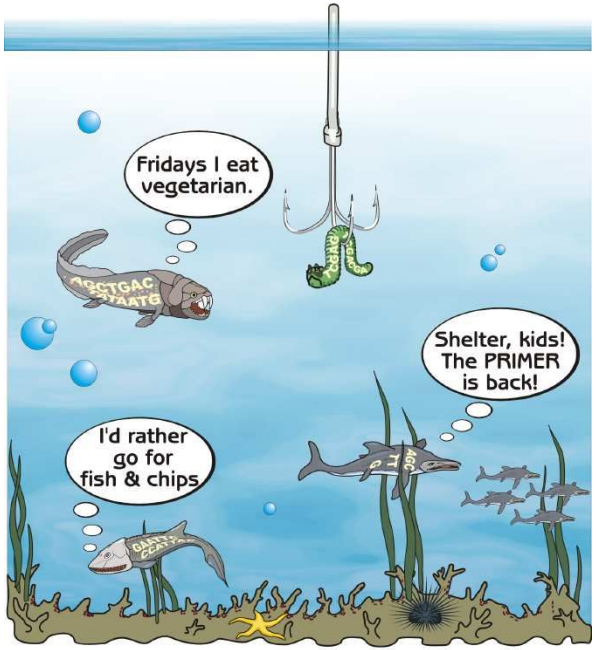
BCB 716 - Sequence Analysis



"The Art of Gene Fishing"

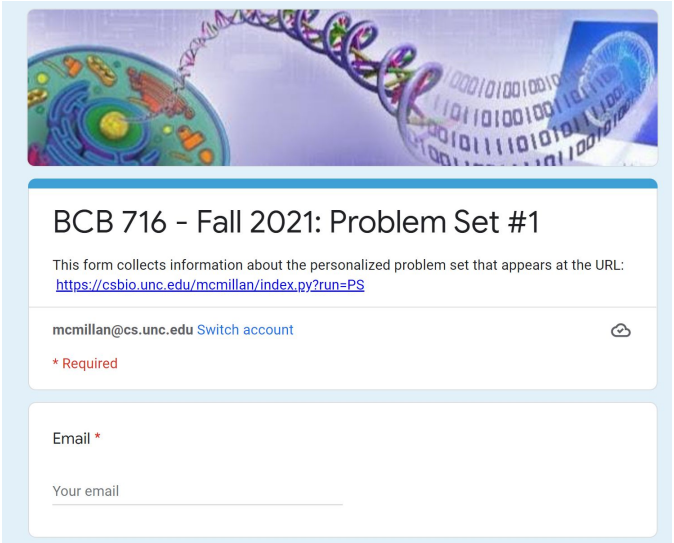
- Problem Set #1
- Problem Set #2
- On Thursday Problem Set #3
- Final exam:
  Taken in 90 mins
  Anytime from 5pm 12/1 to 5pm 12/3

Reference Free DNA Sequence Analysis

Problem Set #1

Google form at:

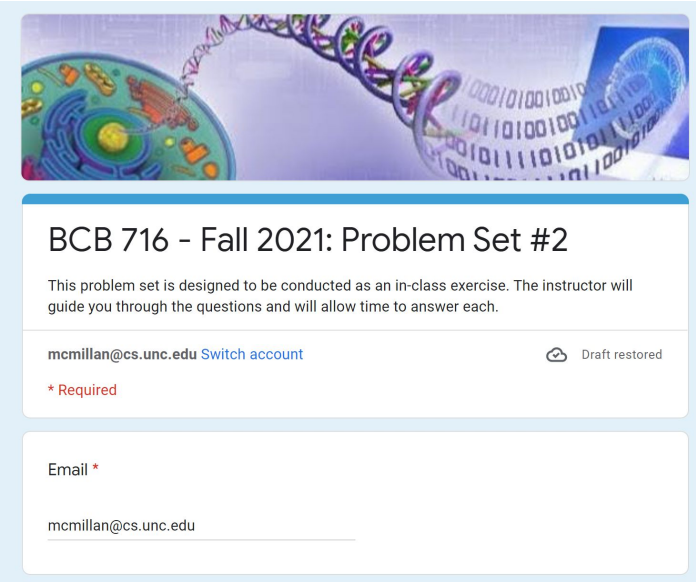
https://forms.gle/9tPueV2Tx9Z7uM7t5



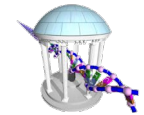
Problem Set #2

Google form at:

https://forms.gle/QVb3wQA7gjLRFxkK8

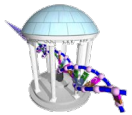


Reference Sequences Introduce bias



- **Relying on a reference sequence makes many assumptions**
  - All samples are assumed to be similarly organized
  - The "reference" allele is assumed to be common when sometimes it isn't
  - The reference is missing things
- **How do you overcome this problem?**
  - Consensus? Depends on population
  - Many references?

