Genome Annotations

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Nov 15, 2014
Adv Sequencing Course

Goal: Genome Annotations

aatgcatgcggctatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaa
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Goal: Genome Annotations

aatgcatgcggctatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaa
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Gene!

Outline

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

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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
```

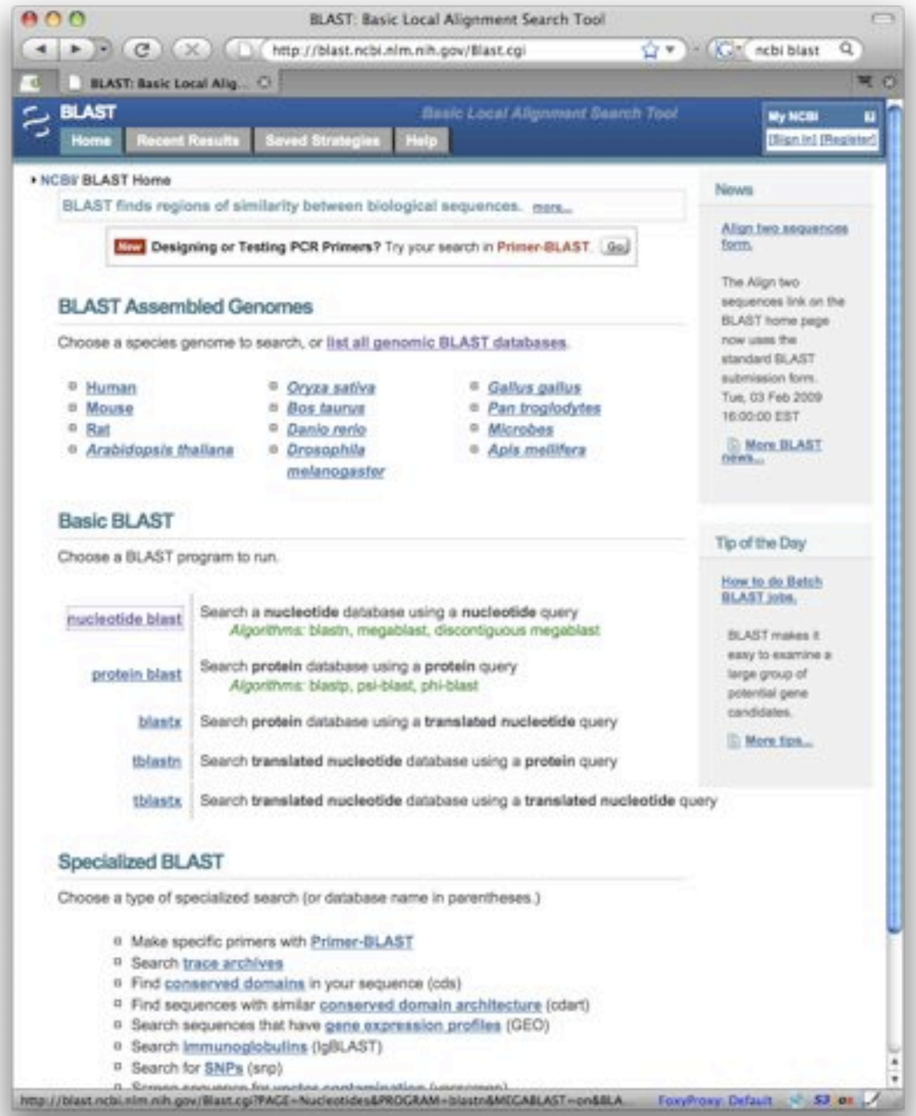
Not similar sequences

```
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 in to protein
TBLASTN	Nucleotide translated in to protein	Protein
TBLASTX	Nucleotide translated in to protein	Nucleotide translated in to protein

NCBI Blast



- Nucleotide Databases
  - nr: All Genbank
  - refseq: Reference organisms
  - wgs: All reads

- Protein Databases
  - nr: All non-redundant sequences
  - Refseq: Reference proteins

Genomic Coordinates

What are coordinates of "TAC" in GATTACA?

1-based coordinates

- Base 4 through 6: [4,6] "closed"
- Base 4 through 7: [4,7) "half-open"
- 3 bases starting at base 4: [4, +3]

```
GATTACA
1234567
```

0-based coordinates

- Position 3 through 5: [3,5] "closed"
- Position 3 through 6: [3,6) "half-open"
- 3 bases starting at position 3: [3, +3]

```
GATTACA
0123456
```

Genomic Conventions

1-based coordinates
- BLAST/MUMmer alignments
- Ensembl Genome Browser
- SAM, VCF, GFF and Wiggle

```
GATTACA
1234567
```

0-based coordinates
- BAM, BCFv2, BED, and PSL
- UCSC Genome Browser
- C/C++, Perl, Python, Java

```
GATTACA
0123456
```

Always double check the manual!
You will get this wrong someday ☹

Outline

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

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).

Start codon

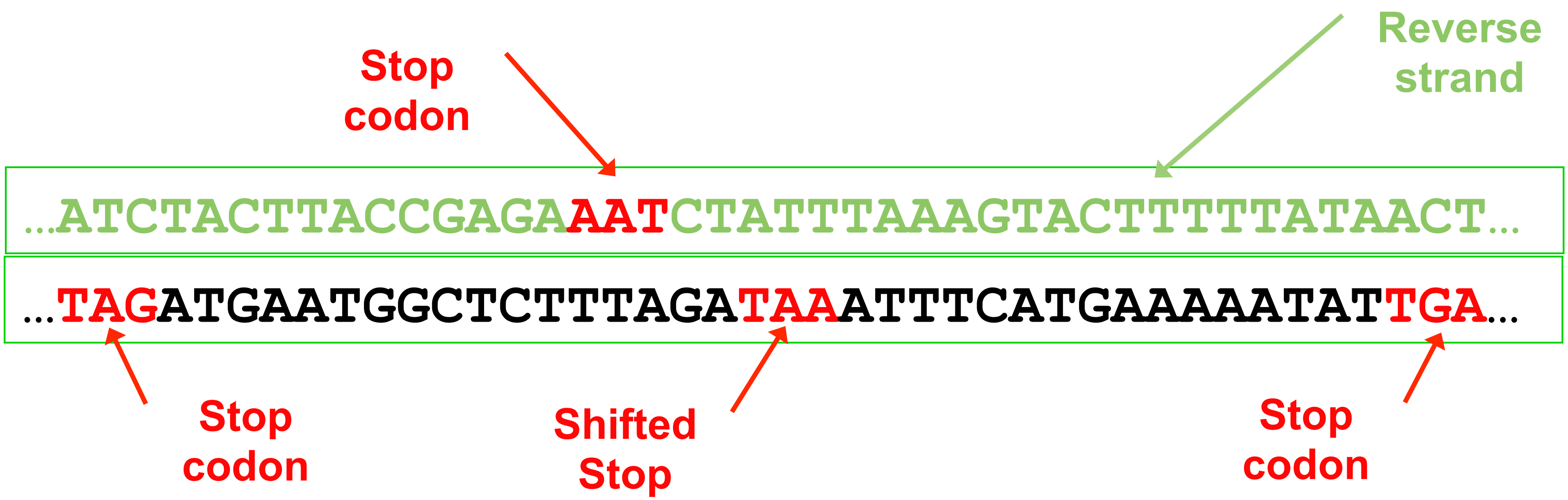
...TAGATGAATGGCTCTTTAGATAAATTTCATGAAAAATATTGA...

Stop codon

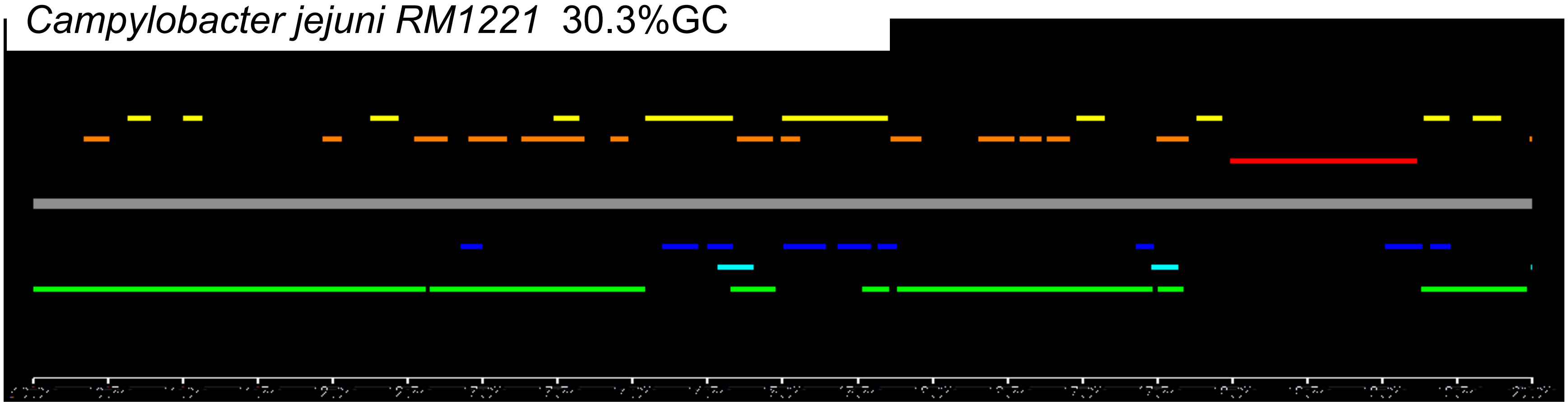
Stop codon

Step One

- Find open reading frames (ORFs).



- But ORFs generally overlap …

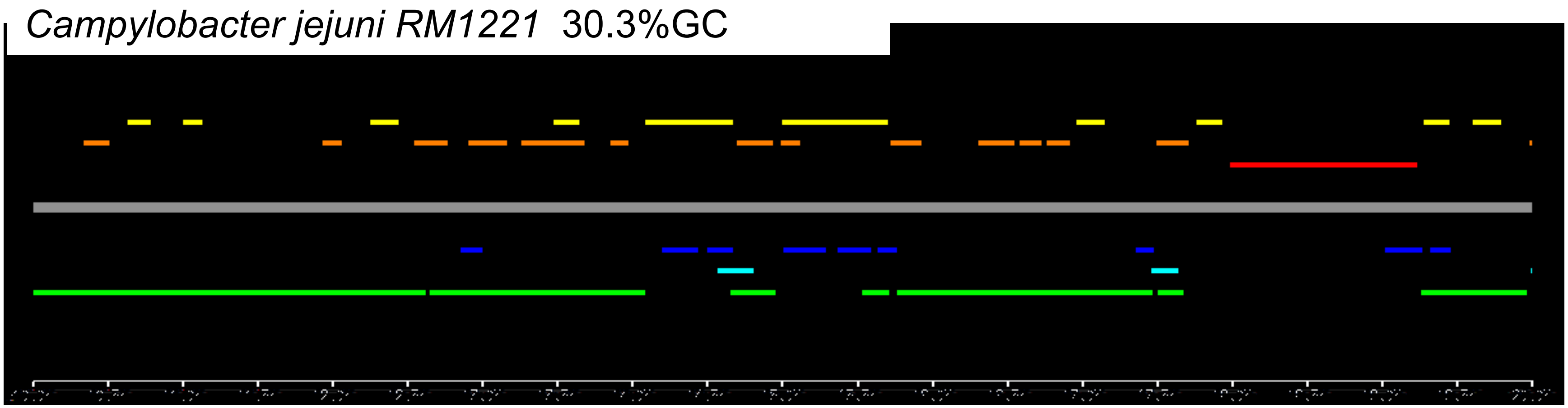


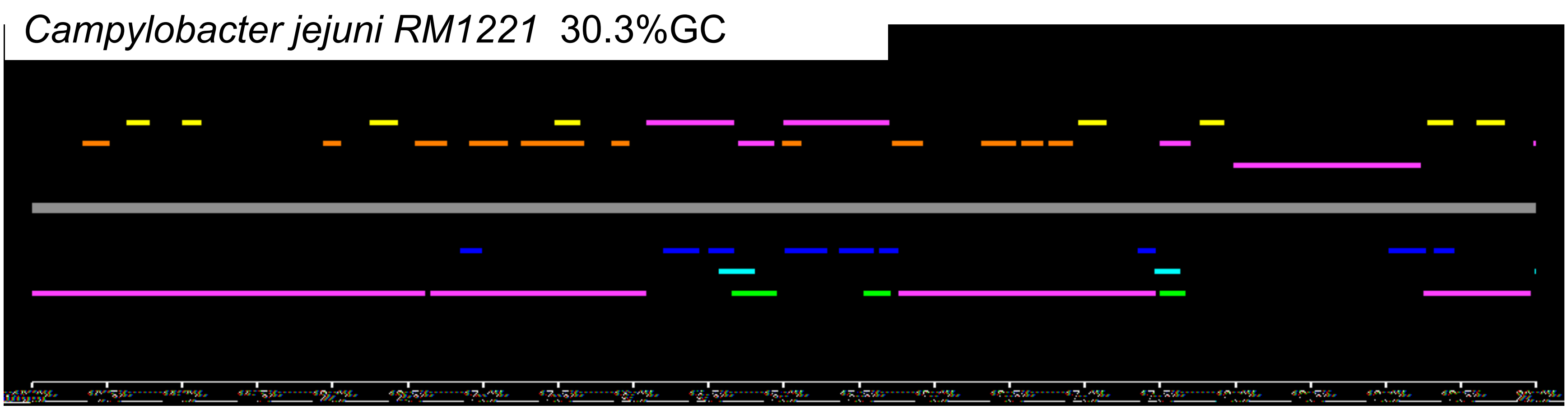
All ORFs longer than 100bp on both strands shown
- color indicates reading frame

