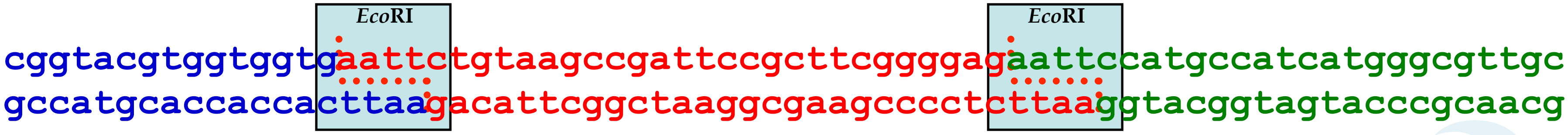
Lecture 4:
DNA Restriction Mapping

Study Chapter 4.1-4.3

Recall Restriction Enzymes

(from Lecture 2)

- Restriction enzymes break DNA whenever they encounter specific base sequences
- They occur reasonably frequently within long sequences (a 6-base sequence target appears, on average, 1:4096 bases)
- Can be used as molecular scissors



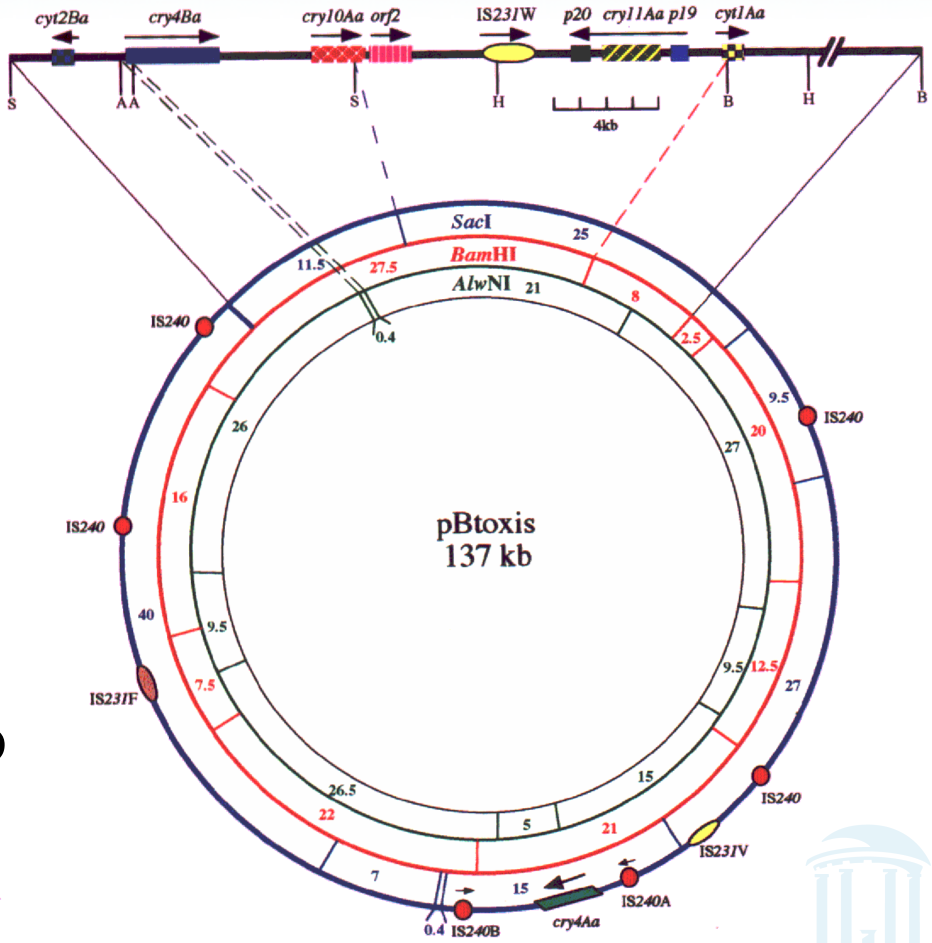
```
cggtacgtggtggtgaattctgtaagccgattccgcttcggggagaattccatgccatcatgggcgttgc
gccatgcaccaccacttaagacattcggctaaggcgaagcccctcttaaggtacggtagtacccgcaacg
```

