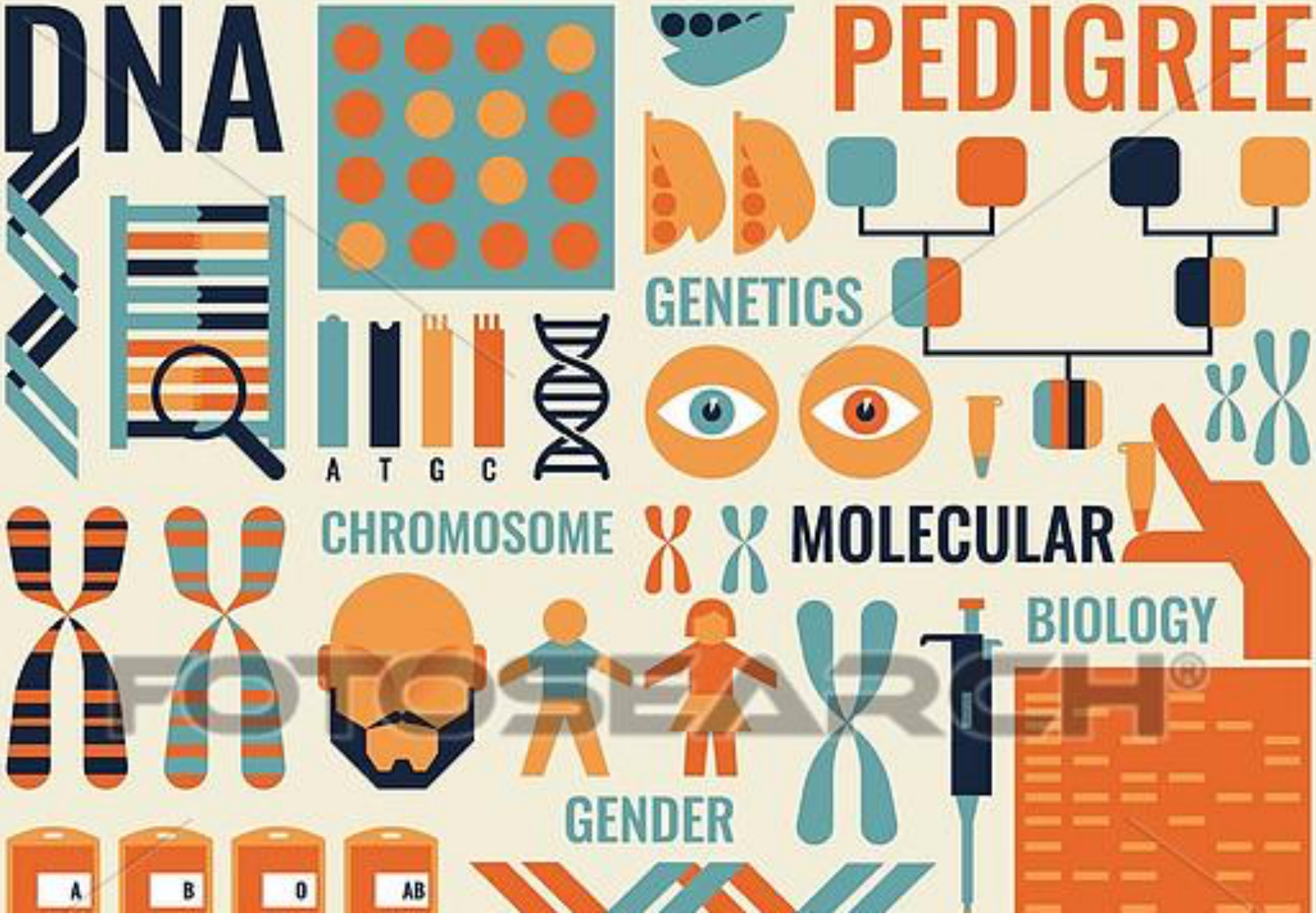

DNA
PEDIGREE
GENETICS
A T G C
CHROMOSOME
MOLECULAR
BIOLOGY
GENDER
A
B
O
AB

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

Theoretical Lections: Prof. Schoeftner (Technologies): 04.10.2021 – 20.01.2021
MONDAY: 08:15 – 10:15
THURSDAY: 08:15 – 09:15

Laboratory exercises: Prof.ssa Bandiera; Turno I – VI
+ 1 experienced supervisor
+ 2 tutors
16 students per turno in lab; social distancing, mascherina

