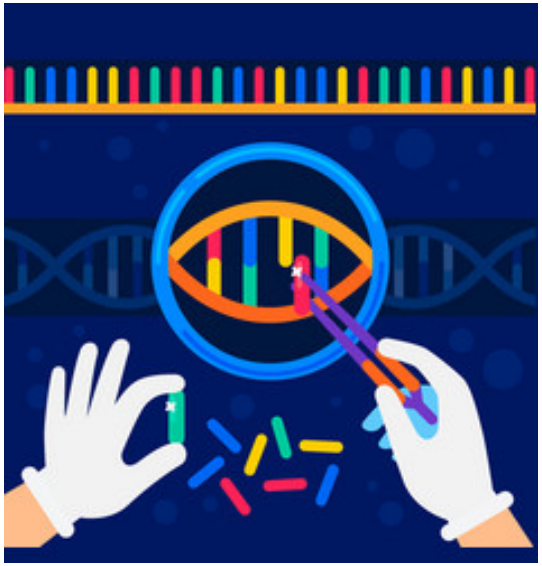
Part III. High-Throughput Sequencing and Applications

Why Sequence DNA?

- All genes available for an organism to use -- a very important tool for biologists
- Not just sequence of genes, but also positioning of genes and sequences of regulatory regions



Evolution of Sequencing Technology

First Generation Sequencing

- *Sanger Sequencing* [**1977**]

Next (or second) Generation Sequencing (NGS)

- *Massively parallel sequencing* [**2006/2007**]

Third Generation Sequencing

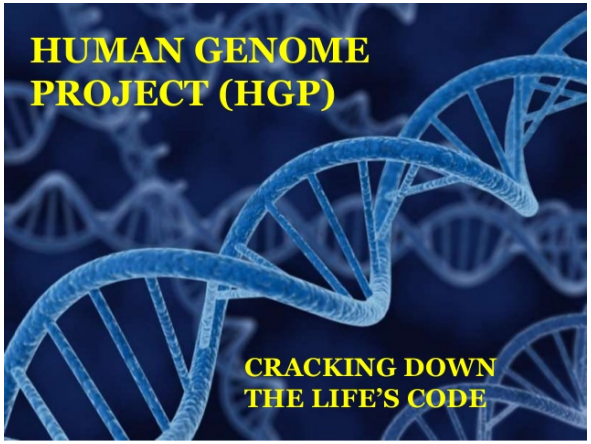
- *Singel molecule sequencing* [**2013**]

Fourth Generation Sequencing?

High-Throughput Sequencing (HTS)

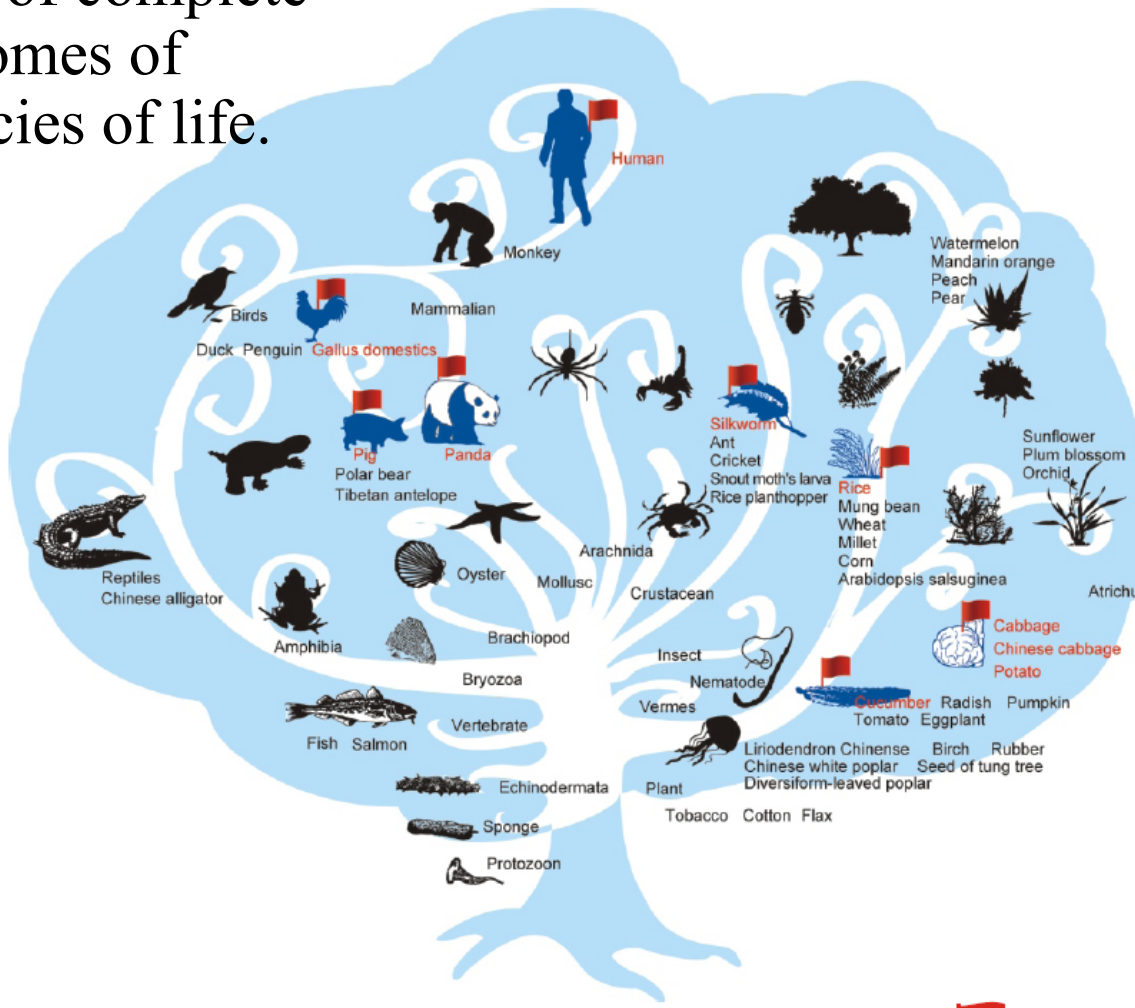
The Advancement of HTS by Human Genome Project

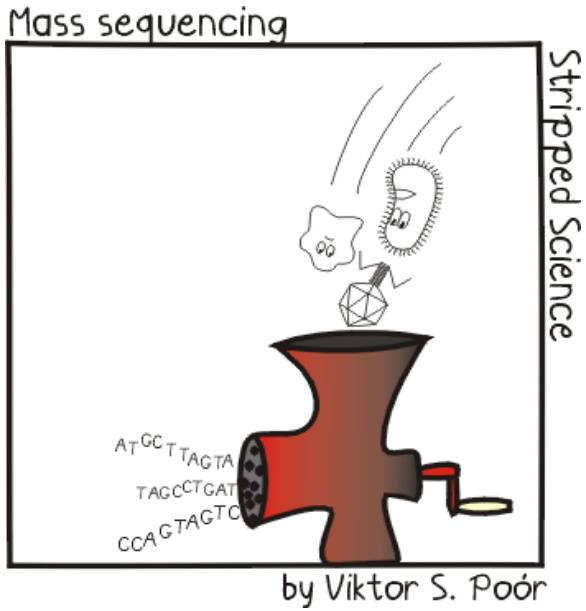
- It all starts with human genome project (1990).
  - What is in “the book of life”?
  - How many genes are there in human genome?
  - The International Human Genome Sequencing Consortium published their analysis of the 'draft' sequence in February 2001; and the finished, 'gold standard' sequence in 2004.



HTS advantages:

- Rapid
- Low cost
- Enables the sequencing of complete DNA sequences, or genomes of numerous types and species of life.





HTS Sequencers

Next generation sequencers

- 454 / Roche sequencing
- Illumina (Solexa) sequencing
- SOLiD systems
- Ion Torrent sequencing

Third generation sequencers

- PacBio RS II (Pacific Biosciences)
- MinION (Oxford Nanopore)

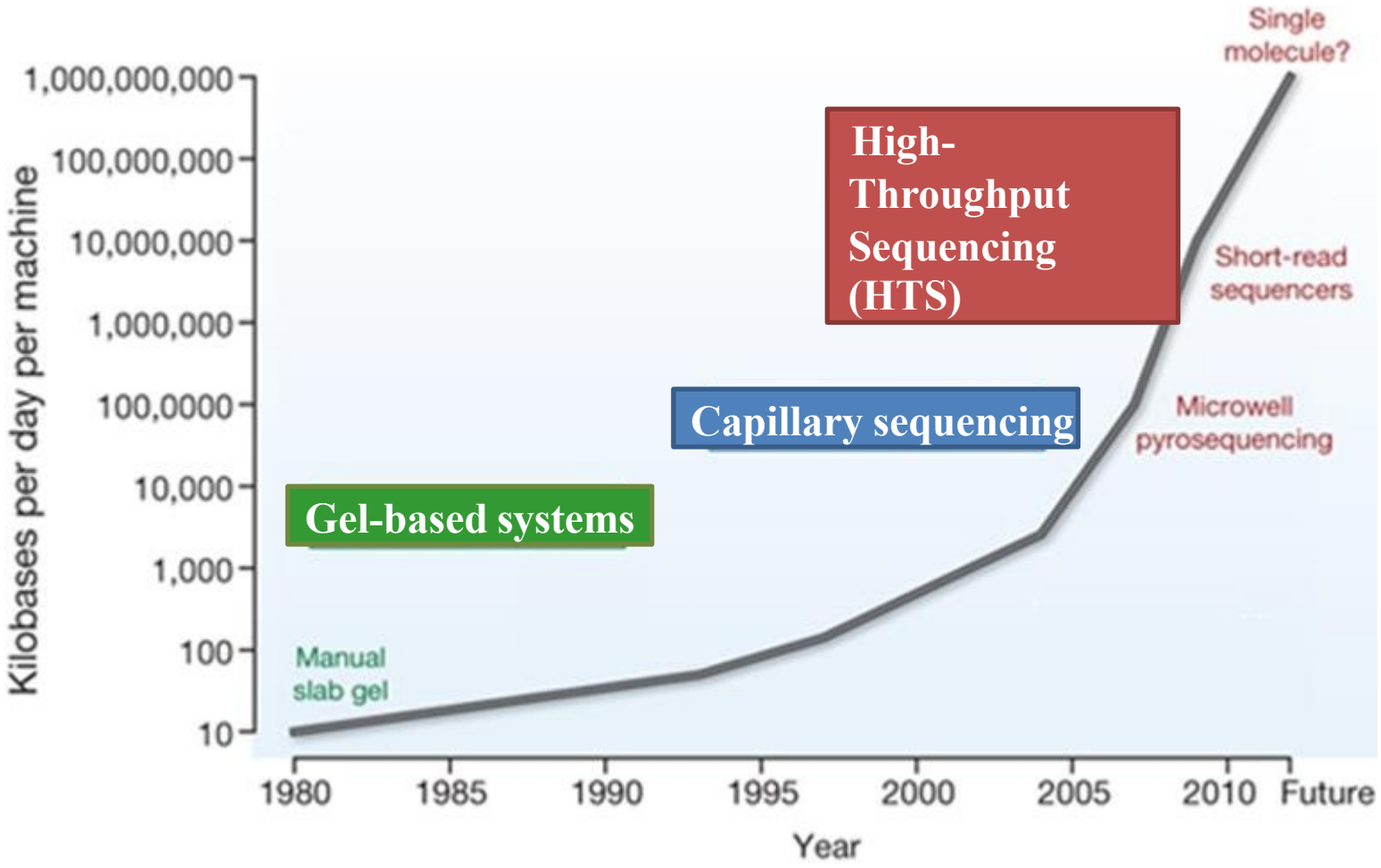
	Focused power.	Flexible power.	Production power.	Population power.
				
	MiSeq Series	NextSeq Series	HiSeq Series	HiSeq X Series
Key Methods	Small genome, amplicon, and targeted gene panel sequencing.	Everyday genome, exome, transcriptome sequencing, and more.	Production-scale genome, exome, transcriptome sequencing, and more.	Population- and production-scale human whole-genome sequencing.

	 HiSeq 2500		 HiSeq 3000	 HiSeq 4000	 HiSeq X Five*	 HiSeq X Ten*
Run Mode	Rapid Run	High-Output	N/A	N/A	N/A	N/A
Flow Cells per Run	1 or 2	1 or 2	1	1 or 2	1 or 2	1 or 2
Output Range	10-300 Gb	50-1000 Gb	125-750 Gb	125-1500 Gb	900-1800 Gb	900-1800 Gb
Run Time	7-60 hours	<1-6 days	<1-3.5 days	<1-3.5 days	<3 days	<3 days
Reads per Flow Cell†	300 million	2 billion	2.5 billion	2.5 billion	3 billion	3 billion
Maximum Read Length	2 x 250 bp	2 x 125 bp	2 x 150 bp	2 x 150 bp	2 x 150 bp	2 x 150 bp
System Overview	Power and efficiency for large-scale genomics.		Maximum throughput and lowest cost for production-scale genomics.	Maximum throughput and lowest cost for production-scale genomics.	Maximum throughput for production-scale human whole-genome sequencing.	Maximum throughput and lowest cost population-scale human whole-genome sequencing.

Comparison of Sequencing Technologies

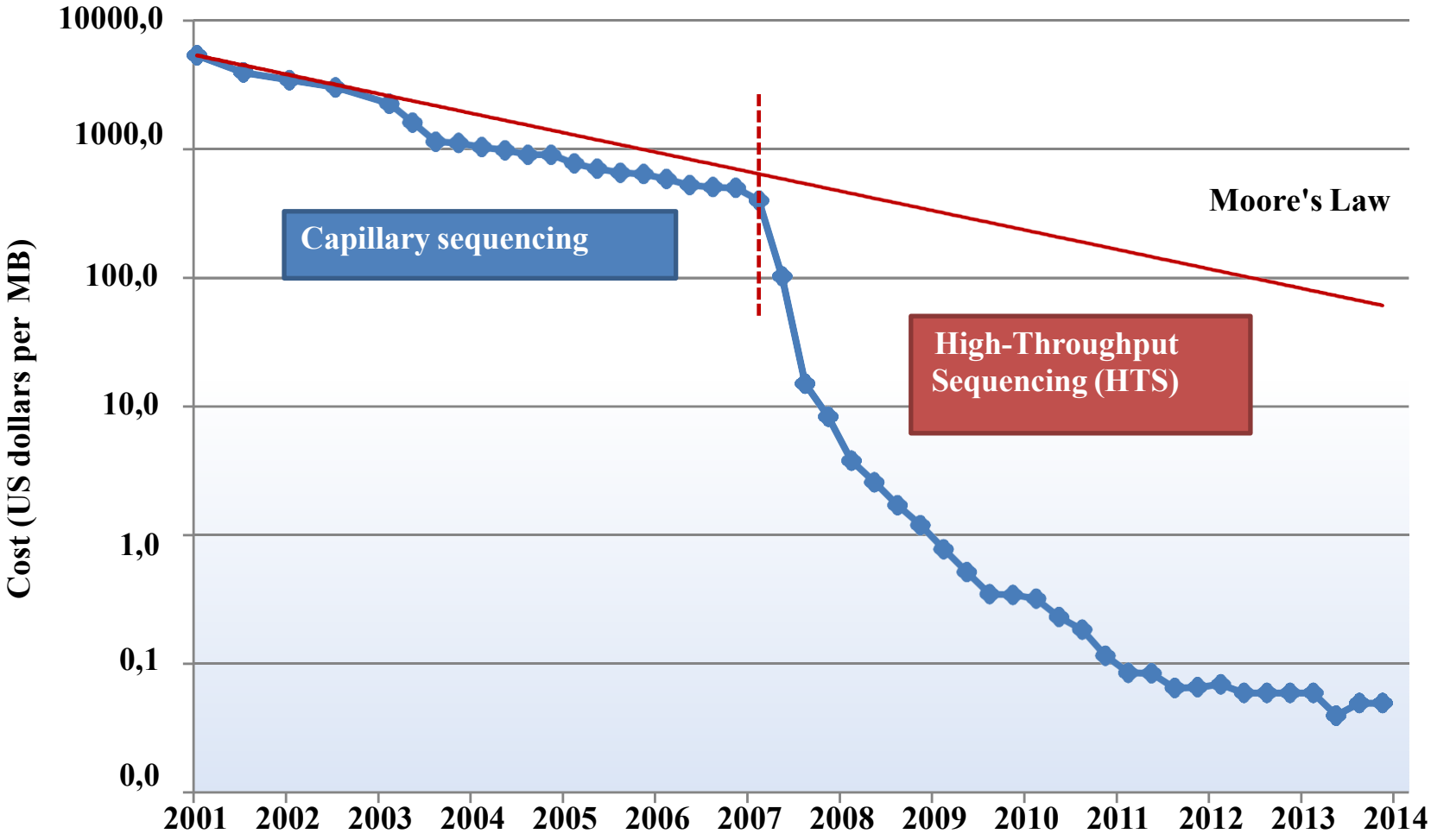
Sequencer	Sanger 3730xl	454 GS	Ion Torrent	SOLiDv 4	Illumina HiSeq 2000	Pac Bio
Mechanism	Dideoxy chain termination	Pyrosequencing	Detection of hydrogen ion	Ligation and two-base coding	Reversible nucleotides	Single molecule real time
Read length	400-900 bp	700 bp	~400 bp	50 + 50 bp	100 bp PE	1000~10000 bp
Error Rate	0.001%	0.1%	2%	0.1%	2%	10-15%
Output data (per run)	100 KB	1 GB	100 GB	100 GB	1 TB	10 GB

