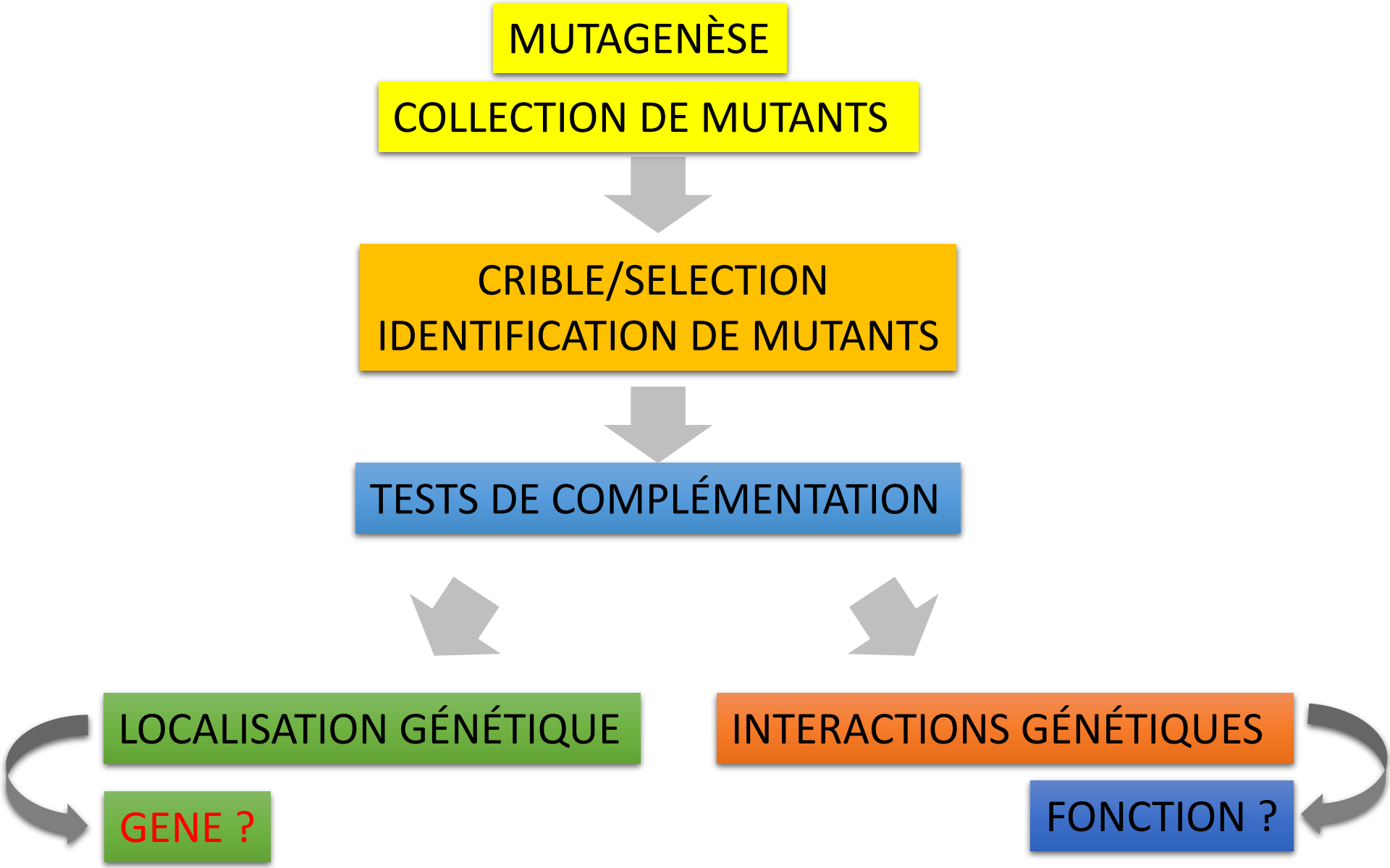
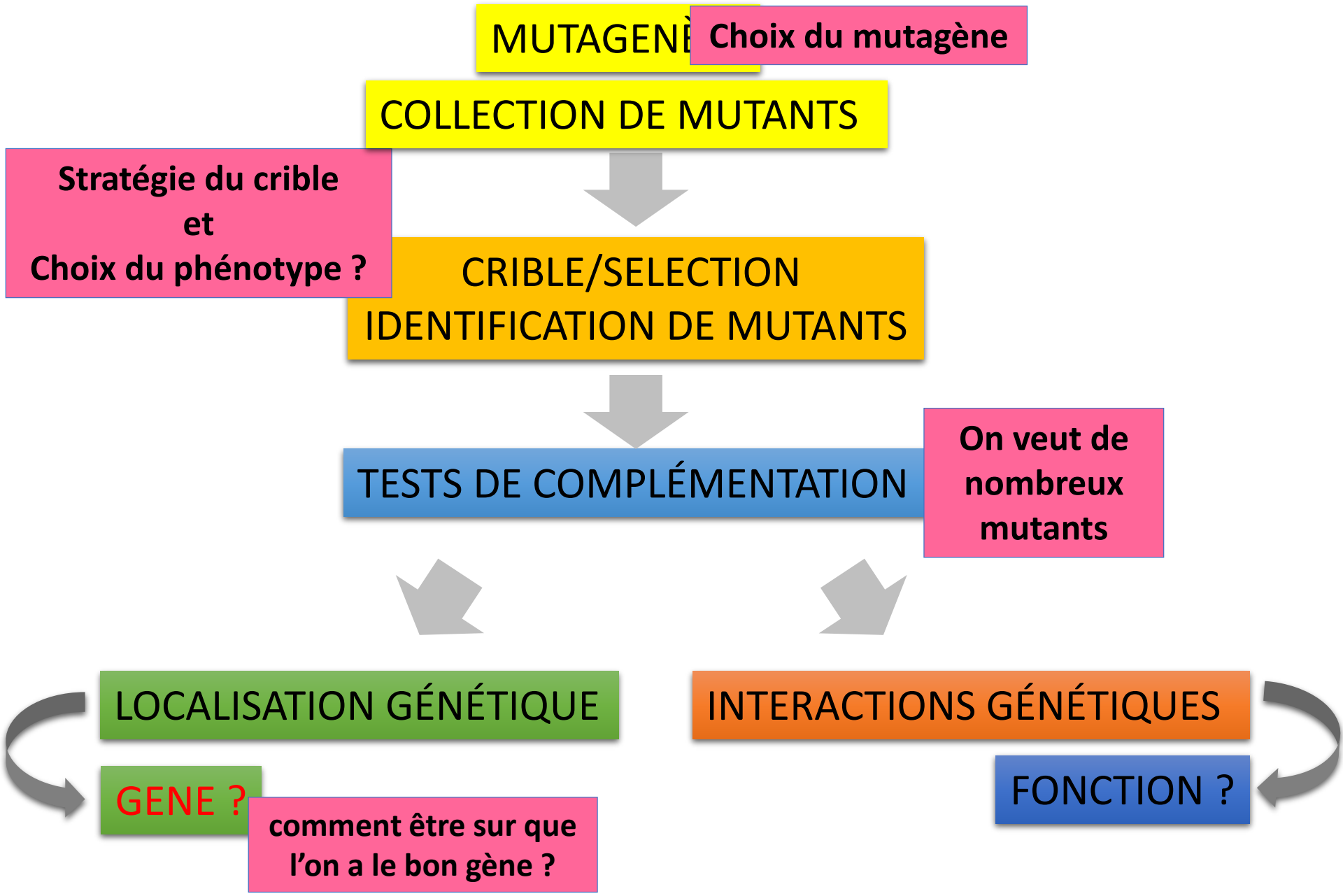
# examen

- Question de cours & problème/excercice


MUTAGENÈSE
COLLECTION DE MUTANTS
CRIBLE/SELECTION
IDENTIFICATION DE MUTANTS
TESTS DE COMPLÉMENTATION
LOCALISATION GÉNÉTIQUE
GENE ?
INTERACTIONS GÉNÉTIQUES
FONCTION ?


MUTAGENÈ
Choix du mutagène
COLLECTION DE MUTANTS
Stratégie du crible
et
Choix du phénotype ?
CRIBLE/SELECTION
IDENTIFICATION DE MUTANTS
TESTS DE COMPLÉMENTATION
On veut de nombreux mutants
LOCALISATION GÉNÉTIQUE
INTERACTIONS GÉNÉTIQUES
GENE ?
comment être sur que l'on a le bon gène ?
FONCTION ?

# II- Mutagenèses et cribles

A. Choix mutagène
B. Collections de mutants
C. Phénotype et crible
D. Stratégies croisements cribles
E. Mutants conditionnels

Bilan : avantages et inconvénients

# A. Mutagenèse: choix de la nature et la dose de mutagène

NATURE

- Chimique
- Physique
- Transposons

DOSE

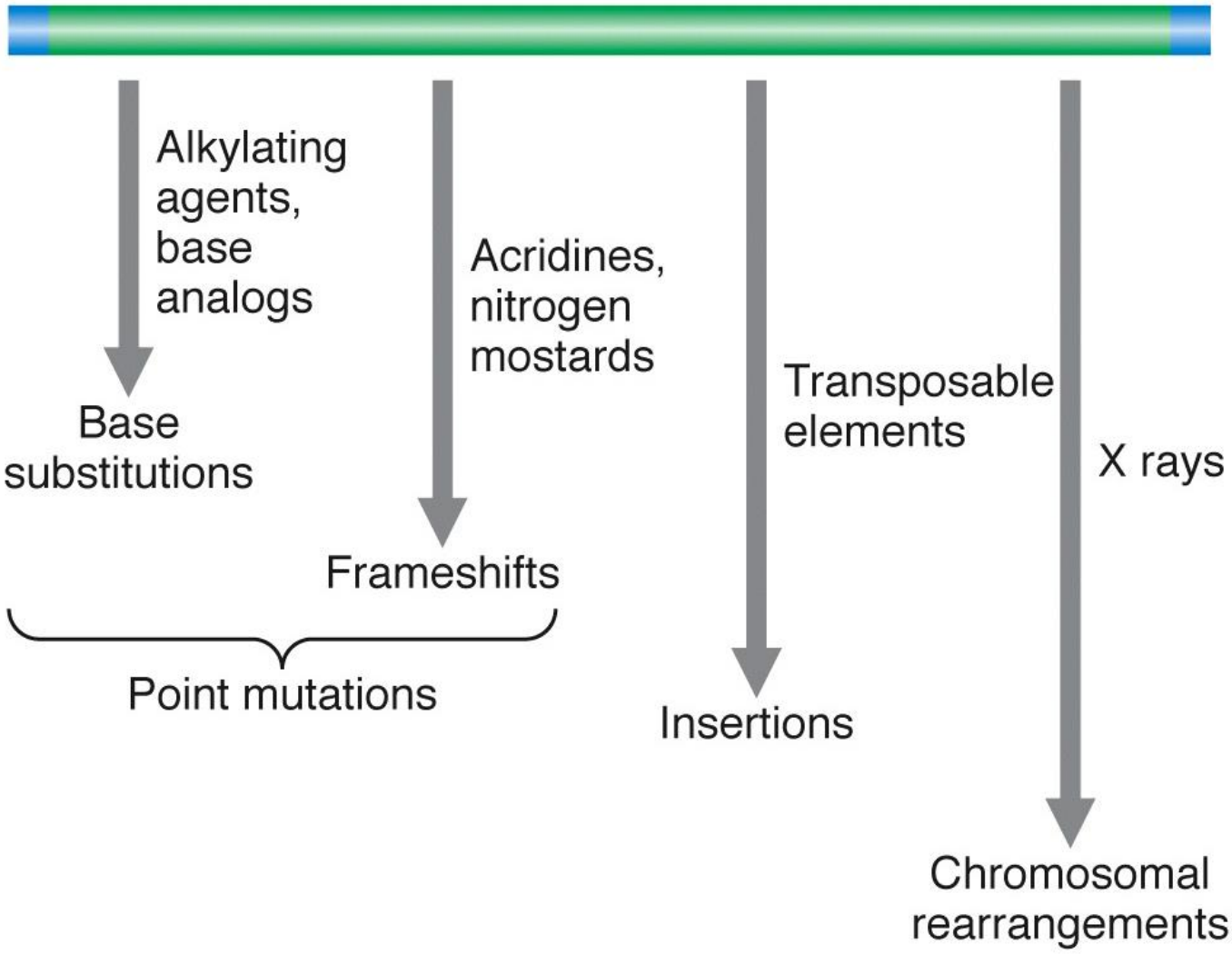
- Ni trop, ni trop peu….

