Alignment of whole genomes using MUMmer

Presentation in Algorithms in Bioinformatics (TÖ111F autumn 2014)

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Motivation

Let’s go back to 1999... (some things that will remind you of that wonderful time)

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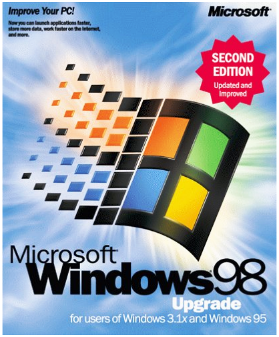
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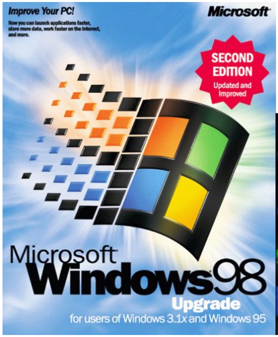
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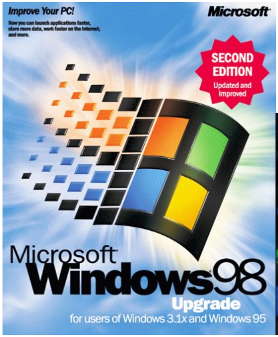
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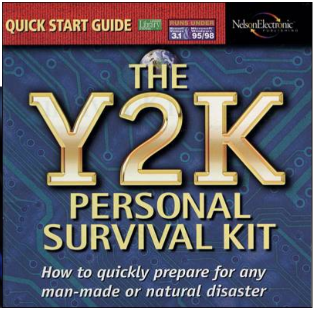
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Motivation

In 1999...

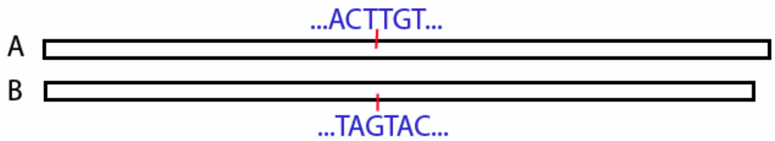
- The number of sequenced genomes was very low but increasing rapidly
- When a new genome is sequenced, one could ask himself:
  - How does this genome align to the other genomes?

Problem:

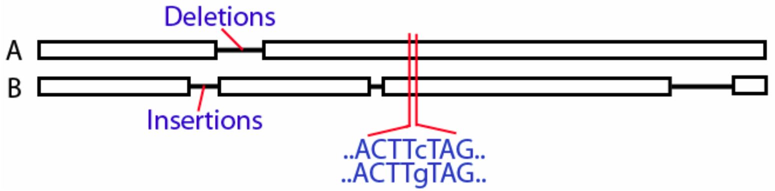
- We had algorithms that were used for single gene sequences (up to 10,000 bp)
- But, they won't work well with whole genomes (can be millions of base pairs or more).
- On the other algorithms they'd either
  - Take up way too much memory or
  - Have unacceptable computational time

Problem description

In Two genomes, A and B. Both could be very large (possibly over 1 Mbp)



Out Align the two genomes using insertions and deletions (or for short, indels) to maximize the matches.



Introduction to MUMmer

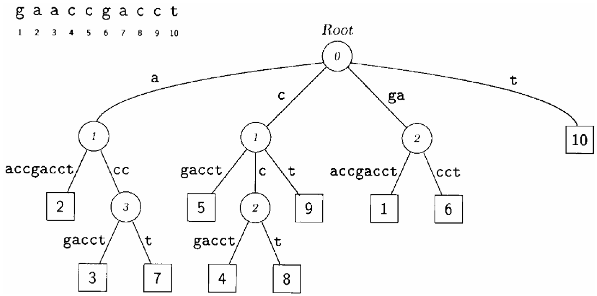
MUMmer:

- Was published in 1999.
- Is a system used to align whole genome sequences.
- Uses suffix trees as a data structure.
- Tries to find large unique chunks of exact matches on both genomes.
  - So it will only work well for two genomes that are similar/related.
- Has been open-source since 2004 (when MUMmer 3 was released).
- Doesn't guarantee the optimal solution, just a good one.
- The algorithm can be split into several steps.

