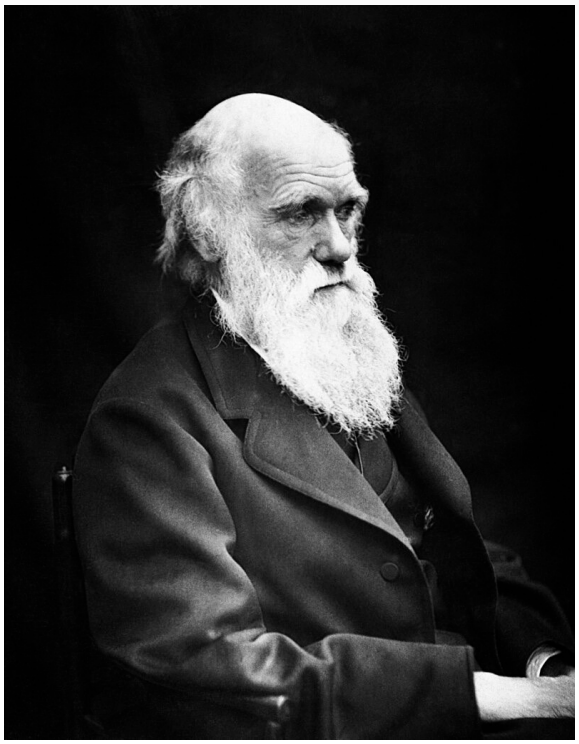
Model systems in biology

Why do we need models in biology?

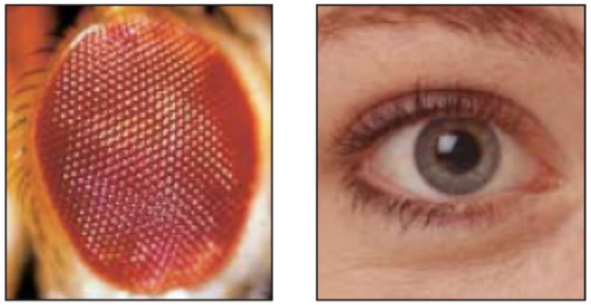
- We can’t perform many experiments on **humans**!
- Also if we could, it would be **too complex**
- We need **NUMBERS** and **REPLICATES**!

The Reductionist approach

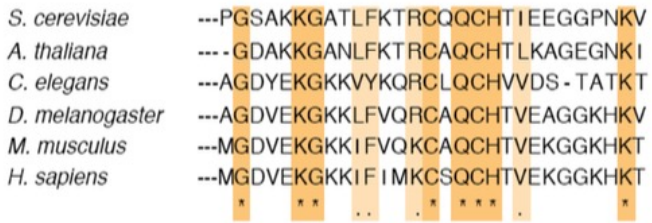
KEY CONCEPT N° 1: EVOLUTION



Charles Darwin



Insect and human eyes have a common origin



An example of protein sequence conservation accross species

- **Basic biological processes** are **shared** accross organisms also if evolutionary really distant
- According to **Charles Darwin's evolution theory**, life has a common origin and many key processes like **metabolism**, **cell cycle** and **embryonic development** are conserved accross species

What is a model system?

Thanks to **evolutionary conservation**, we can study complex human (and non human) processes in ‘simpler’ organism

Things we can do in model systems:

- **Genetic dissection** (e.g direct and reverse genetics, genetic screenings)
- **Biochemistry** (e.g. interactome studies)
- **Genetic manipulation** (e.g. genome editing)
- **Development** (e.g. in vivo)

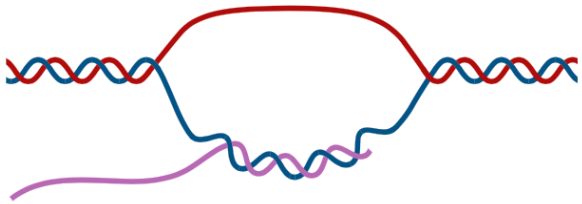
ID card of a model system

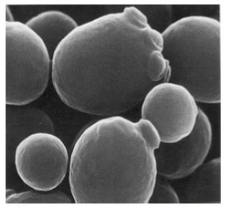
a. Cheap
b. Small
c. Easy to handle
d. Safe
e. Fast development and life cycle

KEY CONCEPT N° 2: BIOLOGICAL QUESTION

- There is no universal model system
- Each model system is fit to answer a specific BIOLOGICAL QUESTION

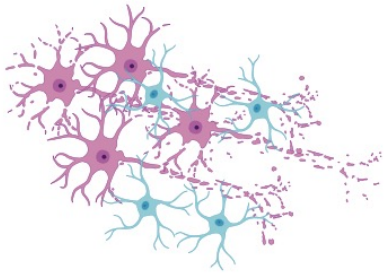
Transcription





S. cerevisiae

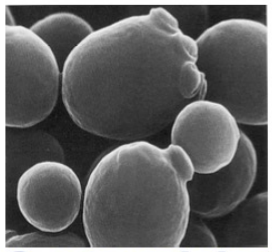
Neurodegeneration



Mus musculus

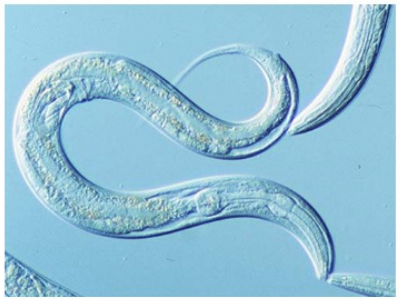
Only ask your model system the answers it is fit to give you!

The most common model systems in biology



S. cerevisiae

D. melanogaster



C. elegans

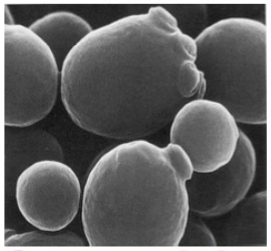
Cell culture

Zebrafish

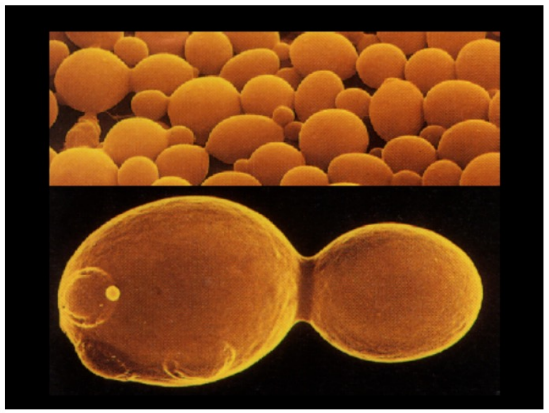
Mus musculus

A. thaliana

Yeast



Saccharomyces cerevisiae (Budding yeast)

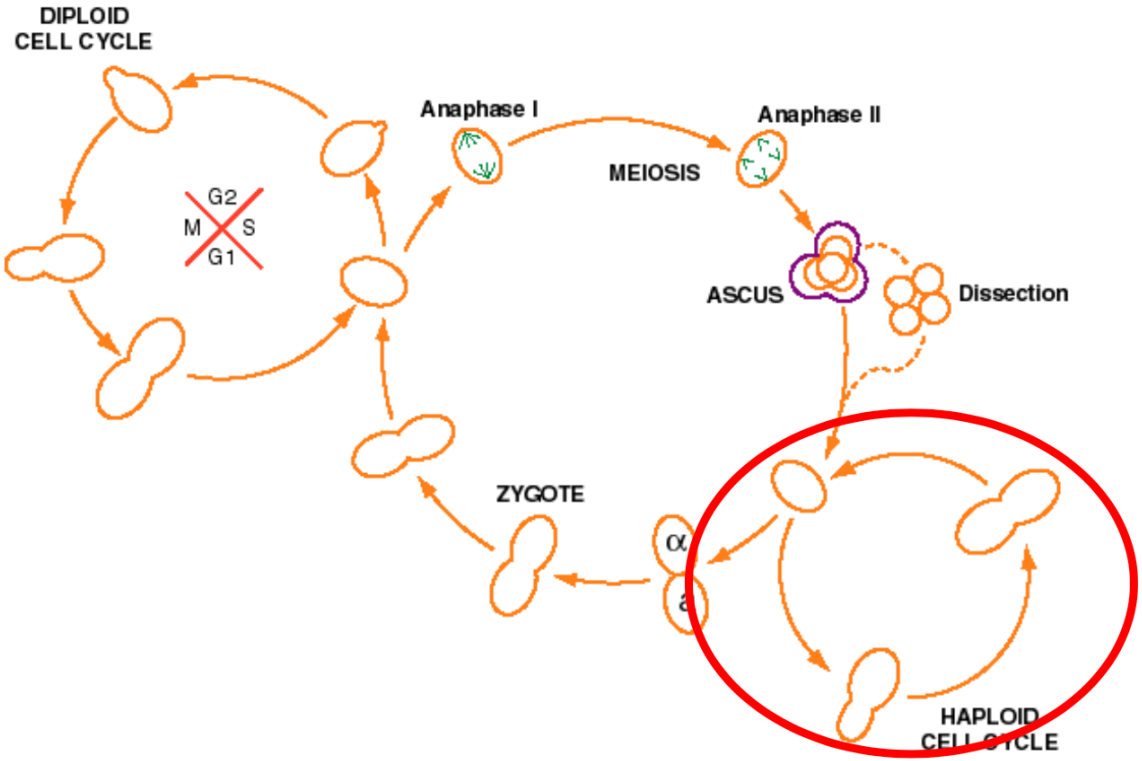


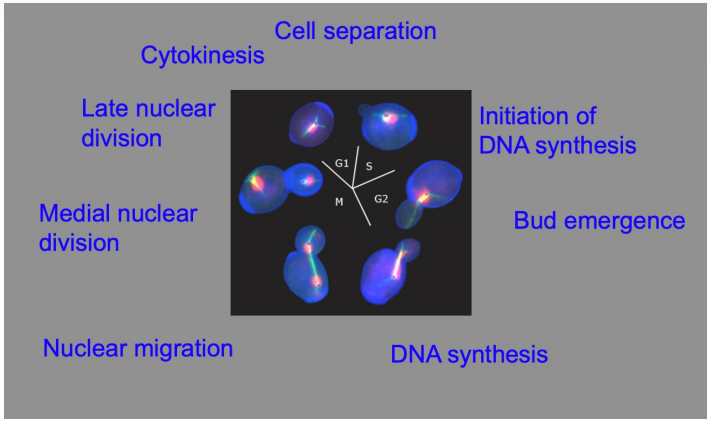
- Genomic DNA 12.052 kb
- 16 chromosomes
- 6000 genes (3.5x a bacteria)
- one gene every ~2 kb), ~50-fold higher than the gene density in the human genome
- Only 3.8% contains introns

ADVANTAGES
- Rapid growht (90' cell cycle)
- Easy to transform with plasmids
- Both aploid and diploid form
- Genic integration with homologous recombination is efficient
- CHEAP

DISADVANTAGES
- No RNAi machinery
- No splicing

The cell cycle



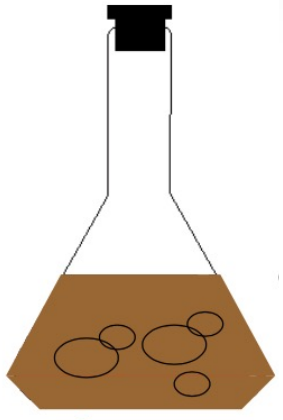


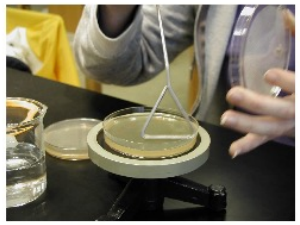
S. cerevisiae cells in nature switch readily between two mating types: haploid **a** cells mate with haploid α cells to form diploids. Under nutrient-poor conditions, diploids can be induced to undergo meiosis and sporulation, forming four haploid spores, two of each mating type

Yeast cells divide as rapidly as once every 90 min under optimal laboratory conditions, through a process of budding in which smaller daughter cells pinch, or bud, off the mother cell. The common name “budding yeast” derives from this notable feature of cell division and distinguishes *S. cerevisiae* from the fission yeast, *Schizosaccharomyces pombe*, also a powerful model organism.

What is it like to work with yeast?

T=30°C





Flask

Agar plates

- Yeast strains are grown in complete medium (YP+sugar) or synthetic minimun medium (SD+sugar), liquid or solid (agar plate)
- Growth is evaluated by OD600 (optical density at 600nm)

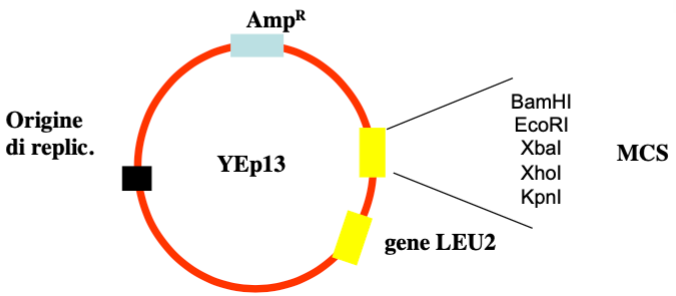
Prototrophy v.s. auxotrophy

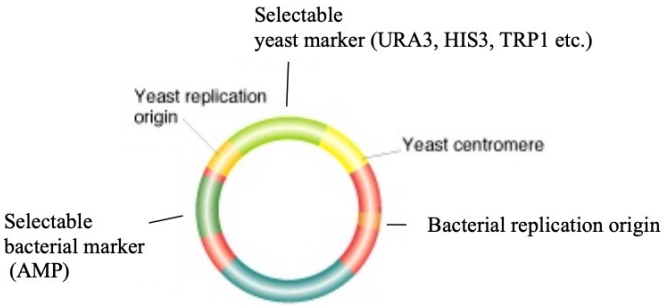
- An organism that is able to synthesize all the essential molecules for its life is AUTOTROPH
- A wild-type yeast only need sugar and vitamins in the medium. Autotrophy for single molecules is defined **PROTOTROPHY** for that molecule
- A yeast that carries a mutation in an enzyme that produces an essential molecule is an **AUXOTROPH** for that molecule
- An auxotrophic yeast for a certain molecule only grows if it can absord it from the medium
- **Laboratory yeast strains are ALWAYS AUXOTROPHIC FOR ESSENTIAL MOLECULES** (like aa or nucleotides)

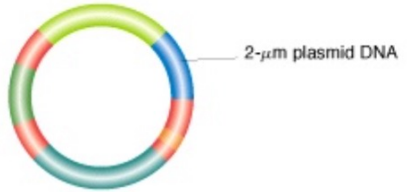
In the genotype of a strain you can read the **mating type** (a or alpha) and the **auxotrophies**

ADE2, URA3, trp1, his3 : prototrophic strain for adenine and uracyl and auxotrophic strain for tryptophan and histidine

Yeast vectors for transformation





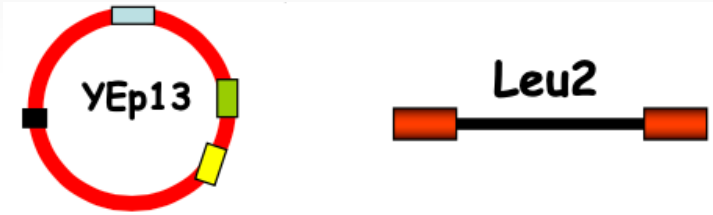


PLASMID
ORI sequence, multicopy replication, unstable, 30/40 per cell

CENTROMERIC VECTOR
Centromere CEN and ARS sequence, behave like stable minichromosomes
½ copies per cell

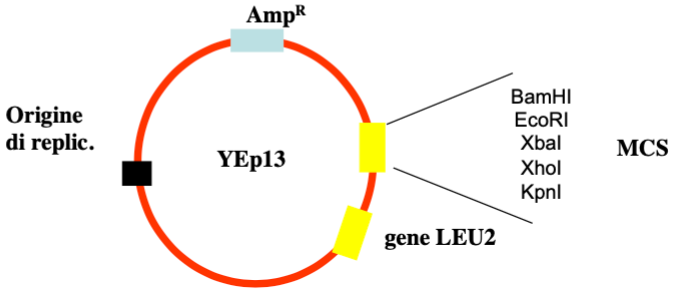
2uM VECTOR
1-10 copies per cell

Selectable markers for yeast



- Yeast vectors usually contain genes for the production of aa or nucleotides tha complement the auxotrophies of the laboratory strains

- **These genes function as selectable markers for transformants**



- **To use a plasmid that contains LEU2 you need a leu2 auxotrophic strain!**

- Grow the yeast in minimal medium with all the auxotrophic aa except leucyne!

How can we study gene function in yeast?

