A Data Analytics View of Genomics

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www.ong-home.my

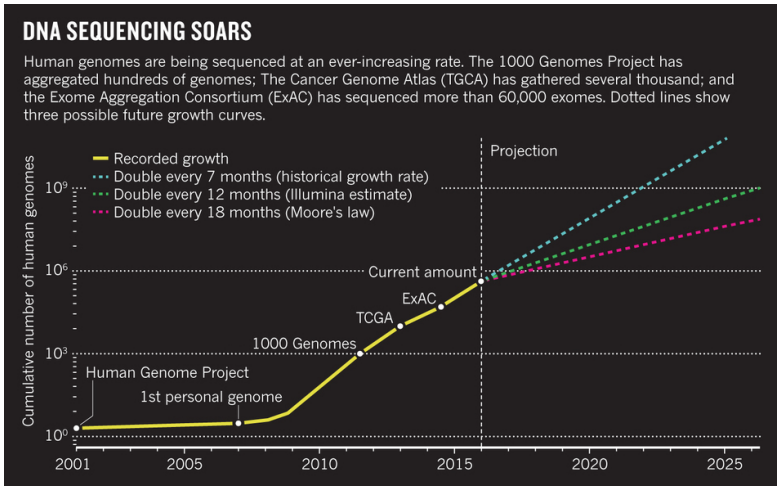
Genomics

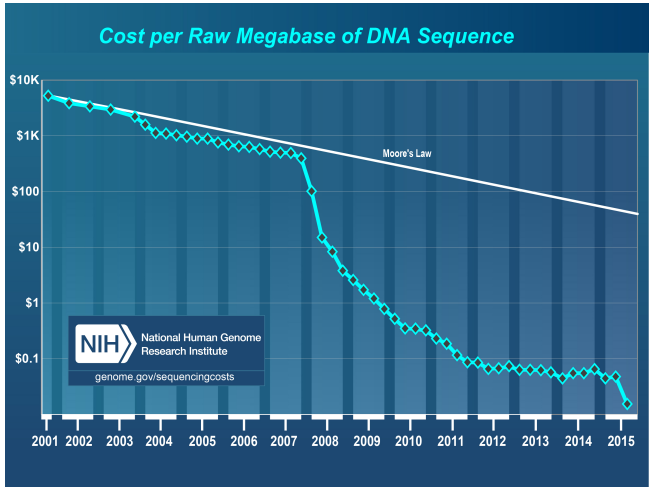
The bottleneck in genome sequencing is no longer data generation – the computational challenges around data analysis, display and integration are now rate limiting.

New approaches and methods are required to meet these challenges.

Green, Guyer and National Human Genome Research Institute

Charting a course for genomic medicine from base pairs to bedside, Nature 2011.





Stephens, Z. D. et al. PLoS Biol.13, e1002195 (2015),

www.genome.gov/sequencingcosts

Why machine learning?

- A lot of data
- Data is noisy
- Large number of features
- No precise biological theory
- Complex relationships

Let the data do the talking!

Outline

Genome wide association studies

Find genetic variation corresponding to an attribute of interest.

The search for genes

A very brief overview of molecular biology

Biological sequencing

The big data revolution in life sciences

Genomics

SNP

Single Nucleotide Polymorphisms or single nucleotide variations (SNVs) are mutations on a single nucleotide (A,C,T or G) in the genome.
For example: AAGCCTA to AAGCTTA.

Alleles

There are two alleles: e.g. C and T.

Major/Minor allele

The nucleotide that occurs commonly in the population is called the major allele (denoted by a capital B) and the nucleotide that occurs more rarely is called the minor allele (denoted by a small letter b).

Diploid

haploid $\Longrightarrow$ one chromosome set
diploid $\Longrightarrow$ two chromosome sets
hexaploid $\Longrightarrow$ six chromosome sets

Genome wide association study

Genotype

The genotype is the specific combination of alleles.

Phenotype

The phenotype is the observable trait or characteristic of an individual, for example whether the individual is healthy or sick.

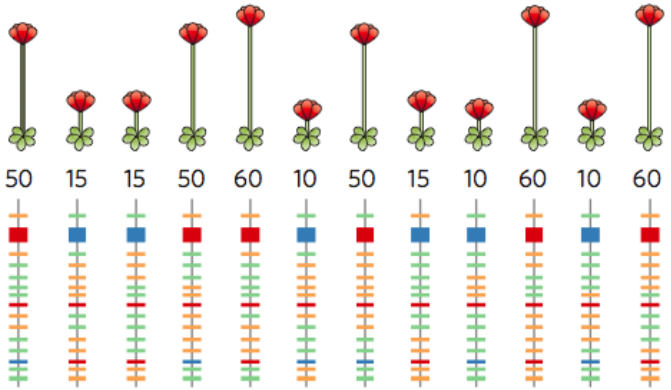
Case-control studies

A cohort of sick individuals (cases) and healthy individuals (controls) are genotyped and their corresponding binary phenotype are recorded.

We use the framework of hypothesis testing

Why Hypothesis Tests?

- Given a case control study, test whether a particular SNP is associated with the phenotype.
- Look through each SNP one by one, and test to see if there is a difference in the frequency of the alleles seen in cases versus controls.
- If difference is statistically significant
  $\Longrightarrow$
  SNP is associated with the phenotype.



General framework

null hypothesis $\mathcal{H}_0$

genotype is independent of the phenotype

alternative hypothesis $\mathcal{H}_1$

SNP is associated with the disease state

hypothesis test can be stated as follows

$$\mathcal{H}_0 : \theta \in \Theta_0 \qquad \text{and} \qquad \mathcal{H}_1 : \theta \in \Theta_1.$$

Important design choices

- How to represent intuition as a probabilistic model?
- How to decide on a test statistic?
- What is the distribution of the random variable?
- What is the level of significance (α)?

Sinsheimer, “Statistics 101” – A Primer for the Genetics of Complex Human Disease, 2011

Agresti, “Categorical Data Analysis”, 2002

Wasserman, “All of Statistics”, 2004

Hypothesis test

- Let X be a random variable with range $\mathcal{X}$.
- $R \subset \mathcal{X}$ called the rejection region
- If $X \in R$ then we reject the null hypothesis, otherwise we do not reject the null hypothesis.

$$R = \{x : T(x) > c\}$$

where T is a test statistic and c is a critical value.

- The p-value is the probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true.

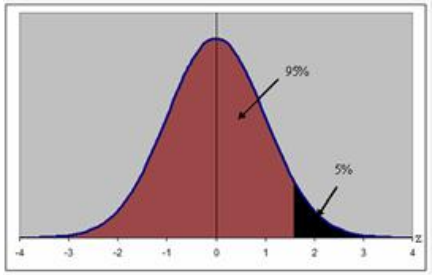
Outcomes

Outcomes of hypothesis tests

	Accept $\mathcal{H}_0$	Reject $\mathcal{H}_0$
$\mathcal{H}_0$ true	correct	type I error
$\mathcal{H}_1$ true	type II error	correct

Significance level

The probability of a rejecting $\mathcal{H}_0$ when it is true is called the *significance level*.



p-value vs significance

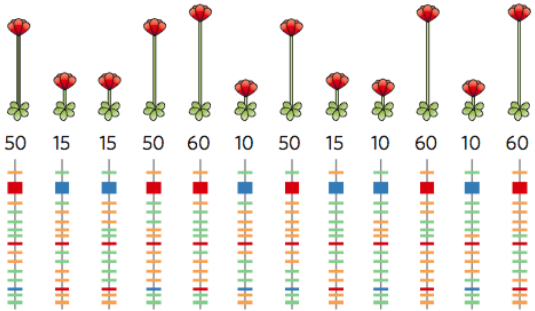
- Reject $\mathcal{H}_0$ when p-value $<$ significance level
- p-value is computed from observation
- significance level is chosen by expert

Allelic test of association

- Single locus, haploid genome
- 200 individuals: 100 cases, 100 controls
- B and b are equally common in the population
- **Null hypothesis**
  No association between the allele and the phenotype

	allele B	allele b
Case	50 ($E_{B,1}$)	50 ($E_{b,1}$)
Control	50 ($E_{B,0}$)	50 ($E_{b,0}$)

Experimental Observation



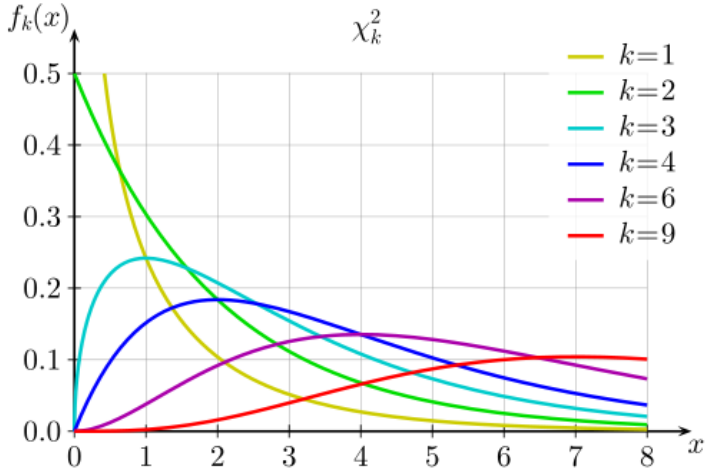
	allele B	allele b
Case	50 ($E_{B,1}$)	50 ($E_{b,1}$)
Control	50 ($E_{B,0}$)	50 ($E_{b,0}$)

	allele B	allele b
Case	23 ($O_{B,1}$)	77 ($O_{b,1}$)
Control	68 ($O_{B,0}$)	32 ($O_{b,0}$)