Increase in Sequencing Capacity



Graph from Macmillan Publishers Ltd: Nature 458, 719-724 (2009)

Decrease in Sequencing Costs



Data from the NHGRI Genome Sequencing Program (GSP)

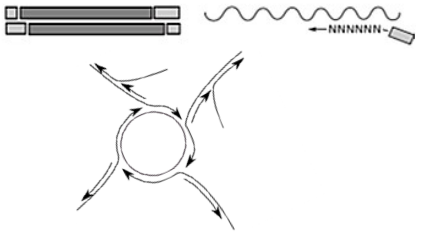
General Methodology for HTS

1. Sampling

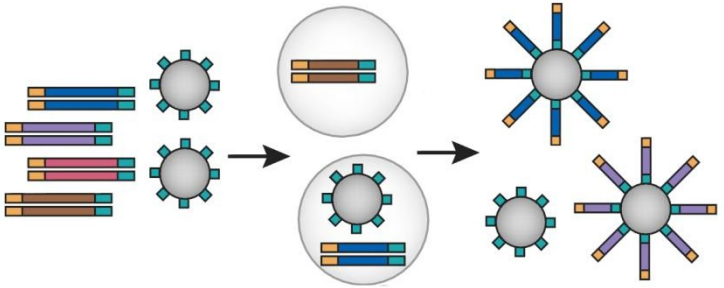
2. Sample preparation

3. Pre-amplification

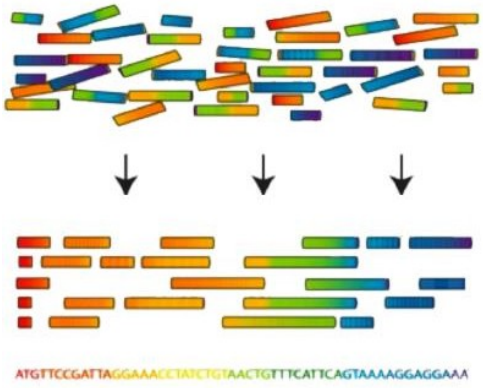
- Random amplification
- Targeted amplification



4. Preparation of sequencing library

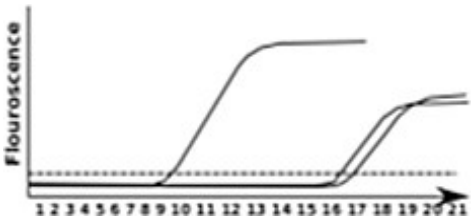


5. High-throughput sequencing



6. Bioinformatics

7. Follow-up



VectorStock

1. Sampling

- Clinical material
  - Collect samples according to applicable recommendations
  - Safe transport & storage of samples (prevent degeneration)
  - Correct & complete documentation
- Cultivated material
  - Normal laboratory procedures

2. Sample Preparation

- Homogenization
- Filtration

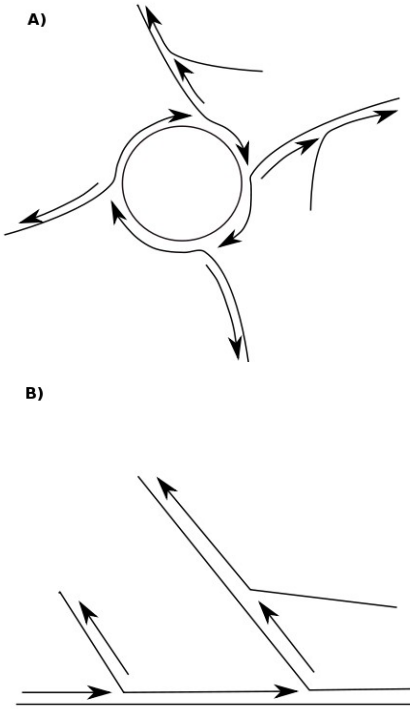
- Enrichment
  - Ultracentrifugation

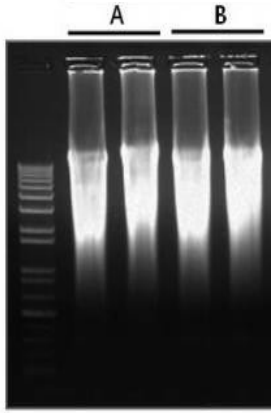
- Extraction (RNA or DNA)

3. Pre-Amplification

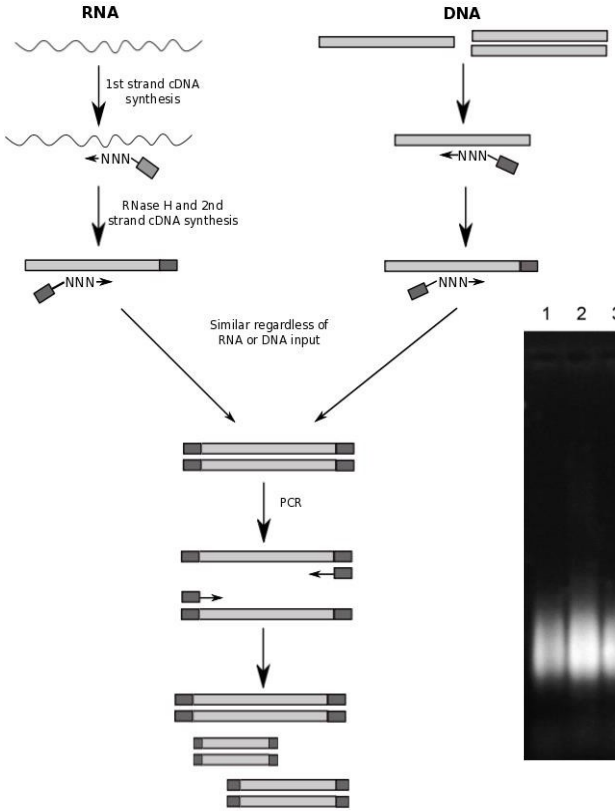
- **Targeted amplification (Amplicon)**
- **Random amplification**

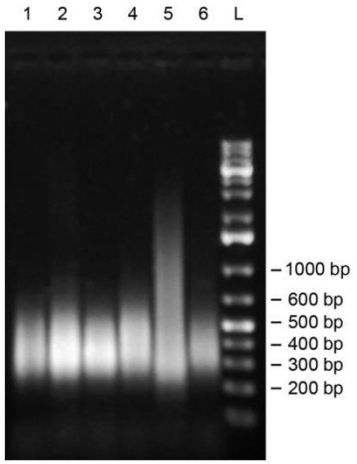
MDA (multiple displacement amplification) by *Phi29* DNA polymerase



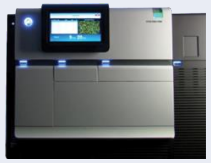


Sequence-independent, single-primer amplification (SISPA)





4 & 5. Library Preparation & Sequencing

Smaller bench-top sequencer	Capacity/Time	Full size sequencer	Capacity/Time
MiSeq Illumina 	~6 Gb (2 x 300) 36 hours	HiSeq Illumina 	~600 Gb (2 x 100) 11 days
Ion Torrent Life Technologies 	~1 Gb (400bp) 4 hours	Ion Proton Life Technologies 	~30 Gb (150bp) 8-10 hours
454 Junior Roche 	35 Mb (up to 400bp) 12 hours	454 GS FLX+ Roche 	700 Mb (up to 1kb) 23 hours
MinION Oxford Nanopore 	(up to 10kb)	PacBio RS II Pacific Biosciences 	~300Mb (up to12kb) 2 hours

Mb = mega base pairs: 1,000,000 bp; Gb = giga base pairs: 1,000,000,000 bp

6. Bioinformatics & Computational Genomics

Sequence data

- Quality control
- Assembly of reads into contigs
- Homology searches

Bacteria

Bacteriophages

Virus

Host animal

Unknow

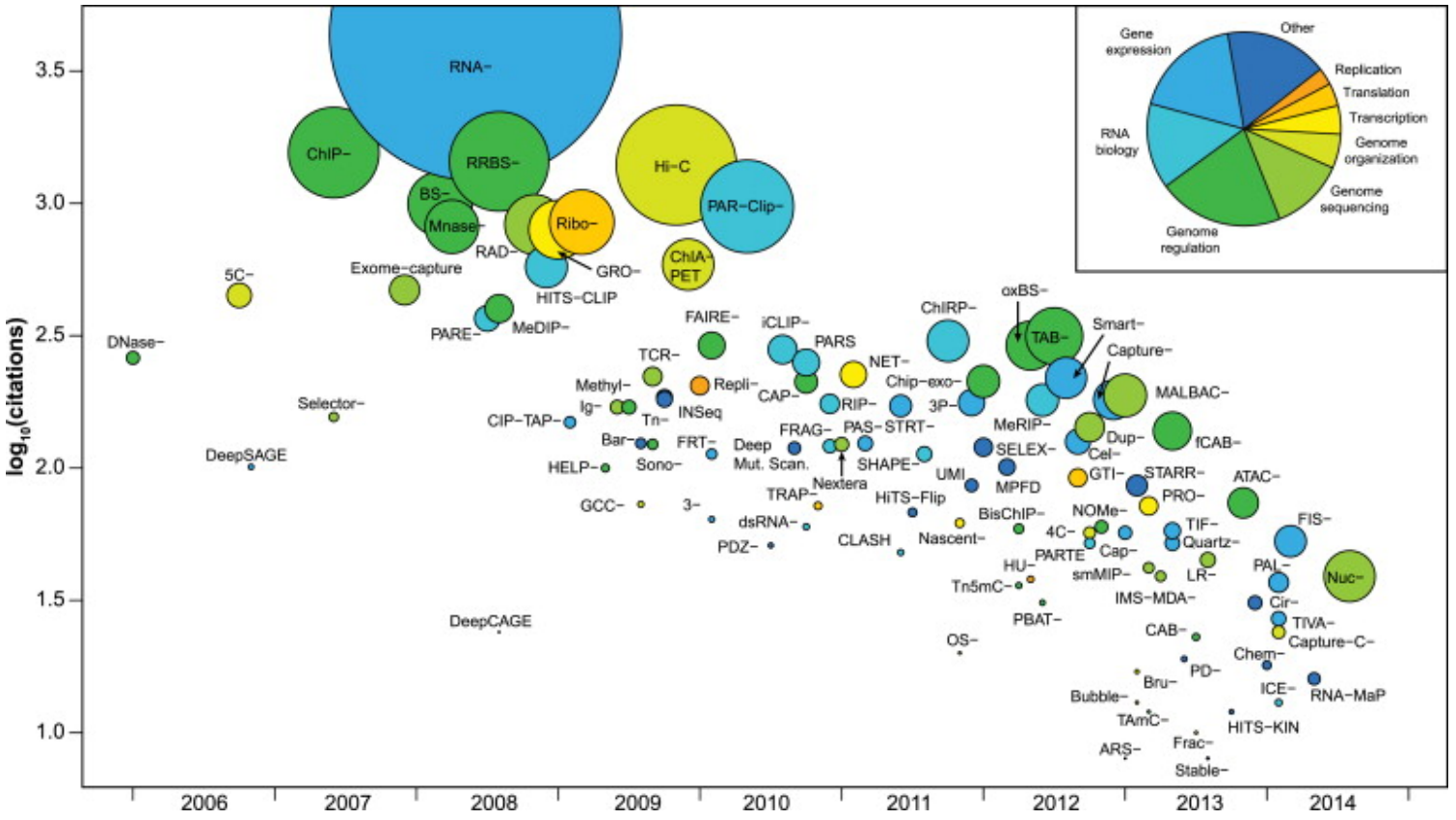
- Bioinformatics filtering, sorting and classification pipeline
- Alignments and annotations

Illumina Sequencing by Synthesis (video)

HTS Applications

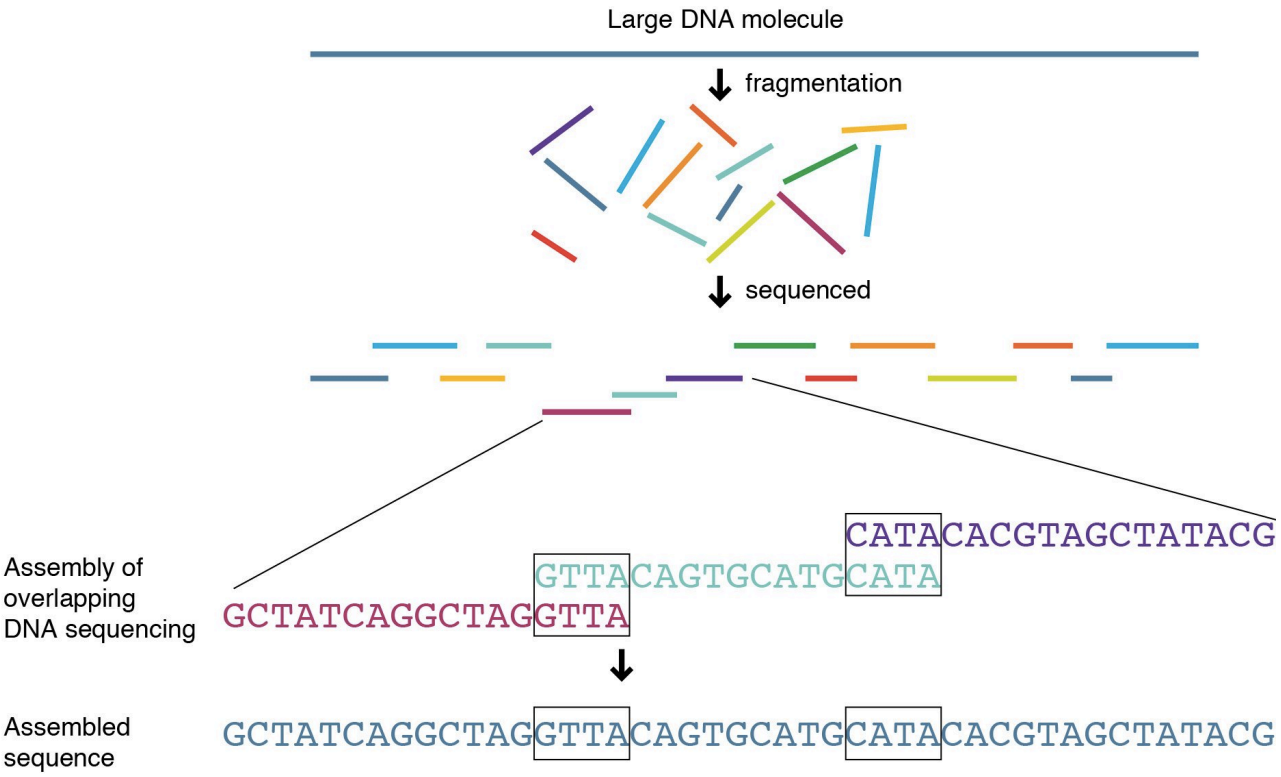
- Whole genome sequencing
- Whole exome sequencing
- RNA sequencing
- ChIP-seq/ChIP-exo
- CLIP-seq
- GRO-seq/PRO-seq
- Bisulfite-Seq
- ……