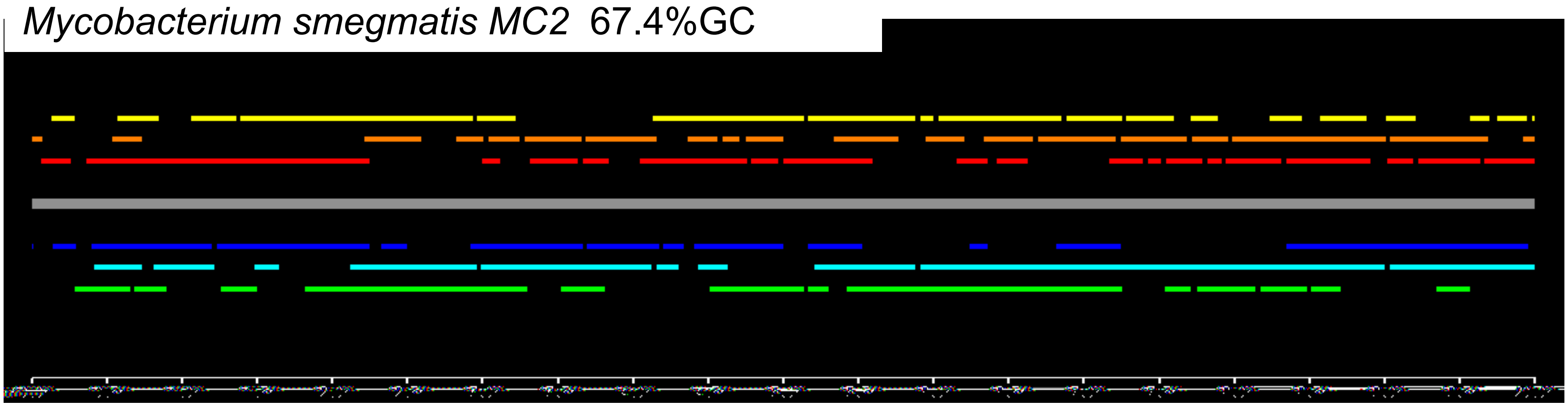
Longest ORFs likely to be protein-coding genes

Note the low GC content

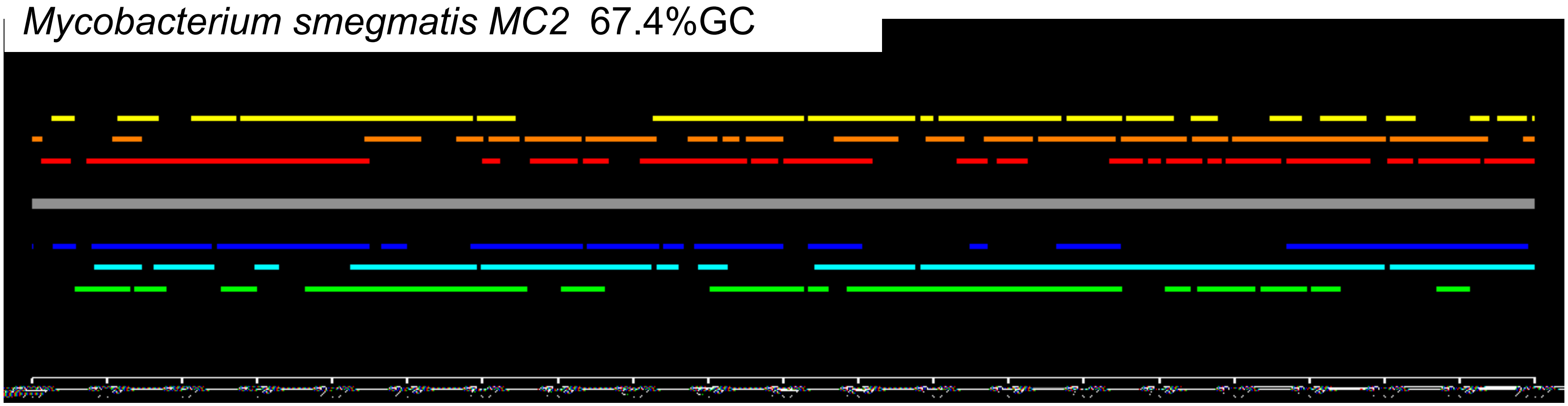
All genes are ORFs but not all ORFs are genes

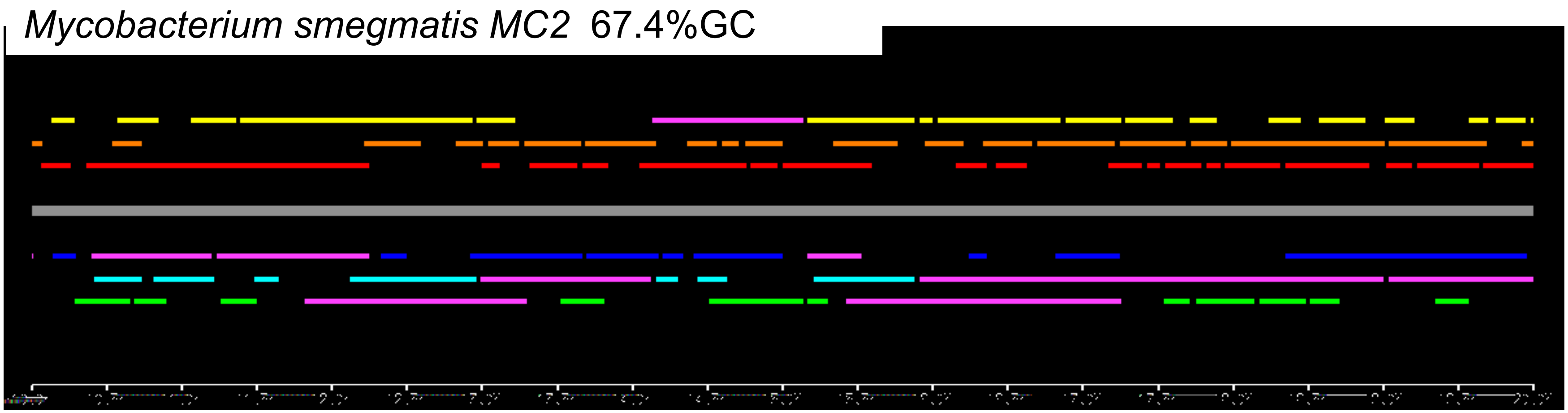

Campylobacter jejuni RM1221 30.3%GC


Campylobacter jejuni RM1221 30.3%GC



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

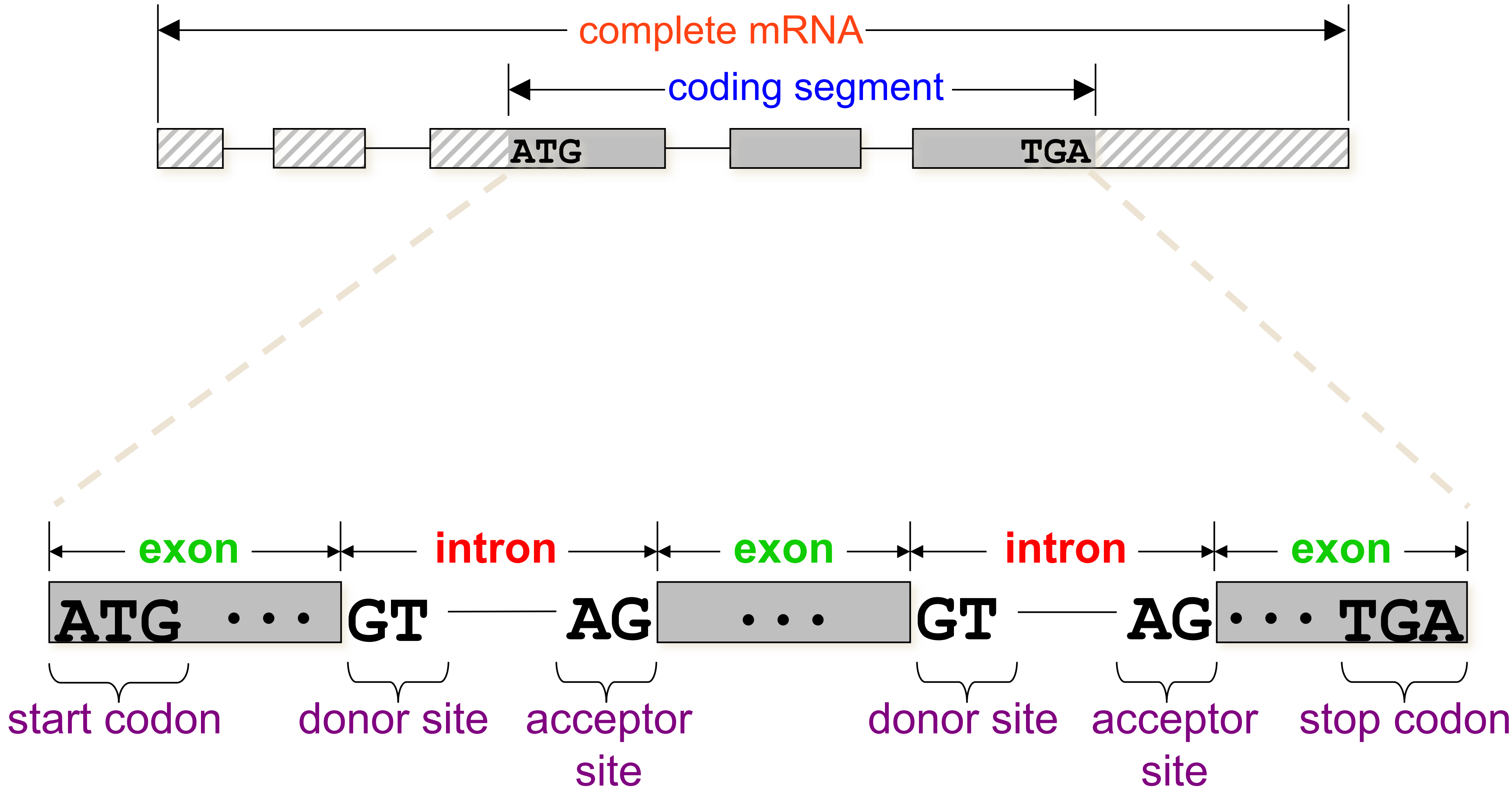
Overview of Eukaryotic Gene Prediction

CBB 231 / COMPSCI 261

W.H. Majoros

Duke UNIVERSITY

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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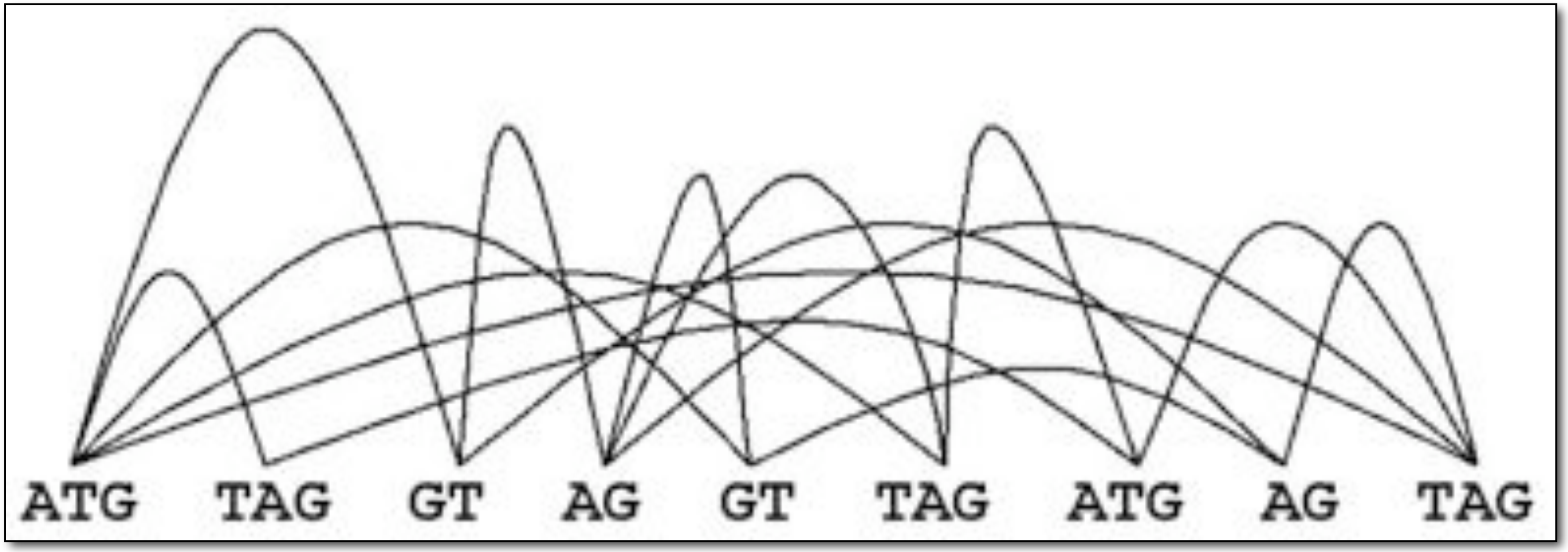
Representing Gene Syntax with ORF Graphs

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*:

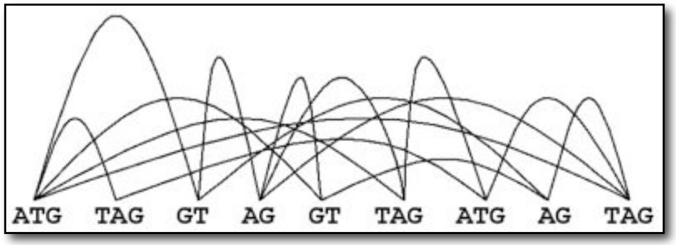


An ORF graph represents all possible *gene parses* (and their scores) for a given set of putative signals. A *path* through the graph represents a single gene parse.

