Clase 1
Biología Molecular aplicada al diagnóstico clínico

Introducción a la biología molecular y su aplicación a la Medicina actual

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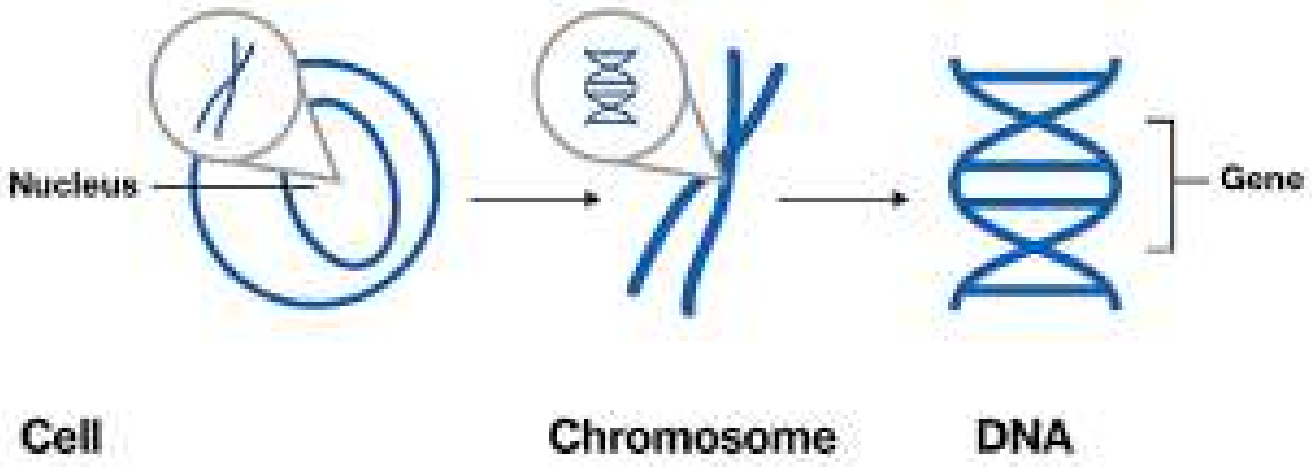
30.07.2020

Conceptos básicos

La **Genética** es la parte de la biología que estudia los genes y los mecanismos que regulan la transmisión de los caracteres hereditarios



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Conceptos básicos

La ***Genética Médica*** estudia los aspectos genéticos en la especie humana y su relación con la salud y la enfermedad, así como su aplicación al Dx, Tx, pronóstico y asesoramiento de enfermos y familiares

- ✓Genética de Poblaciones
- ✓Medicina Forense
- ✓Enfermedades Hereditarias
- ✓Infectología
- ✓Farmacogenética
- ✓Cáncer
- ✓Fertilidad y Reproducción

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Conceptos básicos

La **Medicina Genómica** se encarga de mejorar la calidad de la práctica médica orientando el cuidado pacientes hacia una medicina "a medida", predictiva y preventiva basándose en la información genómica de cada individuo

✓Distrofia muscular Duchenne-Becker

Tx Oligo-antisense (Antisentido)

✓ Fibrosis quística

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Dogma central de la Biología (Pre-Genomic Era)

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. Watson
F. H. C. Crick

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, 171, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, 39, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, 6, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, 9, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, 36, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, 10, 192 (1953).

Molecular Structure of Deoxypentose Nucleic Acids

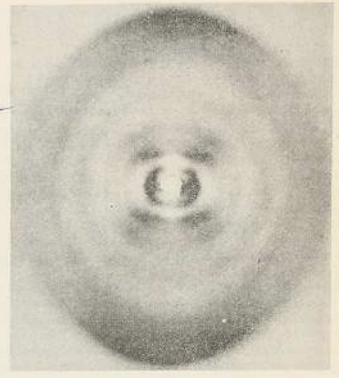


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

While the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[1]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[1–3], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid ('structure *B*' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the internucleotide repeat along the fibre axis. The ~ 34 A. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown[5] (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the nth layer line being proportional to the square of J_n, the nth order Bessel function. A straight line may be drawn approximately through the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_n^2) on the nth layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[6] being to reproduce the intensity distribution about the origin around the new origin, on the nth layer line, corresponding to C in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

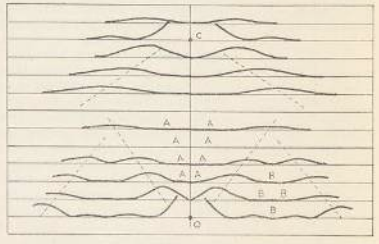
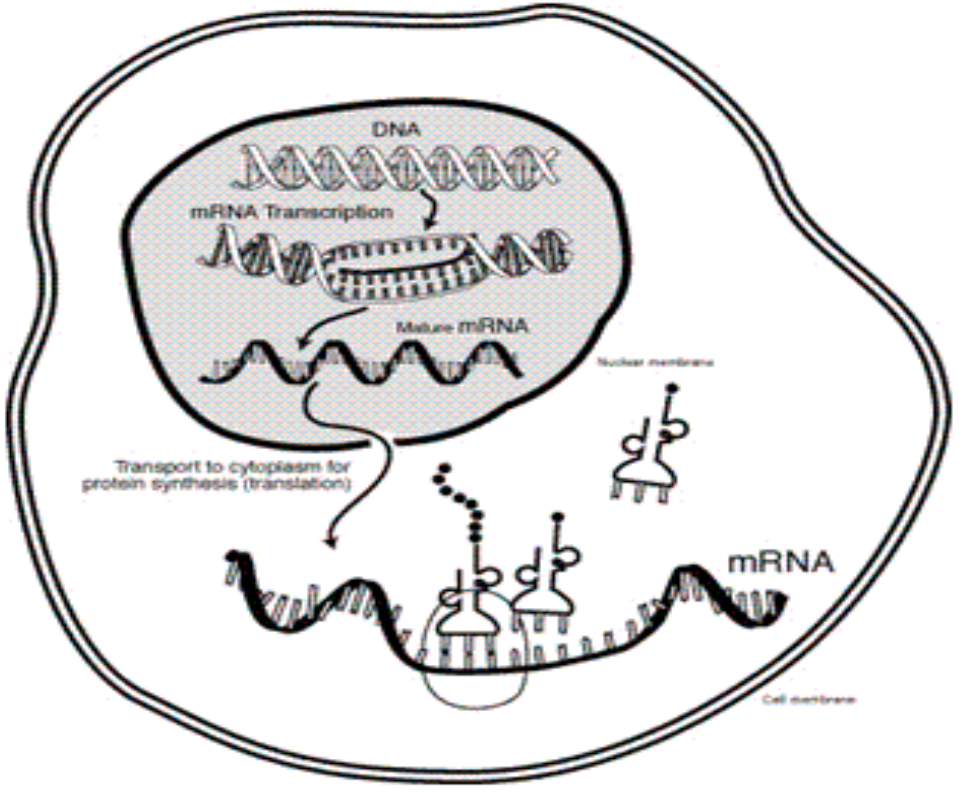
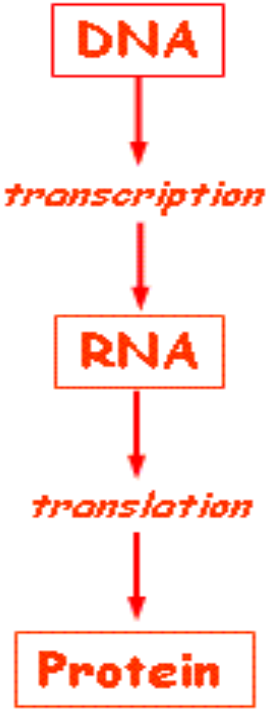


Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About C on the tenth layer line similar functions are plotted for an outer diameter of 12 A.





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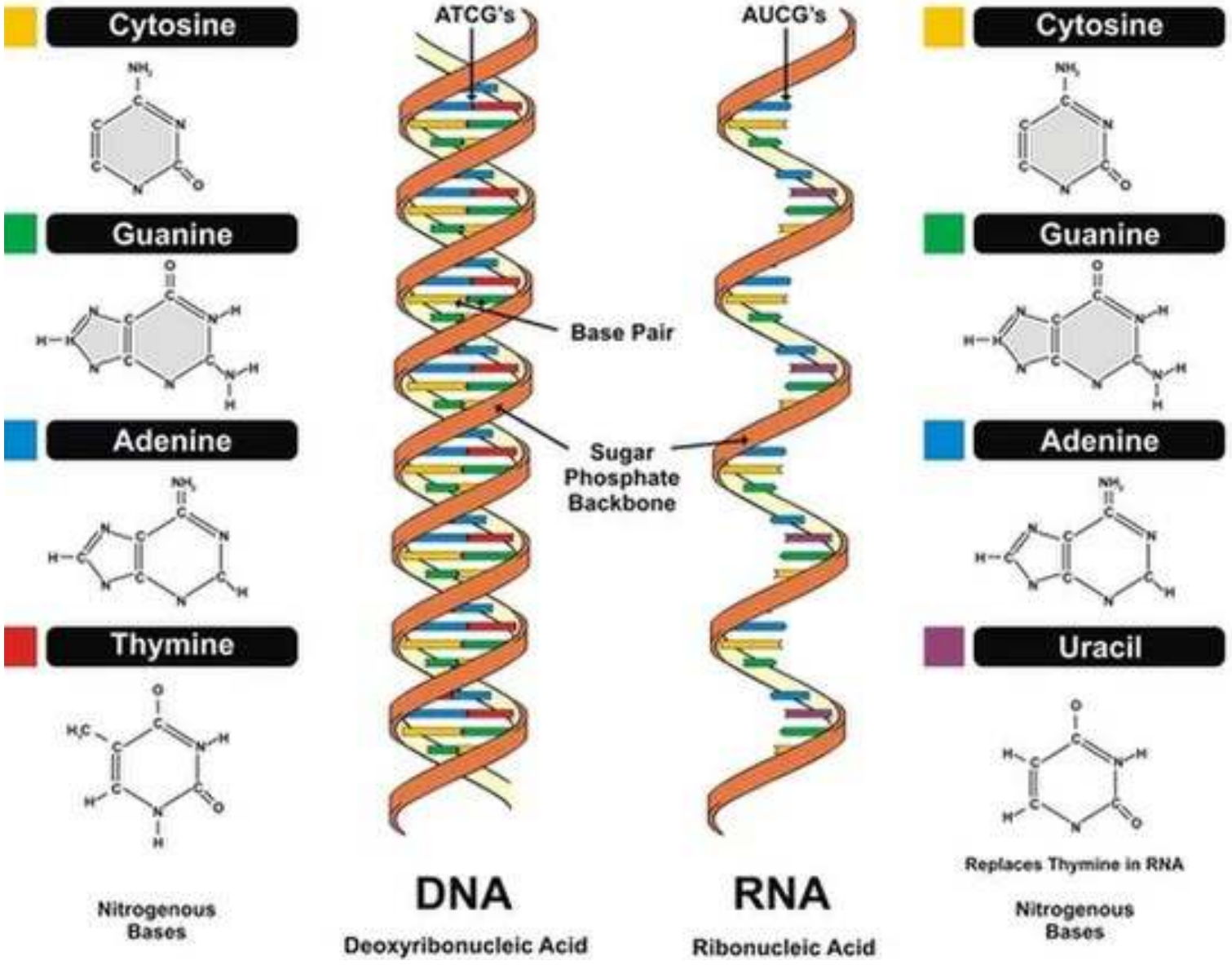
Estructura del DNA/RNA



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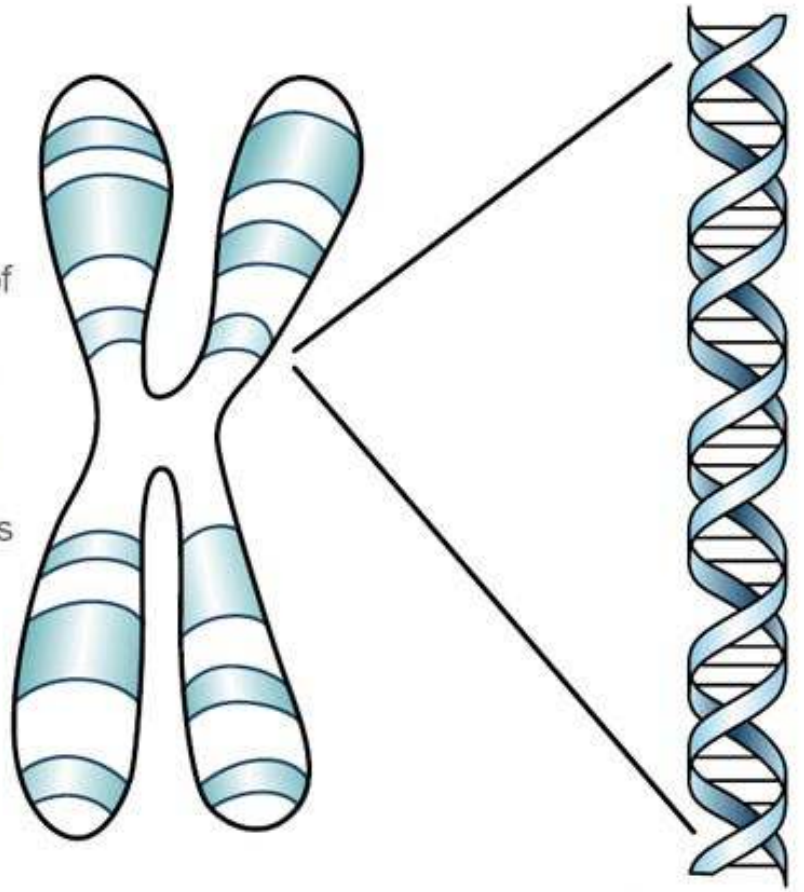
ESTRUCTURA DEL GEN

We all have 23 pairs of chromosomes. One pair of chromosomes determines our sex. The other 22 pairs of chromosomes are non-sex chromosomes and determine things like hair color and our eye color.



Chromosome

DNA

DNA codes the genetic information on a gene.

Gene

Each chromosome is made up of many genes. Genes are made of a section of a long molecule called DNA. Genes carry the genetic information.

