Cellular and Molecular Biology I

Camille Goemans

Cre-LoxP

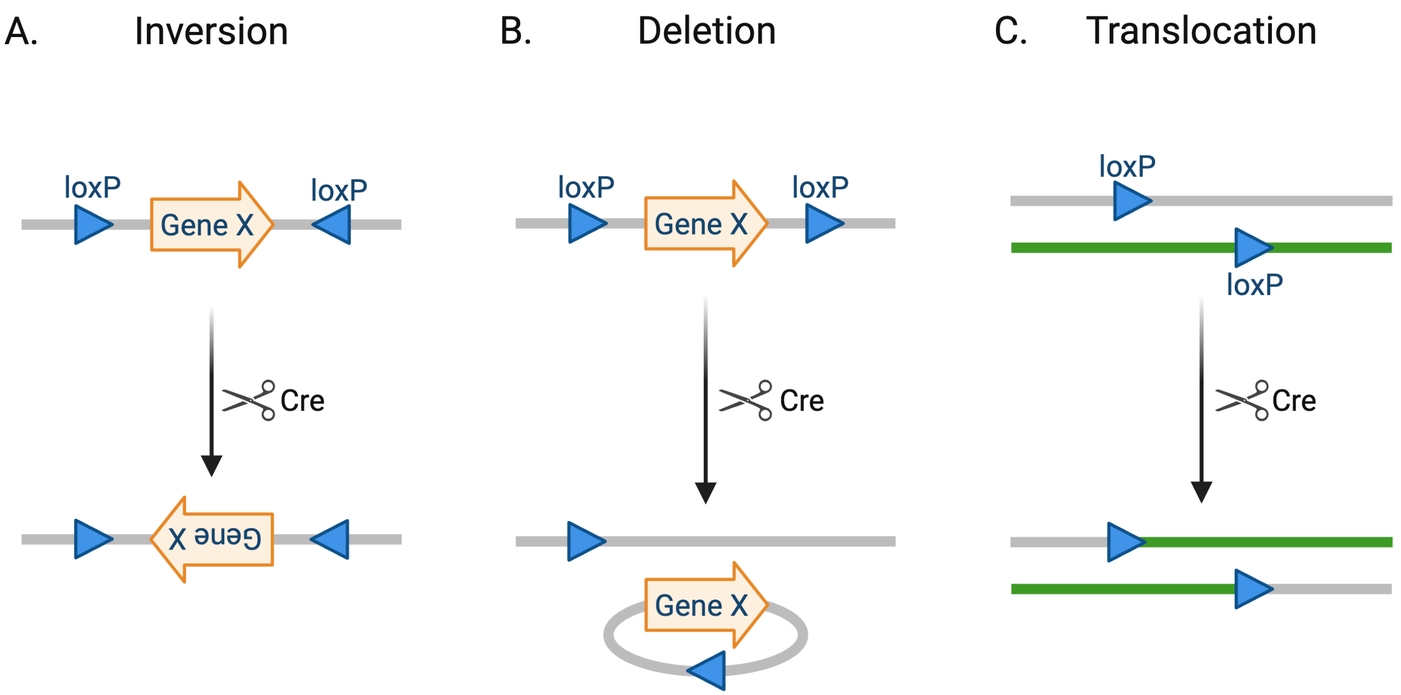
Key Components

1. Cre recombinase

- An enzyme that recognizes specific DNA sequences called **loxP sites**.
- It catalyzes recombination (cutting, inverting, or exchanging DNA) between these sites.

