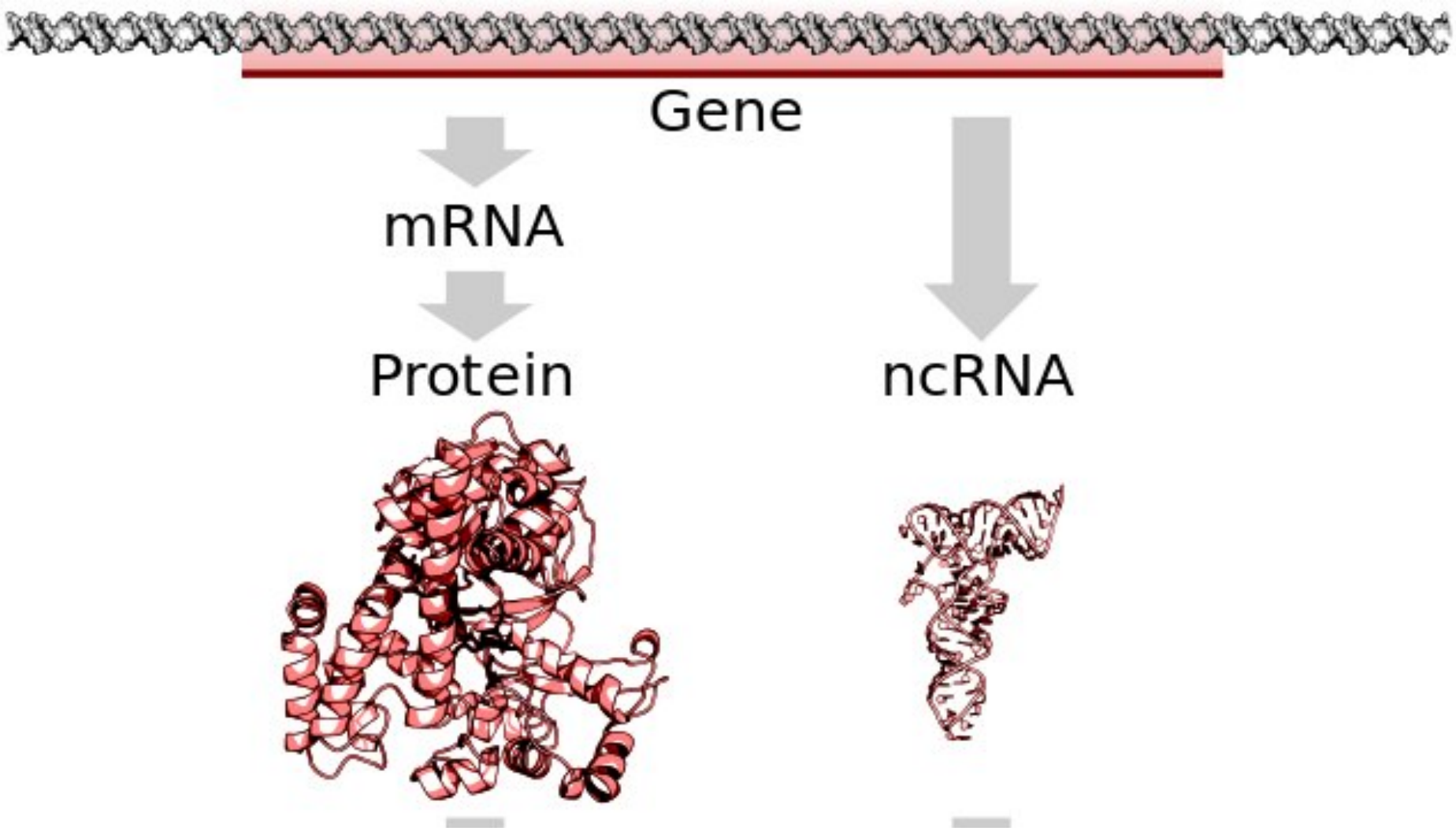
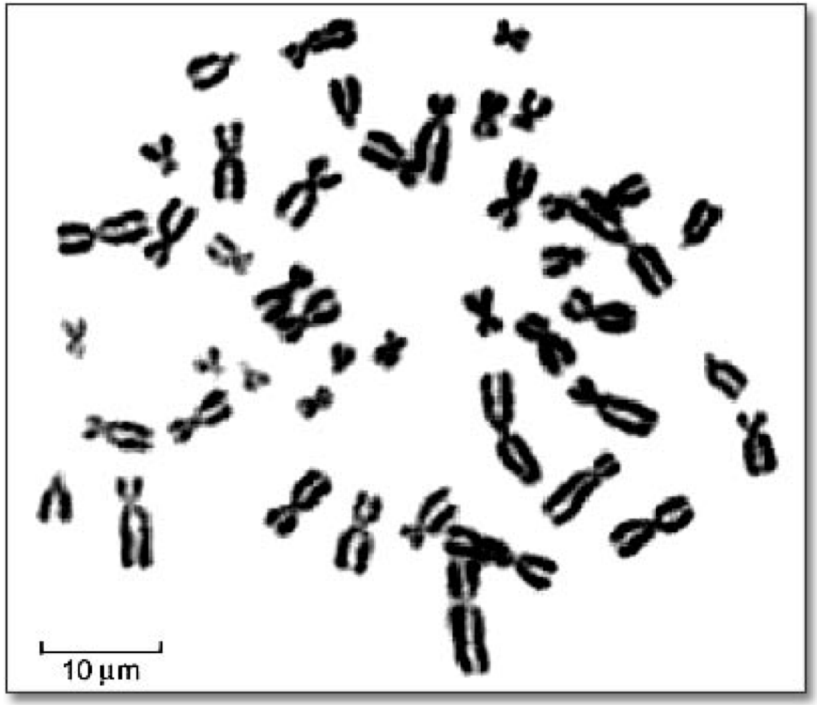
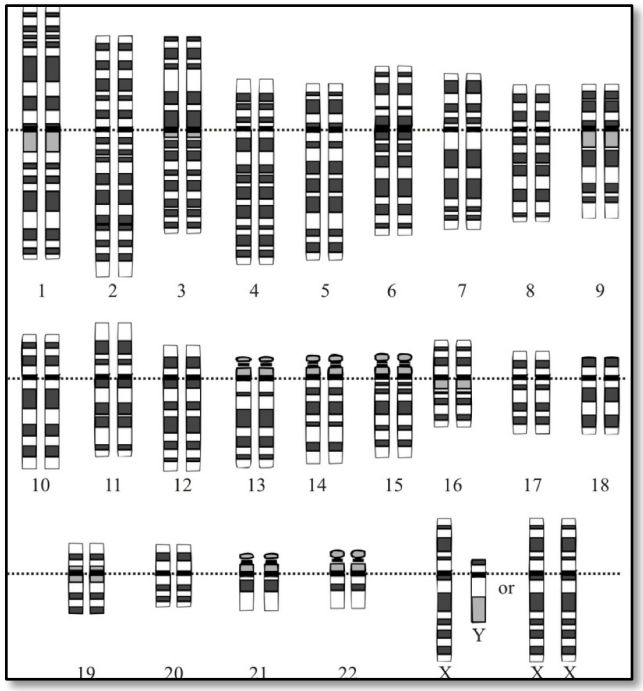
# Non coding RNA Biology

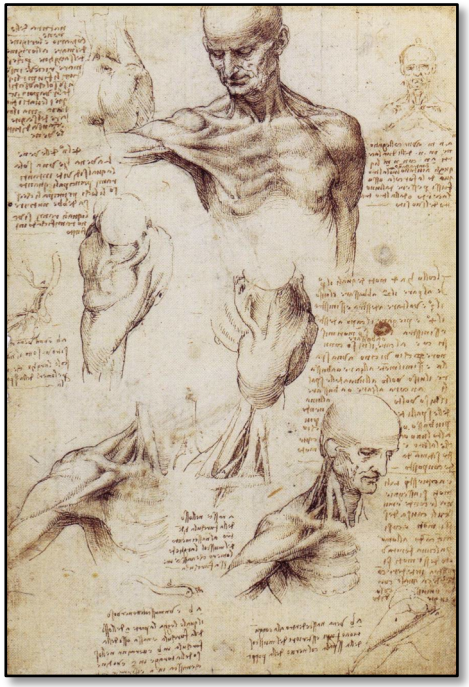
## AA 2021/2022



# The human genome is highly structured



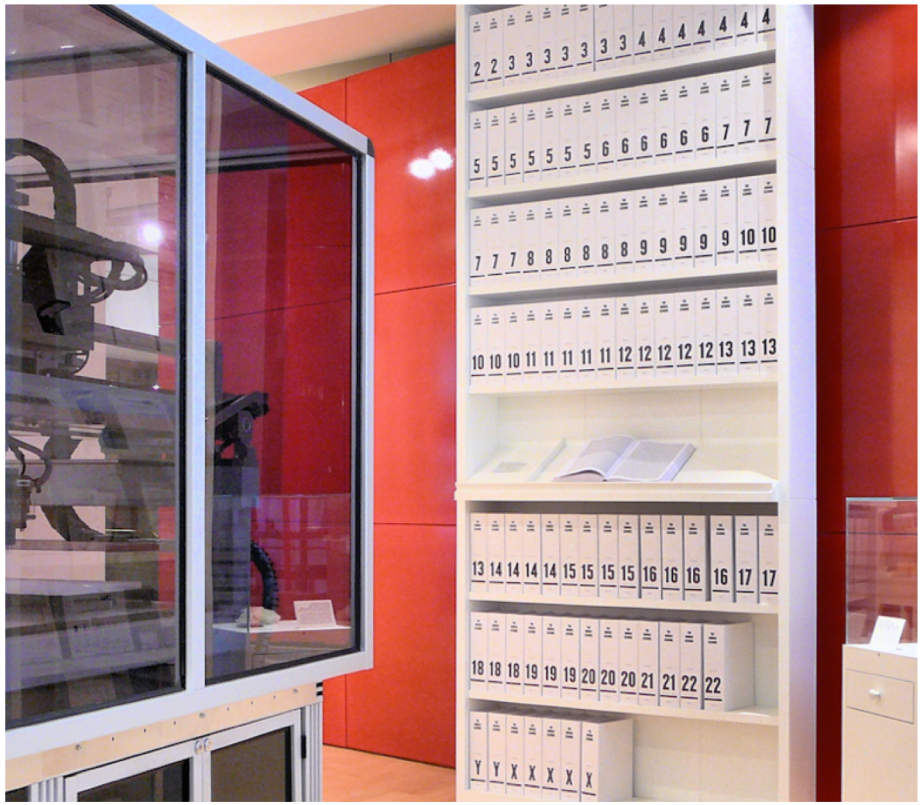
The human genome:

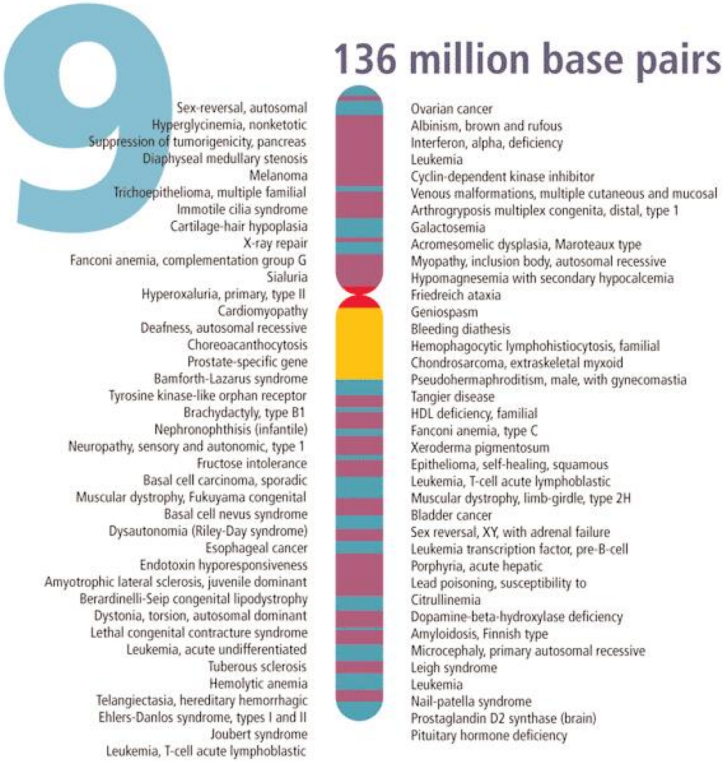
22 autosome paires

2 Sex chromosome pairs (XX o XY)

Total haploid genome $3x10^9$

# The human genome is highly structured






**Haploid human genome: 3.2 x $10^9$ bp (3200000000 bp)**

→ 22 autosomes
→ 2 sex chromosomes (X ed Y)
→ 19797 protein coding genes (ca 20.000)

**Chromosome dimensions: 45-275 Mb;**
→ 3,2 x $10^9$ bp: haploid chromosome set

**Usage of genetic information:**

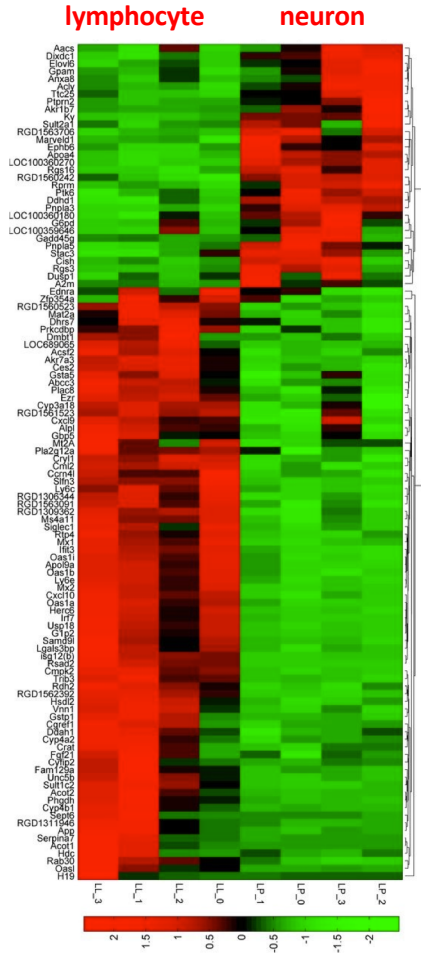
5.000-10.000 geni espressi da ogni cellula
 100.000 different proteins (post- translational modifactions per cell)
 $10^8$ total protein spcecies

***ENORMOUSE COMPLEXITY***

# The human genome encodes information that underlies cell specification in multi-cellular organisms

**GENOMA**
**coding and non-coding genes**

**Specific gene expression programs**




**Cell function**

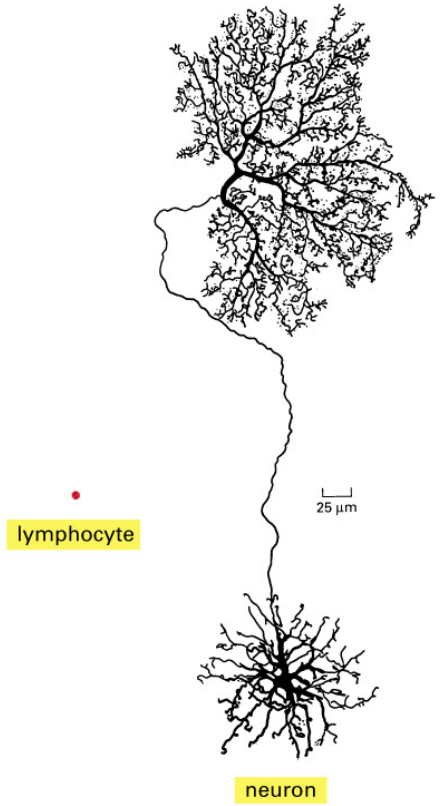



Figure 7–1. Molecular Biology of the Cell, 4th Edition.

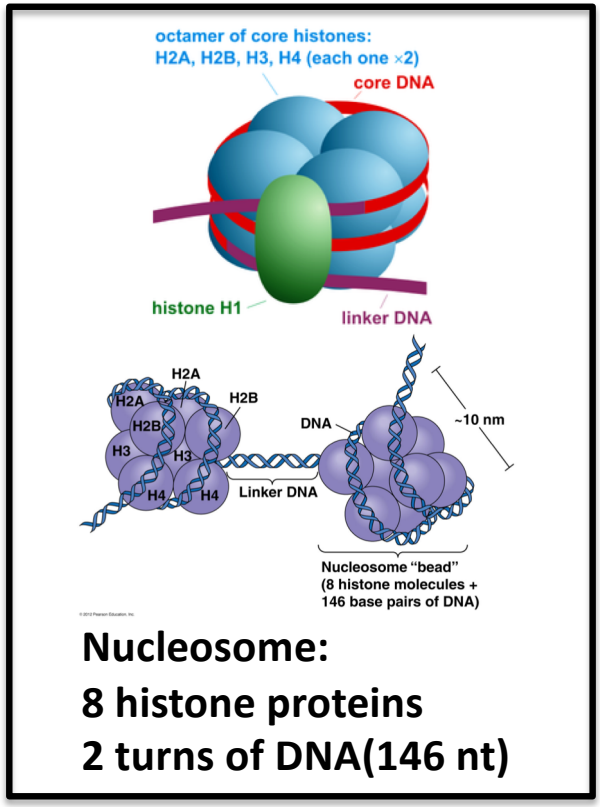
***Genetic information must be highly organized***

# The human genome is highly structured

Chromatin: DNA + protein in nucleus
Organisation of genetic information

**Function:**

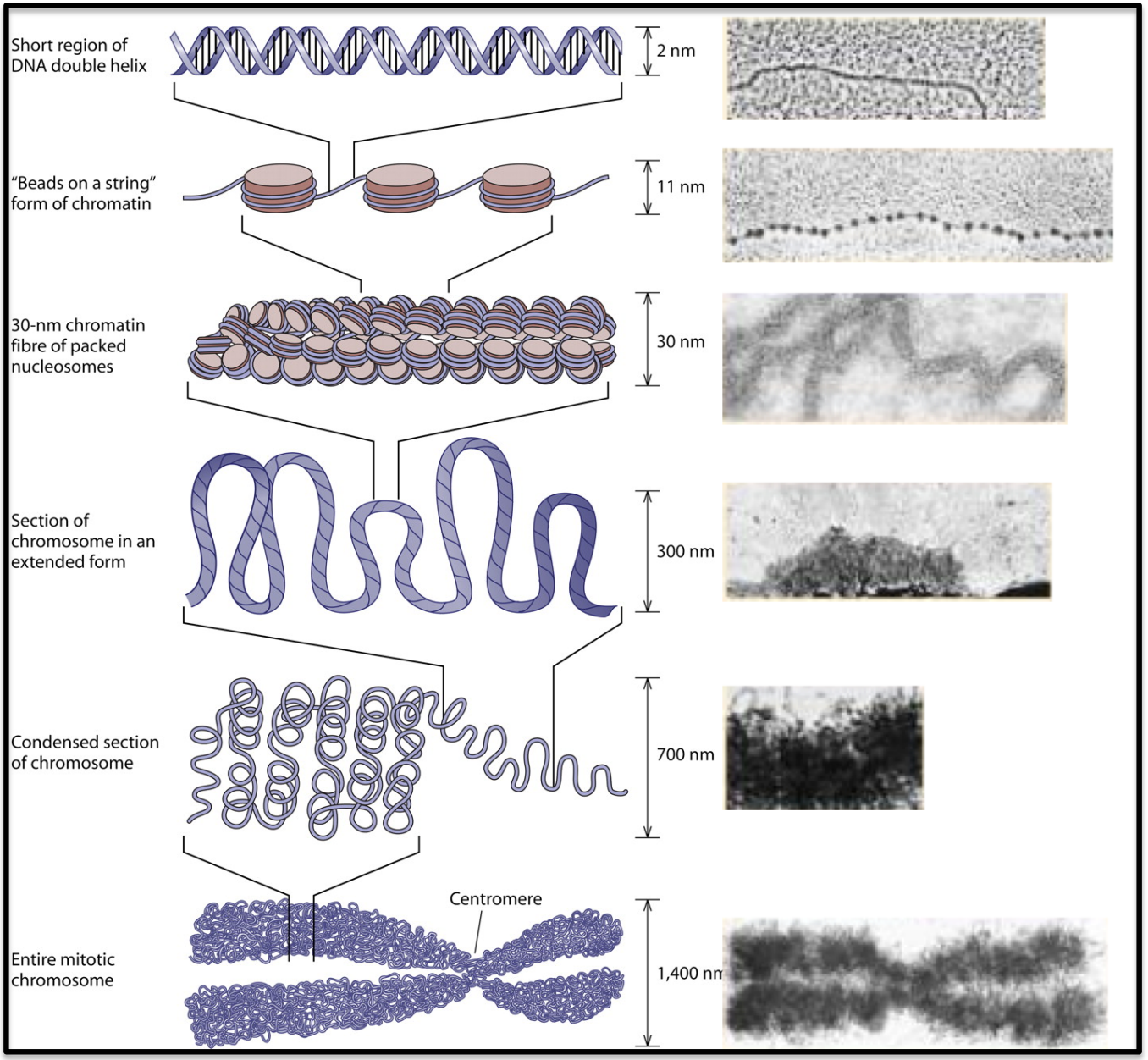
Packaging of DNA
Compaction of DNA
Definition of reagions of gene
Expression (euchromatin) or repression (heterochromatin)
-Increasing stability of DNA
-Prevention of damage
-Control of replication, gene expression
-Cell cycle



