NGS (Next Generation Sequencing) application in Cancer Genomics

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1993	紐約大學西奈山醫學院分子細胞病理學博士
1993 ~ 1996	哈佛大學兒童醫院及霍華修斯研究機構 博士後研究員
1997 ~ 迄今	臺北榮民總醫院教學研究部副研究員
1998 ~ 迄今	陽明大學生物技術研究所兼任副教授

【研究興趣】

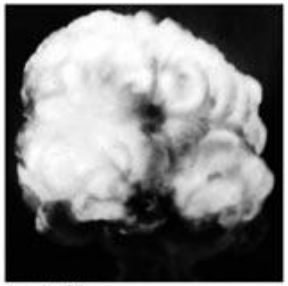
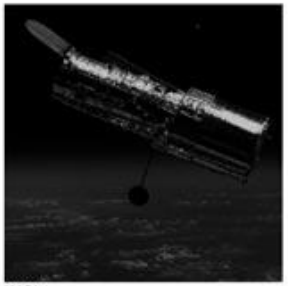
人類基因庫計畫是科學史上最龐大且跨越國界的合作計畫，橫跨廿一世紀。人類染色體上的十萬基因序列將被解碼定序，對於下個世紀有關疾病診斷、治療與藥物開發均將有新的進展與提昇。國人腫瘤疾病相關之基因譜序的研究是本實驗室工作目標，包含：

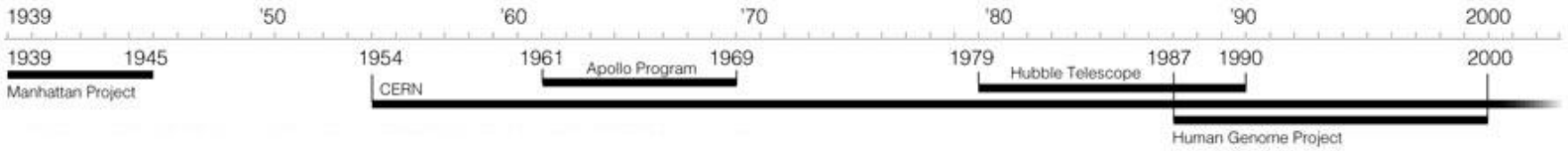
1. 定序基因庫，以建立國人相關疾病EST基因庫。
2. 配合基因庫建立，引進現代化的Bioinformatics的資訊處理。
3. Nuclear Receptor與其Cofactors在癌病變上的篩選與驗證。
4. 肝癌與血液腫瘤科細胞生長的特異基因調控。
5. 分子基因的遺傳調控與選殖。
6. Transgenic mice model 在癌病變上基因的調控與驗證

The Breakthrough Sciences in World

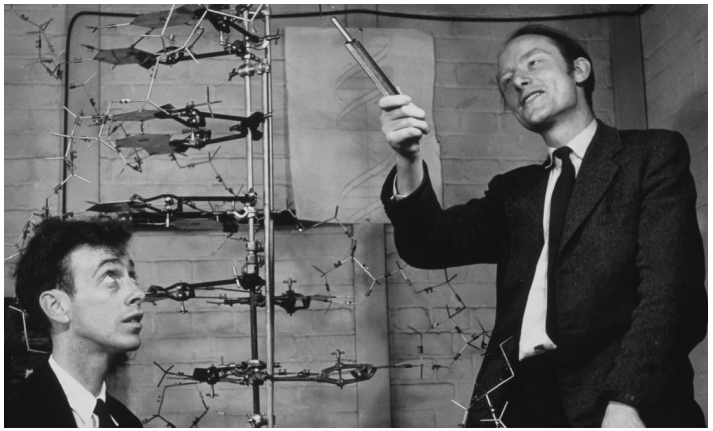
With the Human Genome Project, Biology Joins the Ranks of Big Science

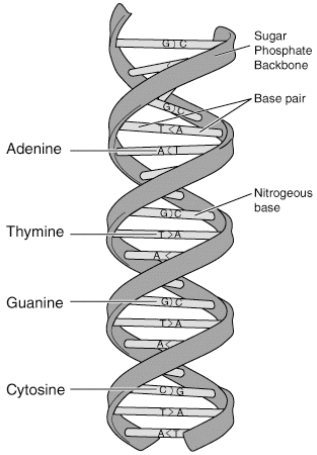
In the last 60 years, physics research like the Manhattan Project, involving billions of dollars and thousands of people, has been labeled Big Science. The Human Genome Project, with an international consortium of 16 public research centers and the privately owned Celera Corporation, has now moved biology into the realm of Big Science.

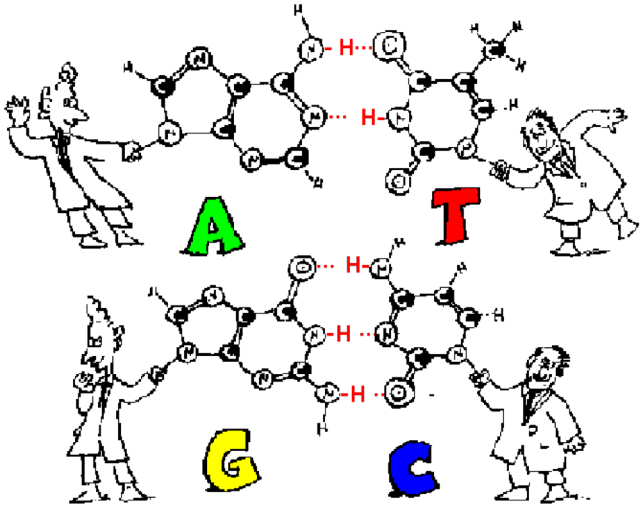
	MANHATTAN PROJECT	CERN (EUROPE)	APOLLO PROGRAM	HUBBLE SPACE TELESCOPE	HUMAN GENOME PROJECT
MISSION	To build an atomic bomb	To perform high-energy particle physics research	To put a man on the moon	To put a powerful optical telescope in orbit	To decode the human genome
COST IN 1999 DOLLARS	$18.5 billion	$638 million a year	$115.3 billion	$3 billion	$250 million from consortium; $200-250 million from Celera
HISTORIC DATE	July 16, 1945, first atomic bomb is detonated in Los Alamos, N.M.	1989, scientists isolate the three families of particles that make up all matter.	July 20, 1969, Neil A. Armstrong and Edwin "Buzz" Aldrin walk on the moon.	April 25, 1990, Hubble is launched into orbit.	June 26, 2000, the first survey of the entire human genome sequence is completed.
	 Associated Press	 CERN	 NASA via Associated Press	 NASA	 Reuters
PEOPLE INVOLVED	129,000 working for the Manhattan District Army Corps of Engineers	6,500 physicists from 57 countries have worked at the labs near Geneva, Switzerland	32,000 employees at NASA's 10 flight centers, plus 377,000 in industry and research	39,000 NASA and European Space Agency employees and contractors	1,100 scientists in the consortium research centers; 542 Celera scientists

