Introduction to NGS Technologies

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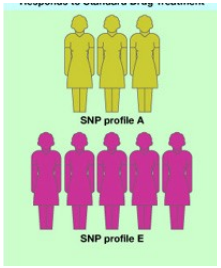
Genetic Research, pre-genomic scenario

Genes in the DNA…

…code for proteins…



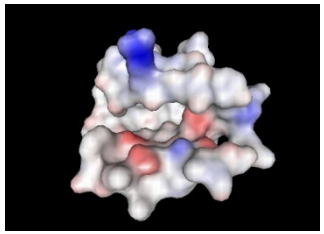
…produces the final phenotype

>protein kinase

acctgttgatggcgacagggactgta
tgctgatctatgctgatgcatgcatgc
tgactactgatgtgggggctattgac
ttgatgtctatc….

From genotype to phenotype.

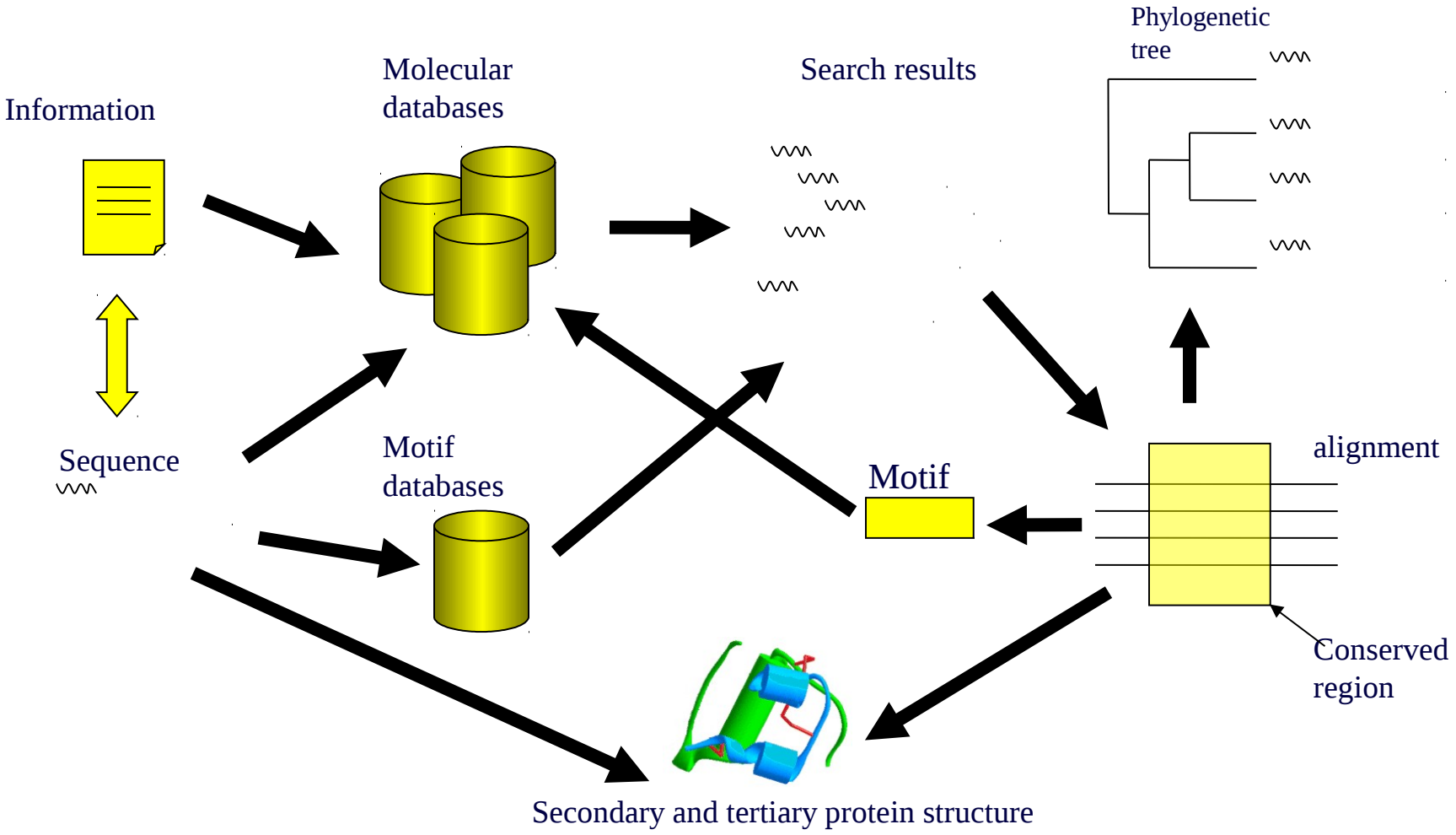
…whose structure accounts for function…

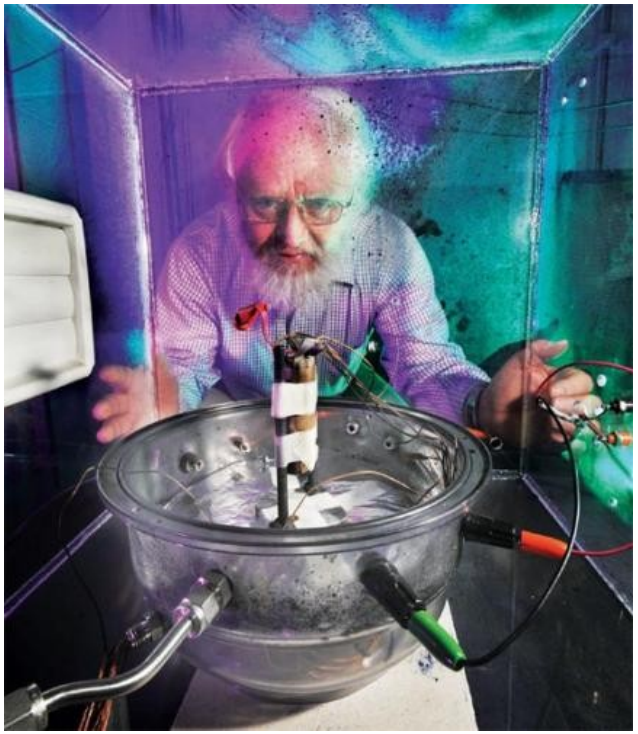


…plus the environment…

Data is information

Bioinformatics tools for pre-genomic sequence data analysis



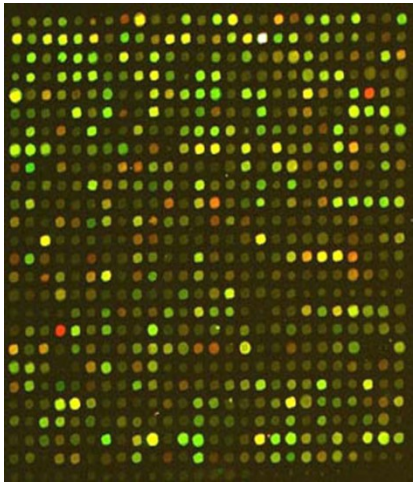


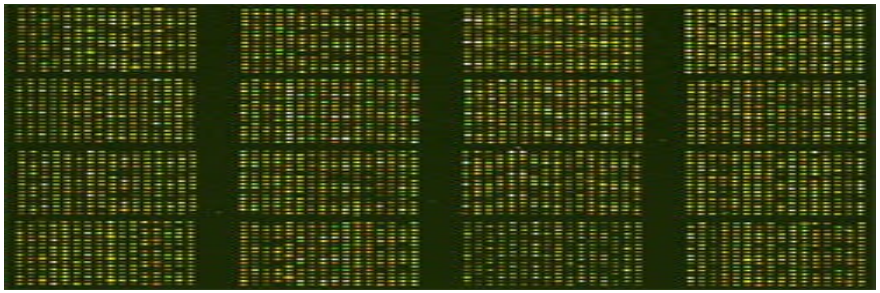
The aim:

Extracting as much information as possible for one single data

High Throughput Technologies

- 1988 arrayed DNAs were used
- 1991 oligonucleotides are synthesized on a glass slide through photolithography (Affymax Research Institute)
- 1995 DNA Microarrays
- 1997 Genome wide Yeast Microarray





Nature Milestones DNA Technologies

Next Generation Sequencing
SOLID **12Gbp** per round

Genes in the DNA…

>protein kunase

acctgttgatggcgacagggactgtatgctg
atctatgctgatgcatgcatgctgactactga
tgtgggggctattgacttgatgtctatc….

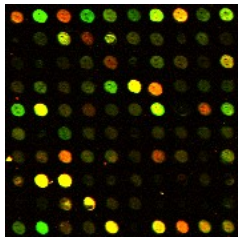
…which can be different because of the variability.

10 million SNPs

…whose final effect configures the phenotype…

…when expressed in the proper moment and place…

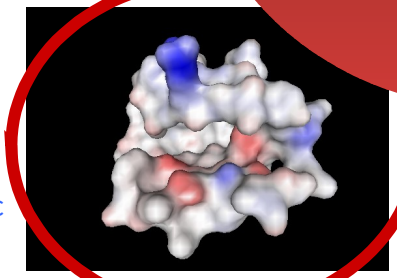
A typical tissue is expressing among **5,000 and 10,000 genes**



Data ≠ Information

…conforming complex interaction networks…

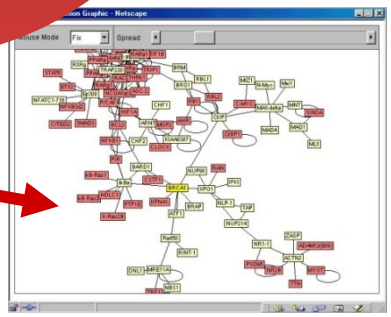
…code for proteins…



That undergo post-translational modifications, somatic recombination…

100K-500K proteins

…whose structures account for function…



Each protein has an average of **8** interactions

…in cooperation with other proteins…

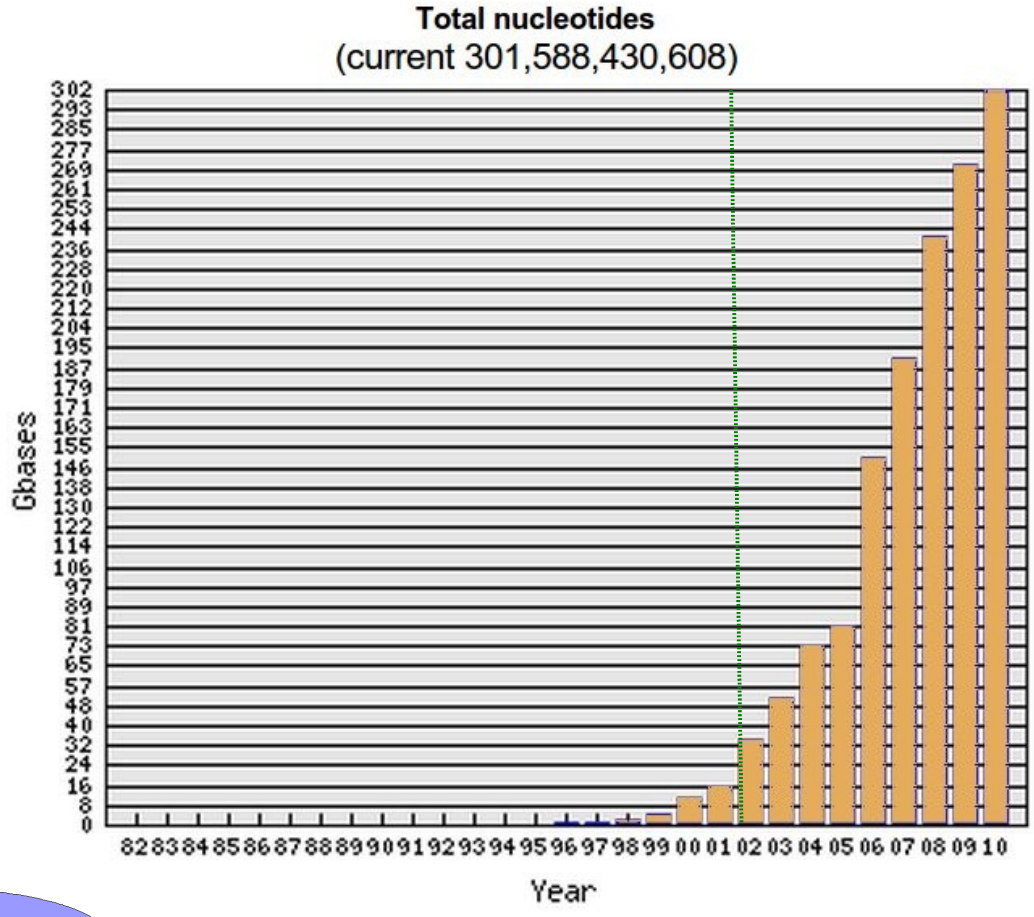
Date	Cost per Mb	Cost per Genome
Sep-01	$5,292.39	$95,263,072
Mar-02	$3,898.64	$70,175,437
Sep-02	$3,413.80	$61,448,422
Mar-03	$2,986.20	$53,751,684
Oct-03	$2,230.98	$40,157,554
Jan-04	$1,598.91	$28,780,376
Apr-04	$1,135.70	$20,442,576
Jul-04	$1,107.46	$19,934,346
Oct-04	$1,028.85	$18,519,312
Jan-05	$974.16	$17,534,970
Apr-05	$897.76	$16,159,699
Jul-05	$898.90	$16,180,224
Oct-05	$766.73	$13,801,124
Jan-06	$699.20	$12,585,659
Apr-06	$651.81	$11,732,535
Jul-06	$636.41	$11,455,315
Oct-06	$581.92	$10,474,556
Jan-07	$522.71	$9,408,739
Apr-07	$502.61	$9,047,003

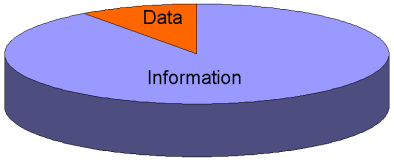
Date	Cost per Mb	Cost per Genome
Jul-07	$495.96	$8,927,342
Oct-07	$397.09	$7,147,571
Jan-08	$102.13	$3,063,820
Apr-08	$15.03	$1,352,982
Jul-08	$8.36	$752,080
Oct-08	$3.81	$342,502
Jan-09	$2.59	$232,735
Apr-09	$1.72	$154,714
Jul-09	$1.20	$108,065
Oct-09	$0.78	$70,333
Jan-10	$0.52	$46,774
Apr-10	$0.35	$31,512
Jul-10	$0.35	$31,125
Oct-10	$0.32	$29,092
Jan-11	$0.23	$20,963
Apr-11	$0.19	$16,712
Jul-11	$0.12	$10,497
Oct-11	$0.09	$7,743
Jan-12	$0.09	$7,666

