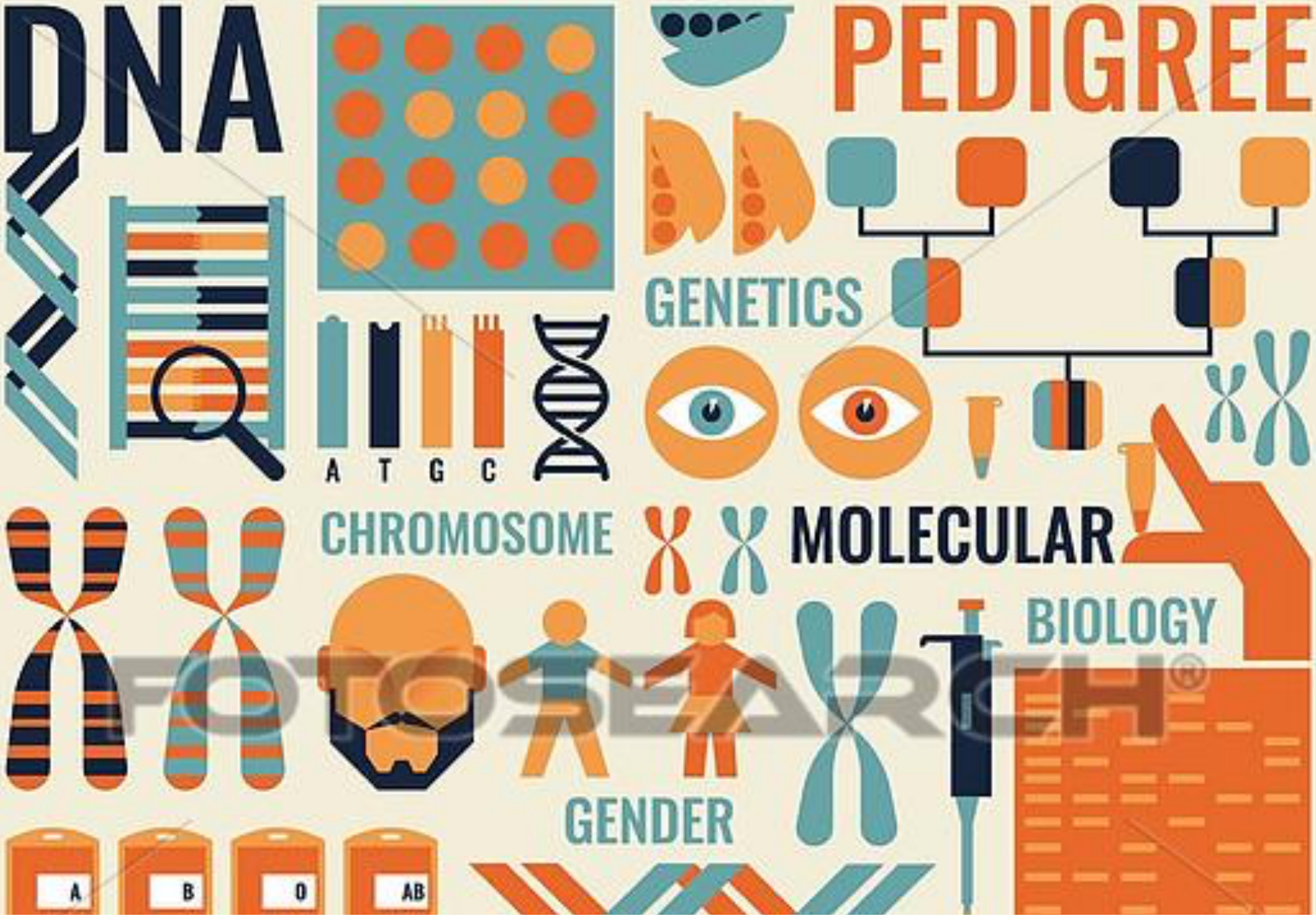

DNA
A T G C
PEDIGREE
GENETICS
CHROMOSOME
MOLECULAR
BIOLOGY
GENDER
A
B
O
AB

Insegnamento Laboratorio Biologia Molecolare

Docenti:

Prof. SCHOEFTNER STEFAN, responsible del corso 3 CFU – Lecture

Prof.ssa BANDIERA ANTONELLA, 2 CFU – Laboratory course

Prof.ssa SCAGGIANTE BRUNA, 1 CFU – Virtual Laboraotry

The course provides theoretical and practical training on techniques and experimental approaches in molecular biology.

- A focus will be set on the molecular biology of nucleic acids and biomolecular techniques.

- Basic techniques for DNA manipulation, gene study, gene cloning, gene expression analysis and recombinant DNA technology will be addressed.

- Laboratory exercises include the teaching of laboratory safety standards the handling of laboratory instruments, the extraction of DNA and its electrophoretic separation on agarose gel, the use of restriction enzymes for plasmid mapping, DNA amplification by PCR and analysis of the amplified products by gel electrophoresis.

- Virtual laboratory course of techniques non applicable in the teaching laboratory

Exam

→ 2 written exams:

Exam 1:

Reports on lab work at the end of each lab practice (Prof. Bandiera).
Reports will be evaluated assessing:
-diligence, attendance, presentation accuracy
-personal skills, synthesis, description and clarity in presentation, technical terms knowledge
-understanding degree, explanation and discussion skills, presence of conceptual errors.
→ A total of 15 points can be reached.
→A minimum of 7,5 points is necessary to participate in the second part of the exam2

Exam 2

Learning progress on the theoretical lectures (Prof. Scaggiante, Prof. Schoeftner) will be monitored in a written exam. Total points: 16.
Exam 2 consists of 12 multiple choice questions (0,5 points per question) and 2 “open questions” (5 points per question, max 1 page answer to question) on broader topics addressed during the theoretical lectures and virtual lab.

