RNA-Sequencing

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Advanced Algorithms for Bioinformatics (P4)
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SoSe 2010
FU Berlin

May 13, 2010

Outline

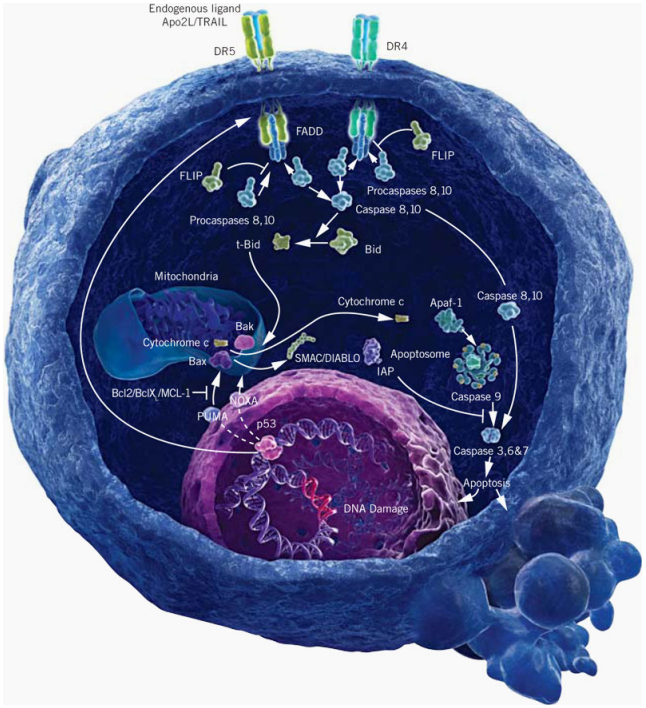
What is RNA-Seq?

- Also called "Whole Transcriptome Shotgun Sequencing"
- Technique to sequence cDNA in order to get information about a RNA sample content

Outline

Why do we use RNA-Seq?

- Crucial to understand the processes inside a cell
- DNA cannot give us all information unlike transcriptomes
  - mRNAs specify cells' identity
  - mRNAs govern cells' activity due to environmental conditions



apoptosis pathway

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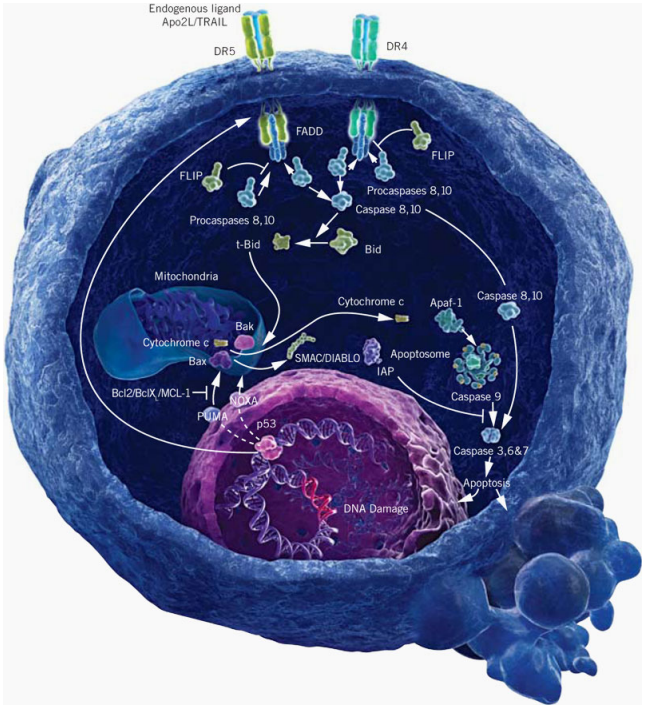

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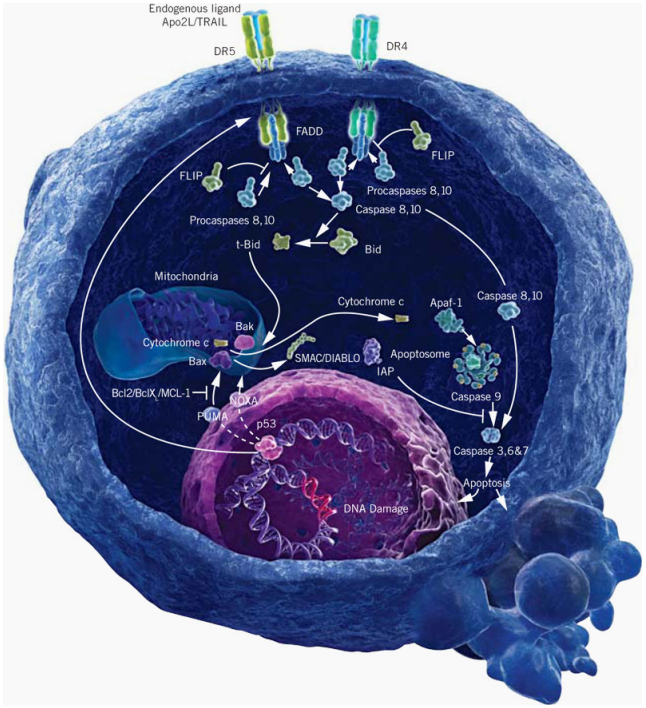

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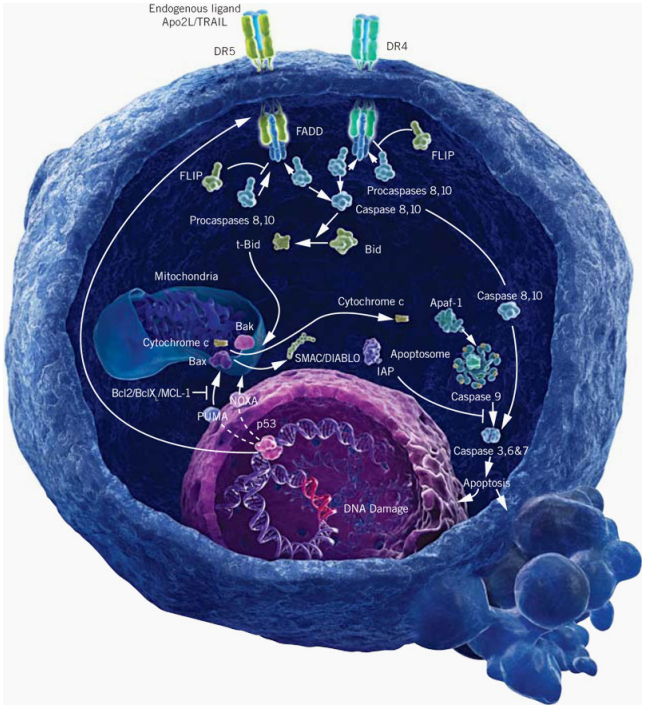

apoptosis pathway

Advantages

- High-throughput sequencing
- No bacterial cloning of cDNA needed (no bacterial cloning constraints)
- High coverage
- Can discover new exons, genes
- Can handle RNA splicing
- Low cost

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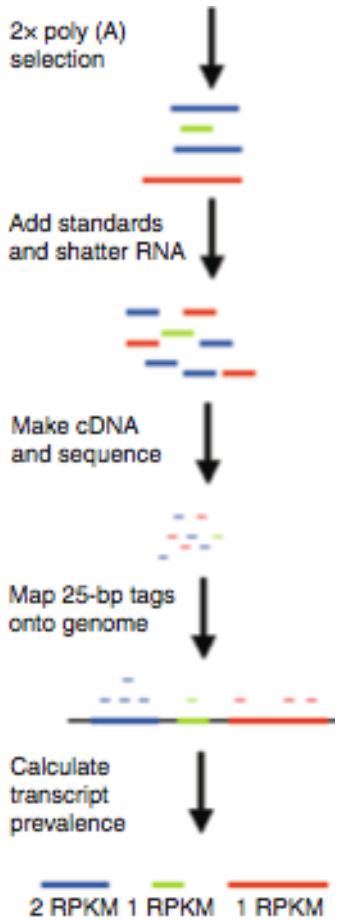
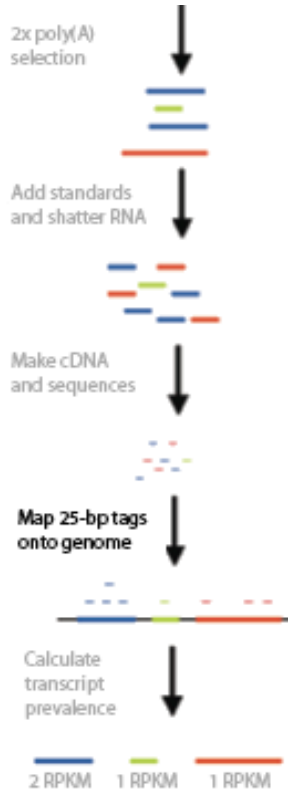
Pipeline

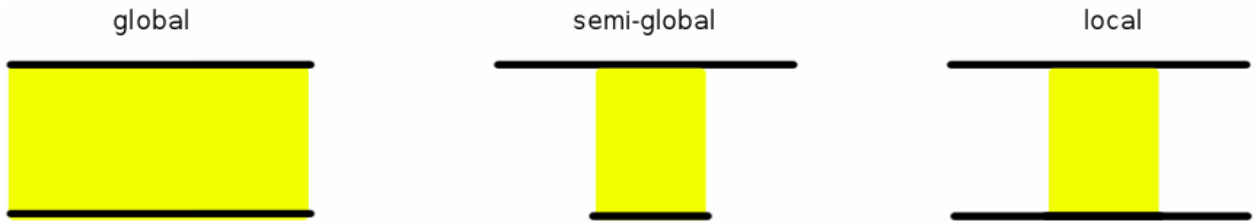
- Poly(A)-selection of RNA
- Fragmentation of RNA to an average length
- Conversion into cDNA
- Sequencing
- Mapping of the reads onto the genome
- Calculation of the transcript prevalence



Mapping



- *Read-mapping* is the semi-global alignment of many (very) short sequences (reads) to a long sequence (the genome)
- Alignment: approximate string matching



Important Questions

Questions regarding choice of algorithm and implementation:

Input
- How long are the reads? How many are there?
- How big is the genome?
- What technology was used?
- Known error-profile or quality values?

Output
- Gapped or ungapped alignments?
- What kind of scoring / distance measurement?

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Tools

Tools designed for short sequences
(limited number of mismatches, short gaps)

- Bowtie
- RazerS
- SOAP
- MAQ
- ZOOM

Overview

Two main phases

1. Filtration / non Filtration (other preprocessing)
2. Verification

$\Rightarrow$ Different (combinations of) algorithms available

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Outline - Lectures

Lecture 1 Non filtering algorithm

Bowtie

- BWT

Lecture 2 Filtering algorithm

Filtering

- Pidgeonhole
- q-gram

RazerS

Lecture 3 Repeat Resolution

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Lecture 3 Repeat Resolution

The paper

Software

Open Access

Ultrafast and memory-efficient alignment of short DNA sequences to the human genome

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Published: 4 March 2009

Genome ***Biology*** 2009, **10:**R25 (doi:10.1186/gb-2009-10-3-r25)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2009/10/3/R25

Received: 21 October 2008
Revised: 19 December 2008
Accepted: 4 March 2009

Abstract

Bowtie is an ultrafast, memory-efficient alignment program for aligning short DNA sequence reads to large genomes. For the human genome, Burrows-Wheeler indexing allows Bowtie to align more than 25 million reads per CPU hour with a memory footprint of approximately 1.3 gigabytes

Bowtie

- Fast, memory-efficient
- Burrows-Wheeler indexing → align >25 million reads per CPU hour (memory footprint aprox. 1.3 GB)
- Extends previous Burrows-Wheeler techniques with a novel quality-aware backtracking algorithm that permits mismatches
- Mismatches → Backtracking

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Bowtie

Goal? → map reads back to reference genome

- Exact match
- Inexact match

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Bowtie

Goal? $\rightarrow$ map reads back to reference genome

- Exact match
- Inexact match

Exact Match

→ Exact string matching → EXACTMATCH

Inexact Match

- Mismatches or indels
- Backtracking

Efficiency

- compress genome $\rightarrow$ space efficient
- indexation of genome $\rightarrow$ fast search
- ideas? $\rightarrow$ suffix array
- or even better $\rightarrow$ Burrows-Wheeler indexing (BWT)

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Suffix Arrays

- array of *integers*
- gives the starting positions of suffixes of a string
- in lexicographical order
- very space economical data structure

Suffix Arrays

Example

Consider the string "'abracadabra$"'

1	2	3	4	5	6	7	8	9	10	11	12	index
a	b	r	a	c	a	d	a	b	r	a	$	string

It has twelve suffixes:
abracadabra$,
bracadabra$,
racadabra$,
...,
a$,
$

Suffix Arrays

Example

index	sorted suffix
12	$
11	a$
8	abra$
1	abracadabra$
4	acadabra$
6	adabra$
9	bra$
2	bracadabra$
5	cadabra$
7	dabra$
10	ra$
3	racadabra$

$\Rightarrow$ For the string "abracadabra$", the suffix array is [12, 11, 8, 1, 4, 6, 9, 2, 5, 7, 10, 3].

