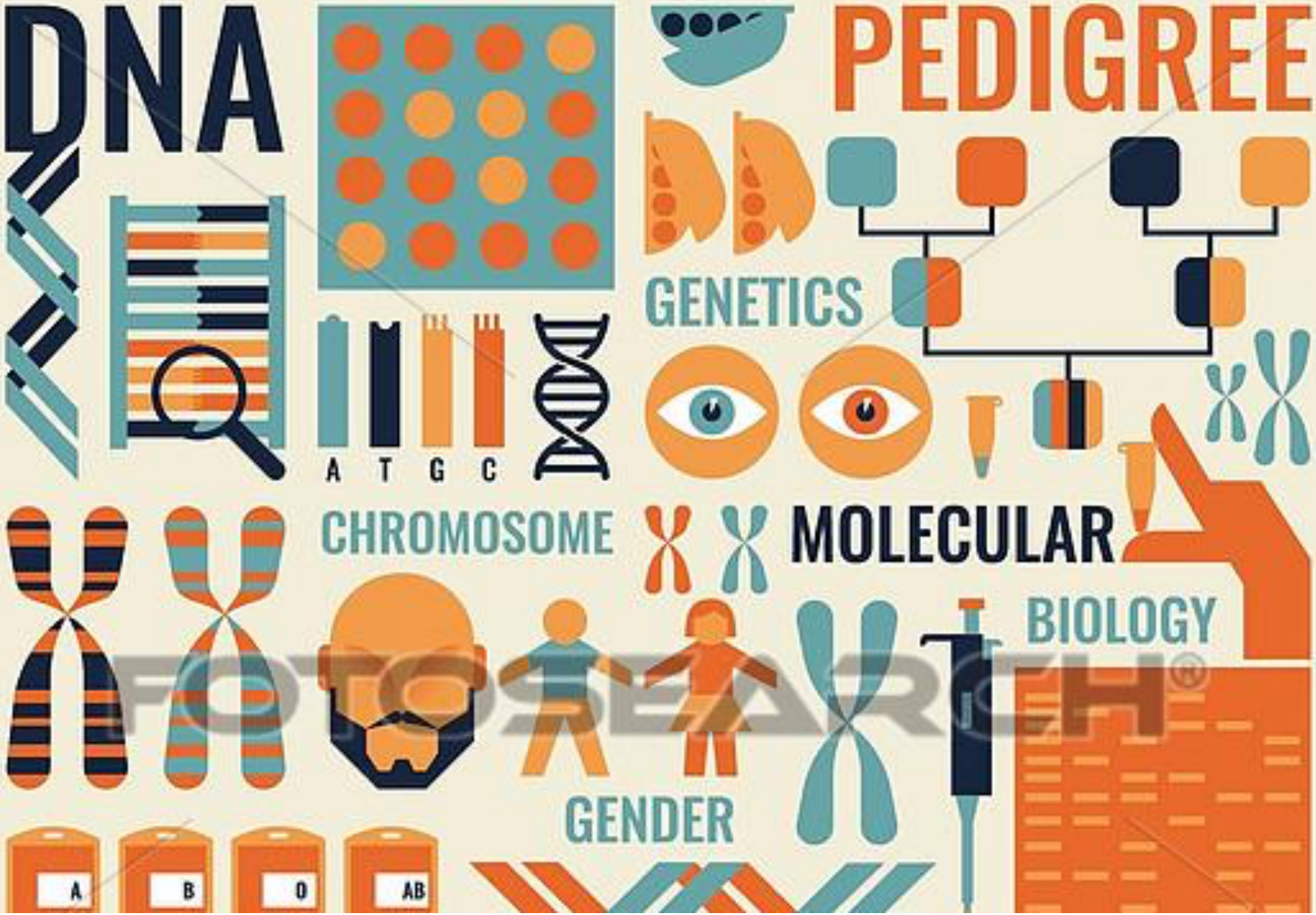

DNA
PEDIGREE
GENETICS
A T G C
CHROMOSOME
MOLECULAR
BIOLOGY
GENDER
A
B
O
AB

Insegnamento: Laboratorio Biologia Molecolare

Docenti:

Prof. SCHOEFTNER STEFAN, responsible corso – Lecture

Prof.ssa BANDIERA ANTONELLA – Laboratory course

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

- A focus will be set on the molecular biology and technologies related to of nucleic acids

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

- Laboratory exercises include the teaching of laboratory safety standards the handling of laboratory instruments, the extraction of DNA from bacteria and human cheak cells, use of restirction enzymes, mapping of plasmids after digest by restriction digest, gel electrophoresis, amplificiation of nucleic acid sequences by PCR, mapping of polymorphisms in "student population"(Alu repeats, disease related SNPs), analysis and interpretation of results in student polylation and overall population.

Organization Course: Laboratorio Biologia Molecolare

Monday 30.09.2019 – 28.10.2019: 11 hours theroretical lections: Prof. Schoeftner (Technologies)

Monday 04.11.2019 – 16.12.2019: Theroretical lections: Prof. Schoeftner (Technologies)

Laboratory course: Prof. Scaggiante

	lunedi 4 novembre	martedi 5 novembre	mercoledi 6 novembre	giovedi 7 novembre		venerdi 8 novembre	
9-10	Alimenti, nutrienti e salute	Alimenti, nutrienti e salute	Fisiologia	Lab. Biol. Bol. II TURNO - Bandiera		Lab. Biol. Bol. IV TURNO -	Fisiologia vegetale
10-11	Alimenti, nutrienti e salute	Alimenti, nutrienti e salute	Fisiologia	Lab. Biol. Bol. II TURNO - Bandiera		Lab. Biol. Bol. IV TURNO -	Fisiologia vegetale
11-12	Lab. di Biologia Molecolare (TEORIA)	Microbiologia	Farmacologia	Lab. Biol. Bol. II TURNO - Bandiera		Lab. Biol. Bol. IV TURNO -	Ecologia
12-13	Lab. di Biologia Molecolare (TEORIA)	Microbiologia	Farmacologia	Lab. Biol. Bol. II TURNO - Bandiera		Lab. Biol. Bol. IV TURNO -	Ecologia
13-14			Lab. Biochimica III e IV turno				
14-15	Microbiologia	Farmacologia	Lab. Biol. Bol. I TURNO - Bandiera	Lab. Biol. Bol. III TURNO -	Ecologia		
15-16	Microbiologia	Farmacologia	Lab. Biol. Bol. I TURNO - Bandiera	Lab. Biol. Bol. III TURNO -	Ecologia		
16-17	Fisiologia	Ecologia	Lab. Biol. Bol. I TURNO - Bandiera	Lab. Biol. Bol. III TURNO -	Fisiologia vegetale		
17-18	Fisiologia	Fisiologia vegetale	Lab. Biol. Bol. I TURNO - Bandiera	Lab. Biol. Bol. III TURNO -	Fisiologia vegetale		

Monday Lecture: ca. 1,5 hours: Theoretical lecture (Schoeftner) – related to topic in weekly laboratory exercise

ca. 0,5 hours: Prof. Scaggiante – practical background to laboratory background

Labortory exercises: Turno I – IV; all "turni will do the same porgram in the respective week Prof. Bandiera + 2 experienced tutors (20-25 stundents per turno; 4-5 students per work station

Contents of Theoretical Lecture (Prof. Schoeftner)

1. Anatomy of the cell, biomolecules, concept of preparation of RNA/Protein/DNA.

2. Recombinant DNA techniques, Cloning vectors, endonucleases, artificial chromosomes, recombinant protein expression, introduction of genes into host-organisms.

3. DNA sequencing, bacterial immunity, manipulation of the genome content of pro- and eukaryotic organisms, siRNA/shRNA mediated knock-down approaches.

