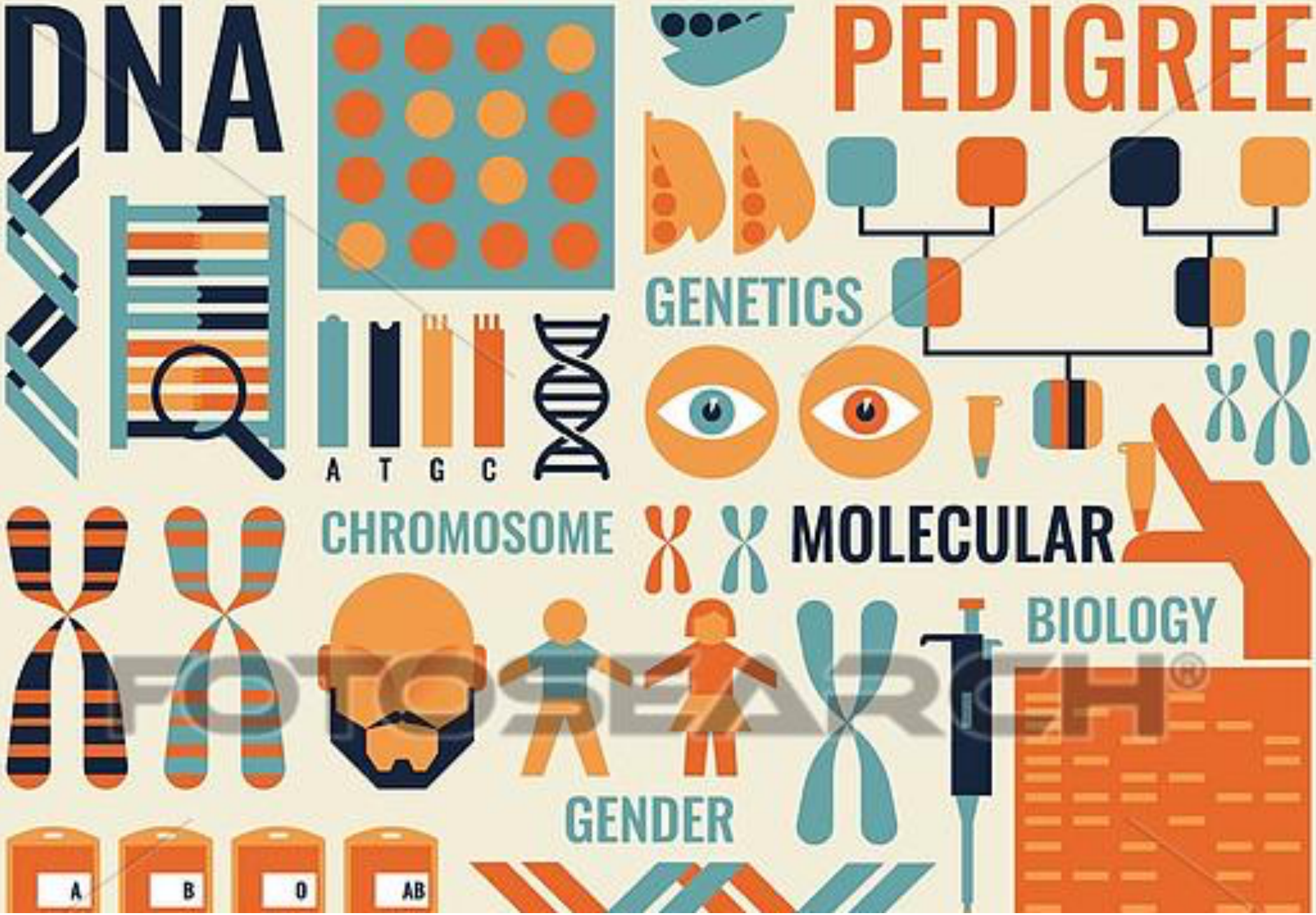

DNA
PEDIGREE
GENETICS
A T G C
CHROMOSOME
MOLECULAR
BIOLOGY
GENDER
A
B
O
AB

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 restriction 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 population.

Organization Course: Laboratorio Biologia Molecolare

Lecture: 24 hours theroretical lections: Prof. Schoeftner (Technologies)
Tuesday/Friday 06.10.2020 – 15.01.2020

Labortory exercises: Prof.ssa Bandiera
Turno I – VII (VIII)
+ 1 experienced supervisor
+ 2 tutors
13 stundents per turno in lab; social distancing, mascerina (91-104 students)

9											
	lunedì 30 novembre		martedì 1 dicembre		mercoledì 2 dicembre		giovedì 3 dicembre		venerdì 4 dicembre	
08.00-09.00			Lab biol mol (Bandiera/Schoeftner) TEORIA STB3	A edif.A	Ecologia (Renzi) STB3	A edif.A	Ecologia (Renzi) STB3	A edif.A	Lab biol mol (Bandiera/Schoeftner) TEORIA STB3	A edif.A
09.00-10.00	Lingua Inglese (liv.B2) STB3/GF1	online su MS Teams	(Sciancalepore/Cingolani) STB3	A edif.A	Ecologia (Renzi) STB3	A edif.A	Fisiologia (Sciancalepore/Cingolani) STB3	A edif.A	Microbiologia (Malfatti) STB3	A edif.A
10.00-11.00	Lingua Inglese (liv.B2) STB3/GF1	online su MS Teams	Fisiologia (Sciancalepore/Cingolani) STB3	A edif.A	Ecologia (Renzi) STB3	A edif.A	Fisiologia (Sciancalepore/Cingolani) STB3	A edif.A	Microbiologia (Malfatti) STB3	A edif.A
11.00-12.00	Lingua Inglese (liv.B2) STB3/GF1	online su MS Teams	Farmacologia (Pacor) STB3	A edif.A	Farmacologia (Pacor) STB3	A edif.A	Microbiologia (Malfatti) STB3	A edif.A	Fisiologia (Sciancalepore/Cingolani) STB3	A edif.A
12.00-13.00	Lecture MS Teams		Farmacologia (Pacor) STB3	A edif.A	Farmacologia (Pacor) STB3	A edif.A	Microbiologia (Malfatti) STB3	A edif.A	Fisiologia (Sciancalepore/Cingolani) STB3	A edif.A
13.00-14.00										
14.00-15.00	*Microbiologia, Igiene e sicurezza alimentare STB3*	A edif.A	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1
15.00-16.00	*Microbiologia, Igiene e sicurezza alimentare STB3*	A edif.A	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1
16.00-17.00	*Alimenti, nutrienti e salute STB3*	A edif.A	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1
17.00-18.00	*Alimenti, nutrienti e salute STB3*	A edif.A	Lab biol mol (Bandiera/Schoeftner) STB3 /Fisiologia vegetale (Nardini)STAN3/STB3	LAB. studenti edif.C-1/ A edif.A	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 /Fisiologia vegetale (Nardini)STAN3/STB3	LAB. studenti edif.C-1/ A edif.A	Lab biol mol (Bandiera/Schoeftner) STB3	LAB. studenti edif.C-1
18.00-19.00	*Alimenti, nutrienti e salute STB3*	A edif.A	Fisiologia vegetale (Nardini) STAN3/STB3	A edif.A			Fisiologia vegetale (Nardini) STAN3/STB3	A edif.A		

