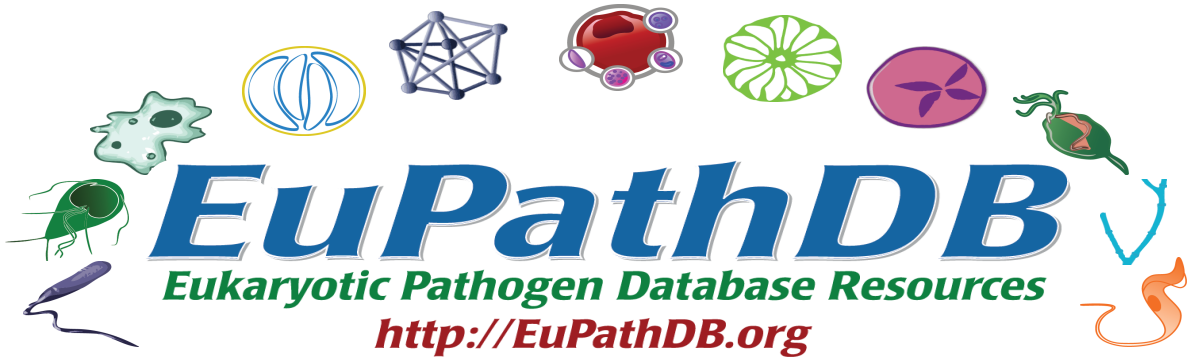
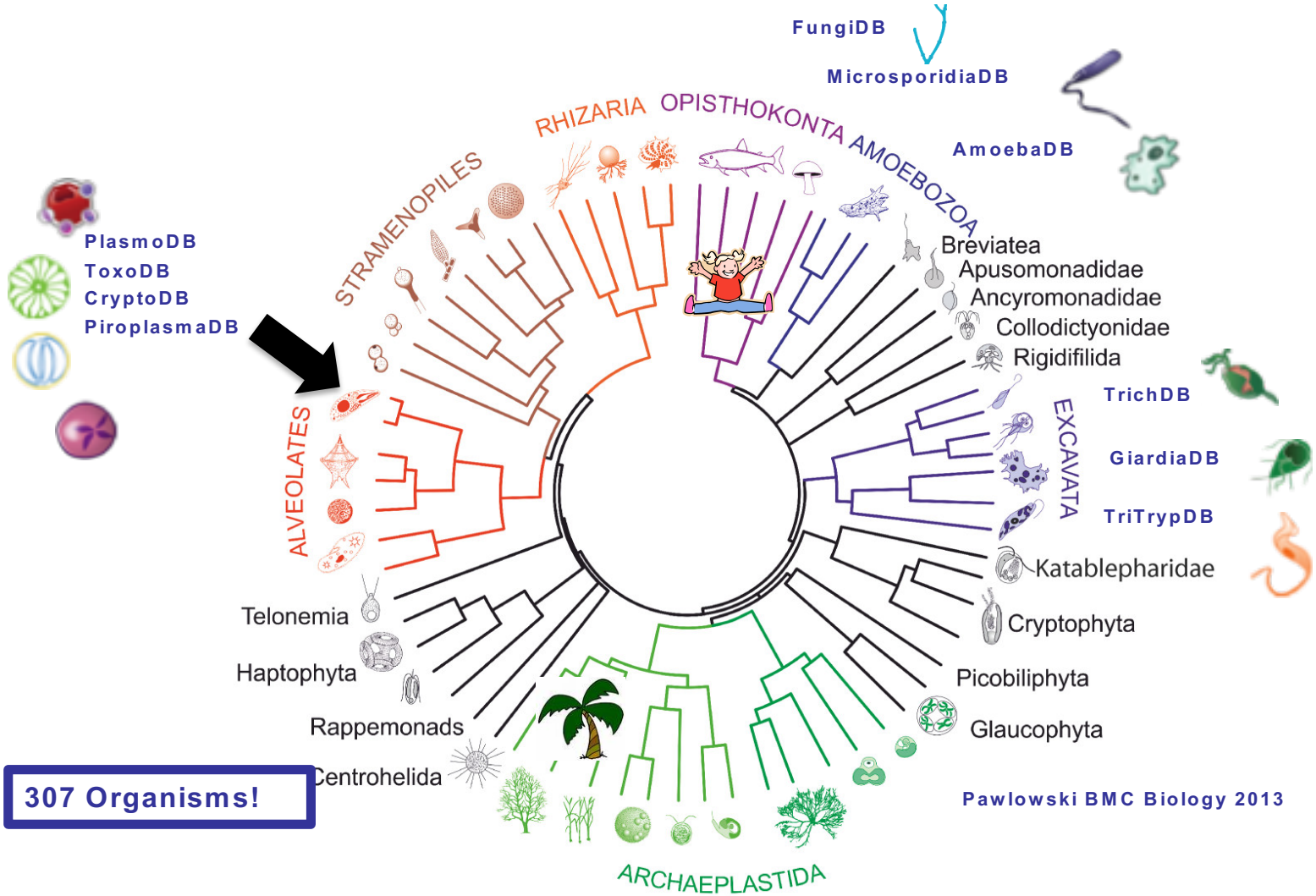


Crash Course in Omics Terminology, Concepts & Data Types

13th Annual EuPathDB Workshop

Jessie Kissinger

June 17th, 2018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MicrosporidiaDB
AmoebaDB
PlasmoDB
ToxoDB
CryptoDB
PiroplasmaDB
RHIZARIA
OPISTHOKONTA
AMOEBOZOA
STRAMENOPILES
ALVEOLATES
EXCAVATA
Breviatea
Apusomonadidae
Ancyromonadidae
Collodictyonidae
Rigidifilida
TrichDB
GiardiaDB
TriTrypDB
Katablepharidae
Cryptophyta
Picobiliphyta
Glaucophyta
Telonemia
Haptophyta
Rappemonads
Centrohelida
ARCHAEPLASTIDA
307 Organisms!
Pawlowski BMC Biology 2013


EuPathDB
OrthoMCL DB

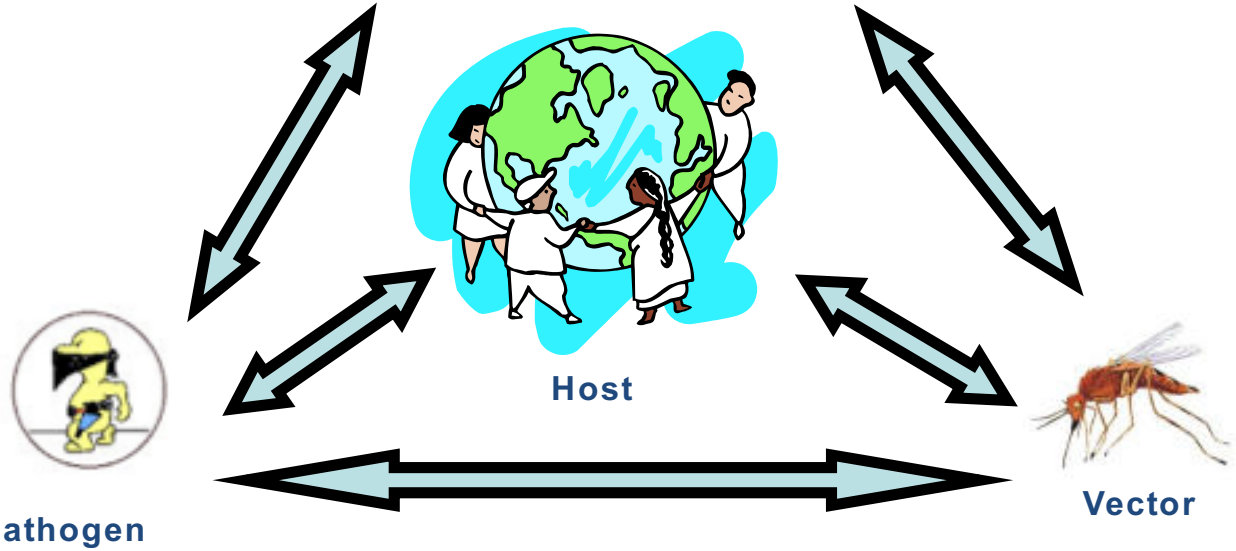

HostDB
SchistoDB
FungiDB
MicrosporidiaDB


AmoebaDB
GiardiaDB
TrichDB
TriTrypDB


PlasmoDB
PiroplasmaDB
ToxoDB
CryptoDB

Infectious Disease Paradigm

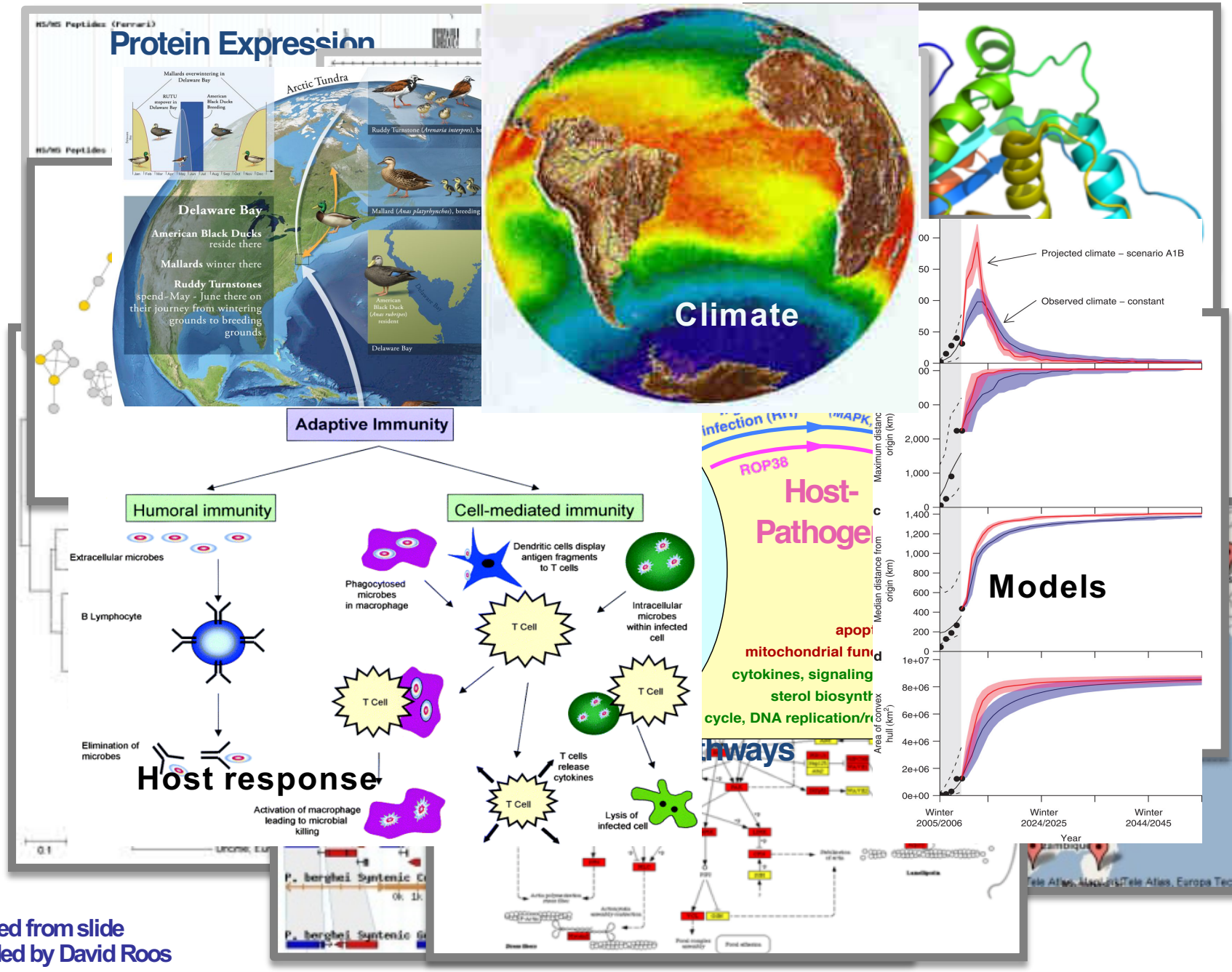
Experimental systems



Host

Pathogen

Vector



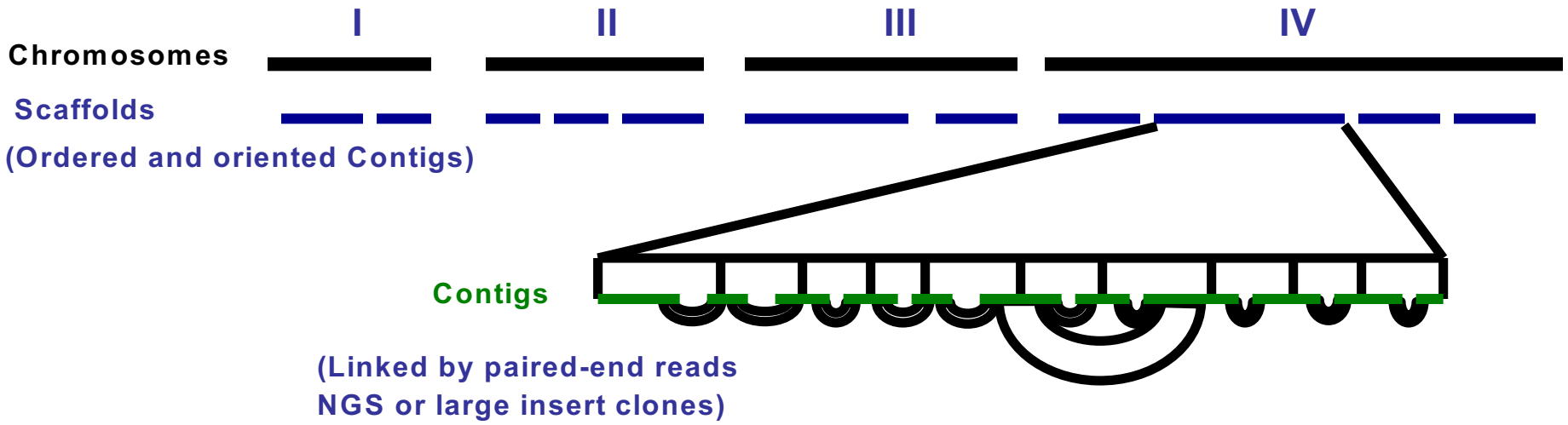
Modified from slide
Provided by David Roos

Sequence Technologies/platforms

- Hybridization (Southern, Northern, Microarray, chip Arrays (Affymetrix), now.."bait + Seq"
- Sanger sequencing, 454, Solexa/Illumina, Solid, Ion torrent, PacBio, Nanopore, 10X Genomics Chromium