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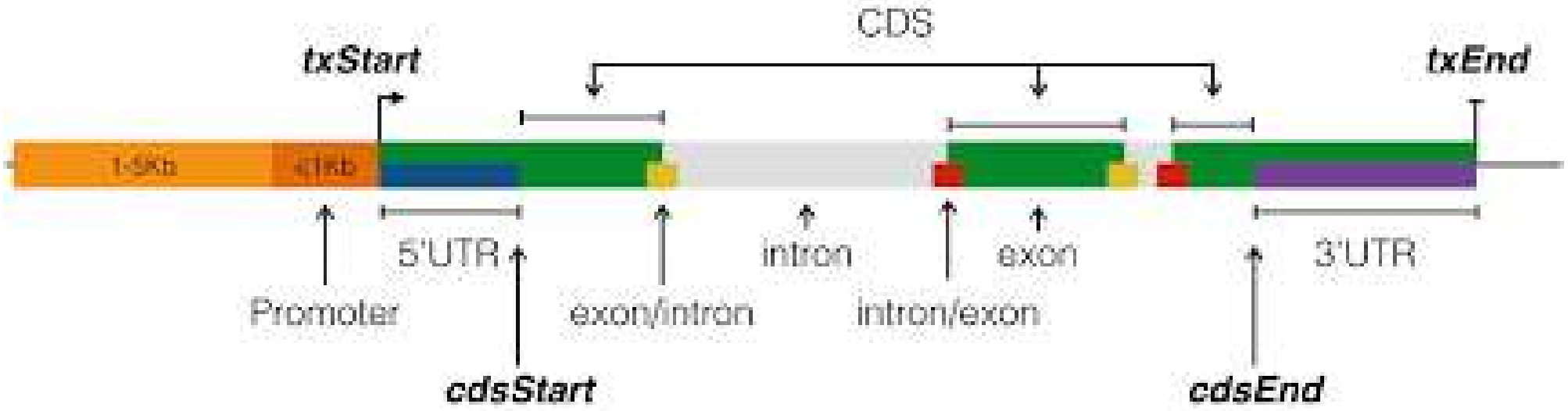
ESTRUCTURA DEL GEN



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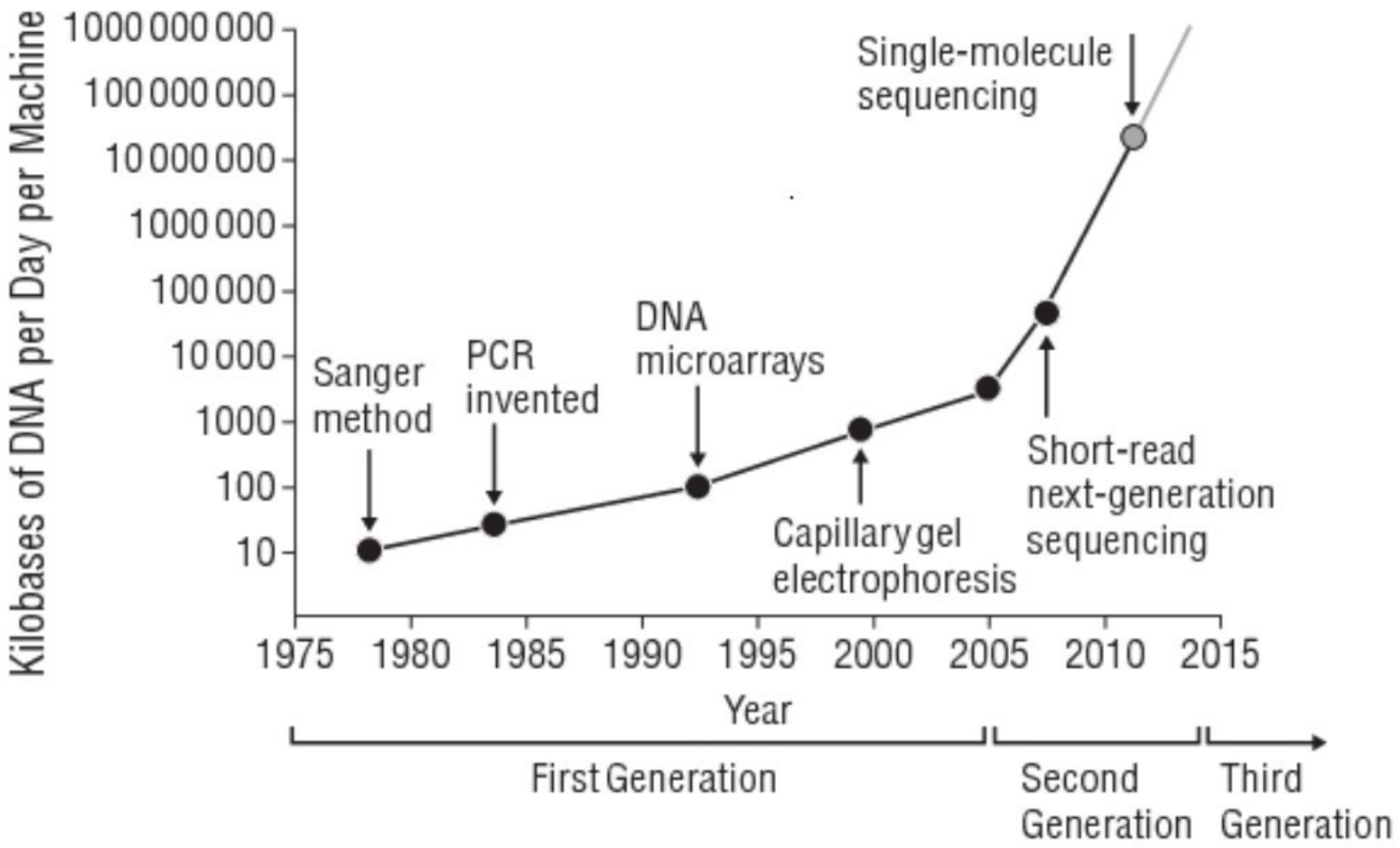
ESTRUCTURA DEL GEN



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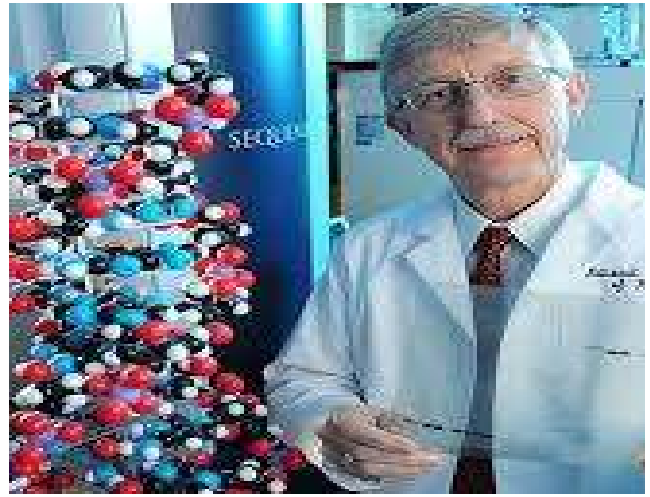
PROYECTO GENOMA HUMANO (1990 - 2000)

OBJETIVOS HGP

"Adquirir la **información** fundamental sobre nuestro material genético para profundizar en el conocimiento de la genética humana y el papel de los distintos genes en la salud y la enfermedad"

Francis Collins
Director *Human Genome Project*



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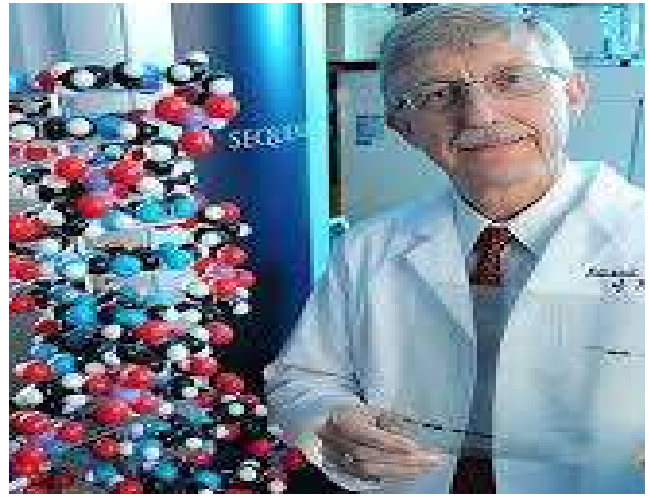
OBJETIVOS HGP

* Identificación de ≈ 30.000 genes
* Determinar la secuencia de ADN (3 billones pb)
* Generación de Bases de Datos públicas
* Mejorar herramientas para análisis de datos
* Transferencia de tecnología asociadas al sector privado
* Definir principios éticos, legales y sociales



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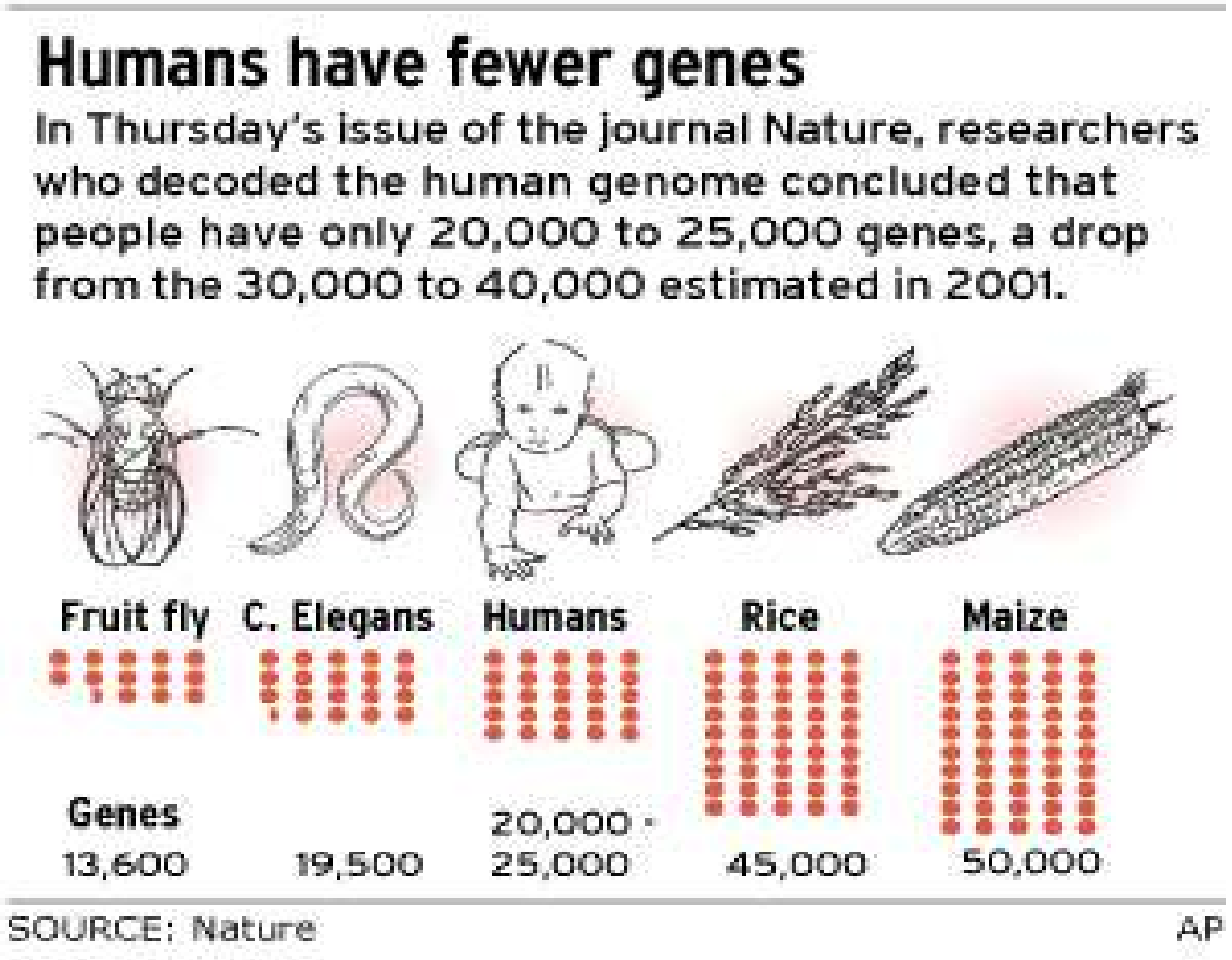
Historia de la Genómica
Proyecto Genoma Humano



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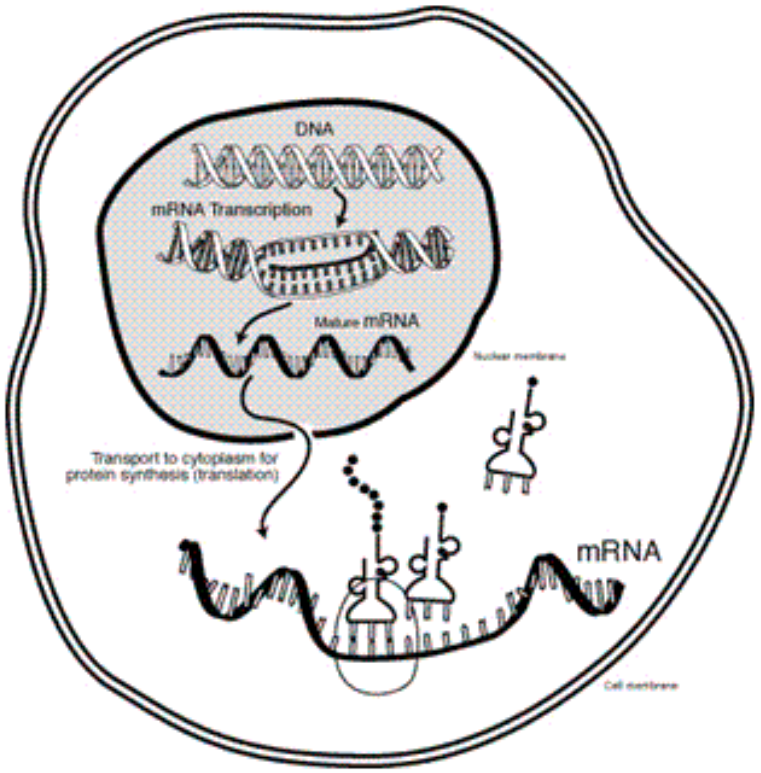
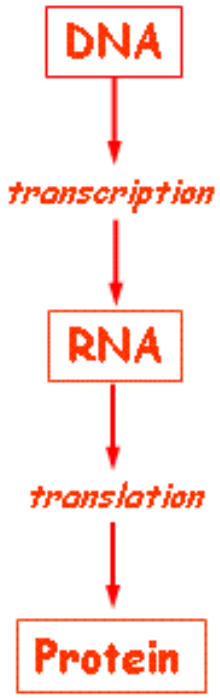
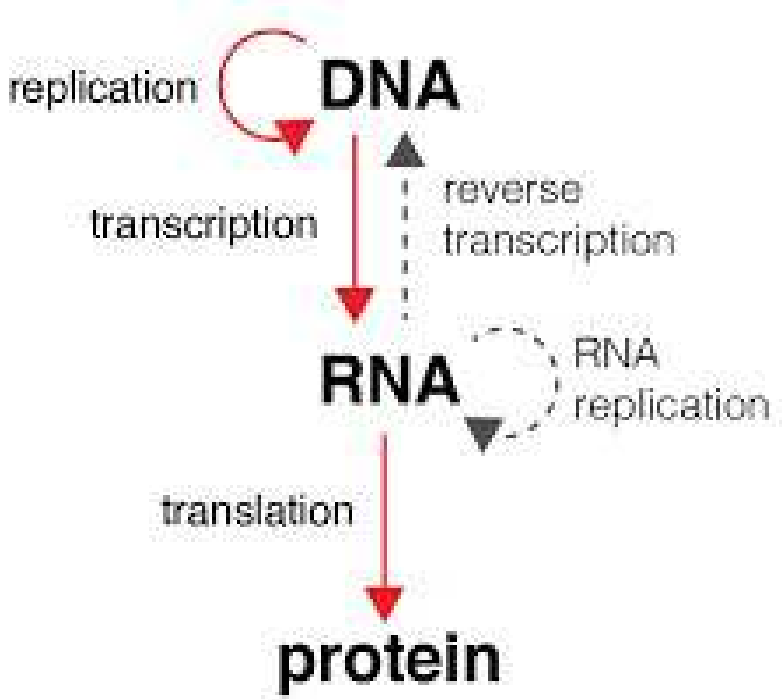
Dogma central de la biología *molecular*