Forward genetics (classic)

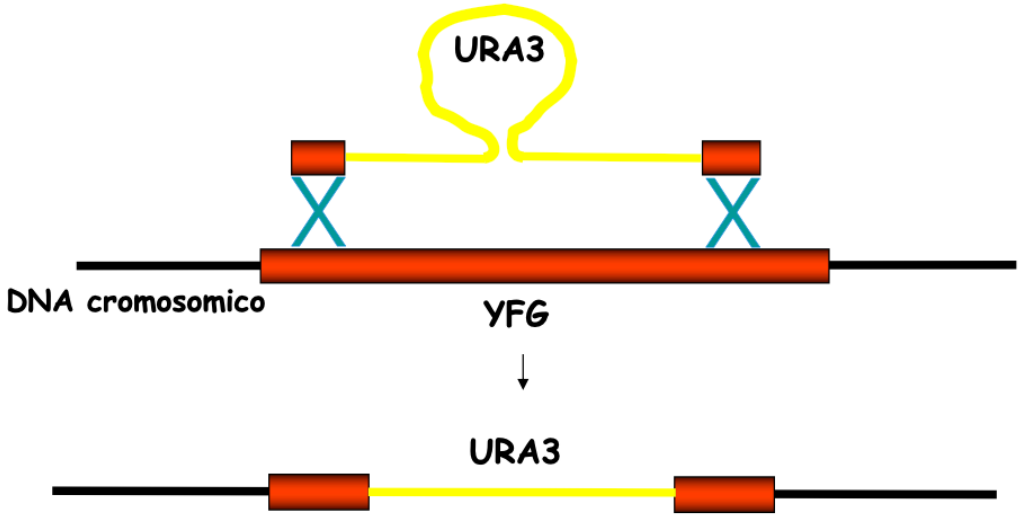
Creating random mutations in an organism's DNA which result in visible changes in the phenotype. The mutation and its associated phenotype are identified, and gene locus on the chromosome are mapped.

Reverse genetics (modern)

Disruption of known genes, followed by screening for mutant phenotypes resulting from these manipulations. Requires the sequence of the genome

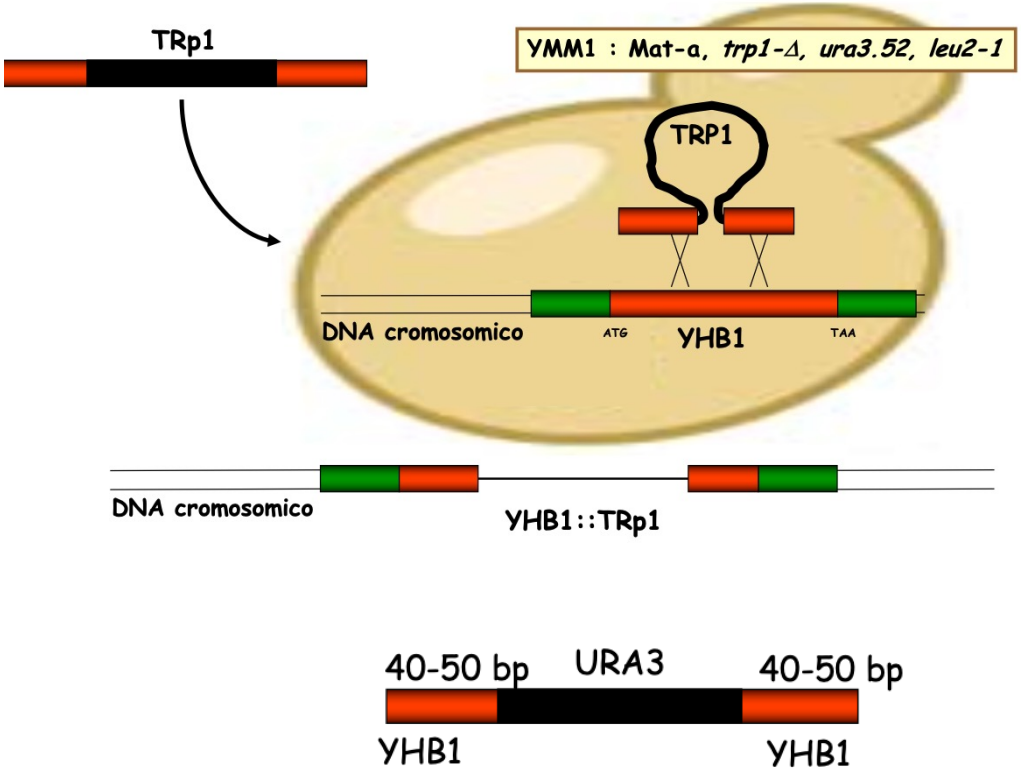
In 1996, the *S. cerevisiae* genome became the first fully sequenced eukaryotic genome

Linear fragment integration by homologous recombination



YFG= your favourite gene

Gene disruption in yeast



The integration is by **homologous recombination**

- The integration produces the disruption of the target gene
- The integration is stable, 'pop-out' never happen

PCR fragment that contains

- Marker gene
- Homology arms for the yeast genome

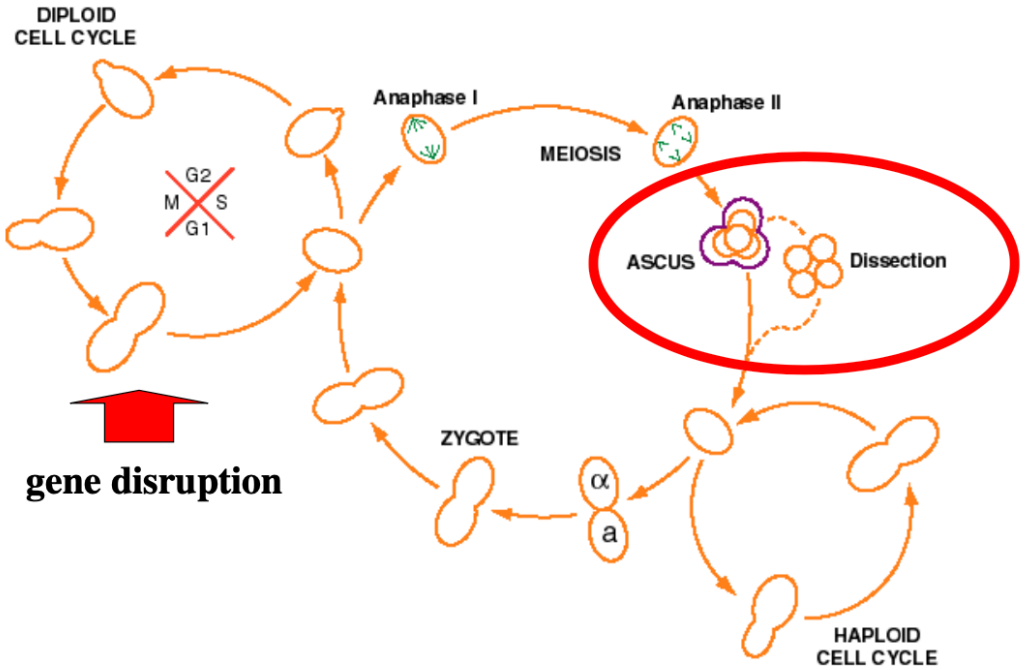
Gene disruption in yeast

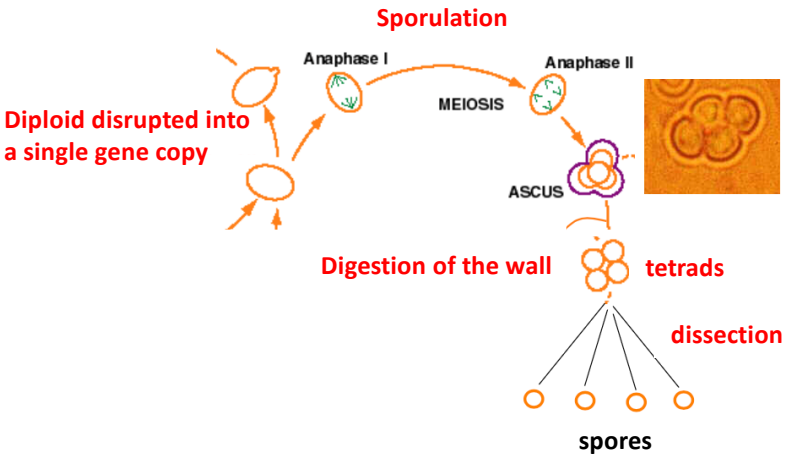
All the *S.cerevisiae* ORFs were disrupted to determine its essentiality for cell survival
(www.yeastgenome.org)

- 6183 ORFs, of which 5700 encode proteins
- 1100 genes are essential for cell growth

How can we handle essential genes?
(they cause lethality)

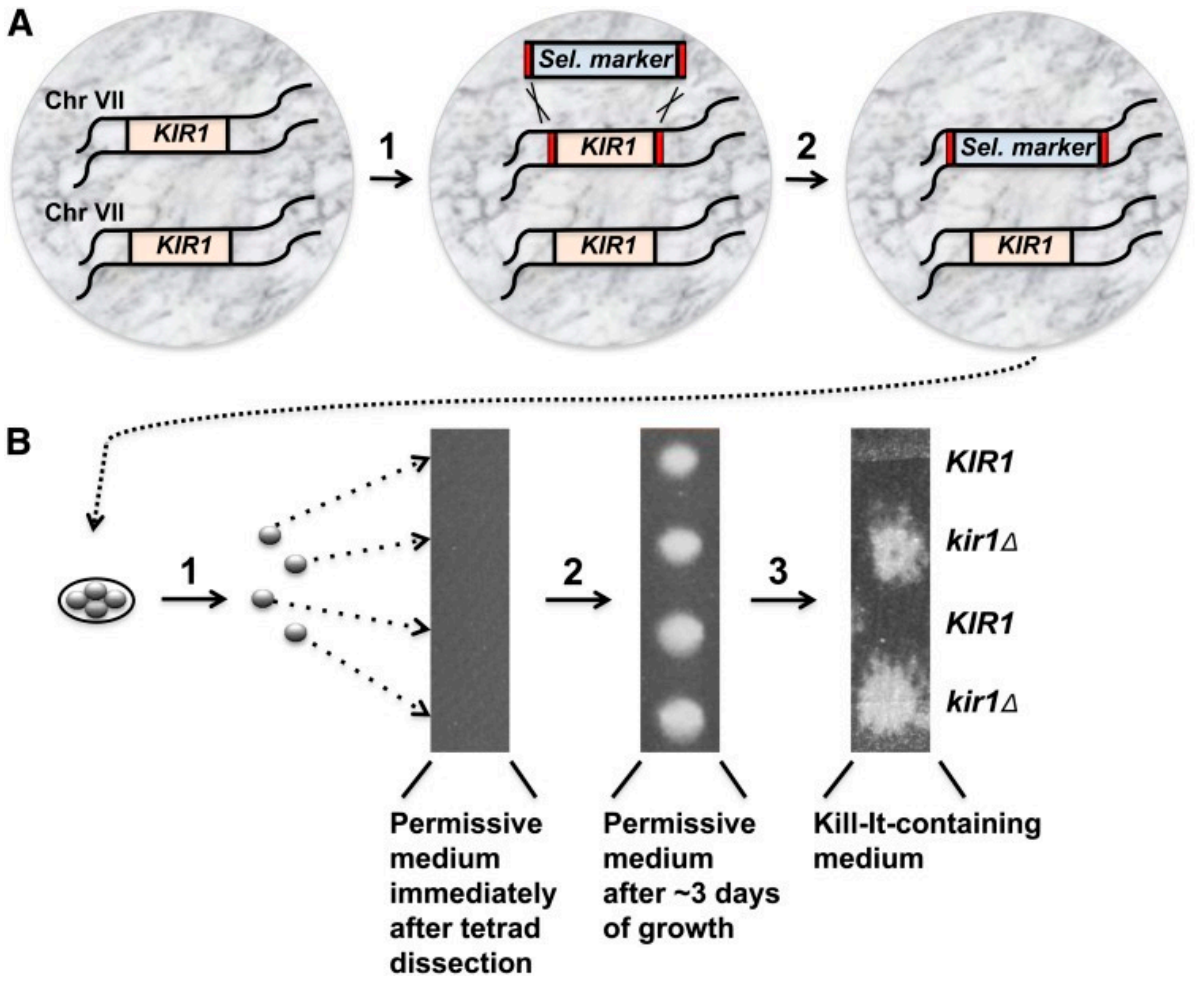
Tetrad analysis



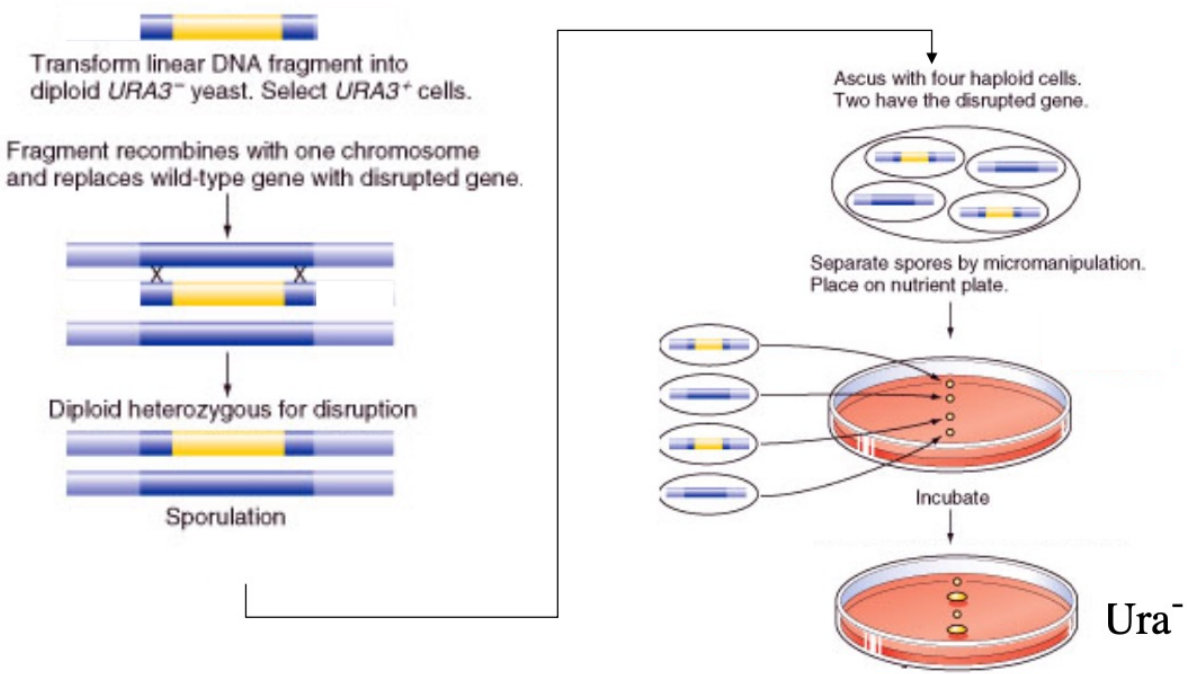


We need **DIPLOID strains** that undergo sporulation (**meiosis**) and produce 4 aploid spores

One-step gene replacement and analysis of meiotic products through tetrad analysis

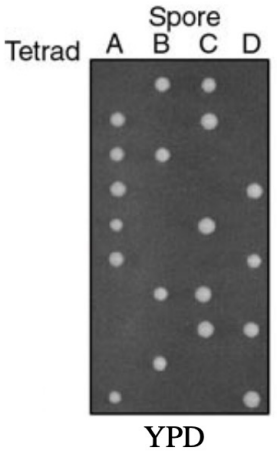


Is a gene essential?

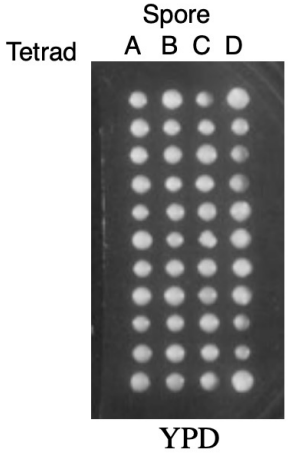


Non essential gene

Essential gene



Non essential gene

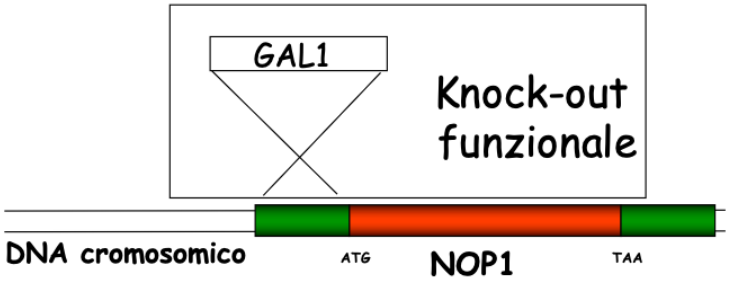


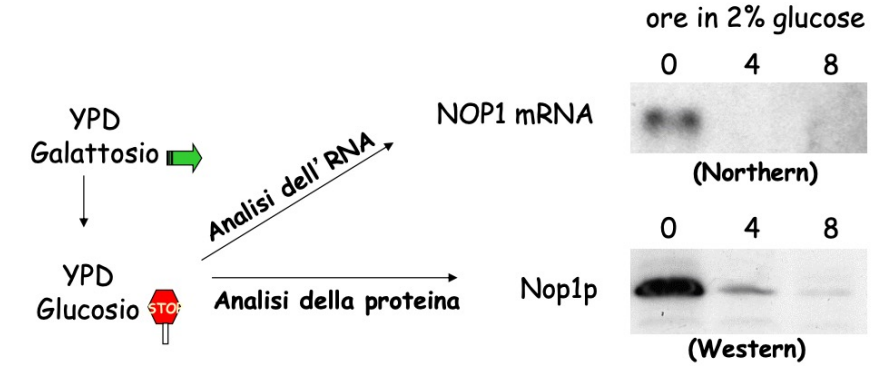
Functional inactivation of genes

If a gene is essential we need to produce a **conditional mutant**:

