UNIVERSITÄT
DES
SAARLANDES

ZBI ZENTRUM FÜR BIOINFORMATIK

Variation and Conservation in MSAs: Positional Prefix and Divergence Arrays

Algorithms for Sequence Analysis

Sven Rahmann

Summer 2021

Overview

Previously

Building **Multiple Sequence Alignments (MSAs)**

Today

How can we **use** multiple sequence alignments once we have them?

- MSAs have the potential to represent **genetic diversity in a population**
- **Calling variants** for each sample relative to a reference genome **implicitly constructs an MSA** with the reference genome at its center

Indexing a Collection of Sequences

In order to **use** an MSA, we need efficient ways of **searching** it.

Applications

- Searching for common sequences
- Compressing the whole collection / MSA
- Finding intervals that are conserved between many samples

Literature

Richard Durbin. *Efficient haplotype matching and storage using the positional Burrows-Wheeler transform (PBWT)*. Bioinformatics, 30(9), pp. 1266-72, 2014.

Tools we have so far

Approach

Use generalized suffix tree, suffix array, or BWT/FM index

Reminder: Generalized Index

1. Concatenate sequences: $S = s_1\$_1 s_2\$_2 \dots s_n\$_n$
2. Build your favorite index on S

Disadvantages

- Does not take alignment of sequences into account,
  i.e. no notion of a **common position** or a **common occurence** of a substring.
- Cannot exploit alignment for compression

Turning an MSA into a Haplotype Panel

```
AGATAGTTGCTTTAA-CCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAAGCCTAATGGACAGATGCTGCTCTGTGAAGCCGGGT
```

Turning an MSA into a Haplotype Panel

```
AGATAGTTGCTTTAA-CCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAAGCCTAATGGACAGATGCTGCTCTGTGAAGCCGGGT
```

Turning an MSA into a Haplotype Panel

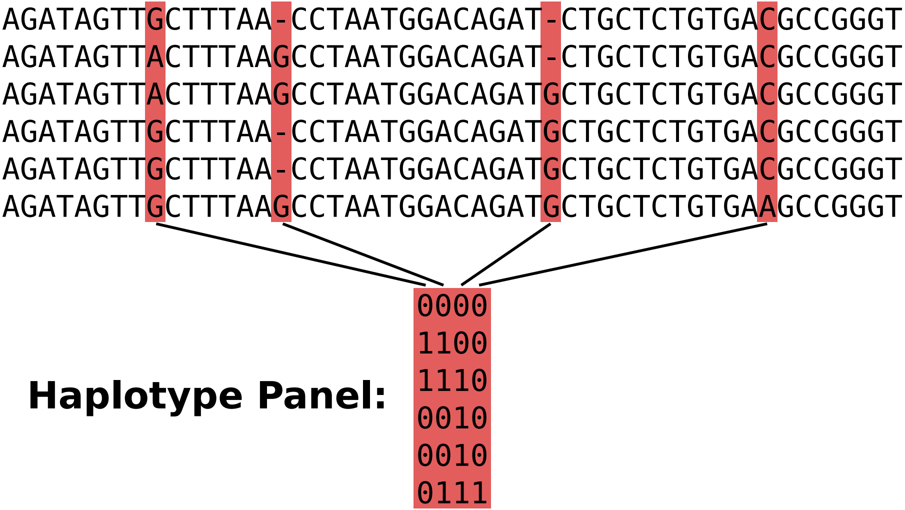
```
AGATAGTTGCTTTAA-CCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAAGCCTAATGGACAGATGCTGCTCTGTGAAGCCGGGT
```

```
********G******-**************-***********C*******
********A******G**************-***********C*******
********A******G**************G***********C*******
********G******-**************G***********C*******
********G******-**************G***********C*******
********G******G**************G***********A*******
```

Turning an MSA into a Haplotype Panel

```
AGATAGTTGCTTTAA-CCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAAGCCTAATGGACAGATGCTGCTCTGTGAAGCCGGGT


        0      0              0           0
        1      1              0           0
        1      1              1           0
        0      0              1           0
        0      0              1           0
        0      1              1           1
```