4. Hybridization related techniques (RNA-FISH, DNA-FISH, Southern blot, Northern blot), Electrophoresis, methods to study DNA:protein interaction (band shift, DNA footprinting, chromatin immunoprecipitation)

5. PCR technologies: standard PCR, RT—PCR and variants

6. Gene expression analysis: array technology and high content sequencing, determination of 3' and 5' ends of RNA, single molecule transcript analysis

7. Exercise session: Students get introduction into the use of the ENSEMBL genome browser and primer construction. Students will do primer design for practical part as homework.

Contents of Practical Course (Prof. Bandiera)

Application of molecular biology techniques for the diagnosis and **monitoring of specific genetic conditions (allelic variants) and genetic variation of Alu repeat in students of the course**.

1. THE MOLECULAR BIOLOGY LABORATORY: Rule 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.

2. PLASMIDS: Plasmid DNA extraction by a commercial kit, evaluation of extraction yield, preparation of samples for electrophoretic analysis. Plasmids will be subjected to control digest using restriction enzymes and will serve as positive and negative control for subsequent PCR.
3. PREPARATION OF GENOMIC DNA:
4. Anonymized preparation of genomic DNA from cheek cells of students and determination of concentration.

5. PCR AMPLIFICATION OF SITE OF GENETIC ALU REPEAT VARIANT: Alu repeats number variation on a locus of chromosome 16 will be determined by specific PCR. Agarose Gel electrophoresis will be used to monitor differences in Alu repeat number.

6. PCR AMPLIFICATION OF SITE OF ALLELIC G6PD VARIANTS:
   A locus harboring allelic variants of the G6PD gene will be amplified by PCR. PCR products will be purified after gel electrophoresis and subjected to digest using restriction enzymes. Digested DNA will be separated by gel electrophoresis.

8. DATA ANALYSIS AND DISCUSSION:
   Chi-square analysis will be used to to compare the Alu genotype frequencies within the class population with those predicted by the Hardy-Weinberg equation. The genotypic frequencies of the class population can also be compared with the genotypic frequencies of another population in
   the database.

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. 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 LABORATORY COURSE

Turni: 5

Inscription in turni via Moodle federato in ca. 2 weeks!

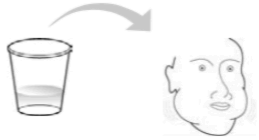
1. Preparation of Genomic DNA

Quick Guide

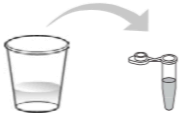
Lesson 1 Cheek Cell DNA Template Preparation

1. Label one 1.5 ml micro test tube with your initials. Label one screwcap tube containing 200 µl of InstaGene matrix with your initials.

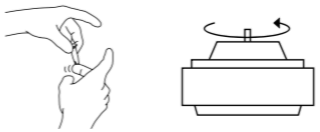
2. Obtain a cup containing saline solution from your instructor. Pour the saline into your mouth and rinse vigorously for 30 seconds. Expel the saline back into the cup.



3. Transfer 1 ml of your saline rinse into the micro test tube (NOT the screwcap tube) with your initials. If a P-1000 micropipet is not available, **carefully** pour ~1 ml of your saline rinse into your micro test tube (use the graduations on the side of the micro test tube to estimate 1 ml).



4. Spin your tube in a balanced centrifuge at full speed for 2 minutes. When the centrifuge has completely stopped, remove your tube. You should see a match-head sized pellet of whitish cells at the bottom of the tube. If you don't see a pellet of this size, decant the saline, refill your tube with more of your oral rinse, and repeat the spin.



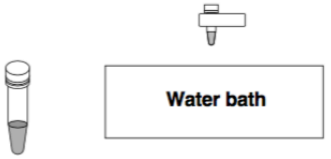
5. After pelleting your cells, pour off the saline. Being careful not to lose your pellet, blot your tube briefly on a paper towel or tissue. It's OK for a small amount of saline (< 50 µl, about the same size as your pellet) to remain in the bottom of the tube.

6. Resuspend the pellet by vortexing or flicking the tube so that no clumps of cells remain.

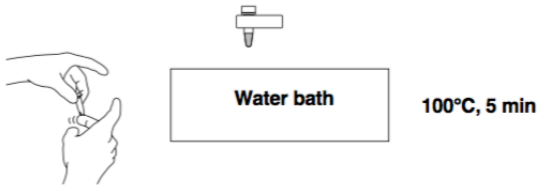
7. Using a 2–20 µl adjustable-volume micropipet set to 20 µl, transfer all of your resuspended cells to the screwcap tube containing InstaGene.

8. Screw the cap tightly on the tube. Shake or vortex to mix the tube contents.

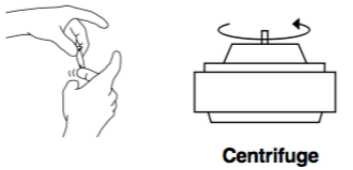
9. When all members of your team have collected their samples, place the tubes in the foam micro test-tube holder, and incubate at 56°C for 10 minutes in a water bath. At the halfway point (5 minutes), shake or vortex the tubes gently, then place back in the 56°C water bath for the remaining 5 minutes.



10. Remove the tubes, shake or vortex, and place the tubes in a boiling water bath (100°C). Incubate at 100°C for 5 minutes.



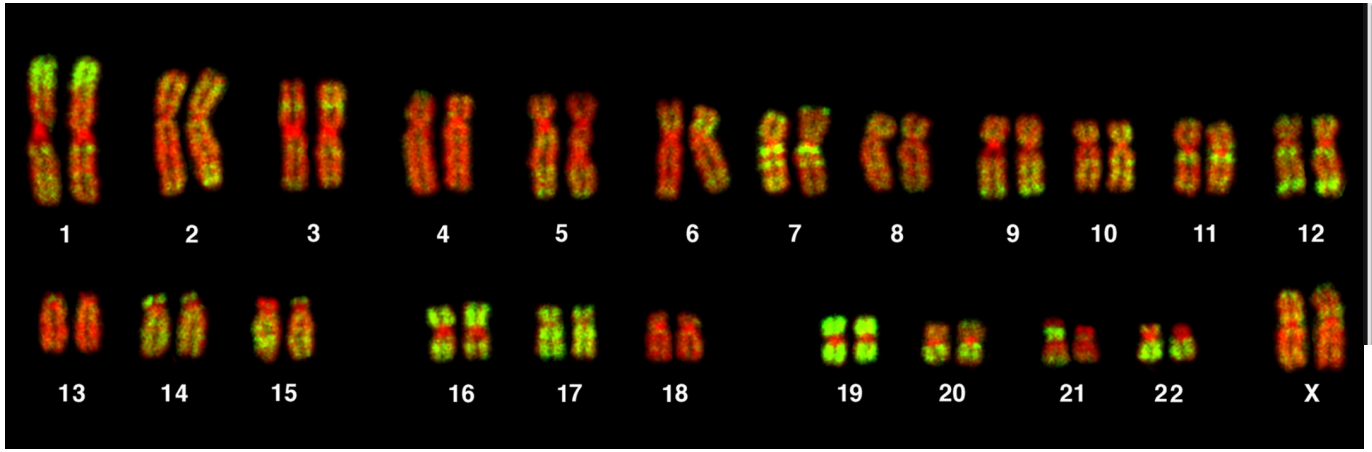
11. Remove the tubes from the boiling water bath and shake or vortex the contents to resuspend. Pellet the matrix by spinning at 6,000 x g for 5 minutes (or 2,000 x g for 10 minutes) in a centrifuge.



12. Store your screwcap tube in the refrigerator until the next laboratory period (or proceed to step 2 of Lesson 2).

2. Determination of presence or absence of Alu insert within the PV92 locus

Alu repeats in humans



Element	Percent of total genome	Copy number
L1 (LINE)	16.9	0.5×10^6
Alu (SINE)	10.6	1.1×10^6
L2 (LINE)	3.2	0.3×10^6
MIR (SINE)	2.5	0.46×10^6
LTR elements	8.3	0.3×10^6
DNA elements	2.8	0.3×10^6
Processed pseudogenes	<1.0	$1–2 \times 10^4$
Total	~45	$\sim 3 \times 10^6$

Karyotype from a female human lymphocyte (46, XX). Chromosomes were hybridized with a probe for Alu elements (green) and counterstained with TOPR (red). Alu elements were used as a marker for chromosomes and chromosome bands rich in genes.

