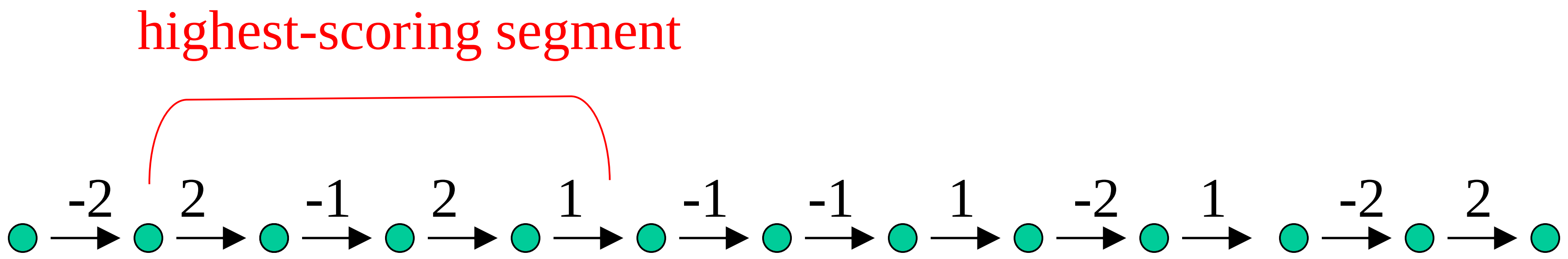
Lecture 7: Weighted linked lists

- Applications (via *sequence graphs*):
  - – regions of atypical residue composition
  - – motif clusters
  - – read count data
- Finding *multiple* high-scoring paths
- “D-segments”
- Statistical significance

Weighted Linked Lists (WLLs)

- *WLL* is linked list with weights on each edge
  - – simplest kind of WDAG.

- Paths = ‘segments’ or ‘regions’



- Find highest-scoring segments by dynamic programming
  - Much better than “brute force” algorithm!

- Beginning & end of best path determine path uniquely, so
  - traceback is unnecessary
  - single pass through list suffices to find best path.

from lecture 6 :

- To reconstruct best path, need "traceback" pointer to immediate predecessor of *v* in best path:

$$T(v) = \begin{cases} v & w(v) = 0 \\ \underset{u \in \text{parents}(v)}{\arg\max} \; (w(u) + w((u,v))) & w(v) \neq 0 \end{cases}$$

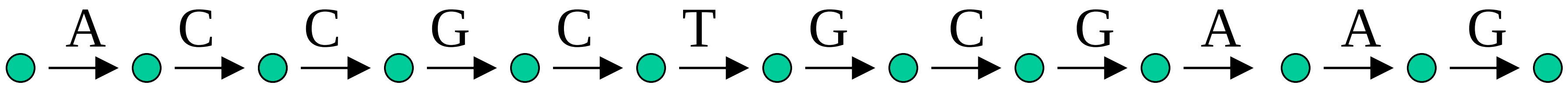
  - in preceding graph, *T*(*v*) is the *parent* on *red edge* coming into *v*
    - if more than one such edge, pick one at random;
    - if no such edge, *T*(*v*) = *v*

- Sometimes useful to record *beginning* of best path:

$$B(v) = \begin{cases} v & w(v) = 0 \\ B(T(v)) & w(v) \neq 0 \end{cases}$$

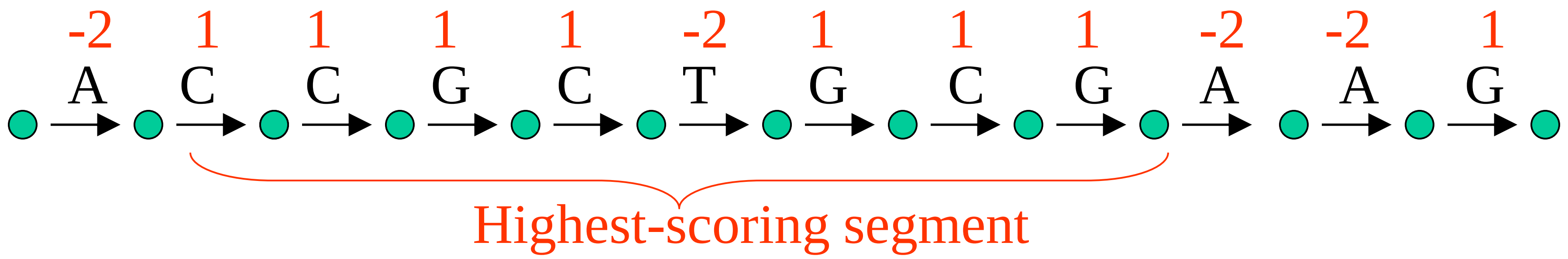
Applications to Sequences

- A *sequence graph* of a sequence is linked list whose edges are labelled by sequence residues (in order):
- e.g. graph for sequence ACCGCTGCGAAG is:



Weighted Sequence Graphs

- If attach weight to each residue, sequence graph becomes a WLL.



- Useful for identifying sequence regions (‘target regions’) with atypical composition:

- In DNA:
  - GC-rich regions in AT-rich thermophile genomes
    - generally correspond to RNA genes (Rob Klein & Sean Eddy)
  - *horizontally transferred* regions
  - isochores (mammalian DNA)
- In proteins:
  - hydrophobic regions (transmembrane segments)
  - hydrophilic regions (loops, intrinsically disordered regions)
  - acidic or basic regions

‘Optimal’ scores

- *Assume* sequence consists of
  - *target regions* with residue freqs t_r
  - *background regions* with residue freqs b_r
  - *independence assumption* applies in both
- *Then* ‘best’ scoring system to detect the target regions uses LLRs:

$$s(r) = \log(t_r / b_r)$$

- if residue freqs are unknown, can usually estimate iteratively

Can use *non-residue-based* scores to find:

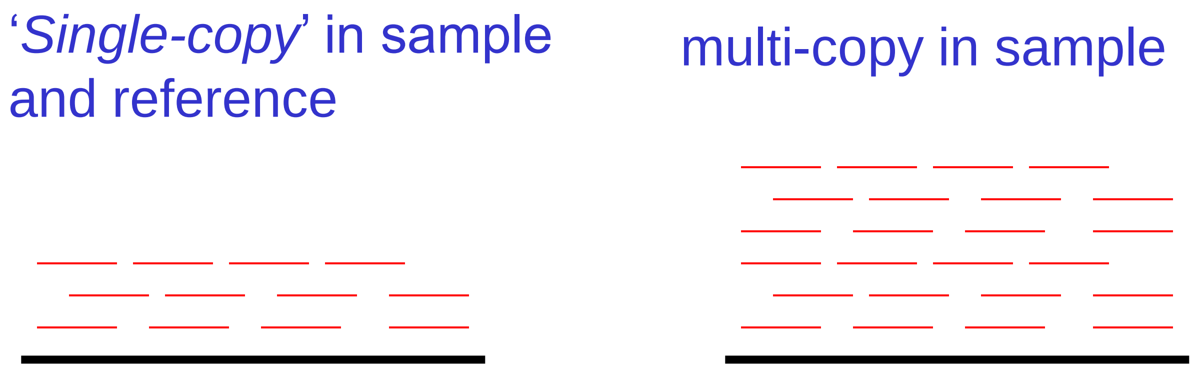
- Regions enriched in particular sequence *motifs*:
  - CpG islands in mammalian genomes
    - positive weight (e.g. +17) to the first C of each CpG, and
    - negative weight (e.g. –1) to every other base

    (This approach was used in *Nature* human genome paper).
  - Regions rich in (known) transcription-factor motifs
  - Optimal scores are LLRs, but now based on 'symbol frequencies' (where symbol = presence/absence of motif)