Turning an MSA into a Haplotype Panel



```
AGATAGTTGCTTTAA-CCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGAT-CTGCTCTGTGACGCCGGGT
AGATAGTTACTTTAAGCCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAA-CCTAATGGACAGATGCTGCTCTGTGACGCCGGGT
AGATAGTTGCTTTAAGCCTAATGGACAGATGCTGCTCTGTGAAGCCGGGT
```

Haplotype Panel:

```
0000
1100
1110
0010
0010
0111
```

Set of Haplotype Sequences (Haplotype Panel)

Given

Set X of M haplotype sequences over $\Sigma = \{0, 1\}$, each of them with N sites

$$
\begin{array}{c|ccccccccc}
x_0 & 0 & 0 & 1 & 0 & \dots & 1 & 1 & 0 & 0 \\
x_1 & 1 & 0 & 1 & 1 & \dots & 1 & 0 & 1 & 1 \\
\vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\
x_{M-2} & 1 & 0 & 1 & 1 & \dots & 1 & 1 & 0 & 0 \\
x_{M-1} & 0 & 1 & 0 & 0 & \dots & 1 & 0 & 1 & 0
\end{array}
$$

Note

Here we assume a binary alphabet, but the ideas extend to larger alphabets as well. (See Mäkinen and Norri, 2019, `https://doi.org/10.1016/j.ipl.2019.02.003`).

Reversed Prefix Sorting

Reminder: Suffix Array

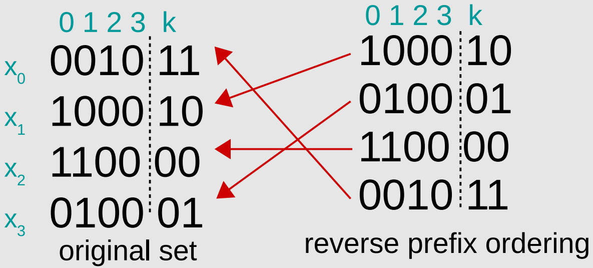
```
    0123456
s = banana$

r   pos[r]   s[pos[r]..]
0   6        $
1   5        a$
2   3        ana$
3   1        anana$
4   0        banana$
5   4        na$
6   2        nana$
```

The **suffix array** contains the starting positions of all suffixes in lexicographic order.

New Concept: Reversed Prefix Sorting

Pick a prefix length k.
Consider the k-prefixes and sort their **reverses** lexicographically.

Example for $k = 4$



0 1 2 3 k

x_0 0010 11

x_1 1000 10

x_2 1100 00

x_3 0100 01

original set

0 1 2 3 k

1000 10

0100 01

1100 00

0010 11

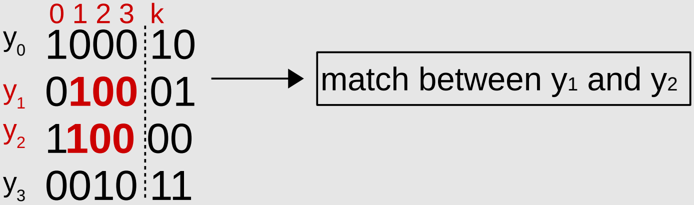
reverse prefix ordering

Reversed Prefix Sorting: Why?

Observations

- **Matches** between two sequences are **adjacent** in the sorting,
- … comparable to the sorted suffixes in the suffix array,
- … but here the matches are **positional/aligned**.

Example

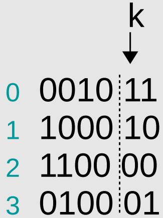


	0	1	2	3	k	
y_0	1	0	0	0	1	0
y_1	0	**1**	**0**	**0**	0	1
y_2	1	**1**	**0**	**0**	0	0
y_3	0	0	1	0	1	1

match between y_1 and y_2

Positional Prefix Array by Example

unsorted:



k

0 0010 11

1 1000 10

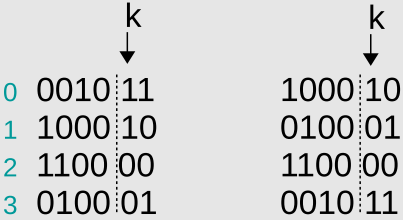
2 1100 00

3 0100 01

Positional Prefix Array by Example

unsorted:

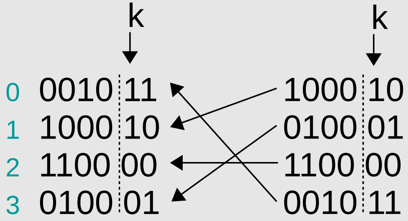
sorted:



Positional Prefix Array by Example

unsorted:

sorted:



k

k

0 0010 11

1 1000 10

2 1100 00

3 0100 01

1000 10

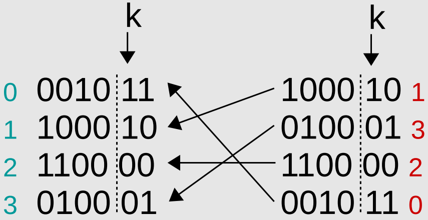
0100 01

1100 00

0010 11

Positional Prefix Array by Example

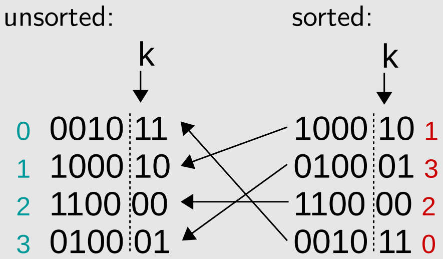
unsorted: sorted:



0 0010 11
1 1000 10
2 1100 00
3 0100 01

1000 10 1
0100 01 3
1100 00 2
0010 11 0

Positional Prefix Array by Example



unsorted:

k

0 0010 11

1 1000 10

2 1100 00

3 0100 01

sorted:

k

1000 10 1

0100 01 3

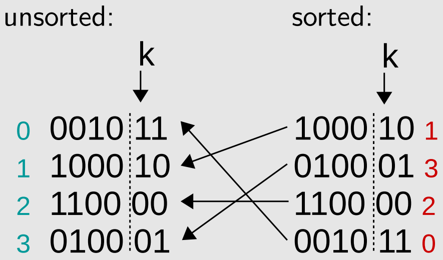
1100 00 2

0010 11 0

$$a_k[0] = 1,\ a_k[1] = 3$$
$$a_k[2] = 2,\ a_k[3] = 0$$

$$a_k = \begin{pmatrix} 1 \\ 3 \\ 2 \\ 0 \end{pmatrix}$$

Positional Prefix Array by Example



unsorted:

k

0 0010 11

1 1000 10

2 1100 00

3 0100 01

sorted:

k

1000 10 1

0100 01 3

1100 00 2

0010 11 0

$a_k[0] = 1,\ a_k[1] = 3$
$a_k[2] = 2,\ a_k[3] = 0$

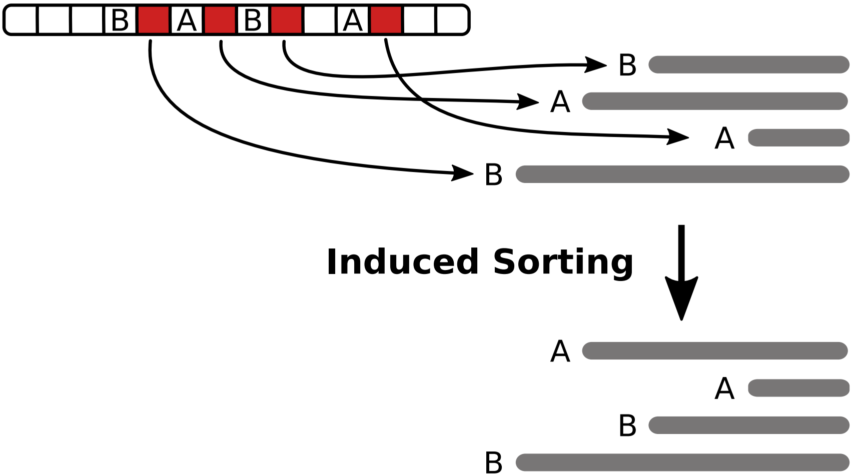
$$a_k = \begin{pmatrix} 1 \\ 3 \\ 2 \\ 0 \end{pmatrix}$$

