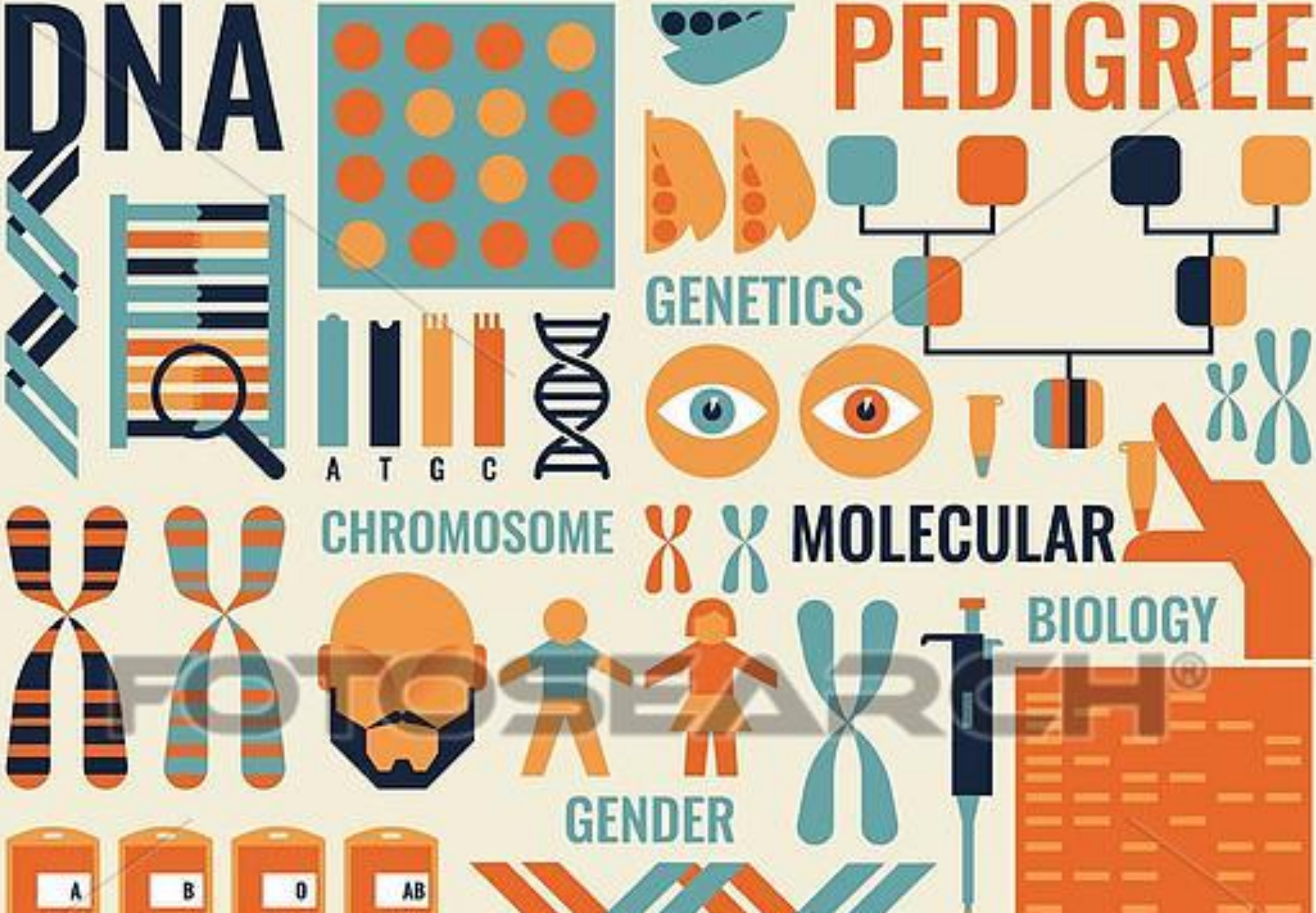

DNA
PEDIGREE
GENETICS
A T G C
CHROMOSOME
MOLECULAR
BIOLOGY
GENDER
A
B
O
AB

Insegnamento: Laboratorio Biologia Molecolare

Docenti:



Prof. SCHOEFTNER STEFAN,
DSV - Laboratory for non-coding RNA and genome stability
Responsaible del corso
Lezioni: 16 ore (2CFU)

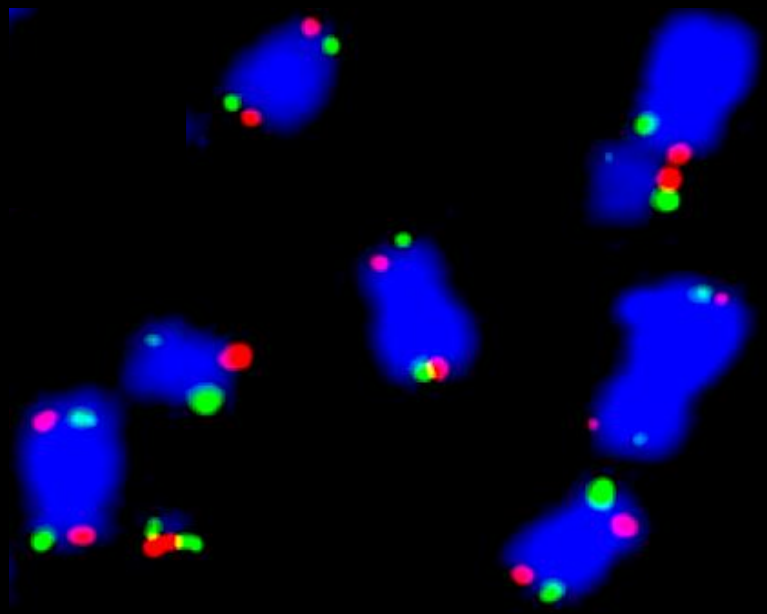


Prof.ssa BANDIERA ANTONELLA
DSV - Laboratory for Protein Engineering – Bioactive Biomimetic Polypeptides
Laboratorio: 72 ore (2 CFU)



Prof.ssa SCAGGIANTE BRUNA
DSV - Laboratory for Liquid Biopsies
Laboratorio: 72 ore (2 CFU)

ncRNA and Genome Instability Laboratory



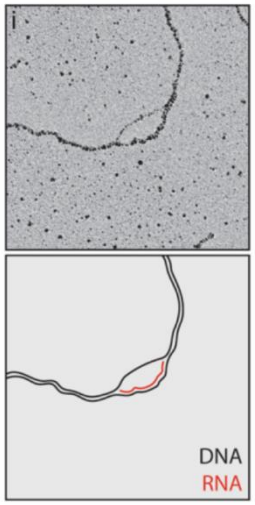
Simone Giaquinto, PhD Student
Andrea Parlante, PhD Student
Francesco Circicioli, PhD Student
Gabriele Cechutti, postgraduate fellow

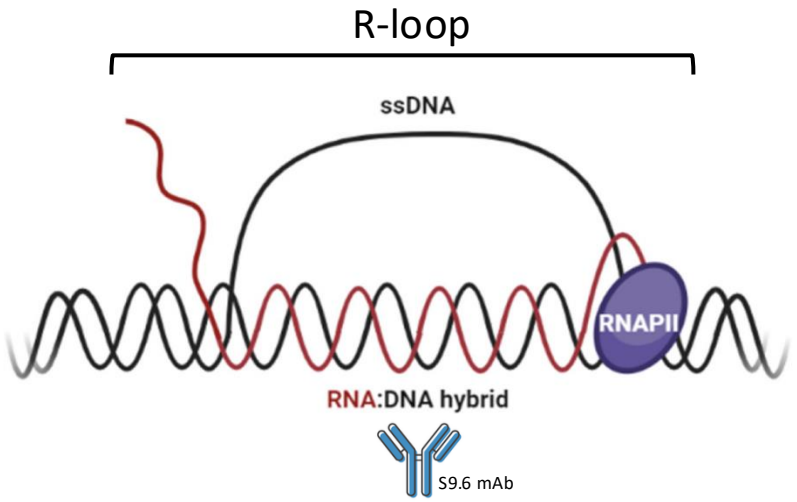


Ed C11
Second floor
Lab 244-247

My Lab: Laboratory for non-coding RNA and genome stability

R-loops are atypical nucleic structures





- *<5% of genome*
- *in cis (transcription)*
- *in trans (recombinase)*
- *GC rich regions*
- *repeats*
- *G-quadruplex regions*
- *long genes (>800kb)*