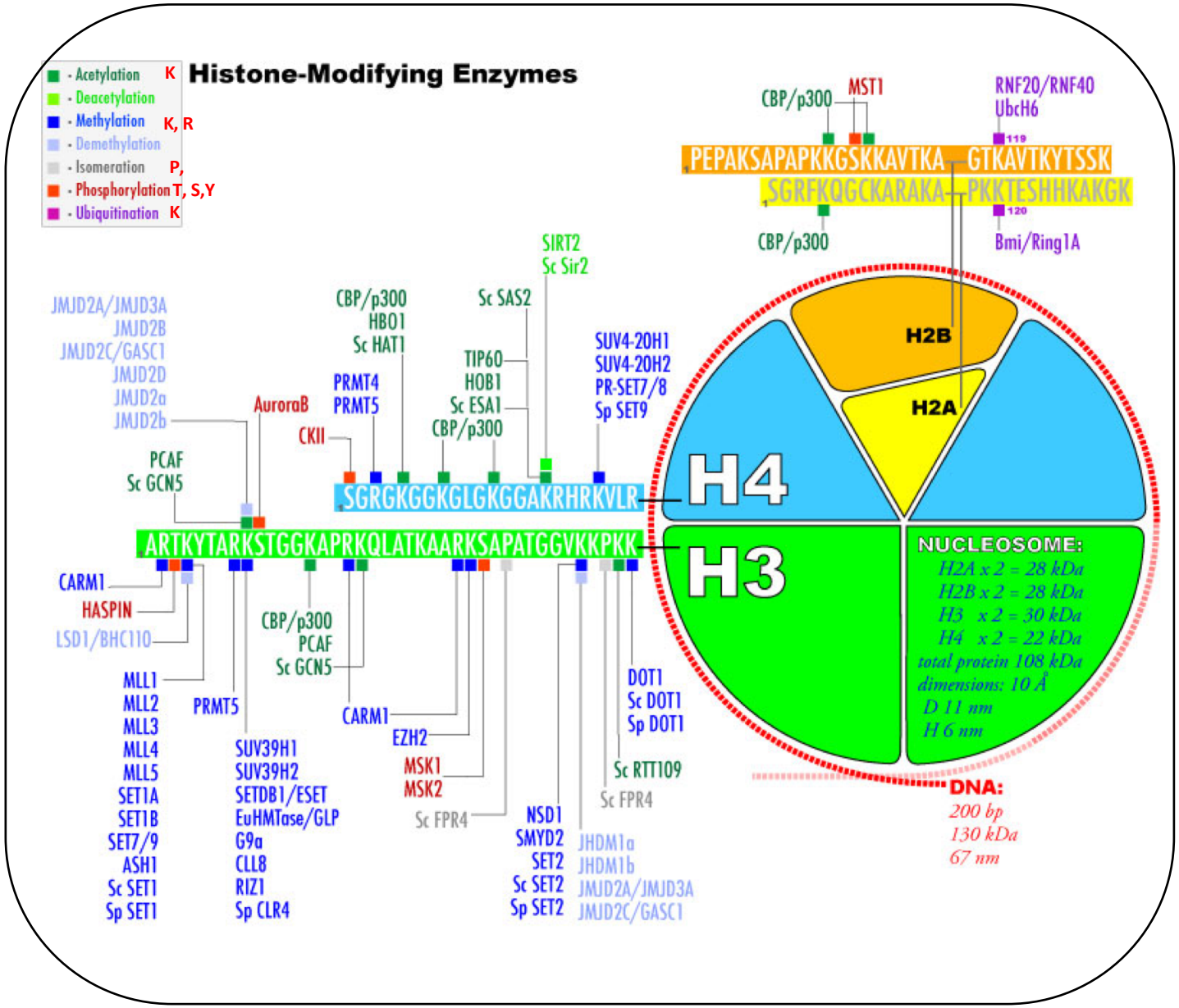

**Nucleosome:**

**8 histone proteins**

**2 turns of DNA(146 nt)**



# POST-TRANSLATIONAL HISTONE MODIFICATIONS



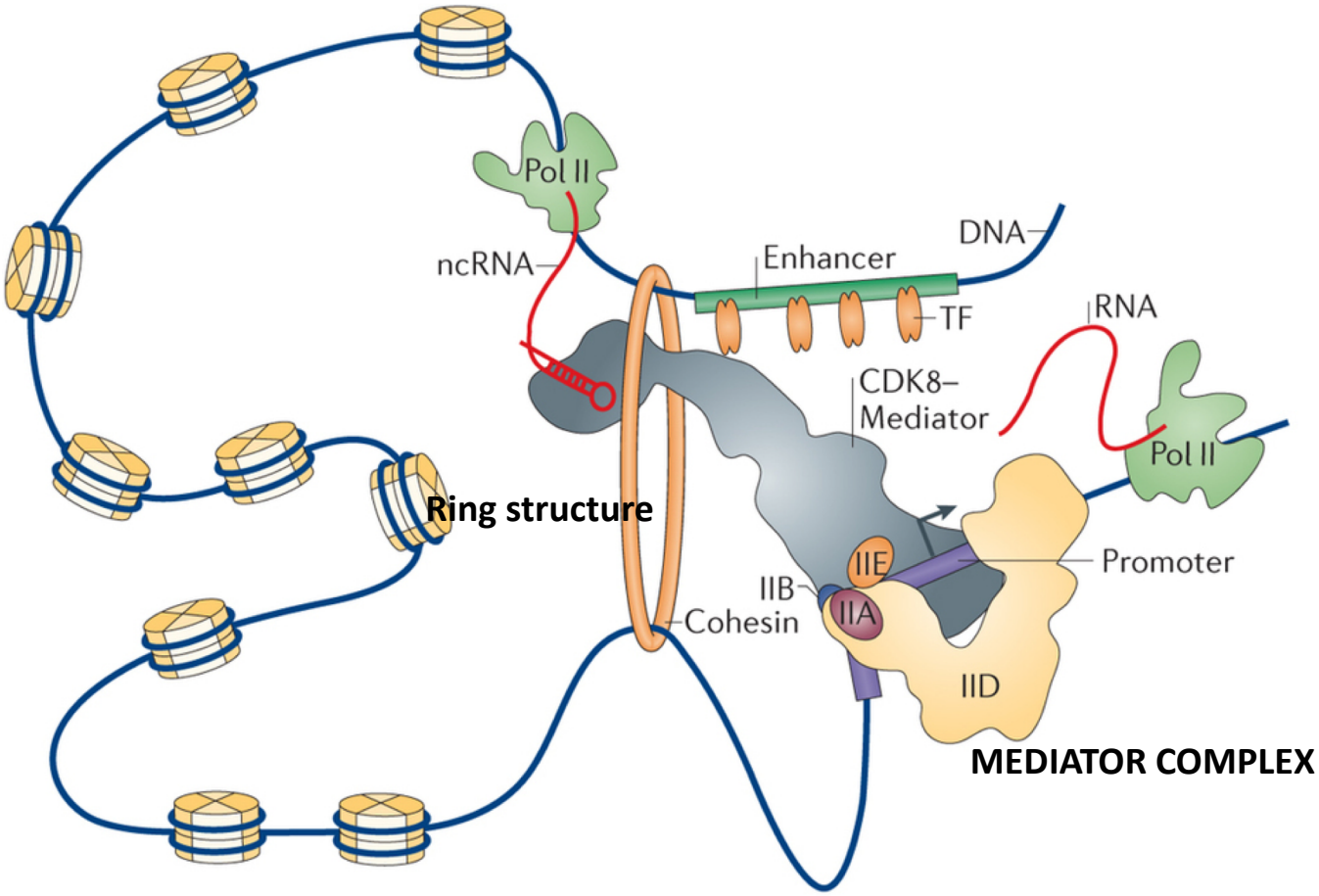

Gene expression
Control by post-translational histone modifications

→Activate transcription (H3K9 acetylation, …)
→Repress transcription (H3K27 trimethylation)
can be cell type specific

**Sum of all modifications = HISTONE CODE**

Specific histone +modifications at promoters Enhancers, along active Genes, site of termination

# The human genome is highly structured

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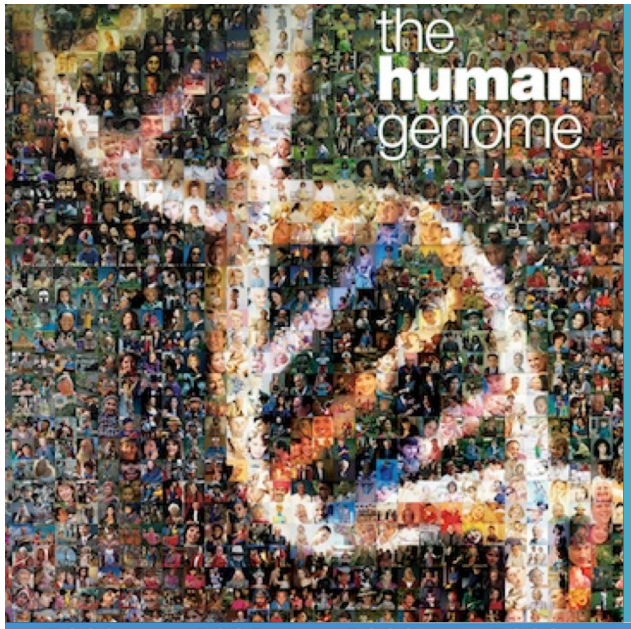
**Specific transcription factors can bind promoters and enhancers**

**RNAs can support the use enhancers**

**Enhancers are brought In vicinity to promoters and other gene regulatory Elements**

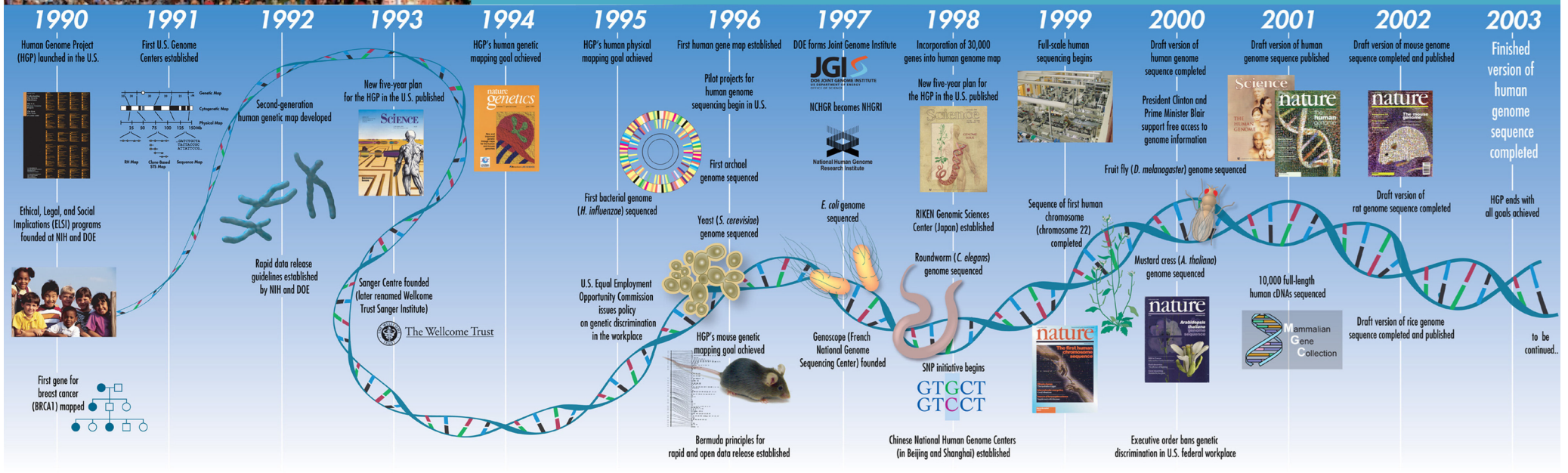
**→ SPECIFIC 3 DIMENTSIONAL STRUCTURE**




# THE GENOME OF MANY ORGANSIMS IS ALREADY SEQUENCED

# THE HUMAN GENOME PROJECT

## SEQEUNCING GENOMIC DNA

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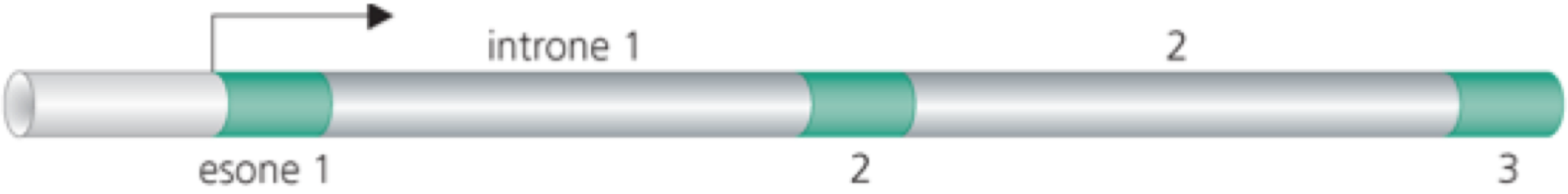
**ISOLATE LARGE PIECES OF DNA AND SEQEUNCE!**



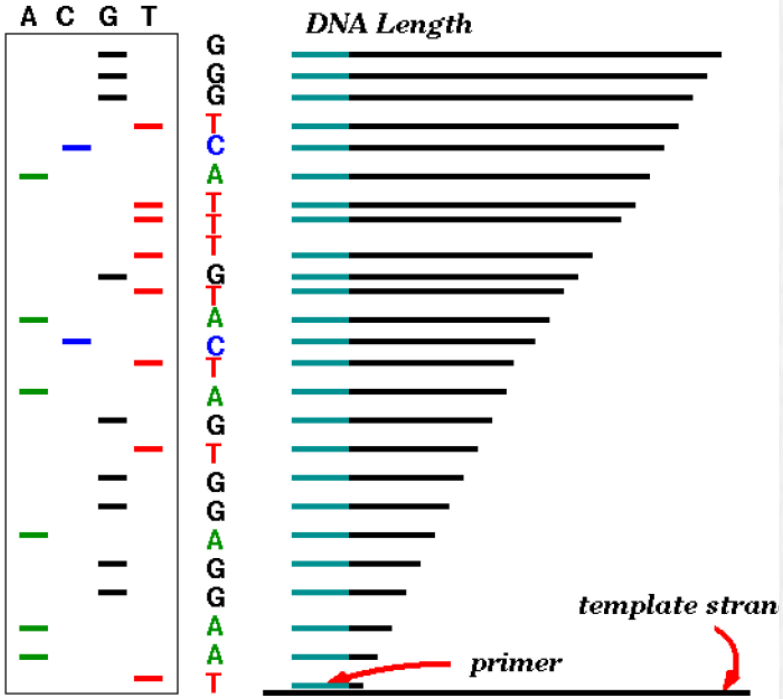
# Dideoxy (Sanger) sequencing

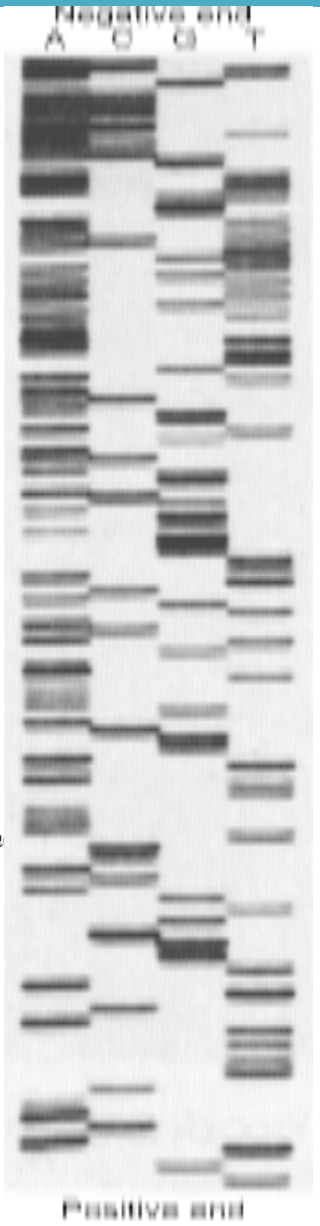
**Principle:**
Gel electrophoresis: discrimination of 1 bp: size range below 300 bp in the lab

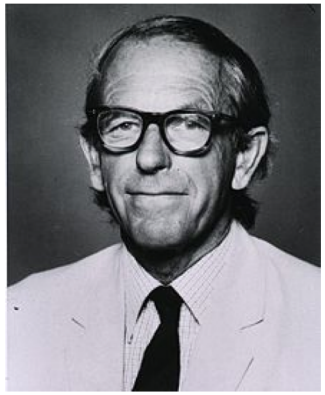
DNA template + 32P-labelled sequencing oligo

4 parallel seqeuncing reactions:
1. dATP, dCTP, dGTP, dTTP + ddATP (low conc)
2. dATP, dCTP, dGTP, dTTP + ddCTP (low conc)
3. dATP, dCTP, dGTP, dTTP + ddGTP (low conc)
4. dATP, dCTP, dGTP, dTTP + ddTTP (low conc)

Synthesis: starts with a32-P labeled DNA oligo
stops after incorporating a (marked) ddNTP



Frederic Sanger
Nobel Prize 1980

# Dideoxy (Sanger) sequencing with Dye termination

**Principle:**

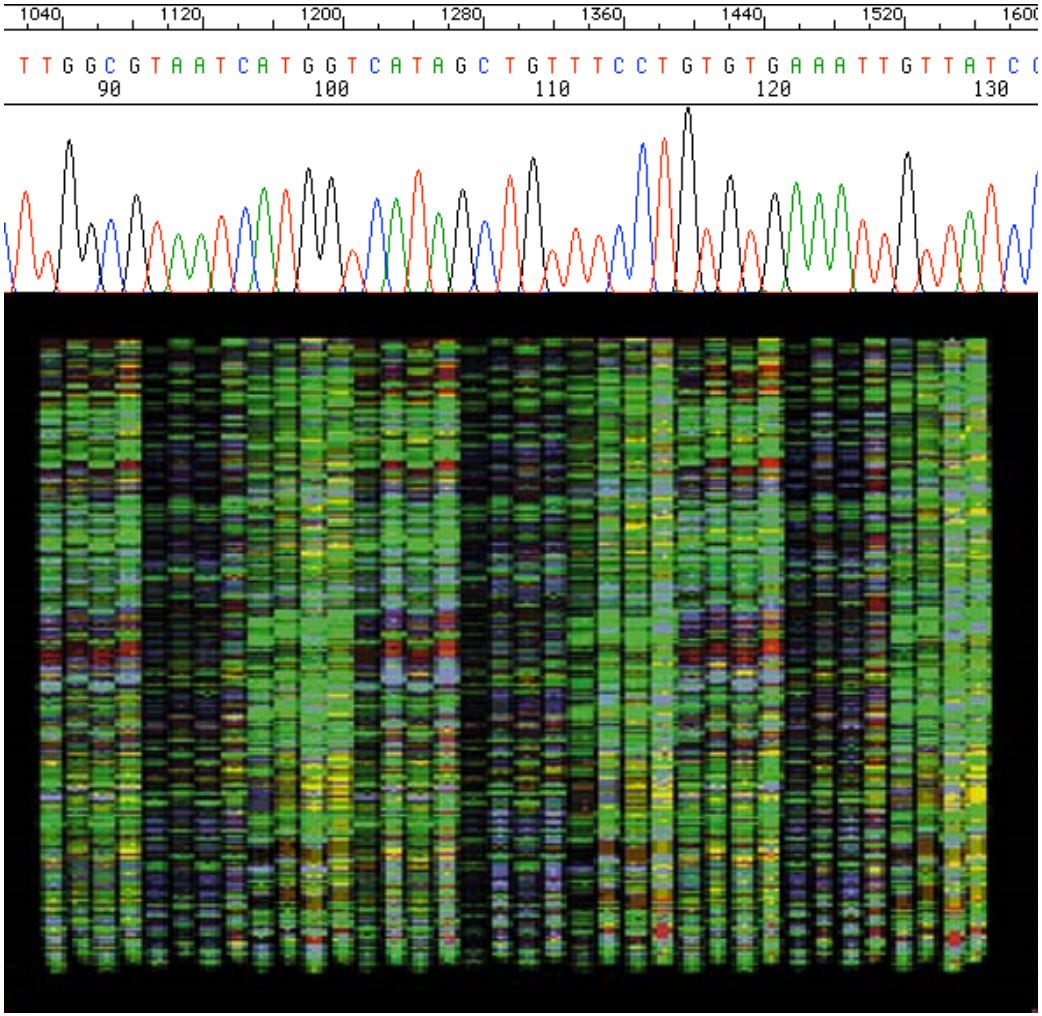
Gel electrophoresis: discrimination of 1 bp: size range below ~1000 bp

DNA template + sequencing oligo

1 seqeuncing reaction:

1. dATP, dCTP, dGTP, dTTP + ddATP-Dye1, ddCTP-Dye2, + ddGTP-Dye3+ddTTP-Dye4 (low conc)

