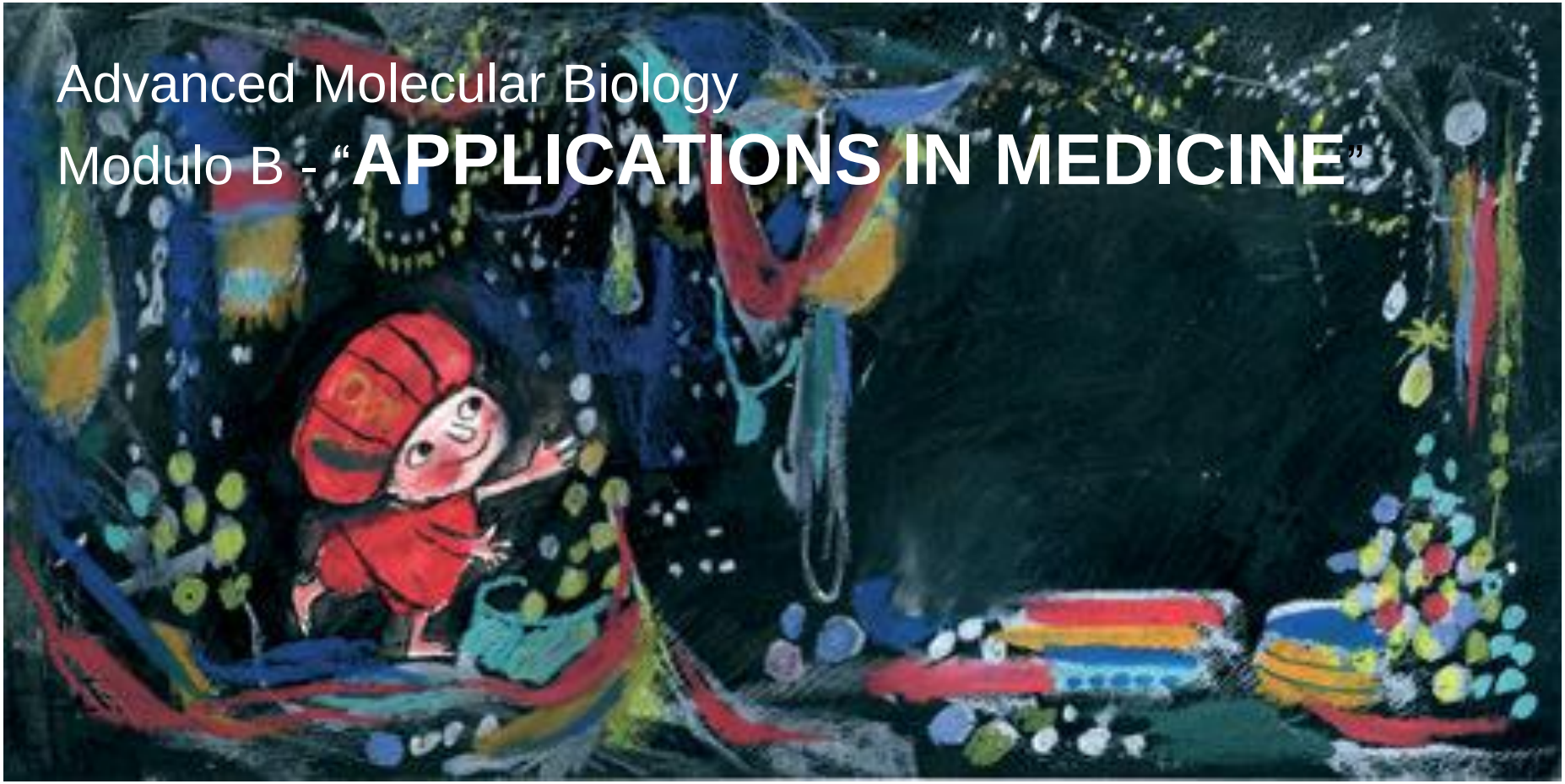


Advanced Molecular Biology

Modulo B - "APPLICATIONS IN MEDICINE"

SANTINA CUTRUPI
Professor of Molecular Biology
University of Turin
Dept. Clinical & Biological Sciences
CIR Molecular Systems Biology
santina.cutrupi@unito.it

" APPLICATIONS IN MEDICINE MODULE"

DEVELOPS SKILLS IN:

- **PROBLEM SOLVING in the application of molecular biology IN MEDICINE**

- **EXPERIMENTAL DESIGN to understand molecular mechanisms linked to disease**

" APPLICATIONS IN MEDICINE MODULE"

How can we improve these skills?

- PROBLEM SOLVING IN MOLECULAR BIOLOGY FIELD
- EXPERIMENTAL DESIGN

- PAPERS ANALYSIS
  - METHODS
  - EXPERIMENT STEPS
  - MEANING OF DATA

- Design experiments and molecular biology methods

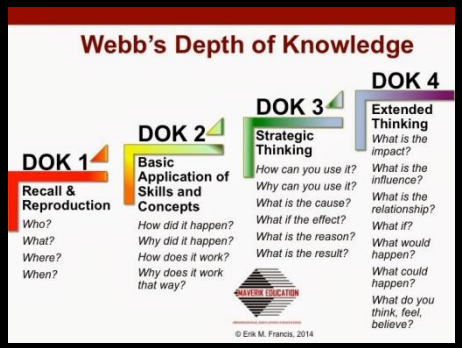
COURSE STRUCTURE:

- **Lesson: presentation/ discussion of main concepts**
- **Quiz: problem solving, experiment design**

We 'll use a "TRAINING TASK"

What is the meaning of this approach?

- Help you to understand the main concept in the deep way
- Help you to remember the main concept
- Help you to apply the main concept to solve problem



In this lesson

- What is the main focus of the course
- Definition of Functional Genomics
- How Functional Genomics is the basis for understanding diseases
- Integration Data
- Application of Functional Genomics and Integration Data

The main focus of this course is
functional genomics

APPLICATIONS ON MEDICINE

Task 1: Search the definition of

FUNCTIONAL GENOMICS

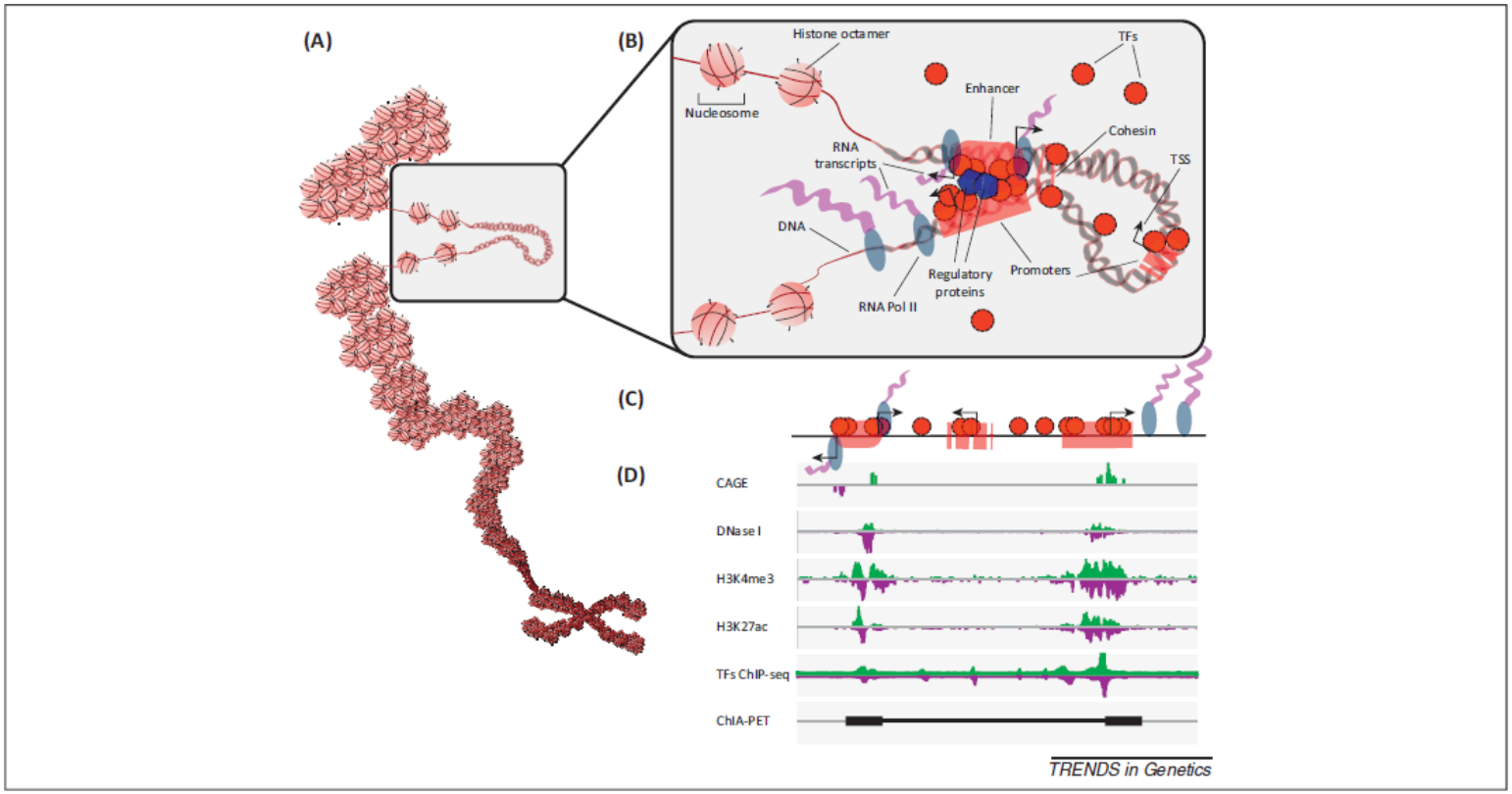
Copy the definition

Answer the questions:

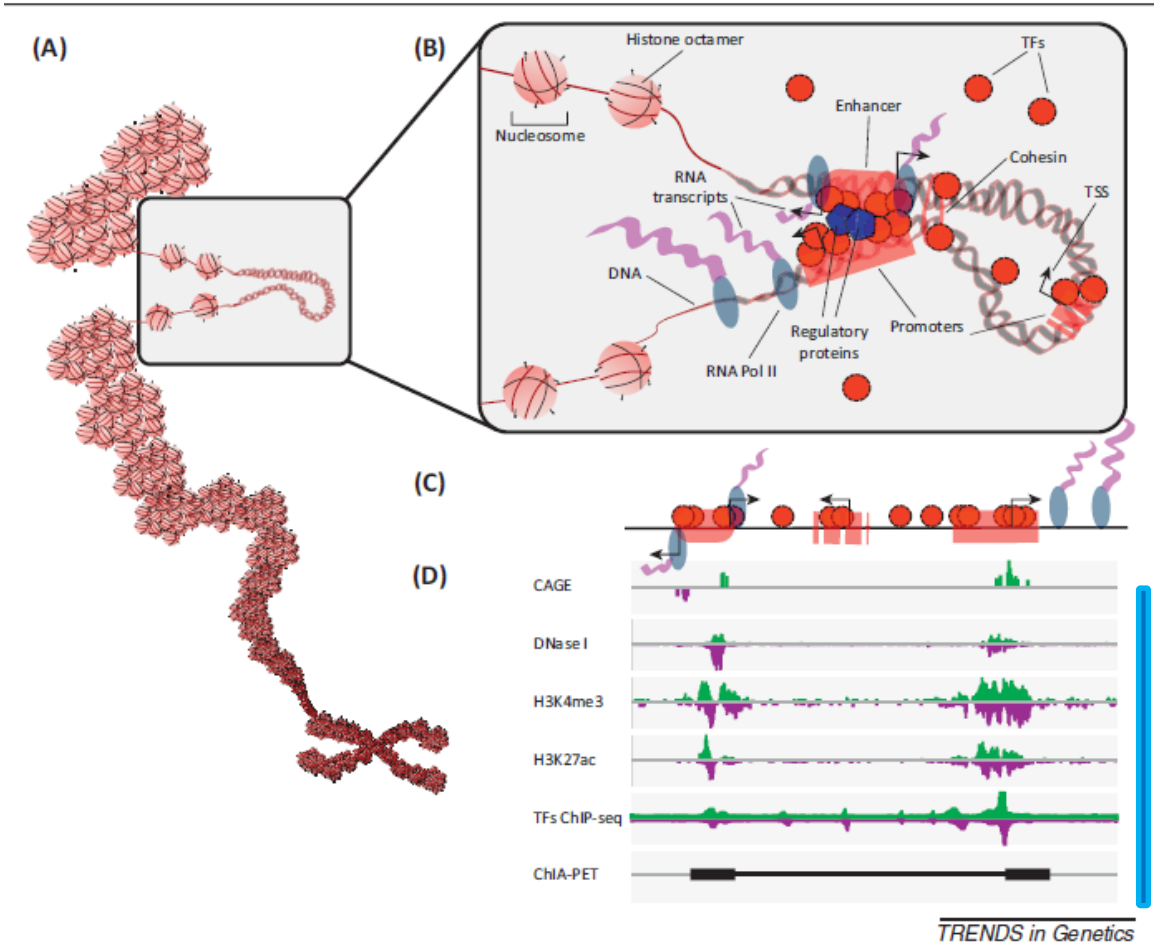
1) **What type of data are used?**
2) **What type of tecniques are used?**
3) **What is the impact?**

Functional genomics

Functional genomics uses genomic data to study gene expression, regulation and biological functions on a global scale (genome-wide or system-wide), focusing on gene transcription, epigenetic modifications, chromatin remodelling enzymes, transcription factors association involving high-throughput methods.



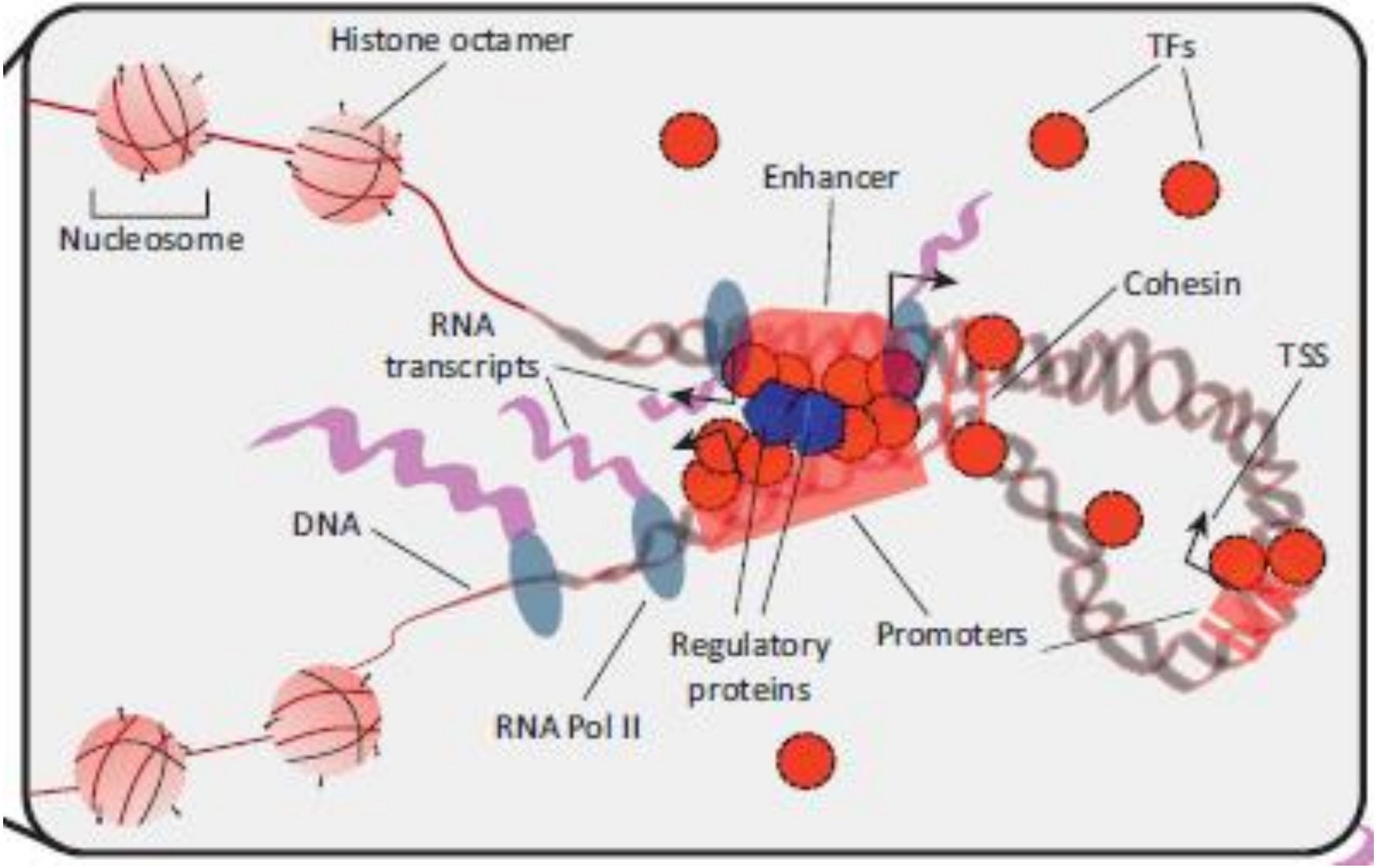
GENOMIC REGULATORY REGIONS



GENE FEATURES

GENE REGULATION

GENOMIC REGULATORY REGIONS



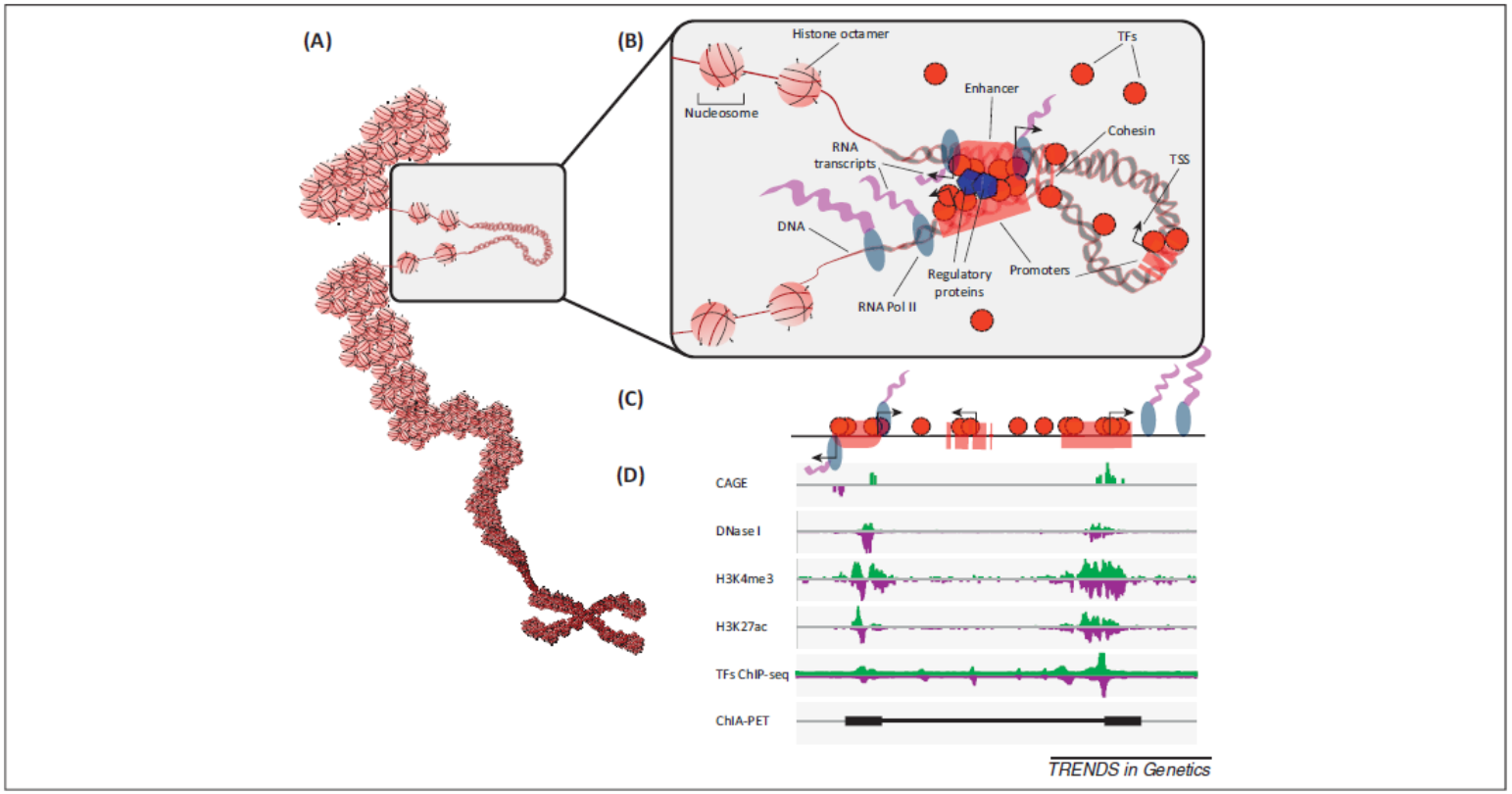
GENE REGULATION
How we can understand gene regulation
Using genome-wide sequencing data

