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The Genetics of Quiescence

Serge Gangloff, Benoit Arcangioli

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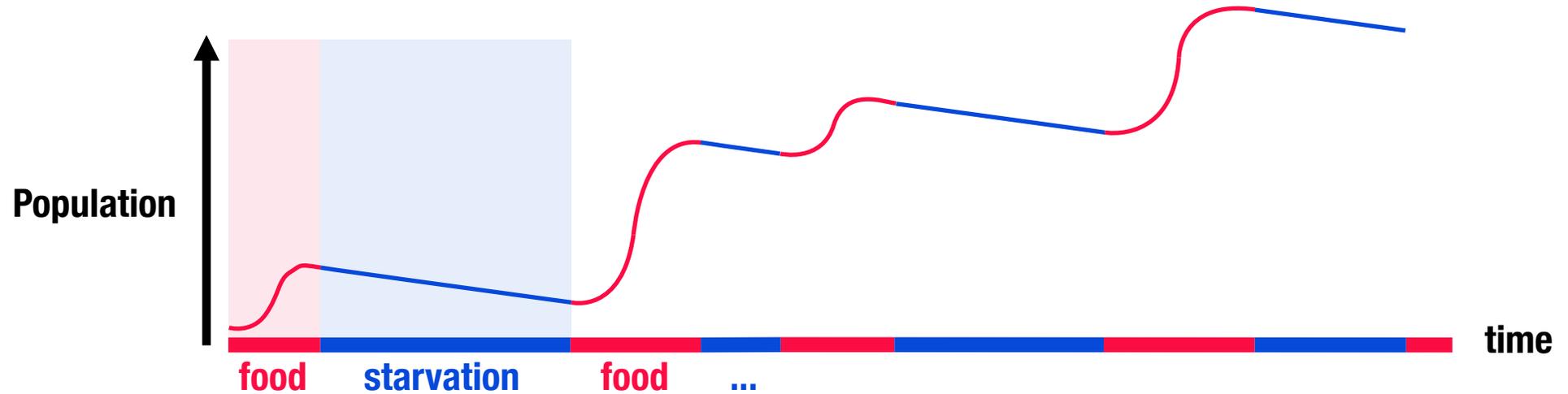
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The Genetics of Quiescence

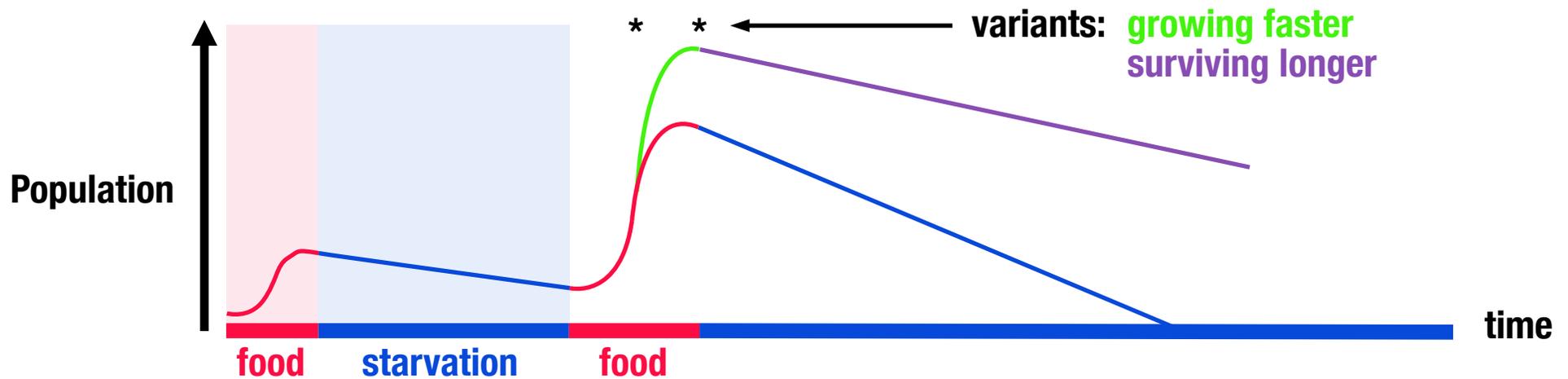
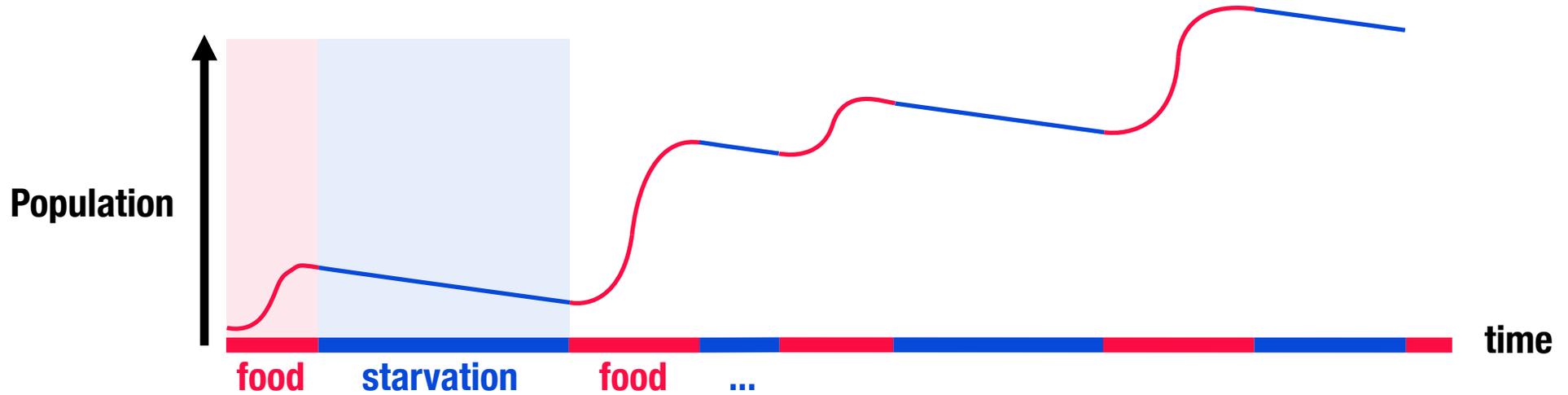
Serge Gangloff

Genome Dynamics Unit – Benoit Arcangioli

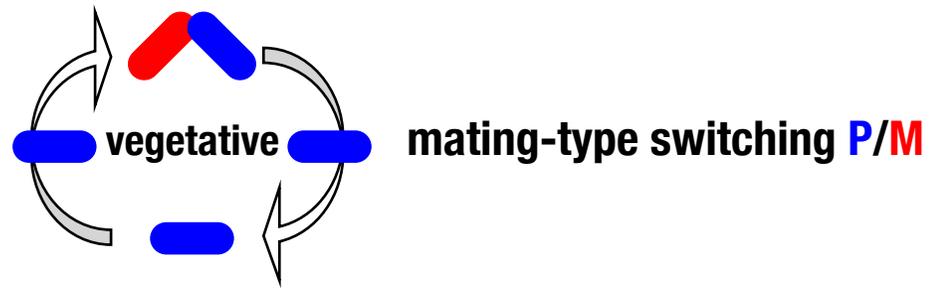
To maintain life across a fluctuating environment, cells alternate between **growth** and **quiescence**



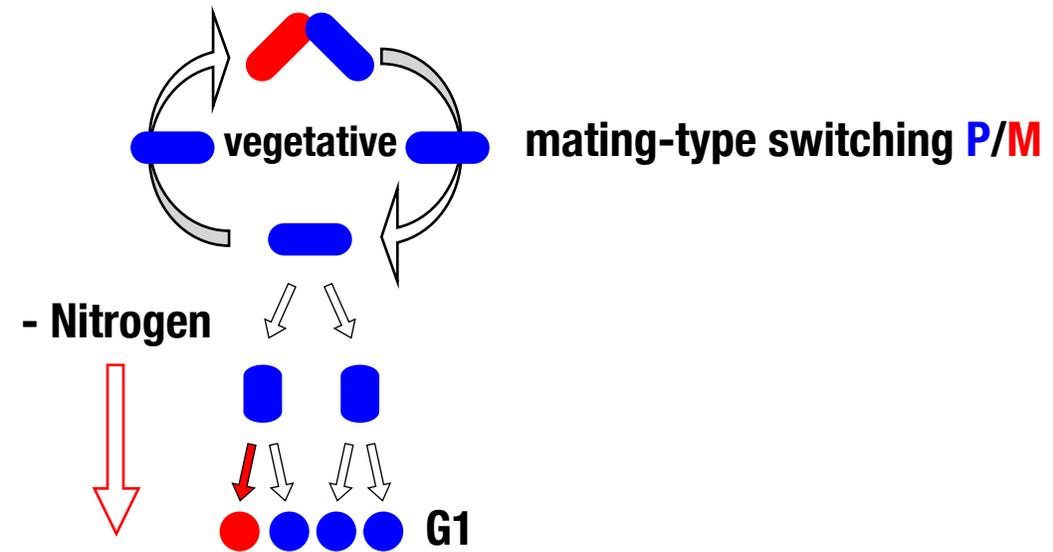
To maintain life across a fluctuating environment, cells alternate between **growth** and **quiescence**



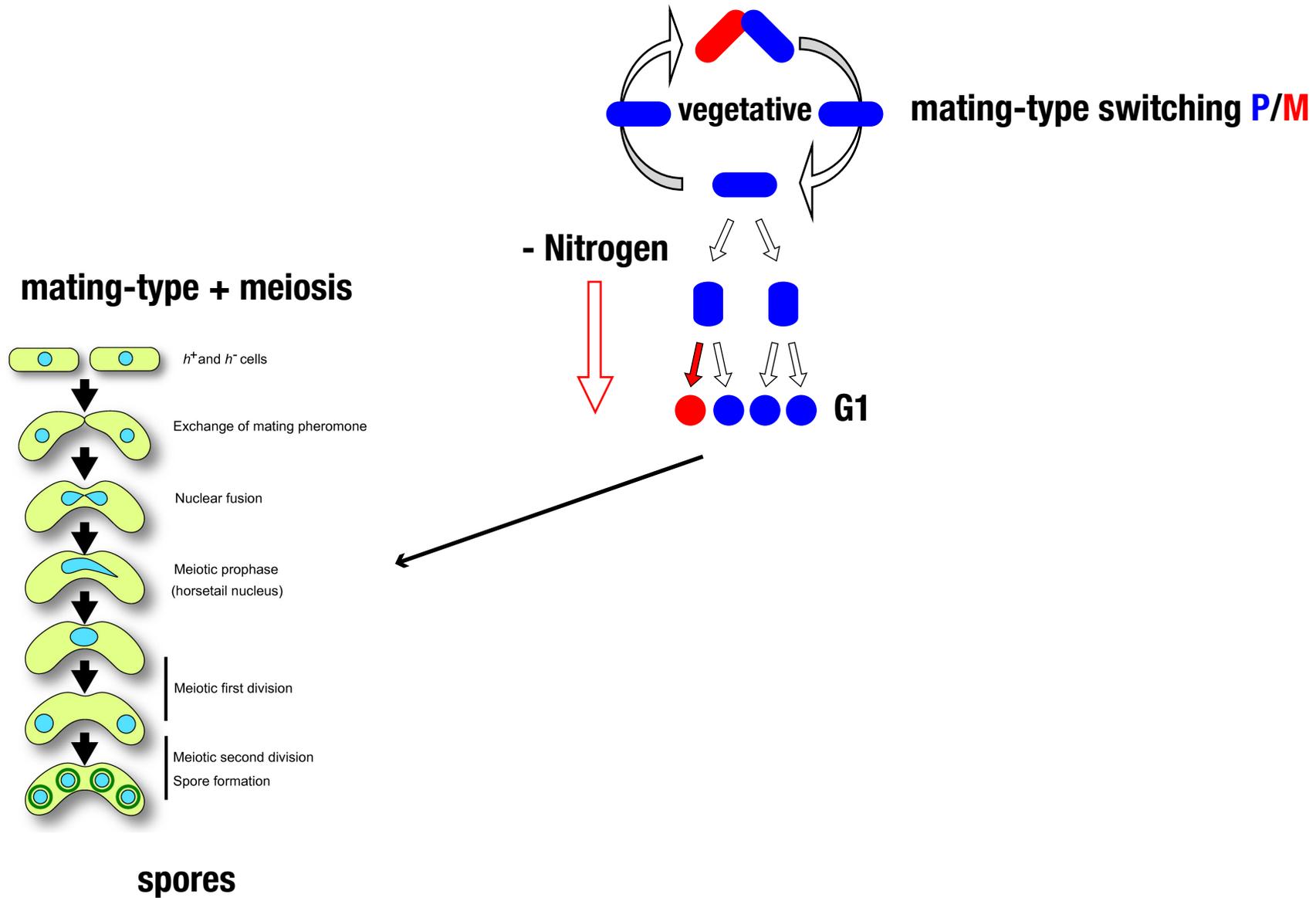
S. pombe: mating-type switching



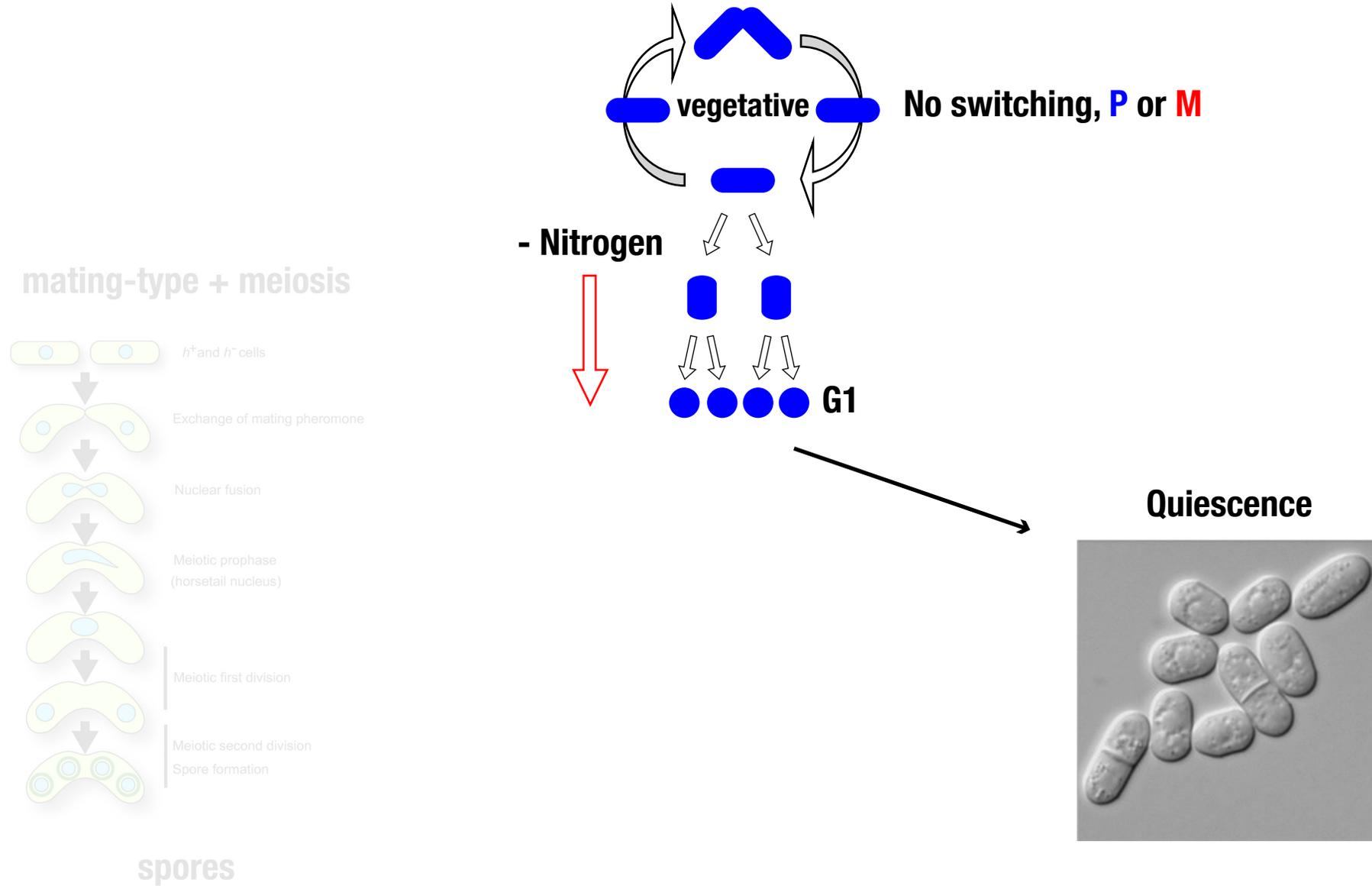
S. pombe: mating-type switching



S. pombe: mating-type switching

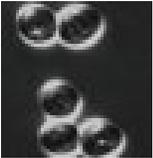


S. pombe: mating-type switching



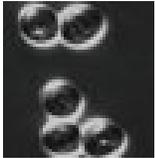
Tools to study quiescence

Single cell

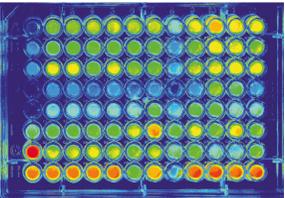


Tools to study quiescence

Single cell

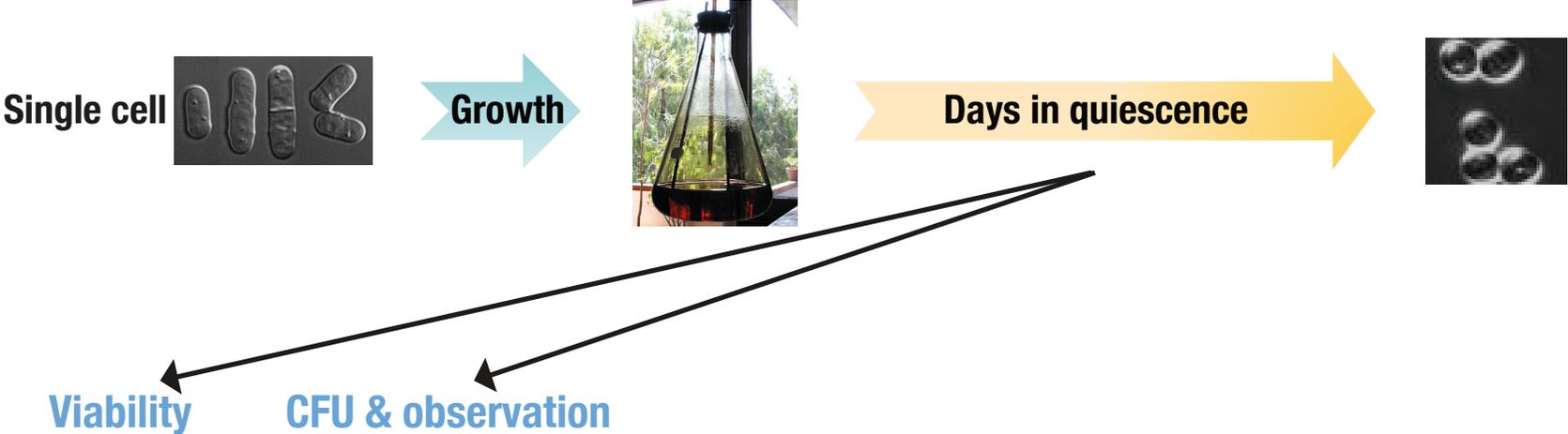


Viability



% of viable cells
in quiescence

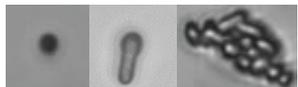
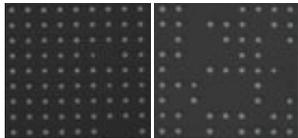
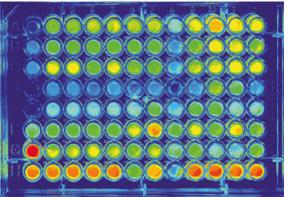
Tools to study quiescence



