

Budding multicellularity in yeast

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Harvard University

FAS Center for Systems Biology

February 19, 2013

Thank you to my advisors

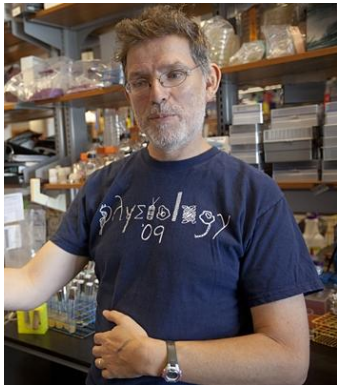


Andrew Murray



Kevin Foster

Thank you to my advisors



Andrew Murray



Kevin Foster



NIH NIGMS K25GM85806

Outline

How could multicellularity have evolved?

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Engineering growth in low sucrose

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Engineering growth in low sucrose

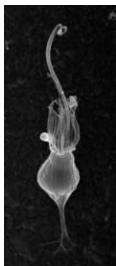
Evolving growth in low sucrose

How could multicellularity have evolved?

Engineering growth in low sucrose

Evolving growth in low sucrose

How could multicellularity have evolved?



Choanoflagellate
www.dayel.com

How could multicellularity have evolved?



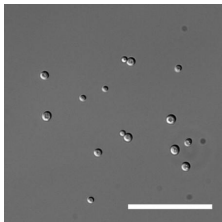
Choanoflagellate
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How could multicellularity have evolved?



Choanoflagellate
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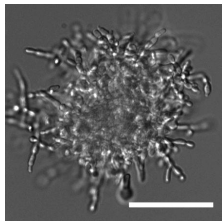
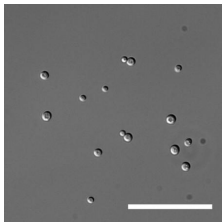


Scale bar = 50 μm

How could multicellularity have evolved?



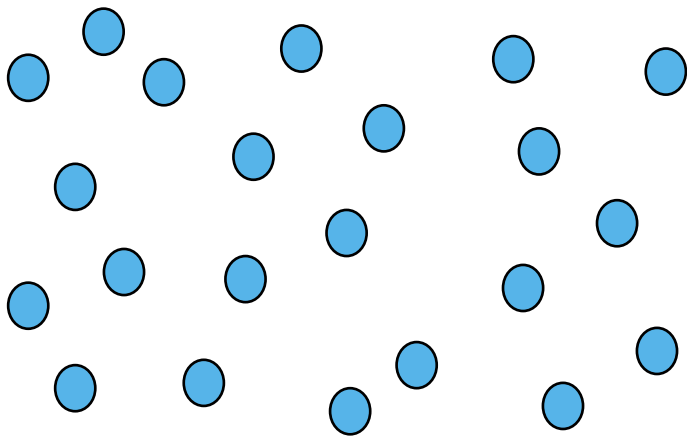
Choanoflagellate
www.dayel.com



Scale bar = 50 μm

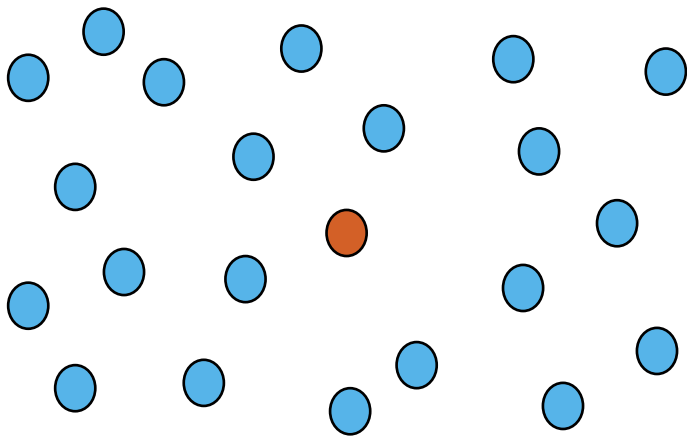
How could multicellularity have evolved?

In a unicellular world...



