VEuPathDB

Eukaryotic Pathogen, Vector & Host Informatics Resources

Crash Course in Omics
Terminology, Concepts & Data Types

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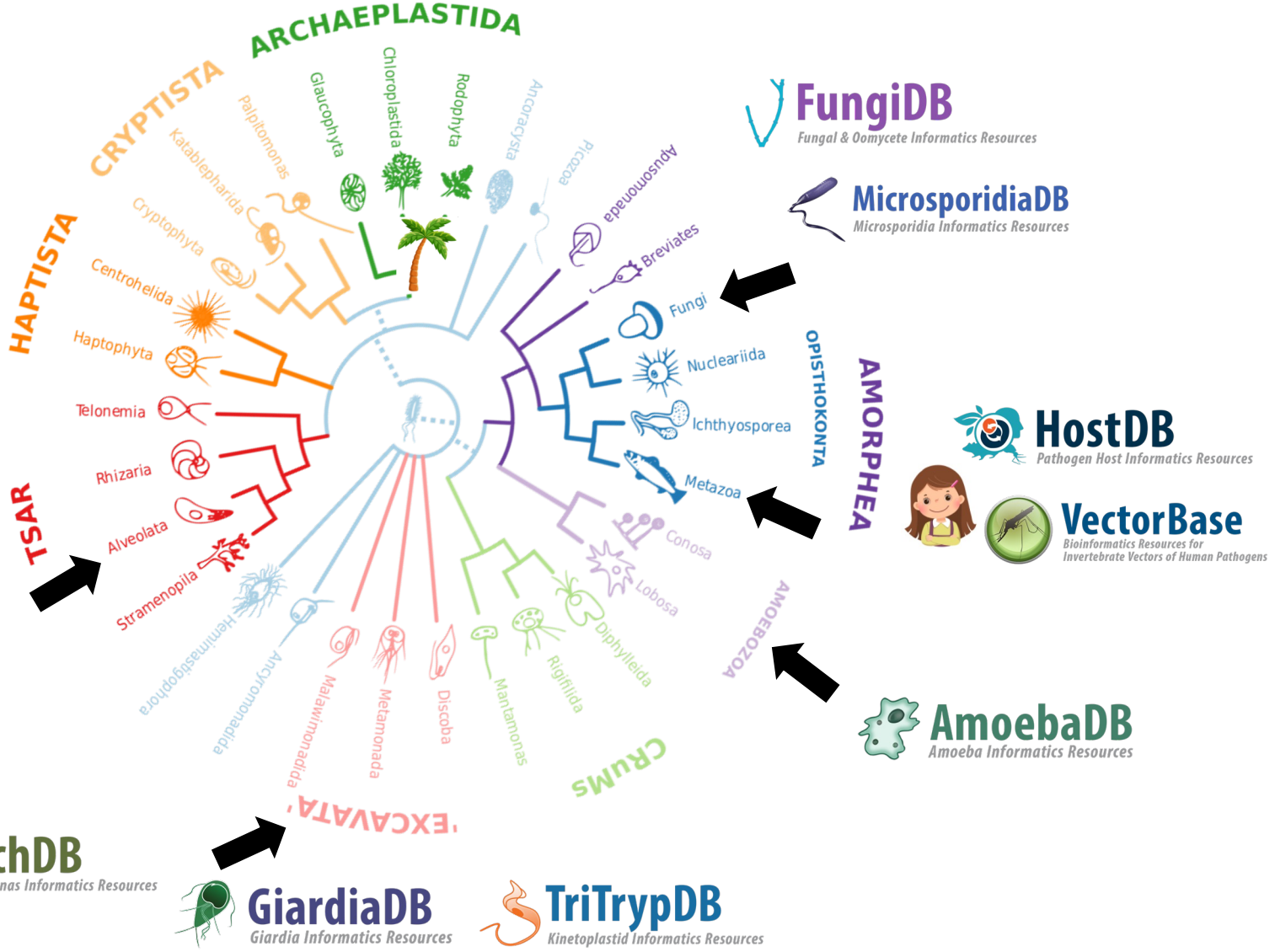
VEuPathDB

Eukaryotic Pathogen, Vector & Host Informatics Resources

758 genome sequences,
3083 datasets in Release 66!



PlasmoDB
Plasmodium Informatics Resources

ToxoDB
Toxoplasma Informatics Resources

CryptoDB
Cryptosporidium Informatics Resources

PiroplasmaDB
Piroplasma Informatics Resources

FungiDB
Fungal & Oomycete Informatics Resources

MicrosporidiaDB
Microsporidia Informatics Resources

HostDB
Pathogen Host Informatics Resources

VectorBase
Bioinformatics Resources for Invertebrate Vectors of Human Pathogens

AmoebaDB
Amoeba Informatics Resources

TrichDB
Trichomonas Informatics Resources

GiardiaDB
Giardia Informatics Resources

TriTrypDB
Kinetoplastid Informatics Resources

MicrobiomeDB
A Microbiome Resource

ClinEpiDB
Clinical Epidemiology Resources

OrthoMCL DB
Ortholog Groups of Protein Sequences

https://commons.wikimedia.org/wiki/File:Eukaryote_Tree_of_Life_2020.svg

Our Advice

¯_(ツ)_/¯

We're still gathering data for this route

Track prices Off

224 of 633 flights

Stops

- Nonstop
- 1 stop $1,117
- 2+ stops $894

Fee Assistant

Carry-on bag 0

Checked bag 0

Book on KAYAK

Show offers instantly bookable on KAYAK.

Times

Take-off | Landing

Take-off from ATL

Cheapest $894 • 18h 37m | **Best** $1,121 • 13h 07m | **Quickest** $1,121 • 13h 07m | Other sort

Best

Delta 3:40 pm – 9:50 am +1 | 1 stop CDG | 12h 10m ATL-TUN
Delta 5:30 am – 1:35 pm | 1 stop CDG | 14h 05m TUN-ATL
Operated by Air France

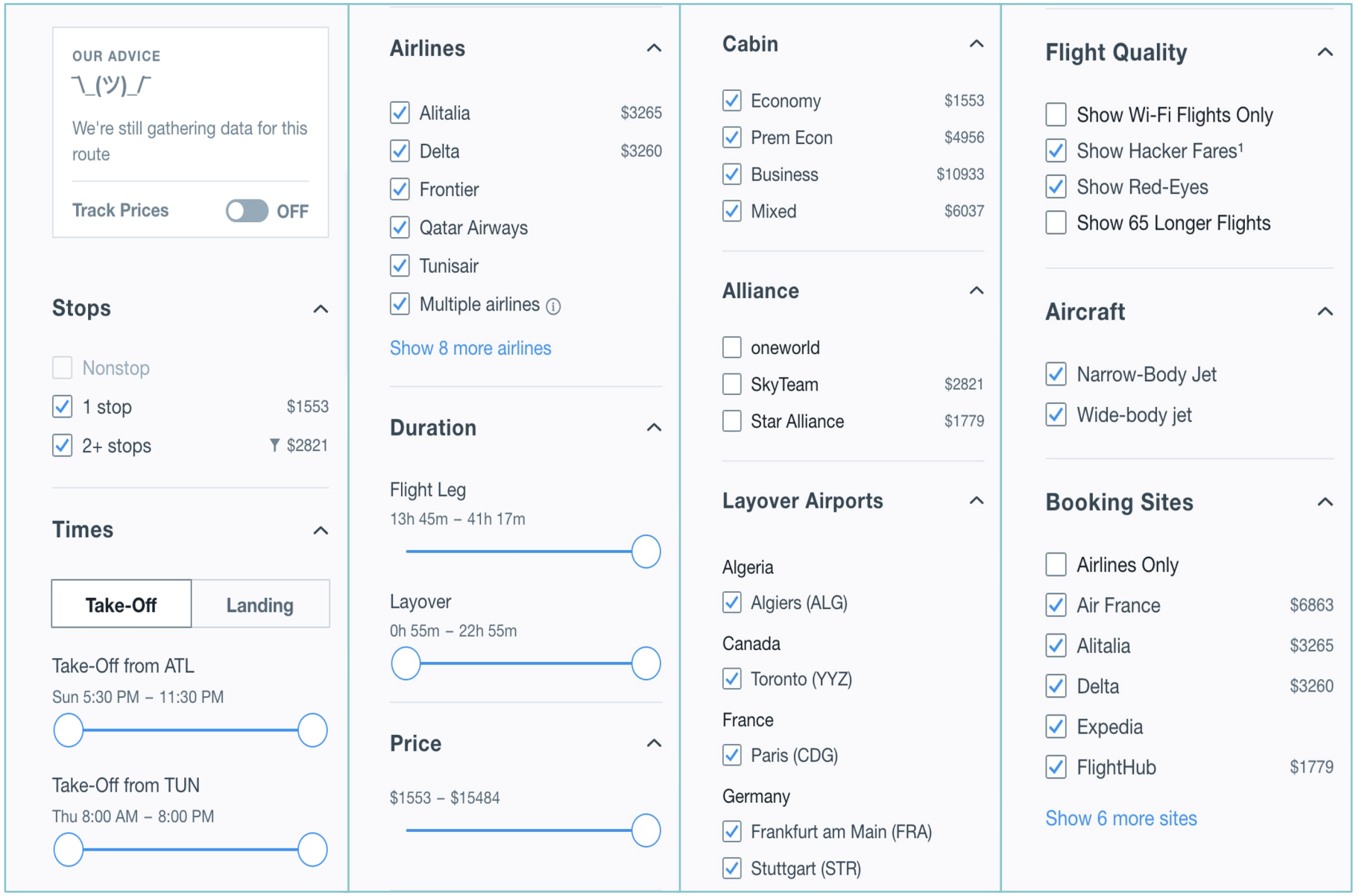
1 0 **$1,121** Basic Economy Delta **View Deal**
Main Cabin $1,301

Cheapest

ITA AIRWAYS 10:10 am – 10:35 am +1 ITA Airways | 2 stops IAD, FCO | 18h 25m ATL-TUN
ITA AIRWAYS 11:25 am – 12:14 am +1 ITA Airways | 2 stops FCO, JFK | 18h 49m TUN-ATL

1 0 **$894** Economy ScholarTrip **View Deal**

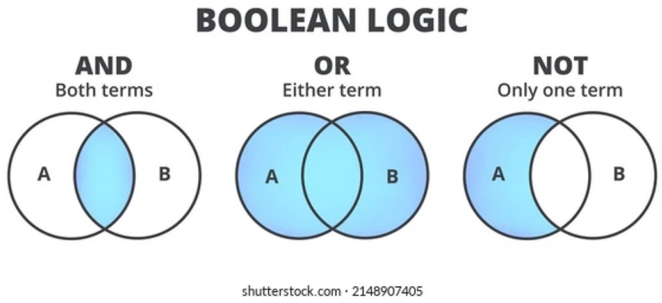
The Travel Site has Very Useful Data Filters!



Filters vs Boolean operators

Filters

- Can only narrow down the original search
- They only return a subset of the original data
- Examples:
  - All genes on chromosome 4
  - All genes with "kinase in their name
  - All genes from *Trypanosoma cruzi*



Boolean operators (and, or & not)

- Intersect, union, subtract
- They can operate on *two different searches!*
- They can narrow down, or, *expand the original search*
- Examples:
  - All genes on Chr 4 that have kinase in their name
  - All genes on chr 4 or chr 8
  - All genes in *T. cruzi* that also have a signal peptide

The Biological Equivalent of Travel Search Engine with Filters and Boolean Logic

- Find all genes that….
  - That are near centromeres
  - That encode a predicted signal protein
  - That encode the amino acid motif *CC..CC*
- Which have evidence of expression …
  - In developmental stage X
  - After treatment with drug Y
- That are phosphorylated in proteomic studies
- That show evidence of diversifying selection in population studies

Searching biological data is difficult because there are so many different technologies!

- Each technology e.g. genomics, transcriptomics, proteomics, metabolomics, etc.. has its own vocabulary that is more complicated than selecting a window or aisle seat.
- So,…to use the databases efficiently, you do not need to be a bioinformatician, rather you need to be an expert on the technologies related to the data you will mine so you can use the filters and Boolean operators well and interpret your results.
- Since nobody can keep up with all of the technologies and terminologies, and because we come from so many different backgrounds, we have created this crash course in omics

Most Genomic terminology in VEuPathDB refers to the following concepts:

Genome assembly: Reads, contigs, scaffolds, chromosomes, genome sequences, gaps, indels rearrangements, sequence

Genome annotation: Genes, sequence, coding and non-coding, intergenic regions, untranslated regions, introns, Promoters

Evolution: Sequence differences, SNPs, SNV, InDels, synonymous, non-synonymous, orthologs, paralogs, homology

Chromatin status: Epigenetics, Methylation, open chromatin, closed chromatin

Gene expression: Transcripts, splicing, alternative splicing, differential expression, expression levels (relative or absolute), transcript modifications. Analyses can bulk on a tissue or population of cells/organisms or can be single-cell

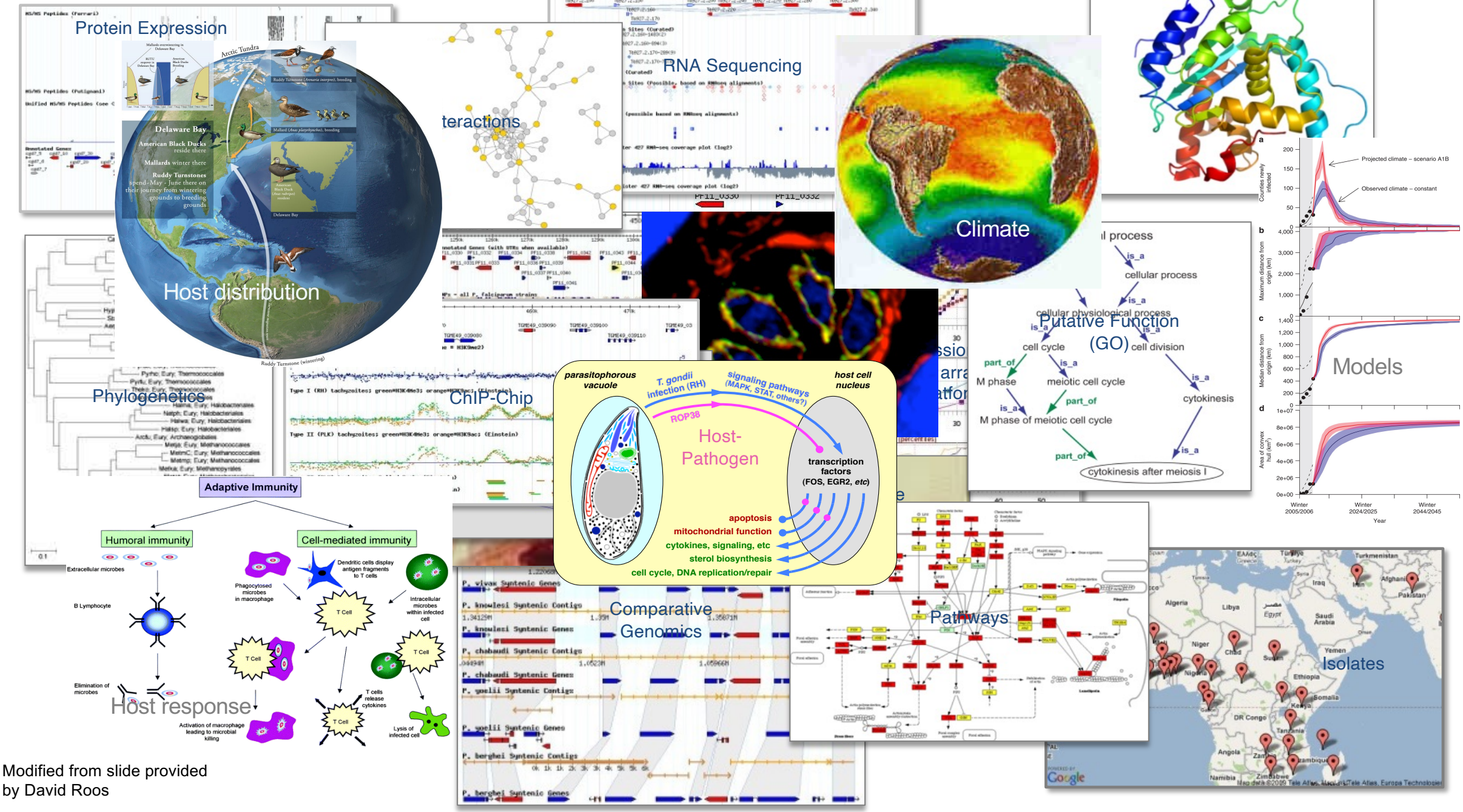
Proteins: Sequence, protein features (motifs, signal peptides, TM domains: chemical properties, chemical modifications (phosphorylation, glycosylation), expression, processing, localization

Metabolites: Chemical compounds, enzymes, pathways, flux

Host(s): Host response, immune responses, gene regulation responses, metabolic responses

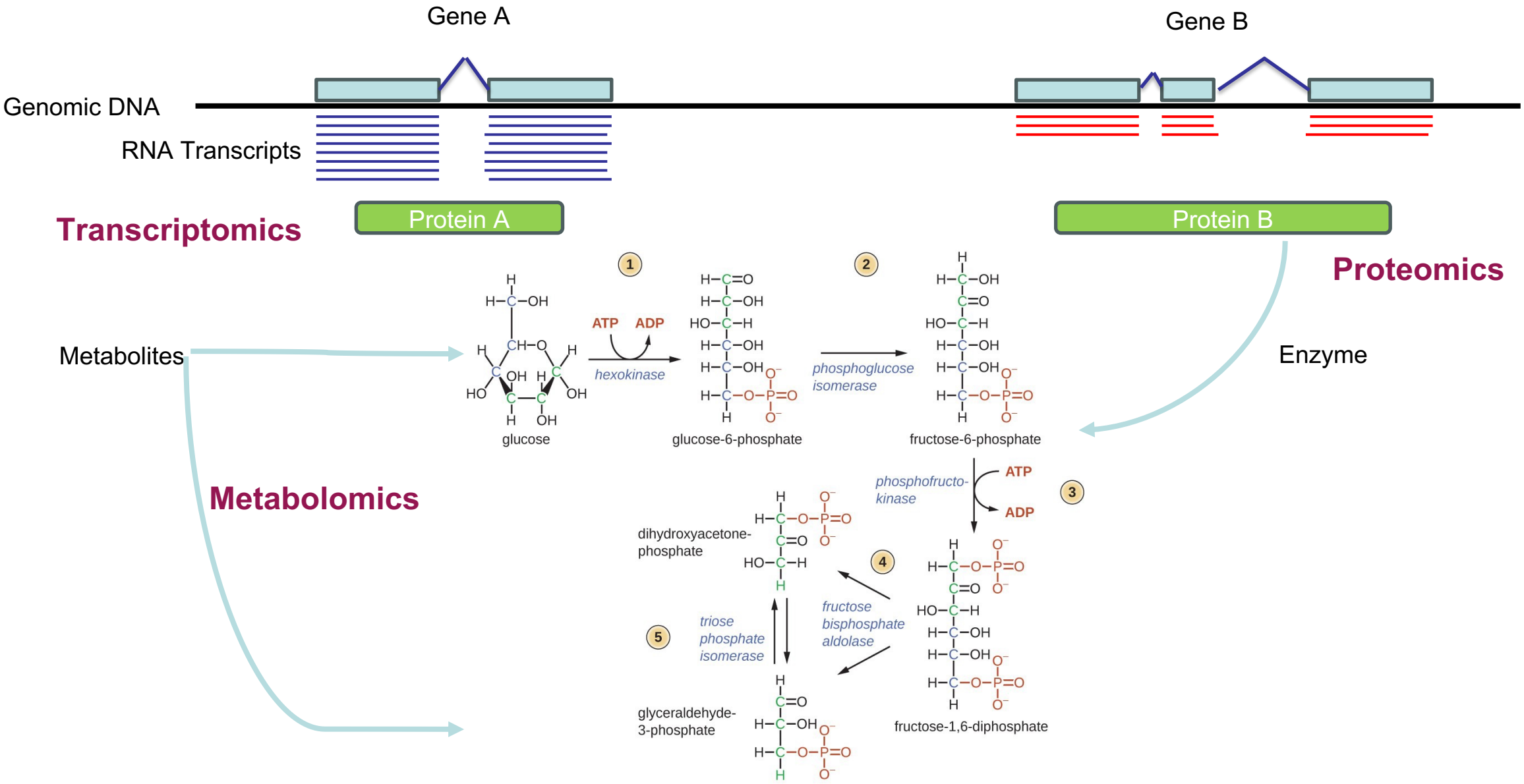
Mutant analysis: Phenotypic response to gene knock-down or knock out, e.g. via CRISPR or other approach, or specific mutations