Definition

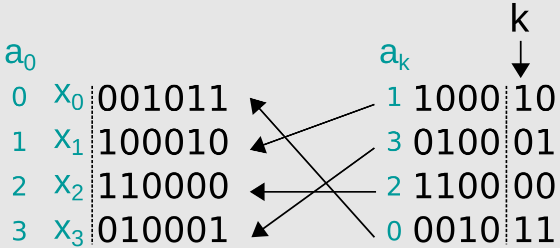
For an input set of strings $x_0, \ldots, x_{M-1} \in \Sigma^N$, the **positional prefix array** a_k for position k lists the sequence indices in lexicographic order with respect to the prefix of the reversed sequences up to k. That is,

$$r\big(x_{a_k[0]}[0..k)\big) \leq r\big(x_{a_k[1]}[0..k)\big) \leq \ldots \leq r\big(x_{a_k[M-1]}[0..k)\big)$$

Reminder: Induced Sorting Idea for Suffix Arrays



Building a_{k+1} from a_k



a_0

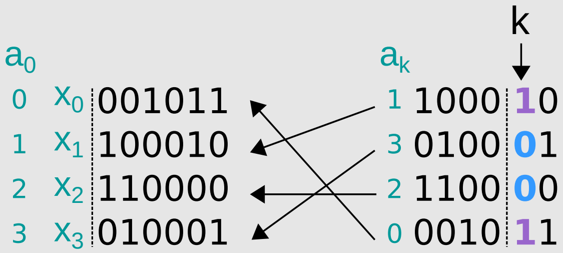
0	x_0	001011
1	x_1	100010
2	x_2	110000
3	x_3	010001

a_k

k

1	1000	10
3	0100	01
2	1100	00
0	0010	11

Building a_{k+1} from a_k



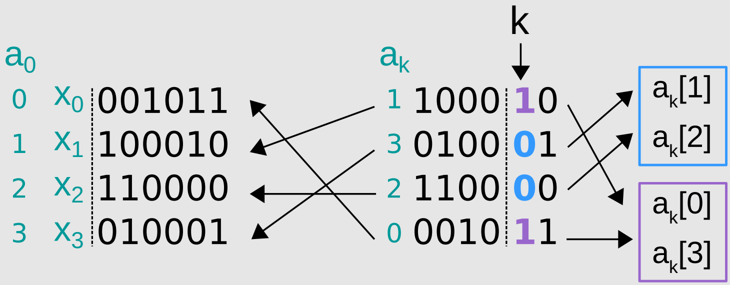
a_0

0	x_0	001011
1	x_1	100010
2	x_2	110000
3	x_3	010001

a_k

1	1000	10
3	0100	01
2	1100	00
0	0010	11

Building a_{k+1} from a_k



a_0

0 x_0 001011

1 x_1 100010

2 x_2 110000

3 x_3 010001

a_k

k

1 1000 10

3 0100 01

2 1100 00

0 0010 11

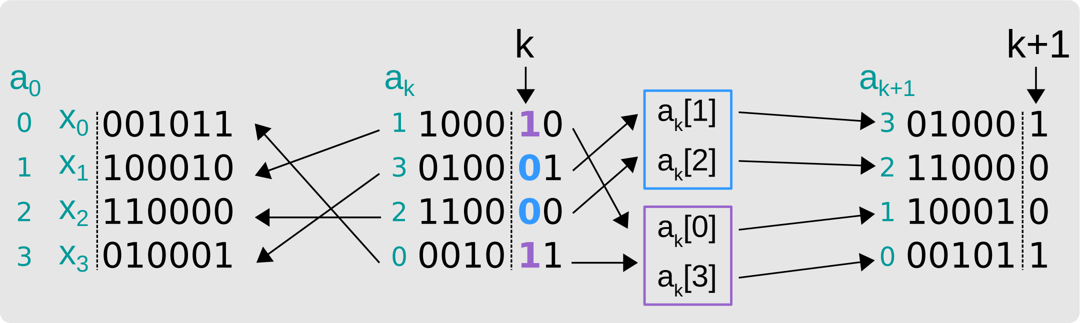
$a_k[1]$

$a_k[2]$

$a_k[0]$

$a_k[3]$

Building a_{k+1} from a_k



Code: Building a_{k+1} from a_k

```python
def build_reverse_prefix_array_column(X, k, a_k):
    """Take a_k and return a_{k+1}."""
    M = len(x)  # number of strings
    a = [[],[]]  # a[0] and a[1] are both empty lists
    for r in range(M):
        i = a_k[r] # index of string at rank r
        j = int(X[i][k])  # 0 or 1?
        a[j].append(i)  # put i into correct list a[j]
    return a[0] + a[1]  # concatenate lists
```