La complejidad del genoma humano no radica en el # de genes, sino en la *interacción* entre ellos (genes reguladores, pleiotropismo, moléculas reguladoras - miARN/siARN)

La mayoría de los genes son **polimórficos**

ESTRATEGIA BIOLÓGICA PARA LA EVOLUCIÓN Y SUPERVIVENCIA DE LAS ESPECIES

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PLEIOTROPISMO

Fenómeno genético en el cual la expresión de un gen afecta en un individuo la manifestación fenotípica de otros caracteres no relacionados

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nature
articles

A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms

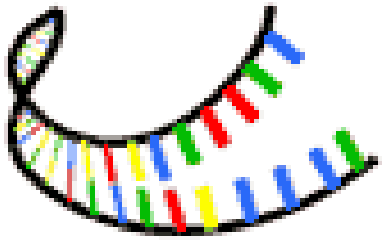
The International SNP Map Working Group*

*A full list of authors appears at the end of this paper.

We describe a map of 1.42 million single nucleotide polymorphisms (SNPs) distributed throughout the human genome, providing an average density on available sequence of one SNP every 1.9 kilobases. These SNPs were primarily discovered by two projects: The SNP Consortium and the analysis of clone overlaps by the International Human Genome Sequencing Consortium. The map integrates all publicly available SNPs with described genes and other genomic features. We estimate that 60,000 SNPs fall within exon (coding and untranslated regions), and 85% of exons are within 5 kb of the nearest SNP. Nucleotide diversity varies greatly across the genome, in a manner broadly consistent with a standard population genetic model of human history. This high-density SNP map provides a public resource for defining haplotype variation across the genome, and should help to identify biomedically important genes for diagnosis and therapy.

Los SNP constituyen hasta el 90% de todas las variaciones genómicas humanas, y aparecen en promedio, cada 1000 pb.

Asociados a la respuesta de los individuos a enfermedades hereditarias, enfermedades infecciosas y respuesta a fármacos



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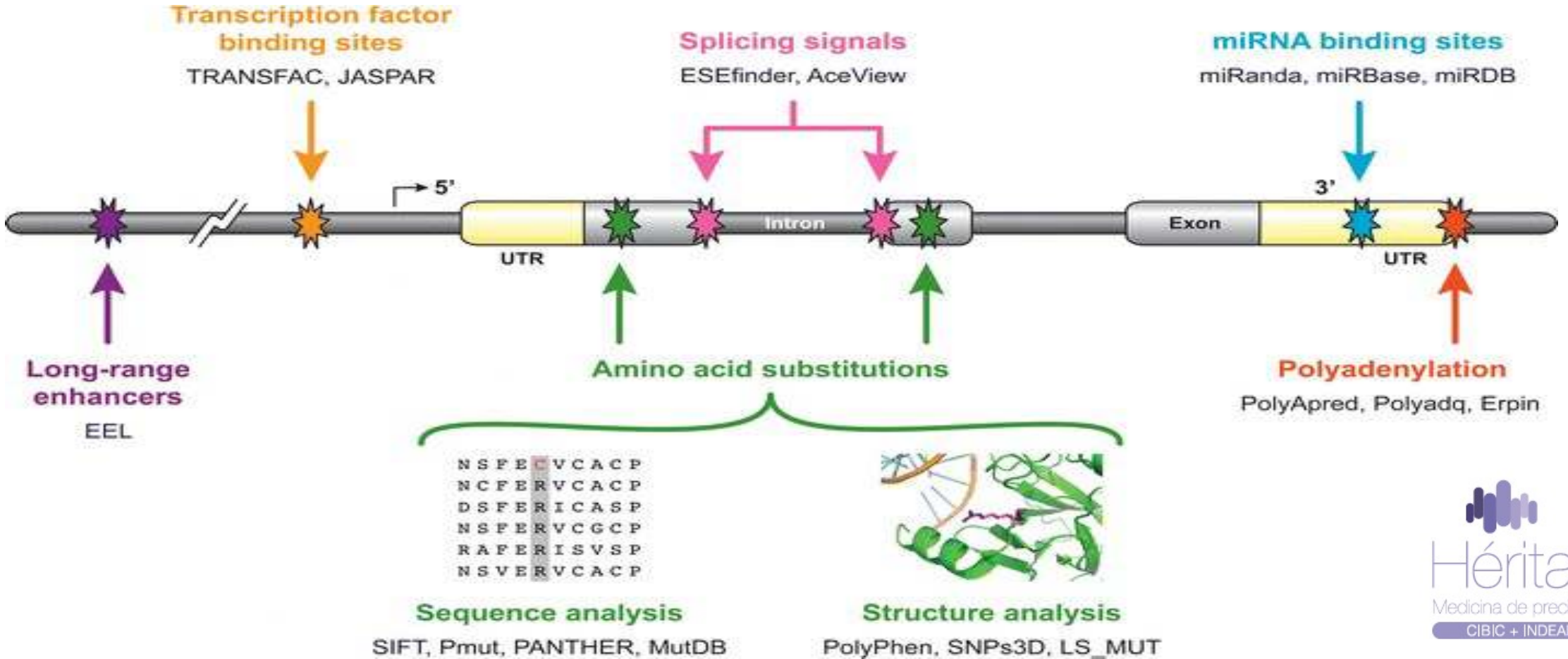
SNP codificantes: se localizan en secuencias codificantes pueden modificar la cadena de aminoácidos de proteínas

SNP no-codificantes: en regiones no codificantes. Consecuencias en el proceso de traducción (*splicing*, factores de transcripción) > Reguladores



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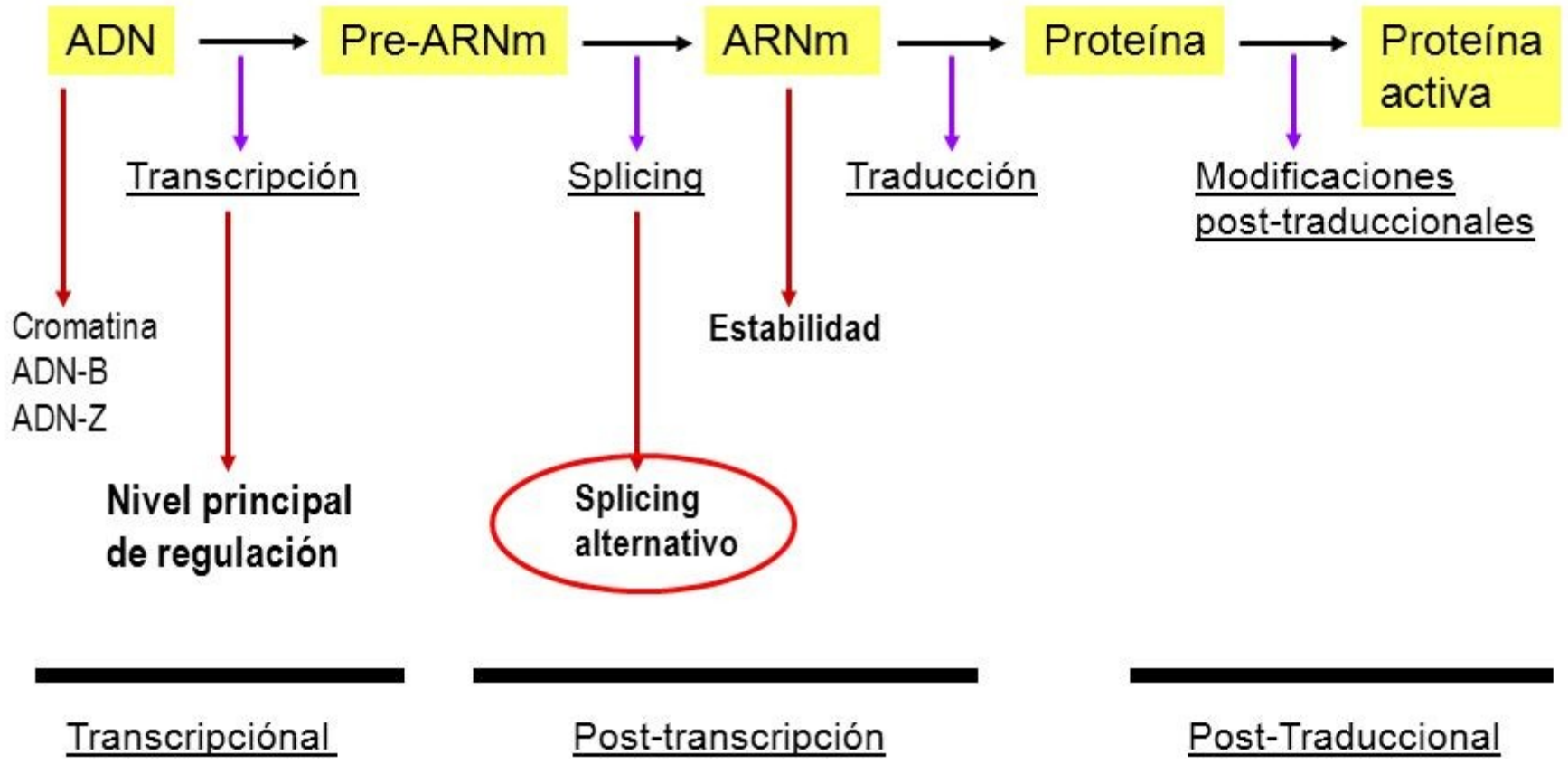
REGULACIÓN GENÉTICA



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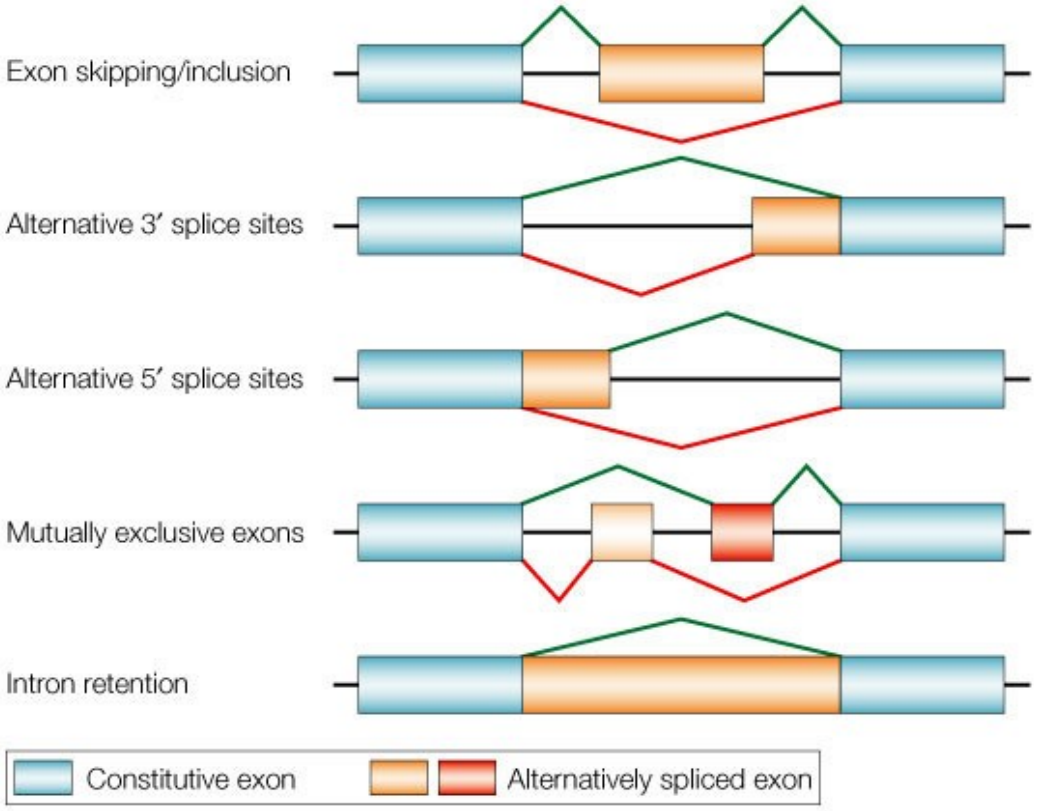
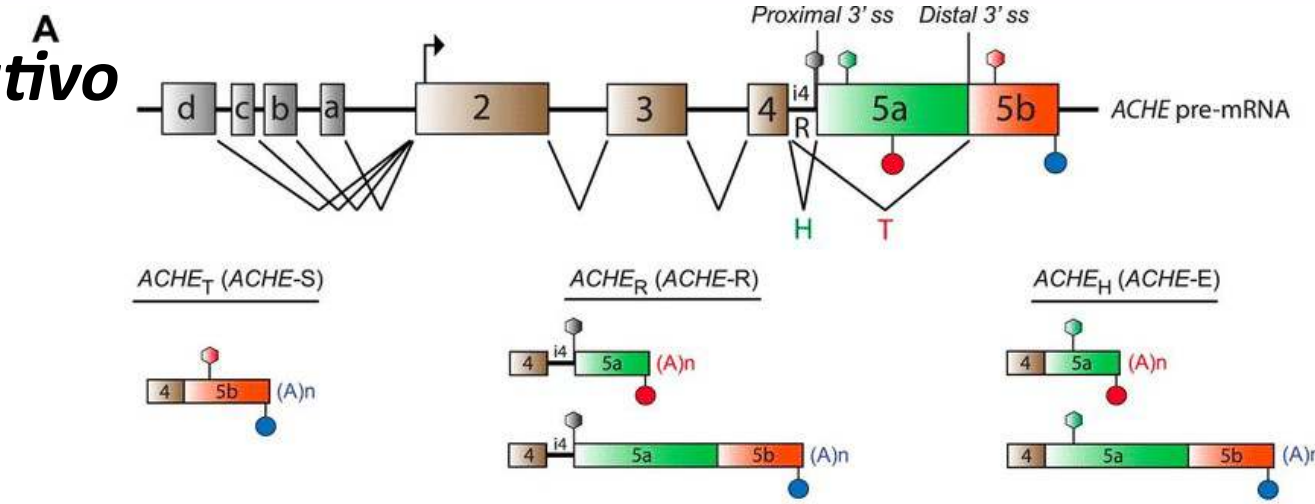
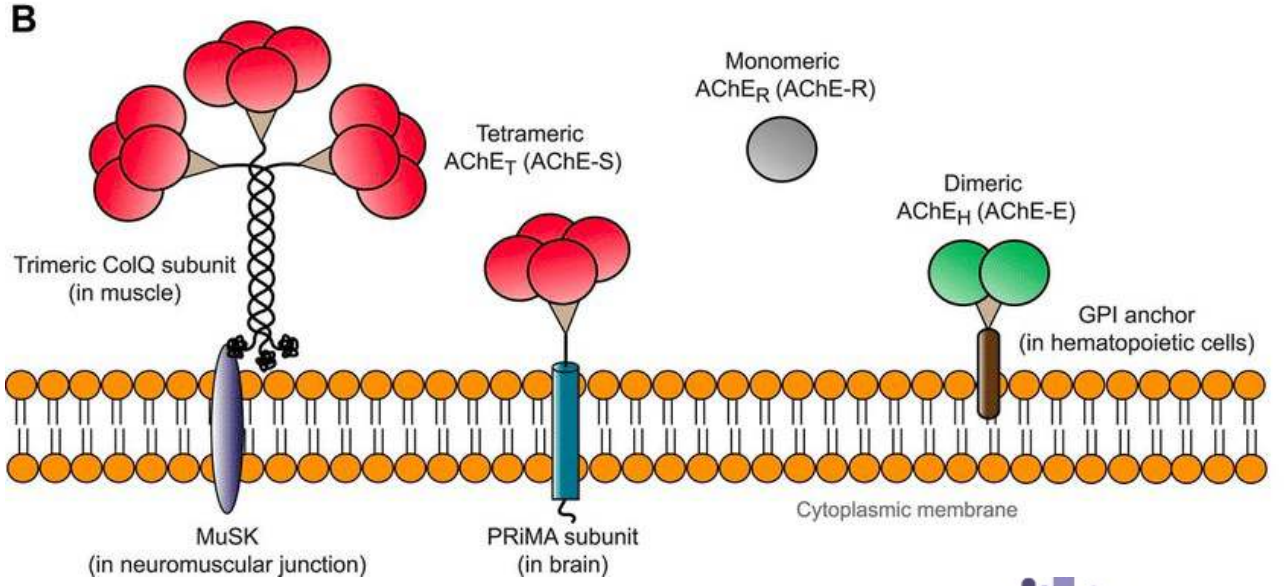
Regulación Genética – *Splicing alternativo*