The final mark of the course results from the sum of both exams.
Maximum points: 31
A minimum of 18 points is required to pass the exam “Laboratorio Biologia Molecolare”.

Contents Of Lecture

Prof. Schoeftner Stefan:

1. Anatomy of the cell, biomolecules, concept of preparation of RNA/Protein/DNA
2. Physical properties of RNA and DNA, Tautomerization of bases, DNA helix types, RNA:DNA hybrids, RNA structure, Aptamers.
3. Recombinant DNA techniques, Cloning vectors, endonucleases, artificial chromosomes, phage technology, recombinant protein expression, introduction of genes into host-organisms
4. DNA sequencing, bacterial immunity, manipulation of the genome content of pro- and eukaryotic organisms (CRISPR, gene targeting)
5. Hybridization related techniques (RNA-FISH, DNA-FISH, Southern blot, Northern blot), Electrophoresis, methods to study DNA:protein interaction (band shift, DNA footprinting, chromatin immunoprecipitation)
6. Gene expression analysis: array technology and high content sequencing, determination of 3’ and 5’ ends of RNA, single molecule transcript analysis

Contents of virtual laboratory

Prof. Bruna Scaggiante:

1. EXTRACTION OF NUCLEIC ACIDS: General principles of extraction of DNA and total RNA. Kits for nucleic acid extraction. Enrichment in mRNA. Qualitative and quantitative evaluation of DNA and RNA. Procedures to avoid RNA degradation. The problem of DNA contamination in RNA preparations. Examples of calculation and qualitative evaluation

2. PCR AS A QUALITATIVE TECHNIQUE. PCR and RT- PCR: general and technical principles. Evaluation of gel products; general principles and amplification curve. The presence of parasitic bands and implications. Distinction between cDNA amplified and contaminating DNA. Quality control. Reaction optimization. Examples of diagnostic applications.

3. PCR AS A QUANTITATIVE TECHNIQUE. General principles of Real-Time PCR and detection of amplifiers with dyes or probes. The value of CoT. The standard curve. The relative and absolute evaluation. Examples of curves and calculation. Advantages and limitations of Real-Time PCR. Competitive PCR: general principles and construction of competitors.

4. THE NEW FRONTIERS OF AMPLIFICATION TECHNIQUES: The digital PCR. General principles. Amplification in droplet and relative and absolute quantification. The application of ddPCR to the analysis of circulating DNA (liquid biopsy). Applications for the study of mutations

Practical laboratory course

Prof. Anonella Bandiera:

1- BIOMOLECULAR LAB EQUIPMENT AND TECHNIQUES
Rules of conduct and safety, hazardous reagents and material safety data sheet; equipment and lab instrumentation. The use of automatic lab pipettes for small volume manipulation. Preparation of gel electrophoresis solutions (running and loading buffers)

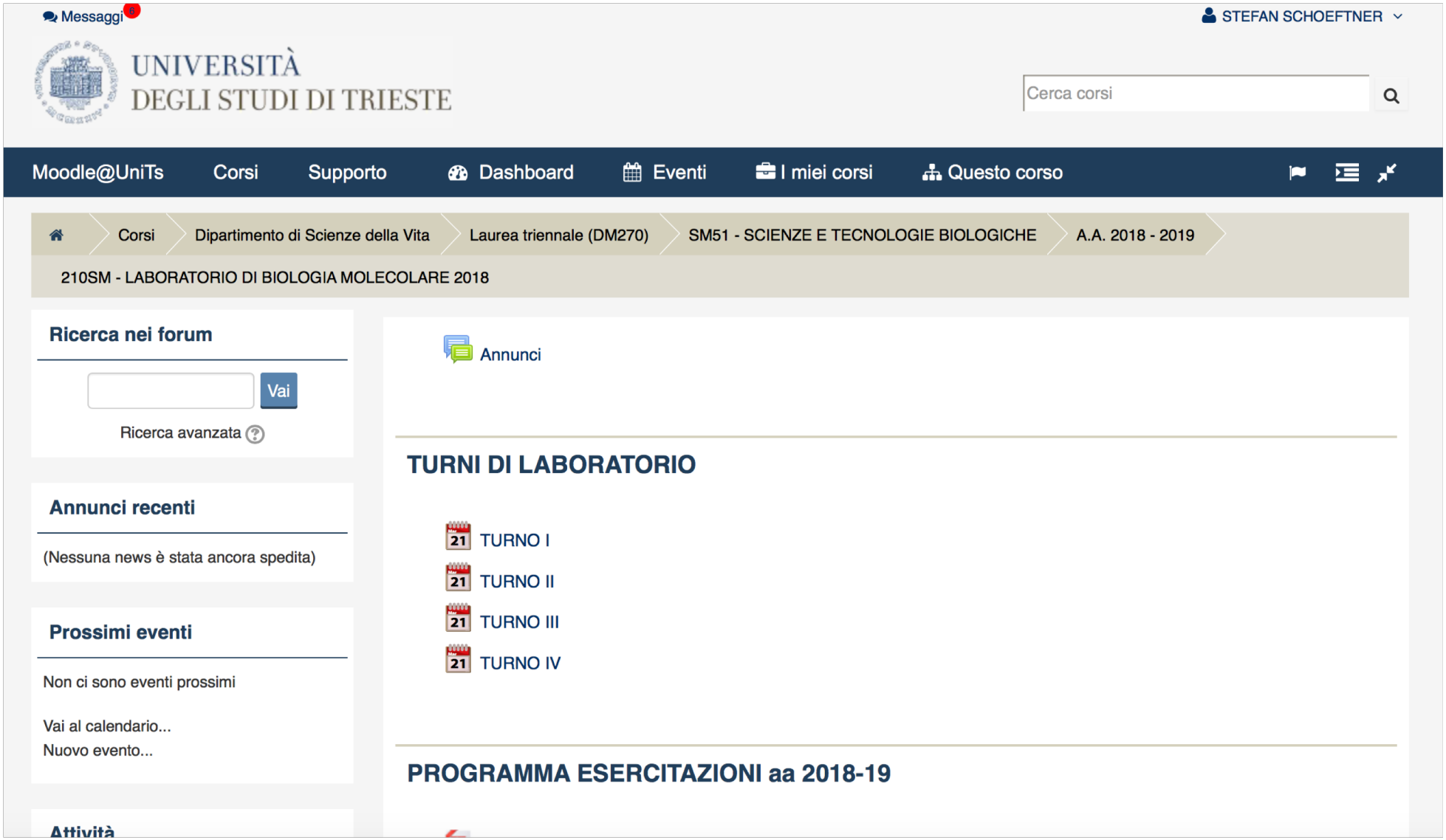
2- PLASMIDS
Plasmid DNA extraction by a commercial kit, evaluation of extraction yield, preparation of samples for electrophoretic analysis

