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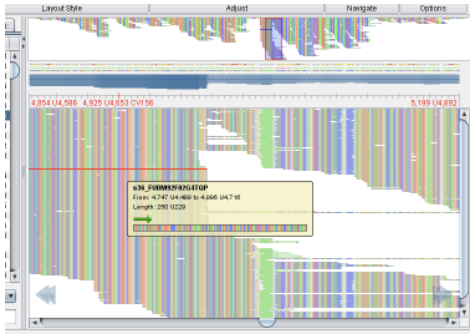
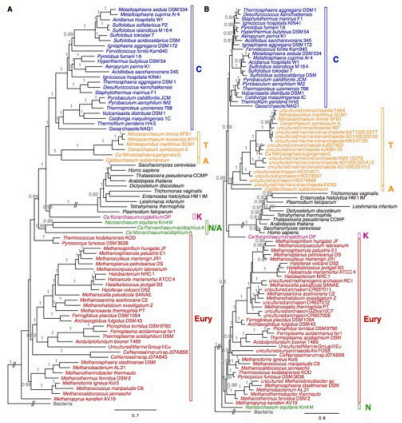
Single-cell genomics pipeline: from raw reads to phylogenomics using Galaxy





Lionel Guy and Thijs Ettema, Department of Cella and Molecular Biology and SciLifeLag, Uppsala University, Sweden

Galaxy Community Conference 2013

Oslo, Norway, 2013-07-01

SciLifeLab

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- Introduction
  - Microbes
  - Single-cell genomics
  - Underlying scientifice question
- Implementation in our lab
  - Sample pipeline
  - Galaxy pipelines
- Conclusions

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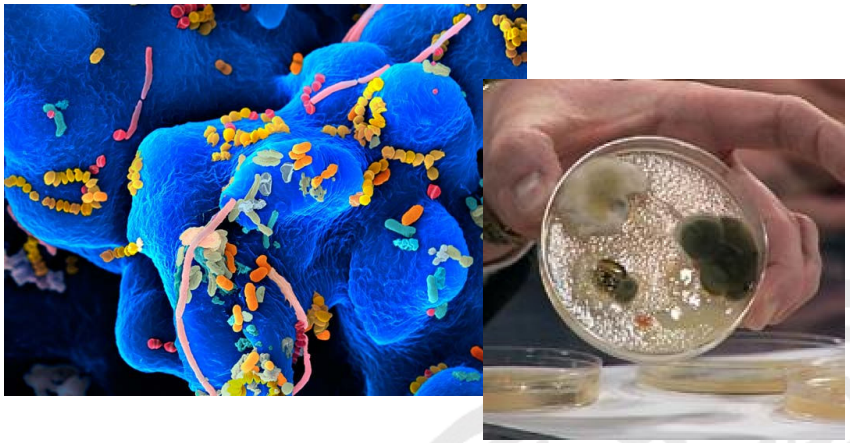
“Microbes rule the world”

Table 5. Number and biomass of prokaryotes in the world

Environment	No. of prokaryotic cells, $\times 10^{28}$	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546



- Microbes are abundant: ~5’000’000’000’000’000’000’000’000’000’000’000 prokaryotic cells ($5x10^{30}$, five million trillion trillion)
- All cells in all humans: $7x10^{9} \times 10^{13} = 7x10^{22}$, i.e. ~70’000’000’000’000’000’000’000 (a mere 70 billion trillion)

Table 6. Relationship of plant and prokaryotic biomass to primary productivity

Ecosystem	Net primary productivity,* Pg of C/yr	Total carbon content, Pg of C		
		Plant*	Soil and aquatic prokaryotes	Subsurface prokaryotes
Terrestrial	48	560	26	22–215
Marine	51	1.8	2.2	303

- Roughly the same biomass as plants (~500 Pg C)

Source: Whitman WB et al, PNAS 1998
Baserga 1985

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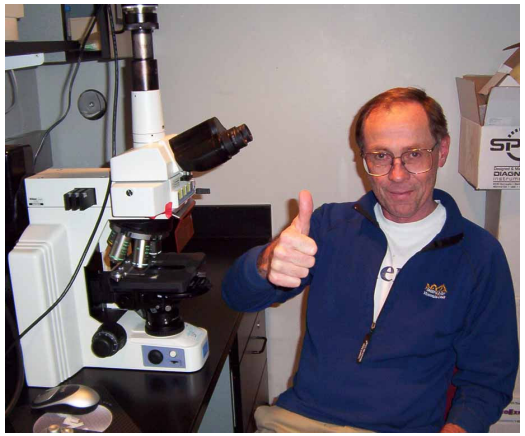
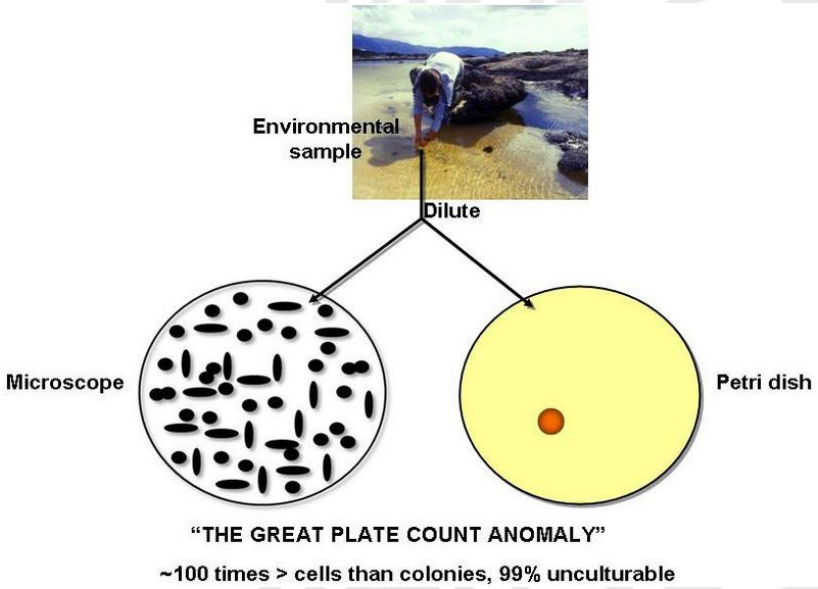
Studying microbes



- Norman Pace (Univ. Colorado, Boulder), 2001:
- "*Imagine if our entire understanding of biology were based on a visit to the **zoo**... And that's exactly the situation we've been at in the **microbial** world until really quite recently*"

- ~1 % of prokaryotes grow on plate ("zoo", aka "The Great Plate Count Anomaly")
- ~99% to be studied!!!



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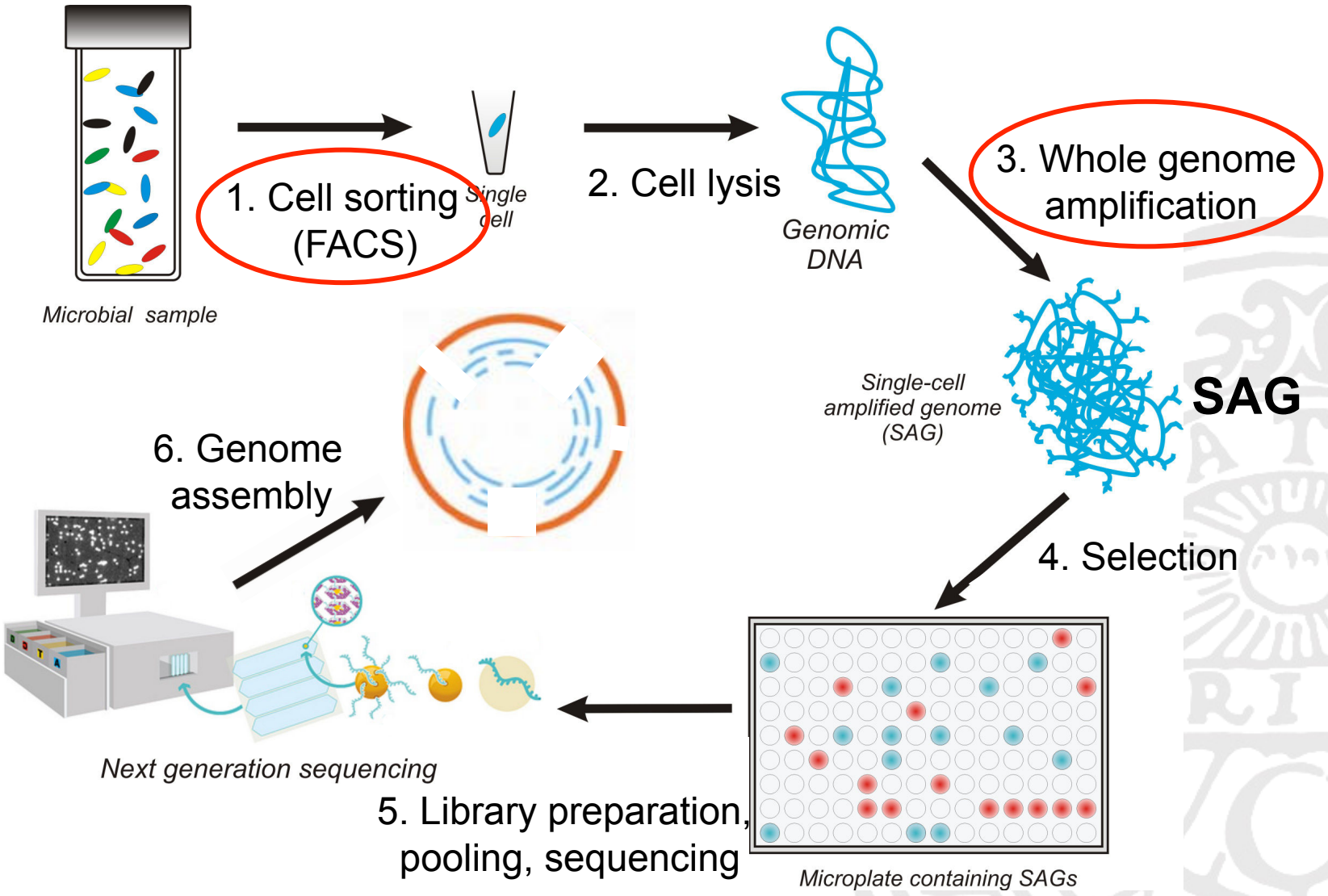
How? Single-cell genomics



