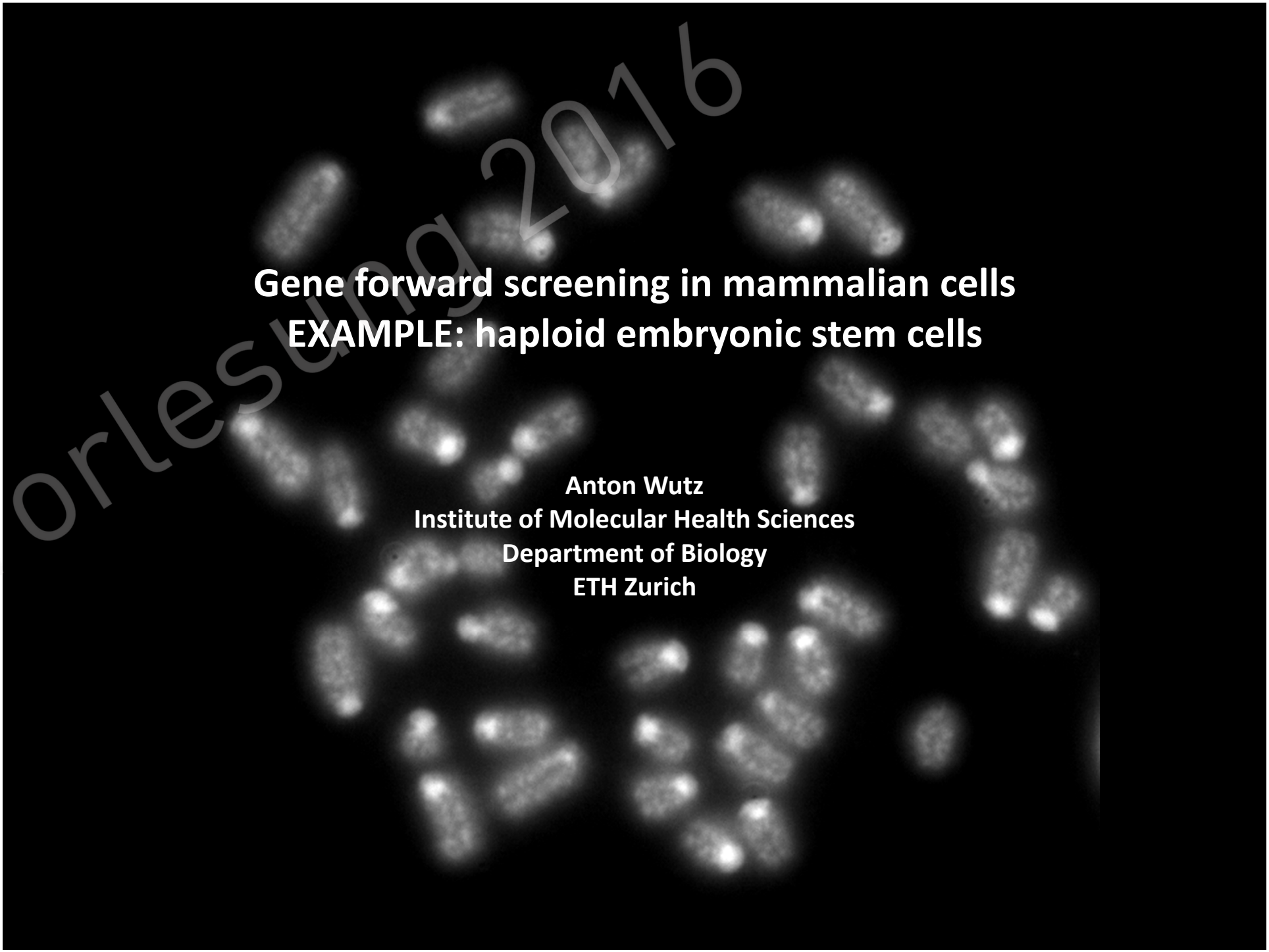
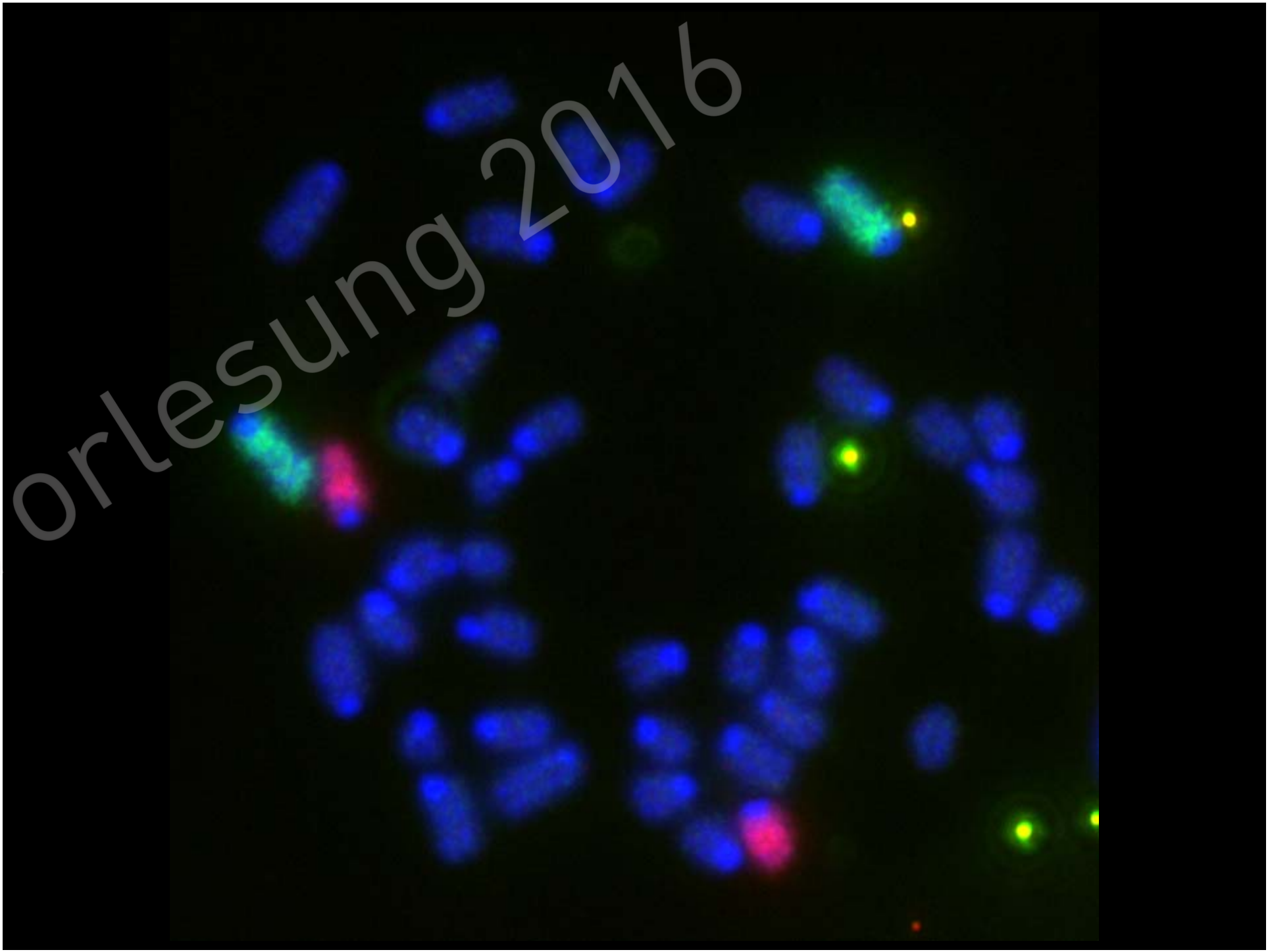


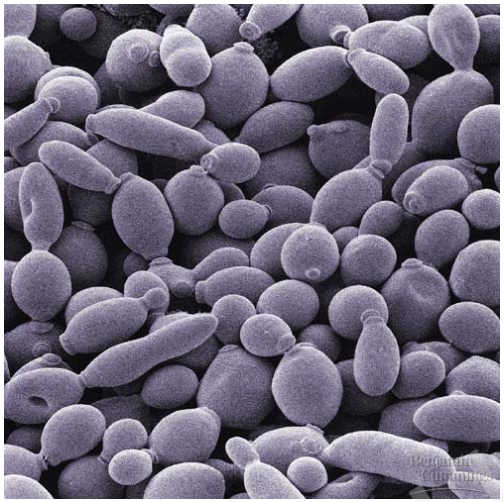
Gene forward screening in mammalian cells
EXAMPLE: haploid embryonic stem cells

Anton Wutz
Institute of Molecular Health Sciences
Department of Biology
ETH Zurich


Vorlesung 2016

CONCEPT I

Cell systems for forward screening



Haploid Superiority

Duncan Greig and Michael Travisano

In common with other higher life forms, the readers of *Science* are diploids, carrying one set of chromosomes from their mother and a second set from their father. Yet many eukaryotes exist quite happily in the haploid state, with only one set of chromosomes and thus only one set of genes. This observation prompts the question: Is there any particular evolutionary advantage to being diploid? On page 555 of this issue, Zeyl *et al.* (*1*) turn this question on its head and instead ask whether haploidy carries an evolutionary advantage. In their experiments, they exploit a valuable eukaryote, the budding yeast *Saccharomyces cerevisiae*, which grows and reproduces in both the haploid and diploid states.

Conventional comparisons between diploids and haploids highlight the hypothetical benefit of having a "backup" copy of each gene in case mutations damage the functional copy (*2*). One problem with this explanation, however, is that a single undamaged gene cannot always fully compensate for the mutant copy, so that mutations carry some deleterious effects (*3*). Nonetheless, such mutations are usually recessive and have only small effects as long as there exists one fully functional gene copy. A more serious problem with this explanation is that doubling the number of gene copies may simply postpone the inevitable. Unless nonfunctional gene copies are somehow purged from the genome, mutations will eventually destroy

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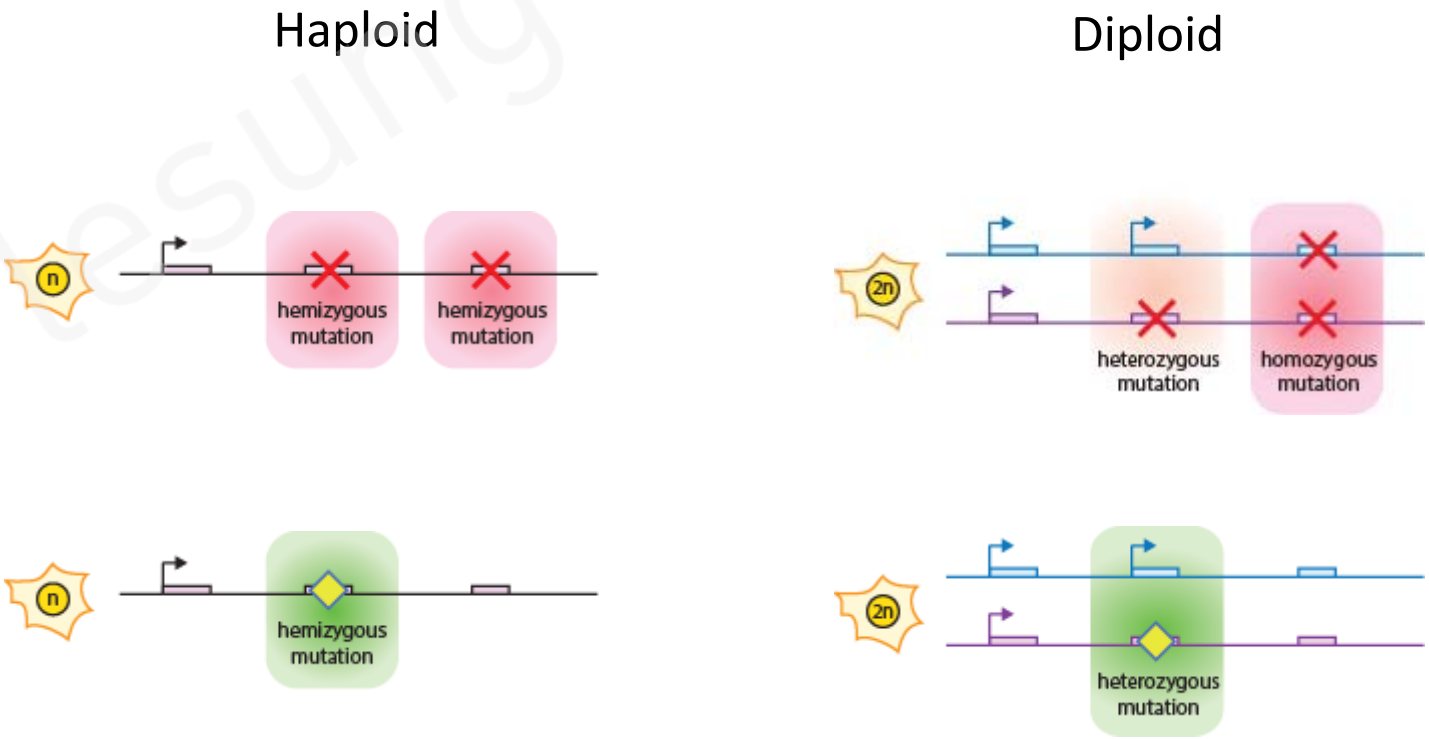
those are truffles, they are made of haploid cells

Tuber melanosporum

Tuber magnatum


Mosses grow as predominately haploid phase with diploid sporophytes that depend on the haploid gametophyte

Diploid genomes protect from deleterious mutations and facilitate dominant beneficial mutations



In a genetic screen we do not want the protection, we really want to see the phenotype

the more complex an animal the less offspring it has.

Haploid cells may be useful for screening recessive mutations

makes little sense for dominant mutations

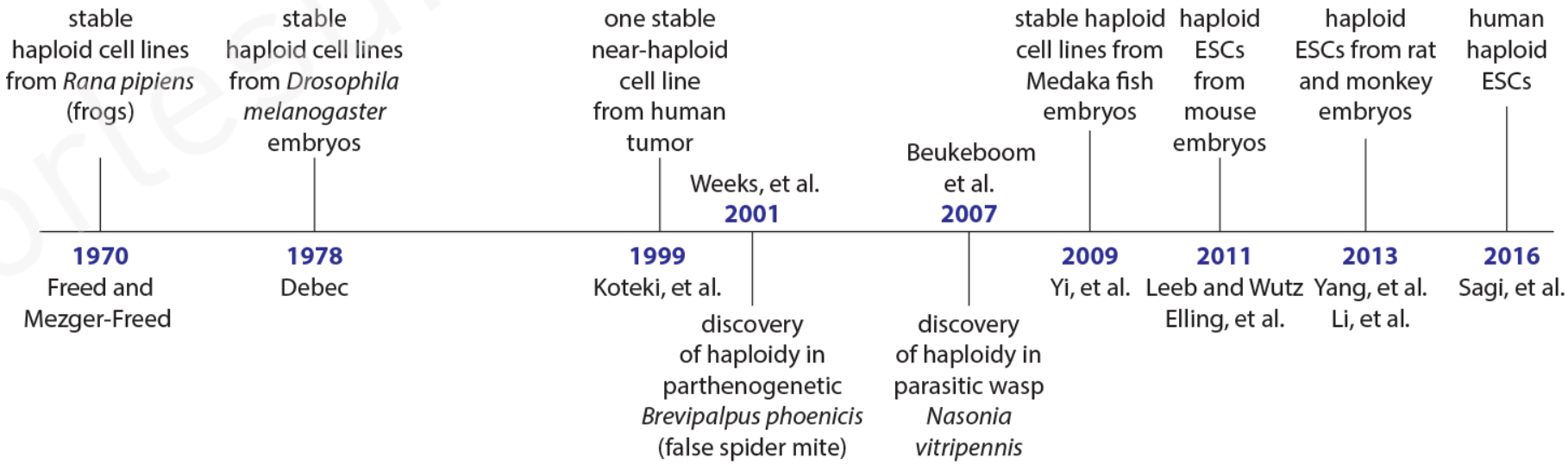
what do we mean by dominance of a mutation ?

what frequency of mutations can you expect to act dominant,
what frequency recessive ?