% of viable cells in quiescence



CFU & cell shape after 3 days of growth



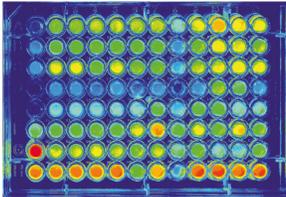
Tools to study quiescence



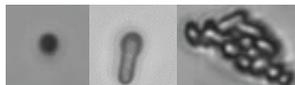
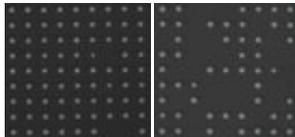
Viability

CFU & observation

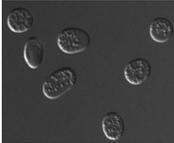
**DNA repair
Rad52-YFP**



**% of viable cells
in quiescence**



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



**Unpaired
lesions**

Tools to study quiescence

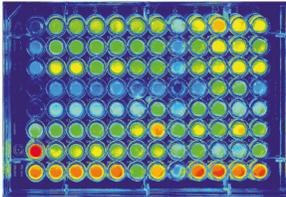


Viability

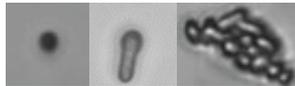
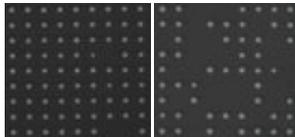
CFU & observation

**DNA repair
Rad52-YFP**

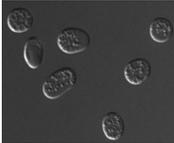
**DNA content
FACS**



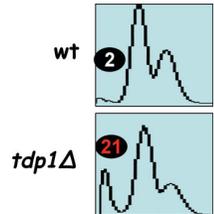
**% of viable cells
in quiescence**



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

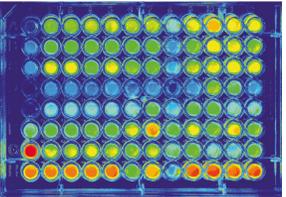
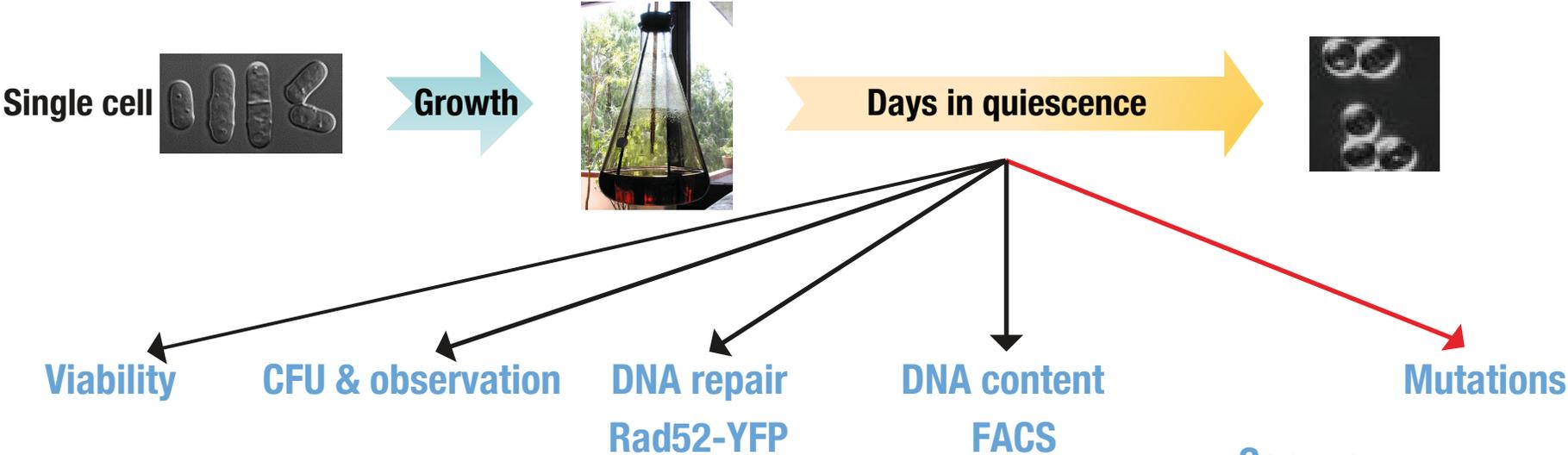


**Unrepaiored
lesions**

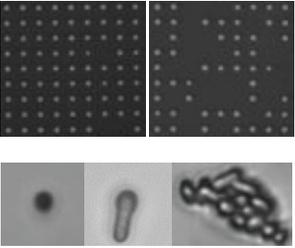


**Anucleated
cells**

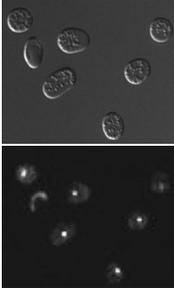
Tools to study quiescence



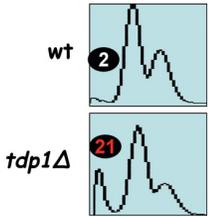
% of viable cells in quiescence



CFU & cell shape after 3 days of growth

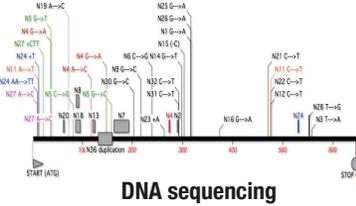
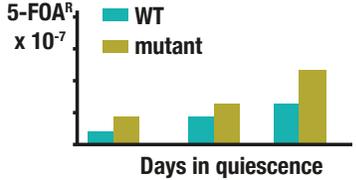


Unpaired lesions



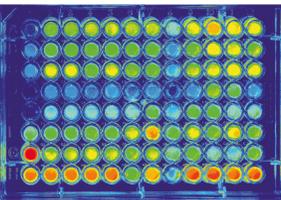
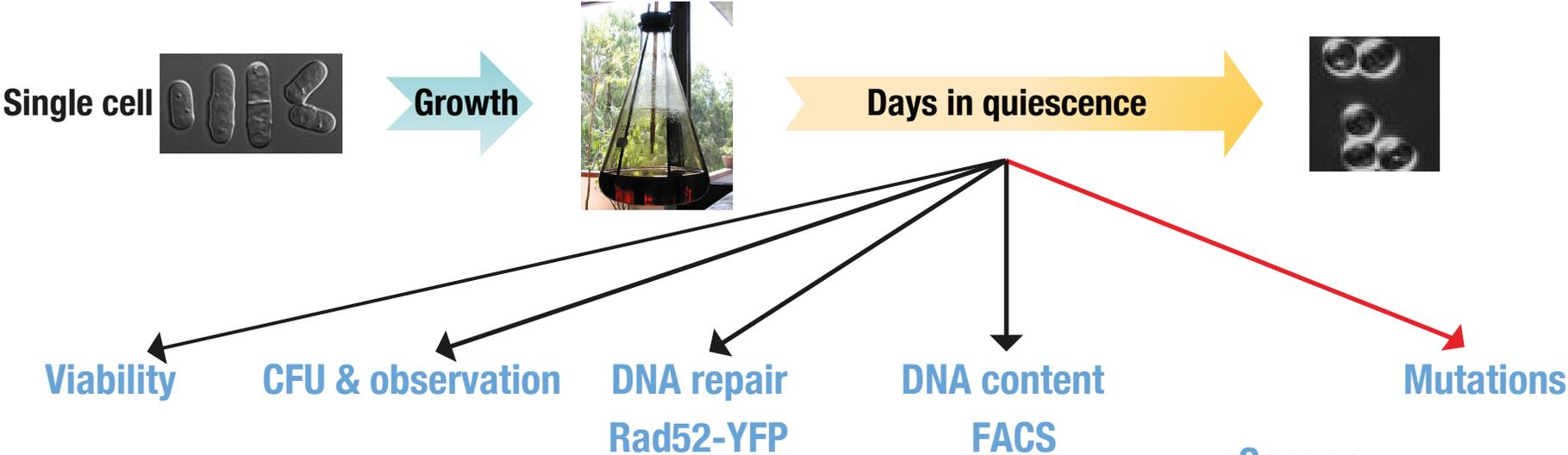
Anucleated cells

Sanger

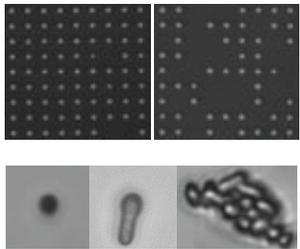


**Mutation rate f(time)
Mutational spectrum**

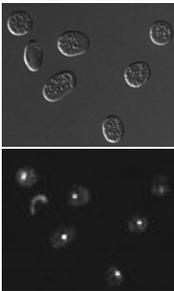
Tools to study quiescence



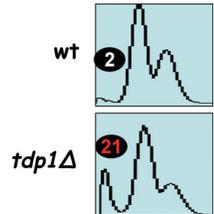
% of viable cells in quiescence



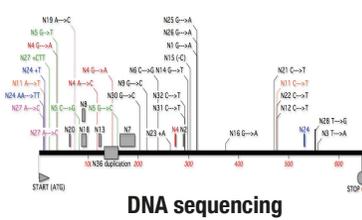
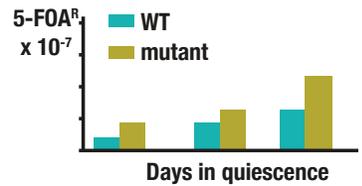
CFU & cell shape after 3 days of growth



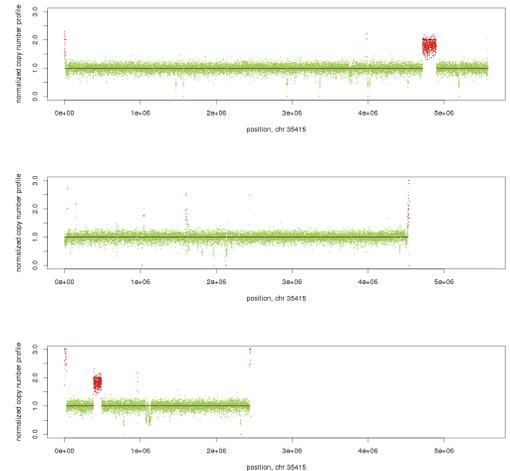
Unrepaired lesions



Anucleated cells



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

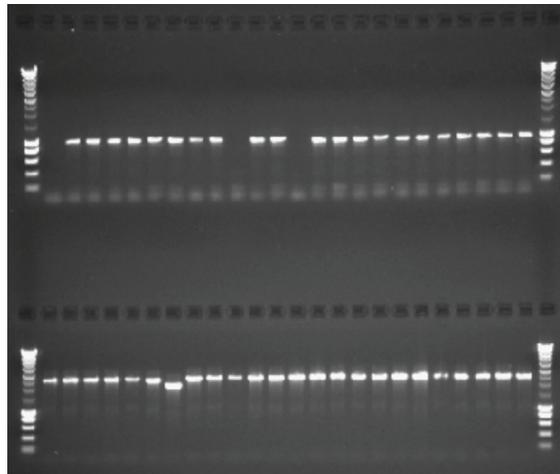


**Mutation rate f(time)
Mutational spectrum**

Prerequisite to the determination of mutations in non-dividing cells

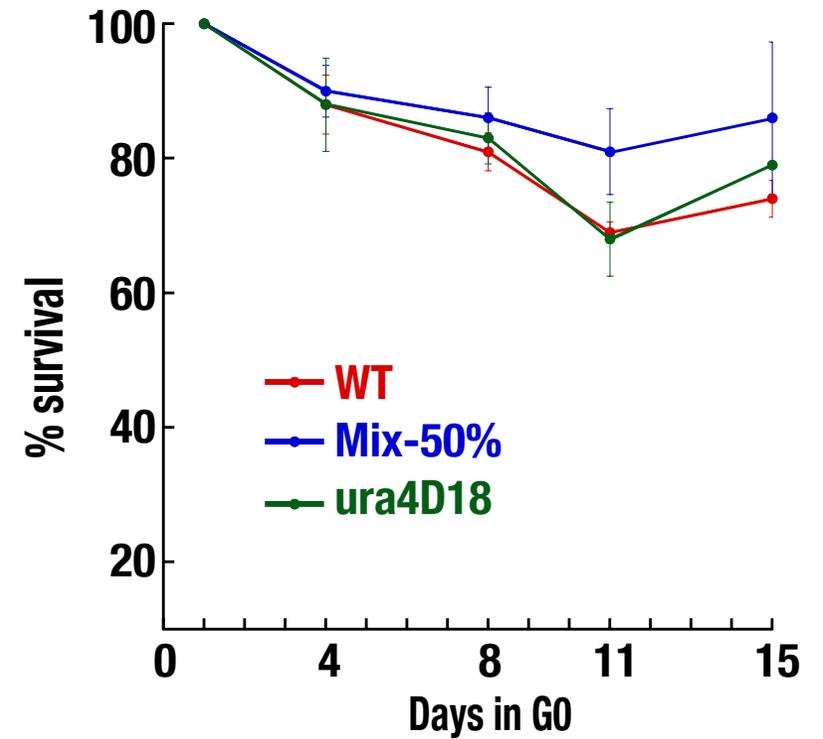
- **Ura mutants should not die during the 2 weeks of the experiment**
- **Growth mutants should be kept as low as possible**
- **All the mutants in the culture must be sequenced**

ura- cells have a similar survival rate in quiescence



ura4 amplification

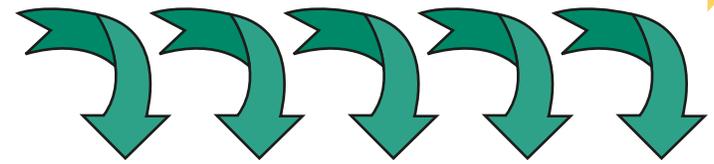
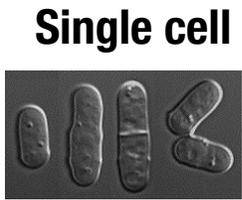
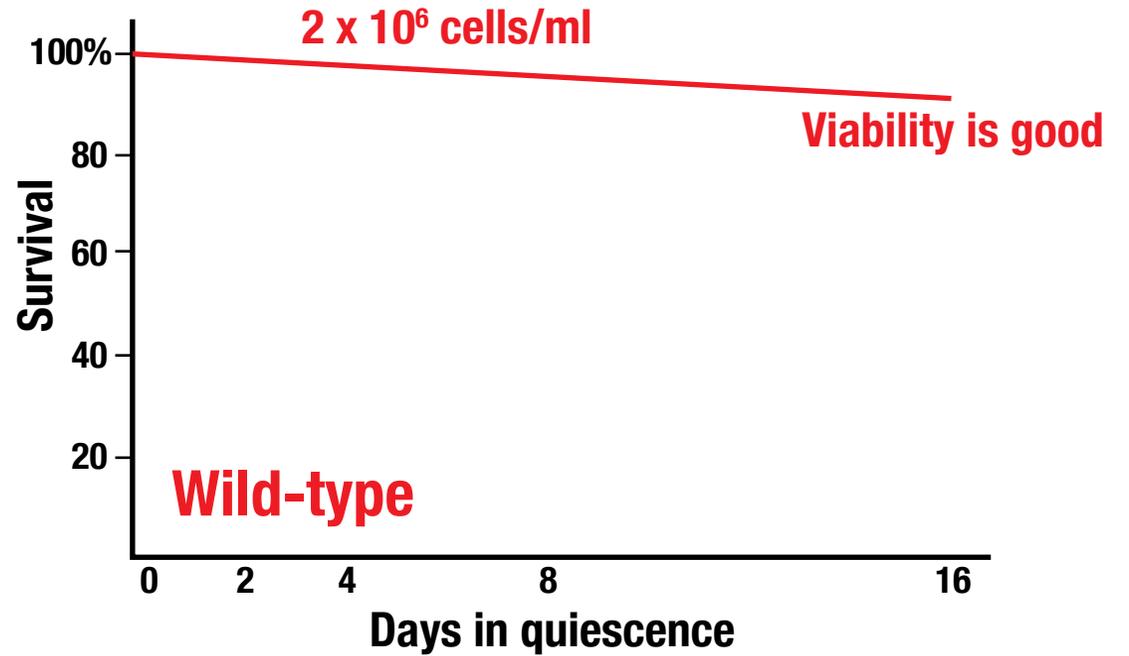
ura5 amplification



Spontaneous mutations in quiescence

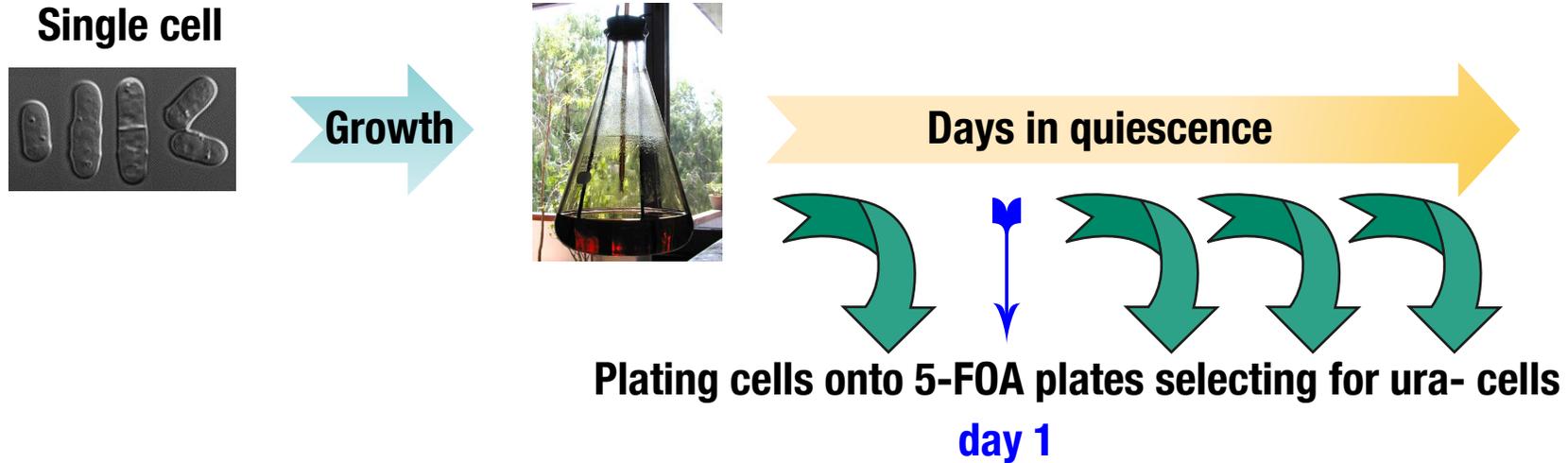
Cells are metabolically active
Transcription, translation, etc.