3- PCR: PRINCIPLES AND PROCEDURE
PCR reactions setting to amplify DNA fragments from plasmid templates previously extracted.

4- ELECTROPHORETIC ANALYSIS ON AGAROSE GEL
DNA analysis methods, technique description, information obtained by the analysis. Electrophoretic run on agarose gel of extracted DNA samples

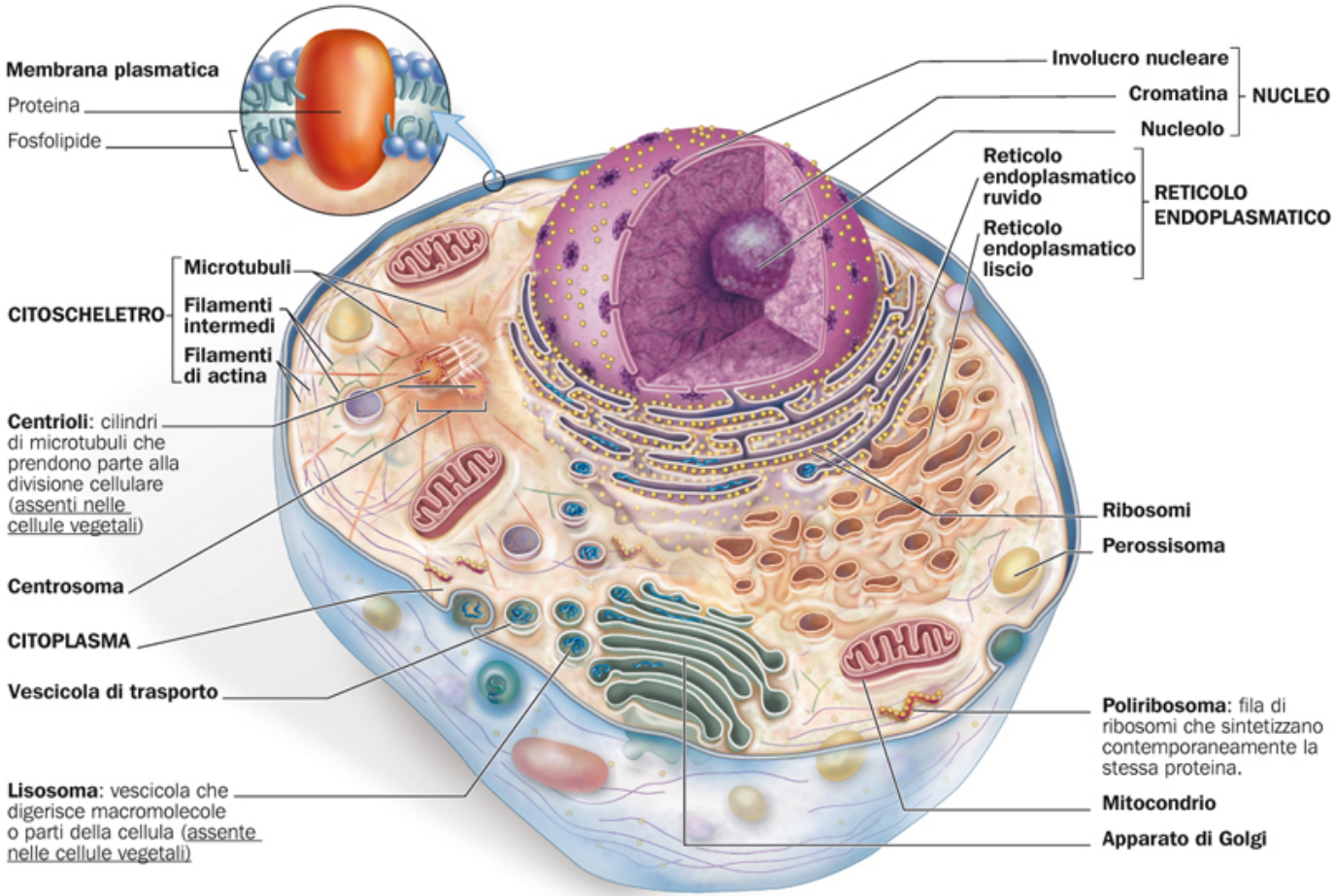
5- RESTRICTION ENZYMES
Description and examples of employment in molecular biology. Restriction maps of plasmids previously extracted with commercial kit and analysis of the DNA fragments obtained

