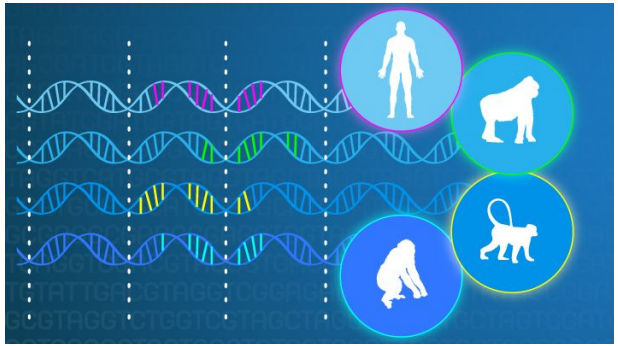
Week 4: Comparative genomics

- Whole genome alignment
  - MUMmer & Suffix trees
- Gene/species trees
  - Phylogenetic trees
  - Gene orthology & functional analysis

What is comparative genomics?



Common features of different organisms

- Conserved DNA.
- Very large distances: gene order & regulatory seqs not conserved.
- Moderate distances: Functional vs. nonfunctional based on negative/purifying selection.

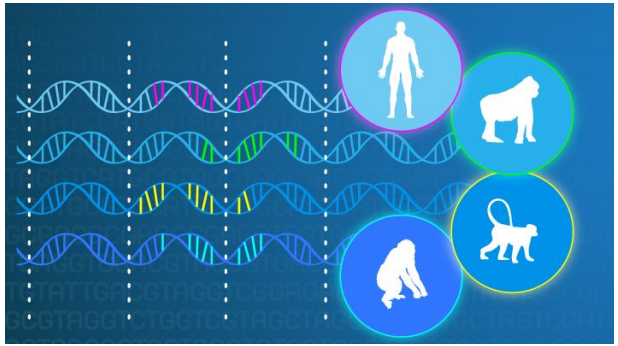
Distinctive features of closely-related species

- Unique genomic elements.

Genetic differences within one species

- Genetic variants with a role in a trait/disease.

Why comparative genomics?



- How many times has a feature (anatomy, trait, cellular/molecular property) arisen? been lost?
- How is a disease evolving to avoid immune system?
- What is the sequence of ancestral proteins?
- What are the most similar species?
- What is the rate of speciation?
- Is there a correlation between gain/loss of traits and environment or geographical events?
- Which features are ancestral to a clade, which are derived?
- What structures are homologous, which are analogous?

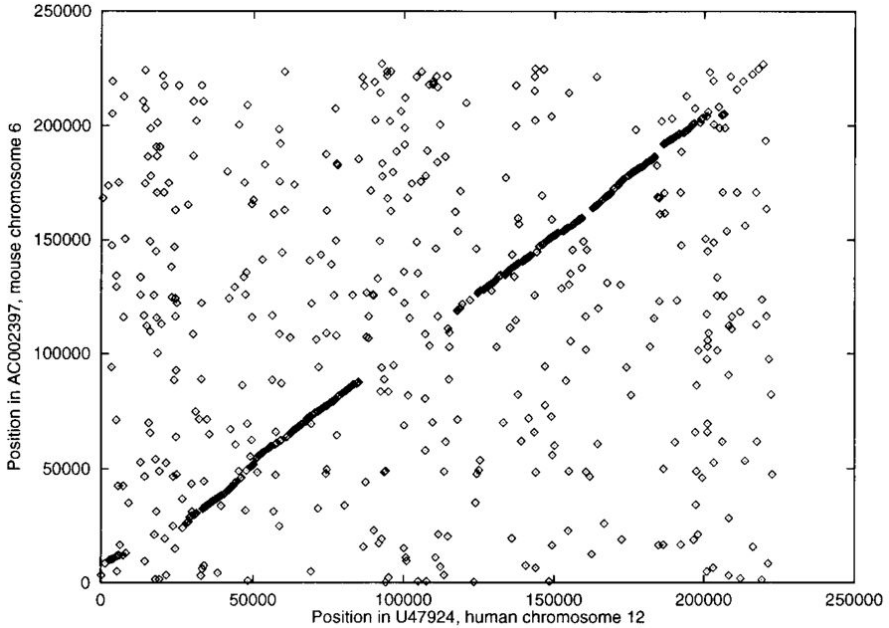
Whole-genome alignment – The problem

Given:

- A pair of large-scale sequences (e.g. chromosomes)
- A method for scoring the alignment (e.g. substitution matrices, insertion/deletion parameters)

Do:

- Construct global alignment: identify all matching positions between the two sequences.



Dechler (1999) Nuc. Acids. Res.

Whole-genome alignment – Need for newer methods

Time & space constraints:

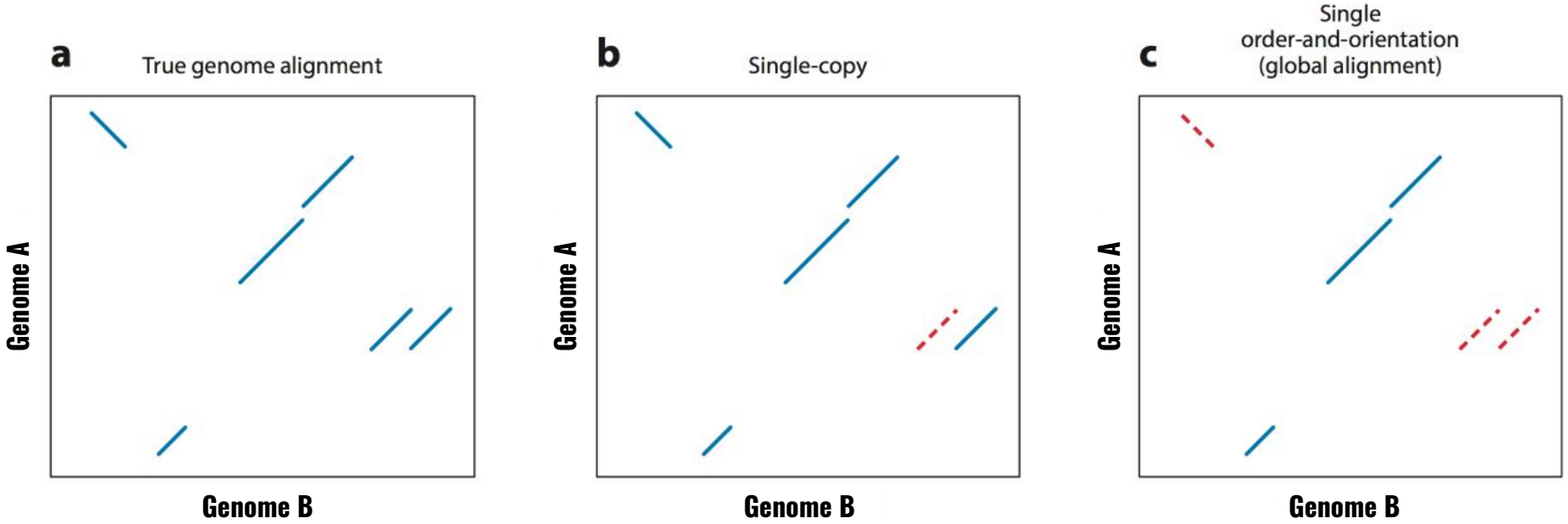
- Traditional DP algorithms require `O(nm)` time and space (`n` and `m` are the lengths of the two sequences).
- As `n` and `m` grow to genome scale, too expensive to solve in practice.

