Lecture 11. Genome Annotation

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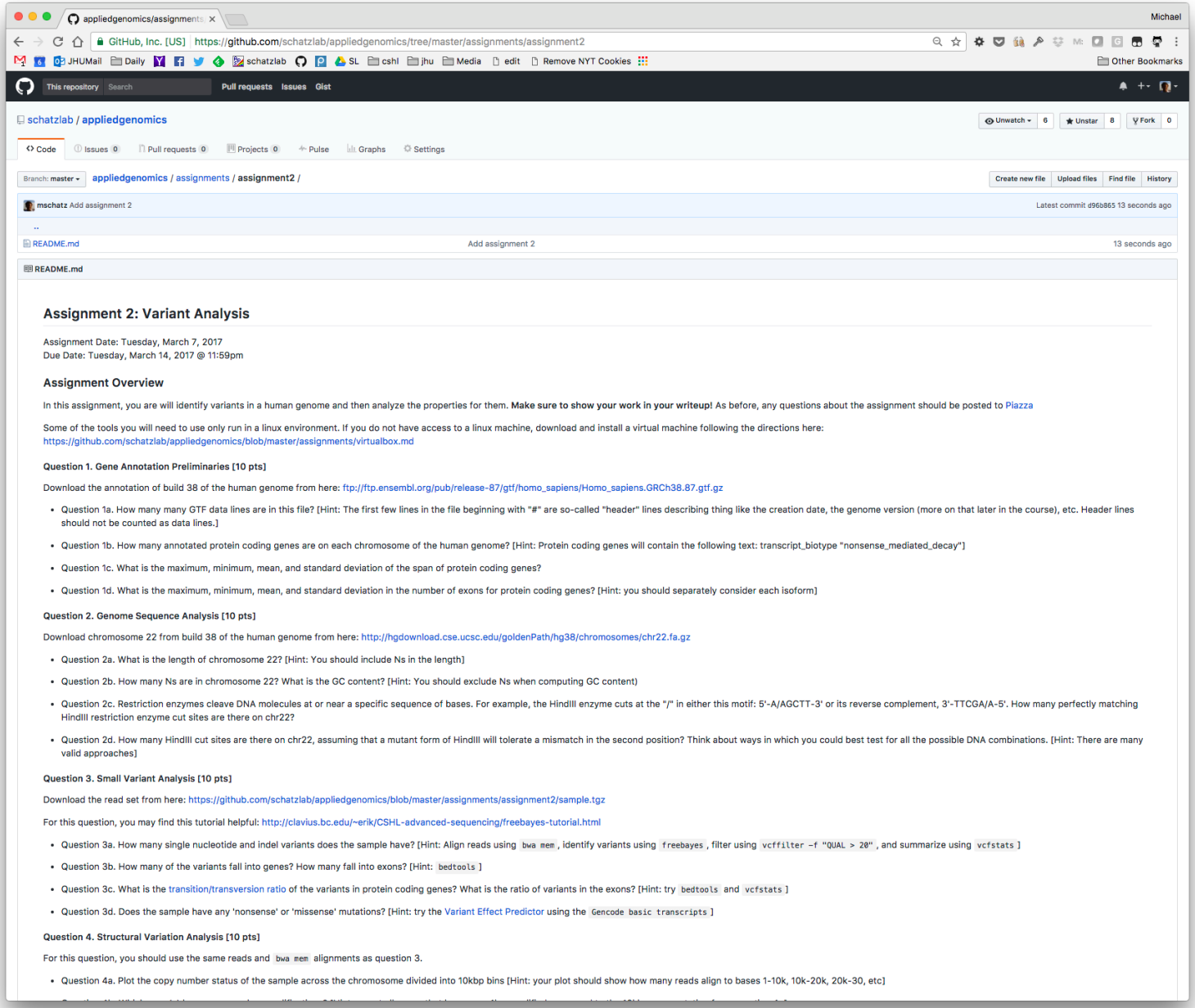
March 7, 2017

JHU 600.649: Applied Comparative Genomics

Assignment 2

Due: Tuesday March 14 @ 11:59pm



appliedgenomics/assignments

GitHub, Inc. [US] https://github.com/schatzlab/appliedgenomics/tree/master/assignments/assignment2

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Assignment 2: Variant Analysis

Assignment Date: Tuesday, March 7, 2017
Due Date: Tuesday, March 14, 2017 @ 11:59pm

Assignment Overview

In this assignment, you are will identify variants in a human genome and then analyze the properties for them. **Make sure to show your work in your writeup!** As before, any questions about the assignment should be posted to Piazza

Some of the tools you will need to use only run in a linux environment. If you do not have access to a linux machine, download and install a virtual machine following the directions here:
https://github.com/schatzlab/appliedgenomics/blob/master/assignments/virtualbox.md

Question 1. Gene Annotation Preliminaries [10 pts]

Download the annotation of build 38 of the human genome from here: ftp://ftp.ensembl.org/pub/release-87/gtf/homo_sapiens/Homo_sapiens.GRCh38.87.gtf.gz

- Question 1a. How many many GTF data lines are in this file? [Hint: The first few lines in the file beginning with "#" are so-called "header" lines describing thing like the creation date, the genome version (more on that later in the course), etc. Header lines should not be counted as data lines.]
- Question 1b. How many annotated protein coding genes are on each chromosome of the human genome? [Hint: Protein coding genes will contain the following text: transcript_biotype "nonsense_mediated_decay"]
- Question 1c. What is the maximum, minimum, mean, and standard deviation of the span of protein coding genes?
- Question 1d. What is the maximum, minimum, mean, and standard deviation in the number of exons for protein coding genes? [Hint: you should separately consider each isoform]

Question 2. Genome Sequence Analysis [10 pts]

Download chromosome 22 from build 38 of the human genome from here: http://hgdownload.cse.ucsc.edu/goldenPath/hg38/chromosomes/chr22.fa.gz

- Question 2a. What is the length of chromosome 22? [Hint: You should include Ns in the length]
- Question 2b. How many Ns are in chromosome 22? What is the GC content? [Hint: You should exclude Ns when computing GC content)
- Question 2c. Restriction enzymes cleave DNA molecules at or near a specific sequence of bases. For example, the HindIII enzyme cuts at the "/" in either this motif: 5'-A/AGCTT-3' or its reverse complement, 3'-TTCGA/A-5'. How many perfectly matching HindIII restriction enzyme cut sites are there on chr22?
- Question 2d. How many HindIII cut sites are there on chr22, assuming that a mutant form of HindIII will tolerate a mismatch in the second position? Think about ways in which you could best test for all the possible DNA combinations. [Hint: There are many valid approaches]

Question 3. Small Variant Analysis [10 pts]

Download the read set from here: https://github.com/schatzlab/appliedgenomics/blob/master/assignments/assignment2/sample.tgz

For this question, you may find this tutorial helpful: http://clavius.bc.edu/~erik/CSHL-advanced-sequencing/freebayes-tutorial.html

- Question 3a. How many single nucleotide and indel variants does the sample have? [Hint: Align reads using `bwa mem`, identify variants using `freebayes`, filter using `vcffilter -f "QUAL > 20"`, and summarize using `vcfstats`]
- Question 3b. How many of the variants fall into genes? How many fall into exons? [Hint: `bedtools`]
- Question 3c. What is the transition/transversion ratio of the variants in protein coding genes? What is the ratio of variants in the exons? [Hint: try `bedtools` and `vcfstats`]
- Question 3d. Does the sample have any 'nonsense' or 'missense' mutations? [Hint: try the Variant Effect Predictor using the `Gencode basic transcripts`]

Question 4. Structural Variation Analysis [10 pts]

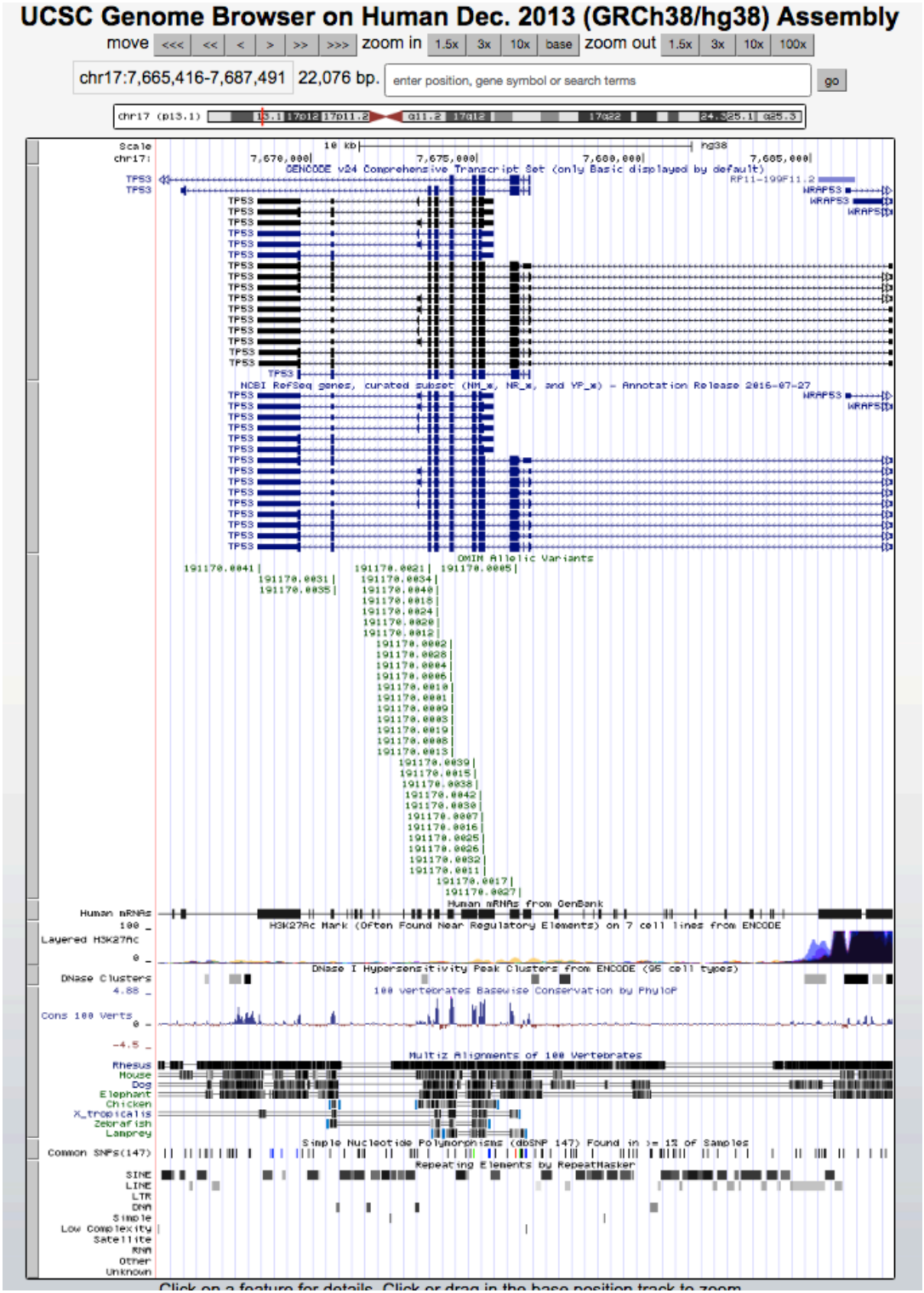
For this question, you should use the same reads and `bwa mem` alignments as question 3.

- Question 4a. Plot the copy number status of the sample across the chromosome divided into 10kbp bins [Hint: your plot should show how many reads align to bases 1-10k, 10k-20k, 20k-30, etc]

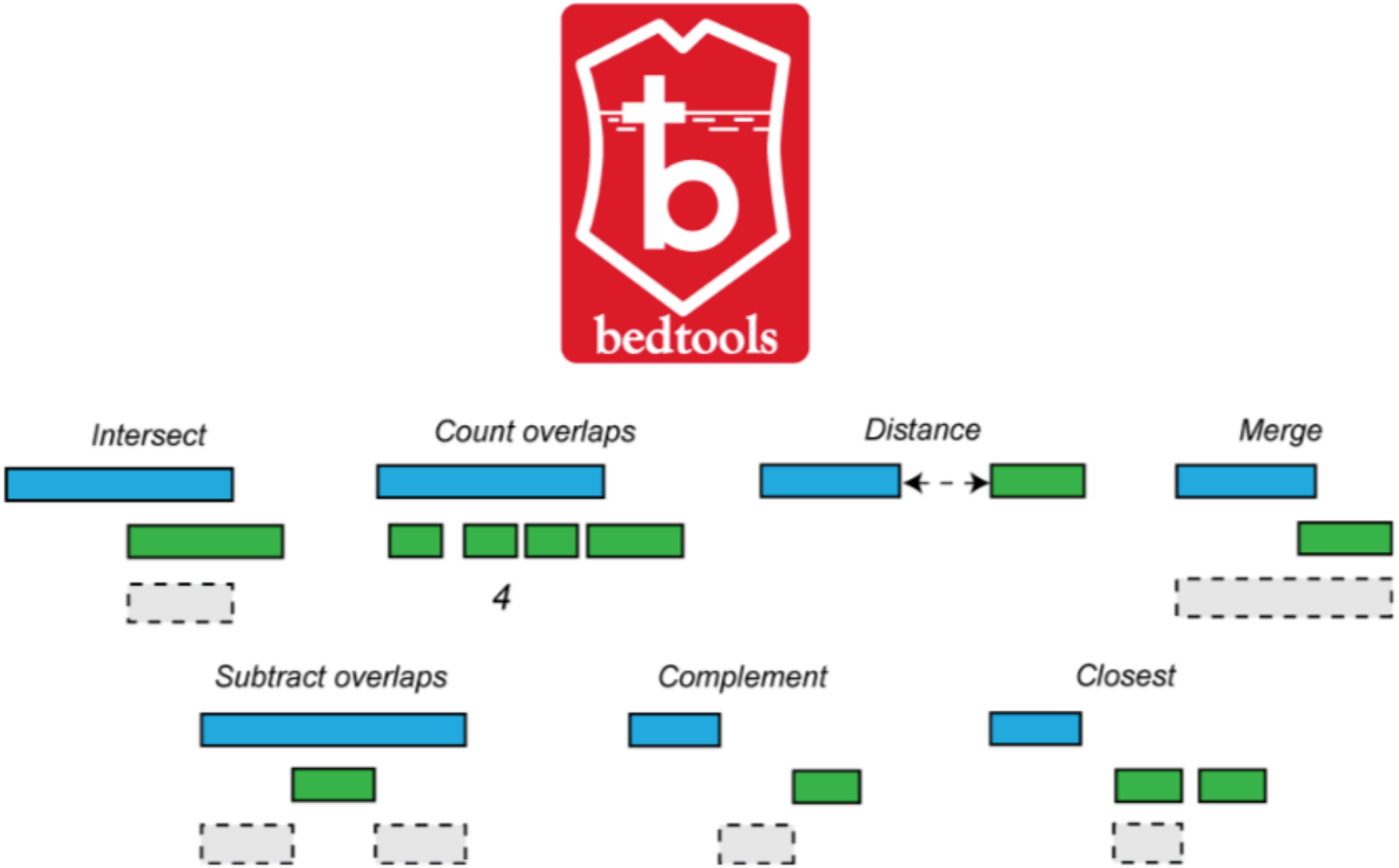
Part 1:
Genome Arithmetic Review

What are genome intervals?

- Genetic variation:
  - SNPs: 1bp
  - Indels: 1-50bp
  - SVs: >50bp
- Genes:
  - exons, introns, UTRs, promoters
- Conservation
- Transposons
- Origins of replication
- TF binding sites
- CpG islands
- Segmental duplications
- Sequence alignments
- Chromatin annotations
- Gene expression data
- …
- ***Your own observations and data: put them into context!***



BEDTools to the rescue!