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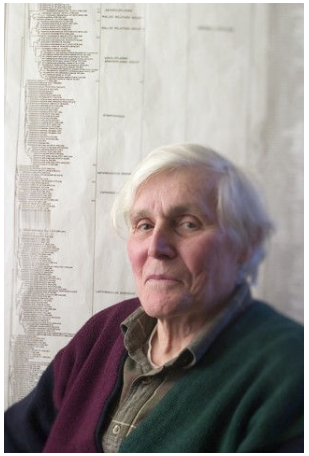
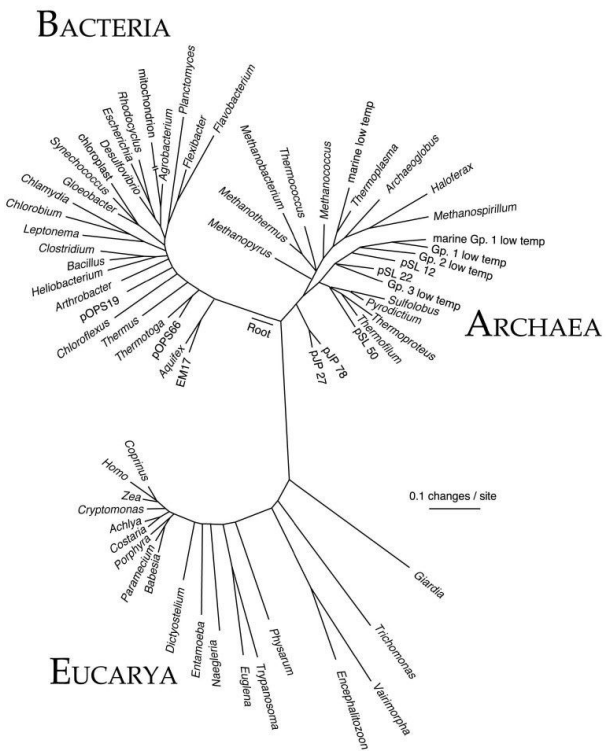
Why? Origin of eukaryotes

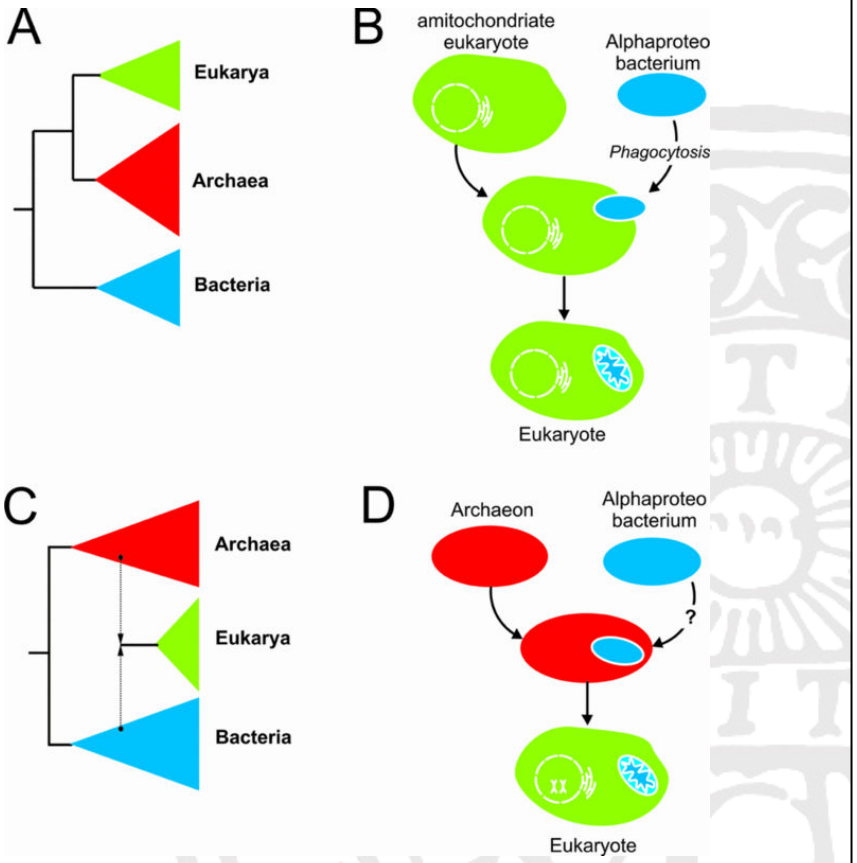


Carl Woese

- Three Domains of Life?



Pace, Science 1997



Martijn & Ettema, Biochem Soc Transac 2013

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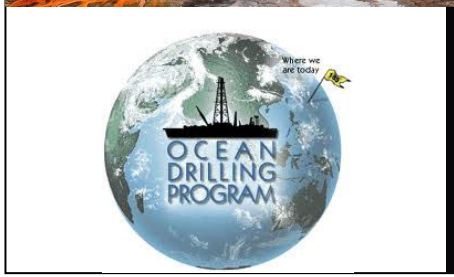
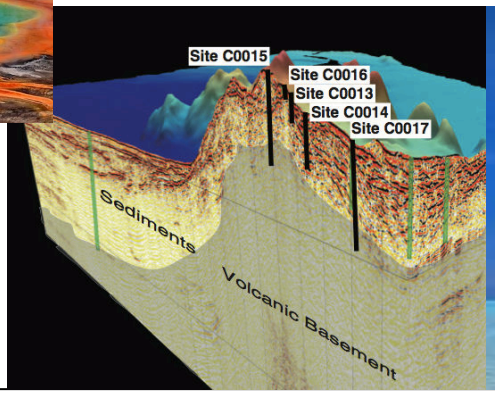
What? Sampling

- Diverse and unexplored environments:
  - New Zealand, 1000 hot springs project (Matthew Scott)
  - Yellowstone hot springs
  - Ocean Drilling Program expeditions (expedition 331, Deep Hot Biosphere)
  - Hawaii oceanic water
  - Sala silver mine, Sweden





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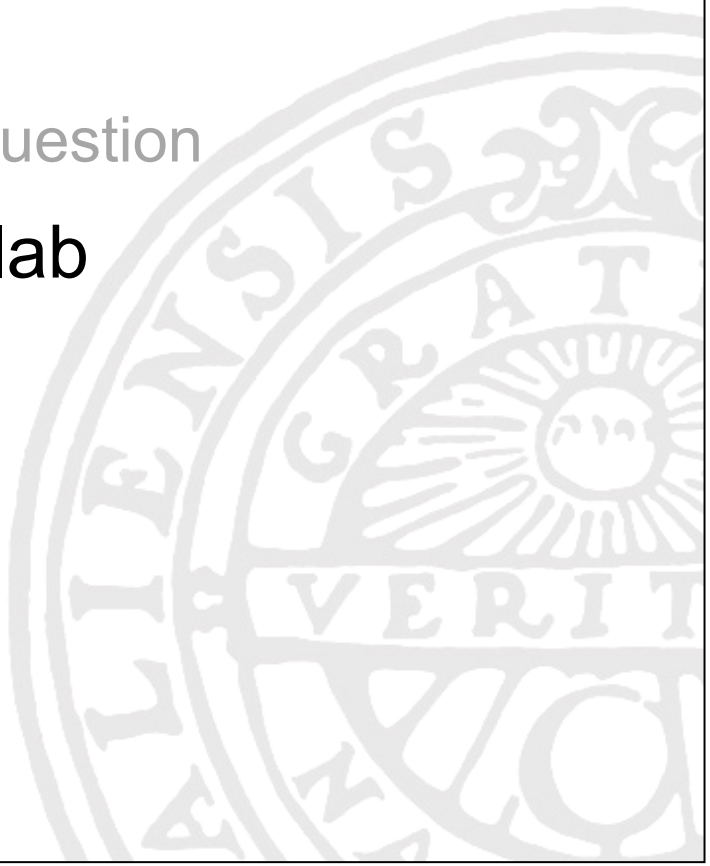