7											
	lunedì 15 novembre		martedì 16 novembre		mercoledì 17 novembre		giovedì 18 novembre		venerdì 19 novembre	
08.15-09.15	Lab biol mol (Bandiera/Schoeftner) TEORIA STB3	aula B edif.A	Ecologia (Renzi) STB3	aula B edif.A	Microbiologia (Malfatti) STB3+STB2	aula magna edif.H3	Lab biol mol (Bandiera/Schoeftner) TEORIA STB3	aula B edif.A		
09.15-10.15	Lab biol mol (Bandiera/Schoeftner) TEORIA STB3	aula B edif.A	Ecologia (Renzi) STB3	aula B edif.A	Microbiologia (Malfatti) STB3+STB2	aula magna edif.H3	Farmacologia (Pacor) STB3	aula B edif.A		
10.15-11.15	Farmacologia (Pacor) STB3	aula B edif.A	Ecologia (Renzi) STB3	aula B edif.A	Fisiologia (Sciancalepore/Cingolani) STB3+STB2	aula magna edif.H3	Farmacologia (Pacor) STB3	aula B edif.A	Fisiologia vegetale (Nardini) STAN3/STB3	aula I edif. C1
11.15-12.15	Farmacologia (Pacor) STB3	aula B edif.A			Fisiologia (Sciancalepore/Cingolani) STB3+STB2	aula magna edif.H3	Comportamento e benessere animale (Chiandetti) STB3	aula B edif.A	Fisiologia vegetale (Nardini) STAN3/STB3	aula I edif. C1
12.15-13.15			Fisiologia vegetale (Nardini) STAN3/STB3	aula I edif. C1	Fisiologia (Sciancalepore/Cingolani) STB3+STB2	aula magna edif.H3			Fisiologia vegetale (Nardini) STAN3/STB3	aula I edif. C1
13.15-14.15	Comportamento e benessere animale (Chiandetti) STB3	aula1B edif.H3	Fisiologia vegetale (Nardini) STAN3/STB3	aula I edif. C1						
14.15-15.15	Comportamento e benessere animale (Chiandetti) STB3	aula1B edif.H3	Microbiologia (Malfatti) STB3+STB2	aula magna edif.H3	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 1	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 2	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 3	LAB. Biologia aula A edif.C-1
15.15-16.15	Alimenti, nutrienti e salute (Di Girolamo) STB3	aula1B edif.H3	Microbiologia (Malfatti) STB3+STB2	aula magna edif.H3	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 1	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 2	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 3	LAB. Biologia aula A edif.C-1
16.15-17.15	Alimenti, nutrienti e salute (Di Girolamo) STB3	aula1B edif.H3	Fisiologia (Sciancalepore/Cingolani) STB3+STB2	aula magna edif.H3	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 1	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 2	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 3	LAB. Biologia aula A edif.C-1
17.15-18.15	Alimenti, nutrienti e salute (Di Girolamo) STB3	aula1B edif.H3	Fisiologia (Sciancalepore/Cingolani) STB3+STB2	aula magna edif.H3	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 1	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 2	LAB. Biologia aula A edif.C-1	Lab biol mol (Bandiera/Schoeftner) STB3 TURNO 3	LAB. Biologia aula A edif.C-1
18.15-19.15	Ecologia (Renzi) STB3	aula1B edif.H3	Fisiologia (Sciancalepore/Cingolani) STB3+STB2	aula magna edif.H3						

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.

Prof.ssa Bandiera:

CONTENTS LABORATORY COURSE

Inscription in turni via Moodle federato – ASAP!!
…this week

Turni: 6: 16 Students / Turno = 96 Students

Background lectures (Prof.ssa Bandiera):

- **Monday 04.10.2021**
- **Monday 11.10.2021**
- **Monday 25.10.2021**
- **Monday 15.11.2021**
- **Monday 29.11.2021**

Turni Laboratorio (Prof.ssa Bandiera):

	Turno 1	Turno 2	Turno 3	Turno 4	Turno 5	Turno 6
Genomic DNA preparation	13-Oct	14-Oct	15-Oct	20-Oct	21-Oct	22-Oct
Restriction digest and gel electrophoresi	27-Oct	28-Oct	29-Oct	10-Nov	11-Nov	12-Nov
PCR set-up	17-Nov	18-Nov	19-Nov	24-Nov	25-Nov	26-Nov
PCR analysos	01-Dec	02-Dec	03-Dec	15-Dec	16-Dec	17-Dec

DNA polymorphism

GOAL: Application of molecular biology techniques for the diagnosis and **monitoring of specific DNA polymorphism in students of the course. Focus on Alu repeats**

Definition: Polymorphism involves one of two or more variants of a particular DNA sequence. The most common type of polymorphism involves variation at a single base pair. Polymorphisms can also be much larger in size and involve long stretches of DNA.