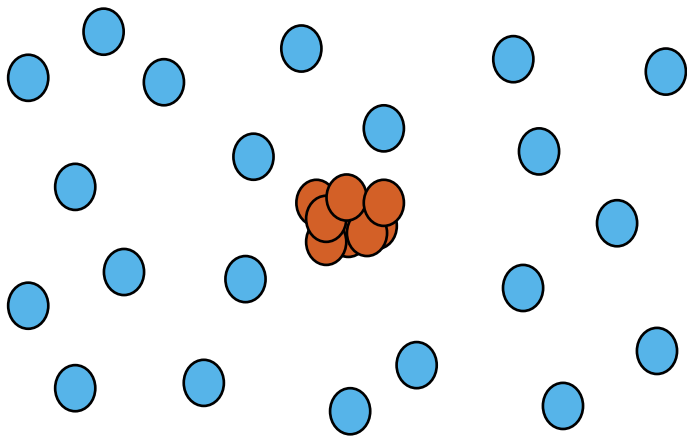
How could multicellularity have evolved?

...a mutation occurs...



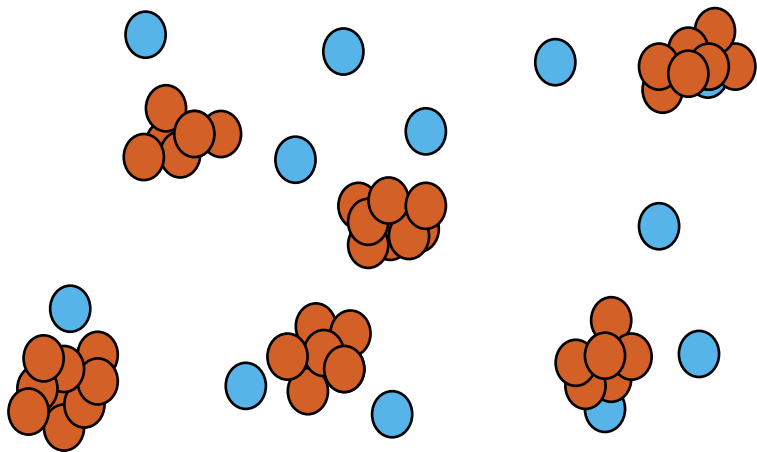
How could multicellularity have evolved?

...that causes the daughters to stay attached to the mother.



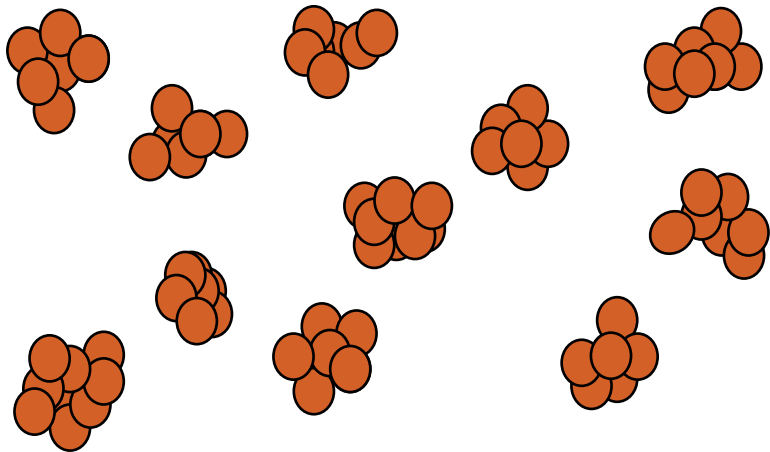
How could multicellularity have evolved?

The clumps outcompete the single cells.

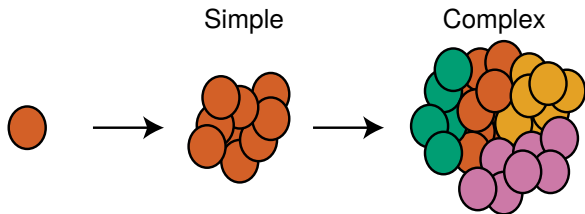


How could multicellularity have evolved?

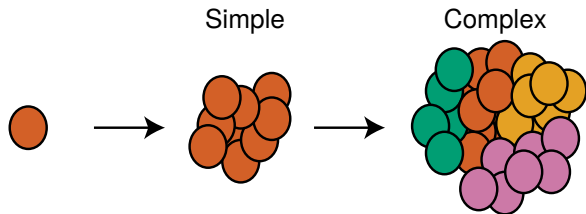
Simple multicellularity evolves.



How could multicellularity have evolved?



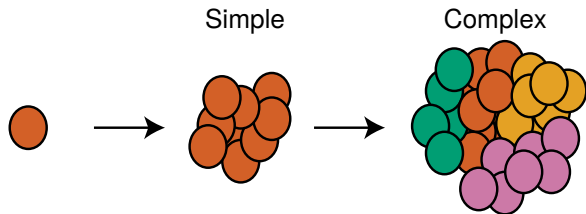
How could multicellularity have evolved?



