inference in the Gene Ontology

True Path Rule

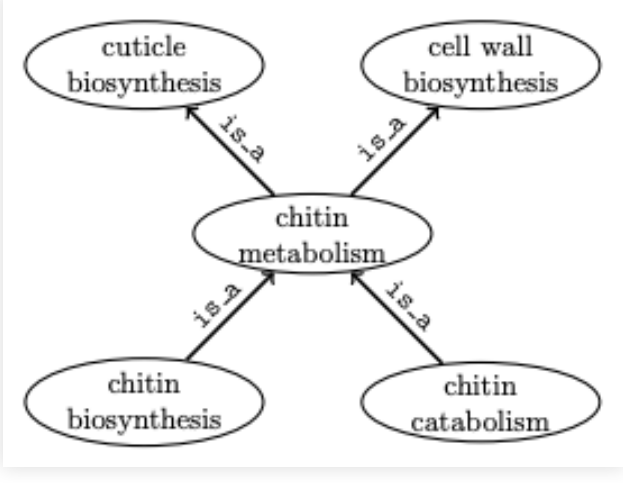
- Gene Products are annotated to the most specific GO term
- Annotations are (implicitly) propagated to ancestor terms (**is_a**) as well as via **part_of**
- True Path Rule: path from a child term through all ancestors back to the root must be biologically accurrate



Inference in the Gene Ontology

Problem Example

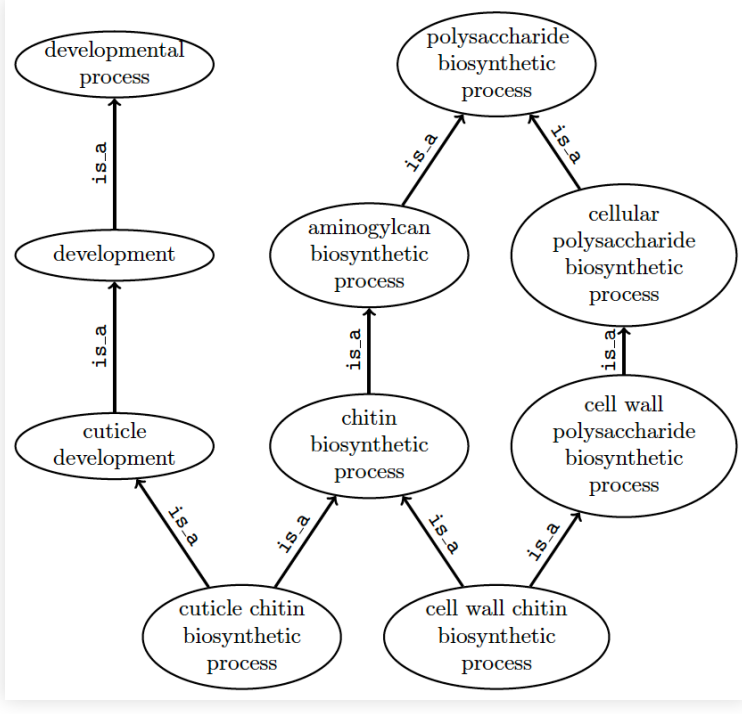
- For one species (flies): chitin metabolism a child of cuticle synthesis
- But chitin metabolism also part of cell wall organization in yeast
- Yeast gene annotated with chitin biosynthesis implies annotation to cuticle biosynthesis, but yeast does not have cuticles



Inference in the Gene Ontology

Problem fix

- Introduction of new GO terms separating the two kinds of processes



Inference in the Gene Ontology

Inferences over GO Edges

- A number of inference rules have been established
- For instance:

$$A \xrightarrow{is\_a} B \wedge B \xrightarrow{part\_of} C \Rightarrow A \xrightarrow{part\_of} C$$