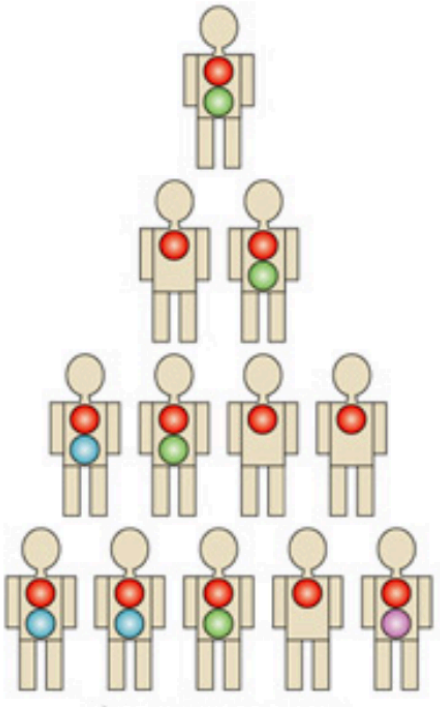


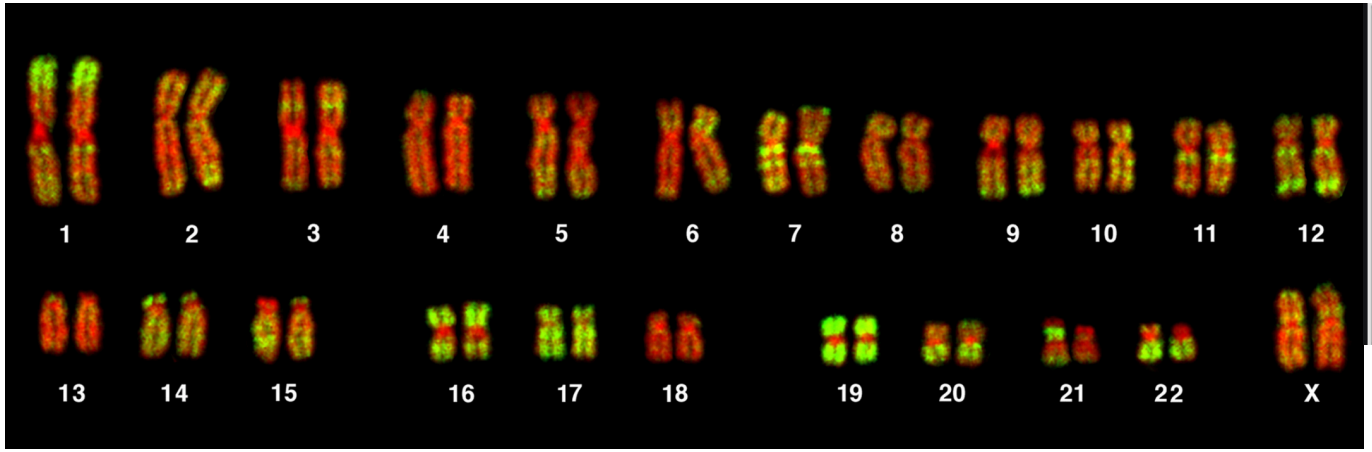
Table 2. Genetic Disease and Complex Variants.*