# Nature du mutagène

- Chimique
- Physique
- Transposons

1. Le type de mutations
2. La facilité que l'on aura à identifier le gène muté

**Each chemical causes a different spectrum of base pair changes, a reflection of chemistry and repair systems.**



# Transposons based mutagenesis

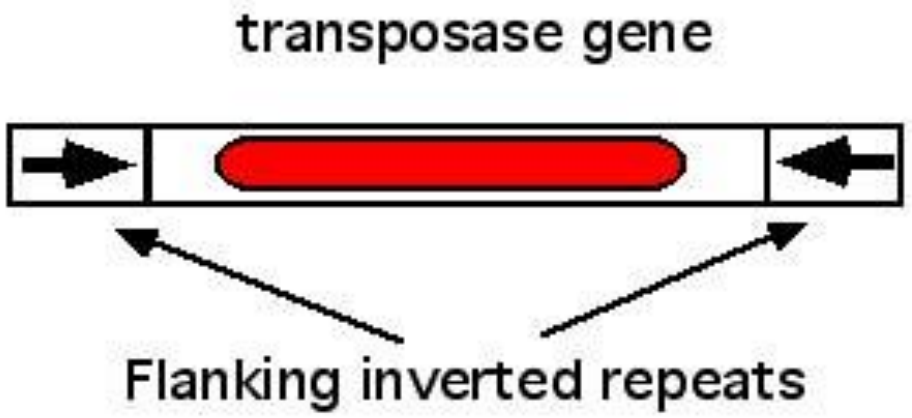
**TRANSPOSONS :**

**DNA elements or Retroelements which move, or transpose, from one site in the genome to another**

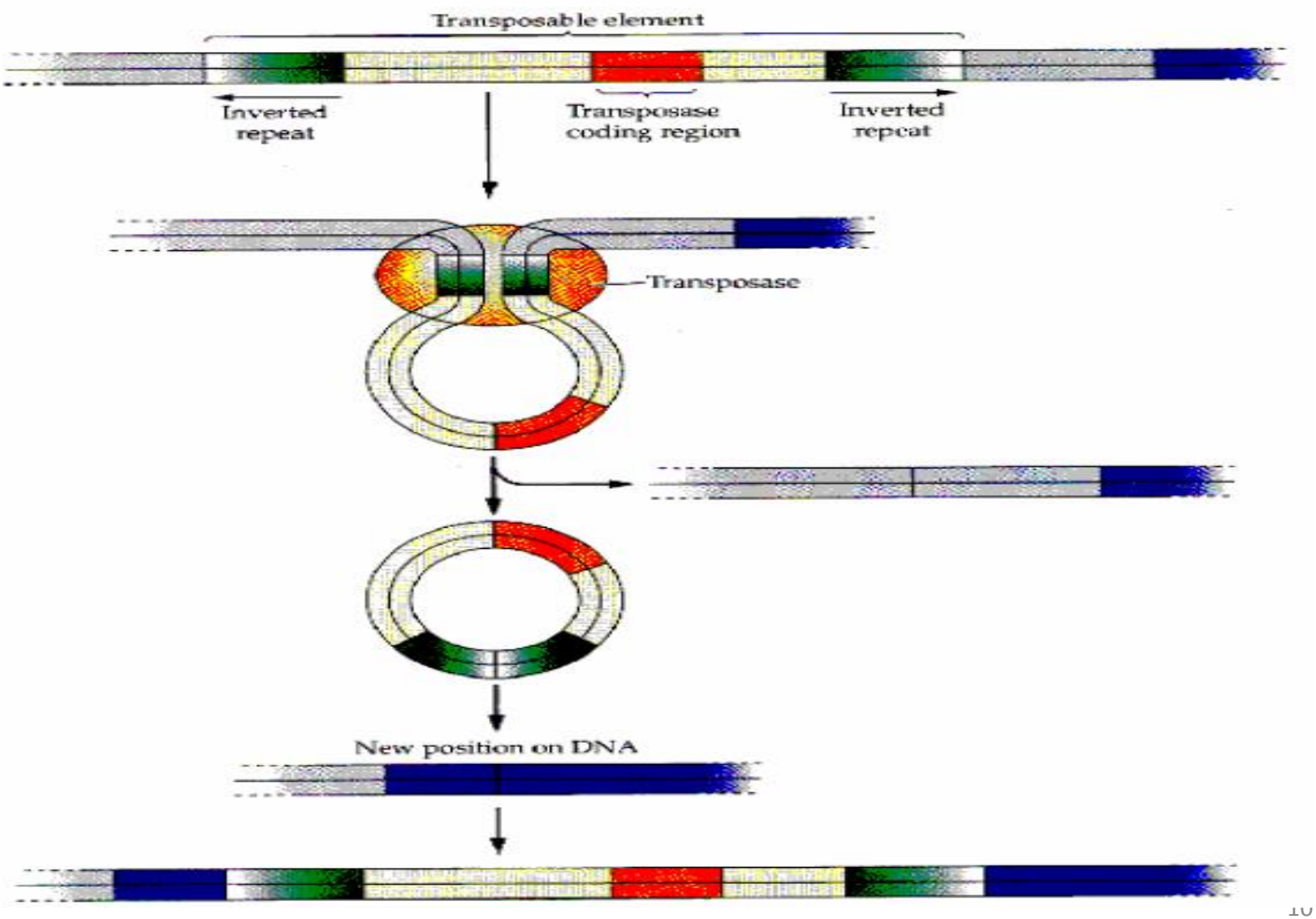
# DNA Mobile Genetic Elements

- transpose via DNA intermediates
- Examples: Ac/Ds and Spm in plants, P elements in animals, Tn in bacteria
- Have Short inverted repeats at the ends of the element

-> substrates for recombination reactions mediated by a transposase



# Structure and transposition of a transposable element



# Tra snposon emdiated mutagenesis

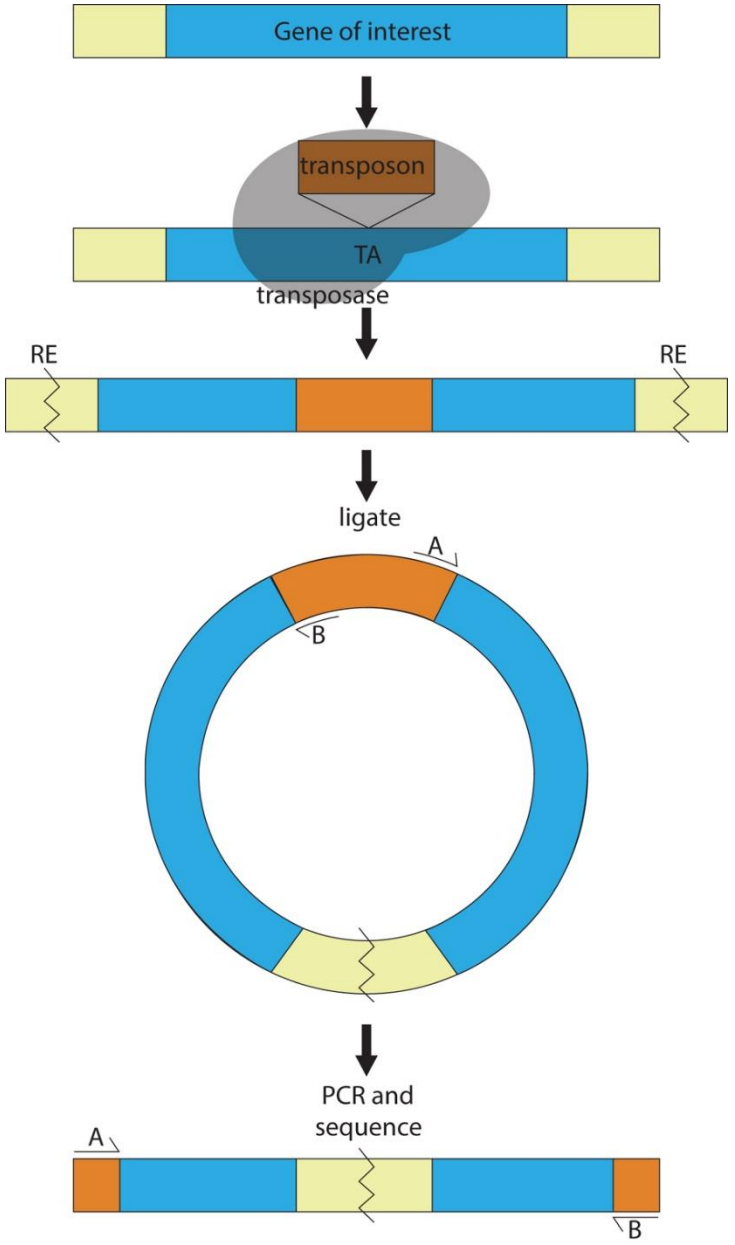
Strain with a transposon

+ Transposase

Mobilization of the transposon

Insertion in other position

# Easy to map/clone



# Transposons based mutagenesis

- **The insertion and excision of transposable elements result in changes to the DNA at the transposition site**
- **The transposition can be identified when a known DNA sequence or selection markers are inserted within the elements**
- **The site of insertion can be “easily” identified**

- Many variants of this method (to be seen later) :
  - Simple
  - Enhancer traps
  - Fusion protein
  - Over/ectopic expression

# Cribler jusqu'à plus soif….

**Spontaneous Mutation**

**$10^{-9}$ per base/generation**

**a 1 Kb ORF gene mutated every $10^{-6}$generation**

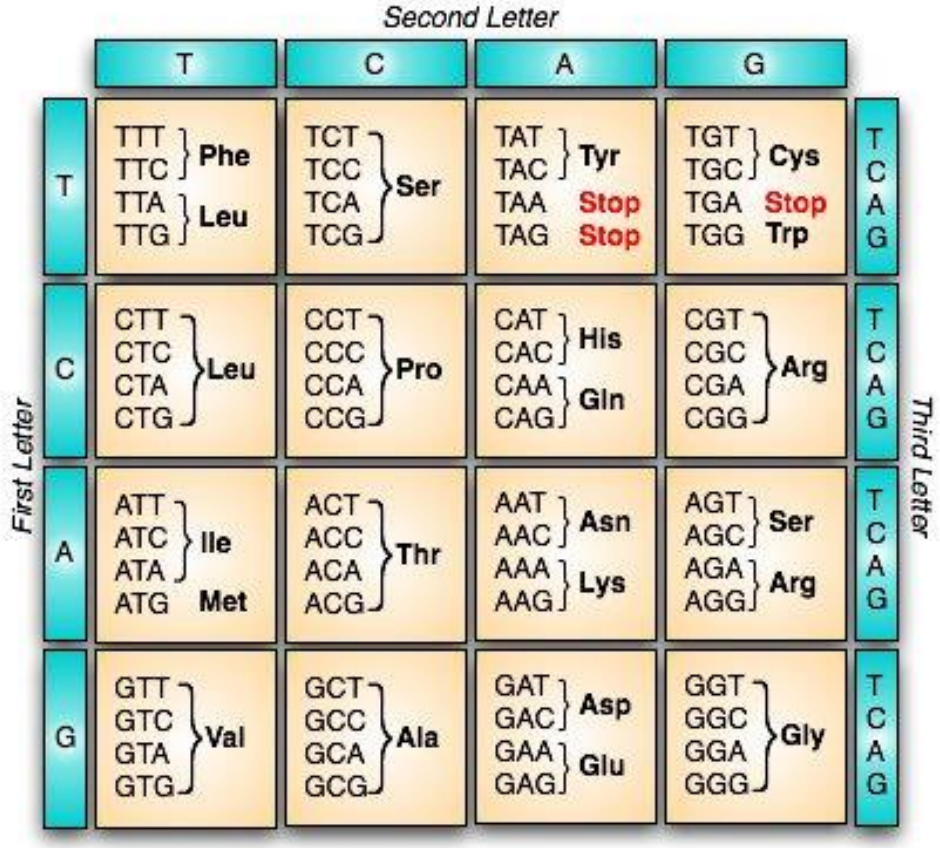
**Chemical mutagenesis can increase mutation rates several orders of magnitude.**

# Cribler jusqu'à plus soif….

**Levure**

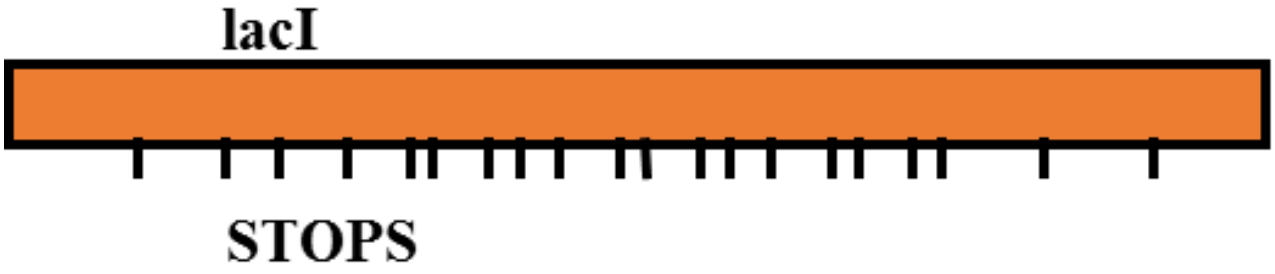
**14 Mb genome**

**$1.4 \times 10^7$ base pairs x 3 = $4.2 \times 10^7$ possible single base changes.**




**Les changements d'aa sont restreints par le code !**

# *E. coli*, lacI gene

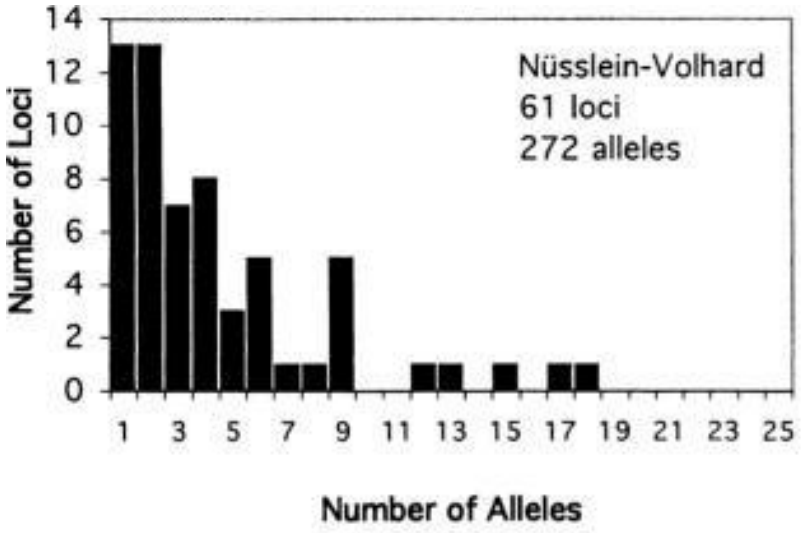


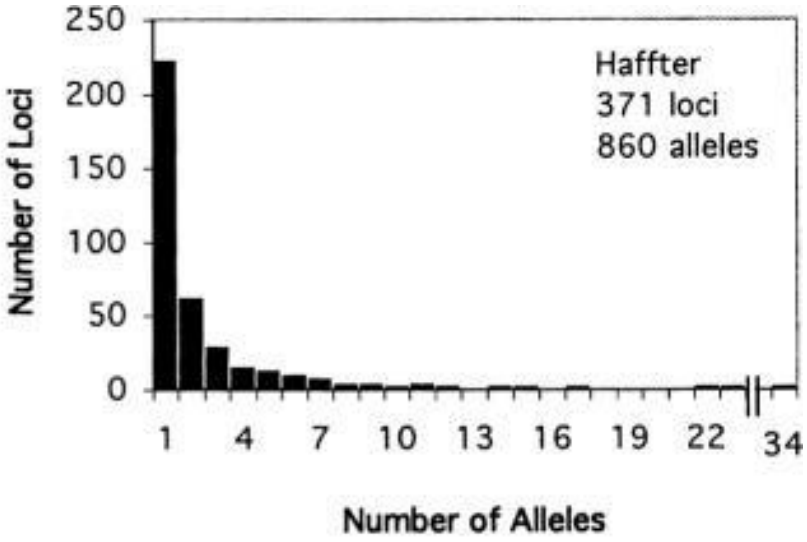

**90% of all positions can tolerate any amino acid**
**10% can tolerate only a few different amino acids**

# Cribler jusqu'à plus soif….

Notions d'indépendance des mutants  et de saturation du crible

- Indépendance mutants: en parallèle
- Saturation
  - Pourquoi ?
  - Comment l'estimer ?