Haplodiploid sex-determination systems

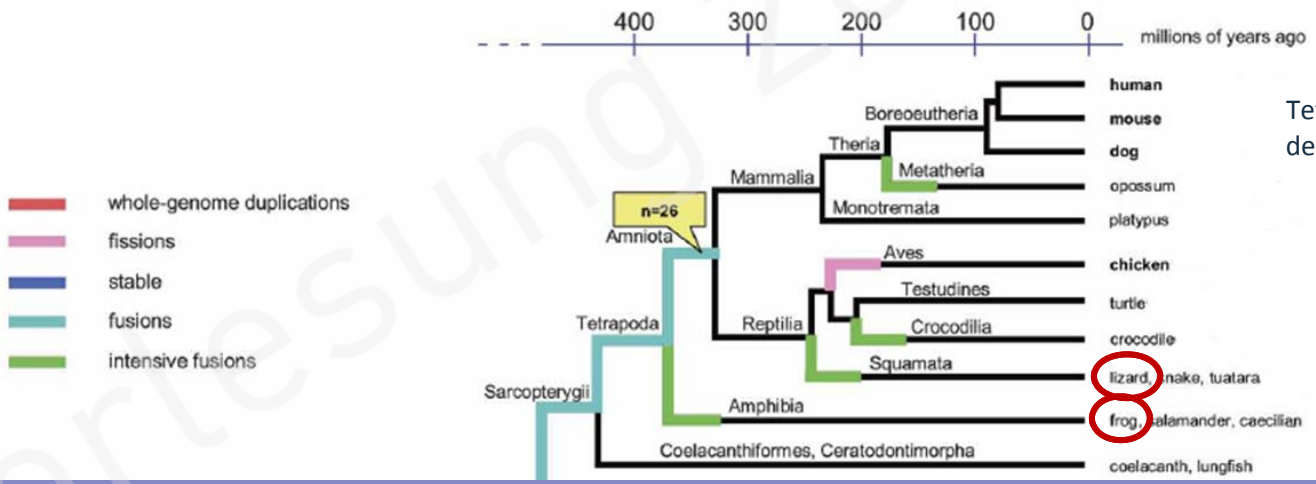
Concise history of haploid animal cells

Haploid animal cell cultures



Discovery of haploidy in animal species

Vertebrate Karyotype Evolution



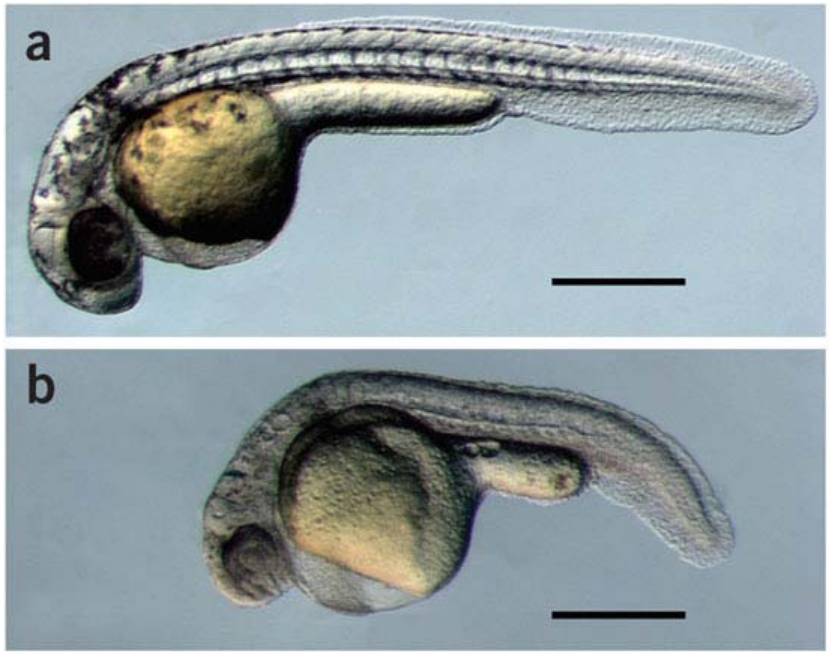
Tetraploidy in mammals is described in *Octodontidae*

Tetraploid lizards AABC type exist

Ploidy elevation in frog and salamander is common

Evidence for a 3rd genome duplication in fish: Haploid medaka and zebrafish development

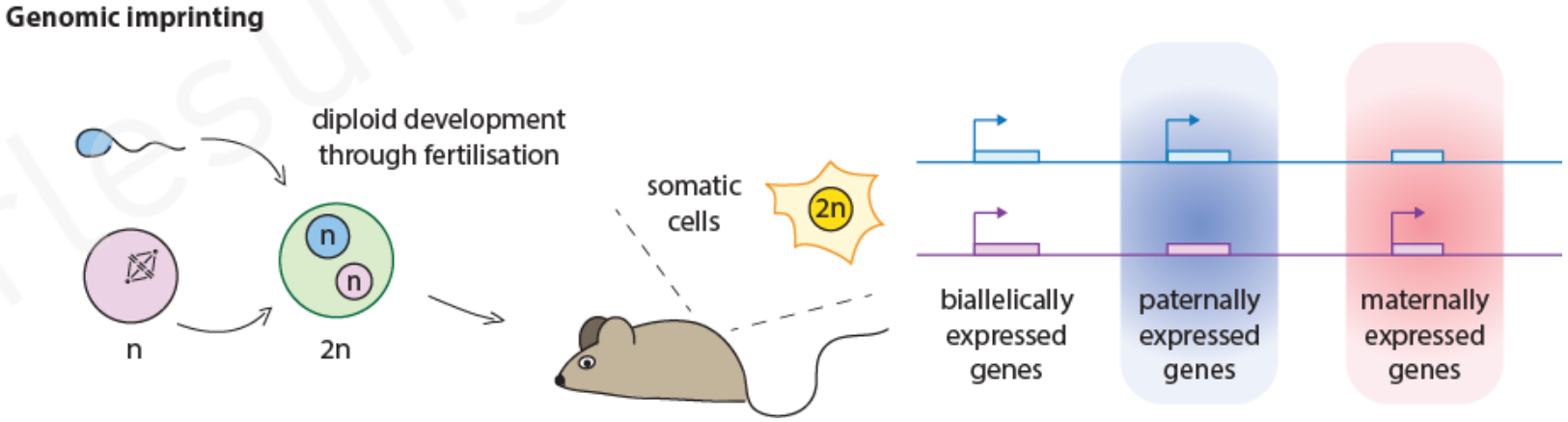
Generation of Medaka Fish Haploid Embryonic Stem Cells
Meisheng Yi *et al.*
Science **326**, 430 (2009);

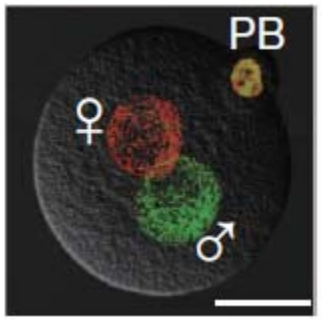


Haploid syndrome in Zebrafish

Nature Protocols 5, 383 - 394 (2010)