2. loxP sites

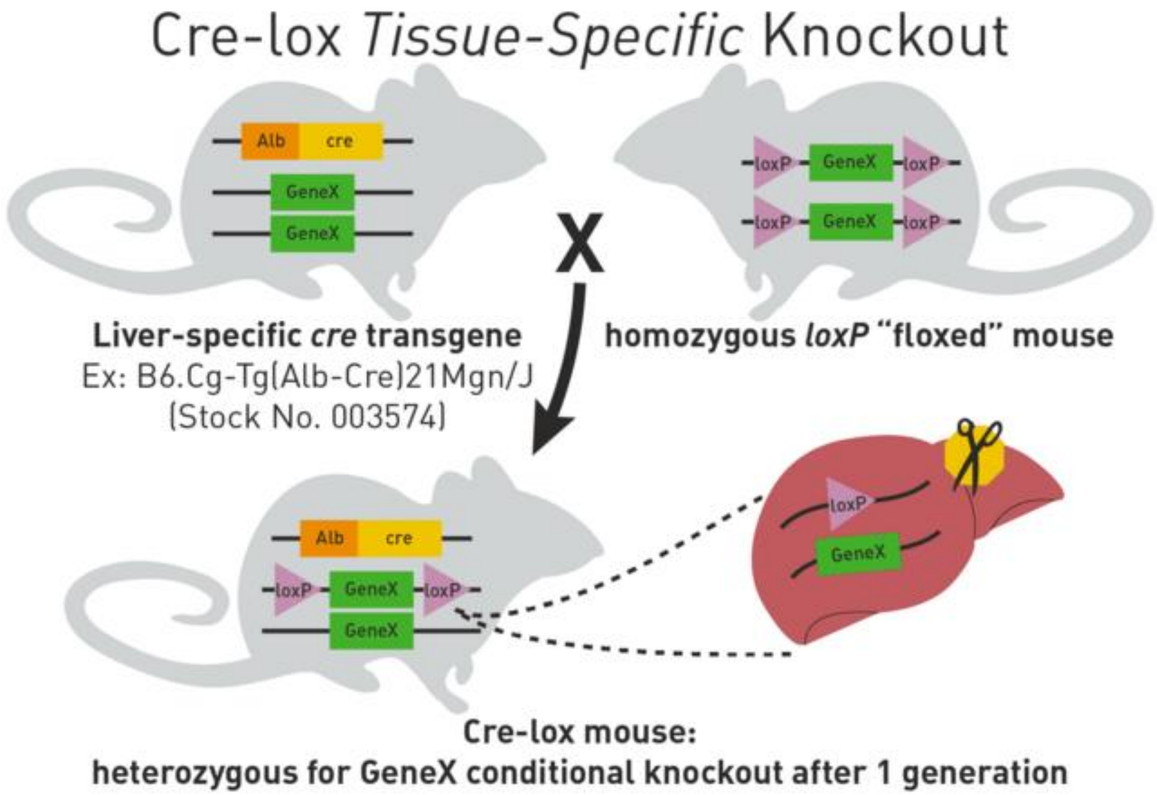
- Short, **34-base-pair** DNA sequences
- The orientation and placement of loxP sites determine the outcome of recombination.



Cre-LoxP

Conditional gene knockout

- A gene can be “floxed” (flanked by loxP sites).
- Cre expression controlled by a **tissue-specific** promoter (e.g., Albumin-Cre for liver, Nestin-Cre for neurons).
- Result: the gene is knocked out **only in certain tissues**.