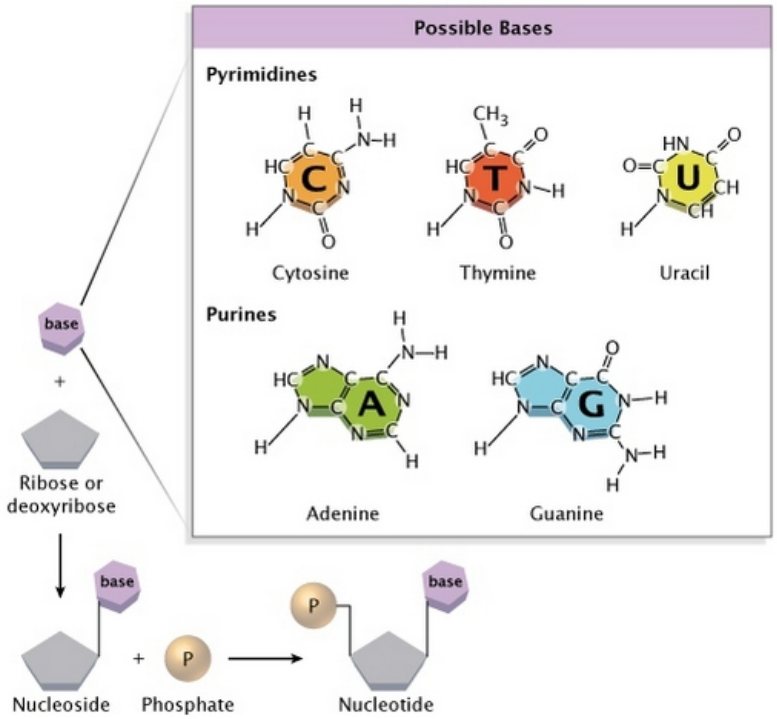


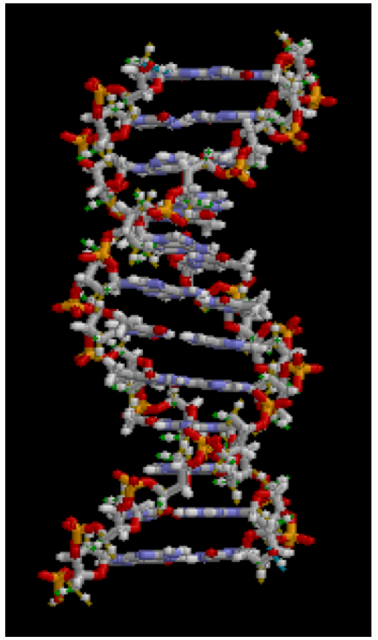
Discovery of DNA Structure and Function
Watson and Crick

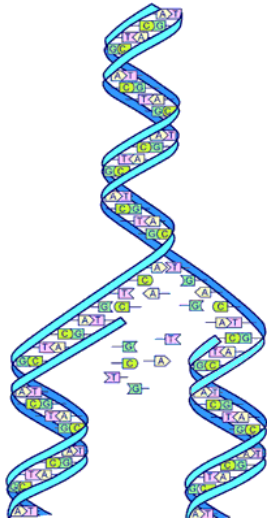








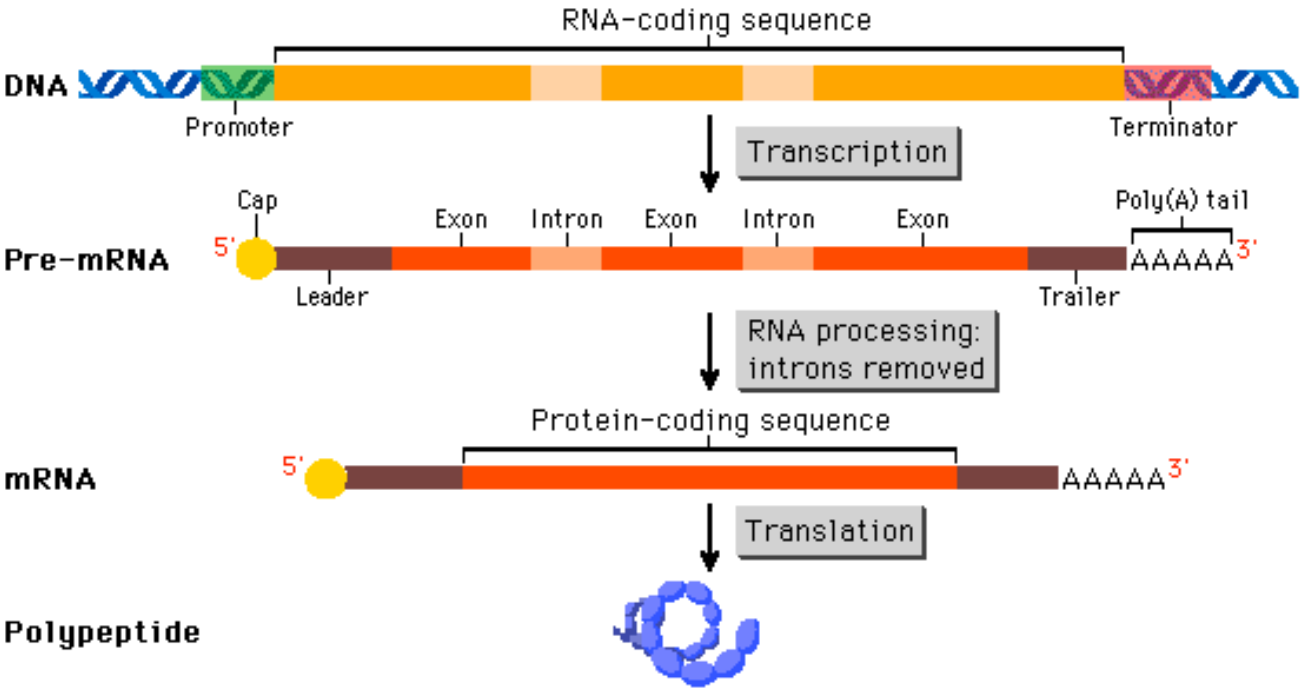


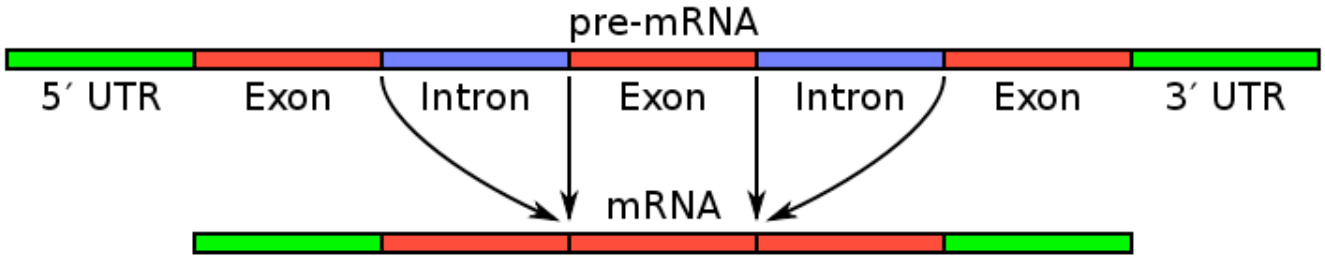


What's the genome?

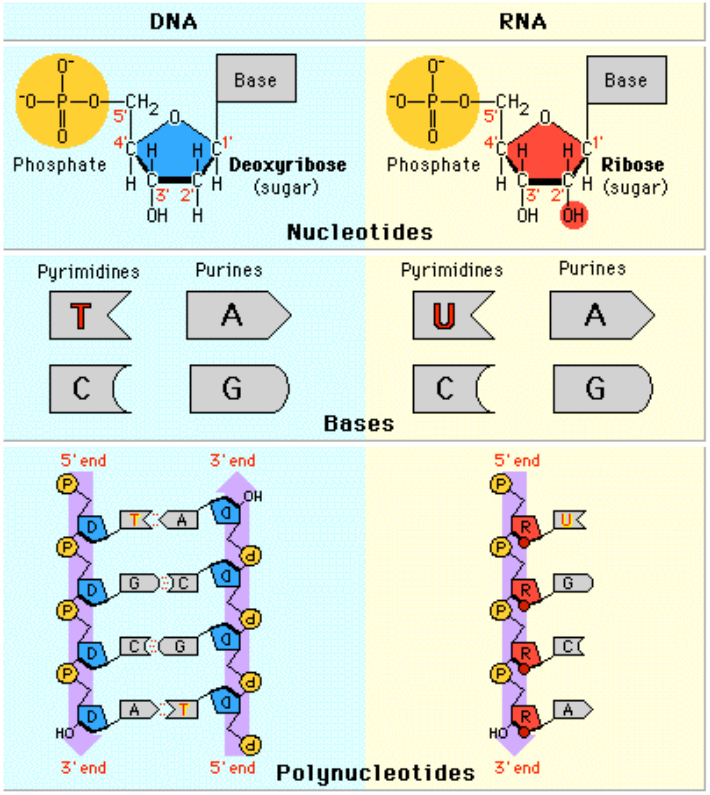
Within a chromosome

The sequence of a eukaryotic protein-coding gene is typically not colinear with the translated mRNA; that is, the transcript of the gene is a molecule that must be processed to remove extra sequences (introns) before it is translated into the polypeptide.





