# Lecture 5: Finding Regulatory Motifs Within DNA Sequences

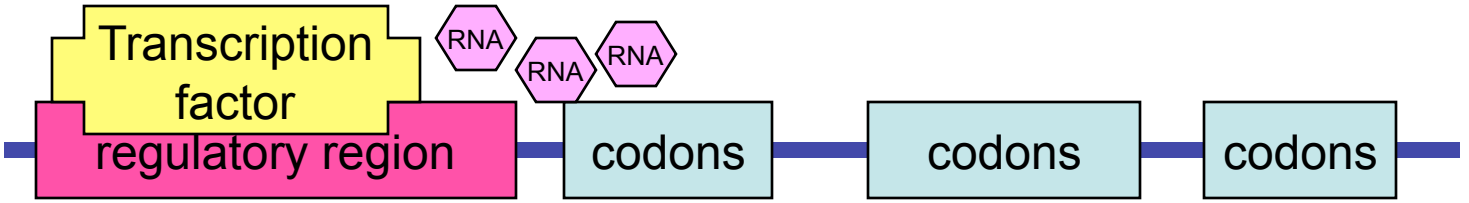
Study Chapter 4.4-4.9

# Initiating Transcription

- As a precursor to transcription (the reading of DNA to construct RNAs, eventually leading to protein synthesis) special proteins bind to the DNA, and separate it to enable its reading.
- 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

- RNA polymerases seek out *regulatory* or *promoting* regions located 100-1000 bp upstream from the coding region
- They work in conjunction with special proteins called *transcription factors* whose presence enables gene expression
- Within these regions are the *Transcription Factor Binding Sites* (TFBS), special DNA sequence patterns known as ***motifs*** that are specific to a given transcription factor

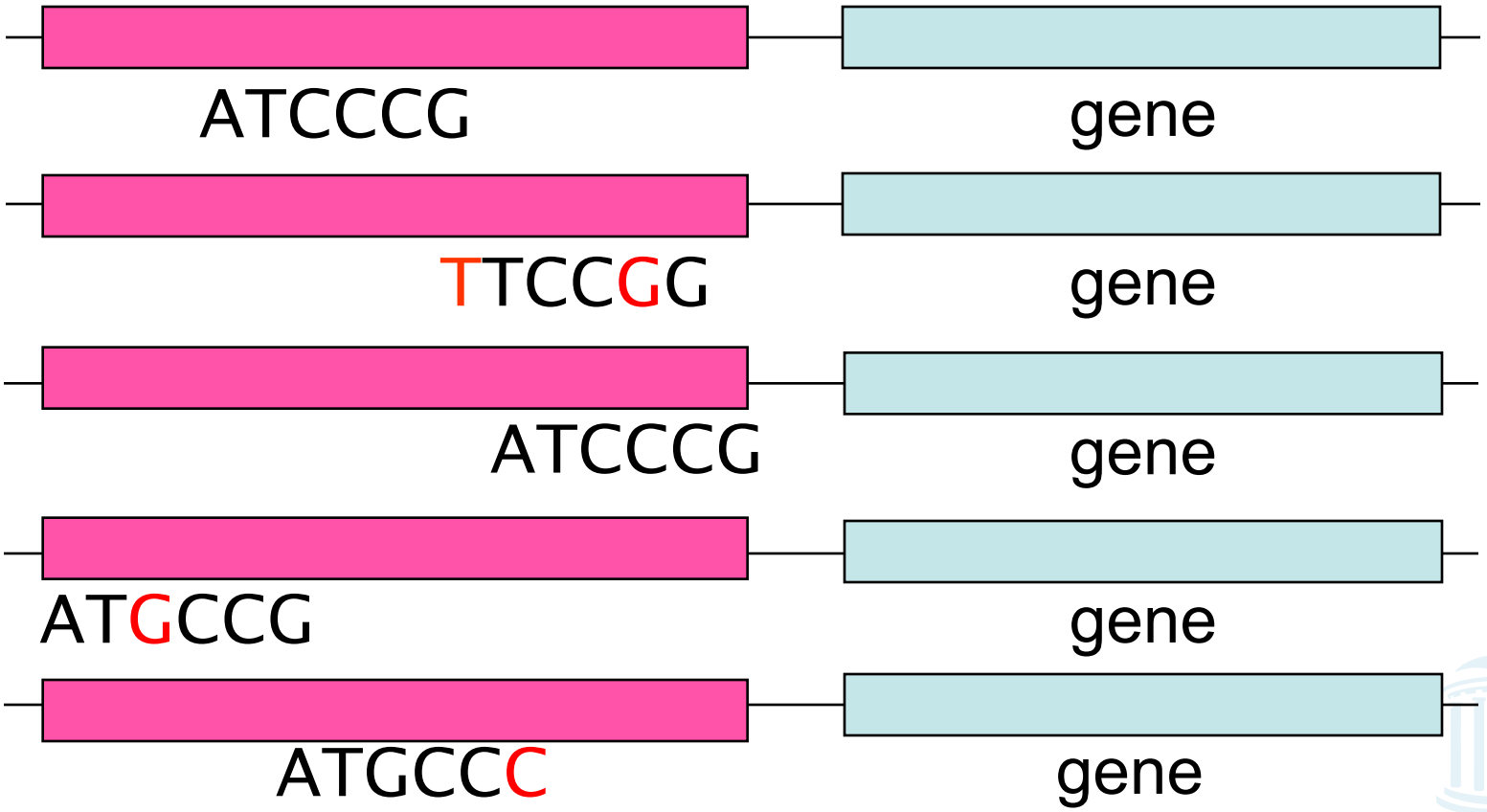


# Transcription Factor Binding Sites

- 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:

  *"By bskq rp klddykr i krhlmrlhy nph rqy kixr pn fypechsopky vlmxysm imsf (F.V.I.). Rqsk krhlmrlhy qik vpgyx nyirlhyk bqsmq ihy pn mpvksfyhioxy ospxpdsmix svryhykr."*