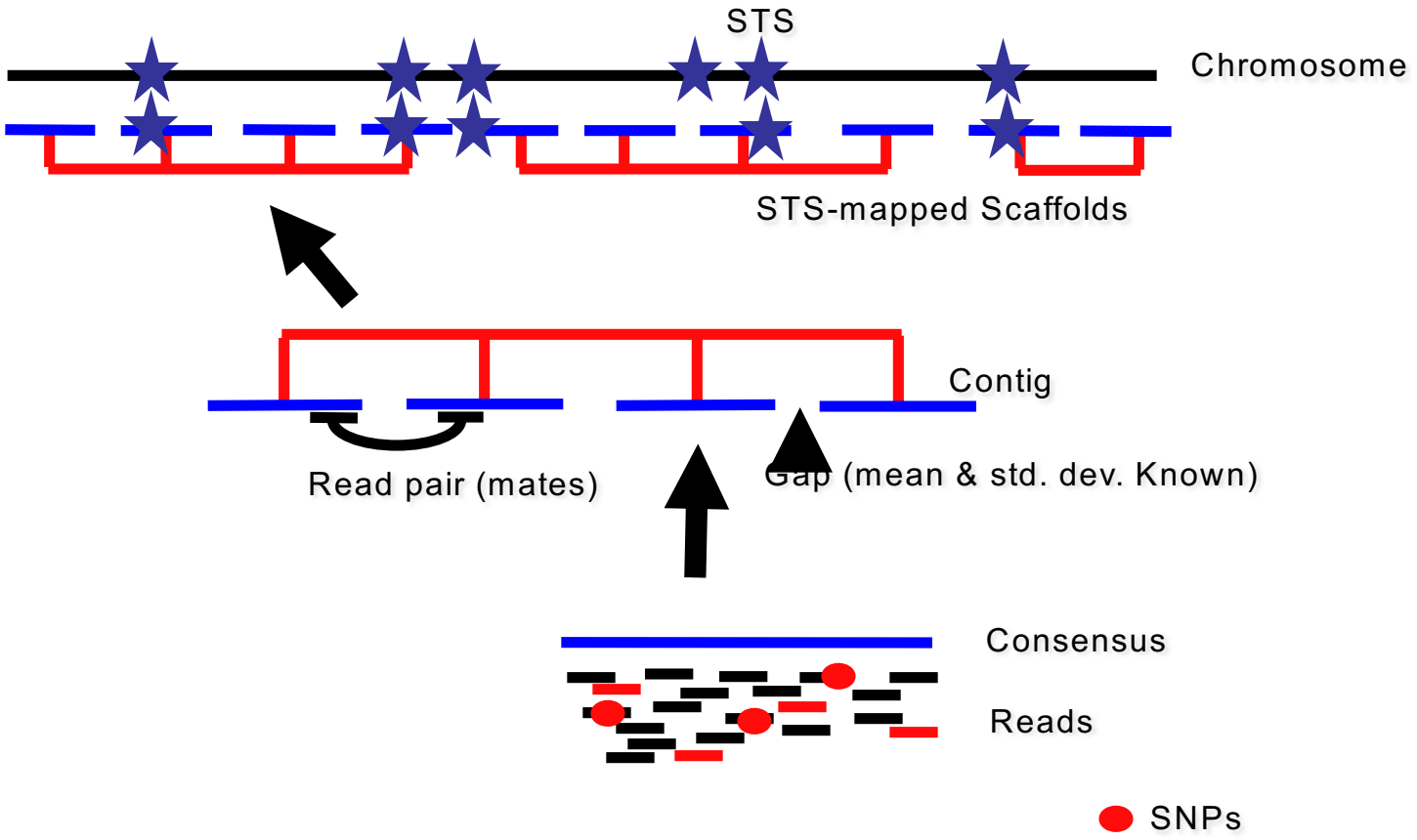
DNA sequencing technologies: 2006–2016 Elaine R Mardis
NATURE PROTOCOLS | VOL.12 NO.2 | 2017 | 213

30,000 ft View – Genome Assembly

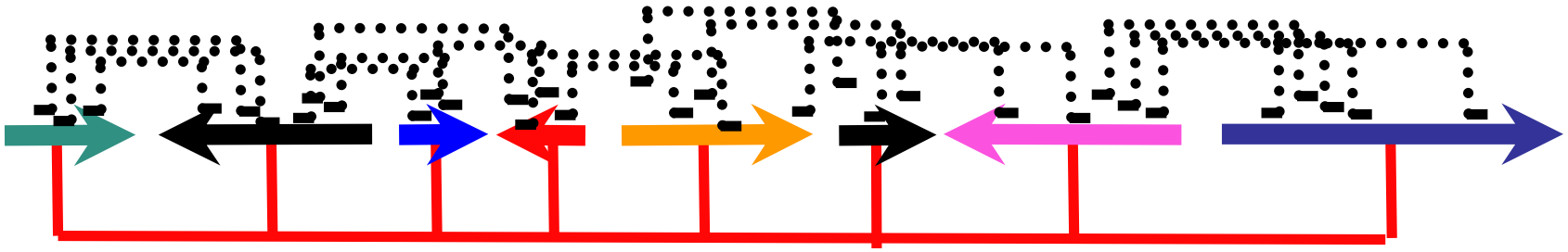


5X genome sequence means that sequences equivalent to 5X the genome size were generate e.g. Genome size = 10 Mbp, then 50Mbp of random sequences were generated

Anatomy of a WGS Assembly

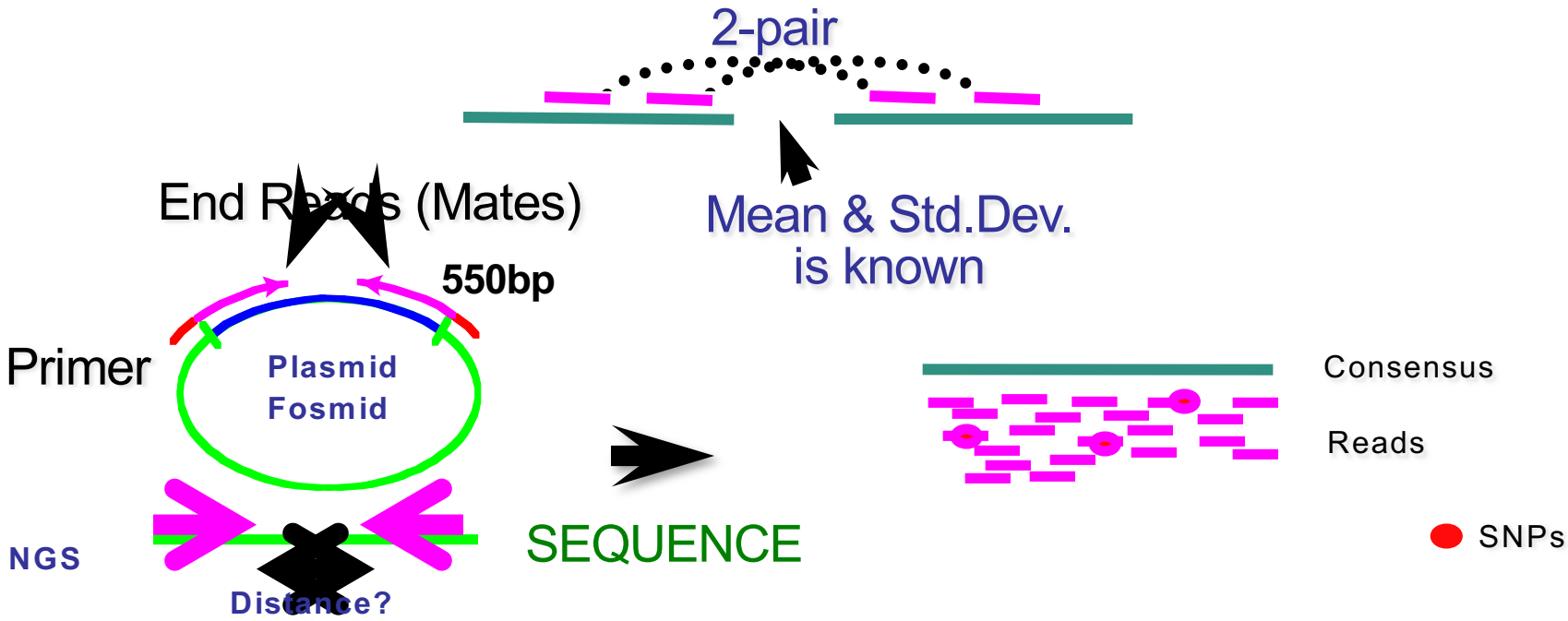


Pairs Give Order & Orientation

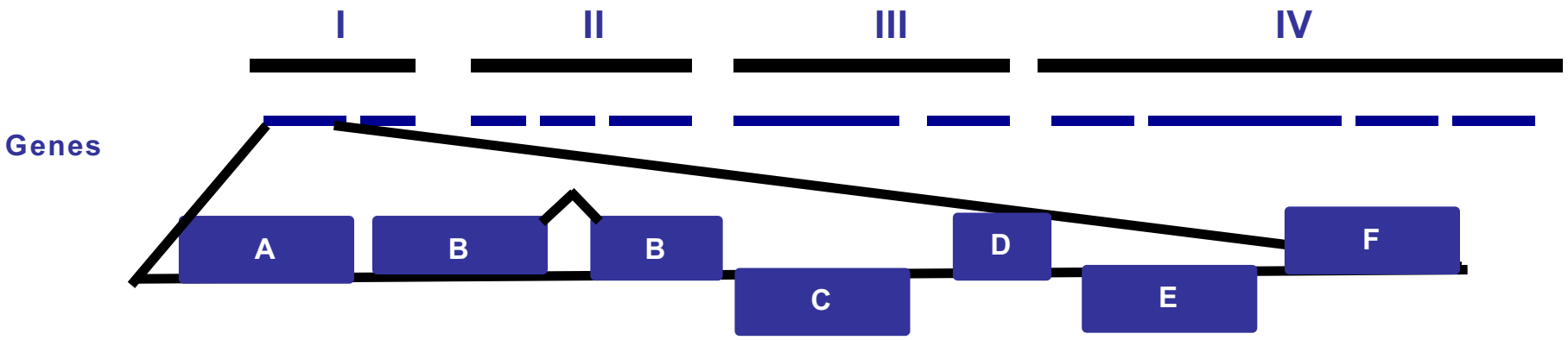


Scaffold

Gaps in scaffolds are traditionally indicated by 100 "N's"



30,000 ft View - Annotation



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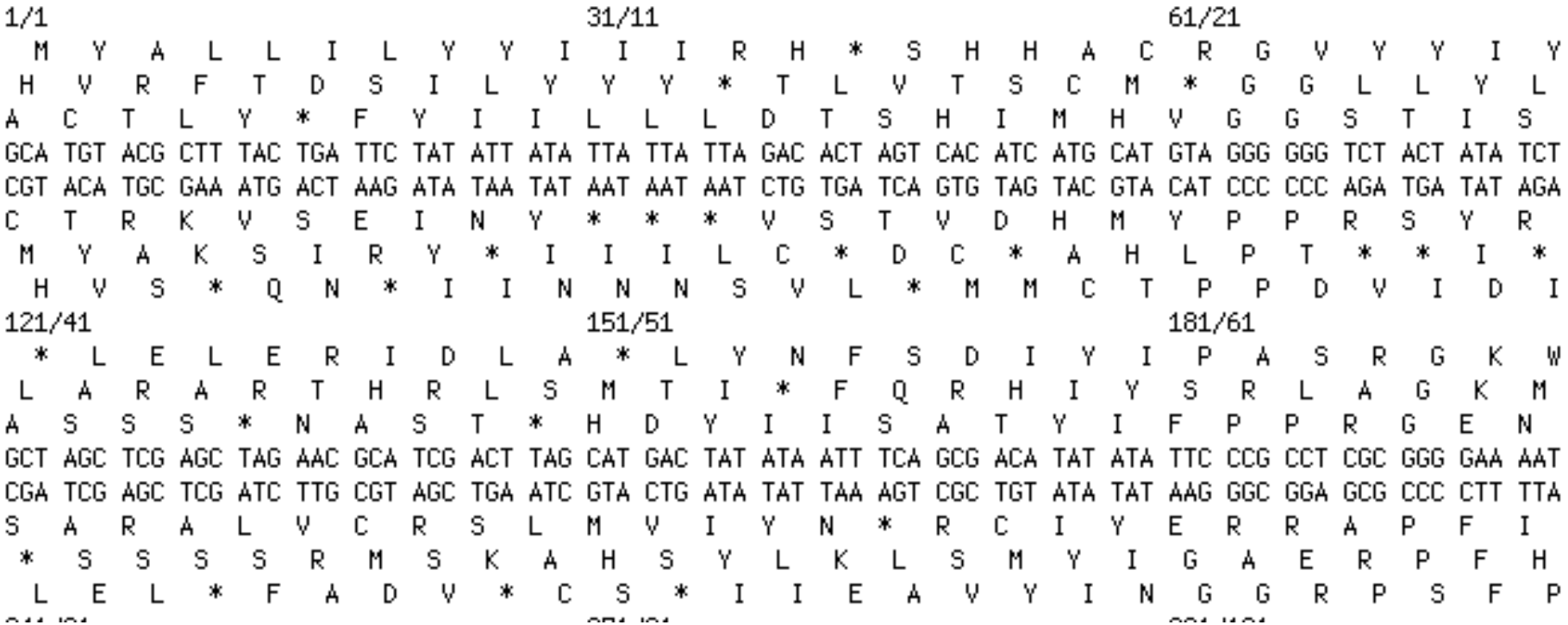
Six Frame Translation ORF-finding



ORFs ≠ Genes

ATGCAGAAACCGGTGTGTCTGGTCGTCGCGATGACCCCCAAGAGGGGCATCGGCATCAACAACGGCCTCCCGTGGCCCC
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GACAACGGGGTACCCTACGACTTTGTGGTTCTCGAGAAGAGAAGGAAGACTGACGACGCAGCCACTGCGGAACCGAGCA
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TGCCCCGGTGTTGGCGTGGATGGACGAAGAAGACCGGAAAAAACGCGAGCAAAAGGAACTGATTCGGGCCGTTCCGCAT
GTTCACTTTAGAGGCCATGAAGAATTCCAGTACCTTGATCTCATTGCCGACATTATTAACAATGGAAGGACAATGGATG
ACCGAACGG

>Translation Frame 1

MQKPVCLVVAMTPKRGIGINNGLPWPHLTTDFKHFSRVTKTTPEEASRLN
GWLPRKFAKTGDSGLPSPSVGKRFNAVVMGRKTWESMPRKFRPLVDRLNI

VVSSSLKEEDIAAEKPQAEGQQRVRVCASLPAALSLLEEEYKDSVDQIFV
VGGAGLYEAALSLGVASHLYITRVAREFPCDVFFPAFPGDDILSNKSTAA
QAAAPAESVFVPFCPELGREKDNEATYRPIFISKTFSDNGVPYDFVVLEK