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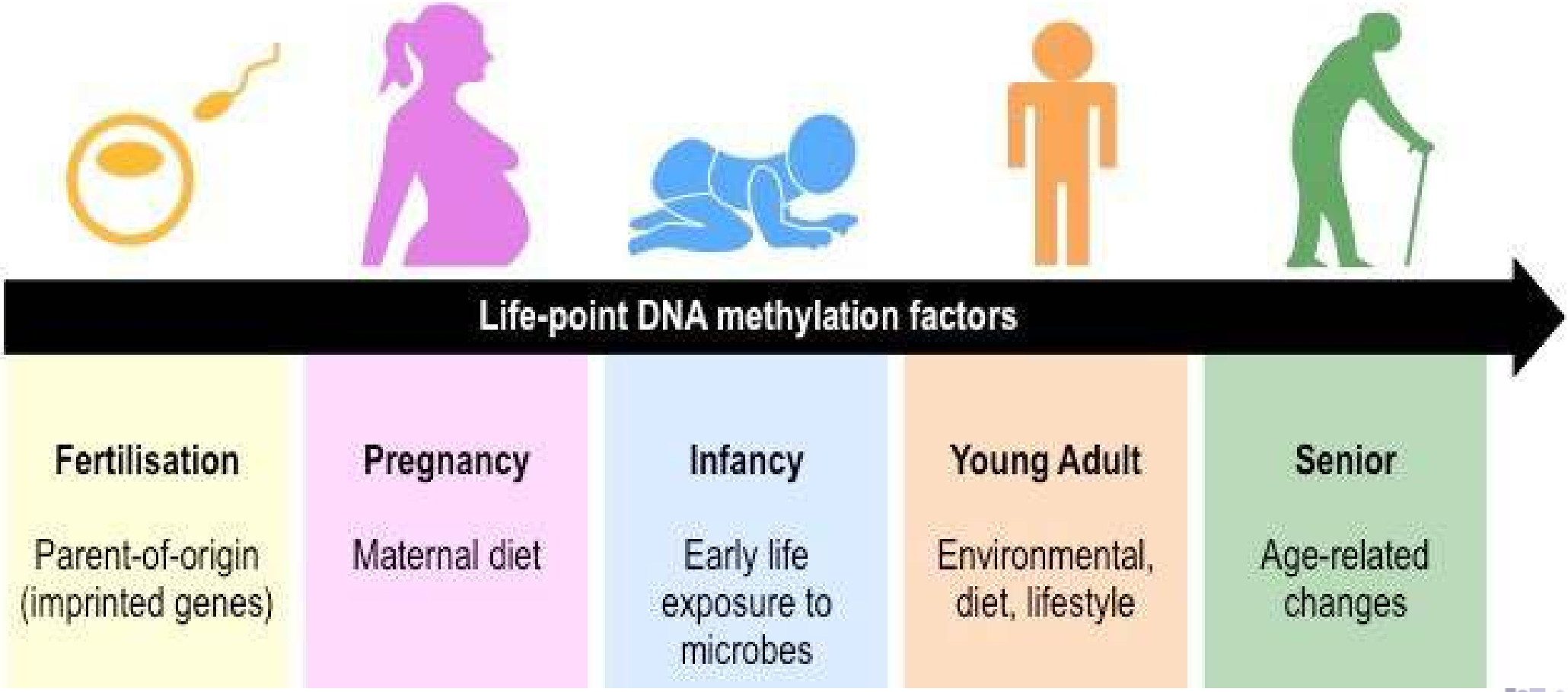
REGULACIÓN EPIGENÉTICA



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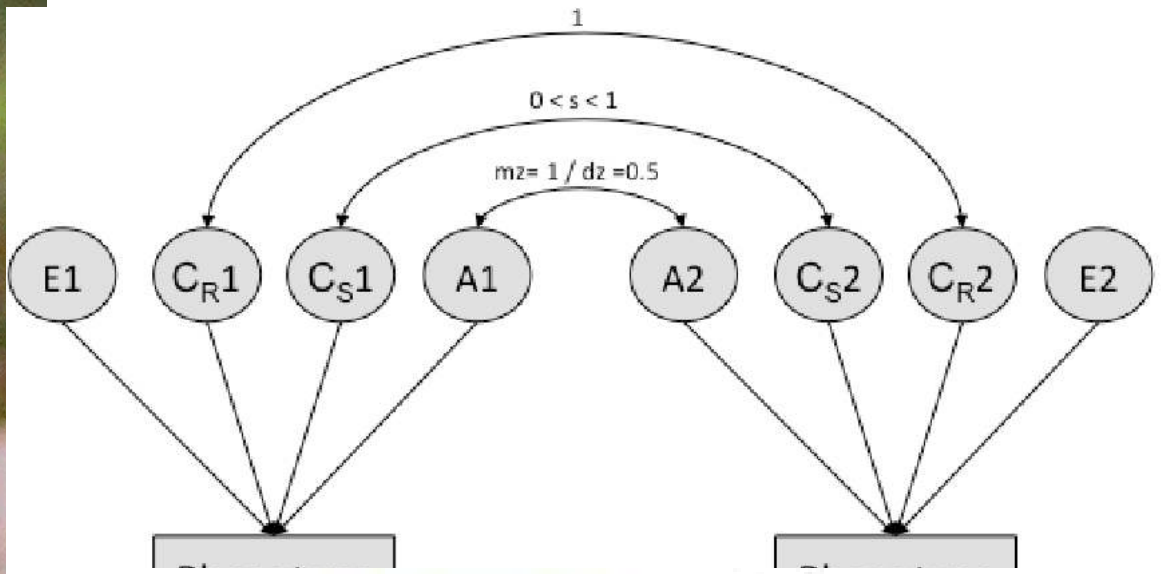
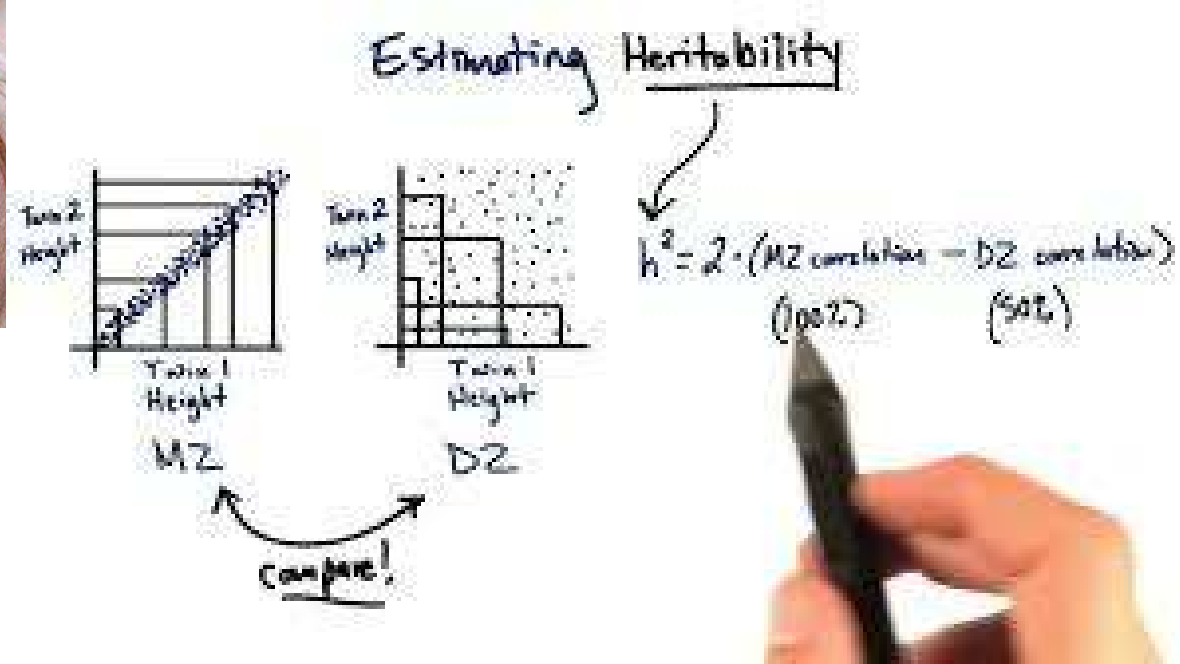
HEREDABILIDAD
Definición