Pearson χ^2 test of independence

$$X^2 = \sum_{i \in \{0,1\}} \sum_{v \in \{B,b\}} \frac{(O_{v,i} - E_{v,i})^2}{E_{v,i}}.$$

Chi squared distribution



$$f(x;k) = \begin{cases} \dfrac{x^{\left(\frac{k}{2}-1\right)} \exp\left(-\dfrac{x}{2}\right)}{2^{\left(\frac{k}{2}\right)}\Gamma\left(\dfrac{k}{2}\right)}, & x \geqslant 0 \\ 0 & \text{otherwise} \end{cases}$$

http://en.wikipedia.org/wiki/Chi-squared_distribution

χ^2 test

	allele B	allele b
Case	50 ($E_{B,1}$)	50 ($E_{b,1}$)
Control	50 ($E_{B,0}$)	50 ($E_{b,0}$)

	allele B	allele b
Case	23 ($O_{B,1}$)	77 ($O_{b,1}$)
Control	68 ($O_{B,0}$)	32 ($O_{b,0}$)

$$X^2 = \frac{(23-50)^2}{50} + \frac{(77-50)^2}{50} + \frac{(68-50)^2}{50} + \frac{(32-50)^2}{50}$$

$$= 42.12$$

What is the probability of observing a value greater than 42.12 of a χ^2 random variable given that the null hypothesis is true?

$$\mathbb{P}(X^2 > 42.12) < 10^{-10}.$$

The p-value is not ...

- ... the probability that the null hypothesis is true.
- ... the probability that a finding is “merely a fluke”.
- ... the probability of falsely rejecting the null hypothesis.
- ... the probability that a replicating experiment would not yield the same conclusion.
- ... indicating the size or importance of the observed effect.
- The significance level of the test is not determined by the p-value.

Family wise error rate

M hypothesis tests

$$\mathcal{H}_{0m} \qquad \text{versus} \qquad \mathcal{H}_{1m}, \qquad m = 1, \ldots, M$$

and let $p_1, \ldots, p_M$ denote the M p-values for these tests.

Bonferroni Method

Reject null hypothesis H_{0m} if

$$p_m < \frac{\alpha}{M}.$$

Outcome

The probability of falsely rejecting any null hypothesis is less than or equal to α.

False discovery proportion

Let M_0 be the number of null hypotheses that are true.

$$M_1 = M - M_0$$

	$\mathcal{H}_0$ accepted	$\mathcal{H}_0$ rejected	Total
$\mathcal{H}_0$ True	U	V	M_0
$\mathcal{H}_0$ False	T	S	M_1
Total	M-R	R	M

Define the *false discovery proportion* (FDP)

$$\text{FDP} = \begin{cases} V/R & \text{if } R > 0 \\ 0 & \text{if } R = 0. \end{cases}$$

False discovery rate

M hypothesis tests

We order the p-values in increasing order.

Benjamini-Hochberg Method

1. For a given α, find the largest k such that

$$p_k \leqslant k\frac{\alpha}{M}.$$

2. Then reject all $\mathcal{H}_{0m}$ for $m = 1, \ldots, k$.

Theorem

$$\text{FDR} = \mathbb{E}(\text{FDP}) \leqslant \frac{M_0}{M}\alpha \leqslant \alpha.$$

Outcome

For a given significance level α, the Benjamini Hochberg method bounds the false discovery rate.

Multiple testing

Suppose 800 of 500,000 variants are significant at 0.05 level.

p-value < 0.05

Expect 0.05 * 500000 = 25000 false positives

false discovery rate < 0.05

Expect 0.05 * 800 = 40 false positives

family wise error rate < 0.05

The probability of at least 1 false positive < 0.05

So far...

The basics of hypothesis testing applied to GWAS

Some Genomics Nomenclature

GWAS, SNPs, Allele, Diploid, Genotype, Phenotype

Hypothesis Testing

- $\mathcal{H}_0$ vs $\mathcal{H}_1$
- Design test statistic and compute p-value
- Reject $\mathcal{H}_0$ if p-value $<$ α.

Multiple Testing

- Bonferroni correction
- Benjamini Hochberg method

http://www.ong-home.my/download/notes-gwas-hypo-test.pdf

Epistatic Interactions

Genome Wide Interaction Search (GWIS)

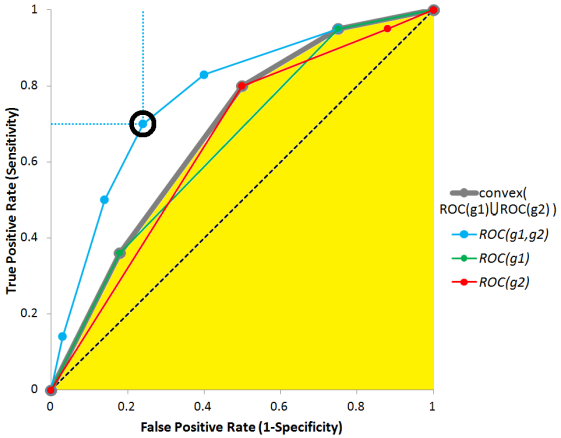
Consider the association of all pairs of genotypes to phenotypes

Large search space

- 5000 individuals, 500,000 SNPs
- Need to tabulate 125 billion contingency tables

Classification based analysis

- Focus on SNPs in case control studies
- New statistical tests
- Consider specificity and sensitivity
- Gain over univariate ROC
- CPU ($\approx$ days) and GPU ($\approx$ hours)



Web service

`http://gwis1.research.nicta.com.au/`

Goudey et. al. BMC Genomics, 2013

Biological Complexity

What is a biomarker?

How to measure?

- Clinical observations
- Whole genome sequencing
- Probes (arrays) for large studies

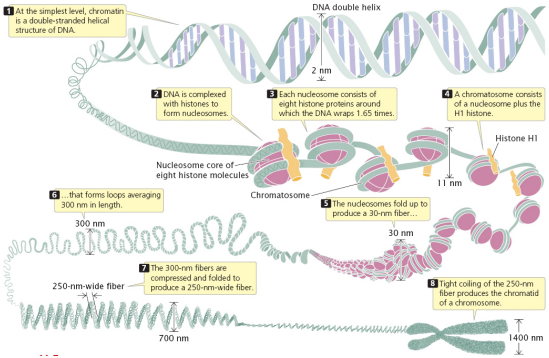
Looking at shadows

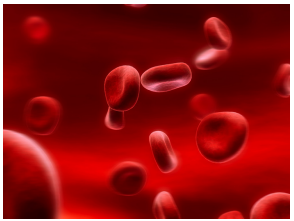
What to measure?

- Assumption: genetic cause
- DNA, RNA, Protein
- SNP, INDEL, CNV, Methylation, ...

Where to measure?

- Non-invasive diagnostic test
- Does tissue show variation?





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The search for genes

A very brief overview of molecular biology

Biological sequencing

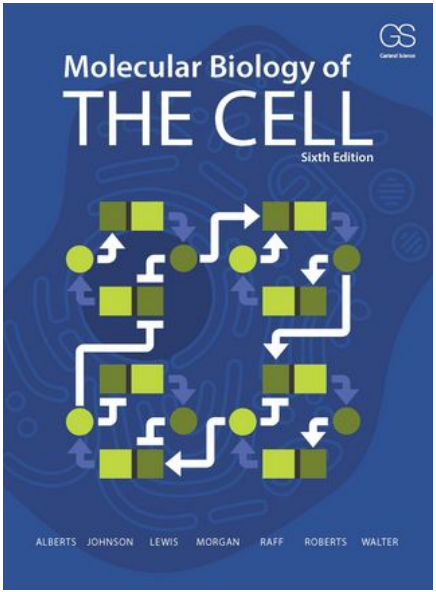
The big data revolution in life sciences

The world is round

https://www.nasa.gov/image-feature/nasa-captures-epic-earth-image

The world is round

Genomics has given us a new perspective that has demanded a complete recasting and expansion of the material on molecular genetics ...

