- Problem set #2 is due tonight
- Problem set #3 should be posted by tonight
- Midterm is set for March 5

Multi-String BWTs
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

<table>
<thead>
<tr>
<th>String1</th>
<th>Sorted</th>
<th>MSBWT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCA$</td>
<td>$ACCA</td>
<td>A</td>
</tr>
<tr>
<td>CCA$A</td>
<td>$CAAA</td>
<td>A</td>
</tr>
<tr>
<td>CA$AC</td>
<td>A$ACC</td>
<td>C</td>
</tr>
<tr>
<td>A$ACC</td>
<td>A$CAA</td>
<td>A</td>
</tr>
<tr>
<td>$ACCA</td>
<td>AA$CA</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>AAA$C</td>
<td>C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>String2</th>
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<th>MSBWT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAAA$</td>
<td>ACCA$</td>
<td>$</td>
</tr>
<tr>
<td></td>
<td>CA$AC</td>
<td>C</td>
</tr>
<tr>
<td>AAA$C</td>
<td>CAAA$</td>
<td>$</td>
</tr>
<tr>
<td>AA$CA</td>
<td>CCA$A</td>
<td>A</td>
</tr>
<tr>
<td>A$CAA</td>
<td>$</td>
<td></td>
</tr>
<tr>
<td>$CAAAA</td>
<td></td>
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</tr>
</tbody>
</table>
MSBWT's FM-index

Identical Definition

- Find k-mer “CA”
- Initialize to full range ("")
- \( \text{lo, hi} = 0, 10 \)
- Find occurrences of 'A'
  - \( \text{lo} = \text{Offset}'A' + \text{FMindex}[\text{lo}]'A' = 2 + 0 = 2 \)
  - \( \text{hi} = \text{Offset}'A' + \text{FMindex}[\text{hi}]'A' = 2 + 5 = 7 \)
- Find occurrences of “CA”
  - \( \text{lo} = \text{Offset}'C' + \text{FMindex}[\text{lo}]'C' = 7 + 0 = 7 \)
  - \( \text{hi} = \text{Offset}'C' + \text{FMindex}[\text{hi}]'C' = 7 + 2 = 9 \)
- Searching and extracting suffixes are identical to a BWT
Incremental MSBWT Construction

- A key tool missing from the BWTs toolbox—adding new strings to an existing msBWT
- You could reconstruct the suffix array of the msBWT using $\text{suffix}(i, \text{fmindex})$ for all $i$, and then insert the suffixes of the new string.
- Variant of find(); Find the insertion point of new string's $j^{th}$ suffix, $s_j$
- Add last character to msBWT
- Update the FMindex
Our original BWT code

```python
In [0]: def FMIndex(bwt):
    fm = [(c: 0 for c in bwt)]
    for c in bwt:
        row = {symbol: count + 1 if (symbol == c) else count for symbol, count in fm[-1].items()}
        fm.append(row)
    offset = {}  
    N = 0
    for symbol in sorted(row.keys()):
        offset[symbol] = N
        N += row[symbol]
    return fm, offset

def recoverSuffix(i, BWT, FMIndex, Offset):
    suffix = ''
    c = BWT[i]
    predec = Offset[c] + FMIndex[i][c]
    suffix = c + suffix
    while (predec != i):
        c = BWT[predec]
        predec = Offset[c] + FMIndex[predec][c]
        suffix = c + suffix
    return suffix

def findBWT(pattern, FMIndex, Offset):
    lo = 0
    hi = len(FMIndex) - 1
    for symbol in reversed(pattern):
        lo = Offset[symbol] + FMIndex[lo][symbol]
        hi = Offset[symbol] + FMIndex[hi][symbol]
    return lo, hi
```
Inserting a new BWT into an existing msBWT

```python
In [9]:
# Constructing a multistring BWT one suffix at a time
# first let's take a look at the implicit suffix array
bwt = "TGAG$TGC$AAASAA"
fm, off = FMIndex(bwt)
for i in range(len(bwt)):
    print("%2d: %s" % (i, recoverSuffix(i, bwt, fm, off)))
print()

# New string to include
new = "TATA$"
inserts = []
for i in range(len(new)):
    rotation = new[i:] + new[:i]
    l, h = findBWT(rotation, fm, off)
    inserts.append((h, new[i-1], rotation))
print(inserts)

# Insert into original BWT in reverse order
for i, c, rot in sorted(inserts, reverse=True):
    bwt = bwt[:i] + c + bwt[i:]

# Look at result
print(bwt)
print()
fm, off = FMIndex(bwt)
for i in range(len(bwt)):
    print("%2d: %s" % (i, recoverSuffix(i, bwt, fm, off)))
```