Restriction Enzyme Uses

- Recombinant DNA technology
  - make novel DNA constructs,
  - add fluorophores
  - add other probes
- Digesting DNA into pieces that can be efficiently and reliably replicated through PCR (Polymerase Chain Reaction)
- Cutting DNA for genotyping via Microarrays
- Sequence Cloning
  - Inserting sequences into a host cell, via vectors
- cDNA/genomic library construction
  - Coding DNA, is a byproduct of transcription
  - Targeted sequencing (ex. RRBS)
- DNA restriction mapping
  - A rough map of a DNA fragment

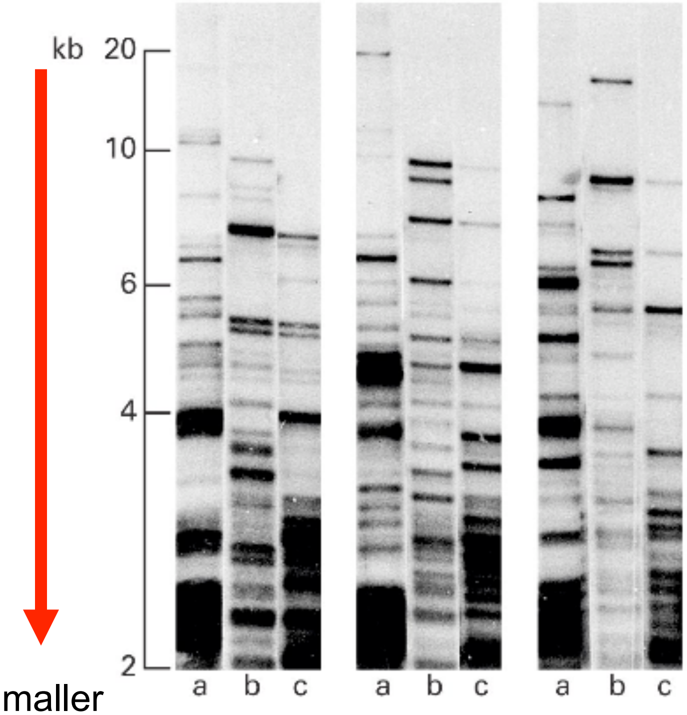
DNA Restriction Maps

- A map of the restriction sites in a DNA sequence
- If the DNA sequence is known, then constructing a restriction map is trivial
- Restriction maps are a cheap alternative to sequencing for unknown sequences



Consider the DNA Mapping Problem

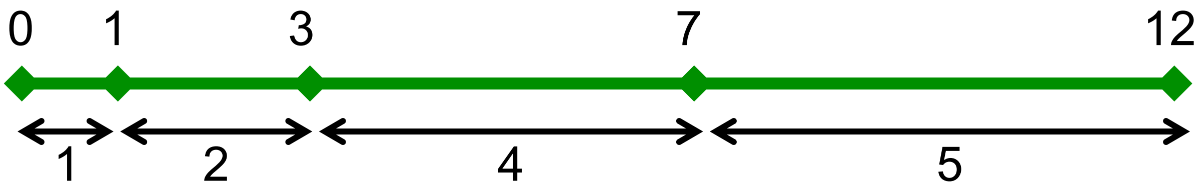
- Begin with an isolated strand of DNA
- *Digest* it with restriction enzymes
  - Breaks strand it in variable length fragments
- Use *gel electrophoresis* to sort fragments according to size
  - Can accurately sort DNA fragments that differ in length by a single nucleotide, and estimate their relative abundance
- Use fragment “lengths” to reassemble a map of the original strand