Code: Complete procedure

```python
def build_reverse_prefix_array(X):
    M = len(X)    # number of strings
    N = len(X[0]) # number of columns
    # column 0 of prefix array: same order as input
    a = [list(range(M))]  # [[0,1,...,M-1]]
    for k in range(N):
        a_next = build_reverse_prefix_array_column(X, k, a[k])
        a.append(a_next)
    return a
```

The Divergence Array

Reminder: LCP Array

r	pos[r]	lcp[r]	s[pos[r]..]
0	6	-1	$
1	5	0	a$
2	3	1	ana$
3	1	3	anana$
4	0	0	banana$
5	4	0	na$
6	2	2	nana$
7		-1	

```
    0123456
s = banana$
```

The **longest common prefix (LCP)** array at position `r` contains the length of the longest common prefix between the suffixes at ranks `r` and `r-1`.

The Divergence Array

Definition

$d_k[i] :=$ the smallest value j such that $y_i[j,k) = y_{i-1}[j,k)$, where $y_r := x_{a_k[r]}$.

The Divergence Array

Definition

$d_k[i] :=$ the smallest value j such that $y_i[j,k) = y_{i-1}[j,k)$, where $y_r := x_{a_k[r]}$.

Example for $k = 4$

$$\begin{array}{ccccc:cc} & 0 & 1 & 2 & 3 & k & \\ y_0 & 1 & 0 & 0 & 0 & 1 & 0 \\ y_1 & 0 & 1 & \mathbf{0} & \mathbf{0} & 0 & 1 \\ y_2 & 1 & \mathbf{1} & \mathbf{0} & \mathbf{0} & 0 & 0 \\ y_3 & 0 & 0 & 1 & \mathbf{0} & 1 & 1 \end{array}$$

$$d_k[i] = \begin{pmatrix} 4 \\ 2 \\ 1 \\ 3 \end{pmatrix}$$

Note

$d_k[0] = k$ by definition, corresponding to an "empty common string".

Common Reverse Prefix between y_i and y_j

Observation

Start of maximal match between y_i and y_j: $\max\limits_{i<m\leq j} d_k[m]$

Example

											1							
	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	k
y_0	0	0	1	1	0	0	0	0	0	1	0	**0**	0	0	0	1	0	0
y_1	0	1	0	0	0	0	0	0	**0**	1	0	**1**	0	0	0	1	0	1
y_2	0	0	1	1	0	0	0	0	**1**	1	**0**	1	0	0	0	1	0	1
y_3	**0**	0	0	1	0	0	0	0	1	0	**1**	1	0	0	0	1	0	0
y_4	**1**	0	0	1	0	0	0	0	1	0	1	1	0	0	0	1	0	0

Common Reverse Prefix between y_i and y_j

Observation

Start of maximal match between y_i and y_j: $\max_{i<m\leq j} d_k[m]$

Example

	0 1 2 3 4 5 6 7 8 9 $\overset{1}{0}$ 1 2 3 4 5 6	k
y_0	00110000010**0**00010	0
y_1	01000000**0**10**1**00010	1
y_2	00110000**1**1**0**100010	1
y_3	**0**0010000010**1**100010	0
y_4	**1**0010000101100010	0