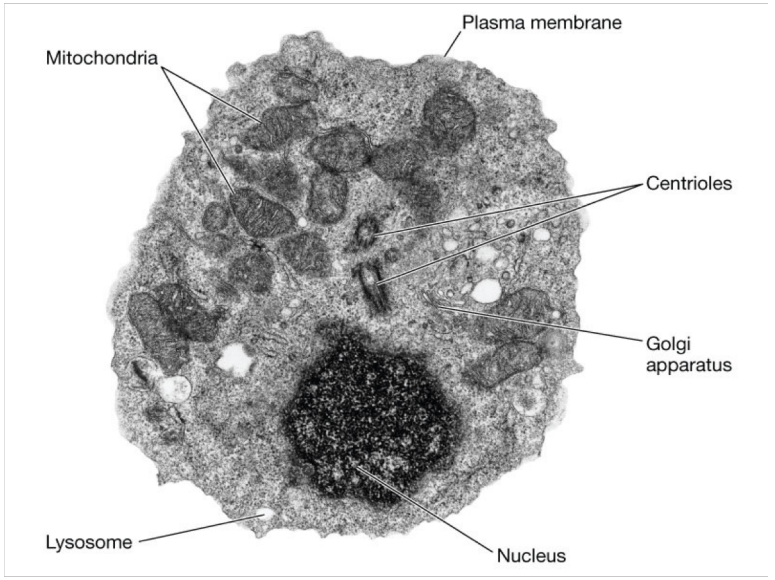
Materiale Didattica



1. RECOMBINANT DNA TECHNIQUES

La cellula eucariote



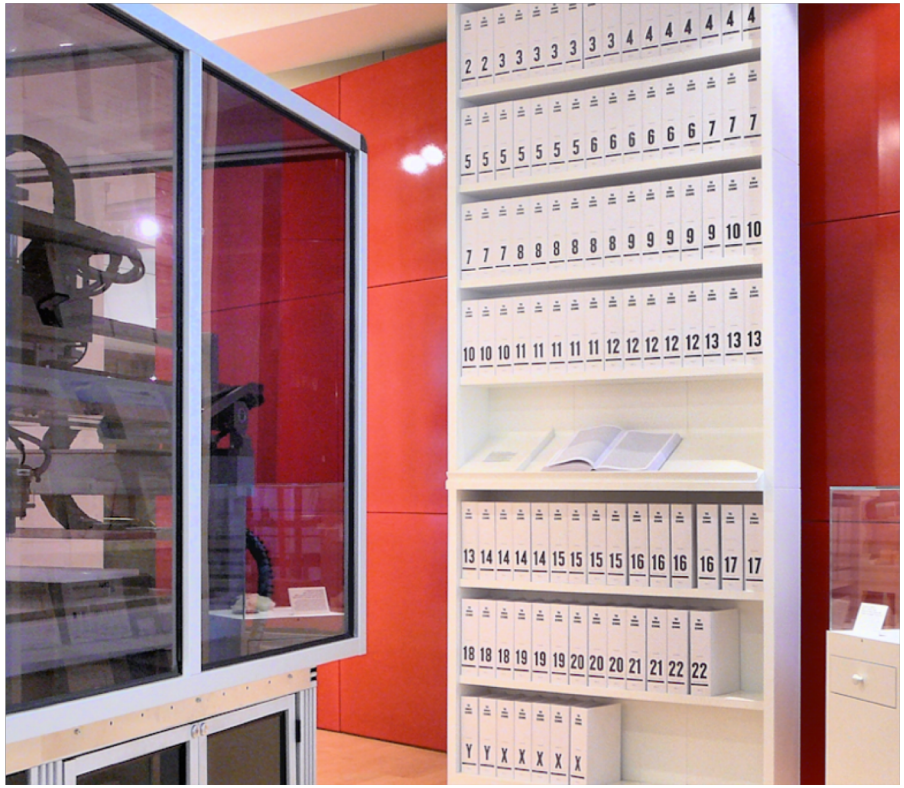


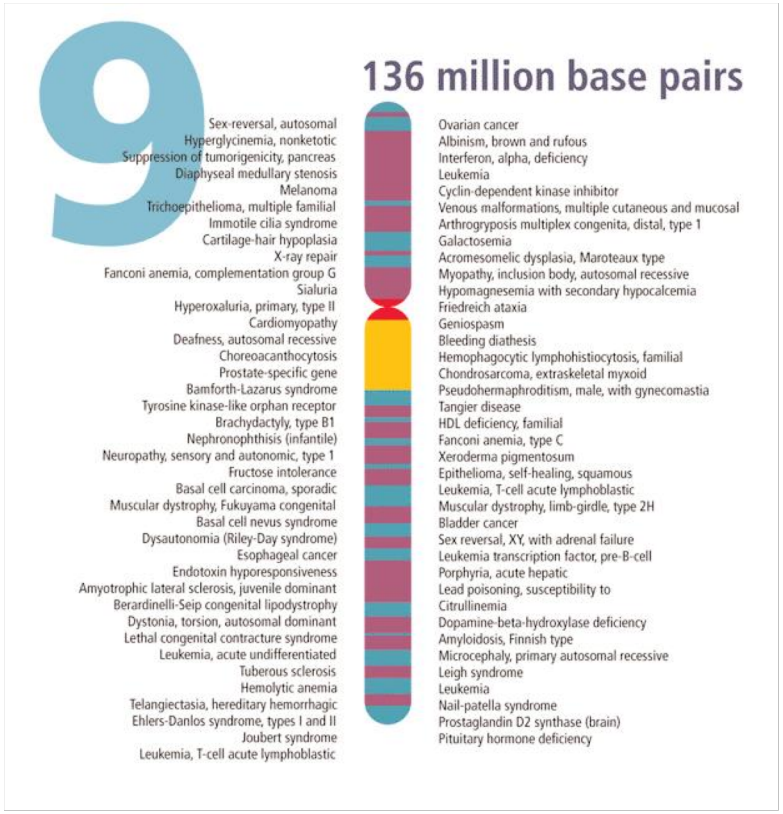
10-100μm

Genoma humano: 3,289,000,000 nucelotidi

- Dimensioni: circa dieci volte piu' grandi delle cellule procariotiche (10-100 μm)
- La **membrana plasmatica** racchiude il materiale cellulare, lo separa dall'ambiente e regola il passaggio di sostanze cellula/esterno
- **Compartimentazione interna**: all'interno della membrana si trova il **citoplasma**, l'insieme del contenuto cellulare, comprendente il **citosol** (soluzione acquosa di piccole e grandi molecole) ed una serie di **organuli**, compartimenti funzionalmente specializzati delimitati da membrana o comunque strutturalmente separati (Apparato di Goghi; Mitocondrio; Reticilo endoplasmatico)

