# Lecture 5: Finding Regulatory Motifs Within DNA Sequences

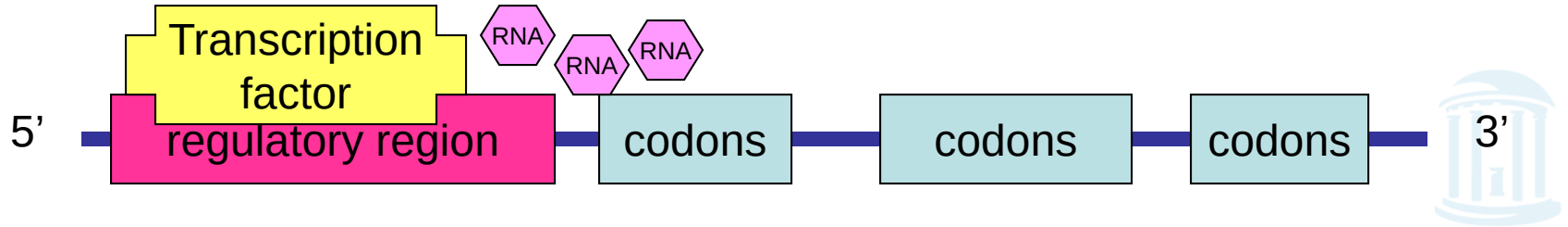
Study Chapter 4.4-4.9

# Initiating Transcription

- To facilitate transcription of a gene, special proteins bind DNA near the gene start and separate the strands.
- How do these proteins know where the coding genes are in order to bind?
- Genes are relatively rare
  - O(1,000,000,000) bases/genome
  - O(10000) genes/genome
  - O(1000) bases/gene
- Approximately 1% of DNA codes for genes ($10^3 10^4 / 10^9$)

# Regulatory Regions

- *Regulatory* or *promoting* regions are located 100-1000 bp upstream from the coding region
- Specific proteins called *transcription factors* bind to matching DNA sequence patterns in the regulatory region known as *Transcription Factor Binding Sites* (TFBS)
- The binding transcription factors separate the DNA strands, enabling RNA polymerases to start transcription
- The DNA sequence patterns in the transcription factor binding sites are known as ***motifs***.

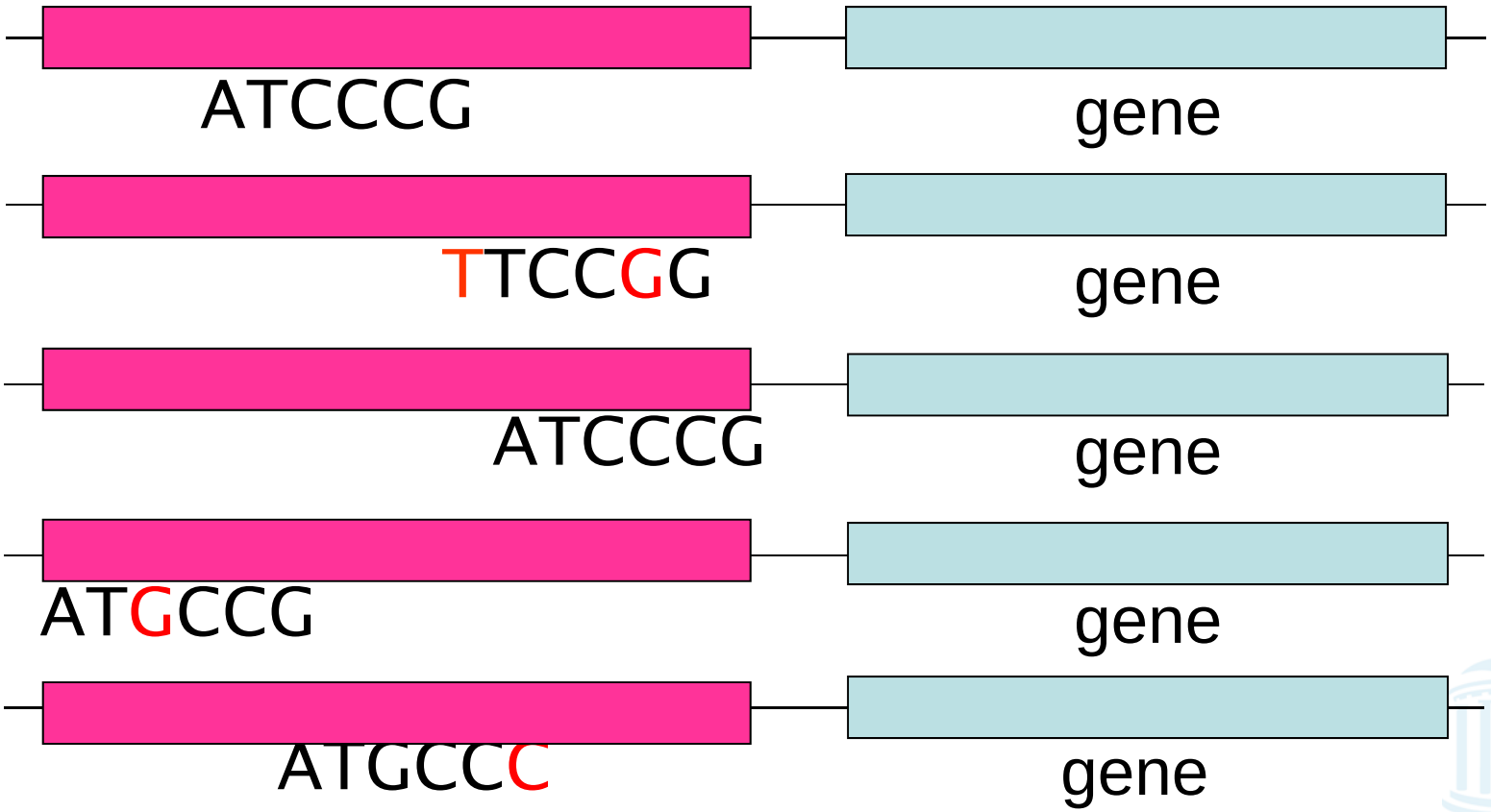


# Transcription Factor Binding Sites

- A TFBS can be located anywhere within the regulatory region.
- TFBS may vary slightly across different regulatory regions since non-essential bases could mutate
- Transcription factors are robust (they will still bind) in the presence of small changes in a few bases

# Motifs and Transcriptional Start Sites

***Motif*** *(n)* - A *repeated* structural element in architecture or decoration



# Identifying Motifs: Complications

- We do not know the motif sequence for every TF
- We do not know where it is located relative to the gene's start
- Motifs can differ slightly from one gene to the next
- We only know that it occurs frequently
- How to discern a motif's frequent "similar" pattern from "random" patterns?