$d_k[1] = 12$
$d_k[2] = 9$
$d_k[3] = 11$
$d_k[4] = 1$

start of maximal match of y_0 and y_4:

$$\max_{0<m\leq 4} d_k[m] = 12$$

Building d_{k+1} from d_k

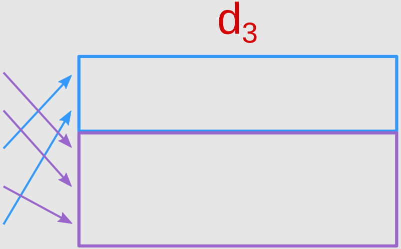
k=2

a_0		r	d_2	a_2	
0	0100	0	2	1	00|11
1	0011	1	1	2	10|10
2	1010	2	2	0	01|00
3	1101	3	0	4	01|10
4	0110	4	1	3	11|01

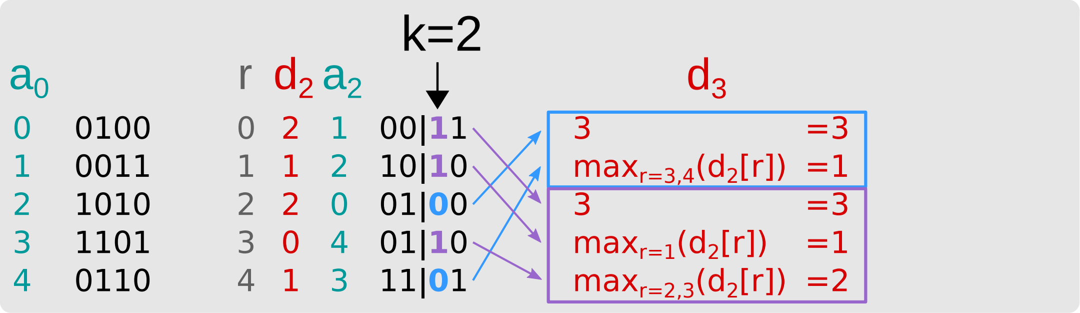
Building d_{k+1} from d_k

k=2

<table>
<tr><th>a₀</th><th></th><th>r</th><th>d₂</th><th>a₂</th><th></th></tr>
<tr><td>0</td><td>0100</td><td>0</td><td>2</td><td>1</td><td>00|11</td></tr>
<tr><td>1</td><td>0011</td><td>1</td><td>1</td><td>2</td><td>10|10</td></tr>
<tr><td>2</td><td>1010</td><td>2</td><td>2</td><td>0</td><td>01|00</td></tr>
<tr><td>3</td><td>1101</td><td>3</td><td>0</td><td>4</td><td>01|10</td></tr>
<tr><td>4</td><td>0110</td><td>4</td><td>1</td><td>3</td><td>11|01</td></tr>
</table>



Building d_{k+1} from d_k



k=2

a_0		r	d_2	a_2	
0	0100	0	2	1	00|11
1	0011	1	1	2	10|10
2	1010	2	2	0	01|00
3	1101	3	0	4	01|10
4	0110	4	1	3	11|01

d_3

$3 = 3$

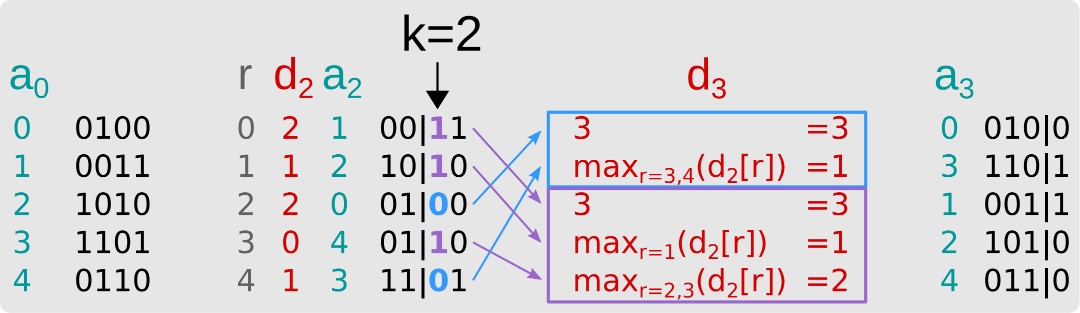
$\max_{r=3,4}(d_2[r]) = 1$

$3 = 3$

$\max_{r=1}(d_2[r]) = 1$

$\max_{r=2,3}(d_2[r]) = 2$

Building d_{k+1} from d_k



k=2

a_0	
0	0100
1	0011
2	1010
3	1101
4	0110

r	d_2	a_2	
0	2	1	00|11
1	1	2	10|10
2	2	0	01|00
3	0	4	01|10
4	1	3	11|01

d_3

3	=3
$\max_{r=3,4}(d_2[r])$	=1
3	=3
$\max_{r=1}(d_2[r])$	=1
$\max_{r=2,3}(d_2[r])$	=2

