Finding TFBS Motifs in our Lifetime

- Recall from last time that the *Brute Force* approach for finding a common 10-mer motif common to 10 sequences of length 80 bases was going to take up roughly 30,000 years
- Today well consider alternative and non-obvious approaches for solving this problem
- We will trade one old man (us) for another (an Oracle)

Recall from last Lecture

- The following set of 10 sequences have an embedded noisy motif, *TAGATCCGAA*.

```
 1 tagtggtcttttgagtg TAGATCTGAA gggaaagtatttccaccagttcggggtcacccagcagggcagggtgacttaat   TAGATCTGAA
 2 cgcgactcggcgctcacagttatcgcacgtttagaccaaaacggagt TGGATCCGAA actggagtttaatcggagtcctt   TGGATCCGAA
 3 gttacttgtgagcctggt TAGACCCGAA atataattgttggctgcatagcggagctgacatacgagtaggggaaatgcgt   TAGACCCGAA
 4 aacatcaggctttgattaaacaatttaagcacg TAAATCCGAA ttgacctgatgacaatacggaacatgccggctccggg   TAAATCCGAA
 5 accaccggataggctgcttat TAGGTCCAAA aggtagtatcgtaataatggctcagccatgtcaatgtgcggcattccac   TAGGTCCAAA
 6 TAGATTCGAA tcgatcgtgtttctccctctgtgggttaacgaggggtccgaccttgctcgcatgtgccgaacttgtaccc   TAGATTCGAA
 7 gaaatggttcggtgcgatatcaggccgttctcttaacttggcggtg CAGATCCGAA cgtctctggaggggtcgtgcgcta   CAGATCCGAA
 8 atgtatactagacattctaacgctcgcttattggcggagaccatttgctccactacaagaggctactgtg TAGATCCGTA   TAGATCCGTA
 9 ttcttacacccttctt TAGATCCAAA cctgttggcgccatcttcttttcgagtccttgtacctccatttgctctgatgac   TAGATCCAAA
10 ctacctatgtaaaacaacatctactaacgtagtccggtctttcctgatctgccctaacctacagg TCGATCCGAA attcg   TCGATCCGAA
                                                                                        9+9+9+9+9
                                                                                        +8+9+9+8+10
                                                                                        = 89
```

Some notes:

1. There are no exact matches
2. The consensus motif gives a good score

Consensus Scoring Function

- We developed a consensus scoring function to address noise
- But, we needed to apply it an exponential number, $O(N^M)$ of times!
- Here's the scoring function...

```
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, 1-based, 0 means ignore
            DNA = list of nucleotide strings
            k = Target Motif length
    """
    score = 0
    for i in xrange(k):
        # loop over string positions
        cnt = dict(zip("acgt",(0,0,0,0)))
        for j, sval in enumerate(s):
            # loop over DNA strands
            base = DNA[j][sval+i]
            cnt[base] += 1
        score += max(cnt.itervalues())
    return score
```

And here's the Score we're looking for...

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

```
print Score([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], seqApprox, 10)
```

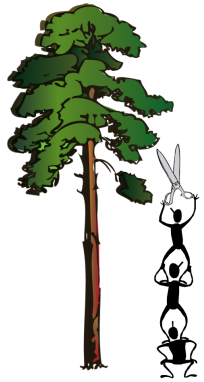
```
89
```

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

```
10000 loops, best of 3: 39.9 µs per loop
```

So even at a blazing $40\mu s$ we'll need many lifetimes to compute the 70^{10} scores

Pruning Trees



• One method for reducing the computational cost of a search algorithm is to *prune* the space of permutations that could not possibly lead to a better answer than the current best answer.

• Pruning decisions are based on ***solutions to subproblems*** that appear early on and offer no hope

• How does this apply to our Motif finding problem?