Benifical role
»scheduled R-loops«

Immunoglobulin class-switch recombination

DNA replication

Activation/Termination of transcription

Chromatin structure

Genome instability
«unscheduled R-loops«

Mutagenesis/Recombination

Replication stress

DNA breaks

Cancer and Neurodegeneration

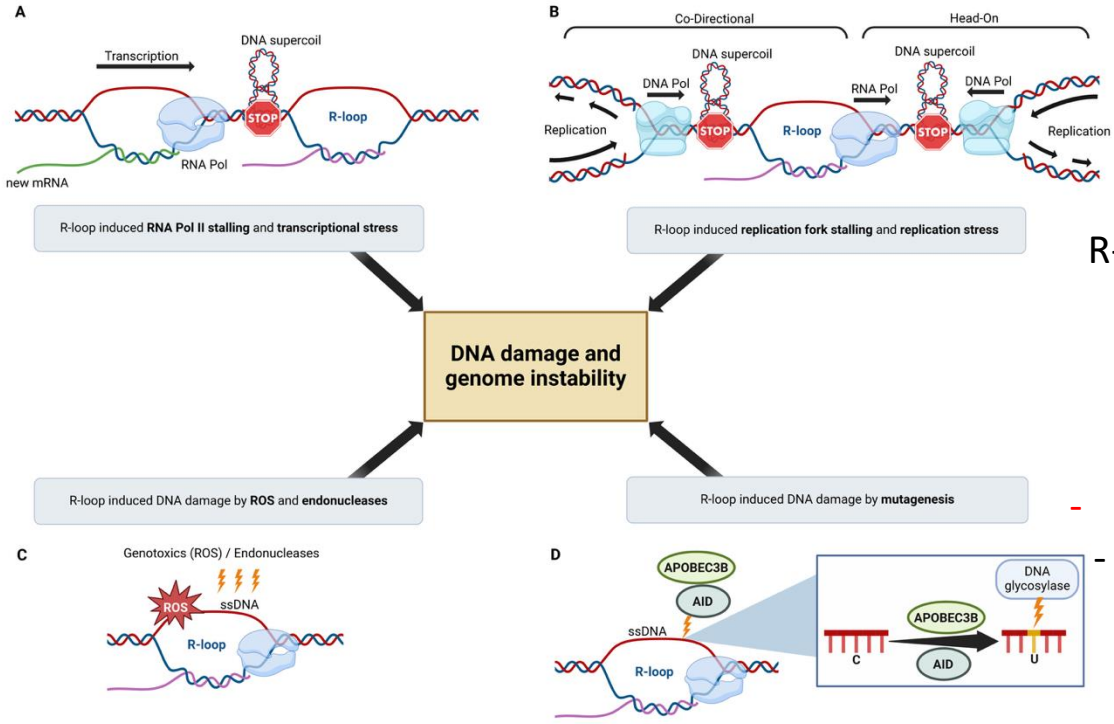
Antineoplastic therapies

Stoy et al. 2022; Methods in Molecular Biology
Modified from: Uruci et al. 2021; International Journal of Molecular Sciences

My Lab: Laboratory for non-coding RNA and genome stability

R-loop mediates replication stress and genome instability → modification of the TME

R-loops
↓
Replication stress
(**ATR**)
↓
DNA damage
↓
Mitotic defects, miconuclei,
cytplasmatic DNA
↓
Innate immunity
↓
TME modification



R-loops suppressors
- ATR
- Helicases
- BRCA1, 2
- FA genes
- Histone chaperons
- toposiomerases
- …..

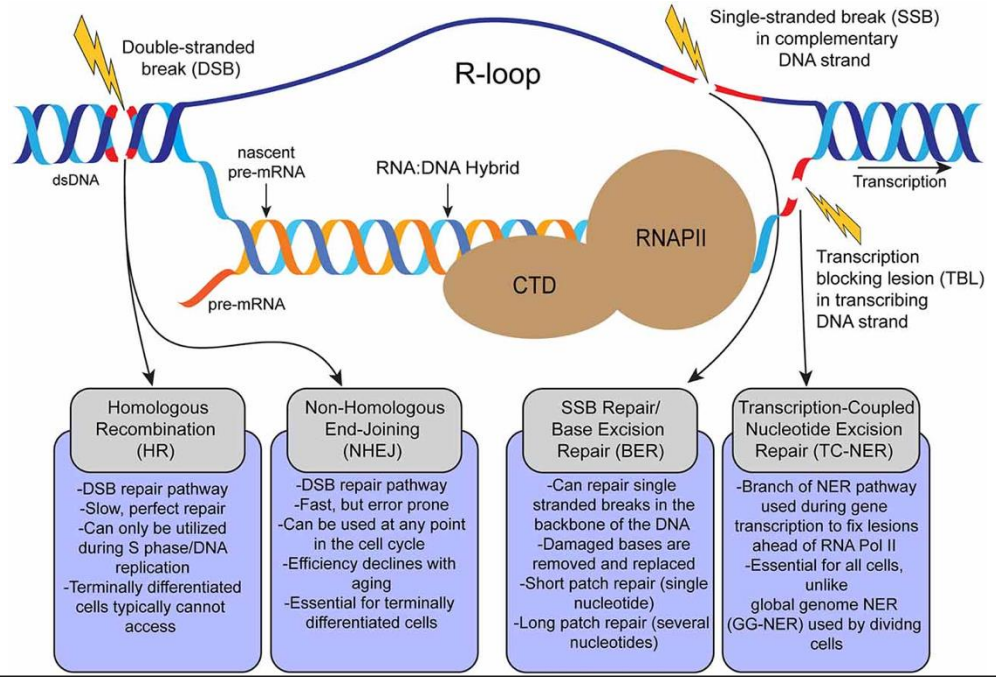
Goals: **Identify novel R-loop sensing and resolving machineries**
Identify cancer pathways that modulate R-loop resolution
Map R-loops and related epigentic alterations

Model systems:

Ferrando et al. NatComms 2026
Giaquinto et al. DNA repair 2025
Gambelli et al. Biochimie 2023
Petti et al. NatComms 2019

My Lab: Laboratory for non-coding RNA and genome stability

1. Control of genome stability by RNA:DNA hybrid management machineries



- **R-loops block DNA and RNA polymerases**
- **Mechanic stress on DNA – cleavage/break of DNA**
- **If DNA damage is not repaired → genome instability**
- **Many factors suppress the formation of R-loops**

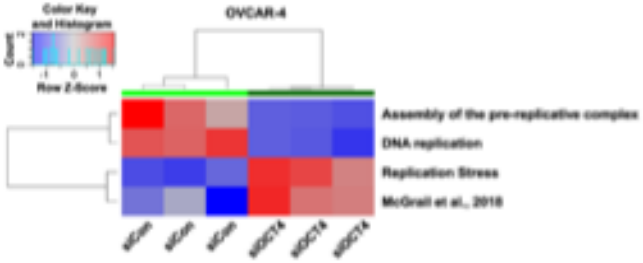
My Lab: Laboratory for non-coding RNA and genome stability

1. MECHANISMS OF R-LOOP RESOLUTION IN CANCER CELLS

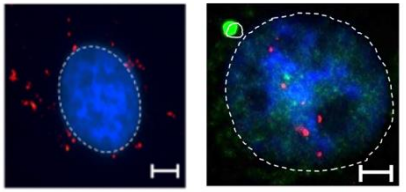
Multiple pathways protect from R-loop mediated genome instability

SFPQ R-loop binding protein	OCT4 stemness	EGFR signalling
↓	↓	↓
DAXX H3.3 chaperon/ATRX helicase	BRCAness related and FA genes	
↓	↓	↓
Chromatin remodelling R-loop resolution	R-loop suppression/resolution	R-loop suppression/resolution
Nat Comms 2026	**Cancer Research submitted**	**Work in progress**

Replication stress

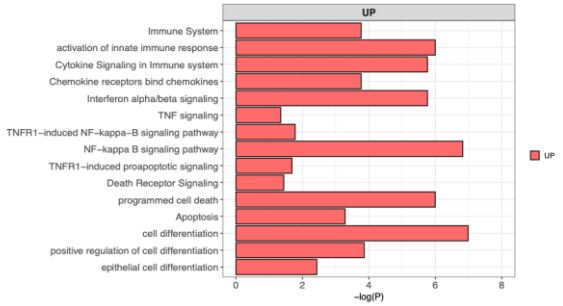


Cytoplasmatic DNA

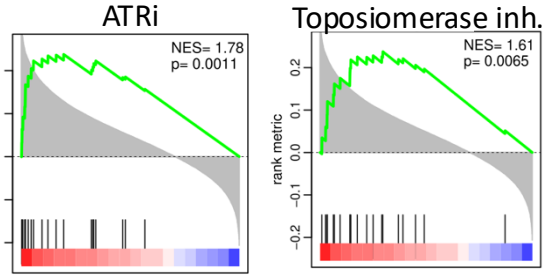


cGAS/ STING

Innate immunity



Drug vulnerability



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

Scienze e Tecnologie Biologiche; Classe L-13: Scienze Biologiche

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

Lecture:
Basic techniques for DNA manipulation, gene study, gene cloning, gene expression analysis and recombinant DNA technology will be addressed; oligo design.

Laboratory:
Laboratory exercises include the teaching of laboratory safety standards the handling of laboratory instruments.

- **PART 1:** the extraction of DNA from bacteria and human 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)

- **PART 2:** gene knock-down in cancer cell lines; use of quantitative RT-PCR for the validation of knock-down efficacy and activation of biological pathway (Interferon signalling); statistical analysis of data with preparation of paper figure

PROGRAM

LABORATORY COURSE:

Ed C1, Monday, Tuesday ,Wednesday 14:00 – 19:00; Start: 05.10.2026; End 16.12.2025; 8 exercises; 3 turni

Calendario esercizi di laboratorio (Prof.sse Bandiera e Scaggiante, 4 CFU)