# An Aside: Solving Cryptograms

- A popular form of word puzzle

  N oucgupju dlgw ynouo nwu sbu ynoho ld n jlzu dlw eupuo, xbhjb, snqup hp swhvmuo, zusuwihpuo vwlsuhp oucgupjuo.

- Based on letter, multi-letter, and word frequency it is not hard to figure out the most likely answer.
- Try solving it using http://rumkin.com/tools/cipher/cryptogram-solver.php

# How's a Motif Like a Cryptogram?

- Nucleotides in motifs encode a message in a "genetic" language. Symbols in a cryptogram, encode messages in English
- In order to solve the problem, we analyze the *frequencies of patterns* in DNA/Cryptogram.
- Knowledge of established regulatory motifs makes the Motif Finding problem simpler. Knowledge of the words in the English dictionary helps to solve cryptograms.

# The Motif Finding Problem

- Given a sample of DNA sequences:

1. cctgatagacgctatctggctatccacgtacgtaggtcctctgtgcgaatctatgcgtttccaaccat
2. agtactggtgtacatttgatacgtacgtacaccggcaacctgaaacaaacgctcagaaccagaagtgc
3. aaacgtacgtgcaccctctttcttcgtggctctggccaacgagggctgatgtataagacgaaaatttt
4. agcctccgatgtaagtcatagctgtaactattacctgccacccctattacatcttacgtacgtataca
5. ctgttatacaacgcgtcatggcggggtatgcgttttggtcgtcgtacgctcgatcgttaacgtacgtc

- Find a pattern that is implanted in each of the individual sequences, namely, the motif

- Additional information:
  - Assume the hidden sequence is of length 8
  - The pattern is not exactly the same in each sequence because random point mutations have been introduced

# Motif Finding Complications

- We don't know the pattern we are looking for
- The pattern isn't identical from occurrence to occurrence
- Only a small fraction of nucleotide sequences encode for motifs
- The size of the genome sequence is enormous

# Motif Finding Example

- Finding motifs if there are **no** mutations
- Probability of a given 8-mer in a random DNA sequence of length $n$ is $n/4^8$ (expect 1 in 65Kb)
- For our five DNA sequences of length 68, there are $5 \cdot (68 - 8+1) \approx 300$ possible 8-mers
- Probability of any one 8-mer is $300/4^8 \approx 0.005$
- So *any* 8-mer repeat is rare

```
cctgatagacgctatctggctatccacgtacgtaggtcctctgtgcgaatctatgcgtttccaaccat
agtactggtgtacatttgatacgtacgtacaccggcaacctgaaacaaacgctcagaaccagaagtgc
aaacgtacgtgcaccctctttcttcgtggctctggccaacgagggctgatgtataagacgaaaatttt
agcctccgatgtaagtcatagctgtaactattacctgccacccctattacatcttacgtacgtataca
ctgttatacaacgcgtcatggcggggtatgcgttttggtcgtcgtacgctcgatcgttaacgtacgtc
```

**acgtacgt**

# The Problem Becomes Harder

- Introduce 2 point mutations into each pattern:

```
cctgatagacgctatctggctatccaGgtacTtaggtcctctgtgcgaatctatgcgtttccaaccat
agtactggtgtacatttgatCcAtacgtacaccggcaacctgaaacaaacgctcagaaccagaagtgc
aaacgtTAgtgcaccctctttcttcgtggctctggccaacgagggctgatgtataagacgaaaatttt
agcctccgatgtaagtcatagctgtaactattacctgccacccctattacatcttacgtCcAtataca
ctgttatacaacgcgtcatggcggggtatgcgttttggtcgtcgtacgctcgatcgttaCcgtacgGc
```

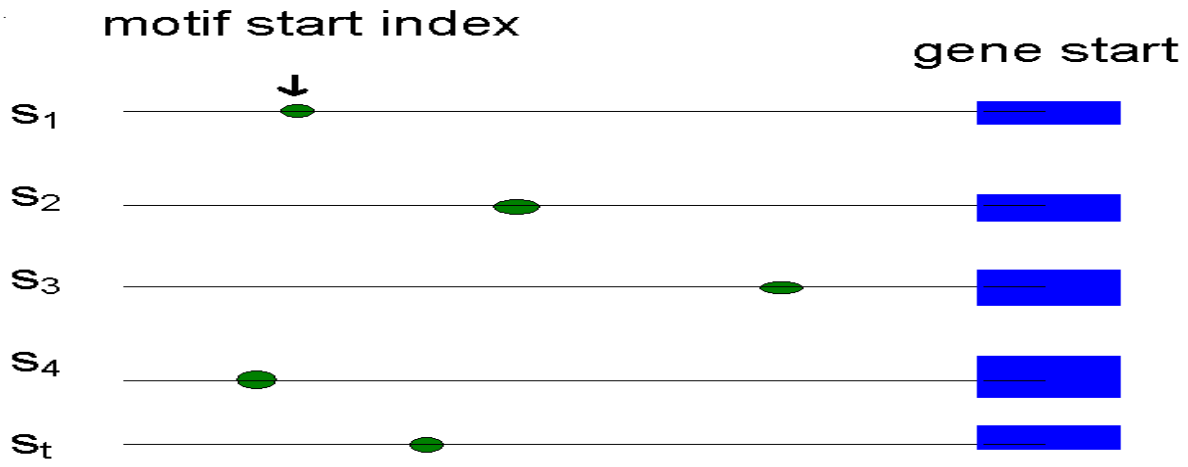
- Our original target pattern no longer appears in any sequence!

**Can we still find the motif?**

# Defining a Motif

- To define a motif, let's assume that we know where the motif starts in each sequence
- The start positions can be represented as

$$\boldsymbol{s} = [s_1, s_2, s_3, \ldots, s_t]$$



# Motifs: Profiles and Consensus

```
                a G g t a c T t
                C c A t a c g t
Alignment       a c g t T A g t
                a c g t C c A t
                C c g t a c g G

                ---------------

          A     3 0 1 0 3 1 1 0
Profile   C     2 4 0 0 1 4 0 0
          G     0 1 4 0 0 0 3 1
          T     0 0 0 5 1 0 1 4

                ---------------

Consensus       A C G T A C G T
```

- Line up the patterns by their start indexes

$$\mathbf{s} = (s_1, s_2, \ldots, s_t)$$

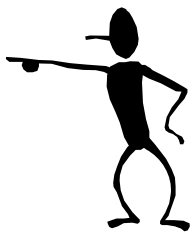
- Construct a matrix profile with the frequencies of each nucleotide in columns

- Consensus nucleotide in each position has the highest score in column

# Consensus

- Think of consensus as an "ancestor" motif, from which mutated motifs emerged
- The *distance* between an actual motif and the consensus sequence is generally less than that for any two actual motifs
- *Hamming distance* is number of positions that differ between two strings

