Analyzing Next Generation Sequencing Data, MSU 2014

Genomic Intervals

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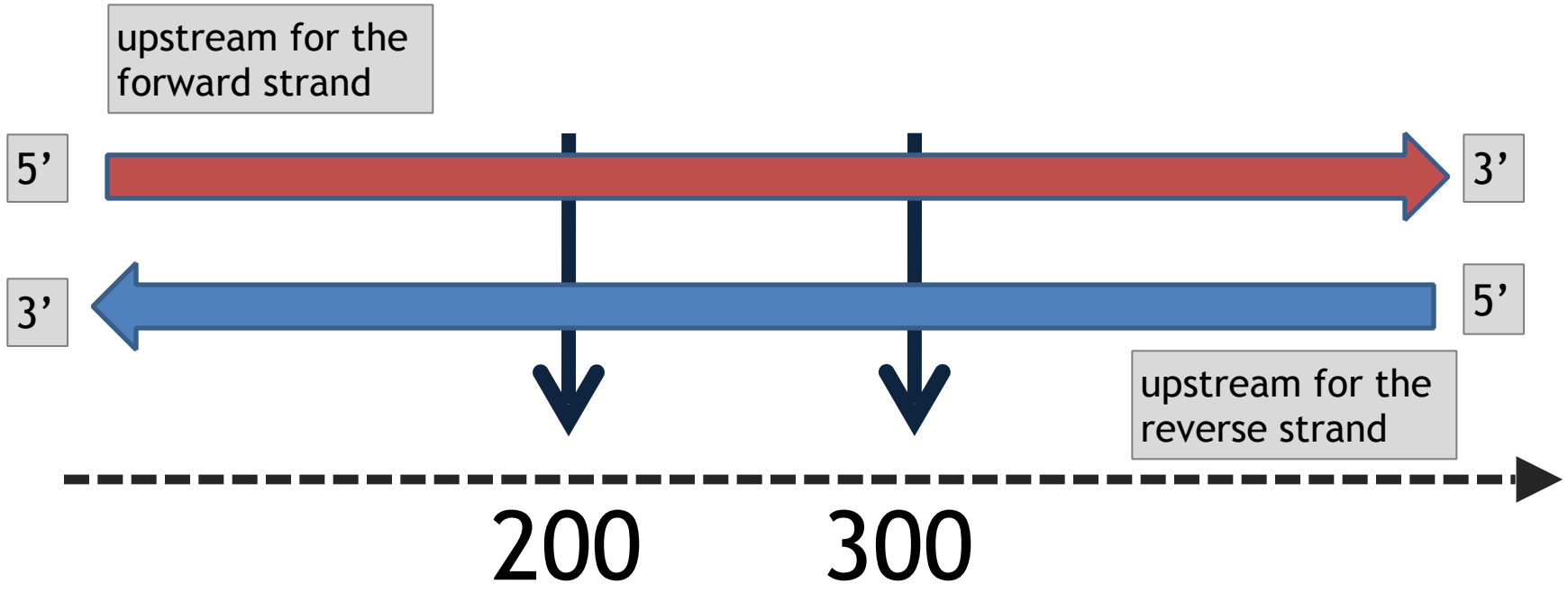
Genome representation concepts

- At the simplest level of abstraction the genome is represented by a one dimensional “space” (lines)
- Genome is two stranded → a line corresponds to each strand
- Each strand has a polarity → each line has a direction
- The genome has strands (lines) are paired
- The smallest unit is one base → one integer on the number line
- Annotations (features) are segments (coordinates) on each line

Genomic coordinates – brief overview

DNA two stranded and directional
But there is only one coordinate system



Standard formats use **start < end** notation even for the reverse strand

The **upstream region** – before the 5’ end relative to the direction of transcription

Coordinate systems

- 0 based → 0, 1, 2, … 9
- 1 based → 1, 2, 3, … 10

Typically

- 0 based are non-inclusive **10:20 → [10, 20)**
- 1 based include both ends **10:20 → [10, 20]**

Comparing coordinate systems

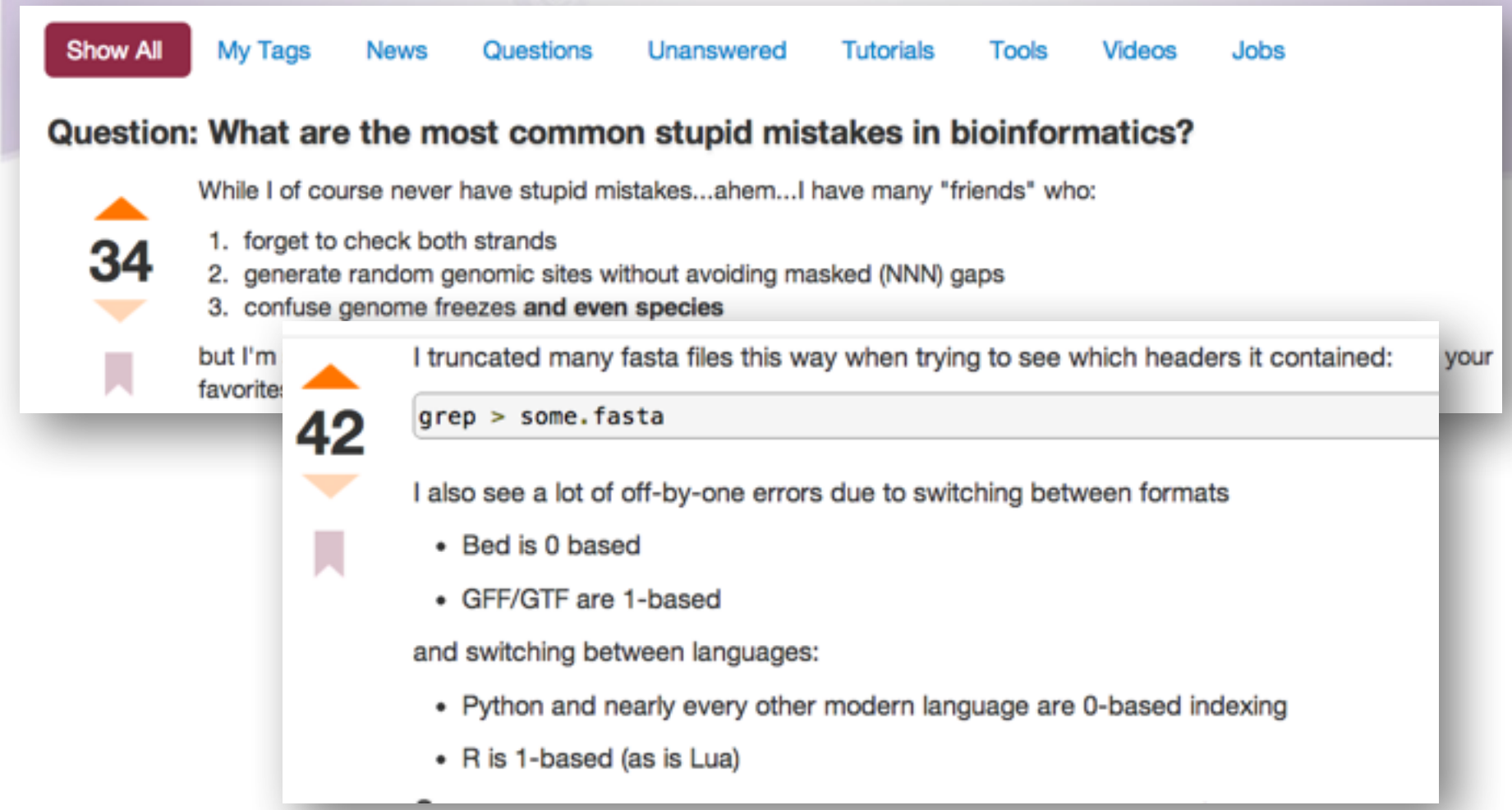
	1 based indexing	0 based indexing
Third element	*3*	*2*
First ten	*1, 10*	*0, 10*
Second ten	*11, 20*	*10, 20*
	21, 30	*20, 30*
One base long starting at 10	*10, 10*	*10, 11*
Length of interval	*end - start + 1*	*end - start*
Five elements starting at	*1000, 1004*	*1000, 1005*
Empty interval	*?*	*start, start (0,0)*

Vote for what you think is better

Fundamental interval formats

- **SAM/BAM** – Sequence Alignment Map
- **BED/GFF** → Gene Annotation representation
- **VCF/BCF** → for variant calls

Pick one coordinate system and stick with it!