- FUNCTIONAL GENOMICS

- INTEGRATION DATA APPROACH

Functional genomics

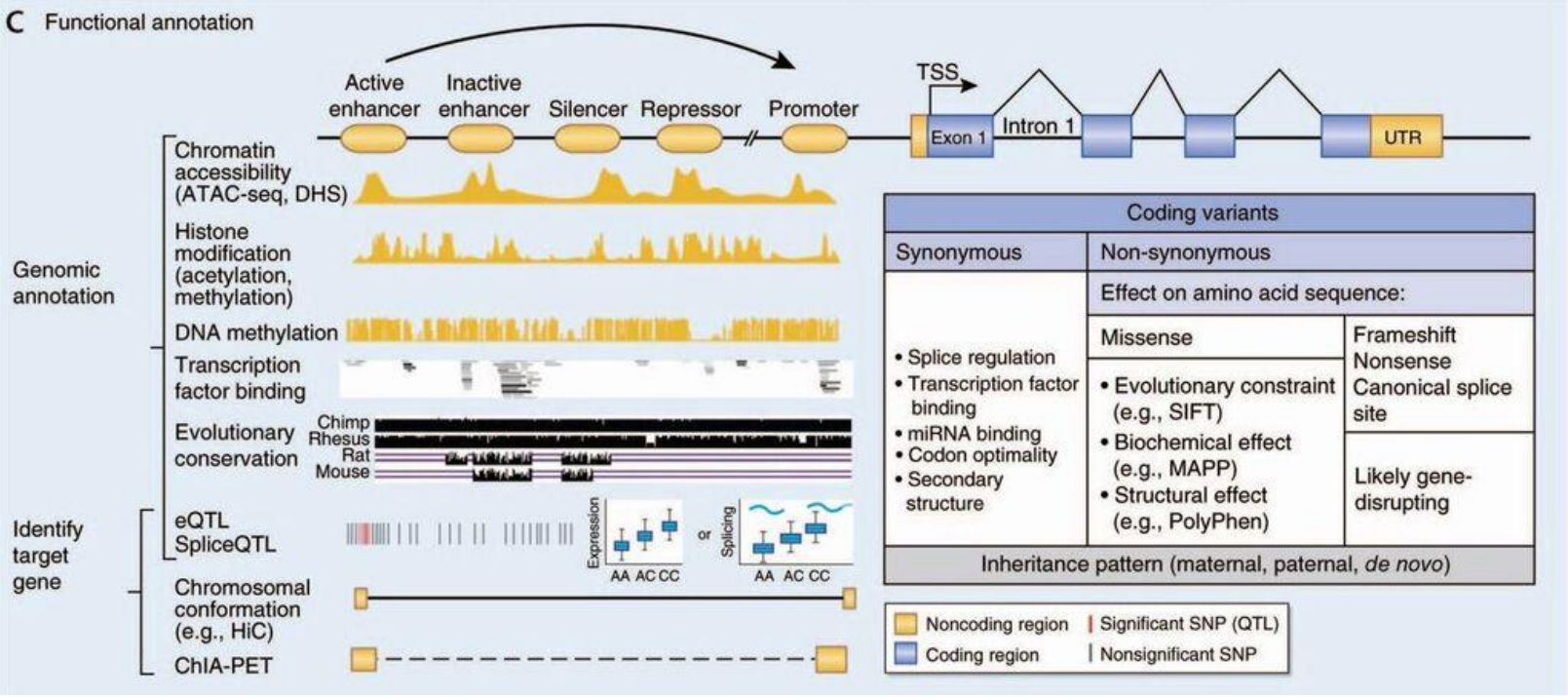
Functional genomics uses genomic data to study gene expression, regulation and biological functions on a global scale (genome-wide or system-wide), focusing on gene transcription, epigenetic modifications, chromatin remodelling enzymes, transcription factors association involving high-throughput methods.



Functional Genomics

Functional genomics is a branch that integrates molecular biology and cell biology studies, and deals with the whole structure, function and regulation of a gene in contrast to the gene-by-gene approach of classical molecular biology technique.

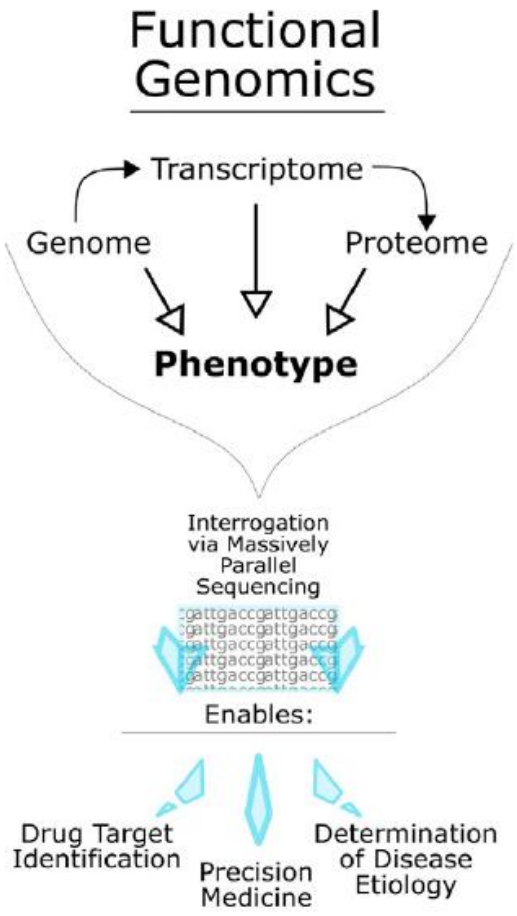
From: Encyclopedia of Bioinformatics and Computational Biology, 2019



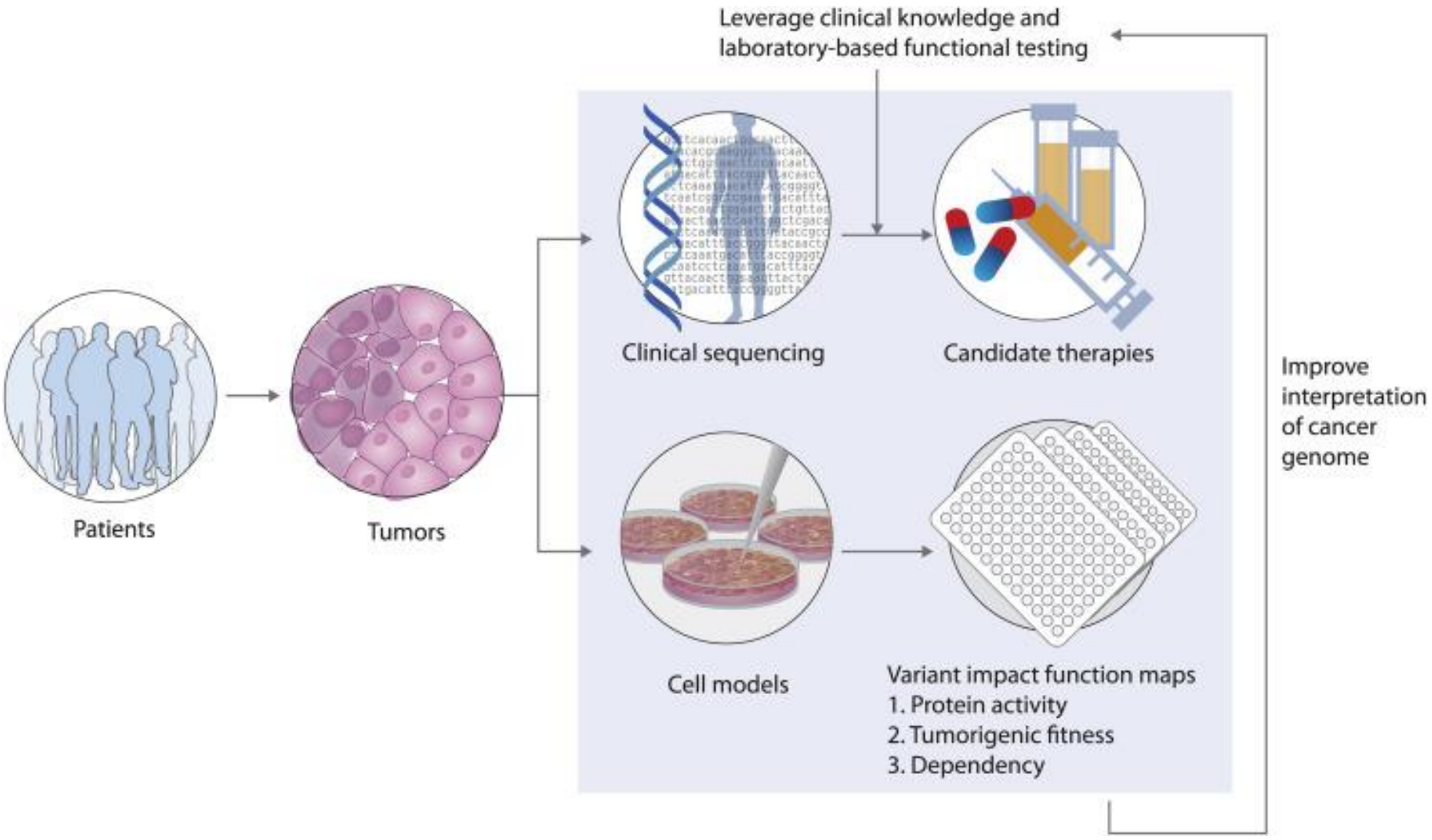
GENE REGULATION

How we can understand gene regulation Using genome-wide sequencing data

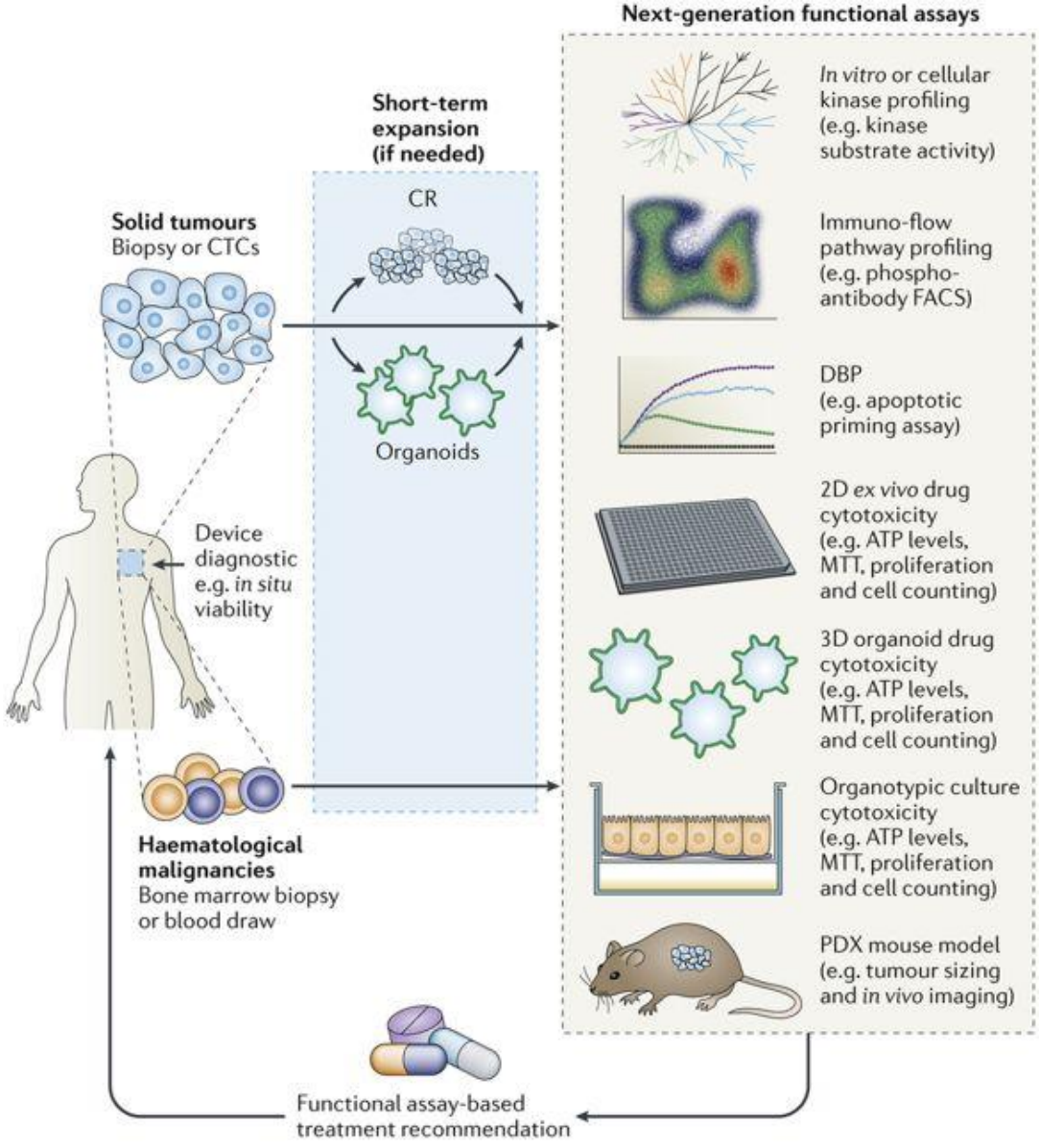
- **FUNCTIONAL GENOMICS**



Functional genomics uses genome-wide data with functional tests



Functional genomics uses genome-wide data with functional tests

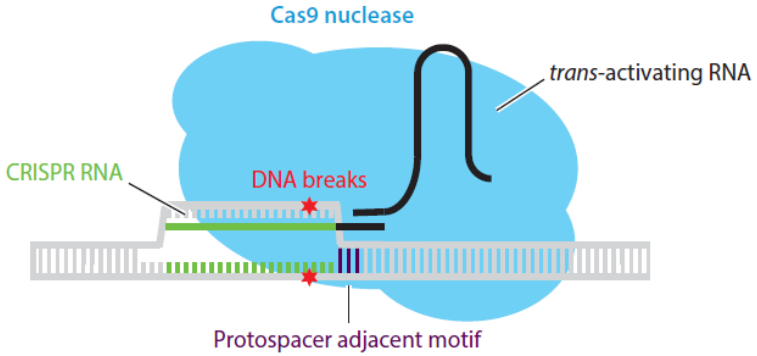


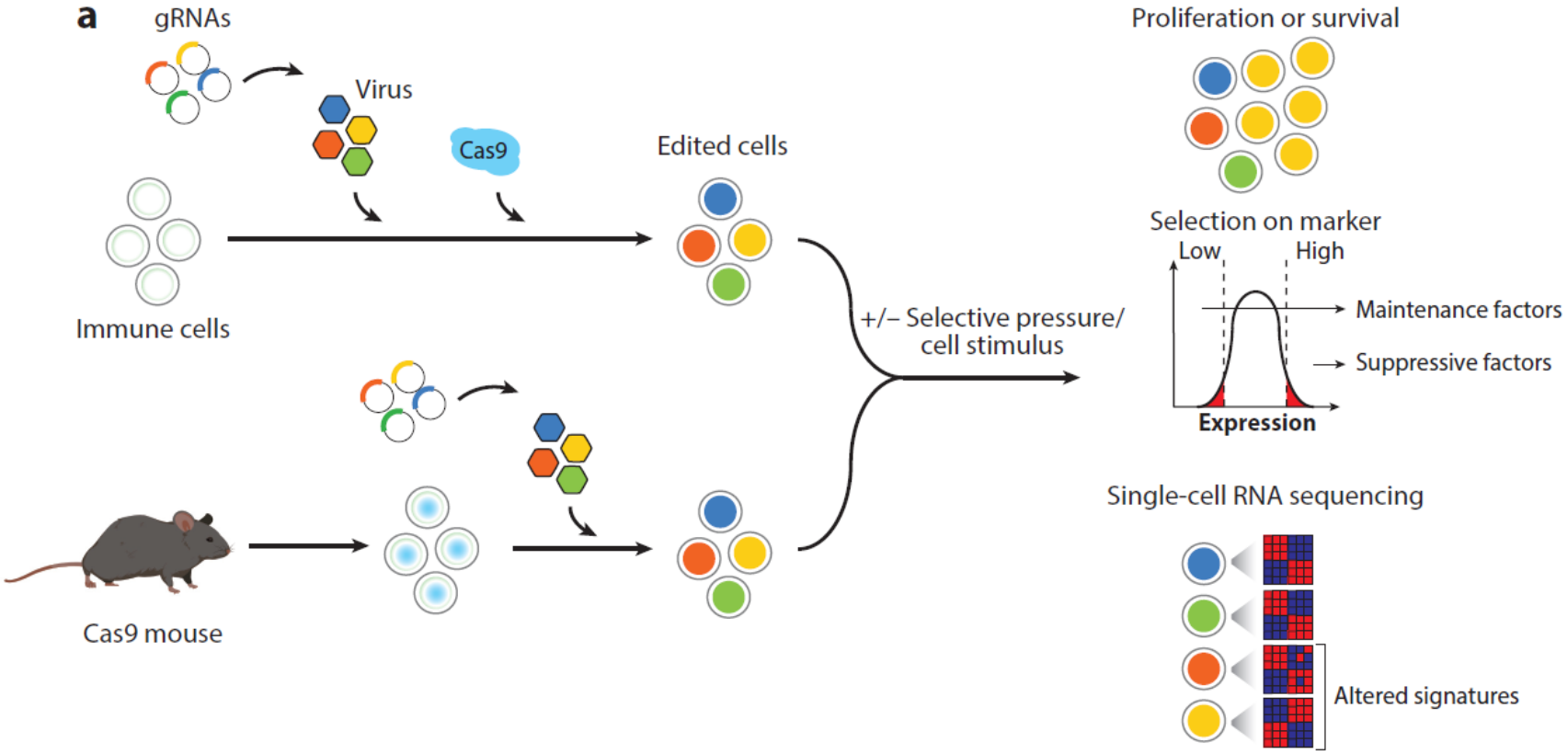
Nature Reviews | Cancer

Annual Review of Immunology

CRISPR-Based Tools in Immunity

Dimitre R. Simeonov[1,2,3] and Alexander Marson[2,3,4,5,6,7]





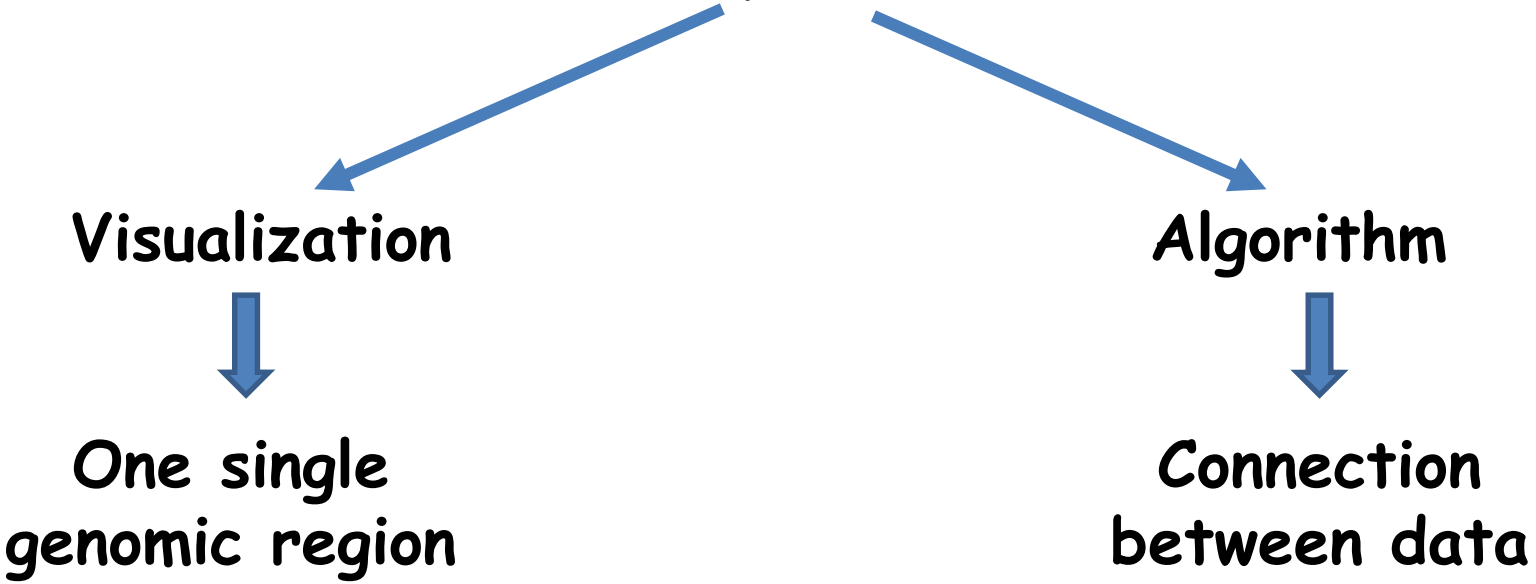
GENE REGULATION
How we can understand gene regulation
Using genome-wide sequencing data

