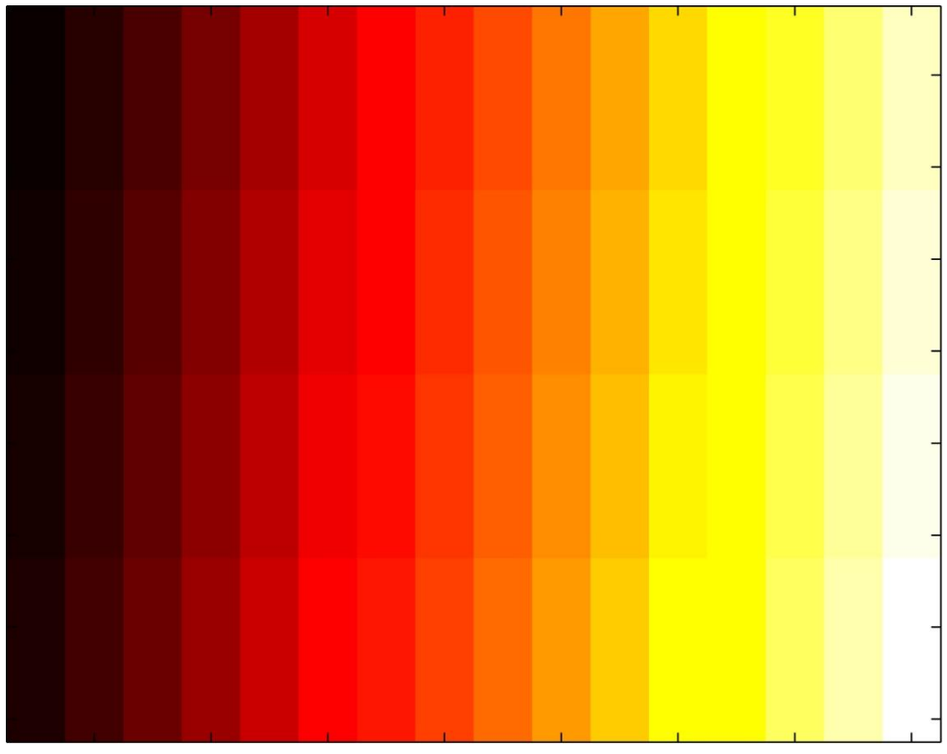
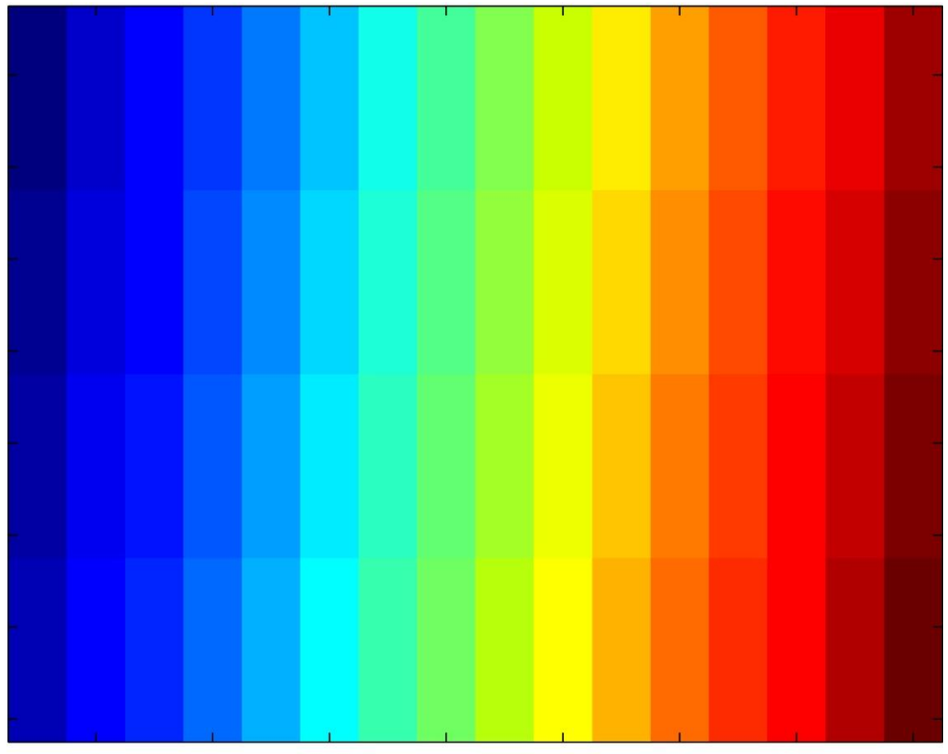
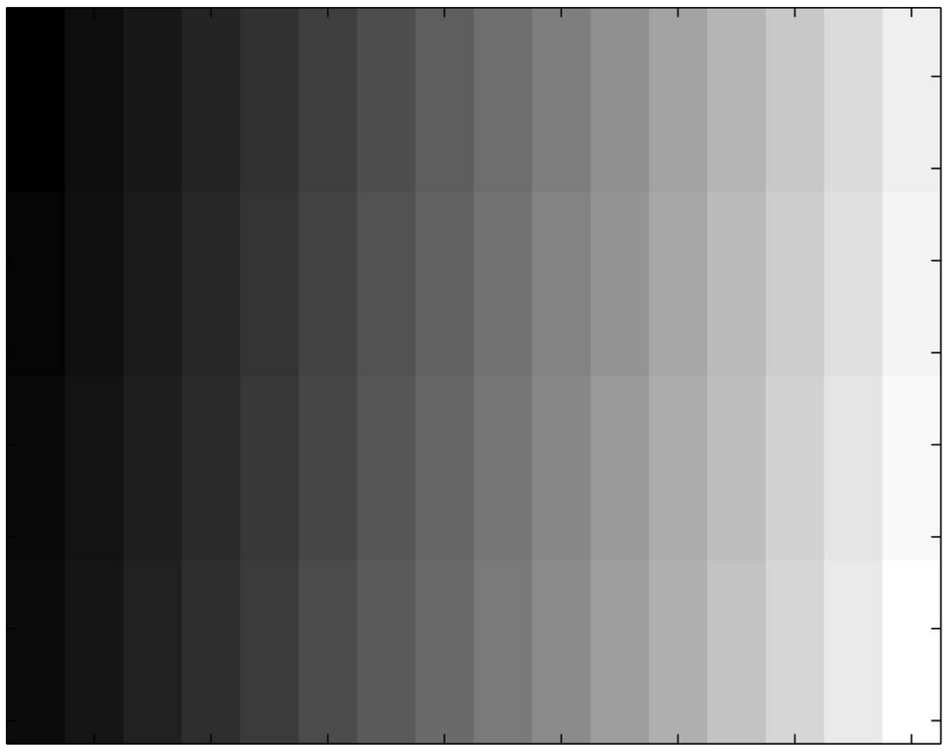
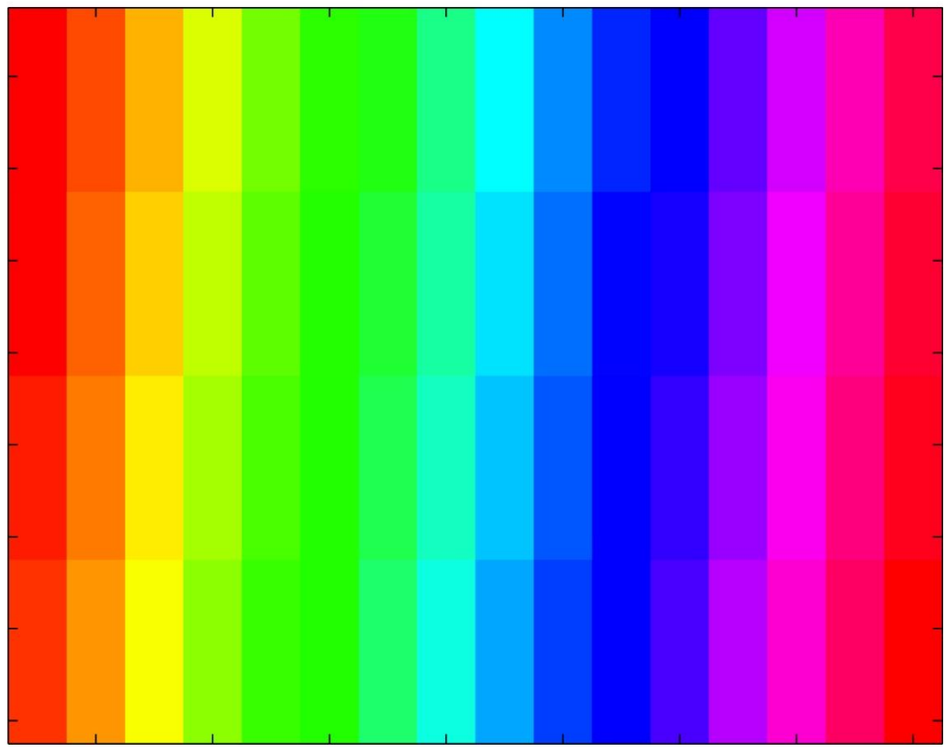




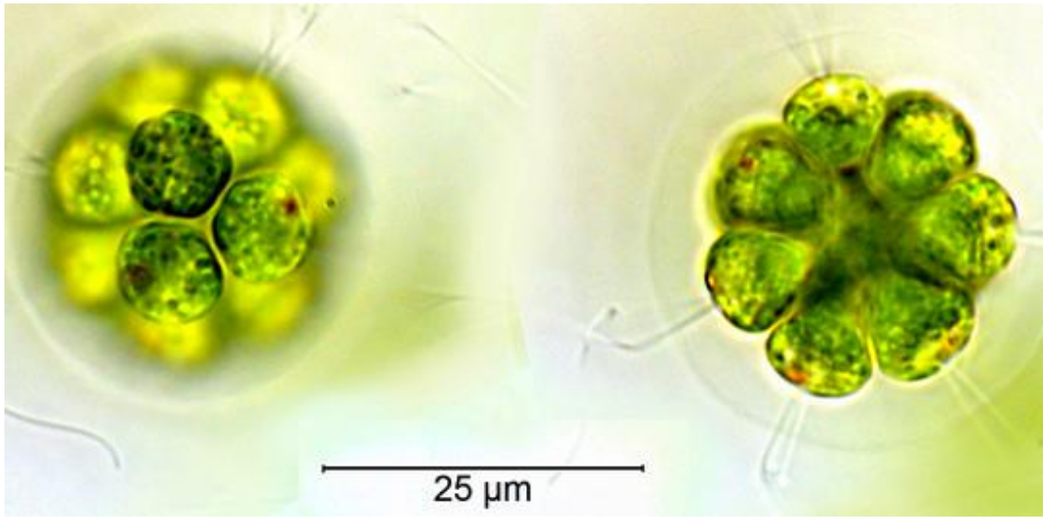




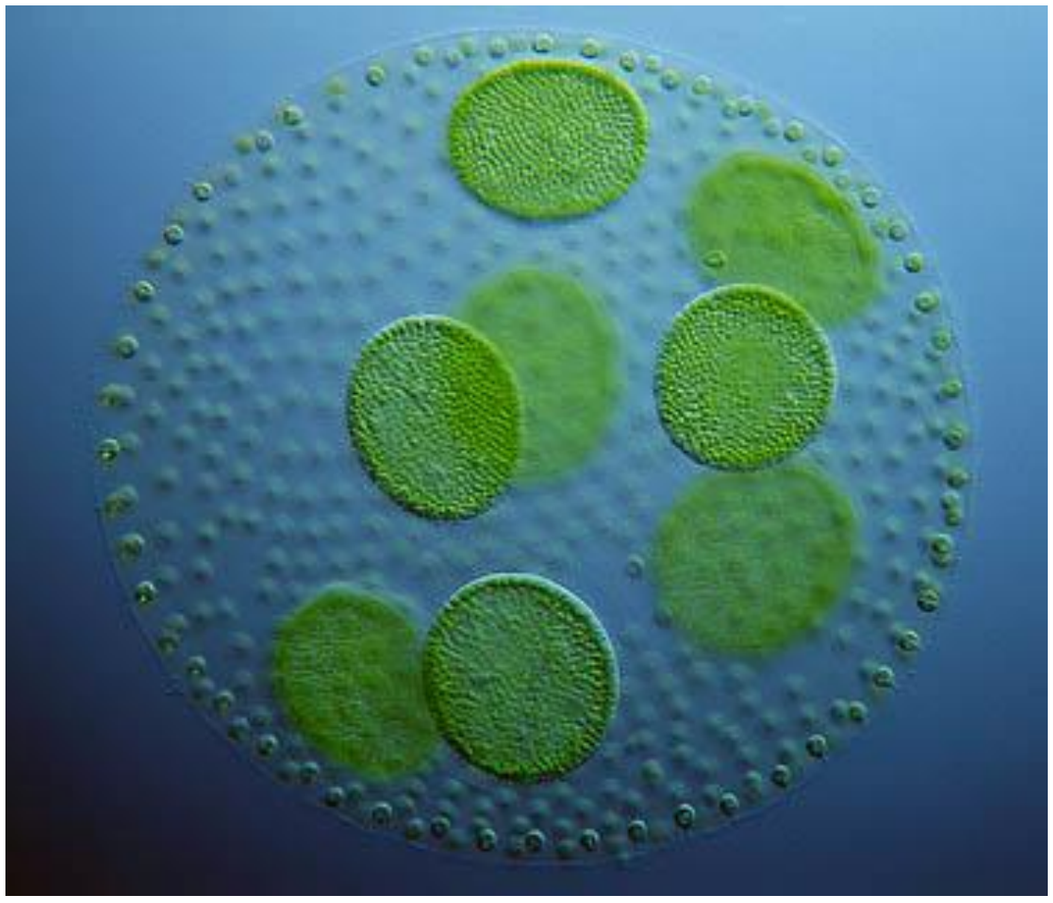
Multicellularity: is there a big idea?

KITP 2013

Who’s multicellular?



Pandorina

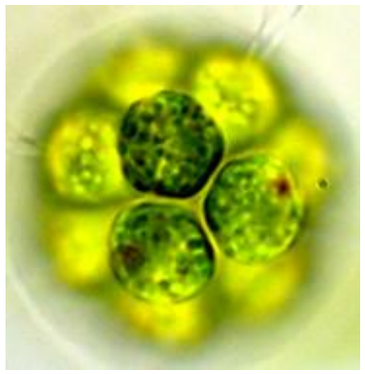


Volvox

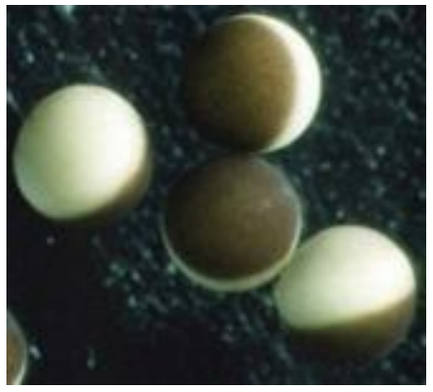
Homo sapiens?

Three aspects of multicellularity

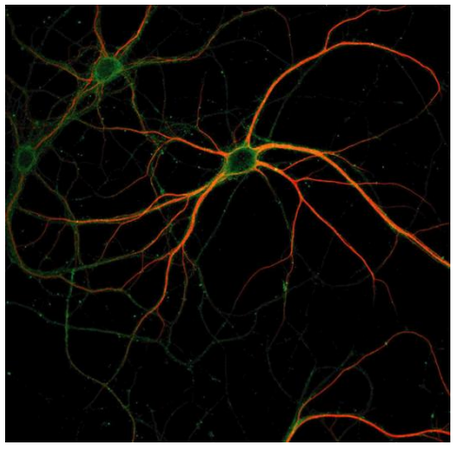
Multiple cells



Single-celled propagules



Division of labor



Questions about multicellularity

What do we mean by multicellularity?

Why did it evolve?

How did it evolve?

How often has it evolved?

What are the roles of experiment and observation in these questions?

What are the roles of experiment and theory in these questions?

Investigating multicellularity by engineering

Hypothesis testing by reconstruction

Partially defines space of possibilities

Lays ground for experimental evolution

& Experimental Evolution

Minimal preconception

Partially defines space of possibilities

Limited by human ingenuity and organismal “cheating”

Issues with inferring evolutionary history

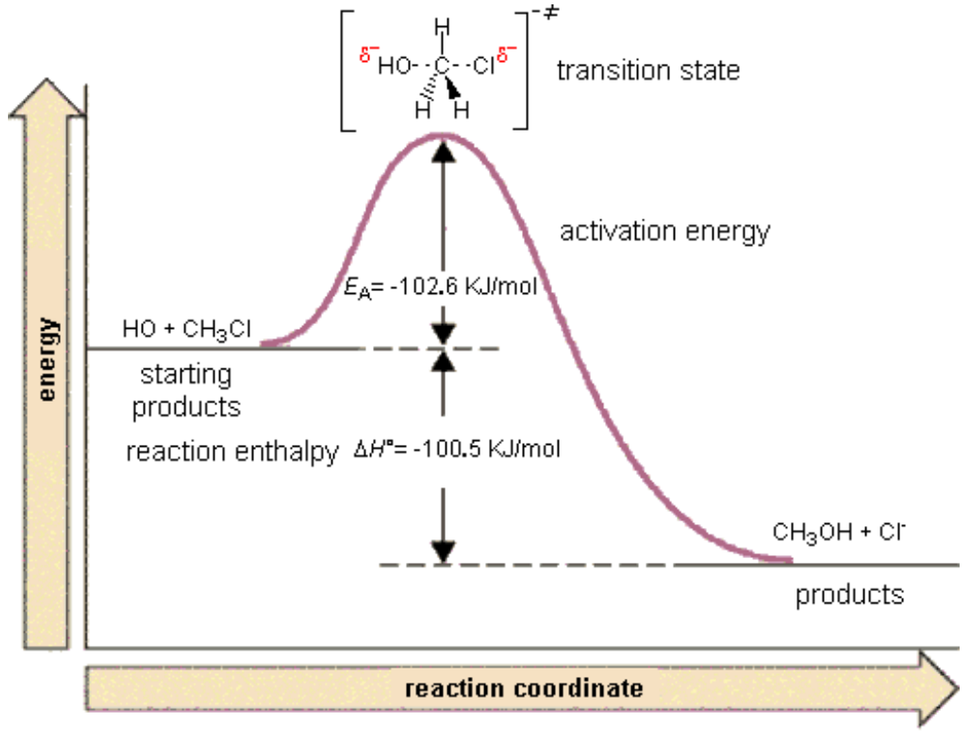
Limited evidence: fossils, imputed DNA sequences etc

Species defined by failure of interbreeding

Exceptions do exist: fruit flies, stickelbacks, cichlid fish

Analysis painstaking

Can lose mutations that drove initial change



In general, each “experiment” only done once

Issues with laboratory experiments

Time and population size limited

Environments too simple: little/no spatial, temporal, organismal variation

Selection usually very strong and unidirectional

Result: unknown relevance to long-term, natural evolution

BUT

Ancestor and frozen fossil record available

Analysis easier

Reconstruction possible

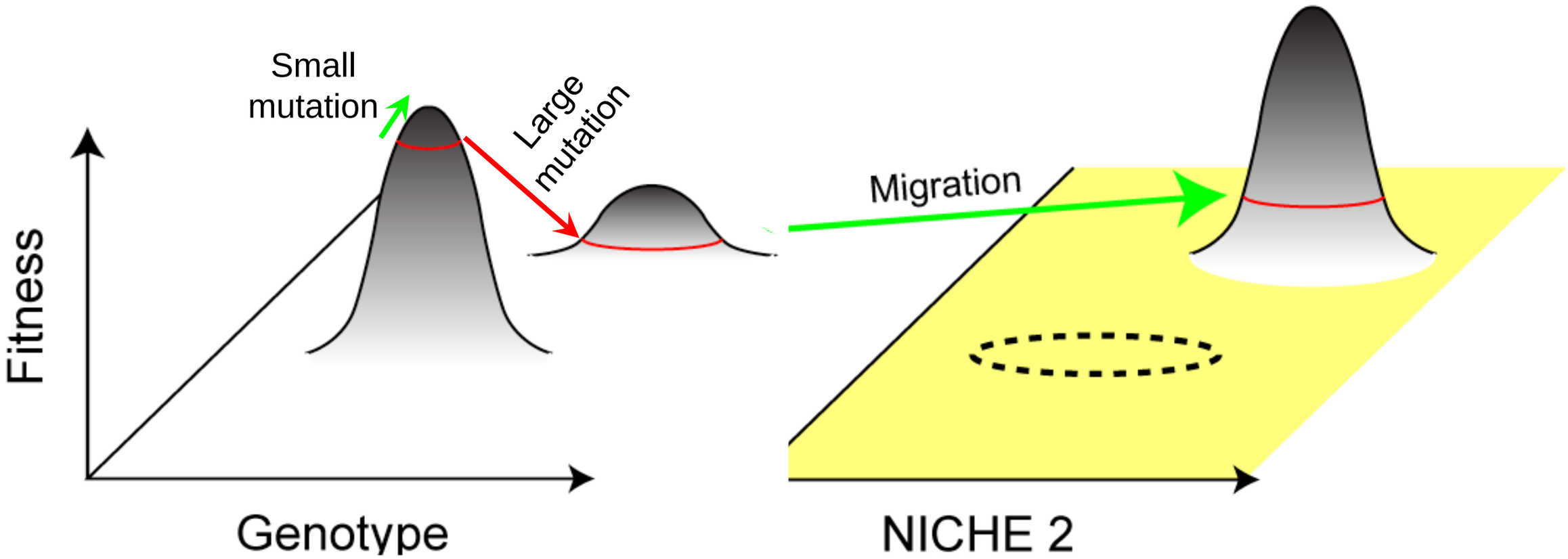
Multiple parallel experiments possible

How does novelty arise?

