Discovery of Novel Metabolic Pathways in PGDBs

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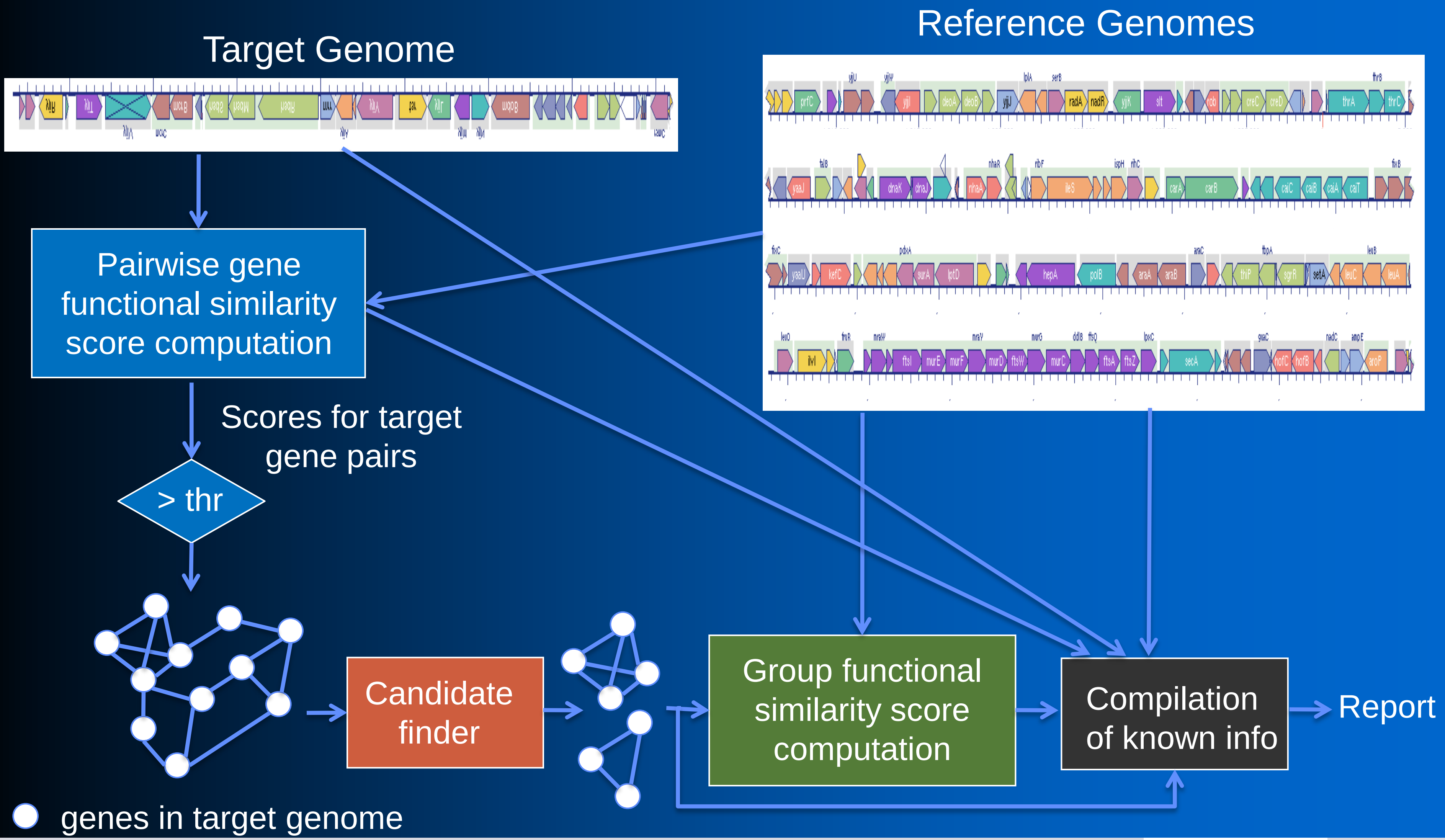
Introduction