1. Pick the standard your group is using and convert every new data to this standard!
2. If you have a choice of what to use pick the one based system! (GFF).

What is a genomic feature?

- Feature: a genomic region (interval) associated with a certain annotation (description).

Typical attributes to describe a feature

1. chromosome
2. start
3. end
4. strand
5. name

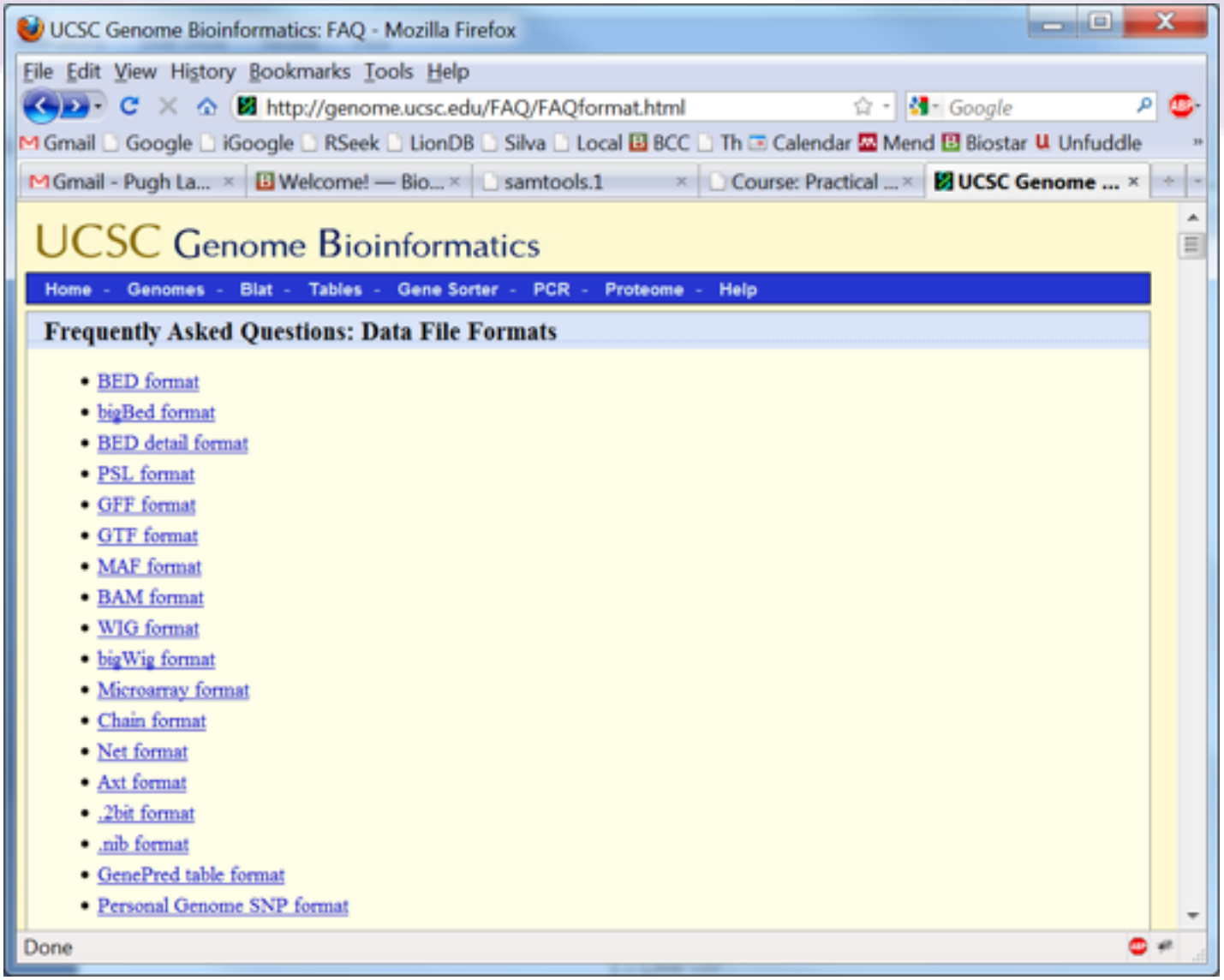
Values over intervals

Two options:

1. A single value characterizes an entire interval → score (value) for the interval

2. Continuous values → different value for each base of the interval

http://genome.ucsc.edu/FAQ/FAQformat.html



Two commonly used formats

- **BED** – UCSC genome browser → 0 based non inclusive → also used to display tracks in the genome browser (US “standard”)
  (variants: **bigBed**, **bedgraph**)

- **GFF** – Sanger institute in Great Britain → 1 based inclusive indexing system (“European standard”),
  (variants: **GTF**, **GFF 2.0**)

BED format

Search for BED format

Tab separated 3 required and 9 optional columns.

1. **chrom** (name of the chromosome, sequence id)
2. **chromStart** (starting position on the chromosome)
3. **chromEnd** (end position of the chromosome, **note** this base is not included
4. **name** (feature name)
5. **score** (between 0 and 1000)
6. **strand** (+ or -)
7. **thickStart** (the starting position at which the feature is drawn thickly)
8. **thickEnd** (the ending position at which the feature is drawn thickly)
9. **itemRGB** (RGB color → 255, 0, 0 display color of the data contained)
10. **blockCount** (the number of blocks (exons) in the BED line.)
11. **blockSizes** (a comma-separated list of the block sizes)
12. **blockStarts** (a comma-separated list of the block starts)