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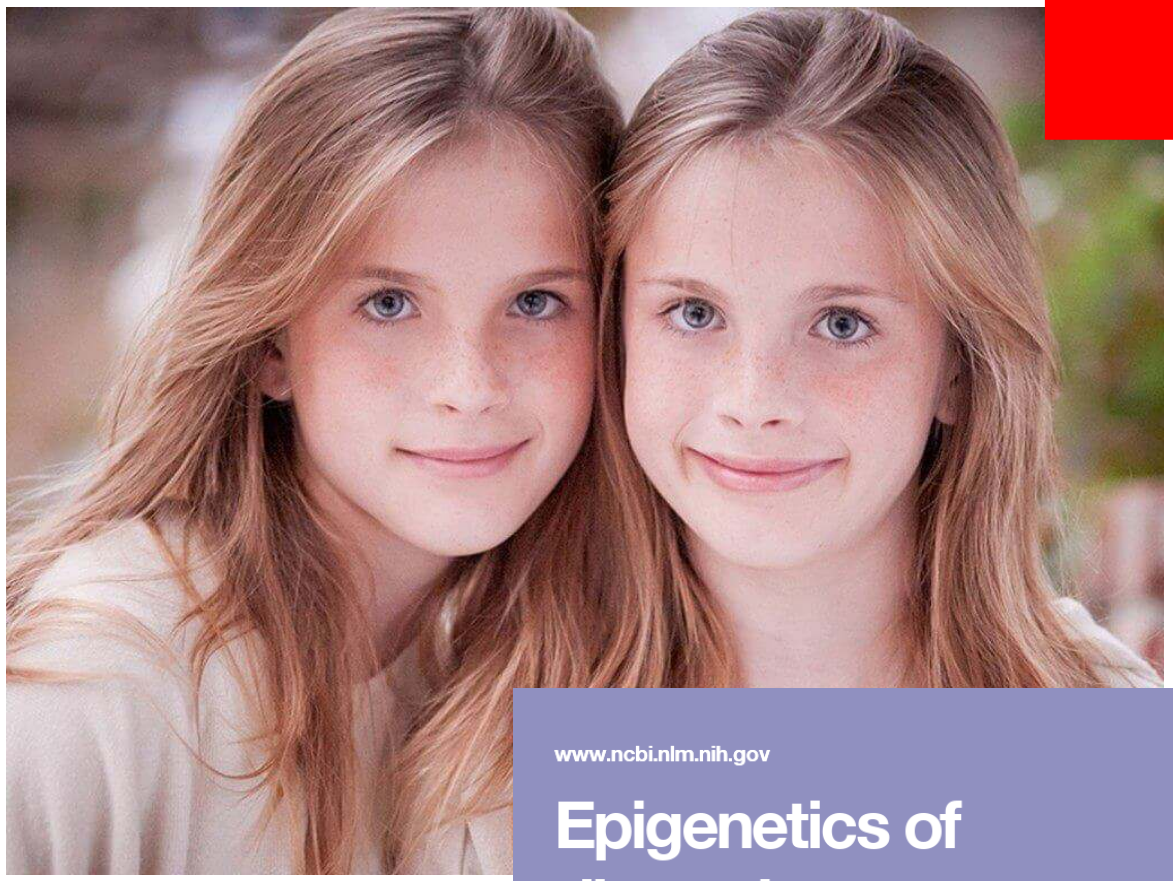
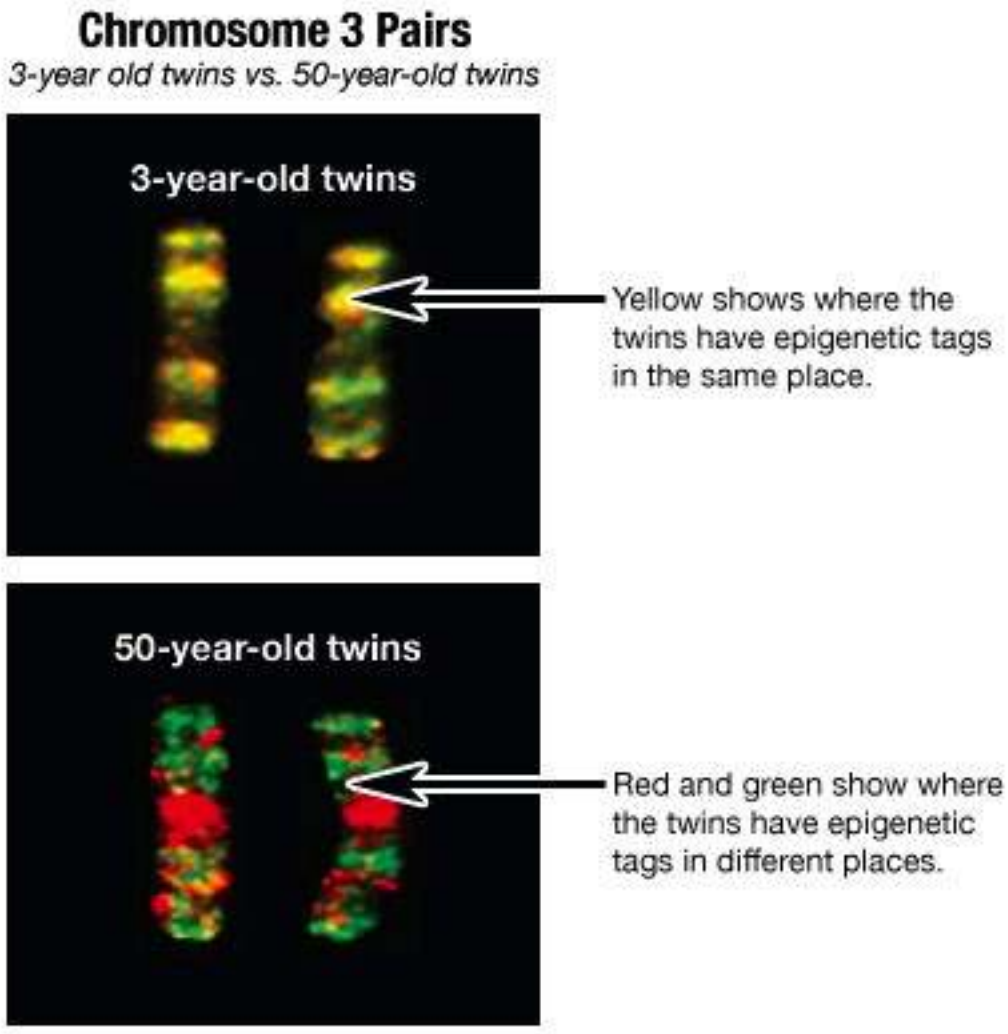
Regulación Epigenética
Definición



www.ncbi.nlm.nih.gov

Epigenetics of discordant monozygotic twins: implications for disease



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Cambios heredables de la expresión génica que ocurren *sin* que se presenten modificaciones en la secuencia de ADN

PRINCIPALES MECANISMO EPIGENÉTICOS

- Metilación del ADN
- Modificación post-traduccional de Histonas
- Silenciamiento de genes mediados por microARNs

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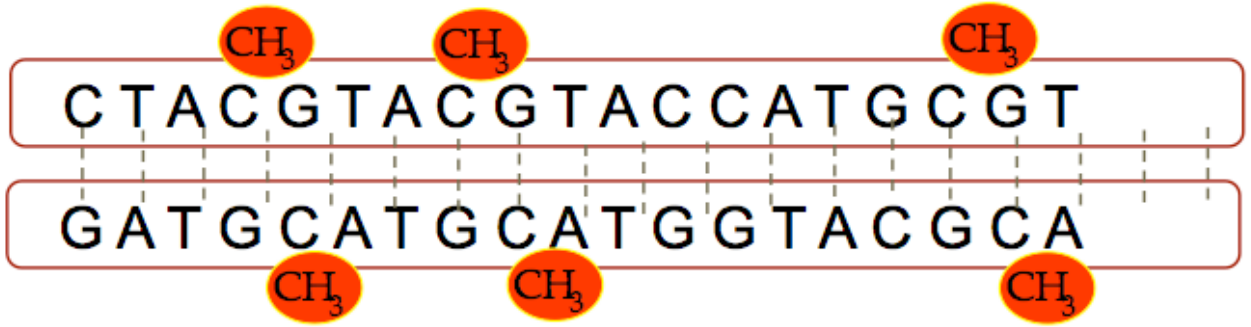
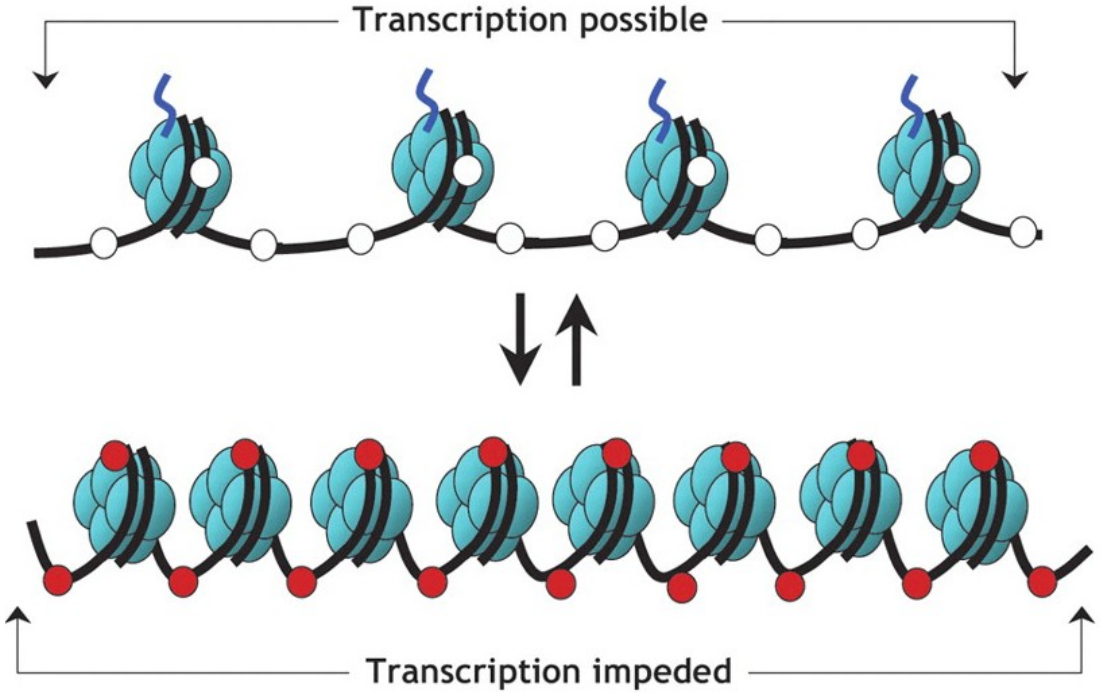
- Metilación del ADN
- Modificación post-traduccional de Histonas
- Silenciamiento de genes mediados por microARN



Ocurre generalmente en Citosinas, especialmente en nucleótidos emparejados CpG (Dímeros metilados CpG)



Químicamente muy estable
Mecanismo de silenciamiento de genes, impronta genómica, inactivación cromosoma X. Hipometilacion de ONCOGENES, Hipermetilación de genes supresores de tumores.

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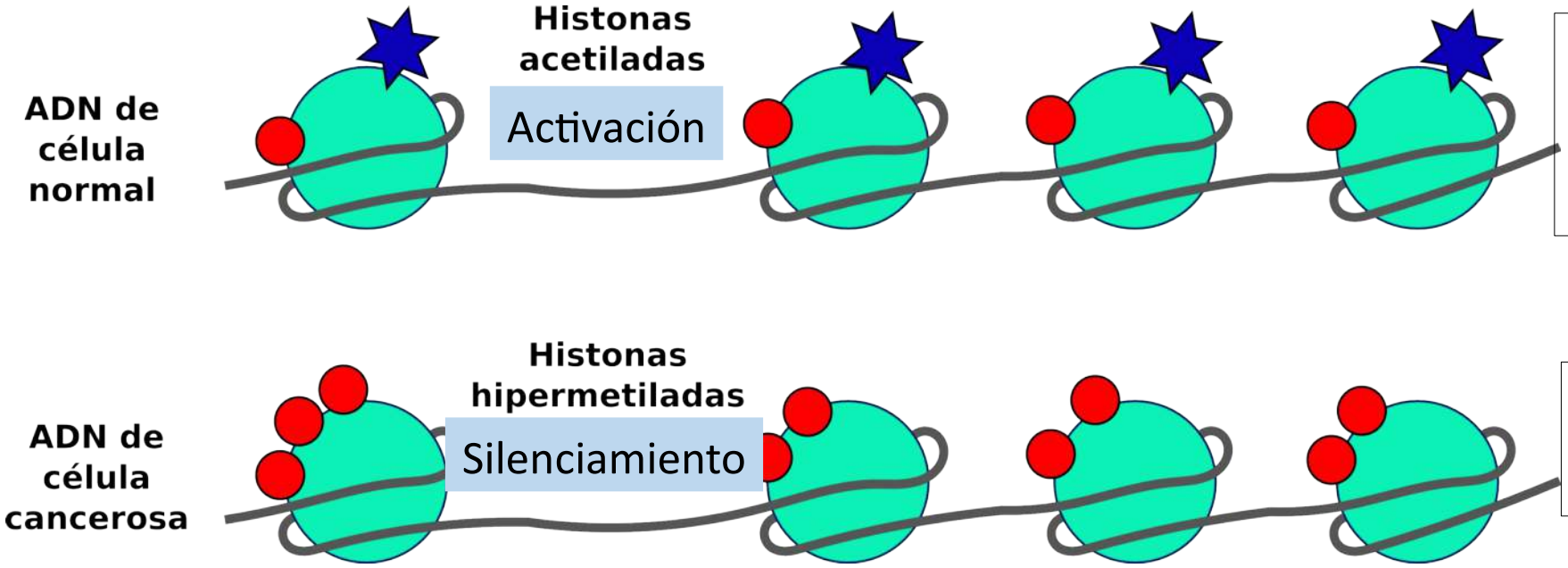
Regulación Epigenética

- Metilación del ADN
- Modificación post-traduccional de Histonas
- Silenciamiento de genes mediados por miARN



ENFERMEDAD DE PARKINSON (PD)

Modelos animales: Tx con inhibidores de inhibidores de acetilación de histonas

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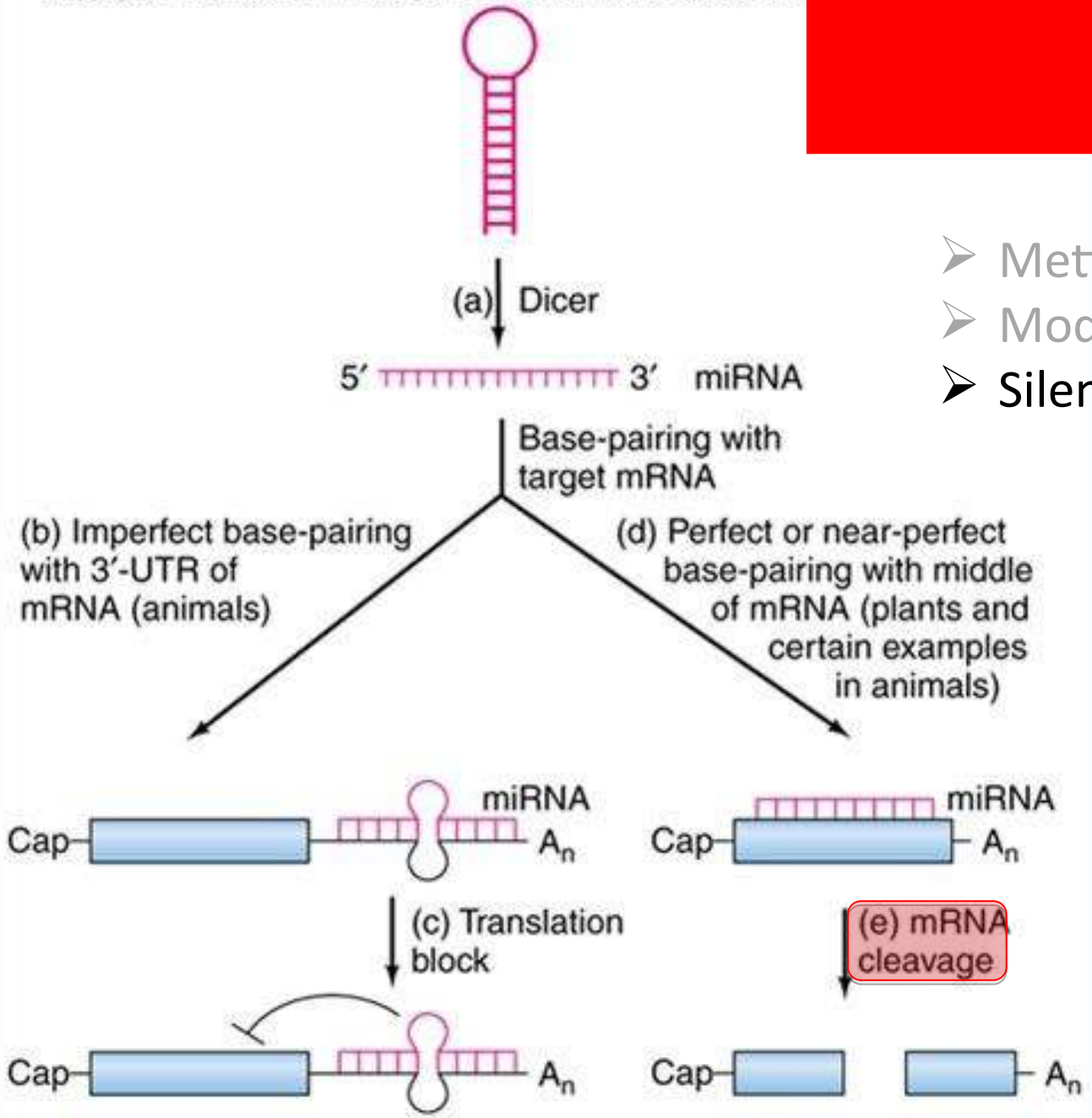
Regulación Epigenética