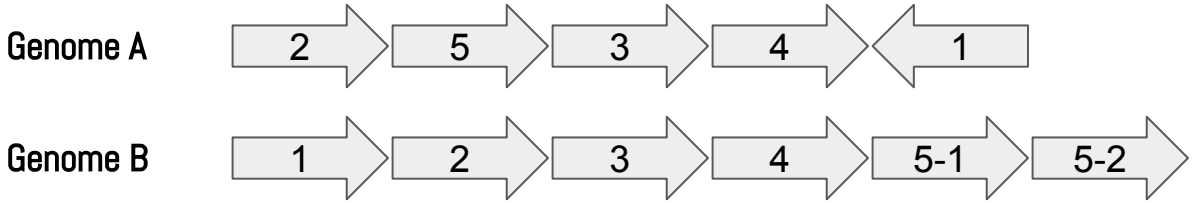
Genomic rearrangements:

- SW & NW ⟶ alignments that have fixed order & orientation.
    - Insertions, deletions, and substitutions are the only allowed edit operations (OK for short or well-conserved sequences, like genes).
- Over large evolutionary distances and within a sufficiently large window, genomes almost always contain more complex rearrangements with respect to each other
    - Inversions, transpositions, and duplications all cause breaks in order and orientation that cannot be captured under constant order and orientation.

Armstrong (2018) Ann. Rev. Anim. Biosci.

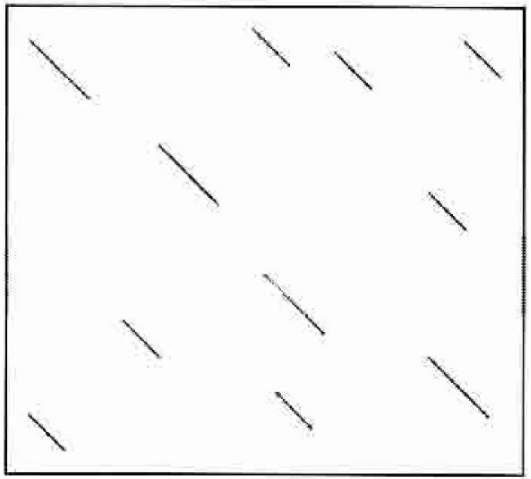
Whole-genome alignment



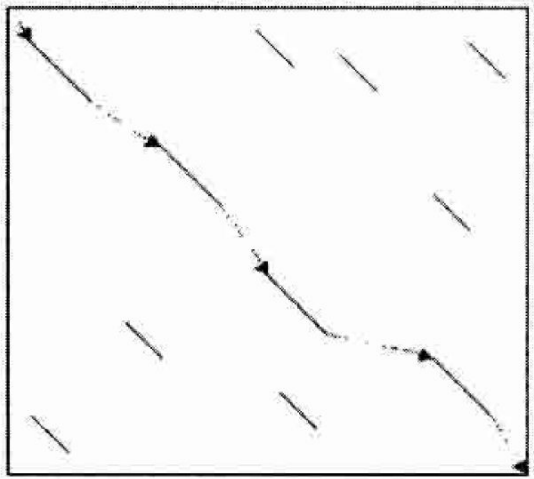


Armstrong (2018) Ann. Rev. Anim. Biosci.

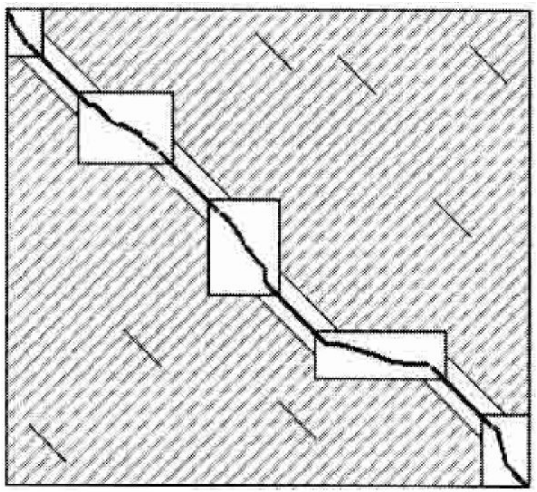
Whole-genome alignment



1. Perform pattern matching to find seeds for global alignment.



2. Find a good chain of anchors.



3. Fill in the gaps with traditional but constrained alignment methods.

Brundo (2003) Genome Res.

The MUMmer system

Delcher et al., *Nucleic Acids Research*, 1999

Given: genomes A and B

1. find all maximal unique matching subsequences (MUMs)
2. extract the longest possible set of matches that occur in the same order in both genomes
3. close the gaps

Step 1: Finding seeds in MUMmer

- *Maximal unique match*:
  - occurs exactly once in both genomes A and B
  - not contained in any longer MUM

- Key insight: a significantly long MUM is certain to be part of the global alignment

Suffix trees

- Substring problem:
  - given text S of length m
  - preprocess S in $O(m)$ time
  - such that, given query string Q of length n, find occurrence (if any) of Q in S in $O(n)$ time