Novel idea

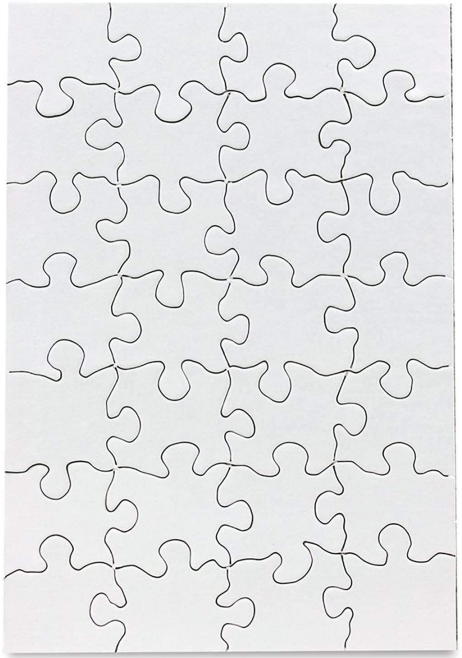


Rather than aligning reads to a reference, why not align them to each other. Not quite an assembly, but just enough to search for all and any sequence substrings.

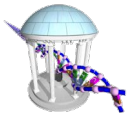
Like assembling a jigsaw puzzle without an image of the assembled version. Or with no image at all!

Let's align every read with all other reads.

Rather than index a reference genome for searching, we build a BWT index of every read.



MSBWT



A BWT of a ***string collection*** instead of just a single string

- Earliest: Mantaci et al. (2005), used concatenation approach
- Bauer et al. (2011) - proposed version we will discuss today

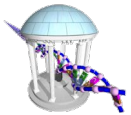
Analogy:

- Instead of searching for a substring within a single book, search every book of a library
  - Each book has its own text, suffix array, and end-of-text delimiter
  - Searching allows us to find how many times a substring appears and in which texts

Bioinformatics?

- Search all genomes? You could, but that's not the main application.
- Search multiple chromosomes of an organism? You should, but even that is not the killer app

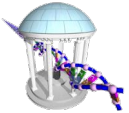
Naive Construction



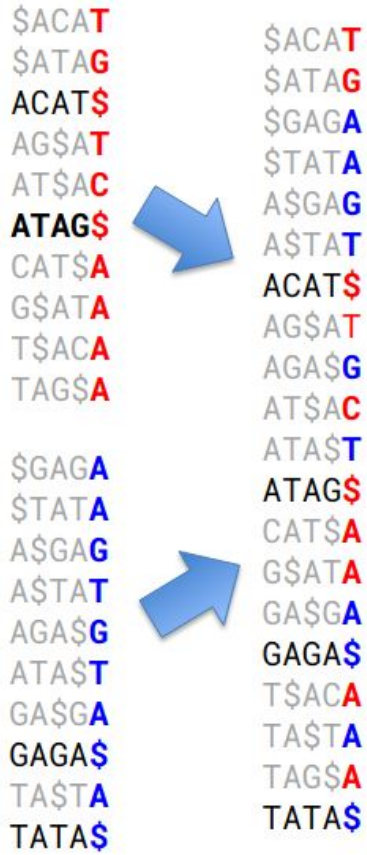
- Create all rotations for all strings in the collection
- Sort all rotations together (Suffix Array)
- Store the predecessor of each suffix
- Strings are “cyclic”
- The predecessor is always from the same string
- Impossible to “jump” from one string to another
- Strings can have different lengths

String1	Sorted	MSBWT
ACCA$	$ACCA	A
CCA$A	$CAAA	A
CA$AC	A$ACC	C
A$ACC	A$CAA	A
$ACCA	AA$CA	A
	AAA$C	C
String2	ACCA$	$
CAAA$	CA$AC	C
AAA$C	CAAA$	$
AA$CA	CCA$A	A
A$CAA		
$CAAA		
(Unsorted suffixes)	(Merged and sorted)	(multi-string BWT Note the 2 '$'s)

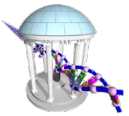
Merging msBWTs



- BETTER YET! Rather than inserting new strings, build a BWT of the new strings and merge the new and old BWTs
- Suffixes of BTWs are already sorted
- BTWs are interleaved
- In the worse case (ties) the entire suffix must be considered, but generally the longest common prefix of suffixes is smaller
- Minimal overhead
- Well suited for divide an conquer approaches (like merge sort)
- Easy to merge multiple data sets!
- Compression improves!