Inference in the Gene Ontology

Example

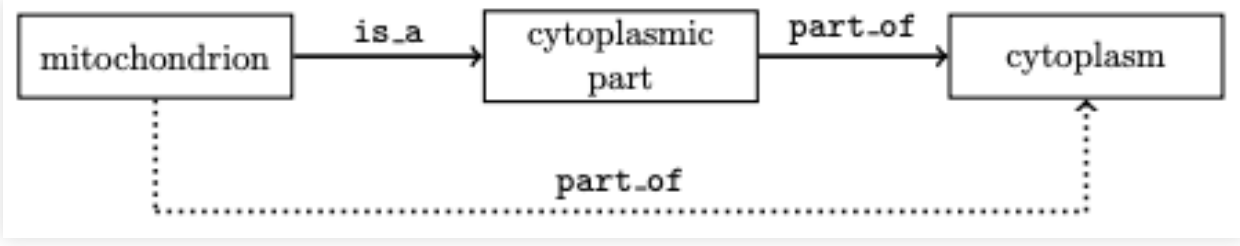
```
[Term]
id: GO:0044444
name: cytoplasmic part
is_a: GO:0044424 ! intracellular part
relationship: part_of GO:0005737 ! cytoplasm

[Term]
id: GO:0005737
name: cytoplasm

[Term]
id: GO:0005739
name: mitochondrion
is_a: GO:0044444 ! cytoplasmic part
```

- We can infer that mitochondrion **part_of** cytoplasm holds

Inference in the Gene Ontology

Example

- We can infer that mitochondrion **part_of** cytoplasm holds



- This is not the same as saying mitochondrion **is_a** cytoplasm

Inference in the Gene Ontology

Other Inference rules

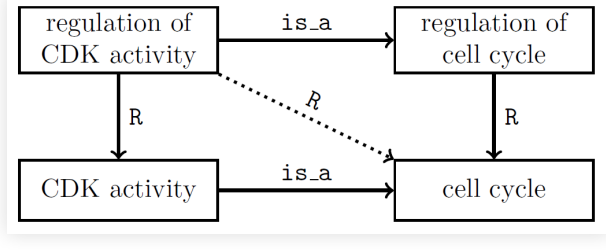
$$A \xrightarrow{part\_of} B \wedge B \xrightarrow{is\_a} C \Rightarrow A \xrightarrow{part\_of} C$$

- Transitivity

$$A \xrightarrow{is\_a} B \wedge B \xrightarrow{is\_a} C \Rightarrow A \xrightarrow{is\_a} C$$

$$A \xrightarrow{part\_of} B \wedge B \xrightarrow{part\_of} C \Rightarrow A \xrightarrow{part\_of} C$$

- Similar rules apply to **has_part**
- As well as to **regulates** and its subproperties **positively_regulates** and **negatively_regulates**



Inference in the Gene Ontology

Intra-GO Cross-Product Definitions

```
[Term]
id: GO:0000152
name: nuclear ubiquitin ligase complex
namespace: cellular_component
def: "A ubiquitin ligase complex found in the nucleus." [GOC:mah]
is_a: GO:0000151 ! ubiquitin ligase complex
is_a: GO:0044428 ! nuclear part
intersection_of: GO:0000151 ! ubiquitin ligase complex
intersection_of: part_of GO:0005634 ! nucleus
```

- Intersections provide an equivalent definition

$$GO:0000152 \equiv GO:0000151 \sqcap \exists part\_of.GO:0005634$$

- Follows the design pattern of a more specific class X (of general class G) differentiated by an additional discriminant D
- X is a G such that D
- GO:0000152 is a GO:0000151 such that $\exists part\_of.GO:0005634$

External Cross-Product Definitions

```
[Term]
id: GO:0001510 ! RNA methylation
intersection_of: GO:0008152 ! metabolic process
intersection_of: OBO_REL:results_in_addition_of CHEBI:32875 !methyl group
intersection_of: OBO_REL:results_in_addition_to CHEBI:33697 !ribonucleic acid
```

- With external terms from the ChEBI ontology (Chemical Entities of Biological Interest)