Californian Tarweed

Hawaiian Silverswords

A model for novelty



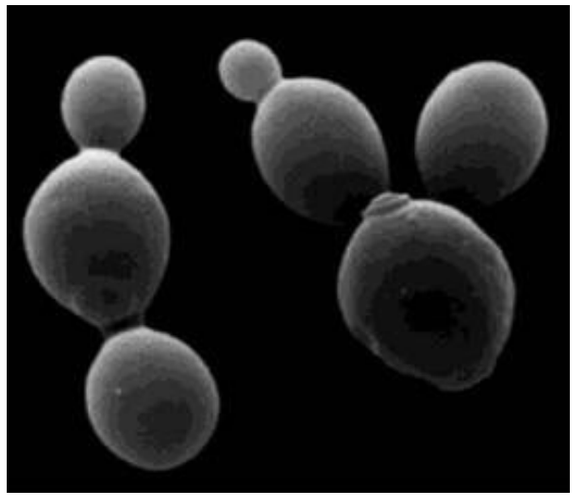
Crowded ecosystems

- Niche 2 occupied
- Mutant outcompeted
- Mutant = hopeless monster

Virgin ecosytems

- Niche 2 empty
- Mutant survives if $w_{abs} > 1$
- Mutant = hopefull monster`

Why study yeast?



Rapid proliferation (t_d = 90 min)

Proliferates sexually or asexually

Excellent genetics

Genome-based tools to find mutations

To a reductionist, it’s multicellular

Reductionism in my eyes

Find something interesting you want to study

Define your terms

State the question in the most general possible form

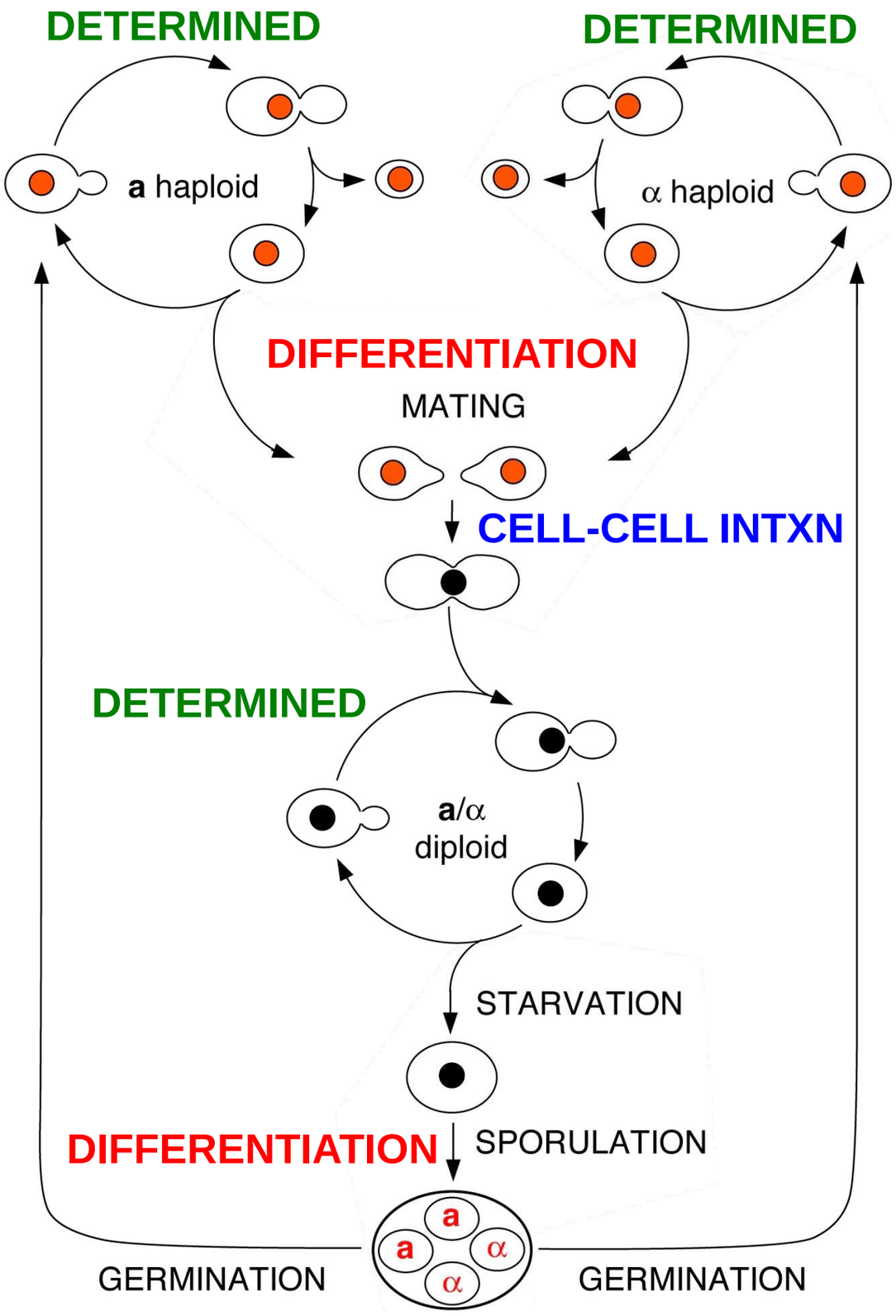
Find the simplest possible example of the problem

Loop: observe, experiment, induce hypotheses, & test predictions

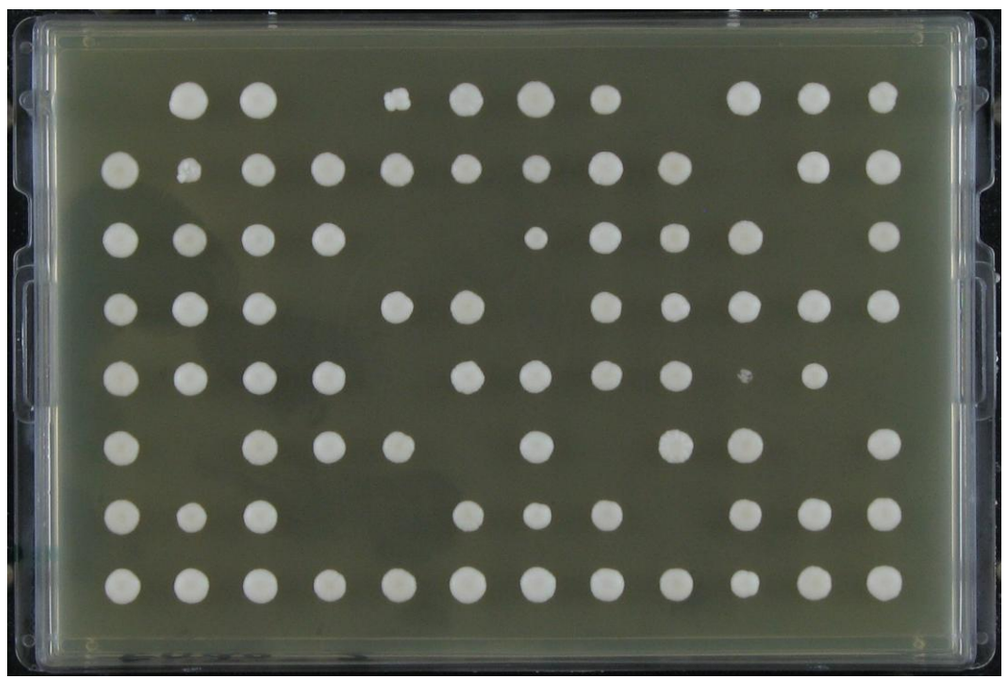
Extrapolate understanding from the specific example to the general problem

Collaborate with theorists and simulators to speed understanding

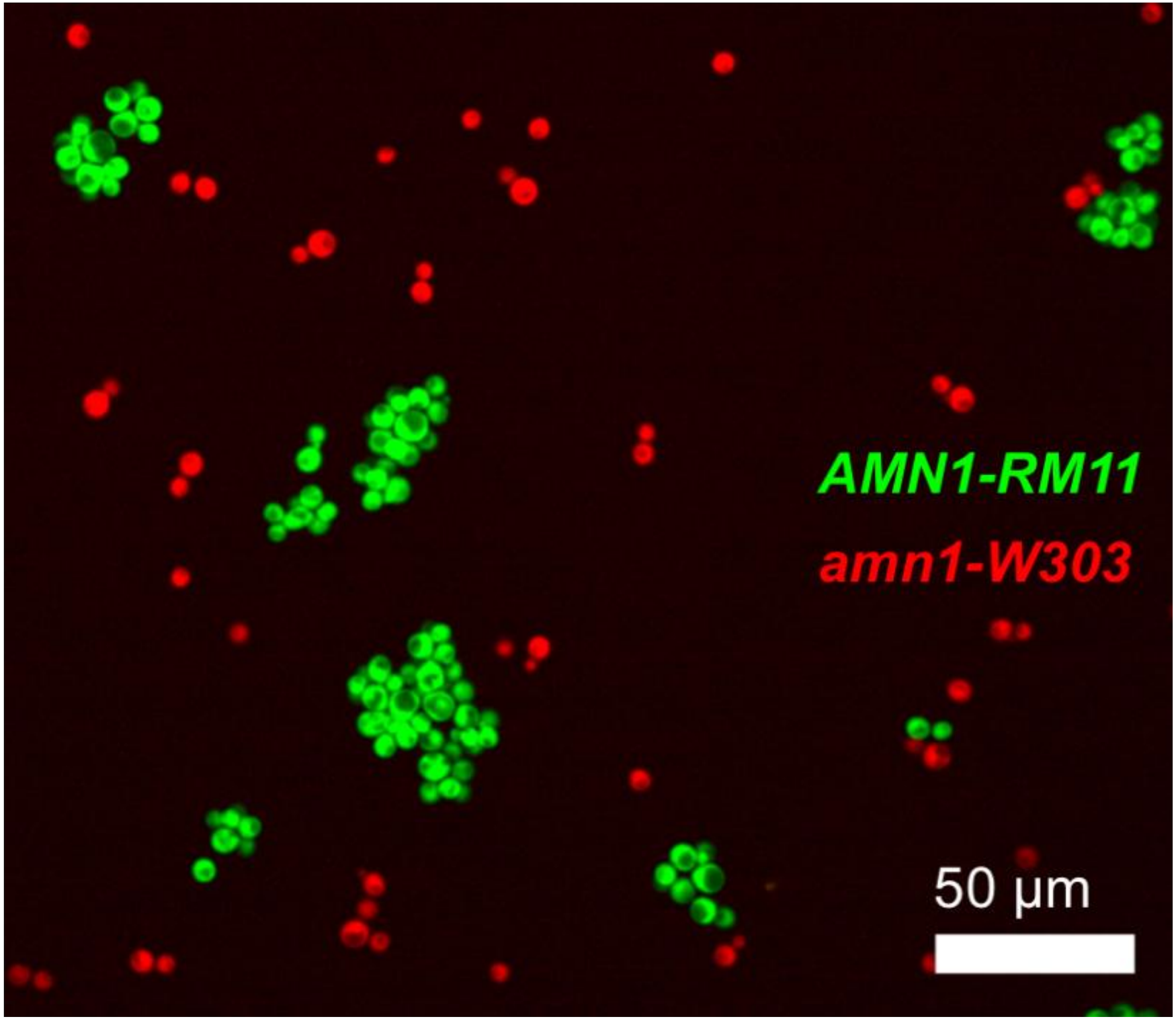
Yeast develops



Taming the wild beast

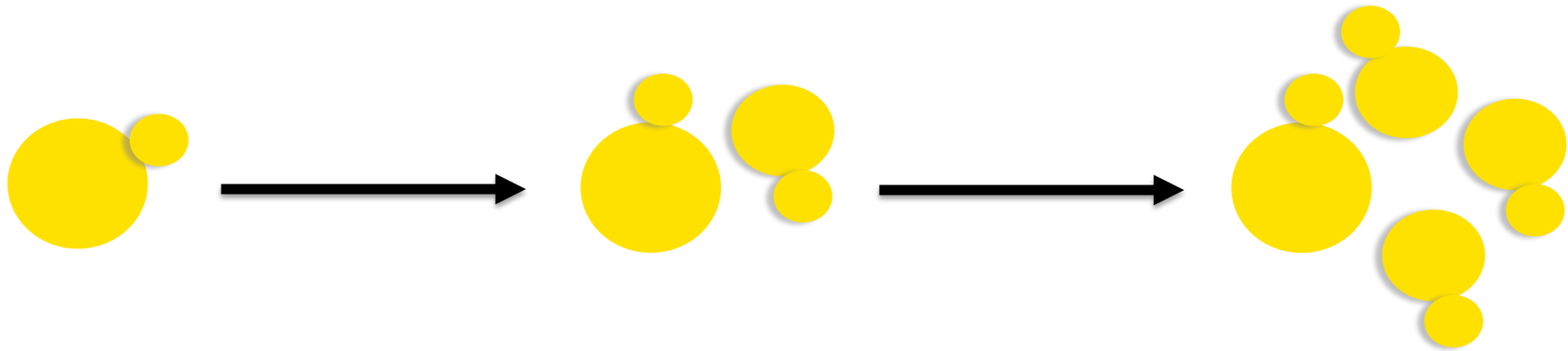


What we lost in the process

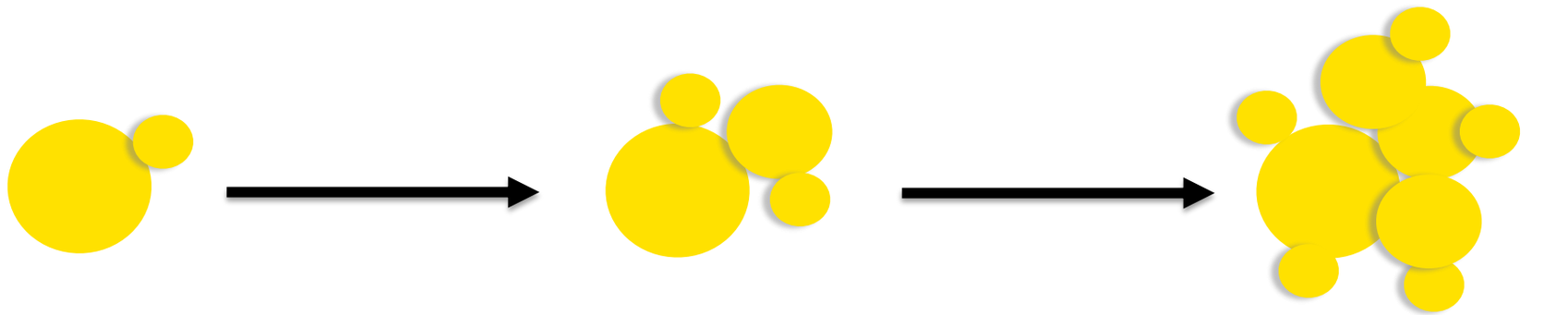


Forms of multicellularity

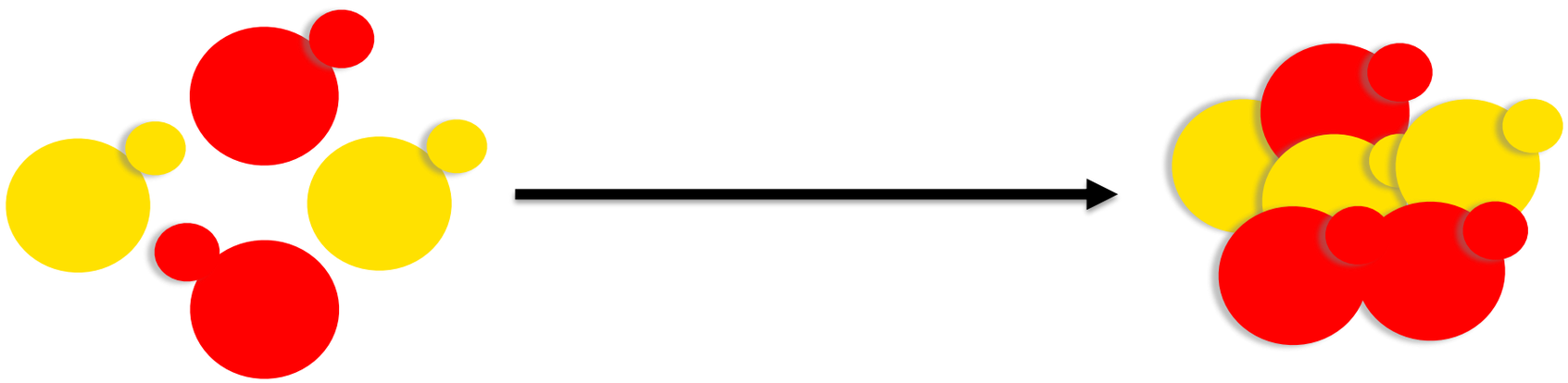
None (lab yeast)



