Next Generation Sequencing Data Techniques: Reference-Based Mapping and de Novo Assembly

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Galaxy: Data intensive biology *for everyone*

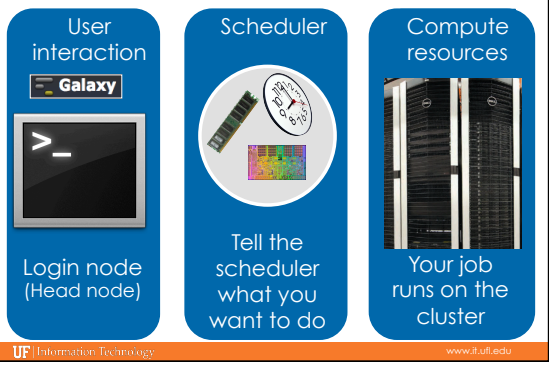
- Accessible, reproducible, transparent computational biology
- galaxy.hpc.ufl.edu
  - Local instance of Galaxy
    - Faster access to storage, easier upload
    - Local compute resources
    - Local control

UNIVERSITY OF FLORIDA | High-Performance Computing

HiPerGator

The University of Florida Supercomputer for Research

Cluster basics

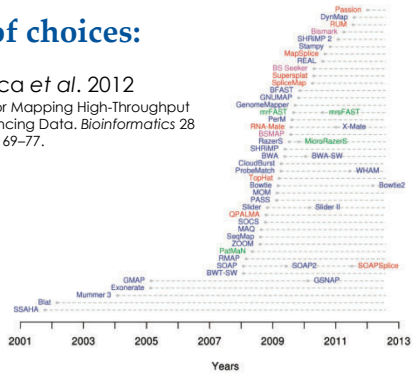


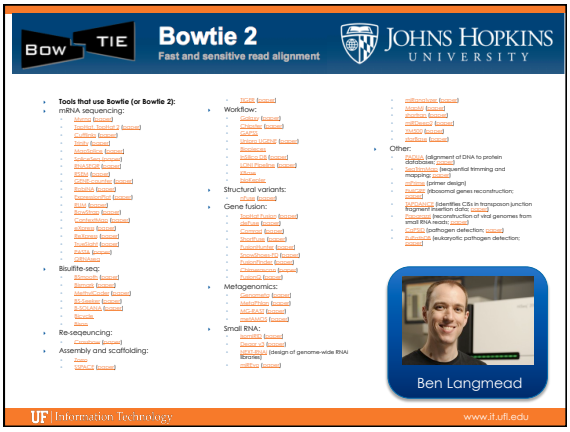
Reference-based mapping

- Map NGS reads onto a reference genome
  - Identify SNPs
  - RNA-seq
  - ChIP-seq
  - Etc.

Lots of choices:

- Fonseca *et al*. 2012
  - Tools for Mapping High-Throughput Sequencing Data. *Bioinformatics* 28 (24): 3169–77.





Bowtie (Langmead *et al.* 2009)

- Pre-built reference genome index
  - Burrows-Wheeler transform
  - Index needs to be computed prior to mapping
    - Either build your own: bowtie-build
    - **Or ask for index to be installed for you**
- Important parameters
  - -v vs. –n
    - Two mapping modes

Bowtie (Langmead *et al.* 2009)

- Mapping mode
  - -v: map reads that have less than v mismatches
    - Ignores quality scores
    - -v can be 0-3

Number of mismatches for SOAP-like alignment policy (-v):
-1
-1 for default MAQ-like alignment policy

Reference ATGCGTAGTACGTCAACGTGTCACGTGACAGACAGT
Read CGAAGTACGACAACGGGTCAC

If number of mismatches <= v, read maps

Bowtie (Langmead *et al.* 2009)

- Mapping mode
  - -n: map using quality scores
    - -n: Mismatches in seed (0-3), ignores quality
    - -l: seed length (default 28bp)
    - -e: max quality score of mismatches across read (default 70)
      - Quality scores range from 0-40

Reference ATGCGTAGTACGTCAACGTGTCACGTGACAGACAGT
Read CGAAGTACGACAACGGGTCAC

Seed: -l 7
-n 1

If sum of quality scores on the mismatches is <=e, read maps here, otherwise not

Bowtie (Langmead *et al.* 2009)

- Mapping mode
  - -n: map using quality scores
    - -n: Mismatches in seed (0-3), ignores quality
    - -l: seed length (default 28bp)
    - -e: max quality score of mismatches across read (default 70)

