INTEGRATION
AND MODELING
for PREDICTIVE
BIOLOGY

KBase
PREDICTIVE BIOLOGY

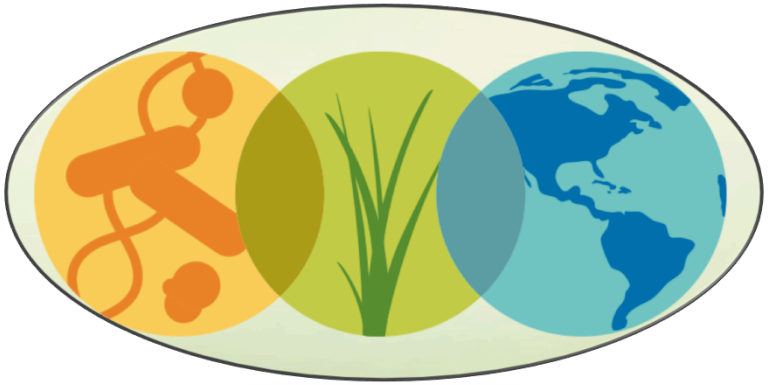
DOE Systems Biology Knowledgebase

Variation & RNA-seq Services

Michael Schatz
Cold Spring Harbor Laboratory

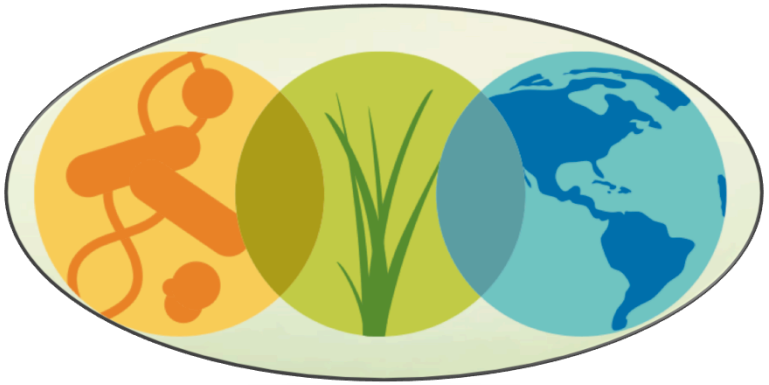
Agenda

1. Getting Started
2. Variation services
3. RNA-seq services

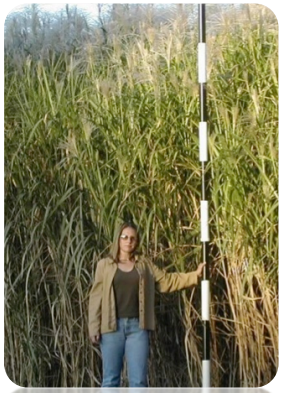


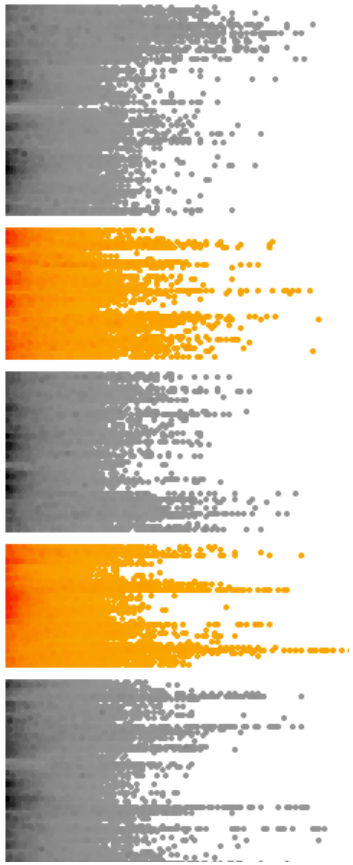
Agenda

1. Getting Started
2. Variation services
3. RNA-seq services



Samples to discoveries





Powered by KBase

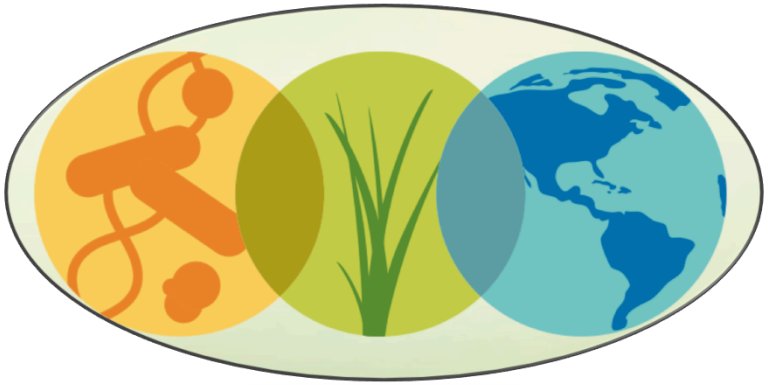
Logging In

- IRIS: command-line access to KBase functionality inside web browser
- Download and install tools on Mac or Linux
- KBase Labs offer early access to web-based interfaces
- Narrative user interface