- Suffix trees solve this problem and others

Suffixes

S = “banana$”

suffixes of S

$ (special character)

a$

na$

ana$

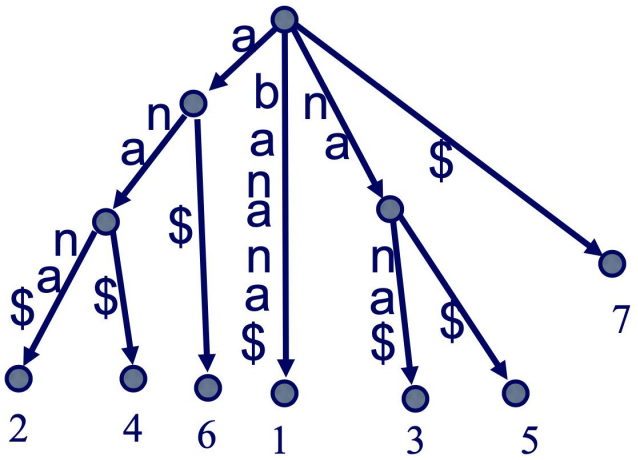
nana$

anana$

banana$

Suffix tree – Example

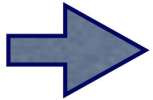
- S = “banana$”
- Add ‘$’ to end so that suffix tree exists (no suffix is a prefix of another suffix)



Suffix tree – Definition

- A suffix tree T for a string S of length m is a tree with the following properties:
  - *rooted* and *directed*
  - m leaves, labeled 1 to m
  - each edge labeled by a substring of S
  - concatenation of edge labels on path from root to leaf i is suffix i of S (we will denote this by $S_{i...m}$)
  - each internal non-root node has at least two children
  - edges out of a node must begin with different characters

key property

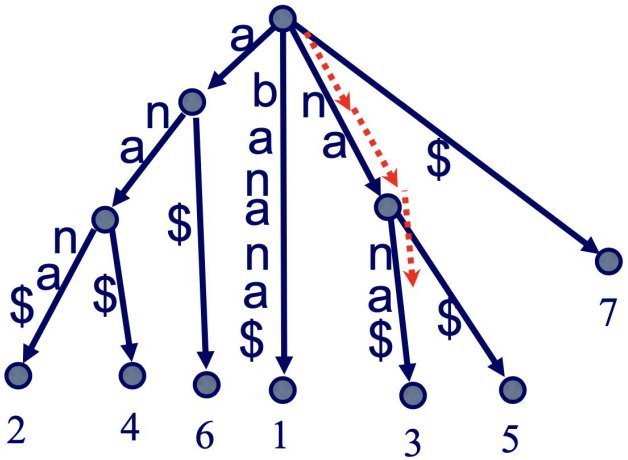


Solving the suffix tree problem

- Assume we have suffix tree T and query string Q
- FindMatch(Q, T):
  - follow (unique) path down from root of T according to characters in Q
  - if all of Q is found to be a prefix of such a path
    return label of some leaf below this path
  - else, return no match found