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: Plasmids will be subjected to control digest and fragments will be analyzed by gel-electrophoresis

3. PREPARATION OF GENOMIC DNA; Anonymized preparation of genomic DNA from cheek cells of students and determination of concentration.

4. 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.

5. DATA ANALYSIS AND DISCUSSION:
   PCR results will be analyzed; discussion on improving PCR; Chi-square analysis will be used to to compare the Alu genotype frequencies within the class population. 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: 7

Inscription in turni via Moodle federato this week!

Exercise	MS Teams Lecture Turno 1-7	turno 1	turno 2	turno 3	turno 4	turno 5	turno 6	turno 7
Genomic DNA prep.	19 ott; 12:00-13:00	mer 21 ott	gio 22 ott	ven 23 ott	mar 27 ott	mer 28 ott	gio 29 ott	ven 30 ott
Restriction digest and Gel-electro	26 ott; 12:00-13:00	mer 4 nov	gio 5 nov	ven 6 nov	mar 10 nov	mer 11 nov	gio 12 nov	ven 13 nov
PCR set-up	16 nov; 12:00-13:00	mer 18 nov	gio 19 nov	ven 20 nov	mar 24 nov	mer 25 nov	gio 26 nov	ven 27 nov
PCR analysis	30 nov; 12:00-13:00	mer 9 dic	gio 10 dic	ven 11 dic	mar 1 dic	mer 2 dic	gio 3 dic	ven 4 dic

CORSO "LABORATORIO DI BIOLOGIA MOLECOLARE" a.a. 2020-2021

PROGRAMMA PARTE PRATICA

1- ESTRAZIONE DEL DNA

Estrazione DNA plasmidico mediante kit commerciale. Estrazione individuale DNA genomico mediante kit commerciale e valutazione della resa. Quantificazione mediante spettrofotometria UV

2- L' ELETTROFORESI SU GEL DI AGAROSIO

L'analisi elettroforetica dei campioni di DNA preparati in precedenza. Preparazione del gel, dei campioni e corsa elettroforetica. Rilevamento e analisi dei risultati.
Allestimento di reazioni di restrizione sui DNA plasmidici per distinguere la sequenza mutata clonata.

3- GLI ENZIMI DI RESTRIZIONE

Corsa elettroforetica dei campioni derivanti dalla digestione con gli enzimi di restrizione e riconoscimento del plasmide contenente la sequenza mutata.
Allestimento della reazione di PCR dal DNA genomico estratto in precedenza

4- I PRINCIPI DELLA PCR

Principio della PCR. Analisi elettroforetica dell'amplificazione mediante PCR di una regione del DNA genomico umano adatta a evidenziare polimorfismo.

Viene richiesta una relazione scritta relativa al lavoro svolto in laboratorio che DEVE essere consegnata alla fine di ogni esercitazione
CIASCUNA RELAZIONE SCRITTA VERRA' VALUTATA E CONCORRERA' A DETERMINARE IL VOTO RELATIVO A QUESTA PARTE DEL CORSO

---

		lezione	Pratica (per ogni turno)
1 ES	Estrazione DNA genomico e plasmidico con kit – analisi UV del DNA estratto	1	4
2 ES	Elettroforesi su gel di agarosio dei campioni di DNA. Allestimento di di reazioni di restrizione sul DNA plasmidico	1	4
3 ES	Analisi di restrizione per riconoscere una mutazione in una sequenza	1	4
4 ES	Analisi mediante PCR di polimorfismi presenti nel genoma umano	1	4
	TOTALE	**4**	**16**

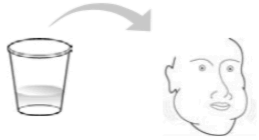
1. Preparation of Genomic DNA

Quick Guide

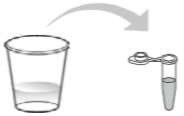
Lesson 1 Cheek Cell DNA Template Preparation

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.

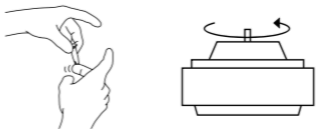
2. Obtain a cup containing saline solution from your instructor. Pour the saline into your mouth and rinse vigorously for 30 seconds. Expel the saline back into the cup.



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.



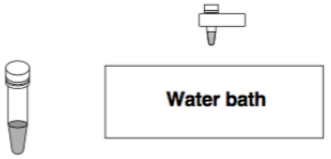
5. After pelleting your cells, pour off the saline. Being careful not to lose your pellet, blot your tube briefly on a paper towel or tissue. It's OK for a small amount of saline (< 50 µl, about the same size as your pellet) to remain in the bottom of the tube.

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

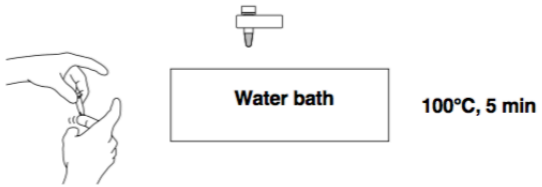
7. Using a 2–20 µl adjustable-volume micropipet set to 20 µl, transfer all of your resuspended cells to the screwcap tube containing InstaGene.

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

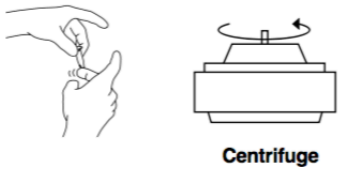
9. When all members of your team have collected their samples, place the tubes in the foam micro test-tube holder, and incubate at 56°C for 10 minutes in a water bath. At the halfway point (5 minutes), shake or vortex the tubes gently, then place back in the 56°C water bath for the remaining 5 minutes.



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.