Throughout evolution, intron sequences have been the target of random insertions by short repetitive interspersed elements, also known as SINEs.7 SINEs have become randomly inserted within our introns over millions of years. One such repetitive element is called the Alu sequence7 (Figure 2). This is a DNA sequence about 300 base pairs long that is repeated, one copy at a time, almost 500,000 times within the human genome.8 The origin and function of such randomly repeated sequences is not yet known. The Alu name comes from the Alu I restriction enzyme recognition site that is found in this sequence.

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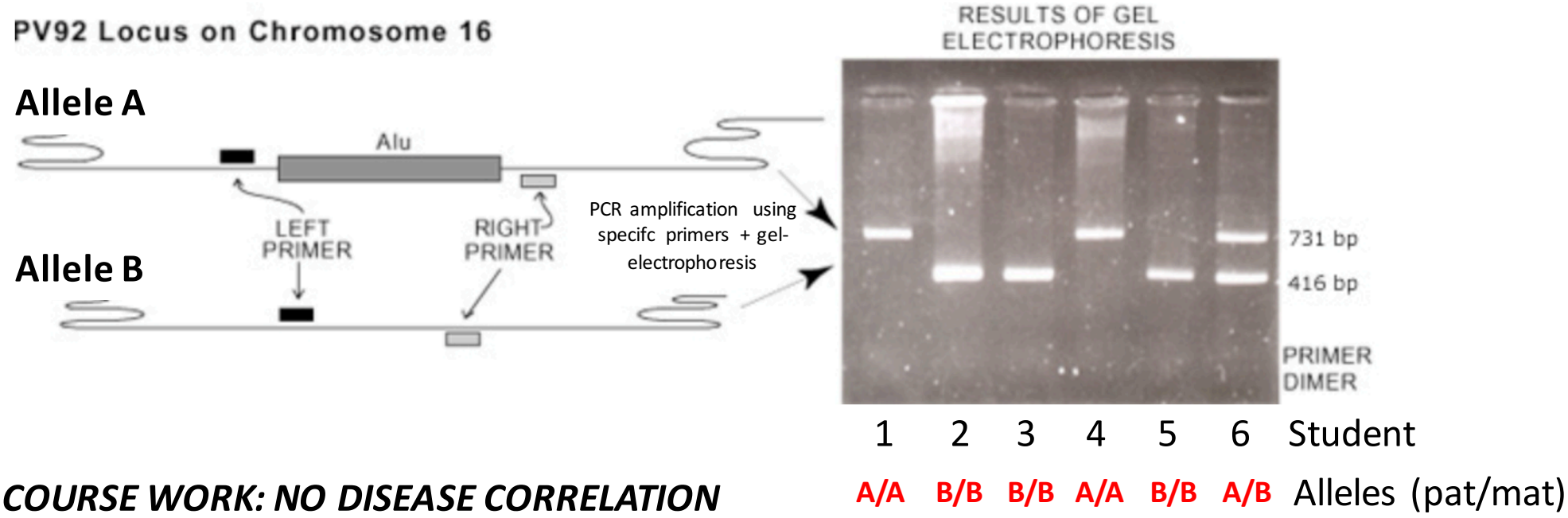
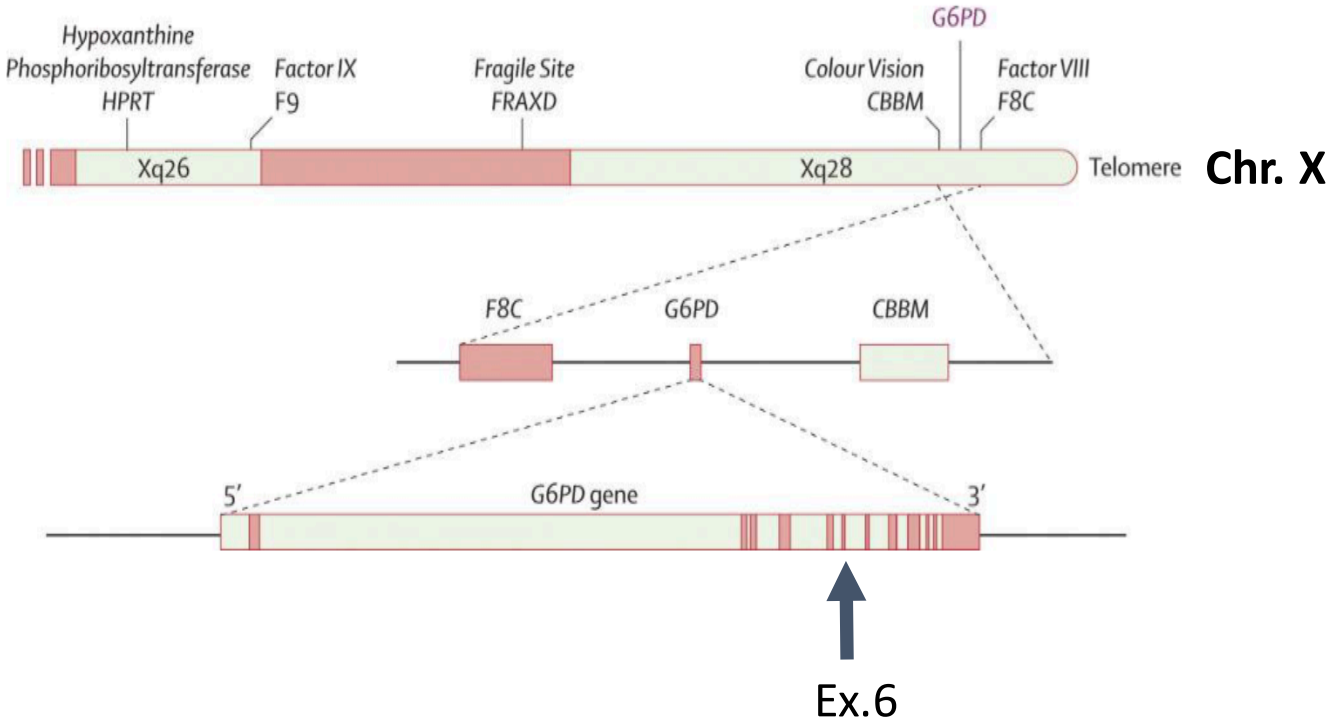


COURSE WORK: NO DISEASE CORRELATION

Students will perform a bioinformatics exercise to investigate the genotypic frequencies for the Alu polymorphism in their class population and compare them with the genotypic frequencies of other populations.