RNA is structurally similar to DNA.

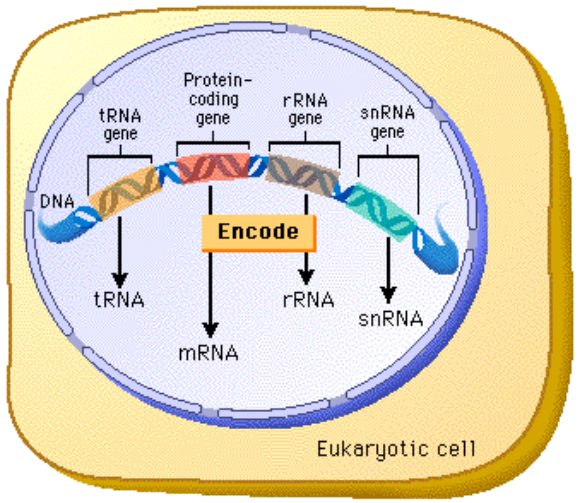


Coding mRNA is the functional gene

There are 4 types of RNA, each encoded by its own type of gene.

The genomic DNA contains all the information for the structure and function of an organism.

In any cell, only some of the genes are expressed, that is, transcribed into RNA.



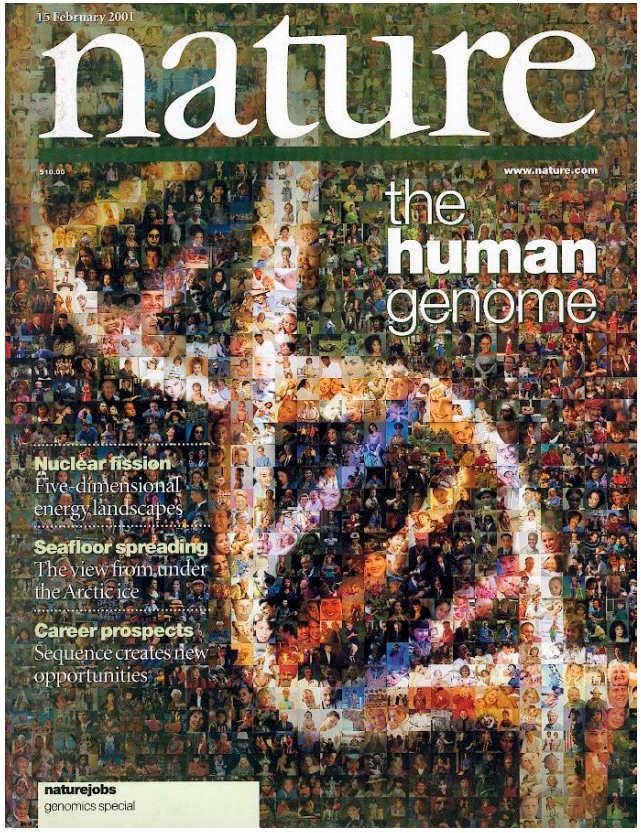
There are 4 types of RNA, each encoded by its own type of gene:

- mRNA - Messenger RNA: Encodes amino acid sequence of a polypeptide.
- tRNA - Transfer RNA: Brings amino acids to ribosomes during translation.
- rRNA - Ribosomal RNA: With ribosomal proteins, makes up the ribosomes, the organelles that translate the mRNA.
- snRNA - Small nuclear RNA: With proteins, forms complexes that are used in RNA processing in eukaryotes. (Not found in prokaryotes.)

