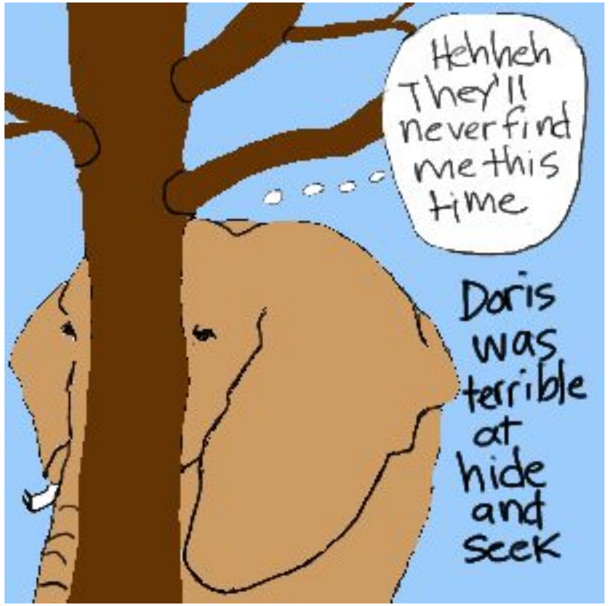
Comp 555 - BioAlgorithms - Spring 2018


Hehheh They'll never find me this time
Doris was terrible at hide and seek

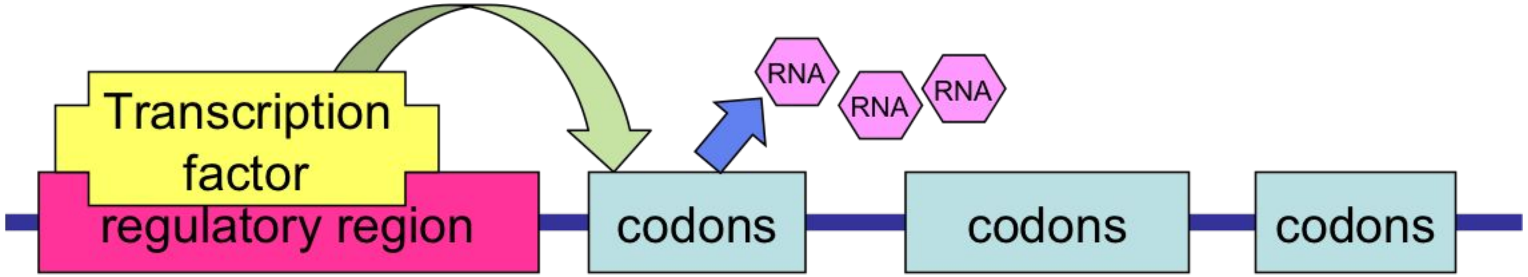
Finding Hidden Patterns in DNA

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 ($10^3 10^4/10^9$)