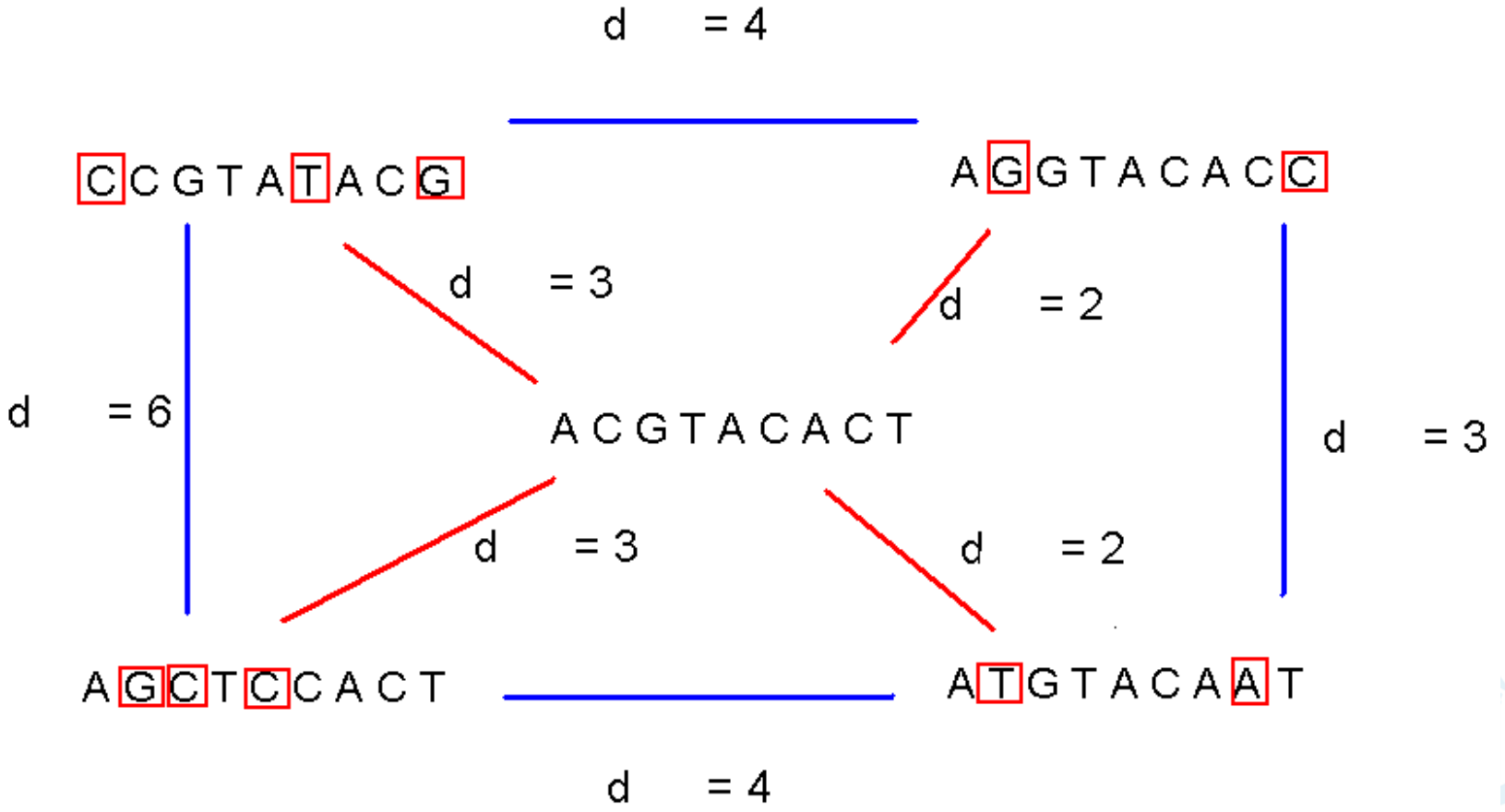
```
G A G A C T C A T
X         X
T A G A C G C A T
```



