Seeding Methods in Homology Search

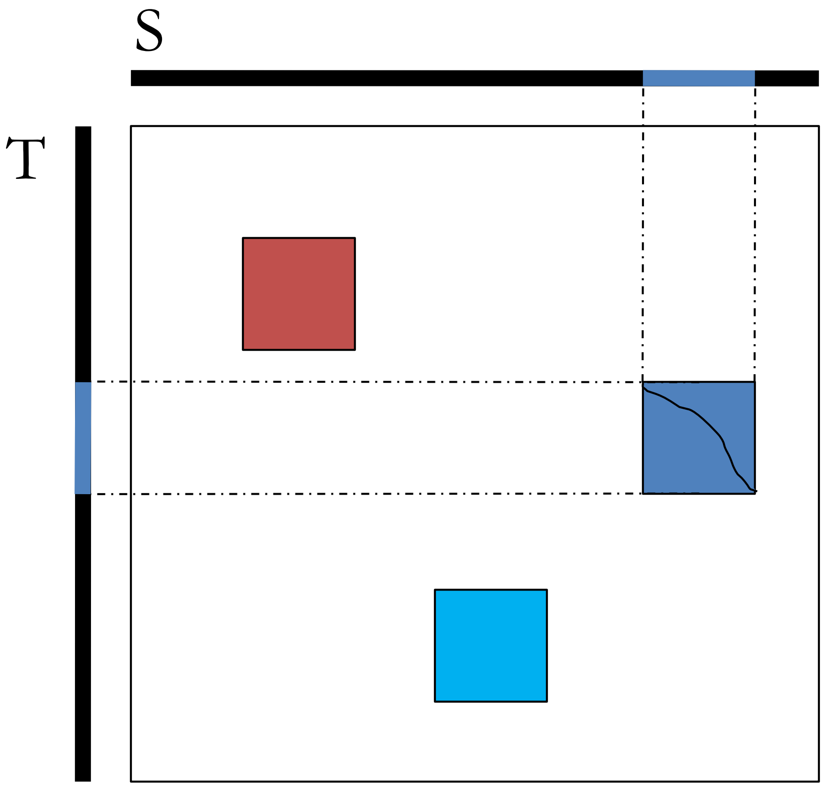
A similarity between mouse and human genomes

```
GCNTACACGTCACCATCTGTGCCACCACNCATGTCTCTAGTGATCCCTCATAAGTTCCAACAAAGTTTGC
|| |||||  | |||  ||||   ||    |||||||||||||||||| | |||||||| |  | |||||
GCCTACACACCGCCAGTTGTG-TTCCTGCTATGTCTCTAGTGATCCCTGAAAAGTTCCAGCGTATTTTGC

GAGTACTCAACACCAACATTGATGGGCAATGGAAAATAGCCTTCGCCATCACACCATTAAGGGTGA----
|| ||||||||| ||||||  | |||||   |||||||| ||| ||||||||  |  | | ||
GAATACTCAACAGCAACATCAACGGGCAGCAGAAAATAGGCTTTGCCATCACTGCCATTAAGGATGTGGG

------------------TGTTGAGGAAAGCAGACATTGACCTCACCGAGAGGGCAGGCGAGCTCAGGTA
                  ||||||||||||| ||| ||||||||||| || ||||||| || ||||   |
TTGACAGTACACTCATAGTGTTGAGGAAAGCTGACGTTGACCTCACCAAGTGGGCAGGAGAACTCACTGA

GGATGAGGTGGAGCATATGATCACCATCATACAGAACTCAC-------CAAGATTCCAGACTGGTTCTTG
||||||| |||| | | |||| ||||| || |||||  ||        |||||| |||||||||||||||
GGATGAGATGGAACGTGTGATGACCATTATGCAGAATCCATGCCAGTACAAGATCCCAGACTGGTTCTTG
```

Smith-Waterman is the most accurate method.

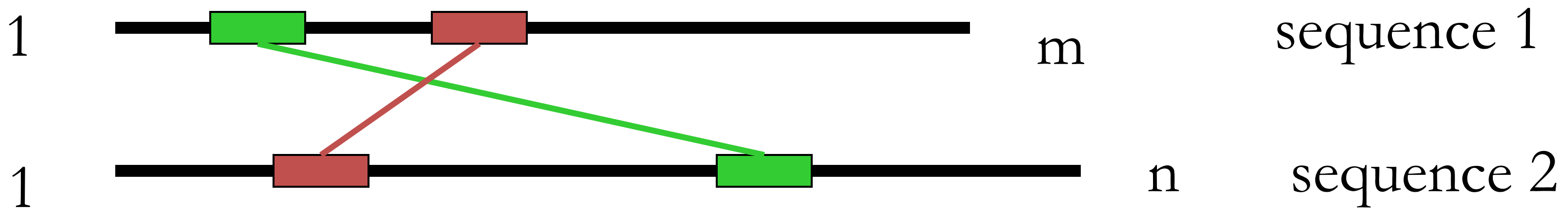
Time complexity：O(mn).

Smith-Waterman Algorithm



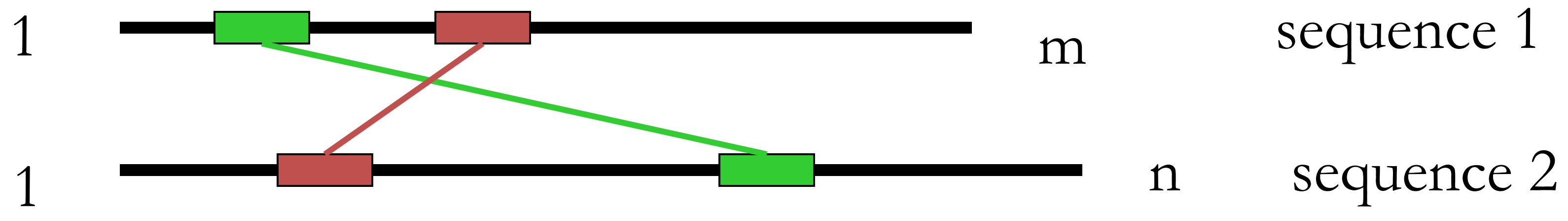
- The old algorithm requires O(mn) and is too slow.
- Human v.s. mouse: $3x10^9x3x10^9=9x10^{18}$

Similarity Search



- Most similarities (local alignments) are very short relative to the genomes.