3. Determination of a G6PD variant by RFLP

Glucose-6-phosphate dehydrogenase deficiency (G6PDD) is an inborn error of metabolism that predisposes to red blood cell breakdown. Most of the time, those who are affected have no symptoms. Following a specific trigger, symptoms such as yellowish skin, dark urine, shortness of breath, and feeling tired may develop. Complications can include anemia and newborn jaundice (yellow pigmentation). Some people never have symptoms



It is an X-linked recessive disorder that results in **defective glucose-6-phosphate dehydrogenase enzyme**. Red blood cell breakdown may be triggered by infections, certain medication, stress, or foods such as fava beans. Depending on the specific mutation the severity of the condition may vary. Diagnosis is based on symptoms and supported by blood tests and **genetic testing (PCR-RFLP)**

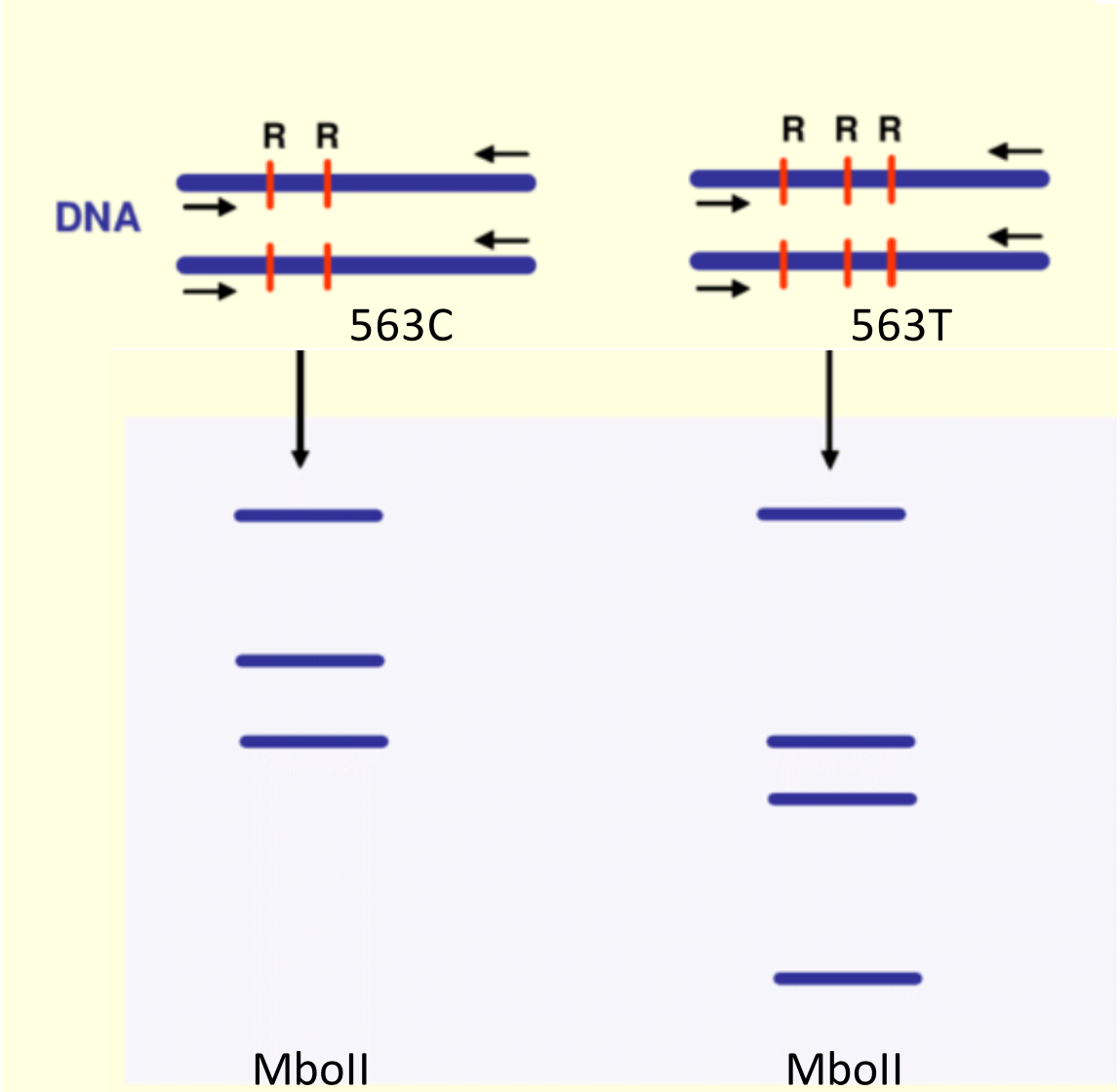
3. Determination of a G6PD variant by RFLP

Many variants have been determined (>190)

Descriptive mutations									
Mutation				Gene			Protein		
Designation	Short name	Isoform G6PD-Protein	OMIM-Code	Type	Subtype	Position	Position	Structure change	Function change
G6PD-A(+)	Gd-A(+)	G6PD A	+305900.0001	Polymorphism nucleotide	A→G	376 (Exon 5)	126	Asparagine→Aspartic acid (ASN126ASP)	No enzyme defect (variant)
G6PD-A(-)	Gd-A(-)	G6PD A	+305900.0002	Substitution nucleotide	G→A	376 (Exon 5) and 202	68 and 126	Valine→Methionine (VAL68MET) Asparagine→Aspartic acid (ASN126ASP)	
G6PD-Mediterranean	Gd-Med	G6PD B	+305900.0006	Substitution nucleotide	C→T	563 (Exon 6)	188	Serine→Phenylalanine (SER188PHE)	Class II
G6PD-Canton	Gd-Canton	G6PD B	+305900.0021	Substitution nucleotide	G→T	1376	459	Arginine→Leucine (ARG459LEU)	Class II
G6PD-Chatham	Gd-Chatham	G6PD	+305900.0003	Substitution nucleotide	G→A	1003	335	Alanine→Threonine (ALA335THR)	Class II
G6PD-Cosenza	Gd-Cosenza	G6PD B	+305900.0059	Substitution nucleotide	G→C	1376	459	Arginine→Proline (ARG459PRO)	G6PD-activity <10%, thus high portion of patients.
G6PD-Mahidol	Gd-Mahidol	G6PD	+305900.0005	Substitution nucleotide	G→A	487 (Exon 6)	163	Glycine→Serine (GLY163SER)	Class III
G6PD-Orissa	Gd-Orissa	G6PD	+305900.0047	Substitution nucleotide	C→G	131	44	Alanine→Glycine (ALA44GLY)	NADP-binding place affected. Higher stability than other variants.
G6PD-Asahi	Gd-Asahi	G6PD A-	+305900.0054	Substitution nucleotide (several)	A→G ± G→A	376 (Exon 5) 202	126 68	Asparagine→Aspartic acid (ASN126ASP) Valine→Methionine (VAL68MET)	Class III.

