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## The Genetics of Quiescence

Serge Gangloff, Benoit Arcangioli

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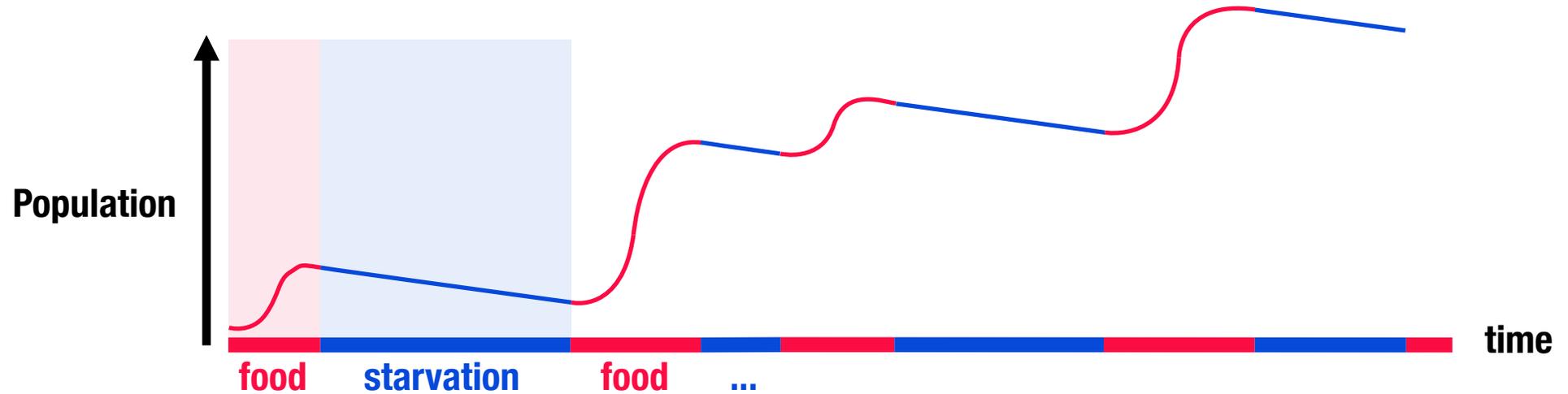
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# **The Genetics of Quiescence**

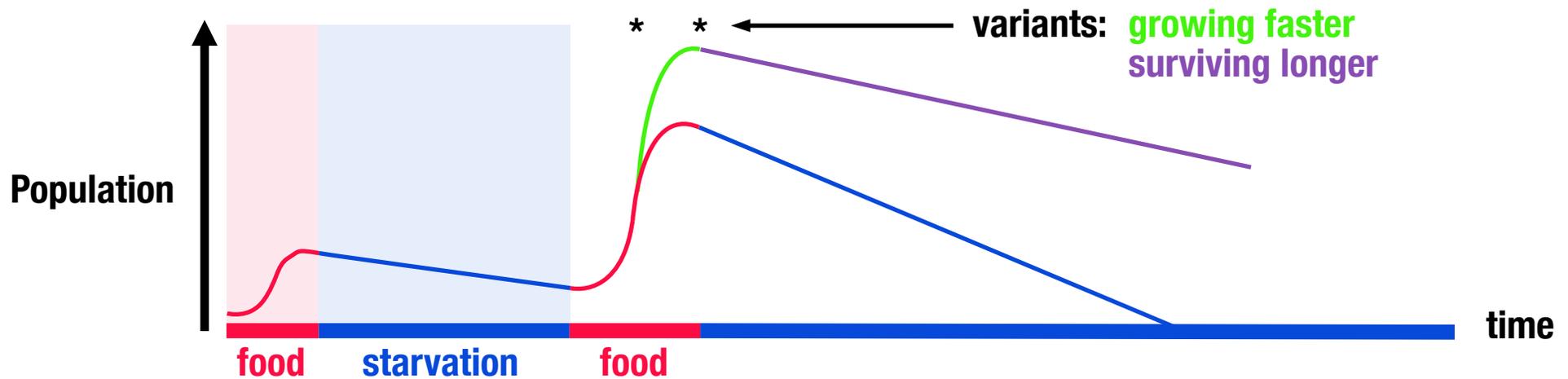
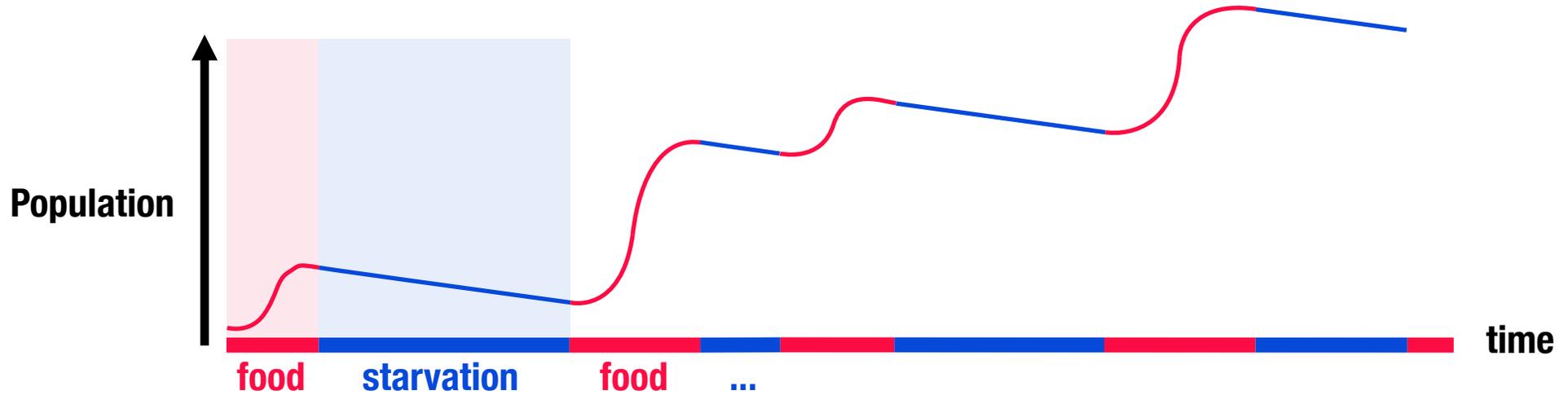
**Serge Gangloff**

**Genome Dynamics Unit – Benoit Arcangioli**

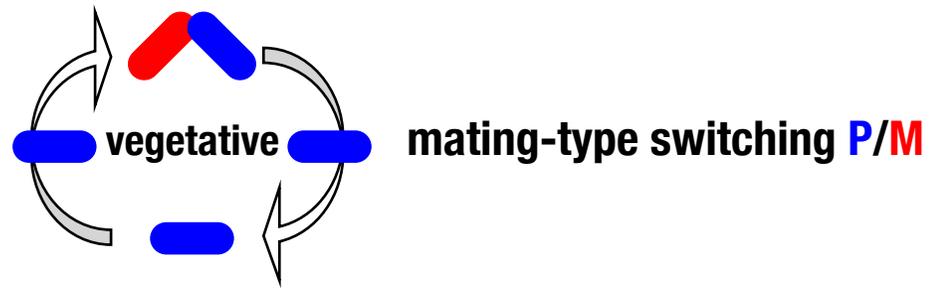
# To maintain life across a fluctuating environment, cells alternate between **growth** and **quiescence**



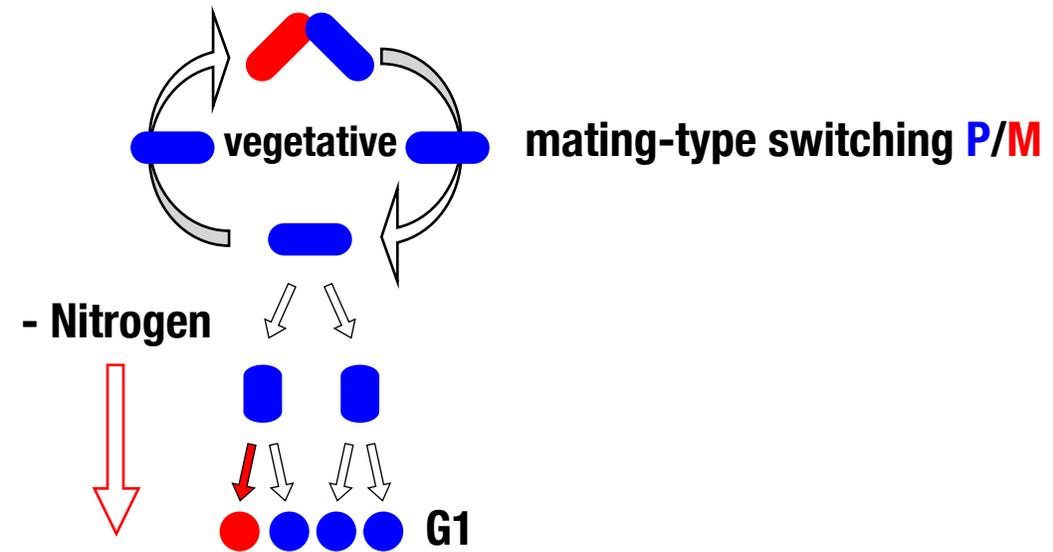
# To maintain life across a fluctuating environment, cells alternate between **growth** and **quiescence**



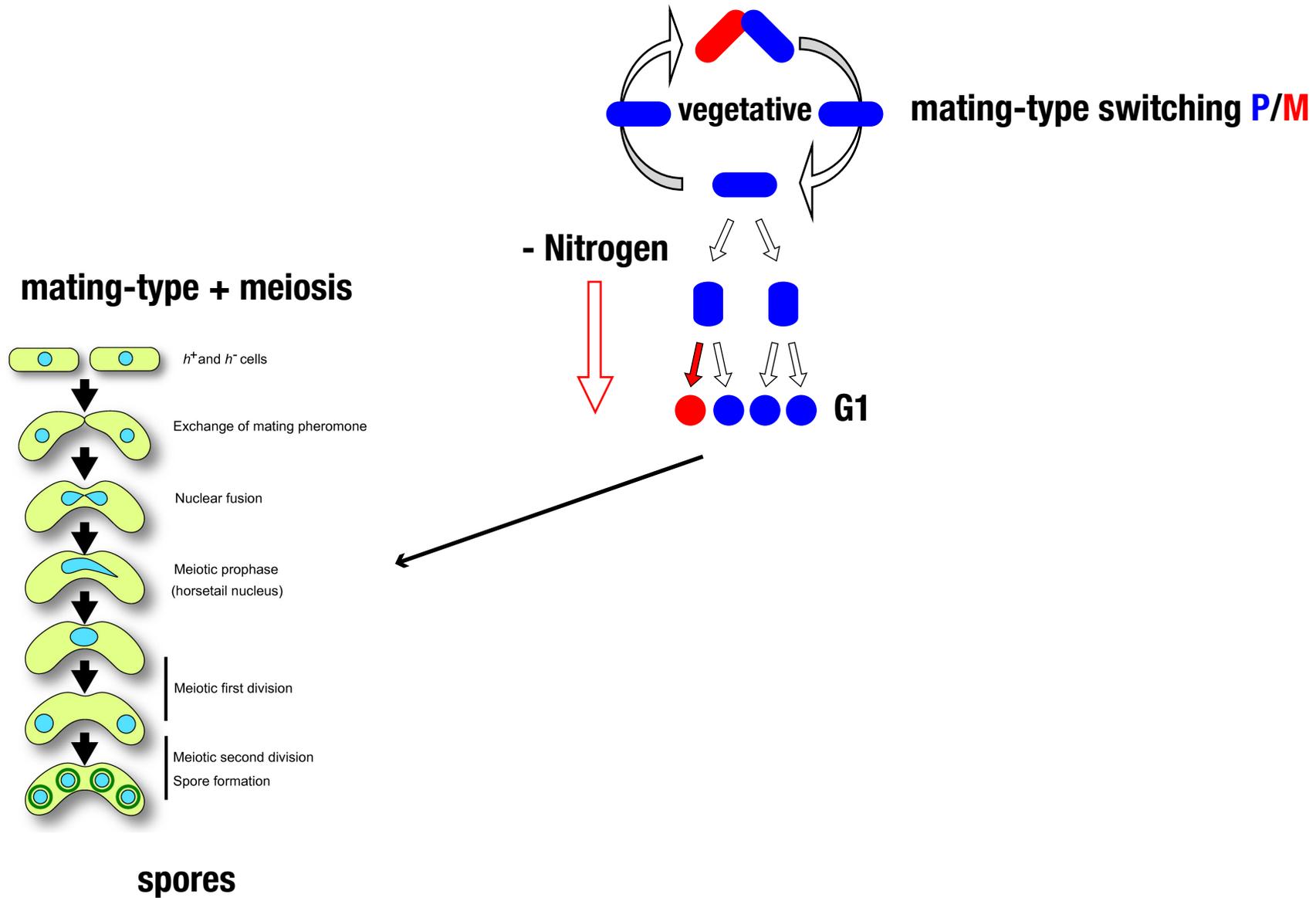
# S. pombe: mating-type switching



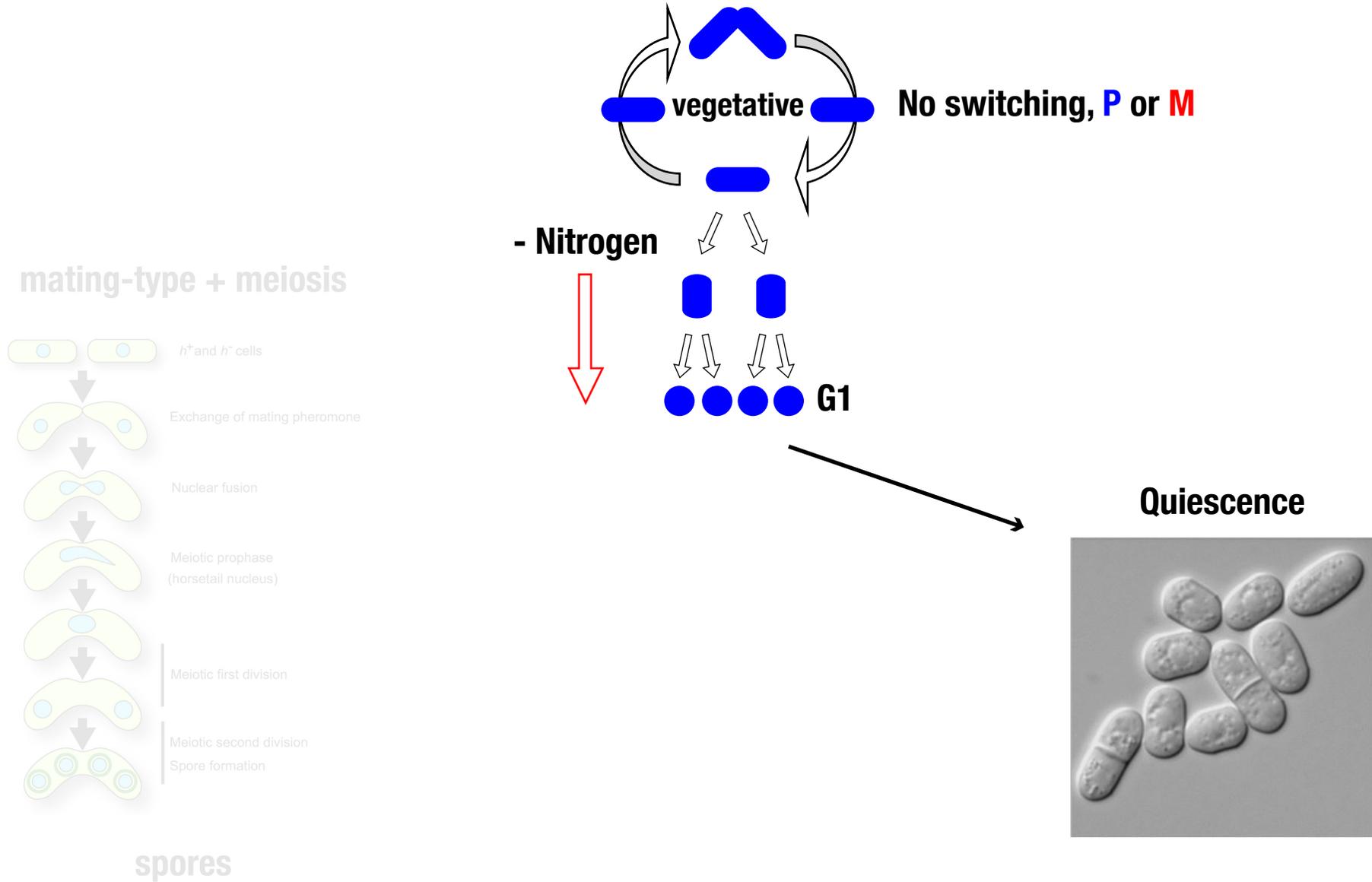
# S. pombe: mating-type switching



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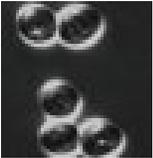


# S. pombe: mating-type switching



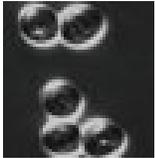
# Tools to study quiescence

Single cell

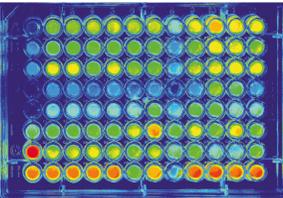


# Tools to study quiescence

Single cell

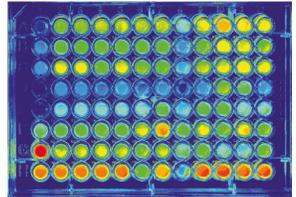
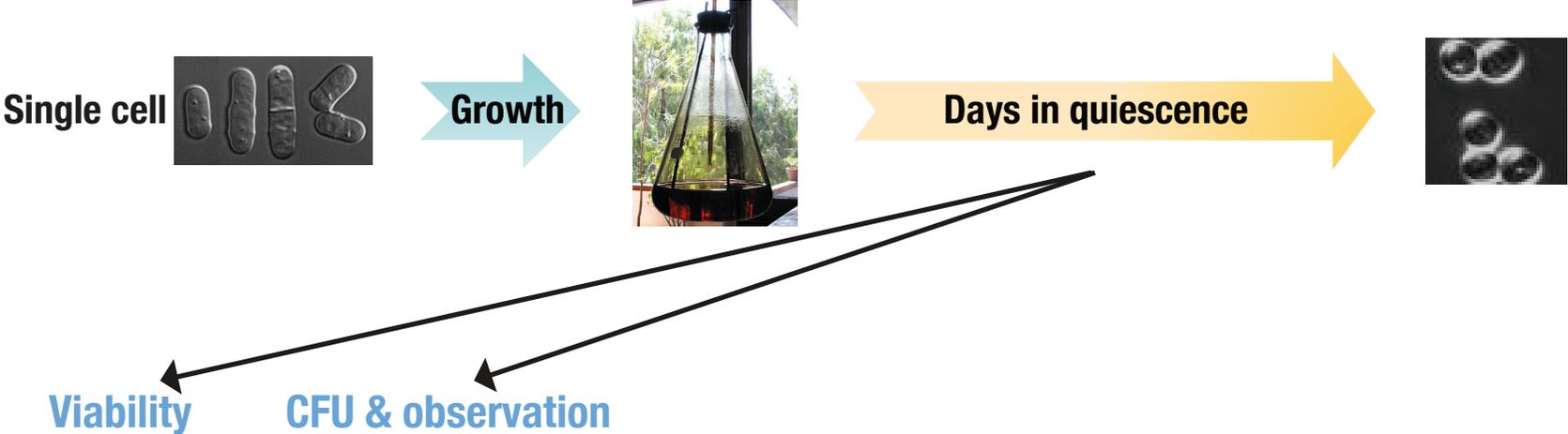


Viability

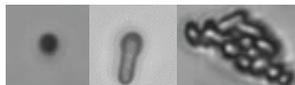
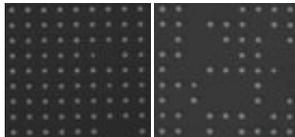


% of viable cells  
in quiescence

# Tools to study quiescence



% of viable cells in quiescence



CFU & cell shape after 3 days of growth

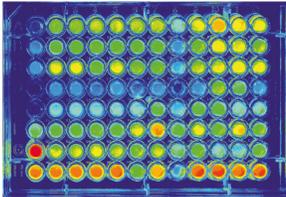
# Tools to study quiescence



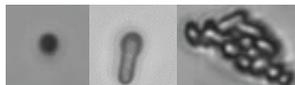
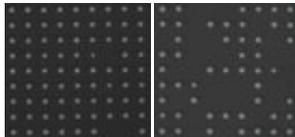
**Viability**

**CFU & observation**

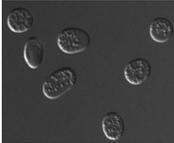
**DNA repair  
Rad52-YFP**



**% of viable cells  
in quiescence**



**CFU & cell shape after  
3 days of growth**



**Unpaired  
lesions**

# Tools to study quiescence

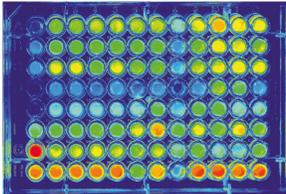


**Viability**

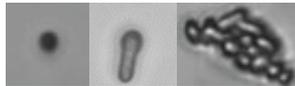
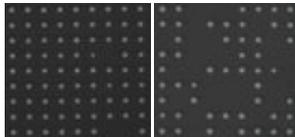
**CFU & observation**

