NGS Reads Mapping and Efficient Indexing Structures

DNA Sequencers

Sanger Sequencing

Sanger sequencing
Maxam and Gilbert
Sanger chain termination

Infer nucleotide identity using dNTPs, then visualize with electrophoresis

500–1,000 bp fragments

Next Generation Sequencing

454, Solexa,
Ion Torrent,
Illumina

High throughput from the parallelization of sequencing reactions

~50–500 bp fragments

Long-read Sequencing

PacBio
Oxford Nanopore

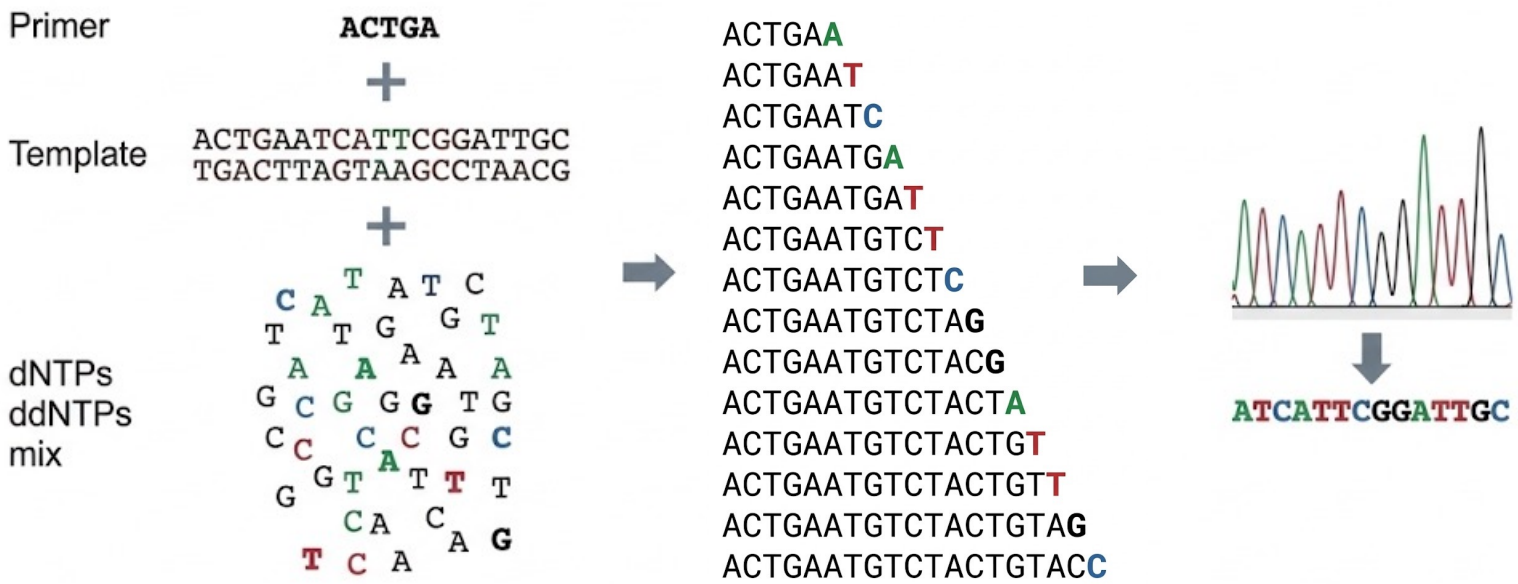
Sequence native DNA in real time with single-molecule resolution

Tens of kb fragments, on average

Short-read sequencing

Long-read sequencing

Sanger Sequencing

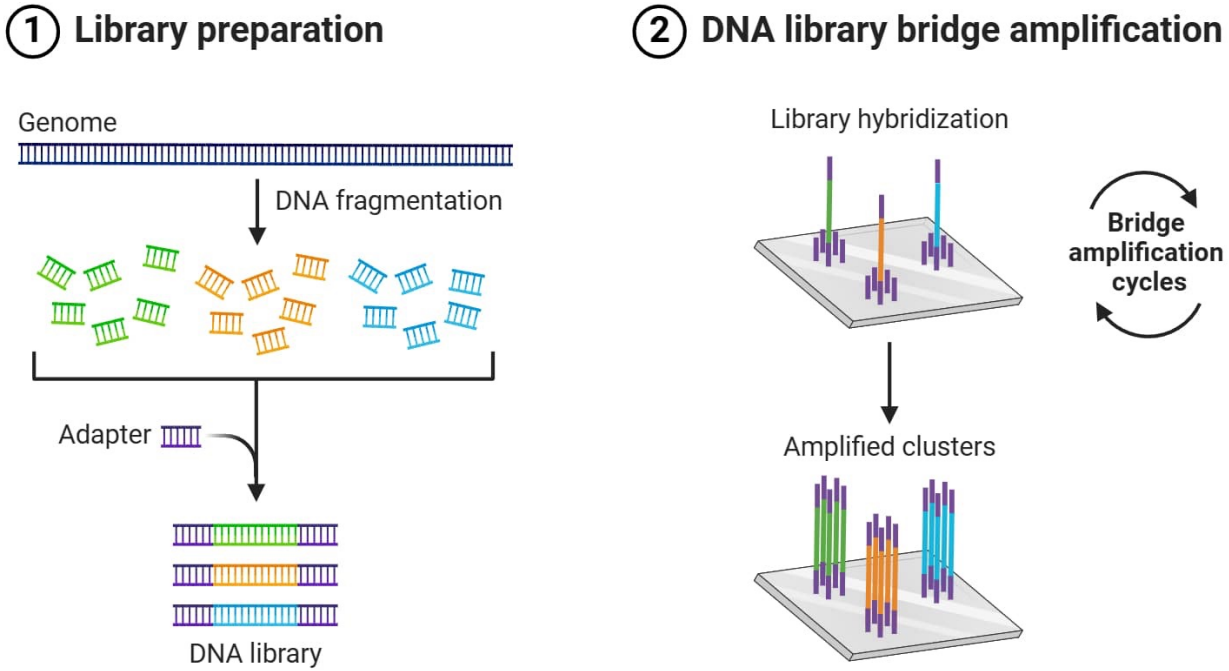


Nucleotides are added like in a normal PCR, and **fluorescently labelled** *ddNTPs* stop the reaction statistically after every nucleotide

Fragments are **separated by size** through a capillary and fluorescence of each molecule is detected

The **basecaller** translates peaks into the sequence of the template

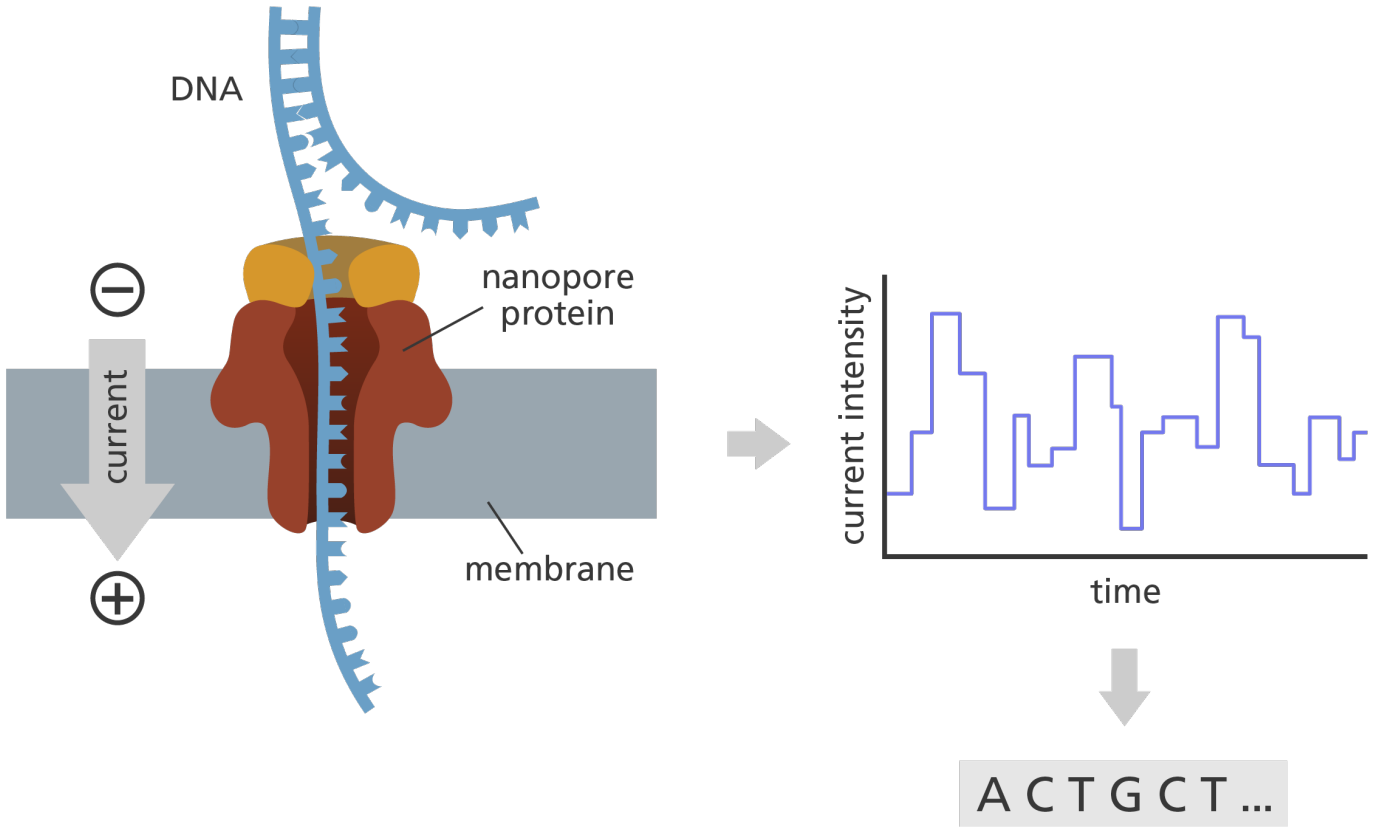
Illuminia Sequencing Steps



- Each cluster of pixels correspond to a DNA fragment.
- Color signal over cycles gives the sequence “read”.

