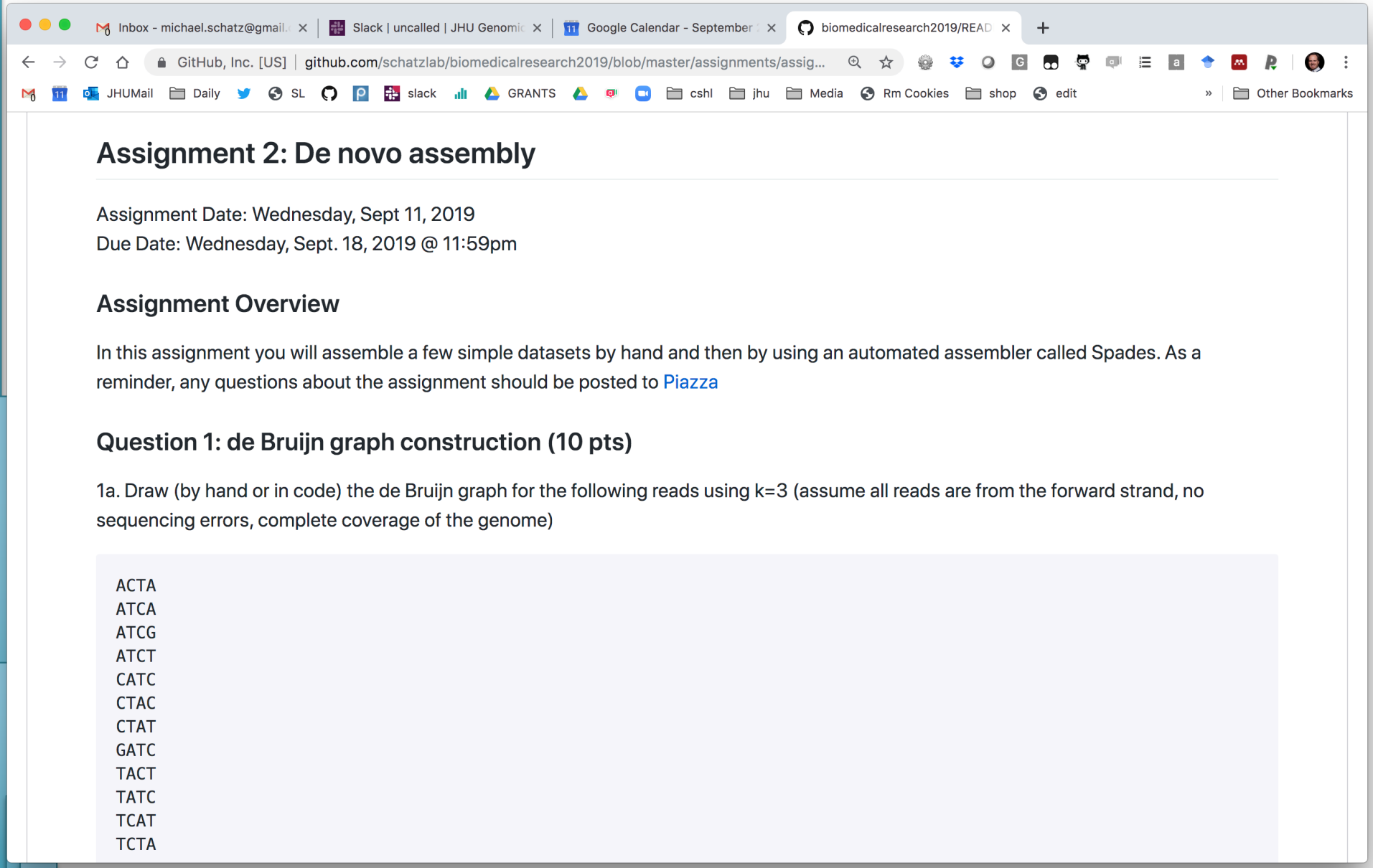
Read mapping

Michael Schatz

Sept 18, 2019

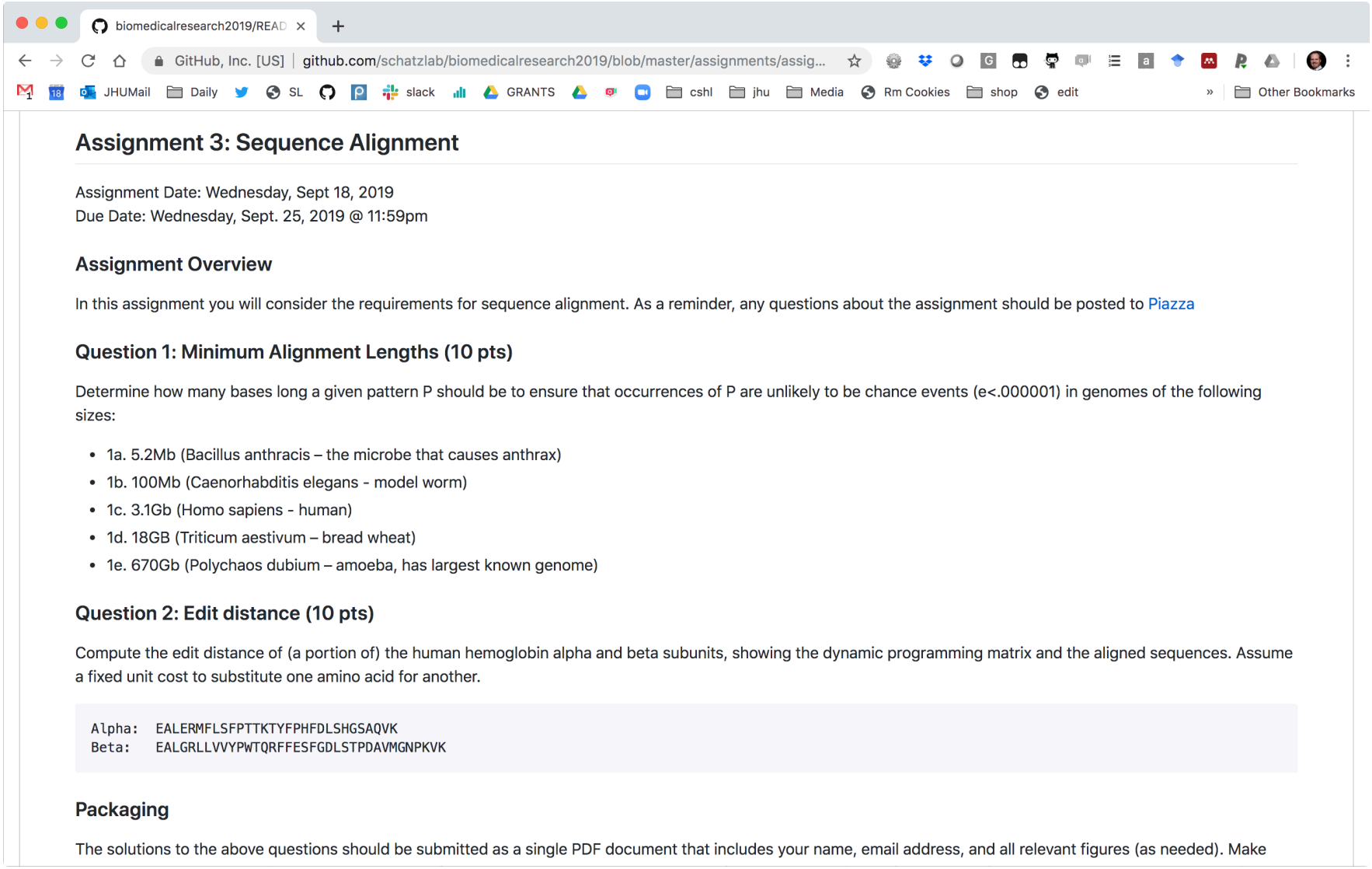
Lecture 6: Computational Biomedical Research