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



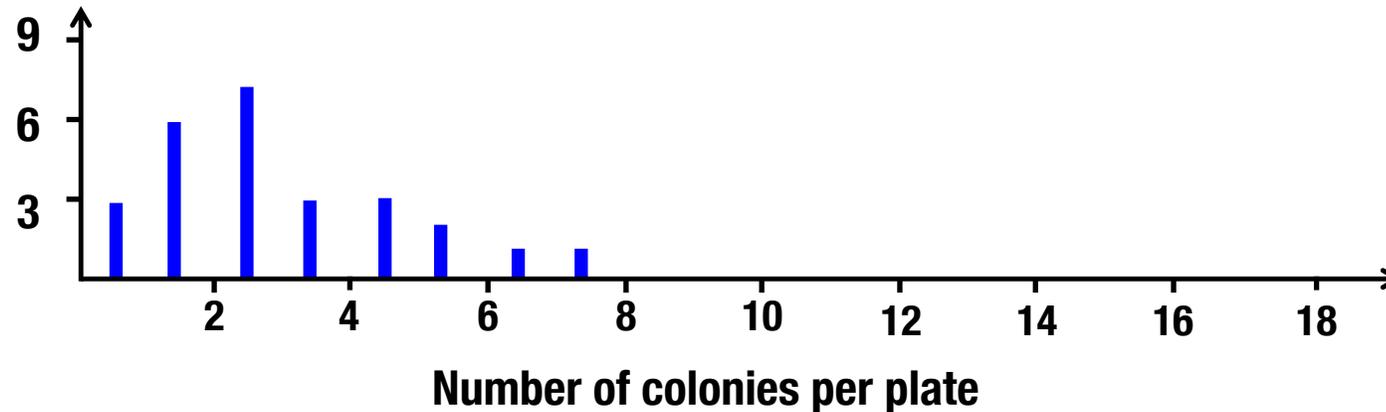
Plating cells onto 5-FOA plates selecting for ura- cells

Spontaneous mutations in quiescence

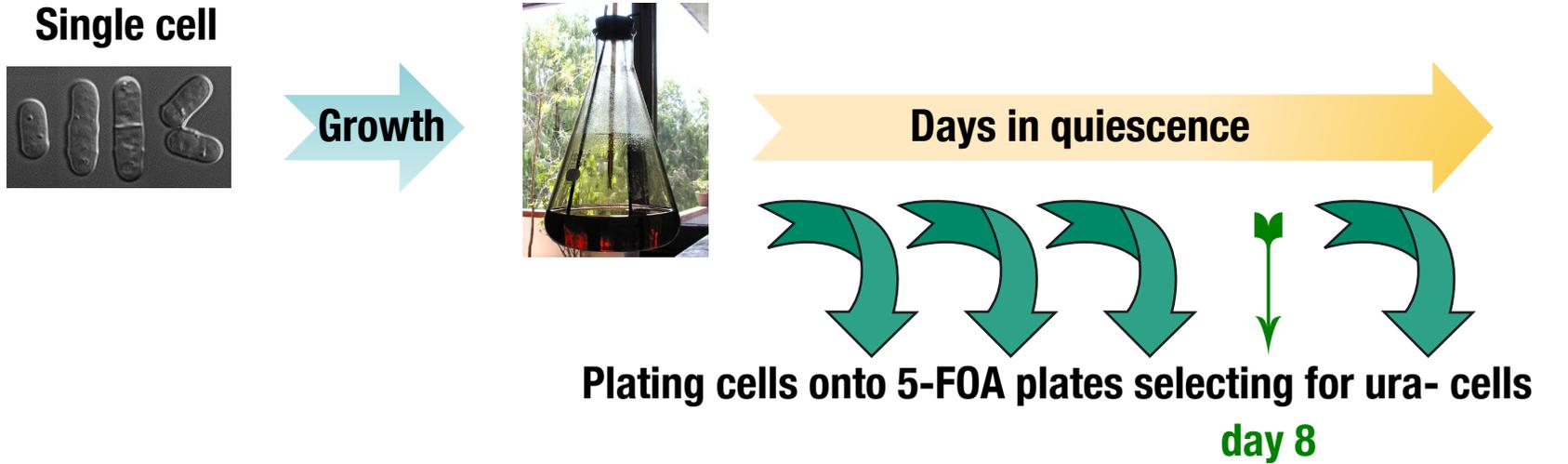


10^7 cells on 5-FOA plates that
select for $ura4^-$ and $ura5^-$ mutants

Number of plates

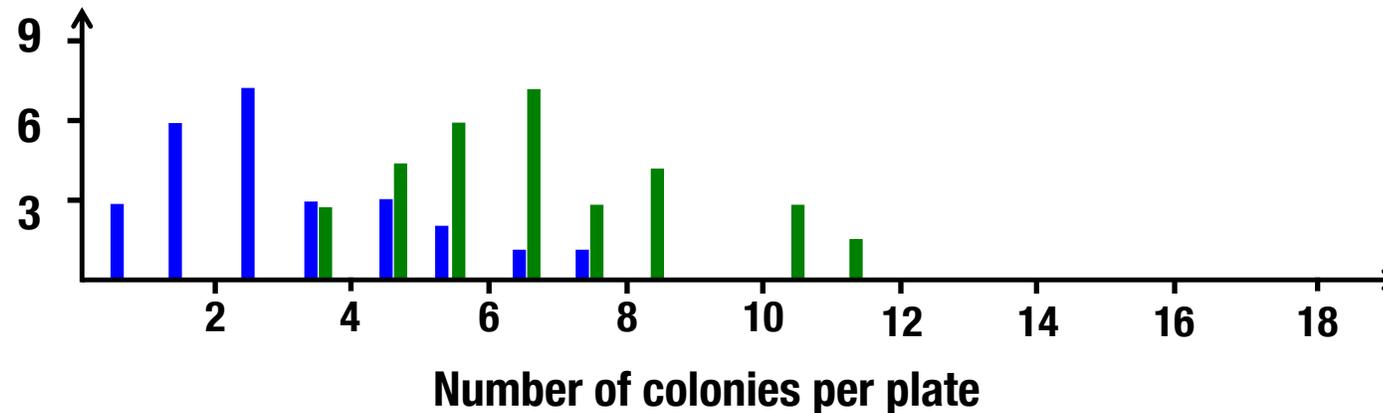


Spontaneous mutations in quiescence

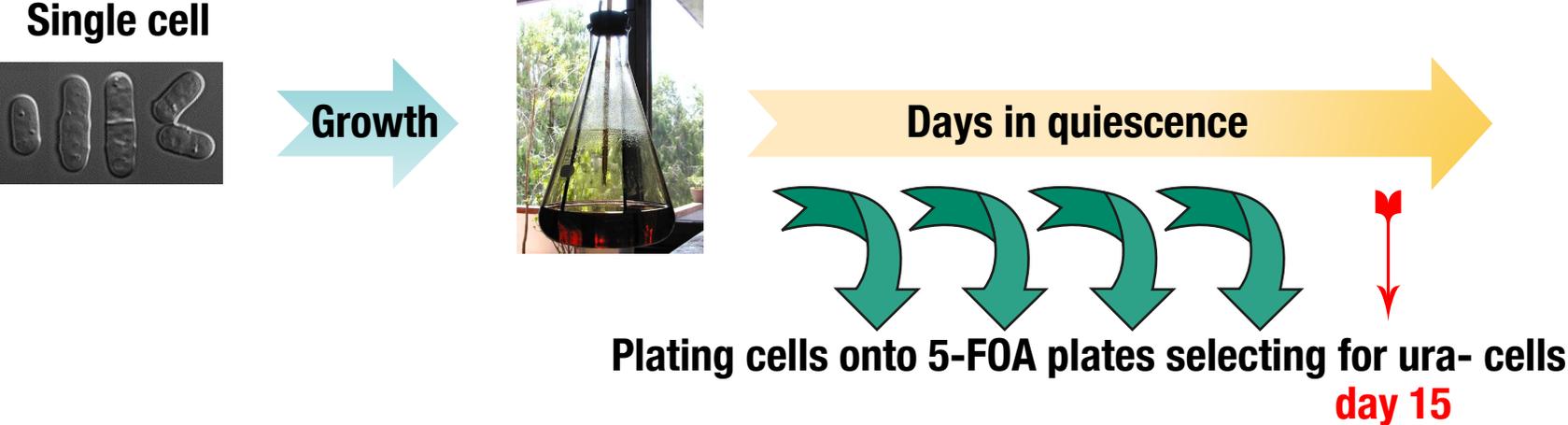


10^7 cells on 5-FOA plates that select for $ura4^-$ and $ura5^-$ mutants

Number of plates

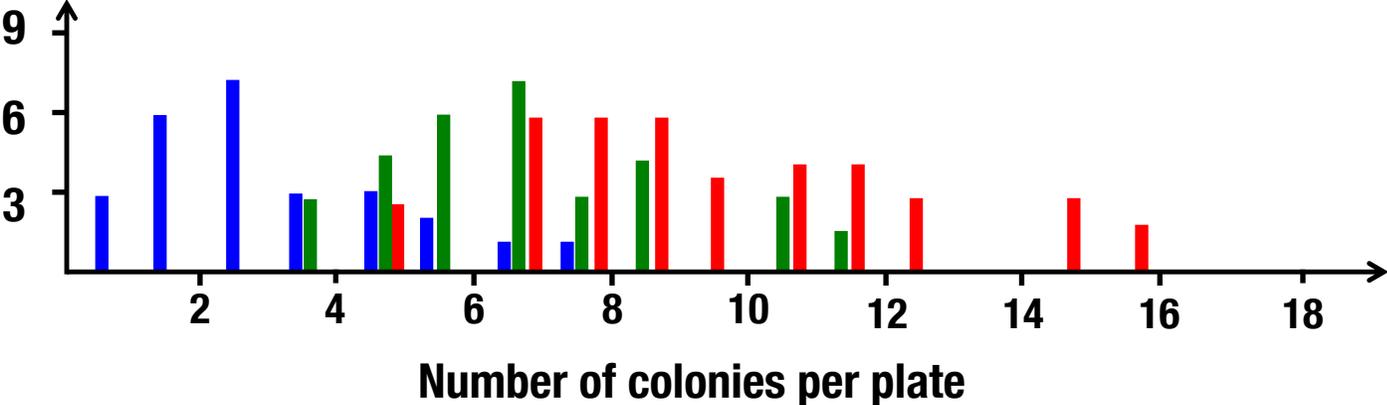


Spontaneous mutations in quiescence



10⁷ cells on 5-FOA plates that select for ura⁴⁻ and ura⁵⁻ mutants

Number of plates



Spontaneous mutations in quiescence

Spontaneous 5-FOA^R
ura mutants

1.3×10^{-8} 5-FOA^R
per generation

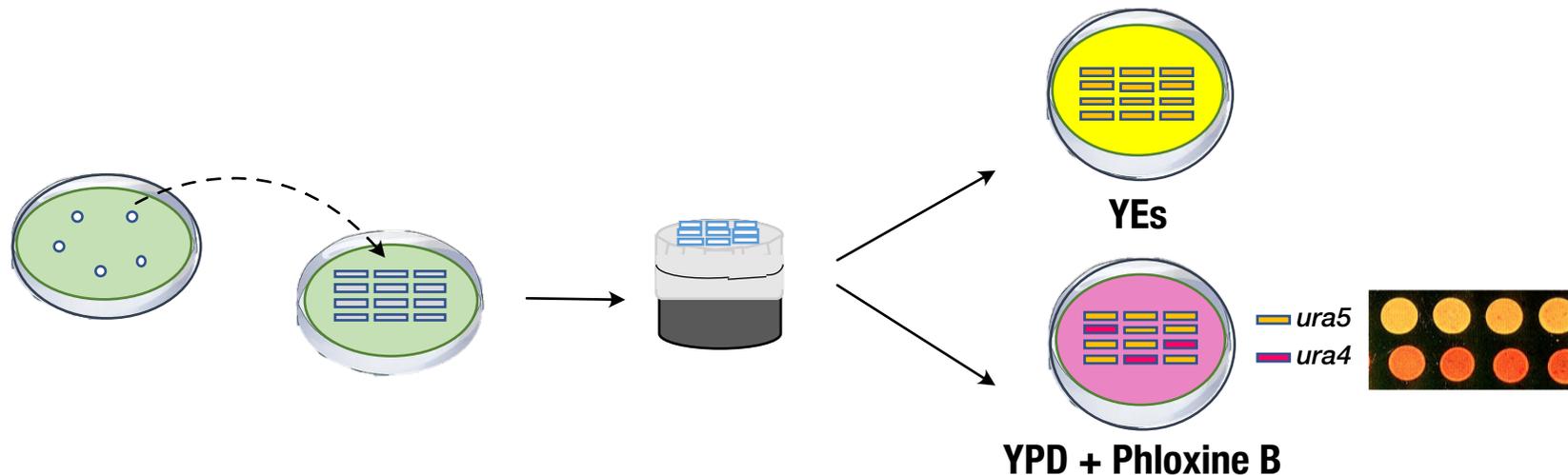


Mutations accumulate as a function of time (constant rate)
1 replication round $\approx 10^{-7}$ 5-FOA^R \approx 3 days of quiescence

5-FOA^R → DNA → PCR → Sequencing

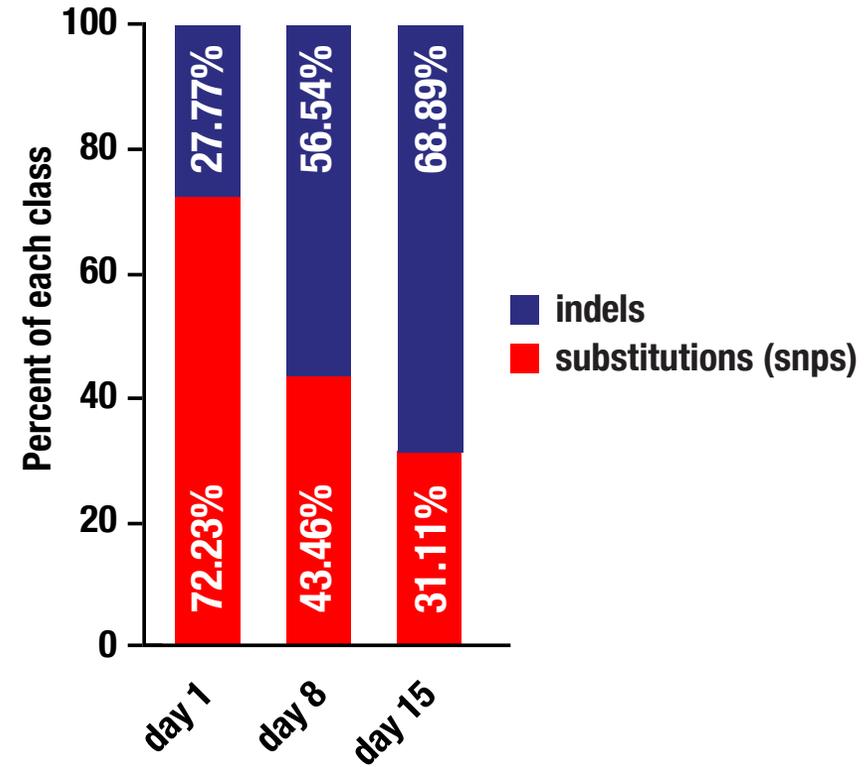
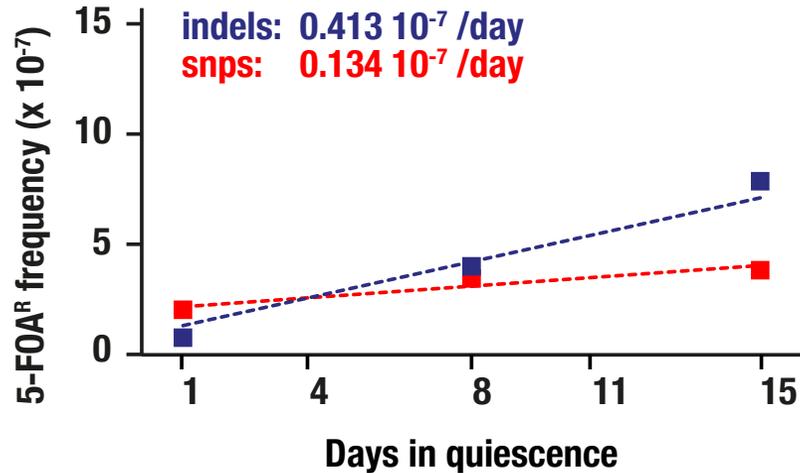
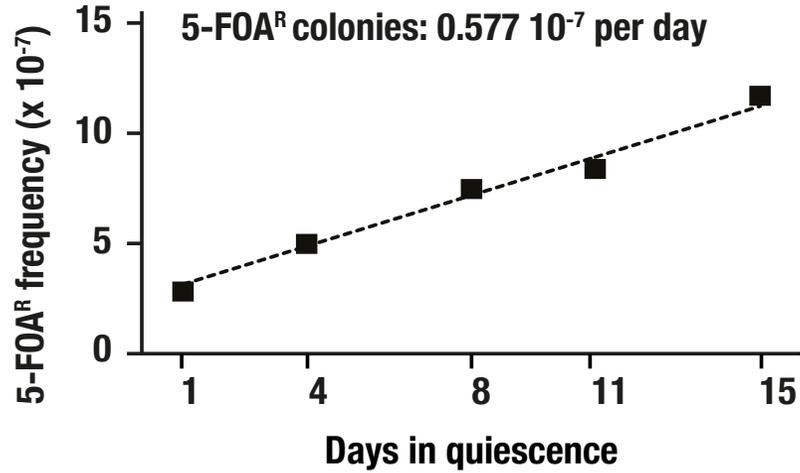
Identification of the ura gene mutated

- 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

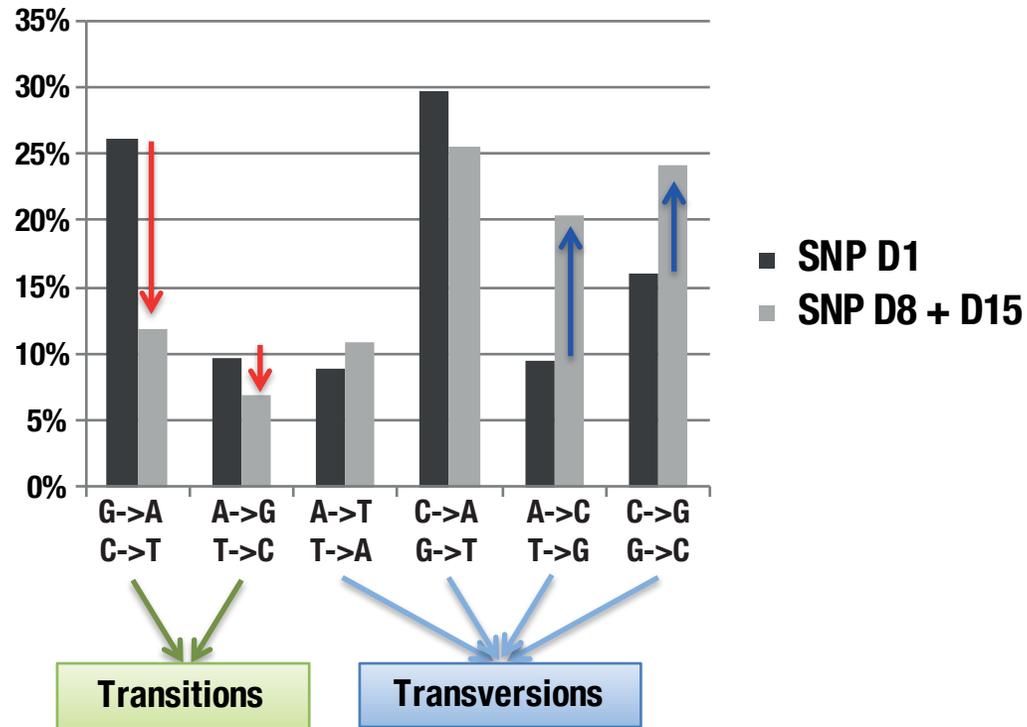


5-FOA^R → DNA → PCR → Sequencing

5-FOA^R 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:

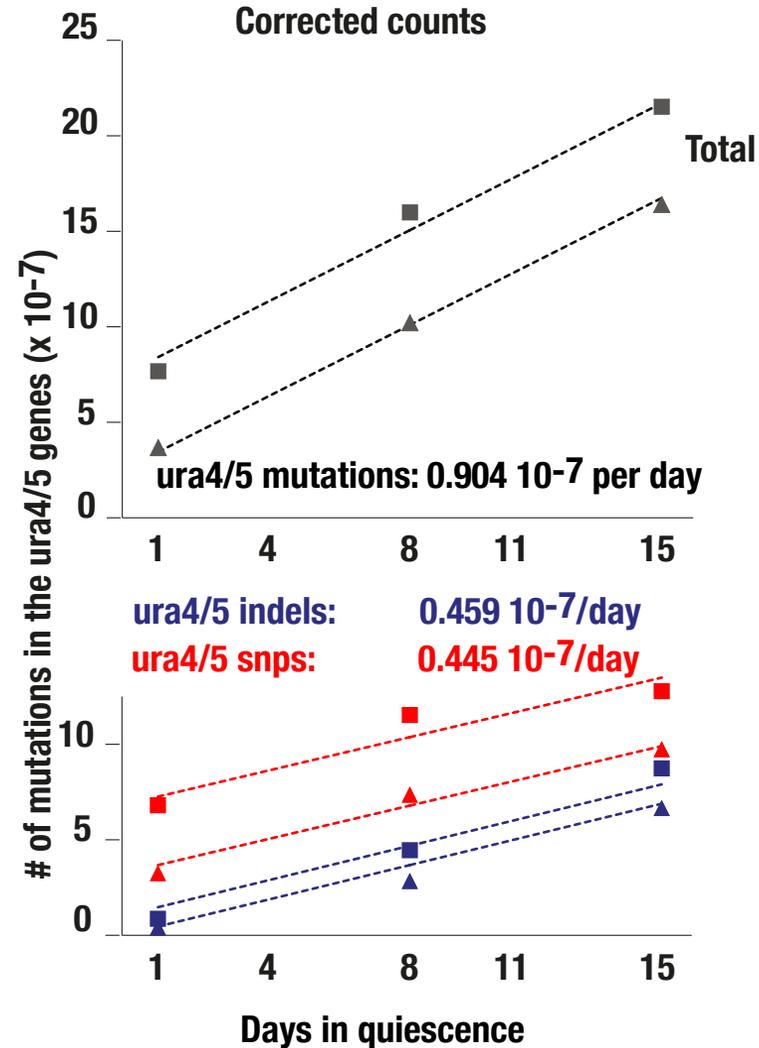
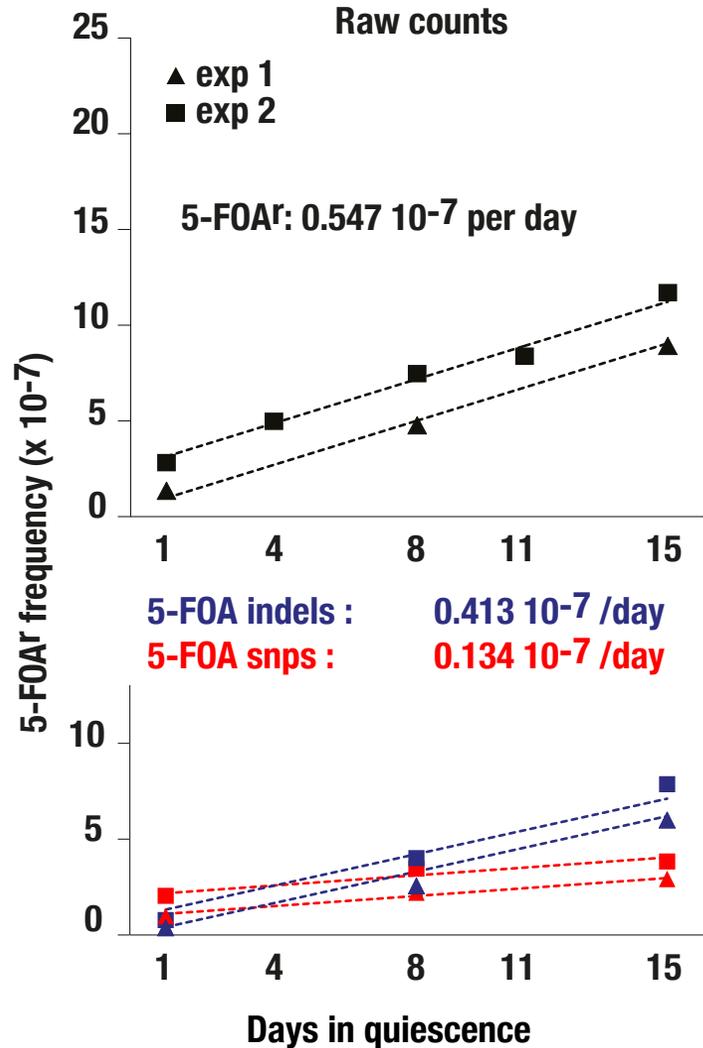
D1: AT bias = 67%

D8+D15: AT bias = 51%

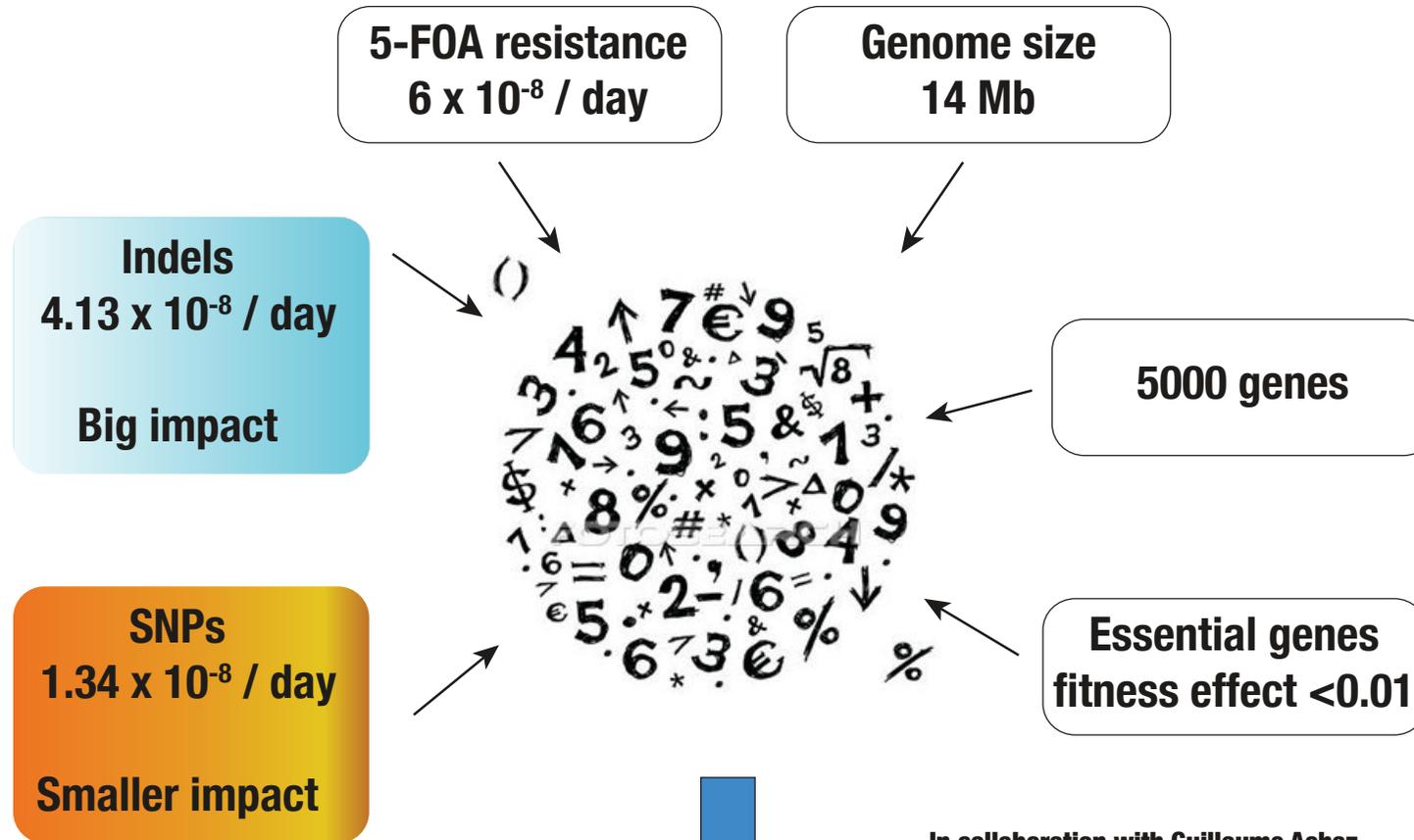
Suggesting that in quiescence:

- 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?

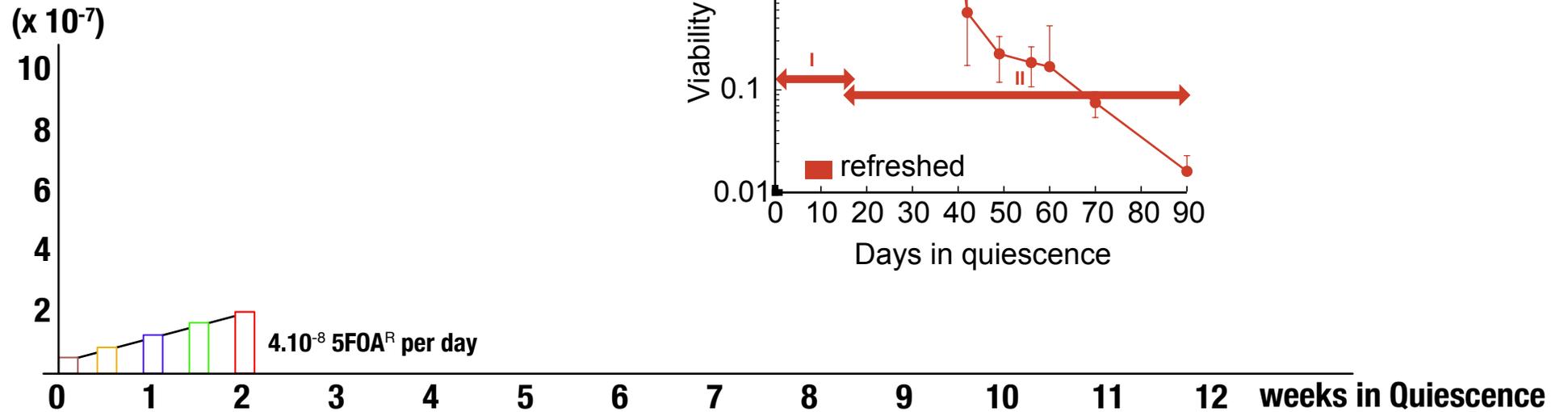
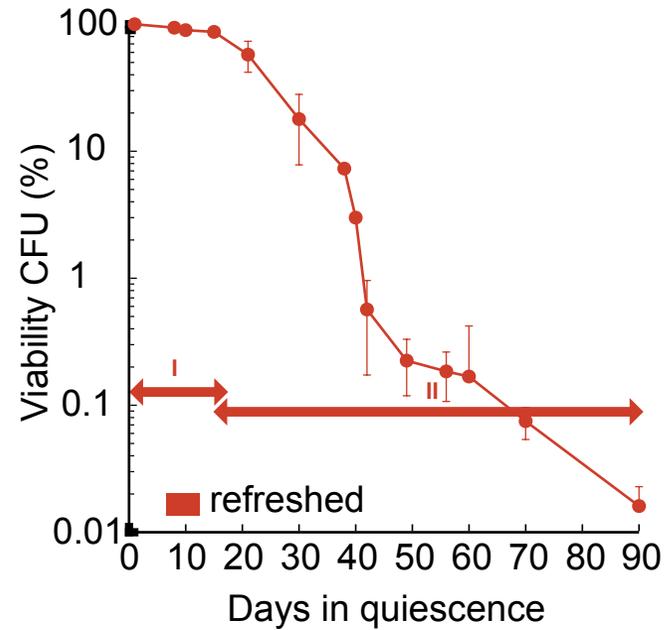


**3 months of quiescence should result in
 ≈ 1 mutation / genome**



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

Refreshed conditions



Spontaneous 5FOA^R mutants

1.3x10⁻⁸ 5FOA^R per generation

4.10⁻⁸ 5FOA^R per day



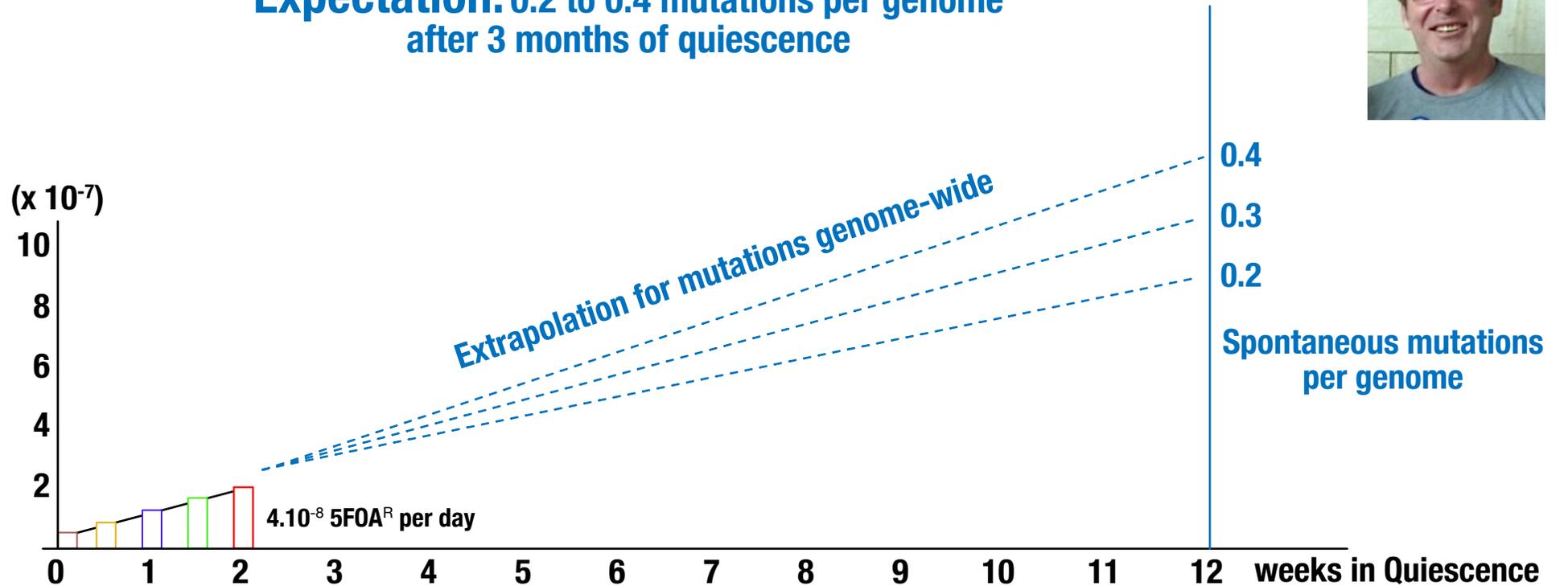
Accumulation of mutations genome-wide (*S. pombe* genome: 14 Mb)

Refreshed conditions

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

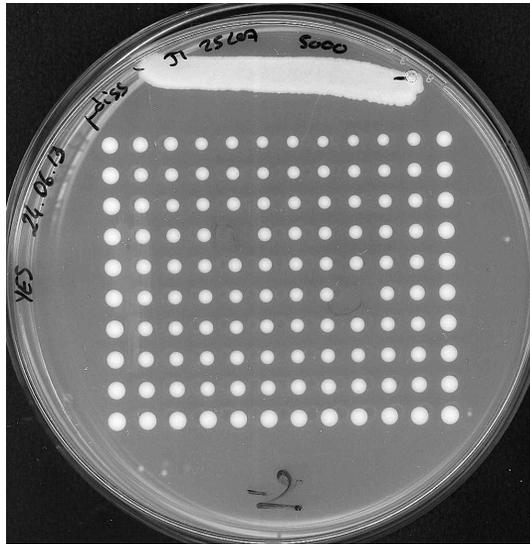
Spontaneous 5FOA^R
mutants

1.3×10^{-8} 5FOA^R
per generation



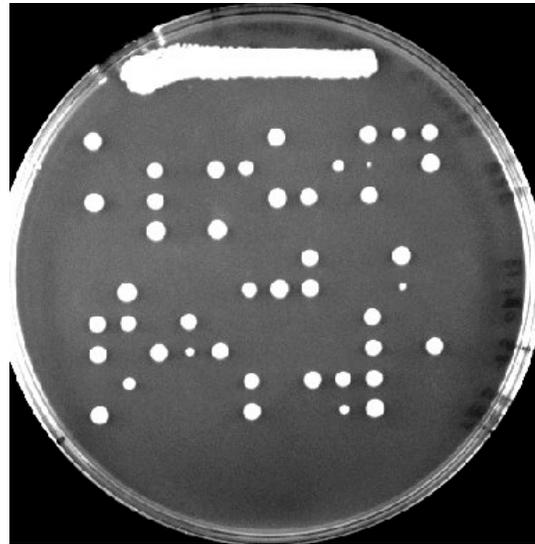
Long term quiescence (up to 3 months)

1 day



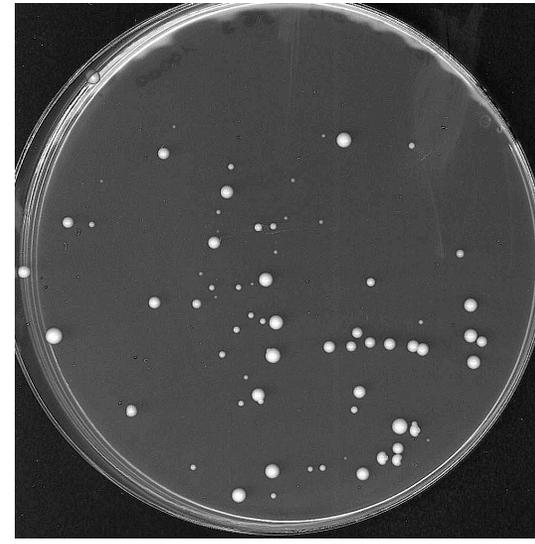
≈ 100%

21 days



≈ 40%

3 months



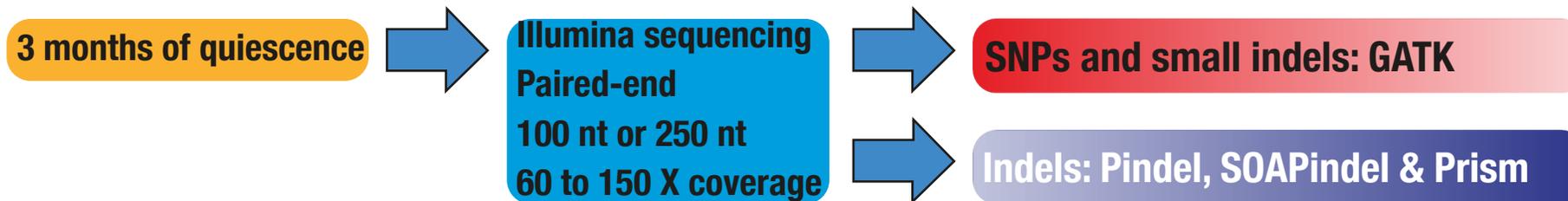
< 1%

**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 ₃) ₂ 0.15M	CaCl ₂ 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



3 months
243 genomes

0.6 mutation per
genome

72 SNPs
48%

77 Indels
52%

False discovery rate (FDR) SNPs < 5%, Indels \approx 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