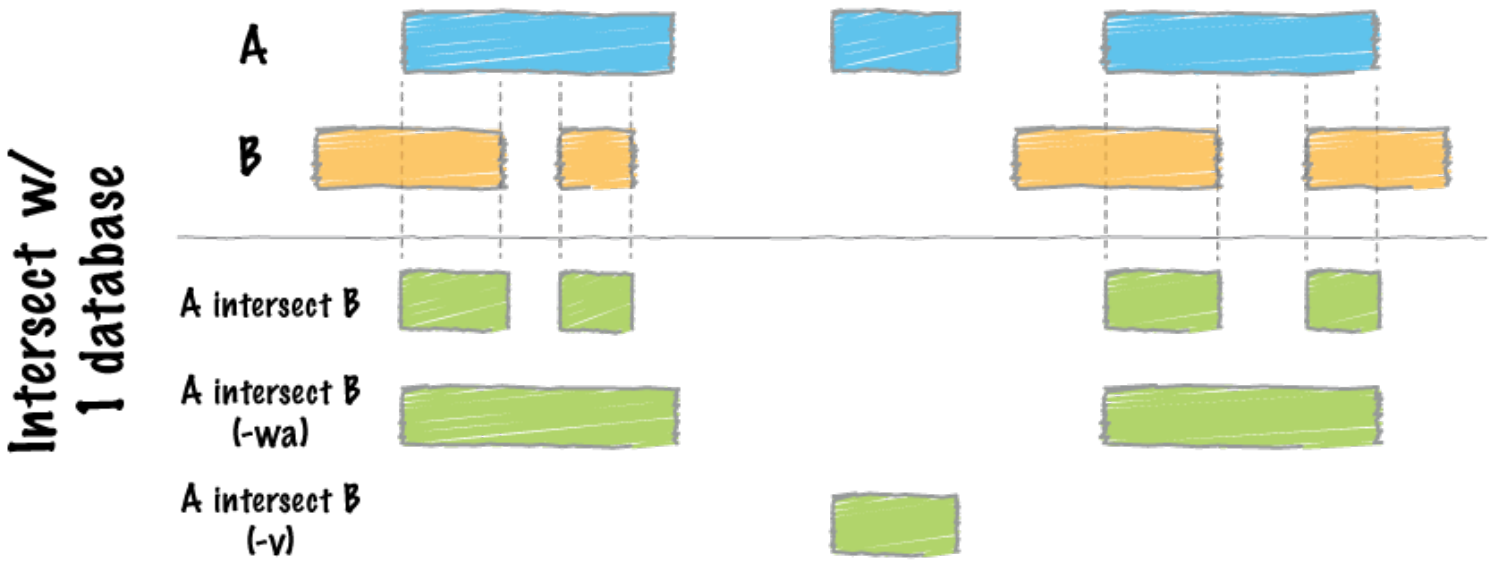


BEDTools commands

```
annotate
bamtobed
bamtofastq
bed12tobed6
bedpetobam
bedtobam
closest
cluster
complement
coverage
expand
flank
fisher
genomecov
getfasta
groupby
groupby
igv
intersect
jaccard
links
makewindows
map
maskfasta
merge
multicov
multiinter
nuc
overlap
pairtobed
pairtopair
random
reldist
shift
shuffle
slop
sort
subtract
tag
unionbedg
window
```

http://bedtools.readthedocs.io/en/latest/content/bedtools-suite.html

BEDTools Intersect



What exons are hit by SVs?

```
$ cat A.bed
chr1  10  20
chr1  30   40

$ cat B.bed
chr1  15  20

$ bedtools intersect -a A.bed -b B.bed -wa
chr1  10   20
```

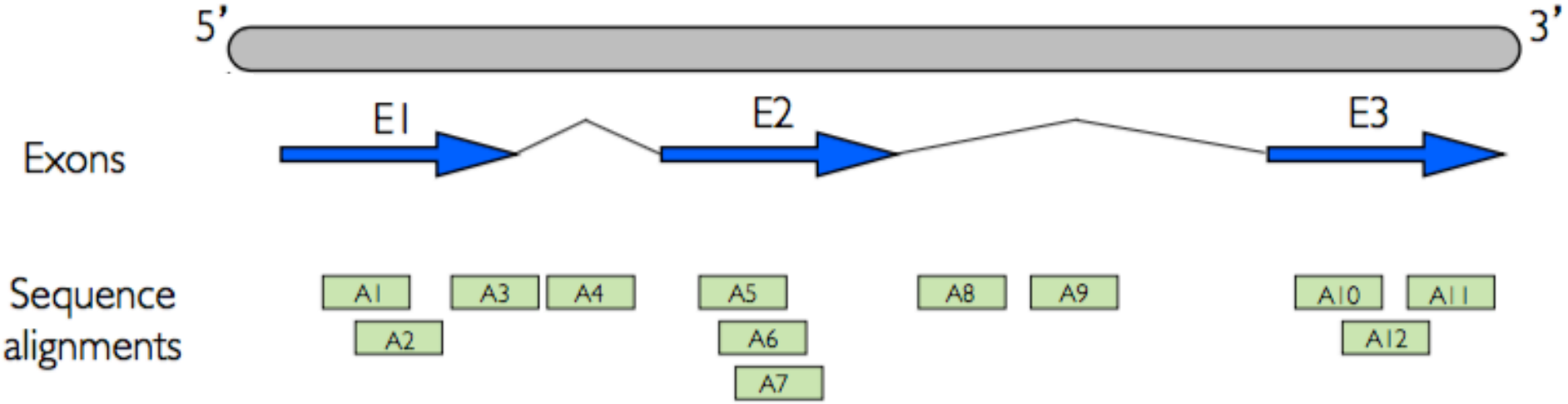
What parts of exons are hit by SVs?

```
$ cat A.bed
chr1  10  20
chr1  30  40

$ cat B.bed
chr1  15   20

$ bedtools intersect -a A.bed -b B.bed
chr1  15   20
```

BEDTools Performance



How many reads are aligned to exonic sequences?

```
$ awk '{if ($3=="exon"){print}}' gencode.v21.annotation.gff3 | wc -l
1162114
```

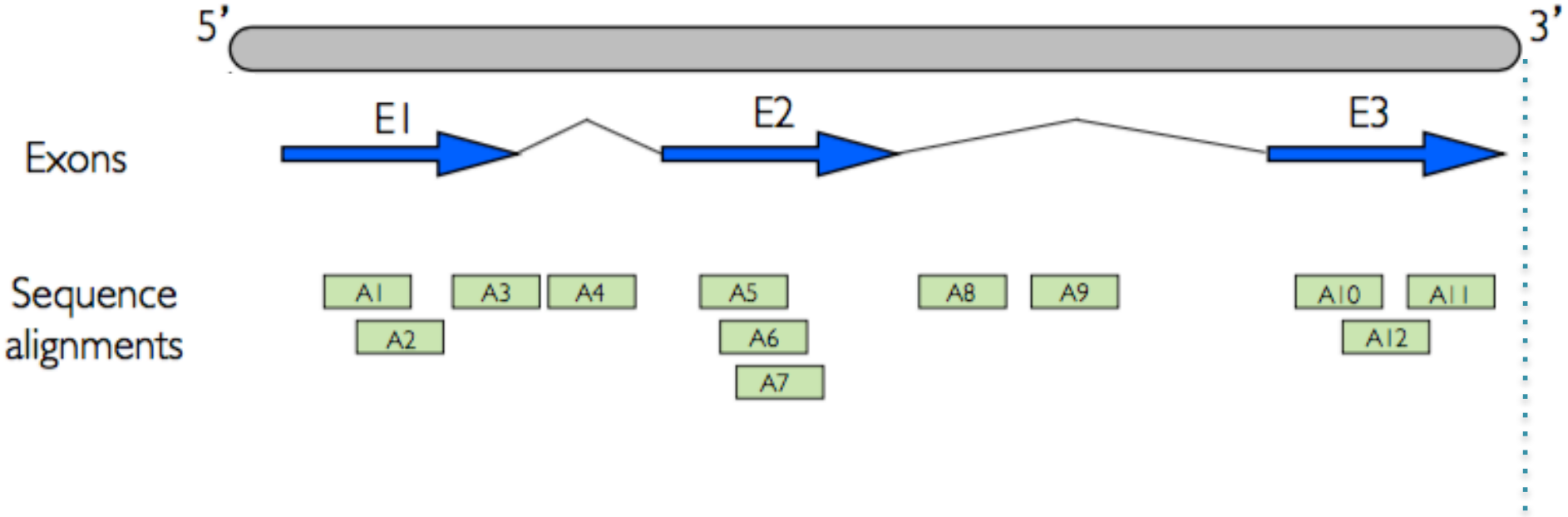
```
if ((read.start <= exon.end) && (read.end >= exon.start)) { print “in exon!”; }
```

How many comparison would a brute force approach take to scan a 30x dataset?

30x3Gb = 90Gbp / 100bp reads = 900M reads

900M reads x 1.1M exons = 990MM comparisons! ☹

Plane Sweep to the Rescue!



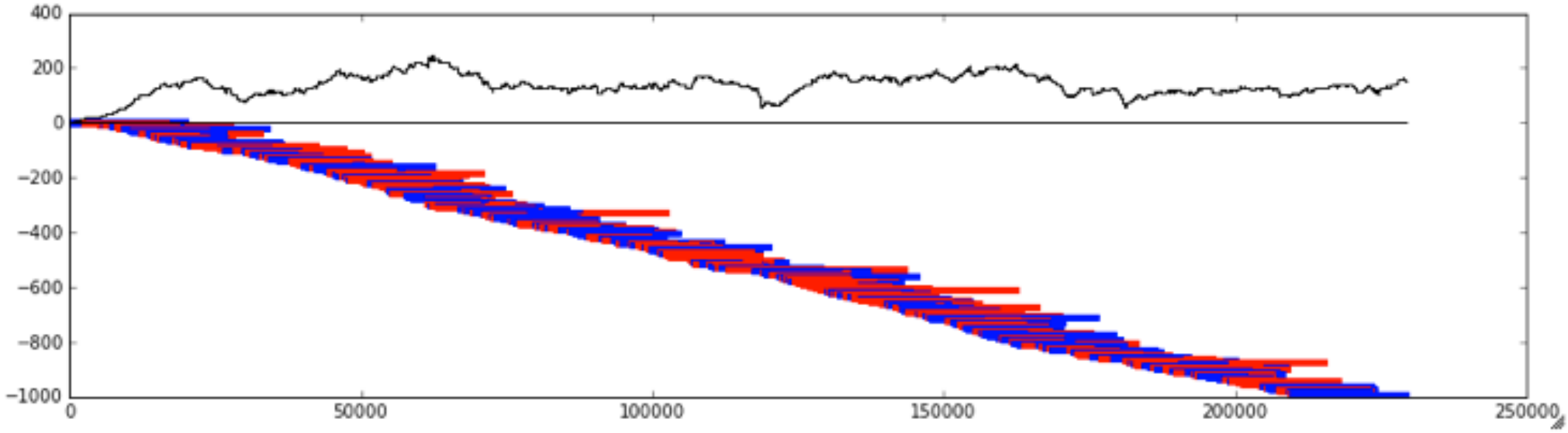
How many comparisons does the plane sweep algorithm make?

Each read is compared to the “active set”

Relatively few exons overlap: average ~1.1 active exons/position

Total comparisons: 900M reads * 1.1 “active exons/read” = 990M comparisons ☺

Coverage across the genome




```
$ head -3 ~/readid.start.stop.txt
1 0 19814
2 799 19947
3 1844 13454

$ tail -3 ~/readid.start.stop.txt
1871 973590 965902
1872 966703 973521
1873 973632 966946
```

Brute Force Coverage Profile

```
print "Brute force computing coverage over %d bp" % (totallen)

starttime = time.time()
brutecov = [0] * totallen

for r in reads:
 # print " -- [%d, %d]" % (r[1], r[2])

  for i in xrange(r[1], r[2]):
    brutecov[i] += 1

brutetime = (time.time() - starttime) * 1000.0

print "  Brute force complete in %0.02f ms" % (brutetime)
print brutecov[0:10]
```

Add 1 to coverage vector at every position the read covers

```
Brute force computing coverage over 973898 bp
  Brute force complete in 4435.00 ms
[1, 1, 1, 1, 1, 1, 1, 1, 1, 1]
```

Notice that it took 4435 ms for this to complete

Delta Encoding

```
deltacov = []
curcov = -1
for i in xrange(0, len(brutecov)):
  if brutecov[i] != curcov:
    curcov = brutecov[i]
    delta = (i, curcov)
    deltacov.append(delta)

## Finish up with the last position
deltacov.append((totallen, 0))
```

Only record those positions when the coverage changes

==> Just like a plane sweep

```
Delta encoding coverage plot
Delta encoding required only 3697 steps, saving 99.62% of the space in 151.32 ms
  0: [0,1]
  1: [799,2]
  2: [1844,3]
  ...
  3694: [973770,2]
  3695: [973779,1]
  3696: [973898,0]
```

Plot Coverage and Read Positions

```
## expand the coverage profile by this amount so that it is easier to see
YSCALE = 5

## draw the layout of reads
for i in xrange(min(MAX_READS_LAYOUT, len(reads))):
  r = reads[i]
  readid = r[0]
  start  = r[1]
  end    = r[2]
  rc     = r[3]
  color  = "blue"

  if (rc == 1):
    color = "red"

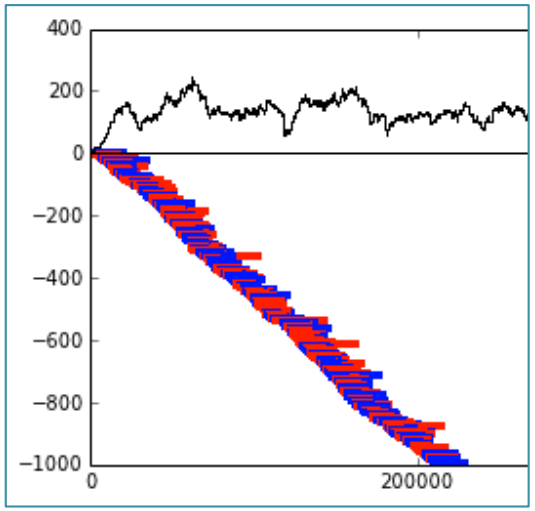
  plt.plot ([start,end], [-2*i, -2*i], lw=4, color=color)

## draw the base of the coverage plot
plt.plot([0, totallen], [0,0], color="black")

## draw the coverage plot
for i in xrange(len(deltacov)-1):
  x1 = deltacov[i][0]
  x2 = deltacov[i+1][0]
  y1  = YSCALE*deltacov[i][1]
  y2  = YSCALE*deltacov[i+1][1]

  ## draw the horizonal line
  plt.plot([x1, x2], [y1, y1], color="black")

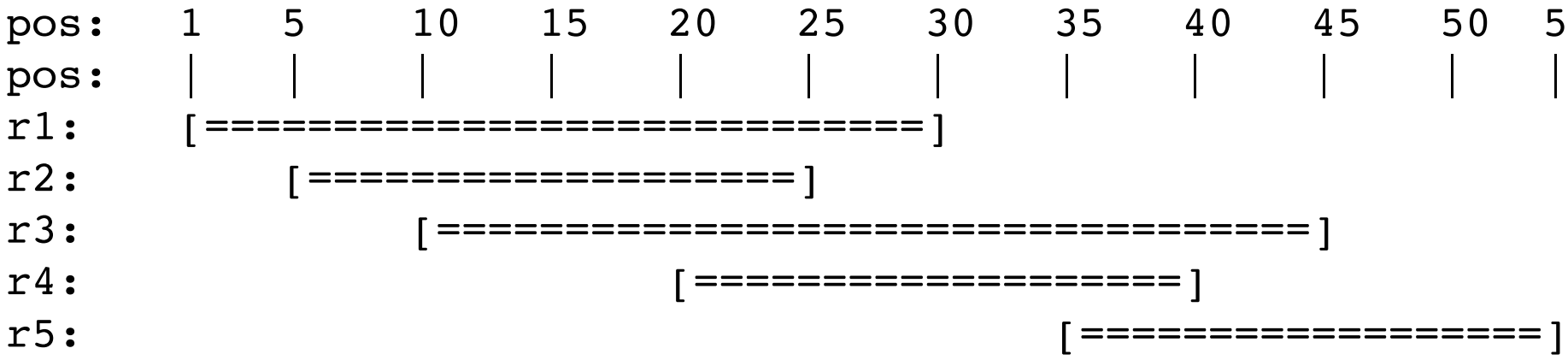
  ## and now the right vertical to the new coverage level
  plt.plot([x2, x2], [y1, y2], color="black")
```