- Thermosensitive mutant
- Genes under control of inducible promoters

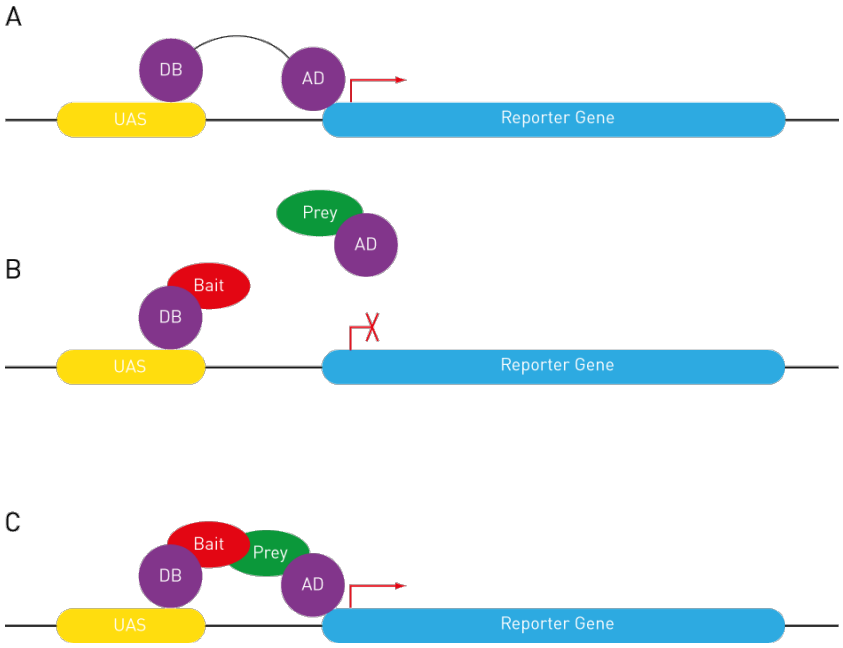
GAL1 inducible promoter





Two-hybrid system in yeast

The yeast two hybrid system is a method to detect the **interaction between two proteins (**from any organism) in yeast

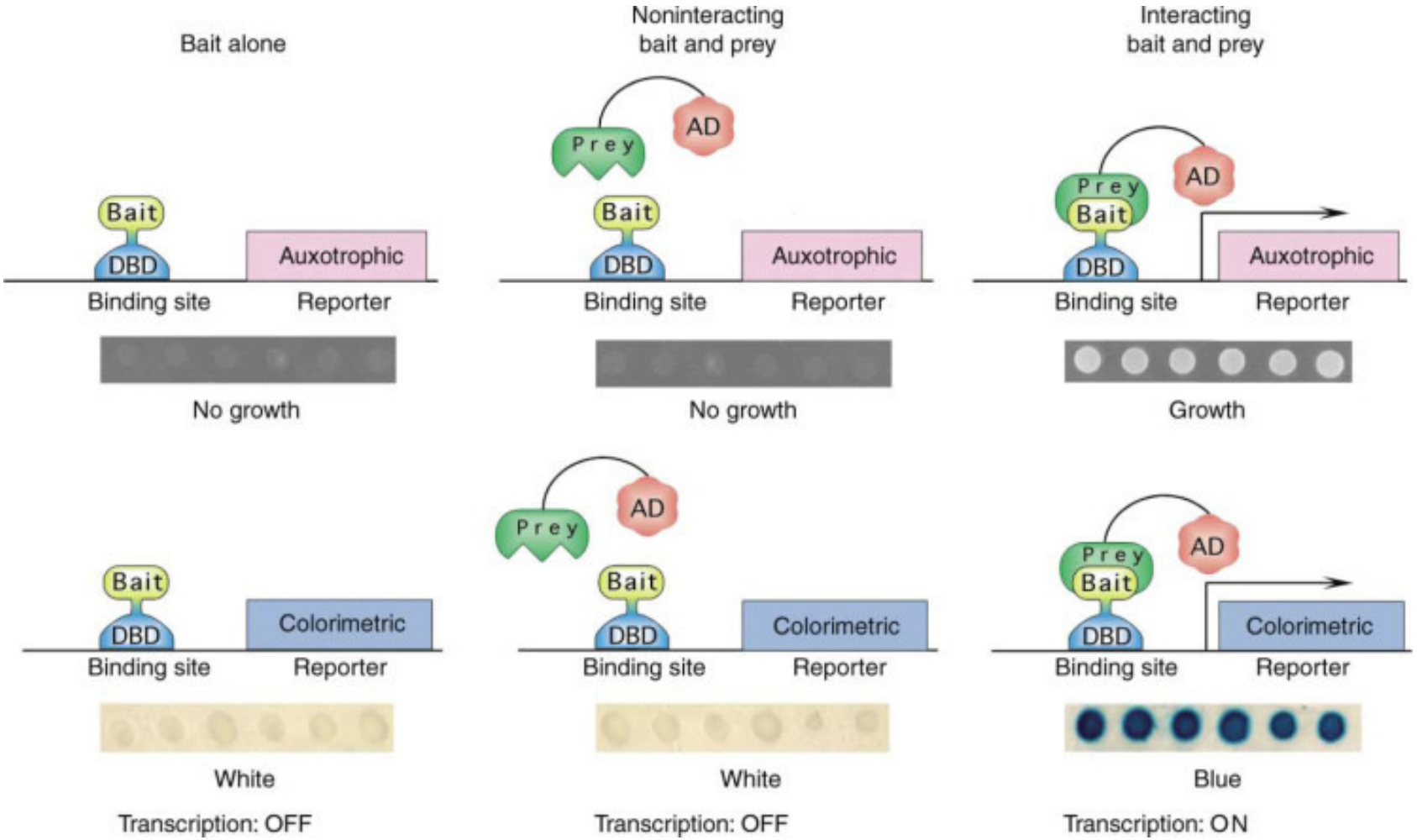


In this method, the yeast transcription factor Gal4 is split into a DNA-binding domain (**DBD**) and an activation domain (AD). Protein pairs are fused to the DBD and AD, and the resulting DBD-X and AD-Y fusions are generally referred to as bait and prey.

The third component is a reporter cassette, which consists of the DNA-binding site for the first hybrid protein in the context of a minimal promoter, upstream of the coding sequence for an easily scored reporter gene

Only when bait and target interact, a reporter gene will be activated

Two-hybrid system in yeast



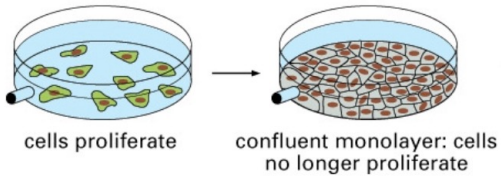
Cell cultures

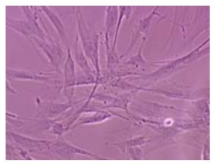
TYPES OF CELL CULTURES

- Primary cultures
- Cell lines
- Stem cells

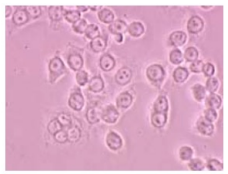
TYPES OF CELL GROWTH

Monolayer



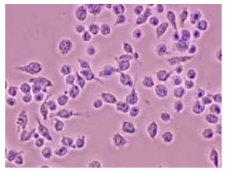


Fibroblasts

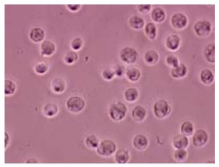


Keratinocytes

Suspension



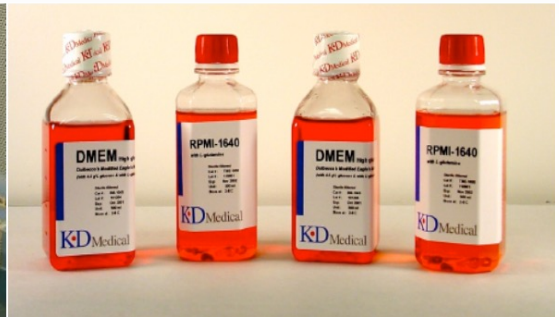
Monocytes



Limphocytes

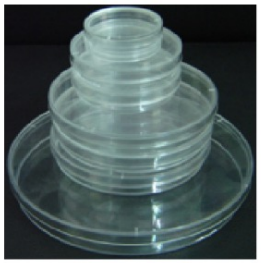
How is it to work with cell cultures?

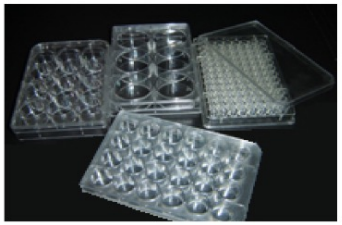
Culture medium



CO2 incubator

Plates (plastic)





Cell hood

How is it to work with cell cultures? (2)

CO_2 incubator

- Temperature 35-37°C
- Humidity 95%
- pH7.2-7.4, maintained by 5% CO2 and NaHCO3 buffer system

Cell medium additives

- Glutammine (very fragile aa)
- Antibiotics (penicillin/streptomicin)
- Serum (complex mixture of growth factors, adhesion factors, albumin, transferrin, fatty acids and enzymatic cofactors

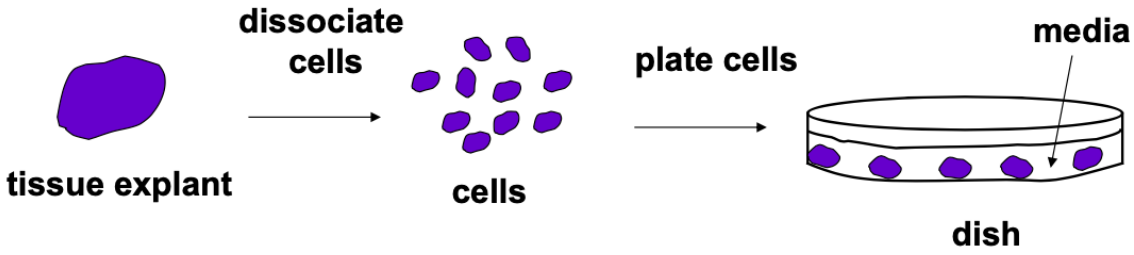
Applications of cell cultures

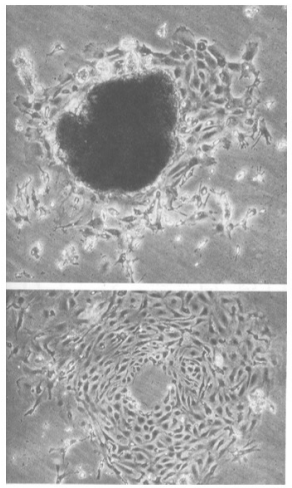
- **CELLULAR ACTIVITIES**: transcription, protein synthesis, metabolism, cell cycle, apoptosis
- **INTRACELLULAR FLUXES:** RNA, hormone receptors, metabolites, calcium, signal transduction, membrane trafficking
- **ENVIRONMENT EFFECTS:** infections, drugs, ligand-receptor interactions, cytotoxicity, mutagenesis, carcinogenesis
- **CELL PRODUCTS:** therapeutical protein production, vaccines, bioreactors, post-translational modifications
- **GENETICS:** genetic analysis, transfections, infections, trasformation, immortalization, senescence
- **CELL-CELL INTERACTIONS AND CELL-ENVIRONMENT INTERACTIONS:** morphogenesis, paracrine control, cell proliferation, cell adhesion, invasion, matrix interaction

Advantages of cell culture

- Controlled physicochemical environment (pH, temperature, osmotic pressure, 02 and CO2 tension)
- Reduced variability and increased reproducibility respect to the *in vivo* condition
- Propagation and freezing of cell lines
- Cheaper than *in vivo* models
- Scale up system
- 2D and 3D cultures
- no ethical issues
- Mechanisation

Primary cell cultures





3 days in vitro

7 days in vitro, after removal of the explant

PROCEDURE

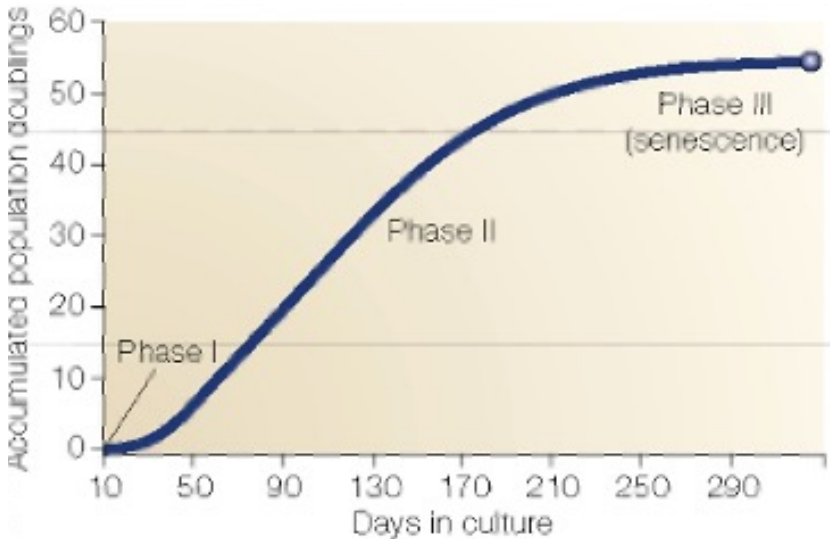
- Organ/tissue removal
- Mechanical or enzymatic dissection
- Isolation of cells

- Grow and replicate in plate
- Require specific medium
- Only last 50-100 division in vitro

Primary cell cultures and senescence

- After several cell division in vitro, primary culttures usually undergo SENESCENCE
- They stop cell division, mainly because of telomere shortening beyond a critical lenght
- Morphology, gene expression and metabolism changes

The Hayflick limit (1961)

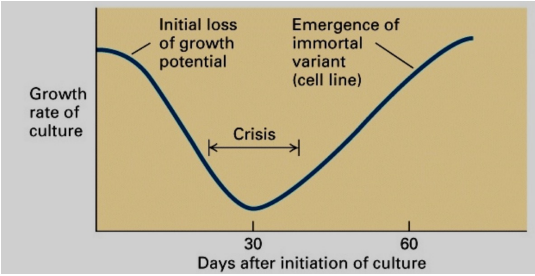


Cell lines

Cell lines can come from different origins

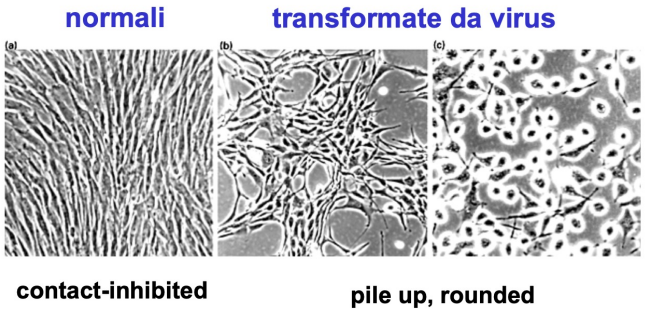
FROM PRIMARY CULTURES

- Rare genetic mutations can immortalize the cells
- Contact inhibited
- Do not form tumors if injected in mice



TRANSFORMED CELLS

- Genetic changes (p53, Rb mutations, OE of telomerase, viral gene as SV40 large T antigen, papilloma virus E6)
- Loose contact inhibition
- Abnormal cariotype
- Form tumors if injected in mice



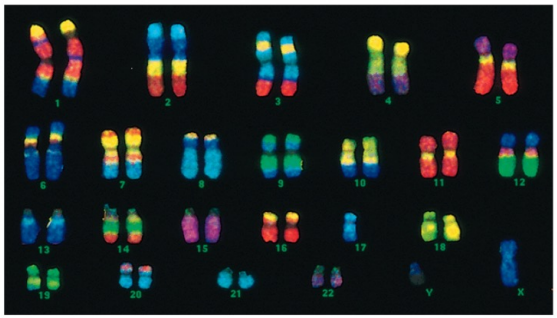
FROM TUMORAL CELLS

- Uncontrolled growth
- Invasion and metastasis
- No contact inhibition an anchoring inhibition
- Abnormal caryotypr

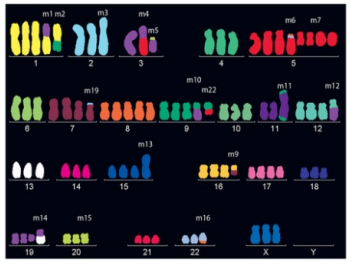
HeLa cells

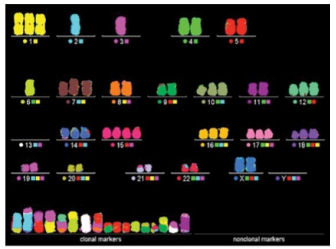
Henrietta Lacks 1920-1951

WT

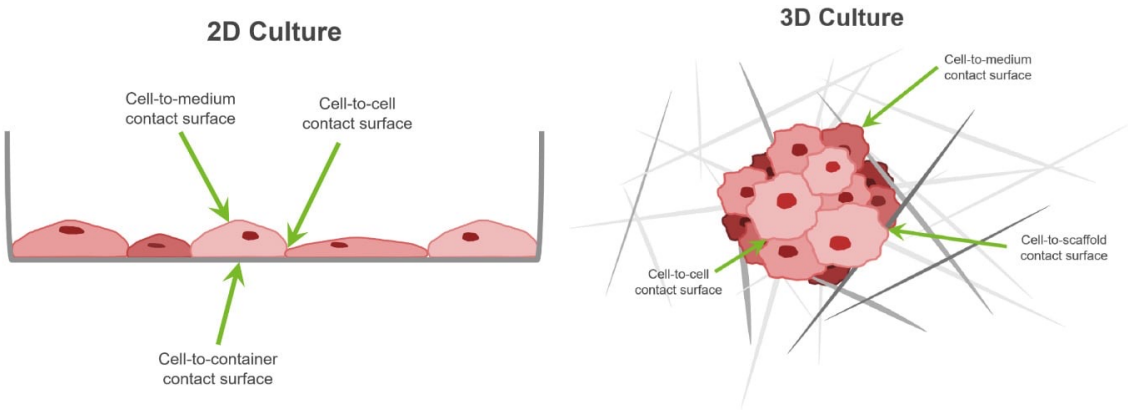


HeLa(s)