Maximum number of mismatches permitted in the seed (-n):
2
May be 0, 1, 2, or 3

Maximum permitted total of quality values at mismatched read positions (-e):
70

Seed length (-l):
28
Minimum value is 5

Bowtie (Langmead *et al.* 2009)

- Dealing with multiple mappings
  - -k: report up to *k* good alignments per read (1)
  - -a: report all alignments for a read (slow!)
  - -m: don't report if more than m alignments exist
  - -M: like –m, but report 1 random alignment
  - --best: guarantees alignment is in best stratum
  - --strata: don't report suboptimal strata

Bowtie (Langmead *et al.* 2009)

- Keeping unmapped/mapped reads
  - --un <filename> unmapped reads
  - --al <filename> mapped reads
  - Can be helpful for downstream analyses
- Use –S for SAM output
  - Most likely will process output using SAM anyway
- -p: Bowtie is threaded, can run using multiple cores on ***one*** node
  - E.g.: nodes=1:ppn=8

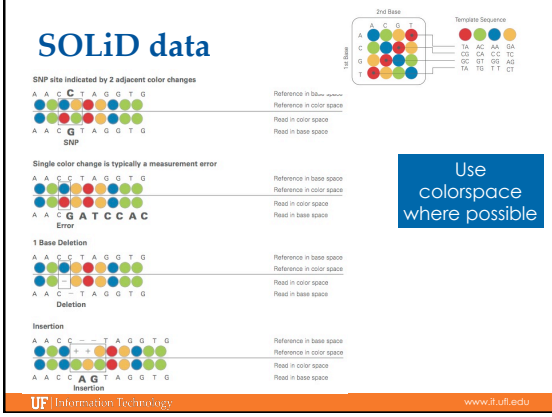
Bowtie2 (Langmead & Salzberg 2012)

- Adds gapped read alignment (indels)
- Faster than Bowtie for reads longer than 50bp
- Supports local alignment
  - Can trim ends that don't map
- Can map reads over Ns in reference
- No colorspace option

Bowtie2 (Langmead & Salzberg 2012)

- Presets for both global and local
  - --very-fast(-local)
  - --fast(-local)
  - **--sensitive(-local) Defaults**
  - --very-sensitive(-local)

SOLiD data



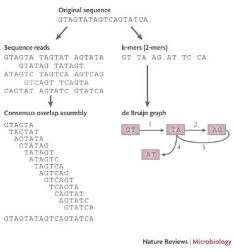
Use colorspace where possible

Other mapping applications

- BWA
- Lastz
- Maq
  - Bowtie is generally faster
- Mosaik
  - Handles gapped alignments relative to reference
- PerM
- SRMA

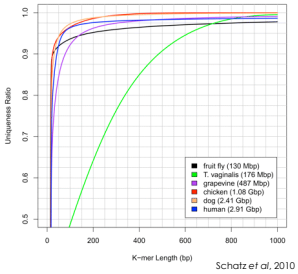
de Novo Assembly

- No reference genome
- Assemble contigs from reads
  - Assemble scaffolds using paired-end data
- Most short-read assemblers are de Buijn graph-based



kmers

- A kmer is a sequence of length *k*
  - Longer kmer
    - More unique
    - Fewer reads/kmer
  - Shorter kmer
    - Less unique
    - More reads/kmer
- The kmer you use does matter!
  - Try different kmers



Schatz *et al*, 2010

Velvet (Zerbino & Birney 2008)

- Two stages
  - velveth
    - Creates the hash table of kmers
  - velvetg
    - Uses the de Bruijn graph to create contigs & scaffolds
- kmer is critical
  - 11-31: Default for Velvet, most memory efficient
  - Up to 249 available.

Velvet (Zerbino & Birney 2008)

- Can use multiple types of sequencing inputs
  - Short, long
  - Paired, single
  - Different insert sizes
  - Reference
- A mix of library types is typically needed for de novo genome assembly
- Many helpful scripts distributed with Velvet
  - VelvetOptimiser—helps pick best kmer

Other de novo assembly applications

- Abyss
- ALLPATHS-LG
  - Has very specific requirements for library types and coverage
- Metavelvet
  - Modified version of Velvet for metagenomics
- Newbler
  - Provided by Roche (454), but can use Illumina data
- SOAPdenovo
- For RNA-seq
  - Oases (builds on after Velvet)
  - SOAPdenovo-TRANS
  - Trinity

UF Research Computing

- Help and Support (Continued)
  - http://wiki.hpc.ufl.edu
    - Documents on hardware and software resources
    - Various user guides
    - Many sample submission scripts
  - http://hpc.ufl.edu/support
    - Frequently Asked Questions
    - Account set up and maintenance