<1%

3. DETECTION OF G6PD 563 C→T VARIANT

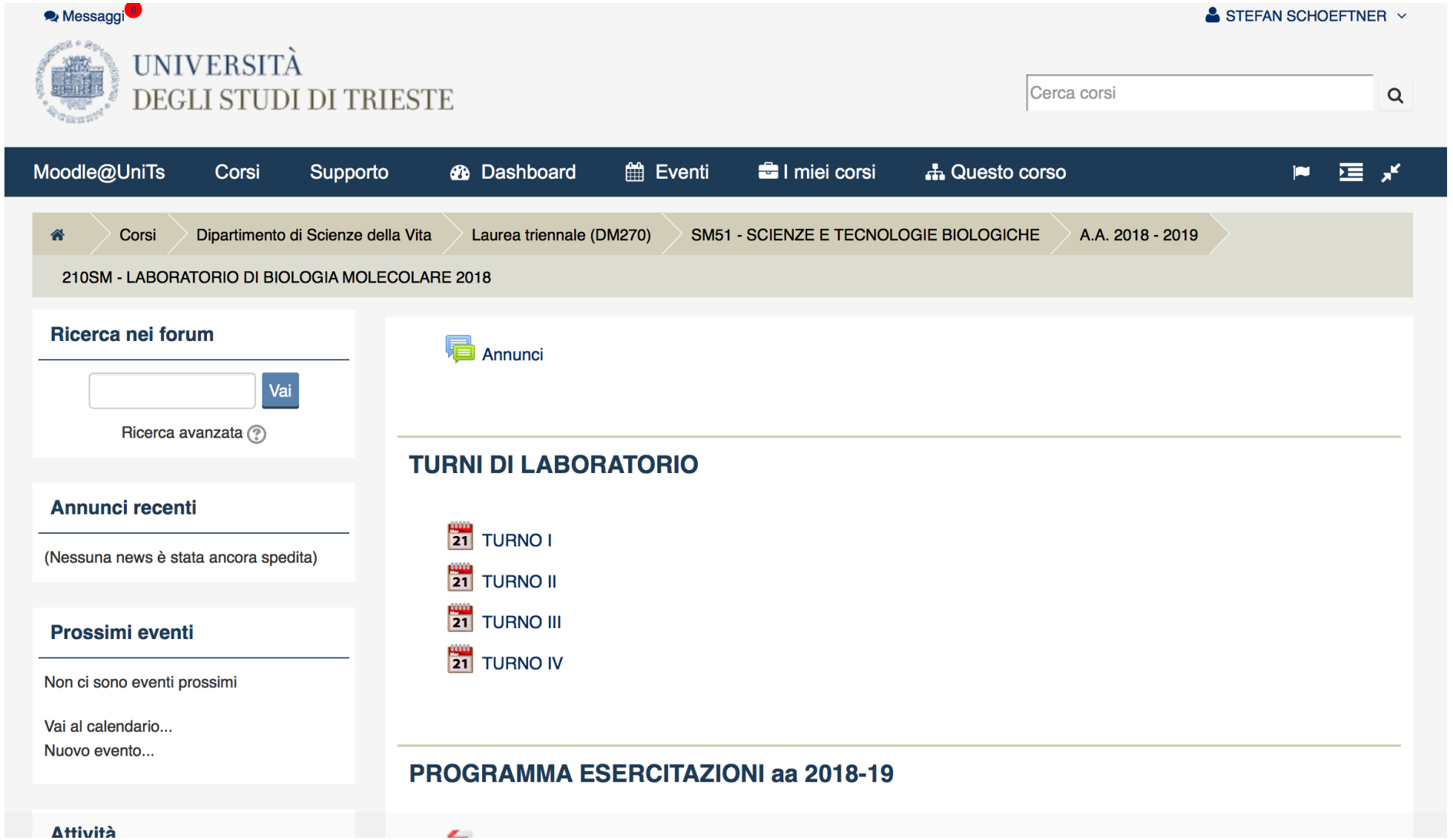


G6PD 563 C→T VARIANT IN EXON 6

1. G6PD Exon 6 specific primers
2. PCR amplify specific region of students
3. Purify PCR product
4. Digest purified DNA using MboII
5. Run agarose gel
6. 563C→T variants results a new MboII site in the PCR fragment
7. Additional band appears in gel

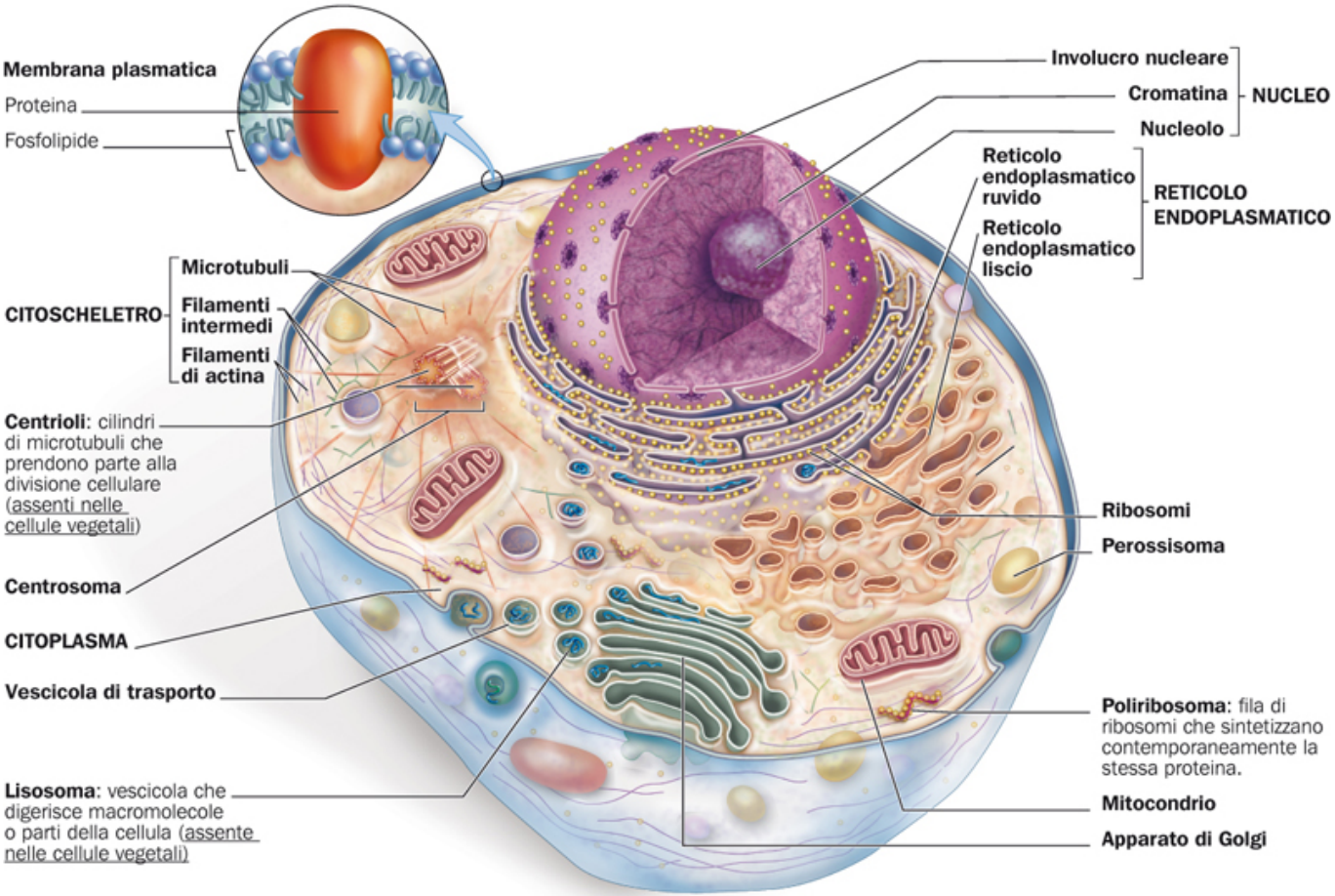
R = sito per enzima di restrizione MboII