Parental genomes are not equivalent in mammals



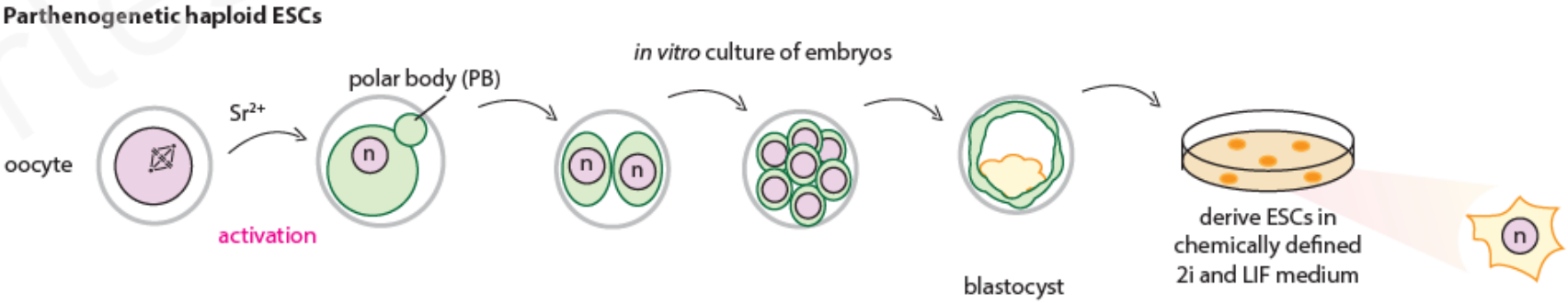


5mC

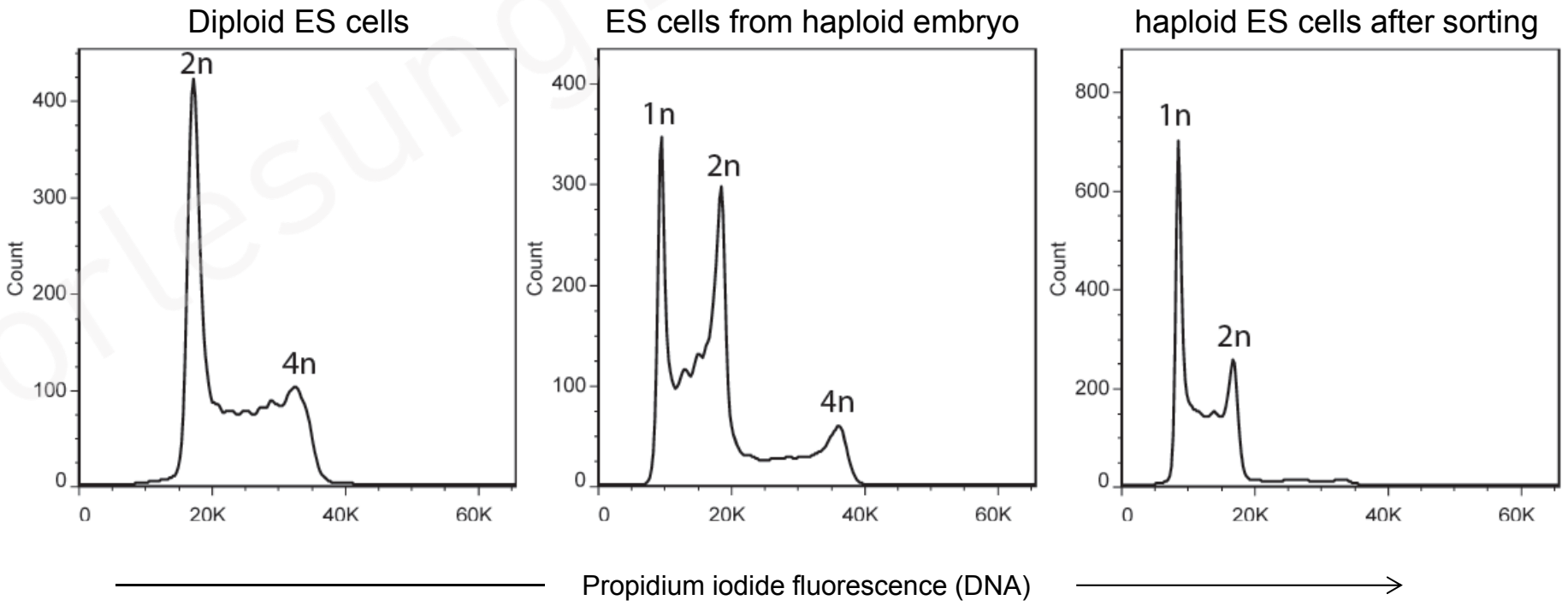
5hmC

Gu et al., Nature 477: 606

Investigation of haploid development through embryonic stem cell derivation

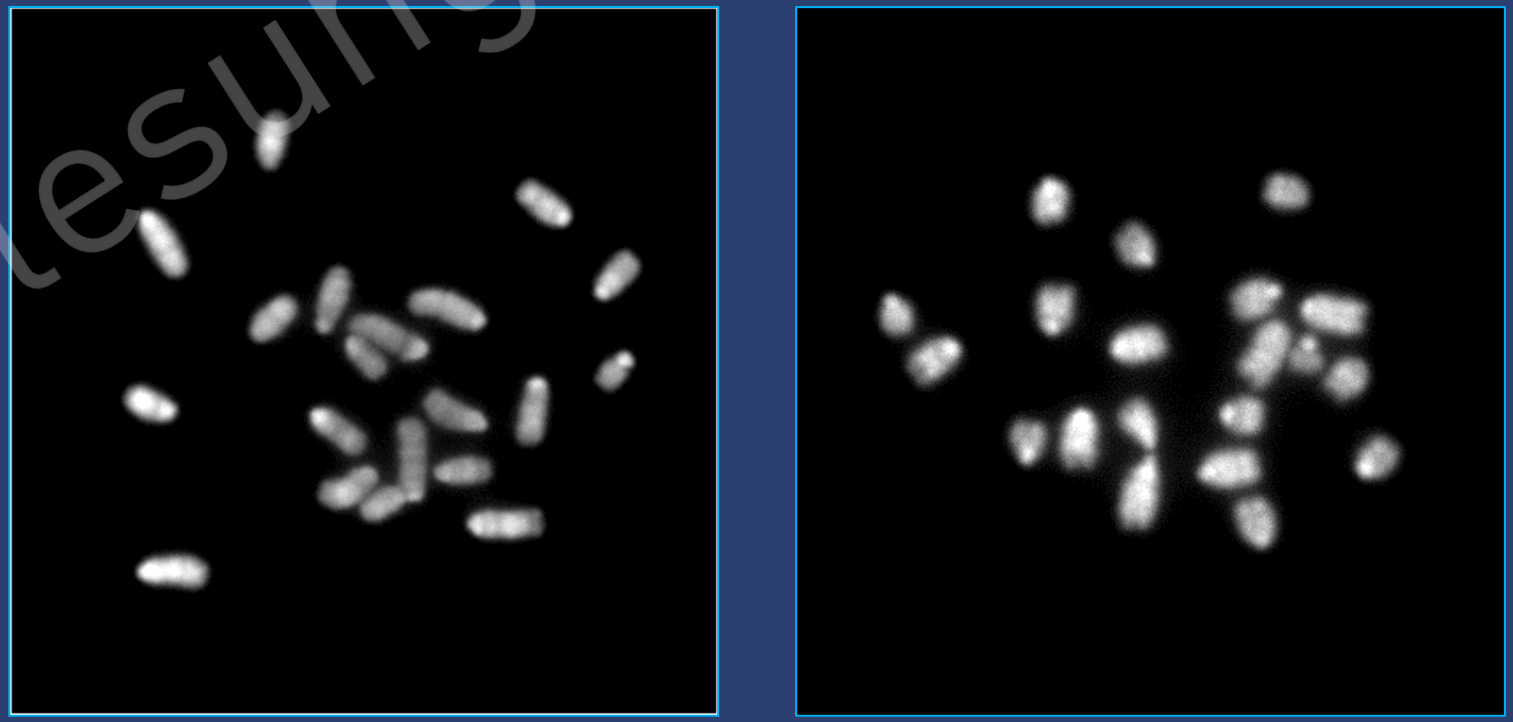


Derivation of haploid embryonic stem cells from 129Sv mice

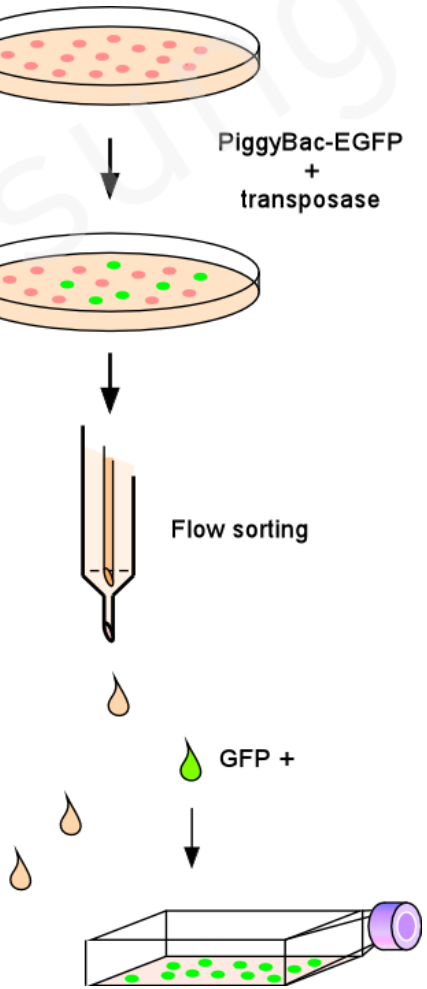


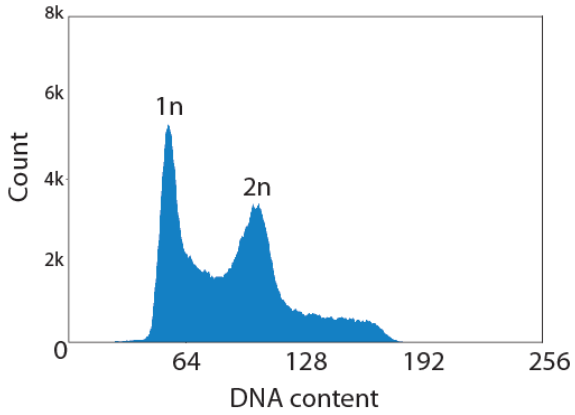
Haploid ES cells

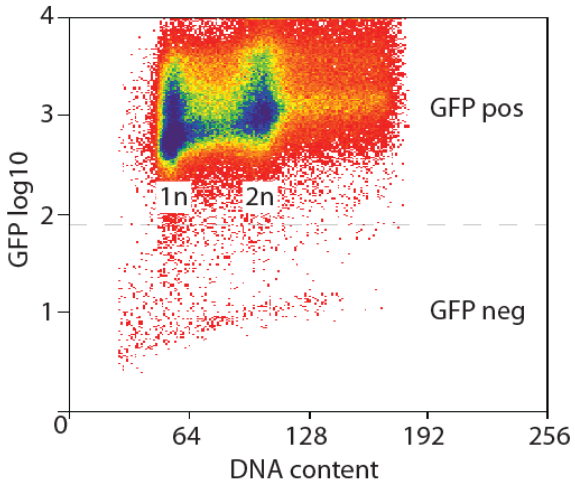
Metaphase chromosome spreads



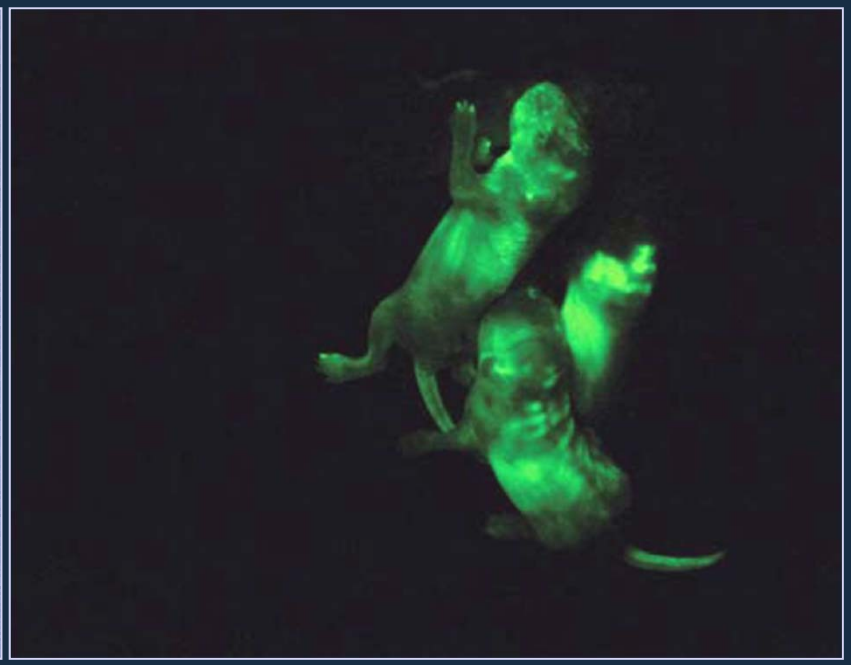
Genetic modification of haploid embryonic stem cells



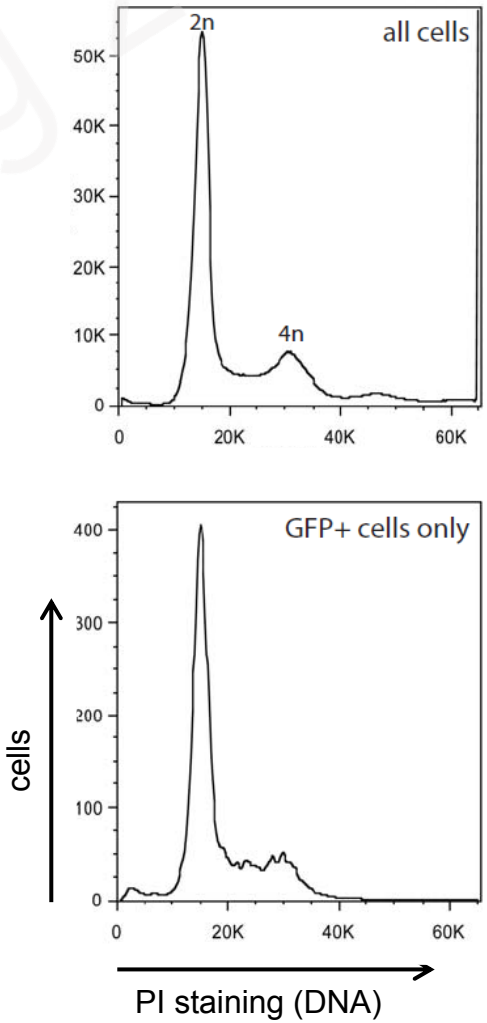




Haploid ES cells can contribute to development



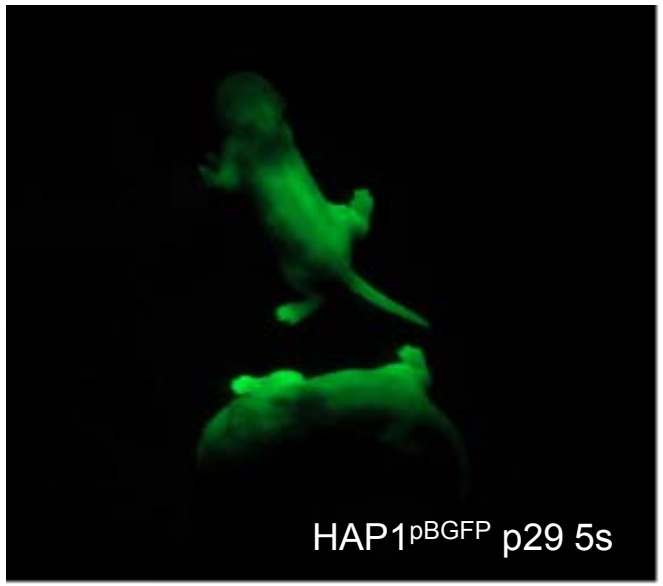
Diploidization is observed in chimeras from haploid ES cells



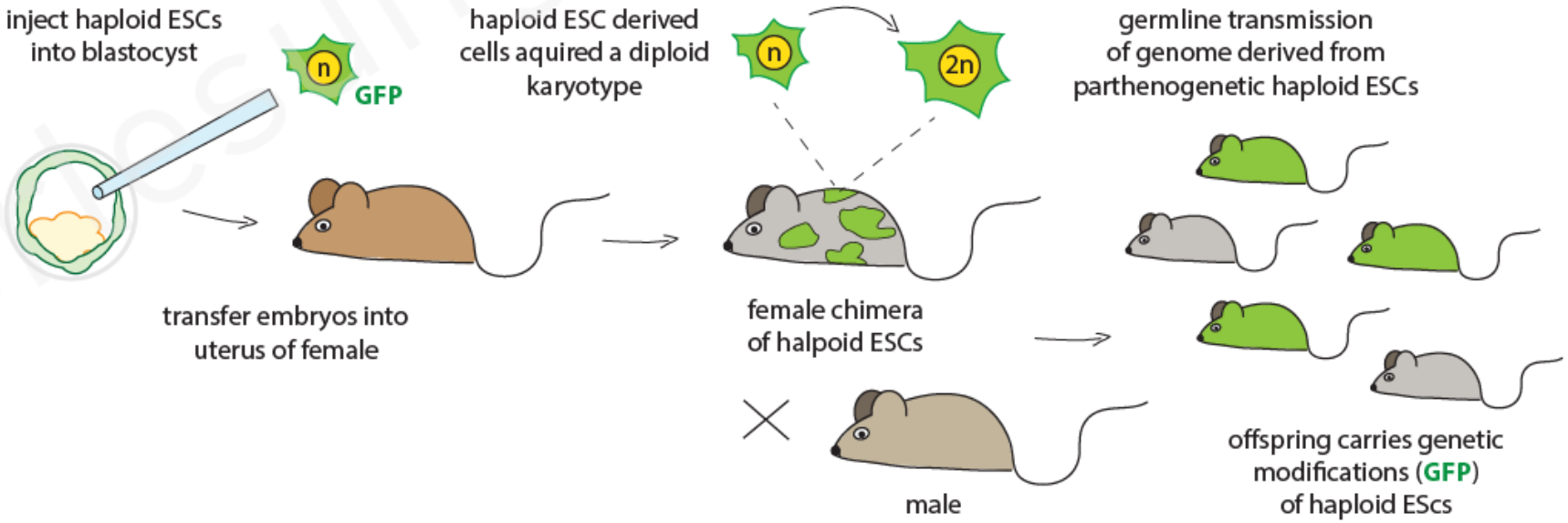
Transgenic mice from haploid ES cells

chimera

germ line transmission

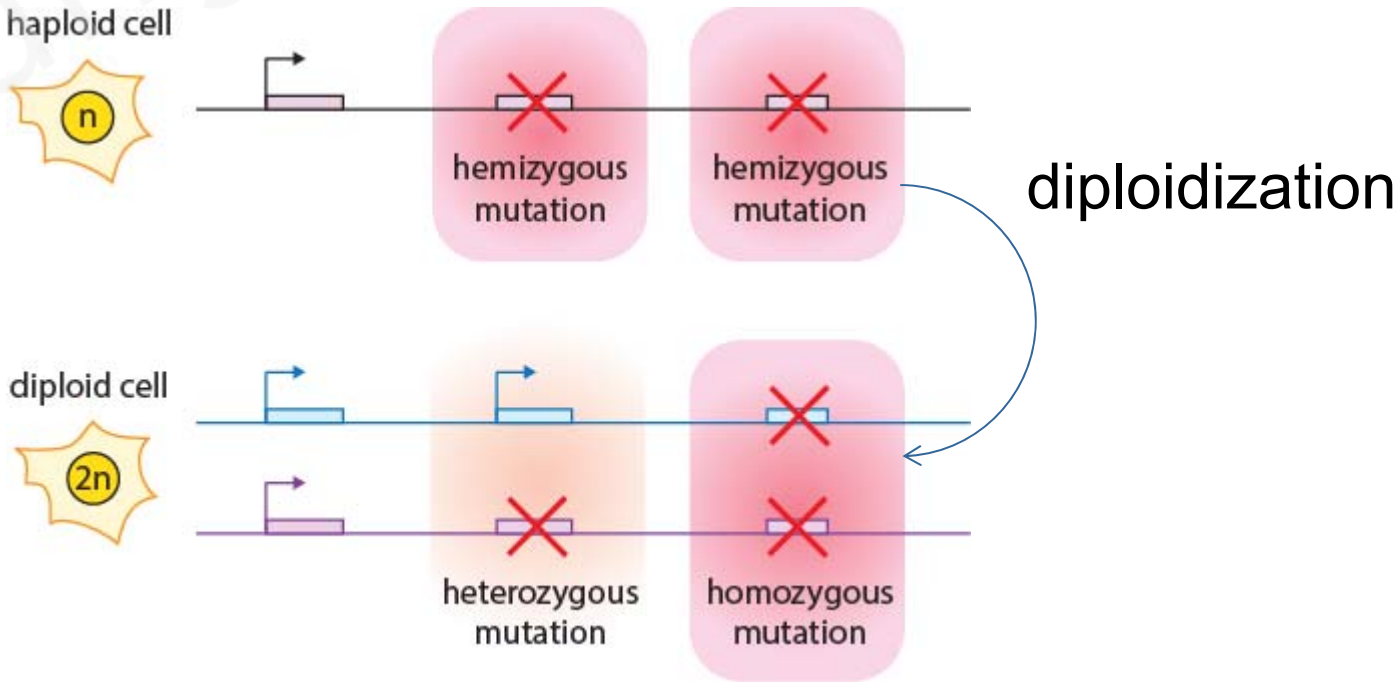


Developmental potential of haploid embryonic stem cells

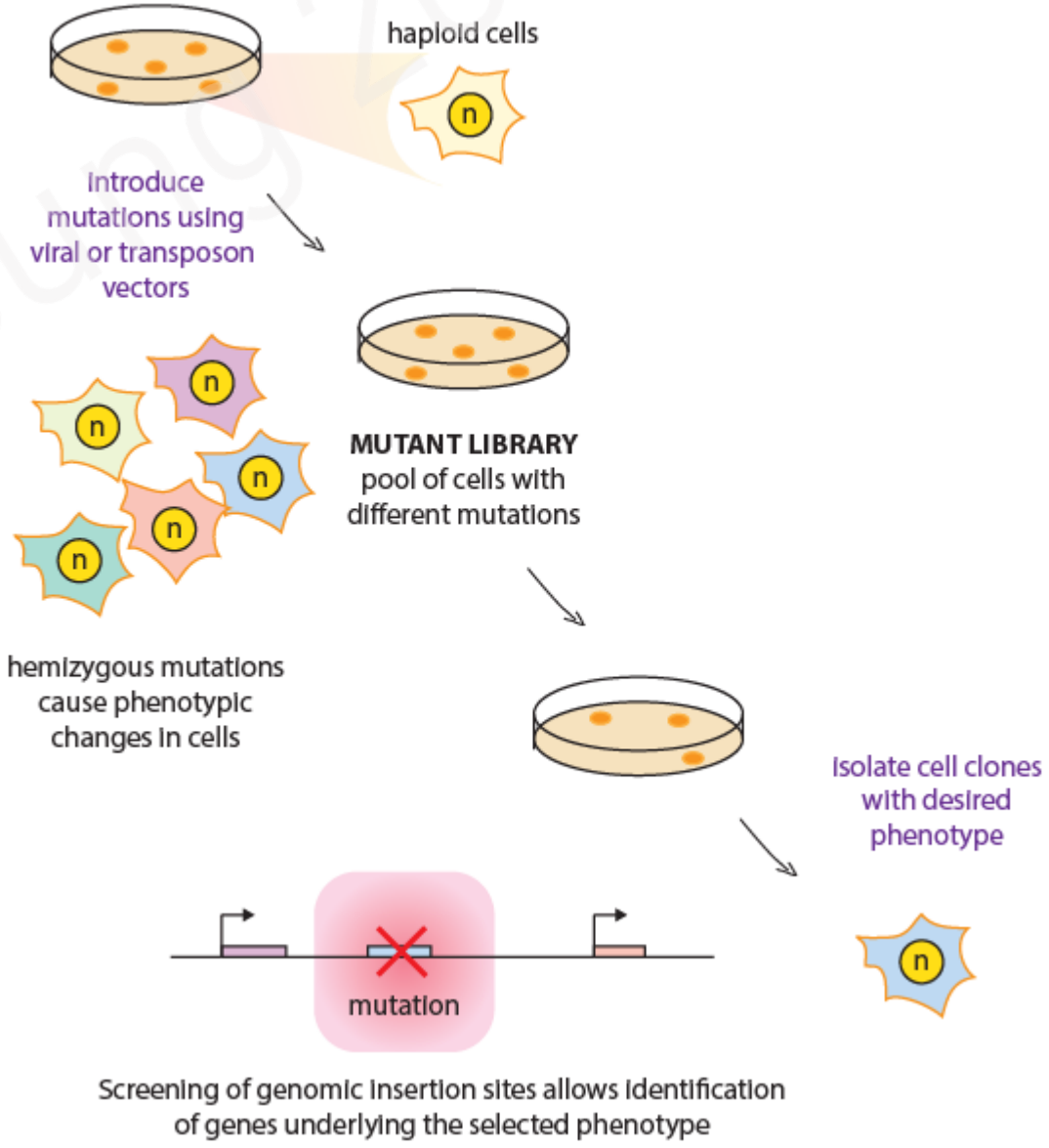


- Haploid ES cell lines can be established from different mouse strains
- Haploid ES cell lines contribute to chimeras
- Haploid ES cell lines possess germline competence allowing the establishment of genetically modified mouse lines