- Based on letter, multi-letter, and word frequency it is not hard to figure out the most likely answer.
- Try solving it using

  http://quipqiup.com

# 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.

# Motif Finding Complications

- We don't have a complete dictionary of motifs
- The "genetic" language doesn't have a standard "grammar"
- Only a small fraction of nucleotide sequences encode for motifs
- The size of the genome sequence is enormous

# The Motif Finding Problem

- Given a random sample of DNA sequences:

```
cctgatagacgctatctggctatccacgtacgtaggtcctctgtgcgaatctatgcgtttccaaccat
agtactggtgtacatttgatacgtacgtacaccggcaacctgaaacaaacgctcagaaccagaagtgc
aaacgtacgtgcaccctctttcttcgtggctctggccaacgagggctgatgtataagacgaaaatttt
agcctccgatgtaagtcatagctgtaactattacctgccacccctattacatcttacgtacgtataca
Ctgttatacaacgcgtcatggcggggtatgcgttttggtcgtcgtacgctcgatcgttaacgtacgtc
```

- Find the 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 might differ slightly in each sequence because random point mutations may have been introduced

# Motif Finding Example

- Finding motifs if there are no mutations
- Probability of a given 8-mer in an infinite sequence is $1/4^8 \approx 1.5 \times 10^{-5}$ (1 every 65Kb)
- Assuming 5 strings of length 68, there are 5 (68 - 8) = 300 distinct 8-mers
- Probability of any one 8-mer is $300/4^8 \approx 0.005$
- So *any* repeat is rare

cctgatagacgctatctggctatcc**acgtacgt**aggtcctctgtgcgaatctatgcgtttccaaccat
agtactggtgtacatttgat**acgtacgt**acaccggcaacctgaaacaaacgctcagaaccagaagtgc
aa**acgtacgt**gcaccctctttcttcgtggctctggccaacgagggctgatgtataagacgaaaatttt
agcctccgatgtaagtcatagctgtaactattacctgccacccctattacatctt**acgtacgt**ataca
ctgttatacaacgcgtcatggcggggtatgcgttttggtcgtcgtacgctcgatcgtta**acgtacgt**c

**acgtacgt**

# The Problem Becomes Harder

- Introduce 2 point mutations into each pattern:

```
cctgatagacgctatctggctatccaGgtacTtaggtcctctgtgcgaatctatgcgtttccaaccat
agtactggtgtacatttgatCcAtacgtacaccggcaacctgaaacaaacgctcagaaccagaagtgc
aaacgtTAgtgcaccctctttcttcgtggctctggccaacgagggctgatgtataagacgaaaatttt
agcctccgatgtaagtcatagctgtaactattacctgccacccctattacatcttacgtCcAtataca
ctgttatacaacgcgtcatggcggggtatgcgttttggtcgtcgtacgctcgatcgttaCcgtacgGc
```

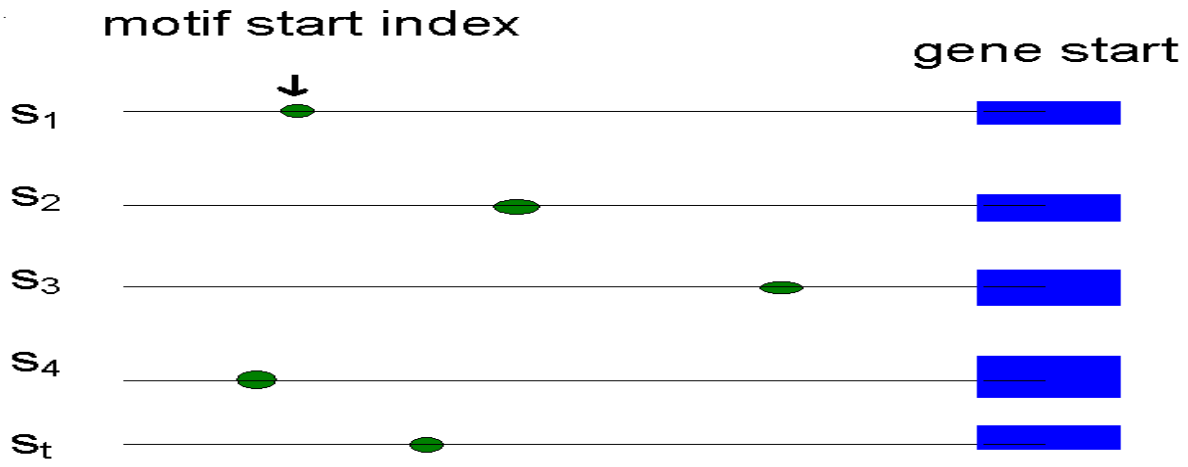
- Our original target pattern no longer appears in any sequence!

**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

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

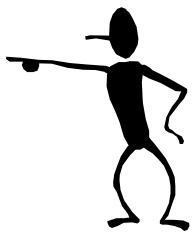
- Construct a matrix profile with the frequencies of each nucleotide in columns