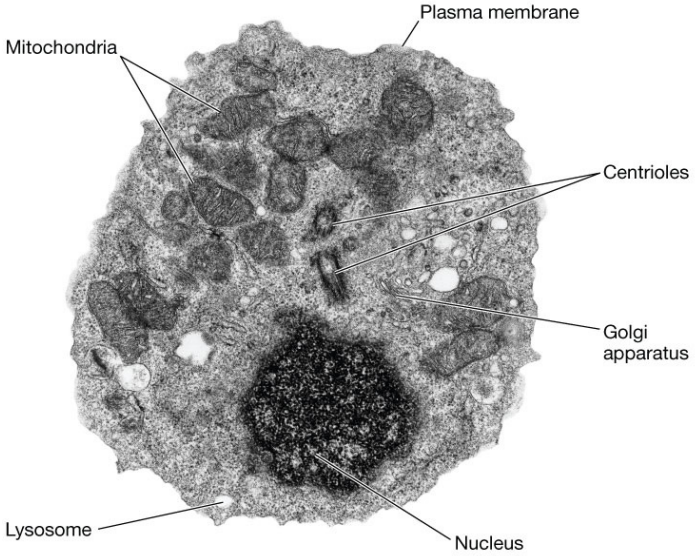
Materiale Didattica



1. RECOMBINANT DNA TECHNIQUES

La cellula eucariote



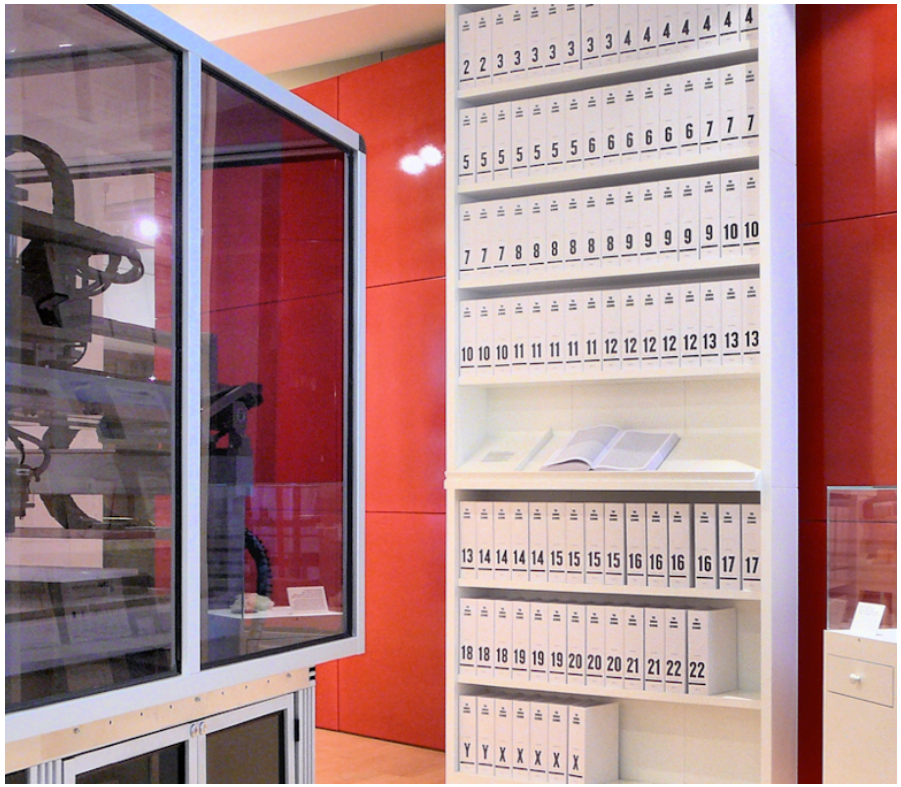


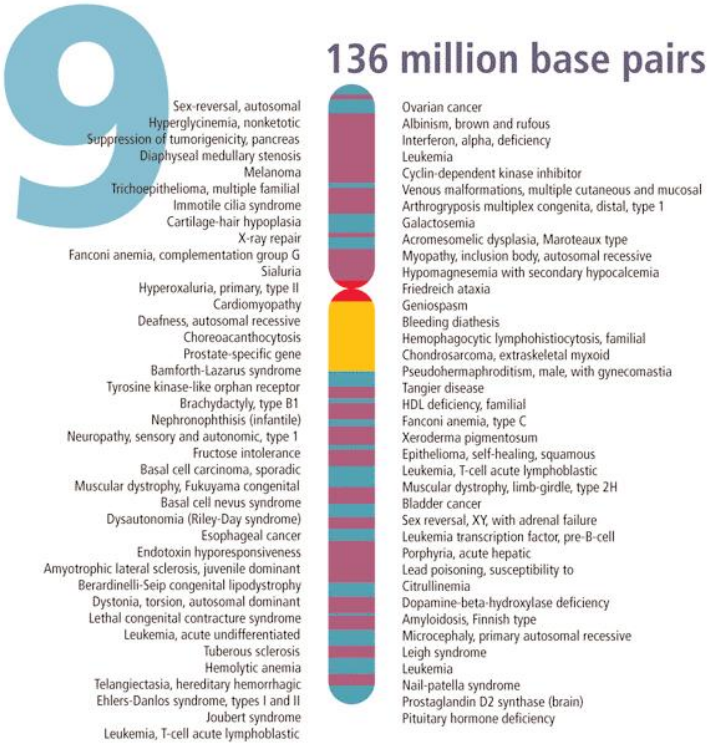
10-100µm

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

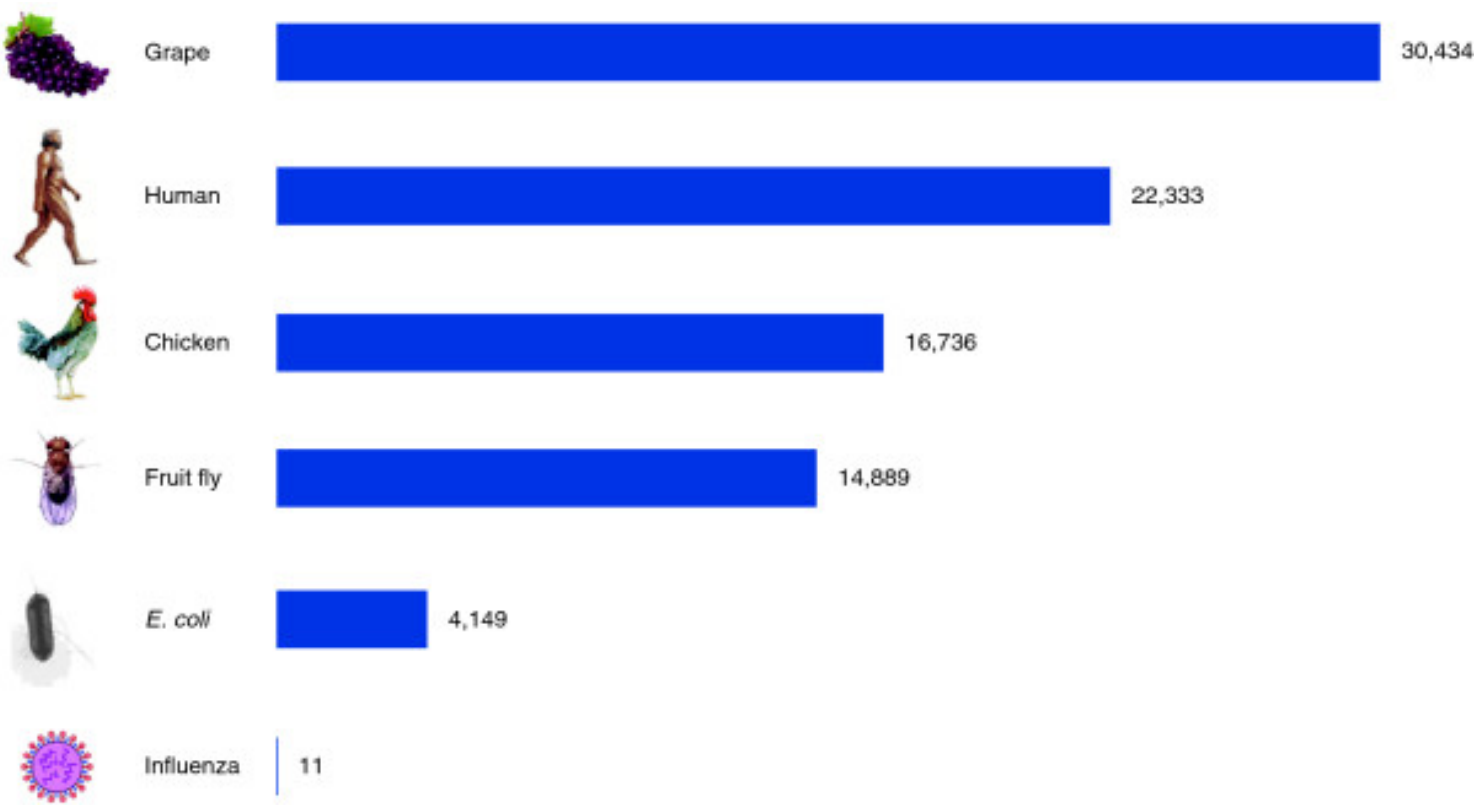
Dimensione dei cromosomi: 45-275 Mb;
→ 2.9 x 109 bp: eucromatina = attivo
→ Genoma noto: >90% dell'eucromatina.