**DNA repair  
Rad52-YFP**

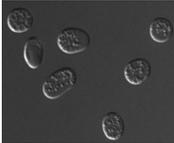
**DNA content  
FACS**



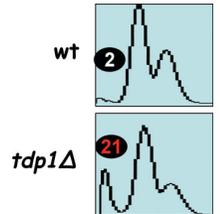
**% of viable cells  
in quiescence**



**CFU & cell shape after  
3 days of growth**

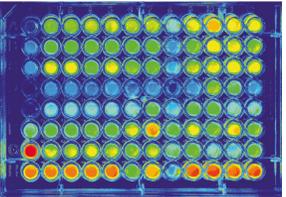
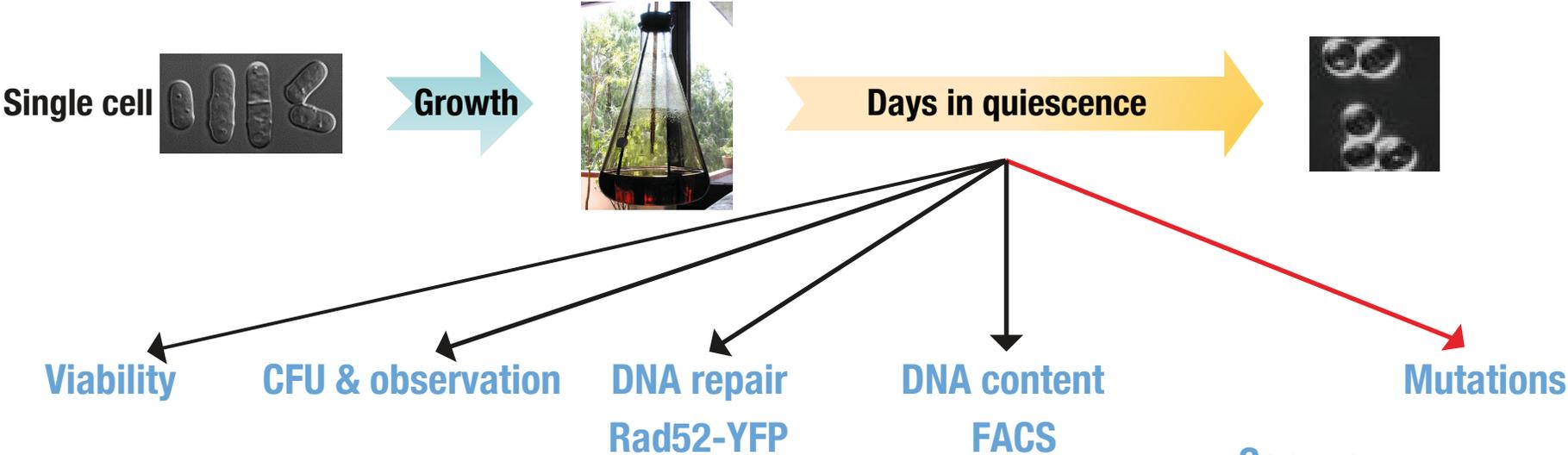


**Unrepaired  
lesions**

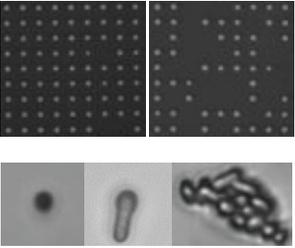


**Anucleated  
cells**

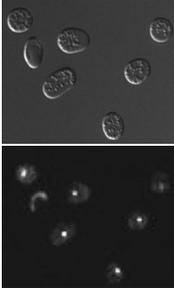
# Tools to study quiescence



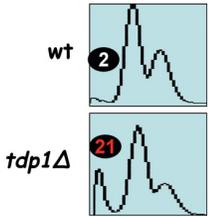
% of viable cells in quiescence



CFU & cell shape after 3 days of growth

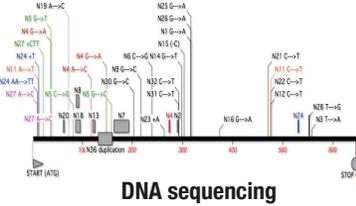
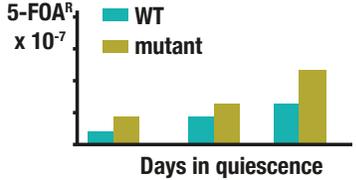


Unpaired lesions



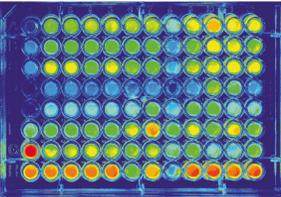
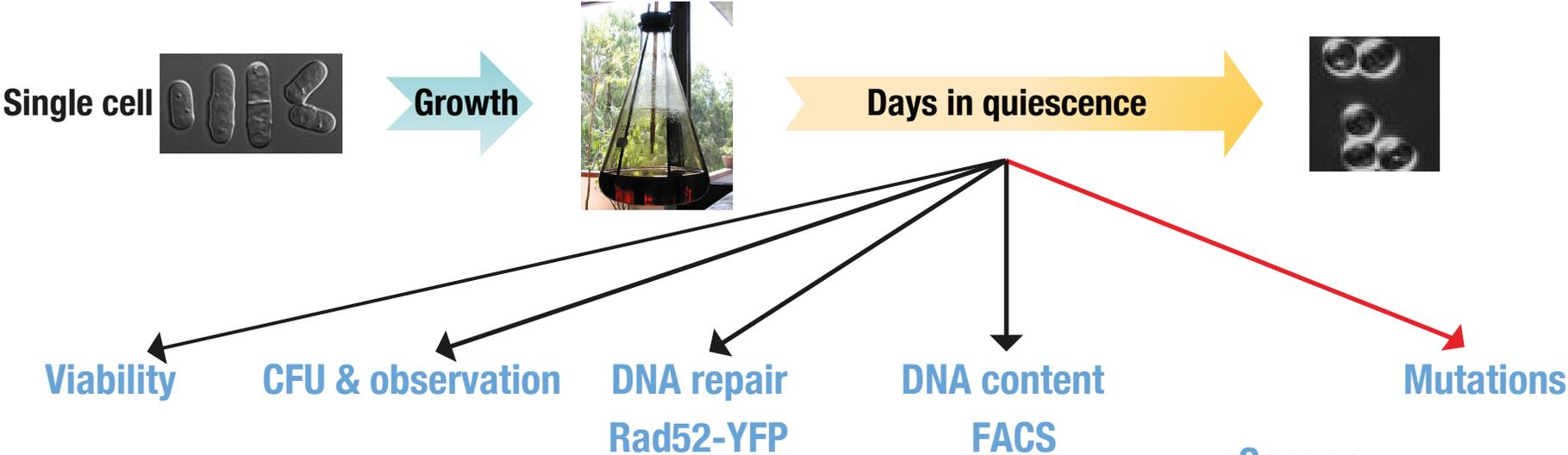
Anucleated cells

## Sanger

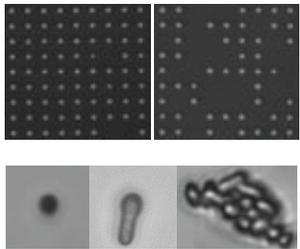


Mutation rate  $f(\text{time})$   
Mutational spectrum

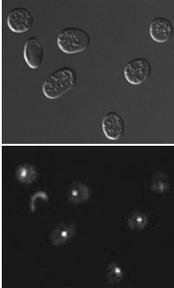
# Tools to study quiescence



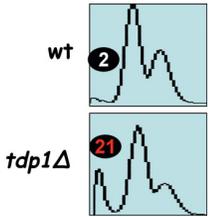
**% of viable cells in quiescence**



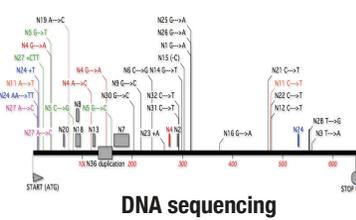
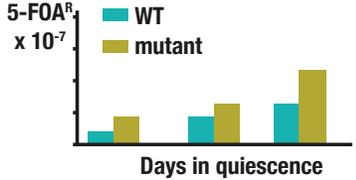
**CFU & cell shape after 3 days of growth**



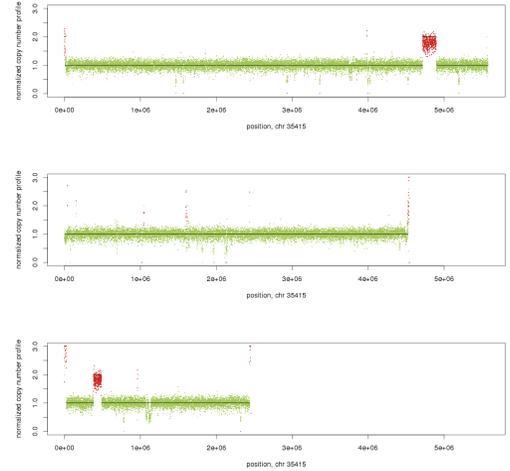
**Unrepaired lesions**



**Anucleated cells**



**Mutation rate f(time)  
Mutational spectrum**

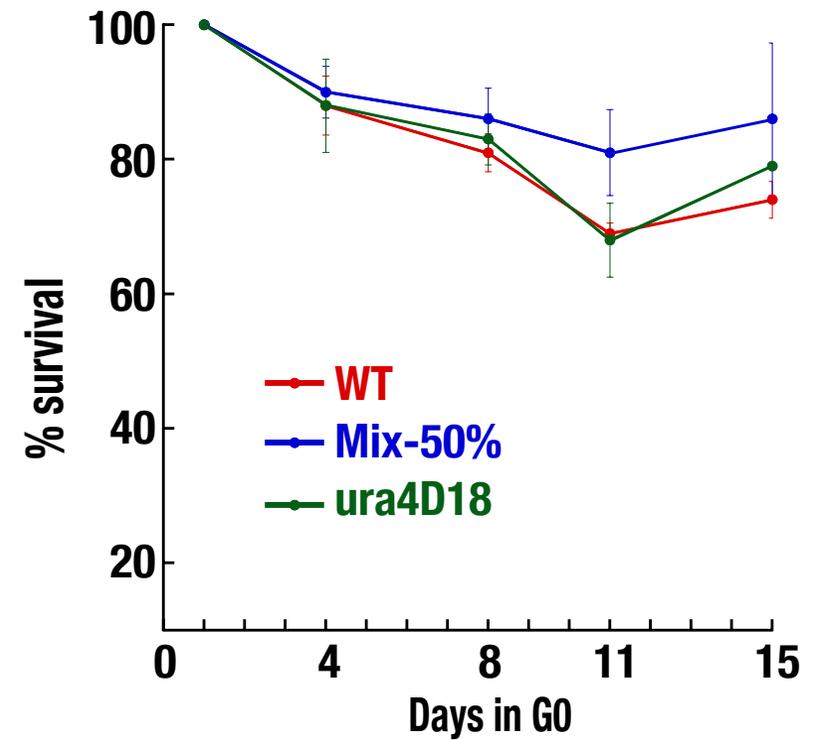
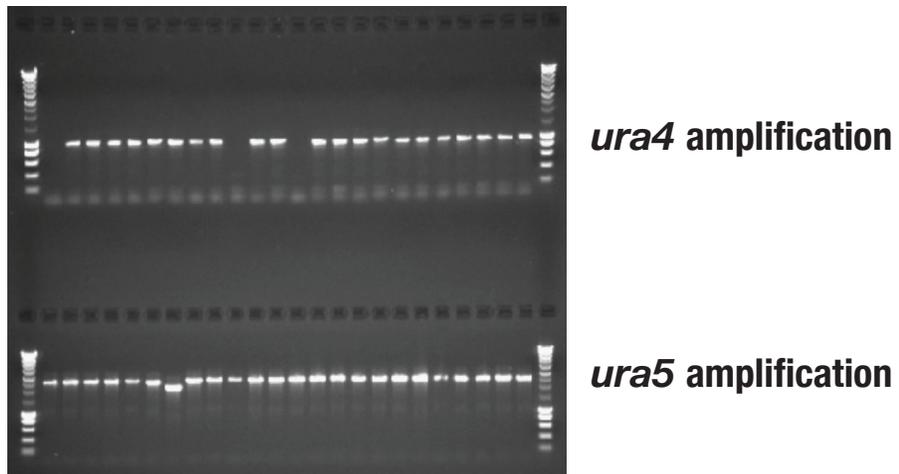


**Mutation rate f(time)  
Mutational spectrum**

# **Prerequisite to the determination of mutations in non-dividing cells**

- **Ura mutants should not die during the 2 weeks of the experiment**
- **Growth mutants should be kept as low as possible**
- **All the mutants in the culture must be sequenced**

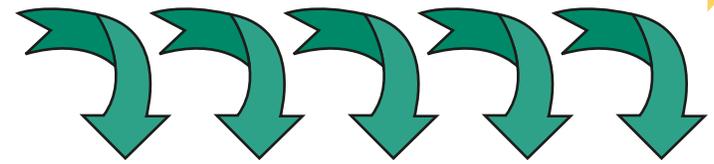
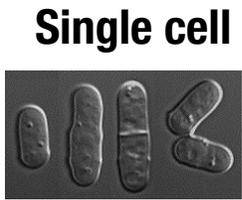
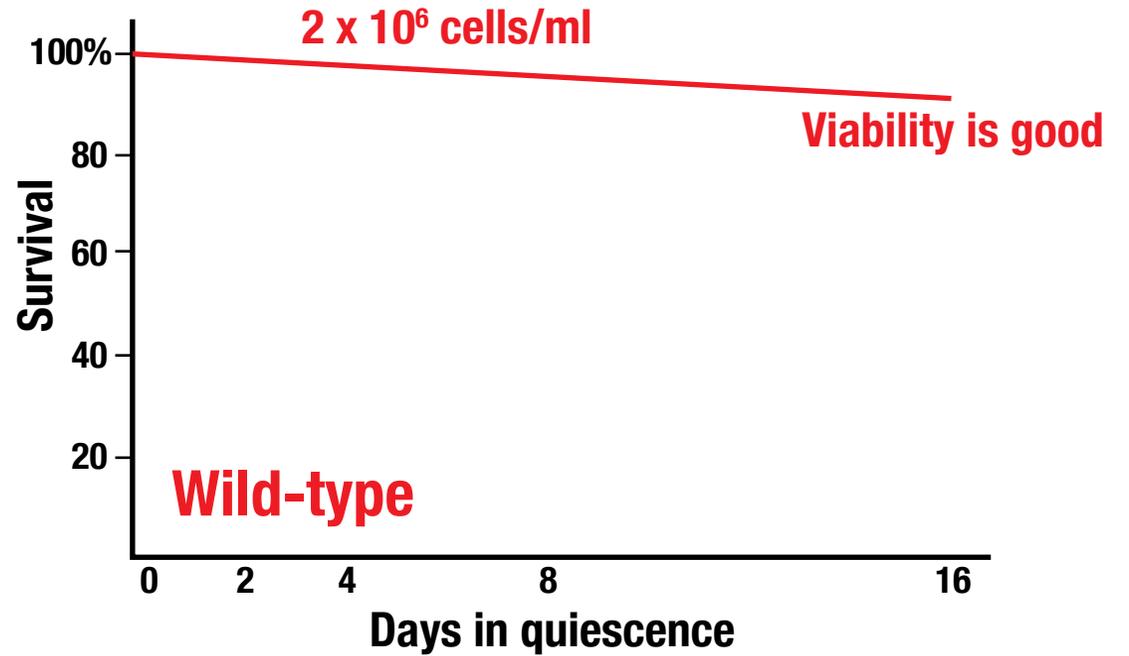
# ura- cells have a similar survival rate in quiescence



# Spontaneous mutations in quiescence

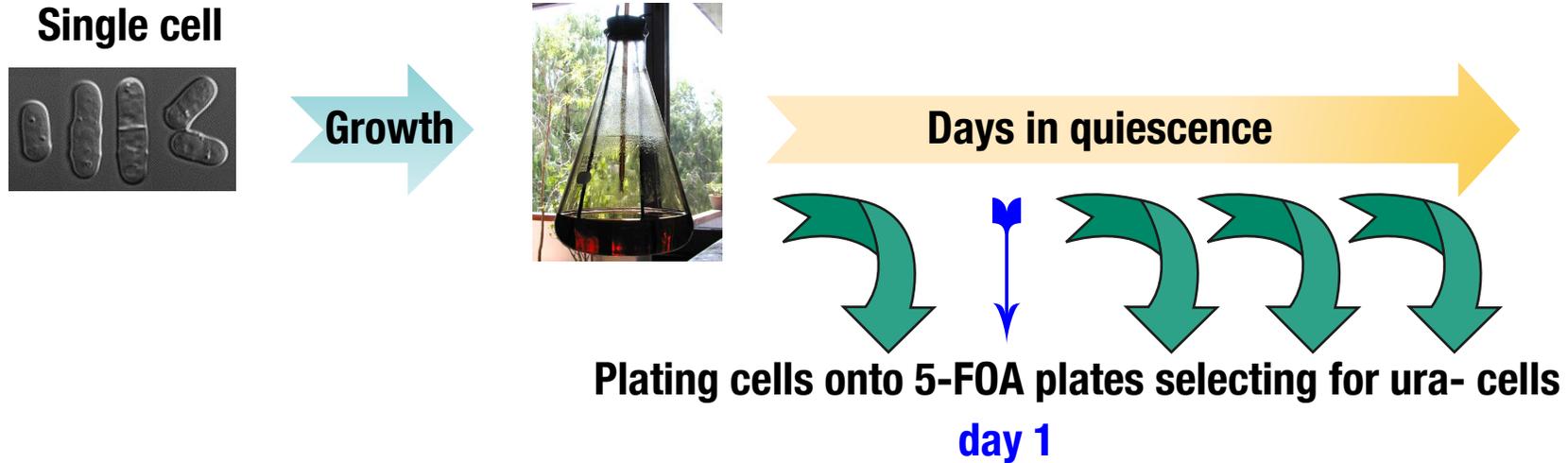
**Cells are metabolically active**  
Transcription, translation, etc.

**Refreshing the medium every week**  
Glucose, trace elements, etc.



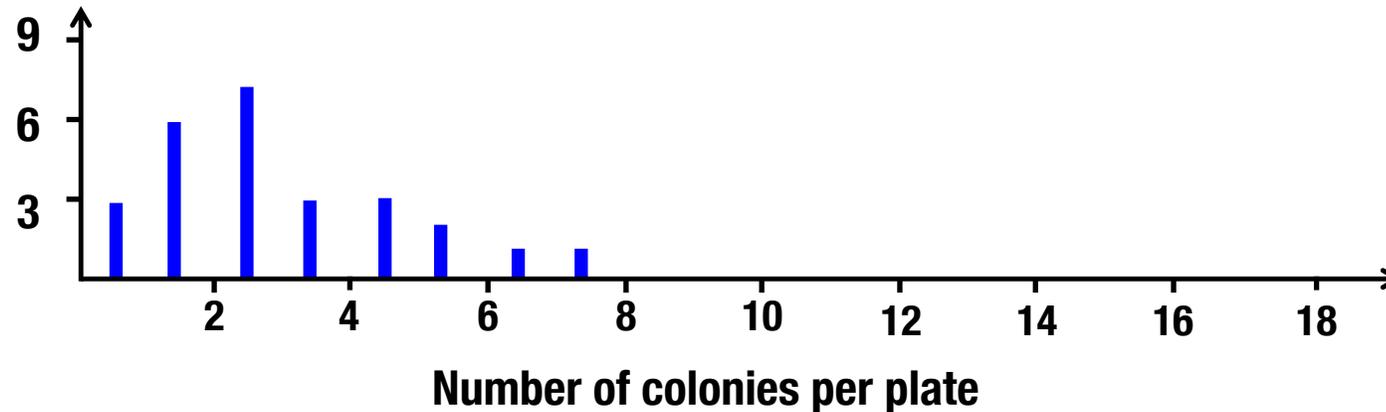
Plating cells onto 5-FOA plates selecting for ura<sup>-</sup> cells

# Spontaneous mutations in quiescence

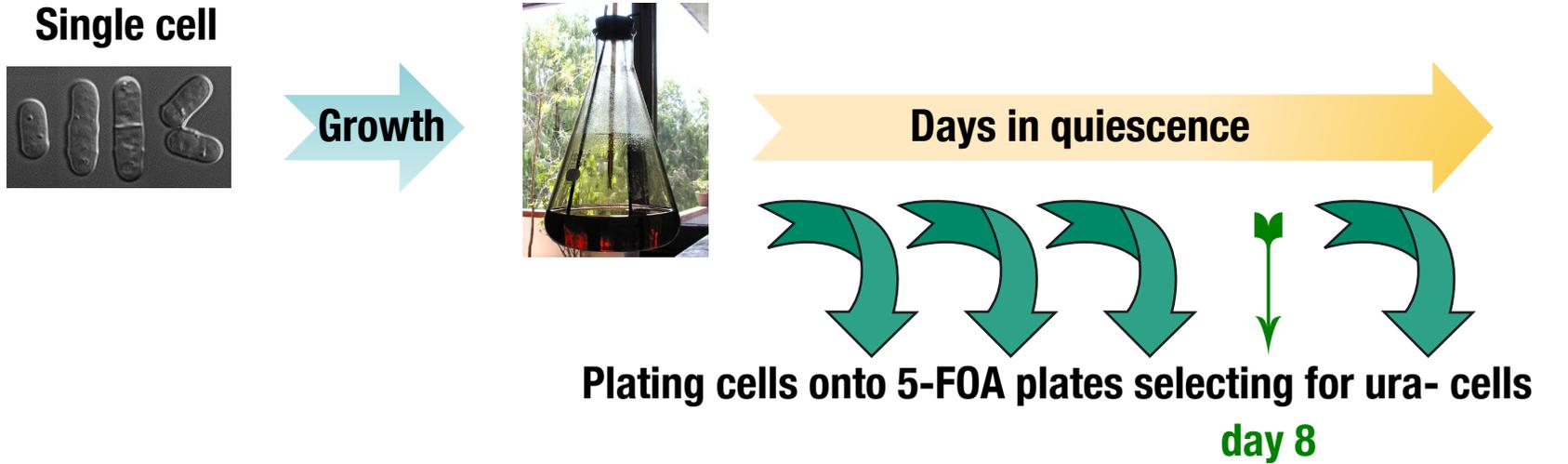


$10^7$  cells on 5-FOA plates that  
select for  $ura4^-$  and  $ura5^-$  mutants