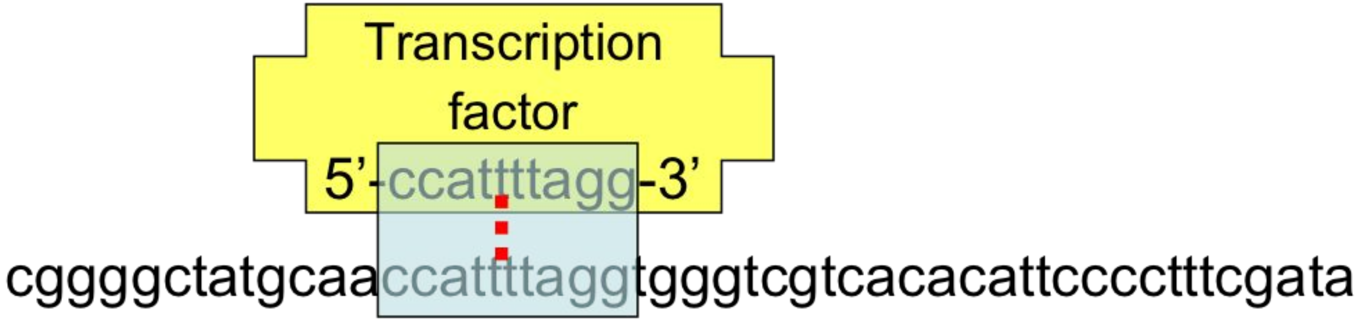
Regulatory Regions

- 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 (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

```
 1 tagtggtcttttgagtgtagatccgaagggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat
 2 cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagttagatccgaaactggagtttaatcggagtcctt
 3 gttacttgtgagcctggttagatccgaaatataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt
 4 aacatcaggctttgattaaacaatttaagcacgtagatccgaattgacctgatgacaatacggaacatgccggctccggg
 5 accaccggataggctgcttattagatccgaaaggtagtatcgtaataatggctcagccatgtcaatgtgcggcattccac
 6 tagatccgaatcgatcgtgtttctccctctgtgggttaacgaggggtccgaccttgctcgcatgtgccgaacttgtaccc
 7 gaaatggttcggtgcgatatcaggccgttctcttaacttggcggtgtagatccgaacgtctctggaggggtcgtgcgcta
 8 atgtatactagacattctaacgctcgcttattggcggagaccatttgctccactacaagaggctactgtgtagatccgaa
 9 ttcttacacccttctttagatccgaacctgttggcgccatcttcttttcgagtccttgtacctccatttgctctgatgac
10 ctacctatgtaaaacaacatctactaacgtagtccggtctttcctgatctgccctaacctacaggtagatccgaaattcg
```