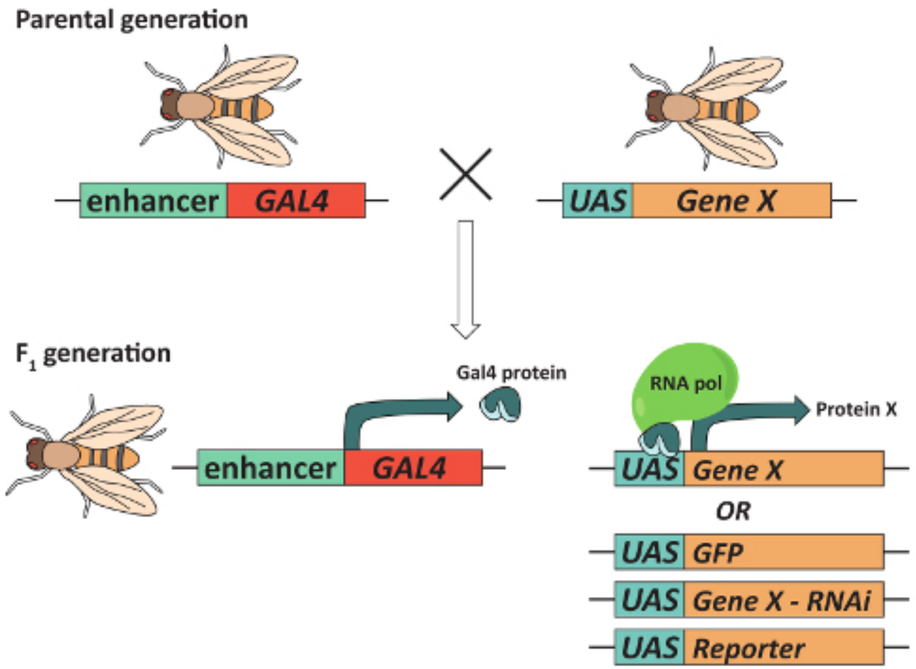
Gal4-AUS

1. Gal4

- A transcription factor that binds a specific DNA sequence.
- Expression is usually driven by a **tissue-specific** or **cell-type-specific** promoter.
- Determines **where** the downstream gene will be turned on.

2. UAS (Upstream Activating Sequence)

- A short DNA sequence recognized and bound by Gal4.
- Placed upstream of any gene you want to express
- When Gal4 binds UAS → transcription is activated.



Genomic imprinting

Genomic imprinting is an epigenetic phenomenon in which certain genes are expressed differently depending on whether they are inherited from the mother or the father.

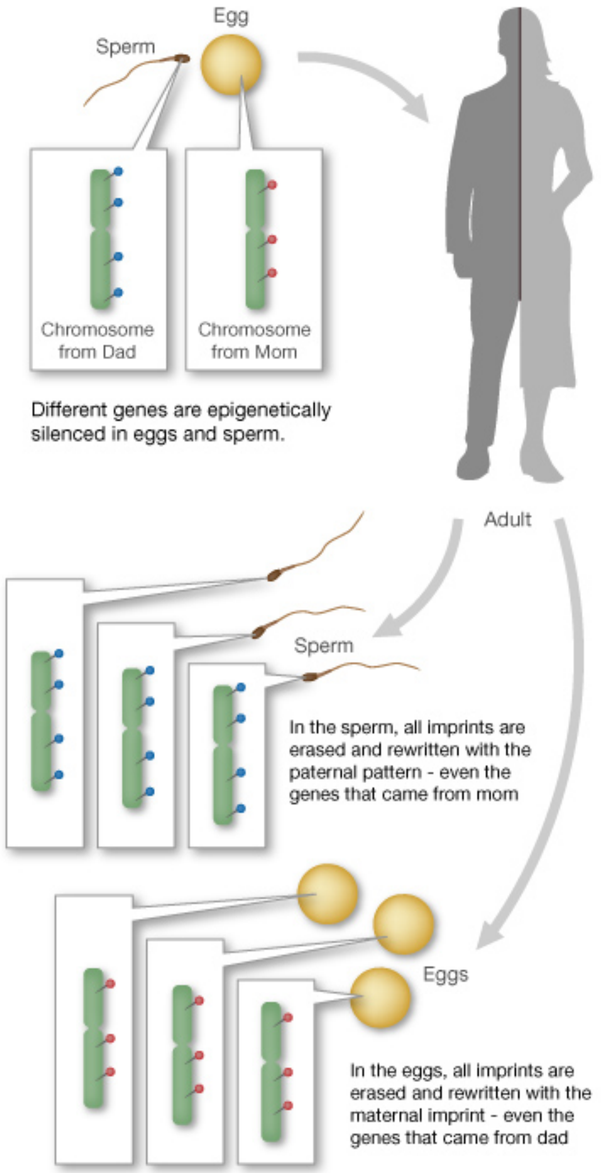
In other words, an imprinted gene remembers which parent it came from, and only one parental copy is active, while the other copy is silenced.