	PART 1 - Prof.ssa Bandiera				PART 2 - Prof.ssa Scaggiante			
Esercizio	**# 1**	**# 2**	**# 3**	**# 4**	**# 5**	**# 6**	**# 7**	**#8**
Docente Laboratorio Ed C1	Bandiera	Bandiera	Bandiera	Bandiera	Scaggiante	Scaggiante	Scaggiante	Scaggiante
Tutor	Tutor 1	Tutor 1	Tutor 1+2	Tutor 1+2	Tutor 2+3	Tutor 2+3	Tutor 3	Tutor 3
GIORNO: Turno 1: 14:00 - 19:00; Lunedì	05-Oct	12-Oct	19-Oct	09-Nov	16-Nov	23-Nov	30-Nov	14-Dic
GIORNO Turno 2: 14:00 - 19:00; Martedì	06-Oct	13-Oct	20-Oct	10-Nov	17-Nov	24-Nov	01-Dic	15-Dic
GIORNO Turno 3: 14:00 - 19:00; Mercoledì	07-Oct	14-Oct	21-Oct	11-Nov	18-Nov	25-Nov	02-Dic	16-Dic
Lezioni introduttiva per esercitazioni 1 - 8 (1 ora); Ed. C1, Aula I; Lunedi 09:45 - 11:00	Prof.ssa Bandiera - Intro Es. 1	Prof.ssa Bandiera - Intro Es. 2	Prof.ssa Bandiera - Intro Es. 3	Prof.ssa Bandiera - Intro Es. 4	Prof.ssa Scaggiante - Intro Es. 5	Prof.ssa Scaggiante - Intro Es. 6	Prof.ssa Scaggiante - Intro Es. 7	Prof.ssa Scaggiante - Intro Es. 8
	02-Oct	09-Oct	16-Oct	06-Nov	13-Nov	20-Nov	27-Nov	11-Dic
	09:45 - 11:00	09:45 - 11:00	09:45 - 11:00	09:45 - 11:00	09:45 - 11:00	09:45 - 11:00	09:45 - 11:00	09:45 - 11:00

LECTURES Prof. SCHOEFTNER: Ed C1; Aula I; Venerdì 08:00 – 11:00 Start: 02.10.2026

Aula I, Edificio C1	Data	Orario	ore	Note:
Lezione 1	02.10.2026	08:00 - 09:45	2	Attenzione: 09:45 - 11:00 Prof.ssa Bandiera
Lezione 2	09.10.2026	08:00 - 09:45	2	Attenzione: 09:45 - 11:00 Prof.ssa Bandiera
Lezione 3	16.10.2026	08:00 - 09:45	2	Attenzione: 09:45 - 11:00 Prof.ssa Bandiera
Lezione 4	23.10.2026	08:00 - 11:00	3	
Lezione 5	06.11.2026	08:00 - 09:45	2	Attenzione: 09:45 - 11:00 Prof.ssa Bandiera
Lezione 6	13.11.2026	08:00 - 09:45	2	Attenzione: 09:45 - 11:00 Prof.ssa Scaggiante
Lezione 7	20.11.2026	08:00 - 09:45	2	Attenzione: 09:45 - 11:00 Prof.ssa Scaggiante
Lezione 8	27.11.2026	08:00 - 09:45	2	Attenzione: 09:45 - 11:00 Prof.ssa Scaggiante
Lezione 9	11.12.2026	08:45- 09:45	1	Attenzione: 09:45 - 11:00 Prof.ssa Scaggiante
eventuale recupero	18.12.2026	08:00 - 11:00	3	

MS TEAMS code for lecture: alhmnas

Lecture slides and Laboratory information: Moodle // Recording of lectures: MS Teams

TURNI DI LABORTORIO

- 3 turni

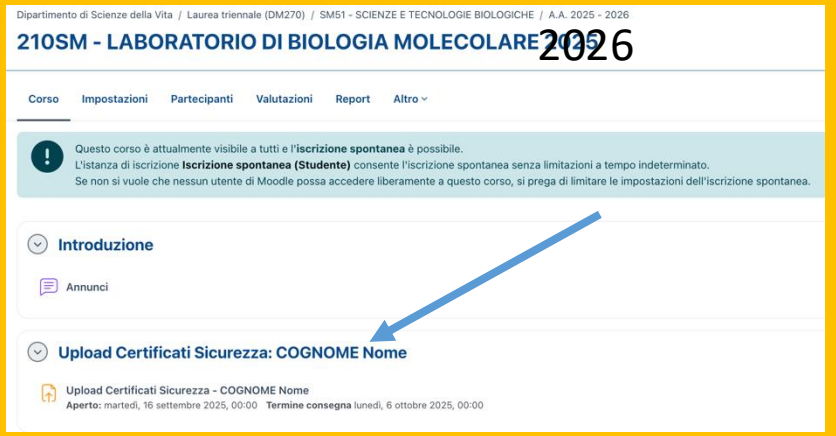
turni laboratori biologia molecolare

	LUNEDÌ 14-19	MARTEDÌ 14-19	MERCOLEDÌ 14 -19
1	Marianna Bomben	Elena Romeo	Alice Grigio
2	Paolo Tremul	Valentina Somma	Emilia Miccio
3	Denise Pianta	Michela Fornasier	Andrea Gemignani
4	Martina Galfo	Giulia Annis	Federica Dal Zilio
5	Martina Contarato	Keiko Sigalotti	Elena Degli Uomini
6	Federica Stefanel	Veronica Guion	Stella Di Gennaro
7	Costanza Rieppi	Luca Serafino	Gaia Barattin
8	Elisa Tomasi	Ludovica Guano de Blasio	Sara Bisetto
9	Celeste Visintini	Nicole Pinatto	Manuel Del Negro
0	Emma Giacinto	Giulia Macchia	Giorgia Monte
1	Elisa Nonis	Nicolas Ronga	Gabriel Burca
2	Camila Teghil	Bechevolo Lucrezia	Mattia Zennaro
3	Alessia Pessotto	Emma Folca-Nash	Giovanni Puissa
4	Francesco Danesin	Francesca Russo	Chiara Scarfiello
5	Margherita Pippa	Nicola Zadro	Lorenzo Gambon
6	Morello Giorgia	Melania Segattini	Elias Lunardelli
7	Ana Tlashadze	Alessio Gaiatto	Alessandro Volpe
8	Matteo Martin	Marta Dal Farra	Giacomo Buoso
9	Lorenzo D'Anna	Riccardo Pizzoni Ardemani	Alice Francescutto
20	Nicola Roman Pognuz	Emma Gava	Chiara Maria Lazzarin
21		Giorgia Toscan	Angelica Oresti
22		Siria Santin	
23		Elisa Orzan	
24			
25			

- Courses for health and safety:

modulo/percorso	chimico	biologico	tecnico	geologia/archeologia
rumore			♦	♦
vibrazioni			♦	♦
laser	♦	♦	♦	
allergie	♦	♦	♦	
cute	♦	♦	♦	
elettricità	♦	♦	♦	
chimica	♦	♦		♦
laboratorio	♦	♦	♦	
lab. biologico		♦		
nebbie polveri				♦
biologico		♦		
prevenzione biologico		♦		
dpi	♦	♦		♦
primo soccorso	♦	♦	♦	♦
meccanica			♦	
chimica in officina	♦		♦	
chimica in laboratorio	♦			
attività esterne		♦		♦

Attestati Sicurezza Studenti need to be uploaded as SINGLE PDF file to moodle until 05.10.2026:



ATTENTION: STUDENTS THAT TO NOT UPLOAD THE DOCUMENTS CANNOT ENTER THE LABORATORY

Exam

PART 1 - LABORATORY

Reports on lab work at the end of each lab practice (Prof. Bandiera, Prof.ssa Scaggiante).
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 16 points can be reached.**
→**A minimum of 7,5 points is necessary to participate in the second part of the exam**

PART 2 - LECTURE

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.
Gli studenti che **non** possono partecipare al corso di laboratorio ricevono 3 ulteriori "domande aperte" relative al contenuto del corso di laboratorio (5 punti per domanda).

The final mark of the course results from the sum of both exams.
Maximum points: 31 (= 30L)

A minimum of 18 points (total) is required to pass the exam "Laboratorio Biologia Molecolare".

Guidelines MS Teams AA2026-2027

POLICY OF THE UNIVERSITY OF TRIESTE:

- **ALL LECTURES “IN PRESENZA”, recording of lectures provided on MS Teams**
- **IF STUDENT PRESENCE IS SIGNIFICANTLY DECREASING; RECORDING OF LECTURES MAY BE STOPPED**