Step 1: Creating a suffix tree

A suffix tree is a compact representation that stores all possible suffixes of an input sequence.

- Square nodes are leaves.
  - Store information about the starting position of the suffix.
- Circular nodes are internal nodes.
  - That means two or more sequences share the same prefix.
  - Store information about the length of the shared prefix.



Creating a suffix tree takes $O(n)$ time and space.

Step 2: MUM decomposition

Let us first define what a MUM is

Definition

A subsequence is a MUM (Maximal Unique Matches) if and only if:

- The subsequence has an exact match on both genomes
- It is not a subsequence of another matched sequence
  - This means the sequence is surrounded be mismatches
- It is unique
  - It appears exactly once in both genomes
- Almost always uses some minimum sequence length.

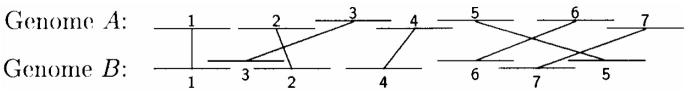
Example

```
tcgatcGACGATCGCGGCCGTAGATCGAATAACGAGAGAGCATAAcgactta
gcattaGACGATCGCGGCCGTAGATCGAATAACGAGAGAGCATAAtccagag
```

We can find these MUMs using the suffix tree (further details later).

Step 3: Sorting the matches found in the MUM alignment

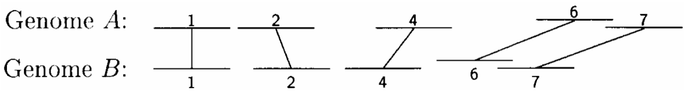
Once we have found the MUMs, we enumerate them like this:



Problem: We cannot align all MUMs because they aren't in the same order in both genomes.

Solution: Align as many MUMs as we can:

- We find the longest increasing sequence for genome B.
  - In this case the sequence is <1,3,2,4,6,7,5>.
- We can do this in $O(K \log K)$ time, where K is the number of MUMs. Since in general $K << N/\log N$ this step takes $O(n)$ time.



Step 4: Closing the gaps

Everything in between the MUMs is called **gaps**.

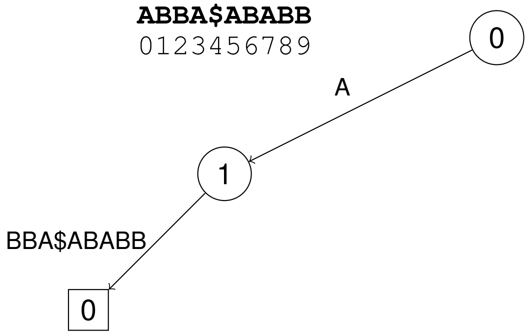
- To find alignment for the gaps we use any alignment algorithm.
- The first version of MUMmer uses the Smith-Waterman alignment algorithm (which takes $O(n^2)$ time).
- If the gaps are too large for the alignment algorithm we need to recursively find new MUMs in that region using a smaller minimum sequence length than before.

Step 5: Output results

The final alignment is the alignment of MUMs and gaps combined.

- If the two genomes are very similar, then the MUMs sequences will...:
  - ...be long.
  - ...cover most of the genome.
  - ...rarely be a random match. Therefore few or no errors.
  - ...make the algorithm run fast (almost linear time)
- If the genomes are very different, then the MUMs sequences will...:
  - ...be short.
  - ...cover a small part of the genome.
  - ...often be a random match. Therefore many errors.
  - ...make the algorithm run slow.