Imprinting is controlled by chemical marks that do not change DNA sequence, including:

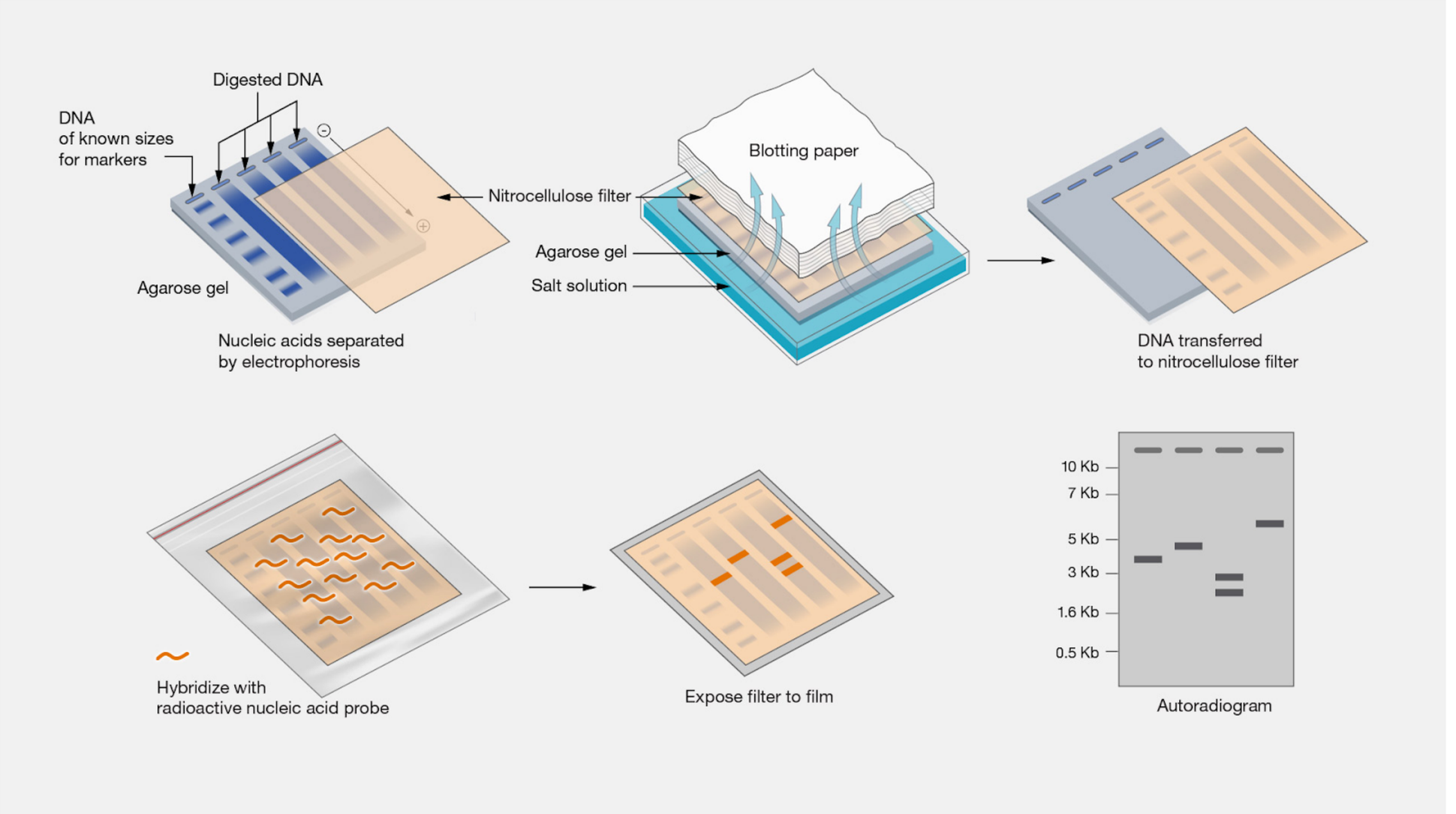
- DNA methylation (especially at imprinting control regions, ICRs)
- Histone modifications
- Non-coding RNAs

These marks are laid down in the germline (sperm or oocyte), erased in the early embryo, and then re-established in a sex-specific pattern.