Reasoning with Cross-Product Definitions

```
[Term]
id: GO:0030223 ! neutrophil differentiation
intersection_of: GO:0030154 ! cell differentiation
intersection_of: OBO_REL:results_in_acquisition_of_features_of\ CL:0000775
   ! neutrophil

[Term]
id: GO:0030851 ! granulocyte differentiation
intersection_of: GO:0030154 ! cell differentiation
intersection_of: OBO_REL:results_in_acquisition_of_features_of\ CL:0000094
   ! granulocyte

[Term]
id: CL:0000775
name: neutrophil
is_a: CL:0000094 ! granulocyte
```

- We can infer that neutrophil differentiation is a subclass of granulocyte differentiation

Overrepresentation Analysis

Overrepresentation Analysis

Overview

- High-throughput technologies in molecular biology

- Allow to measure all genes in the genome experimentally
- DNA microarrays with thousands of probes
- Quantify the amount of corresponding sequences in the sample

- Typical microarray experiments:

- Compare gene expression profiles (their concentrations) under two or more biological conditions
- E.g., comparison between healthy and diseased tissue or different developmental stages
- Several replicate microarray experiments for each biological condition
- Statistical analysis for significant differences for each gene
- Outcome often a list of hundreds/thousands of differentially expressed genes

Overrepresentation Analysis

Idea

- Question: One or more specific GO term annotates more of the differentially expressed genes than one would expect by chance?
- Example

- Say 221 of 6000 yeast genes (3.7%) represented on a microarray are annotated to the GO term sporulation
- If we perform some experiment and observe 100 differentially expressed genes, 3 or 4 should be annotated with sporulation, merely by chance
- Suppose that 35 of 100 are annotated to sporulation
- We conclude that sporulation is overrepresented among differentially expressed genes

Overrepresentation Analysis

Issue

- We may based on such observations develop hypotheses to justify the outcome
  - Determine subsequent experiments to test the hypothesis
- Multiple overrepresented GO terms may be indentified, inflating the number of significantly overrepresented terms
  - List of 50 to 100 GO terms is not helpful in principle for determining which of the terms is the most characteristic

Overrepresentation Analysis

Solution

- Several algorithms based on hypergeometric distribution and related concepts

- Some on a term for term basis
- Irrelevant terms are omitted upfront (filtered)
- Still often many corrolated terms in results - propagation rule - which one is the most suitable?
- Being annotated to a given GO term, also implies annotation to its ancestors
- Tests for overrepresentation of similar terms are not statistically independent

Overrepresentation Analysis

Solution

- Parent-child algorithms

- Takes the propagation rule into account for determining overrepresentation

- Topology-based algorithms

- Find the most specific overrepresented terms

- Other model-based approaches

- Rather than a term for term analysis, an optimization problem is created that associates a score to a set of GO terms, trying to find an optimal combination of GO terms that together best explain the observed pattern

Semantic Similarity

Semantic Similarity

Overview

- Concepts in ontologies are connected by semantic relations
- Measures of similiarity for terms can be defined based on that
- Used for

- Validating results of gene expression clustering
- Predicting molecular interactions
- Disease gene prioritization
- Clinical diagnostics

Basic Idea - Information Content

- For ontologies of is_a relations
- Define a propability function p such that $p(C_i)$ is the probability of encountering an instance of class C_i
- Recall that $x\ instance\_of\ A$ and $A\ is\_a\ B$ implies $x\ instance\_of\ B$, i.e., p is monotonically increasing as we move to more general concepts
- Unique root C has $p(C) = 1$
- Information Content of a term t:

$$IC(t) = -log\ p(t)$$

- More general concepts provide less information

Semantic Similarity

Probability of a term

- Intrinsic: uses internal structure of the ontology

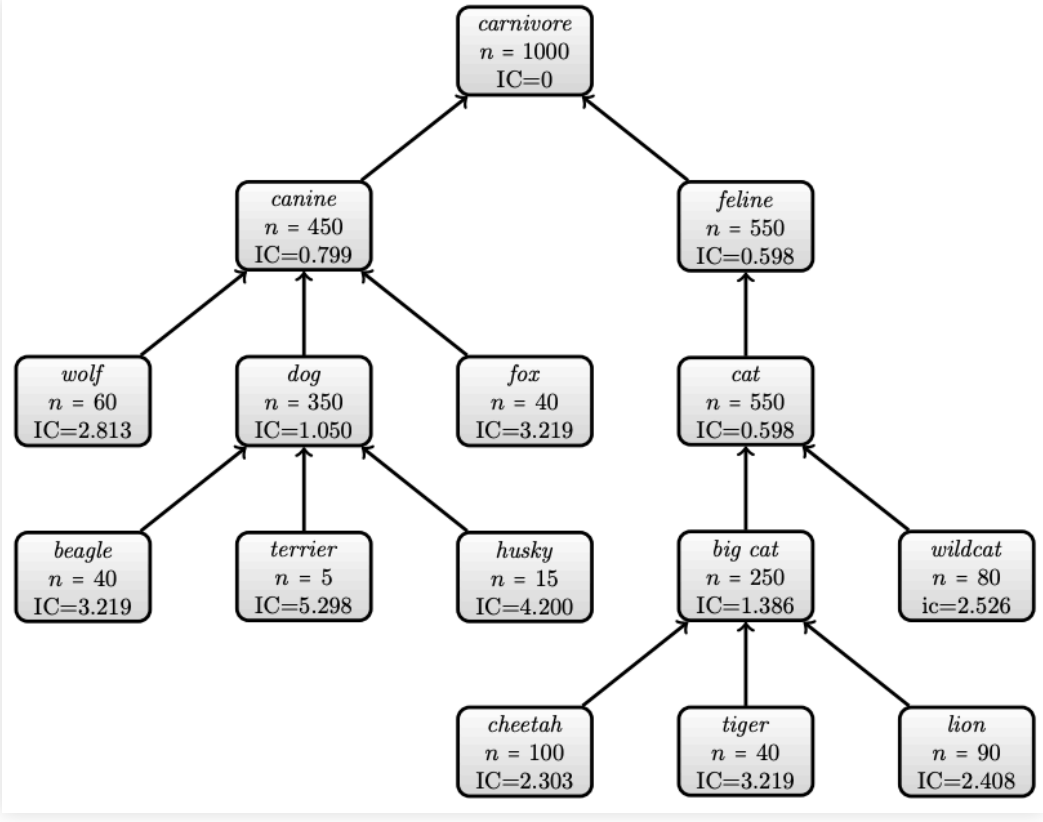
- e.g. number of descendants / total number of Entities

- Extrinsic: uses frequency on a dataset

- probability that a randomly chosen protein is annotated to t, if we choose the protein from the set of all proteins under consideration
- Terms that annotate many genes have low information content
- Terms that annotate few genes have high information content