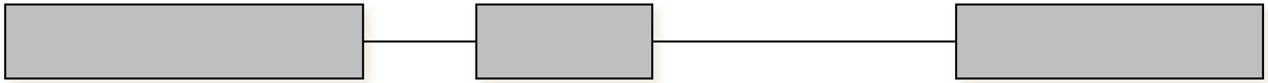
Conceptual Gene-finding Framework

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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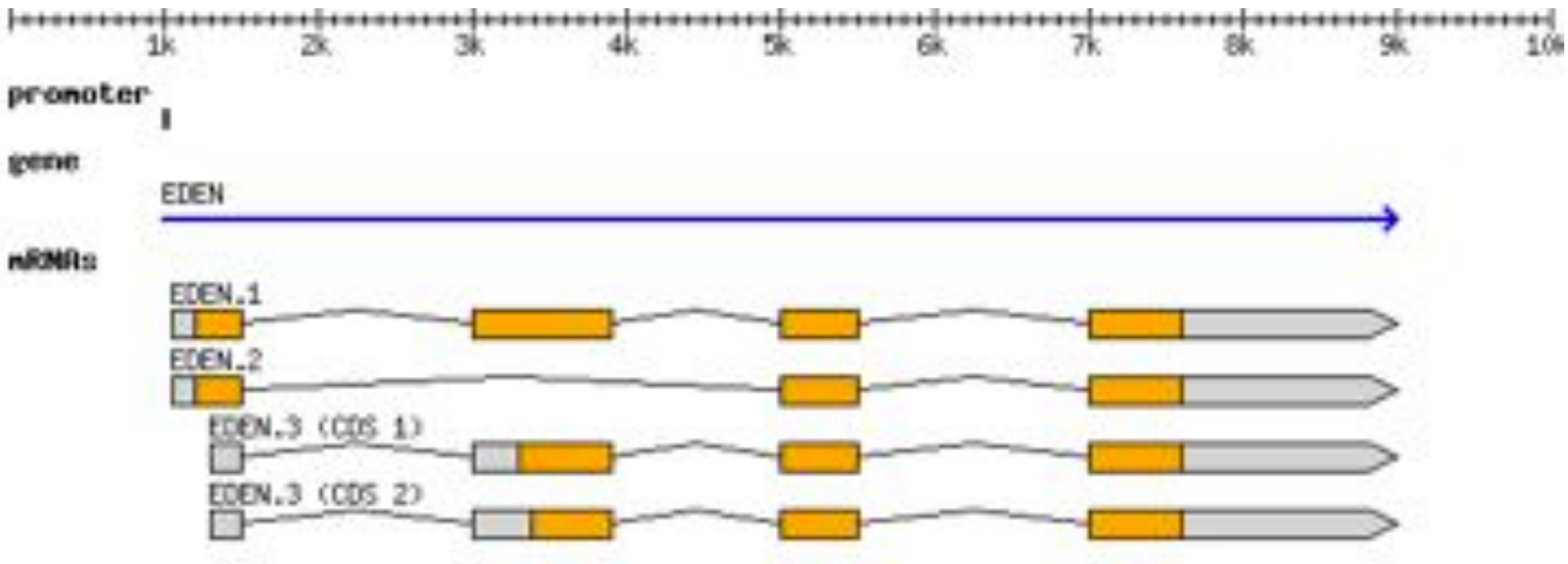
Gene Finding Overview

- 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

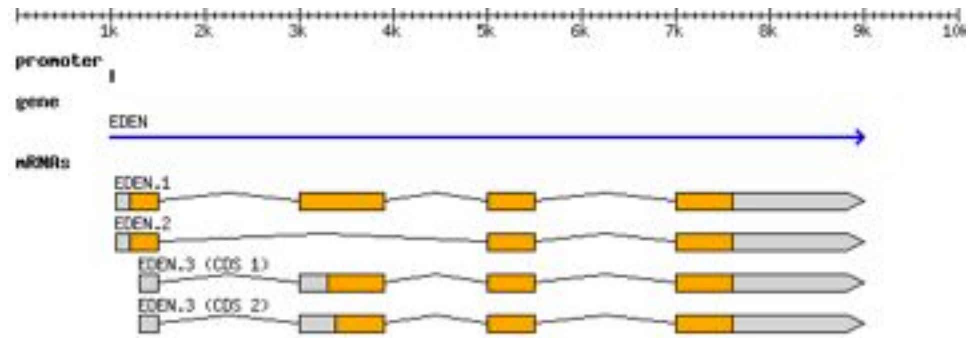
8. phase: For "CDS", where the feature begins with reference to the reading frame (0,1,2)