About 300 genes in human cells



Western, southern, northern Blot



Western, southern, northern Blot

	Southern Blot	Northern Blot	Western Blot
Target molecule	DNA	RNA	Protein
Sample preparation	DNA extraction enzymatic digestion	RNA isolation	Protein extraction
Separation	Electrophoresis	Electrophoresis	Electrophoresis
Membrane material	Nylon	Nylon	Nitrocellulose or PVDF
Probe	Nucleic acid probe with sequence homologous to target	RNA, DNA, or oligodeoxynucleotide	Primary antibody
Probe label	Radiolabel, enzyme	Radiolabel, enzyme	Enzyme
Detection methods	X-ray film, chemiluminescence	X-ray film, chemiluminescence	Film, cooled CCD, camera, LED, or infrared imaging system

Table 1: Comparing Southern, Northern, and Western Blots.

ChiP (genome-wide chromatin immunoprecipitation)

Chromatin Immunoprecipitation (ChIP) is a molecular biology technique used to study protein–DNA interactions inside cells.

It tells scientists:

- Which regions of DNA are bound by a specific protein (e.g., transcription factors, histones)
- How chromatin modifications (like histone marks) relate to gene regulation
- How gene expression is controlled in different conditions or cell types

ChIP works by:

1. Fixing protein–DNA interactions in place
2. Capturing (immunoprecipitating) a protein of interest using an antibody
3. Recovering the DNA that was bound to that protein
4. Identifying those DNA regions using sequencing or PCR

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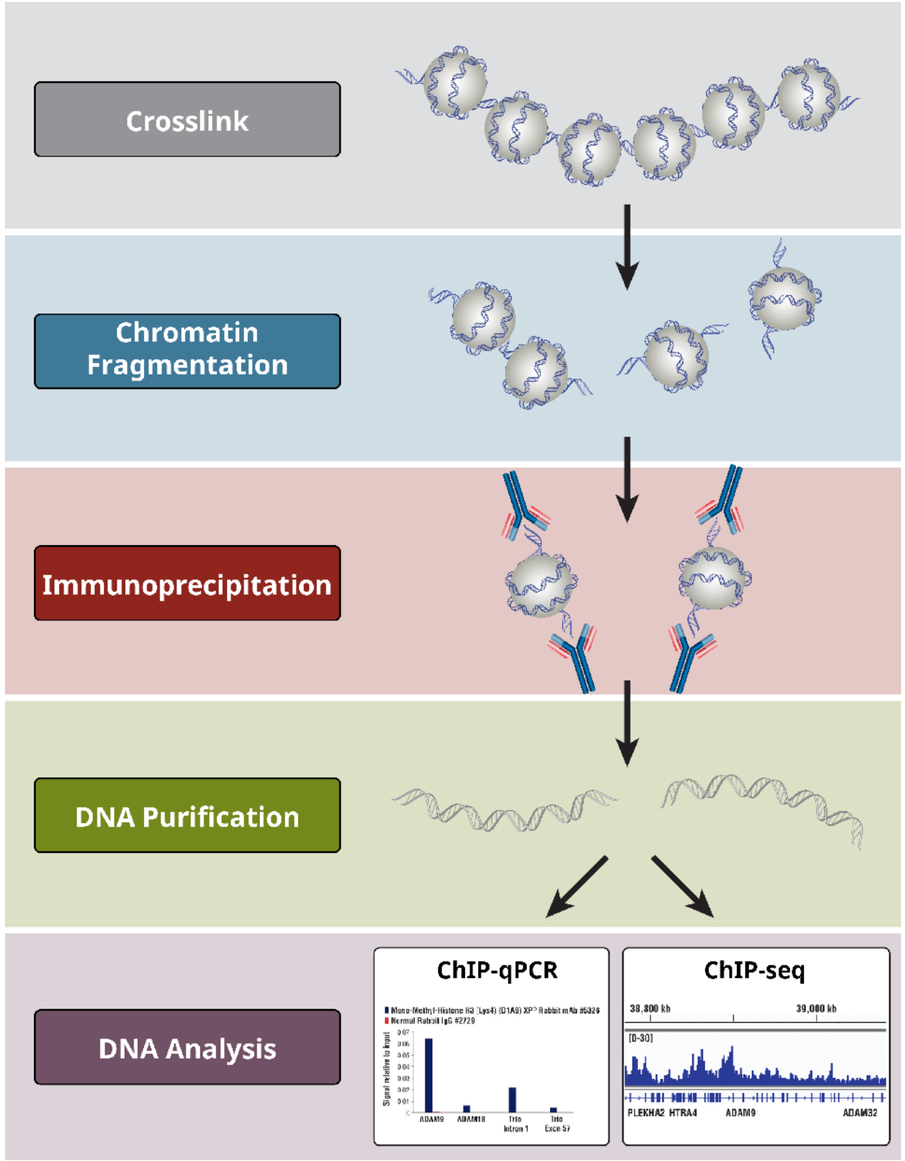