Similarity Search



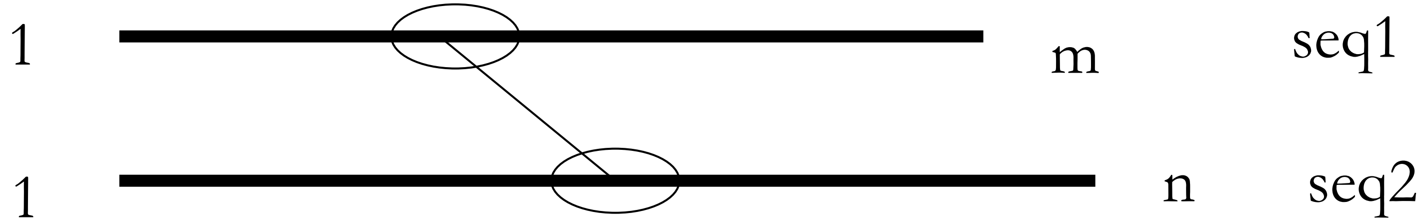
- For every pairs of (i, j), build a local alignment around it.
    - O(mnT)
    - Not better than Smith-Waterman.
- But this leads to an important idea…

Main Idea

- Most pairs of (i, j) are useless. We only want to try local alignments on the “promising” pairs of (i, j).
- In the context of sequence similarity search in bioinformatics, these “promising” pairs are called “seeds” or “hits”.
- We need
  - a proper definition of hits.
  - some efficient way to enumerate the hits faster than trying every pair of (i, j).

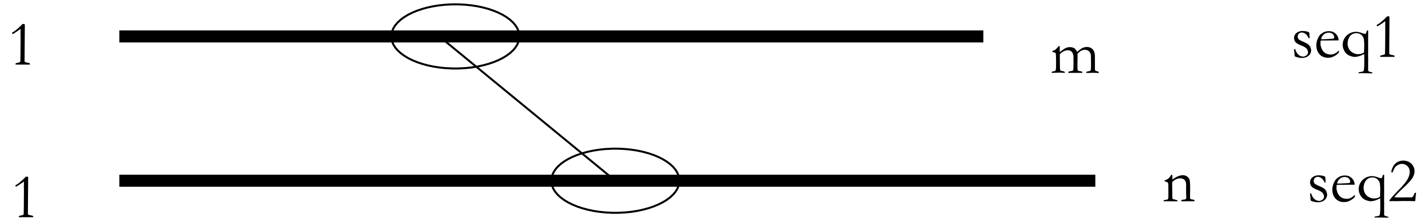
BLAST Uses Short Consecutive Match as Hits

```
GCNTACACGTCACCATCTGTGCCACCACNCATGTCTCTAGTGATCCCTCATAAGTTCCAACAAAGTTTGC
|| |||||  | |||  ||||   ||    |||||||||||||||||| | |||||||| |  | |||||
GCCTACACACCGCCAGTTGTG-TTCCTGCTATGTCTCTAGTGATCCCTGAAAAGTTCCAGCGTATTTTGC
```



BLAST Uses Short Consecutive Match as Hits

```
GCNTACACGTCACCATCTGTGCCACCACNCATGTCTCTAGTGATCCCTCATAAGTTCCAACAAAGTTTGC
|| |||||  | |||  ||||   ||    |||||||||||||||||| | |||||||| |  | |||||
GCCTACACACCGCCAGTTGTG-TTCCTGCTATGTCTCTAGTGATCCCTGAAAAGTTCCAGCGTATTTTGC
```



- Majority of (i,j) are random and probability of generating a random hit is small.
- For length-k seed, time complexity becomes $O(4^{-k}mnT)$
- By default, BLAST used k=11.
- What's the speed up factor for k=11?

The Idea behind Seeding

- A true similarity has a high chance of being hit.
- A random pair (i, j) has low chance of being hit.
- Thus, if we use hit to filter (i, j), we will
  - Detect most true similarities.
  - Not wasting time on random pairs of (i, j).

The Data Structure for Finding Hit?

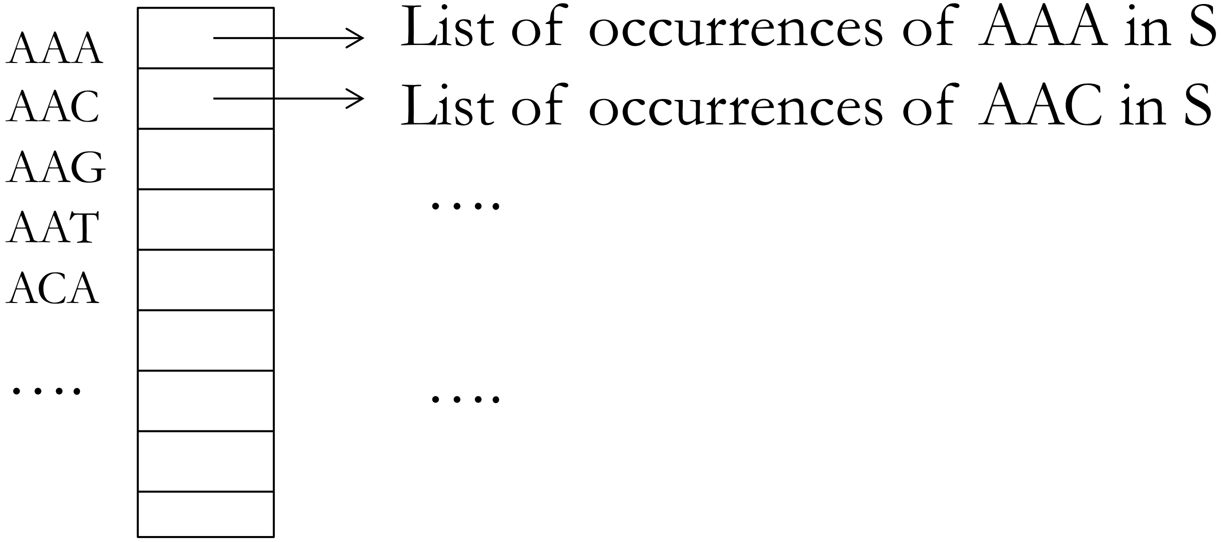
- for each *k*-mer, index table to remember all its occurrences in S.
- for each *k*-mer of T, find its hits in the index table.
- The index table can be a trie or a hash table.