- INTEGRATION DATA APPROACH
- FUNCTIONAL GENOMICS

GENE REGULATION
How we can understand gene regulation Using genome-wide sequencing data

- **INTEGRATION DATA APPROACH**

Is based on the comparison of different data



GENE REGULATION
How we can understand gene regulation
Using genome-wide sequencing data

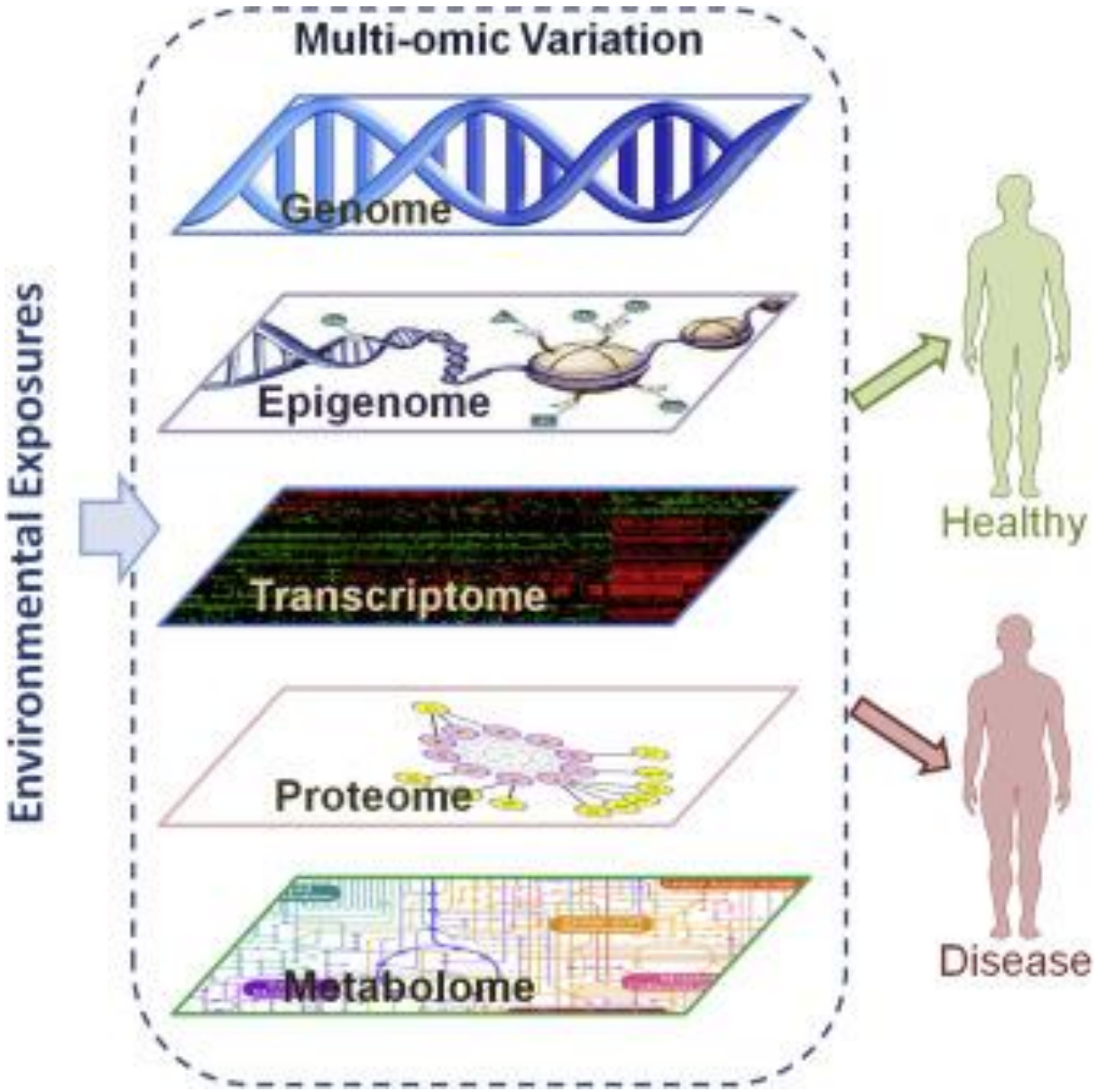
- **INTEGRATION DATA APPROACH**

Integrative omics for health and disease

Konrad J. Karczewski[1,2] and Michael P. Snyder[3]

Abstract | Advances in omics technologies — such as genomics, transcriptomics, proteomics and metabolomics — have begun to enable personalized medicine at an extraordinarily detailed molecular level. Individually, these technologies have contributed medical advances that have begun to enter clinical practice. However, each technology individually cannot capture the entire biological complexity of most human diseases. Integration of multiple technologies has emerged as an approach to provide a more comprehensive view of biology and disease. In this Review, we discuss the potential for combining diverse types of data and the utility of this approach in human health and disease. We provide examples of data integration to understand, diagnose and inform treatment of diseases, including rare and common diseases as well as cancer and transplant biology. Finally, we discuss technical and other challenges to clinical implementation of integrative omics.

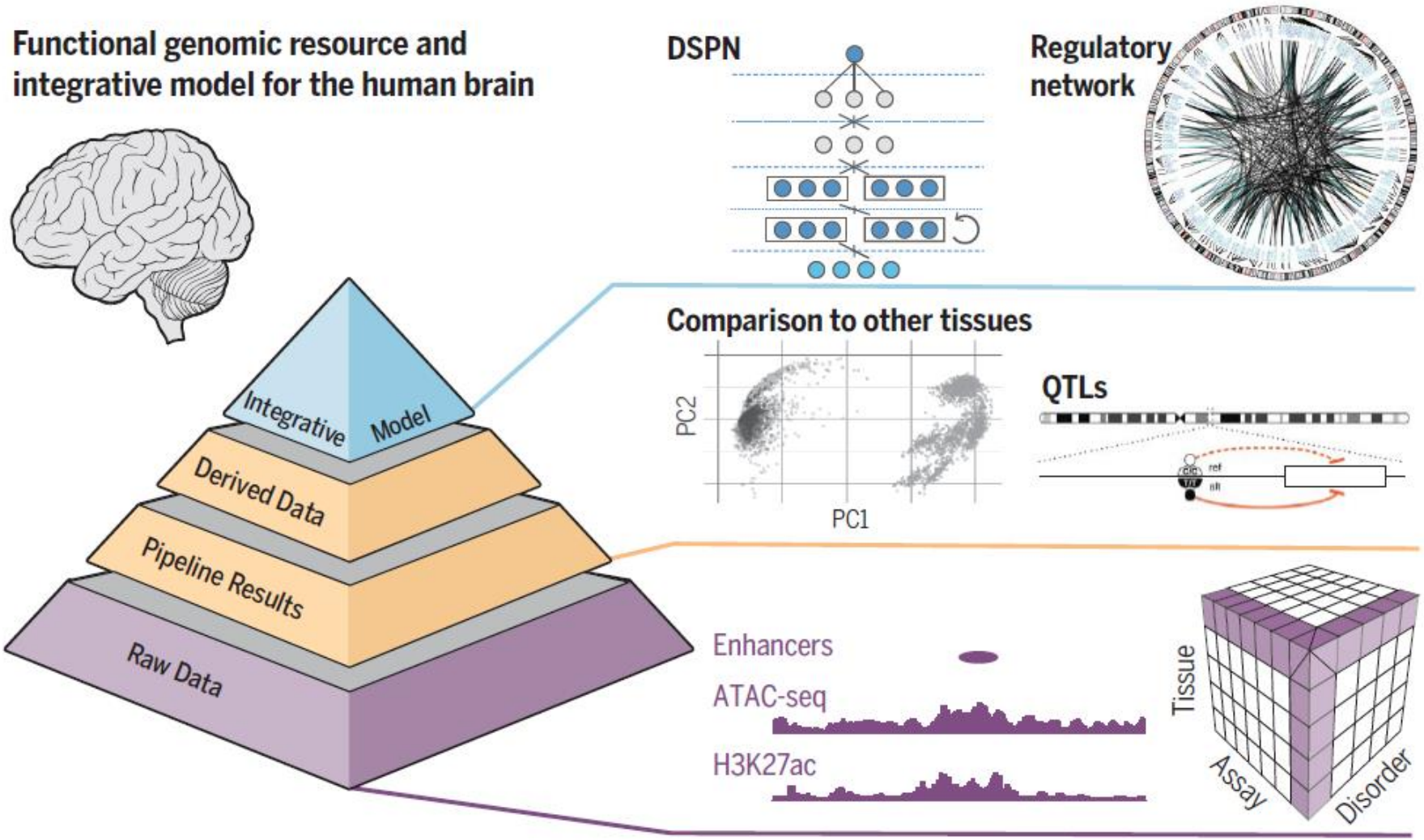
Integrative Omics for health and disease



PSYCHIATRIC GENOMICS

Comprehensive functional genomic resource and integrative model for the human brain

Daifeng Wang*, Shuang Liu*, Jonathan Warrell*, Hyejung Won*, Xu Shi*, Fabio C. P. Navarro*, Declan Clarke*, Mengting Gu*, Prashant Emani*, Yucheng T. Yang, Min Xu, Michael J. Gandal, Shaoke Lou, Jing Zhang, Jonathan J. Park, Chengfei Yan, Suhn Kyong Rhie, Kasidet Manakongtreecheep, Holly Zhou, Aparna Nathan, Mette Peters, Eugenio Mattei, Dominic Fitzgerald, Tonya Brunetti, Jill Moore, Yan Jiang, Kiran Girdhar, Gabriel E. Hoffman, Selim Kalayci, Zeynep H. Gümüş, Gregory E. Crawford, PsychENCODE Consortium†, Panos Roussos, Schahram Akbarian, Andrew E. Jaffe, Kevin P. White, Zhiping Weng, Nenad Sestan, Daniel H. Geschwind‡, James A. Knowles‡, Mark B. Gerstein‡


Functional genomic resource and integrative model for the human brain
Integrative Model
Derived Data
Pipeline Results
Raw Data
DSPN
Regulatory network
Comparison to other tissues
PC2
PC1
QTLs
Enhancers
ATAC-seq
H3K27ac
Tissue
Assay
Disorder

PSYCHIATRIC GENOMICS

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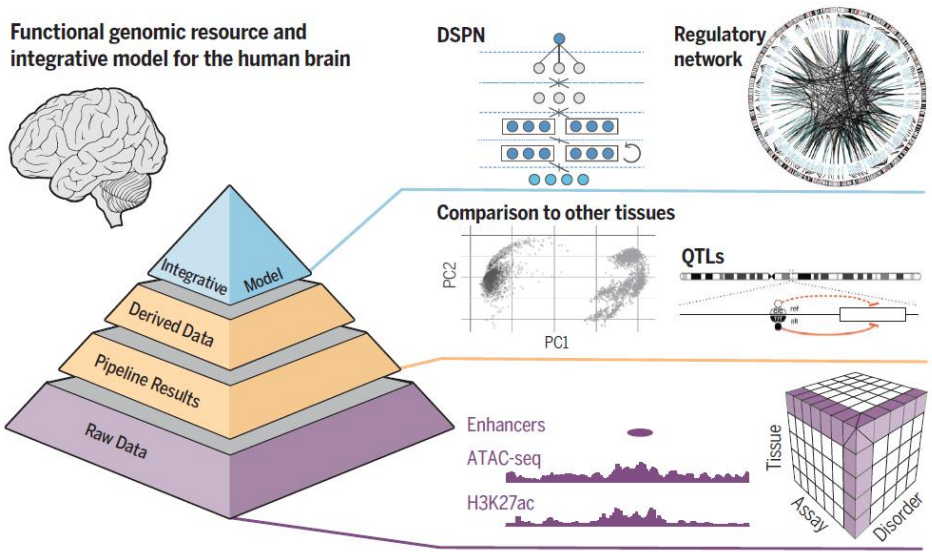
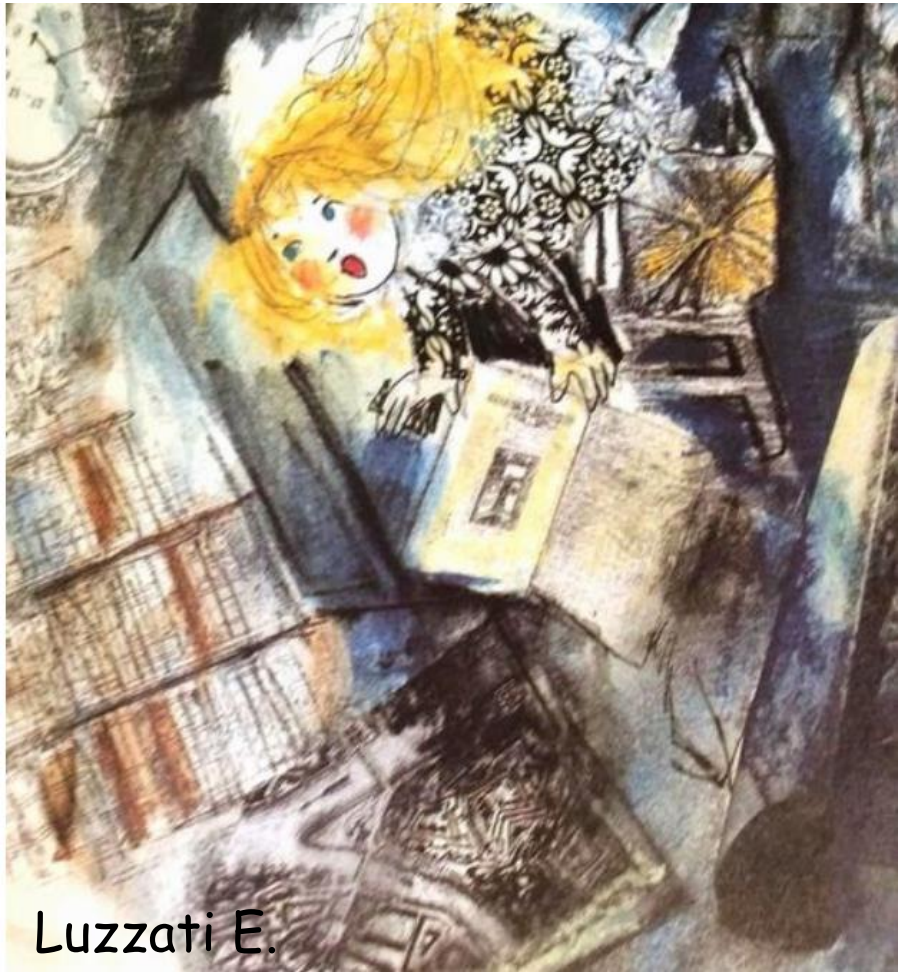


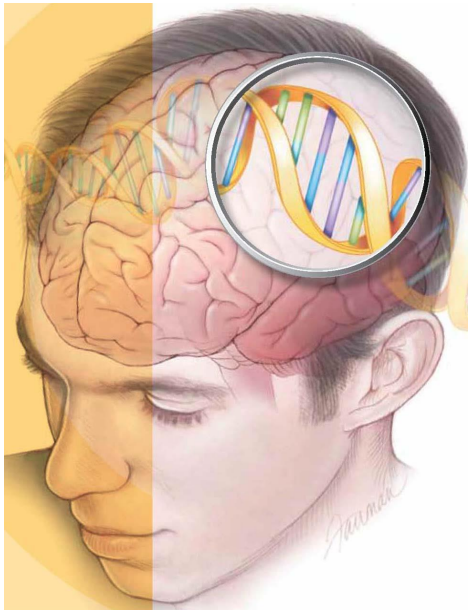


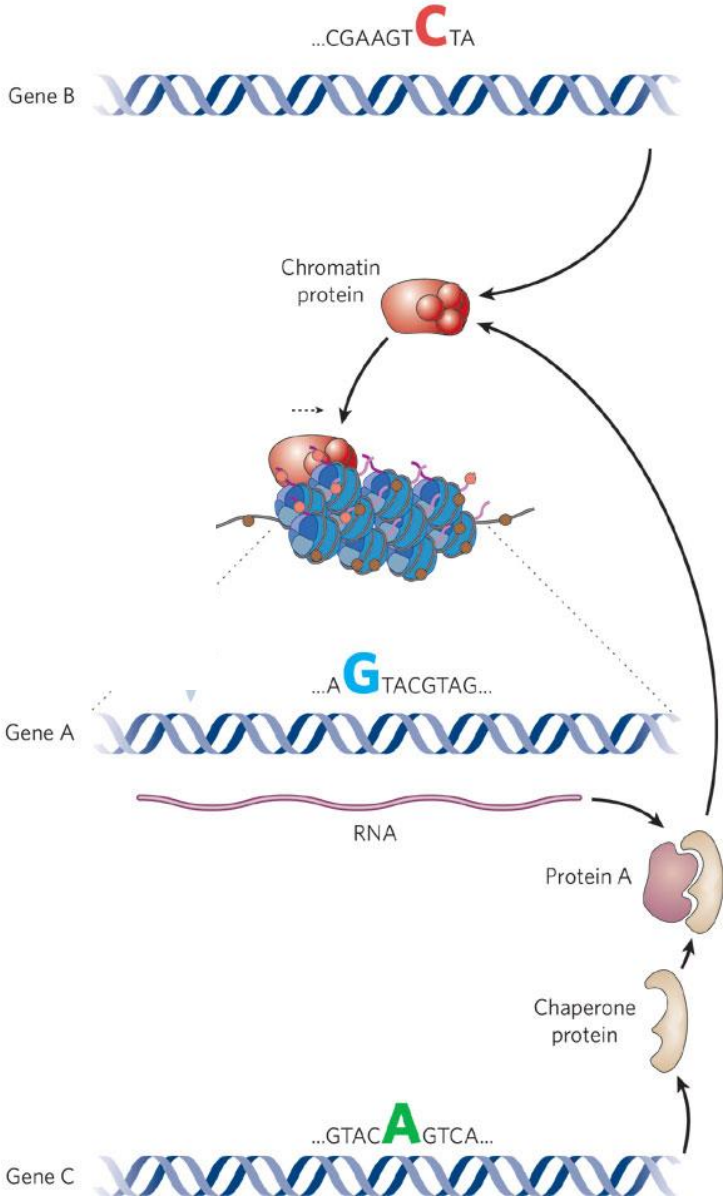
Don't worry

We will learn to understand this complex article

INTRODUCTION: Strong genetic associations have been found for a number of psychiatric disorders. However, understanding the underlying molecular mechanisms remains challenging.

RATIONALE: To address this challenge, the PsychENCODE Consortium has developed a comprehensive online resource and integrative models for the functional genomics of the human brain.

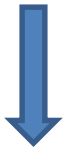




RESULTS: The base of the pyramidal resource is the datasets generated by PsychENCODE, including bulk transcriptome, chromatin, genotype, and Hi-C datasets and single-cell transcriptomic data from ~32,000 cells for major brain regions. We have merged these with data from Genotype-Tissue Expression (GTEx), ENCODE, Roadmap Epigenomics, and single-cell analyses. Via uniform processing, we created a harmonized resource, allowing us to survey functional genomics data on the brain over a sample size of 1866 individuals.

1. New genome-wide data

2. Comparison of New genome-wide data with data derived from several databases



What is the meaning of this statement?

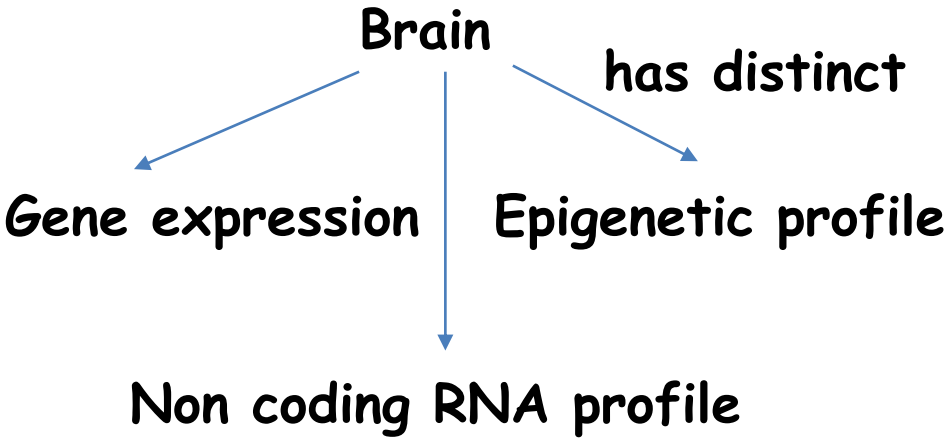
From this uniformly processed dataset, we created derived data products. These include lists of brain-expressed genes, coexpression modules, and single-cell expression profiles for many brain cell types; ~79,000 brain-active enhancers with associated Hi-C loops and topologically associating domains; and ~2.5 million expression quantitative-trait loci (QTLs) comprising ~238,000 linkage-disequilibrium–independent single-nucleotide polymorphisms and of other types of QTLs associated with splice isoforms, cell fractions, and chromatin activity. By using these, we found that >88% of the cross-population variation in brain gene expression can be accounted for by cell fraction changes. Furthermore, a number of disorders and aging are associated with changes in cell-type proportions. The derived data also enable comparison between the brain and other tissues. In particular, by using spectral analyses, we found that the brain has distinct expression and epigenetic patterns, including a greater extent of noncoding transcription than other tissues.