Genomes





Genoma umano aploide: 3.2 x 10^9 bp (3200000000 bp)

→ 22 autosomi
→ eterocromosomi (X ed Y)
→ 23000 geni

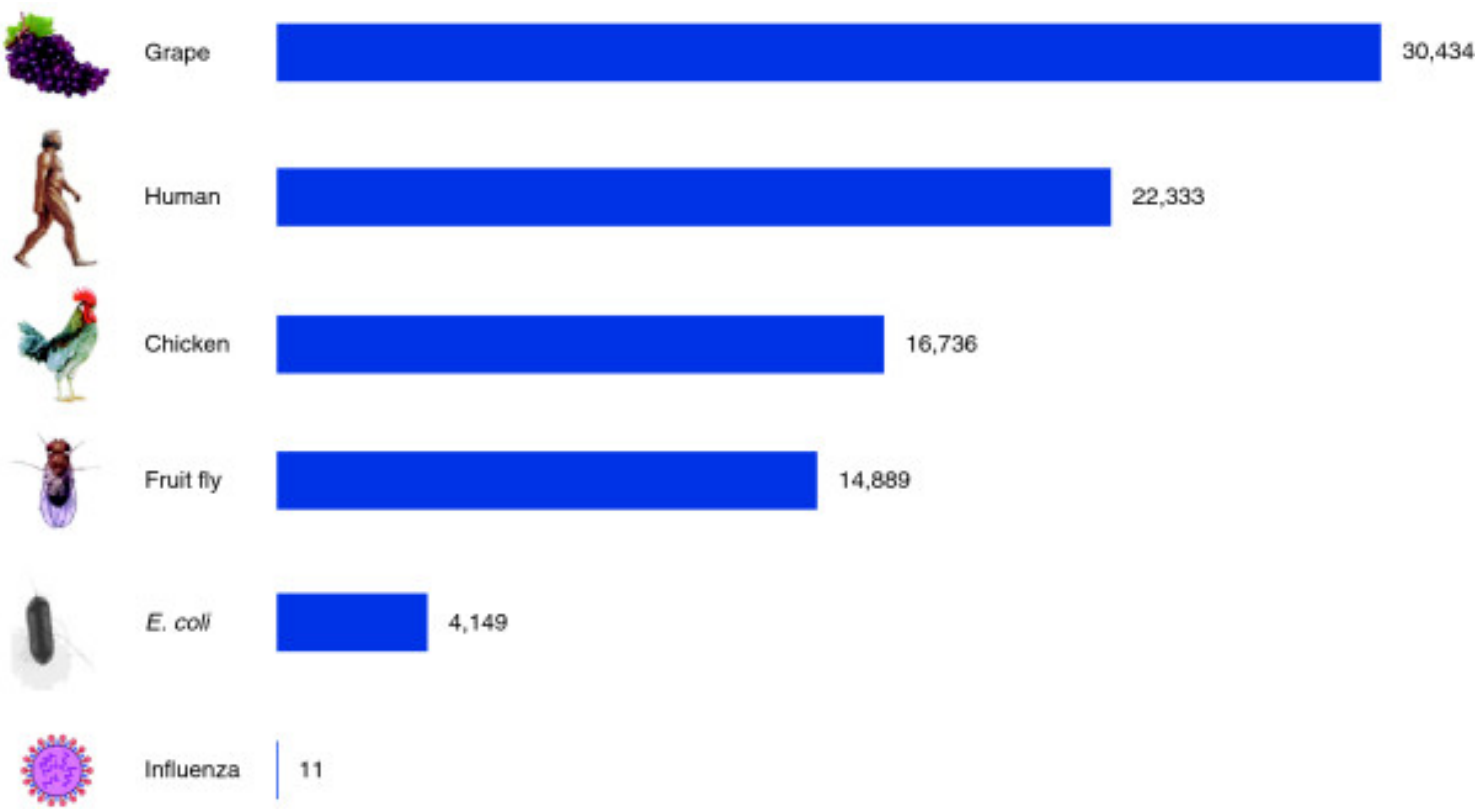
Dimensione dei cromosomi: 45-275 Mb;
→ 2.9 x 109 bp: eucromatina = attivo
→ Genoma noto: >90% dell'eucromatina.