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

# Consensus

- One can think of the 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

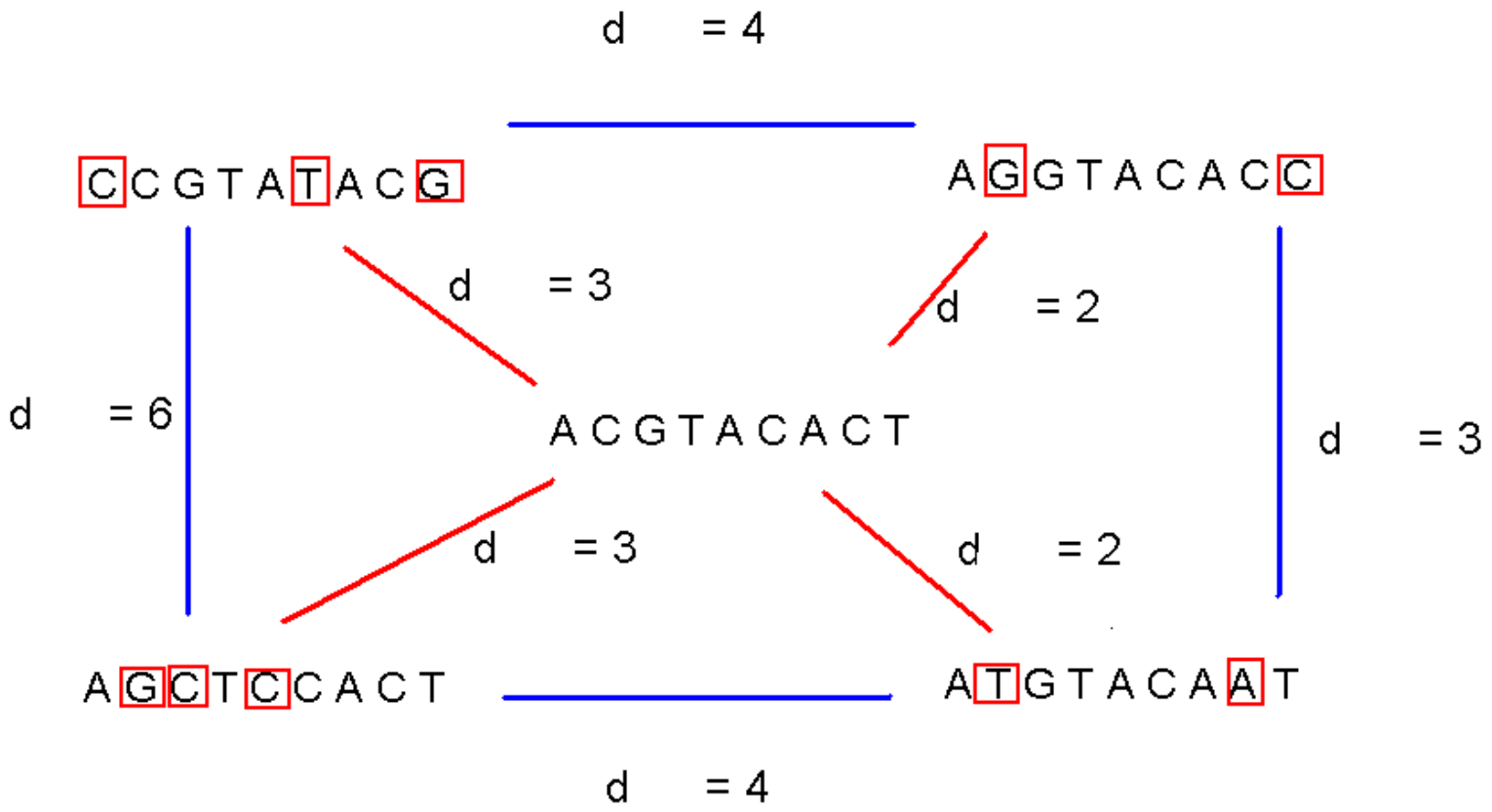
```
G A G A C T C A T
X         X
T A G A C G C A T
```



A Hamming distance of 2

# Consensus Properties

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

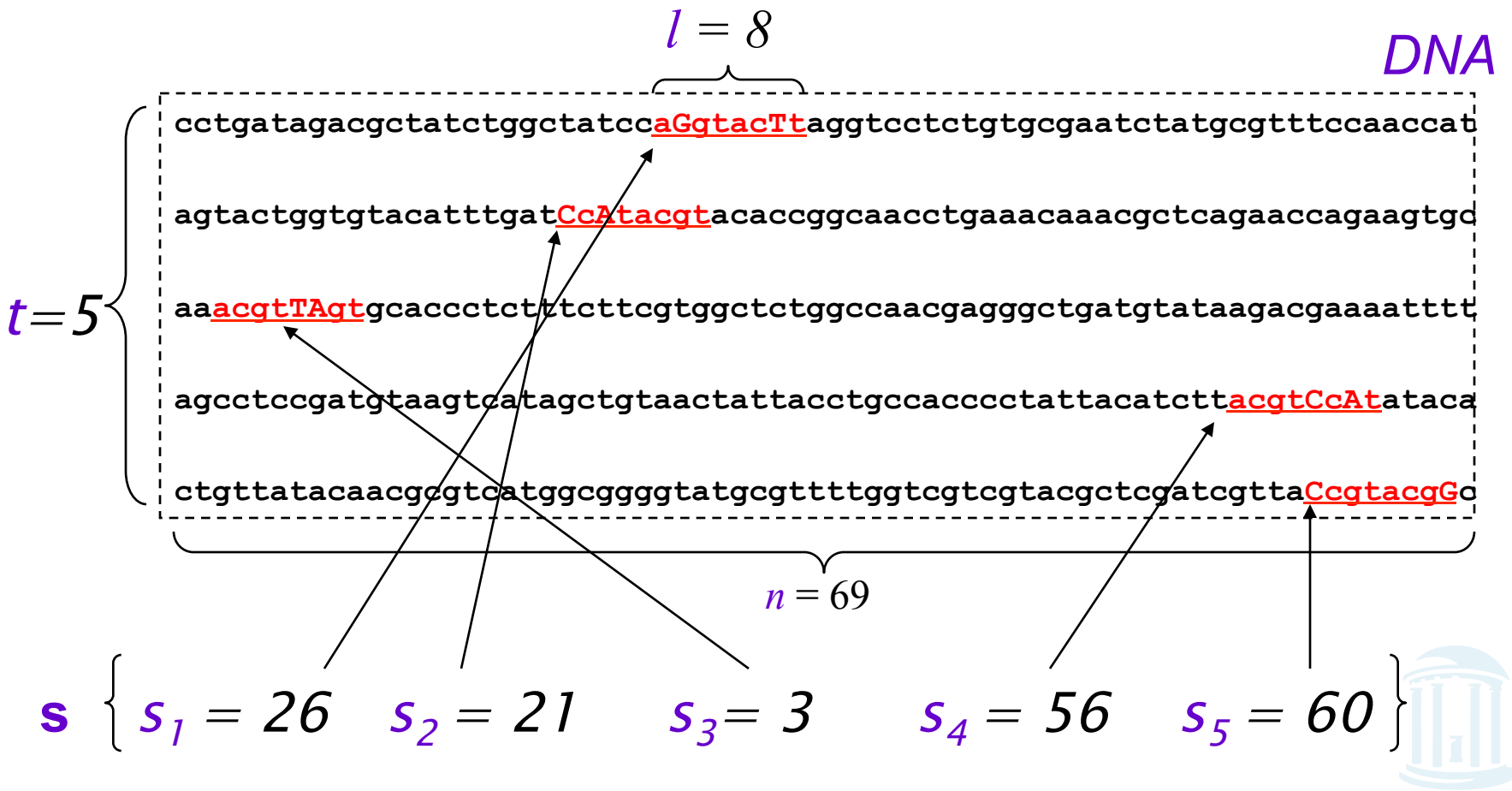


# Defining Some Terms

- ***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 $i$
- **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***:

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

$l$

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 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

1. BruteForceMotifSearch(DNA, t, n, $l$)
2. bestScore ← 0
3. for each s = ($s_1$, $s_2$, …, $s_t$) from (1, 1, …, 1)
   to (n- $l$+1, n- $l$+1, …, n- $l$+1)
4. if score(s, DNA, $l$) > bestScore
5. bestScore ← score(s, DNA, $l$)
6. bestMotif ← ($s_1$, $s_2$, …, $s_t$)
7. return bestMotif

# Running Time of BruteForceMotifSearch

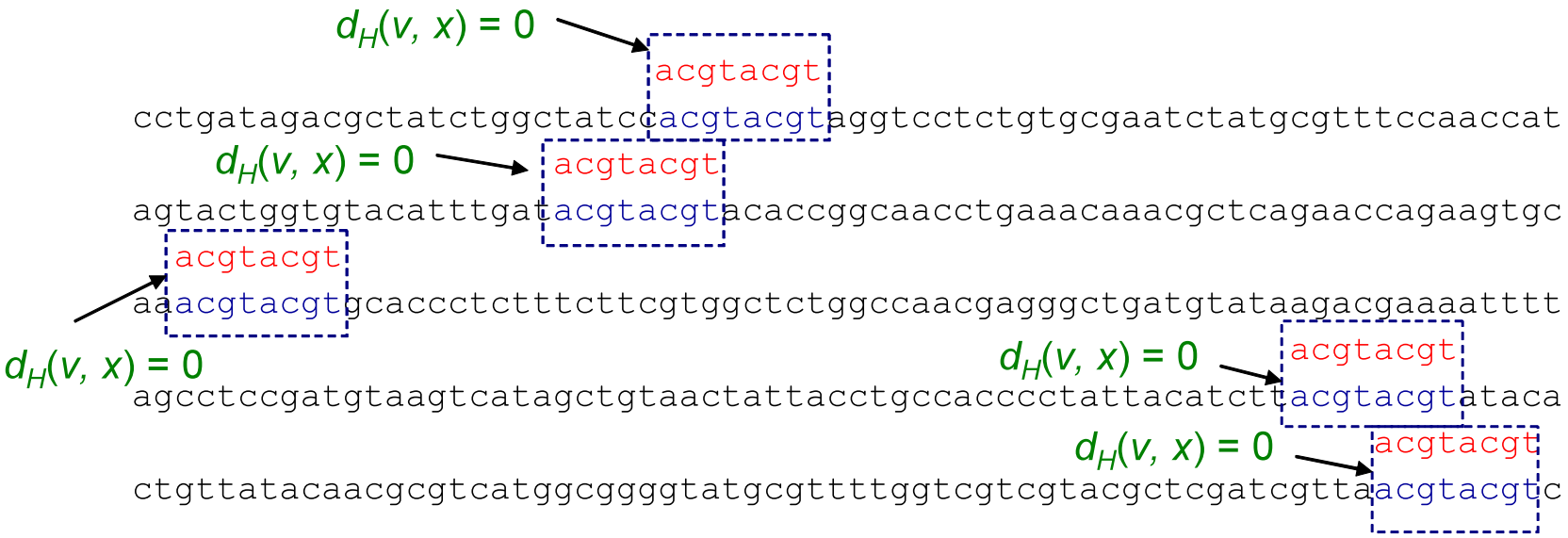
- Search $(n - \ell + 1)$ positions in each of $t$ sequences, by examining $(n - \ell + 1)^t$ sets of starting positions
- For each set of starting positions, the scoring function makes $\ell$ operations, so complexity is $\ell(n - \ell + 1)^t = O(\ell n^t)$
- That means that for $t = 8$, $n = 1000$, $\ell = 10$ 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 the minimal distance string