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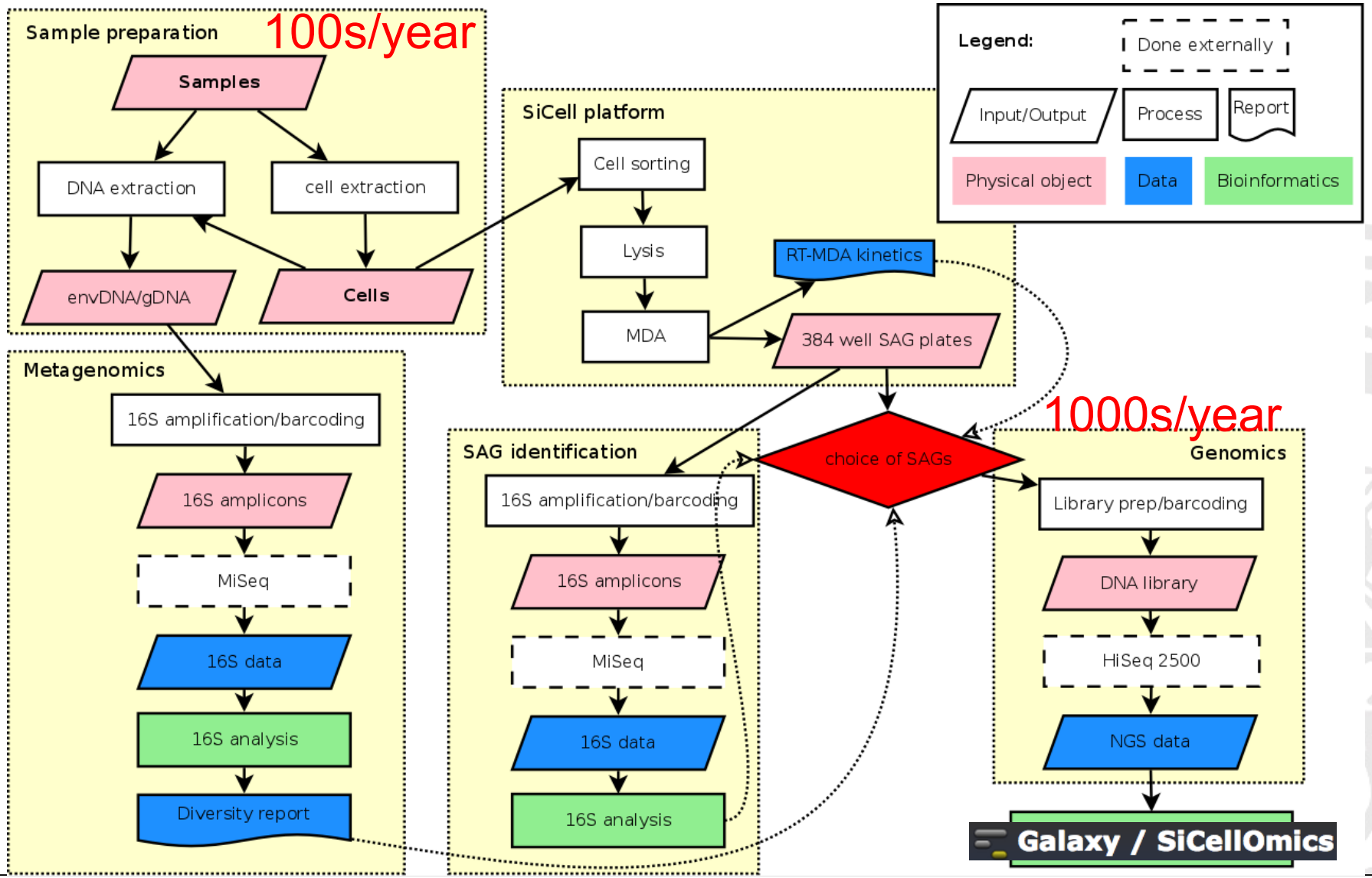
Sample pipelines



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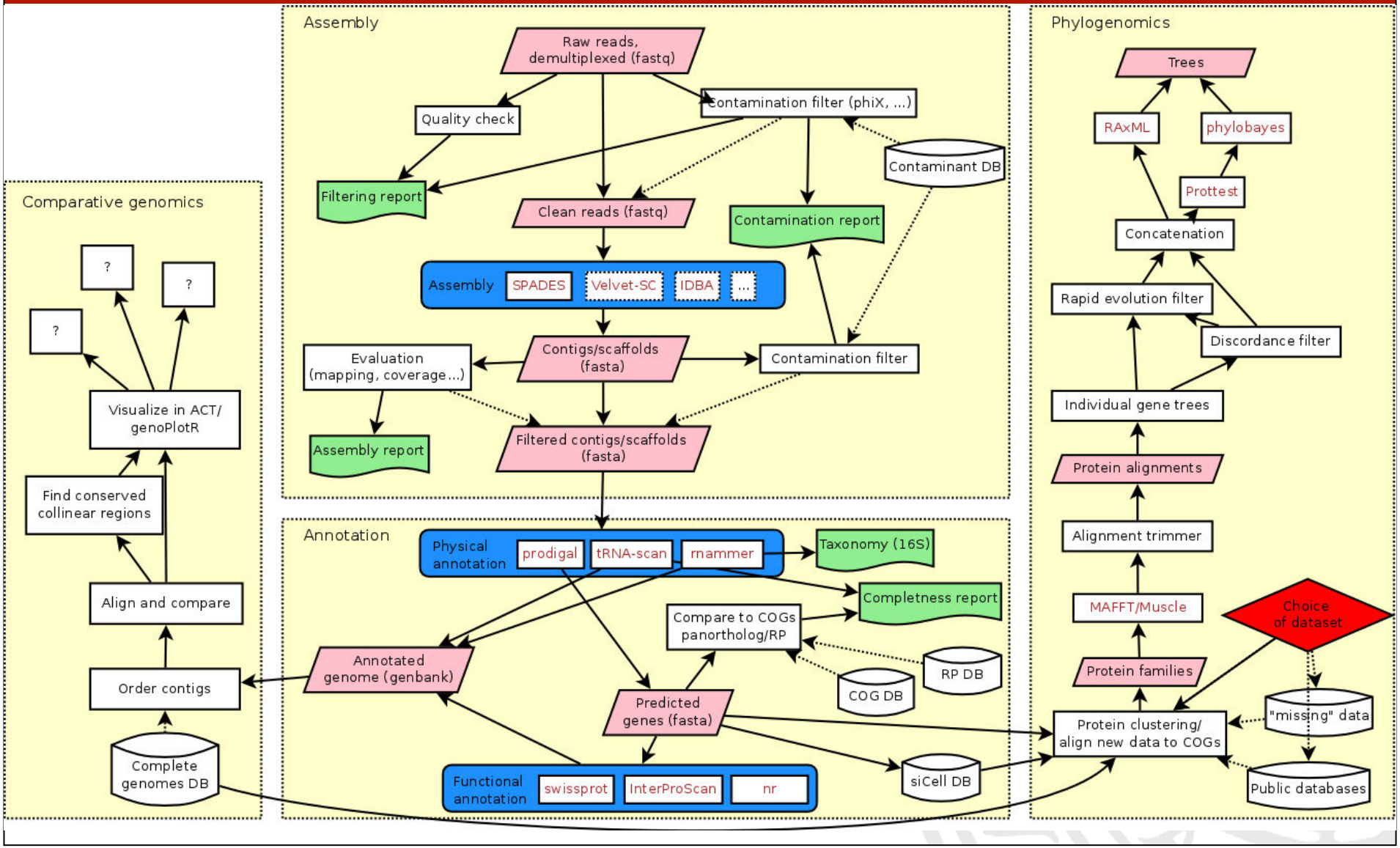
Galaxy pipelines