Modelling physiological events in 2D vs 3D cell culture

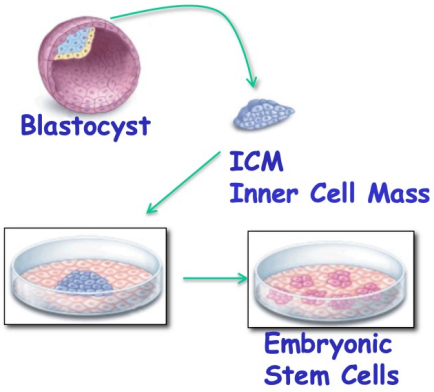


Advantages of 2D cell culture	Advantages of 3D cell culture
Fast proliferation and colony formation (minutes to hours)	3D mimics tissue and organ structures
Simpler procedures	*In vivo*-like cell-cell and cell-environment interactions
Lower reagent cost	*In vivo*-like concentration gradients of essential compounds
More traditionally performed and accepted	Preserved morphology and molecular mechanisms
Very good reproducibility	Heterogenous cell polarities and phenotypes present, more representative of native tissue architecture
Suitable for high throughput	Mimics tissue stiffness
	Good reproducibility
	Some models are suitable for high throughput applications

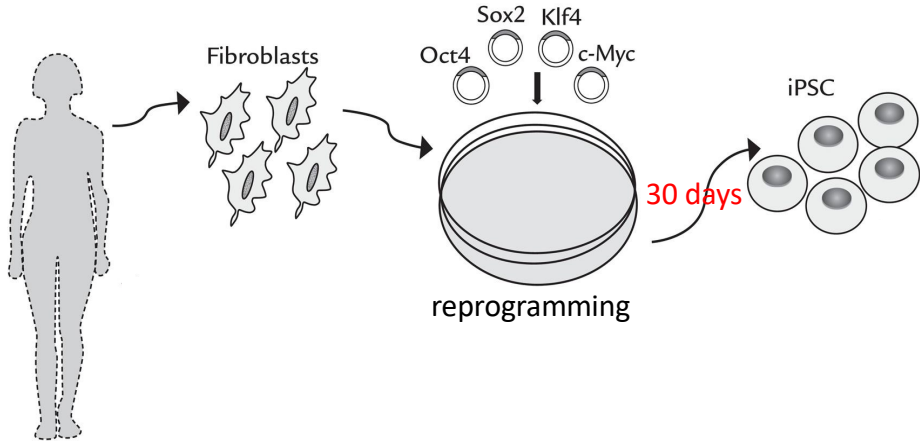
Disadvantages of 2D cell culture	Disadvantages of 3D cell culture
Cells forced into planar shape, does not mimic native structures	Slower culture formation due to physical restraints of the matrix (hours to days)
No cellular microenvironment	More complex procedures
Lacking complex cell-cell and cell-environment interactions	Higher reagent cost
Unrestricted access to essential compounds, unlike *in vivo*	Fewer commercially available tests
Different cell morphology and molecular mechanisms compared to in vivo	
Stiffness of surrounding tissue not replicated	

Stem cells

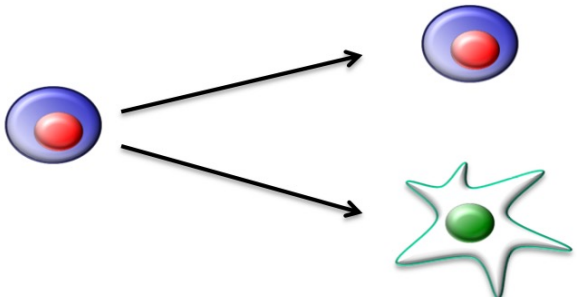
Embryonic stem cells (ESCs)



Induced pluripotent stem cells (IPSCs)

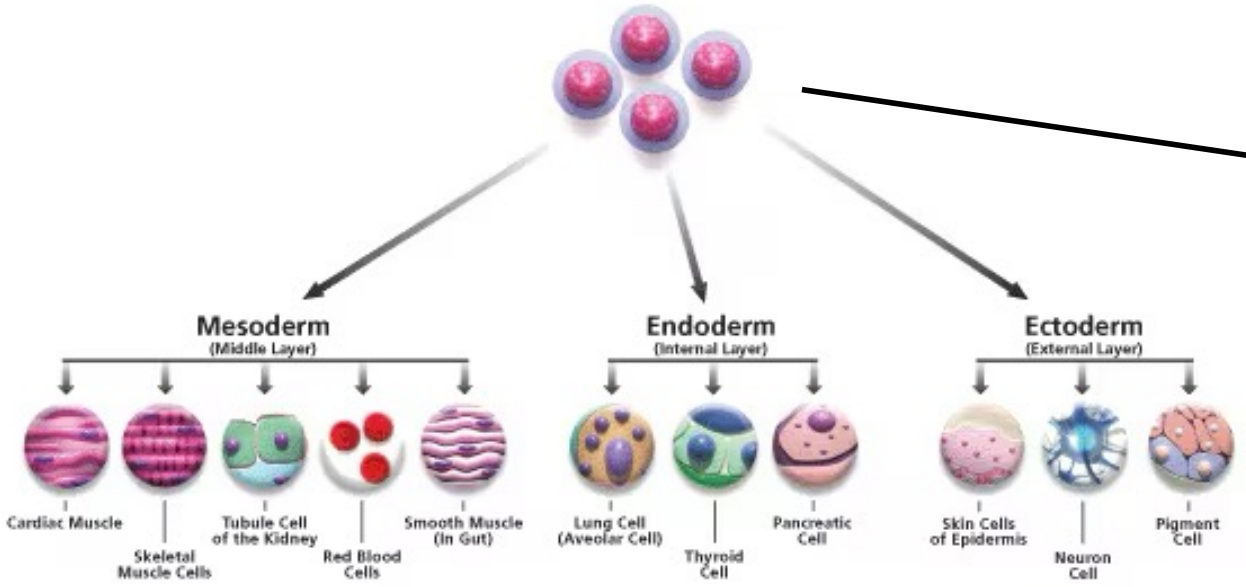


Adult stem cells



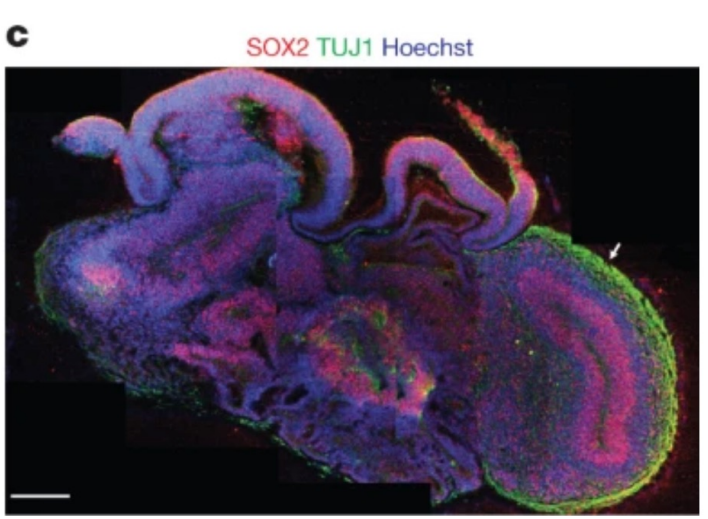
What cell cultures can we derive from stem cells?

Directing differentiation of ESC/IPSC



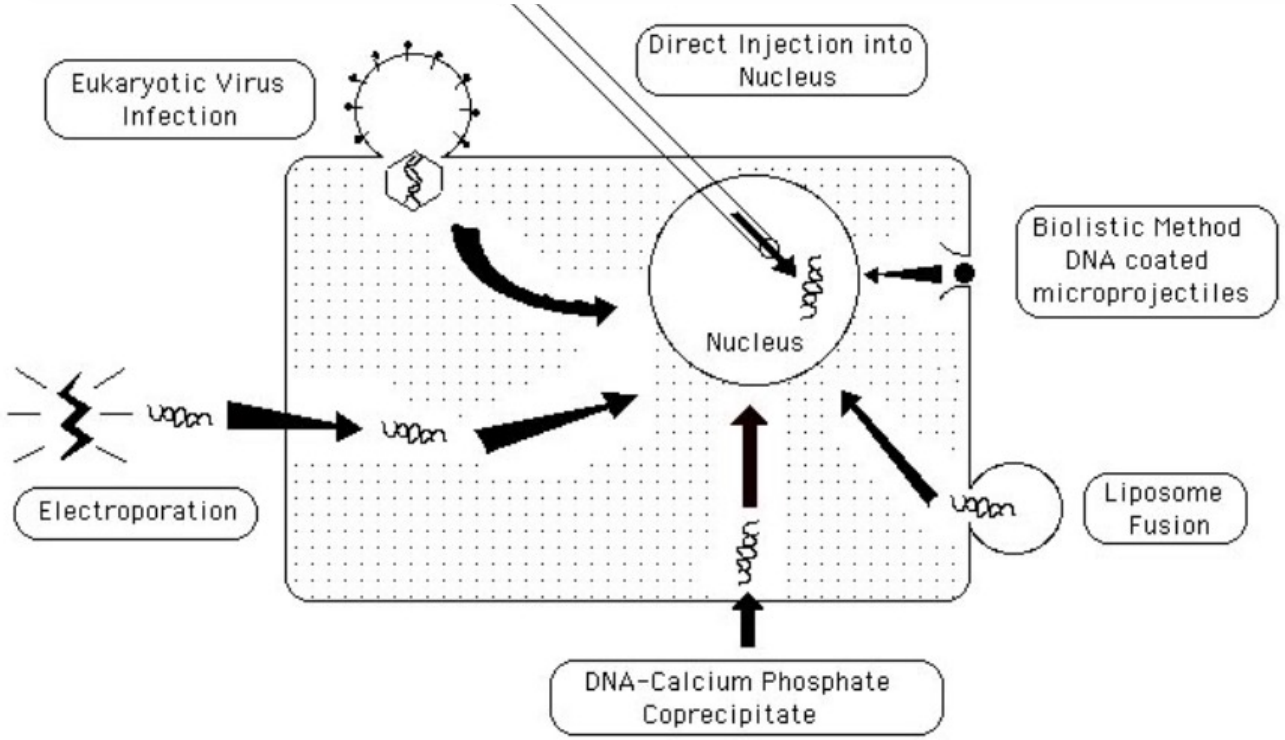
2D

3D



Organoids

Genic tranfer in mammalian cells



DIRECT TRANSFER
- Microinjection
- Gene gun (plants)

TRASFECTION
- DEAE-dextran
- Calcium phosphate
- Electroporation
- Lipofection

TRASDUCTION
- Virus

Trasfection

CO-PRECIPITATION

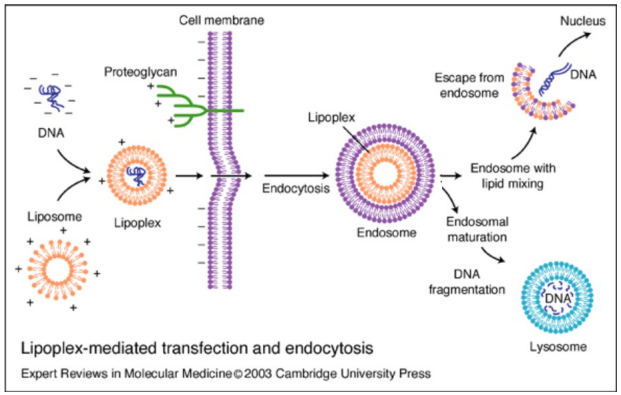
- DEAE-dextrane or Calcium phosphate
- DNA forms insoluble precipitates that are uptaken by endocytosis
- Requires a lot of DNA
- Anchored cells
- Not so efficient

ELECTROPORATION

- Electric pulse opens holes in the membranes

LIPOFECTION

- DNA is incubated in vescicles of liposomes that bind the anionic surface of cells
- DNA enters by endocytosis or membrane fusion



Applications of transfection

Easy way to perform experiments without (time consuming) gene editing:

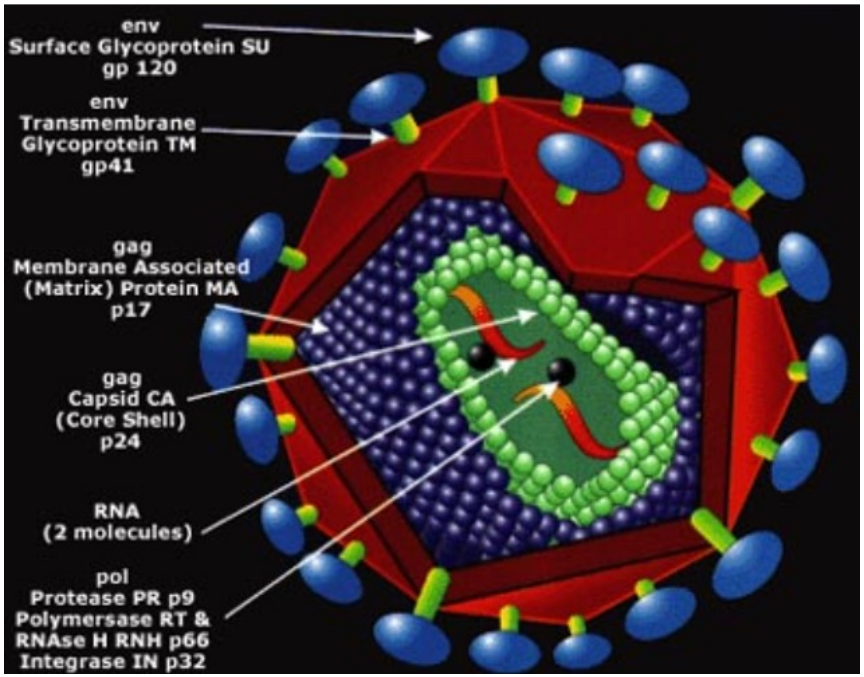
- **Overexpression** experiments (proteins, ncRNA molecules…)
- **RNAi** (knock-down potentially any gene to study function)
- **Biochemistry** (overexpress two molecules e.g. two proteins and perform biochemistry to analise interactions)
- **Molecular biology** (e.g. luciferase assay, tethering experiments…)

Use cell as ‘boxes’ to perform you experiments and analyses

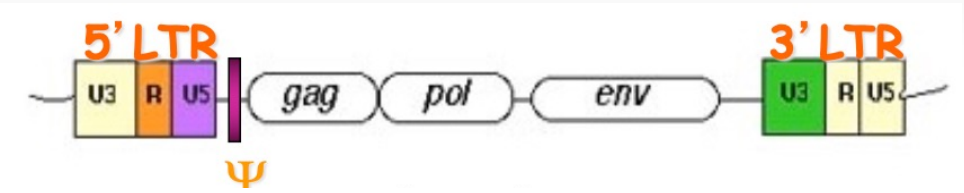
Genic integration

Normally transfection is transient (>100 copies per cell) and it is diluted through replication

To obtain stable expression we can use viral vectors



Retrovirus



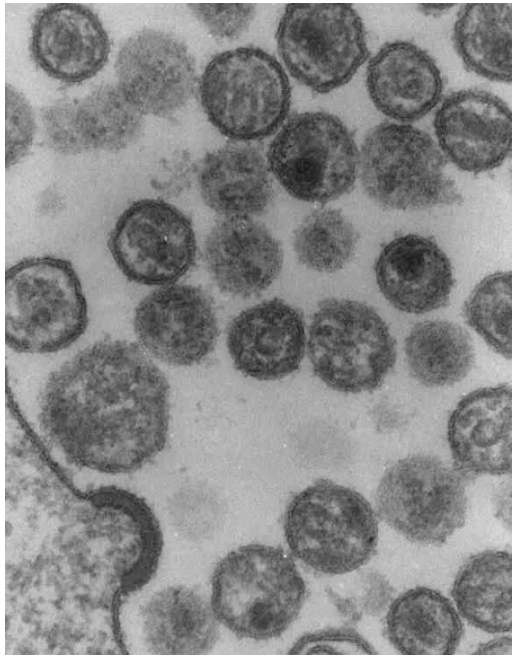
gag-: encodes for the 'core' proteins
pol-: encodes for multifunctional proteins (RT, DNApol, protease and integrase)
env-: encodes for the capsid proteins

LTR: Long Terminal Repeats, contains U3, R and U5, the elements transcription, retrotranscription and integration control