Burrows-Wheeler transformation

- published by Michael Burrows and David Wheeler in 1994
- reversible transformation algorithm to a input text
- does no data compression itself, but might lead to effective compression

Outline

Principle of BWT

- Input: **string** S of length N with characters of ordered alphabet Σ
- i.e.: $S = mississippi$, $N = 11$, $\Sigma = \{i, m, p, s\}$

- Output: **string** L of length $N + 1$ with characters of ordered alphabet $\Sigma \cup$ special character and index I
- i.e.: $L = ipssm\#pissii$, $I = 5$

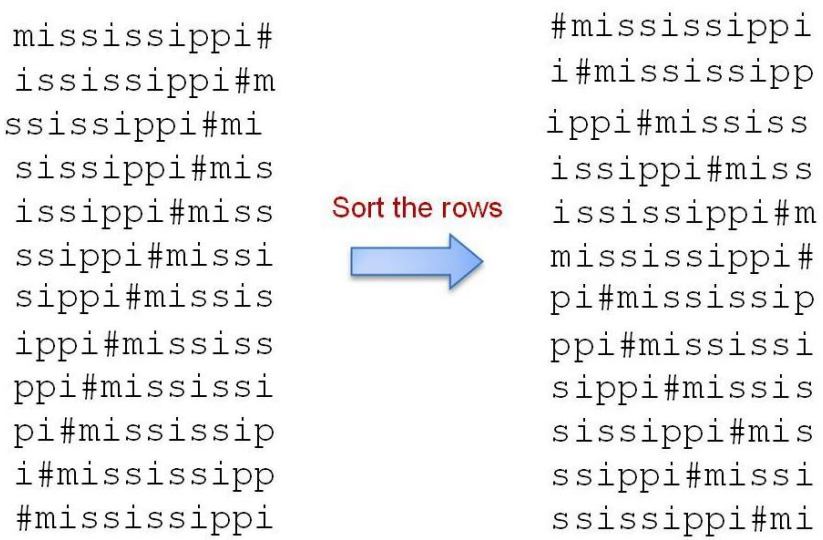
Principle of BWT

1. sort rotations
   - append an special character $\notin \Sigma$ like $\#$ to S, $\#$ is lexicographically minimal
   - build a conceptual $N + 1 \times N + 1$ Matrix M whose rows are cyclic shifts of S

```
mississippi#
ississippi#m
ssissippi#mi
sissippi#mis
issippi#miss
ssippi#missi
sippi#missis
ippi#mississ
ppi#mississi
pi#mississip
i#mississipp
#mississippi
```

Principle of BWT

1. sort rotations
   - append an special character $\notin \Sigma$ like $'\#'$ to S, $\#$ is lexicographically minimal
   - build a conceptual $N + 1 \times N + 1$ Matrix M whose rows are cyclic shifts of S
   - sort rows lexicographically



Principle of BWT

2. find last characters of rotations

- string L or F is the last respectively first column of matrix M
  i.e.:
  $L = ipssm\#pissii$
- characters in $L[i]$ are predecessors of characters in $F[i]$

F		L
#	mississipp	i
i	#mississip	p
i	ppi#missis	s
i	ssippi#mis	s
i	ssissippi#	m
m	ississippi	#
p	i#mississi	p
p	pi#mississ	i
s	ippi#missi	s
s	issippi#mi	s
s	sippi#miss	i
s	sissippi#m	i

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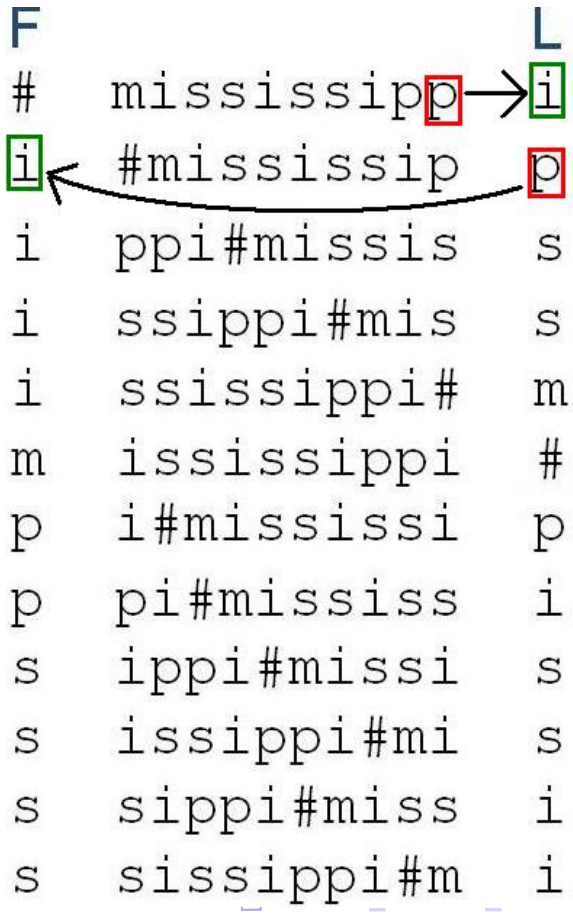
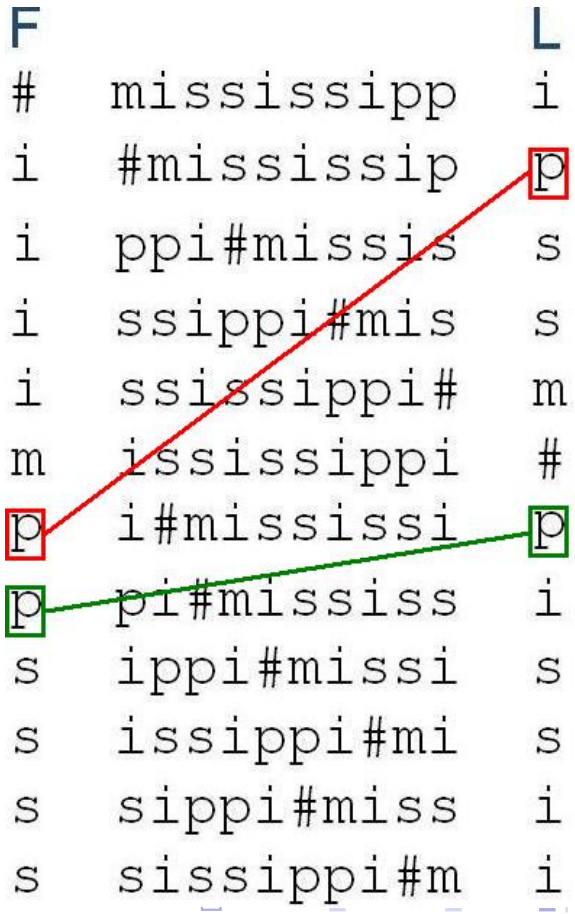


Principle of BWT

2. find last characters of rotations

- rank preserving property: $pos_L(c_1) < pos_L(c_2) \Leftrightarrow pos_F(c_1) < pos_F(c_2)$, whereas $pos_X(c)$: position of character c in string X
- index I is row number of input $S\cup$ special character (i.e. $I = 5$)



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F		L
#	mississipp	i
i	#mississip	p
i	ppi#missis	s
i	ssippi#mis	s
i	ssissippi#	m
m	ississippi	#
p	i#mississi	p
p	pi#mississ	i
s	ippi#missi	s
s	issippi#mi	s
s	sippi#miss	i
s	sissippi#m	i

Principle of BWT

Fig. 7: Part of an BWT ouput example from the original BWT paper.

final char (L)	sorted rotations
a	n to decompress. It achieves compression
o	n to perform only comparisons to a depth
o	n transformation} This section describes
o	n transformation} We use the example and
o	n treats the right-hand side as the most
a	n tree for each 16 kbyte input block, enc
a	n tree in the output stream, then encodes
i	n turn, set $L[i]$ to be the
i	n turn, set $R[i]$ to the
o	n unusual data. Like the algorithm of Man
a	n use a single set of probabilities table
e	n using the positions of the suffixes in
i	n value at a given point in the vector $R
e	n we present modifications that improve t
e	n when the block size is quite large. Ho
i	n which codes that have not been seen in
i	n with ch appear in the {\em same order
i	n with ch. In our exam
o	n with Huffman or arithmetic coding. Bri
o	n with figures given by Bell~\cite{bell}.

Advantages of BWT

- due to many repetitions in a big input S string L has regions of same characters
- $\rightarrow$ good for compression algorithms (may be talked about next lecture)
- BWT stores string of characters instead of integer indexes like suffix arrays! $\rightarrow$ less space for big text input like DNA

Outline

Retransformation

- Input: string L of length $N + 1$ with characters of ordered alphabet $\Sigma \cup$ special character and index I
- i.e.: $L = ipssm\#pissii$, $N = 11$, $\Sigma = \{i, m, p, s\}$, special character $= \#$ and $I = 5$

- Output: string S of length N with characters of ordered alphabet Σ
- i.e.: $S =mississippi$

Retransformation

1. find F using L
   - every column in matrix M is permutation of string $S\#$
   - first column F and last column L also permutations of $S\#$ and of one another
   - M has sorted rows and F is first column $\Rightarrow$ F is sorted
   - get F by sorting L

F		L
#	mississipp	i
i	#mississip	p
i	ppi#missis	s
i	ssippi#mis	s
i	ssissippi#	m
m	ississippi	#
p	i#mississi	p
p	pi#mississ	i
s	ippi#missi	s
s	issippi#mi	s
s	sippi#miss	i
s	sissippi#m	i

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F	L
#	i
i	p
i	s
i	s
i	m
m	#
p	p
p	i
s	s
s	s
s	i
s	i

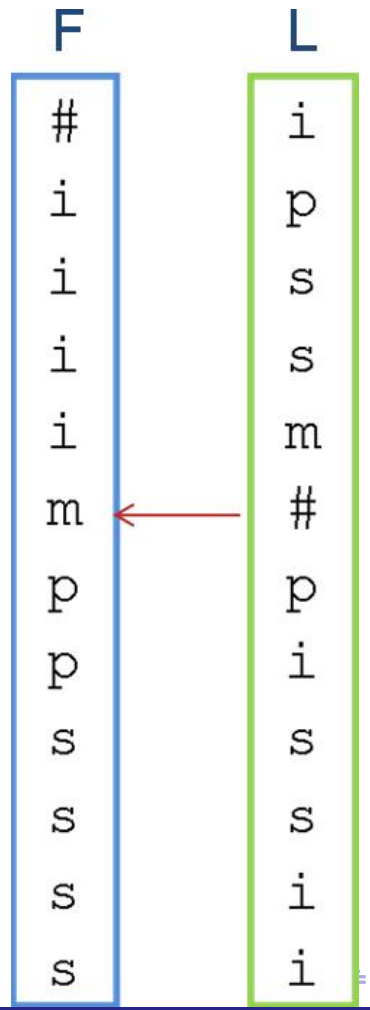
Retransformation

2. F to L mapping
   - build mapping vector T where $T[i]$ is position of the character in L corresponding to character $F[i]$ (bijective mapping)
   - i.e.: $T = \{5, 0, 7, 10, 11, 4, 1, 6, 2, 3, 8, 9\}$ (rank preserving!)