Creating a suffix tree



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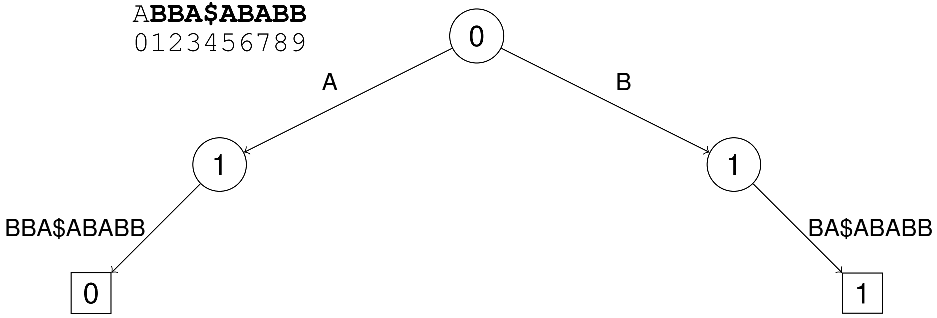
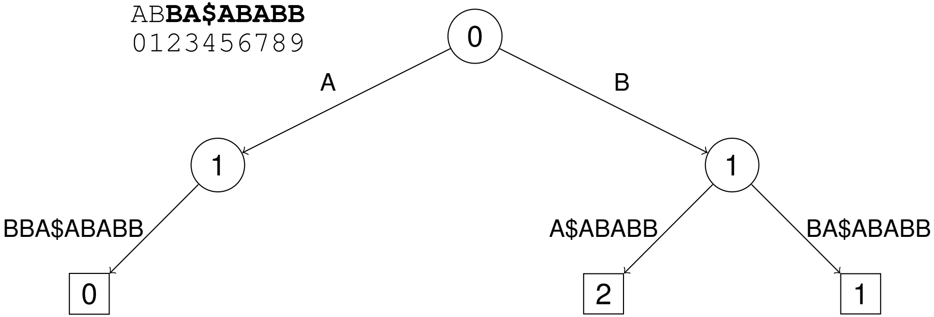


Creating a suffix tree

AB**BA$ABABB**
0123456789



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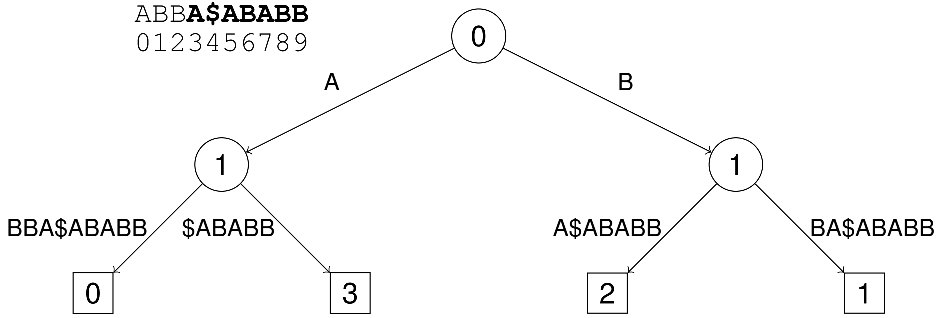

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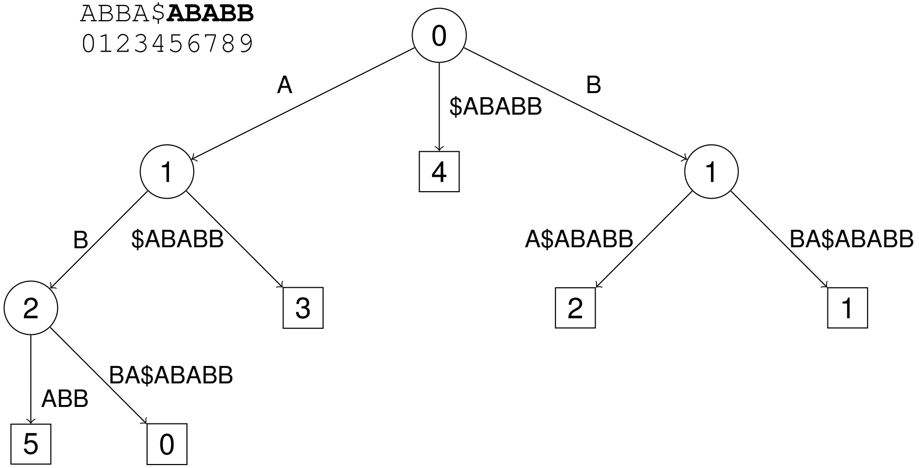