0: $ACAT
1: $ATAG
2: $GAGA
3: A$GAG
4: ACAT$
5: AG$AT
6: AGA$G
7: AT$AC
8: ATAG$
9: CAT$A
10: G$ATA
11: GA$GA
12: GAGA$
13: T$ACA
14: TAG$A
[(15, 'S', 'TATA$'), (8, 'T', 'ATA$T'), (14, 'A', 'TA$TA'), (4, 'T', 'A$TAT'), (3, 'A', 'TATA$)]

TGAAGT$TGCT$AAA$AAA$

0: $ACAT
1: $ATAG
2: $GAGA
3: $GAGA
4: ACAT$
5: AG$AT
6: AG$G
7: AT$AC
8: ATAG$
9: CAT$A
10: G$ATA
11: GA$GA
12: GAGA$
13: T$ACA
14: TAG$A
15: GAGA$
16: T$ACA
17: TA$TA
18: TAG$A
19: TATA$

That was a little tricky...
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 general 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!
Merging Steps

msBWT merging alternates between sorting and interleaving

1. Consider the BWTs as a tuple of (character, BWTid) pairs
2. Sort these tuples
3. Based on the BWTids after the sort, select a new character
   For each tuple from the original msBWTs
4. Repeat from Step 2 until the sort is stable
5. The resulting characters are the merged msBWT
6. Number of passes is proportional to largest LCP value.
In [12]:

    def mergeBWT(bwt1, bwt2):
        interleave = [(c, 0) for c in bwt1] + [(c, 1) for c in bwt2]
        passes = min(len(bwt1), len(bwt2))
        for p in range(passes):
            i, j = 0, 0
            nextInterleave = []
            for c, k in sorted(interleave, key=lambda x: x[0]):
                if (k == 0):
                    b = bwt1[i]
                    i += 1
                else:
                    b = bwt2[j]
                    j += 1
                nextInterleave.append((b, k))
                if (nextInterleave == interleave):
                    break
        interleave = nextInterleave
        return ''.join([c for c, k in interleave])

bwt1 = "TG$TCS$AAAA"
bwt2 = "AAGTGTASA$"
bwt12 = mergeBWT(bwt1, bwt2)
print(bwt12)
FM, Offset = FMIndex(bwt12)
for i in range(len(bwt12)):
    j = (i>>2)+(i&3)*(len(bwt12)//4)
print("%d: %s" % (j, recoverSuffix(j, bwt12, FM, Offset)), "\n" if (i % 4 == 3) else "", end='"')

TGAAGT$T6CT$AAA$AAS
0: $ACAT 5: ASTAT 10: AT$TA 15: GAGA$
1: $ATAG 6: ACAT$ 11: ATAG$ 16: T$ACA
2: $GAGA 7: AG$AT 12: CAT$A 17: TA$TA
3: $TATA 8: AGASA 13: GSATA 18: TAGSA
4: $AGAG 9: AT$AC 14: GA$GA 19: TATA$
MSBWT Applications

- Instead of building a BWT of a reference genome, build a MSBWT of every sequenced reads
- Arbitrary exact-match k-mer queries
- $O(k)$ time
- Enables fast searches/counting
- Recover an arbitrary read of length $L$ from MSBWT
- $O(L)$ time
- Enables extraction of user-selected reads
Compression of high-throughput sequencing

- Using Run-length encoding again
- Reasons we expect compression:
  - True genomic repeats: gene families, long repeats, etc.
  - Over-sampling: 30x coverage means we expect 30 copies of every k-mer pattern
- Sequencing errors may break up runs
- Technical errors may cause biases for or against a particular pattern
- Real Mouse DNA-seq:
  - 368654191 × 151 × 2 = ~112 Giga-bases
  - Compresses to ~15.3 GB using RLE (1.09 bits/base)
- Real Mouse RNA-seq:
  - ~8.9 Giga-bases
  - ~1.2 GB using RLE (1.05 bits/base)
K-mer Search & Read Extraction

Basic Use:

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

Comp 555 - Spring 2020
Moving over 12 bases shows a different story

CC Genome: CC001M4363 UNC NYGC
799,869,982 strings with 121,580,237,264 bases and index size of 16,944,071,172 bytes (1.11 bits per base)
Target: CGAATTCTGAAAAACGTGAAAAATGAGAAATGCACCTGCGAGACCTGG

Found 46 times (25 forward, 21 reverse-complemented)
Reference-based Searches

- Given a reference genome and region of that genome
- Split reference into k-mers
- Count the abundance of each k-mer and plot
- Fast - $O(k)$ time per k-mer
- Similar to a post-alignment pileup
Iterative Reference Correction

CAST/EiJ DNA-seq for annotated gene Igf2
Summary

- Burrows-Wheeler Transform
  - Permutation of characters that represents a suffix array
  - Run-length encoded for compression

- FM-index
  - Derived from BWT
  - Exploits LF-mapping property
  - O(k) search time for arbitrary k-mer, independent of BWT’s size
  - Used in many fast aligners

- MSBWT
  - Applies to string collections
  - Enables database-like access to reads via k-mer searches