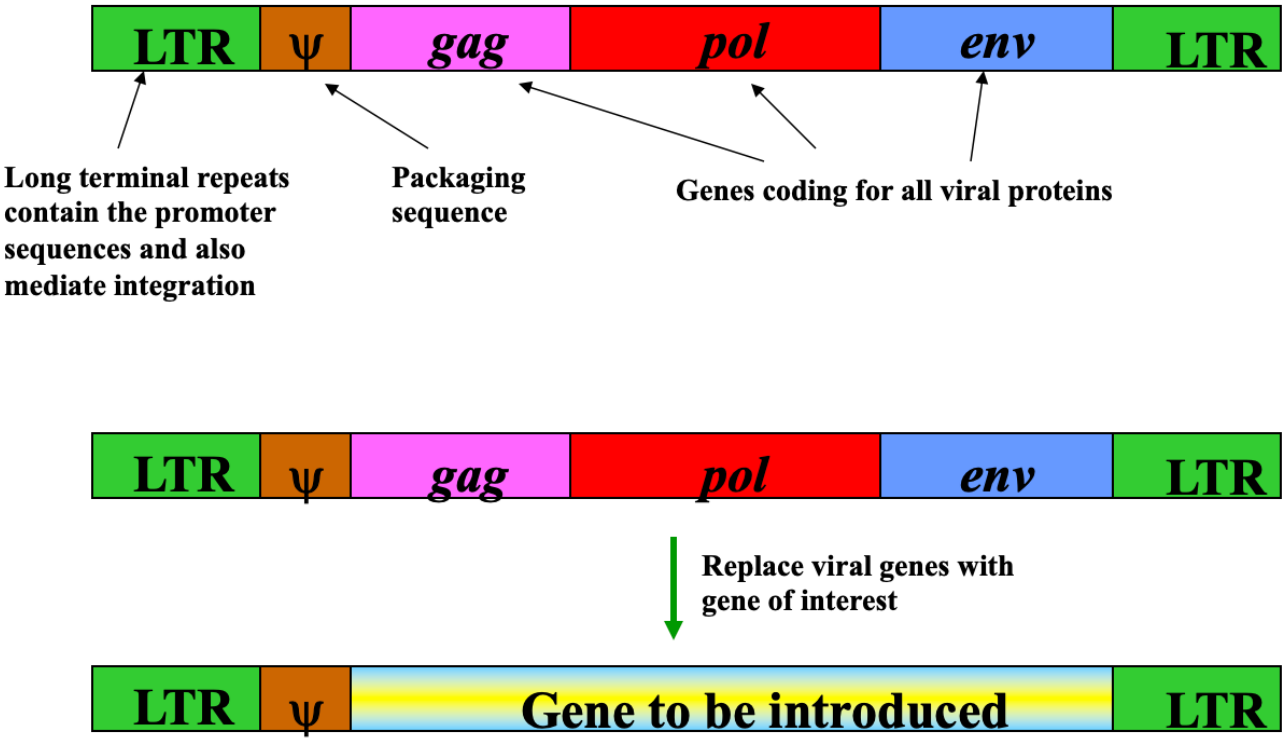
Psi: packaging sequence

Genic transfer with retrovirus

- Genic transfer of long sequences (up to 7.5kb)
- Stable expression thanks to genome integration
- They infect dividing cells
- Low immunogenicity



Genic transfer with retrovirus

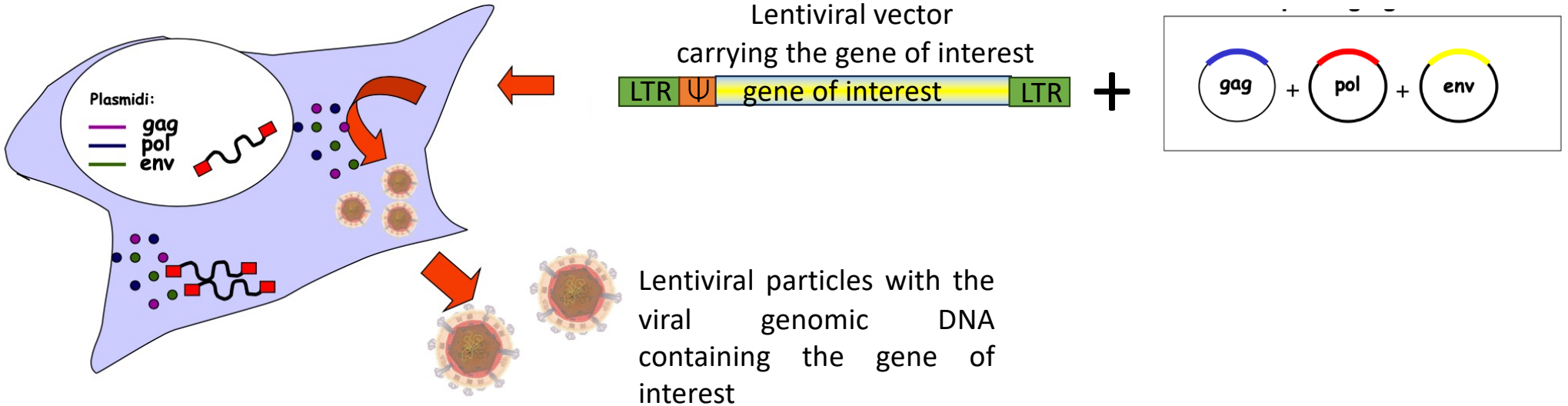


Recombinant (defective) retroviral DNA (a.k.a. retroviral vector DNA)

Lentiviral vectors

Lentivirus can infect both dividing and non dividing cells (also neuron, macrophages, hemotopoietic stem cells, muscle, hepatic cells)

Packaging plasmids (gag, po, env) and the vector with viral constructs are transfected in packaging cells



Lentiviral particles with the viral genomic DNA containing the gene of interest

Drosophila, the golden bug

REVIEWS

DROSOPHILA, THE GOLDEN BUG, EMERGES AS A TOOL FOR HUMAN GENETICS

Ethan Bier

Abstract | *Drosophila melanogaster* is emerging as one of the most effective tools for analyzing the function of human disease genes, including those responsible for developmental and neurological disorders, cancer, cardiovascular disease, metabolic and storage diseases, and genes required for the function of the visual, auditory and immune systems. Flies have several experimental advantages, including their rapid life cycle and the large numbers of individuals that can be generated, which make them ideal for sophisticated genetic screens, and in future should aid the analysis of complex multigenic disorders. The general principles by which *D. melanogaster* can be used to understand human disease, together with several specific examples, are considered in this review.

- 75% of all human disease genes have related sequences in *D. melanogaster*.

- nearly a third of all human disease genes have matches in the *D. melanogaster;* on the basis of the current list of 2,309 human disease-gene entries, ~700 human disease genes are estimated to have sufficiently well-conserved homologues to be analysed in *D. melanogaster*.

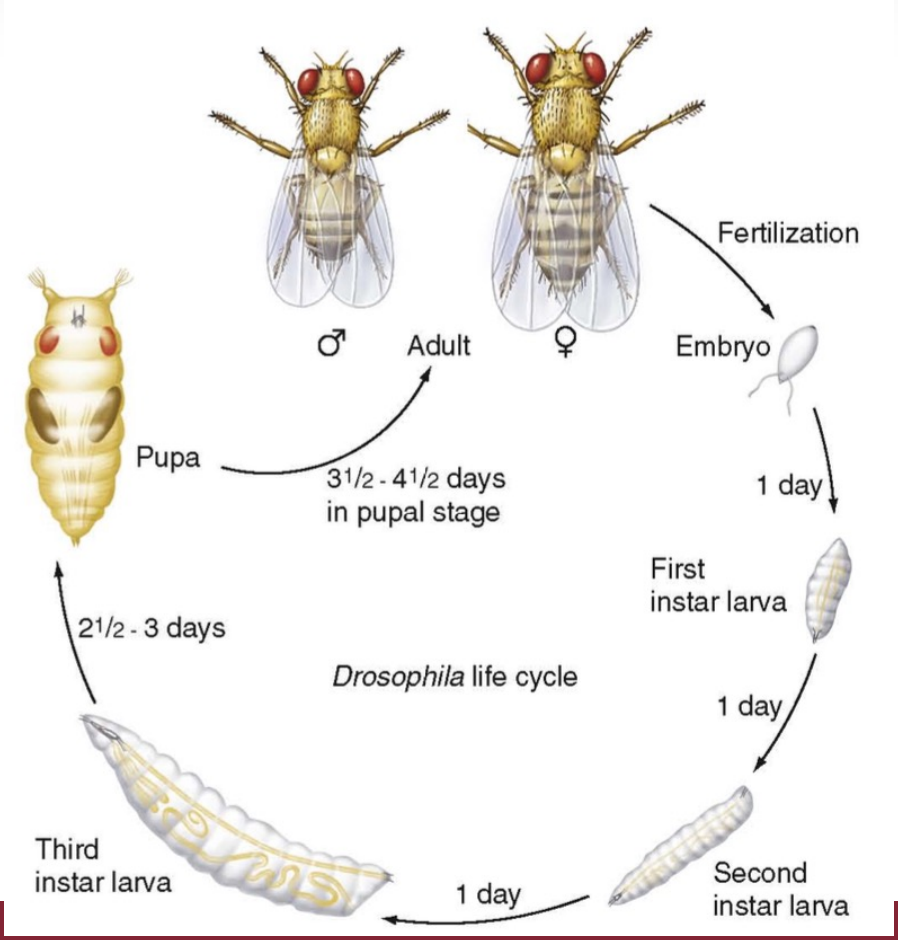
- *D. melanogaster* has homologues of genes that, when disrupted, cause a broad spectrum of human diseases such as neurological disorders, cancer, developmental disorders, metabolic and storage disorders and cardiovascular disease, as well as homologues of genes required for the visual, auditory, and immune systems.

- This and other bioinformatic analyses indicate that *D. melanogaster* can serve as a complex multicellular assay system for analysing the function of a wide array of gene functions involved in human disease.

Drosophila melanogaster

- 4 pair of chromosomes (1 pari of sex chromosomes)
- Genome completely sequenced

The life cycle



10 days at 25°C
18 days at 18°C

What is it like to work with Drosophila?

The fly room at Columbia University

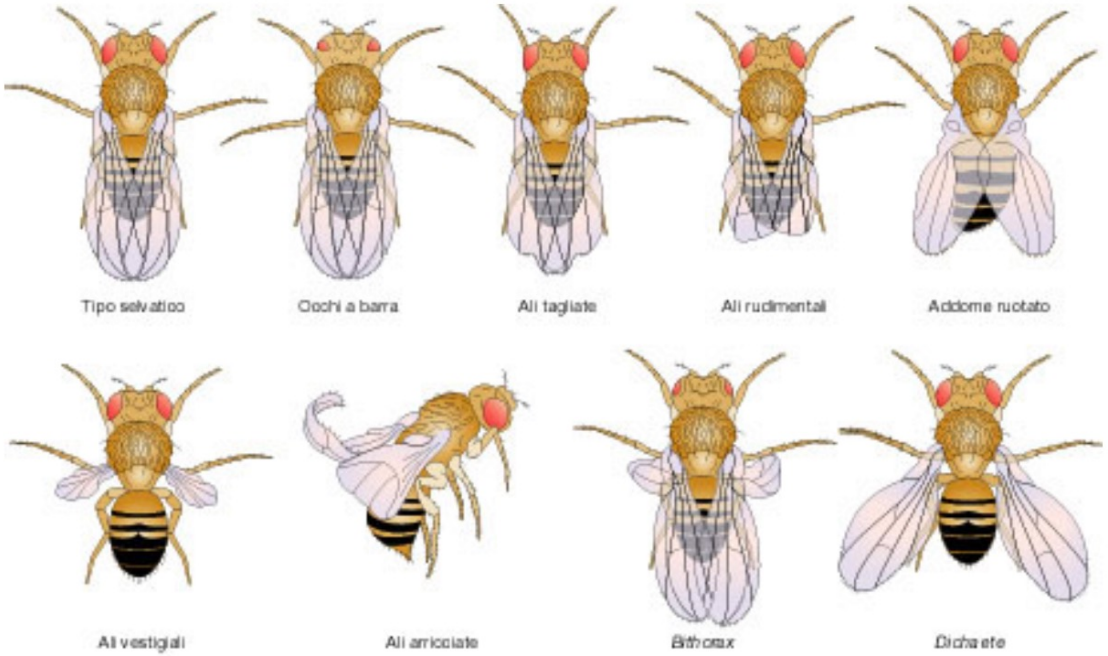
T.H. Morgan, H.J. Muller, C.B. Bridges, A.H. Sturtevant

A modern fly room

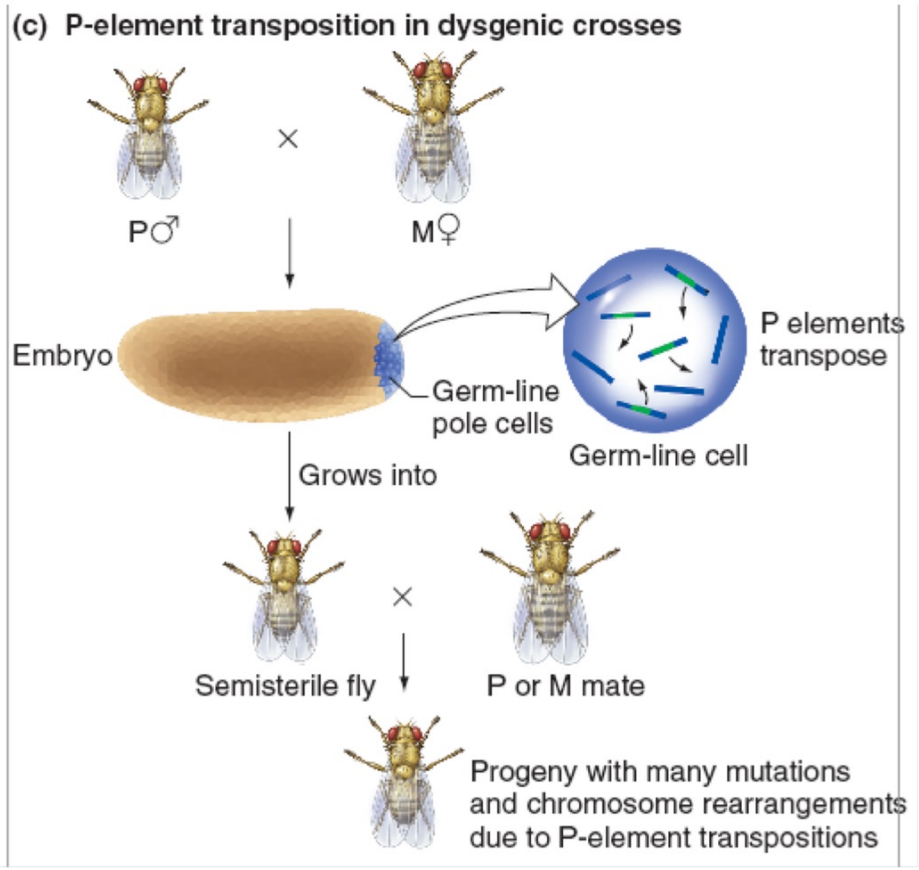
- Fruit flies grow in these bottles
- **There is no way to freeze strains!** You have to propagate lines continuously

Phenotypic markers

Phenotypic markes can be assessed visually



Transposable elements in Drosophila: P-elements



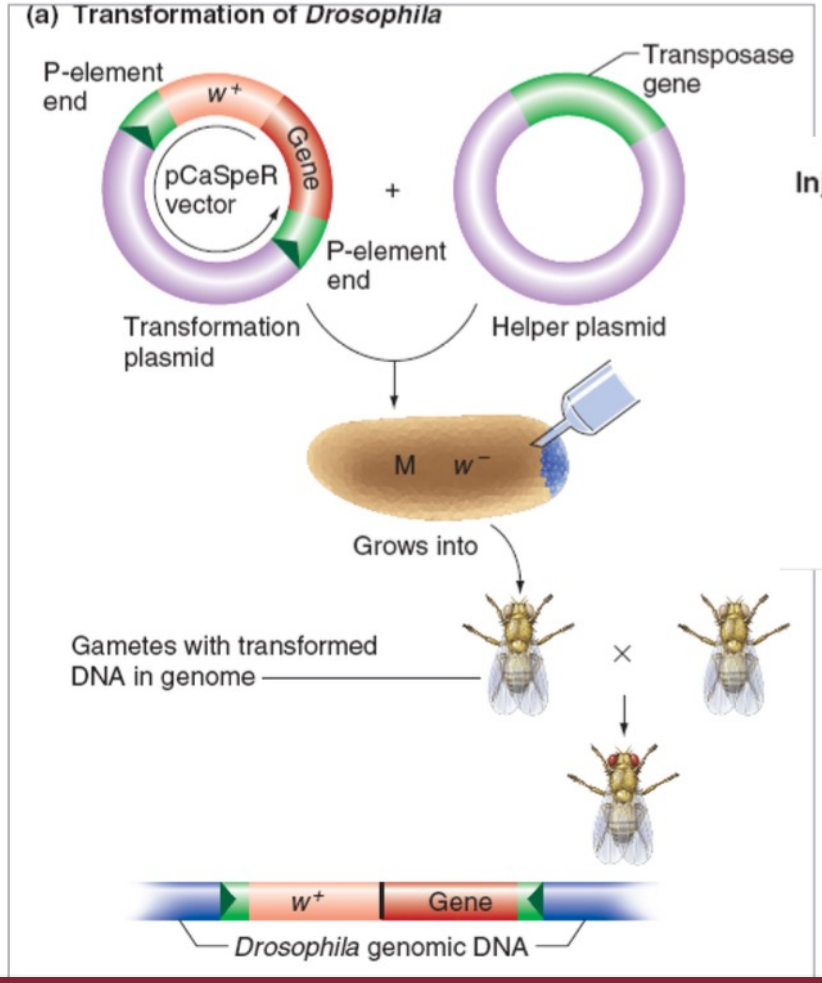
P-elements are transposons discovered in Drosophila observing the hybrid dysgenesis crossings

Trasposition only happens in the germ line, in somatic tissues it is repressed by a mechanism that control the splicing of trasposase

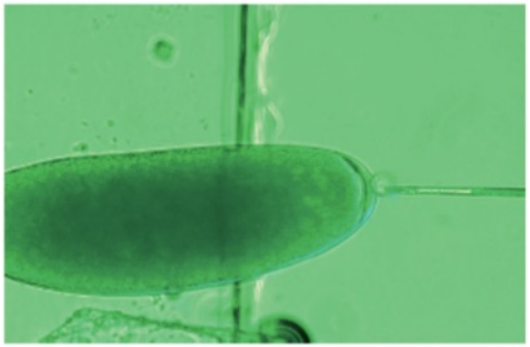
Transposable elements are a powerful tool for functional analysis of genomes

- **Transgenic lines**
- **Insertional mutagenesis** with engineered transposable elements

Transgenic flies with P-elements



Injecting a transgene into a *Drosophila* embryo



Transposon-mediated mutagenesis

Transposon-mediated gene-disruption screens

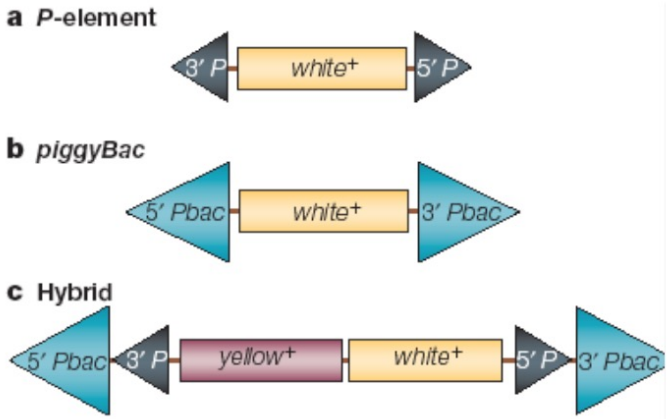


Figure 1 | **Transposon-mediated gene-disruption screens.** The gene-disruption projects used two types of transposable element as JUMP-STARTER ELEMENTS, the *P*-element (**a**) and the *piggyBac* transposon (**b**). Both transposable elements contain 5′ and 3′ ends that are necessary for efficient transposition: *P*-elements encode a 3′ *P*-transposase (3′ *P*) and a 5′ *P*-transposase (5′ *P*) site, whereas *piggyBac* elements contain a 3′ *piggyBac* transposase (3′ *Pbac*) and a 5′ *piggyBac* transposase (5′ *Pbac*) site. A hybrid element (**c**) that contains the features of both the *P*-element and *piggyBac* transposon should combine the advantages of both: precise excision and random hopping using *piggyBac* transposase, and imprecise excision using *P*-transposase. If two dominant markers are used, *yellow*+ and *white*+, it should be possible to screen for imprecise excisions resulting in unidirectional deletions.