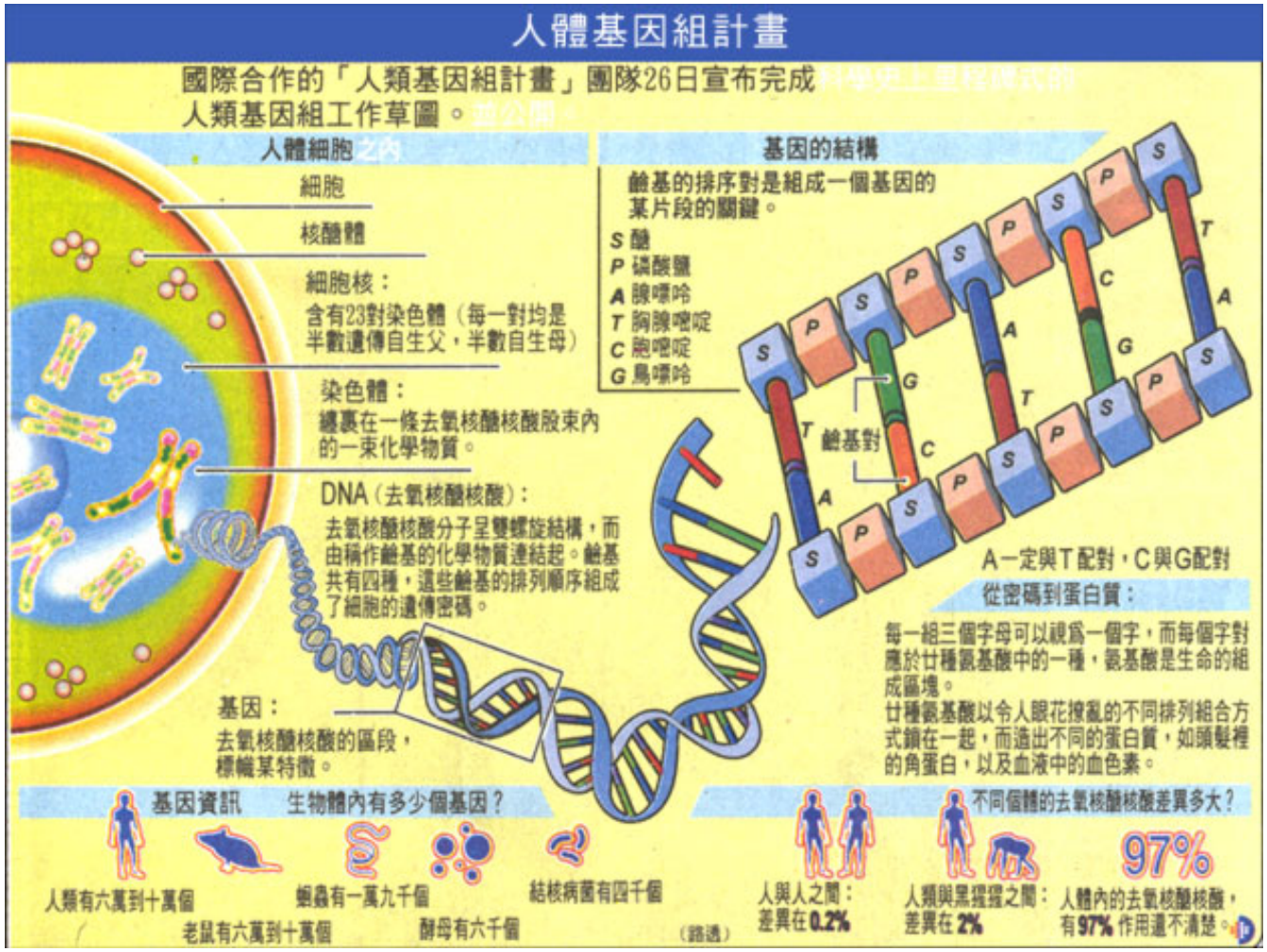
接下來的挑戰：從序列資訊到基因

Sequence data to Functional genes

- 2001年，人類基因體草圖完成

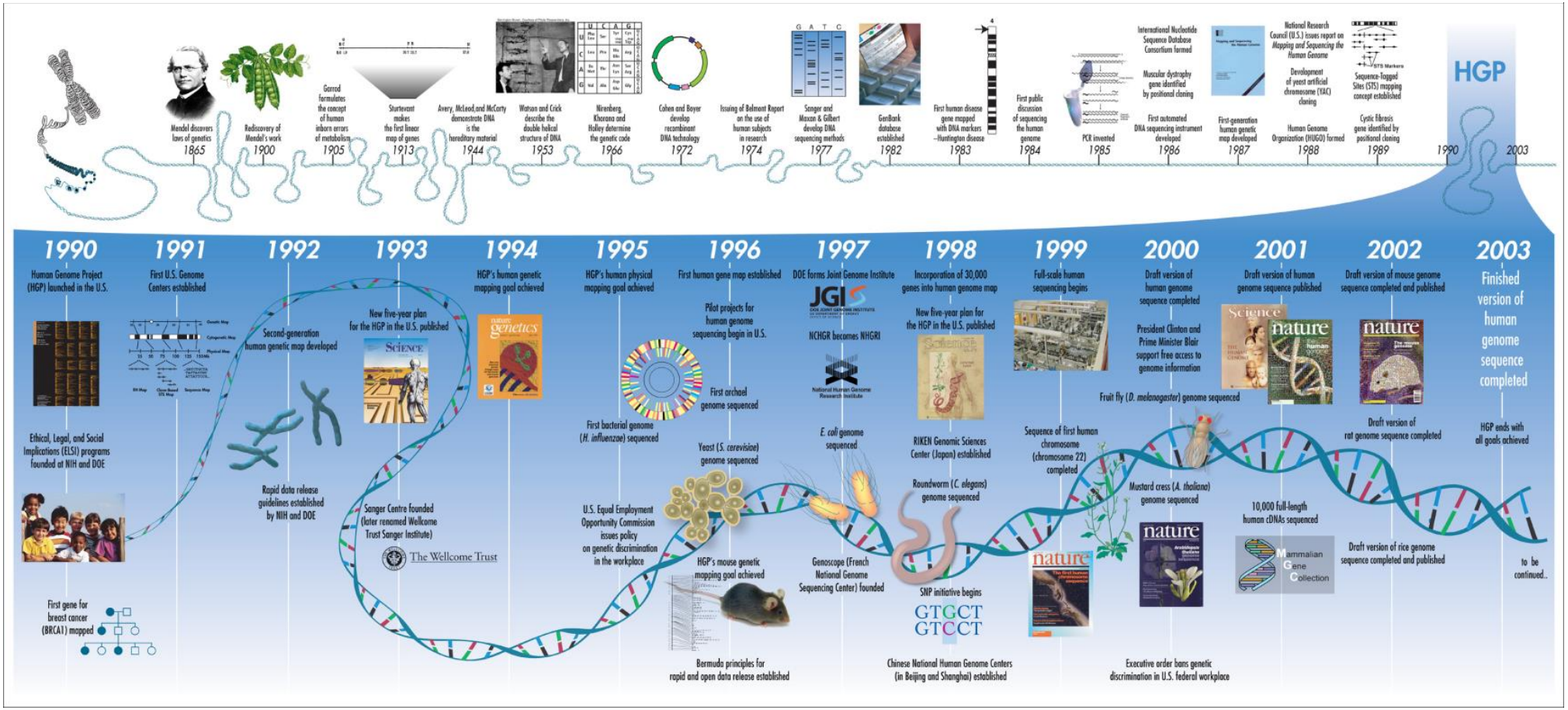


Human Genome Project

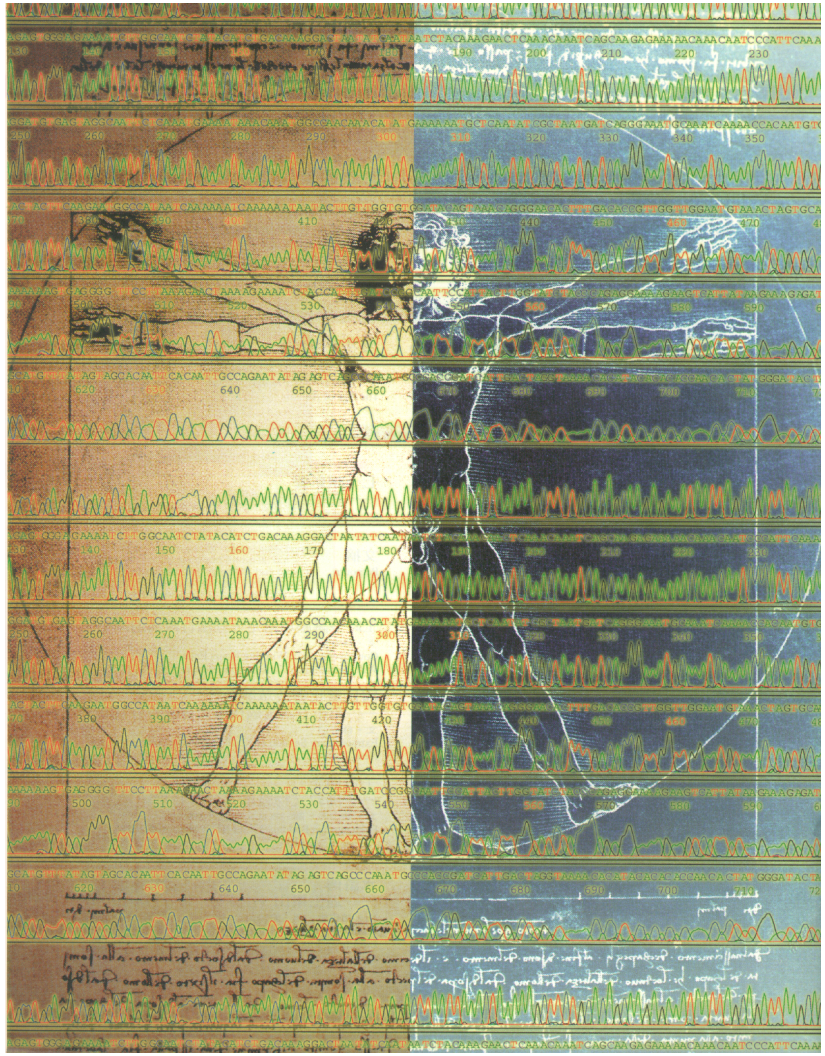


Human Genome Project progress

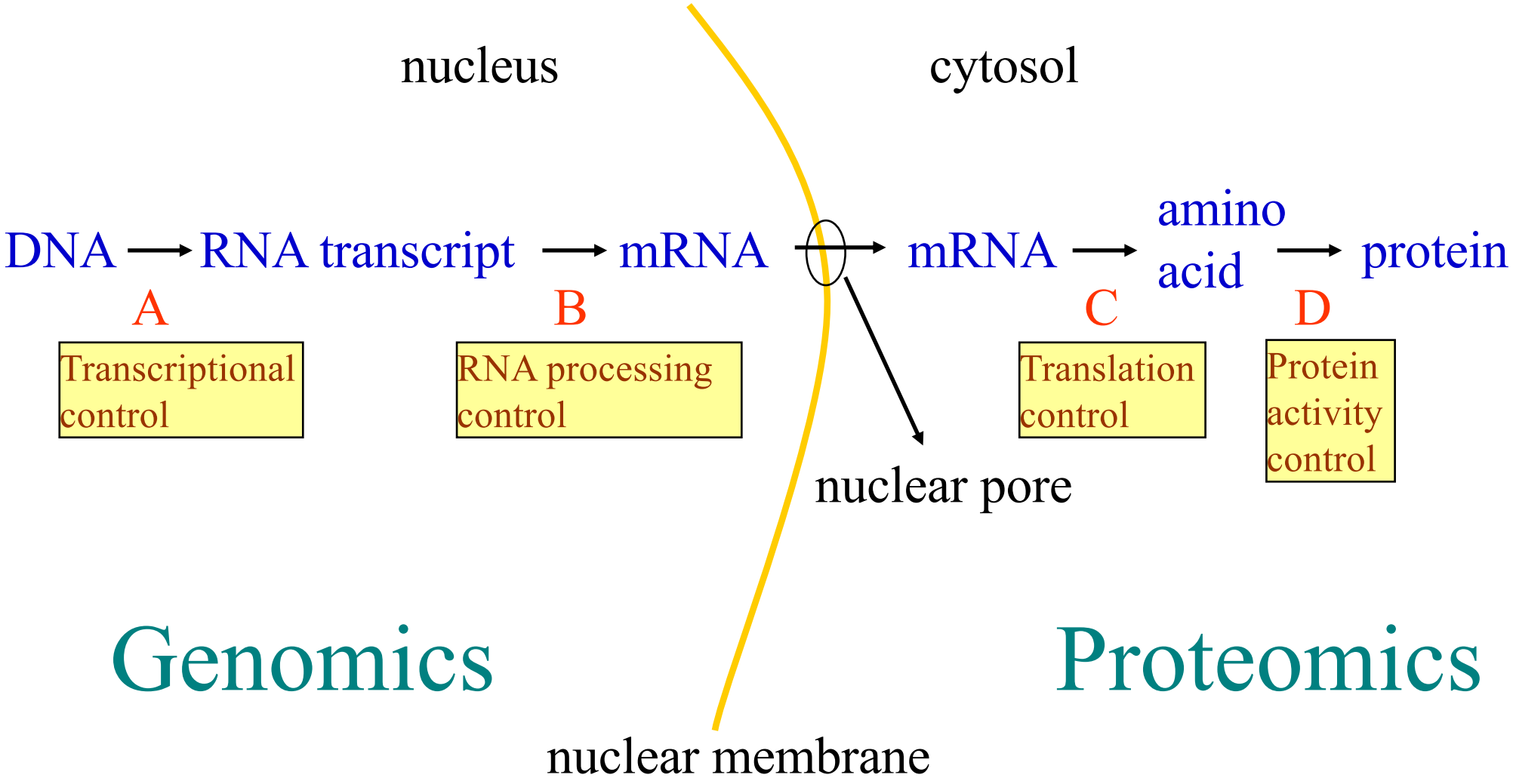
- The HGP is one of the greatest endeavors of humanity and was accomplished by a sequencing technology developed in 1977 (Chain termination or Sanger Sequencing)
- 1st Revolution in the Sequencing world
- Parallel sequencing through Single Chain Termination + capillary electrophoresis + shotgun sequencing



The Study of Human Cancer Genome Project



Regulation of Gene Expression Control



History of Biotech Research

- 1950 Structure of double strand DNA