F	L
#	i
i	p
i	s
i	s
i	m
m	#
p	p
p	i
s	s
s	s
s	i
s	i

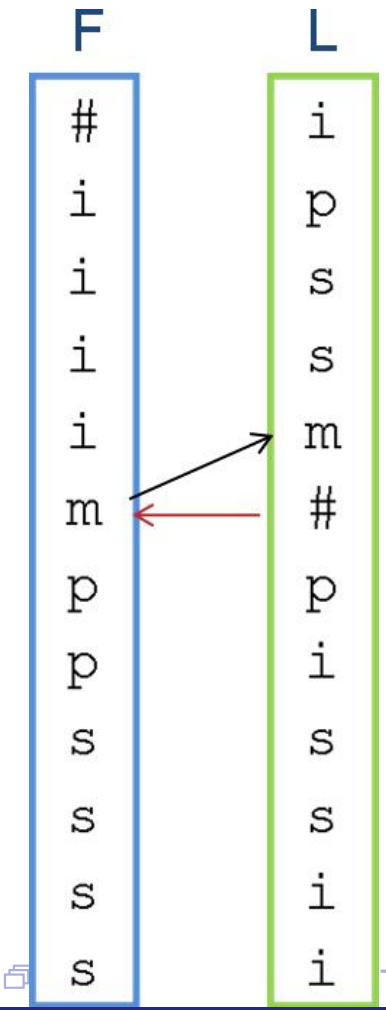
Retransformation

2. *F* to *L* mapping
   - recover *S* by starting at position of # in *L*, which is the last character in $S \cup \#$
   - successor of # is the character of *F* in the same row
   - i.e.: *m* is first character of *S*



Retransformation

2. *F* to *L* mapping
   - use mapping vector *T* to get back from *m* in *F* to *m* in *L*
   - jump from last character of this row to first character to get next successor character of *S*
   - i.e.: we get *mi*



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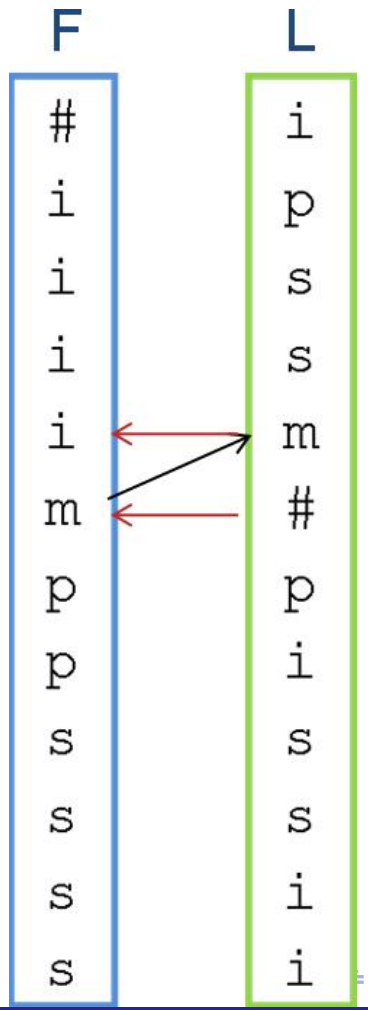
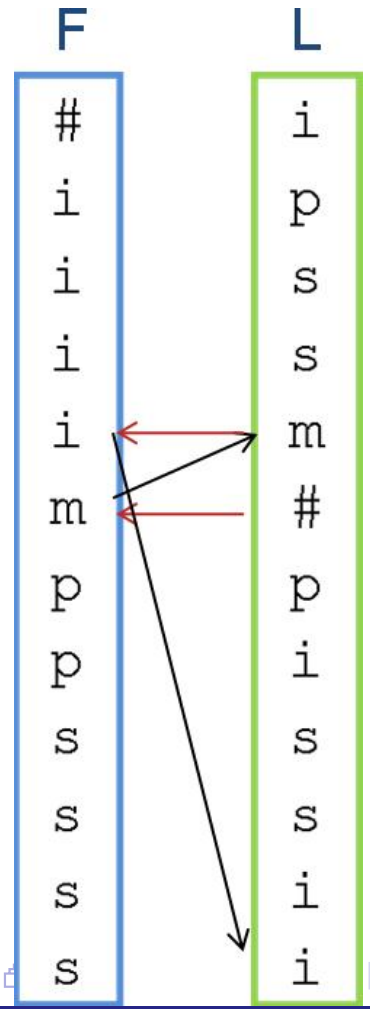


Retransformation

2. *F* to *L* mapping
   - use mapping vector *T* to get back from *i* in *F* to *i* in *L*
   - jump to front
   - i.e.: we get *mis*
   - go on until # in *F* reached



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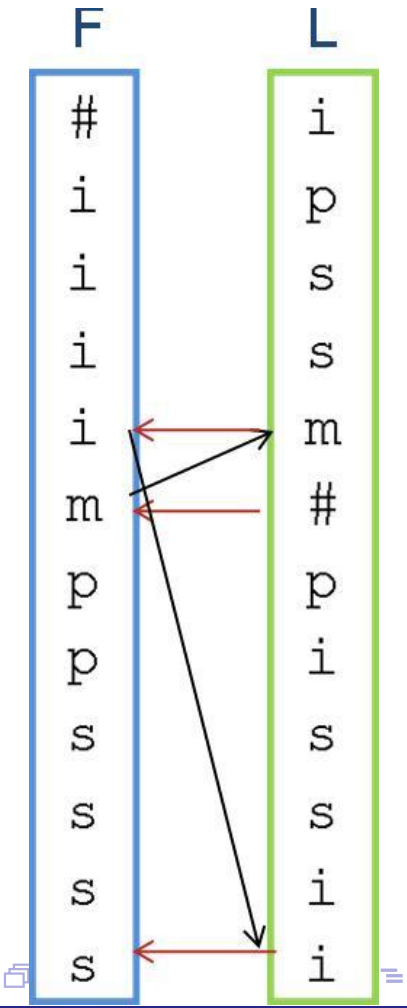

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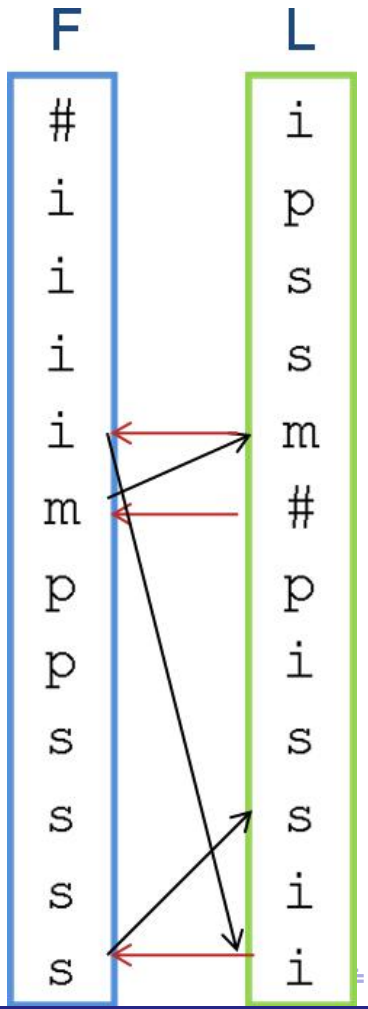

Where are we?

We have

- Reads
- Index of the reference genome (BWT)

We want

- Exact matches

si

psi

F		L
#	mississipp	i
i	#mississip	p
i	ppi#missis	s
i	ssippi#mis	s
i	ssissippi#	m
m	ississippi	#
p	i#mississi	p
p	pi#mississ	i
s	ippi#missi	s
s	issippi#mi	s
s	sippi#miss	i
s	sissippi#m	i

FM-Index

Full-Text Index In Minute Space

- Motivation: Finding reads by looking at only a small portion of the compressed text
- Use the relation between the Burrows-Wheeler compression algorithm and the suffix array data structure
- FM-Index = Compressed suffix array that connects both the compressed text and the full-text indexing information

- Supports two basic operations:
  - **Count** - return number of occurrences of P in T
  - **Locate** - find all positions of P in T

Outline

Backward-search algorithm

= Algorithm that counts the number of occurrences of P in T

- Input: L, C, Occ()
- Output: Number of occurrences

Example:

- si $\rightarrow$ 2
- pssi $\rightarrow$ 0

F		L	
#	mississipp	i	1
i	#mississip	p	2
i	ppi#missis	s	3
i	ssippi#mis	s	4
i	ssissippi#	m	5
m	ississippi	#	6
p	i#mississi	p	7
p	pi#mississ	i	8
s	ippi#missi	s	9
s	issippi#mi	s	10
s	sippi#miss	i	11
s	sissippi#m	i	12

Backward-search algorithm

- C[1,...,|Σ|]: C[c] contains the total number of chars in T which are alphabetically smaller than c (including char repetitions)

Example

- C[] for *mississippi#*

i	m	p	s

Backward-search algorithm

- C[1,...,|Σ|]: C[c] contains the total number of chars in T which are alphabetically smaller than c (including char repetitions)

Example

- C[] for *mississippi#*

i	m	p	s
I			

Backward-search algorithm

- C[1,...,|Σ|]: C[c] contains the total number of chars in T which are alphabetically smaller than c (including char repetitions)

Example

- C[] for *mississippi#*

i	m	p	s
1	5	6	8

Backward-search algorithm

- Occ(c,q): Number of occurrences of char c in prefix L[1,q]

Example

Occ(s,5) =

F		L	
#	mississipp	i	1
i	#mississip	p	2
i	ppi#missis	s	3
i	ssippi#mis	s	4
i	ssissippi#	m	5
m	ississippi	#	6
p	i#mississi	p	7
p	pi#mississ	i	8
s	ippi#missi	s	9
s	issippi#mi	s	10
s	sippi#miss	i	11
s	sissippi#m	i	12

Backward-search algorithm

- Occ(c,q): Number of occurrences of char c in prefix L[1,q]

Example

Occ(s,5) = 2

F		L	
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i	#mississip	p	2
i	ppi#missis	s	3
i	ssippi#mis	s	4
i	ssissippi#	m	5
m	ississippi	#	6
p	i#mississi	p	7
p	pi#mississ	i	8
s	ippi#missi	s	9
s	issippi#mi	s	10
s	sippi#miss	i	11
s	sissippi#m	i	12

Backward-search algorithm

- Occ(c,q): Number of occurrences of char c in prefix L[1,q]

Example

Occ(s,5) = 2
Occ(s,12) =

F		L	
#	mississipp	i	1
i	#mississip	p	2
i	ppi#missis	s	3
i	ssippi#mis	s	4
i	ssissippi#	m	5
m	ississippi	#	6
p	i#mississi	p	7
p	pi#mississ	i	8
s	ippi#missi	s	9
s	issippi#mi	s	10
s	sippi#miss	i	11
s	sissippi#m	i	12

Backward-search algorithm

- Occ(c,q): Number of occurrences of char c in prefix L[1,q]

Example

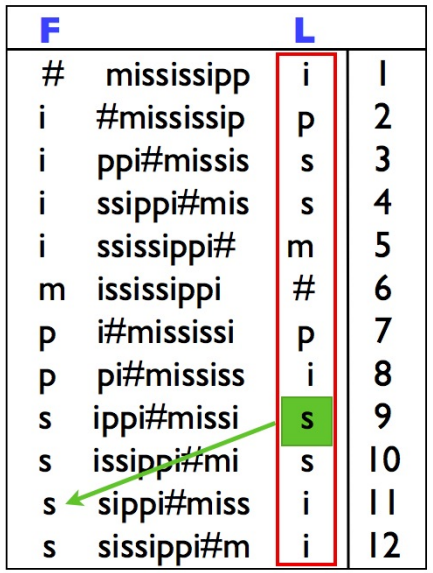
Occ(s,5) = 2
Occ(s,12) = 4

F		L	
#	mississipp	i	1
i	#mississip	p	2
i	ppi#missis	s	3
i	ssippi#mis	s	4
i	ssissippi#	m	5
m	ississippi	#	6
p	i#mississi	p	7
p	pi#mississ	i	8
s	ippi#missi	s	9
s	issippi#mi	s	10
s	sippi#miss	i	11
s	sissippi#m	i	12

L-to-F Mapping

- Formula to determine the position of char L[i] in F:
  pos(i) = C[L[i]] + Occ(L[i], i)
- Why?
  - C[L[i]] = Number of characters before the first L[i] in F
  - Occ(L[i], i) = Occurrence of character L[i] in string L[1,i]
- Example: i=9

$$pos(9) = C[s] + Occ(s, 9)$$
$$= 8 + 3 = 11$$



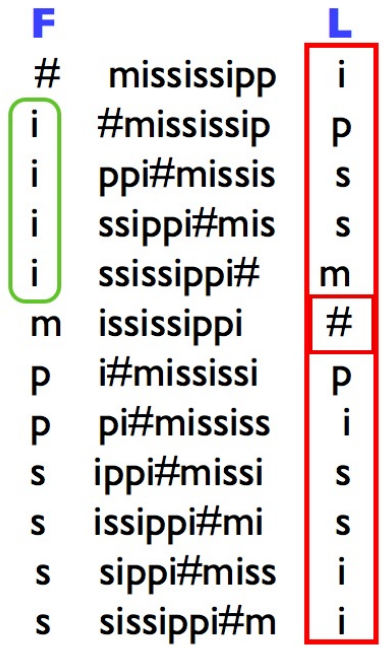
i	m	p	s
1	5	6	8

Occ(s, 9)= 3

Backward-search algorithm

How do we find a pattern of length = 1?