Plot Each Read

Plot Each Coverage Step

Plane Sweep



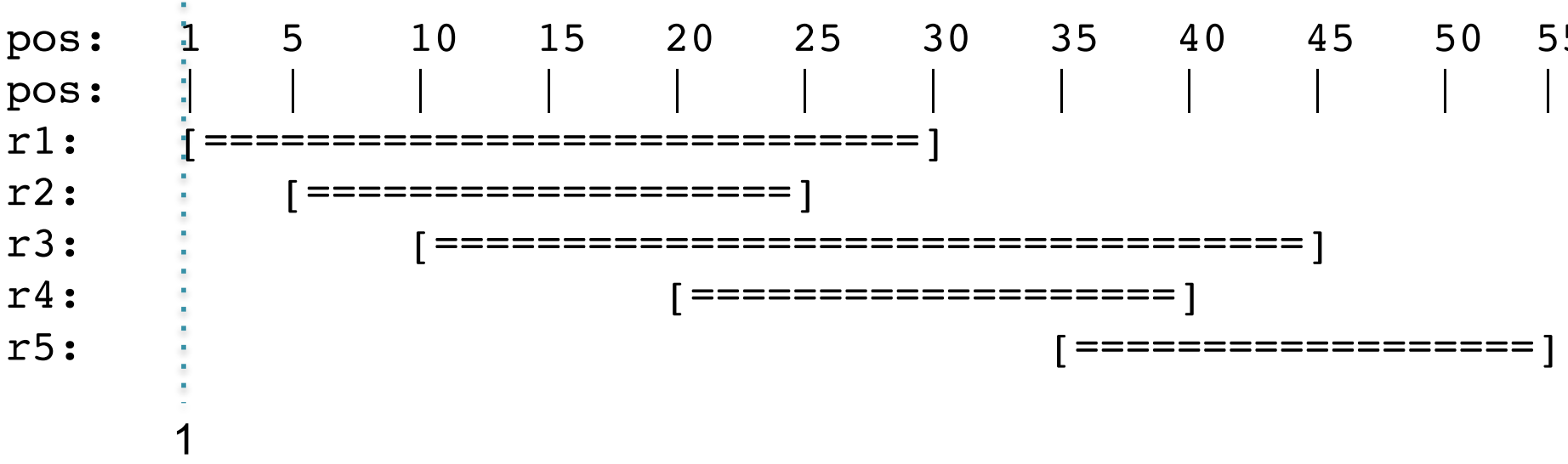
The basic algorithm works like this:

- Assume layout is in sorted order by start position (or explicitly sort by start position)

- use a “list” to track how many reads currently intersect the plane keyed by end coord
  - the number of elements in the list corresponds to the current depth

- walking from start position to start position
  - check to see if we past any read ends
  - coverage goes down by one when a read ends
  - coverage goes up by one when new read is encountered

Plane Sweep

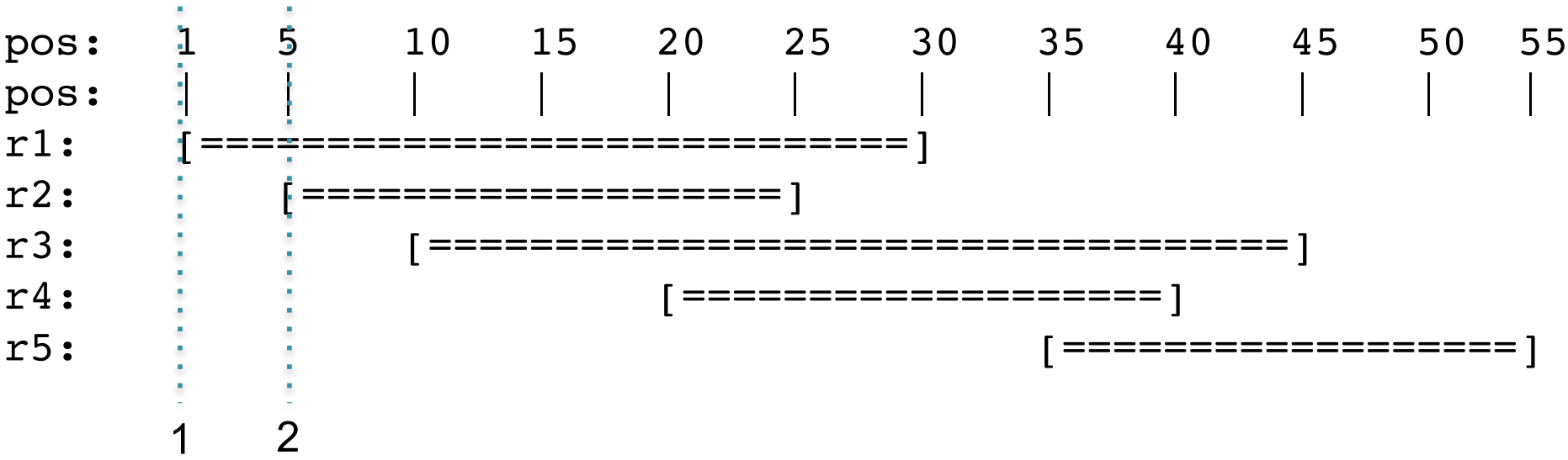


arrive at r1 [1,30]:

active set is empty; add to active set: 30

output (1,1)

Plane Sweep



arrive at r1 [1,30]:

active set is empty; add to active set: 30

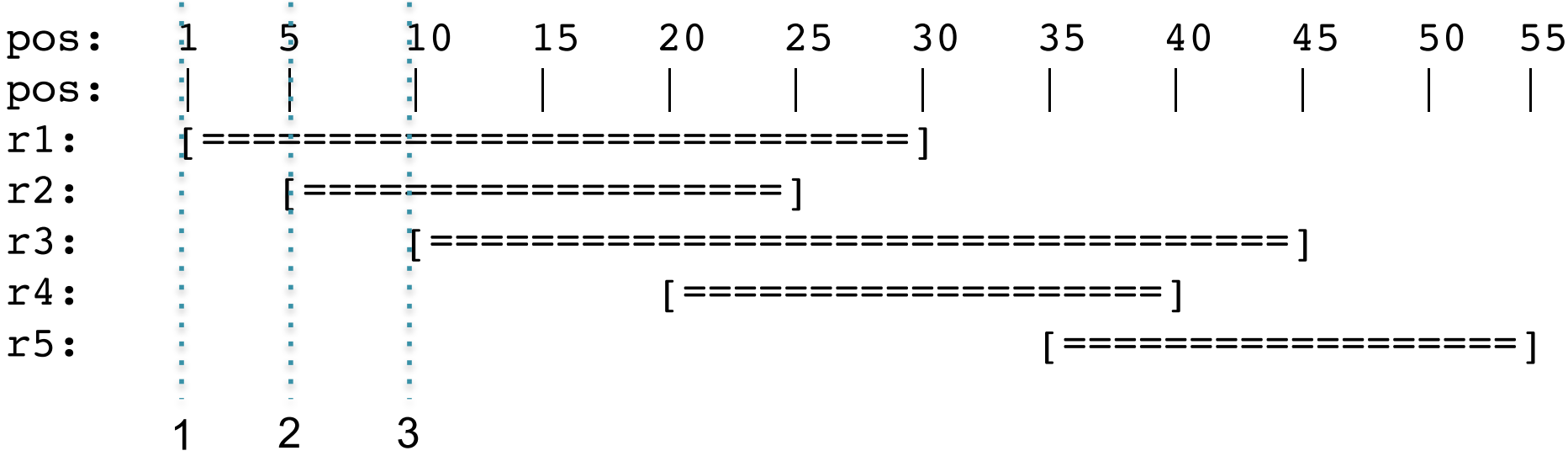
output (1,1)

arrive at r2 [5,25]:

5 < 30: add to active set: 25, 30 <- notice insert out of order

output (5, 2)

Plane Sweep



arrive at r1 [1,30]:

active set is empty; add to active set: 30

output (1,1)

arrive at r2 [5,25]:

5 < 30: add to active set: 25, 30 <- notice insert out of order

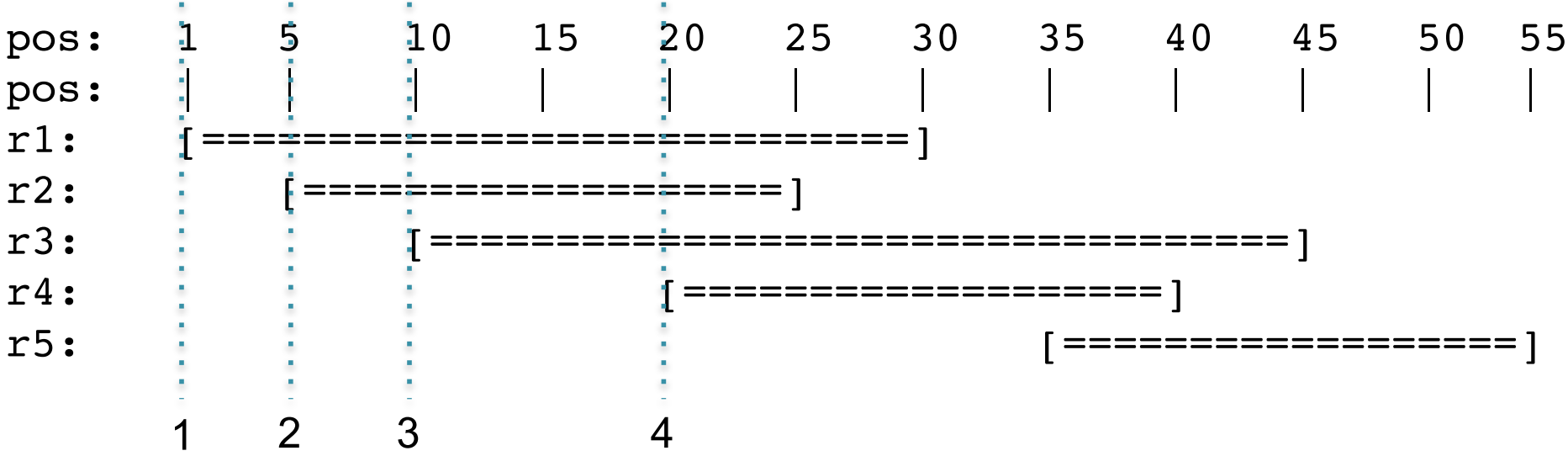
output (5, 2)

arrive at r3 [10,45]:

10 < 25; add to active set: 25, 30, 45

output (10, 3)

Plane Sweep



arrive at r3 [10,45]:

10 < 25; add to active set: 25, 30, 45

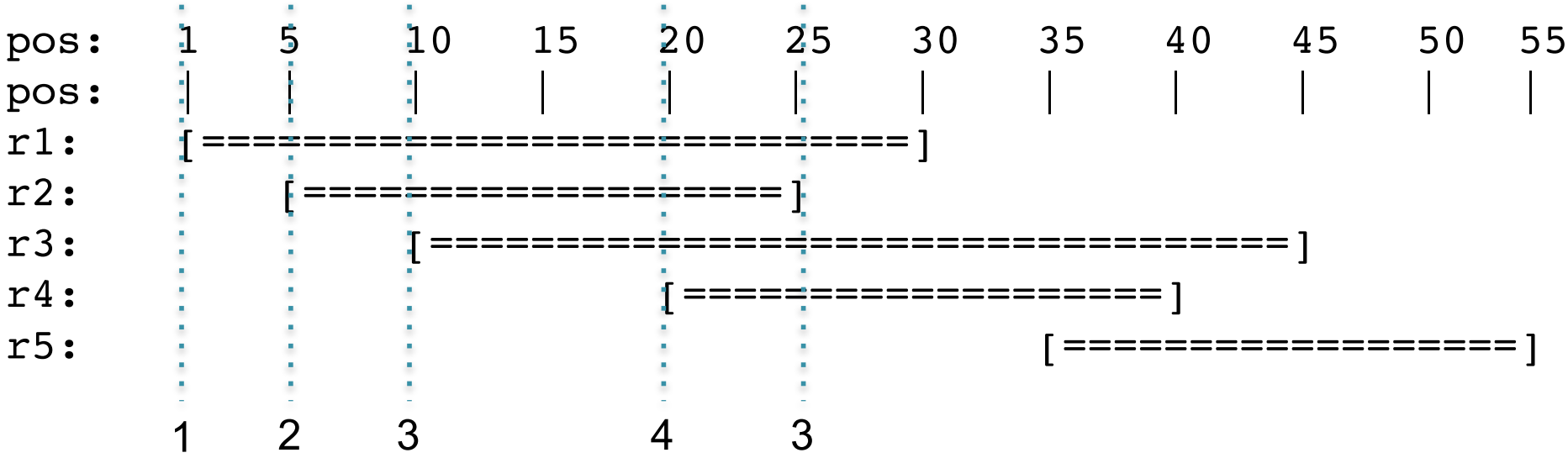
output (10, 3)

arrive at r4 [20,40]:

20 < 25; add to active set: 25, 30, 40, 45 <- out of order again

output (20, 4)

Plane Sweep

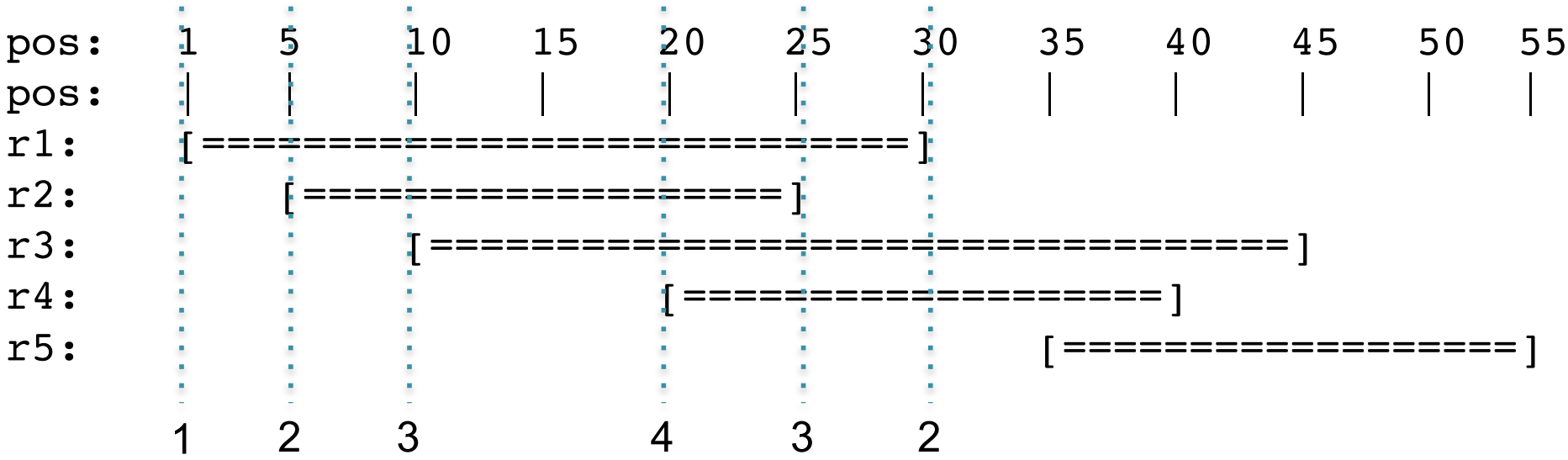


arrive at r5[35,55]:

35 > 25: step down at 25; active set: 30, 40, 45

output (25, 3)

Plane Sweep

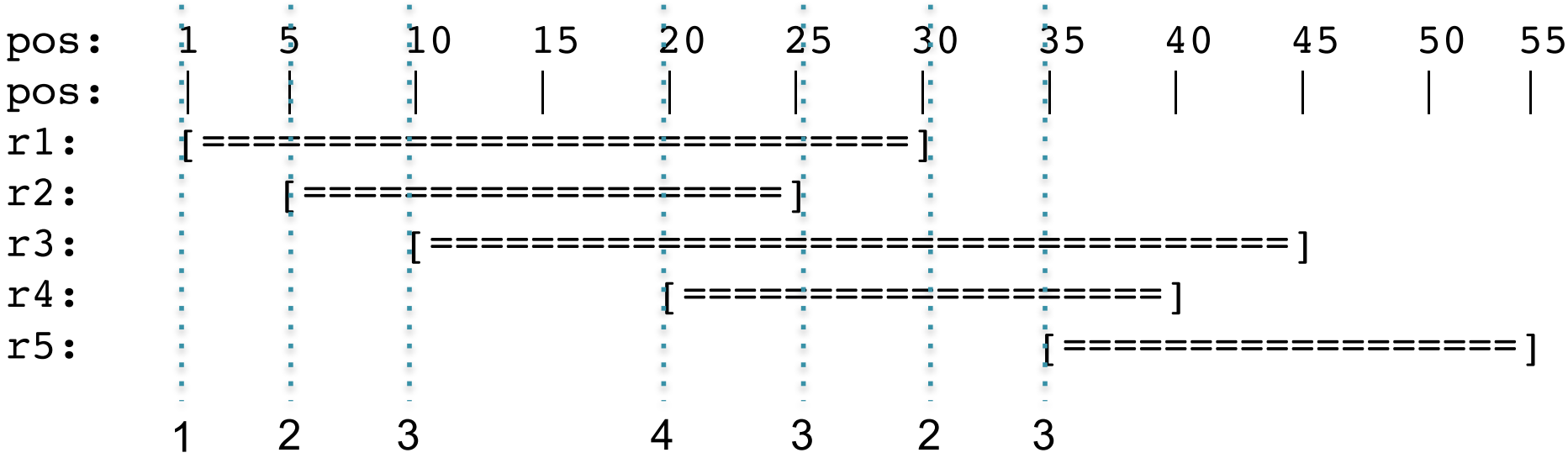


arrive at r5[35,55]:

35 > 25: step down at 25; active set: 30, 40, 45
output (25, 3)

35 > 30: step down at 30; active set: 40, 45
output (30, 2)

Plane Sweep



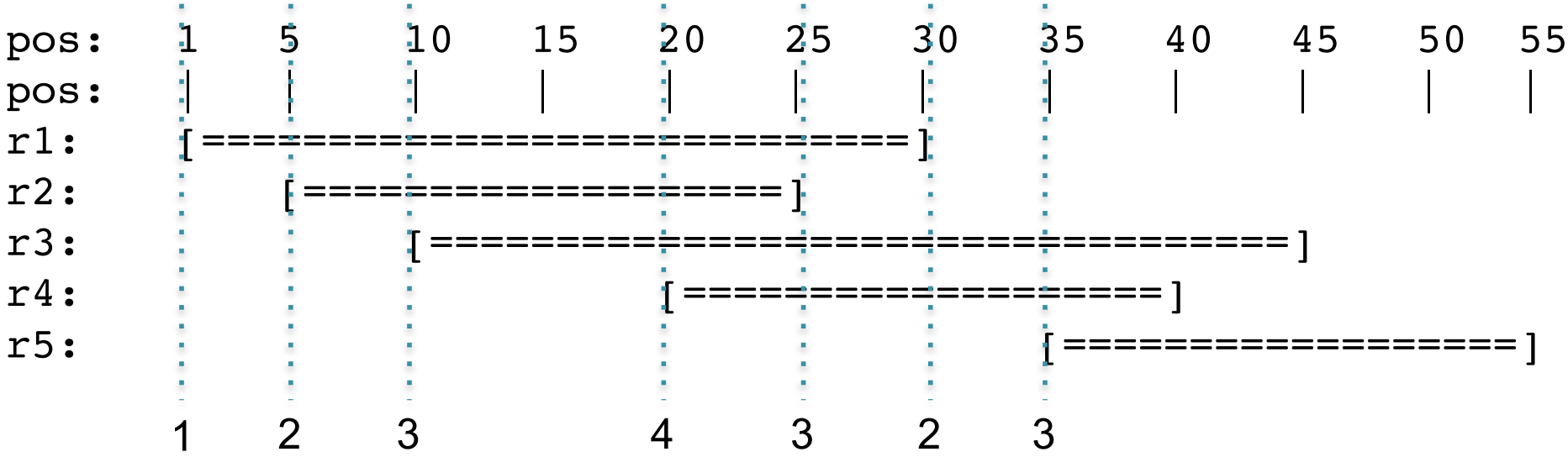
arrive at r5[35,55]:

35 > 25: step down at 25; active set: 30, 40, 45
output (25, 3)

35 > 30: step down at 30; active set: 40, 45
output (30, 2)

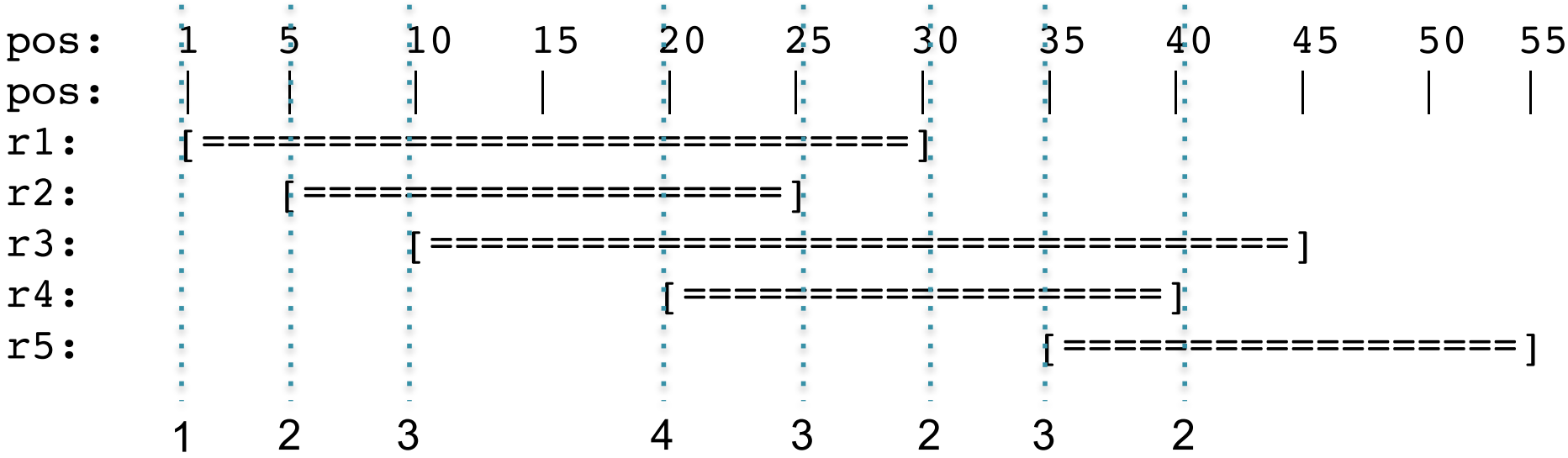
35 < 40: add to active set: 40, 45, 55
output (35, 3)

Plane Sweep



Flush:

Plane Sweep

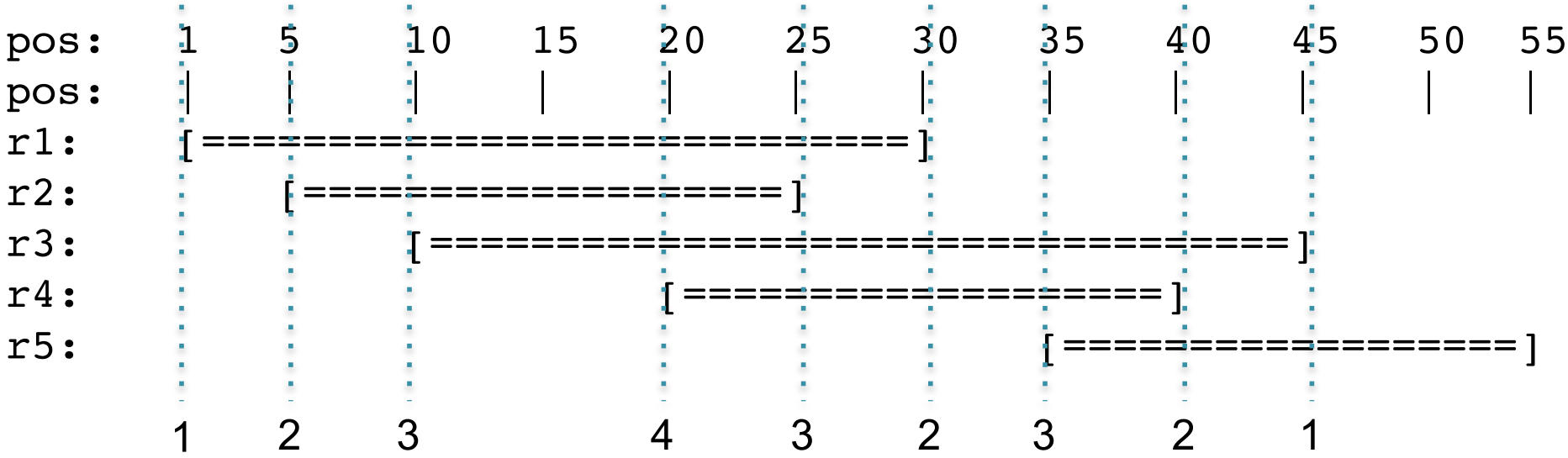


Flush:

step down at 40; active set: 45, 55

output (40, 2)

Plane Sweep

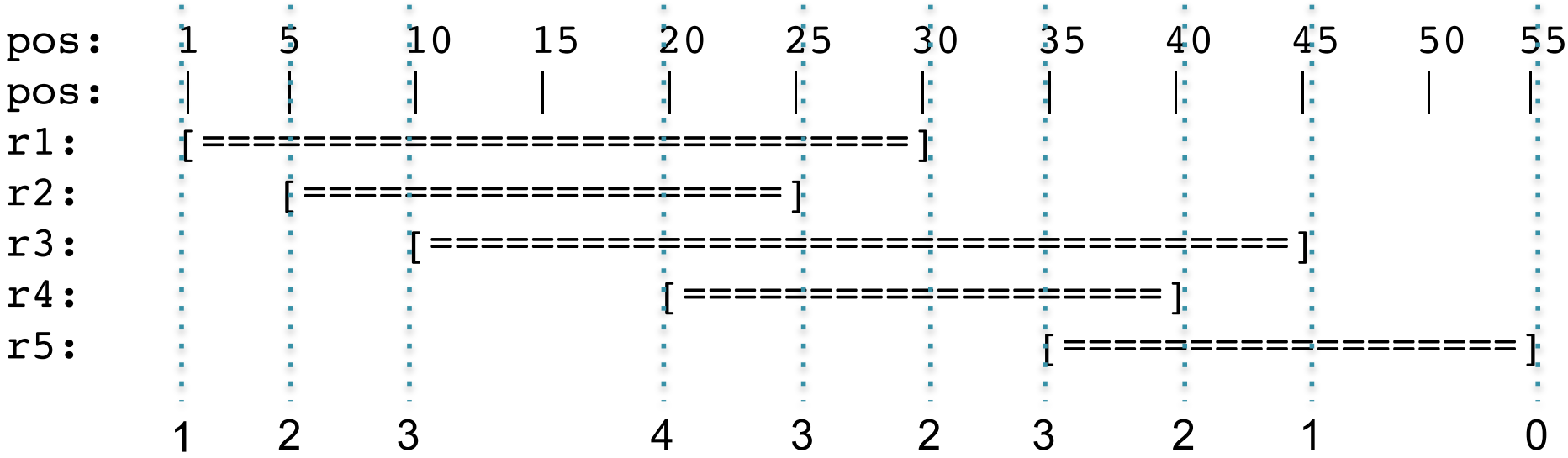


Flush:

step down at 40; active set: 45, 55
output (40, 2)

step down at 45: active set: 55
output (45, 1)

Plane Sweep



Flush:

step down at 40; active set: 45, 55
output (40, 2)

step down at 45: active set: 55
output (45, 1)

step down at 55: active set: {}
output (55, 0)

Plane Sweep

```
## record the delta encoded depth using a plane sweep
deltacovplane = []

## use a list to record the end positions of the elements currently in plane
planelist = []

## BEGIN SWEEP
for r in reads:
  startpos = r[1]
  endpos   = r[2]

  ## clear out any positions from the plane that we have already moved past
  while (len(planelist) > 0):

    if (planelist[0] <= startpos):
      ## the coverage steps down, extract it from the front of the list
      oldend = planelist.pop(0)
      deltacovplane.append((oldend, len(planelist)))
    else:
      break

  ## Now insert the current endpos into the correct position into the list
  insertpos = -1
  for i in xrange(len(planelist)):
    if (endpos < planelist[i]):
        insertpos = i
        break

  if (insertpos > 0):
    planelist.insert(insertpos, endpos)
  else:
    planelist.append(endpos)


  ## Finally record that the coverage has increased
  deltacovplane.append((startpos, len(planelist)))


## Flush any remaining end positions
while (len(planelist) > 0):
  oldend = planelist.pop(0)
  deltacovplane.append((oldend, len(planelist)))
```

Keep track of end positions of reads that have been seen so far

Check to see if any reads have ended before the start of this one

Add the end of the current read to the sweep plane in sorted order

Why sorted?

```
Beginning list-based plane sweep over 1873 reads
Plane sweep found 3746 steps, saving 99.62% of the space in 48.90 ms (90.69 speedup)!
```

Plane Sweep

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while (len(planelist) > 0):
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```

Keep track of end positions of reads that have been seen so far

Check to see if any reads have ended before the start of this one

Add the end of the current read to the sweep plane in sorted order

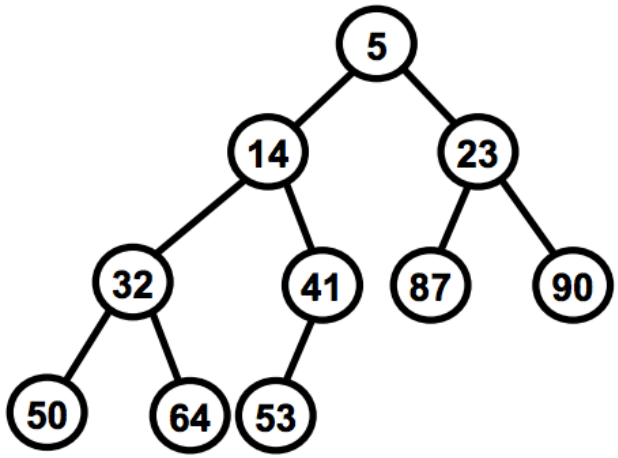
Do we really need the whole list to be sorted?

```
Beginning list-based plane sweep over 1873 reads
Plane sweep found 3746 steps, saving 99.62% of the space in 48.90 ms (90.69 speedup)!
```

Heaps & Priority Queues

Binary Min Heap: Binary tree such that the value of a node is less than or equal to the value of its 2 children

Similar to a binary search tree, although there are no guarantees about the relationships of the left and right children

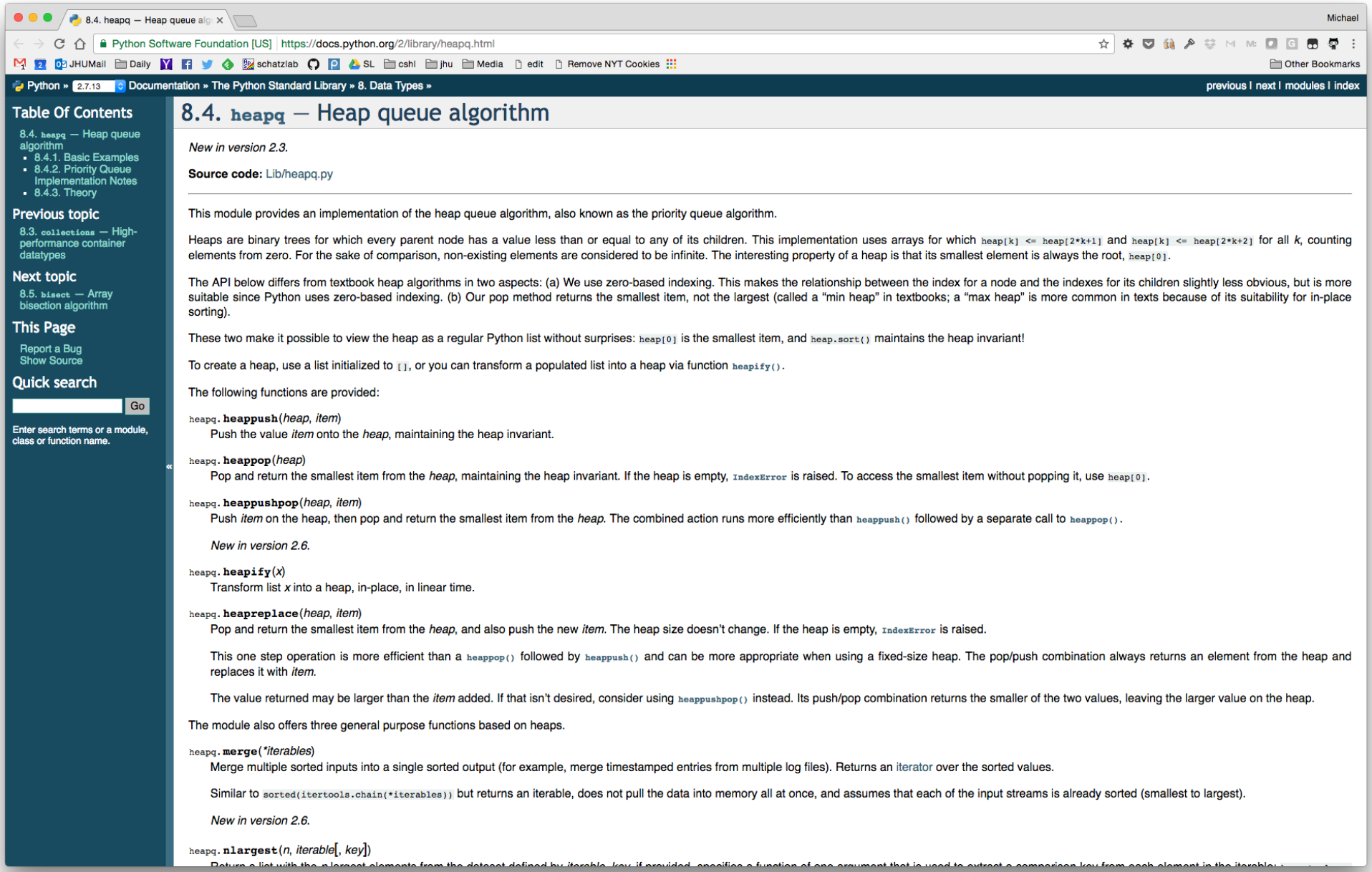


Very efficient data structure for dynamically maintaining a set of element while allowing you to find the minimum (or maximum) very fast:

Insert: O(lg(n)) <- super fast
Remove: O(lg(n)) <- super fast
Find-min: O(1) <- instantaneous

Key to fast performance derives from ***heap shape property***: the tree is guaranteed to be a complete binary tree, meaning it will remain balanced and the height will always be log(n)

Heaps In Python



Heap-based Plane-Sweep

```
## record the delta encoded depth using a plane sweep
deltacovplane = []

## use a list to record the end positions of the elements currently in plane
planeheap = []

## BEGIN SWEEP (note change to index based so can peek ahead)
for rr in xrange(len(reads)):
  r = reads[rr]
  startpos = r[1]
  endpos   = r[2]

  ## clear out any positions from the plane that we have already moved past
  while (len(planeheap) > 0):

    if (planeheap[0] <= startpos):
      ## the coverage steps down, extract it from the front of the list
      ## oldend = planelist.pop(0)
      oldend = heapq.heappop(planeheap)

      nextend = -1
      if (len(planeheap) > 0):
        nextend = planeheap[0]

      ## only record this transition if it is not the same as a start pos
      ## and only if not the same as the next end point
      if ((oldend != startpos) and (oldend != nextend)):
        deltacovplane.append((oldend, len(planeheap)))
    else:
      break

  ## Now insert the current endpos into the correct position into the list
  heapq.heappush(planeheap, endpos)


  ## Finally record that the coverage has increased
  ## But make sure the current read does not start at the same position as the next
  if ((rr == len(reads)-1) or (startpos != reads[rr+1][1])):
    deltacovplane.append((startpos, len(planeheap)))

  ## if it is at the same place, it will get reported in the next cycle


## Flush any remaining end positions
while (len(planeheap) > 0):
  ##oldend = planelist.pop(0)
  oldend = heapq.heappop(planeheap)
  deltacovplane.append((oldend, len(planeheap)))
```

Heaps in python are built from regular lists

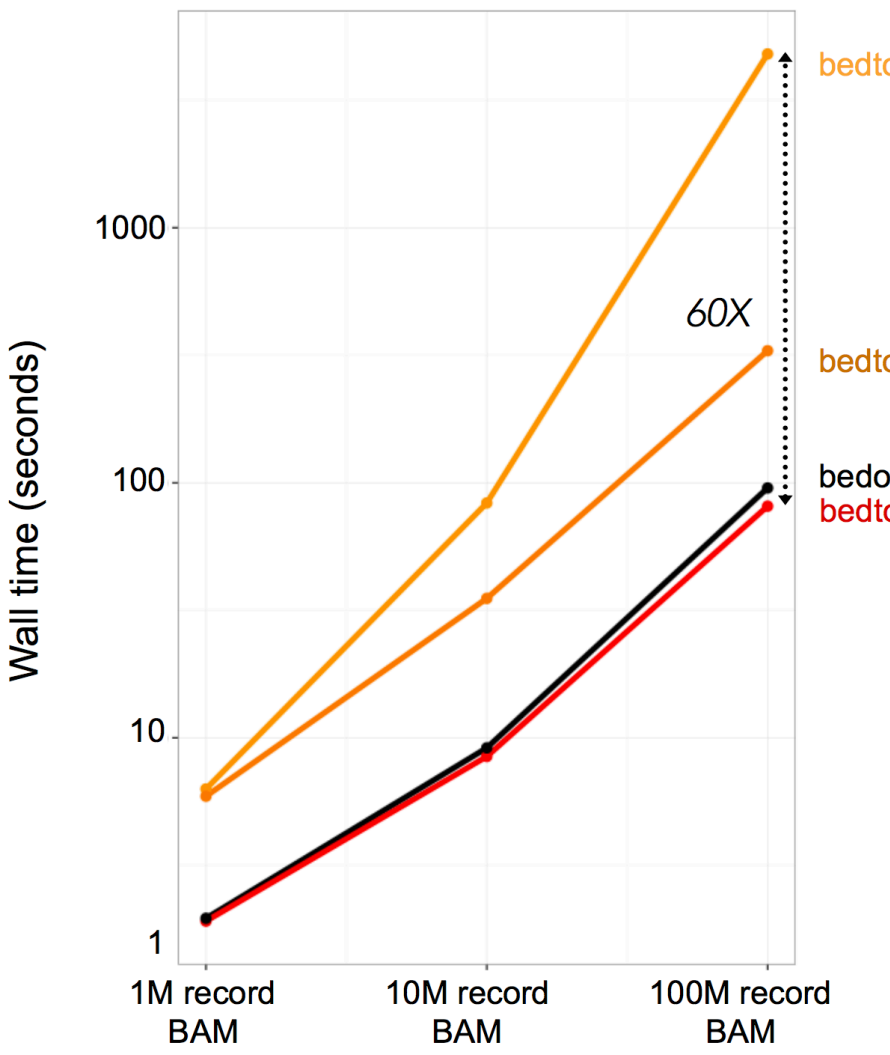
planeheap[0] is min

heapq.heappop() removes from heap

heapq.heappush() adds to heap

```
Beginning heap-based plane sweep over 1873 reads
Heap-Plane sweep found 3698 steps, saving 99.62% of the space in 14.26 ms (311.08 speedup)!
```

BEDTools Performance



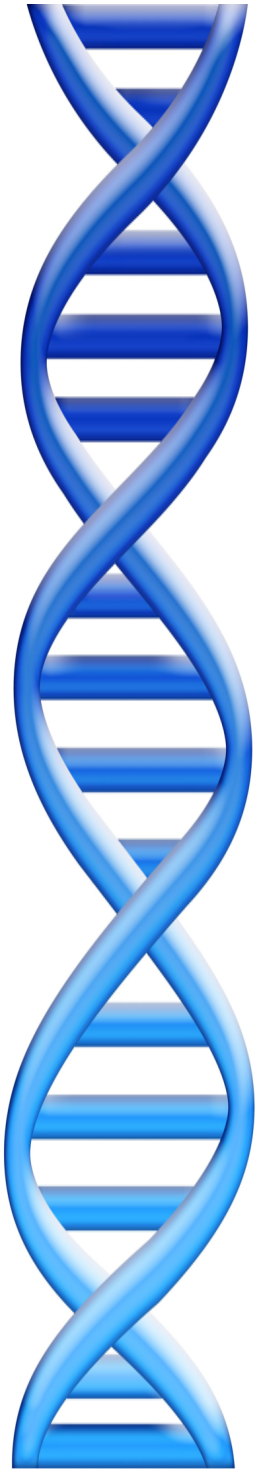

```
bedtools intersect -a gencode.exons.bed -b exome.bam -sorted -c
bedmap --echo --count gencode.exons.bed exome.bam
```

Part 2:
Genome Annotation

Goal: Genome Annotations

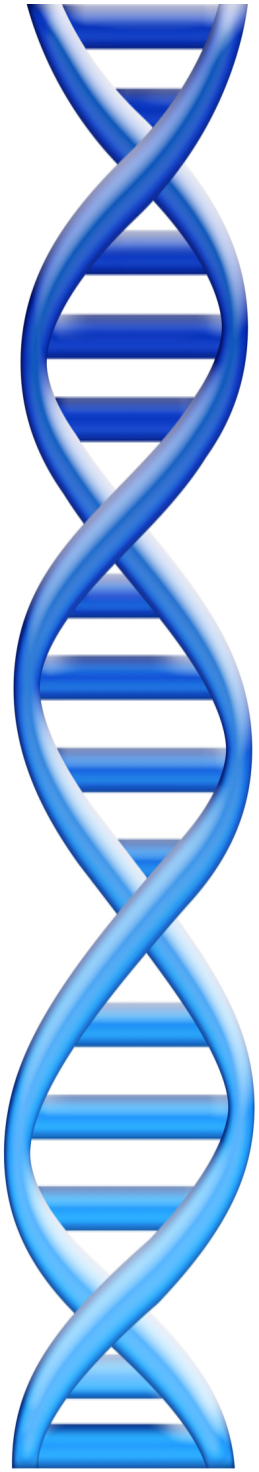
aatgcatgcggctatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaat
gcatgcggctatgcaagctgggatccgatgactatgctaagctgggatccgatgacaatgcatgcggctatgct
aatgaatggtcttgggatttaccttggaatgctaagctgggatccgatgacaatgcatgcggctatgctaatgaa
tggtcttgggatttaccttggaatatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcg
gctatgctaatgcatgcggctatgcaagctgggatccgatgactatgctaagctgcggctatgctaatgcatgcg
gctatgctaagctgggatccgatgacaatgcatgcggctatgctaatgcatgcggctatgcaagctgggatcct
gcggctatgctaatgaatggtcttgggatttaccttggaatgctaagctgggatccgatgacaatgcatgcggct
atgctaatgaatggtcttgggatttaccttggaatatgctaatgcatgcggctatgctaagctgggaatgcatgcg
gctatgctaagctgggatccgatgacaatgcatgcggctatgctaatgcatgcggctatgcaagctgggatccg
atgactatgctaagctgcggctatgctaatgcatgcggctatgctaagctcatgcggctatgctaagctgggaat
gcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaatgcatgcggctatgcaagctg
ggatccgatgactatgctaagctgcggctatgctaatgcatgcggctatgctaagctcggctatgctaatgaatg
gtcttgggatttaccttggaatgctaagctgggatccgatgacaatgcatgcggctatgctaatgaatggtcttgg
gatttaccttggaatatgctaatgcatgcggctatgctaagctgggaatgcatgcggctatgctaagctgggatc
cgatgacaatgcatgcggctatgctaatgcatgcggctatgcaagctgggatccgatgactatgctaagctgcg
gctatgctaatgcatgcggctatgctaagctcatgcgg

Goal: Genome Annotations



Outline

1. Alignment to other genomes
2. Prediction aka “Gene Finding”
3. Experimental & Functional Assays



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Basic Local Alignment Search Tool

- Rapidly compare a sequence Q to a database to find all sequences in the database with an score above some cutoff S.
  - Which protein is most similar to a newly sequenced one?
  - Where does this sequence of DNA originate?

- Speed achieved by using a procedure that typically finds "most" matches with scores > S.
  - Tradeoff between sensitivity and specificity/speed
    - Sensitivity – ability to find all related sequences
    - Specificity – ability to reject unrelated sequences

(Altschul et al. 1990)

Seed and Extend

```
FAKDFLAGGVAAAISKTAVAPIERVKLLLQVQHASKQITADKQYKGIIDCVVRIPKEQGV
F  D  +GG AAA+SKTAVAPIERVKLLLQVQ ASK I  DK+YKGI+D ++R+PKEQGV
FLIDLASGGTAAAVSKTAVAPIERVKLLLQVQDASKAIAVDKRYKGIMDVLIRVPKEQGV
```

- Homologous sequences are likely to contain a short high scoring word pair, a seed.
  - Smaller seed sizes make the sense more sensitive, but also (much) slower
  - Typically do a fast search for prototypes, but then most sensitive for final result

- BLAST then tries to extend high scoring word pairs to compute high scoring segment pairs (HSPs).
  - Significance of the alignment reported via an e-value

BLAST E-values

E-value = the number of HSPs having alignment score S (or higher) expected to occur by chance.

→ Smaller E-value, more significant in statistics

→ Bigger E-value, less significant

→ Over 1 means expect this totally by chance

(not significant at all!)

The expected number of HSPs with the score at least S is :

$$E = K*n*m*e^{-\lambda S}$$

K, λ are constant depending on model

n, m are the length of query and sequence

E-values quickly drop off for better alignment bits scores

Very Similar Sequences

```
Query: HBA_HUMAN Hemoglobin alpha subunit
Sbjct: HBB_HUMAN Hemoglobin beta subunit

Score =  114 bits (285),  Expect = 1e-26
Identities = 61/145 (42%), Positives = 86/145 (59%), Gaps = 8/145 (5%)

Query  2   LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF------DLSHGSAQV 55
           L+P +K+ V A WGKV  +  E G EAL R+ + +P T+ +F  F      D   G+ +V
Sbjct  3   LTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKV 60

Query  56  KGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPA 115
           K HGKKV  A ++ +AH+D++    + LS+LH  KL VDP NF+LL + L+  LA H
Sbjct  61  KAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGK 120

Query  116 EFTPAVHASLDKFLASVSTVLTSKY 140
           EFTP V A+  K +A V+  L  KY
Sbjct  121 EFTPPVQAAYQKVVAGVANALAHKY 145
```

Quite Similar Sequences

```
Query: HBA_HUMAN Hemoglobin alpha subunit
Sbjct: MYG_HUMAN Myoglobin

Score = 51.2 bits (121), Expect = 1e-07,
Identities = 38/146 (26%), Positives = 58/146 (39%), Gaps = 6/146 (4%)

Query  2   LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF------DLSHGSAQV  55
           LS  +   V   WGKV A    +G E L R+F   P T   F  F      D    S  +
Sbjct  3   LSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKASEDL  62

Query  56  KGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPA  115
           K HG  V  AL   +          +  L+  HA K ++     + +S C++  L +  P
Sbjct  63  KKHGATVLTALGGILKKKGHHEAEIKPLAQSHATKHKIPVKYLEFISECIIQVLQSKHPG  122

Query  116 EFTPAVHASLDKFLASVSTVLTSKYR  141
            +F      +++K L      + S Y+
Sbjct  123 DFGADAQGAMNKALELFRKDMASNYK  148
```

Not similar sequences

```
Query: HBA_HUMAN Hemoglobin alpha subunit
Sbjct: SPAC869.02c [Schizosaccharomyces pombe]

 Score = 33.1 bits (74),  Expect = 0.24
 Identities = 27/95 (28%), Positives = 50/95 (52%), Gaps = 10/95 (10%)

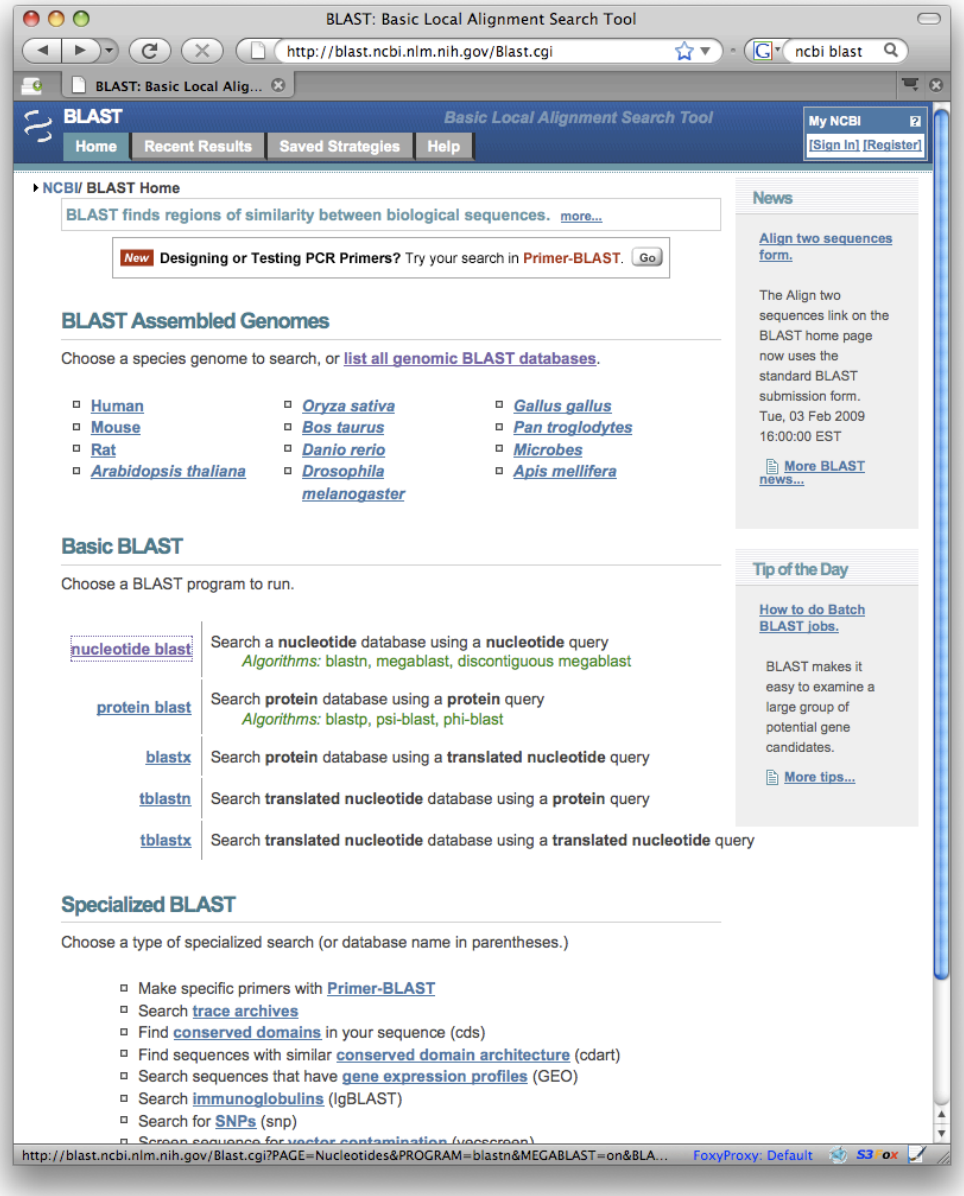
Query  30  ERMFLSFPTTKTYFPHFDLSHGSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAH  89
           ++M  ++P      P+F+ +H  +      + +A AL N   ++DD+  +LSA  D
Sbjct  59  QKMLGNYPEV---LPYFNKAHQISL--SQPRILAFALLNYAKNIDDL-TSLSAFMDQIVV 112

Query  90  K---LRVDPVNFKLLSHCLLVTLAAHLPAEF-TPA  120
           K   L++   ++ ++ HCLL T+   LP++  TPA
Sbjct  113 KHVGLQIKAEHYPIVGHCLLSTMQELLPSDVATPA  147
```