A Hamming distance of 2

# Consensus Properties

- A consensus string has a minimal Hamming distance to all source strings

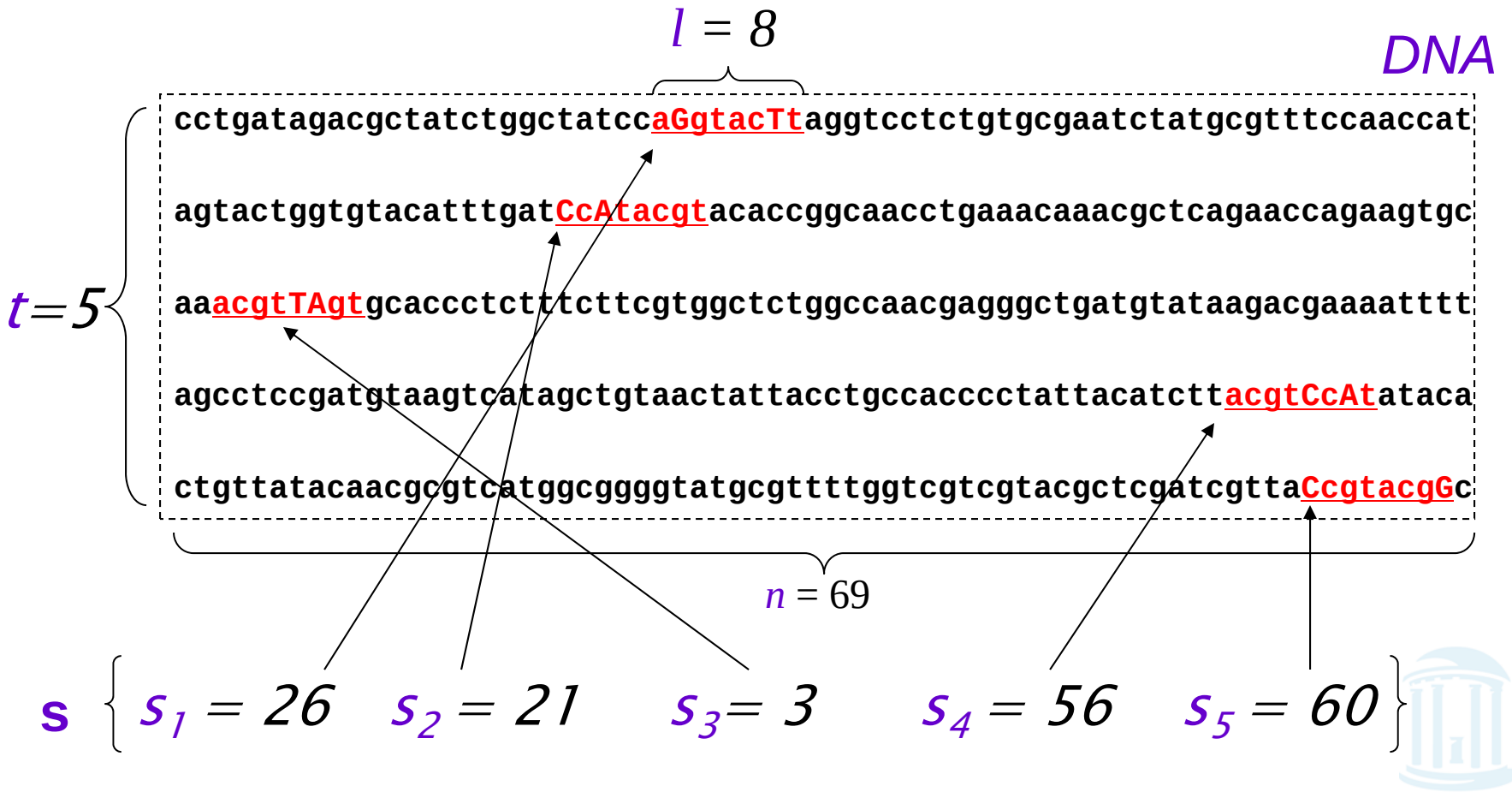


# Defining Some Terms

- ***DNA*** – array of sequence fragments
- $t$ - number of sample DNA sequences
- $n$ - length of each DNA sequence

- $\ell$ - length of the motif ($\ell$-mer)
- $s_i$ - starting position of an $\ell$-mer in sequence $i$
- $\mathbf{s}=(s_1, s_2, \ldots s_t)$ - array of motif's starting positions

# Illustration of Terms



# Scoring Motifs

- Given **s** = $(s_1, \ldots s_t)$ and ***DNA***:

$$Score(\mathbf{s}, DNA) = \sum_{i=1}^{l} \left( \max_{k \in \{A,C,G,T\}} count(k,i) \right)$$

$l$

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | a | G | g | t | a | c | T | t |
| | C | c | A | t | a | c | g | t |
| | a | c | g | t | T | A | g | t |
| | a | c | g | t | C | c | A | t |
| | C | c | g | t | a | c | g | G |

$t$

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 3 | 0 | 1 | 0 | 3 | 1 | 1 | 0 |
| C | 2 | 4 | 0 | 0 | 1 | 4 | 0 | 0 |
| G | 0 | 1 | 4 | 0 | 0 | 0 | 3 | 1 |
| T | 0 | 0 | 0 | 5 | 1 | 0 | 1 | 4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Consensus | a | c | g | t | a | c | g | t |

**Score** 3+4+4+5+3+4+3+4=**30**

# The Motif Finding Problem

- Goal: Given a set of DNA sequences, find a set of $\ell$-mers, one from each sequence, that maximizes the consensus score

- Input: A $t$ x $n$ matrix of $DNA$, and $\ell$, the length of the pattern to find

- Output: An array of $t$ starting positions $\mathbf{s} = (s_1, s_2, \ldots s_t)$ maximizing $Score(\mathbf{s}, DNA)$

# Brute Force Solution

- Compute the scores for all possible combinations of starting positions **s**
- The best score determines the best profile and the consensus pattern in ***DNA***
- The goal is to maximize *Score*(**s**,***DNA***) by varying the starting positions $s_i$, where:

$$s_i = [1, \ldots, n\text{-}l+1]$$
$$i = [1, \ldots, t]$$

# Brute Force Pseudocode

```
BruteForceMotifSearch(DNA, t, n, l )
bestScore ← 0
for each s = (s1, s2, …, st) from (1, 1, …, 1)
                              to (n- l+1, n- l+1, …, n- l+1)
   if score(s, DNA, l ) > bestScore
      bestScore ← score(s, DNA, l )
      bestMotif ← (s1, s2, …, st)
return bestMotif
```

# Running Time of BruteForceMotifSearch

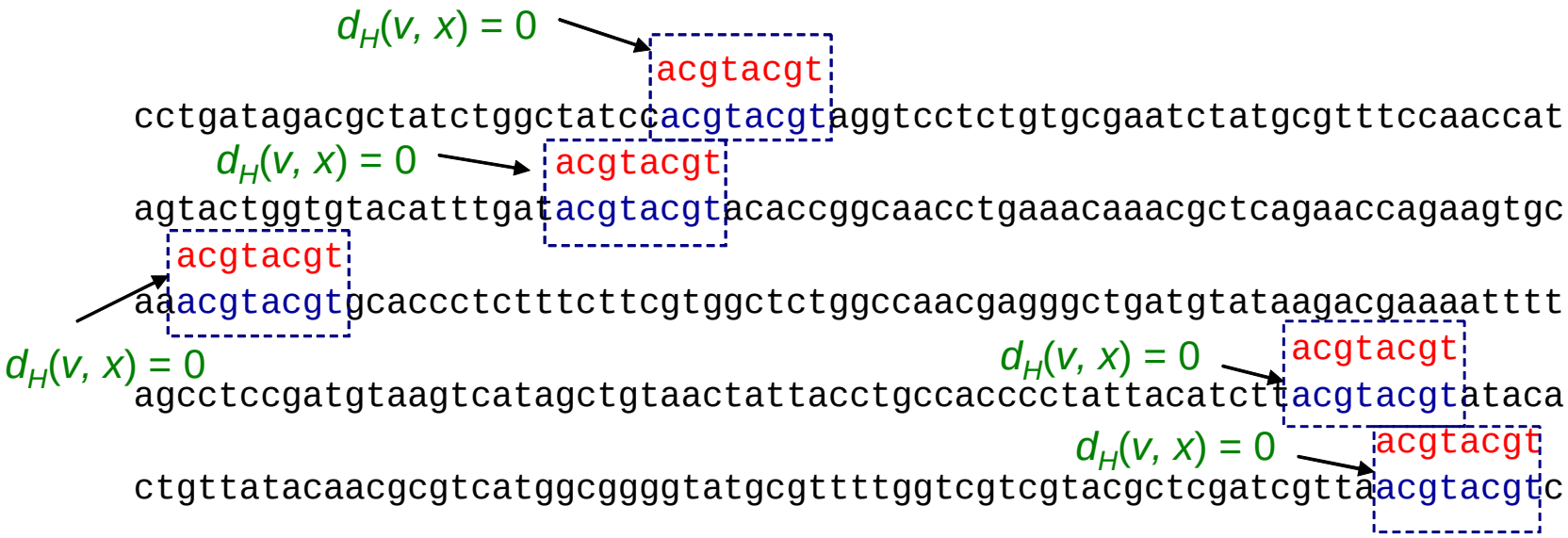
- Search $(n - \ell + 1)$ positions in each of $t$ sequences, by examining $(n - \ell + 1)^t$ sets of starting positions
- For each set of starting positions, the scoring function makes $\ell t$ operations, so complexity is $\ell(n - \ell + 1)^t = O(\ell t n^t)$
- That means that for $t = 8$, $n = 1000$, $\ell = 10$ we must perform approximately $10^{25}$ computations
- Generously assuming $10^9$ comps/sec it will require only $10^{16}$ secs
- $10^{16}/(60 * 60 * 24 * 365)$ → millions of years