Nüsslein-Volhard
61 loci
272 alleles
Number of Loci
Number of Alleles
0
2
4
6
8
10
12
14
1 3 5 7 9 11 13 15 17 19 21 23 25


Haffter
371 loci
860 alleles
Number of Loci
Number of Alleles
0
50
100
150
200
250
1 4 7 10 13 16 19 22 34

# D Collections de mutants

- Collection de « délétions KO »
- Banques d'insertions de transposons
- Banques ARN interférence

etc

# 1- La collection de disruptants de levure

# Séquencage S. cerevisiae

## 1996: 1er génome eucaryote séquencé

• The project got started in 1989

•May 1992, the complete nucleotide sequence (315 kb) of an entire chromosome ( yeast chromosome III ) was published by 35 European laboratories.

•1994, the sequence of two more chromosomes was published: chromosome II of 820 kb and chromosome XI of 666 kb.

• Spring 1996 the entire sequence of the yeast genome known (More than 100 laboratories from Europe, USA, Canada and Japan took part of the joined effort.)

# Analyse fonctionnelle

Trois points critiques :

- Identification séquences codantes (analyse bioinformatique)

- Attribuer fonction nouvelles gènes putatifs (approches globales + analyse bioinfo)

- Gérer les informations (banques de données)

Science magazine



# Life with 6000 Genes

**A. Goffeau, * B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, E. J. Louis, H. W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin, S. G. Oliver**

The genome of the yeast *Saccharomyces cerevisiae* has been completely sequenced through a worldwide collaboration. The sequence of 12,068 kilobases defines 5885 potential protein-encoding genes, approximately 140 genes specifying ribosomal RNA, 40 genes for small nuclear RNA molecules, and 275 transfer RNA genes. In addition, the complete sequence provides information about the higher order organization of yeast's 16 chromosomes and allows some insight into their evolutionary history. The genome shows a considerable amount of apparent genetic redundancy, and one of the major problems to be tackled during the next stage of the yeast genome project is to elucidate the biological functions of all of these genes.

→ 5885 protein-encoding genes initially found in the *S.cerevisiae*

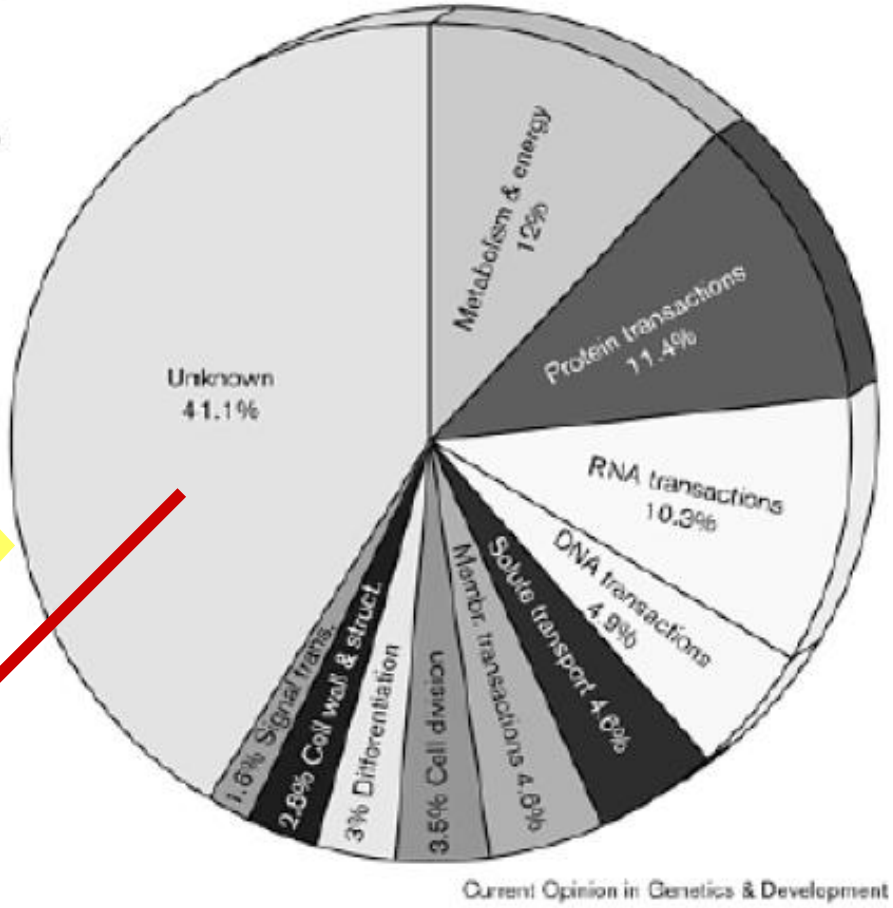
**TABLE 1. The yeast genome broken down into its various identified genetic elements**

| Genetic element | Total number | Density[a] (kb per element) | Average size (kb) | Total kb | Contribution to yeast genome[b] (%) |
|---|---|---|---|---|---|
| **Protein-coding elements** | | | | | |
| ORFs (total[c]) | 6183 | 2.0 | 1.450 | 8965.0 | 68.03 |
| Actual protein-coding genes[d] | 5800 | 2.1 | 1.450 | 8410.0 | 65.82 |
| Introns | 233 | 52.0 | 0.500 | 116.0 | 0.88 |
| **RNA-coding elements** | | | | | |
| rDNA repeats | 120–130 | – | 9.000 | 1125.0 | 8.54 |
| tRNA genes | 262 | 46.0 | 0.080 | 21.0 | 0.16 |
| Introns in tRNA genes | 80 | – | 0.020 | 1.6 | 0.01 |
| Other RNA species | 37 | 326.0 | 0.500 | 18.0 | 0.14 |
| **Mobile elements[e]** | | | | | |
| Complete Ty elements | 53 | 227.0 | 5.600 | 297.0 | 2.25 |
| Solo LTRs | 363 | 33.0 | 0.330 | 119.0 | 0.90 |
| **Chromosomal elements** | | | | | |
| ARS consensus | 750 | 16.0 | 0.020 | 15.0 | 0.11 |
| Centromeres | 16 | – | 0.095 | 1.5 | 0.01 |
| Subtelomeric elements (Y′)[e] | 21 | – | 5.800 | 121.0 | 0.92 |
| Subtelomeric elements (X)[e] | 31 | – | 0.400 | 12.0 | 0.09 |
| Telomeric ($C_{1-3}A$) repeats | 32 | – | 0.300 | 9.6 | 0.07 |
| **'Intergenic' regions** | | | | | |
| Including promoters, terminators, regulatory sequences, and all unidentified elements | – | – | 0.500 | 2912.0 | 22.10 |

[a]Density is calculated for the whole yeast genome, except rDNA.
[b]Relative contribution is calculated from the whole yeast genome, including rDNA.
[c]ORFs as defined in the European sequencing program[11].
[d]Predicted number of actual protein-coding genes, assuming that 6–7% of ORFs are not actual genes.
[e]Actual number of such elements varies from strain to strain.

-environ 6000 gènes
-seulement 4% avec des introns
-organisation compacte avec 70%
-du génome contenant des gènes
-et très peu de séquences répétées

**Comparaison autres organismes**
**Identification séquences consensus**




1/3 ORF non annotées
« orphelines »

# Analyse fonctionnelle du génome

**Analyse systématique, à grande échelle (automatisation, outils informatique)**

Profil d'expression «Transcriptome, Protéome »

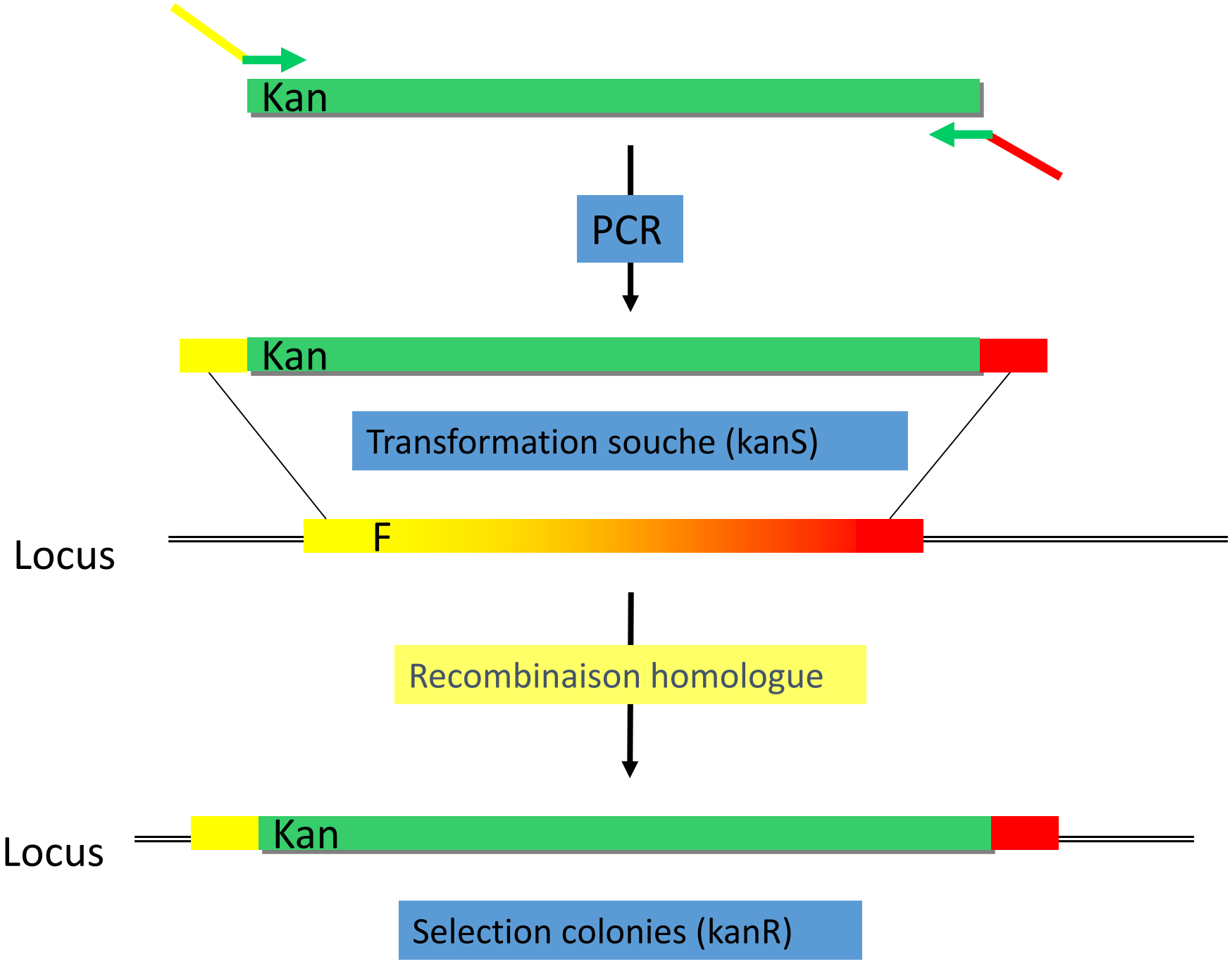
Phénotype de disruptants simples « Phénome »

Localisation « Localisome »

Partenaires physiques « Interactome»

# Elaboration d'une collection de disruptants

- But: inactiver chacun des gènes ->


Kan
PCR
Kan
Transformation souche (kanS)
Locus
F
Recombinaison homologue
Locus
Kan
Selection colonies (kanR)

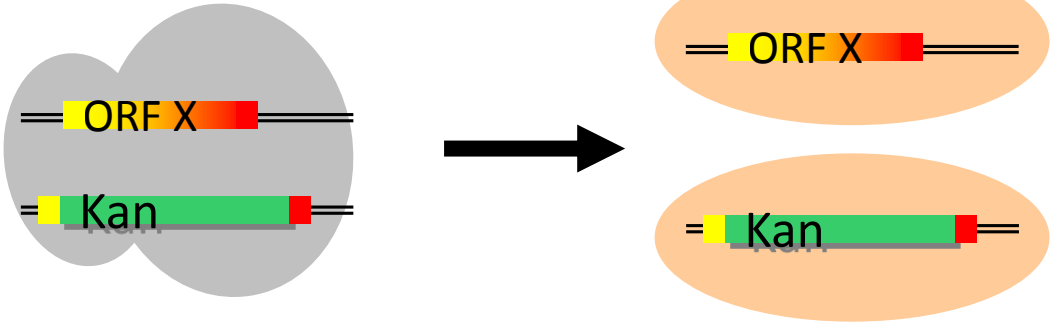
- Repose sur rec homologue
- Disruption faite chez diploides (un seul allèle disrupté à la fois)

-> collection de plus de 6000 mutants

# Cribles de la collection de disruptants

- 1- crible de viabilité
- 2- mutants viables -> autres cribles
- 3- cribles de co-létalité

# crible de viabilité




Diploïde X+/x::kan

Tétrades :

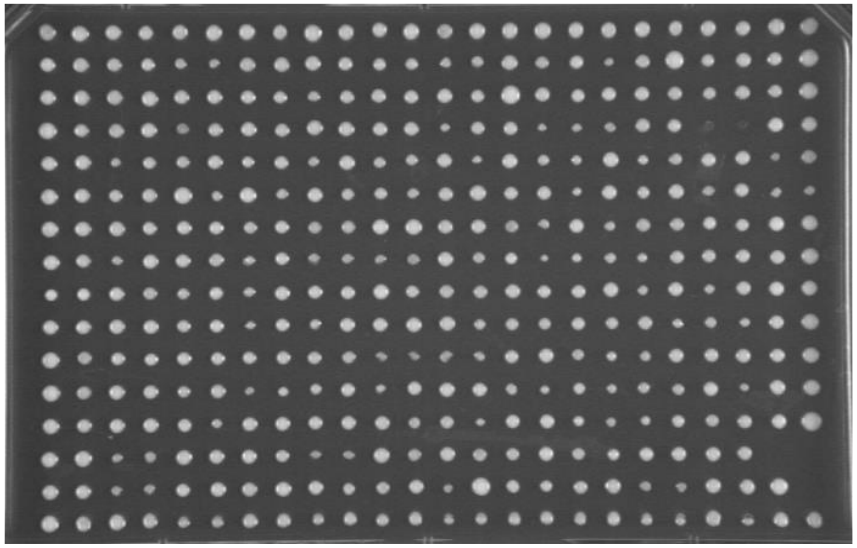
2 spores X+ (kanS, +)

2 spores x::kan (kanR, ?)