Number of plates

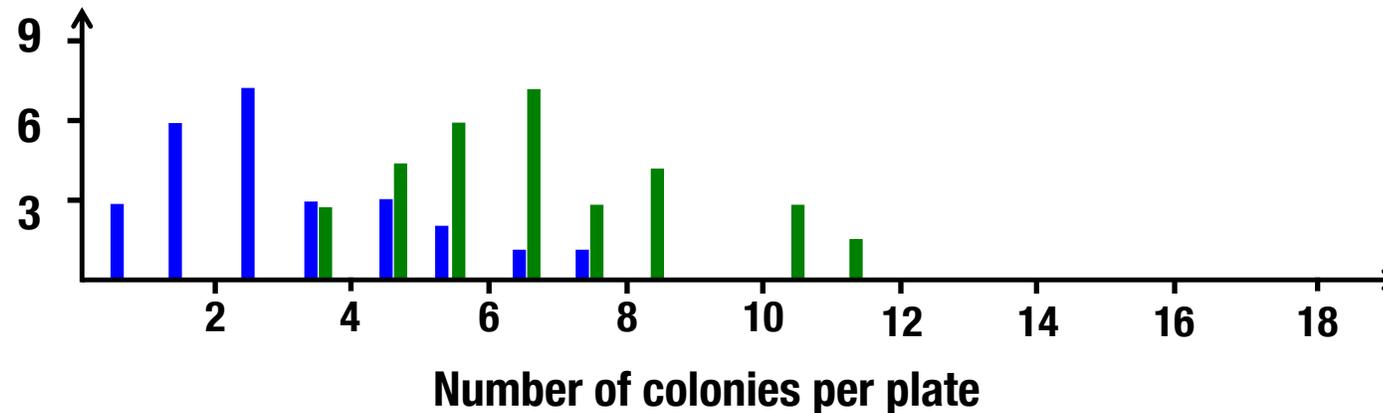


# Spontaneous mutations in quiescence

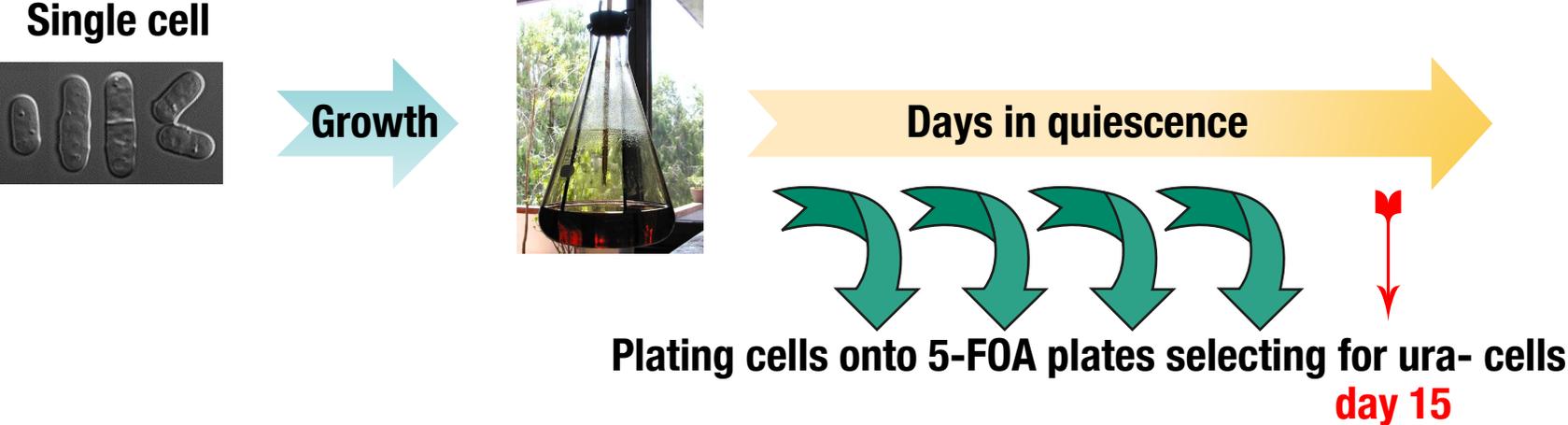


**$10^7$  cells on 5-FOA plates that select for  $ura4^-$  and  $ura5^-$  mutants**

Number of plates

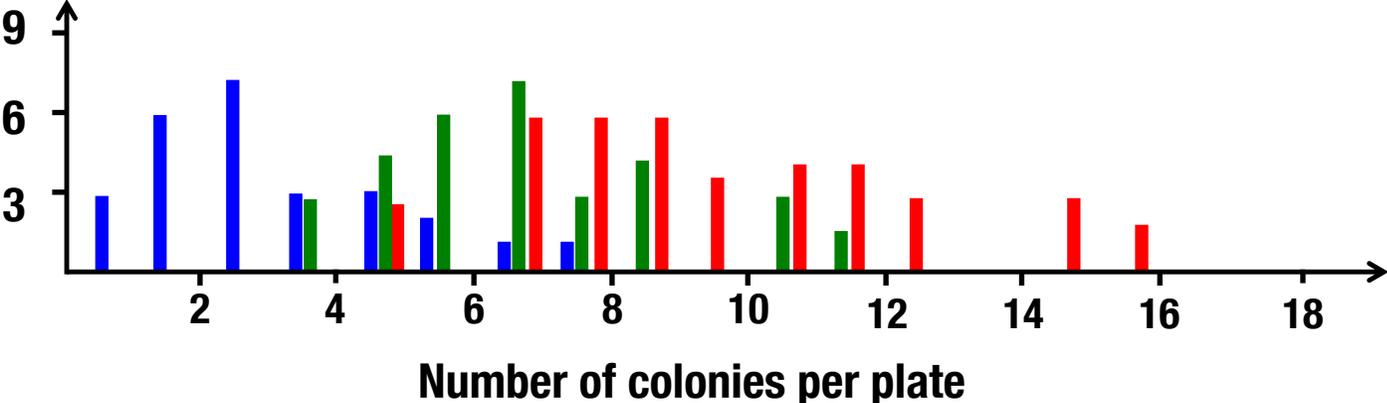


# Spontaneous mutations in quiescence



**10<sup>7</sup> cells on 5-FOA plates that select for ura<sup>4-</sup> and ura<sup>5-</sup> mutants**

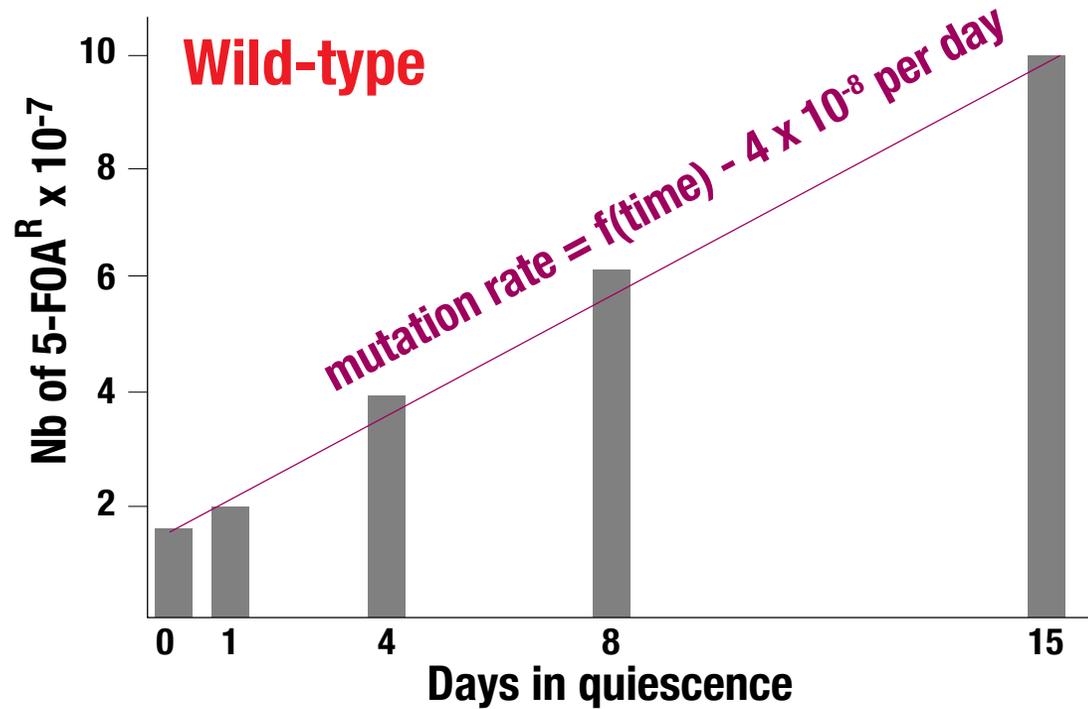
Number of plates



# Spontaneous mutations in quiescence

Spontaneous 5-FOA<sup>R</sup>  
ura mutants

$1.3 \times 10^{-8}$  5-FOA<sup>R</sup>  
per generation

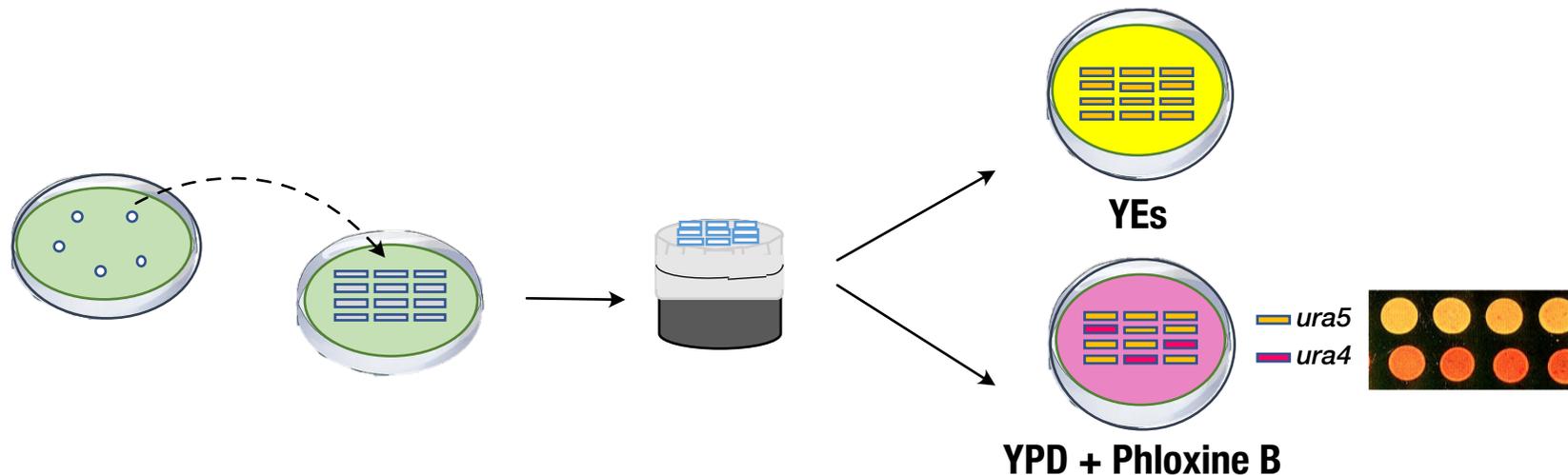


Mutations accumulate as a function of time (constant rate)  
1 replication round  $\approx 10^{-7}$  5-FOA<sup>R</sup>  $\approx$  3 days of quiescence

5-FOA<sup>R</sup> → DNA → PCR → Sequencing

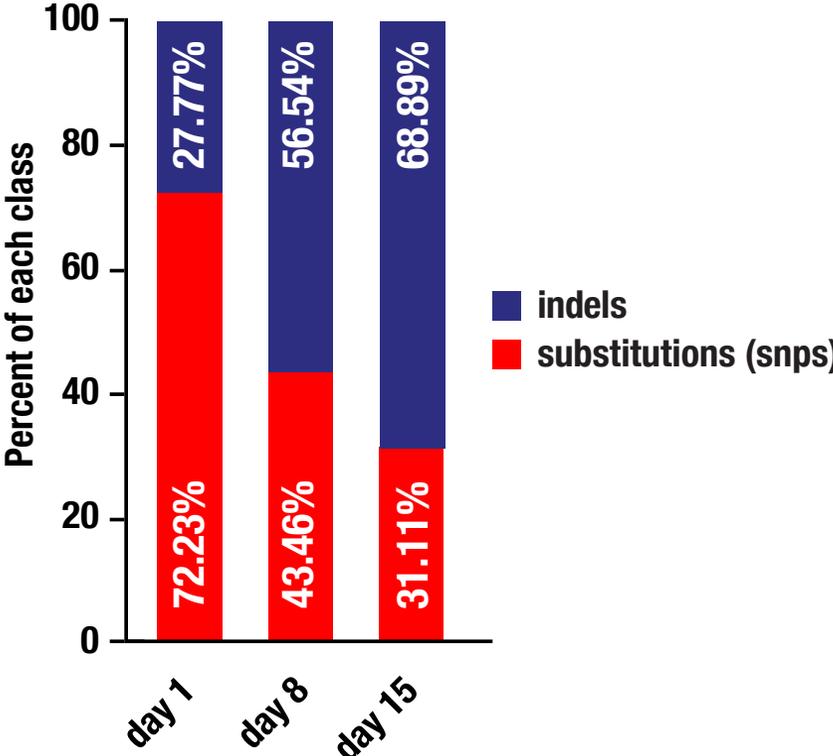
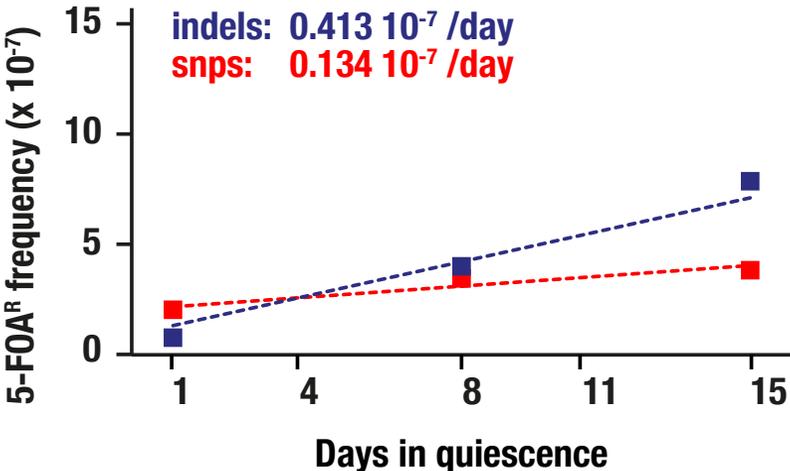
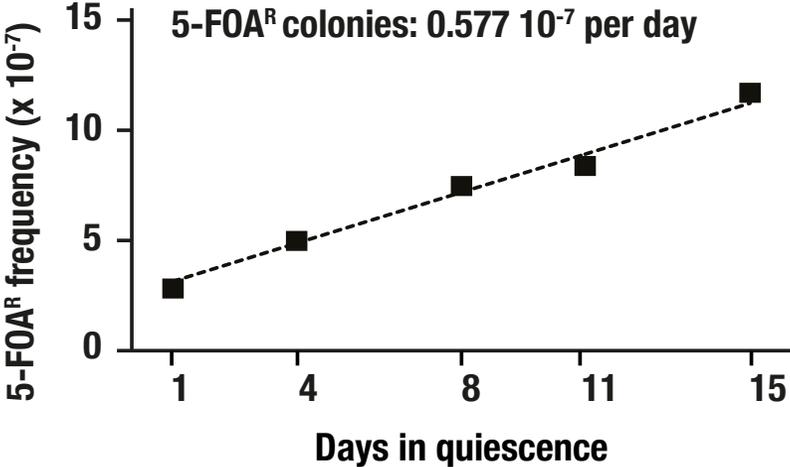
# Identification of the *ura* gene mutated

- Complementation test with a strain of the opposite mating type mutated in one or the other gene
- Replicate the colonies purified on 5-FOA onto a Phloxine B containing YPD plate

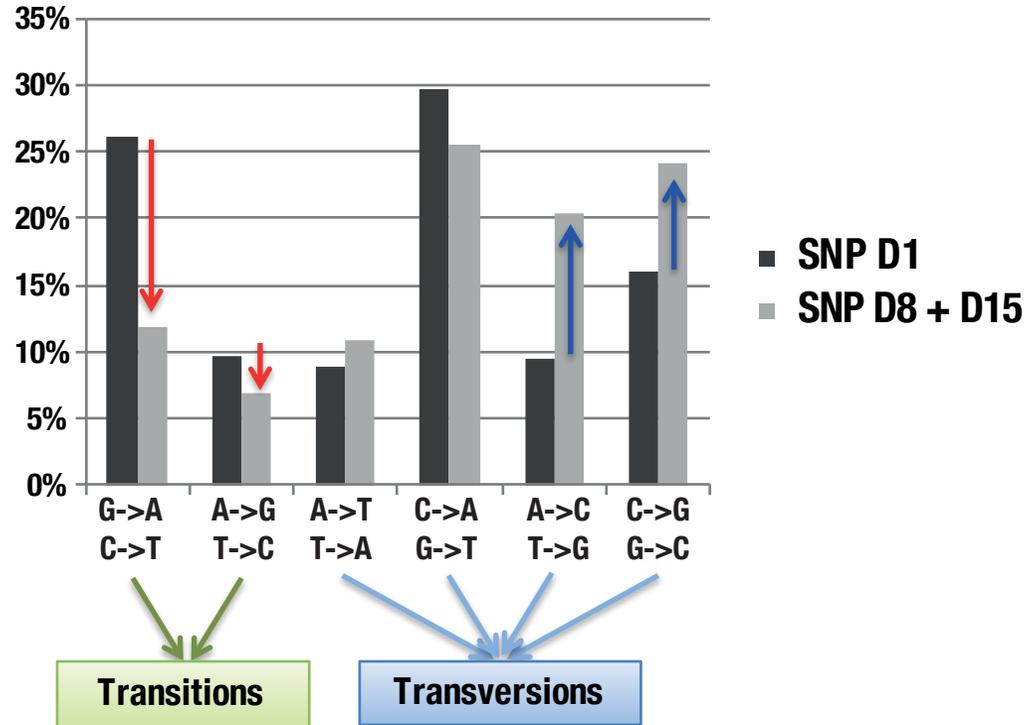


**5-FOA<sup>R</sup> → DNA → PCR → Sequencing**

# 5-FOA<sup>R</sup> cells accumulate as a function of time in G0



# Substitutions in quiescence



Vegetative:  $T_s/T_v = 1/2$

Quiescence:

D1:  $T_s/T_v = 1/2$

D8+D15:  $T_s/T_v = 1/4$

Vegetative: AT bias = 61%

Quiescence:

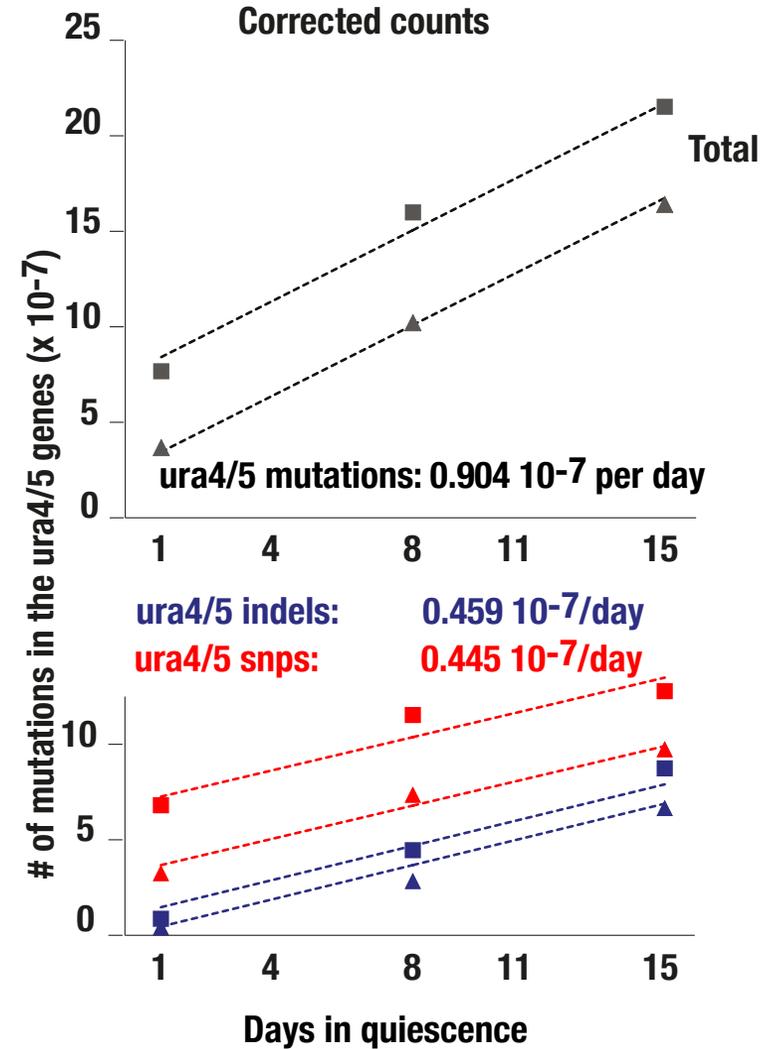
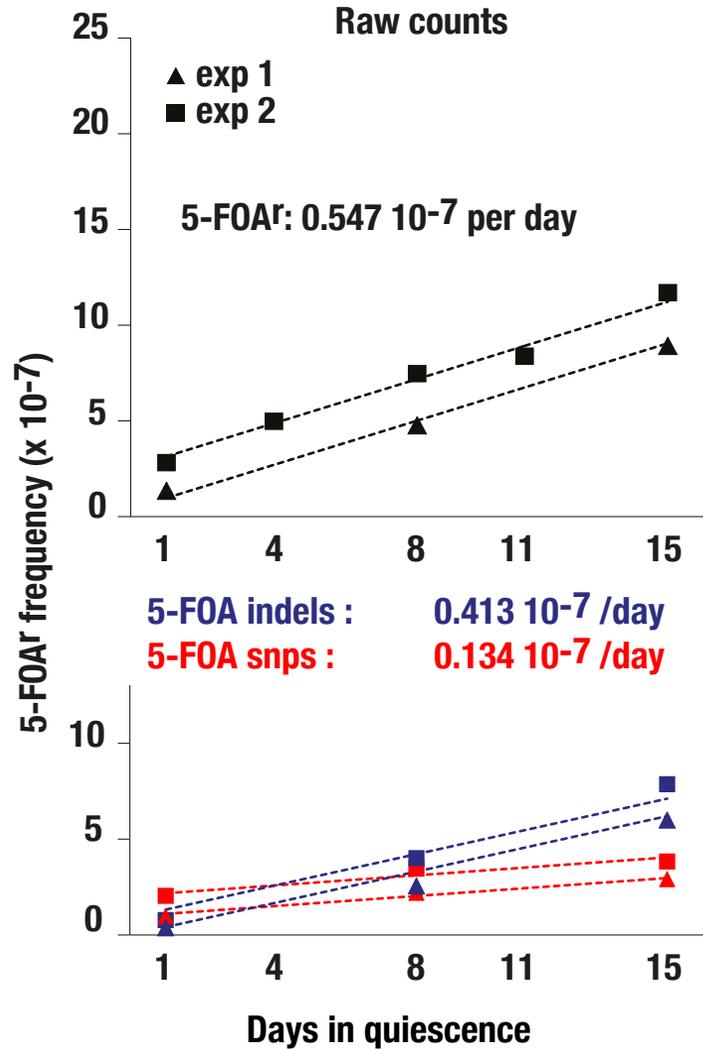
D1: AT bias = 67%

D8+D15: AT bias = 51%

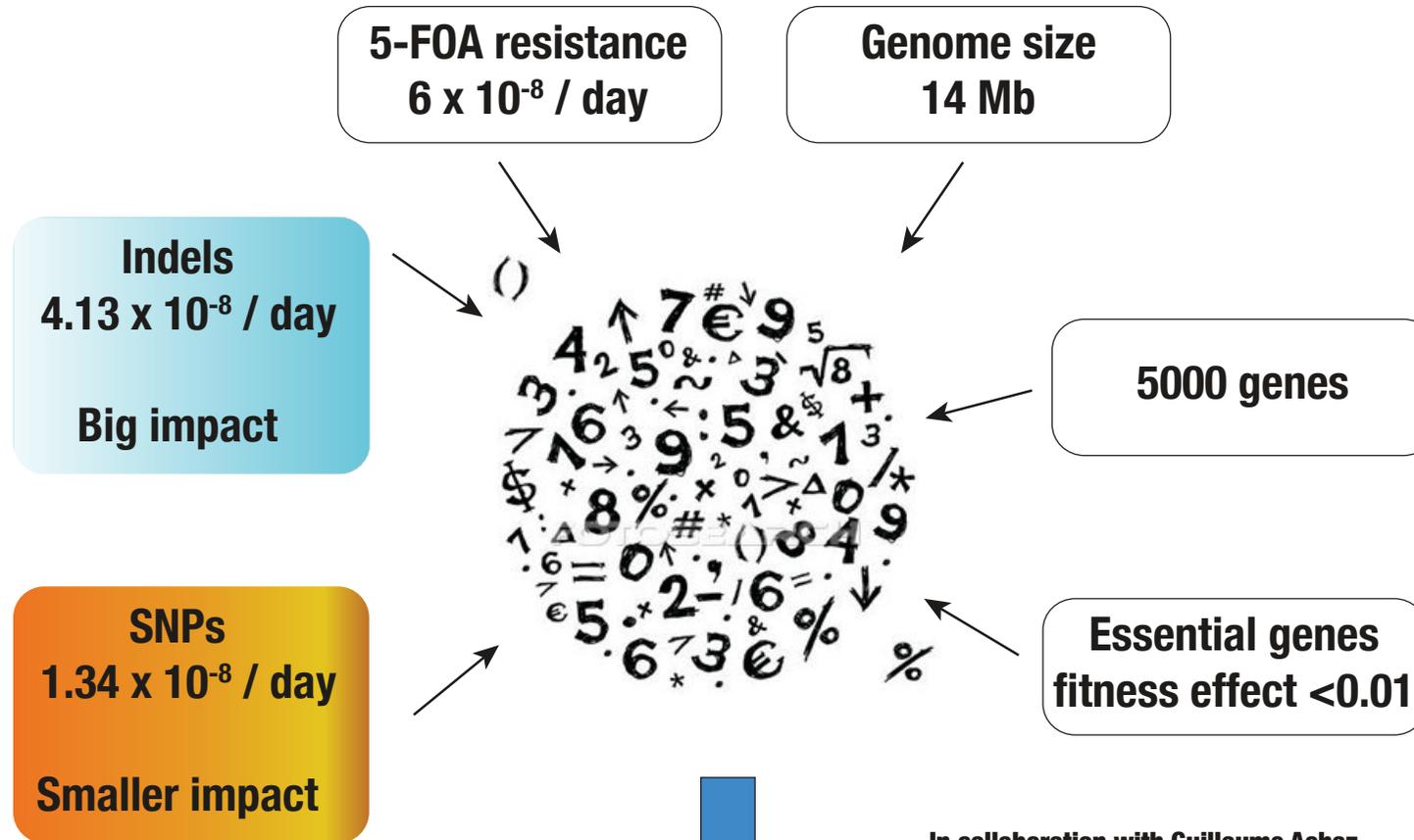
## Suggesting that in quiescence:

- i) cytosine deamination is processed differently (from G:C → A:T to C:G → G:C)
- ii) an increase of dU incorporation during repair (A:T to C:G)
- iii) an increase of the GC content that counter balances the somatic AT bias

# Extrapolation to whole genomes



# Can we extrapolate genome-wide?

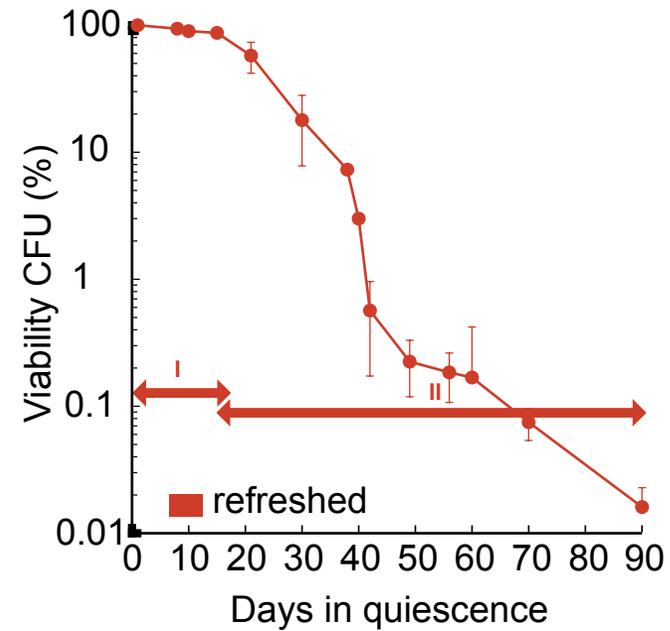


**3 months of quiescence should result in  
 $\approx 1$  mutation / genome**



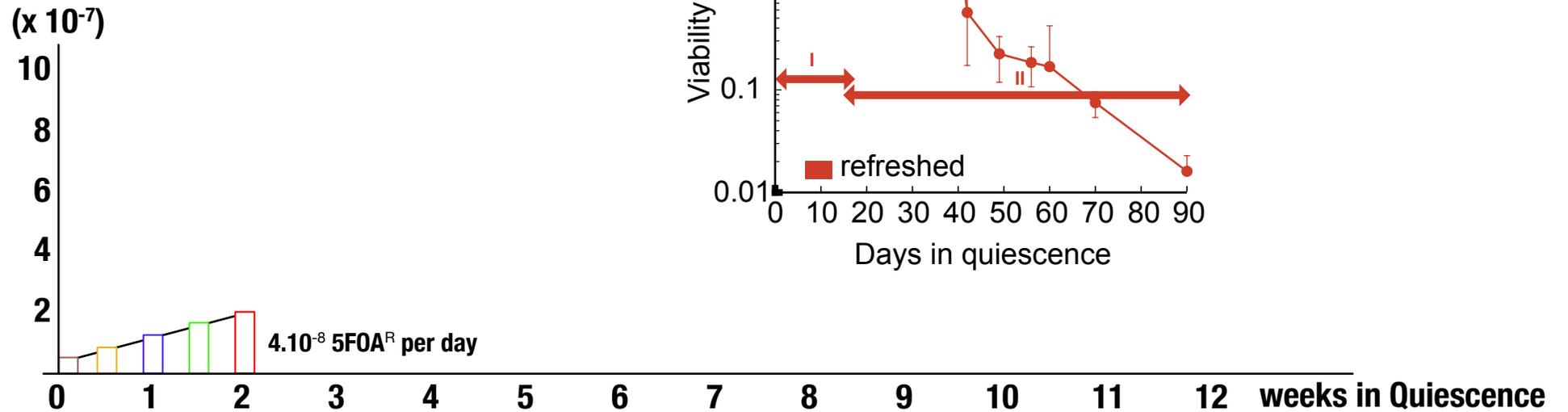
# Accumulation of mutations genome-wide (*S. pombe* genome: 14 Mb)

Refreshed conditions



Spontaneous 5FOA<sup>R</sup> mutants

$1.3 \times 10^{-8}$  5FOA<sup>R</sup> per generation





# Accumulation of mutations genome-wide (*S. pombe* genome: 14 Mb)

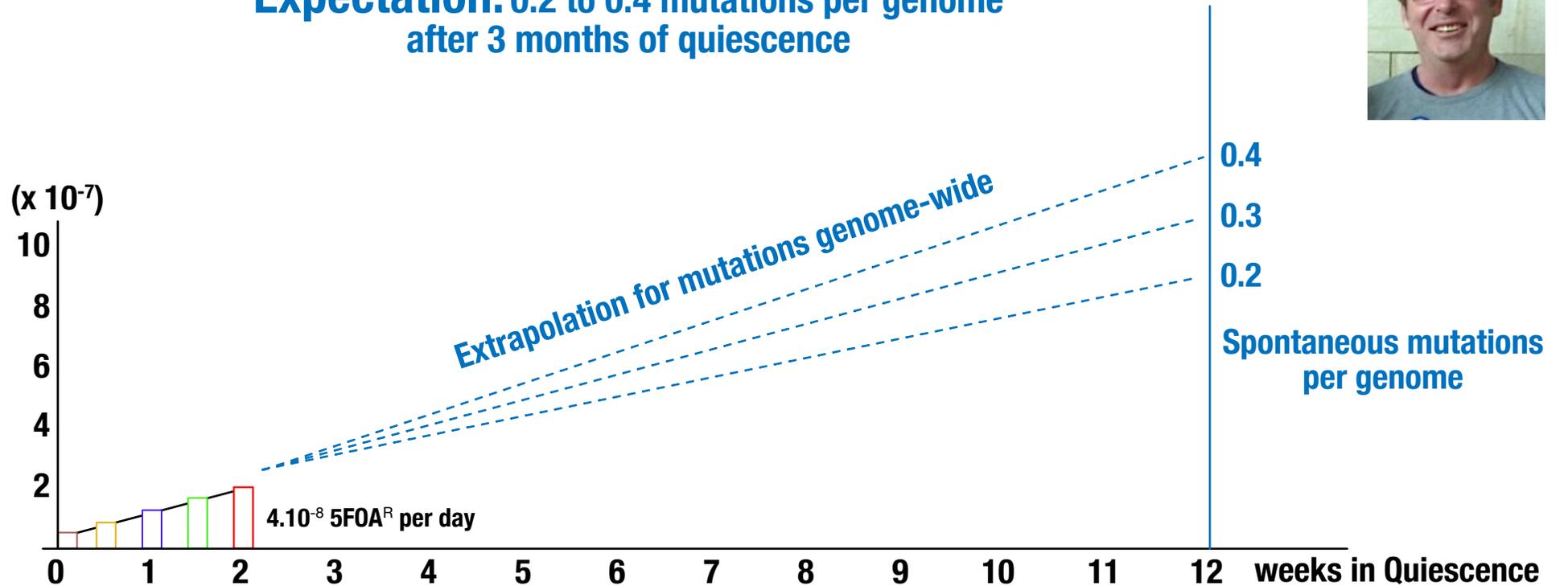


Refreshed conditions

Expectation: 0.2 to 0.4 mutations per genome  
after 3 months of quiescence

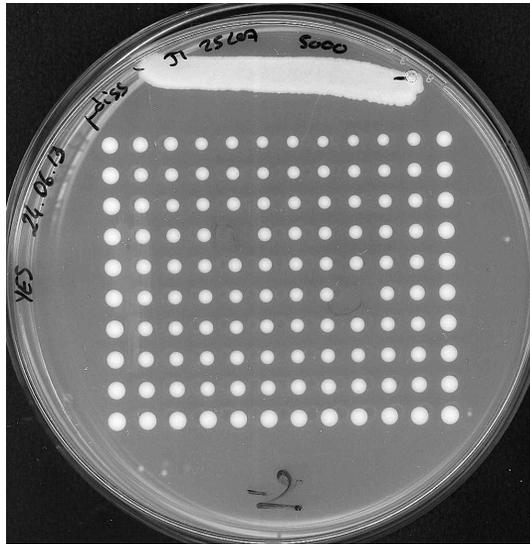
Spontaneous 5FOA<sup>R</sup>  
mutants

$1.3 \times 10^{-8}$  5FOA<sup>R</sup>  
per generation



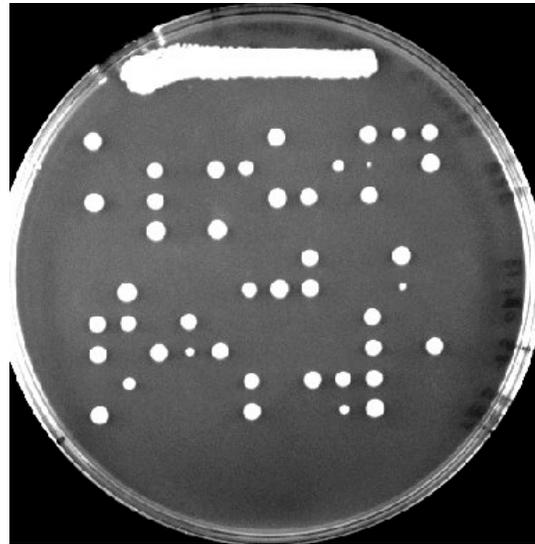
# Long term quiescence (up to 3 months)

1 day



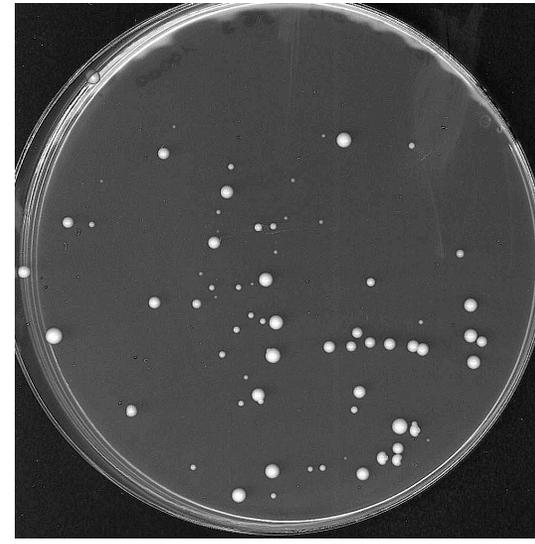
≈ 100%

21 days



≈ 40%

3 months



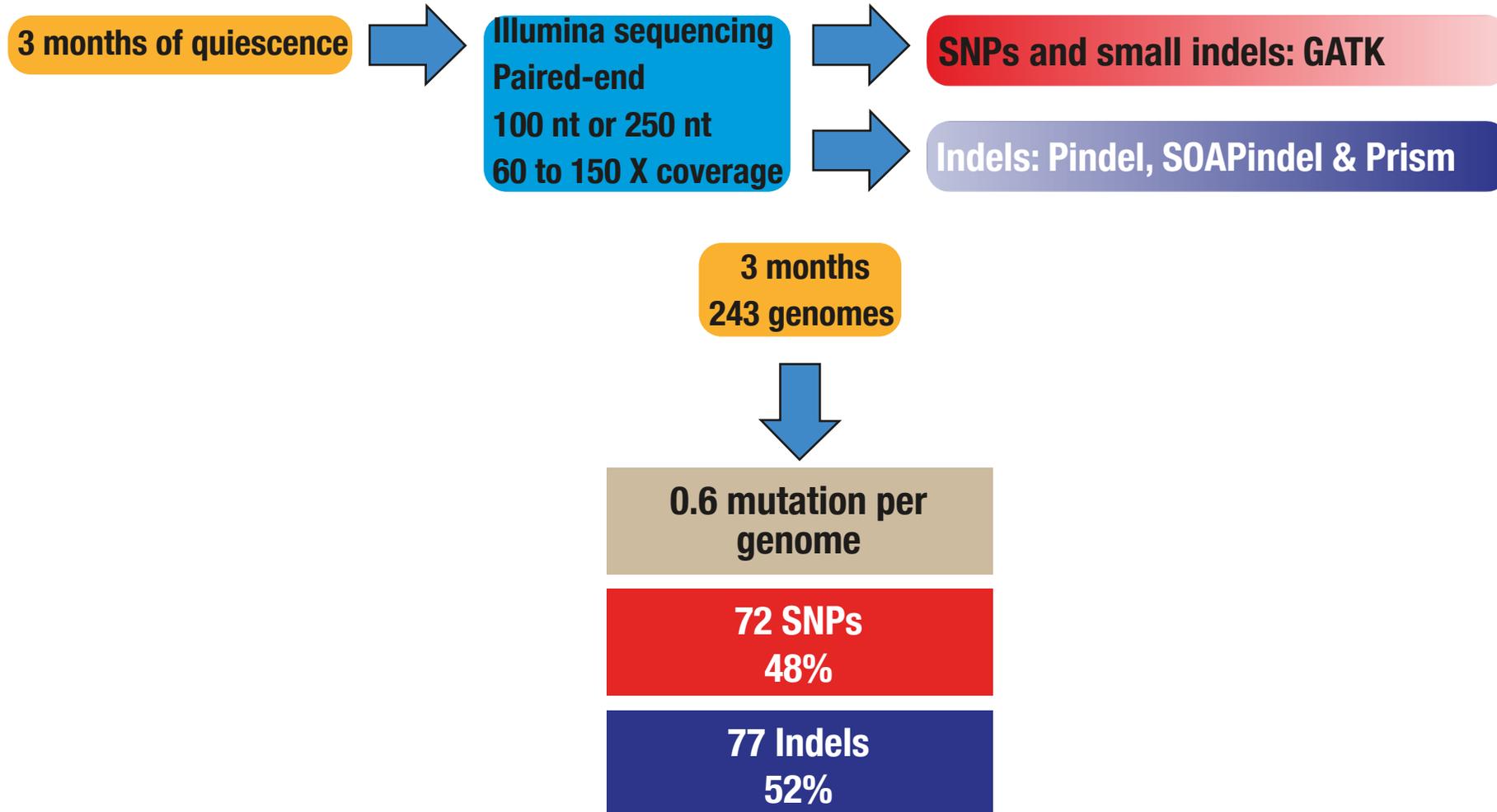
< 1%

**Colony size heterogeneity  
suggesting the presence of  
mutations**

# Phenotypic alterations as a function of time spent in quiescence

time in G0	# of colonies w/ phenotype	18°C	37°C	KCl 1M	Ca(NO <sub>3</sub> ) <sub>2</sub> 0.15M	CaCl <sub>2</sub> 0.3M	TBZ 15 µg/ml	HU 4 mM	SDS 0.01%
1 day	0/384	-	-	-	-	-	-	-	-
1 month	0/384	-	-	-	-	-	-	-	-
2 months	4/376	-	-	1	1	4	-	-	-
3 months	6/334	1	1	1	1	3	3	2	1