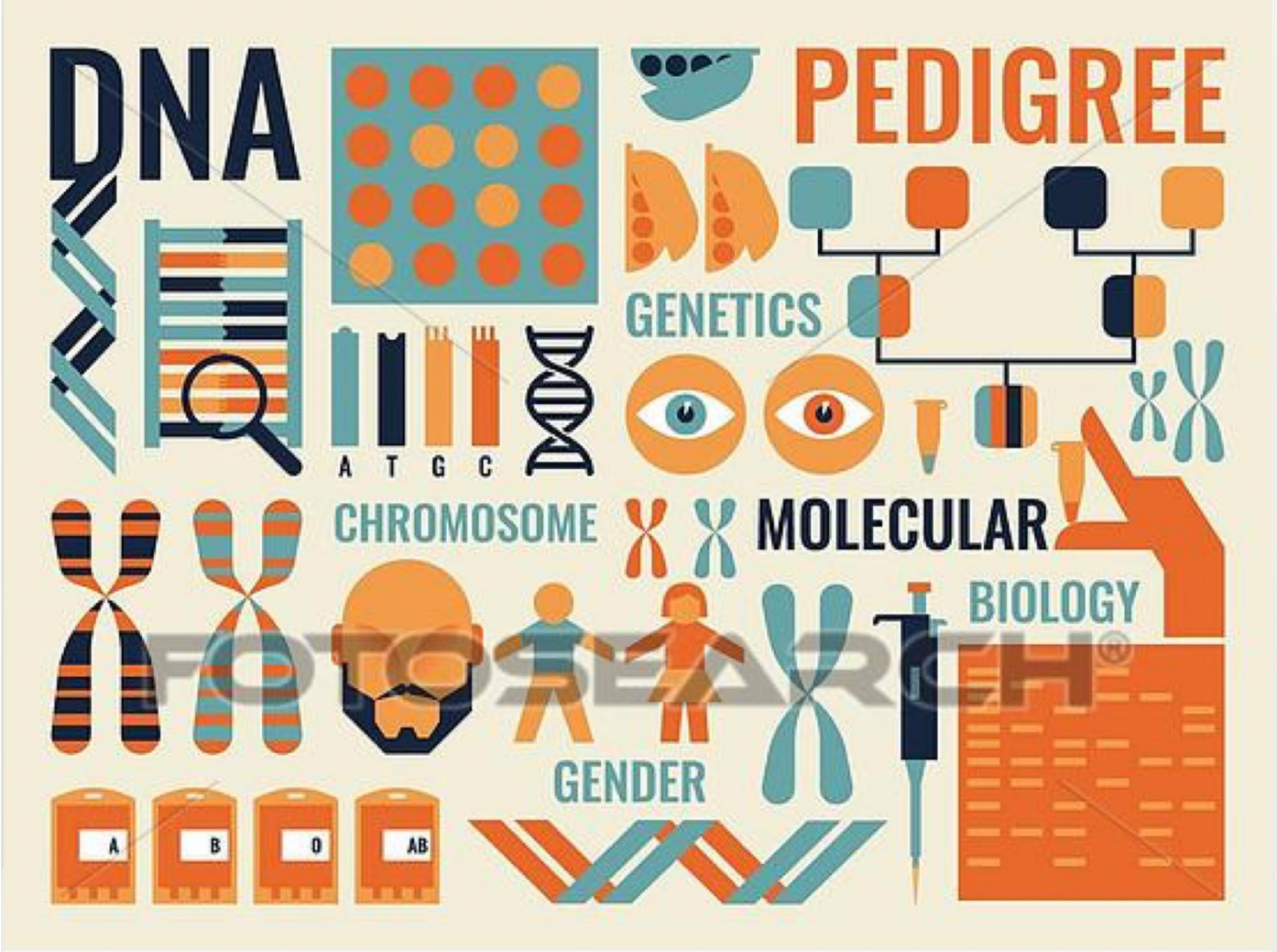
WHAT IS YOUR EXPECTION ON THE LECTURE….

WHAT DO YOU THINK YOU SHOULD LEARN….

Form groups, discuss 5-7 minutes, individuate 2-3 of your goals

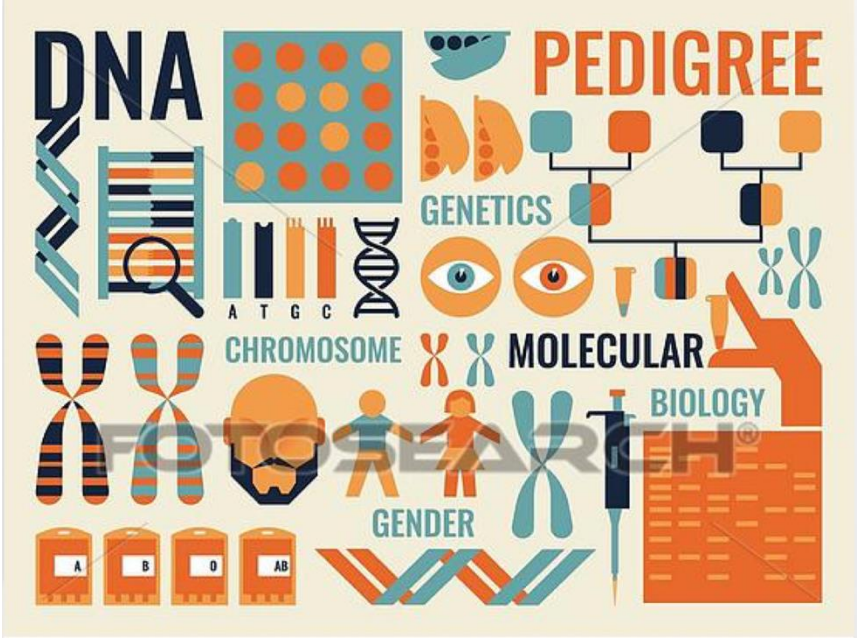
Choose speaker of group

Present result



WHAT IS MY EXPECTION ON THE LECTURE….

WHAT I THINK YOU SHOULD LEARN….



A good overview on basic methods in molecular biology

Knowing what method to use to solve a biological question

Understand the concept of a method and initial knowledge in trouble shooting

Learn how to use pipettes (precision)

Learn how to set up an experiment

Learn how to read a result

Interpretation

Contents of Theoretical Lecture (Prof. Schoeftner)

LECTURES: on Friday lecture slots (08:00 – 11:00); Ed C1, Aula I
MS TEAMS: code: alhmnas
PPT files, protocols will be stored on Moodle
Recording of lectures will be stored on MSTeams (if student presence in maintained)

1. Preparation of RNA/Protein/DNA.

2. PCR technologies: standard PCR, PCR oligo design (with exercise)

3. Gene expression analysis, quantitative real-time PCR (Northern Blot, RNase Protection Assay)

4. Recombinant DNA techniques, Cloning vectors, Endonucleases, Recombinant protein production, introduction of genes into host-organisms.

5. Loss and gain of function approaches in vertebrate cells (siRNA, overexpression)

6. Methods to study DNA:protein interaction (band shift, DNA footprinting)

Contents of Practical Course Part 1 (Prof.ssa 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. PLASMIDS: Plasmids will be subjected to control digest and fragments will be analyzed by gel-electrophoresis

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

3. 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.

4. DATA ANALYSIS AND DISCUSSION: PCR results will be analyzed; discussion on improving PCR; discuss 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.

Contents of Practical Course Part 2 (Prof.ssa Scaggiante)

Quantitative RT-PCR:

Molecular analysis of loss of function effects by gene expression analysis

Evaluation of transient knock-down of gene; evaluation of biological effect using molecular marker gene expression.

1. Transient, **siRNA mediated knock-down** of gene of interest (SFPQ) in U-2 OS cells.
2. Preparation of **total RNA**
3. **Reverse transcription** to produce cDNA
4. Performing **quantitative real-time PCR** using gene specific primers: SFPQ and reference gene to evaluate knock-down efficacy; other primer set to evaluate activation of Interferon signaling as biological consequence of loss of SFPQ.
5. Discussion of RT-PCR data: amplification blot, melting curve, quantification

BACKGROUND PART 1 - 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.

Classic polymorphisms also comprise the insertion of a transposable elements (such as Alu elements) or repeat expansions/retractions

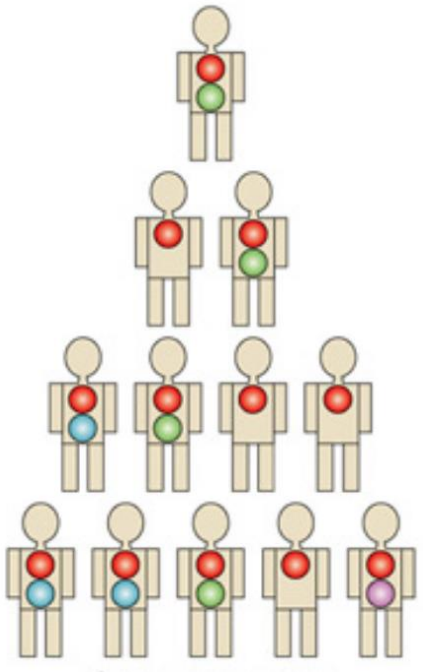


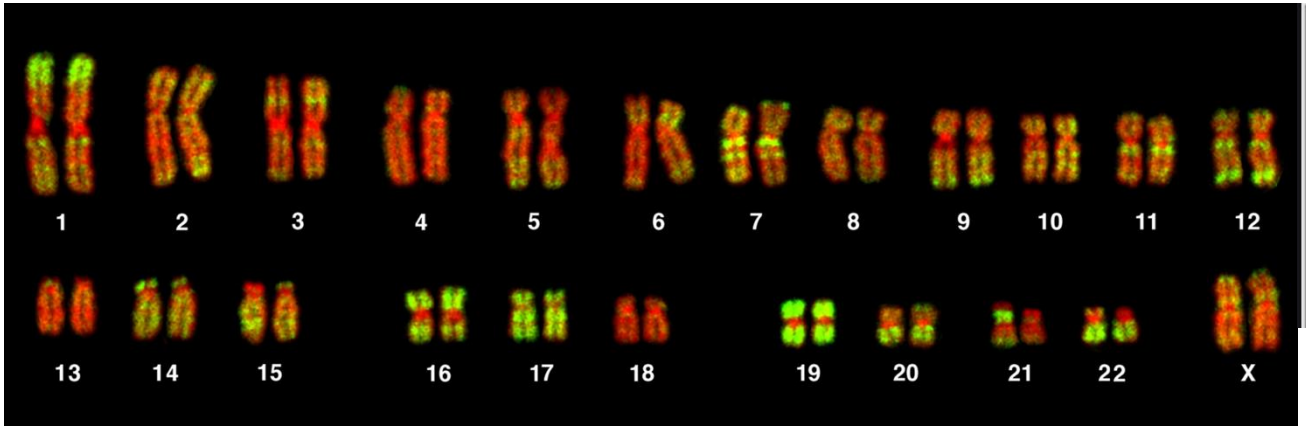
Table 2. Genetic Disease and Complex Variants.*

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.