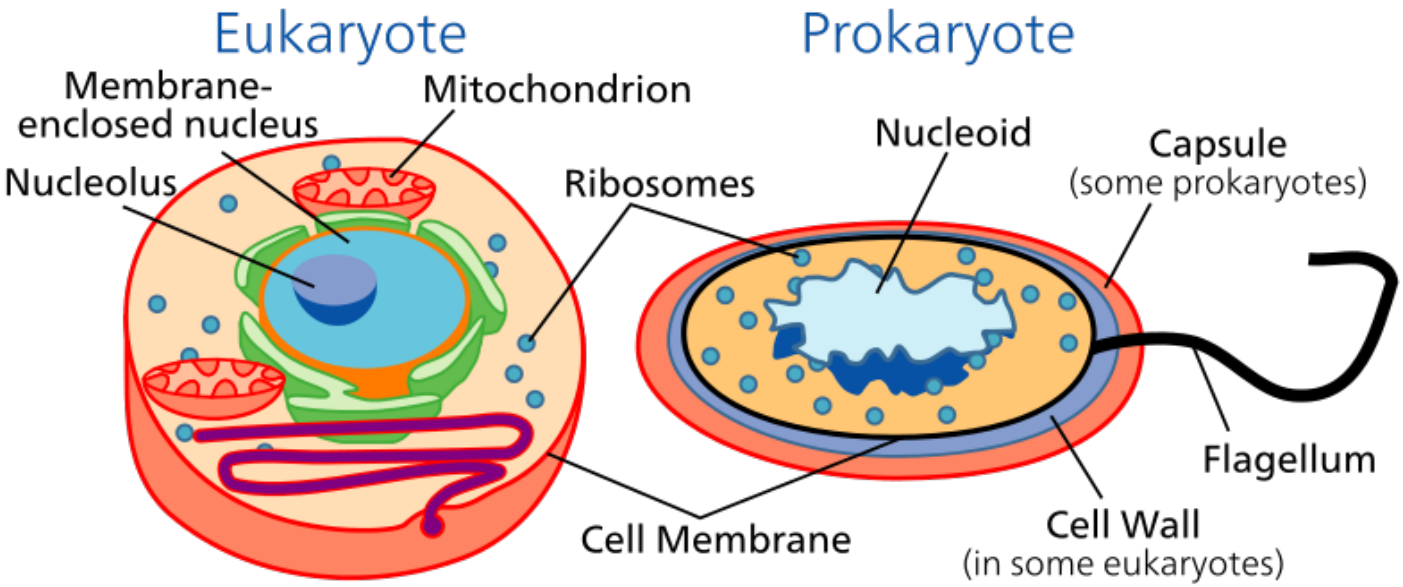


The traditionally explanatory cartoons that we show on nearly every page of the book generally represent only the primitive first step toward an explanation.

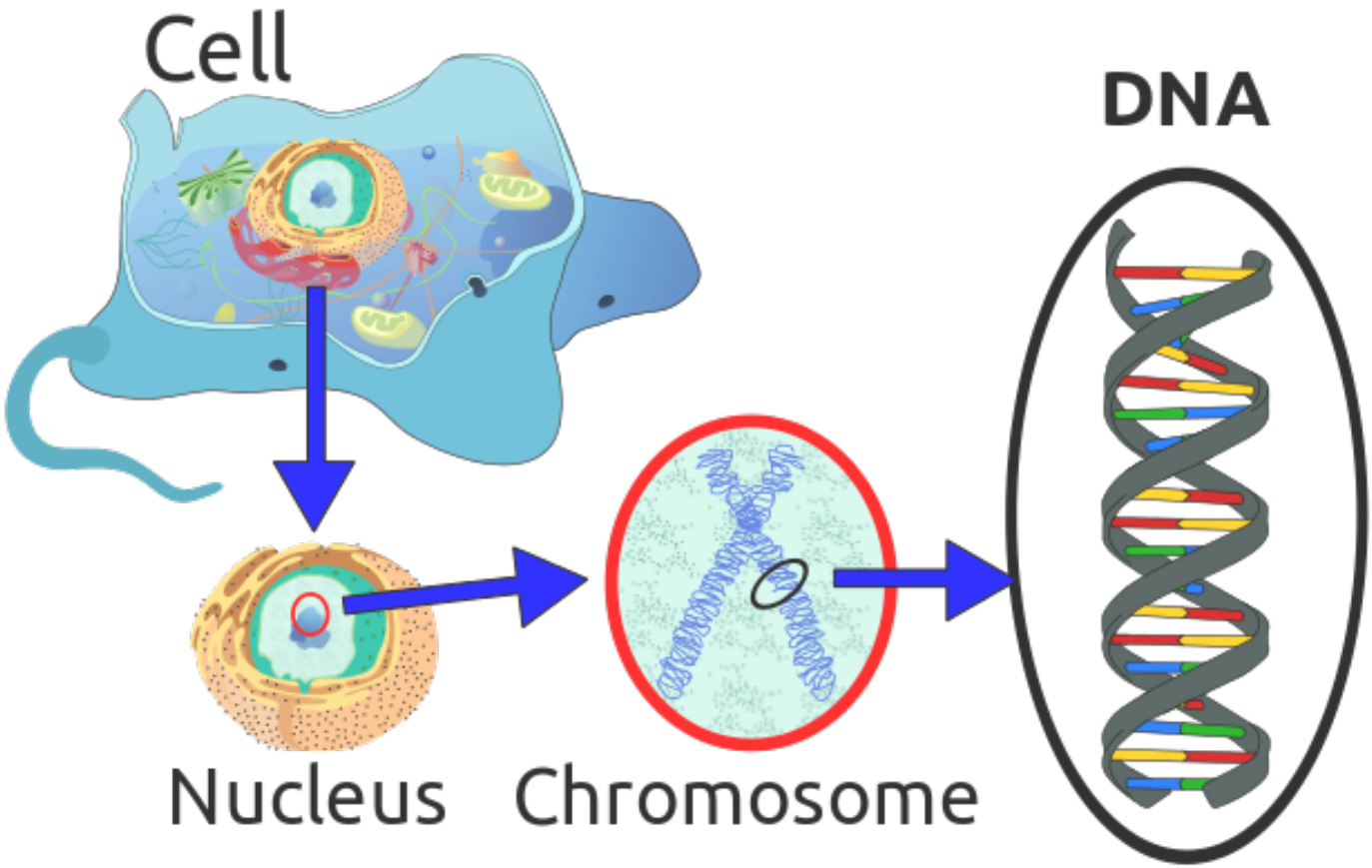
Preface: Alberts et. al. 2002

Tree of life

Bacteria, Archea, Eukaroyte

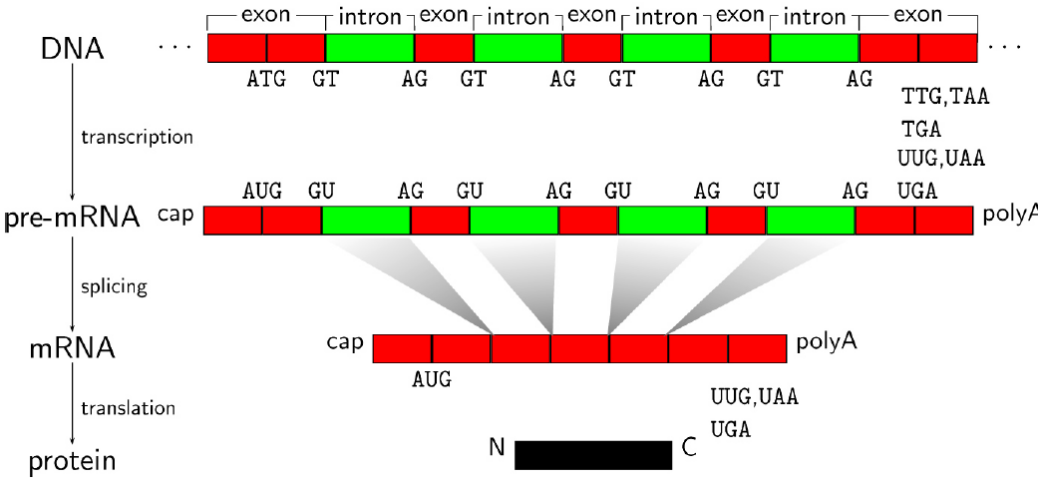


Inside a cell



https://en.wikipedia.org/wiki/DNA

Central Dogma



DNA Positive strand, written 5' to 3'.
e.g. AATCGAAGTTA

RNA T $\Rightarrow$ U
e.g. AAUCGAAGUUA

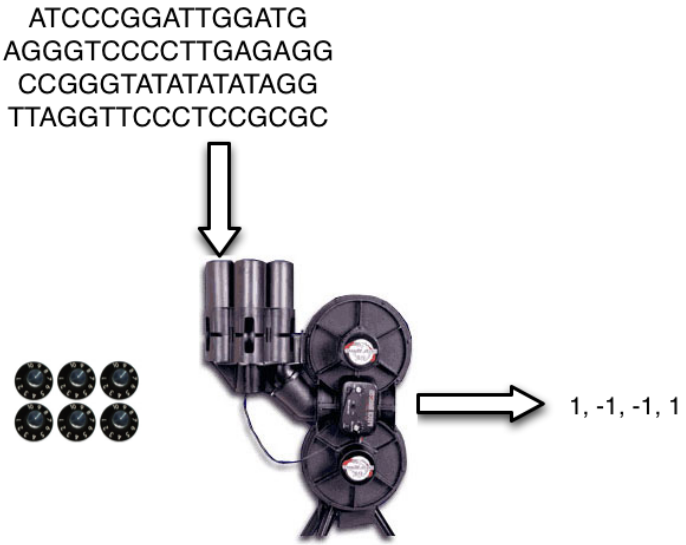
Amino acid 3 letters of RNA (codon) $\Rightarrow$ amino acid, 20 letter alphabet.

Lewin, Genes

Classification of Sequences

Example: Recognition of splice sites

- Every 'AG' is a possible acceptor splice site
- Computer has to learn what splice sites look like
  - given some known genes/splice sites . . .
- Prediction on unknown DNA



From Sequences to Features

- Many algorithms depend on numerical representations.
  - Each example is a vector of values (features).
- Use background knowledge to design good features.