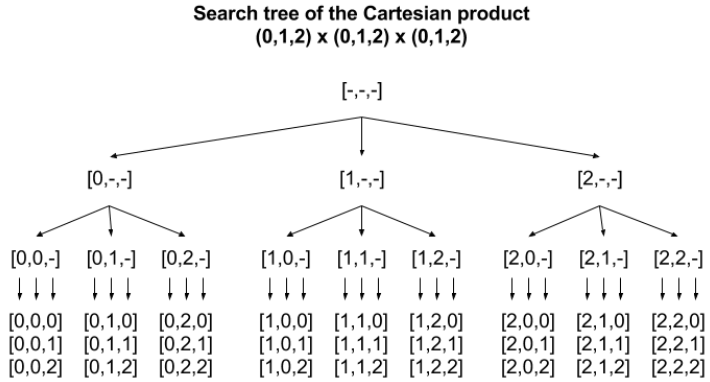
Consider any permutation of offsets that begins with the indices [25, 63, 10, 43, ...]. Just based on the first 4 indices the largest possible score is 17 + 6*10 = 87, which assumes that all 6 remaining strings match perfectly at all 10 positions.

```
DNA[0][25:35]        a  a  g  g  g  a  a  a  g  t
DNA[1][63:73]        g  t  t  t  a  a  t  c  g  g
DNA[2][10:20]        a  g  c  c  t  g  g  t  t  a
DNA[3][43:53]        t  t  g  a  c  c  t  g  a  t
                 a [2, 1, 0, 1, 1, 2, 1, 1, 1, 1]
      Profile    c [0, 0, 1, 1, 1, 1, 0, 1, 0, 0]
                 g [1, 1, 2, 1, 1, 1, 1, 1, 2, 1]
                 t [1, 2, 1, 1, 1, 0, 2, 1, 1, 2]
                   [2, 2, 2, 1, 1, 2, 2, 1, 2, 2] Score = 17
```

If the best answer so far is 89, there is no need to consider the 70^6 offset permuations that start with these 4 indices.

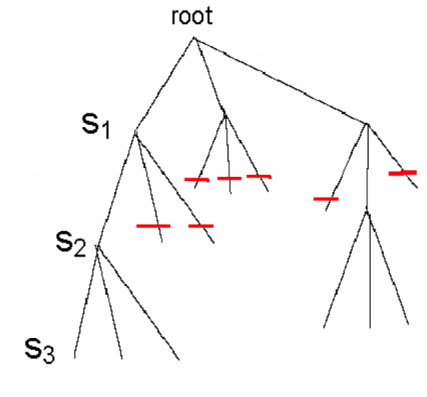
Search Trees

- Our standard method for enumerating permutations can be considered as a traversal of leaf nodes in a search tree
- Suppose after checking the first few offsets could know already that *any* score of children nodes could not beat the best score seen so far?



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 $(N-k+1)^{t-depth}$ leaves
- Note our enumeration of tree-branches is *depth-first*
- We'll formulate of trimming algorithm as a recursive algorithm



A Recursive Exploration of a Search Tree

```
bestAlignment = []
prunedPaths = 0

def exploreMotifs(DNA,k,path,bestScore):
    """ Search for a k-length motif in the list of DNA sequences by exploring
        all paths in a search tree. Each call extends path by one. Once the
        path reaches the number of DNA strings a score is computed. """
    global bestAlignment, prunedPaths
    depth = len(path)
    M = len(DNA)
    if (depth == M):            # here we have an index in all M sequences
        s = Score(path,DNA,k)
        if (s > bestScore):
            bestAlignment = [p for p in path]
            return s
        else:
            return bestScore
    else:
        # Let's consider if an optimistic best score can beat the best score so far
        if (depth > 1):
            OptimisticScore = k*(M-depth) + Score(path,DNA,k)
        else:
            OptimisticScore = k*M
        if (OptimisticScore < bestScore):
```

```
[17, 47, 18, 33, 21, 0] 53 8615931
CPU times: user 5min 17s, sys: 533 ms, total: 5min 17s
Wall time: 5min 17s
```

Observations

- For our problem instance, Branch-and-Bound Motif finding is significantly faster
  - It found a motif in the first 6 strings in less time than the Brute Force approach found a solution in the first 4 strings
  - More than $70^2 \approx 5000$ times faster
  - It did so by trimming more than 8 Million paths
  - Trimming added extra calls to Score (basically doubling the worst-case number of calls), but ended up saving even more *hopeless* calls along longer paths.
  - In practice, Branch-and-Bound, significantly improved the average performance
- Does this improve the worst-case performance from $O(kN^M)$?
  - What if all of our motifs were found at the end of each DNA string?
  - How do we avoid these worse case data sets?
  - Randomize the search-tree tranversal order