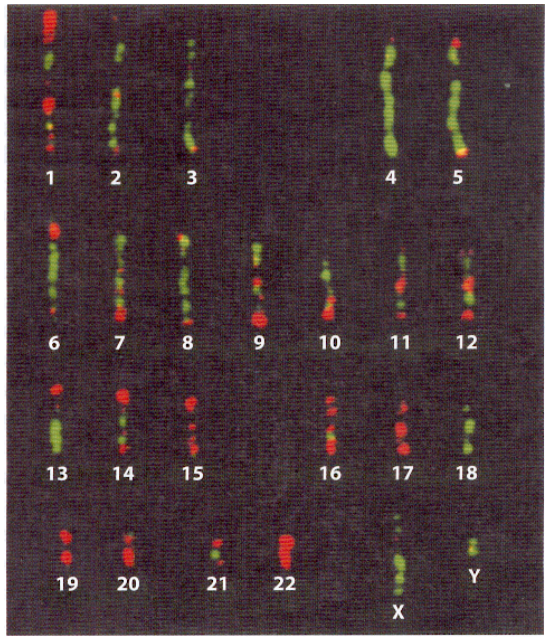
L'utillizo della infromazione genetica:

5.000-10.000 geni espressi da ogni cellula
 100.000 specie proteiche diverse per modificazioni post-traduzionali
 10^8 specie proteiche diverse nel genere umano (plasma: ***proteoma di proteomi)***

ENORME COMPLESSITA

Gene numbers in different organisms





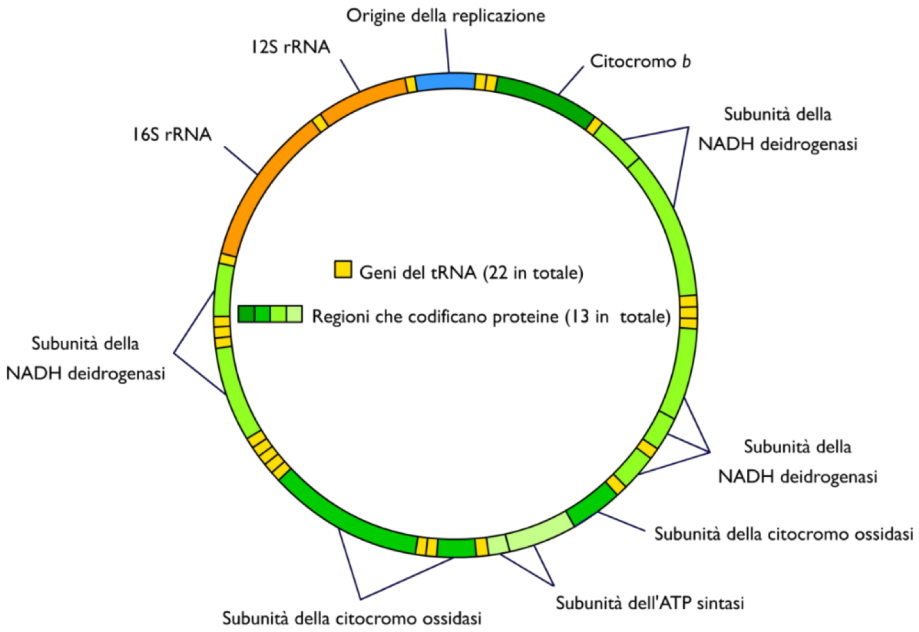
Mappa genica umana

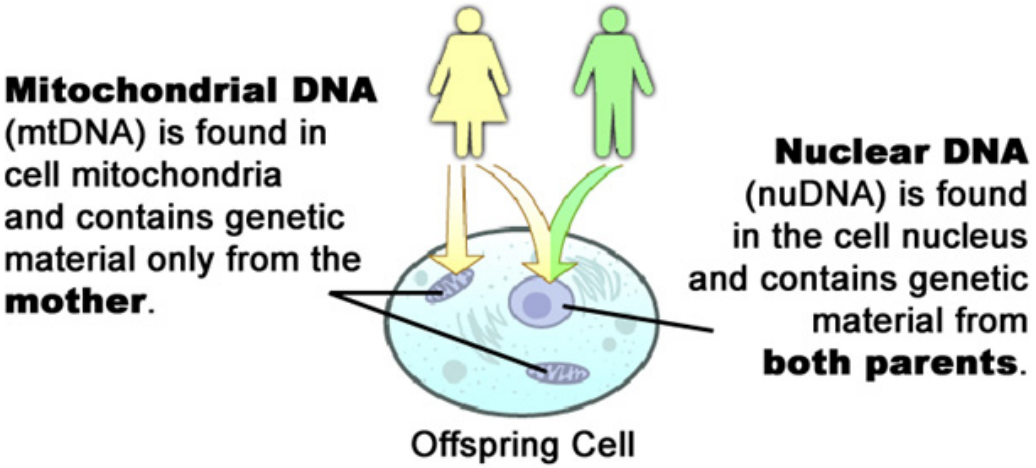
Le regioni in rosso indicano porzioni dei cromosomi ad alta densità genica (ad esempio i cromosomi 15, 16, 17, 19, 20 e 22).

Altri cromosomi come 4, 18, X e Y mostrano una colorazione rossa molto debole e sono poveri di geni.

MITOCHONDRIAL DNA

DNA mitocondriale dell'uomo:
16569 paia di basi e 37 geni (codificano per 13 polipeptidi sintetizzati dal ribosoma mitocondriale
22 tRNA e 2 rRNA), coinvolti nella produzione di proteine necessarie alla respirazione cellulare.



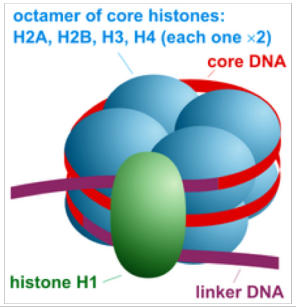


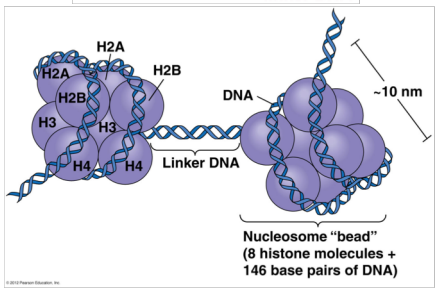
LA CROMATINA

La **cromatina** è la forma in cui gli acidi nucleici si trovano nella cellula.