Selected HTS Applications



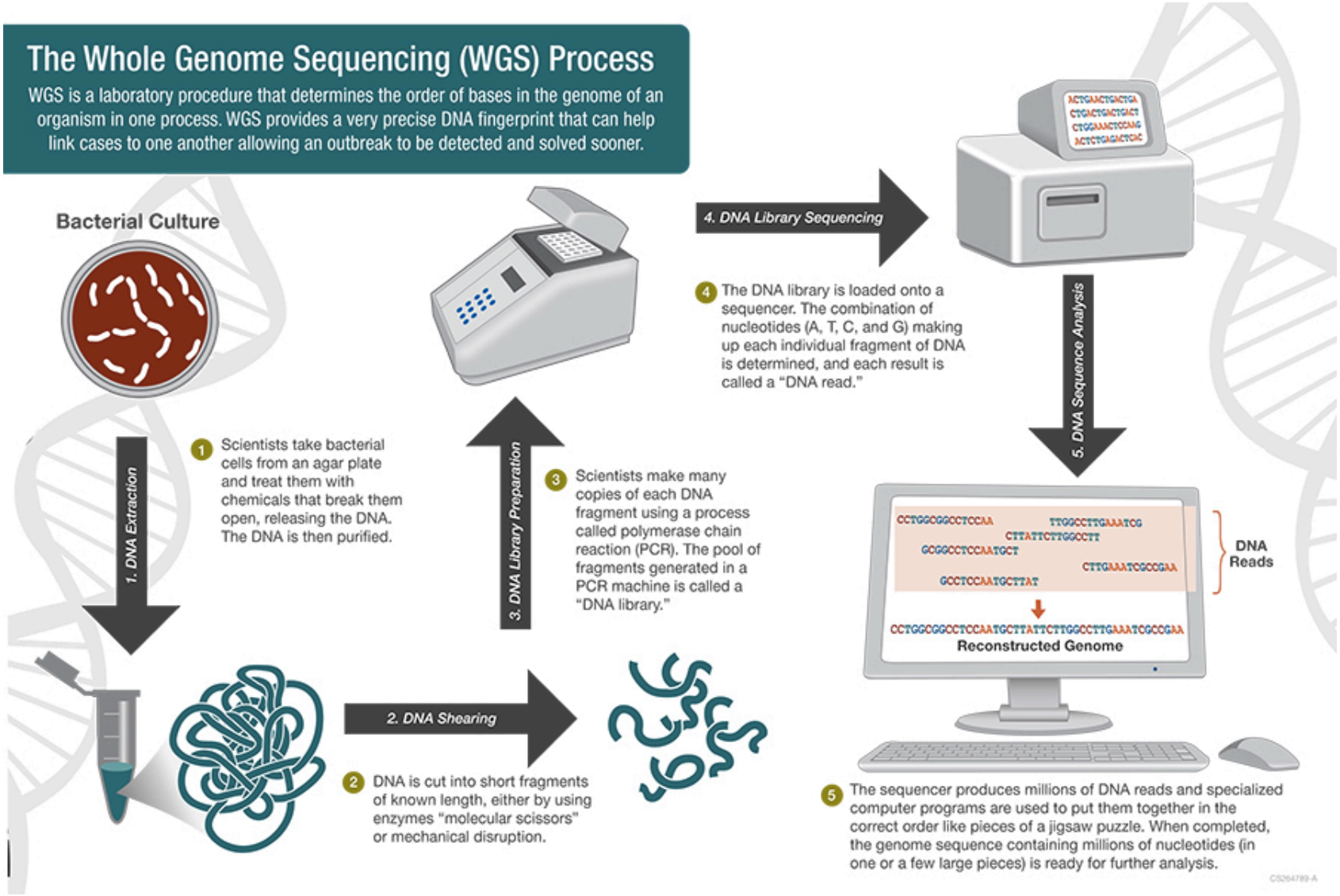
Molecular Cell, 58, 586–597 (May, 2015)

HTS application: whole genome sequencing



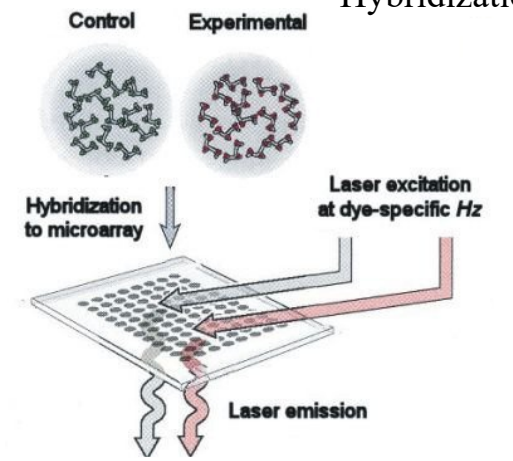
The Whole Genome Sequencing (WGS) Process

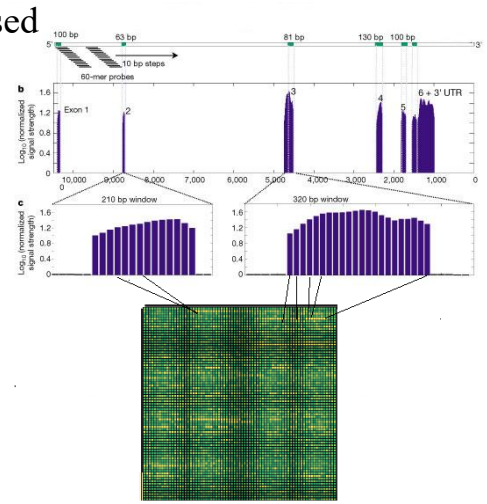
WGS is a laboratory procedure that determines the order of bases in the genome of an organism in one process. WGS provides a very precise DNA fingerprint that can help link cases to one another allowing an outbreak to be detected and solved sooner.

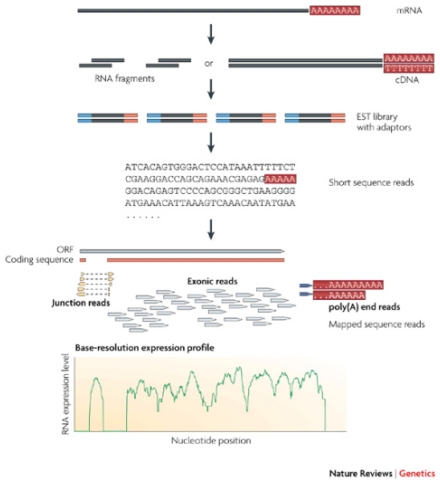


HTS Application: RNAseq
The evolution of transcriptomics

Hybridization-based







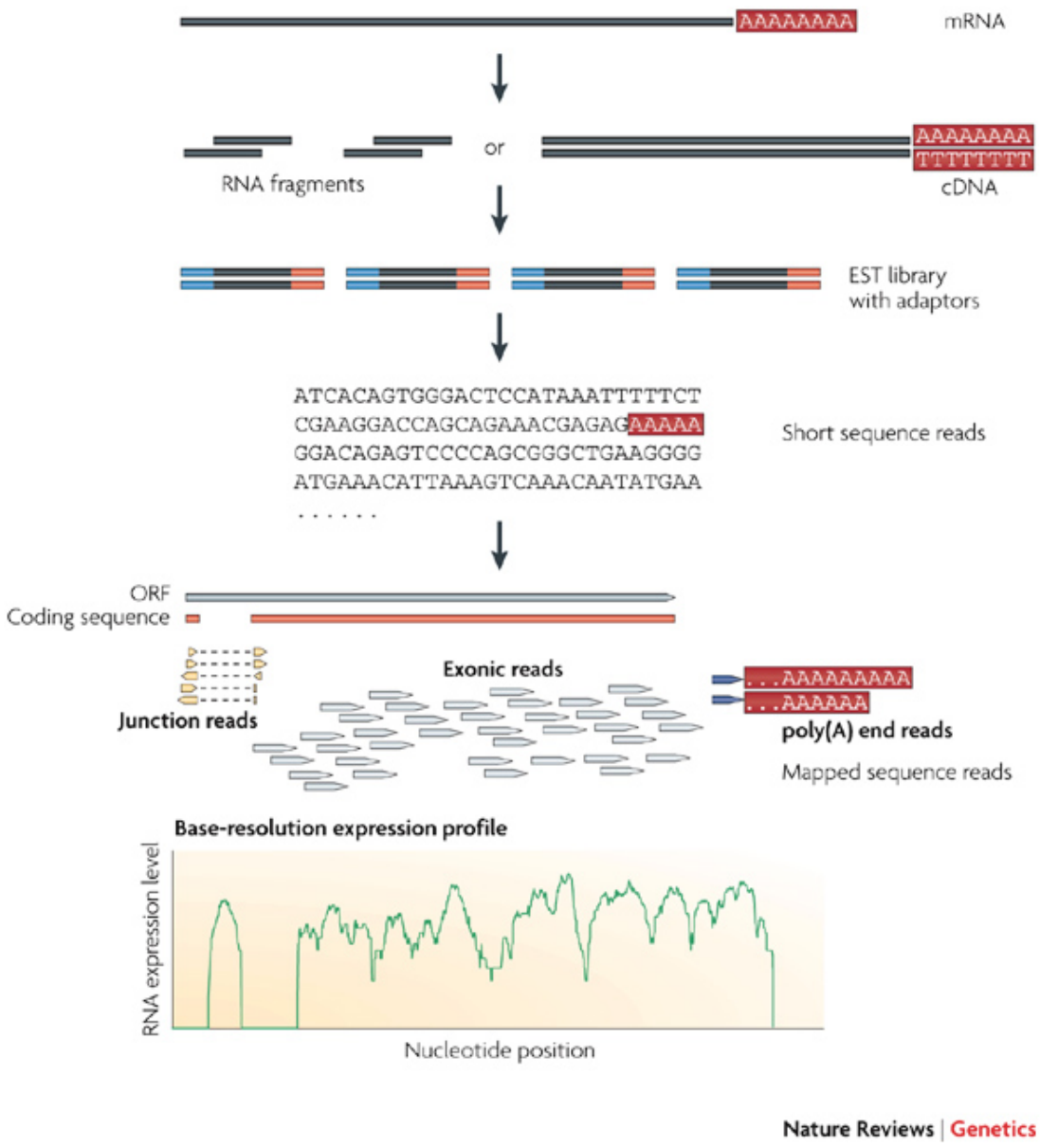
RNA-seq is still a technology under active development

1995 P. Brown, et. al.
Gene expression profiling using spotted cDNA microarray: expression levels of known genes

2002 Affymetrix, whole genome expression profiling using tiling array: identifying and profiling novel genes and splicing variants

2008 many groups, mRNA-seq: direct sequencing of mRNAs using next generation sequencing techniques (NGS)

How RNA-seq works



Sample preparation

Next generation sequencing (NGS)

Data analysis:
- ✓Mapping reads
- ✓Visualization
- ✓De novo assembly
- ✓Quantification

Figure from Wang et. al, **RNA-Seq: a revolutionary tool for transcriptomics,** Nat. Rev. Genetics 10, 57-63, 2009)**.**

RNA-seq Application

- Differential expression
- Gene fusion
- Alternative splicing
- Novel transcribed regions
- Allele-specific expression
- RNA editing
- Transcriptome for non-model organisms

Benefits & Challenges of RNA-seq

Benefits:

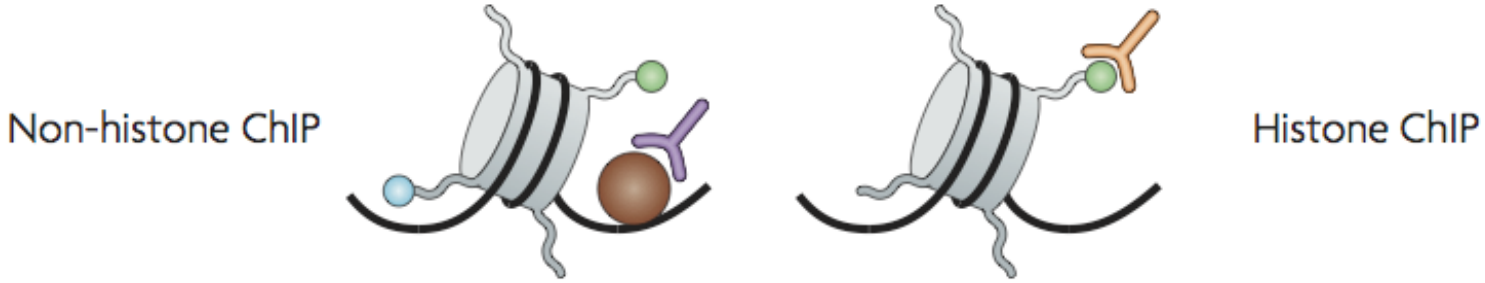
- Independence on prior knowledge
- High resolution, sensitivity and large dynamic range
- Unravel previously inaccessible complexities

Challenges:

- Interpretation is not straightforward
- Procedures continue to evolve

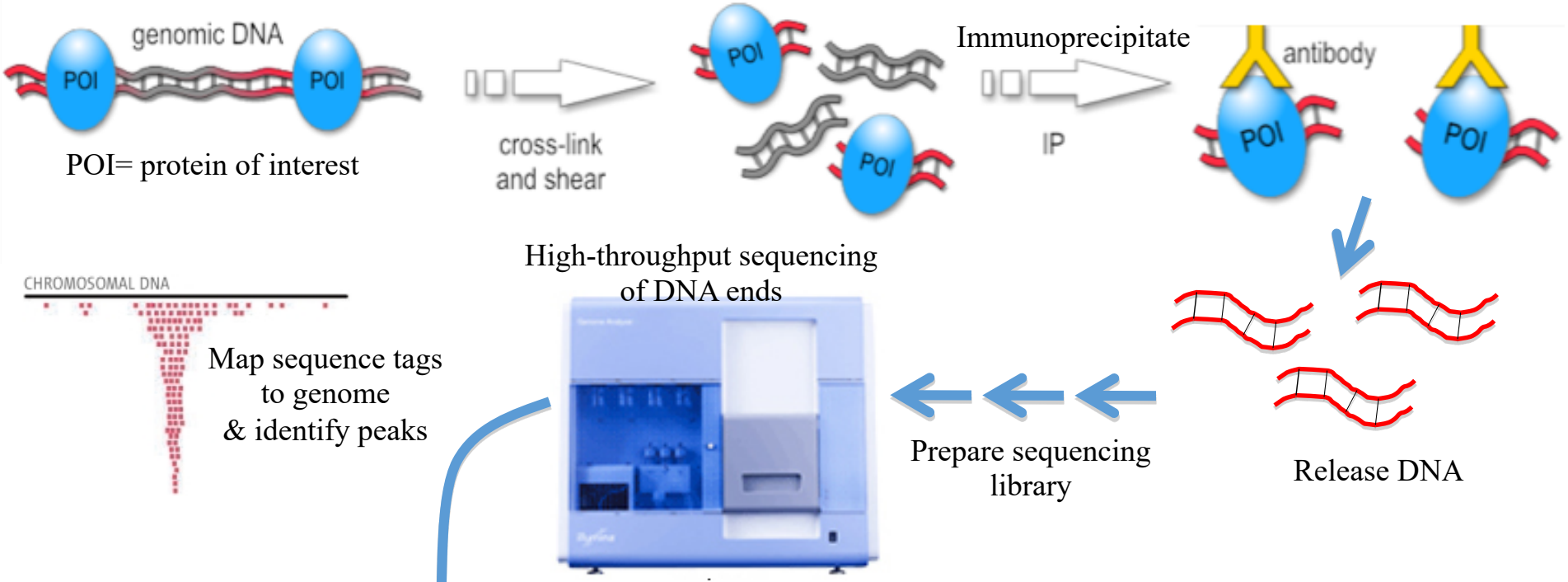
ChIP-seq is Key to Epigenome Mapping

- Binding site identification and discovery of binding sequence motifs (Non-histone ChIP)
- Epigenomic gene regulation and chromatin structure (Histone ChIP)



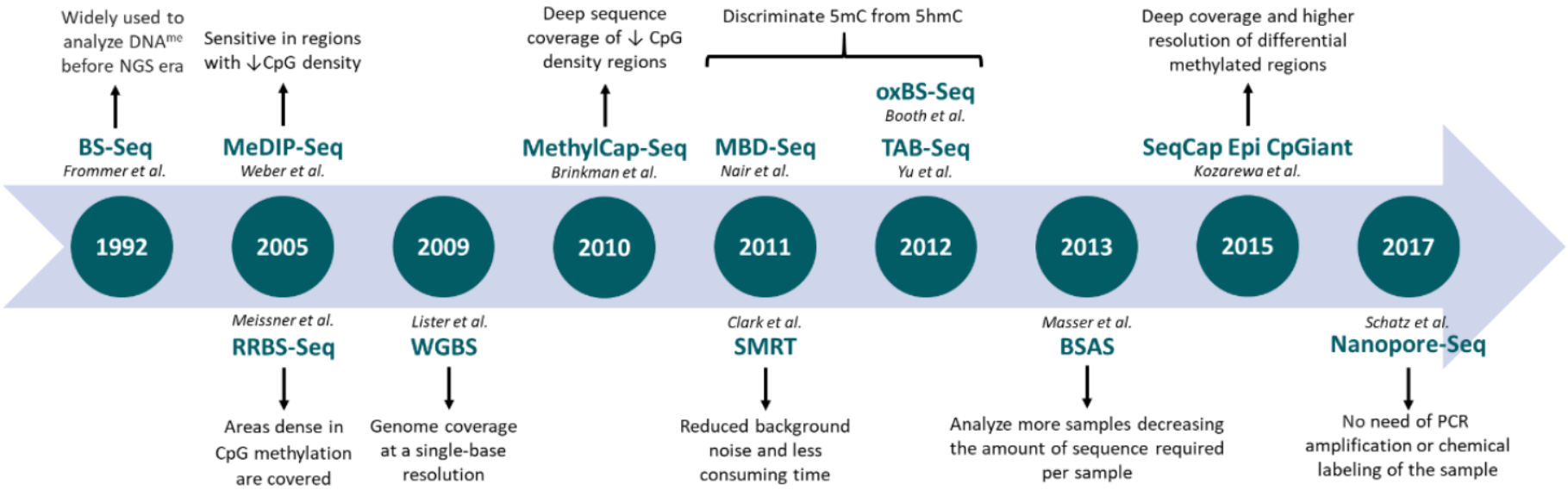
- **Transcription Factors**
  - Where are they?
  - Sequence preference
  - Correlation with gene expression
- **Chromatin Marks**
  - Where are they?
  - What combinations do they come in
  - How do they relate to biological status