Mutations in Haploid Genomes: hemizygous mutations



Forward genetics using haploid embryonic stem cells



Forward genetics using haploid embryonic stem cells

Aim of the screen	Cell system	Strategy	genes identified	Reference
Mismatch repair pathway	mouse haploid ESCs	gene trap piggyBac transposon	*Msh2*	(Leeb & Wutz 2011)
Ricin toxicity	mouse haploid ESCs	gene trap virus	*Gpr107*	(Elling et al 2011)
Olaparib toxicity	mouse haploid ESCs	gene trap piggyBac transposon	*Parp1*	(Pettitt et al 2013)
Exit from ESC self-Renewal	mouse haploid ESCs	gene trap piggyBac transposon	*Zfp706*, *Pum1*	(Leeb et al 2014)

Haploid cells:

advantages for genetic screening, not new but only very recent opportunity for mammals

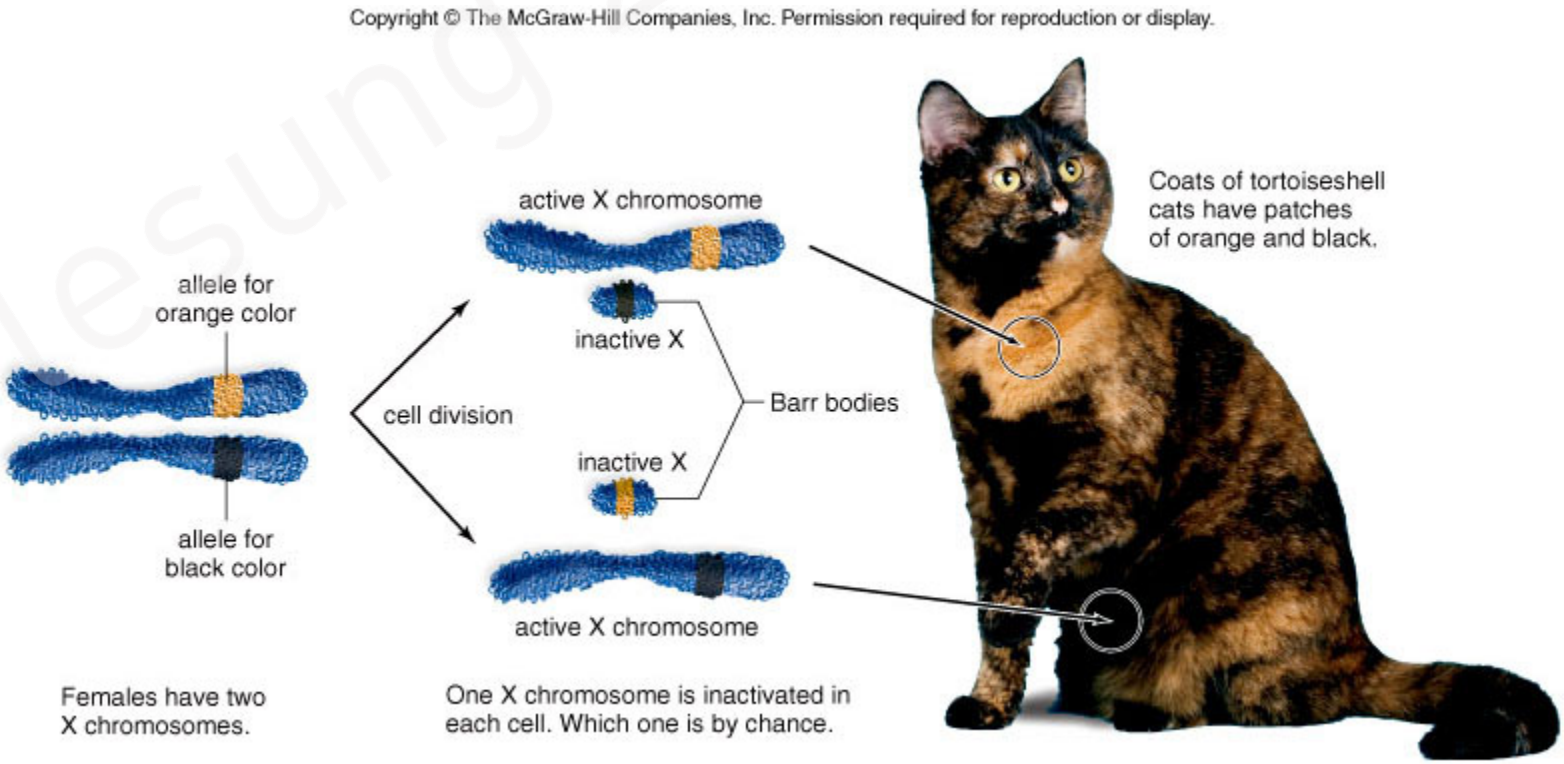
recapitulate some characteristics of developing embryo

clearly not an organism but an experimental system
usefulness for exploring genomic questions at a cellular level but no substitute for studies on organism (tissue, organ function)

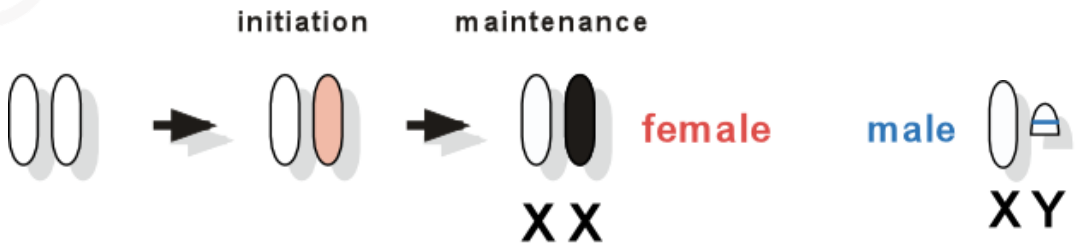
complement weakness of organismal studies for molecular details (cells express the genome – often hard to make out in multicelluar organisms)

X chromosome inactivation and Mammalian Dosage Compensation

BRIEF INTRODUCTION



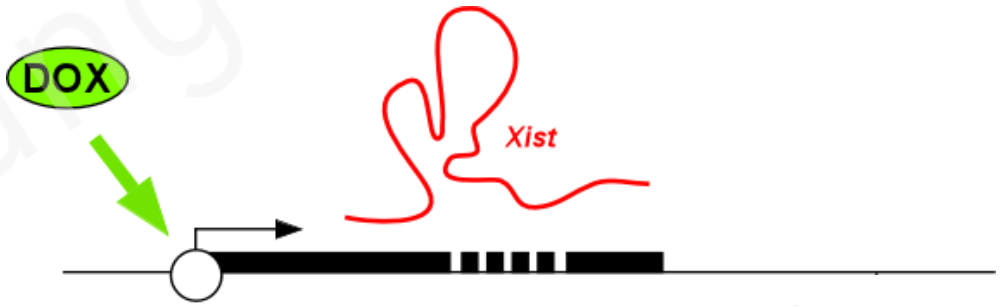
X chromosome inactivation in mammals provides a mechanism for silencing a single X chromosome in female cells for dosage compensation towards the male single X(y) karyotype



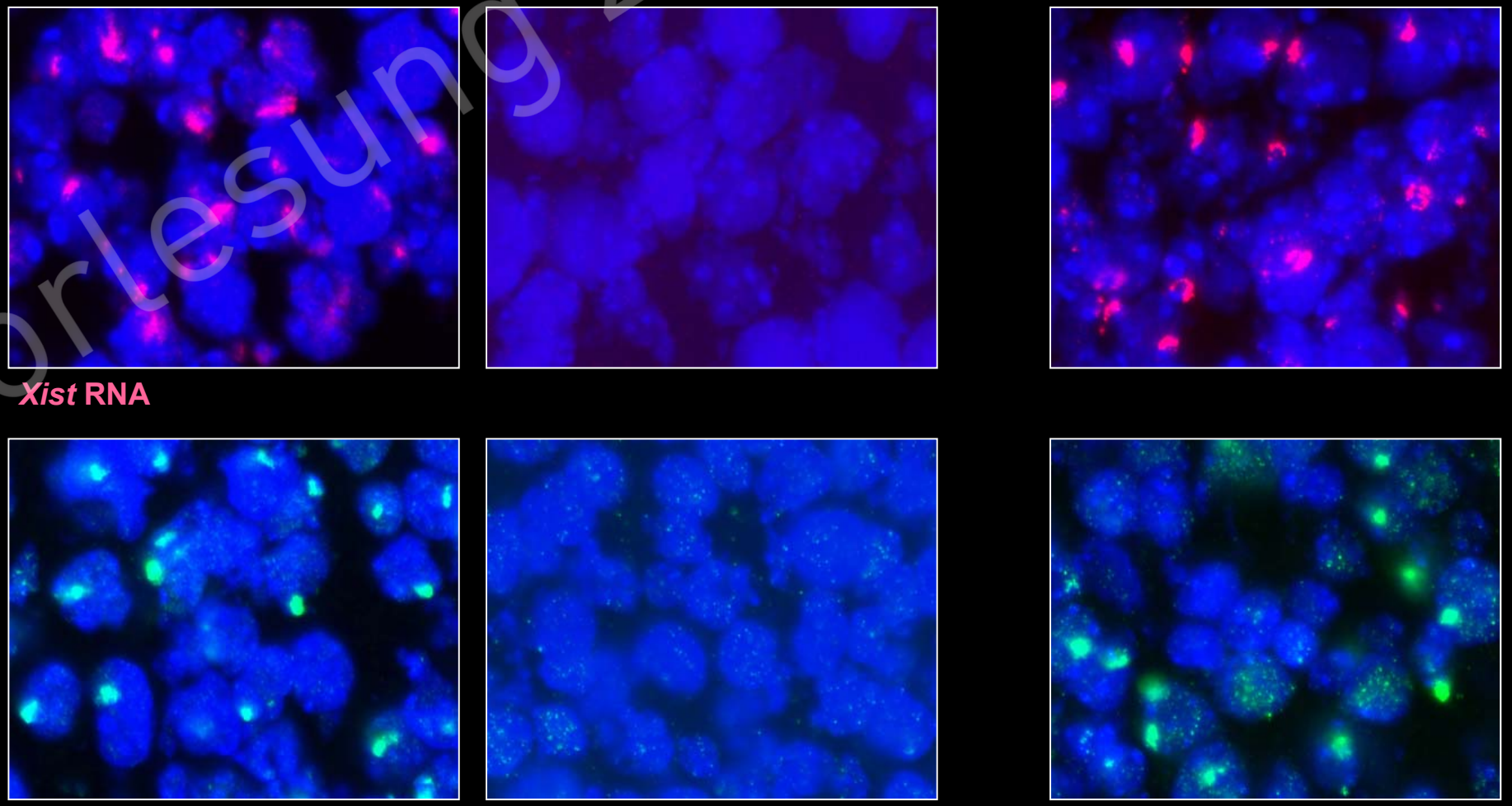
both male and female cells have a single transcriptionally active X

The noncoding RNA *Xist* is the trigger for chromosome wide repression

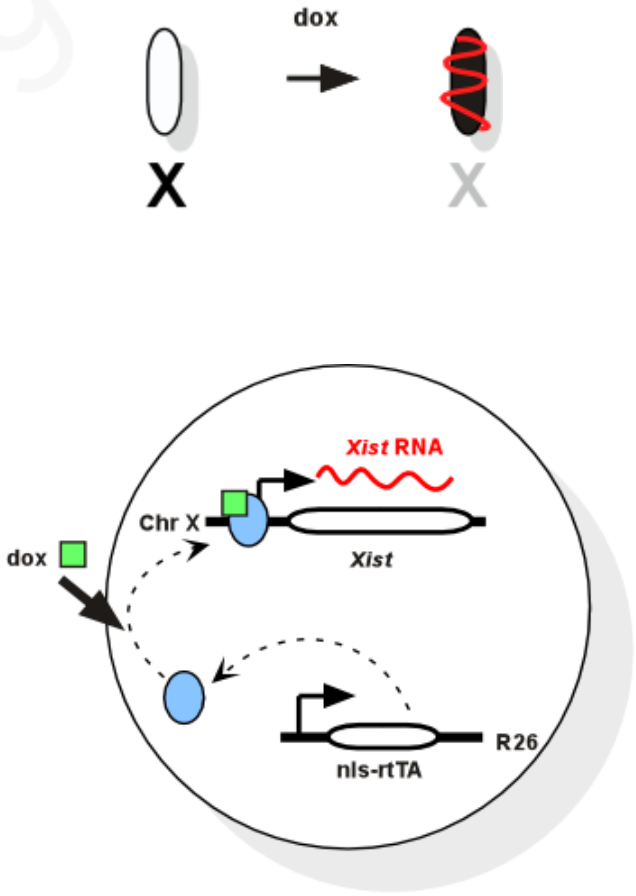
To make an advance on understanding chromosome-wide gene repression in X inactivation mice carrying an inducible promoter inserted in the *Xist* gene were generated



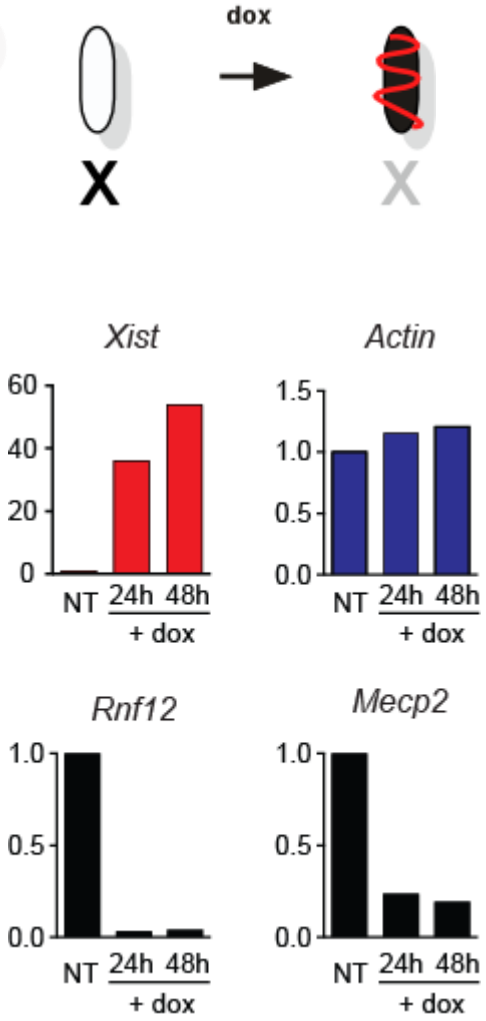
Xist induction from the TX allele in the embryo



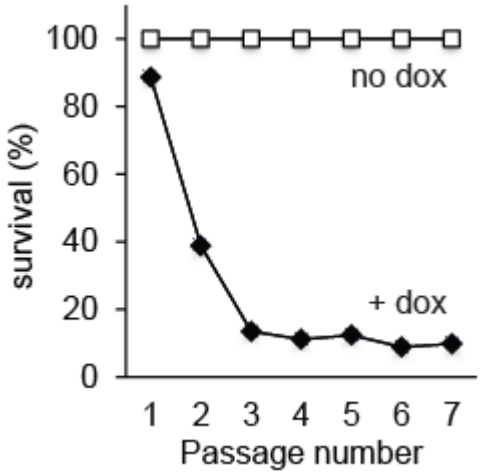
Establishment of haploid ES cells for screening for silencing factors



