Finding Hidden Patterns in DNA
Initiating Transcription

- As a precursor to transcription (the reading of DNA to construct RNAs, that 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 ($10^310^4/10^9$)
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 (TFs) 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.
- A Single TF can influence the expression of many genes. Through biological experiments one can infer, at least a subset of these affected genes.
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 sequence differences by a few bases.
Identifying Motifs: Complications

- We don’t know the motif sequence for every TF
- We don’t know where it is located relative to a gene’s start
- Moreover, motifs can differ slightly from one gene to the next
- We only know that it occurs somewhere near genes that share a TF
- How to discern a Motif’s frequent similar pattern from random patterns?
- How is this problem different that finding frequent k-mers from Lecture 2?
Let's look for an Easy Motif

Problem: Given M sequences of length N find any k-mer that appears in each sequence.

How would you go about finding a 10-mer that appears in every one of these 10 strings?
Sneak Peek at the Answer

1. tagtggtcttttgagtgTAGATCCGAAgggaaagtattttccaccagttcggggtcacccacgcagggcagggtgacttaat
2. cgccgactcggcgcctacacagtatttcgcacgtttagaccaaaacggaggtTAGATCCGAAactggagttaatatcggaatctctt
3. gtatctttgtgagcctgtgTAGATCCGAAatataatgttggctgcatagcggagctgacatagcagtaggagggaaatggt
4. aacatcaggcttttgattaacatattaagcagcTAGATCCGAAttgacctgatgacaataacggaacatgcctccggg
5. accacggataggctgctttatTAGATCCGAAaggtagtatcgtaataatggctcagccatgtcataatgtgcgcaggatccac
6. TAGATCCGAAactgatcgggtttctccccctctgtggtgtaacgagggtcgcacccctgcagcatctgaccggaactttgtaccc
7. gaaatggttccgagtatcaggccgcttacctcttaacctggcggtgTAGATCCGAActgctctgaggggtgctctgcgcta
8. atgtagattcactagactctcaacgcctcagctttattgcgggagacattttgctccactacaagaggctactgtgTAGATCCGAA
9. ttcttaacccctctttTAGATCCGAActgtttggcgcactctttttcagtcctttagctcctcttgatcgtactgac
10. ctacctatgttaaaacaacatctactaacgtagtcgggtcttttcctgtctgcctctctaaccctacaggTAGATCCGAAattcg

Now that you've seen the answer, how would you find it?
Meet Mr Brute Force

- He's often the best starting point when approaching a problem
- He'll also serve as a straw-man when designing new approaches
- Though he's seldom elegant, he gets the job done
- Often, we can't afford to wait for him

For our current problem a brute force solution would consider every k-mer position in all strings and see if they match. Given M sequences of length N, there are:

\[(N-k+1)^M\]

position combinations to consider.

How do you write M nested loops when M is a variable?
A Library of Helper Functions

- There's a tendency to approach this problem with a series of nested for-loops, while the approach is valid, it doesn't generalize. It assumes a specific number of sequences.
- What we need is an iterator that generates all permutations of a sequence.
- This nested-for-loop iterator is called a Cartesian Product over sets.
- Python has a library to accomplish this
Using itertools

**itertools: 3 loops over 2 things**

```python
In [4]:
1  import itertools
2  
3  for number in itertools.product(range(2), repeat=3):
4  print(number, sum(2**(len(number)-i-1)*bit for i, bit in enumerate(number)))
```

```
(0, 0, 0) 0
(0, 0, 1) 1
(0, 1, 0) 2
(0, 1, 1) 3
(1, 0, 0) 4
(1, 0, 1) 5
(1, 1, 0) 6
(1, 1, 1) 7
```