http://kbase.us/ https://gologin.kbase.us/SignUp

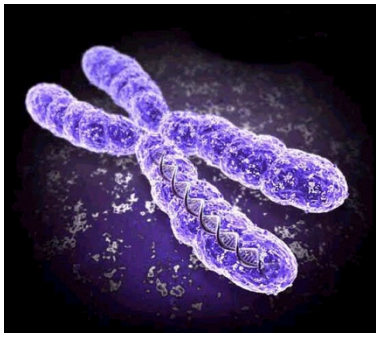
Agenda

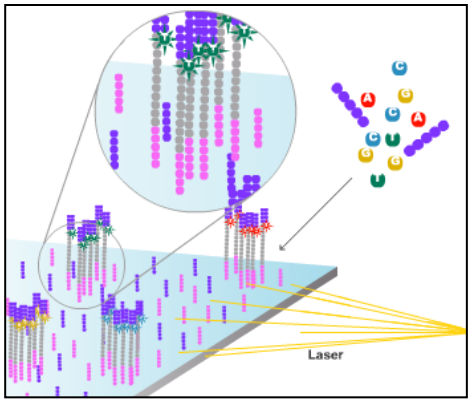
1. Getting Started
2. Variation services
3. RNA-seq services



Resequencing & Variations

How does your sample compare to the reference?



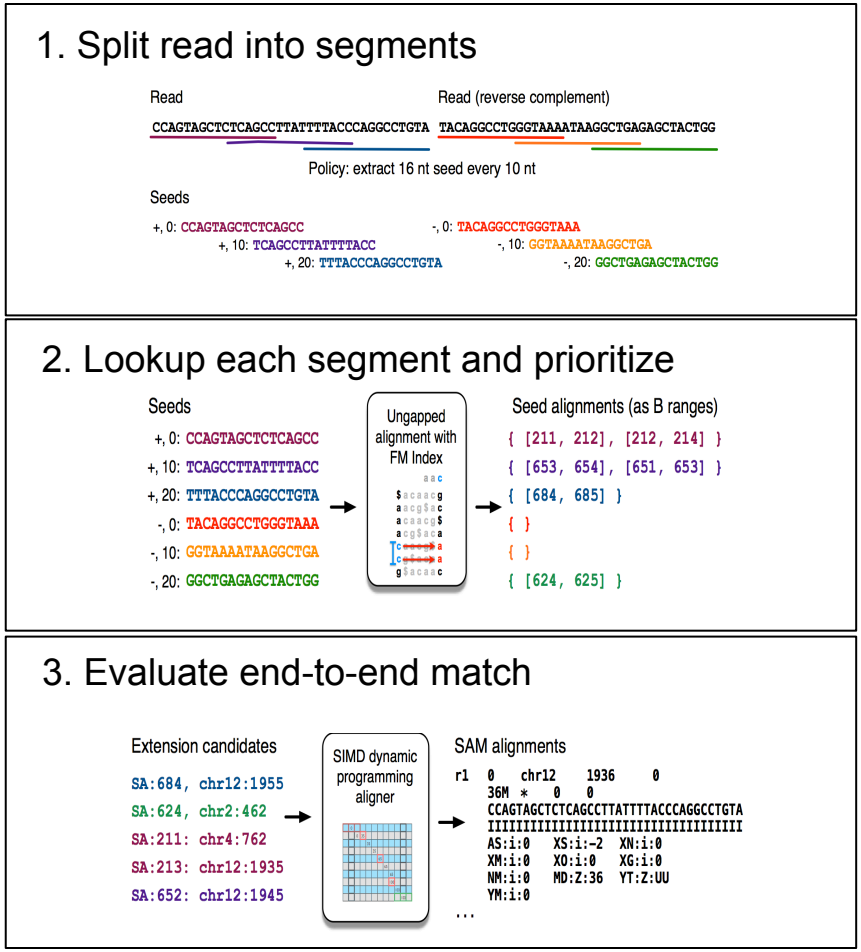


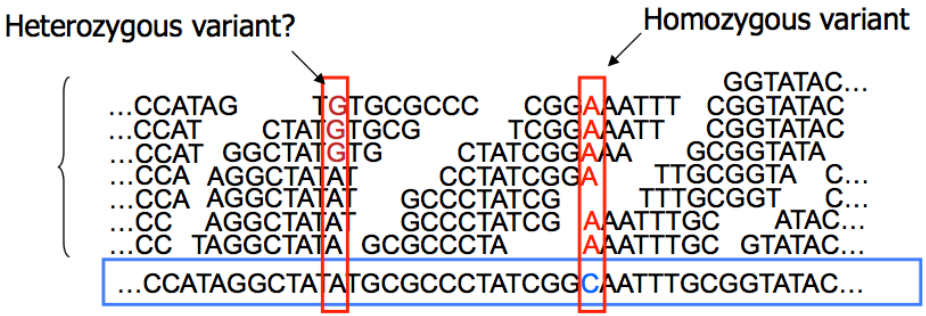
Plant Height

Drought Resistance

Biomass production

Algorithms for Mapping & Genotyping





- Distinguishing SNPs from sequencing error typically a likelihood test of the coverage
  - Hardest to distinguish between errors and heterozygous SNP.
  - Coverage is the most important factor!
    - Target at least 10x, 30x more reliable

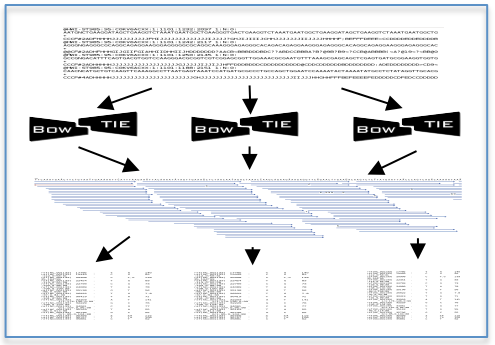
Fast gapped-read alignment with Bowtie 2
Langmead & Salzberg. (2012) Nature Methods. 9:357-359.