GFF format

Search for GFF3 → http://www.sequenceontology.org/gff3.shtml

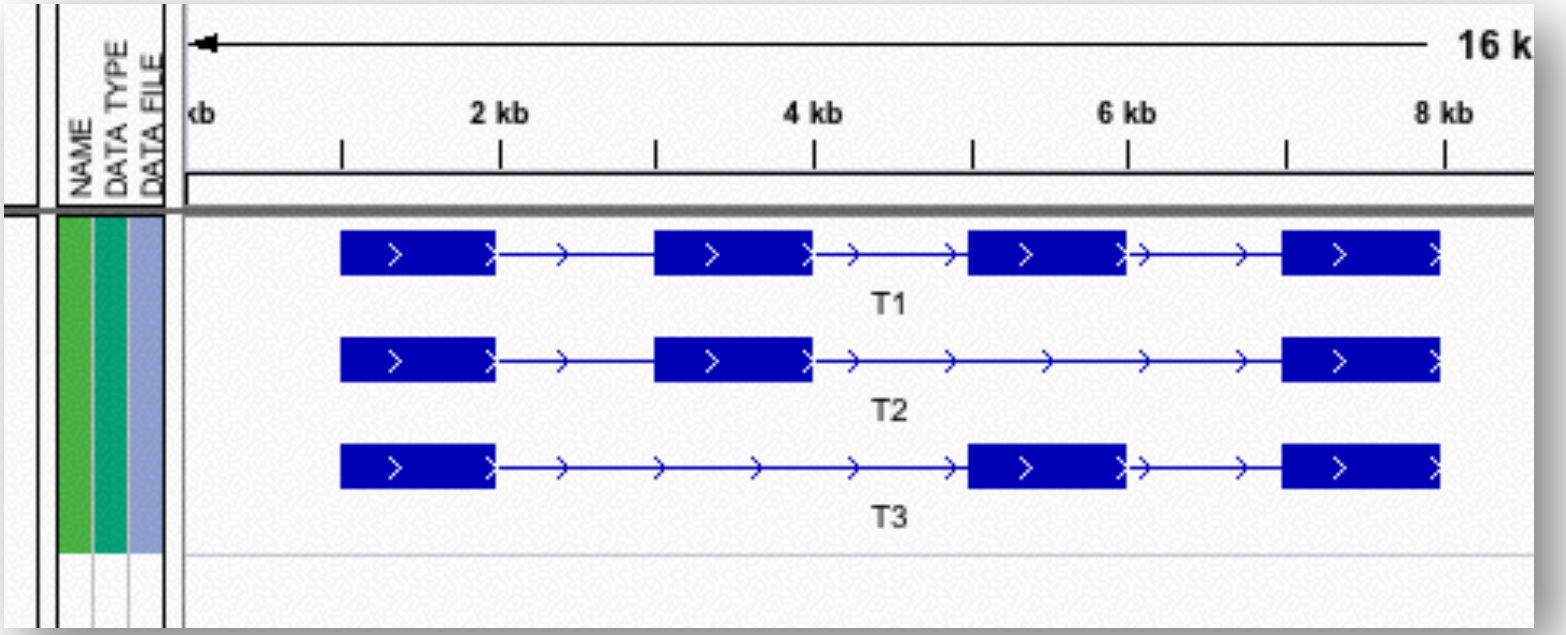
Tab separated with 9 columns. Missing attributes may be replaced with a dot →

1. **Seqid** (usually chromosome)
2. **Source** (where is the data coming from)
3. **Type** (usually a term from the sequence ontology)
4. **Start** (interval start relative to the seqid)
5. **End** (interval end relative to the seqid)
6. **Score** (the score of the feature, a floating point number)
7. **Strand** (+ or -)
8. **Phase** (used to indicate reading frame for coding sequences)
9. **Attributes** (semicolon separated attributes → Name=ABC;ID=1)

people like to stuff a lot of information here

Representing interval relationships

We have a gene with three splicing variants



Note: each exon is 1kb separated by multiples of 1kb

How to represent this data in a simple text file?

Data representation

- Both BED and GFF files can represent them
- Two common versions of GFF → **GTF2** and **GFF3**
  (note: tool documentation can often wrong and shows a weird combination of these two formats)
- In GFF the content of the ATTRIBUTE (9^{th}) column specifies the relationship between features

The GTF formats

GTF attributes:

- **gene_id value;**
  a globally unique identifier for the genomic source of the transcript
- **transcript_id value**
  a globally unique identifier for the predicted transcript.

gene_id “G1” transcript_id “T1”

GFF attributes:

ID=exon1; Parent=T1

See the GFF3 site for exact specification of the these mean.
Important: More than one parent may be listed!

The GFF formats

GFF attributes:

ID=exon1; Parent=T1

Example interval as GTF

example.gtf (~/work/lec26)

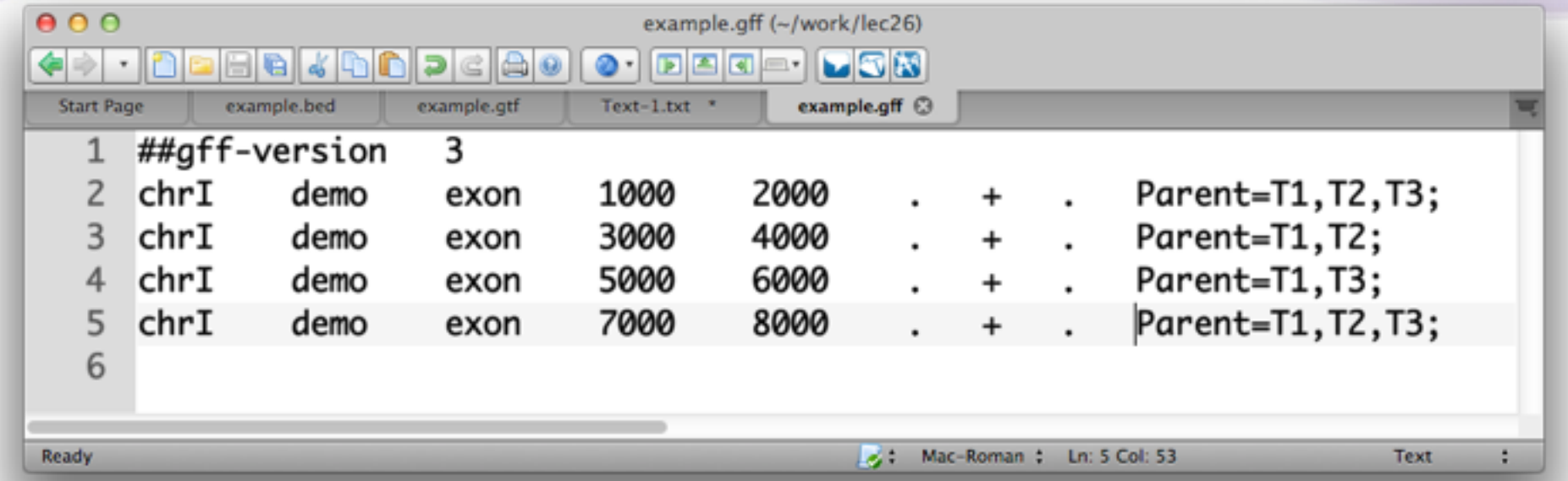
Start Page | example.gff | example.gtf | Text-1.txt

```
1  chrI  demo  exon  1000  2000  .  +  .  gene_id "G1"; transcript_id "T1";
2  chrI  demo  exon  3000  4000  .  +  .  gene_id "G1"; transcript_id "T1";
3  chrI  demo  exon  5000  6000  .  +  .  gene_id "G1"; transcript_id "T1";
4  chrI  demo  exon  7000  8000  .  +  .  gene_id "G1"; transcript_id "T1";
5  chrI  demo  exon  1000  2000  .  +  .  gene_id "G1"; transcript_id "T2";
6  chrI  demo  exon  3000  4000  .  +  .  gene_id "G1"; transcript_id "T2";
7  chrI  demo  exon  7000  8000  .  +  .  gene_id "G1"; transcript_id "T2";
8  chrI  demo  exon  1000  2000  .  +  .  gene_id "G1"; transcript_id "T3";
9  chrI  demo  exon  5000  6000  .  +  .  gene_id "G1"; transcript_id "T3";
10 chrI  demo  exon  7000  8000  .  +  .  gene_id "G1"; transcript_id "T3";
```