Assignment 2: De novo Assembly
Due Wed Sept 18 @ 11:59pm

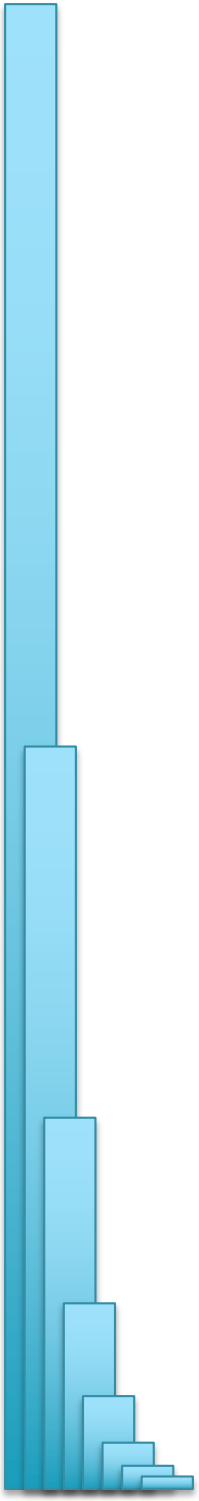


https://github.com/schatzlab/biomedicalresearch2019

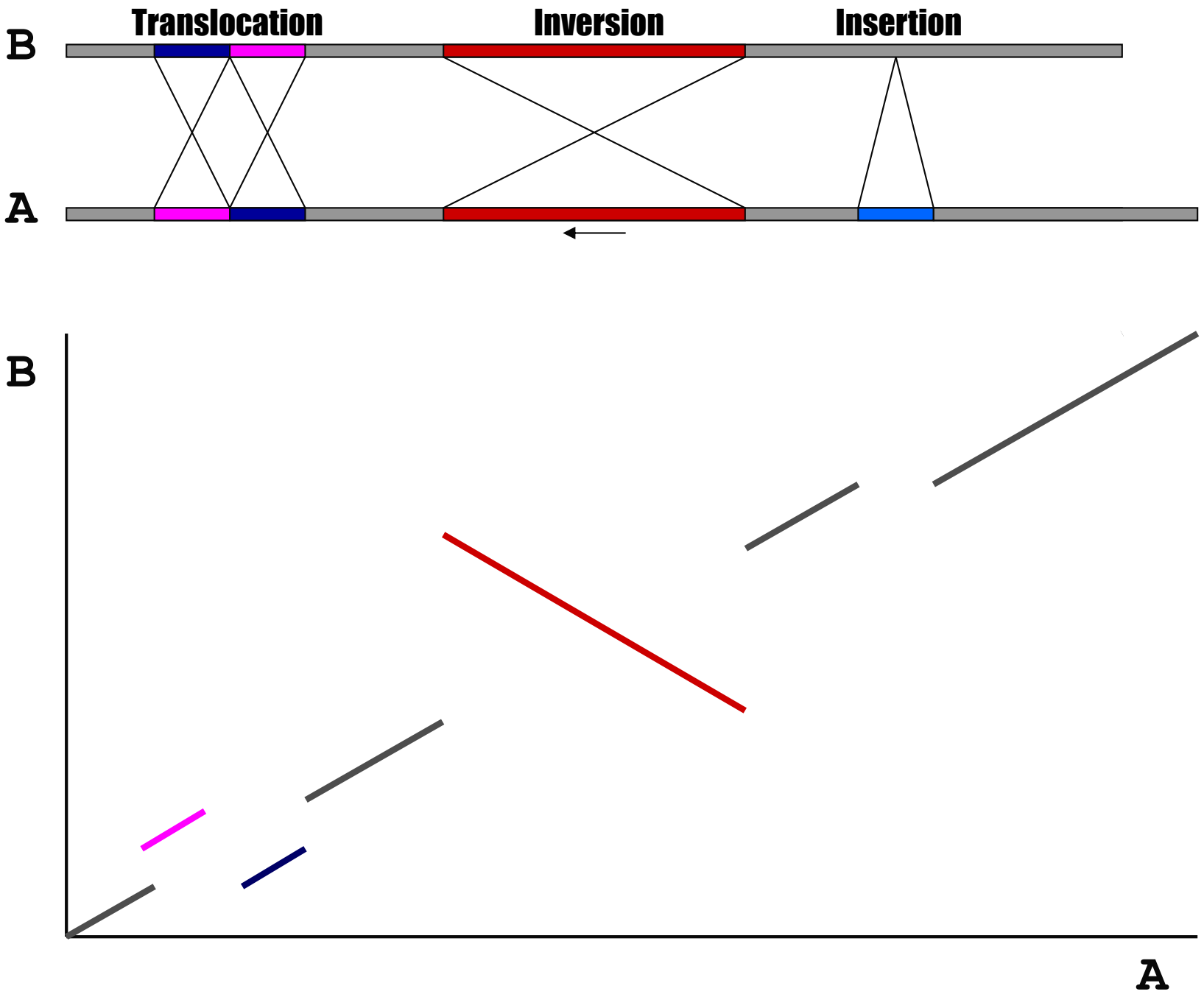
Assignment 3: Sequence Alignment
Due Monday Sept 30 @ 11:59pm