AA → 0, 6
AC
AG
AT → 1
CA
CC
CG
CT → 3
GA
GC
GG
GT
TA → 5
TC → 2
TG
TT → 4

S: `AATCTTAA`
`01234567`

T: `GAACTTA`

The Data Structure for Finding Hit?



Space complexity?

Overall runtime

- Build the index using S: O(n) time.
- Find matches between the index and sequence T: O(m) time to scan T, plus we need to examine all of the N hits found. Let t be the per-hit examination time. Then
  O(m+Nt).
- Overall runtime: O(n+m+Nt).
- The term Nt is the most expensive part. Indexing overhead is small.
- In practice, most of the hits encountered are random hits.

Filtration can have multiple rounds

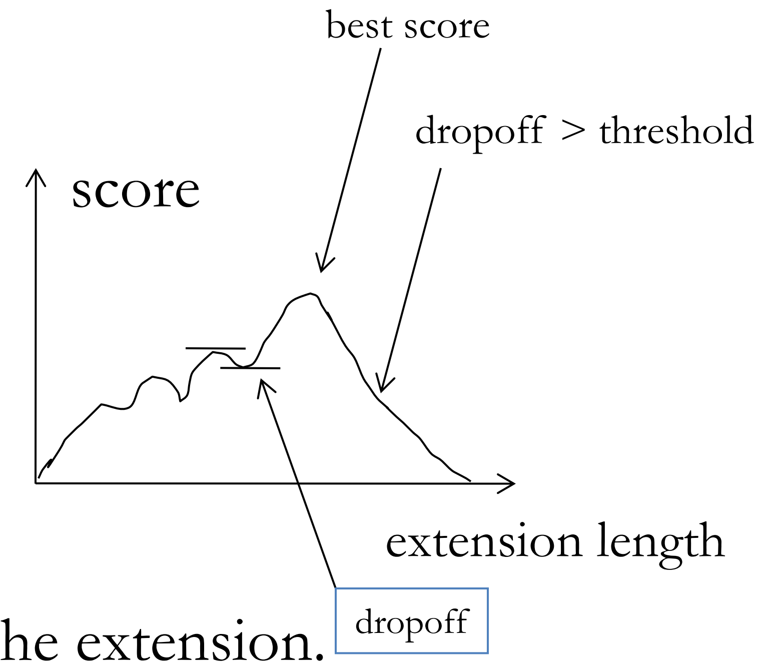
```
GCNTACACGTCACCATCTGTGCCACCAGCCATGTCTCTAGTGATCCCTCATGGTGGCCAACAAAGTTTGC
          |  |   |      || ||  ||||||||||| |||||| |      ||| |  | |||||
TGCCTACACACCGCCAGTTGTGTTCCTGCTATGTCTCTAGTTATCCCTGAAAAGTTCCAGCGTATTTTGC
```

- After finding a hit, instead of trying to build a local alignment directly, BLAST uses another round of filtration to determine if a hit is a "good" or "bad" hit.
- Quick search in both directions; if most symbols match, it's a good hit. Otherwise it's bad.
  - More precisely, use ungapped extension to find HSPs.
- If an HSP is above a certain score threshold, build a local alignment around it.

HSP extension

```
GCNTACACGTCACCATCTGTGCCACCAGCCATGTCTCTAGTGATCCCTCATGGTGGCCAACAAAGTTTGC
          |  |   |      || || ||||||||||| |||||| |      ||| |  | |||||
TGCCTACACACCGCCAGTTGTGTTCCTGCTATGTCTCTAGTTATCCCTGAAAAGTTCCAGCGTATTTTGC
```

```
for k from 0 to …
    score += sc(S[i+k],T[j+k])
for k from 1 to …
    score += sc(S[i-k],T[j-k])
```



- But when to stop?
- Score will increase and decrease during the extension.
- Extension stops when score greater than reporting threshold, or drop off greater than drop threshold.

HSP Extension

- How long will the extension continue after reaching best score?
- Assumptions:
  - After reaching best score, sequence becomes random.
  - match=1 and mismatch=-1
- Expected score on each additional base is -0.5.
- If dropoff=k, then after 2k bases, the expected dropoff will reach k.
- Conclusion: Not too long.

Example of missing a target

- Fail:

```
GAGTACTCAACACCAACATTAGTGGGCAATGGAAAAT
|| ||||||||| |||||| | ||||||   ||||||
GAATACTCAACAGCAACATCAATGGGCAGCAGAAAAT
```

- Dilemma
  - **Sensitivity** – needs shorter seeds
    - the success rate of finding a homology
  - **Speed** – needs longer seeds
    - Mega-BLAST uses seeds of length 28.

PatternHunter uses “spaced seeds”

- 111*1**1*1**11*111 (called a spaced seed)
    - Eleven required matches (**weight**=11, **length** = 18)
    - Seven “don’t care” positions

```
GAGTACTCAACACCAACATTAGTGGCAATGGAAAAT…
|| ||||||||| ||||| || |||||   ||||||
GAATACTCAACAGCAACACTAATGGCAGCAGAAAAT…
       111*1**1*1**11*111
```

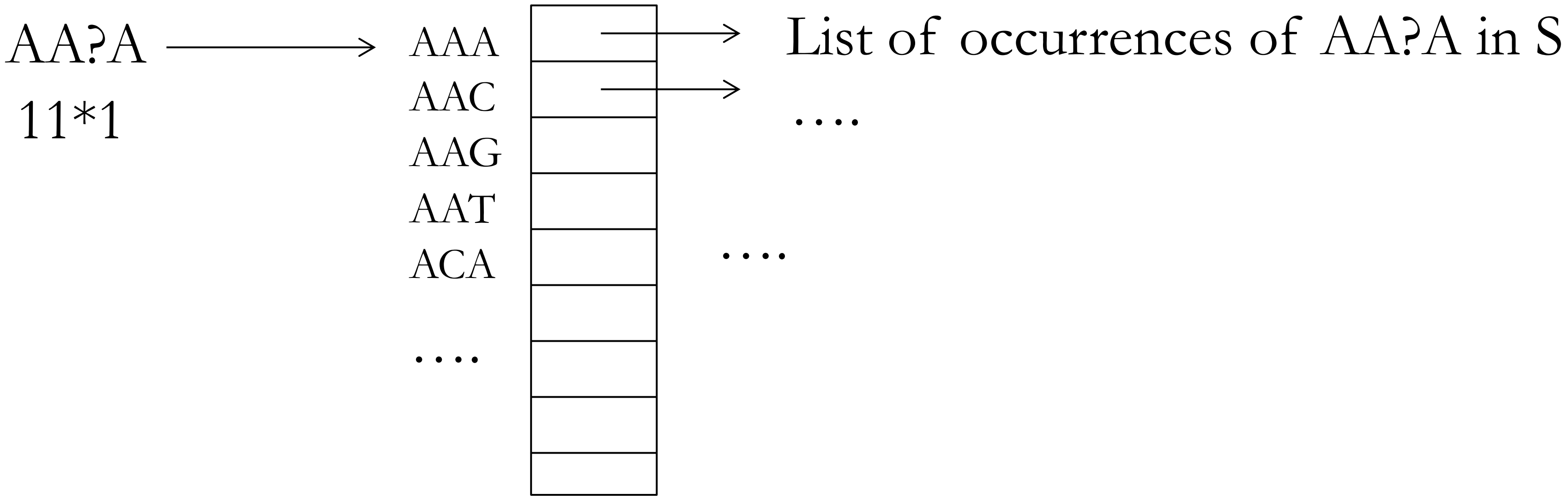
- Hit = all the required matches are satisfied.
- BLAST’s seed = 11111111111