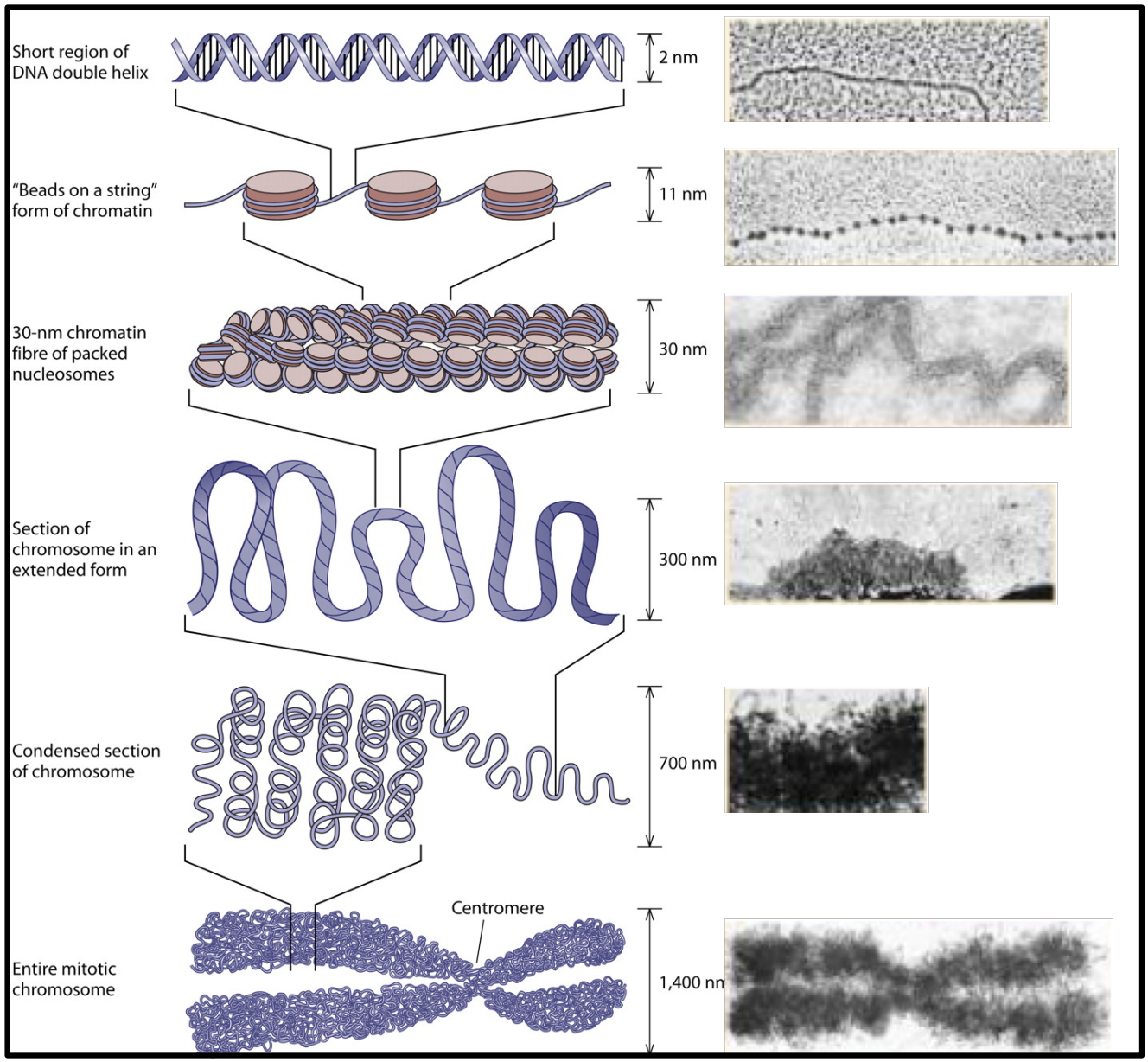
Funzione:

- impacchettamento del DNA
-rafforzare il DNA per permettere la mitosi
- prevenire danni al DNA
-controllare la replicazione del DNA e l'espressione (attivita) del gene