Metadata: Data about the data, e.g. the patient, source, environment or experimental condition

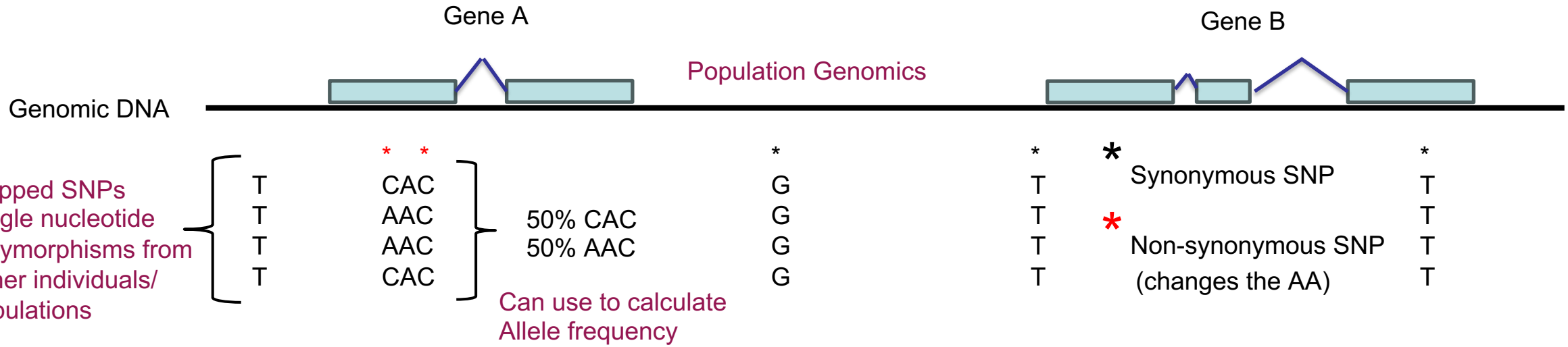


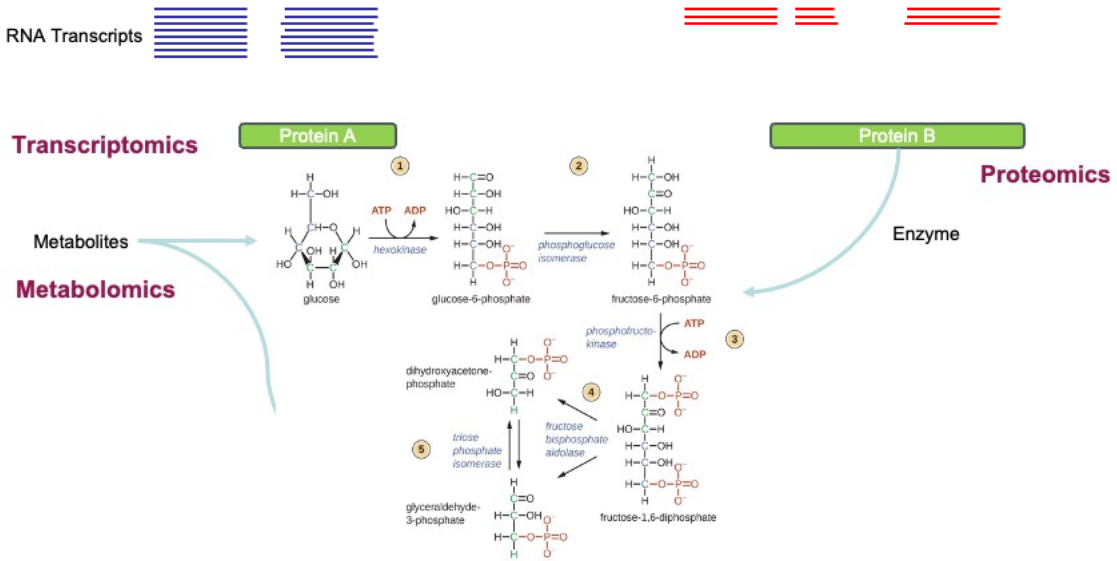
Modified from slide provided by David Roos

Conceptually, how are the data integrated?



Conceptually, how are the data integrated?





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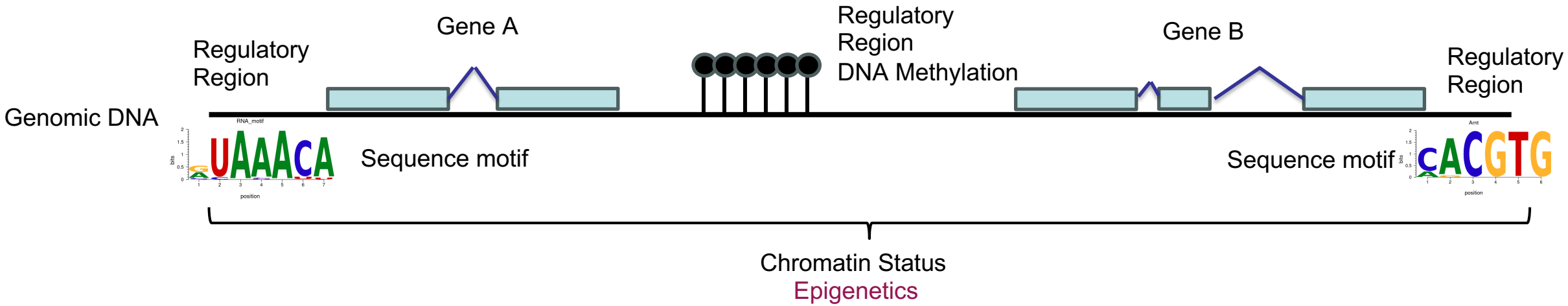
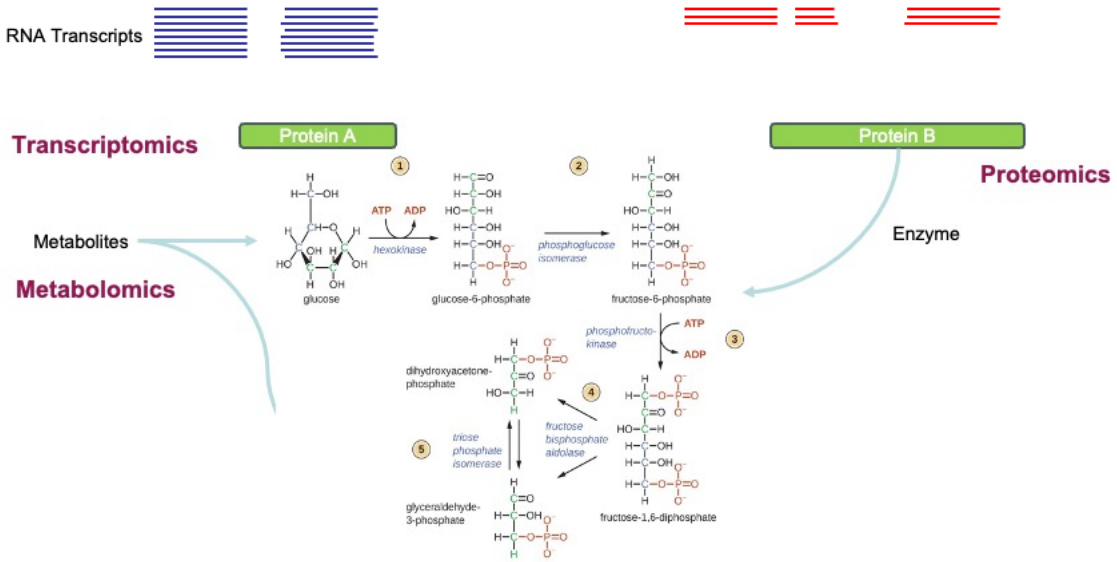
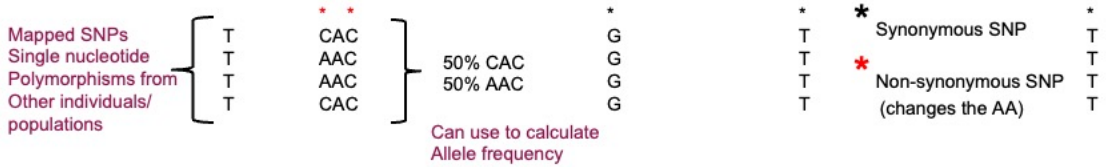


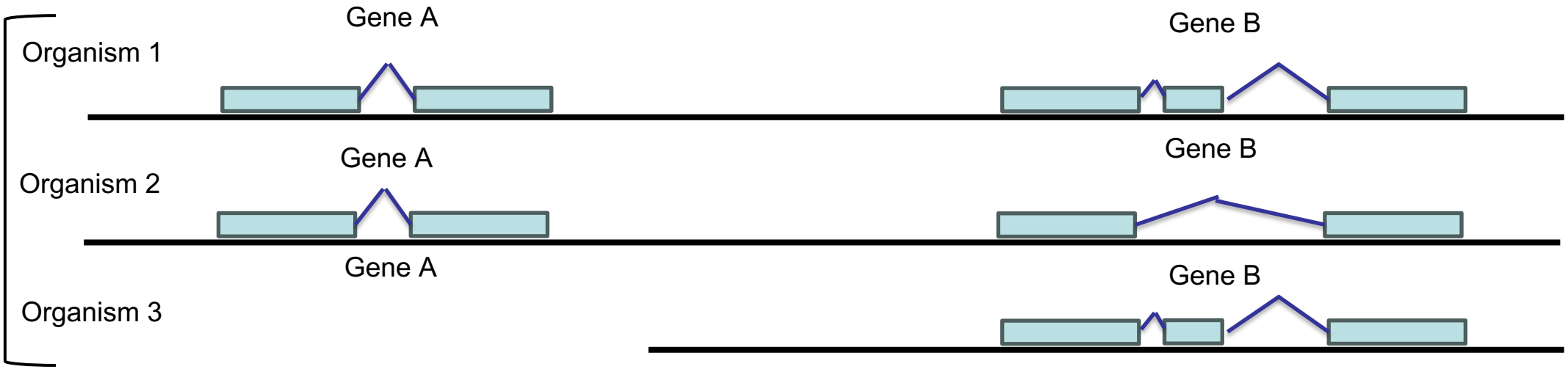


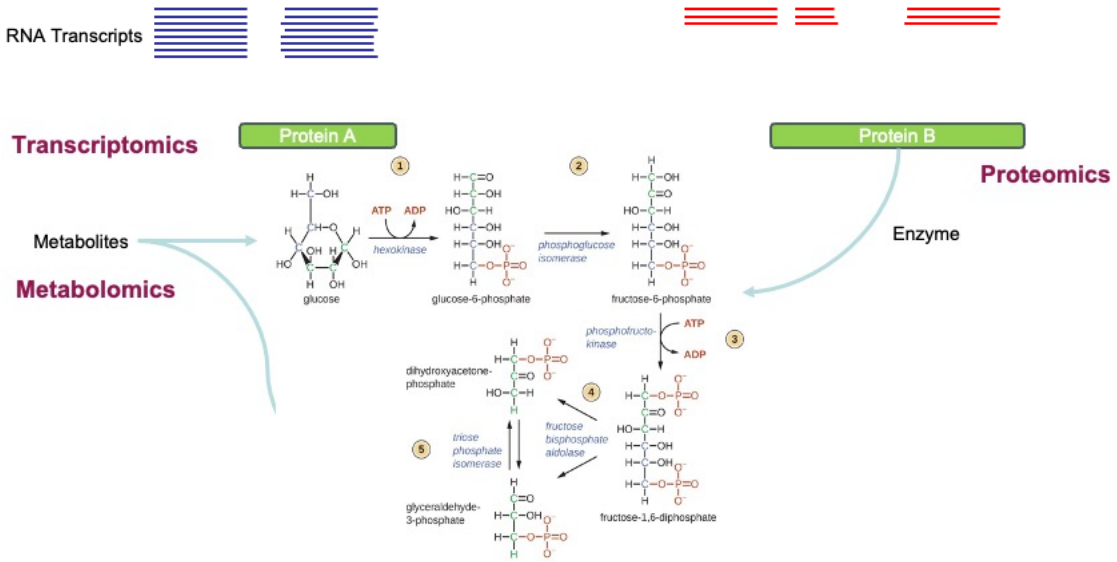
Population Genomics

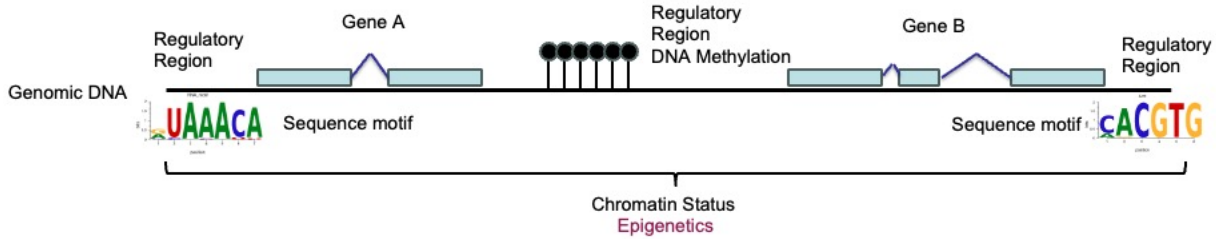


Conceptually, how are the data integrated?

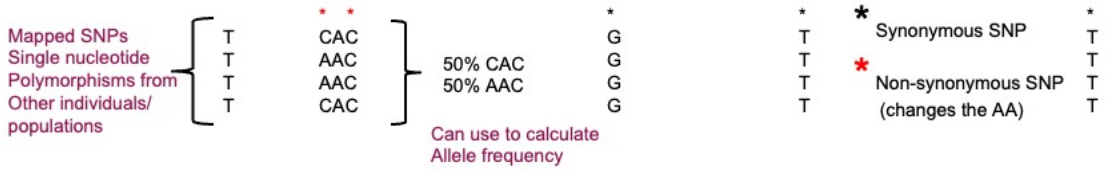
Evolution, Synteny, And Annotation





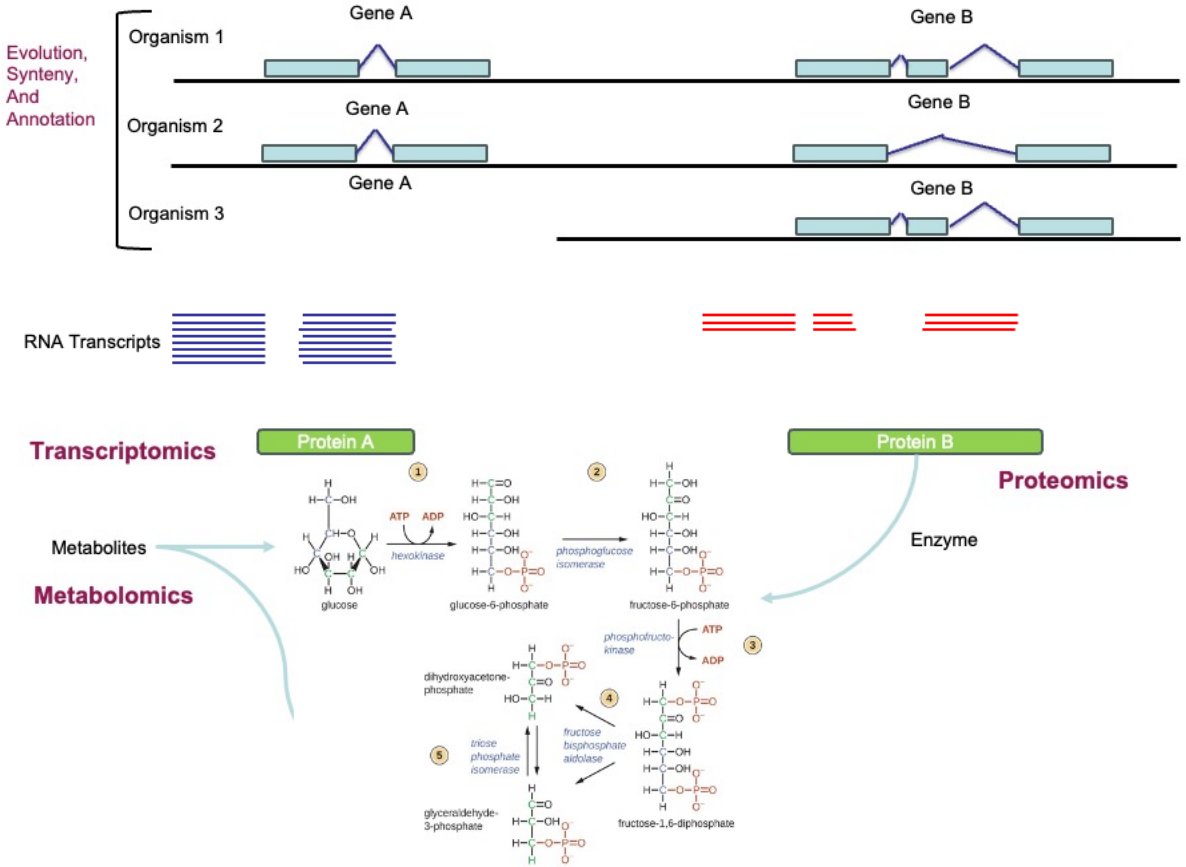


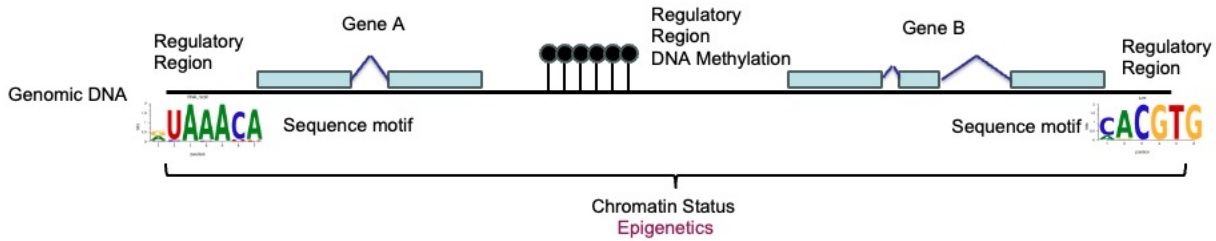
Population Genomics



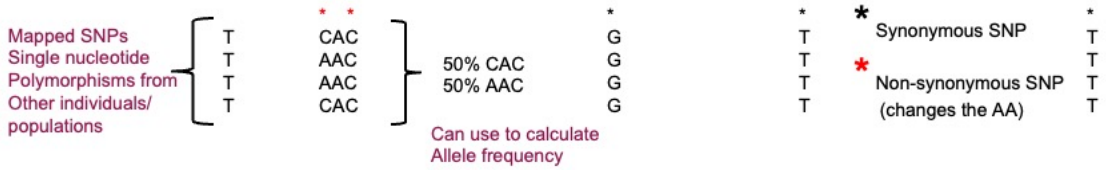
Conceptually, how are the data integrated?