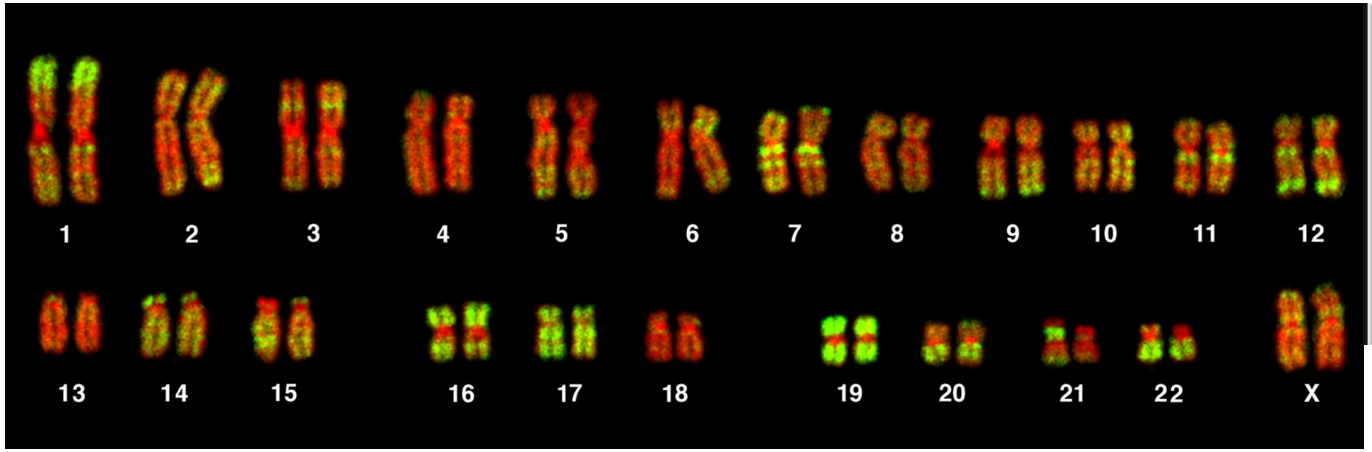
11. Remove the tubes from the boiling water bath and shake or vortex the contents to resuspend. Pellet the matrix by spinning at 6,000 x g for 5 minutes (or 2,000 x g for 10 minutes) in a centrifuge.



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



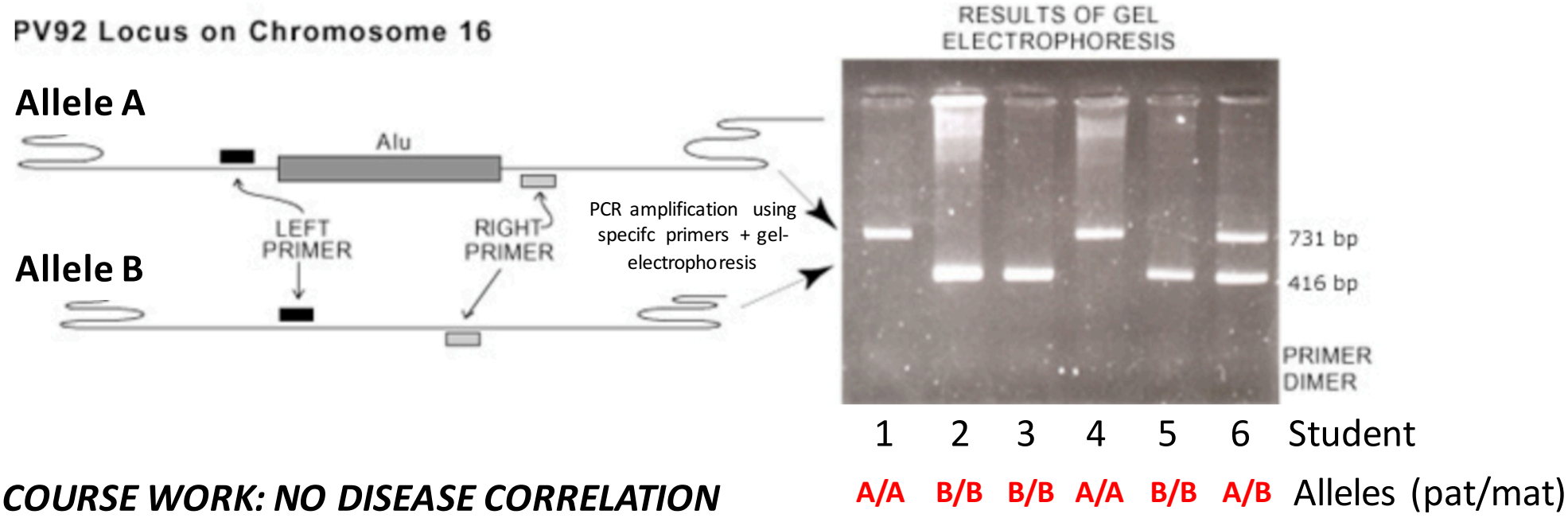
Element	Percent of total genome	Copy number
L1 (LINE)	16.9	0.5×10^6
Alu (SINE)	10.6	1.1×10^6
L2 (LINE)	3.2	0.3×10^6
MIR (SINE)	2.5	0.46×10^6
LTR elements	8.3	0.3×10^6
DNA elements	2.8	0.3×10^6
Processed pseudogenes	<1.0	$1–2 \times 10^4$
Total	~45	$\sim 3 \times 10^6$

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.