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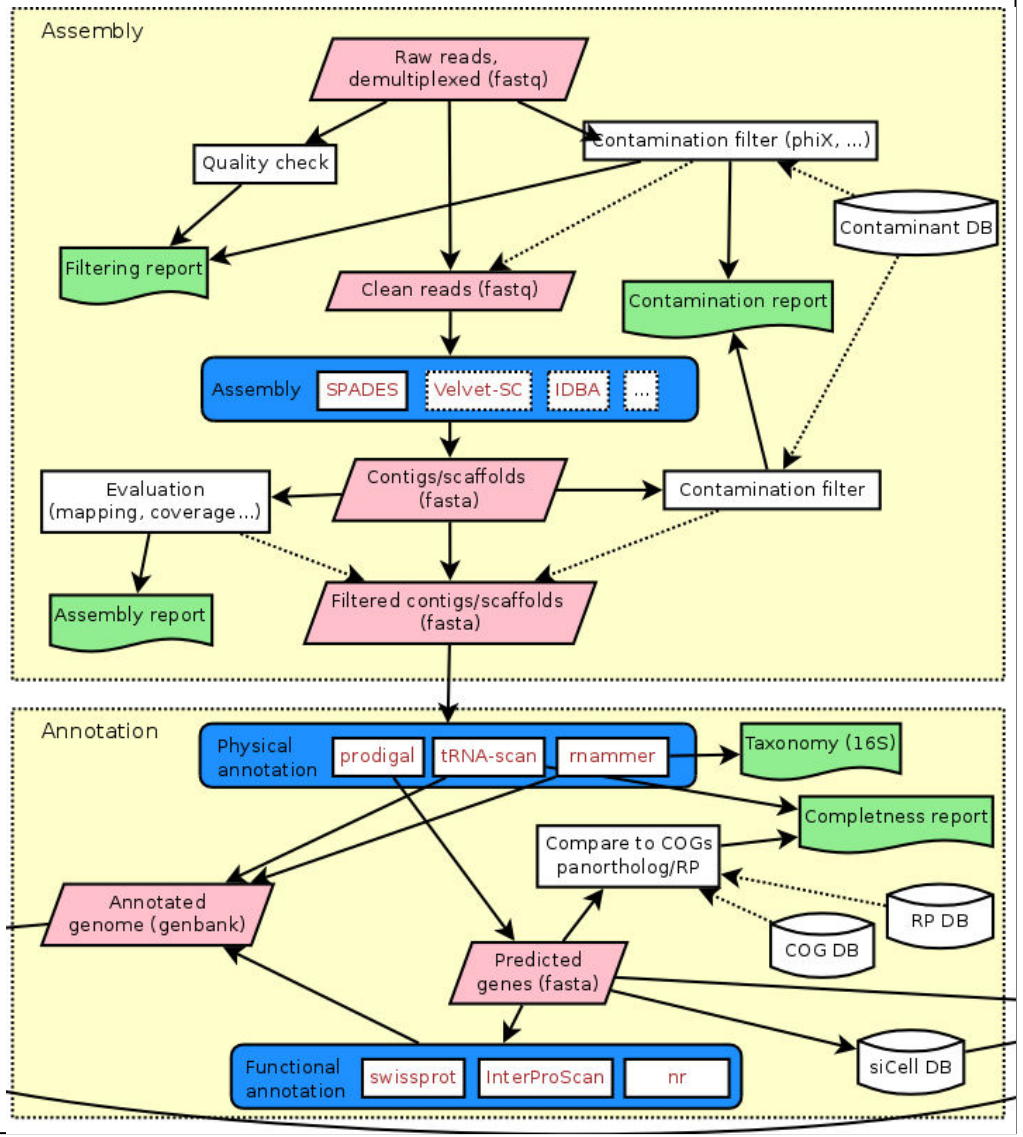
Assembly/annotation

- Input: raw reads from sequencing center
- Output: annotated genome
- Reports:
  - Reads quality
  - Contaminations
  - Closest sequenced organism
  - Completeness



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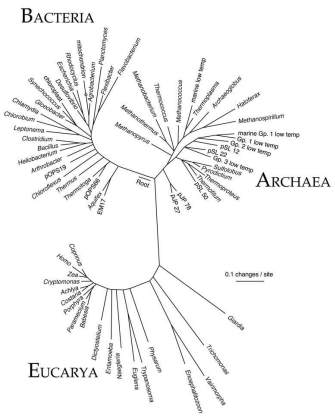
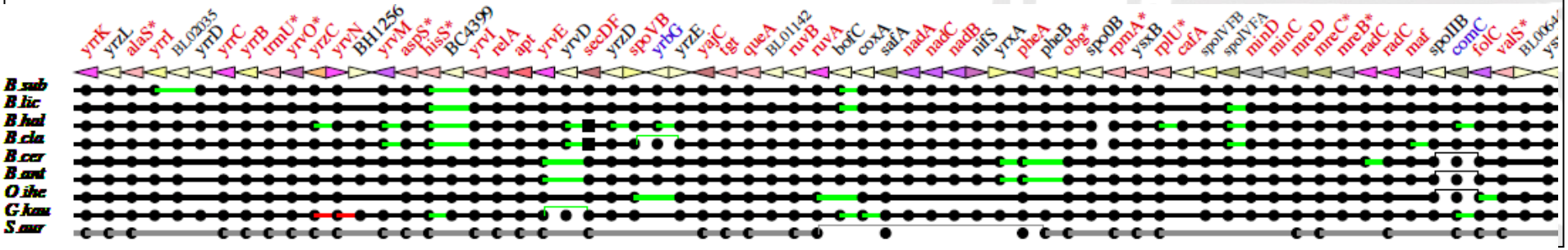
Phylogenomics



- Ribosomal RNA: 1.5 kb (SSU/16S/18S), 3 kb (LSU)
- Use proteome: in average ~2000 genes per genome (range 150-12000):
  - Find orthologous genes, align, concatenate, run phylogeny
- Issues:
  - Tree of species ≠ Tree of genes: paralogs, horizontal gene transfers (HGT)
  - Distant homologies hard to assess
  - Very few genes conserved in all genomes small (~30)
  - Phylogenies computationally costly
  - SAGs are incomplete (20-90% of the genome)



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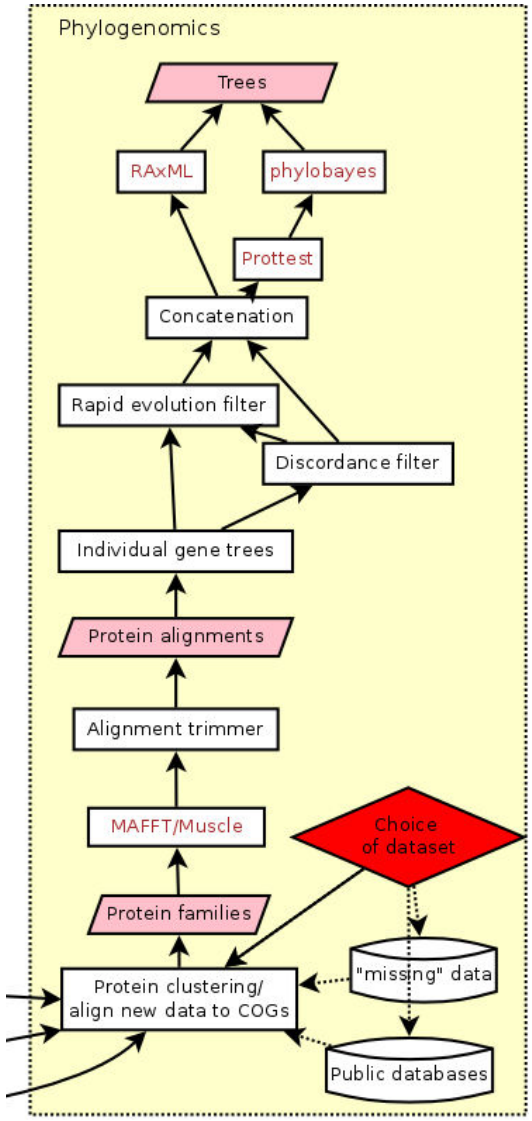
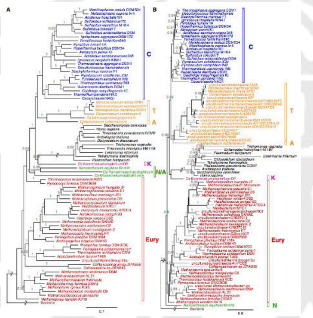
Phylogenomics pipeline



- Task: find a set of orthologous genes and organisms with the following properties:
  - Few HGTs
  - Little (and evenly spread) missing data
  - Representative genomes (<100)

Genomes

Genes	**A**	**B**	**C**
x	1	1	1
y	1	0	1
z	1	1	1



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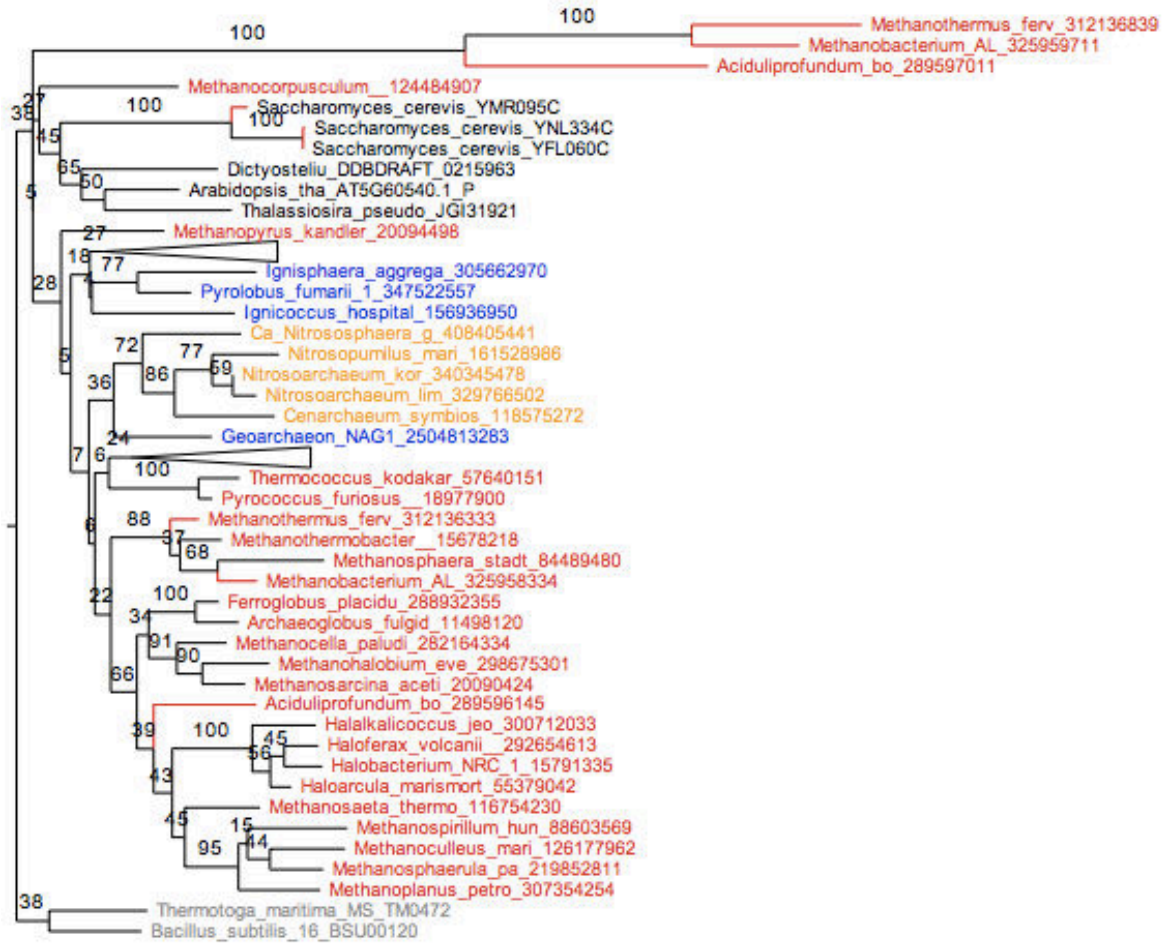
Phylogenomics – data seletcion