# Genome-wide analysis of quiescence



**False dicoverly rate (FDR) SNPs < 5%, Indels ≈ 20% (Validated by Sanger-sequencing)**

# Accumulation of mutations genome-wide (*S. pombe* genome: 14 Mb)

Behringer et al. 2015; Farlow et al. 2015

## Growth

1700 generations

96 genomes sequenced

6 mutations per genome  
( $2.10^{-10}$  mutation per generation per nucleotide)

422 SNPs  
78%

119 indels  
22%  
+101 bp

Gangloff et al. 2017

## Quiescence

3 months

243 genomes sequenced

0.6 mutations per genome

72 SNPs  
48%

77 indels  
52%  
-215 bp

There are two **antagonistic** mutational forces during growth and quiescence that affect the **size** and the **composition** of the *S. pombe* genome



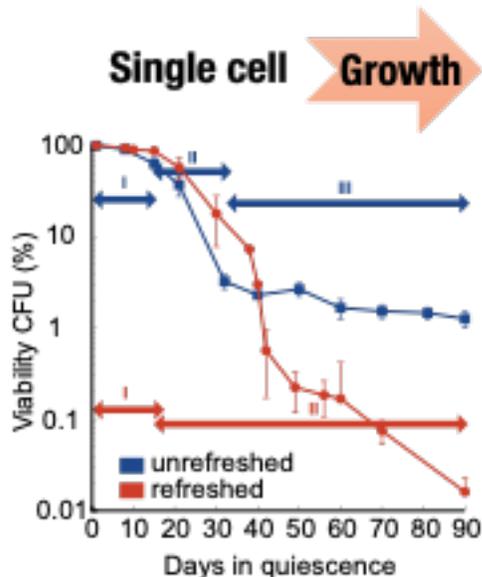
4.10<sup>7</sup> cells



**Unrefreshed conditions**

1% survivors

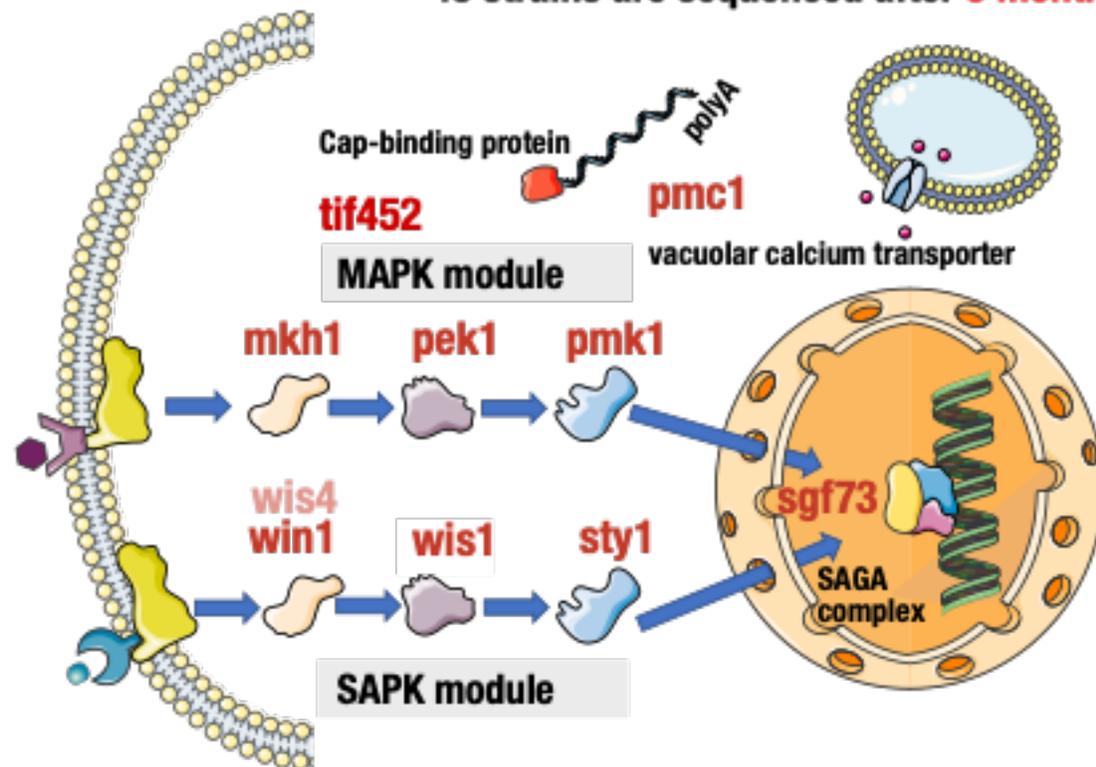
2-3 months of quiescence



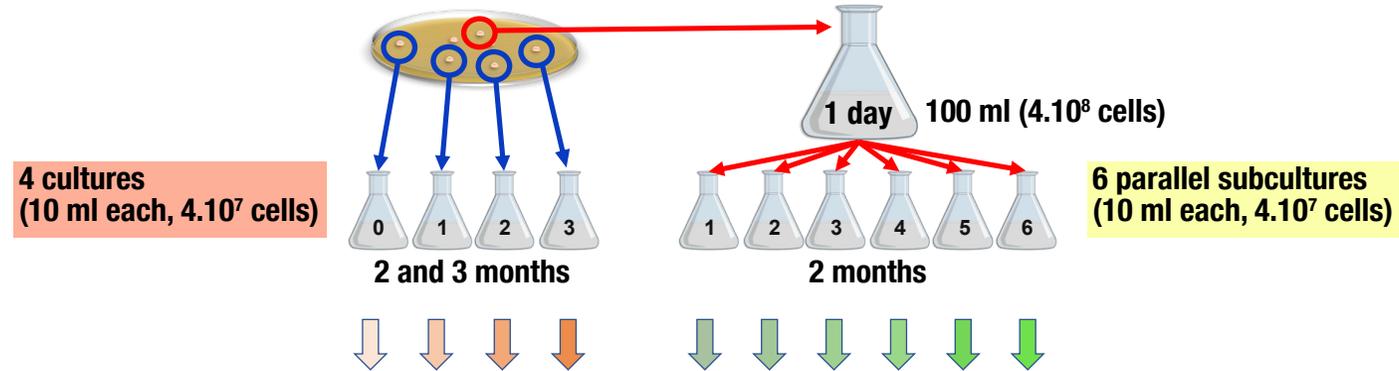
52 mutations (>1 per genome), including 25 mutants in the MAPK and SAPK pathways

Genes	Times found	Function	Human homologues
win1	3	SAPKKK	MAP3K4
wis1	2	SAPKK	MAP2K1
sty1	2	SAPK	p38
mkh1	4	MAPKKK	MEKK1
pek1	1	MAPKK	MEK2
pmk1	2	MAPK	ERK
pmc1	6	Calcium efflux	PMCA
sgf73	4	SAGA subunit	ATXN7
tif452	1	CAP binding	eIF4E

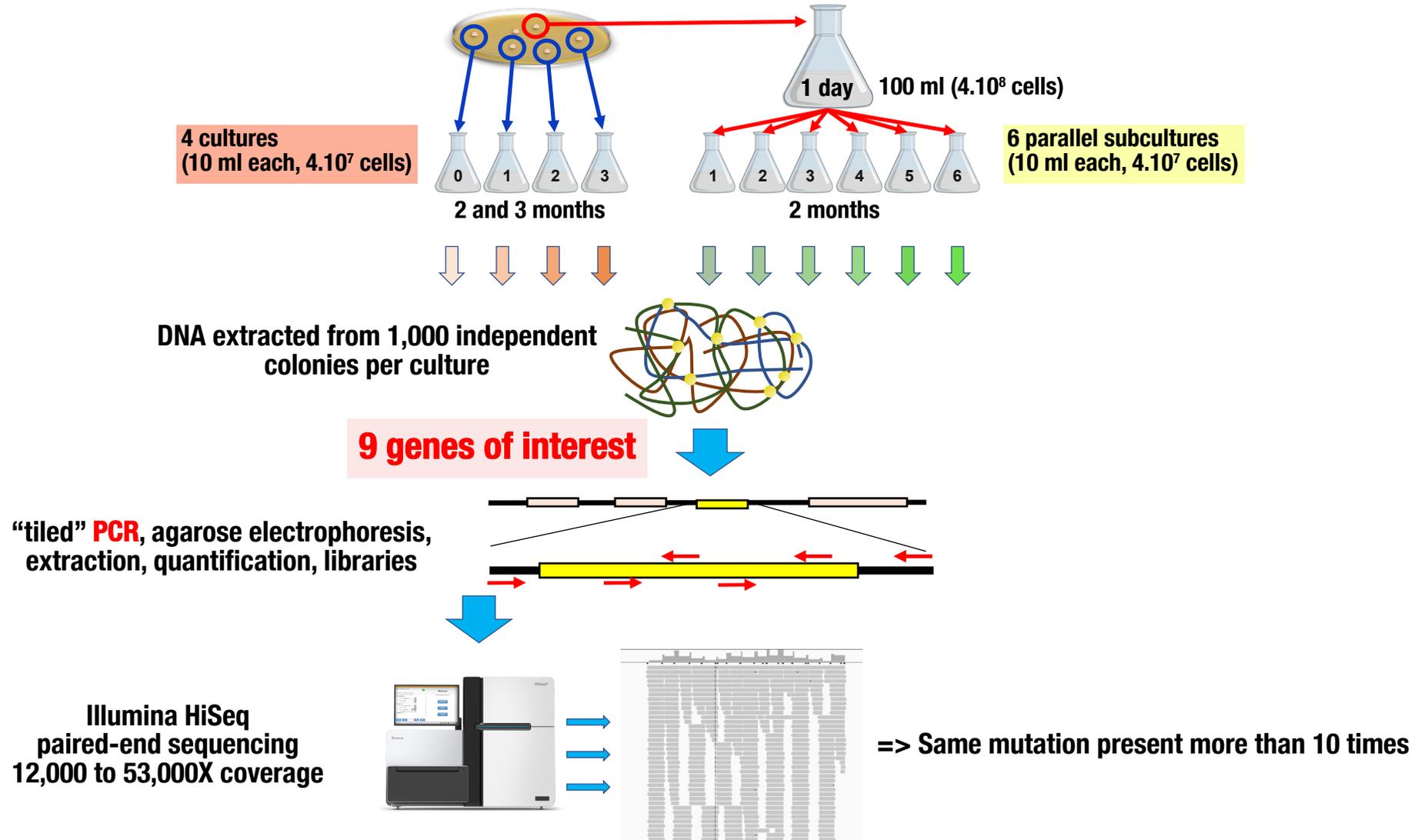
48 strains are sequenced after 3 months



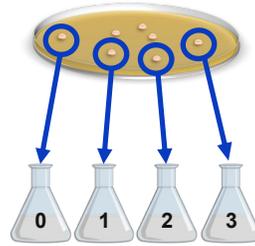
# Distribution of the S/MAPK mutants at the population level: Targeted sequencing approach



# Distribution of the S/MAPK mutants at the population level: Targeted sequencing approach



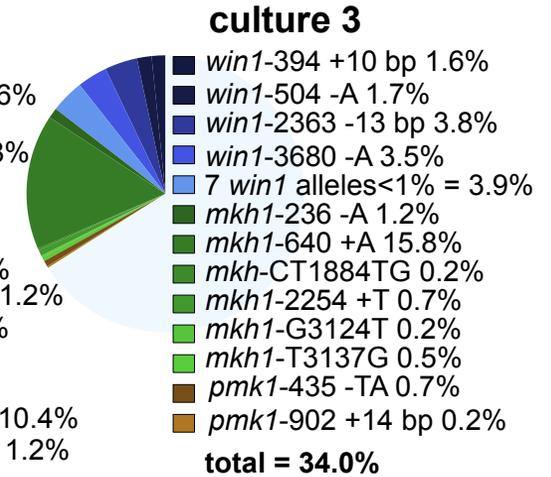
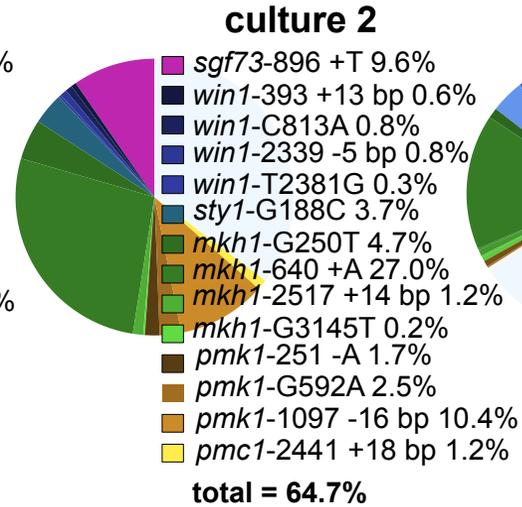
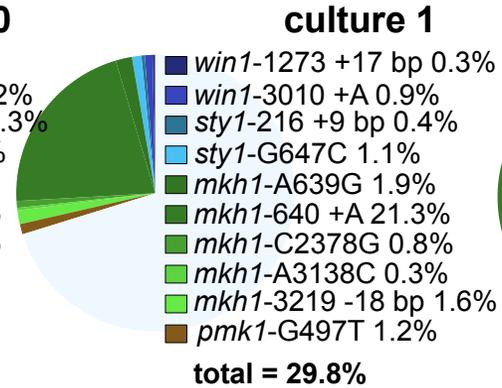
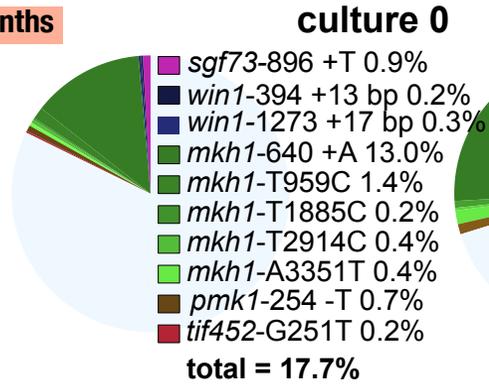
# Targeted sequencing



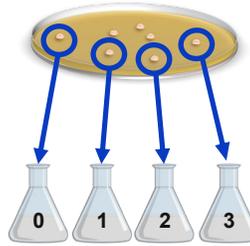
4 clonal cultures

PCR and sequencing

2 months

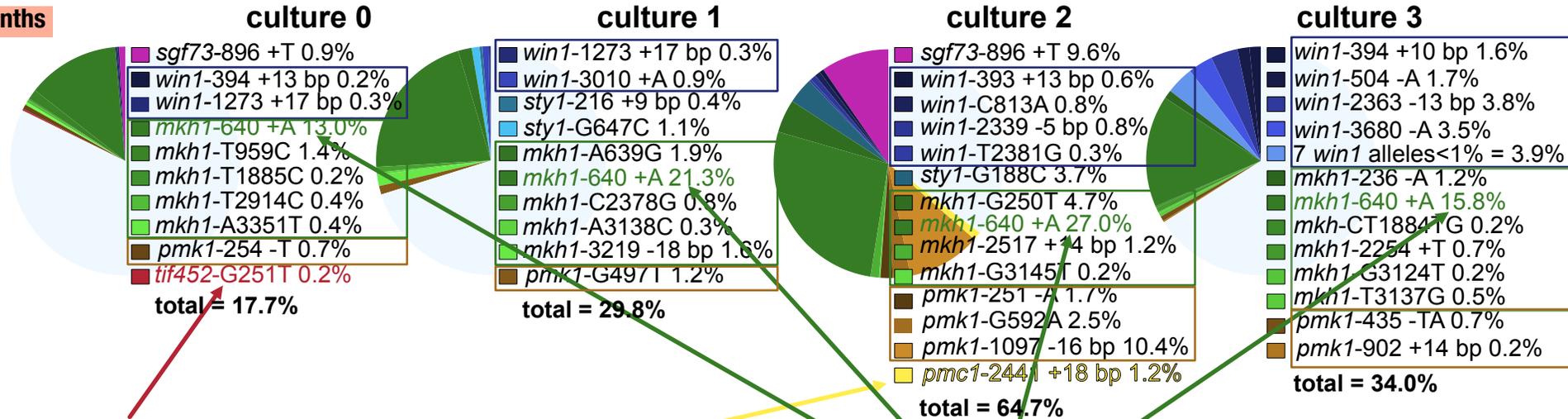


# Targeted sequencing



PCR and sequencing

2 months



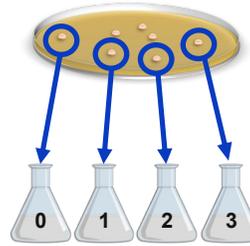
*tif452-G251T* is found only once

*pmc1-2440+18* is found only once

*mkh1-640+A* is an hot-spot of mutation (stretch of 8 As)

*mkh1*, *pmk1* and *win1* are mutated in all cultures

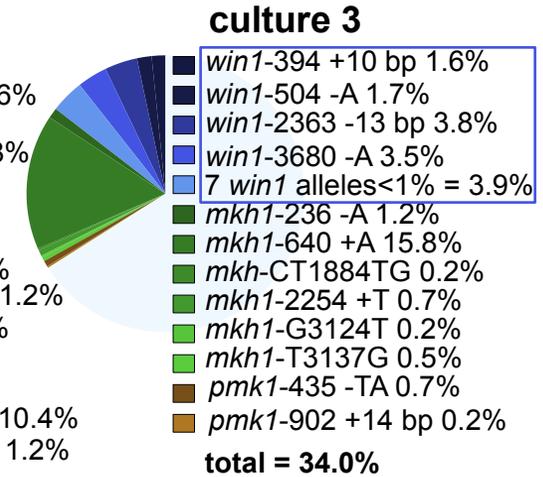
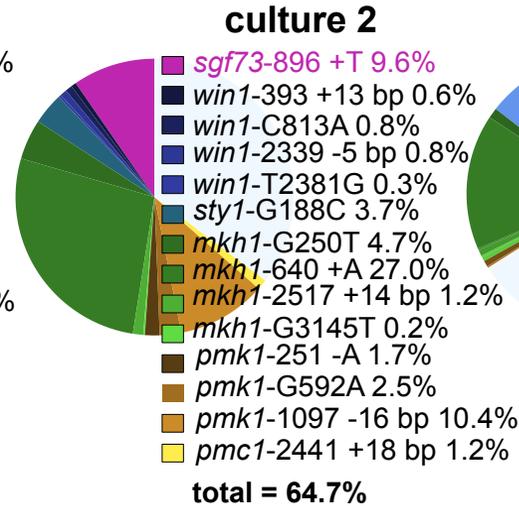
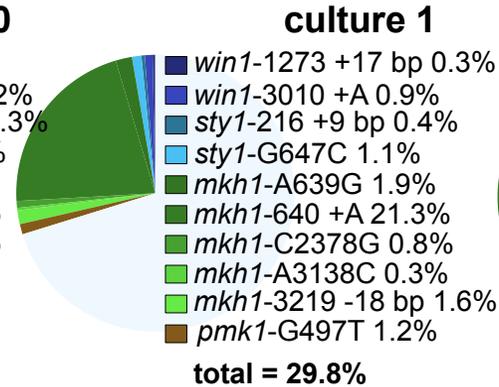
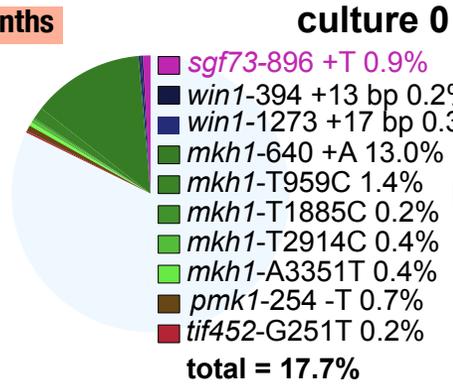
# Targeted sequencing



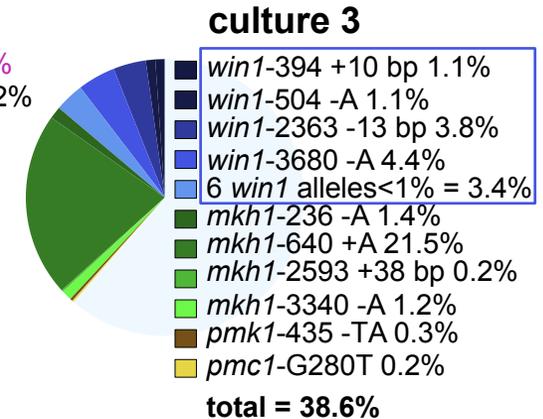
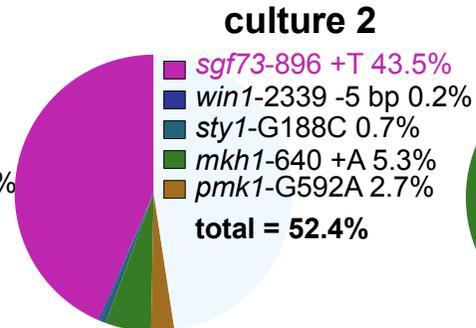
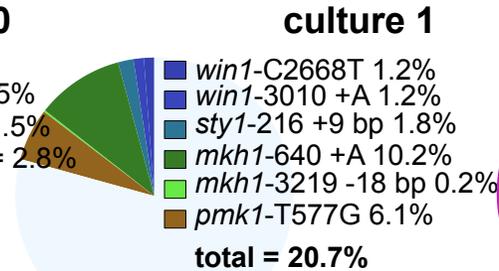
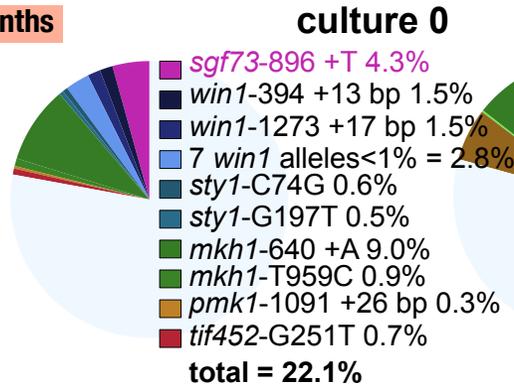
4 clonal cultures