Basic ChIP-Seq Workflow



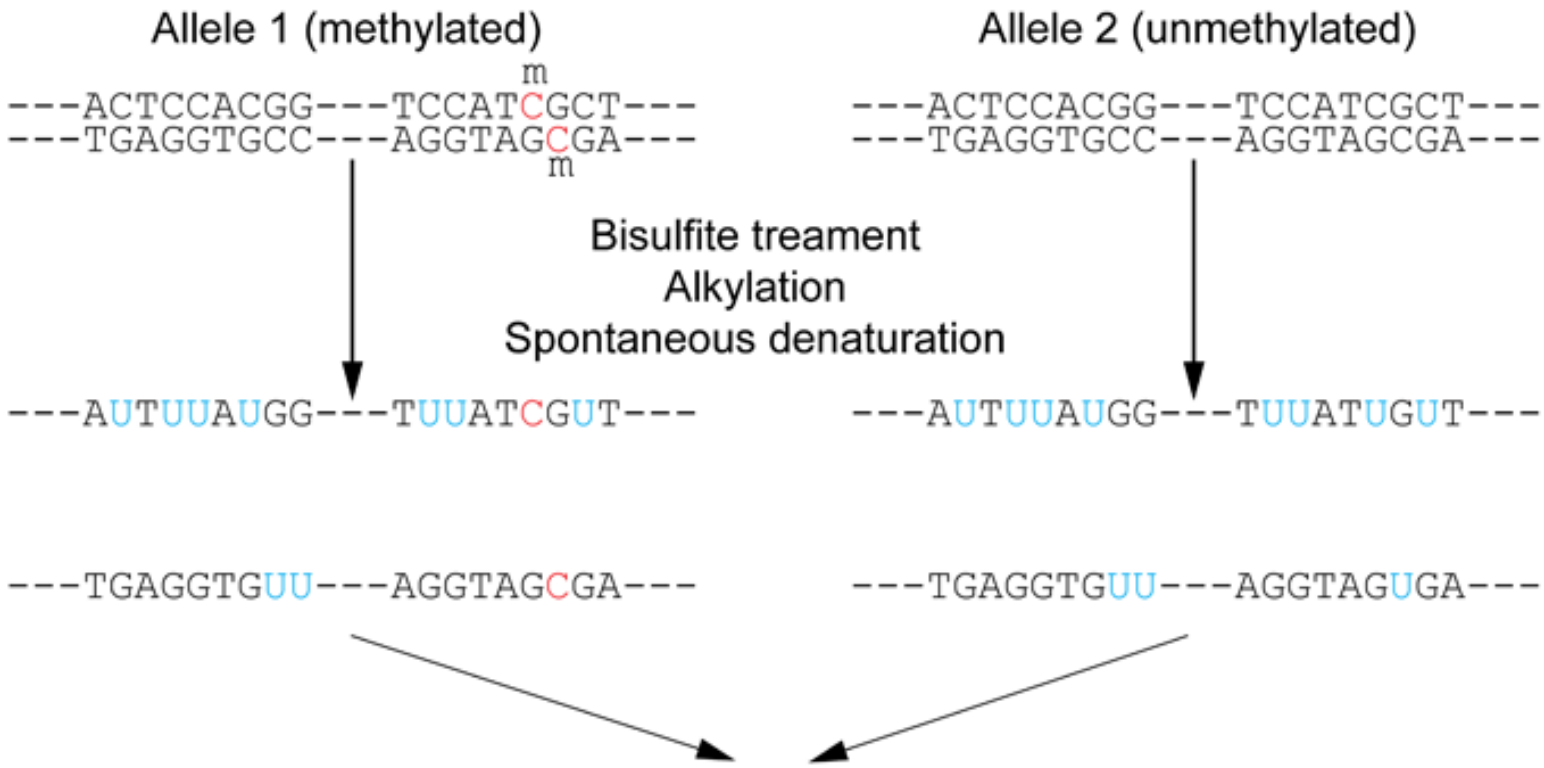
Adapted from slide set by: Stuart M. Brown, Ph.D.,
Center for Health Informatics & Bioinformatics, NYU School of Medicine

DNA Methylation Profiling (Methylome) by HTS



BS-Seq: bisulfite sequencing;
MeDIP-Seq: methylated DNA immunoprecipitation sequencing;
RRBS-Seq: reduced representation bisulfite sequencing;
WGBS: whole genome bisulfite sequencing;
MethylCap-Seq: methylation capture sequencing;
MBD-Seq: methyl-CpG binding domain sequencing;
oxBS-Seq: oxidative bisulfite sequencing;
TAB-Seq: TET-associated bisulfite sequencing;
BSAS: bisulfite amplicon sequencing

Bisulfite-seq for Methylome Study

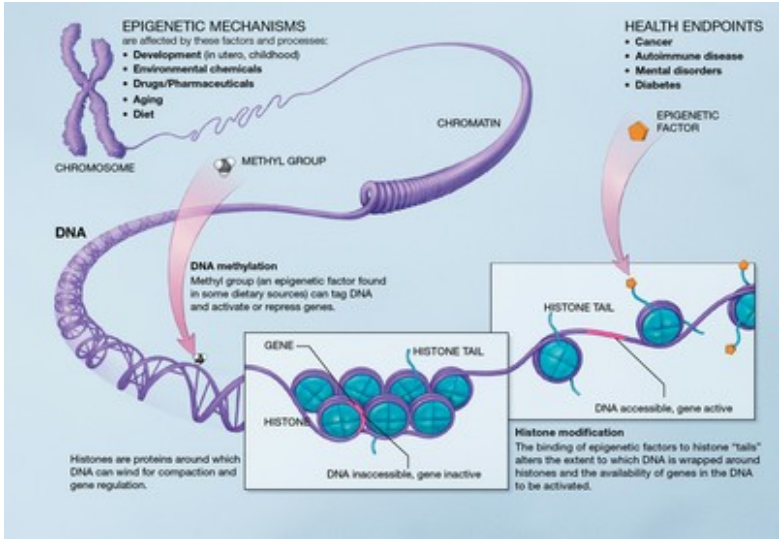


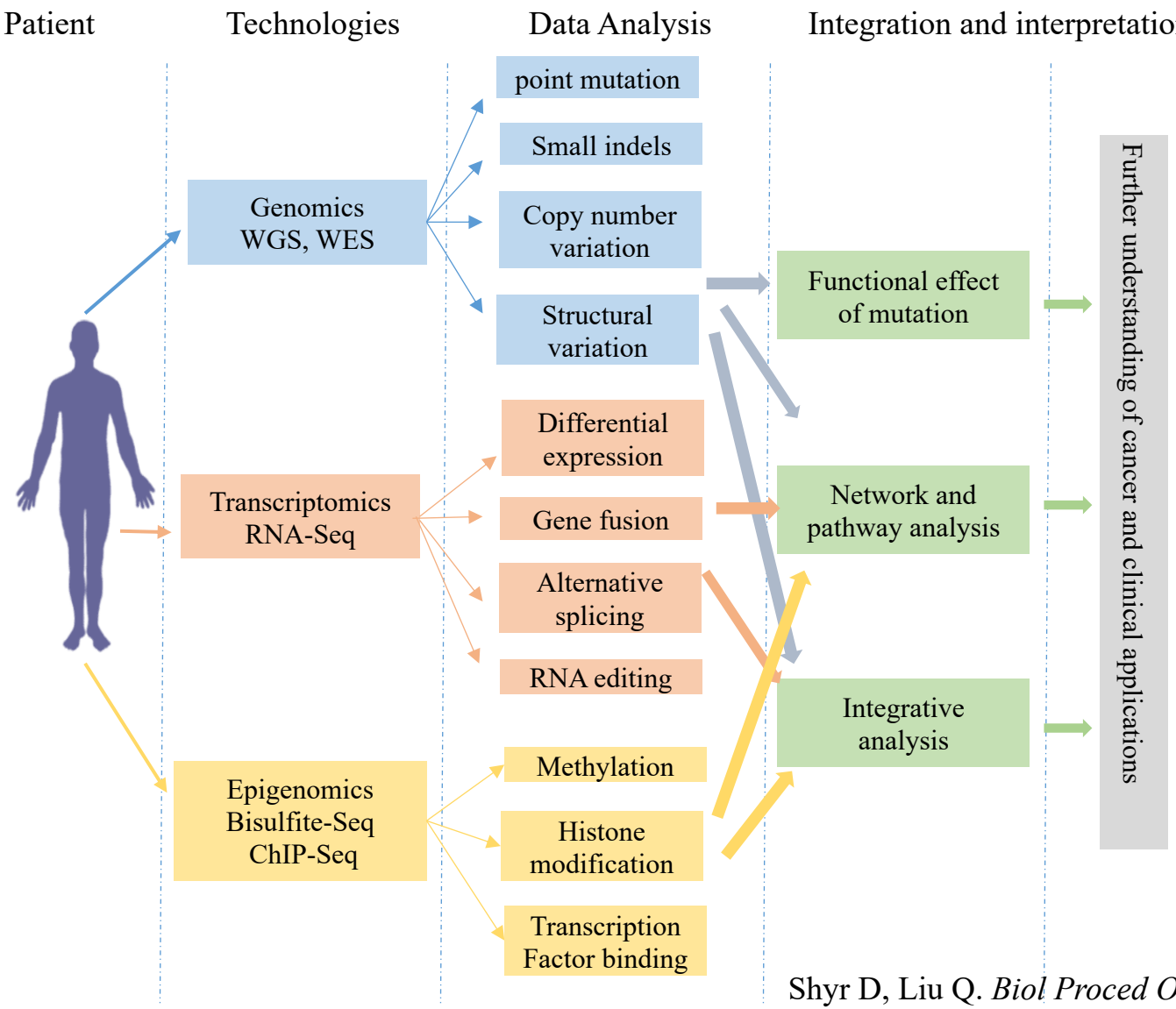
Sequencing: unemethylated cytosines read as thymidine in sense strand; adenine in the anti-sense strand. Other technologies evolved from here.

HTS Advances Human (Epi)Genome Project

- Human Genome Project (1990-2003)
  - Sequenced all ~20,000 genes in our DNA
  - 3 billion basepairs, ~3 billion dollars
  - Only 1.5% of genome codes for proteins!

- Epigenomics Road Map (2008-Present)
  - Goal: Create map of epigenome in multiple tissue types and cancers





Shyr D, Liu Q. *Biol Proced Online*. (2013)15,4

Application of HTS in Clinic

- Genomic testing
- Precision medicine

GENETICS VS. GENOMICS

GENETICS

- **Genetics** is the study of heredity.
- "Gene" refers to a specific sequence of DNA on a single chromosome.
- Genetics involves the study of functions and composition of the single gene.

GENOMICS

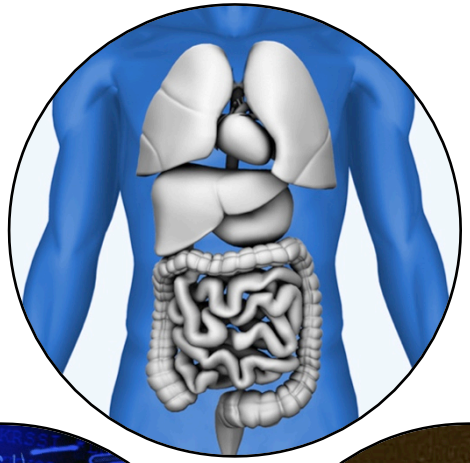
- **Genomics** is the study of the entirety of an organism's genes.
- "Genome" refers to an organism's entire genetic makeup.
- Genomics addresses all genes and their inter relationships.

Unlike genetics, genomics is not constrained to inheritable mutations. It identifies how your genetic makeup influences the course of a disease and, conversely, how environment, lifestyle, and drug treatments can trigger mutations that alter that course.

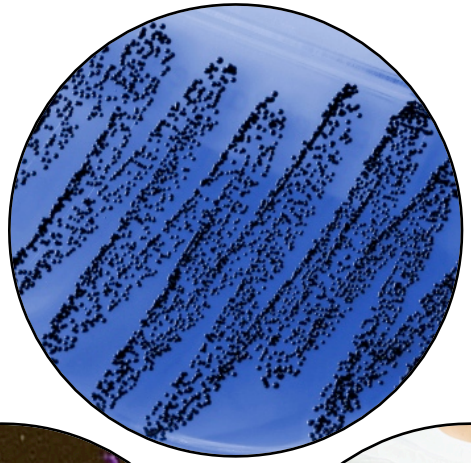
By understanding these ever-changing variables, doctors can make more informed choices in treatment, often preemptively.

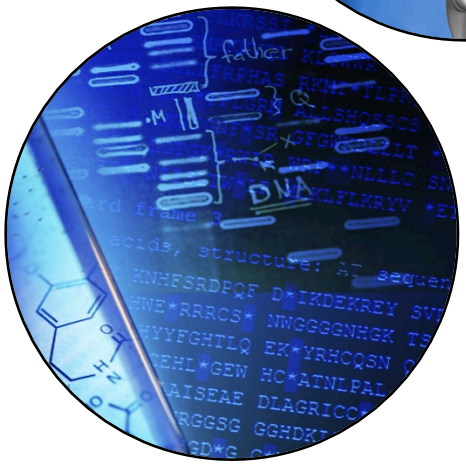
Applications of Genomic Testing in Medicine

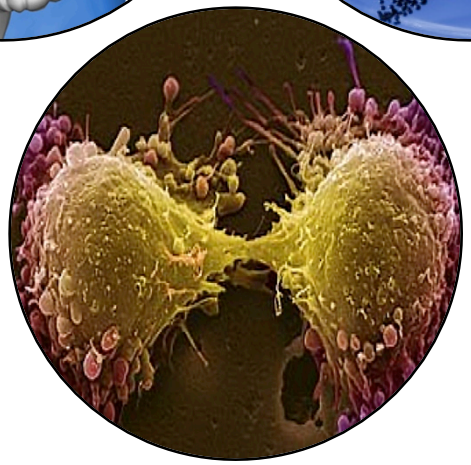
Transplant Medicine



Microbiology







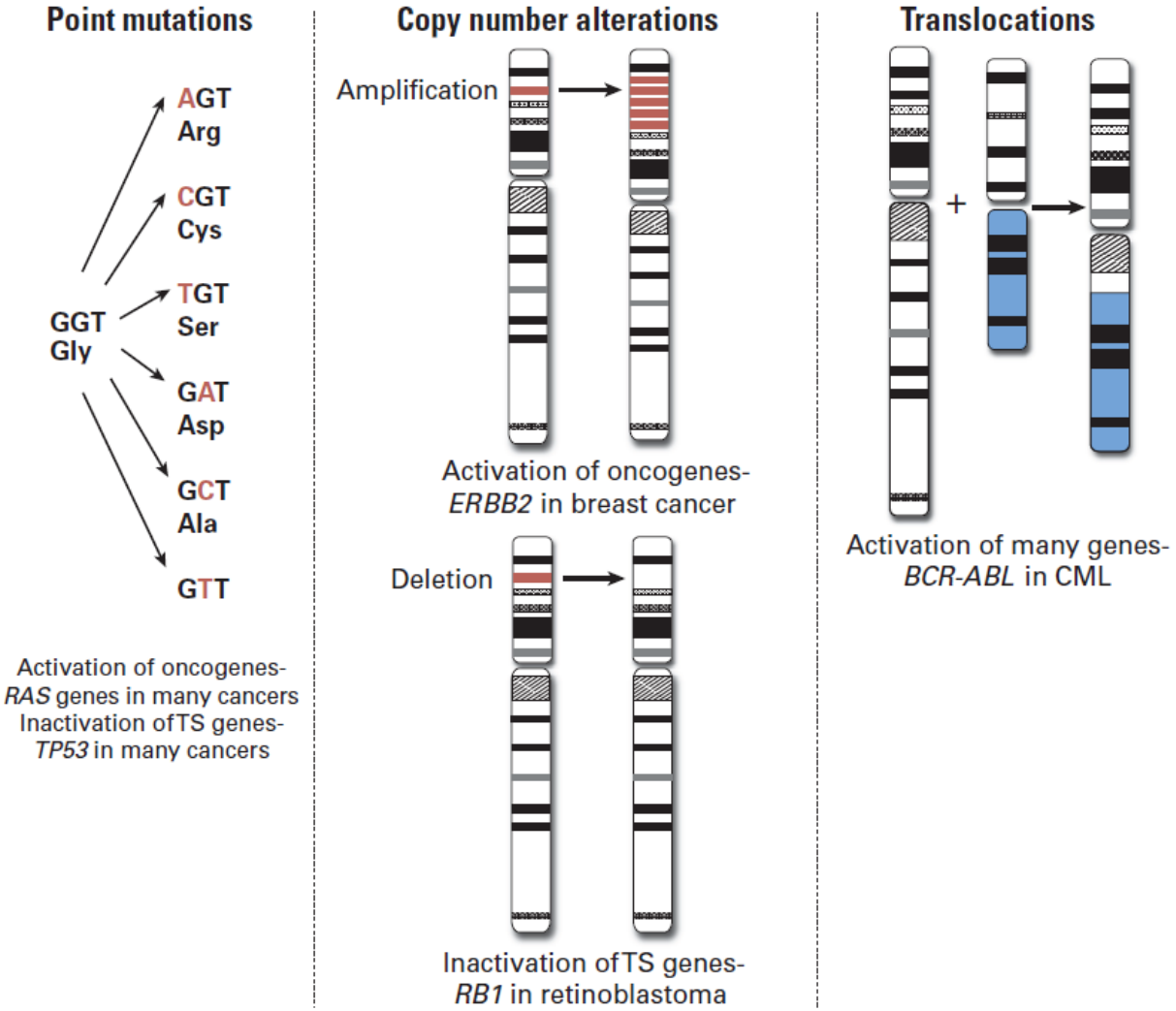
Inherited Disease

Cancer

Reproductive Health

Genomic Alterations in Cancer

Major classes



Macconaill LE, Garraway LA. Clinical implications of the cancer genome. Journal of Clinical Oncology, 28(35), 5219-5228 (2010).