- 1960 Development of biochemistry
- 1970 Tools development for DNA science
- 1980 Foundation base for biotechnology industry

Current Trends of Cancer Genome Study

- Characterization of disease genes
- Development novel gene library
- Proteomics analysis
- Bioinformatics
- Pharmacogenomic and pharmacogenetics studies

Impact of Cancer Genome Study

- New biotechnology tools
- New medicine and diagnosis methods
- Personalized therapy
- Agricultural improvements
- Forensic identification
- Ethical, legal and social issues

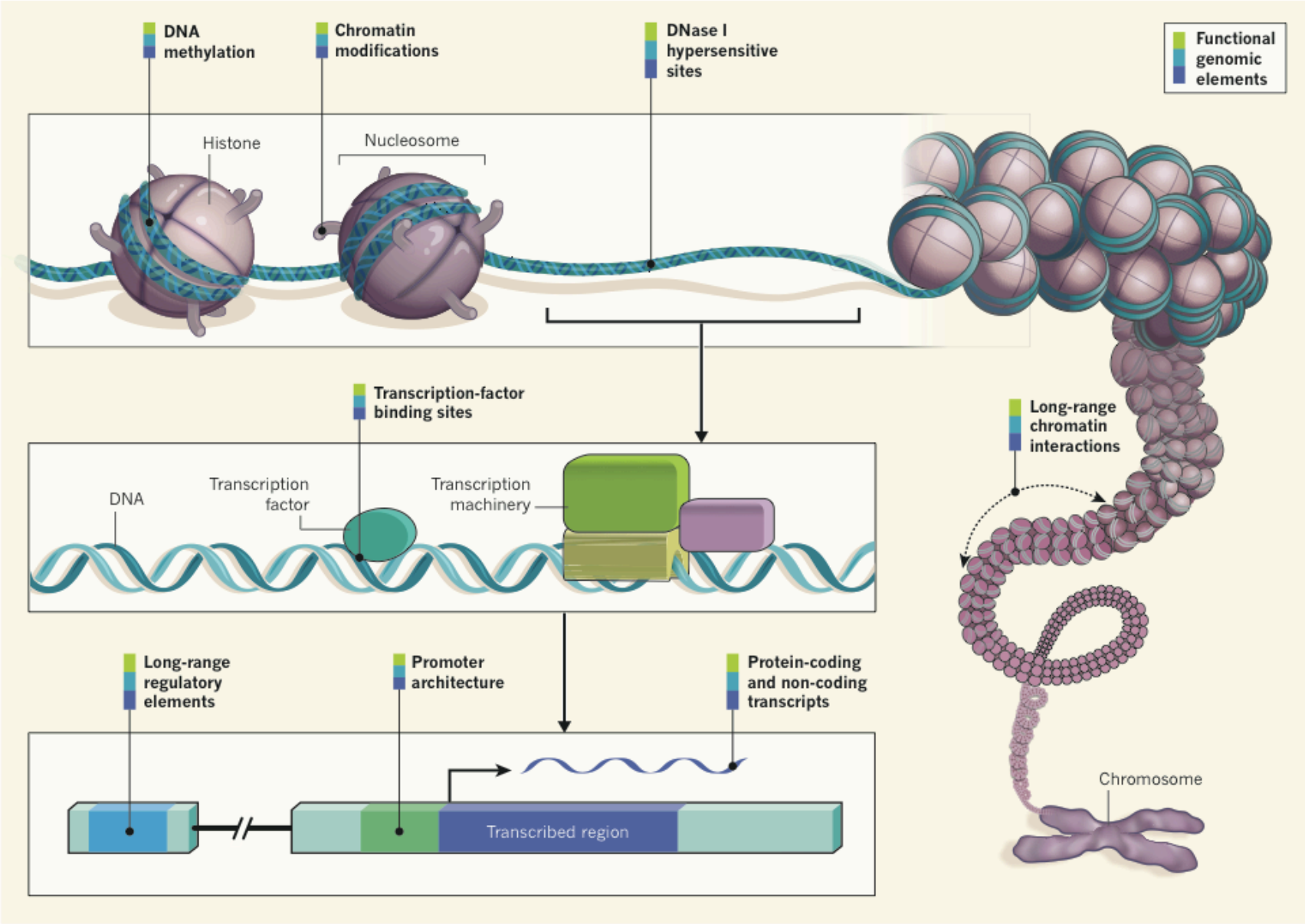
Technologies applied in Cancer genomics

- Functional Genomics—NGS etc.
- Bioinformatics
- Drug Discovery
- Stem Cell
- Gene Targeting
- Gene Therapy