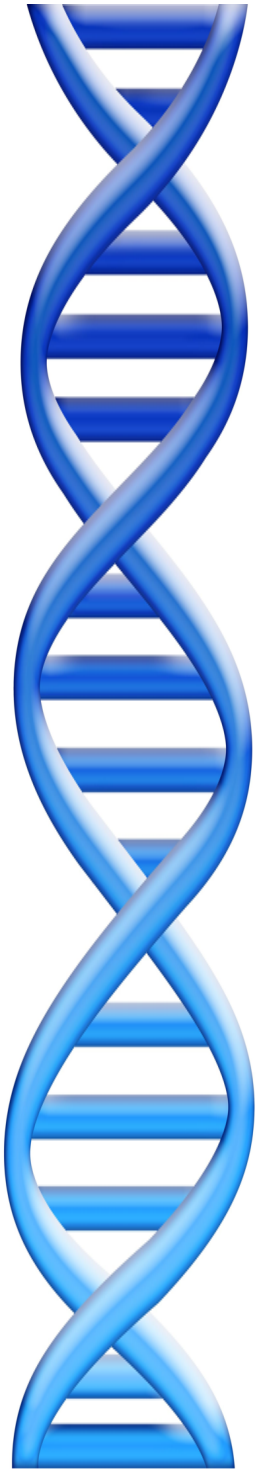
Blast Versions

Program	Database	Query
BLASTN	Nucleotide	Nucleotide
BLASTP	Protein	Protein
BLASTX	Protein	Nucleotide translated into protein
TBLASTN	Nucleotide translated into protein	Protein
TBLASTX	Nucleotide translated into protein	Nucleotide translated into protein

NCBI Blast



- Nucleotide Databases
  - nr: All Genbank
  - refseq: Reference organisms
  - wgs: All reads
- Protein Databases
  - nr: All non-redundant sequences
  - Refseq: Reference proteins



Outline

1. Alignment to other genomes
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Bacterial Gene Finding and Glimmer

(also Archaeal and viral gene finding)

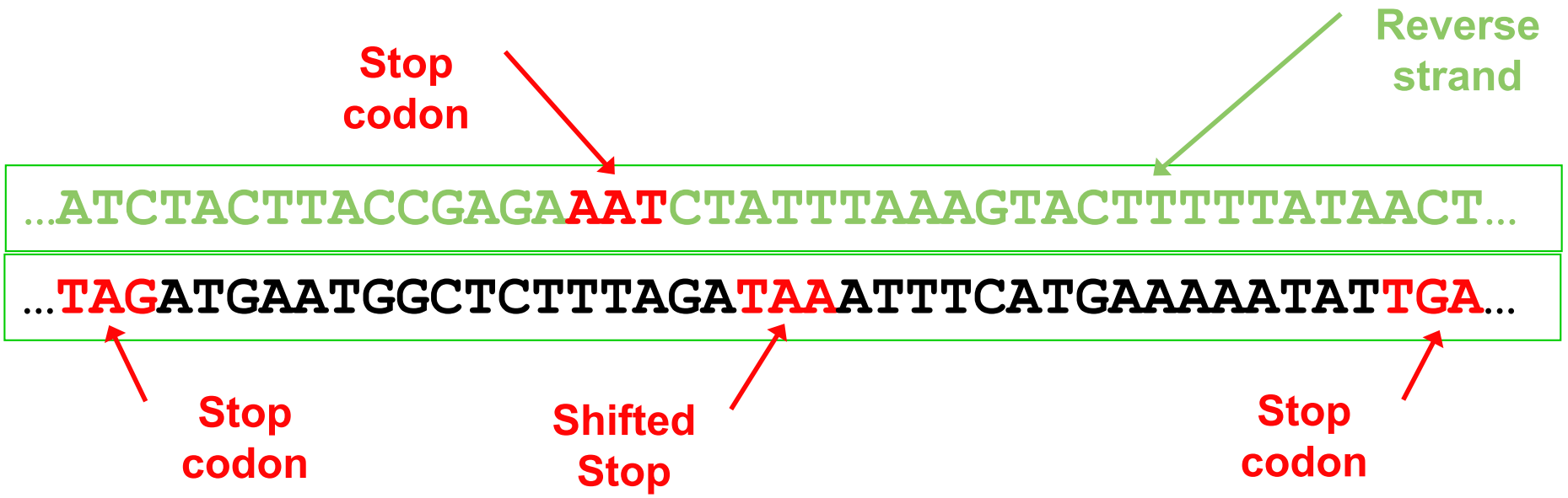
Arthur L. Delcher and Steven Salzberg
Center for Bioinformatics and Computational Biology
Johns Hopkins University School of Medicine

Step One

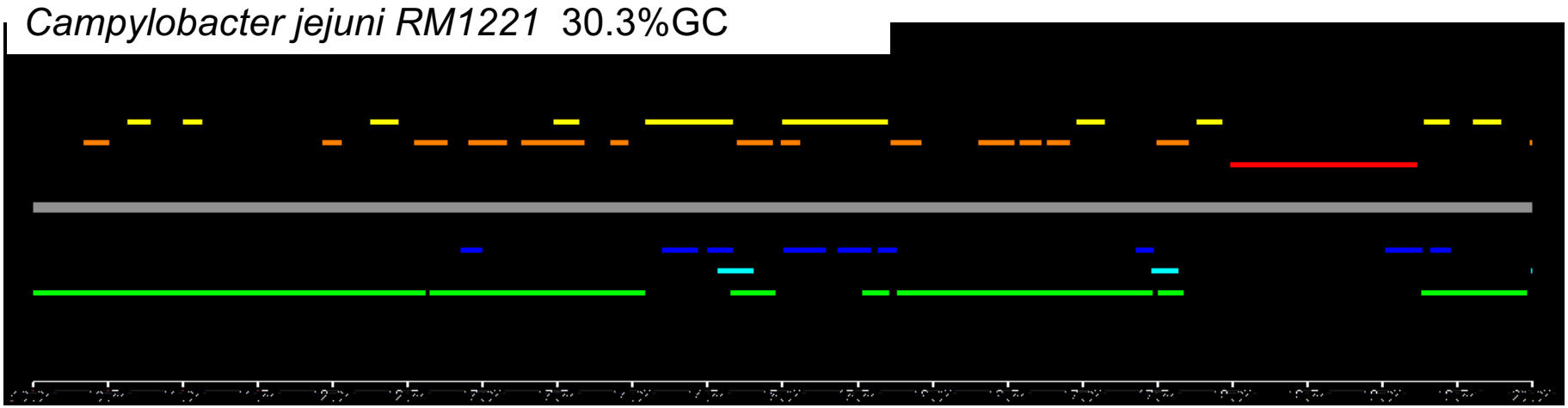
- Find open reading frames (ORFs).

Step One

- Find open reading frames (ORFs).



- But ORFs generally overlap …

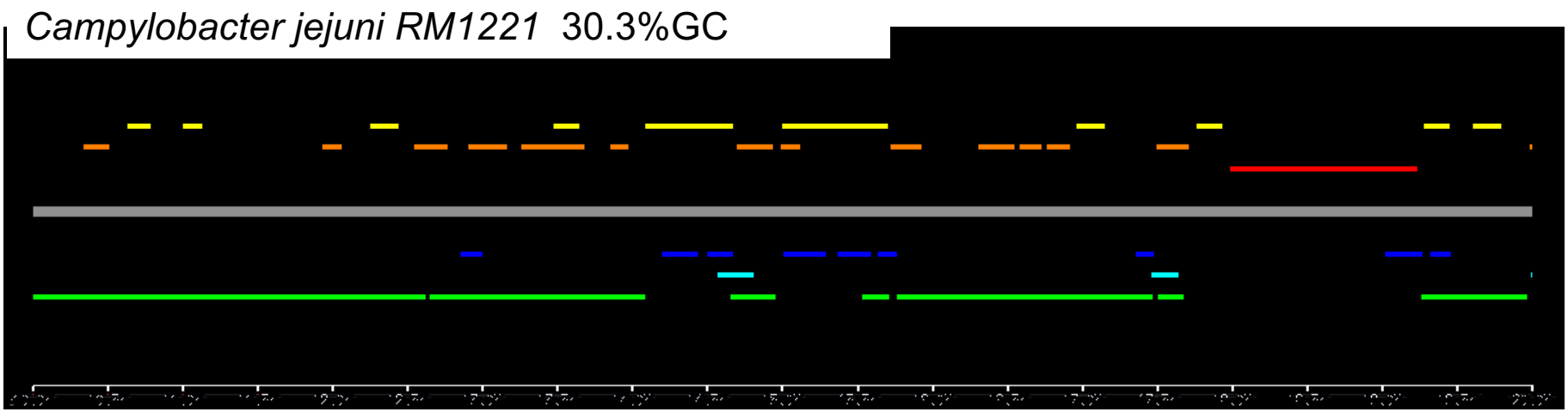


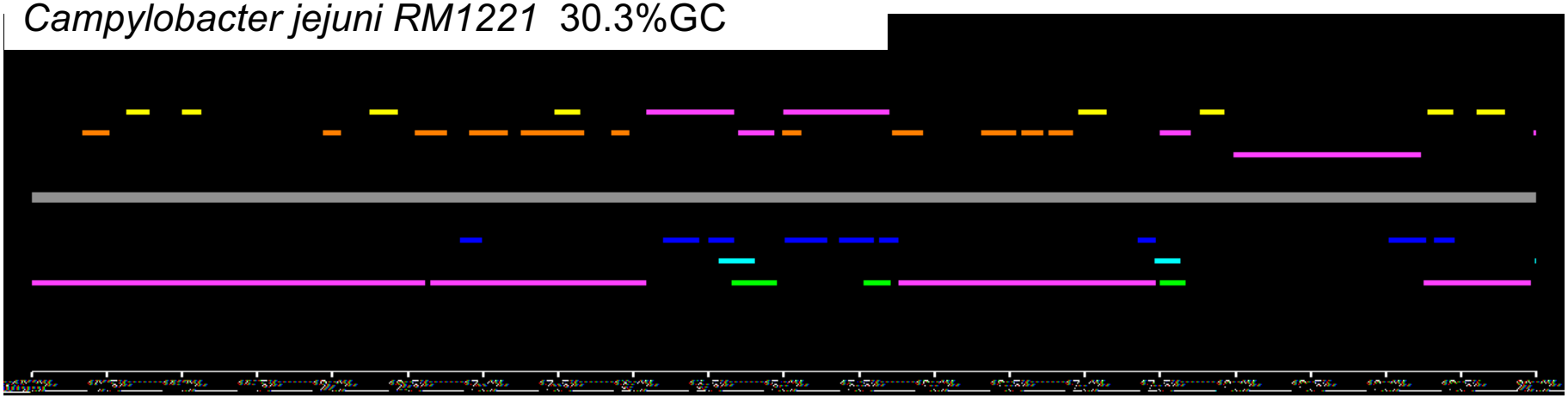
All ORFs longer than 100bp on both strands shown
- color indicates reading frame