Discovering unique therapies that treat an individual's cancer based on the specific genetic abnormalities of that person's tumor.

www.cancer.gov


I SAW ON THE INTERNET THAT IT WAS MY GENES THAT WERE MAKING ME FAT AND THOUGHT YOU COULD GET ME A NEW PAIR!
SIGH!
© Original Artist
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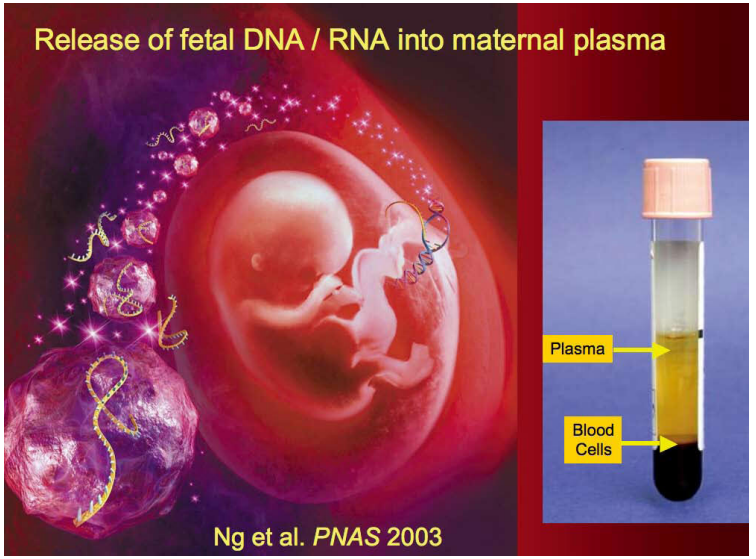
www.CartoonStock.com

HTS Application in Clinics: NIPT (Non-invasive Prenatal Testing

Prof. Dennis Lo, CUHK



無創產檢獲獎 港教授與霍金齊名

◀本報訊 十五年前率先發現孕婦血漿存有胎兒DNA，推出無創產前診斷技術的中文大學醫學院教授盧煜明，科研貢獻揚威海外，獲美國權威組織頒發「傑出研究獎」，是首名華人獲此殊榮。盧教授昨稱能將科學發現變成臨床測試，令人鼓舞，望港府及慈善團體增加撥款及捐助，繼續推動本港科研。

免流產風險測遺傳病

盧煜明為中大醫學院講座教授兼化學病理學系系主任，研究成果蜚聲國際。美國臨床化學協會和美國國家臨床生物化學學院本月中在洛杉磯向盧頒發傑出研究獎，過去曾有兩名諾貝爾得獎者獲獎。盧去年七月已獲英國皇家學會院士名銜，與光纖之父高錕、著名物理學家霍金等齊名。

盧煜明與科研團隊在九七年率先發現孕婦血漿存有胎兒DNA，一改醫學界認為母體與胎兒血液不相連的說法。一〇年再突破，成功破解胎兒基因圖譜，有助及早發現是否先天性遺傳病。他推出名為「敏兒安T21」無創產前診斷技術，診斷唐氏綜合症準確度達百分之九十九點一，免卻高危孕婦接受抽羊胎水或絨毛的入侵性檢查，毋須面對百分之〇點五至百分之一的流產風險。

中大醫學院為技術申請多項專利，授權本港私家醫院或診所搜集孕婦血液樣本測試。但盧早前公開批評有內地機構涉嫌侵權，使用同一技術檢測。盧教授昨表示，中大醫學院仍要諮詢法律意見是否追究涉嫌侵權事件。

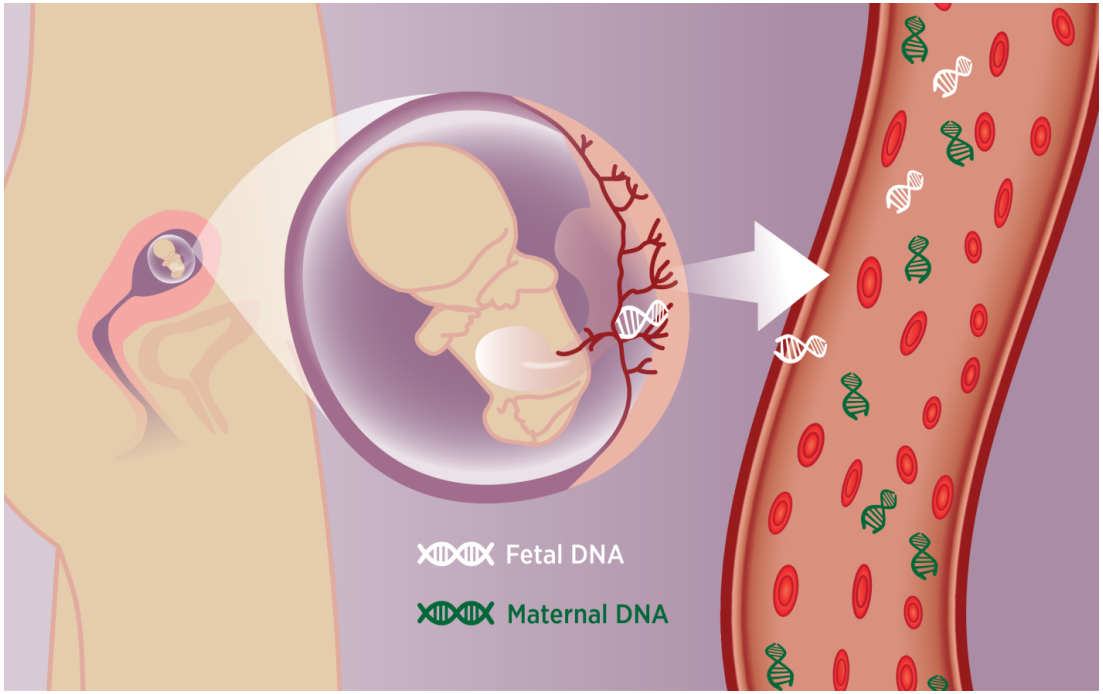
●盧煜明成功破解胎兒基因圖譜，為產前檢查技術領域的重要突破。

NAS

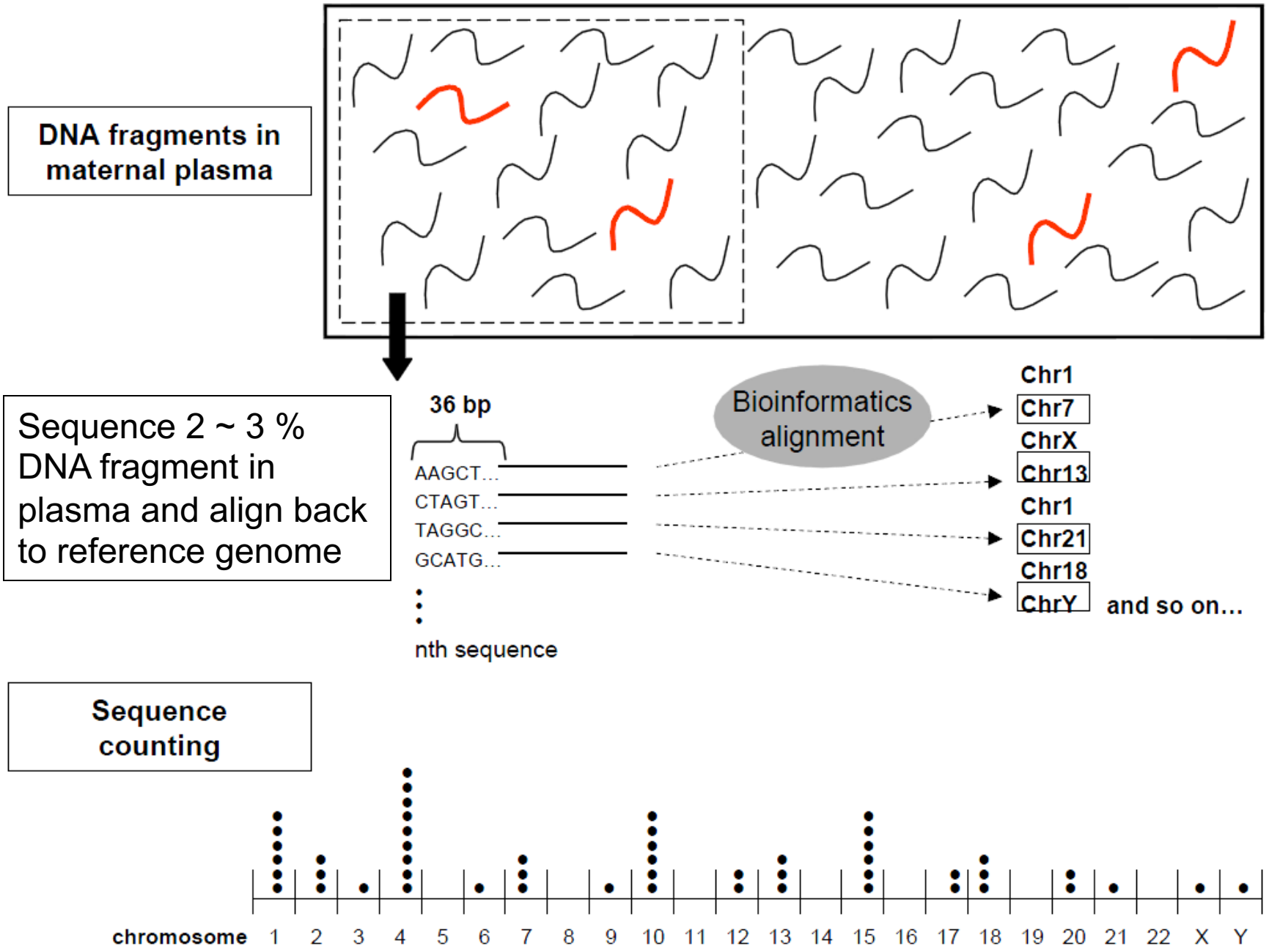
Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma

Rossa W. K. Chiu[a,b], K. C. Allen Chan[a,b], Yuan Gao[c,d], Virginia Y. M. Lau[a,b], Wenli Zheng[a,b], Tak Y. Leung[e], Chris H. F. Foo[f], Bin Xie[c], Nancy B. Y. Tsui[a,b], Fiona M. F. Lun[a,b], Benny C. Y. Zee[f], Tze K. Lau[e], Charles R. Cantor[g,1], and Y. M. Dennis Lo[a,b,1]

Cell Free Fetal DNA (cff DNA) in Maternal Blood



- < 1 % of total DNA in maternal circulation is fetal
- 5-30 % of cell-free DNA in maternal circulation is fetal

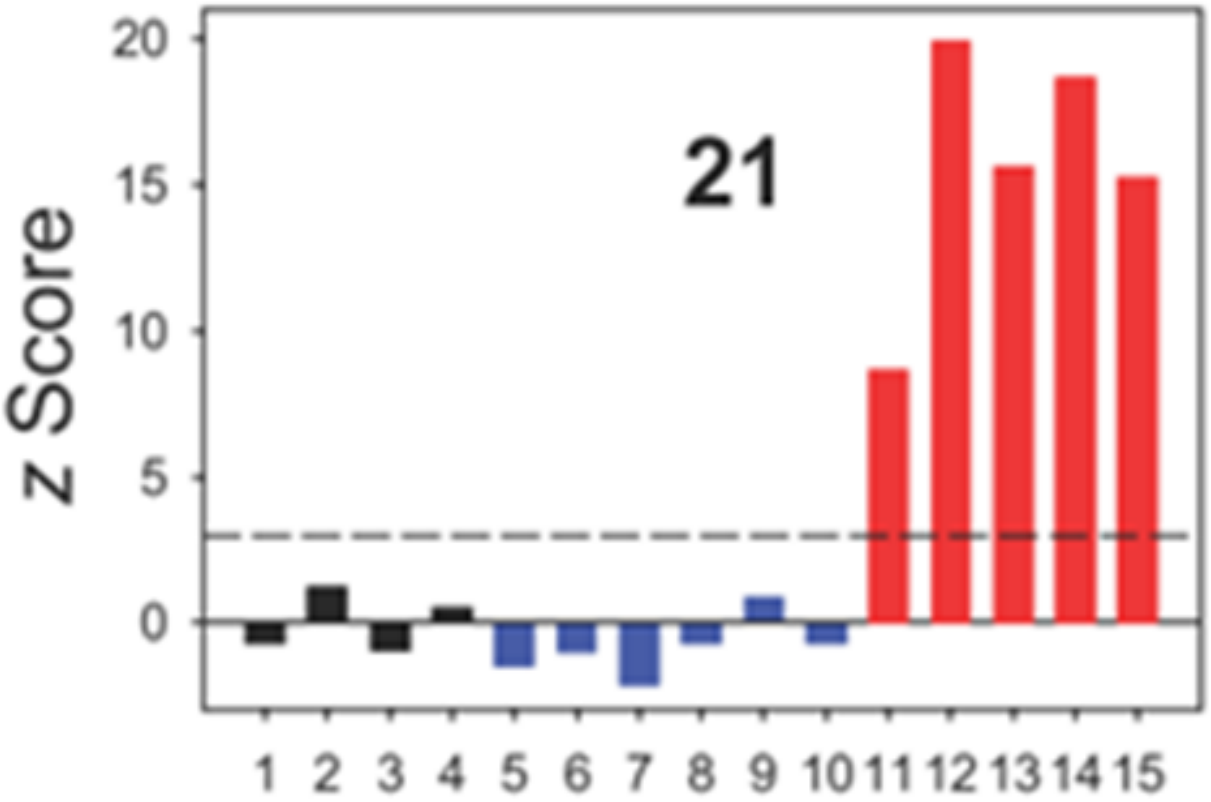


Chiu, PNAS, 2008

Sequenced Raw data

Alignment

Counts of reads on each chromosomes



5 Down syndrome

10 normal

100% accuracy

Earliest: 11 weeks

Chiu et al., Clinical Chemistry, 2010

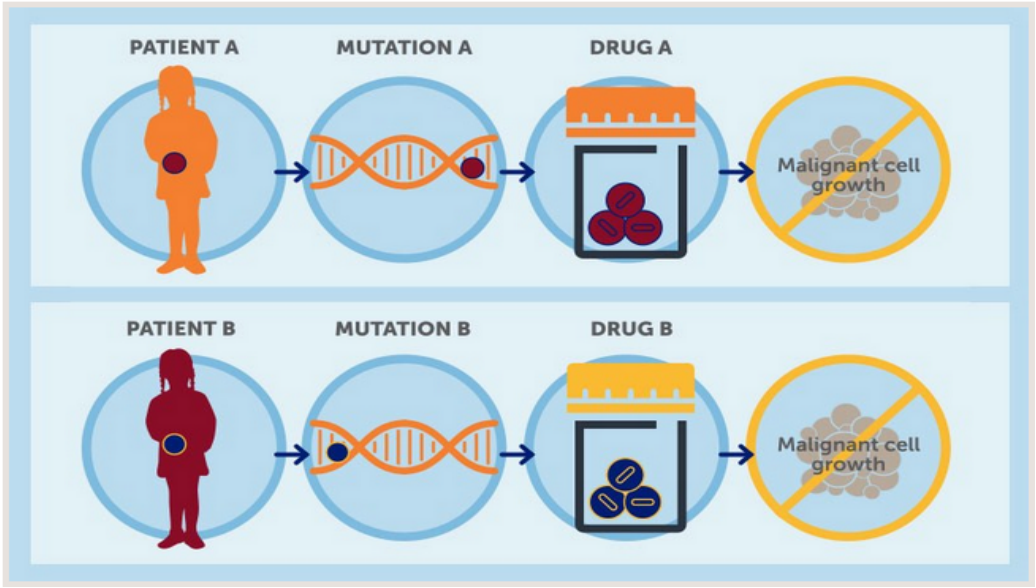
HTS Application in Oncology

Cancer diagnosis

- Biopsy or sample removed during surgery can help identify the precise genetic origin.
- Liquid biopsies—samples of blood or urine—from which cell-free DNA (cfDNA) is isolated, avoiding the need for invasive procedures.