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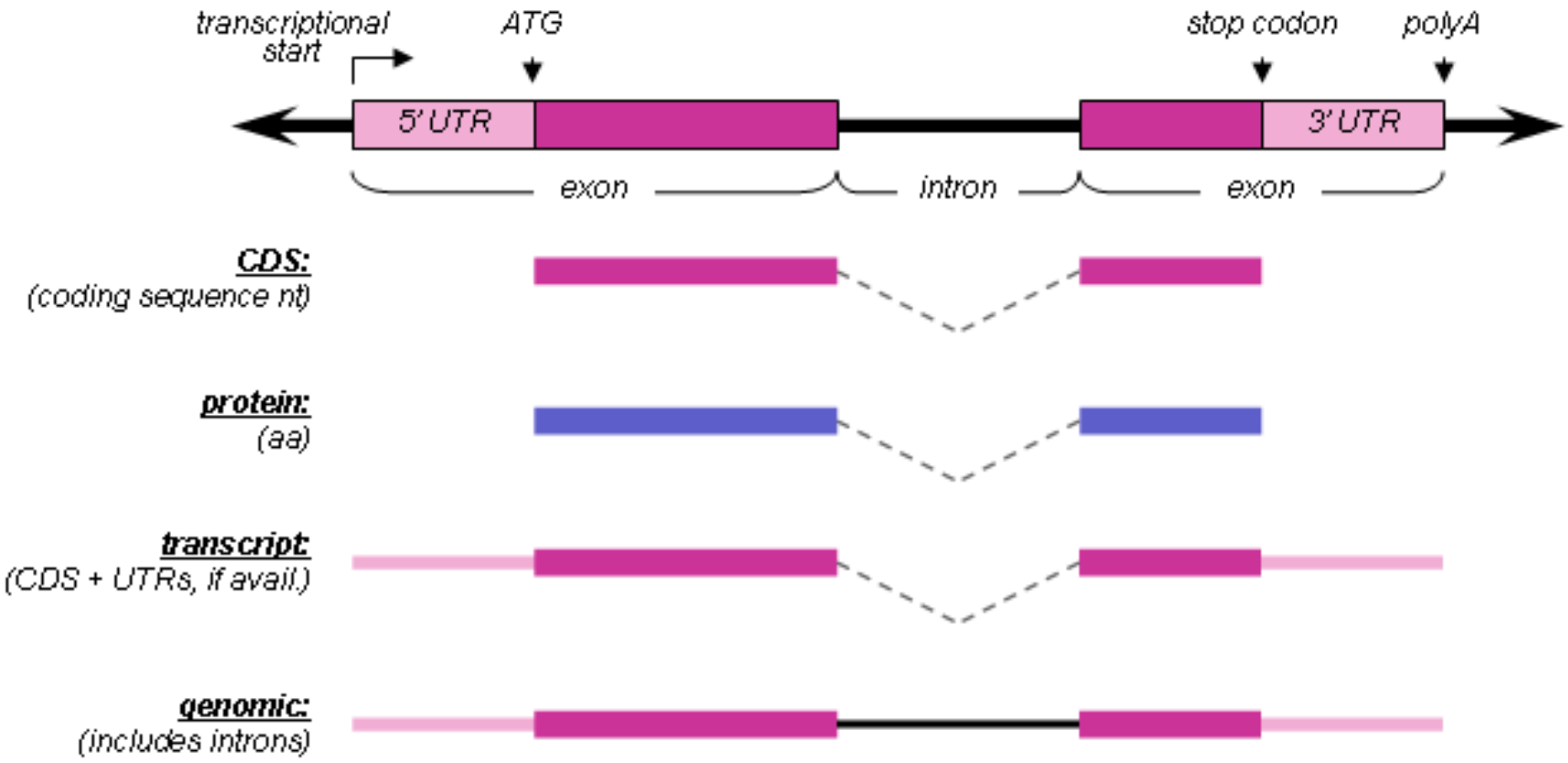
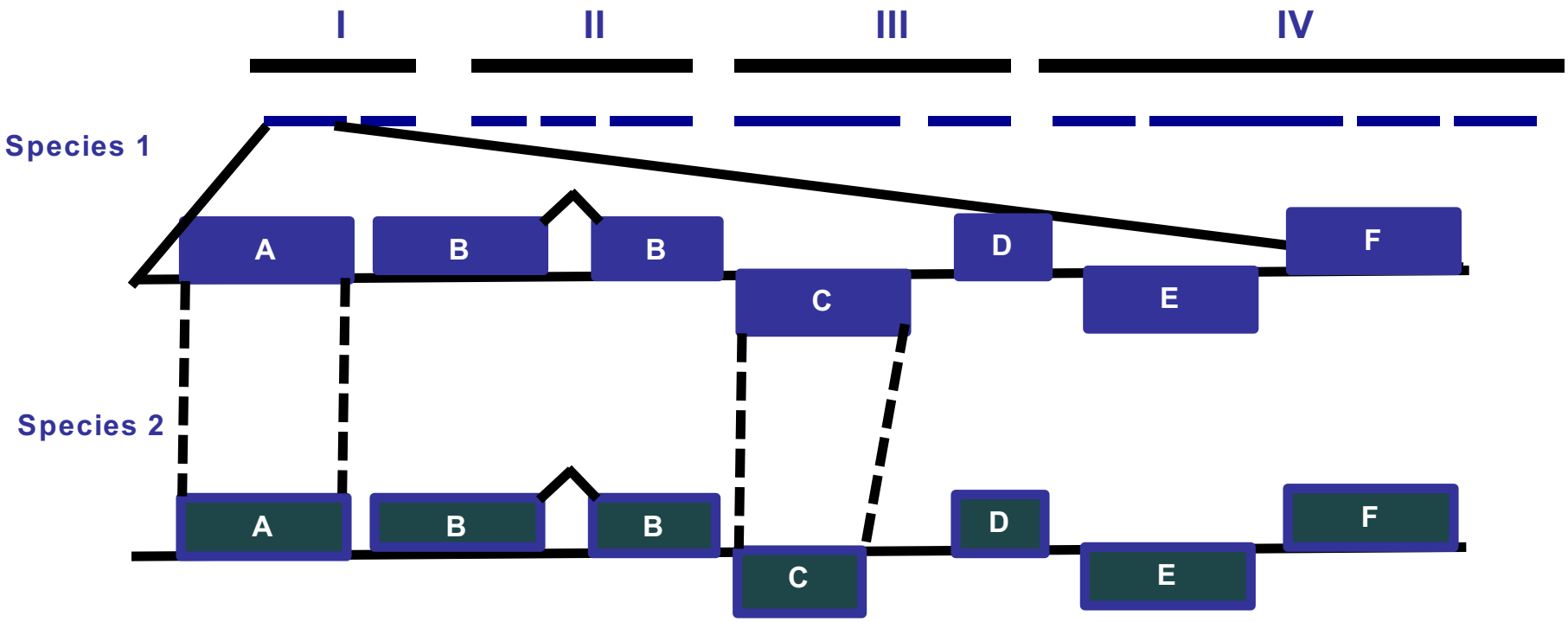
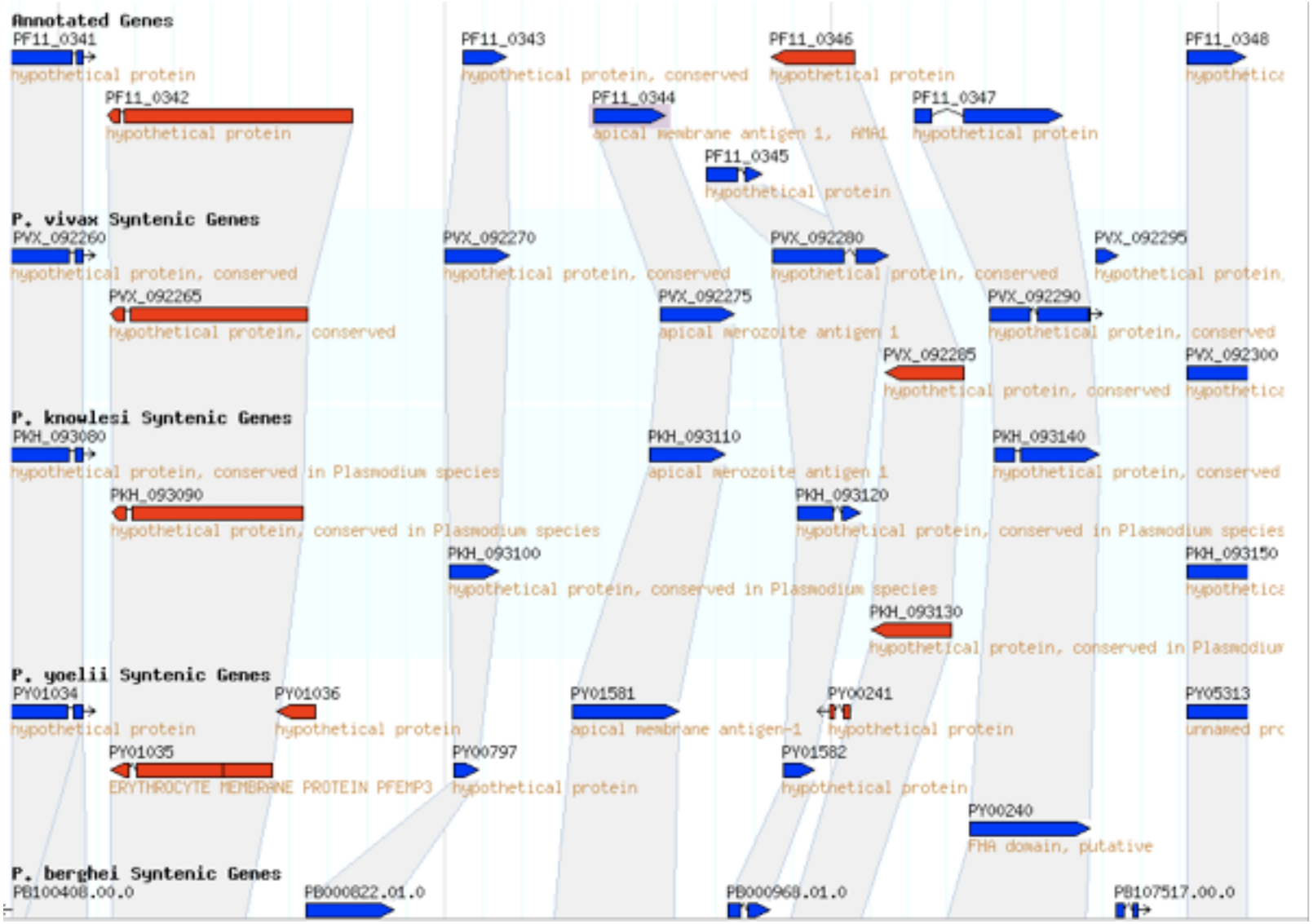
Terminology



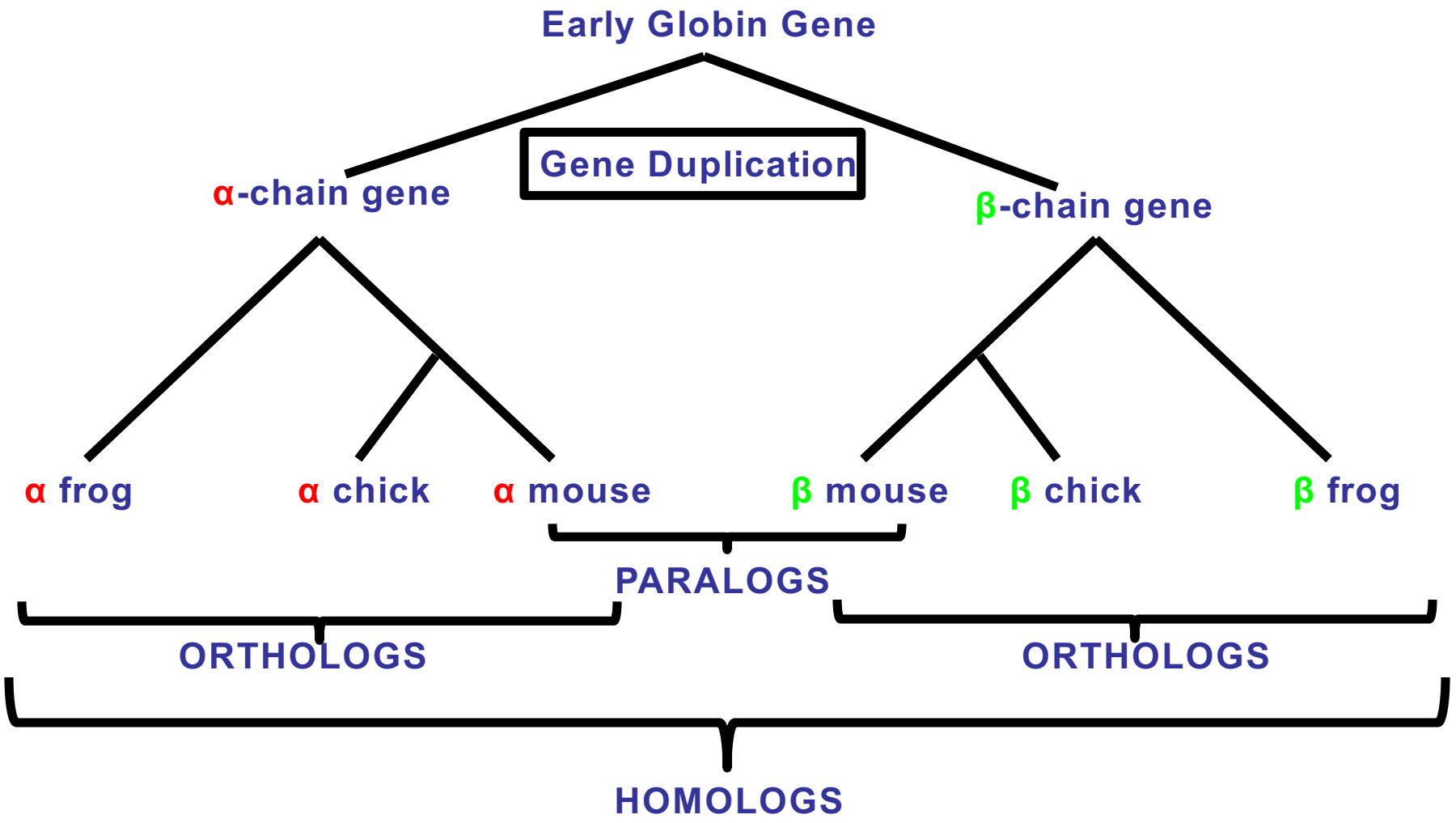
30,000 ft View - Synteny



Synteny among Plasmodia



Homology


Early Globin Gene
Gene Duplication
α-chain gene
β-chain gene
α frog
α chick
α mouse
β mouse
β chick
β frog
PARALOGS
ORTHOLOGS
ORTHOLOGS
HOMOLOGS

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:

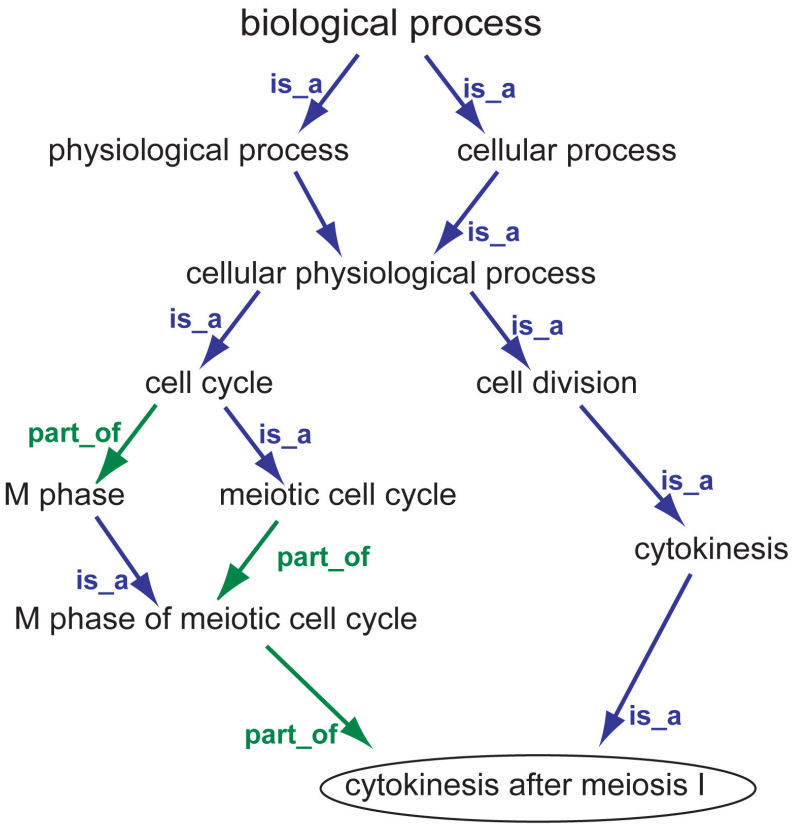
D. melanogaster gene CG3340 annotated as: "*Kruppel*" and *P. falciparum* gene PF3D7_1209300 annotated a "putative KROX1"