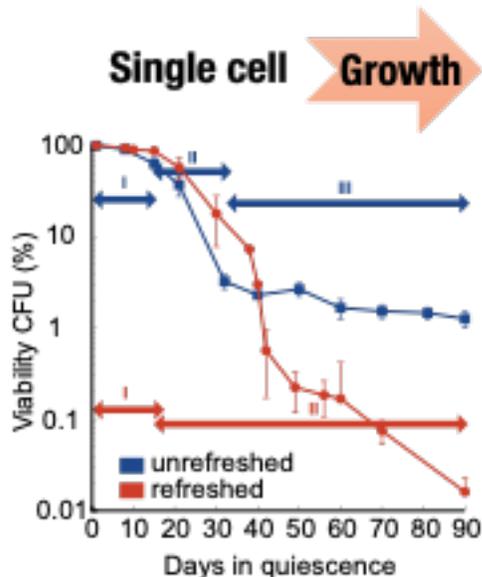
4.10⁷ cells



Unrefreshed conditions

1% survivors

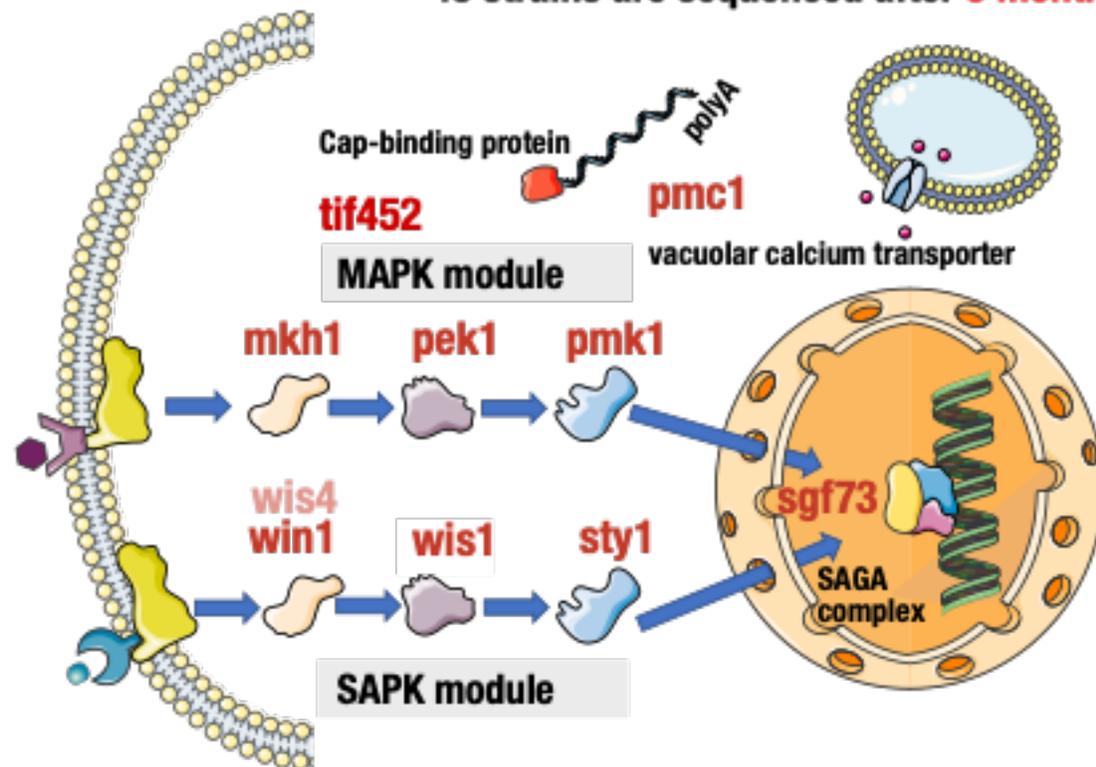
2-3 months of quiescence



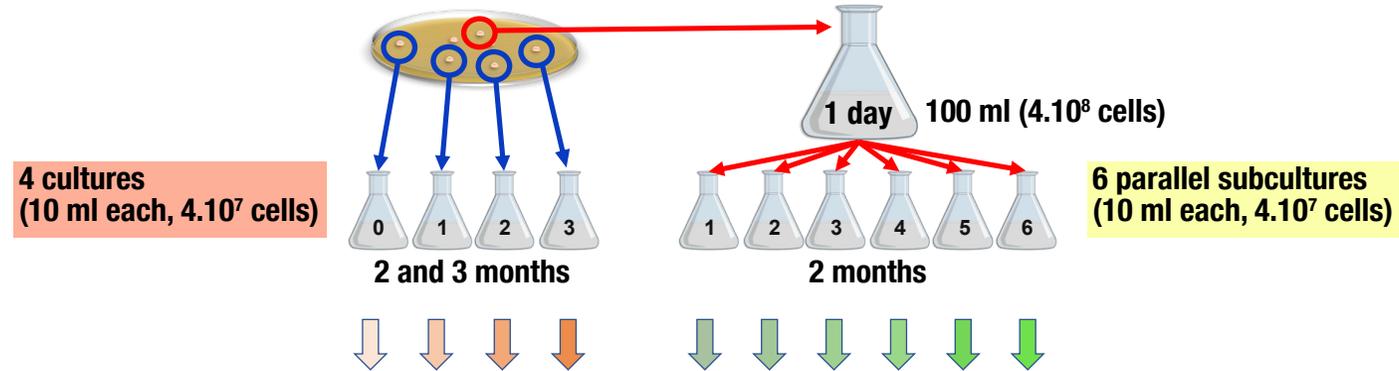
52 mutations (>1 per genome), including 25 mutants in the MAPK and SAPK pathways

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

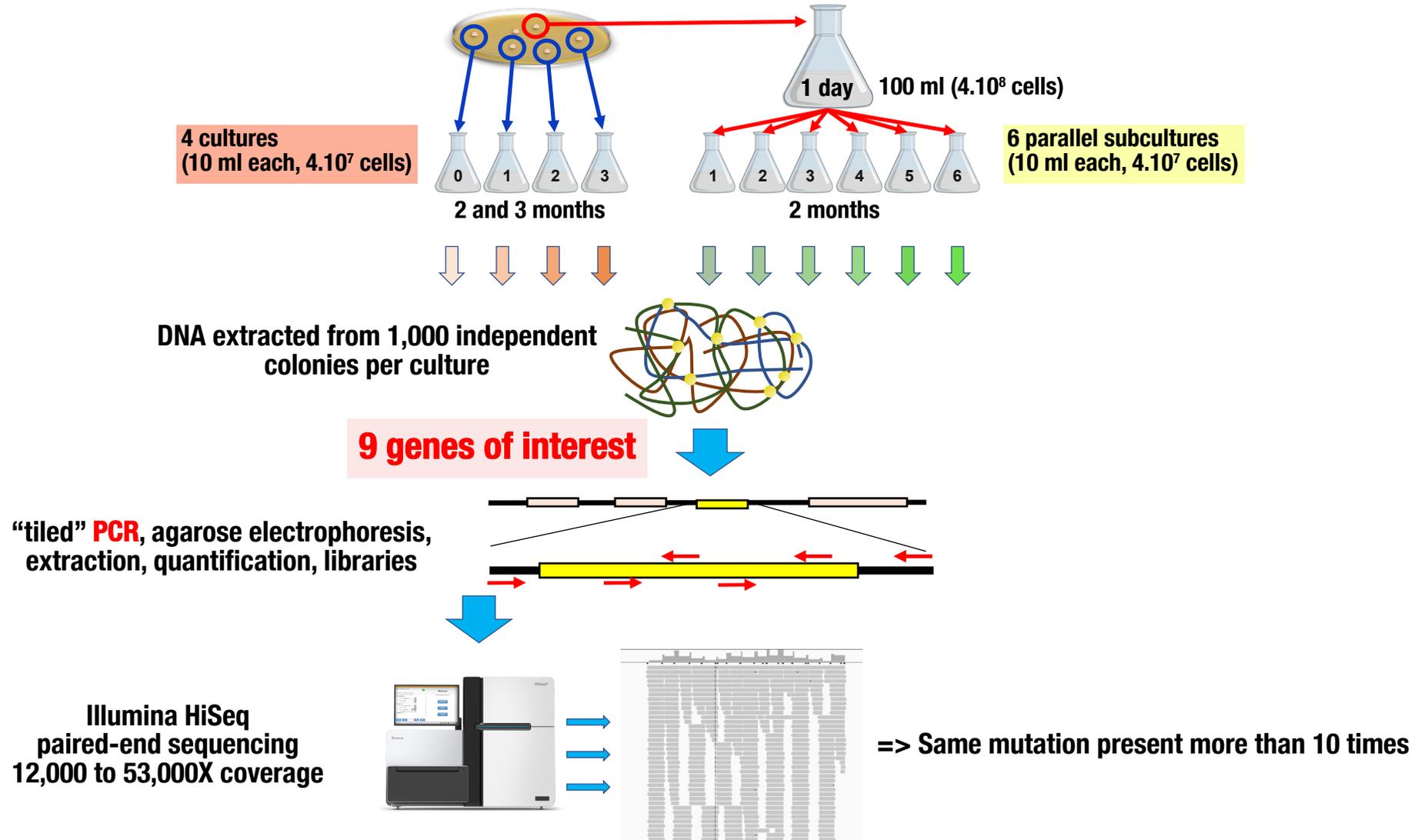
48 strains are sequenced after 3 months



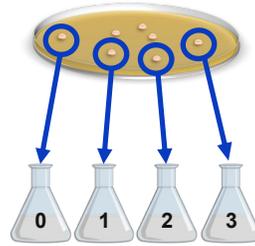
Distribution of the S/MAPK mutants at the population level: Targeted sequencing approach



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



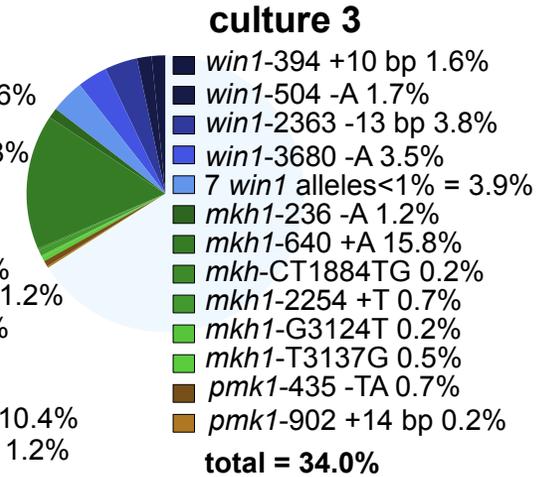
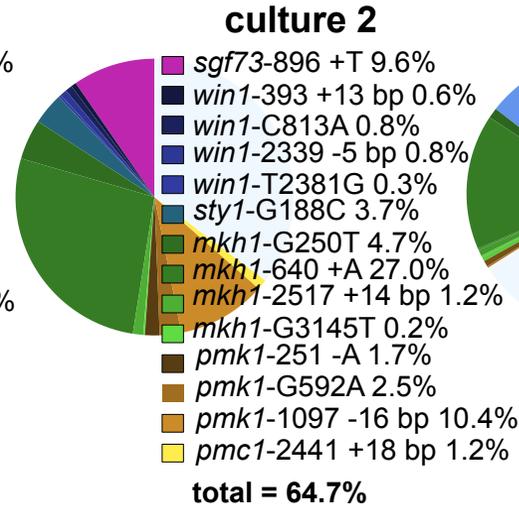
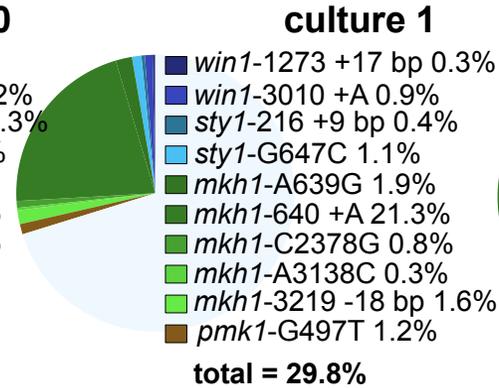
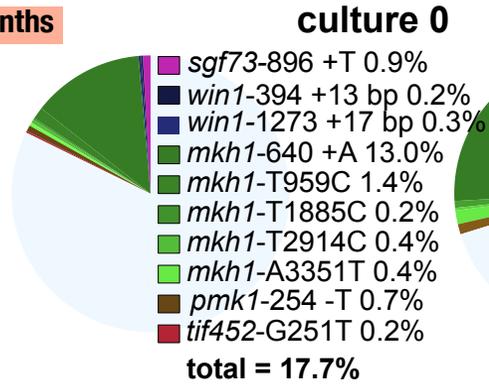
Targeted sequencing



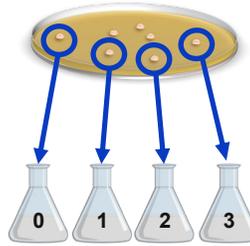
4 clonal cultures

PCR and sequencing

2 months

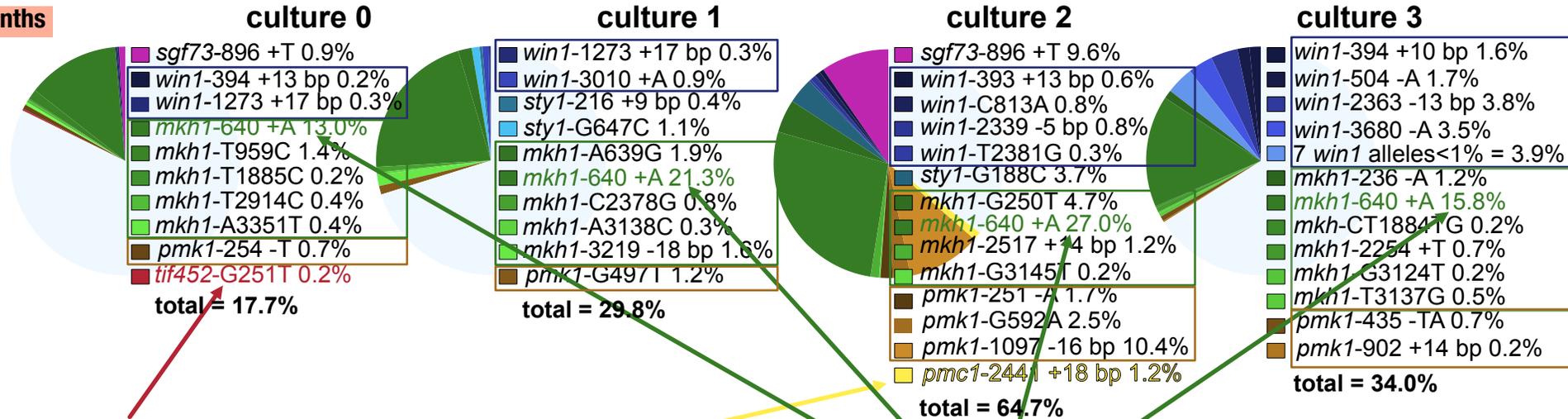


Targeted sequencing



PCR and sequencing

2 months



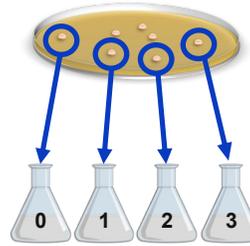
tif452-G251T is found only once

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

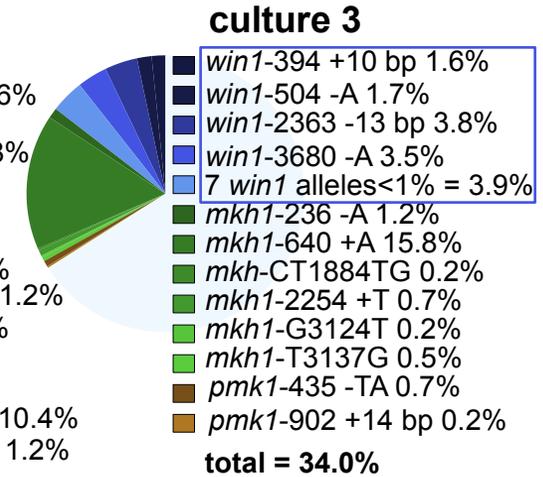
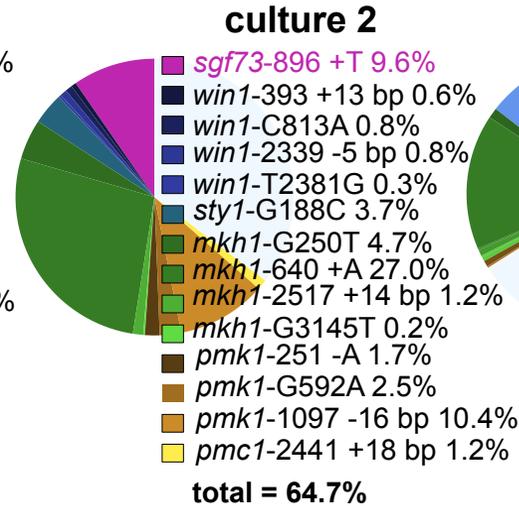
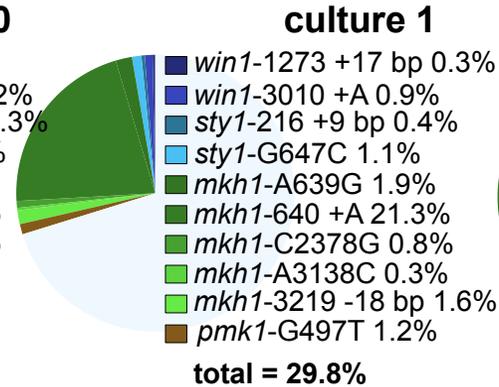
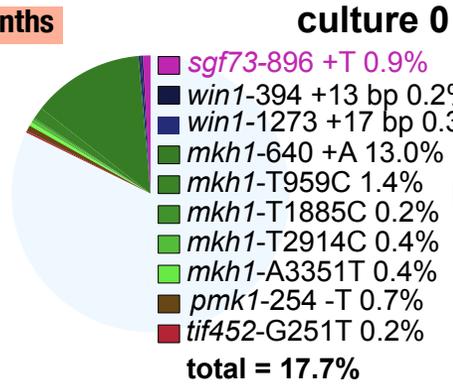
Targeted sequencing



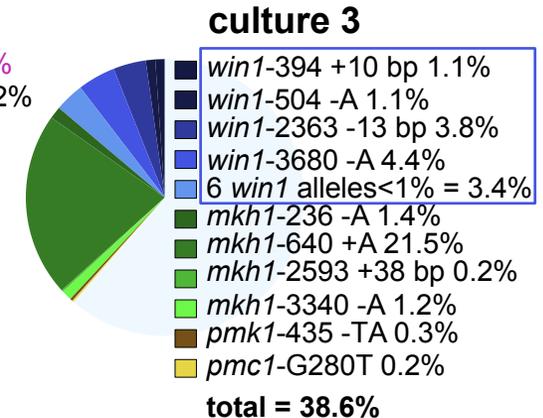
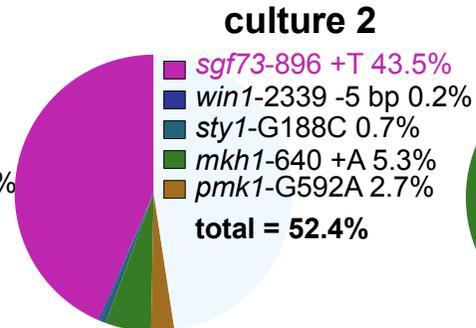
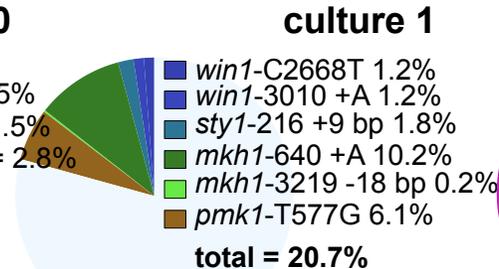
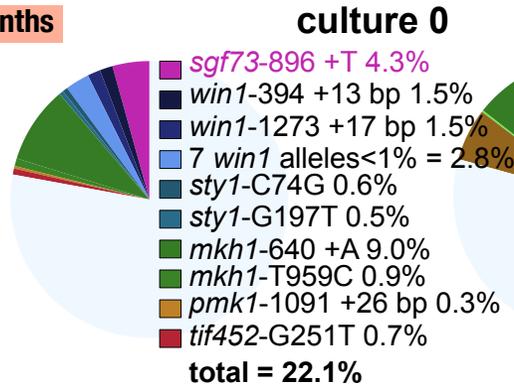
4 clonal cultures