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

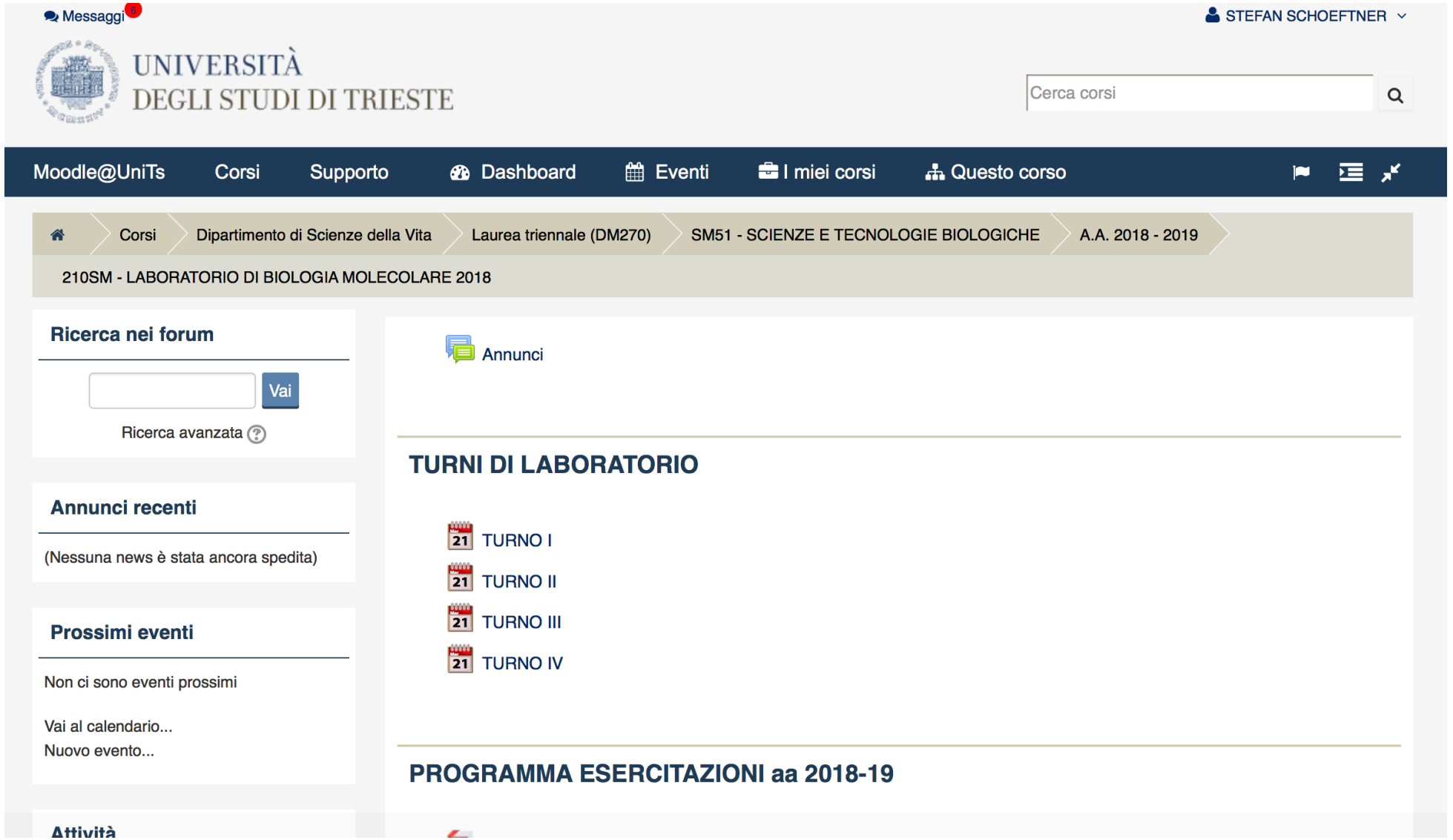
Alu repeats: Throughout evolution, intron sequences have been the target of random insertions by short repetitive interspersed elements, also known as SINEs.7 SINEs have become randomly inserted within our introns over millions of years. One such repetitive element is called the Alu sequence7 (Figure 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.



COURSE WORK: NO DISEASE CORRELATION

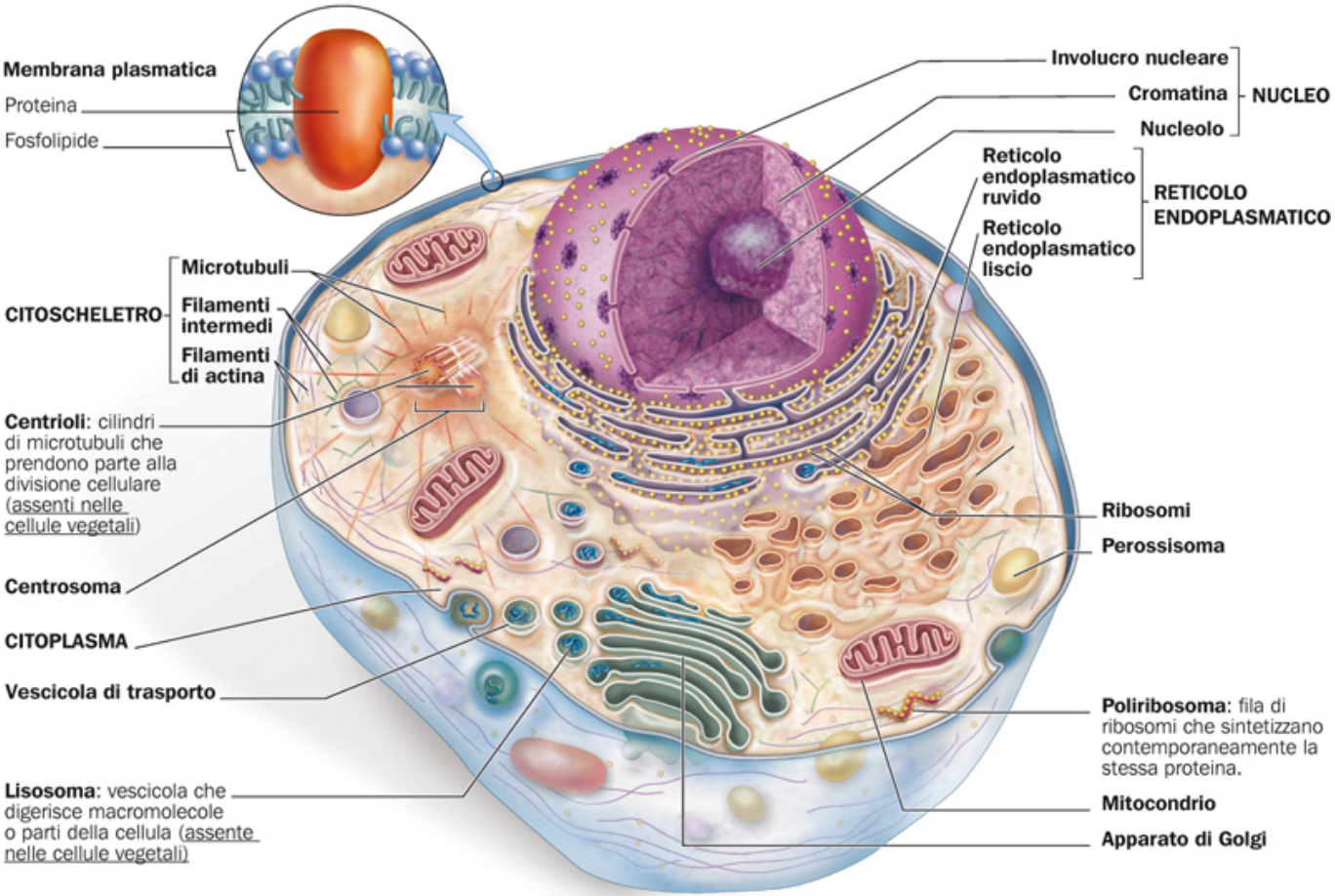
Students will perform a bioinformatics exercise to investigate the genotypic frequencies for the Alu polymorphism in their class population and compare them with the genotypic frequencies of other populations.