Notes about the notation

- A homology/similarity region's actual sequences do not matter, the match/mismatch matters.
- Therefore, a region is often denoted by a binary 0-1 sequence, `11011111001110111011111`
- A hit is then as follows:
- `11011111001110111011111`
- `   111*1**1*1**11*111`

The Data Structure for Finding Hit

- The same as consecutive seed. Except that now we have a length *l*, weight *w* seed. E.g. 11*1.
  - Each *l*-mer, take the *w* letters out and put in index table.
- The index table can be a hash table.



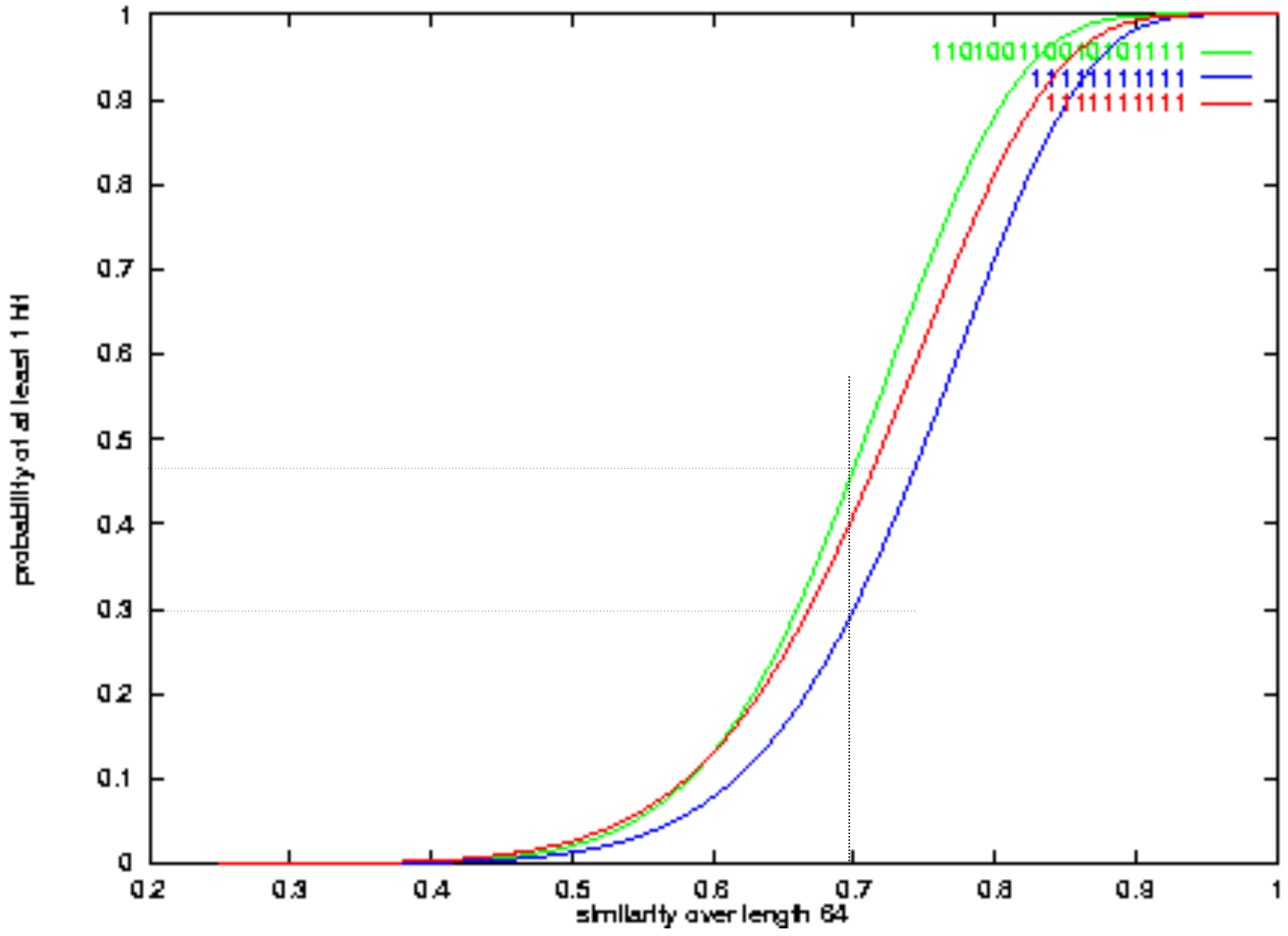
Time Complexity Comparison

- Lemma: for random sequence S and T with lengths m and n, the expected number of hits for weight w, length l seed is

