Methods and Techniques in Bioinfomatics

(From DNA bases to image databases)

Stephen Taylor

MRC WIMM Centre of Computational Biology

MRC WIMM Centre for Computational Biology

Using computational biology to help understand complex biological systems and combat diseases, from blood disorders to cancer and diabetes.

IN THIS SECTION

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Contact us

Do you have a query or would like to find out who to contact within the Centre?

Email us at
ccb@imm.ox.ac.uk

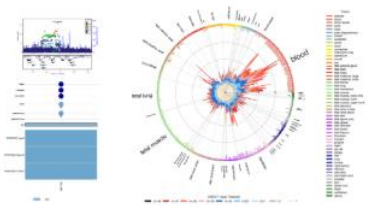
From the bench to VR

Computational Biologist Stephen Taylor and his team were awarded an Innovation grant to develop a software package that allows researchers to use virtual reality for scientific research and public engagement.

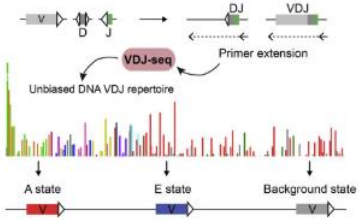
Read more

Research Groups

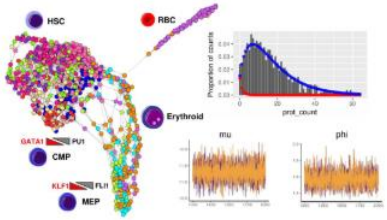
Hughes Group



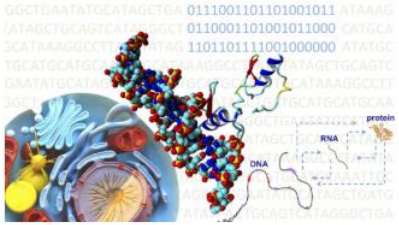
Iotchkova Group: Statistical Genetics



Koohy Group: Machine Learning and Integrative Approaches in Immunology

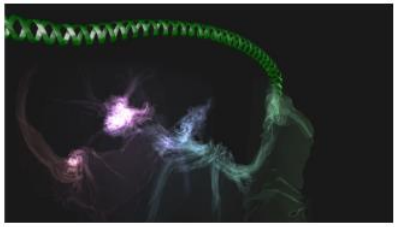


Morrissey Group: Quantitative biology of cell fate and tissue dynamics



Sahakyan Group: Integrative Computational Biology and Machine Learning

Sims Group: Computational Genomics



Taylor Group: Analysis, Visualisation and Informatics

Computational Biology and Bioinformatics is all about data…

- Definition
  - Bioinformatics is the computational analysis and storage of biological data
- Derivation
  - informatique – French for ‘data processing’
- Goal
  - To discover new biological insights using computers and biology

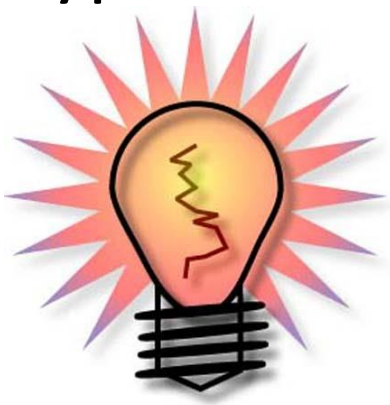
What is bioinformatics?

Experiment

Analysis

Sequence
Structure
Function
Evolution
Pathway
Interaction
Mutation
Expression

Hypothesis



Why use bioinformatics?

- Find an answer quickly
  - Most *in silico* biology is faster than *in vitro*
- Massive amounts of data to analyse
  - Need to make use of all information
  - Not possible to do analysis by hand
  - Can't organise and store information only using lab note books
  - Automation is key
- However!
  - All results of computer analysis should to be verified by biologists

Bioinformatics databases

- Public databases are the most important entity in bioinformatics
- Store knowledge about
  - Sequence e.g. EMBL/Genbank
  - HT Sequencing Experiments e.g. GEO
  - Structure e.g. PDB
  - Pathways e.g. KEGG, Metacore
  - Diseases e.g. OMIM

- Can be searched in a variety of ways

  e.g. keyword, sequence, pattern,

Keyword

NCBI Resources How To bioinfbloke My NCBI Sign Out

Search NCBI databases Help

p53 Search

About 1,673,010 search results for "p53"

Literature

Books	1,279	books and reports
MeSH	158	ontology used for PubMed indexing
NLM Catalog	108	books, journals and more in the NLM Collections
PubMed	71,937	scientific & medical abstracts/citations
PubMed Central	93,434	full-text journal articles

Health

ClinVar	225	human variations of clinical significance
dbGaP	22	genotype/phenotype interaction studies
GTR	110	genetic testing registry
MedGen	72	medical genetics literature and links
OMIM	583	online mendelian inheritance in man
PubMed Health	71	clinical effectiveness, disease and drug reports

Genomes

Assembly	1	genomic assembly information
BioProject	642	biological projects providing data to NCBI
BioSample	307	descriptions of biological source materials
Clone	0	genomic and cDNA clones
dbVar	1,464	genome structural variation studies
Epigenomics	0	epigenomic studies and display tools
Genome	5	genome sequencing projects by organism
GSS	36	genome survey sequences
Nucleotide	24,181	DNA and RNA sequences
Probe	3,507	sequence-based probes and primers
SNP	6,592	short genetic variations
SRA	440	high-throughput DNA and RNA sequence read archive
Taxonomy	0	taxonomic classification and nomenclature catalog

Genes

EST	796	expressed sequence tag sequences
Gene	7,879	collected information about gene loci
GEO DataSets	8,899	functional genomics studies
GEO Profiles	1,403,459	gene expression and molecular abundance profiles
HomoloGene	38	homologous gene sets for selected organisms
PopSet	94	sequence sets from phylogenetic and population studies
UniGene	414	clusters of expressed transcripts

Proteins

Conserved Domains	120	conserved protein domains
Protein	29,695	protein sequences
Protein Clusters	15	sequence similarity-based protein clusters
Structure	1,082	experimentally-determined biomolecular structures

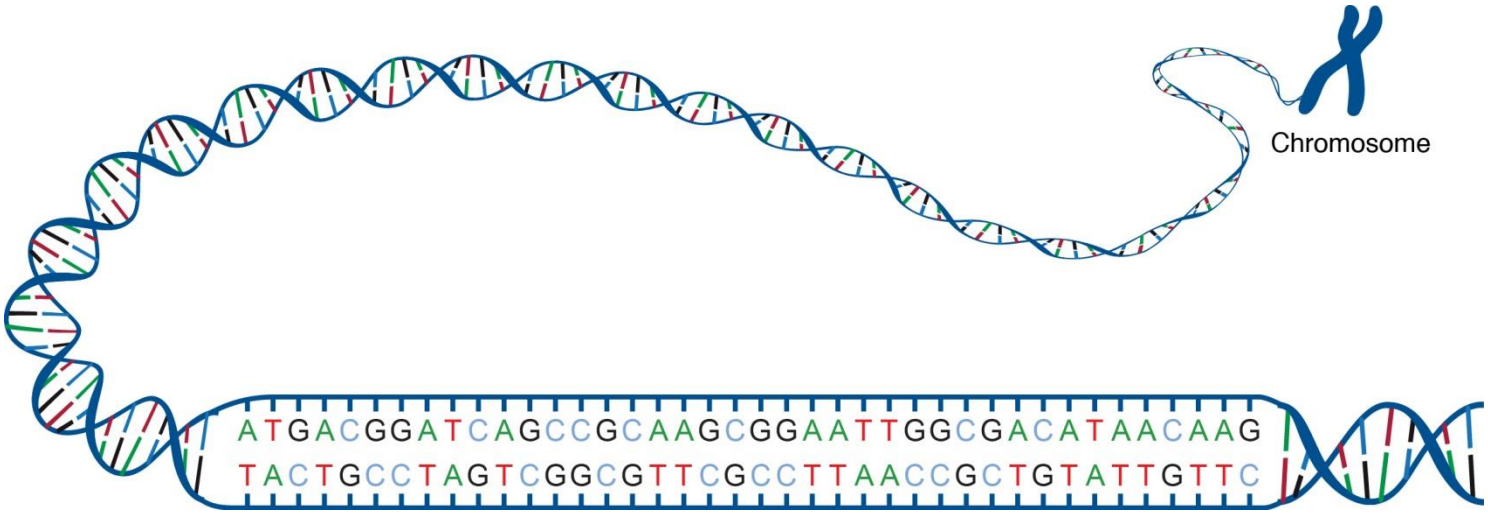
Chemicals

BioSystems	3,799	molecular pathways with links to genes, proteins and chemicals
PubChem BioAssay	10,848	bioactivity screening studies
PubChem Compound	8	chemical information with structures, information and links
PubChem Substance	650	deposited substance and chemical information

Bioinformatics Tools

- Hundreds of computer programs
- Many freely available
- Generally available on UNIX or LINUX
- Often interact with bioinformatics databases
- Many accessible via the WWW
- Some require very powerful computers to run on
- CCB provide a environment to do this