# The Median String Problem

- Given a set of ***t*** DNA sequences find a pattern that appears in all ***t*** sequences with the minimum number of mutations
- This pattern will be the motif
- Rather than finding the maximal consensus string, this approach attempts to find the minimal distance string
  - The former enumerates possible starting positions
  - The latter enumerates possible strings

# Total Distance: An Example

- Given ***v*** = "acgtacgt" and **s**




*v* is the sequence in red, *x* is the sequence in blue

- $TotalDistance(\mathbf{v}, \mathbf{DNA}) = 0$

# Total Distance: An Example

- Given ***v*** = "acgtacgt" and **s**


*v* is the sequence in red, *x* is the sequence in blue

- $TotalDistance(\mathbf{v},\mathbf{DNA}) = 1+0+2+0+1 = 4$

# Total Distance: Definition

- For each DNA sequence $\boldsymbol{i}$, compute all $d_H(\boldsymbol{v}, \boldsymbol{x})$, where $\boldsymbol{x}$ is an $\boldsymbol{\ell}$-mer with starting position $s_i$

  $(1 \leq s_i \leq \boldsymbol{n} - \boldsymbol{\ell} + 1)$
- Find minimum of $d_H(\boldsymbol{v}, \boldsymbol{x})$ among all $\boldsymbol{\ell}$-mers in sequence $\boldsymbol{i}$
- *TotalDistance*$(\boldsymbol{v}, \boldsymbol{DNA})$ is the sum of the minimum Hamming distances for each DNA sequence $\boldsymbol{i}$
- *TotalDistance*$(\boldsymbol{v}, \boldsymbol{DNA}) = min_{\mathbf{s}}\, d_H(\boldsymbol{v}, \boldsymbol{s})$, where $\boldsymbol{s}$ is the set of starting positions $s_1, s_2, \ldots s_t$

# The Median String Problem

- Goal: Given a set of DNA sequences, find a median string
- Input: A $t \times n$ matrix DNA, and $\ell$, the length of the pattern to find
- Output: A string $v$ of $\ell$ nucleotides that minimizes $TotalDistance(v,DNA)$ over all strings of that length

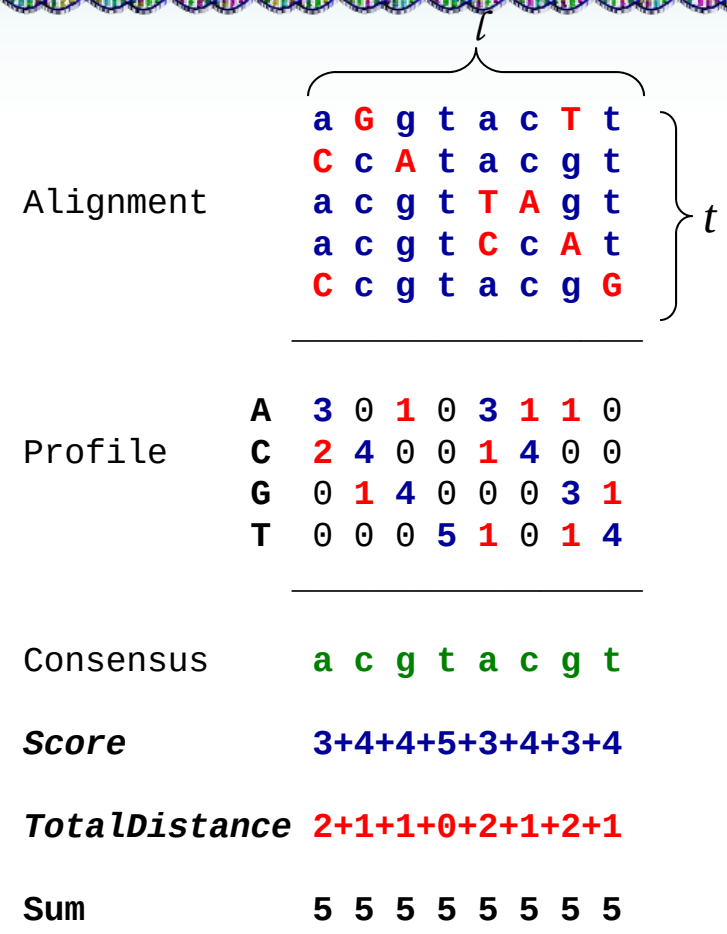
# Median String Search Algorithm

```
MedianStringSearch(DNA, t, n, l )
bestMotif ← “”
bestDistance ← t × l
for each l –mer, s, from “aaa…a” to “ttt…t”
   if TotalDistance(s, DNA) < bestDistance
        bestDistance ← TotalDistance(s, DNA)
        bestMotif ← s
return bestMotif
```

# Equivalent Problems!

- Motif Finding Problem ≡ Median String Problem
- The *Motif Finding* is a maximization problem while *Median String* is a minimization problem
- However, the *Motif Finding* problem and *Median String* problem are computationally equivalent (they give the same output for a common input)
- Need to show that minimizing *TotalDistance* is equivalent to maximizing *Score*

# We're looking for the same thing




- At any column $i$
  $Score_i + TotalDistance_i = t$
- Because there are $l$ columns
  $Score + TotalDistance = l * t$
- Rearranging:
  $Score = l * t - TotalDistance$
- $l * t$ is constant the minimization of the right side is equivalent to the maximization of the left side

# Why Bother?

- What is the point of reformulating the Motif Finding problem as the Median String problem?
  - The Motif Finding Problem needs to examine all the combinations for **s**. That is $(n - l + 1)^t$ combinations!!!
  - The Median String Problem needs to examine all $4^l$ combinations for $v$. This number is relatively smaller

n=1000, $l$=10, t=8

→ $8(1000-10+1)4^{10} \approx 8.3 \times 10^9$

→ $(1000-10+1)^8 \approx 9.3 \times 10^{23}$

# Improving both algorithms

- … using branch and bound

# Improving Motif Finding

BruteForceMotifSearch(DNA, t, n, *l* )

bestScore ← 0

for each s = ($s_1$, $s_2$, …, $s_t$) from (1, 1, …, 1)
to (n- *l*+1, n- *l*+1, …, n- *l*+1)

if score(s, DNA, l) > bestScore

bestScore ← score(s, DNA, *l* )

bestMotif ← ($s_1$, $s_2$, …, $s_t$)

return bestMotif

# How to Structure the Search?

- How can we perform the line

**for** each **s**=($s_1$,$s_2$ , . . ., $s_t$) from (1,1 . . . 1) to ($n$–$l$+1, . . ., $n$–$l$+1) ?

- We need a method to more efficiently examine the many possible motifs locations
- This is not very different than exploring all "*t*-digit base *(n-l+1)*" numbers