Biologically! Most data can be mapped directly to a genome sequence or to a feature that is mapped to a genome sequence thus, we can use the genomic backbone of coordinates to relate genes, to transcripts and proteins and SNPs. We have also linked the meta data about the genomes, e.g. organisms, taxonomy and geographic location. Together the system is powerful.





Population Genomics



So let's look at a few data types in more detail

Genome sequences & Assembly

FASTQ format for reads

Figure 2 – Flowchart of an NGS workflow

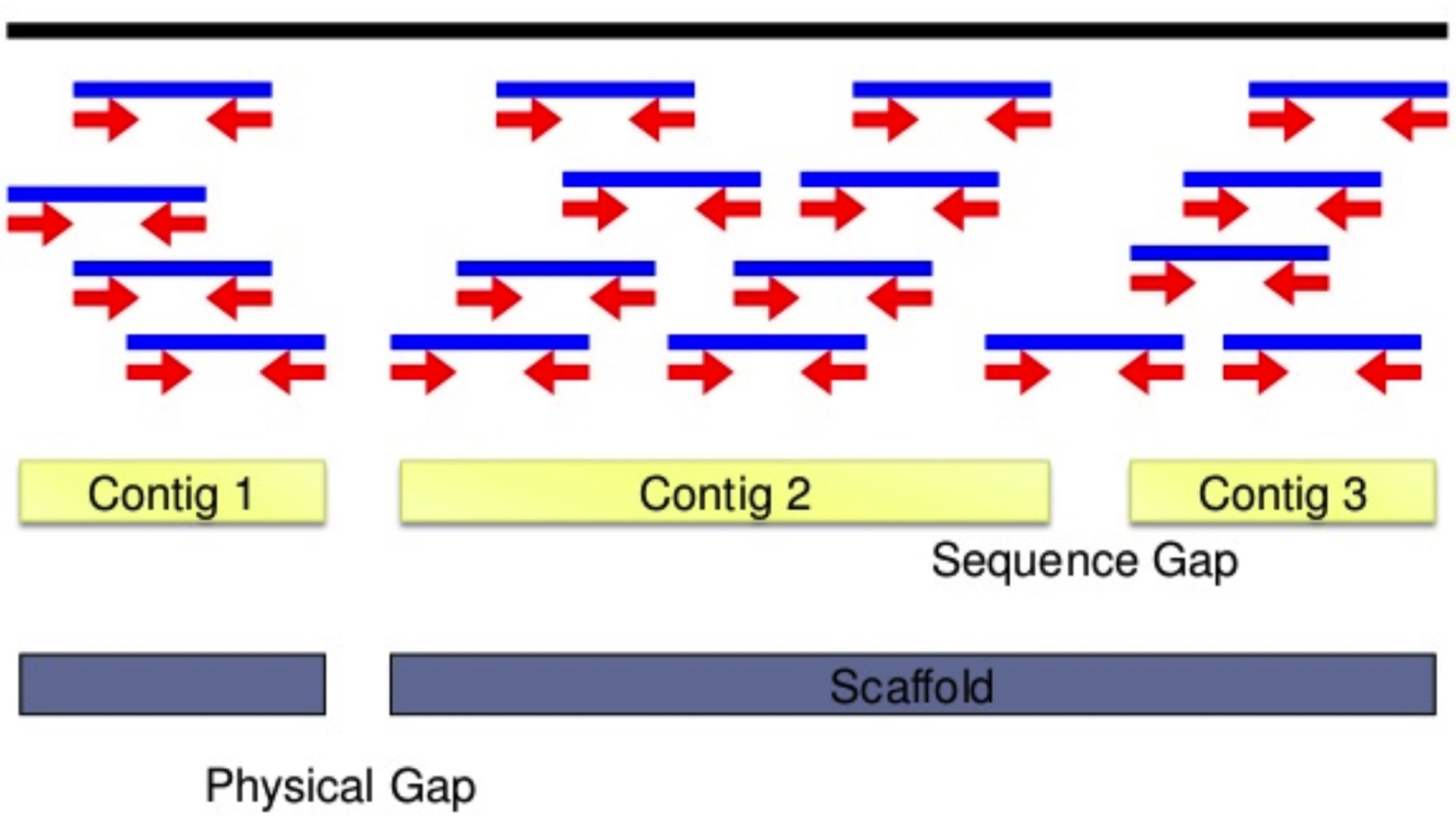
$$Q = -10 \log_{10} P$$

Phred Quality Score	Probability of Incorrect Base Call	Base Call Accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Figure 3 – Phred quality score chart

https://www.abmgood.com/marketing/knowledge_base/next_generation_sequencing_data_analysis.php

A *de novo* Short-Read Paired-End Genome Assembly



DNA Chromosome e.g. “truth”

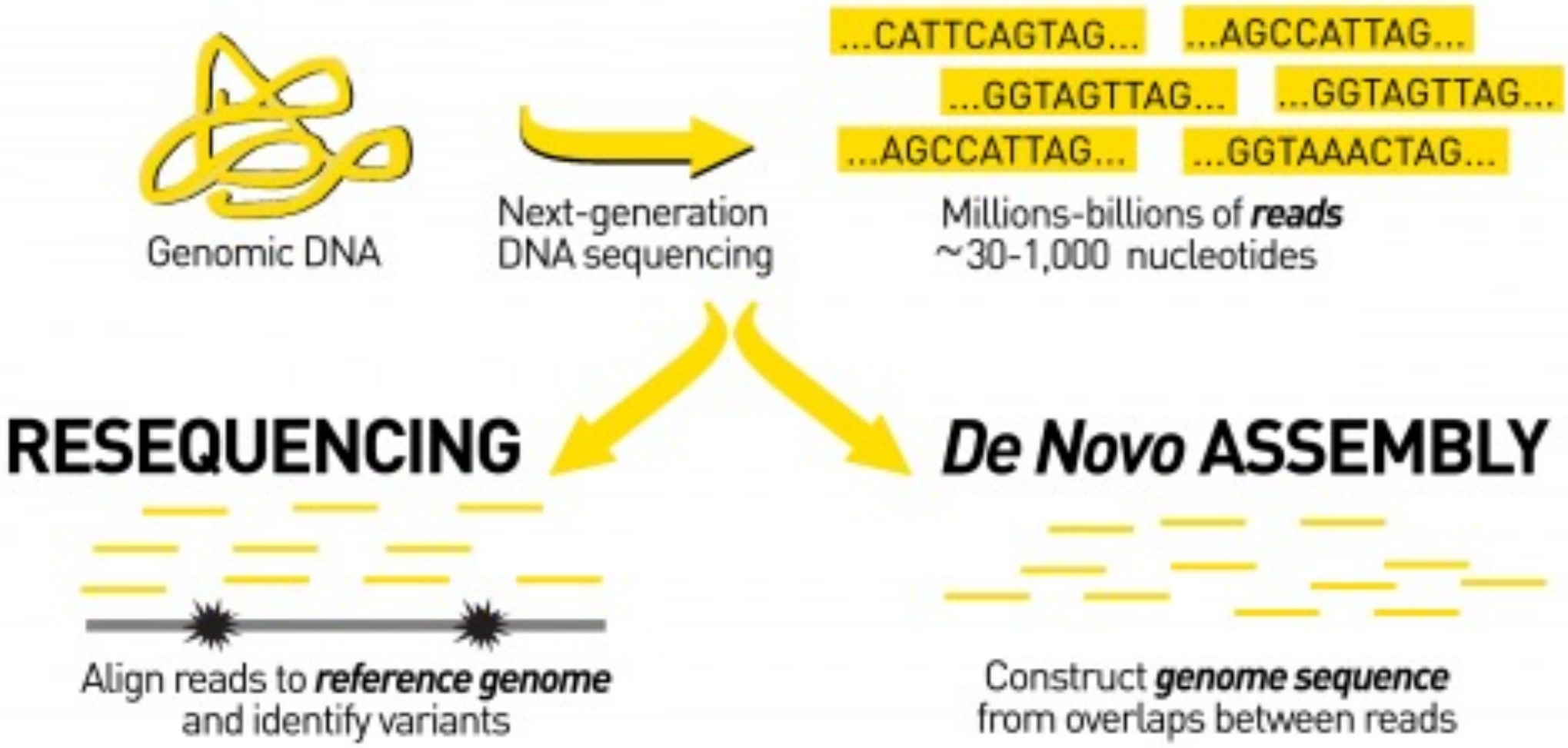
Paired-End Reads, Note the direction of the arrows

Assembled Contigs

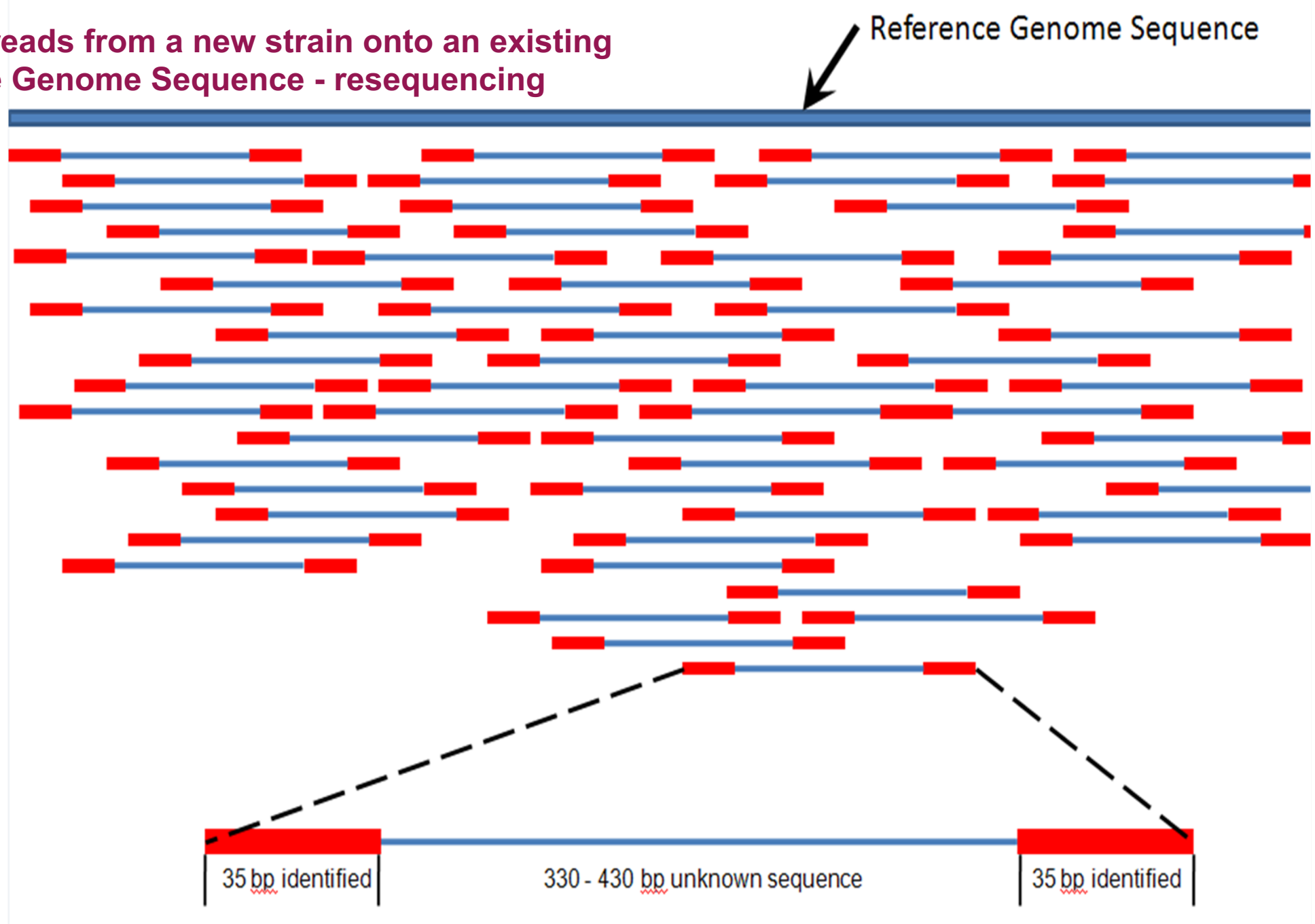
Assembled Scaffolds

https://github.com/Ecological-and-Evolutionary-Genomics/eeg2016/wiki/Mar-21-Exercise-7----SPAdes-assembler

We use genome sequences in 2 ways



Mapping reads from a new strain onto an existing Reference Genome Sequence - resequencing

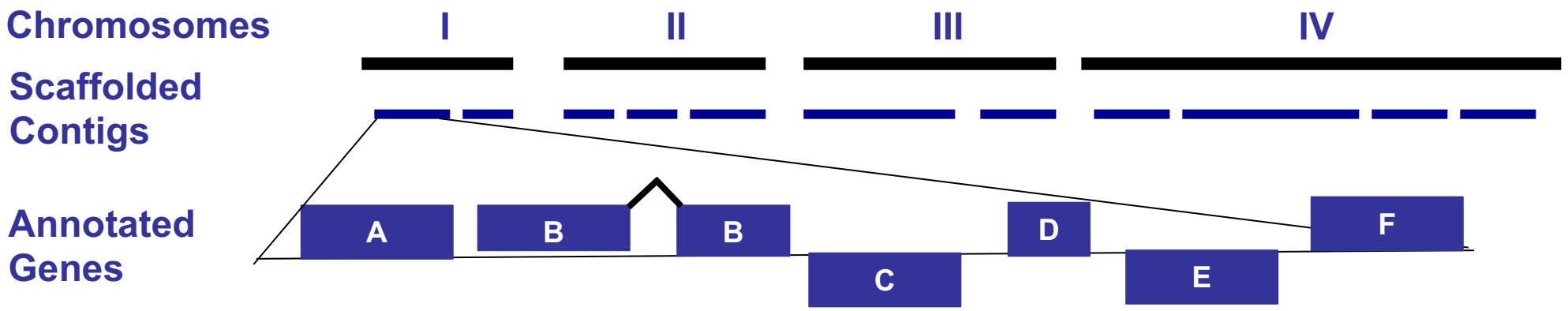


https://www.biostars.org/p/104218/

RNA or DNA reads mapped to a reference genome sequence provide insight into “coverage”, the number of reads mapping to a specific region

https://github.com/trinityrnaseq/NaplesWorkshop2016/wiki/Day_2

30,000 ft View – Genome Annotation



The Genome Sequence

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TTACCATCGAGTCACCACTCATAGTTGCGTGTGTCTACATGTTTTCTAGAACGTCCGTTGTGTTGCCTCGTGGCGACCGGCGCGAGTGTATGTACCCTGCGCTGTCAGAAGTTGATCCTT

Genes can be located on either DNA strand Convention - Gene location = non-template strand, i.e. the sequence of the gene is the same as the mRNA (except U = T in DNA)

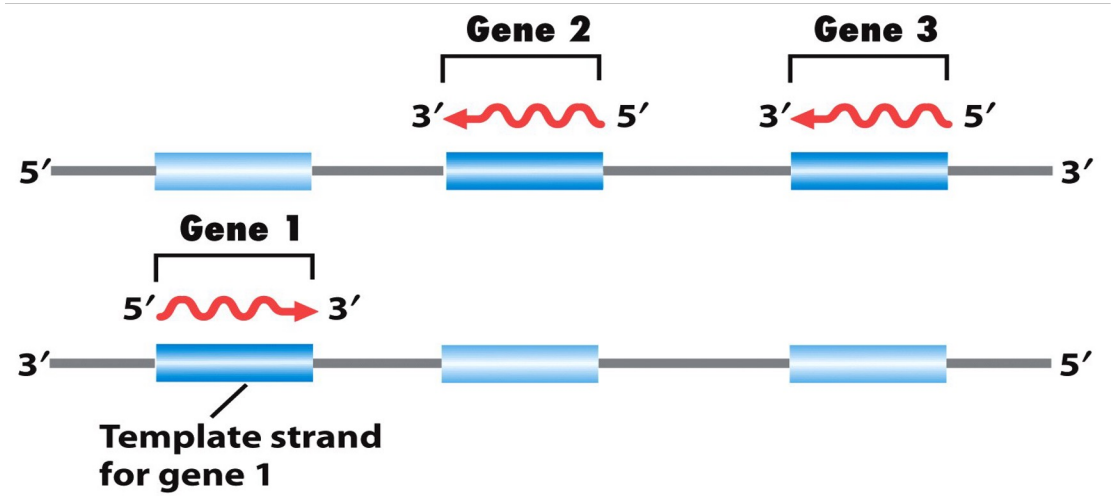


Figure 8-3
Introduction to Genetic Analysis, Ninth Edition

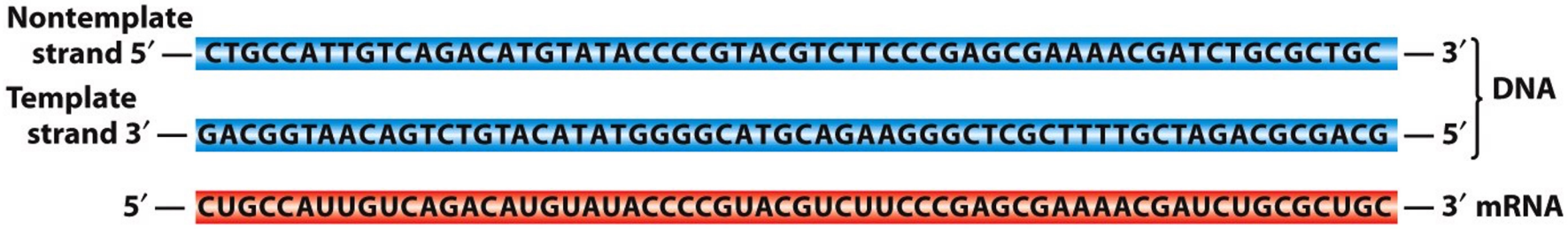
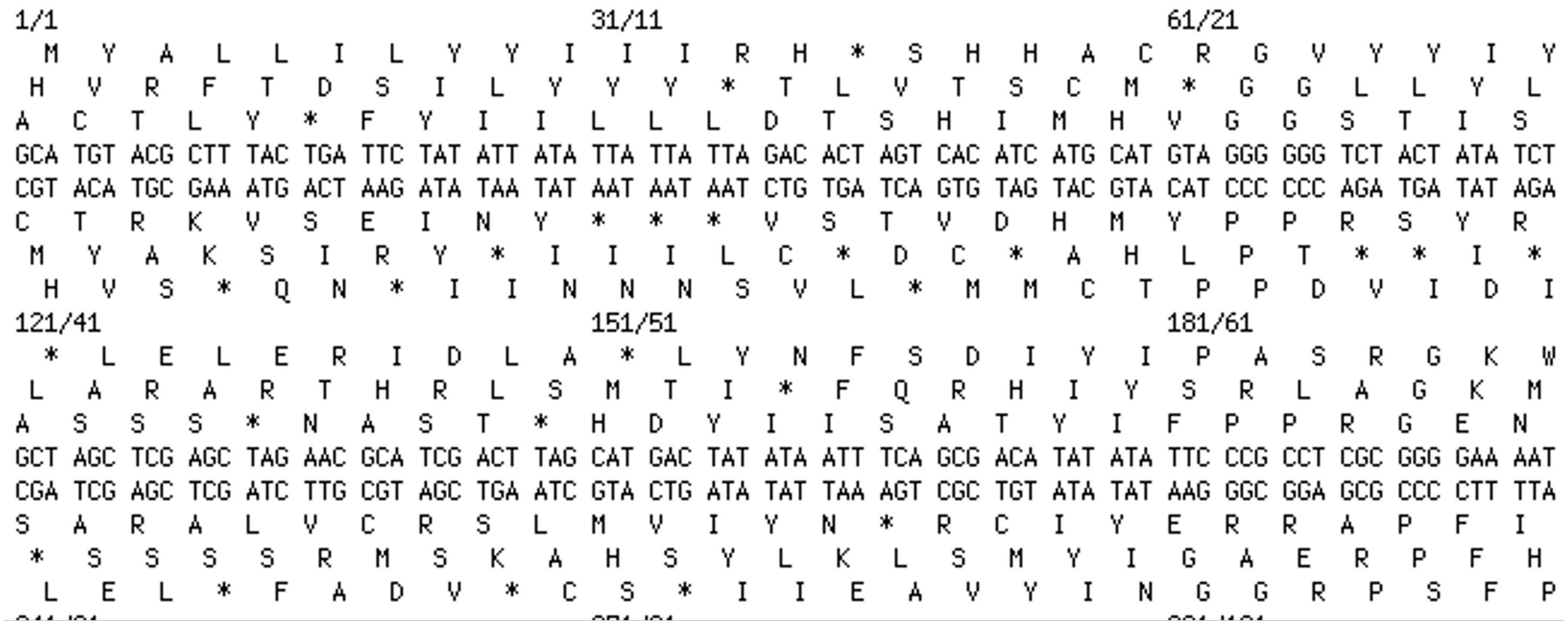