Longest ORFs likely to be protein-coding genes

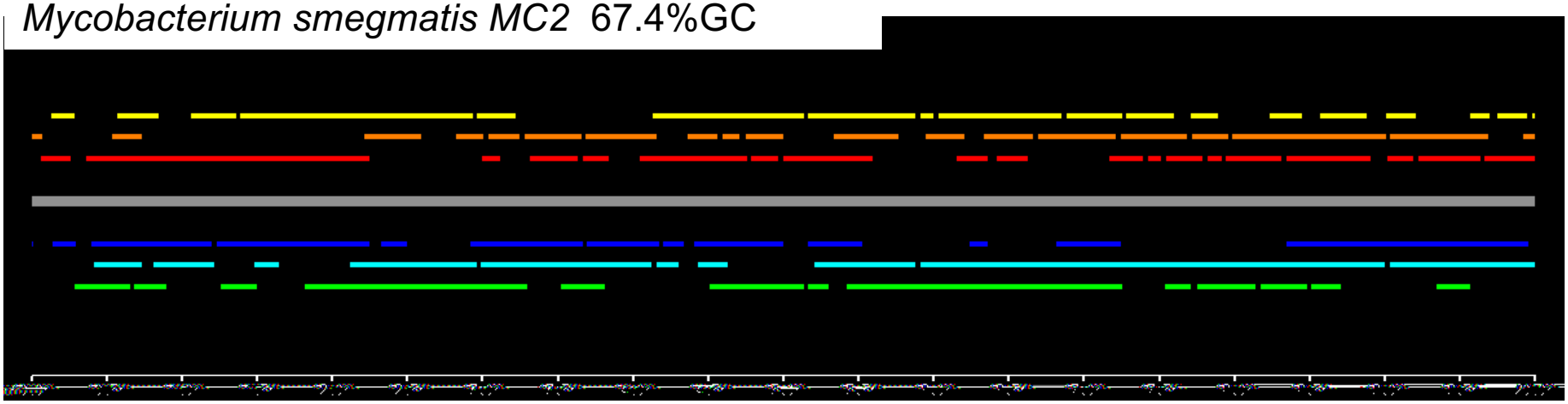
Note the low GC content

All genes are ORFs but not all ORFs are genes


Campylobacter jejuni RM1221 30.3%GC

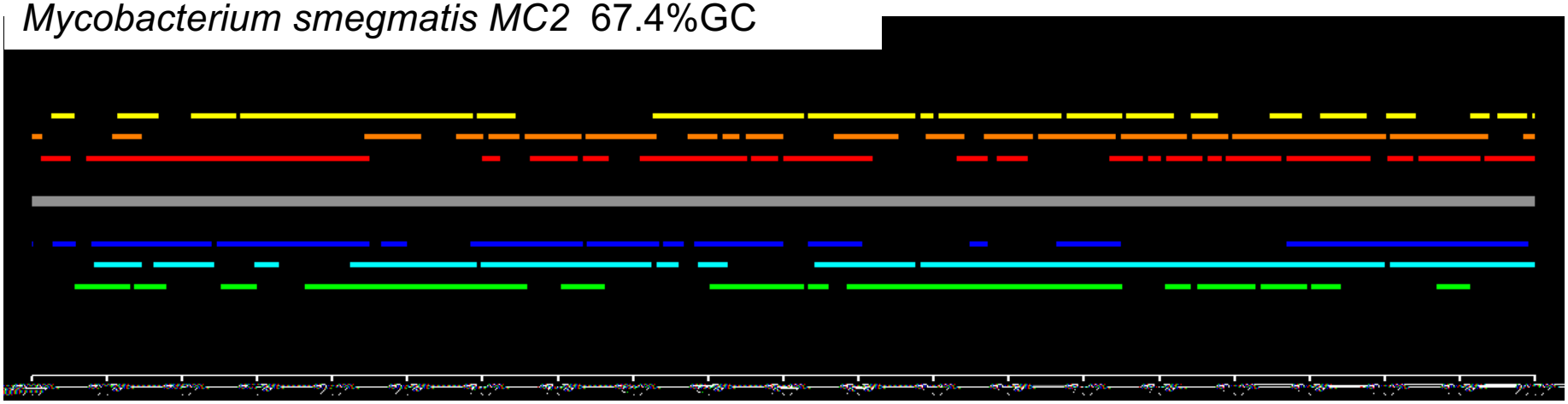

Campylobacter jejuni RM1221 30.3%GC

Mycobacterium smegmatis MC2 67.4%GC

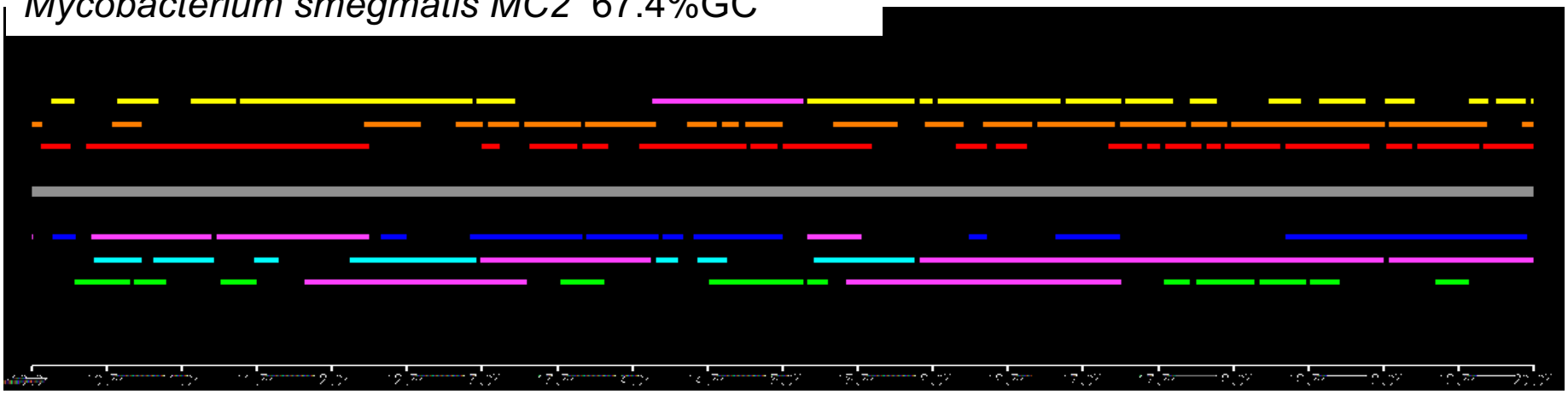


Note what happens in a high-GC genome

Mycobacterium smegmatis MC2 67.4%GC



Mycobacterium smegmatis MC2 67.4%GC



Probabilistic Methods

- Create models that have a probability of generating any given sequence.
  - Evaluate gene/non-genome models against a sequence
- Train the models using examples of the types of sequences to generate.
  - Use RNA sequencing, homology, or "obvious" genes
- The "score" of an orf is the probability of the model generating it.
  - Most basic technique is to count how kmers occur in known genes versus intergenic sequences
  - More sophisticated methods consider variable length contexts, "wobble" bases, other statistical clues

Overview of Eukaryotic Gene Prediction

CBB 231 / COMPSCI 261

W.H. Majoros

Duke
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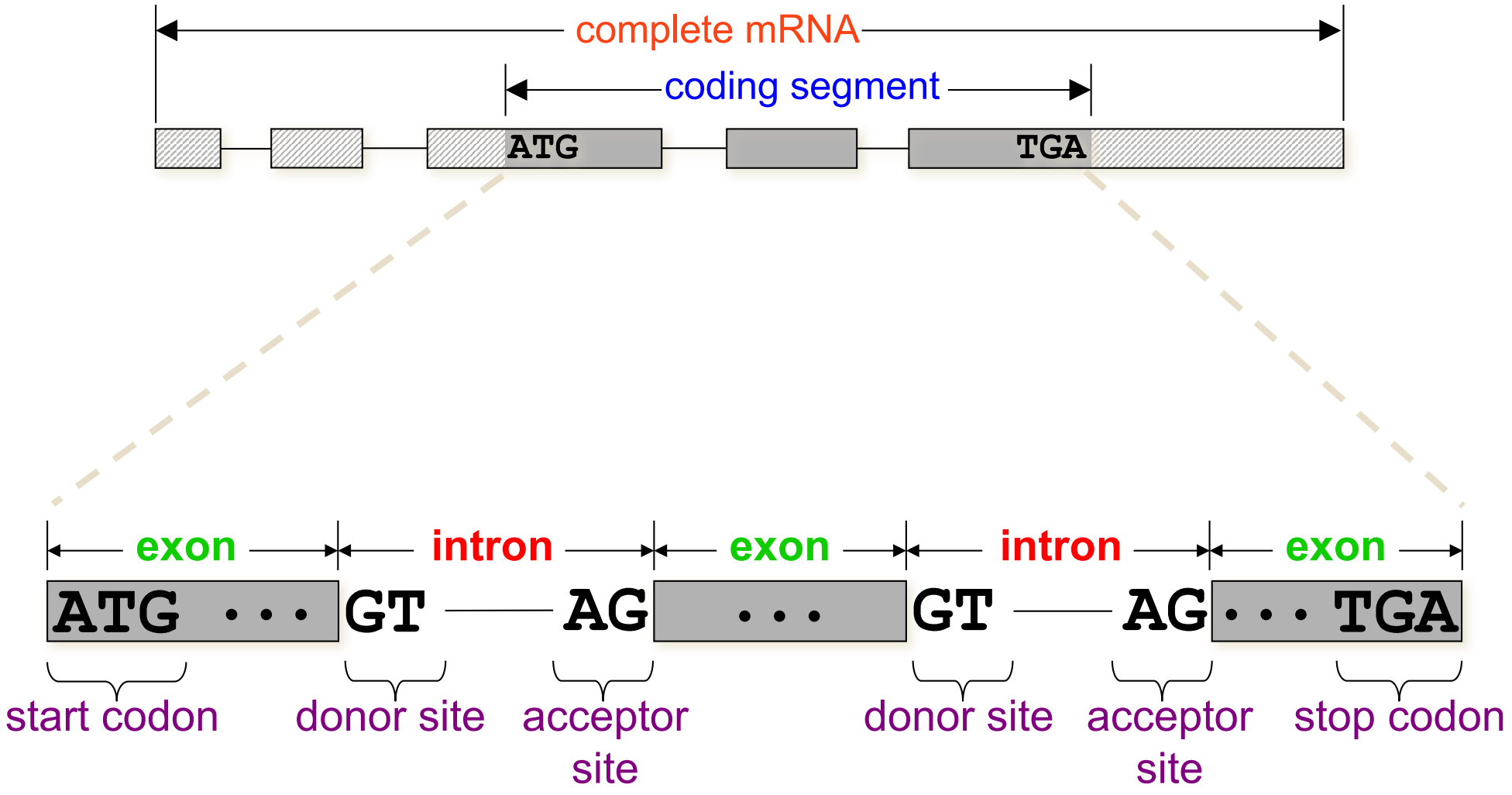
Eukaryotic Gene Syntax



Regions of the gene outside of the CDS are called *UTR*'s (*untranslated regions*), and are mostly ignored by gene finders, though they are important for regulatory functions.

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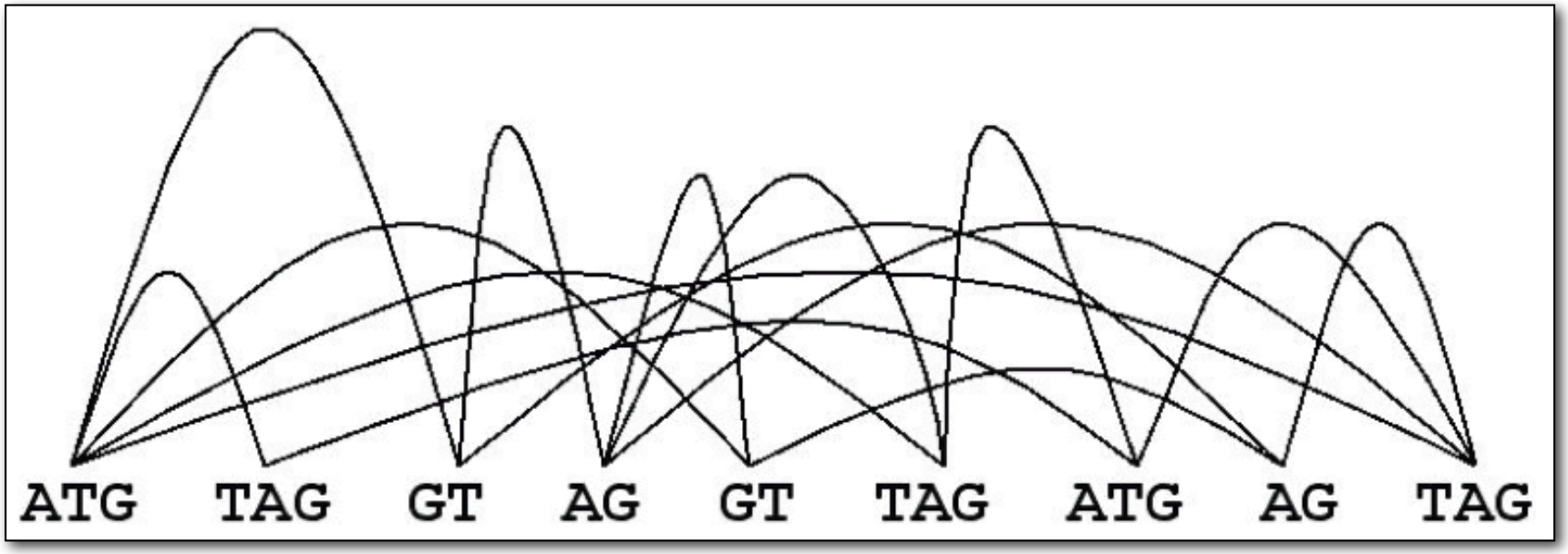
Representing Gene Syntax with ORF Graphs

After identifying the most promising (i.e., highest-scoring) signals in an input sequence, we can apply the gene syntax rules to connect these into an *ORF graph*:

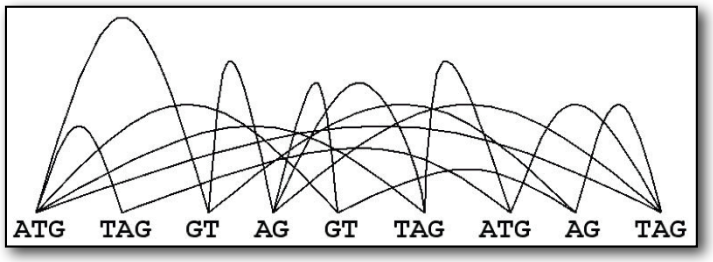


An ORF graph represents all possible *gene parses* (and their scores) for a given set of putative signals. A *path* through the graph represents a single gene parse.

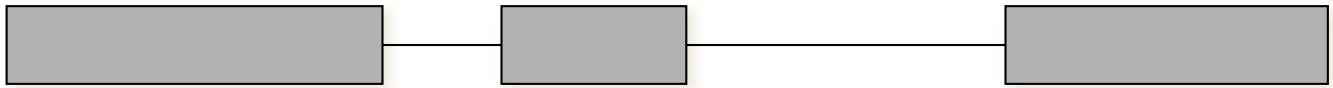
Conceptual Gene-finding Framework

```
TATTCCGATCGATCGATCTCTCTAGCGTCTACG
CTATCATCGCTCTCTATTATCGCGCGATCGTCG
ATCGCGCGAGAGTATGCTACGTCGATCGAATTG
```

identify most promising signals, score signals and content regions between them; induce an ORF graph on the signals



find highest-scoring path through ORF graph; interpret path as a gene parse = gene structure



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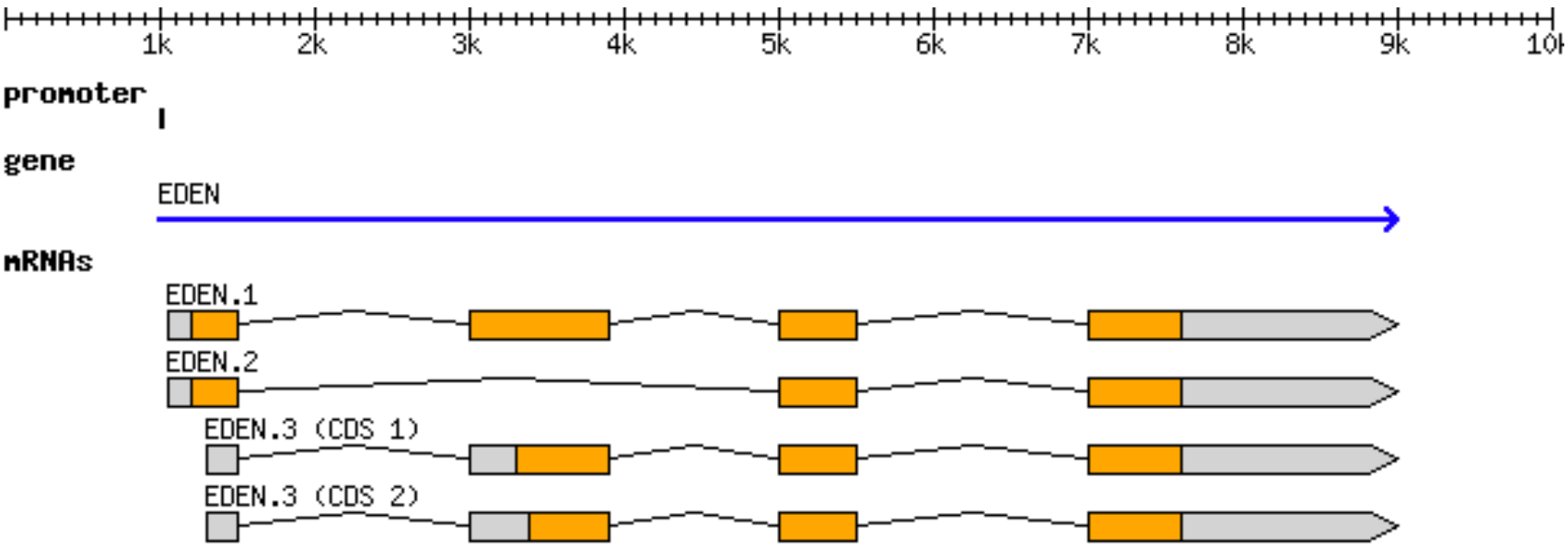
Gene Finding Overview

- Prokaryotic gene finding distinguishes real genes and random ORFs
  - Prokaryotic genes have simple structure and are largely homogenous, making it relatively easy to recognize their sequence composition

- Eukaryotic gene finding identifies the genome-wide most probable gene models (set of exons)
  - "Probabilistic Graphical Model" to enforce overall gene structure, separate models to score splicing/transcription signals
  - Accuracy depends to a large extent on the quality of the training data

Gene Models



- "Generic Feature Format" (GFF) records genomic features
  - Coordinates of each exon
  - Coordinates of UTRs
  - Link together exons into transcripts
  - Link together transcripts into gene models

http://www.sequenceontology.org/gff3.shtml

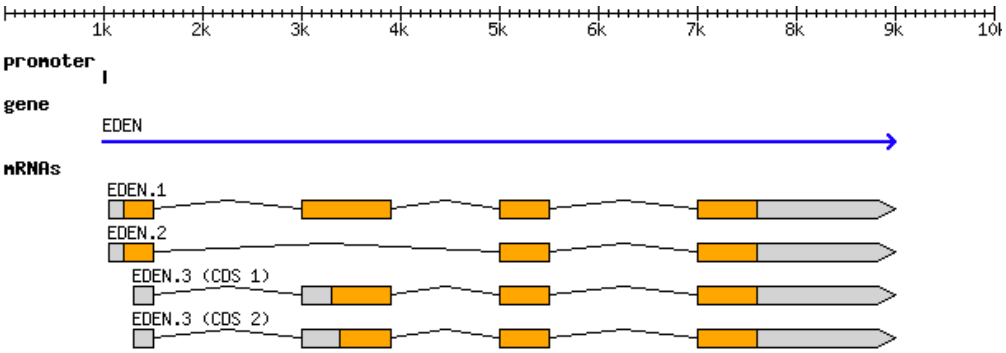
GFF File format

GFF3 files are nine-column, tab-delimited, plain text files

1. seqid:	The ID of the sequence
2. source:	Algorithm or database that generated this feature
3. type:	*gene/exon/CDS/etc…*
4. start:	1-based coordinate
5. end:	1-based coordinate
6. score:	E-values/p-values/index/colors/…
7. ***strand:***	"+' for positive "-" for minus, "." not stranded
8. phase:	For "CDS", where the feature begins with reference to the reading frame (0,1,2)
9. attributes:	A list of tag=value features
	Parent: Indicates the parent of the feature (group exons into transcripts, transcripts into genes, …)