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
```

Break

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2. Prediction aka “Gene Finding”
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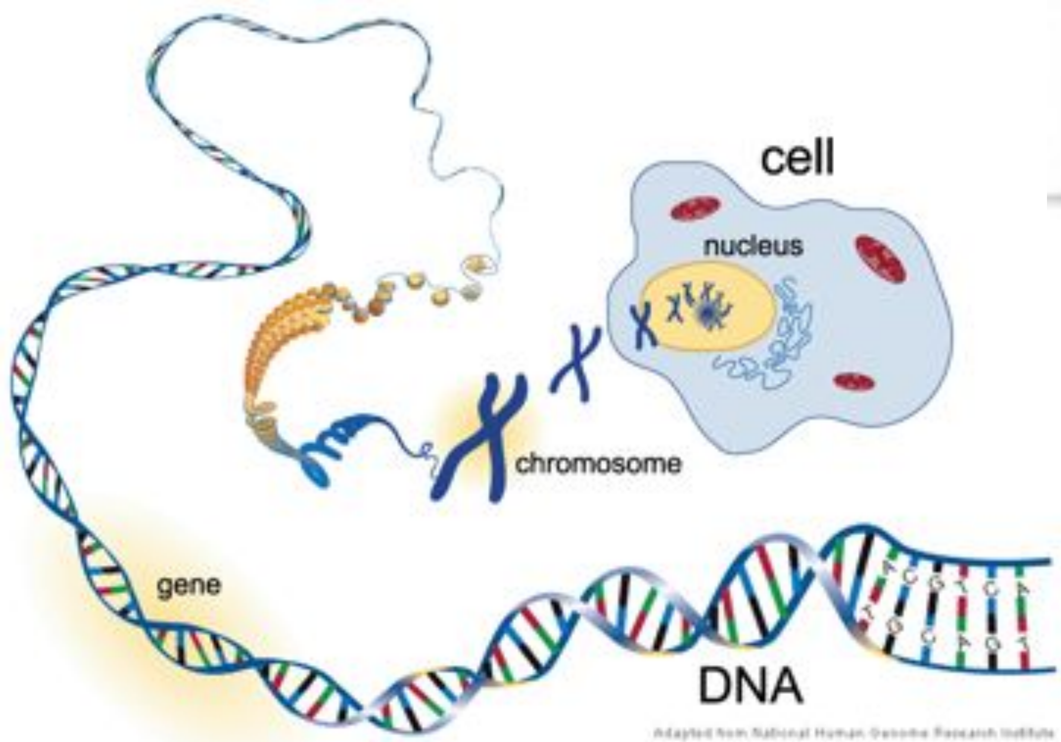
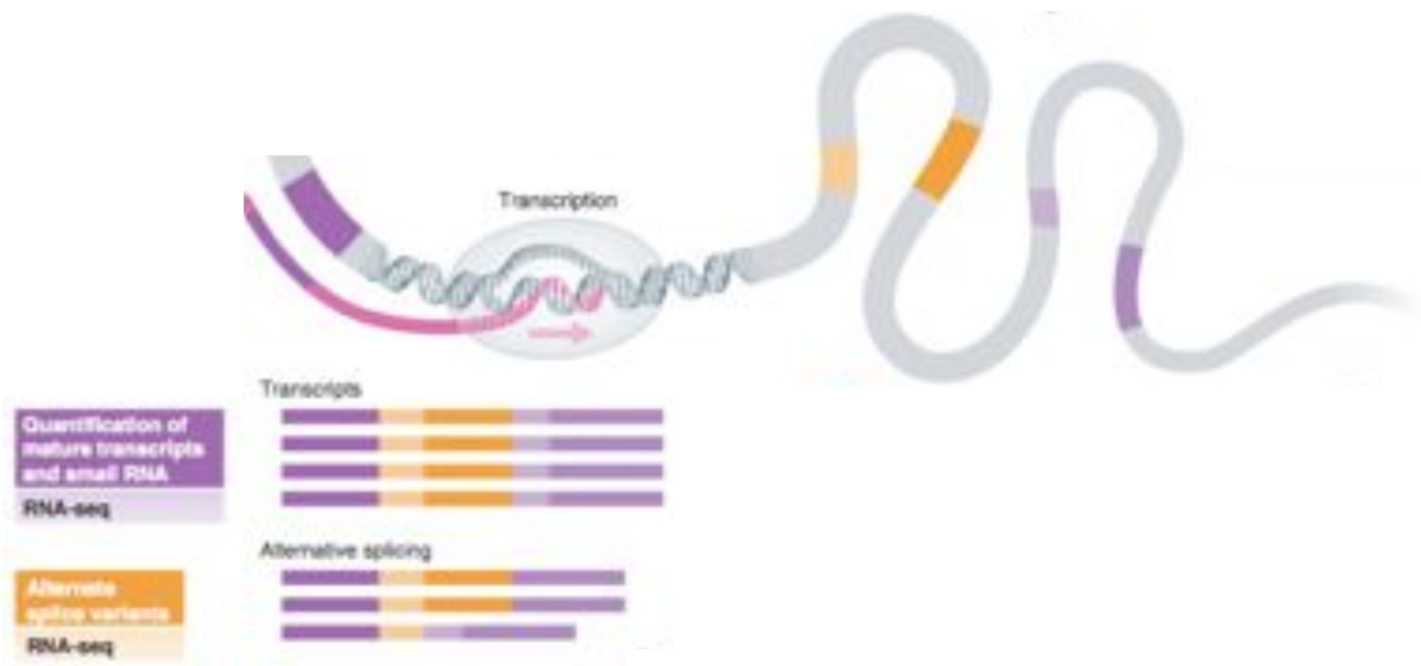
Sequencing techniques

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), doi:10.1101/gr.

What is a *Seq assay?

Molecular Biology

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

Creativity

Chemistry & Physics

Statistics

Mathematics & Computer Science

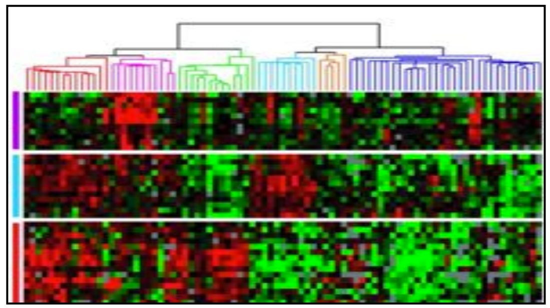
Biology

Computational Biology

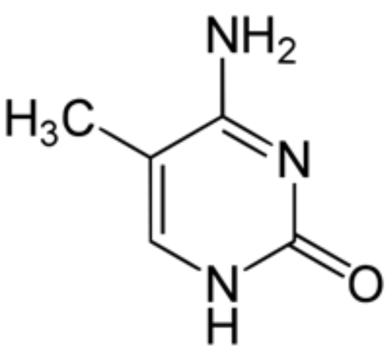
$$\mathbb{P}(f = (p,t,l)) \approx \frac{\lambda_l \cdot \frac{\tau_t}{\tilde{l}(t)} \cdot w_{p|t,l} \cdot \phi_{f|p,t,l}}{\sum_{(q,r,m)\in\mathcal{A}(f)} \lambda_m \cdot \frac{\tau_r}{\tilde{l}(r)} \cdot w_{q|r,m} \cdot \phi_{f|q,r,m}}$$

*-seq in 3 short vignettes

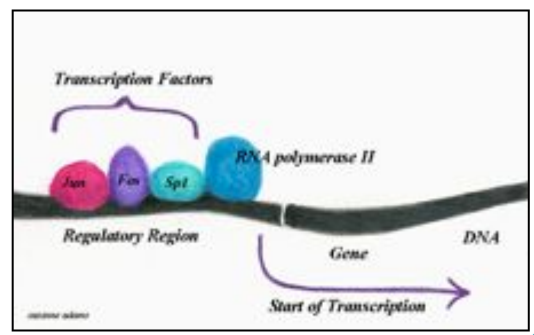
RNA-seq



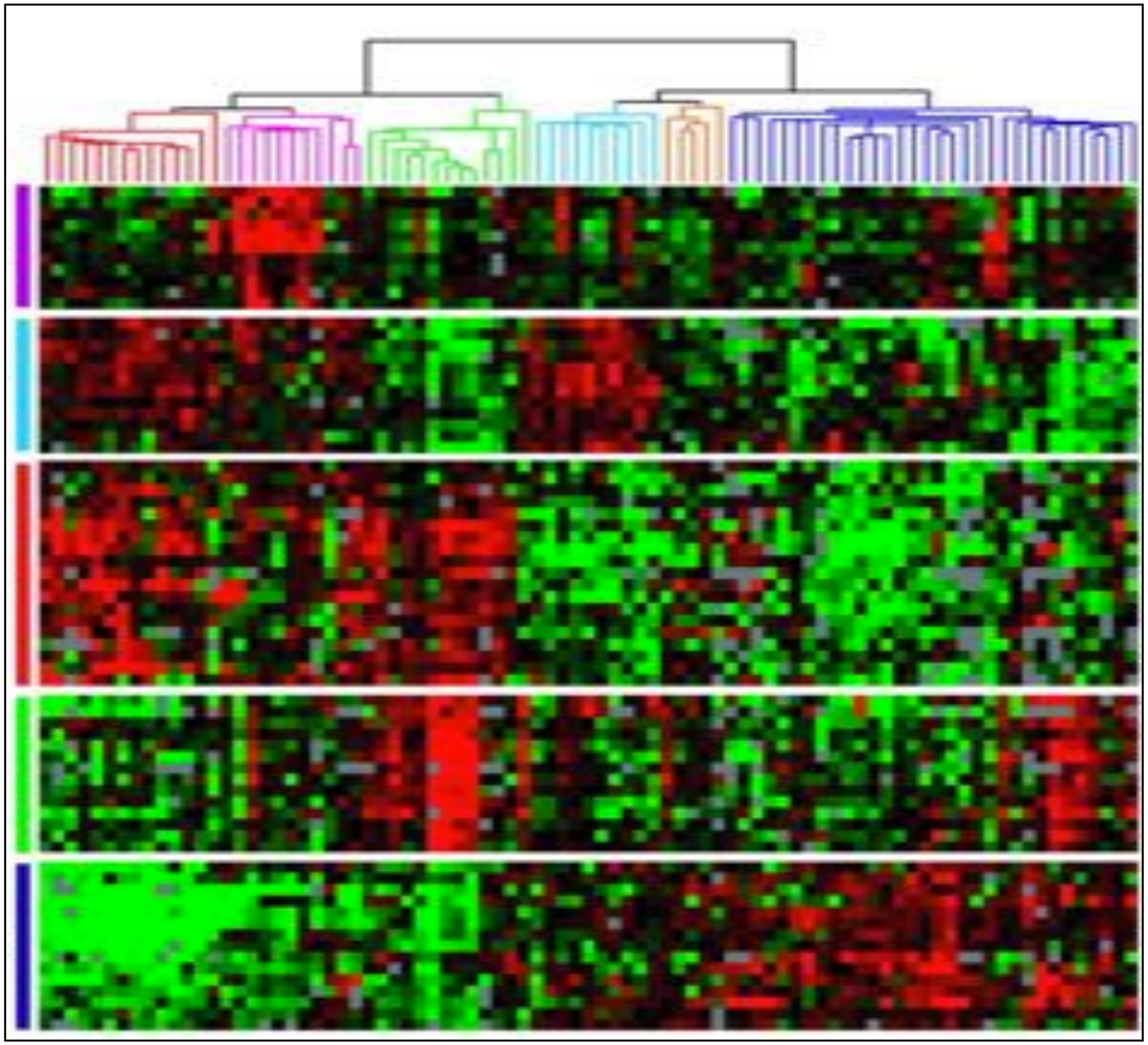
Methyl-seq



ChIP-seq

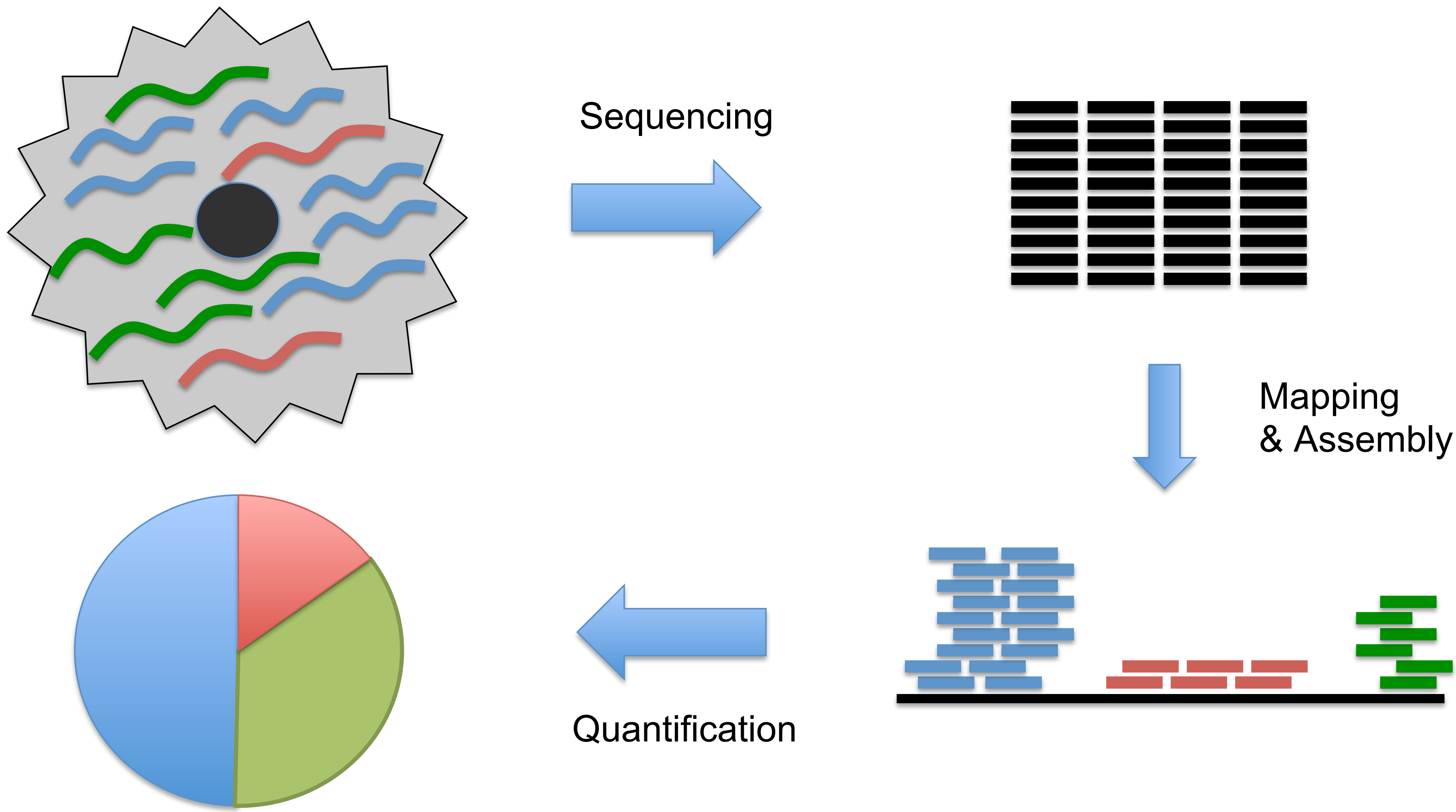


RNA-seq

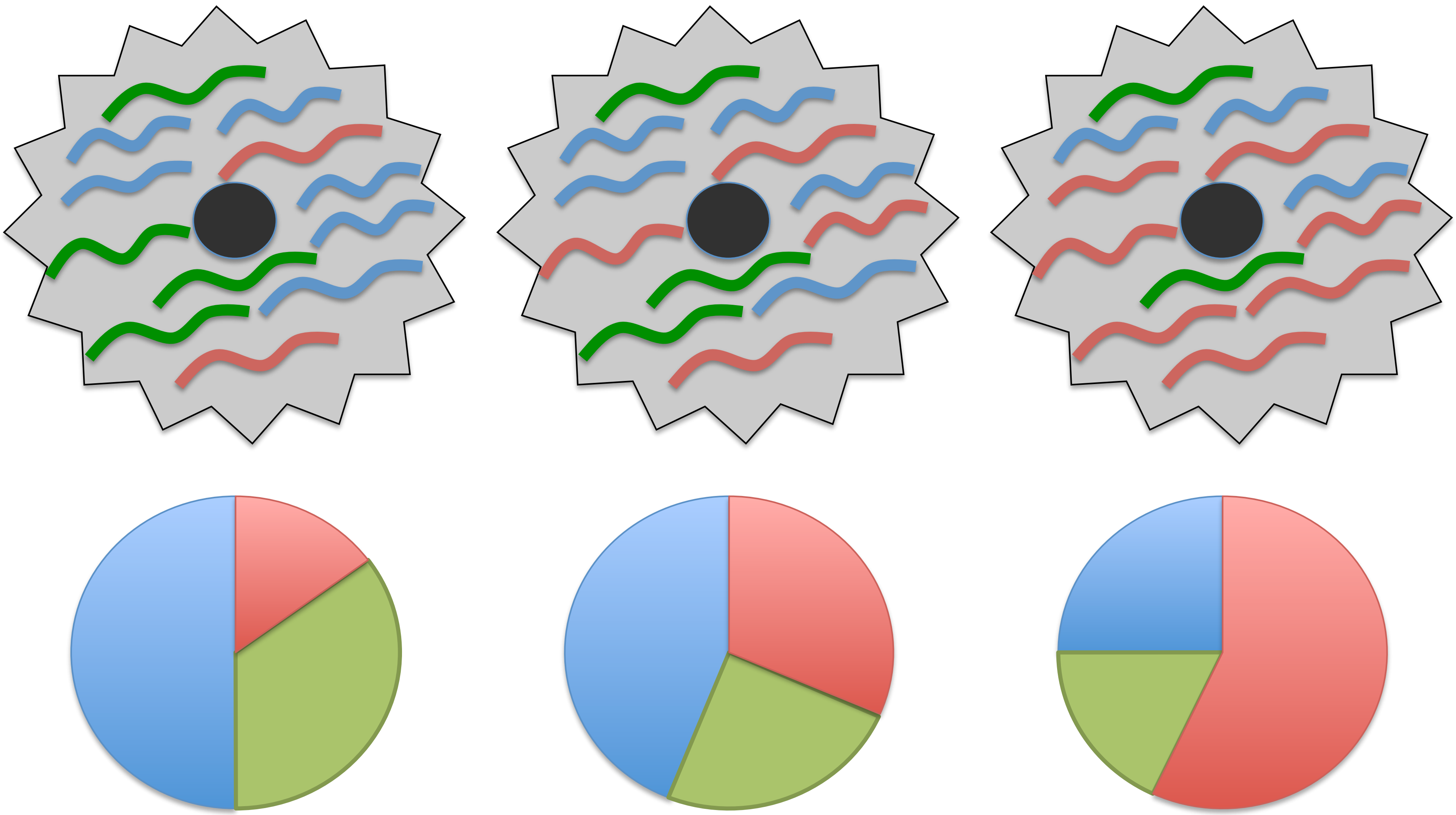


Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.
Sørlie et al (2001) *PNAS*. 98(19):10869-74.

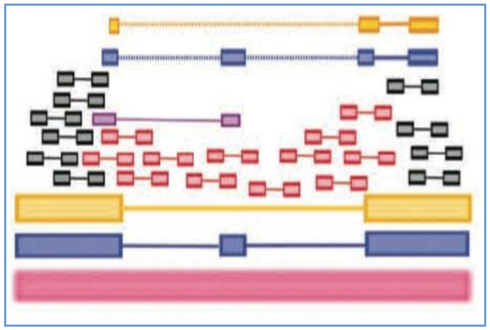
RNA-seq Overview



RNA-seq Overview



RNA-seq Challenges

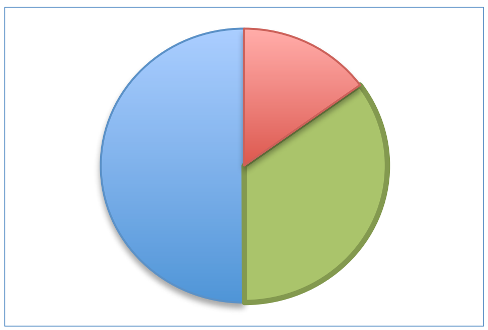


Challenge 1: Eukaryotic genes are spliced

Solution: Use a spliced aligner, and assemble isoforms

TopHat: discovering spliced junctions with RNA-Seq.

Trapnell et al (2009) *Bioinformatics*. 25:0 1105-1111

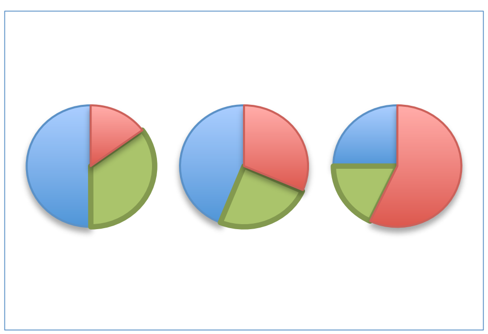


Challenge 2: Read Count != Transcript abundance

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?

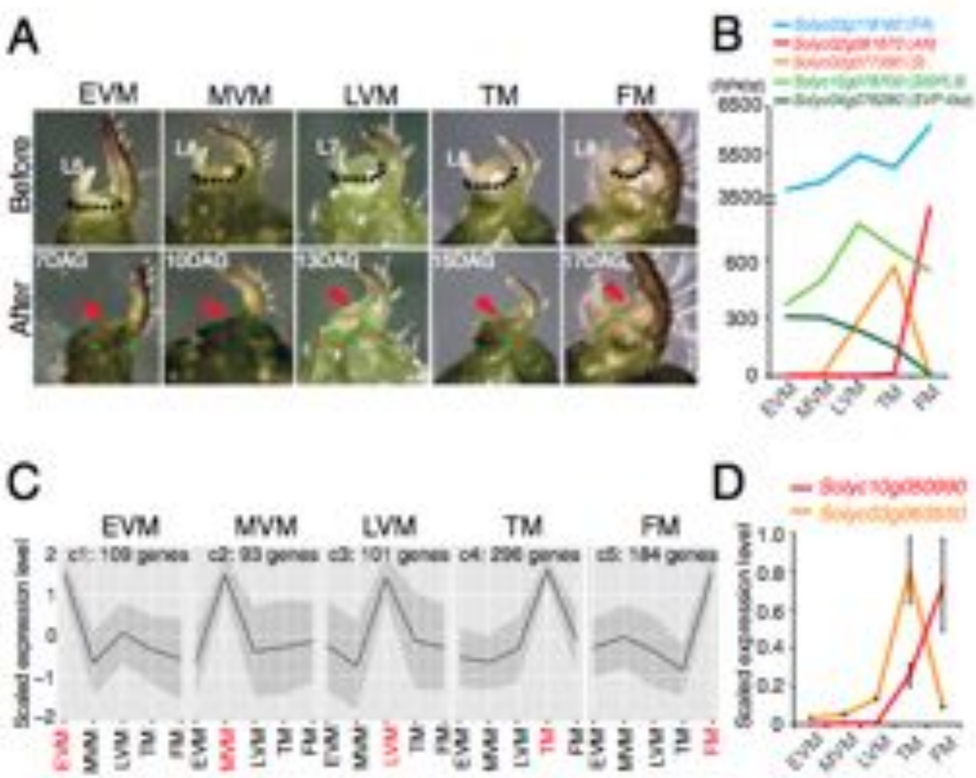
Liu et al (2013) *Bioinformatics*. doi:10.1093/bioinformatics/btt688

Rate of meristem maturation determines inflorescence architecture in tomato

Soon Ju Park[1], Ke Jiang[1], Michael C. Schatz, and Zachary B. Lippman[2]

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

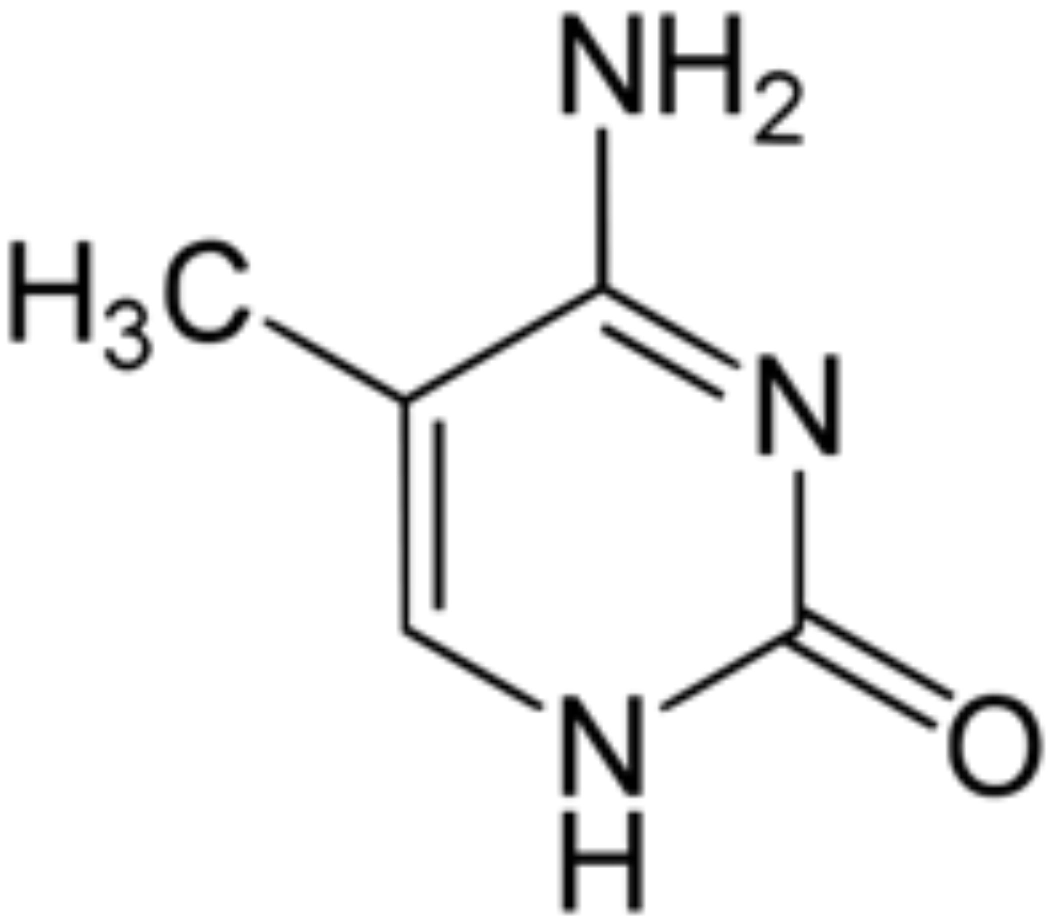
Edited by Maarten Koornneef, Wageningen University and Research Centre, Cologne, Germany, and approved November 28, 2011 (received for review September 12, 2011)



RNA-seq to determine the expression dynamics during development

- Laser microdissection to precisely extract tissue from developing organs
- Use RNA-seq to watch different classes of genes become activated at different stages of development
- When those genes are delayed or interupted, tomato mutants take on very different branching patterns.

Methyl-seq



Finding the fifth base: Genome-wide sequencing of cytosine methylation
Lister and Ecker (2009) *Genome Research.* 19: 959-966

OPEN ACCESS Freely available online PLoS BIOLOGY

The Honey Bee Epigenomes: Differential Methylation of Brain DNA in Queens and Workers

Frank Lyko[1¶], Sylvain Foret[2¶], Robert Kucharski[3], Stephan Wolf[4], Cassandra Falckenhayn[1], Ryszard Maleszka[3*]

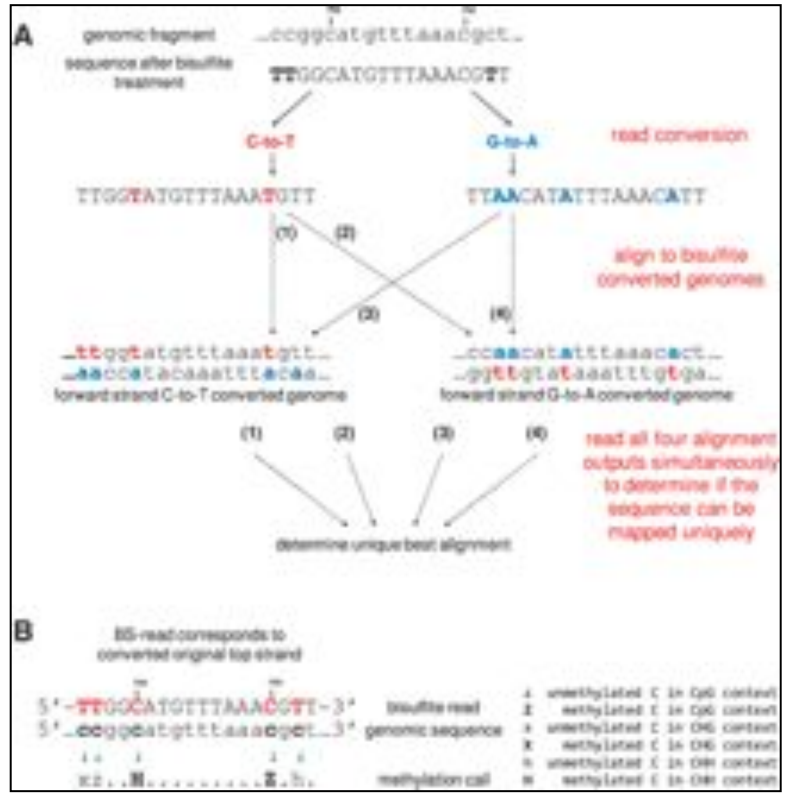
1 Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center, Heidelberg, Germany, 2 ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Australia, 3 Research School of Biology, the Australian National University, Canberra, Australia, 4 Genomics and Proteomics Core Facility, German Cancer Research Center, Heidelberg, Germany