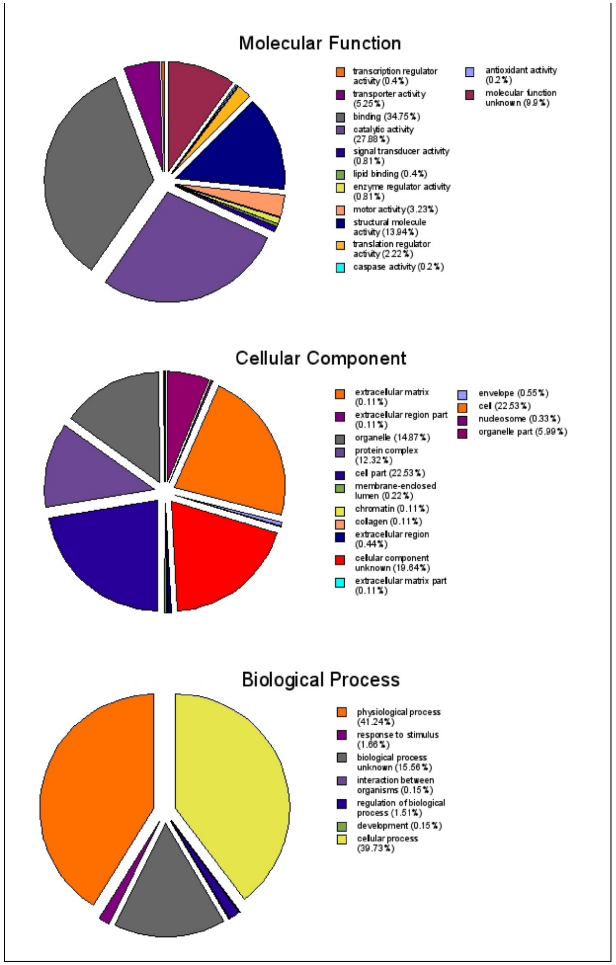
Can both be annotated with GO term:

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





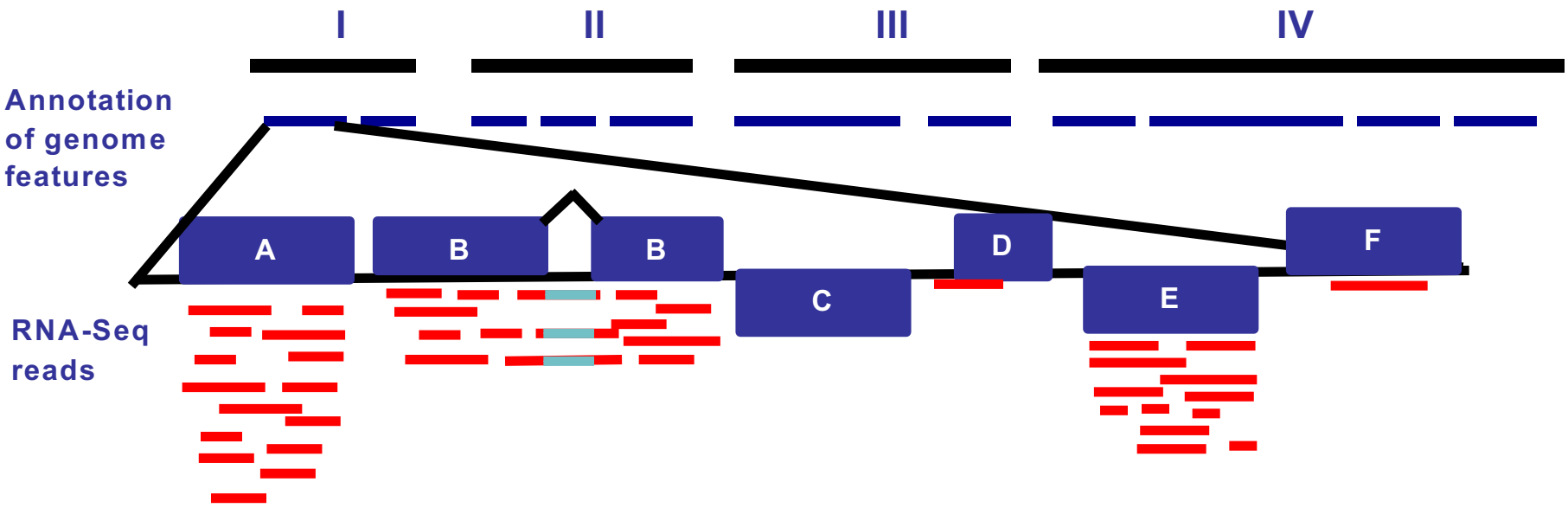
Expression Profiles (RNA and Protein)

- 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

RNA expression

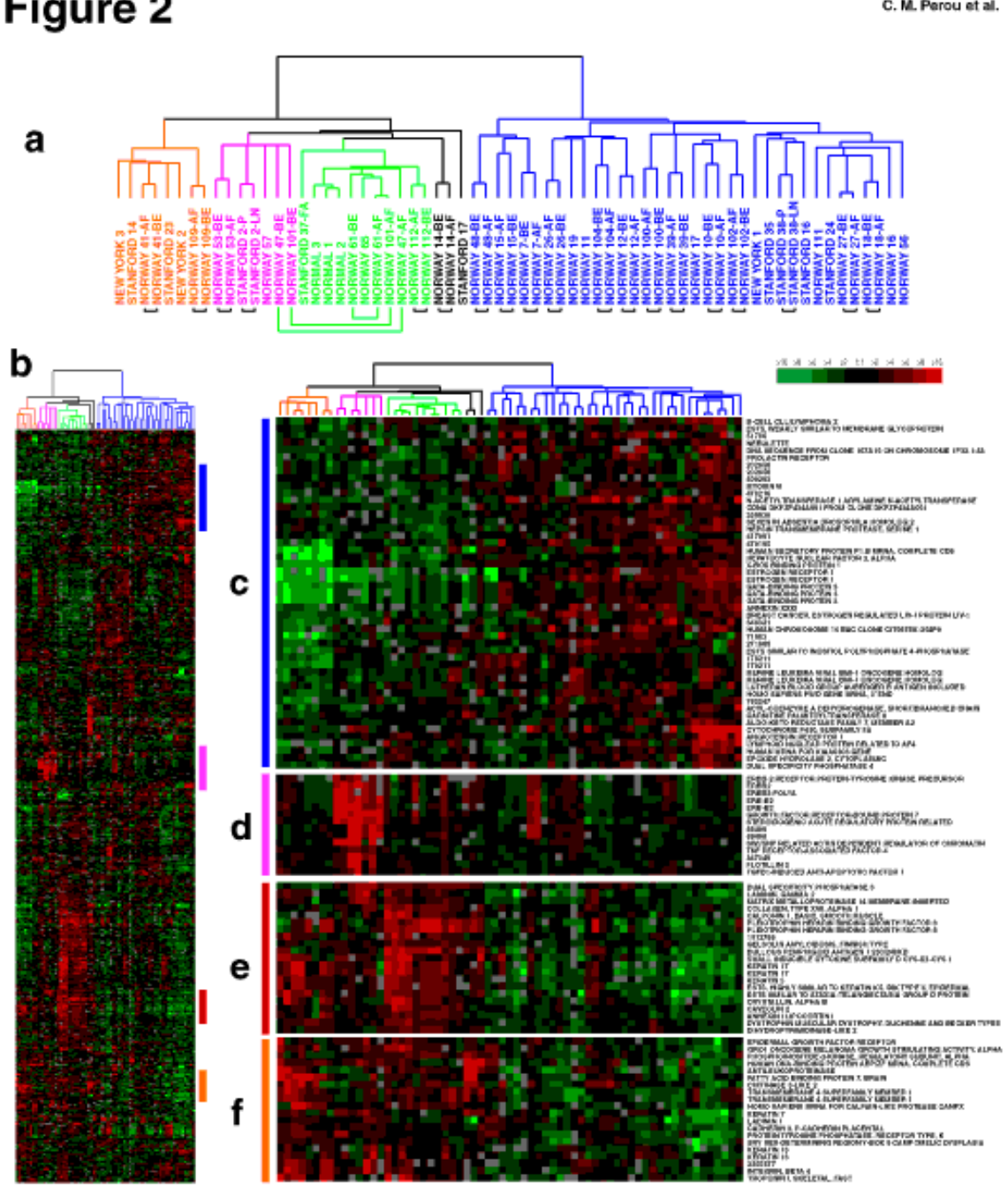
- RNA-Seq (NGS)
  - Little sequence bias
  - Quantitative
  - Usually are strand-specific
  - Can be used to identify UTR's and exon splice junctions
- Expressed Sequence Tags, ESTs
  - Usually represent partial cDNA
  - Often clustered
  - Come from libraries that may, or may not be normalized
  - Often used to identify genes in genomes and locations of introns
- SAGE tags
  - Serial Analysis of Gene Expression

30,000 ft View – RNA-Seq



FPKM = Fragments per kilobase of exon per million fragments mapped

Clustered Microarray Data Genes with Similar Expression Profiles are Grouped together



Genes can be located on either DNA strand
Convention - Gene location = non-template strand, i.e. same as the mRNA