- Regions targeted by *next-gen read experiments* (symbols = *read counts*)
  - CNVs (Homework 5)
  - Hypersensitive sites
  - CHIP-seq
- Conserved regions in *sequence alignments* (symbols = *alignment columns*)

CNVs & Read Depth

- CNV = ‘copy number variant’– e.g. region that is single copy in reference sequence but duplicated in sample
- One way to detect: map reads from sample onto reference, look for regions of atypical coverage depth



HW 5: finding CNVs using D-segments

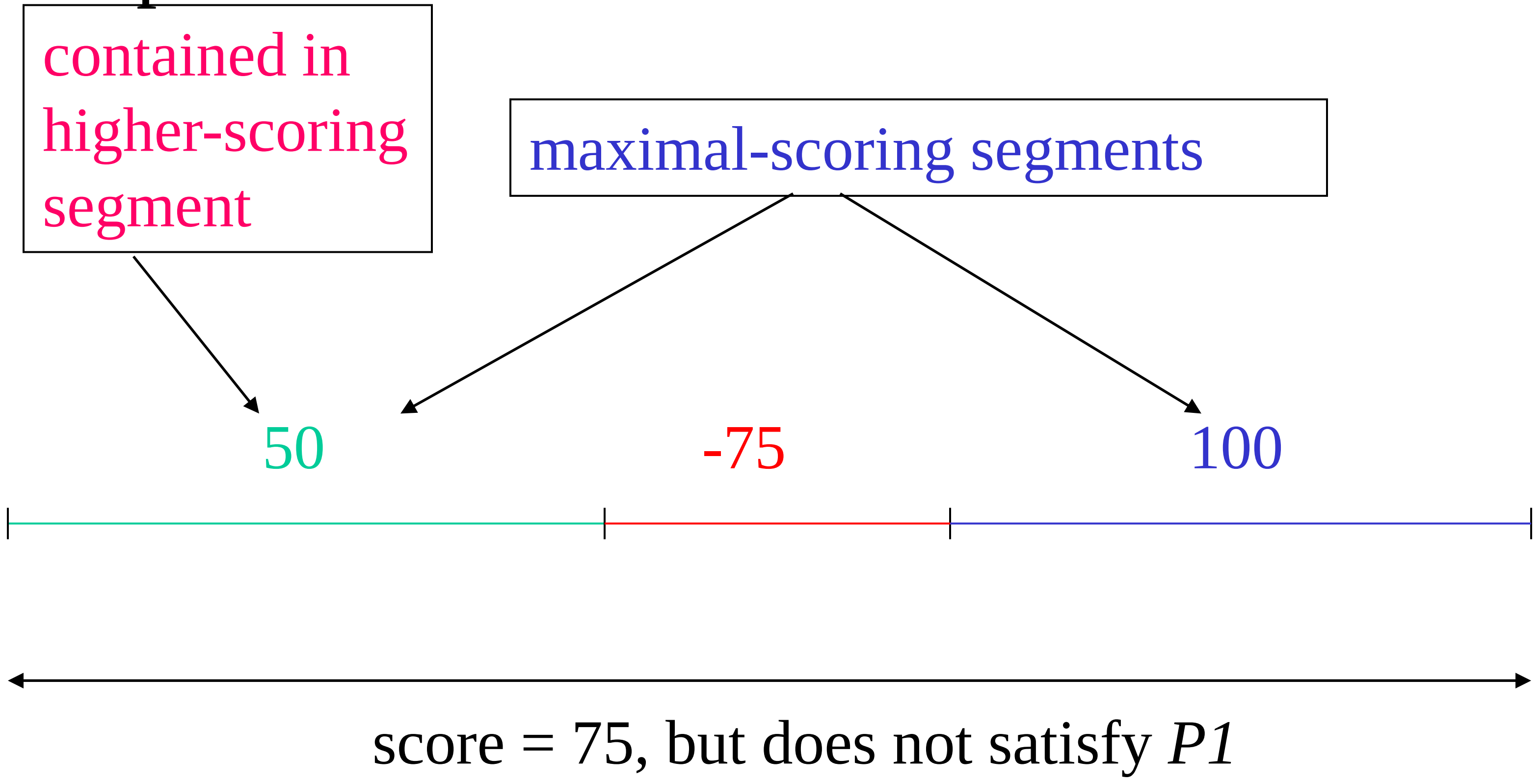
- ***data***: next-gen read alignments to genome
- observed symbols: *counts* of **# *read starts*** at each position (0, 1, 2, ≥ 3)
  - *frequencies* from ***Poisson dist'n*** with appropriate mean
- target regions: ***heterozygous duplications***
  - One chrom = reference allele, other is dup
  - Poisson mean = **1.5** X background mean

