Module 2: Mapping

Caveat from previous exercise

- Algorithms are not perfect
  - fastx_clipper problems
- you can not write everything from scratch
  - well most people can not (some do when they learn of stuff like this)
- But you should do you due dillegence when using any algorithm; even very common/popular ones.

Introduction

Non-mapping cases

- Exercise pipeline
- k-mer based methods
  - Kallisto, Sailfish

Most of time need to do alignment

- Most bioinformatics pipeline have a mapping step
- Mapping is just sub-string find:

```
Given string s and G over some common alphabet
    Len(s)<=Len(G)
    find pos of s in G
```

which is conceptually a pretty simple problem.

2 complications

- But there are two wrinkles (complications):
  - Size (N): len(G)==N is enormouse (3,000,000+ for human) and while len(s)==m is usually tiny (50–200) we have 10^8 to 10^9 of them
  - Imperfect and/or multiple matches: we want to find the best closest match
    - actually want both best and sub-optimal
    - sometimes just one, a few, all
    - most algorithm trade of speed for ability to find all

Indexes to manage search space

- Dealing with size issue (and mismatches partially)
  - Standard solution when searching in a large space; build index
  - For searching strings the index is usually a suffix tree

  In computer science, a suffix tree (also called PAT tree or, in an earlier form, position tree) is a compressed trie containing all the suffixes of the given text as their keys and positions in the text as their values. Suffix trees allow particularly fast implementations of many important string operations. (https://en.wikipedia.org/wiki/Suffix_tree)