$$(m-l+1)(n-l+1)4^{-w}$$

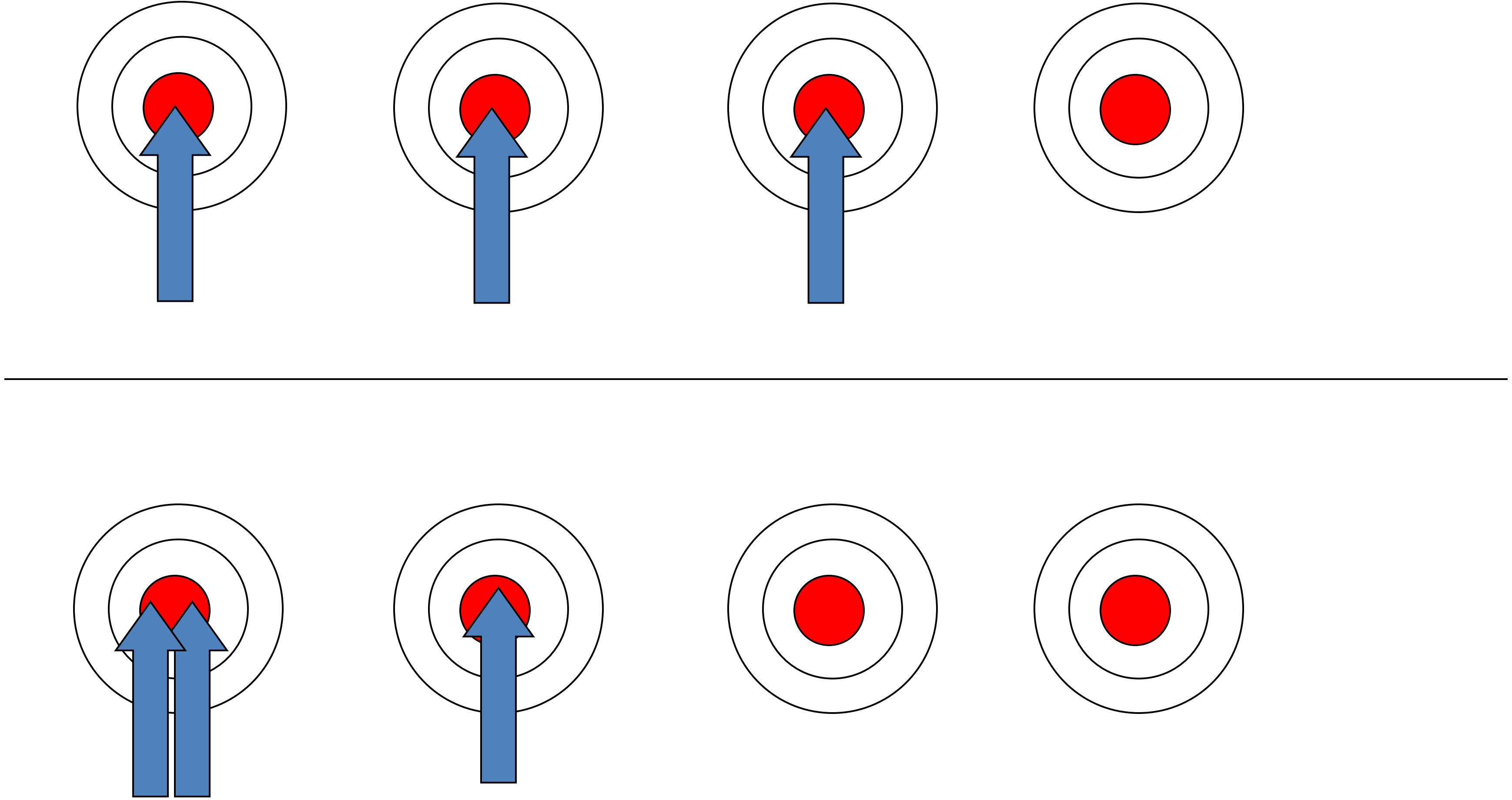
- Because usually l is much shorter than m and n, this is approximately $4^{-w}mn$
- That is, the expected number of hits in random regions only depends on the weight, but not the shape of the seed. So does the running time.
- So, speed-wise, spaced seed is similar to consecutive seed.
- What about the sensitivity?

Simulated sensitivity curves



Why spaced seeds are more sensitive?

- Consider a simple scenario where there are n HSPs to be detected. Each HSP has length L. Each position of the HSP is a match with probability p independently. L is much larger than seed length.
- Compare weight-w spaced seed s and contingous seed t.
- At each position, probability of hit is p^w for both seeds.
- $E[\text{\# of hits}] = n \cdot (L - l + 1) \cdot p^w$, here l is the length of the seed.
- Two seeds have comparable number of hits.
- But why spaced seeds are more sensitive?



Why spaced seeds are more sensitive?

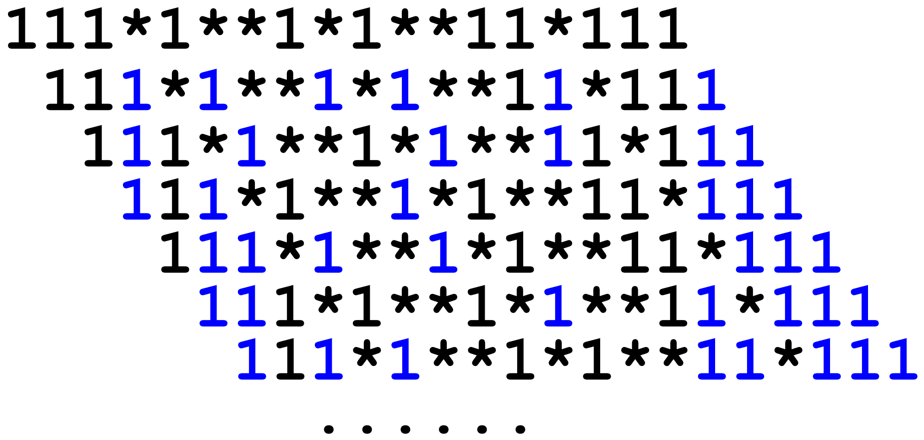
```
TTGACCTCACC?
|||||||||||?
TTGACCTCACC?
11111111111
 11111111111
```

```
CAA?A??A?C??TA?TGG?
|||?|??|?|??||?|||?
CAA?A??A?C??TA?TGG?
111*1**1*1**11*111
 111*1**1*1**11*111
```

- BLAST’s seed usually uses more than one hits to detect one homolog (wasteful)
- A spaced seed uses fewer hits to detect one homolog (efficient)

Good spaced seed does not overlap much

- The following spaced seed does not overlap heavily when shifts:



```
111*1**1*1**11*111
 111*1**1*1**11*111
  111*1**1*1**11*111
   111*1**1*1**11*111
    111*1**1*1**11*111
     111*1**1*1**11*111
      111*1**1*1**11*111
         ......
```

- The hits at different positions are independent.
- The probability of having the second hit is $3*p^6$ + …
  - compare to BLAST's seed $p + p^2 + p^3 + p^4$ + …

Lossless Filtration

- When seeds are short enough and HSP similarity is high enough, lossless filtration is also possible.
- For example, seed 111 can guarantee to match when a sufficiently long HSP has similarity 66.7%.
- Proof: To fail being hit by 111, the HSP must have a mismatch in every 3 adjacent positions.
- On the other hand, 110110110…, which has 66.6% similarity, will fail the seed 111.