- Remember: BWT matrix rows = sorted suffixes of T
  - All suffixes prefixed by P occupies in a set of rows
  - Possible to calculate the first and last position
  - Occurrences = Last - First +1
- How do we search?



Backward-search algorithm

How do we find a pattern of length = 1?

- Remember: BWT matrix rows = sorted suffixes of T
  - All suffixes prefixed by P occur in a consecutive set of rows
  - Possible to calculate the first and last position
  - Occurrences = Last - First +1
- How do we search?
  - Use C
  - Find *First* and *Last*

Backward-search algorithm

How do we find a pattern of length = 1?

- Remember: BWT matrix rows = sorted suffixes of T
  - All suffixes prefixed by P occur in a consecutive set of rows
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  - Use C

i	m	p	s
1	5	6	8

  - Find *First* and *Last*

F		L
#	mississipp	i
i	#mississip	p
i	ppi#missis	s
i	ssippi#mis	s
i	ssissippi#	m
m	ississippi	#
p	i#mississi	p
p	pi#mississ	i
s	ippi#missi	s
s	issippi#mi	s
s	sippi#miss	i
s	sissippi#m	i

Backward-search algorithm

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- Remember: BWT matrix rows = sorted suffixes of T
  - All suffixes prefixed by P occur in a consecutive set of rows
  - Possible to calculate the first and last position
  - Occurrences = Last - First +1
- How do we search?
  - Use C

i	m	p	s
1	5	6	8

  - Find *First* and *Last*

F		L
#	mississipp	i
i	#mississip	p
i	ppi#missis	s
i	ssippi#mis	s
i	ssissippi#	m
m	ississippi	#
p	i#mississi	p
p	pi#mississ	i
s	ippi#missi	s
s	issippi#mi	s
s	sippi#miss	i
s	sissippi#m	i

Backward-search algorithm

How do we find a pattern **ssi**?

- Find *First* and *Last* of P[j]
- Determine L[*First*, *Last*]
- Find the first P[j-1]in L[*First*, *Last*]
  Find the last P[j-1]in L[*First*, *Last*]
- Use L-to-F mapping
- j = j-1 $\rightarrow$ Repetition

- Termination by finding **ssi**
- Occurrence = 12-11+1 = 2

Backward-search algorithm

How do we find a pattern **ssi**?

- Find *First* and *Last* of P[j]
- Determine L[*First*, *Last*]
- Find the first P[j-1]in L[*First*, *Last*]
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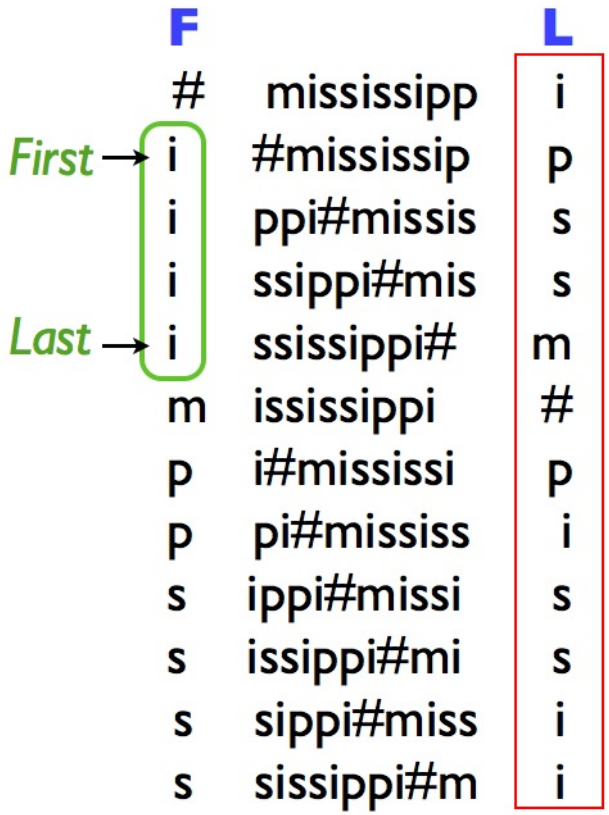

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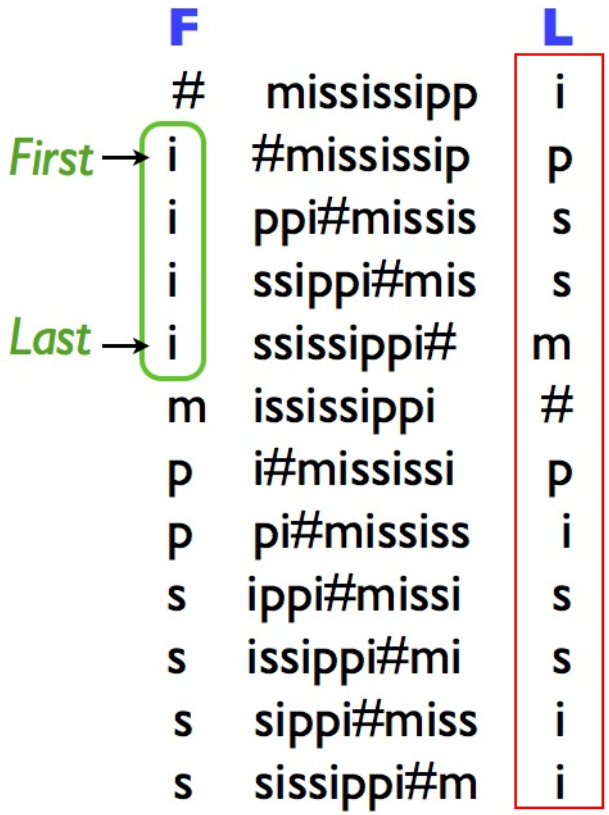

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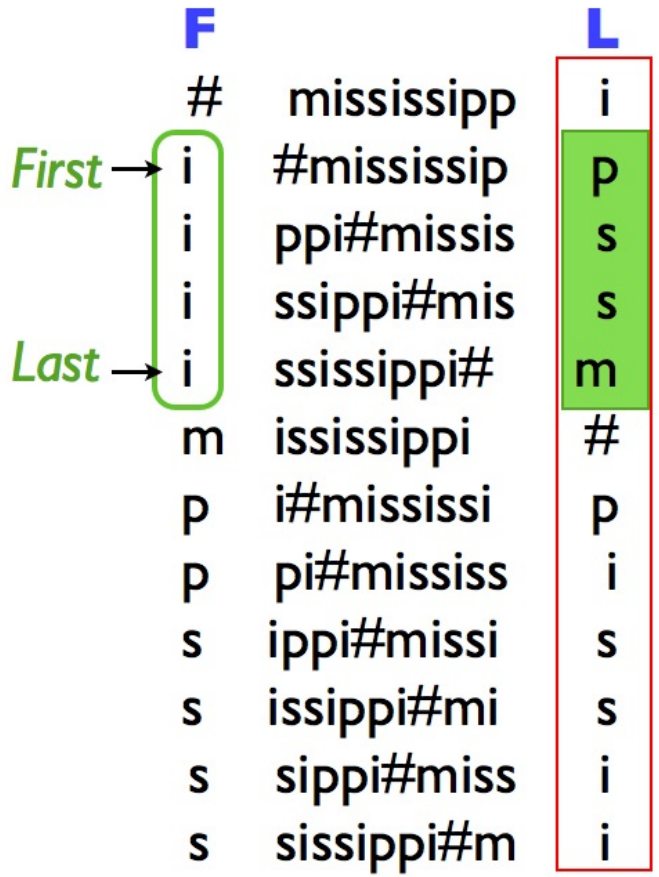

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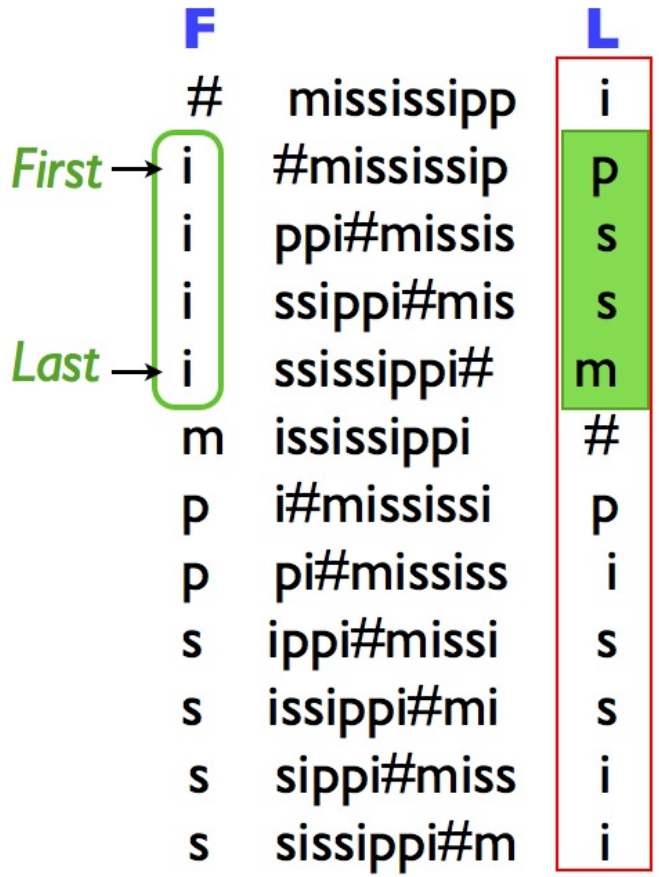

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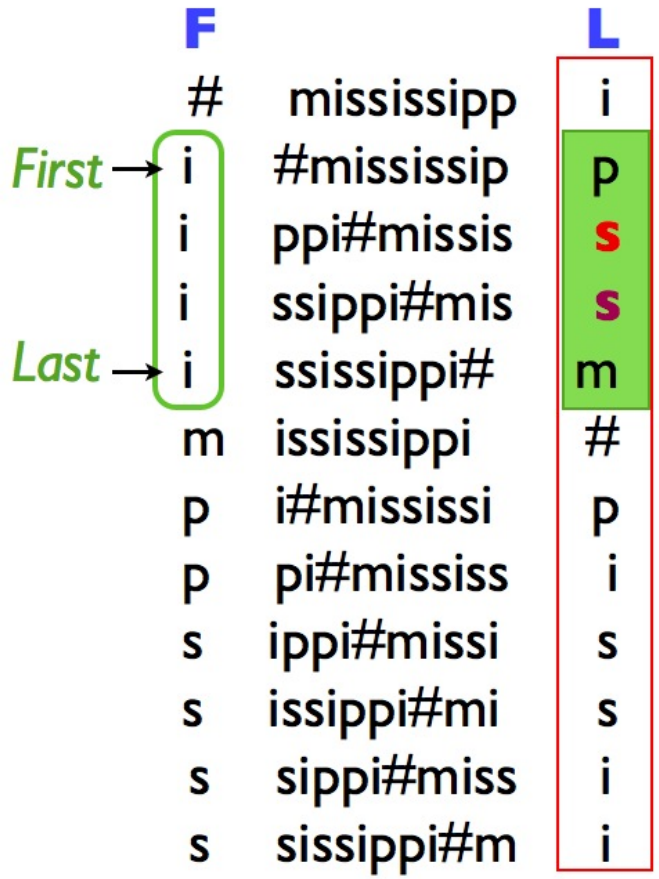