We need a new approach

- Enumerating every possible permuation of motif positions is *still* not getting us the speed we want.
- Let's try another tried-and-tested approach to algorithm design, *mixing up the problem*
  - Suppose that some *Oracle* could tell us what the motif is
  - How long would it take us to find its position in each string?
  - We could compute the Hamming Distance from our given motif to the k-mer at every position of each DNA sequence and keep track of the smallest distance and its position on each string.
  - These positions are our best guess of where the motif can be found on each string
- Let's call this approach *scanning-and-scoring* to find a given motif.

Scanning-and-Scoring a Motif

```
def ScanAndScoreMotif(DNA, motif):
    totalDist = 0
    bestAlignment = []
    k = len(motif)
    for seq in DNA:
        minHammingDist = k+1
        for s in xrange(len(seq)-k+1):
            HammingDist = sum([1 for i in xrange(k) if motif[i] != seq[s+i]])
            if (HammingDist < minHammingDist):
                bestS = s
                minHammingDist = HammingDist
        bestAlignment.append(bestS)
        totalDist += minHammingDist
    return bestAlignment, totalDist
```

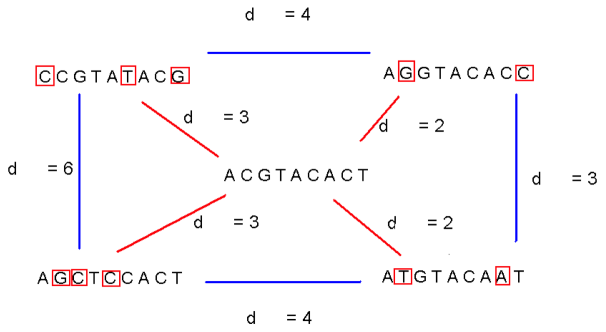
```
print ScanAndScoreMotif(seqApprox, "tagatccgaa")
%timeit ScanAndScoreMotif(seqApprox, "tagatccgaa")
```

```
([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11)
1000 loops, best of 3: 1.41 ms per loop
```

Wow, we can test over 650 motifs per second!

Scan-and-Score Motif Performance

- There are $M(N - k + 1)$ positions to test the motif, and each test requires k tests.
- So each scan is $O(MNk)$.



- So where where do we get candidate motifs?
- Can we try all of them? There are $4^{10} = 1048576$ in our example.
  - Do the math, 1048576 motifs × 2 mS ≈ 35 mins
  - Not fast, but less than a lifetime

- This approach is called a ***Median String Motif Search***
- Recall from last Lecture that a string that minimizes *Hamming distance* is like finding a middle or median string that is closer to all instances than the instances are to each other.

Let's Do It

```
import itertools

def MedianStringMotifSearch(DNA,k):
    """ Consider all possible 4**k motifs"""
    bestAlignment = []
    minHammingDist = k*len(DNA)
    kmer = ''
    for pattern in itertools.product('acgt', repeat=k):
        motif = ''.join(pattern)
        align, dist = ScanAndScoreMotif(DNA, motif)
        if (dist < minHammingDist):
            bestAlignment = [p for p in align]
            minHammingDist = dist
            kmer = motif
    return bestAlignment, minHammingDist, kmer

%time MedianStringMotifSearch(seqApprox,10)
```

```
CPU times: user 26min 35s, sys: 613 ms, total: 26min 35s
Wall time: 26min 35s

([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
```

Should we declare victory and move on? Do you find anything uncomfortable about an algorithm that requires $O(MNk4^k)$ steps?

Notes on Median String Motif Search

- Similarities between finding and alignment with minimal Hamming Distance and maximizing a Motif's consensus score.
- In fact, if instead of counting mismatches as in the code fragment:

```
HammingDist = sum([1 for i in xrange(k) if motif[i] != seq[s+i]])
```

  we had counted matches

```
Matches = sum([1 for i in xrange(k) if motif[i] == seq[s+i]])
```

  and found the *maximum(TotalMatches)* instead of the *min(TotalHammingDistance)* we would be using the same measure as *Score()*.
- Thus, we expect *MedianStringMotifSearch()* to give the same answer as either *BruteForceMotifSearch()* or *BranchAndBoundMotifSearch()*.
- However, the 4^k term raises some concerns. If k were instead 20, then we'd have to Scan-and-Score more than 10^{12} times. Another *not-in-a-lifetime* algorithm
- We can also apply the *Branch-and-Bound* approach to the Median string method, but, as before it would only improve the average case.