ON OUR WEBSITE

Read the full article at http://dx.doi.org/10.1126/science.aat8464

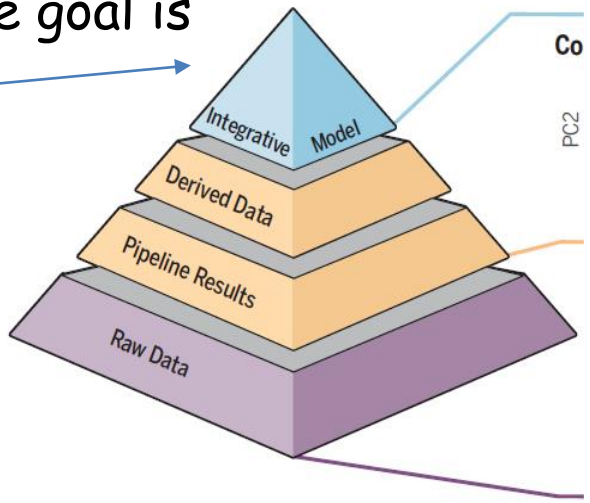
1. New genome-wide data

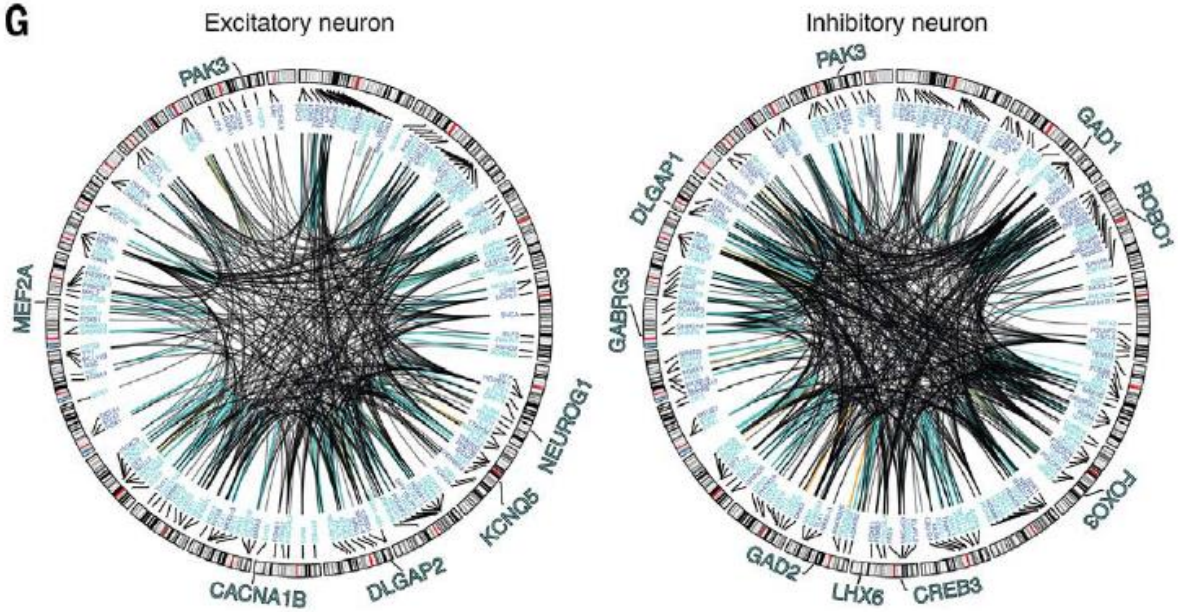
88% crosspopulation variation in brain gene expression



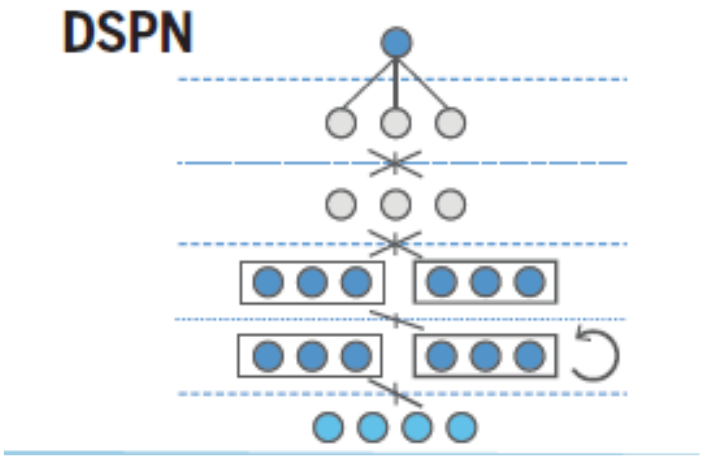
The top level of the resource consists of integrative networks for regulation and machine-learning models for disease prediction. The networks include a full gene regulatory network (GRN) for the brain, linking transcription factors, enhancers, and target genes from merging of the QTLs, generalized element-activity correlations, and Hi-C data. By using this network, we link disease genes to genome-wide association study (GWAS) variants for psychiatric disorders. For schizophrenia, we linked 321 genes to the 142 reported GWAS loci. We

The goal is



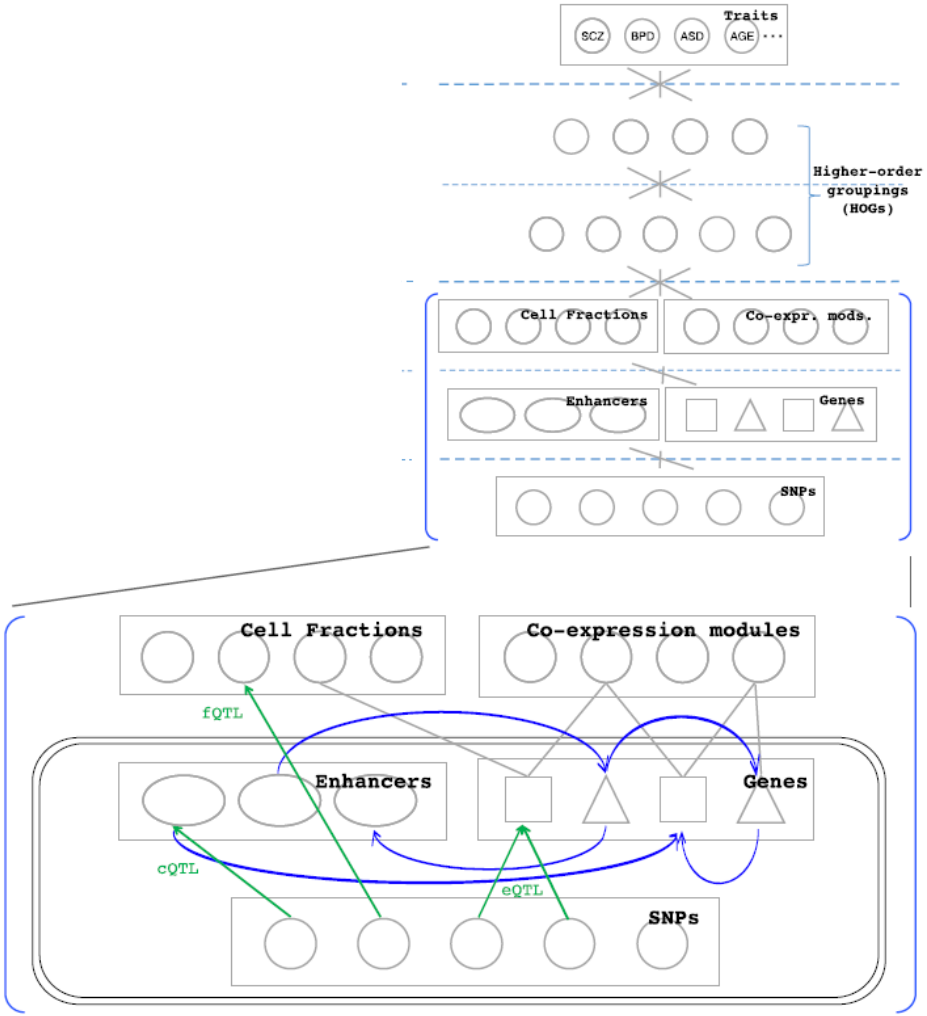


321 genes to the 142 reported GWAS loci. We then embedded the regulatory network into a deep-learning model to predict psychiatric phenotypes from genotype and expression. Our model gives a ~6-fold improvement in prediction over additive polygenic risk scores. Moreover, it achieves a ~3-fold improvement over additive models, even when the gene expression data are imputed, highlighting the value of having just a small amount of transcriptome data for disease prediction. Lastly, it highlights key genes and pathways associated with disorder prediction, including immunological, synaptic, and metabolic pathways, recapitulating de novo results from more targeted analyses.



What is integrative analysis?

- Gene regulatory network
- **Deep-learning model**



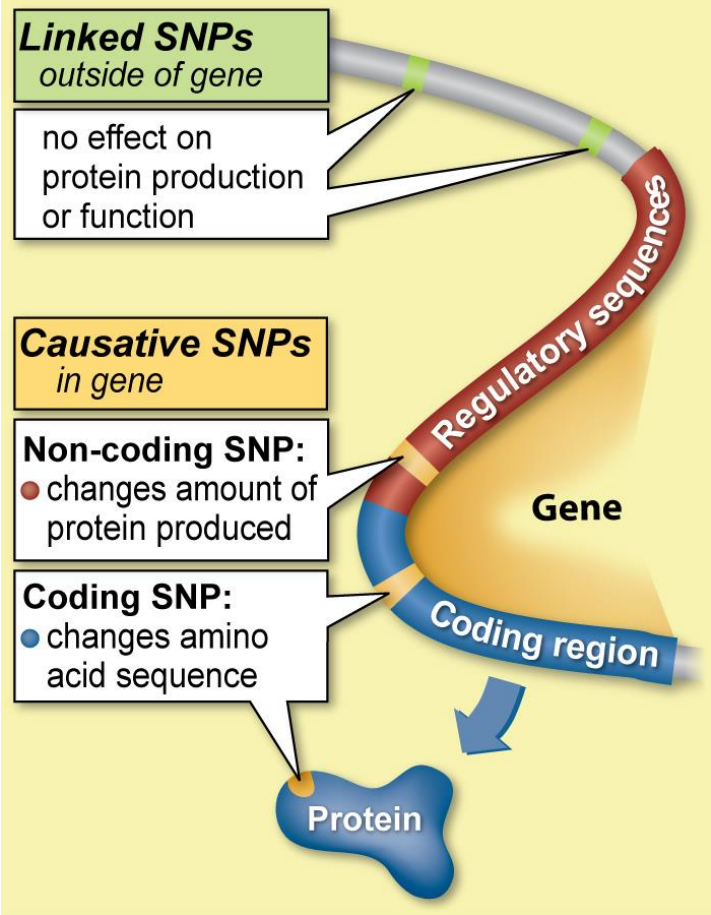
GENOMIC REGULATORY REGIONS

are defined by:

- → **EPIGENETIC MARKS**
- → **TRANSCRIPTION FACTORS BINDING**
- → **CHROMATIN REMODELLING ENZYMES**
- → **NUCLEOSOME POSITIONING**

Task 2- What is the impact of single nucleotide variants

Describe how the change in the single nucleotide in the DNA sequence has an impact in the biological functions.



- What is the main focus of the course
- Definition of Functional Genomics
- **Focus: DNA genomic elements as cell-type specific regulatory regions**
- How Functional Genomics is the basis for undestanding diseases
- Genome-wide sequencing methods to annotate DNA genomic elements. Storing in Databases.

GENE EXPRESSION REGULATION

BY

ENHACERS

PROMOTER

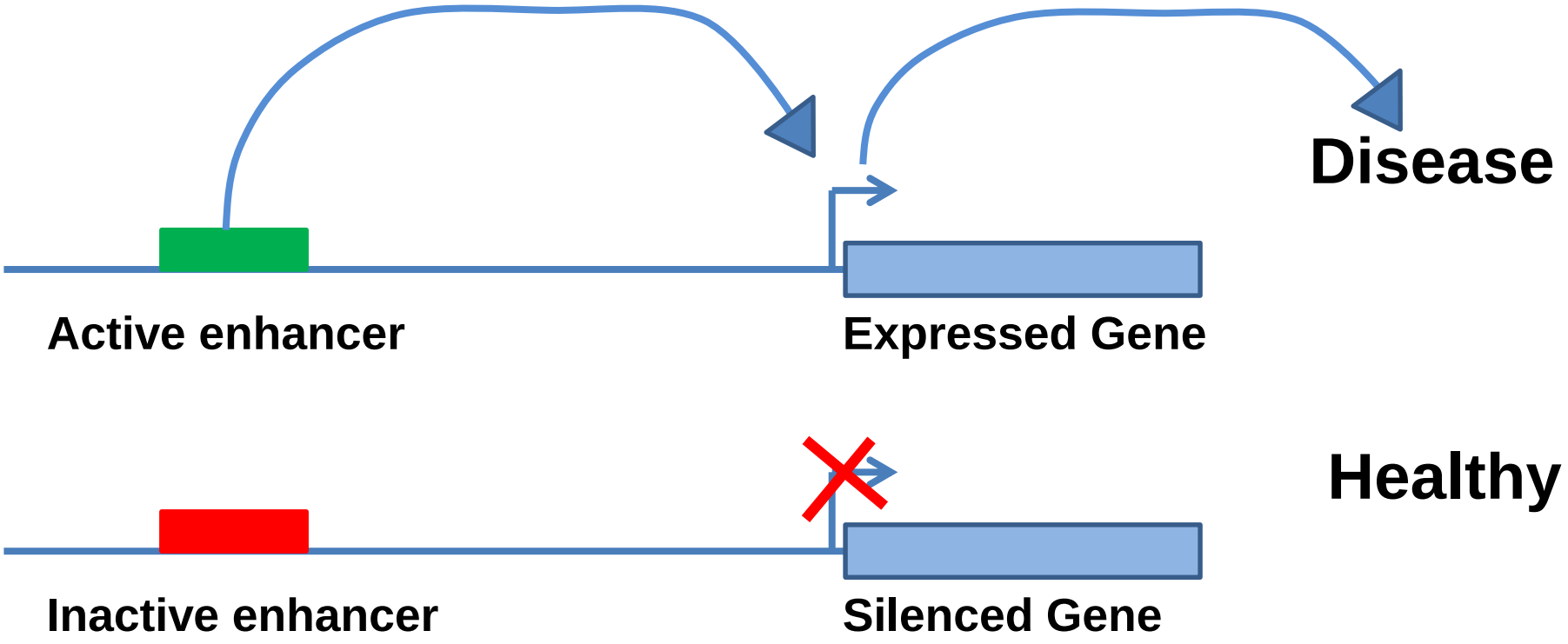
GENOMIC REGULATORY REGIONS

are defined by:

- EPIGENETIC MARKS
- TRANSCRIPTION FACTORS BINDING
- CHROMATIN REMODELLING ENZYMES
- NUCLEOSOME POSITIONING

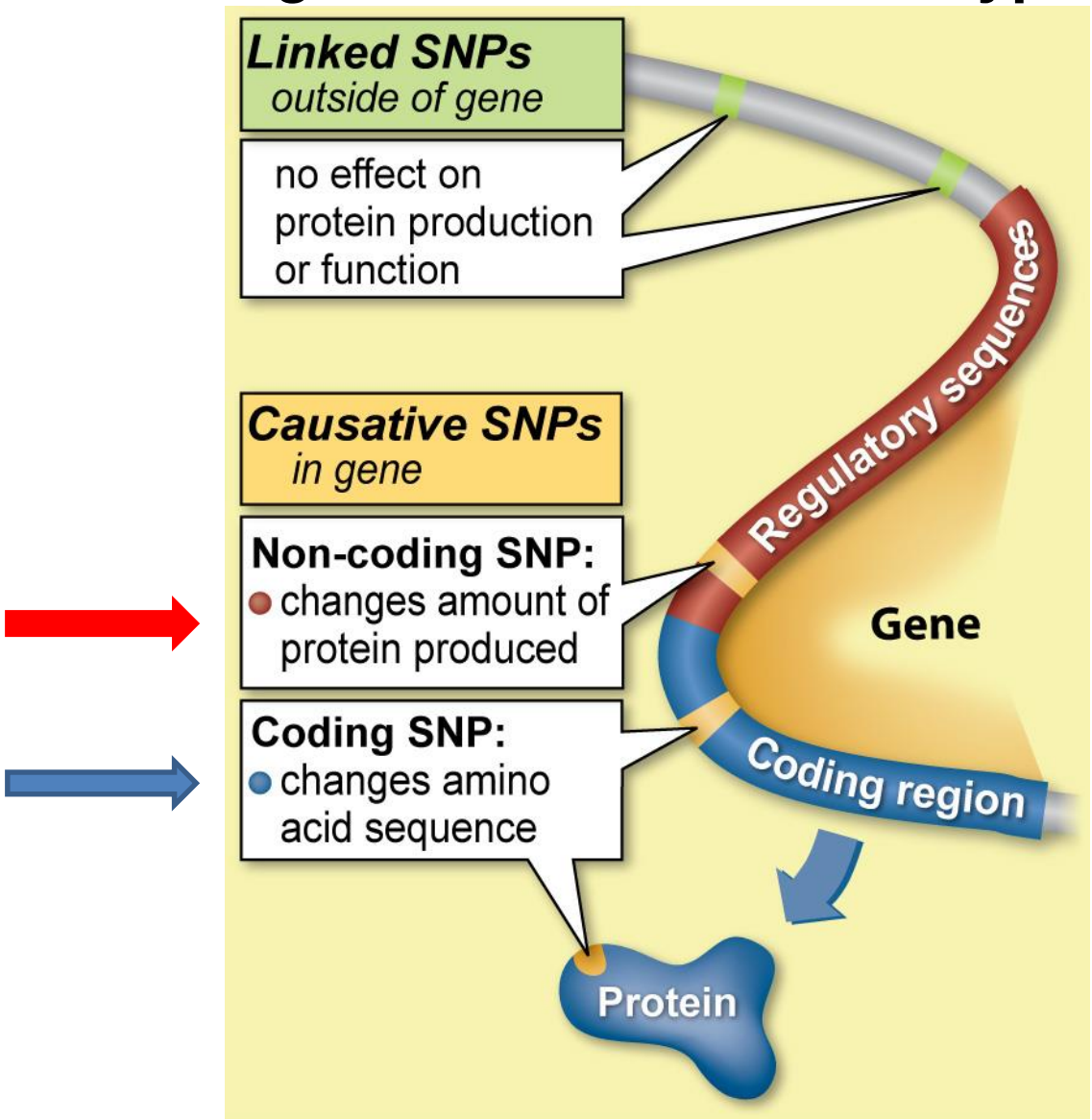
FROM GENOMIC REGULATORY REGIONS TO MOLECULAR MECHANISMS

Genomic regulatory regions control gene expression and specific activation may be associated with disease: One possible Scenario



FROM GENOMIC REGULATORY REGIONS TO MOLECULAR MECHANIMS

Single nucleotide variants types



FROM GENOMIC REGULATORY REGIONS TO MOLECULAR MECHANISMS

What types of alterations in the molecular mechanism could induce diseases:

- **Single nucleotide variations into the genomic regulatory regions change the consensus sequences for transcription factors binding**

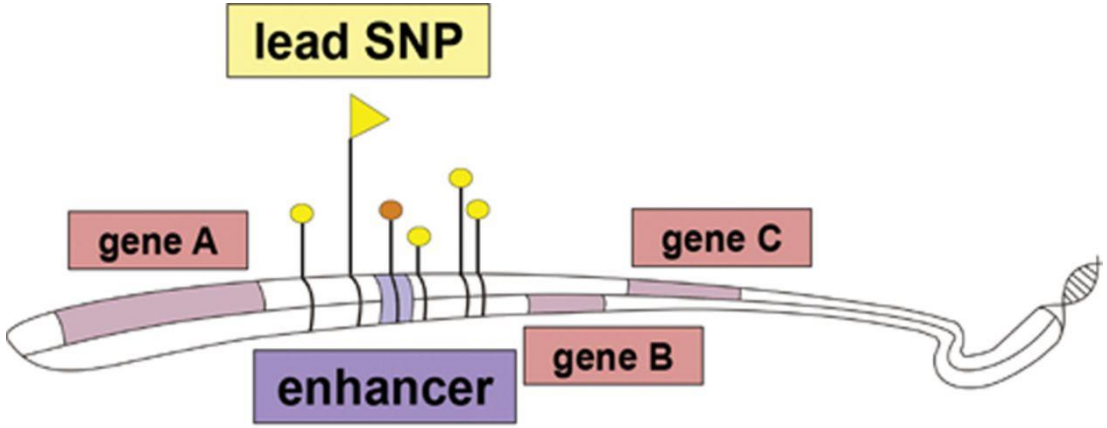
- **Single nucleotide variations into the genomic regulatory regions change long range interactions between two regulatory regions**