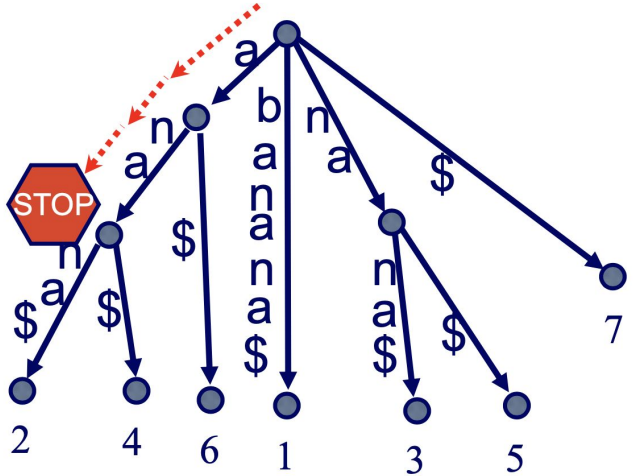
Solving the substring problem

Q = nan



return 3

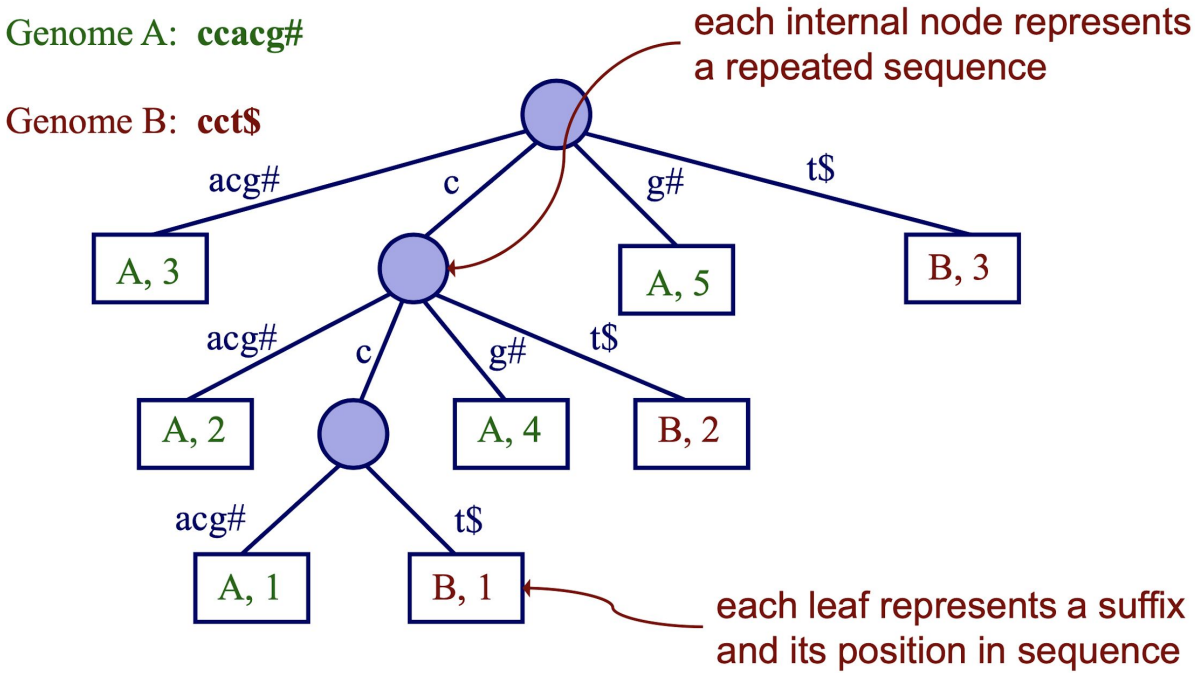
Q = anab



return no match found

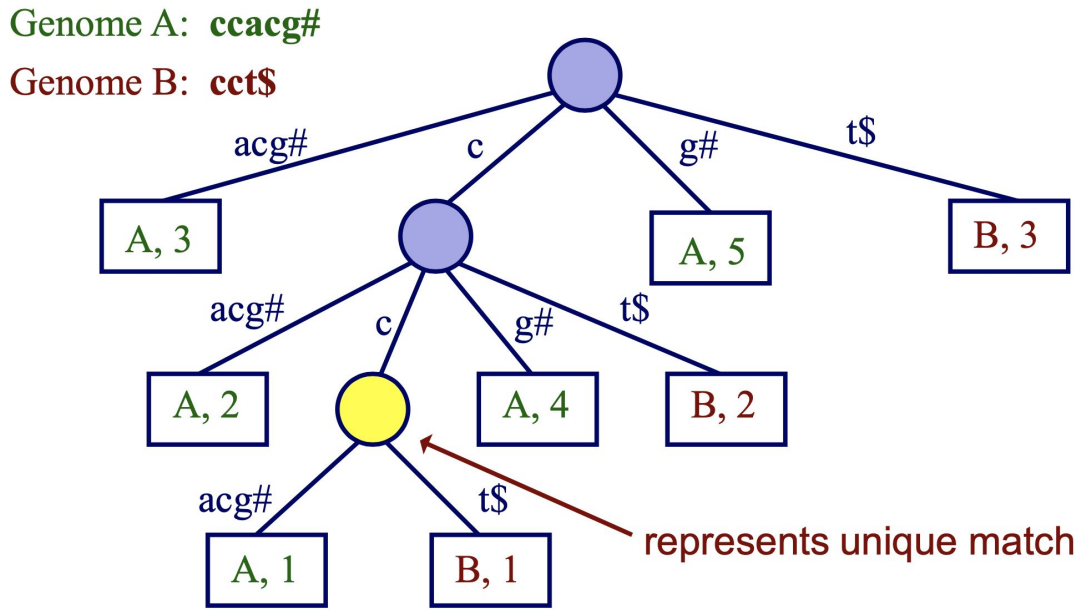
MUMs and Generalized suffix trees

- Build one suffix tree for both genomes A and B
- Label each leaf node with genome it represents



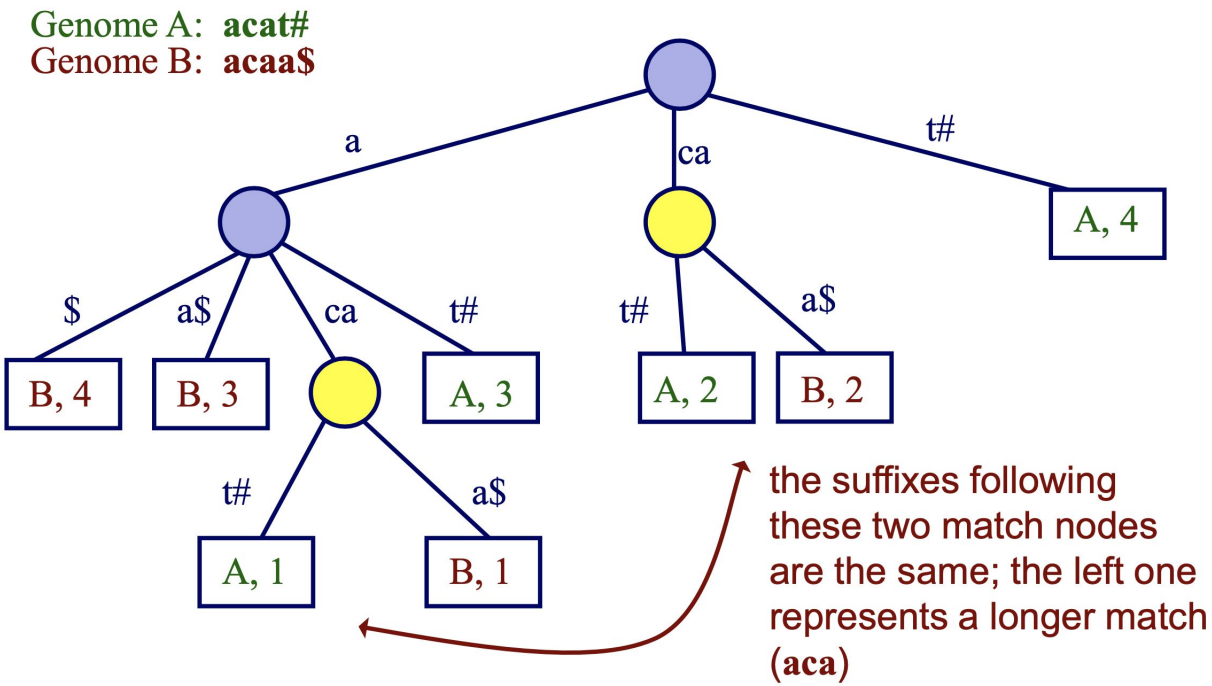
MUMs and Suffix trees

- Unique match: internal node with 2 children, leaf nodes from different genomes
- But these matches are not necessarily maximal



MUMs and Suffix trees

- To identify maximal matches, can compare suffixes following unique match nodes



Using suffix trees to find MUMs

- O(n) time to construct suffix tree for both sequences (of lengths ≤ n)
- O(n) time to find MUMs - one scan of the tree (which is O(n) in size)
- O(n) possible MUMs in contrast to O(n^2) possible exact matches

- Main parameter of approach: length of shortest MUM that should be identified (20 – 50 bases)

Step 2: Chaining in MUMmer

- Sort MUMs according to position in genome A
- Solve variation of *Longest Increasing Subsequence* (LIS) problem to find sequences in ascending order in both genomes