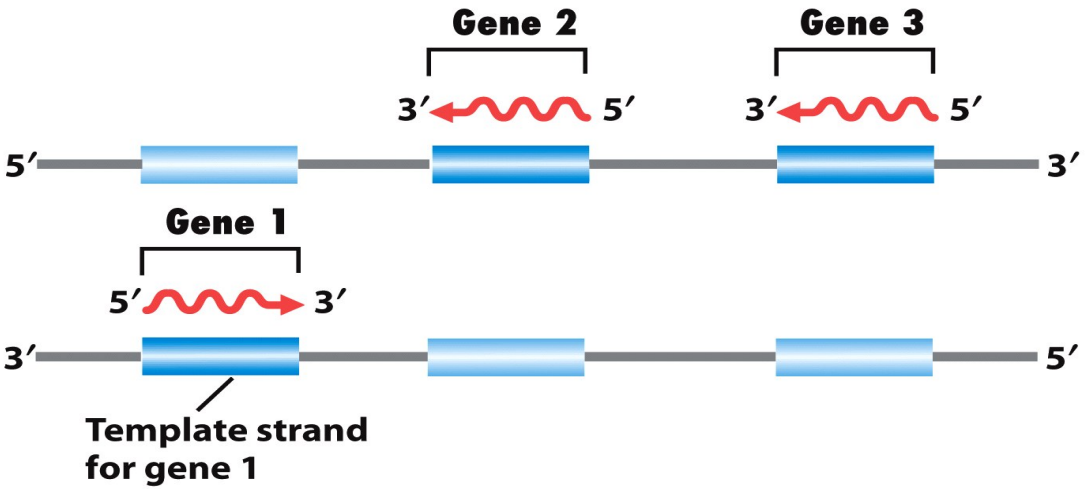


Figure 8-3
Introduction to Genetic Analysis, Ninth Edition

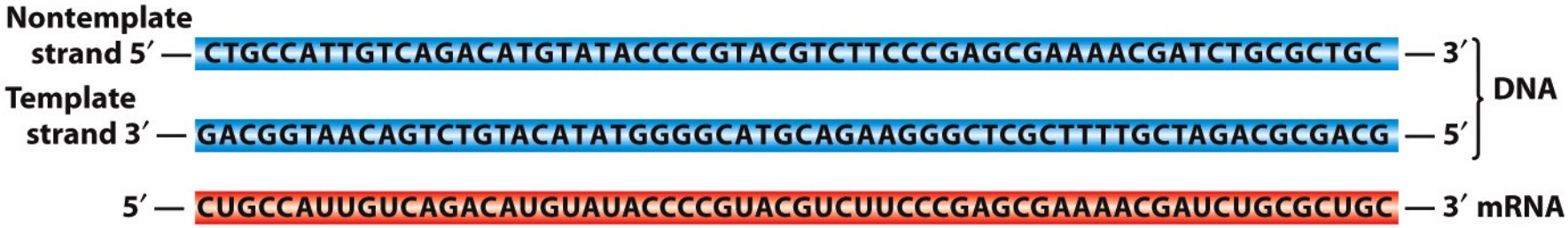


Figure 8-6
Introduction to Genetic Analysis, Ninth Edition

Overview of transcription: Either strand can serve as a template for a gene

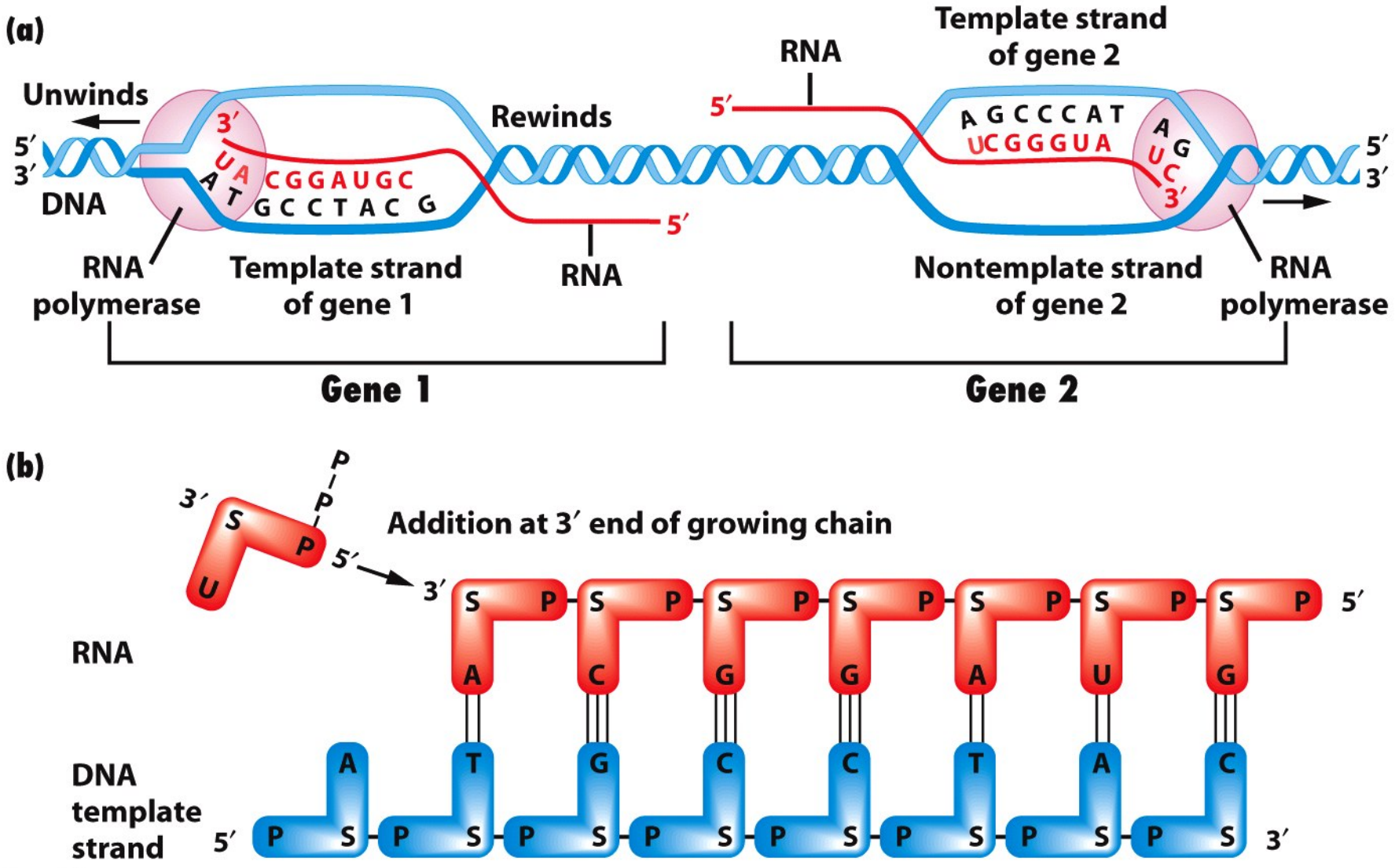


Figure 8-4
Introduction to Genetic Analysis, Ninth Edition

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

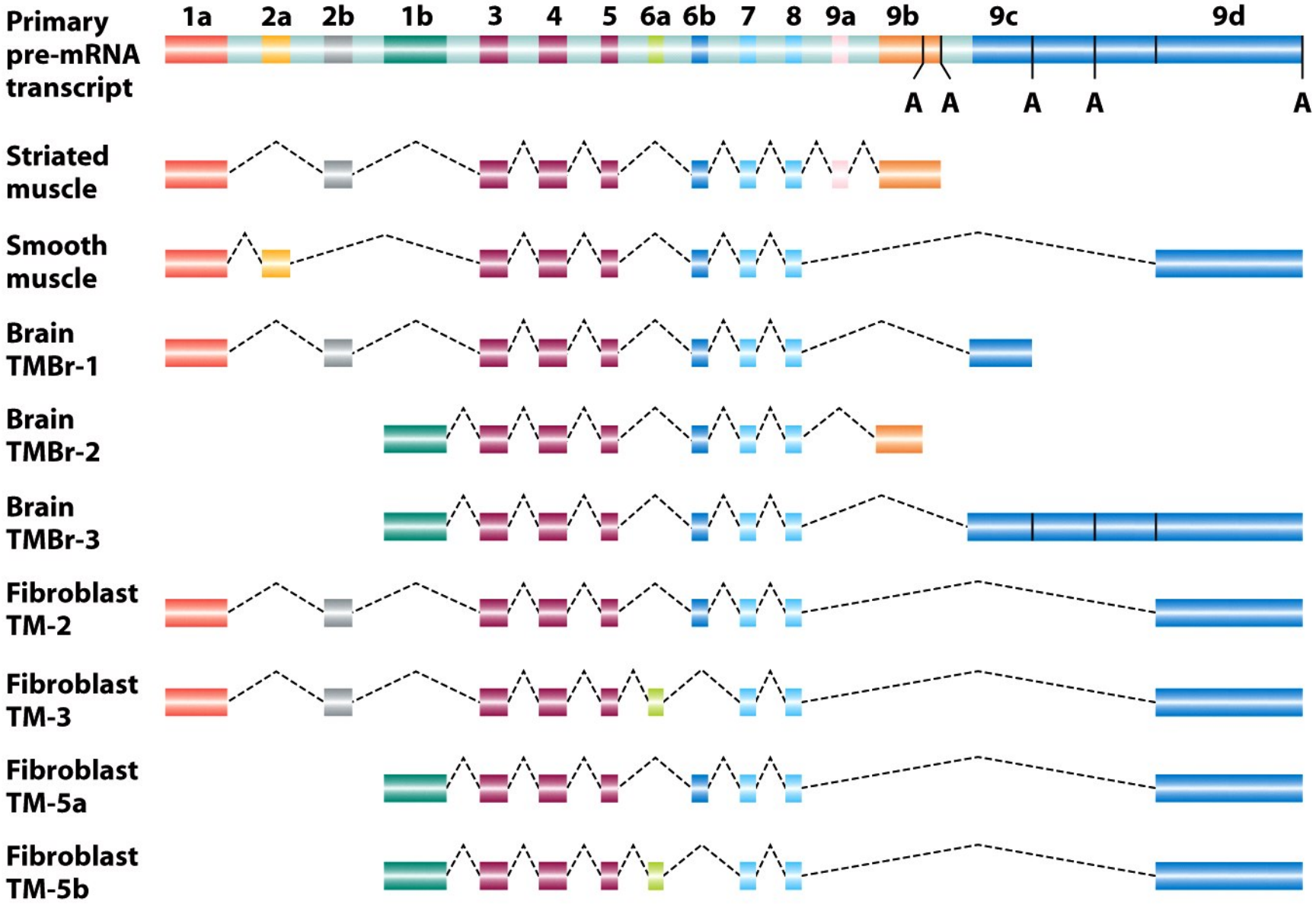
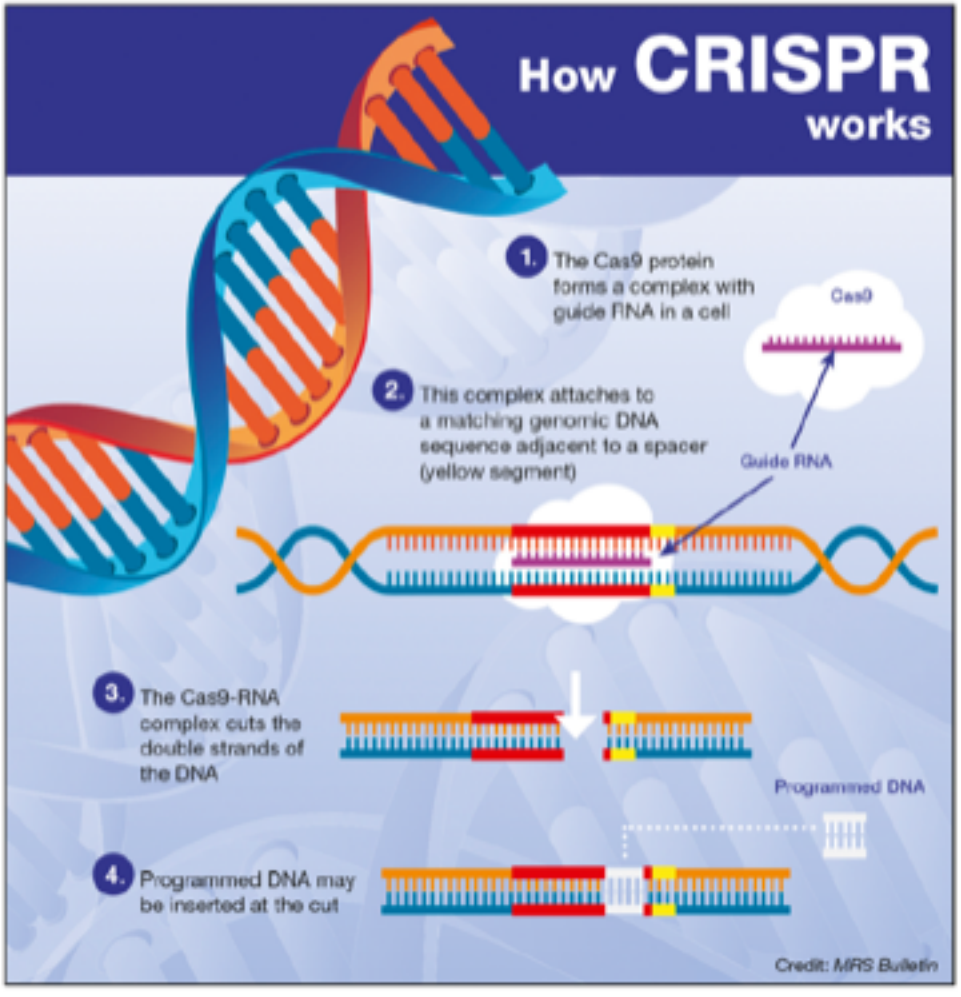


Figure 8-14
Introduction to Genetic Analysis, Ninth Edition

CRISPR-CAS



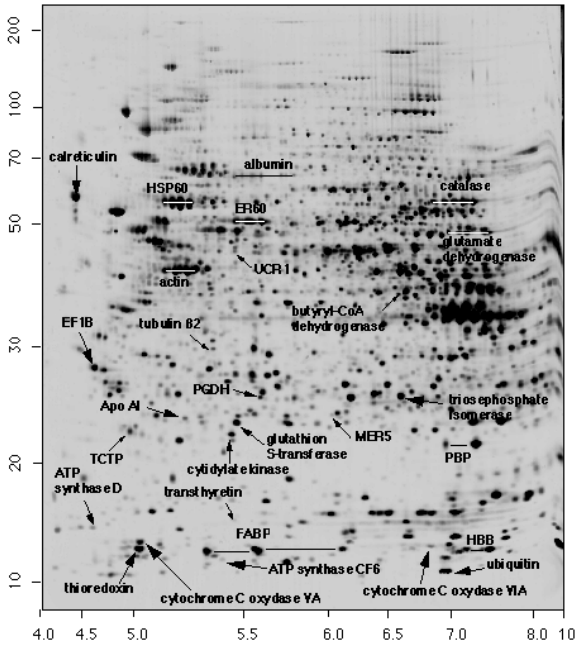
- Need to provide both the enzyme and the guide RNA to the cell
- Need to design the guide RNA to the gene of interest, ideally at multiple target locations per gene

Ball et al., MRS Bulletin November 2016

Protein Expression/Sequence

Data

- MW-Isoelectric point
- MW
- Sequence/spans

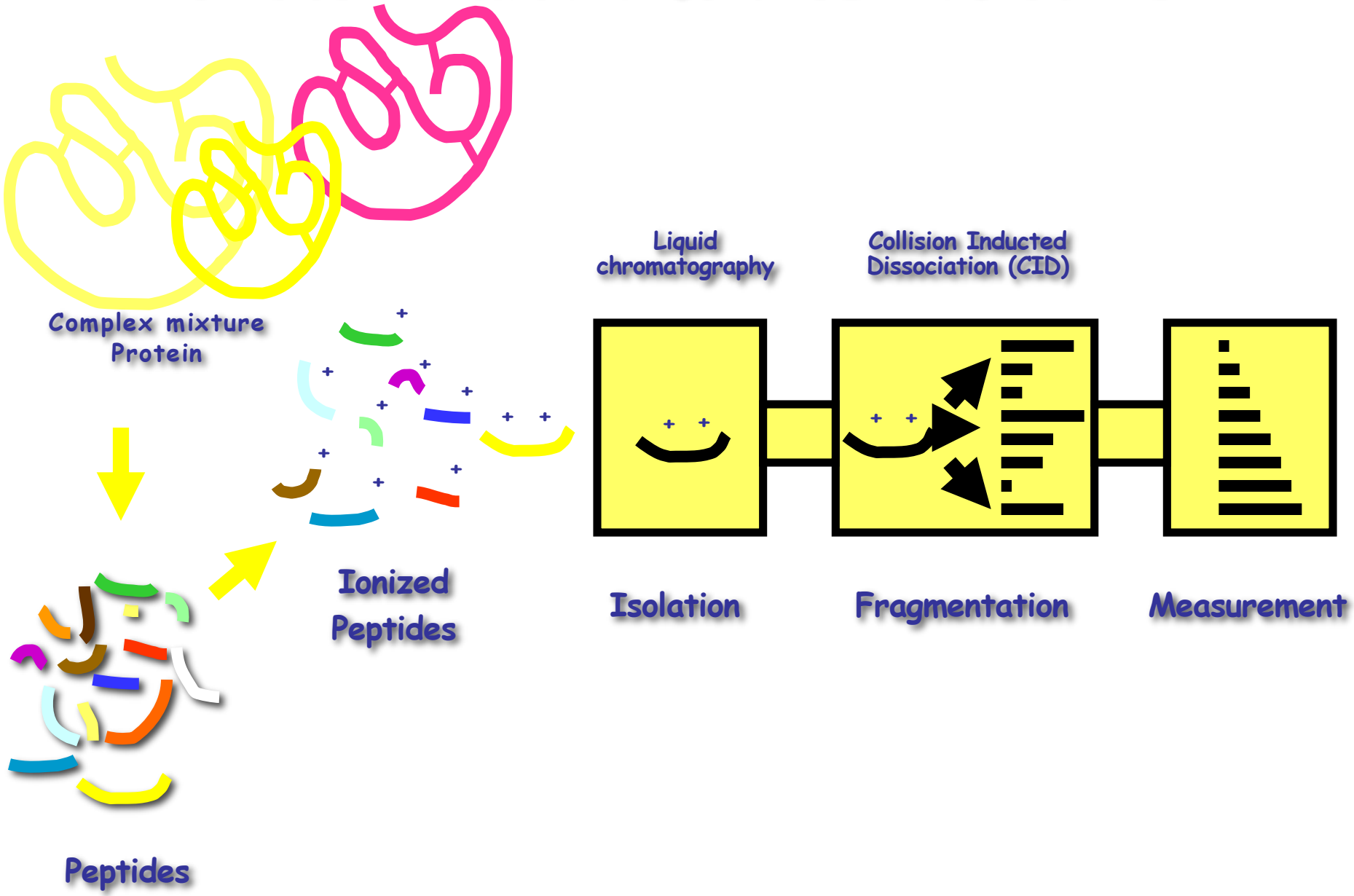


Technology

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

Typical 2 D gel

How Tandem MS Works



Tandem MS protein data

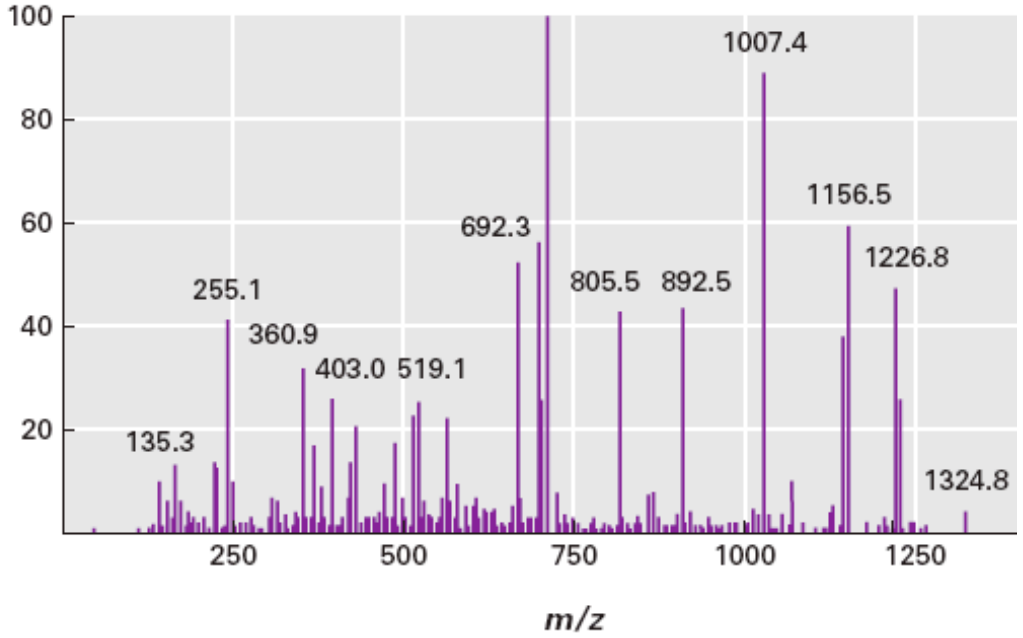
a)