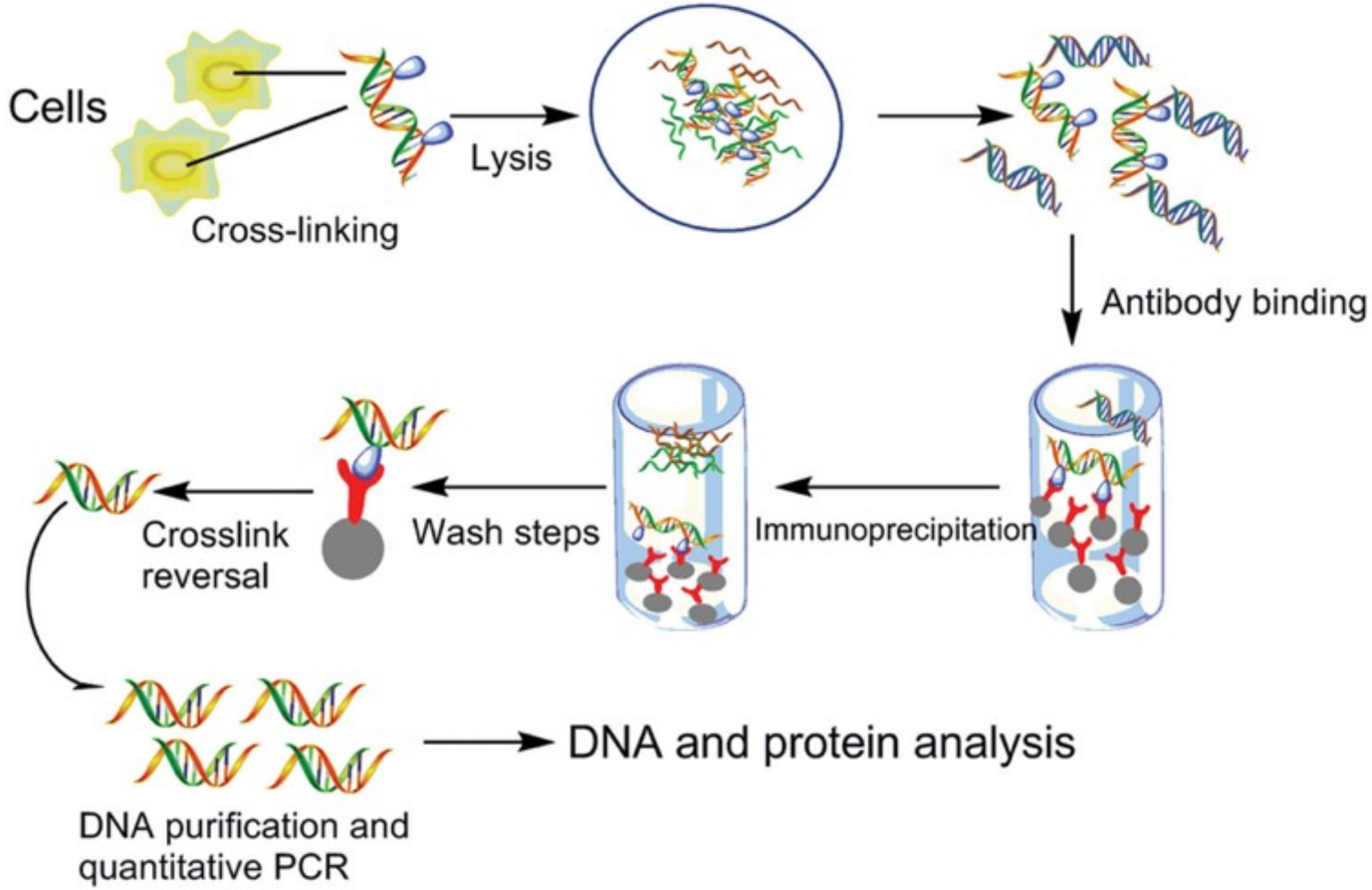