- We propose a computational method for the discovery of functional gene groups from annotated genomes
- The method can potentially be used for finding
  - Novel pathways
  - Protein complexes or other kinds of functional groups
  - Genes that are functionally related to a starting gene of interest
- The method relies on sequence information only
- For now, restricted to prokaryotes

Method Overview



Target Genome

Reference Genomes

Pairwise gene functional similarity score computation

Scores for target gene pairs

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Candidate finder

Group functional similarity score computation

Compilation of known info

Report

● genes in target genome

Method Overview

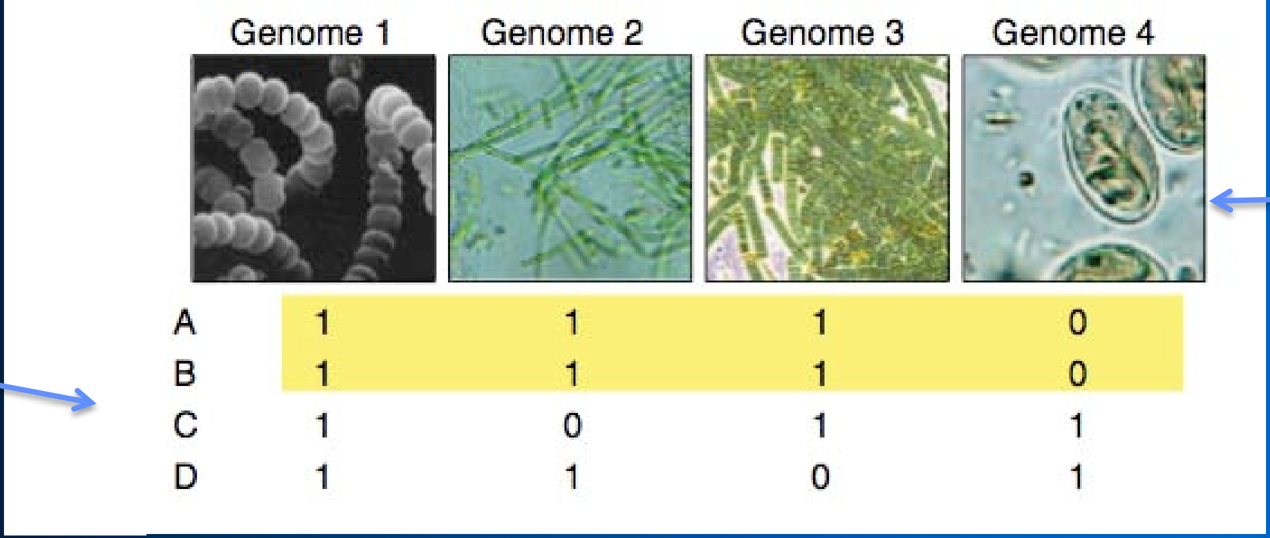
1. Pairwise functional similarity scores: For all pairs of genes in the target genome find a measure of the probability that the genes are functionally related
2. Candidate finder: Find all cliques (set of nodes linked to all others) in a network where
   - nodes are genes and,
   - edges are given when the above scores are above a threshold.
3. Group functional similarity scores: For each *candidate group* find a measure of the functional relatedness of its members. Optionally filter out groups with low score.
4. Generate Report: For each candidate group gather all available information to facilitate analysis

Pairwise functional similarity scores

- Estimated using Genome Context (GC) methods
  - Use assumptions about the evolutionary processes to find associations between genes that might point to functional interactions
  - Uses the set of reference genomes to infer interactions (currently 623 bacterial genomes from BioCyc version 14.5)
  - Methods: Phylogenetic profiles, Gene neighbor, Gene fusion, Gene cluster
- Currently using only Gene Neighbor method, which is by far the best performing of the four

Phylogenetic Profile Method

- Assumption: *Genes whose products function together tend to evolve in a correlated fashion*
  - they tend to be preserved or eliminated together in a new species
- For each gene in the target genome create a binary vector with
  - a 1 in component i if the gene has a homolog in genome i
  - a 0 otherwise