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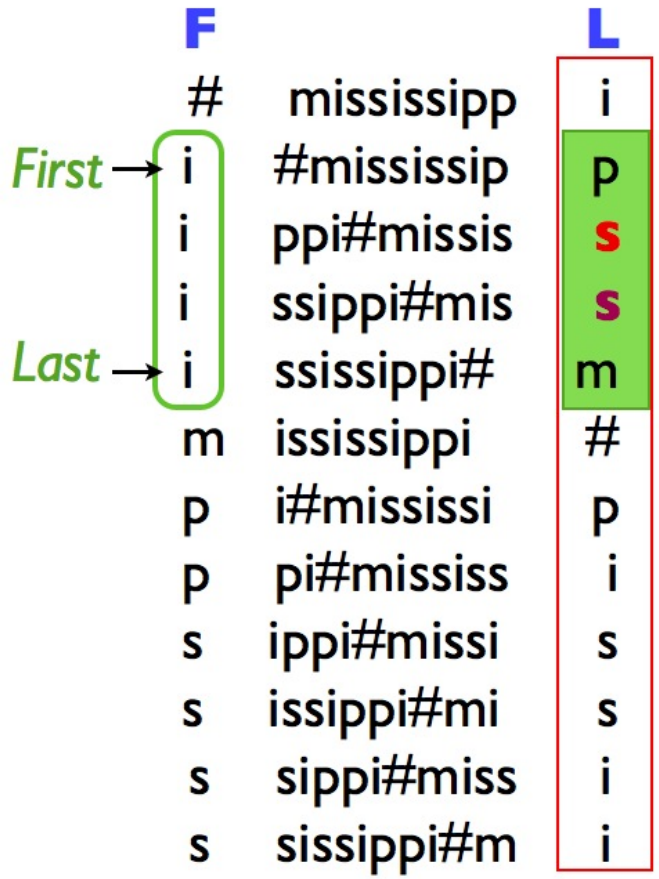

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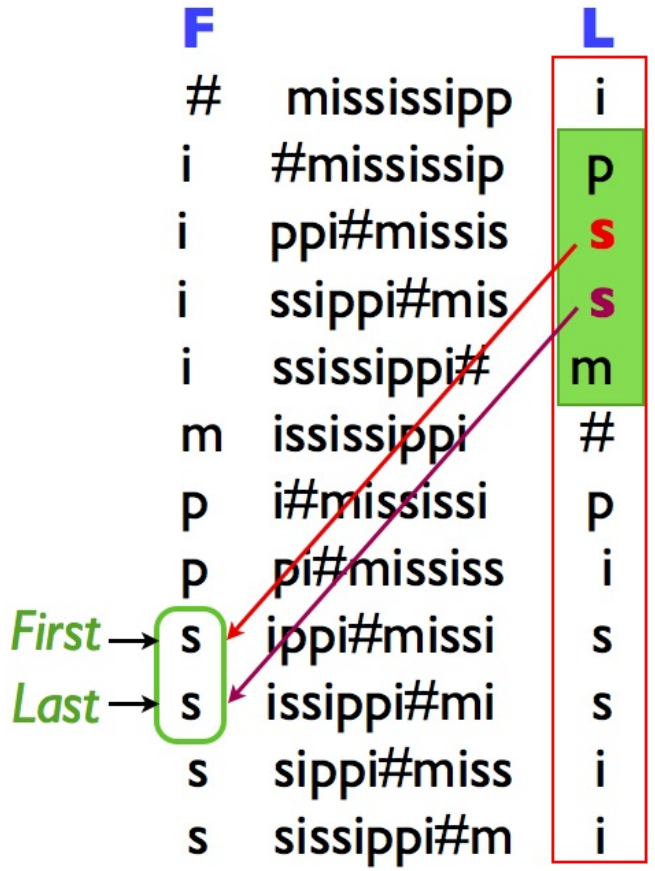

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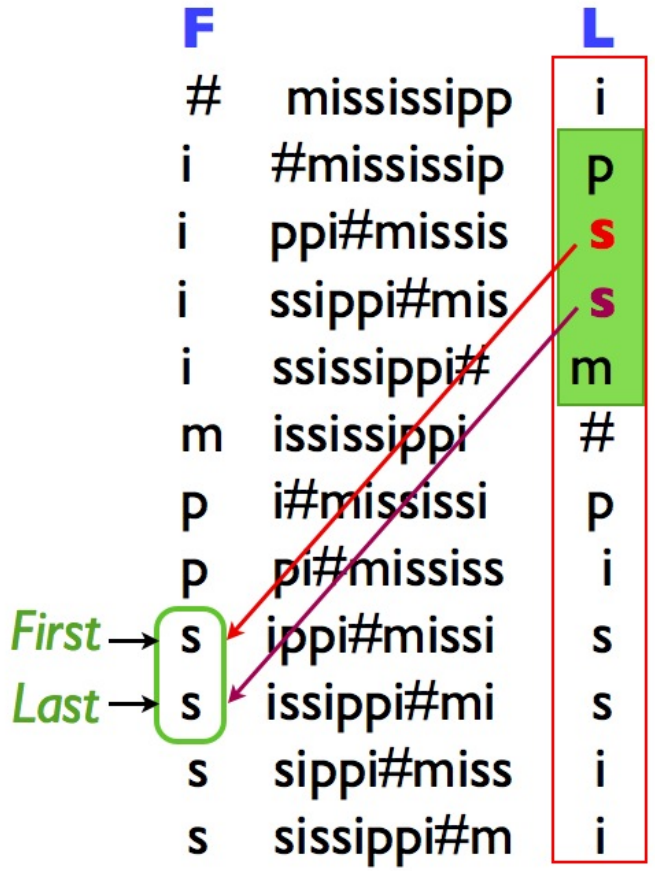

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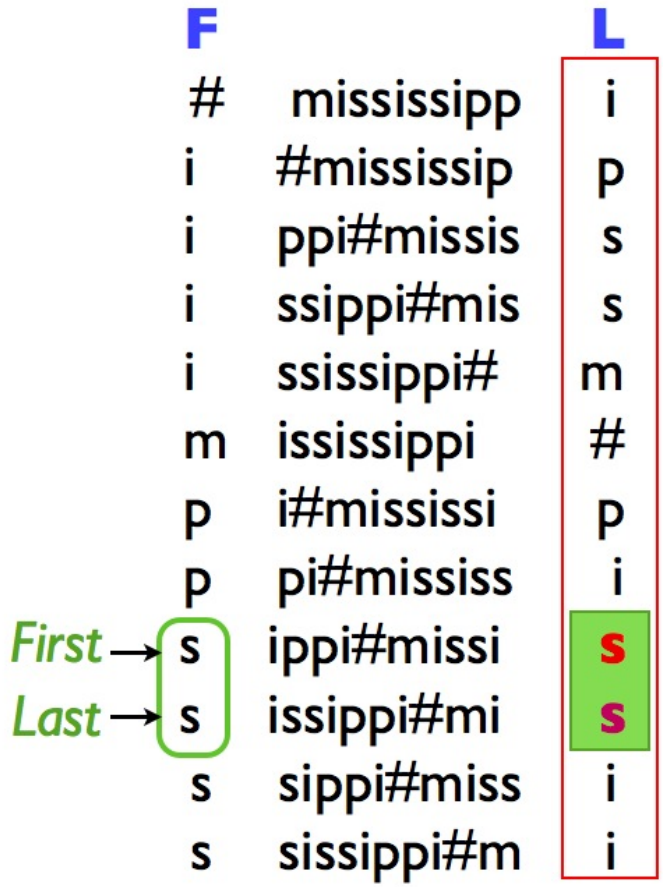

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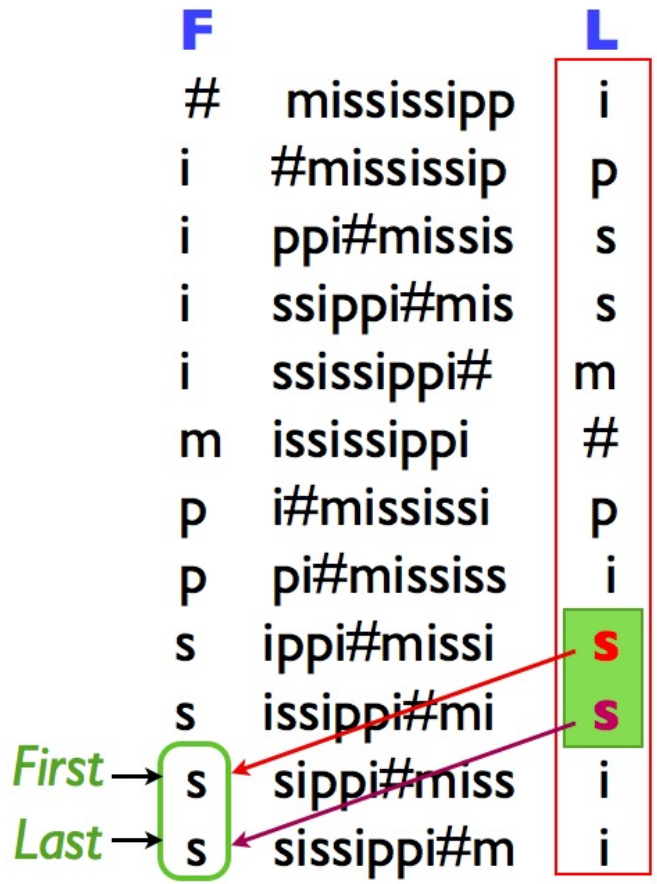
- Find *First* and *Last* of P[j]
- Determine L[*First*, *Last*]
- Find the first P[j-1]in L[*First*, *Last*]
  Find the last P[j-1]in L[*First*, *Last*]
- Use L-to-F mapping
- j = j-1 $\rightarrow$ Repetition

- Termination by finding **ssi**
- Occurrence = 12-11+1 = 2



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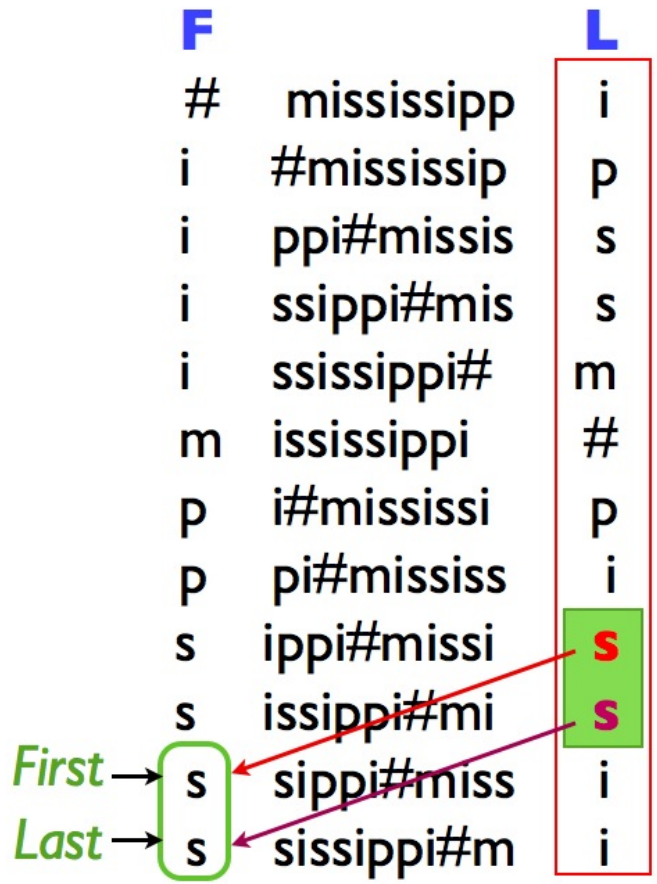
- Find *First* and *Last* of P[j]
- Determine L[*First*, *Last*]
- Find the first P[j-1]in L[*First*, *Last*]
  Find the last P[j-1]in L[*First*, *Last*]
- Use L-to-F mapping
- j = j-1 $\rightarrow$ Repetition

- Termination by finding **ssi**
- Occurrence = 12-11+1 = 2

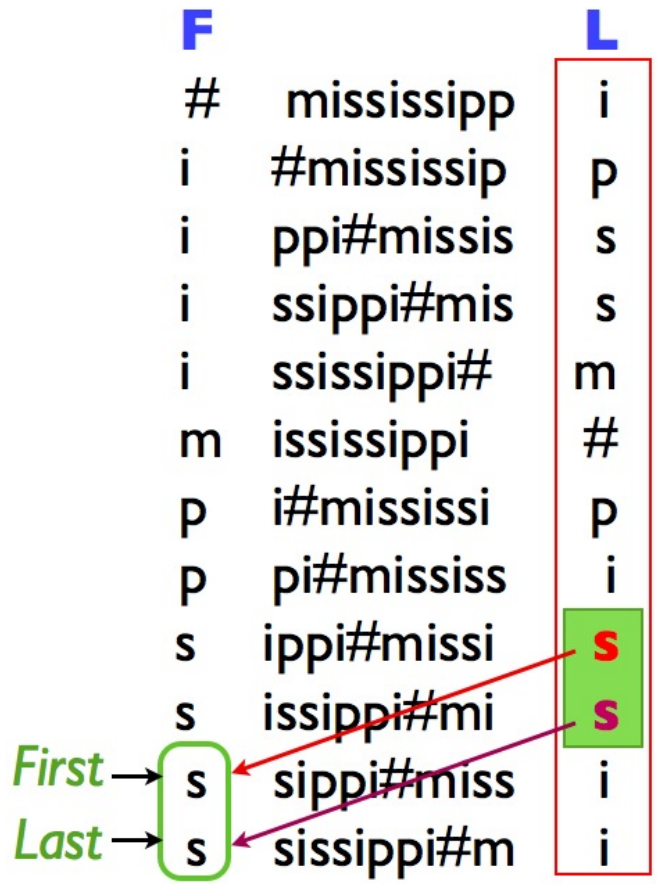


Backward-search algorithm

How do we find a pattern **ssi**?