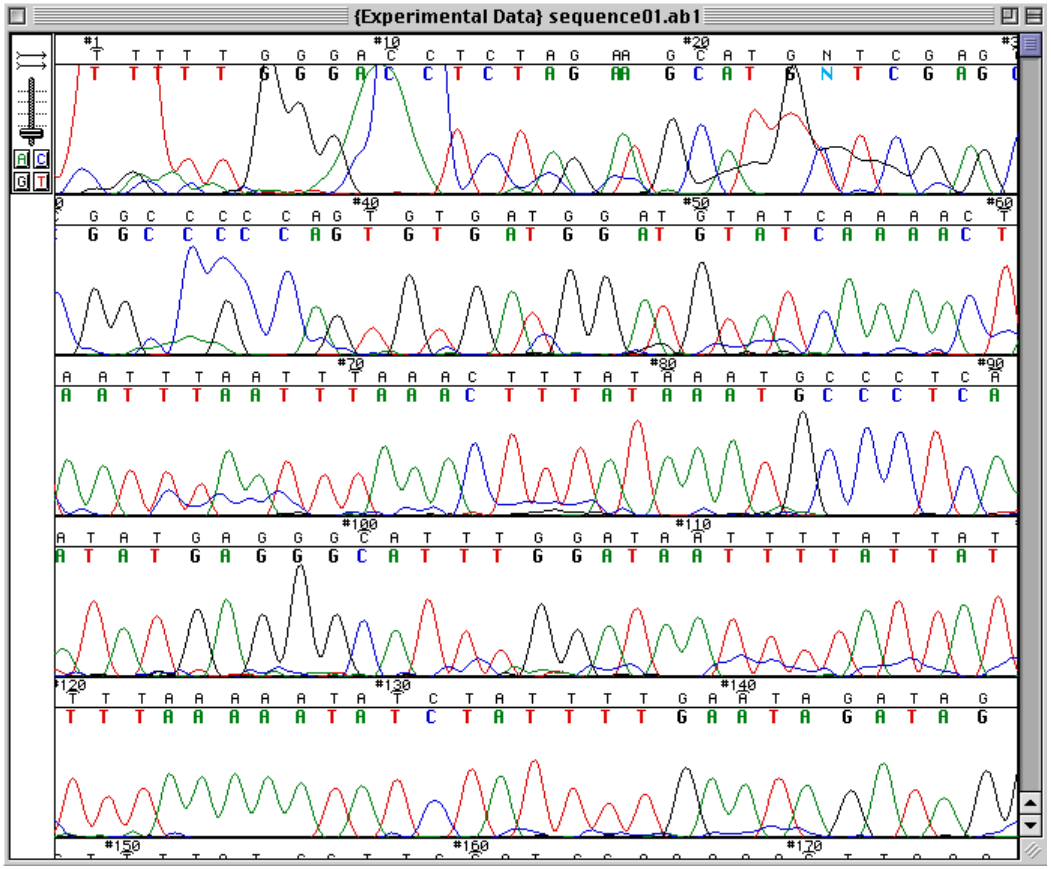
DNA


Chromosome
ATGACGGATCAGCCGCAAGCGGAATTGGCGACATAACAAG
TACTGCCTAGTCGGCGTTCGCCTTAACCGCTGTATTGTTC

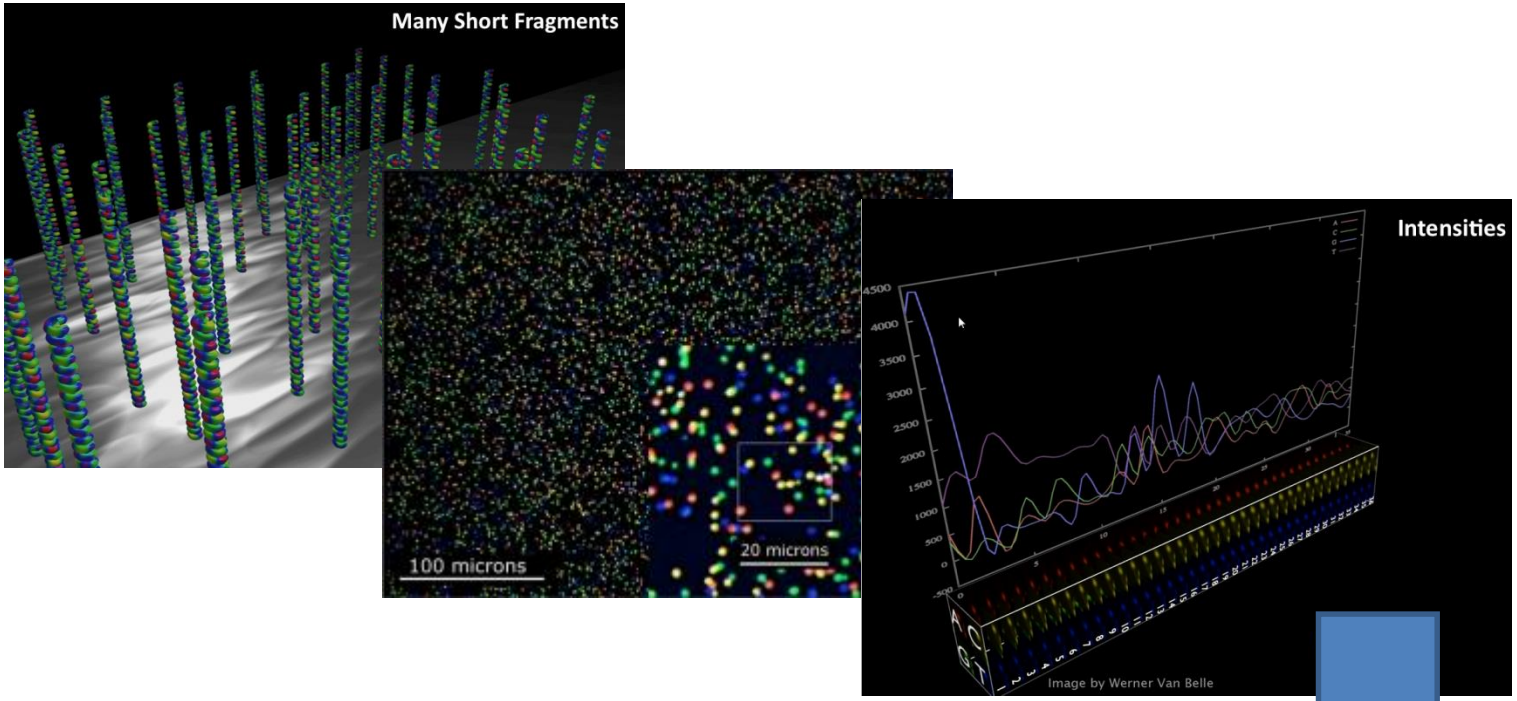
The Human Genome Project (1990-2003)

- Cost $3 billion
- Could not have been achieved without bioinformatics
- Goals
  - *identify* all the 20,500 genes in human DNA,
  - *determine* the sequences of the 3 billion chemical base pairs that make up human DNA
  - *store* this information in databases
  - *improve* tools for data analysis
  - *transfer* related technologies to the private sector, and
  - *address* the ethical, legal, and social issues (ELSI) that may arise from the project.
- Need to bring together and store vast amounts of information from
  - Lab equipment and experiments
  - Computer Analysis
  - Human Analysis
  - Make visible to the world's scientists
- Now (2019) can sequence a genome for less than $1000