National Human Genome Research Institute

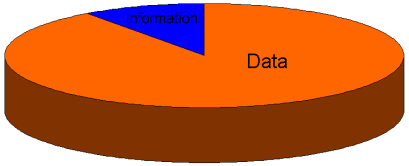
genome.gov/sequencingcosts

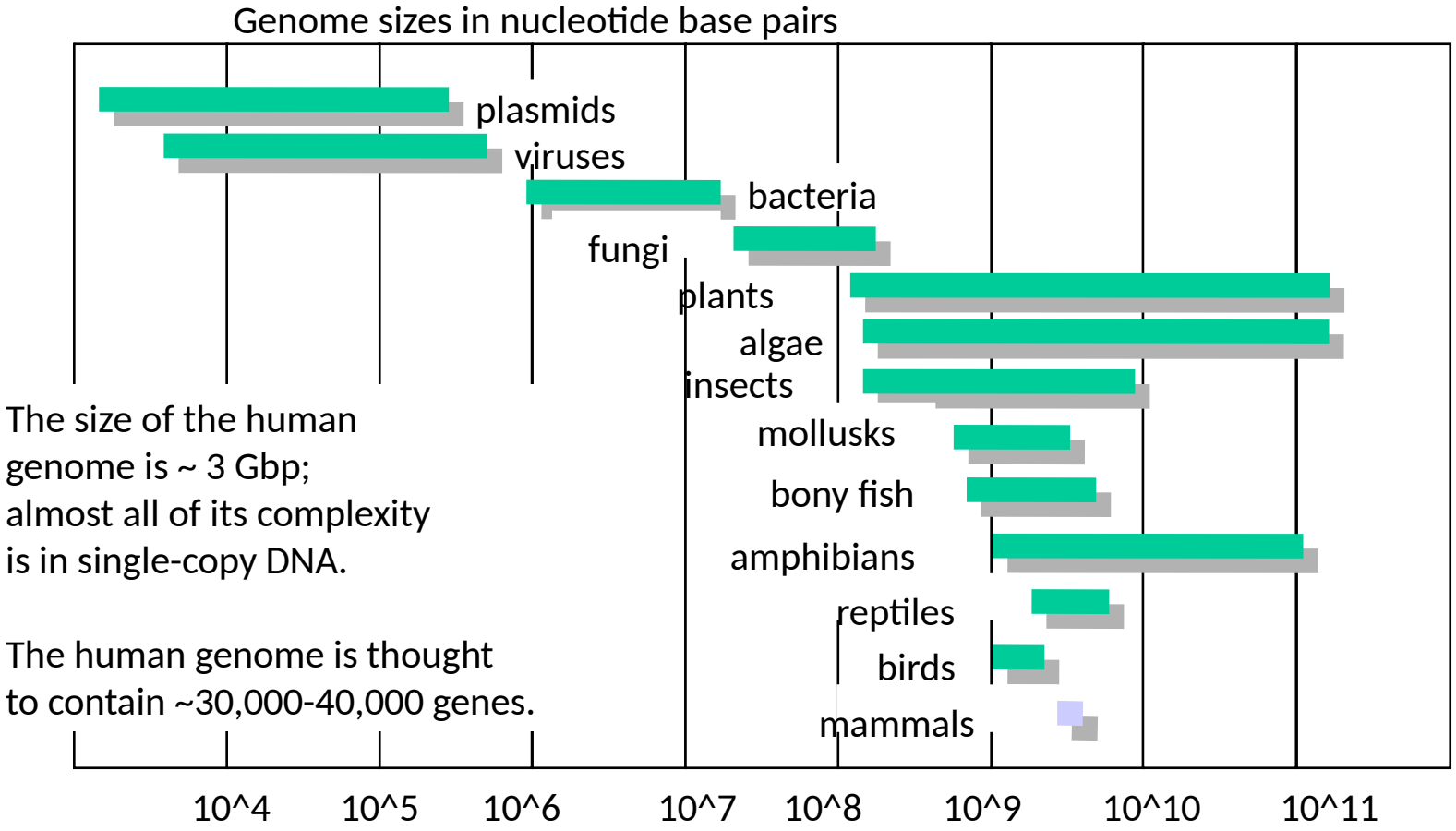
Pre & Post-genomic databases





EMBL database growth (March 2011)



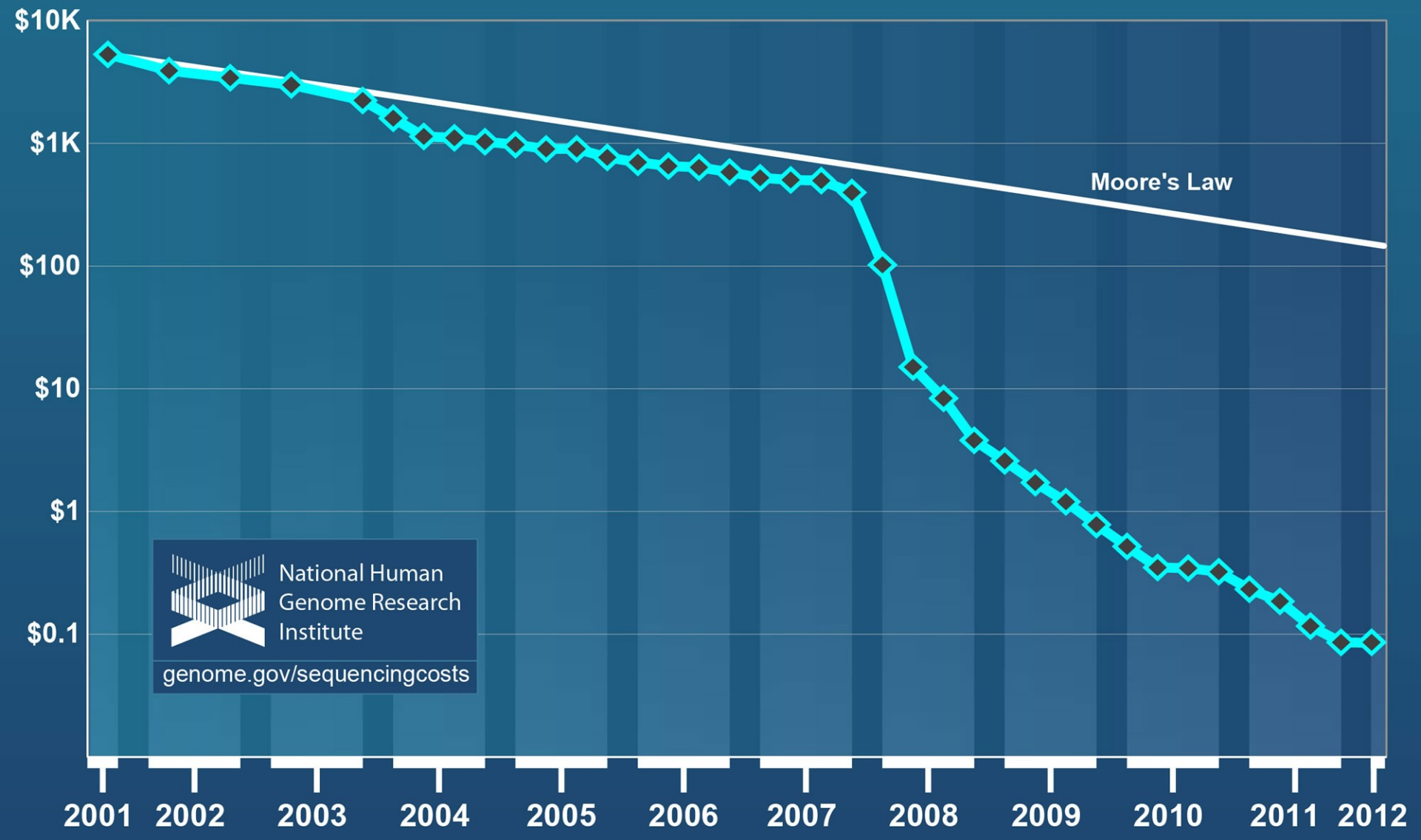


http://www3.kumc.edu/jcalvet/PowerPoint/bioc801b.ppt

Computing capabilities (*CPU power doubles in ~18-24 moths, hard drive capacity doubles in ~12 moths, network bandwidth doubles in ~20 moths*) should increase : **7-10x** in 5 years. Follows **Moors's law**

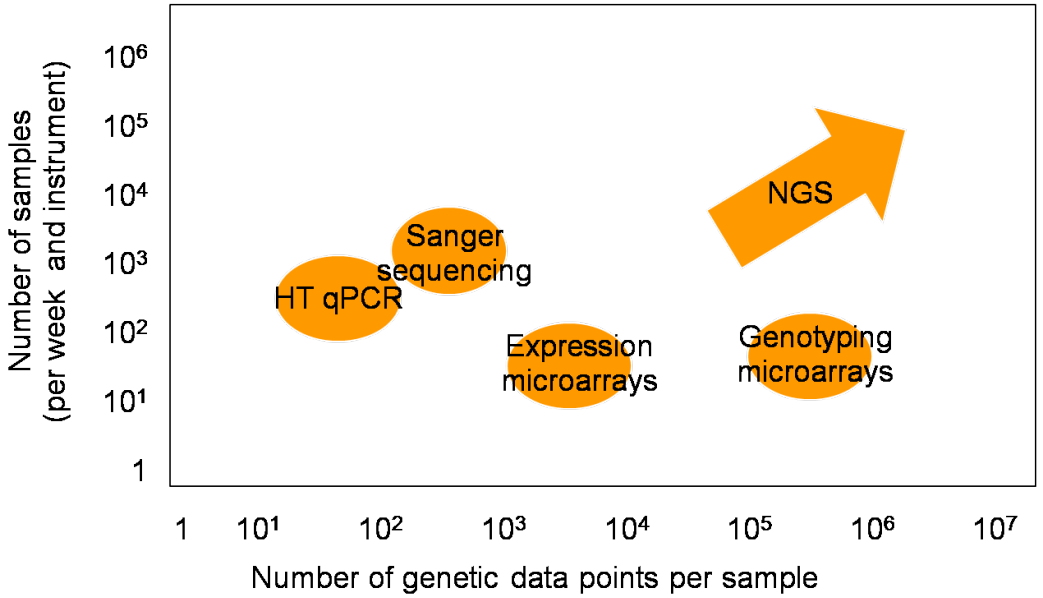
Data projection in 3-5 years: **100x** increase in sequencing volume. Still new technologies with higher throughput to come very soon !!!

Cost per Raw Megabase of DNA Sequence



Relative throughput of the different HT technologies

NGS emerges with a potential of data production that will, eventually wipe out conventional HT technologies in the years coming



Too many sequences to be handled in a standard computer

	Sanger (1st-gen) Sequencing	Next-Gen Sequencing, and 3rd generation
Whole Genome	Human (early drafts), model organisms, bacteria, viruses and mitochondria (chloroplast), low coverage	New human (!), individual genome, exomes, 2,500 normal (1K genome project), 25,000 cancer (TCGA and ICGC initiatives), CNV, matched control pairs, time course, rare-samples
RNA	cDNA clones, ESTs, Full Length Insert cDNAs, other RNAs	RNA-Seq: Digitization of transcriptome, alternative splicing events, miRNA, allele specific transcripts
Communities	Environmental sampling, 16S RNA populations, ocean sampling,	Human microbiome, deep environmental sequencing, Bar-Seq
Other		Epigenome, rearrangements, ChIP-Seq

NGS technologies

Cost-effective
Fast
Ultra throughput
Cloning-free
Short reads

Differences between the various platforms:

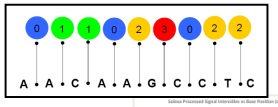
- Nanotechnology used.
- Resolution of the image analysis.
- Chemistry and enzymology.
- Signal to noise detection in the software
- Software/images/file size/pipeline
- Cost
- Applications

Similarities- LOTS of DATA
General ways of dealing at the sequences

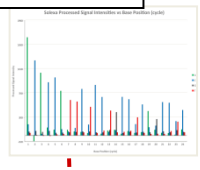
- Assemble them and look at what you have
- You map them (align against a known genome) and then look at what you have.
- Or a mixture of both!
- Sometimes you select the DNA you are sequencing
- or you try to sequence everything
- Depends on biological question, sequencing machine you have, and how much time and money you have.
- **NGS is relatively cheap but think what you want to answer, because the analysis won't do magic**

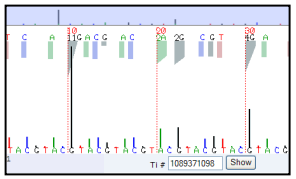
Next-gen sequencers

From John McPherson, OICR



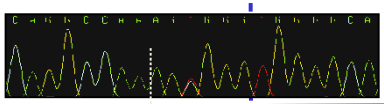
Illumina/HiSeq short-read sequencers 10-600Gb,
150/150 bp, 11 days
AB/SOLiD5500, 200+Gb,75/35 bp reads, 10 days





Roche 454 GS FLX+ 2.8
(0.2-1Gb in 800-1000 bp reads,
1M reads, 20 hours)

ABI capillary sequencer
(0.04-0.08 Mb in 450-800 bp reads,
96 reads, 1-3 hours)



100 Gb
bases per
machine run
10 Gb
1 Gb
100 Mb
10 Mb
1 Mb

10 bp
100 bp
1,000 bp
read length

Next Generation Sequencers

3 main platforms:

- **Solexa/illumina**
- **Roche 454**
- **ABI SOLiD**

- Follow an approach similar to Sanger sequencing, but do away with separation of fragments by size and "read" the sequence as the reaction occurs
- Several different "next generation" sequencing platforms developed and commercialized, more on the way.
- Simultaneously sequence entire libraries of DNA sequence fragments

454 (Roche)

- First next generation method to be commercially available

- Uses a “sequencing by synthesis” (SBS) approach:
  - DNA is broken into pieces of 500-1,400 bp, ligated to adaptors, and amplified on tiny beads by PCR (emulsion PCR)
  - Beads (with DNA attached) are placed into tiny wells (one bead per well) on a PicoTiter Plate that has millions of wells. Each well is connected to an optical fibre.
  - DNA is sequenced by adding polymerase and DNA bases containing pyrophosphate. The different bases (A,C,G,T) are added sequentially in a flow chamber
  - When a base complementary to the template is added, the pyrophosphate is released and a burst of light is produced
  - The light is detected and used to call the base

- Initially 100-150 bp, but they have been improved to 600-1000 bp

- >1 million, filter-passed reads per run (20 hours)

- 1 billion bases per day

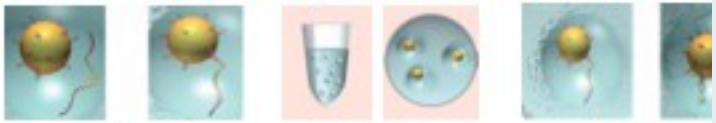
Roche 454 pyrosequencing

Principel

Preparation of the DNA includes : DNA fragmentation (nebulization), DNA size selection, Fragment end polishing, Adaptor ligation, Library immobilization, fill in reaction and ssDNA library isolation. At the end of these steps, the DNA fragments are ready for the emulsion PCR (emPCR).

fragmentation | size selection | Polishing | Adaptor | Immobilization | Fill in reaction | ssDNA library

emPCR include the immobilisation of the DNA fragments on ca
indirect enrichment resulting in an immobilized and amplified lib



Immobilisation on capture beads | emulsification | amplification

Sequencing includes a prewash, the loading DNA library bead
end of these steps you get your data.