PCR and sequencing

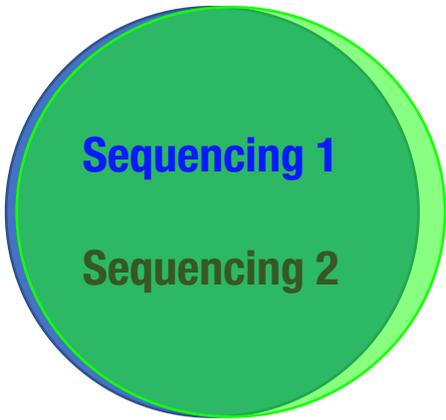
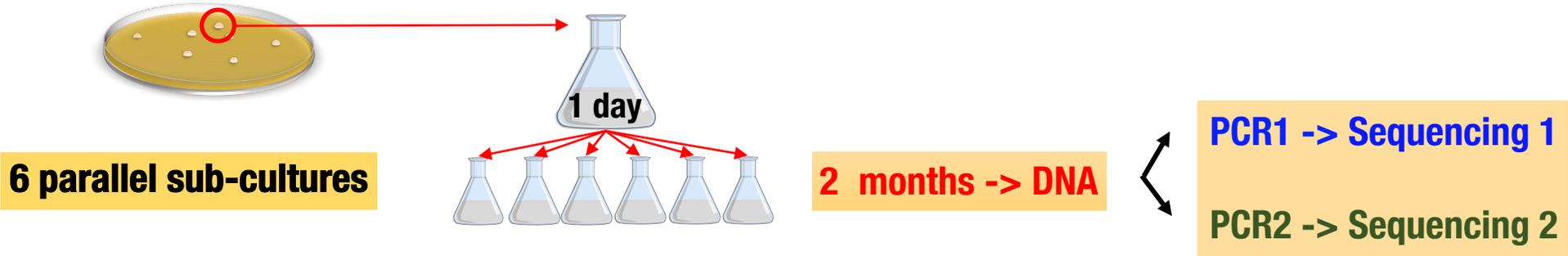
2 months



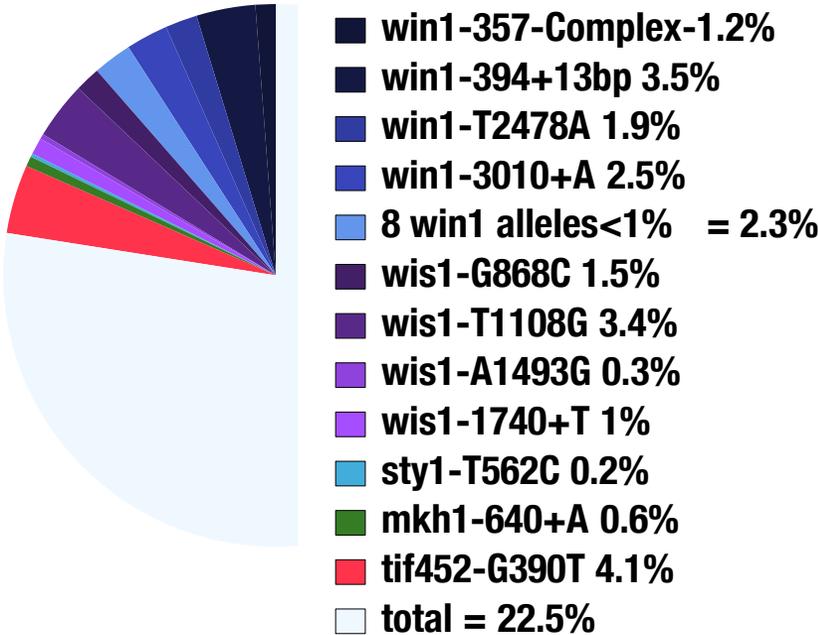
3 months



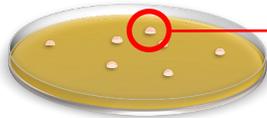
# Targeted sequencing



subculture 1



# Targeted sequencing



1 day



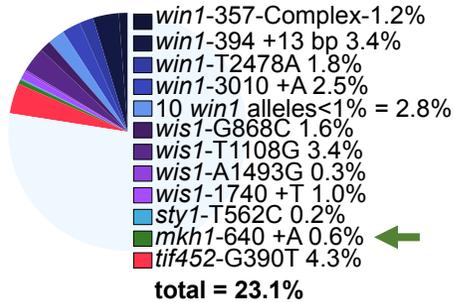
6 parallel sub-cultures

2 months -> DNA

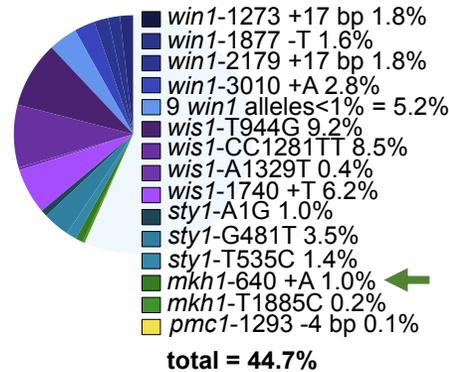
PCR1 -> Sequencing 1

PCR2 -> Sequencing 2

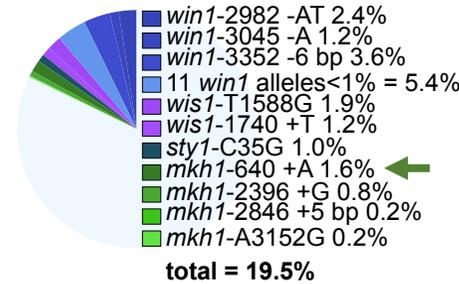
subculture 1



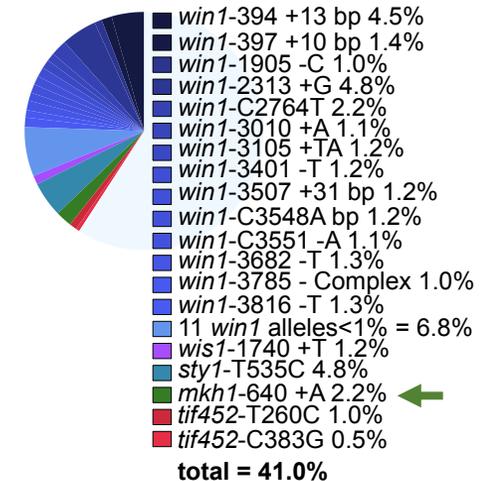
subculture 3



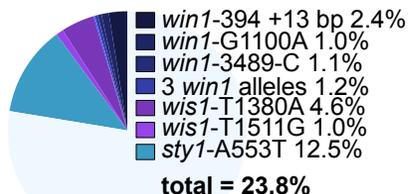
subculture 4



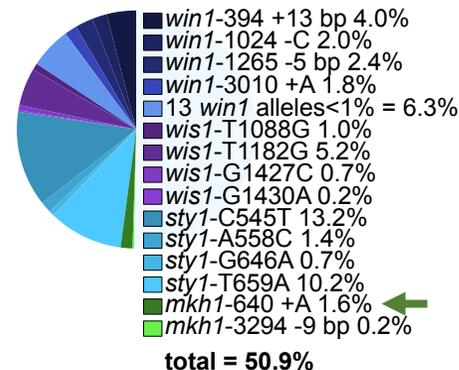
subculture 6



subculture 2

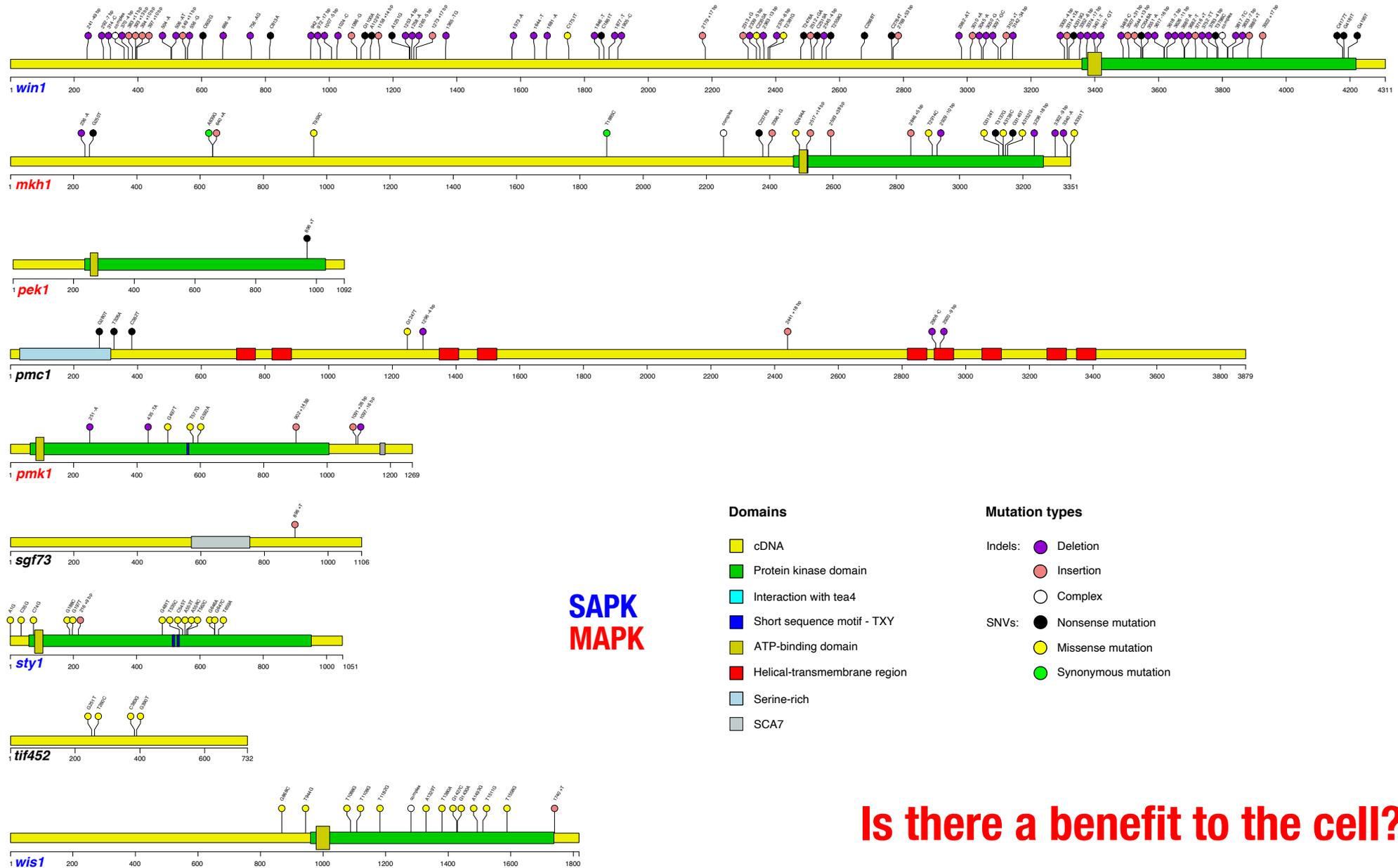


subculture 5



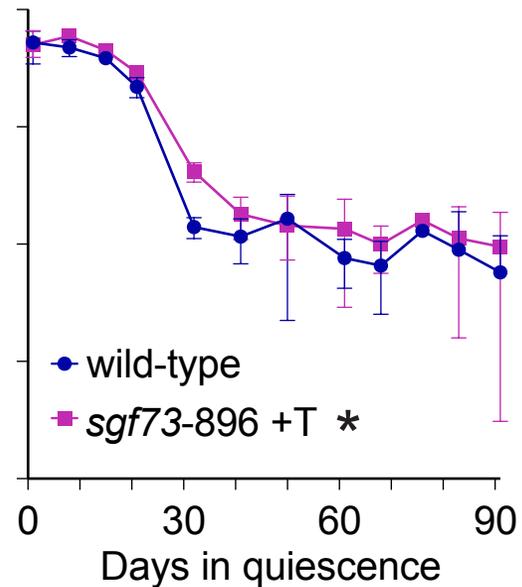
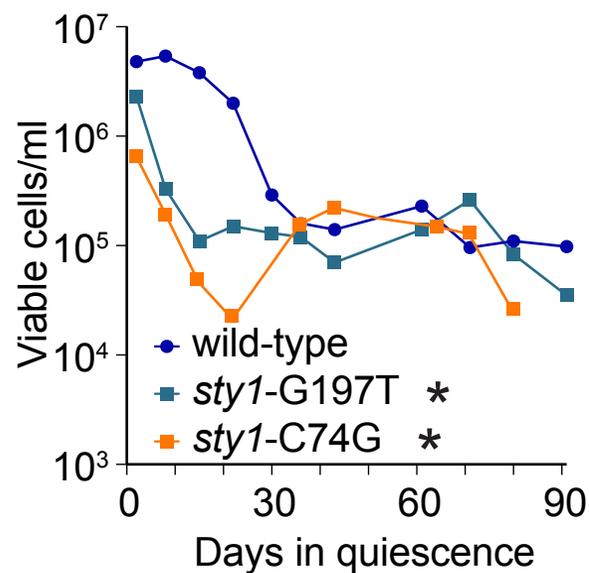
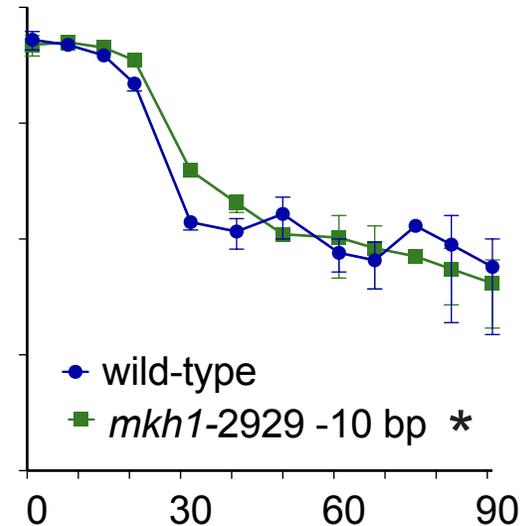
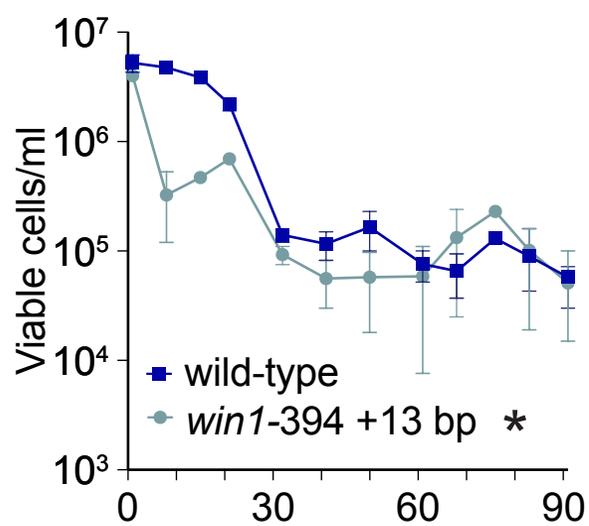
95% of mutations are different with mkh1640+A hot spot

# Detail of the 195 individual mutations recovered

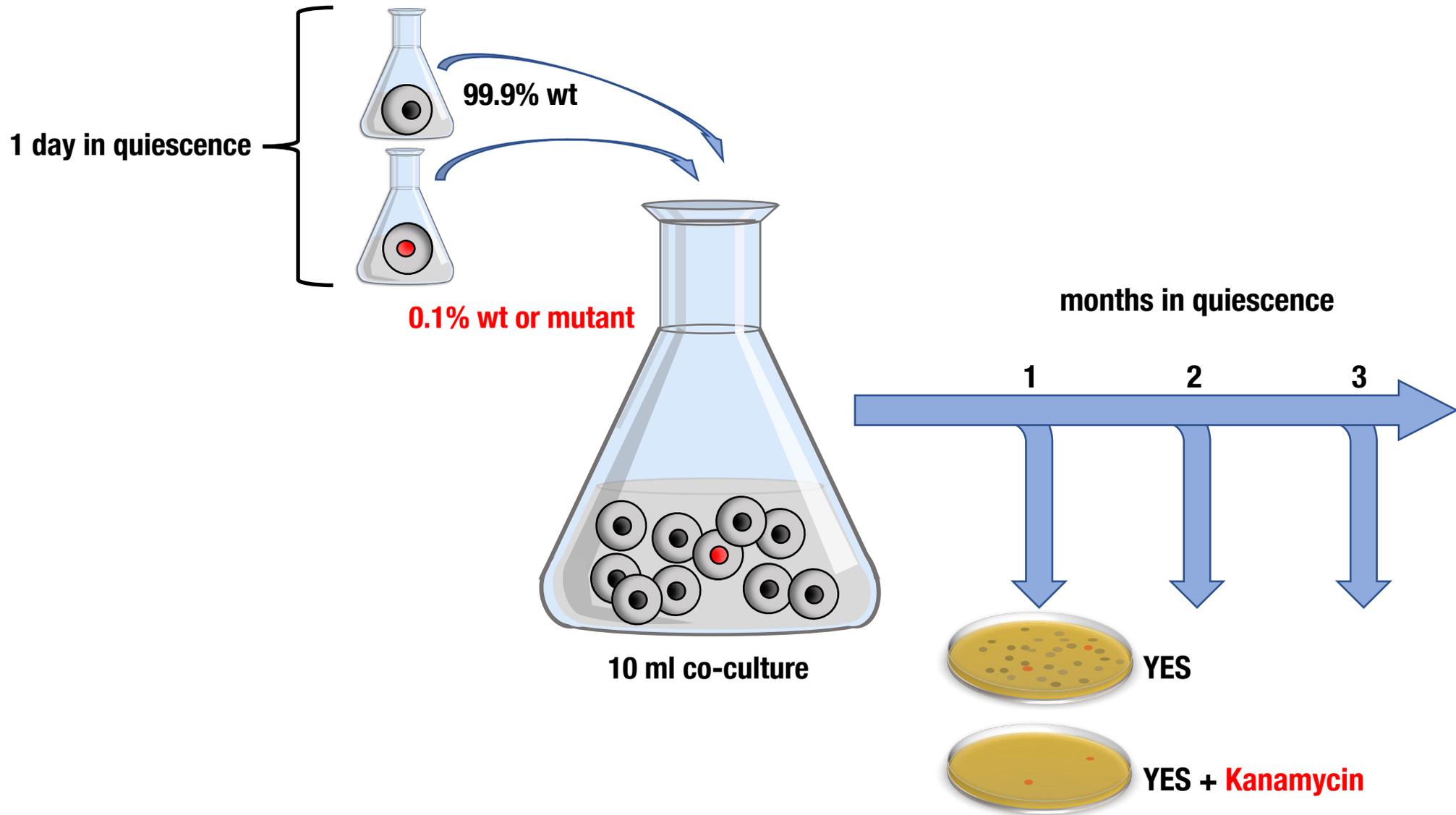


Is there a benefit to the cell?