"The queen honey bee and her worker sisters do not seem to have much in common. Workers are active and intelligent, skillfully navigating the outside world in search of food for the colony. They never reproduce; that task is left entirely to the much larger and longer-lived queen, who is permanently ensconced within the colony and uses a powerful chemical influence to exert control. Remarkably, these two female castes are generated from identical genomes. The key to each female's developmental destiny is her diet as a larva: future queens are raised on royal jelly. This specialized diet is thought to affect a particular chemical modification, methylation, of the bee's DNA, causing the same genome to be deployed differently."

Bisulfite Conversion

Treating DNA with sodium bisulfite will convert <u>un</u>methylated C to T

- 5-MethyC will be protected and not change, so can look for differences when mapping
- Requires great care when analyzing reads, since the complementary strand will also be converted (G to A)
- Typically analyzed by mapping to a "reduced alphabet" where we assume all Cs are converted to Ts once on the forward strand and once on the reverse

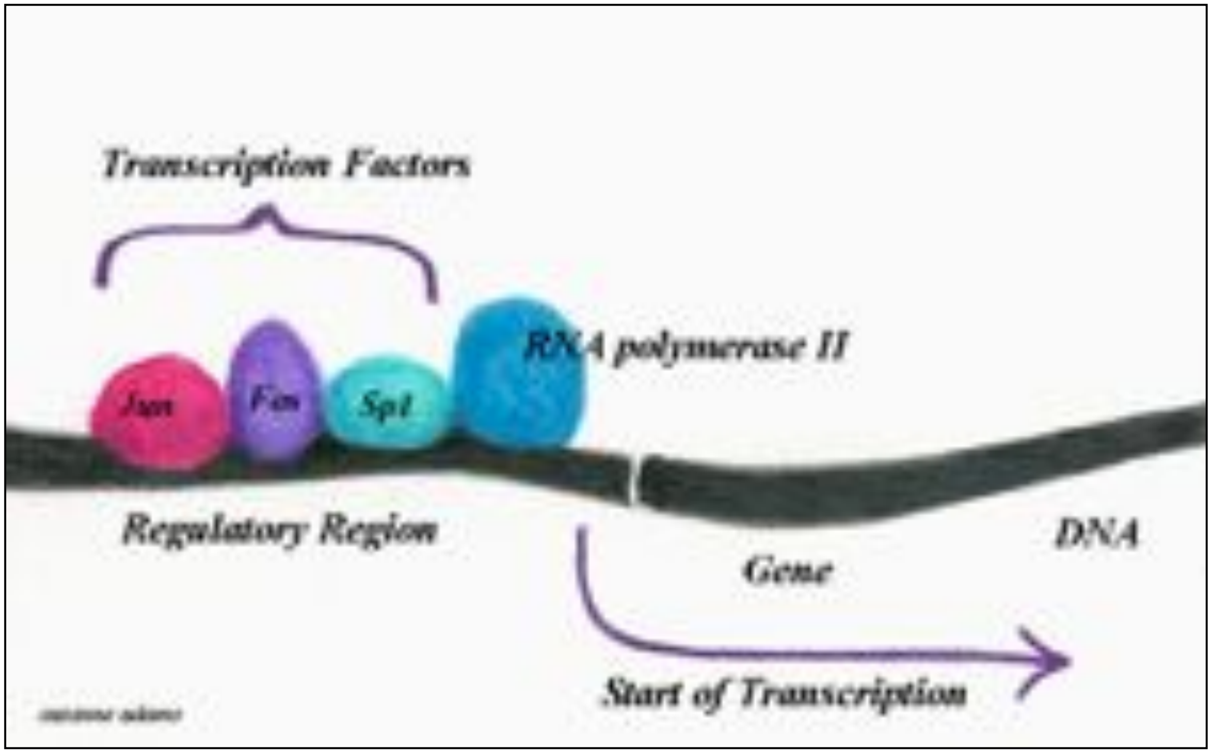


Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications
Krueger and Andrews (2010) *Bioinformatics*. 27 (11): 1571-1572.

Bisulfite Conversion

Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications
Krueger and Andrews (2010) *Bioinformatics*. 27 (11): 1571-1572.

ChIP-seq

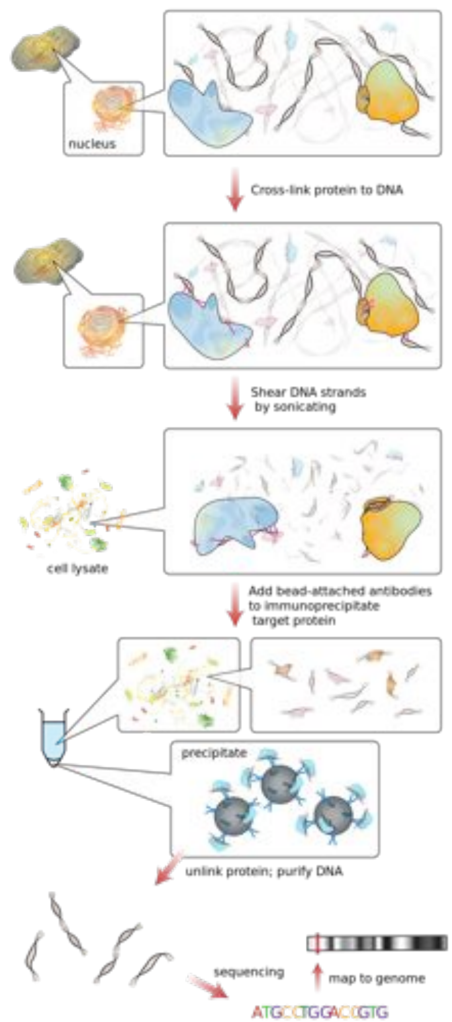


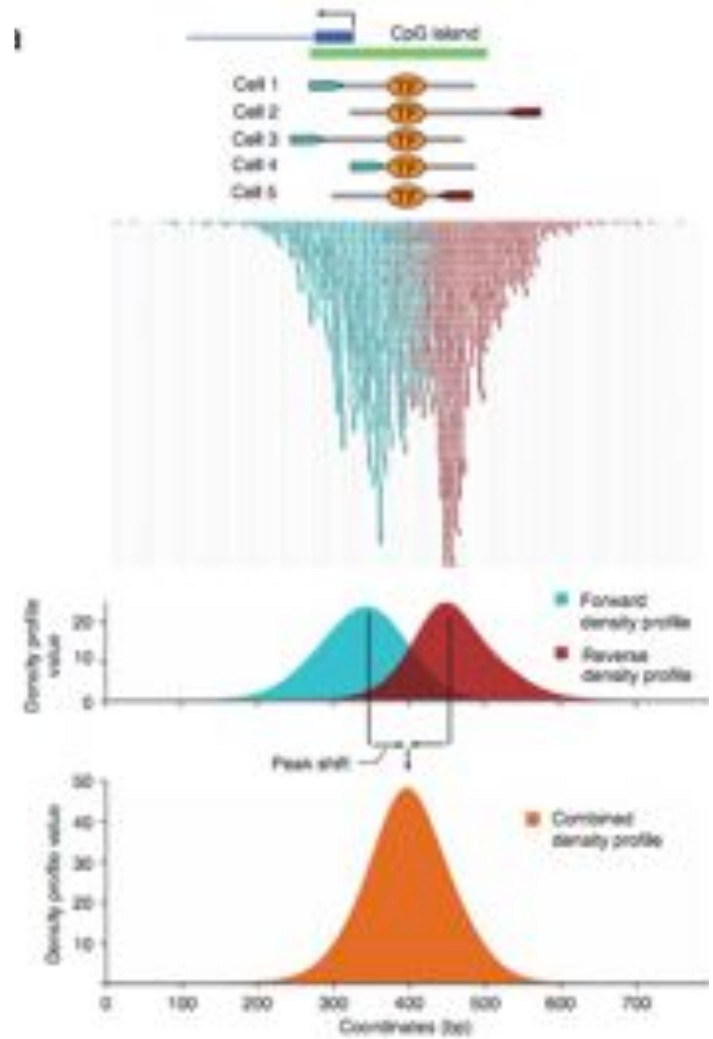
Genome-wide mapping of in vivo protein-DNA interactions.
Johnson *et al* (2007) *Science*. 316(5830):1497-502

ChIP-seq

Goals:

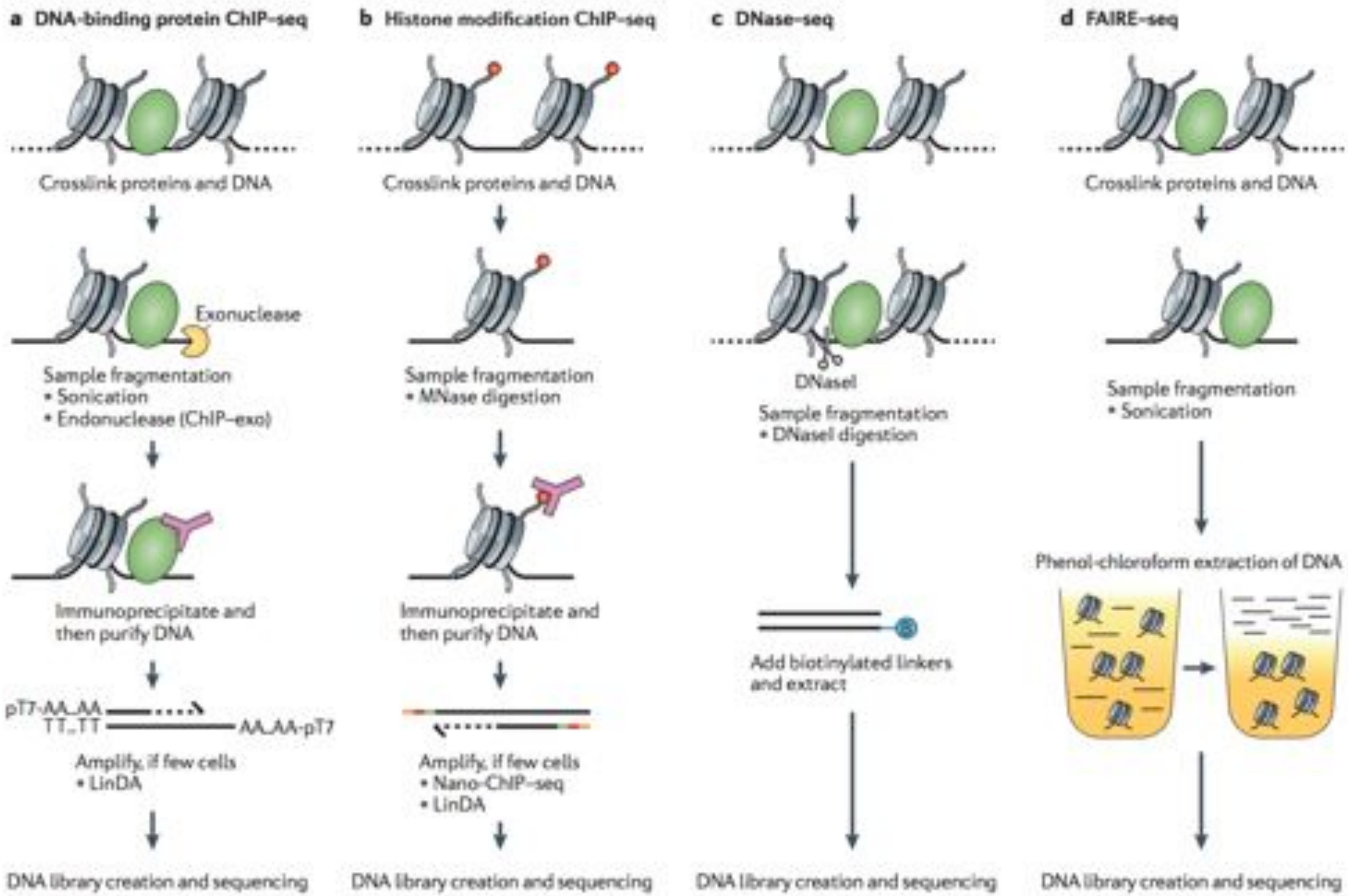
- Where are transcription factors and other proteins binding to the DNA?
- How strongly are they binding?
- Do the protein binding patterns change over developmental stages or when the cells are stressed?





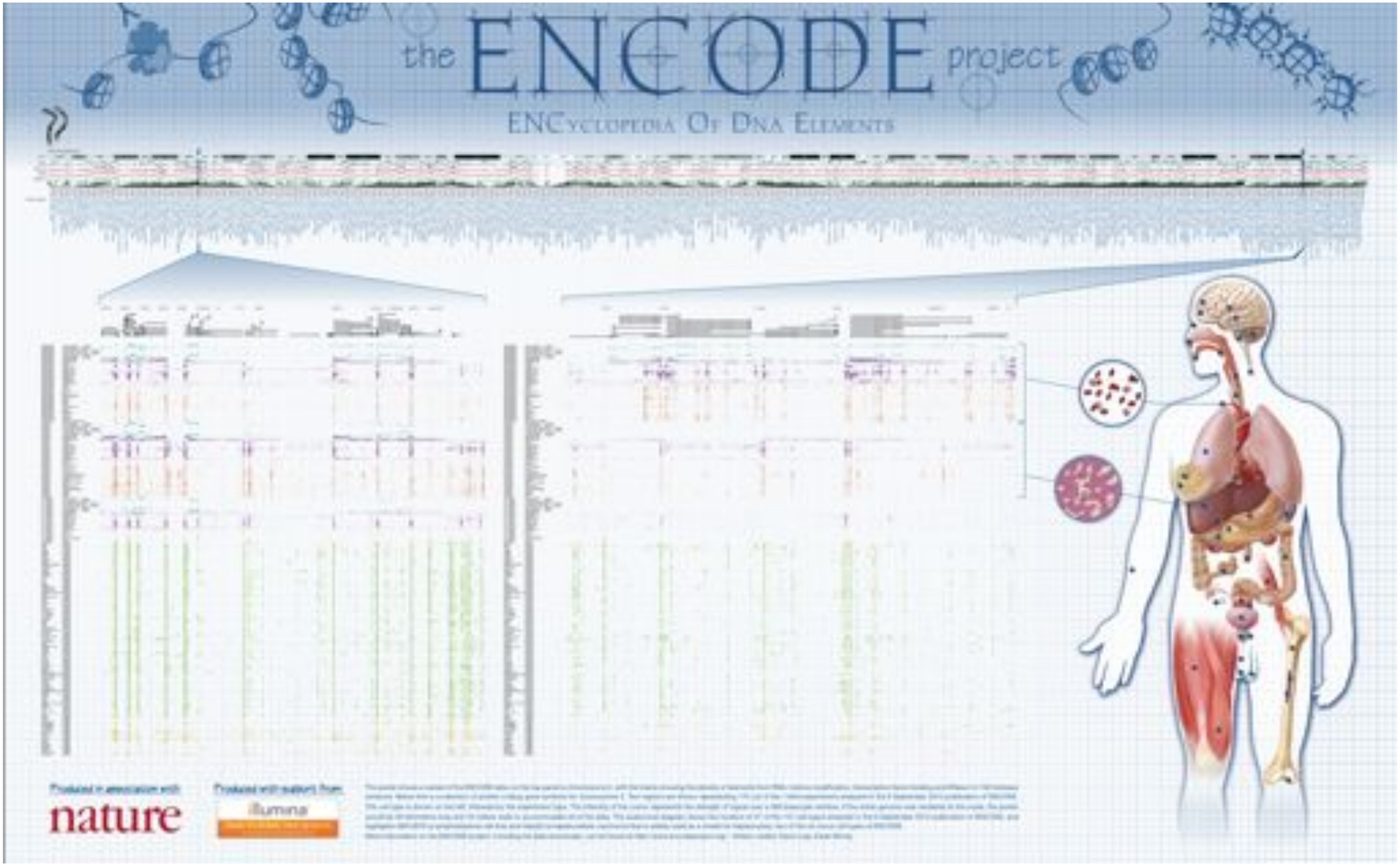
Genome-wide analysis of transcription factor binding sites based on ChIP-Seq data
Valouev et al (2008) *Nature Methods*. 5, 829 - 834

Related Assays



ChIP–seq and beyond: new and improved methodologies to detect and characterize protein–DNA interactions
Furey (2012) *Nature Reviews Genetics*. 13, 840-852

ENCODE Data Sets



1,640 data sets total over 147 different cell types

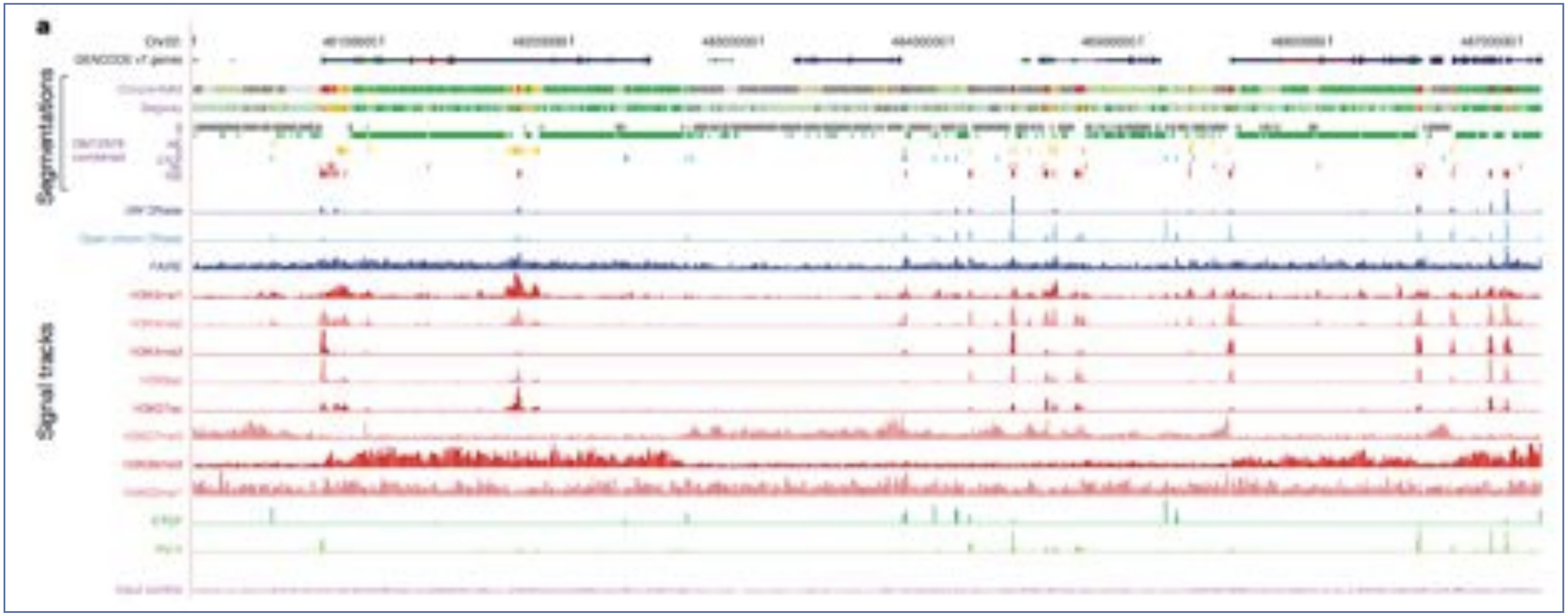
Summary of ENCODE elements

"Accounting for all these elements, a surprisingly large amount of the human genome, 80.4%, is covered by at least one ENCODE-identified element"

- 62% transcribed
- 56% enriched for histone marks
- 15% open chromatin
- 8% TF binding
- 19% At least one DHS or TF Chip-seq peak
- 4% TF binding site motif
- (Note protein coding genes comprise ~2.94% of the genome)

"Given that the ENCODE project did not assay all cell types, or all transcription factors, and in particular has sampled few specialized or developmentally restricted cell lineages, ***these proportions must be underestimates of the total amount of functional bases."***