DNA library loading | PPiase + enzyme + packing beads loading | assembly of the P

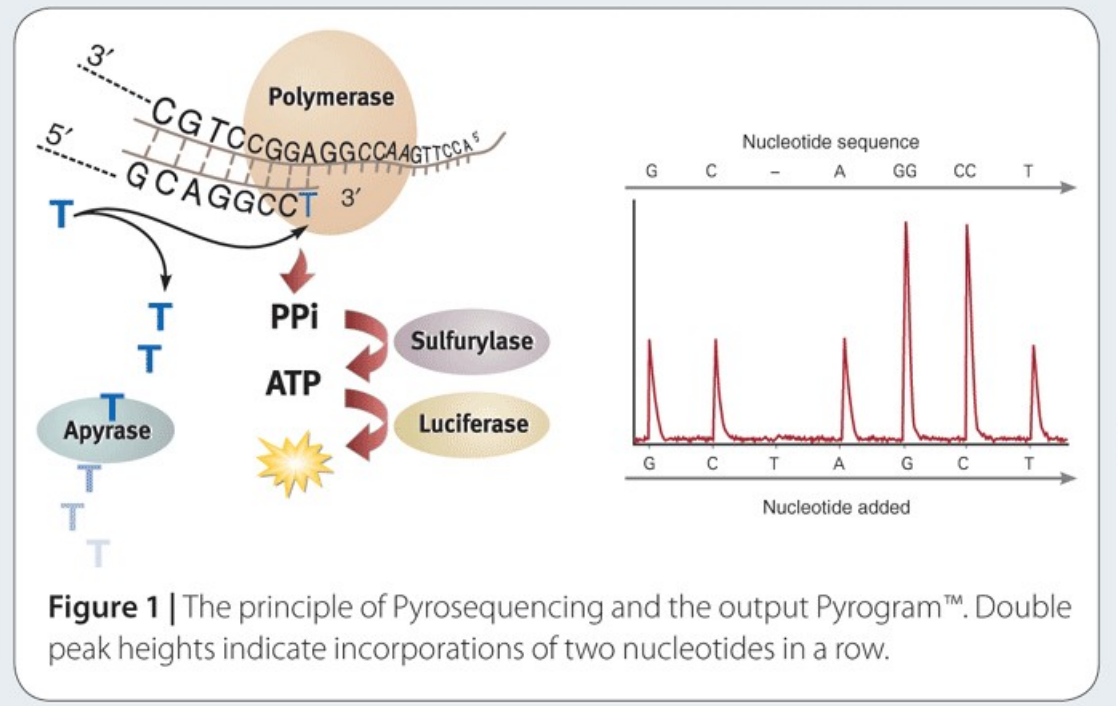
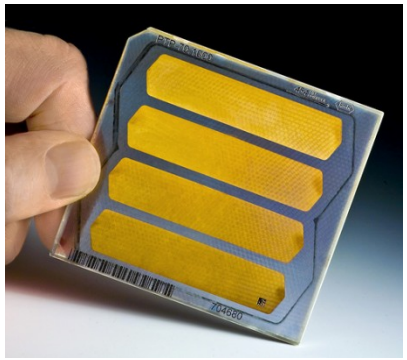


Figure 1 | The principle of Pyrosequencing and the output Pyrogram™. Double peak heights indicate incorporations of two nucleotides in a row.

Roche / 454 : GS FLX

- Good for
  - “de novo“ sequencing (longer reads).
  - Resequencing (expensive)
  - New bacterial genomes.
  - Amplicons
- Pyrosequencing. Bias with long polinucleotide streches

Roche 454



Throughput	400-600 million high-quality, filter-passed bases per run* 1 billion bases per day
Run Time	10 hours
Read Length	Average length = 400 bases
Accuracy	Q20 read length of 400 bases (99% at 400 bases and higher for prior bases)
Reads per run	>1 million high-quality reads
Data	Trace data accepted by NCBI since 2005
Computing Requirements	Cluster recommended (Roche GS FLX Titanium Cluster available)
Robustness	No complex optics or lasers; reagents have long shelf life

GS Junior, benchtop

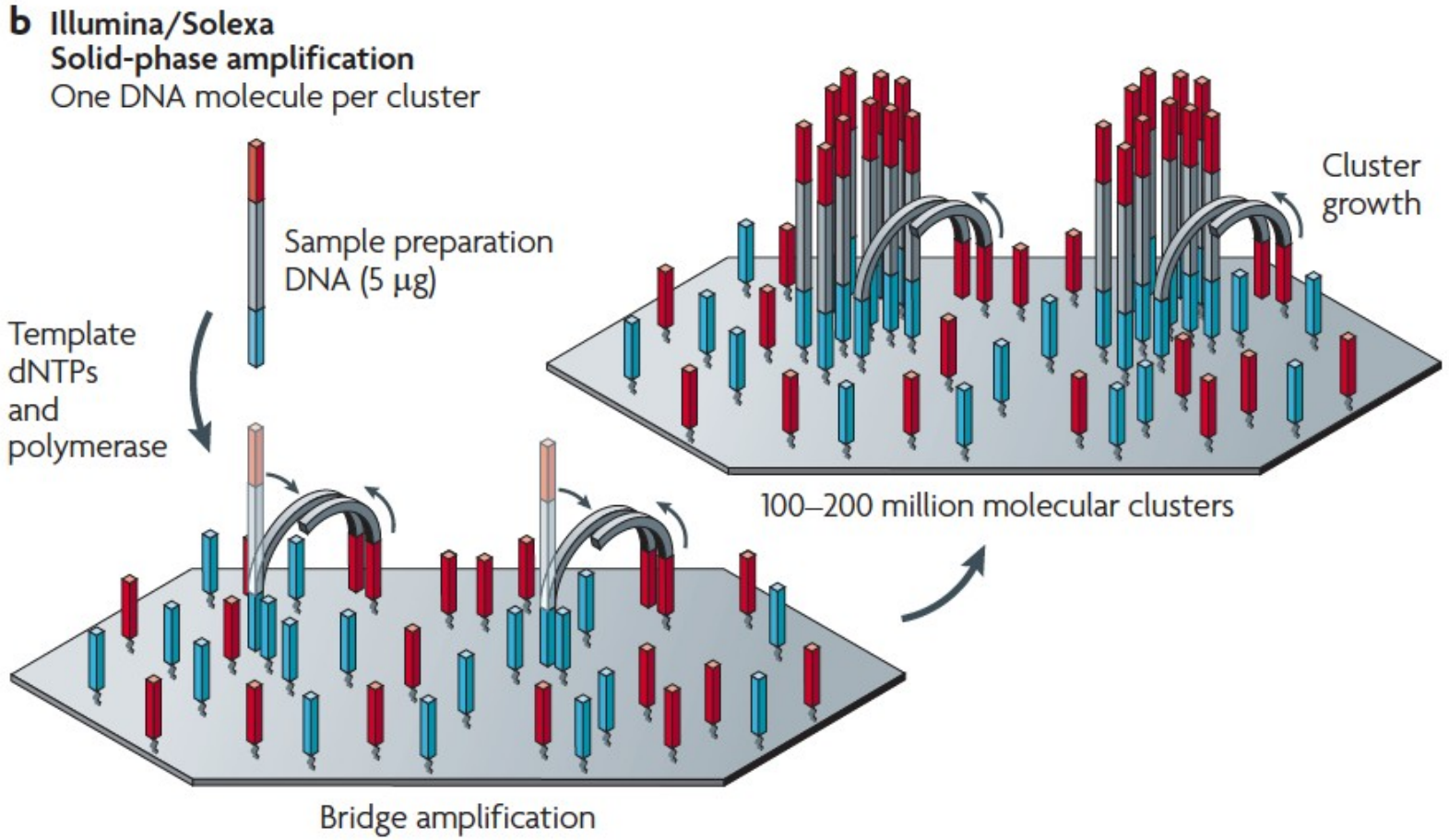
System Performance	
Throughput	35 million high-quality, filtered bases per run*
Run Time	10 hours sequencing 2 hours data processing
Avg. Read Length	400 bases*
Accuracy	Q20 read length of 400 bases (99% accuracy at 400 bases)
Reads per Run	100,000 shotgun, 70,000 amplicon
Sample Input	gDNA, amplicons, cDNA, or BACs depending on the application
Physical Dimensions	40 cm wide x 60 cm deep x 40 cm high (the size of a laser printer) Weight = 55 lbs.
Computing	Linux-based OS on HP desktop computer included. All software is point-and-click.

***Typical results**. Average read length and number of reads depend on specific sample and genomic characteristics*

Solexa (Illumina)

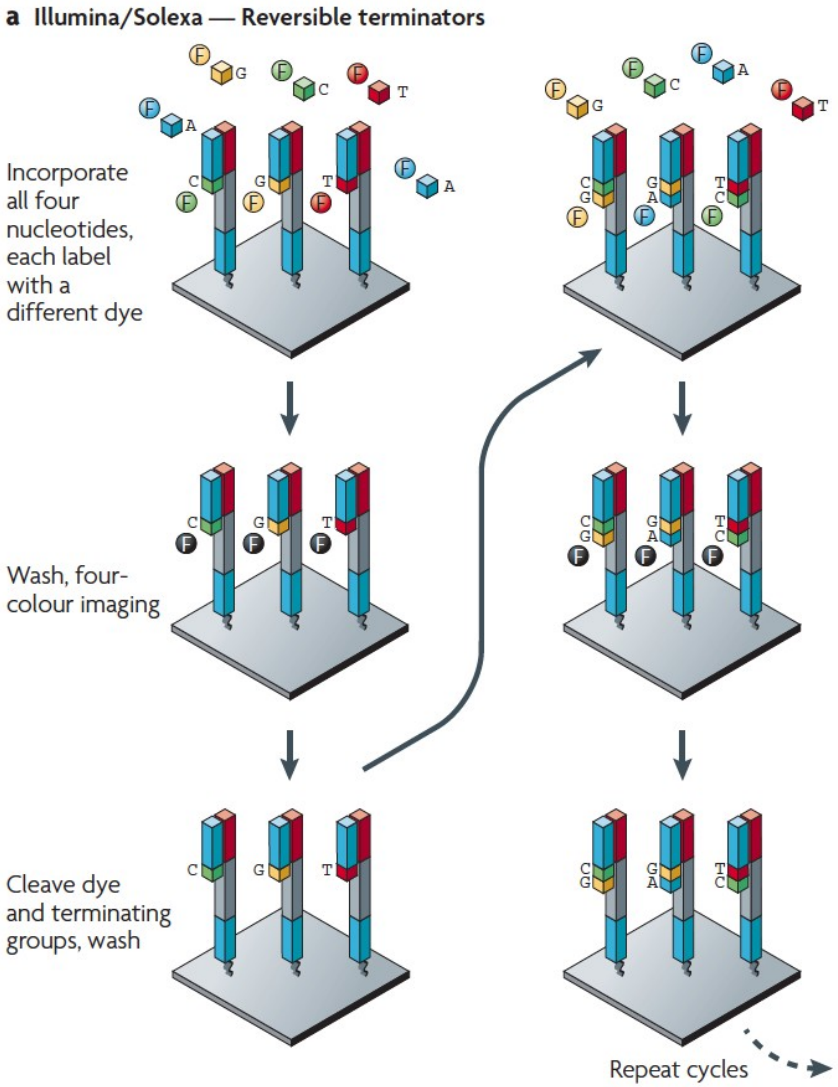
- Over 90% of all sequencing data is produced on Illumina systems.
- Uses a “sequencing by synthesis” approach:
  - DNA is broken into small fragments and ligated to an adaptor.
  - The fragments are attached to the surface of a flow cell and amplified.
  - DNA is sequenced by adding polymerase and labeled reversible terminator nucleotides (each base with a different color).
  - The incorporated base is determined by fluorescence.
  - The fluorescent label is removed from the terminator and the 3’ OH is unblocked, allowing a new base to be incorporated
- Started with 35 bp, increased now to up to 150 bp
- One run can give up to 10-600 Gb, 300-6000 million paired-end reads
- 75-85% of bases at or above Q30

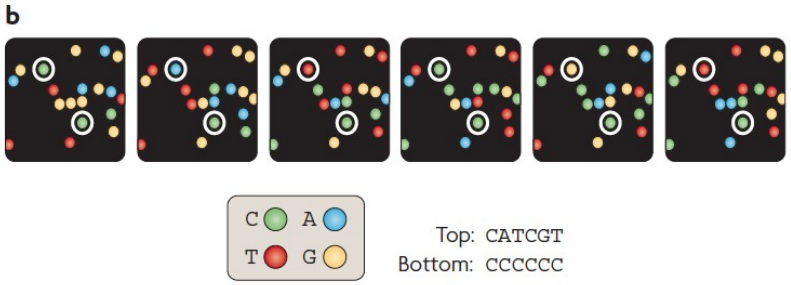
Solexa / illumina



From Michael Metzker, http://view.ncbi.nlm.nih.gov/pubmed/19997069

Solexa / illumina





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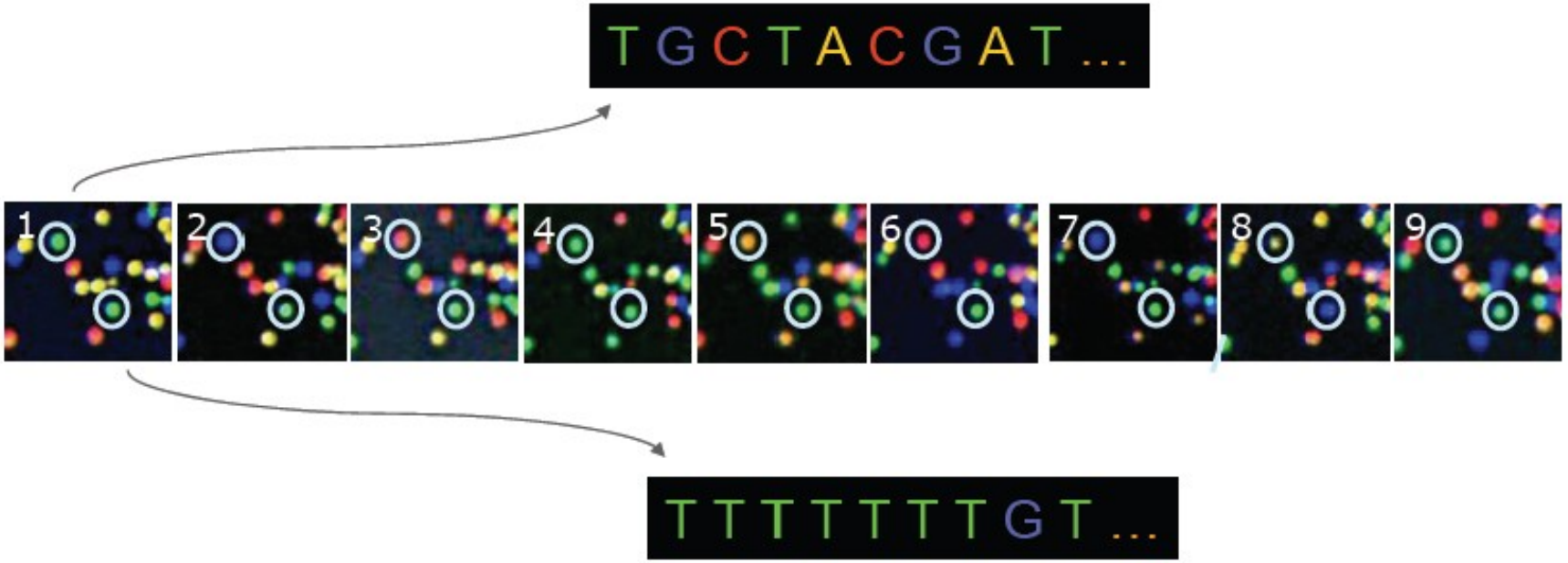
Solexa / illumina

Base calling from raw data



From Debbie Nickerson, Department of Genome Sciences, University of Washington, http://tinyurl.com/6zbzh4

The identity of each base of a cluster is read off from sequential images

Illumina-HiSeq 2500

600 Gb/run in 11 days
2x100 bp fragments
6 billion reads per run

Illumina-MiSeq

175-245 Mb 4h 1x 36bp

1.5-2.0 Gb 27h 2x150 bp

SOLiD (ABI / Life Technologies)