# Total Distance: An Example

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

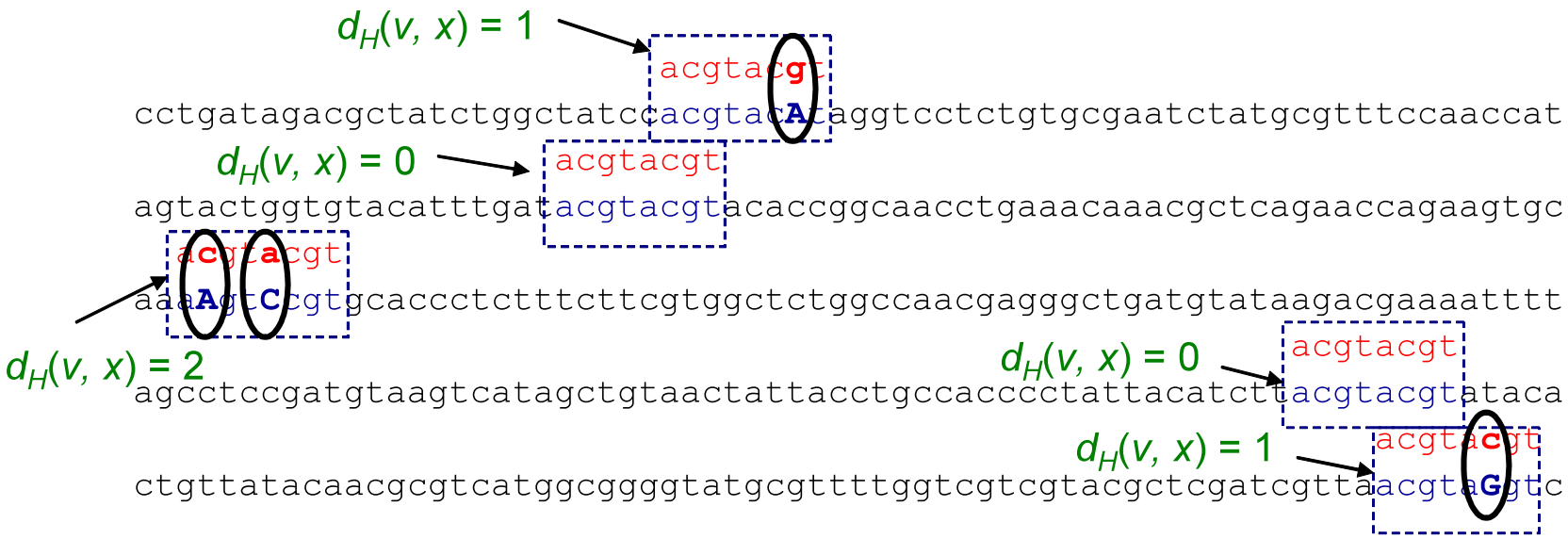



*v* is the sequence in red, *x* is the sequence in blue

- $TotalDistance(v,DNA) = 0$

# Total Distance: An Example

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




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

- $TotalDistance(v,DNA) = 1+0+2+0+1 = 4$

# 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(**v**,**DNA**)* is the sum of the minimum Hamming distances for each DNA sequence $\boldsymbol{i}$
- *TotalDistance(**v**,**DNA**)* = $min_{\mathbf{s}}\, d_H(\boldsymbol{v}, \mathbf{s})$, where $\mathbf{s}$ 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 $t$ x $n$ 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

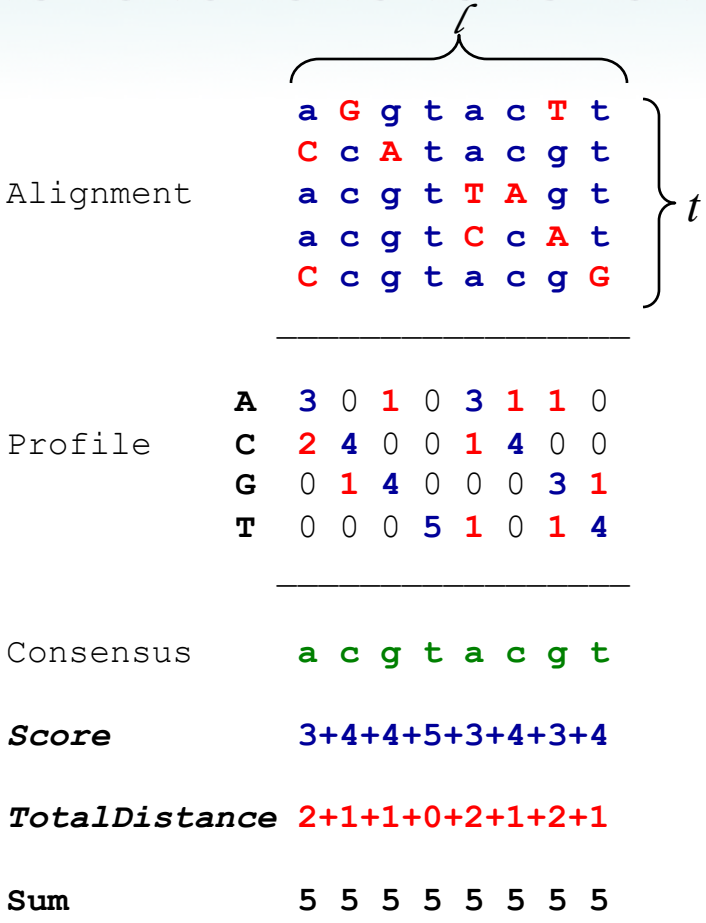
1. MedianStringSearch(DNA, t, n, *l*)
2. bestMotif ← “”
3. bestDistance ← t × *l*
4. for each *l*–mer, s, from “AAA…A” to “TTT…T”
5. if TotalDistance(s, DNA) < bestDistance
6. bestDistance ← TotalDistance(s, DNA)
7. bestMotif ← s
8. return bestMotif

# Are these Equivalent Problems?

- Motif Finding Problem ≡ Median String Problem?
- Note: *Motif Finding* is a maximization problem while *Median String* is a minimization problem
- If *Motif Finding* problem and *Median String* problem are computationally equivalent they must give the same output for a common input
- How do you prove it?

Need to show that minimizing *TotalDistance* is equivalent to maximizing *Score*

# They're looking for the same thing




- At any column $i$
  $Score_i + TotalDistance_i = t$
- Because there are $\ell$ columns
  $Score + TotalDistance = \ell * t$
- Rearranging:
  $Score = \ell * t - TotalDistance$
- $\ell * t$ 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 ($l < t$ and $4 < n$)

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

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

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

# Improving Motif Finding

1. BruteForceMotifSearch(DNA, t, n, $l$)
2. bestScore ← 0
3. for each s = ($s_1$, $s_2$, …, $s_t$) from (1, 1, …, 1)
   to (n- $l$+1, n- $l$+1, …, n- $l$+1)
4. if score(s, DNA, l) > bestScore
5. bestScore ← score(s, DNA, $l$)
6. bestMotif ← ($s_1$, $s_2$, …, $s_t$)
7. return bestMotif

# How to Structure the Search?

- How can we perform the line

for each $s=(s_1,s_2, \ldots, s_t)$ from $(1,1 \ldots 1)$ to $(n-\ell+1, \ldots, n-\ell+1)$ ?

| | | | |
|---|---|---|---|
| (1,1,1,1) | (1,1,2,1) | … (1,2,2,1) | … (60,60,60,1) |
| (1,1,1,2) | (1,1,2,1) | … (1,2,2,2) | … (60,60,60,1) |
| : | : | : | : |
| (1,1,1,60) | (1,1,2,60) | … (1,2,2,60) | … (60,60,60,60) |

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

# Improving Median String

```
1. MedianStringSearch(DNA, t, n, l)
2. bestMotif ← ""
3. bestDistance ← t × l
4. for each l–mer, s, from "aaa…a" to "ttt…t"
5.    if TotalDistance(s, DNA) < bestDistance
6.        bestDistance ← TotalDistance(s, DNA)
7.        bestMotif ← s
8. return bestMotif
```