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 tagtggtcttttgagtgTAGATCCGAAgggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat
 2 cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagtTAGATCCGAAactggagtttaatcggagtcctt
 3 gttacttgtgagcctggtTAGATCCGAAatataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt
 4 aacatcaggctttgattaaacaatttaagcacgTAGATCCGAAttgacctgatgacaatacggaacatgccggctccggg
 5 accaccggataggctgcttatTAGATCCGAAaggtagtatcgtaataatggctcagccatgtcaatgtgcggcattccac
 6 TAGATCCGAAtcgatcgtgtttctccctctgtgggttaacgaggggtccgaccttgctcgcatgtgccgaacttgtaccc
 7 gaaatggttcggtgcgatatcaggccgttctcttaacttggcggtgTAGATCCGAAcgtctctggaggggtcgtgcgcta
 8 atgtatactagacattctaacgctcgcttattggcggagaccatttgctccactacaagaggctactgtgTAGATCCGAA
 9 ttcttacacccttcttTAGATCCGAAcctgttggcgccatcttcttttcgagtccttgtacctccatttgctctgatgac
10 ctacctatgtaaaacaacatctactaacgtagtccggtctttcctgatctgccctaacctacaggTAGATCCGAAattcg
```

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:

$$(N-k+1)^M$$

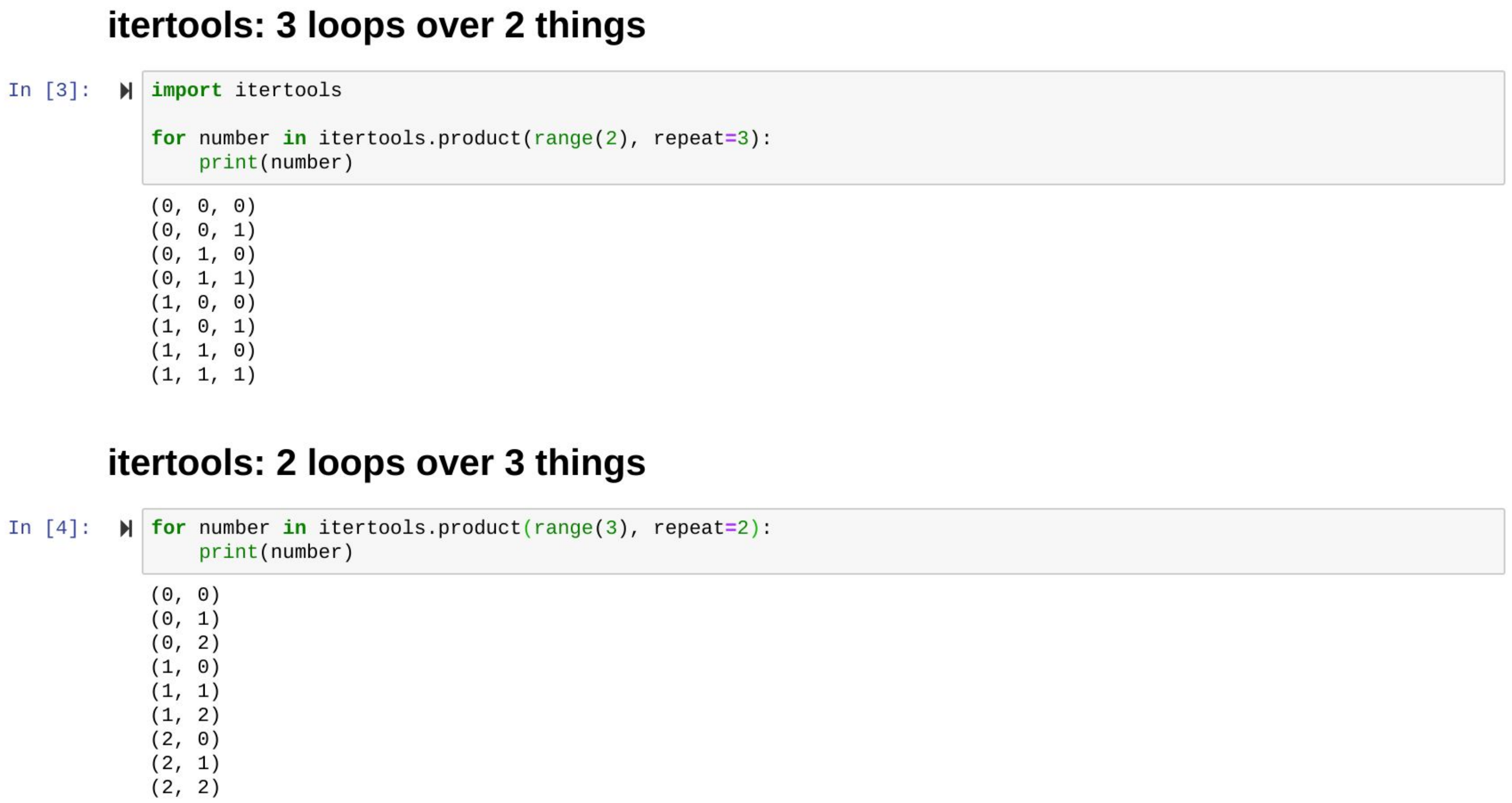
position combinations to consider.

How do you write M nested loops when M is a variable?

A Library of Helper Functions

- There's a tendancy 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

In [3]:

```
import itertools

for number in itertools.product(range(2), repeat=3):
    print(number)
```

```
(0, 0, 0)
(0, 0, 1)
(0, 1, 0)
(0, 1, 1)
(1, 0, 0)
(1, 0, 1)
(1, 1, 0)
(1, 1, 1)
```

itertools: 2 loops over 3 things

In [4]:

```
for number in itertools.product(range(3), repeat=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)
```

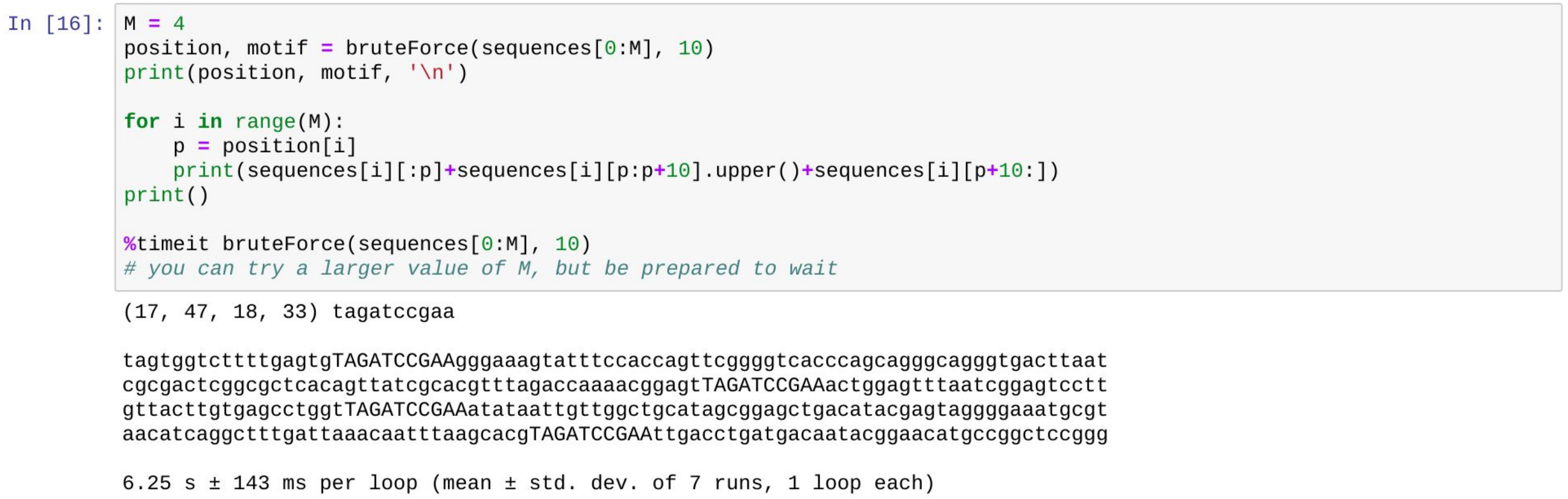
```
('I', 'A', 1)
('I', 'A', 2)
('I', 'B', 1)
('I', 'B', 2)
('I', 'C', 1)
('I', 'C', 2)
('II', 'A', 1)
('II', 'A', 2)
('II', 'B', 1)
('II', 'B', 2)
('II', 'C', 1)
('II', 'C', 2)
('III', 'A', 1)
('III', 'A', 2)
('III', 'B', 1)
('III', 'B', 2)
('III', 'C', 1)
('III', 'C', 2)
('IV', 'A', 1)
('IV', 'A',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

Bruteforce Exact Search

```
In [15]: sequences = [
             'tagtggtcttttgagtgtagatccgaagggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat',
             'cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagttagatccgaaactggagtttaatcggagtcctt',
             'gttacttgtgagcctggttagatccgaaatataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt',
             'aacatcaggctttgattaaacaatttaagcacgtagatccgaattgacctgatgacaatacggaacatgccggctccggg',
             'accaccggataggctgcttattagatccgaaaggtagtatcgtaataatggctcagccatgtcaatgtgcggcattccac',
             'tagatccgaatcgatcgtgtttctccctctgtgggttaacgaggggtccgaccttgctcgcatgtgccgaacttgtaccc',
             'gaaatggttcggtgcgatatcaggccgttctcttaacttggcggtgtagatccgaacgtctctggaggggtcgtgcgcta',
             'atgtatactagacattctaacgctcgcttattggcggagaccatttgctccactacaagaggctactgtgtagatccgaa',
             'ttcttacacccttctttagatccgaacctgttggcgccatcttcttttcgagtccttgtacctccatttgctctgatgac',
             'ctacctatgtaaaacaacatctactaacgtagtccggtctttcctgatctgccctaacctacaggtagatccgaaattcg']

         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]
```