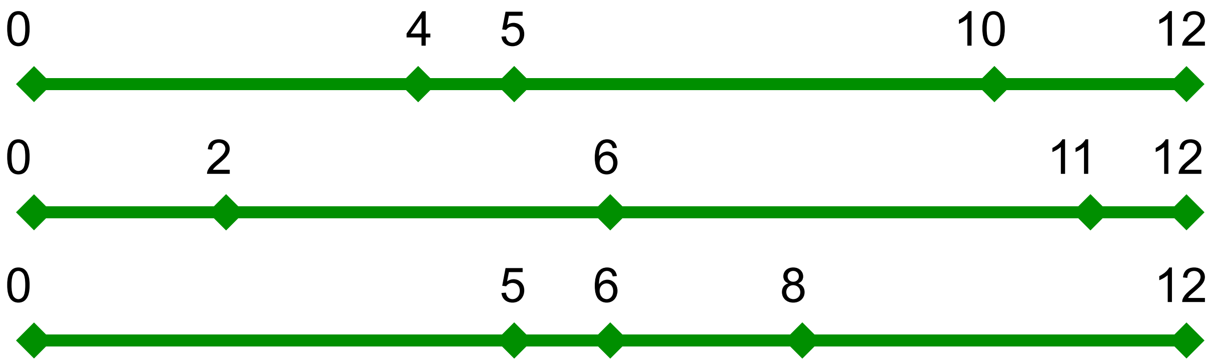
Smaller fragments move farther

Single Enzyme Digestion

- What can be learned from a single complete digest?

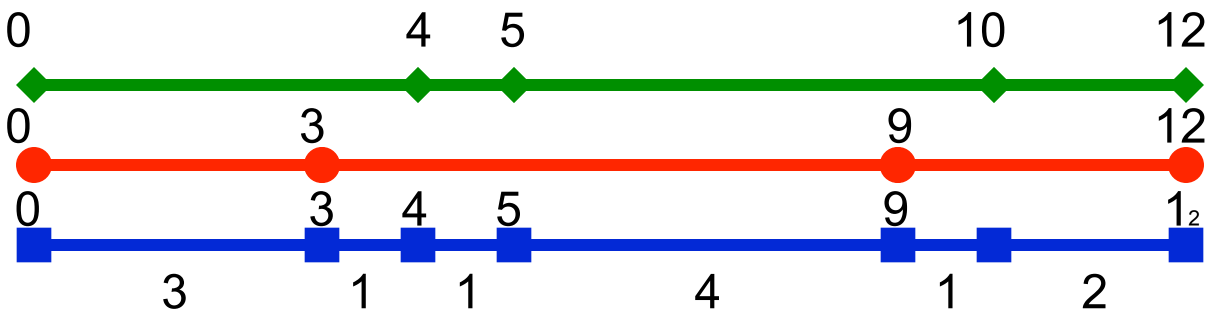


- Not much. There are many possible answers



Double Enzyme Digestion

- An alternative approach is to digest with two different enzymes in three stages
  - First, with restriction enzyme A
  - Second, with restriction enzyme B
  - Third, with both enzymes, A & B



- The inputs are three sets of restriction fragment lengths [1,2,4,5], [3,3,6], [1,1,1,2,3,4]

Double Digest Problem

- Given two sets of intervals on a common line segment between two disjoint interior point sets, and a third set of intervals between all points, reconstruct the positions of the points.

Input:

dA – fragment lengths from the digest with enzyme ***A***.

dB – fragment lengths from the digest with enzyme ***B***.

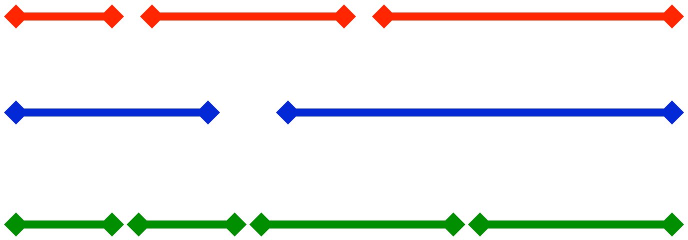
dX – fragment lengths from the digest with *both* ***A*** and ***B***.

Output: ***A*** – location of the cuts for the enzyme ***A***.

B – location of the cuts for the enzyme ***B***.

Class Exercise

- Suppose you are asked to assemble a map from three digests
  - A = [1,2,3]
  - B = [2,4]
  - AB = [1,1,2,2]



- How do you solve for the map?
- How do you state your strategy as a general purpose algorithm?