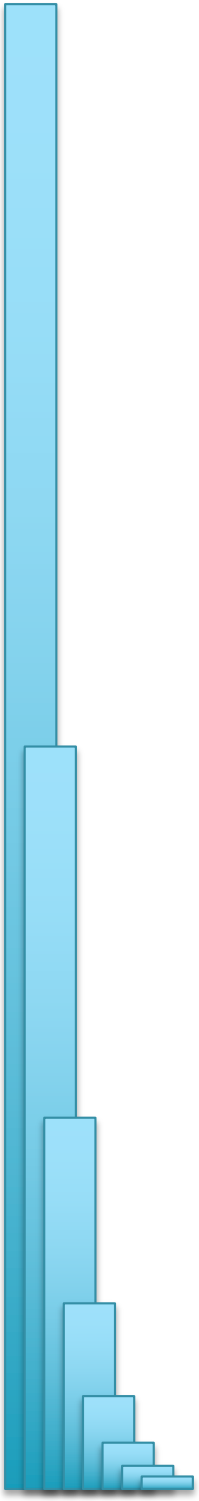


https://github.com/schatzlab/biomedicalresearch2019



Part 1: Recap

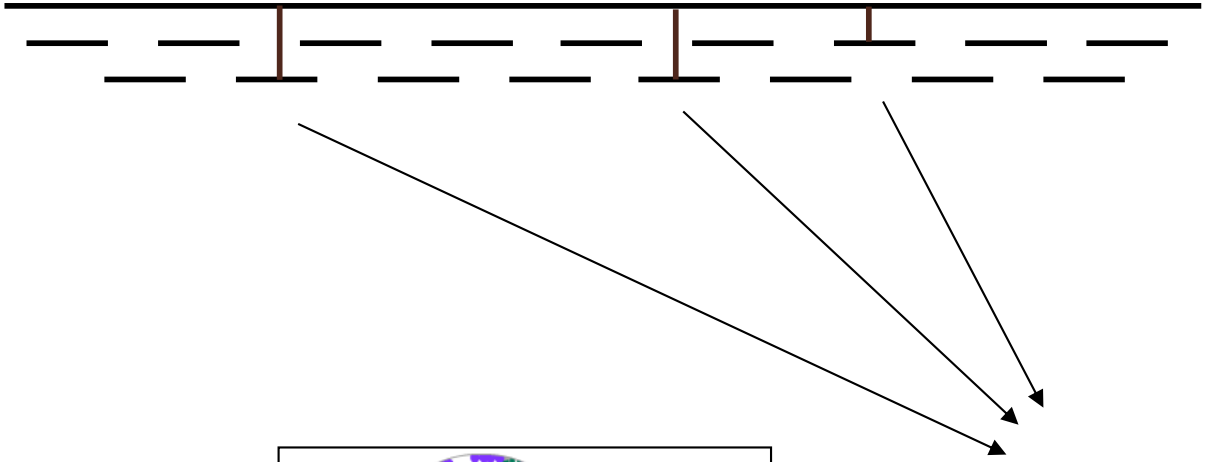

Translocation
Inversion
Insertion
B
A
B
A

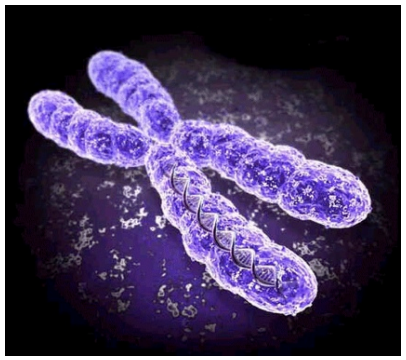


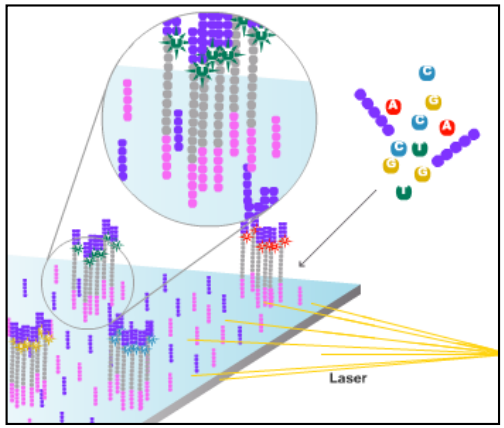
Part 2: Read Mapping

Personal Genomics

How does your genome compare to the reference?







Heart Disease

Cancer

Presidential Smile

Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
G	A	T	T	A	C	A									

No match at offset 1

Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
	G	A	T	T	A	C	A								

Match at offset 2

Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
		G	A	T	T	A	C	A	…						

No match at offset 3…

Searching for GATTACA

- Where is GATTACA in the human genome?
- Strategy 1: Brute Force

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
								G	A	T	T	A	C	A	

No match at offset 9 <- Checking each possible position takes time

Brute Force Analysis

- Brute Force:
  - At every possible offset in the genome:
    - Do all of the characters of the query match?

- Analysis
  - Simple, easy to understand
  - Genome length = n [3B]
  - Query length = m [7]
  - Comparisons: (n-m+1) * m [21B]

- Overall runtime: O(nm)

[How long would it take if we double the genome size, read length?]