Ready | Mac-Roman | Ln: 1 Col: 81 | Text

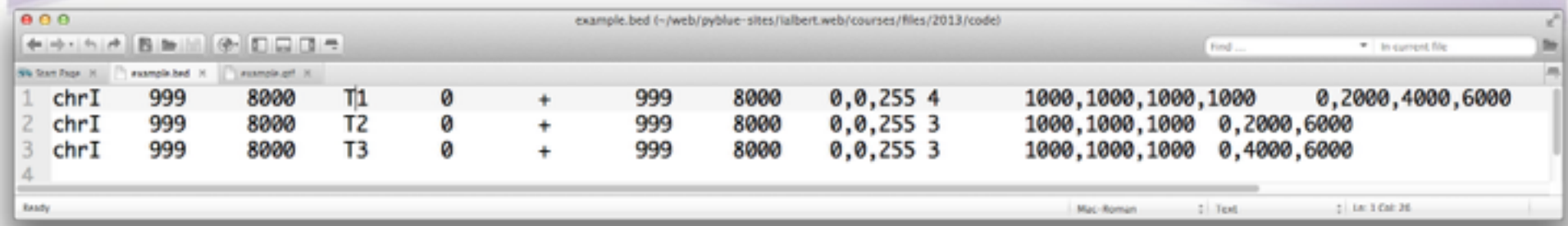
A distinct line is entered for each exon, repeated for each transcript

Example interval as GFF 3



The same exon may be part of different transcripts (parents)

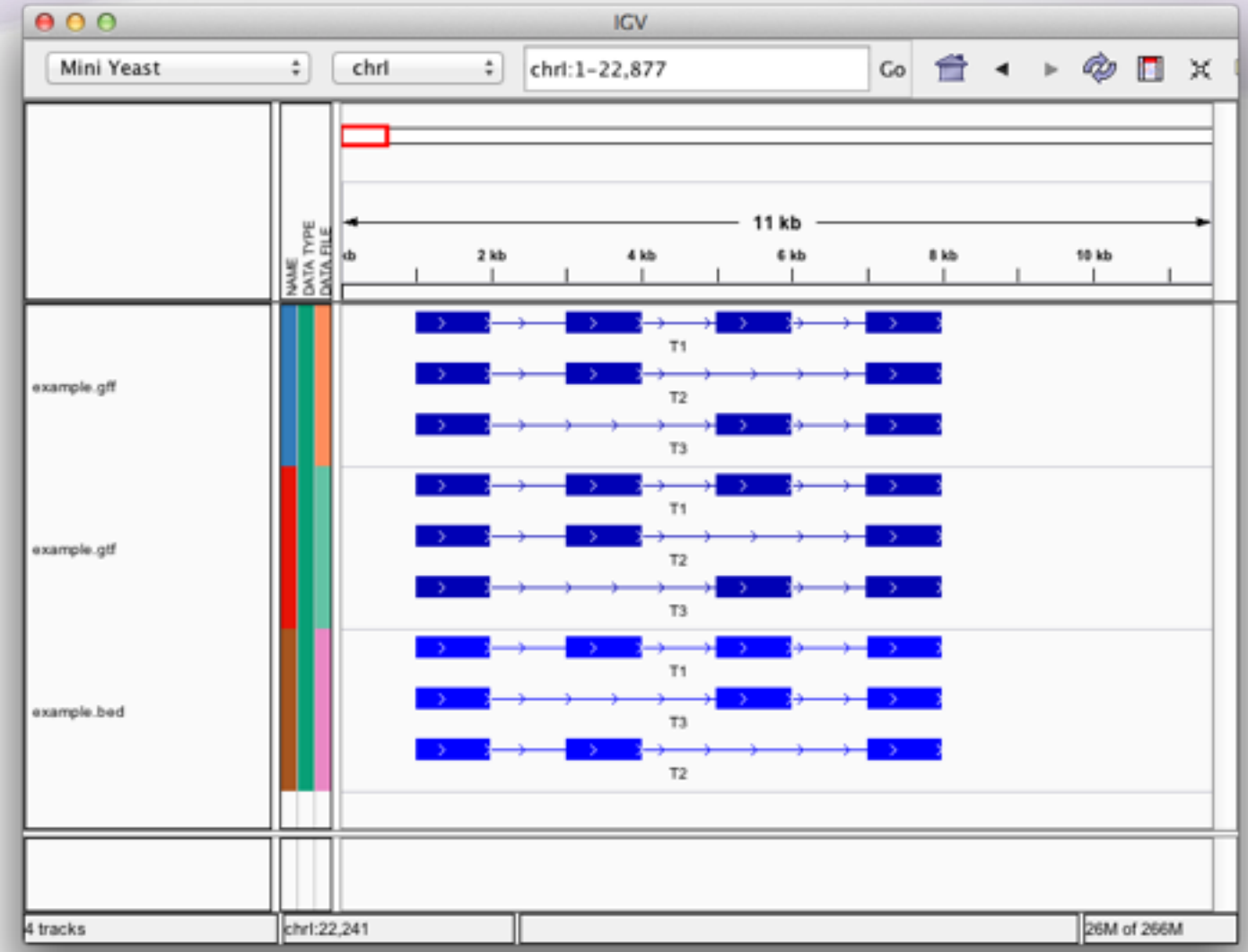
Example interval in BED



6. **strand** - Defines the strand - either '+' or '-'.
7. **thickStart** - The starting position at which the feature is drawn thickly (for example, the start codon in gene displays).
8. **thickEnd** - The ending position at which the feature is drawn thickly (for example, the stop codon in gene displays).
9. **itemRgb** - An RGB value of the form R,G,B (e.g. 255,0,0). If the track line *itemRgb* attribute is set to "On", this RBG value will determine the display color of the data contained in this BED line. NOTE: It is recommended that a simple color scheme (eight colors or less) be used with this attribute to avoid overwhelming the color resources of the Genome Browser and your Internet browser.
10. **blockCount** - The number of blocks (exons) in the BED line.
11. **blockSizes** - A comma-separated list of the block sizes. The number of items in this list should correspond to *blockCount*.
12. **blockStarts** - A comma-separated list of block starts. All of the *blockStart* positions should be calculated relative to *chromStart*. The number of items in this list should correspond to *blockCount*.