PCR and sequencing

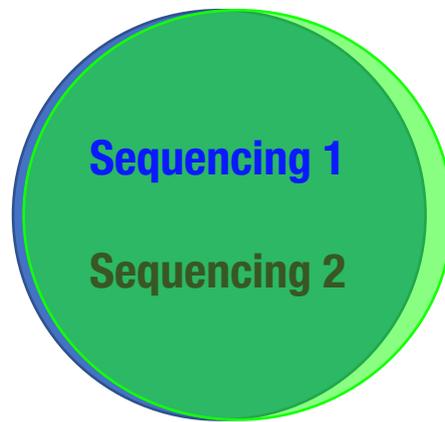
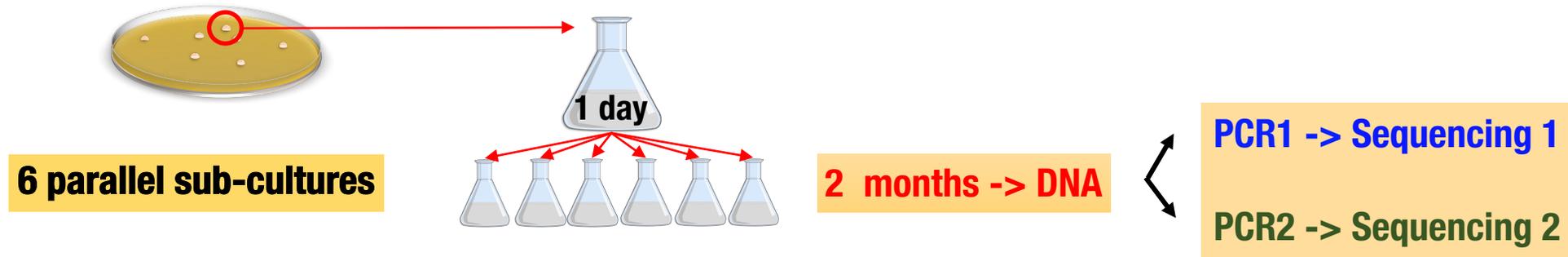
2 months



3 months

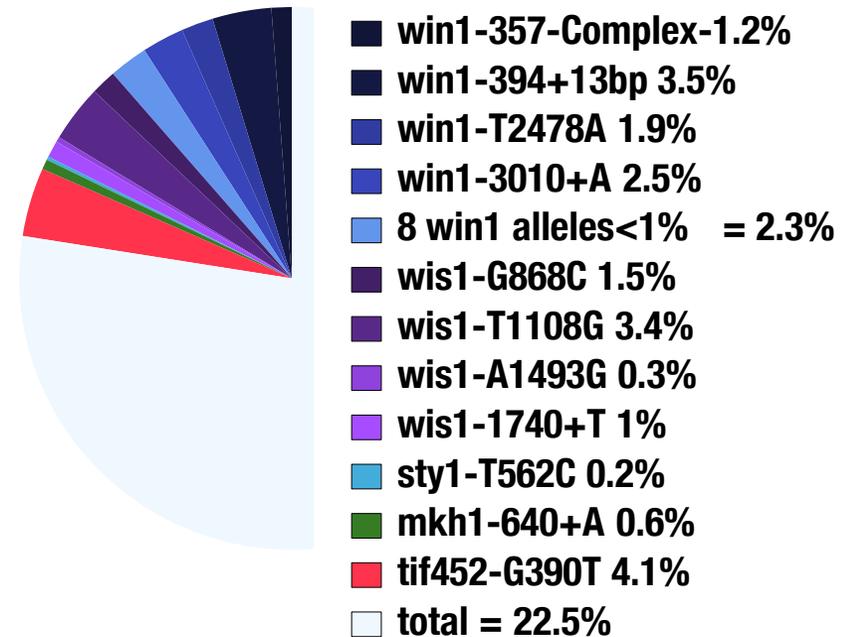


Targeted sequencing

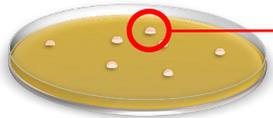


very similar

subculture 1



Targeted sequencing



1 day

6 parallel sub-cultures

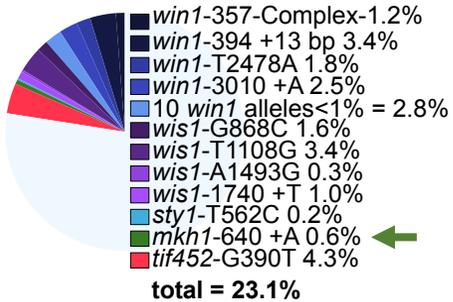


2 months -> DNA

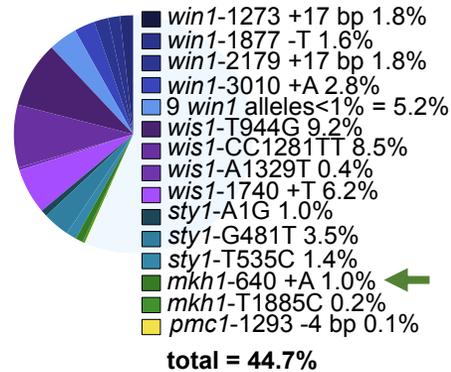
PCR1 -> Sequencing 1

PCR2 -> Sequencing 2

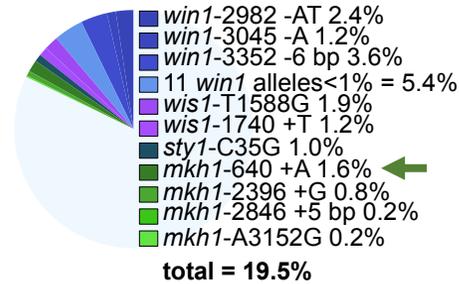
subculture 1



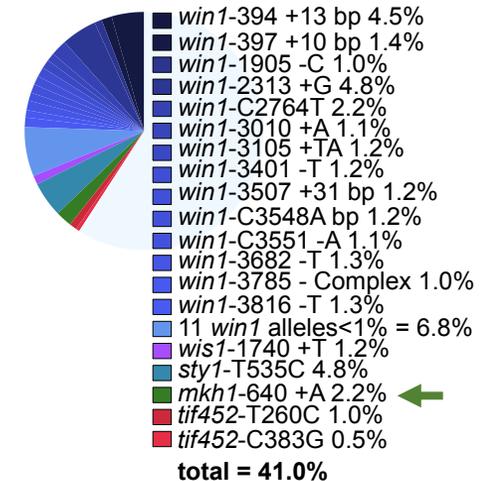
subculture 3



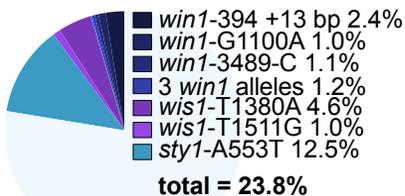
subculture 4



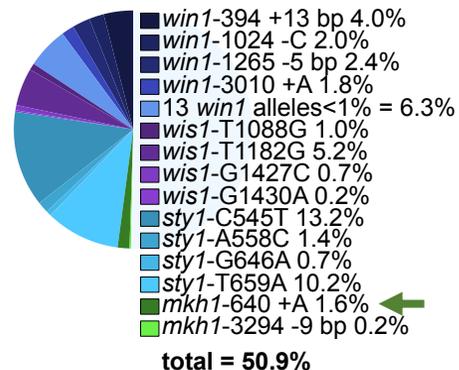
subculture 6



subculture 2

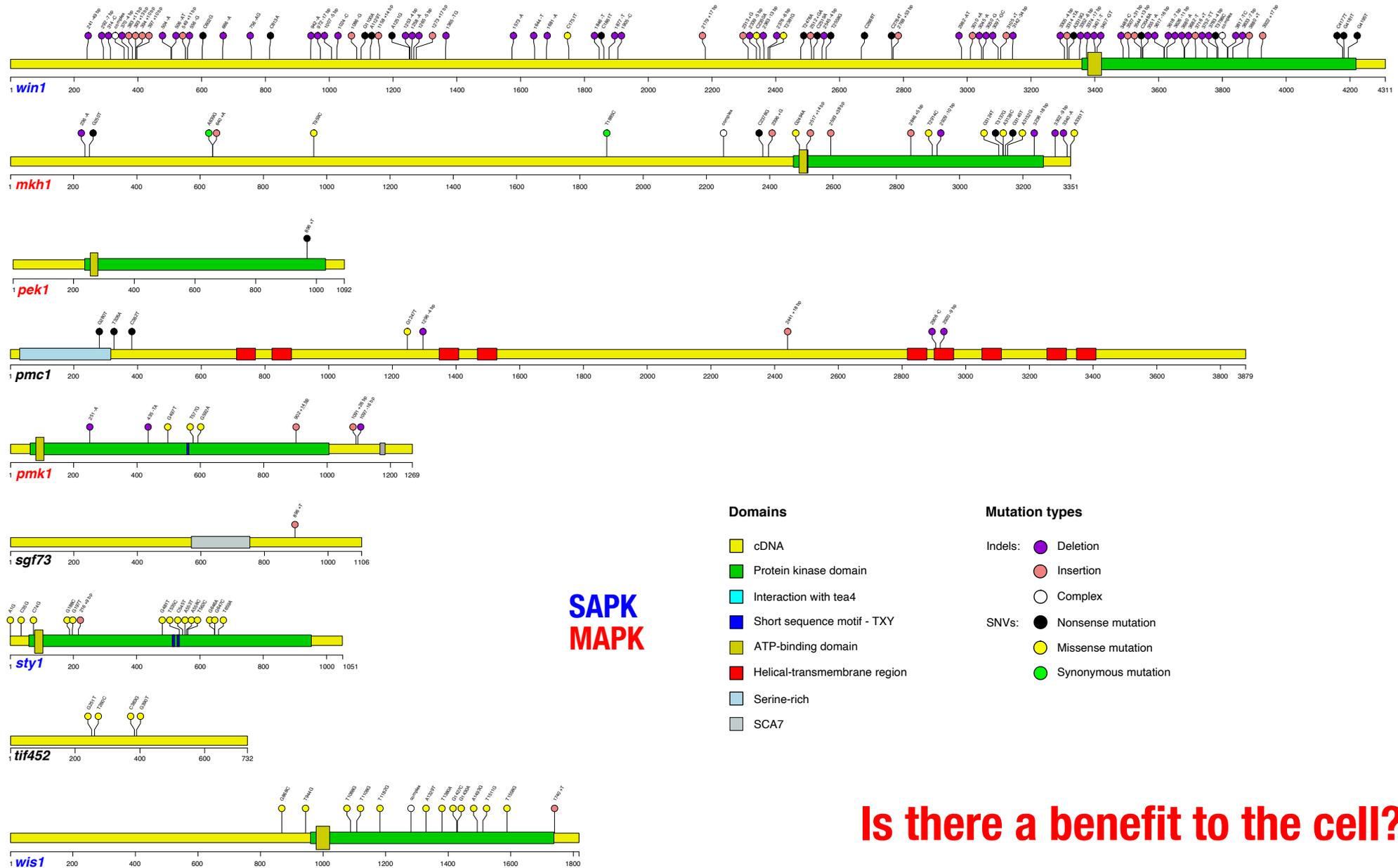


subculture 5



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

Detail of the 195 individual mutations recovered



**SAPK
MAPK**

Domains

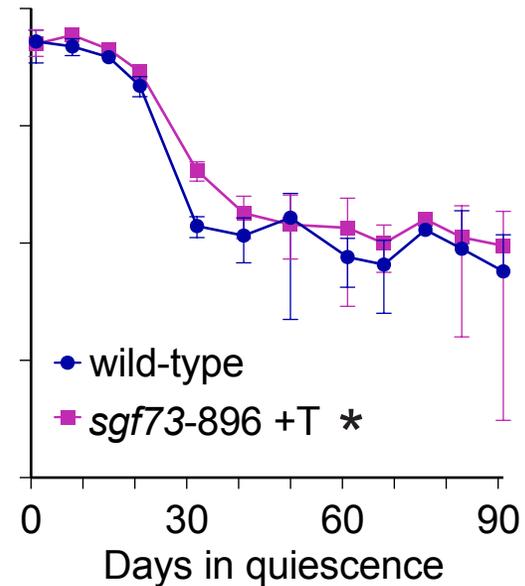
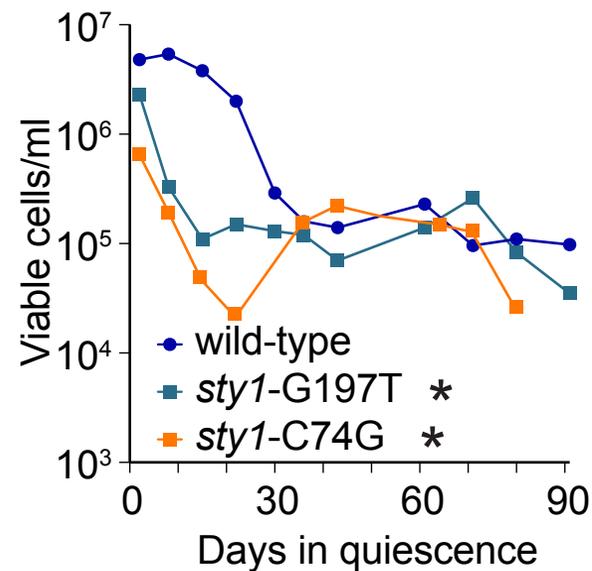
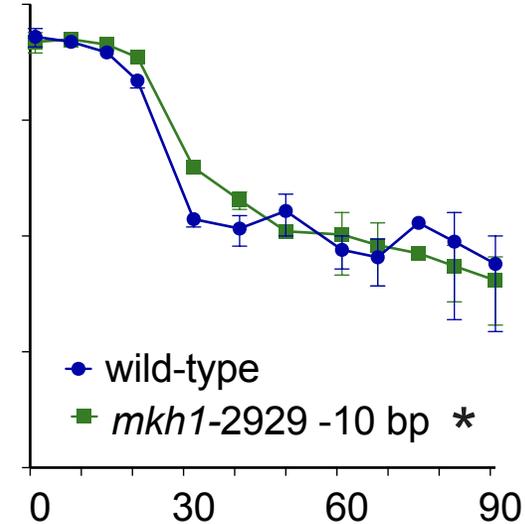
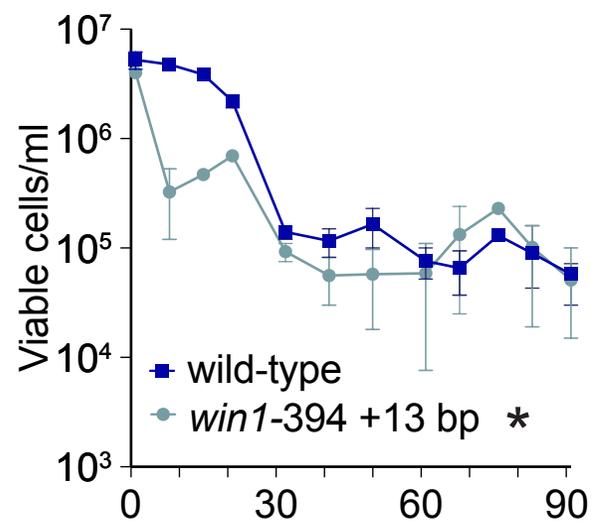
- cDNA
- Protein kinase domain
- Interaction with tea4
- Short sequence motif - TXY
- ATP-binding domain
- Helical-transmembrane region
- Serine-rich
- SCA7

Mutation types

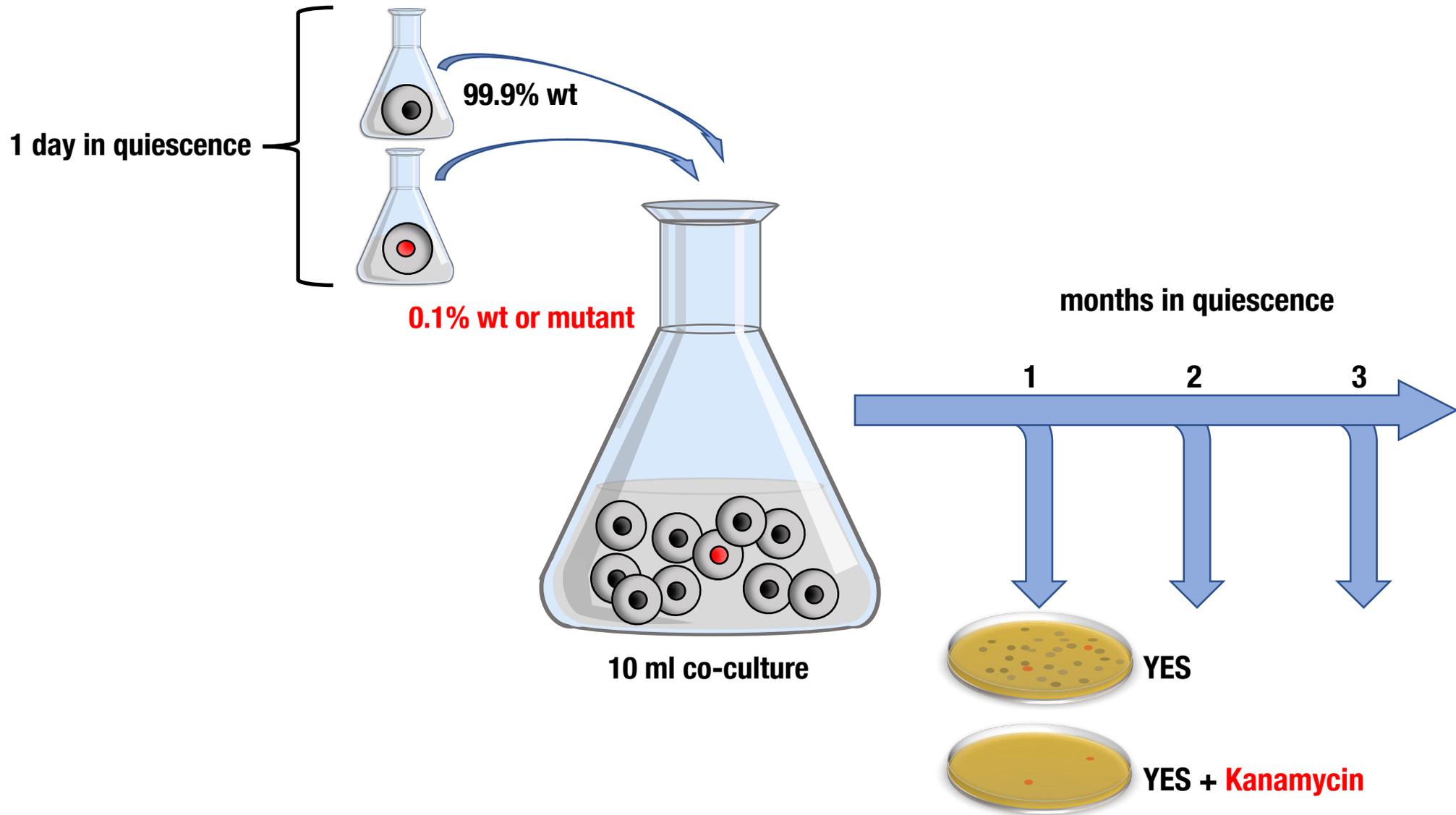
- Indels: Deletion
- Insertion
- Complex
- SNVs: Nonsense mutation
- Missense mutation
- Synonymous mutation

Is there a benefit to the cell?