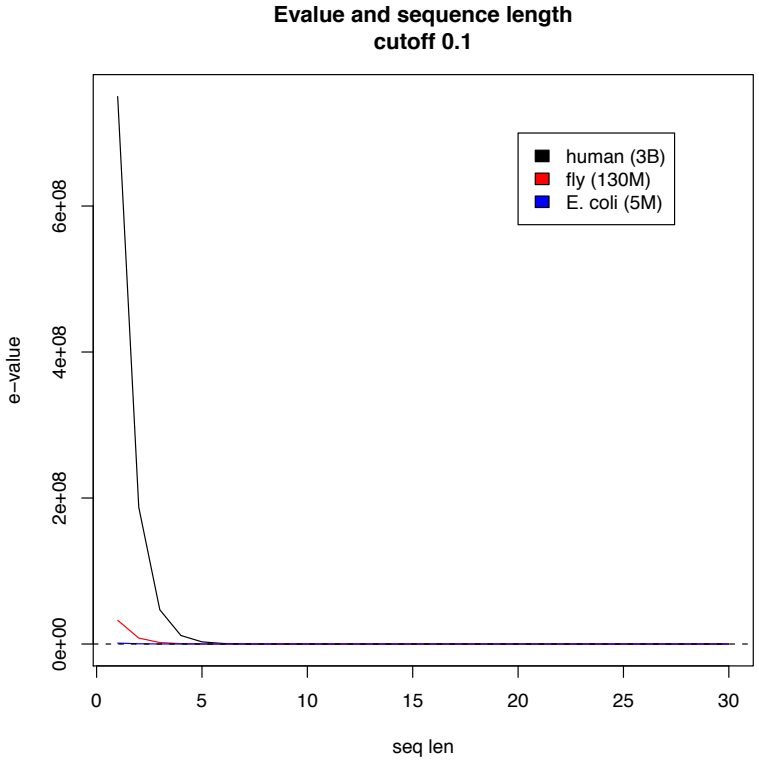
[How long would it take if we double both?]

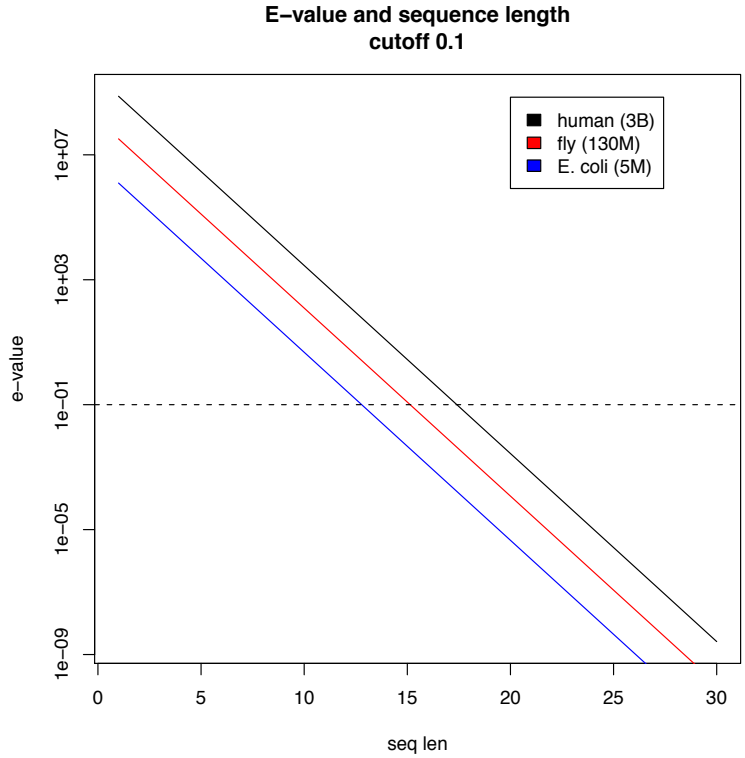
Expected Occurrences

The expected number of occurrences (e-value) of a given sequence in a genome depends on the length of the genome and inversely on the length of the sequence

- 1 in 4 bases are G, 1 in 16 positions are GA, 1 in 64 positions are GAT, …
- 1 in 16,384 should be GATTACA
- $E=n/(4^m)$ [183,105 expected occurrences]

[How long do the reads need to be for a significant match?]





Brute Force Reflections

Why check every position?

– GATTACA can't possibly start at position 15 [WHY?]

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	…
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	…
								G	A	T	T	A	C	A	

– Improve runtime to O(n + m) [3B + 7]
  - If we double both, it just takes twice as long
  - Knuth-Morris-Pratt, 1977
  - Boyer-Moyer, 1977, 1991

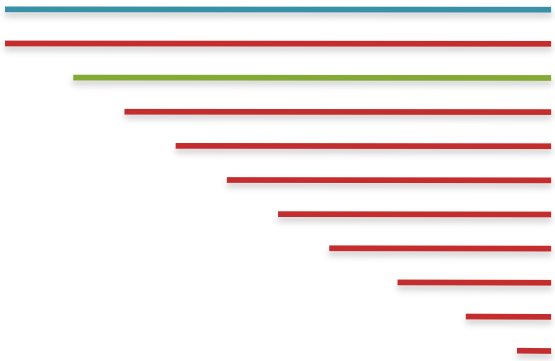
– For one-off scans, this is the best we can do (optimal performance)
  - We have to read every character of the genome, and every character of the query
  - For short queries, runtime is dominated by the length of the genome

How can we make this go faster?

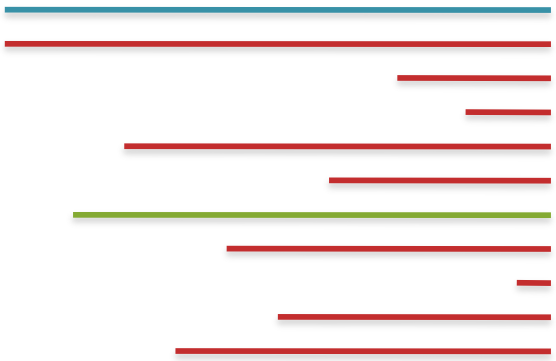
Suffix Arrays: Searching the Phone Book

- What if we need to check many queries?
  - We don't need to check every page of the phone book to find 'Schatz'
  - Sorting alphabetically lets us immediately skip 96% (25/26) of the book *without any loss in accuracy*

- Sorting the genome: Suffix Array (Manber & Myers, 1991)
  - Sort every suffix of the genome



Split into n suffixes



Sort suffixes alphabetically

[Challenge Question: How else could we split the genome?]