- **Colorspace**
- "**sequencing by ligation**" method
- Does not use polymerase, instead uses DNA ligase for sequencing:
  - DNA is broken into small fragments and ligated to an adaptor.
  - The fragments are attached to beads and amplified by emulsion PCR. Beads are attached to the surface of a glass slide.
  - DNA is sequenced by adding 8-mer fluorescently labelled oligonucleotides
  - If an oligo is complementary to the template, it will be ligated and 2 of the bases can be called.
  - The attached oligo is then cut to remove the label and the next set of labelled oligos are added
  - The process is repeated from different starting points (using different universal primers) so that each base is called twice
- 200 Gb, 1.8 billion reads per run, 35bp-75bp, 10 days

5500XL SOLiD

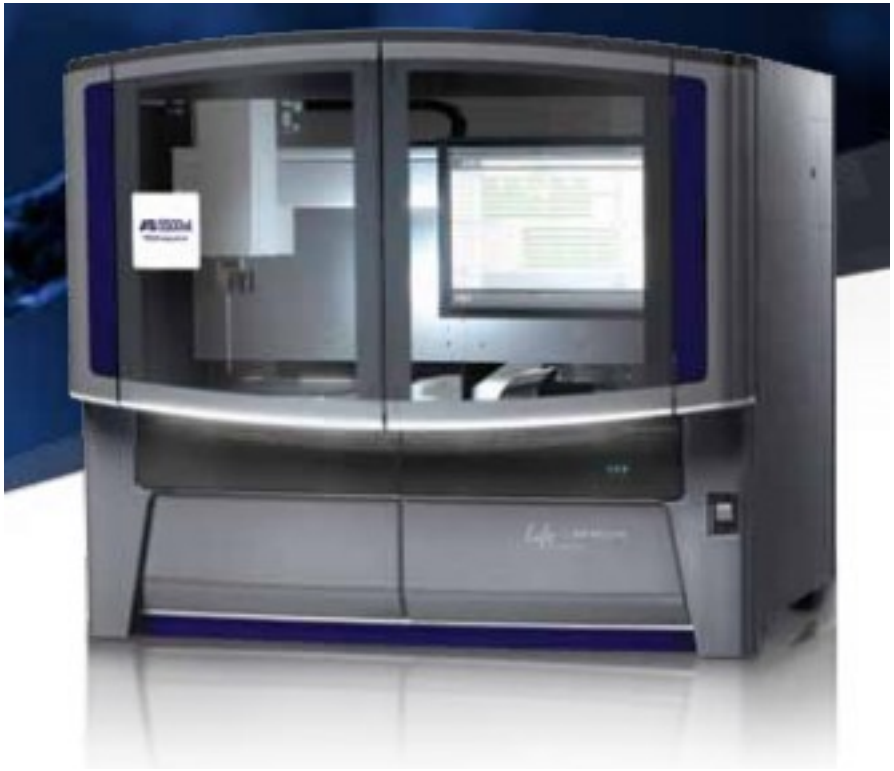
200 Gb/run (microbeads)
300 Gb/run (nanobeads)

35-75 bp fragments

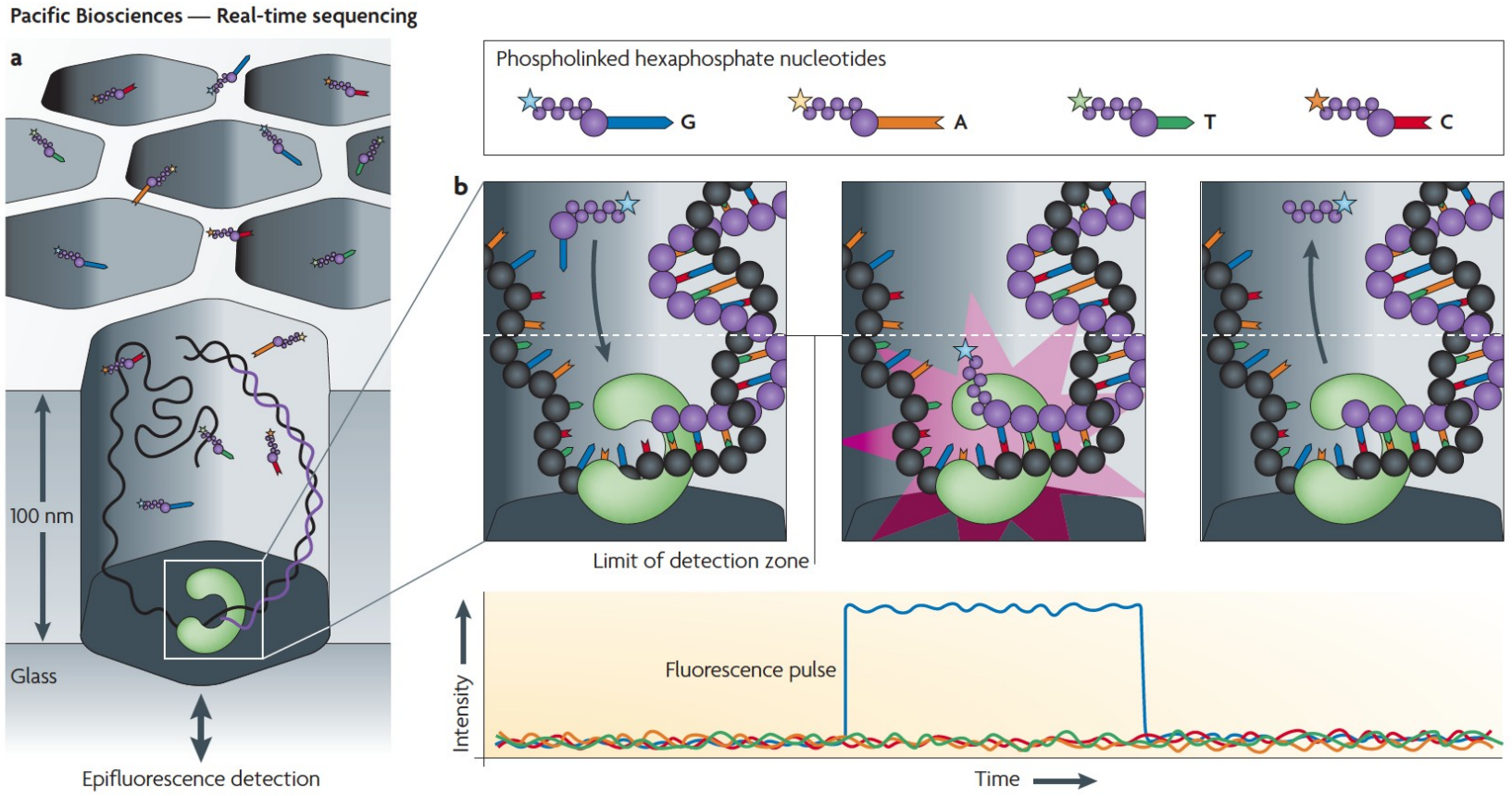
1.8 - 4.8 billion reads/run

2x6 lanes/run
96 bar-codes

ECC: 99.99% accuracy



PacBio

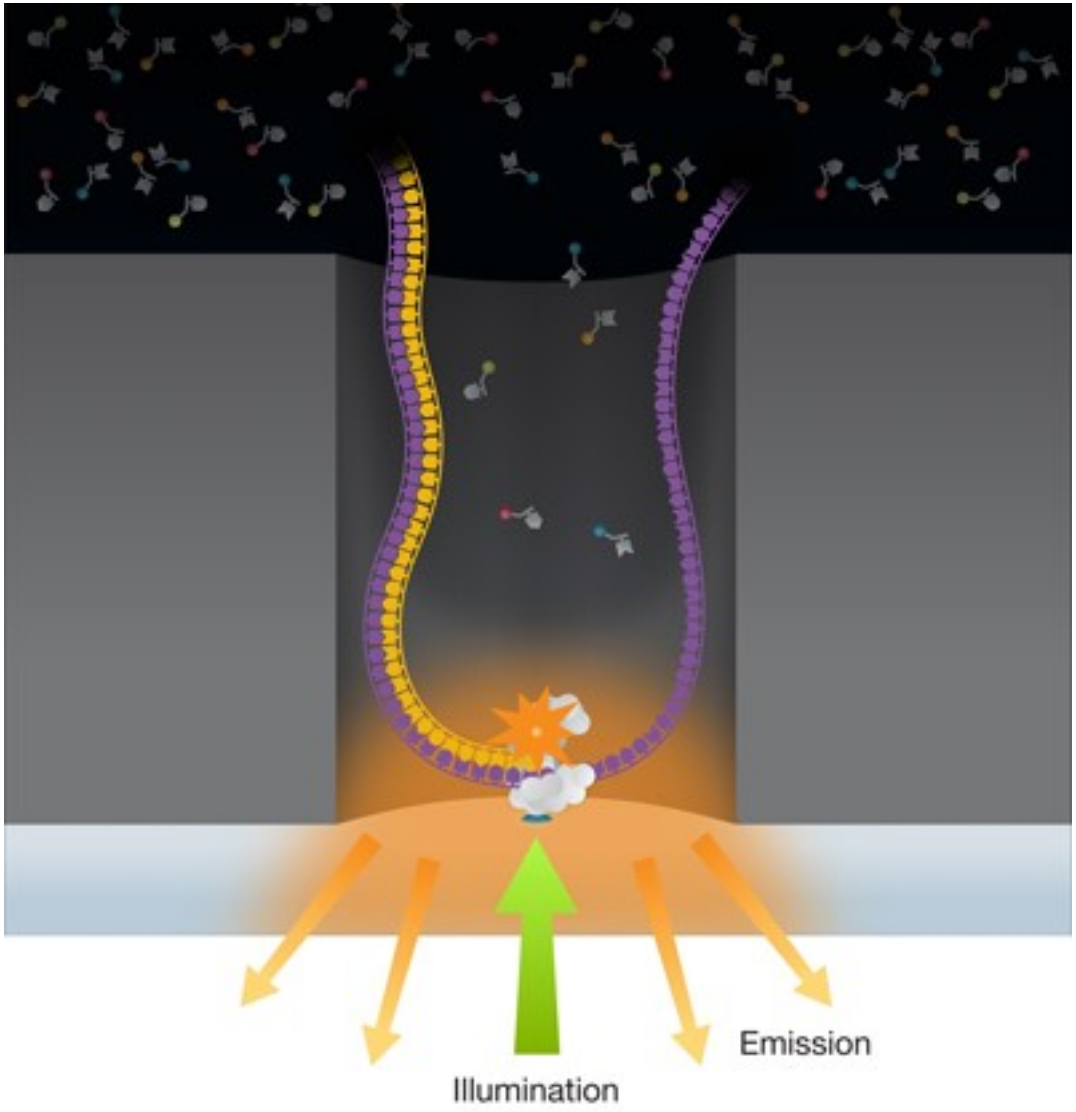


From Michael Metzker, http://view.ncbi.nlm.nih.gov/pubmed/19997069

Pacific Bioscience

SMRT: Singel Molecule Real time DNA synthesis
Up to 12000 nt
50 bases/second

ZMW: Zero Mode Waveguide



Ion Torrent

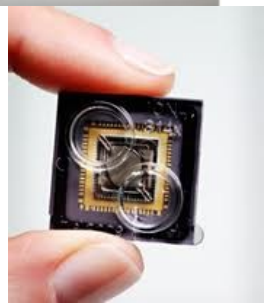
$ 50.000

$ 500 /sample

1 hour/run

> 200 nt lengths

Reads H+ released by DNA polymerase



Comparison

Roche 454

- Long fragments
- Errors: poly nts
- Low throughput
- Expensive

- De novo sequencing
- Amplicon sequencing
- RNASeq

Illumina

- Short fragments
- Errors: Hexamer bias
- High throughput
- Cheap

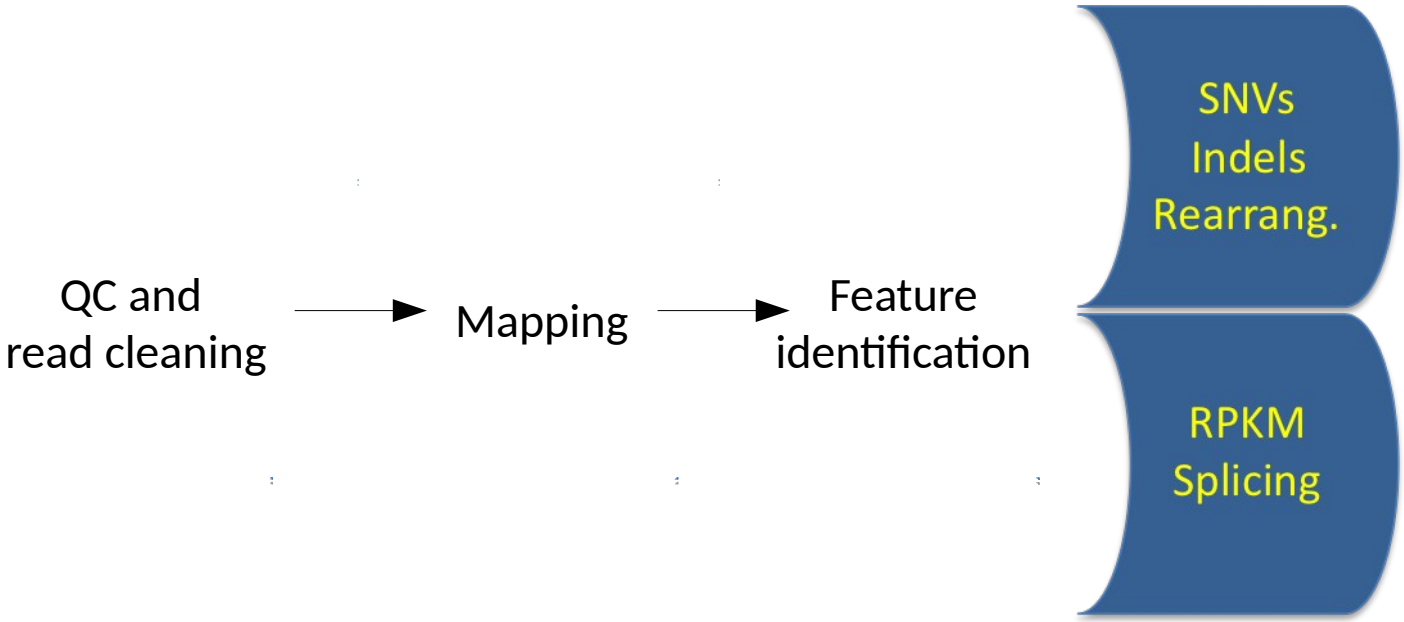
- Resequencing
- De novo sequencing
- ChipSeq
- RNASeq
- MethylSeq