The Sequence Alignment/Map format and SAMtools
Li H et al. (2009) Bioinformatics. 25:16 2078-9

Variation Services API 1.0

Genotyping API

- **Bowtie**: Launch alignment task with Bowtie
- **BWA**: Launch alignment task with BWA
- **SNPCalling**: Launch SNPcalling task with SAMTools
- **SortAlignments**: Launch task to sort by chromosome



Data API

- **List**: List files in a directory
- **Fetch**: Fetch files from HDFS
- **Put**: Put files into HDFS
- **RM**: Delete files on HDFS
- **FetchBAM**: On-the-fly conversion to BAM
- **PutFastq**: Put reads into HDFS with conversion

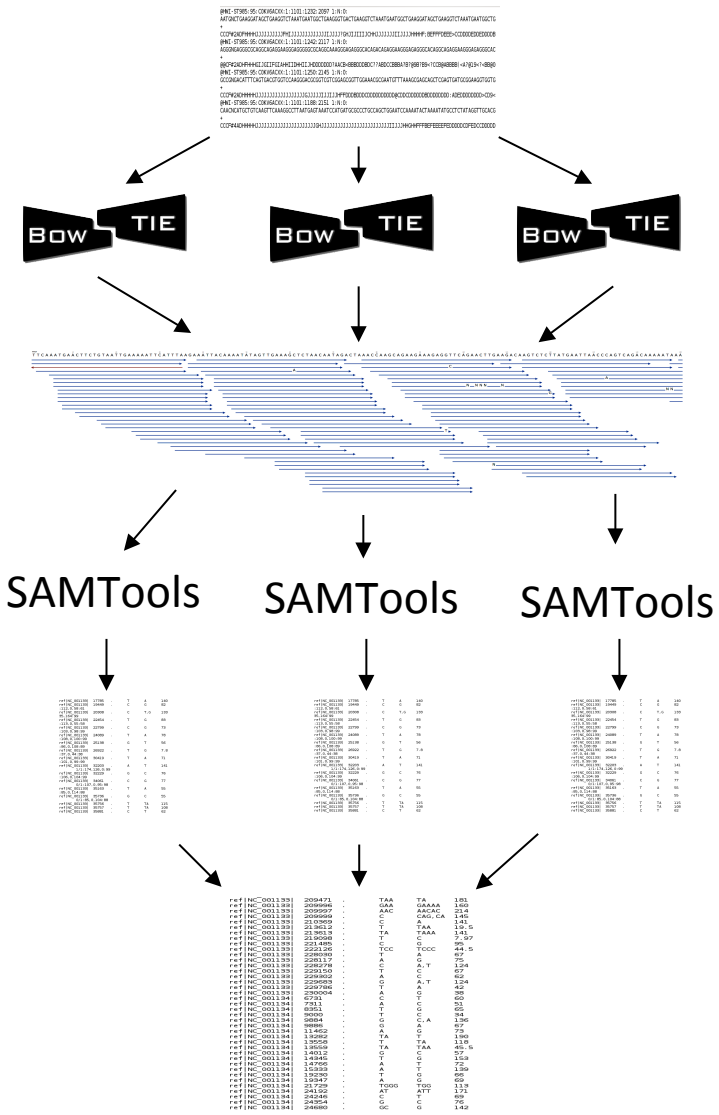
Job API

- **ClusterStatus**: return basic status of cluster (jobs running, nodes available, etc)
- **JobStatus**: Given a JobID, returns current status
- **ListJobs**: List JobID running with a given username
- **KillJob**: Kills a given JobID

Notes:

- All calls are authenticated with KBase username/password

Reads to SNPs in 5 easy steps



1.Identify reference genome

```
$ all_entities_Genome -f scientific_name | grep -i 'Populus'
```

2.Upload Reads to KBase cloud

```
$ jk_fs_put_pe populus.1.fq.gz populus.2.fq.gz populus
```

3.Align Reads with Bowtie2

```
$ jk_compute_bowtie -in=populus.pe \
  -org=populus -out=populus_align
```

4.Call SNPs with SAMTools

```
$ jk_compute_samtools_snp -in=populus_align \
   -org=populus -out=populus_snps
```

5.Merge and Download VCF files

```
$ jk_compute_vcf_merge -in=populus_snps \
   --alignments=populus_align -out=populus.vcf
$ jk_fs_get populus.vcf
```

http://kbase.us/for-users/tutorials/analyzing-data/variation-service/

Maize population analysis

Align & call SNPs from 131 maize samples

1TB fastq / 408Gbp input data

	Serial	KBase cloud (small)	KBase Cloud (large)
Config	1 core (1 node)	210 cores (15 nodes)	854 cores (61 nodes)
Bowtie2	1311 hr*	19.5 hr	5 hr
Sort	58 hr*	N/A	N/A
Samtools	58 hr*	3.5 hr	1.5 hr
End-to-End Speedup	1427 hr* 1x	23 hr 62x	6.5 hr 219x