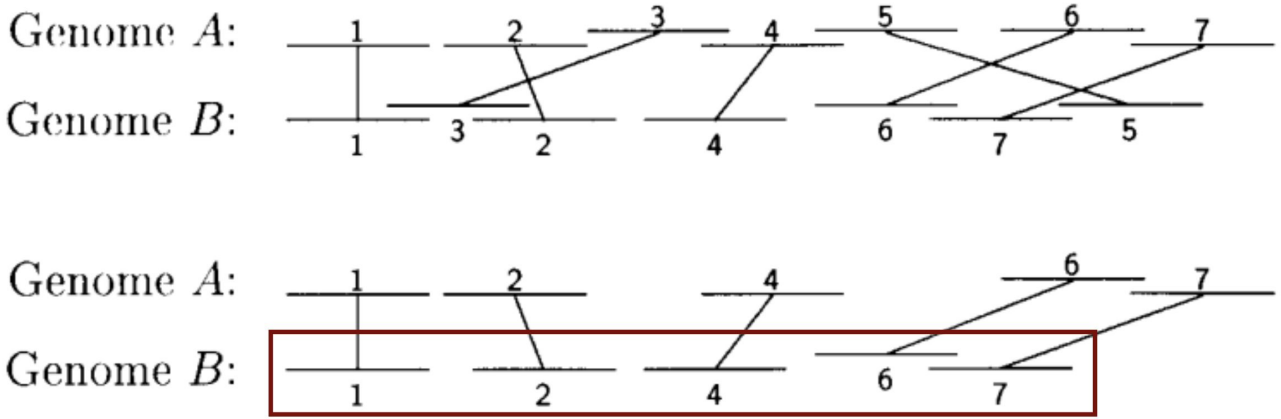
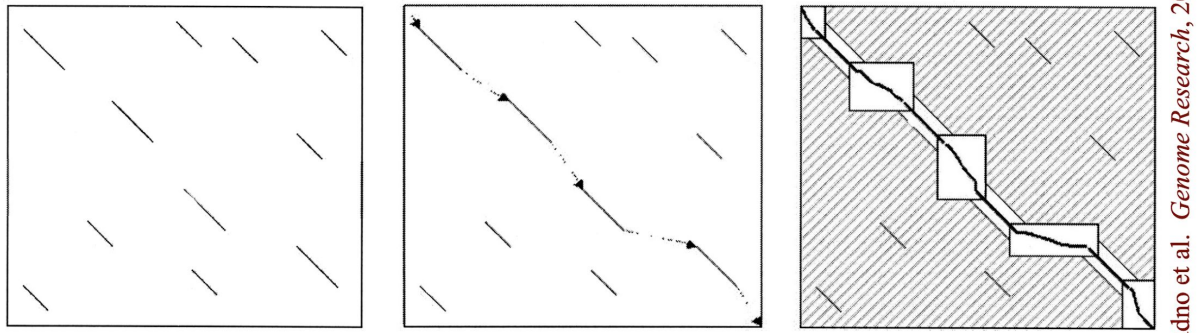


Figure from: Delcher et al., *Nucleic Acids Research* 27, 1999

Finding longest subsequence

- Unlike ordinary LIS problems, MUMmer takes into account
  - lengths of sequences represented by MUMs
  - overlaps
- Requires $O(k \log k)$ time where k is number of MUMs

Recall: Three main steps of large-scale alignment



Brudno et al. *Genome Research*, 20

General

1. Pattern matching to find seeds for global alignment
2. Find a good chain of anchors
3. Fill in with standard but constrained alignment

MUMmer

1. Suffix trees to obtain MUMs
2. LIS to find colinear MUMs
3. Smith-Waterman and recursive MUMmer for gap filling

Types of gaps in a MUMmer alignment

1. SNP: exactly one base (indicated by ^) differs between the two sequences. It is surrounded by exact-match sequence.

```
Genome A:  cgtcatgggcgttcgtcgttg
Genome B:  cgtcatgggcattcgtcgttg
                     ^
```

2. Insertion: a sequence that occurs in one genome but not the other.

```
Genome A:  cggggtaaccgc.................cctggtcggg
Genome B:  cggggtaaccgcgttgctcggggtaaccgccctggtcggg
                     ^^^^^^^^^^^^^^^^^
```

3. Highly polymorphic region: many mutations in a short region.

```
Genome A:  ccgcctcgcctgg.gctggcgcccgctc
Genome B:  ccgcctcgccagttgaccgcgcccgctc
                   ^ ^^ ^^^
```

4. Repeat sequence: the repeat is shown in uppercase. Note that the first copy of the repeat in Genome B is imperfect, containing one mismatch to the other three identical copies.

```
Genome A:  cTGGGTGGGACAACGTaaaaaaaaaTGGGTGGGACAACGTc
Genome B:  aTGGGTGGGGCgACGTgggggggggTGGGTGGGACAACGTa
           ^          ^                          ^
```

Figure from: Delcher et al., *Nucleic Acids Research* 27, 1999

Step 3: Close the gaps

- SNPs:
  - between MUMs: trivial to detect
  - otherwise: handle like repeats
- Insertions
  - simple insertions: trivial to detect
  - transpositions (subsequences that were deleted from one location and inserted elsewhere): look for out-of-sequence MUMs

Step 3: Close the gaps

- Polymorphic regions
  - short ones: align them with dynamic programming method
  - long ones: call MUMmer recursively with reduced minimum MUM length
- Repeats
  - detected by overlapping MUMs