- Metilación del ADN
- Modificación post-traduccional de Histonas
- Silenciamiento de genes mediados por miARN

Enfermedad de Huntington (HD)

- Elevada variabilidad en miARN en región 3´en pacientes con HD
- miARN-34b upregulated (plasma) en pacientes HD asintomáticos

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ENFERMEDADES HEREDITARIAS

Enfermedades genéticas cuya característica principal es su supervivencia de generación en generación, transmitiéndose de padres a hijos

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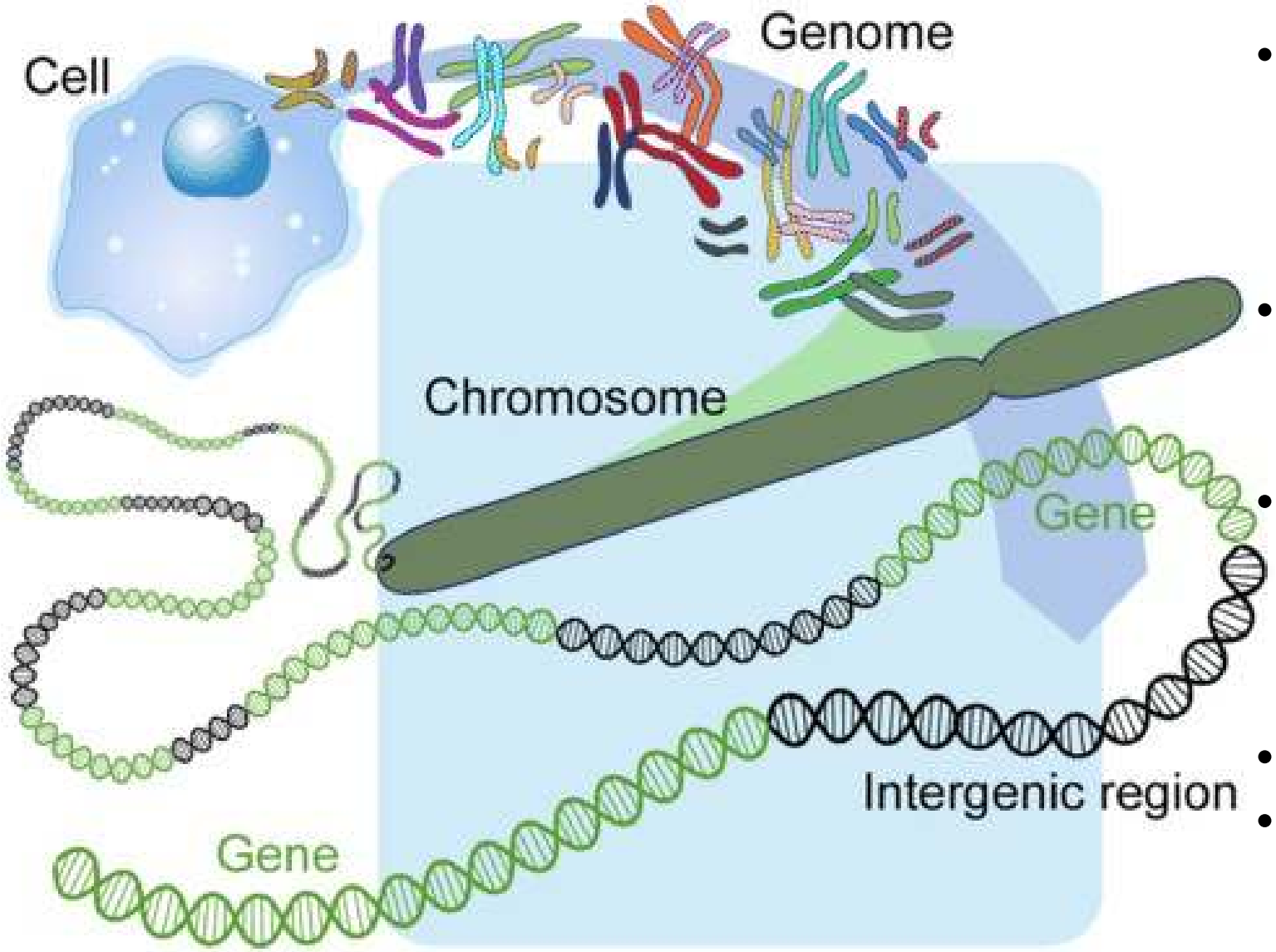

- **Cromosómicas**
  Anormalidades numéricas
  Anormalidades estructurales
- **Monogénicas**
  Herencia Mendeliana
- **Multifactoriales**
  Poligénicas
  GxE
- **Mitocondriales**
- (herencia materna)

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Enfermedades hereditarias Mendelianas

Árbol genealógico

El *pedigree* es una forma de análisis genético en donde el médico genetista hace un diagrama que muestra a un individuo con una característica estudiada y todos sus familiares conocidos.

El *pedigree* indica la presencia o ausencia de esta característica y si es aplicable la variación de expresión de la misma

Propósito facilitar el análisis genético de una característica examinando su posible **patrón de herencia** en una familia en particular

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EXISTEN DESVIACIONES DE LOS PATRONES MENDELIANOS CLÁSICOS…

- Penetrancia incompleta
- Expresividad variable
- Mutaciones de-novo
- Mosaicismo germinal
- Impronta genética
- Heterogeneidad de locus
- Mutaciones dinámicas
- Pleiotropismo

Generalmente los patrones de herencia no pueden ser definidos

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COMO SE ESTUDIABAN LAS ENFERMEDADES MENDELIANAS PREVIO A LA FINALIZACIÓN DEL PROYECTO GENOMA HUMANO?

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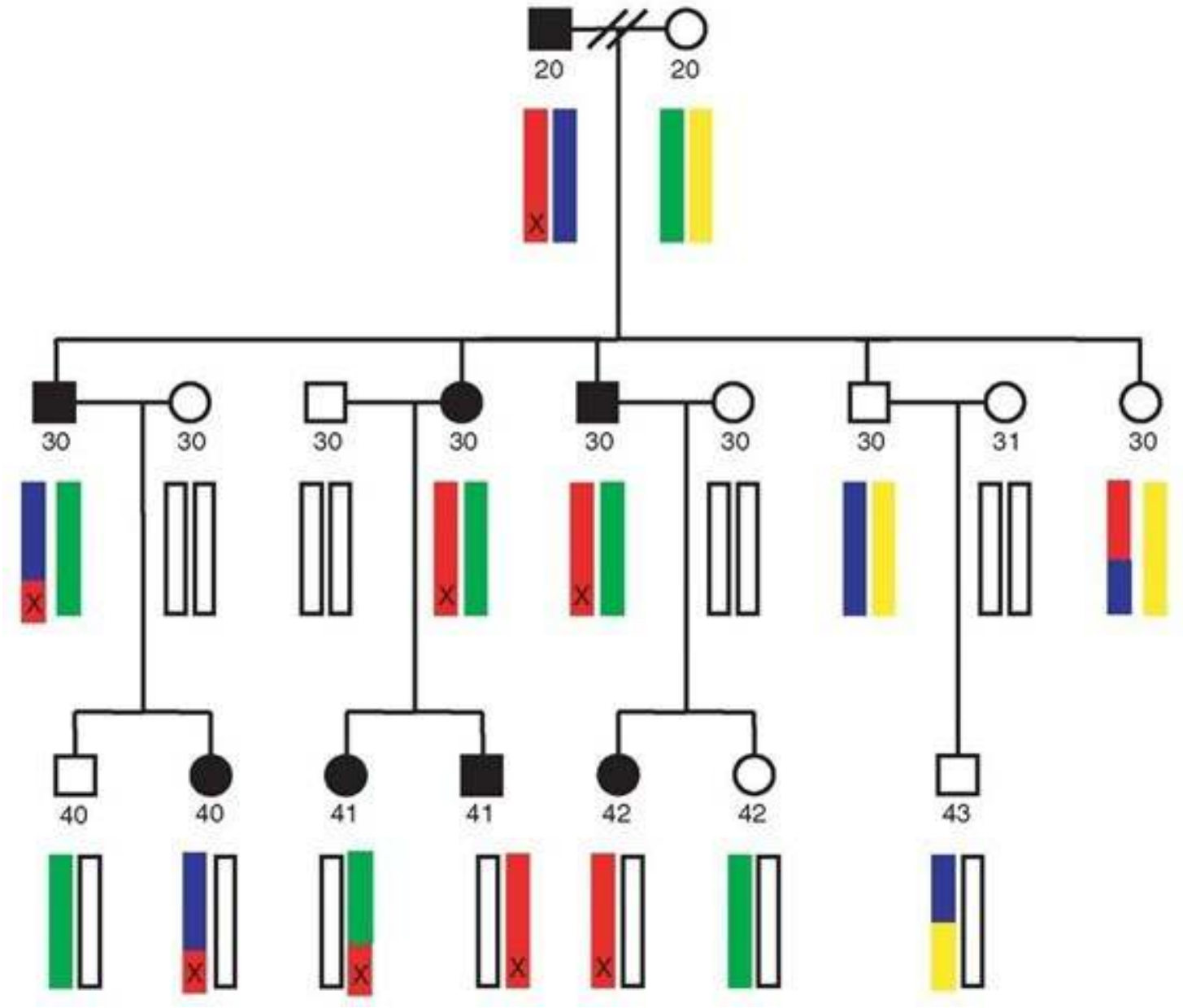
Enfermedades hereditarias Mendelianas - Estudios de Ligamiento



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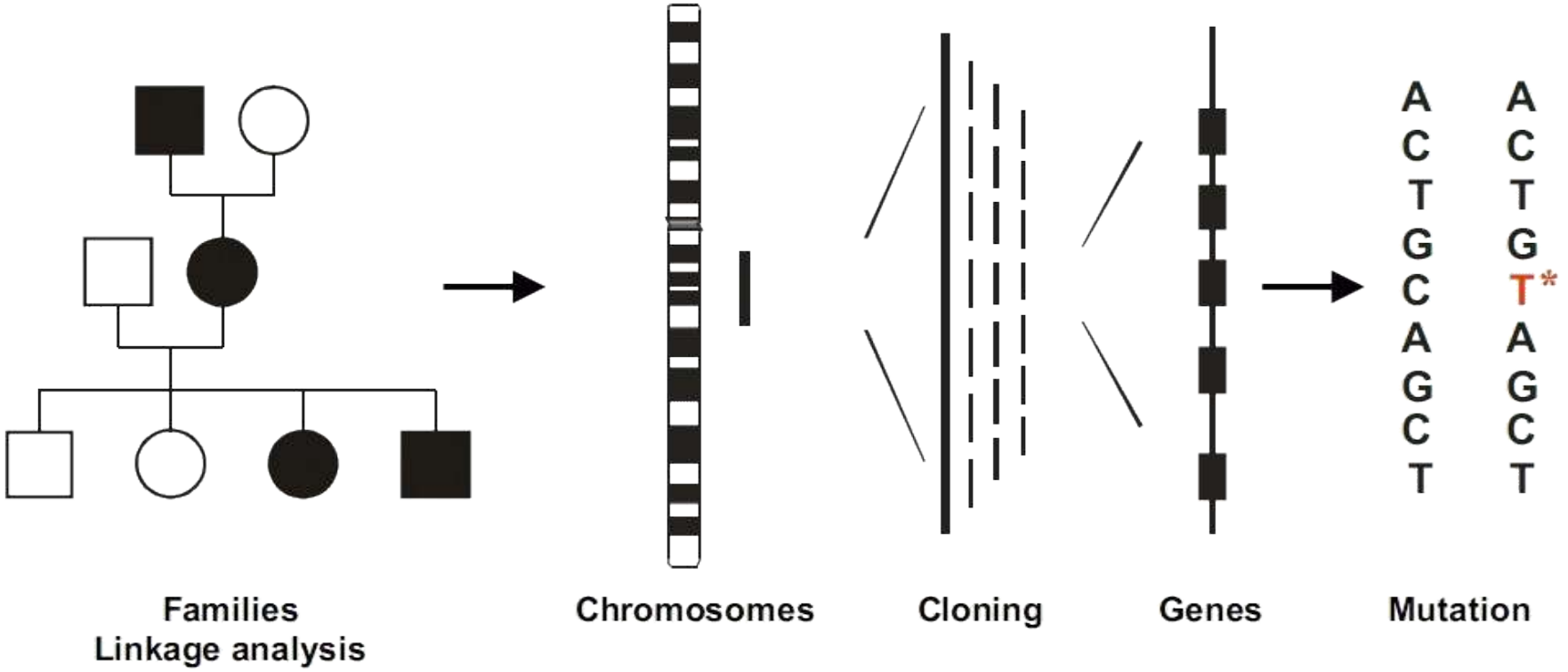
Gene mapping

Gene identification

Functional studies



Estudios funcionales para **confirmar la causalidad** de la VARIANTE GENÉTICA identificada

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Estudios de Ligamiento

Limitaciones

- Tiempo de realización: 3-6 meses
- Altamente laborioso
- Familias numerosas
- Polimorfismos informativos (microsatélites)
- Meiosis informativas (por lo menos 3 generaciones)
- Requiere *finemapping* para posterior secuenciación de genes candidatos

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COMO ESTUDIAMOS LAS ENFERMEDADES MENDELIANAS EN LA ERA POSTGENOMICA?