# How to Best Explore Permutations?

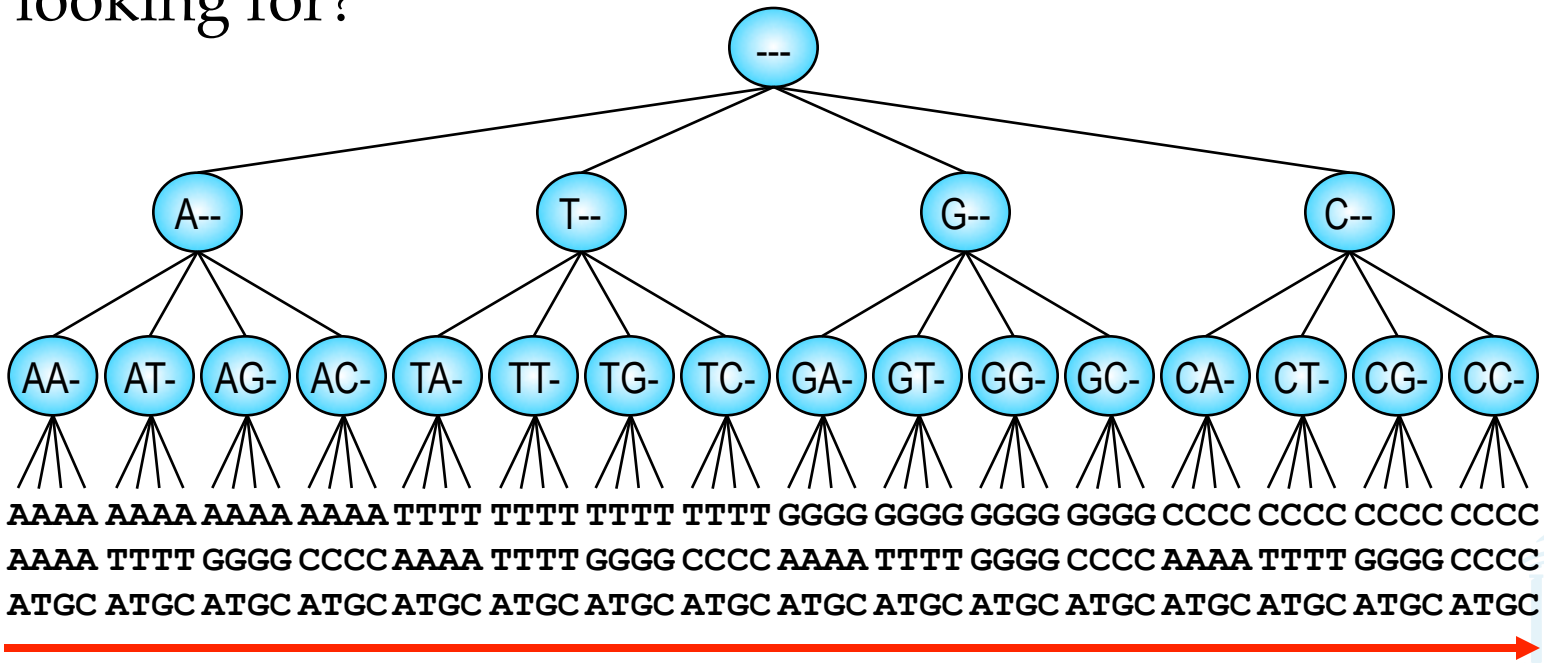
- For the Median String Problem we need to consider all $4^{\ell}$ possible $\ell$-mers:

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

How to organize this search?

# Search Tree

- Our standard method for enumerating permutations can be considered as a traversal of leaf nodes in a search tree
- 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 for many examples thus far (e. g. BruteForceChange)

```
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
```

- Is there another way to search permutations?

# How does NextLeaf work?

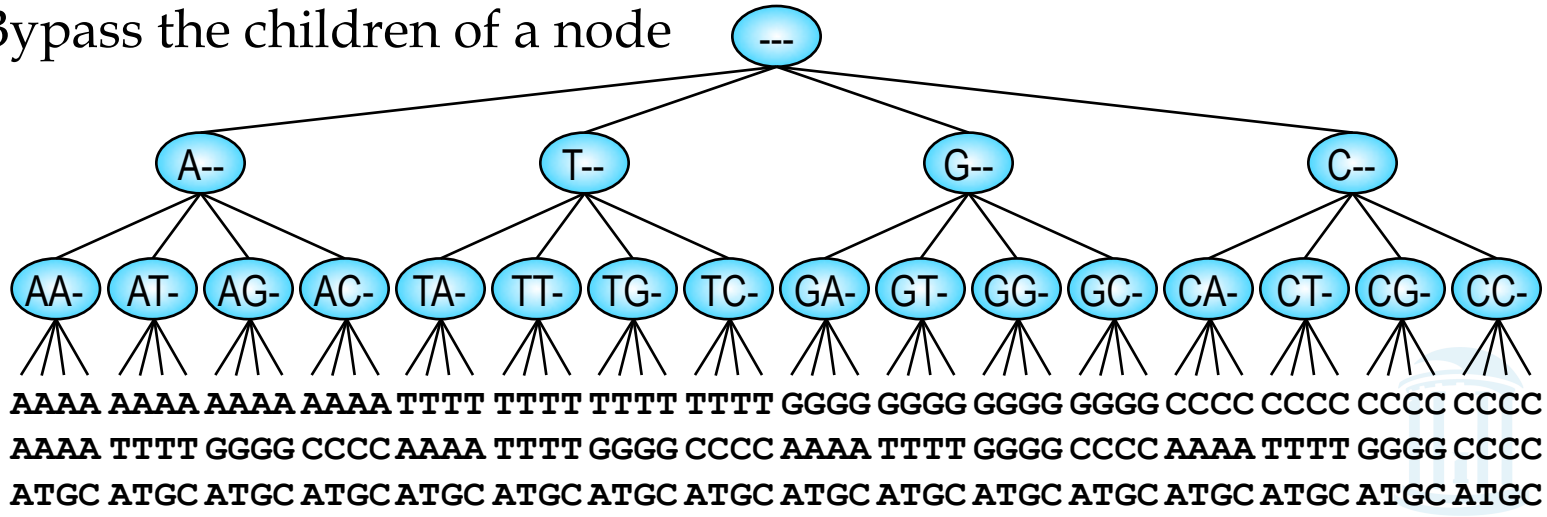
- Code for NextLeaf is the same logic as counting