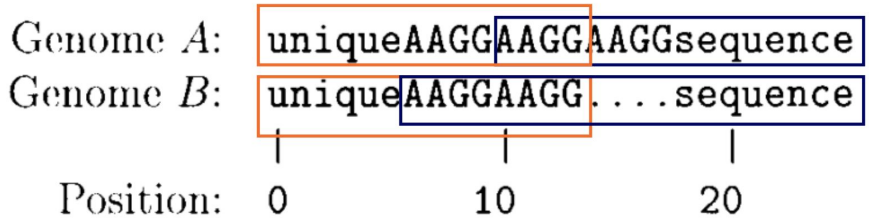


Figure from: Delcher et al. Nucleic Acids Research 27, 1999

MUMmer performance

FASTA on 1000 base pair segments

MUMmer

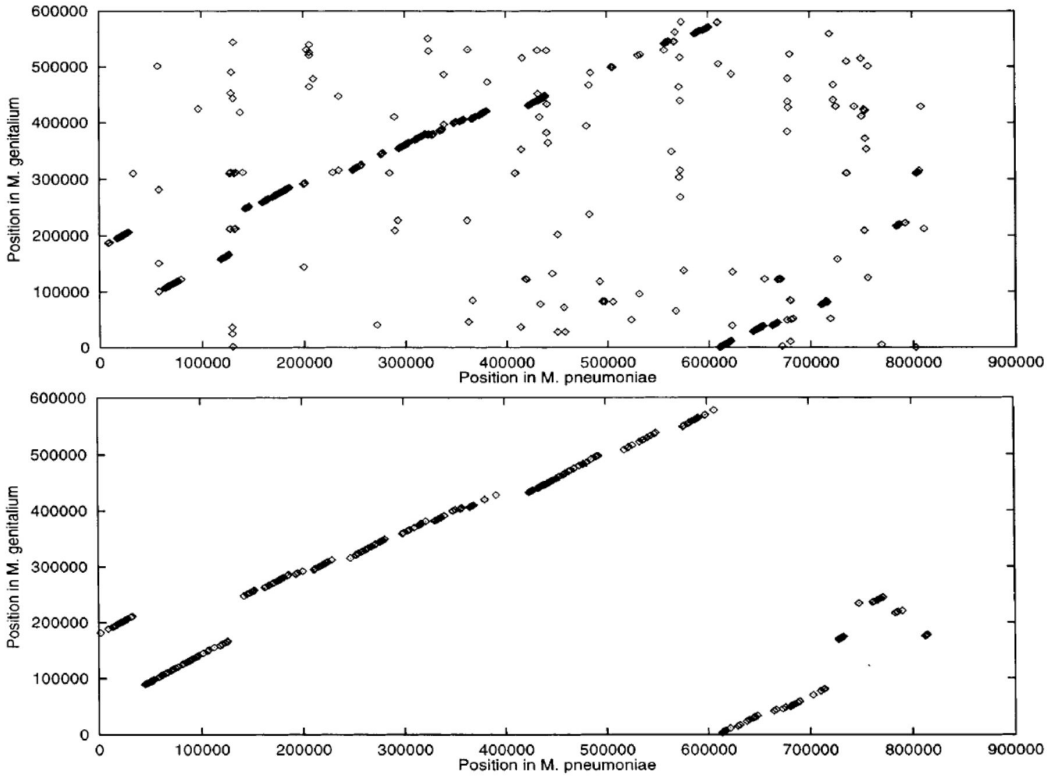


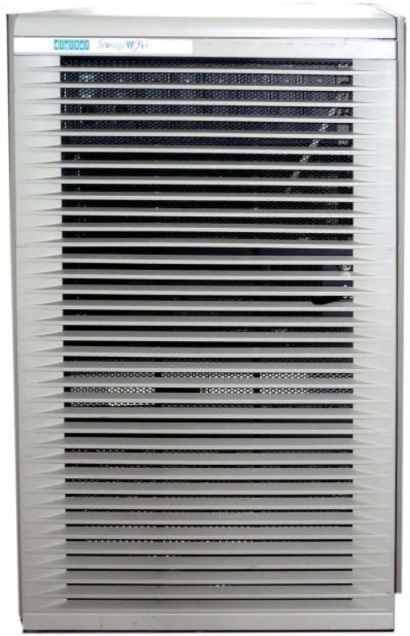
Figure from: Delcher et al. Nucleic Acids Research 27, 1999

MUMmer performance

- *Mycoplasma* test case
- Suffix tree: 6.5s
- LIS: 0.02s
- Smith-Waterman: 116s

- FASTA baseline: many hours

DEC Alpha 4100

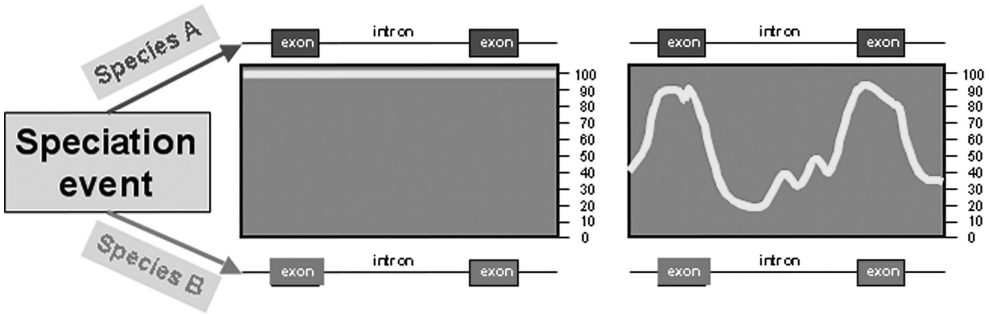


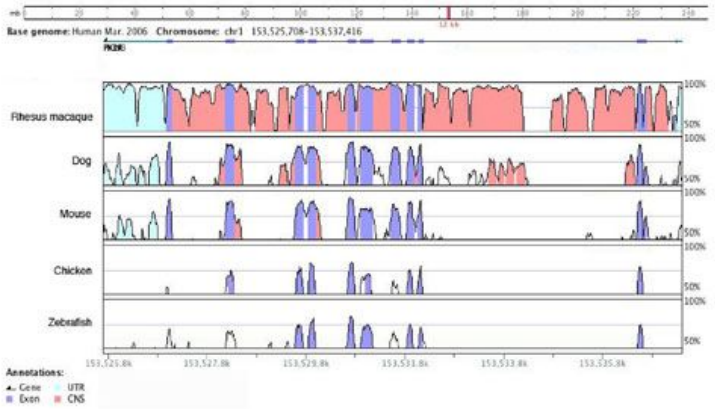
Centre for Computing History

Limitations of MUMmer

- MUMs are perfect matches, typically ≥ 20-50 base pairs
- Evolutionarily distant may not have sufficient MUMs to anchor global alignment
- How can we tolerate minor variation in the seeds?