Figure 8-6
Introduction to Genetic Analysis, Ninth Edition

Six Frame Translation
Looking for Open Reading Frames, ORFs



ORFs ≠ Genes – but they can be part of a gene

Green = UTRs Red = CDS Pink = Intron

The "Coding Sequence" - CDS

ATGCAGAAACCGGTGTGTCTGGTCGTCGCGATGACCCCCAAGAGGGGCATCGGCATCAACAACGGCCTCCCGTGGCCCC
ACTTGACCACAGATTTCAAACACTTTTCTCGTGTGACAAAAACGACGCCCGAAGAAGCCAGTCGCCTGAACGGGTGGCT
TCCCAGGAAATTTGCAAAGACGGGCGACTCTGGACTTCCCTCTCCATCAGTCGGCAAGAGATTCAACGCCGTTGTCATG
GGACGGAAAACCTGGGAAAGCATGCCTCGAAAGTTTAGACCCCTCGTGGACAGATTGAACATCGTCGTTTCCTCTTCCC
TCAAAGAAGAAGACATTGCGGCGGAGAAGCCTCAAGCTGAAGGCCAGCAGCGCGTCCGAGTCTGTGCTTCACTCCCAGC
AGCTCTCAGCCTTCTGGAGGAAGAGTACAAGGATTCTGTCGACCAGATTTTTGTCGTGGGAGGAGCGGGACTGTACGAG
GCAGCGCTGTCTCTGGGCGTTGCCTCTCACCTGTACATCACGCGTGTAGCCCGCGAGTTTCCGTGCGACGTTTTCTTCC
CTGCGTTCCCCGGAGATGACATTCTTTCAAACAAATCAACTGCTGCGCAGGCTGCAGCTCCTGCCGAGTCTGTGTTCGT
TCCCTTTTGTCCGGAGCTCGGAAGAGAGAAGGACAATGAAGCGACGTATCGACCCATCTTCATTTCCAAGACCTTCTCA
GACAACGGGGTACCCTACGACTTTGTGGTTCTCGAGAAGAGAAGGAAGACTGACGACGCAGCCACTGCGGAACCGAGCA
ACGCAATGAGCTCCTTGACGTCCACGAGGGAGACAACTCCCGTGCACGGGTTGCAGGCTCCTTCTTCGGCCGCAGCCAT
TGCCCCGGTGTTGGCGTGGATGGACGAAGAAGACCGGAAAAAACGCGAGCAAAAGGAACTGATTCGGGCCGTTCCGCAT
GTTCACTTTAGAGGCCATGAAGAATTCCAGTACCTTGATCTCATTGCCGACATTATTAACAATGGAAGGACAATGGATG
ACCGAACGG

>Translation Frame 1 **The Protein**

MQKPVCLVVAMTPKRGIGINNGLPWPHLTTDFKHFSRVTKTTPEEASRLN

GWLPRKFAKTGDSGLPSPSVGKRFNAVVMGRKTWESMPRKFRPLVDRLNI

VVSSSLKEEDIAAEKPQAEGQQRVRVCASLPAALSLLEEEYKDSVDQIFV

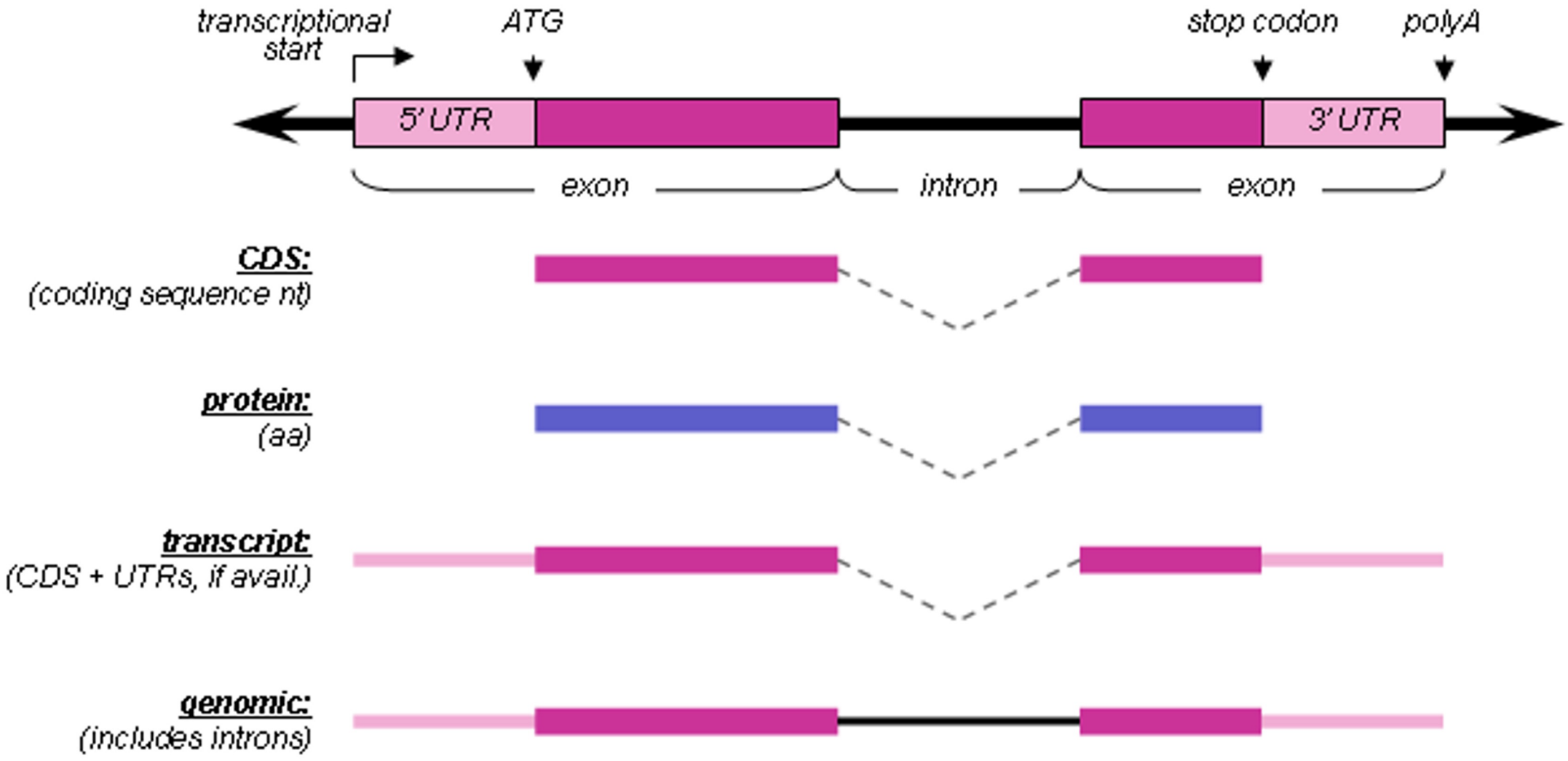
VGGAGLYEAALSLGVASHLYITRVAREFPCDVFFPAFPGDDILSNKSTAA

QAAAPAESVFVPFCPELGREKDNEATYRPIFISKTFSDNGVPYDFVVLEK

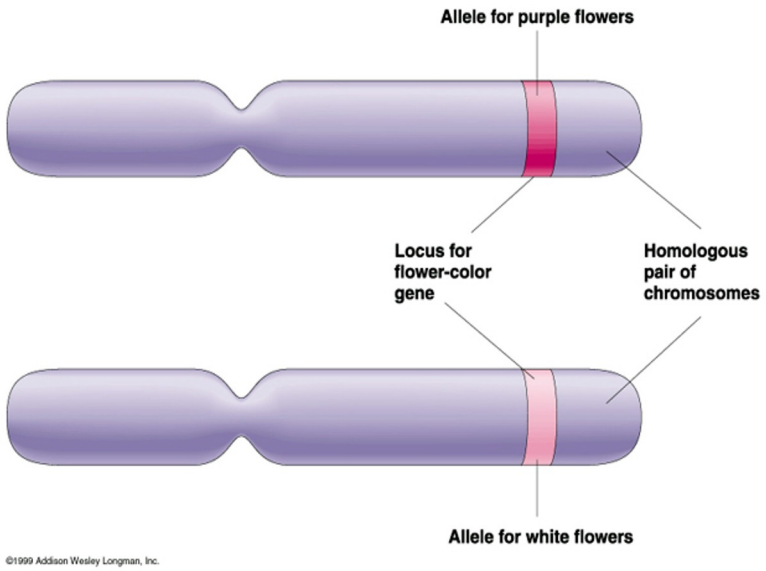
RRKTDDAATAEPSNAMSSLTSTRETTPVHGLQAPSSAAAIAPVLAWMDEE

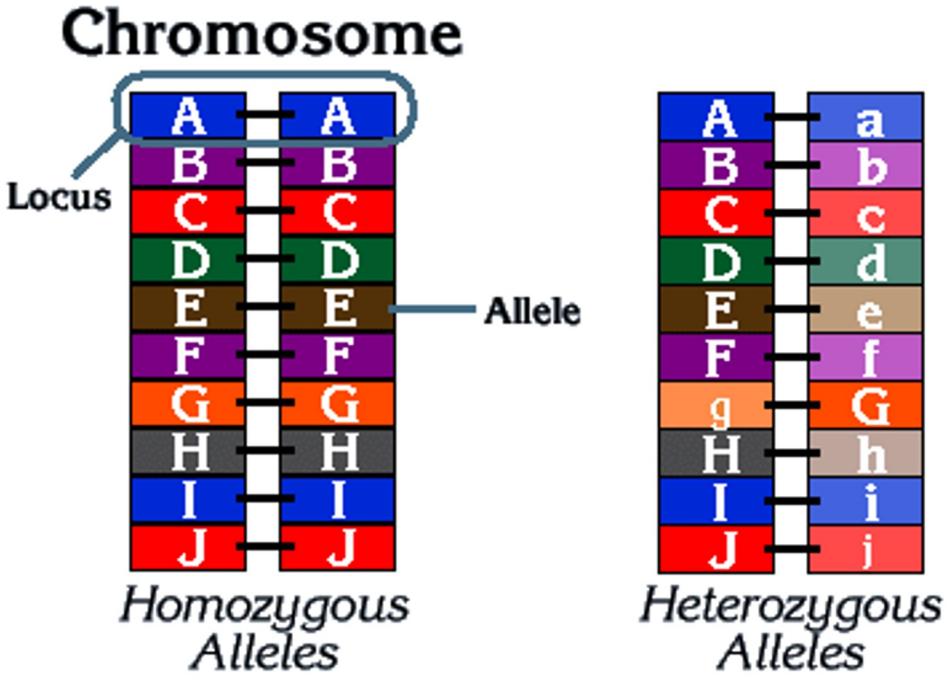
DRKKREQKELIRAVPHVHFRGHEEFQYLDLIADIINNGRTMDDRT

Terminology



Evolution Homologous chromsomes (in a diploid)



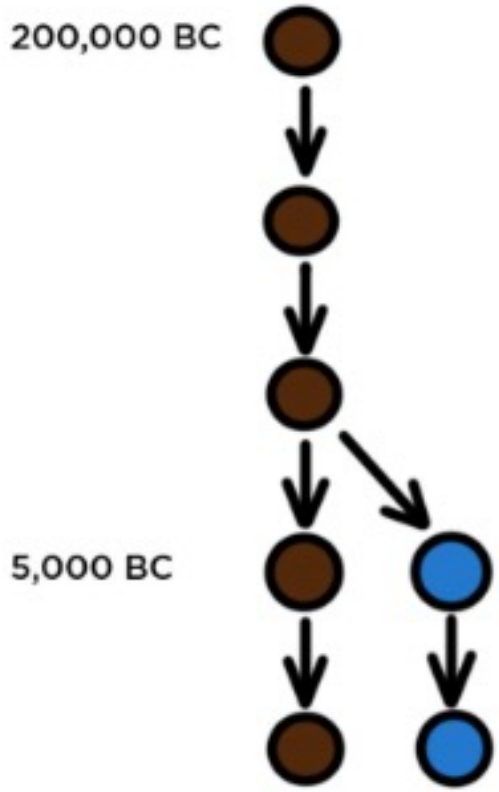


A AAGCCTCATC

a ACGCCTCATC

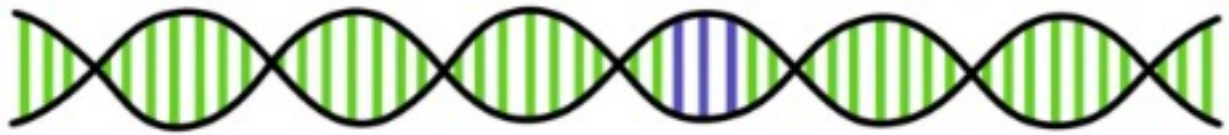
SNP =Single Nucleotide Polymorphism (a variant)

How Do Mutations Alter Allele Frequencies?



At the start of the human race, brown was the only eye color people could have.

The allele frequency for the allele that caused brown eyes was 1.0 (100%).



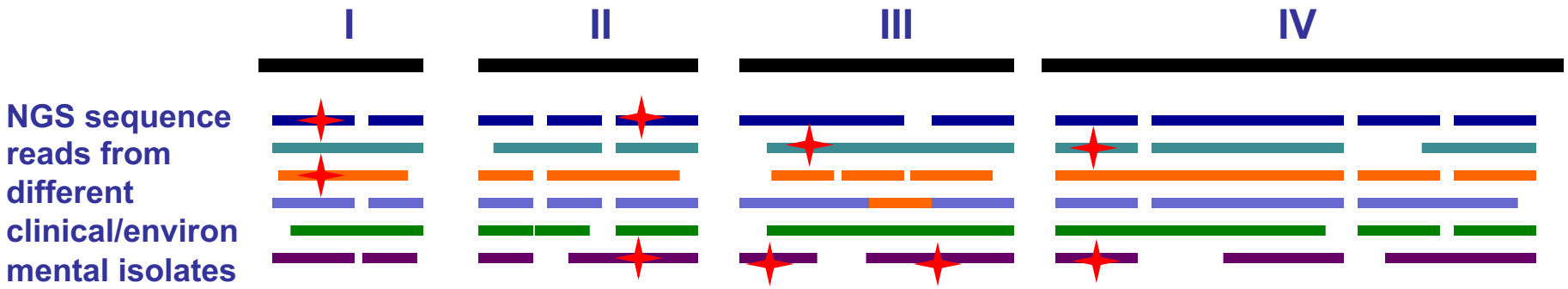
A mutation in the main gene for eye color created a new phenotype: blue eyes!

Slowly, the allele frequency shifted.

Since then, many variations of eye color have emerged, though brown eyes remain the most common.