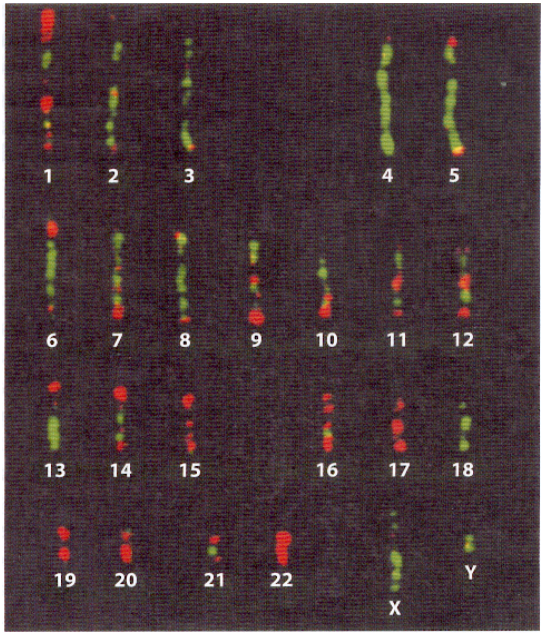
L'utillizo della infromazione genetica:

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





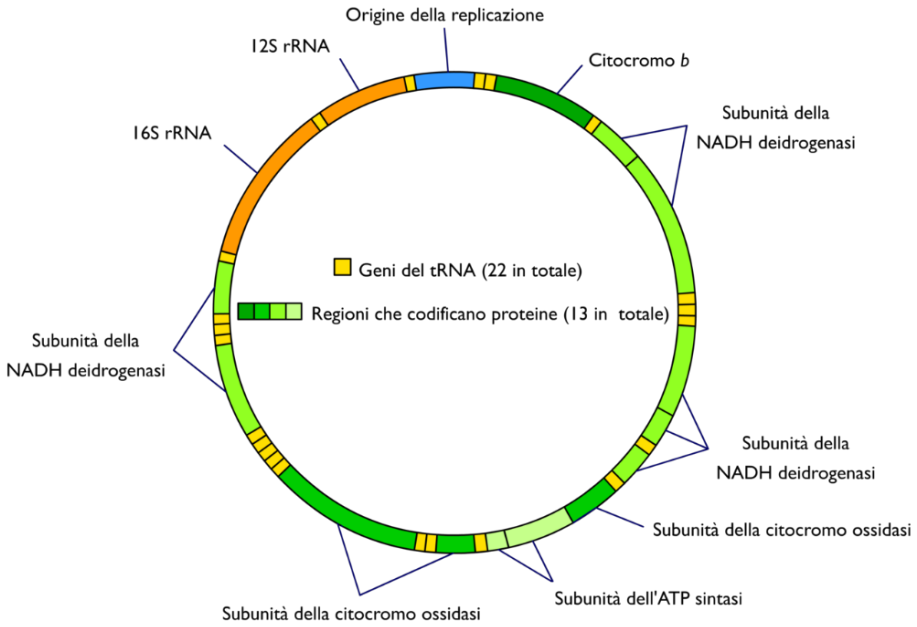
Mappa genica umana

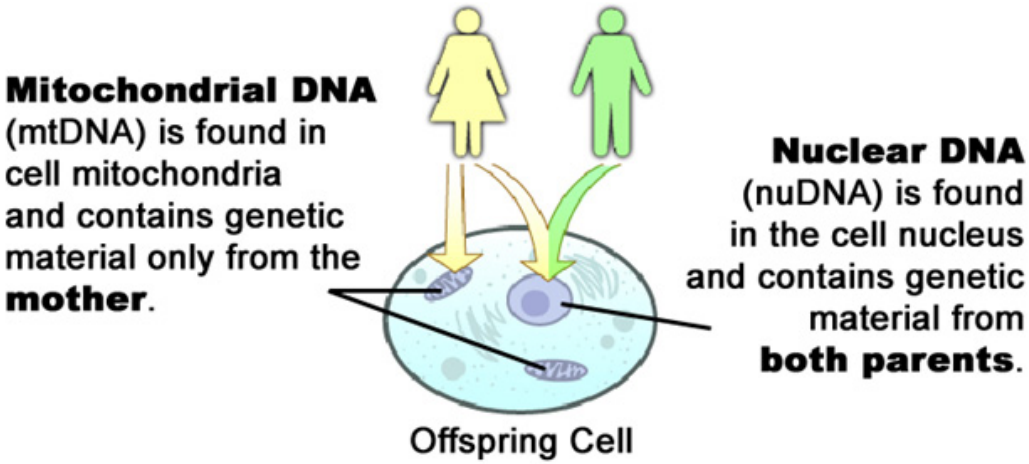
Le regioni in rosso indicano porzioni dei cromosomi ad alta densità genica (ad esempio i cromosomi 15, 16, 17, 19, 20 e 22).

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.



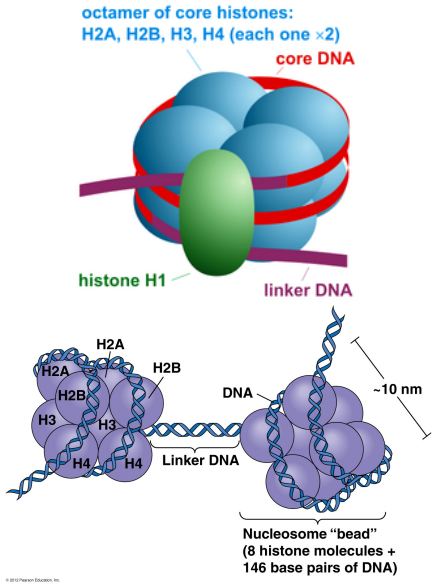


LA CROMATINA

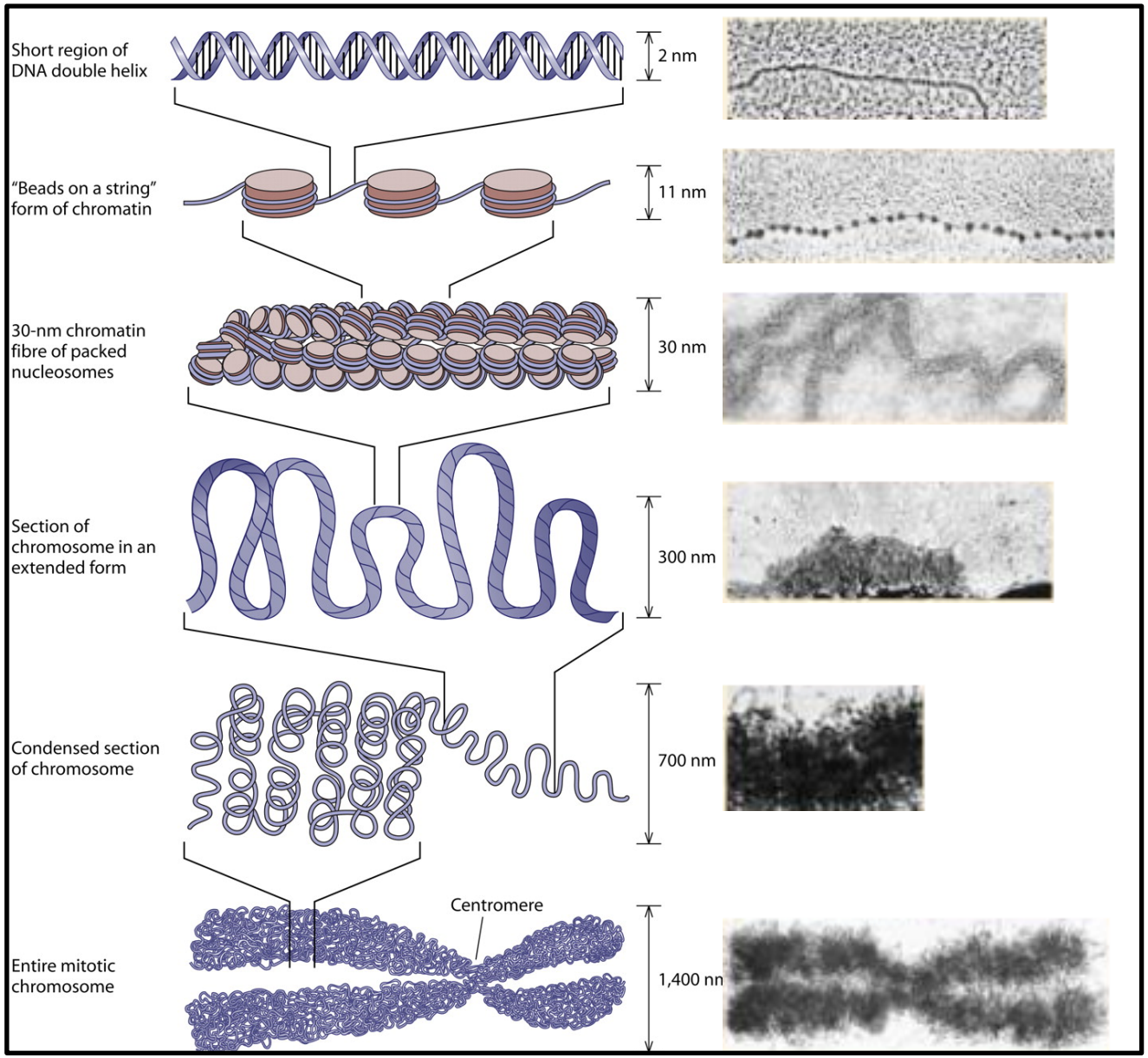
La **cromatina** è la forma in cui gli acidi nucleici si trovano nella cellula.