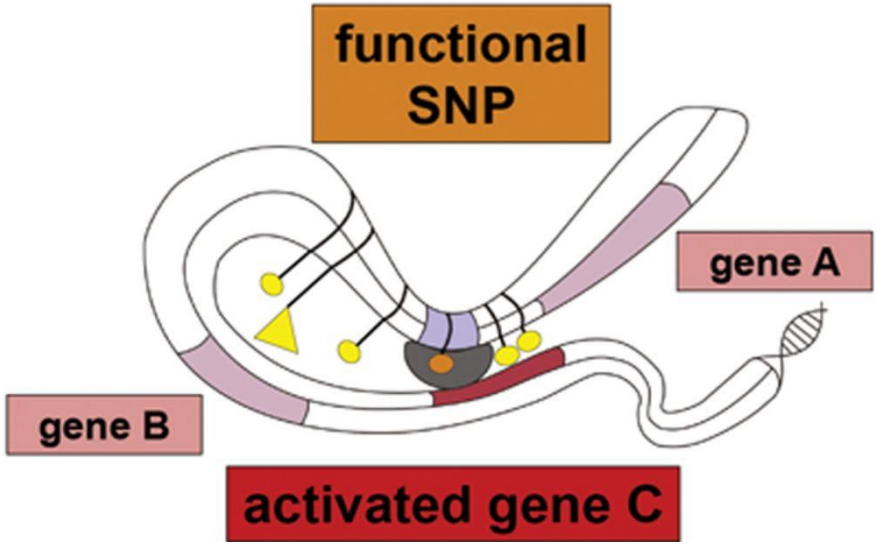
- **Single nucleotide variations in the coding sequence of proteins change:**

a) **Enzimatic activity**
b) **Protein-protein interactions**
c) **Cofactors binding**

FROM GENOMIC REGULATORY REGIONS TO MOLECULAR MECHANIMS

Single nucleotide variants in genomic regulatory regions

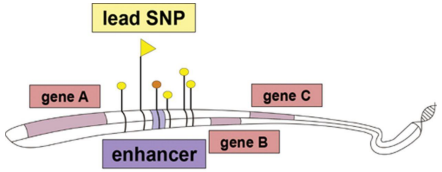




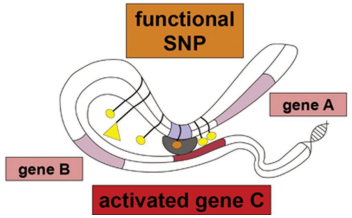
IDENTIFICATION AND CHARCTERIZATION

MOLECULAR MECHANISMS

GENOMIC REGULATORY REGIONS



GENE EXPRESSION REGULATION



CELL IDENTITY

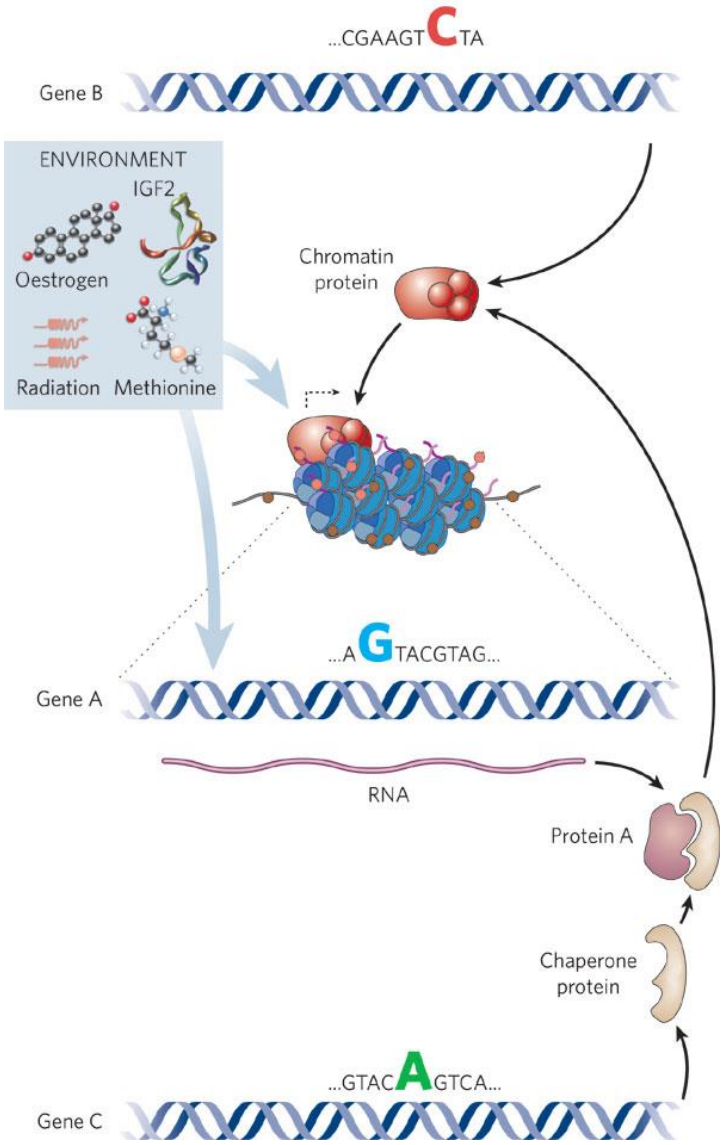
BIOLOGICAL FUNCTIONS

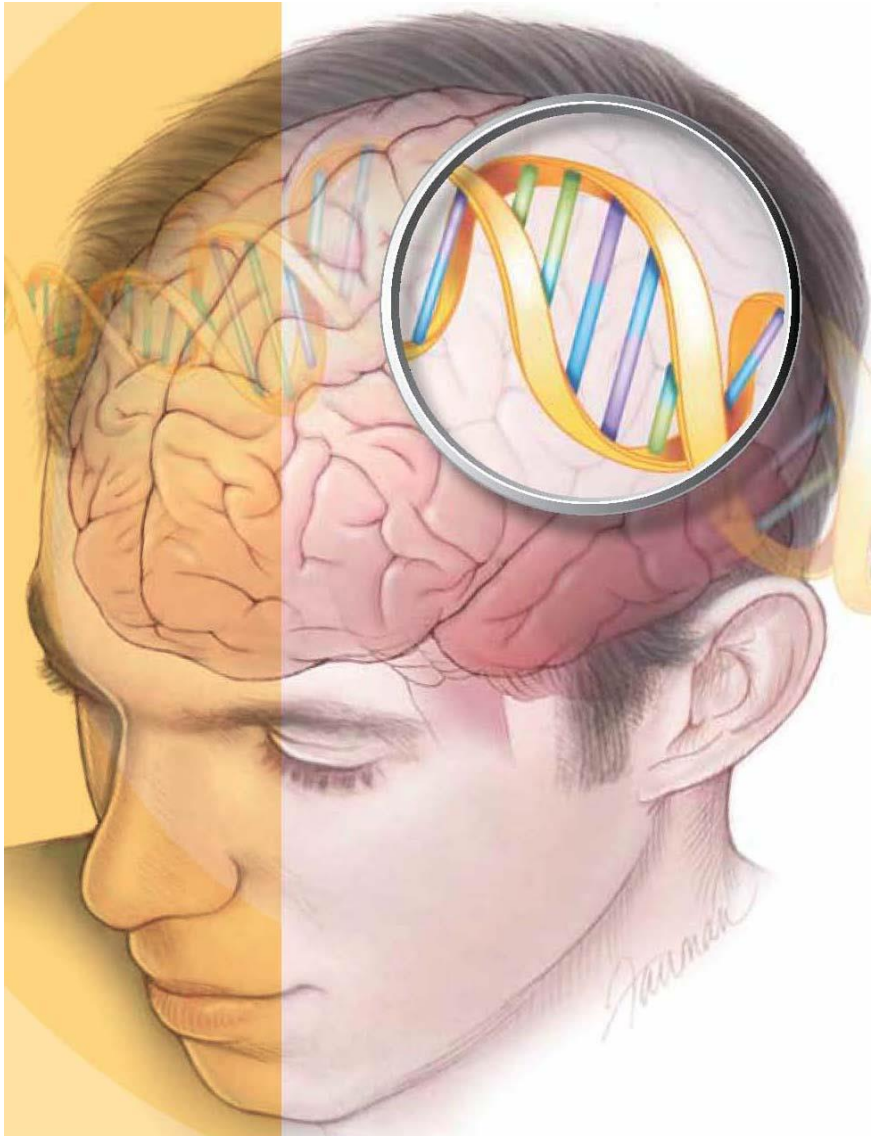
TO UNDERSTAND DISEASES

Genome-wide association studies (GWAS) have capitalized on the millions of common single nucleotide polymorphisms (SNPs) to identify those SNPs that are genome-wide significantly associated with a disease or trait.

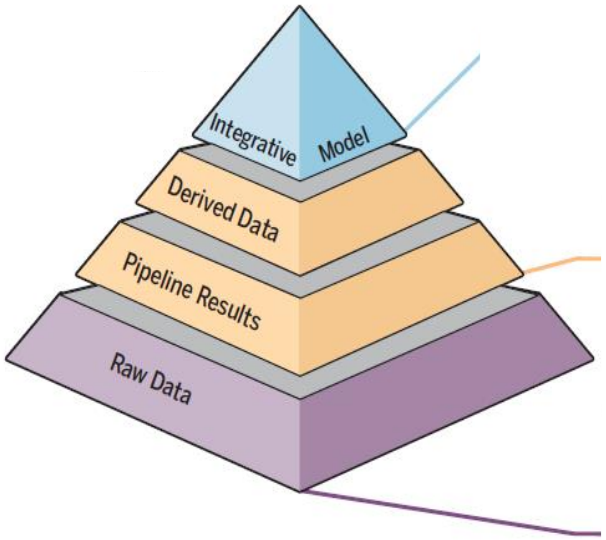
- What is the main focus of the course
- Definition of Functional Genomics
- Focus: DNA genomic elements as cell-type specific regulatory regions
- **How Functional Genomics is the tool to understand diseases**
- Genome-wide sequencing methods to annotate DNA genomic elements. Storing in Databases.

SNPs may have an impact on chromatin remodelling to control neuron activity and network

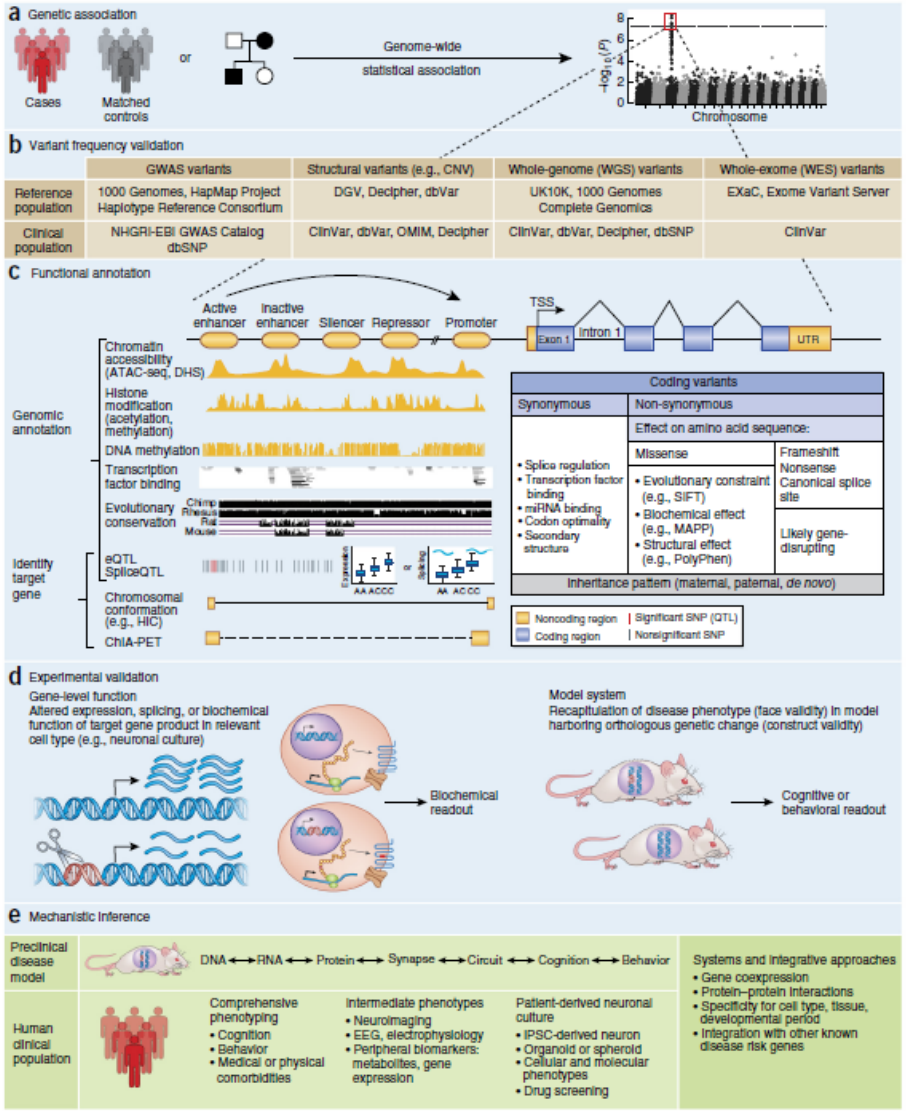




Which are the steps to understand the SNPs meaning?

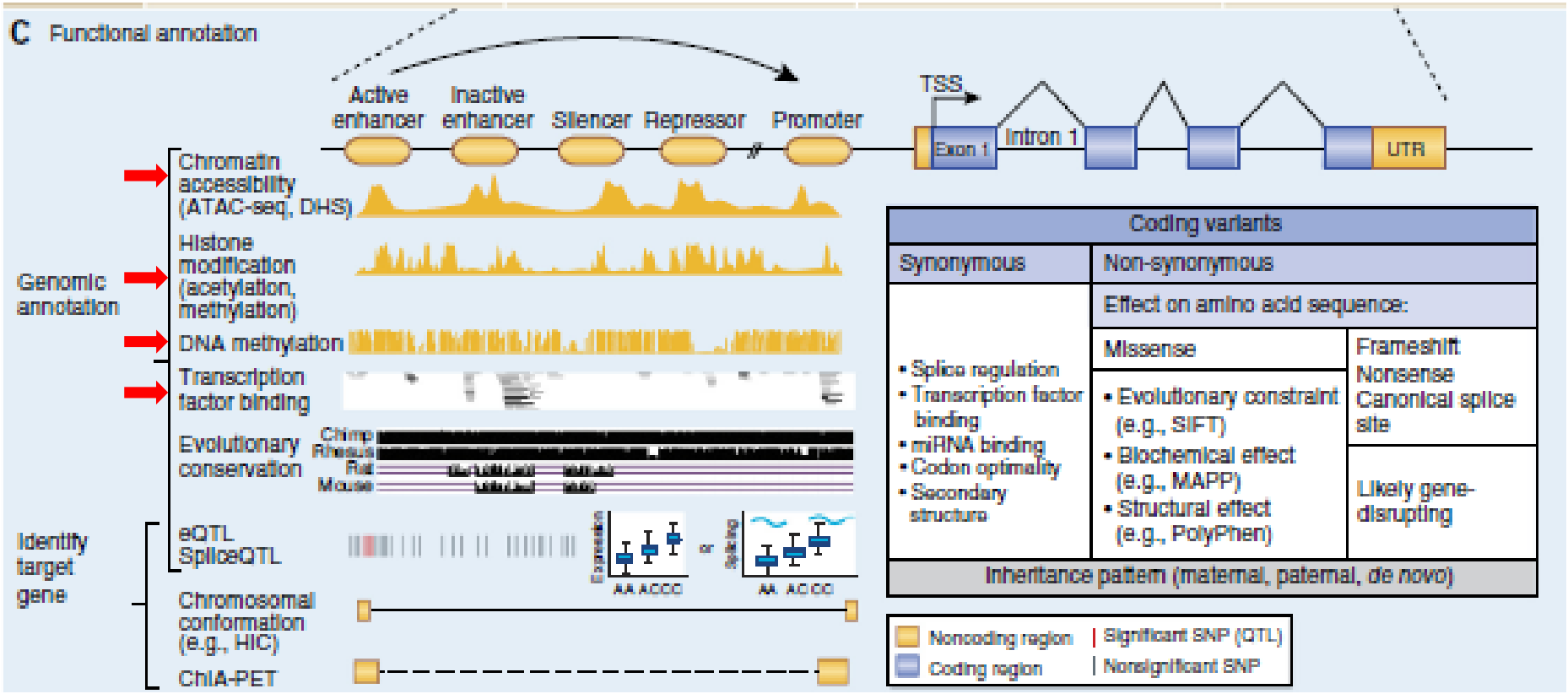


- **Experimental validation**
- **Disease Animal models**
- **Correlation between molecular mechanisms and disease symptoms**
- **Drug Discovery**