Set Permutations

- Given a set [A,B,C,D] find all permutations

[A,B,C,D]
[A,B,D,C]
[A,C,B,D]
[A,C,D,B]
[A,D,B,C]
[A,D,C,B]

[B,A,C,D]
[B,A,D,C]
[B,C,A,D]
[B,C,D,A]
[B,D,A,C]
[B,D,C,A]

[C,A,B,D]
[C,A,D,B]
[C,B,A,D]
[C,B,D,A]
[C,D,A,B]
[C,D,B,A]

[D,A,B,C]
[D,A,C,B]
[D,B,A,C]
[D,B,C,A]
[D,C,A,B]
[D,C,B,A]

- How many?
  - 1st choice = n
  - 2nd choice = n-1
  - 3rd choice = n-2

N! permutations of N elements

10! = 3628800
24! = 620448401733239439360000

A Brute Force Solution

- Test all permutations of A and B checking they are compatible with some permuation of AB

```
def doubleDigest(seta, setb, setab, circular = False):
    a = Permute(seta)
    while (a.permutationsRemain()):
        ab = Permute(setab)
        while (ab.permutationsRemain()):
            if compatible(a.order, ab.order):
                b = Permute(setb)
                while (b.permutationsRemain()):
                    if (circular):
                        for i in xrange(len(setab)):
                            abShift = shift(ab.order, i)
                            if compatible(b.order, abShift):
                                return (a.order, b.order, ab.order, i)
                    else:
                        if compatible(b.order, ab.order):
                            return (a.order, b.order, ab.order, 0)
    return (aState, bState, abState, -1)
```

len(a)!

len(b)!

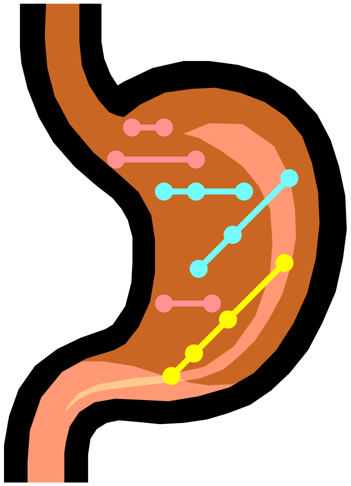
len(ab)!

How to Improve Performance?

- What strategy can we use to solve the double restriction map problem faster?
- Is there a branch-and-bound strategy?
  - Does the given code *really* test every permutation?
  - How does compatible( ) help?
  - Does the order of the loops help?
- Could you do all permutations of A and B, then compute the intervals and compare to AB?
- The double digest problem is truly a hard problem (NP-complete). No one knows an algorithm whose execution time does not grow slower than some exponent in the size of the inputs. If one is found, then an entire set of problems will suddenly also be solvable in less than exponential time.

Partial Digestion Problem

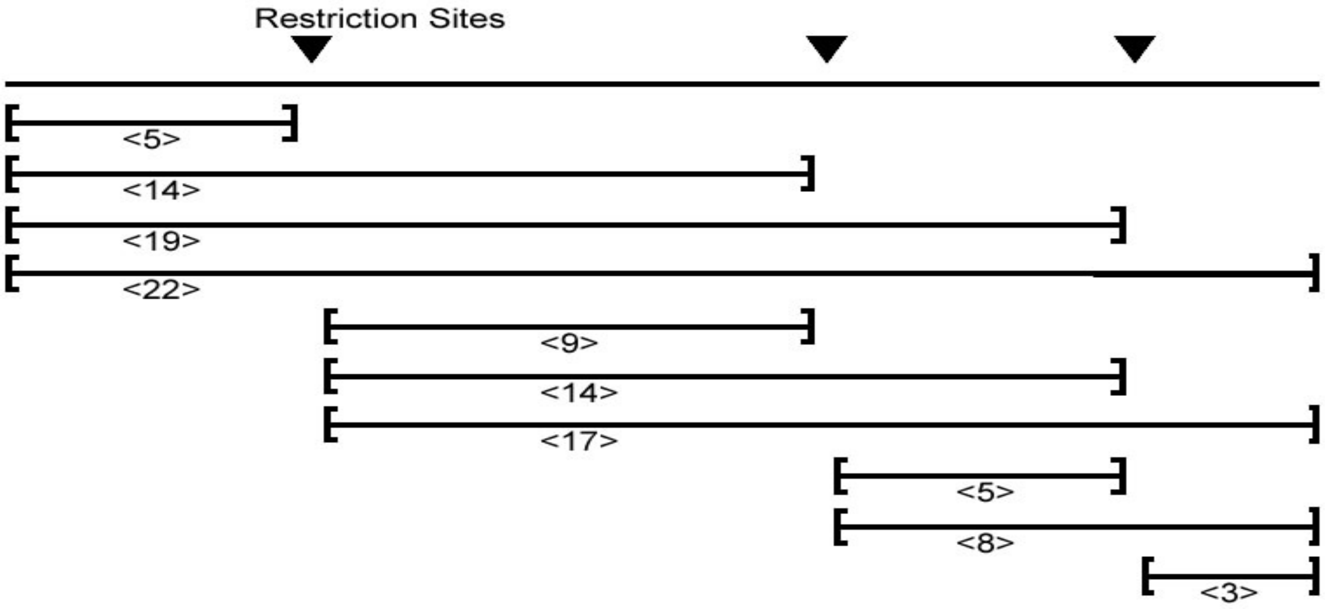
- Another way to construct a restriction map
- Expose DNA to the restriction enzyme for a limited amount of time to prevent it from cutting at all restriction sites (partial digestion)
- Generates the set of all possible restriction fragments between every pair of (not necessarily consecutive) points
- The set of fragment sizes is used to determine the positions of the restriction sites
- We assume that the multiplicity of a repeated fragment can be determined, i.e., multiple restriction fragments of the same length can be determined (e.g., by observing twice as much fluorescence for a double fragment than for a single fragment)