Finding *multiple* high-scoring segments

- In general, expect several regions of particular type in a given sequence – not just one!
- So want to find multiple high-weight paths in a WDAG
- But not interested in slight perturbations of previously found paths
- One strategy:
  - Find highest-weight path
  - 'Mask it' (remove its edges from graph)
  - Repeat above two steps until scores no longer 'interesting'

- Is there a more efficient algorithm not requiring repeated scans?
  - Ruzzo & Tompa solved for WLLs
  - ∃ solution for arbitrary WDAGs?

- A (*locally*-)*maximal*(*-scoring*) *segment* I is one such that
  - *P1:* no subsegment of I has a higher score than I
  - *P2:* no segment properly containing I satisfies *P1*
- Example:

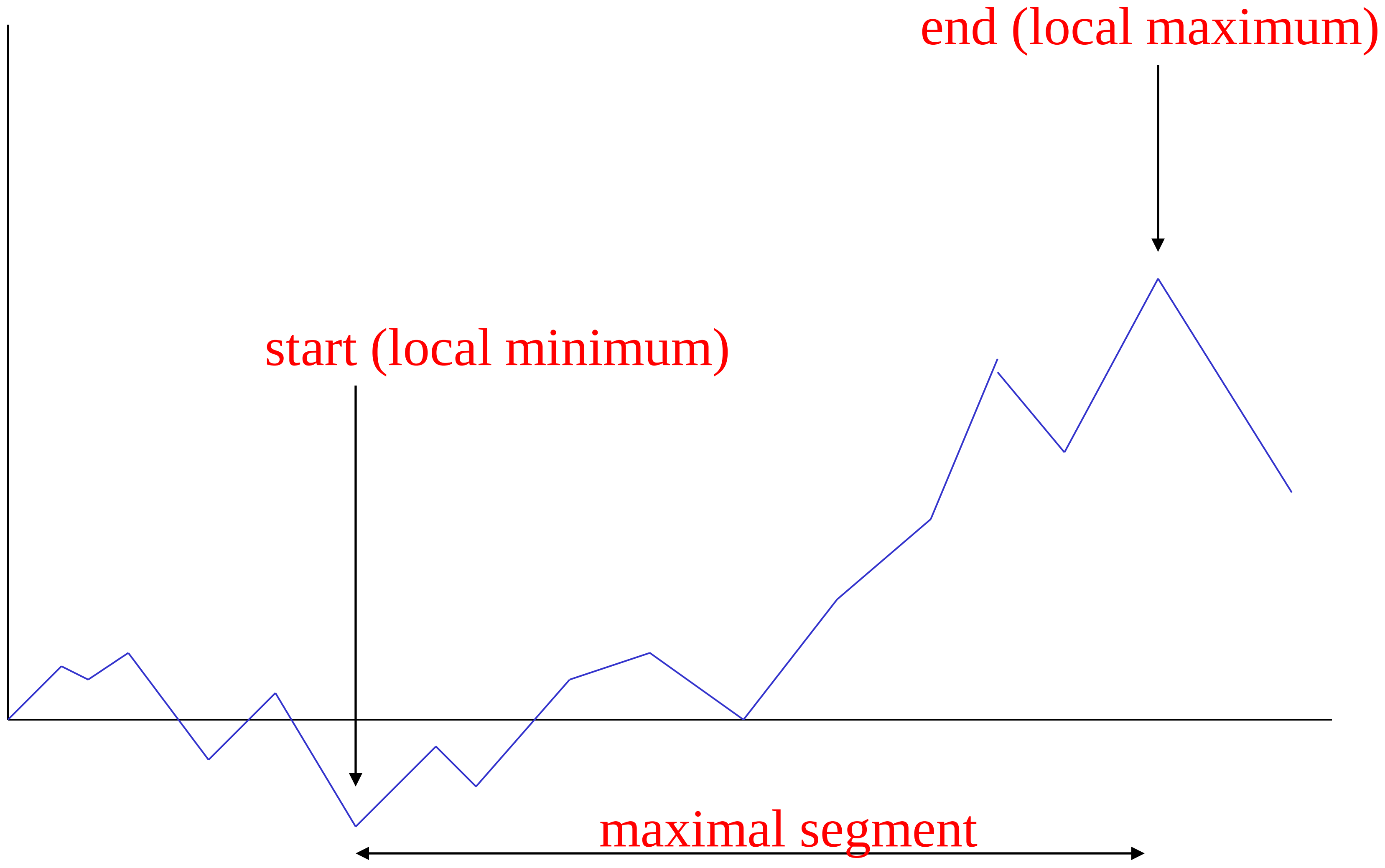

contained in higher-scoring segment
maximal-scoring segments
50
-75
100
score = 75, but does not satisfy P1

- ***Problem***: given $S > 0$, find all maximal segs of score $\geq S$
- Segments are *paths* in a linked-list WDAG with $N+1$ vertices and N edges
- ***Highest weight path*** is found by dynamic programming;

  in (pseudo-)pseudocode:

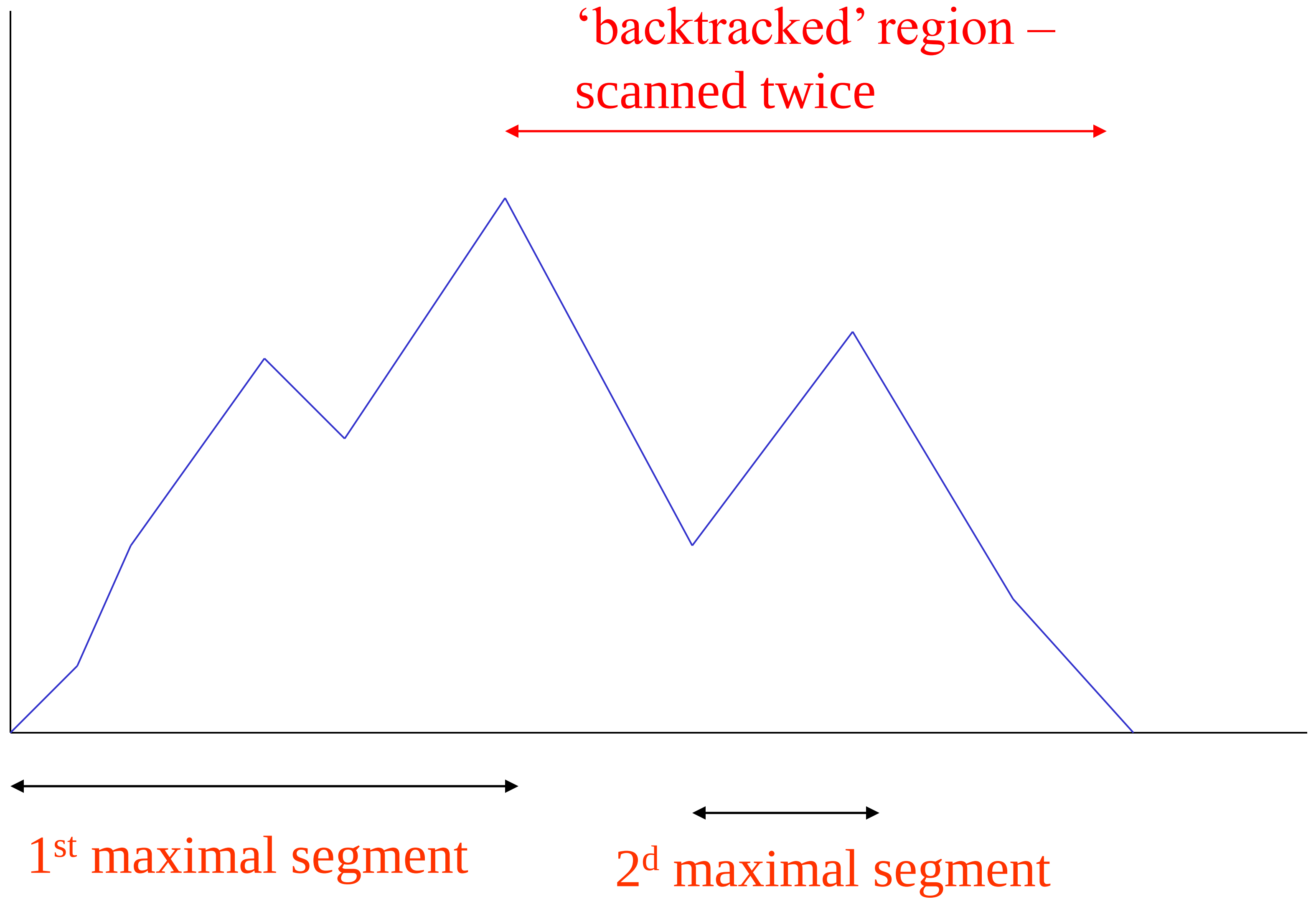
```
cumul = max = 0;  start = 1;
for (i = 1; i ≤ N; i++)  {
    cumul += s[i];
    if (cumul ≤ 0)
          {cumul = 0;  start = i + 1;}  /* NOTE RESET TO ZERO */
    else if (cumul ≥ max)
          {max = cumul;  best_end = i;  best_start = start;}
}
if (max ≥ S) print best_start, best_end, max
```

Maximal segments – from cumulative score plot (*without* 0 resets)