	Genome 1	Genome 2	Genome 3	Genome 4
A	1	1	1	0
B	1	1	1	0
C	1	0	1	1
D	1	1	0	1

Genes from target genome

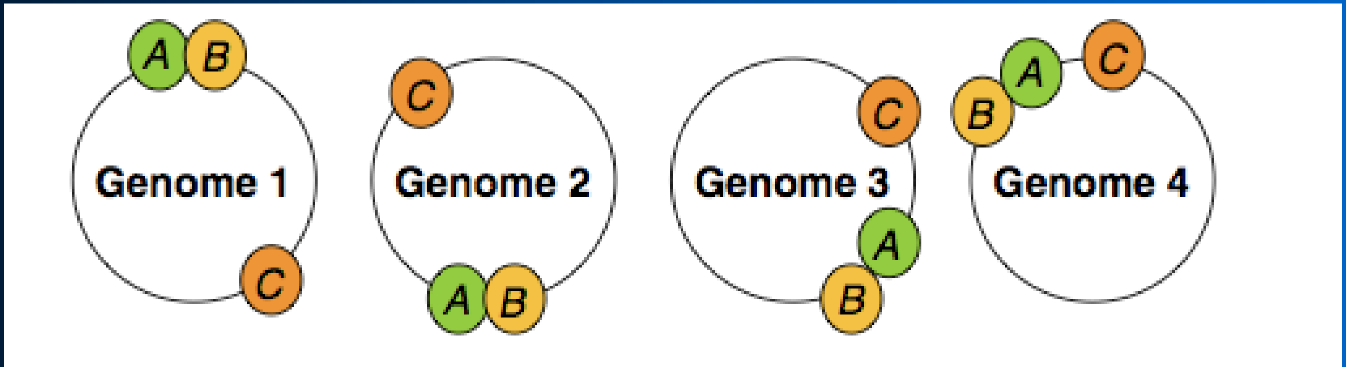
Reference Genomes

- Score: similarity between these vector

Gene Neighbor Method

(Bowers 2004)

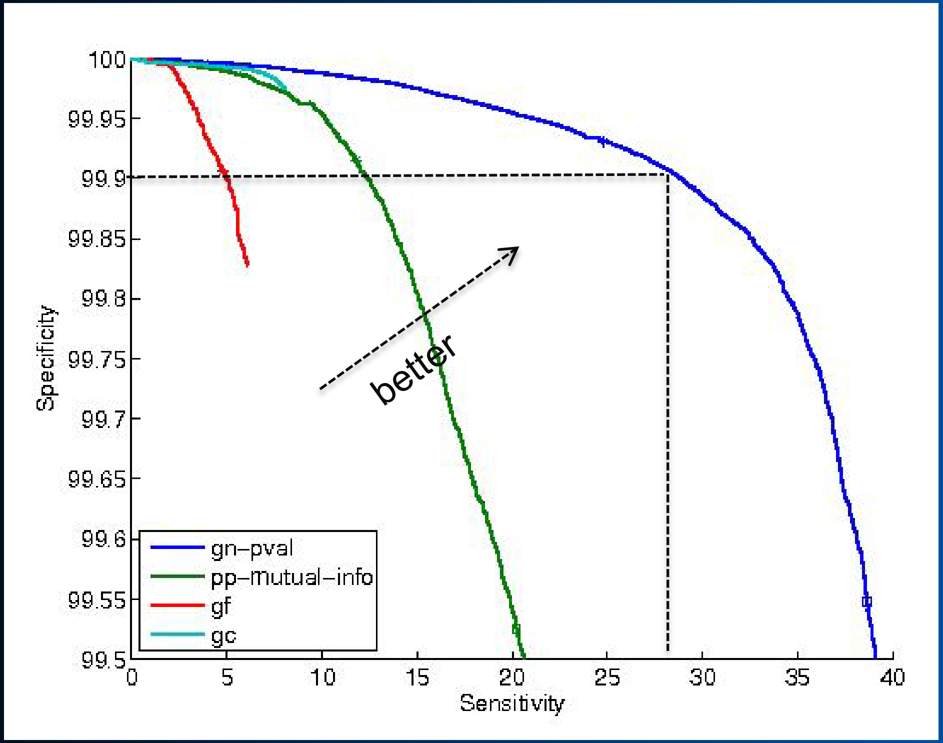
- Assumption: Genes whose products function together tend to appear nearby, at least in some genomes