Example on Information Content

- Annotations on 1000 documents about carnivores



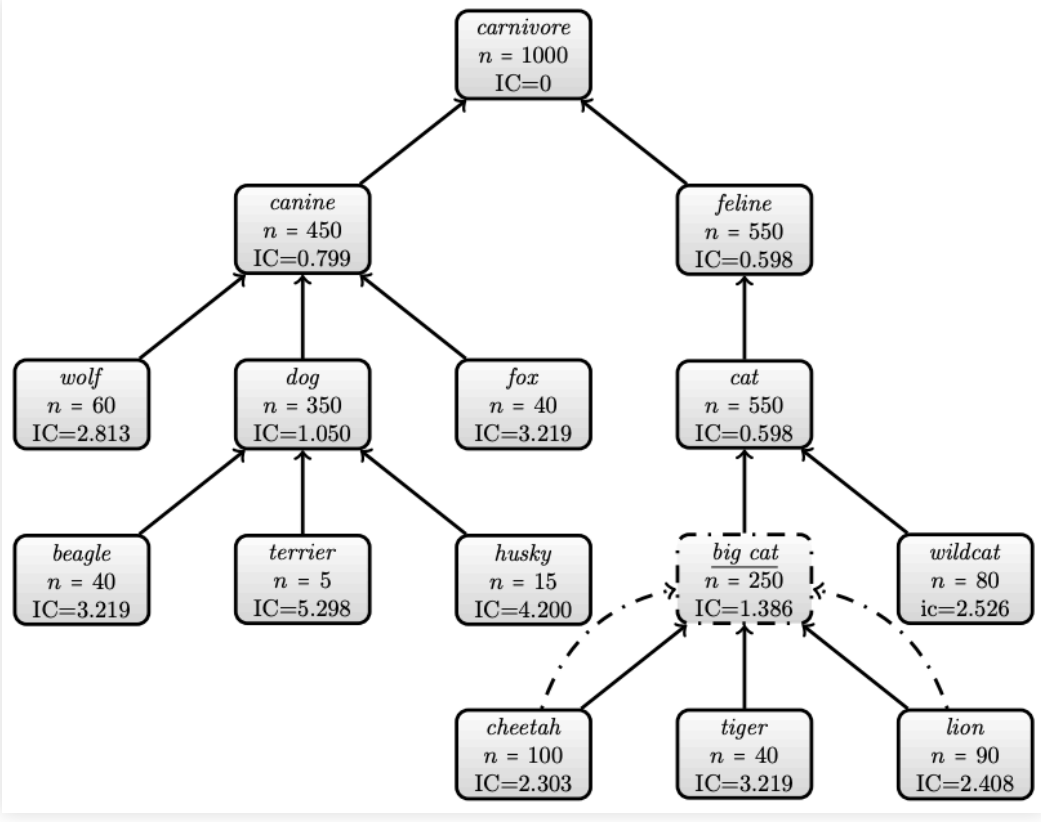
Resnik Semantic Similarity

- The more information two terms share, the more similar they are
- Information shared between two terms is indicated by the information content of their Most Informative Common Ancestor (MICA)
- Similarity between two terms determined as follows:

$$sim(t_1, t_2) = IC(MICA(t_1, t_2)) = max_{t \in Anc(t_1) \cap Anc(t_2)} IC(t)$$