From the BED format specification

Visualizing in IGV



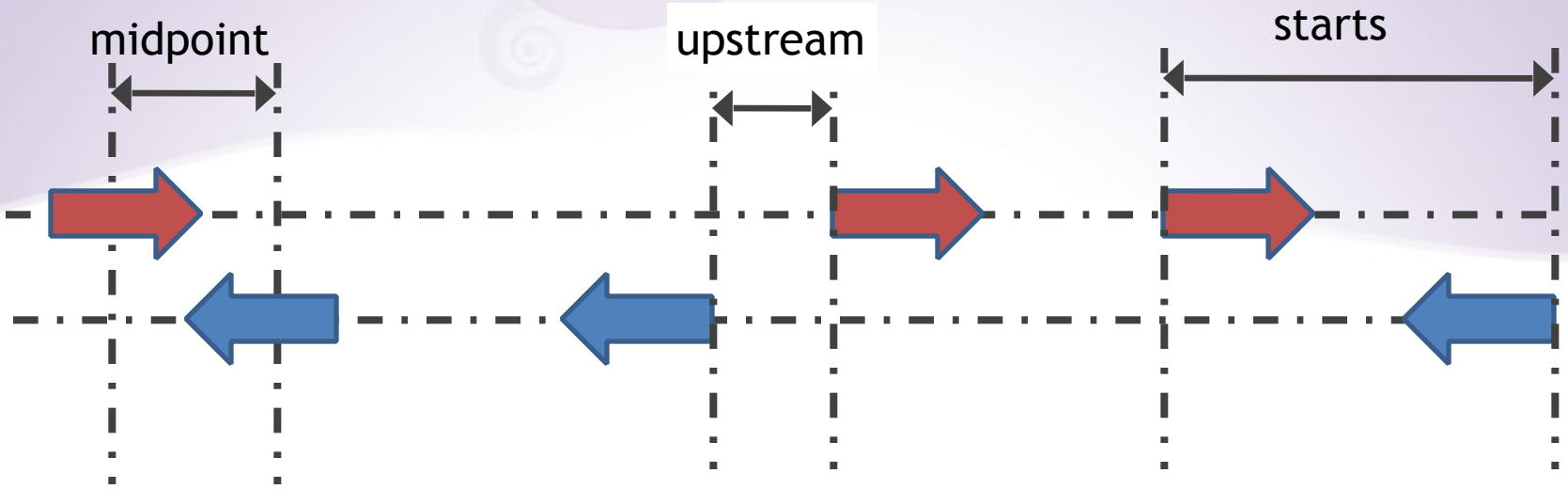
Interval related tasks

An intervals are not one-dimensional points! –

We need to specify tasks more precisely than for one dimensional points. For example:

- For each feature find the intervals from another dataset that are overlapping with it
- For each interval on one strand find the closest on the other strand

Important details

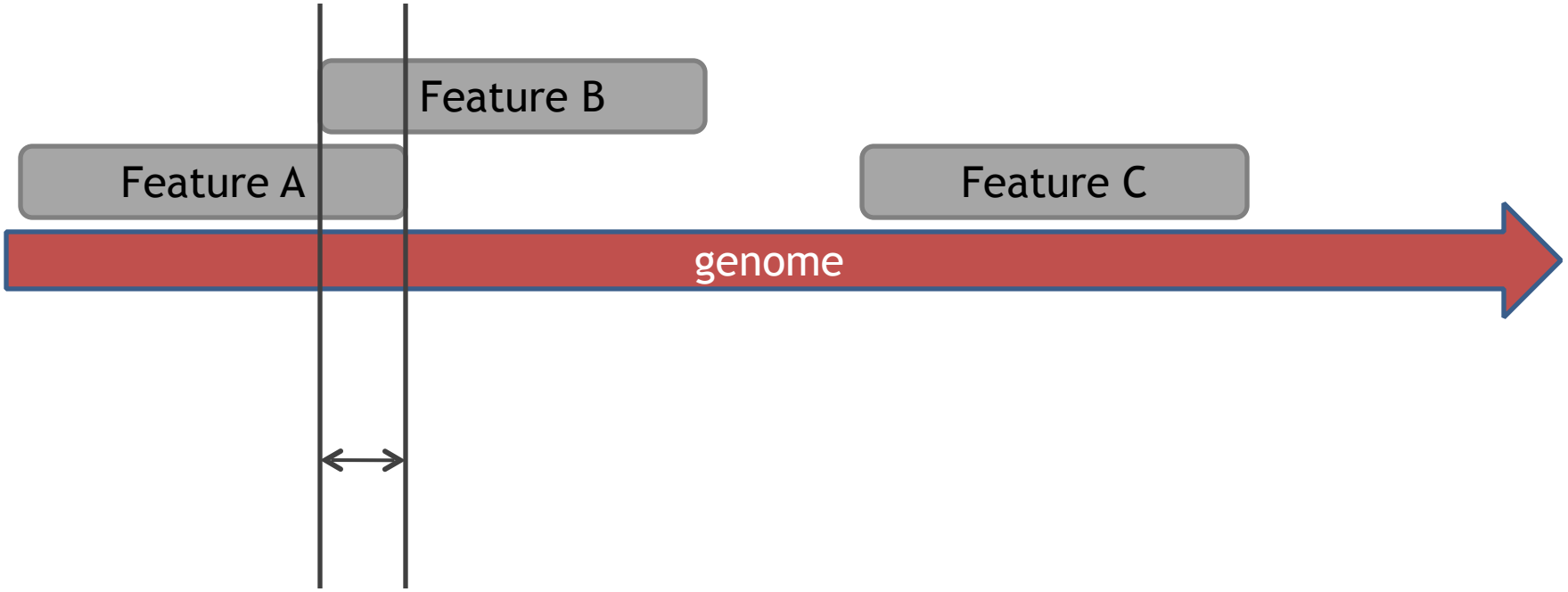


- What are the anchor points (the locations that represent the intervals)
- Which direction does the comparison proceed – upstream, downstream?
- What gets reported?

Often we need to create another transformed interval data that conforms to what we actually need

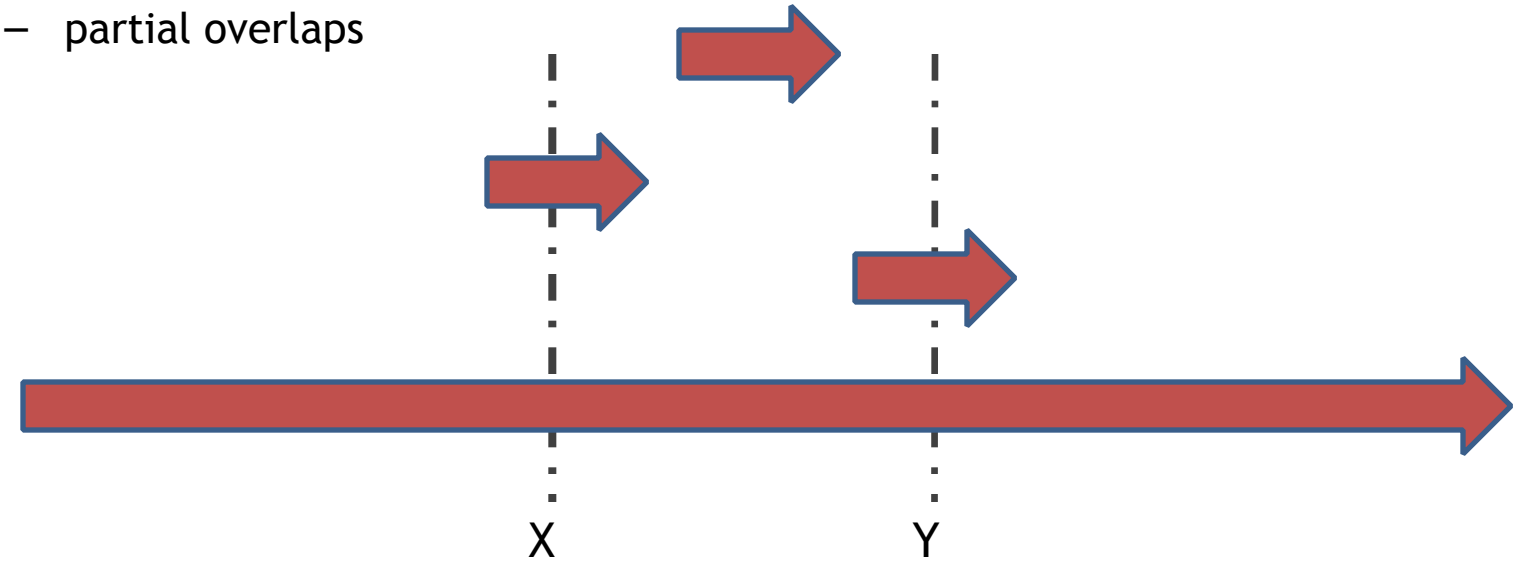
Overlap/intersect

- Two features are said to overlap or intersect if they share at least one base in common.