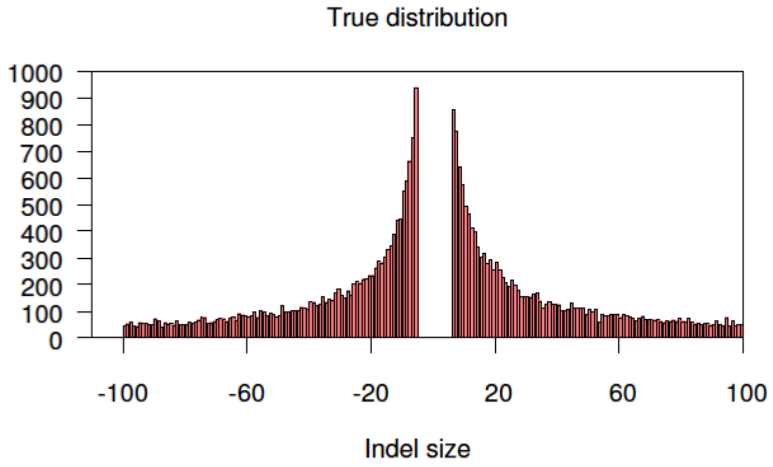
*estimated time

Variation Services 2.0 Sneak peak

SNPs + Short Indels

High precision and sensitivity

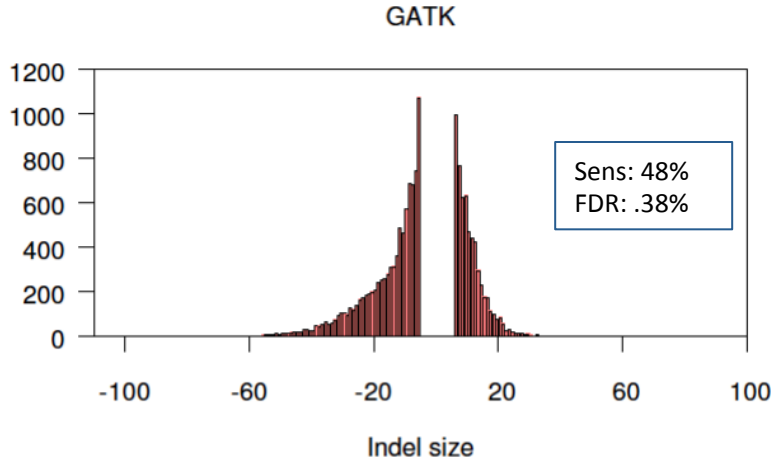
```
..TTTAGAATAG-CGAGTGC...
      ||||||| ||||
      AGAATAGGCGAG
```



"Long" Indels (>5bp)

Reduced precision and sensitivity

```
..TTTAG--------AGTGC...
   |||||         |||||
   TTTAGAATAGGC  |||||
         ATAGGCGAGTGC
```



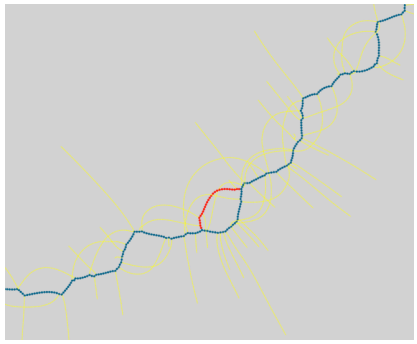
Analysis confounded by sequencing errors, localized repeats, allele biases, and mismapped reads

Scalpel: Haplotype microassembly

DNA sequence **micro-assembly** pipeline for accurate detection and validation of *transmitted* and *de novo* mutations

Features

1. Combine mapping and assembly
2. Exhaustive search of haplotypes
3. De novo mutations

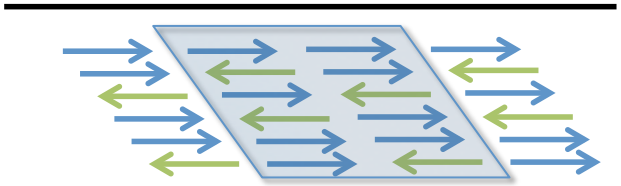


NRXN1 *de novo* SNP
(auSSC12501 chr2:50724605)

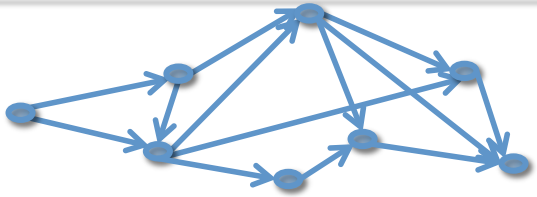
Accurate detection of de novo and transmitted INDELs within exome-capture data using micro-assembly
Narzisi, G et al (2014) *bioRxiv* doi: 10.1101/001370

Algorithm Overview

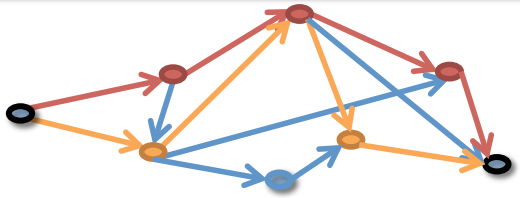
Extract reads mapping within the exon including (1) well-mapped reads, (2) soft-clipped reads, and (3) anchored pairs



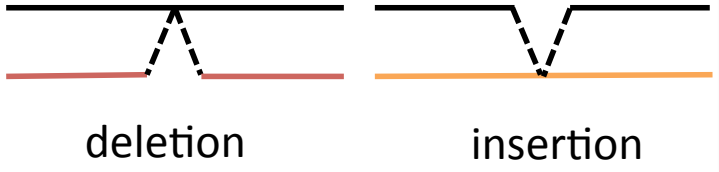
Decompose reads into overlapping *k*-mers and construct de Bruijn graph from the reads