Synthesis: starts with DNA oligo
stops after incorporating a (marked) ddNTP

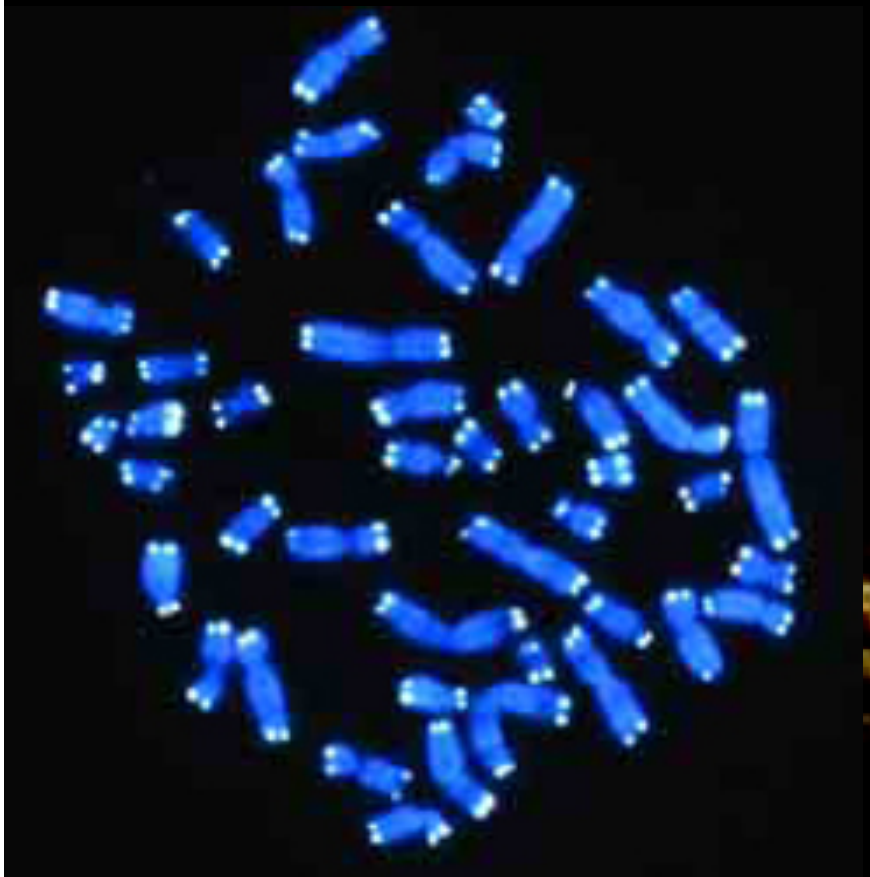


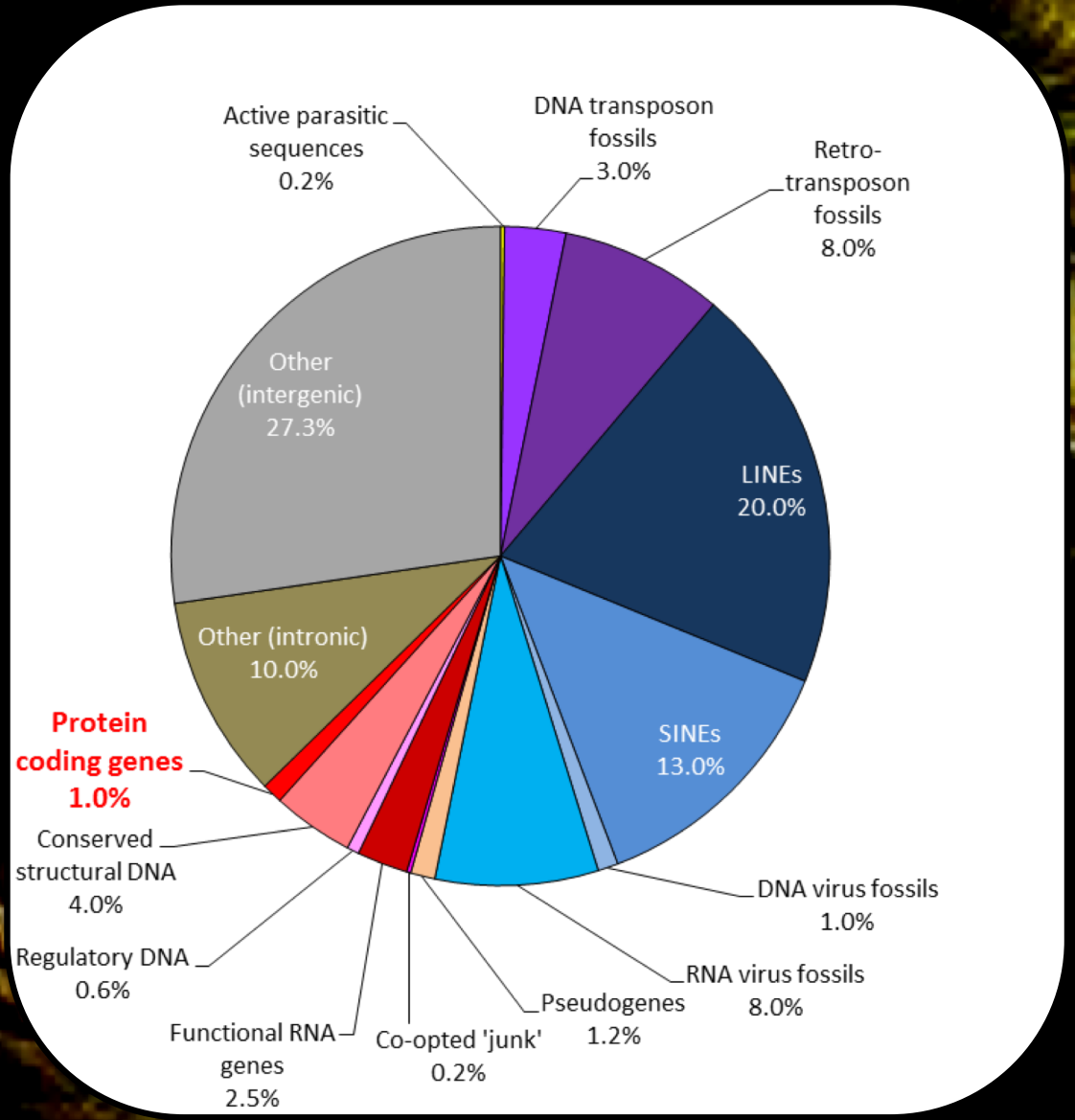
# 98% OF GENOMIC DNA DOES NOT ENCODE FOR PROTEINS

ca 50% transposable elements

1-2% protein coding genes

0.5-1% pseudogenes

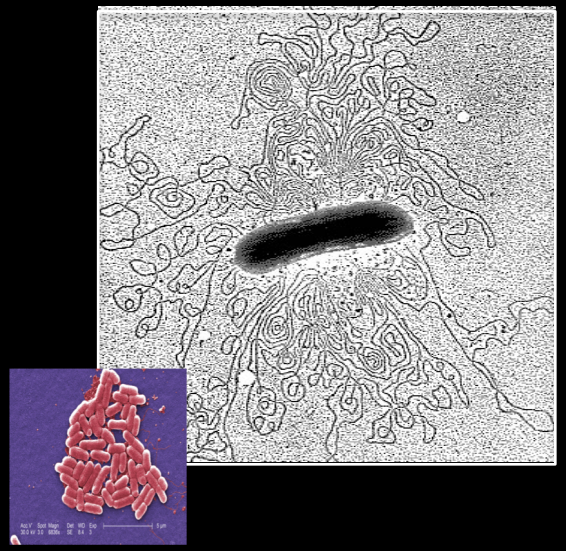





*Almost all genomic sequences are subjected to transcription*

# THE NUMBER OF PROTEIN CODING GENES IS RELATVLY LOW

*E.coli*



*C. elegans*

*H. sapiens*

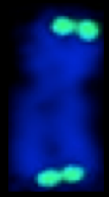
| | | | |
|---|---|---|---|
| Genome | $5x10^6$ bp | $1x10^8$ bp | $3x10^9$ bp |
| Chromosomes | 1 | 6 | 23 |
| Coding genes | 6692 | 20541 | 21995 |
| ncDNA | | | |
| non-coding RNA genes | | ?????????????? | |
| miRNAs | | | |
| pseudogenes | | | |

**WHAT INFORMATION INCREASES ORGNAISMAL COMPLEXITY**

**ncDNA derived information?**

## Why to study ncRNAs
## 1. There are things proteins cannot do

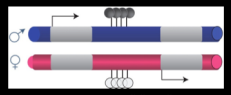
AGING-CANCER



Telomere function

hTR lncRNA

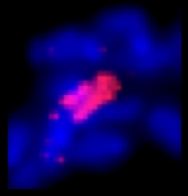
DEVELOPMENTAL DEFECTS



mono-allelic Gene expression

Airn lncRNA

Xist lncRNA



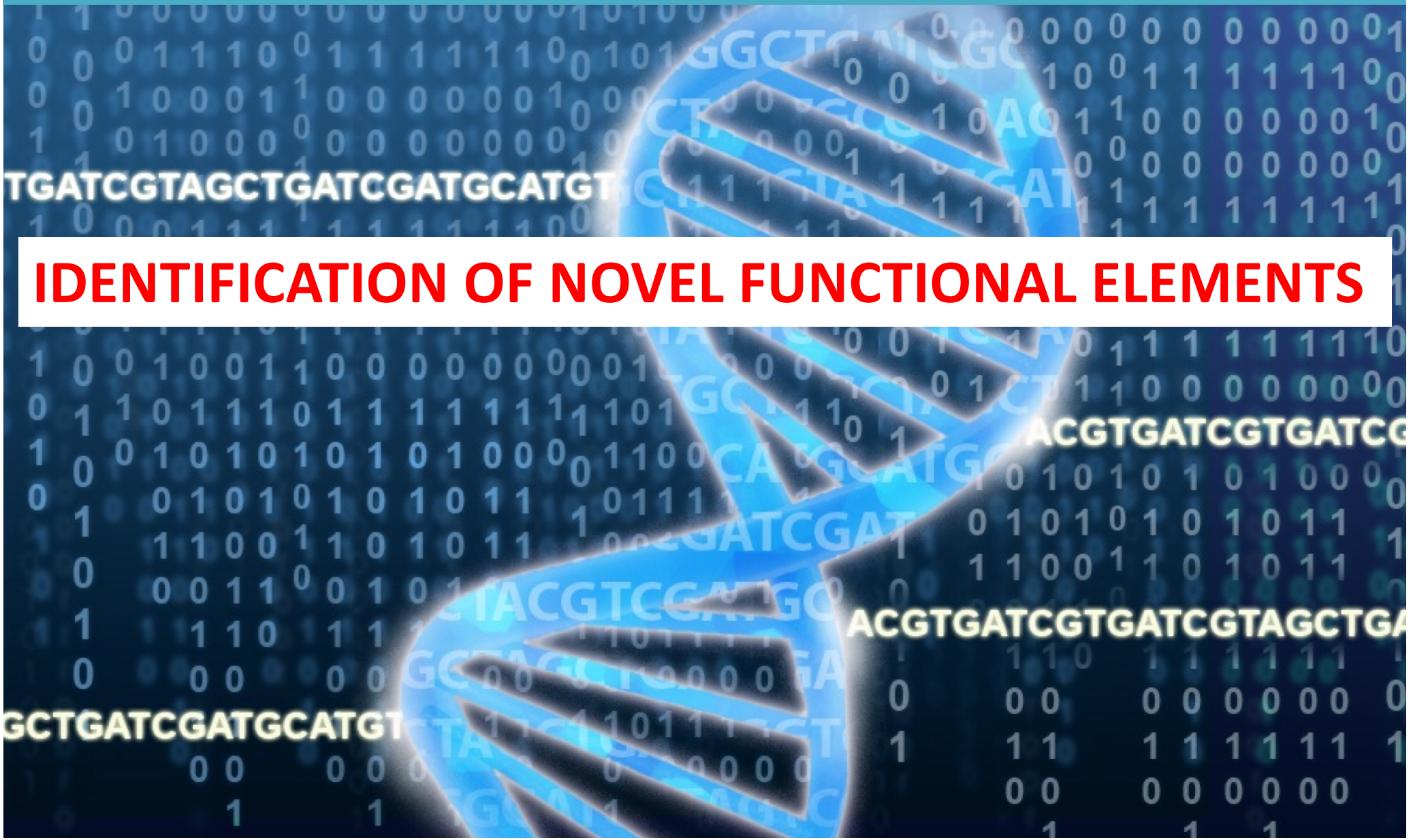
X inactivation Silencing 1000 genes on the Xi

LETHAL

## 2. they have high relevance for development and pathology

Classic Sanger sequencing is inefficient and slow:
→Establishement of massive parallel sequencing

## NEXT GENERATION SEQEUNCING OF DNA AND RNA




## IDENTIFICATION OF NOVEL FUNCTIONAL ELEMENTS

# NEXT GENERATION SEQEUNCING OF DNA AND RNA

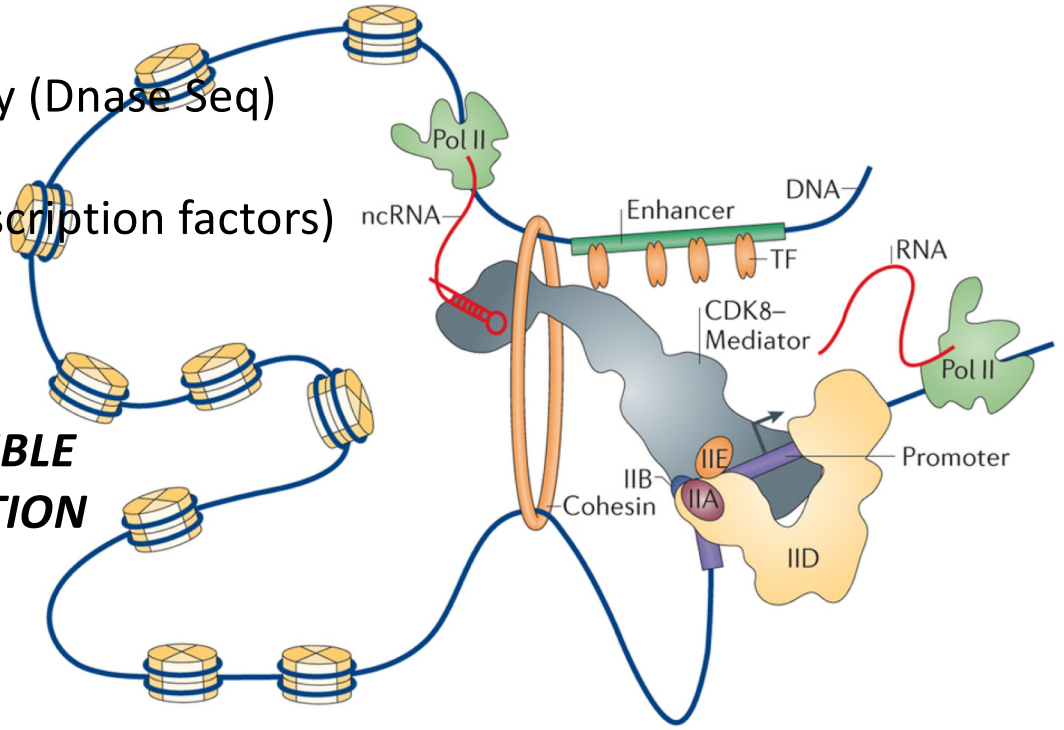
→IDENTIFICATION OF ALL GENES
→ IDENTIFICATION OF ALL CODING AND NON-CODING TRANSCRIPTS
→IDENTIFICATION OF REGUALTORY ELEMENTS

## HOW CAN "NEW" = *FUNCTIONAL ELEMENTS* - (GENES/TRANSCRIPTS) BE IDENTIFIED?

1. DNA Seqeuncing (Human genome project, DNA-Seq)
2. Landscape of transcription: Seqeuncing of RNA (total RNA, small/large RNA, CAGE)
3. DNA methylation: High representation reduced representation bisulfite sequencing (RRBS)
4. Local chromatin structure:

- determination of DNAseI hypersensitivity (Dnase Seq)
- nucelosome occupancy (MNase-seq)
- ChIP-seq (chromatin modifications, transcription factors)
- 3 Dimensional space interaction

***GENE REGUALTION AS INDICATOR OF POSSIBLE FUNCTIONAL RELEVANCE OF lncRNA FUNCTION***

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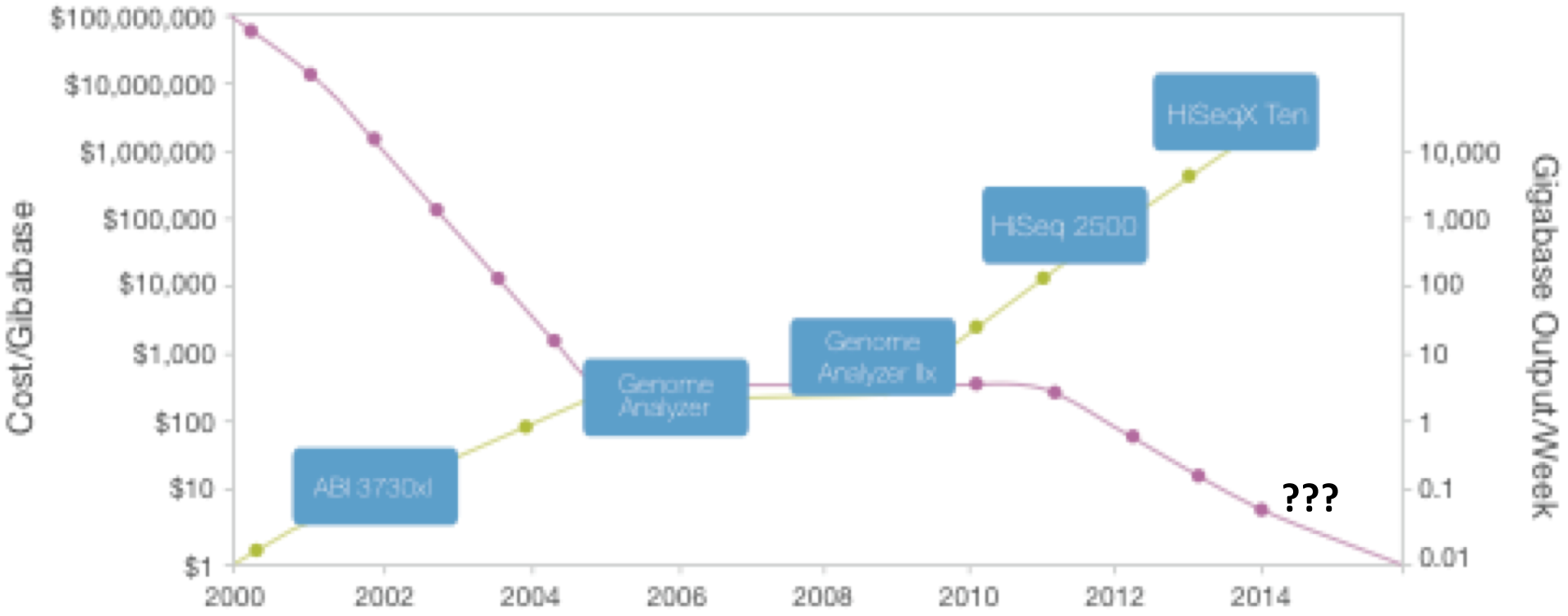
# PROGRESS IN SEQUENCING POWER



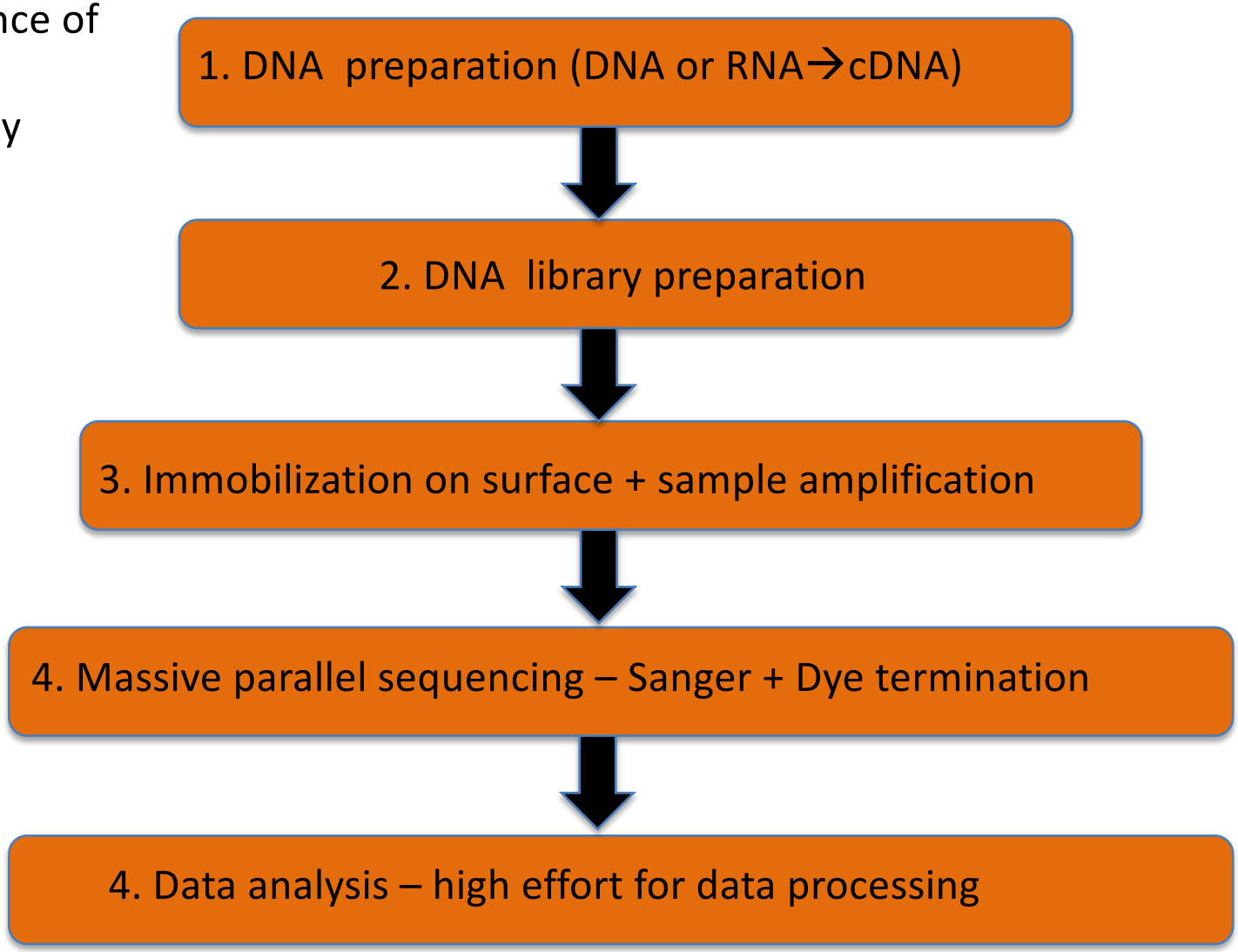

**BIOINFORMATICS EFFORT**
**= PROCESING OF DATA**

# Next generation sequencing:

# MASSIVE PARALLEL SEQUENCING (ILLUMINA)

**DNA SEQ** – genome sequence of many organisms
**RNA SEQ** – all RNAs of many organisms – also at low anbunance

**ChiP seq…..**



# Illumina: massive parallel sequencing Genomic DNA

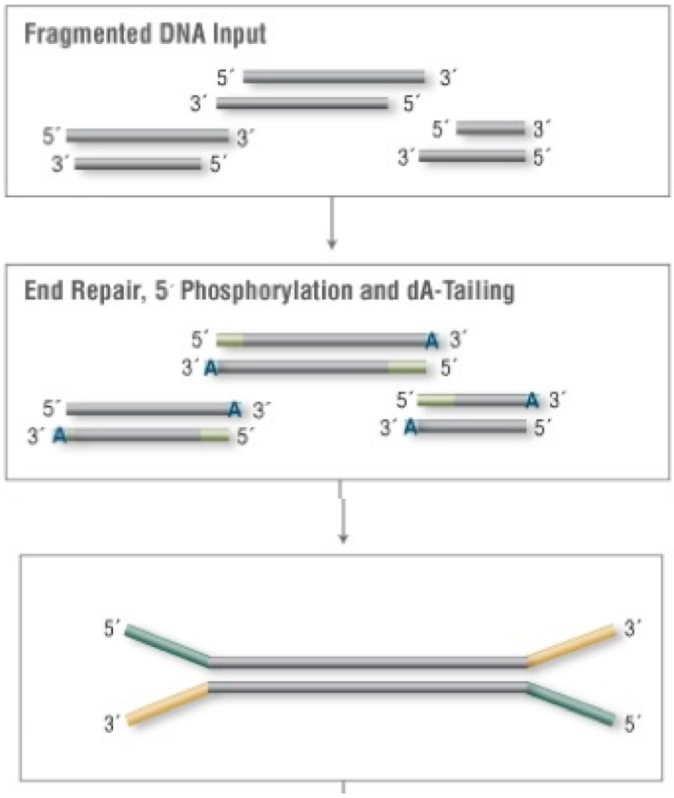
**Generation of DNA libraries:**

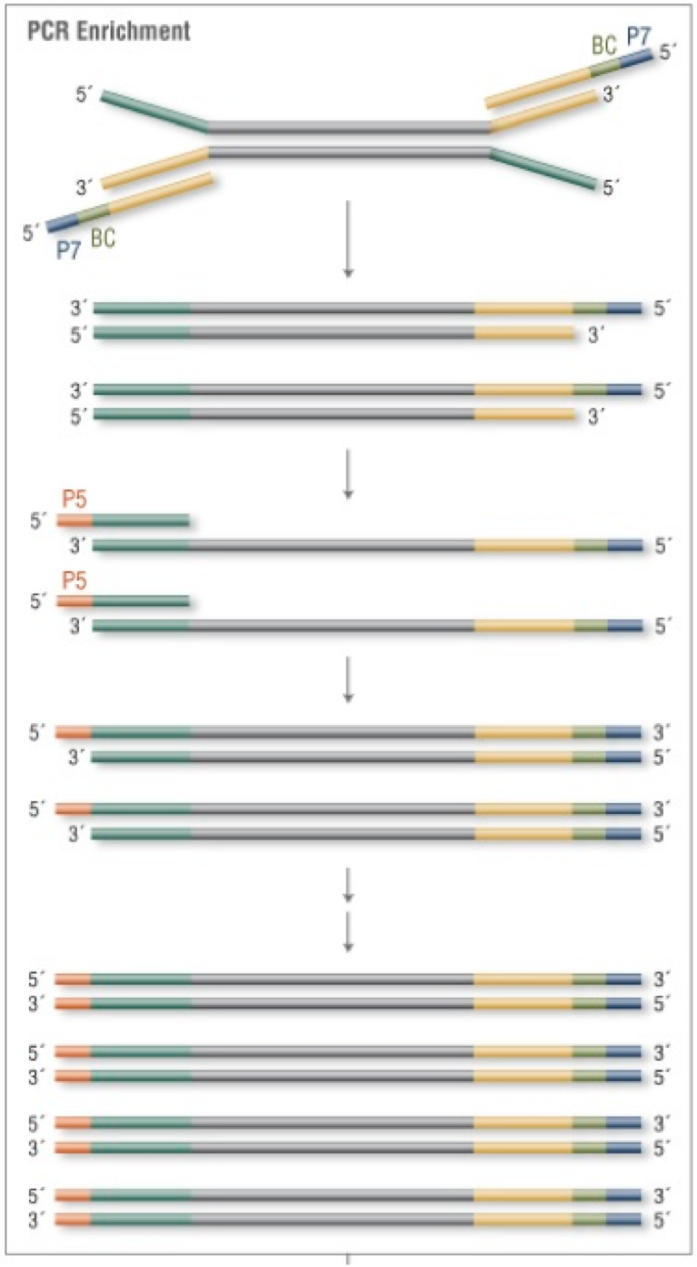
Application:

ChIP Seq

Genome Seq

Methyl Seq



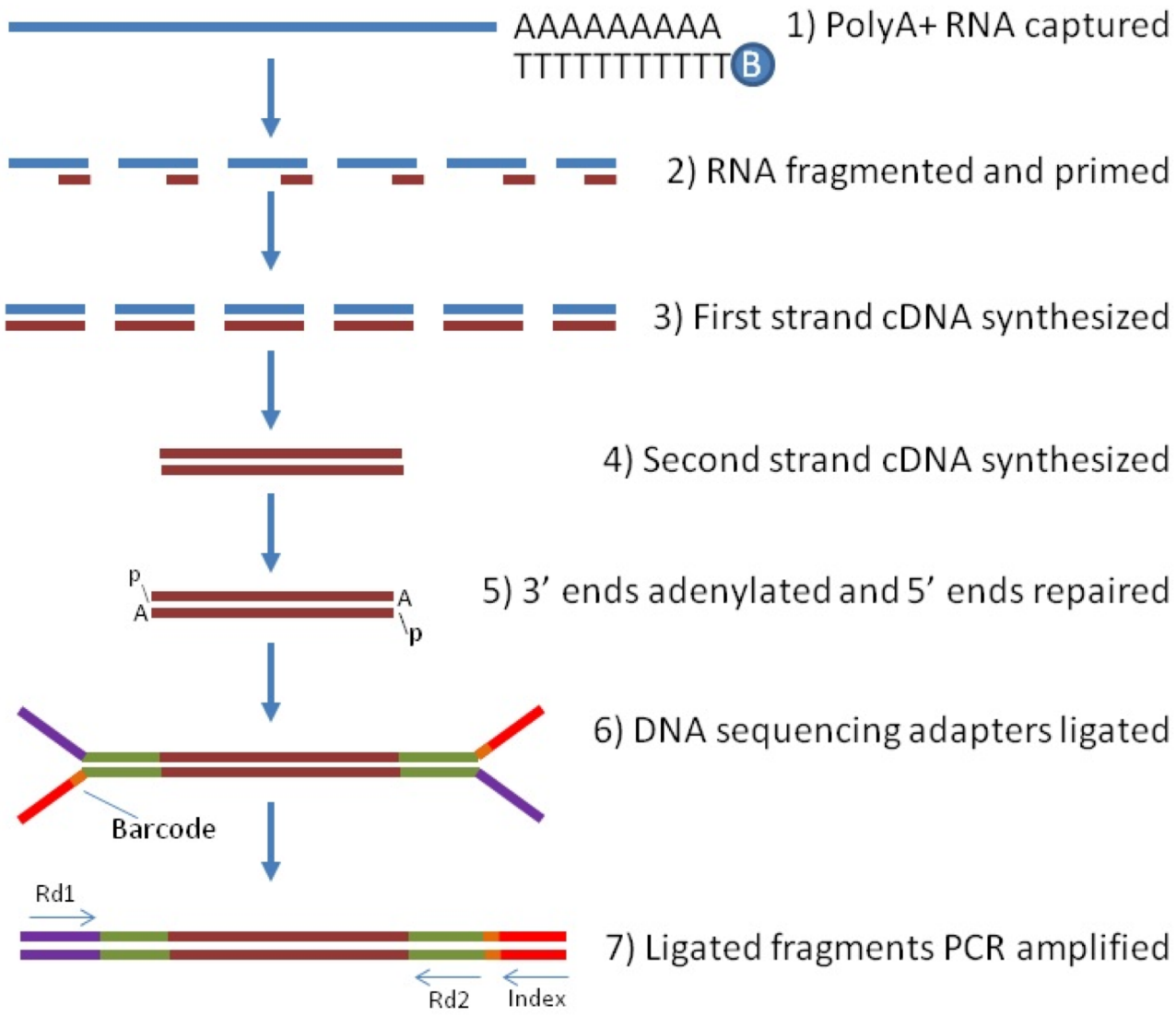



# Illumina: massive parallel sequencing: ALL TRANSCRIPTS

**Generation of RNA libraries:**

Application:
RNA Seq

**Important:**
Involves cDNA synthesis



# Illumina: massive parallel sequencing:

**Illumina Massively Parallel Sequencing**

https://www.illumina.com/company/video-hub/pfZp5Vgsbw0.html


**The heart of the Illumina Massive Parallel Sequencer is the "FLOW-CELL". A surface with millions of small wells that allow thousands of Sanger-sequencing reaction In parallel = "massive parallel sequencing". In each well a SINGLE MOLECULE of DNA Is amplified and sequenced**

**Illumina offers the most potent massive sequencing instruments – leader on the market**

# Illumina: massive parallel sequencing:

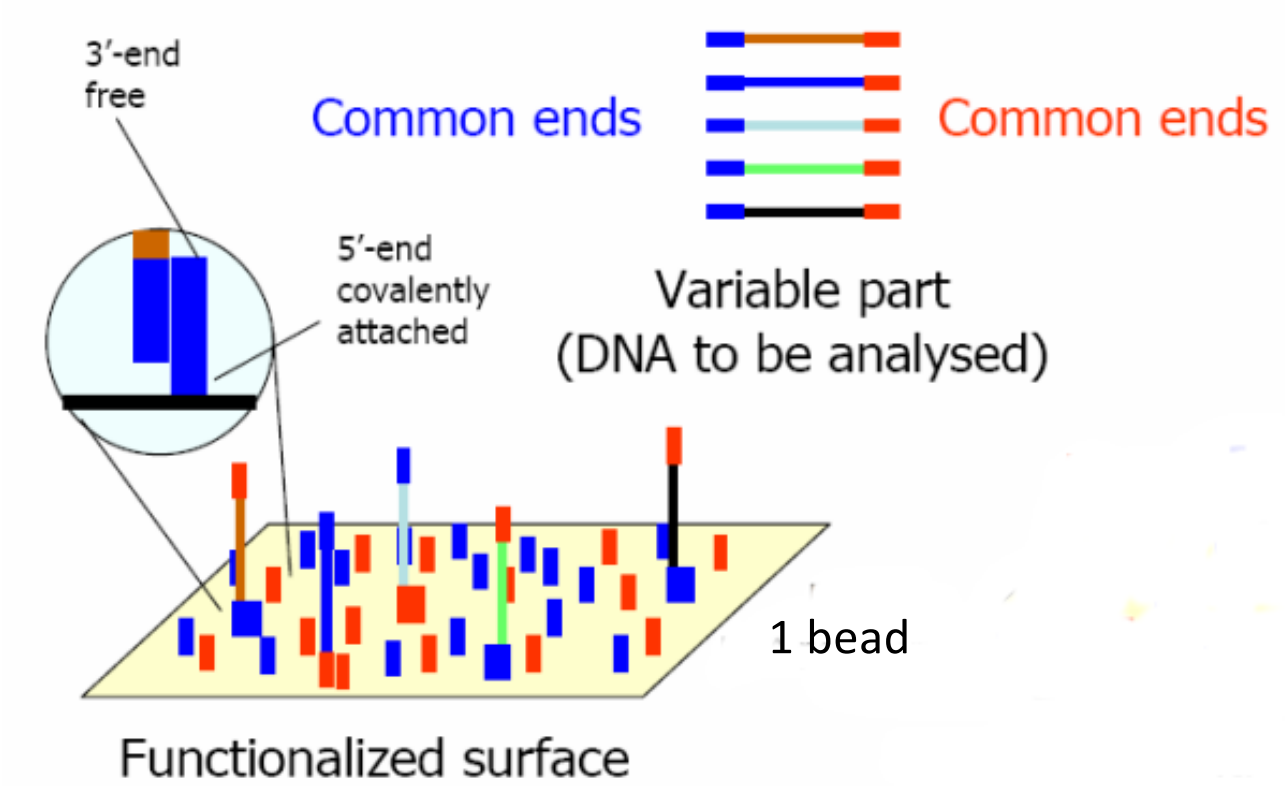
**Flow cell contains surface with millions of wells**

**→Each well contains beads mounted with 2 species of oligonucleotides that hybridize with adaptor oligos of DNA library**

**→DNA library will be loaded onto the flow cell in a determined concentration:**
**ONLY ONE MOLECULE PER WELL**

# Illumina: massive parallel sequencing:

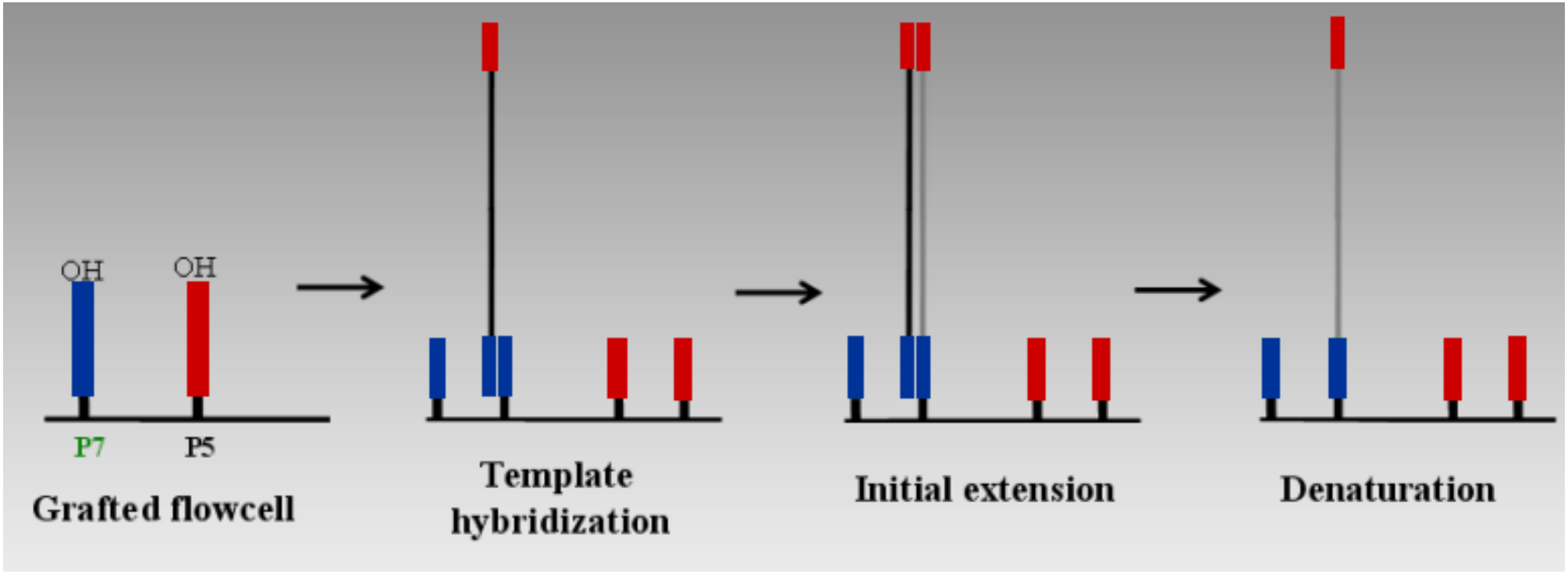
-making DNA library (~300bp fragments)
-ligation of adapters **A** and **B** to the fragments




-binding the ssDNA randomly to the flow cell surface
-***complementary*** primers are ligated to the surface

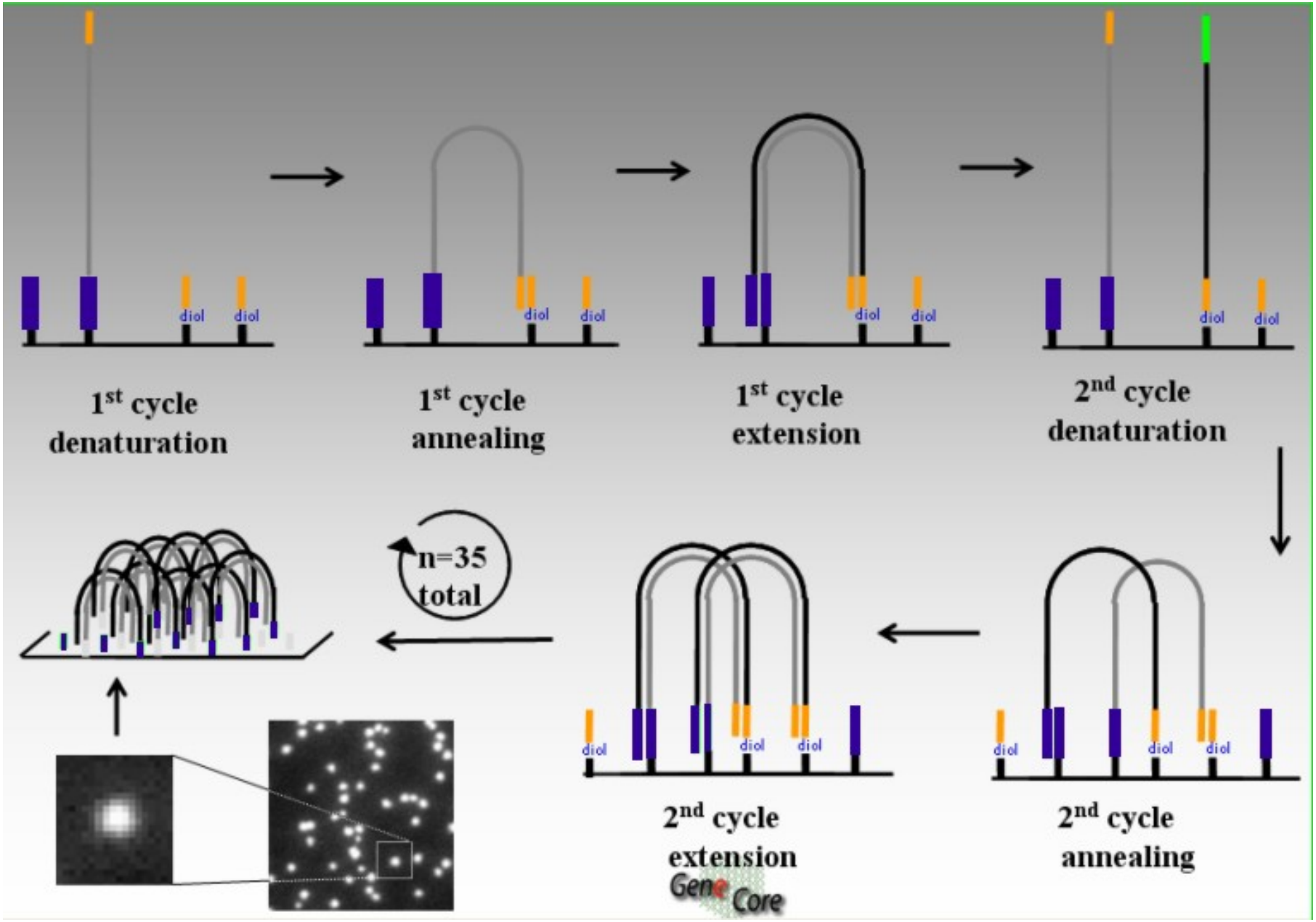
# Illumina: massive parallel sequencing:

Bridge amplification:
initiation



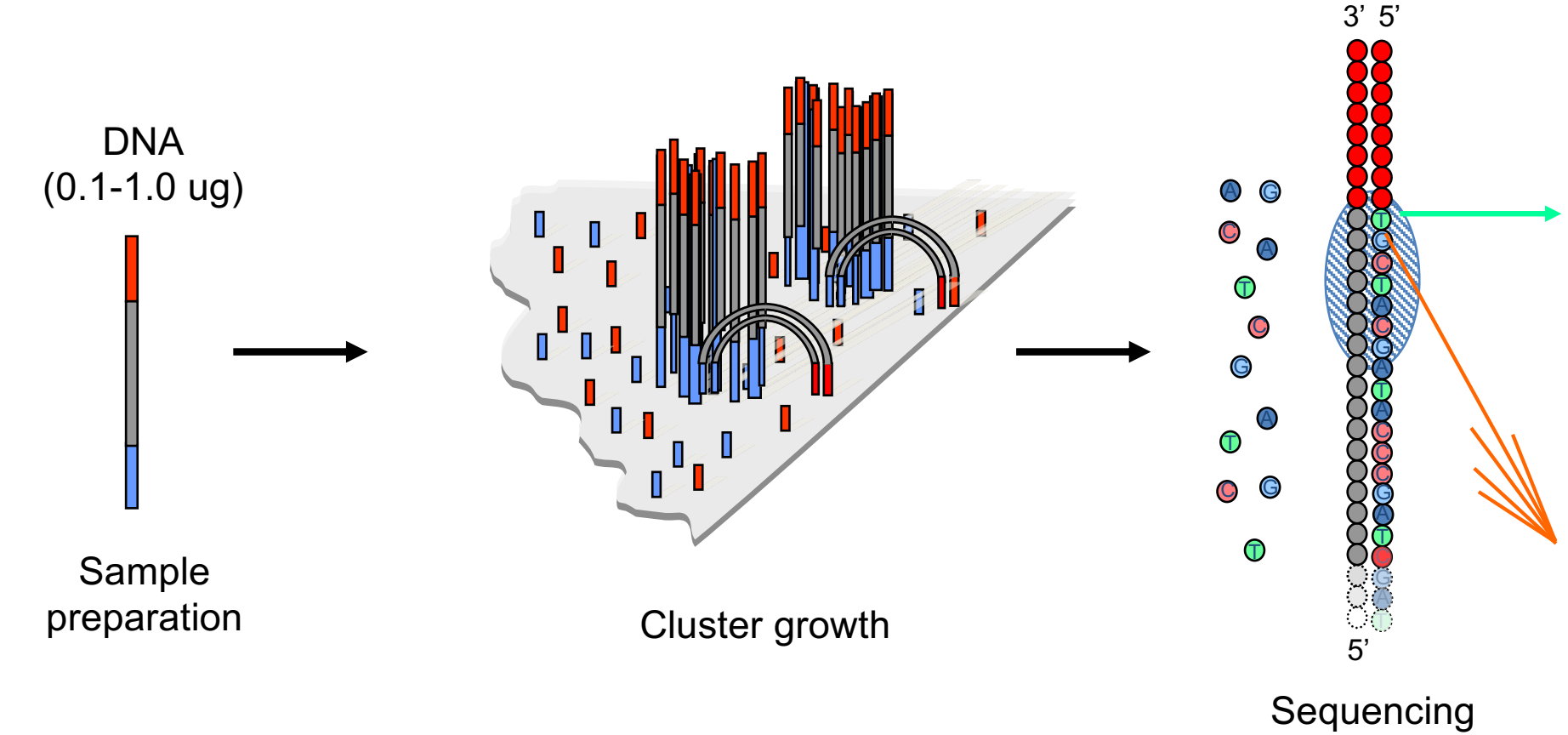

On the surface: complementary oligos

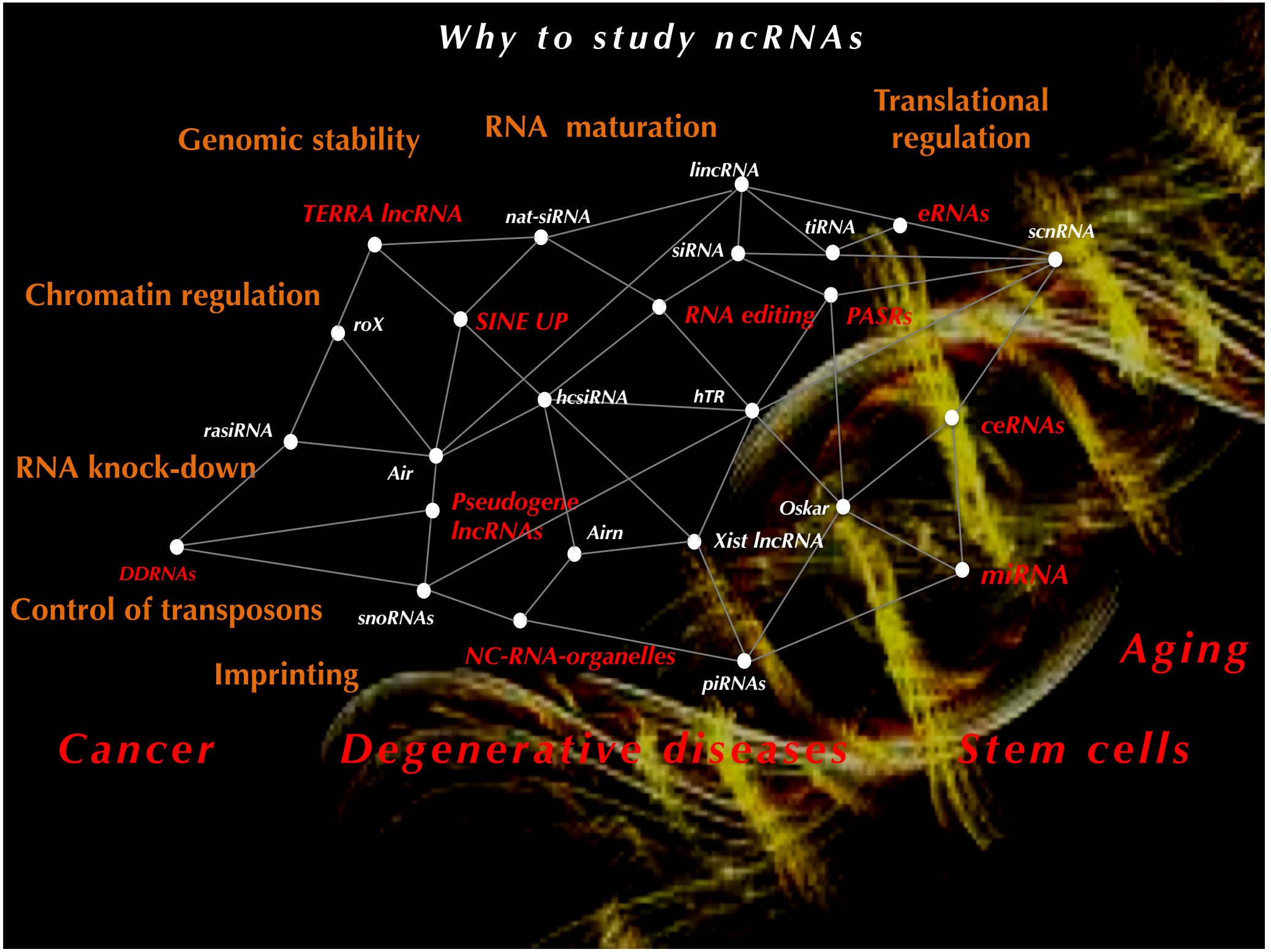
# Illumina: massive parallel sequencing:



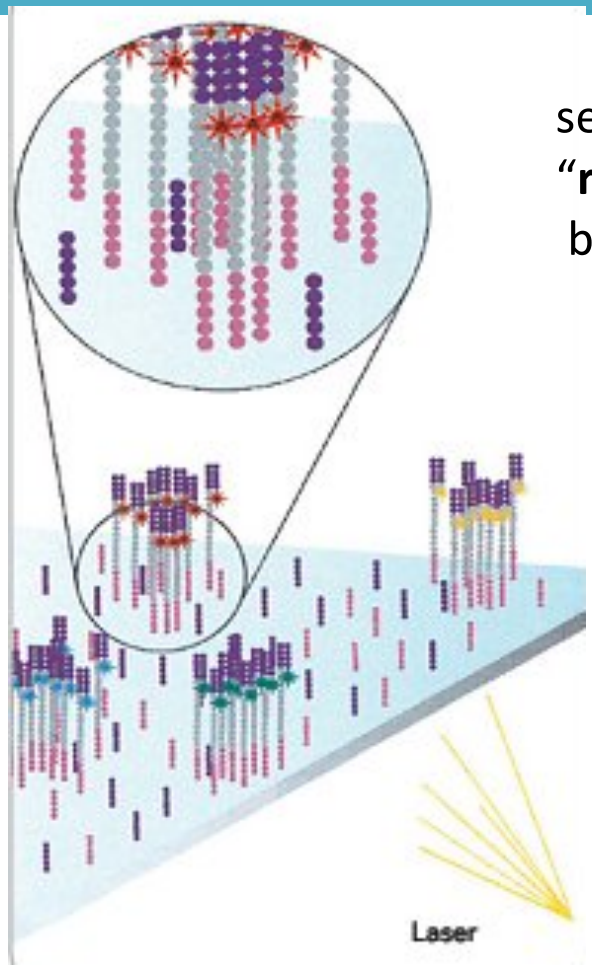
# Illumina Sequencing Technology

*Robust Reversible Terminator Chemistry Foundation*

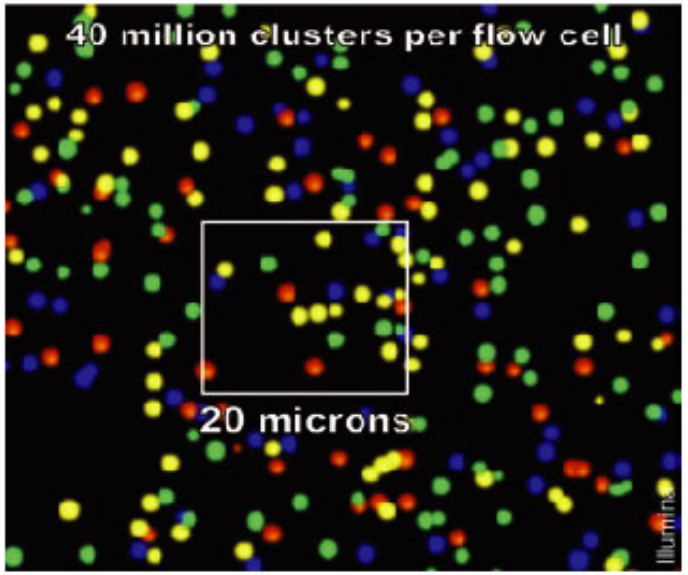



Why to study ncRNAs
Genomic stability
RNA maturation
Translational regulation
lincRNA
TERRA lncRNA
nat-siRNA
tiRNA
eRNAs
scnRNA
siRNA
Chromatin regulation
roX
SINE UP
RNA editing
PASRs
hcsiRNA
hTR
rasiRNA
ceRNAs
RNA knock-down
Air
Pseudogene lncRNAs
Oskar
Airn
Xist lncRNA
DDRNAs
miRNA
Control of transposons
snoRNAs
NC-RNA-organelles
Aging
Imprinting
piRNAs
Cancer
Degenerative diseases
Stem cells

# Illumina: massive parallel sequencing:




sequencing by synthesis:
"**reverible terminator**" nucleotides
blocked + fluorescently labeled



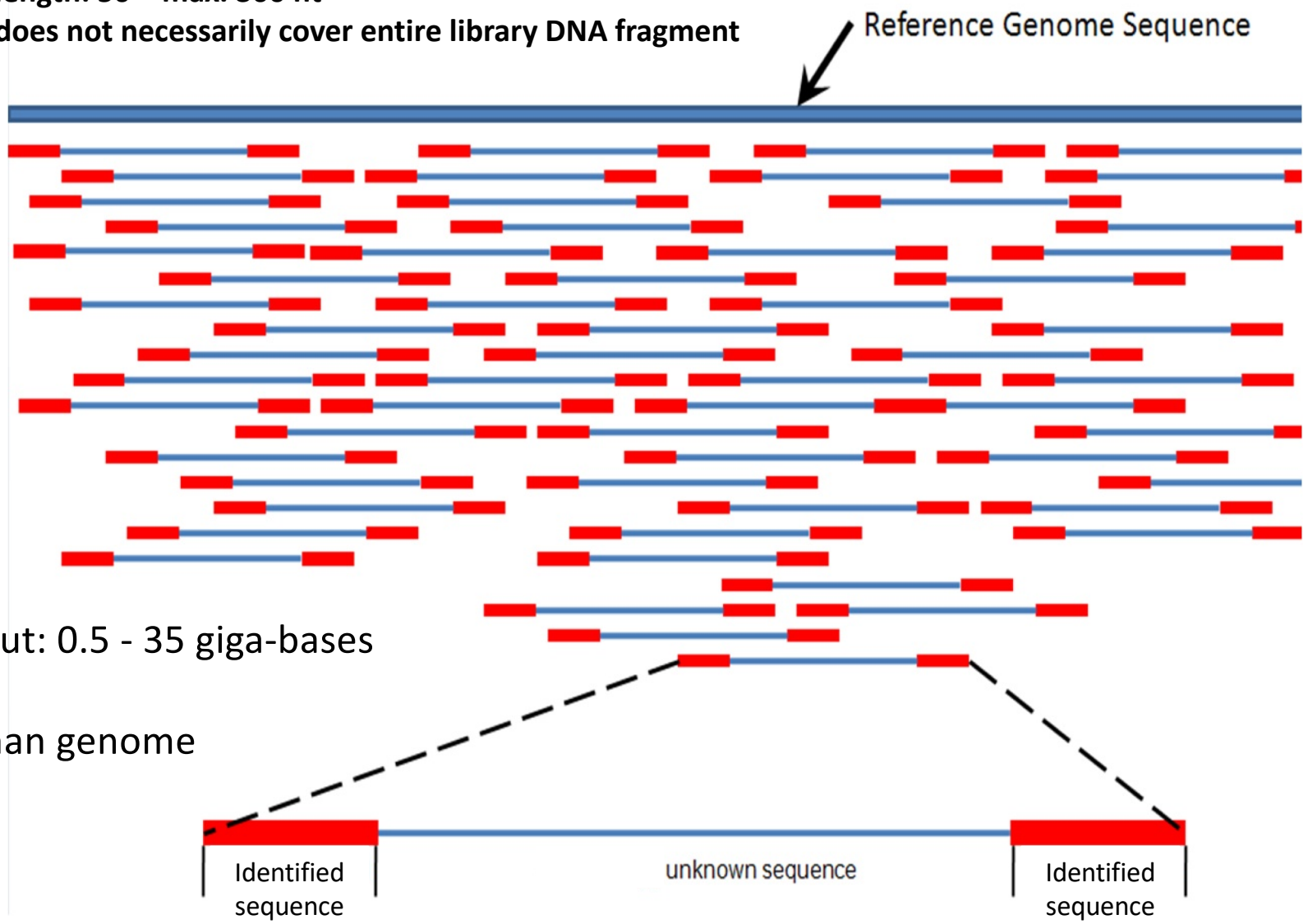

**1. Synthesis = incorporation of fluorescent nucleotide: blocking synthesis**
**2. dye cleavage + elimination**
**3. wash step**
**4. Scanning of fluorescent signal**
**1. Synthesis = incorporation of fluorescent nucleotide: blocking synthesis**

**READ LENGTH: ca: 150nt from each primer (2x150nt = 300nt)**

illumina.com

**Data analysis: obtained sequence reads are aligned along genomic DNA sequence → high number of reads necessary to obtain full sequence coverage**

**Read length: 50 – max. 300 nt**
**Read does not necessarily cover entire library DNA fragment**

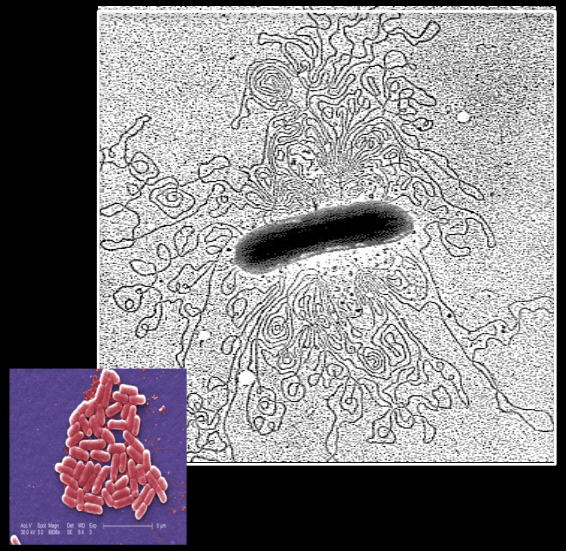



Max. output: 0.5 - 35 giga-bases
$=3.5*10^{10}$
= 10x human genome

***Sequence derived from one amplified cluster***

# Reason 1:
# The non-coding genome (r)evolution

*E.coli*



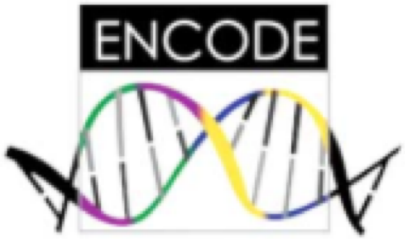
*C. elegans*

*H. sapiens*

| | | | |
|---|---|---|---|
| Genome | $5x10^6$ bp | $1x10^8$ bp | $3x10^9$ bp |
| Chromosomes | 1 | 6 | 23 |
| Coding genes | 6692 | 20541 | 21995 |
| ncDNA | 5% | 60% | **98%** |
| non-coding RNA genes | 15 | 23136 | ca. 40000 |
| miRNAs | 0 | 224 | 4274 |
| pseudogenes | 21 | 1522 | 10616 |

# The ENCODE PROJECT: IDENTIFCATION OF ALL FUNCTIONAL ELEMENTS IN THE REMAINING 98% OF THE HUMAN GENOME (2003)

The Encyclopedia of DNA Elements (ENCODE) is a public research project launched by the US National Human Genome Research Institute (NHGRI) in September 2003.



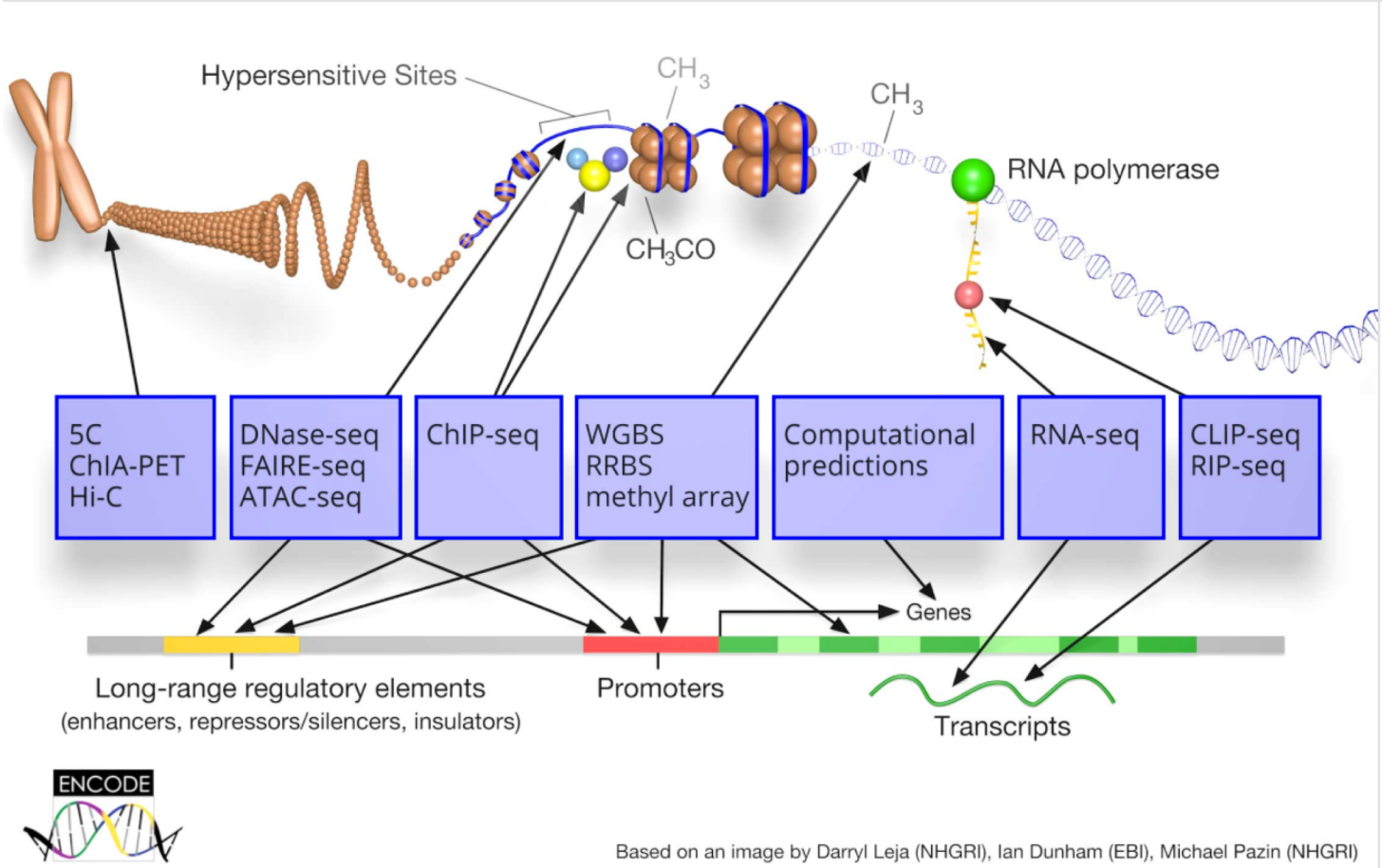

**Intended as a follow-up to the Human Genome Project (Genomic Research), the ENCODE project aims to identify all functional elements in the human genome.**

The project involves a worldwide consortium of research groups, and data generated from this project can be accessed through public databases.

NCODE is implemented in three phases: the pilot phase, the technology development phase and the production phase.

Along the pilot phase, the ENCODE Consortium evaluated strategies for identifying various types of genomic elements. The goal of the pilot phase was to identify a set of procedures that, in combination, could be applied cost-effectively and at high-throughput to accurately and comprehensively characterize large regions of the human genome. The pilot phase had to reveal gaps in the current set of tools for detecting functional sequences, and was also thought to reveal whether some methods used by that time were inefficient or unsuitable for large-scale utilization. Some of these problems had to be addressed in the ENCODE technology development phase (being executed concurrently with the pilot phase), which aimed to devise new laboratory and computational methods that would improve our ability to identify known functional sequences or to discover new functional genomic elements. The results of the first two phases determined the best path forward for analysing the remaining 99% of the human genome in a cost-effective and comprehensive production phase.

# ENCODE: Encyclopedia of DNA Elements




The ENCODE (Encyclopedia of DNA Elements) Consortium is an international collaboration of research groups funded by the National Human Genome Research Institute (NHGRI). The goal of ENCODE is to build a comprehensive parts list of functional elements in the human genome, including elements that act at the protein and RNA levels, and regulatory elements that control cells and circumstances in which a gene is active.