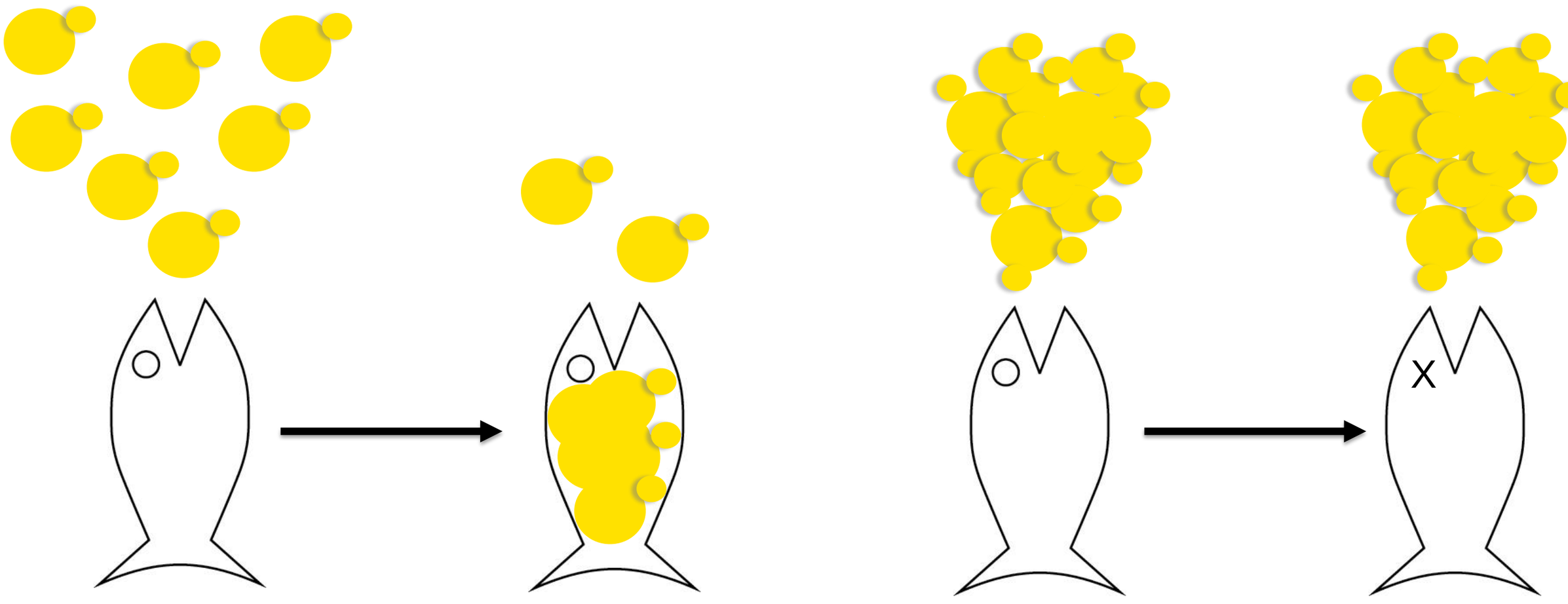
Inseparable



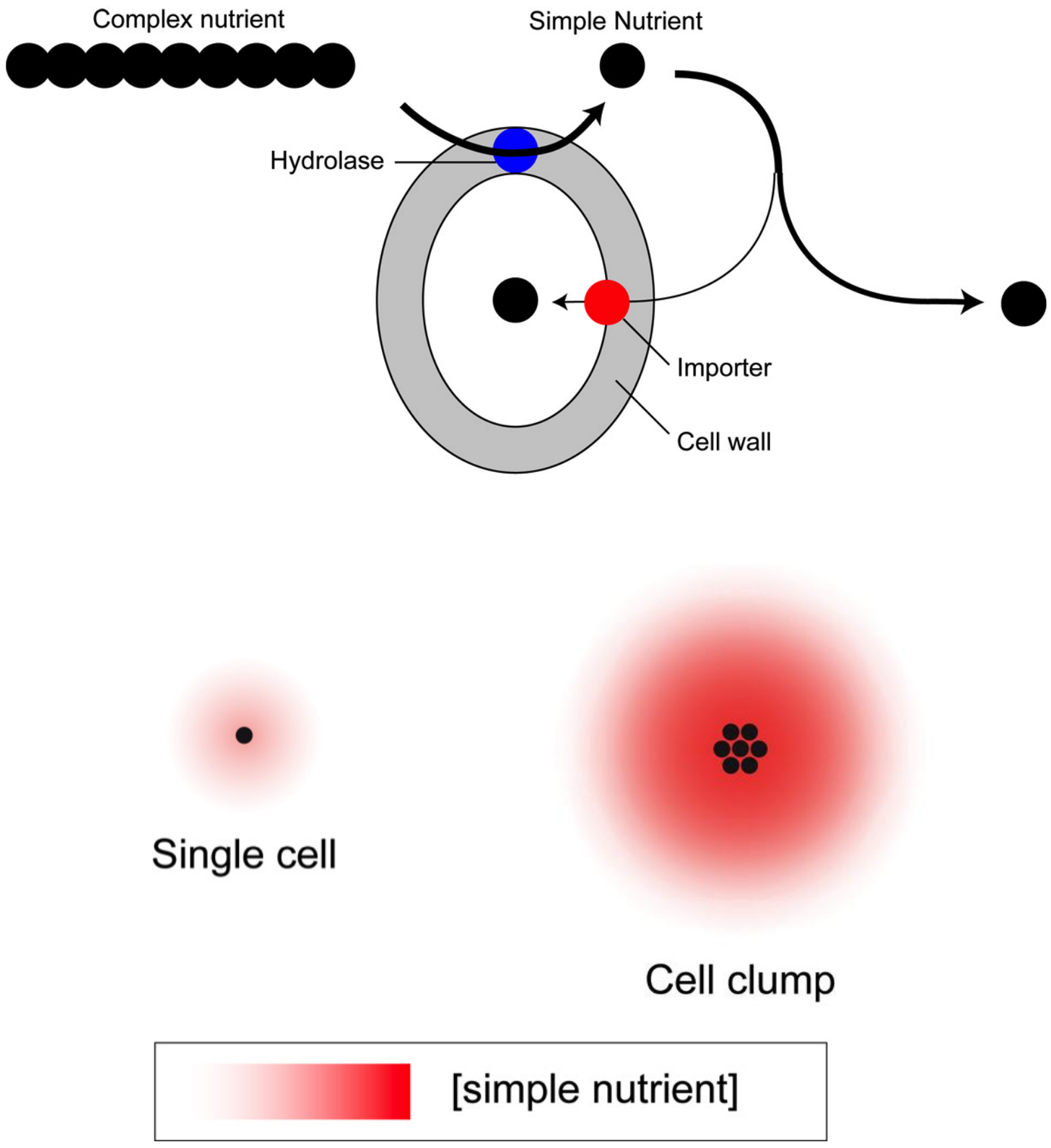
Flocculation



Why multicellular? Don’t be lunch

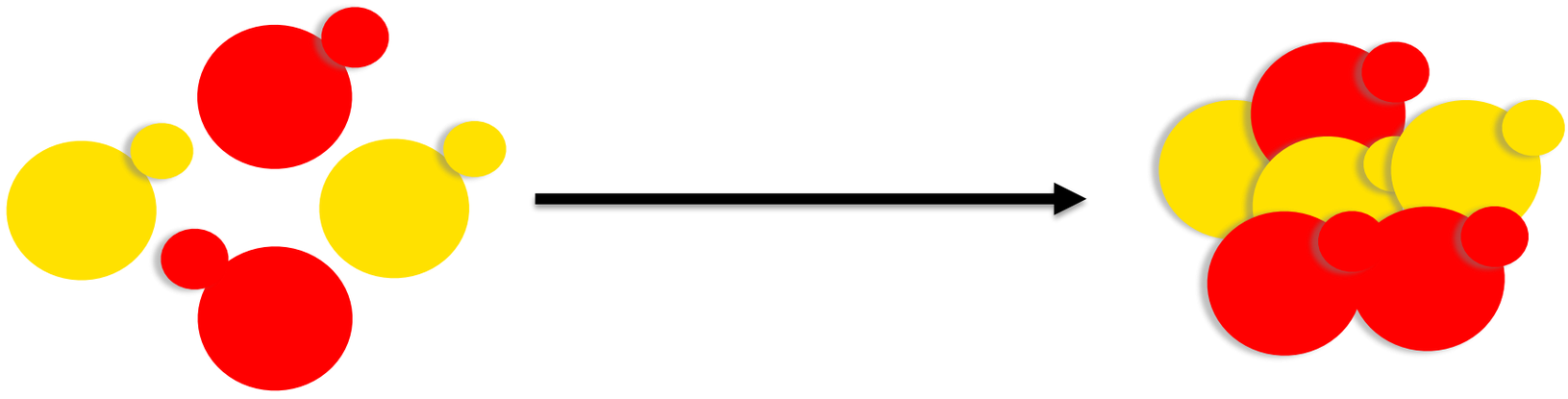


Why multicellular? Utilize public goods

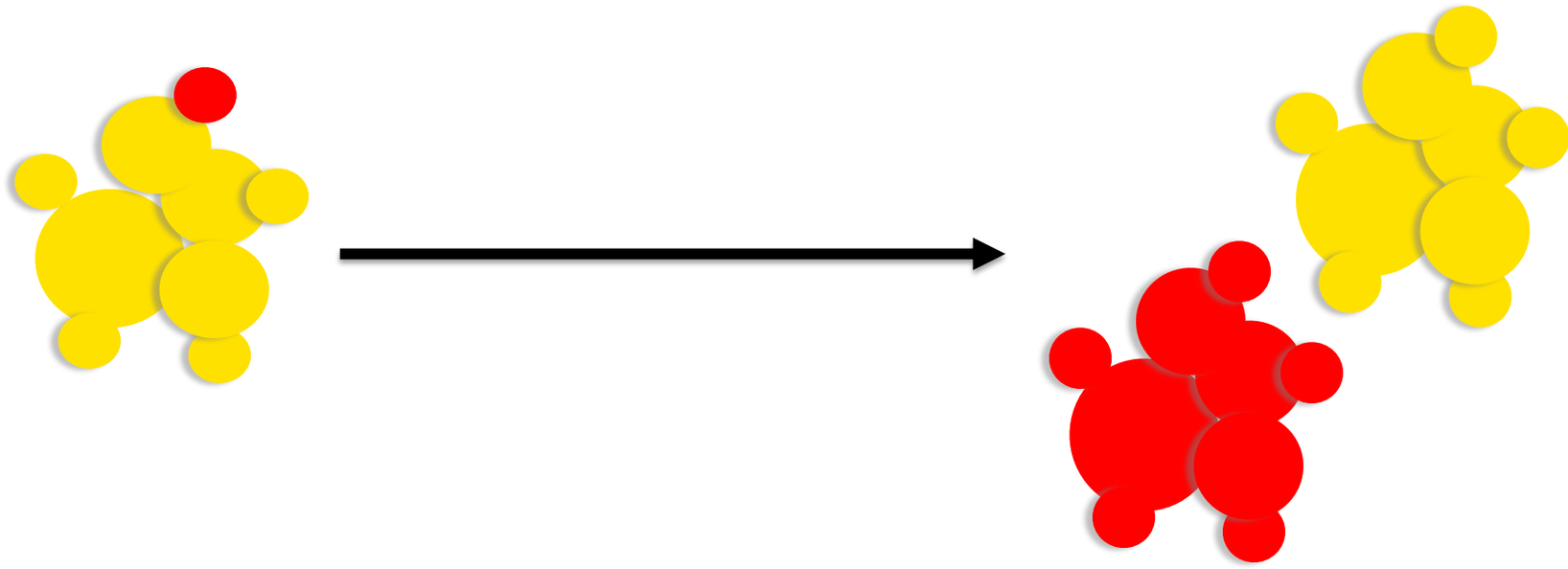


Why multicellular? Fend off freeloaders

Mixed society



Clonal growth

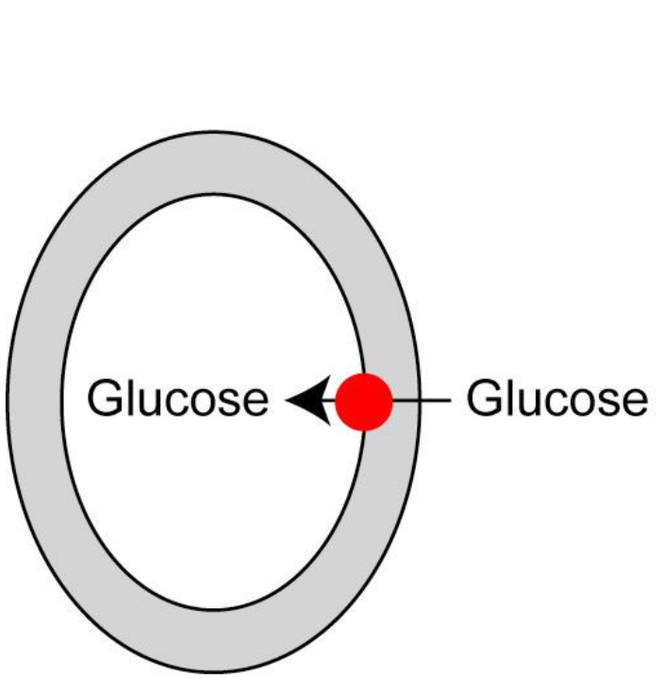


Multicellularity improves access to some nutrients

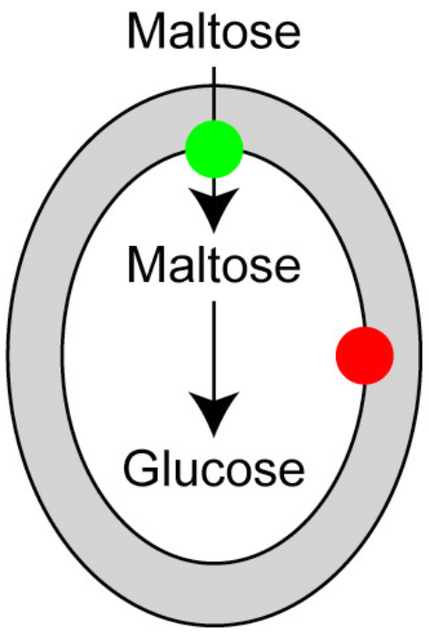
John Koschwanez

Kevin Foster

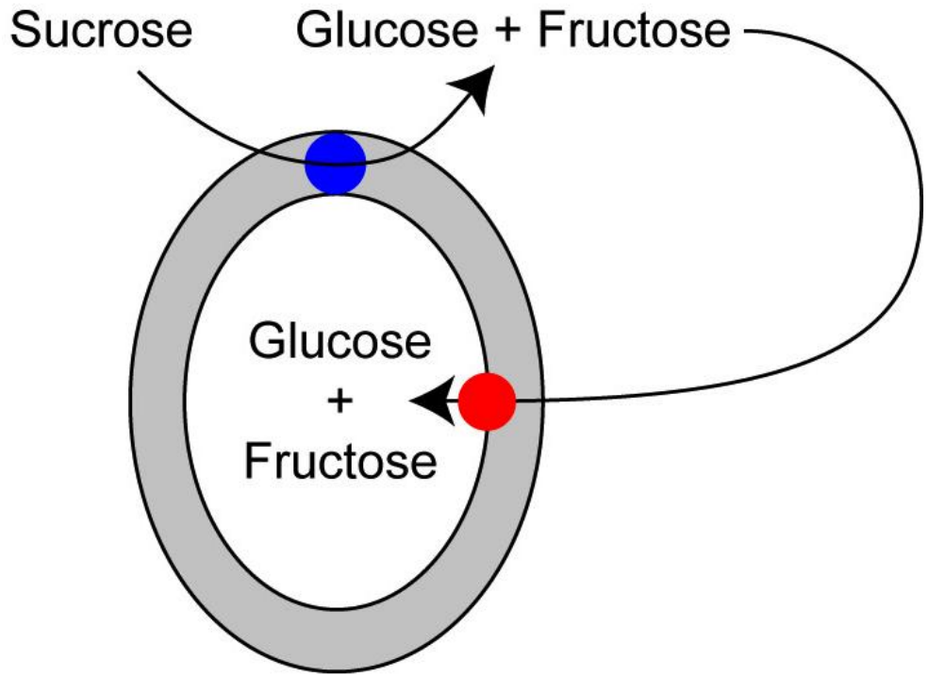
Yeast grow on various carbon sources



LAB



BEER



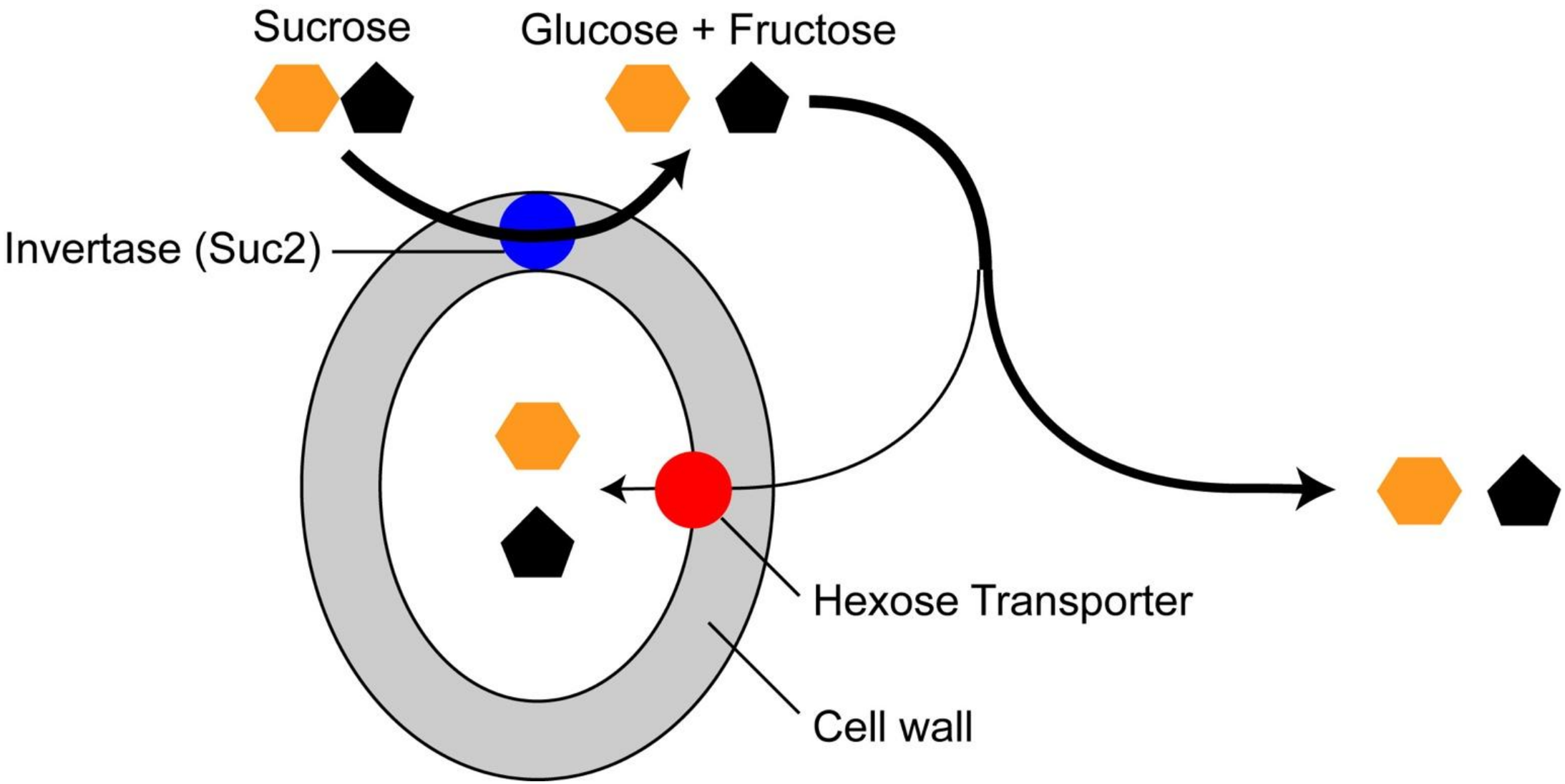
WINE

Glucose (and fructose) transporter

Maltose transporter

Invertase

Can cells capture enough of the glucose they make?