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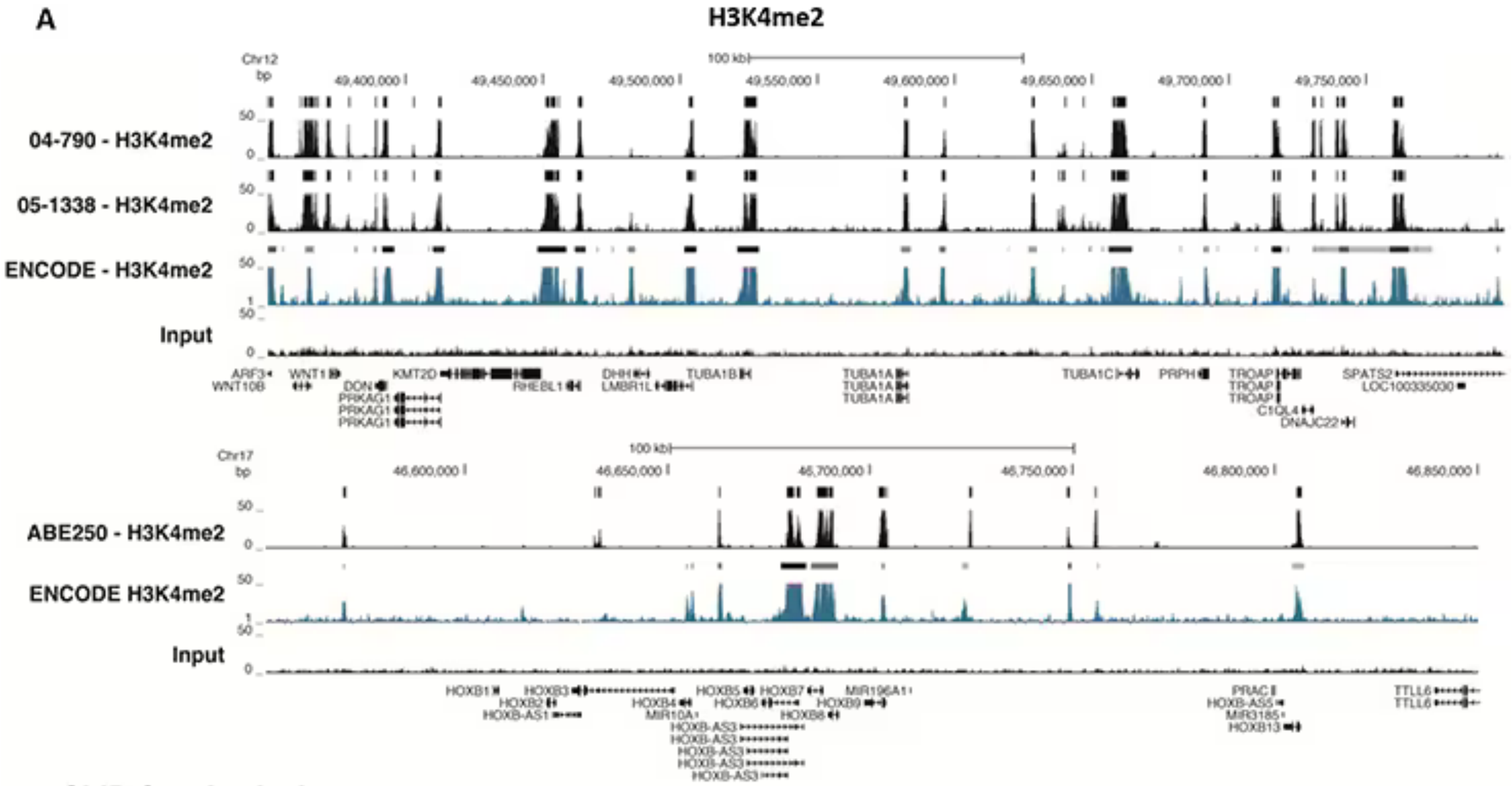