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

- "a" is the current permutation list ([1,1,1,15,7]), "L" is the # of variables (5 in this case) and "k" is the upper range (60 here)

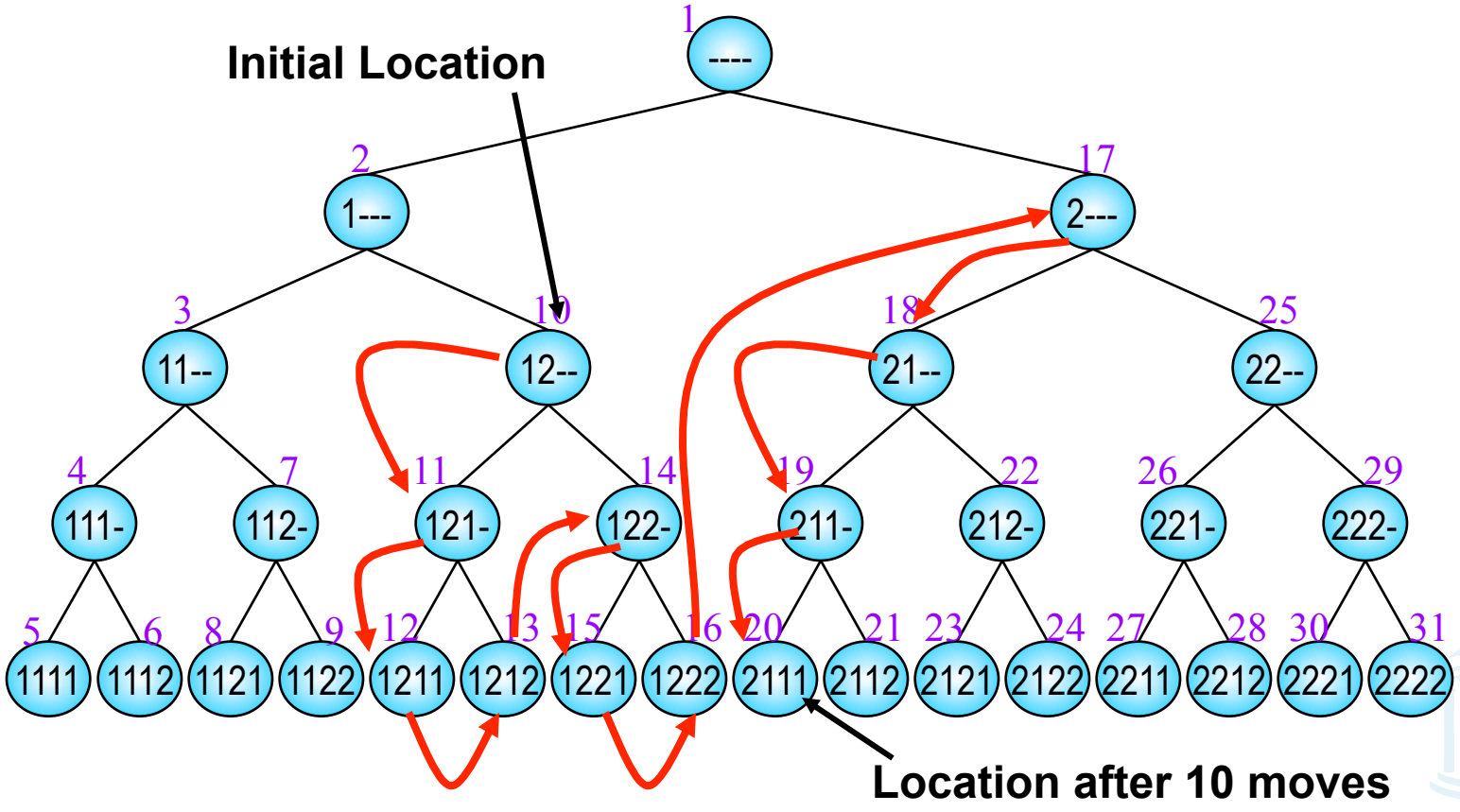
# Analyzing Search Trees

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



# Depth First Traversal

- Start from the root and explore down to the bottom one path at a time, backup and explore unvisited children