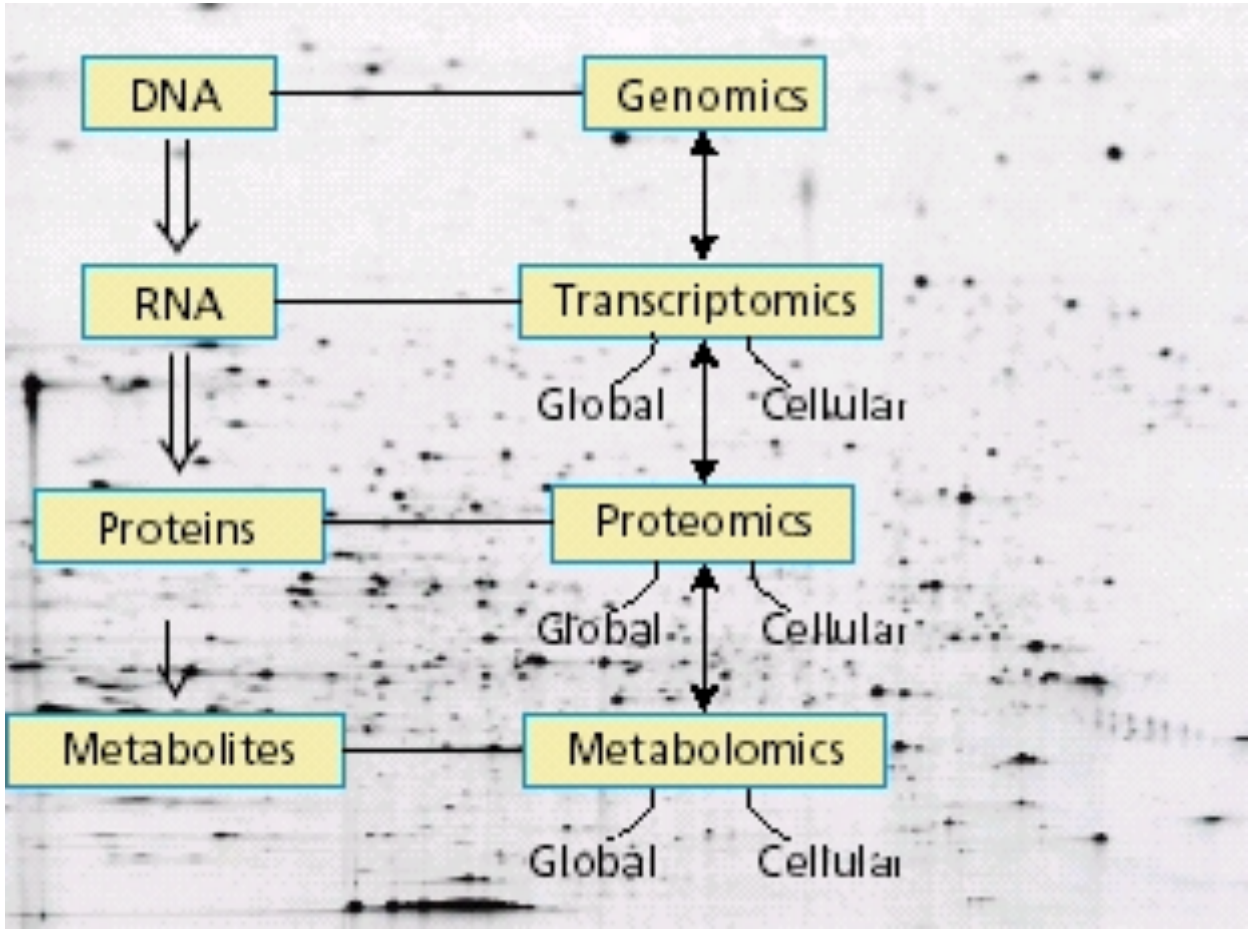
Functional Genomics

Genomics: ENCODE explained

Encyclopedia of DNA Elements (ENCODE) project

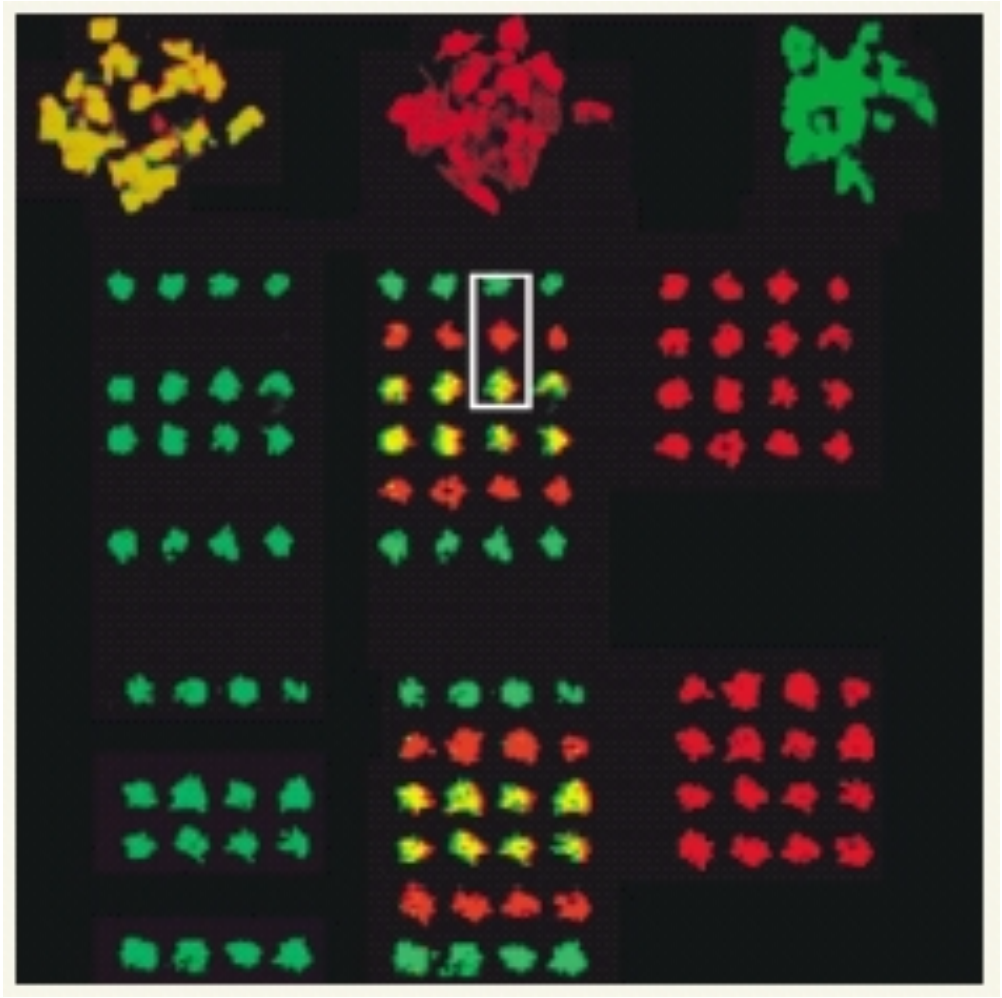


The Taxonomy of Genomic Biology