# Improving Median String

```
MedianStringSearch(DNA, t, n, l )
bestMotif ← ""
bestDistance ← t × l
for each l –mer, s, from "aaa…a" to "ttt…t"
    if TotalDistance(s, DNA) < bestDistance
        bestDistance ← TotalDistance(s, DNA)
        bestMotif ← s
return bestMotif
```

# How to Enumerate the Candidates

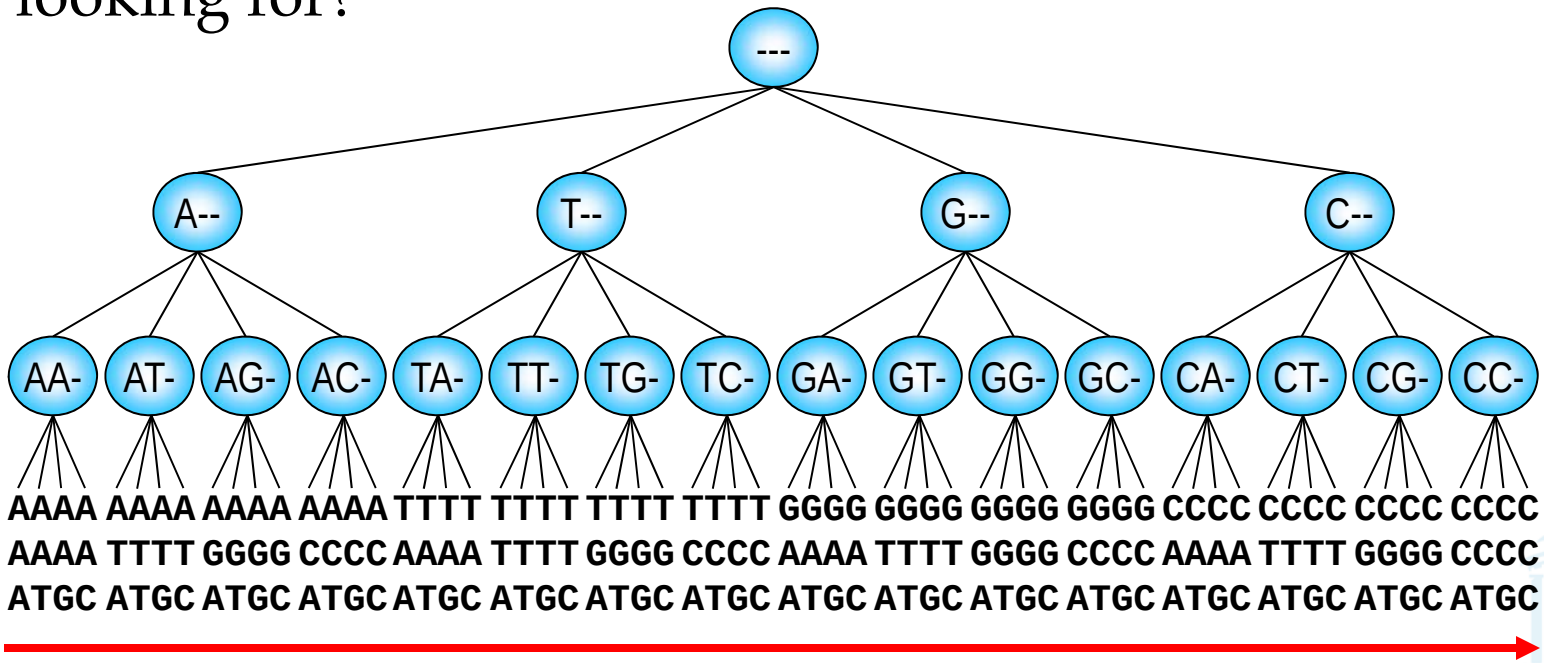
- For the Median String Problem we need to consider all $4^{\ell}$ possible $\ell$-mers:

aa… aa
aa… ac
aa… ag
aa… at
aa… ca
.
.
tt… tt

How to organize this search?

# Search Tree

- Our standard method for enumerating candidates just traverses the leaf nodes
- Suppose after checking the first or second letter we already know the solution could not be the one we are looking for?



# NextLeaf Usage

- This is the basic loop structure that we have used to explore the search tree thus far

```
def AllLeaves(L, k):
    a = [1 for i in xrange(L)]
    while True:
        print a
        a = NextLeaf(a, L, k)
        if (sum(a) == L)
            return
```

- Enumerates values in L-digit "odometer" order (each digit cycles 1..k instead of 0..k-1).

# How does nextLeaf work?

- Code for NextLeaf is the same logic as counting

```
def NextLeaf(a, L, k):
    # generates all k^L candidates
    for i in reversed(xrange(L)):
        if (a[i] < k):
            a[i] += 1
            break
        else:
            a[i] = 1
    return a
```