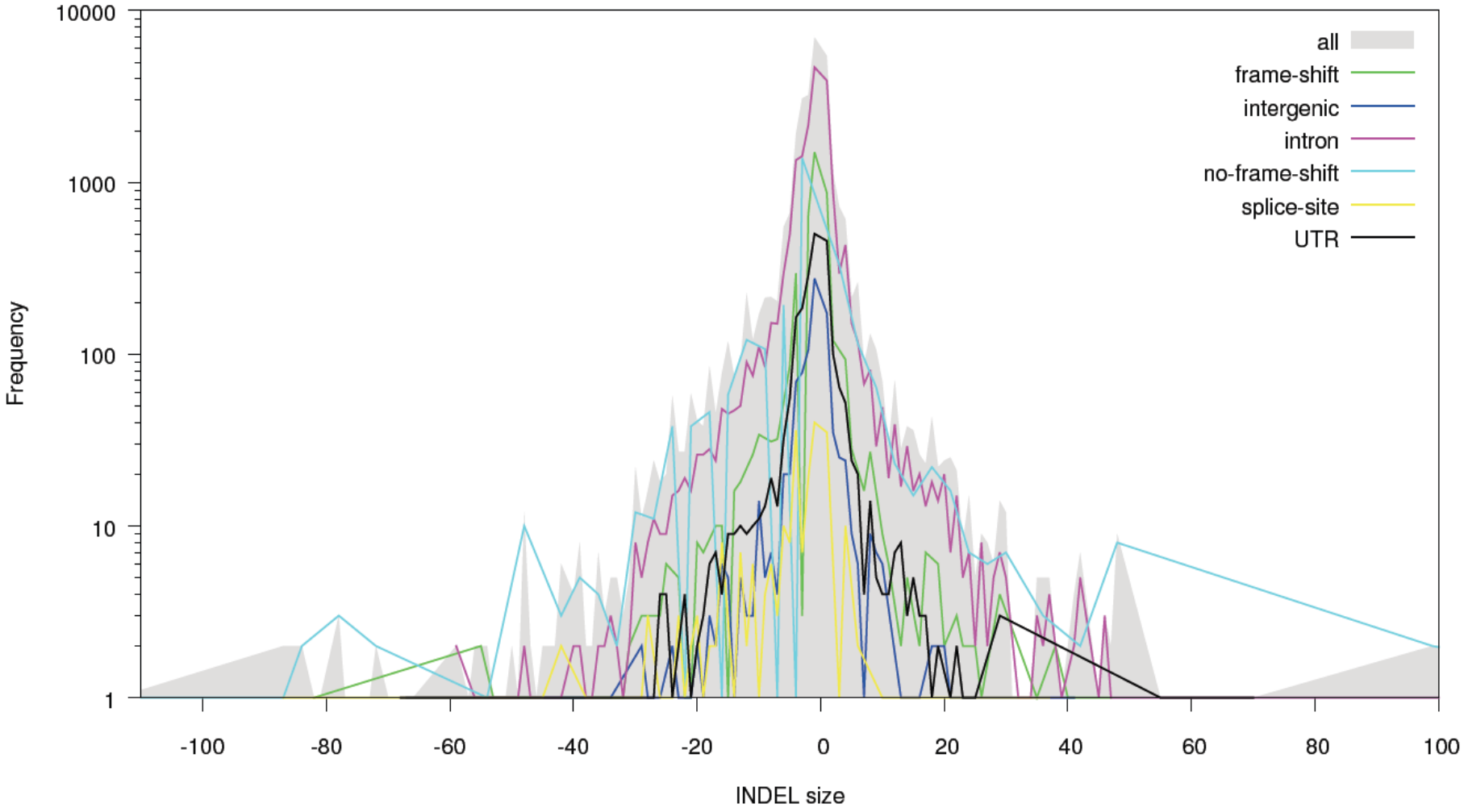
Find end-to-end haplotype paths spanning the region