K-mer Search & Read Extraction



Basic Use:

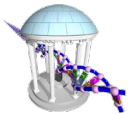
CC MRCAs: CC010_MRCA_2020
730,665,362 strings with 110,330,469,662 bases and index size of 14,976,208,773 bytes (1.09 bits per base)
Target: CTTGTCCTTGGAAGGAAGATATATG
Found 27 times (15 forward, 12 reverse-complemented)

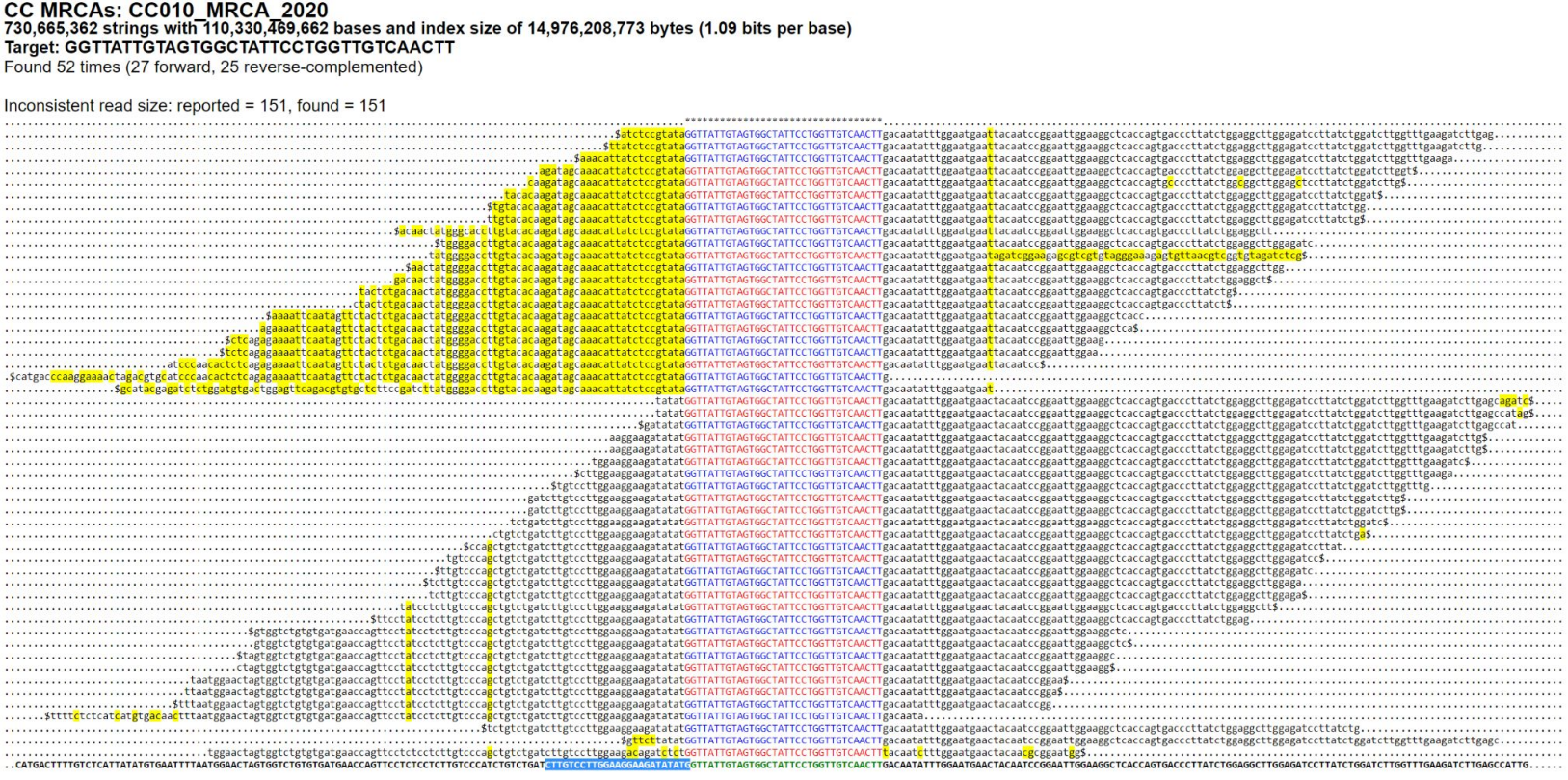
Inconsistent read size: reported = 151, found = 151

Green: query k-mer. Red: forward reads. Blue: reverse-complement reads. Yellow: sequencing errors

- Search for all reads with a given k-mer
- Extract all reads with that k-mer and its reverse-complement
- Build a consensus