Lossless Filtration

- Now consider spaced seed 11*1.
- Claim: For any $\epsilon > 0$, seed 11*1 will hit every sufficiently long region with similarity $0.6 + \epsilon$.

Proof

- Suppose there is a sufficiently long region not hit by 11*1.
- We can break the region into blocks of 1^a0^b. Besides the last block that can have at most three 1s, each of the other blocks is one of the following three cases:
  - 10^b for b>=1
  - 110^b for b>=2
  - 1110^b for b>=2
- In each block, similarity <= 0.6.
- So the long region's similarity is $< 0.6 + \epsilon$.

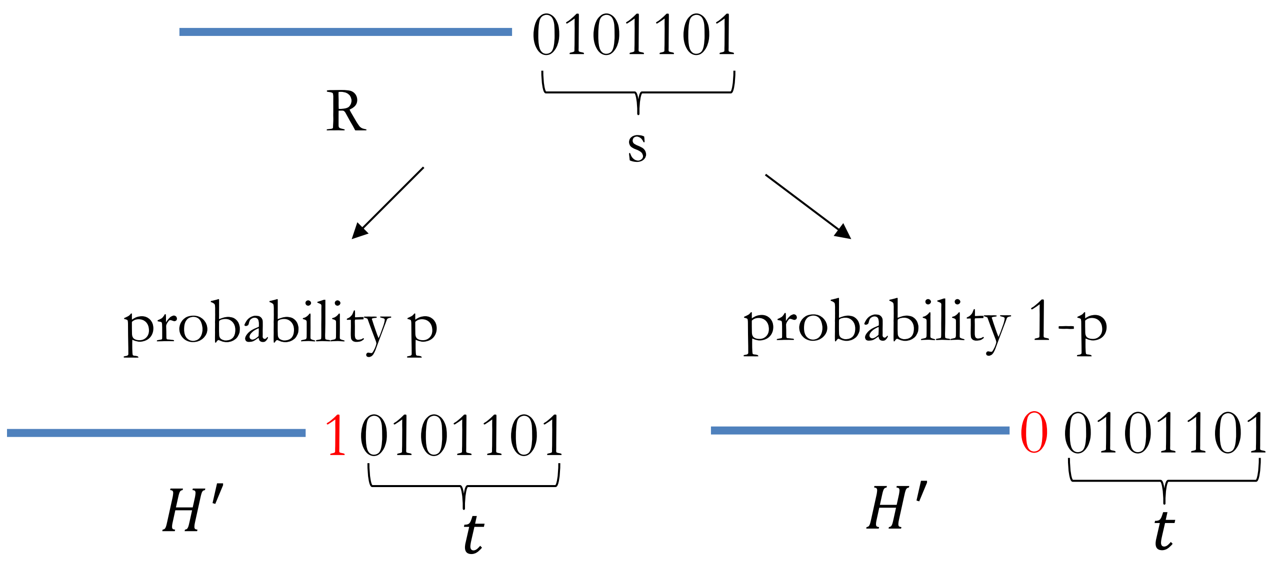
Compute a Seed's Sensitivity

- H: A probabilistic distribution of HSP, $\Pr(H[i] = 1) = p$;
- We want Pr(length-n such HSP is hit by a seed s). $\text{len}(s) = l$
- t: A length-l 0-1 string.
- Ht: The concatenation of H and t.
- Let $D[i, t]$ be the probability Ht is hit by t for $\text{len}(H) = i$.

- By total probability law, answer is $\sum_t (p(t) \cdot D[n-k, t])$. Note the summation is over all length l binary string t, and
- $p(s) = p^{\#1\ in\ t}(1-p)^{\#0\ in\ t}$

Dynamic Programming

- Case I: t is hit by s. Then $D[i,t] = 1$.
- Case II: t is not hit by s:



R $\underbrace{0101101}_{s}$

probability p

probability 1-p

H' 1 $\underbrace{0101101}_{t}$

H' 0 $\underbrace{0101101}_{t}$

H' is the length-(i-1) distribution. t' is the length-(l-1) prefix of t.

$$D[i,s] = p \cdot D[i-1,1t'] + (1-p) \cdot D[i-1,0t']$$

Dynamic Programming

```
Initialize D[0,$t$]
For $i$ from 1 to n
    for every length-$l$ binary string $t$
        if $t$ is hit by $s$
            $D[i,t] = 1$
        else
            $D[i,t] = p \cdot D[i-1,1t'] + (1-p) \cdot D[i-1,0t']$
Return $\sum_t p(t) \cdot D[n-l,t]$
```

Here $p(t) = p^{\#1\ in\ t}(1-p)^{\#0\ in\ t}$.

Time complexity $O\left(n \cdot 2^l\right)$

More efficient algorithm exists (not lectured here). $O\left(n \cdot 2^{\#*\ in\ \mathrm{s}}\right)$.

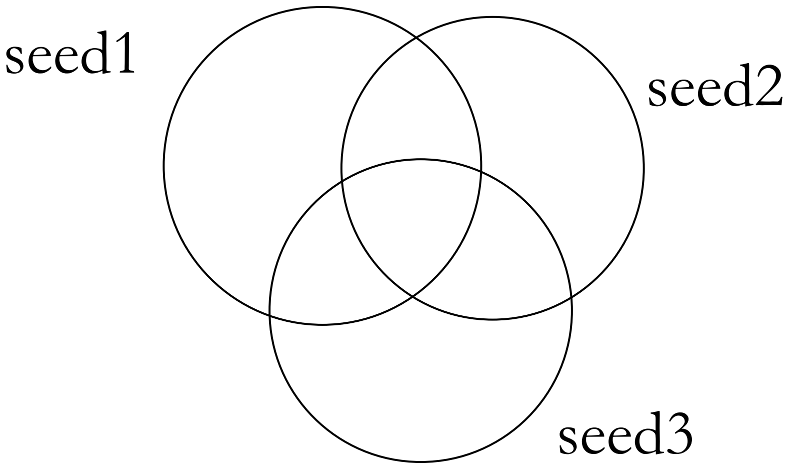
The “algorithm” to select the optimal spaced seed

- Enumerate all spaced seeds with weight 11 and no longer than 18, calculate the sensitivity of each, and output the one with the highest sensitivity.
- This is the ONLY known algorithm that guarantees the finding of optimal seed.
- Many heuristics exist to find suboptimal seeds.