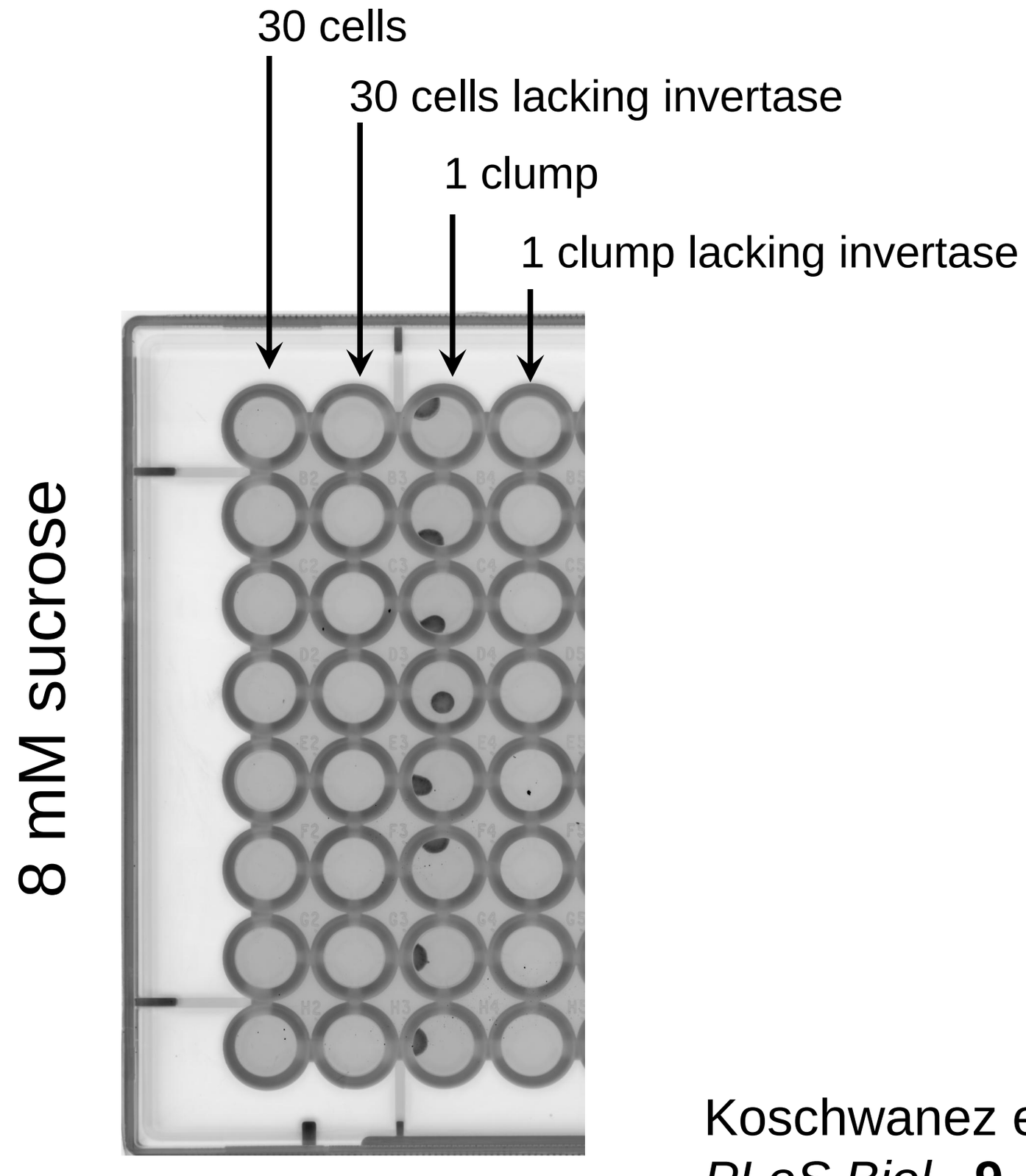


Prediction from free parameter-free simulations

Single cells *can't* form colonies at low [sucrose], clumps can

Koschwanez et al.
PLoS Biol., **9**, e1001122

Single cells *don't* grow on sucrose, clumps *do*



Koschwanez et al.
PLoS Biol., **9**, e1001122

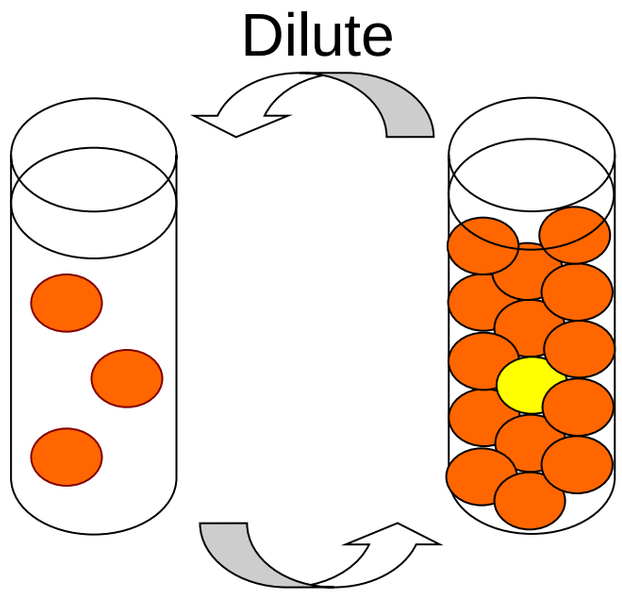
Three engineered ways to grow in low sucrose

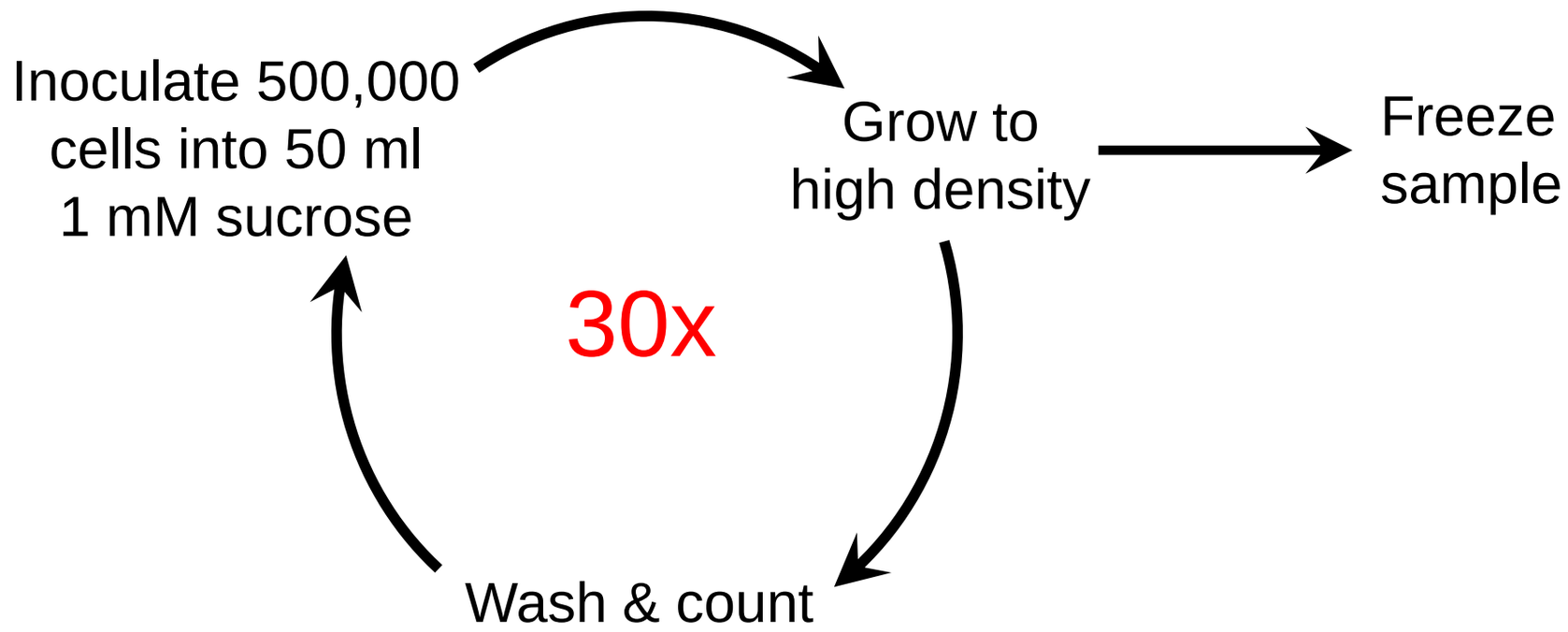
Multicellular clump

Make more invertase

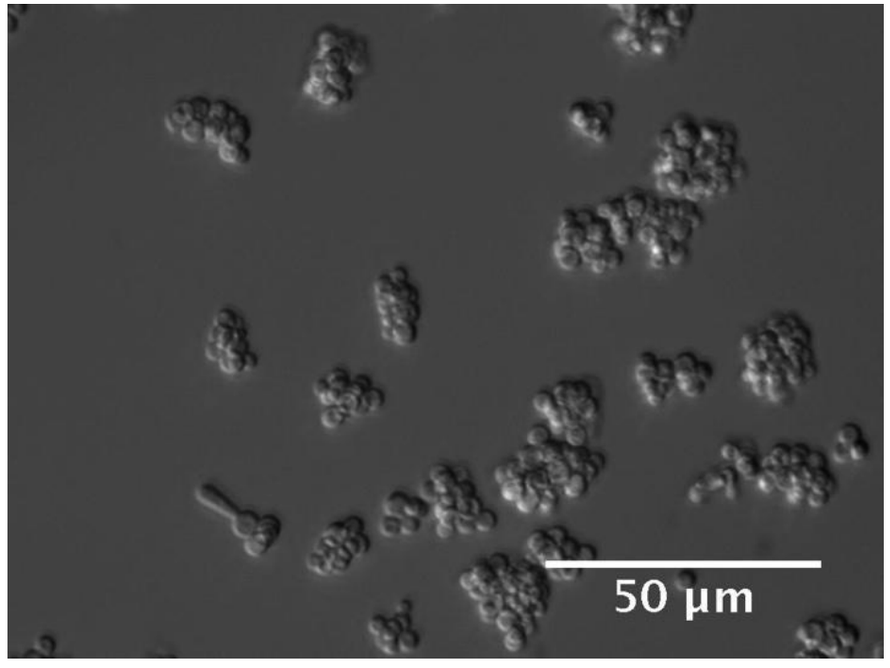
Import sucrose, hydrolyze internally

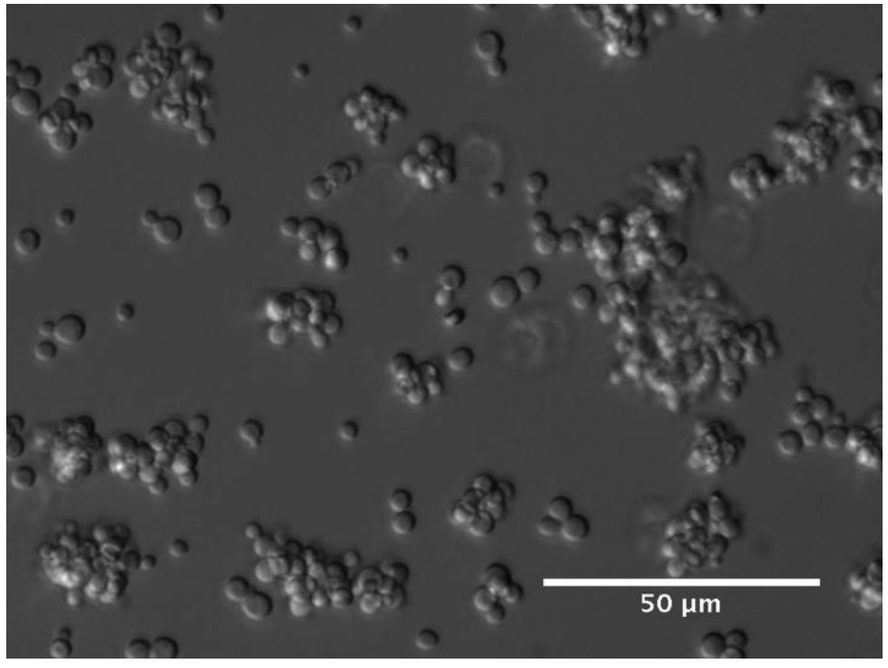
WWED? :Evolving multicellularity

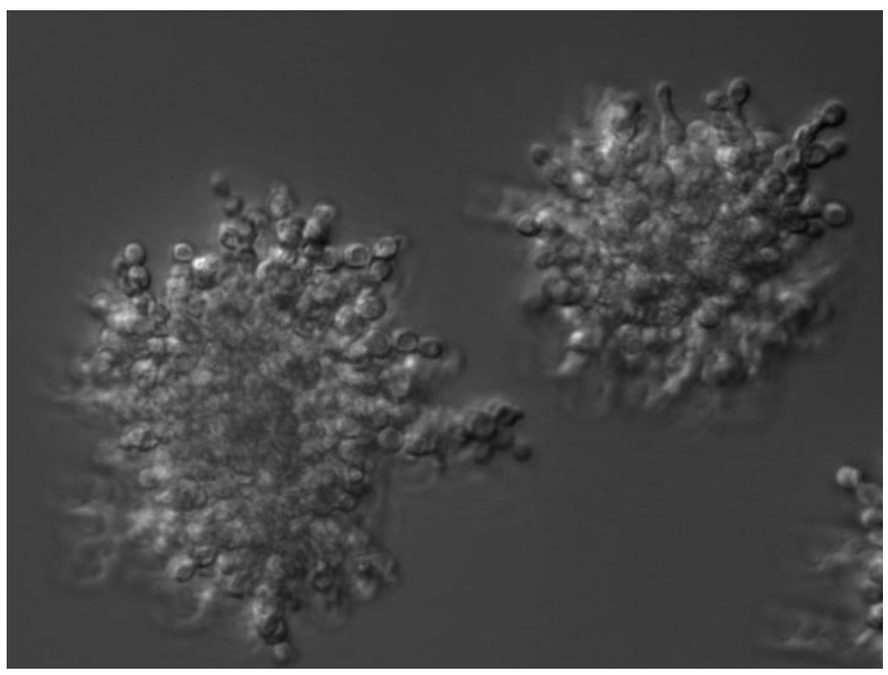


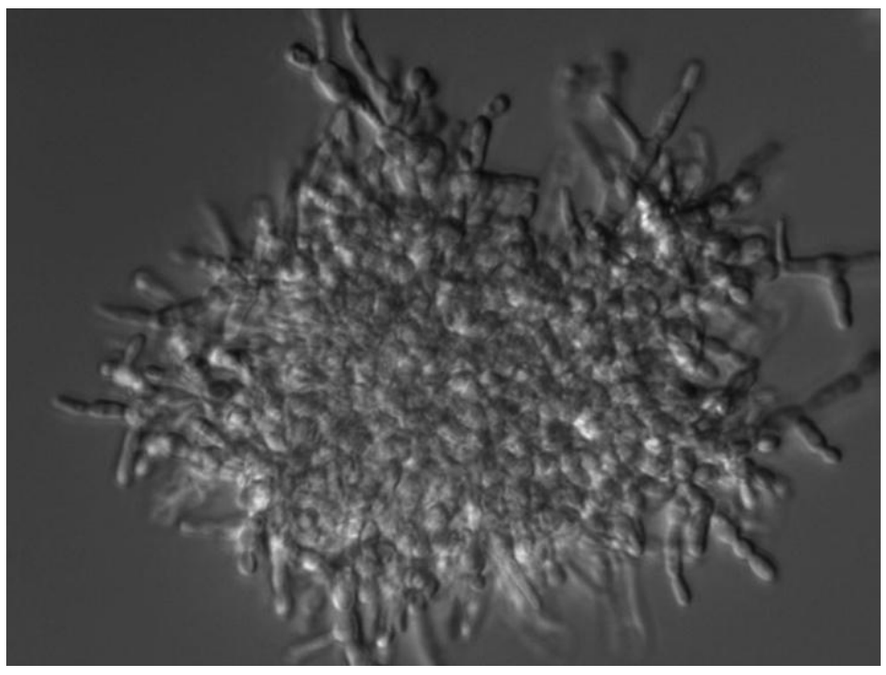


Experimentally evolved multicellularity









Who’s mutated?