Get Started

HUMAN | MOUSE | WORM | FLY

**https://www.encodeproject.org**

# NEXT GENERATION SEQEUNCING OF DNA AND RNA

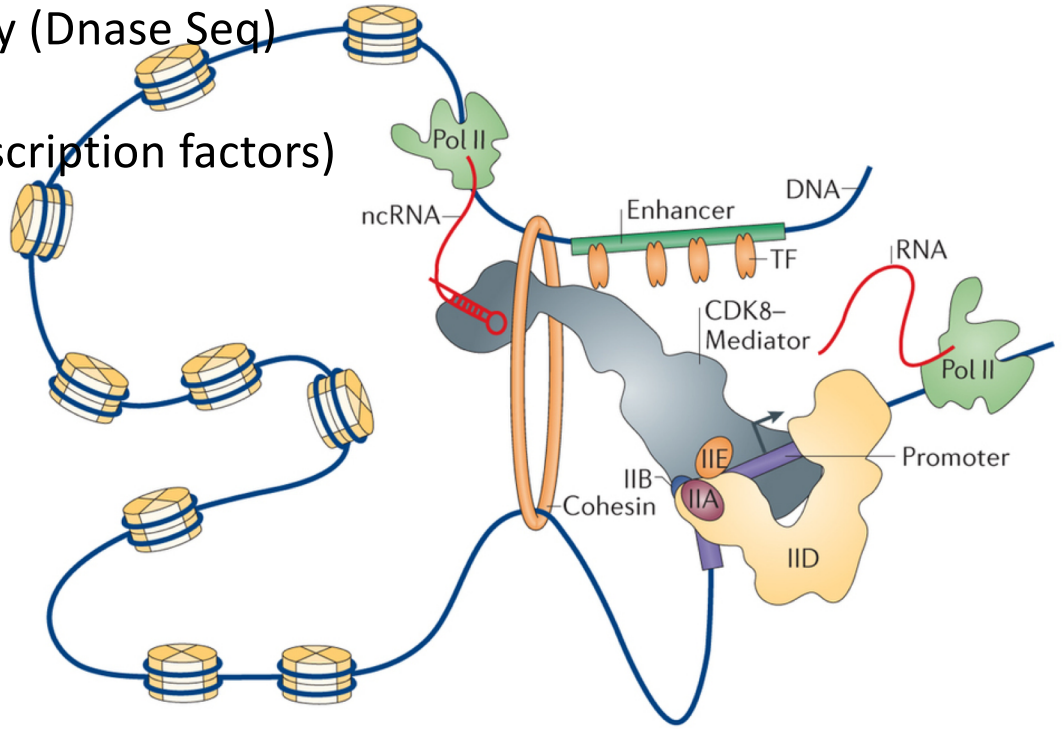
## →IDENTIFICATION OF ALL GENES
## → IDENTIFICATION OF ALL CODING AND NON-CODING TRANSCRIPTS

### HOW CAN GENES/TRANSCRIPTS BE DEFINED?

1. DNA Seqeuncing (Human genome project, DNA-Seq)
2. Landscape of transcription: Sequencing of RNA (total RNA, small/large RNA, CAGE)
3. DNA methylation: High representation reduced representation bisulfite sequencing (RRBS)
4. Local chromatin structure:

- determination of DNAseI hypersensitivity (Dnase Seq)
- nucelosome occupancy (MNase-seq)
- ChIP-seq (chromatin modifications, transcription factors)
- 3 Dimensional space interaction

**chromatin structure is combined with RNA expression data and DNA sequence to identify all genes/functional elements**
**The presence of regulated chromatin indicates the presence of a real functional element**




Nature Reviews | Molecular Cell Biology

# ENCODE MASSIVE EXPERIMENTAL INPUT

**Table 1 Summary of ENCODE experiments**

| Experiment | Description |
|---|---|
| DNA methylation | In 82 human cell lines and tissues:<br>A549, Adrenal gland, AG04449, AG04450, AG09309, AG09319, AG10803, AoSMC, BE2 C, BJ, Brain, Breast, Caco-2, CMK, ECC-1, Fibrobl, GM06990, GM12878, GM12891, GM12892, GM19239, GM19240, H1-hESC, HAEpiC, HCF, HCM, HCPEpiC, HCT-116, HEEpiC, HEK293, HeLa-S3, Hepatocytes, HepG2, HIPEpiC, HL-60, HMEC, HNPCEpiC, HPAEpiC, HRCEpiC, HRE, HRPEpiC, HSMM, HTR8svn, IMR90, Jurkat, K562, Kidney, Left Ventricle, Leukocyte, Liver, LNCaP, Lung, MCF-7, Melano, Myometr, NB4, NH-A, NHBE, NHDF-neo, NT2-D1, Osteoblasts, Ovcar-3, PANC-1, Pancreas, PanIslets, Pericardium, PFSK-1, Placenta, PrEC, ProgFib, RPTEC, SAEC, Skeletal muscle, Skin, SkMC, SK-N-MC, SK-N-SH, Stomach, T-47D, Testis, U87, UCH-1 and Uterus |
| TF ChIP-seq | A total of 119 TFs:<br>ATF3, BATF, BCLAF1, BCL3, BCL11A, BDP1, BHLHE40, BRCA1, BRF1, BRF2, CCNT2, CEBPB, CHD2, CTBP2, CTCF, CTCFL, EBF1, EGR1, ELF1, ELK4, EP300, ESRRA, ESR1, ETS1, E2F1, E2F4, E2F6, FOS, FOSL1, FOSL2, FOXA1, FOXA2, GABPA, GATA1, GATA2, GATA3, GTF2B, GTF2F1, GTF3C2, HDAC2, HDAC8, HMGN3, HNF4A, HNF4G, HSF1, IRF1, IRF3, IRF4, JUN, JUNB, JUND, MAFF, MAFK, MAX, MEF2A, MEF2C, MXI1, MYC, NANOG, NFE2, NFKB1, NFYA, NFYB, NRF1, NR2C2, NR3C1, PAX5, PBX3, POLR2A, POLR3A, POLR3G, POU2F2, POU5F1, PPARGC1A, PRDM1, RAD21, RDBP, REST, RFX5, RXRA, SETDB1, SIN3A, SIRT6, SIX5, SMARCA4, SMARCB1, SMARCC1, SMARCC2, SMC3, SPI1, SP1, SP2, SREBF1, SRF, STAT1, STAT2, STAT3, SUZ12, TAF1, TAF7, TAL1, TBP, TCF7L2, TCF12, TFAP2A, TFAP2C, THAP1, TRIM28, USF1, USF2, WRNIP1, YY1, ZBTB7A, ZBTB33, ZEB1, ZNF143, ZNF263, ZNF274 and ZZZ3 |
| Histone ChIP-seq | A total of 12 types:<br>H2A.Z, H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K9me1, H3K9me3, H3K27ac, H3K27me3, H3K36me3, H3K79me2 and H4K20me1 |
| DNase-seq | In 125 cell types or treatments:<br>8988T, A549, AG04449, AG04450, AG09309, AG09319, AG10803, AoAF, AoSMC/serum_free_media, BE2_C, BJ, Caco-2, CD20, CD34, Chorion, CLL, CMK, Fibrobl, FibroP, Gliobla, GM06990, GM12864, GM12865, GM12878, GM12891, GM12892, GM18507, GM19238, GM19239, GM19240, H7-hESC, H9ES, HAc, HAEpiC, HA-h, HA-sp, HBMEC, HCF, HCFaa, HCM, HConF, HCPEpiC, HCT-116, HEEpiC, HeLa-S3, HeLa-S3_IFNa4h, Hepatocytes, HepG2, HESC, HFF, HFF-Myc, HGF, HIPEpiC, HL-60, HMEC, HMF, HMVEC-dAd, HMVEC-dBl-Ad, HMVEC-dBl-Neo, HMVEC-dLy-Ad, HMVEC-dLy-Neo, HMVEC-dNeo, HMVEC-LBl, HMVEC-LLy, HNPCEpiC, HPAEC, HPAF, HPDE6-E6E7, HPdLF, HPF, HRCEpiC, HRE, HRGEC, HRPEpiC, HSMM, HSMMemb, HSMMtube, HTR8svn, Huh-7, Huh-7.5, HUVEC, HVMF, iPS, Ishikawa_Estr, Ishikawa_Tamox, Jurkat, K562, LNCaP, LNCaP_Andr, MCF-7, MCF-7_Hypox, Medullo, Melano, MonocytesCD14+, Myometr, NB4, NH-A, NHDF-Ad, NHDF-neo, NHEK, NHLF, NT2-D1, Osteobl, PANC-1, PanIsletD, PanIslets, pHTE, PrEC, ProgFib, PrEC, RPTEC, RWPE1, SAEC, SKMC, SK-N-MC, SK-N-SH_RA, Stellate, T-47D, Th0, Th1, Th2, Urothelia, Urothelia_UT189, WERI-Rb-1, WI-38 and WI-38_Tamox |
| DNase footprint | In 41 cell types:<br>AG10803, AoAF, CD20+, CD34+ Mobilized, fBrain, fHeart, fLung, GM06990, GM12865, HAEpiC, HA-h, HCF, HCM, HCPEpiC, HEEpiC, HepG2, H7-hESC, HFF, HIPEpiC, HMF, HMVEC-dBl-Ad, HMVEC-dBl-Neo, HMVEC-dLy-Neo, HMVEC-LLy, HPAF, HPdLF, HPF, HRCEpiC, HSMM, Th1, HVMF, IMR90, K562, NB4, NH-A, NHDF-Ad, NHDF-neo, NHLF, SAEC, SkMC and SK-N-SH RA |
| MNase-seq | In GM12878 and K562 |
| 3C-carbon copy (5C) | In GM12878, K562, HeLa-S3 and H1-hESC |
| GWAS SNP targeting | 296 noncoding GWAS SNPs were assigned a target promoter |

**Ca.**
**400 Mio $**

**GENCODE:**
**Project that uses ENCODE data for the annotation of functional elements in the genome**

**http://www.gencodegenes.org/**

GENCODE | Data | Stats | Browser | Blog

## Statistics about all Human GENCODE releases

* The statistics derive from the gtf files that contain only the annotation of the main chromosomes.

For details about the calculation of these statistics please see the README_stats.txt file.

Version 23 (March 2015 freeze, GRCh38) - Ensembl 81, 82

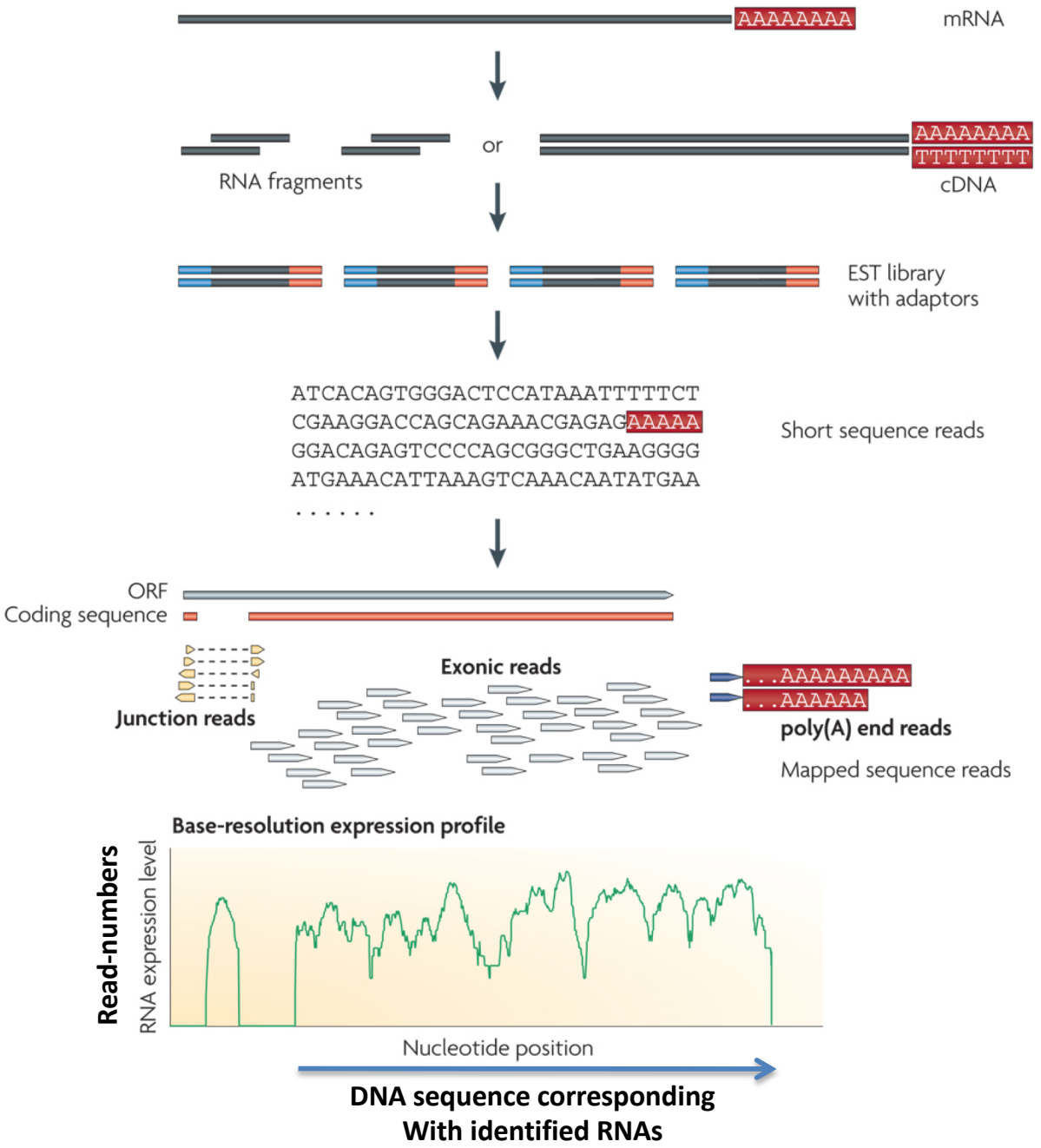
Download release

### General stats

| | | | |
|---|---|---|---|
| Total No of Genes | 60498 | Total No of Transcripts | 198619 |
| Protein-coding genes | 19797 | Protein-coding transcripts | 79795 |
| Long non-coding RNA genes | 15931 | - full length protein-coding: | 54775 |
| Small non-coding RNA genes | 9882 | - partial length protein-coding: | 25020 |
| Pseudogenes | 14477 | Nonsense mediated decay transcripts | 13307 |
| - processed pseudogenes: | 10727 | Long non-coding RNA loci transcripts | 27817 |
| - unprocessed pseudogenes: | 3271 | | |
| - unitary pseudogenes: | 172 | | |
| - polymorphic pseudogenes: | 59 | | |
| - pseudogenes: | 21 | Total No of distinct translations | 59774 |
| Immunoglobulin/T-cell receptor gene segments | | Genes that have more than one distinct translations | 13556 |
| - protein coding segments: | 411 | | |
| - pseudogenes: | 227 | | |

# 2. RNA SEQ – TO IDENTIFY ALL SORTS OF TRANSCRIPTS

**Serial Analysis of Gene Expression (SAGE, superSAGE)**

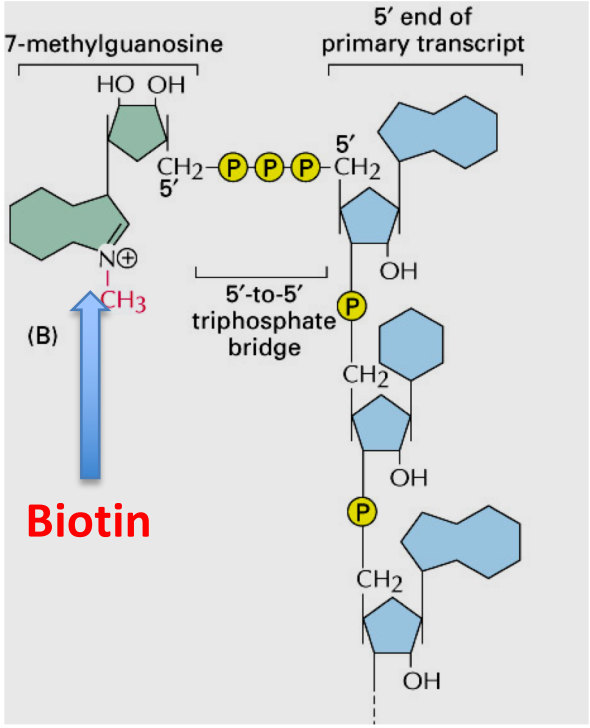


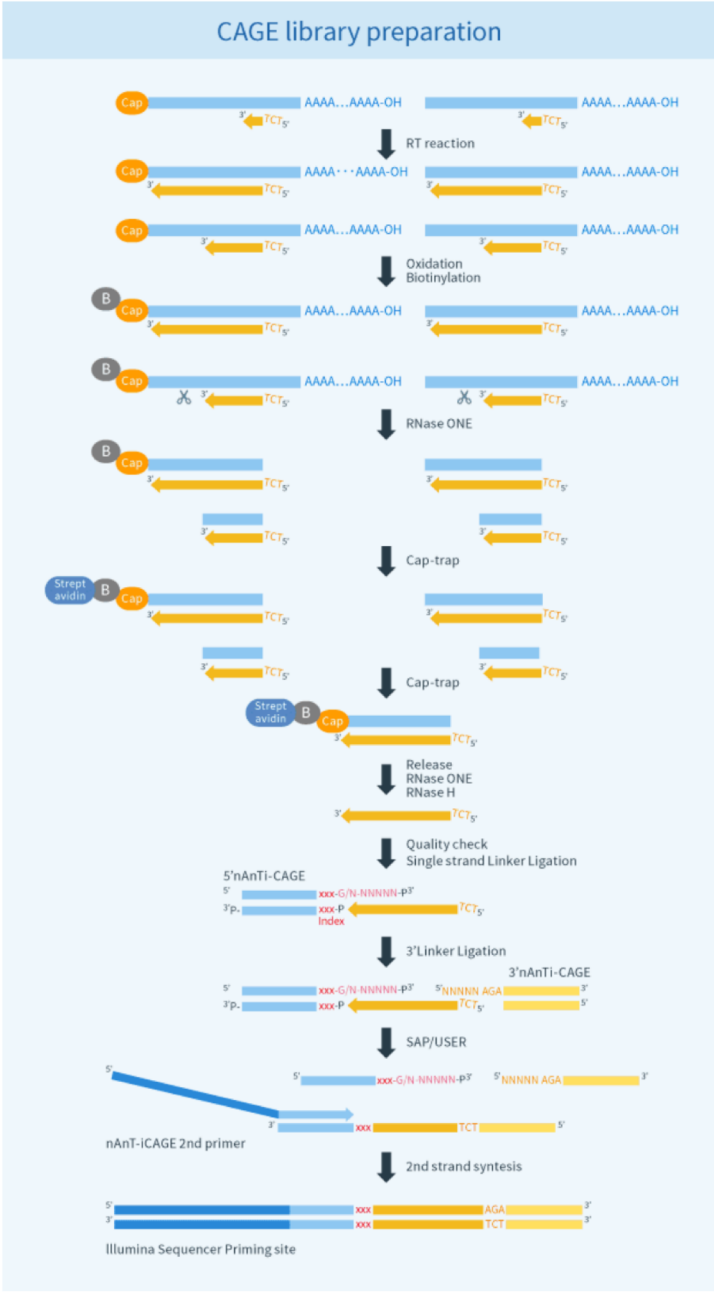

Method can also be used
for all transcripts
When using a random
Primers for reverse
transcription

# 2. RNA Seq variant technology: CAGE (Cap Analysis of Gene Expression)

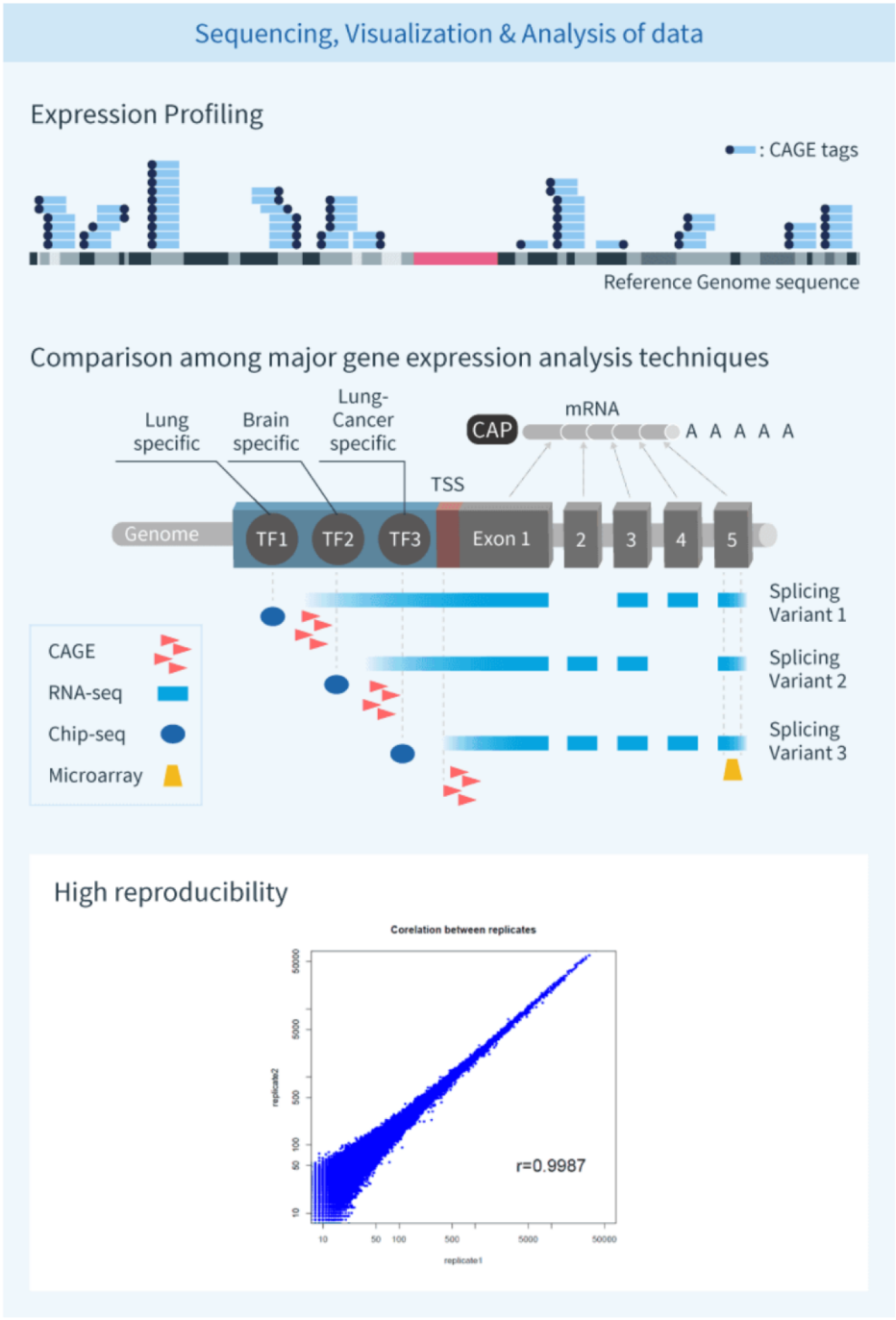
http://www.osc.riken.jp/english/activity/cage/basic/

Unlike a similar technique Serial Analysis of Gene Expression (SAGE, superSAGE) in which tags come from other parts of transcripts, CAGE is primarily used to locate an exact transcription start sites in the genome. This knowledge in turn allows a researcher to investigate promoter structure necessary for gene expression.