	$\mathbf{x}_1$	$\mathbf{x}_2$	$\mathbf{x}_3$	$\mathbf{x}_4$	$\mathbf{x}_5$	$\mathbf{x}_6$	$\mathbf{x}_7$	$\mathbf{x}_8$	...
GC before	0.6	0.2	0.4	0.3	0.2	0.4	0.5	0.5	...
GC after	0.7	0.7	0.3	0.6	0.3	0.4	0.7	0.6	...
AG**AG**AAG	0	0	0	1	1	0	0	1	...
TTT**AG**	1	1	1	0	0	1	0	0	...
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋱
Label	+1	+1	+1	−1	−1	+1	−1	−1	...

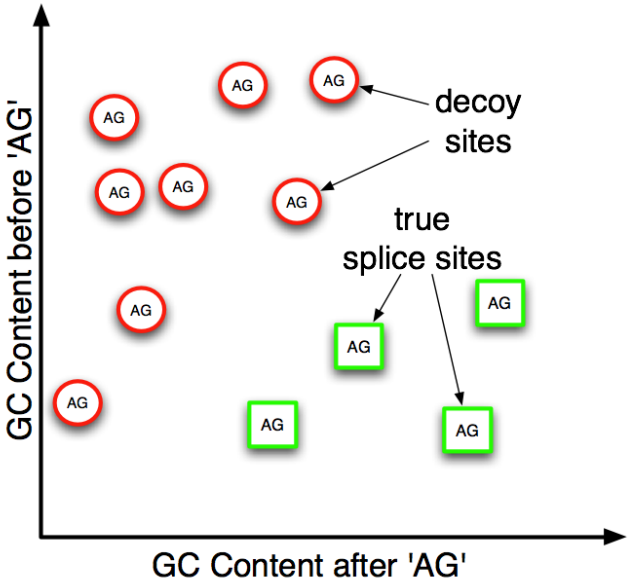
Recognition of Splice Sites

- Given: Potential acceptor splice sites

```
AAACAAATAAGTAACTAATCTTTTAGGAAGAACGTTTCAACCATTTTGAG
AAGATTAAAAAAAAACAAATTTTTAGCATTACAGATATAATAATCTAATT
CACTCCCCAAATCAACGATATTTTAGTTCACTAACACATCCGTCTGTGCC
TTAATTTCACTTCCACATACTTCCAGATCATCAATCTCCAAAACCAACAC
```

intron **exon**

- Goal: Rule that distinguishes true from false ones

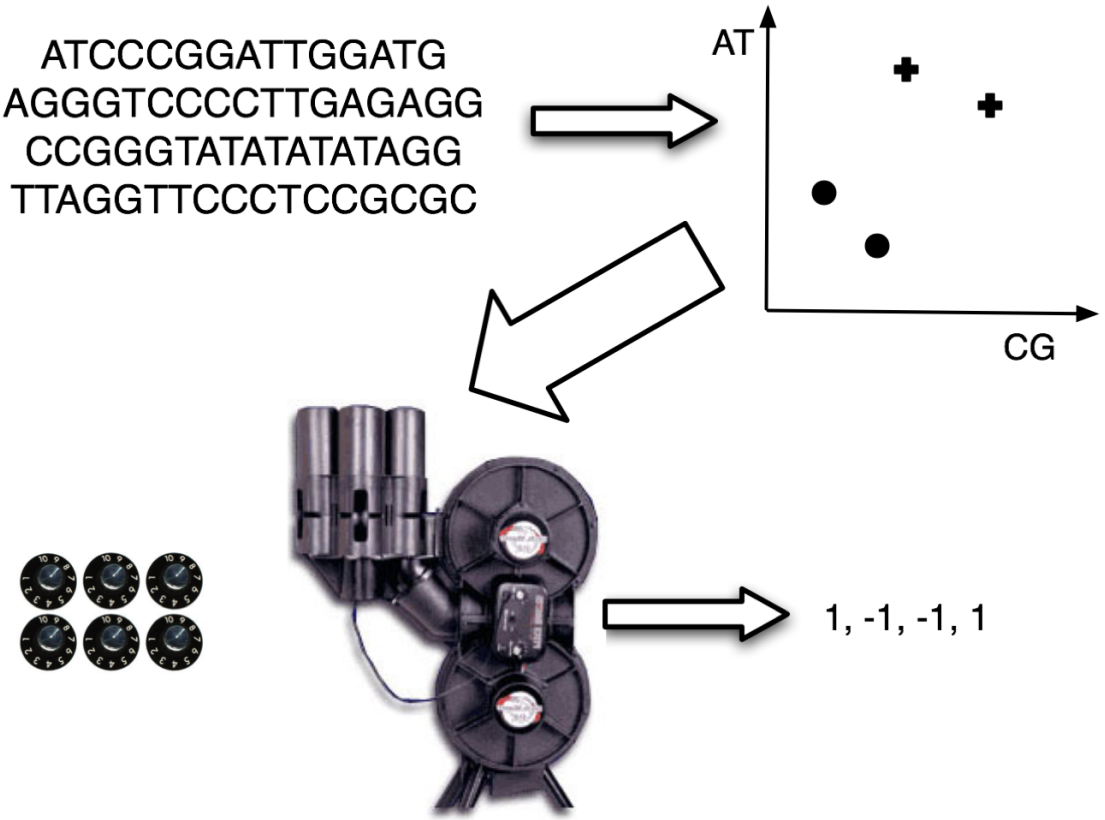


e.g. exploit that exons have higher GC content

or

that certain motifs are located nearby

Numerical Representation

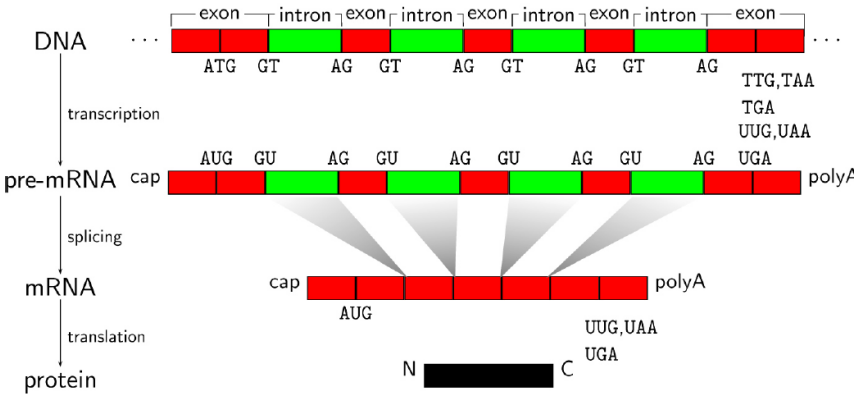


Support vector machines and kernels for computational biology, PLoS Comp. Bio. 2008

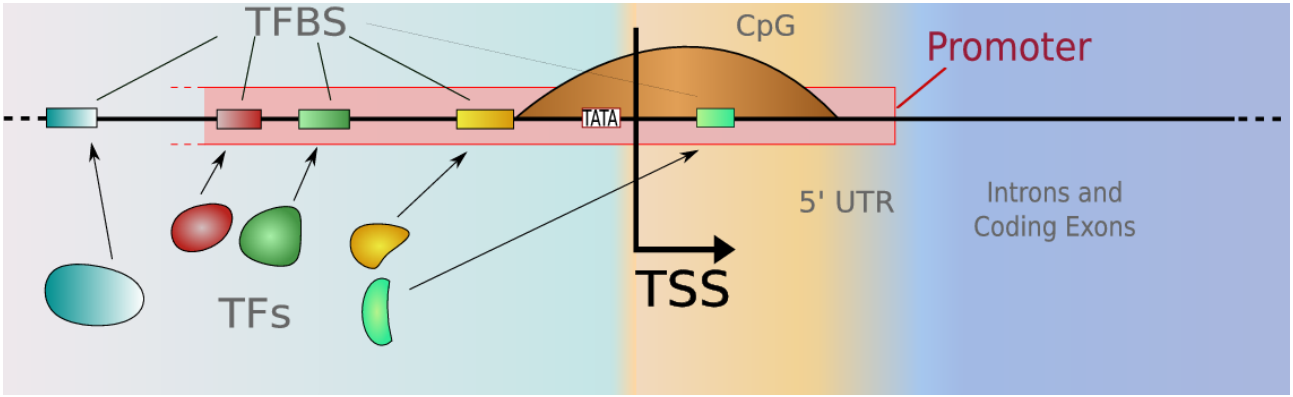
Gene Finding

Given the DNA, predict resulting mRNA and protein

- Requires very accurate identification of
  - splice sites, translation & transcription starts & stops
  - sites of regulation (transcription, splicing, etc.)
- Develop methods to integrate single site predictions
  - usually HMMs
  - Novel learning methods for structured outputs