Now let’s Test and Time it



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

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

tagtggtcttttgagtgTAGATCCGAAgggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat
cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagtTAGATCCGAAactggagtttaatcggagtcctt
gttacttgtgagcctggtTAGATCCGAAatataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt
aacatcaggctttgattaaacaatttaagcacgTAGATCCGAAttgacctgatgacaatacggaacatgccggctccggg

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.

```
 1 tagtggtcttttgagtgTAGATCTGAAgggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat
 2 cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagtTGGATCCGAAactggagtttaatcggagtcctt
 3 gttacttgtgagcctggtTAGACCCGAAatataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt
 4 aacatcaggctttgattaaacaatttaagcacgTAAATCCGAAttgacctgatgacaatacggaacatgccggctccggg
 5 accaccggataggctgcttatTAGGTCCAAAaggtagtatcgtaataatggctcagccatgtcaatgtgcggcattccac
 6 TAGATTCGAAtcgatcgtgtttctccctctgtgggttaacgaggggtccgaccttgctcgcatgtgccgaacttgtaccc
 7 gaaatggttcggtgcgatatcaggccgttctcttaacttggcggtgCAGATCCGAAcgtctctggaggggtcgtgcgcta
 8 atgtatactagacattctaacgctcgcttattggcggagaccatttgctccactacaagaggctactgtgTAGATCCGTA
 9 ttcttacacccttctttTAGATCCAAAcctgttggcgccatcttcttttcgagtccttgtacctccatttgctctgatgac
10 ctacctatgtaaaacaacatctactaacgtagtccggtctttcctgatctgccctaacctacaggTCGATCCGAAattcg
```

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

$$s = (s_1, s_2, \dots, s_t)$$

- Construct a matrix profile with the frequencies of each nucleotide in columns
- Consensus nucleotide in each position has the highest score in each column