**itertools: 2 loops over 3 things**

```python
In [4]:
1  for number in itertools.product(range(3), repeat=2):
2  print(number)
```

```
(0, 0)
(0, 1)
(0, 2)
(1, 0)
(1, 1)
(1, 2)
(2, 0)
(2, 1)
(2, 2)
```
Permutations of mixed types

```
In [14]: for section in itertools.product(("I", "II", "III", "IV"), "ABC", range(1,3)):
    print(section)

('I', 'A', 1)
('I', 'A', 2)
('I', 'B', 1)
('I', 'B', 2)
('I', 'C', 1)
('I', 'C', 2)
('II', 'A', 1)
('II', 'A', 2)
('II', 'B', 1)
('II', 'B', 2)
('II', 'C', 1)
('II', 'C', 2)
('III', 'A', 1)
('III', 'A', 2)
('III', 'B', 1)
('III', 'B', 2)
('III', 'C', 1)
('III', 'C', 2)
('IV', 'A', 1)
('IV', 'A', 2)
('IV', 'B', 1)
('IV', 'B', 2)
('IV', 'C', 1)
('IV', 'C', 2)
```
Bruteforce Exact Search

```python
In [15]:
sequences = [
    'tagtggtccccagttcagttagctccccccaaggggtaagtttttctcaccacaggttttgcagaggtgacttaat',
    'cgcgcctgcccatcagttacgctttagacacaaaacgagtttagcatccccagagctctggttttaattctcttcttt','
    'gattgccttgagccctggactaatgtcattatgtcagcctggctcagcttagctgacactgcagacagttgaggaatgct',
    'aaccacaggttagtctagctttattaaggtagatcagccagttttaactgagttagctcagcctggctgctcggg',
    'accacccgagagtctgctttattatagatcagccagacttttagactatgtgtcagcctagccttacac',
    'tagctagcagatcggctttctctcttctggttacacgaggggctccaggtttcctctctcgtgacactgcagacagttgaggaatgct',
    'aaccacaggttagtctagctttattaaggtagatcagccagttttaactgagttagctcagcctggctgctcggg',
    'accacccgagagtctgctttattatagatcagccagacttttagactatgtgtcagcctagccttacac',
    'tagctagcagatcggctttctctcttctggttacacgaggggctccaggtttcctctctcgtgacactgcagacagttgaggaatgct',
    'aaccacaggttagtctagctttattaaggtagatcagccagttttaactgagttagctcagcctggctgctcggg',
    'accacccgagagtctgctttattatagatcagccagacttttagactatgtgtcagcctagccttacac',
    'tagctagcagatcggctttctctcttctggttacacgaggggctccaggtttcctctctcgtgacactgcagacagttgaggaatgct',
    'aaccacaggttagtctagctttattaaggtagatcagccagttttaactgagttagctcagcctggctgctcggg',
    'accacccgagagtctgctttattatagatcagccagacttttagactatgtgtcagcctagccttacac',
    'tagctagcagatcggctttctctcttctggttacacgaggggctccaggtttcctctctcgtgacactgcagacagttgaggaatgct',
    'aaccacaggttagtctagctttattaaggtagatcagccagttttaactgagttagctcagcctggctgctcggg',
    'accacccgagagtctgctttattatagatcagccagacttttagactatgtgtcagcctagccttacac',
    'tagctagcagatcggctttctctcttctggttacacgaggggctccaggtttcctctctcgtgacactgcagacagttgaggaatgct',
]

def bruteForce(dna,k):
    """Finds a *k*-mer common to all sequences from a list of *dna* fragments with the same length"""
    M = len(dna)        # how many sequences
    N = len(dna[0])     # length of sequences
    for offset in itertools.product(range(N-k+1), repeat=M):
        for i in range(1,len(offset)):
            if dna[0][offset[0]:offset[0]+k] != dna[i][offset[i]:offset[i]+k]:
                break
        else:
            return offset, dna[0][offset[0]:offset[0]+10]
```

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Now let’s Test and Time it

In [16]:
M = 4
position, motif = bruteForce(sequences[0:M], 10)
print(position, motif, '
')

for i in range(M):
    p = position[i]
    print(sequences[i][p:]+sequences[i][p+10:].upper()+sequences[i][p+10:]):
print()