Image source: By Gabi Slizewska

30,000 ft View- NGS SNPs



= SNP
= SNV

```
reference     TGGTGATACT AAGCTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTCA
303.1         TGGTGATACT AAGCTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTTA
309.1         TGATAATNCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
RV_3600       TGGTGATACT AAACTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTCA
RV_3606       TGATAATNCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
RV_3610       TGATGATTCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
SenT119.09    TGGTGATACT AAACTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTCA
SenT123.09    TGATRATTCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
SenT140.08    TGGTGATACT AAACTGGGAA CTCCACTTCC TTTTCTACTG CGGTGCTTCA
SenT142.09    TGGTGATACT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
SenT175.08    TGGTGATACT AAACTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTTA
```

Reference = A
6 isolate seq = A
2 isolate seq = T
2 isolate seq = N (no call)
% with base call = 80
Major allele = A
Major allele freq = 75% (6/8)

Reference = G
9 isolate seq = A
1 isolate seq = G
% with base call = 100
Major allele = A
Major allele freq = 90% (9/10)

Reference = C
8 isolate seq = C
2 isolate seq = T
% with base call = 100
Major allele = C
Major allele freq = 80% (8/10)

Alleles have frequencies in different populations

Population variation data

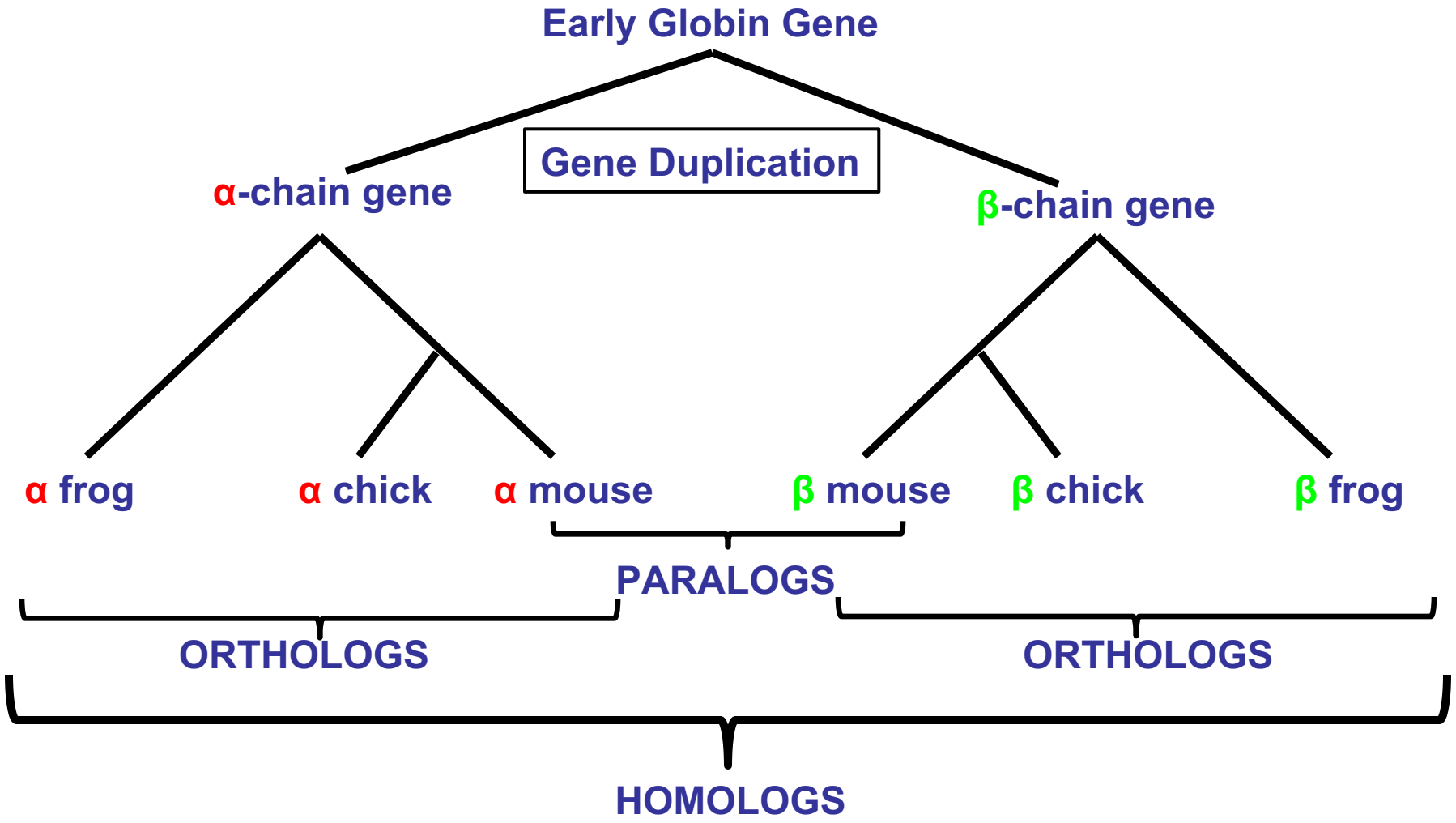
Data

- Single Nucleotide Polymorphisms, SNPs. SNVs
- Rearrangements
- Alleles
- Allele frequency
- Haplotypes (an organism's collection of variants)

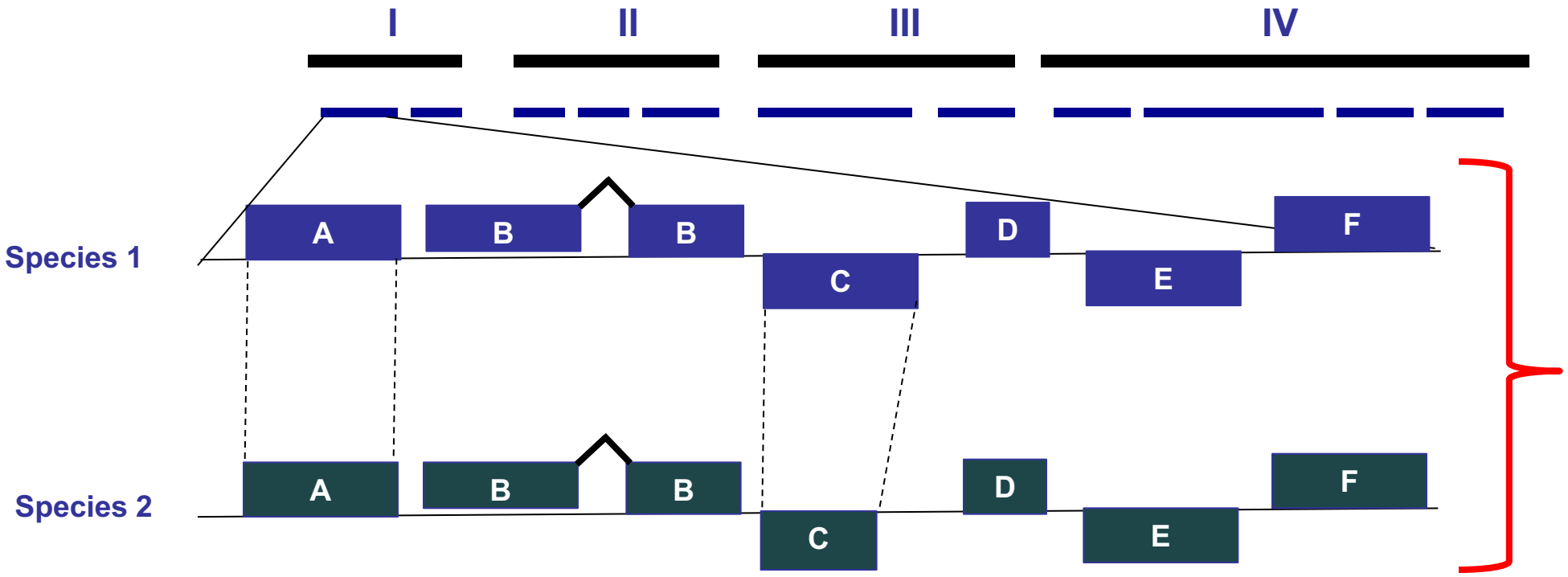
Technology

- Next Generation Sequencing, NGS
- Synteny (conserved positions on chromosomes)

Homology – a vocabulary for different types of evolutionary relationships



30,000 ft View - Synteny



Synteny = the majority of the same genes are present in the same order and orientation in another species. The chromosomal regions are evolutionarily related

Synteny among *Plasmodium* species

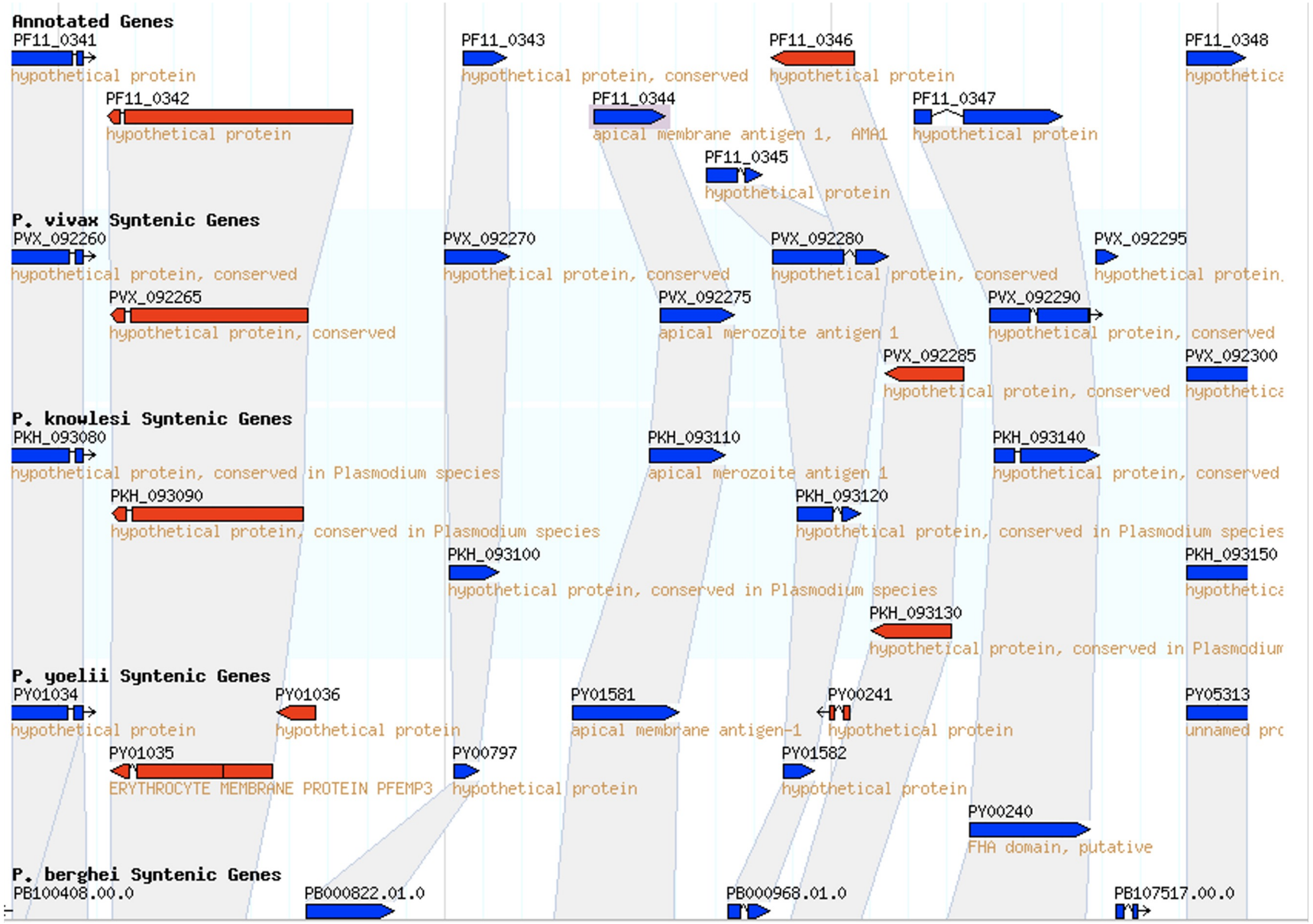
P. falciparum

P. vivax

P. knowlesi

P. yoelii

P. berghei



Synteny shows relationships in positioning: Ontologies show relationships in meaning

- The Gene Ontology – GO provides terms to link genes with similar functions and/or locations in the cell.

- An ontology was needed because the cultural traditions in different organisms led to different gene naming schemes that made it difficult to identify orthologous genes with the same function.

For Example:

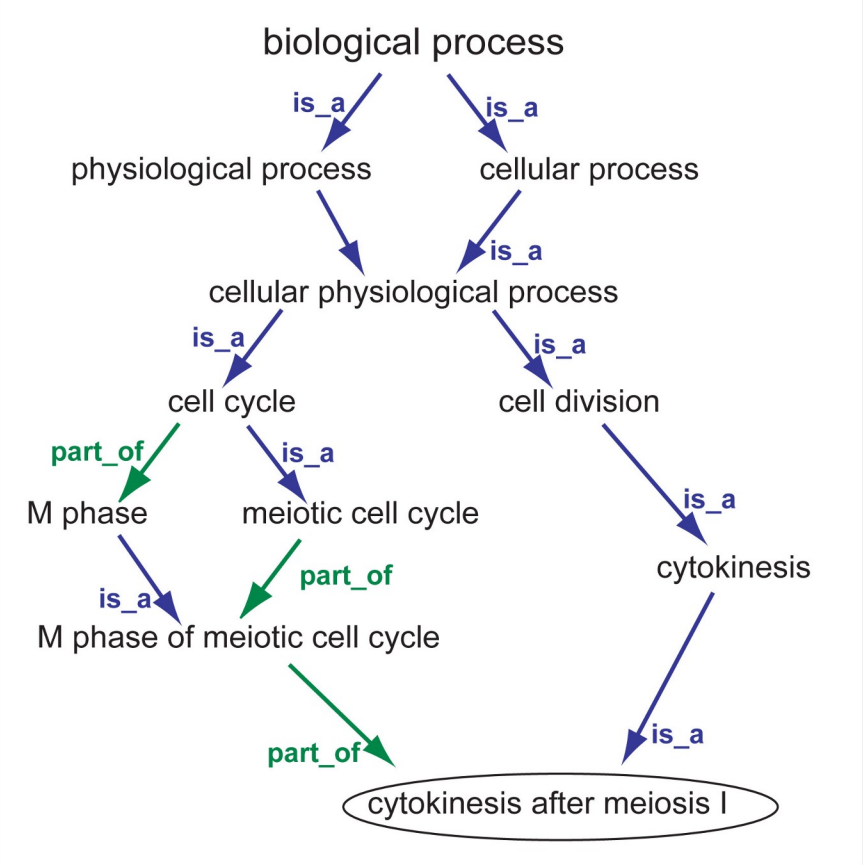
D. melanogaster gene CG3340 annotated as: "*Kruppel*" and *P. falciparum* gene PF3D7_1209300 annotated a "putative KROX1"

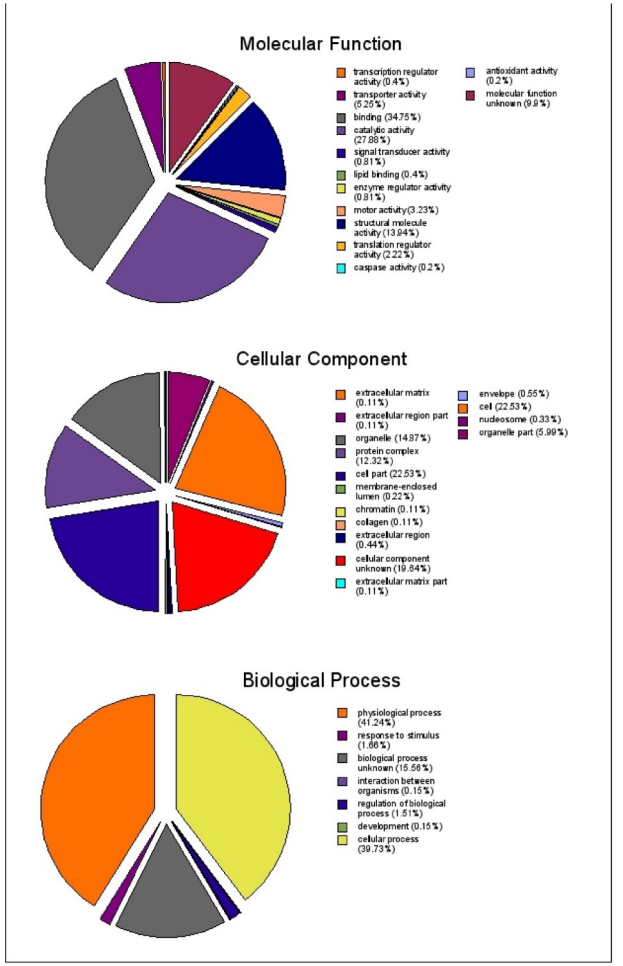
Both can be annotated with GO term:

GO:0003705 (RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity)

Both proteins, functionally, are Zinc Fingers despite their different names

Note that the Gene Ontologies themselves contain only information about terms in the ontology and their relationships to other terms





Gene expression

Expression Profiles (RNA and Protein)

- The pattern of expression of one or more genes over time or a set of experimental conditions, e.g. during development or a drug treatment or in a genetic mutant such as a gene knock-out.
- Always… has a time and location component, much like a photograph

RNA expression

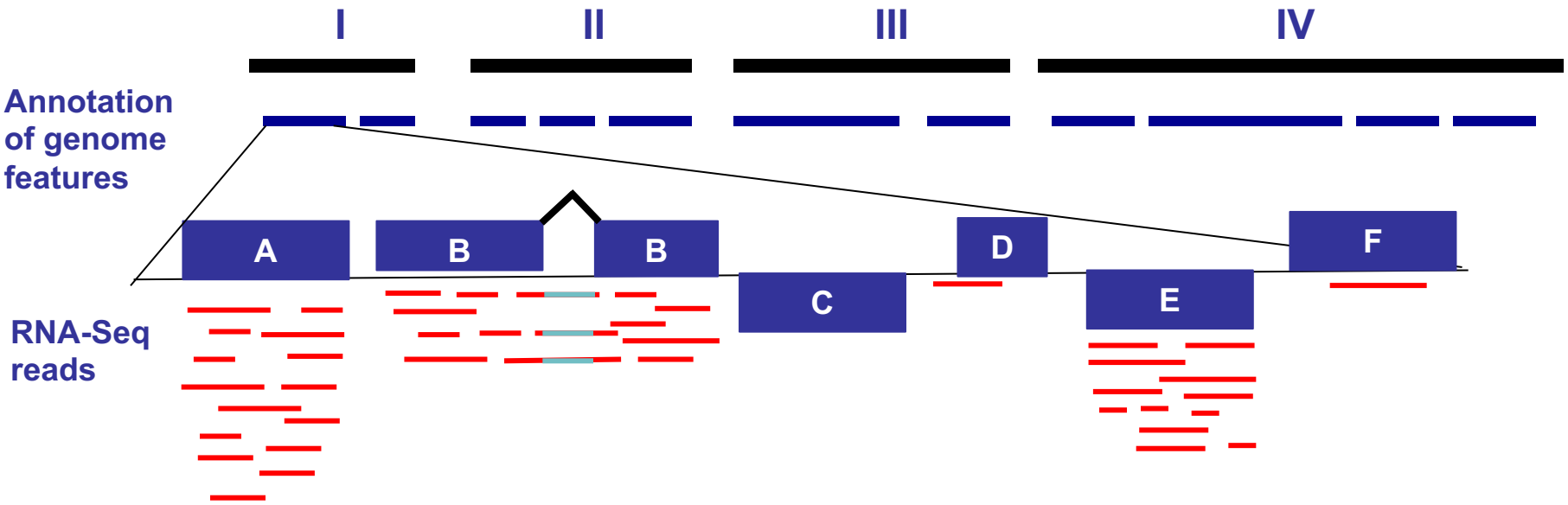
Bulk sequencing from many cells

- RNA-Seq (NGS)
  - Little sequence bias
  - Quantitative
  - Usually are strand-specific
- PacBio ISO-seq
  - Full-length transcripts from single molecules
- ONT Direct seq
  - Single-molecule, direct sequencing of RNA (or can sequence cDNA)
- All of these methods can be used to identify UTR's and exon splice junctions