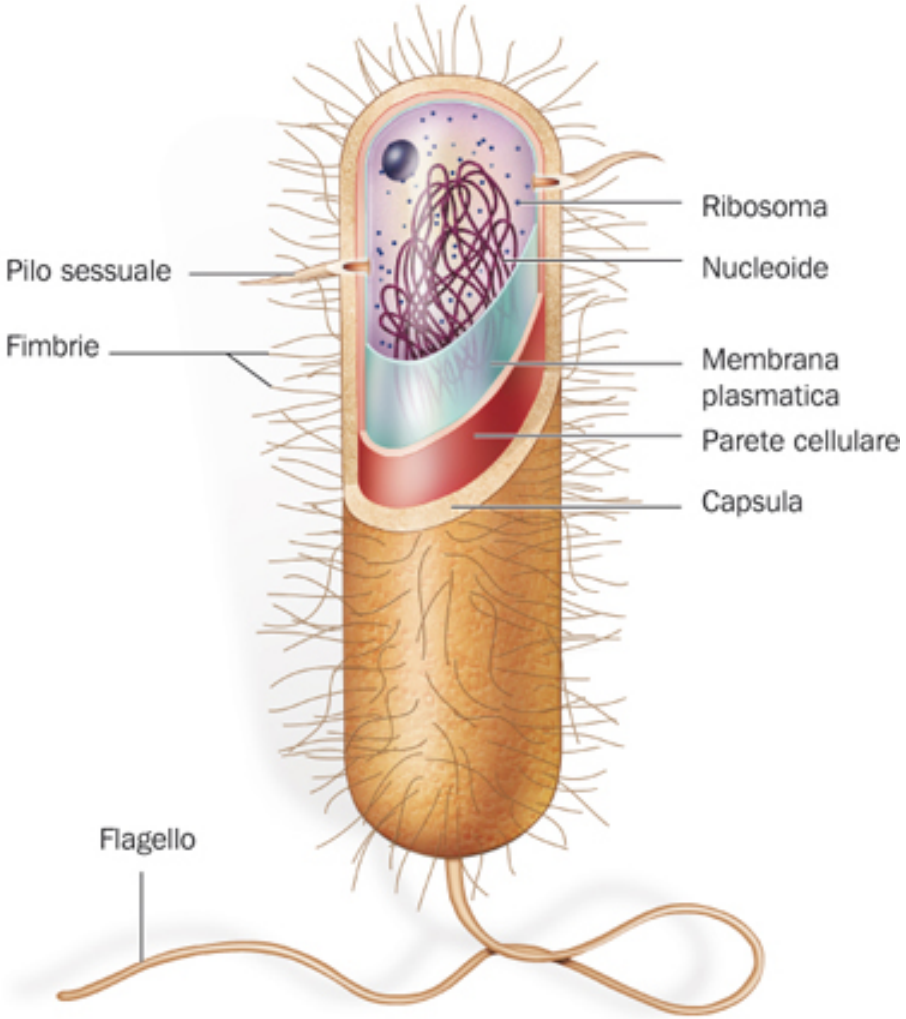




Nucleosome:
8 histone proteins
2 giri di DNA (146 nt)



PROCARIOTI



Le cellule procariotiche (da *pro*, prima e *karyon*, nucleo) sono **prive di un nucleo** racchiuso da una membrana.

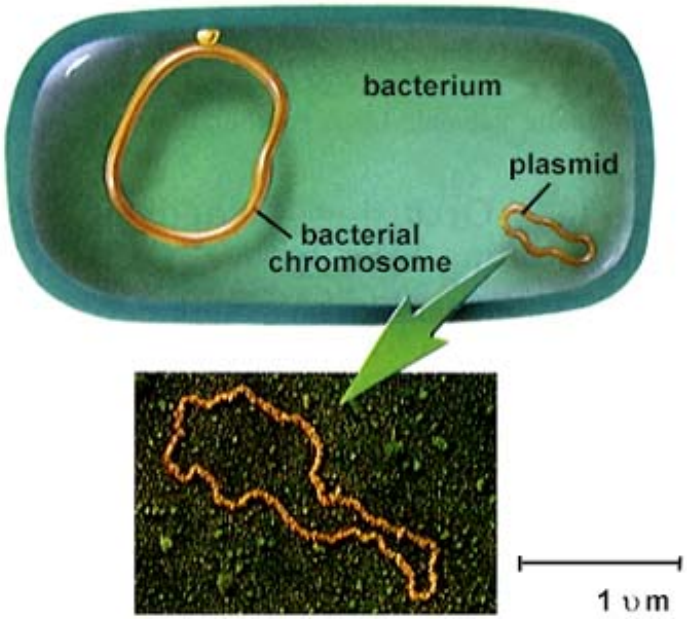
Gli organismi unicellulari costituiti da cellule procariotiche, i **procarioti**, sono classificati in due domini:

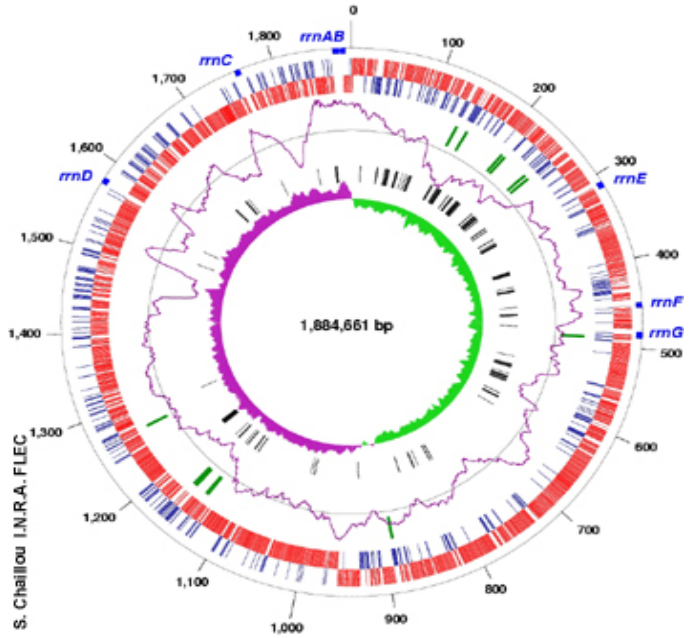
- ***Archaea* (archei);**
- ***Bacteria* (batteri).**

PROCARIOTI - EUCARIOTI

Il materiale genetico, il DNA, e' organizzato in un **singolo cromosoma circolare,** localizzato nell'area nucleare o **nucleoide**, una regione della cellula non delimitata da membrana.

1-2 µm (1.000.000 µm = 1m)





- In aggiunta al DNA principale i batteri possono contenere piccole molecole di DNA circolare, dette **plasmidi**, che codificano per enzimi catabolici, per la resistenza ad antibiotici o legati a meccanismi per lo scambio di materiale genetico tra organismi.
- **Genoma: 130.000 – 14.000.000 nucleotidi**

DNA RICOMBINANTE

tecnica che permette di

- **ottenere brevi segmenti di DNA clonati e di studiarne la sequenza nucleotidica**
- **di trasferirli nel genoma di altre cellule**
- **di controllare l'incorporazione e l'espressione del DNA clonato**
- **di introdurre mutazioni nel DNA e di studiarne gli effetti**

A General Strategy to study or use recomobinant DNA