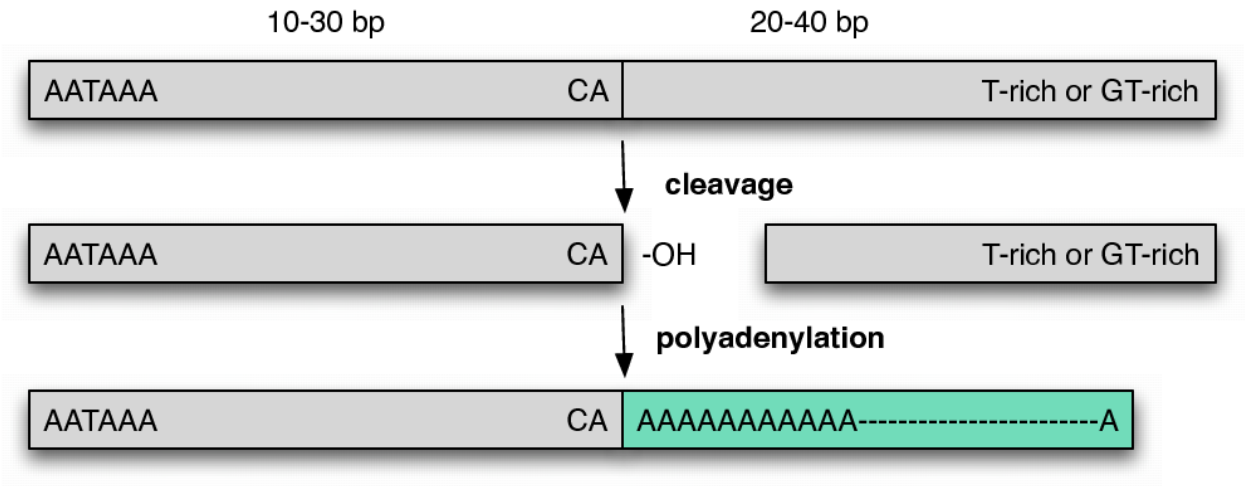


Gene Finding I: Transcription Start



- POL II binds to a rather vague region of $\approx [-20, +20]$ bp
- Upstream of TSS: promoter containing transcription factor binding sites
- Downstream of TSS: 5' UTR, and further downstream coding regions and introns (different statistics)
- 3D structure of the promoter must allow the transcription factors to bind

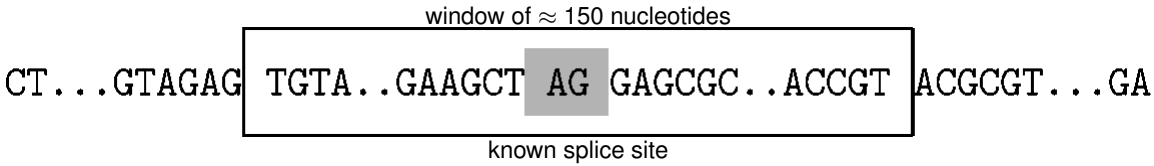
Gene Finding II: Transcr. Termination



- Polyadenylation signal (AATAAA or variants) 10-30 bp upstream
- T-rich or GT-rich elements 20-40 bp downstream
- Transcription end is several hundreds of bp after 3' cleavage site, mechanism not yet understood

Gene Finding III: Splice Sites

Finding Intron-Exon junctions



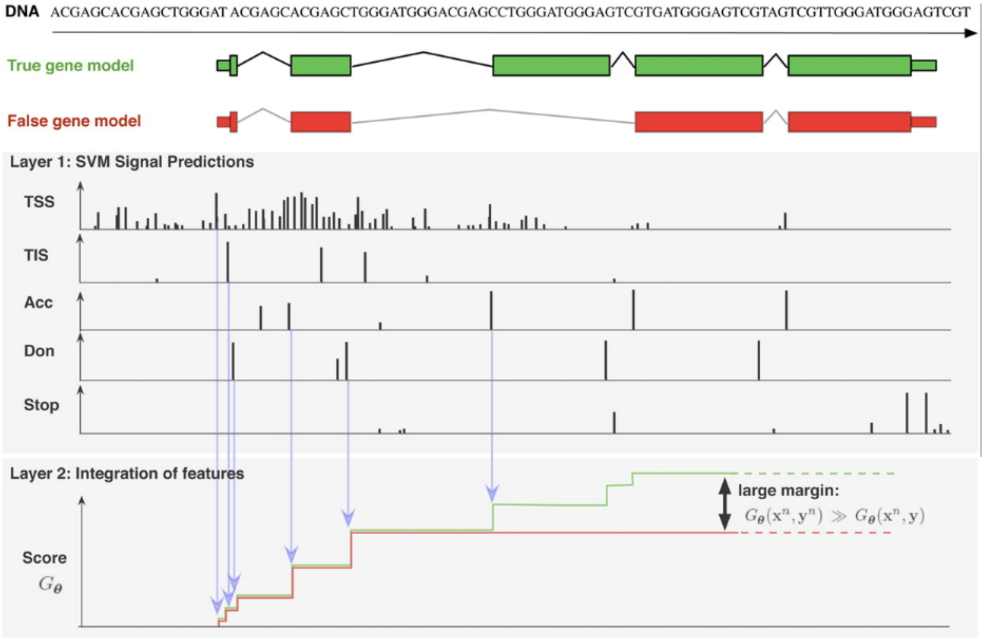
- true sites: fixed-length window around splice site
- decoys sites: generated by shifting the window

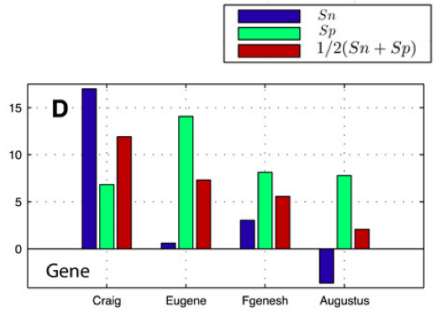
```
AAACAAATAAGTAACTAATCTTTTAGGAAGAACGTTTCAACCATTTTGAG
AAGATTAAAAAAAAAACAAATTTTTAGCATTACAGATATAATAATCTAATT
CACTCCCCAAATCAACGATATTTTAGTTCACTAACACATCCGTCTGTGCC
TTAATTTCACTTCCACATACTTCCAGATCATCAATCTCCAAAACCAACAC
```

$\Rightarrow$ Very unbalanced problem (1:200)
$\Rightarrow$ Millions of points from EST databases
$\Rightarrow$ Large scale methods necessary

Gene Finding IV: Splice Forms

Predict a sequence of binary decisions



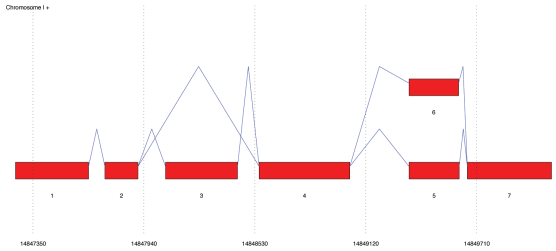


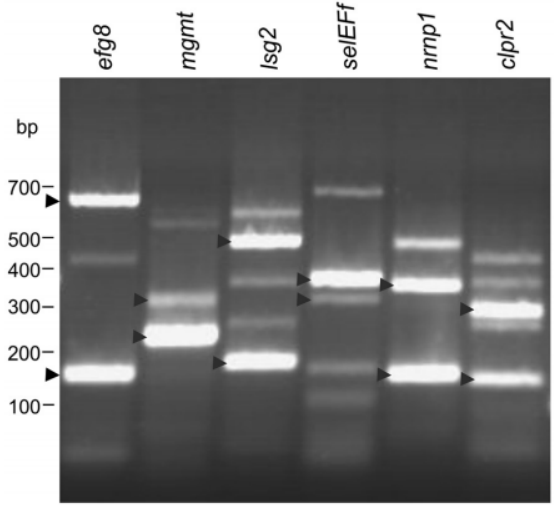
www.mgene.org/web

Gene Finding V: Alt. Splicing

Goal: Find sites of alternative splicing, conditions and regulating genes

- Understand differences between alternative and constitutive splicing
- Predict yet unknown alternative splicing events
- Predict on newly sequenced organisms
- Experimentally verify predictions via RT-PCR.

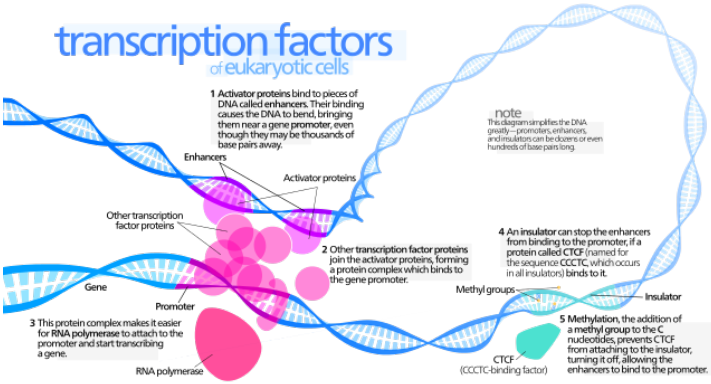


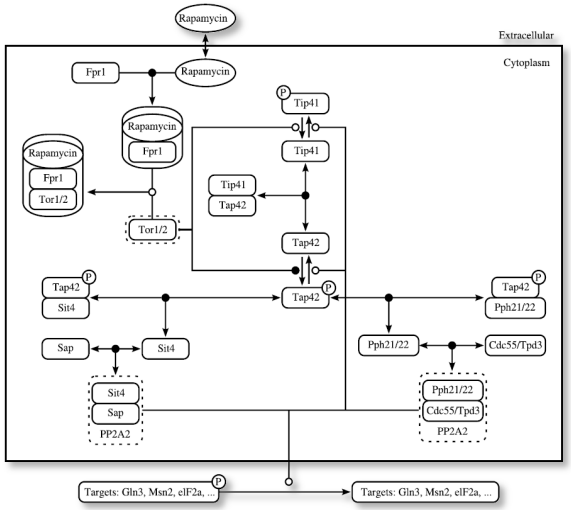


Kianianmomeni et. al. Genome-wide analysis of alternative splicing in Volvox carteri, 2014