Figure 1 | **Transposon-mediated gene-disruption screens.**

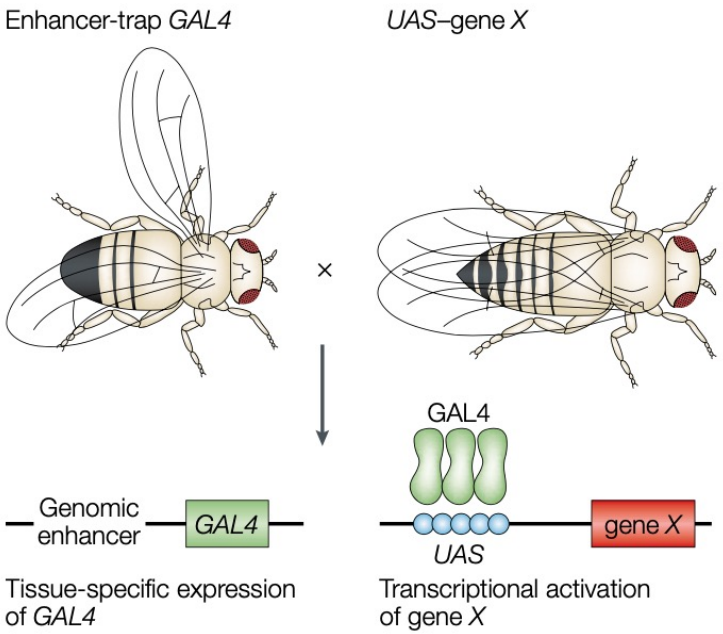
- P-element: imprecise excision
- piggyBac: precise excision and random hopping

BDGP (Berkeley Drosophila Genome Project): the goal is to obtain at least one P-element insertion in each gene

The GAL4-UAS system

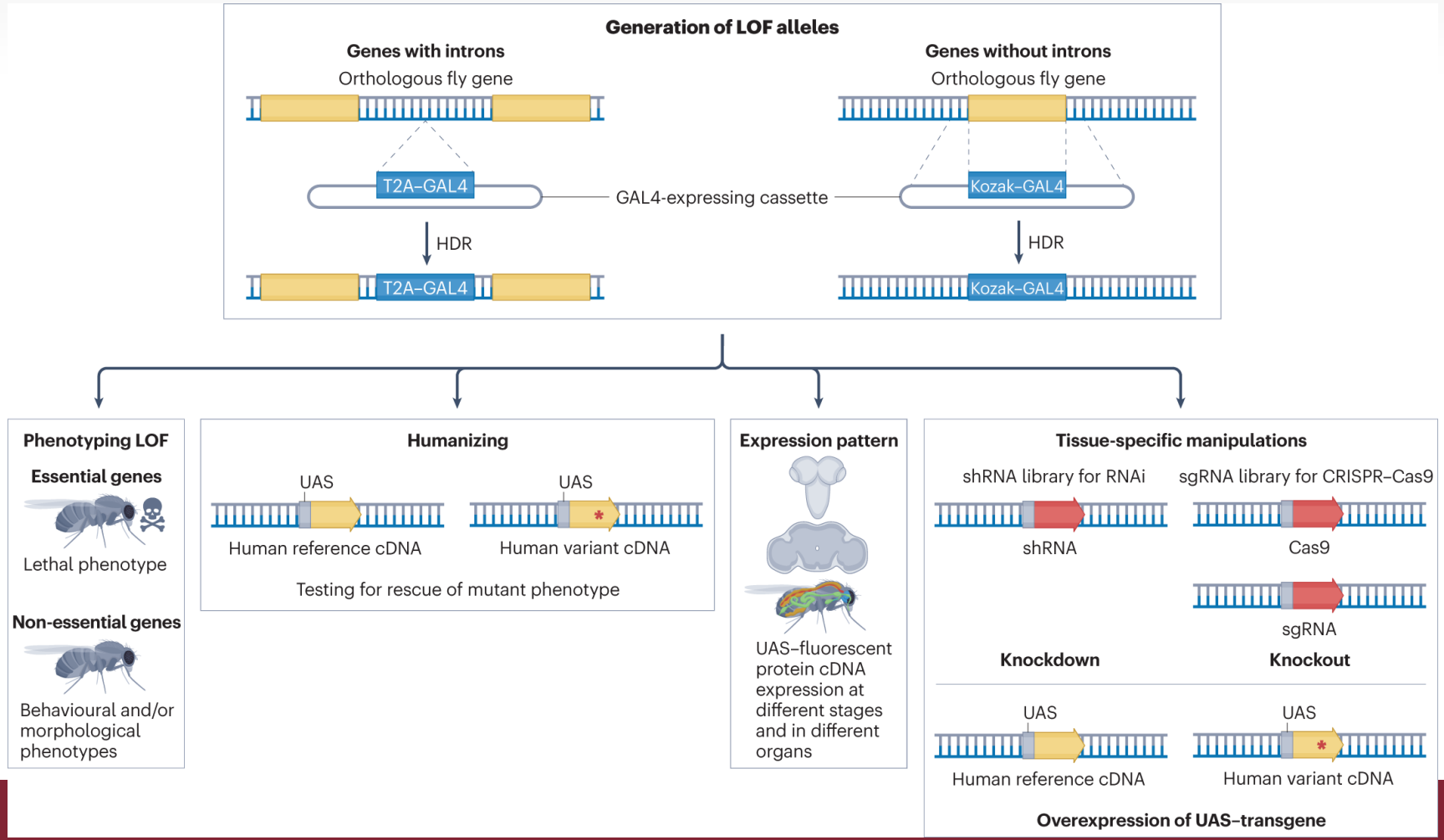
Box 2 | **The *GAL4–UAS* system for directed gene expression**

The yeast transcriptional activator Gal4 can be used to regulate gene expression in *Drosophila* by inserting the upstream activating sequence (*UAS*) to which it binds next to a gene of interest (gene *X*)[96]. The *GAL4* gene has been inserted at random positions in the *Drosophila* genome to generate 'enhancer-trap' lines that express *GAL4* under the control of nearby genomic enhancers, and there is now a large collection of lines that express *GAL4* in a huge variety of cell-type and tissue-specific patterns[97]. Therefore, the expression of gene *X* can be driven in any of these patterns by crossing the appropriate *GAL4* enhancer-trap line to flies that carry the *UAS*–gene *X* transgene. This system has been adapted to carry out genetic screens for genes that give phenotypes when misexpressed in a particular tissue (modular misexpression screens)[79].

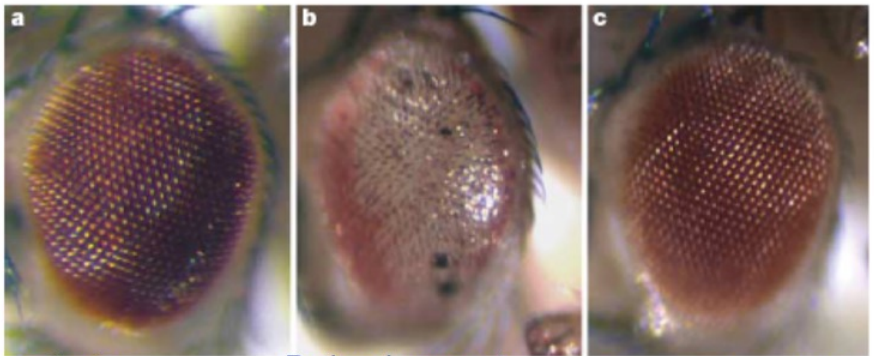


Driver lines Reporter lines

The GAL4-UAS system



APPLICATION 1: ectopic expression of disease genes

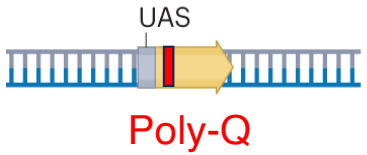


Wild-type

Ectopic PolyQ expansion in the eye: retinal degeneration

Suppression of the phenotype

Expanded tracts of Poly-CAG or Poly-Q repeats are associated with several hereditary disorders in humans including Huntington's Chorea and Muscular Dystrophy



- Generate flies with UAS-repeat expansion encoding artificial gene insertion
- Drive the transgene expression with a eye-specific GAL4 line
- Screen for mutation that suppress the phenotype

APPLICATION 2: RNAi in Drosophila

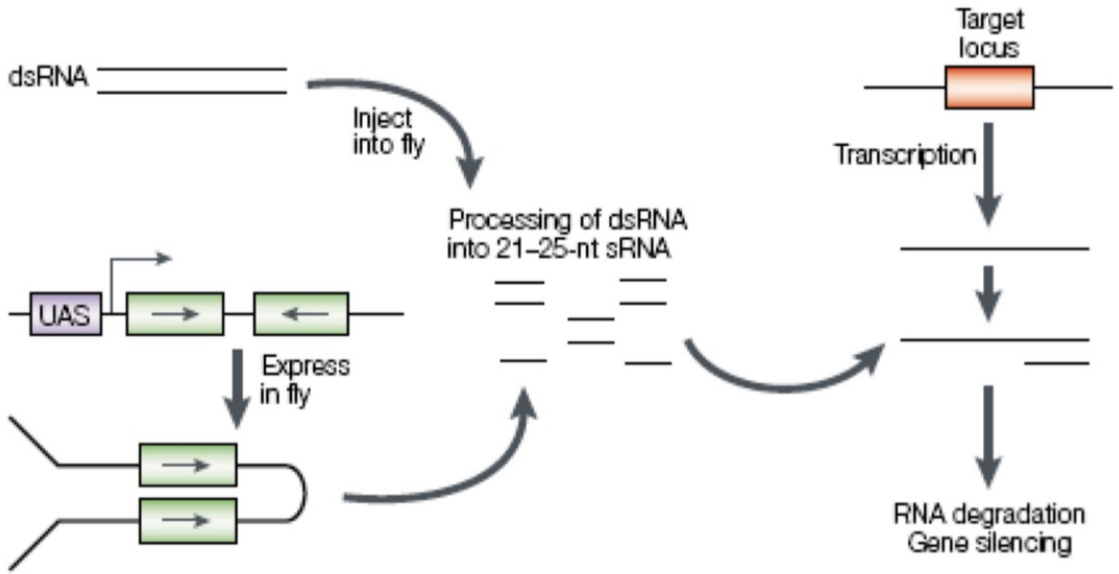
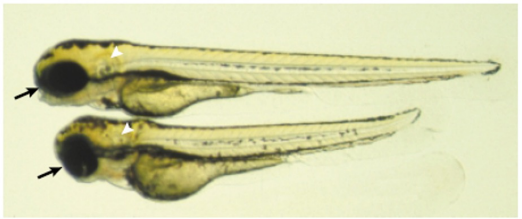


Figure 5 | **RNA interference in *Drosophila*.** Double-stranded RNA (dsRNA) that is homologous to the target gene is delivered by either of two methods: injection of *in vitro*-transcribed RNA into individual embryos (upper left), or expression of an inverted repeat RNA *in vivo* (lower left). The dsRNA is processed into 21–25-nucleotide (nt) small interfering RNAs (siRNA) by the Dicer ribonuclease[75]. The siRNA is used to guide the sequence-specific degradation of mRNA, leading to post-transcriptional silencing of the target locus. UAS, upstream activating sequence.

Zebrafish



- Vertebrate model!
- External development in translucid embryos
- Short generation time
- Large progeny allows genetic screening
- Small dimension and easy to handle

Zebrafish as an animal model for biomedical research

Zebrafish

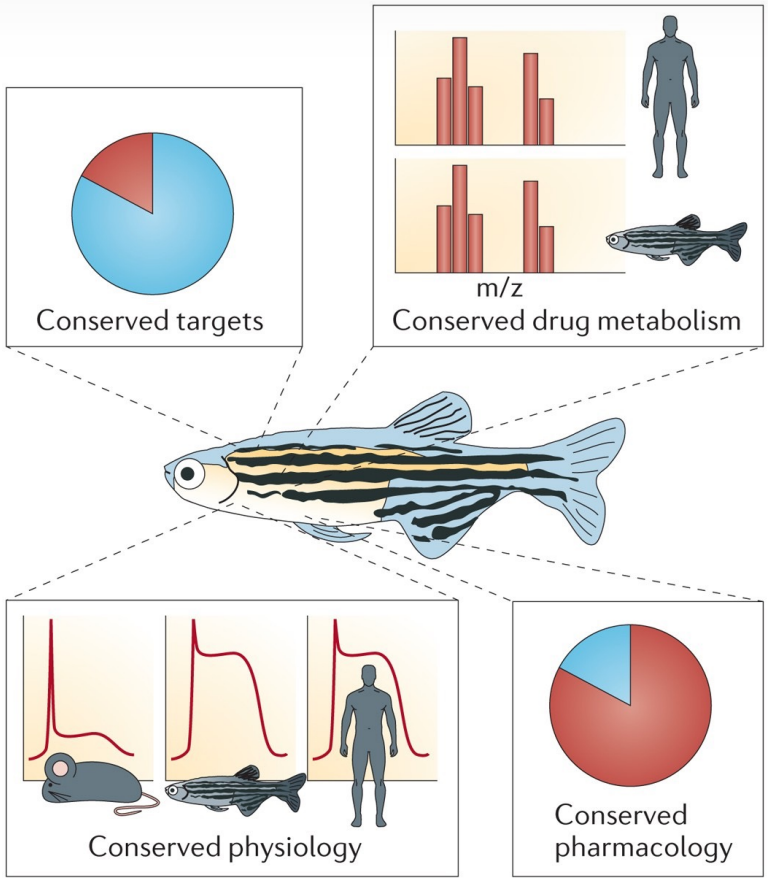
- The high level of genome structure shared between zebrafish and humans (~70% of human genes have at least one obvious zebrafish ortholog, compared to 80% of human genes with mouse orthologs) has facilitated the use of zebrafish for understanding human genetic diseases.