Sanger Sequencing



Next Generation Sequencing

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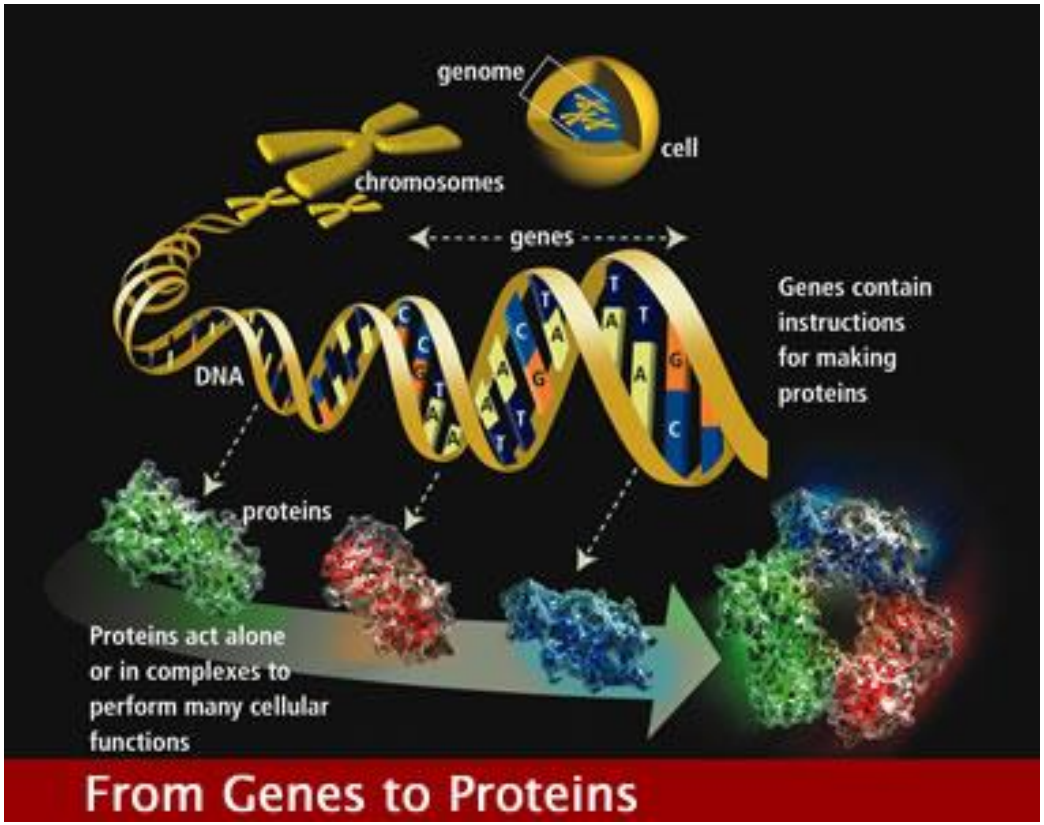
@HWI-EAS209_0006_FC706VJ:5:58:5894:21141#ATCACG/1
TTAATTGGTAAATAAATCTCCTAATAGCTTAGATNTTACCTTNNNNNNNNNNTAGTTTCTTGAGATTTGTTGGGGGAGACATTTTTGTGATTGCCTTGAT
+HWI-EAS209_0006_FC706VJ:5:58:5894:21141#ATCACG/1
efcfffffcfeefffcffffffddf`feed]`]_Ba_^__[YBBBBBBBBBRTT\]][]dddd`ddd^dddadd^BBBBBBBBBBBBBBBBBBBBBBB
```

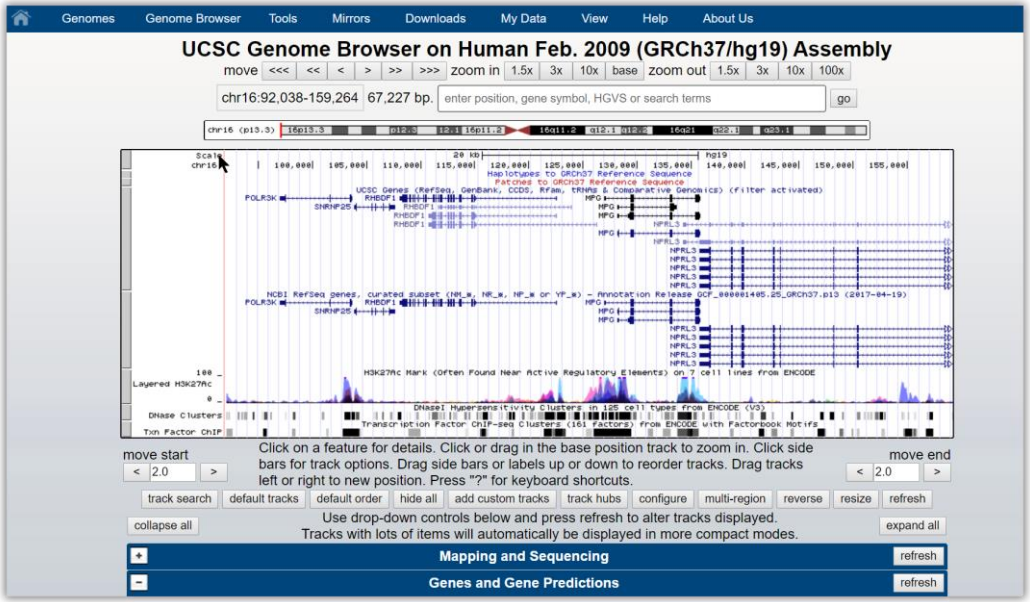
http://werner.yellowcouch.org/Papers/pippres0802/index.html

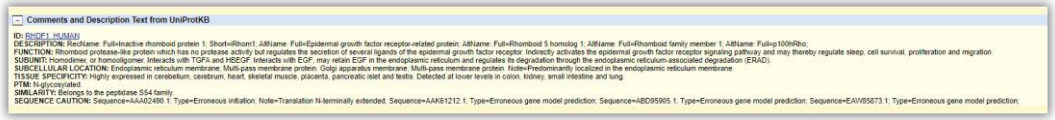
Central Dogma of Molecular Biology

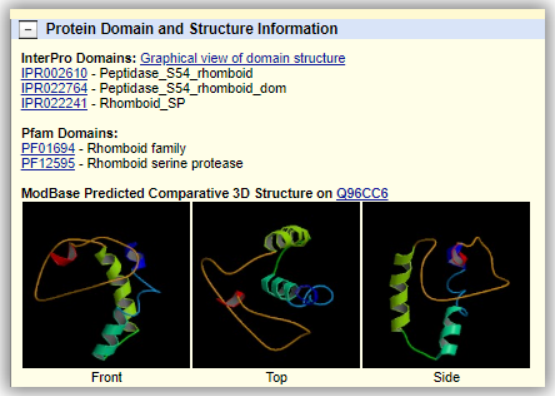


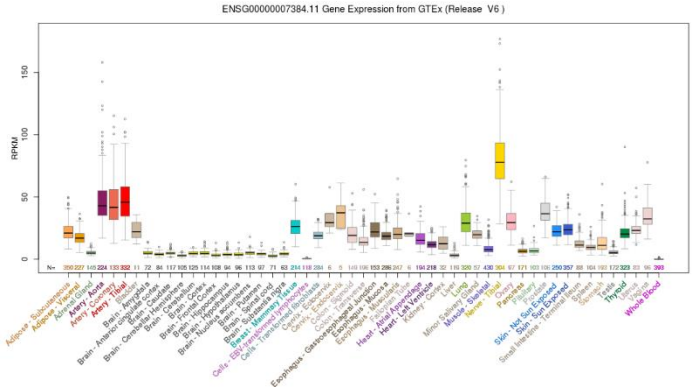
(http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml)

UCSC Genome Browser (http://genome.ucsc.edu/)





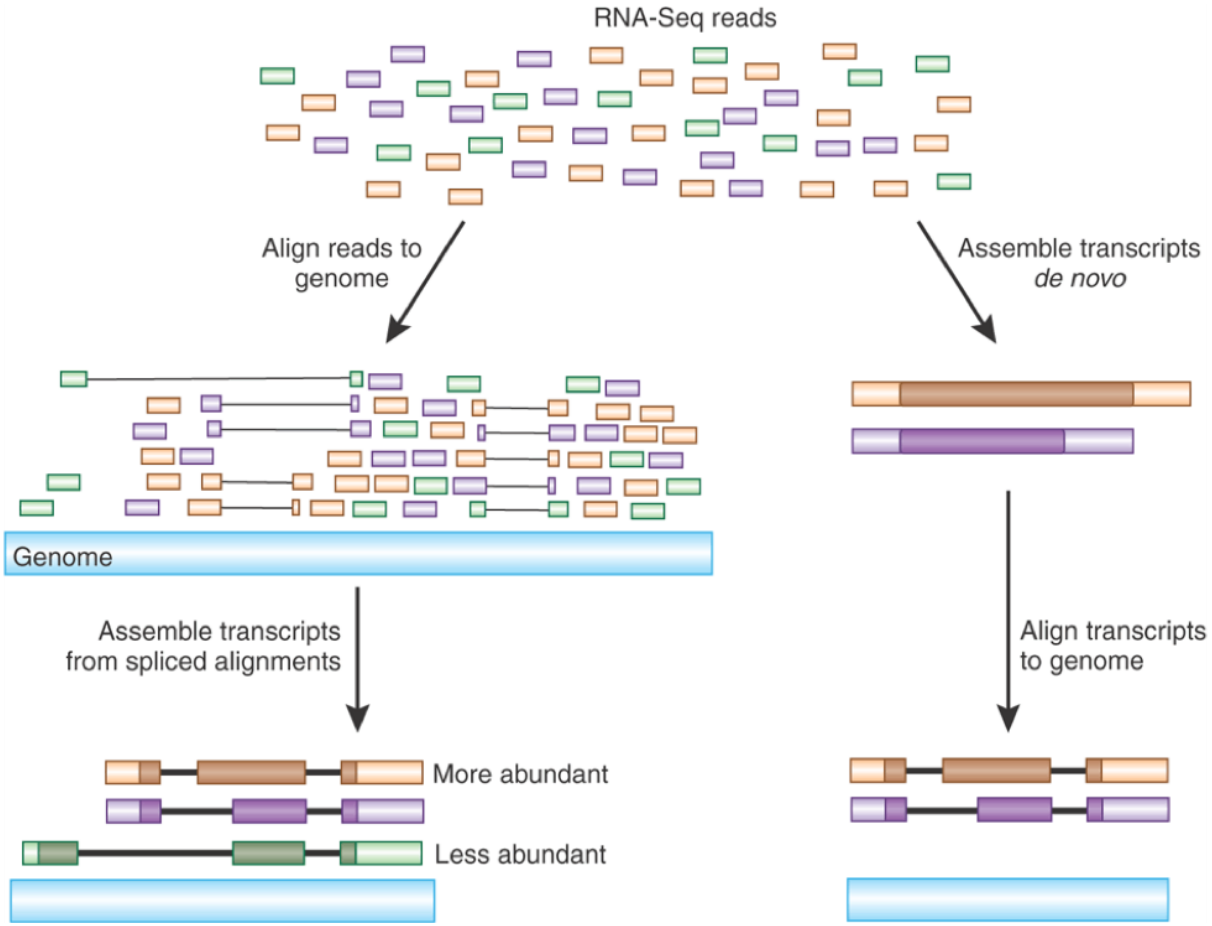




Post Genome (19 years on)

- What do all the genes do?
  - How do they interact?
  - How to cells specialise?
- Junk DNA – is not junk after all…
  - 2% Genome contains genes
  - Between 80% (ENCODE) and 25% (Graur et al, 2017) genome seems to have function, usually regulation

Expression Analysis (RNA-Seq)



Hass and Zody, Advancing RNA-Seq analysis, Nature Biotechnology 28:421-423

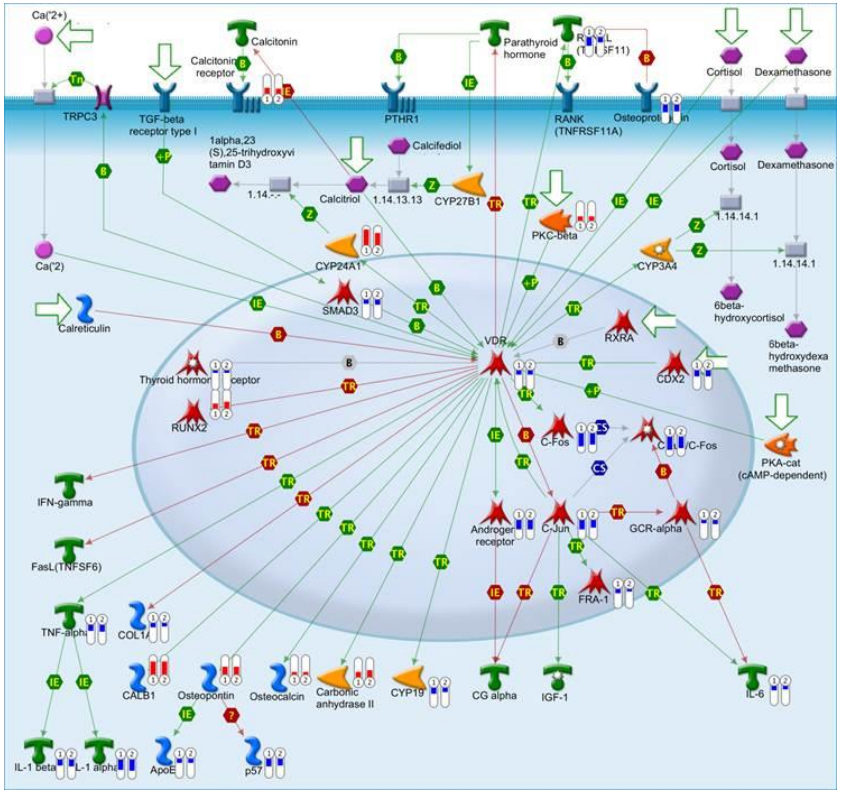
Tools for RNA-Seq

- **STAR (Spliced Transcripts Alignment to a Reference).** Fast, but uses a lot of memory.
  - Alexander Dobin, Carrie A. Davis, Felix Schlesinger, Jorg Drenkow, Chris Zaleski, Sonali Jha, Philippe Batut, Mark Chaisson, Thomas R. Gingeras, STAR: ultrafast universal RNA-seq aligner, *Bioinformatics*, Volume 29, Issue 1, January 2013, Pages 15–21

- Normalisation and quantification of read counts use:
  - **edgeR**
    - edgeR: a Bioconductor package for differential expression analysis of digital gene expression data." *Bioinformatics*, **26**(1), 139-140)or DESeq2
  - **DESeq2**
    - Love MI, Huber W, Anders S (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." *Genome Biology*, **15**, 550

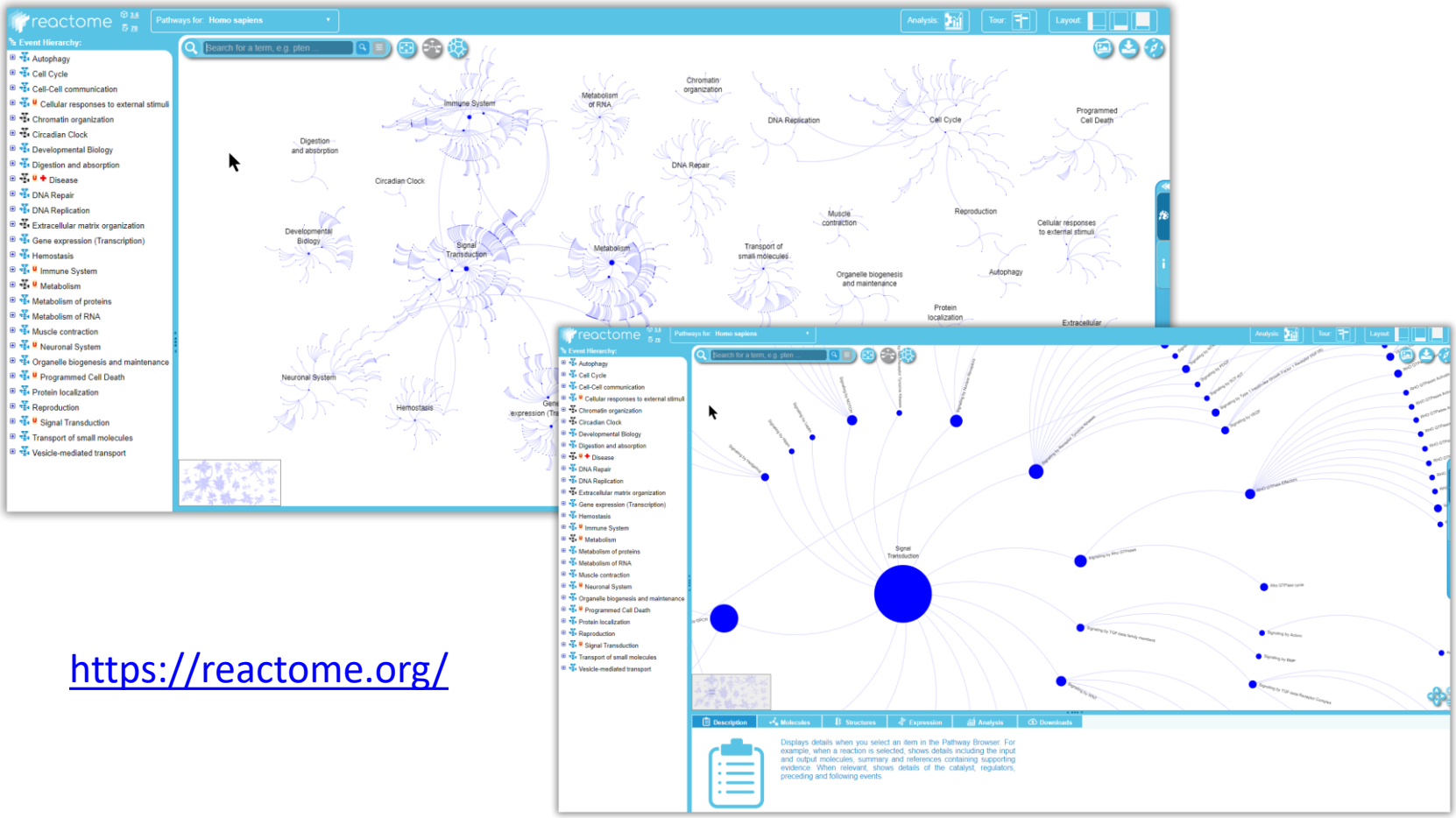