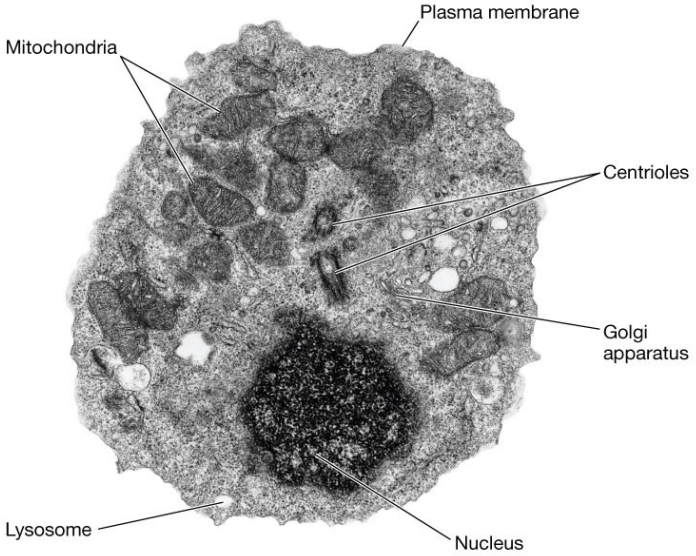
Materiale Didattica



1. RECOMBINANT DNA TECHNIQUES

La cellula eucariote



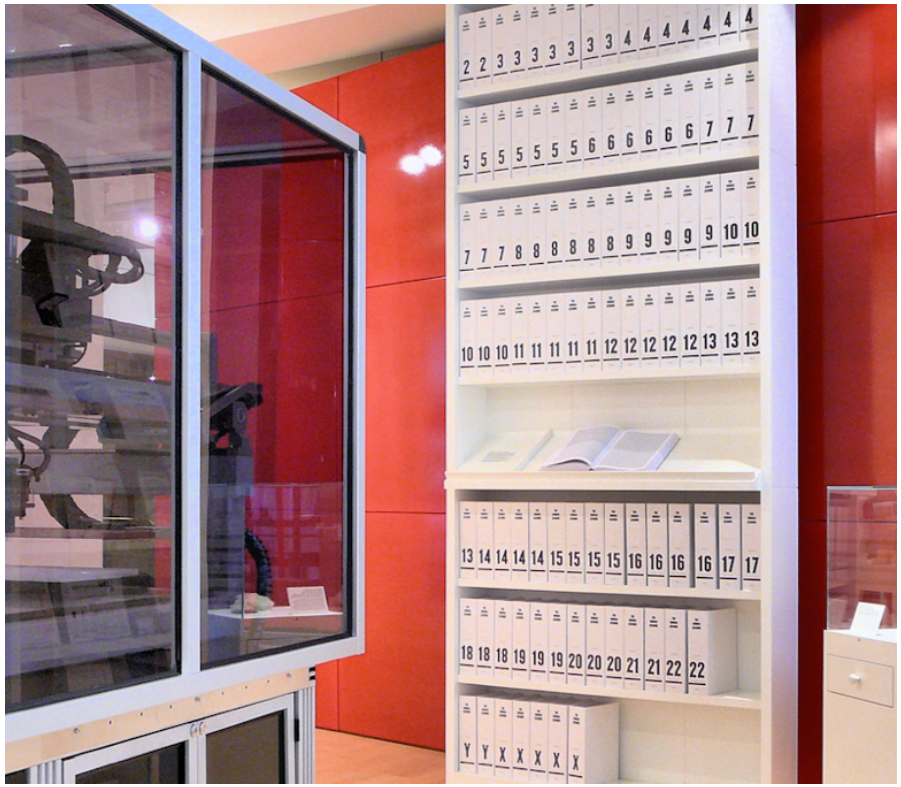


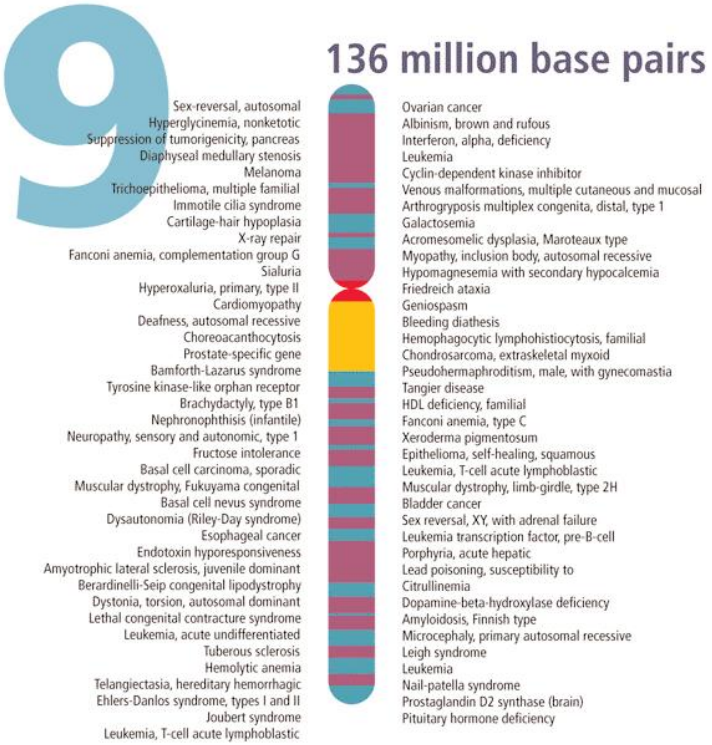
10-100µm

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