Genomes

Genes	A	B	C
x	1	1	3
y	0	1	1
z	1	2	0

Genomes

Genes	A	B	C
x	1	1	1
y	0	1	1
~~z~~	~~1~~	~~2~~	~~0~~



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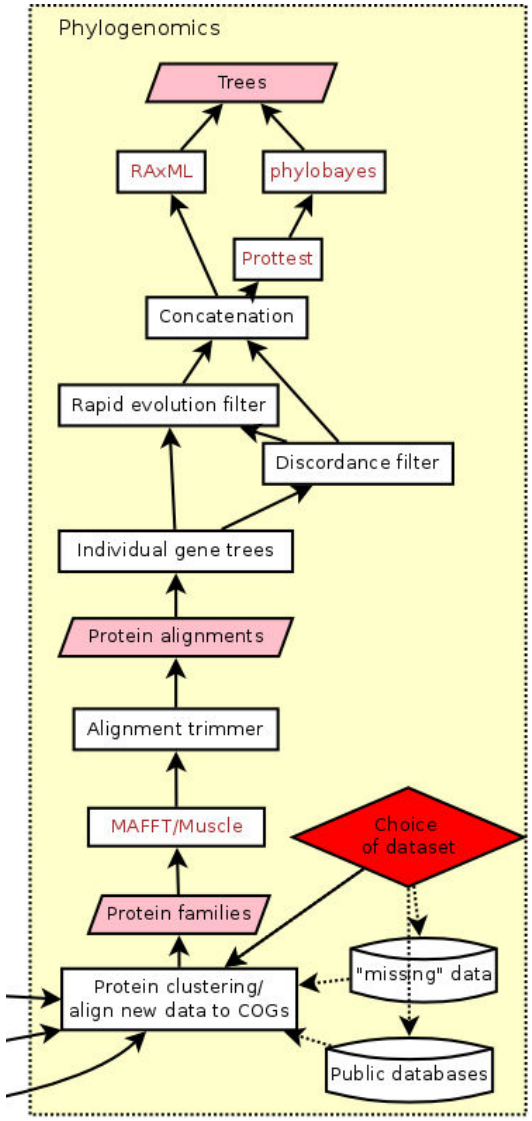
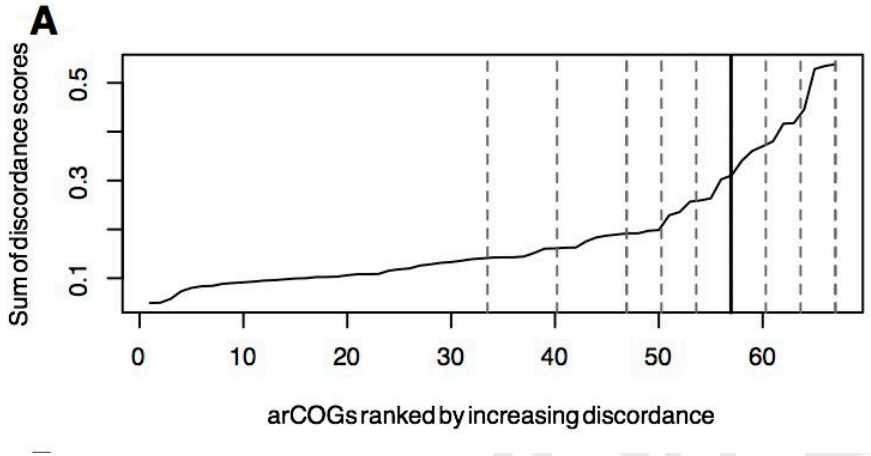
Phylogenomics – filtering



- Per gene: discordance filter
  - Compare all gene trees to all, count conflicts
  - Rank genes by sum of conflicts



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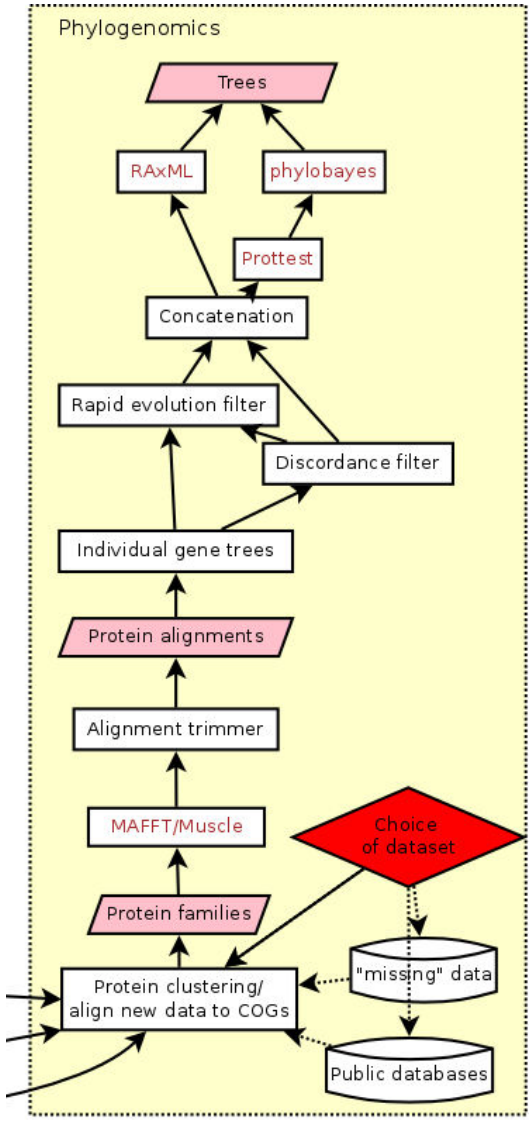
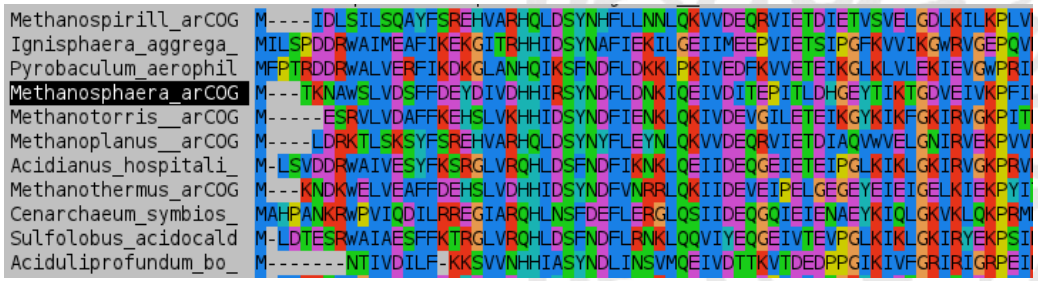
Phylogenomics – filtering



- Per site:
  - Calculate the global amino-acid biases in a protein alignment
  - Remove one site (column), recalculate
  - Rank sites by how much they contribute to the global bias