Searching the Index

- Strategy 2: Binary search
  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15;

Lo →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Hi →

Searching the Index

- Strategy 2: Binary search
  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC

Lo →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
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  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
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    => Higher: Lo = Mid + 1

Lo →

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11	TACAGATTACC…	5
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13	TGATTACAGATTACC…	1
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Hi →

Searching the Index

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- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15;

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo →

Hi →

Searching the Index

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  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo →

Hi →

Searching the Index

- Strategy 2: Binary search
  - Compare to the middle, refine as higher or lower

- Searching for GATTACA
  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11;

Lo →

Hi →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
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15	TTACC…	11

Searching the Index

- Strategy 2: Binary search
  - Compare to the middle, refine as higher or lower

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  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11; Mid = (9+11)/2 = 10
  - Middle = Suffix[10] = GATTACC

Lo →

Hi →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Searching the Index

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  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
  - Middle = Suffix[8] = CC
    => Higher: Lo = Mid + 1

  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11; Mid = (9+11)/2 = 10
  - Middle = Suffix[10] = GATTACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 9;

Lo →
Hi →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
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  - Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
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    => Higher: Lo = Mid + 1

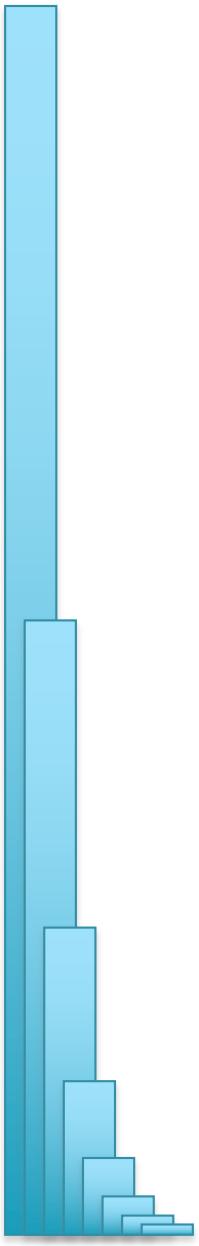
  - Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
  - Middle = Suffix[12] = TACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 11; Mid = (9+11)/2 = 10
  - Middle = Suffix[10] = GATTACC
    => Lower: Hi = Mid - 1

  - Lo = 9; Hi = 9; Mid = (9+9)/2 = 9
  - Middle = Suffix[9] = GATTACA…
    => Match at position 2!

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Binary Search Analysis



- Binary Search

  Initialize search range to entire list

  mid = (hi+lo)/2; middle = suffix[mid]

  if query matches middle: done

  else if query < middle: pick low range

  else if query > middle: pick hi range

  Repeat until done or empty range [WHEN?]

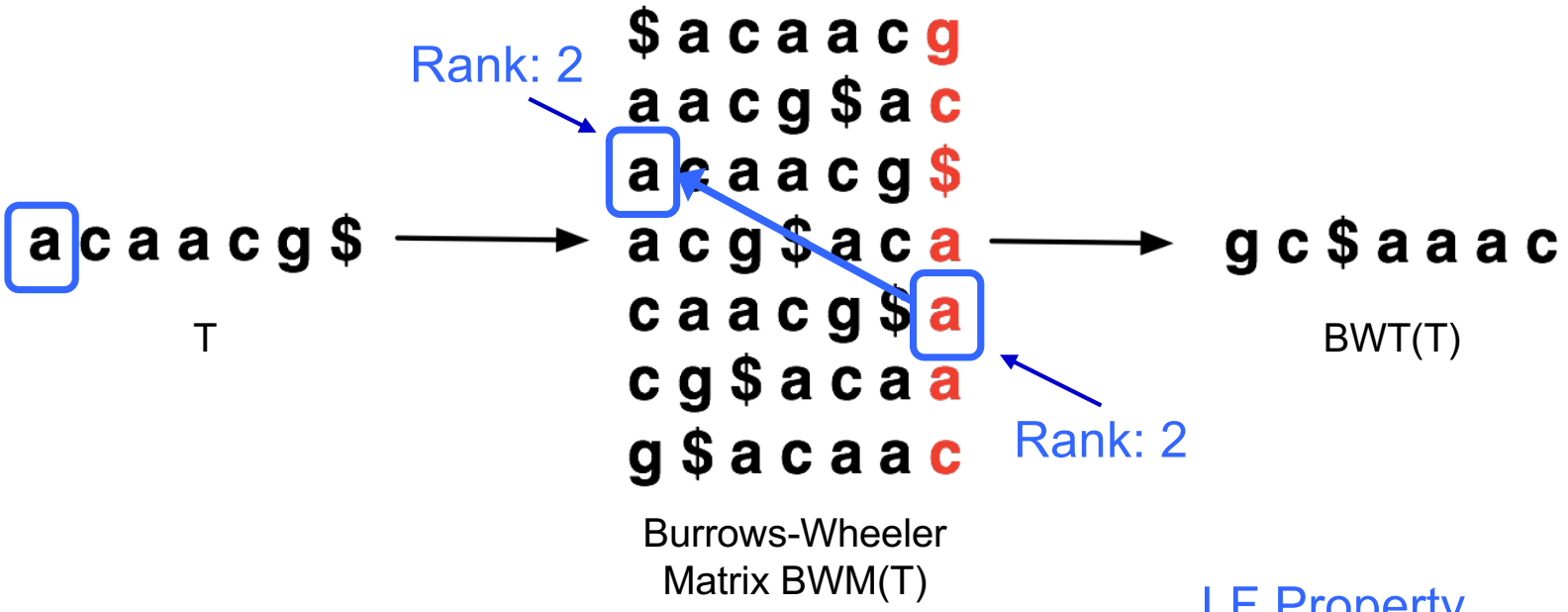
- Analysis
  - More complicated method
  - How many times do we repeat?
    - How many times can it cut the range in half?
    - Find smallest x such that: $n/(2^x) \leq 1$; $x = \lg_2(n)$ [32]

- Total Runtime: O(m lg n)
  - More complicated, but much faster!
  - Looking up a query loops 32 times instead of 3B

[How long does it take to search 6B or 24B nucleotides?]