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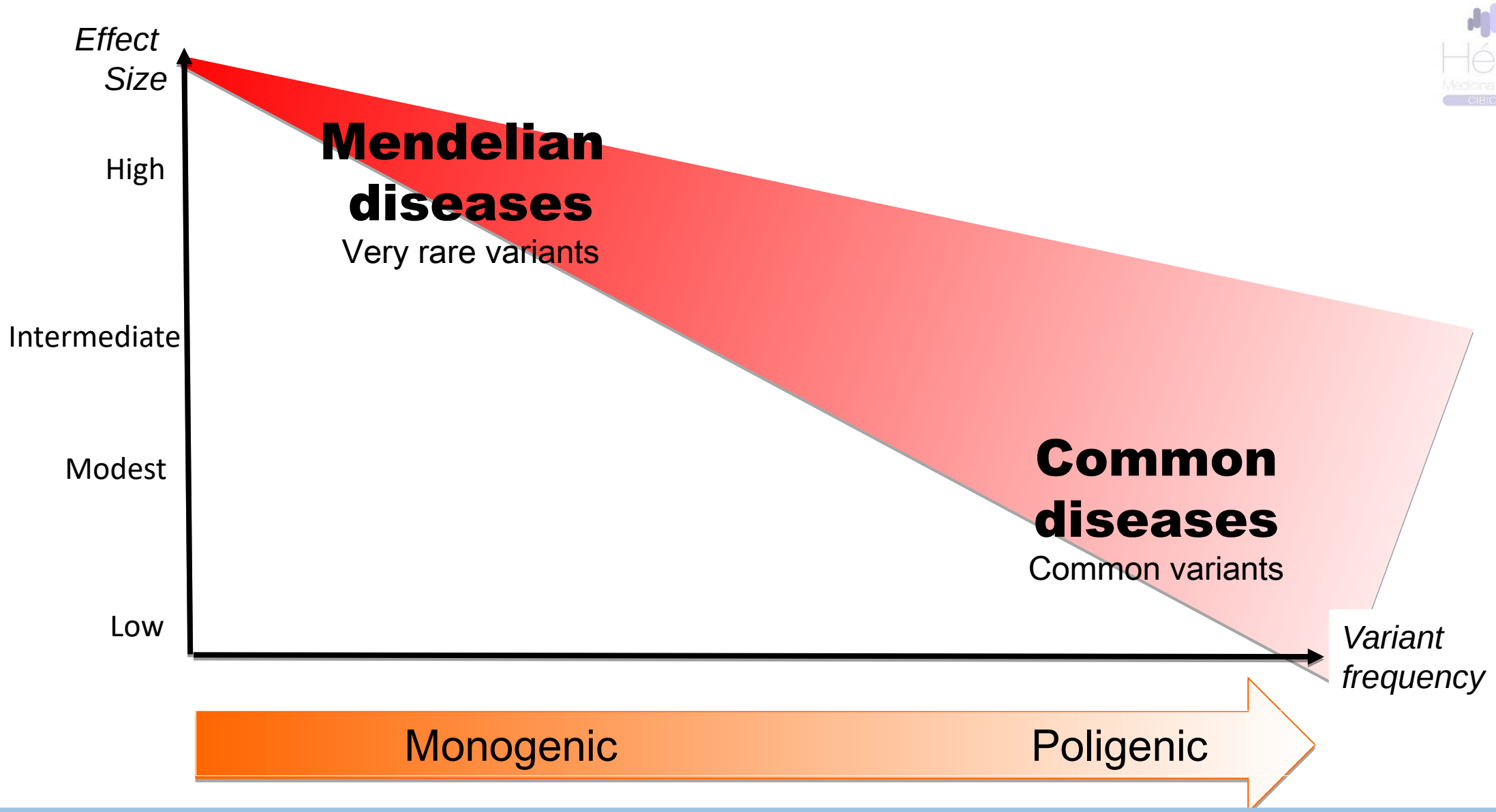
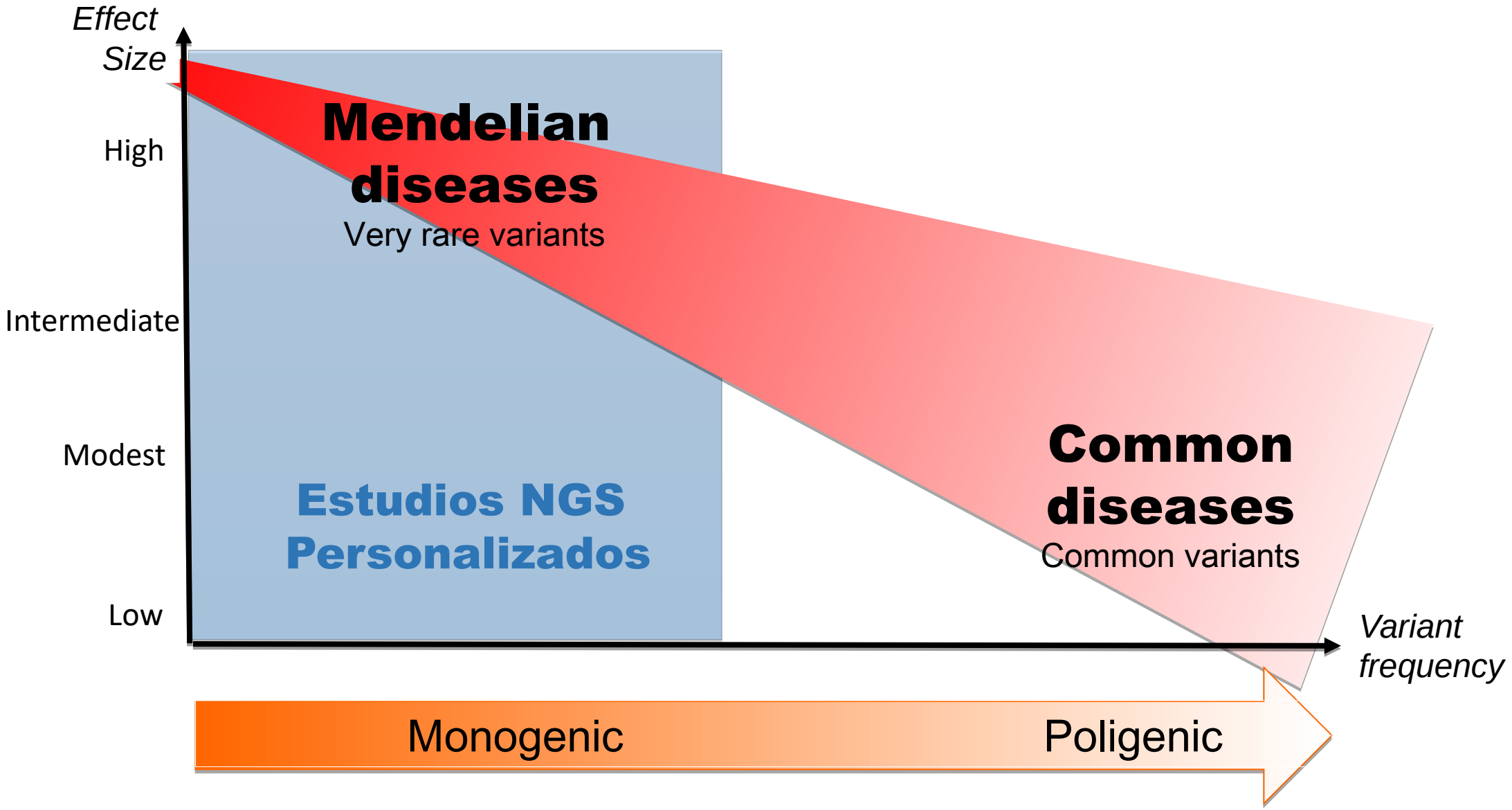
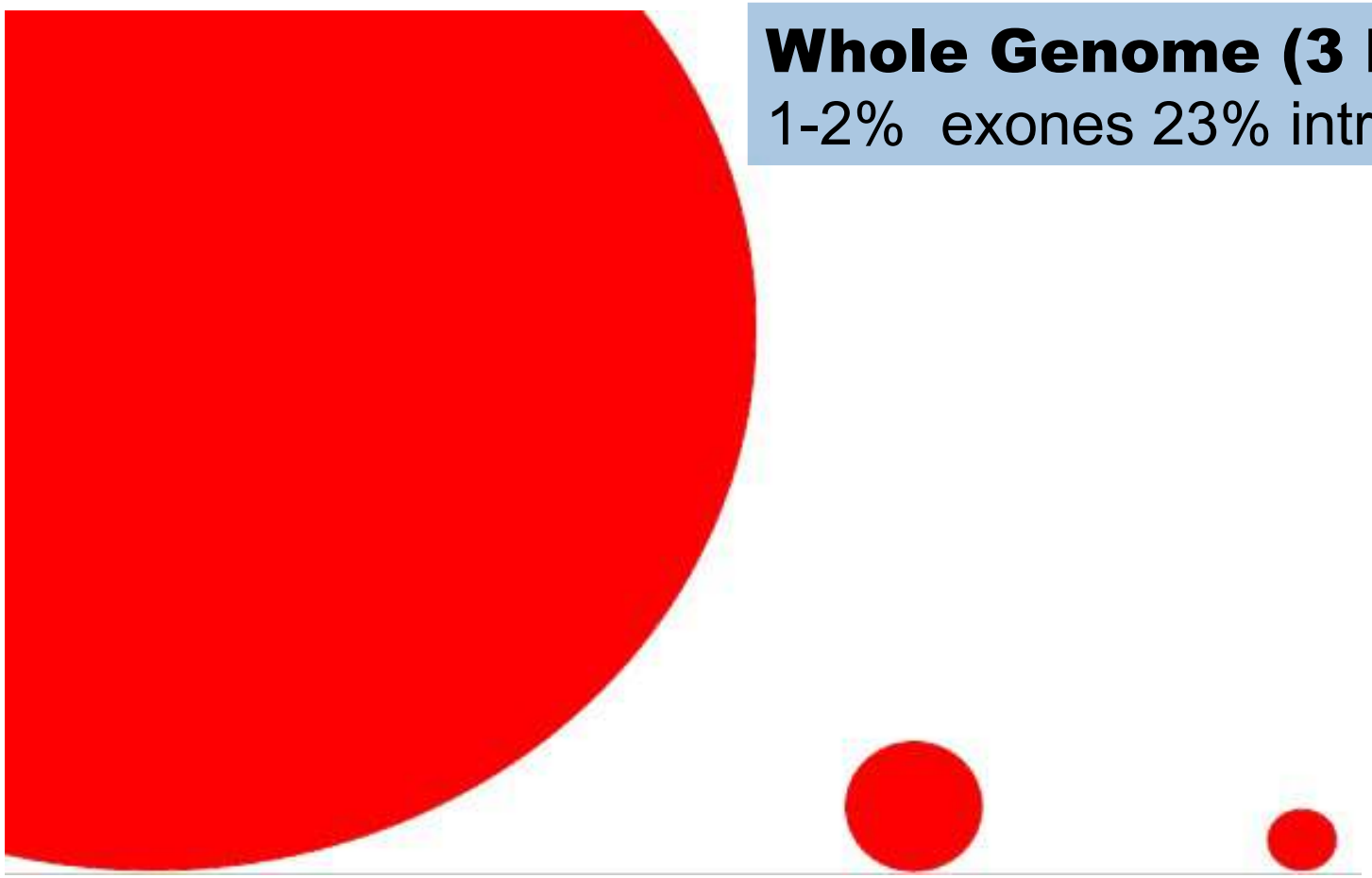


En función al tipo de patología sospechada, se utilizarán diferentes técnicas de biología molecular


Effect Size
High
Intermediate
Modest
Low
Mendelian diseases
Very rare variants
Estudios NGS Personalizados
Common diseases
Common variants
Variant frequency
Monogenic
Poligenic

PROYECTO HGP

Whole Genome (3 billones pb) 25,000 genes
1-2% exones 23% intrones 75% intergenómico



GENOMA
3000Mb

EXOMA COMPLETO
30Mb

EXOMA CLÍNICO
12-17Mb

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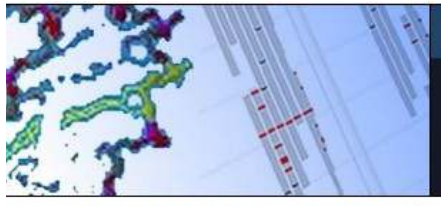
OMIM Entry Statistics

Number of Entries in OMIM (Updated July 24th, 2020) :

MIM Number Prefix	Autosomal	X Linked	Y Linked	Mitochondrial	Totals
Gene description *	15,489	743	51	37	16,320
Gene and phenotype, combined +	34	0	0	0	34
Phenotype description, molecular basis known #	5,463	346	5	33	5,847
Phenotype description or locus, molecular basis unknown %	1,419	117	4	0	1,540
Other, mainly phenotypes with suspected mendelian basis	1,669	103	3	0	1,775
Totals	24,074	1,309	63	70	25,516

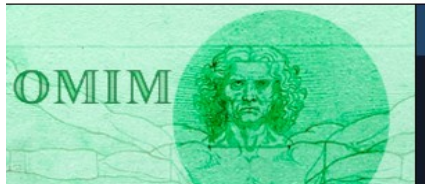


dbSNP

Database of single nucleotide polymorphisms (SNPs) and multiple small-scale variations that include insertions/deletions, microsatellites, and non-polymorphic variants.

ClinVar

ClinVar aggregates information about genomic variation and its relationship to human health.



OMIM

OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. OMIM is authored and edited at the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, under the direction of Dr. Ada Hamosh. Its official home is omim.org.