ChromHMM: Signal Integration



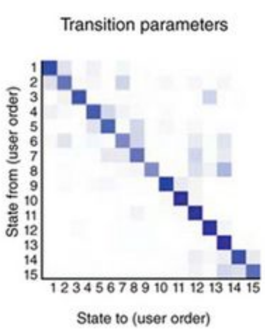


Table 3 | Summary of the combined state types

Label	Description	Details*	Colour
CTCF	CTCF-enriched element	Sites of CTCF signal lacking histone modifications, often associated with open chromatin. Many probably have a function in insulator assays, but because of the multifunctional nature of CTCF, we are conservative in our description. Also enriched for the cohesin components RAD21 and SMC3; CTCF is known to recruit the cohesin complex.	Turquoise
E	Predicted enhancer	Regions of open chromatin associated with H3K4me1 signal. Enriched for other enhancer-associated marks, including transcription factors known to act at enhancers. In enhancer assays, many of these (>50%) function as enhancers. A more conservative alternative would be cis-regulatory regions. Enriched for sites for the proteins encoded by *EP300, FOS, FOSL1, GATA2, HDAC8, JUNB, JUND, NFE2, SMARCA4, SMARCB1, SIRT6* and *TAL1* genes in K562 cells. Have nuclear and whole-cell RNA signal, particularly poly(A)− fraction.	Orange
PF	Predicted promoter flanking region	Regions that generally surround TSS segments (see below).	Light red
R	Predicted repressed or low-activity region	This is a merged state that includes H3K27me3 polycomb-enriched regions, along with regions that are silent in terms of observed signal for the input assays to the segmentations (low or no signal). They may have other signals (for example, RNA, not in the segmentation input data). Enriched for sites for the proteins encoded by *REST* and some other factors (for example, proteins encoded by *BRF2, CEBPB, MAFK, TRIM28, ZNF274* and *SETDB1* genes in K562 cells).	Grey
TSS	Predicted promoter region including TSS	Found close to or overlapping GENCODE TSS sites. High precision/recall for TSSs. Enriched for H3K4me3. Sites of open chromatin. Enriched for transcription factors known to act close to promoters and polymerases Pol II and Pol III. Short RNAs are most enriched in these segments.	Bright red
T	Predicted transcribed region	Overlap gene bodies with H3K36me3 transcriptional elongation signal. Enriched for phosphorylated form of Pol II signal (elongating polymerase) and poly(A)+ RNA, especially cytoplasmic.	Dark green
WE	Predicted weak enhancer or open chromatin cis-regulatory element	Similar to the E state, but weaker signals and weaker enrichments.	Yellow

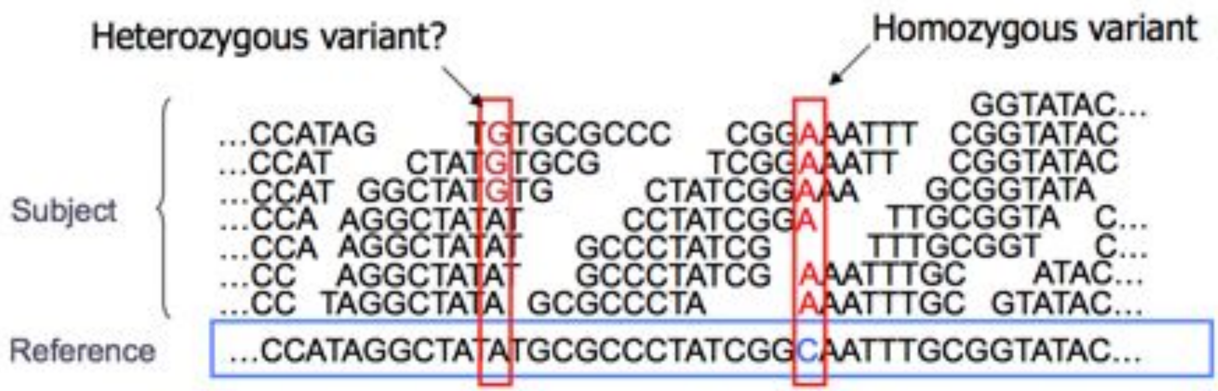
- Summarize the individual assays into 7 functional/ regulatory states using an HMM across the genome

ChromHMM: automating chromatin-state discovery and characterization

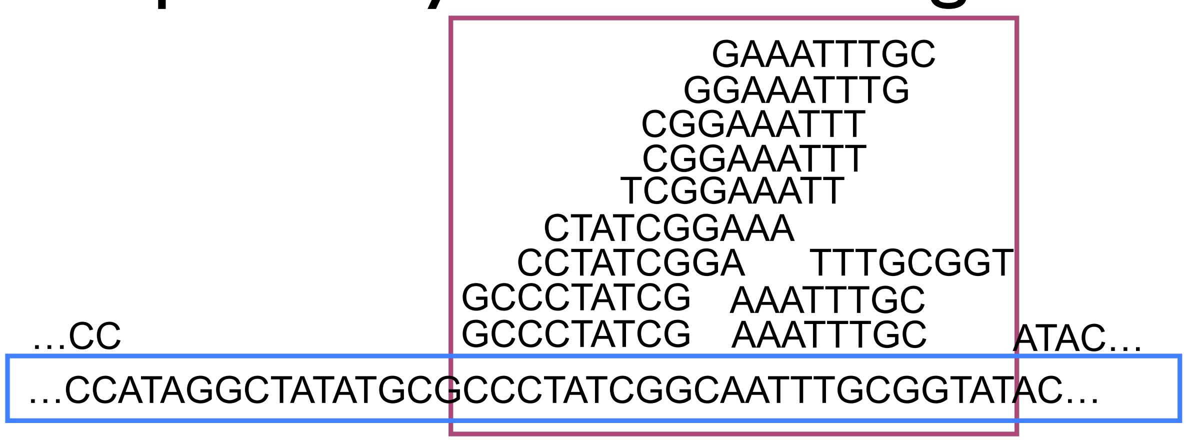
Ernst & Kellis (2012) *Nature Methods*. doi:10.1038/nmeth.1906

Genotyping vs *-seq

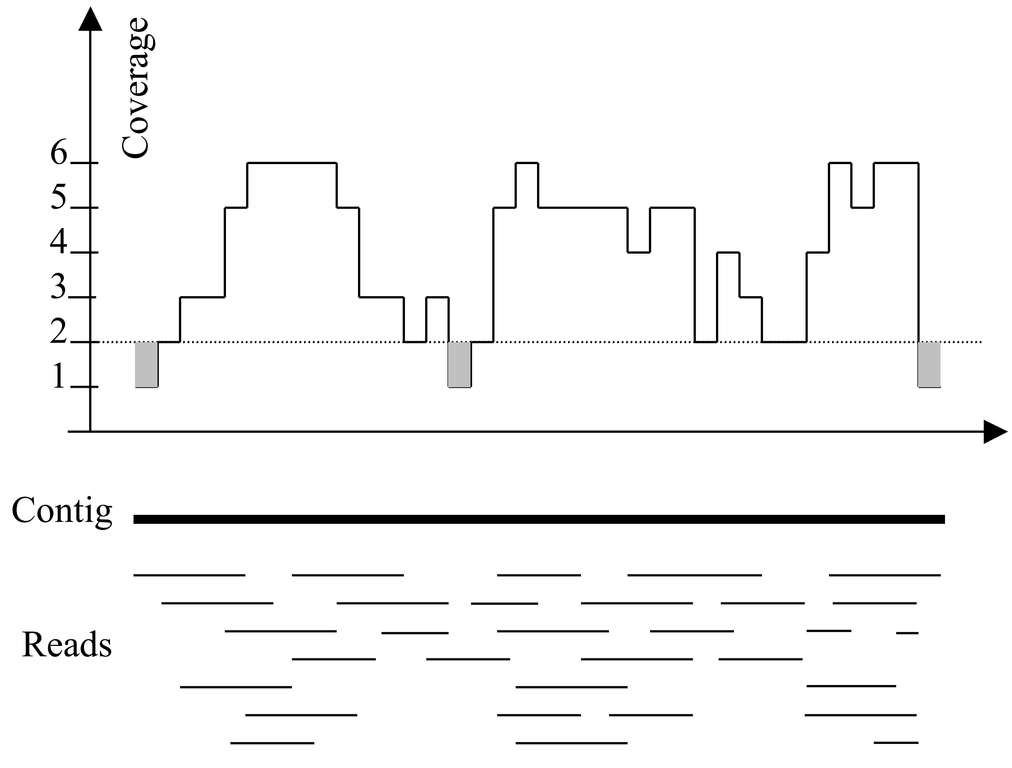
- Genotyping: Identify Variations



- *-seq: Classify & measure significant peaks



WIG/bigWIG Format



- Coverage can change at every single position (3B integers)
- But we often want to summarize to every 100th or every 1000th
- WIG format to the rescue!

WIG/bigWIG Format

Wiggle format is line-oriented, 1st line must be a track definition, followed by declaration lines and data lines

fixedStep is for data with regular intervals between new data values