Partial Digestion Illustration

- A complete set of pairwise distances between points. In the following example a set of 10 fragments is generated.

L = {3, 5, 5, 8, 9, 14, 14, 17, 19, 22}



Pairwise Distance Matrix

- Often useful to consider partial digests in a distance matrix form
- Each entry is the distance between a pair of point positions labeled on the rows and columns

	0	5	14	19	22
0	-	5	14	19	22
5		-	9	14	17
14			-	5	8
19				-	3
22					-

- The distance matrix for *n* points has *n(n-1)/2* entries, therefore we expect that many digest values as inputs
- Largest value in L establishes the segment length
- Actual non-zero point values are a subset of L

Partial Digest Problem

- Given all pairwise distances between points on a line, reconstruct the positions of those points.

Input: A multiset of pairwise distances L, containing $\frac{n(n-1)}{2}$ elements

Output: A set X, of *n* integers, such that the set of pairwise distances $\Delta X = L$

Homometric Solutions

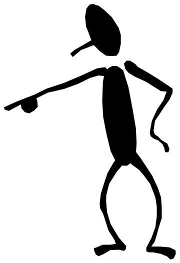
	0	1	3	4	5	7	12	13	15
0		1	3	4	5	7	12	13	15
1			2	3	4	6	11	12	14
3				1	2	4	9	10	12
4					1	3	8	9	11
5						2	7	8	10
7							5	6	8
12								1	3
13									2
15									

	0	1	3	8	9	11	12	13	15
0		1	3	8	9	11	12	13	15
1			2	7	8	10	11	12	14
3				5	6	8	9	10	12
8					1	3	4	5	7
9						2	3	4	6
11							1	2	4
12								1	3
13									2
15									

- The solution of a PDP is not always unique
- Two distinct point sets, A and B, can lead to indistinguishable distance multisets, ΔA = ΔB

Brute Force PDP Algorithm

- Basic idea: Construct all combinations of *n* - 2 integers between 0 and max(L), and check to see if the pairwise distances match.



Compare this Python code to the pseudocode on page 88 in the book

```
def bruteForcePDP(L, n):
    L.sort()
    M = max(L)
    X = intsBetween(0,M,n-2)
    while (X.combinationsRemain()):
        dX = allPairsDist(X.intSet())
        dX.sort()
        if (dX == L):
            print "X =", X.intSet()
```

Set Combinations

- Combinations of A things taken B at a time
- Order is unimportant
  [A,B,C] ≡ [A,C,B] ≡ [B,A,C] ≡ [B,C,A] ≡ [C,A,B] ≡ [C,B,A]
- All combinations of *n* items in *k* positions
  [1,1,0,0], [1,0,1,0],[1,0,0,1],[0,1,1,0],[0,1,0,1],[0,0,1,1]
- Smaller than a factorial

$$\binom{n}{k} = \frac{n!}{k!(n-k)!}$$

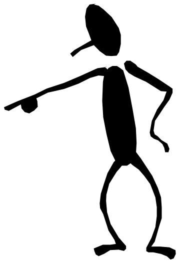
- Interesting relation $\sum_{k=0}^{n} \binom{n}{k} = 2^n$

BruteForcePDP Performance

- BruteForcePDP takes $O(\boldsymbol{max(L)}^{n-2})$ time since it must examine all possible sets of positions.
- The problem scales with the size of the largest pairwise distance
- Suppose we multiply each element in *L* by a constant factor?
- Should we consider *every* possible combination of *n* - 2 points? (Consider our observations concerning distance matrices)

Another Brute Force PDP Approach

- Recall that the actual point values are a subset of L's values. Thus, rather than consider all combinations of possible points, we need only consider n – 2 combinations of values from L.