- Find *First* and *Last* of P[j]
- Determine L[*First*, *Last*]
- Find the first P[j-1]in L[*First*, *Last*]
  Find the last P[j-1]in L[*First*, *Last*]
- Use L-to-F mapping
- j = j-1 $\rightarrow$ Repetition

- Termination by finding **ssi**
- Occurrence = 12-11+1 = 2



Backward-search algorithm

Algorithm backward_search(P[1,p])

(1) i←p, c←P[p], **First**←C[c]+1, **Last**←C[c+1]
(2) **while** ((First≤Last) **and** (i≥ 2)) **do**
  (3) c←P[i-1];
  (4) **First**←C[c]+ Occ(c,**First**-1)+1;
  (5) **Last**←C[c]+ Occ(c,**Last**);
  (6) i←i-1;
(7) **if** (Last<First)
  then return „no rows prefixed by P[1,p]“
  else return ⟨First, Last⟩

- Running time: O(p)

Outline

Algorithm to locate P in T

- Every i= *First, First+1, ..., Last* presents the pattern
- Possible because all characters have the same order in L and F
- Locate the position in T for every i

m	i	s	s	i	s	s	i	p	p	i	#
1	2	3	4	5	6	7	8	9	10	11	12

	F		L	
	#	mississipp	i	1
	i	#mississip	p	2
	i	ppi#missis	s	3
	i	ssippi#mis	s	4
	i	ssissippi#	m	5
	m	ississippi	#	6
	p	i#mississi	p	7
	p	pi#mississ	i	8
First →	s	ippi#missi	s	9
Last →	s	issippi#mi	s	10
	s	sippi#miss	i	11
	s	sissippi#m	i	12

Algorithm to locate P in T

Backward-step

- Locate the position $pos_T(i)$ in T for every i

m	i	s	s	i	s	s	i	p	p	i	#
1	2	3	4	5	6	7	8	9	10	11	12

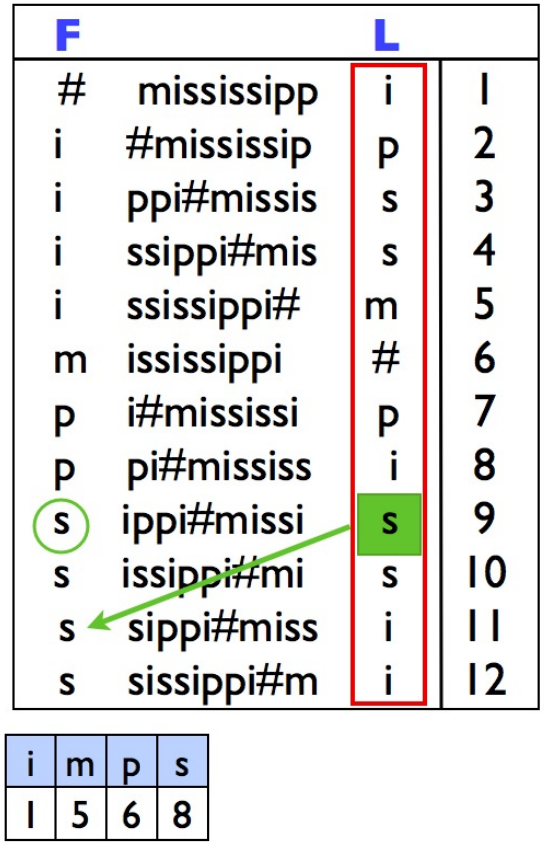
- Problem: Can't find pos_T(i) in T directly
  But: It's possible to find it indirectly
- Given index j and it's position in T
  $\rightarrow$ Can find pos_T(i) such that
  $pos_T(i) = pos_T(j) - 1$
- Backward_step algorithm

	F		L	
	#	mississipp	i	1
	i	#mississip	p	2
	i	ppi#missis	s	3
	i	ssippi#mis	s	4
	i	ssissippi#	m	5
	m	ississippi	#	6
	p	i#mississi	p	7
	p	pi#mississ	i	8
First →	s	ippi#missi	s	9
Last →	s	issippi#mi	s	10
	s	sippi#miss	i	11
	s	sissippi#m	i	12

Algorithm to locate P in T

Backward-step

- How does it work?
  - *You have:* j and $pos_T(j)$, a compressed L
  - *You want:* index i of L of T[$pos_T(j)$-1]
  - Determine L[j]:
    Compare Occ(c, j) with Occ(c,j-i) for every c $\in \Sigma \cup \{\#\}$
  - Use L-to-F mapping:
    pos(i)=C[L[j]] + Occ(L[j], i)
- Calling Occ() is O(1)
  $\rightarrow$ Backward_step in O(1)



Algorithm to locate P in T

Preprocessing

- Mark every x-th character from T and its corresponding position in L
- Store them in S
- Example: pos(r_3) = 8

	F		L	
	#	mississipp	i	1
	i	#mississip	p	2
	i	ppi#missis	s	3
	i	ssippi#mis	s	4
	i	ssissippi#	m	5
	m	ississippi	#	6
	p	i#mississi	p	7
	p	pi#mississ	i	8
First →	s	ippi#missi	s	9
Last →	s	issippi#mi	s	10
	s	sippi#miss	i	11
	s	sissippi#m	i	12

m	i	s	s	i	s	s	i	p	p	i	#
1	2	3	4	5	6	7	8	9	10	11	12

Algorithm to locate P in T

- Find position of row i:
  - **If r_i is marked**, then return pos(r_i) from S
  - **Otherwise** use Backward_step(i)
    - Repeat t times until you find a marked row j
    - $pos_T(i) = pos_T(j) + t$

F		L	
#	mississipp	i	1
i	#mississip	p	2
i	ppi#missis	s	3
i	ssippi#mis	s	4
i	ssissippi#	m	5
m	ississippi	#	6
p	i#mississi	p	7
p	pi#mississ	i	8
First → s	ippi#missi	s	9
Last → s	issippi#mi	s	10
s	sippi#miss	i	11
s	sissippi#m	i	12

m	i	s	s	i	s	s	i	p	p	i	#
1	2	3	4	5	6	7	8	9	10	11	12

Algorithm to locate P in T

Finding **si**

- Determine all positions for i = First, First+1, ..., Last
- First = 9, Last = 10
- i= 10
  - r_{10} is marked
  - pos(10) = 4

m	1
i	2
s	3
s	4
i	5
s	6
s	7
i	8
p	9
p	10
i	11
#	12

	F	L	
	#	mississipp	i	1
	i	#mississip	p	2
	i	ppi#missis	s	3
	i	ssippi#mis	s	4
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	p	i#mississi	p	7
	p	pi#mississ	i	8
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Algorithm to locate P in T

Finding **si**

- Determine all positions for i = First, First+1, ..., Last
- First = 9, Last = 10
- i= 10
  - r_{10} is marked
  - pos(10) = 4

m	1
i	2
s	3
s	4
i	5
s	6
s	7
i	8
p	9
p	10
i	11
#	12

	F	L	
	#	mississipp	i	1
	i	#mississip	p	2
	i	ppi#missis	s	3
	i	ssippi#mis	s	4
	i	ssissippi#	m	5
	m	ississippi	#	6
	p	i#mississi	p	7
	p	pi#mississ	i	8
	s	ippi#missi	s	9
i →	s	issippi#mi	s	10
	s	sippi#miss	i	11
	s	sissippi#m	i	12

Algorithm to locate P in T

Finding **si**

- Determine all positions for i = First, First+1, ..., Last
- First = 9, Last = 10
- i= 10
  - r_{10} is marked
  - pos(10) = 4

m	1
i	2
s	3
s	4
i	5
s	6
s	7
i	8
p	9
p	10
i	11
#	12

	F	L	
	#	mississipp	i	1
	i	#mississip	p	2
	i	ppi#missis	s	3
	i	ssippi#mis	s	4
	i	ssissippi#	m	5
	m	ississippi	#	6
	p	i#mississi	p	7
	p	pi#mississ	i	8
	s	ippi#missi	s	9
i →	s	issippi#mi	s	10
	s	sippi#miss	i	11
	s	sissippi#m	i	12

Algorithm to locate P in T

Finding **si**

- i = 9
  - r_9 is not marked
  - Backward_step(9), t=1
  - r_{11} is not marked
  - Backward_step(11), t=2
  - r_4 is not marked
  - Backward_step(4), t=3
  - r_{10} is marked
  - pos(9) = pos(10) +3 = 4 + 3 = 7

m	1
i	2
s	3
s	4
i	5
s	6
s	7
i	8
p	9
p	10
i	11
#	12

	F	L	
	#	mississipp	i	1
	i	#mississip	p	2
	i	ppi#missis	s	3
	i	ssippi#mis	s	4
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i →	s	ippi#missi	s	9
	s	issippi#mi	s	10
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	s	sissippi#m	i	12

Algorithm to locate P in T

Finding **si**

- i = 9
  - r_9 is not marked
  - Backward_step(9), t=1
  - r_{11} is not marked
  - Backward_step(11), t=2
  - r_4 is not marked
  - Backward_step(4), t=3
  - r_{10} is marked
  - pos(9) = pos(10) +3 = 4 + 3 = 7

m	1
i	2
s	3
s	4
i	5
s	6
s	7
i	8
p	9
p	10
i	11
#	12

	F	L	
	# mississipp	i	1
	i #mississip	p	2
	i ppi#missis	s	3
	i ssippi#mis	s	4
	i ssissippi#	m	5
	m ississippi	#	6
	p i#mississi	p	7
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	s issippi#mi	s	10
	s sippi#miss	i	11
	s sissippi#m	i	12

Algorithm to locate P in T

Finding **si**

- i = 9
  - r_9 is not marked
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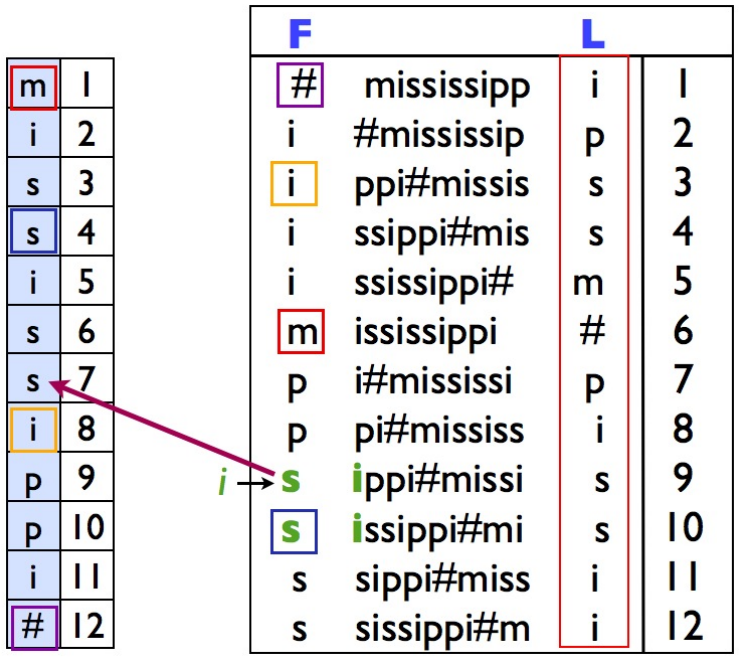