```
fixedStep  chrom=chrN  start=position  step=stepInterval  [span=windowSize]
dataValues

fixedStep chrom=chr3 start=400601 step=100
11
22
33
```

variableStep is for data with irregular intervals

```
variableStep  chrom=chrN  [span=windowSize]
chromStartA  dataValueA

variableStep chrom=chr2
300701 12.5
300702 12.5
300703 12.5
300704 12.5
300705 12.5
```

WIG Example

```
browser position chr19:49304200-49310700
browser hide all
#     150 base wide bar graph at arbitrarily spaced positions,
#     threshold line drawn at y=11.76
#     autoScale off viewing range set to [0:25]
#     priority = 10 positions this as the first graph
#     Note, one-relative coordinate system in use for this format
track type=wiggle_0 name="variableStep" description="variableStep format" visibility=full autoScale=off
viewLimits=0.0:25.0 color=50,150,255 yLineMark=11.76 yLineOnOff=on priority=10
variableStep chrom=chr19 span=150
49304701 10.0
49304901 12.5
49305401 15.0
49305601 17.5
49305901 20.0
49306081 17.5
49306301 15.0
49306691 12.5
49307871 10.0

#     200 base wide points graph at every 300 bases, 50 pixel high graph
#     autoScale off and viewing range set to [0:1000]
#     priority = 20 positions this as the second graph
#     Note, one-relative coordinate system in use for this format
track type=wiggle_0 name="fixedStep" description="fixedStep format" visibility=full autoScale=off
viewLimits=0:1000 color=0,200,100 maxHeightPixels=100:50:20 graphType=points priority=20
fixedStep chrom=chr19 start=49307401 step=300 span=200
1000
 900
 800
 700
 600
 500
 400
 300
 200
 100
```

WIG Example

```
browser position chr19:49304200-49310700
browser hide all
#     150 base wide bar graph at arbitrarily spaced positions,
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variableStep chrom=chr19 span=150
49304701 10.0
49304901 12.5
49305401 15.0
49305601 17.5
49305901 20.0
49306081 17.5
49306301 15.0
49306691 12.5
49307871 10.0
```
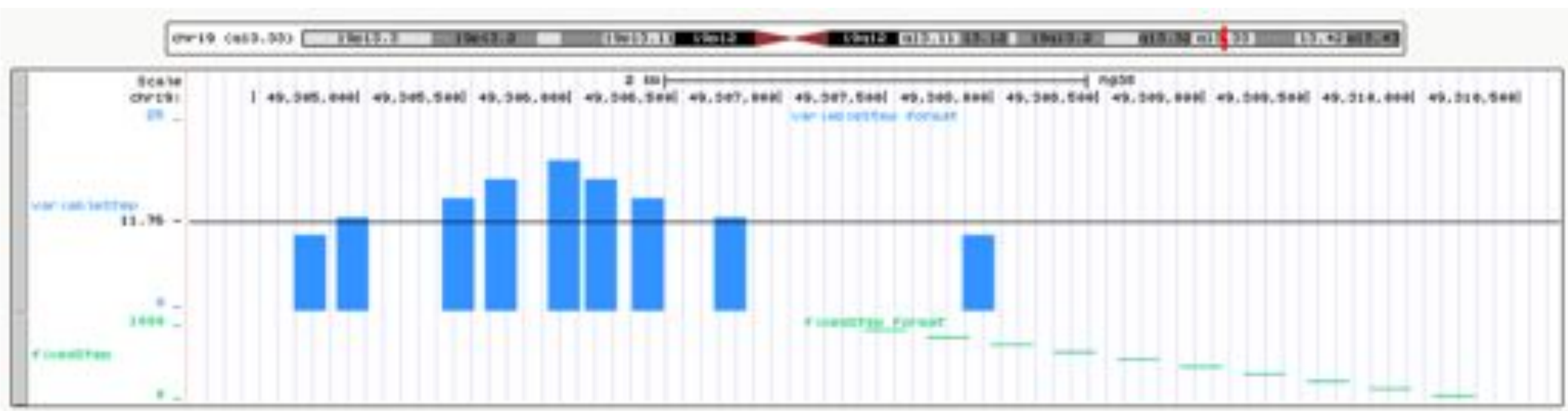
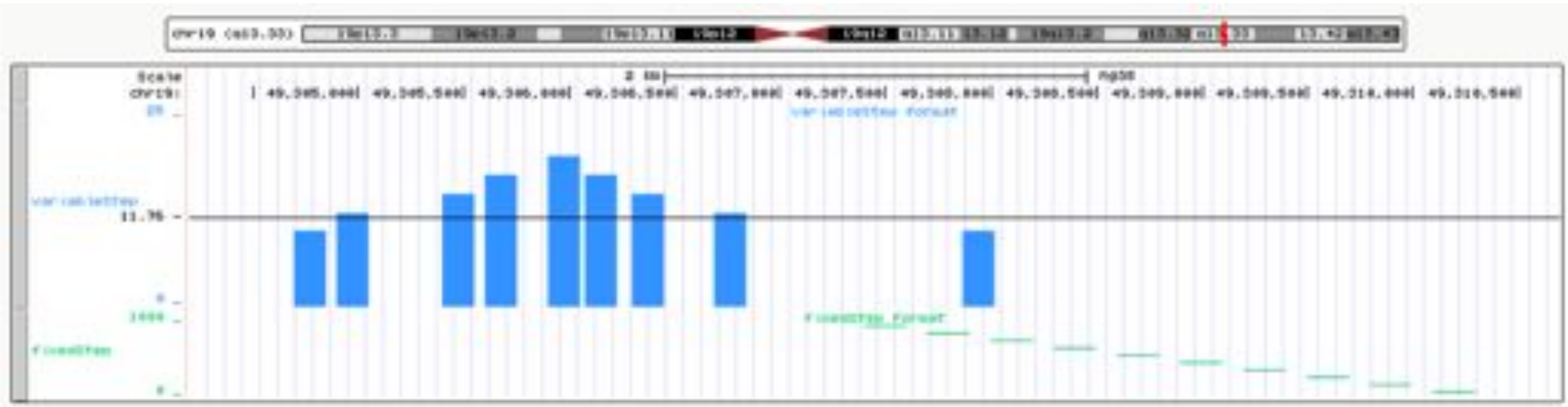



```
 100
```

WIG Example

```
browser position chr19:49304200-49310700
browser hide all
#     150 base wide bar graph at arbitrarily spaced positions,
#     threshold line drawn at y=11.76
```



```
#     200 base wide points graph at every 300 bases, 50 pixel high graph
#     autoScale off and viewing range set to [0:1000]
#     priority = 20 positions this as the second graph
#     Note, one-relative coordinate system in use for this format
track type=wiggle_0 name="fixedStep" description="fixedStep format" visibility=full autoScale=off
viewLimits=0:1000 color=0,200,100 maxHeightPixels=100:50:20 graphType=points priority=20
fixedStep chrom=chr19 start=49307401 step=300 span=200
1000
 900
 800
 700
 600
 500
 400
 300
 200
 100
```

BED Format

Simple tab-delimited general format for recording "intervals"

Required fields:

1. chrom:	The name of the sequence
2. chromStart:	The 0-based starting position
3. chromEnd:	The 0-based half-open ending position

The first 100 bases of a sequence are defined as chromStart=0, chromEnd=100, and span the bases numbered 0-99.

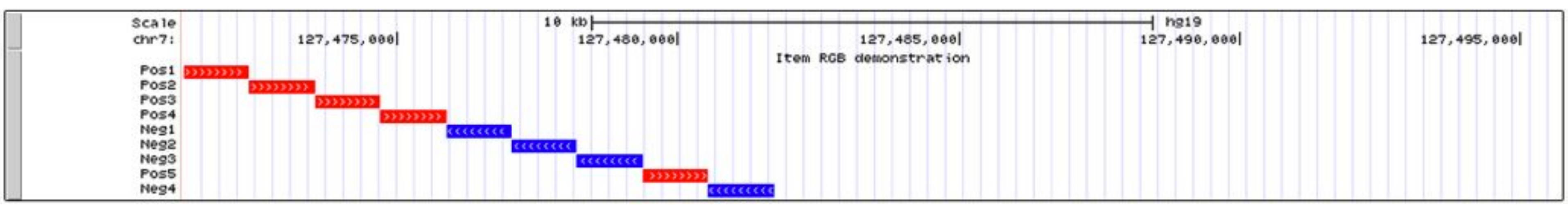
The 9 additional optional BED fields are:

4. name:	Defines the name of the BED line
5. score:	A score value (typically between 0 and 1000)
6. strand:	Defines the strand - either '+' 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:	An RGB value of the form R,G,B (e.g. 255,0,0).
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 block starts.

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

BED Example