Cancer screening

- For people at higher risk of cancer, genetic testing for cancer predisposition can play an important role in reducing cancer mortality through prevention.
- For people with a family history of these cancers, NGS-based testing is well-suited to identifying any inherited harmful variants



HTS and Precision Medicine

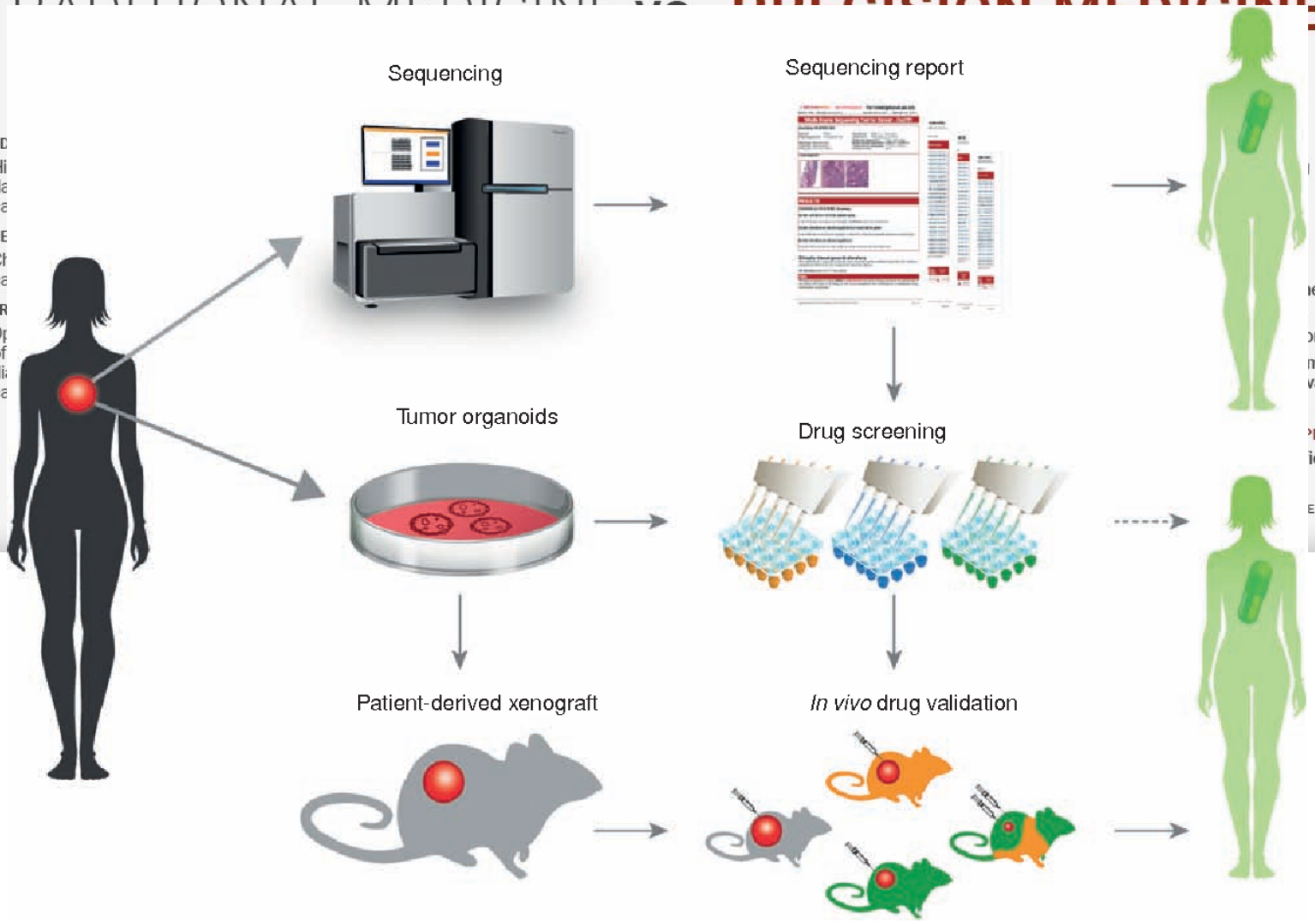


Figure 2. Personalized models to guide precision medicine in advanced cancer. Illustration of our precision medicine program depicting the workflow,

HTS Application in Mendelian Diseases-Genomic Testing

- Originate from single-gene variants, for example single nucleotide polymorphisms (SNPs) and indels, that parents can pass on to their offspring, and can be dominant or recessive in nature.
- HTS can be used to efficiently analyze multiple genes simultaneously, making it well-suited to searching for rare variants in a pool of many genes.
- HTS reduces the time to find the cause of a condition, contributing substantially to the well-being of patients and their relatives


Angelina Jolie tested positive for BRCA gene.
getty images
FIND OUT IF YOU SHOULD BE TESTED:
BeAwareFoundation.org/ask-the-doctor

HTS Application in Microbiology and Infectious Diseases

- **Primary diagnostics – Detection**
  - Detection, identification & characterization of previously unidentified microorganisms
  - Molecular marker profiles directly from clinical samples

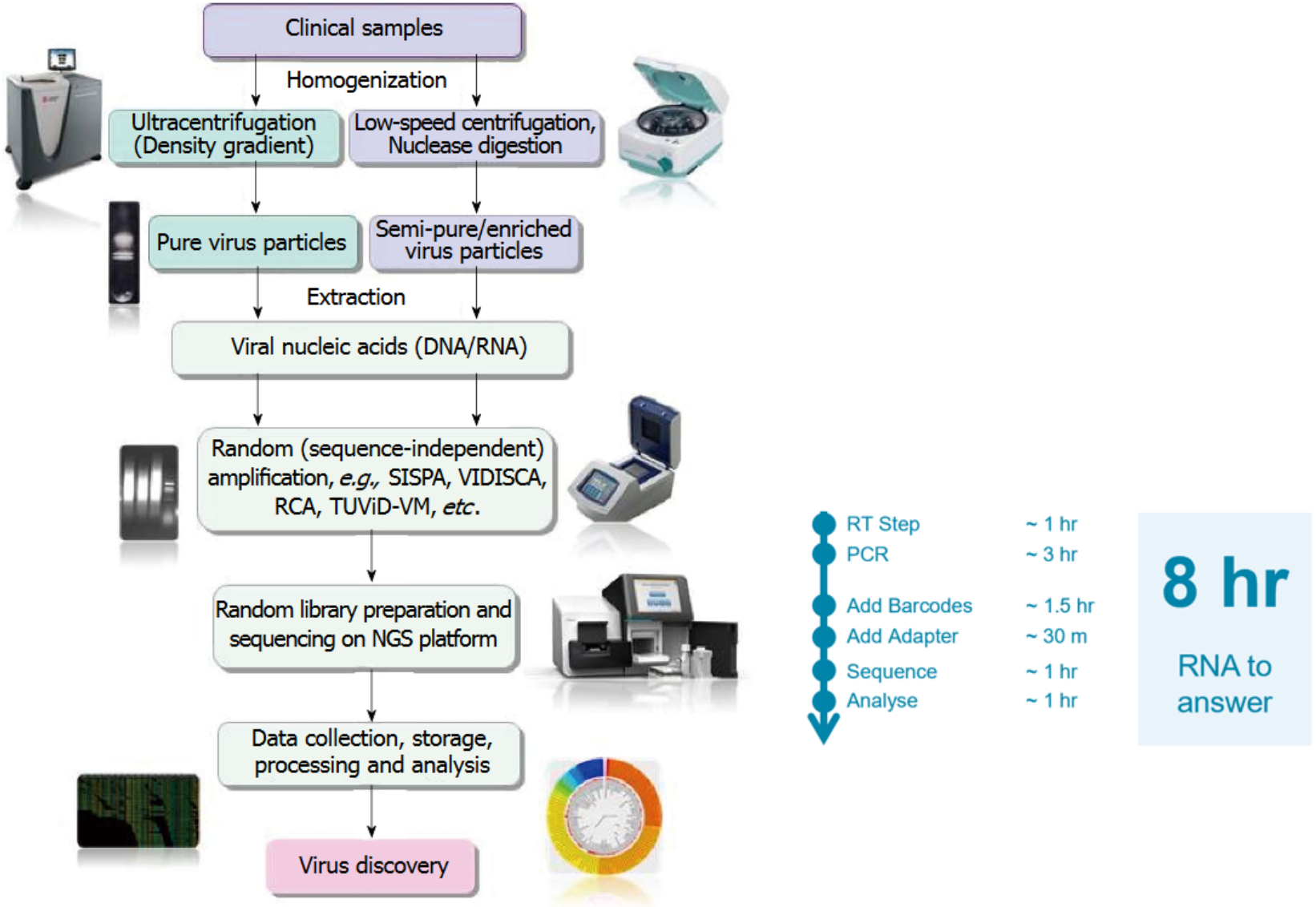
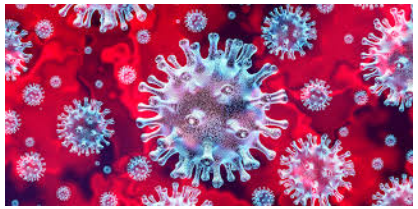


Figure 1 Diagrammatic representation of main steps of clinical virus discovery by next-generation sequencer based technologies.

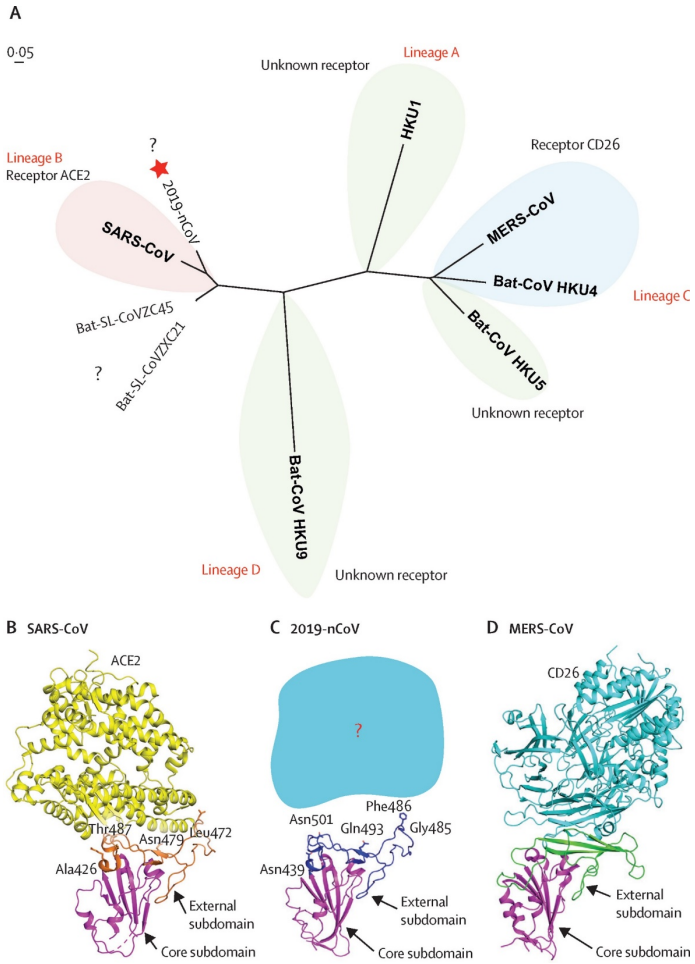
Coronavirus Discovery and Detection

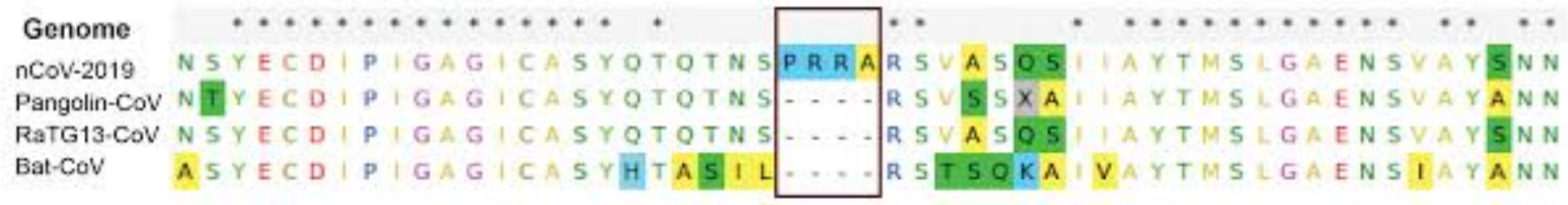


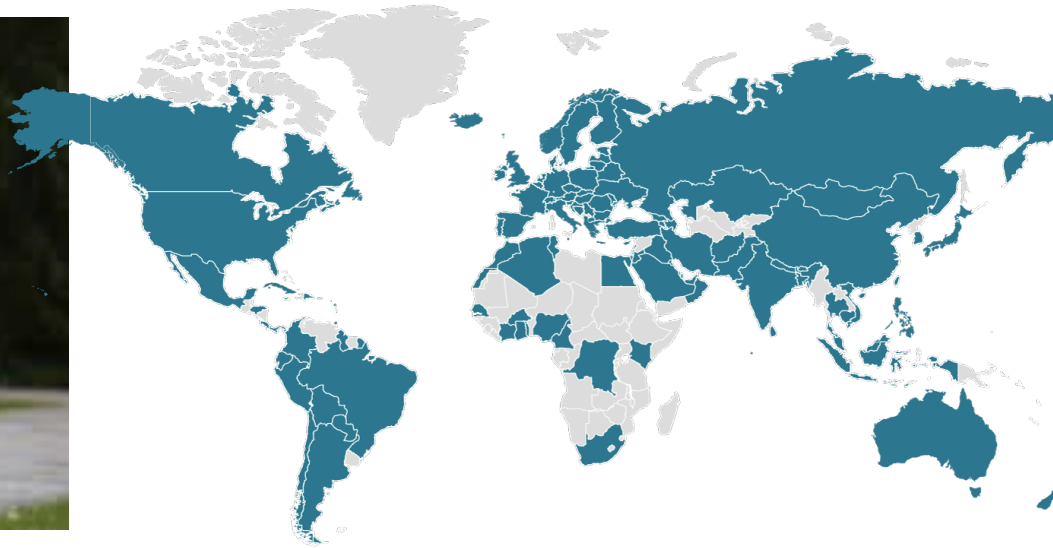
SARS-CoV-2
COVID-19
nCov-2019
Wuhan virus
Chinese virus

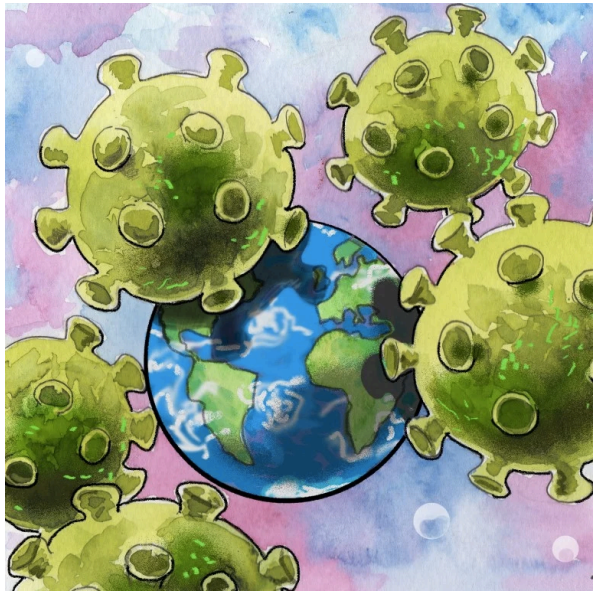
Phylogenetic analysis of full-length genomes of nCoV-2019 and representative viruses of the genus Beta-coronavirus