Computing Interval Overlaps

- Unexpectedly complex task as it needs to account for various types of positioning:
  - full containment of either interval
  - partial overlaps



Neat and useful formulas (X,Y is the query interval):

- **midpoint = (start + end) // 2** (with integer division)
- **overlap condition: (start < Y) and (end > X)**

BedTools: Interval Arithmetics

- High performance software package that operates on multiple interval oriented data formats: BED, GFF, SAM, BAM and VCF

  http://bedtools.readthedocs.org/en/latest/

Quinlan AR and Hall IM,
BEDTools: a flexible suite of utilities for comparing genomic features.
Bioinformatics. 26, 6, (2010)

BedTools concepts

- There are many (25 and growing) tools/actions with different names
- Most tools write to the standard output
- The – (minus) character specifies the standard input
- Can be chained with *pipes* like all UNIX commands
- Most tools write their help when invoked, others need –h flag
- Flag options can substantially change the output format

BedTools has an excellent documentation

bedtools: *a powerful toolset for genome arithmetic*

Collectively, the **bedtools** utilities are a swiss-army knife of tools for a wide-range of genomics analysis tasks. The most widely-used tools enable *genome arithmetic*: that is, set theory on the genome. For example, **bedtools** allows one to *intersect*, *merge*, *count*, *complement*, and *shuffle* genomic intervals from multiple files in widely-used genomic file formats such as BAM, BED, GFF/GTF, VCF. While each individual tool is designed to do a relatively simple task (e.g., *intersect* two interval files), quite sophisticated analyses can be conducted by combining multiple bedtools operations on the UNIX command line.

Interesting usage examples

To whet your appetite, here are a few examples of ways in which bedtools has been used for genome research. If you have interesteding examples, please send them our way and we will add them to the list.

- Coverage analysis for targeted DNA capture. Thanks to Stephen Turner.
- Measuring similarity of DNase hypersensitivity among many cell types
- Extracting promoter sequences from a genome
- Comparing intersections among many genome interval files
- RNA-seq coverage analysis. Thanks to Erik Minikel.
- Identifying targeted regions that lack coverage. Thanks to Brent Pedersen.

Basic concepts

- For any operation that requires **two files** the tools asks for file **A** and file **B**
- Each element in file **A** is matched against each element in file **B**
- File **B** is loaded into memory – try to make that the **smaller** file

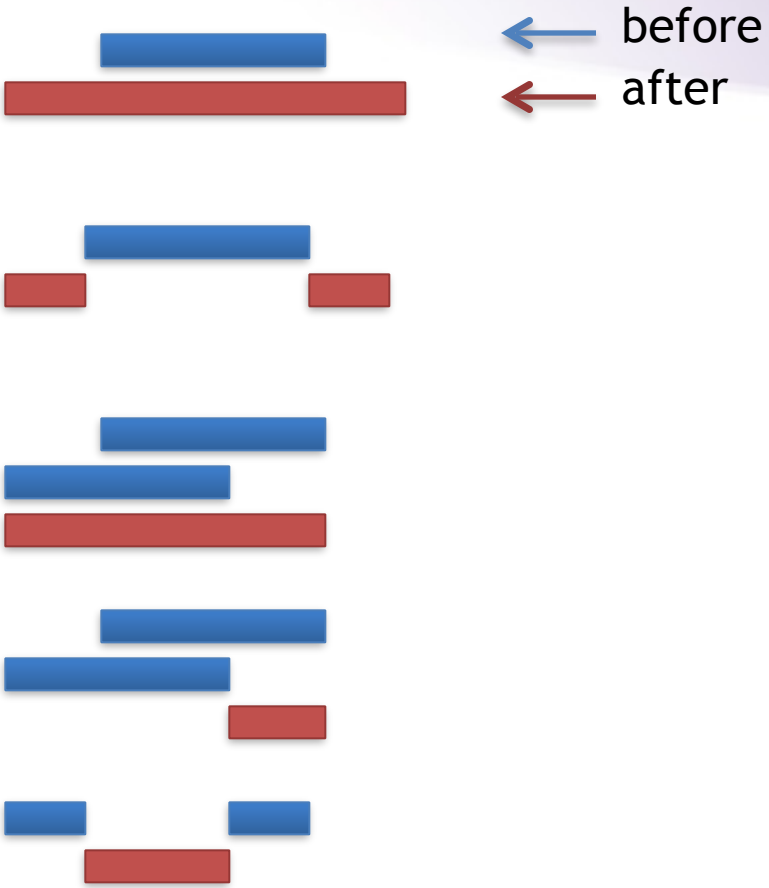
(make file –A the reads file and file –B the feature file)

Bedtools concepts

- The **old style** mode contains a different tool for each task (the manual covers these tools):
  - **intersectBed**
  - **windowBed**
  - **closestBed**
- A **new style** mode that contains only one tool that takes commands like **samtools**:
  - **bedtools** intersect
  - **bedtools** window
  - **bedtools** closest

BedTools operators

- slop (extend)
- flank
- merge
- subtract
- complement



Essential feature: Strand Awareness

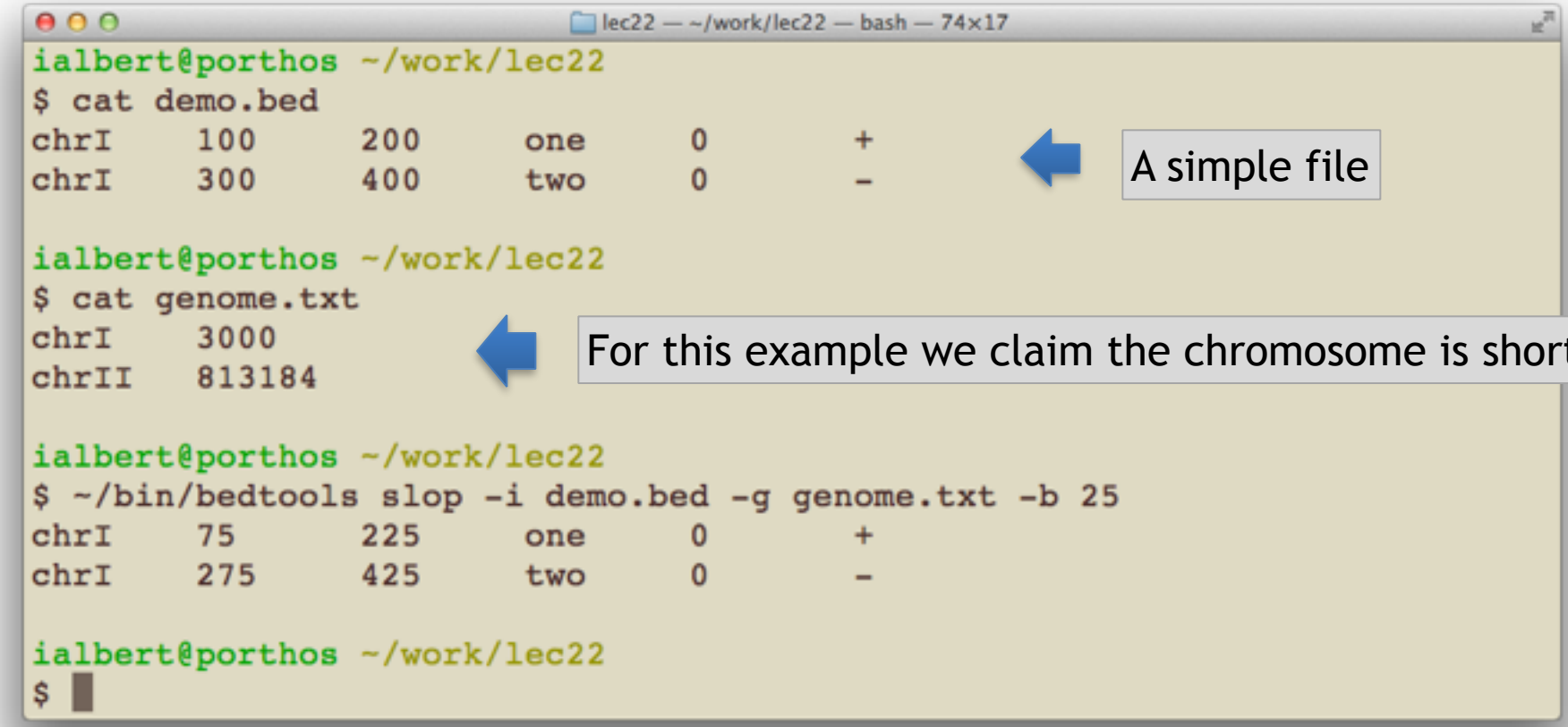
- Some tools take a –l (left), -r (right) parameter that will have a different effect if the “stranded” mode is turned on