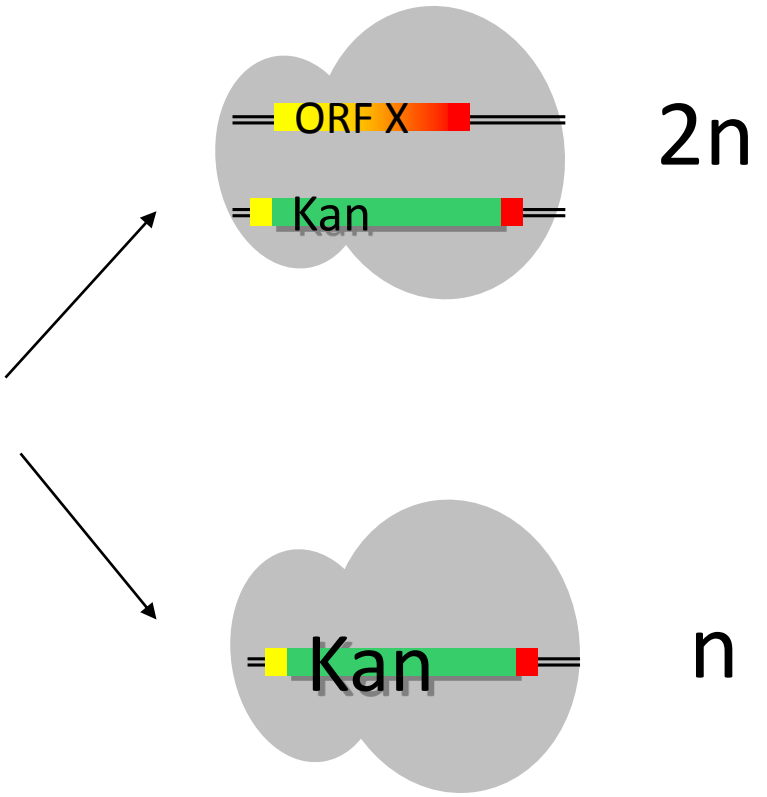
-> Environ 800 gènes « essentiels »

2 spores kanS /2 non viables

# Analyse des gènes non essentiels



**384 délétants par plaque**




La collection Knock-Out : environ 5000 souches (gènes non-essentiels)

**-> tests croissance sur différents milieux**

(sucres, drogues, pression osmotique etc)

2n, n et compétions

**->  tests spécifiques**

(trafic, épissage, activité biochimique…  etc)

**-> analyse des doubles mutatiants**

# 2- La collection de P chez la Drosophile

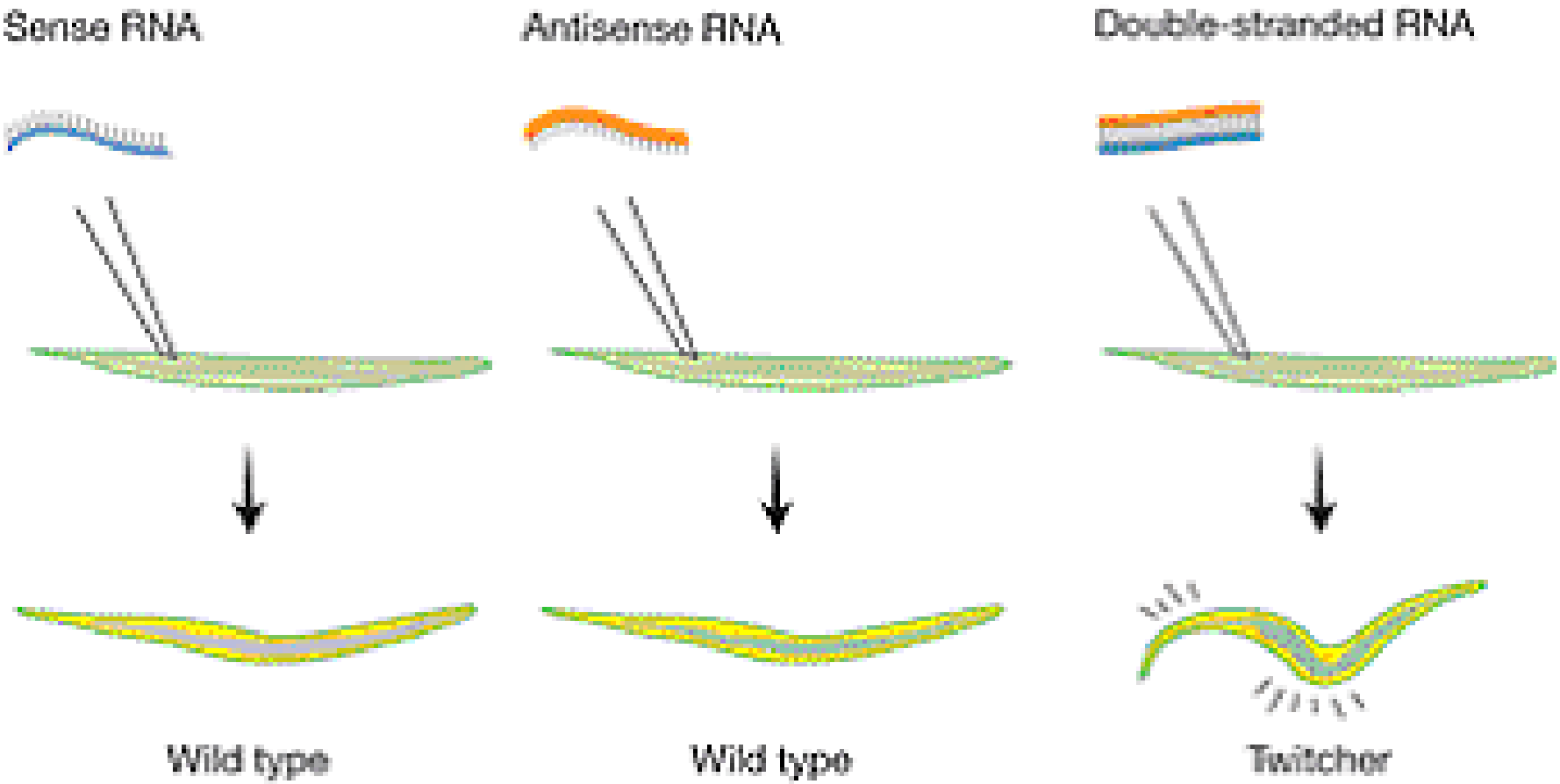
# The Drosophila Genome Project

- 13,600 known or predicted genes present
- One gene every 9 kb
- Half of fly proteins homologous with mammalian proteins
- One third homologous to nematodes
- 61% of human disease genes have homologues in flies
- 30% of genes unrelated to genes in other organisms
- Only 4000 genes essential for viability

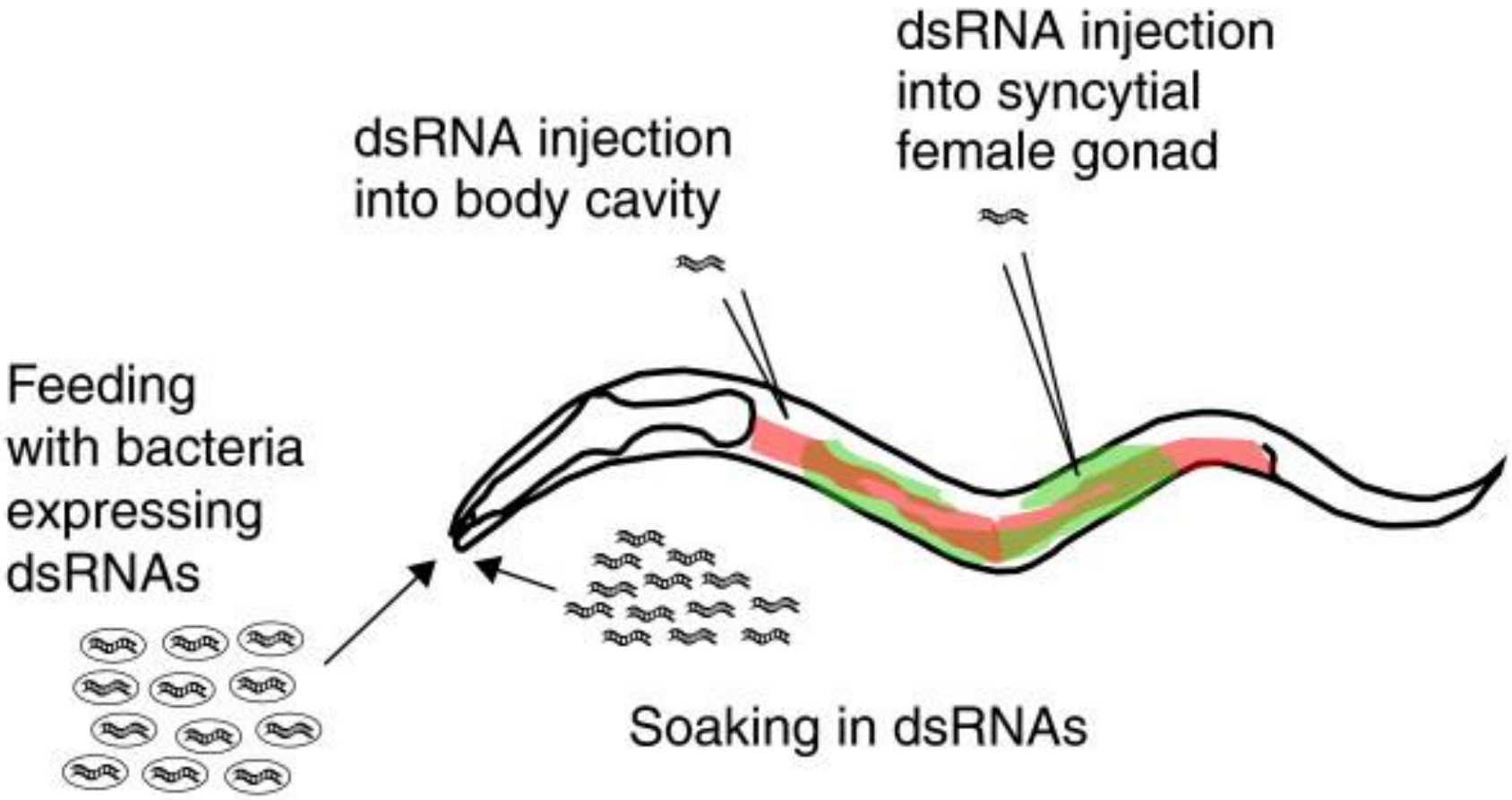
# A New Direction for Drosophila Genome Project: Obtaining Mutations in All Fly Genes

- Isolate as many P element insertion alleles as possible of as many genes as possible
- To date – P element disruptions for 65% of genes
- New methods of gene knockout such as RNAi to circumvent difficulties with knockouts by homologous recombination

# 3- La collection de RNAi chez C. elegans


Sense RNA
Antisense RNA
Double-stranded RNA
Wild type
Wild type
Twitcher

*C. elegans*


dsRNA injection
into syncytial
female gonad
dsRNA injection
into body cavity
Feeding
with bacteria
expressing
dsRNAs
Soaking in dsRNAs

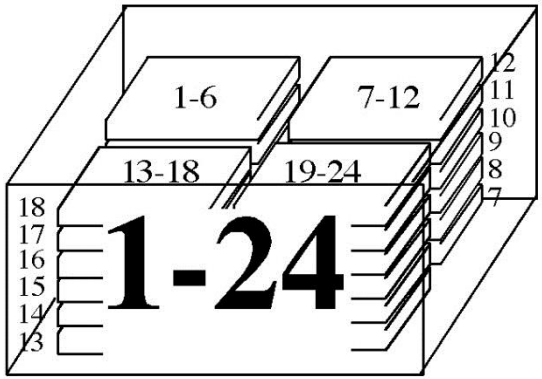
# Deletion Library Arrangement in the -80° freezer

Always keep the plates meticulously in order! This protects the library from warming by minimizing the amount of time it takes to retrieve plates.

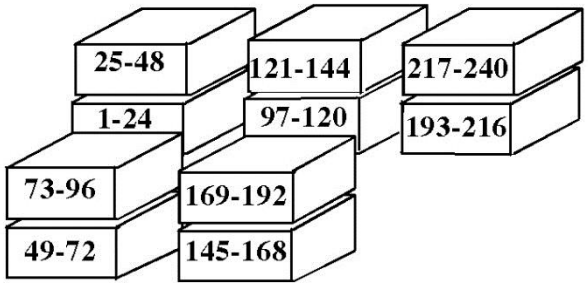
The library has two parts housed on separate freezer shelves:

1. a set of soft plastic plates with frozen worms (upper shelf)
2. a set of hard plastic plates with single-well lysates (lower shelf)

Each shelf is arranged identically in a set of 10 Tupperware-style containers, each container holding 24 plates. Each container is labelled on the front with the plate numbers it contains. The plates are arranged within a box as shown in this example:




Boxes are arranged on the freezer shelves as follows:



# C Crible: Choix du phénotype

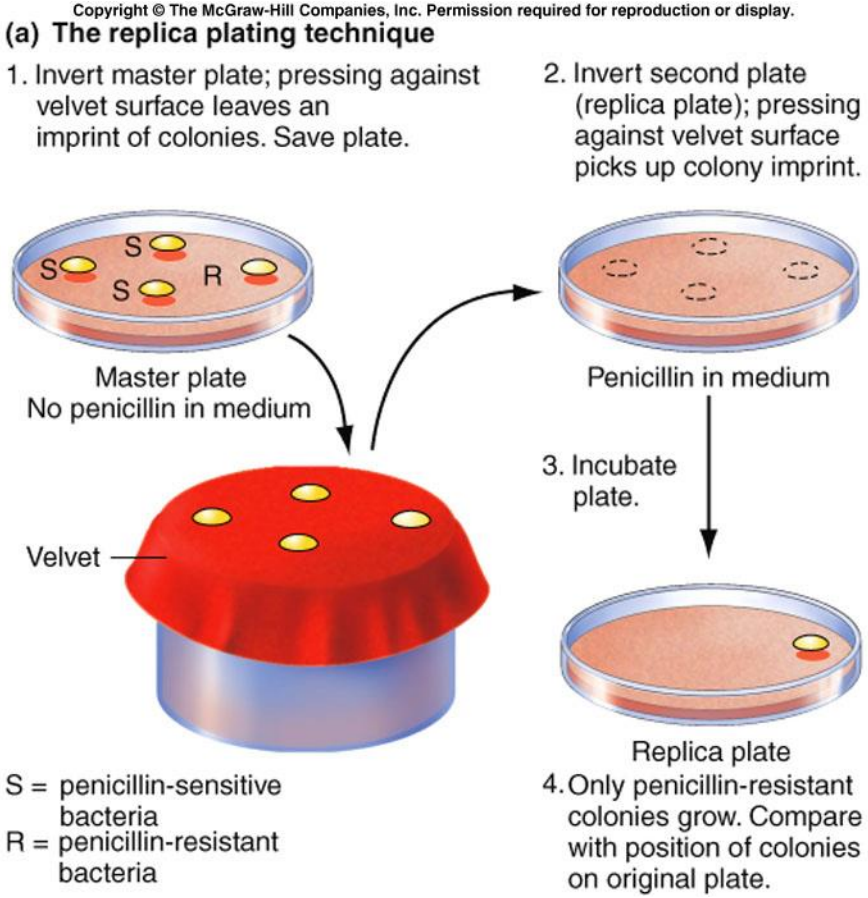
## Genetic screens can be carried out for a wide variety of biological functions (phenotypes):

- Growth
- Biochemical
- Morphological
- Anatomical
- Behavioral
- Lethality

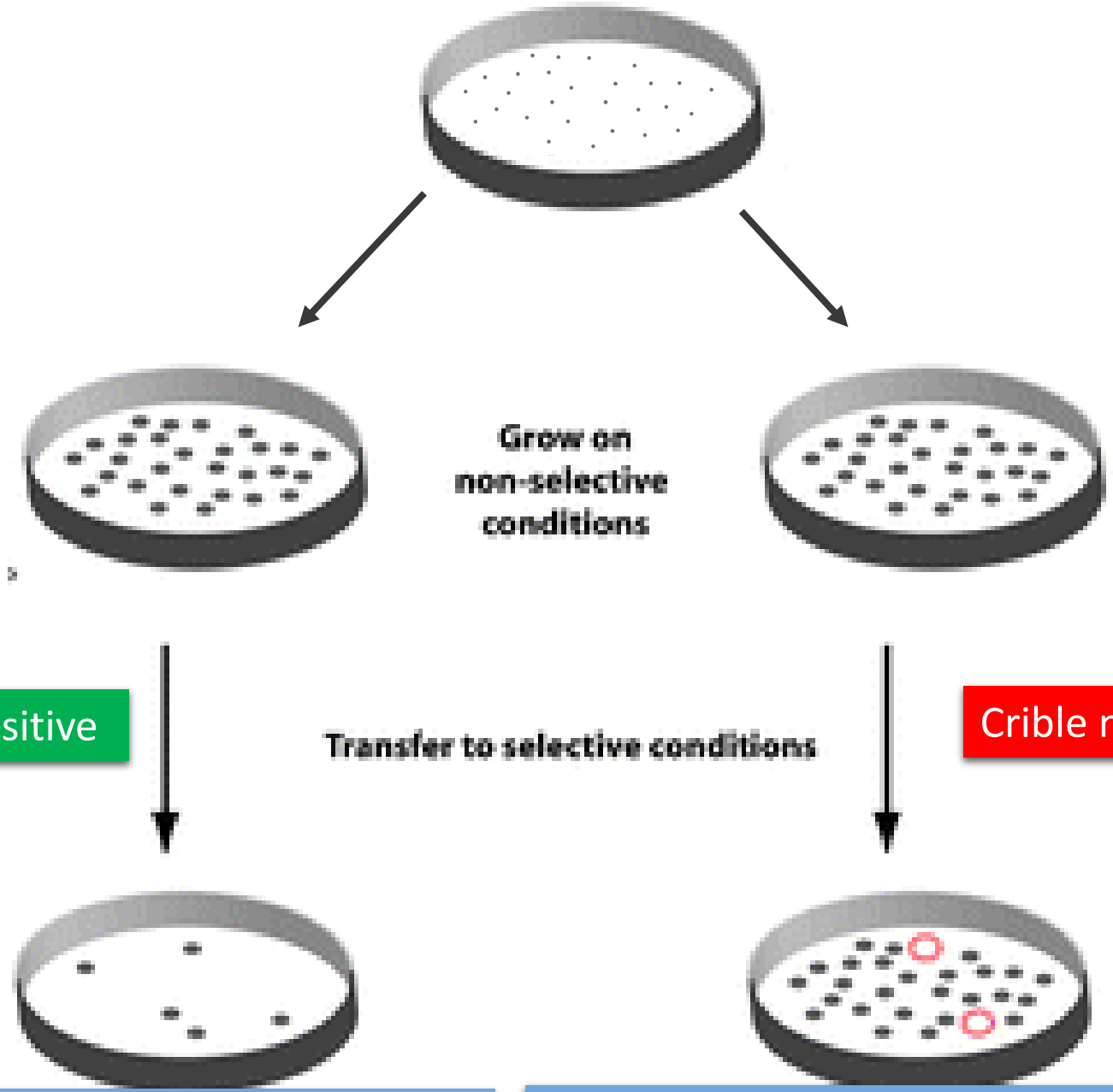
# Genetic screening *versus* selection

**Genetic screen: produce and sort through many non-mutant individuals to find the rare desired mutation**

**Genetic selection: only the desired mutation survives**




7.5a



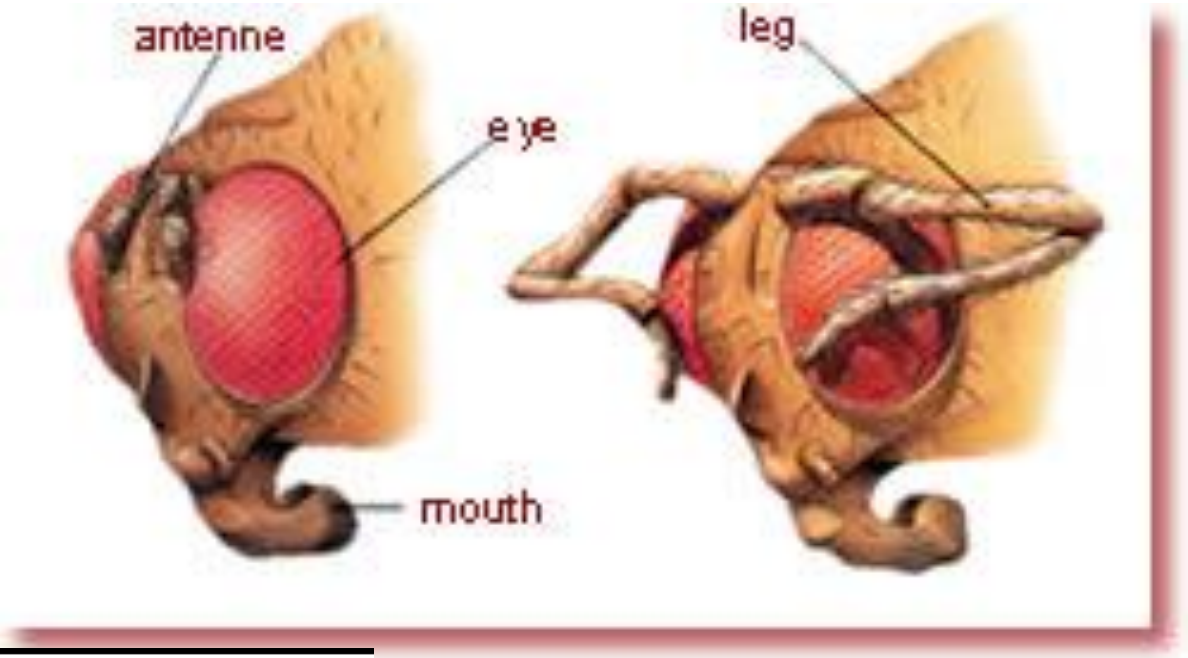

Sélection positive

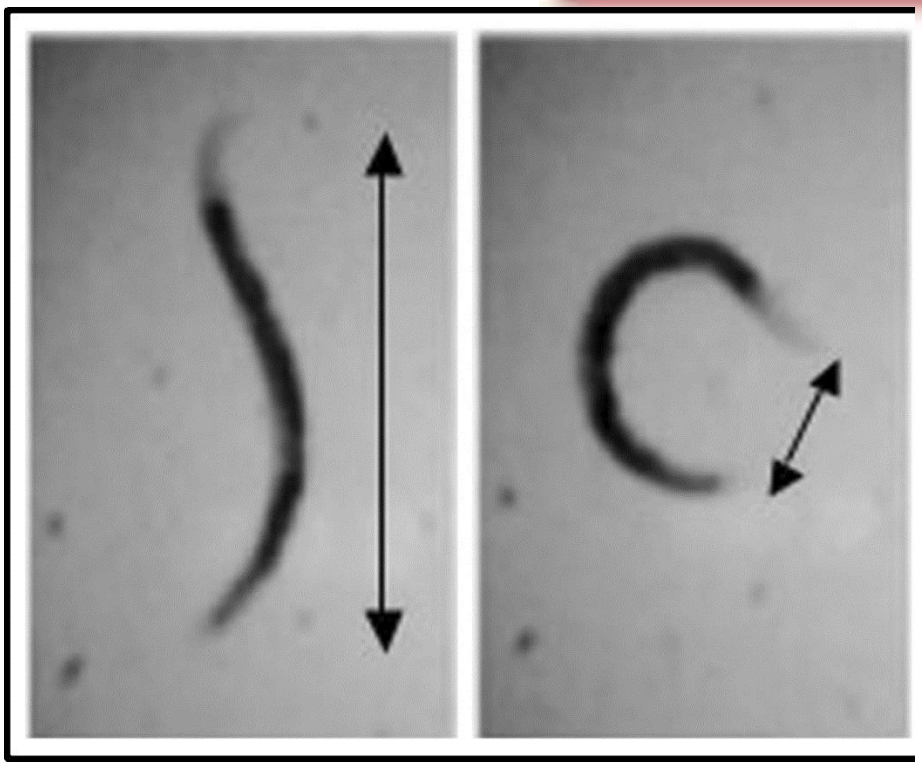
Crible négatif

- « Résister à » drogue, toxine, virus…

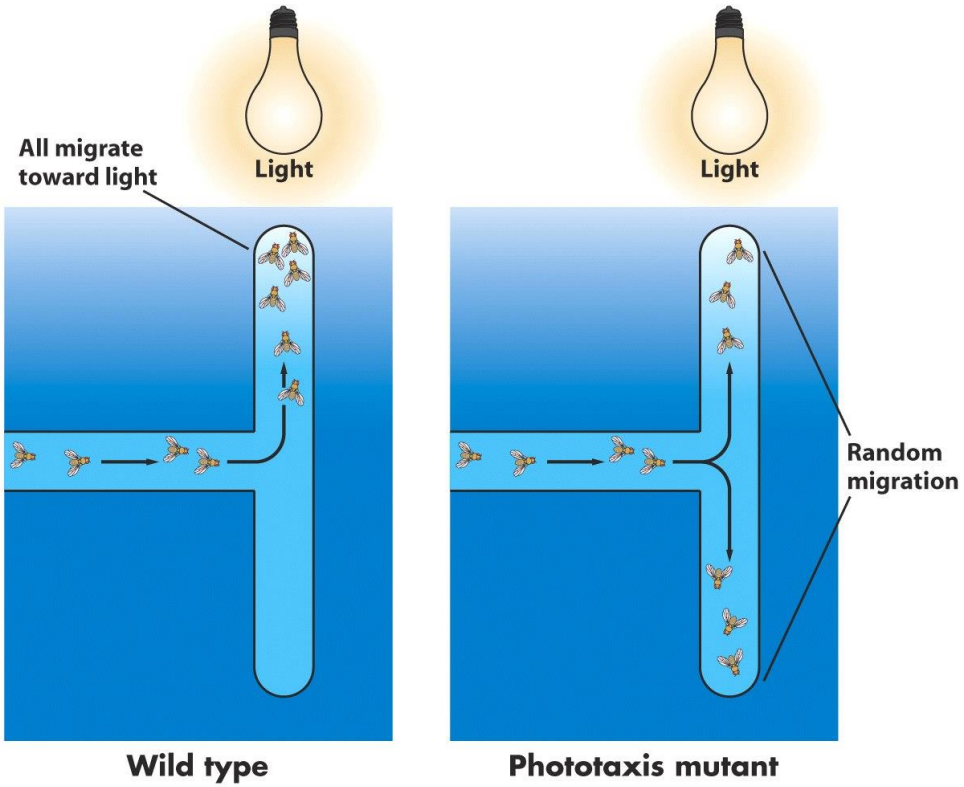
- Auxotrophie: pouvoir produire aa, base
- Capacité à utiliser cetains sucres…