Phylogenetic analysis and homology modelling of the receptor-binding domain of the 2019-nCoV, SARS-CoV, and MERS-CoV









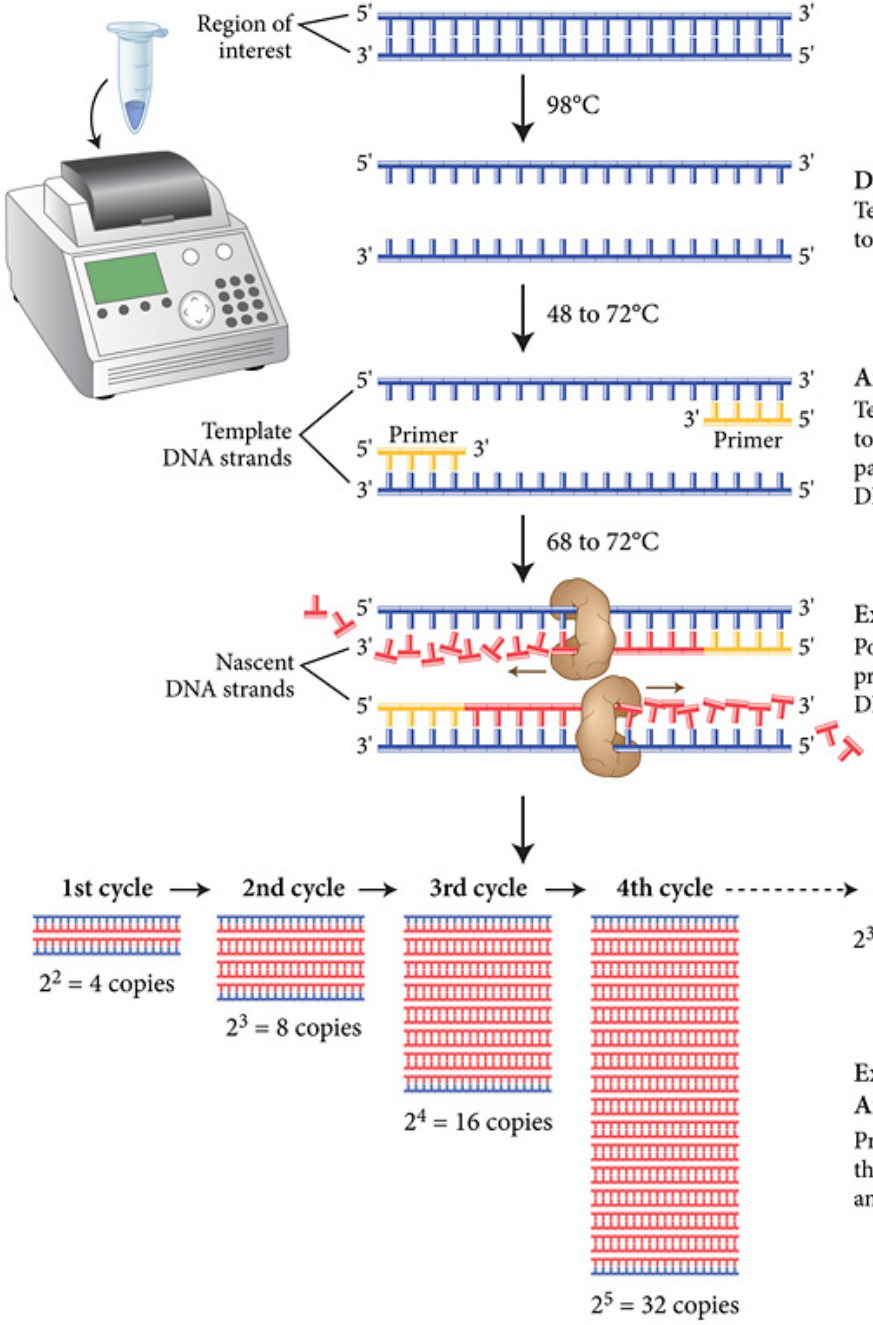

MAKE BRAZIL
GREAT AGAIN

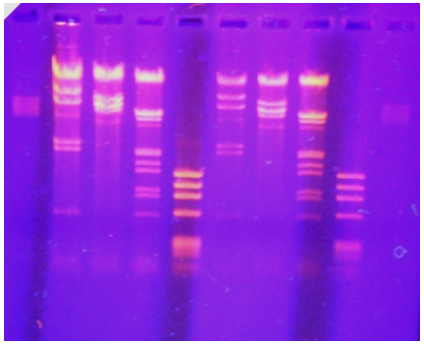
Old techniques for genetic testing

- Karyotyping
- FISH
- PCR
- Array Comparative Genomic Hybridisation (aCGH)

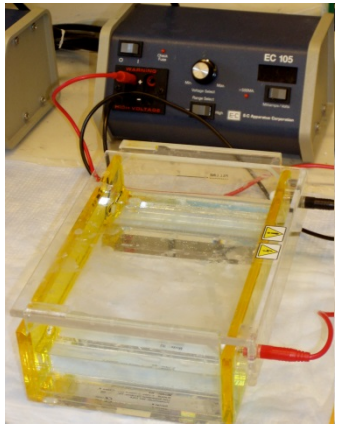
Polymerase chain reaction (PCR)

- Amplify specific DNA and RNA fragments
- Once nucleotide sequence of a region of DNA strand is known, complimentary oligonucleotides & polymerase are added to single strand DNA
- Repeat process 30 times to get adequate DNA
- PCR identify specific DNA sequence for gene mutation.





Visualization of the DNA



Gel electrophoresis

a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge.

Huntington gene

```
  1 ttg ctg tgt gag gca gaa cct gcg ggg gca
    ggg gcg ggc tgg ttc cct ggc cag cca ttg
 61 gca gag tcc gca ggc tag ggc tgt caa tca
    tgc tgg ccg gcg tgg ccc cgc ctc cgc cgg
121 cgc ggc ccc gcc tcc gcc ggc gca cgt ctg
    gga cgc aag gcg ccg tgg ggg ctg ccg gga
181 cgg gtc caa gat gga cgg ccg ctc agg ttc
    tgc ttt tac ctg cgg ccc aga gcc cca ttc
241 att gcc ccg gtg ctg agc ggc gcc gcg agt
    cgg ccc gag gcc tcc ggg gac tgc cgt gcc
301 ggg cgg gag acc gcc atg gcg acc ctg gaa
    aag ctg atg aag gcc ttc gag tcc ctc aag
361 tcc ttc cag cag cag cag cag cag cag cag
    cag cag cag cag cag cag cag cag cag cag
421 cag cag cag caa cag ccg cca ccg ccg ccg
    ccg ccg ccg ccg cct cct cag ctt cct cag
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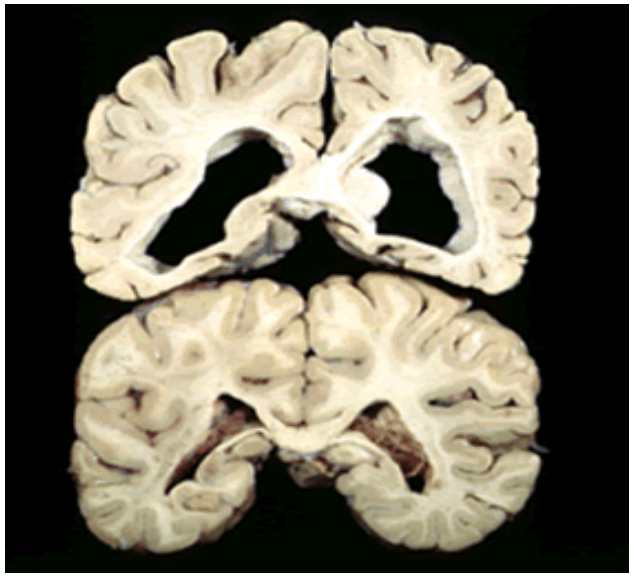
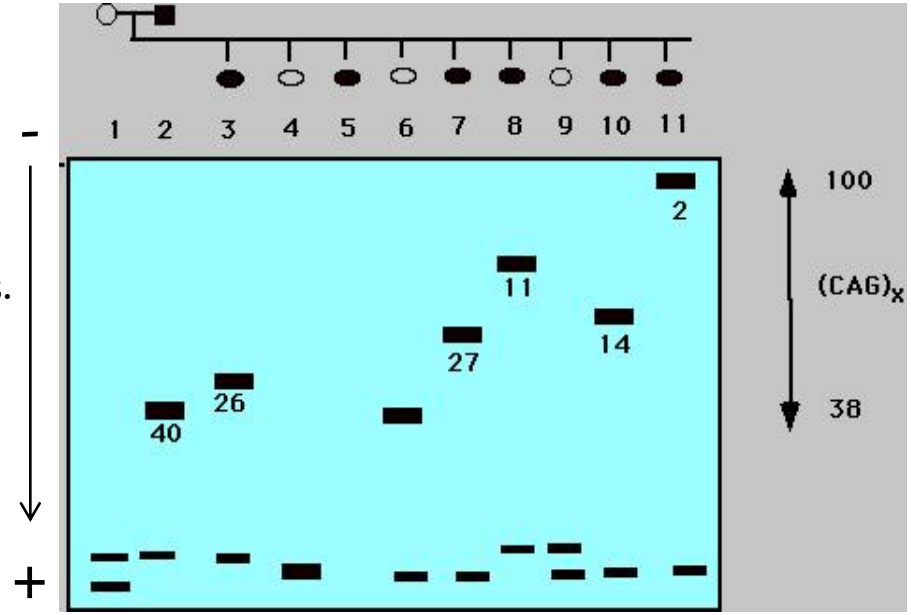


Encodes a run of 11-34 glutamine amino acid residues in the HD protein. A run of > 34 glutamine residues causes the protein to aggregate in the brain cells and cause progressive cell death.

The data below shows the results of electrophoresis of PCR fragments amplified using probes for the site which has been shown to be altered in Huntington's disease. The male parent, as shown by the black box, got Huntington's disease when he was 40 years old. His children include 6 (3,5,7,8,10,11) with Huntington's disease, and the age at which the symptoms first began is shown by the number above the band from the PCR fragment.

DNA migrates from – to +
the larger the fragment is, the slower it runs.

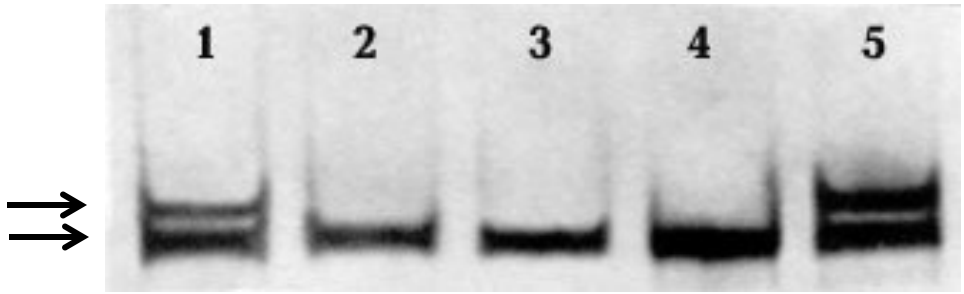


Quiz:

A. No relationship between age of onset of disease and the migration rate of PCR fragments.
B. A shorter PCR fragment predicts early onset of Huntington's disease.
C. Increased length of the amplified PCR fragment predicts early onset of Huntington's disease.
D. Huntington's disease must be contagious since many of the children have the disease.

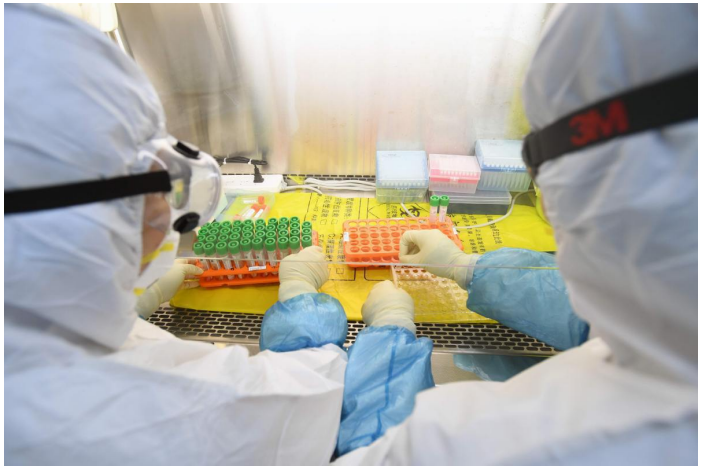
BRCA1 and BRCA2 (breast cancer , early onset) are normally expressed in the cells of breast and other tissue, where they help repair damaged DNA or destroy cells if DNA cannot be repaired. They are involved in the repair of chromosomal damage with an important role in the error-free repair of DNA double-strand breaks.

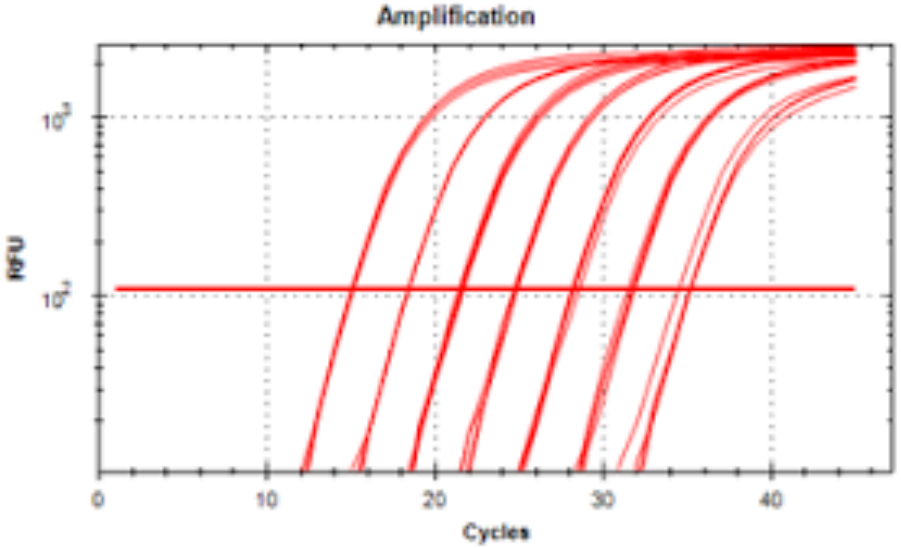
Population or subgroup	BRCA1 mutation(s)[57]	Reference(s)
African-Americans	943ins10, M1775R	[58]
Afrikaners	E881X	[59]
Ashkenazi Jewish	185delAG, 188del11, 5382insC	[54][55]
Austrians	2795delA, C61G, 5382insC, Q1806stop	[60]
Belgians	2804delAA, IVS5+3A>G	[61][62]
Dutch	Exon 2 deletion, exon 13 deletion, 2804delAA	[61][63][64]
Finns	3745delT, IVS11-2A>G	[65][66]
French	3600del11, G1710X	[67]
French Canadians	C4446T	[68]
Germans	5382insC, 4184del4	[69][70]
Greeks	5382insC	[71]
Hungarians	300T>G, 5382insC, 185delAG	[72]
Italians	5083del19	[73]
Japanese	L63X, Q934X	[74]
Native North Americans	1510insG, 1506A>G	[75]
Northern Irish	2800delAA	[76]
Norwegians	816delGT, 1135insA, 1675delA, 3347delAG	[77][78]
Pakistanis	2080insA, 3889delAG, 4184del4, 4284delAG, IVS14-1A>G	[79]
Polish	300T>G, 5382insC, C61G, 4153delA	[80][81]
Russians	5382insC, 4153delA	[82]
Scottish	2800delAA	[76][83]
Spanish	R71G	[84][85]
Swedish	Q563X, 3171ins5, 1201del11, 2594delC	[58][86]



Test for 5382insC mutation presence (lanes 1 and 5) or absence (lanes 2 - 4). PCR amplified fragments of exon 20 of the BRCA1 gene were subjected to electrophoresis and DNA bands were visualized by silver staining.

nCov-2019 PCR detection