Establishment of haploid HATX ES cells for screening for silencing factors



Establishment of haploid ES cells for screening for silencing factors



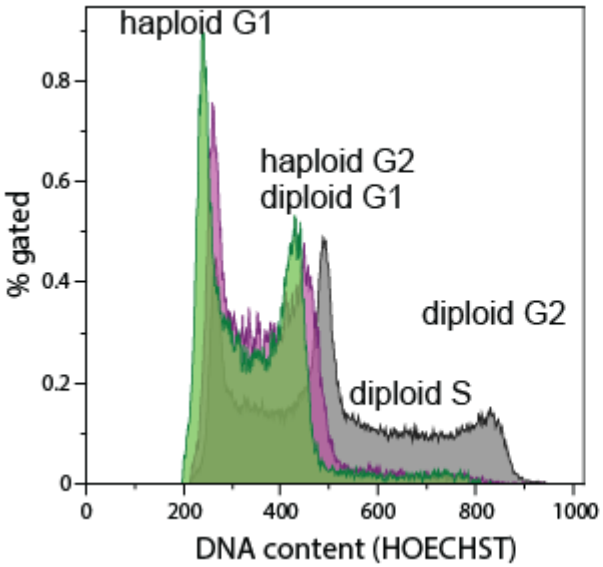
CONCEPT II

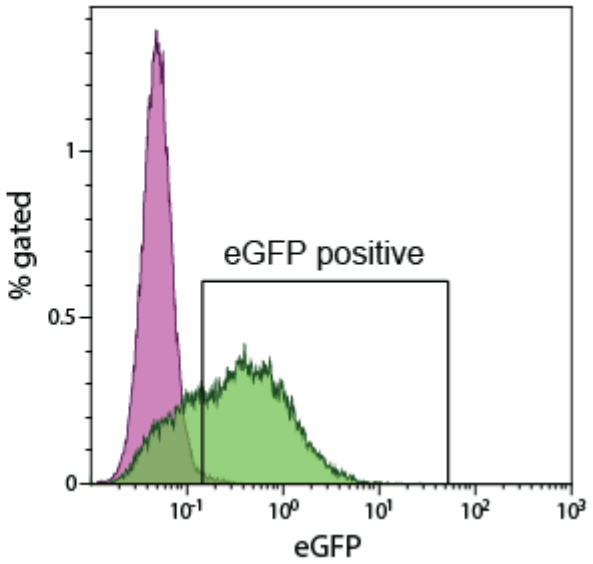
Mutagens and mutant cells in screening process

Infection of haploid HATX ES cells with gene trap viruses

GFP is encoded in gene trap vector:

structure of integrated provirus





infected
uninfected
haploid
diploid
mixed
control

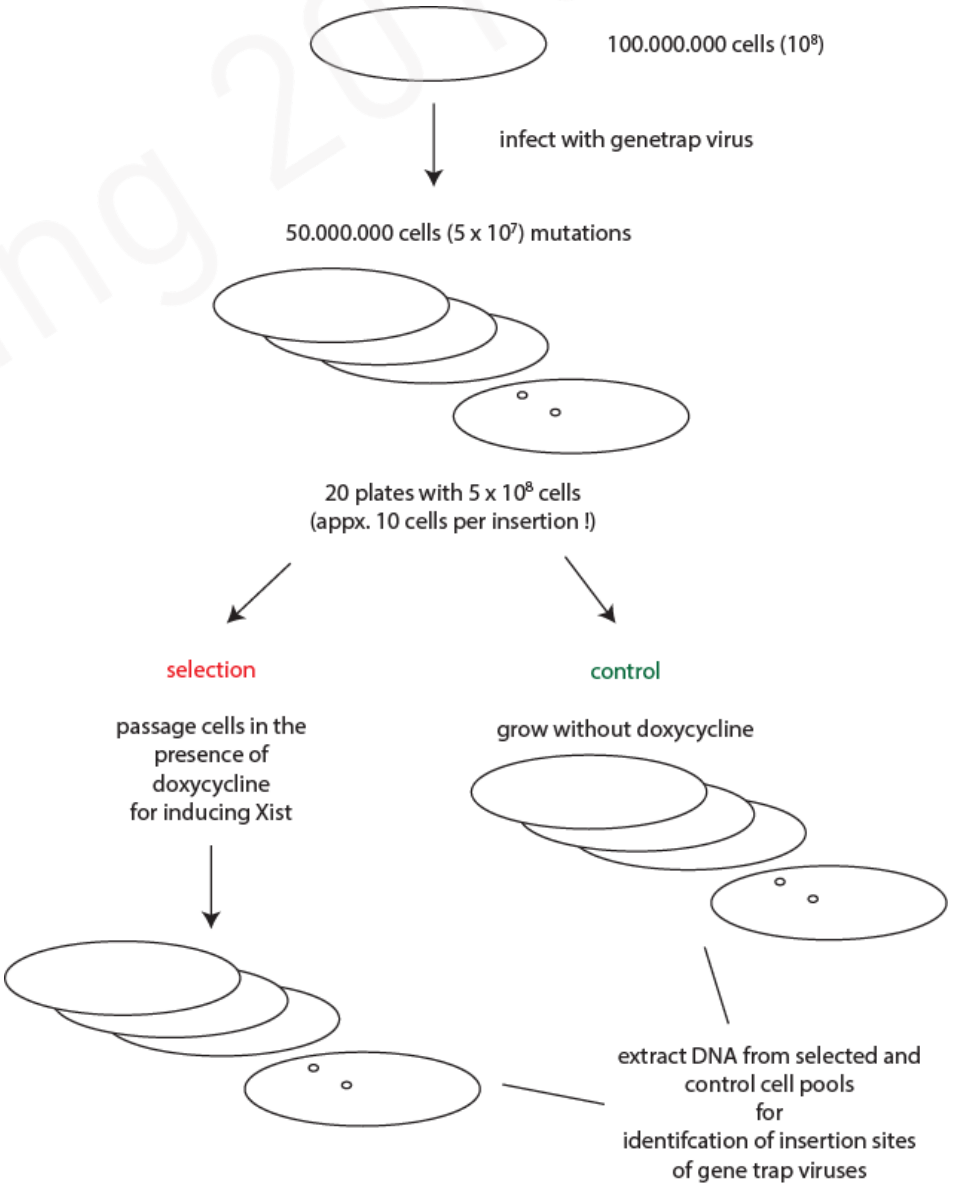
Gene trap vectors:

splice acceptor can trap transcripts in an intron

high mutagenic effect dirupting reading frame or termination
(nonsense mediated decay)

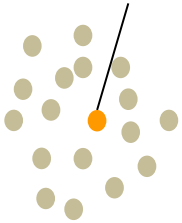
orientation dependent activity of splice acceptor
(works only in one direction)

Screening strategy with random genetrap virus insertions: OVERVIEW

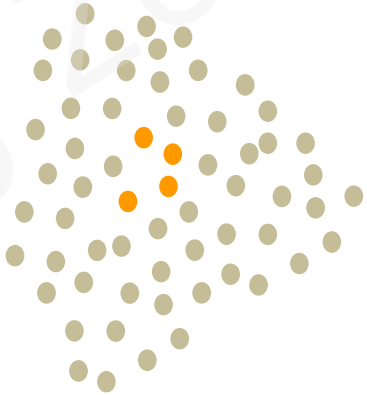


Mutant library representation

following one particular mutation

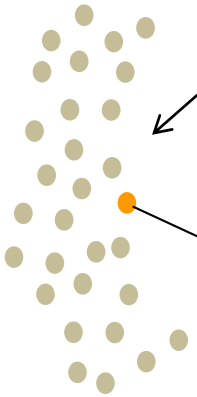


initially one cell per virus insertion



proliferation increases the number of
cells carrying a particular mutation

as cell numbers increase
cells need be passaged

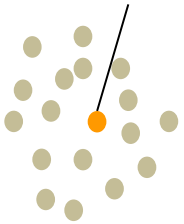


what if it dies ?

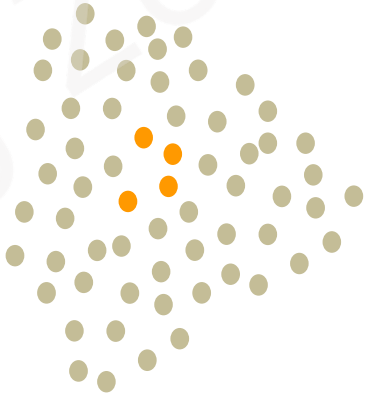
at low numbers of cells carrying
a particular insertion potential loss
of the mutation due to unequal spliting
or cell death is high

Mutant library representation

following one particular mutation

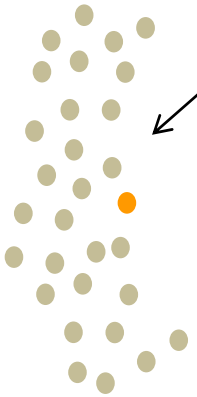


initially one cell per virus insertion



proliferation increases the number of cells carrying a particular mutation

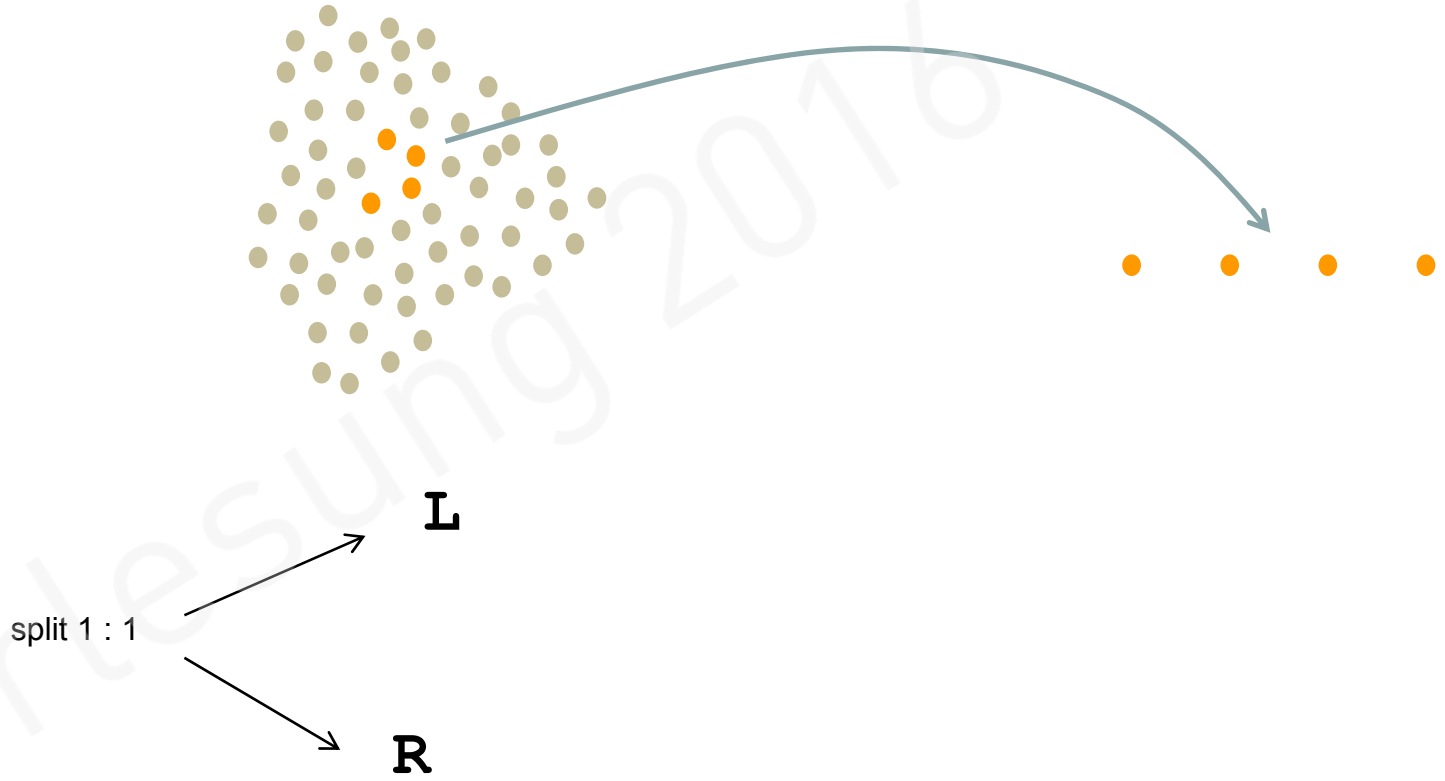
as cell numbers increase cells need be passaged

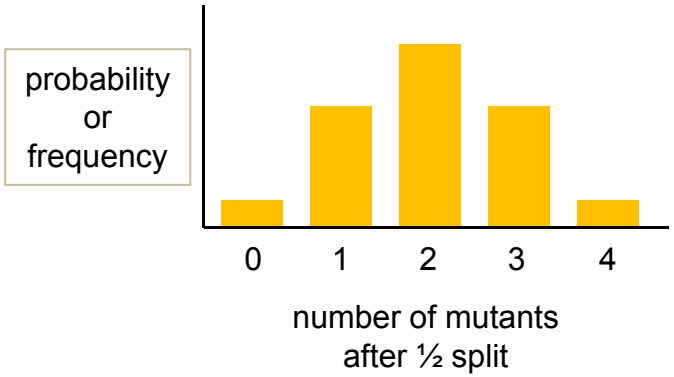


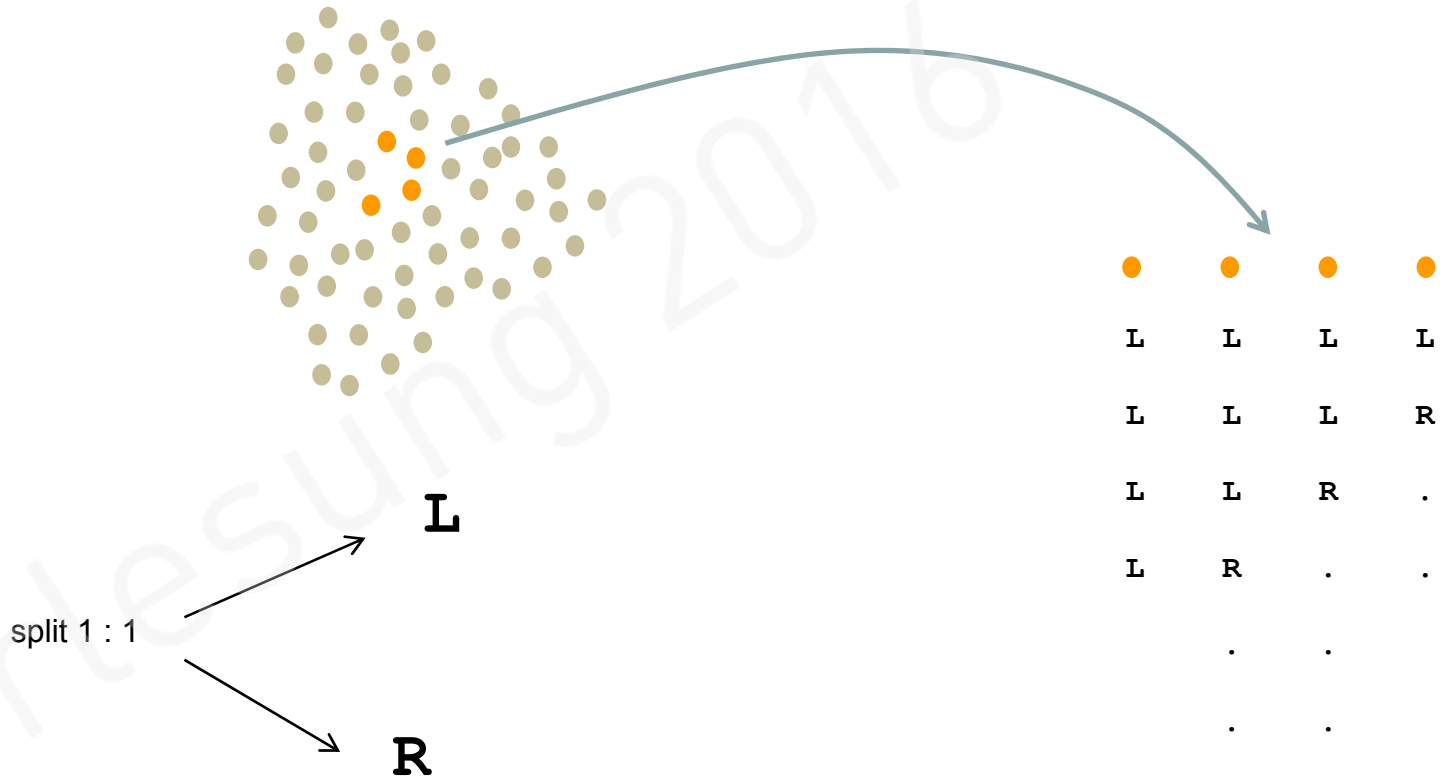
probability or frequency of outcome we expect if a large number ... statistics ☹ ... here of mutations ! ☺