Suffix Tree

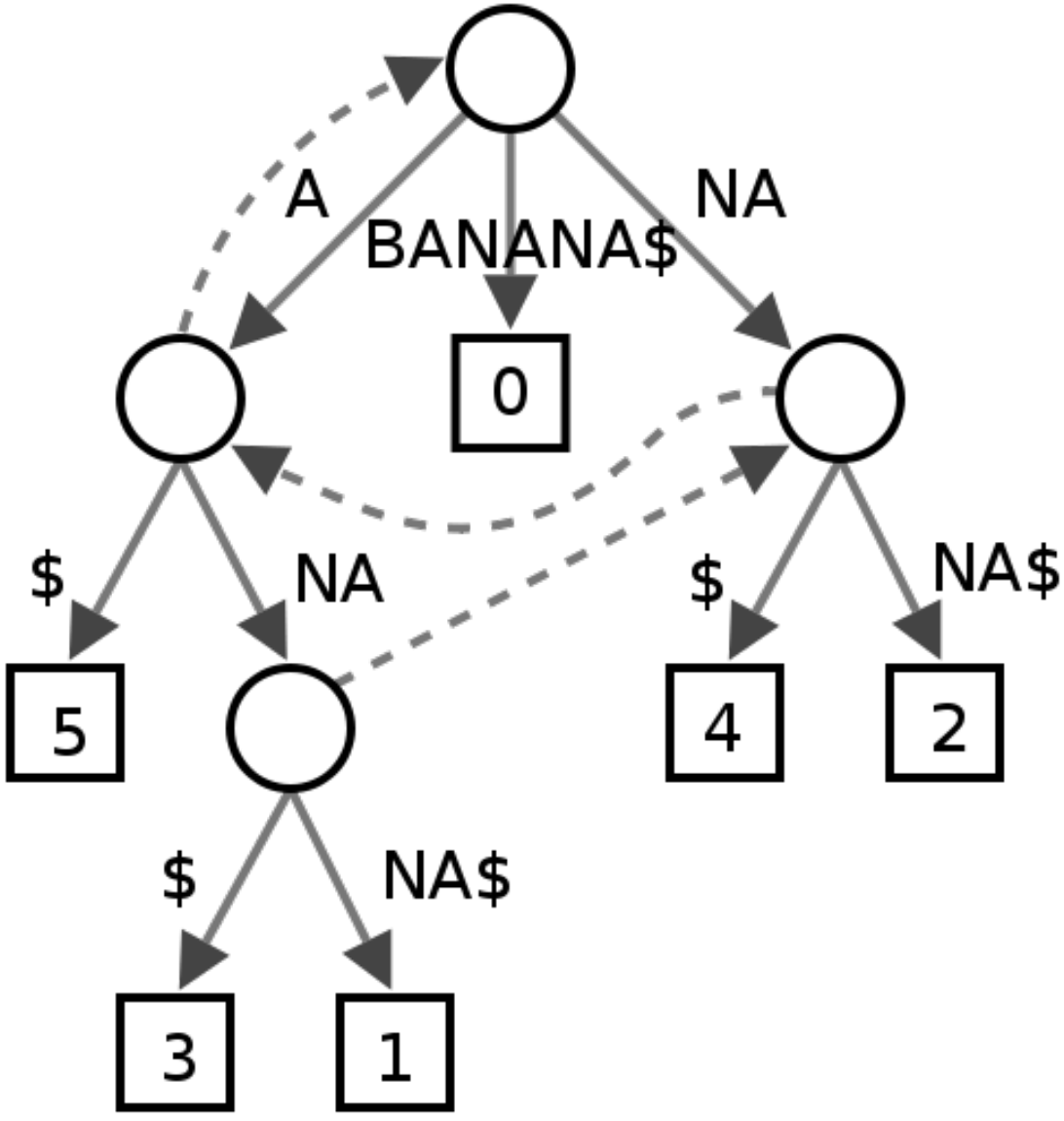


Figure 1:Suffix Tree (wiki)

Partial matches

- Imperfect partial matches
  - Long history of work on this; the original sequence (proteins between species) searches look at highly divergent sequences.
  - Read Durbin for serious details:
    - Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids
- Pairwise alignments, with mismatches and in/dels
  - Can be stated as finding optimal path through scoring matrix:
    - Dynamic Programming
  - Global: Needleman-Wunsch
  - Local: Smith-Waterman

Alignment Algorithms

Pre-next gen methods

BLAST (Basic Local Alignment Search Tool)

http://blast.ncbi.nlm.nih.gov/Blast.cgi

- Very good a searching for extrememly fuzzy (imperfect matches)
- Can index a massive amount of sequence data:
  - Many (all) genomes and known sequence
- Great for things like homology seach.
- Much to slow to use for most next-gen applications
  - We use if for diagnostics to figure out contamination problems