- **Salmon** Very fast and does quantification. Uses *quasi-mapping* but no alignments to visualise.
  - Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods.

Functional Annotation



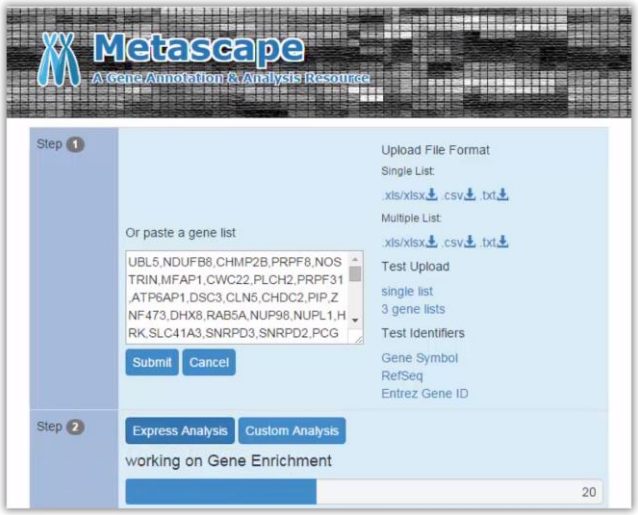
- **Metacore**
- Ingenuity

Reactome

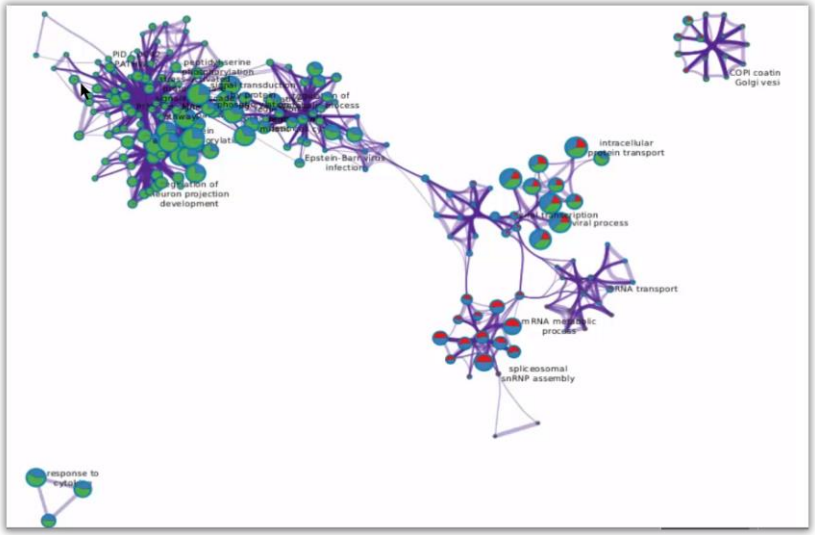


https://reactome.org/

Metascape

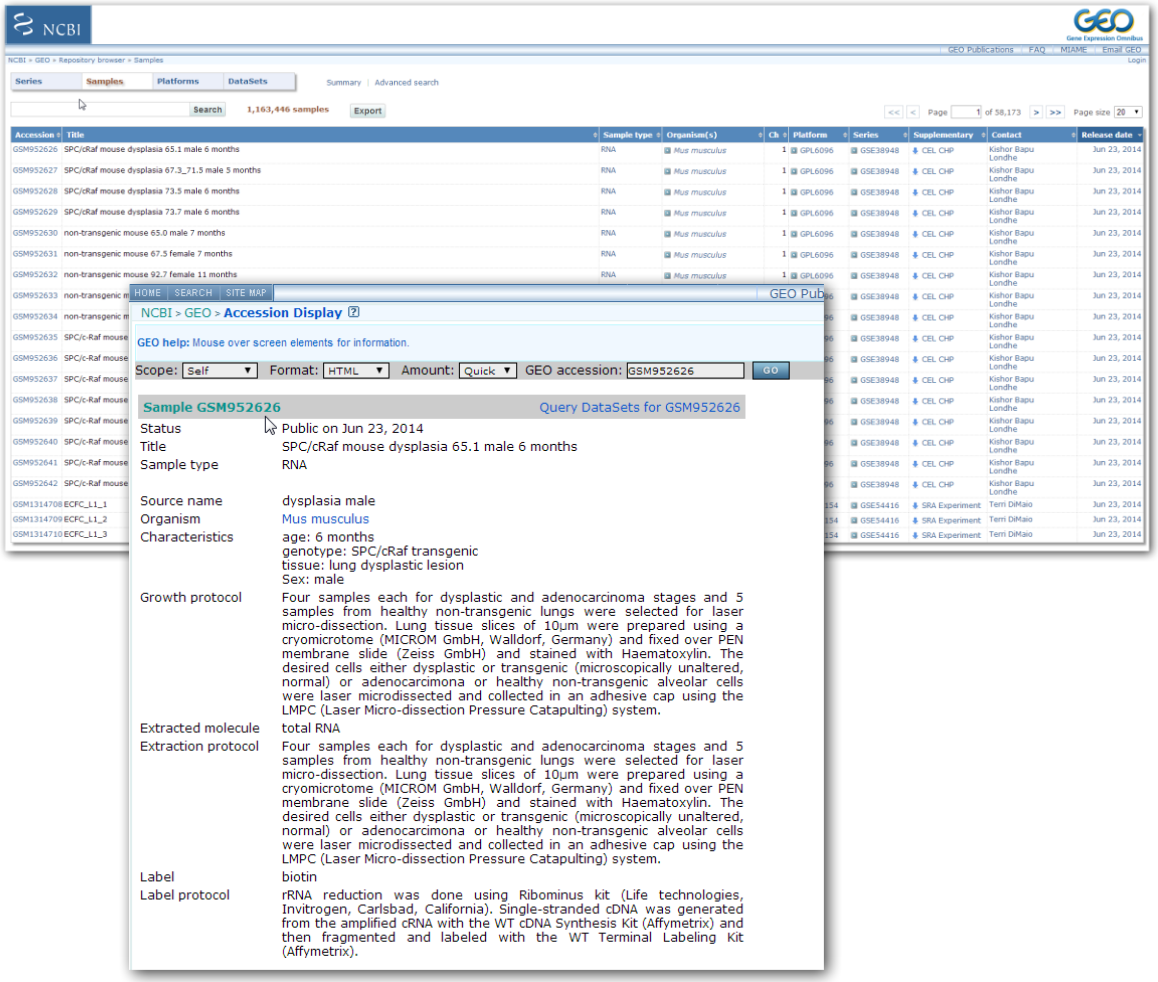


	A	B	C	D	E	F	G	H
1	GroupID	Category	Term	Description	LogP	InTerm_InList	Genes	Symbols
2	1_Summary	GO Biological Processes	GO:0016032	viral process	-18.851	49/771	156,527,2033,	ADRBK1,ATP
3	1_Member	GO Biological Processes	GO:0016032	viral process	-18.851	49/771	156,527,2033,	ADRBK1,ATP
4	1_Member	GO Biological Processes	GO:0044764	multi-organism cellular process	-18.549	49/784	156,527,2033,	ADRBK1,ATP
5	1_Member	GO Biological Processes	GO:0044419	interspecies interaction between organisms	-18.086	50/838	156,527,1536,	ADRBK1,ATP
6	1_Member	GO Biological Processes	GO:0044403	symbiosis, encompassing mutualism through parasitism	-18.086	50/838	156,527,1536,	ADRBK1,ATP
7	1_Member	GO Biological Processes	GO:0051351	positive regulation of ligase activity	-12.291	16/101	5347,5682,56	PLK1,PSMA1,
8	1_Member	GO Biological Processes	GO:0051437	positive regulation of ubiquitin-protein ligase activity invo	-12.099	14/72	5347,5682,56	PLK1,PSMA1,
9	1_Member	GO Biological Processes	GO:000020	protein polyubiquitination	-11.608	1/212	331,4734,568	XIAP,NEDD4,
10	1_Member	GO Biological Processes	GO:0051443	positive regulation of ubiquitin-protein transferase activity	-11.468	15/96	5347,5682,56	PLK1,PSMA1,
11	1_Member	GO Biological Processes	GO:2000060	positive regulation of protein ubiquitination involved in ub	-10.921	14/87	5347,5682,56	PLK1,PSMA1,
12	1_Member	KEGG Pathway	hsa03050	Proteasome	-10.903	11/44	5682,5683,56	PSMA1,PSM
13	1_Member	GO Biological Processes	GO:0051436	negative regulation of ubiquitin-protein ligase activity invo	-10.714	14/90	5347,5682,56	PLK1,PSMA1,
14	1_Member	GO Biological Processes	GO:2000058	regulation of protein ubiquitination involved in ubiquitin-	-10.322	14/96	5347,5682,56	PLK1,PSMA1,