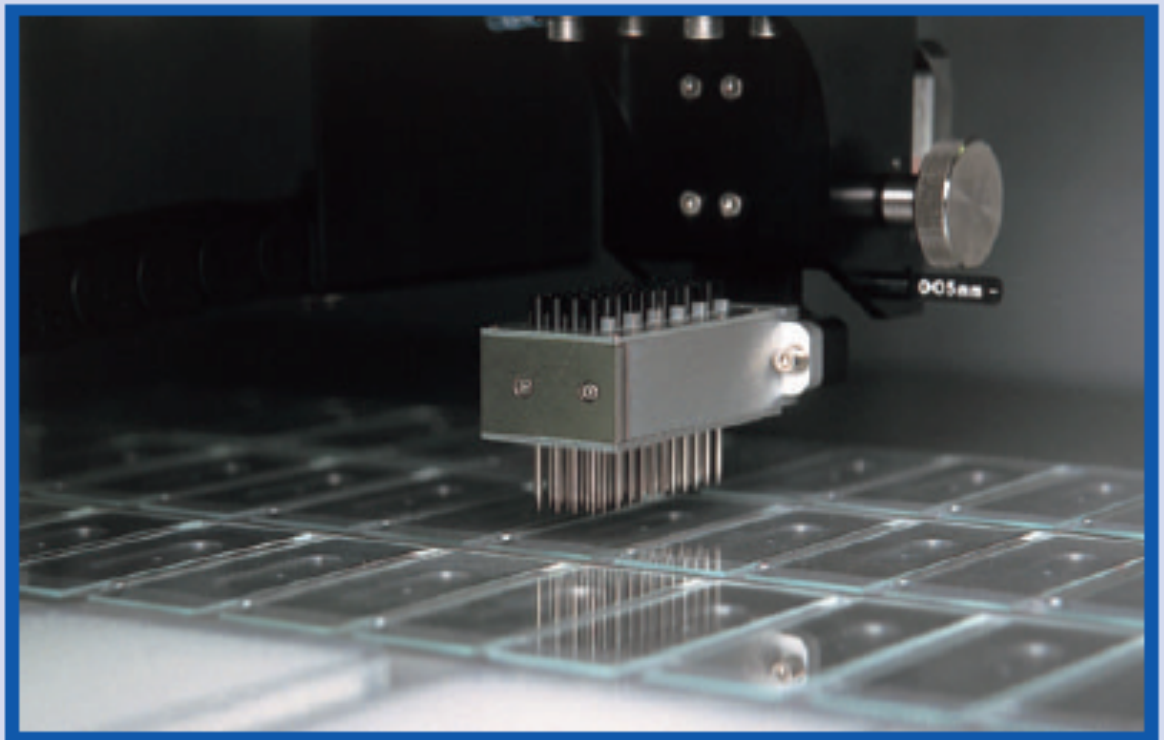


High Throughput

Microarrays Go Live

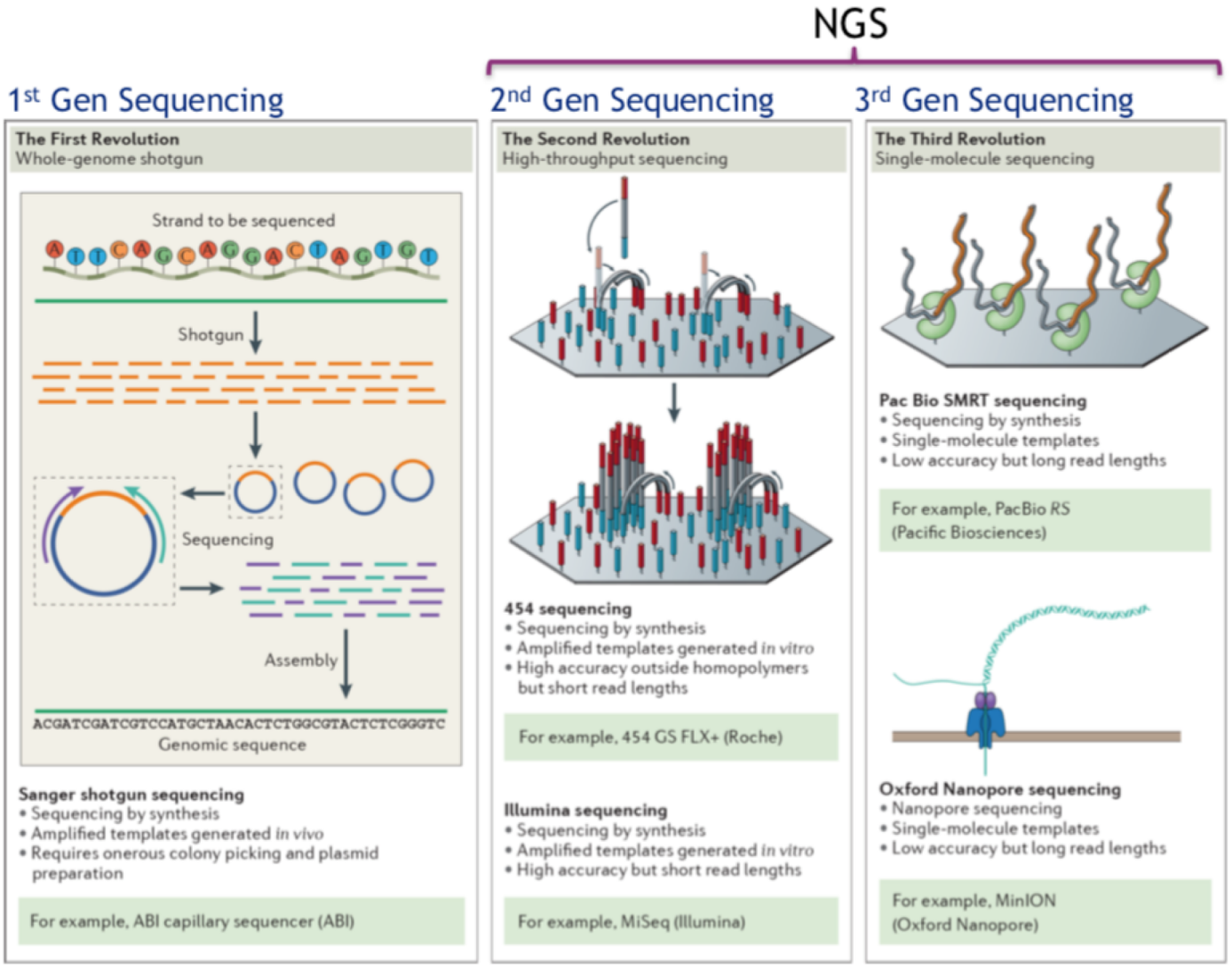


GENETIX



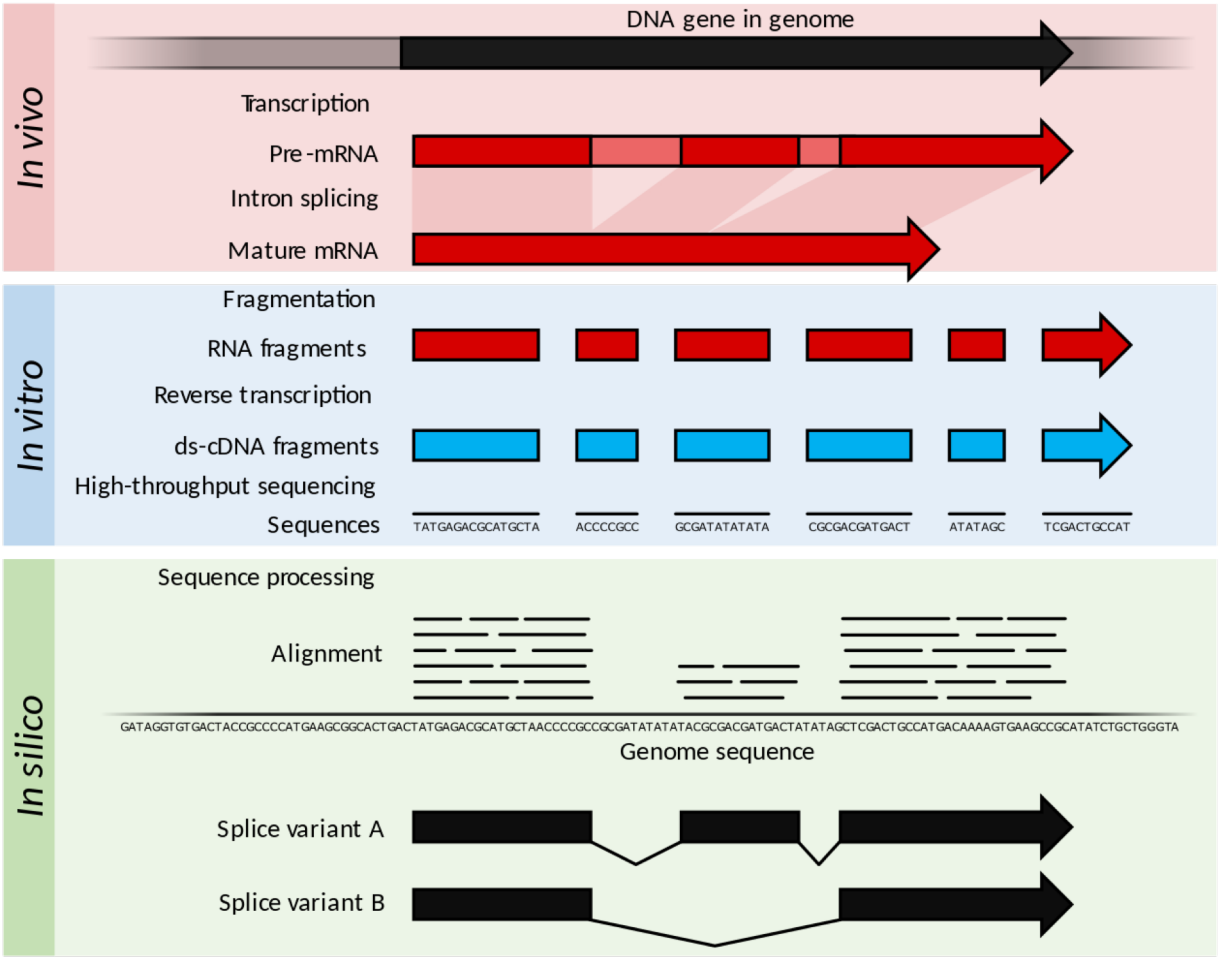
Ready-to-go: QArraymini gets you printing.