Alu elements - 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

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

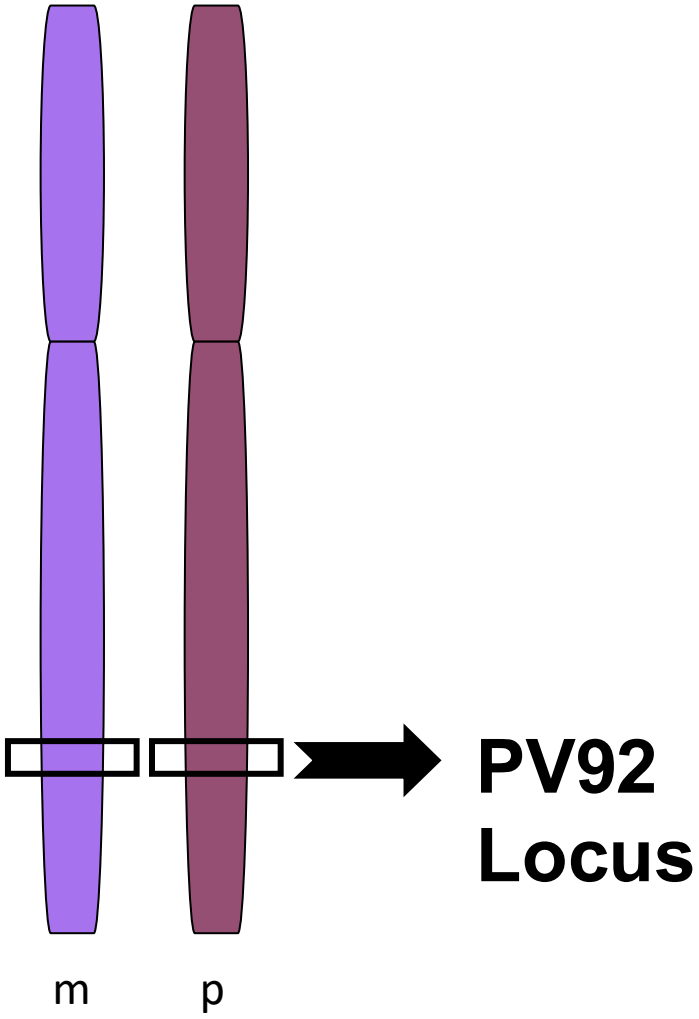
Gene	Position	Subfamily	Mechanism	Disease	Reference
ACE	Chr 17	*AluY*a5	Insertion	Alzheimer's disease	[39]
ALMS1	Chr 2	*AluY*a5	Insertion	Alström syndrome	[40]
BMPR2	Chr 2	*AluY* *AluS*	ARMD_NAHR NHEJ	Pulmonary arterial hypertension	[41]
CDSN	Chr 6	*AluS*	ARMD_NHEJ	Peeling skin disease	[42]
COL4A5	Chr 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 AA2025-2026

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: +/-

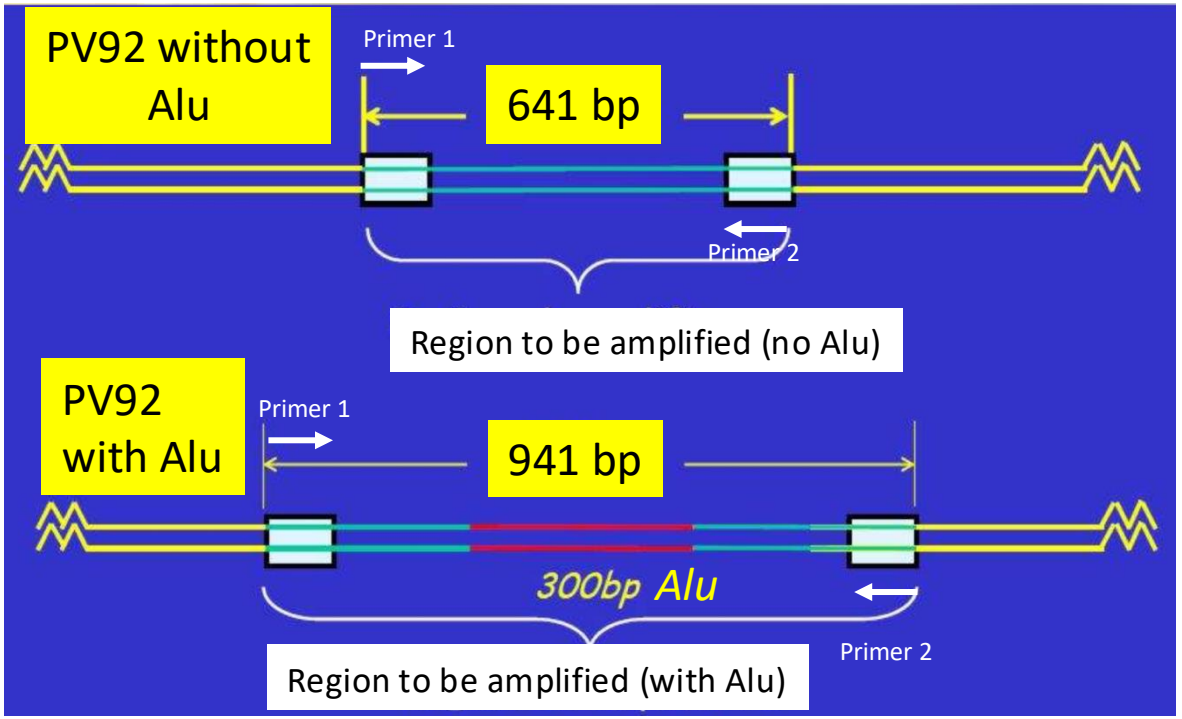
- Alu sequence insertion in PV92 locus is not diagnostic for any disease or disorder!

- Useful for forensic tests

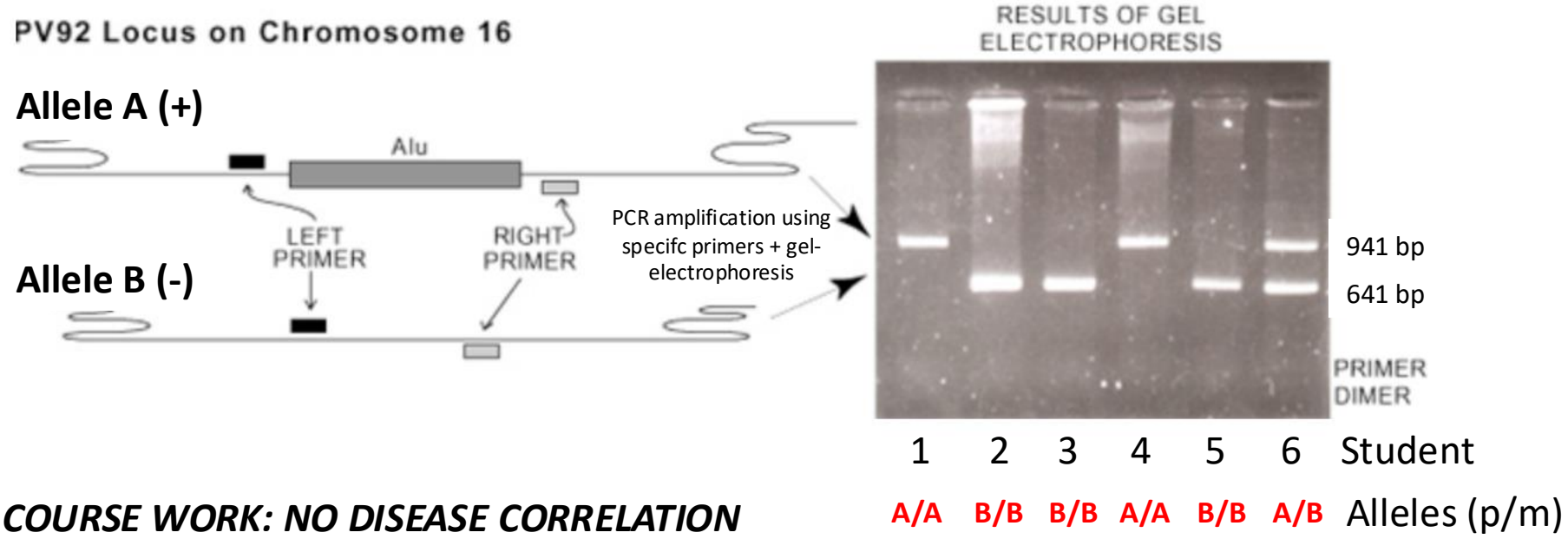
Transposable elements in the genome

PCR fragment amplified by PCR
Options for fragment size from maternal/paternal allele:

p+/m+ = 941 / 941 bp
p+/m- = 941 / 641 bp
p-/m+ = 641 / 641 bp
p-/m- = 641 / 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.

Esercitazioni di LABORATORIO – 1 PARTE (prof. A. BANDIERA)

1° esercitazione:
ESTRAZIONE DI DNA GENOMICO e PLASMIDICO



Preparazione di campioni

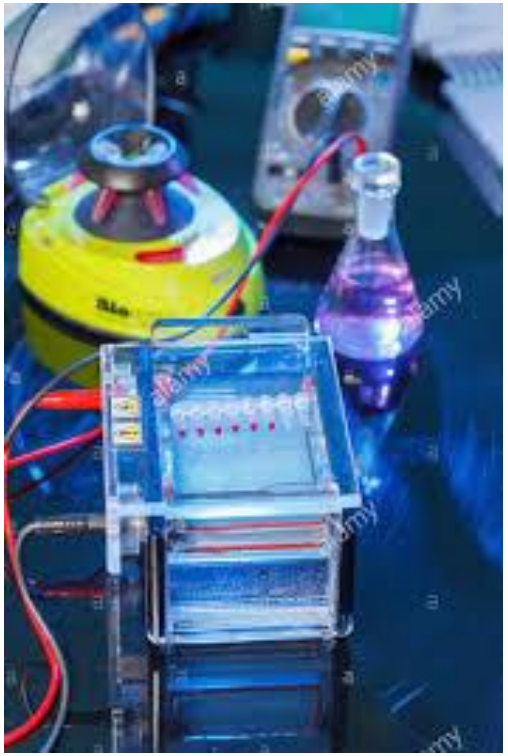
- DNA genomico studente
- DNA plasmidico

che verranno analizzati nelle successive esercitazioni

Plasmidi:
Due plasmidi diversi, contenenti la regione genomica umana PV92:
- Un plasmide con l'elemento Alu;
- l'altro senza l'elemento Alu.