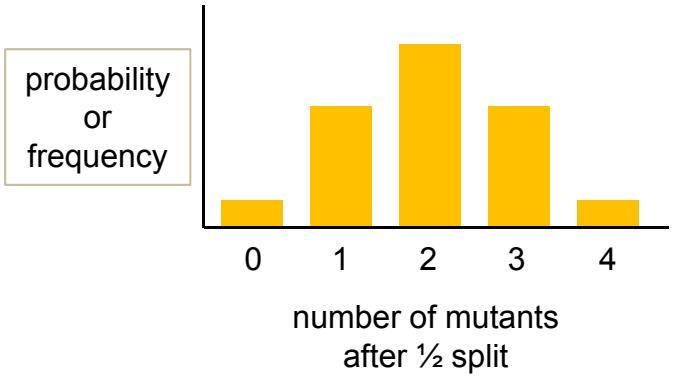
number of mutants after ½ split →

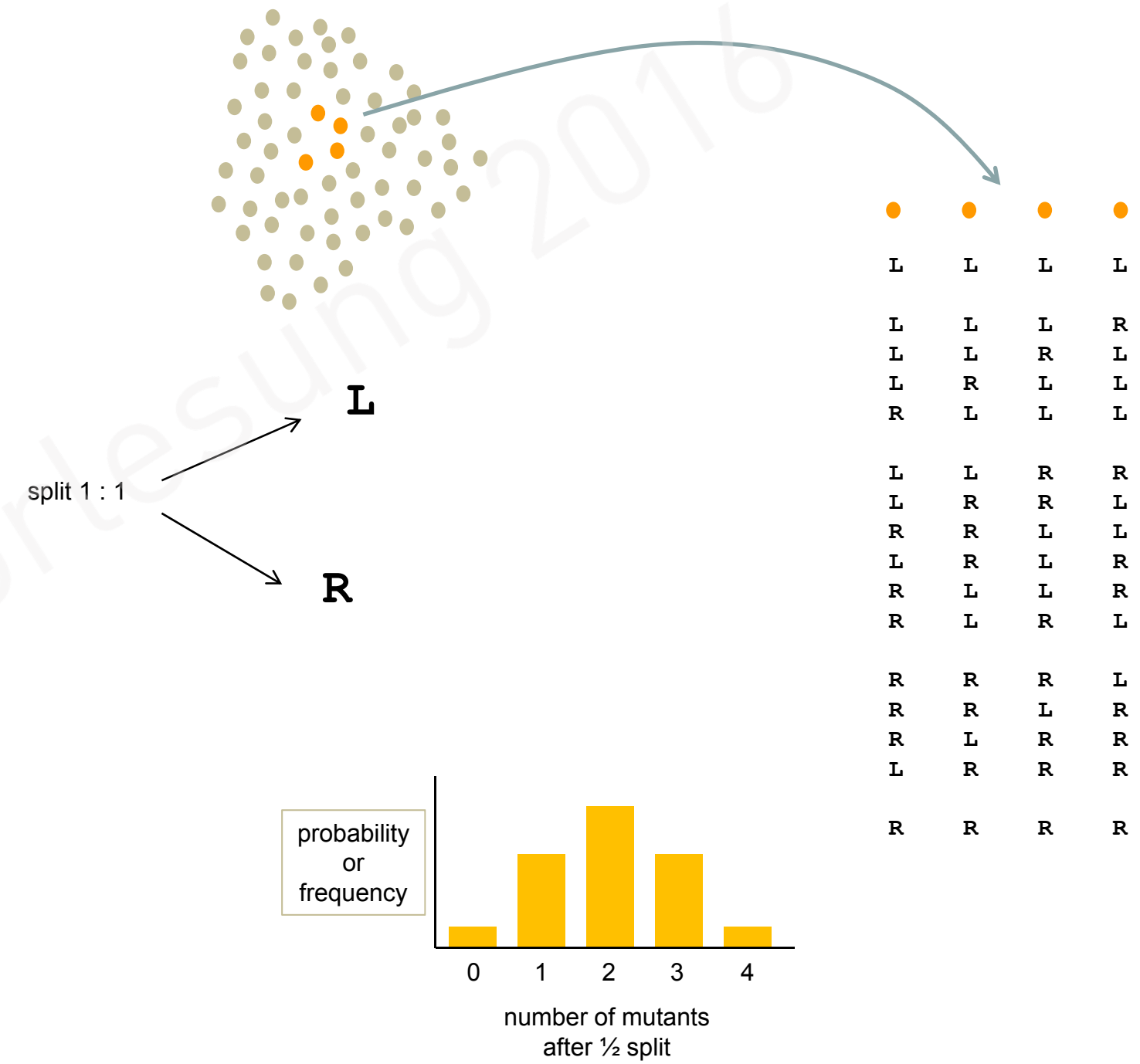
at low numbers of cells carrying a particular insertion potential loss of the mutation due to unequal spliting or cell death is high

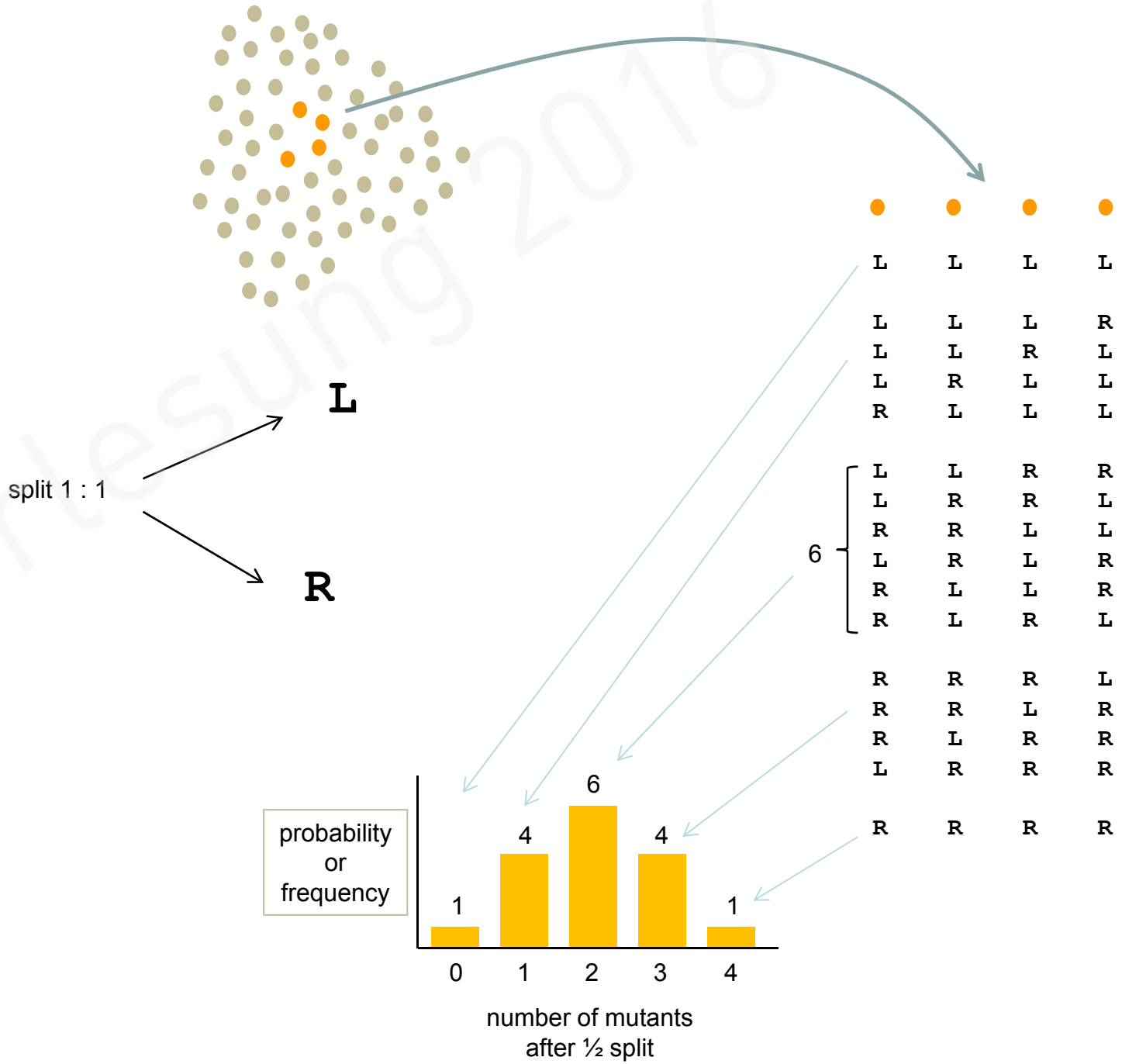











L
split 1 : 1
R
L L L L
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L L R L
L R L L
R L L L
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R L R R
L R R R
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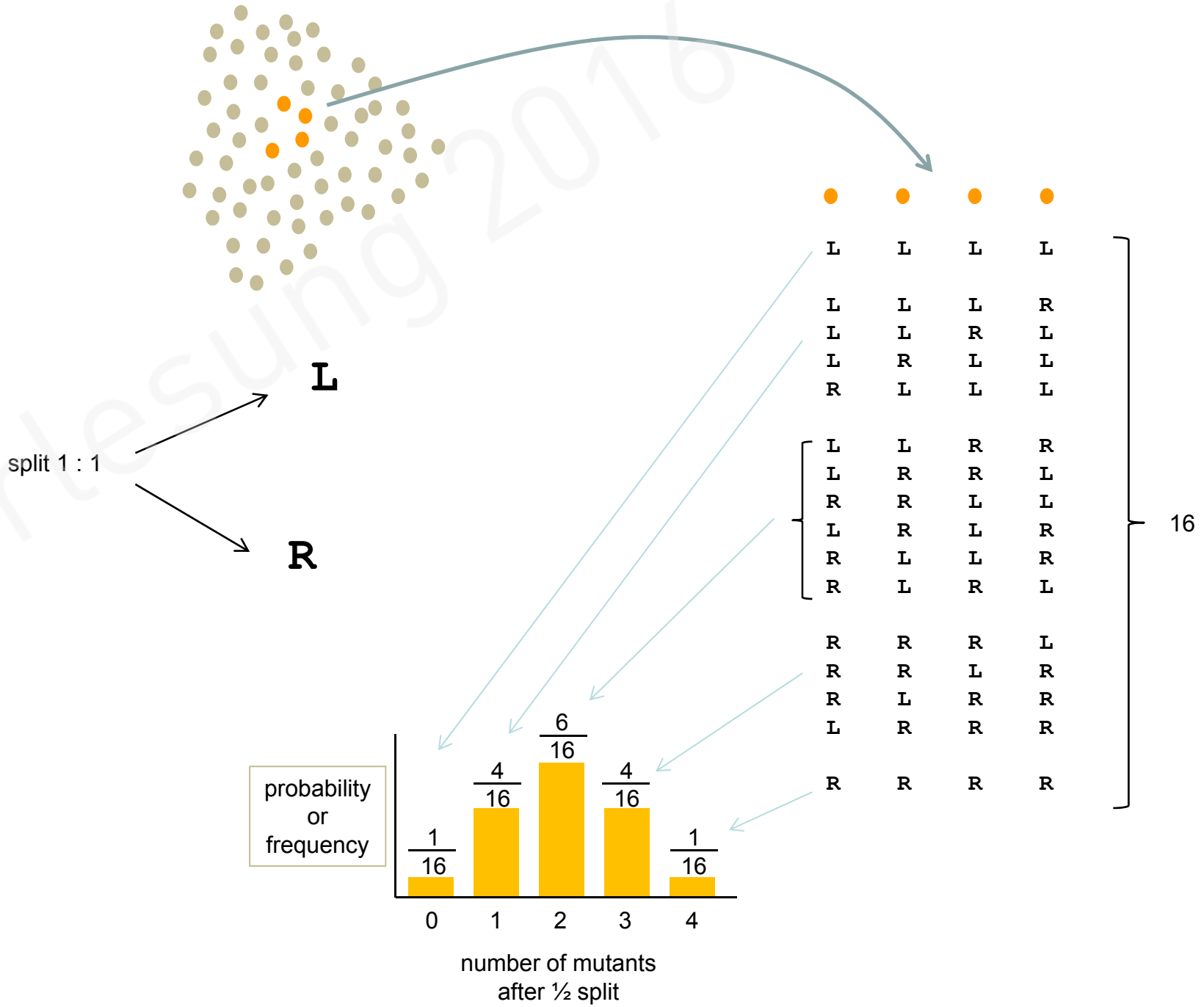
6
probability
or
frequency
1
4
6
4
1
0
1
2
3
4
number of mutants
after ½ split


L
split 1 : 1
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L R L R
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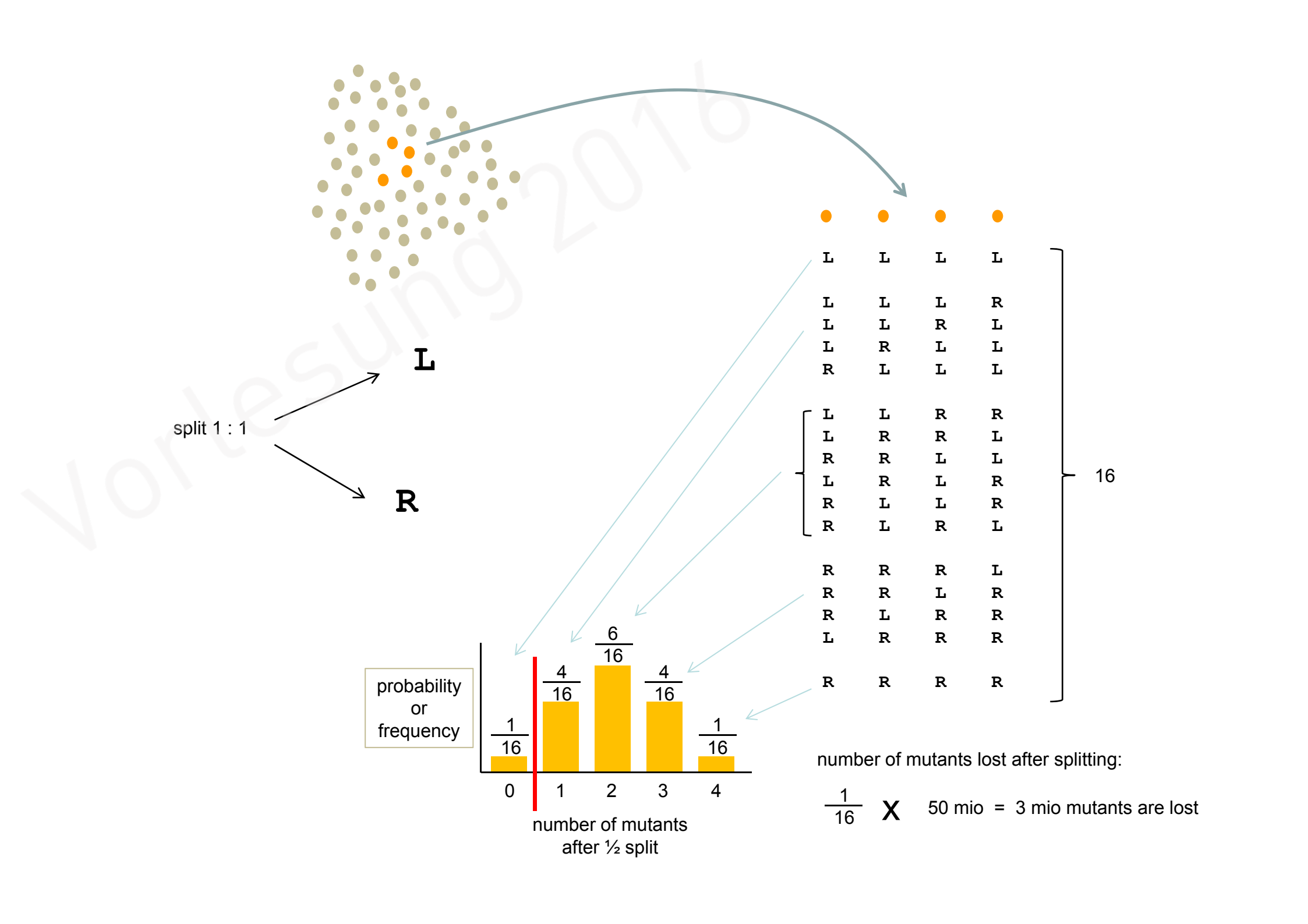
R L R L
R R R L
R R L R
R L R R
L R R R
R R R R
16
probability
or
frequency
1/16
4/16
6/16
4/16
1/16
0
1
2
3
4
number of mutants
after ½ split