Disease	Variant or Variants and Location	Gene or Genes	Locus Structure Represented in Human Reference Genome†	Variant Detectable by Whole-Exome Sequencing	Variant Detectable by Whole-Genome Sequencing‡	Method of Discovery
X-linked dystonia–parkinsonism	SVA insertion, noncoding region[21]§	*TAF1*	Yes	No	No	Long-read transcript sequencing
Bipolar disorder and schizophrenia	VNTR composition, noncoding region[20]	*CANA1C*	No	No	No	Long-read sequencing
Schizophrenia	Complex structural variant of C4 genes, coding and noncoding regions[48]	*C4A, C4B*	Yes/No	No	Yes/No	Digital droplet PCR
Benign adult familial myoclonic epilepsy	TTTTA expansion, noncoding region[22]	*SAMD12*	No	No	No	Long-read sequencing
Baratela–Scott syndrome	CCG expansion, noncoding region[49]	*XYLT1*	No	No	Yes	Southern blot and Illumina sequencing
Fascioscapulohumeral muscular dystrophy	Macrosatellite D4Z4 contraction and permissive SNVs, coding and noncoding regions[26]	*FSHD1*	Yes/No	No	Yes/No	Southern blot
Amyotrophic lateral sclerosis–frontal temporal dementia	GGGGCC repeat expansion, noncoding region[50,51]	*c9ORF72*	No	No	Yes/No	Southern blot, FISH, and repeat-primed PCR

* FISH denotes fluorescence in situ hybridization, PCR polymerase chain reaction, SNV single-nucleotide variant, and VNTR variable-number tandem repeat.
† "Yes/No" indicates that the locus structure was incompletely represented in the human reference genome.
‡ "Yes/No" indicates that the variant could be partially detected (depending on the size of the allele — i.e., sequences of the larger alleles are not completely resolved).
§ SVA (SINE-VNTR-Alu) is a class of retrotransposon found in humans and great apes.

Determination of presence or absence of Alu insert within the PV92 locus in student DNA

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 fluorescence in situ hybridization probe for Alu elements (green). DNA is counterstained with TOPRO-3 (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. SINEs have become randomly inserted within our introns over millions of years. One such repetitive element is called the Alu sequence

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.

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.

Transposable elements in the genome

- 306 base pair segment of DNA, Classified as a **SINE** (**S**hort **In**terspersed Repetitive **E**lement)
- Named for the **Alu I** restriction site within the sequence (**AGCT**)
- Human-specific **Alu** insertion
- Approx. 1 million **Alu** copies per haploid genome = 11% of the genome: role in genetic architecture and genetic disorders
- Intron: Found in a non-coding region of your DNA

GGCCGGGCGCGGTGGCTCACGCCTGT
AATCCCAGCACTTTGGGAGGCCGAGG
CGGGCGGATCACGAGGTCAGGAGATC
GAGACCATCCCGGCTAAAACGCTGAAA
CCTCGTCTCTACTAAAAATACAAAAAAT
TAGCCGGGCGTAGTGGCGGGCGCCTG
TAGTCCC***AGCT***ACTTGGGAGGCTGAG
GCAGGAGAATGGCGTGAACCCGGGAG
GCGG***AGCT***TGCAGTGAGCCGAGATCC
TGCCACTGCACTCCAGCGTGGGCG
ACAGAGCGAGACTCCGTCTCAAAA
AAAAAAAAAAAAAAAAAAAA

306 base pairs long: This sequence remains the same, no matter where it is found in the genome