47 genes have non-synonymous coding mutations

Some common mutations (*ace2* in 7 clones, *ubr1* in 6)

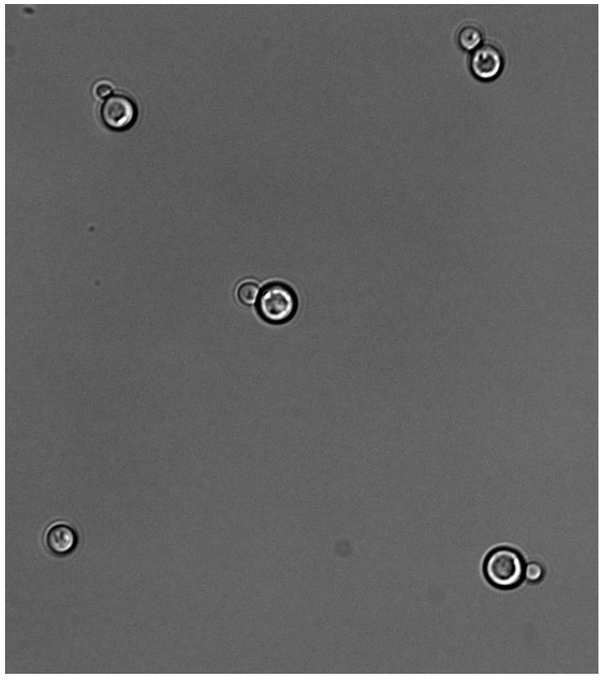
Some genes mutated in 2 (8 genes) or 3 (3 genes) clones

34 genes mutated in a single clone

3 pathways frequently mutated

- Catabolite repression (8/12 clones)
- Transcription (Mediator Complex) (5/12 clones)
- Growth control via cAMP (4/12 clones)

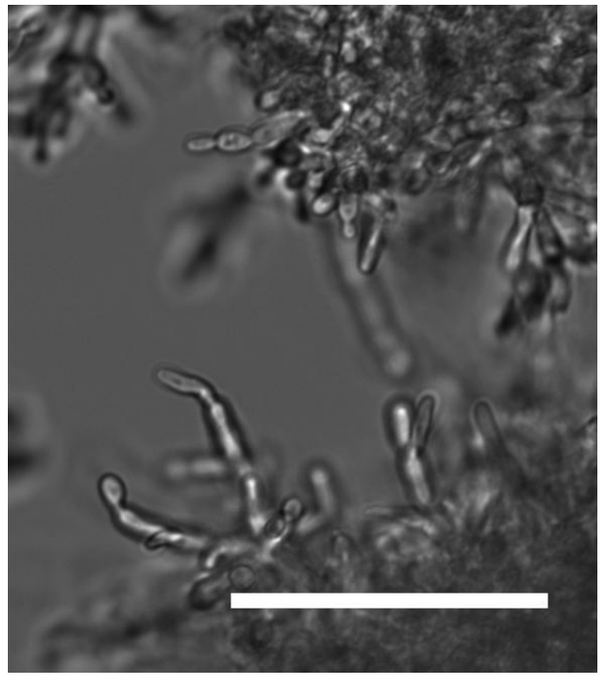
Reconstruct evolved & ancestral phenotypes



Engineer **in** evolved alleles

Engineer **out** evolved alleles

Scale bar = 50 µm

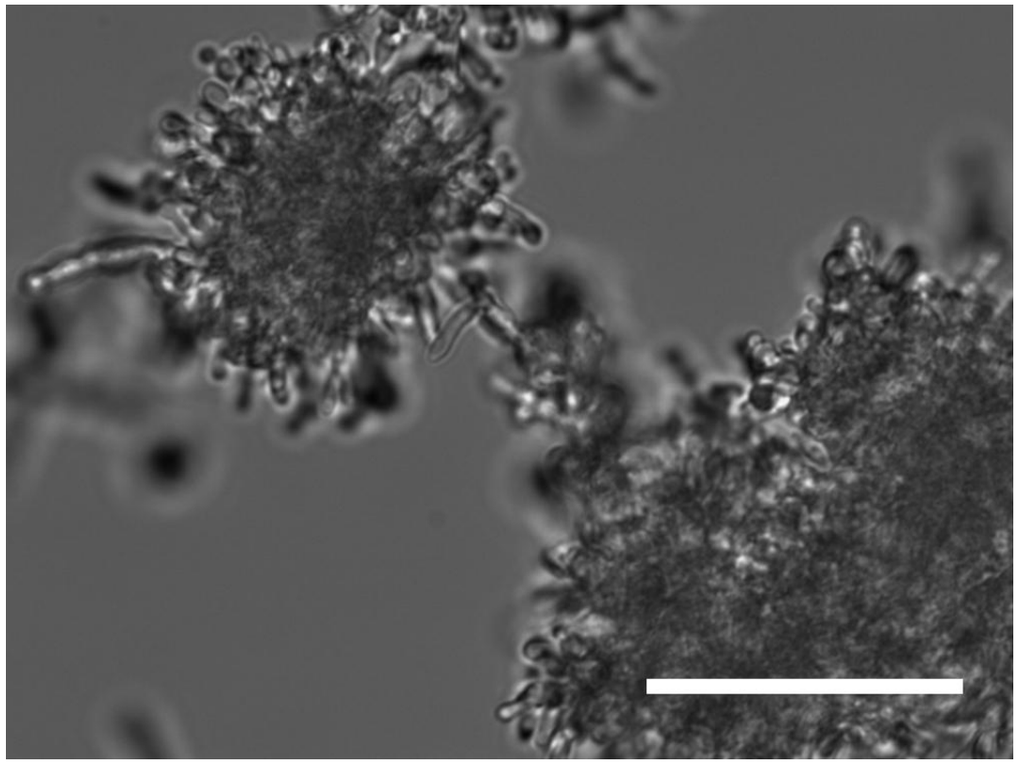


Ancestor

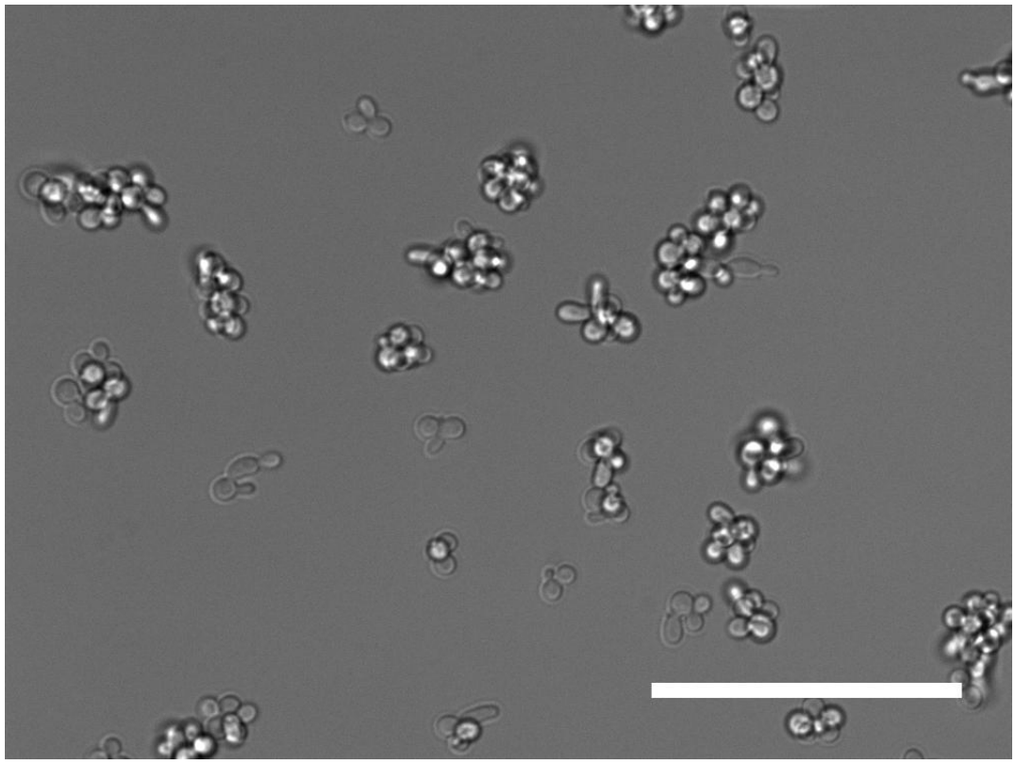
Evolved

A spandrel: one clone regulates clump size

Scale bar = 50 µm



1 mM sucrose



1 mM glucose + 1 mM fructose

Why use extracellular hydrolysis?

Historical Constraint

Selection

Tick, tock, evolve a clock

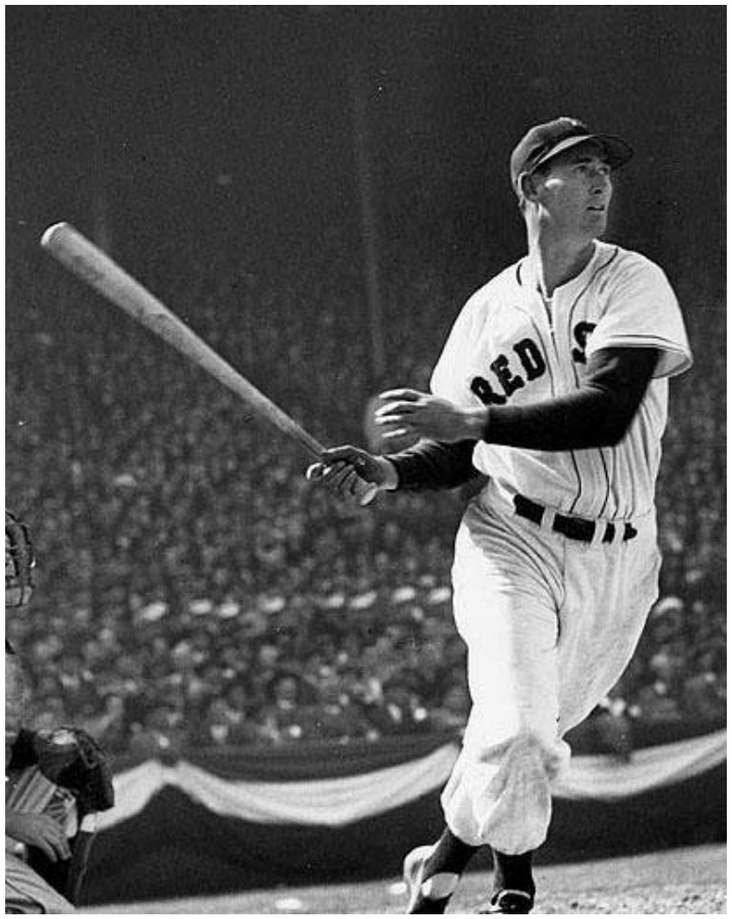
Gregg Wildenberg

Evolution is a selection for computation

Computation: a rule based transformation of symbols

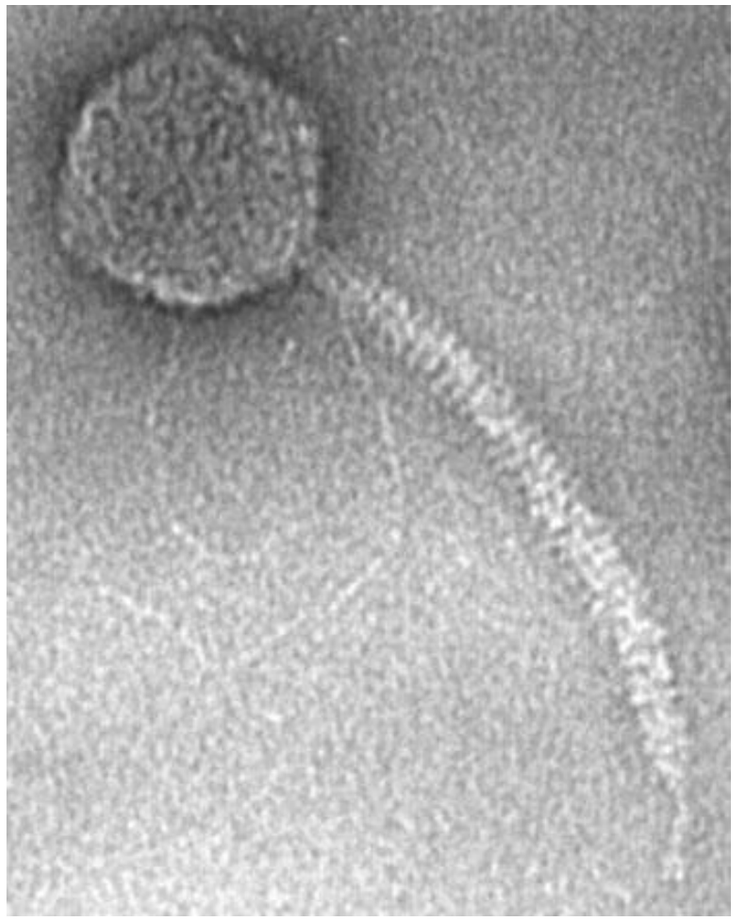
filer à l’anglaise ⟷ take French leave

Organisms are selected to predict and prepare



Theodore Williams, f_{HIT} = 0.406, 1946

Harrison's H1 chronometer, 1735

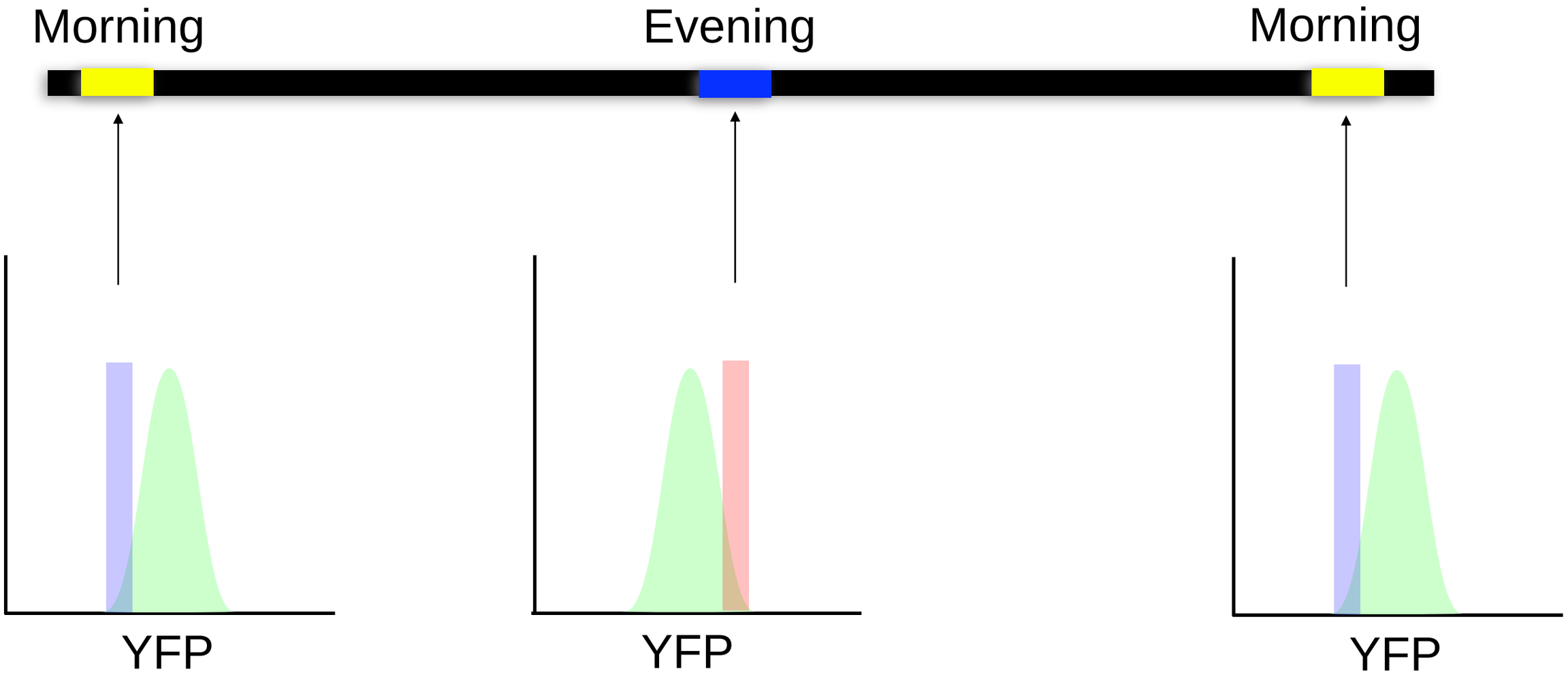


Kourilsky, Mol Gen Genet, 1973

Tagkopoulos et al Science 2008
Mitchell et al Nature 2009

Selection outline

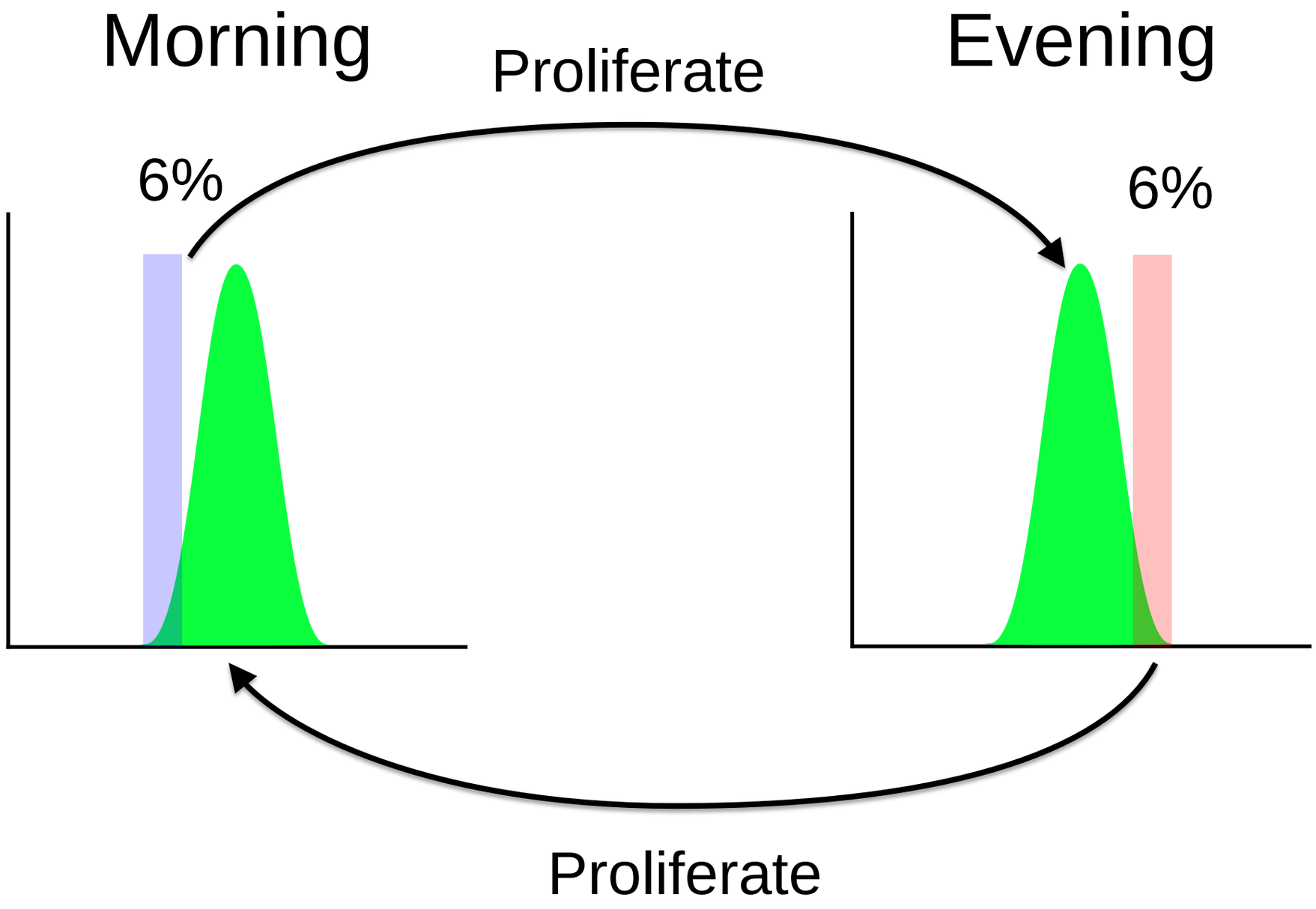
Select → Proliferate → Select → Proliferate → Select



Selection details

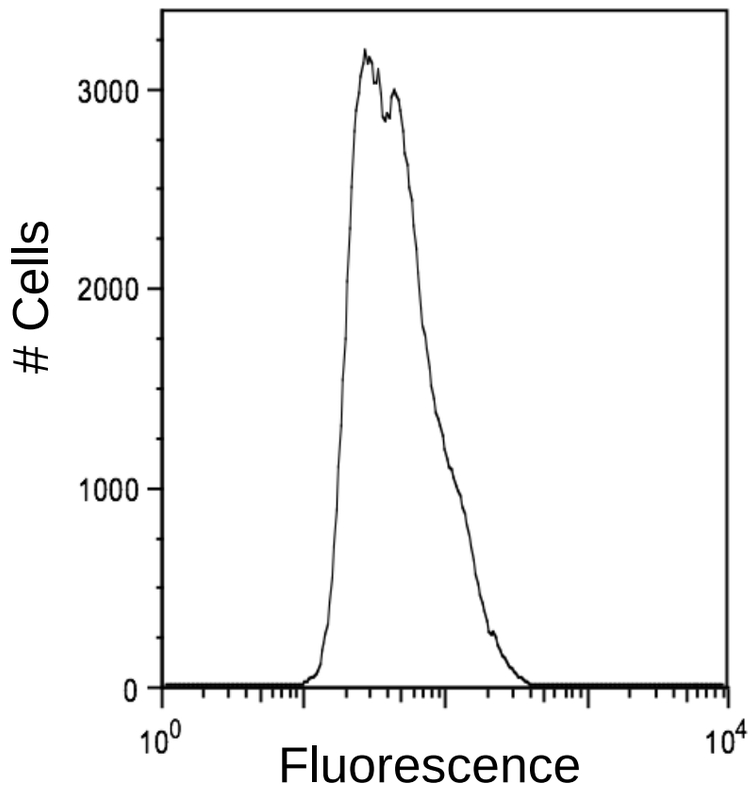
Strain: *MAT***a** P_{FLO1}-*ymCitrine POL3-L523D*