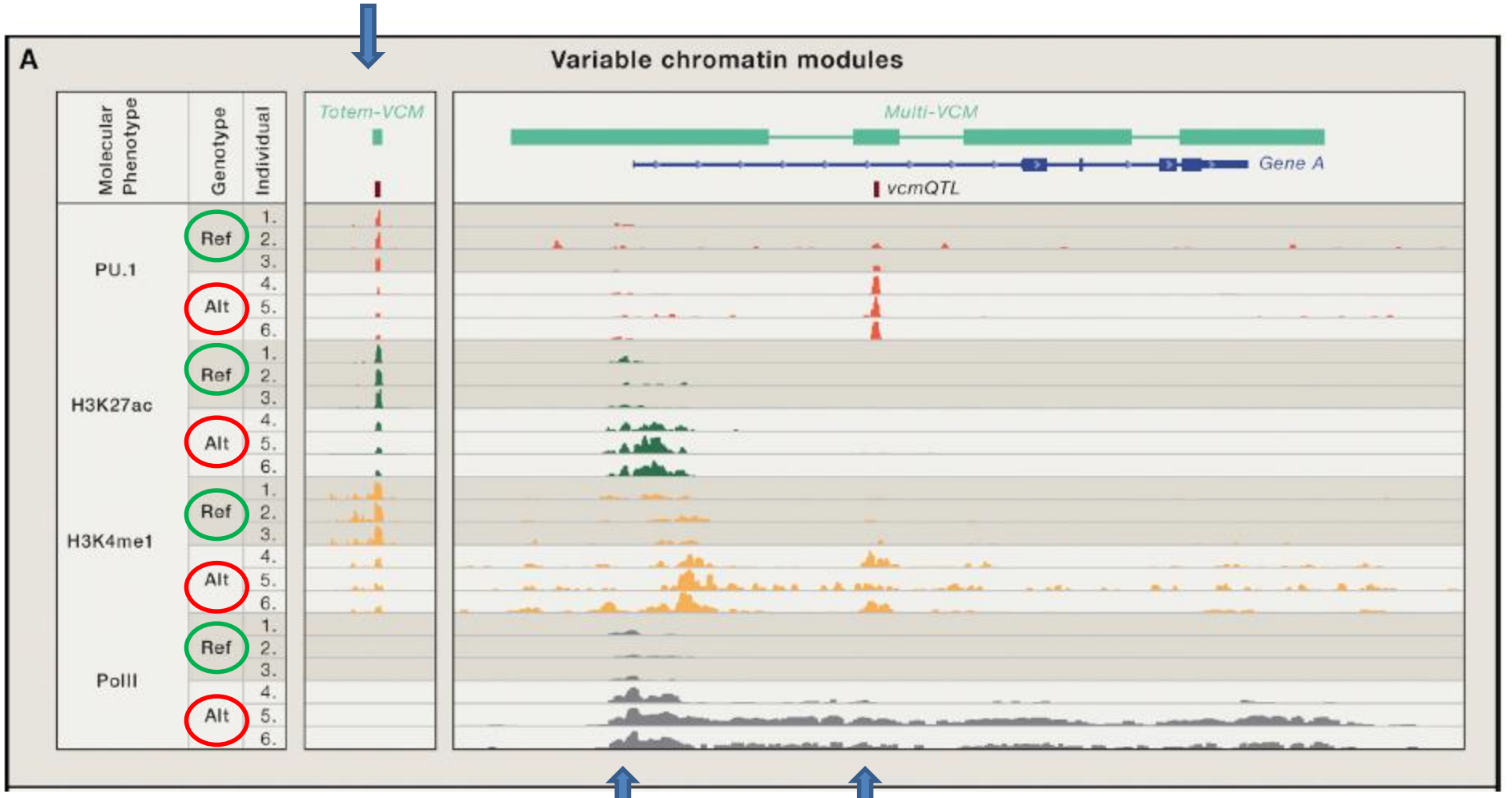


Framework for interpretation of individual disease-associated variants

FUNCTIONAL ANNOTATION

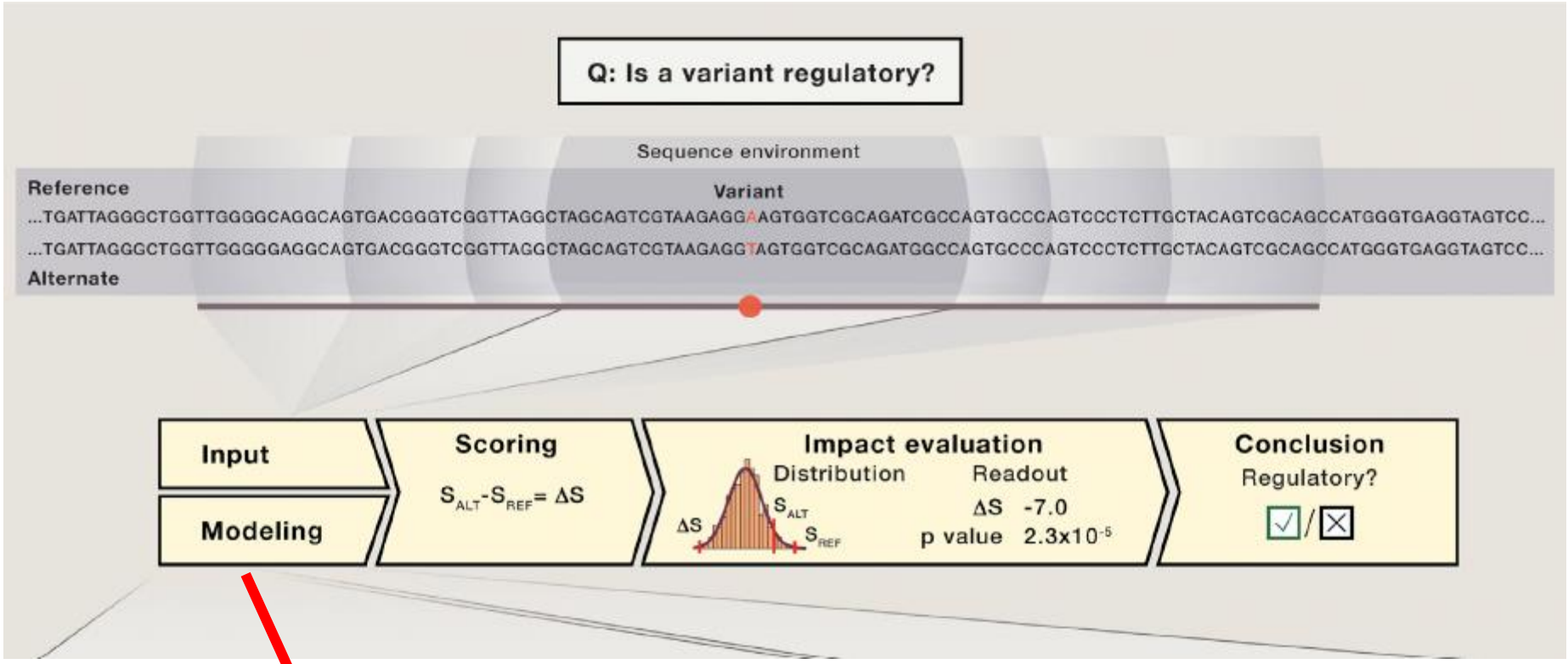


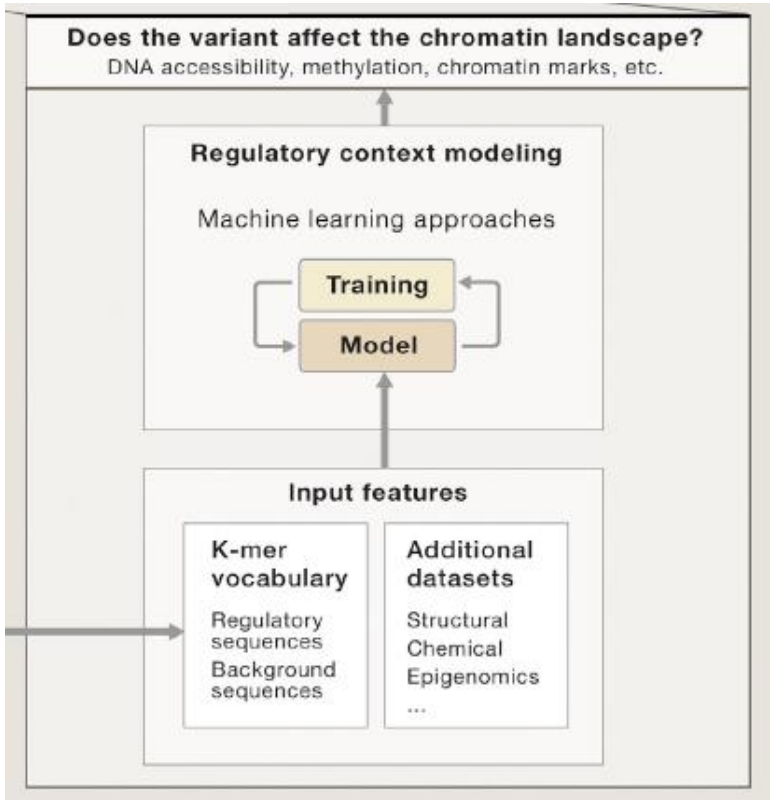
SNPs in the genomic regions may alter a binding site of a specific TFs, such as PU.1 and chromatin states change in the same region

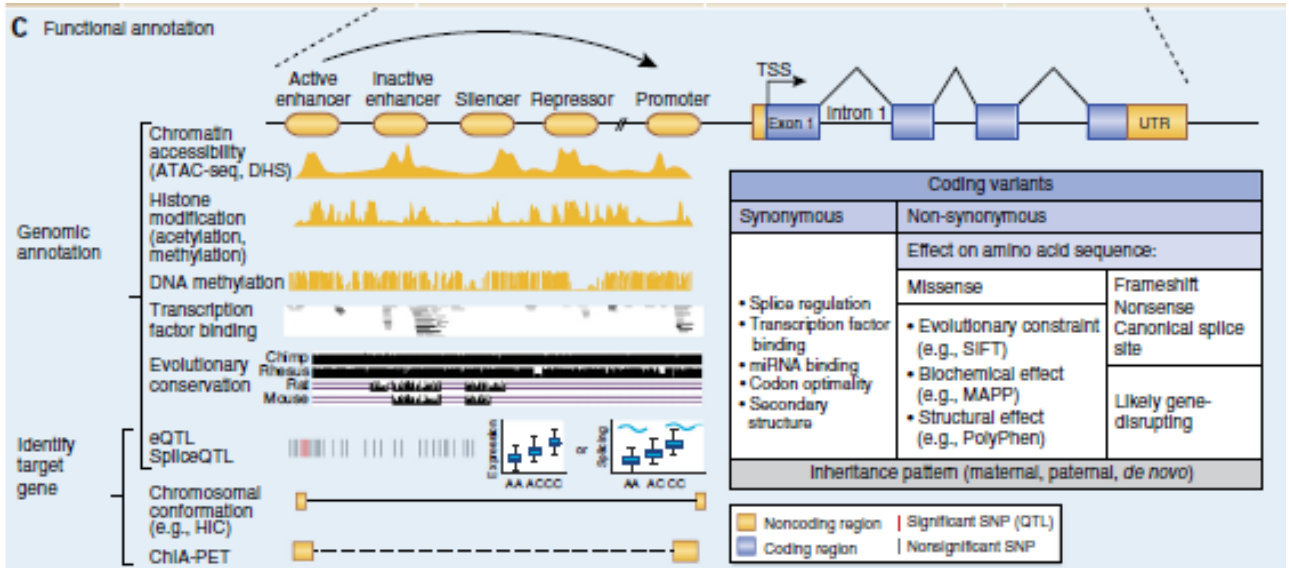


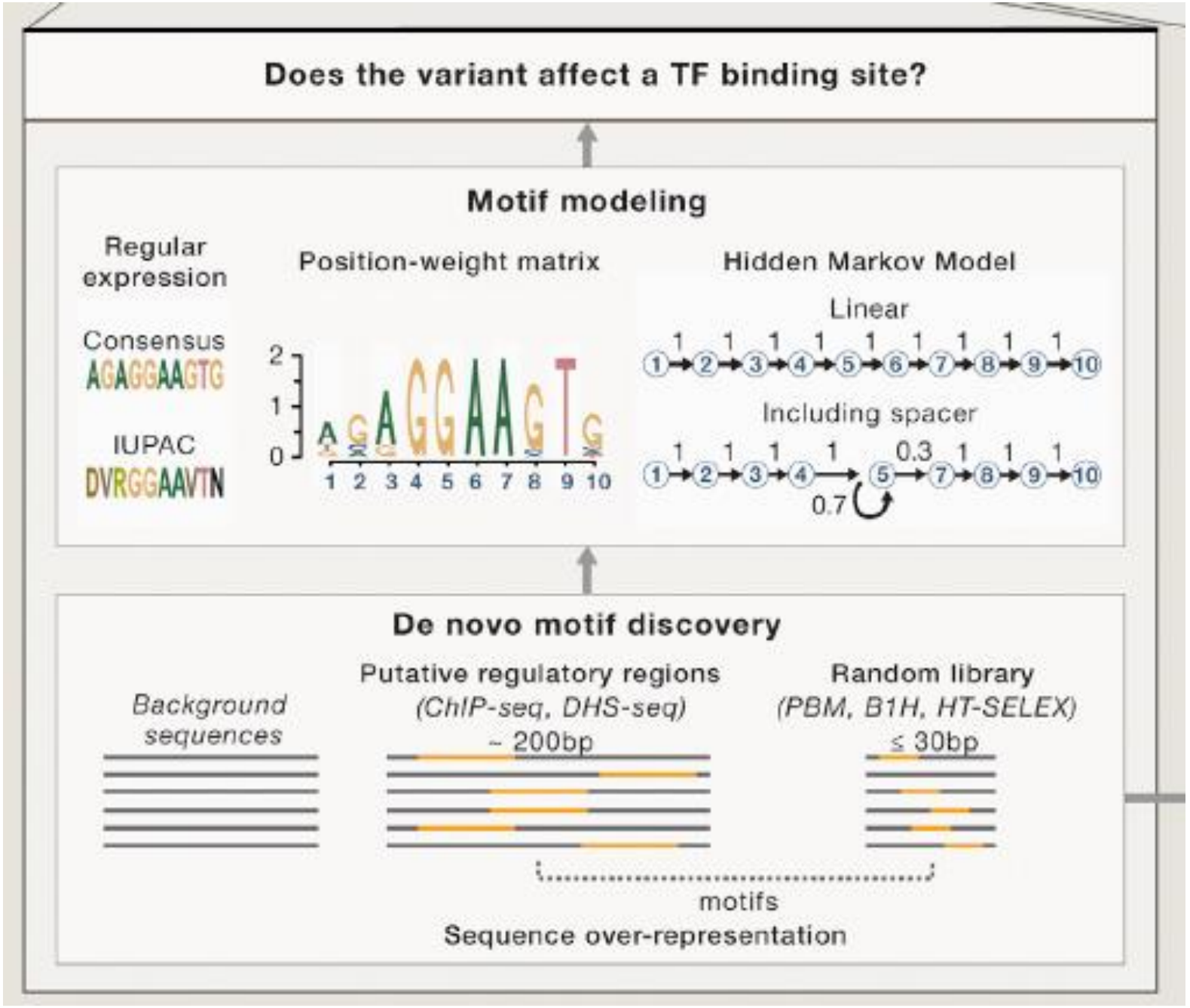
SNPs in the genomic regions may alter a binding site of a specific TFs, such as PU.1 while chromatin states change a whidespread region

SNPs in the genomic regulatory regions DEFINITION

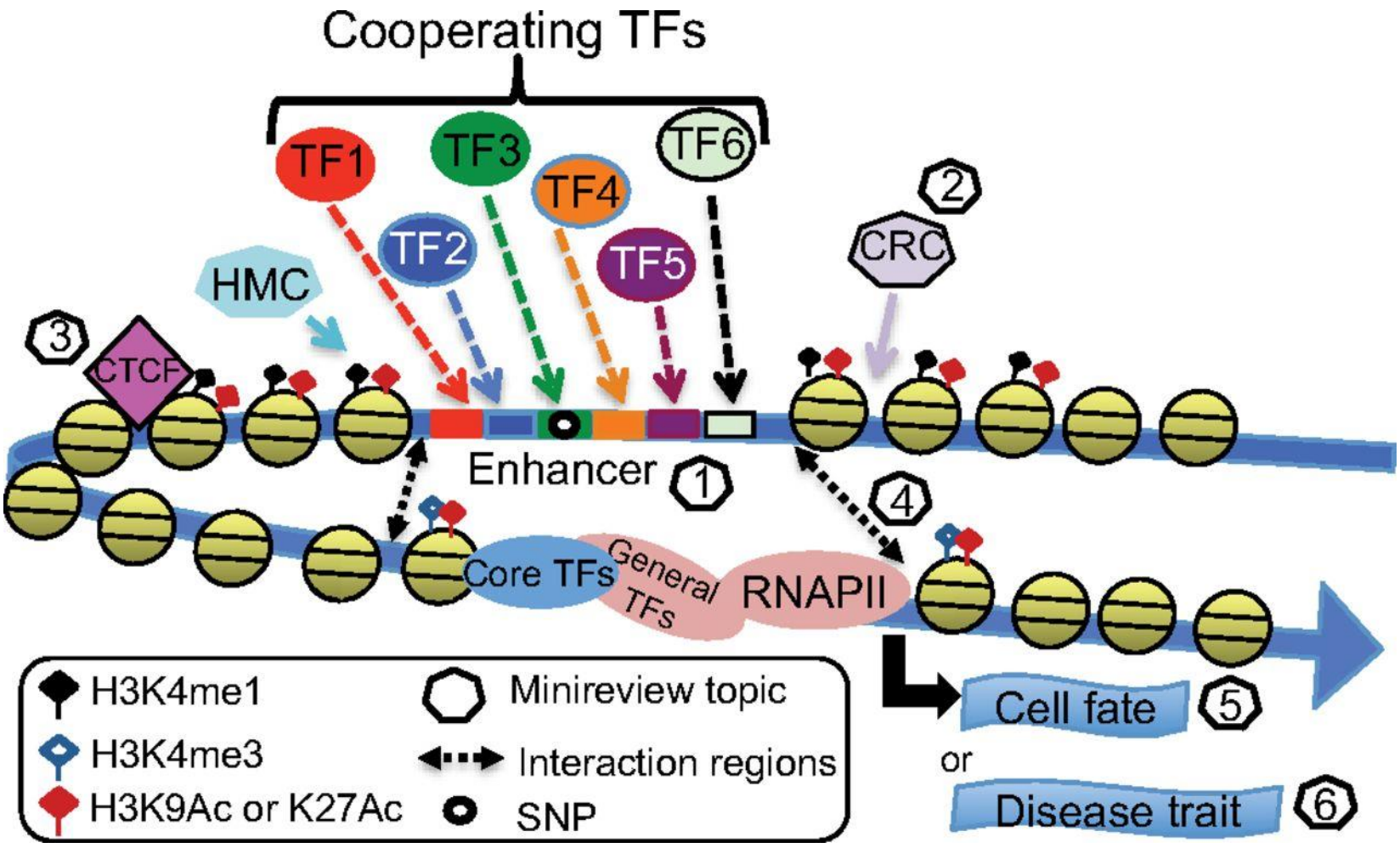



Does the variant affect the chromatin landscape?
DNA accessibility, methylation, chromatin marks, etc.
Regulatory context modeling
Machine learning approaches
Training
Model
Input features
K-mer vocabulary
Regulatory sequences
Background sequences
Additional datasets
Structural
Chemical
Epigenomics
...


C Functional annotation
Active enhancer
Inactive enhancer
Silencer
Repressor
Promoter
TSS
Exon 1
Intron 1
UTR
Genomic annotation
Chromatin accessibility (ATAC-seq, DHS)
Histone modification (acetylation, methylation)
DNA methylation
Transcription factor binding
Evolutionary conservation
Chimp
Rhesus
Rat
Mouse
Identify target gene
eQTL
SpliceQTL
Expression
or
Splicing
AA ACCC
AA ACCC
Chromosomal conformation (e.g., HIC)
ChIA-PET
Coding variants
Synonymous
Non-synonymous
Effect on amino acid sequence:
Missense
Frameshift
Nonsense
Canonical splice site
• Splice regulation
• Transcription factor binding
• miRNA binding
• Codon optimality
• Secondary structure
• Evolutionary constraint (e.g., SIFT)
• Biochemical effect (e.g., MAPP)
• Structural effect (e.g., PolyPhen)
Likely gene-disrupting
Inheritance pattern (maternal, paternal, de novo)
Noncoding region
Coding region
Significant SNP (QTL)
Nonsignificant SNP

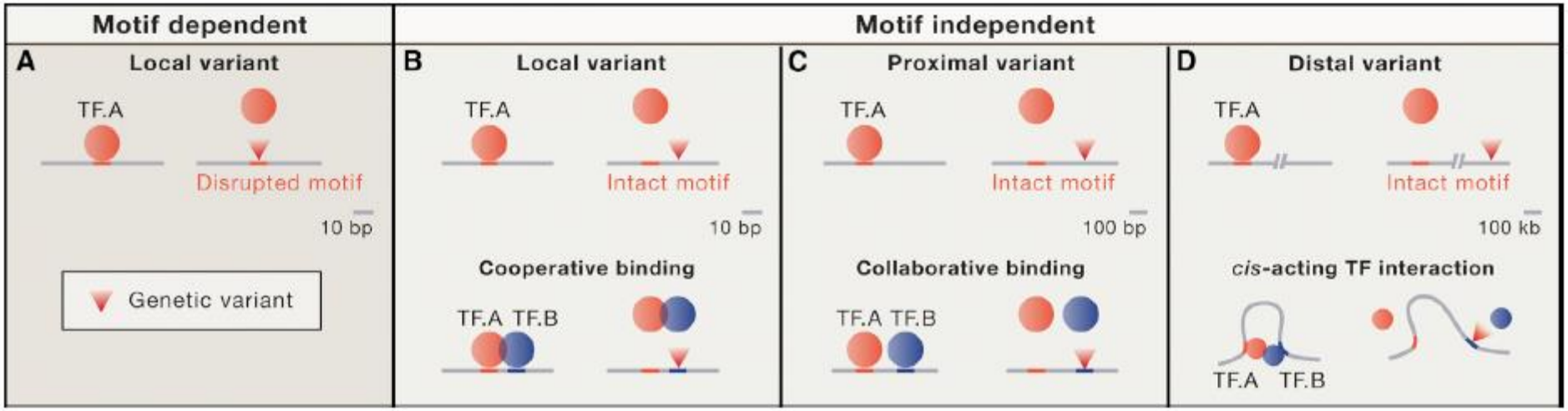

Does the variant affect a TF binding site?
Motif modeling
Regular expression
Consensus
AGAGGAAGTG
IUPAC
DVRGGAAVTN
Position-weight matrix
2
1
0
1 2 3 4 5 6 7 8 9 10
Hidden Markov Model
Linear
1 1 1 1 1 1 1 1 1 1
1 2 3 4 5 6 7 8 9 10
Including spacer
1 1 1 1 0.3 1 1 1
1 2 3 4 5 7 8 9 10
0.7
De novo motif discovery
Background sequences
Putative regulatory regions
(ChIP-seq, DHS-seq)
~ 200bp
Random library
(PBM, B1H, HT-SELEX)
≤ 30bp
motifs
Sequence over-representation

Genome-wide characterizations of regulatory regions.