TaxBLAT

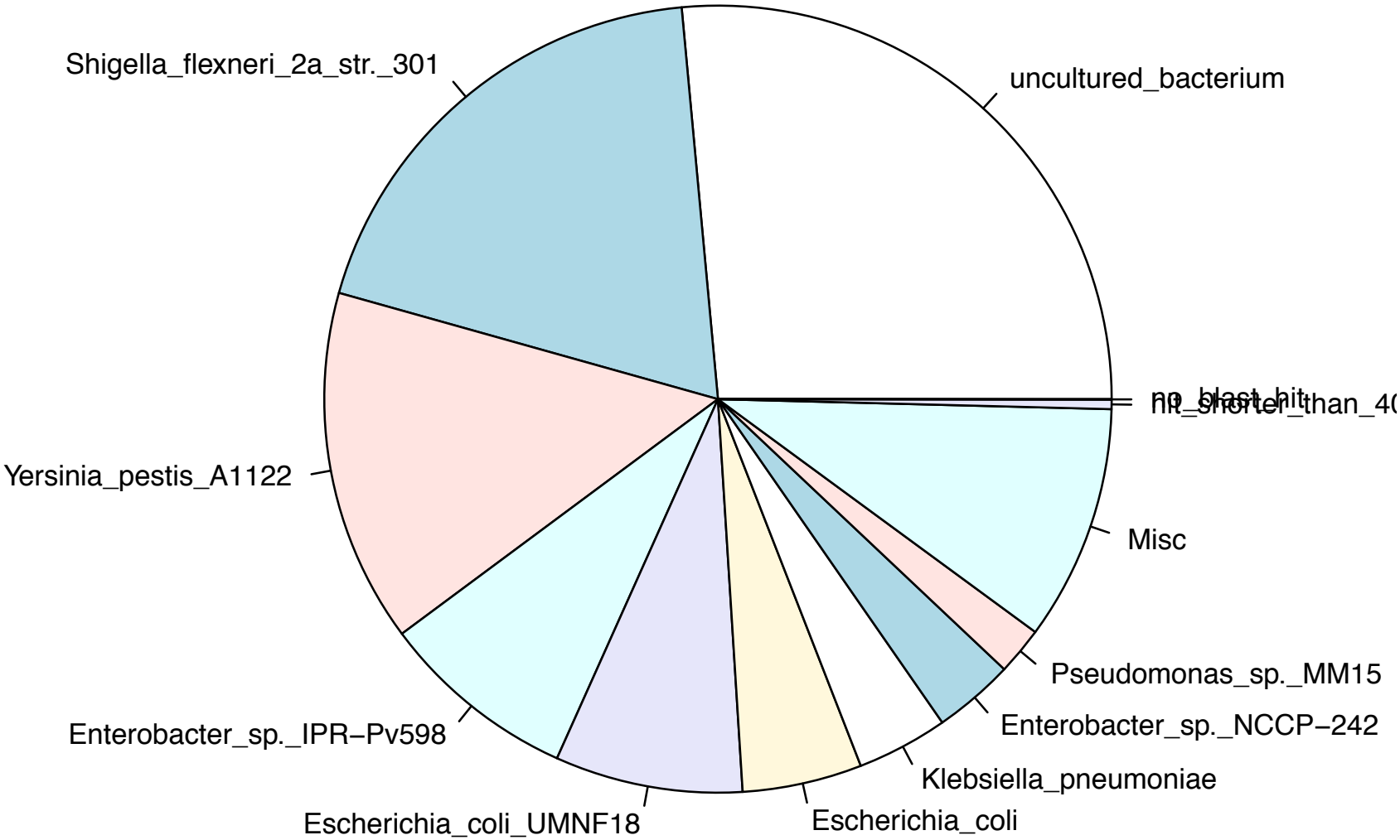


Figure 2:BLAST of what was suppose to be human data

Pre-next gen methods

Pre-next gen methods

BLAT (BLAST-like alignment tool)

- Designed map large numbers of sequences to a single genome.
- Sequences that are likely to be from that genome
- Much faster than BLAST.
- Also chains alignments into one consistent piece
  - BLAST will give many individual pieces

Ultra-fast methods

- MAQ
  - Definition of MAPQ
  - No indels
- Bowtie
  - Many algorithms have Bowtie as their mapping kernel (TopHat; Express)
  - more tweakable (or more easily so) then BWA
  - MAPQ score do not follow "expected" form/formula
  - Original version did not allow indels
- BWA (successor of MAQ)
  - Work horse in variant detection community
- Bowtie2