Single-Cell Sequencing

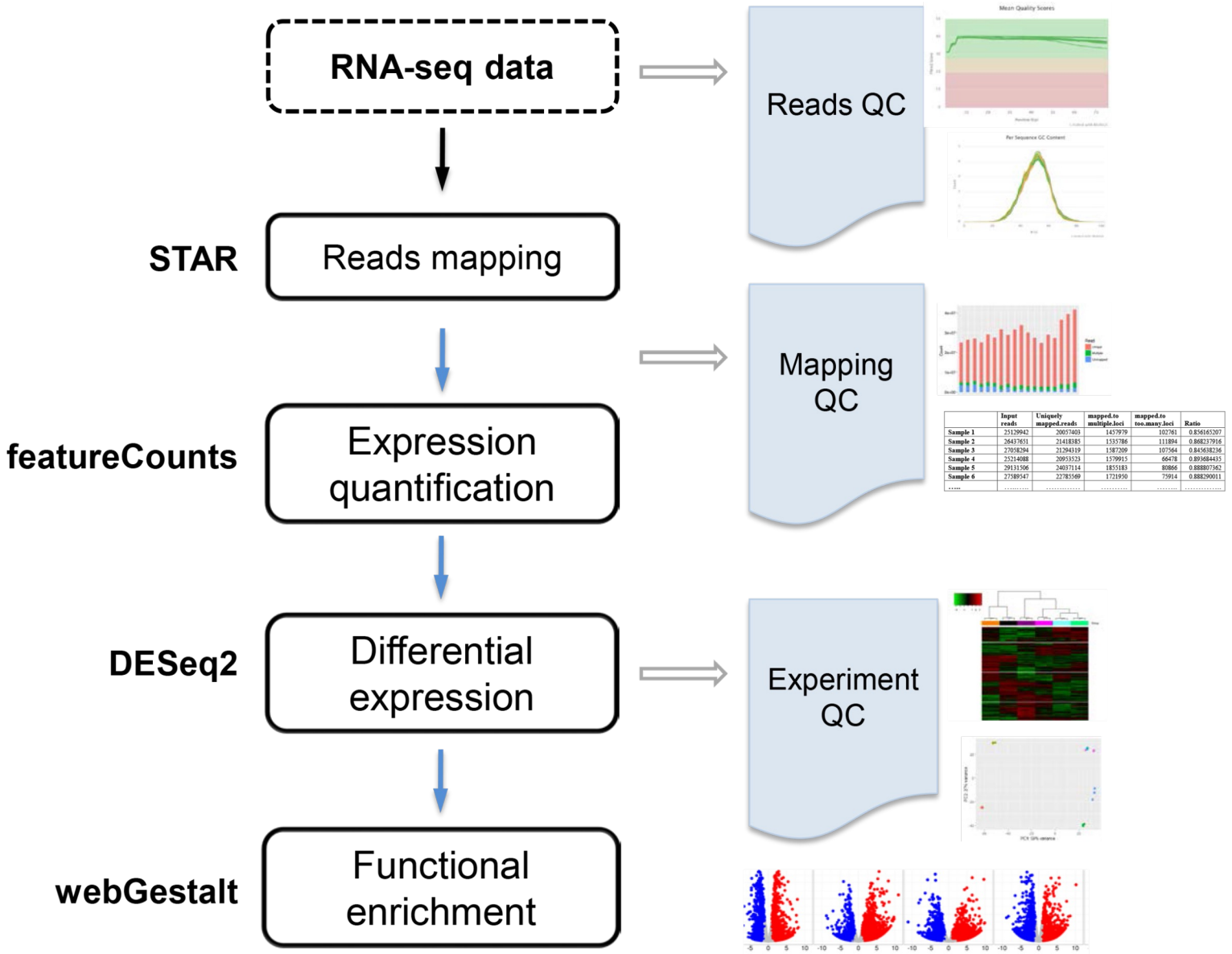
- Examines the transcriptome inside each cell analyzed
- Often only detects a fraction of the transcripts within a cell
- Often analyzed with tSNE plots to categorize cells that have similar transcriptional profiles.
- Excellent for detecting cellular heterogeneity or differentiation

30,000 ft View – RNA-Seq



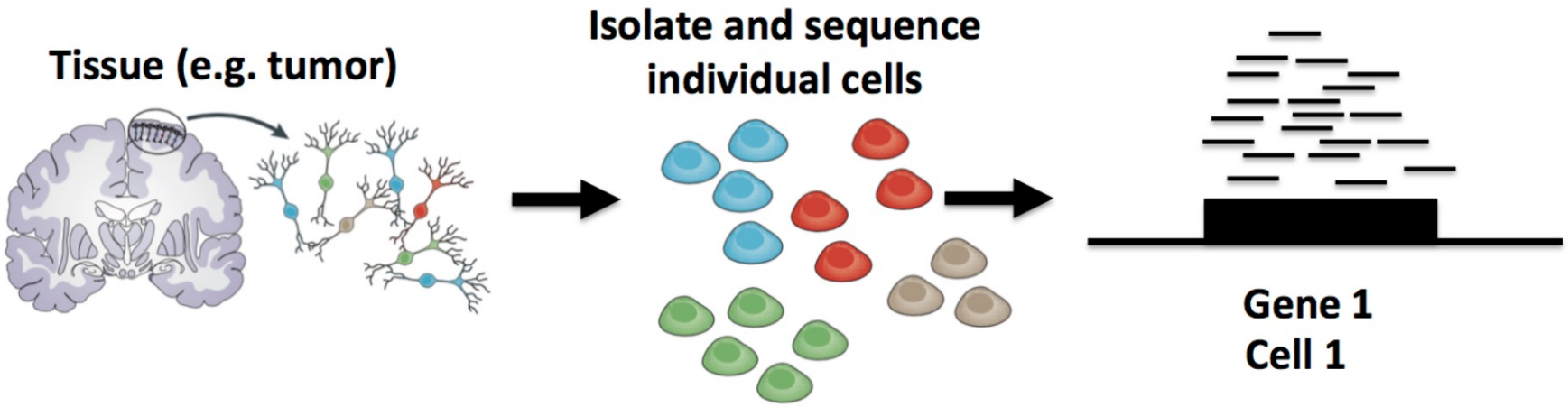
FPKM = Fragments per kilobase of exon per million fragments mapped (old calculation)
TPM = Transcripts per kilobase million (counts per length of transcript (kb) per million reads mapped

RNA-seq data are very powerful – you can see how expression changes over time or different conditions



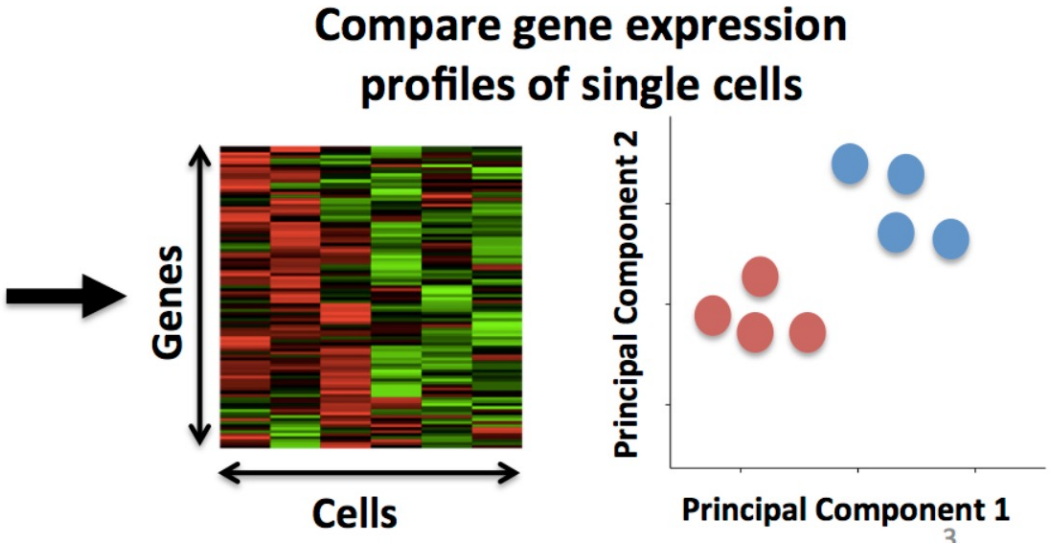
http://bioinfo.vanderbilt.edu/vangard/services-rnaseq.html

Single-cell RNA-Seq (scRNA-Seq)



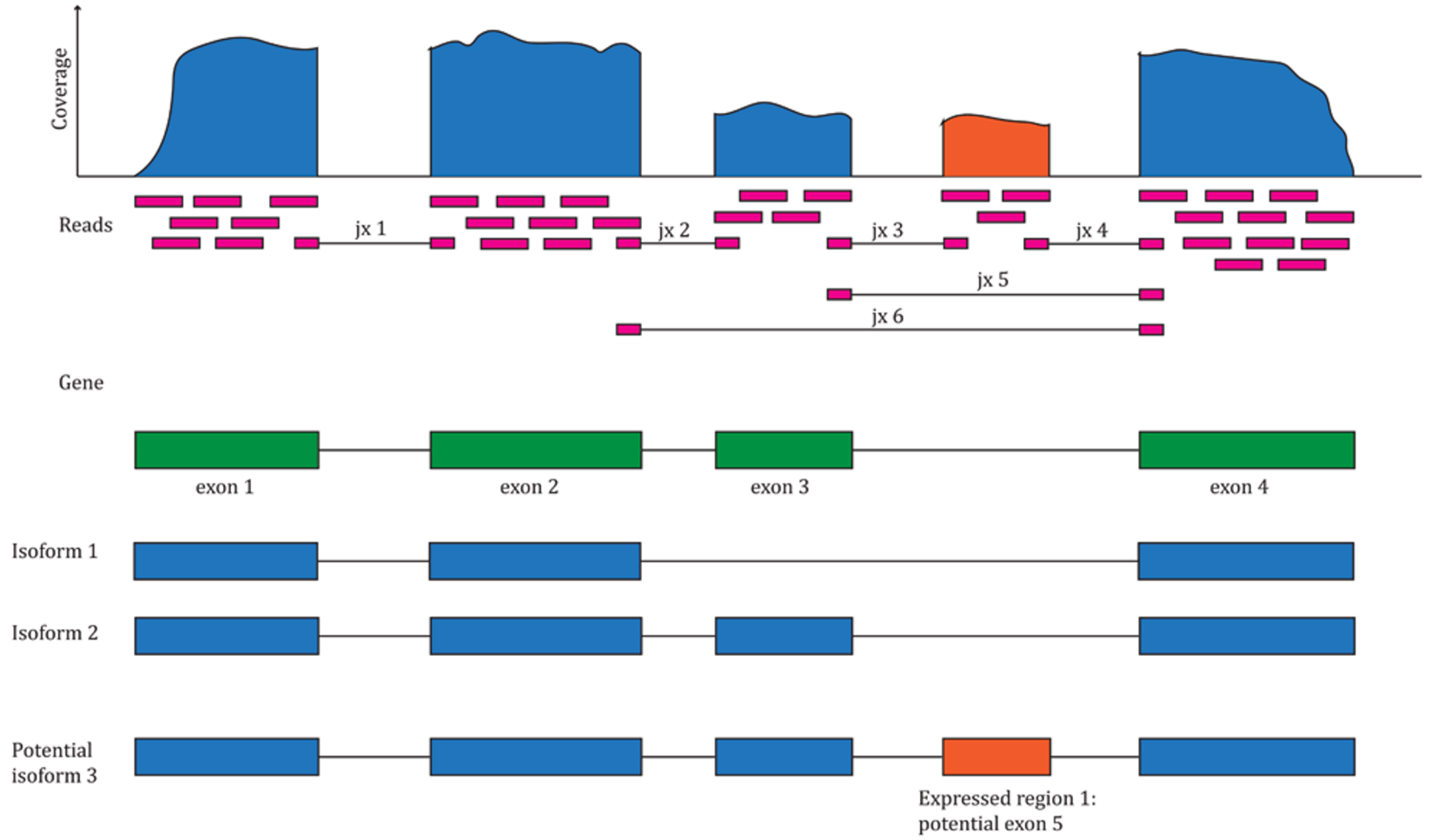
Read Counts

	Cell 1	Cell 2	…
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
…			



https://www.rna-seqblog.com/wp-content/uploads/2020/12/Single-Cell-RNA-Seq.jpg

RNA-seq identifies splice junctions if present (remember context dependent)



https://bioconductor.org/packages/devel/workflows/vignettes/recountWorkflow/inst/doc/recount-workflow.html

Complex patterns of eukaryotic mRNA splicing: What is a Gene?

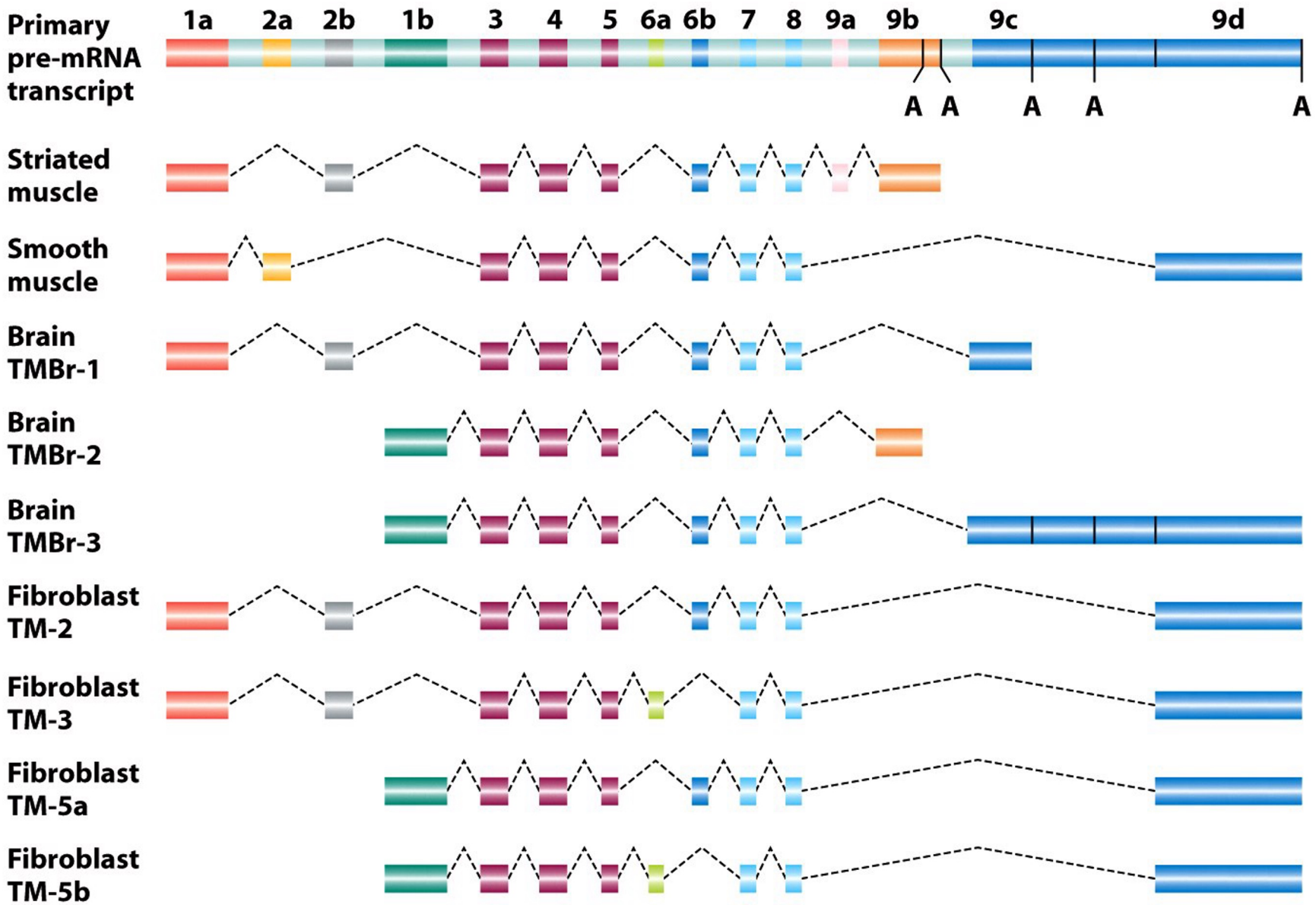
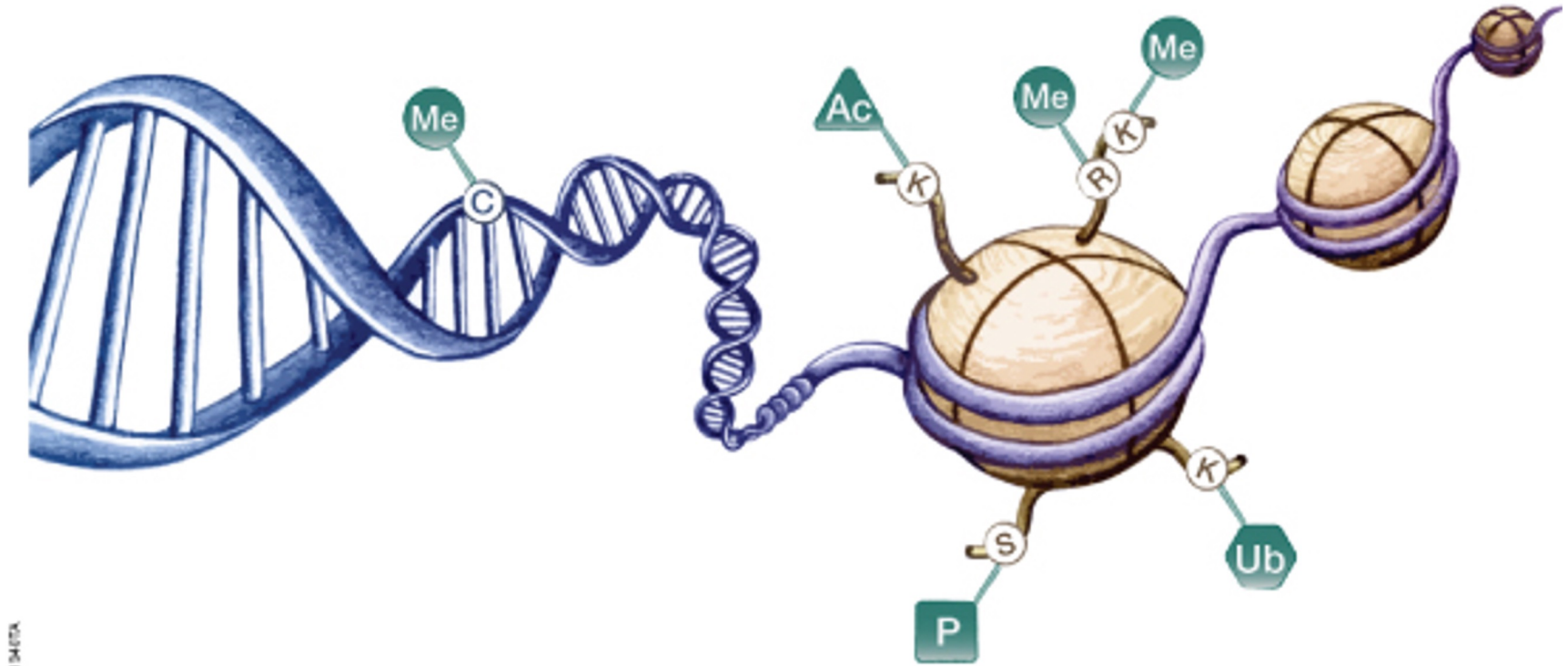