Multiple Seeds – PatternHunter II:

Multiple Spaced Seeds

- Seeds with different shapes can detect different homologies.
  - Some seeds *may* detect more homologies than others. This leads to the use of optimized spaced seed.
  - Can use several seeds simultaneously to hit more homologies
    - Approaching 100% sensitive homology search



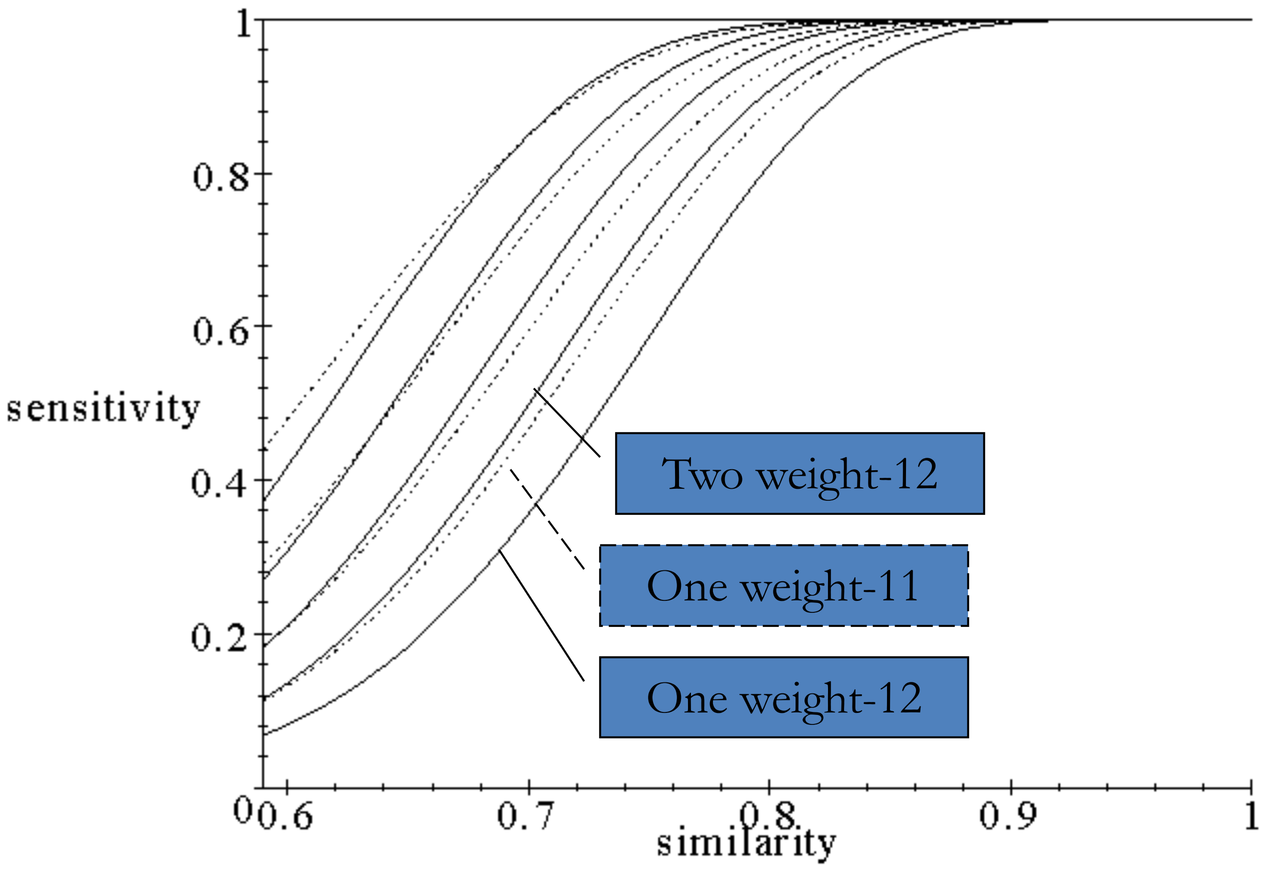
Multiple Seeds Example

(homology identity = 0.7, homology length=64)

```
111*11**1*11*1*111
1111***1***1**11*1*111
11**11*1**1*1***11*111
111*1***1111**1***11*1
```

- To use multiple seeds, one only needs to search multile times with different seeds, and combine results. Of course, you can search with them simultaneously.
- In either case, this slows down approximately k times if k seeds are used.
- Is it worth it? How does it compare with using one shorter seed?

Simulated sensitivity curves:



- Solid curves: Multiple (1, 2, 4, 8, 16) weight-12 spaced seeds.
- Dashed curves: Optimal spaced seeds with weight = 11, 10, 9, 8.
- Typically, "Doubling the seed number" gains better sensitivity than "decreasing the weight by 1".