WT and mutants behave alike in pure cultures

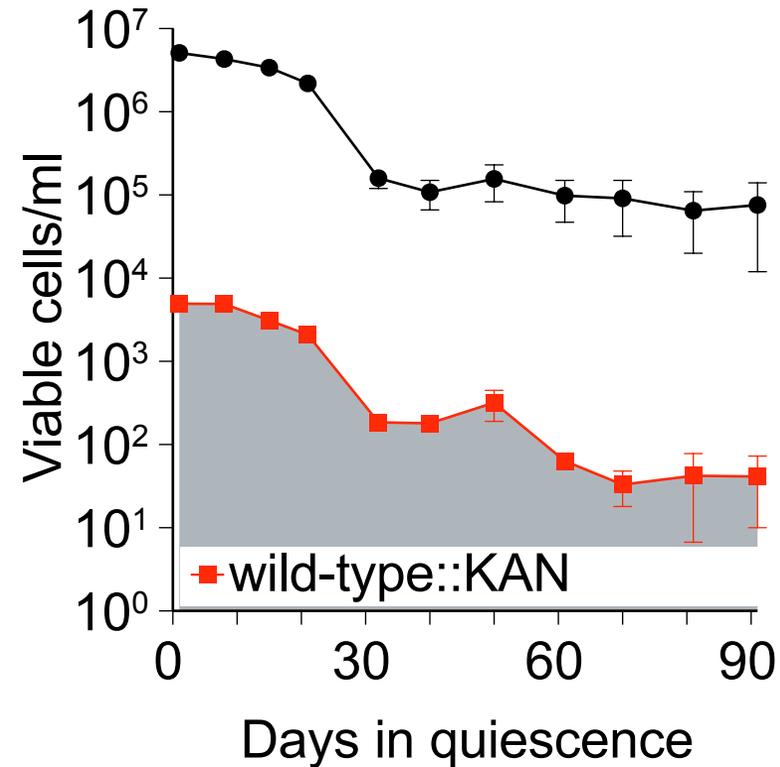


Co-cultures in quiescence



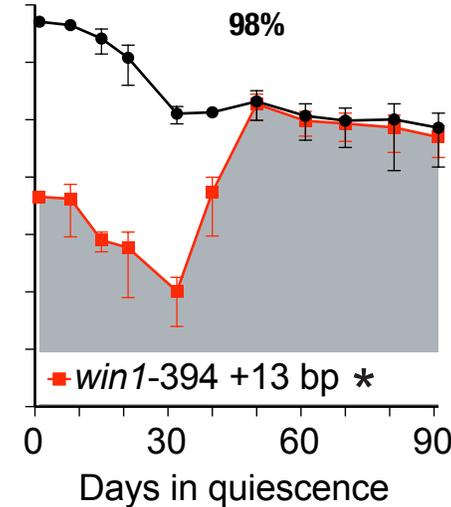
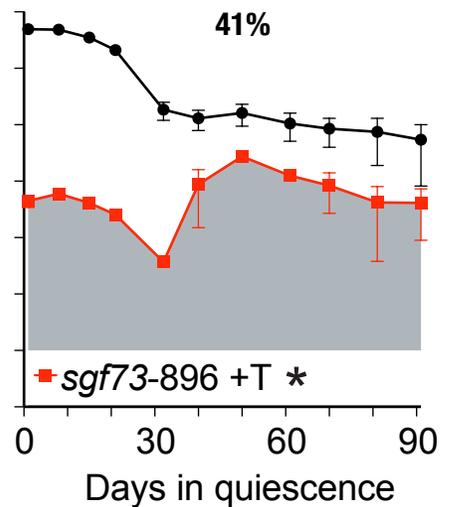
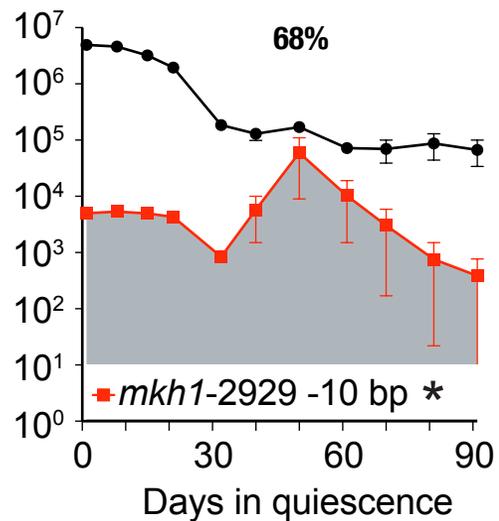
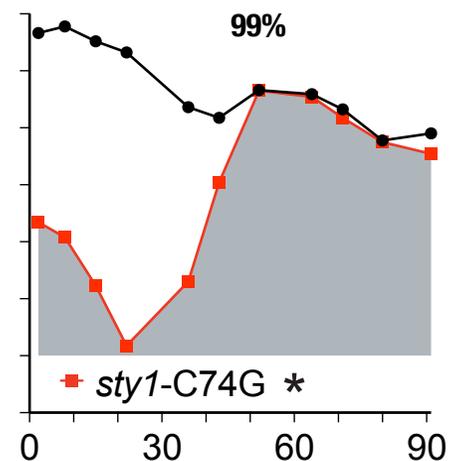
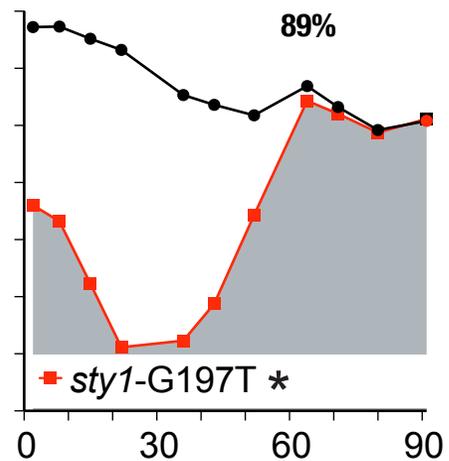
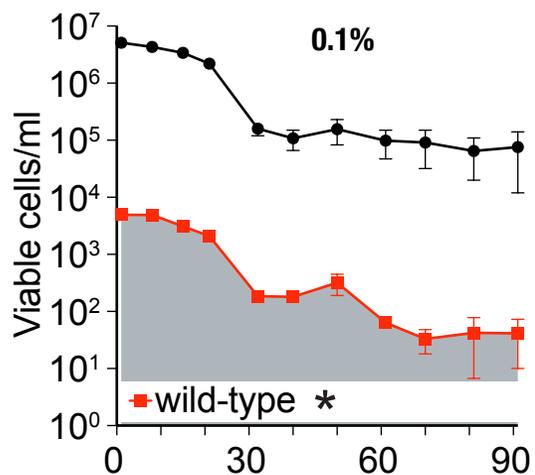
Co-cultures in quiescence

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



The proportion of KAN resistant cells is constant throughout the experiment

Co-cultures in quiescence

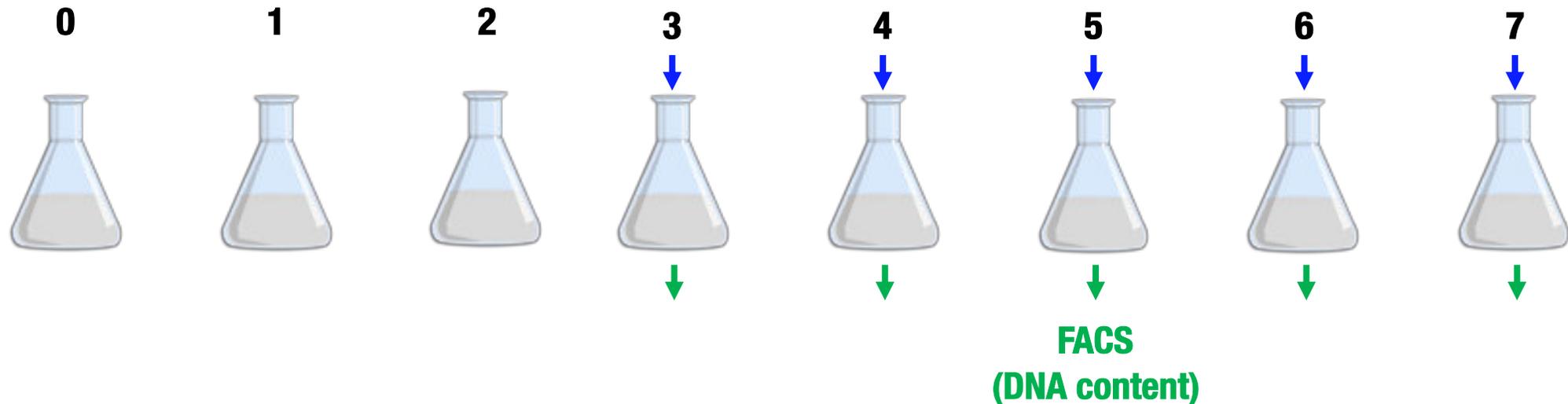


Is a slow release of nitrogen responsible for cell death?

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

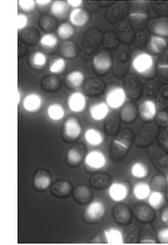
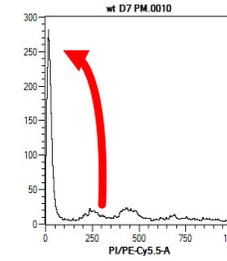
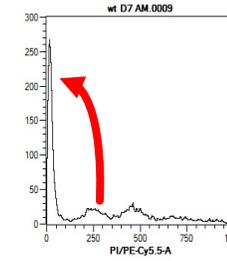
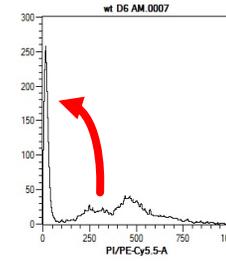
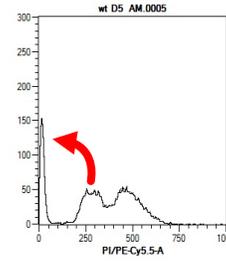
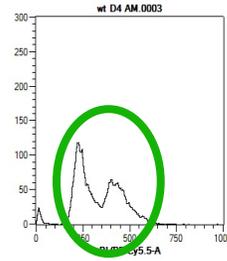
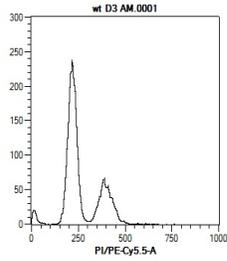
5

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

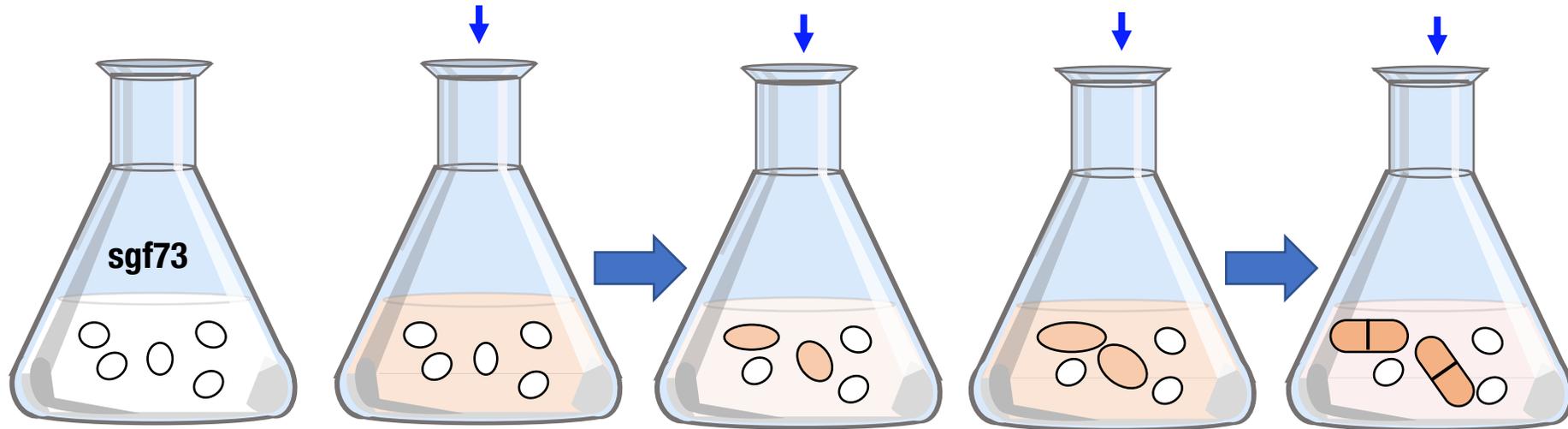
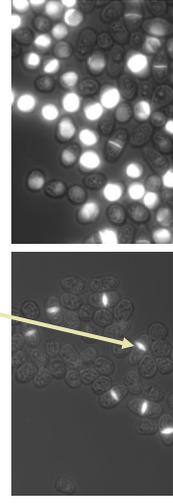
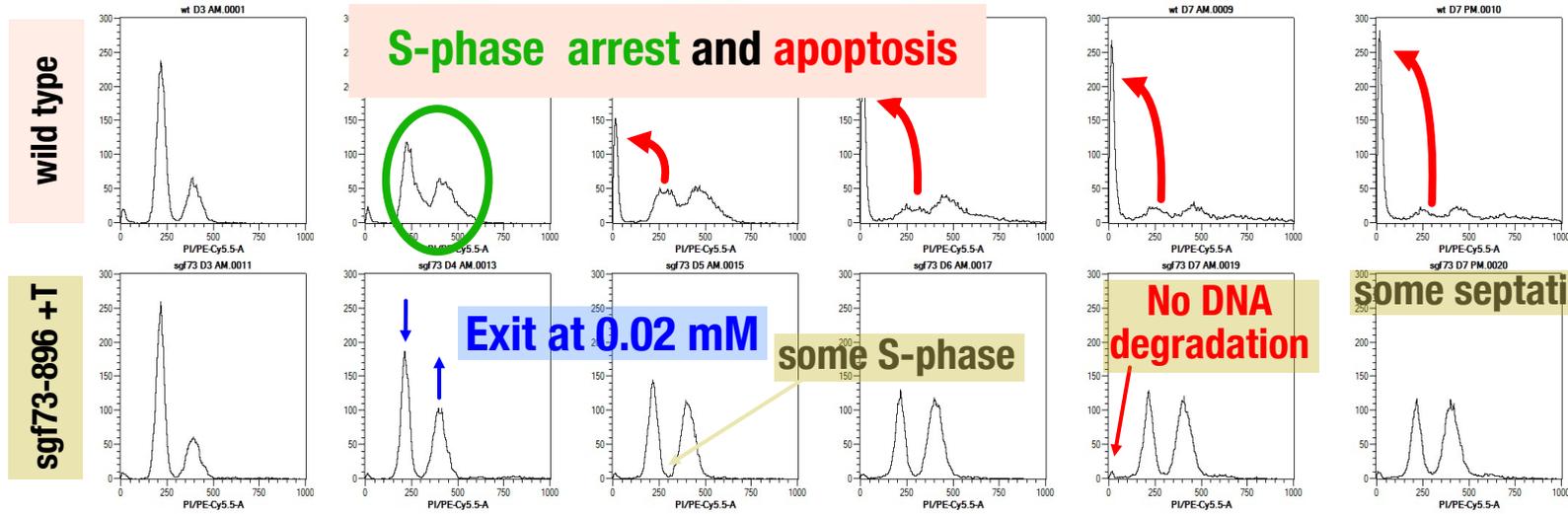
4

5

6

7

8



+ 0.02 mM Glutamate (every day)

Calcofluor staining at day 6

Days in GO

3

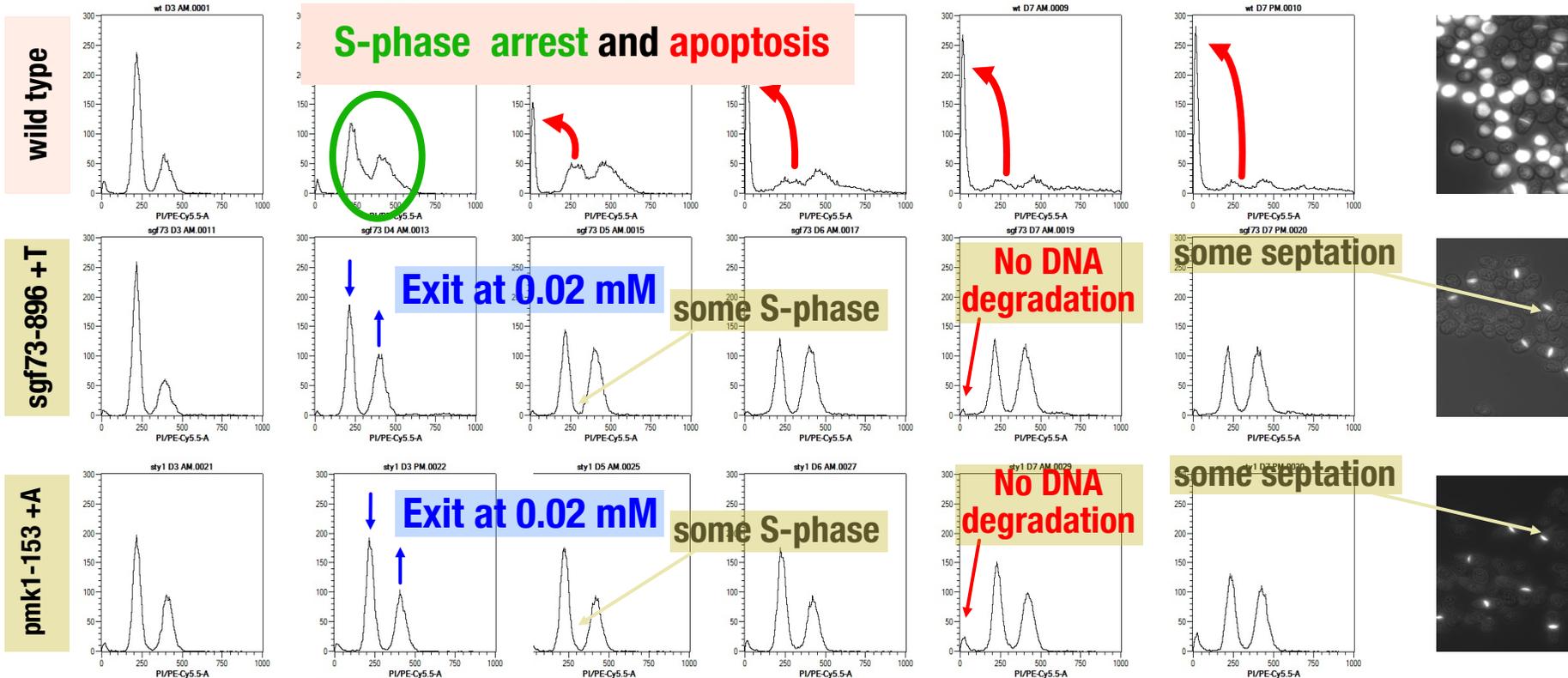
4

5

6

7

8



+ 0.02 mM Glutamate (every day)

Calcofluor staining at day 6

Days in G0

3

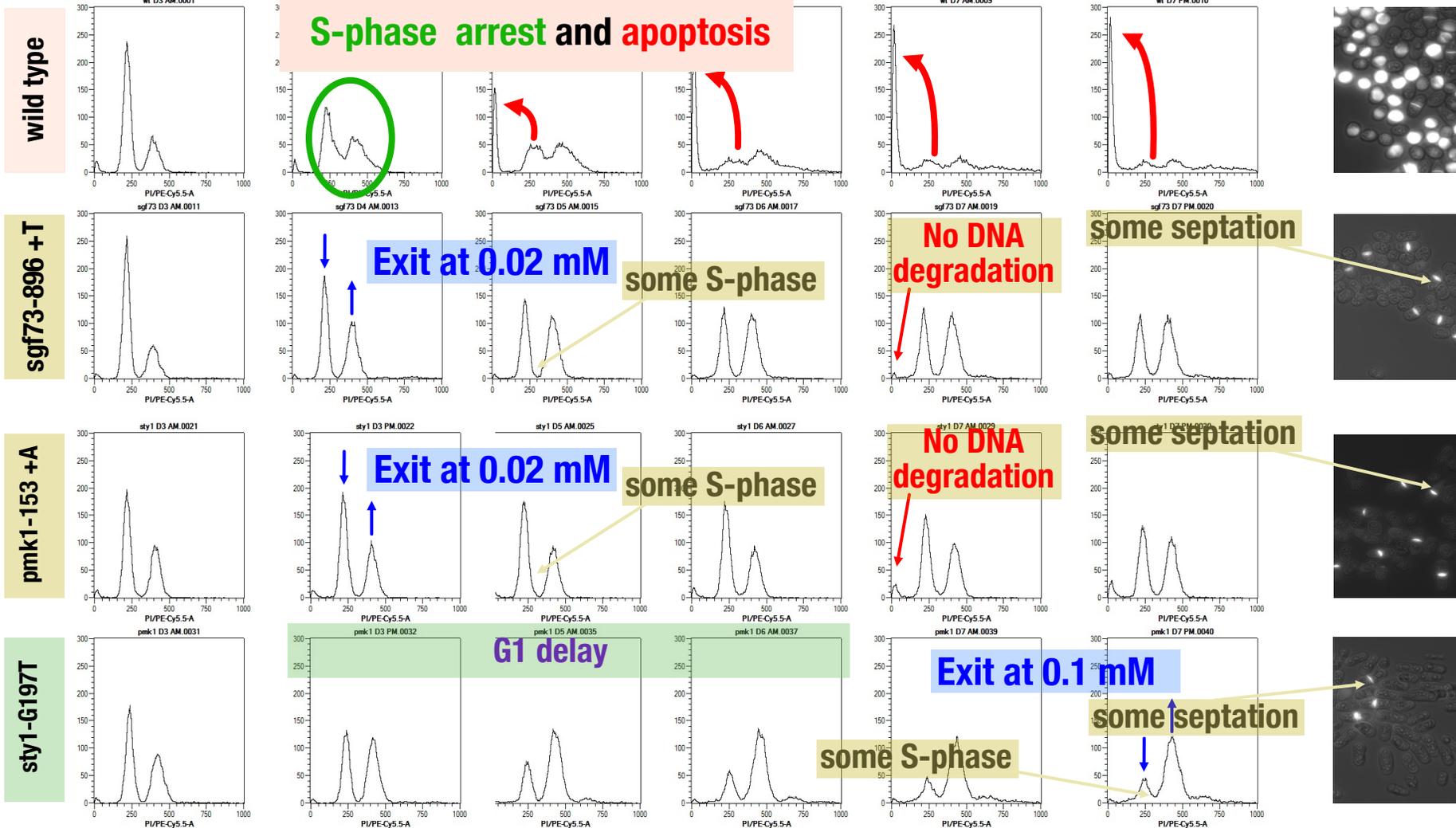
4

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.