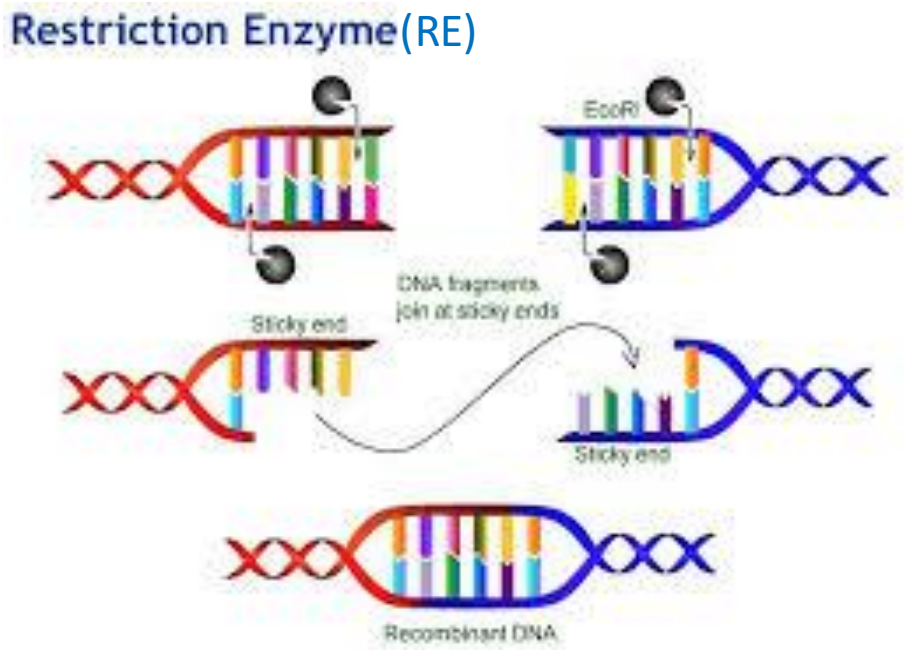
Questi plasmidi servono come controllo nella reazione di PCR a valle.

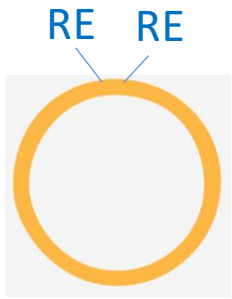
2° esercitazione:
ELETTROFORESI SU GEL DI AGAROSIO

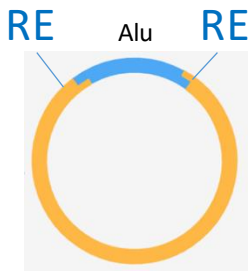


Metodo elettroforetico per l'analisi dei campioni di DNA genomico e plasmidico preparati nell'esercitazione precedente

3° esercitazione:
ANALISI DI RESTRIZIONE DEL DNA PLASMIDICO







Analisi dei campioni di DNA plasmidico preparati e analizzati nelle esercitazioni precedenti per controllare la presenza/assenza del inserto (Alu element)

4° esercitazione:
PCR - Polymerase Chain Reaction





Analisi del campione di DNA genomico preparato nella prima esercitazione

BACKGROUND of Practical Course Part 2 (Prof.ssa Scaggiante)

Quantitative RT-PCR: Evaluation of transient knock-down of gene; evaluation of biological effect using molecular marker gene expression.

1. Transient, **siRNA mediated knock-down** of gene of interest (SFPQ) in U-2 OS cells.

2. Preparation of **total RNA**

3. **Reverse transcription** to produce cDNA

4. Performing **quantitative real-time PCR** using gene specific primers

   - SFPQ and reference gene to evaluate knock-down efficacy
   - primer set to evaluate activation of Interferon signaling CCL5 as biological consequence of loss of SFPQ

5. Discussion of RT-PCR data: amplification blot, melting curve, quantification

The experiment we will perform is part of a scientific publication currently under revision:

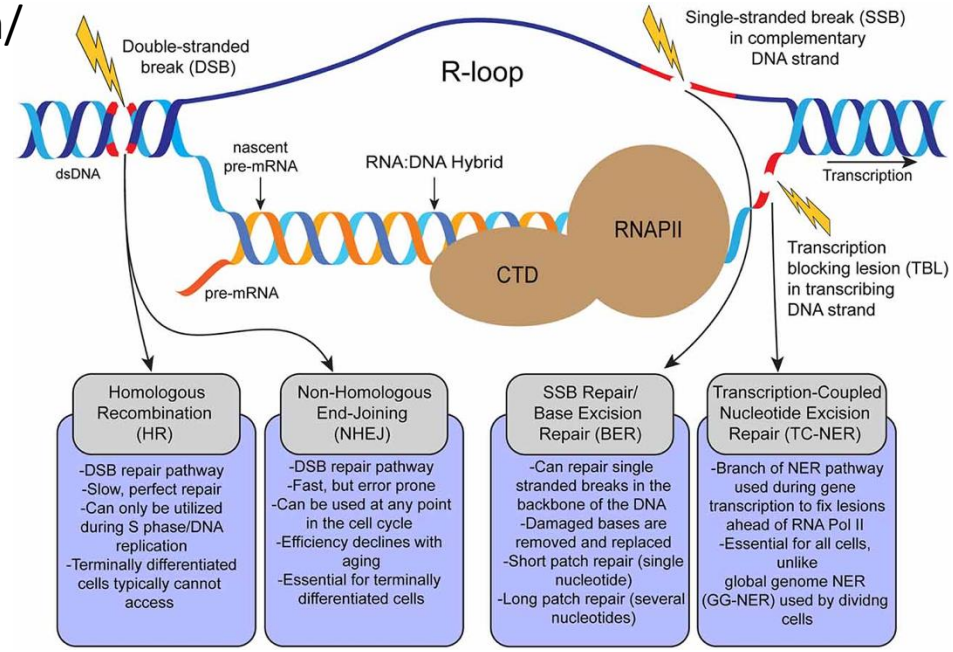
"SFPQ Directs Histone H3.3 Deposition to R-Loops in DNA Repeats to Protect Genome Stability"

→ **Read the pre-print paper and individuate the experiment we will do (page 1-13 and figures)**

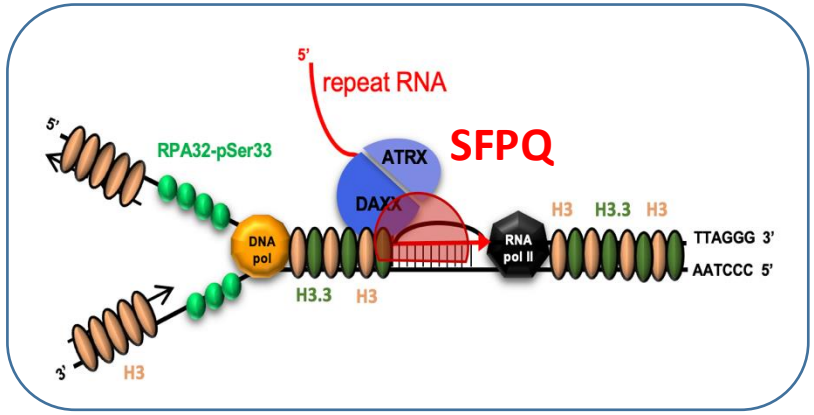
→ **https://sciety.org/articles/activity/10.21203/rs.3.rs-5721144/v1**

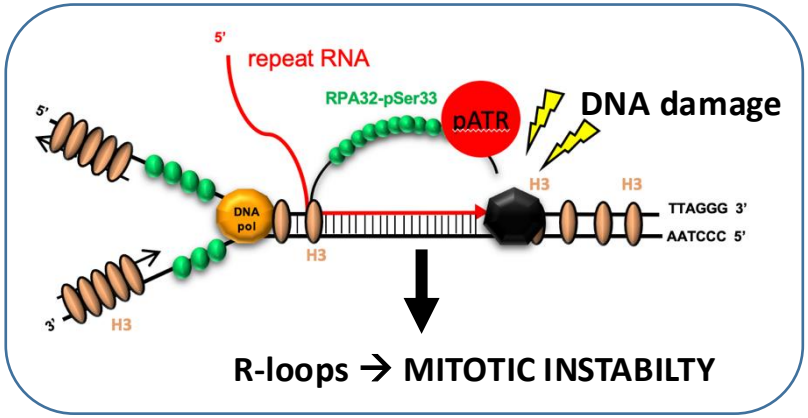
Long hybrid between RNA and DNA drive R-loop formation cause DNA damage