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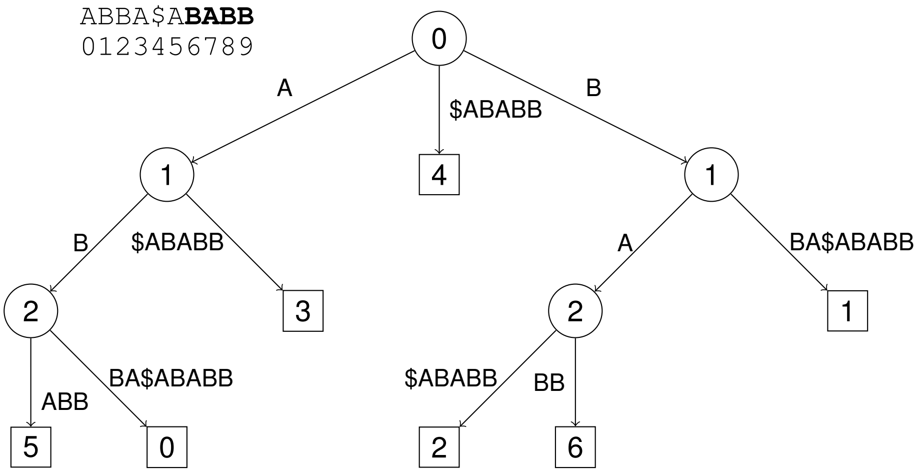

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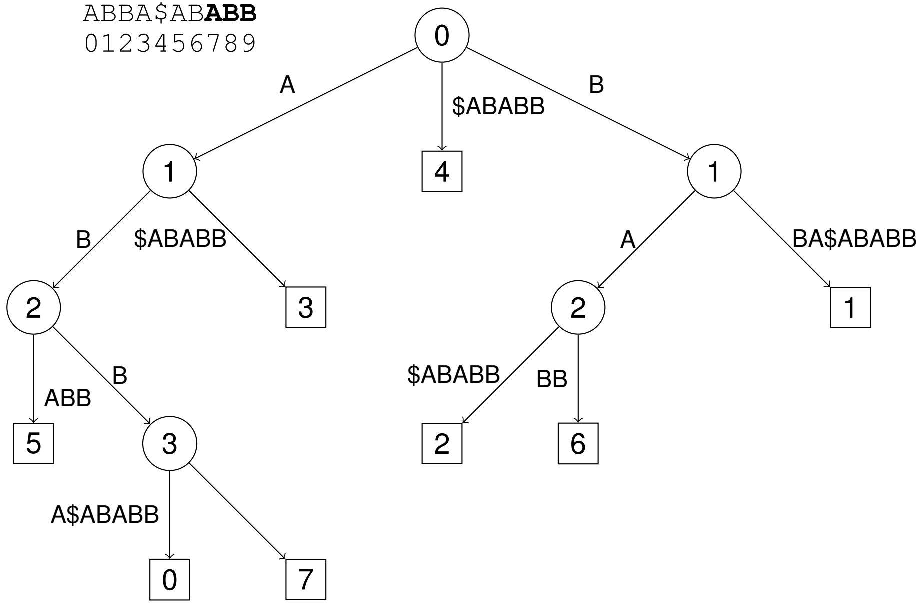

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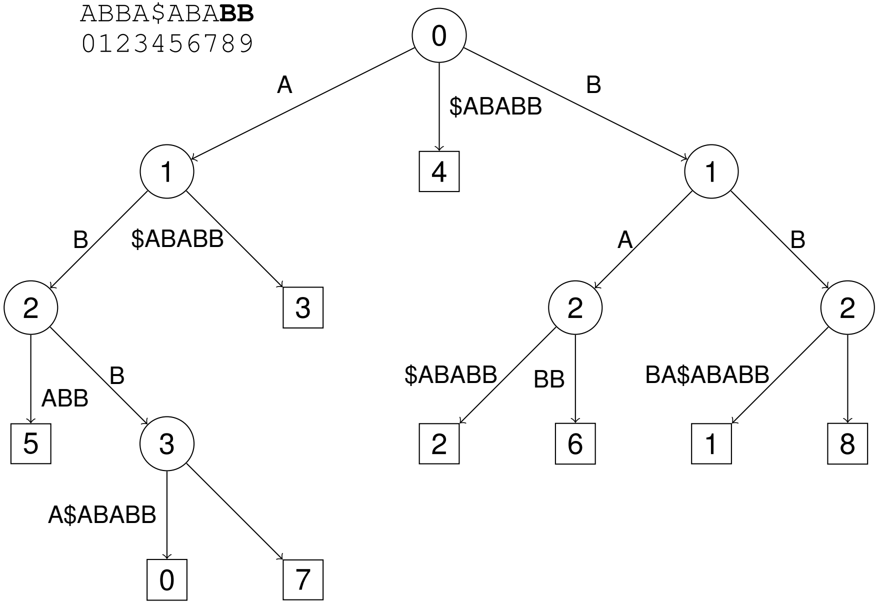