- Can find *all* maximal segs of score ≥ S using following practical (but ***non-optimal***) algorithm:

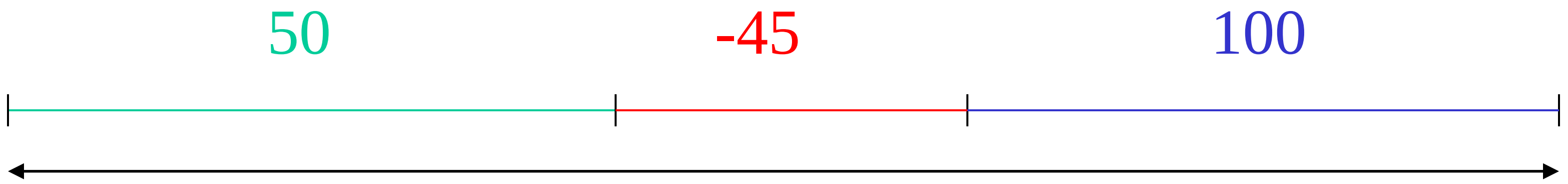
```
cumul = max = 0;  start = 1;
for (i = 1; i ≤ N; i++) {
    cumul += s[i];
    if (cumul ≥ max)
        {max = cumul; end = i;}
    if (cumul ≤ 0 or i == N) {
        if (max ≥ S)
          {print start, end, max;   i = end; }  /* N.B. MUST BACKTRACK! */
        max = cumul = 0;  start = end = i + 1;
    }
}
```