Regulation and control

- Genes are regulated by proteins called transcription factors
- Environment, e.g. metabolism (internal), temperature (external)





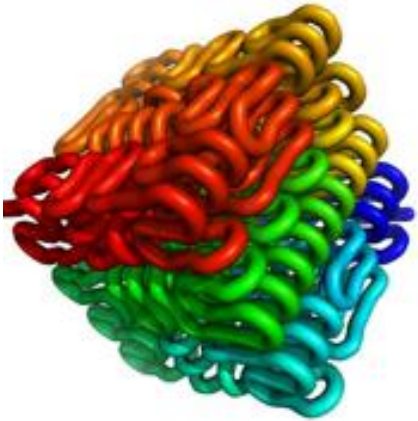
https://en.wikipedia.org/wiki/Transcription_factor

Alon, An Introduction to Systems Biology, 2007

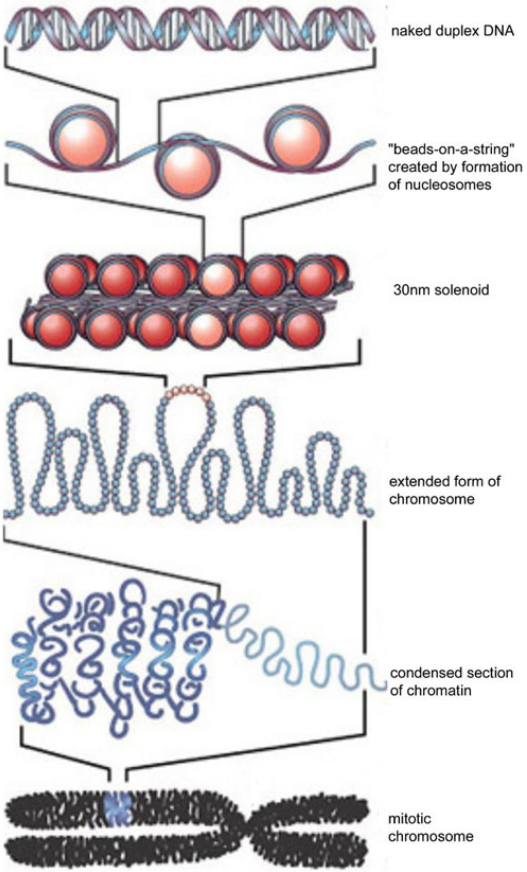
Lawrence et. al. Learning and Inference in Computational Systems Biology, 2010

Chromatin structure

- DNA packed tightly in nucleus
- DNA wrapped around histones to form nucleosomes
- Nucleosomes organised into chromatin fibres
- Transcription accessibility
- DNA repair

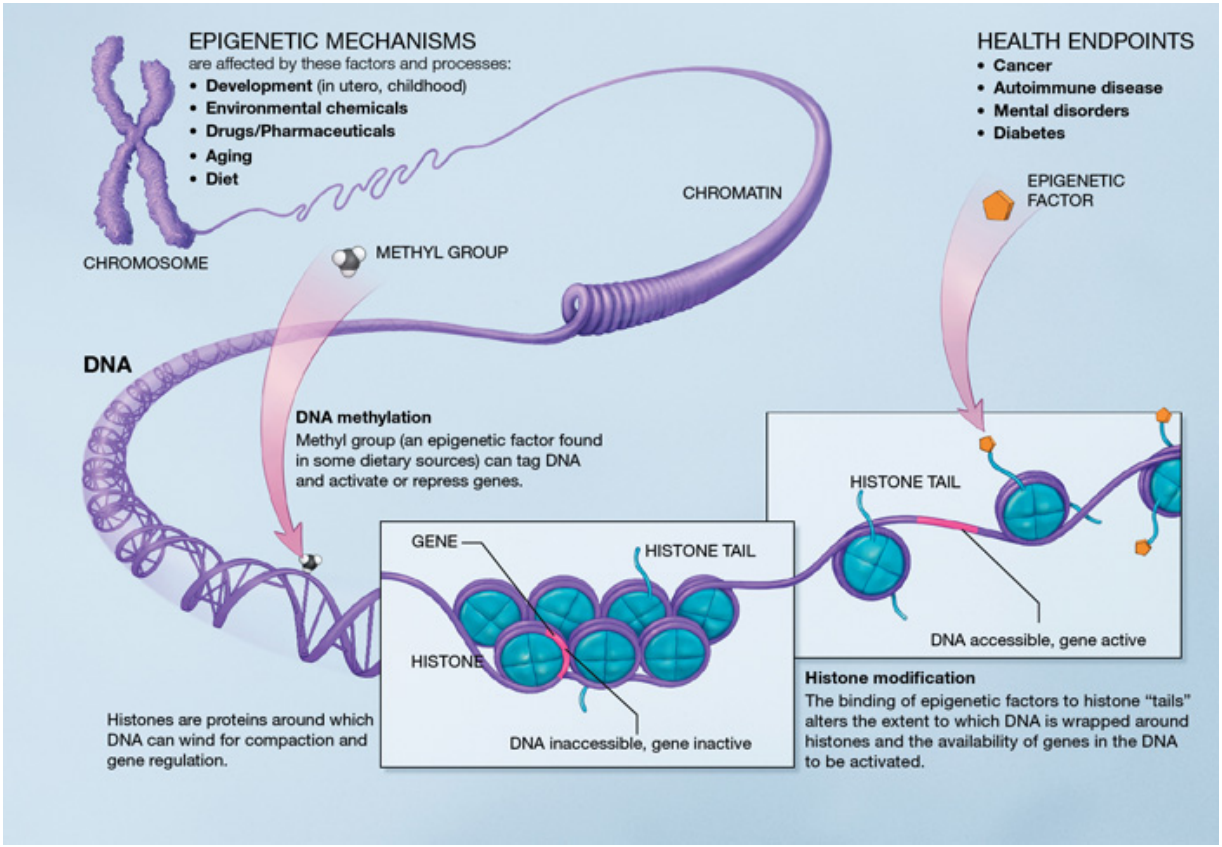


http://dx.doi.org/10.1103/PhysRevLett.114.178102



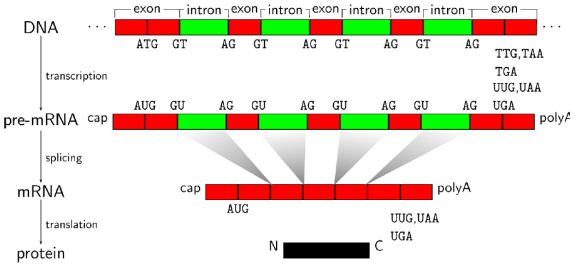
https://en.wikipedia.org/wiki/Nucleic_acid_struc

Methylation



https://theconversation.com/explainer-what-is-epigenetics-13877

Glimpse of molecular biology



DNA Positive strand, written 5' to 3'.
e.g. AATCGAAGTTA

RNA T $\Rightarrow$ U
e.g. AAUCGAAGUUA

Amino acid 3 letters of RNA (codon) $\Rightarrow$ amino acid, 20 letter alphabet.

Splicing pre-mRNA to mature mRNA

Transcription factor Regulate expression of gene, through promoters and repressors

Epigenetics Methylation, Chromatin marks

Outline

Genome wide association studies

Find genetic variation corresponding to an attribute of interest.