Other ways to guess the motif?

- If we *knew* that the motif that we are looking for was contained *somewhere* in our DNA sequences we could test the $(N - k + 1)t$ motifs from our DNA, giving a $O(N^2 t^2)$ algorithm.
- Unfortunately, as you may recall ***our motif*** did not appear actually appear in our data.
- You could keep track of a few good *motif candidates* using a manageable and perhaps random subsets of the given DNA sequences, and use them as your candidate motifs.

Let's try considering only Motifs seen in the DNA

```
def ContainedMotifSearch(DNA,k):
    """ Consider only motifs from the given DNA sequences"""
    motifSet = set()
    for seq in DNA:
        for i in xrange(len(seq)-k+1):
            motifSet.add(seq[i:i+k])
    print "%d Motifs in our set" % len(motifSet)
    bestAlignment = []
    minHammingDist = k*len(DNA)
    kmer = ''
    for motif in motifSet:
        align, dist = ScanAndScoreMotif(DNA, motif)
        if (dist < minHammingDist):
            bestAlignment = [s for s in align]
            minHammingDist = dist
            kmer = motif
    return bestAlignment, minHammingDist, kmer

%time ContainedMotifSearch(seqApprox,10)
```

```
709 Motifs in our set
CPU times: user 1.33 s, sys: 16 ms, total: 1.34 s
Wall time: 1.33 s

([17, 31, 18, 33, 21, 0, 46, 70, 16, 65], 17, 'tagatccaaa')
```

Not exactly the motif we were looking for (off by a 'g'), [17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa', but boy was it fast! Where's a good Oracle when you need one?

Insights from the consensus score matrix

If we call Score([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], seqApprox, 10)

```
DNA[0][17:27]    t  a  g  a  t  c  t  g  a  a
DNA[1][31:41]    t  a  g  a  c  c  a  a  a  a
DNA[2][18:28]    t  a  g  a  c  c  c  g  a  a
DNA[3][33:43]    t  a  a  a  t  c  c  g  a  a
DNA[4][21:31]    t  a  g  g  t  c  c  a  a  a
DNA[5][ 0:10]    t  a  g  a  t  t  c  g  a  a
DNA[6][46:56]    c  a  g  a  t  c  c  g  a  a
DNA[7][70:80]    t  a  g  a  t  c  c  g  t  a
DNA[8][16:26]    t  a  g  a  t  c  c  a  a  a
DNA[9][65:75]    t  c  g  a  t  c  c  g  a  a
              a [0, 9, 1, 9, 0, 0, 1, 3, 9,10]
              c [1, 1, 0, 0, 2, 9, 8, 0, 0, 0]
              g [0, 0, 9, 1, 0, 0, 0, 7, 0, 0]
              t [9, 0, 0, 0, 8, 1, 1, 0, 1, 0]
                [9, 9, 9, 9, 8, 9, 8, 7, 9,10]  Score = 87
Consensus        t  a  g  a  t  c  c  g  a  a   Our motif!
```

Any Ideas?

Contained Consensus Motif Search

```
def Consensus(s, DNA, k):
    """ compute the consensus k-Motif of an alignment given offsets into each DNA string.
            s = list of starting indices, 1-based, 0 means ignore, DNA = list of nucleotide strings,
            k = Target Motif length """
    consensus = ''
    for i in xrange(k):
        # loop over string positions
        cnt = dict(zip("acgt",(0,0,0,0)))
        for j, sval in enumerate(s):
            # loop over DNA strands
            base = DNA[j][sval+i]
            cnt[base] += 1
        consensus += max(cnt.iteritems(), key=lambda tup: tup[1])[0]
    return consensus

def ContainedConsensusMotifSearch(DNA,k):
    bestAlignment, minHammingDist, kmer = ContainedMotifSearch(DNA,k)
    motif = Consensus(bestAlignment,DNA,k)
    newAlignment, HammingDist = ScanAndScoreMotif(DNA, motif)
    return newAlignment, HammingDist, motif

%time ContainedConsensusMotifSearch(seqApprox,10)
```

```
709 Motifs in our set
CPU times: user 1.06 s, sys: 23 ms, total: 1.08 s
Wall time: 1.06 s

([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
```

Now we're cooking!