		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

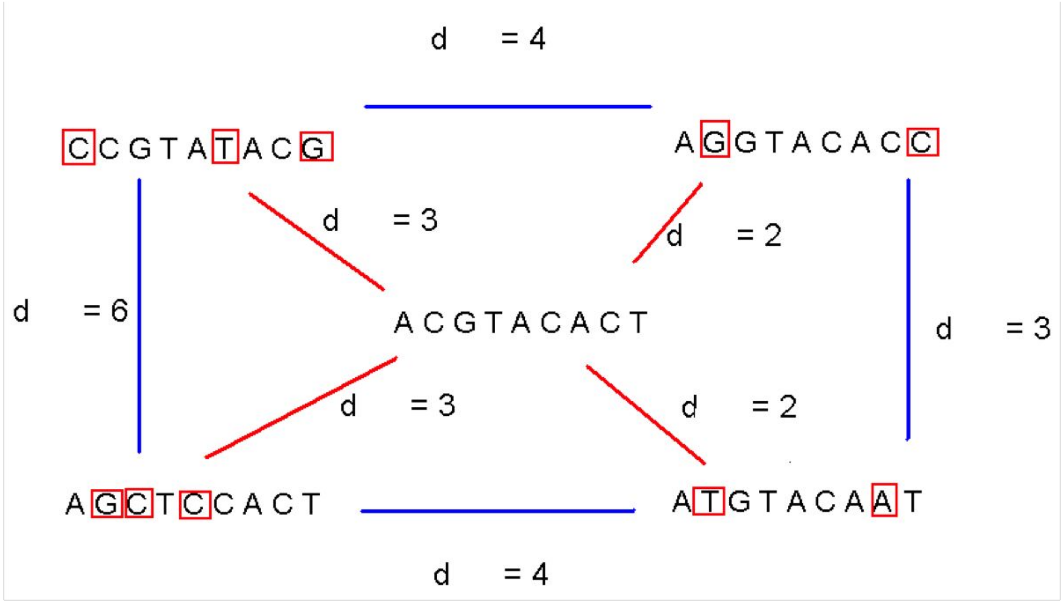
Consensus

- 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
```

Consensus Properties

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

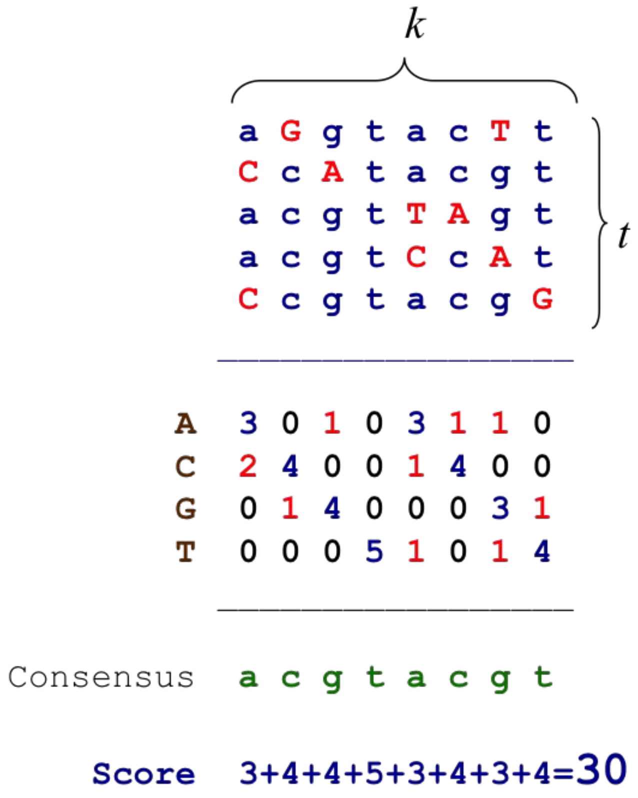


Scoring Motifs

- **Given s = (s_1, s_2, … , s_t) and DNA**

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

- **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



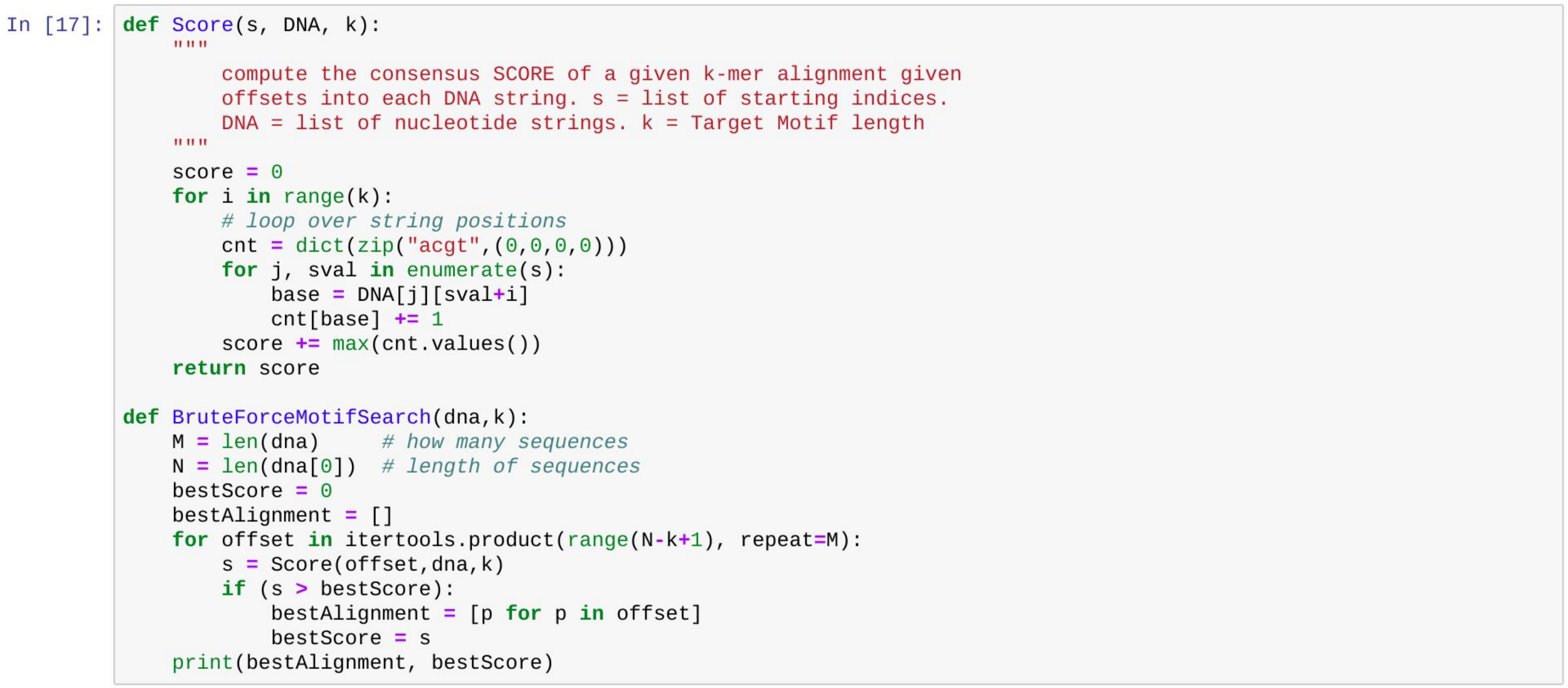
```
a G g t a c T t
C c A t a c g t
a c g t T A g t
a c g t C c A t
C c g t a c g G
```

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

Let's try again allowing for errors