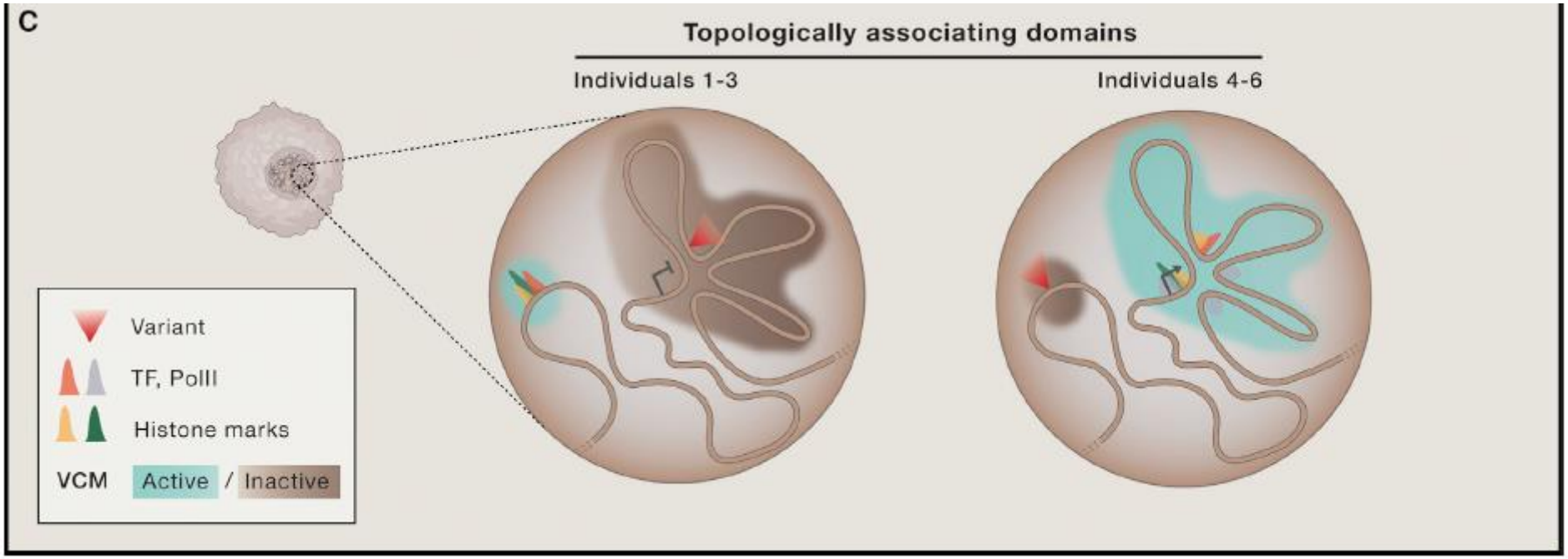


Peggy J. Farnham J. Biol. Chem. 2012;287:30885-30887

SNPs mechanims for alteration of regulatory transcrption factors complexes



SNPs may change long range interactions



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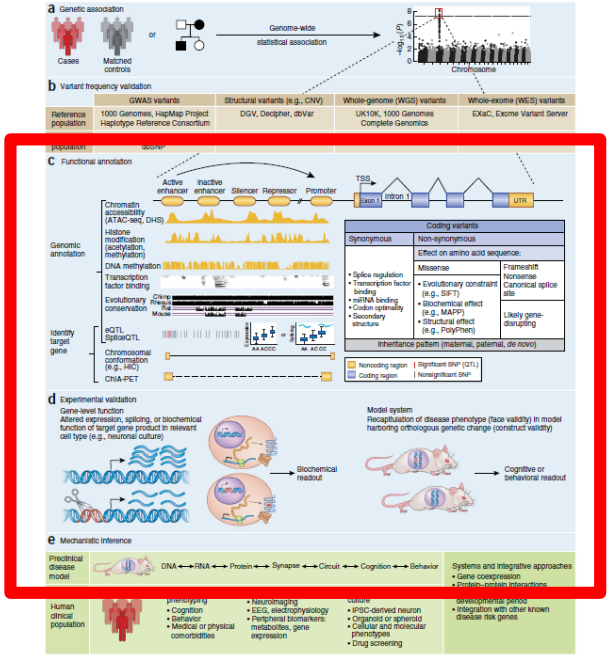


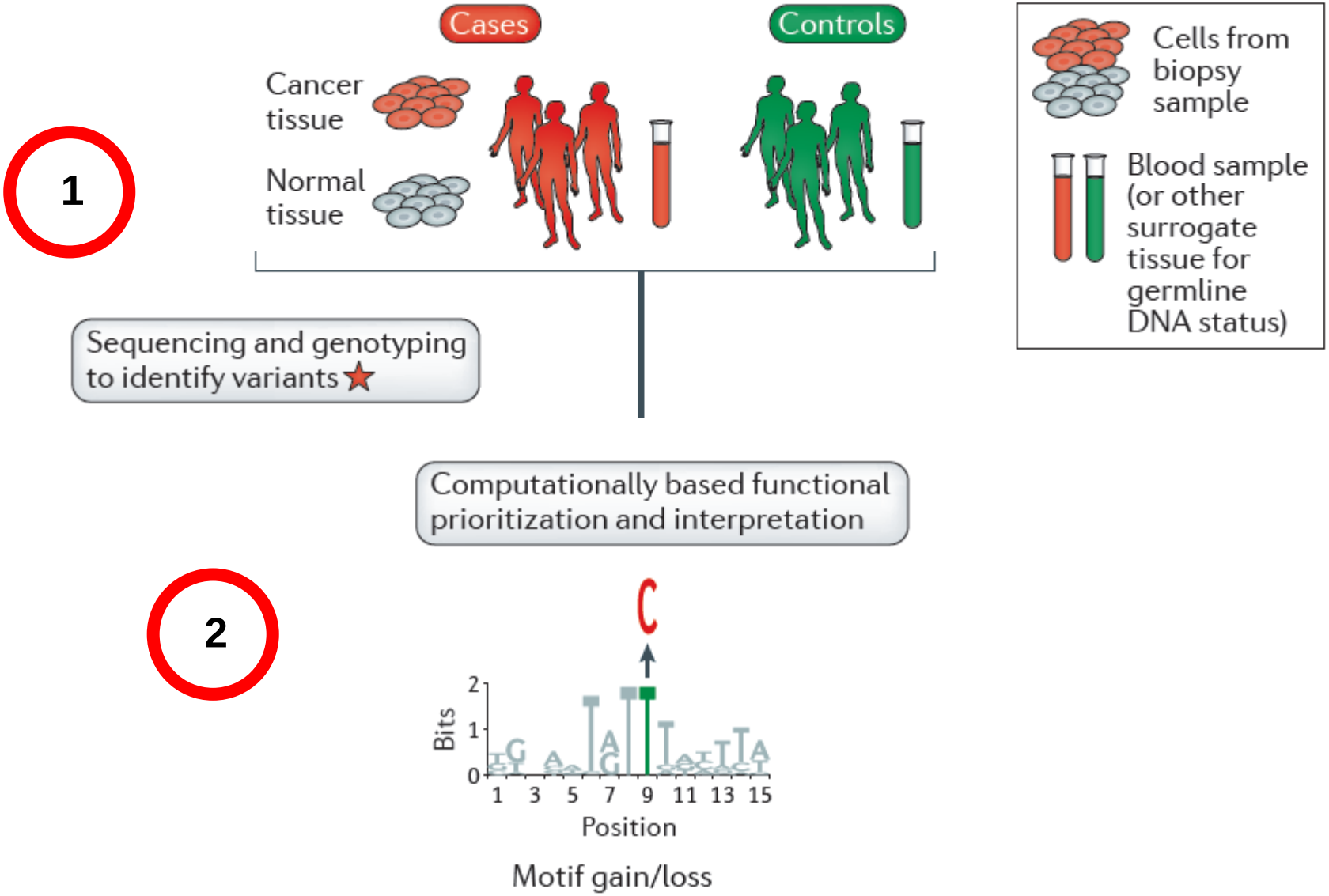
Role of non-coding sequence variants in cancer

Ekta Khurana[1–4], Yao Fu[5], Dimple Chakravarty[2,6], Francesca Demichelis[2,3,7], Mark A. Rubin[1,2,6] and Mark Gerstein[8–10]

Abstract | Patients with cancer carry somatic sequence variants in their tumour in addition to the germline variants in their inherited genome. Although variants in protein-coding regions have received the most attention, numerous studies have noted the importance of non-coding variants in cancer. Moreover, the overwhelming majority of variants, both somatic and germline, occur in non-coding portions of the genome. We review the current understanding of non-coding variants in cancer, including the great diversity of the mutation types — from single nucleotide variants to large genomic rearrangements — and the wide range of mechanisms by which they affect gene expression to promote tumorigenesis, such as disrupting transcription factor-binding sites or functions of non-coding RNAs. We highlight specific case studies of somatic and germline variants, and discuss how non-coding variants can be interpreted on a large-scale through computational and experimental methods.

SNPs with an impact in tumorigenesis

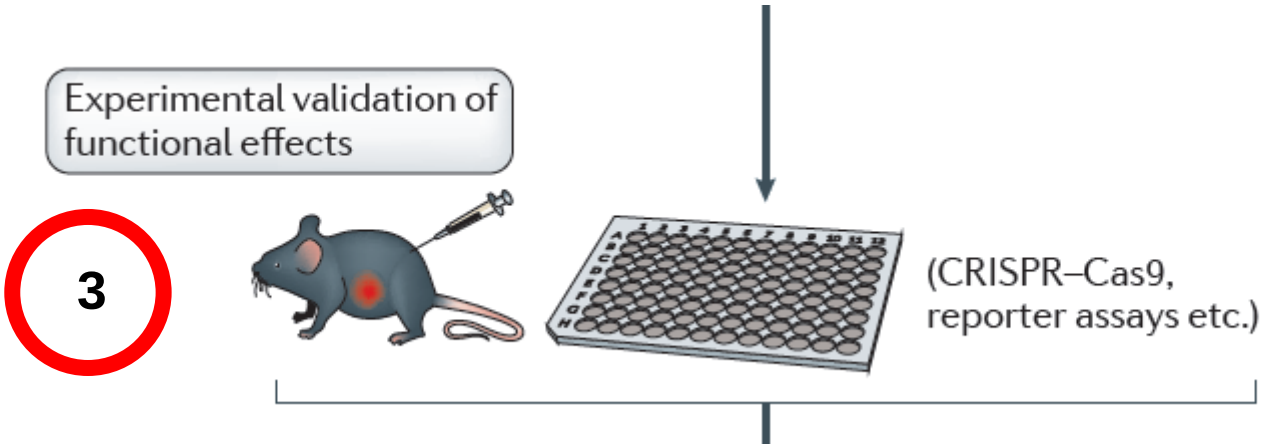
Steps for studying the role of SNP



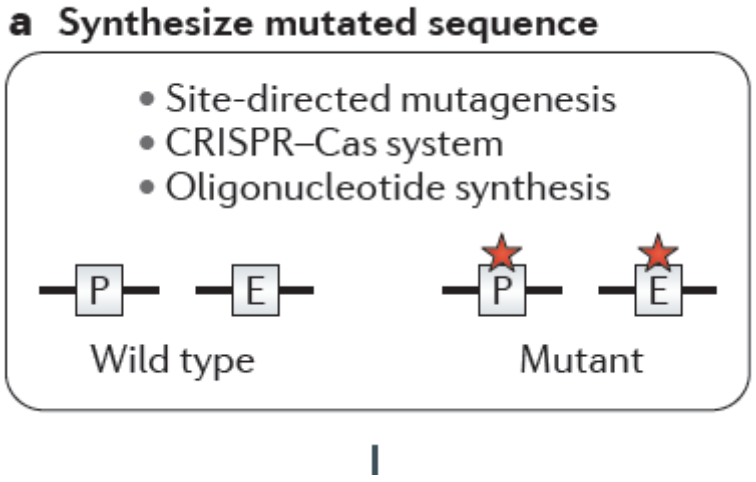
SNPs may have an impact in tumorigenesis

2

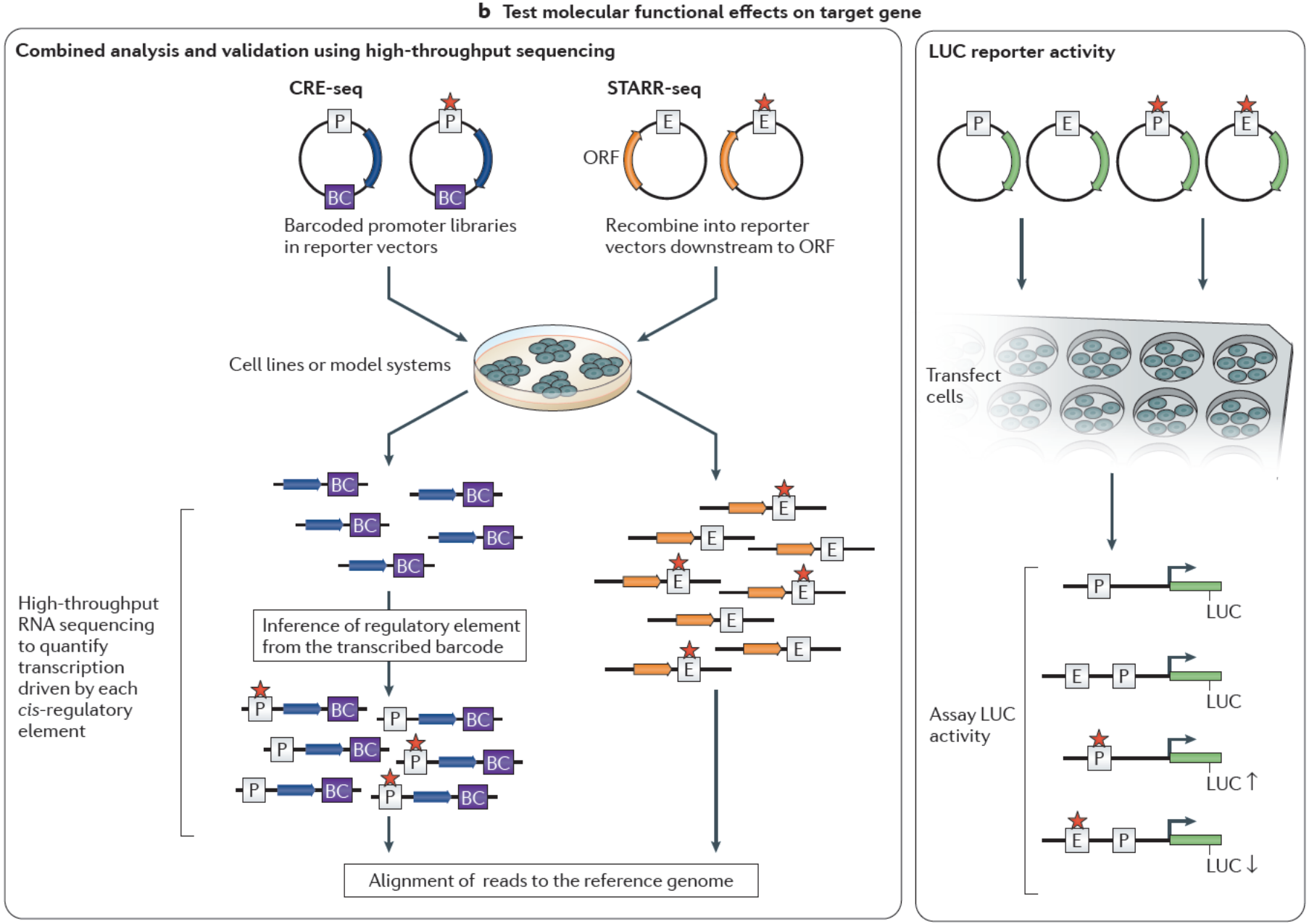
FUNCTIONAL ANNOTATION OF SNPs



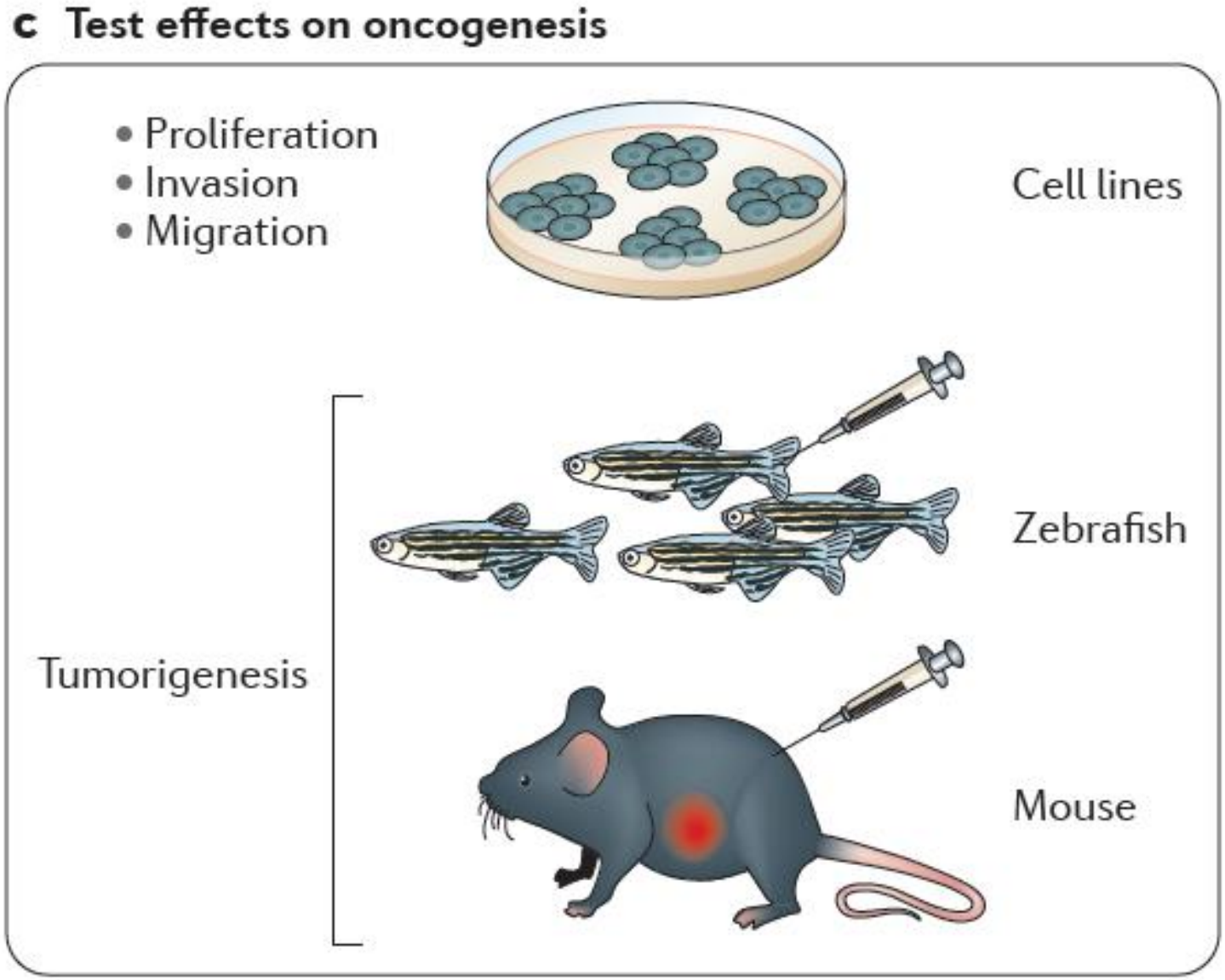
SNPs ESPERIMENTAL VALIDATIONS



MOLECULAR FUNCTIONAL EFFECTS



BIOLOGICAL FUNCTION TESTS



Task 3- Design an experiment by using plasmid with luciferase reporter

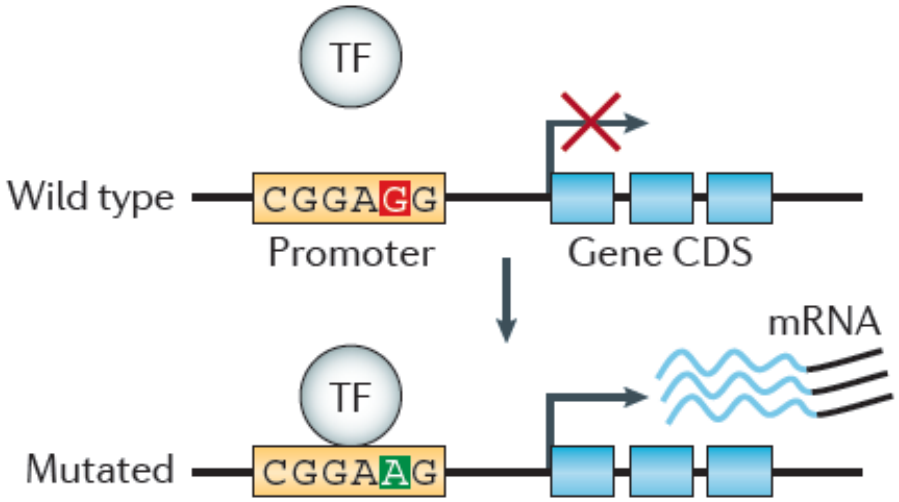
- How you create the mutation in the plasmid

- Which are the samples of your experiment? Positive control and negative control