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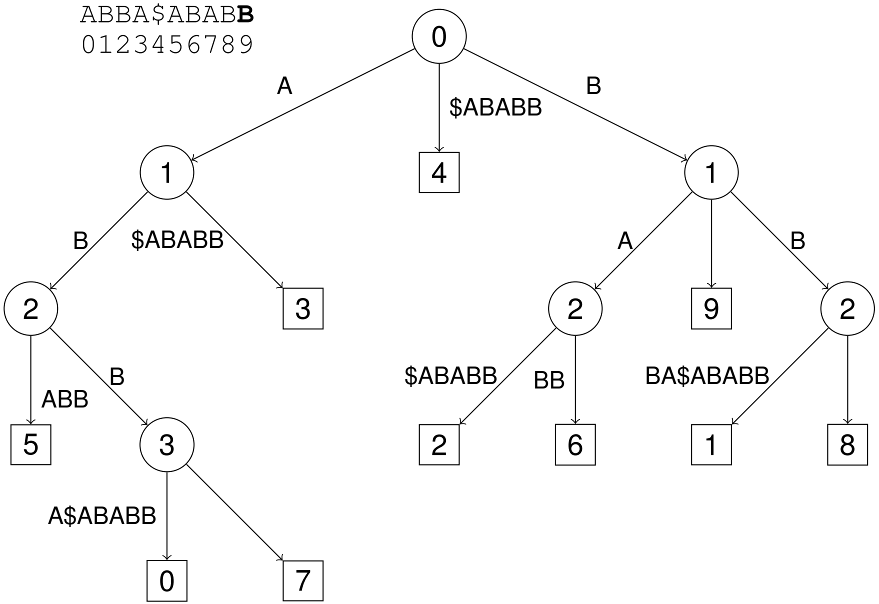


Finding MUMs from suffix tree

Let's recall what condition MUMs had to have:

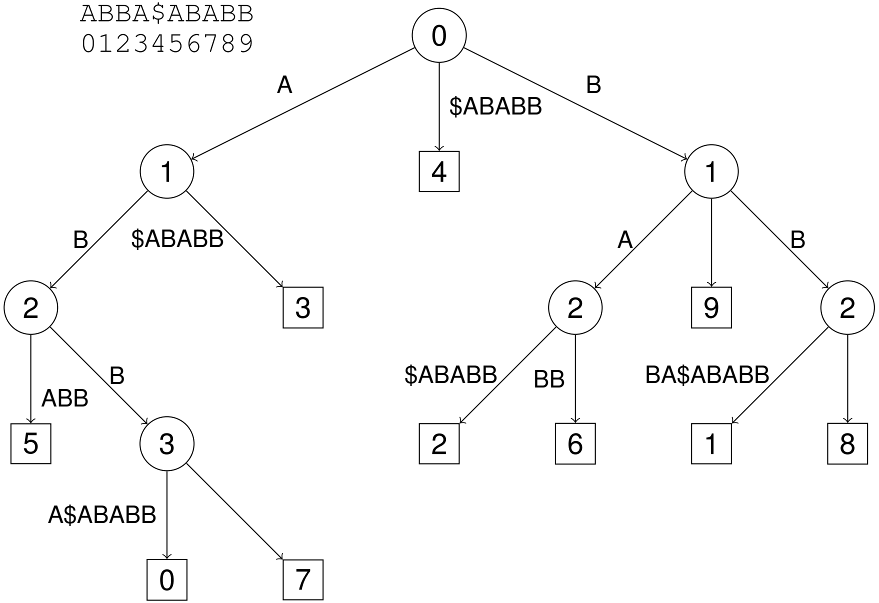
1. Exact matches on both genomes.
2. Surrounded by mismatches.
3. Unique.