%timeit bruteForce(sequences[0:M], 10)
# you can try a larger value of M, but be prepared to wait

(17, 47, 18, 33) tagatccgaa
tagtggctttttgagtagTAGATCCGAAagggaaagtatatttccaccagttcgggtacaccgcagggcaggtgaccttaat
cgggacgcggggctcacagtattatatcgacgttttagaccaaaaccggagtTAAGATCCGAAactgggtttaatcggaggtccttt
gttacctttagacgcttggtTAGATCCGAAatatataatttggtgtgctgcatacgagctgacatagcaggtaggggaatgcgt
aacatcagggctttgattaaacaatattaagcagTAGATCCGAAttgacctgtgataatcaggaacatatggcgggtccggg

6.25 s ± 143 ms per loop (mean ± std. dev. of 7 runs, 1 loop each)
Approximate Matching

Now let's consider a more realistic motif finding problem, where the binding sites do not need to match exactly.

Actually, *none* of the sequences have an unmodified copy of the original motif.
Profile and Consensus

How to find approximate string matches?

- Align candidate motifs by their start indexes
  \[ s = (s_1, s_2, \ldots, s_t) \]

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

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

Alignment:
- \( a \quad G \quad g \quad t \quad a \quad c \quad T \quad t \)
- \( C \quad c \quad A \quad t \quad a \quad c \quad g \quad t \)

Profile:
- \( A \quad 3 \quad 0 \quad 1 \quad 0 \quad 3 \quad 1 \quad 1 \quad 0 \)
- \( C \quad 2 \quad 4 \quad 0 \quad 0 \quad 1 \quad 4 \quad 0 \quad 0 \)
- \( G \quad 0 \quad 1 \quad 4 \quad 0 \quad 0 \quad 0 \quad 3 \quad 1 \)
- \( T \quad 0 \quad 0 \quad 0 \quad 5 \quad 1 \quad 0 \quad 1 \quad 4 \)

Consensus:
- \( A \quad C \quad G \quad T \quad A \quad C \quad G \quad T \)
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.

\[\begin{align*}
\text{G} & \quad \text{A} & \quad \text{G} & \quad \text{A} & \quad \text{T} & \quad \text{C} & \quad \text{A} & \quad \text{T} \\
\text{X} & \quad & \text{X} & \quad & & \\
\text{T} & \quad \text{A} & \quad \text{G} & \quad \text{A} & \quad \text{C} & \quad \text{G} & \quad \text{C} & \quad \text{A} & \quad \text{T}
\end{align*}\]

A Hamming distance of 2.
Consensus Properties

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

- Given \( s = (s_1, s_2, \ldots, s_t) \) and DNA

\[
Score(s, DNA) = \sum_{i=1}^{k} \max_{j \in \{A,C,G,T\}} \text{count}(j, i)
\]

- So our approach is back to brute force
  - We consider every candidate motif in every string
  - Return the set of indices with the highest score

\[
\begin{array}{cccccccc}
A & 3 & 0 & 1 & 0 & 3 & 1 & 1 & 0 \\
C & 2 & 4 & 0 & 0 & 1 & 4 & 0 & 0 \\
G & 0 & 1 & 4 & 0 & 0 & 0 & 3 & 1 \\
T & 0 & 0 & 0 & 5 & 1 & 0 & 1 & 4 \\
\end{array}
\]