The search for genes

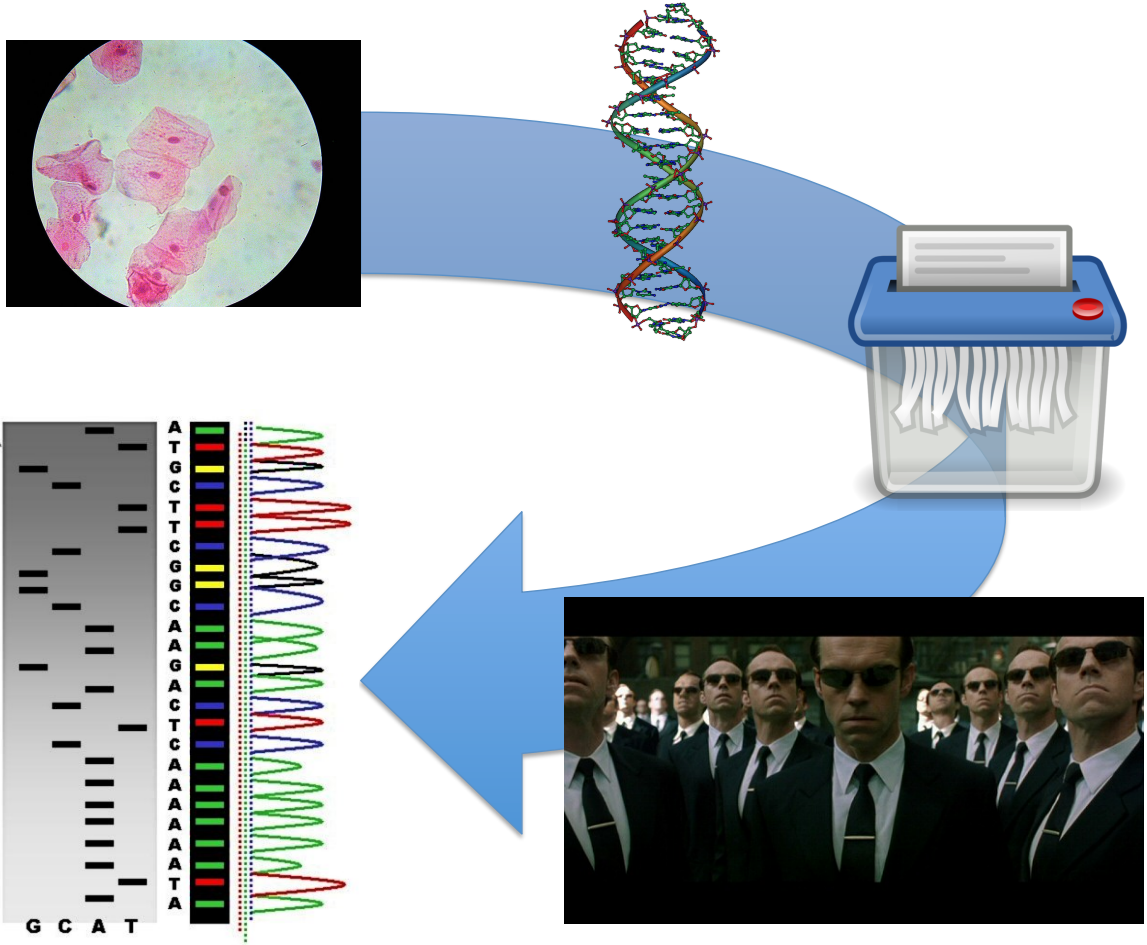
A very brief overview of molecular biology

Biological sequencing

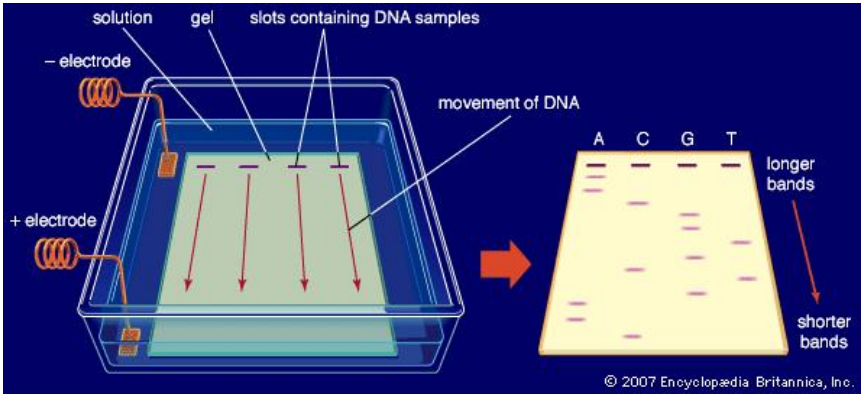
The big data revolution in life sciences

- Identifying biomarkers
- Bottleneck: data analysis
- Open area of research

Sequencing



History of sequencing



- 1960s: DNA - properties, proto sequencing
- 70s-90s: Manual sequencing - Sanger, Maxam-Gilbert
- 90s: Automated Sanger - flourescent, clones, colony picking
- 2003: Human genome - 25 cents per 1000 bases
- 00s: NGS, Clusters - 454-Roche, Solexa-Illumina, Ion Torrent
- Illumina HiSeq X Ten: 6 billion 150 base sequences in 3 days

http://phylogenomics.blogspot.com.au/2015/10/evolution-of-dna-sequencing-talk-2015.html

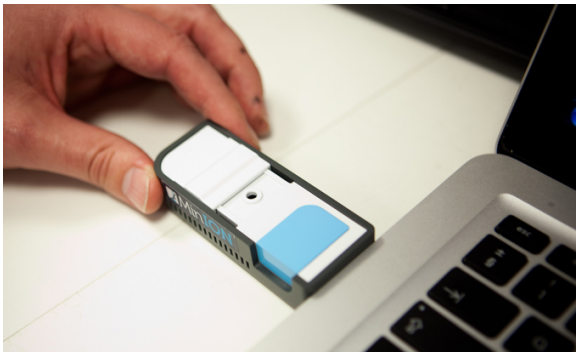
USD 1000 genome

Data volume

- HiSeq X Ten: 12 GB per hour
- 700MB per human genome
  $\sim$ 200GB reads

Work in progress

- Multiplexing - tag sequences
- Capture: Enrich a particular set
- Paired Ends: sequence from both ends
- Small amounts of DNA
- Longer reads



Small sequencers

- Single cell sequencing: PacBio
- Real time sequencing:
  Oxford Nanopore

6 billion 150 base sequences

SO WHAT?

Apply cheap sensor

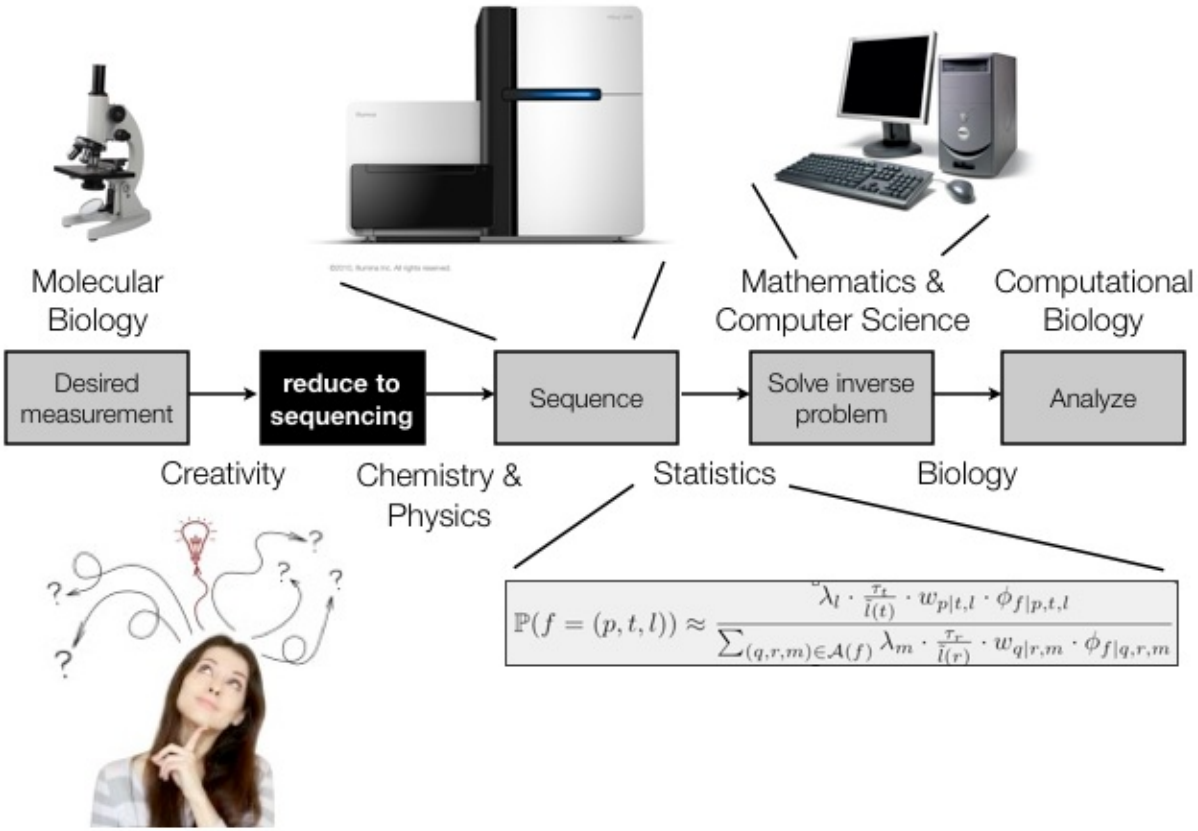


Image from Lior Pachter's ISMB 2013 keynote

DNA Sequencing

Analogy: Shotgun sequencing

Take many copies of a text, split at random points, reconstruct.