https://www.nature.com/articles/s41467-026-69479-w



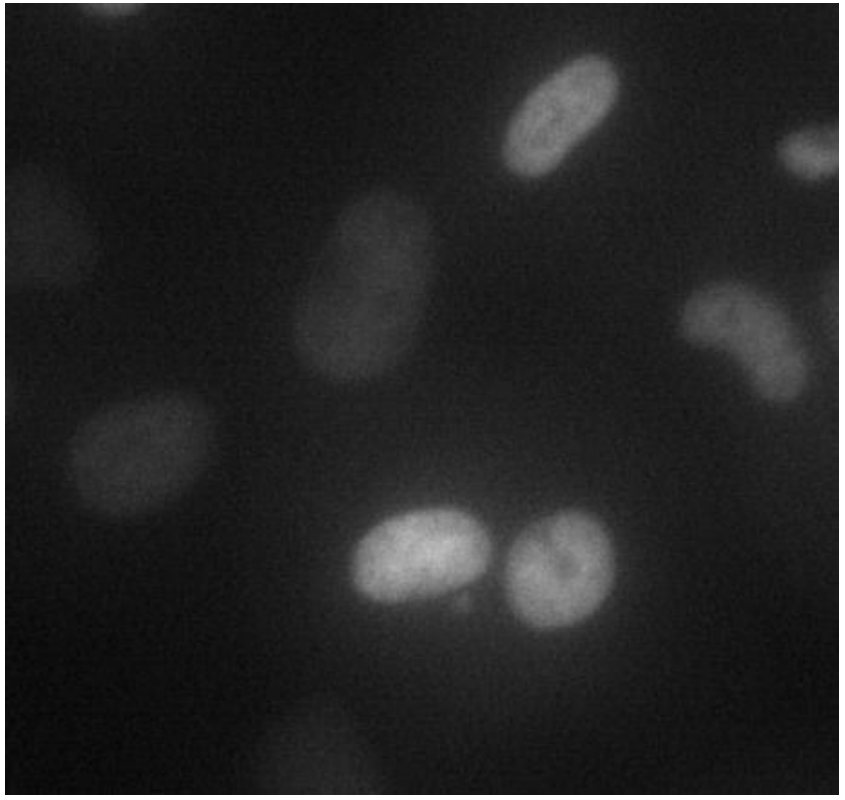
- R-loops block DNA and RNA polymerases
- Mechanic stress on DNA – cleavage/break of DNA
- If DNA damage is not repaired → genome instability
- Many factors suppress the formation of R-loops
- SFPQ is one of these factors





Loss of SFPQ increases R-loop levels and genome instability in cancer cells

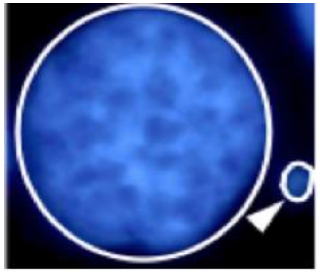
Loss of SFPQ results mitotic defects that lead to genome instability



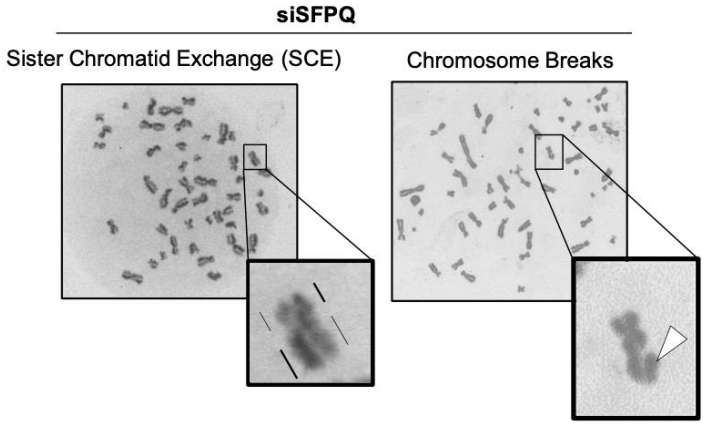
U-2 OS osteosarcoma cancer cells after RNAi mediated depletion of SFPQ
- Cells stably express GFP-tagged histone H2B (integrates into nucleosomes → fluorecent nuclei)
- Live microscopy on living cells

Formation of micronuceli
(a chromosome fragment localized in the cytoplasma

siSFPQ



Defects in metaphase chromosomes



Loss of SFPQ results mitotic defects that lead to genome instability

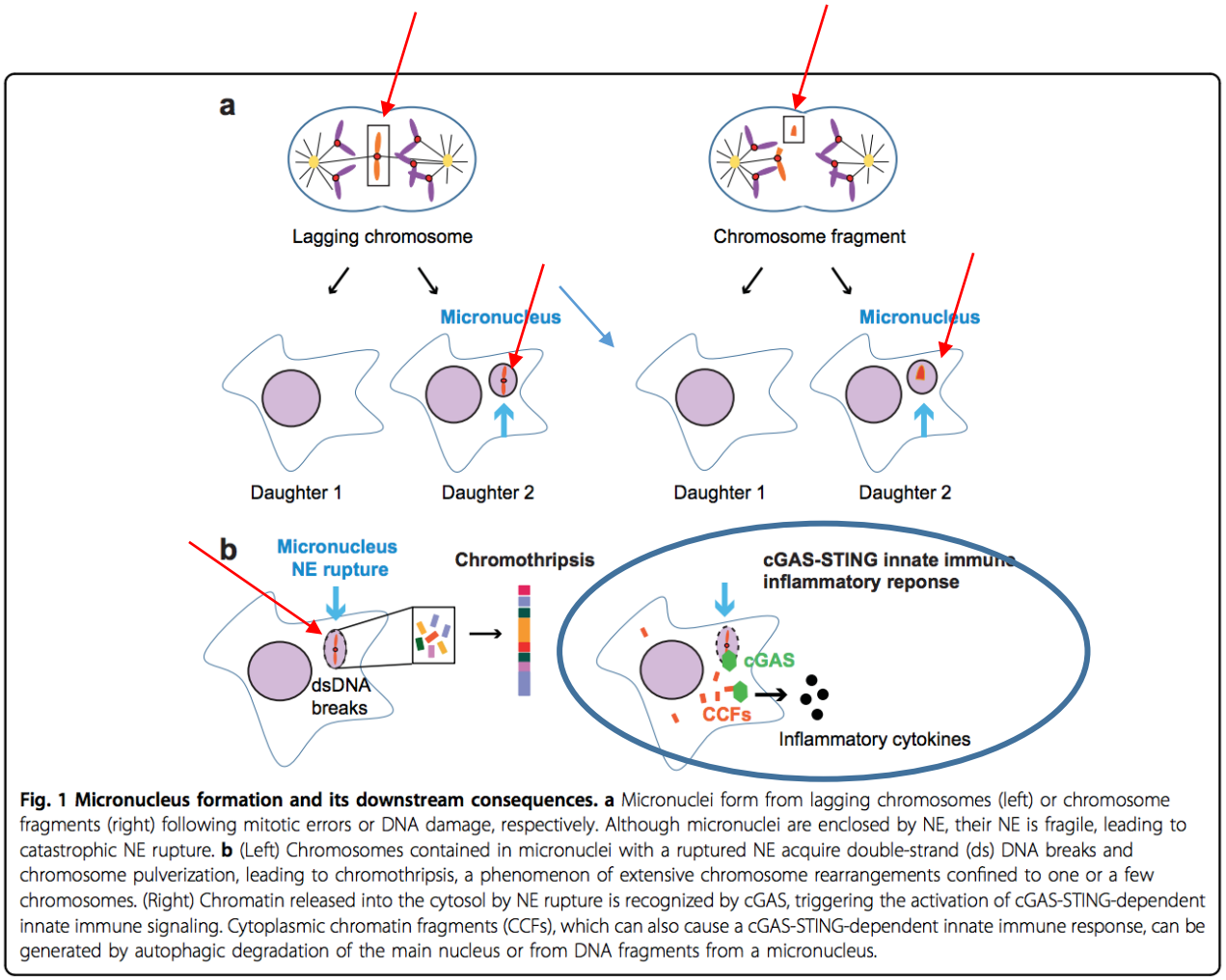
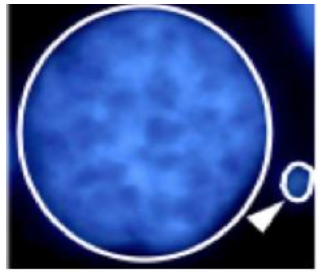


Fig. 1 Micronucleus formation and its downstream consequences. a Micronuclei form from lagging chromosomes (left) or chromosome fragments (right) following mitotic errors or DNA damage, respectively. Although micronuclei are enclosed by NE, their NE is fragile, leading to catastrophic NE rupture. **b** (Left) Chromosomes contained in micronuclei with a ruptured NE acquire double-strand (ds) DNA breaks and chromosome pulverization, leading to chromothripsis, a phenomenon of extensive chromosome rearrangements confined to one or a few chromosomes. (Right) Chromatin released into the cytosol by NE rupture is recognized by cGAS, triggering the activation of cGAS-STING-dependent innate immune signaling. Cytoplasmic chromatin fragments (CCFs), which can also cause a cGAS-STING-dependent innate immune response, can be generated by autophagic degradation of the main nucleus or from DNA fragments from a micronucleus.

micronucleus



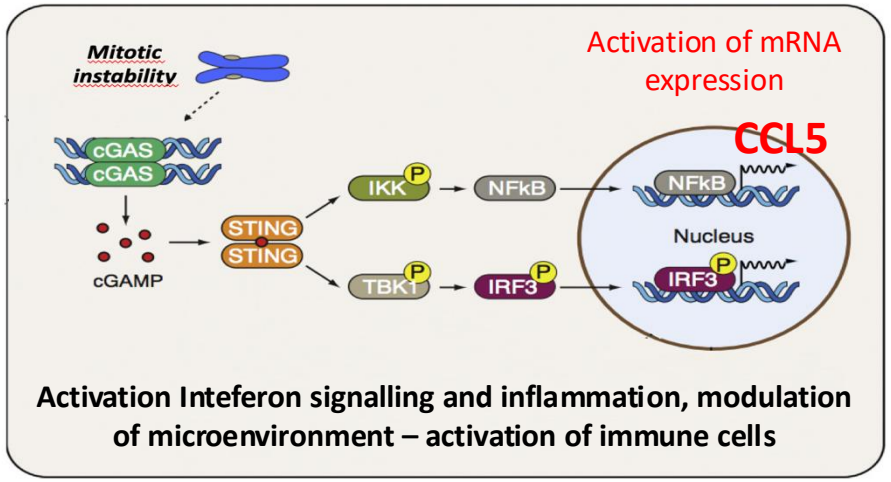
NE rupture – cytoplasmatic DNA

cGAS/STING pathway

Inflammatory response

Recruitment of immune cells

Activation of IFN signalling by promoting genomic instability



Activation Inteferon signalling and inflammation, modulation of microenvironment – activation of immune cells