‘backtracked’ region –
scanned twice
1st maximal segment
2d maximal segment

- In worst case this is $O(N^2)$ (because of backtracking),
  - but in practice usually $O(N)$ because a given base is usually traversed only a few times
- Ruzzo-Tompa algorithm ***guarantees*** $O(N)$

- undesirable aspect of maximal segments as defined:
  - single maximal seg may contain *two* (or more) high-scoring regions, separated by significant negative-scoring regions
  - i.e. two possibly biologically distinct target occurrences get merged into one maximal segment

- Example:

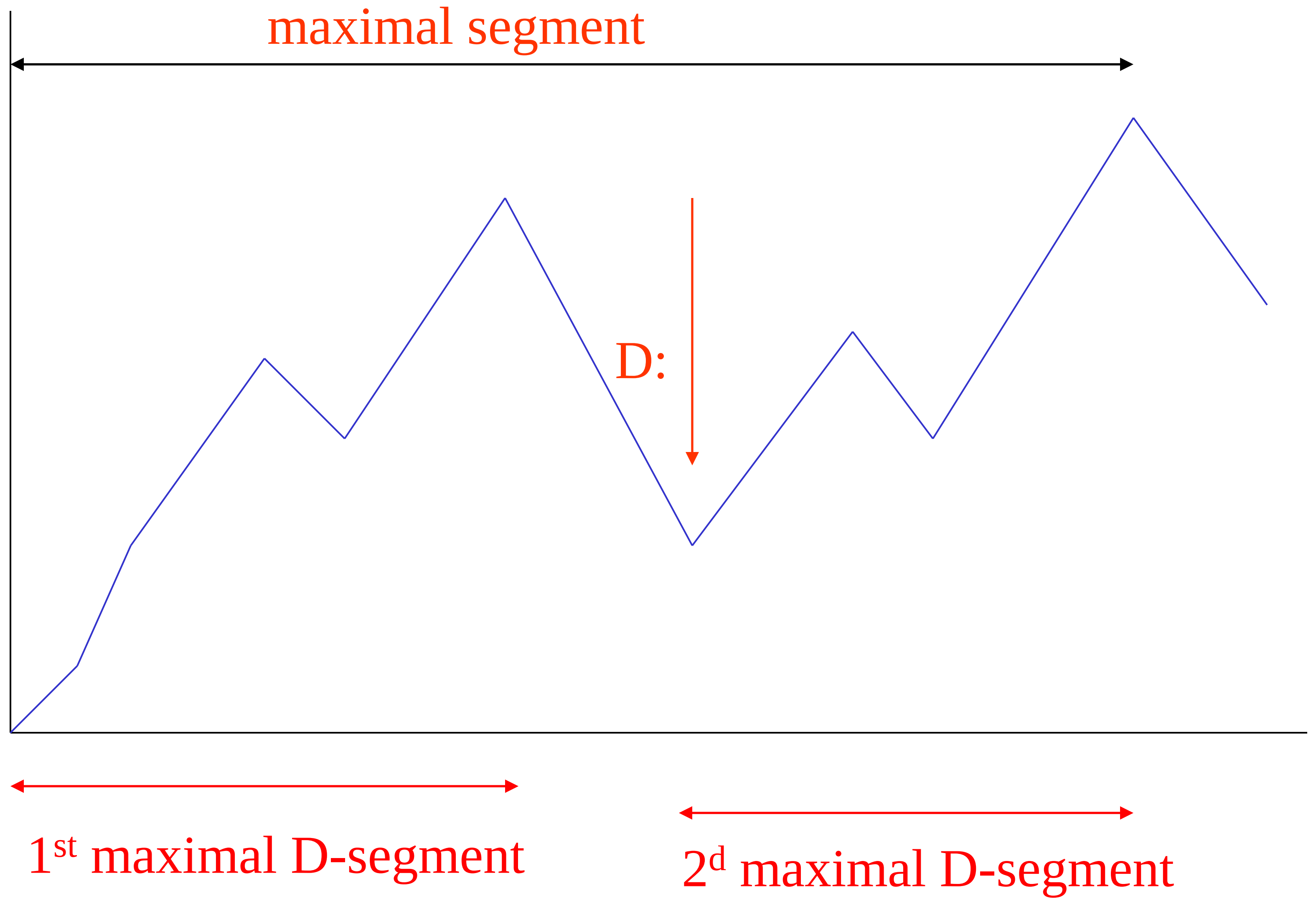


now entire segment has score = 105, & satisfies *P1* ***and*** *P2*

A better problem!

- to avoid this, have max allowed ‘dropoff’ D < 0
- *D-segment* is segment without any subsegments of score < D
- *maximal D-segment* is D-segment I such that
    - *P1:* no subsegment of I has higher score than I
    - *P2:* no D-segment properly containing I satisfies *P1*
- Problem: given S ($\geq$ –D), find all maximal D-segs of score $\geq S$
  - (algorithm fails if $S <$ –D)

Maximal D-segments



- $O(N)$ algorithm to find all maximal D-segs:

```
cumul = max = 0; start = 1;
for (i = 1; i ≤ N; i++) {
    cumul += s[i];
    if (cumul ≥ max)
        {max = cumul; end = i;}
    if (cumul ≤ 0 or cumul ≤ max + D or i == N) {
        if (max ≥ S)
            {print start, end, max; }
        max = cumul = 0; start = end = i + 1; /* NO BACKTRACKING
            NEEDED! */
    }
}
```

- So *more biologically relevant* problem is also *computationally simpler*!
- what are appropriate S and D?
  - mainly an empirical question (based on known examples); altho
    - interpretation via 2-state HMM can be useful
    - Karlin-Altschul theory tells when they are ‘statistically significant’

D-Segments

- Powerful tool for analyzing ‘linear’ data