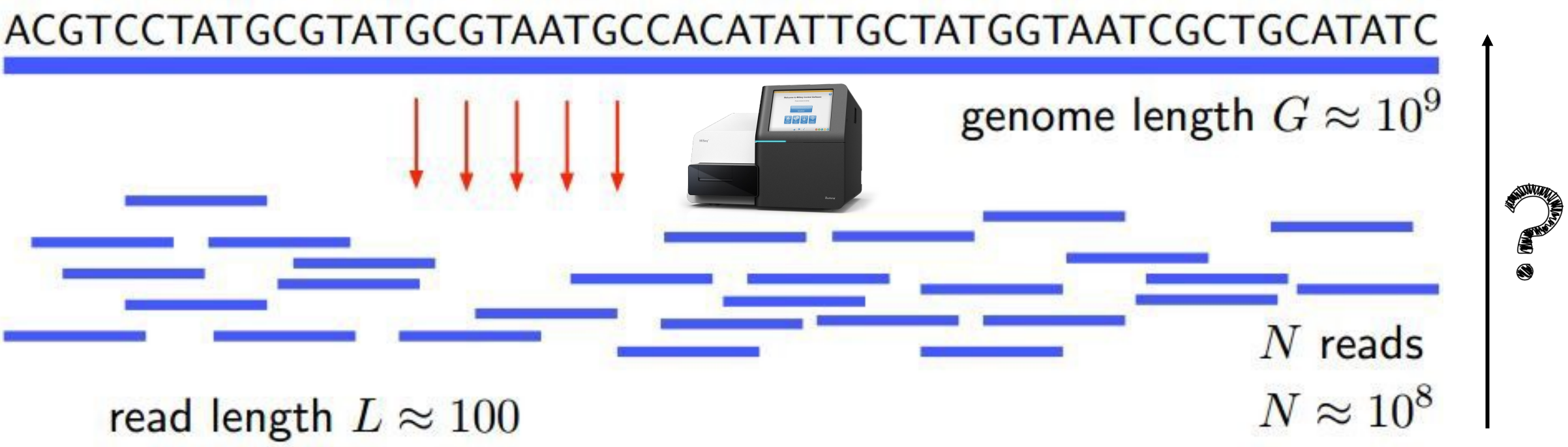
Sequencing by synthesis

Nanopore Sequencing



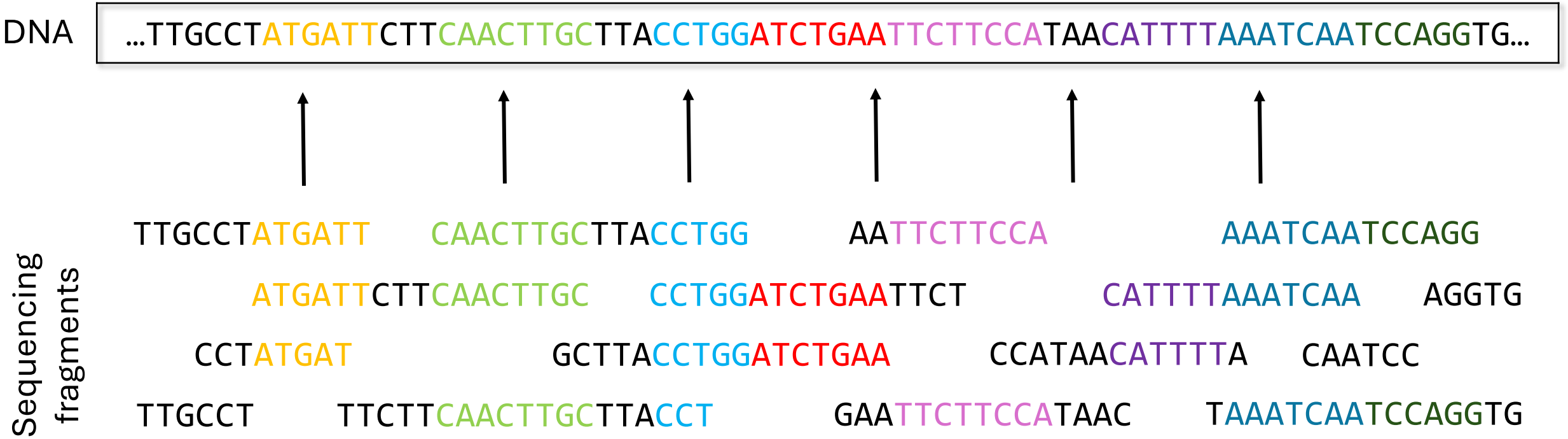
Single molecule sequencing

NGS generate short sequencing fragments/reads



Computational Problems on Sequencing Data

- Genome Assembly
  - ➢ Reconstruct full chromosomes from short/long sequencing reads/fragments



Overlaps can be found by sequence alignment

Computational Problems on Sequencing Data

- Read Mapping/Alignments
  - ➢ Map/align reads/fragments back to a known genome

Computational Problems on Sequencing Data

- Variant Calling
  - ➢ Detect positions varying from a reference population

Computational Problems on Sequencing Data

- Genome Assembly
  - ➢ Reconstruct full chromosomes from short/long sequencing reads/fragments
- Read Mapping/Alignments
  - ➢ Map/align reads/fragments back to a known genome
- Variant Calling
  - ➢ Detect positions varying from a reference population

- Problem: hundreds of millions of reads of short length
  - ➢ Computational challenge that needs efficient algorithms

The Core Problem

- For each read find its target regions on the reference genome such that there are at most k mutations between the read and the target
  - ➢ Global/local alignment of whole reads is computationally prohibitive
  - ➢ Seed-and-extend: Build alignment from seed regions

Things To Study

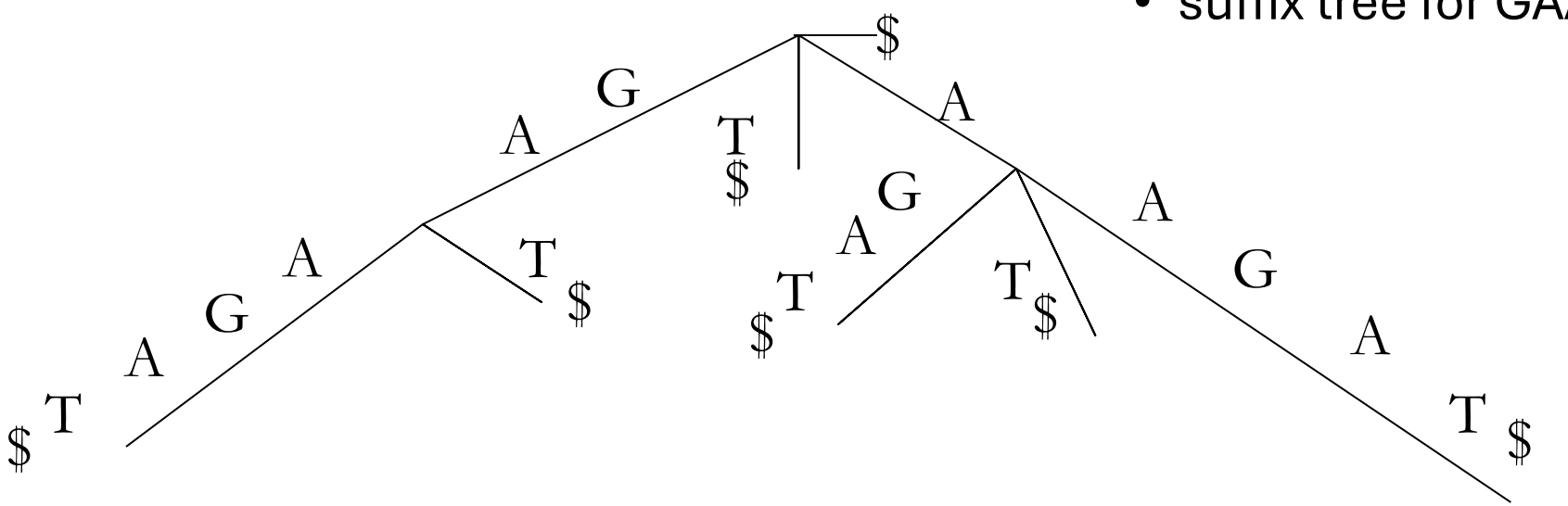
- Suffix Tree
  - Data structure
  - A few examples of using suffix tree to solve practical problems.
- Suffix Array
  - Data structure
  - The algorithms for constructing suffix array.
- FM Index
  - Data structure
- These are very related data structures

A Little History

- 1973, Weiner introduced the concept of suffix tree (position tree), which Donald Knuth subsequently characterized as "Algorithm of the Year 1973".
- 1990, Gene Myers and Udi Manber proposed suffix array.
  - Gene Myers: former VP Informatics Research at Celera Genomics
  - Udi Manber: VP engineering, Google.
- 1992, Gonnet, Baeza-Yates & Snider independently discovered suffix array (called PAT array).
  - Gaston Gonnet: cofounders Maplesoft and OpenText.
  - Baeza-Yates: VP for Yahoo! Europe and Latin America.