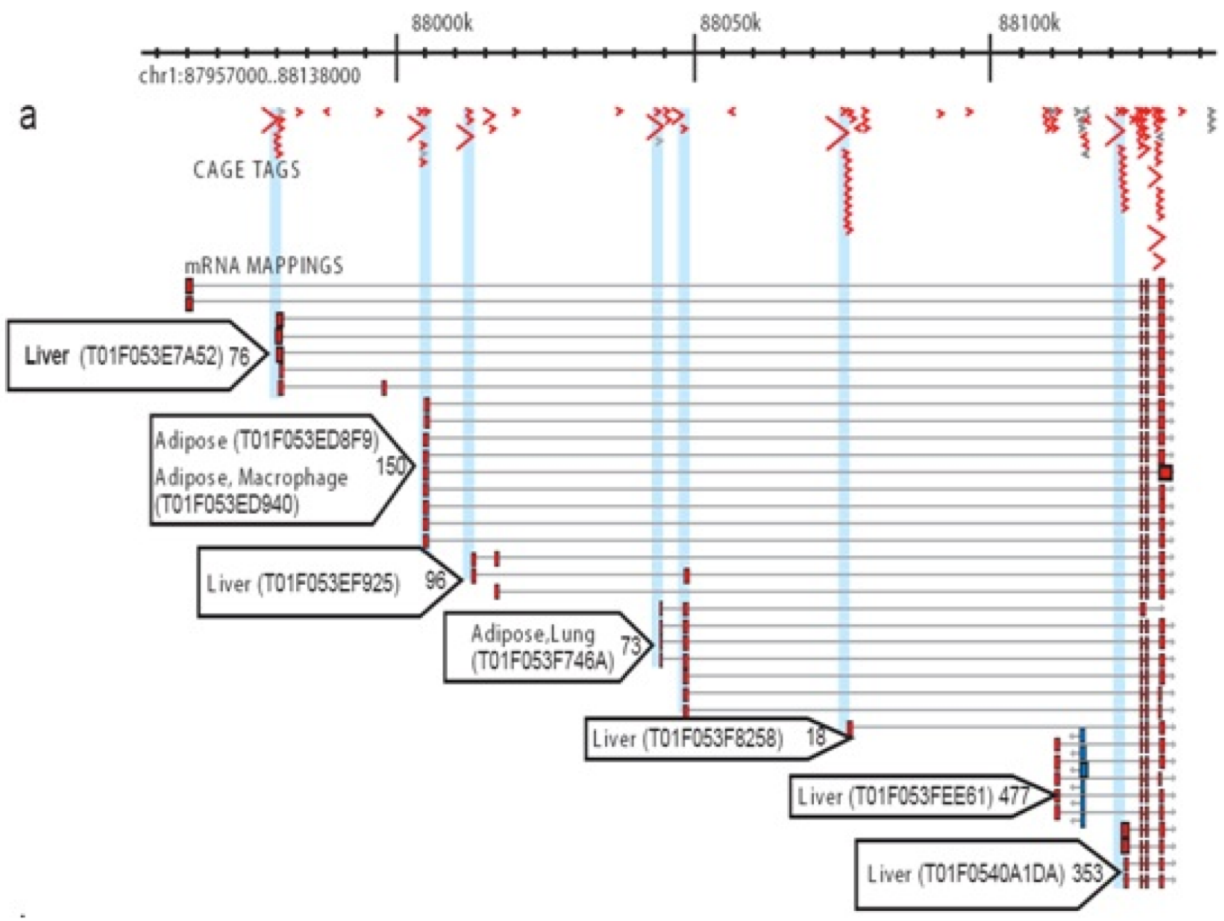


# 2. RNA Seq variant technology: CAGE (Cap Analysis of Gene Expression)



# 2. RNA Seq variant technology: CAGE (Cap Analysis of Gene Expression)

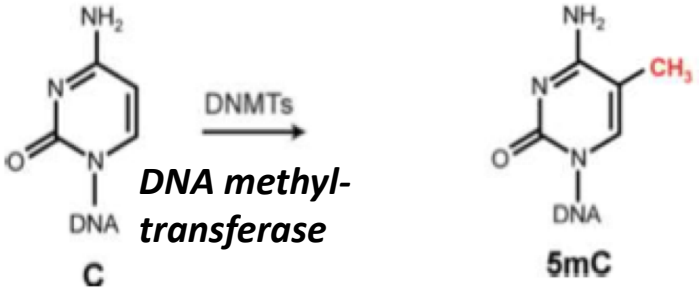



***Excellent tool***
***To identify transcriptional start sites***

***Help to identify up-stream regulatory sequences = PROMOTERS RELEVANT CpG***

# 2. DNA methylation: educed representation bisulfite sequencing (RRBS)

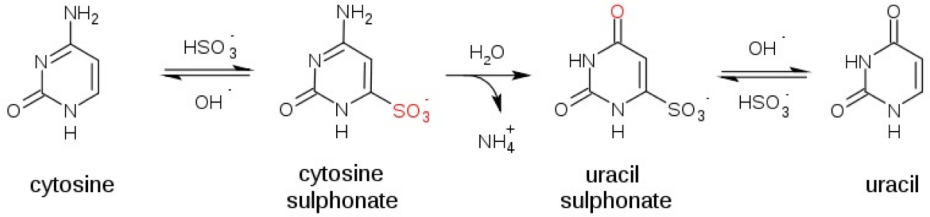
**Methylation of cytosine at CpG dinucleotides is an important epigenetic regulatory modification in many eukaryotic genomes.**

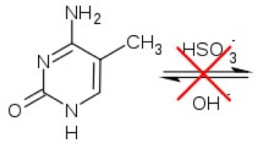



active gene

Silenced gene

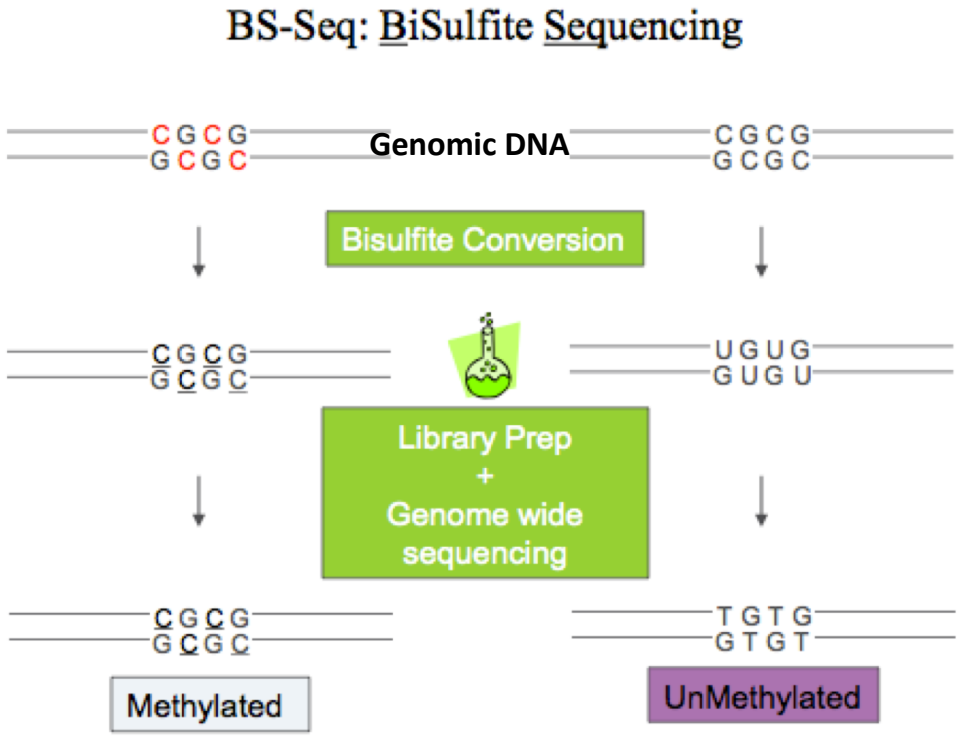
## Bi-sulfite conversion: C→U conversion



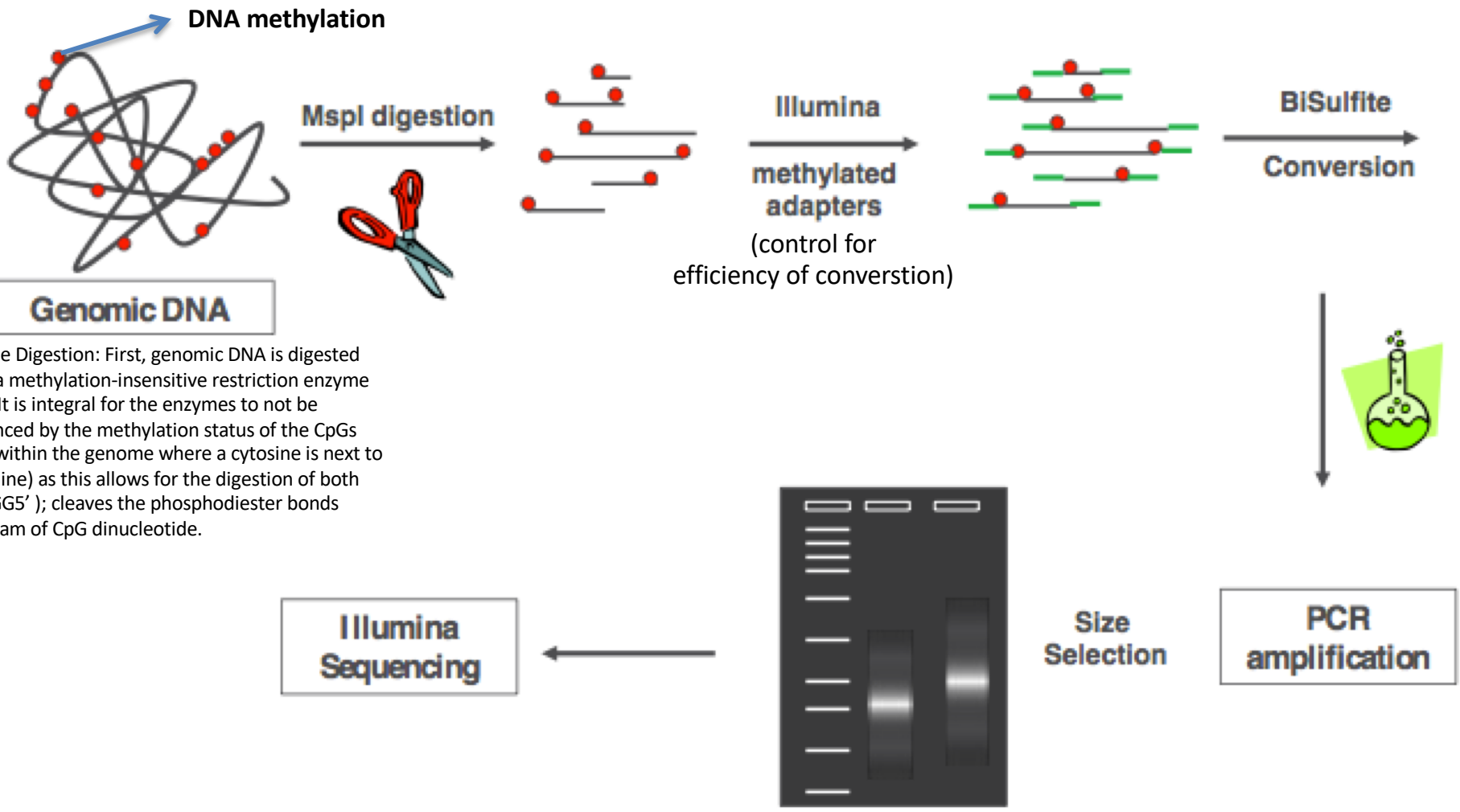

5-methylcytosine

**methylated C cannot be converted!!**



# 2. DNA methylation: Reduced representation bisulfite sequencing (RRBS)

Reduced representation bisulfite sequencing (RRBS) is an efficient and high-throughput technique used to analyze the genome-wide methylation profiles on a single nucleotide level. This technique combines restriction enzymes and bisulfite sequencing in order to enrich for the areas of the genome that have a high CpG content. Due to the high cost and depth of sequencing needed to analyze methylation status in the entire genome. The fragments that comprise the reduced genome still include the majority of promoters, as well as regions such as repeated sequences that are difficult to profile using conventional bisulfite sequencing approaches.



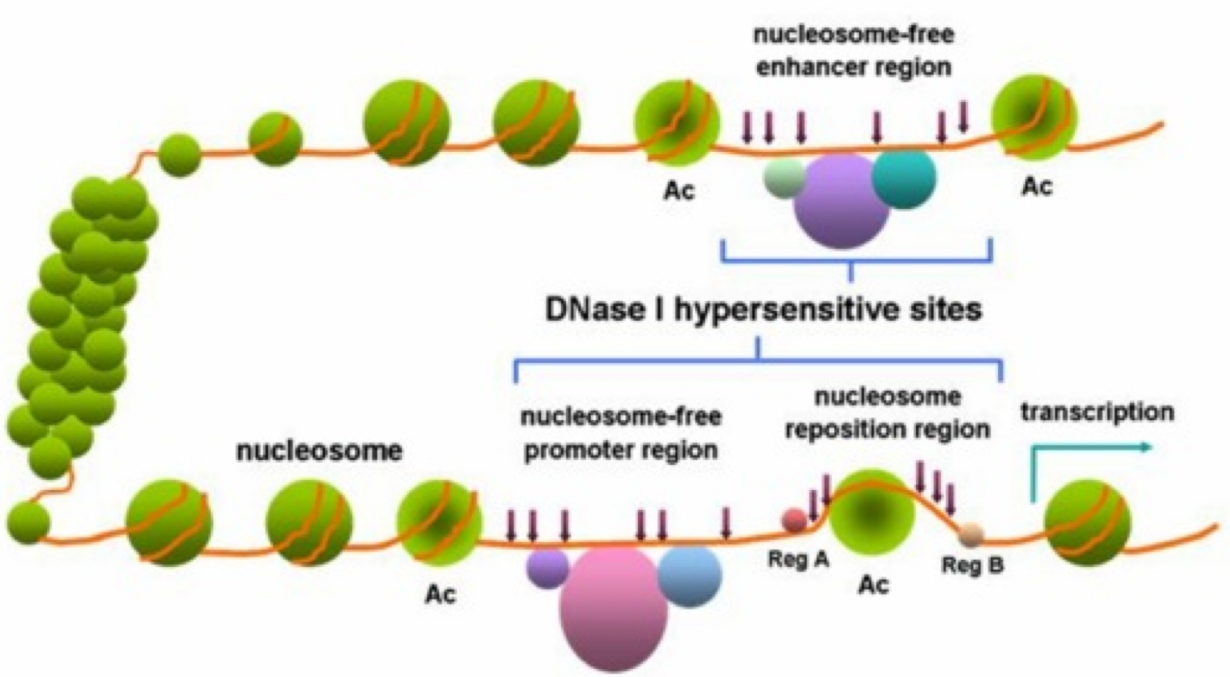

Enzyme Digestion: First, genomic DNA is digested using a methylation-insensitive restriction enzyme MspI. It is integral for the enzymes to not be influenced by the methylation status of the CpGs (sites within the genome where a cytosine is next to a guanine) as this allows for the digestion of both (3'CCGG5' ); cleaves the phosphodiester bonds upstream of CpG dinucleotide.

# 4. Local chromatin structure: determination of DNAse I hypersensitivity (DNase Seq)

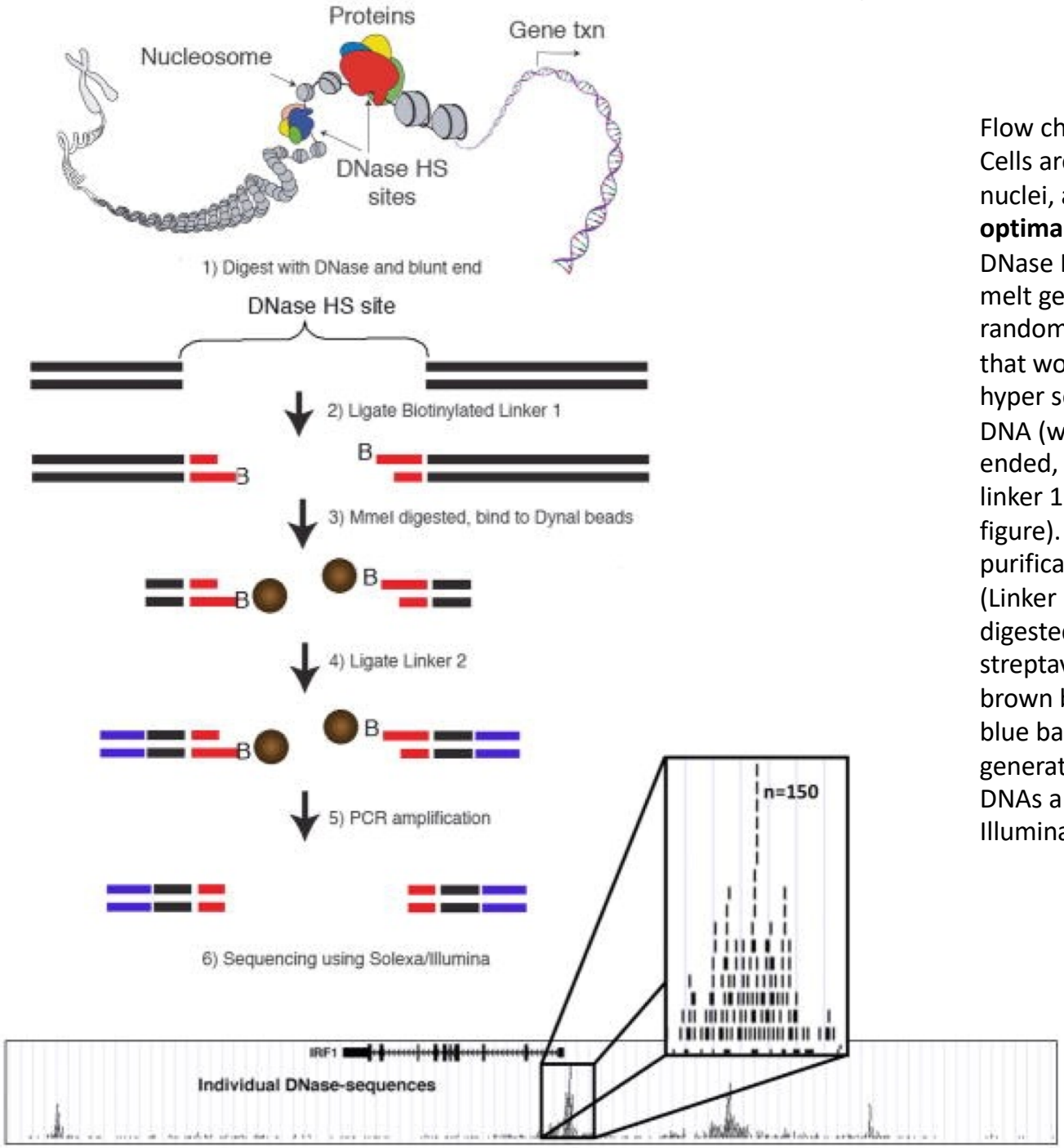
- determination of DNAse I hypersensitivity (DNase Seq)
- Nucleosome occupancy (MNase-seq)
- ChIP-seq (chromatin modifications, transcription factors)
- 3 Dimensional space interaction

## DNase hypersensitive sites mark sequences involved in gene regulation

DNase I hypersensitive sites (DHSs) are regions of chromatin that are sensitive to cleavage by the DNase I enzyme. In these specific regions of the genome, chromatin has lost its condensed structure, exposing the DNA and making it accessible. This raises the availability of DNA to degradation by enzymes, such as DNase I. These accessible chromatin zones are functionally related to transcriptional activity, since this remodeled state is necessary for the binding of proteins such as transcription factors.

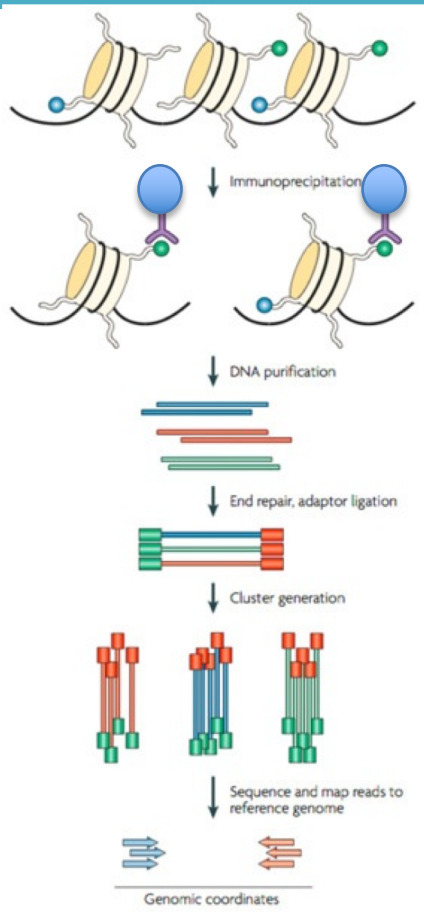


# 4. Local chromatin structure: determination of DNAse I hypersensitivity (DNase Seq)




Flow chart of DNase-seq protocol.
Cells are lysed with detergent to release nuclei, and the nuclei are **digested with optimal concentrations of DNase I**.
DNase I digested DNA is immobilized in low-melt gel agarose plugs to reduce additional random shearing. (pipetting can cause breaks that would cause "false positive" DNase hyper sensitive sites).
DNA (while still in the plugs) are then blunt-ended, extracted and ligated to biotinylated linker 1 (represented by red bars in the figure). Excess linker is removed by gel purification, and biotinylated fragments (Linker 1 plus 20 bases of genomic DNA) are digested with MmeI, and captured by streptavidin-coated beads (represented by brown balls). Linker 2 (represented by the blue bars) is ligated to the 2 base overhang generated by MmeI, and the ditagged 20 bp DNAs are amplified by PCR and sequenced by Illumina/Solexa.