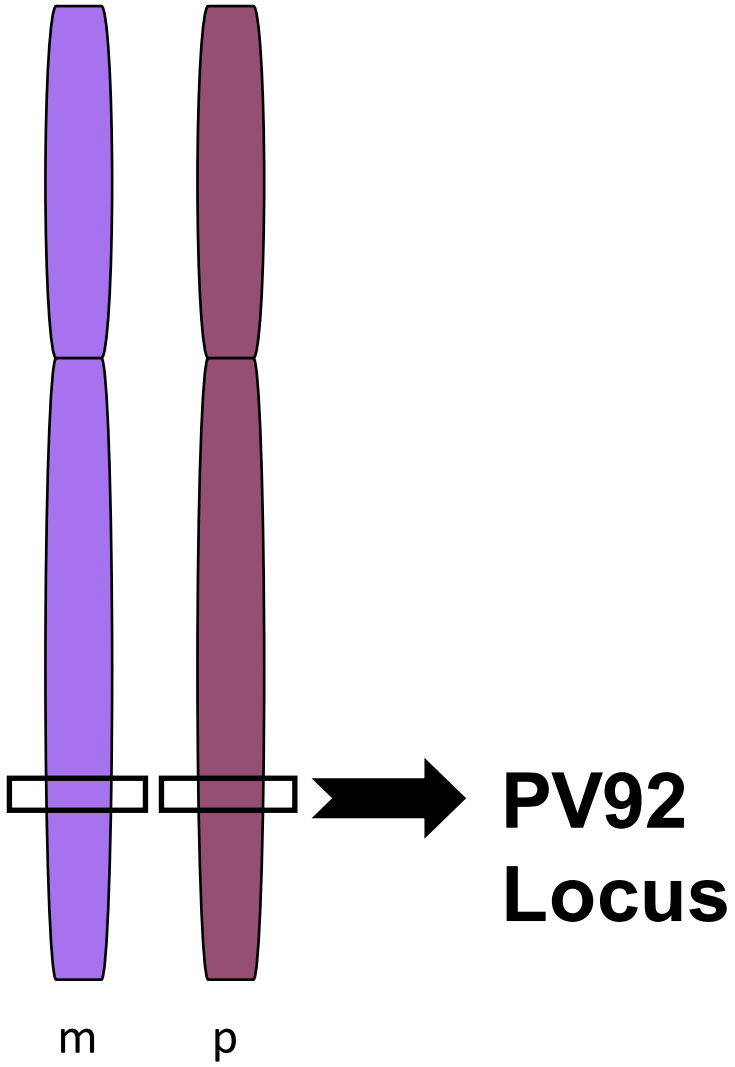
Gene	Position	Subfamily	Mechanism	Disease	Reference
ACE	Chr 17	*AluY*a5	Insertion	Alzheimer's disease	[39]
ALMS1	Chr 2	*AluY*a5	Insertion	Alström syndrome	[40]
BMPR2	Chr 2	*AluY* *AluS*	ARMD_NAHR NHEJ	Pulmonary arterial hypertension	[41]
CDSN	Chr 6	*AluS*	ARMD_NHEJ	Peeling skin disease	[42]
COL4A5	Chr X	*AluY*	Insertion	Alport syndrome	[43]
FA	Chr X	*AluY*	ARMD_NAHR	Fanconi anemia	[44]
GBA1	Chr 1	*AluS*x	ARMD_NAHR	Gaucher disease	[45]
GGA	Chr 17	*AluS*	ARMD_NAHR	Pomp disease	[46]
GLA	Chr X	*Alu*	Insertion mediated deletion	Fabry disease	[47]
MUTYH	Chr 1	*AluY*b8	Insertion	Breast cancer/gastric cancer	[48]
PMP22	Chr 17	*AluY*/*AluS*c	ARMD_NAHR	Charcot-Marie-Tooth disease	[49]
SOX10	Chr 22	*AluS*	FoSTes/MMBIR	Waardenburg syndrome type 4	[50]
SPAST	Chr 2 Chr 2	*AluY*/*AluS* *AluY*	FoSTes/MMBIR	Hereditary spastic paraplegia	[51]
SPG11	Chr 15 Chr 15	*AluY*/*AluS* *AluS*	ARMD_NAHR	Spastic paraplegias	[52]
STK11	Chr 19	*AluY*	ARMD_NAHR	Peutz-Jeghers syndrome	[53]

ARMD, *Alu* recombination-mediated deletions; NAHR, nonallelic homologous recombination; NHEJ, nonhomologous end-joining mediated deletion; FoSTeS/MMBIR, fork stalling and template switching/microhomology-mediated break-induced replication.