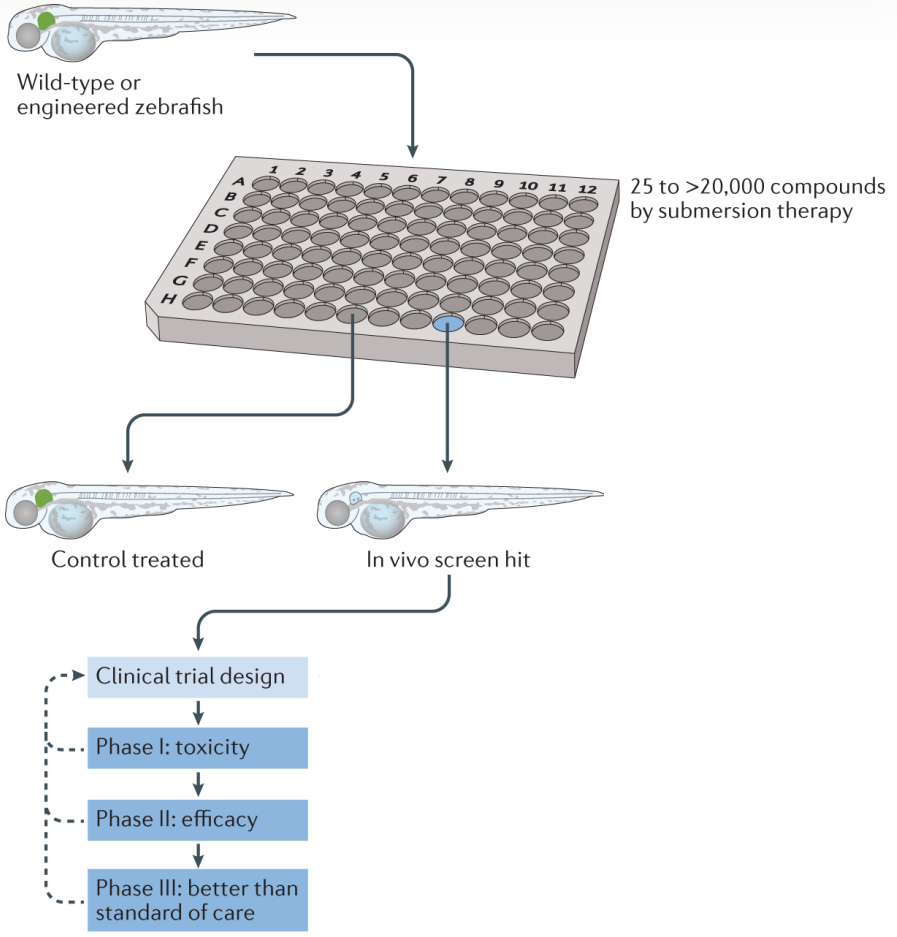
- Recent advancements in next-generation sequencing (NGS) coupled with the demand for personalized medicine has further driven zebrafish uses in identifying causal relationships between the genotype and phenotype of various human diseases.

- Zebrafish possess several advantages over rodent models in the study of vertebrate development and disease. These include hundreds of embryos in a single clutch and optical clarity of the developing embryo, which allows live imaging at the organism level.

- The use of tissue-specific transgenic animals can be easily generated under the control of various selected gene promoters.

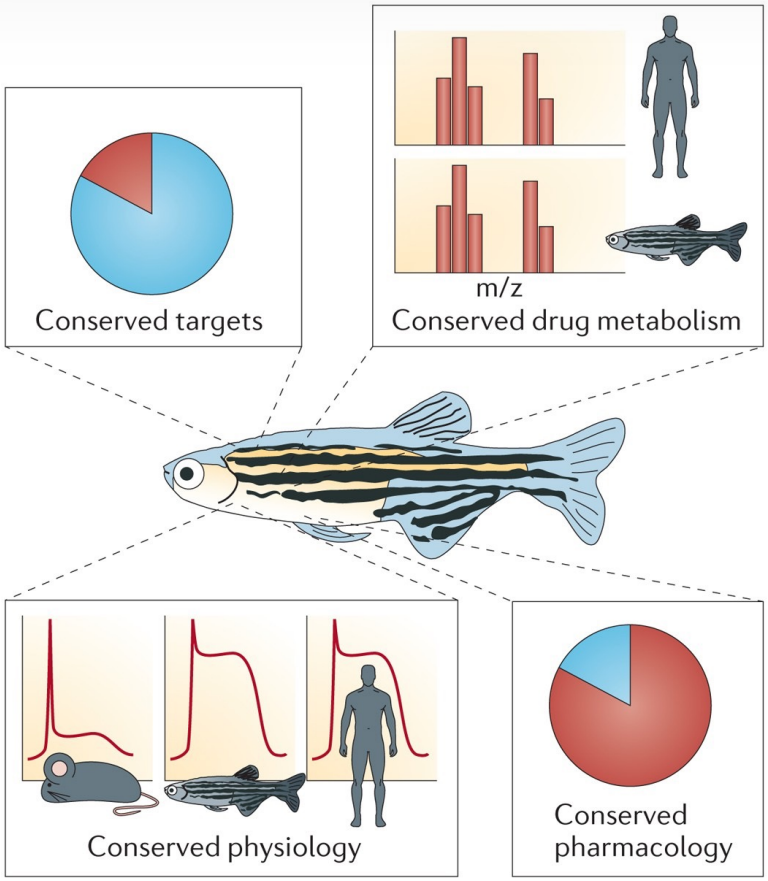
Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials



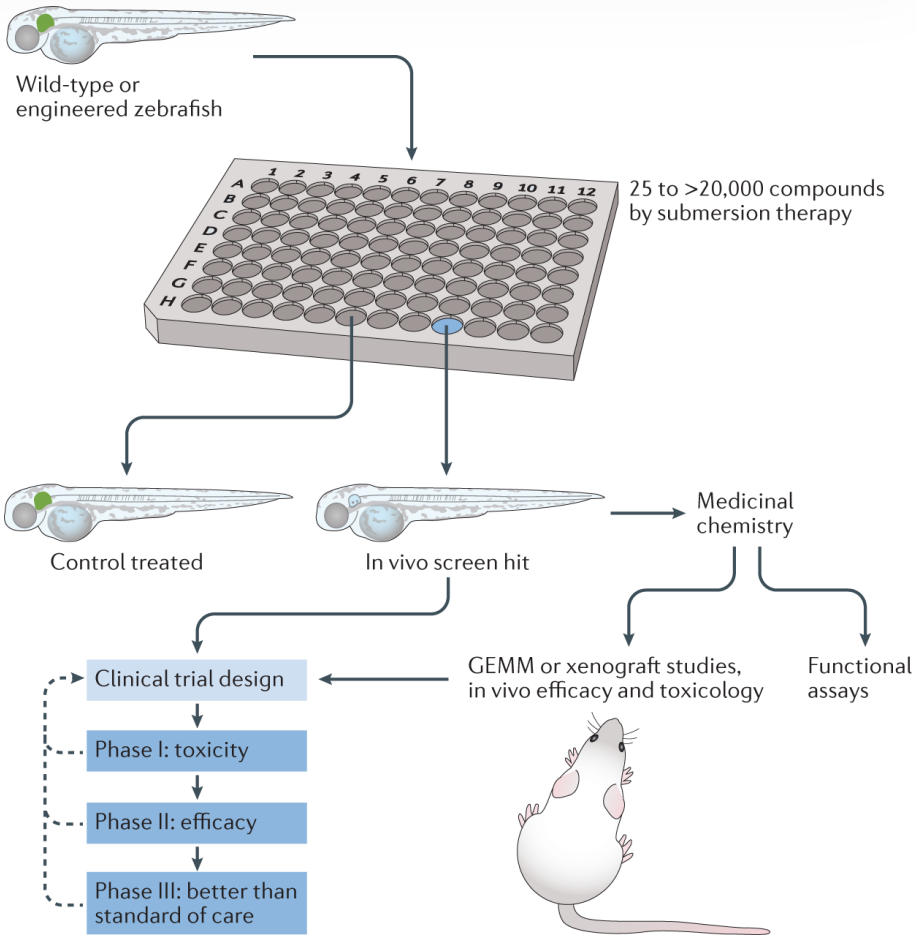


Nature Reviews | Drug Discovery

Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials



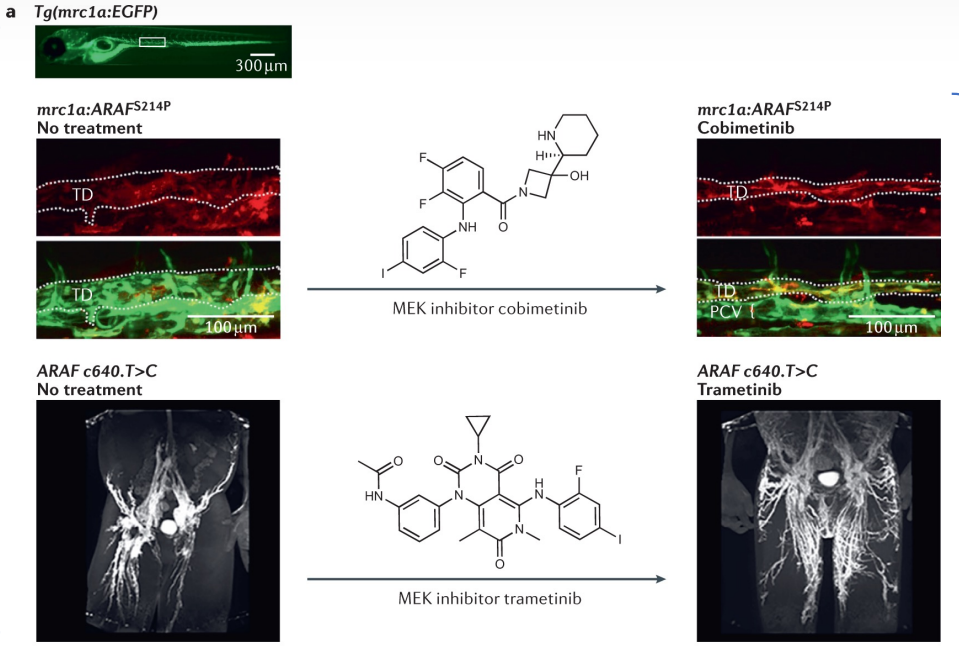
Nature Reviews | Drug Discovery



Compassionate drug treatment: from zebrafish to child.

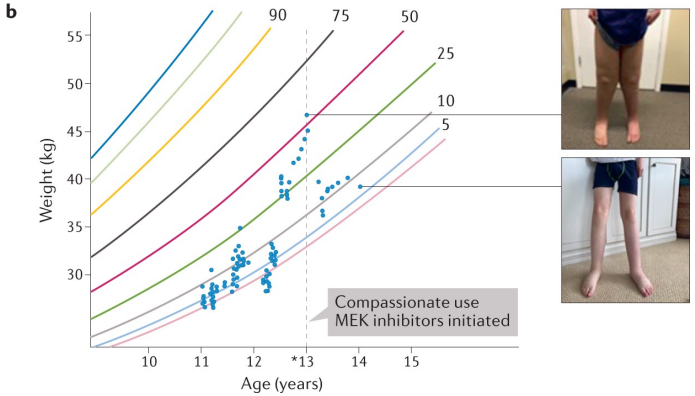
Transgenic zebrafish embryo expressing *ARAF*^{S214P} mutation (red) in the lymphatic endothelial cells (green), and lymphangiograms of the 12-year-old child with *ARAF*^{S214P} before treatment.

In zebrafish, lymphatic vessels become dilated with *ARAF*^{S214P} expression and, in the child, there are inadequate ducts in the thighs and dilation of the lymphatic ducts, resulting in significant swelling in the legs.



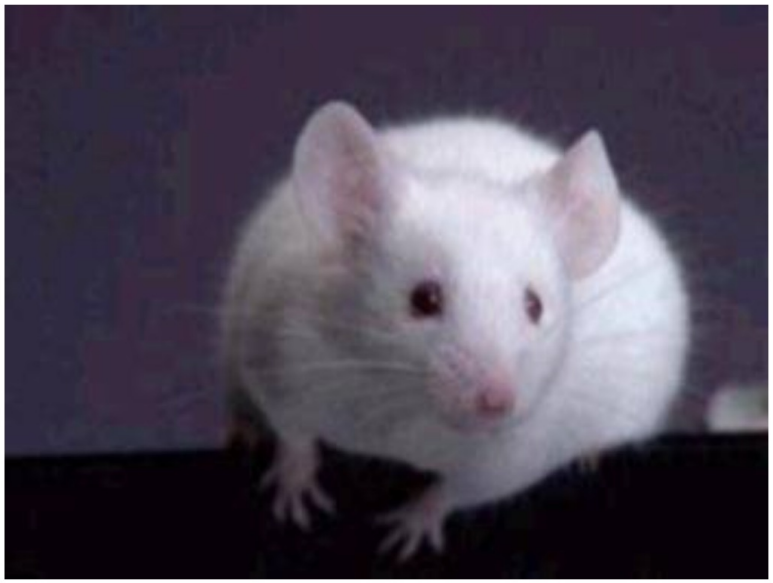
Treatment with MEK inhibitors rescues the zebrafish phenotype and resolves abnormal dilated ducts and normalizes lymphatic networks in the child

Standard growth chart, with measurements from the patient indicated as dots plotted on a graph that shows growth centiles by age. Treatment was initiated just before age 13. PCV, posterior cardinal vein;



The improved lymphoedema is clearly seen in the patient's legs (inset photos from the indicated times) and weight.

Mus musculus

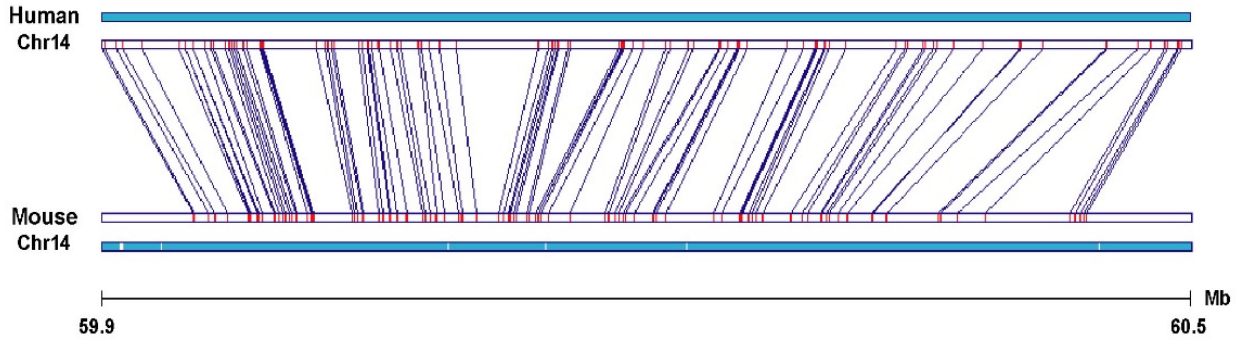


ADVANTAGES

- Small
- Lots of pups
- Mutant availability
- Sequenced genome
- It’s a MAMMAL!

Mouse genome

Extremely high conservation: 560,000 “anchors”



Mouse-Human Comparison

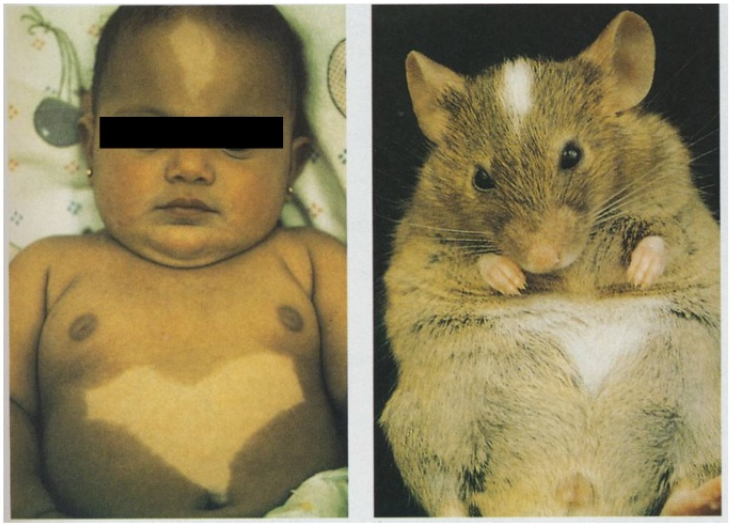
both genomes 2.5-3 billion bp long
> 99% of genes have homologs
> 95% of genome “syntenic”

19 autosomes, X and Y

20.000 genes

A model for human disease

ADVANTAGES



Kit mutation

DISADVANTAGES

- Differences in cognitive functions, behaviour, genic expression
- Long gestation compared to other model systems
- **Requires animal facility**

Inbred strains

Is defined as "*one that has been maintained for more than 20 generations of brother-sister matings and is essentially homozygous at all genetic loci, except for mutations arising spontaneously*"

Therefore, these strains are genetically identical in all other aspects except at the locus of interest - useful for studying cancer, inheritance of visible traits, histocompatibility etc…