L
split 1 : 1
R
L L L L
L L L R
L L R L
L R L L
R L L L
L L R R
L R R L
R R L L
L R L R
R L L R
R L R L
R R R L
R R L R
R L R R
L R R R
R R R R
16
probability
or
frequency
1/16
4/16
6/16
4/16
1/16
0
1
2
3
4
number of mutants
after ½ split
number of mutants lost after splitting:
1/16 X 50 mio = 3 mio mutants are lost

$\frac{1}{16}$

if roughly 6% of mutations are lost from the pool each split ...

```
starting library ..................... 50 mio

 1. split  ........................... 47 mio
 2. split  ........................... 44 mio
 3. split  ........................... 42 mio
 4. split  ........................... 39 mio
 5. split  ........................... 37 mio
 6. split  ........................... 34 mio
 7. split  ........................... 32 mio
 8. split  ........................... 30 mio
 9. split  ........................... 29 mio
10. split  ........................... 27 mio
11. split  ........................... 25 mio
```

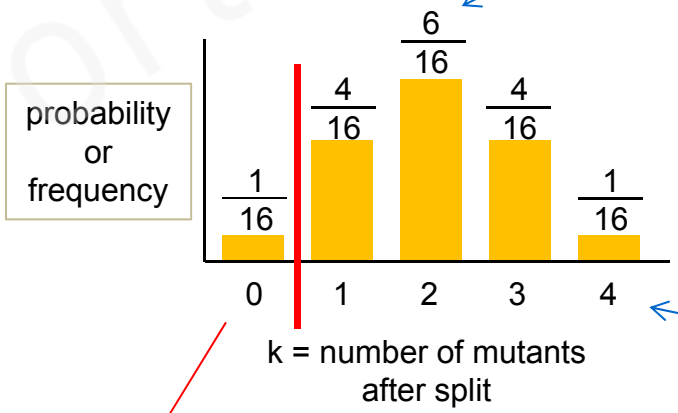
.... cell death and increased loss frequency for mutations with few cells exacerbates the problem with successive rounds of splitting

Ideally 10 fold more cells would be required to keep loss of mutations from pools negligible.

Binominal distribution:

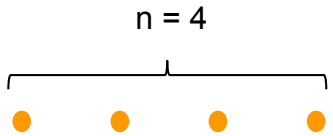
$$\Pr(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

$$\binom{n}{k} = \frac{n!}{k!(n-k)!}$$



k = 0: cathegory for loss
no cells left for this mutation after split

n ... number of cells for a given mutation



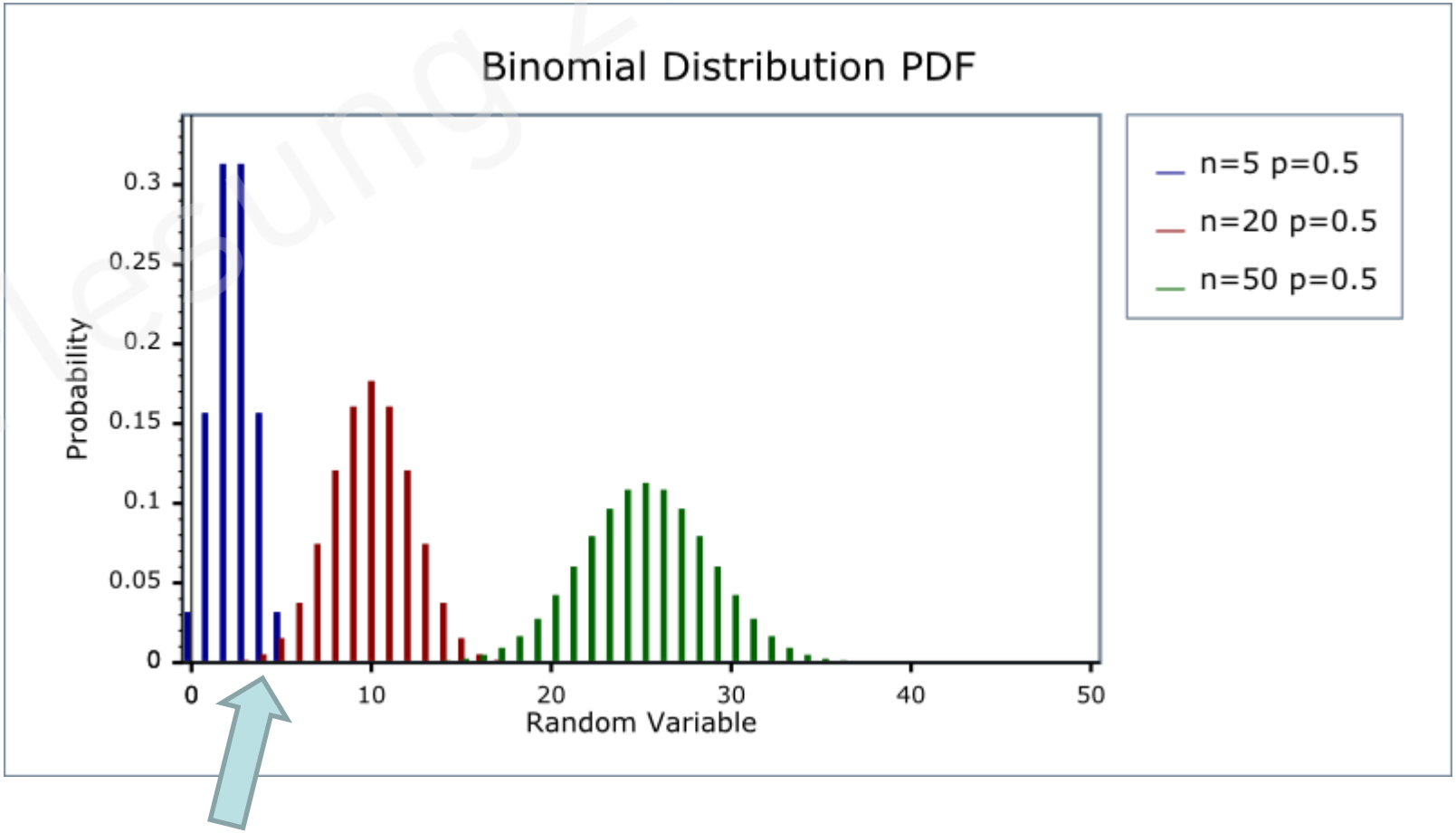
k = 0, 1, 2, ..., n: number of cells after split

p ... probability of recovery after split:

p = 0.5 for split into 2 (p = 1/2)

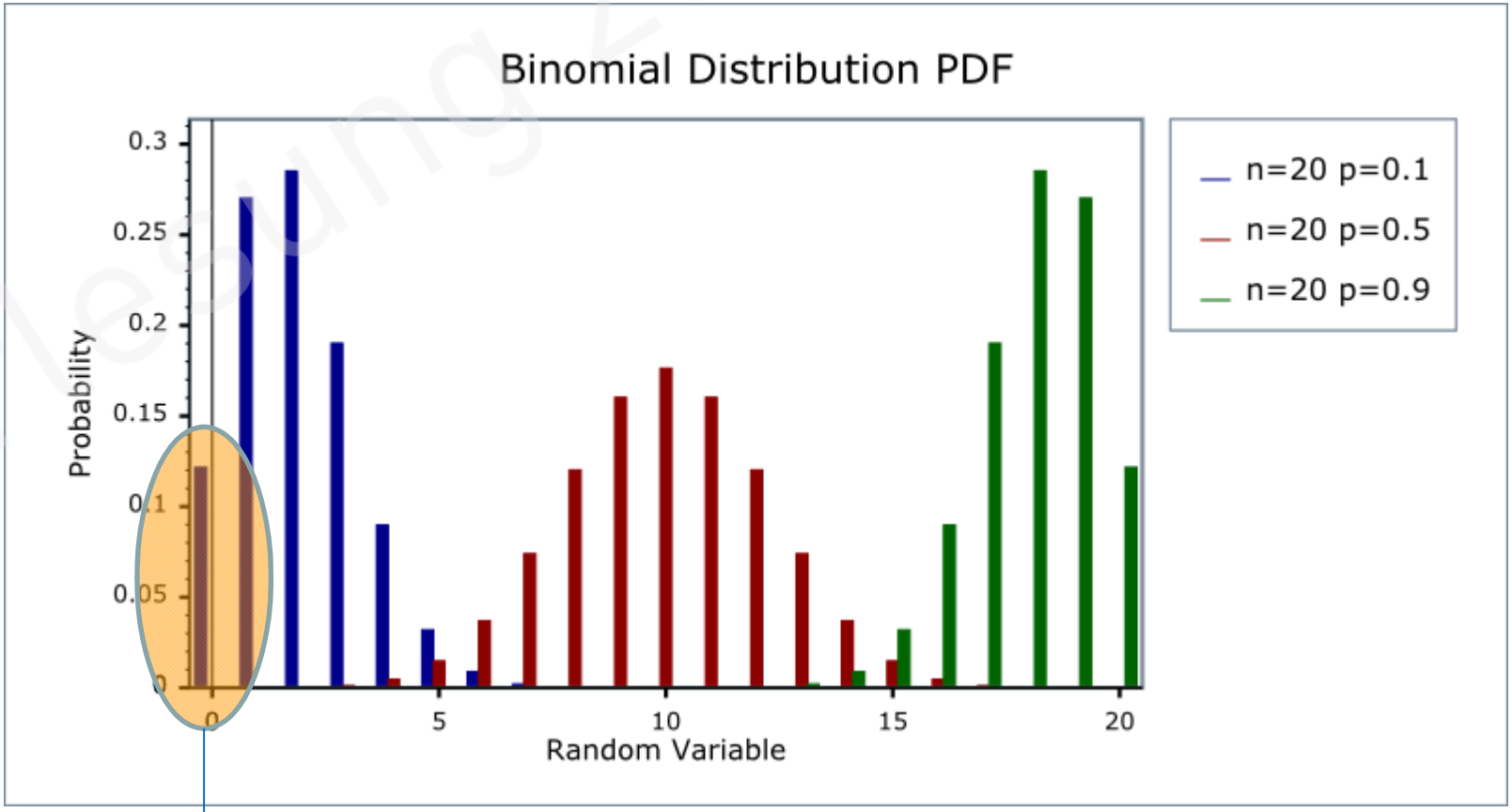
1/3 for split into 3 (or in case 33% of cells die !)
1/10 for split into 10 plates, etc.

! account for cell death and plating efficiency with p < 0.5



ca. 2% of mutations are represented
by only 4-5 cells after ½ split of 20 cells

... subsequent splits could risk loss



loss at 1 : 10 split with 20 cells per mutation

Probability density (or mass) function for the binomial distribution

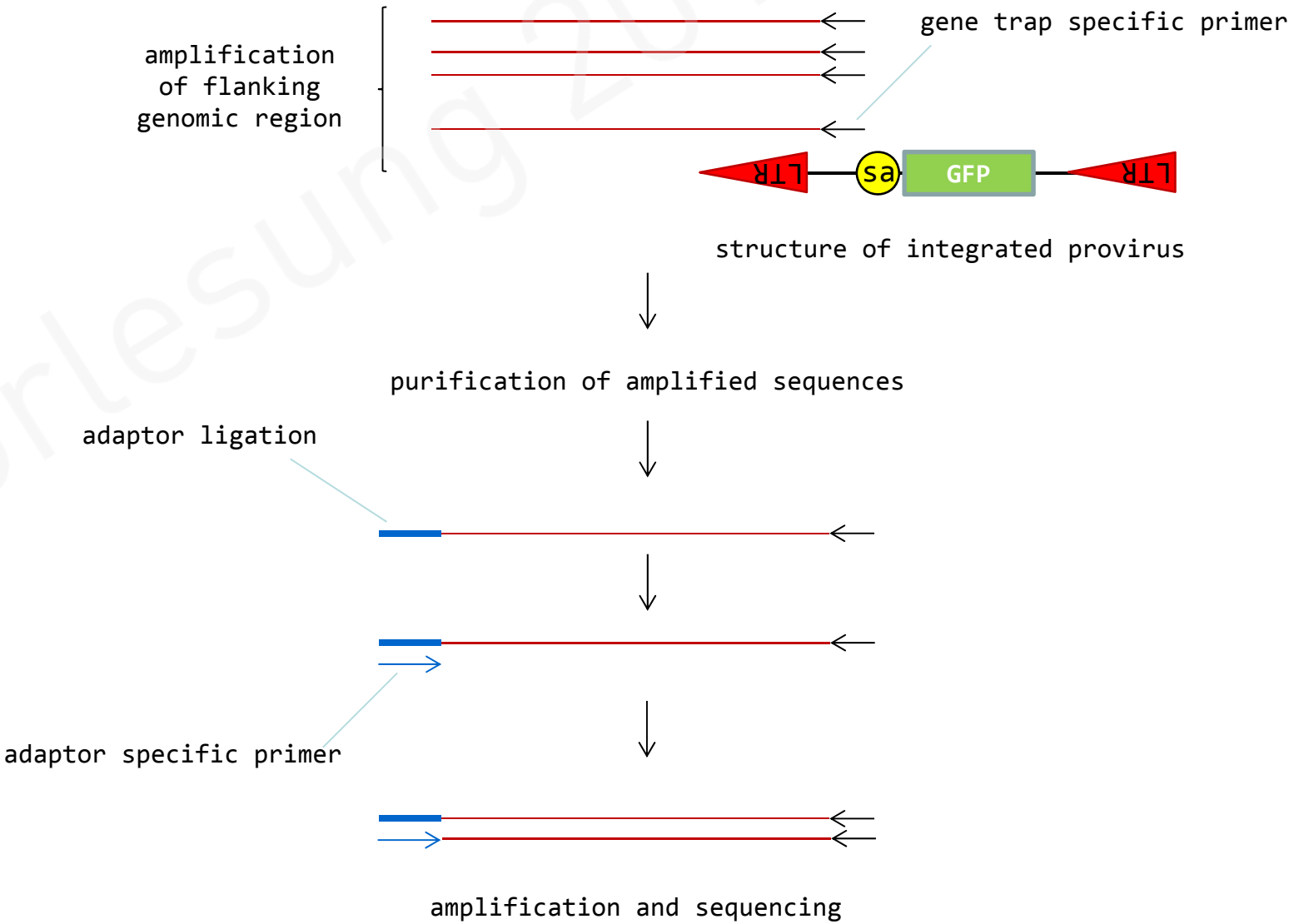
C++

```
#include <boost/math/distributions/binomial.hpp>
namespace math = boost::math;
prob_k = math::pdf(math::binomial(n, p), k);
```