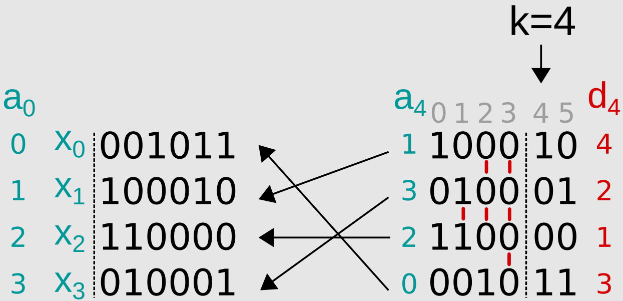
a_3	
0	010|0
3	110|1
1	001|1
2	101|0
4	011|0

Code: Efficiently Building Divergence Arrays

```python
def build_divergence_array(X, a):
  M, N = len(x), len(x[0])  # number of strings, columns
  D = [[0] * M]  # [[0,...,0]]
  for k in range(N):
    d = [ [] for _ in range(2) ]  # d[0], d[1]: empty lists
    next_d = [k+1] * 2  # [k+1, k+1]
    for r in range(M):
      i = a[k][r]  # index of string at rank r
      next_d = [max(D[k][r], next_d[q]) for q in range(2)]
      j = int(X[i][k])
      d[j].append(next_d[j])
      next_d[j] = 0
    D.append(d[0] + d[1])
  return D
```

Application:
Finding all length-L matches within X

Idea: Finding all length-L matches



k=4

a_0		
0	x_0	001011
1	x_1	100010
2	x_2	110000
3	x_3	010001

a_4	0 1 2 3	4 5	d_4
1	1000	10	4
3	0100	01	2
2	1100	00	1
0	0010	11	3

- Matches are adjacent in the reverse prefix sorting
- For each column k:
  - Iterate through d_k to find runs of matches of length L

Code: Finding all length-L matches

```
def length_L_matches(a, d, L):
    M = len(a[0])  # number of strings
    for k, (a_k, d_k) in enumerate(zip(a,d)):
        start = None
        for r in range(M):
            # match between row r-1 and r ending in column k-1?
            if d_k[r] <= k - L:
                if start is None: start = r-1
            else:
                if start is not None:
                    # yield (interval, [row indices]):
                    yield ( (k-L, k),
                      [a_k[r] for r in range(start, r)] )
                    start = None
        # any matches that include the last row?
        if start is not None:
            yield ((k-L,k), [a_k[r] for r in range(start, M)])
```

Running time: Finding all length-L matches

```
for k, (a_k, d_k) in enumerate(zip(a,d)):
  ...
  for r in range(M):
    ...
      if start is not None:
        yield (k-L, k), [a_k[r] for r in range(start, r)]
        ...
  if start is not None:
    yield (k-L,k), [a_k[r] for r in range(start, M)]
```

$O(NM + Z)$, where

- N: number of sites
- M: number of sequences in X
- Z: number of length-L matches (output size)

Summary

- MSA gives rise to **2D matrix** of (today: binary) characters
- Index structures:
  - **Positional prefix array** a_k is related to **suffix arrays**, but with reversed prefixes instead
  - **Divergence array** d_k is related to **longest common prefix arrays**, also with reversed prefixes
- Application: Length-L matches

Possible Exam questions

- How are haplotype panels related to multiple sequence alignments?
- What is a positional prefix array?
- What are commonalities/differences to a suffix array?
- Explain how to build the positional prefix arrays for all columns in $O(MN)$ time.
- How does this algorithm relate to induced sorting?
- Define the divergence array.
- What are commonalities and differences between the divergence array and the LCP array?
- How can the divergence array be constructed efficiently?
- Explain how to find all positional length-L matches in a binary matrix X.