SOLiD

- Short fragments
- Color-space
- High throughput
- Cheap

- Resequencing
- ChipSeq
- RNASeq
- MethylSeq

Basic steps NGS data processing



File formats

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1       XO:i:0  XG:i:0  MD:Z:8A29
```

fastq: sequence data and qualities

SAM/BAM: mapping data and qualities

Most common applications of NGS

RNA-seq /Transcriptomics
- Quantitative
- Descriptive
  - Alternative splicing
  - miRNA profiling

Resequencing
- Mutation calling
- Profiling
- Genome annotation

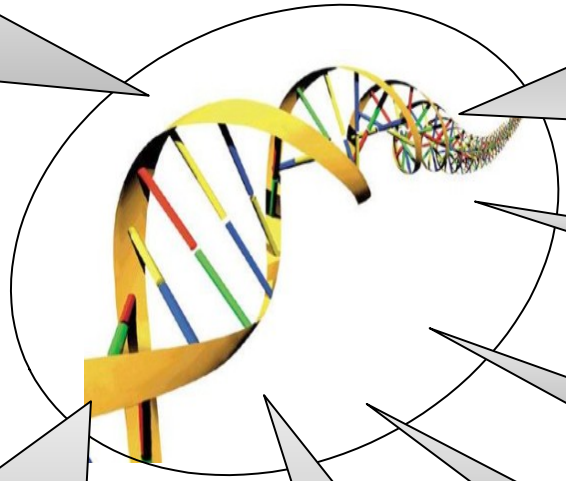
***De novo* sequencing**

Exome sequencing
Targeted sequencing

Copy number variation

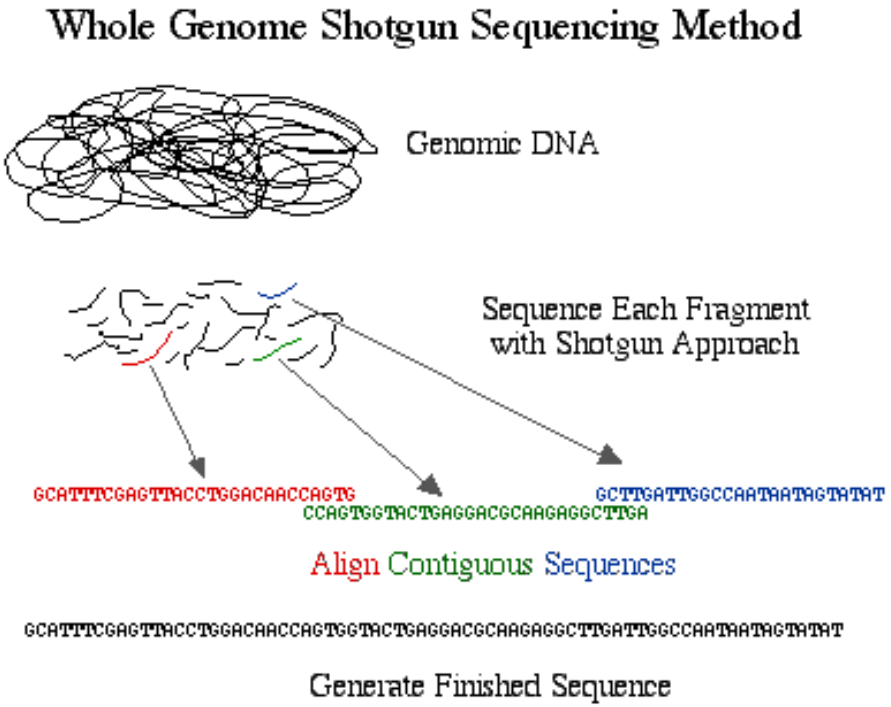
Metagenomics
Metatranscriptomics

ChIP-seq /Epigenomics
- Protein-DNA interactions
- Active transcription factor binding sites
- Histone methilation



DNA sequencing - 1

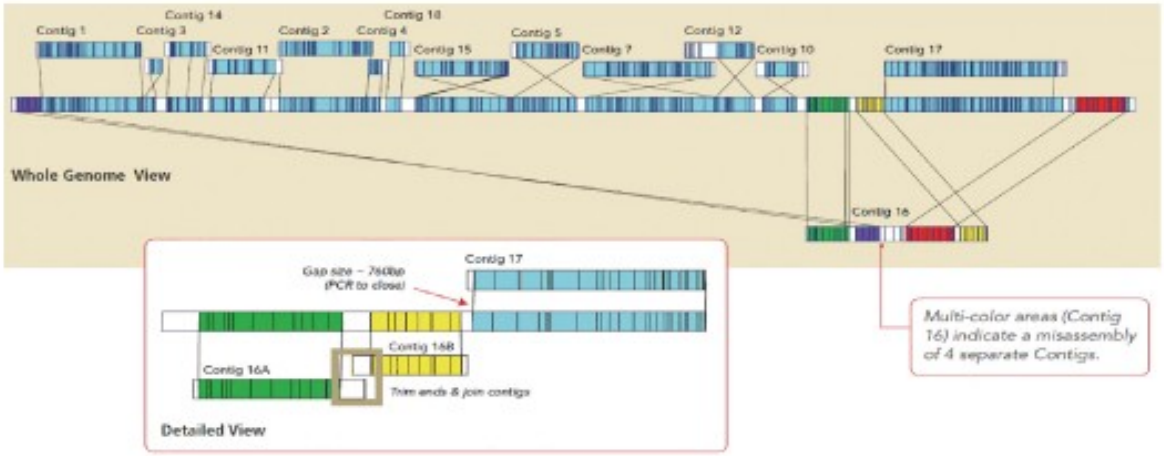
- **Whole GENOME Resequencing**
  - Need reference genome
  - Variation discovery



DNA sequencing - 2

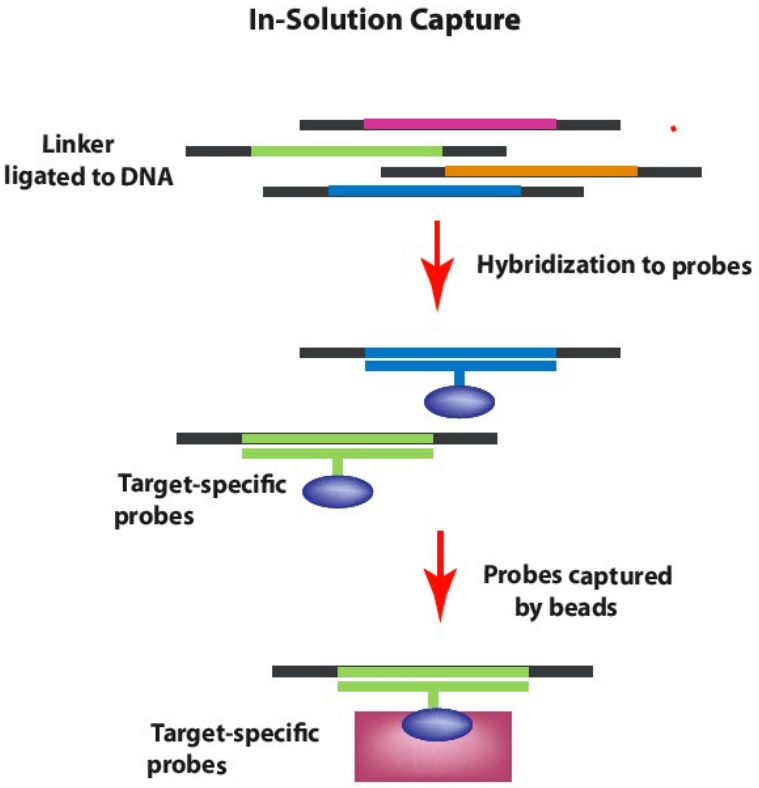
- **Whole GENOME “de novo” sequencing**
  - Uncharacterized genomes with no reference genome available
  - known genomes where significant structural variation is expected.
  - Long reads or mate-pair libraries. Sequencing mostly done by Roche 454 and also Illumina.
  - Assembly of reads is needed: Computational intensive

  - E.g. Genome bacteria sequencing



DNA sequencing - 3

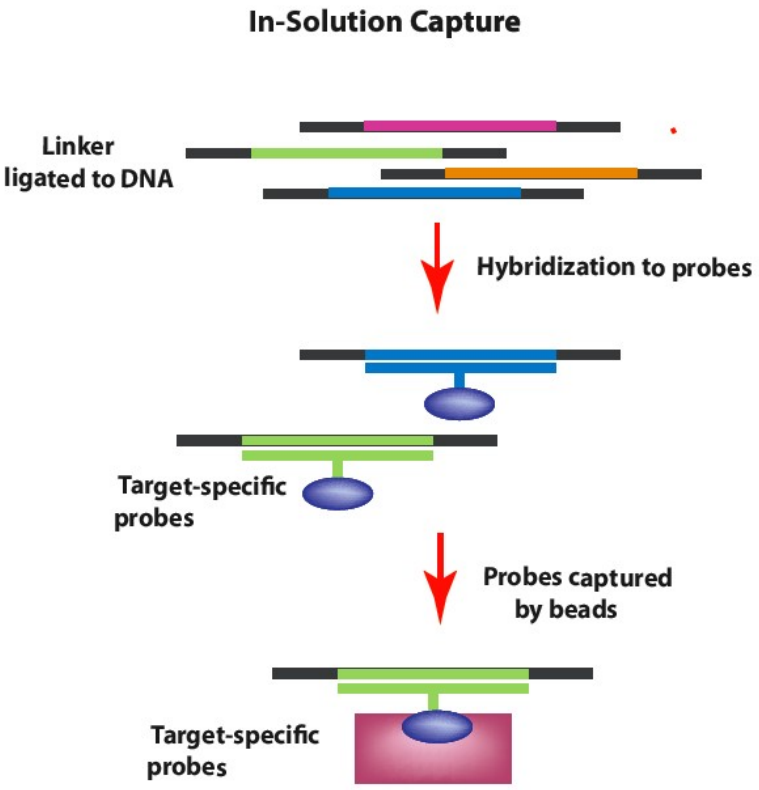
- **Whole EXOME Resequencing**
  - Need reference genome
    - Available for Human and Mouse
  - Variation discovery on ORFs
    - 2% of human genome (lower cost)
    - 85% disesease mutation are in the exome
  - Need probes complementary to exons
    - Nimblegen
    - Agilent



- E.g. Human exome

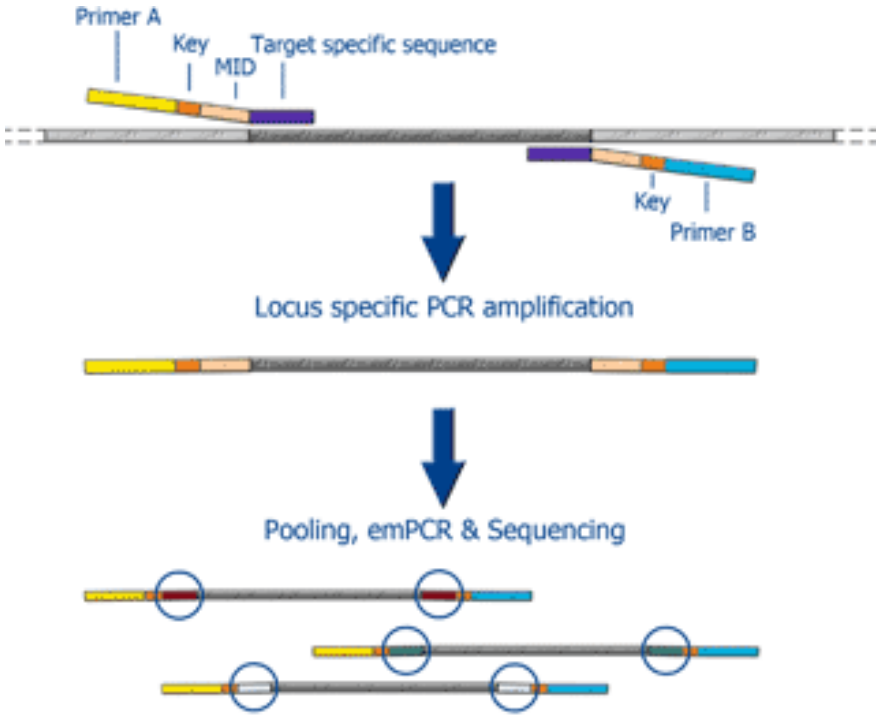
DNA sequencing - 4

- **Targeted Resequencing**
  - Capture of specific regions in the genome
- **Custom genes panel sequencing**
  - Allows to cover high number of genes related to a disease
  - *E.g. Disease gene panel*
- Low cost and quicker than capillary sequencing
- Multiplexing is possible
- Need custom probes complementary to the genomic regions
  - Nimblegen
  - Agilent



DNA sequencing - 5

- **Amplicon sequencing**
  - Sequencing of regions amplified by PCR.
  - Shorter regions to cover than targeted capture
  - No need of custom probes
  - Primer design is needed
  - High fidelity polymerase
  - Multiplexing is needed



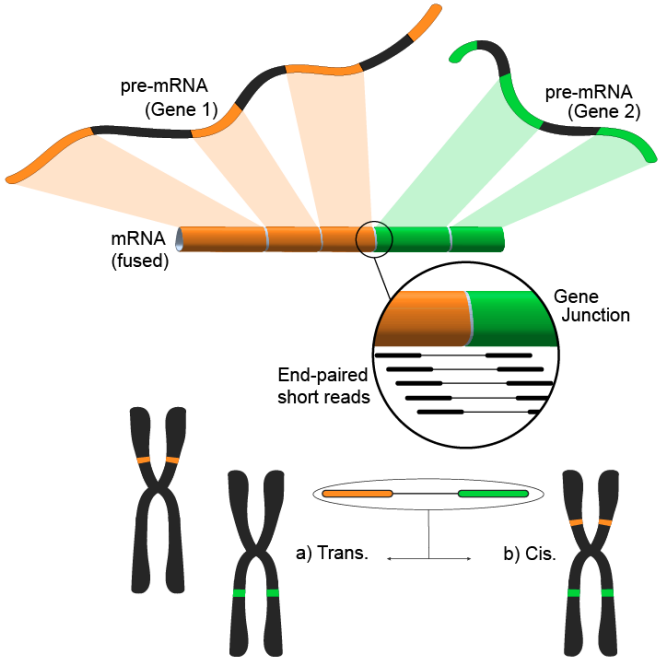
- *E.g. P53 exon amplicon sequencing*

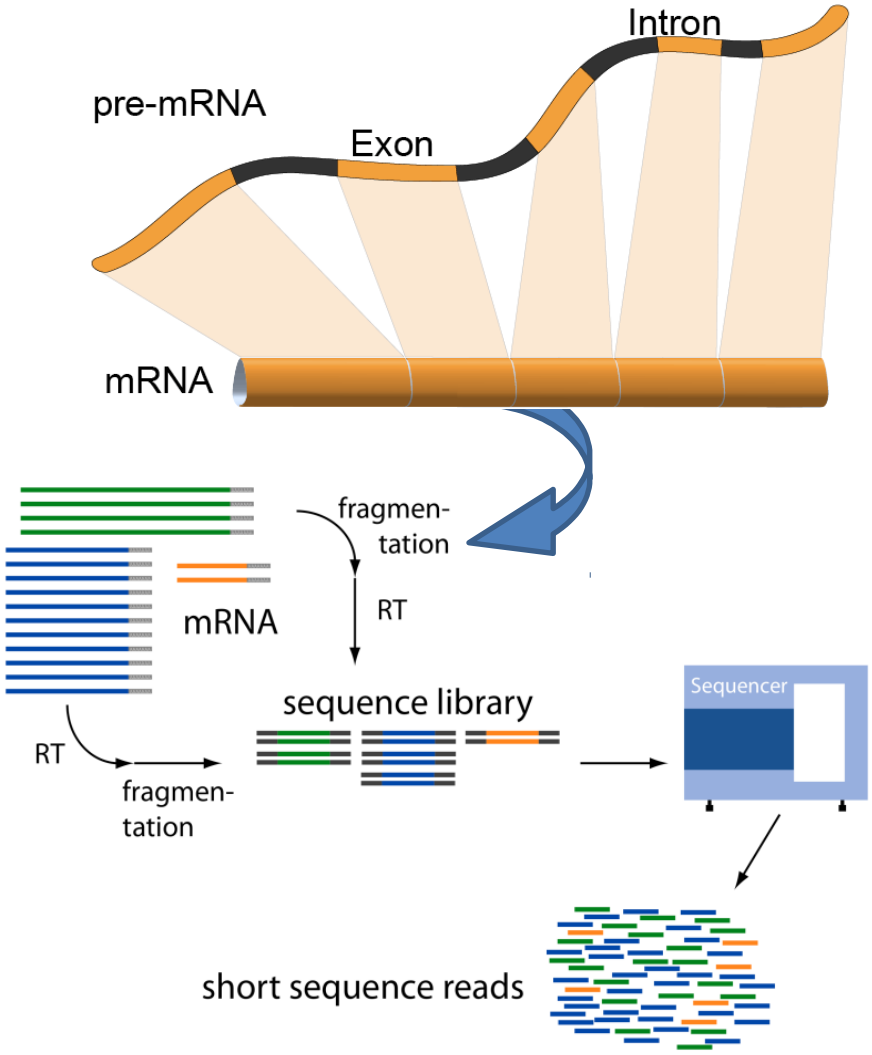
Transcriptomics - 1

- **RNA-Seq**
  - Sequencing of mRNA
  - rRNA depleted samples
  - Very high dynamic range
  - No prior knwoledge of expressed genes
  - Gives information about (richer than microarrays)