```
                                                  20                  40
SBRp3          :  CTTTCTGATGATGCCGTCGTGTGCTACAACAGTAACTACG :40
SBRs           :  CTTTCTGATGATGCCGTTGTGTGCTATAACAGTAACTATG :40
SHT or2        :  CTTTCTGATGATGCCGTTGTGTGCTATAACAGTAACTATG :40
SC             :  CTTTCTGATGATGCCGTTGTGTGCTATAACAGTAACTATG :40
SB             :  CTTTCTGATGATGCCGTTGTGTGCTATAACAGTAACTATG :40
SBRf1          :  CTTTCTGATGATGCCGTTGTGTGCTACAATAGTAACTATG :40
SBRm1          :  CTTTCTGATGATGCCGTTGTGTGCTACAATAGTAACTATG :40
SBHKU3-1:  CTTTCTGACGATGCCGTTGTGTGCTATAATAGTAATTACG :40
SB273          :  CTTTCTGATGATGCCGTTGTGTGCTACAATAGTAACTATG :40
SB279          :  CTTTCTGATGATGCCGTTGTGTGCTACAATAGTAACTATG :40
11FW-mod:  -----TGATGATGCCGTCGTGTGCTACAA---------- :24
Consensus seq. CTTTCTGATGATGCCGTTGTGTGCTACAACAGTAACTATG
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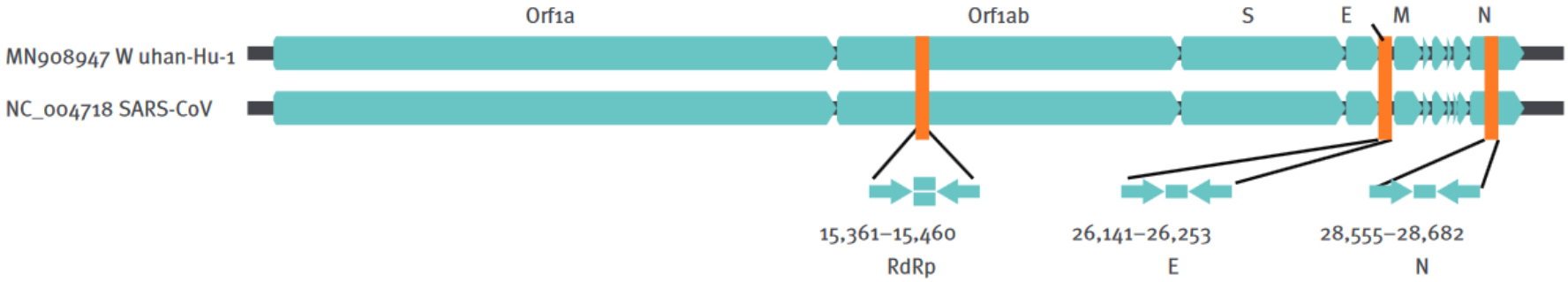
https://www.youtube.com/watch?v=tgyzdgf66eM

Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome



E: envelope protein gene; M: membrane protein gene; N: nucleocapsid protein gene; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene.

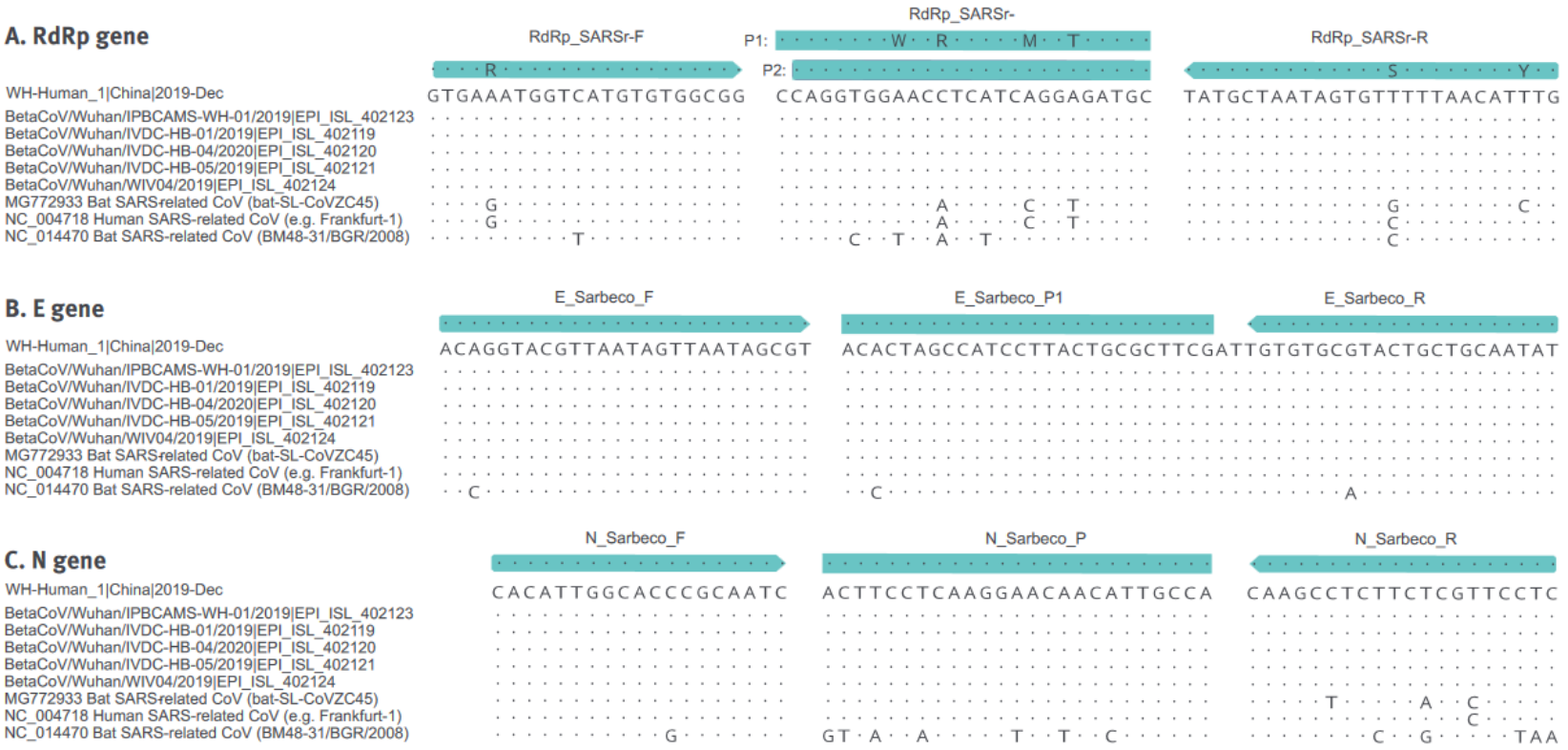
Primers and probes, real-time RT-PCR for 2019 novel coronavirus

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

Partial alignments of oligonucleotide binding regions, SARS-related coronaviruses (n = 9)

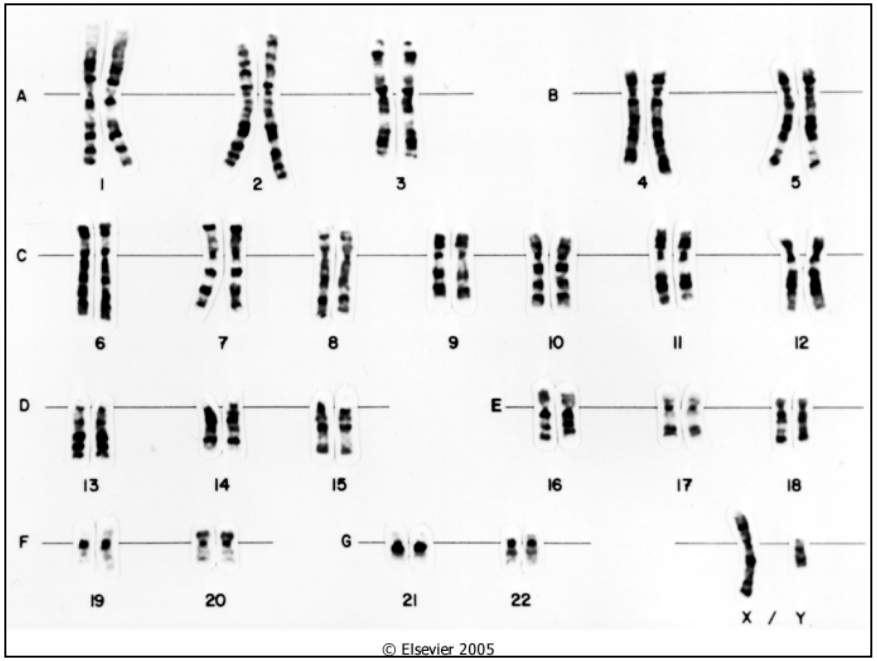


Banded karyotyping

- A karyotype is the number and appearance of chromosomes in the nucleus of a eukaryotic cell.

- Karyotypes describe the number of chromosomes, and what they look like under a light microscope.
  - Abnormal number of chromosomes
  - Large duplications and deletions
  - Balanced rearrangements (translocations, inversions)

G-banding is obtained with Giemsa stain following digestion of chromosomes with trypsin. It yields a series of lightly and darkly stained bands — the dark regions tend to be heterochromatic, late-replicating and AT rich. The light regions tend to be euchromatic, early-replicating and GC rich.



In normal diploid organisms, autosomal chromosomes are present in two copies.

Downloaded from: Robbins & Cotran Pathologic Basis of Disease (on 18 July 2005 09:03 PM)
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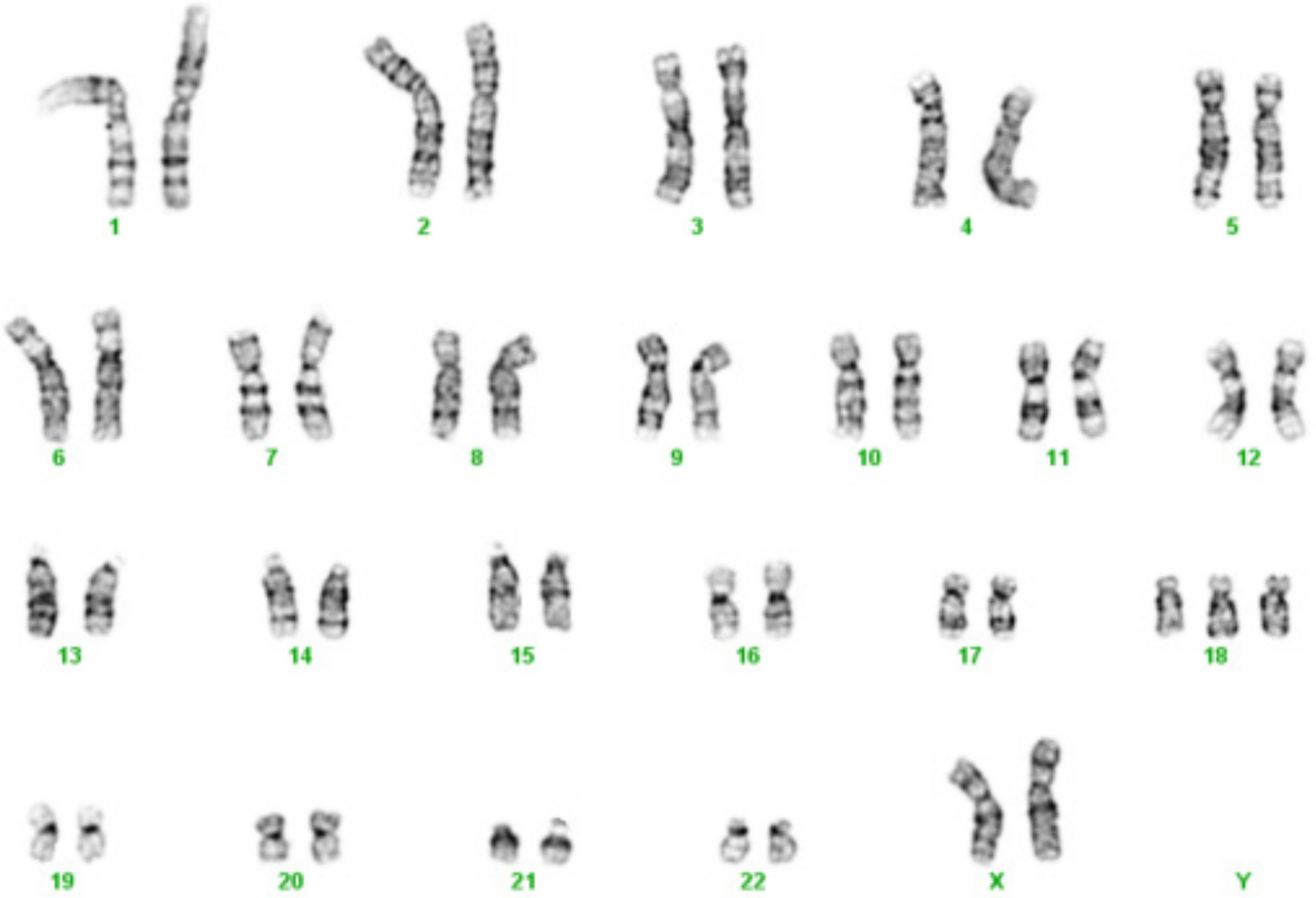
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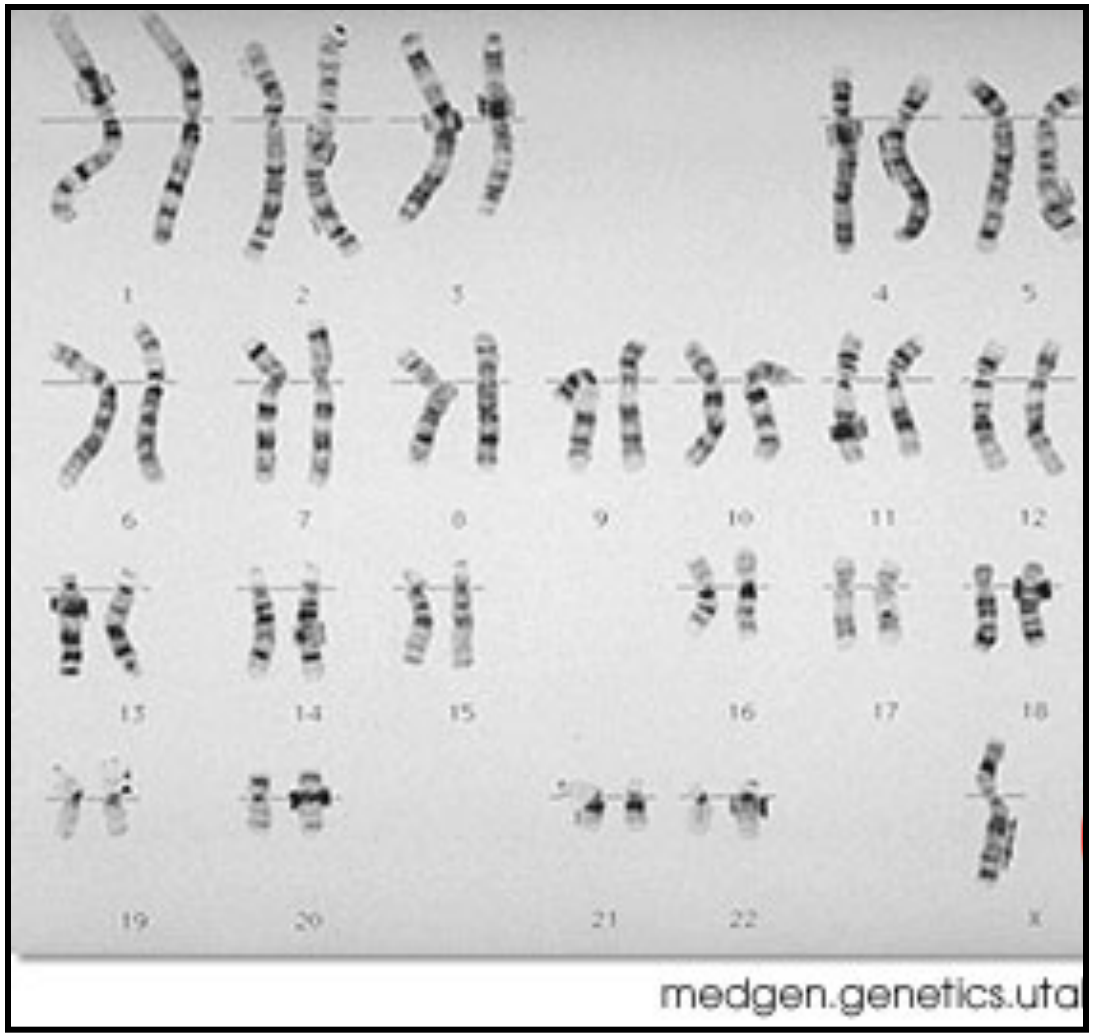
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