S–P–A–F–D–S–I–M–A–E–T–L–K

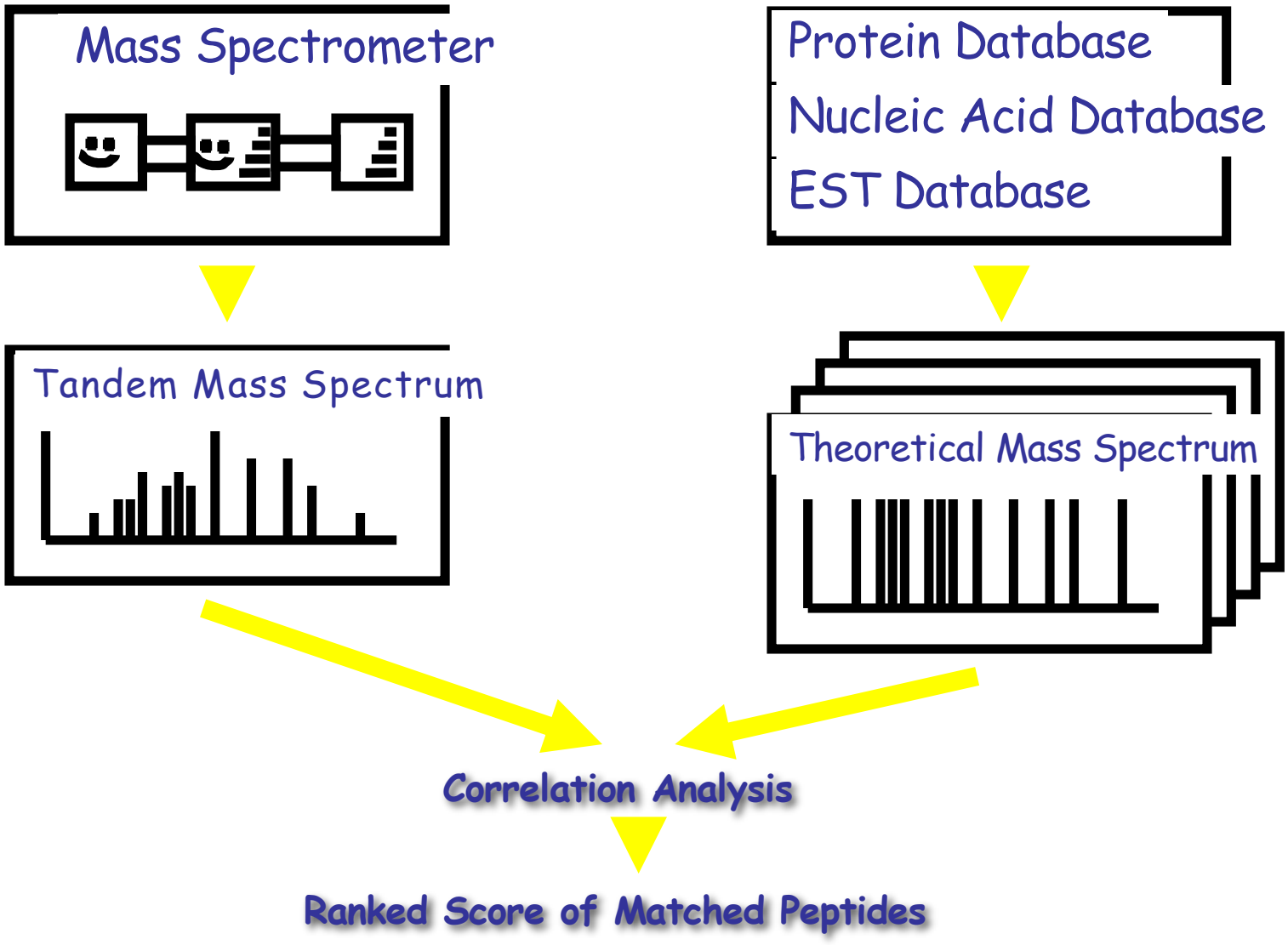
(protonated mass 1410.6)

Mass$^+$	b-ions	y-ions	Mass$^+$
81.1	S	PAFDSIMAETLK	1323.6
185.2	SP	AFDSIMAETLK	1226.4
256.3	SPA	FDSIMAETLK	1155.4
403.5	SPAF	DSIMAETLK	1008.2
518.5	SPAFD	SIMAETLK	893.1
605.6	SPAFDS	IMAETLK	806.0
718.8	SPAFDSI	MAETLK	692.3
850.0	SPAFDSIM	AETLK	561.7
921.1	SPAFDSIMA	ETLK	490.6
1050.2	SPAFDSIMAE	TLK	361.5
1151.3	SPAFDSIMAET	LK	260.4
1264.4	SPAFDSIMAETL	K	147.2

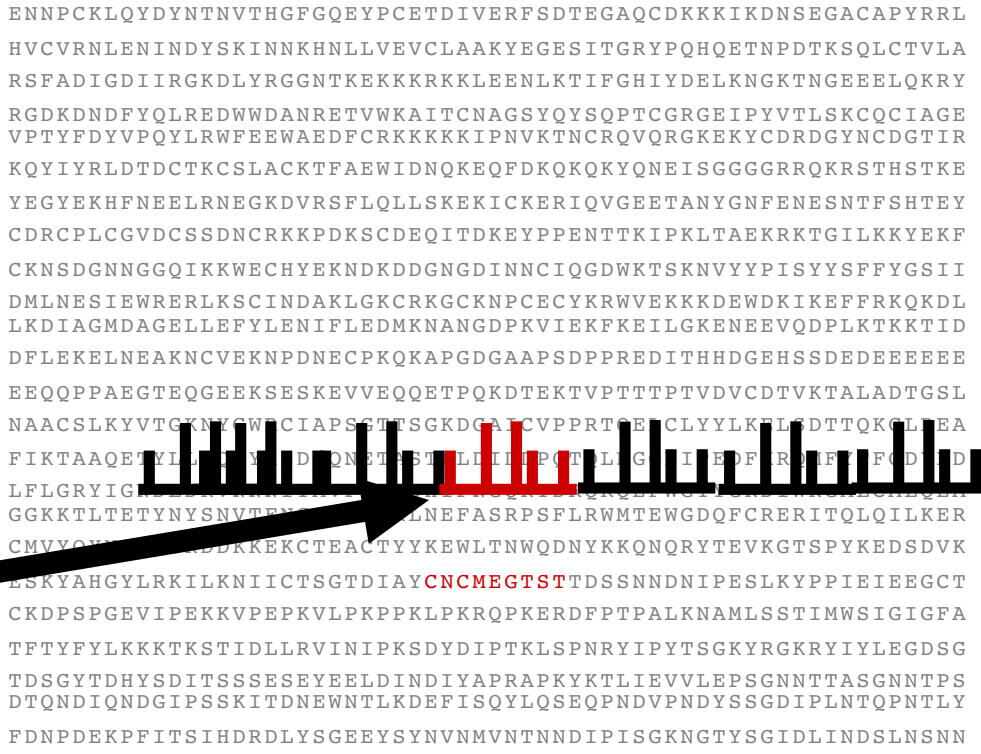
b)