# WT and mutants behave alike in pure cultures

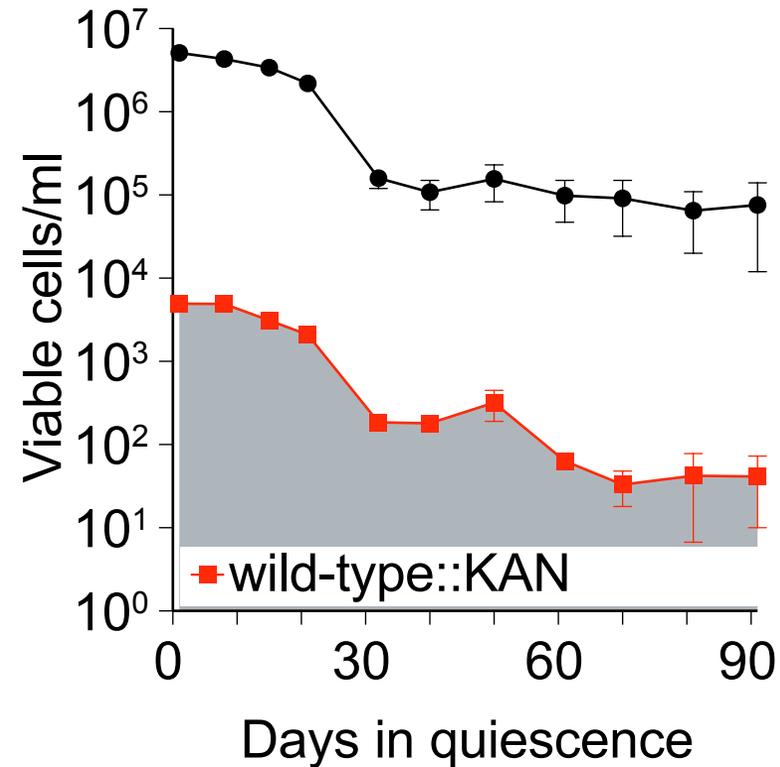


# Co-cultures in quiescence



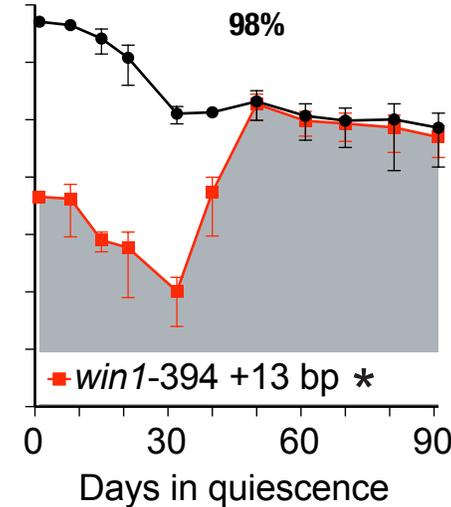
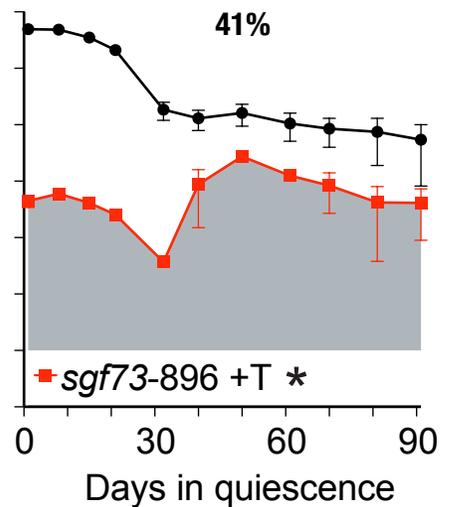
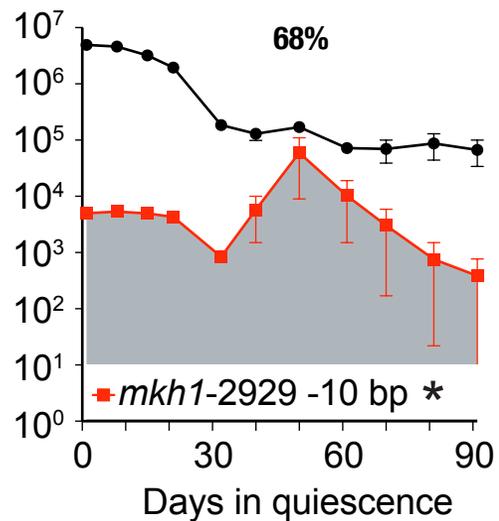
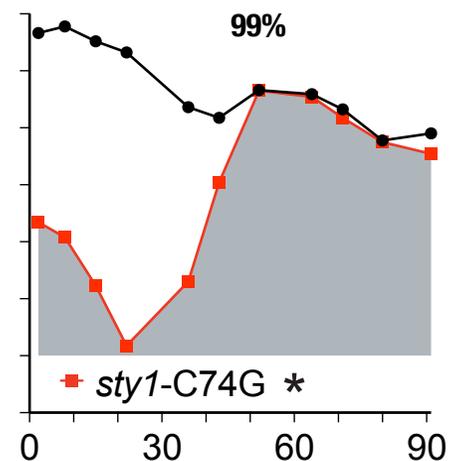
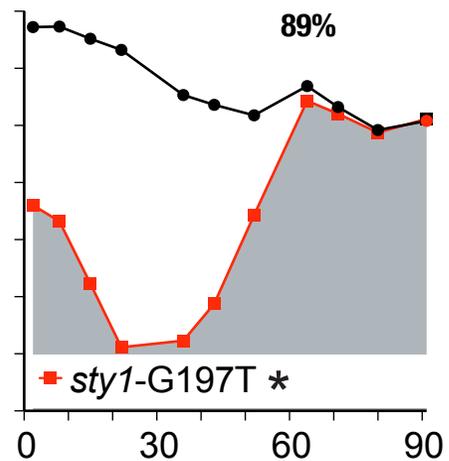
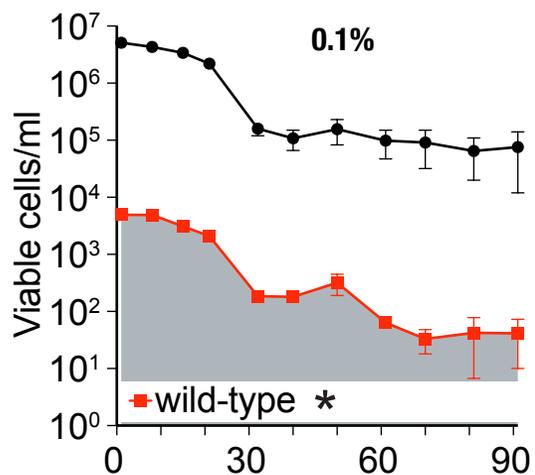
# Co-cultures in quiescence

**WT::KAN in co-culture with WT (0.1%)**



**The proportion of KAN resistant cells is constant throughout the experiment**

# Co-cultures in quiescence

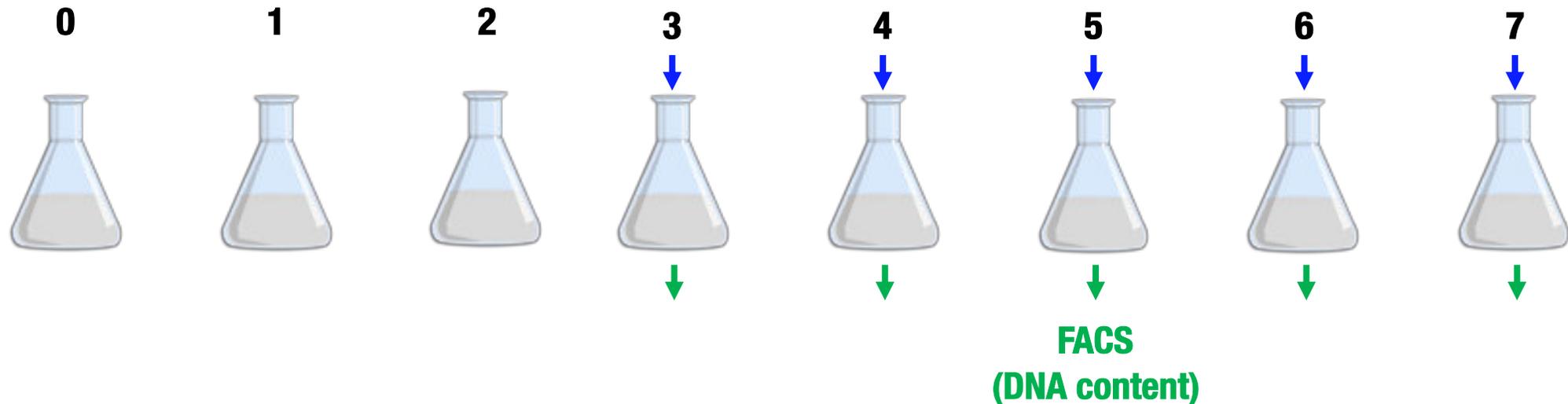


# Is a slow release of nitrogen responsible for cell death?

We determined that 0.2 mM of glutamate is required to replicate the genome of  $4 \cdot 10^6$  cells

We added 1/10th (0.02 mM) this amount every day to a 3-day-old quiescent culture to mimic slow nitrogen release

Days in G0



**+ 0.02 mM Glutamate (every day)**

**Calcofluor staining  
at day 6**

**Days in G0**

**3**

**4**

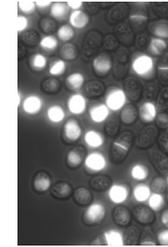
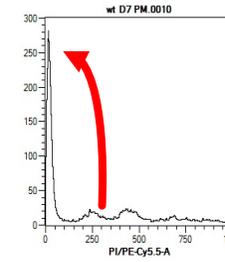
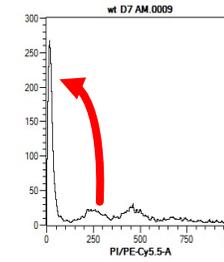
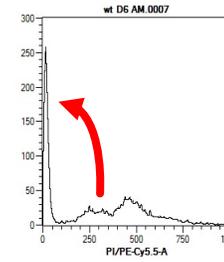
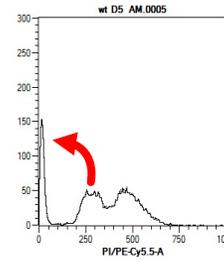
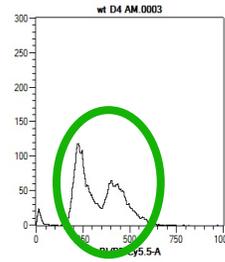
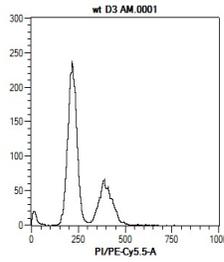
**5**

**6**

**7**

**8**

**wild type**



**S-phase arrest and apoptosis**

+ 0.02 mM Glutamate (every day)

Calcofluor staining at day 6

Days in GO

3

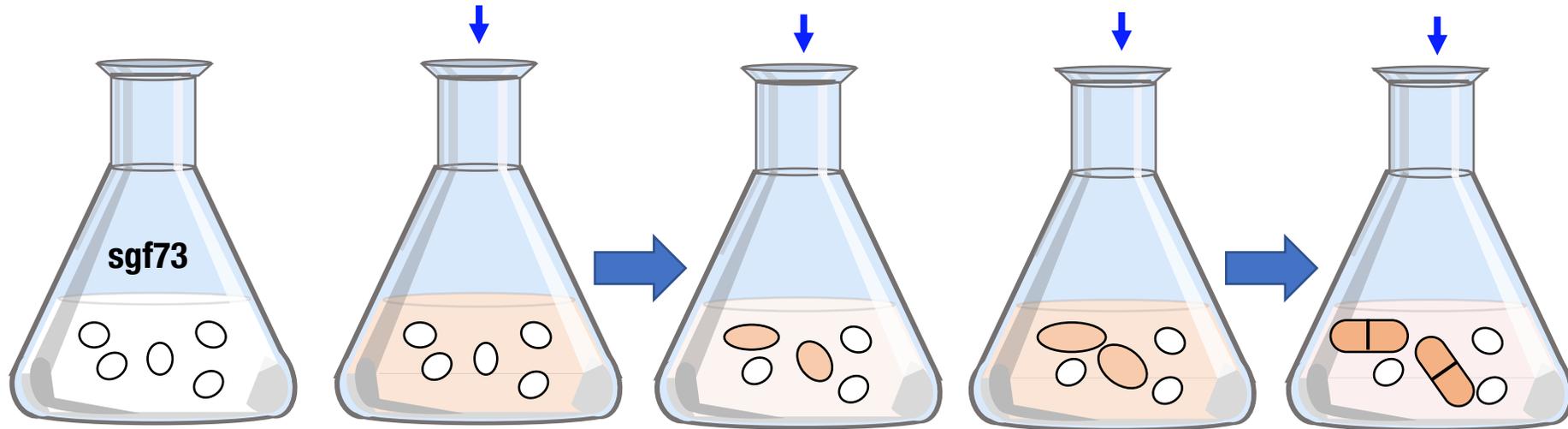
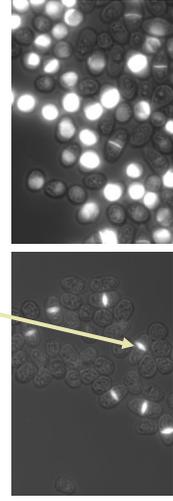
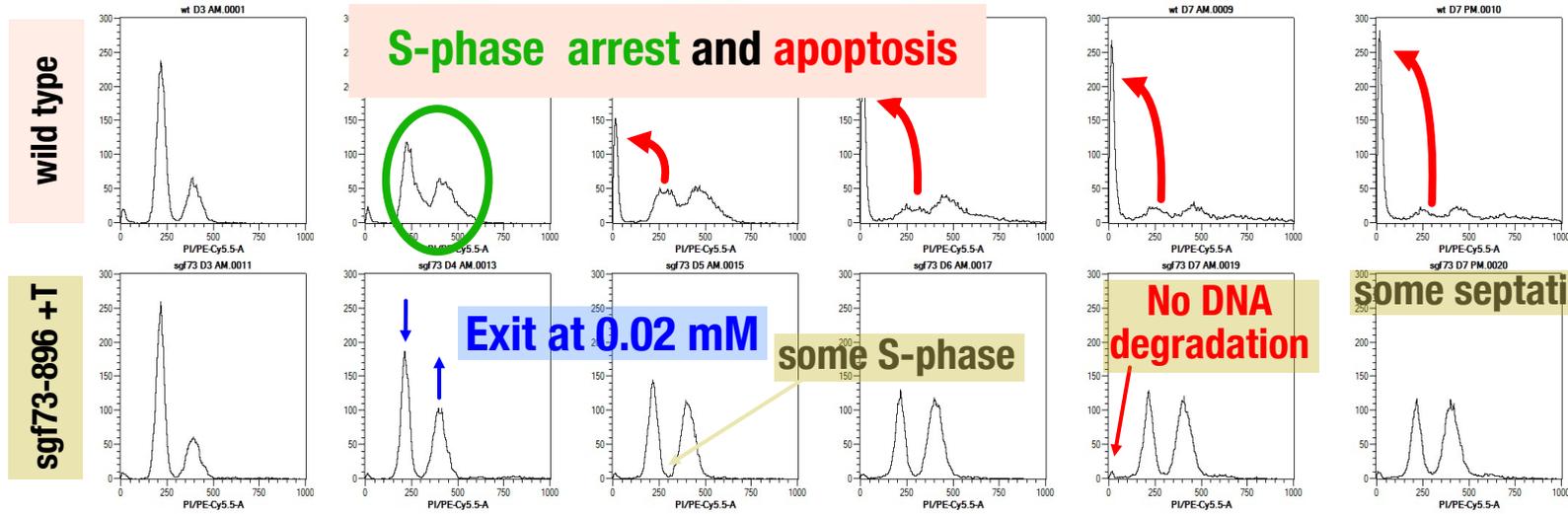
4

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+ 0.02 mM Glutamate (every day)

Calcofluor staining at day 6

Days in GO

3

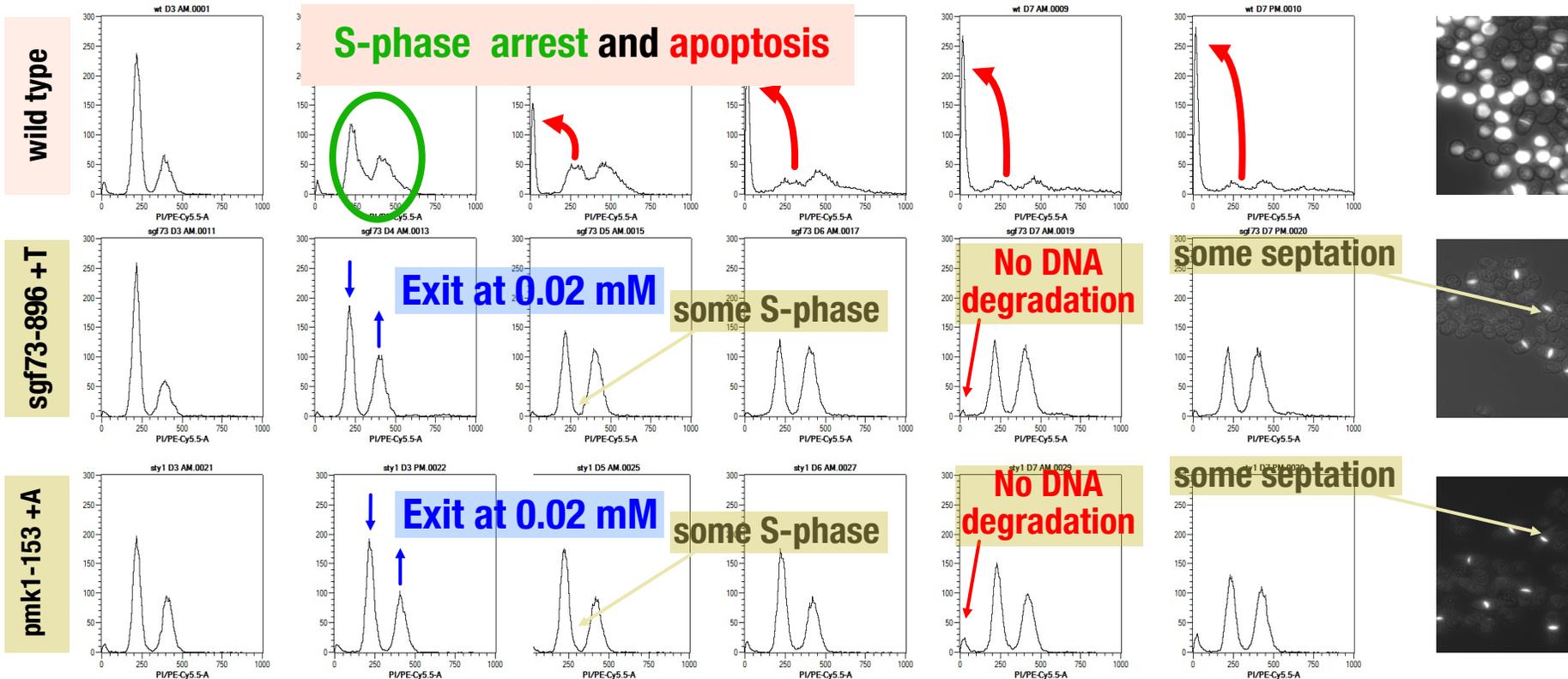
4

5

6

7

8



+ 0.02 mM Glutamate (every day)

Calcofluor staining at day 6

Days in G0

3

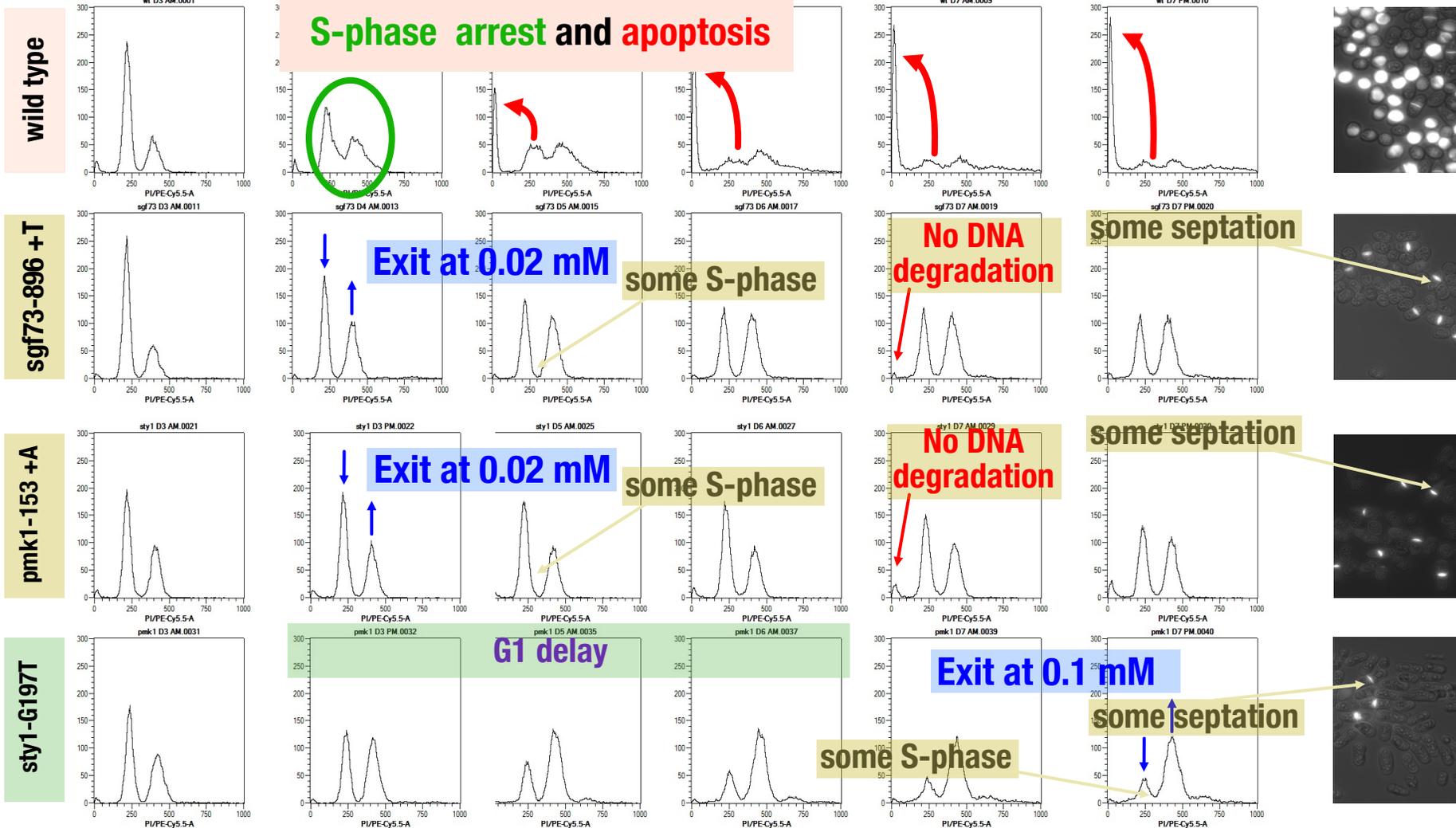
4

5

6

7

8



# Conclusions

## Suboptimal nitrogen concentrations trigger:

- In wild-type cells
  - **rapid exit** from quiescence (**Sty1- dependent**)
  - **S-phase arrest** and **apoptosis** (**Sgf73 and Pmk1- dependent**)
- In the **(S/MAPK) mutants**
  - slow proliferation
  - **pmk1/sgf73 exit quiescence at a low concentration of nitrogen in a binary mode**
  - **sty1 delays quiescence exit until a higher concentration of nitrogen becomes available**

HOW? Remains an open question

Non-dividing conditions promote genetic diversity and generate a social cellular environment prone to kin selection (sacrifice of many for the benefit of some to preserve the species)

Science. 1993 Mar 19;259(5102):1757-60.