Mating Gene expression 100x Mutator

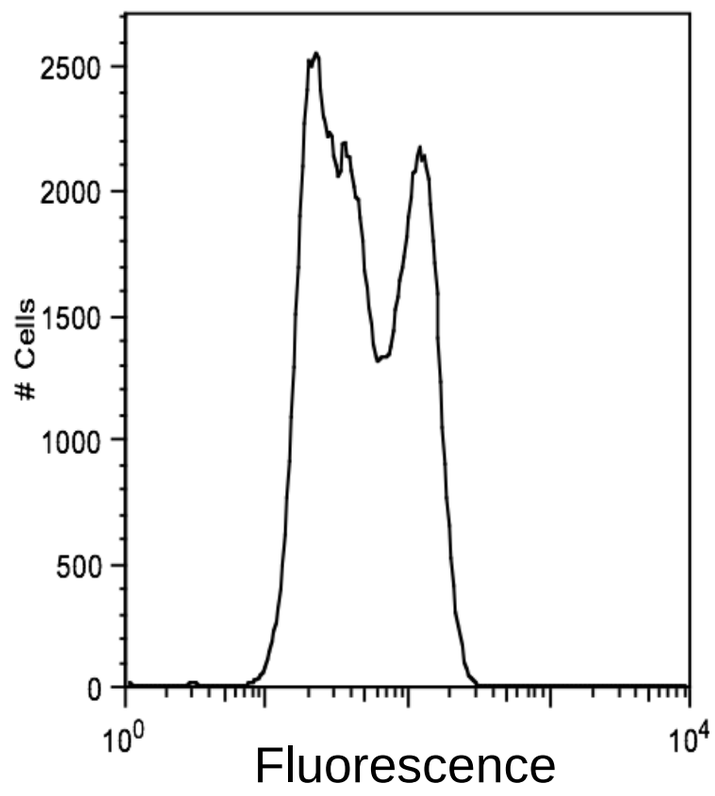


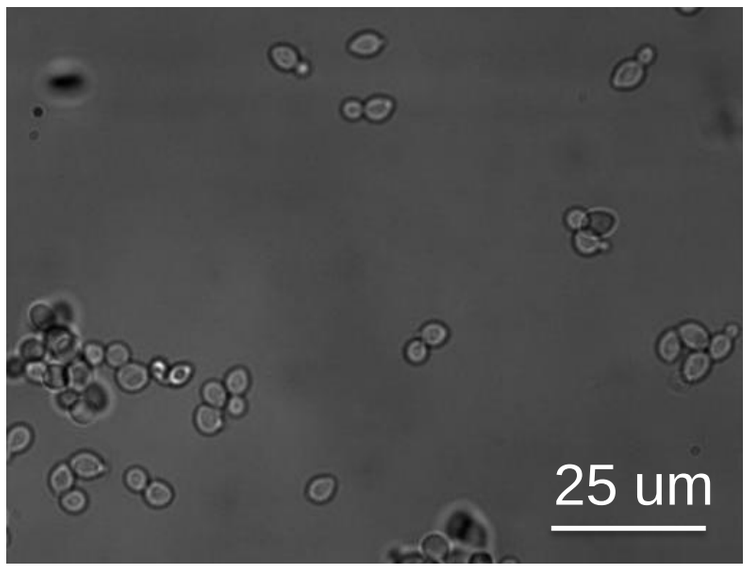
Study cells not FACS profiles!

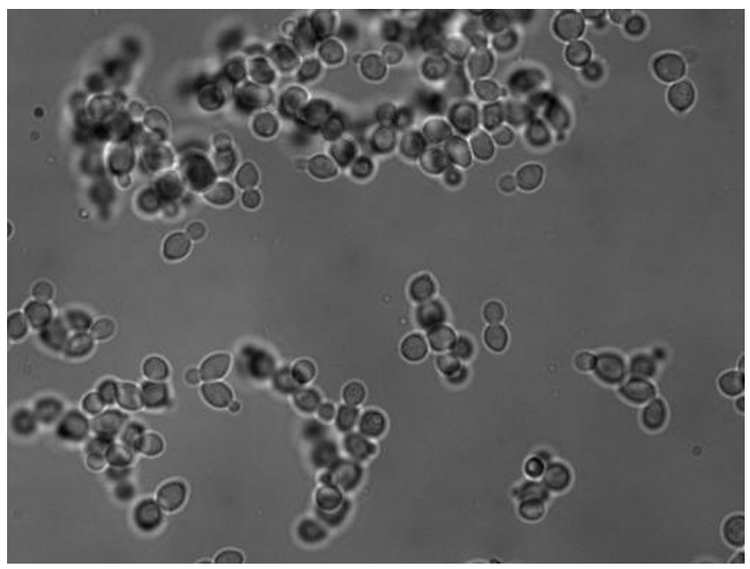
Morning



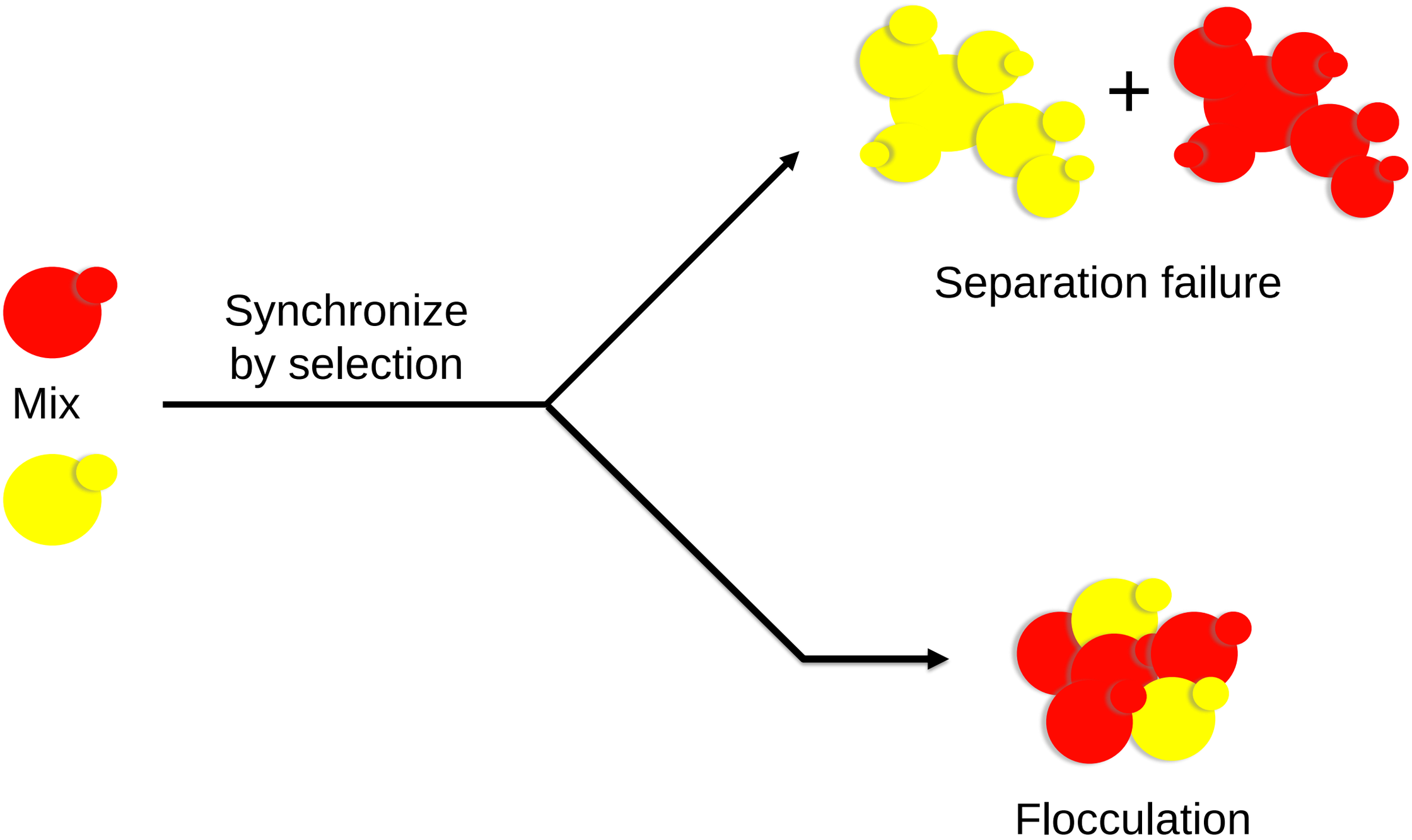
Evening







Testing the two clump hypotheses



Divide and Conquer

Mary Wahl

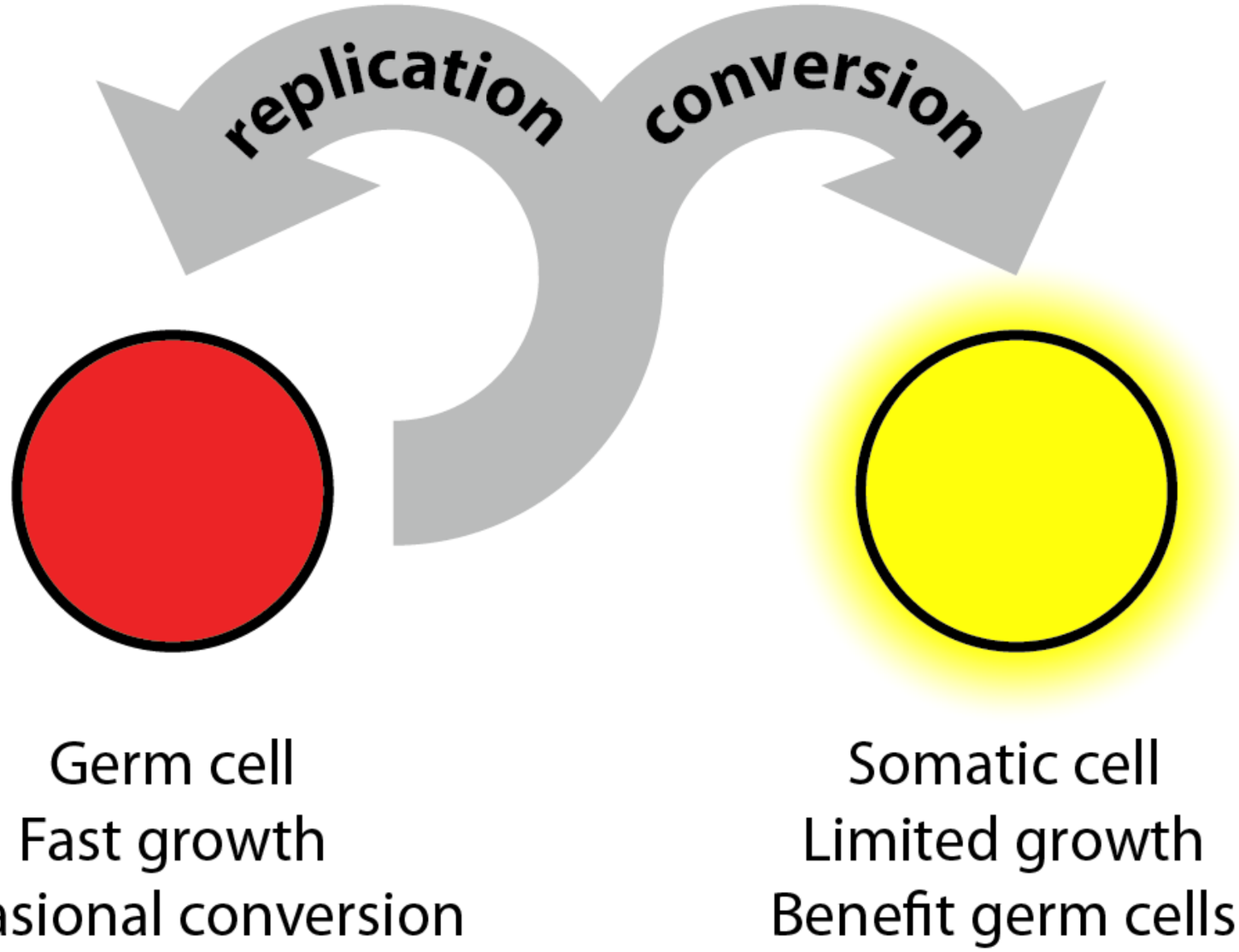
Engineering divided labor

We need:

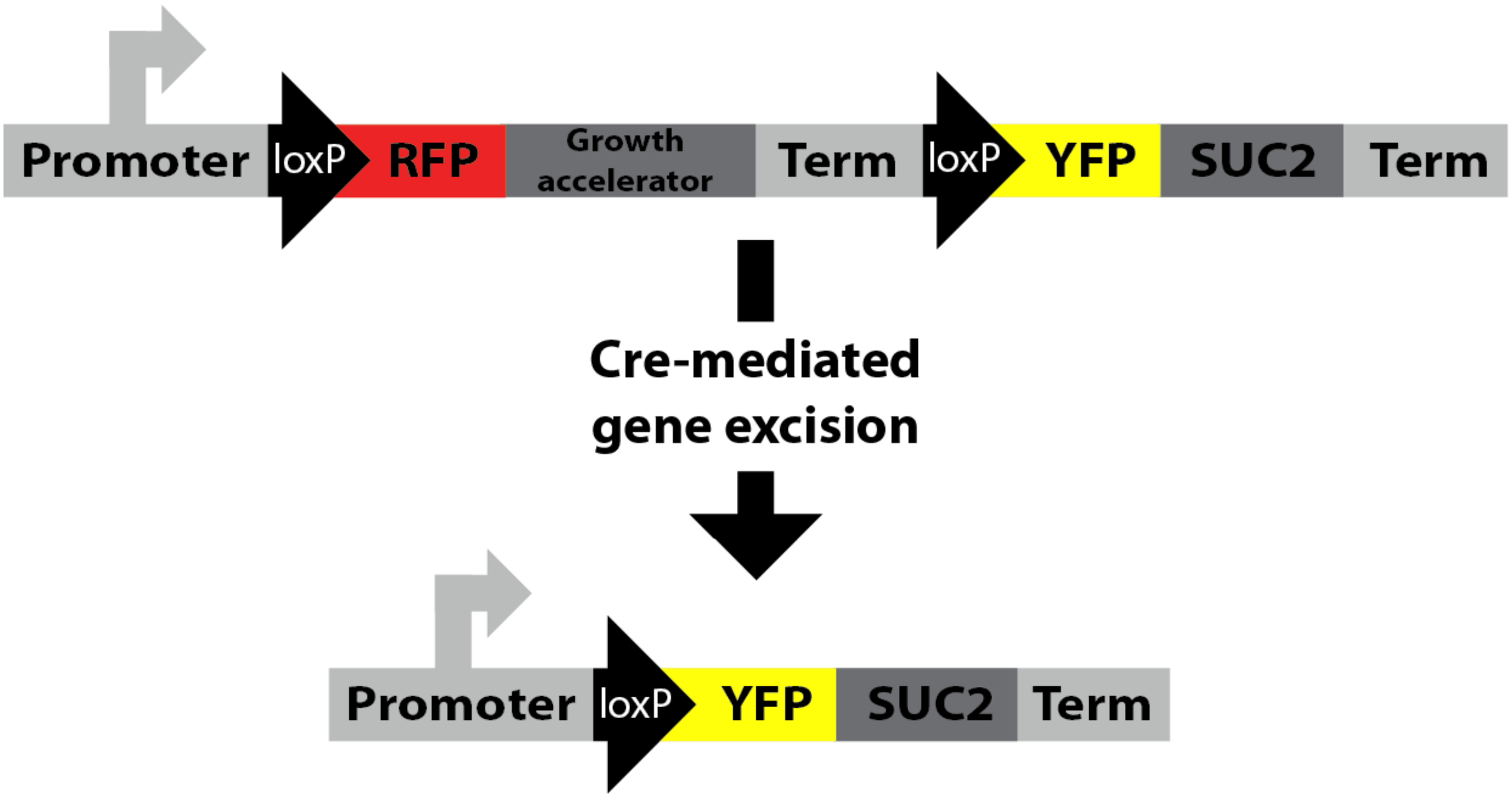
Irreversible differentiation

Limited somatic cell growth

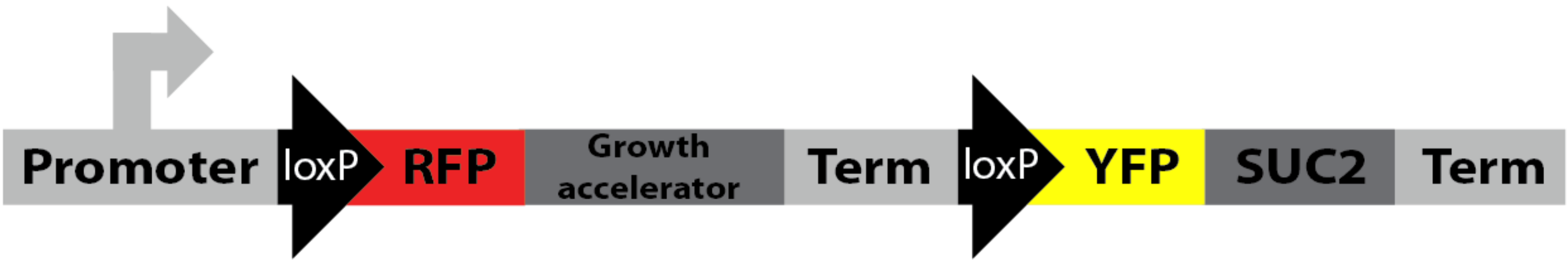
Somatic cells help germ cells



Gene excision-based differentiation



“Growth accelerator” makes germ cells grow faster

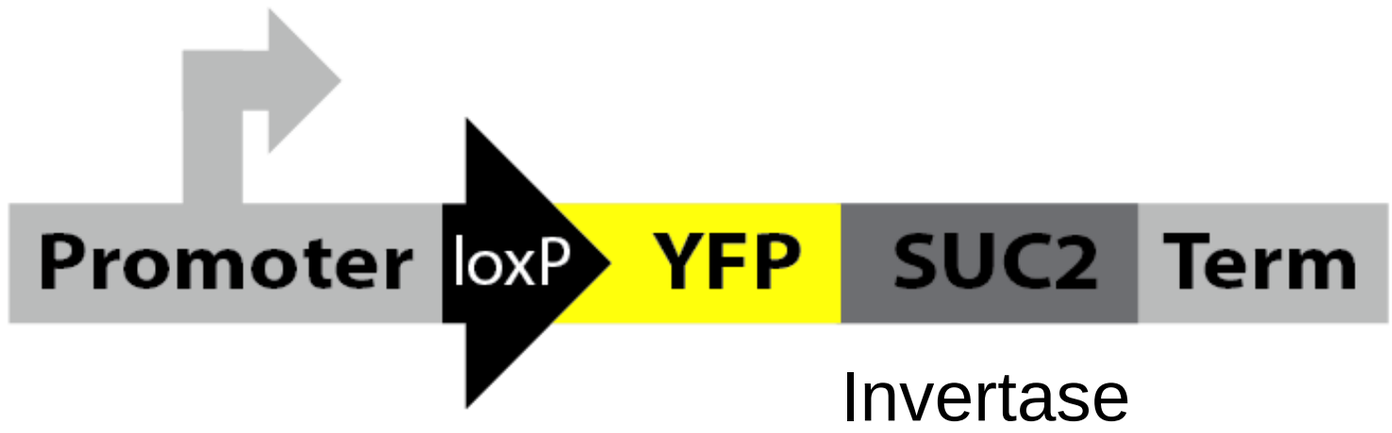


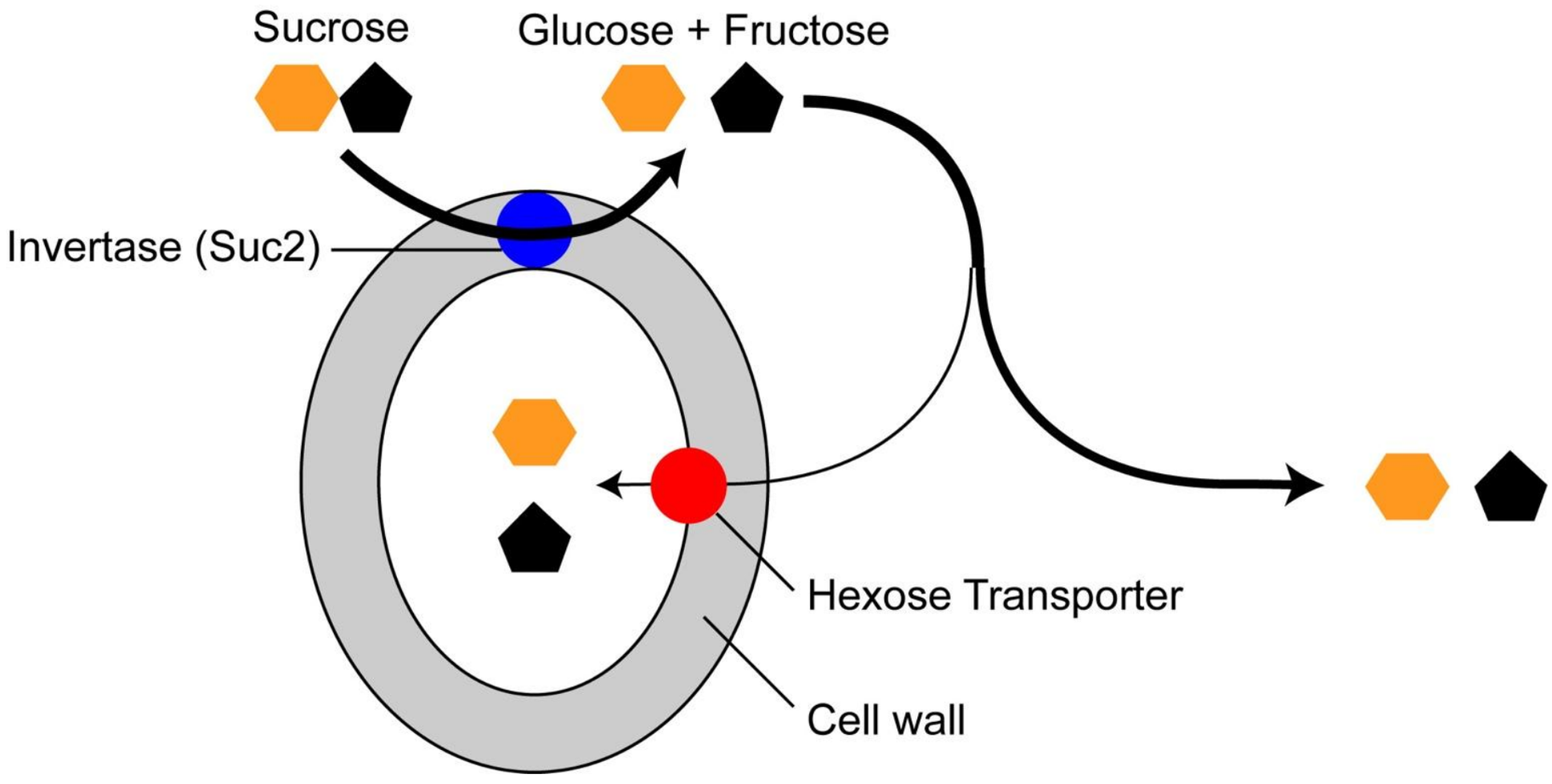
2 Growth Accelerators

CDC28 – essential cell cycle gene; excision halts growth

cyh2^{r} – excision makes cells grow slower in cycloheximide

Somatic cells do work by digesting sucrose externally





Regulating division of labor (recombinase activity)

Cre transcribed only in daughter cells (P_{SCW11})

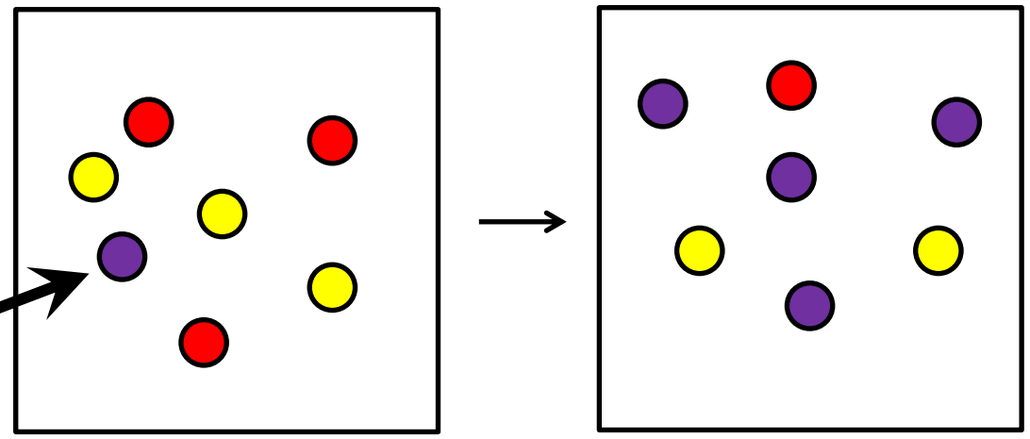
Estrogen binding domain (EBD) keeps Cre inactive

Inducer (b-estradiol) gives graded Cre activity

Lindstrom et al., Genetics, 2009

Hypothesis: cellularity regulates strategy fitness

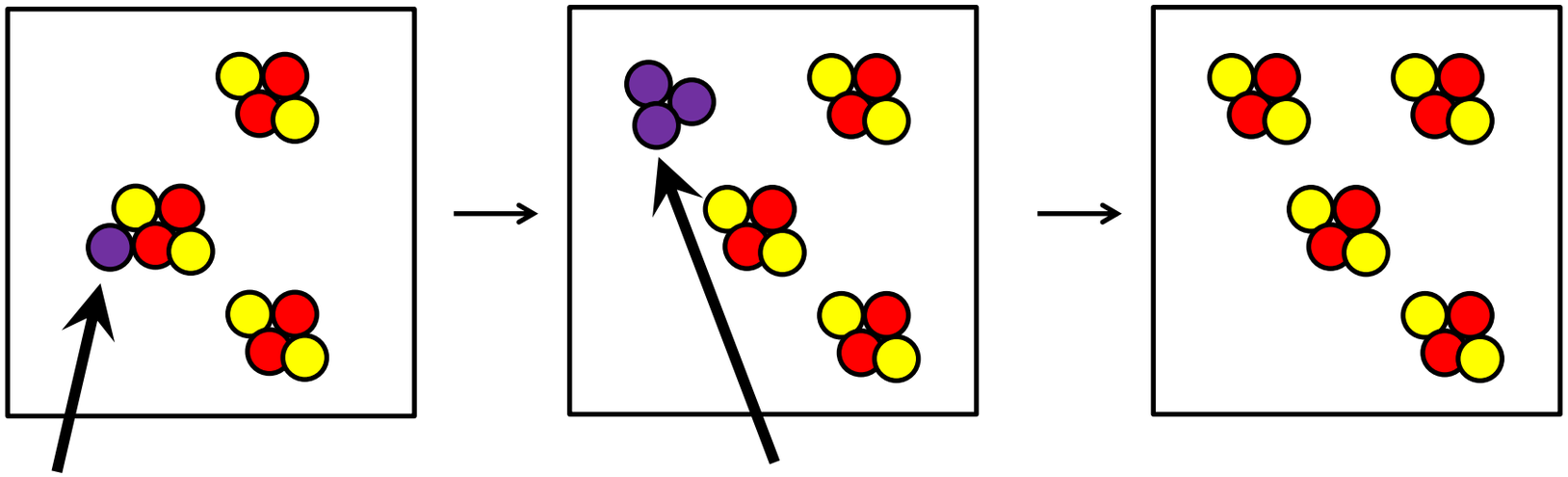
Unicellular



Cheat: “Germ cell” that never differentiates

Culture eventually crashes (too few Suc^+ cells)

Multicellular



Cheat grows faster initially (close to Suc+ cells), but eventually forms own clumps

Now farther from Suc^+ cells than true germ cells: grows slower

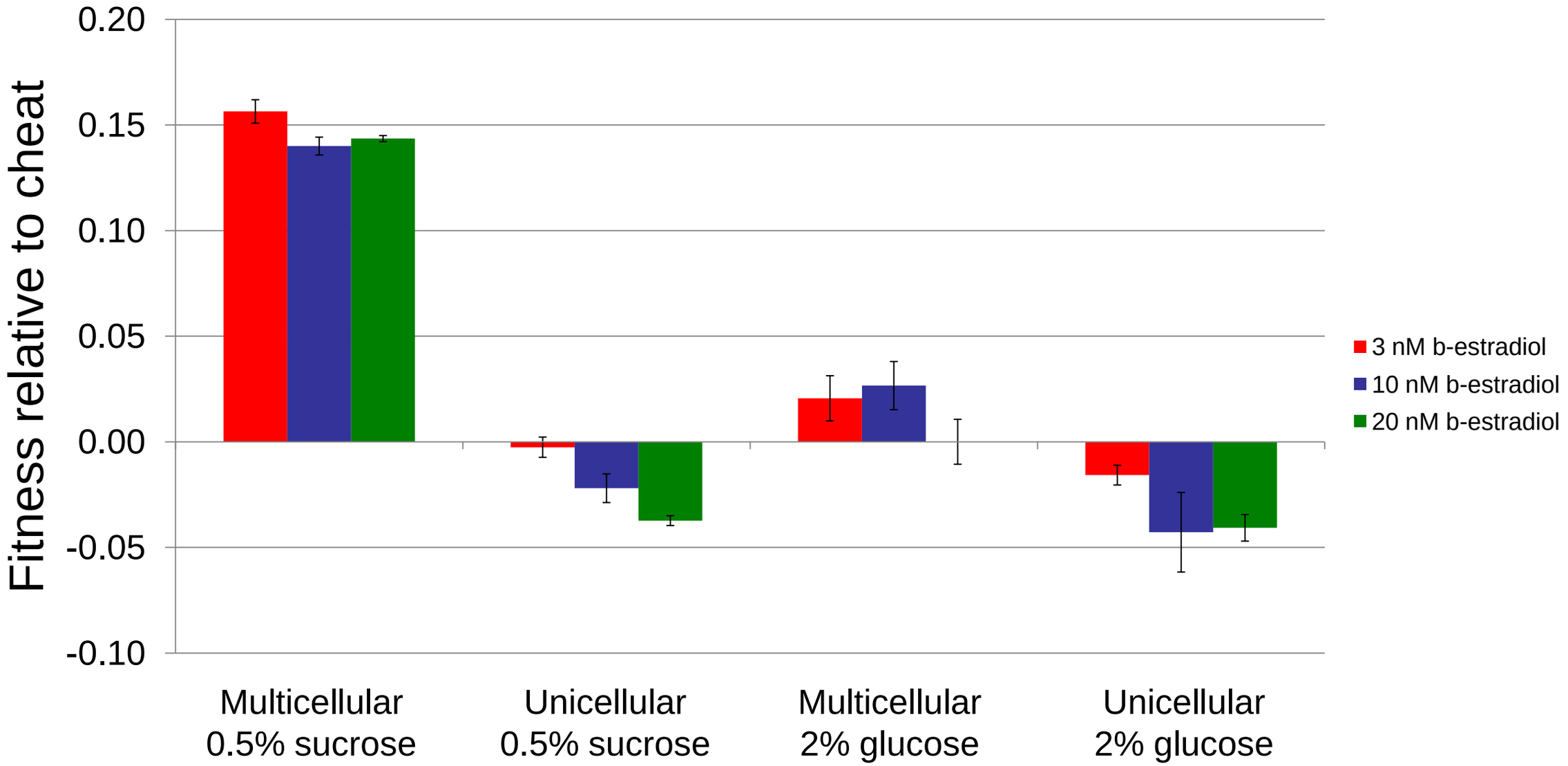
Original genotype wins

Cheats win as single cells, lose as clumps

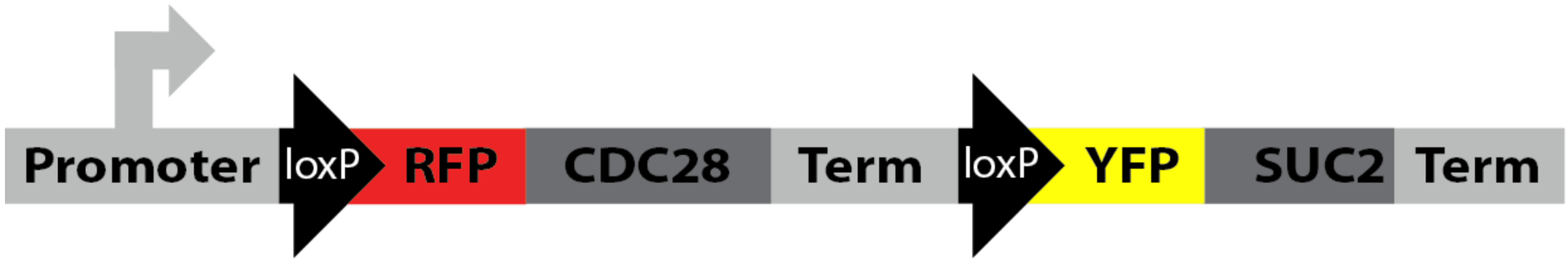
Engineer cheats (no recombinase, thus no soma)

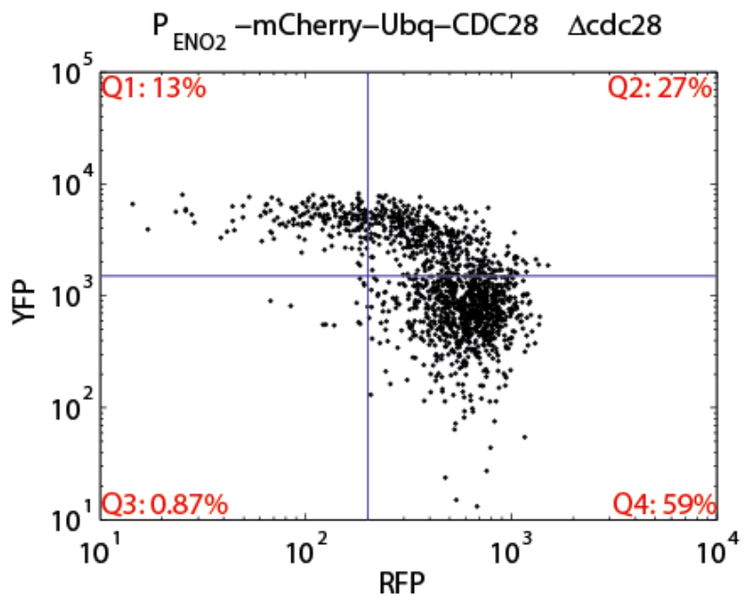
Make multicellular versions by inhibiting cell separation (*ace2*Δ mutant)

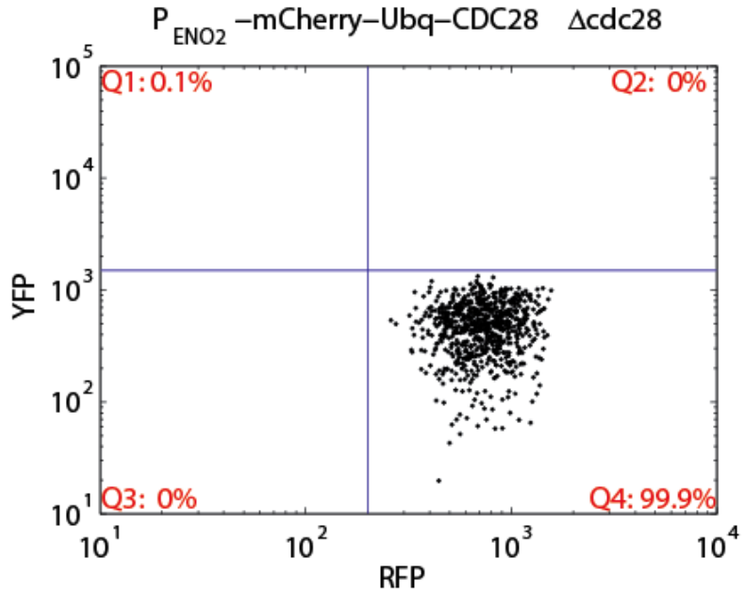
Measure fitness of converting vs. cheating strain ($s > 0$: cheater loses)