- For each gene pair
  - Find the location of the *best* homologs of both genes in each of the reference genomes
  - For genomes that contain homologs of both genes, compute the relative distance between them
  - Score: a p-value for the observed distances

Results of Genome Context Methods

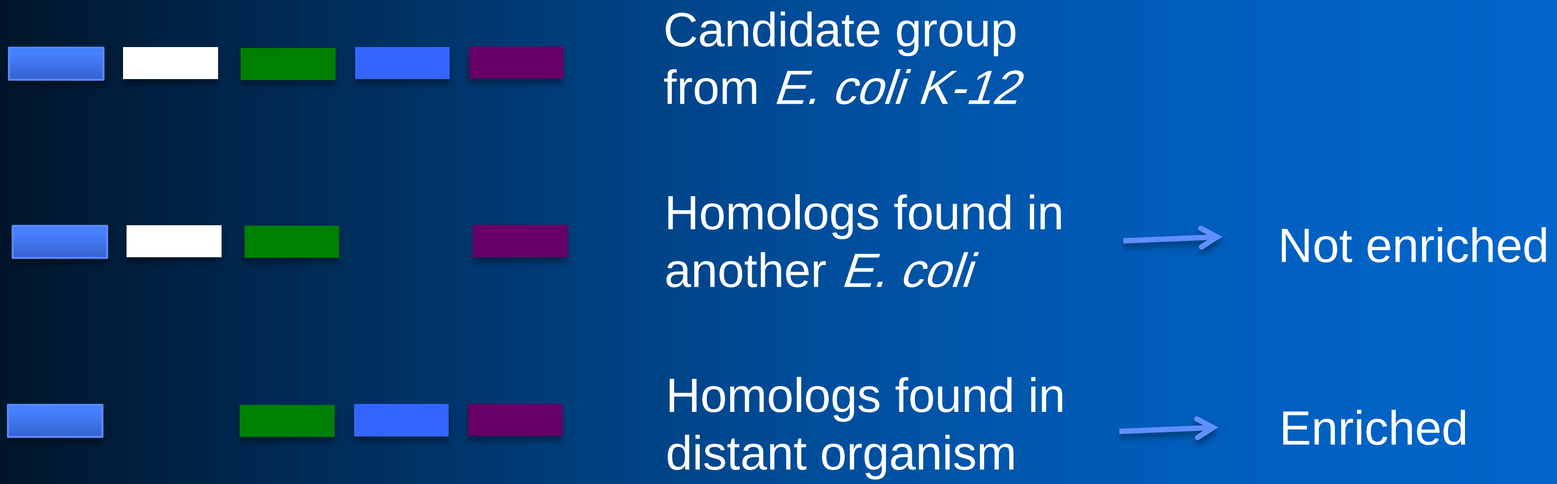
- Results on *E. coli K12*
  - Positive examples are gene-pairs in the same metabolic or signaling pathway or the same protein complex
  - All other pairs of genes of known-function are negative examples



- At this operating point:
  - 6869 pairs are labeled as positives
  - Around 28% of the positives are found
  - Only 0.1% of the negative samples are labeled as positives
  - But, this percent corresponds to 5044 negatives

Group Functional Similarity Scores

- For each candidate group find the reference genomes G that are enriched for the genes in the group
- A genome G will be enriched for the group if
  - A large fraction of the genes in the group have homologs in G, and
  - A small fraction of all the genes in the target genome have homologs in G



Candidate group from *E. coli K-12*

Homologs found in another *E. coli* → Not enriched

Homologs found in distant organism → Enriched

Report

- List of genes with all known info about each
- List of organisms enriched for group
- List of organisms depleted for group
- Phylogenetic similarity with known pathways from Metacyc
  - As phylogenetic profile method for genes but now for gene groups
  - Create binary vectors with a 1 if the organism is enriched for the candidate group
  - For each Metacyc pathway or complex, create a binary vector with a 1 for organisms that contain it
  - Compare these vectors with the one for the candidate

Report

- Genome context scores between gene pairs in the group
- BLAST E-values between gene pairs in the group
- Known pathways or complexes involving at least two genes from the group
- Genome context information
  - For each gene, list the relative position in all the organisms for which it has a homolog