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A. The retroelement reduces the gene expression
B. The retroelement enhances the gene expression
C. The retroelement disrupts the coding sequence
D. The gene expression is down-regulated because of insertion in a cis-regulatory sequence
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2. Loss of function mutations change the activity of histone methylation transferase proteins (HMT). What is the effect on histone methylation?

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B. Increase in histone-3-lysine-27 methylation
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3. What is the major difference between the Sanger sequencing and next generation sequencing methods?

A. Only with the next-generation sequencing methods is possible to sequence non-coding regions
B. Only next-generation sequencing methods use modified nucleotides
C. Sanger method allows sequencing multiple samples in parallel in the same reaction
D. The next generation sequencing methods use BAC clones.
E. Whole exome sequencing is faster with next generation methods than with the Sanger sequencing

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4. To positively select bacteria containing a plasmid of interest, what sequence should be present in the plasmid?

A. A gene encoding eukaryote antibiotic resistance
B. An origin of replication
C. A gene encoding prokaryote antibiotic resistance
D. A Beta-galactosidase gene
E. A multi-cloning site

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5. A single nucleotide polymorphism (SNP) for gene A is present in 10% of the population and is associated with Alzheimer's disease. You sequence the DNA of patient Z and you find this polymorphism, what can you conclude?

A. Patient Z will develop Alzheimer.
B. Patient Z will never develop Alzheimer
C. Patient Z is predisposed to develop Alzheimer.
D. Gene A is always mutated in patients with Alzheimer.
E. None of the above answers is correct.

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6. The mutation profile of 5 cell lines reveals that 3 cell lines have loss of function mutations in gene A and 2 cell lines have gain of function mutation in gene B. You test a pharmacological inhibitor that blocks the activity of gene A in the cell lines with the mutation in gene B and all cells die. Considering the information that you have above, what can you conclude?

A) The gene A and gene B are not essential
B) The gene A is essential for cell survival
C) The drug is not specific and blocks the activity of gene B as well
D) The activity of gene B is enhanced by the treatment with the drug.
E) Mutations in gene A are synthetic lethal with mutation in gene B

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7) A patient with a myeloproliferative disease arrives at the hospital. You sequence the DNA of the patient and you discover a single point mutation that causes a premature stop codon in a gene encoding a transcription factor. You also sequence the DNA of his biological parents and find that this mutation is not present in their genome. Considering the above information, only one of the following statements is correct. Which one?

A) The mutation must be recessive.
B) This patient could be cured by Gleevec.
C) The mutation is likely to have been provoked by excess exposure to X-rays.
D) There is a 25% probability that the patient's daughter will suffer from this disease.
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A. Disrupt the 3D conformation of proteins by reducing disulfide bonds
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2. It is possible to obtain a transgenic animal in one generation by genetic modification of ES cells.

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Have a nice holiday/exam prep !