```
browser position chr7:127471196-127495720
browser hide all
track name="ItemRGBDemo" description="Item RGB demonstration" visibility=2 itemRgb="On"
chr7 127471196 127472363 Pos1 0    +    127471196 127472363 255,0,0
chr7 127472363 127473530 Pos2 0    +    127472363 127473530 255,0,0
chr7 127473530 127474697 Pos3 0    +    127473530 127474697 255,0,0
chr7 127474697 127475864 Pos4 0    +    127474697 127475864 255,0,0
chr7 127475864 127477031 Neg1 0    -    127475864 127477031 0,0,255
chr7 127477031 127478198 Neg2 0    -    127477031 127478198 0,0,255
chr7 127478198 127479365 Neg3 0    -    127478198 127479365 0,0,255
chr7 127479365 127480532 Pos5 0    +    127479365 127480532 255,0,0
chr7 127480532 127481699 Neg4 0    -    127480532 127481699 0,0,255
```



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

Outline

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

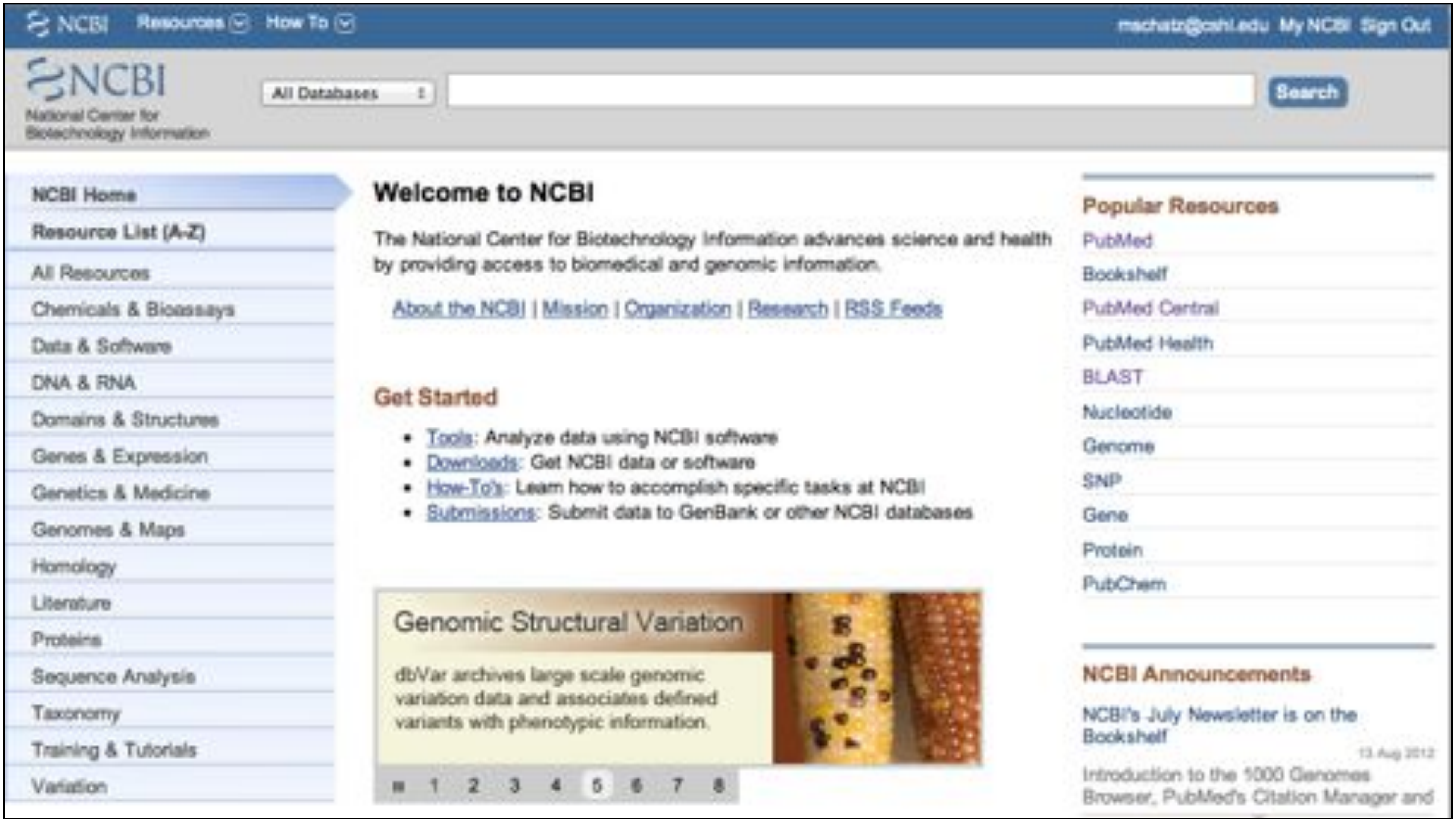
Common Genomics Questions

- What is the closest gene to this ChIP-seq peak?
- Is my latest discovery novel?
- Is there strand bias in my data?
- How many genes does this mutation affect?
- Where did I fail to collect sequence coverage?
- Is this feature significantly correlated with some other feature?

Solution is to integrate (many) online resources
with your own data!

NCBI

http://www.ncbi.nlm.nih.gov/

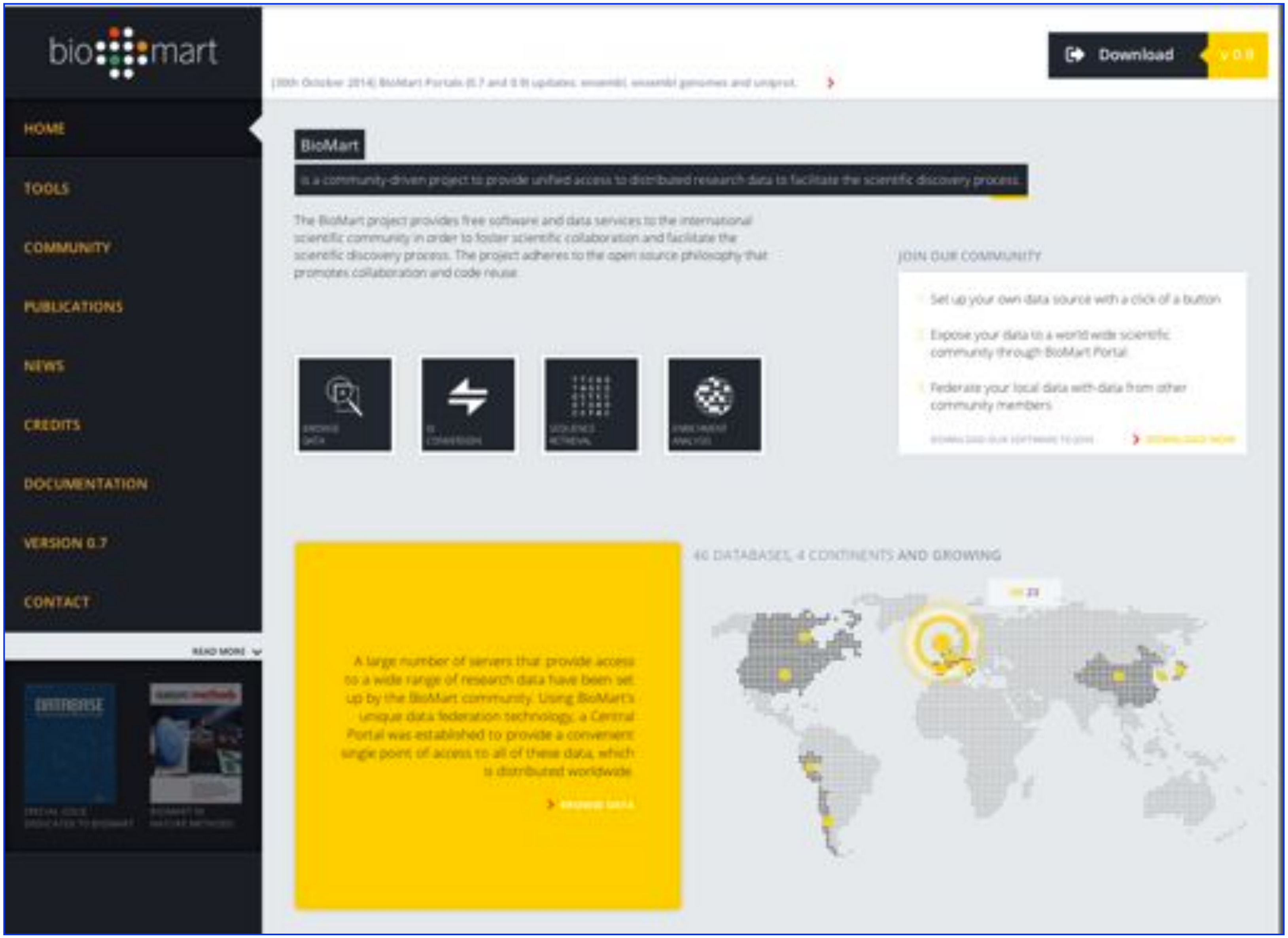


Ensembl

http://www.ensembl.org

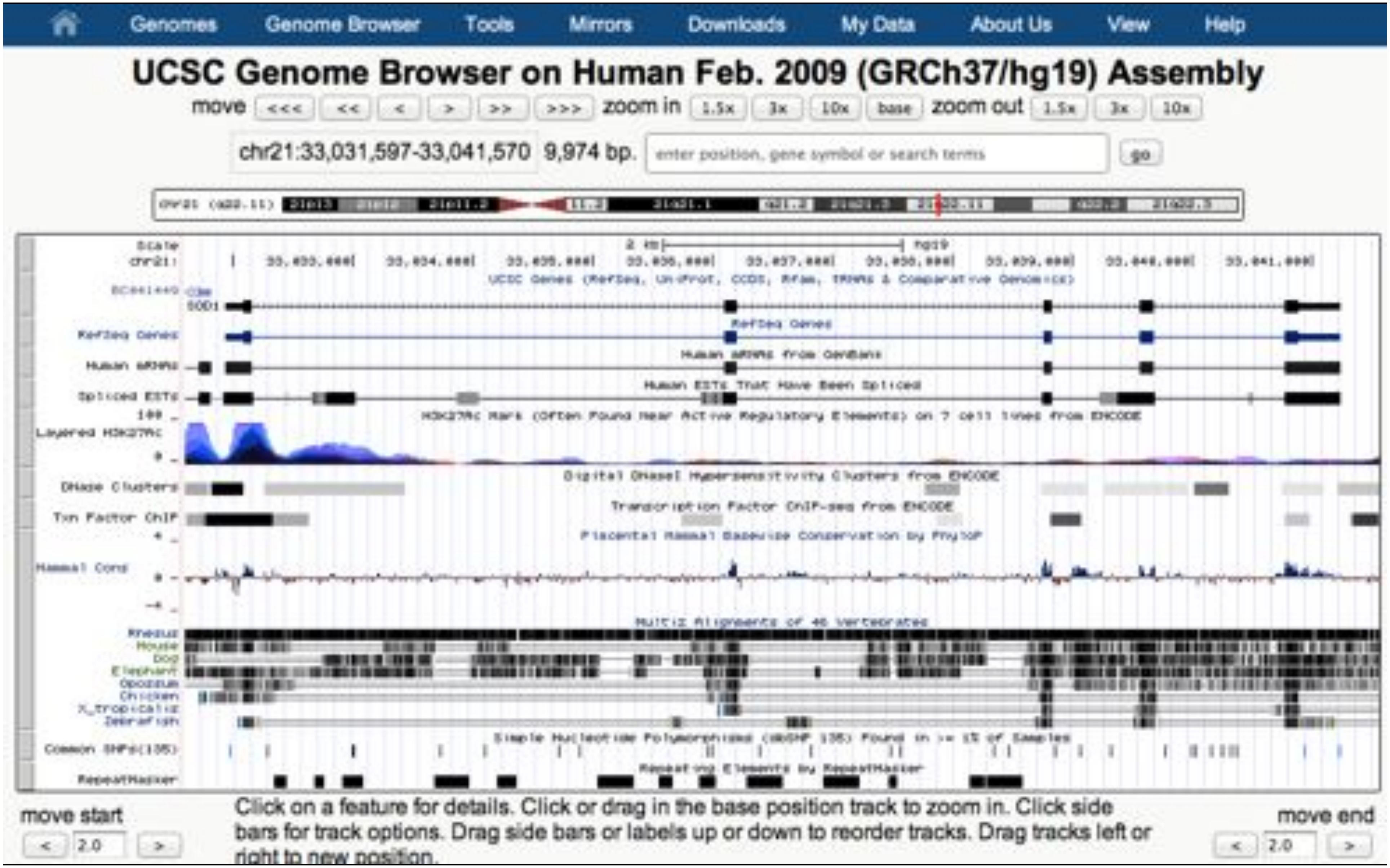
Biomart

http://www.biomart.org



UCSC Genome Browser

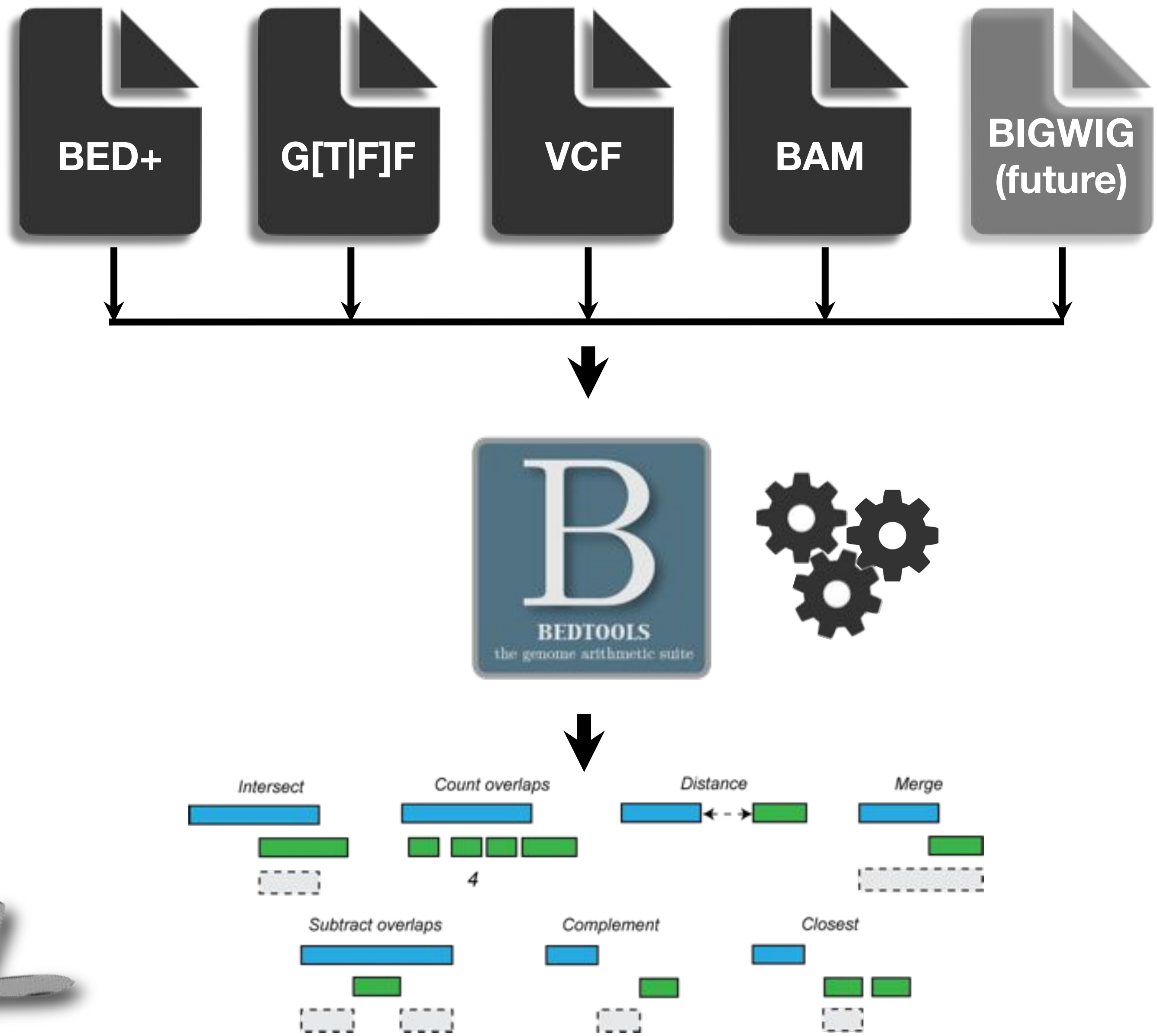
http://genome.ucsc.edu/



UCSC Genome Browser / Table Browser

http://genome.ucsc.edu/cgi-bin/hgTables?command=start

BEDTools to the rescue!



Find SNPs that have the potential to alter gene expression regulation by affecting methylation at CpG islands.

Wednesday @ 1pm

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 ☺
- Coming up:
  - IGV, QC, Variant Analysis, De novo assembly, Transcriptome, etc…

Thank you!

http://schatzlab.cshl.edu
@mike_schatz