Alu polymorphisms can be linked to disease

Detection of Alu polymorphisms in student population AA2021-2022

Chromosome 16
Homologous Chromosomes



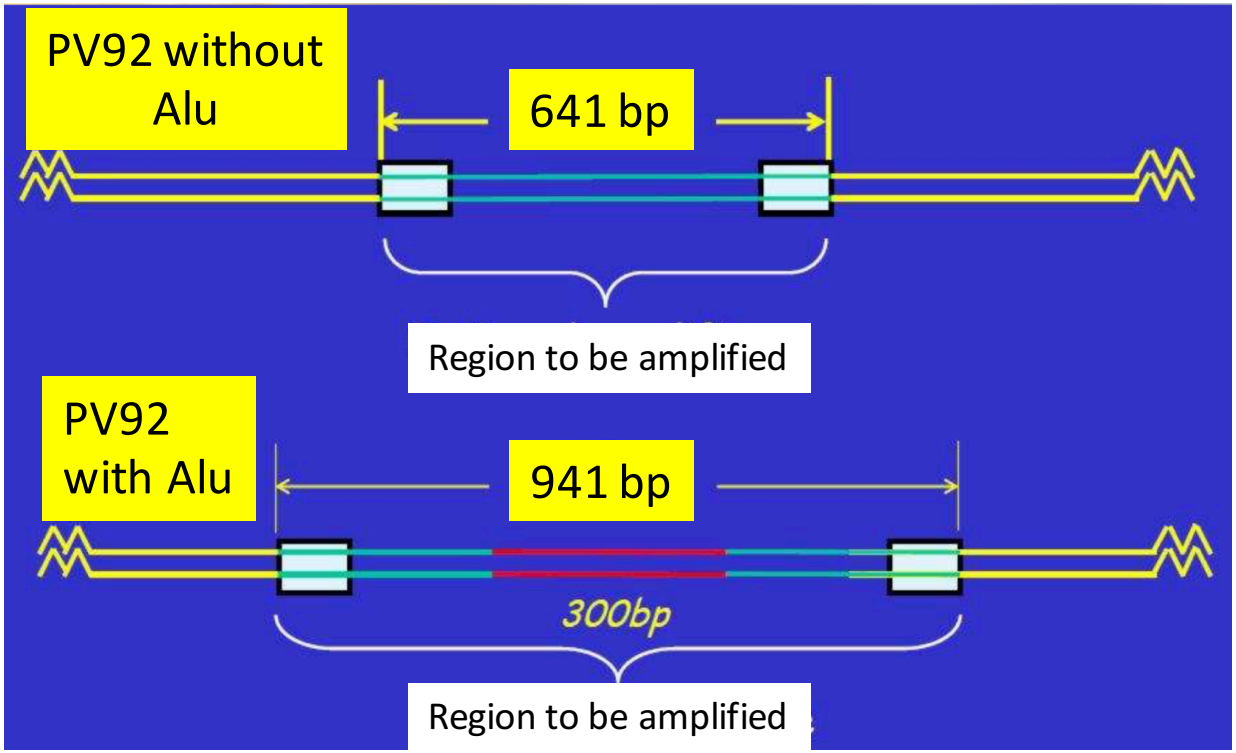
- Each gene locus has a particular form of the gene, or **allele**
- What are the possible alleles for the Alu insert at each locus?
  - +, Alu present
  - -, Alu not present
- What are the possible genotypes for the Alu insert for any given person?
  - Homozygous positive: +/+
  - Homozygous negative: -/-
  - Heterozygous: +/-

- Alu sequence insertion in PV92 locus is not diagnostic for any disease or disorder!
- Useful for forensic tests

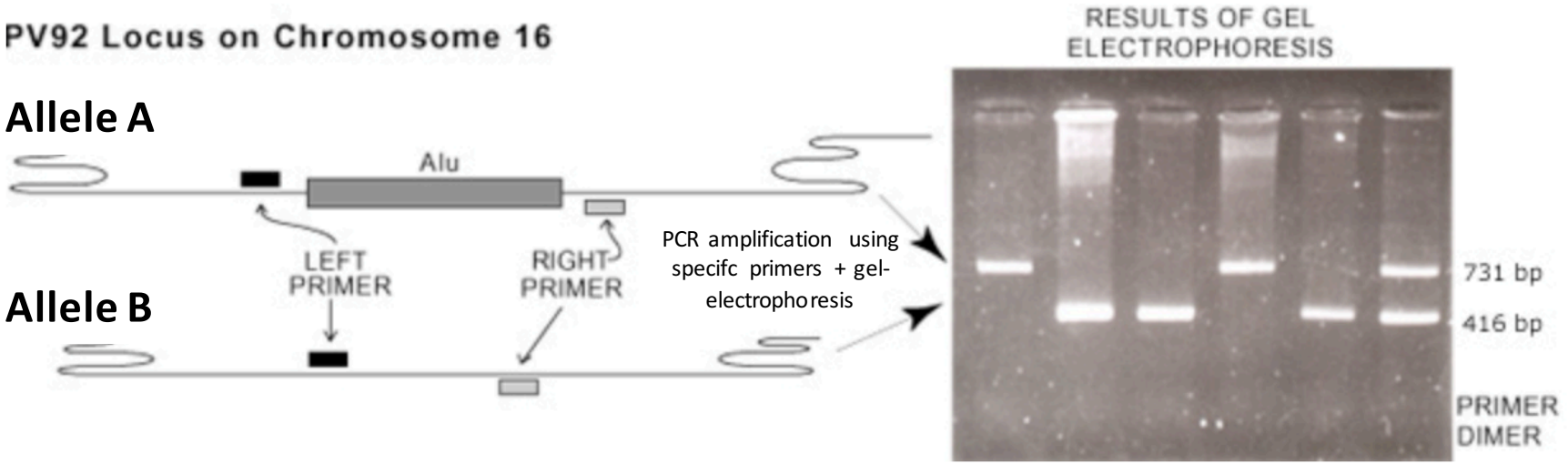
Transposable elements in the genome

Options:

+/+ = 941 / 941 bp
+/- = 941 / 641 bp
-/- = 941 / 641 bp



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



COURSE WORK: NO DISEASE CORRELATION

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

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".

Guidelines MS Teams AA2021-2022

POLICY OF THE UNIVERSITY OF TRIESTE:

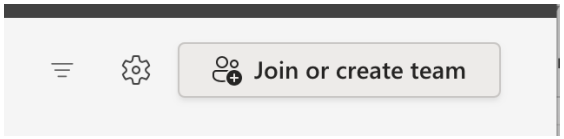
- **ALL LECTURES "IN PRESENZA", recording of lectures provided on MS Teams**
- **ONLY IN EXCEPTIONAL CASES, students can opt for the didattica "IN PARALLELA"**

Guidelines MS Teams AA2021-2022

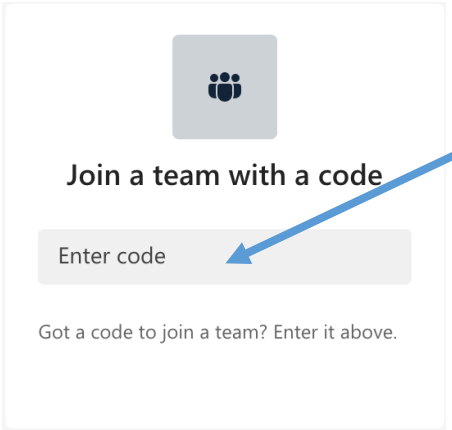
For ALL students (in presenza e didattica in parallela):

Use the code to partecipate to the MSTeam "Laboratorio di Biologia Molecolare":

1.



2.



clj2rn1

(code provided in Moodle and saved lecture ppt)

This code enables you to become a participant of our Teams (only using your UniTS account without requesting the OK from owners of the Team

Once member of the Team you get access to the video recording of the lectures and eventual other supporting material. You CANNOT join the lecture in direct streaming

Note: videos will be automatically cancelled 180 days after the end of the lecture

Note: the code DOES NOT give access to the lectures in direct streaming!!

Guidelines MS Teams AA2021-2022

For SELECTED students that have the OK to follow the lectures in direct streaming

NORMATIVA DSV:
Gli studenti di CdS del DSV che hanno la facoltà chiedere la modalità di frequenza (anche temporanea) tramite didattica parallela sono:

- **gli studenti affetti da particolari disabilità,**

- **studenti genitori,**

- **gli studenti detenuti** e

- **gli studenti che per ragioni sanitarie legate all'emergenza covid19** sono temporaneamente esclusi dall'accesso alle lezioni in presenza.

IMPORTANTE:
Gli studenti che si riconoscono in queste categorie per accedere alla didattica parallela **devono** presentare la richiesta alla **Segreteria didattica che, valutata l'ammissibilità delle domande**, la inoltrerà al coordinatore del CdS (Prof. Martellos) interessato ed ai docenti degli insegnamenti (Prof. Bandiera, Schoeftner) di cui si chiede la didattica parallela.

HOW TO GET ACCESS:
When lecture channel has been opened (NOT EARLIER), ask permission to join the lecture channel. Lecturer will allow to join the session.

THIS IS AN EXCEPTION: THE POLICY OF UNI TS IS TO OFFER ALL LECTURES "IN PRESENZA"

Guidelines MOODLE AA2021-2022

Supporting material for the lectures and laboratory activtity will be provided on Moodle

- **INSCRIPTION TO LABORATORY "TURNI"**
- **PROTOCOLS**
- **LECTURE SLIDES**
- **OTHER SUPPORTING MATERIAL**
- **RESULTS OF EXAMS**
- **NO VIDEO RECORDINGS (they are stored for 180 days on MS Teams)**
- **NOTE: Material on Moodle will be stored for at least 2 years, accessible to students inscribed into the Lecture**

Materiale Didattica