# 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):  # if not a leaf
        a[i] = 1 # we go down a level
        return (a, i+1)
    else:        # otherwise count
        for j in reversed(xrange(L)):
            if (a[j] < k):
                a[j] += 1
                return (a, j+1)
            a[j] = 0
    return (a, 0)
```

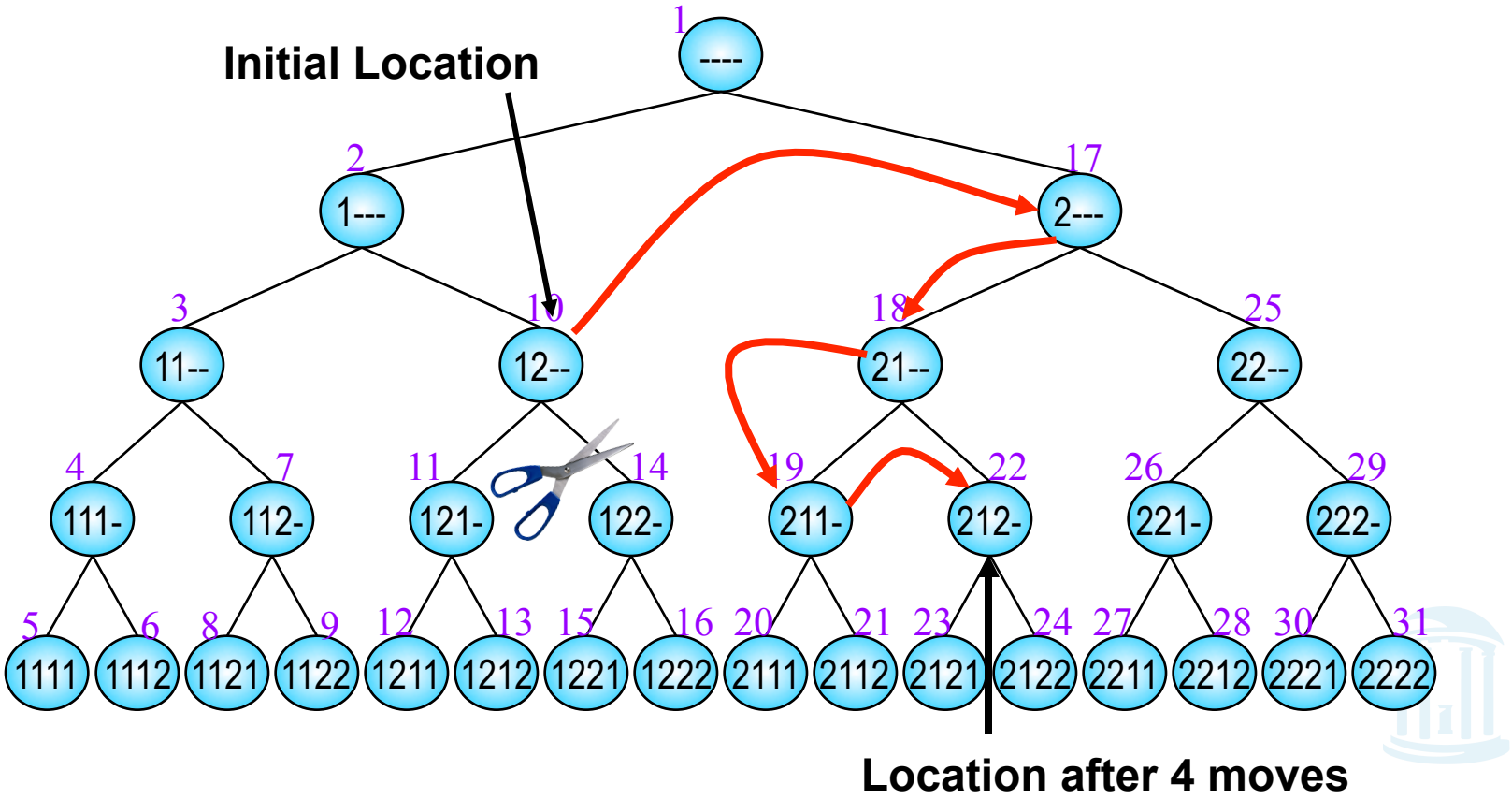
# Bypass Nodes

- Given a prefix (internal vertex), find next vertex 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
- Optimism: if all subsequent *(t-i)* positions ($s_{i+1}$, …$s_t$) match, we'll 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()**

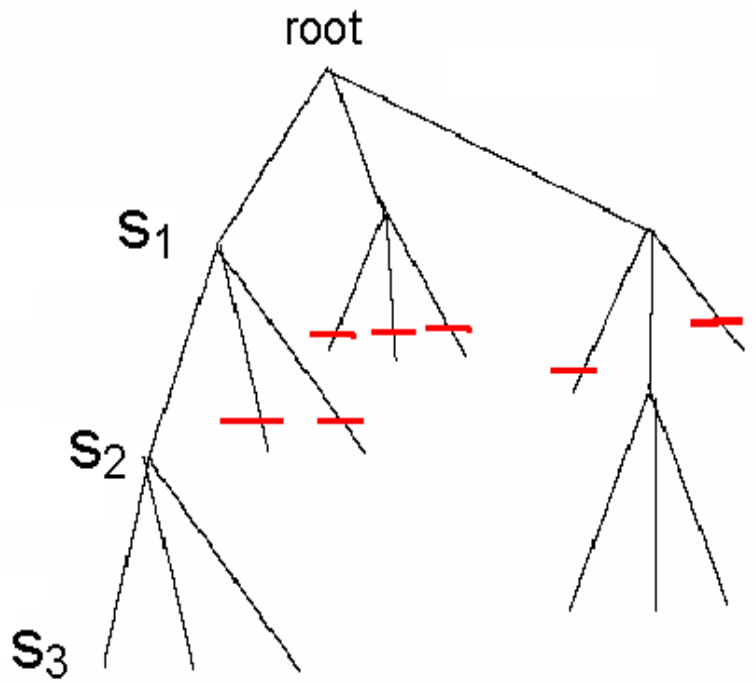
# Rewrite Using Tree Traversal

- 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 $(n-\ell+1)^t$ combinations for **s**.
  - The Median String Problem needs to examine $4^\ell$ combinations of $v$. 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:

  TotalDistance (*prefix*, *DNA*) > *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)
            optimisticDistance = TotalDistance(prefix, DNA)
            if (optimisticDistance > 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
```

# Today's Bad Example

- An embarrassing confession. I got bitten by a bug in the online notes for the book!

```
cctgatagacgctatctggctatccaGGgtacTtaggtcctctgtgcgaatctatgcgtttccaaccat
agtactggtgtacatttgatCCATacgtacaccggcaacctgaaacaaacgctcagaaccagaagtgc
aaacgtTAgtgcaccctctttcttcgtggctctggccaacgagggctgatgtataagacgaaaatttt
agcctccgatgtaagtcatagctgtaactattacctgccacccctattacatcttacgtCCATataca
ctgttatacaacgcgtcatggcggggtatgcgttttggtcgtcgtacgctcgatcgttaCCgtacgGc
```

- The target motif has a consensus score of 30
- But [2, 5, 46, 4, 1] = 31 and [2, 5, 46, 6, 1] = 34
- >30 solutions with consensus of 30 or better
- Which is the real Motif?

# Further Improvements

- More improvements to Motif searching
  - Why just prune based on prefixes? Can you consider suffixes too?
  - Consider a random subset of $t$ strings, or $l$ characters
  - Consider multiple letters at a time?
- How do you really find a TFBS?
  - Multiple answers
  - Near optimal and approximate answers (later on)
  - Motifs are just a starting point
- Next Time
  - We revisit greedy algorithms