Align assembled sequences to reference to detect mutations



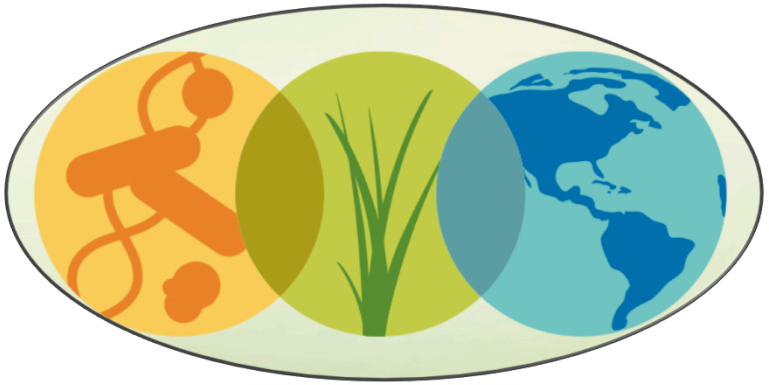
Population Analysis



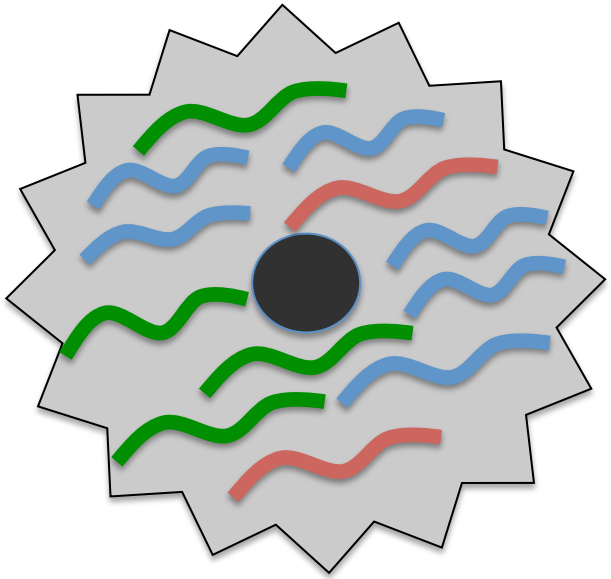
See Ranjan's Talk at 5:40

Agenda

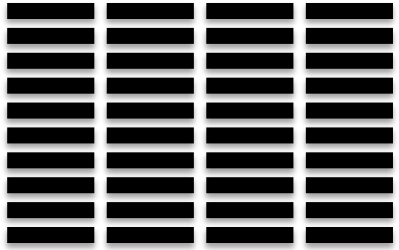
1. Getting Started
2. Variation services
3. RNA-seq services