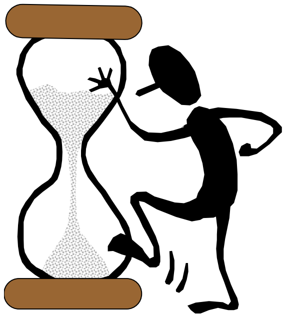


Compare this Python code to the pseudocode on page 88 in the book

```
def anotherBruteForcePDP(L, n):
    L.sort()
    M = max(L)
    X = intsFromL(L,n-2)
    while (X.combinationsRemain()):
        dX = allPairsDist(X.intSet())
        dX.sort()
        if (dX == L):
            print "X = ", X.intSet()
```

Efficiency of AnotherBruteForcePDP

- It's more efficient, but still slow
- If $\boldsymbol{L}$ = {2, 998, 1000} ($\boldsymbol{n}$ = 3, $\boldsymbol{M}$ = 1000), BruteForcePDP will be extremely slow, but AnotherBruteForcePDP will be quite fast
- Fewer sets are examined, but runtime is still exponential: $O(\boldsymbol{n}^{2n-4})$
- Is there a better way?



A Practical PDP Algorithm

1. Begin with ***X*** = {0}
3. Remove the largest element in ***L*** and place it in ***X***
5. See if the element *fits* on the right or left side of the restriction map
7. When it fits, find the other lengths it creates and remove those from ***L***
9. Go back to step 3 until ***L*** is empty

Defining delta(y, X)

- Before describing PartialDigest, we first define a helper function:

  delta(***y***, ***X***)

  as the multiset of all distances between point ***y*** and the points in the set ***X***

  $\text{delta}(y, X) = \{|y - x_1|, |y - x_2|, \ldots, |y - x_\text{n}|\}$

  ex. [3,6,11] = delta(8,[5,14,19])

An Example

$L = \{ 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 \}$

$X = \{ 0 \}$

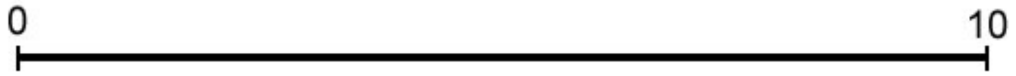
An Example

L = { 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 }
X = { 0 }

Remove 10 from ***L*** and insert it into ***X***. We know this must be the total length of the DNA sequence because it is the largest fragment.

An Example

$L = \{ 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 \}$
$X = \{ 0, 10 \}$



An Example

L = { 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 }
X = { 0, 10 }

Remove 8 from ***L*** and make ***y*** = 2 or 8. But since the two cases are symmetric, we can assume ***y*** = 2.

An Example

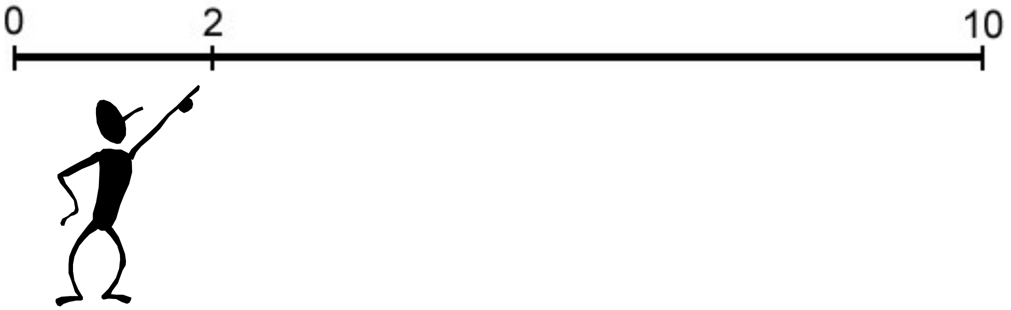
L = { 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 }
X = { 0, 10 }

Find the distances from *y* = 2 to other elements in *X*.
D(***y***, ***X***) = {8, 2}, so we remove {8, 2} from ***L*** and add 2 to ***X***.