# Morhphologie



# Comportement



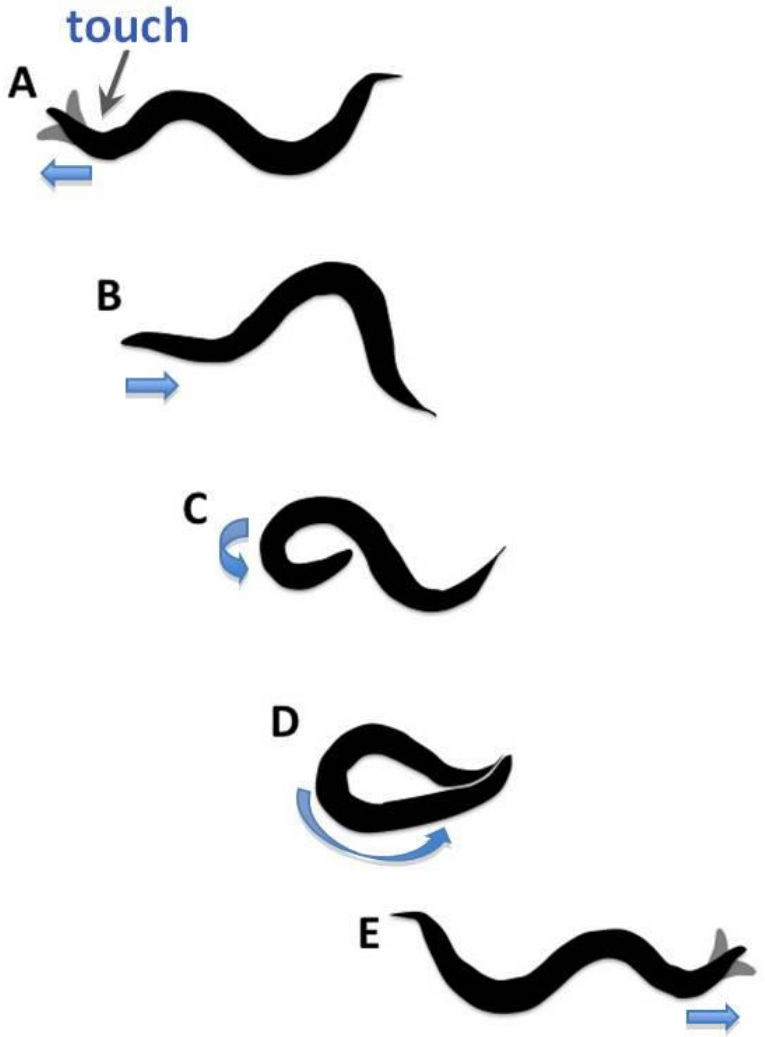




Fig. 16-7

# D. Crible: Stratégie

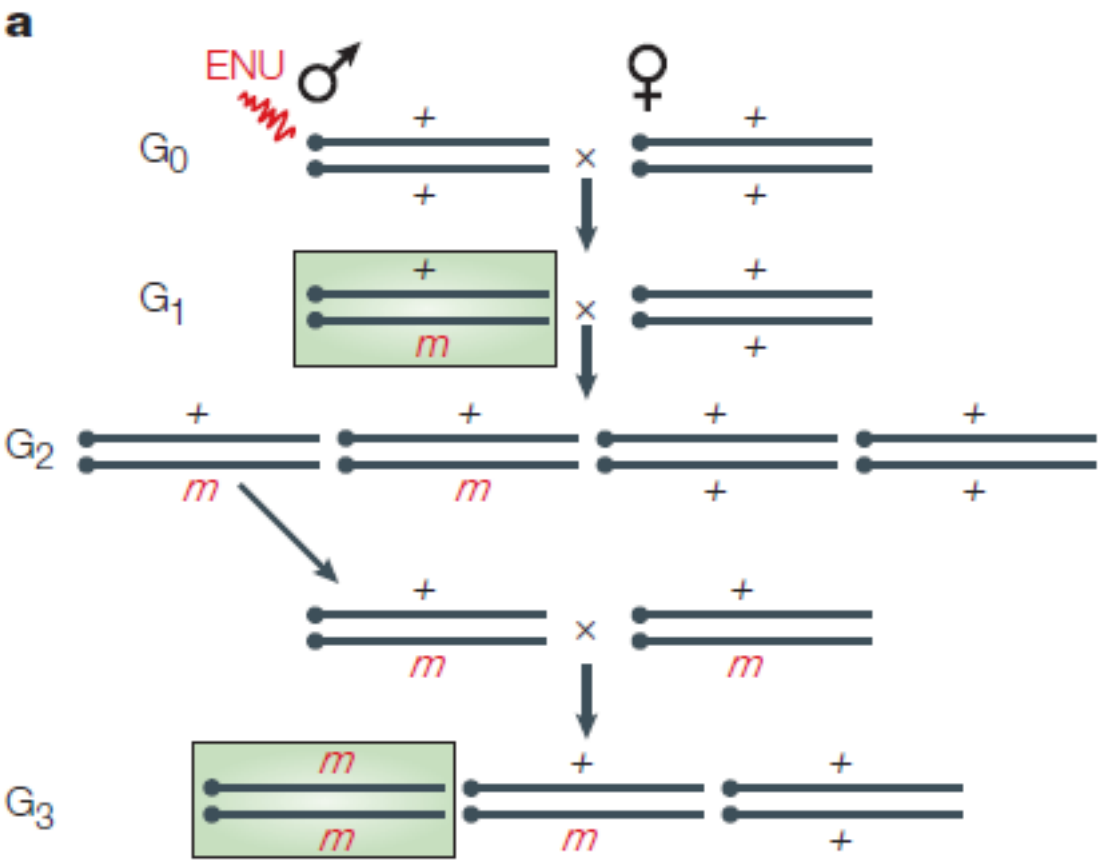
PREMIERS CRITERES À CONSIDERER

- Organisme (haploide vs diploide, hermaphrodisme…)
- Mutation à effet dominant ou récessif
- Létalité, stérilité etc

# D. Crible: Stratégie

- Crible pour mutations à effet dominant
- Crible pour mutations à effet récessif
- Crible pour mutations à effet maternel
- Cribles mutants conditionnels
- Cribles de collection/banques de mutants

Crible en F1 (G1)




Crible en F3 (G3)

Héritable ???

Nguyen et al. 2011 doi: 10.1152

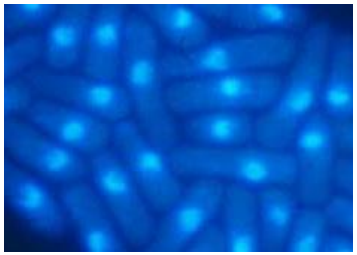
# Les grands cribles

- Cycle cellulaire levure
- Heidelberg Mouche
- Poisson zèbre
- Cribles souris
- Cribles *C. elegans*
- Cribles *Arabidopsis*

# Yeasts: cell cycle regulation screens

**2001 Nobel Prize in Physiology or Medicine to Lee Hartwell, Paul Nurse, and Tim Hunt**

Saccharomyces cerevisiae



Schizosaccharomyces pombe

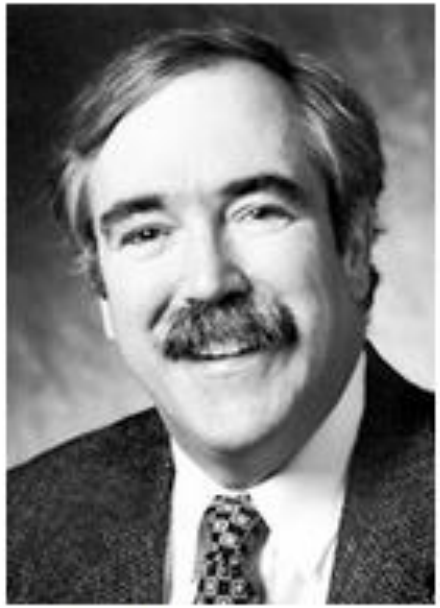
# The Nobel Prize in Physiology or Medicine 1995

Edward B. Lewis

Prize share: 1/3

Christiane Nüsslein-Volhard

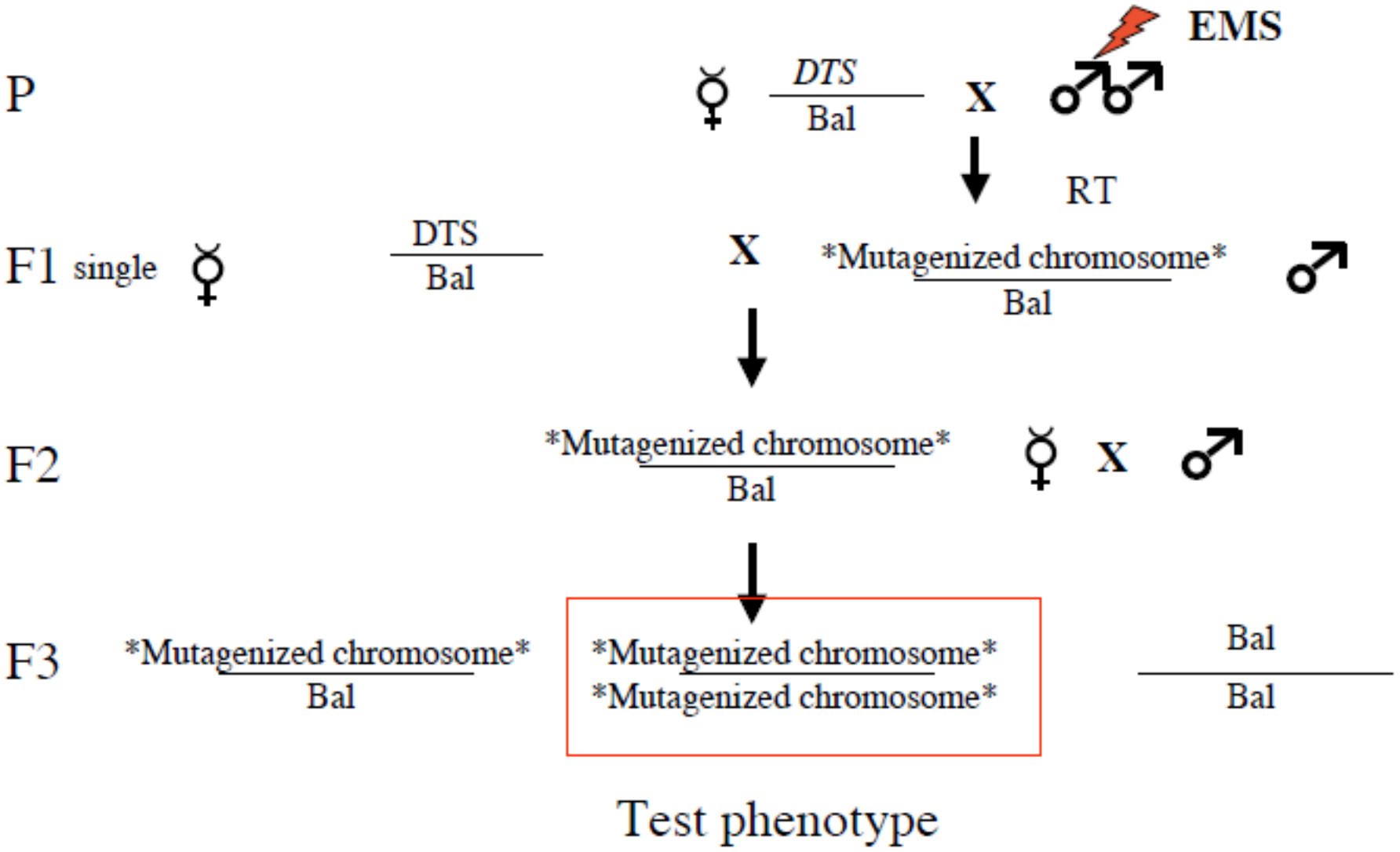
Prize share: 1/3



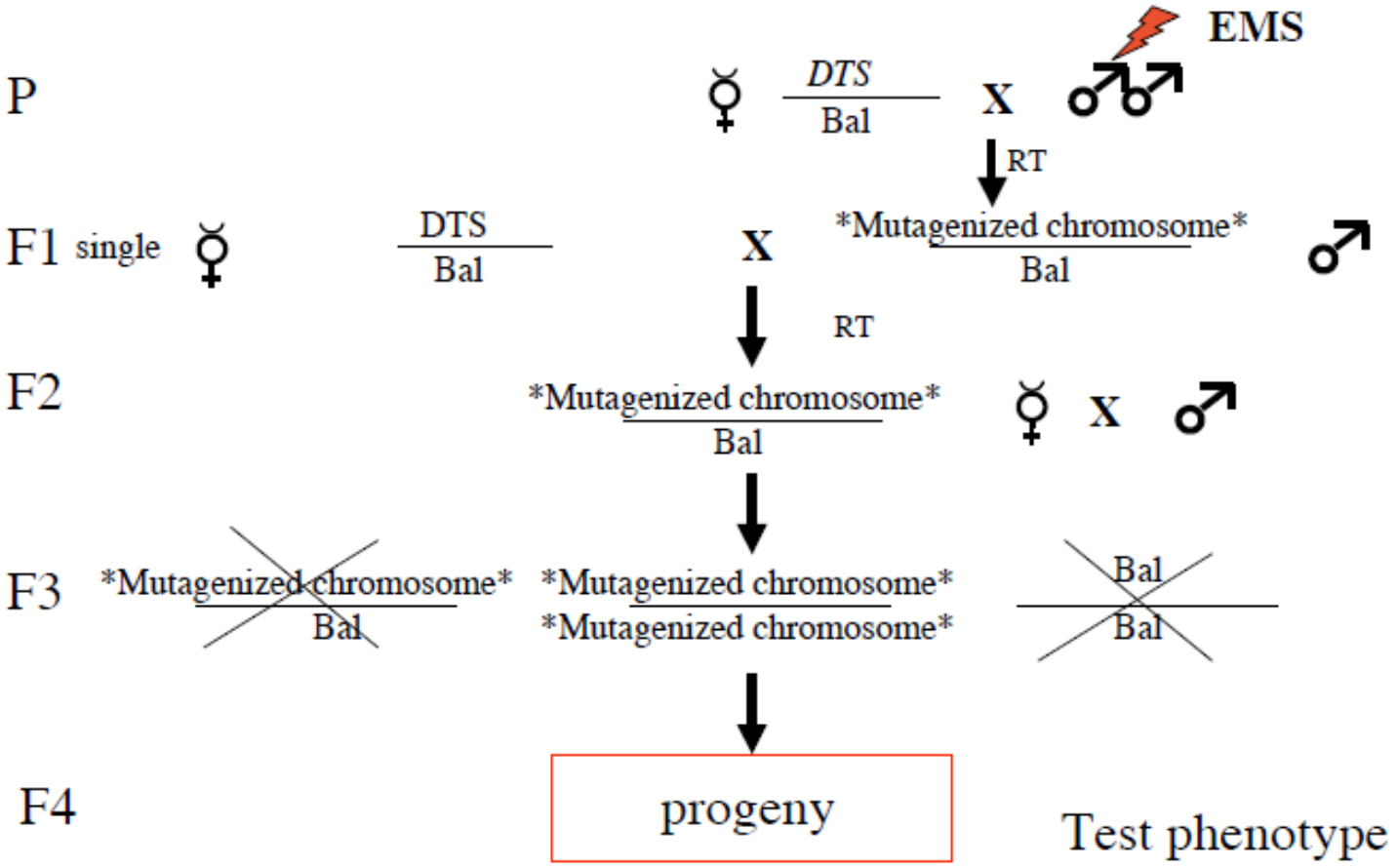
Eric F. Wieschaus

Prize share: 1/3

# General mutagenesis approach to isolate zygotic genes



# Screen for maternal effect mutants



# C. Elegans screens

## The Nobel Prize in Physiology or Medicine 2002

Sydney Brenner
**Prize share:** 1/3

H. Robert Horvitz
**Prize share:** 1/3

John E. Sulston
**Prize share:** 1/3

The Nobel Prize in Physiology or Medicine 2002 was awarded jointly to Sydney Brenner, H. Robert Horvitz and John E. Sulston *"for their discoveries concerning genetic regulation of organ development and programmed cell death'".*