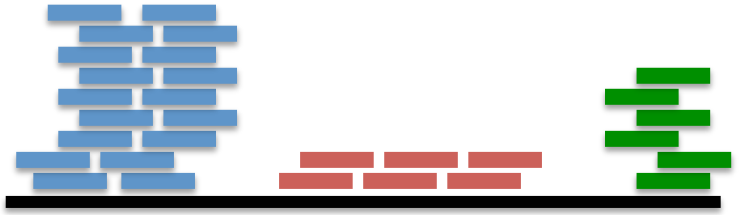
RNA-seq Overview



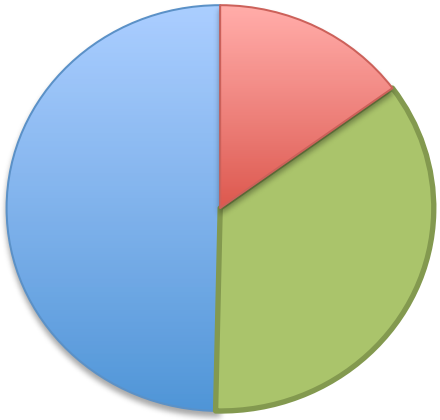
Sequencing



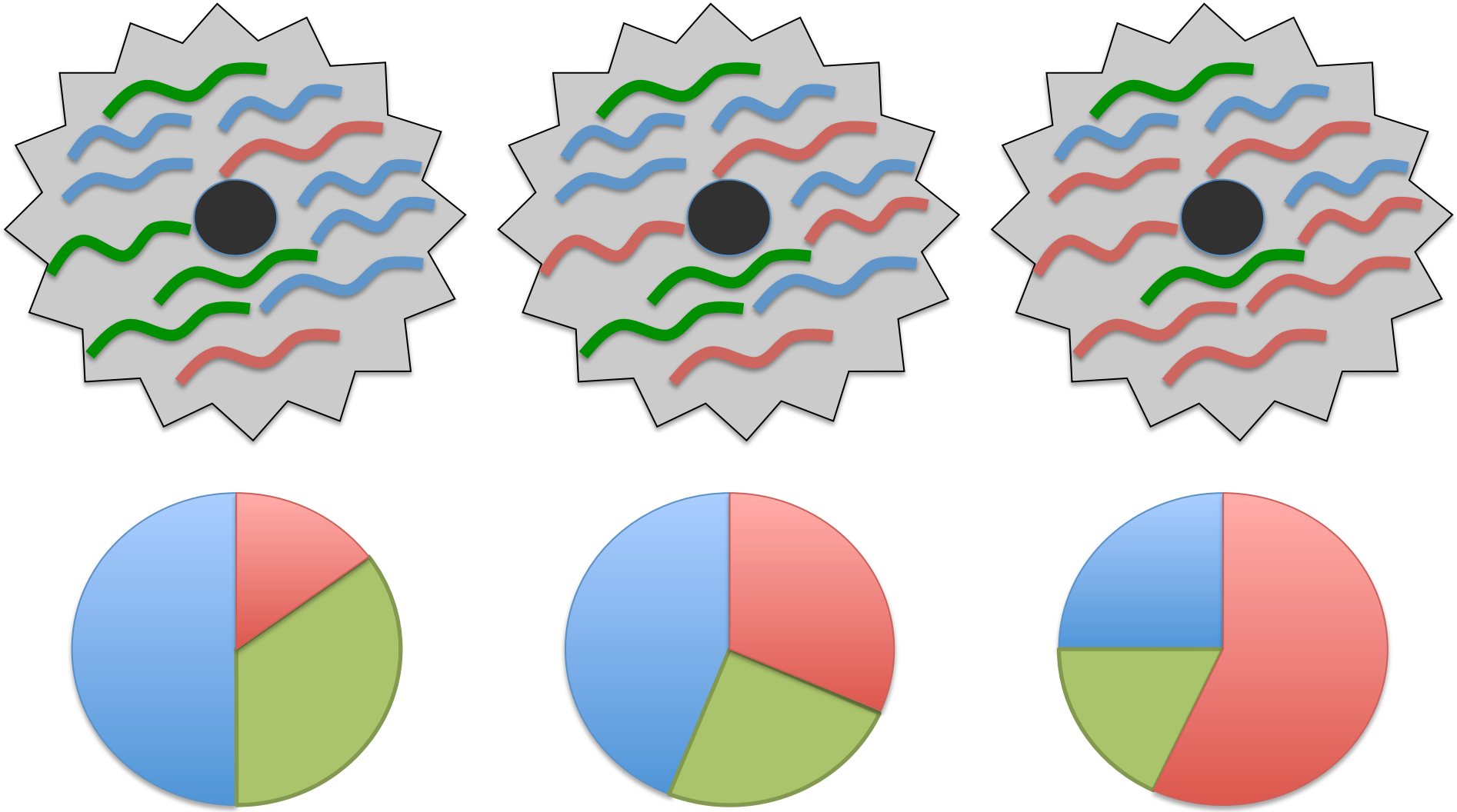
Mapping
& Assembly



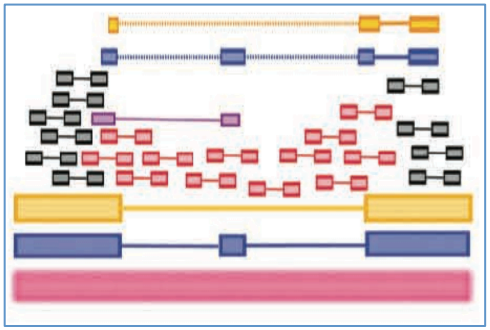
Quantification



RNA-seq Overview



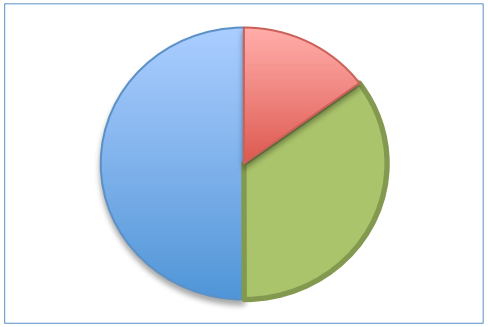
RNA-seq Challenges



Challenge 1: Eukaryotic genes are spliced

Solution: Use a spliced aligner, and assemble isoforms

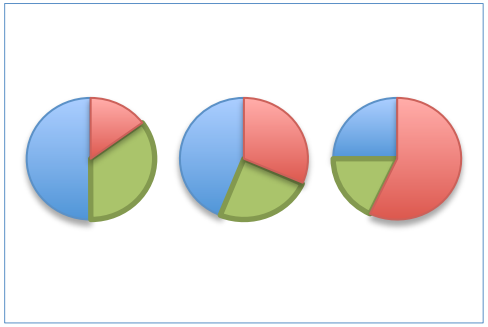
TopHat: discovering spliced junctions with RNA-Seq.
Trapnell et al (2009) *Bioinformatics*. 25:0 1105-1111



Challenge 2: Read Count != Transcript abundance

Solution: Infer underlying abundances (e.g. FPKM)

Transcript assembly and quantification by RNA-seq
Trapnell et al (2010) *Nat. Biotech.* 25(5): 511-515

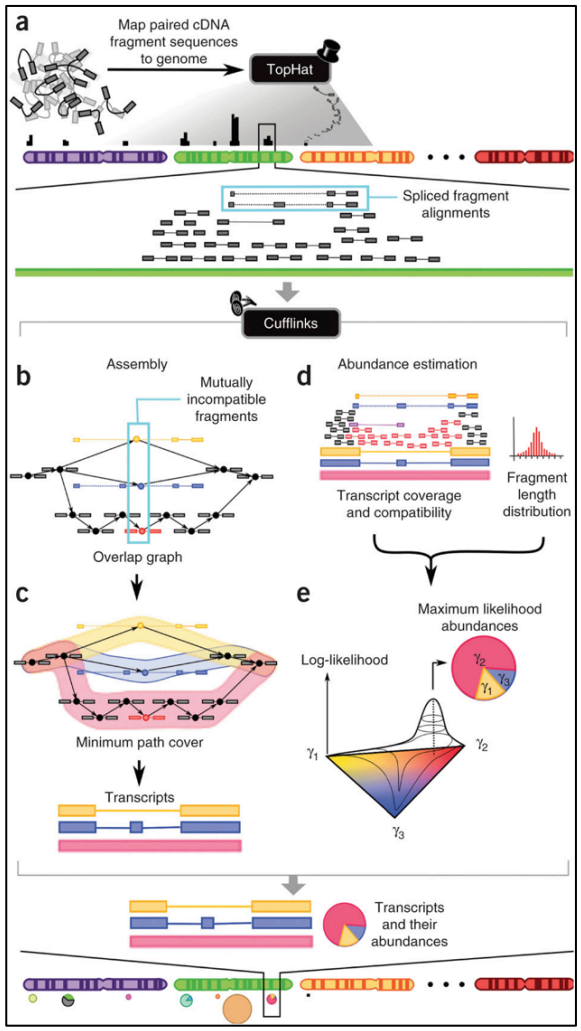


Challenge 3: Transcript abundances are stochastic

Solution: Replicates, replicates, and more replicates

RNA-seq differential expression studies: more sequence or more replication?
Liu et al (2013) *Bioinformatics.* doi:10.1093/bioinformatics/btt688