Viklund et al, MBE 2012

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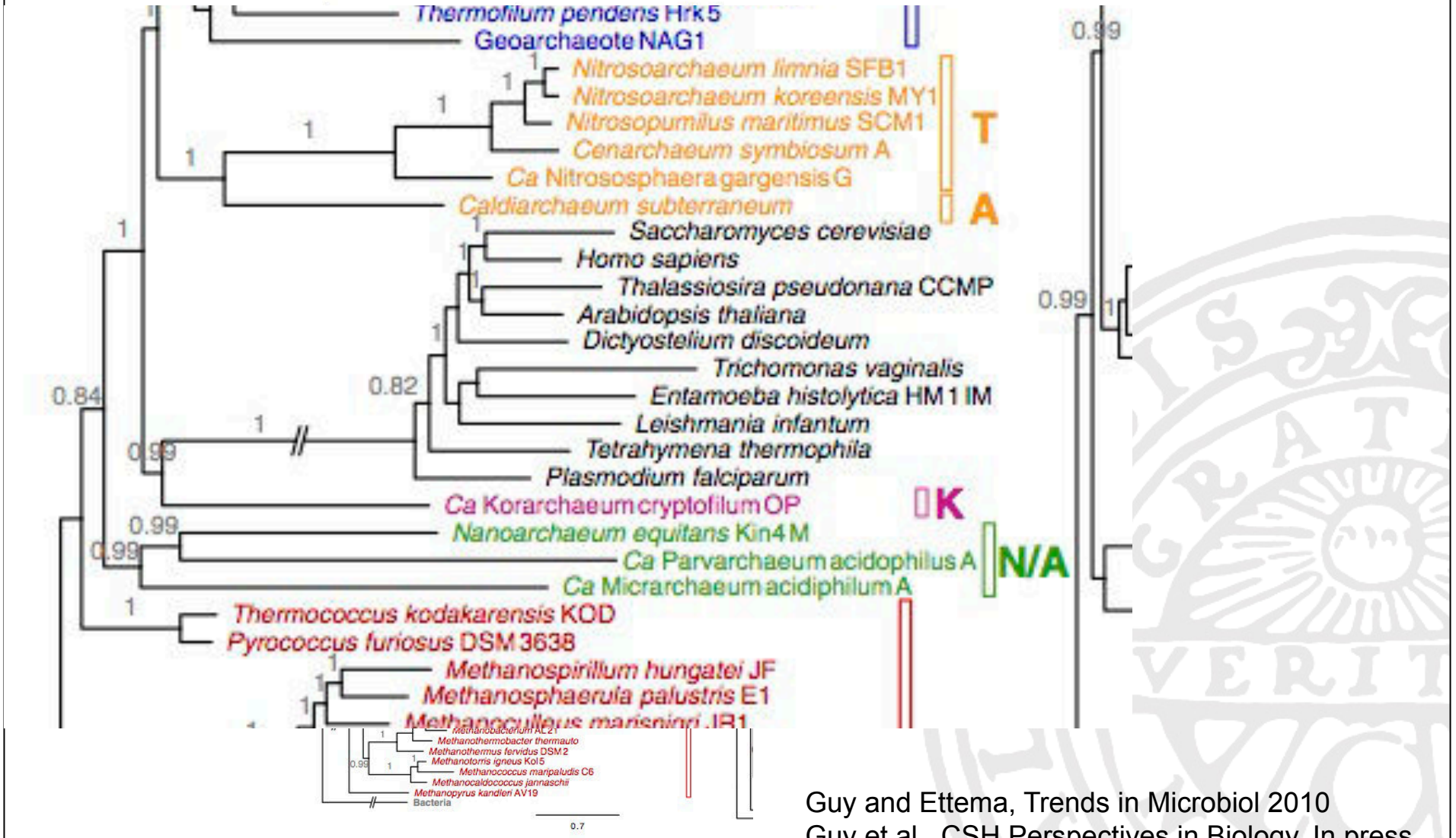
A robust Tree of Life



Guy and Ettema, Trends in Microbiol 2010
Guy et al., CSH Perspectives in Biology, In press

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Future work

- Automatic generation of clusters (OrthoMCL, TribeMCL, …)
- Automated trimming of paralogs (hard)
- Complete automated pipeline, incremental database

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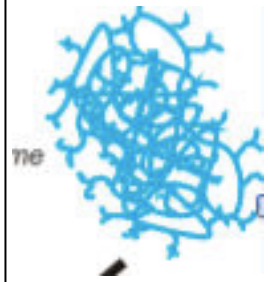
Summary



Galaxy / SiCellOmics

- Sequencing genomes from single-cell in a high-througput manner
- Exploring genomics outside the 1% “zoo” is now possible
- Galaxy is the tool of choice!

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Acknowledgments

- SiCell:
  - Single-cell genomics (test) platform from SciLifeLab, Uppsala Unviersity.
  - First in Europe!
  - Thijs Ettema & Stefan Bertilsson, co-directors
  - Claudia Bergin & Anna-Maria Divne
- Ettema group (ICM, Uppsala University)
  - Thijs Ettema (PI)
  - Jimmy Saw
  - Anja Spang
  - Anders Lind
  - Joran Martijn
  - Janko Tackmann
  - Santhanam Kulasekara

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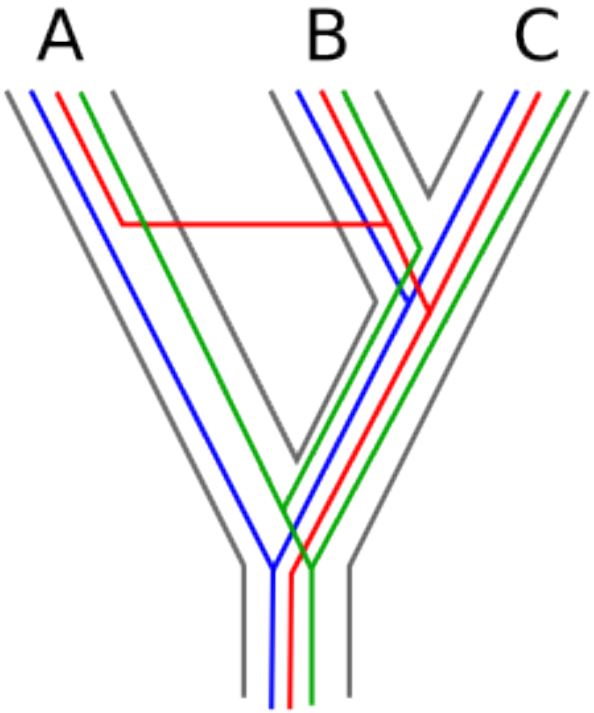
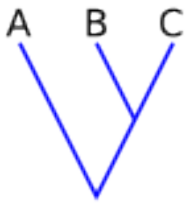
Gene tree =/= Species tree





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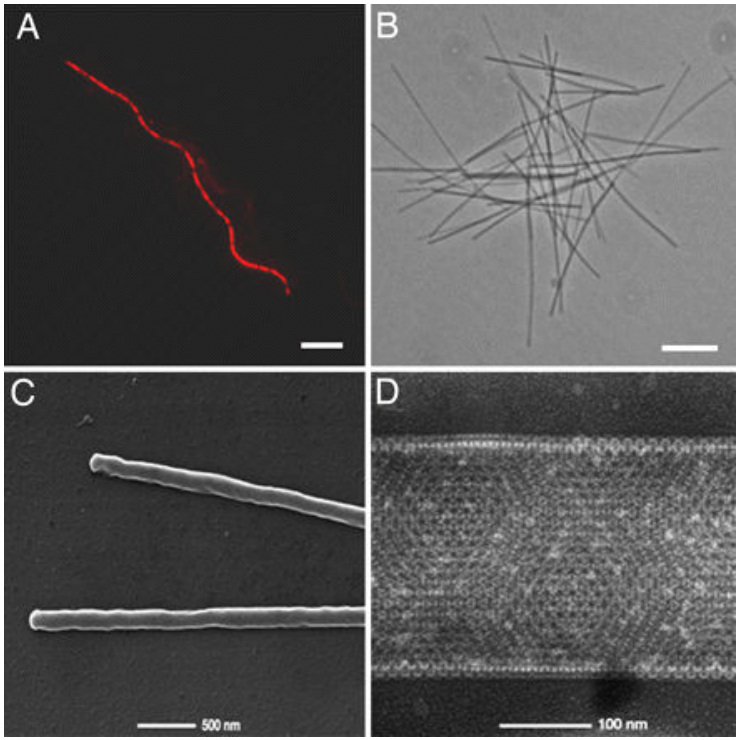
Korarchaeota



- “Ultrathin filamentous morphology”
- Obligate anaerobes
- Heterotrophes
- Peptides as principal carbon and energy source
- Our ancestor?

Elkins et al, PNAS 2008

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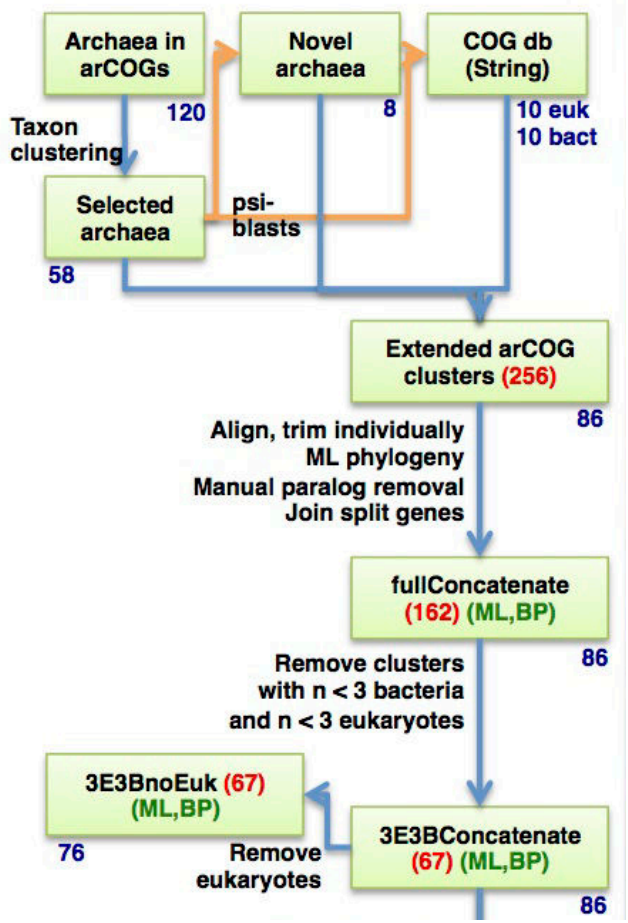
Pipeline – Data selection