Performance on E. coli K-12

- EcoCyc version 14.5 contains 944 protein complexes and 340 pathways curated from the literature
  - Of which 103 complexes and 175 pathways contain more than four genes
- Decide a candidate is correct if at least 70% of its genes are in a known pathway or protein complex
- We declare a pathway or complex as found by our method if at least 70% of its genes are included in some candidate
- Only consider candidates and pathways/complexes with more than 4 genes
  - Algorithm is less reliable for smaller groups
  - For candidates of size 2, it's only as reliable as the genome neighbor method alone

Results at Different Operating Conditions

Percent of edges in network	Minimum number of enriched orgs	Number of candidates	Percent of correct candidates	Number of pathways found
0.15%	0	1130	13%	96
	5	312	19%	69
	20	155	25%	42
0.07%	0	413	22%	65
	5	150	29%	38
	20	86	35%	13

- The percent of edges in the “actual” network for E. coli is 0.07%
- The predicted 0.07% contains some of those edges, but also many false positives
- So, you might want to include more edges to catch more of the positives

Example 1: Rediscovered Pathways

Some examples of *E. coli K-12* pathways or complexes that are found by the proposed method

Pathway or Complex	# genes in pathway or complex	# matching genes in candidate	
Histidine biosynthesis	8	8	Perfect match
Tryptophan biosynthesis	5	5	Perfect match
ATP synthase	8	8	Perfect match
NADH:ubiquinone oxidoreductase I	13	13	Five additional genes: hycE/D/F and hyfH/G
Flavin biosynthesis I	6	5	One missing gene: ribF

Example 2: Nascent Biosynthetic Pathway

Gene	Product
moaA b0781	molybdopterin biosynthesis protein A
moaB b0782	molybdopterin biosynthesis protein B
moaC b0783	molybdopterin biosynthesis protein C
moaE b0785	molybdopterin synthase large subunit

- Missed getting moaD by very little (a slightly lower score on the pairwise functional similarity scores would have allowed us to find it)
- This a known biosynthetic pathway, but the exact pathway has not been elucidated yet and, hence, does not exist in EcoCyc
- This is one case that would count as an error in our statistics though it is really not an error

Example 3

Gene		Product
dacA	b0632	D-alanyl-D-alanine carboxypeptidase, fraction A; penicillin-binding protein 5
dacC	b0839	penicillin-binding protein 6
dacD	b2010	DD-carboxypeptidase, penicillin-binding protein 6b
lipA	b0628	lipoate synthase monomer
rlpA	b0633	rare lipoprotein RlpA

- A RlpA-RFP fusion accumulates at cell division sites
- dacACD involved in peptidoglycan biosynthesis and cell morphology

Example 4

Gene	Product
rsxE b1632	integral membrane protein of SoxR-reducing complex
rsxG b1631	member of SoxR-reducing complex
rsxD b1630	integral membrane protein of SoxR-reducing complex
rsxB b1628	member of SoxR-reducing complex
nth b1633	endonuclease III; specific for apurinic and/or apyrimidinic sites

- rsxABCDGE predicted to form a membrane-associated complex
- Involved in regulation of soxS which participates in removal of superoxide and nitric oxide and protection from organic solvents
- nth has been shown to act in the process of base-excision DNA repair

Future Work

Two main obvious directions

- Instead of using a single genome context method, use them all in combination
  - Not trivial, we need training data (a gold standard) to find the combination function
  - Have an initial solution that is about to get into the system
- Relax the condition of the candidates being cliques in the network
  - Maybe some genes in the pathways are only related to some percent of the other genes in the pathway

Candidates for E. coli K-12

- Reports for the *E. coli K-12* candidates available in: http://brg.ai.sri.com/pwy-discovery/ecoli.html
- More documentation on the method and reports available from that page
- Better Web interfaces will be available in the future
- Applicable to other organisms
- Contact us if you are interested in more information

  pkarp@ai.sri.com, lferrer@ai.sri.com

References

- Paper on genome context methods:

  http://www.biomedcentral.com/1471-2105/11/493

- A paper on the pathway discovery method is under preparation