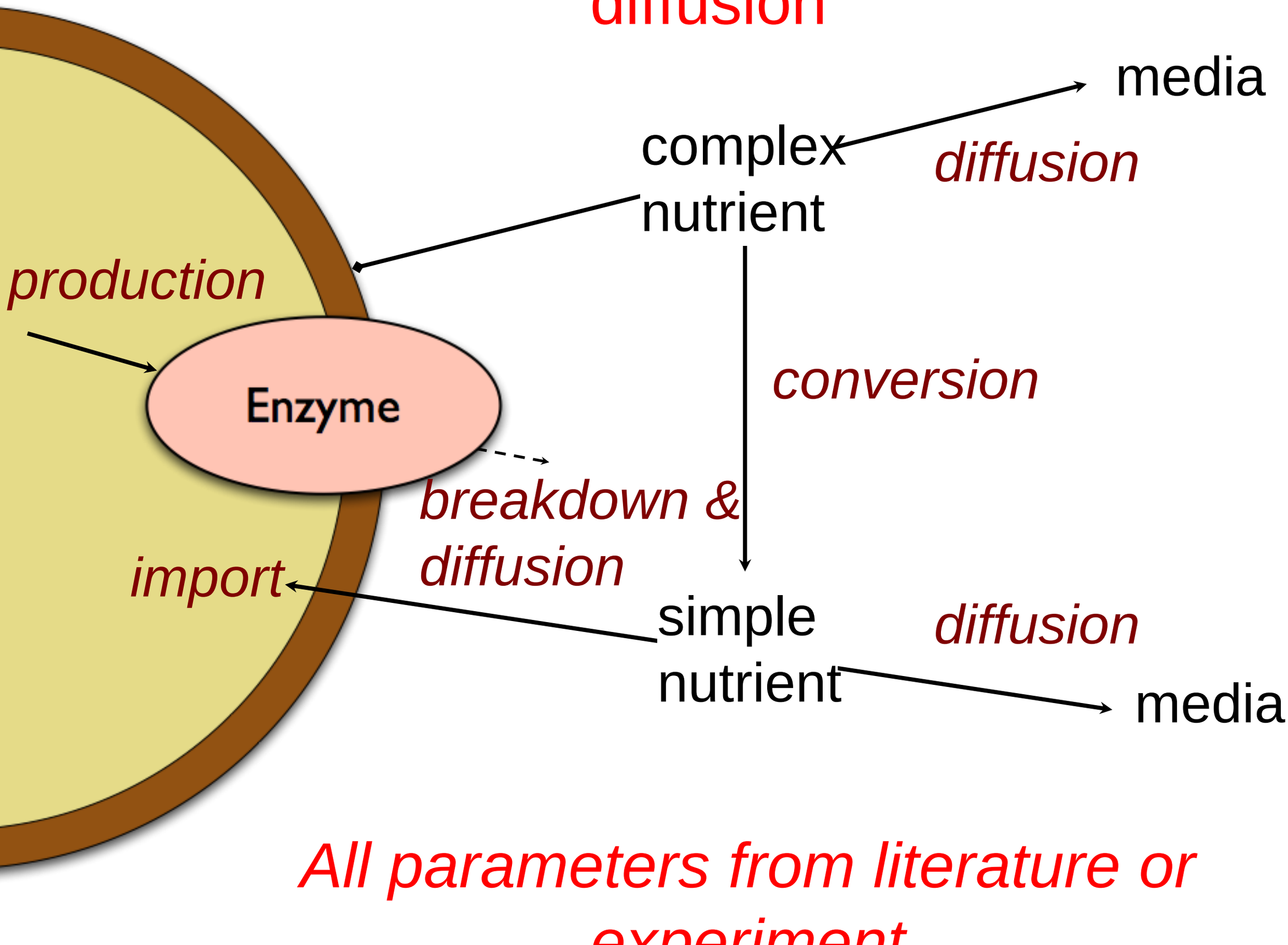
CDC28 loopout strategy





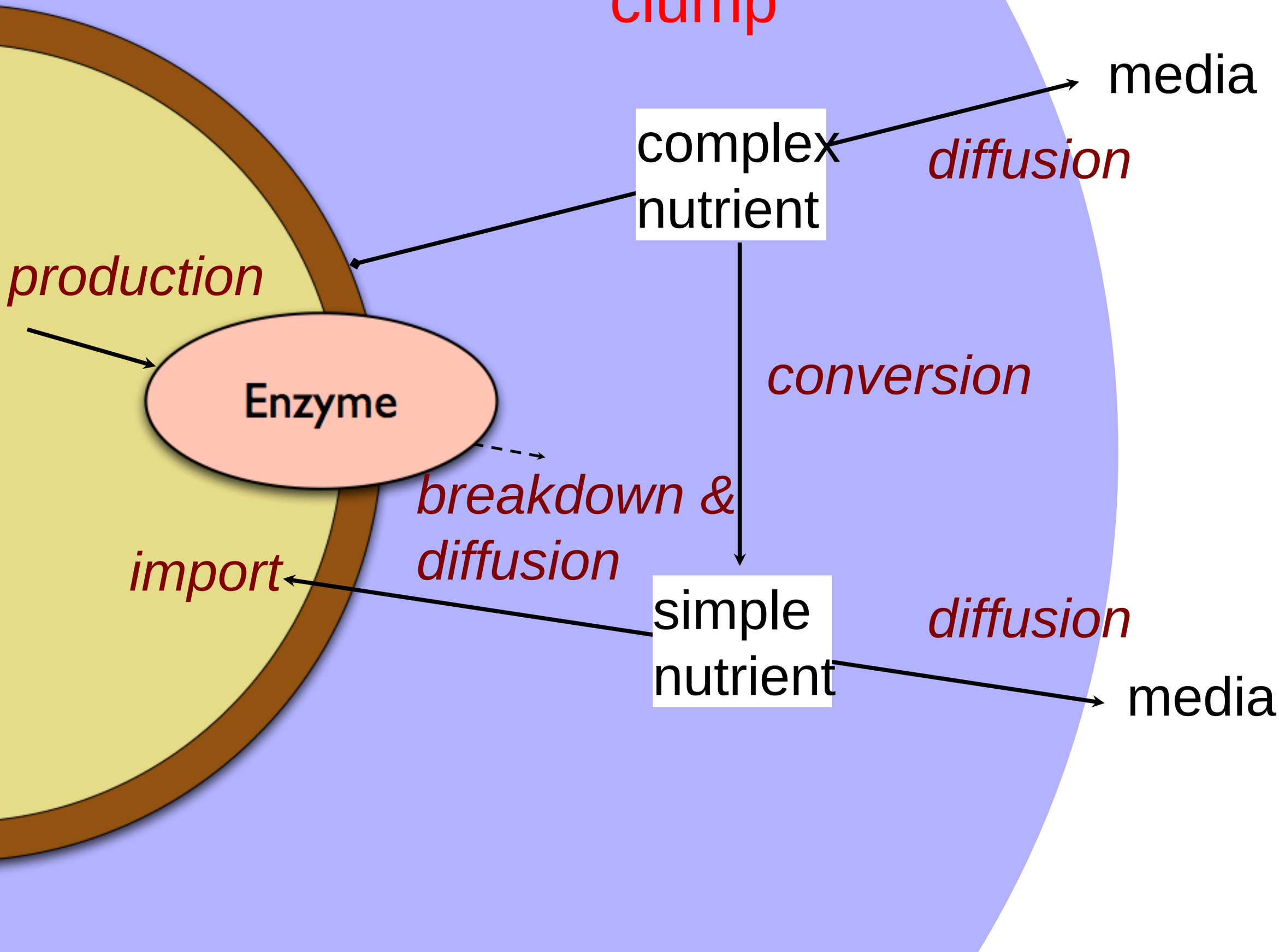


Simulation of single-cell nutrient diffusion

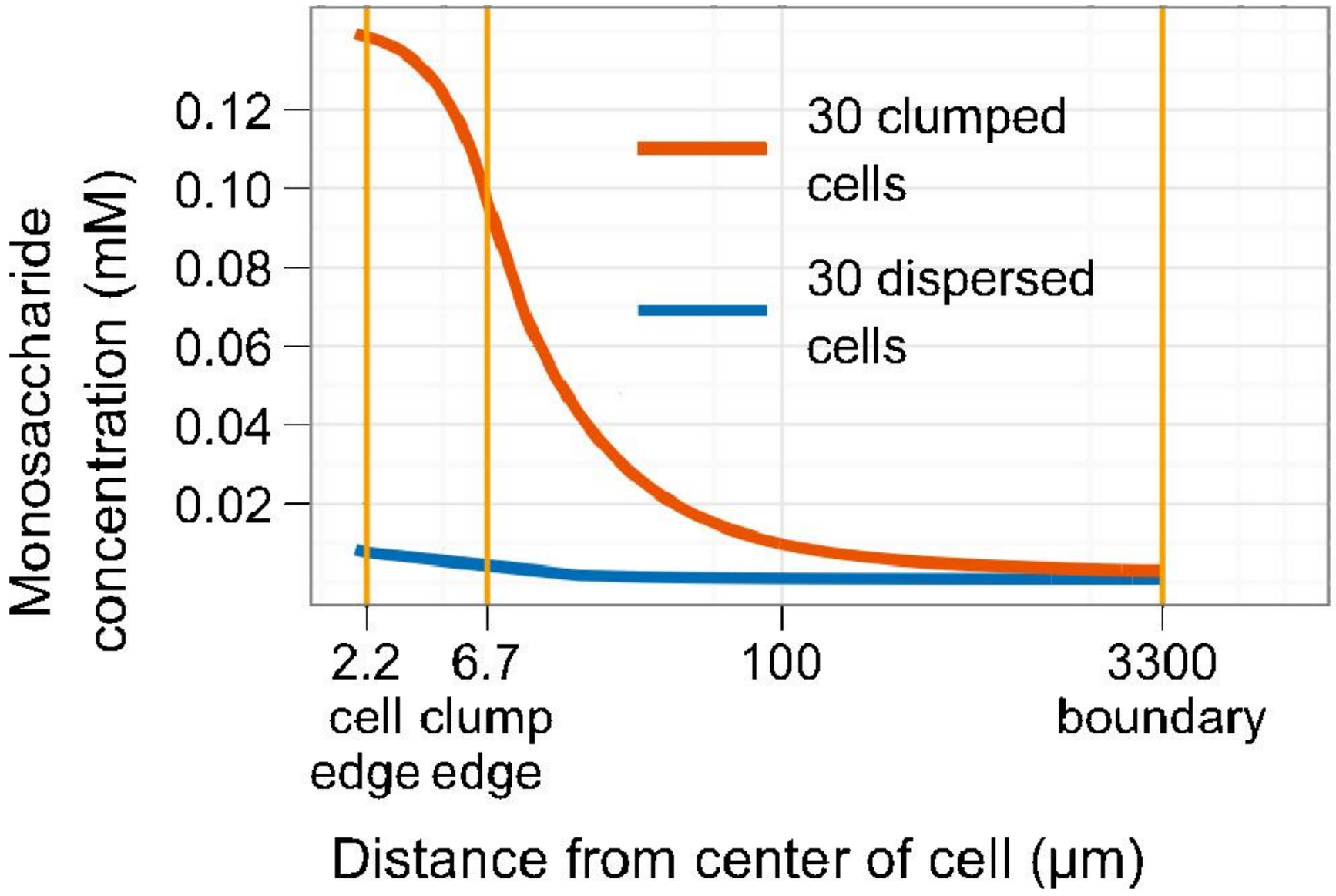


All parameters from literature or experiment

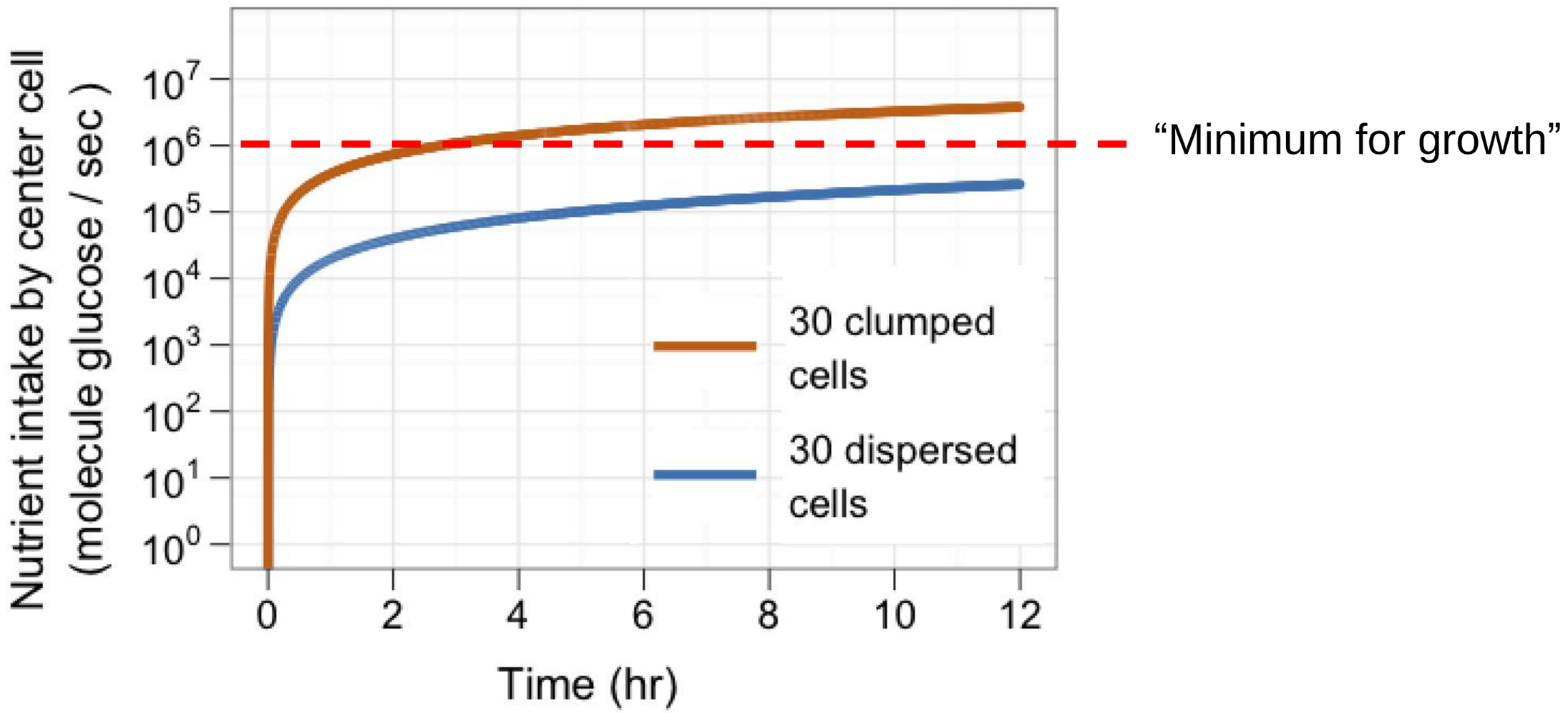
Simulation: glucose economy of a clump



Simulation: steady state [Monosaccharide] with 8 mM sucrose



Simulation: Monosaccharide uptake rate with 8 mM sucrose



Why not study the Royals?

Multicellular

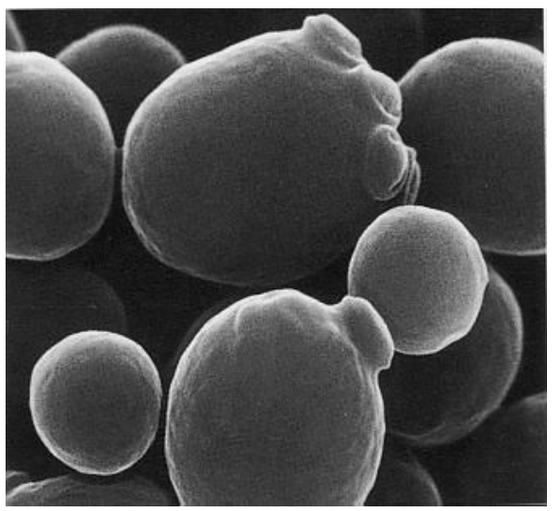
Excellent geneological records

Proliferates legitimately or illegitimately

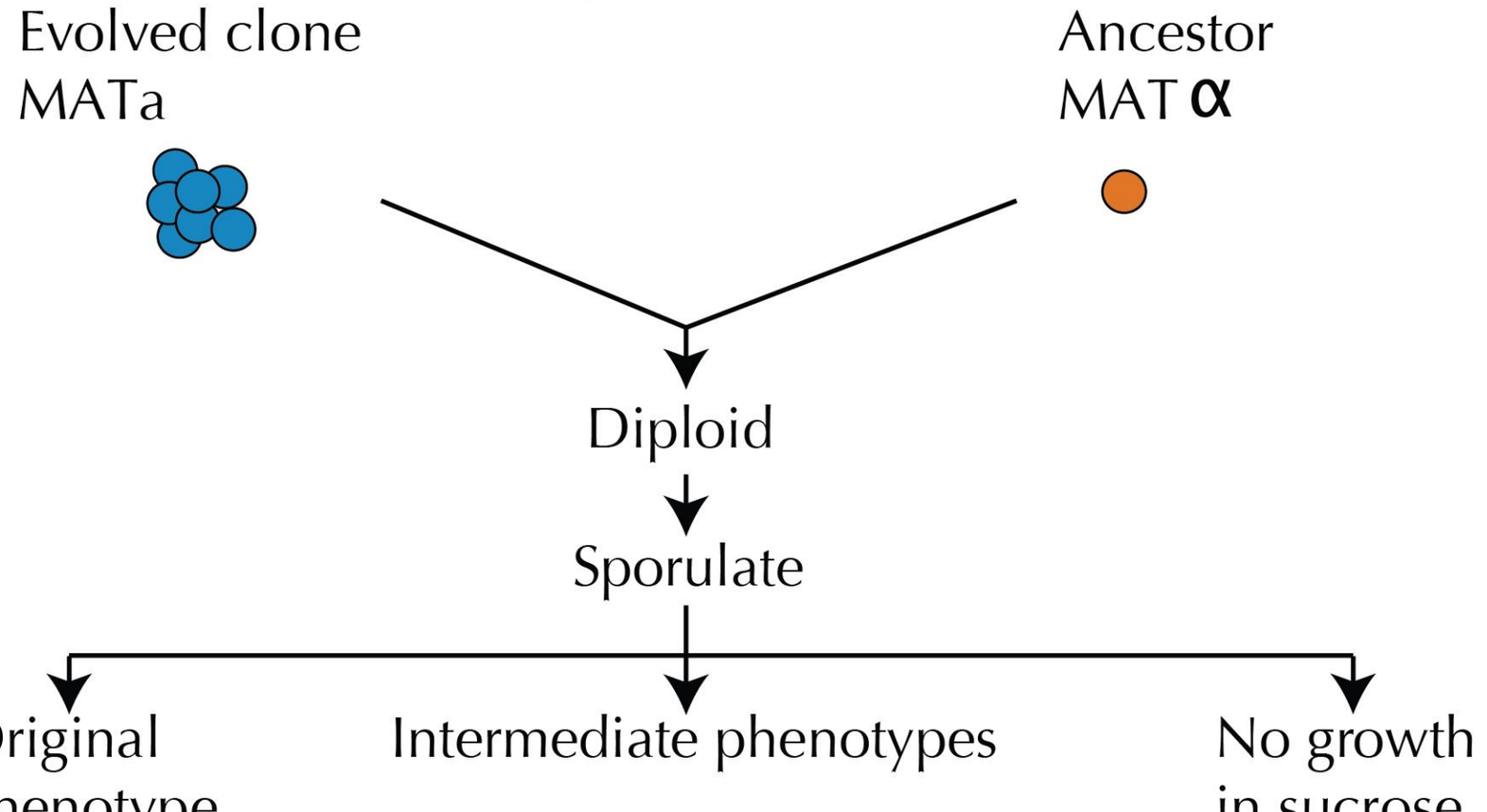
Alcohol tolerant and dependent varieties

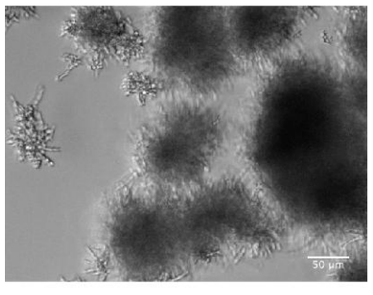
Wide public interest

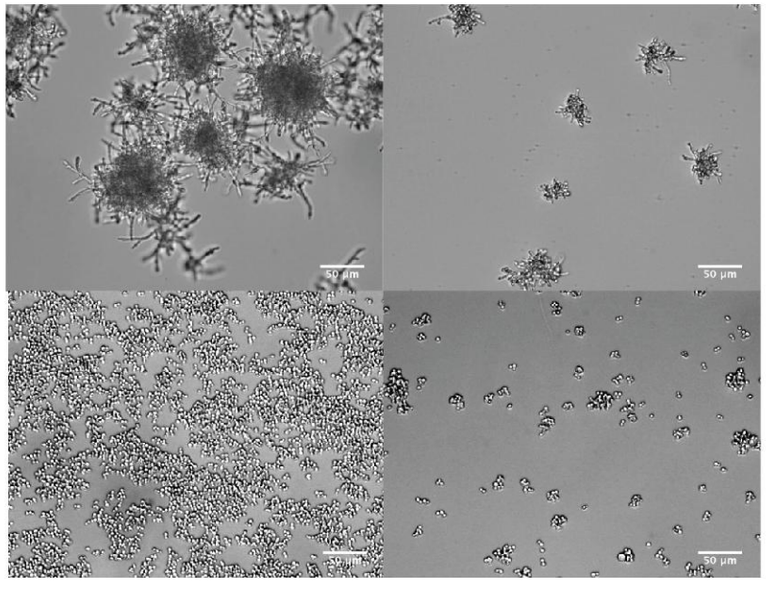
Budding yeast: *Saccharomyces cerevisiae*



Finding the causal mutations by genetics: part 1







Finding the causal mutations by genetics: part 2