    - Differential expression of **known or unknown** transcripts during a treatment or condition
    - **Isoforms** and
    - New **alternative splicing** events
    - **Non-coding** RNAs
    - Post-transcriptional mutations or **editing**,
    - **Gene fusions**.

Transcriptomics - 2

- **RNA-Seq**
  - Sequencing of **mRNA**
  - **Detecting gene fusions**





Applications of RNAseq

Qualitative:
* Alternative splicing
* Antisense expression
* Extragenic expression
* Alternative 5’ and 3’ usage
* Detection of fusion transcripts

….

Tophat/Cufflinks
Scripture
Alexa

Quantitative:
* Differential expression
* Dynamic range of gene expression

….

edgeR
DESeq
baySeq
NOISeq

Advantages of RNAseq?

RNAseq

* Non targeted transcript detection
* No need of reference genome
* Strand specificity
* Find novels splicing sites
* Larger dynamic range
* Detects expression and SNVs
* Detects rare transcripts

....

microarrays

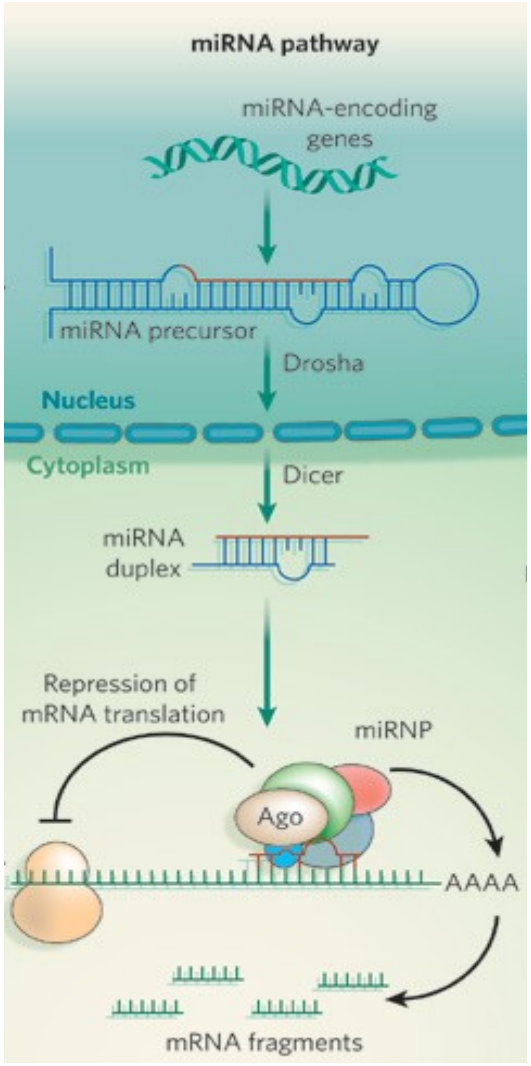
* Restricted to probes on array
* Needs genome knowledge
* Normally, not strand specific
* Exon arrays difficult to use
* Smaller dynamic range
* Does not provide sequence info
* Rare transcripts difficult

....

and.... are there any disadvantages?????

Transcriptomics - 3

- **miRNA/small nonCoding RNA sequencing**
  - RNA Size selection step
    - 18-40 bp
  - Profiling of known miRNAs
  - miRNA discovery

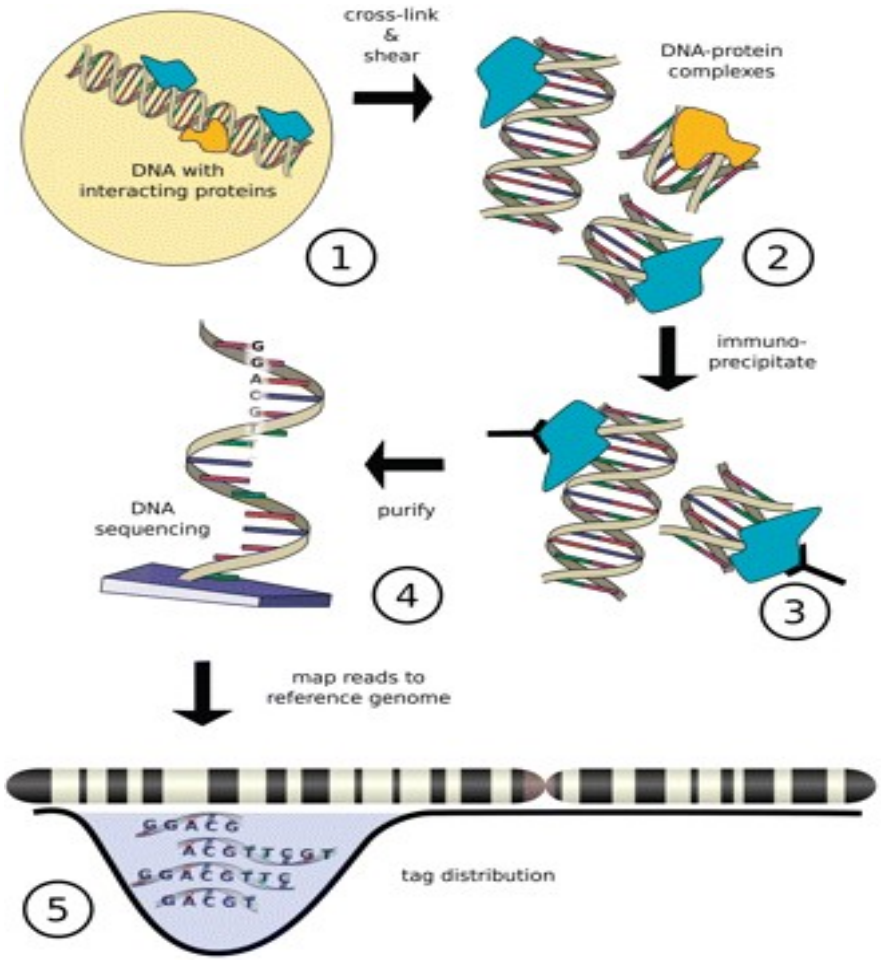


TFBS detection

ChIP-Seq

- Identification of genomic region for gDNA binding proteins:

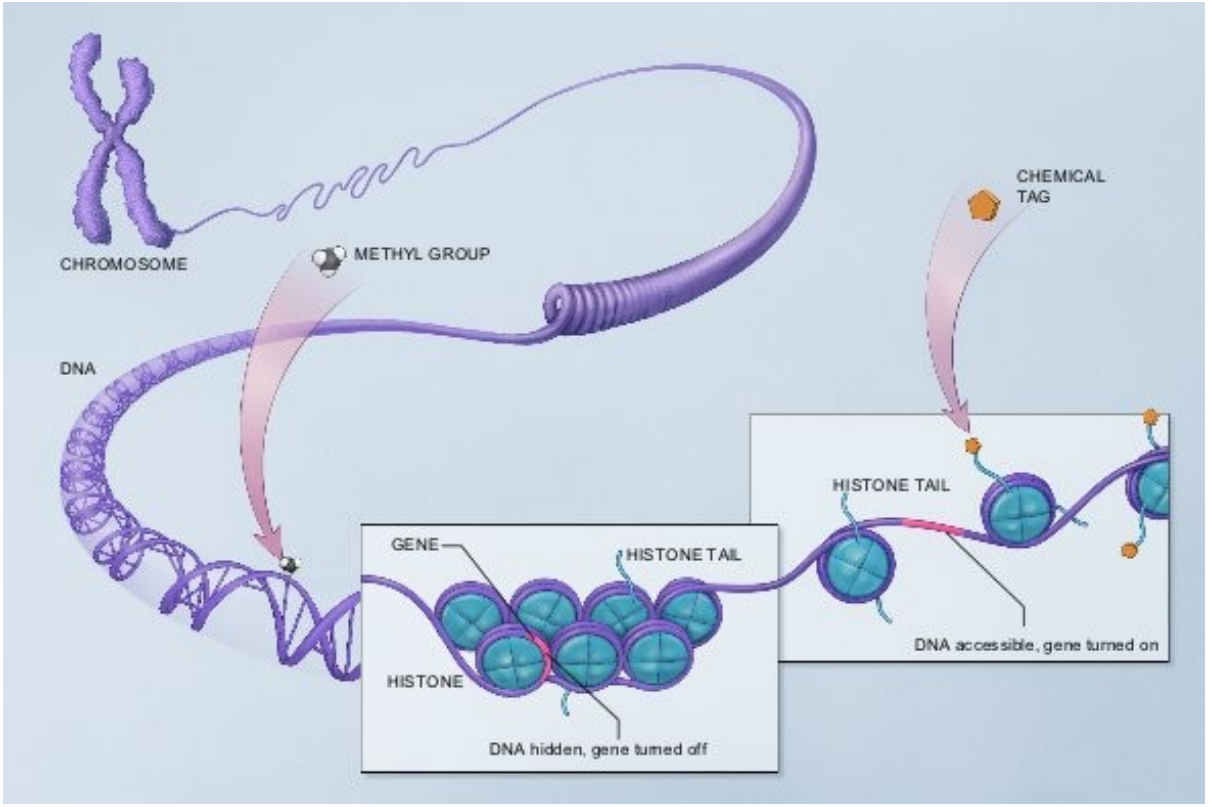
  - Transcription Factor binding site detection



Epigenomics - I

Epigenomics refers to functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence

- *Play a role in turning genes off or on*



Epigenomic Marks.

a) Methyl groups attach to the backbone of a DNA molecule.

b) A variety of chemical tags attach to the tails of histones. This action affects how tightly DNA is wound around the histones.

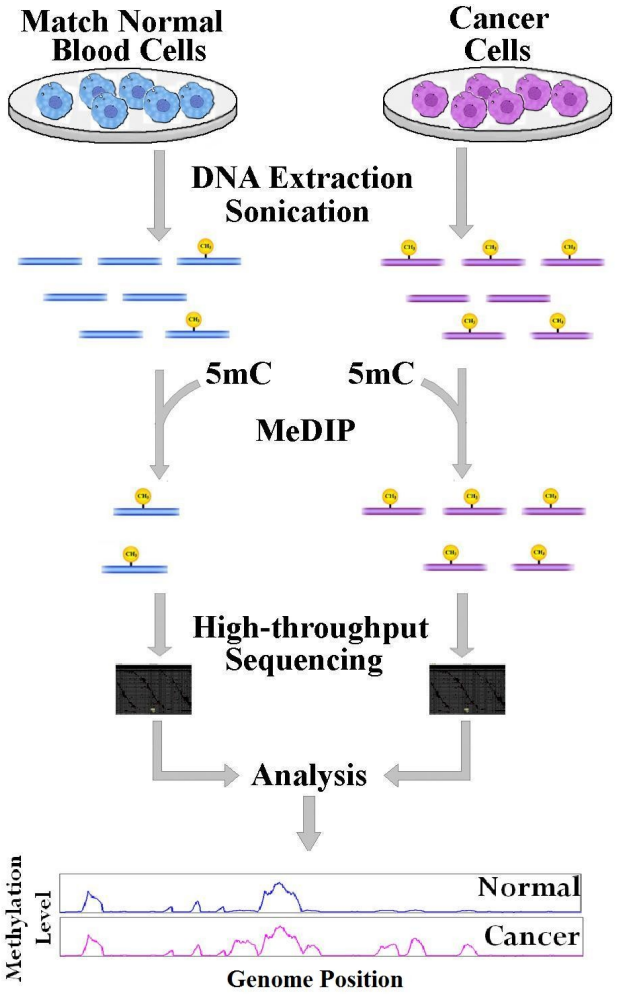
ChIP-Seq: Histone methylation detection

Epigenomics - 2

- **Methyl-Seq**
  - CpG island methylation
  - Bisulfite sequencing-based method

> E.g. Cancer studies.

- Different degree of chromatin methylation affects expression of genes



Successful NGStories

NATURE | ARTICLE OPEN

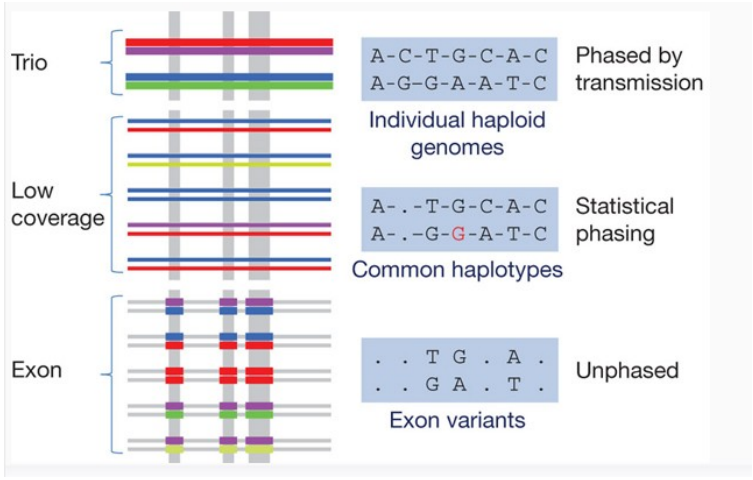
previous article next article

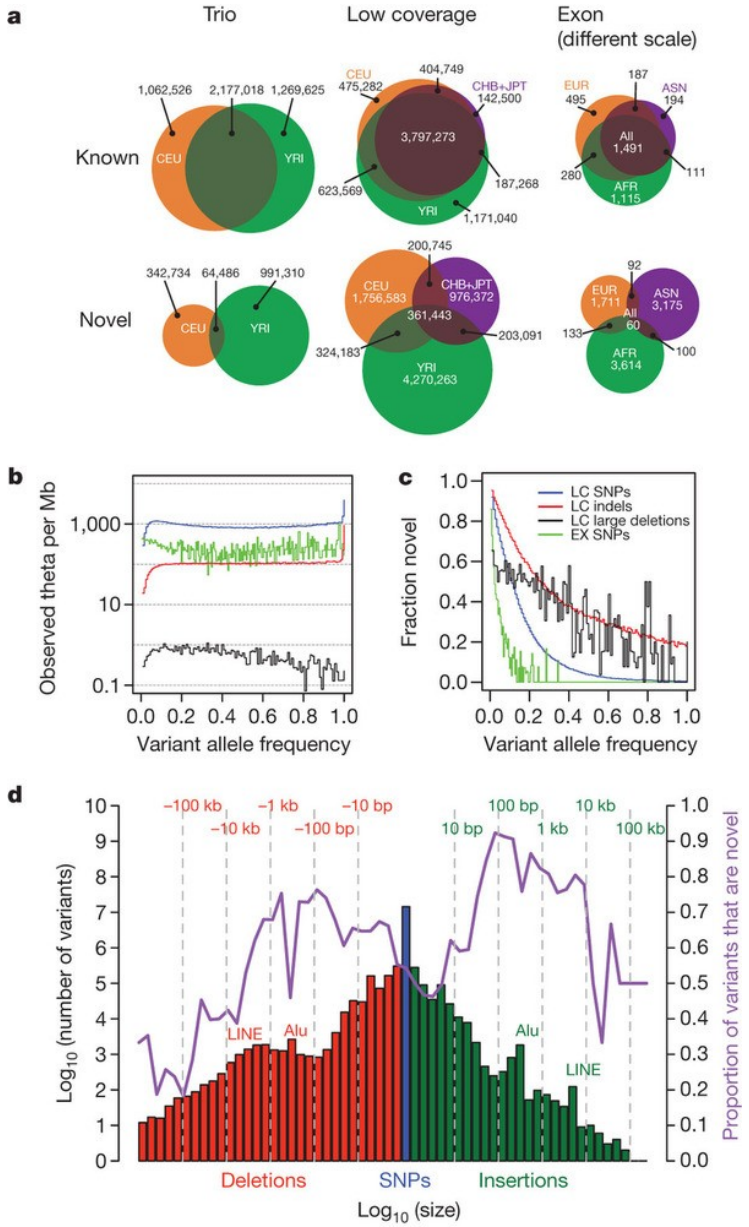
A map of human genome variation from population-scale sequencing

The 1000 Genomes Project Consortium

Affiliations | Contributions | Corresponding author

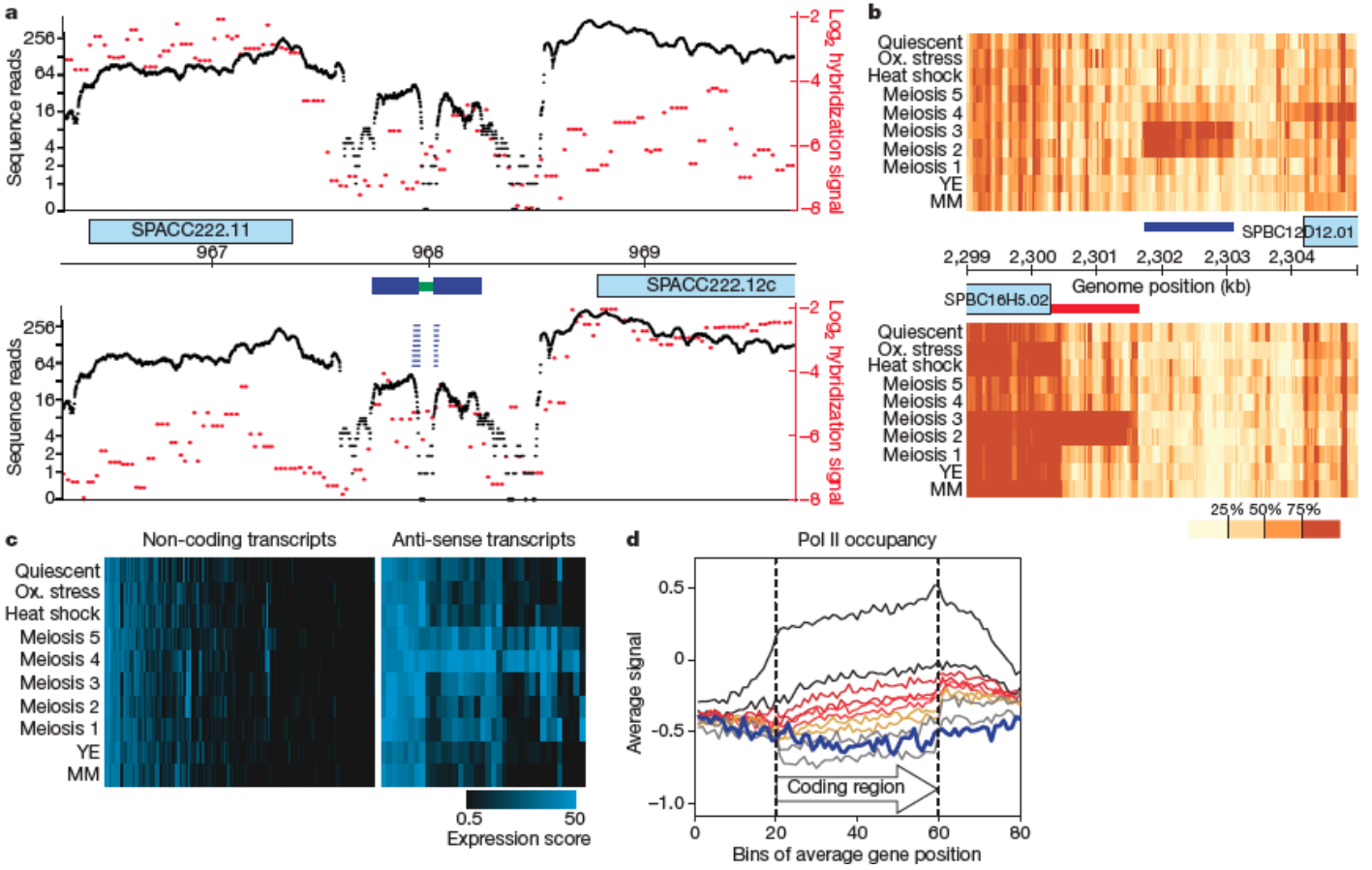
Nature **467**, 1061–1073 (28 October 2010) | doi:10.1038/nature09534
Received 20 July 2010 | Accepted 30 September 2010 | Published online 27 October 2010





Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution

Brian T. Wilhelm[1]*†, Samuel Marguerat[1]*†, Stephen Watt[1]†, Falk Schubert[1]†, Valerie Wood[1], Ian Goodhead[1]†, Christopher J. Penkett[1]†, Jane Rogers[1] & Jürg Bähler[1]†



Published in final edited form as:
Nat Genet. 2010 January ; 42(1): 30–35. doi:10.1038/ng.499.

Exome sequencing identifies the cause of a Mendelian disorder

Sarah B. Ng[1,*], **Kati J. Buckingham**[2,*], **Choli Lee**[1], **Abigail W. Bigham**[2], **Holly K. Tabor**[2], **Karin M. Dent**[3], **Chad D. Huff**[4], **Paul T. Shannon**[5], **Ethylin Wang Jabs**[6,7], **Deborah A. Nickerson**[1], **Jay Shendure**[1,†], and **Michael J. Bamshad**[1,2,8,†]

[1]Department of Genome Sciences, University of Washington, Seattle, Washington, USA [2]Department of Pediatrics, University of Washington, Seattle, Washington, USA [3]Department of Pediatrics, University of Utah, Salt Lake City, Utah, USA [4]Department of Human Genetics, University of Utah, Salt Lake City, Utah, USA [5]Institute of Systems Biology, Seattle WA, USA [6]Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, New York, USA [7]Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland [8]Seattle Children's Hospital, Seattle, Washington, USA

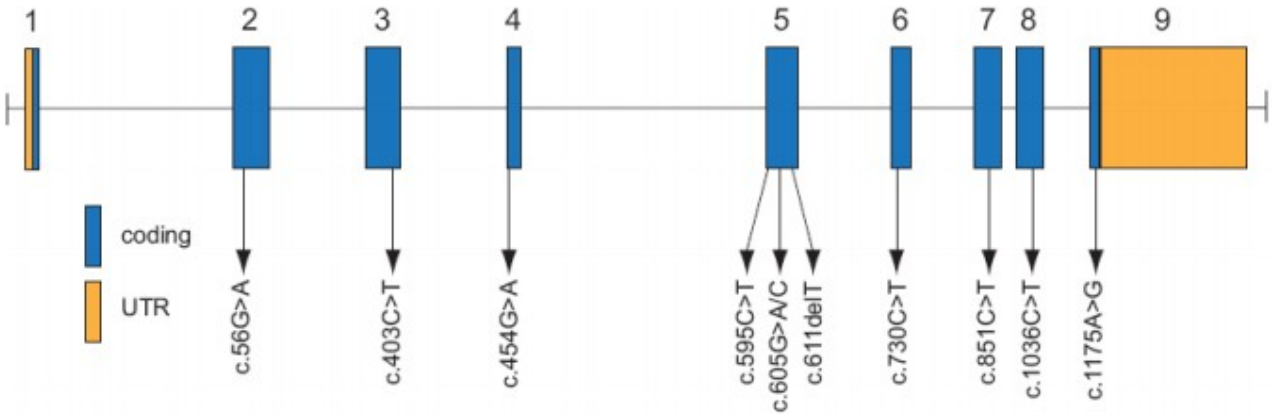


Figure 2. Genomic structure of the exons encoding the open reading frame of *DHODH*

DHODH is composed of 9 exons that encode untranslated regions (orange) and protein coding sequence (blue). Arrows indicate the locations of 11 different mutations found in 6 families with Miller syndrome.

Miller syndrome

Method

Open Access

Targeted next-generation sequencing of a cancer transcriptome enhances detection of sequence variants and novel fusion transcripts

Joshua Z Levin*, Michael F Berger†, Xian Adiconis*, Peter Rogov*, Alexandre Melnikov*, Timothy Fennell‡, Chad Nusbaum*, Levi A Garraway†§ and Andreas Gnirke*

Addresses: *Genome Sequencing and Analysis Program, Broad Institute of MIT and Harvard, 320 Charles Street, Cambridge, MA 02141, USA. †Cancer Program, Broad Institute of MIT and Harvard, 5 Cambridge Center, Cambridge, MA 02142, USA. ‡Sequencing Platform, Broad Institute of MIT and Harvard, 320 Charles Street, Cambridge, MA 02141, USA. §Department of Medical Oncology and Center for Cancer Genome Discovery, Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Boston, MA 02115, USA.

Figure 3
Sequences from NUP214-XKR3 fusion transcripts detected after hybrid selection. After hybrid selection, 152 reads were aligned to the transcriptome and detected as *NUP214-XKR3* fusions. From top to bottom, we observed 137, four, eight, and three reads for these transcripts. The *NUP214* (exon 27) to *XKR3* (exon 4) has a stop codon downstream (not shown). Only *NUP214* (exon 29) to *XKR3* (exon 4) retains an open reading frame downstream of the fusion. Before hybrid selection, eight reads were aligned to the transcriptome and detected as *NUP214-XKR3* fusions; only the *NUP214* (exon 29) to *XKR3* (exon 2) transcript was detected. Sequence from *NUP214* DNA is shown as lower case, and from *XKR3*, as bold and upper case.