  - Single sequences (incl. motifs, numerical data)
  - Fixed alignment
- Strengths:
  - Very simple to program
  - Very fast, even for mammalian genomes
- Main limitation:
  - Only allows two types of segments (‘target’ and ‘background’)
    - Essentially a generalization of 2-state HMMs
    - multi-state HMMs are more flexible

Statistical significance of segment scores

- How often does a given score occur by chance in background sequence?
- Can suggest (but not prove!) biological significance

Methods for Assessing Significance of Maximal Segment Scores

1. exact prob dist’n
2. approximate formula (Karlin-Altschul)
3. from simulated sequences
4. from real biological ‘background’ sequences
   - i.e. not having feature in question

1, 2, 3 require probability model approximating biological reality; 4 requires an appropriate dataset

2 is faster than 1 or 3 (and gives ‘intuition’), but involves add’l approximations (ignores ‘edge effects’)

1 requires more complex algorithm

Karlin / Altschul approximation

- for $s(r) = \log_b(t_r / b_r)$, expected # segments of score $\geq S$ in (random) backgd seq of length N

  $\approx$ $NK\,b^{-S}$

- for some constant K (not depending on S)
- Note that $b^{-S} = b^{-LLR} = 1 / LR$

  so (apart from K) this is essentially the observation in lecture 5:

(from lecture 5) *Average* likelihood ratios

- ***average LR*** (for sites) ≈ ***average spacing*** between occurrences of 'site-like' sequences ***in background***
- So e.g. for 3' splice sites
  - if the average *LR* is 1000, then one expects 'splice-site-like' sequences to occur on average once per kb *in background sequence*
  - ***N.B.*** This says nothing about the frequency of *actual* splice sites! (which could be greater or smaller than 1 per kb), and so doesn't by itself provide the probability that an *apparent* splice site is an *actual* site.