# C. Elegans screens

- Morphology, locomotion, muscle …: uncorodinated, rolled
- PAR genes
- Apoptosis pathway voir TD

## 1ère Limitation majeure

Une grande proportion des gènes agissent dans de multiples processus

## 2ème Limitation majeure

A large fraction of genes have no obvious knockout phenotypes

# Des solutions

- Mutations conditionnelles
- Fond génétique « sensibilisé pour détecter des pertes de fonctions ou activation partielles
- Cribles modificateurs
- Synthetic screens
- Cribles surexpression
- Cribles dans des tissus spécifiques ou en utilisant des mosaïques génétiques
- Crible pour expression gène (avec “reporters”)

# D. Mutants conditionnels

- **Ts, Cs, Salt-sensitive, degron….**

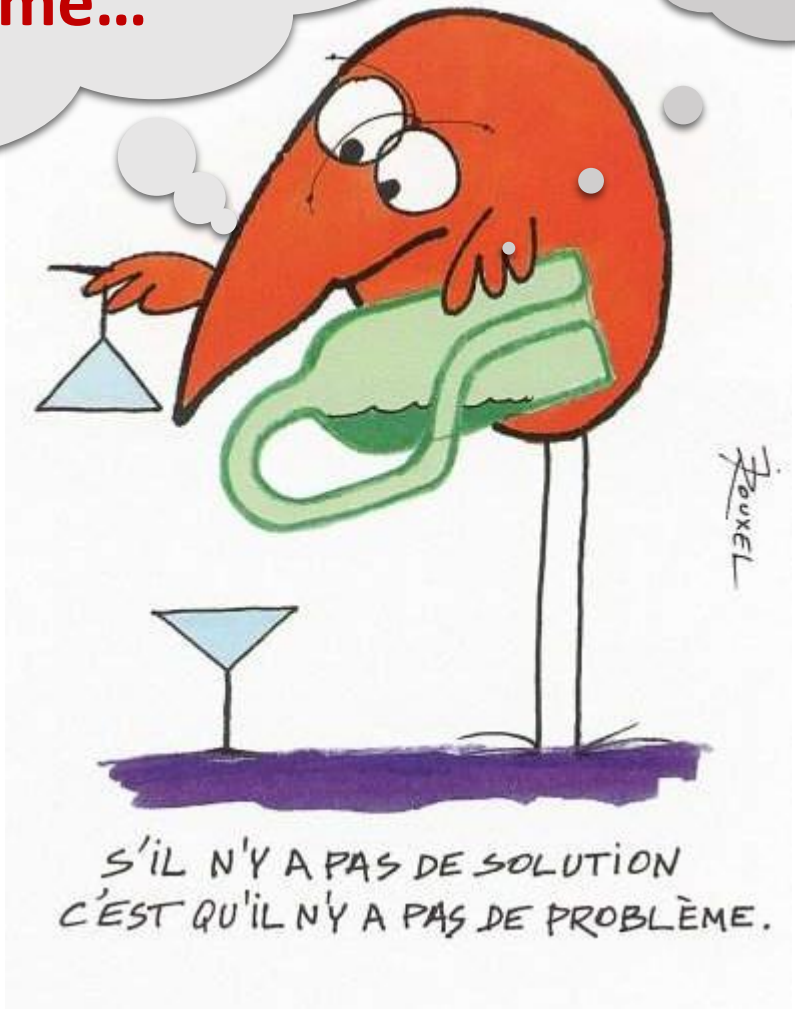
- They allow us to
  - easily work with mutations in essential genes (voir above cdc $^{ts}$ in yeast).
  - To study phenotypes at late stages
  - to observe changes in the organism as we change from permissive to non-permissive conditions.

# D. Mutants conditionnels

- **Temperature sensitive (ts), cold sensitive (cs) , Salt-sensitive,**
- They allow us to
  - easily work with mutations in essential genes (excdc $^{ts}$ in yeast).
  - To study phenotypes at late stages o fdevelopment (fly)
  - to observe changes in the organism as we change from permissive to non-permissive conditions.

**Avec la thermosensibilité, plus de probléme…**

**ts… ts… ts…**



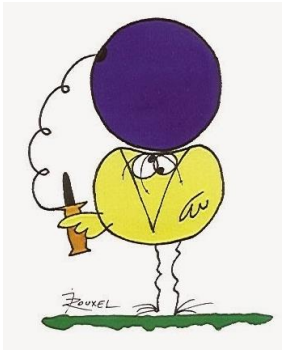
# What does a conditional phenotype mean at a molecular level?

1) ts-synthesis.

2) ts-activity.

3) ts-stability.

4) ts-process.




30°C

**Température permissive**

42°C

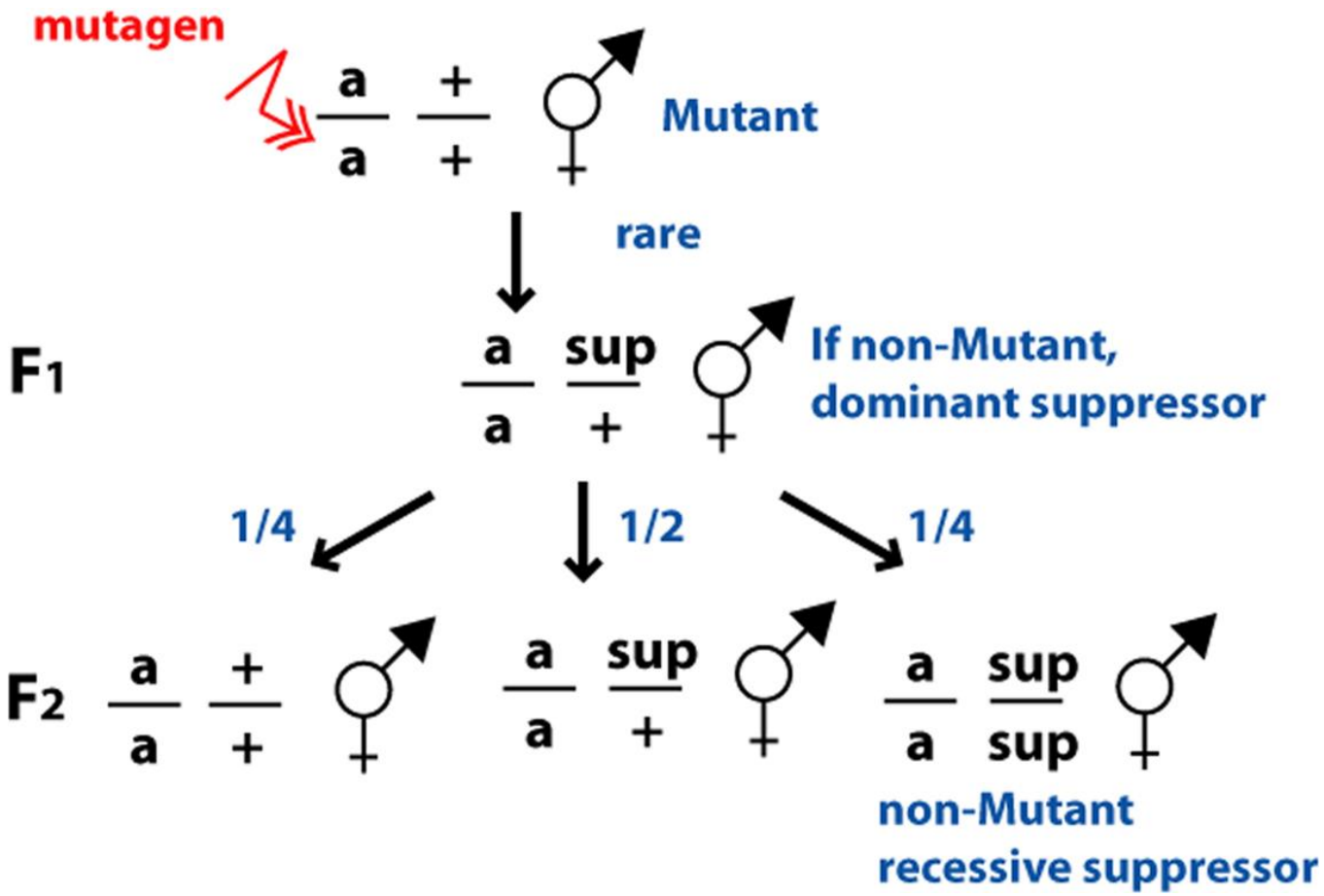
**Température non permissive**

# D. Cribles basés sur interactions génétique

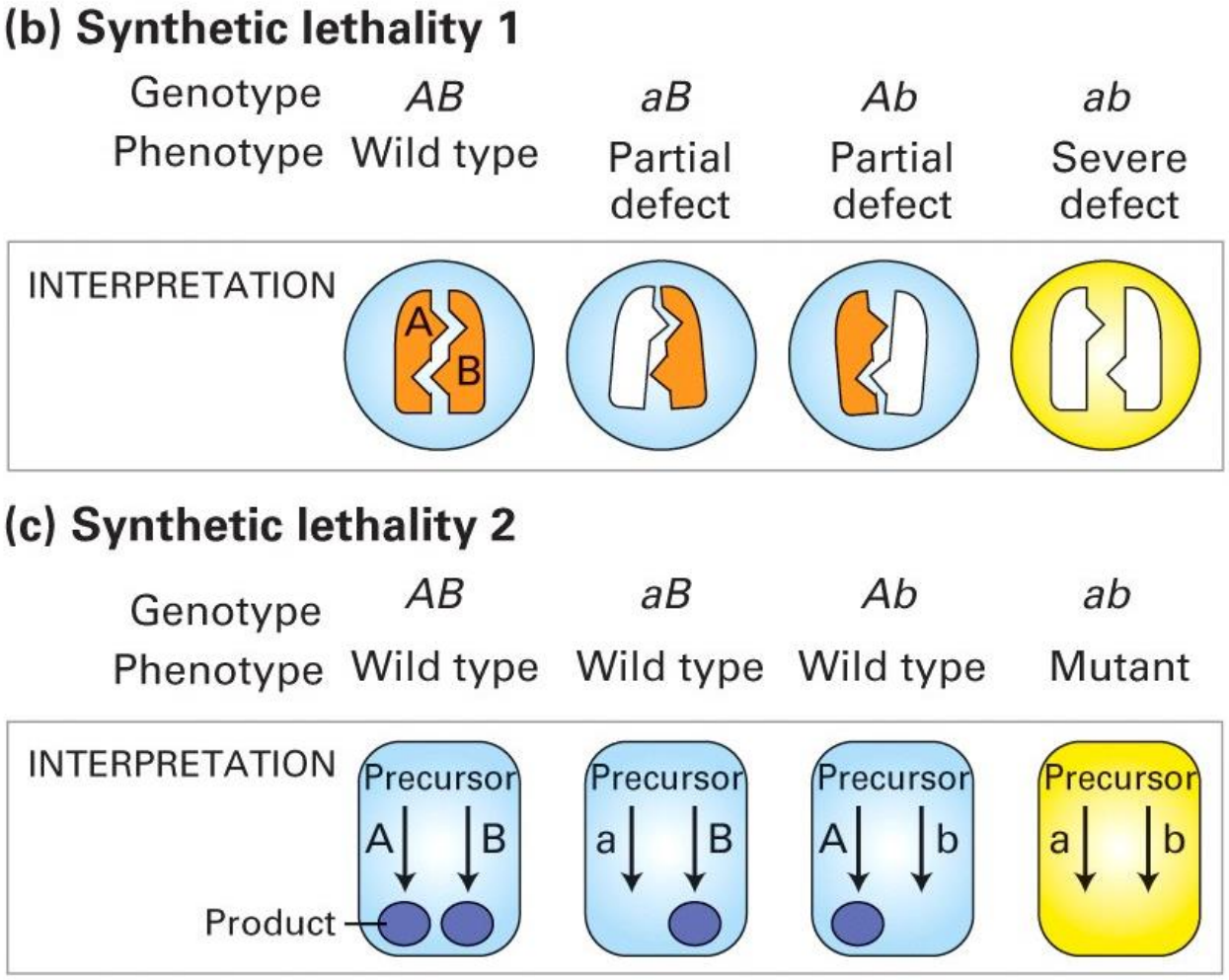
- Suppression
- Agravation
- Colétalité

# Pourquoi étudier l'effet de double-mutations?

- **Genetic interactions reflect functional interaction between genes**
- **Relationship of complex genotypes to complex phenotypes**
- **Conserved genetic interactions may be applicable to humans**