An Example Suffix Tree

- suffix tree for GAAGAT$



- An edge is labelled with a substring of the original string.
- A **node**'s label is the concatenation of all edge labels for the path leading to that node.
- The path from the root, *r*, to any leaf *x* is a suffix of the string S. Meanwhile, every suffix corresponds to such a path.
- Suppose there is a special "end-of-string" character, each suffix will end at the leaf.
- Each internal node has at least 2 children.
- Edge labels to the child nodes of an internal node start with different letters.

Application I. Search for a substring.

- Any substring of S is a **prefix** of a **suffix**.

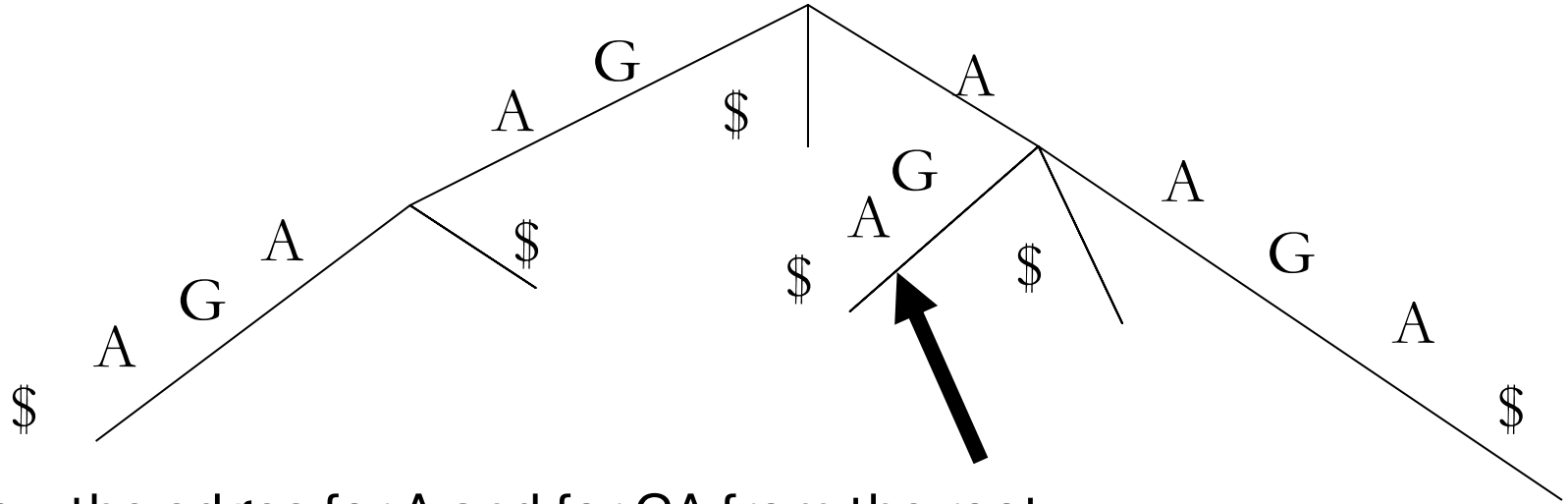
- Example of using this: Is the string *x* a substring of S?
  - Start at the root, and follow paths labelled by the characters of *x*. If you can get to the end of *x*, then yes, it is.

How to construct a suffix tree?

- There is a linear time algorithm to construct a suffix tree. (We will not study it.)
- We'll examine a quadratic-time algorithm (quite intuitive).
- The idea is to
  - Start with an empty tree.
  - Iteratively add more suffices into the tree (from shortest to longest).

One round

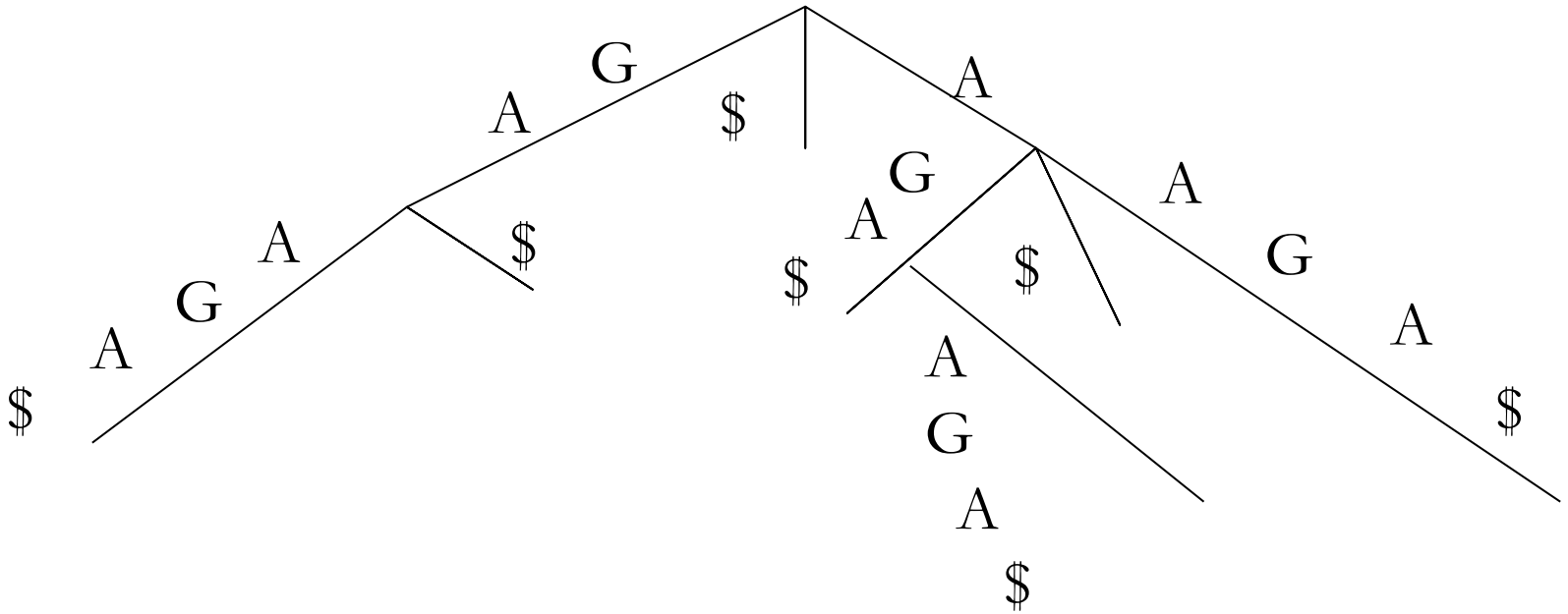
- Suppose the following is the suffix tree for GAAGA$, add another suffix AGAAGA$.



- First, follow the edges for A and for GA from the root.
- Then split after the A since the only path in the tree is for $, and we have an A, instead.
- Add a new edge for AGA$.