Burrows-Wheeler Transform

- Reversible permutation of the characters in a text



LF Property implicitly encodes Suffix Array

- BWT(T) is the index for T

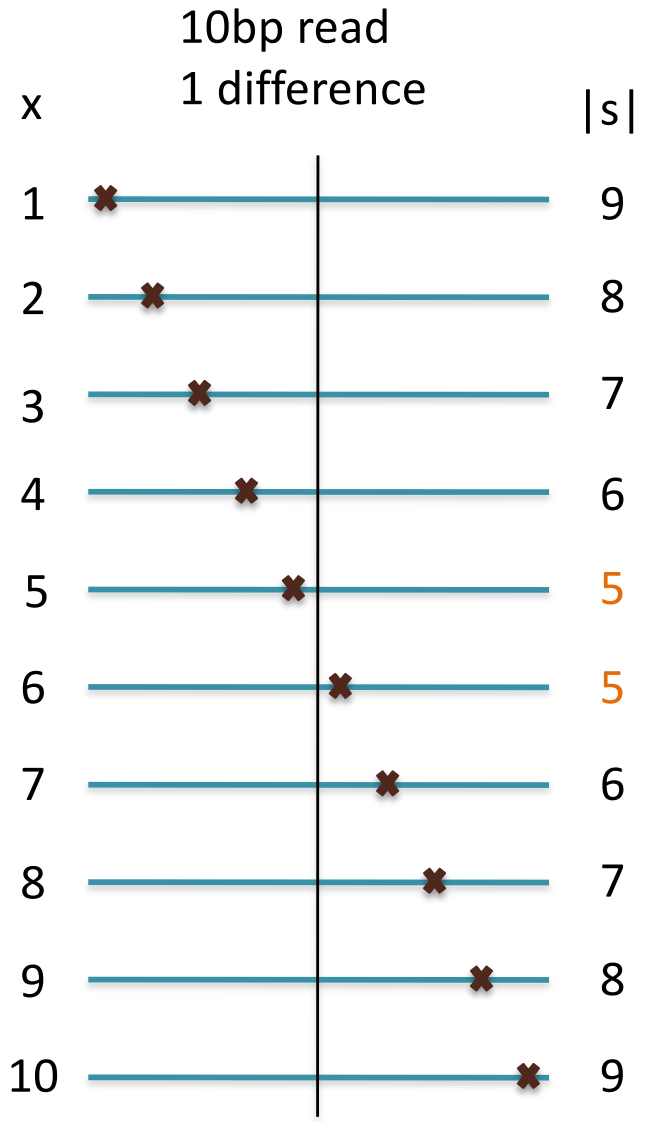
A block sorting lossless data compression algorithm.
Burrows M, Wheeler DJ (1994) *Digital Equipment Corporation.* Technical Report 124

What about in-exact alignment?

Seed-and-Extend Alignment

Theorem: An alignment of a sequence of length *m* with at most *k* differences ***must*** contain an exact match at least $s=m/(k+1)$ bp long

(*Baeza-Yates* and Perleberg, 1996)



- Proof: Pigeonhole principle
  - 1 pigeon can't fill 2 holes

- Seed-and-extend search
  - Use an index to rapidly find short exact alignments to seed longer in-exact alignments
    - BLAST, MUMmer, Bowtie, BWA, SOAP, …
  - Specificity of the depends on seed length
    - Guaranteed sensitivity for k differences
    - Also finds some (but not all) lower quality alignments <- heuristic

Similarity metrics

- Hamming distance
  - Count the number of substitutions to transform one string into another

```
MIKESCHATZ
||X||XXXX|
MICESHATZZ
     5
```

- Edit distance
  - The minimum number of substitutions, insertions, or deletions to transform one string into another

```
MIKESCHAT-Z
||X||X|||X|
MICES-HATZZ
     3
```

Edit Distance Example

AGCACACA → ACACACTA in 4 steps

AGCACACA → (1. change G to C)
ACCACACA → (2. delete C)
ACACACA → (3. change A to T)
ACACACT → (4. insert A after T)
ACACACTA → done

[Is this the best we can do?]

Edit Distance Example

AGCACACA → ACACACTA in 3 steps

AGCACACA → (1. change G to C)
ACCACACA → (2. delete C)
ACACACA → (3. insert T after 3rd C)
ACACACTA → done

[Is this the best we can do?]

Reverse Engineering Edit Distance

D(AGCACACA, ACACACTA) = ?

Imagine we already have the optimal alignment of the strings, the last column can only be 1 of 3 options:

…**M**	…**I**	…**D**
…A	…-	…A
…A	…A	…-

The optimal alignment of last two columns is then 1 of 9 possibilities

…**MM**	…**IM**	…**DM**	…**MI**	…**II**	…**DI**	…**MD**	…**ID**	…**DD**
…CA	…-A	…CA	…A-	…--	…A-	…CA	…-A	…CA
…TA	…TA	…-A	…TA	…TA	…-A	…A-	…A-	…--