15	1_Member	GO Biological Processes	GO:0051340	regulation of ligase activity	-10.312	16/135	5347,5682,56	PLK1,PSMA1,
16	1_Member	GO Biological Processes	GO:0051444	negative regulation of ubiquitin-protein transferase activit	-10.260	14/97	5347,5682,56	PLK1,PSMA1,
17	1_Member	GO Biological Processes	GO:0051352	negative regulation of ligase activity	-10.198	14/98	5347,5682,56	PLK1,PSMA1,
18	1_Member	GO Biological Processes	GO:0051439	regulation of ubiquitin-protein ligase activity involved in n	-10.137	14/99	5347,5682,56	PLK1,PSMA1,
19	1_Member	GO Biological Processes	GO:0032446	protein modification by small protein conjugation	-9.776	37/821	331,4734,492	XIAP,NEDD4,
20	1_Member	GO Biological Processes	GO:0031398	positive regulation of protein ubiquitination	-9.579	17/174	331,5347,568	XIAP,PLK1,P
21	1_Member	GO Biological Processes	GO:0031145	anaphase-promoting complex-dependent proteasomal ub	-9.565	14/109	5347,5682,56	PLK1,PSMA1,
22	1_Member	GO Biological Processes	GO:0002479	antigen processing and presentation of exogenous peptide	-9.412	12/75	1536,5682,56	CYBB,PSMA1
23	1_Member	GO Biological Processes	GO:0051438	regulation of ubiquitin-protein transferase activity	-9.302	15/135	5347,5682,56	PLK1,PSMA1,
24	1_Member	GO Biological Processes	GO:0042590	antigen processing and presentation of exogenous peptide	-9.140	12/79	1536,5682,56	CYBB,PSMA1
25	1_Member	GO Biological Processes	GO:1903322	positive regulation of protein modification by small protei	-9.091	17/187	331,5347,568	XIAP,PLK1,P
26	1_Member	GO Biological Processes	GO:0044265	cellular macromolecule catabolic process	-9.055	38/913	3146,3837,41	HMGB1,KPNI
27	1_Member	GO Biological Processes	GO:0006521	regulation of cellular amino acid metabolic process	-9.021	11/64	5682,5683,56	PSMA1,PSM
28	1_Member	GO Biological Processes	GO:0042787	protein ubiquitination involved in ubiquitin-dependent pr	-8.962	16/166	4734,5347,56	NEDD4,PLK1,
29	1_Member	GO Biological Processes	GO:0006977	DNA damage response, signal transduction by p53 class me	-8.872	11/66	5682,5683,56	PSMA1,PSM