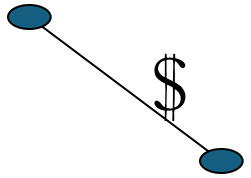
New tree

- This yields this new tree for AGAAGA$



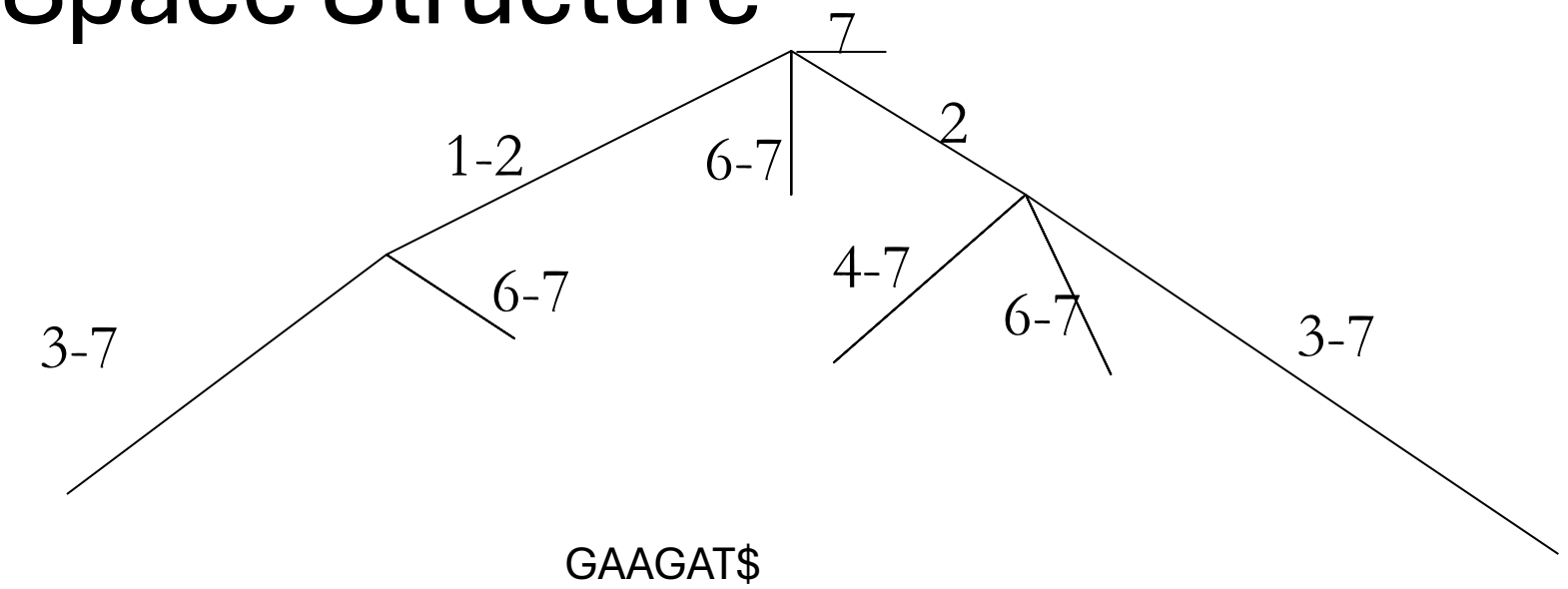
Quadratic Time Construction

- Given: A string S of length *m* over a finite alphabet. The last character of S is a unique $ character.
- We'll build the suffix tree from right to left.
  - S[m..m], S[m-1..m], S[m-2..m], ……
- Begin with this tree:



- Then, for *i* = *m* downto 1:
- Follow the letters of S[*i*…*m*] along the edges of the tree T.
- When we reach a point where no path exists, break the current edge and add a new edge for what is left.
- Time complexity: O(m^2). (Remember: The best algorithm has linear time.)

Linear Space Structure



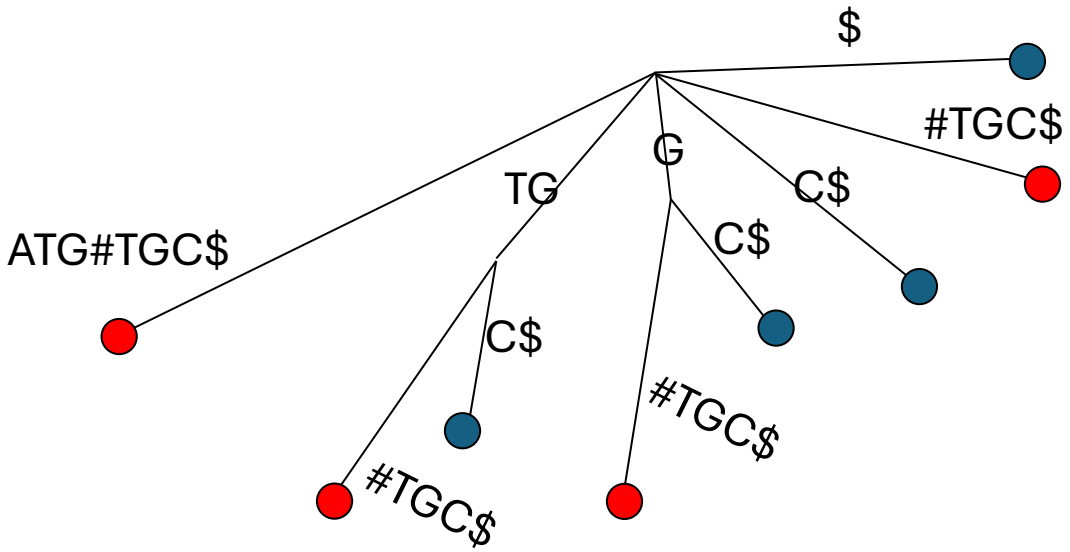
- Each edge doesn’t need to be labelled with a string, but just with starting and ending in the sequence.
- This is the same suffix tree as before, but in **linear space**.

Application II: Longest Common Substring

- What's the longest substring common to both S_1 and S_2?
- Straightforward algorithm will try to compare all substrings of equal length. This takes cubic time.
- Can we do better?

Longest Common Substring with Suffix Tree

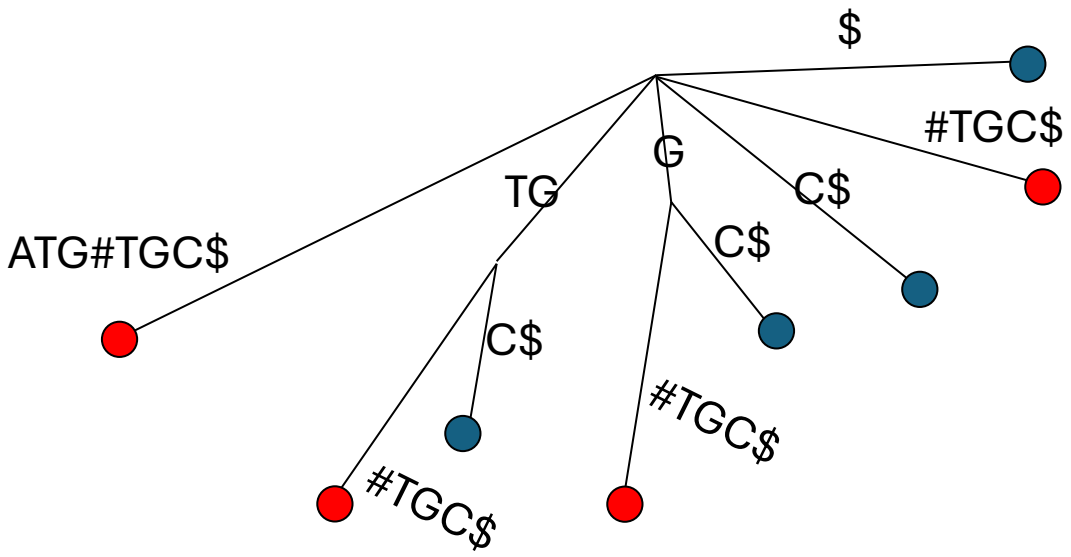
- Build a suffix tree for $S=S_1\#S_2\$$, where # and $ are unique characters.
- All suffixes of S_1 end with an edge including $\#S_2\$$. So we can label whether a leaf belongs to S_1 or S_2
- Substrings are prefixes of suffixes, i.e. internal and leaf nodes of the tree.
- Each common substring is the prefix of at least two suffixes, each from an input string (S_1 or S_2).
- Longest?



Example

ATG#TGC$

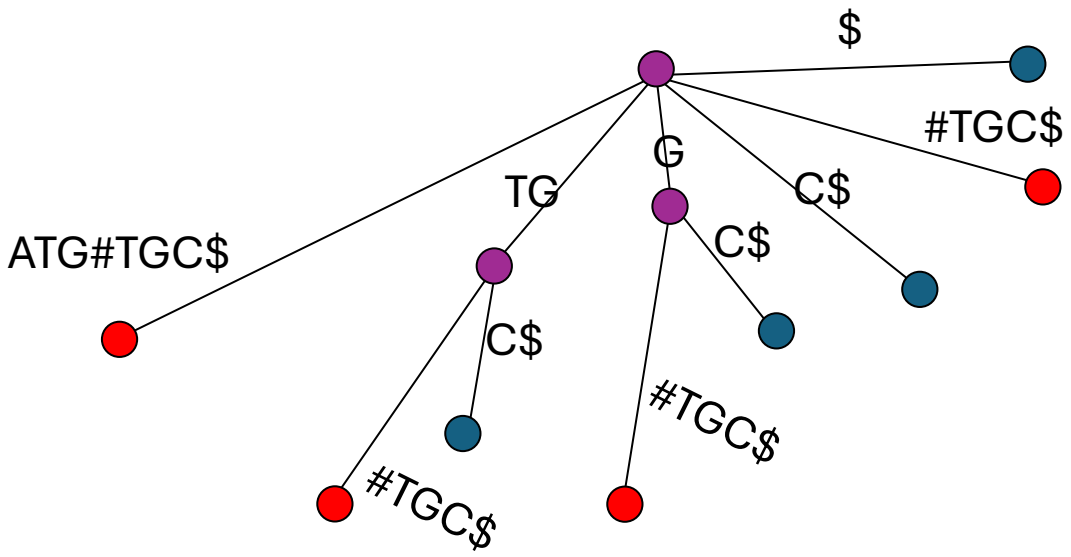
Step 1. Label leaves as red or blue, depending on whether it is a suffix starting in first or second string.