Sequest Database Search

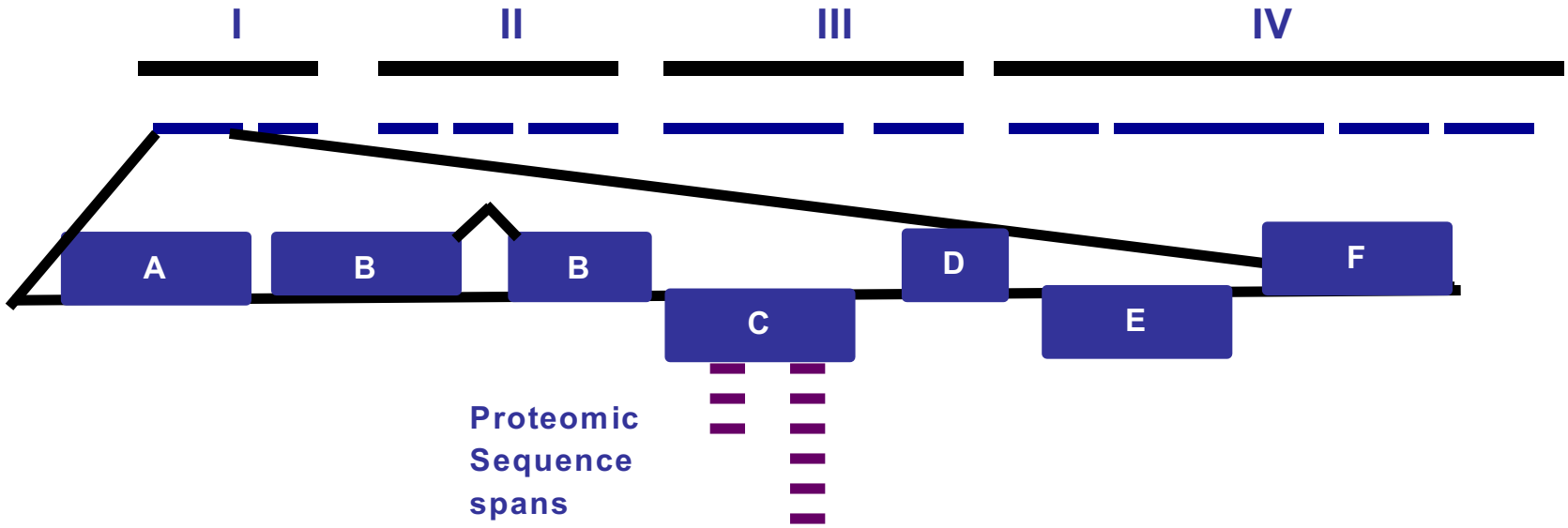


Peptide database



Note: ORFs in addition to predicted Genes must be searched

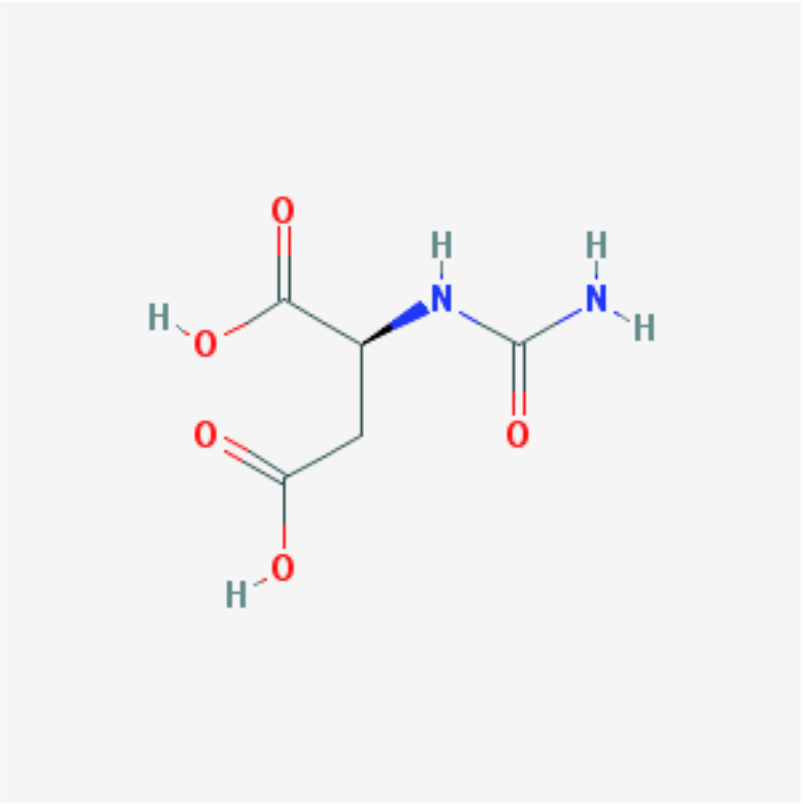
30,000 ft View - Proteomics



Overview

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

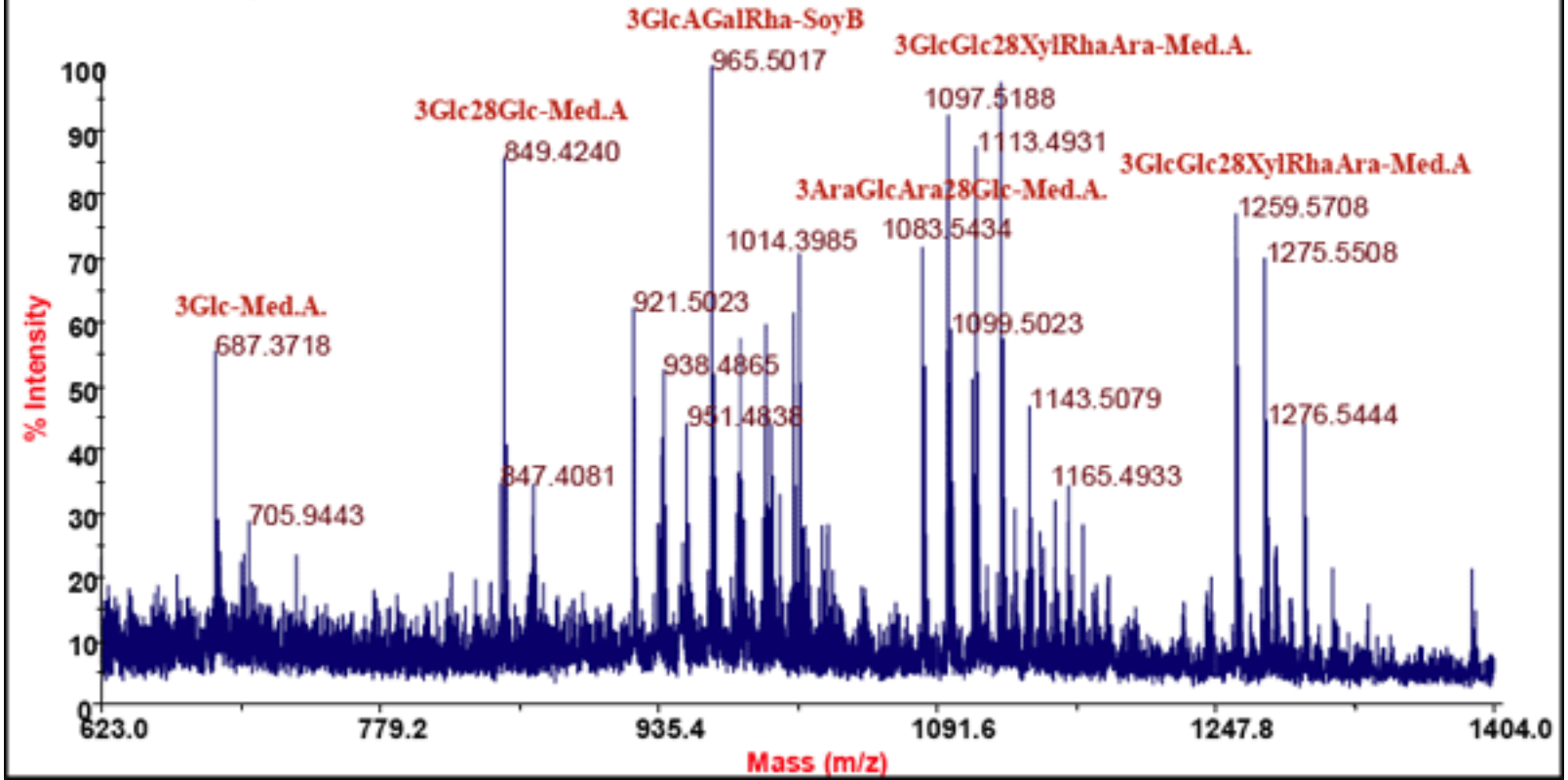
2D Structure



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

Complex mixtures can be analyzed and interpreted

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



Metabolites can be linked to metabolic pathways and enzymes

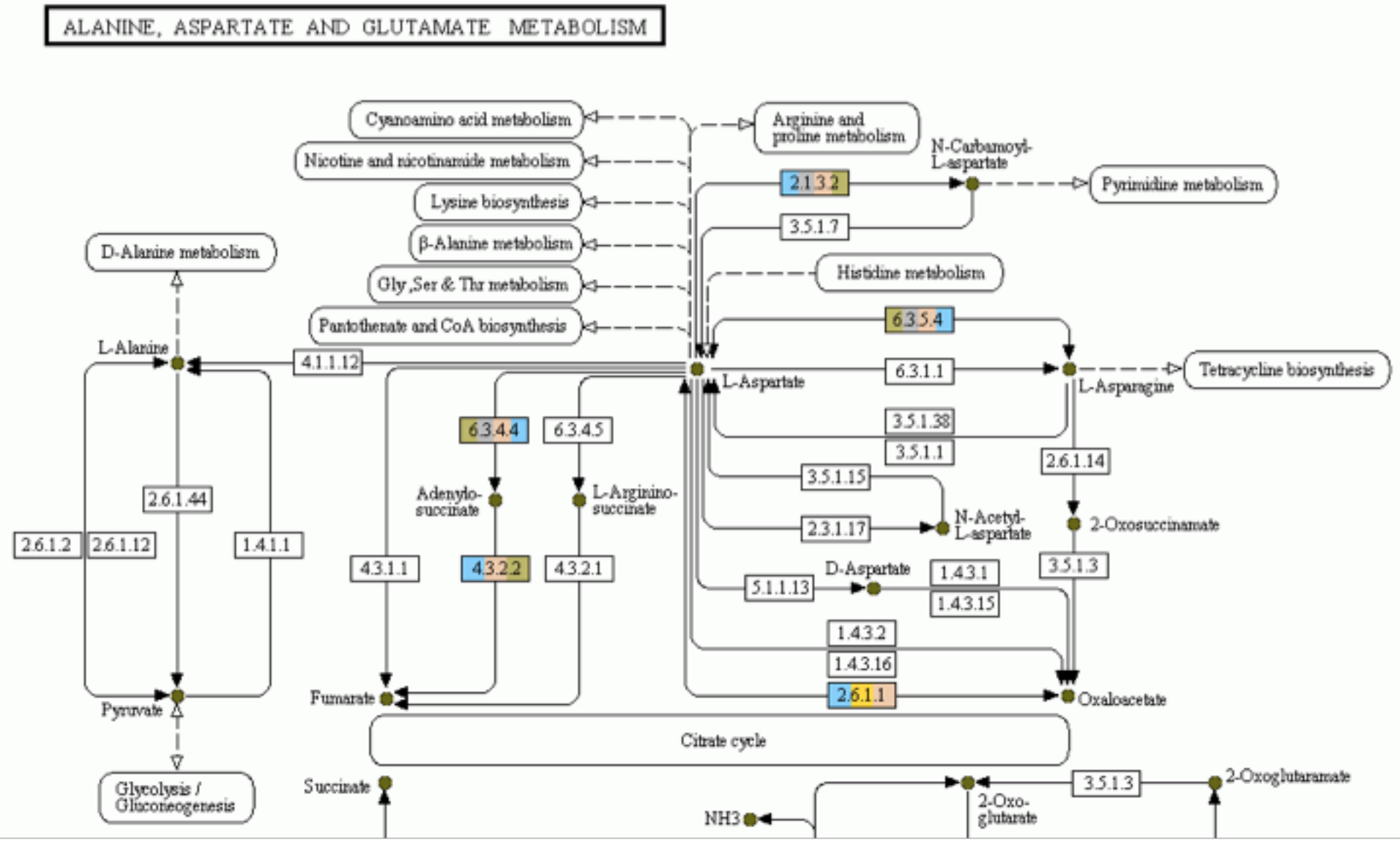
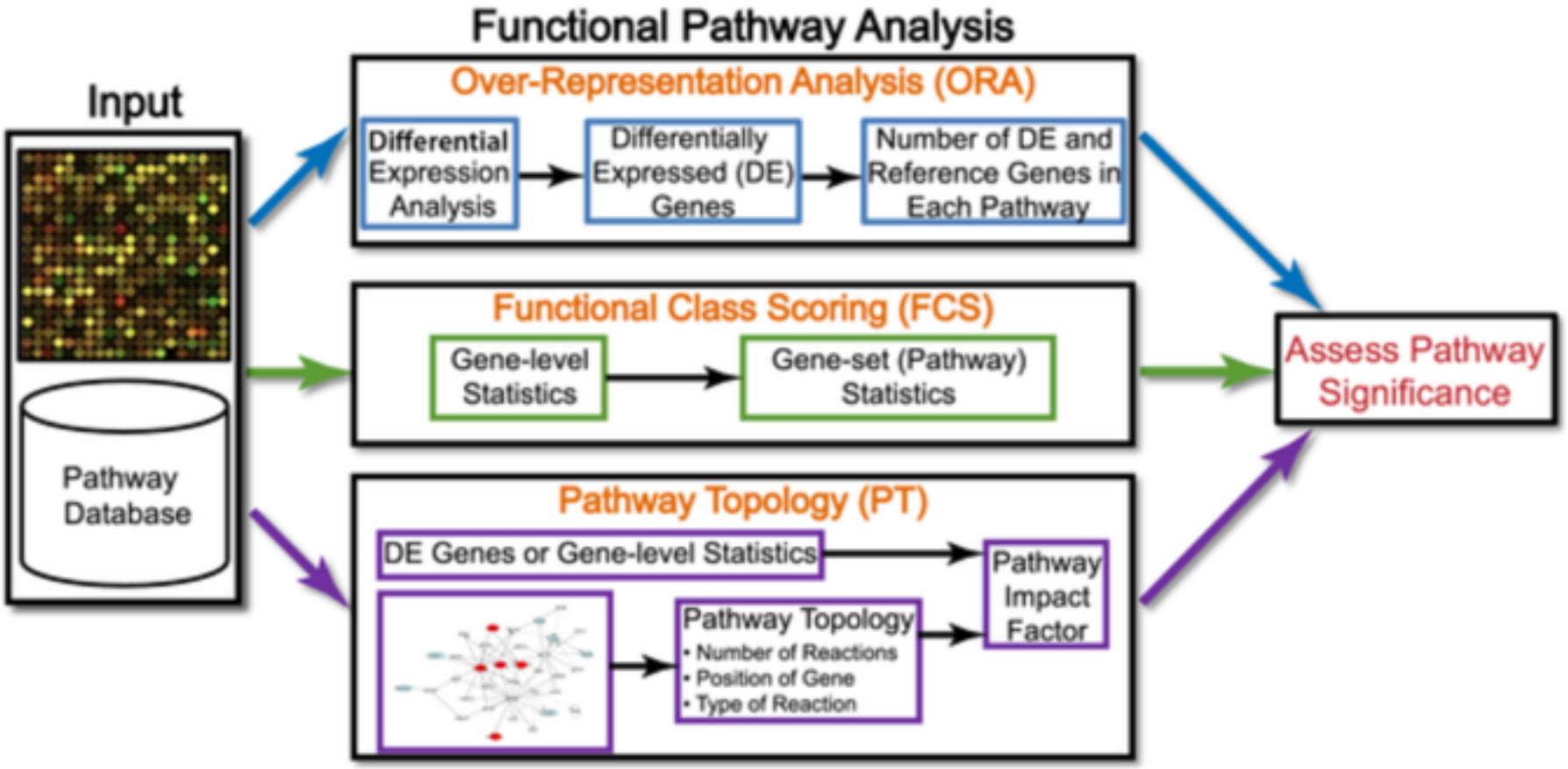


Figure 1. Overview of existing pathway analysis methods using gene expression data as an example.



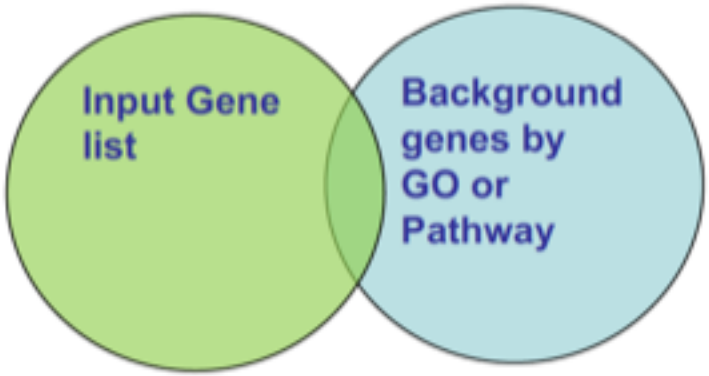
Khatri P, Sirota M, Butte AJ (2012) Ten Years of Pathway Analysis: Current Approaches and Outstanding Challenges. PLOS Computational Biology 8(2): e1002375. https://doi.org/10.1371/journal.pcbi.1002375
http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1002375

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

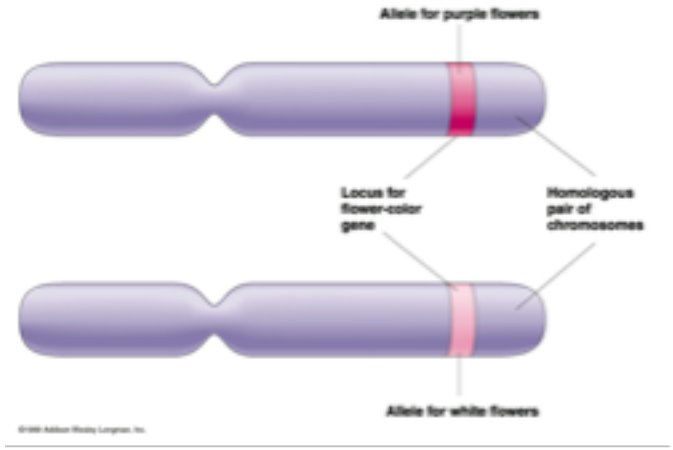
Statistics: Are more genes observed than expected (P-value)
Multiple hypothesis testing (Bonferroni, Benjamini-Hochberg)

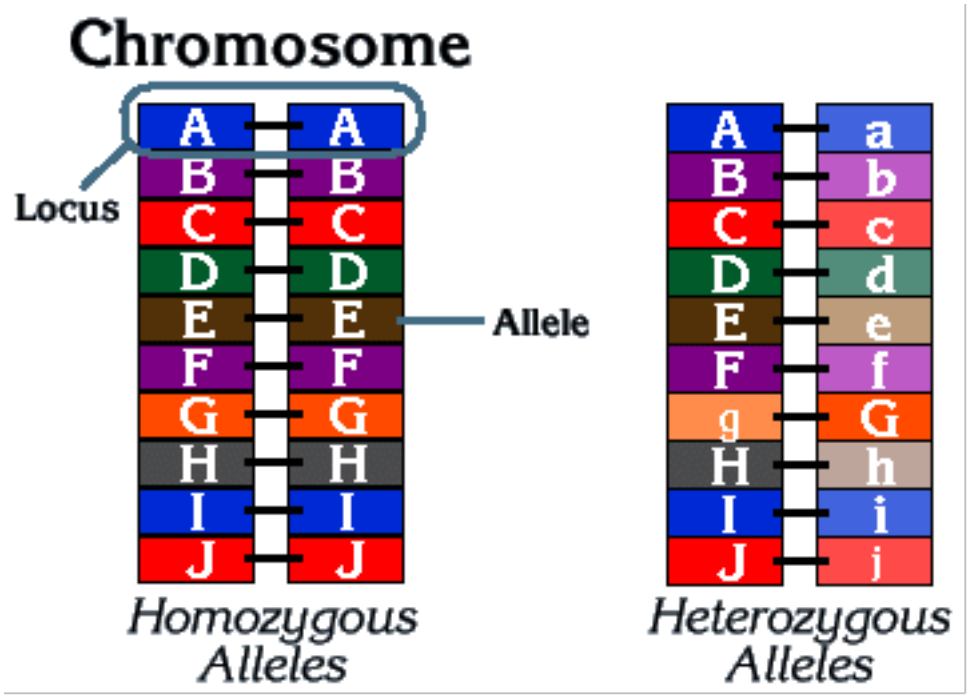
Atul Butte Review: http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1002375

Alleles and Phenotype

- Some phenotypes are caused by a single locus in the genome and a single allele at that locus (e.g. some flower colors, or *Drosophila* eye color)
- Other phenotypes (Type-I diabetes, heart disease are multi-locus or "complex" (i.e. many genes are involved, each potentially with many alleles)

Homologous chromsomes (in a diploid)