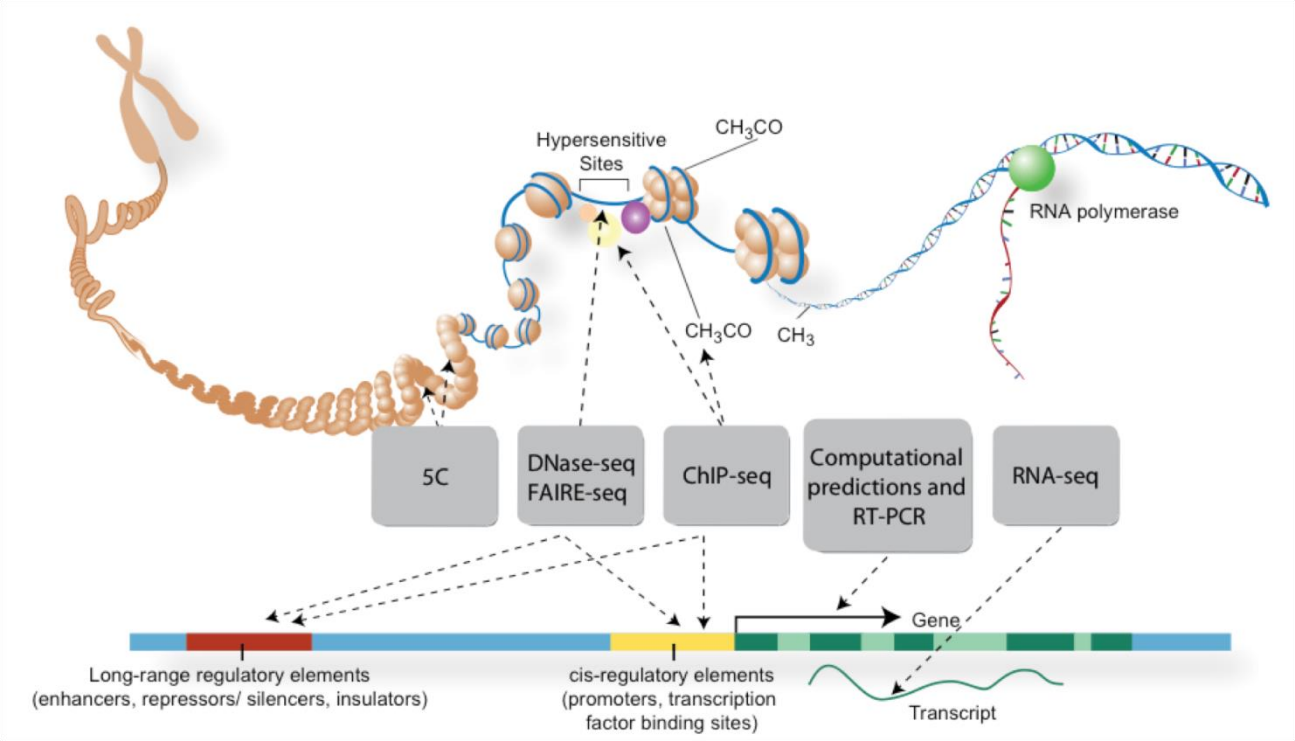


http://metascape.org/

Gene Expression Omnibus (GEO)



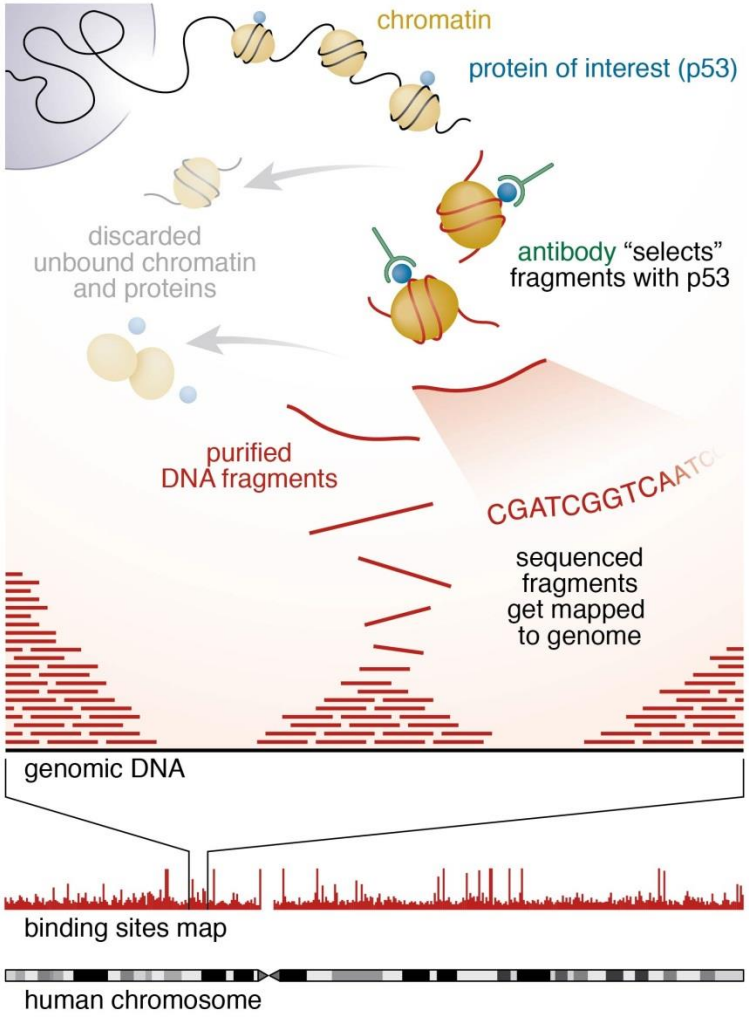
ENCODE (Encyclopedia of DNA Elements)



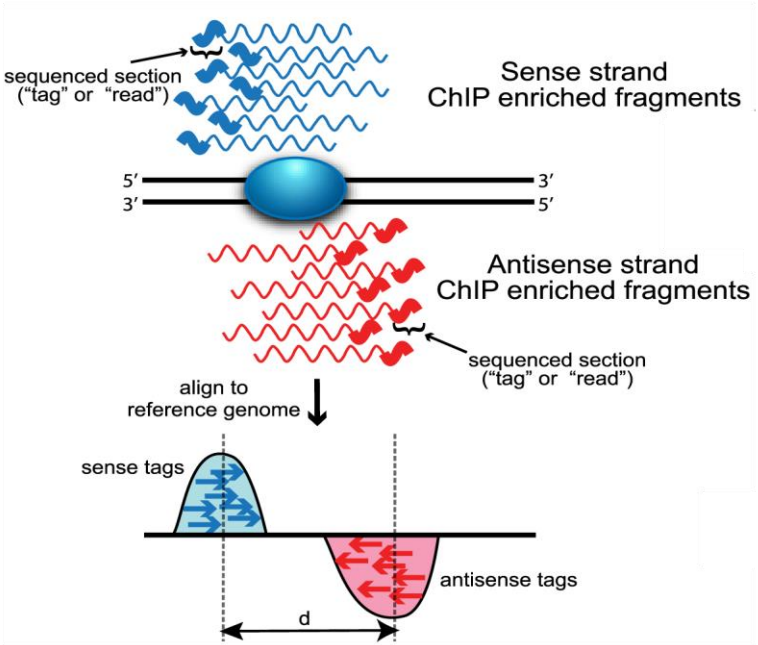
http://genome.ucsc.edu/ENCODE/

What Controls Expression?

ChIP-Seq



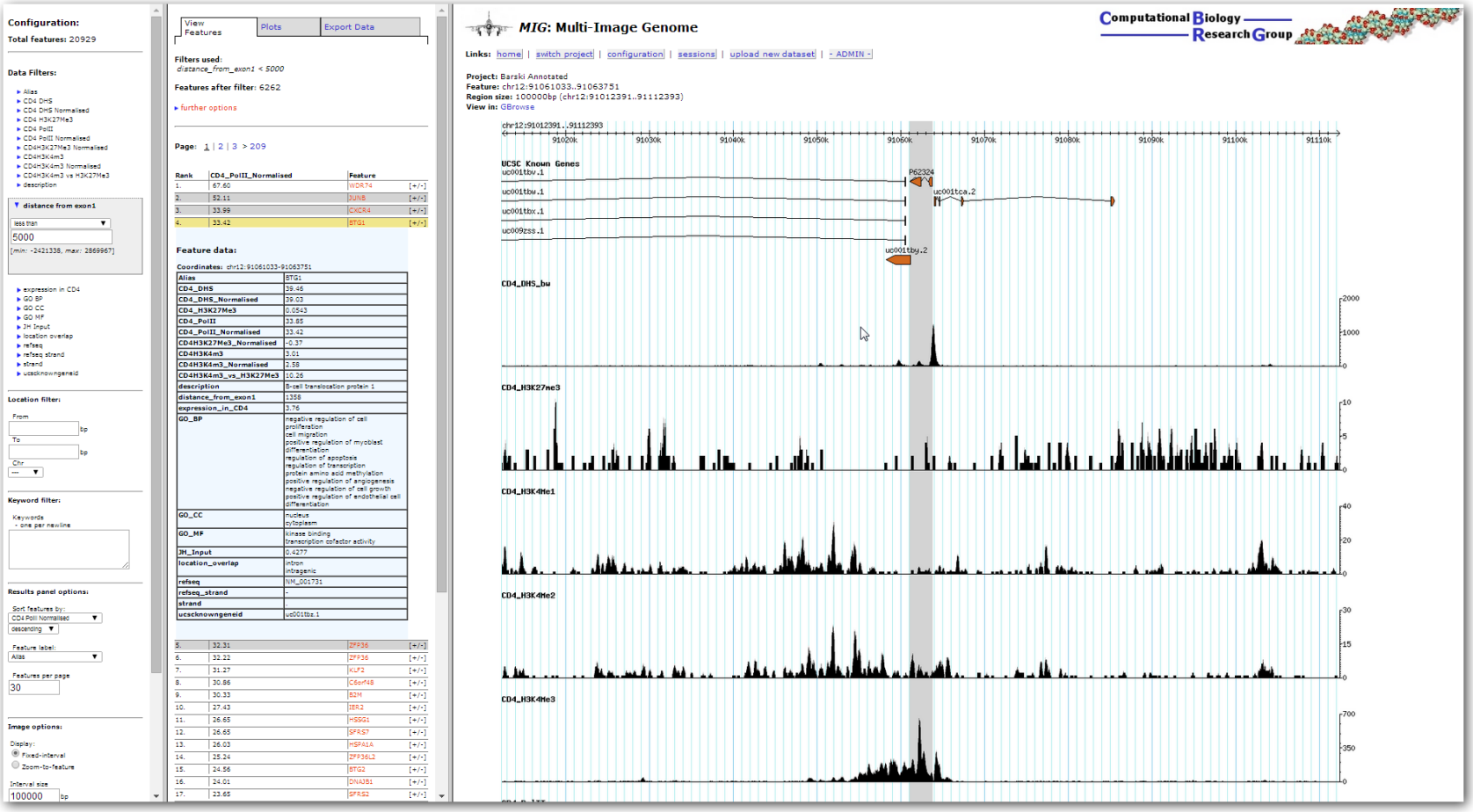
Tools for ChIP-Seq



1. Align using Bowtie
2. Peak call using Model-based Analysis of ChIP-seq (MACS)
3. Look for motif enrichment using HOMER
4. Functional annotation using GREAT

1. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10:R25.
2. Zhang, Y., Liu, T., Meyer, C.A. *et al.* Model-based Analysis of ChIP-Seq (MACS). *Genome Biol* **9,** R137 (2008) doi:10.1186/gb-2008-9-9-r137
3. Heinz S, Benner C, Spann N, Bertolino E et al. Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. Mol Cell 2010 May 28;38(4):576-589.
4. Cory Y McLean, Dave Bristor, Michael Hiller, Shoa L Clarke, Bruce T Schaar, Craig B Lowe, Aaron M Wenger, and Gill Bejerano. "GREAT improves functional interpretation of *cis*-regulatory regions". *Nat. Biotechnol.* **28**(5):495-501, 2010

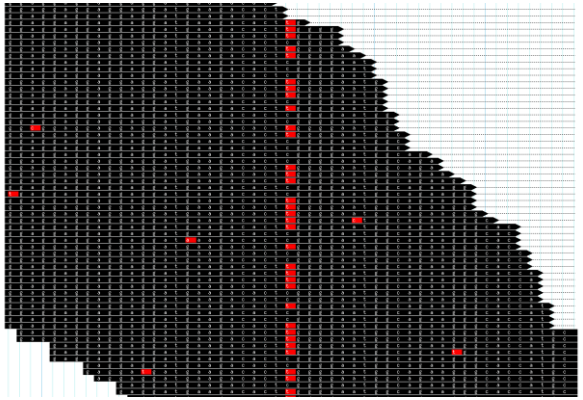
Multi-Image Genome Viewer



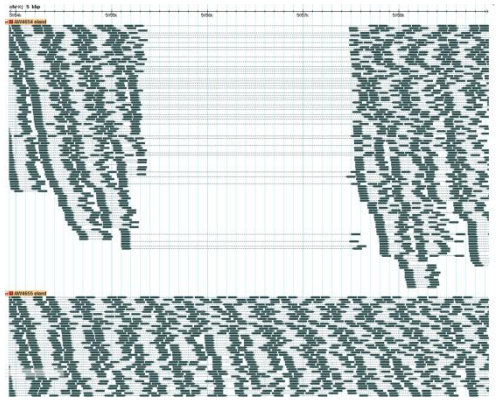
McGowan SJ, Hughes JR, Han ZP, Taylor S MIG: Multi-Image Genome viewer. *Bioinformatics* (2013) **29**: 2477-8

DNA Mutations

Single base mutation



Insertion



FAULTY GENE

The Single Nucleotide Polymorphism database (**dbSNP**) is a public-domain archive for a broad collection of simple genetic polymorphisms.

(http://www.ncbi.nlm.nih.gov/SNP/)

Tools for variant calling

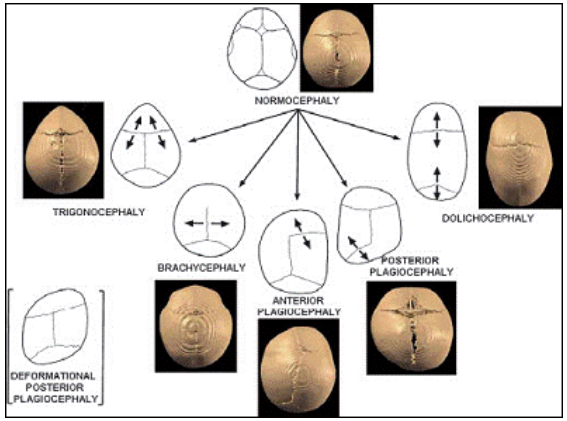
SAMTOOLS

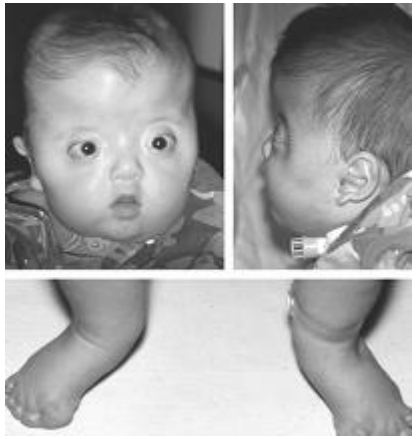
A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Li H Bioinformatics. 2011 Nov 1;27(21):2987-93.

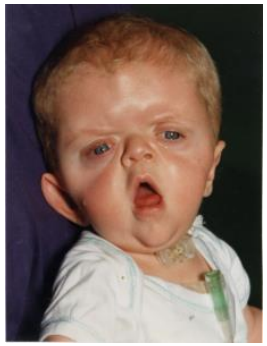
The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA, 2010 *GENOME RESEARCH 20:1297-303*

A unified haplotype-based method for accurate and comprehensive variant calling