Example

ATG#TGC$

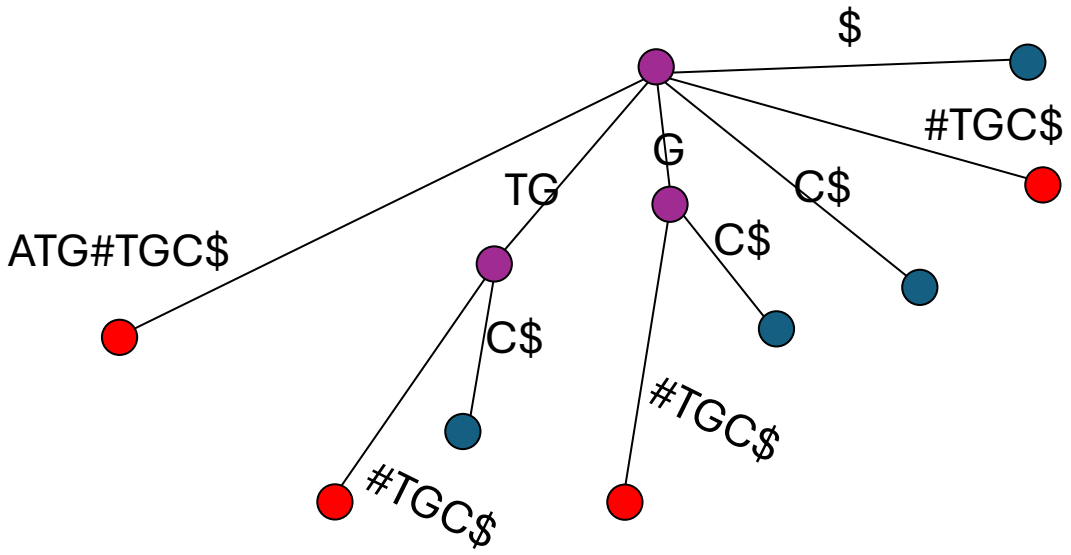
Step 2. In a bottom up order, label internal nodes. If all child nodes have the same color, label it with the same color; If not, label it with purple.



Example

ATG#TGC$

Step 3. Find the purple node with the longest path to the root.



Algorithm Summary

- 1. Build suffix tree of $S_1\#S_2\$$
- 2. Color all leaf nodes
  - red if *v*'s label is a substring of S_1
  - blue if it's a substring of S_2
- 3. Color all internal nodes from bottom up
  - red (or blue) if all child nodes are red (or blue)
  - purple if otherwise
- 4. Find the purple node with longest path label.
- Complexity: Linear time, linear space.
- Sketch proof of correctness:
  - Let t be the longest common substring. Follow the path label t starting from the root. The path can't stop in the middle of the edge – otherwise t is not the longest. Then the path has to stop at an internal node. And it has to be purple.

Application III: Maximal Unique Matches

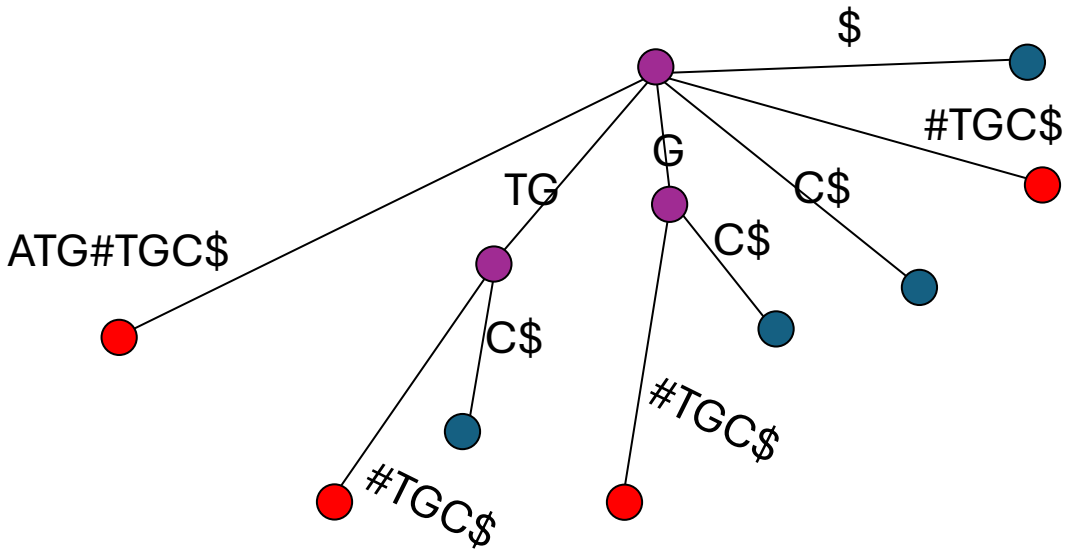
- Given two strings, a MUM (Maximal Unique Match) is a string that occurs exactly once in each string, and is maximal (can't be extended either way and still be a match).
- E.g. ATGAATC vs. AGATC
  - AT is not.
  - G is not.
  - GA is a MUM.
  - ATC is a mum.

How to find mums?

- Build a suffix tree for $S_1\#S_2\$$
- Color the nodes as in the longest common substring algorithm.
- Each MUM must be a purple internal node that has exactly two leaf children: one red and one blue.
  - It is shared by the two strings.
  - It can't extend to the right by an additional letter and still be shared.
  - It must be unique.

Example:

ATG#TGC$

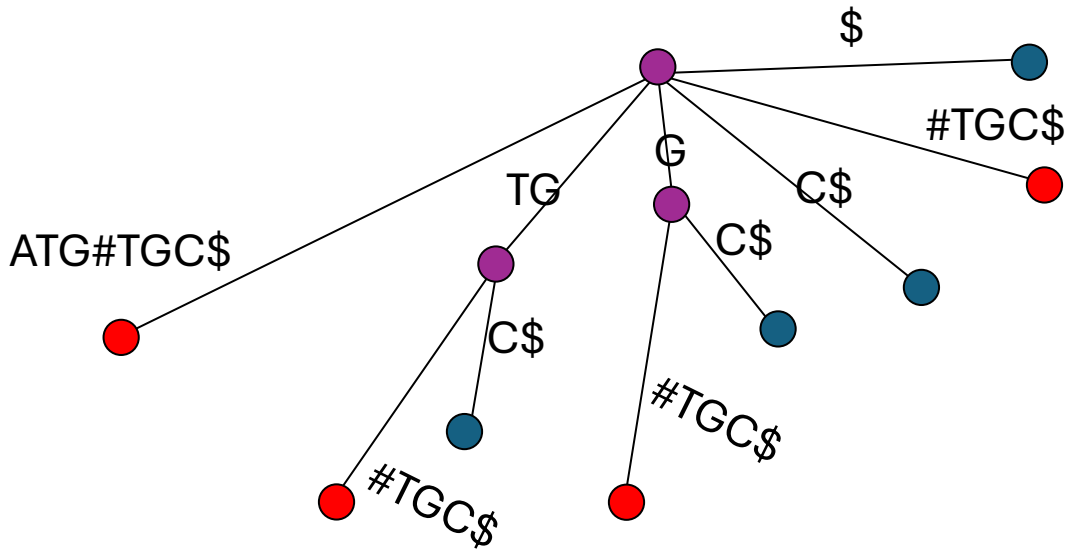


How to find mums?

- But a purple internal node may not be a MUM: only because the two occurrences may still extend to the left.
  - Node G is not: For G's two occurrences, the left character are both T.
  - Node TG is: For TG's two occurrences, the left characters are A and #, respectively.
- But it is easy to compute the left character of each leaf
  - It is a suffix, and we know its path's starting position in the original string.

Example:

ATG#TGC$



Summary

- Build a suffix tree for $S_1\#S_2\$$.
- For each leaf v, define left(v) be the letter at left of suffix v.
- Find the internal nodes that
  - Have exactly two child leaves
  - The two child leaves are two suffixes from S1 and from S2, respectively.
  - The two child leaves must have two different left characters.
- Linear time.
- After find all MUMs, use them as anchor to speed up global alignment.

MUMMER: Large-scale Global Alignment

- Large-scale global alignment

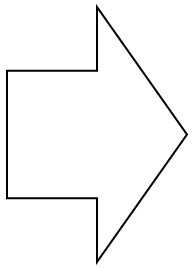
- Idea:
- Pick some “anchors” through which the true alignment is very likely to fall.
- Align the regions between the anchors either recursively or just using classical global alignment tools.

- MUMs are good anchors: maximal, unique, match.
- First program that does so: MUMMER by Delcher et al.

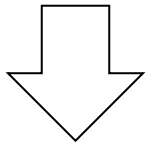
Suffix Array

- AGAAGAT$

1 = AGAAGAT$
2 = GAAGAT$
3 = AAGAT$
4 = AGAT$
5 = GAT$
6 = AT$
7 = T$
8 = $



8 = $
3 = AAGAT$
1 = AGAAGAT$
4 = AGAT$
6 = AT$
2 = GAAGAT$
5 = GAT$
7 = T$



8, 3, 1, 4, 6, 2, 5, 7

String Matching

- Given a string P of length m, find its occurrence in T.
- Fact: If matched, P is a prefix of some suffix.
- Binary search in suffix array to find P.
- Each time, compare T[SA[i]:] with P

Time Complexity

- O(m log n) if implemented straightforwardly.
- Practical trick: remember the lcp (longest common prefix) between query and low (l1), and between query and high (l2). Skip first min(l1, l2) letters when comparing with mid.
- This saves time in practice but there is no theoretical guarantee.
- Counter example?

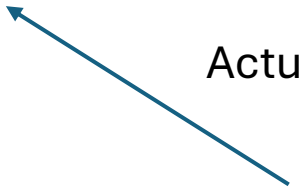
```
while low < high
    mid = (low + high) / 2
    compare query with T[SA[mid]:]
    ...
```

Counter Example

low `AAAAAAAAAAAAAA...`

`BBBBBBBBBBBAAA...`

`BBBBBBBBBBBBAA...`



Actual location of query

mid `BBBBBBBBBBBBBB...`

query `BBBBBBBBBBBAAA...`

high `BBBBBBBBBBBBBB...`

LCP Array

- The worst case scenario can be solved if we know lcp(low, mid) and lcp(mid, high) as well.
- With lcp(query, low) and lcp(query, high), we can figure out exactly how many letters to skip when compared with mid.
- LCP[i] = lcp(T[SA[i]:], T[SA[i-1]:]) for i>0. LCP[0] = 0.
- LCP can be constructed in linear time after or together with suffix array (we will not study this).
- With LCP, one can precompute all the needed lcp(low, mid) and lcp(mid, high). There are only O(n) possible combinations needed during binary search.