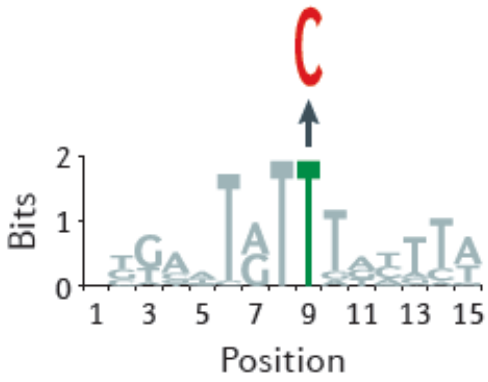
- Data interpretation

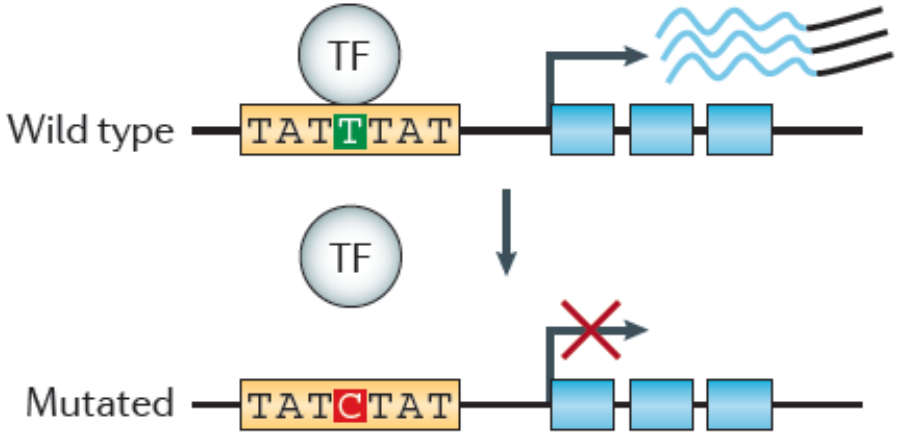
SNPs types functions:

Ba Gain of motif



Bb Loss of motif





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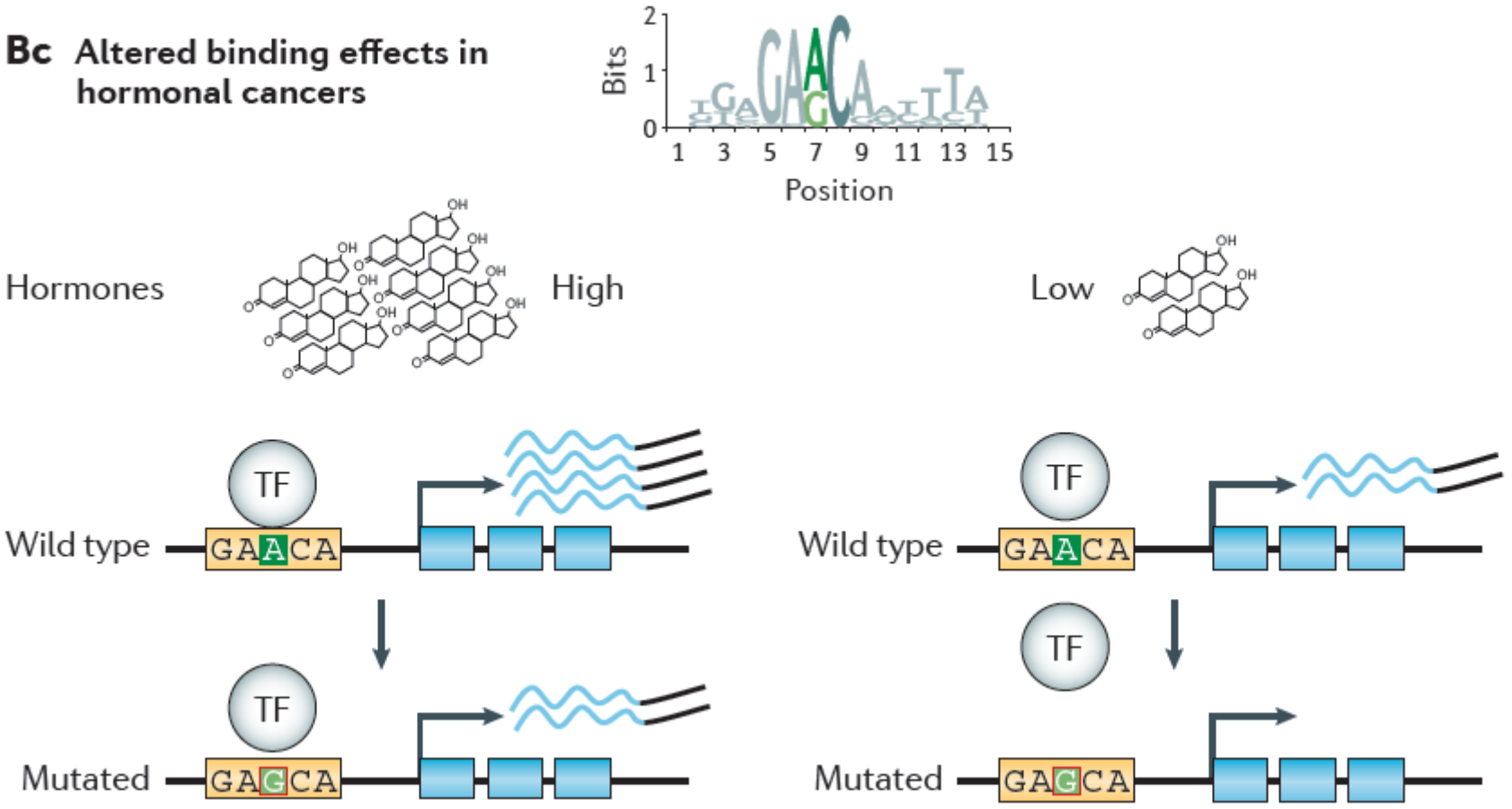
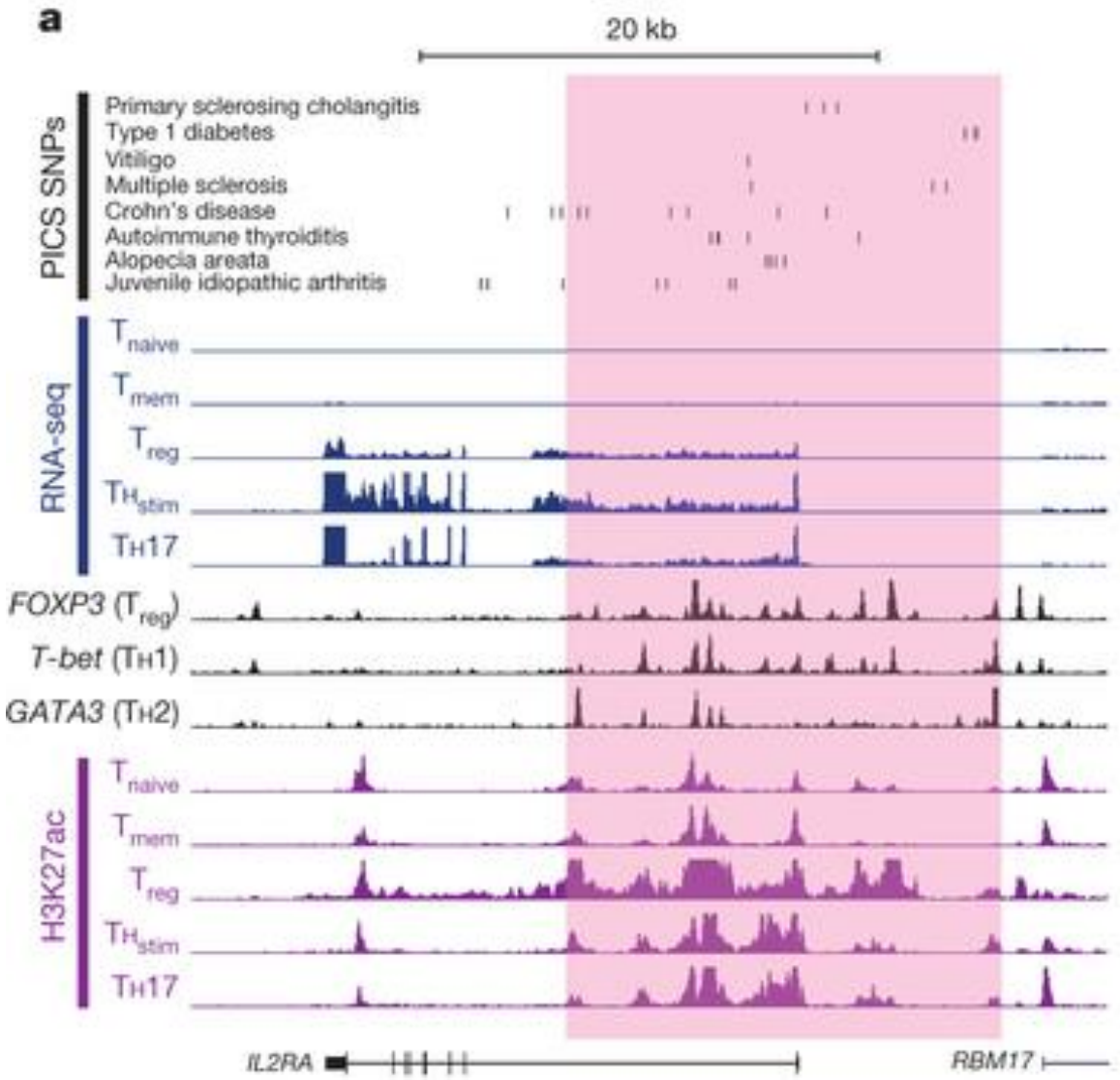


Genome-wide data describe activation state of specific gene locus and the correlation of these features with disease open the way to understand disease outcome



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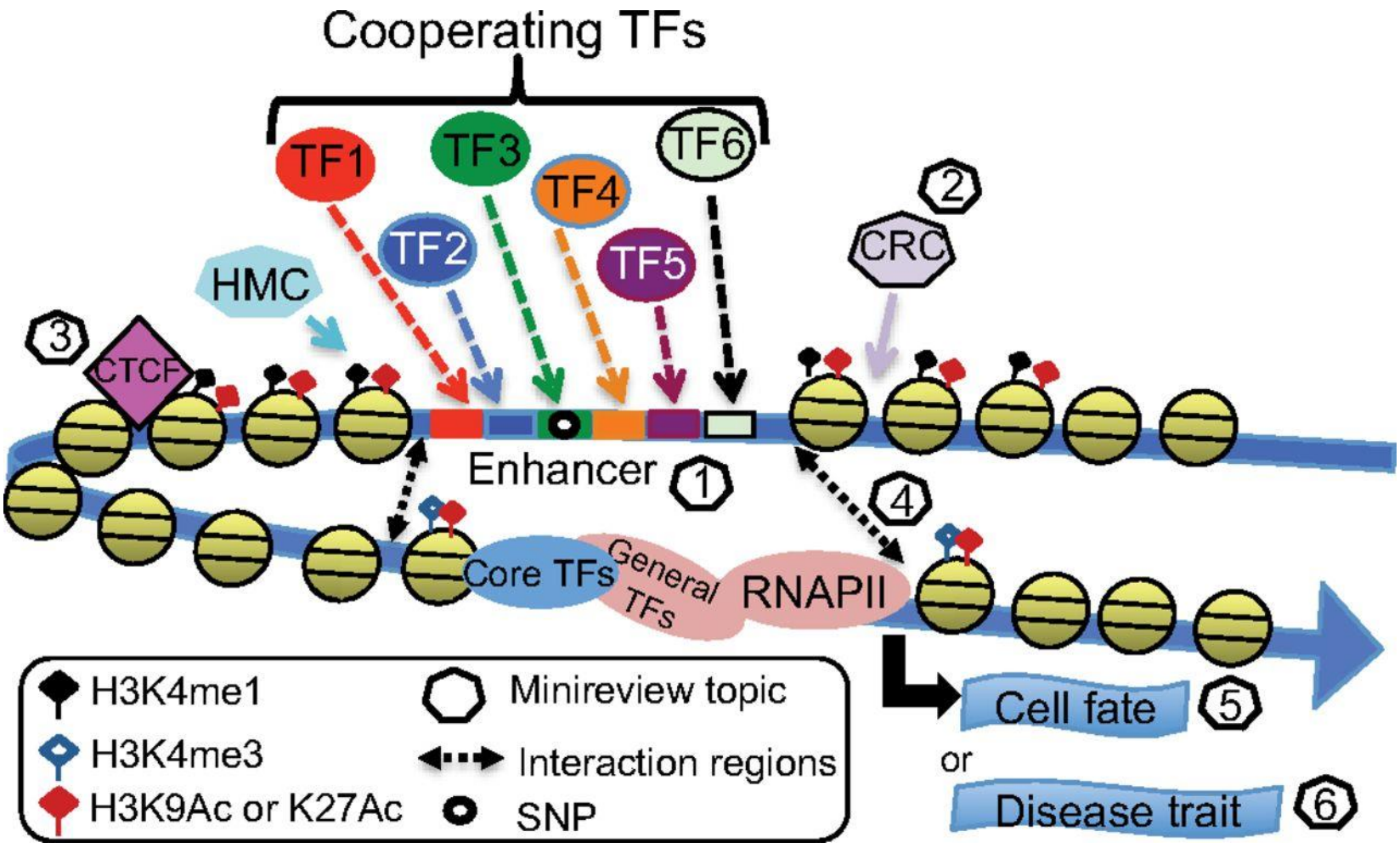

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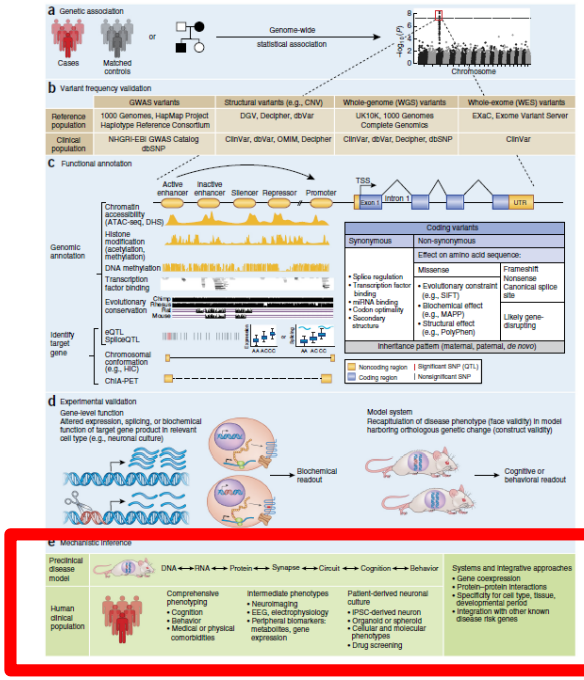
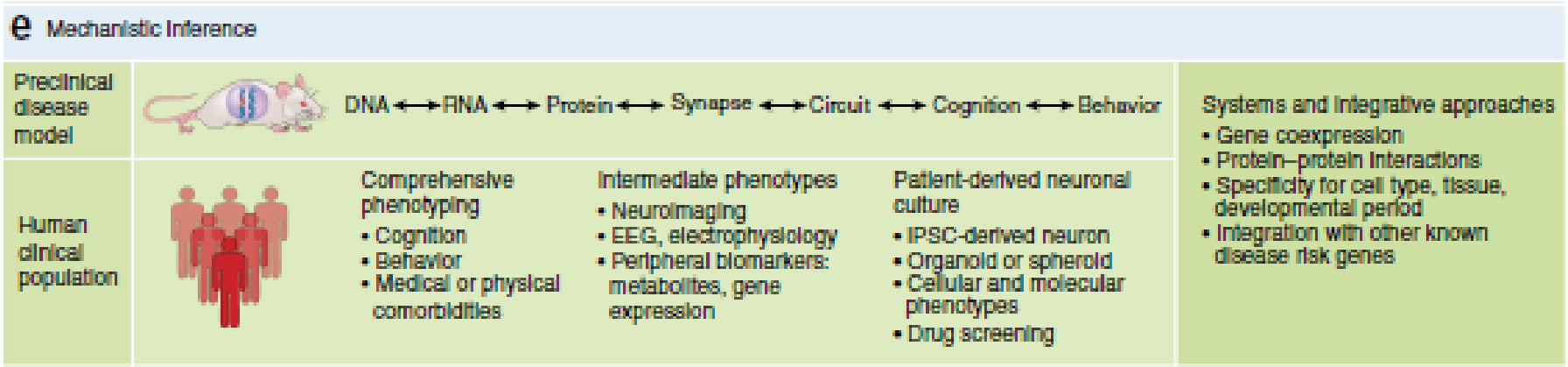


Correlation of SNP/functions with several clinical analysis

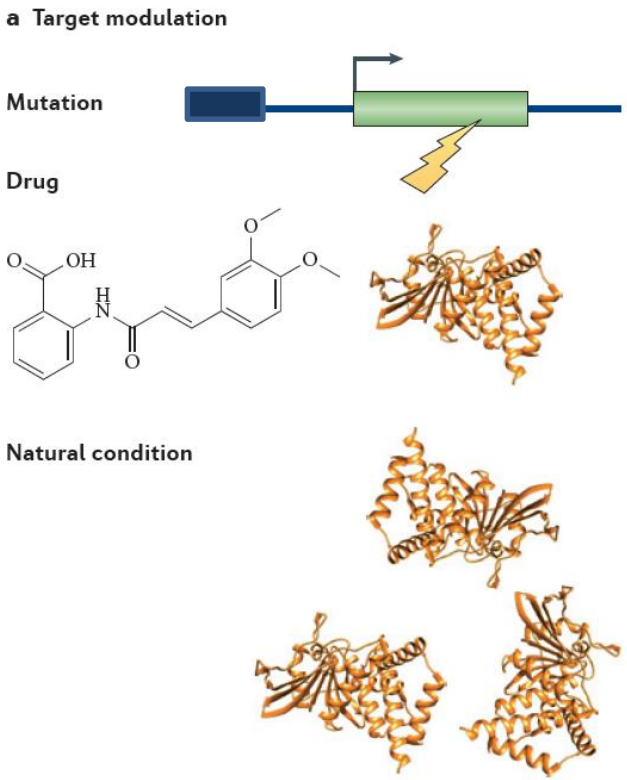


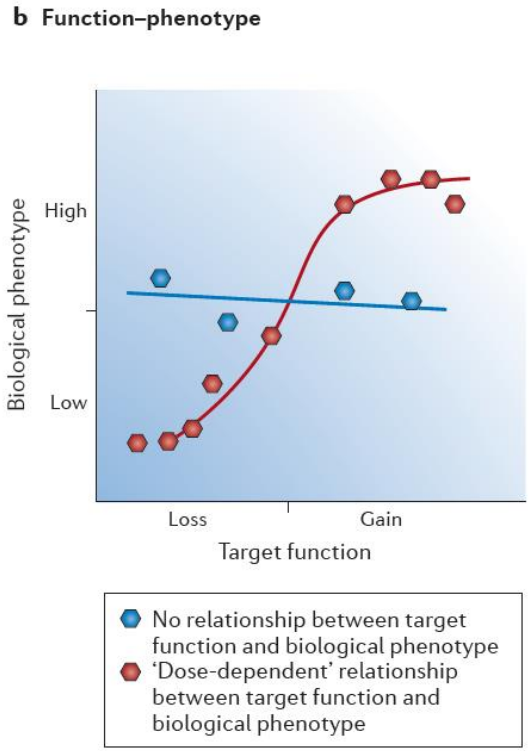
How can we use these knowledge?

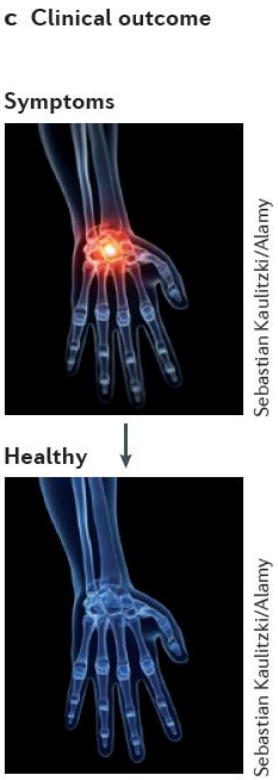
EXAMPLE

Gene expression alteration in disease May be used as BIOMARKERS (molecules acting as sensor of disease)

Gene expression alteration in disease May be used as DRUG TARGET (drug discovery to stop disease and restore health)







In Summary:

- **Functional genomics is a field of molecular biology based on genome-wide sequencing data.**
- **Genome-wide sequencing data describe genomic regulatory regions that control gene expression**
- **Gene expression disregulation may be linked to the disease**
- **Understanding molecular mechanisms of disease outcome opens the way to discovery drug and identify biomarkers**

http://biologia.i-learn.unito.it/:

1. Lecture PDFs: the slides we used during the class
2. Textbook: *reviews* that will give the necessary background and lessons first part
3. Research Papers: articles that we will analyze
4. Bibliography: scientific literature concerning the subject
5. Audio and Main Concept Lessons

EXAM

Students are expected to demonstrate:

1. Knowledge of **basic** concepts
2. Understanding of **specific** concepts
3. Comprehension of experimental **methodology**
4. **Solving problem** that we have discuss during lesson

Evaluation:

EXAMS is based on lessons and is composed to multiple choice questions and two open questions.