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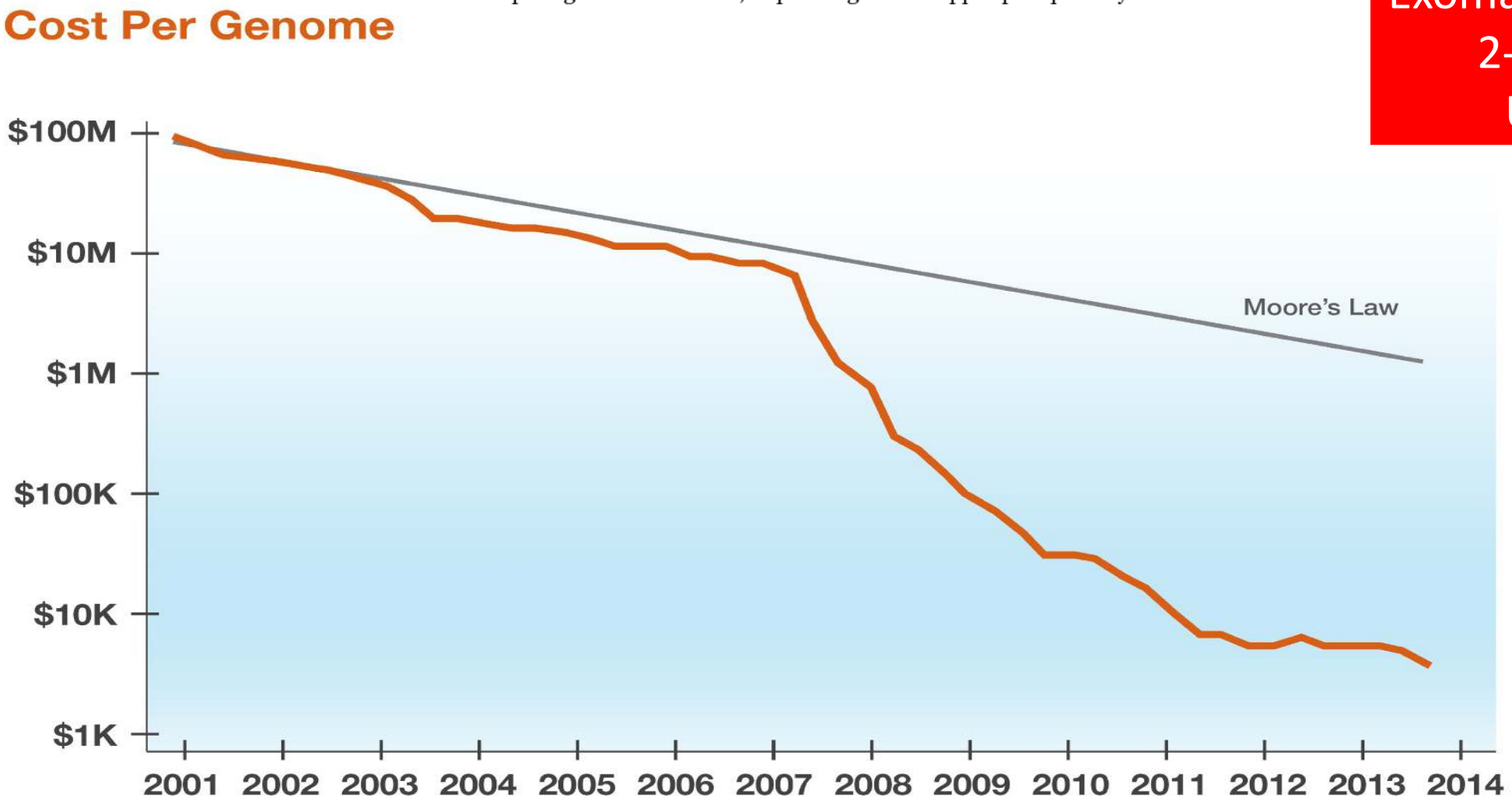
1990
PGH – 13 años
U$D 3.000.000.000

Falling fast

In the first few years after the end of the Human Genome Project, the cost of genome sequencing roughly followed Moore's law, which predicts exponential declines in computing costs. After 2007, sequencing costs dropped precipitously.

2020
Exoma Clínico dirigido
2-4 semanas
U$D < 700



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exac.broadinstitute.org

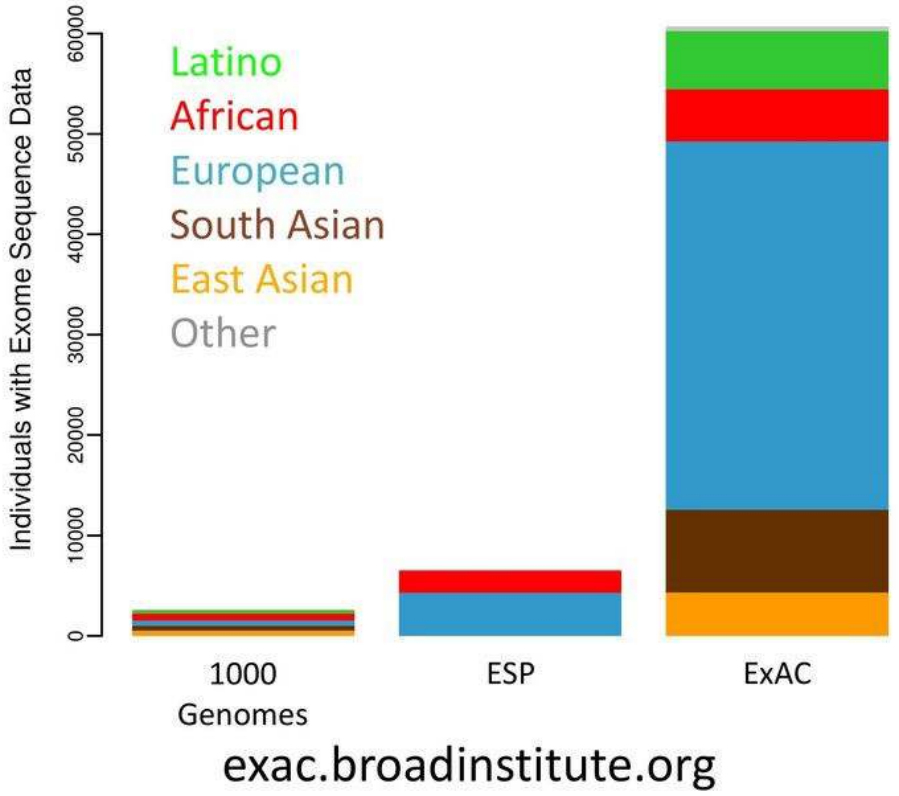
ExAC Browser

ESTANDARIZACIÓN DE DATASETS de proyectos de secuenciación existentes

Generación de grandes grupos control

Herramienta indispensable para interpretación de variantes en el contexto de estudios NGS

Exome Aggregation Consortium (ExAC)



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Análisis bioinformático / **Interpretación de variantes**

- ✓ **OMIM (≅4000 genes, >6200 fenotipos GENES)**
- ✓ **Databases ExAc / GnomAD**
- ✓ **Clasificación de variantes (ACMG)**

- ☑ **Benign**
- ☑ **Likely-benign**
- ☑ **Variant of unknown significance**
- ☑ **Likely-patogenic**
- ☑ **Pathogenic**

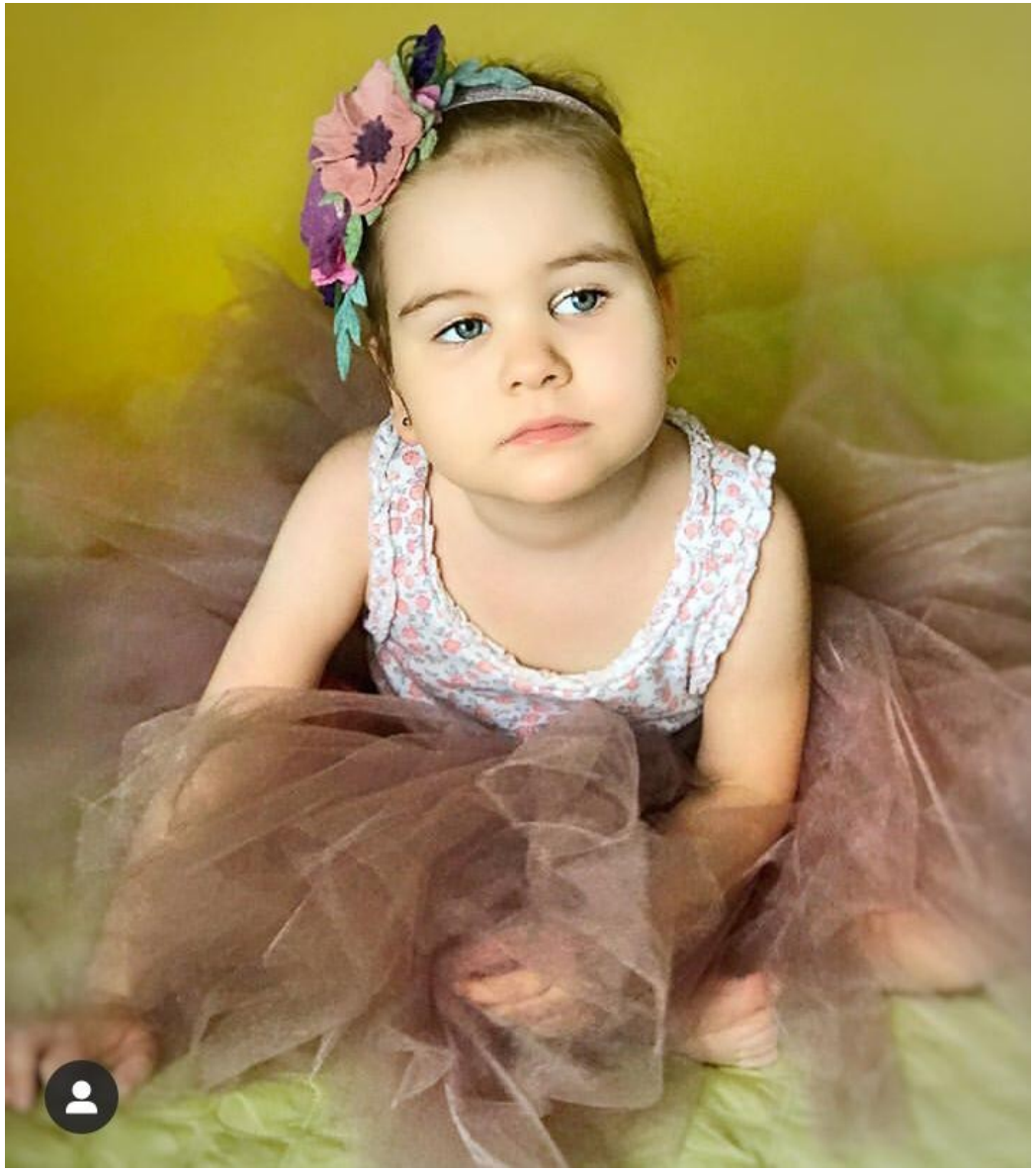
Benign		Pathogenic			
		Moderate	Strong	Very strong	
Computational and predictive data	Multiple lines of computational evidence suggest no impact on gene /gene product BP4 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
		missense variants and path. missenses common PP2	hal hot spot or well studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
			De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
	Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		

Programas de Controles externos para validacion de estudios NGS

EMQN The European Molecular Genetics Quality Network

COLLEGE of AMERICAN PATHOLOGISTS

Diagnóstico molecular = Tratamiento ? SINDROME DE RETT



#rettsyndrome
abretesp

Diagnóstico molecular = Tratamiento? ENFERMEDAD DE BATTEN

A custommade drug appears to be helping Mila, a 7-year-old born with Batten disease. JULIE AFFLERBAUGH

A tailormade drug developed in record time may save girl from fatal brain disease

By **Jocelyn Kaiser** | Oct. 19, 2018 , 9:00 PM

"N-of-one" clinical Trials

2016
Primeros síntomas

2017 Enero
Consulta Boston Children´s Hospital

2018 Enero
Oligo antisense "Milasen"

Diagnóstico molecular = Tratamiento? SINDROME DE CANTÚ

Conclusiones

- Costos de estudios moleculares cada vez más accesibles
- Algoritmos diagnósticos clínicos → **QUÉ**
- Algoritmos diagnósticos moleculares → **CÓMO**
- Equipos interdisciplinarios para diagnóstico de pacientes

DESARROLLO DE TRATAMIENTOS !!!

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Gracias !!!

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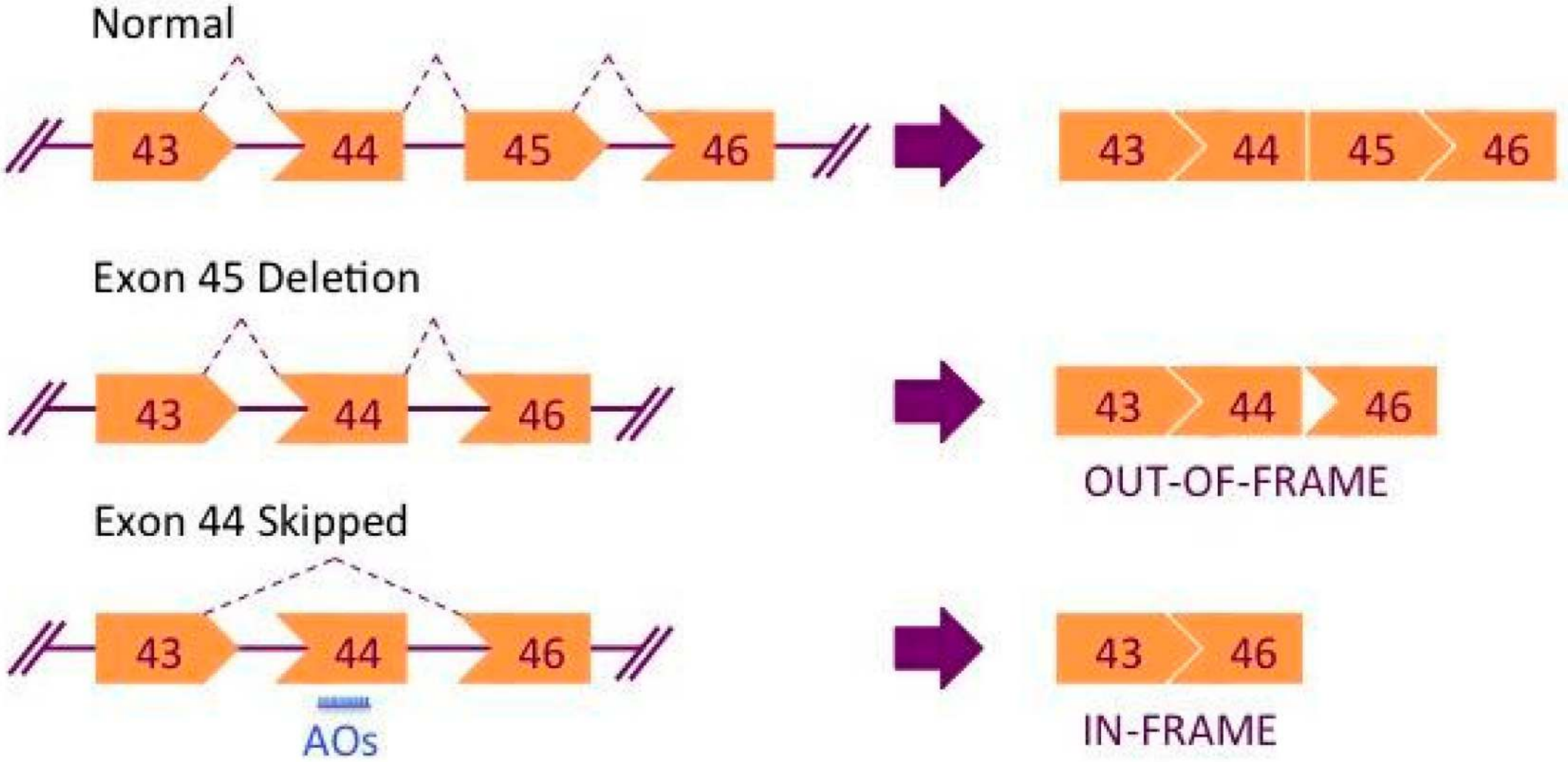

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