The optimal alignment of the last three columns is then 1 of 27 possibilities…

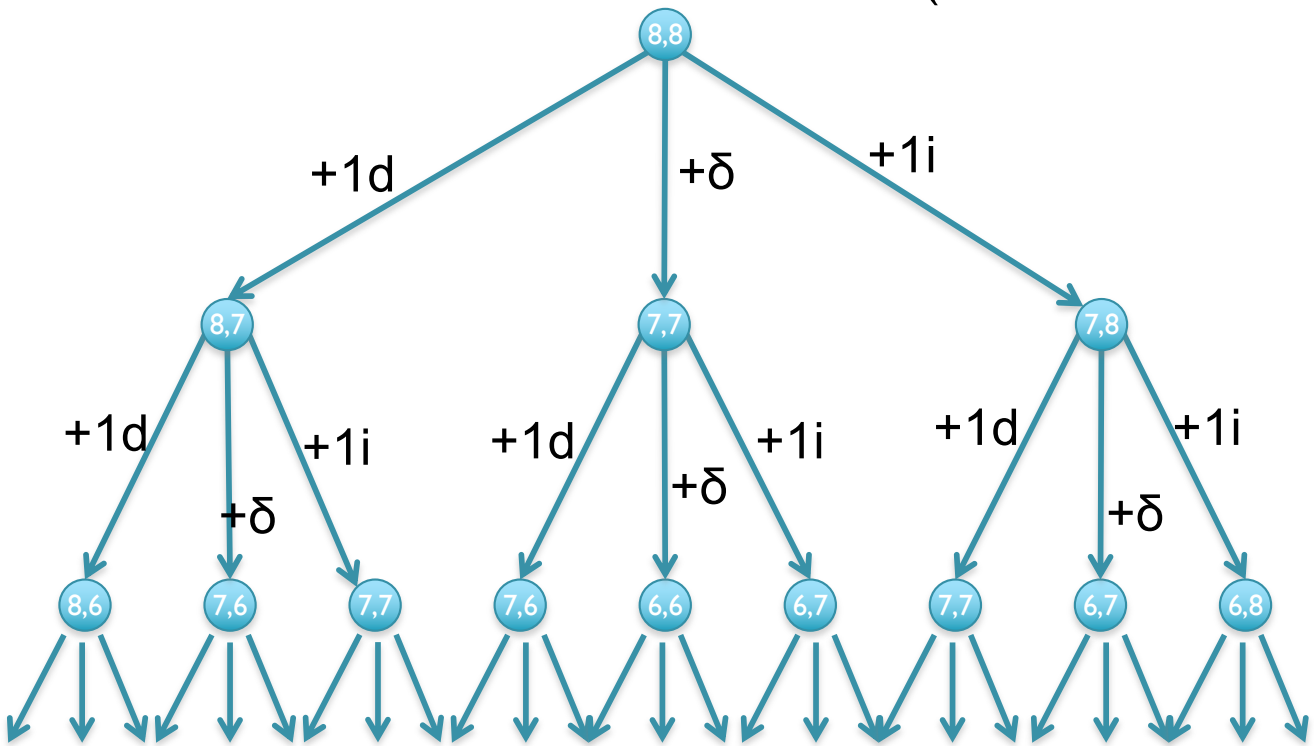
…**M**…	…**I**…	…**D**…
…X…	…-…	…X…
…Y…	…Y…	…-…

Eventually spell out every possible sequence of {I,M,D}

Recursive solution

- Computation of D is a recursive process.
  - At each step, we only allow matches, substitutions, and indels
  - D(i,j) in terms of D(i', j') for i' ≤ i and j' ≤ j.

D(AGCACACA, ACACACTA) = min{D(AGCACACA, ACACACT) + 1,
D(AGCACAC, ACACACTA) + 1,
D(AGCACAC, ACACACT) +δ(A, A)}



[What is the running time?]

Dynamic Programming

- We could code this as a recursive function call...

  ...with an exponential number of function evaluations

- There are only (n+1)x(m+1) pairs i and j
  - We are evaluating D(i,j) multiple times

- Compute D(i,j) bottom up.
  - Start with smallest (i,j) = (1,1).
  - Store the intermediate results in a table.
    - Compute D(i,j) *after* D(i-1,j), D(i,j-1), and D(i-1,j-1)

Recurrence Relation for D

Find the edit distance (minimum number of operations to convert one string into another) in O(mn) time

•Base conditions:

- D(i,0) = i, for all i = 0,...,n
- D(0,j) = j, for all j = 0,...,m

•For i > 0, j > 0:

$$
\begin{aligned}
D(i,j) = \min \{ & \\
& D(i-1,j) + 1, && \text{// align 0 chars from S, 1 from T} \\
& D(i,j-1) + 1, && \text{// align 1 chars from S, 0 from T} \\
& D(i-1,j-1) + \delta(S(i),T(j)) && \text{// align 1+1 chars} \\
\} &
\end{aligned}
$$

[Why do we want the min?]

Dynamic Programming Matrix

		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1								
G	2								
C	3								
A	4								
C	5								
A	6								
C	7								
A	8								

[What does the initialization mean?]

Dynamic Programming Matrix

		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1	0							
G	2								
C	3								
A	4								
C	5								
A	6								
C	7								
A	8								

$$D[A,A] = \min\{D[A,]+1,\ D[,A]+1,\ D[,]+\delta(A,A)\}$$

Dynamic Programming Matrix

		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1	0	1						
G	2								
C	3								
A	4								
C	5								
A	6								
C	7								
A	8								

$$D[A,AC] = \min\{D[A,A]+1,\ D[,AC]+1,\ D[,A]+\delta(A,C)\}$$

Dynamic Programming Matrix

		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1	0	1	2					
G	2								
C	3								
A	4								
C	5								
A	6								
C	7								
A	8								

D[A,ACA] = min{D[A,AC]+1, D[,ACA]+1, D[,AC]+δ(A,A)}

Dynamic Programming Matrix

		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1	0	1	2	3	4	5	6	7
G	2								
C	3								
A	4								
C	5								
A	6								
C	7								
A	8								

D[A,ACACACTA] = 7

```
-------A
*******|
ACACACTA
```

[What about the other A?]

Dynamic Programming Matrix

		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1	0	1	2	3	4	5	6	7
G	2	1	1	2	3	4	5	6	7
C	3								
A	4								
C	5								
A	6								
C	7								
A	8								