- Start from arCOGs (Wolf et al, Biology direct 2012)
- Cluster taxa (CD-HIT, threshold = 70%)
- Add bacteria/eukaryotes (psi-blasts to restricted databases)
- Draw trees, manual check and remove paralogs
- Remove clusters with little or no eukaryotes

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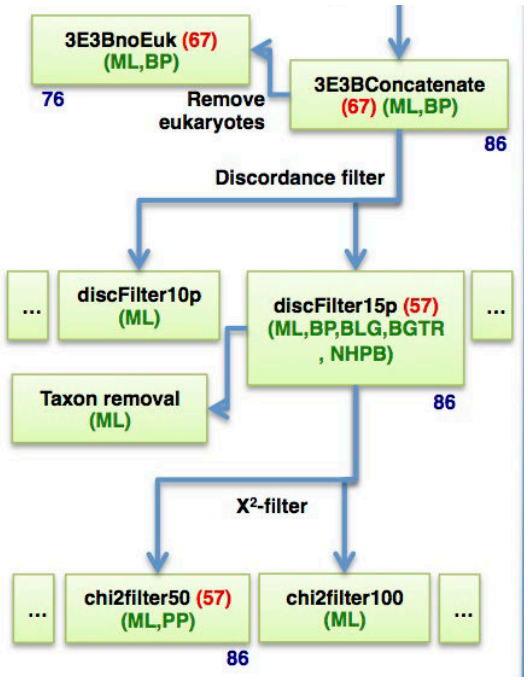
Position of eukaryotes



- Run ML and Bayesian trees with all eukaryotes
- Test different filters
  - Remove HGT
  - Remove sites with strong amino-acid composition bias
- Test the effect of removing taxa on the position of eukaryotes

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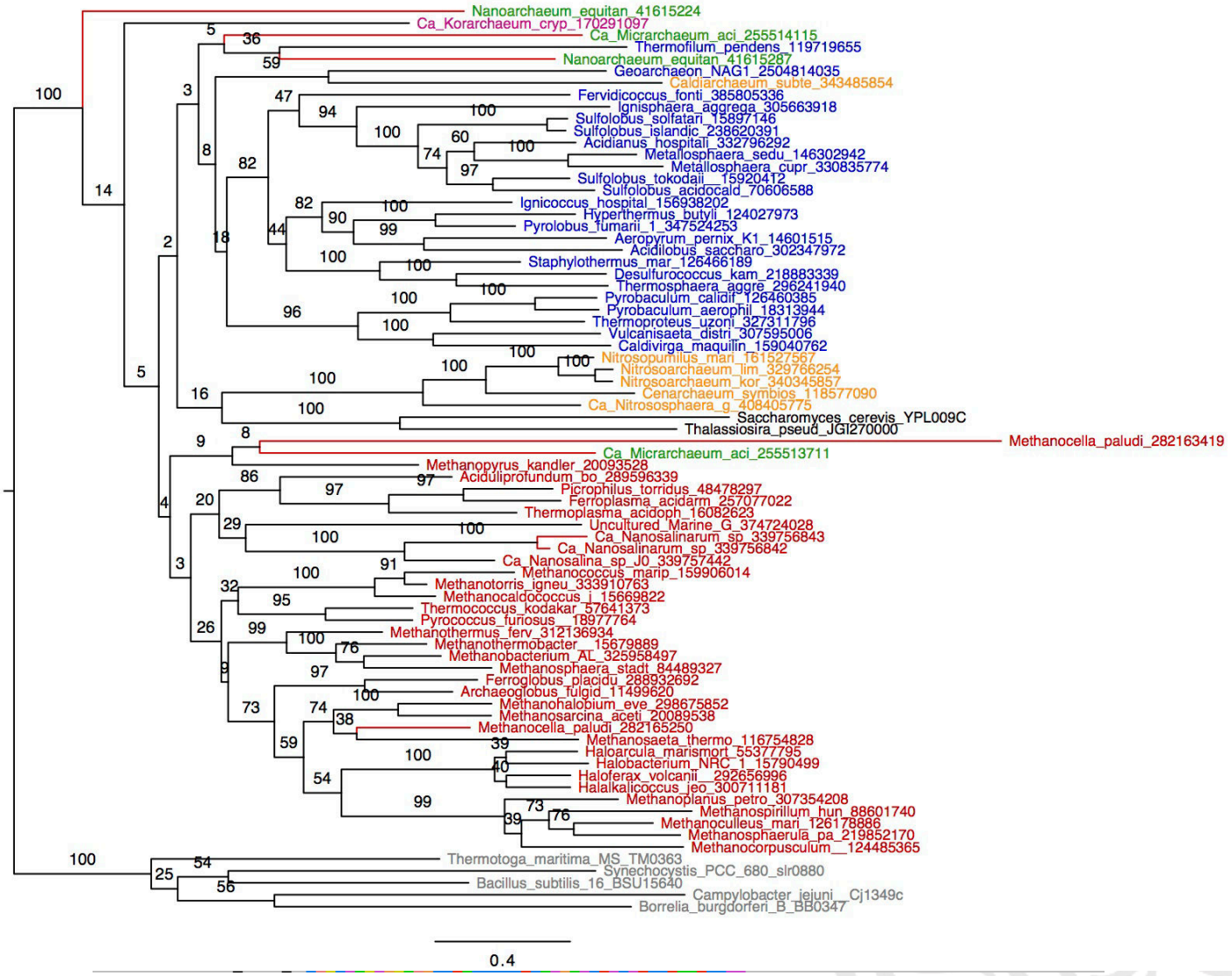
Manual curation of trees



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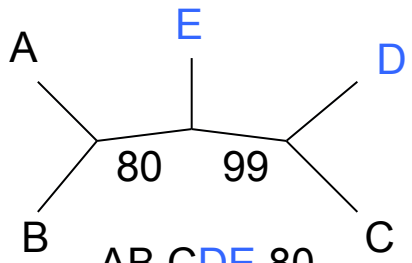
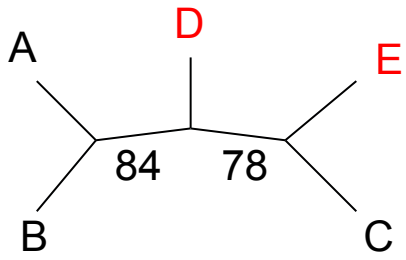
Discordance filter



AB,CDE 80
ABE,CD 99
ABD,CE 12

AB,CDE 84
ABD,CE 80
AE,BCD 2



Disc. score = 2 / (2 * 2) = 0.5

- Goal: remove clusters with significant HGT
- Starting point: 67 clusters (3E3B dataset)
- For each possible pairwise comparison:
  - Get bipartitions for bootstrap trees in both trees
  - Keep only high-support bipartitions (HSB), >75
  - Sum the number of incompatible HSB
  - Divide that by the product of HSBs in each of the two trees
- The discordance score of one tree is the sum of all its pairwise discordance scores

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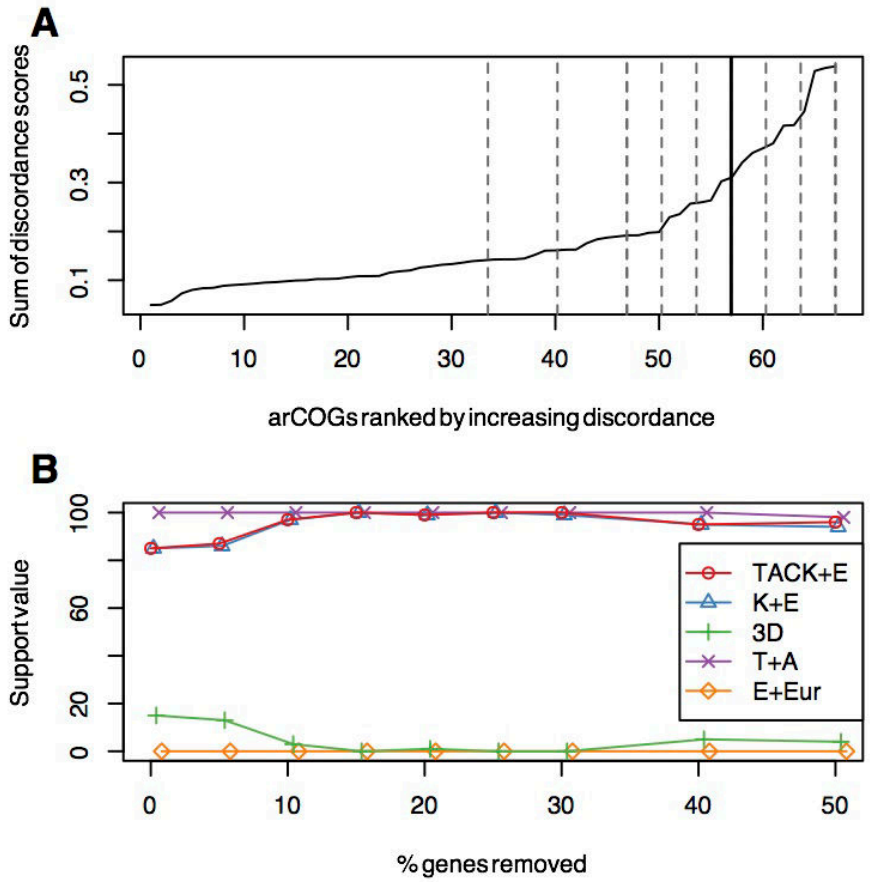
Discordance filter