Daniel P Cooke, David C Wedge, Gerton Lunter
bioRxiv 456103; doi: https://doi.org/10.1101/456103

Craniosynostosis

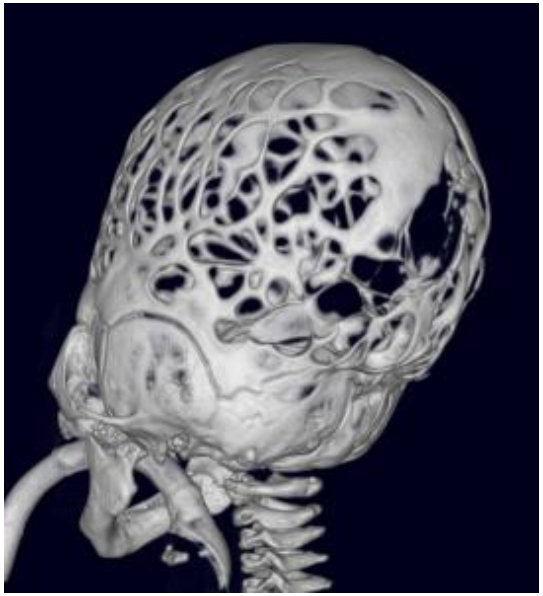


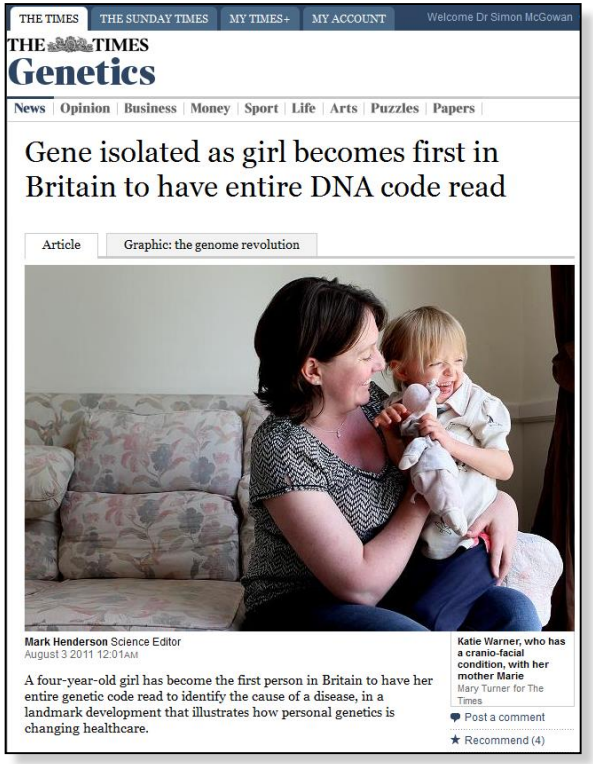




Andrew Wilkie, WIMM

Craniosynostosis





THE TIMES | THE SUNDAY TIMES | MY TIMES+ | MY ACCOUNT | Welcome Dr Simon McGowan

THE TIMES
Genetics

News | Opinion | Business | Money | Sport | Life | Arts | Puzzles | Papers

Gene isolated as girl becomes first in Britain to have entire DNA code read

Article | Graphic: the genome revolution

Mark Henderson Science Editor
August 3 2011 12:01AM

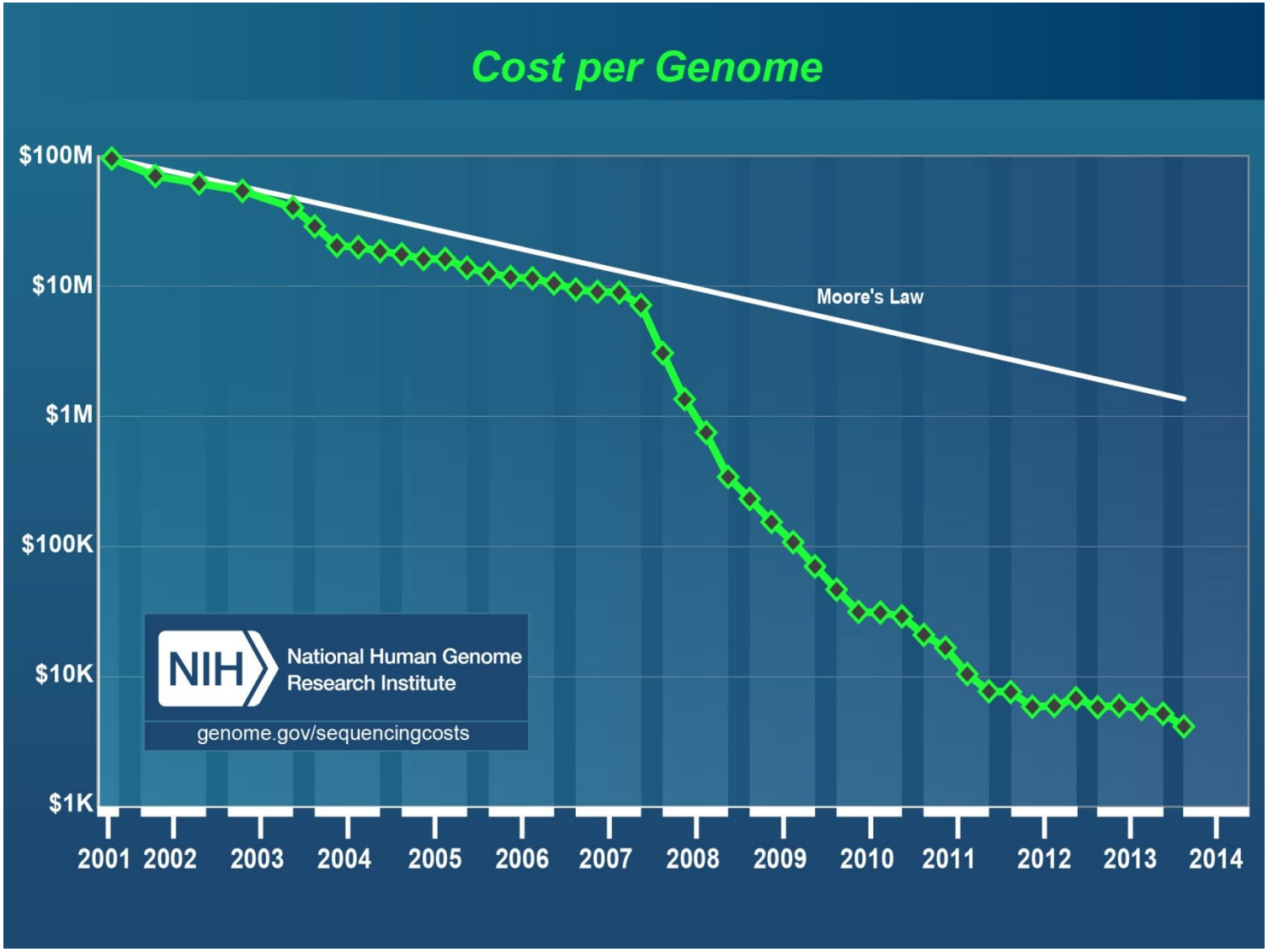
A four-year-old girl has become the first person in Britain to have her entire genetic code read to identify the cause of a disease, in a landmark development that illustrates how personal genetics is changing healthcare.

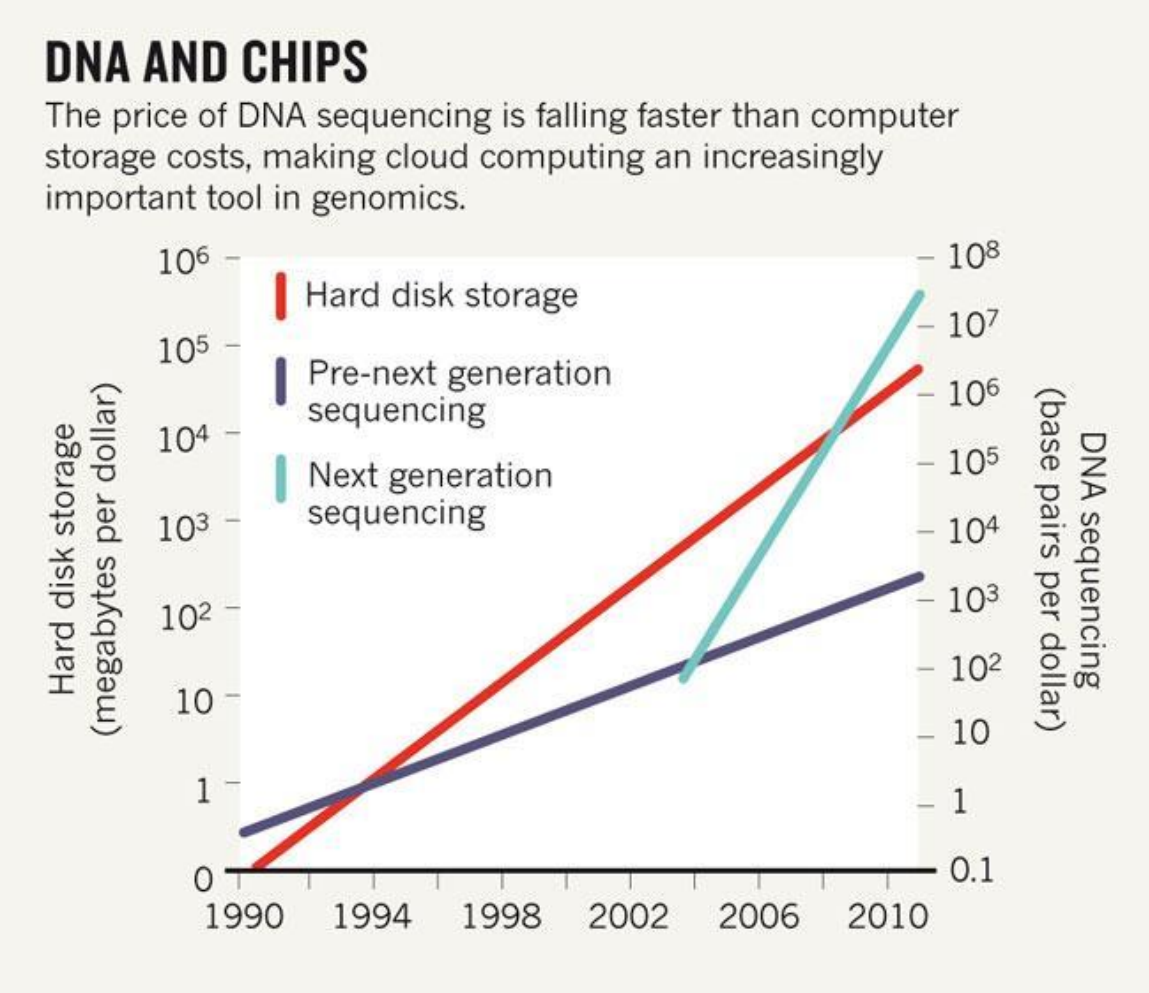
Katie Warner, who has a cranio-facial condition, with her mother Marie
Mary Turner for The Times

Post a comment

Recommend (4)

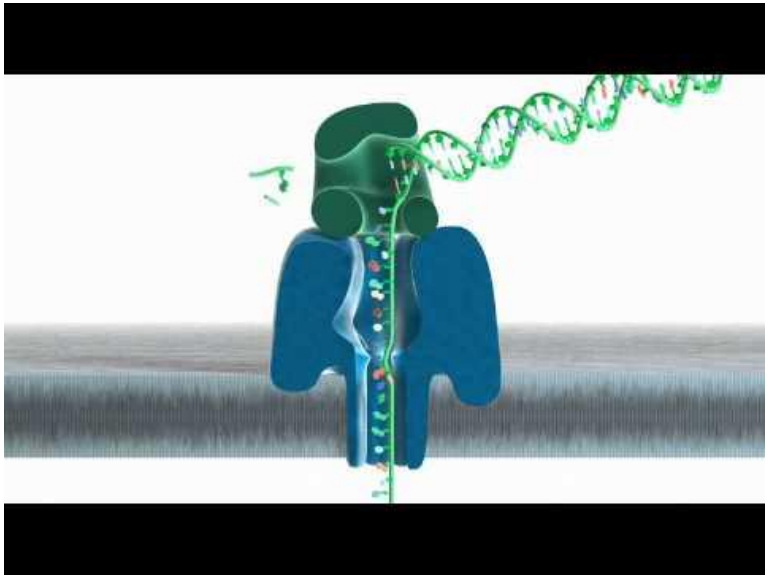
- 100,000 patients with rare inherited disease, common cancers and pathogens from the NHS in England
- Whole Genome Sequencing
- http://www.genomicsengland.co.uk/


Cost per Genome
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014



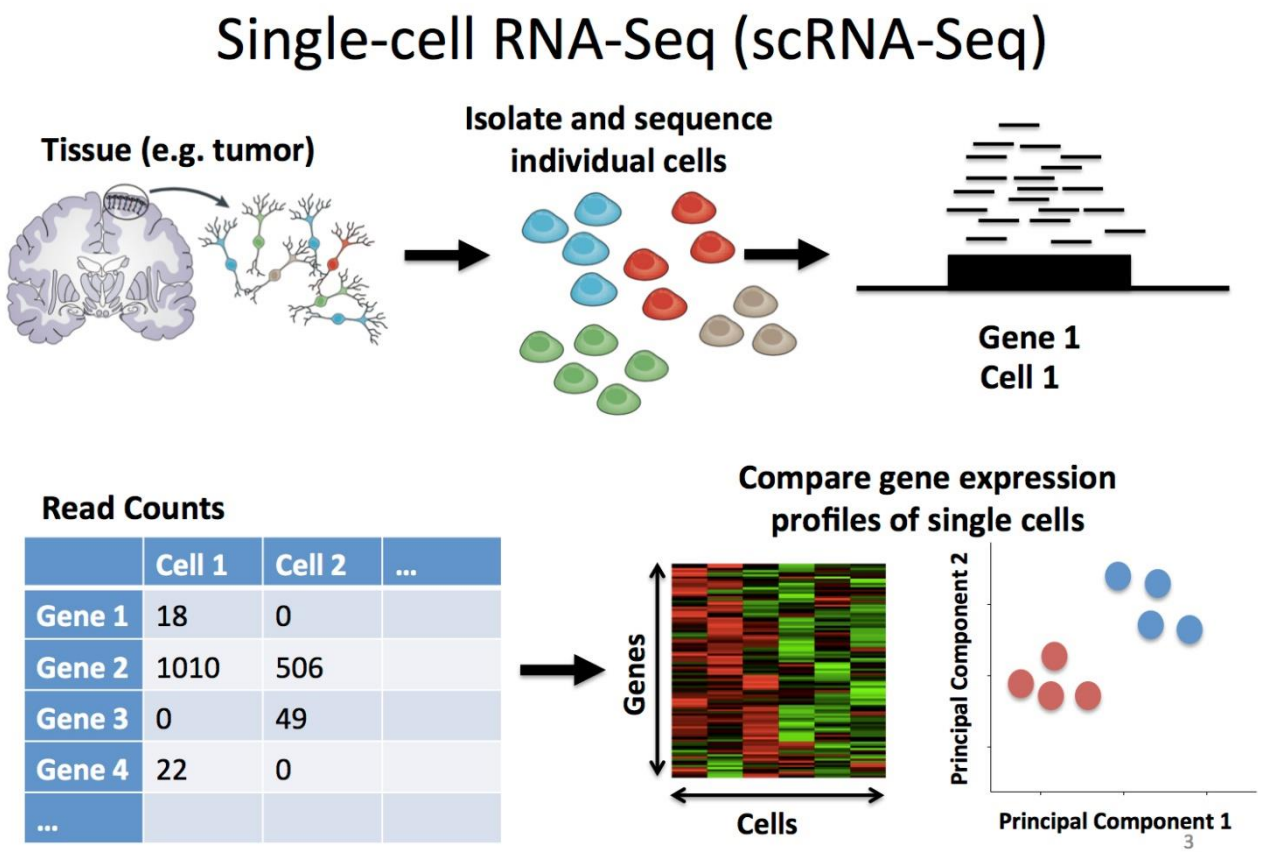
Source: L. D. Stein Genome Biol. 11, 207 (2010)

Nanopore sequencing



200KB Read Lengths, genome assembly, direct RNA sequencing…

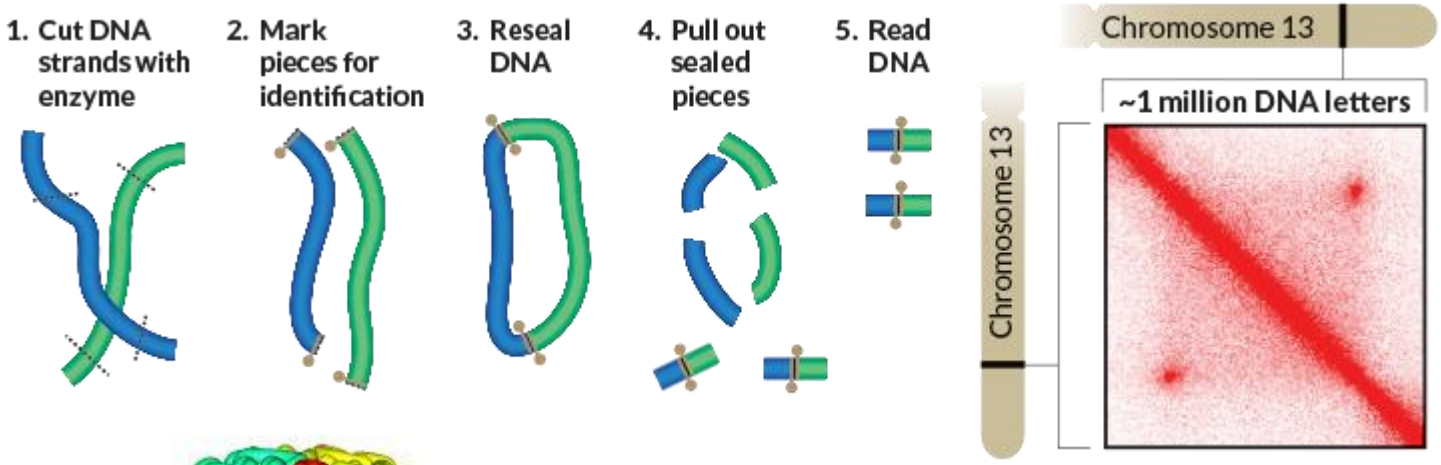
Single Cell Sequencing

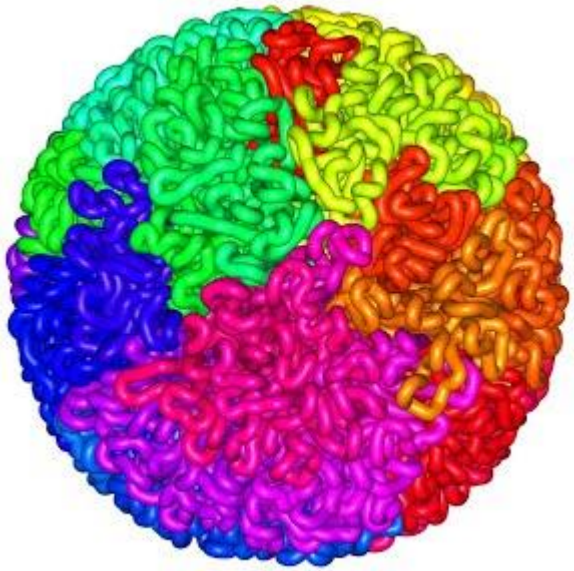


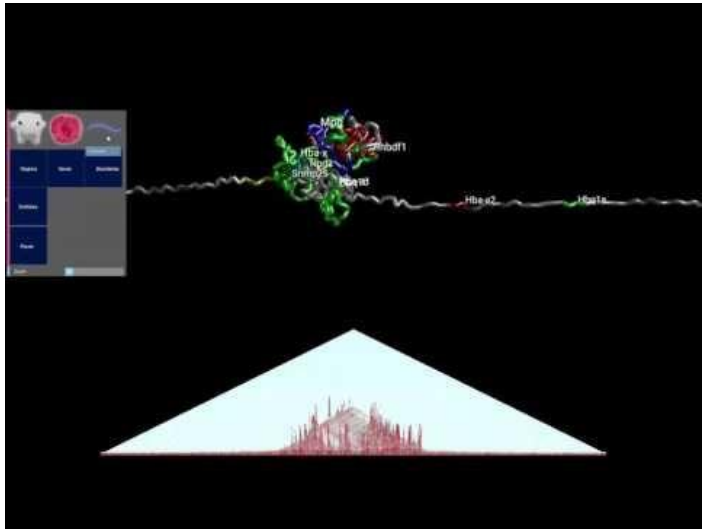
	Cell 1	Cell 2	...
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
...			

https://learn.gencore.bio.nyu.edu/single-cell-rnaseq/

Genome Modelling

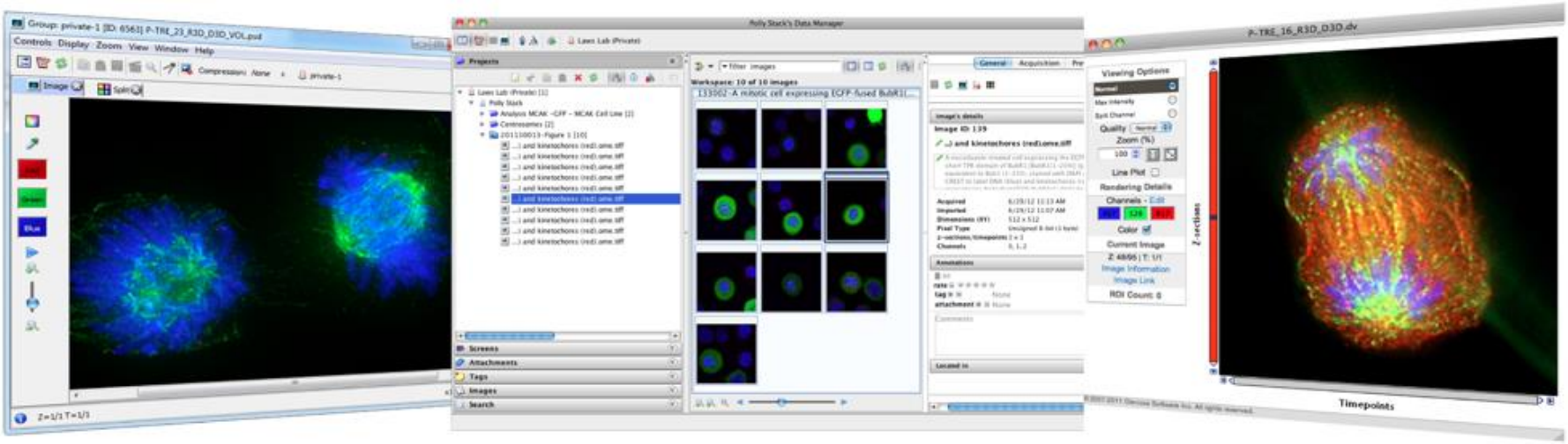




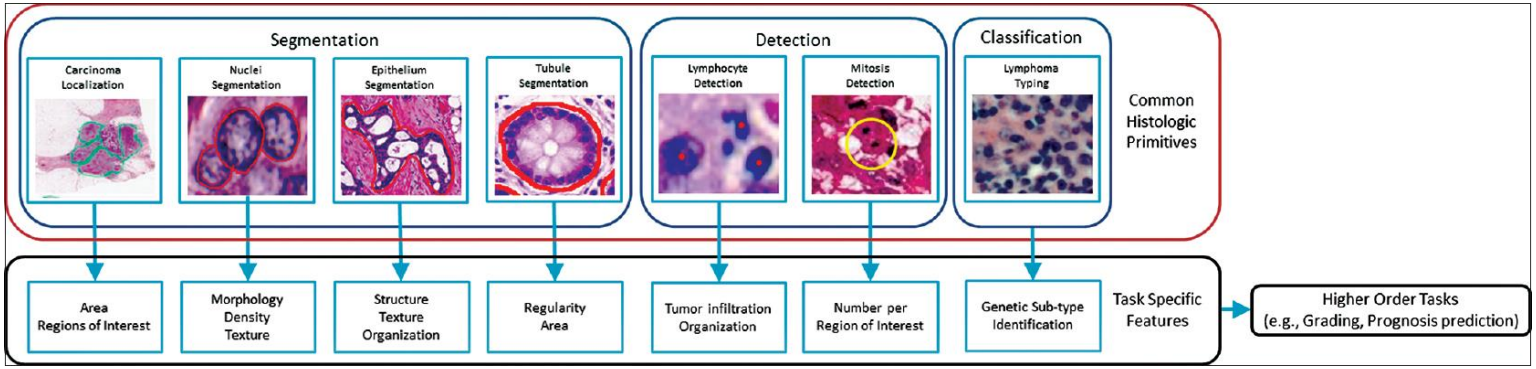


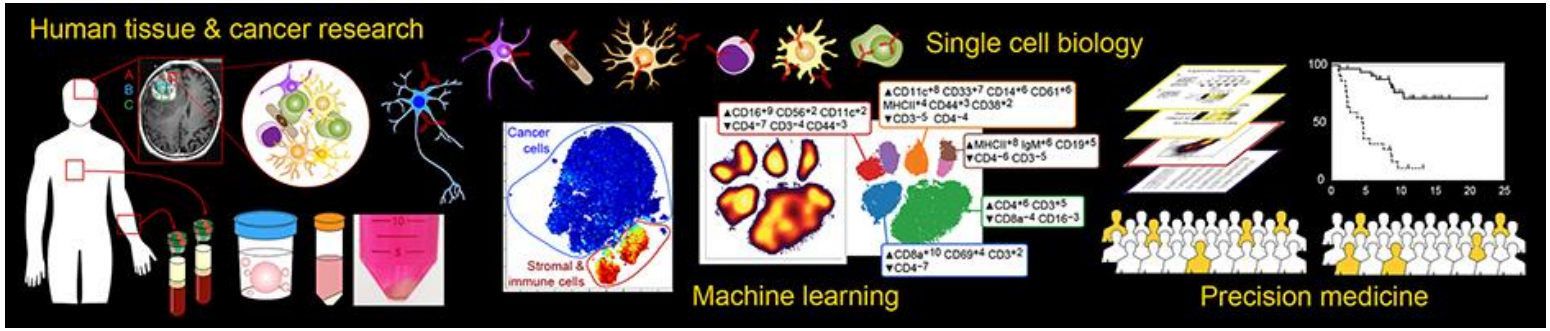
CSynth
Bio-Visualisation made interactive

OMERO Image Database



Machine Learning



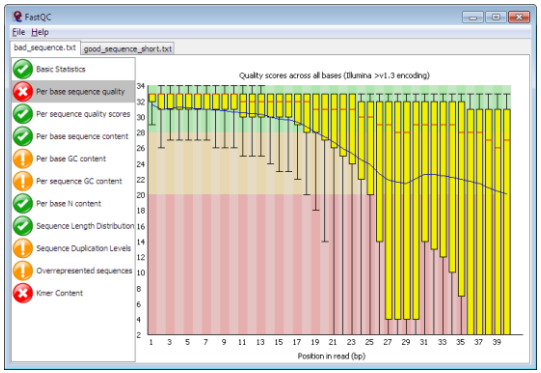


https://my.vanderbilt.edu/irishlab/