Python

```
from scipy.stats import binom
prob_k = binom.pmf(k, n, p)
```

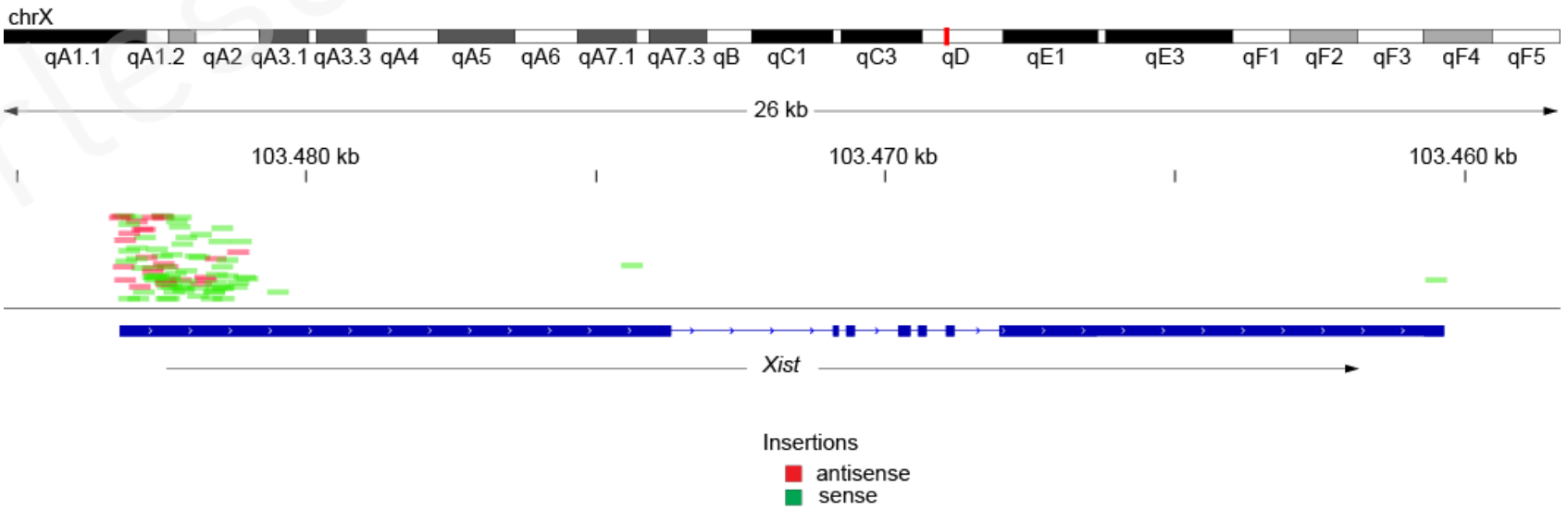
Identification of genomic sites of insertion of gene trap viruses



Identification of insertions by massive parallel sequencing

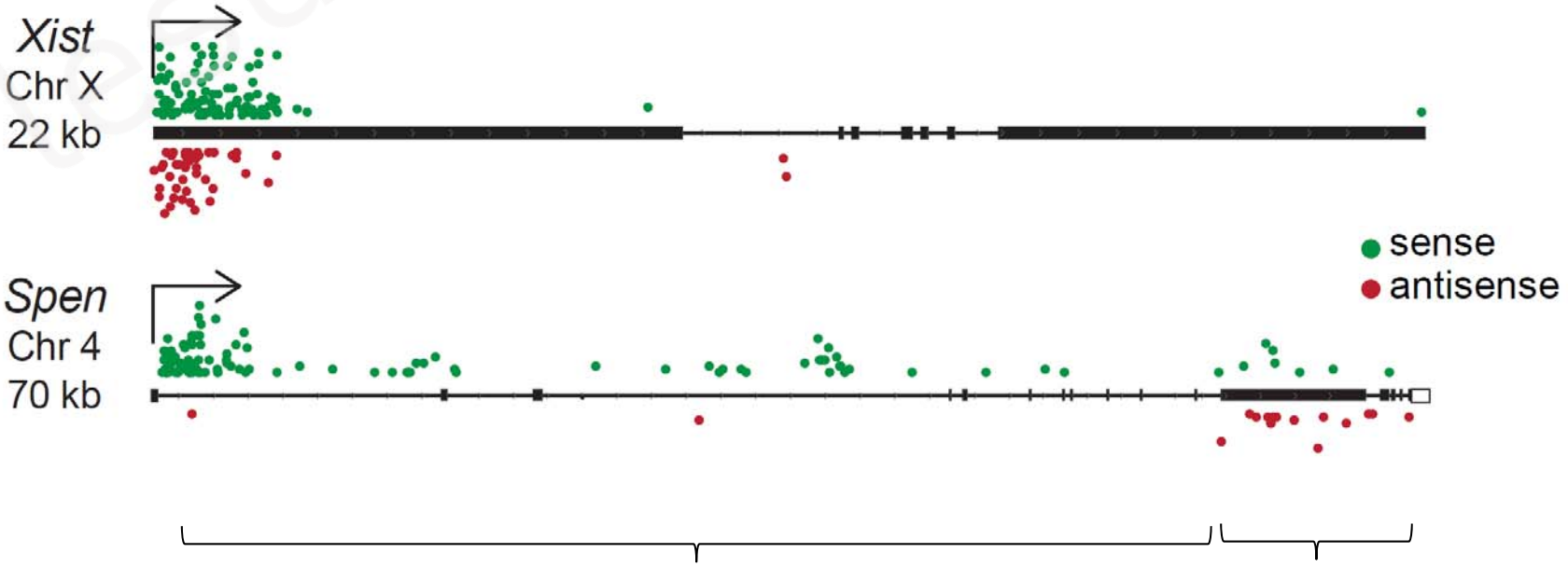

```
_global_ _const_ char * ADAPTOR = "ATGTTGGATTATTAG"

_global_ _const_ int ADAPTOR_LEN = 15

_global_ void TrimmAdaptor(char * dDataArray, char * read_length, int
reads, int MAX_READ_LEN)
{
    for (idx = blockIdx*blockDim + threadIdx.x; idx < reads; idx +=
blockDim * gridDim)
    {
        if (idx<reads)
        {
            char * sequence = &dDataArray[idx*MAX_READ_LEN];
            int rl=read_length[idx];
            int p=0;
            while (p < rl-ADAPTOR_LEN)
            {
                if (sequence[p]==ADAPTOR[0])
                {
                    int mm=0;
                    for (int n=1; n<ADAPTOR_LEN; n++)
                    {
                        if (sequence[p+n]!=ADAPTOR[n])
                        {
                           mm++;
                           if (mm > 2) break;
                        }
                    }
                    if (mm < 3)
                    {
                        read_length[idx]=p;
                        break;
                    }
                }
                p++;
            }
            if (mm < 3 ) break;
            if (p < rl-ADAPTOR_LEN
        }
    }
}
```

Expected Mutations:
Xist gene trap mutations



READOUT: gene trap orientation bias

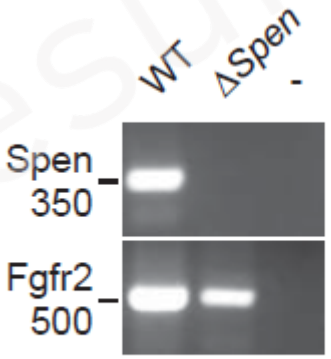
splice acceptor only works if inserted
in this direction into a transcription unit



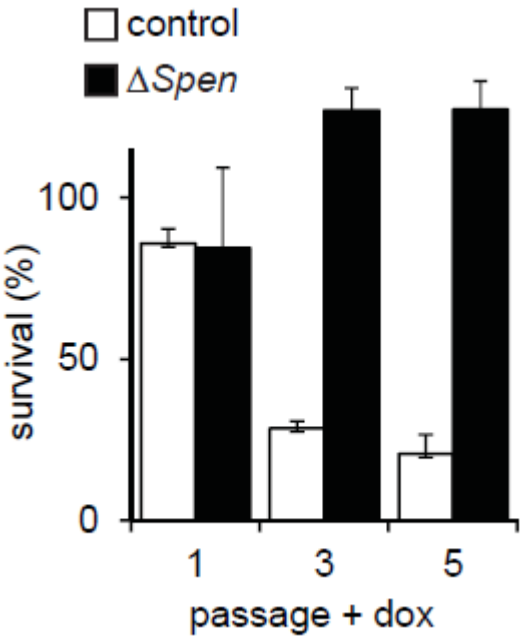
orientation bias in intronic insertions:
<splice acceptor works orientation dependent>

exonic region
<no bias>

Candidate gene validation through REVERSE GENETICS
gene deletion using CRISPR/Cas nuclease pairs



genotyping confirms absence of *Spen* sequences in Δ*Spen* cells



ΔSpen cells survive well induction of *Xist*

CONCLUSION:
Spen is required for *Xist* mediated gene repression

CONCEPT III

Developing screening strategies

EXERCISE 1: Define criteria and strategy for a genetic screen in cell lines for identifying components in signal transduction

1. Consider the problem and pick a concrete example of a signaling pathway

2. Discuss in groups how you can define a selection strategy for interesting mutations

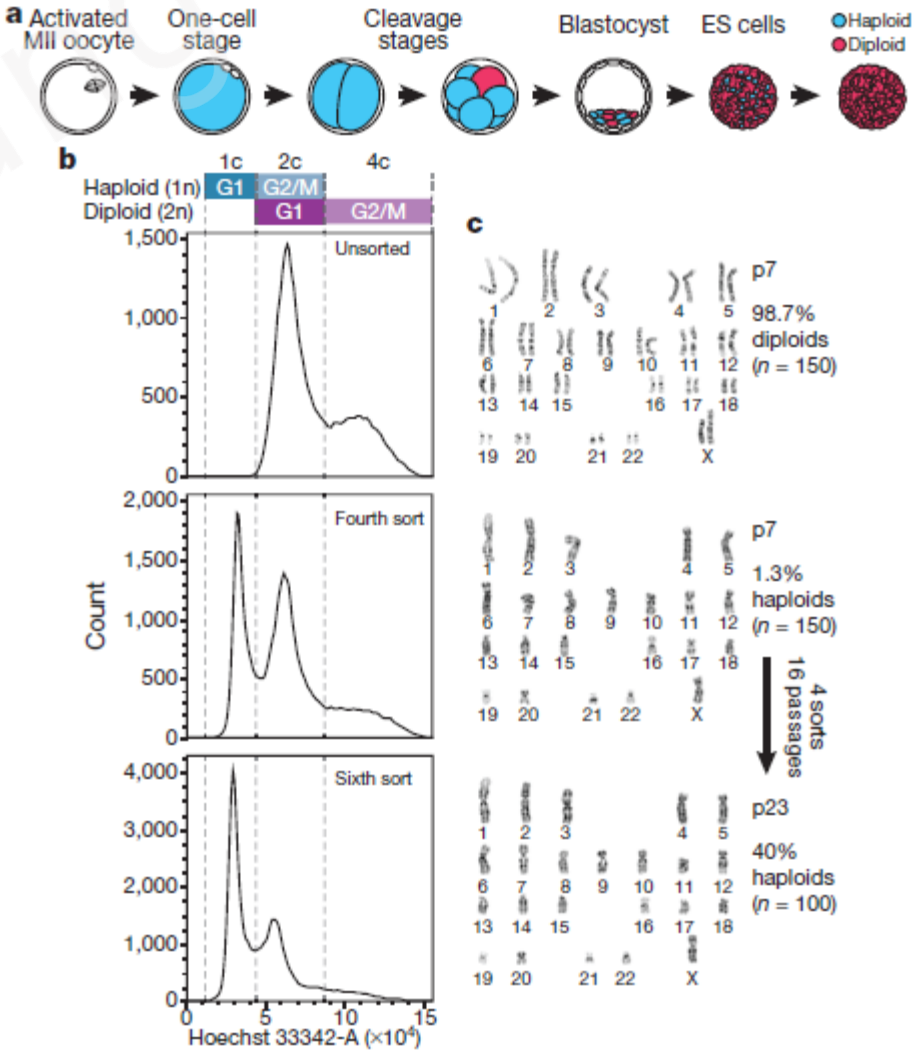
3. What are considerations on the cell system?

4. What are considerations on the mutagenesis?

5. What classes of mutations can you expect to isolate

Derivation and differentiation of haploid human embryonic stem cells

Ido Sagi[1], Gloryn Chia[2], Tamar Golan-Lev[1], Mordecai Peretz[1], Uri Weissbein[1], Lina Sui[2], Mark V. Sauer[3], Ofra Yanuka[1], Dieter Egli[2,4] & Nissim Benvenisty[1]



human oocytes can be activated with an electric pulse or Ca-ionophore to generate parthenogentic embryos with varying amounts of haploid cells

Nature 523: 107-111

use of cell sorting for enriching human ES cells with a haploid genome content

EXERCISE 2: Synthetic lethal mutations hold promise new avenues in cancer treatment

1. Consider the problem: What is the idea behind?

2. Discuss in groups how you possibly could apply screens in cell lines to identify mutations that are synthetic lethal with tumor suppressor gene mutations

3. What are considerations on the cell system?

4. What are considerations on the mutagenesis?

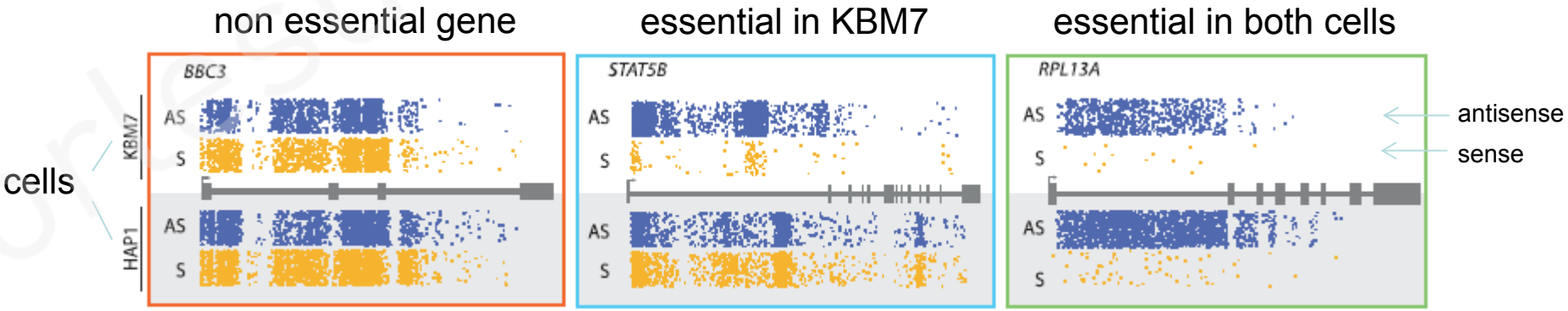
5. What classes of mutations can you expect to isolate

GENOMICS

Gene essentiality and synthetic lethality in haploid human cells

Vincent A. Blomen,[1*] Peter Májek,[2*] Lucas T. Jae,[1*] Johannes W. Bigenzahn,[2] Joppe Nieuwenhuis,[1] Jacqueline Staring,[1] Roberto Sacco,[2] Ferdy R. van Diemen,[1] Nadine Olk,[2] Alexey Stukalov,[2] Caleb Marceau,[3] Hans Janssen,[1] Jan E. Carette,[3] Keiryn L. Bennett,[2] Jacques Colinge,[2,4†] Giulio Superti-Furga,[2,5†] Thijn R. Brummelkamp[1,2,6†]

Science 350: 1092-1096



KBM7 are tumor derived haploid cells that carry a t(8:22) philadelphia chromosomal translocation (BCR/ABL oncofusion gene)

HAP1 are an adherent cell type derived from KBM7 that has lost several tumor associated genetic abnormalities.

Selective sensitivity of KBM7 cells can indicate potential tumor vunerabilities.