GFF Example

Gene "EDEN" with 3 alternatively spliced transcripts, isoform 3 has two alternative translation start sites




```
##gff-version 3
##sequence-region   ctg123 1 1497228
ctg123 . gene            1000  9000  .  +  .  ID=gene00001;Name=EDEN

ctg123 . TF_binding_site 1000  1012  .  +  .  ID=tfbs00001;Parent=gene00001

ctg123 . mRNA            1050  9000  .  +  .  ID=mRNA00001;Parent=gene00001;Name=EDEN.1
ctg123 . mRNA            1050  9000  .  +  .  ID=mRNA00002;Parent=gene00001;Name=EDEN.2
ctg123 . mRNA            1300  9000  .  +  .  ID=mRNA00003;Parent=gene00001;Name=EDEN.3

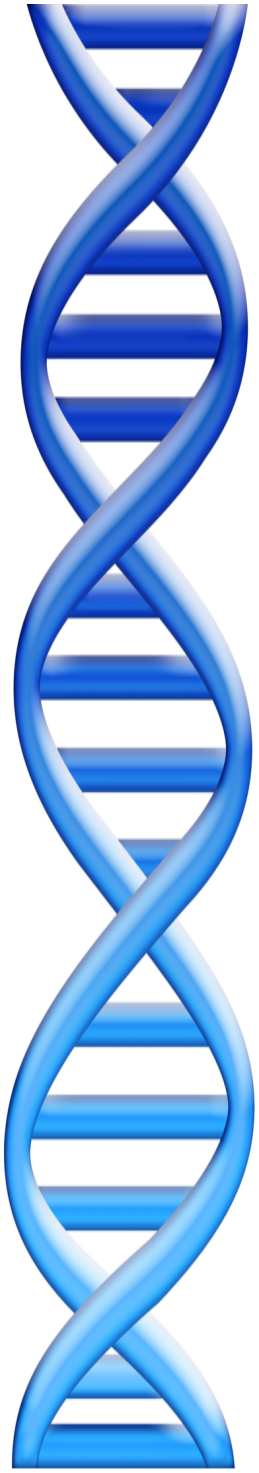
ctg123 . exon            1300  1500  .  +  .  ID=exon00001;Parent=mRNA00003
ctg123 . exon            1050  1500  .  +  .  ID=exon00002;Parent=mRNA00001,mRNA00002
ctg123 . exon            3000  3902  .  +  .  ID=exon00003;Parent=mRNA00001,mRNA00003
ctg123 . exon            5000  5500  .  +  .  ID=exon00004;Parent=mRNA00001,mRNA00002,mRNA00003
ctg123 . exon            7000  9000  .  +  .  ID=exon00005;Parent=mRNA00001,mRNA00002,mRNA00003

ctg123 . CDS             1201  1500  .  +  0  ID=cds00001;Parent=mRNA00001;Name=edenprotein.1
ctg123 . CDS             3000  3902  .  +  0  ID=cds00001;Parent=mRNA00001;Name=edenprotein.1
ctg123 . CDS             5000  5500  .  +  0  ID=cds00001;Parent=mRNA00001;Name=edenprotein.1
ctg123 . CDS             7000  7600  .  +  0  ID=cds00001;Parent=mRNA00001;Name=edenprotein.1

ctg123 . CDS             1201  1500  .  +  0  ID=cds00002;Parent=mRNA00002;Name=edenprotein.2
ctg123 . CDS             5000  5500  .  +  0  ID=cds00002;Parent=mRNA00002;Name=edenprotein.2
ctg123 . CDS             7000  7600  .  +  0  ID=cds00002;Parent=mRNA00002;Name=edenprotein.2

ctg123 . CDS             3301  3902  .  +  0  ID=cds00003;Parent=mRNA00003;Name=edenprotein.3
ctg123 . CDS             5000  5500  .  +  1  ID=cds00003;Parent=mRNA00003;Name=edenprotein.3
ctg123 . CDS             7000  7600  .  +  1  ID=cds00003;Parent=mRNA00003;Name=edenprotein.3

ctg123 . CDS             3391  3902  .  +  0  ID=cds00004;Parent=mRNA00003;Name=edenprotein.4
ctg123 . CDS             5000  5500  .  +  1  ID=cds00004;Parent=mRNA00003;Name=edenprotein.4
ctg123 . CDS             7000  7600  .  +  1  ID=cds00004;Parent=mRNA00003;Name=edenprotein.4
```

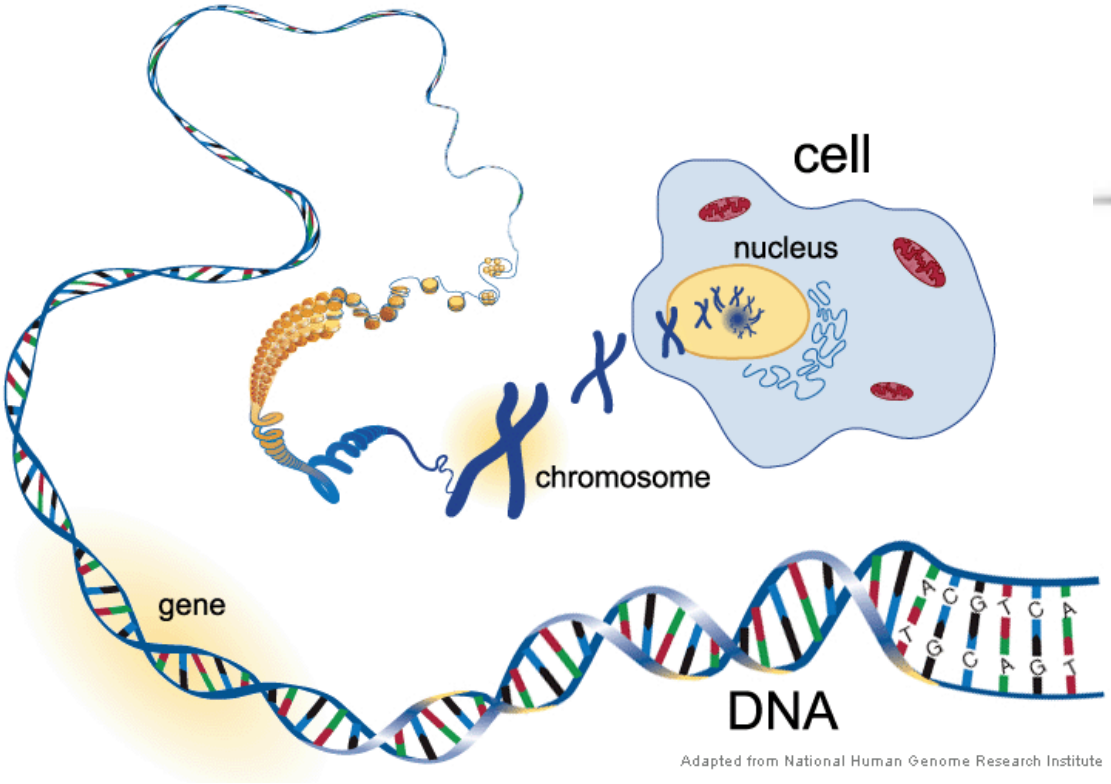


Outline

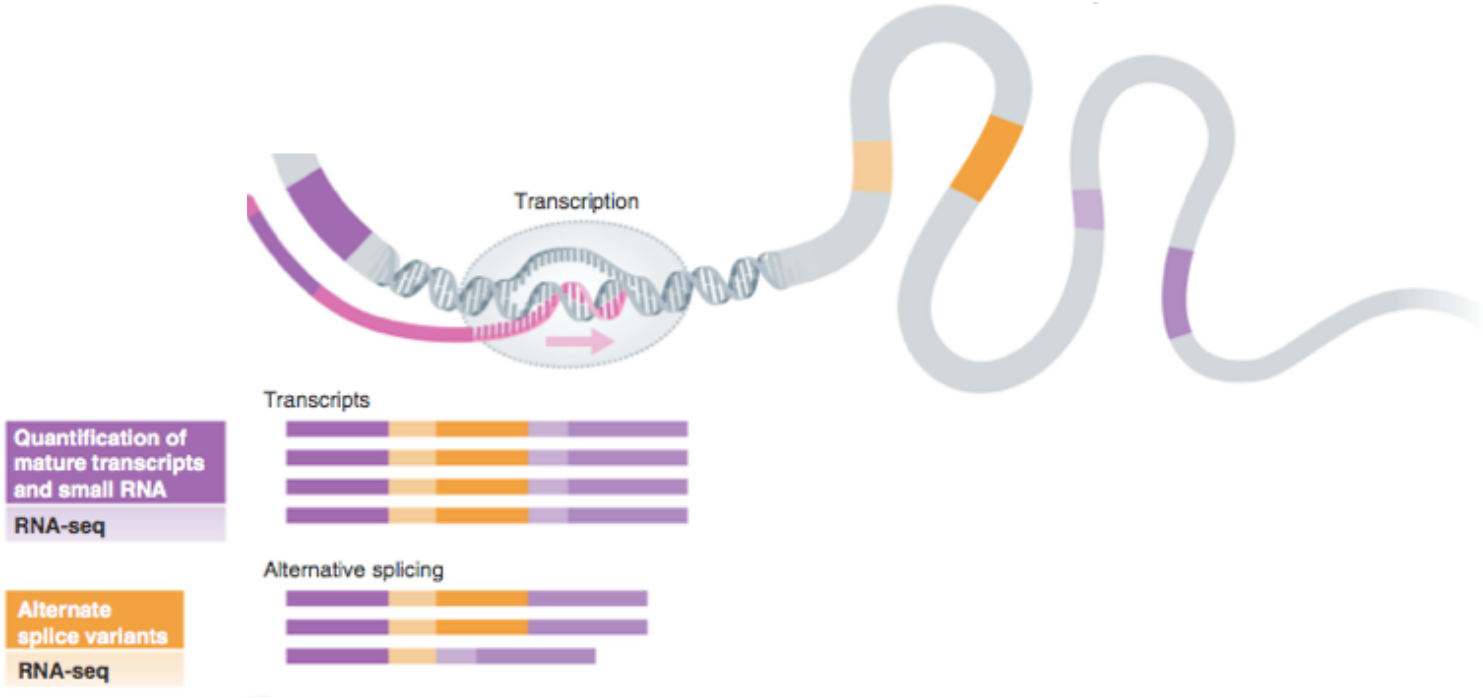
1. Alignment to other genomes
2. Prediction aka "Gene Finding"
3. Experimental & Functional Assays

Sequencing techniques

Much of the capacity is used to sequence genomes (or exomes) of individuals…



… but biology is much more than just genomes…



Soon et al., Molecular Systems Biology, 2013

Sequencing Assays

The *Seq List (in chronological order)

1. Gregory E. Crawford et al., "Genome-wide Mapping of DNase Hypersensitive Sites Using Massively Parallel Signature Sequencing (MPSS)," Genome Research 16, no. 1 (January 1, 2006): 123–131, doi:10.1101/gr.4074106.
2. David S. Johnson et al., "Genome-Wide Mapping of in Vivo Protein-DNA Interactions," Science 316, no. 5830 (June 8, 2007): 1497–1502, doi:10.1126/science.1141319.
3. Tarjei S. Mikkelsen et al., "Genome-wide Maps of Chromatin State in Pluripotent and Lineage-committed Cells," Nature 448, no. 7153 (August 2, 2007): 553–560, doi:10.1038/nature06008.
4. Thomas A. Down et al., "A Bayesian Deconvolution Strategy for Immunoprecipitation-based DNA Methylome Analysis," Nature Biotechnology 26, no. 7 (July 2008): 779–785, doi:10.1038/nbt1414.
5. Ali Mortazavi et al., "Mapping and Quantifying Mammalian Transcriptomes by RNA-Seq," Nature Methods 5, no. 7 (July 2008): 621–628, doi:10.1038/nmeth.1226.
6. Nathan A. Baird et al., "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers," PLoS ONE 3, no. 10 (October 13, 2008): e3376, doi:10.1371/journal.pone.0003376.
7. Leighton J. Core, Joshua J. Waterfall, and John T. Lis, "Nascent RNA Sequencing Reveals Widespread Pausing and Divergent Initiation at Human Promoters," Science 322, no. 5909 (December 19, 2008): 1845–1848, doi:10.1126/science.1162228.
8. Chao Xie and Martti T. Tammi, "CNV-seq, a New Method to Detect Copy Number Variation Using High-throughput Sequencing," BMC Bioinformatics 10, no. 1 (March 6, 2009): 80, doi:10.1186/1471-2105-10-80.
9. Jay R. Hesselberth et al., "Global Mapping of protein-DNA Interactions in Vivo by Digital Genomic Footprinting," Nature Methods 6, no. 4 (April 2009): 283–289, doi:10.1038/nmeth.1313.
10. Nicholas T. Ingolia et al., "Genome-Wide Analysis in Vivo of Translation with Nucleotide Resolution Using Ribosome Profiling," Science 324, no. 5924 (April 10, 2009): 218–223, doi:10.1126/science.1168978.
11. Alayne L. Brunner et al., "Distinct DNA Methylation Patterns Characterize Differentiated Human Embryonic Stem Cells and Developing Human Fetal Liver," Genome Research 19, no. 6 (June 1, 2009): 1044–1056, doi:10.1101/gr.088773.108.
12. Mayumi Oda et al., "High-resolution Genome-wide Cytosine Methylation Profiling with Simultaneous Copy Number Analysis and Optimization for Limited Cell Numbers," Nucleic Acids Research 37, no. 12 (July 1, 2009): 3829–3839, doi:10.1093/nar/gkp260.
13. Zachary D. Smith et al., "High-throughput Bisulfite Sequencing in Mammalian Genomes," Methods 48, no. 3 (July 2009): 226–232, doi:10.1016/j.ymeth.2009.05.003.
14. Andrew M. Smith et al., "Quantitative Phenotyping via Deep Barcode Sequencing," Genome Research (July 21, 2009).

What is a *Seq assay?

Molecular Biology

Mathematics & Computer Science

Computational Biology

Desired measurement → **reduce to sequencing** → Sequence → Solve inverse problem → Analyze

Creativity

Chemistry & Physics

Statistics

Biology

$$\mathbb{P}(f=(p,t,l)) \approx \frac{\tilde{\lambda}_l \cdot \frac{\tau_t}{\tilde{l}(t)} \cdot w_{p|t,l} \cdot \phi_{f|p,t,l}}{\sum_{(q,r,m)\in\mathcal{A}(f)} \lambda_m \cdot \frac{\tau_r}{\tilde{l}(r)} \cdot w_{q|r,m} \cdot \phi_{f|q,r,m}}$$

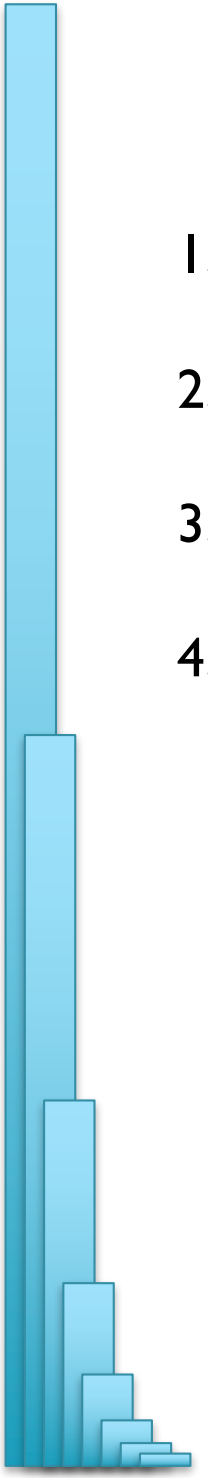
Annotation Summary

- Three major approaches to annotate a genome
  1. Alignment:
     - Does this sequence align to any other sequences of known function?
     - Great for projecting knowledge from one species to another
  2. Prediction:
     - Does this sequence statistically resemble other known sequences?
     - Potentially most flexible but dependent on good training data
  3. Experimental:
     - Lets test to see if it is transcribed/methylated/bound/etc
     - Strongest but expensive and context dependent

- Many great resources available
  - Learn to love the literature and the databases
  - Standard formats let you rapidly query and cross reference
  - Google is your number one resource ☺

Next Steps

1. See Lecture Notes for Full Details
2. Review Bedtools docs: http://bedtools.readthedocs.io/
3. Get ready for assignment 2
4. Check out the course webpage




Welcome to Applied Comparative Genomics
https://github.com/schatzlab/appliedgenomics

Questions?