Comparative genomics



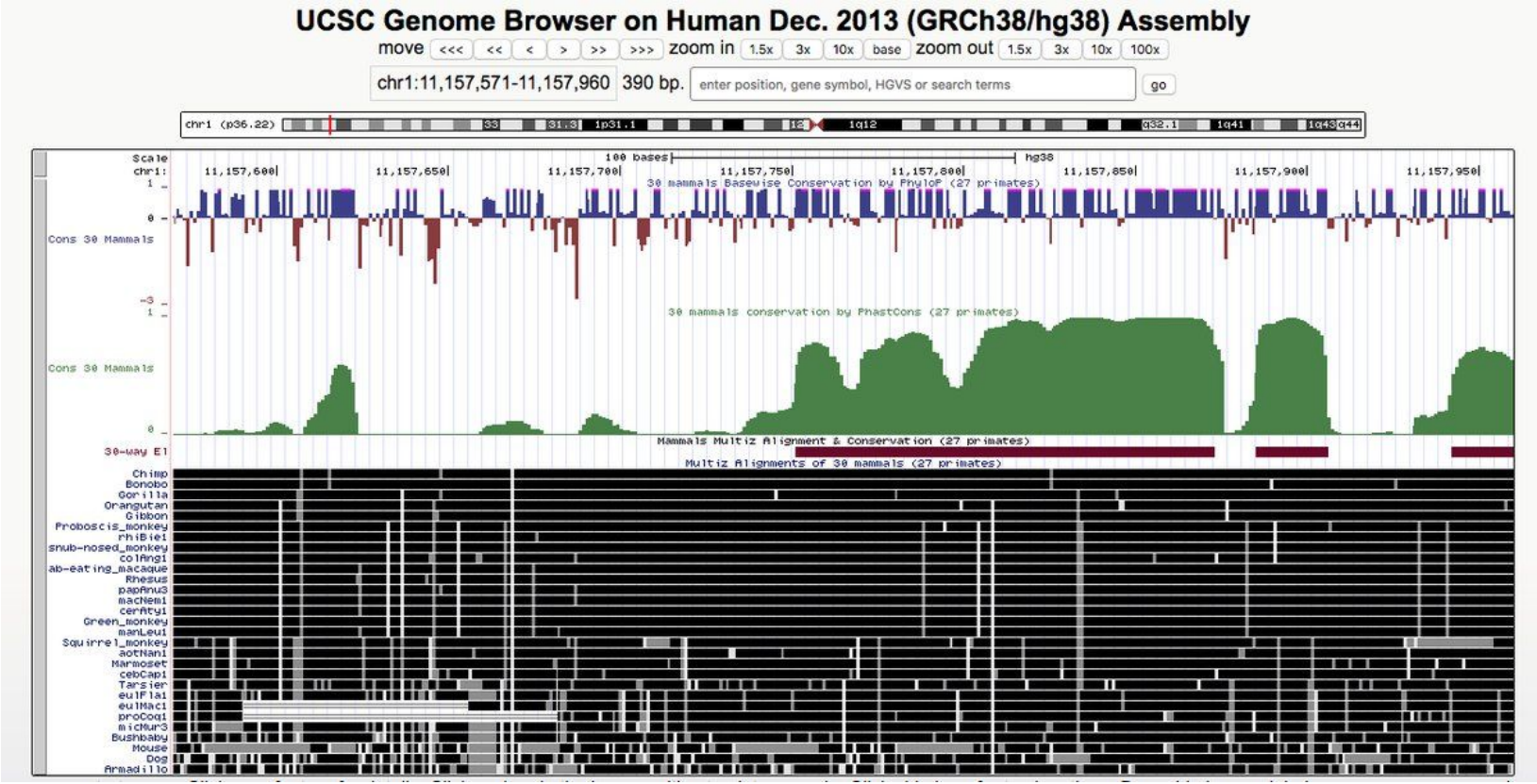


Miller et al., (2004) Ann. Rev. Genomics & Hum. Gen.

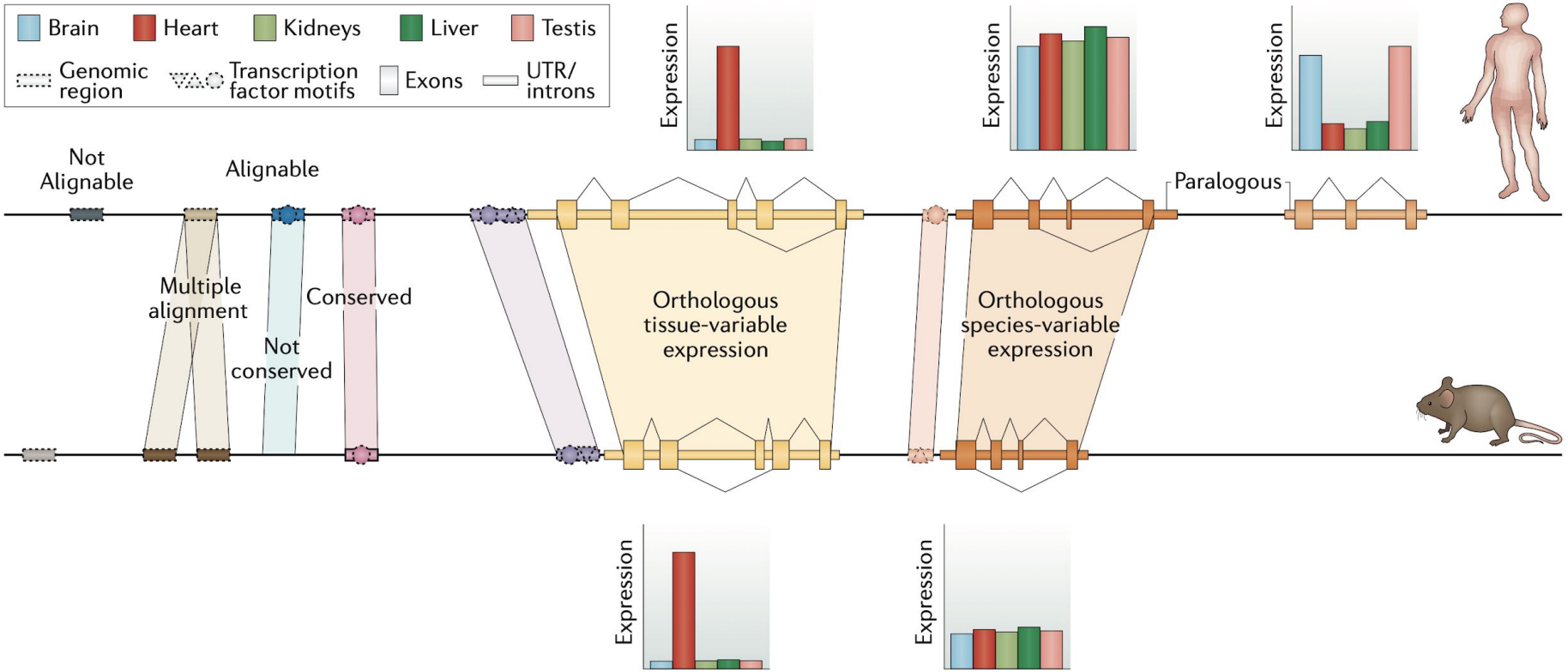
Nature Scitable

Comparative genomics

Multiple alignments and measurements of evolutionary conservation for 30 species (27 primates)