Dad, are we there yet?

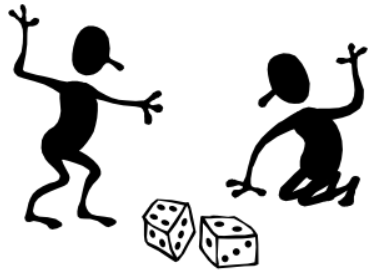
We got the answer that we were looking for, but

- How can we be sure it will always give the correct answer?
  - Our other methods were exhaustive, they examined every possibility
  - This method considers only a subset of solutions, picks the best one in a *greedy* fashion
  - What if there had been ties amoung the candidate motifs?
  - What if the consensus score (87% matches) had been lower
  - Would we, should we, be satisfied?

- It's one thing to be greedy, and another to be both *greedy and biased*
  - Our method is greedy in that it considers only the best contained motif, greedy methods are subject to falling into *local minimums*
  - Since consider only subsequences as motifs we introduce bias

- Note that Consensus can generate motifs not seen in our data

A randomized approach to motif finding



- One way to avoid bias and local minima is to introduce *randomness*
- We can generate candidate motifs from our data by treating it as distribution
  - likely motif candidates from this distribution are those generated by Consensus
  - Consensus strings can be tested by Scan-and-Score and their alignments lead to new consensus strings
  - Eventually, we should converge to some local minimal answer
- To avoid finding a local minimum, we try several random starts, and search for the best score amongst all these starts.
- A randomized algorithm does not guarantee an optimal solution. Instead it promises a good/plausible answer on average, and it is not susceptible to a worse-case data sets as our greedy/biased method was.

Code for Randomized Motif Search

```
import random

def RandomizedMotifSearch(DNA,k):
    """ Searches for a k-length motif that appears
    in all given DNA sequences. It begins with a
    random set of candidate consensus motifs
    derived from the data. It refines the motif
    until a true consensus emerges."""

    # Seed motifs from random alignments
    motifSet = set()
    for i in xrange(500):
        randomAlignment = [random.randint(0,len(DNA[j])-k) for j in xrange(len(DNA))]
        motif = Consensus(randomAlignment, DNA, k)
        motifSet.add(motif)

    bestAlignment = []
    minHammingDist = k*len(DNA)
    kmer = ''
    testSet = motifSet.copy()
    while (len(testSet) > 0):
        print len(motifSet),
        nextSet = set()
        for motif in testSet:
            align, dist = ScanAndScoreMotif(DNA, motif)
            # add new motifs based on these alignments
```

Let's try it

```
%time RandomizedMotifSearch(seqApprox,10)
```

```
500 749 822 839 842CPU times: user 1.43 s, sys: 23 ms, total: 1.45 s
Wall time: 1.56 s

([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
```

Randomized algorithms should be restarted multiple times to insure a stable solution.

```
for i in xrange(10):
    print RandomizedMotifSearch(seqApprox,10)
```

```
500 751 820 836 837 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
500 750 825 838 844 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
500 755 837 856 859 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
499 745 814 831 834 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
500 760 837 859 862 863 864 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
500 744 813 825 827 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
498 746 830 846 850 851 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
500 766 848 864 866 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
500 728 800 810 811 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
500 750 833 851 852 ([17, 47, 18, 33, 21, 0, 46, 70, 16, 65], 11, 'tagatccgaa')
```

Lessons Learned

- We can find Motifs in our lifetime
  - Practical exhaustive search algorithm for small k, MedianStringMotifSearch()
  - Practical fast algorthim RandomizedMotifSearch(DNA,k)
- Three algorithm design approaches "Branch-and-Bound", "Greedy", and "Randomized"
- Reversing the objective, pretending that you know the answer, and validating it
- The power of randomness
  - Not susceptable to worse case data
  - Avoids local minimums that plague some greedy algorithms