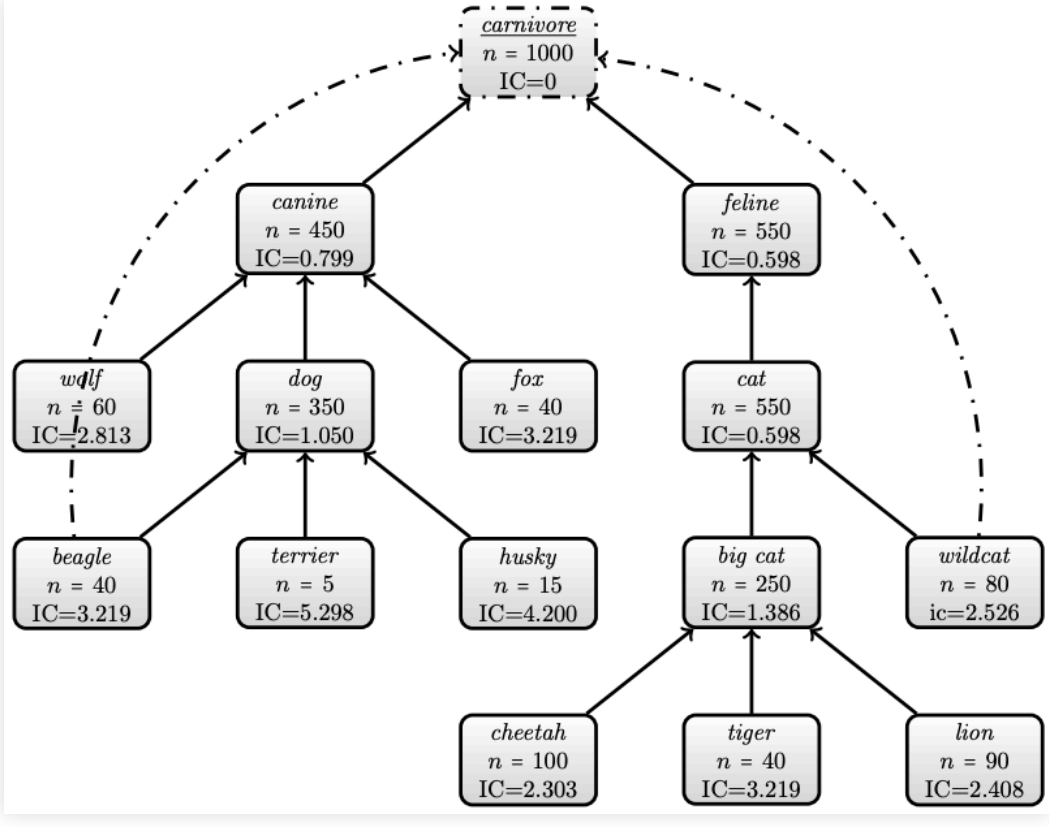
Example on similarity

- Similarity between cheetah and lion



Example on similarity

- Similarity between beagle and wildcat



Semantic Similarity

Improvements

- Variants of similarity measure have been developed

- Take also into account the distance between the terms
- Otherwise wolf and fox are are as similar as beagle and fox
- Can be measured by path length
- But depends on the ontology
- Maximum similarity is reached if the terms are identical

$$sim_{Lin}(t_1, t_2) = (2 \times max_{t \in Anc(t_1) \cap Anc(t_2)} IC(t))/(IC(t_1) + IC(t_2))$$

- Further notions have been defined

Semantic Similarity

Applied to GO

- Focus is on similarity between genes annotated by terms - not similarity between terms
- Similarity measures defined based on the similarity of its annotations

- Maximum value of all pairs
- Average
- Also graph-based approaches relying on counting edges
- Set-based measures, e.g., intersection of annotations/union of annotations

Usage of Gene Ontology

Summary

- Inference in the Gene Ontology
- Overrepresentation Analysis
- Semantic Similarity

Further reading:

- Robinson and Bauer, Introduction to Bio-Ontologies, Chapters 8, 10, 12
- Dessimoz and Skunca, The Gene Ontology Handbook
- GO webpage http://geneontology.org/

Exercises

Inference

```
[Term]
id: GO:0007519
name: skeletal muscle tissue development
is_a: GO:0014706 ! striated muscle tissue development
relationship: part_of GO:0060538 ! skeletal muscle organ\development

[Term]
id: GO:0014706
name: striated muscle tissue development
is_a: GO:0060537 ! muscle tissue development

[Term]
id: GO:0060537
name: muscle tissue development
is_a: GO:0009888 ! tissue development
relationship: part_of GO:0007517 ! muscle organ development
```

- The CSRPP3 gene is annotated to the GO term skeletal muscle tissue development. What other annotations can we infer for this protein and why?

Exercises

Inference

```
[Term]
id: GO:0006310
name: DNA recombination
is_a: GO:0006259 ! DNA metabolic process
[Term]
id: GO:0042148
name: strand invasion
is_a: GO:0006259 ! DNA metabolic process
relationship: part_of GO:0006310 ! DNA recombination
[Term]
id: GO:0060542
name: regulation of strand invasion
is_a: GO:0000018 ! regulation of DNA recombination
relationship: regulates GO:0042148 ! strand invasion
[Term]
id: GO:0060543
name: negative regulation of strand invasion
is_a: GO:0045910 ! negative regulation of DNA recombination
is_a: GO:0060542 ! regulation of strand invasion
relationship: negatively_regulates GO:0042148 ! strand invasion
```

- MPH1 protein is annotated to GO:0060543. What other ...