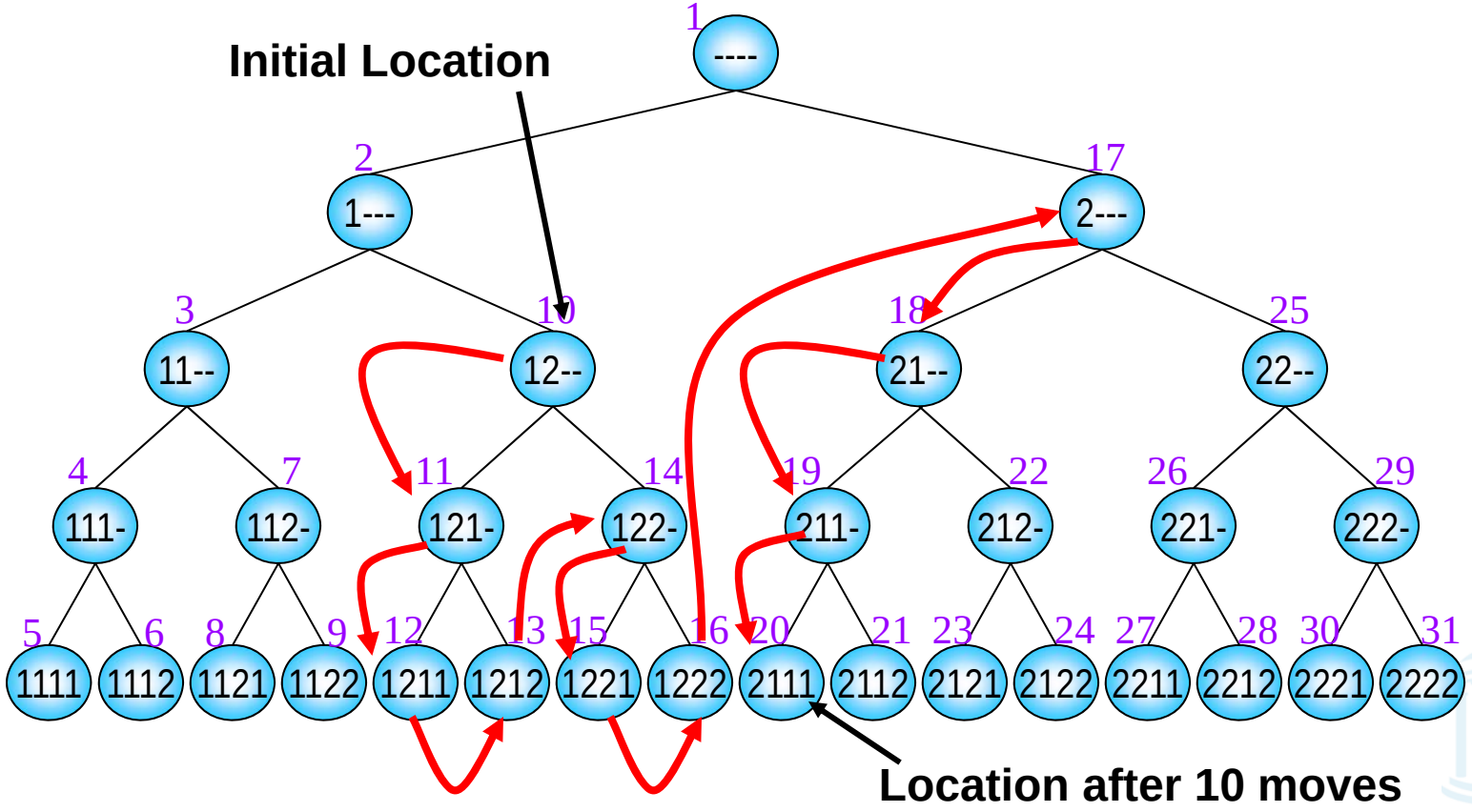
- "a" is the current candidate list ([2,1,3])
- "k" is the largest value (k = 4 for {A,C,T,G})
- "L" is the # of variables (L = 3 for 3-mers)

# Analyzing Search Trees

- Characteristics of the search trees:
  - The unique candidates reside at leaves
  - A parent node is a common prefix of its children
- How can we traverse the tree?
- Things we'd like to do:
  - Visit all the nodes (interior and leaves)
  - Visit the next leaf (in an ordered way)
  - Bypass the children of a node

# Depth First Search

- Start from the root and visit nodes in preorder
  - First parent, then visit subtrees in left to right order



# Visiting the Next Vertex

- Uses 0s to encode unspecified part of interior nodes (the dashes in our figure)

```
def NextVertex(a, i, L, k):
    if (i < L):
        # not at leaf, go down a level
        a[i] = 1
        return (a, i+1)
    else:
        # at leaf, go to next leaf
        for j in reversed(xrange(L)):
            if (a[j] < k):
                a[j] += 1
                return (a, j+1)
            a[j] = 0
        return (a, 0)
```

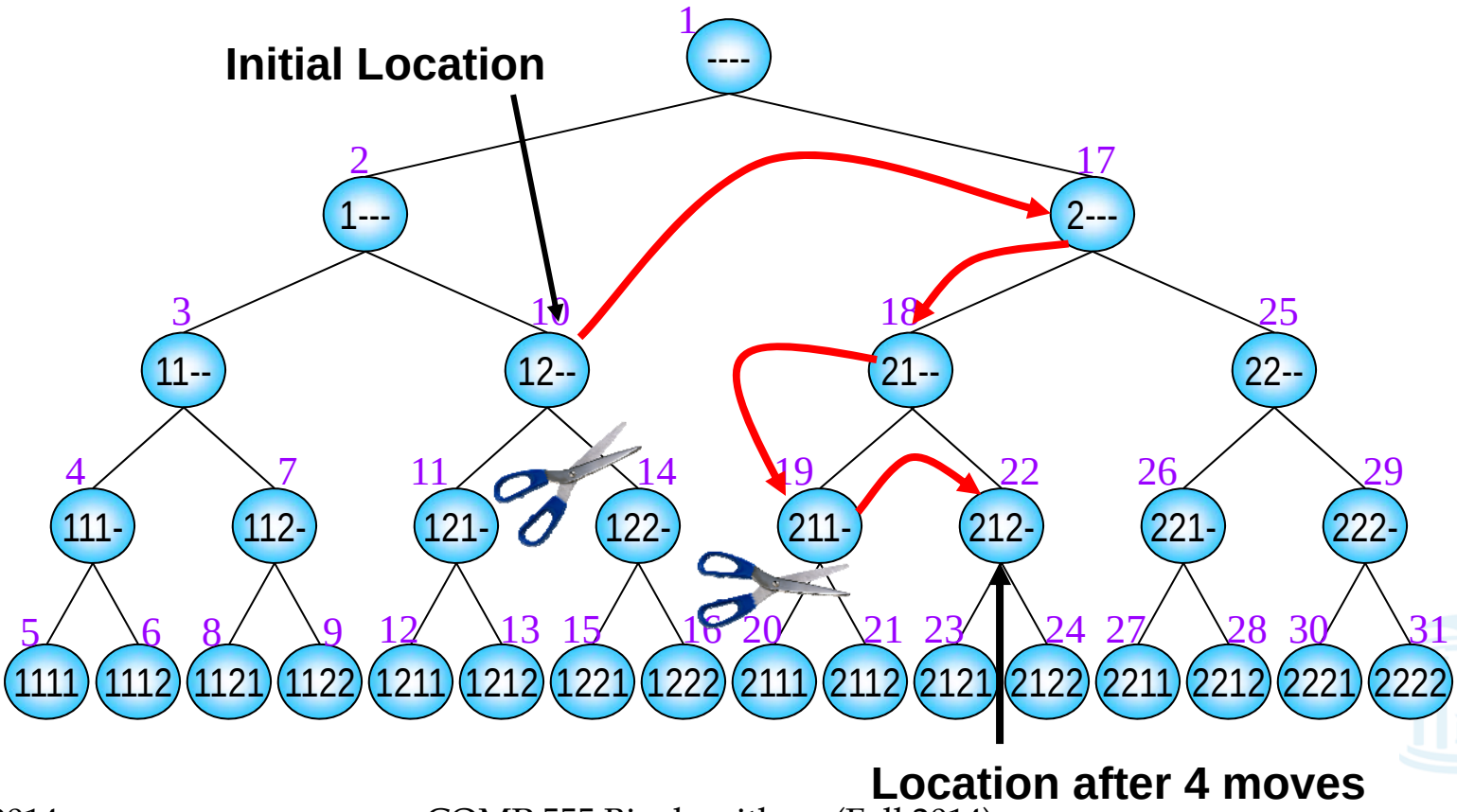
# Bypass Subtrees

- Given an internal node find next node after skipping all of the current vertex's children

```
def Bypass(a, i, L, k):
    for j in reversed(xrange(i)):
        if (a[j] < k):
            a[j] += 1
            return (a, j+1)
        a[j] = 0
    return (a, 0)
```