# 4. Local chromatin structure: Chromatin immunoprecipitation sequencing (ChIP-seq)




H3K4me3 (active chromatin mark)

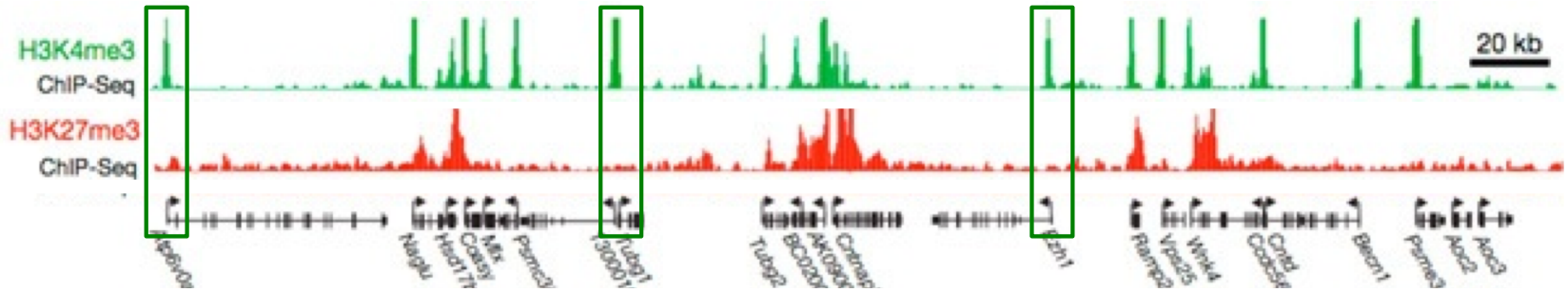
H3K27me3 (repressive chromatin mark)

magnetic beads covered with specific antibody

1. Cell fixation-proteins and DNA are crosslinked
2. Sonication of DNA (fragmentation)
3. Immunoprecipitation of chromatin using

Specific antibodies: histone modifications or transcription Factors

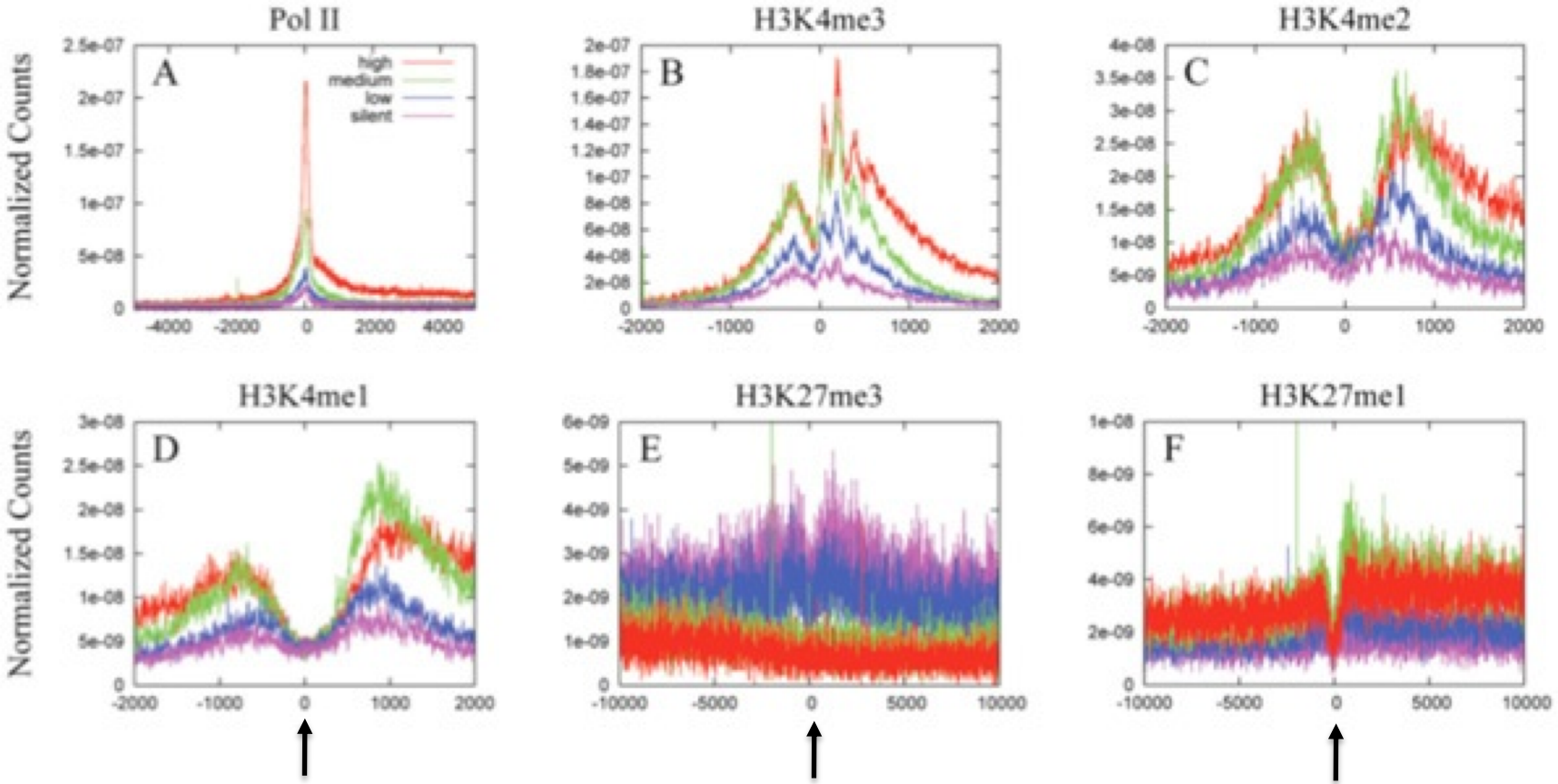
4. Purify beads (magnet), washing of beads + elution of immunoprecipitated material
5. Library construction
6. Massive parallel sequencing
7. Align sequencing results to genomic sequence
8. Increase in read-number for a particular sequence indicates Enrichment for the histone modification or transcription factor

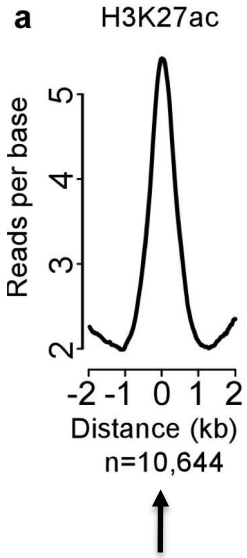



The results indicate that some modifications (H3K4me) are correlated with increased gene expression, while others (H3K27me3) correlate with decreases gene expression. The peaks observed in the H3K4me3 for genes at high expression levels occur at +50, +210, and +360 based which correlates well with the known spacing interval for nucleosome positioning. Furthermore, the dip in abundance at the transcriptional start site is consistent with local nucleosome depletion of actively expressed genes.

# 4. Local chromatin structure: Chromatin immunoprecipitation sequencing (ChIP-seq)

***A special chromatin code marks the transcriptional start site of Pol II target genes***



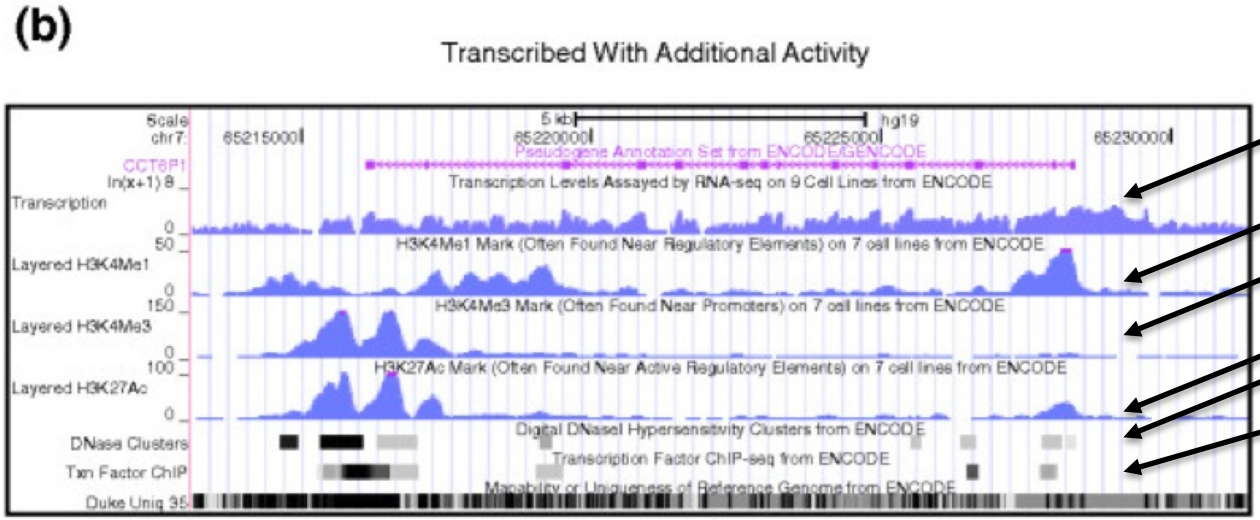




Position 0:
RNA Polymerase II: peak
H4K4me3: peak
H3K4me2: drop
H3K4me1: drop
H3K27me3: low
H3K27me1: drop

transcriptional start site = position 0
Regulatory elements

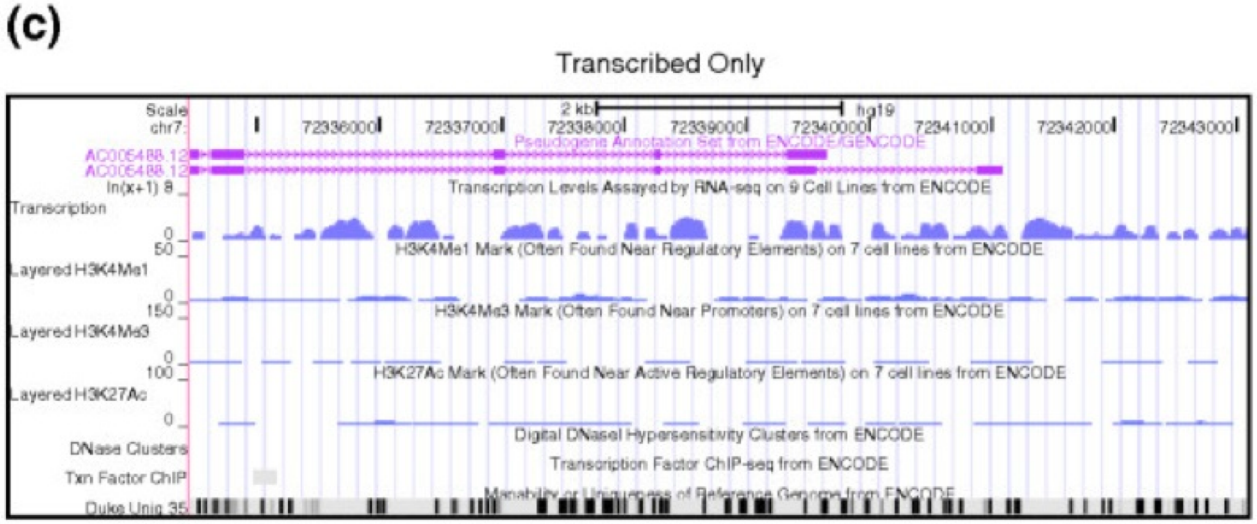
**Same method can be used to localize transcription factors**

# AN EXAMPLE:ORGANISATION OF A FUNCTIONAL ELEMENT: PSEUDOGENES




## Pseudogene CCT6P1

RNA expression: PRESENT
RNA Polymerase II: not shown
H4K4me1: near regulatory elements
H3K4me3: near promoters
H3K27Ac: near regulatory elements
DNAse hypersensitive sites: at regulatory elements
Transcription factor (TF) binding: Near promoter

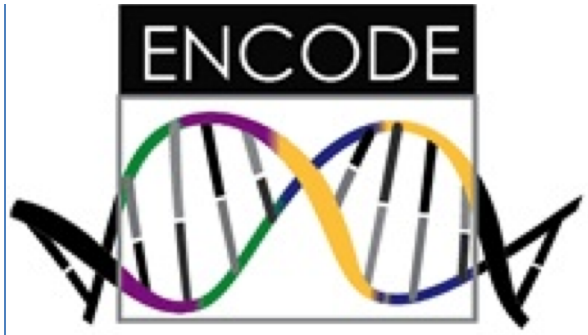



## Pseudogene AC0064BB12

RNA expression: PRESENT
Chromatin shows actve marks
Poor definition

**Summary of pseudogene annotation and case studies**. **(a)** A heatmap showing the annotation for transcribed pseudogenes including active chromatin segmentation, DNaseI hypersensitivity, active promoter, active Pol2, and conserved sequences. Raw data were from the K562 cell line. **(b)** A transcribed duplicated pseudogene (Ensembl gene ID: ENST00000434500.1; genomic location, chr7: 65216129-65228323) showing consistent active chromatin accessibility, histone marks, and TFBSs in its upstream sequences. **(c)** A transcribed processed pseudogene (Ensembl gene ID: ENST00000355920.3; genomic location, chr7: 72333321-72339656) with no active chromatin features or conserved sequences. **(d)** A non-transcribed duplicated pseudogene showing partial activity patterns (Ensembl gene ID: ENST00000429752.2; genomic location, chr1: 109646053-109647388). **(e)** Examples of partially active pseudogenes. E1 and E2 are examples of duplicated pseudogenes. E1 shows *UGT1A2P* (Ensembl gene ID: ENST00000454886), indicated by the green arrowhead. *UTG1A2P* is a non-transcribed pseudogene with active chromatin and it is under negative selection. Coding exons of protein-coding paralogous loci are represented by dark green boxes and UTR exons by filled red boxes. E2 shows *FAM86EP* (Ensembl gene ID: ENST00000510506) as open green boxes, which is a transcribed pseudogene with active chromatin and upstream TFBSs and Pol2 binding sites. The transcript models associated with the locus are displayed as filled red boxes. Black arrowheads indicate features novel to the pseudogene locus. E3 and E4 show two unitary pseudogenes. E3 shows *DOC2GP* (Ensembl gene ID: ENST00000514950) as open green boxes, and transcript models associated with the locus are shown as filled red boxes. E4 shows *SLC22A20* (Ensembl gene ID: ENST00000530038). Again, the pseudogene model is represented as open green boxes, transcript models associated with the locus as filled red boxes, and black arrowheads indicate features novel to the pseudogene locus. E5 and E6 show two processed pseudogenes. E5 shows pseudogene *EGLN1* (Ensembl gene ID: ENST00000531623) inserted into duplicated pseudogene *SCAND2* (Ensembl gene ID: ENST00000541103), which is a transcribed pseudogene showing active chromatin but no upstream regulatory regions as seen in the parent gene. The pseudogene models are represented as open green boxes, transcript models associated with the locus are displayed as filled red boxes, and black arrowheads indicate features novel to the pseudogene locus. E6 shows a processed pseudogene *RP11-409K20* (Ensembl gene ID: ENST00000417984; filled green box), which has been inserted into a CpG island, indicated by an orange arrowhead. sRNA, small RNA.

Pei *et al. Genome Biology* 2012 **13**:R51 doi:10.1186/gb-2012-13-9-r51




**Aim: Identify functional elements of the genome (ENCODE)**

**WORK STILL IN PRGRESS** ***http://www.genome.gov/encode/***


**Aim: a catalog of manually curated list of genes/transcripts (GENCODE)** ***http://www.gencodegenes.org/***

**Release ENCODE7 (2012)**; new release expected 12/2015)

ARTICLE

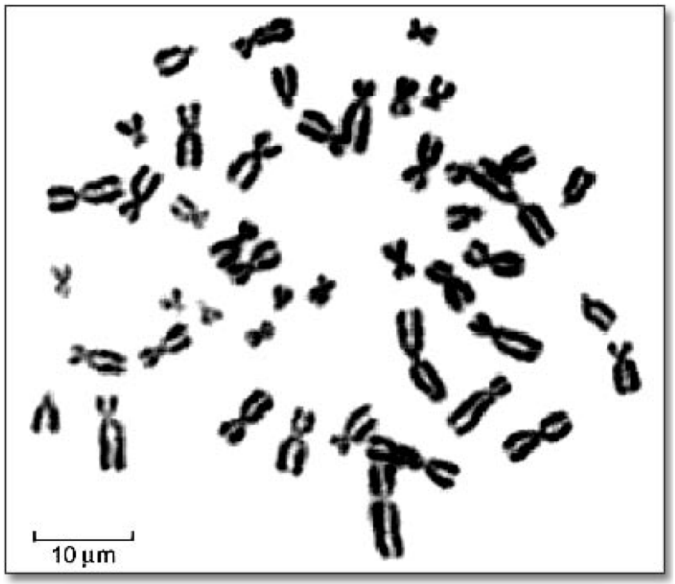
doi:10.1038/nature11247

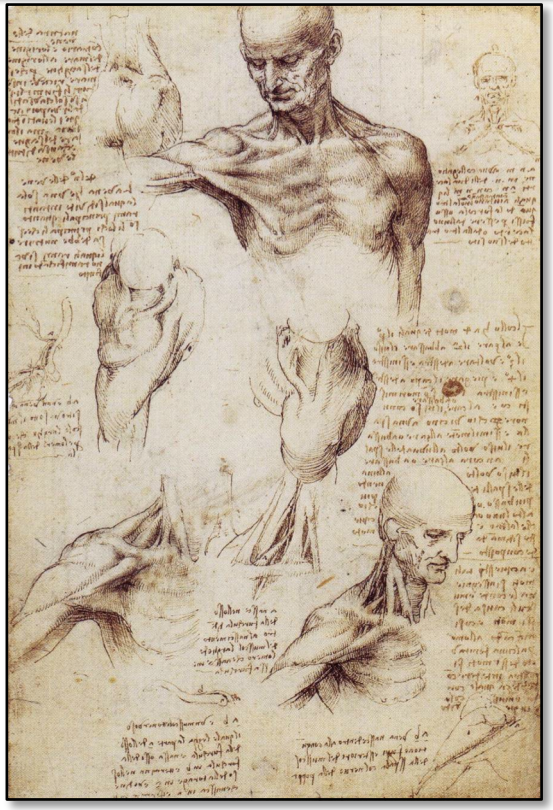
# An integrated encyclopedia of DNA elements in the human genome

The ENCODE Project Consortium*

The human genome encodes the blueprint of life, but the function of the vast majority of its nearly three billion bases is unknown. The Encyclopedia of DNA Elements (ENCODE) project has systematically mapped regions of transcription, transcription factor association, chromatin structure and histone modification. These data enabled us to assign biochemical functions for 80% of the genome, in particular outside of the well-studied protein-coding regions. Many discovered candidate regulatory elements are physically associated with one another and with expressed genes, providing new insights into the mechanisms of gene regulation. The newly identified elements also show a statistical correspondence to sequence variants linked to human disease, and can thereby guide interpretation of this variation. Overall, the project provides new insights into the organization and regulation of our genes and genome, and is an expansive resource of functional annotations for biomedical research.

# Almost all regions in the genome are subjecte to regualtion and transcription



The vast majority (80.4%) of the human genome participates in at least one biochemical RNA and/or chromatin associated event in at least one cell type. Much of the genome lies close to a regulatory event: 95% of the genome lies within 8kb of a DNA-protein interaction (as assayed by bound ChIP-seq motifs or DNaseI footprints), and 99% is within 1.7kb of at least one of the biochemical events measured by ENCODE.

Classifying the genome into seven chromatin states suggests an initial set of 399,124 regions with enhancer-like features and 70,292 regions with promoter-like features, as well hundreds of thousands of quiescent regions. High-resolution analyses further subdivide the genome into thousands of narrow states with distinct functional properties.

It is possible to quantitatively correlate RNA sequence production and processing with both chromatin marks and transcription factor (TF) binding at promoters, indicating that promoter functionality can explain the majority of RNA expression variation.

Many non-coding variants in individual genome sequences lie in ENCODE-annotated functional regions; this number is at least as large as those that lie in protein coding genes.

SNPs associated with disease by GWAS are enriched within non-coding functional elements, with a majority residing in or near ENCODE-defined regions that are outside of protein coding genes. In many cases, the disease phenotypes can be associated with a specific cell type or TF.

# GENCODE – STATUS 09.11.2015:

## Project that uses ENCODE for the annotation of functional elements in the genome

**http://www.gencodegenes.org/**

**http://www.gencodegenes.org/**

Release 23 (GRCh38.p3)

Blog

Statistics about all Human GENCODE releases

* The statistics derive from the gtf files that contain only the annotation of the main chromosomes.

For details about the calculation of these statistics please see the README_stats.txt file.

**Long ncRNAs: >200nt**

**Short ncRNAs:<200nt**

Version 23 (March 2015 freeze, GRCh38) - Ensembl 81, 82

Download release

General stats

| | |
|---|---|
| Total No of Genes | 60498 |
| Protein-coding genes | 19797 |
| Long non-coding RNA genes | 15931 |
| Small non-coding RNA genes | 9882 |
| Pseudogenes | 14477 |
| - processed pseudogenes: | 10727 |
| - unprocessed pseudogenes: | 3271 |
| - unitary pseudogenes: | 172 |
| - polymorphic pseudogenes: | 59 |
| - pseudogenes: | 21 |
| Immunoglobulin/T-cell receptor gene segments | |
| - protein coding segments: | 411 |
| - pseudogenes: | 227 |

| | |
|---|---|
| Total No of Transcripts | 198619 |
| Protein-coding transcripts | 79795 |
| - full length protein-coding: | 54775 |
| - partial length protein-coding: | 25020 |
| Nonsense mediated decay transcripts | 13307 |
| Long non-coding RNA loci transcripts | 27817 |
| Total No of distinct translations | 59774 |
| Genes that have more than one distinct translations | 13556 |

# ANNOTATED TRANSCRIPT TYPES (ENCODE ; 11/2015)

Further details on this version's gene and transcript types

| biotype | genes | transcripts |
|---|---|---|
| 3prime_overlapping_ncrna | 29 | 33 |
| all IG_genes | 216 | 246 |
| all other pseudogenes | 14477 | 14516 |
| all RNA pseudogenes | 0 | 0 |
| all RNA_genes | 13460 | 19109 |
| antisense | 5565 | 11203 |
| IG_C_gene | 14 | 31 |
| IG_C_pseudogene | 9 | 9 |
| IG_D_gene | 37 | 37 |
| IG_J_gene | 18 | 18 |
| IG_J_pseudogene | 3 | 3 |
| IG_V_gene | 147 | 160 |
| IG_V_pseudogene | 181 | 181 |
| lincRNA | 7678 | 13301 |
| macro_lncRNA | 1 | 1 |
| miRNA | 4093 | 4093 |
| misc_RNA | 2298 | 2312 |
| Mt_rRNA | 2 | 2 |
| Mt_tRNA | 22 | 22 |
| non_stop_decay | 0 | 77 |
| nonsense_mediated_decay | 0 | 13307 |
| polymorphic_pseudogene | 59 | 73 |
| processed_pseudogene | 10285 | 10287 |
| processed_transcript | 497 | 26945 |
| protein_coding | 19797 | 79795 |
| pseudogene | 21 | 44 |
| retained_intron | 0 | 26616 |
| ribozyme | 8 | 8 |

# ANNOTATED TRANSCRIPT TYPES (ENCODE ; 11/2015)

| | | |
|---|---|---|
| rRNA | 544 | 544 |
| scaRNA | 49 | 49 |
| sense_intronic | 917 | 976 |
| sense_overlapping | 194 | 344 |
| snoRNA | 949 | 961 |
| snRNA | 1896 | 1896 |
| sRNA | 20 | 20 |
| TEC | 1050 | 1137 |
| TR_C_gene | 6 | 23 |
| TR_D_gene | 4 | 4 |
| TR_J_gene | 79 | 79 |
| TR_J_pseudogene | 4 | 4 |
| TR_V_gene | 106 | 108 |
| TR_V_pseudogene | 30 | 30 |
| transcribed_processed_pseudogene | 442 | 442 |
| transcribed_unitary_pseudogene | 2 | 2 |
| transcribed_unprocessed_pseudogene | 668 | 667 |
| translated_unprocessed_pseudogene | 1 | 1 |
| unitary_pseudogene | 170 | 170 |
| unprocessed_pseudogene | 2602 | 2603 |
| vaultRNA | 1 | 1 |

***NOTE: These are annotated ncRNA transcripts/gene: they are subjected to gene Regulatory mechanisms.***

***NOTE: ncRNAs can also be generated outside of defined transcription units!!!***
***Example: DNA damage repair RNAs (DDRNA)***