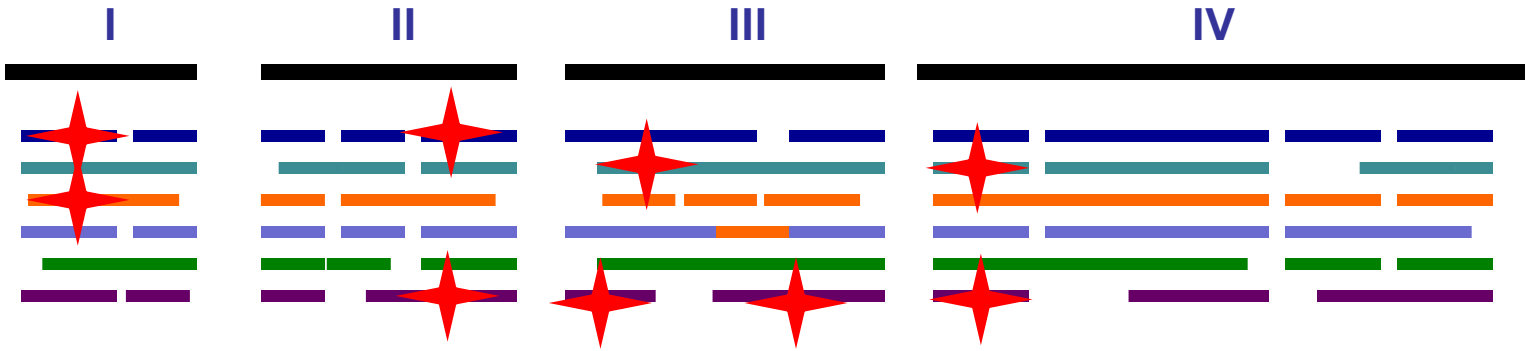


A AAGCCTCATC

a ACGCCTCATC

SNP =Single Nucleotide Polymorphism

30,000 ft View- NGS SNPs



NGS sequence reads from different clinical/environmental isolates

= SNP

```
reference     IGGTGATACT AAGCTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTCA
  303.1       IGGTGATACT AAGCTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTTA
  309.1       IGATAATNCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
 RV_3600      IGGTGATACT AAACTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTCA
 RV_3606      IGATAATNCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
 RV_3610      IGATGATTCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
SenT119.09    IGGTGATACT AAACTGGGAA CTCCACTTCT TTTTCTACTG CGGTGCTTCA
SenT123.09    IGATRATTCT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
SenT140.08    IGGTGATACT AAACTGGGAA CTCCACTTCC TTTTCTACTG CGGTGCTTCA
SenT142.09    IGGTGATACT AAACTGGGAA CTCCACTTCC TTTTCTACTG CAGTGCTTCA
SenT175.08    IGGTGATACT 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)

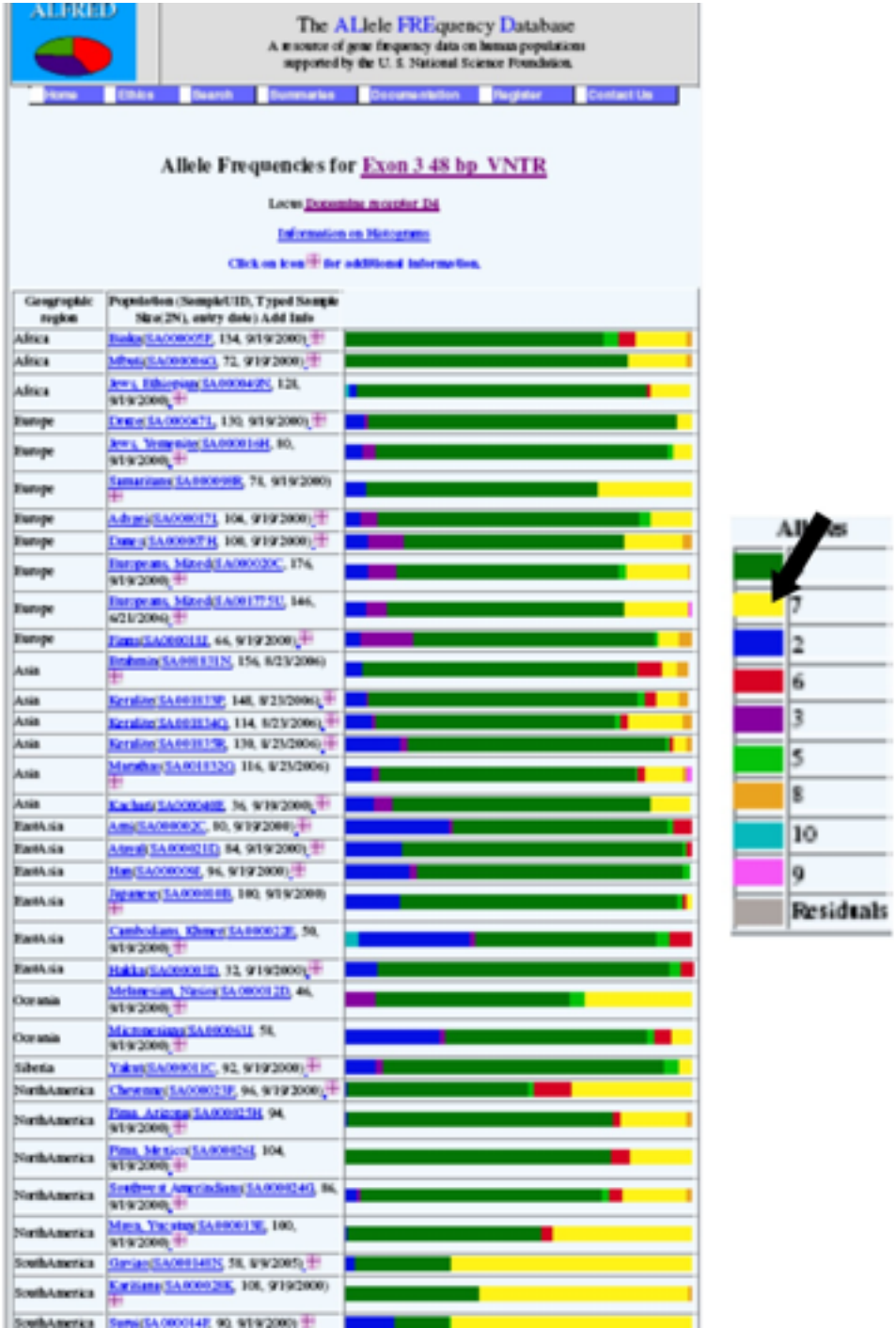
Population data

Data

- Single Nucleotide Polymorphisms, SNPs
- Alleles
- Allele frequency
- Haplotypes

Technology

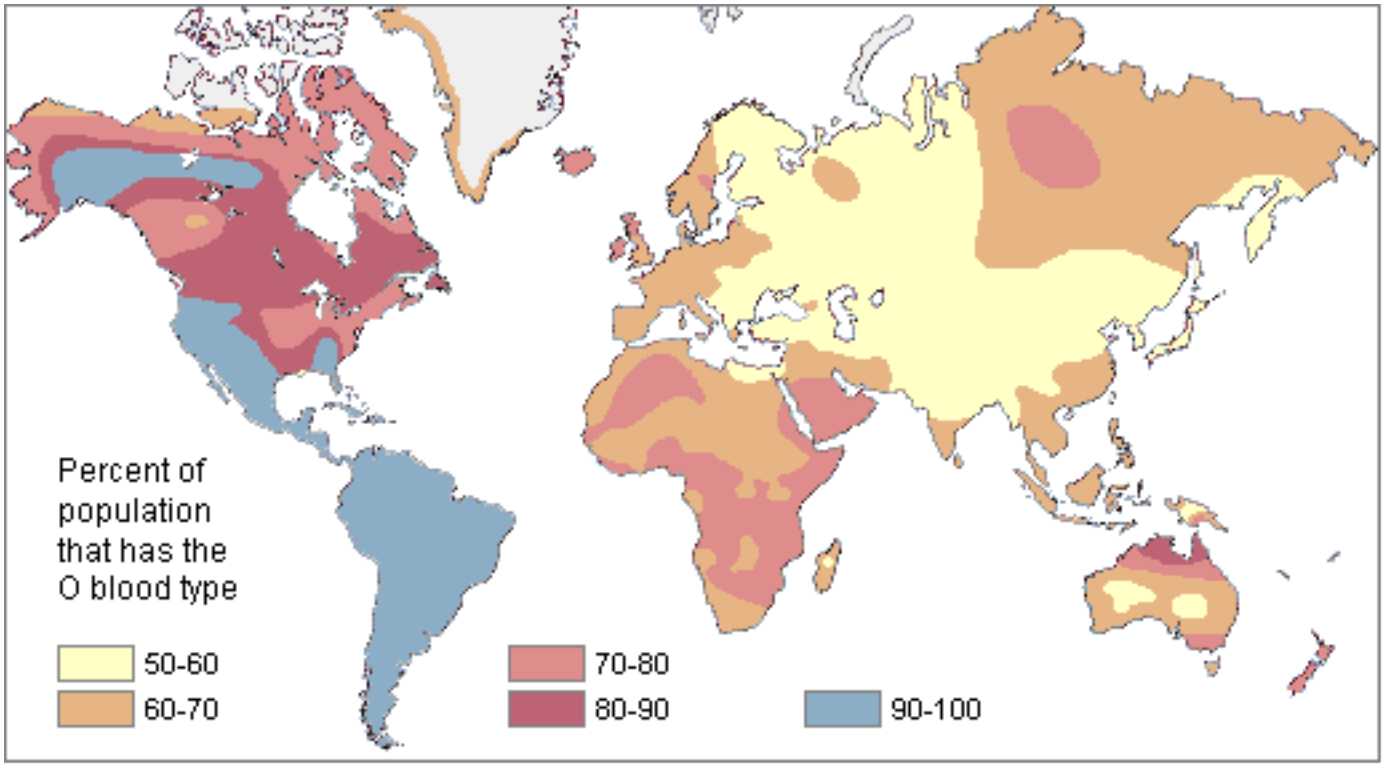
- Chip-Seq
- NGS



Alleles have frequencies in different populations

Populations and alleles have geographic boundaries

A parasite isolate comes from a particular population, a particular location and will have a specific haplotype (e.g. representation of alleles) often characterized via SNPs



Bioinformatics uses algorithms

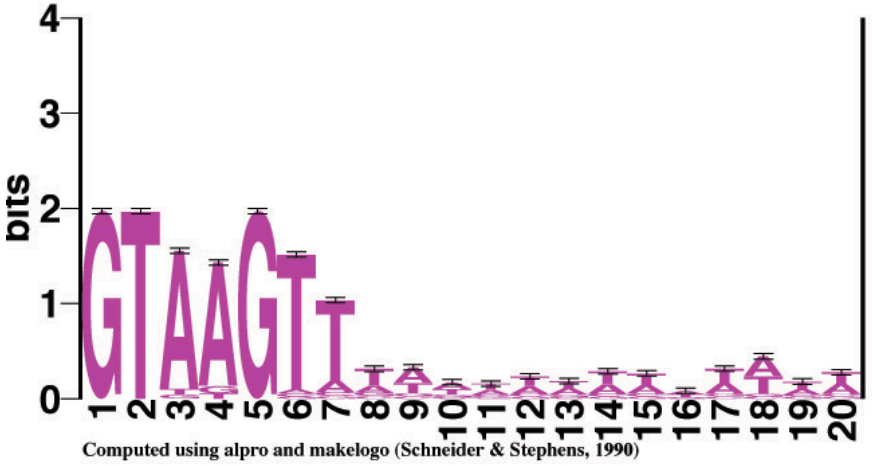
- Algorithms are sets of rules for solving problems or identifying patterns
- Algorithms can be general or case specific and often need to be trained
- Computational analysis, like wet-bench analyses are only as good as the tools, techniques and material allow, and all interpretations come with caveats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

How to find an intron

- Usually begins with GT and end with AG
- Must be longer than 19 nucleotides
- Must contain a branchpoint "A"
- Donor GT often followed by a sequence pattern. This pattern is species-specific
- Acceptor AG often proceeded by pyrimidine stretch
- Has a mean length of "X" as is observed in this species

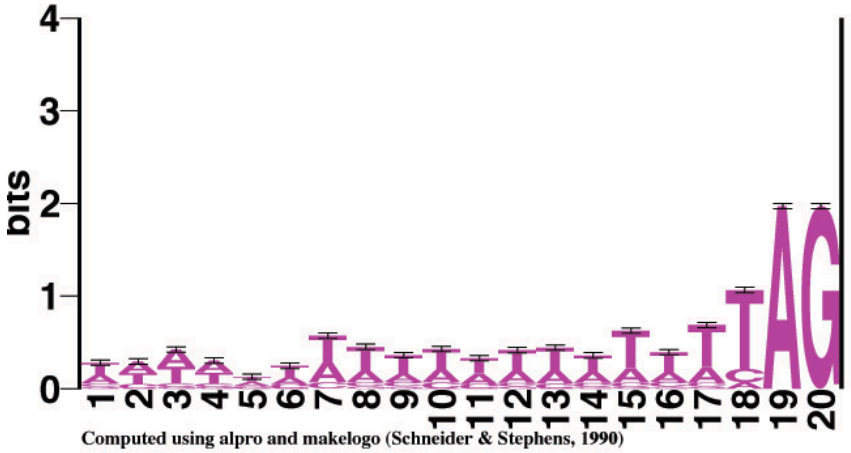
Donor Site

Generated by http://www.bio.cam.ac.uk/seqlogo/log

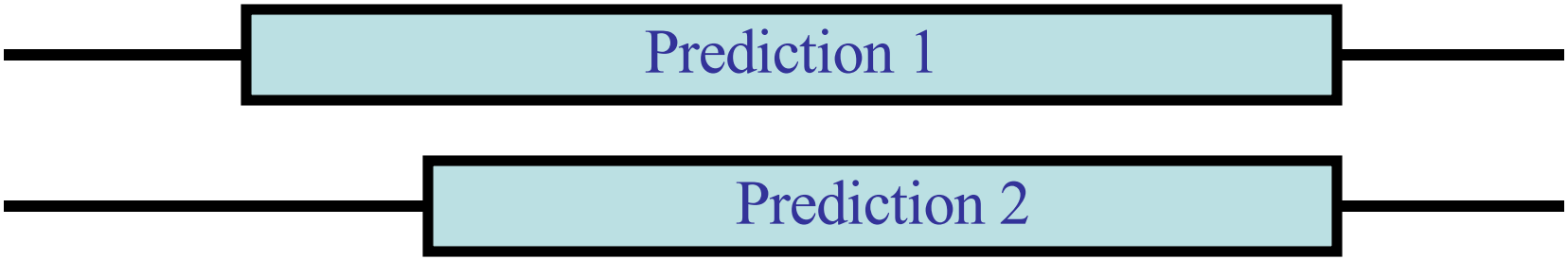


Acceptor site

Generated by http://www.bio.cam.ac.uk/seqlogo/logo.



Different prediction methods often generate different results

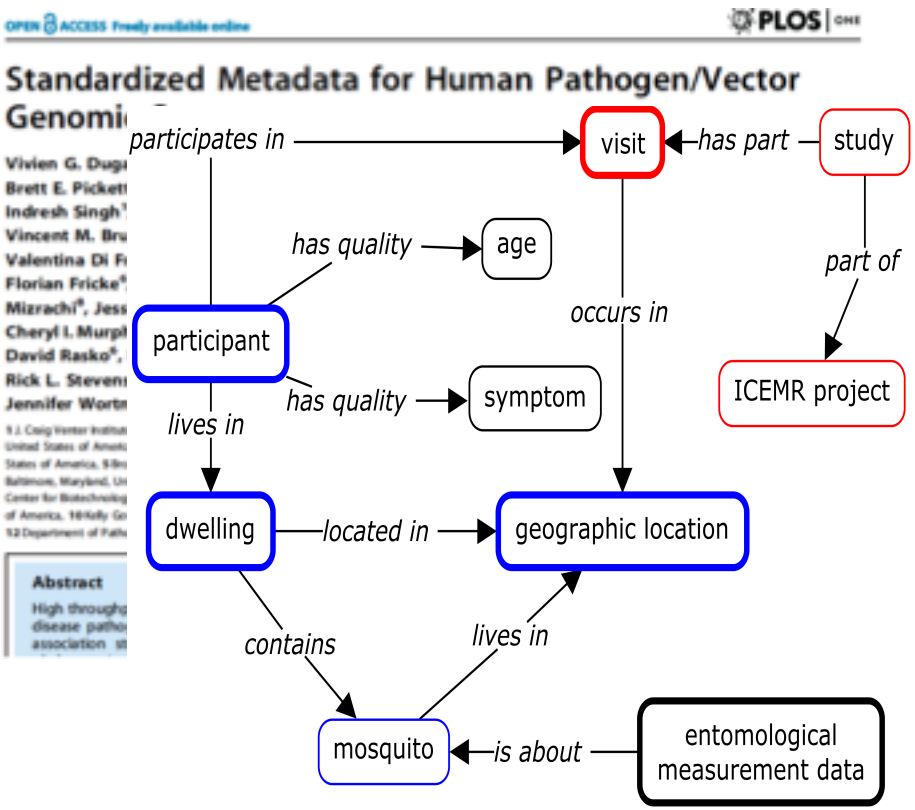


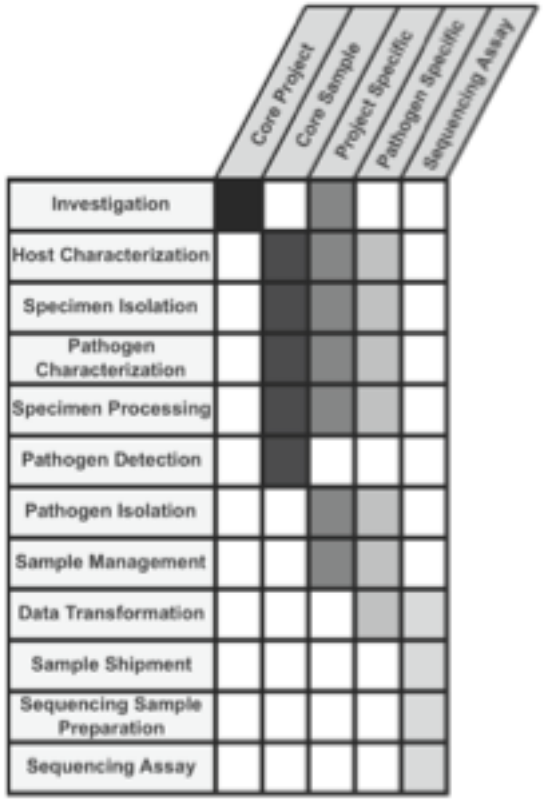
We provide lots evidence so that you can decide or design an experiment to confirm!

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?
  - Data generator
  - Clinical outcome
  - Geographic location
  - Phenotype

Data sharing standards





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.