Consensus \( a \ c \ g \ t \ a \ c \ g \ t \)

\[
\text{Score} = 3+4+4+5+3+4+3+4 = 30
\]
Let's try again allowing for errors

```python
In [17]: def Score(s, DNA, k):
     """
     compute the consensus SCORE of a given k-mer alignment given
     offsets into each DNA string. s = list of starting indices.
     DNA = list of nucleotide strings. k = Target Motif length
     """
     score = 0
     for i in range(k):
         # loop over string positions
         cnt = dict(zip("acgt",(0,0,0,0)))
         for j, sval in enumerate(s):
             base = DNA[j][sval+1]
             cnt[base] += 1
             score += max(cnt.values())
     return score

def BruteForceMotifSearch(dna,k):
    M = len(dna)  # how many sequences
    N = len(dna[0])  # length of sequences
    bestScore = 0
    bestAlignment = []
    for offset in itertools.product(range(N-k+1), repeat=M):
        s = Score(offset,dna,k)
        if (s > bestScore):
            bestAlignment = [p for p in offset]
            bestScore = s
    print(bestAlignment, bestScore)
```
Test and time this one

```
In [13]: seqApprox = [
    ...:   'tagtggtcttcttgagtagtagatctgaaagagaagtaattctaccacagtctggggtacccagacgagggggtgacttaat',
    ...:   'cgcgactcggcgctcagttatcgcacgtttagaccakaacaacgggaagttggatccggaaactggagtttaacgctgcctt',
    ...:   'gttactttggacgctttggttagacgcgaaatattaatgttgctgcatagcagagctacagtagagggagaatagct',
    ...:   'aacacaaggcttggattaaaccataatttaagcaggttaatccgaaatgtgacctctgatgacaataacgggaacatgccagtcggtcggg',
    ...:   'accacccgtagtagttccctttttaggcctaaagagttagatgcgtaataatggtgctacgcatctgtaatgtgctgccatcctcacc',
    ...:   'tagattcgaatcggatctgtttctctctctttggtttaaagagggggtccagaccttgctgcactgtgcggcagcttacc',
    ...:   'ggtaaatgttacggtgcgaatacggcgctttttaacacttgctgtcgatccggaaactggatctttcttgcattgtacgctgcgta',
    ...:   'agttatactagacattcagctcthtattggtgacgagcttttttttttcttctttcttctgatctcttcttgatcgcacgcaacag',
    ...:   'ctactcattgtaaaacacatctactaagctgatcgcggtctttttctgtgatcgcctaatctacacggtcagtcgctacgaggtctacc',
]
```

```
%timeit Score([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], seqApprox, 10)
%time BruteForceMotifSearch(seqApprox[:4], 10)
```

47.4 µs ± 5.52 µs per loop (mean ± std. dev. of 7 runs, 10000 loops each)

[17, 47, 18, 33] 36
CPU times: user 12min 57s, sys: 50.2 ms, total: 12min 57s
Wall time: 12min 57s
Running Time of BruteForceMotifSearch

- Search \((N - k + 1)\) positions in each of \(M\) sequences, by examining \((N - k + 1)^M\) sets of starting positions
- For each set of starting positions, the scoring function makes \(O(Mk)\) operations, so the complexity is:

\[
Mk(N-k+1)^M = O(MkN^M)
\]

- That means that for \(M = 10, N = 80, k = 10\) we must perform approximately \(10^{21}\) computations
- Generously assuming \(10^9\) comps/sec it will require only \(10^{12}\) secs

\[
\frac{10^{12}}{(60 \times 60 \times 24 \times 365)} > 30000 \text{ years}
\]

- Want to wait?
How conservative is this estimate?

- For the example we just did $M = 4, N = 80, k = 10$
- So that gives $\approx 4.0 \times 10^9$ operations
- Using our $10^9$ operations per second estimate, it should have taken only 4 secs.
- Instead it took closer to 700 secs, which suggests we are getting around 5.85 million operations per second.
- So, in reality it will even take longer!
How can we find Motifs in our lifetime?

- Should we give up on Python and write in C? Assembly Language?
- Will biological insights save us this time?
- Are there other ways to find Motifs?
- Consider that if you knew what motif you were looking for, it would take only 
  
  \[ k(N-k+1)M = O(kNM) \]

  to find its indices in each string.

- Is that significantly better?