Older methods

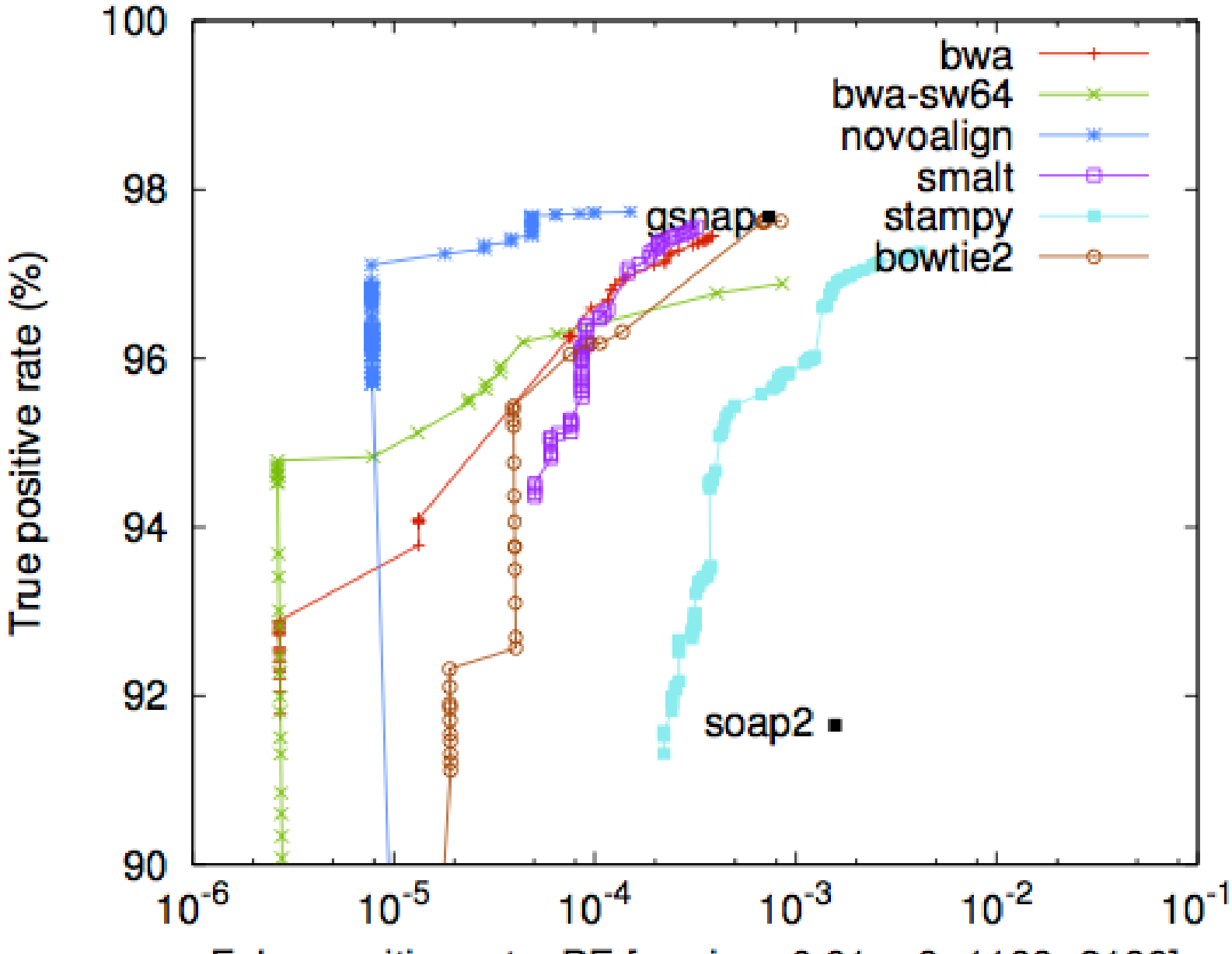
- SHRiMP (no longer developed but old favorite)
  - Very tweakable
  - Native COLOR space support (ie reads in FASTA genome; no double encodeing non-sense)
  - Actually has a useful non-standard output mode (alignment); more easily parsed cigar string
  - work well for intermediate length reads
  - very, very slow compared to BWA/BOWTIE

Many, many other methods

From the BWA ALN paper:

- novoalign
  - very good performance but very slow
  - also commercial
- smalt
- stampy
- soap (soap2)
- gsnap

Comparison


100
98
96
94
92
90
True positive rate (%)
bwa
bwa-sw64
novoalign
smalt
stampy
bowtie2
gsnap
soap2
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}

BWA vs Bowtie2

- First note we are comparing Bowtie2; Bowtie (version 1) did not do indels (will not map) so much less useful variant calling

Comments from bwa programmer

- From lh3 (this is Heng Li BWA creator) (http://seqanswers.com/forums/showthread.php?t=15200)

  Basically bowtie2 chooses a nice balance point where it is the fastest without much loss of accuracy in comparison to others, but for variant calling for Illumina data, novoalign/smalt/bwa/gsnap may still be the mapper of choice. Things may change in future of course. Bowtie2 is still in beta, while bwa and bwa-sw are mature (i.e. not many improvements can be made).

http://lh3lh3.users.sourceforge.net/alnROC.shtml

Tradoffs

Nearly all aligners use heuristics. Few of them can guarantee to find the best hit even if the top hit is clearly (i.e. in all sensible scoring schemes) better than other hits. Here are several examples.

Comparison

matric	BWA ALN (v6)	Bowtie2
Index HG19	9.4Gb	3.8Gb
Speed (from lh3)	230.1	154
Specificity	Better	.
Sensitivity	Better	.

Bowtie2 faster and less memory resources but less sensitive and specific (in some cases 10x). Specificity is key in variant calling. These were for 100mers

Bowtie2 was better at aligning longer reads (like from the 454)

BWA (now up to version 7) has BWA MEM which is suppose to improve both short read and long read and BWASW for long queries

Summary (very rough)

- BLAST super sensitive; massive search space; but by far the slowest
- BLAT faster nearly as senitive; constructs chains; but single genome
- Bowtie
  1. Fastest of the bunch; but no indels; accuracy issues
  2. Faster with indels and long reads
- BWA
  - ALN Fast / more accurate
  - BWASW Long reads, can be much slower
  - MEM optimised for most cases; make sensible tradoffs; accuracy paramount
- Faster not always better