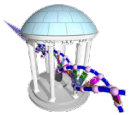
Moving over a little bit



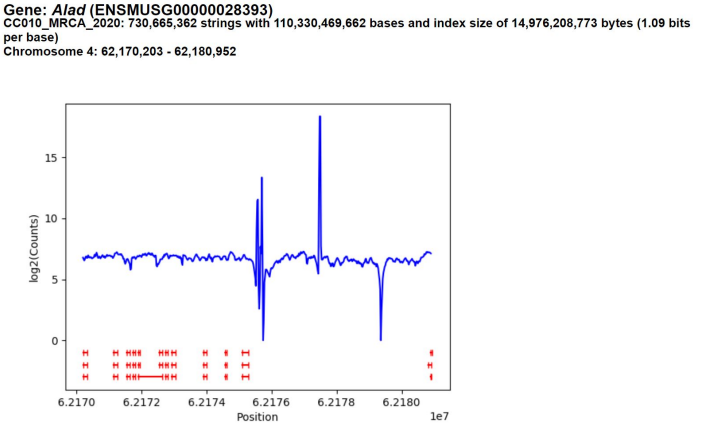


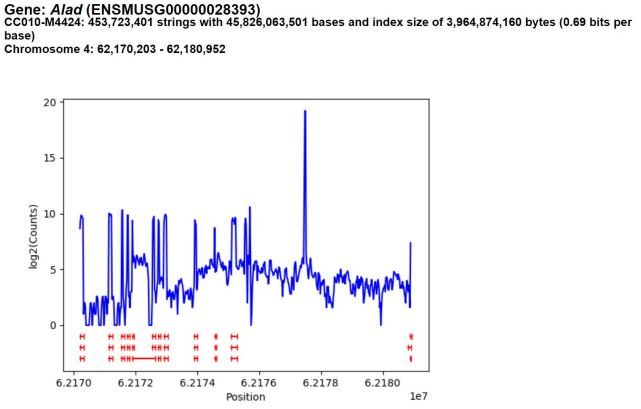
Moving over 24 bases shows a different story

Reference-based Searches

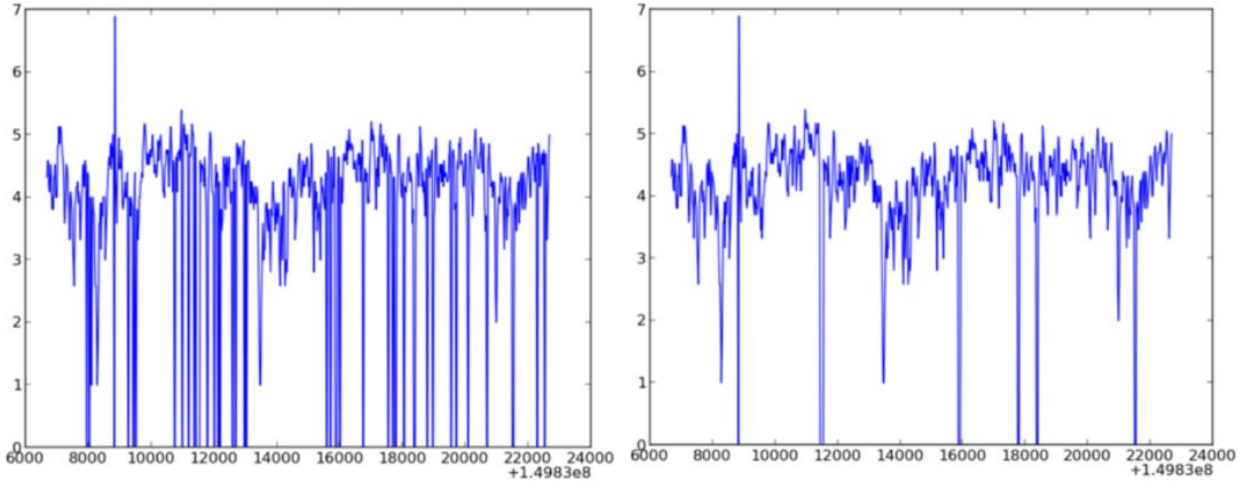


- Rather than align reads to a reference search for reference k-mers in the read dataset.
- Given a reference genome and region
- Split reference into overlapping k-mers
- Count the abundance of each k-mer and plot
- Fast - O(k) time per k-mer
- With preprocessing (sorting k-mers in suffix order) it can be even faster approaching O(1) for many k-mers
- Similar to an alignment pileup





Iterative Reference Correction



CAST/EiJ DNA-seq for annotated gene *Igf2*

Next Time

Reference-free RNAseq Analysis Approaches