Time Complexity for String Matching with SA

- O(m log n) if implemented straightforwardly
- O(m + log n) if with LCP.

Suffix Array Construction

- The construction of suffix array is also referred to as suffix sorting.
- What is the time complexity of the straightforward sorting algorithm?
- Linear time suffix sorting algorithm exists.
- LCP array also takes linear time to construct
- We studies an O(n log n) algorithm first (suffix doubling) and then a linear time algorithm (skew algorithm).

Prefix Doubling

- Idea:
  - We sort length L substrings for L=1, 2, 4, …, n
  - T[i:i+l] is the prefix of the suffix T[i:].
  - The hope is that the sorting at length L will make the sorting at length 2L easier.
- More specifically, let S^l contains the indices of text T, we want $T[S^l[i]:S^l[i]+l] < T[S^l[i+1]:S^l[i+1]+l]$
- The sorting of L=1 is trivially O(n log n).

Bucket Sort

- For an array T of length n containing objects with int keys and key values is between 0 to n-1, bucket sort works:
  - For i from 0 to n-1, map to T[i] to bucket T[i].key.
  - Gather the objects sequentially from bucket 0, 1, …, n-1
- Linear time, linear space.
- If we do it carefully to maintain the original order in T in each bucket, this is a stable sorting (the original order of objects with the same key is preserved).
- From now on, we assume our bucket sort is stable.

Radix Sort

- Now suppose the key is a tuple, e.g. (K1, K2), and each key is between 0..n-1.
- Radix Sort
  - Bucket sort according to K2.
  - Bucket sort according to K1.
- Properties:
  - The objects are sorted by K1.
  - If two objects have the same K1, their order is determined by K2.
  - If two objects have the same (K1, K2), their order is determined by their original order in the input.
- Linear time, linear space.
- This works if the key is a k-tuple for constant k.

Prefix Doubling (L=2)

- We cannot use bucket sort for L=1, because the keys are not bounded by n.
- But with some trick, we can use it for L=2. How?

Renaming

- We want to replace each token with an integer between 0..n-1, while maintaing the token order.
- The suffix array (sorted indice) before and after the renaming will be the same.
- But after renaming, we can use bucket or radix sort.
- How?

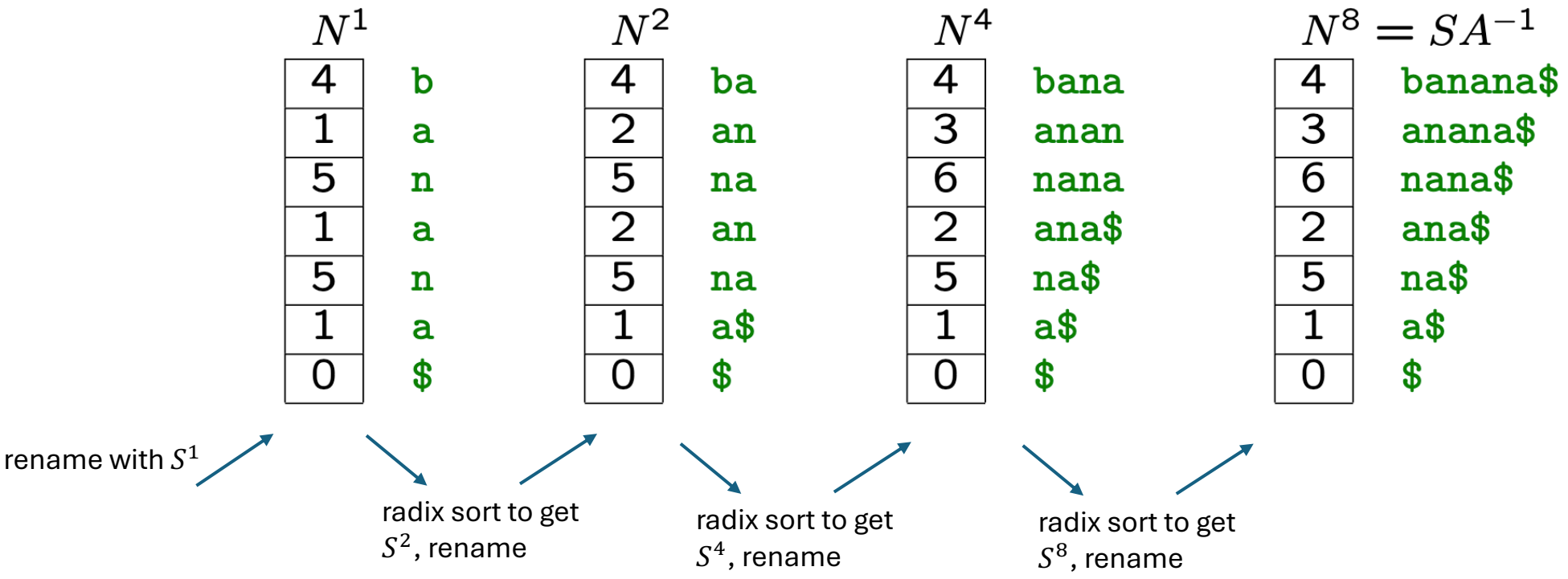
Renaming

- After we get the sorted indices S^1, the tokens are sorted now.
- We just scan through S^1, count the number of different tokens we have encountered, and use the counter as the new name for each token.
- This works because the tokens are sorted according to S^1
  - the identical tokens are clustered together so they get the same name
  - smaller tokens get smaller names

```
S = S^1
name = 0
N[S[0]] = 0
for i = 1..n-1
   if T[S[i]] != T[S[i-1]]
       name = name +1
   N[S[i]] = name
```

Prefix Doubling Algorithm

Renaming example for T=banana$



Time Complexity

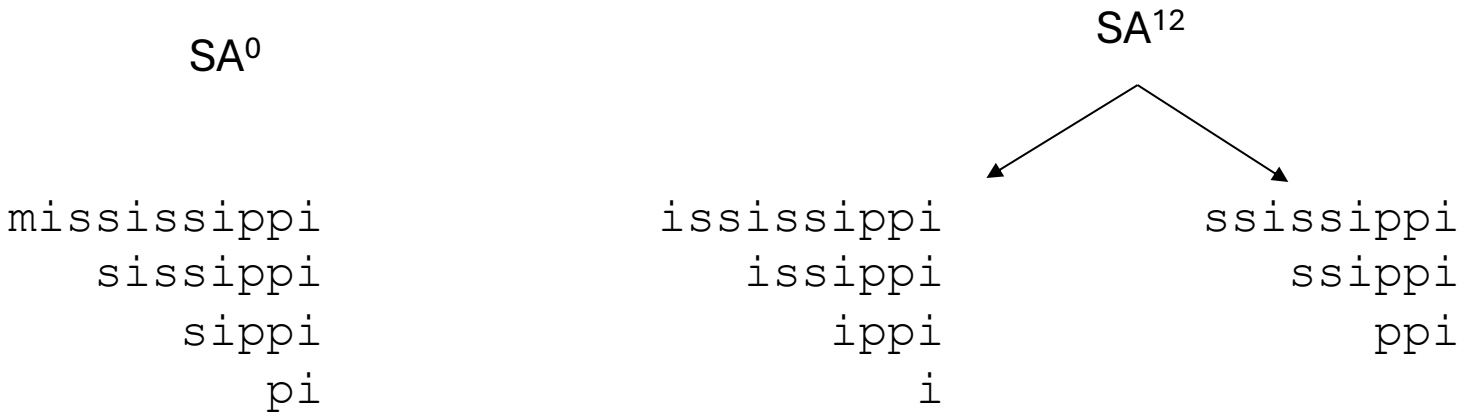
- O(n log n)
- Getting S^1 takes O(n log n) time. Every other rounds of radix sort and renaming takes linear time. There are log n rounds.

Skew Algorithm For Suffix Sorting

- Let S_0, S_1, S_2, …, S_{n-1} be all the n suffixes. S_i starts at i-th position.
- Skew algorithm uses divide and conquer. But it divides the problem into unequally sized parts.
- Two sets $SA^0 = \{S_i : i = 0 \bmod 3\}$ and $SA^{12} = \{S_i : i=1 \text{ or } 2 \bmod 3\}$.