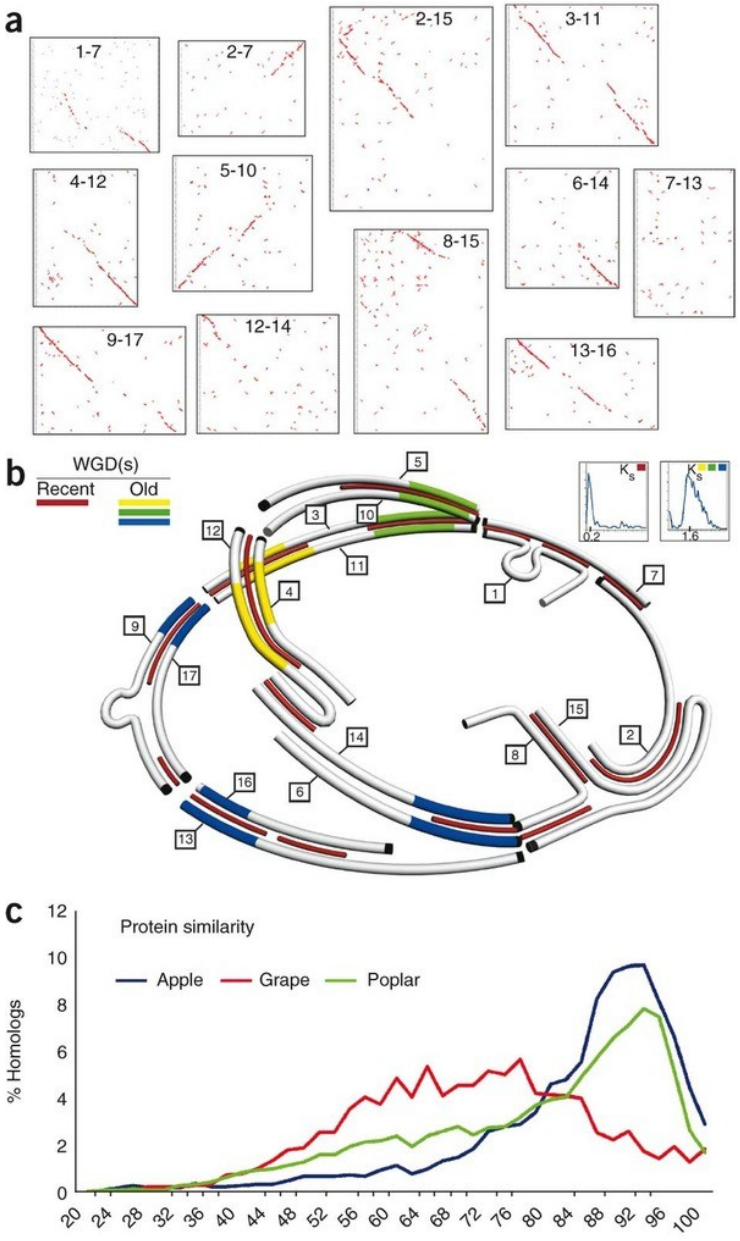
NATURE GENETICS | ARTICLE

The genome of the domesticated apple (*Malus* × *domestica* Borkh.)

Riccardo Velasco, Andrey Zharkikh, Jason Affourtit, Amit Dhingra, Alessandro Cestaro, Ananth Kalyanaraman, Paolo Fontana, Satish K Bhatnagar, Michela Troggio, Dmitry Pruss, Silvio Salvi, Massimo Pindo, Paolo Baldi, Sara Castelletti, Marina Cavaiuolo, Giuseppina Coppola, Fabrizio Costa, Valentina Cova, Antonio Dal Ri, Vadim Goremykin, Matteo Komjanc, Sara Longhi, Pierluigi Magnago, Giulia Malacarne, Mickael Malnoy + *et al.*

Affiliations | Contributions | Corresponding author

Nature Genetics **42**, 833–839 (2010) | doi:10.1038/ng.654
Received 19 November 2009 | Accepted 03 August 2010 | Published online 29 August 2010



Letter

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Sequencing the nuclear genome of the extinct woolly mammoth

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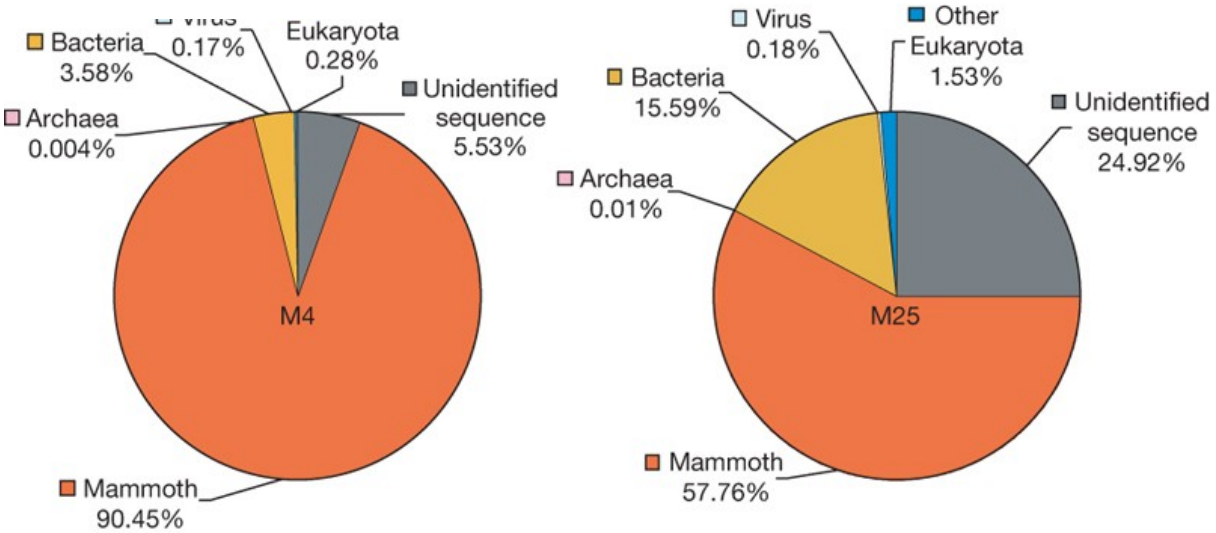


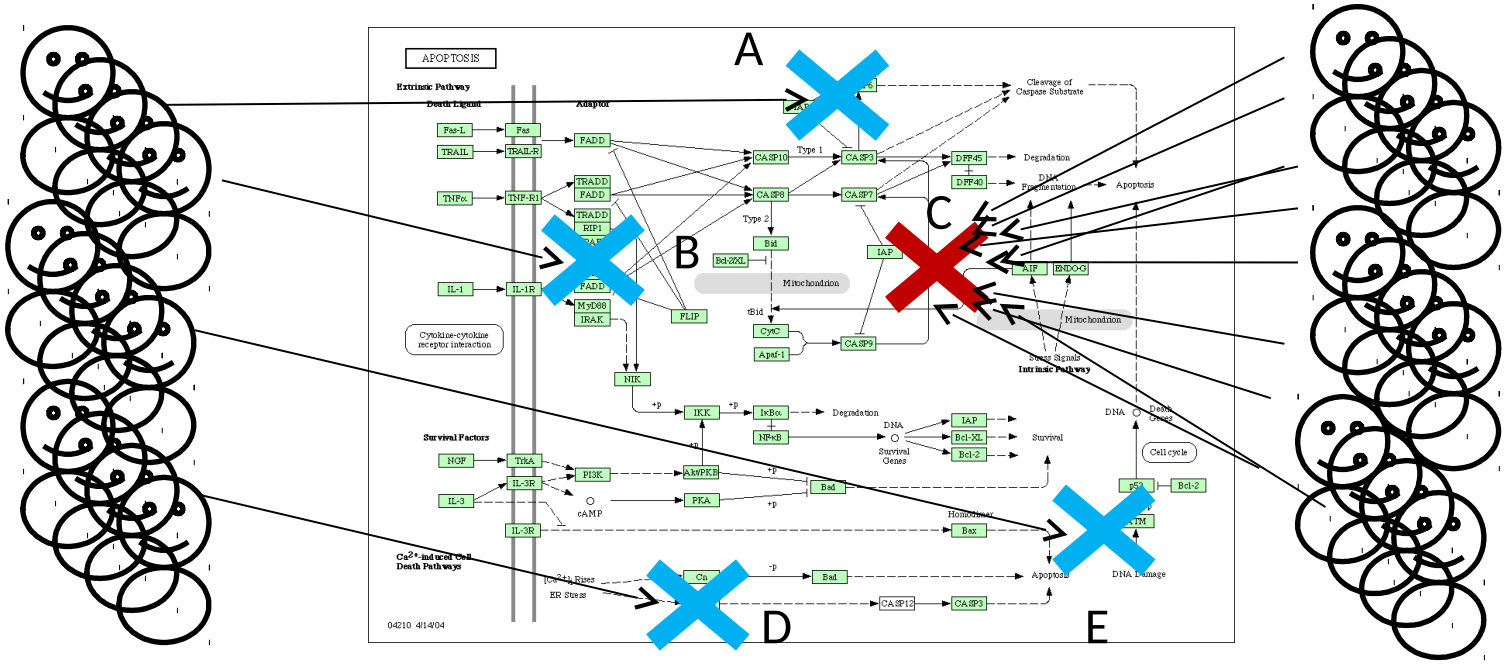
Species composition of metagenomic DNA extracted from mammoth hair

nature

Not that easy, some challenges

Secondary analysis:
Finding the mutations causative of diseases

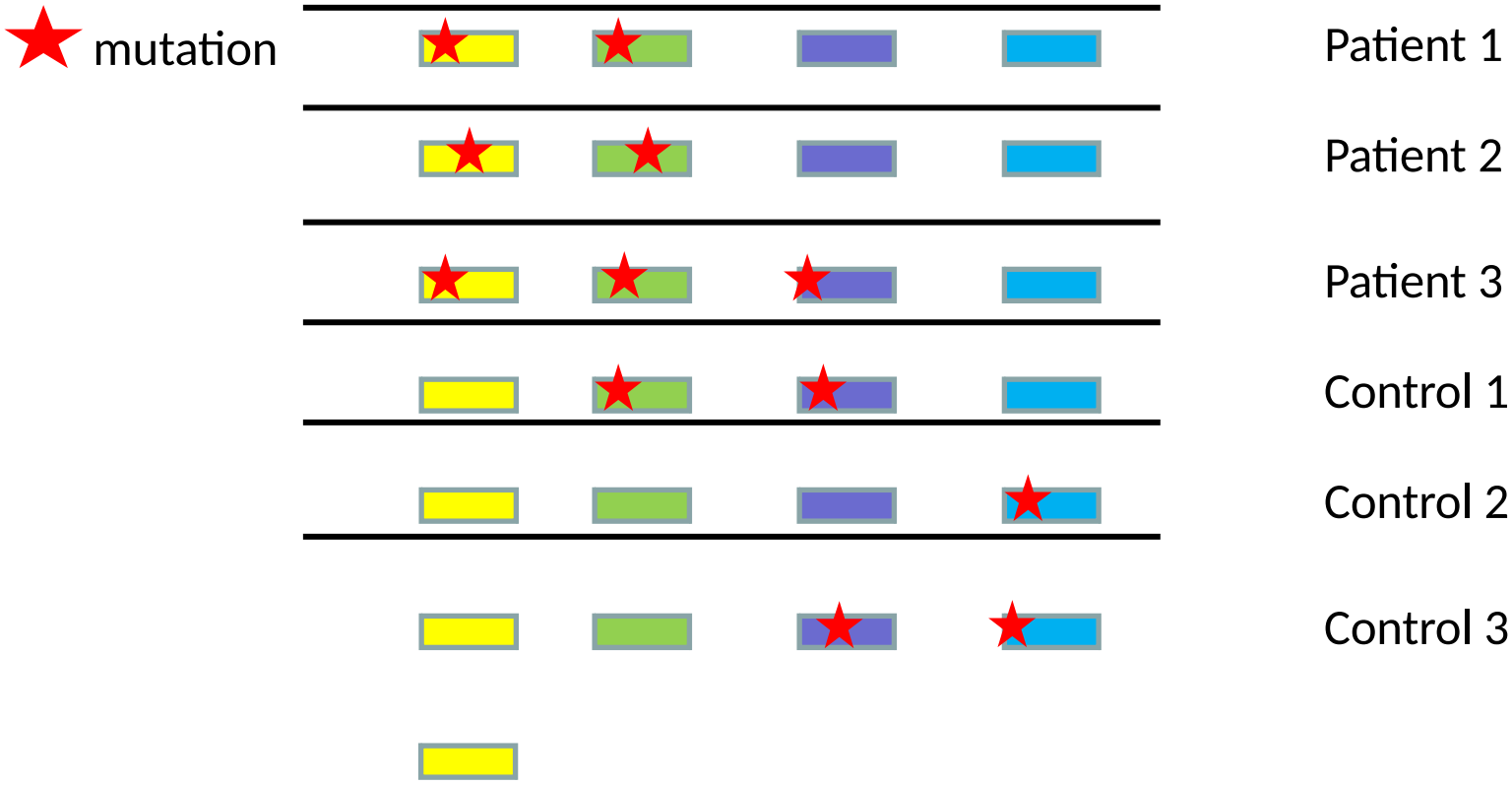
The simplest case: monogenic disease due to a single gene



Controls

Cases

The principle: comparison of patients (or families) and reference controls



candidate gene (shares mutation for all patients but no controls)

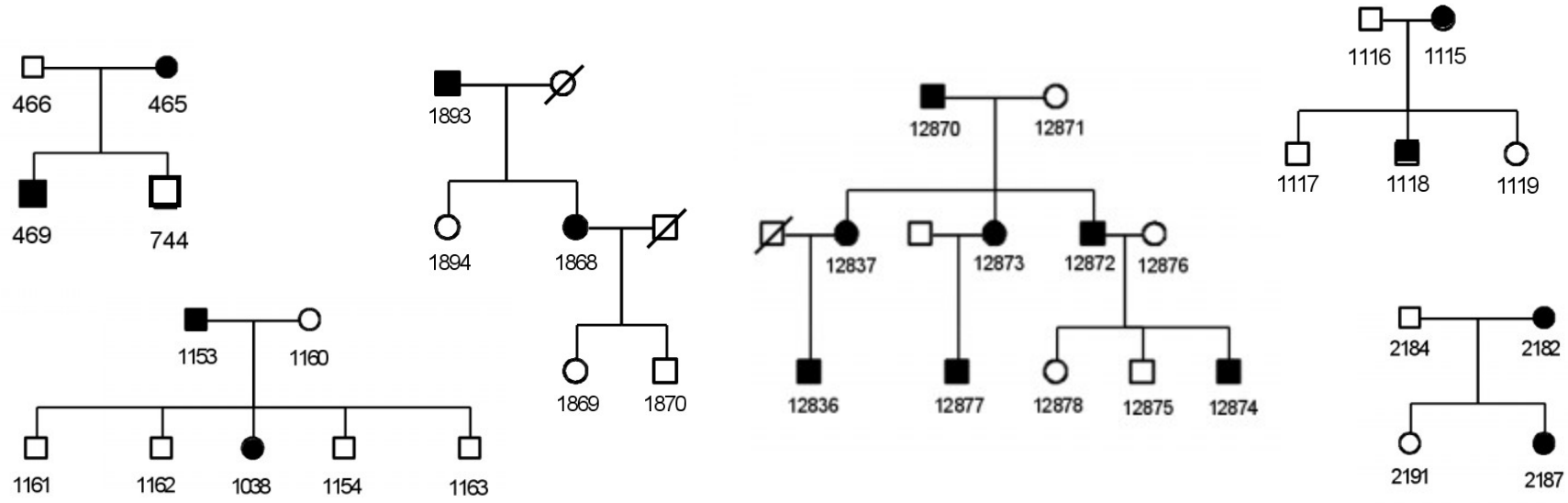
Is this approach realistic?
Can we detect such rare variants so easily?

a) Interrogating 50Mb produces too many variants

b) In many cases we are not hunting new but known variants

c) Same phenotype can be due to different mutations and different genes

Filtering with multiple family information



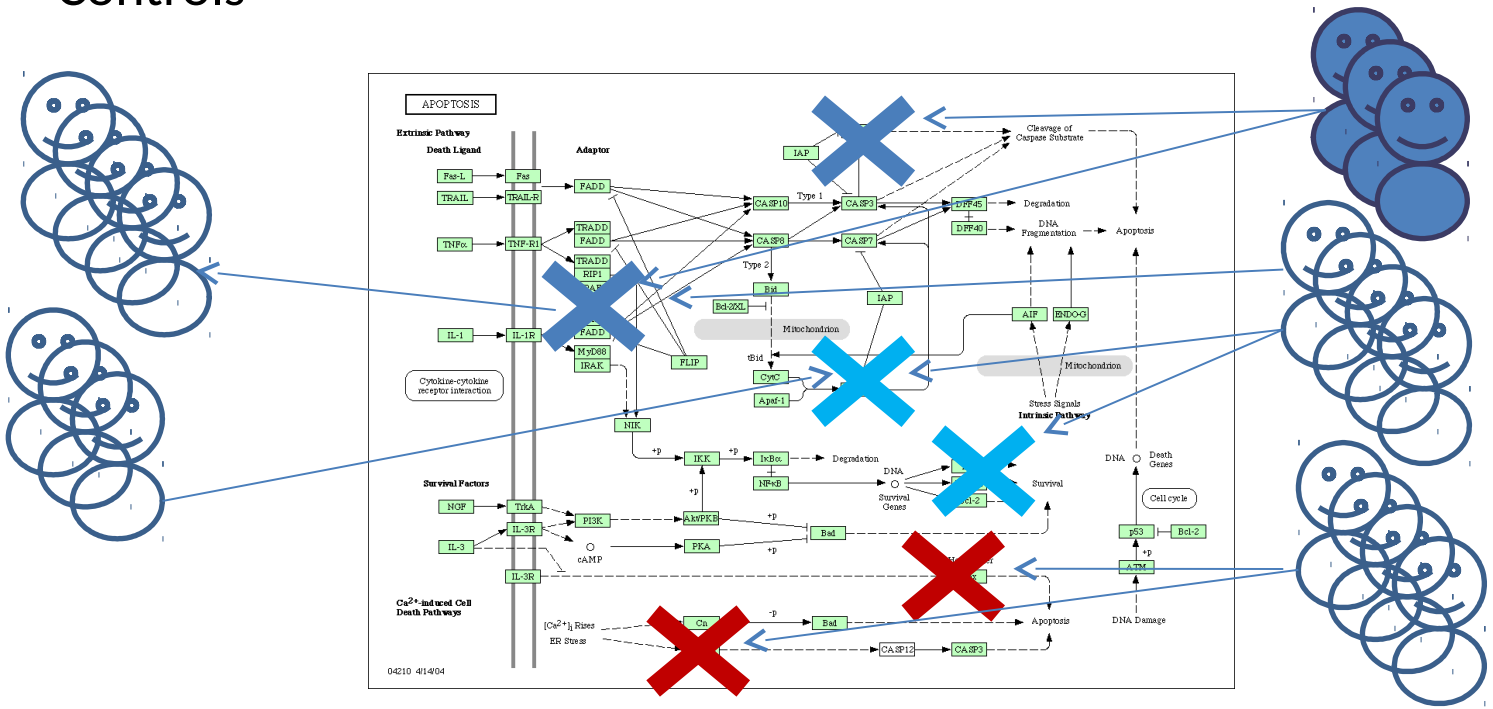
	Families					
	1	2	3	4	5	6
Variants	3403	82	4	0	0	0
Genes	2560	331	35	8	1	0

Problem: how to prioritize putative candidate genes

Clear individual gene associations are difficult to find in some diseases

Controls

Cases



They can have different mutations (or combinations).

Many cases have to be used to obtain significant associations to many markers.

The only common element is the pathway (yet unknow) affected.

Conclusions

NGS is revolutionizing how we do genome research

But it will also revolutionize our lives….

If we manage to process and analyze ALL the DATA