We can achieve conditions 1 and 3 by searching the tree for a internal with exactly two leafs which have to start on each side of the dollar sign (on each genome).

We'll need to check for condition 2 separately.

Finding potential MUMs

```
ABBA$ABABB
0123456789
```



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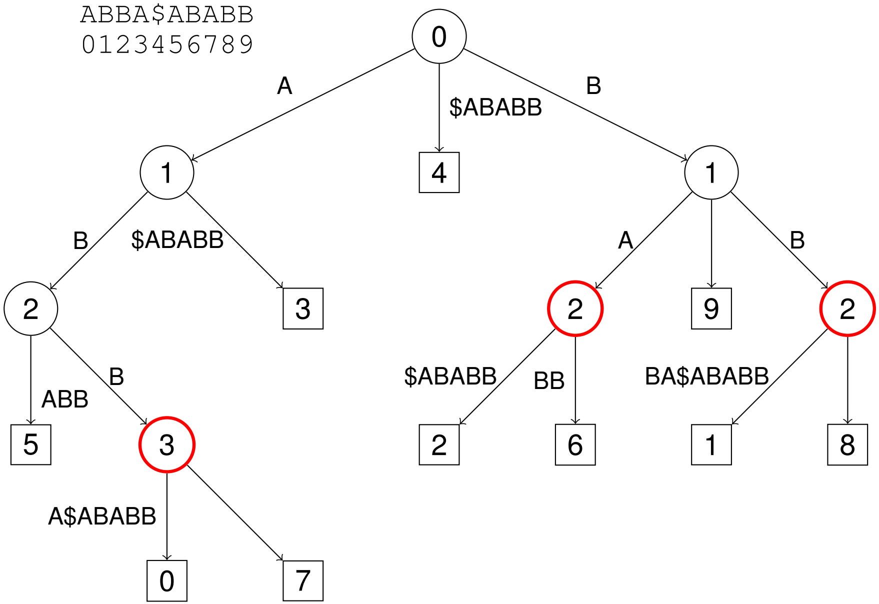

Potential MUMs to MUMs

We have the following potential MUMs:

- `ABB` at starting positions 0 and 7.
- `BA` at starting positions 2 and 6.
- `BB` at starting positions 1 and 8.

Our string: `ABBA$ABABB`

- `ABB` achieves condition 2 (it's surrounded by mismatches).
- `BA` achieves condition 2 as well.
- `BB` does NOT achieve condition 2. In both cases it is preceded by a `A`.

Resulting MUMs: **`ABB`** and **`BA`**.

Results of using MUMmer

MUMmer was put to the test on various genomes:

- Two strains of tuberculosis (bacteria) that are >99% identical
    - 5 seconds to create the suffix tree.
    - 45 seconds to sort the MUMs.
    - 5 seconds to generate the Smith-Waterman alignments of the gaps.

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- Two 'cousin' genomes. Genome of *M.genitalium* (580,074 nucleotides) and *M.pneumoniae* (816,394 nucleotides)
  - 6.5 seconds to create the suffix tree.
  - 0.02 seconds to sort the MUMs.
  - 116 seconds to generate the Smith-Waterman alignments of the gaps.

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    - 116 seconds to generate the Smith-Waterman alignments of the gaps.
- Subsequence of a human chromosome 12p13 and mouse chromosome 6 (both rougly 230,000 nucleotides)
    - 1.6 seconds to create the suffix tree.
    - $\approx$ 0 seconds to sort the MUMs.
    - 27.4 seconds to generate the Smith-Waterman alignments of the gaps.

MUMmer 3

- MUMmer 3 is the latest version of MUMmer
- It was released in 2004 and is open-source.
- Requires less than half the memory and more than twice as fast than the initial MUMmer.
- Most notable problems fixed and options added since the initial MUMmer:
  - You can allow tolarence for mismatches when finding MUMs.
  - Can handle MUMs that are not necessarily unique.
  - All sorts of visualization tools.
- Could successfully compare all human chromosomes to each other. Tests were performed on a single computer with 950 MHz processor.
  - Computation time: 4.5 days
  - Memory used: 3.9 GB