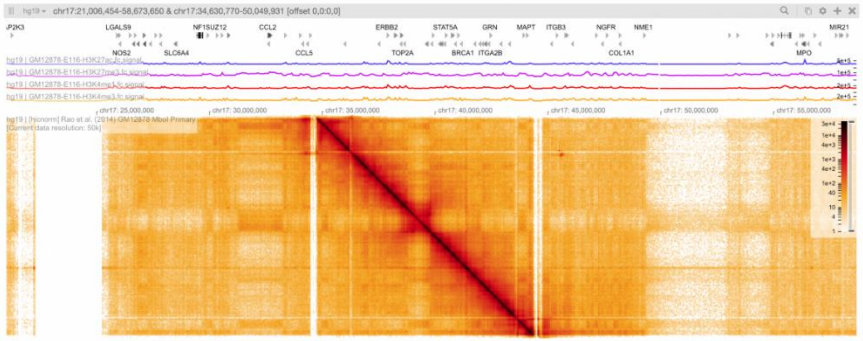
Also being applied increasing to all data types e.g. health records, DNA sequences

Visualisation

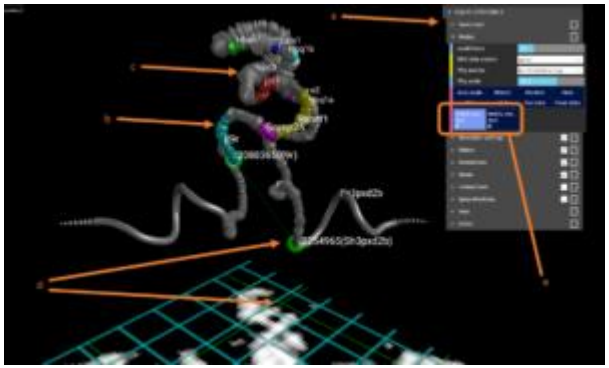
FASTQC



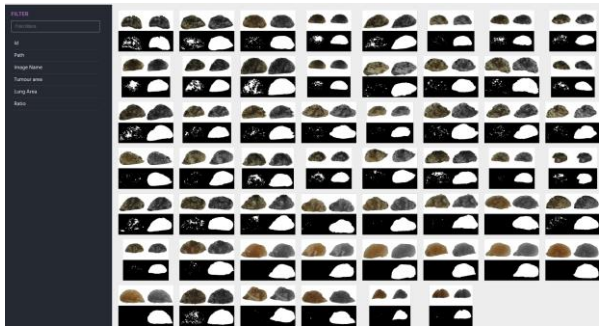
HiGlass



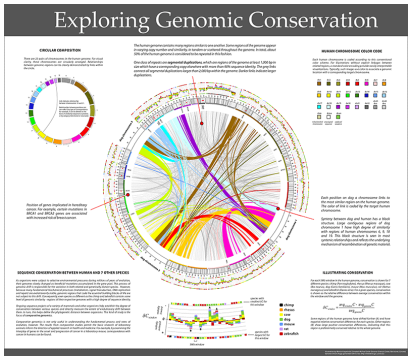
CSynth



Zegami



Circos



BabelVR

Coding in R

- Very good for statistics
- Libraries
  - CRAN (12000 packages)
  - Bioconductor (1823 packages)
- Lots of methods for communicating results
- Rstudio is nice graphical environment

Coding in Python

- Most popular language in bioinformatics (and probably data science)
- Used in industry and academic settings
- Very readable
- Great ‘glue’ for automation
- Lots of libraries for using matrices, machine learning, plotting etc
- https://biopython.org/

Python vs R

Parameter	R	Python
Objective	Data analysis and statistics	Deployment and production
Primary Users	Scholar and R&D	Programmers and developers
Flexibility	Easy to use available library	Easy to construct new models from scratch. I.e., matrix computation and optimization
Learning curve	Difficult at the beginning	Linear and smooth
Popularity of Programming Language. Percentage change	4.23% in 2018	21.69% in 2018
Average Salary	$99.000	$100.000
Integration	Run locally	Well-integrated with app
Task	Easy to get primary results	Good to deploy algorithm
Database size	Handle huge size	Handle huge size
IDE	Rstudio	Spyder, Ipthon Notebook
Important Packages and library	tydiverse, ggplot2, caret, zoo	pandas, scipy, scikit-learn, TensorFlow, caret
Disadvantages	Slow High Learning curve Dependencies between library	Not as many libraries as R
Advantages	• Graphs are made to talk. R makes it beautiful • Large catalog for data analysis • GitHub interface • RMarkdown • Shiny	• Jupyter notebook: Notebooks help to share data with colleagues • Mathematical computation • Deployment • Code Readability • Speed • Function in Python

Learn both!

See review https://www.guru99.com/r-vs-python.html

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