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m	1
i	2
s	3
s	4
i	5
s	6
s	7
i	8
p	9
p	10
i	11
#	12

	F	L	
	# mississipp	i	1
	i #mississip	p	2
	i ppi#missis	s	3
	i ssippi#mis	s	4
	i ssissippi#	m	5
	m ississippi	#	6
	p i#mississi	p	7
	p pi#mississ	i	8
i →	s ippi#missi	s	9
	s issippi#mi	s	10
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F		L	
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i	ssippi#mis	s	4
i	ssissippi#	m	5
m	ississippi	#	6
p	i#mississi	p	7
p	pi#mississ	i	8
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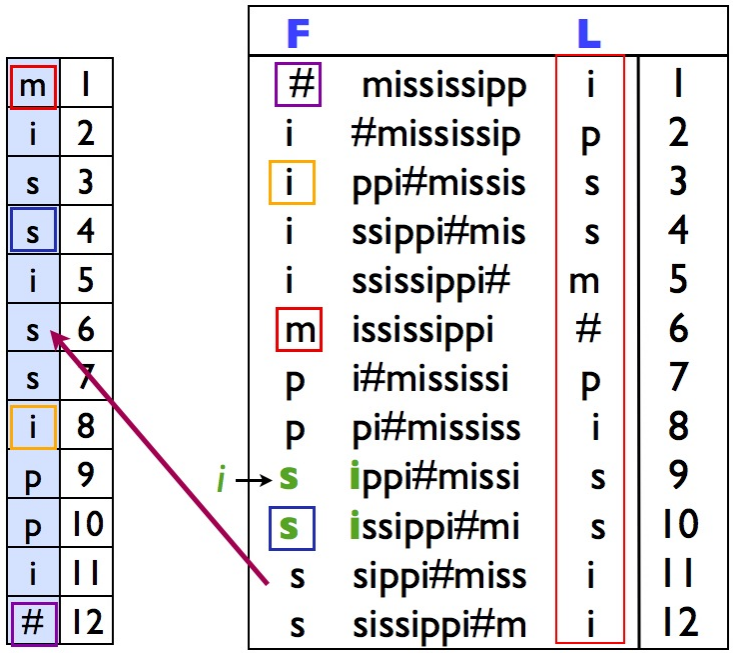

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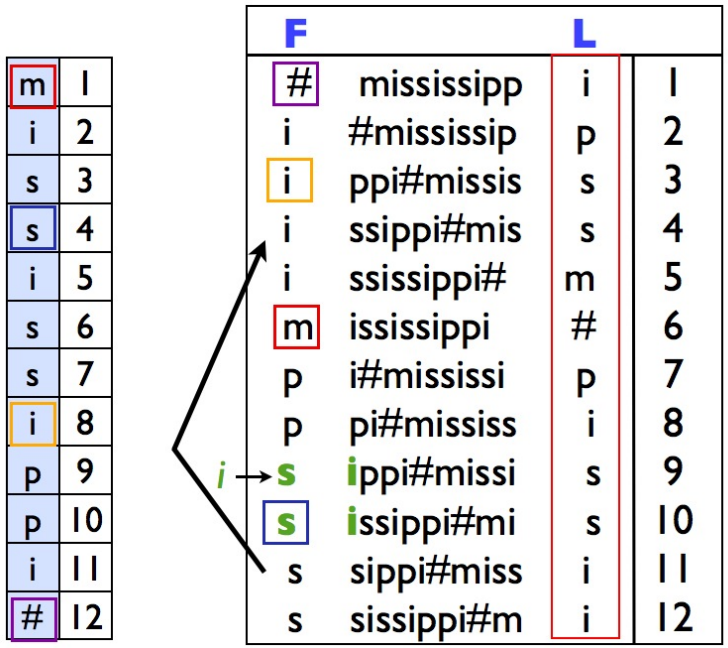

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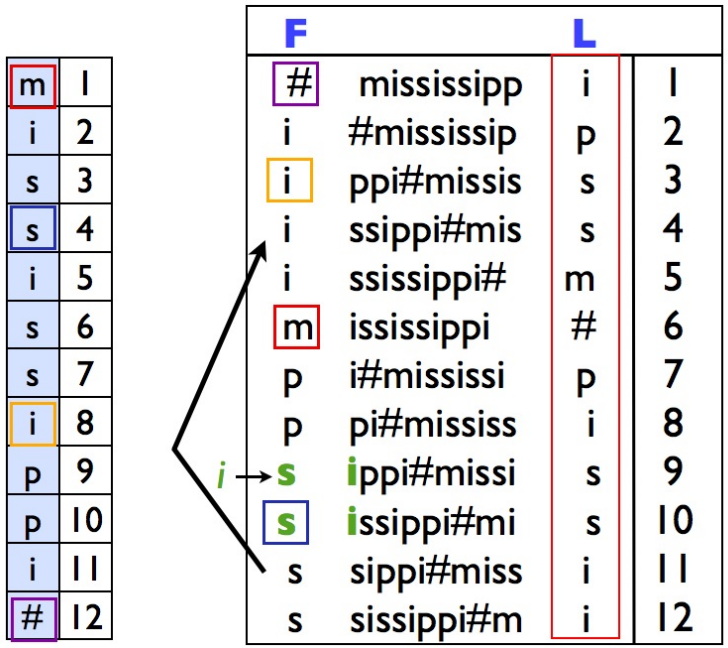

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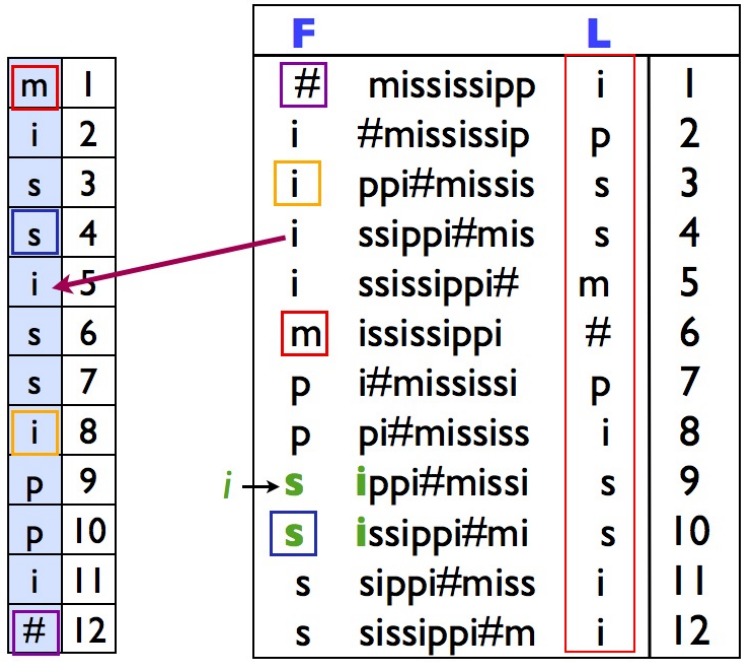

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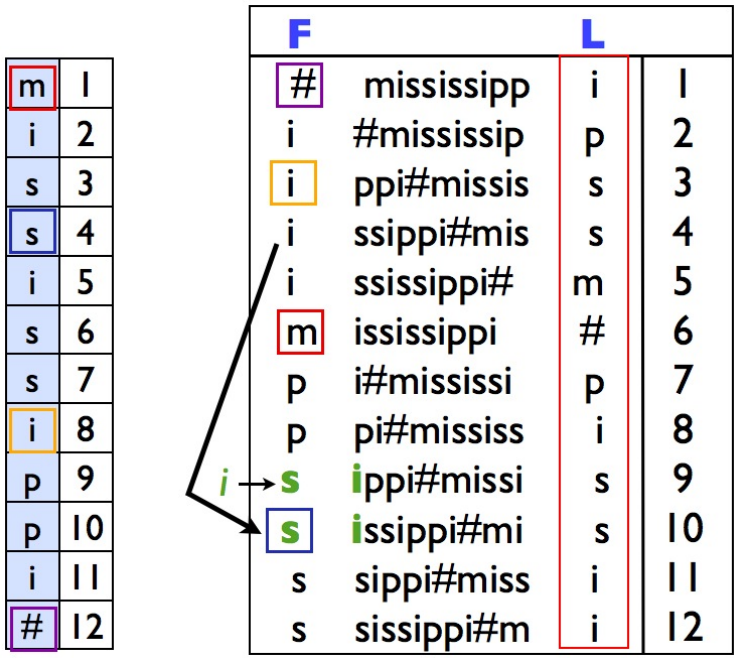

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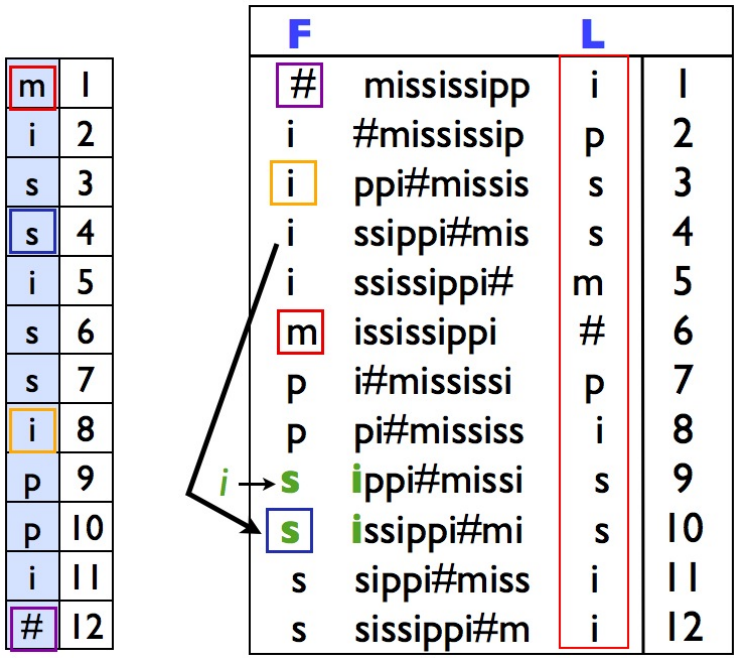

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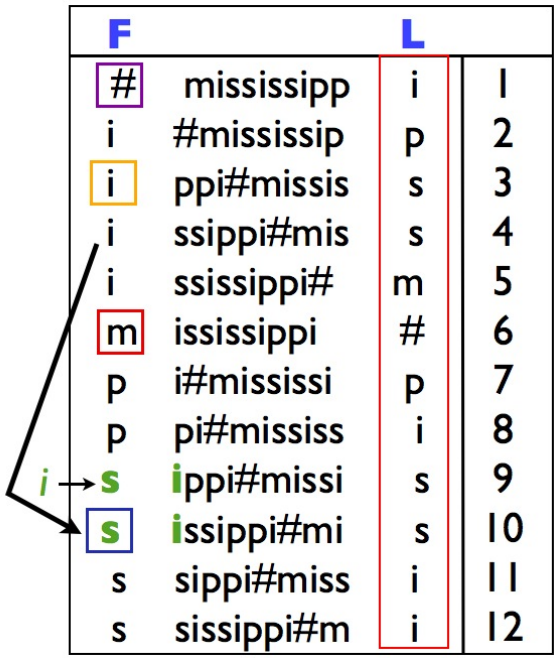


Outline

Mismatches

Due to:

- sequencing errors
- differences between reference and query organisms

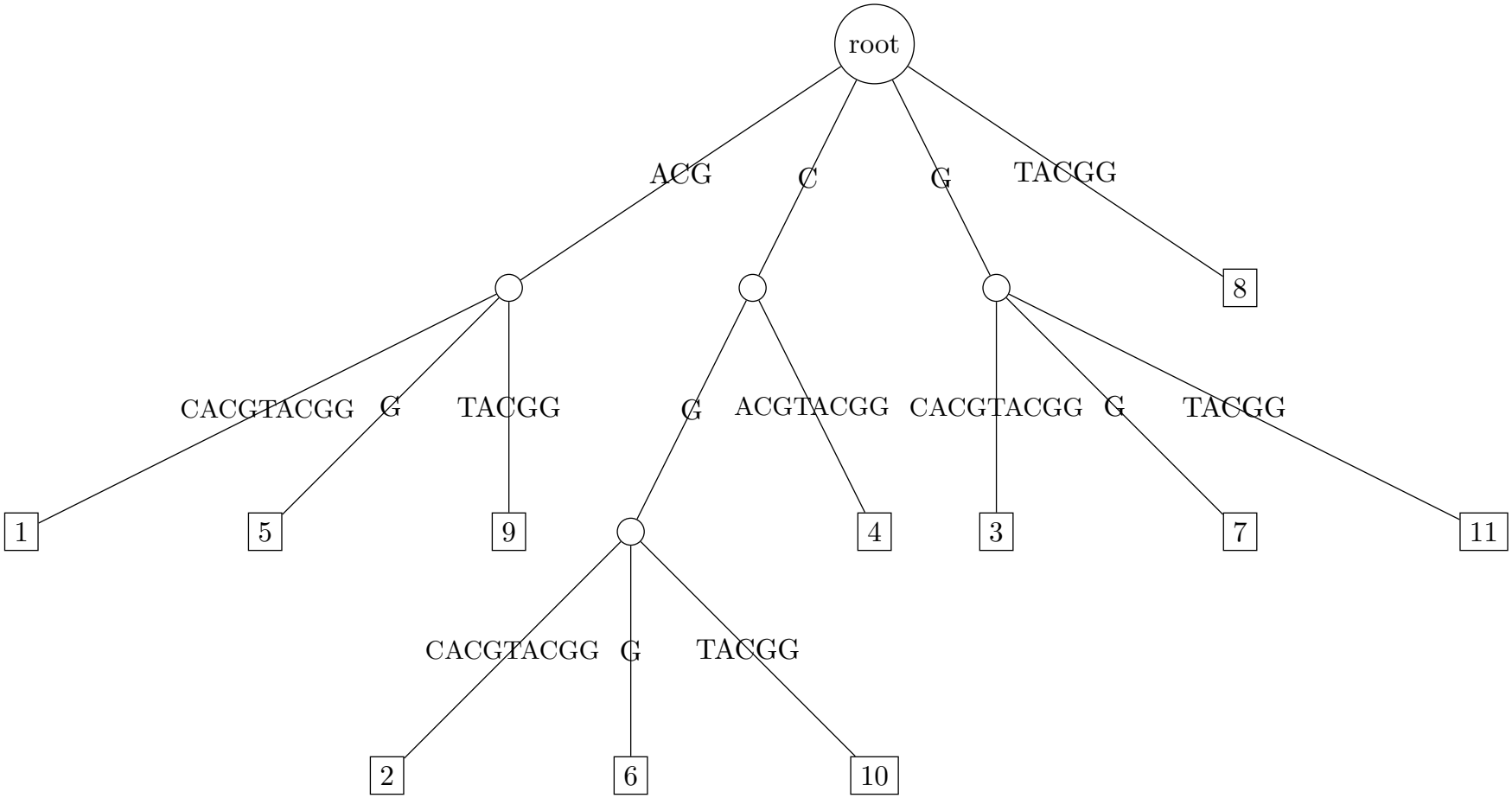
EXACTMATCH insufficient

Backtracking algorithm

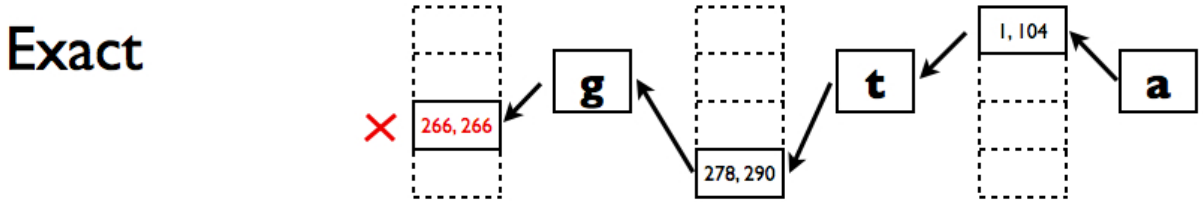
- Greedy
- Depth-First Search
- Allowed mismatches $\uparrow \Rightarrow$ runtime $\uparrow$

Example

ACGCACGTACGG

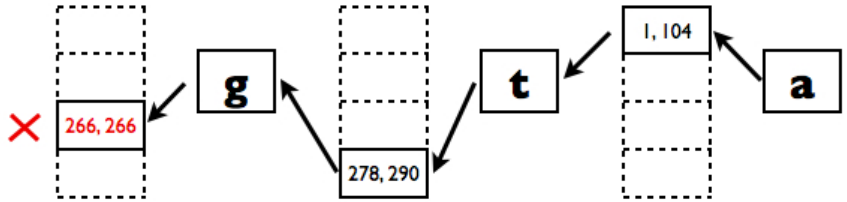


ggta

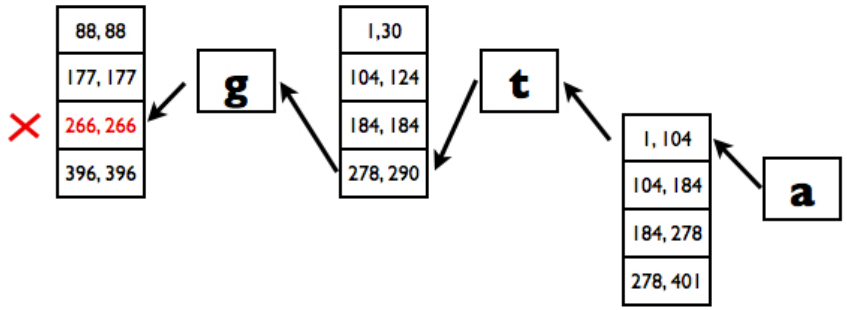


ggta

Exact



Inexact



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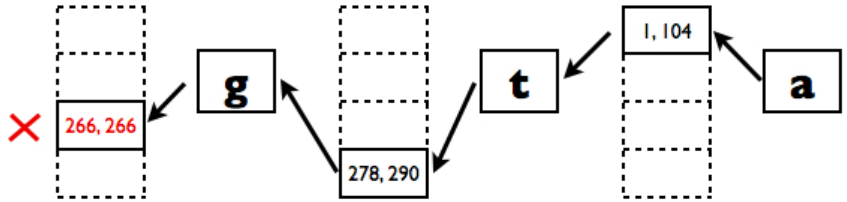
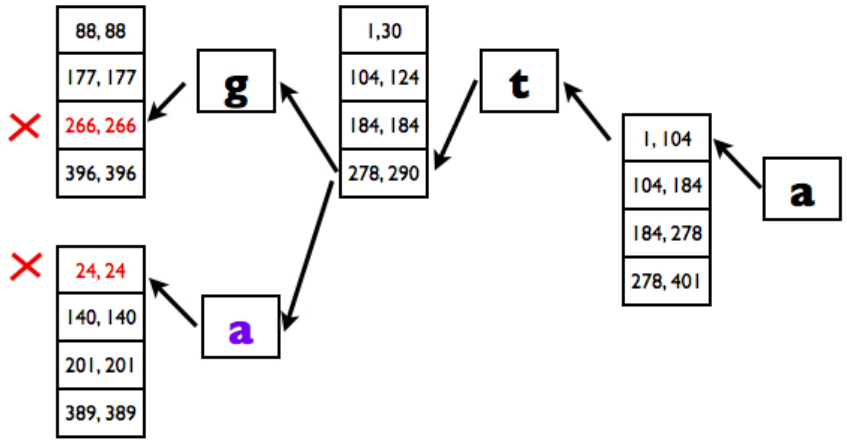
Exact



Inexact



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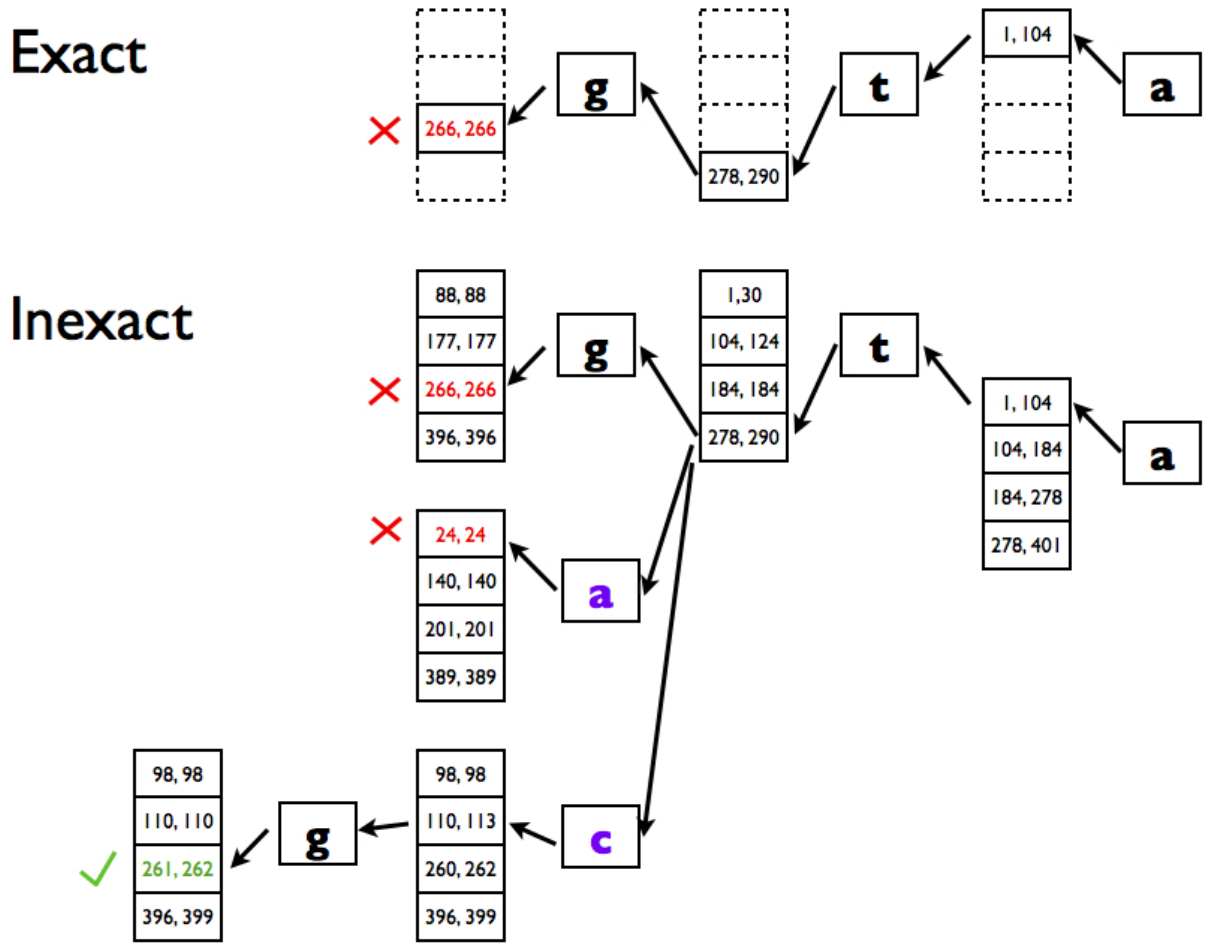
Exact
266, 266
g
278, 290
t
1, 104
a
Inexact
88, 88
177, 177
266, 266
396, 396
g
1,30
104, 124
184, 184
278, 290
t
1, 104
104, 184
184, 278
278, 401
a
24, 24
140, 140
201, 201
389, 389
a
98, 98
110, 110
261, 262
396, 399
g
98, 98
110, 113
260, 262
396, 399
c

Outline

Excessive backtracking

- Avoid excessive backtracking
- Solution $\rightarrow$ double indexing
- and backtracking ceiling

Sources


Ben Langmead, Cole Trapnell, Mihai Pop & Steven L Salzberg.
Ultrafast and memory-efficient alignment of short DNA sequences to the human genome.
Genome Biology 2009, 10:R25.


M. Burrows & D.J. Wheeler
A block-sorting lossless data compression algorithm.
Digital SRC Research Report 1994.

Thank you

Questions?