Exercises

Gene Ontology (Python)

- Download basic version of GO ontology

- http://current.geneontology.org/ontology/go-basic.obo

- Download GO Semantic Similarity file
  http://labs.rd.ciencias.ulisboa.pt/dishin/go202104.db.gz

- Install:

- pip install goatools ssmpy

Gene Ontology (Python) - Basics

```
from goatools import obo_parser

go_obo = 'go-basic.obo'
# create a dictionary of the GO terms
go = obo_parser.GODag(go_obo)

go_id = 'GO:0048528'
go_term = go[go_id]
print(go_term)
print('GO term name: {}'.format(go_term.name))
print('GO term namespace: {}'.format(go_term.namespace))

for term in go_term.parents:
    print(term)
for term in go_term.children:
    print(term)

rec = go[go_id]
parents = rec.get_all_parents()
children = rec.get_all_children()
for term in parents.union(children):
    print(go[term])
```

Gene Ontology (Python) - common ancestors

- Find the nearest common ancestor of GO:0048527 and GO:0097178

```
def common_parent_go_ids(terms, go):
    # Find candidates from first
    rec = go[terms[0]]
    candidates = rec.get_all_parents()
    candidates.update({terms[0]})

    # Find intersection with second to nth term
    for term in terms[1:]:
        rec = go[term]
        parents = rec.get_all_parents()
        parents.update({term})

        # Find the intersection with the candidates, and update.
        candidates.intersection_update(parents)

    return candidates
```

Gene Ontology (Python) - common ancestors

```
def deepest_common_ancestor(terms, go):
    # Take the element at maximum depth.
    return max(common_parent_go_ids(terms, go), key=lambda t: go[t].depth)


...
go_id_id1_dca = deepest_common_ancestor([go_id, go_id1], go)
print('The nearest common ancestor of\n\t{} ({})\nand\n\t{} ({})\nis\n\t{} ({})
      .format(go_id, go[go_id].name,
              go_id1, go[go_id1].name,
              go_id_id1_dca, go[go_id_id1_dca].name))
```

Exercises

Gene Ontology (Python)

- What is the name of the term GO:0097192?
- What is the most specific term that is parent of both GO:0097191 and GO:0038034?

Exercises

Gene Ontology (Python) - semantic similarity

- Calculate the semantic similarity between GO:0048364 (root development) and GO:0048486 (parasympathetic nervous system development) based on the number of branches separating them.

```
def min_branch_length(go_id1, go_id2, go):
    # First get the deepest common ancestor
    dca = ...

    # Then get the distance from the DCA to each term
    dca_depth = go[dca].depth
    d1 = go[go_id1].depth - dca_depth
    d2 = go[go_id2].depth - dca_depth

    # Return the total distance - i.e., to the deepest common ancestor and back
    return ...
```

Gene Ontology (Python) - semantic similarity

```
def semantic_distance(go_id1, go_id2, go):
    return min_branch_length(go_id1, go_id2, go)

def semantic_similarity(go_id1, go_id2, go):
    return 1.0 / float(semantic_distance(go_id1, go_id2, go))

...
print('The semantic similarity between terms {} and {} is {}.'.format(
      go_id1, go_id2, sim))
```

Exercises

Gene Ontology (Python) - semantic similarity

- Using DiShIn: https://dishin.readthedocs.io/
- Download and uncompress:
  http://labs.rd.ciencias.ulisboa.pt/dishin/go202104.db.gz

```
import ssmpy
 ssmpy.semantic_base("go.db")
 ssmpy.ssm.intrinsic = True
 e1 = ssmpy.get_id(go_id1.replace(":", "_"))
 e2 = ssmpy.get_id(go_id2.replace(":", "_"))
 print(e1, e2)
 print(ssmpy.ssm_resnik(e1,e2))
 print(ssmpy.ssm_lin(e1,e2))
```

Gene Ontology (Python) - semantic similarity

- Similarity between proteins

```
e1 = ssmpy.get_uniprot_annotations("Q12345")
e2 = ssmpy.get_uniprot_annotations("Q12346")
ssmpy.ssm_multiple(ssmpy.ssm_resnik, e1, e2)
ssmpy.ssm_multiple(ssmpy.ssm_lin, e1, e2)
```