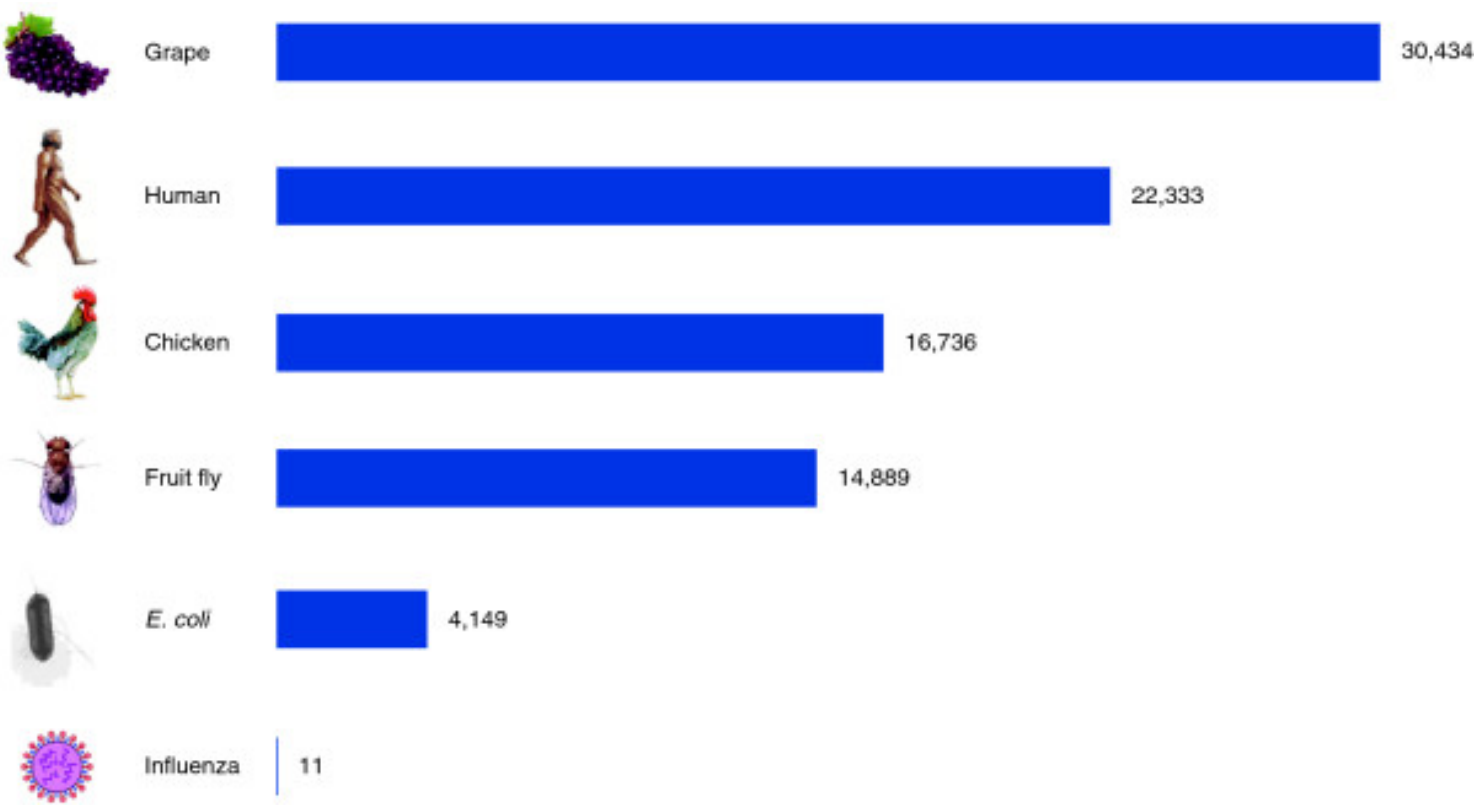
Dimensione dei cromosomi: 45-275 Mb;
→ 2.9 x 109 bp: eucromatina = attivo
→ Genoma noto: >90% dell'eucromatina.

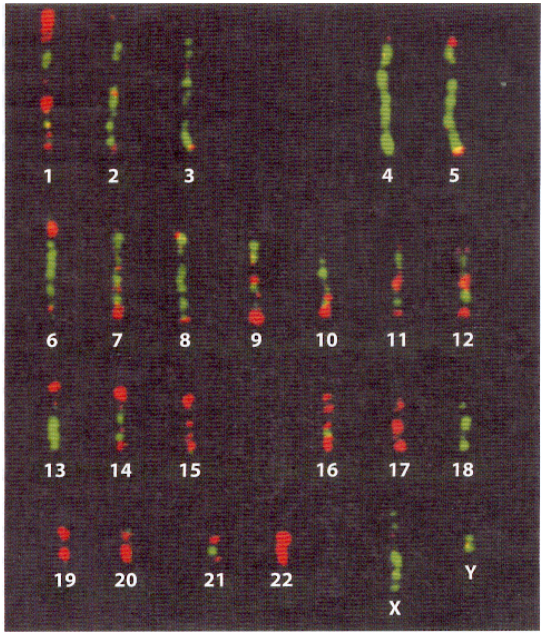
L'utillizo della infromazione genetica:

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





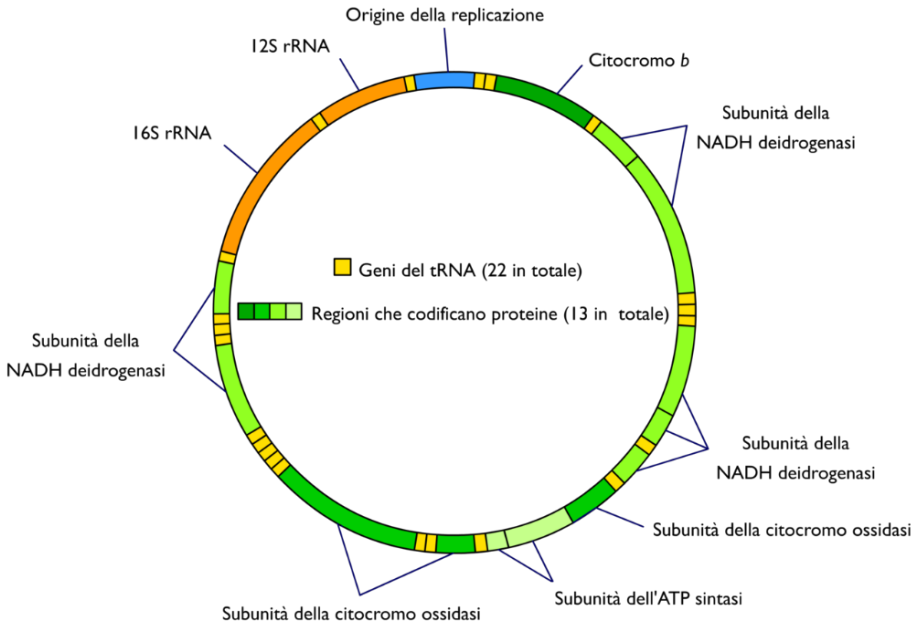
Mappa genica umana

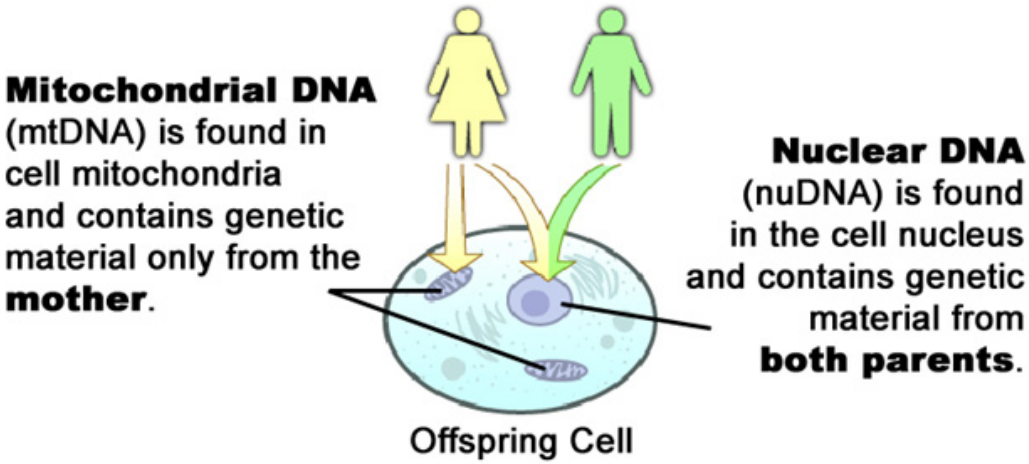
Le regioni in rosso indicano porzioni dei cromosomi ad alta densità genica (ad esempio i cromosomi 15, 16, 17, 19, 20 e 22).

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.



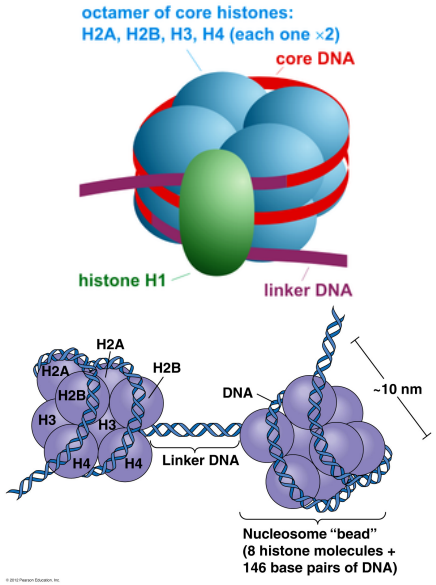


LA CROMATINA

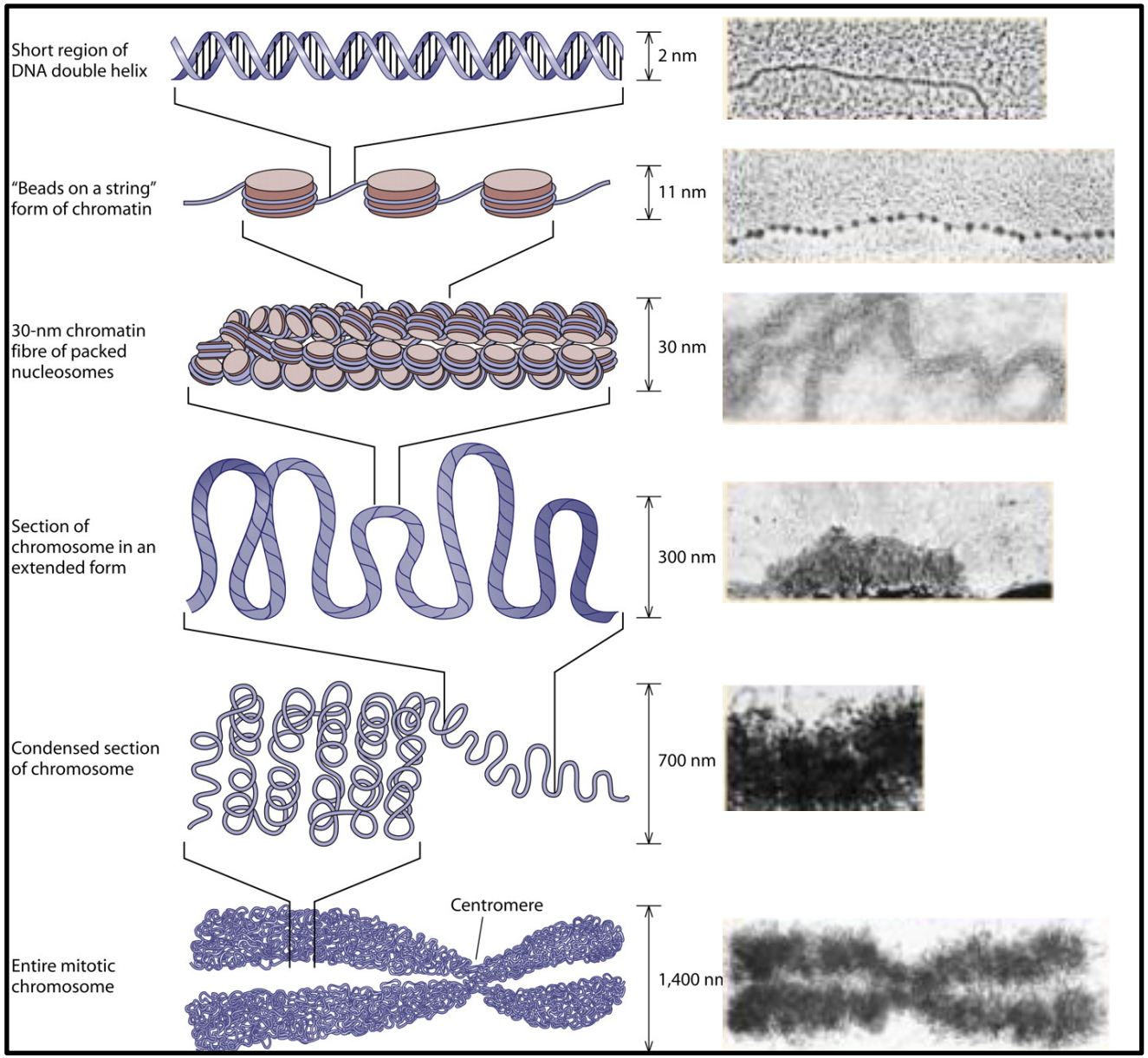
La **cromatina** è la forma in cui gli acidi nucleici si trovano nella cellula.