An Example

$L = \{\ 2, 2, 3, 3, 4, 5, 6, 7, 8, 10\ \}$

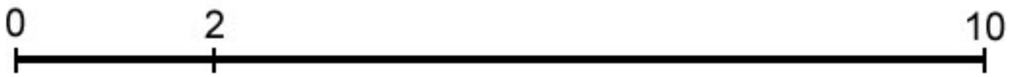
$X = \{\ 0, 2, 10\ \}$



An Example

$L = \{ 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 \}$
$X = \{ 0, 2, 10 \}$

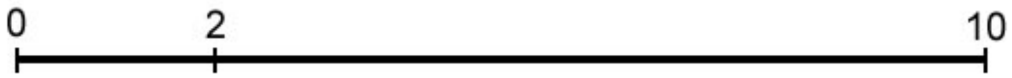
Next, remove 7 from $\boldsymbol{L}$ and make $y = 7$ or $y = 10 – 7 = 3$.
We explore $\boldsymbol{y} = 7$ first, so delta(y, X) = {7, 5, 3}.



An Example

$L = \{ 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 \}$
$X = \{ 0, 2, 10 \}$

For ***y*** = 7 first, delta(y, X) = {7, 5, 3}. Therefore, we remove {7, 5 ,3} from ***L*** and add 7 to ***X***.

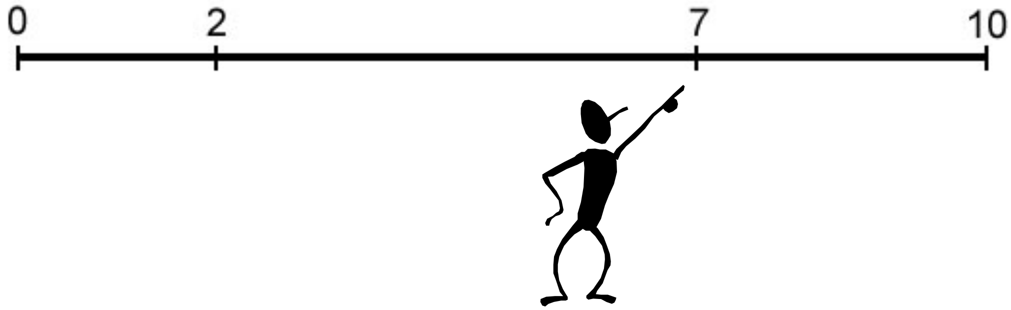


$D(y, X) = \{7, 5, 3\} = \{ |7 - 0|, |7 - 2|, |7 - 10| \}$

An Example

$L = \{\ 2, 2, 3, 3, 4, 5, 6, 7, 8, 10\ \}$

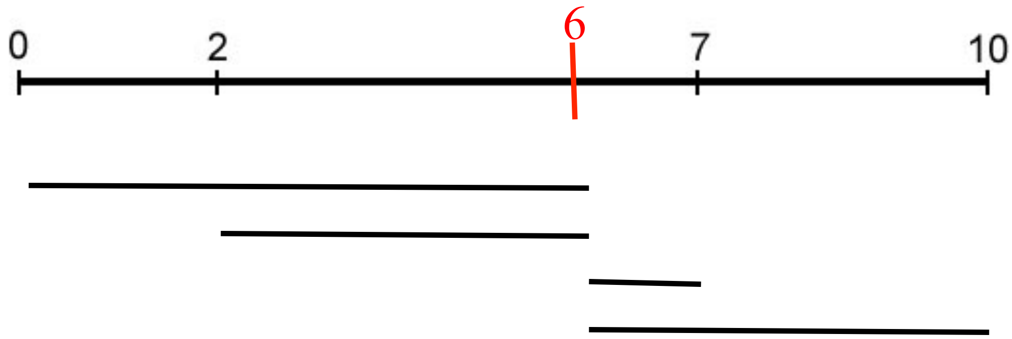
$X = \{\ 0, 2, 7, 10\ \}$



An Example

L = { 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 }
X = { 0, 2, 7, 10 }

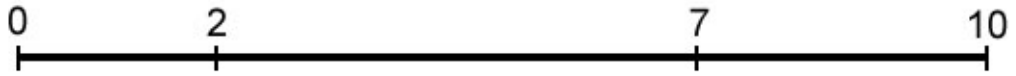
Next, take 6 from ***L*** and make ***y*** = 6. Unfortunately, delta(*y*, *X*) = {6, 4, 1 ,4}, which is not a subset of ***L***. Therefore, we won't explore this branch.