D[AG,ACACACTA] = 7

```
----AG--
****|***
ACACACTA
```

Dynamic Programming Matrix

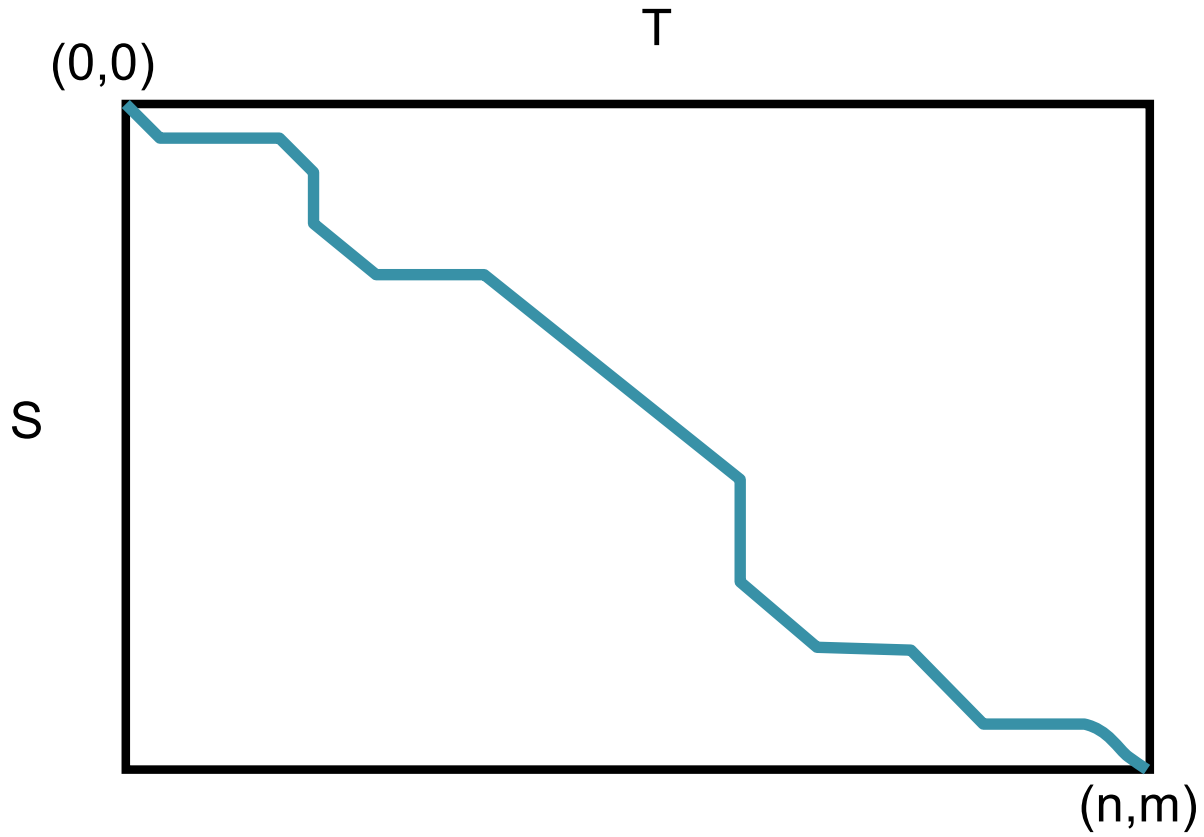
		A	C	A	C	A	C	T	A
	0	1	2	3	4	5	6	7	8
A	1	0	1	2	3	4	5	6	7
G	2	1	1	2	3	4	5	6	7
C	3	2	1	2	2	3	4	5	6
A	4	3	2	1	2	2	3	4	5
C	5	4	3	2	1	2	2	3	4
A	6	5	4	3	2	1	2	3	3
C	7	6	5	4	3	2	1	2	3
A	8	7	6	5	4	3	2	2	2

D[AGCACACA,ACACACTA] = 2

```
AGCACAC-A
| * | | | | | * |
A-CACACTA
```

[Can we do it any better?]

Global Alignment Schematic



- A high quality alignment will stay close to the diagonal
  - If we are only interested in high quality alignments, we can skip filling in cells that can't possibly lead to a high quality alignment
  - Find the global alignment with at most edit distance d: O(2dn)

Alignment Ambiguity

Notice that the edit distance of GATTTACA and GATACA is 2,
but there are multiple possible optimal alignments:

```
GATTTACA      GATTTACA      GATTTACA
GAT--ACA      GA-T-ACA      GA--TACA
```

Which one is right?

Algorithm Overview

1. Split read into segments

Read

CCAGTAGCTCTCAGCCTTATTTTACCCAGGCCTGTA

Read (reverse complement)

TACAGGCCTGGGTAAAATAAGGCTGAGAGCTACTGG

Policy: extract 16 nt seed every 10 nt

Seeds

+, 0: CCAGTAGCTCTCAGCC

+, 10: TCAGCCTTATTTTACC

+, 20: TTTACCCAGGCCTGTA

-, 0: TACAGGCCTGGGTAAA

-, 10: GGTAAAATAAGGCTGA

-, 20: GGCTGAGAGCTACTGG

2. Lookup each segment and prioritize

Seeds

+, 0: CCAGTAGCTCTCAGCC

+, 10: TCAGCCTTATTTTACC

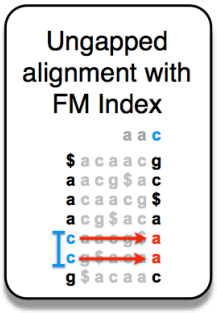
+, 20: TTTACCCAGGCCTGTA

-, 0: TACAGGCCTGGGTAAA

-, 10: GGTAAAATAAGGCTGA

-, 20: GGCTGAGAGCTACTGG

→



→

Seed alignments (as B ranges)

{ [211, 212], [212, 214] }

{ [653, 654], [651, 653] }

{ [684, 685] }

{ }

{ }

{ [624, 625] }

3. Evaluate end-to-end match

Extension candidates

SA:684, chr12:1955

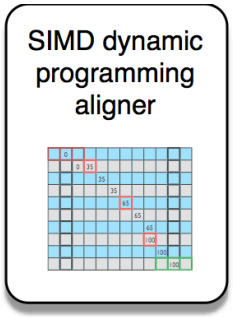
SA:624, chr2:462

SA:211: chr4:762

SA:213: chr12:1935

SA:652: chr12:1945

→



→

SAM alignments

```
r1   0    chr12    1936     0
     36M  *    0    0
     CCAGTAGCTCTCAGCCTTATTTTACCCAGGCCTGTA
     IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
     AS:i:0    XS:i:-2   XN:i:0
     XM:i:0    XO:i:0    XG:i:0
     NM:i:0    MD:Z:36   YT:Z:UU
     YM:i:0
...
```

(Langmead & Salzberg, 2012)

Next Steps

1. Reflect on the magic and power of DNA ☺
2. Check out the course webpage
3. Submit HW2
4. Work on HW3