1. **default mode**: left, right are interpreted on the forward strand’s coordinate system

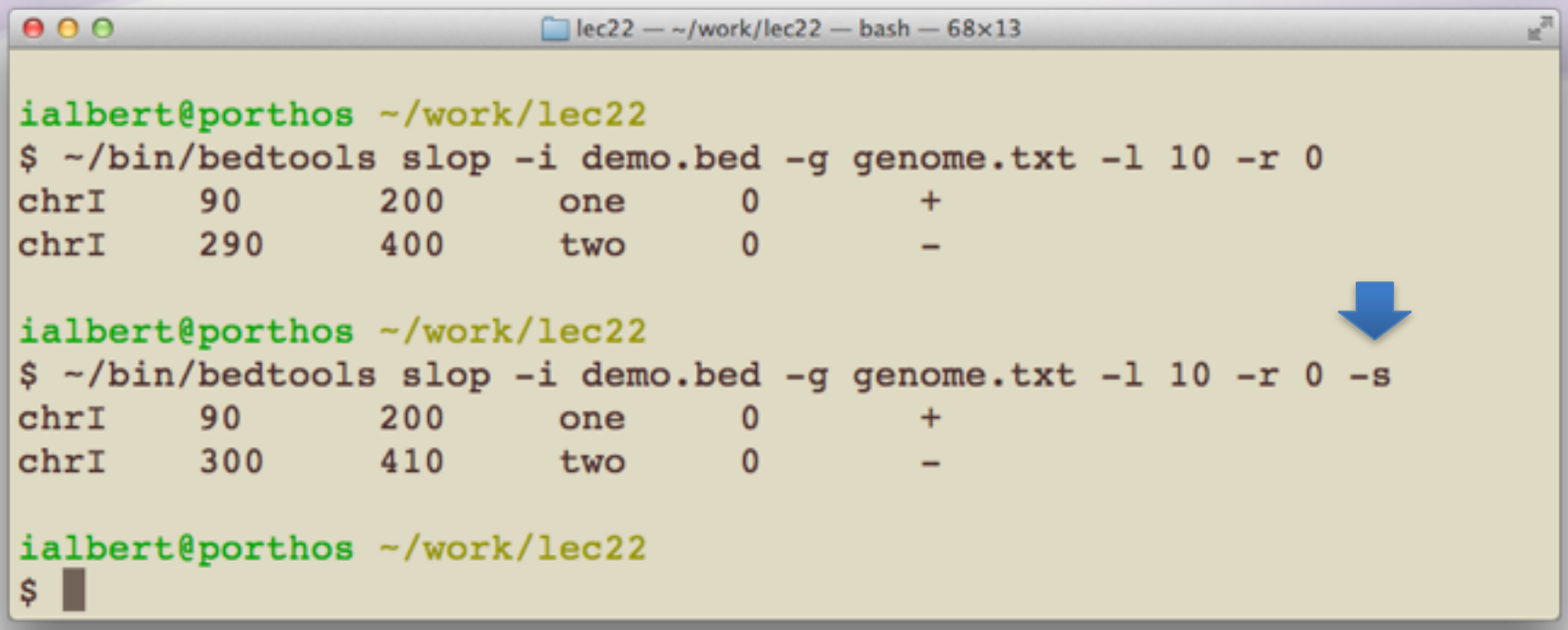
2. **stranded mode**: left, right are interpreted in the transcriptional direction 5’ to 3’