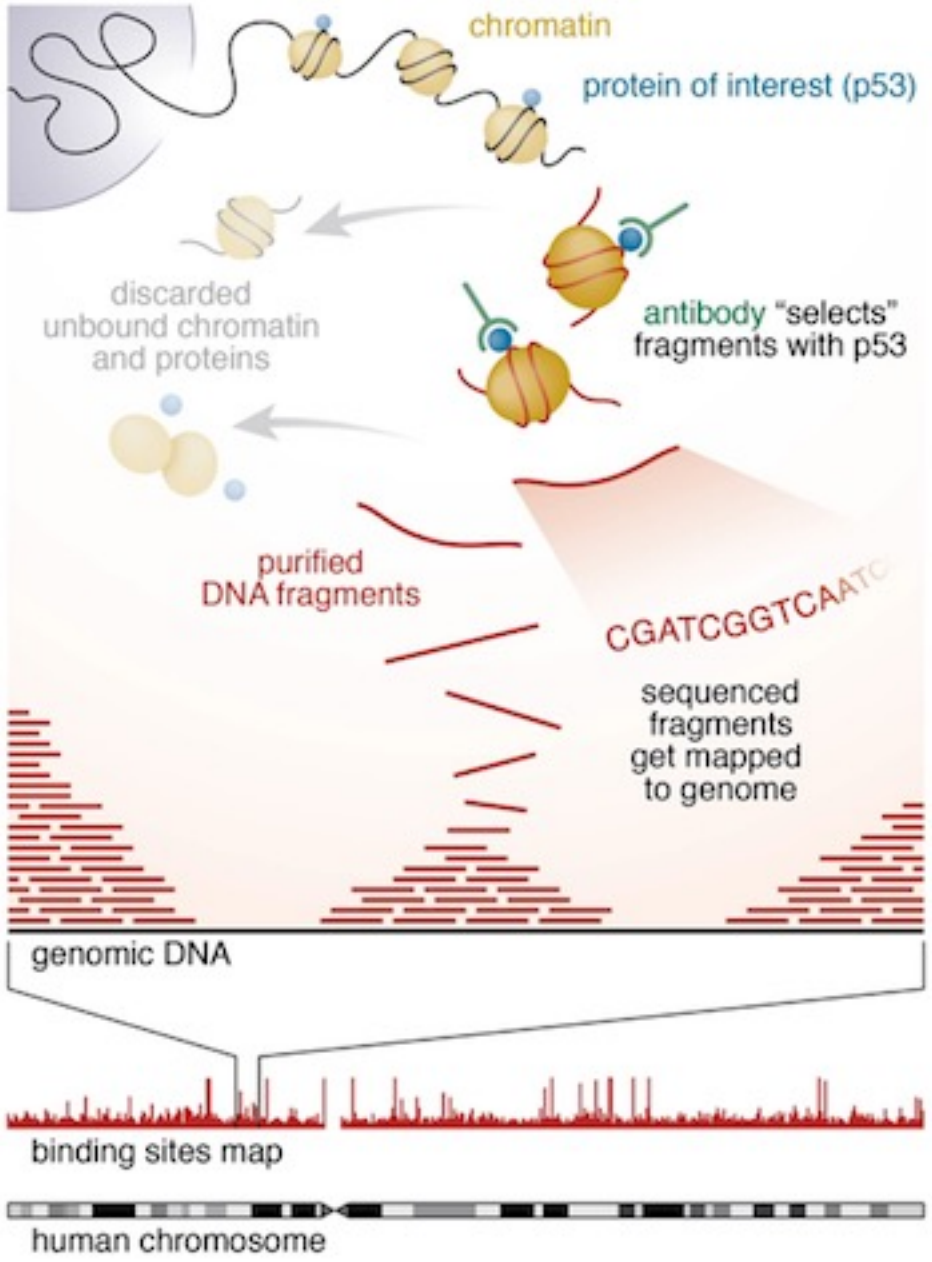
Figure 8-14
Introduction to Genetic Analysis, Ninth Edition

Chromatin Status and Epigenetic Gene Regulation



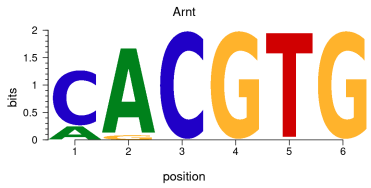
- DNA methylation at CpG islands –Bisulfite sequencing is a common assay
- H3K4me3 = transcriptionally active chromatin
- H3K27me3 = compact chromatin
- There are MANY other histone modifications
- ChIP-Seq (Chromatin ImmunoPrecipitation) is a common assay for histone markers

https://www.promega.com/resources/guides/nucleic-acid-analysis/introduction-to-epigenetics/



ChIP -Seq

- Chromatin Immuno Precipitation
- Can identify regulatory sequence motifs, e.g. transcription factor binding sites



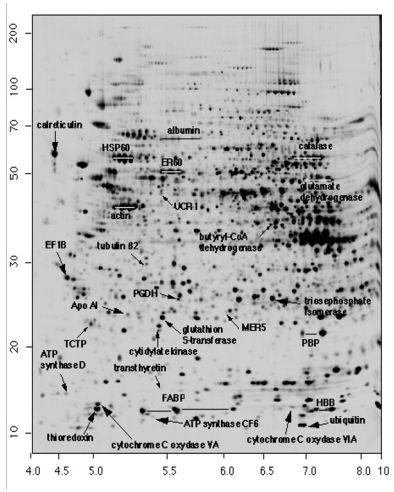
https://hbctraining.github.io/Intro-to-ChIPseq/lessons/01_Intro_chipseq_data_organization.html

Protein Expression/Sequence

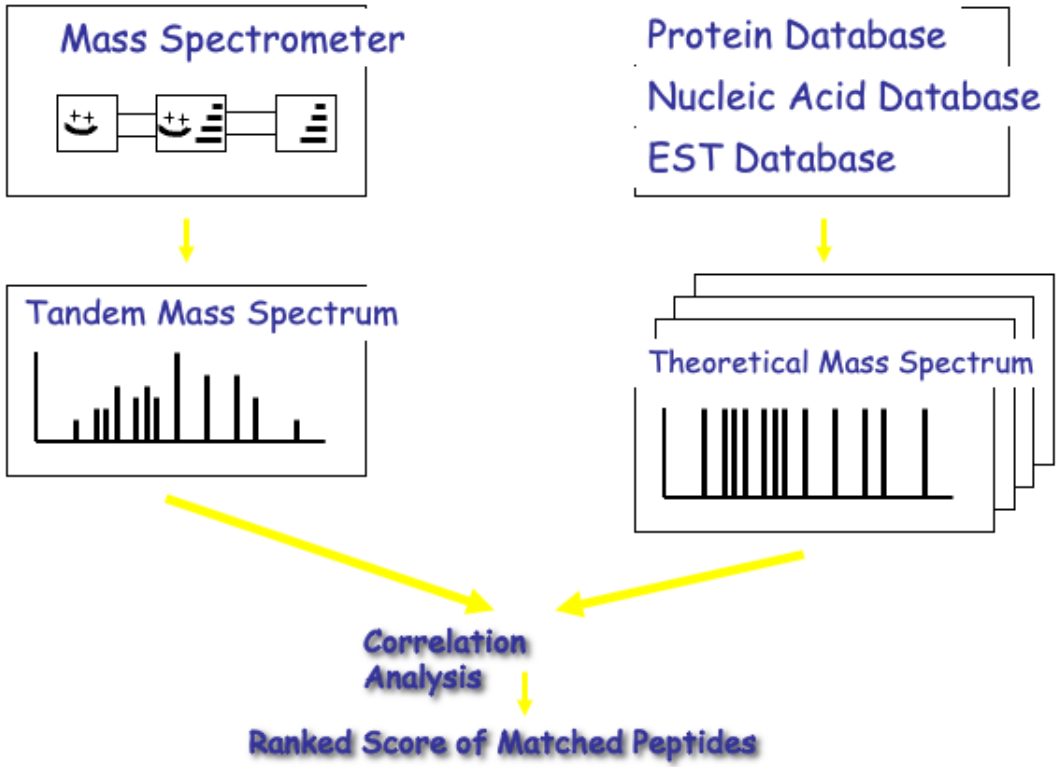
Technology

- 2D gel electrophoresis
- Mass spectrometry
- Tandem MS (MS-MS, LC MS-MS etc)

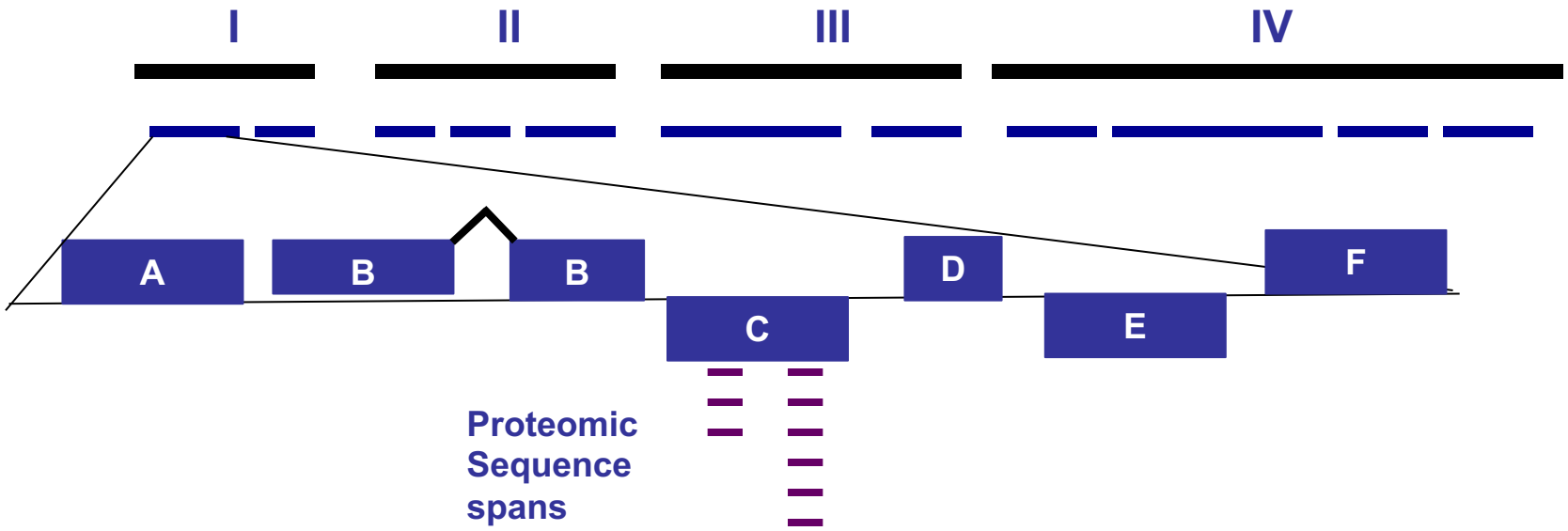
Typical 2 D gel



Tandem Mass Spectrometry analysis



30,000 ft View - Proteomics



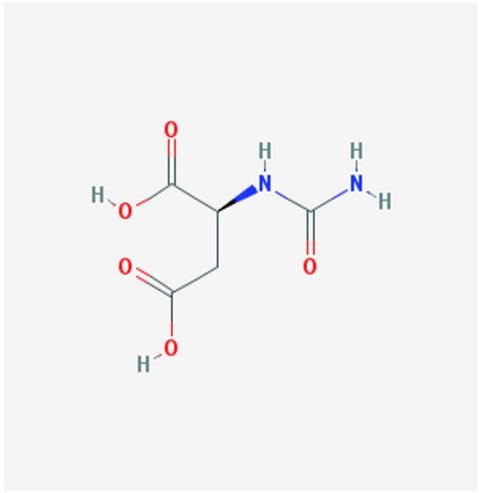
When looking at protein mass-spec sequences it is common to only detect parts of proteins. Some regions are refractory to detection, so don't be alarmed.

Metabolomics measures all metabolites

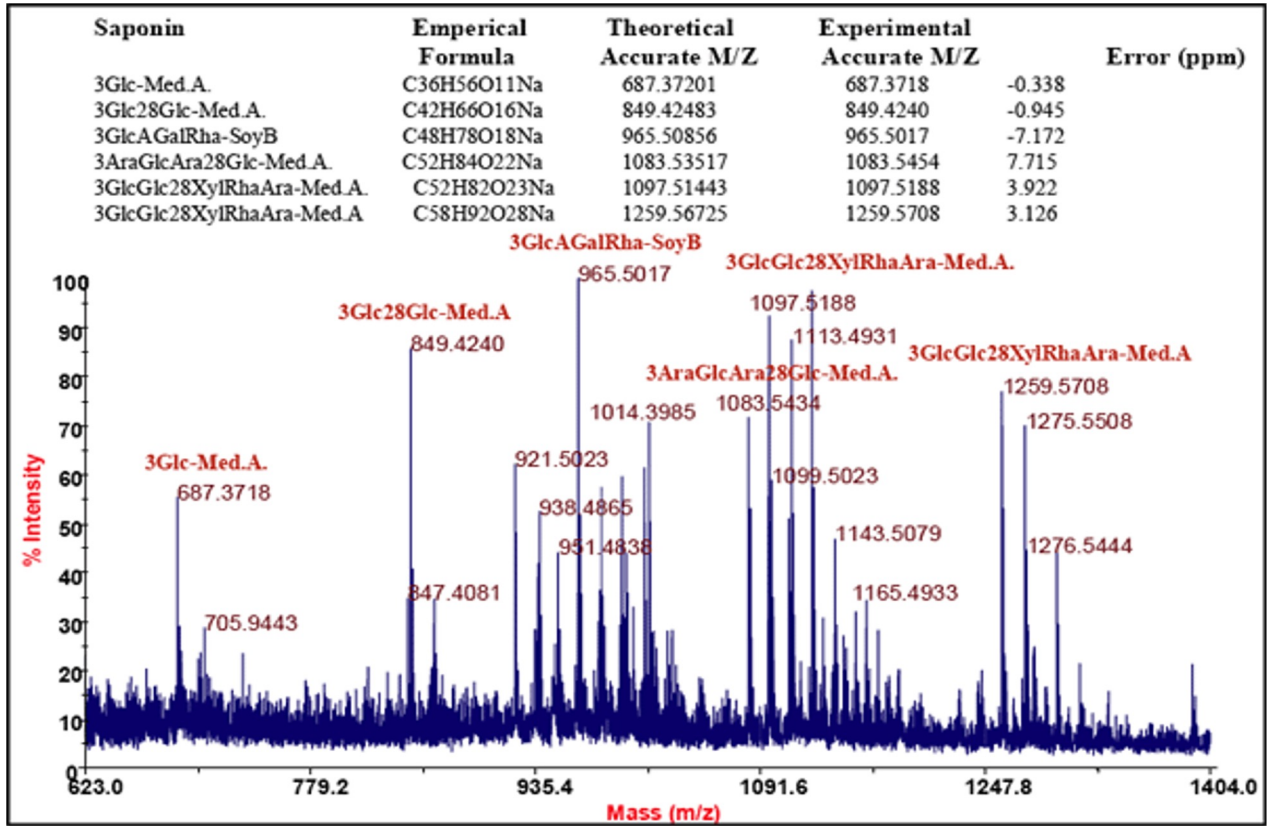
Overview

PubChem Compound ID:	CID:93072
PubChem Substance ID(s):	3727
Synonyms:	N-Carbamoyl-L-aspartate
Molecular Weight:	176.12742
Molecular Formula:	$C_5H_8N_2O_5$

2D Structure

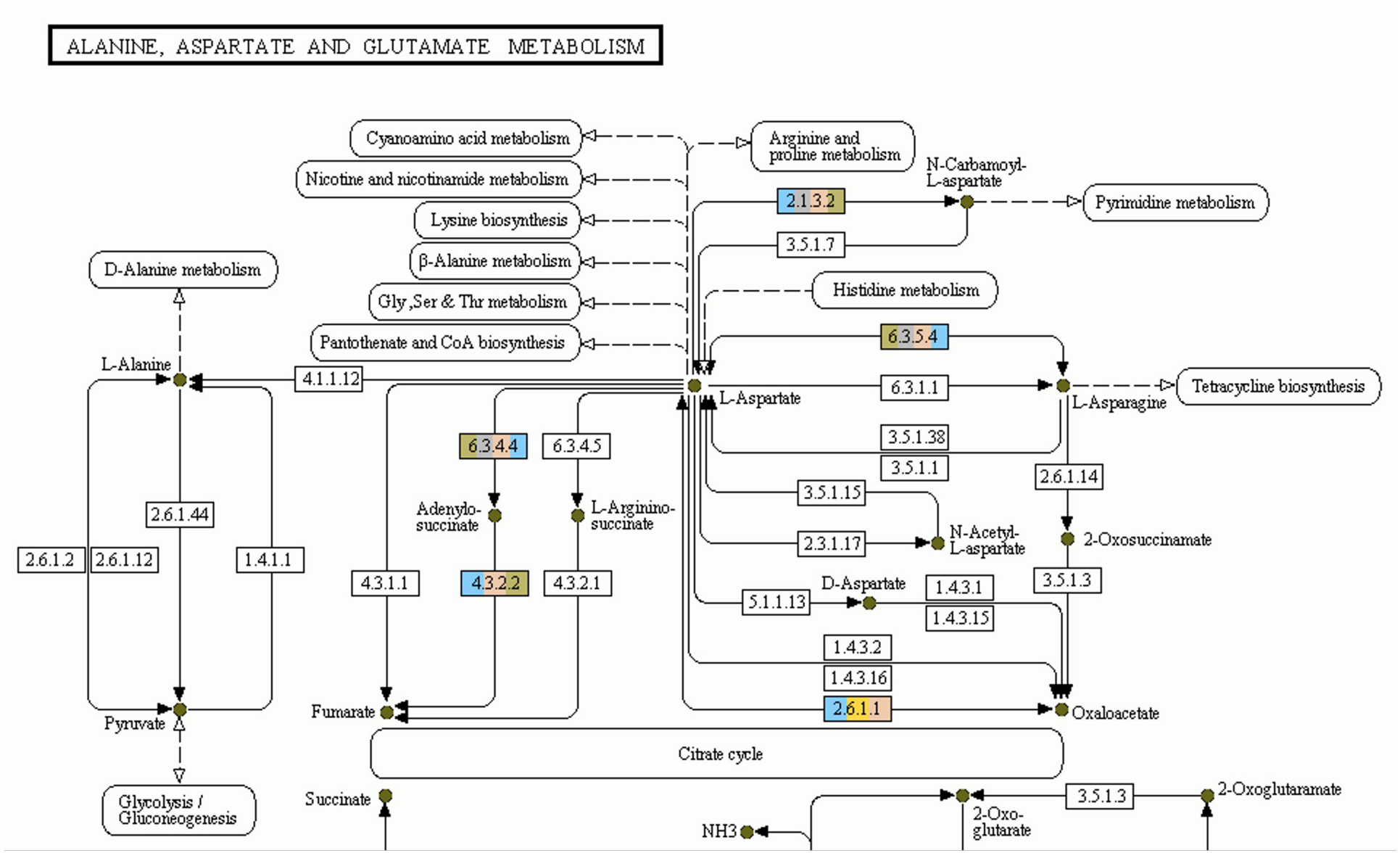


Saponin	Emperical Formula	Theoretical Accurate M/Z	Experimental Accurate M/Z	Error (ppm)
3Glc-Med.A.	C36H56O11Na	687.37201	687.3718	-0.338
3Glc28Glc-Med.A.	C42H66O16Na	849.42483	849.4240	-0.945
3GlcAGalRha-SoyB	C48H78O18Na	965.50856	965.5017	-7.172
3AraGlcAra28Glc-Med.A.	C52H84O22Na	1083.53517	1083.5454	7.715
3GlcGlc28XylRhaAra-Med.A.	C52H82O23Na	1097.51443	1097.5188	3.922
3GlcGlc28XylRhaAra-Med.A	C58H92O28Na	1259.56725	1259.5708	3.126