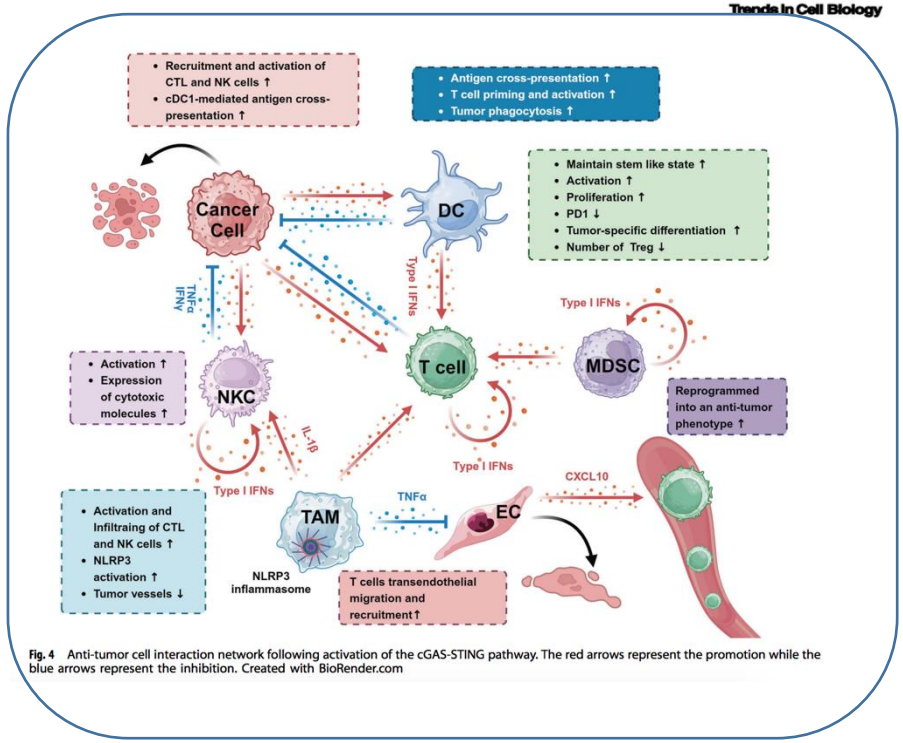
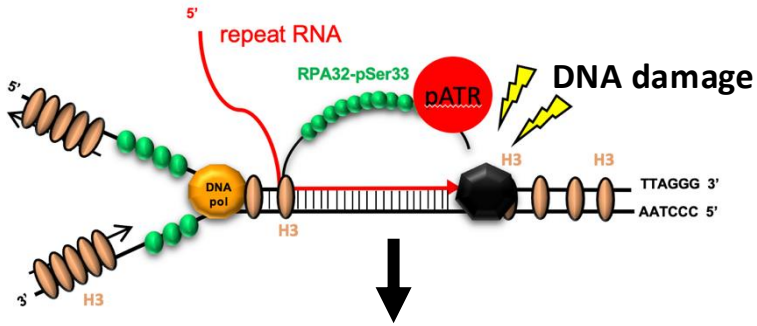


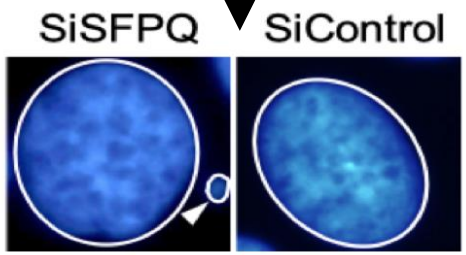
Fig. 4 Anti-tumor cell interaction network following activation of the cGAS-STING pathway. The red arrows represent the promotion while the blue arrows represent the inhibition. Created with BioRender.com

Modified from: Zierhut and Funabiki, 2020

Transient knock-down of SFPQ
→ Replication stress



DNA damage and mitotic instability

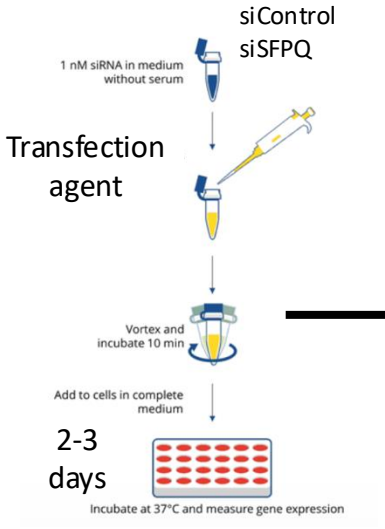


Micronuclei → cytoplasmatic DNA → innate immunity pathways

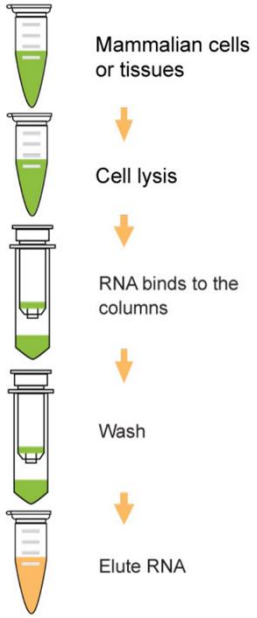
Monitor increased expression of CCL5:
proinflammatory chemokine, recruiting leukocytes to the site of inflammation. It is chemotactic for T cells, eosinophils, and basophils, but also for monocytes, natural-killer (NK) cells, dendritic cells and mastocytes.

Laboratory Exercises: Montoring the activation of innate immunity by real-time PCR

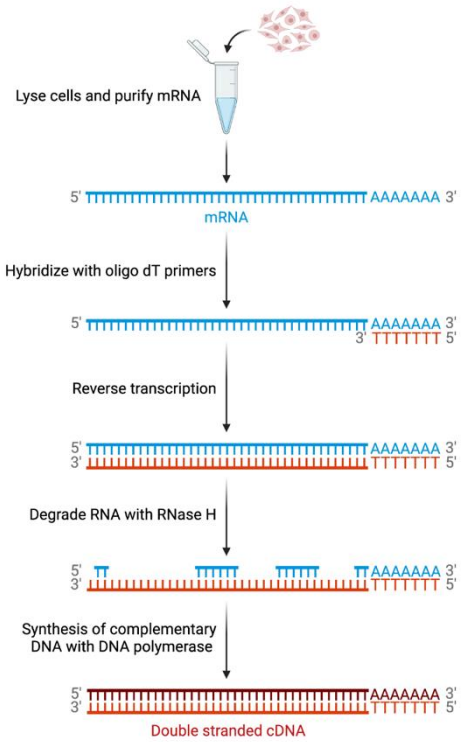
Transfection of U-2 OS cells



total RNA preparation



cDNA synthesis



Exercise 5
siCON
siSFPQ

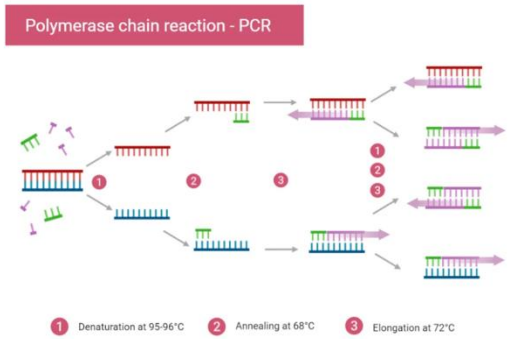
Exercise 6
siCON
siSFPQ

Exercise 7
siCON
siSFPQ

Activation of IFN signalling by promoting genomic instability

Quantitative RT-PCR

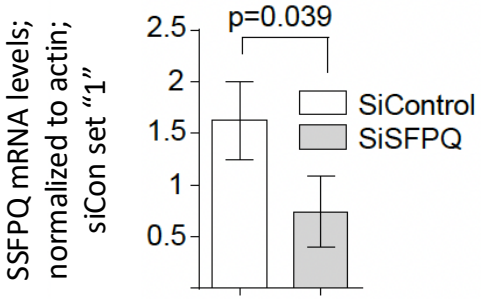




Exercise 7
siCON
siSFPQ

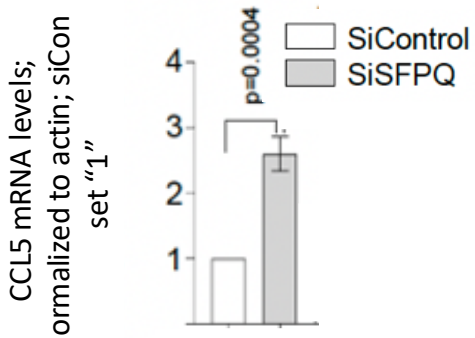
- Processing of data, with statistical analysis
- Preparation of data in style of a scientific publication
- Interpretation

Control knock-down (siSFPQ)



qRT-PCR 1

Read out on IFN stimulated gene (CCL5)



qRT-PCR 2

Exercise 8
Statistical Analysis
Preparation of figure