Next Generation Sequencing (NGS)

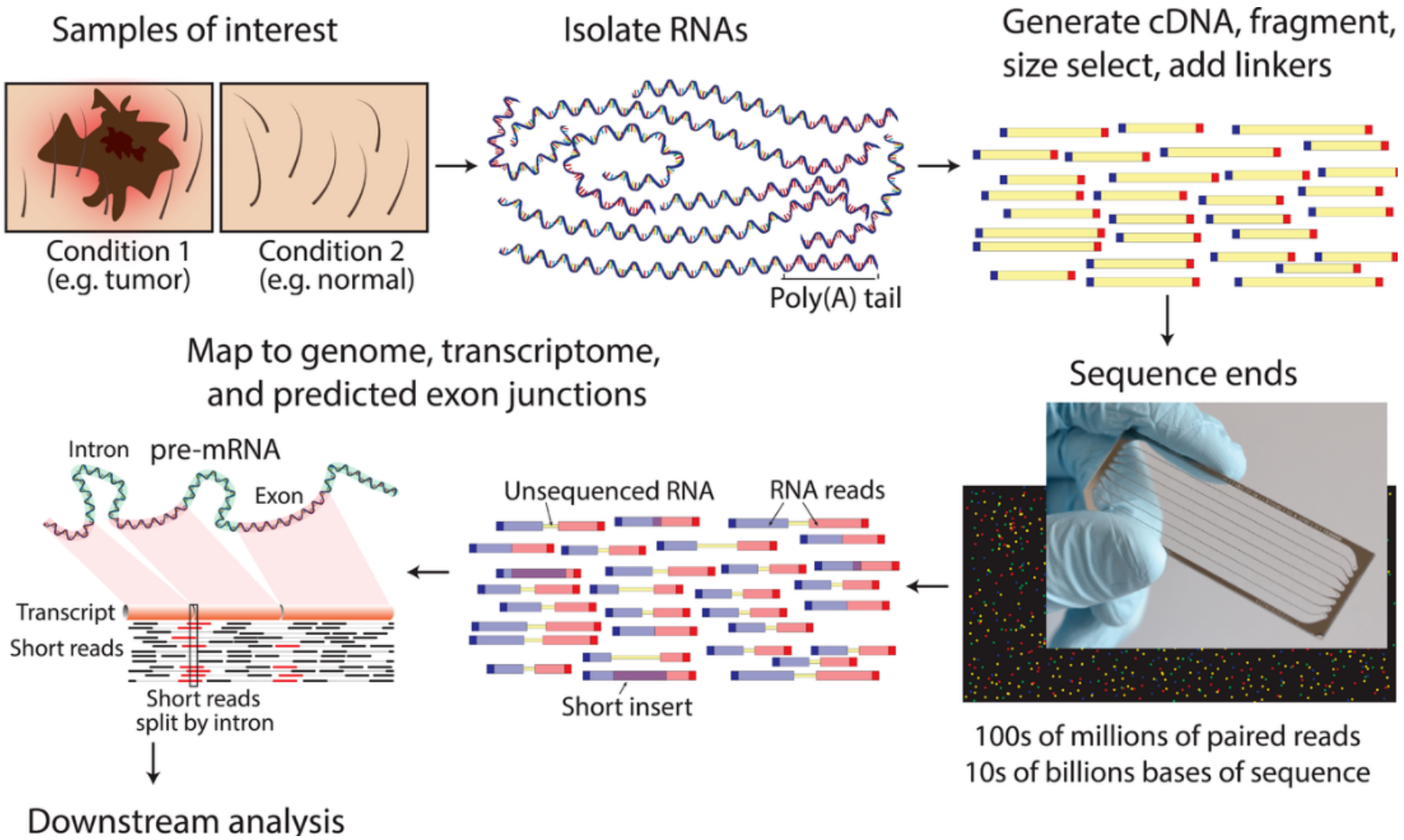


Nat Rev Microbiol. 2017 Dec;13(12):787-94

RNA-Seq



Summary of RNA-Seq. Within the organism, genes are transcribed and (in an eukaryotic organism) spliced to produce mature mRNA transcripts (red). The mRNA is extracted from the organism, fragmented and copied into stable ds-cDNA (blue). The ds-cDNA is sequenced using high-throughput, short-read sequencing methods. These sequences can then be aligned to a reference genome sequence to reconstruct which genome regions were being transcribed. This data can be used to annotate where expressed genes are, their relative expression levels, and any alternative splice variants



https://en.wikipedia.org/wiki/RNA-Seq#/media/File:Journal.pcbi.1004393.g002.png

Current NGS Technologies

- 2nd Generation NGS (short reads / clonal amplification)
  1. Discontinued or Almost: 454 (Roche), SOLiD
  2. Reigning kings: Illumina (Solexa)
  3. Gaining Market: Complete Genomics (BGI), Ion Torrent
  4. Just Arrived or Upcoming: Qiagen (Intelligent Biosystems), Agilent , Illumina Firefly

- 3rd Generation NGS (single molecule seq. / long reads)
  - Pacific Biosystems (not anymore a Roche partner) – SMRT
  - Oxford Nanopore
  - Genia (Roche)

- Synthetic Long Reads:
  - 10x Genomics
  - Illumina Synthetic Long Reads (formerly Moleculo)

- Failed platforms: Helicos Biosciences, VisiGen, Genizon Biosciences, Starlight, etc.

Predictive molecular biomarkers in oncotherapy

Gene	*Pathway*	*Cancer types*	*Anticancer Agent*
ERBB2 (HER2)	Receptor tyrosine kinase (ERBB2)	Breast, bladder, gastric & lung cancer	ERBB2 inhibitors ERBB2 antibodies
MET	RTK (MET)	Bladder, gastric & renal cancer	MET inhibitors MET antibodies
DDR2	RTK	Lung adenoid cystic carcinoma & lung large cell carcinoma	Some tyrosine kinase inhibitors
PIK3CA, PIK3R1	PI3K	Breast, colorectal & endometrial cancer	PI3K inhibitors
PTEN	PI3K	Numerous cancers	PI3K inhibitors
MTOR & TSC1	mTOR	Tuberous sclerosis & bladder cancer	mTOR inhibitors
FGFR1	FGFR1	Myeloma, sarcoma, bladder, bresast, ovarian, lung, endometrial & myeloid cancer	FGFR inhibitors FGFR antibodies
BRCA1 & BRCA2	(DNA damage repair signaling) HR repair pathway	Breast & ovarian cancer	PARP inhibitors
MRN Complex: (MRE11- RAD50- NBS1)	(DNA damage repair signaling)	Breast, ovarian, colorectal, gastric, prostate cancer, leukemia & melanoma	MRN complex inhibitors
ERCC2 (XPD)	NER (Nucleotide Excision Repair Pathway) with ATPase and helicase activity	Breast, ovarian, lung & bladder cancer	Specific DNA repair pathway inhibitors
KRAS (Also known as V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog)	RAS/MEK/ERK & PI3K/AKT	Pancreatic, colon, lung, biliary tract, endometrial, cervical, bladder, liver, myeloid leukemia & breast cancer	RAF inhibitors PI3K inhibitors MEK inhibitors

Predictive biomarkers from discovery to clinical practice of personalized oncotherapy have been approved for the management of five diseases: chronic my- eloid leukemia, colon, breast, lung cancer and melanoma