Funzione:

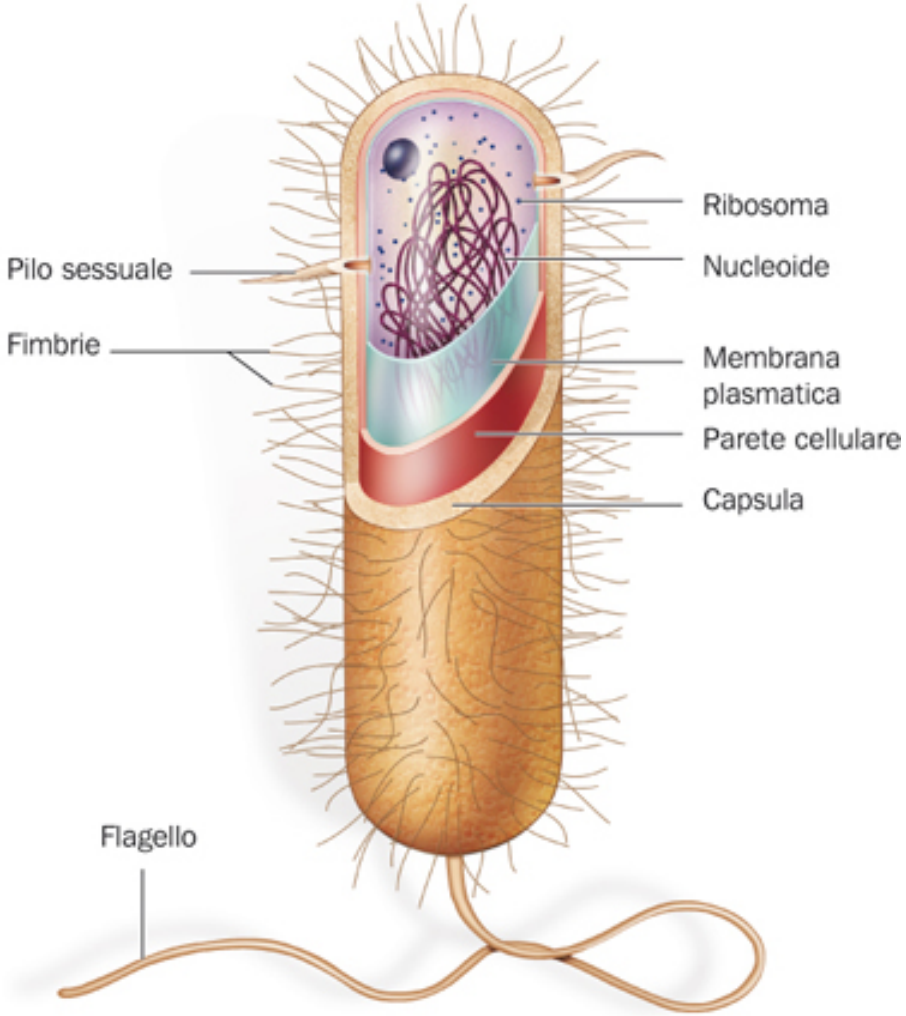
- impacchettamento del DNA
-rafforzare il DNA per permettere la mitosi
- prevenire danni al DNA
-controllare la replicazione del DNA e l'espressione (attivita) del gene



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.

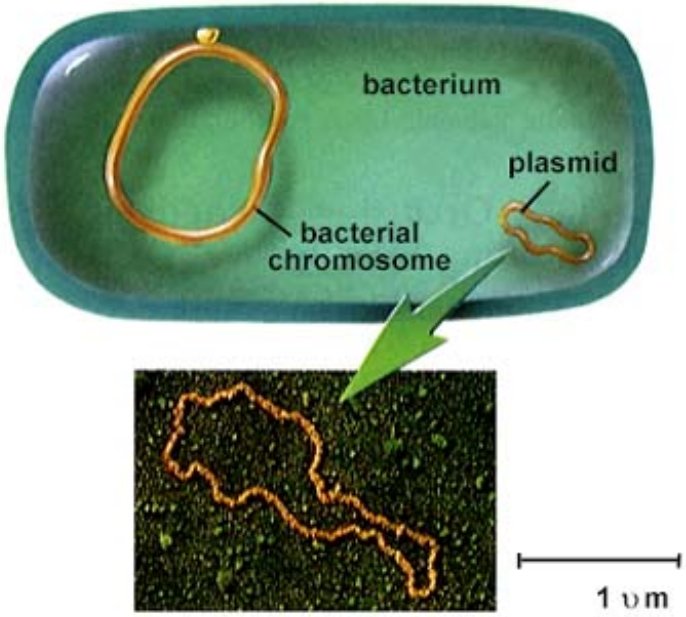
Gli organismi unicellulari costituiti da cellule procariotiche, i **procarioti**, sono classificati in due domini:

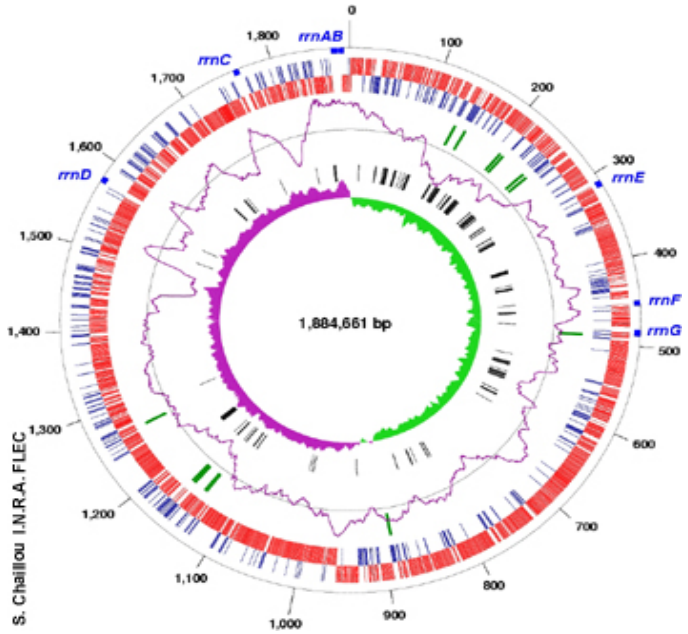
- ***Archaea* (archei);**
- ***Bacteria* (batteri).**

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