# Bypass Example

- Bypassing descendents of nodes "12—" and "211-"



# Revisiting Brute Force Search

- Now that we have method for navigating the tree, lets convert our pseudocode version of BruteForceMotifSearch to real code

```
def BruteForceMotifSearchAgain(DNA,t,n,l):
    s = [1 for i in xrange(t)]
    bestScore = Score(s, DNA)
    while (True):
        s = NextLeaf(s,t,n-l+1)
        if (Score(s, DNA) > bestScore):
            bestScore = Score(s, DNA)
            bestMotif = [x for x in s]
        if (sum(s) == t):
            break
    return bestMotif
```

# Can We Do Better?

- Sets of **s=**($s_1$, $s_2$, …,$s_t$) may have a weak profile for the first $i$ positions ($s_1$, $s_2$, …,$s_i$)
- Every row of alignment may add at most $\ell$ to Score
- Best possible outcome: all subsequent $(t-i)$ positions ($s_{i+1}$, …$s_t$) add

$$(t - i) * \ell \text{ to } \text{Score}(\mathbf{s}, i, DNA)$$

- If $\text{Score}(\mathbf{s}, i, DNA) + (t - i) * \ell < \text{BestScore}$, it makes no sense to search subtrees of the current vertex
  - Use **ByPass()**

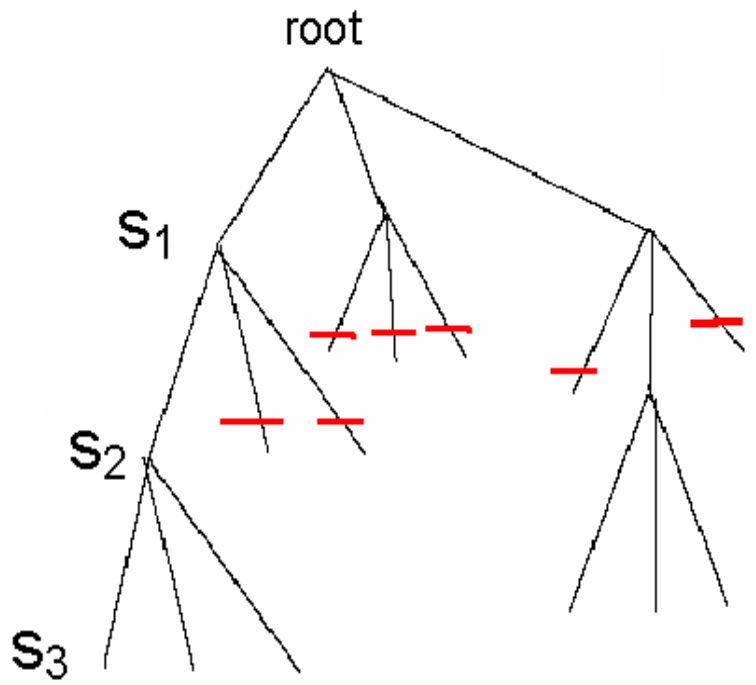
# Rewrite Using Tree Traversal

- Before we apply a branch-and-bound strategy let's rewrite the brute-force algorithm using a search tree

```
def SimpleMotifSearch(DNA,t,n,l):
    s = [0 for i in xrange(t)]
    bestScore = 0
    i = 0
    while (True):
        if (i < t):
            s, i = NextVertex(s,i,t,n-l+1)
        else:
            if (Score(s, DNA, l) > bestScore):
                bestScore = Score(s, DNA, l)
                bestMotif = [x for x in s]
            s, i = NextVertex(s,i,t,n-l+1)
            if (sum(s) == 0):
                break
    return bestMotif
```

# Branch and Bound Motif Search

- Since each level of the tree goes deeper into search, discarding a prefix discards all following branches




- This saves us from looking at $(n - \ell + 1)^{t-i}$ leaves
  - Use **NextVertex()** and **ByPass()** to navigate the tree

# Branch-and-Bound Motif Code

```
def BranchAndBoundMotifSearch(DNA,t,n,l):
    s = [0 for i in xrange(t)]
    bestScore = 0
    i = 0
    while (True):
        if (i < t):
            optimisticScore = Score(s, DNA, l) + (t-i)*l
            if (optimisticScore < bestScore):
                s, i = Bypass(s,i,t,n-l+1)
            else:
                s, i = NextVertex(s,i,t,n-l+1)
        else:
            score = Score(s, DNA, l)
            if (score > bestScore):
                bestScore = score
                bestMotif = [x for x in s]
            s, i = NextVertex(s,i,t,n-l+1)
        if (sum(s) == 0):
            break
    return bestMotif
```

# Improving Median Search

- Recall the computational differences between motif search and median string search
  - The Motif Finding Problem needs to examine all $(\boldsymbol{n}\text{-}\boldsymbol{\ell}+1)^t$ combinations for **s**.
  - The Median String Problem needs to examine $4^{\ell}$ combinations of $\boldsymbol{v}$. This number is relatively small
- We want to use median string algorithm with the Branch and Bound trick!

# Insight for Improving Median Search

- Note that if, at any point, the total distance for a prefix is greater than that for the best word so far:

  $$\text{TotalDistance}(\textit{prefix}, \textit{DNA}) > \textit{BestDistance}$$

  there is no use exploring the remaining part of the word

- We can eliminate that branch and BYPASS exploring that branch further

# Bounded Median String Search

```
def BranchAndBoundMedianSearch(DNA,t,n,l):
    s = [1 for i in xrange(t)]
    bestDistance, bestWord = l*t, ''
    i = 1
    while (i > 0):
        if (i < l):
            prefix = NucleotideString(s, i)
            atLeastDistance = TotalDistance(prefix, DNA)
            if (asLeastDistance > bestDistance):
                s, i = Bypass(s,i,l,t)
            else:
                s, i = NextVertex(s,i,l,t)
        else:
            word = NucleotideString(s, l)
            if (TotalDistance(word, DNA) < bestDistance):
                bestDistance = TotalDistance(word, DNA)
                bestWord = word
            s, i = NextVertex(s,i,l,t)
    return bestWord
```

# Final remarks

- Motif Search
  - What if there are multiple consensus strings (or median strings) with similar score/distance?
    - It can easily happen, how to define significance?
    - Many k-mers nearby as hamming distance increases
  - Is substitution the correct error model?
    - insertions or deletions are possible/likely as well
    - The algorithms will need to change very substantially
- How do you really find a TFBS?
  - Motifs are just a starting point
- Next Time
  - We revisit greedy algorithms