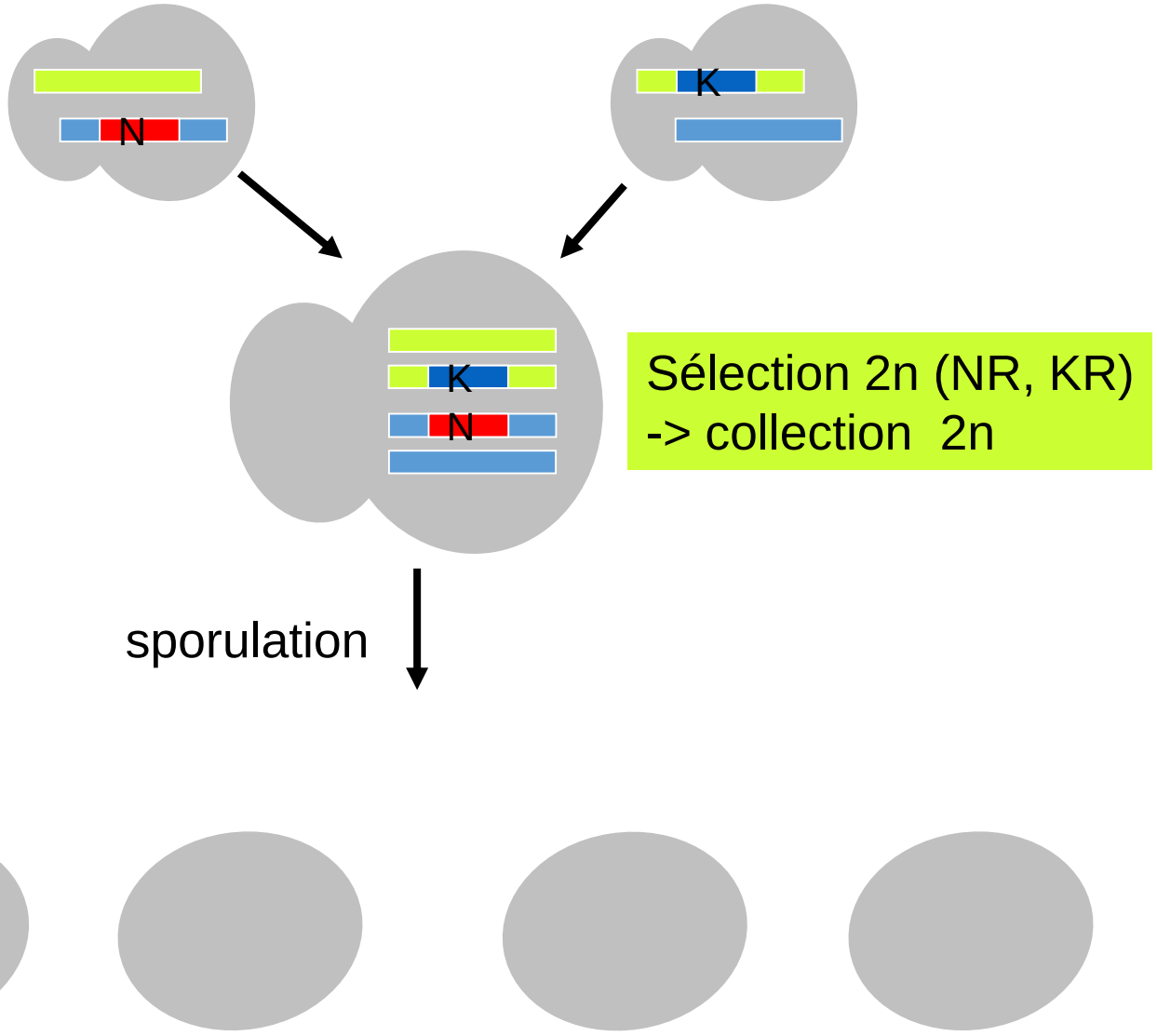

mutagen
a/a +/+
Mutant
rare
F1
a/a sup/+
If non-Mutant,
dominant suppressor
1/4
1/2
1/4
F2
a/a +/+
a/a sup/+
a/a sup/sup
non-Mutant
recessive suppressor

# Synthetic Lethal Mutations



KO gène connu, étiqueté NR

Collection KO étiquetés KR Ordonnée

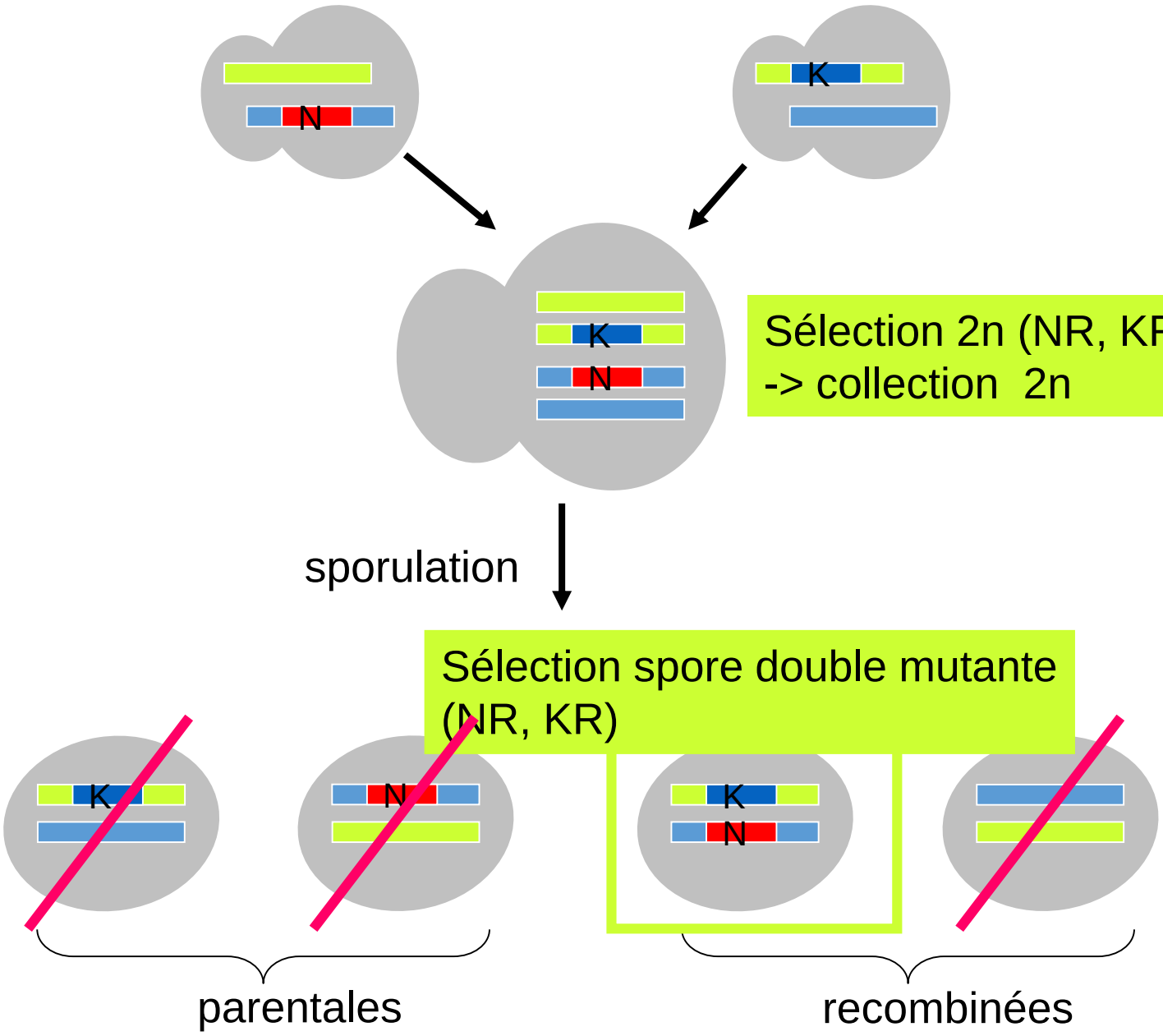



Sélection 2n (NR, KR) -> collection 2n

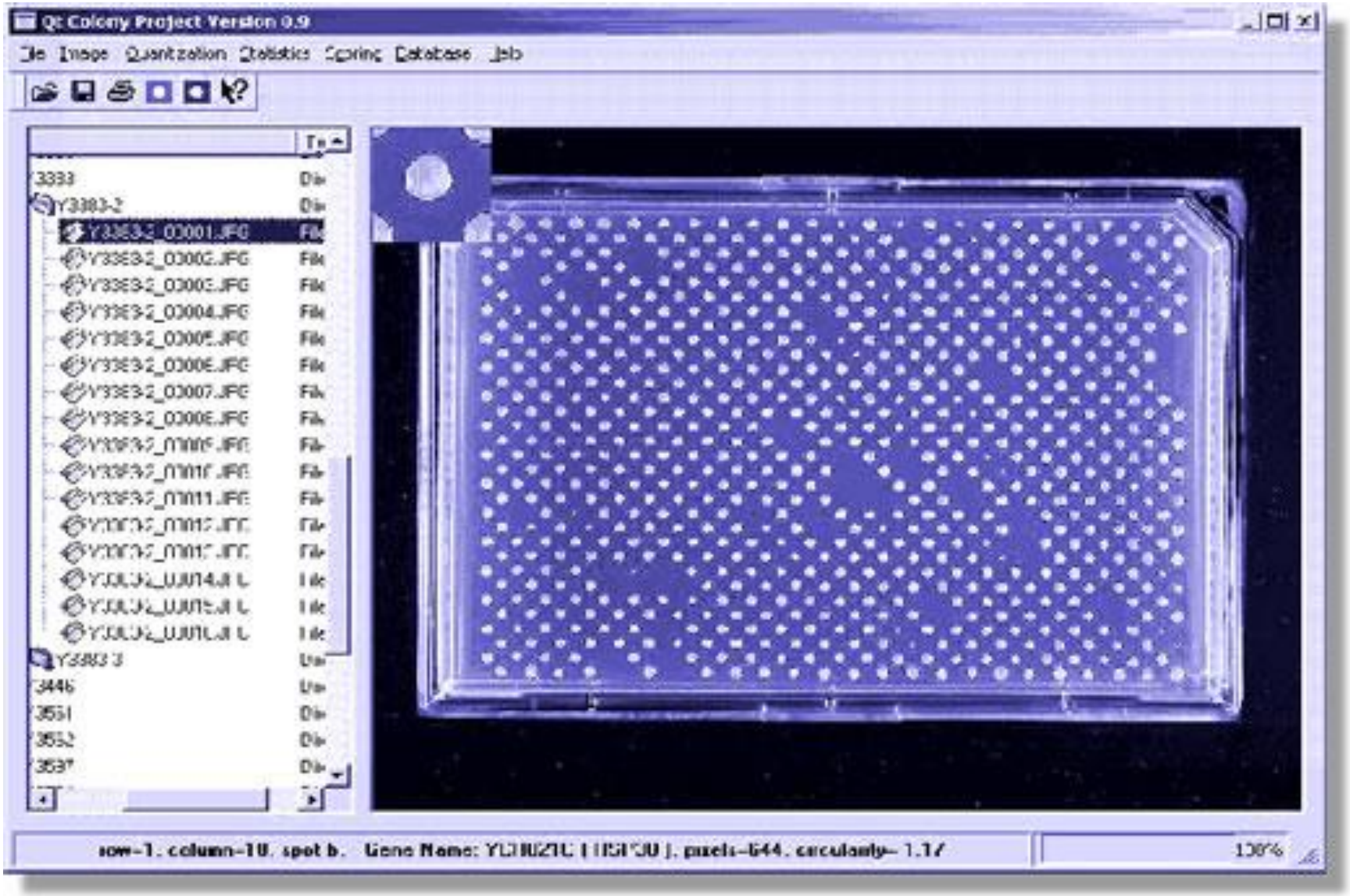
Sélection haploides

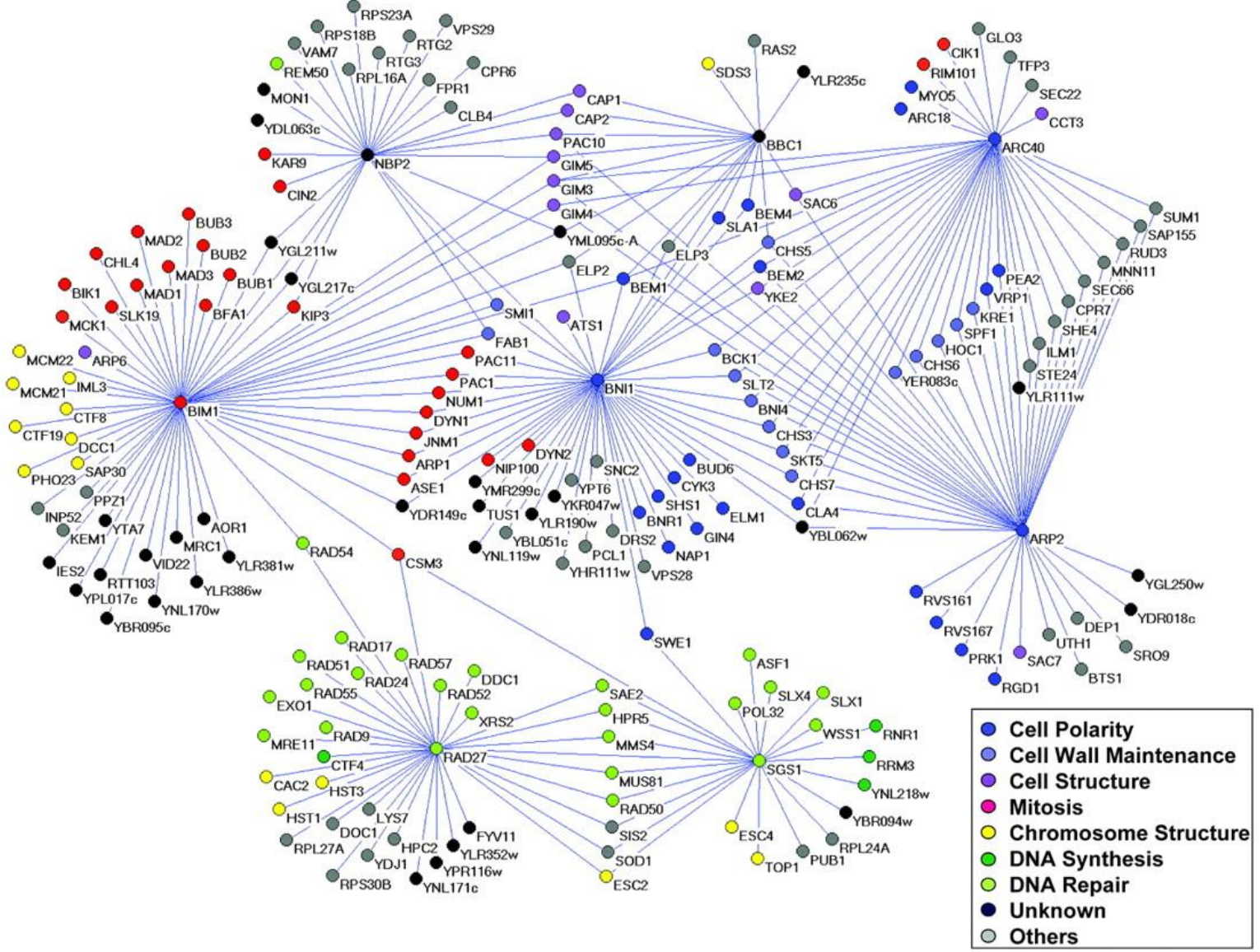
KO gène connu, étiqueté NR

Collection KO étiquetés KR Ordonnée



# Automated scoring






132 délétants croisés avec la collection knock-out

1000 interactions SL soit: **34 interactions par gène!!!**

**Tong et al. Science 2001**

## Advantages:

The potential to systematically survey the entire genome for any desired genetic interactions

Can be largely automated

## Disadvantages:

High false positive rate (e.g. sporulation/germination defects)

Linked genes will be underrepresented (or false positives)

Only uses complete nulls

Essential genes not included