Mass Spectrometry can be used to measure metabolic and other chemical compounds

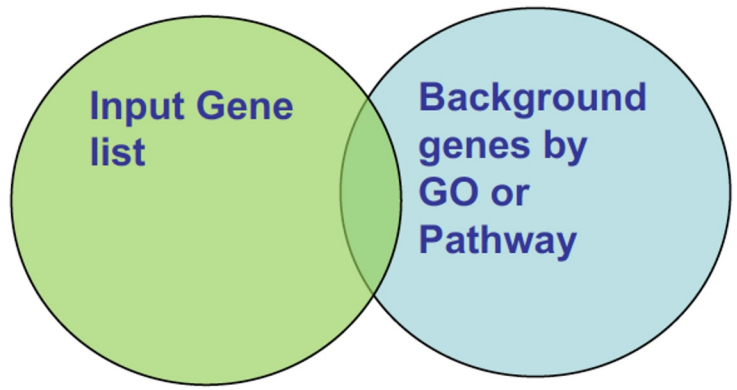
Metabolites can be linked to metabolic pathways and enzymes



Gene & Pathway Enrichment

Gene list:

Up/Down-regulated based on some experiment, e.g. RNA-Seq



Background-Pathway information: All genes known to be involved in some process, e.g. glycolysis or cell signaling. ALL pathways are examined

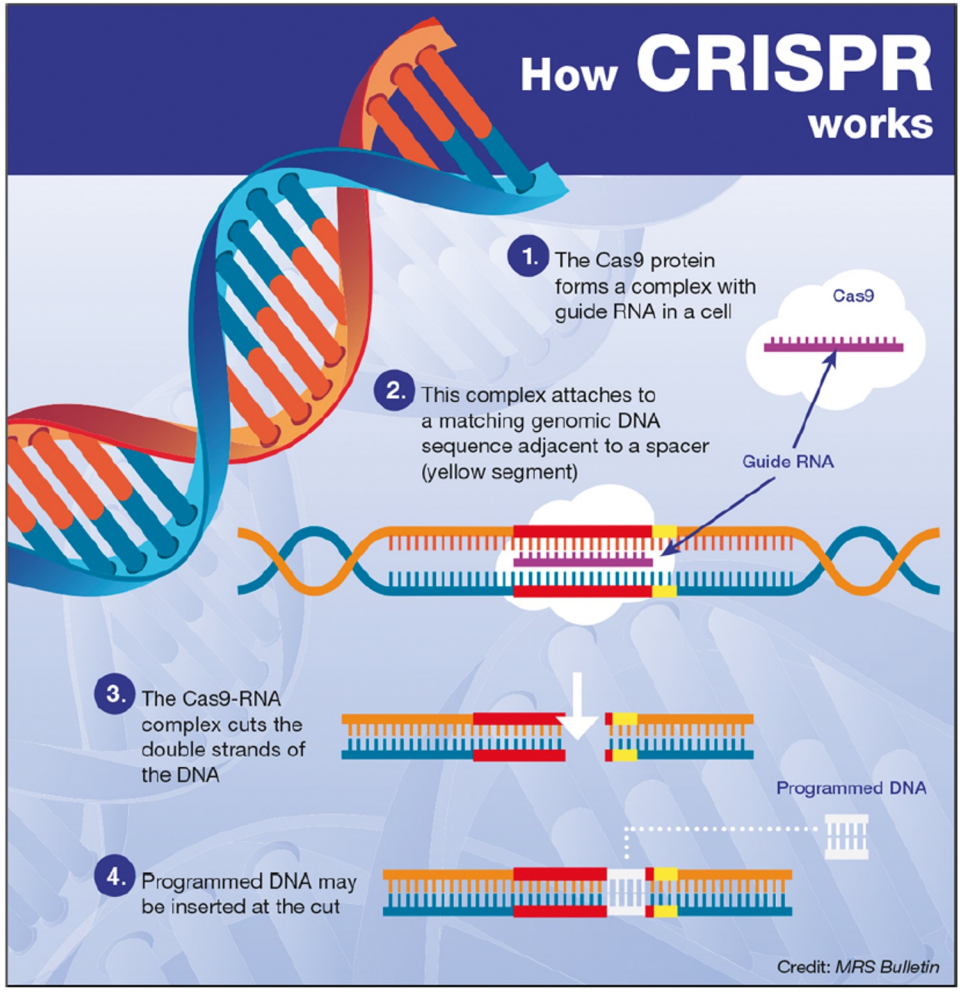
Result: GO:ID or Pathway ID that is enriched

Statistics: Are more genes observed than expected (P-value)
Multiple hypothesis testing (Bonferroni, Benjamini-Hochberg)

Atul Butte Review: http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1002375

CRISPR-CAS

Mutant analysis of one gene or <u>all</u> genes!



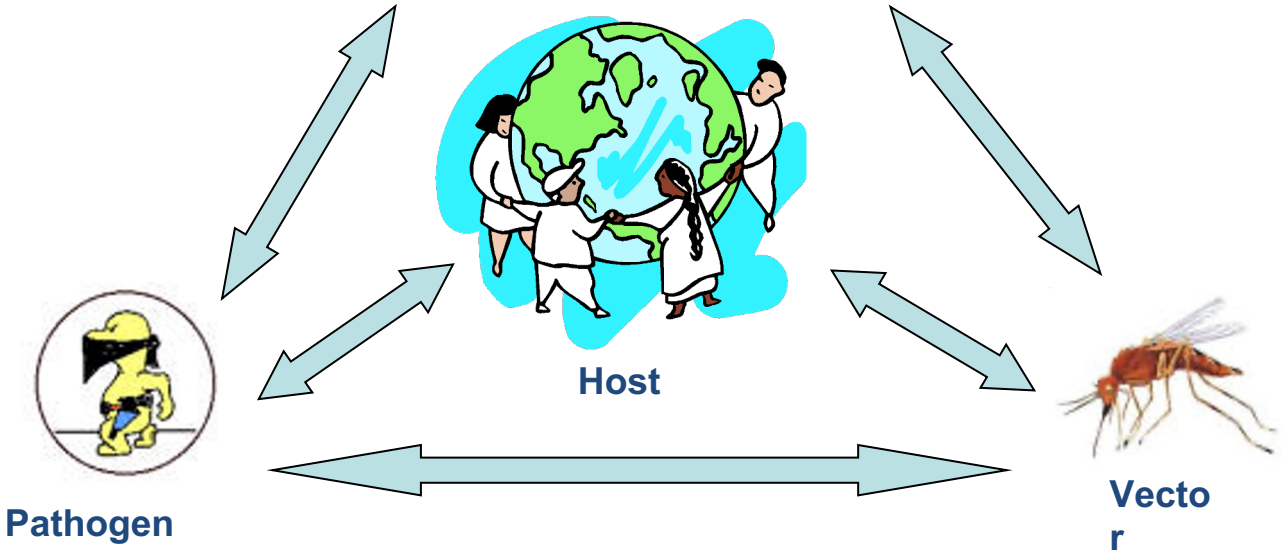
- Need to provide both the enzyme and the guide RNA to the cell
- Need to design the guide RNA to the gene of interest, ideally at multiple target locations per gene

Ball et al., MRS Bulletin November 2016

Host(s)

Infectious Disease Paradigm of Host-Pathogen Interactions

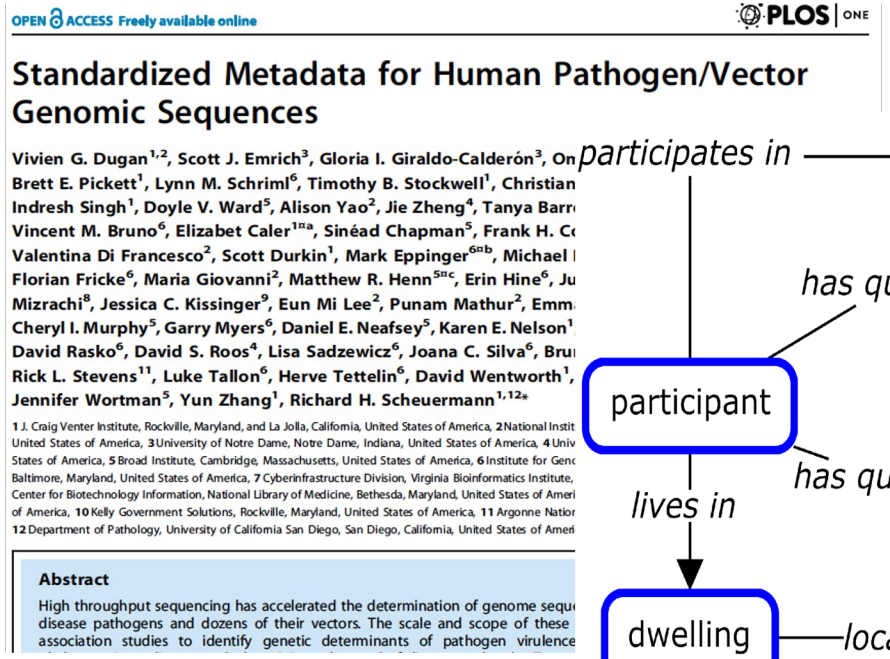
Experimental systems



Metadata – The next Frontier

- Data about the data are critical
- What makes a data set valuable? (The reason it was generated…but often this is missing)
- Introducing the "data set"
- How can you find the data set you need? Pull down Menu? A search of data set properties?
  - Person and technology that generated the data
  - Clinical outcome
  - Geographic location
  - Phenotype

Data sharing standards



OPEN ACCESS Freely available online

PLOS | ONE

Standardized Metadata for Human Pathogen/Vector Genomic Sequences

Vivien G. Dugan[1,2], Scott J. Emrich[3], Gloria I. Giraldo-Calderón[3], On
Brett E. Pickett[1], Lynn M. Schriml[6], Timothy B. Stockwell[1], Christian
Indresh Singh[1], Doyle V. Ward[5], Alison Yao[2], Jie Zheng[4], Tanya Barr
Vincent M. Bruno[6], Elizabeth Caler[1¤a], Sinéad Chapman[5], Frank H. C
Valentina Di Francesco[2], Scott Durkin[1], Mark Eppinger[6¤b], Michael
Florian Fricke[6], Maria Giovanni[2], Matthew R. Henn[5¤c], Erin Hine[6], Ju
Mizrachi[8], Jessica C. Kissinger[9], Eun Mi Lee[2], Punam Mathur[2], Emm
Cheryl I. Murphy[5], Garry Myers[6], Daniel E. Neafsey[5], Karen E. Nelson[1]
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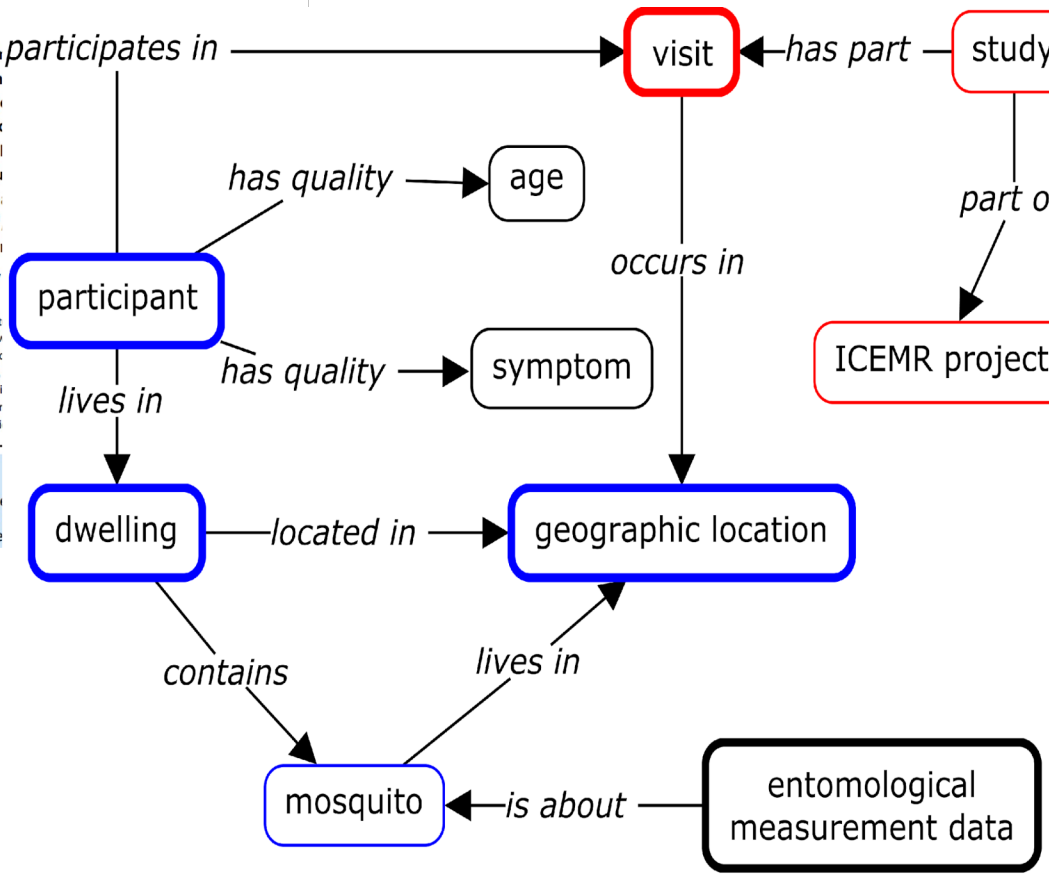
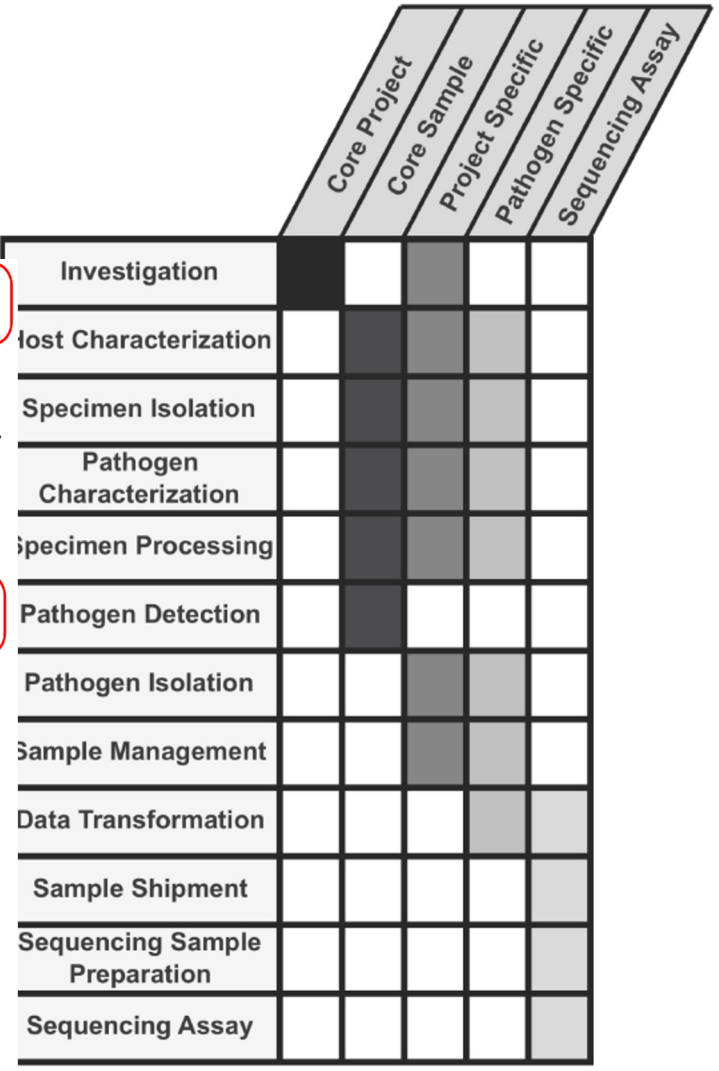
Abstract

High throughput sequencing has accelerated the determination of genome sequ
disease pathogens and dozens of their vectors. The scale and scope of these
association studies to identify genetic determinants of pathogen virulence

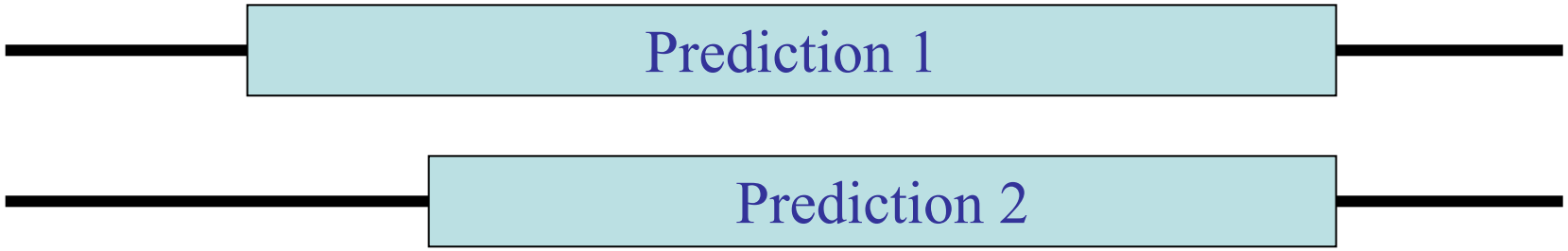




Bioinformatics uses algorithms

- Algorithms are sets of rules for solving problems or identifying patterns
- Algorithms can be general or case specific and often need to be trained
- Computational analysis, like wet-bench analyses are only as good as the tools, techniques and material allow, and all interpretations come with caveats (like the experimental conditions, often call parameters in bioinformatics.

Different algorithms often generate different results



We provide lots evidence so that you can decide or design an experiment to confirm!

Garbage in Garbage out!

- The algorithms will almost always return a result, it is up to you, the scientist to evaluate if it has made a mistake. Much of the data in the archival databases have errors. Not intentional errors but errors
- If you can't find the gene or answer it does NOT mean that it does not exist. It may be in a gap, or never have been annotate, or discovered after the annotation e.g. lncRNAs. Interpret carefully

Bioinformatics Resource Center Community Evolution

Browsing ⇨ Mining ⇨ Integrating ⇨ Facilitating

2004 **2024**

The End

- If you have questions, I and the other instructors will be around and we are happy to talk to you.
- These slides are available to you as a PDF on the workshop web site.

VEuPathDB

Eukaryotic Pathogen, Vector & Host Informatics Resources

National Institute of Allergy and Infectious Diseases

wellcome

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Our goal: enabling end users in the lab, field & clinic
to make effective and appropriate use of large-scale datasets, expediting discovery research and translational application by making data FAIR

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