Identifying Differentially Expressed Genes



1. Spliced alignment with TopHat

$ jk-compute-tophat -in=t1.1.fq.gz,t1.2.fq.gz -ref=ecoli -out=t1-tophat -align_opts=-p8

2. Assemble and quantify expression with Cufflinks

$ jk-compute-cufflinks -in=t1-tophat/accepted_hits.bam -out=t1-cufflinks \
-assembly_opts=-p8

3. Merge samples

$ jk-compute-cuffmerge -in=t1-cufflinks/transcripts.gtf,t2-cufflinks/transcripts.gtf \
-ref=ecoli -out=cuffmerge-out -assembly_opts=-p8

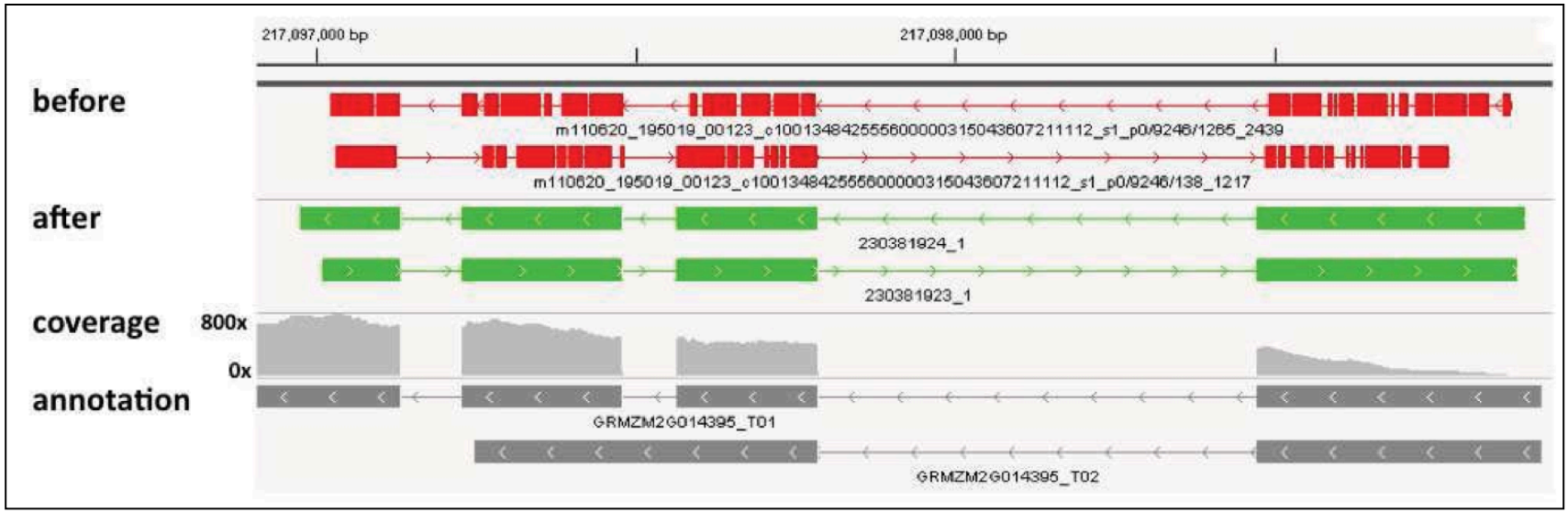
4a. Identify DE genes

$ jk-compute-cuffdiff -in=t1-tophat/accepted_hits.bam,t2-tophat/accepted_hits.bam \
-out=cuffdiff-out -ref=ecoli -assembly_opts=-p8 -condn_labels=T1,T2 \
-merged_gtf=cuffmerge-out/merged.gtf

4b. Discover novel genes & isoforms

$ jk-compute-cuffcompare -in=t1-cufflinks/transcripts.gtf,t2-cufflinks/transcripts.gtf \
-out=cuffcompare-out -ref_gtf=cuffmerge-out/merged.gtf

RNA-seq 2.0: Long Read Analysis



- Long-read single-molecule sequencing has potential to directly sequence full length transcripts
  - Error corrected reads almost perfectly match the genome, pinpointing splice sites, identifying alternative splicing

Hybrid error correction and de novo assembly of single-molecule sequencing reads.
Koren, S, Schatz, MC, et al. (2012) *Nature Biotechnology*. doi:10.1038/nbt.2280

Additional Resources

Resource	URL
KBase	http://kbase.us/
Getting Started	http://kbase.us/for-users/user-home/
Variation Services	http://kbase.us/for-users/tutorials/analyzing-data/variation-service/
RNA-seq Services	http://kbase.us/for-users/tutorials/analyzing-data/plant-genome-analysis/plant-expression-service/
Bowtie2	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
BWA	http://bio-bwa.sourceforge.net/
SAMTools	http://samtools.sourceforge.net/
Cufflinks	http://cufflinks.cbcb.umd.edu/
KBase Contact	http://kbase.us/contact-us/
Survey	https://www.surveymonkey.com/s/KB-user-info

Thank you!

http://schatzlab.cshl.edu
@mike_schatz / @DOEKBase

Office of Biological and Environmental Research
U.S. DEPARTMENT OF ENERGY
Office of Science
KBase PREDICTIVE BIOLOGY
DOE Systems Biology Knowledgebase