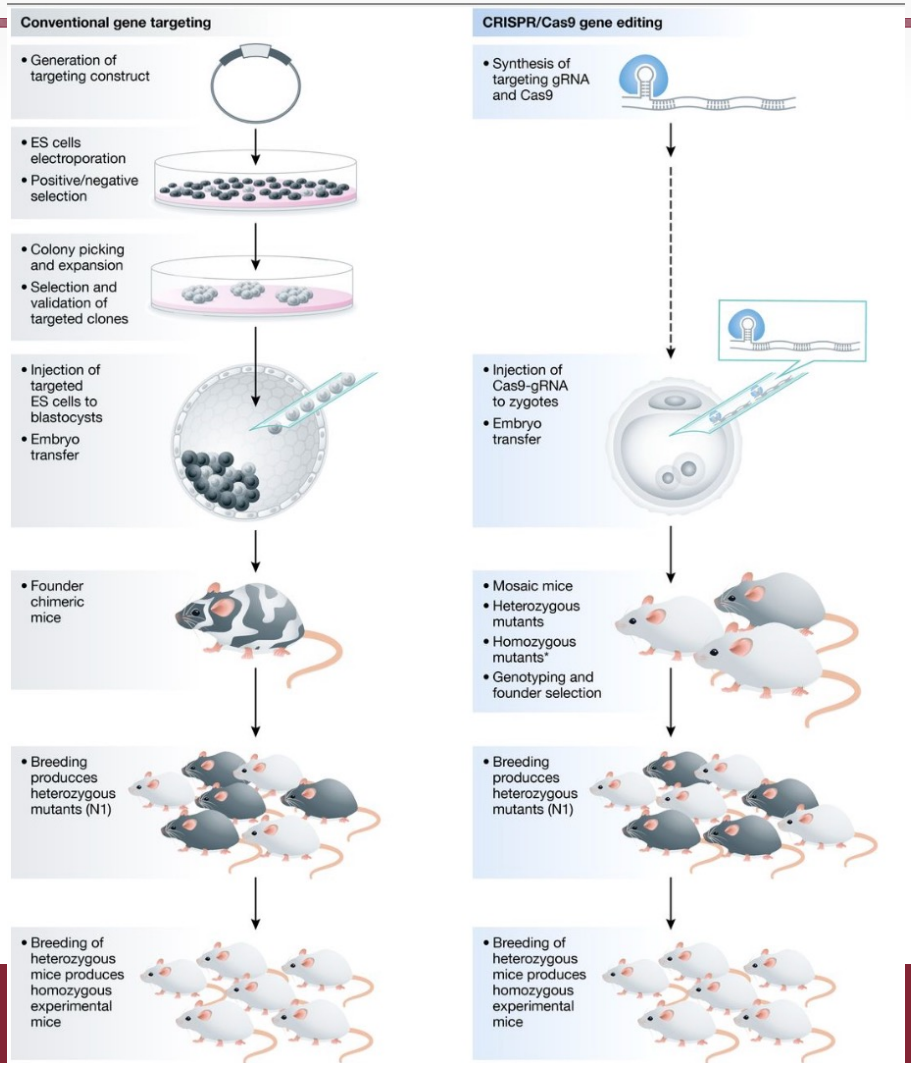
The Jackson Laboratory is the major supplier of inbred strains

Insertional mutagenesis in mouse

- Random mutagenesis with retrovirus
- Knock-out mouse models
- Knock-in mouse models
- Inducible systems
- Transposon mutagenesis

How to modify the genome of mouse

Gene targeting (homologous recombination)

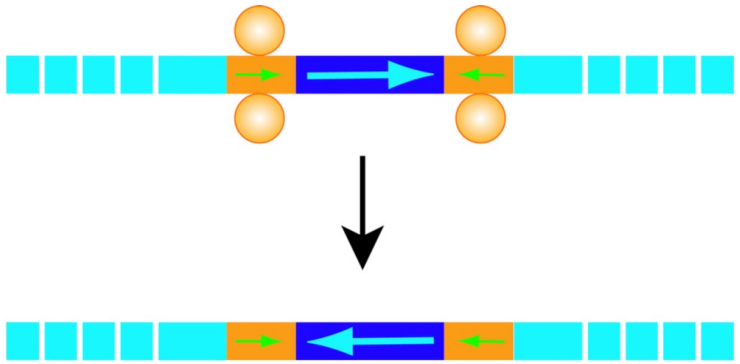


CRISPR/CAS9 gene editing

Cre-Lox system

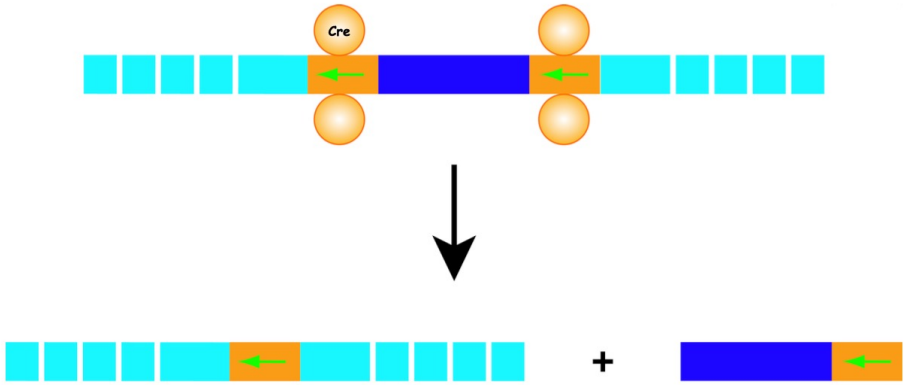
- Cre is a ricombinase from P1 phage
- Cre recognises a 34bp site loxP and induced reciprocal and conservative recombination between two loxP sites

INVERTED REPEATS



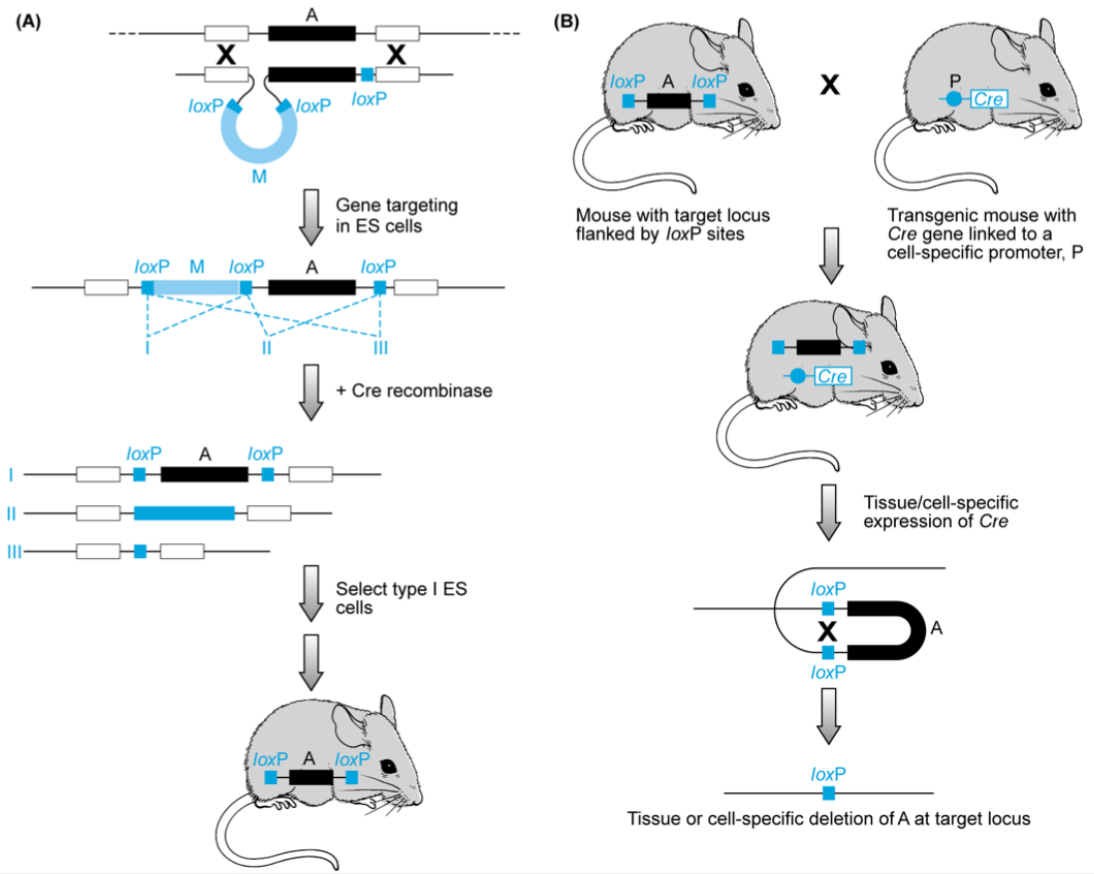
Result: inversion of the loxP sequence

TANDEM REPEATS



Result: excision of the loxP sequence

Conditional or tissue specific knock-out



Other applications

Human-Animal Chimeras

The primary goal of chimera research is to produce human cellular characters in model animals

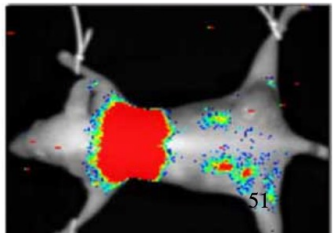
- **Human cell line or tumor transplantation**
- **Primary cell transplantation**
- **Organ transplantation**
- **hES transplantation**

Xenograft models

Are produced by grafting human cells into an immune-deficient animal

1. The **immune-deficient athymic nude mouse** is the most utilized for xenograft. ***Nude*** is a recessive mutation that results in loss of T cells. They still have B cells and NK cells.
2. Mice with the **sever combined immunodeficinecy (SCID)** crossed with **nonobese diabetic (NOD)**, NOD-SCID mouse, show geater human engraftment.

Useful to understand cancer mechanism and to test therapies against tumors



In 2014, the Encyclopedia of DNA Elements (ENCODE) program published a comparative analysis of the genomes of *Homo sapiens* and *Mus musculus* , as well as an in-depth analysis of the differences in the regulatory landscape of the genomes of these species . ENCODE, a follow-up to the Human Genome Project, was implemented by the National Human Genome Research Institute (NHGRI) at the National Institutes of Health in order to develop a comprehensive catalog of protein-encoding and nonprotein-coding genes and the regulatory elements that control gene expression in a number of species. This was achieved using a number of genomic approaches (e.g., RNA-seq, DNase-seq, and ChIP-seq) to assess gene expression in over 100 mouse cell types and tissues; the data were then compared with the human genome.

Overall, these studies showed that although gene expression is fairly similar between mice and humans, considerable differences were observed in the regulatory networks controlling the activity of the immune system, metabolic functions, and responses to stress, all of which have important implications when using mice to model human disease. In essence, **mice and humans demonstrate genetic similarity with regulatory divergence**. Specifically, there is a high degree of similarity in transcription factor networks but a great deal of divergence in the cis-regulatory elements that control gene transcription in the mouse and human genomes. Moreover, the chromatin landscape in cell types of similar lineages in mouse and human is both developmentally stable and evolutionarily conserved

The 3Rs

The 3Rs are principles of good science designed by scientists to improve animal welfare and scientific accuracy.

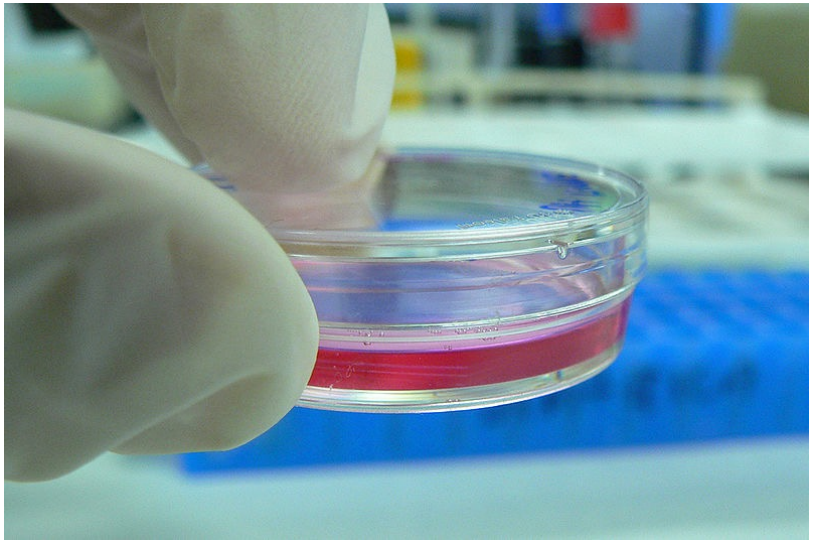
Refinement – Finding ways of making animals' lives better in labs, this can include toys for animals or better training for technicians

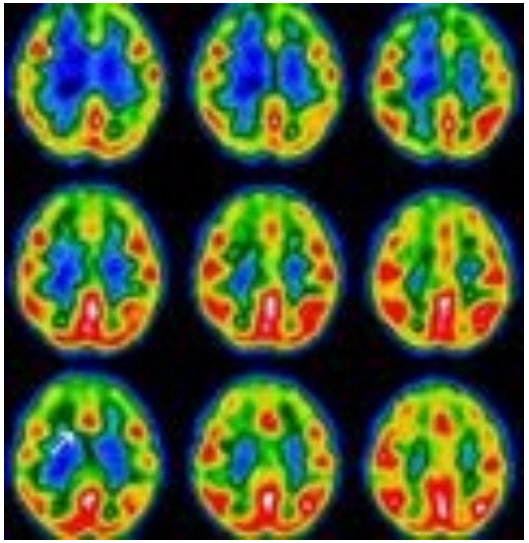
Reduction – Using as few animals as possible to get good results

Replacement – Using non-animal alternatives wherever they exist

Alternatives

Scientists use many ways to try to replace animals used in research. These include using cell cultures, computer modelling and human studies. Researchers **must,** by law, use these techniques if they would be as effective as using animals.



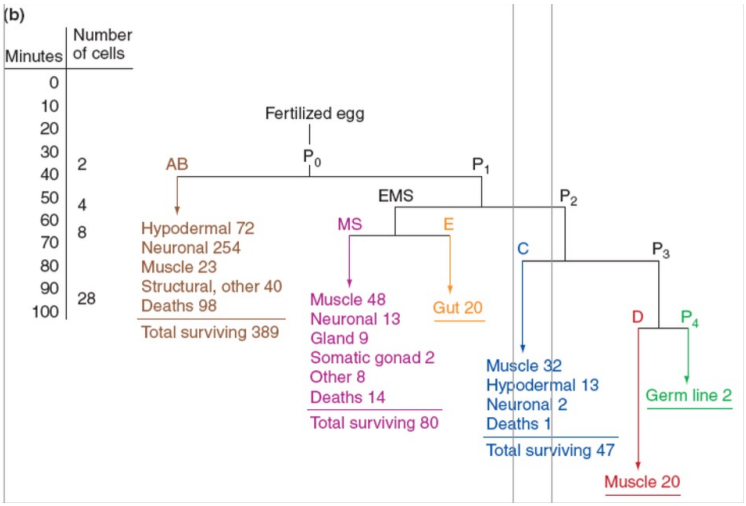


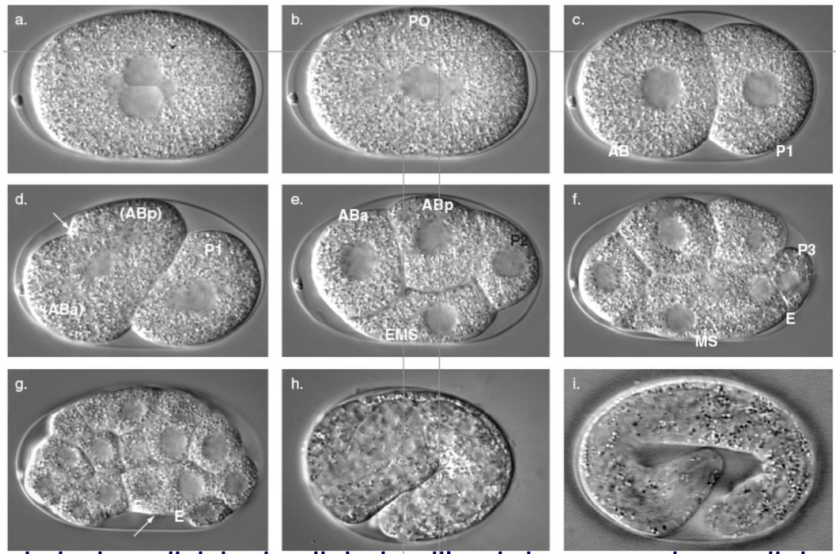
C.elegans

- Harmless Nematode
- Can grow in petri dish, economical, easy to maintain in laboratory settings, easy manipulation
- Adult *C. elegans* are 1 mm long self-fertilizing hermaphrodites with a 2.5–4 days reproductive cycle at room temperature, and a mean lifespan of approximately 18–20 days when cultured at 20°C
- 97Mb genome, 6 holocentric chromosomes
- The C. elegans genome possesses homologs of about two-thirds of all human disease genes

Why C.elegans?

- Several development, physiology similar to Drosophila and humans
- Useful to understand development pathways and conserved functions through evolution
- Invariance of spatiotemporal position and cell fate. Genetic control of development
- Apoptosis phenomena during development





RNAi in C-elegans

Performing RNAi interference experiments in C.elegans is easy

You can just feed the worms with siRNA!

How to explore genic function

- **Morpholino (MOs)** : Gene knockdown by Oligonucleotidi antisenso, modificati chimicamente che inibiscono la traduzione in modo specifico. Utilizzati per interazioni genetiche

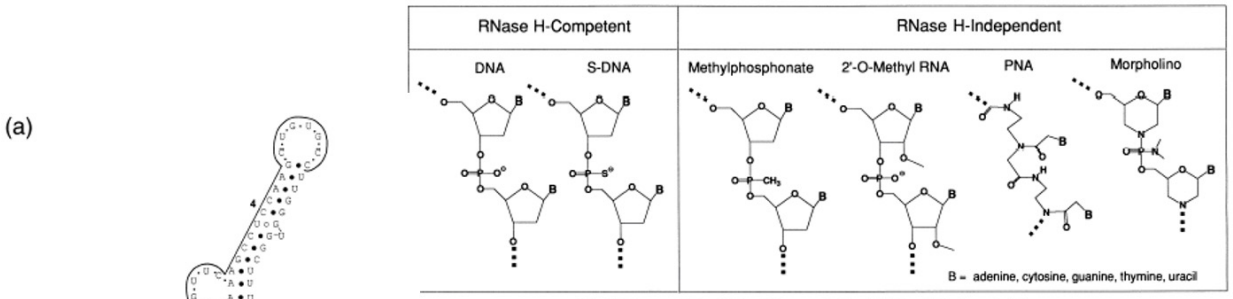


Fig. 1. Representative RNase H-competent and RNase H-independent types.