Alignment

- Dynamic programming
- Needleman-Wunsch and Smith-Waterman

https://en.wikipedia.org/wiki/List_of_sequence_alignment_software

Assembly

- reference genome vs de-novo
- grouping: reads $\rightarrow$ contigs $\rightarrow$ scaffold
- Bridges of Königsberg $\rightarrow$ de Bruijn graphs

http://www.nature.com/nmeth/journal/v9/n4/full/nmeth.1935.html

Cohort

Single Nucleotide Variation

- Recall two copies of chromosomes
- at every location: AA, AB, BB
- Noise free, high coverage $\Rightarrow$ frequency = probability
- Probabilistic methods for maximum a posteriori estimation
- Correlations along the genome

https://en.wikipedia.org/wiki/SNV_calling_from_NGS_data

Structural variation

- copy number variation
- insertions, deletions
- inversion, translocation

http://www.ncbi.nlm.nih.gov/dbvar/content/overview/

Study cohort germline vs somatic mutations

http://www.bioplanet.com/gcat

Replication

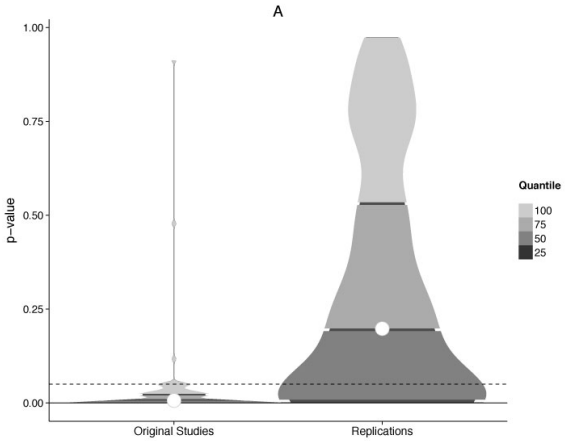
Multiple samples to estimate noise

Techical

- Effect of measurement instrument
- Different days, researcher
- Usually same biological sample

Biological

- Effect of biological development
- Different individuals of same “species”



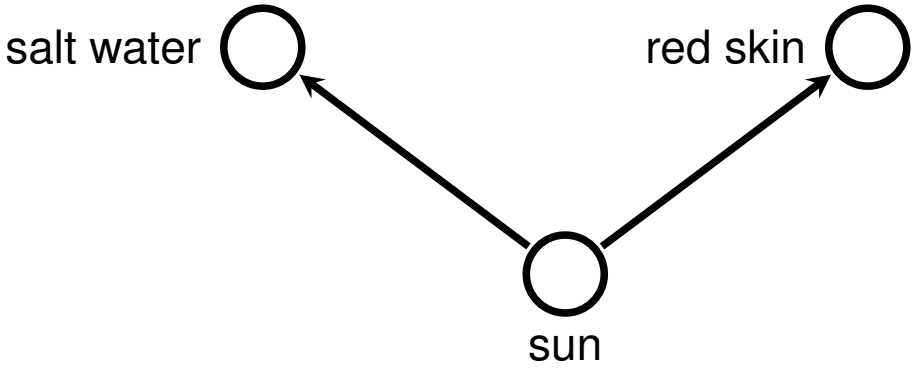
Reproducibility crisis?

- Psychology: https://osf.io/ezcuj/
- Cancer biology: underway
  http://elifesciences.org/collections/reproducibility-project-cancer-biology

Experimental Design

confounding

Common variable affecting two variables of interest.



batch effect

There is a hidden confounding variable for the effect, e.g. time

- Randomisation: randomly allocate samples to cases/controls
- Stratification: age, gender, group, geography

Lambert, Black: Learning from our GWAS mistakes, 2011

Bisulfite Seq

Methylation - epigenetics

- Identify methylated bases
- Regulates gene expression

Chemistry

- Bisulfite conversion converts unmethylated C to U
- AAC^{M}GGTC^{M}CCAGT
- AAC^{M}GGTC^{M}UUAGT

Algorithm

- Align converted sequence to reference
- Need to disambiguate unmethylated C from T
- AAC^{M}GGUC^{M}UUAGU
- E.g. latent variable models

http://www.epigenome.org/

RNA Seq

Chemistry Convert RNA to DNA

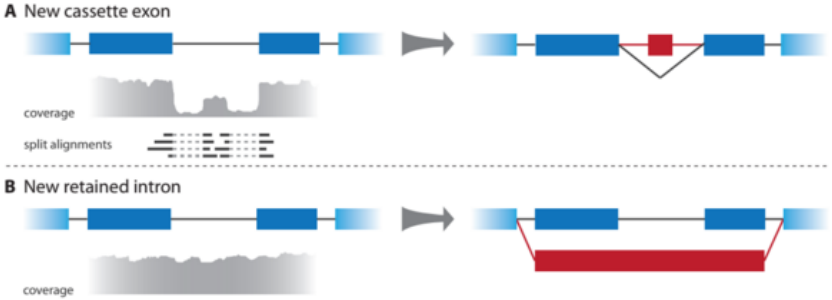
Gene Expression

- Recall: mRNA translated to proteins
- Which genes are expressed in what tissues at which levels?
- What are the regulators of a particular gene?
- How does treatment change expression (differential expression)?

https://www.encodeproject.org/

Splicing

Align expressed RNA to reference genome



http://biorxiv.org/content/early/2015/03/26/017095

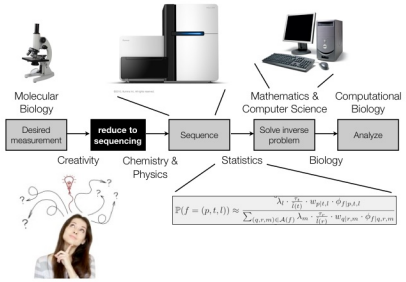
*-Seq

- dsRNA-Seq
- FRAG-Seq
- SHAPE-Seq
- PARTE-Seq
- PARS-Seq
- DMS-Seq
- ⋮

- Nucleo-Seq
- DNAse-Seq
- Sono-Seq
- ChIA-PET-Seq
- FAIRE-Seq
- NOMe-Seq
- ATAC-Seq
- ⋮

- GRO-Seq
- Quartz-Seq
- CAGE-Seq
- Nascent-Seq
- Cel-Seq
- 3P-Seq
- ⋮

https://liorpachter.wordpress.com/seq/

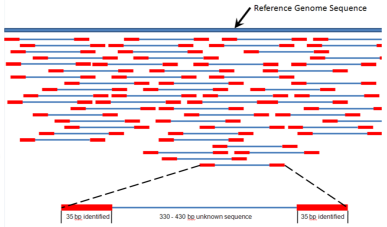


Putting things together

Association Study

	A	B	C	D	E	F	G	H	I	J	K	L
ID3023												
ID4454												
ID7675												
ID2283												

Sequence Analysis



Variation

- SNP
- Structural
- Methylation
- Expression
- ...

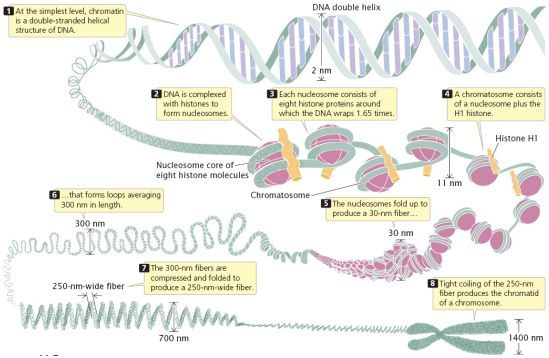
Shameless plug

What is a biomarker?

How to measure?

Use adaptive experimental design to identify important time series.

Busetto et. al. Near-optimal experimental design for model selection in systems biology, 2013



What to measure?

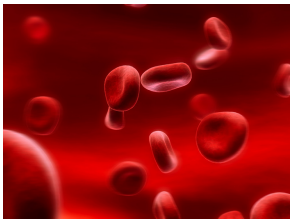
Combine various sources of information for robust decision making.

Macintyre et. al. Associating disease-related genetic variants in intergenic regions to the genes they impact, 2014

Where to measure?

Use expert domain knowledge to construct dynamical models.

Brodersen et. al. Generative embedding for model-based classification of fMRI data, 2011



Open Source

Machine Learning Open Source Software

mloss.org mldata.org

Do We Need Hundreds of Classifiers to Solve Real World Classification Problems?

jmlr.org/papers/v15/delgado14a.html

Spoiler: No

Usability and Reproducibility

- (too much) focus on new algorithms
- Documentation, modularity issues
- Literate programming

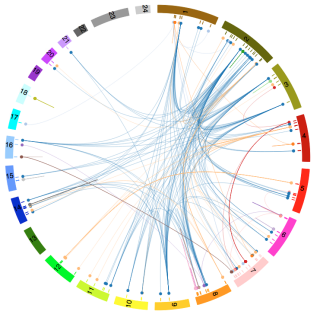
  rmarkdown.rstudio.com yihui.name/knitr jupyter.org
- Scientific computing workflows

  galaxyproject.org www.taverna.org.uk

Dream: App Bazaar for data science

Summary

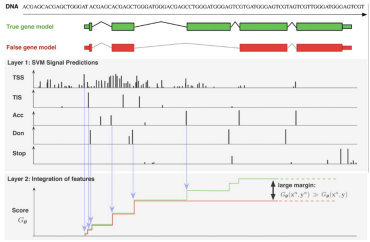
A Data Analytics View of Genomics



Genome wide association studies

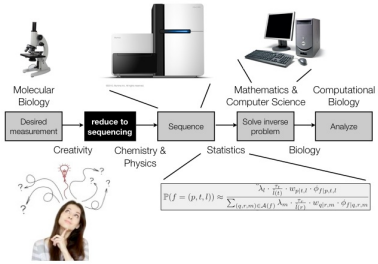
- Find genetic variation corresponding to an attribute of interest
- Hypothesis testing framework
- Batch effects and experimental design

The search for genes



- Glimpse of molecular biology
- Machine learning on sequences

Biological sequencing



- Bottleneck is analysis
- Sequence assignment and deconvolution

Please make your research open

Any questions?

http://www.ong-home.my/download/ai2015-genomics-tutorial.pdf