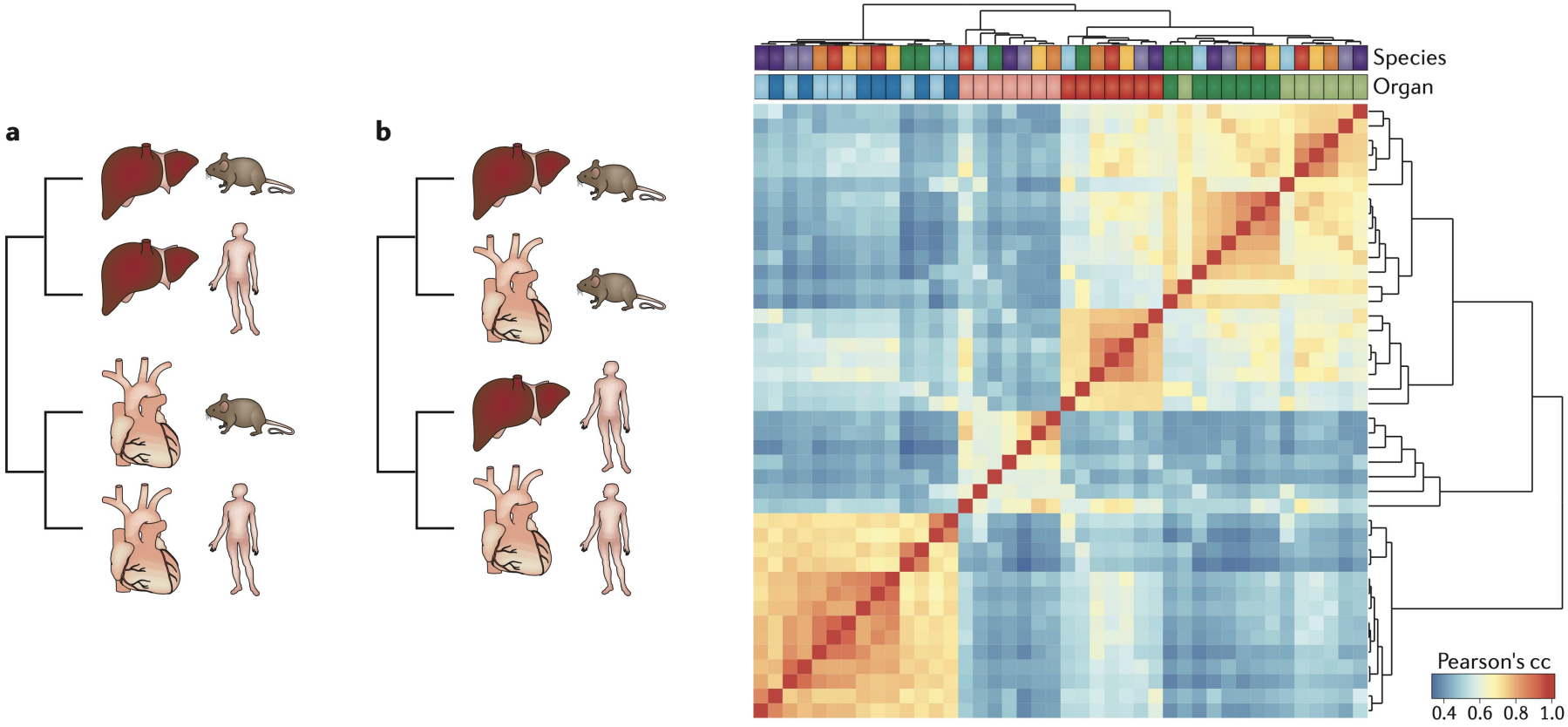


Comparative transcriptomics



Breschi (2017) Nat. Rev. Genet.

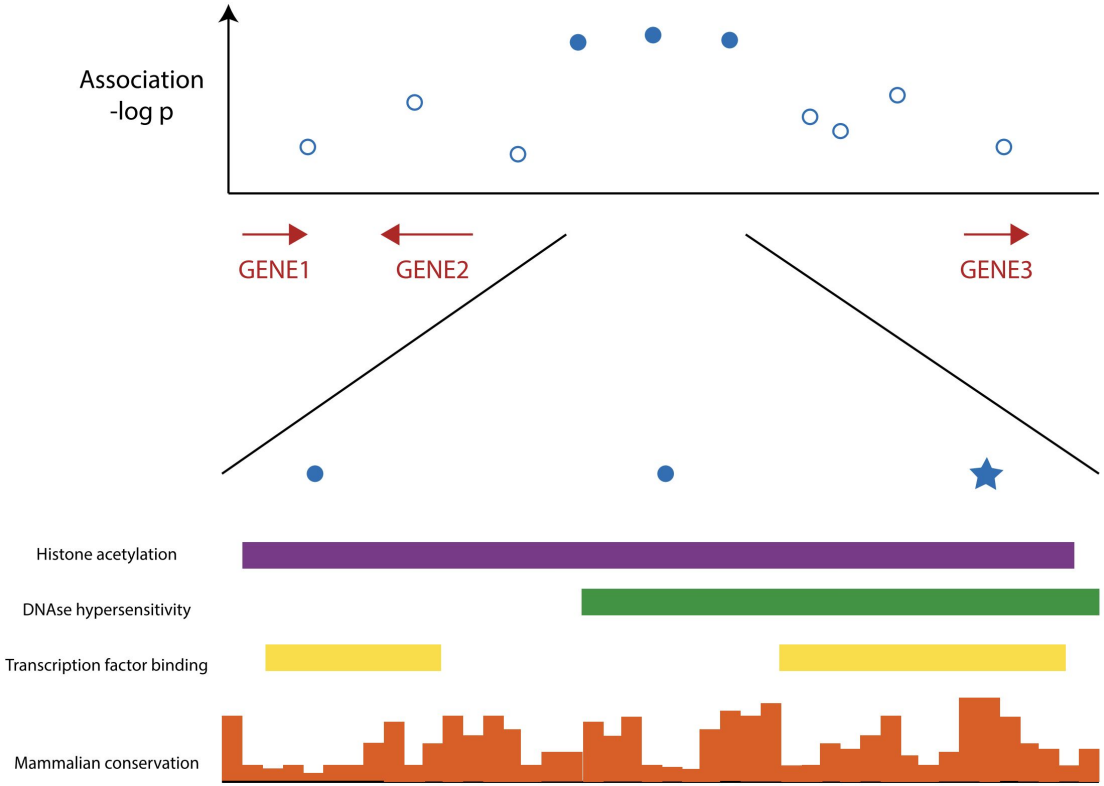
Comparative transcriptomics



Breschi (2017) Nat. Rev. Genet.

Comparative –omics

How conservation can be used to identify the most likely functional variants in a disease locus?



Alföldi & Lindblad-Toh (2013) Genome Res.

Paper report & discussion: Longevity of MUMmer

	Biological / conceptual	Technological / experimental	Algorithmic / methodological	Hardware / software
MUMer v1				
MUMer v2				
MUMer v3				
MUMer v4				