Strategy: generate a simple file then study what happens

Some tools require a genome file, tab delimited list of chromosome sizes

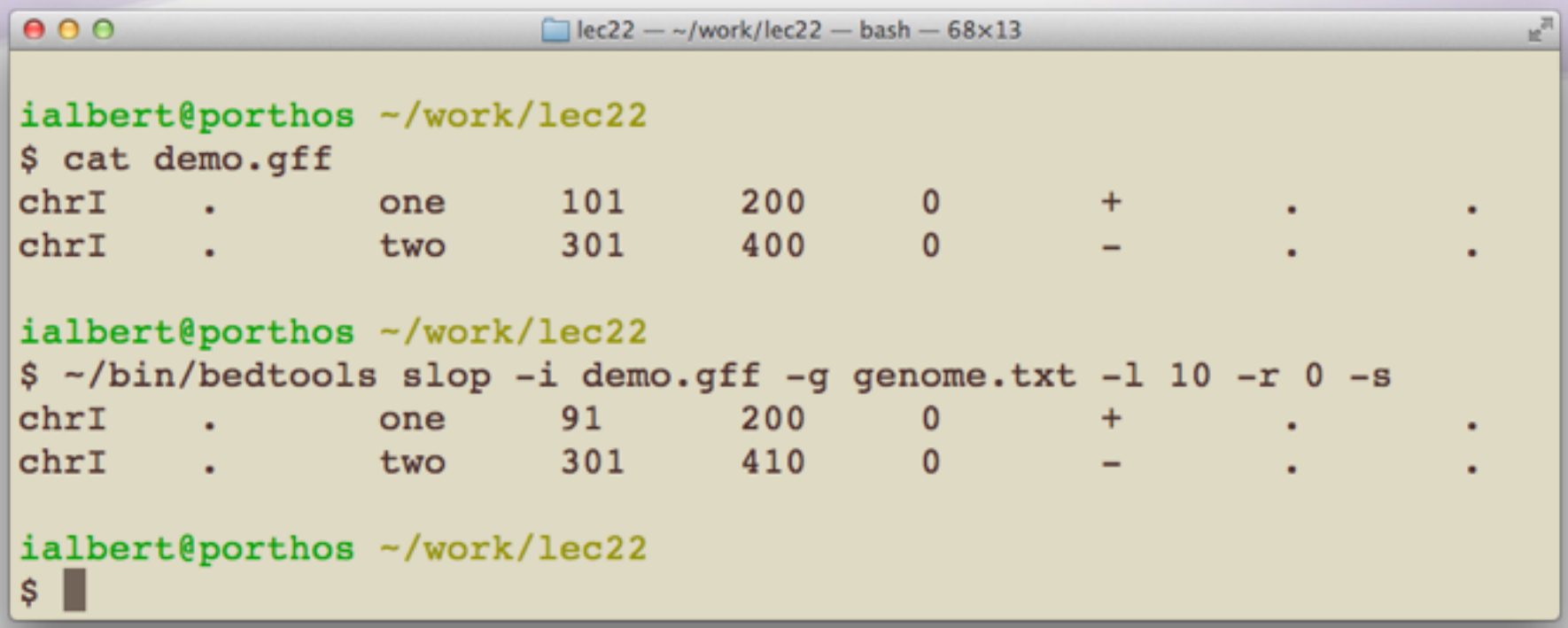


Stranded mode



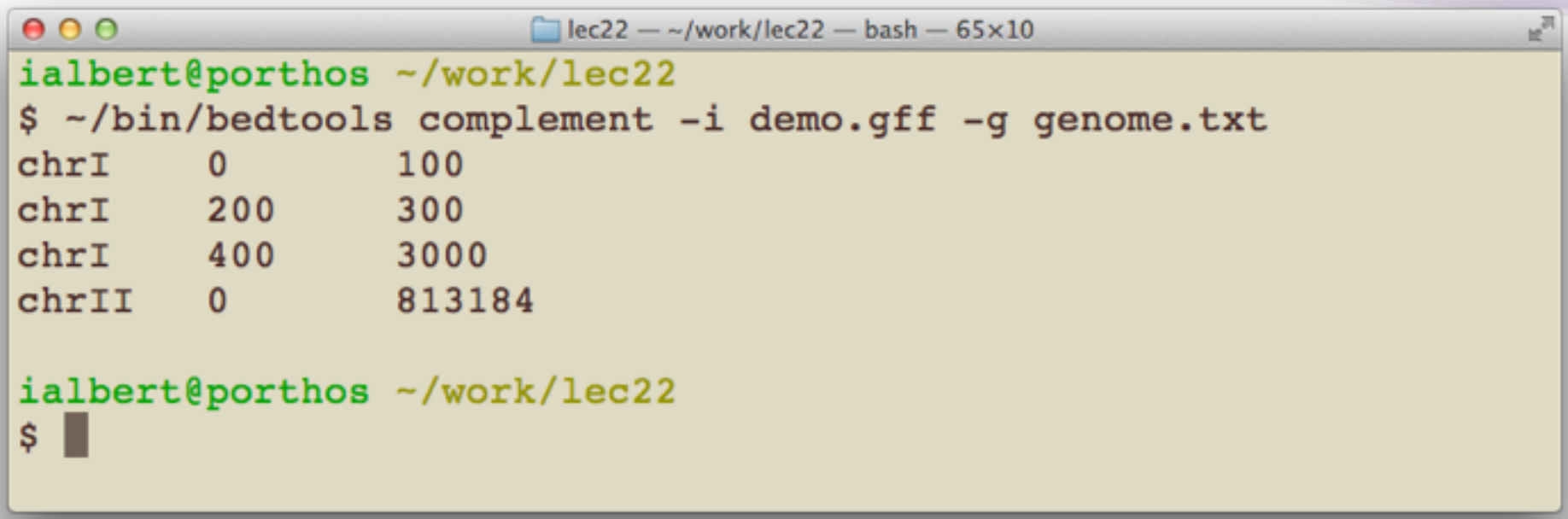
It is very important to understand what happens here.
It can be occasionally feel counterintuitive

BedTools is format aware for input



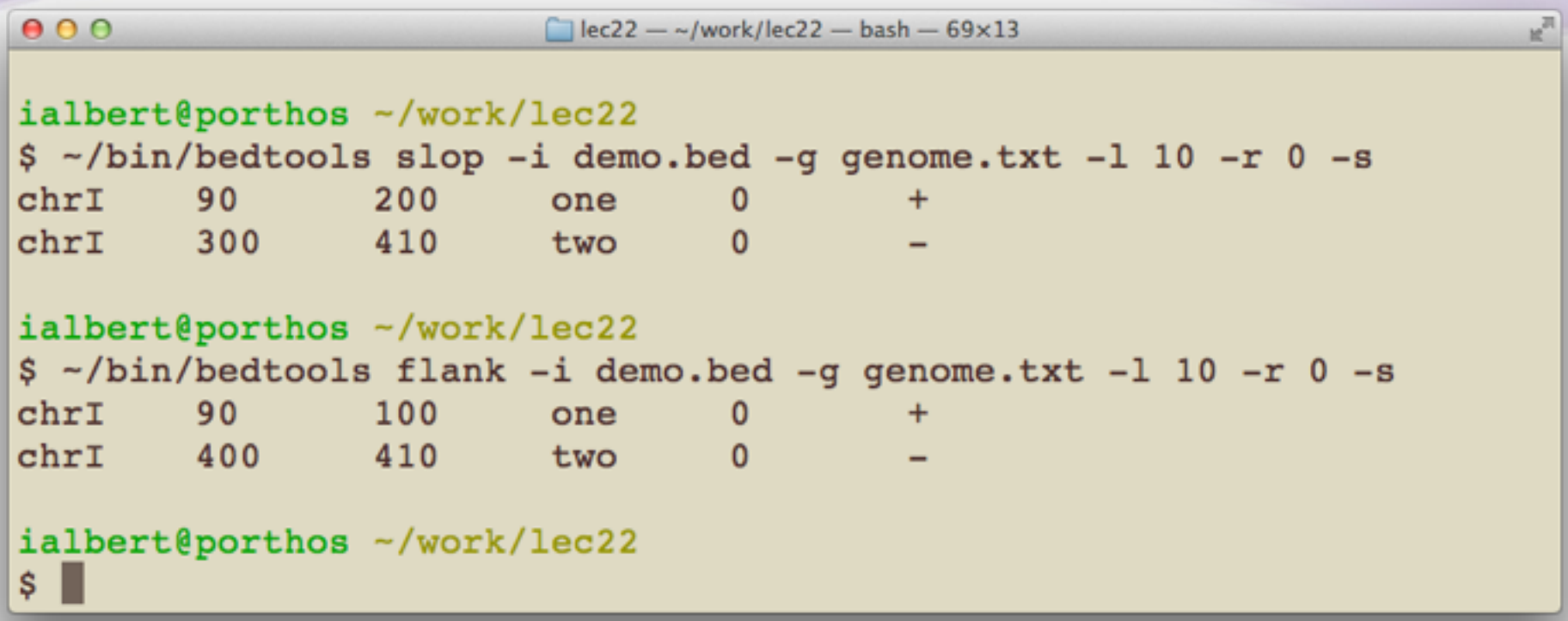
But some tools may produce output that is in different format!

This changed the output format!



Note that the output is in BED format! Moreover it is a 3 column BED format!

Slop vs Flank



The best is to draw the intervals and track what each tool does

Visualize your intervals

Prepare toy examples and explore what the tool does.

Pay close attention to the directionality

Think in terms of “interval operations” as they were “mathematical operations”