An Example

L = { 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 }
X = { 0, 2, 7, 10 }

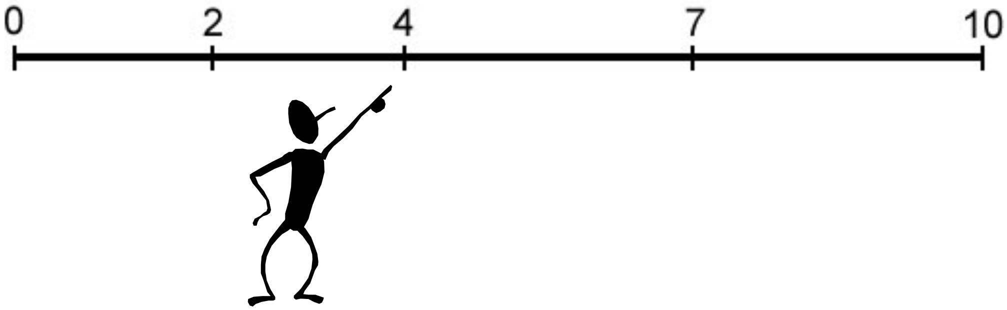
This time make *y* = 4. delta(*y*, *X*) = {4, 2, 3 ,6}, which is a subset of ***L,*** so we explore this branch. We remove {4, 2, 3 ,6} from ***L*** and add 4 to ***X***.



An Example

$L = \{ 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 \}$

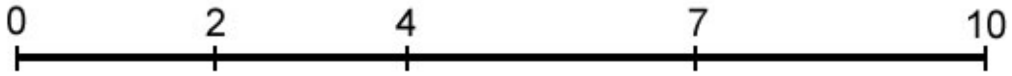
$X = \{ 0, 2, 4, 7, 10 \}$



An Example

L = { 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 }
X = { 0, 2, 4, 7, 10 }

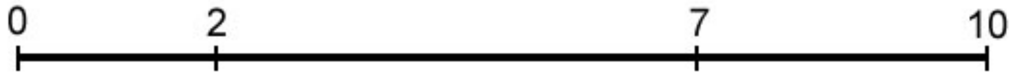
L is now empty, so we have a solution, which is *X*.



An Example

L = { 2, 2, 3, 3, 4, 5, 6, 7, 8, 10 }
X = { 0, 2, 7, 10 }

To find other solutions, we backtrack (remove old insertions and try different ones).

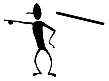


Implementation

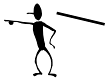
```
def partialDigest(L):
    width = max(L)
    L.remove(width)
    X = [0, width]
    Place(L, X)

def Place(L, X):
    if (len(L) == 0):
        print X
        return
    y = max(L)
    dyX = delta(y, X)
    if (dyX.subset(L)):
        X.append(y); map(L.remove, dyX.items)
        Place(L, X)
        X.remove(y); map(L.append, dyX.items)
    w = max(X) - y
    dwX = delta(w, X)
    if (dwX.subset(L)):
        X.append(w); map(L.remove, dwX.items)
        Place(L,X)
        X.remove(w); map(L.append, dwX.items)
    return
```

This PDP algorithm outputs all solutions. In fact, it might even repeat solutions



Checks distances from the "0" end



Checks distances from the "width" end

Analysis

- Let *T(n)* be the maximum time that partialDigest takes to solve an n-point instance of PDP
- If, at every step, there is only one viable solution, then partialDigest reduces the size of the problem by one on each recursive call

$$T(n) = T(n-1) + O(n) \rightarrow O(n^2)$$

- However, if there are two alternatives then

$$T(n) = 2T(n-1) + O(n) \rightarrow O(2^n)$$

Comments & Next Time

- In the book there is a reference to a polynomial algorithm for solving PDP (pg. 115). The authors of this paper have since posted a clarification that their solution does not suggest a polynomial algorithm. Therefore, the complexity of the PDP is still unknown.
- Next Time: More Exhaustive Search problems
- Next Time: The Motif Finding Problem