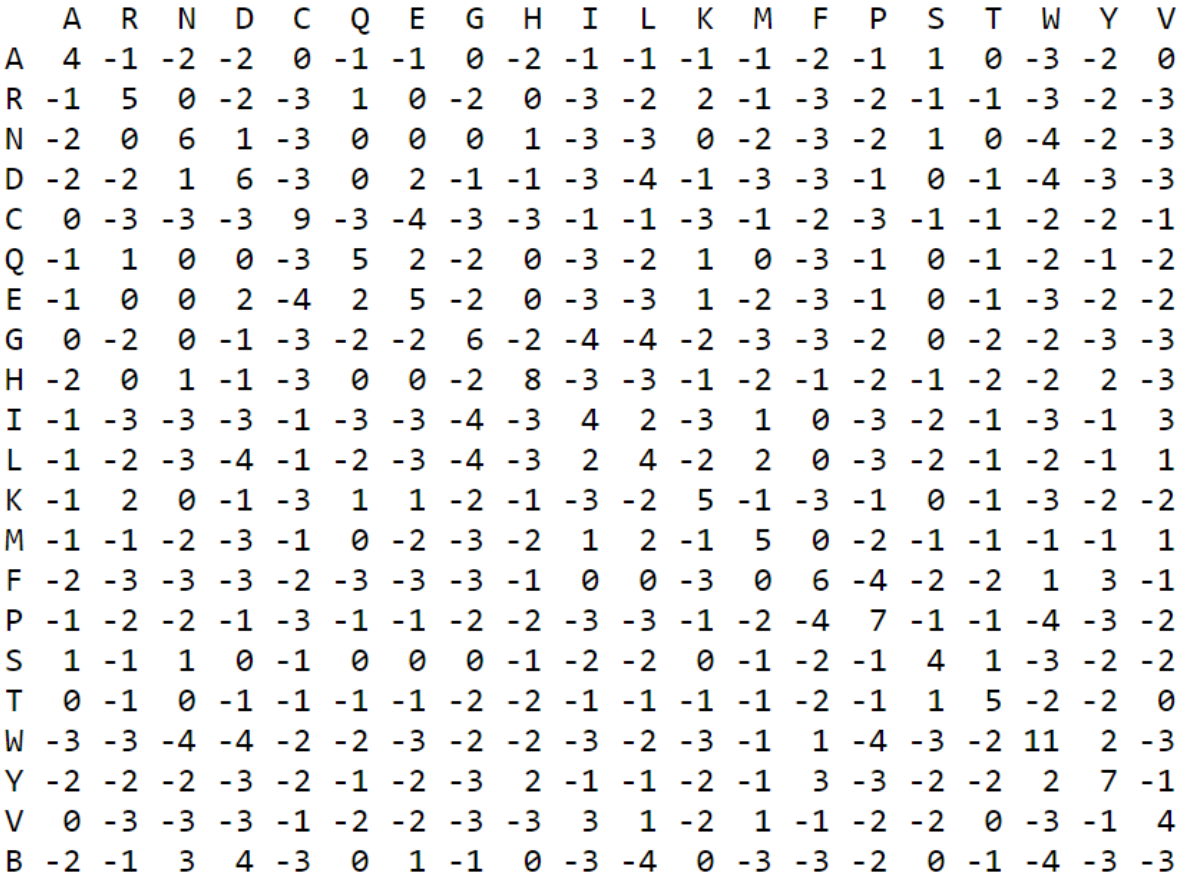
Seeding for Proteins - BLASTP

- With nucleotides, we're requiring *k* positions with exact matches.

- For proteins, that's not really reasonable: some amino acids mutate to another one very often.
- So BLASTP looks for 3- or 4-letter protein sequences that are "very close" to each other, and then builds matches from them.
- Where very close ➔ total BLOSUM score in the short window is at least +13 (or +11 for 3 mer).

Excercise

- For query RRR, threshold 11, what are the other 3-mers that can generate hits?



```
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V
A  4 -1 -2 -2  0 -1 -1  0 -2 -1 -1 -1 -1 -2 -1  1  0 -3 -2  0
R -1  5  0 -2 -3  1  0 -2  0 -3 -2  2 -1 -3 -2 -1 -1 -3 -2 -3
N -2  0  6  1 -3  0  0  0  1 -3 -3  0 -2 -3 -2  1  0 -4 -2 -3
D -2 -2  1  6 -3  0  2 -1 -1 -3 -4 -1 -3 -3 -1  0 -1 -4 -3 -3
C  0 -3 -3 -3  9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1
Q -1  1  0  0 -3  5  2 -2  0 -3 -2  1  0 -3 -1  0 -1 -2 -1 -2
E -1  0  0  2 -4  2  5 -2  0 -3 -3  1 -2 -3 -1  0 -1 -3 -2 -2
G  0 -2  0 -1 -3 -2 -2  6 -2 -4 -4 -2 -3 -3 -2  0 -2 -2 -3 -3
H -2  0  1 -1 -3  0  0 -2  8 -3 -3 -1 -2 -1 -2 -1 -2 -2  2 -3
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4  2 -3  1  0 -3 -2 -1 -3 -1  3
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4 -2  2  0 -3 -2 -1 -2 -1  1
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5 -1 -3 -1  0 -1 -3 -2 -2
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5  0 -2 -1 -1 -1 -1  1
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6 -4 -2 -2  1  3 -1
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7 -1 -1 -4 -3 -2
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4  1 -3 -2 -2
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5 -2 -2  0
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11  2 -3
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7 -1
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4
B -2 -1  3  4 -3  0  1 -1  0 -3 -4  0 -3 -3 -2  0 -1 -4 -3 -3
```

How to implement that?

- With BLASTP:
  - Build an automaton that reflects all string close to short strings in T (the short sequence)
  - Scan S (the longer sequence), looking for matches.
- We do not study the classic ways to match multiple patterns efficiently. If interested, search for Aho–Corasick algorithm.

A Simpler Way

- There is another way:

1) For every 3-mer, find all “neighboring” 3-mers that, score at least +11 (or whatever). Build these into a data structure NeighborList.
2) Build a hash table H for S of its 3-mers, just like for the nucleotide case
3) For every 3-mer *x* in T, retrieve all neighbors from NeighborList. For each neighbor, query H to find hits in S.

NeighborList is a small structure: there are only 8000 3-mers.

Which sequence to index?

- That's actually a tough question.

- Here's a typical scenario:
- S is the human genome (length n)
- P_1 is a short protein sequence (length m_1)
- P_2 is another short sequence (length m_2)

- If we're smart, build an index for S, *once*, and then look up the short sequences in it.
- Added time for P_2 is more like $O(m_2)$, not $O(n+m_2)$.

More on indexing

- But memory is a concern:
- Indexing the human genome is expensive!
- Oh, wait. No, it isn't, not anymore… you probably should index the longer sequence.
- BLASTN (1990) indexes the query, not the database.
- BLAT (2000) indexes the database, not the query.

- BLASTP also indexes the query.

Extensions to this idea

- Two-hit BLAST:
- Require two seeds (probably shorter) that are nearer than *k* from each other, and base the alignment on their enclosing box.
- Potentially even fewer false positives, but one has to use shorter seeds. There's quite a tradeoff here.

Wrap-up

- Local alignment slow when sequences are large
- Use 11 consecutive matches as hits
    - How these hits are found efficiently
    - What to do after hits are found
- Spaced seeds better
    - How sensitivity is computed and how optimal seed is found
    - How hits are found for spaced seed
- Multiple spaced seed.
- Protein seeds.
- Two hits.