- Rank clusters by increasing discordance score
- Remove increasing fractions of the most discordant genes
- Assess the effect on key splits
- After removing 15% of the data, most nodes are supported either with 0 or 100

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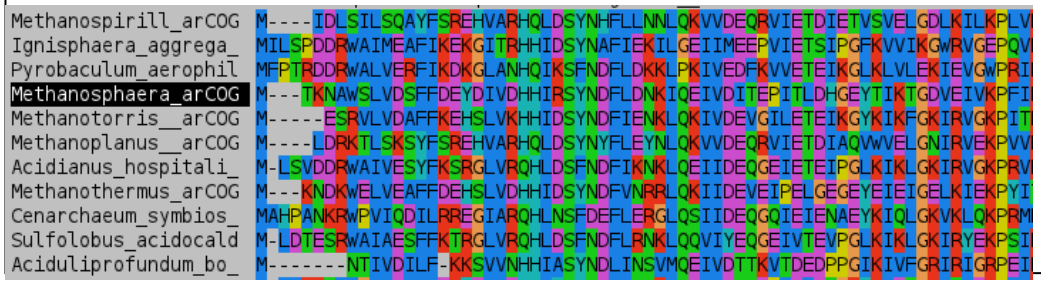
Chi2 filter

$$\chi^2 = \sum_t \sum_{i=1}^{20} \frac{(O_i(t) - E_i)^2}{E_i},$$

- Goal: remove sites with high amino-acid bias
- Principle:
  - Calculate χ^2-score for the whole alignment: aa composition difference between a row and the whole alignment, summed over aa and rows.
  - Trim one position, redo the calculation, get the difference between the trimmed and the complete alignment
  - Repeat for each site
- Estimates how much each site contributes to aa composition bias



Viklund et al, MBE 2012

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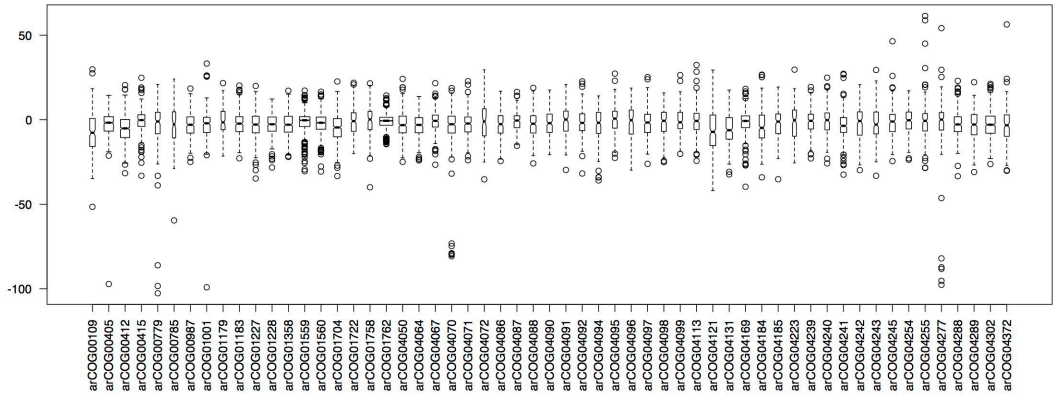
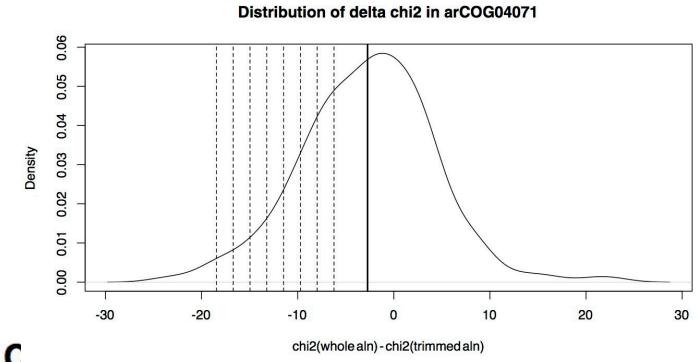
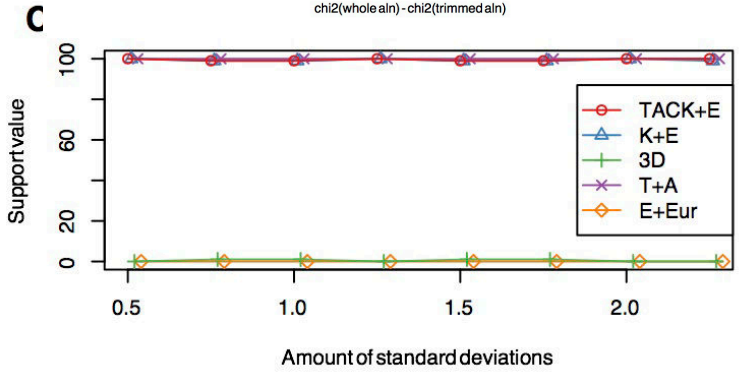
Chi2 filter







- Removing sites in a gene-specific manner: removing the most discordant sites, in terms of mean – fractions of sd
- Assess the effect on critical nodes
- No noticeable effect…

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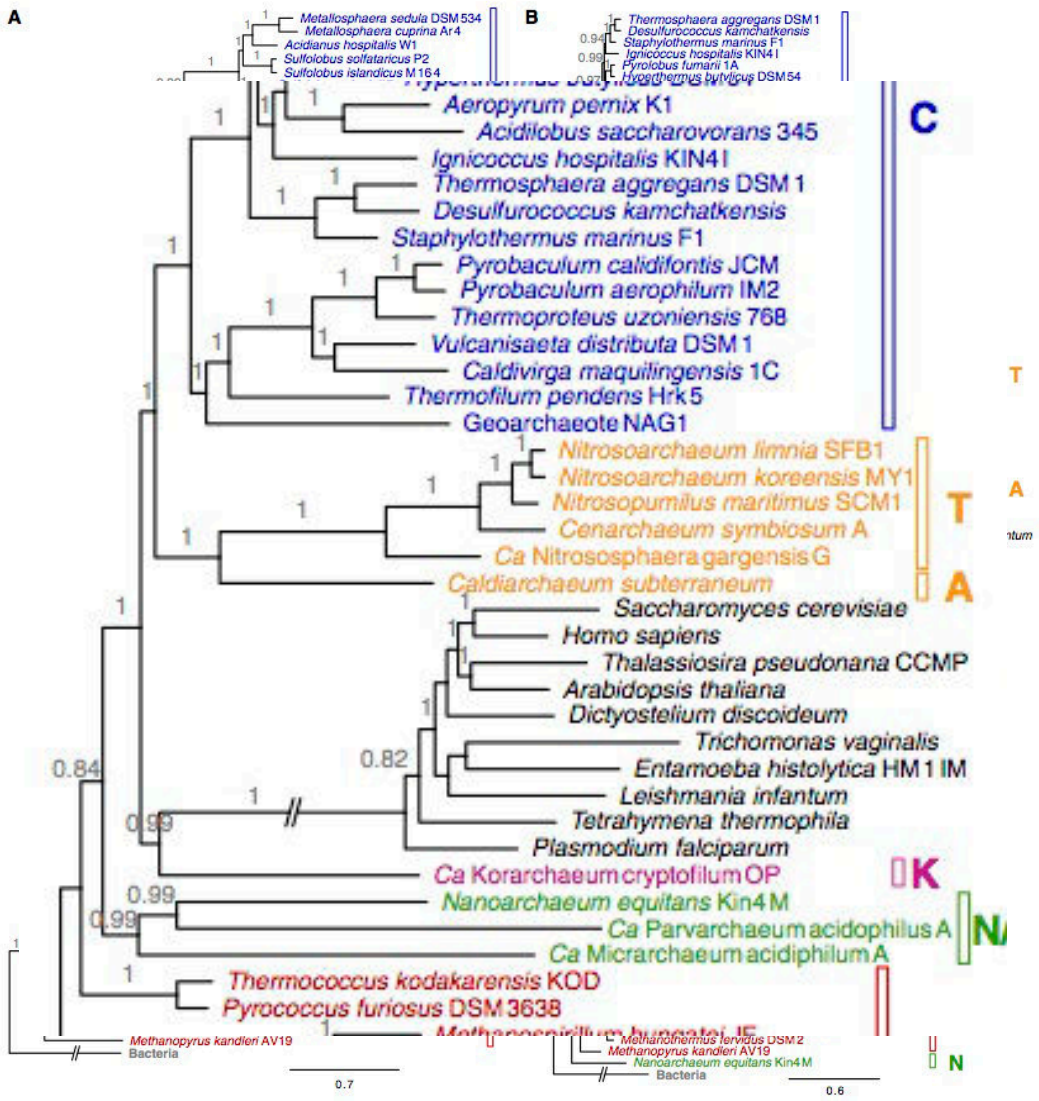
“Best” tree



- A:
  - disc15p dataset
  - Phylobayes
  - CAT-Poisson
  - Kind of converged
- B: (for comparison)
  - Concatenate SSU/ LSU rDNA
  - Same program/ methods
  - Made by Jimmy Saw