Skew Algorithm Example

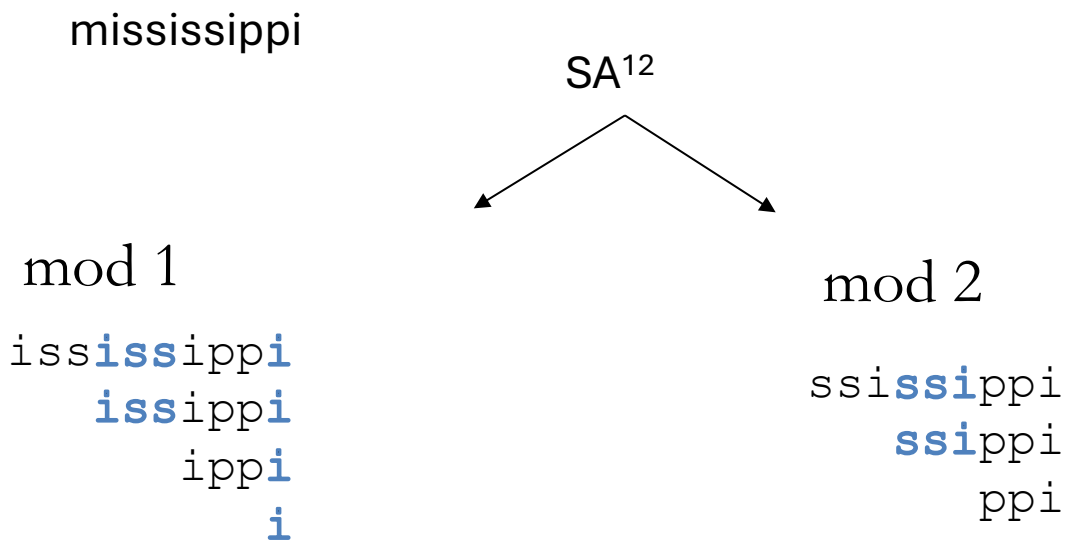
- Example: mississippi



Skew Algorithm For Suffix Sorting

- Plan:
  - 1. Sort SA^{12} recursively.
  - 2. Sort SA^{0} with the help of the sorted SA^{12}.
  - 3. Merge sort SA^{0} and SA^{12}.
- Our goal is to do step 2 and 3 in linear time. If this can be achieved, then the time complexity is
  - $T(n) = O(n) + T(2n/3)$.
  - This leads to $T(n)=O(n)$.
  - Compare with merge sort.

How to sort SA^{12} recursively



- We need to know the order of these suffixes.
- In order to solve it recursively, we need to reduce the problem to a smaller suffix sorting problem.

Reduction to a smaller suffix sorting problem

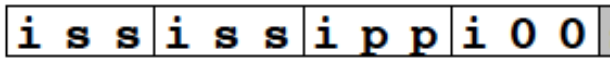
SA^{12}

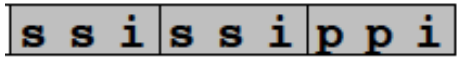
mod 1

```
ississippi00
  issippi00
    ippi00
      i00
```

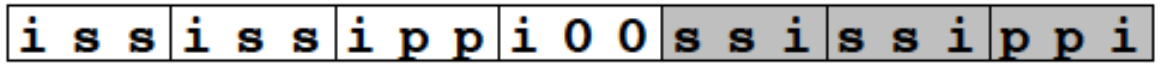
mod 2

```
ssissippi
  ssippi
    ppi
```





- Pad 0 to make their length multiple of 3. Then treat each string as a string of “triplets”. Each subset is the suffixes of the “triplet string”.
- We connect the two “triplet strings” together to make a longer string. We put the one with padding at the left.



Reduction

SA^{12}

mod 1

ississippi00
issippi00
ippi00
i00

mod 2

ssissippi
ssippi
ppi

ississippi00ssissippi
issippi00ssissippi
ippi00ssissippi
i00ssissippi

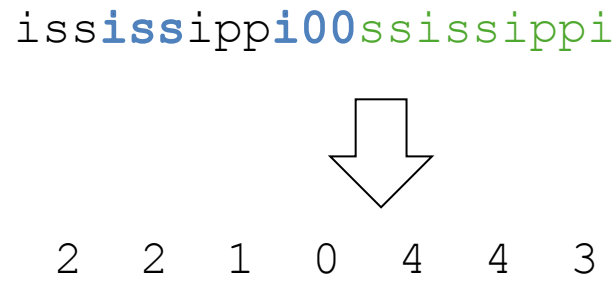
ssissippi
ssippi
ppi

- Now check all the suffixes of the concatenated triplet string. Their relative order can be used to build the relative order of SA^{12} easily.
- We are almost there, except that keeping tripling the size (number of bytes) of the “character” is a problem.

Renaming

- We solve the unlimited expansion problem by renaming. It maps each unique triplet to a single unique integer.
- To rename, we first sort the triplets, and then assign integer values sequentially to unique triplets. Sorting triplets can be done in linear time by radix sort.
- This ensures
  - The max value is always bounded by the length of array.
  - The suffix order is unchanged.

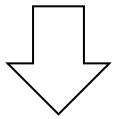
```
i00 -> 0
ipp -> 1
iss -> 2
ppi -> 3
ssi -> 4
```



Renaming Example

```
i00 -> 0
ipp -> 1
iss -> 2
ppi -> 3
ssi -> 4
```

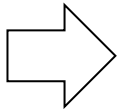
```
ississippi00ssissippi
```



```
2  2  1  0  4  4  3
```

```
ississippi00ssissippi
   issippi00ssissippi
      ippi00ssissippi
         i00ssissippi

            ssissippi
               ssippi
                  ppi
```

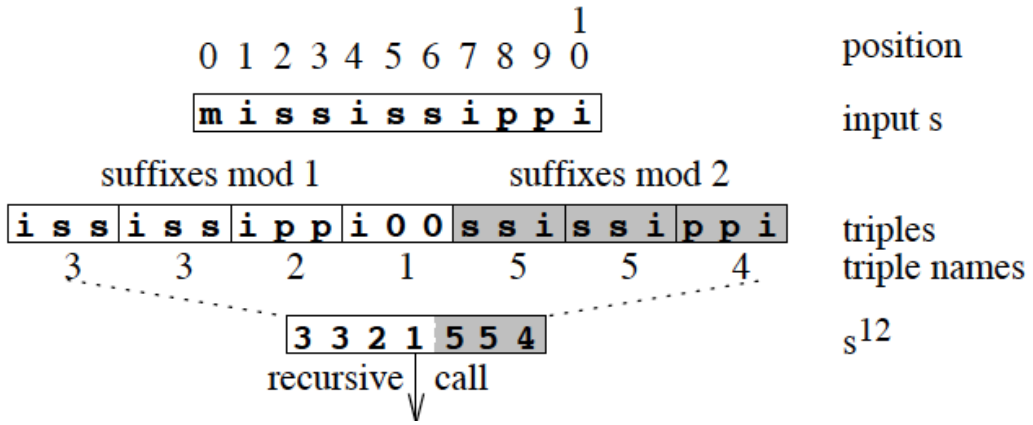


```
2 2 1 0 4 4 3
  2 1 0 4 4 3
    1 0 4 4 3
      0 4 4 3
        4 4 3
          4 3
            3
```

Recursion

- After renaming, we just suffix sort the new integer string, which has length approximately 2n/3. This can be done by recursion.
- The time complexity of renaming is dominated by sorting the triplets. This can be solved in linear time with radix sort.

Recap Sort S^{12} recursively



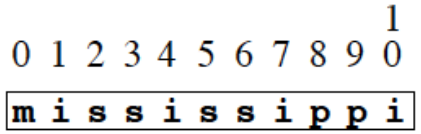
1. Padding and concatenation to get string of triplets.
2. Radix sort the triplets to get an ID (name) of each triple.
3. Recursion to get the suffix order on the string of IDs.

Sort S^0 in linear time

- $S_i = s[i]\ S_{i+1}$.
- For all S_i in SA^0, S_{i+1} has been sorted already. Use s[i] to do another pass of radix sorting will give us the right order of SA^0. This takes linear time.

Sorted SA12

```
10: i
 4: issippi
 1: ississippi
 7: ippi
 8: ppi
 5: ssippi
 2: ssissippi
```



To sort SA0

```
0: mississippi
3: sissippi
6: sippi
9: pi
```

Merge

Sorted SA12

```
10: i
 4: issippi
 1: ississippi
 7: ippi
 8: ppi
 5: ssippi
 2: ssissippi
```