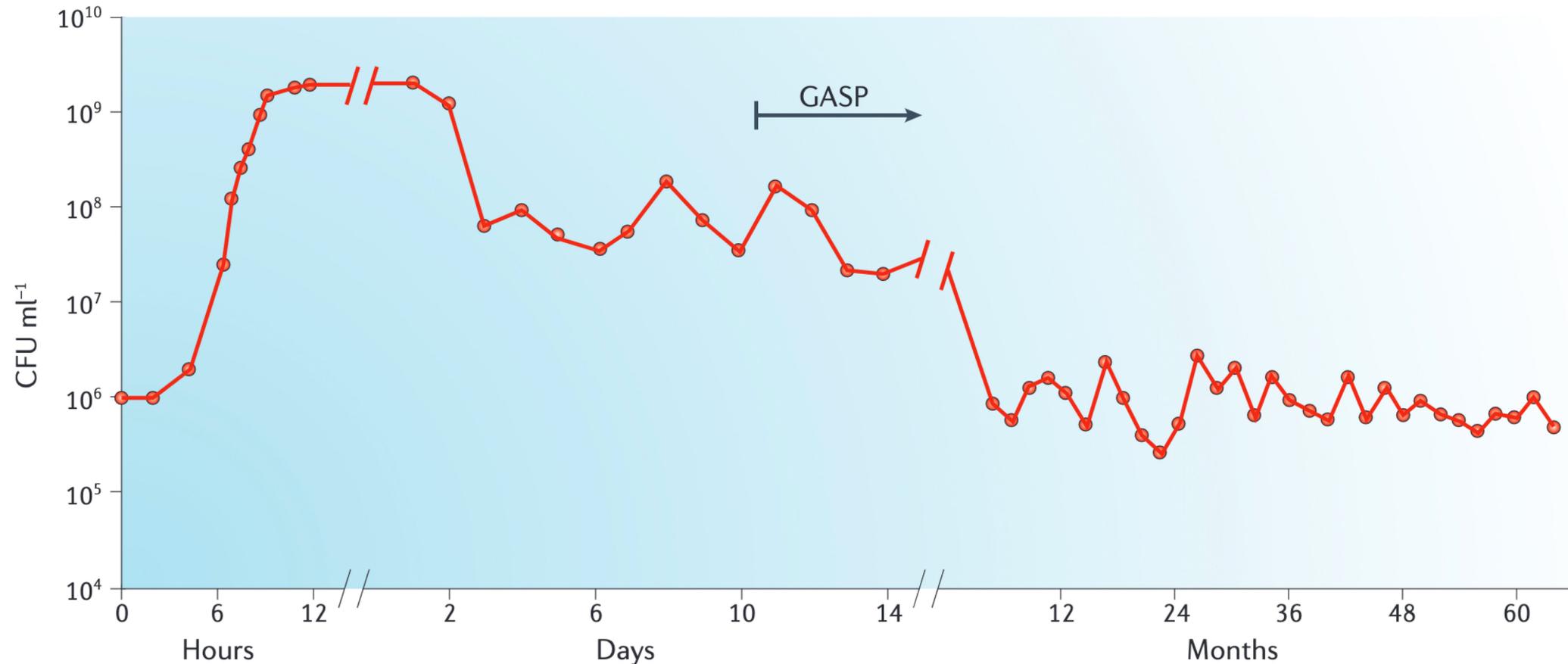
## **Microbial competition: Escherichia coli mutants that take over stationary phase cultures.**

Zambrano MM<sup>1</sup>, Siegele DA, Almirón M, Tormo A, Kolter R.

**GASP** phenotype: **G**rowth **A**dvantage in **S**tationary **P**hase

- During starvation most of the mutations in *E. coli* are found in **RpoS**
- **RpoS** (Sigma S) is induced during starvation and protects the cells against stress by helping to scavenge nutrients

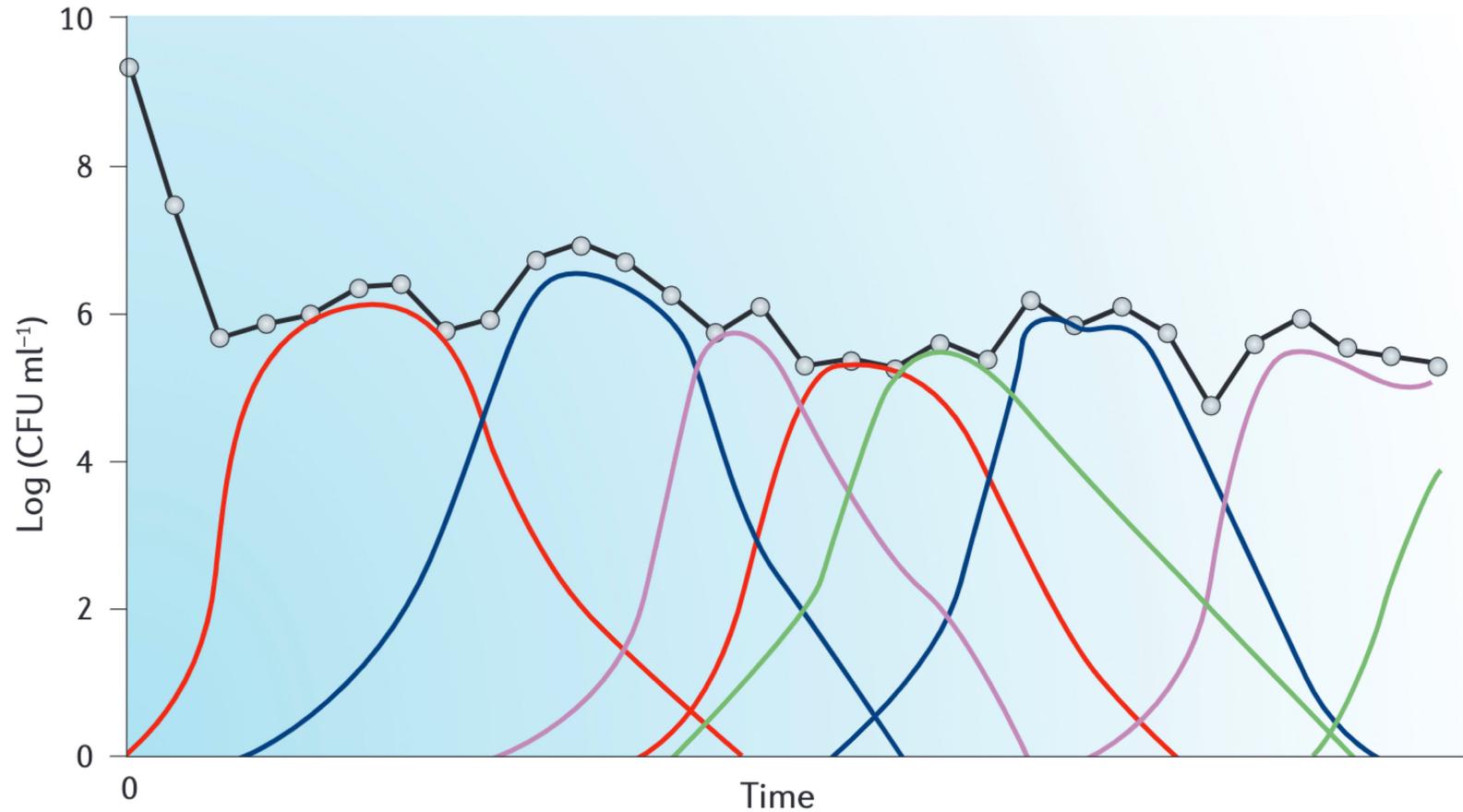
# The five phases of the bacterial life cycle



Once bacteria are inoculated into fresh medium, there is an initial lag period **(1)** followed by exponential-phase growth **(2)**. After remaining at high density for 2 or 3 days - stationary phase **(3)**, cells enter death phase **(4)**. After ~99% of the cells die, the survivors **(5)** can be maintained under long-term stationary-phase culture conditions for months or years.

**Finkel SE. (2006) Nature Reviews Microbiology, 4(2), 113–120. doi: 10.1038/nrmicro1340**

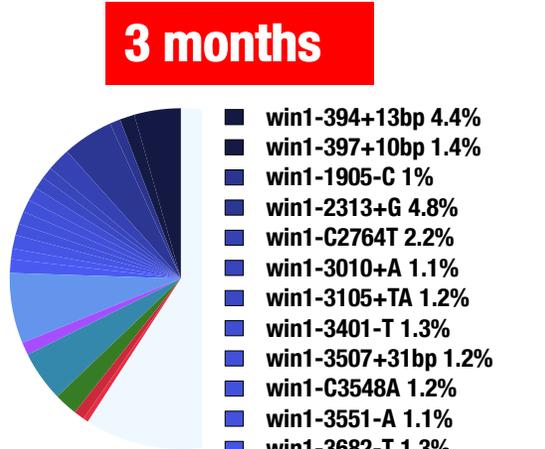
# Population dynamics of long-term stationary-phase cultures



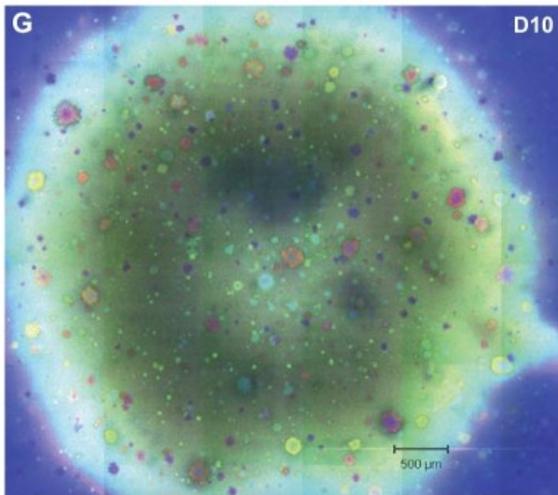
Finkel SE. (2006) *Nature Reviews Microbiology*, 4(2), 113–120. doi: 10.1038/nrmicro1340

# Mutations as a function of time/age

**S/MAPK**  
Genotype diversity  
in quiescence  
(*S. pombe*)  
Makarenko et al. Nat Comm 2020

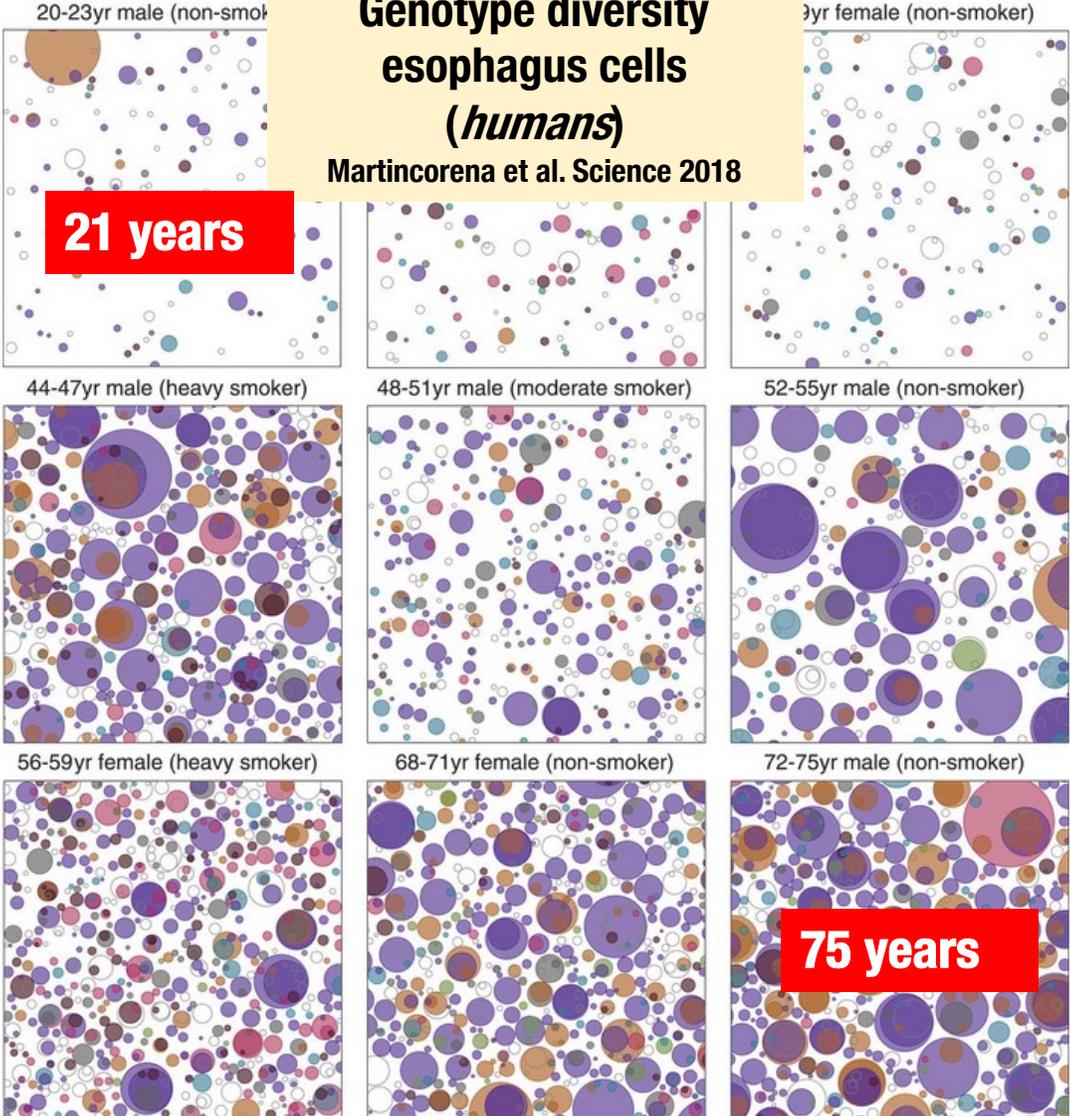


**10 days**

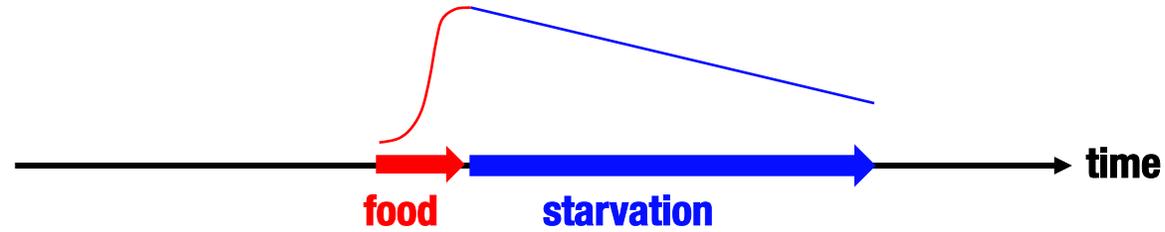


**RpoS**  
Genotype diversity  
in old colony  
(*E. coli*)  
Saint-Ruf et al. J. Bact 2014

**NOTCH1**  
Genotype diversity  
esophagus cells  
(*humans*)  
Martincorena et al. Science 2018



TP53 NOTCH1 NOTCH2 NOTCH3 FAT1 ARID1A  
KMT2D / CUL3 / AJUBA / PIK3CA / ARID2 / TP63 / NFE2L2 / CCND1      Other genes



## Take home message

Mutations: **f(division)** + **f(time)**

Potential impact on:

Evolution (molecular clock)

Heterogamy/Anisogamy (**Male** and **Female** sexes)

Somatic cells (**embryo**/postmitotic)

Aging-related diseases (**cancer** and neuro **degeneration**)

ARTICLE

DOI: [10.1038/s41467-017-01894-6](https://doi.org/10.1038/s41467-017-01894-6)

OPEN

# Eroded telomeres are rearranged in quiescent fission yeast cells through duplications of subtelomeric sequences

Laetitia Maestroni<sup>1</sup>, Julien Audry<sup>1</sup>, Samah Matmati<sup>1</sup>, Benoit Arcangioli<sup>2</sup>, Vincent Géli <sup>1</sup> & Stéphane Coulon<sup>1</sup>

Nature Communications (2017), 8(1), 1684. doi: [10.1038/s41467-017-01894-6](https://doi.org/10.1038/s41467-017-01894-6)



# Leo1 is essential for the dynamic regulation of heterochromatin and gene expression during cellular quiescence

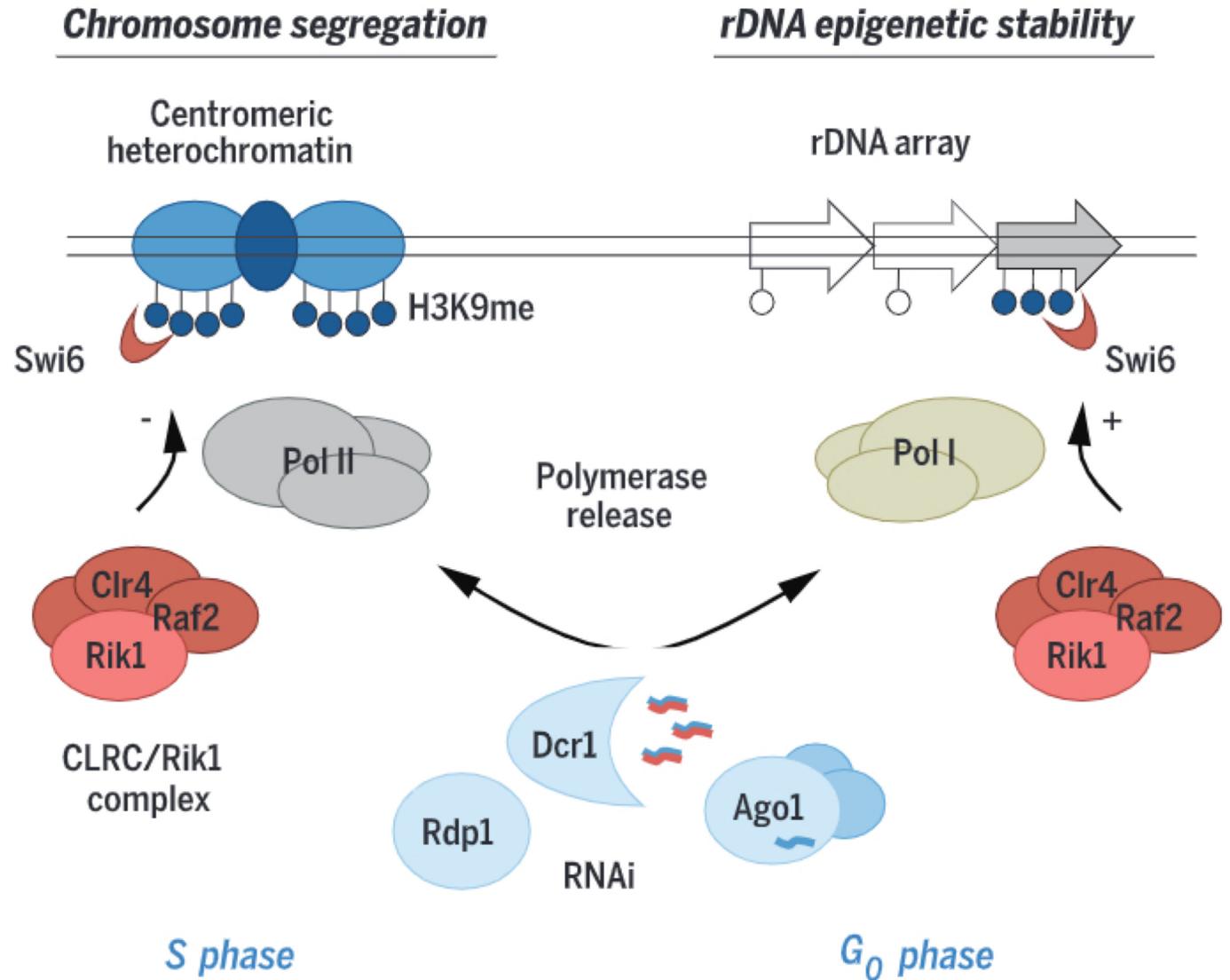
Eriko Oya<sup>1</sup>, Mickaël Durand-Dubief<sup>1</sup>, Adiel Cohen<sup>2</sup>, Vladimir Maksimov<sup>1</sup>, Catherine Schurra<sup>3</sup>, Jun-ichi Nakayama<sup>4</sup>, Ronit Weisman<sup>2</sup>, Benoit Arcangioli<sup>3</sup> and Karl Ekwall<sup>1\*</sup> 

## RESEARCH ARTICLE SUMMARY

CELL CYCLE

# RNA interference is essential for cellular quiescence

B. Roche, B. Arcangioli, R. A. Martienssen\*



Science (2016), 354(6313), aah5651.  
doi: 10.1126/science.aah5651

# Genome Dynamics Unit

## Present

**Benoit Arcangioli**

**Serge Gangloff**

**Claire Denis**

**Stefania Francesconi**

**Catherine Schurra**

**Balveer Singh**

## Past

**Rostyslav Makarenko**

**Célia Raimondi**

**Samia Miled**

# Plateforme de Bioanalyse génomique

**Adrien Villain\***

## UPMC - Interface Biologie, Informatique et Mathématiques

**Guillaume Achaz**