```
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+i]
                     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 = [
             'tagtggtcttttgagtgtagatctgaagggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat',
             'cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagttggatccgaaactggagtttaatcggagtcctt',
             'gttacttgtgagcctggttagacccgaaatataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt',
             'aacatcaggctttgattaaacaatttaagcacgtaaatccgaattgacctgatgacaatacggaacatgccggctccggg',
             'accaccggataggctgcttattaggtccaaaaggtagtatcgtaataatggctcagccatgtcaatgtgcggcattccac',
             'tagattcgaatcgatcgtgtttctccctctgtgggttaacgaggggtccgaccttgctcgcatgtgccgaacttgtaccc',
             'gaaatggttcggtgcgatatcaggccgttctcttaacttggcggtgcagatccgaacgtctctggaggggtcgtgcgcta',
             'atgtatactagacattctaacgctcgcttattggcggagaccatttgctccactacaagaggctactgtgtagatccgta',
             'ttcttacacccttctttagatccaaacctgttggcgccatcttcttttcgagtccttgtacctccatttgctctgatgac',
             'ctacctatgtaaaacaacatctactaacgtagtccggtctttcctgatctgccctaacctacaggtcgatccgaaattcg']

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

$$Mk(N-k+1)^M = O(MkN^M)$$

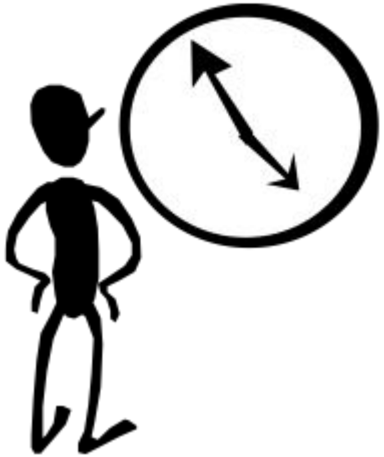
- That means that for *$M = 10$, $N = 80$, $k = 10$* we must perform approximately *10^{21}* computations
- Generously assuming *10^9* comps/sec it will require only *10^{12}* secs

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

- Want to wait?

How conservative is this estimate?

- For the example we just did M = 4, N = 80, k = 10
- So that gives $\approx 4.0\times10^9$ 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?