Sorted SA0

```
0: mississippi
9: pi
6: sippi
3: sissippi
```

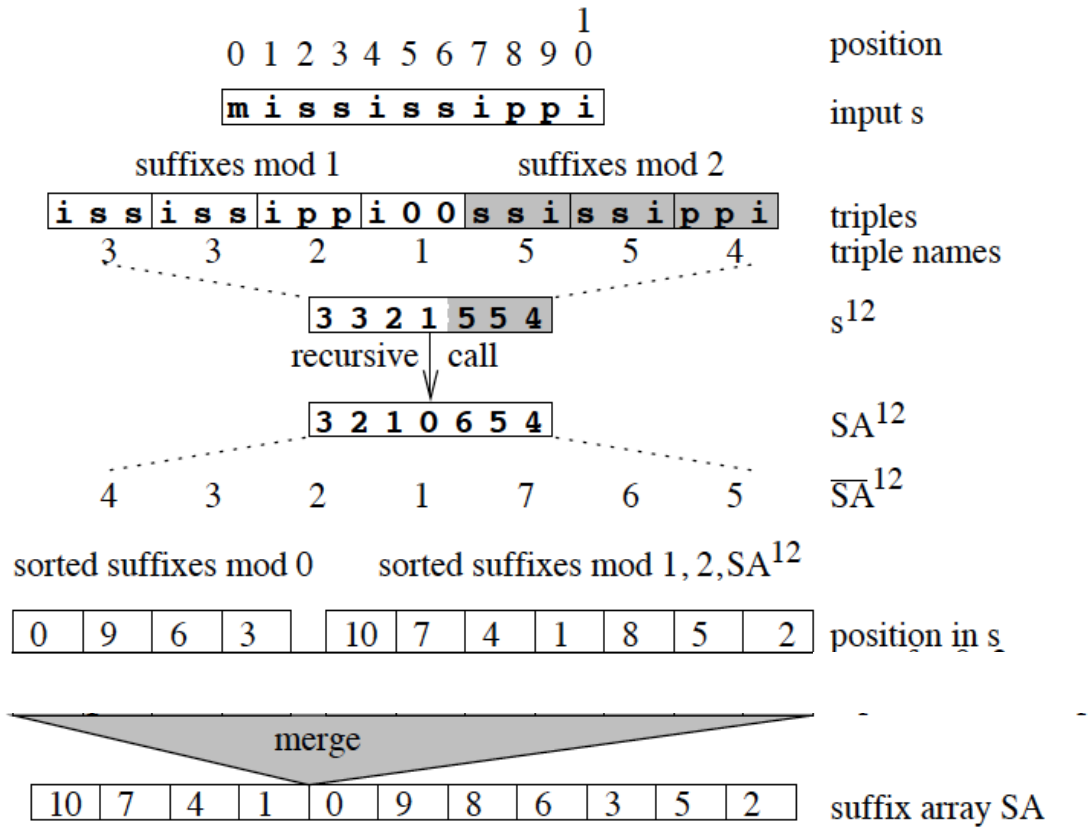
- Would be a simple merge if comparison of two takes constant time.
- Trouble is when two suffices share a long prefix, which takes more than constant time to compare.

E.g. what if S5 = aaaa… and S6=aaaa…

Merge S^0 and S^{12}

- Merging only requires to compare a suffix S_j with j mod 3 = 0 with a suffix S_i with i mod 3 != 0. :
- Case 1: If i mod 3 = 1, we write S_i as (s[i], S_{i+1}) and S_j as (s[j], S_{j+1}).
  - Since (i +1) mod 3 = 2 and (j + 1) mod 3 = 1, the relative order of S_{j+1} and S_{i+1} can be determinded from their position in SA^{12}.
- Case 2: If i mod 3 = 2, we compare the triples (s[i], s[i + 1], S_{i+2}) and (s[j], s[j + 1], S_{j+2}).

Recap


0 1 2 3 4 5 6 7 8 9 10
position
m i s s i s s i p p i
input s
suffixes mod 1
suffixes mod 2
i s s i s s i p p i 0 0 s s i s s i p p i
triples
3 3 2 1 5 4
triple names
3 3 2 1 5 5 4
s^{12}
recursive call
3 2 1 0 6 5 4
SA^{12}
4 3 2 1 7 6 5
$\overline{SA}^{12}$
sorted suffixes mod 0
sorted suffixes mod 1, 2, SA^{12}
0 9 6 3
10 7 4 1 8 5 2
position in s
merge
10 7 4 1 0 9 8 6 3 5 2
suffix array SA

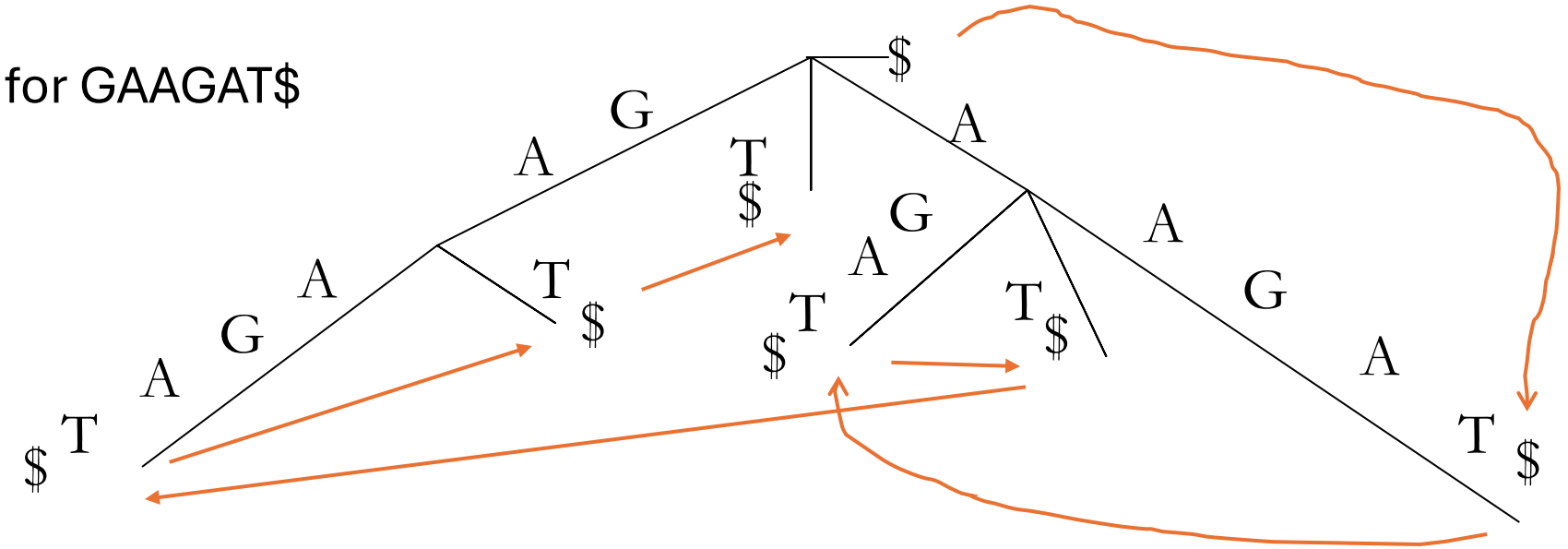
C codes

- 50 lines of C++ codes were given in J.C.M. Baeten et al. (Eds.): ICALP 2003, LNCS 2719, pp. 943–955, 2003.

- http://www.mpi-inf.mpg.de/~sanders/programs/suffix/

Relationship between Suffix Tree and Array

- suffix tree for GAAGAT$



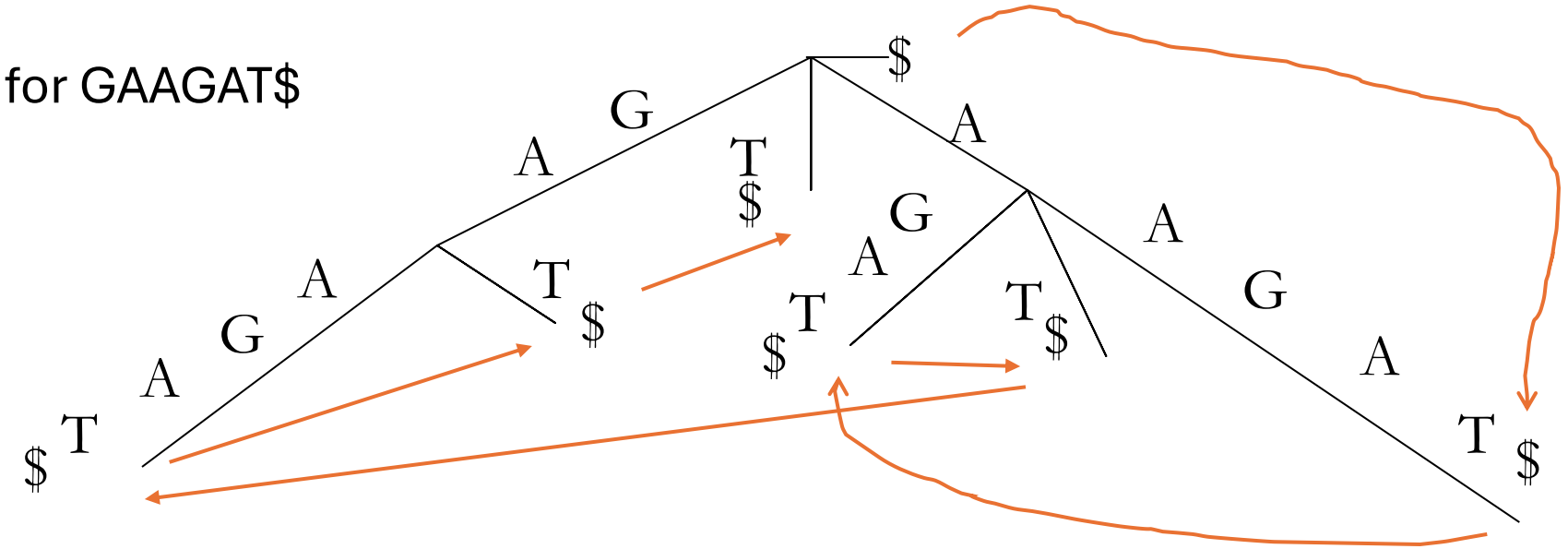
Suffix array order corresponds to the depth-first traversal of the leaves of the suffix tree.

$	6
AAGAT$	1
AGAT$	2
AT$	4
GAAGAT$	0
GAT$	3
T$	5

← Suffix Array

Suffix Tree Construction from SA

- suffix tree for GAAGAT$



- Start from the first suffix in the SA.
- Add suffix to the tree sequentially while simulating the depth-first traversal on the tree.

$	6
AAGAT$	1
AGAT$	2
AT$	4
GAAGAT$	0
GAT$	3
T$	5

← Suffix Array