Funzione:

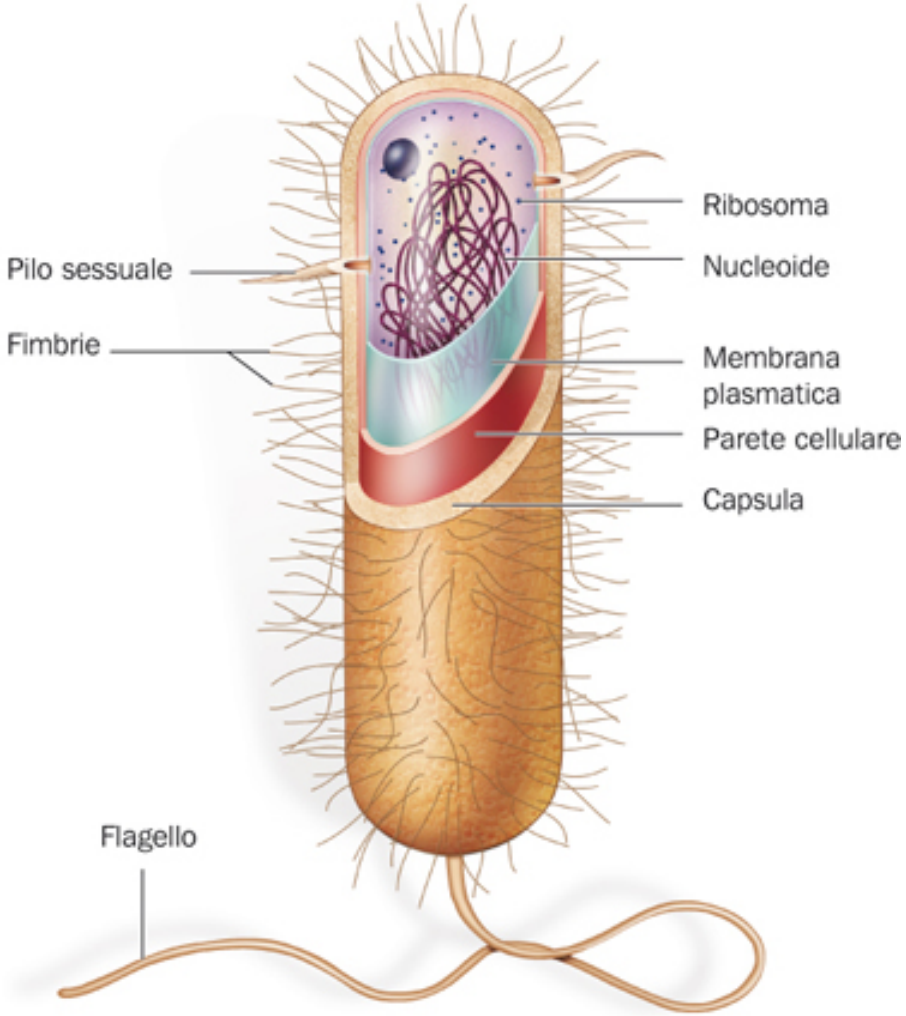
- impacchettamento del DNA
-rafforzare il DNA per permettere la mitosi
- prevenire danni al DNA
-controllare la replicazione del DNA e l'espressione (attivita) del gene



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.

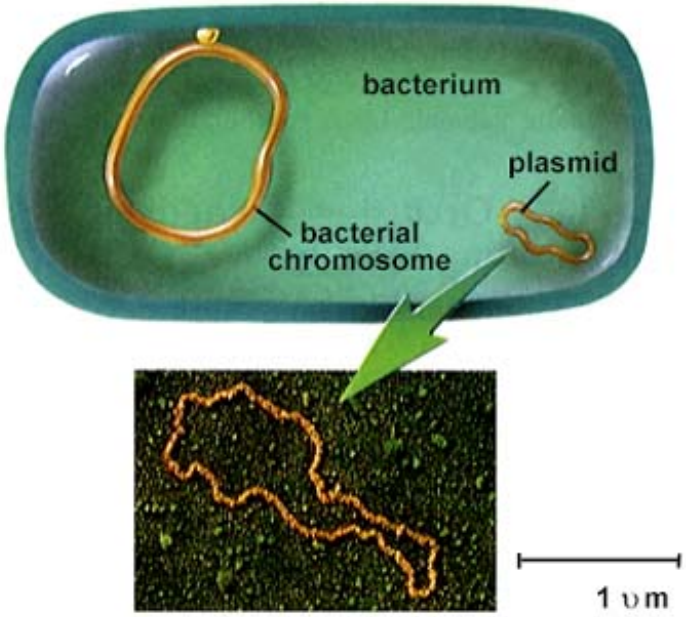
Gli organismi unicellulari costituiti da cellule procariotiche, i **procarioti**, sono classificati in due domini:

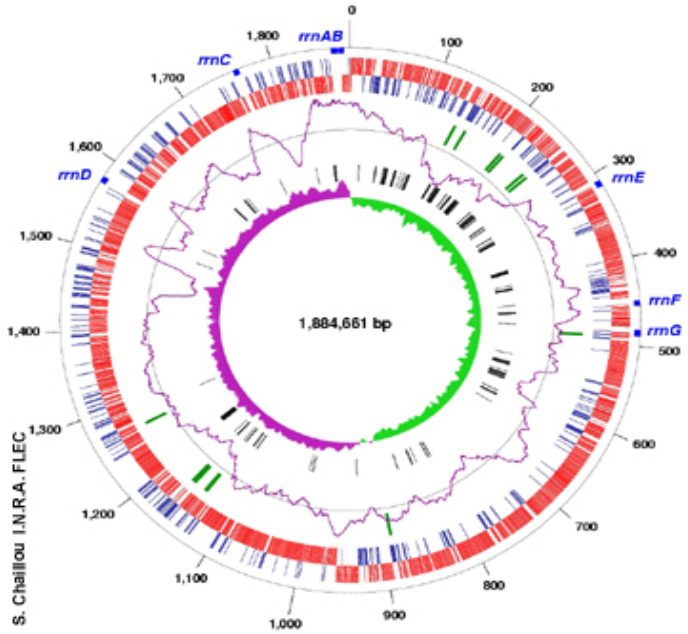
- ***Archaea* (archei);**
- ***Bacteria* (batteri).**

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