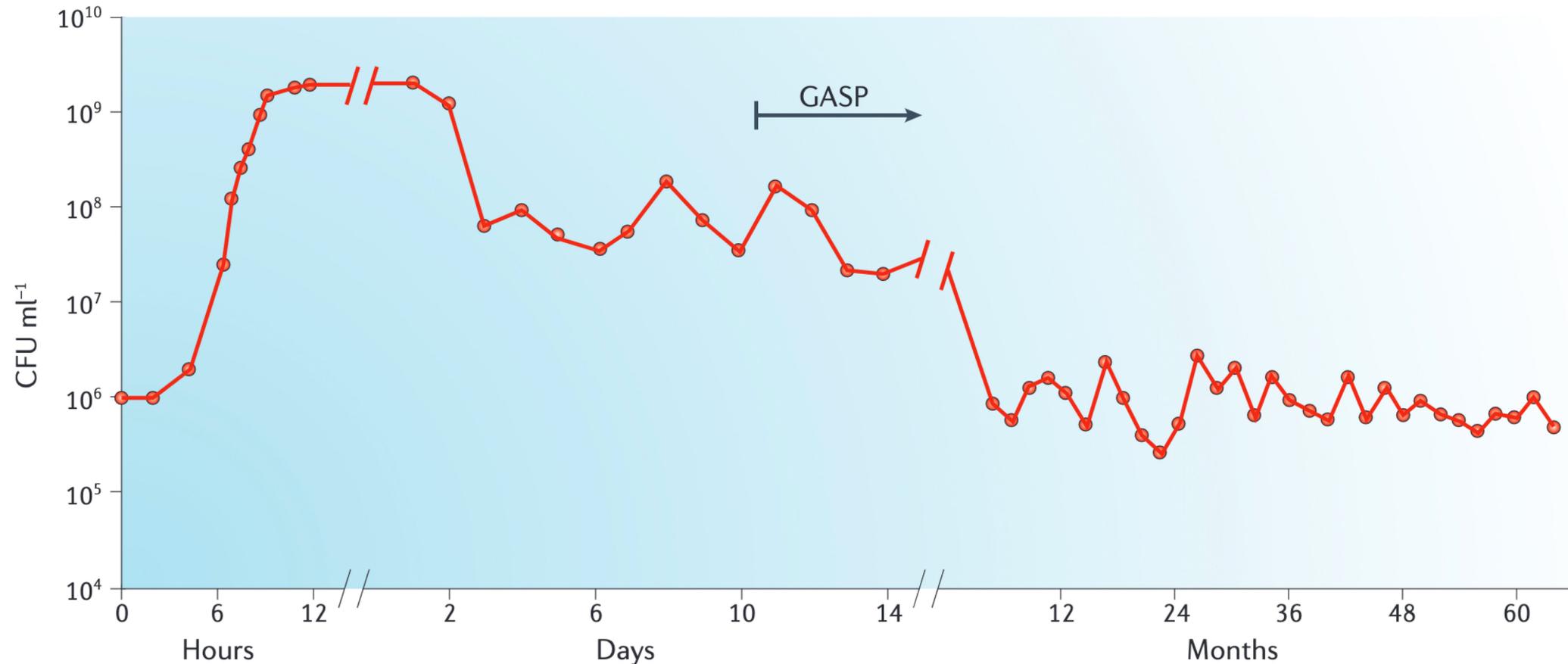
Microbial competition: Escherichia coli mutants that take over stationary phase cultures.

Zambrano MM¹, 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

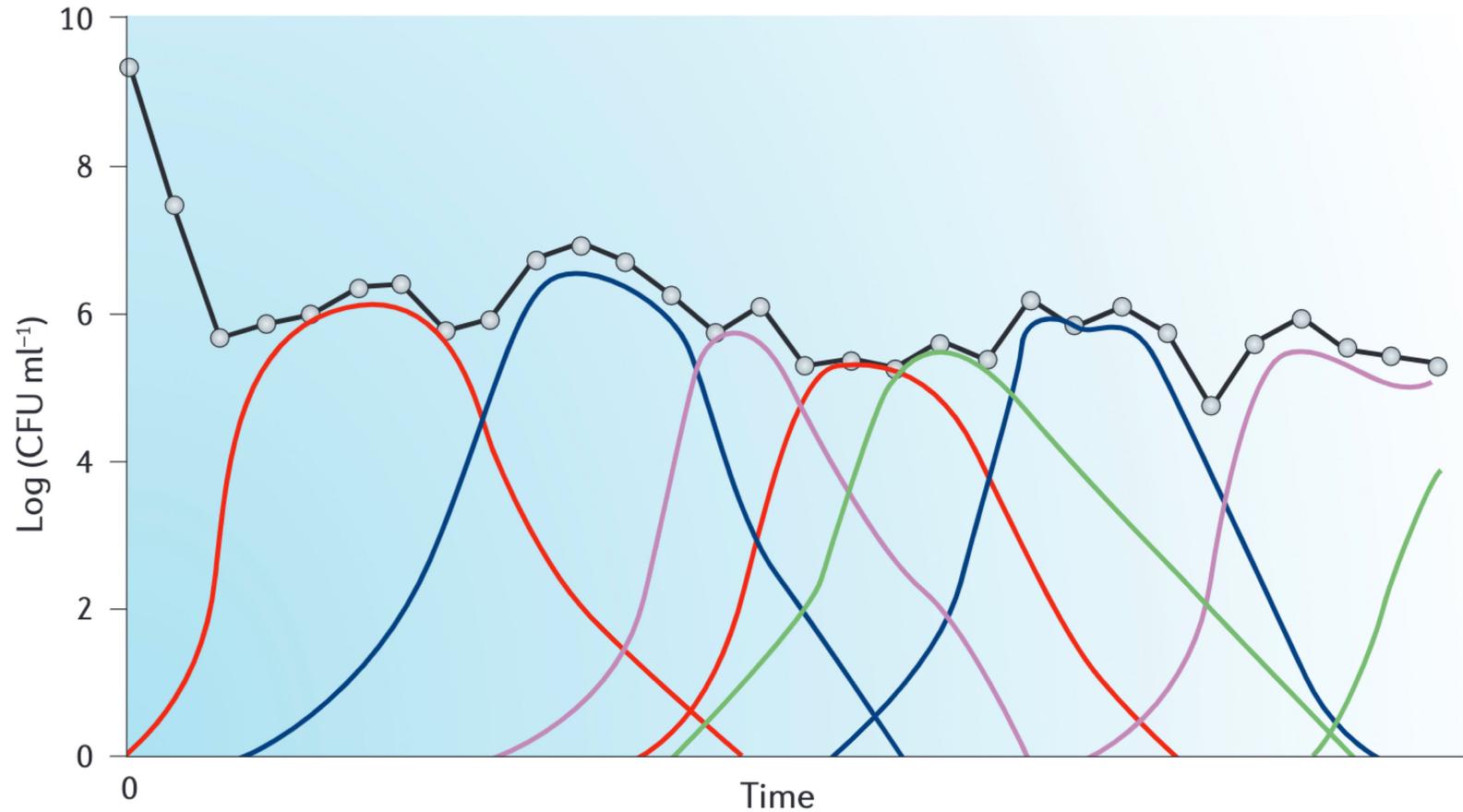
The five phases of the bacterial life cycle



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

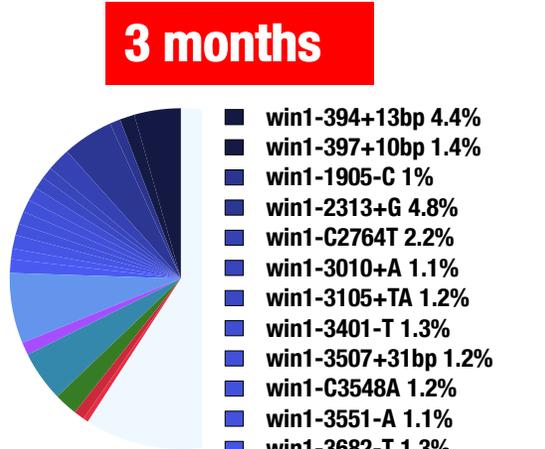
Population dynamics of long-term stationary-phase cultures



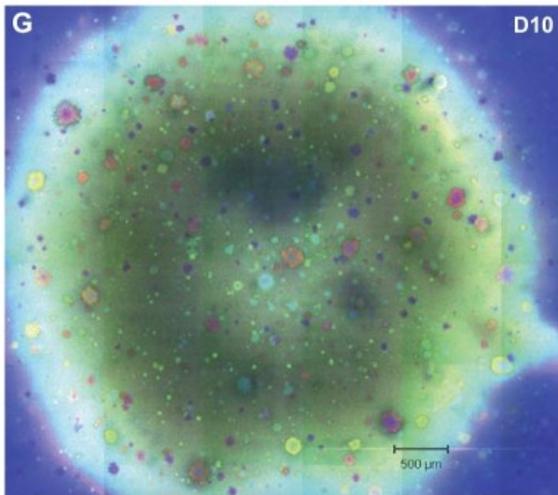
Finkel SE. (2006) Nature Reviews Microbiology, 4(2), 113–120. doi: 10.1038/nrmicro1340

Mutations as a function of time/age

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

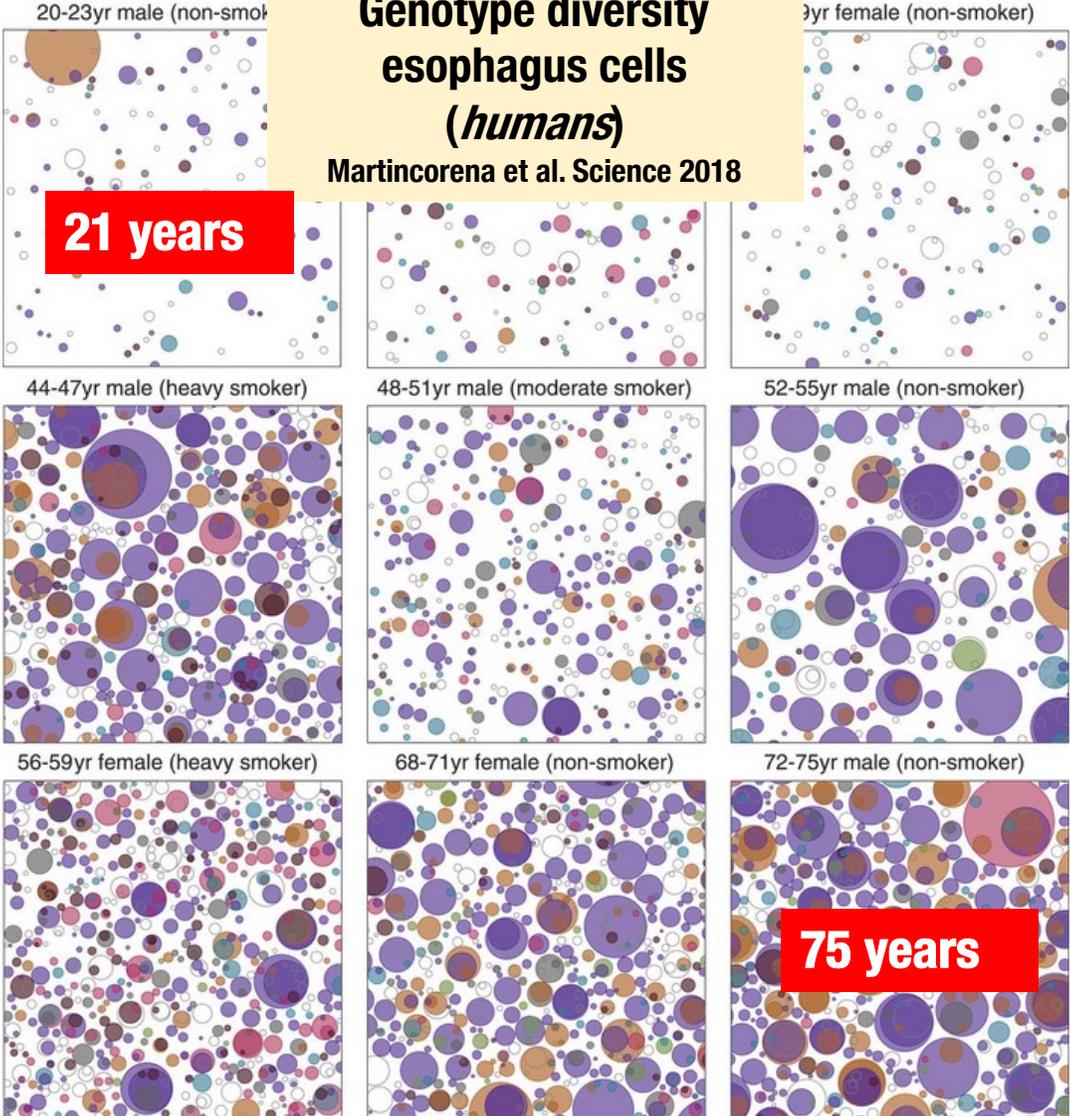


10 days

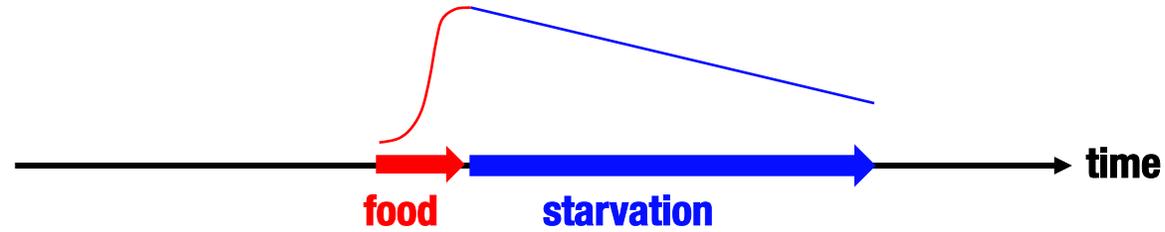


RpoS
Genotype diversity
in old colony
(*E. coli*)
Saint-Ruf et al. J. Bact 2014

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 Maestroni¹, Julien Audry¹, Samah Matmati¹, Benoit Arcangioli², Vincent Géli ¹ & Stéphane Coulon¹

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

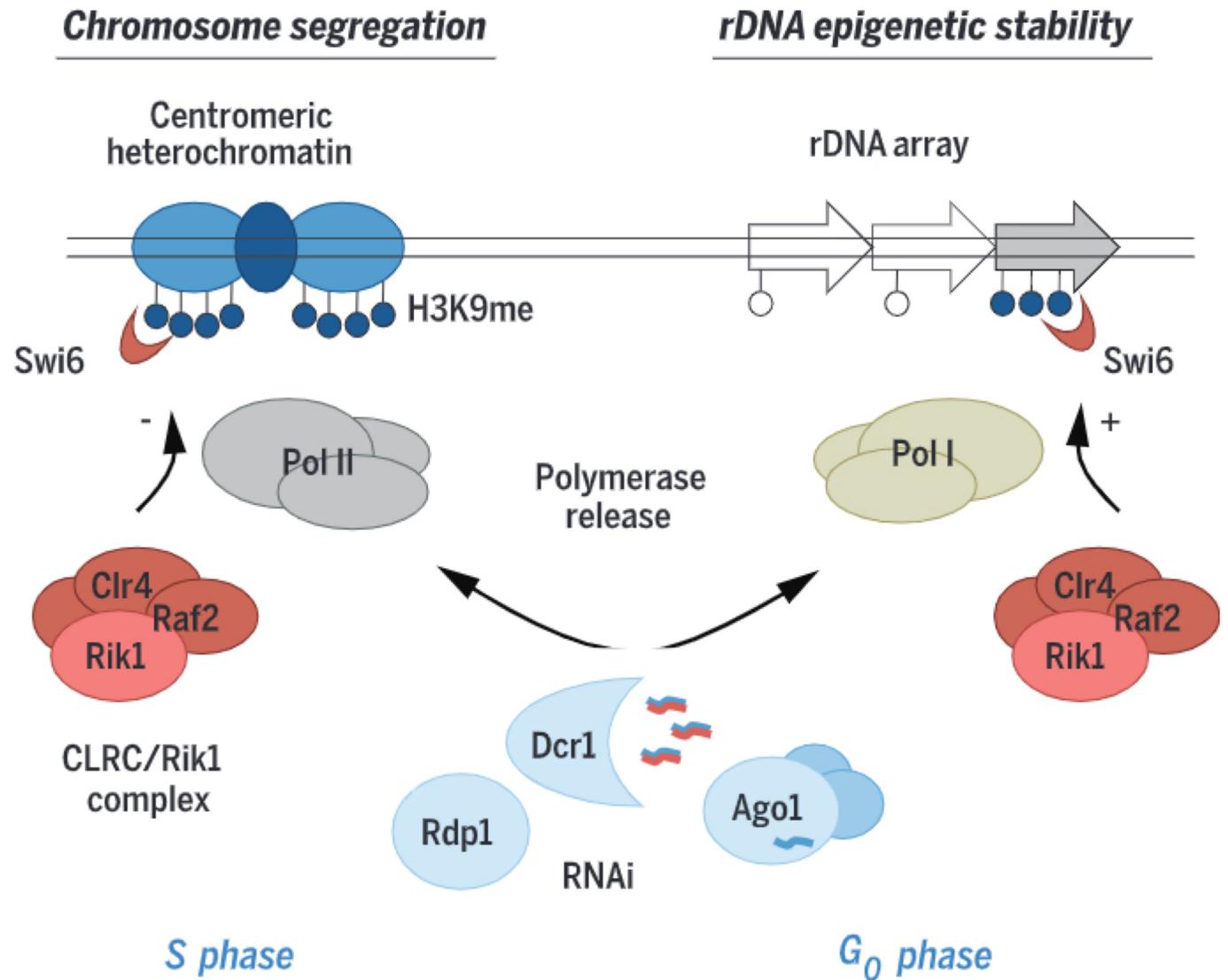
Eriko Oya¹, Mickaël Durand-Dubief¹, Adiel Cohen², Vladimir Maksimov¹, Catherine Schurra³, Jun-ichi Nakayama⁴, Ronit Weisman², Benoit Arcangioli³ and Karl Ekwall^{1*} 

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

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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