RNA (splicing read) aligners

- Engstrom, et, al. Nature Methods 2013

Systematic evaluation of spliced alignment programs for RNA-seq data

Pär G Engström[1,13], Tamara Steijger[1], Botond Sipos[1], Gregory R Grant[2,3], André Kahles[4,5], The RGASP Consortium[6], Gunnar Rätsch[4,5], Nick Goldman[1], Tim J Hubbard[7], Jennifer Harrow[7], Roderic Guigó[8,9] & Paul Bertone[1,10–12]

High-throughput RNA sequencing is an increasingly accessible method for studying gene structure and activity on a genome-wide scale. A critical step in RNA-seq data analysis is the alignment of partial transcript reads to a reference genome sequence. To assess the performance of current mapping software, we invited developers of RNA-seq aligners to process four large human and mouse RNA-seq data sets. In total, we compared 26 mapping protocols based on 11 programs and pipelines and found major performance differences between methods on numerous benchmarks, including alignment yield, basewise accuracy, mismatch and gap placement, exon junction discovery and suitability of alignments for transcript reconstruction. We observed concordant results on real and simulated RNA-seq data, confirming the relevance of the metrics employed. Future developments in RNA-seq alignment methods would benefit from improved placement of multimapped reads, balanced utilization of existing gene annotation and a reduced false discovery rate for splice junctions.

examines the density of independent reads at those loci. Many algorithms also consider base-call quality scores and use sophisticated indexing schemes to decrease runtime.

Here we assess the performance of 26 RNA-seq alignment protocols on real and simulated human and mouse transcriptomes. We adopted a competitive evaluation model applied in other areas of bioinformatics[11–14]. Developers were invited to run their software and submit results for evaluation as part of the RNA-seq Genome Annotation Assessment Project (RGASP). Programs included six spliced aligners GSNAP[7], MapSplice[4], PALMapper[8], ReadsMap, STAR[9] and TopHat[5,6]) and four alignment pipelines (GEM[3], PASS[15], GSTRUCT and BAGET). GSTRUCT is based on GSNAP, whereas BAGET uses a contiguous DNA aligner to map reads to the genome as well as to exon junction sequences derived from reference gene annotation. For comparison, the contiguous aligner SMALT was also tested. SMALT can map reads in a split manner, but it lacks several features of dedicated spliced aligners, such as precise determination of exon-intron boundaries. We demonstrate that choice of align-

Spliced aligners

- TopHat
- STAR
  - Two pass from Engstrom
  - Fast alone is not a great thing; but in this case (see paper) STAR does not seem to sacrifice accuracy or sensitivity and is much faster (much faster)
- Mappless aligners
  - k-mer
    - Sailfish
    - Kallisto
  - Much faster still but at least Sailfish seems to have problems; jury still out on Kallisto but looks promising

Other aligning algorithms

- long read (Roche 454)
  - bwa bwasa
    - 454 and IonTorren have problem with homopolymers
  - lastz (http://www.bx.psu.edu/~rsharris/lastz/)
- very long: genome to genome
  - mummer (http://mummer.sourceforge.net)
  - lastz

Genomes (the very big string)

Builds / version

- Chromosome naming annoyance
  - Disagreements
    - (chr1 vs 1)
    - chrM vs chrMT in human (not just different name)
  - Who thought this was a good idea (chrI, chrII)

Genome Build Details

- Full builds (random / unassembled)
  - USE THEM!!
  - Problem with UCSC
    - (haplotype blocks)
- Decoy (for variant)
- Hybrid genomes (xenografts)
  - With 64bit indexs can build 4Gb+ genomes

Sources

- UCSC (old favorite; lots of annotation easily available)
  - Extensive annotation; perhaps the most complete for some genomes/builds
- NCBI/ENSEMBLE (less easy to use; but seems be becoming more standard)
  - Have agreed on genome names; not chromosome names
- iGenome; incomplete and sometimes out of date but if they have your organism and build then they usually have almost everything you need
  - Sequence (both full and broken into chromosomes)
  - Indexes (but not STAR)
  - Annotation (for some like human, versioned annotation)

Indexes

- specific to aligner and usually version specific
- Spliced aligners also have Gene Model Dependence
  - Star has read length also