For each transition:

1. What is the selection pressure?
2. What strategies can answer the pressure?
3. What are the mutations underlying each strategy?

How could multicellularity have evolved?



For each transition:

1. What is the selection pressure?
2. What strategies can answer the pressure?
3. What are the mutations underlying each strategy?

Use modeling, engineering, and experimental evolution.

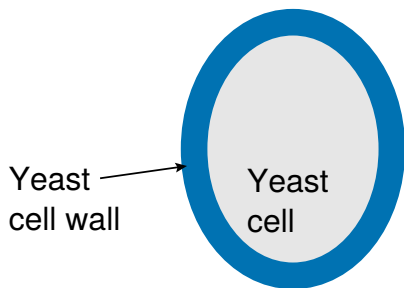
Outline

How could multicellularity have evolved?

Engineering growth in low sucrose

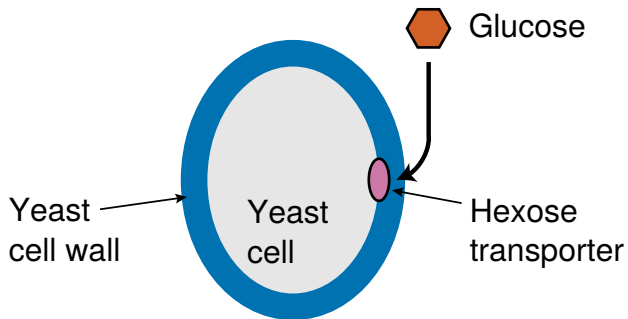
Evolving growth in low sucrose

Yeast sucrose digestion



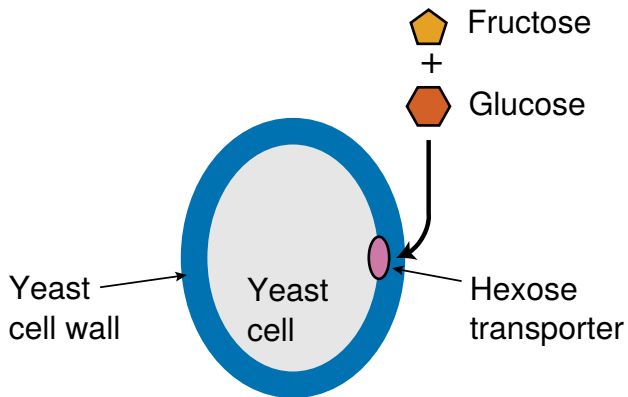
Yeast sucrose digestion

Yeast can directly import glucose...



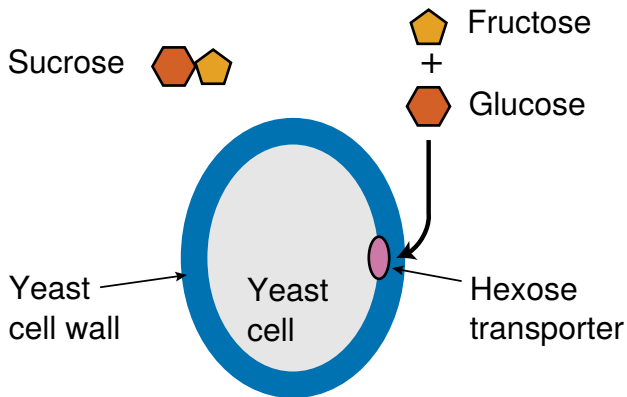
Yeast sucrose digestion

...and directly import fructose.



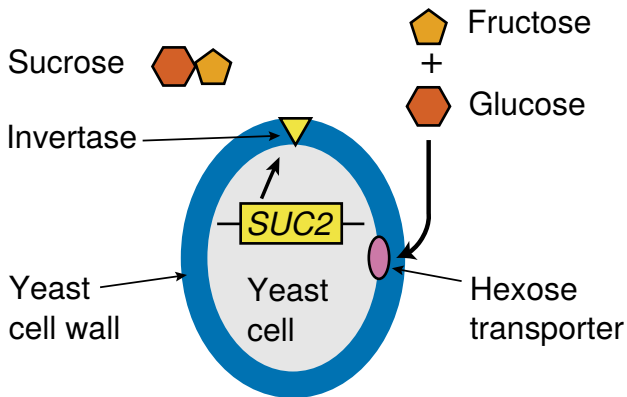
Yeast sucrose digestion

Sucrose cannot be directly imported in the lab.



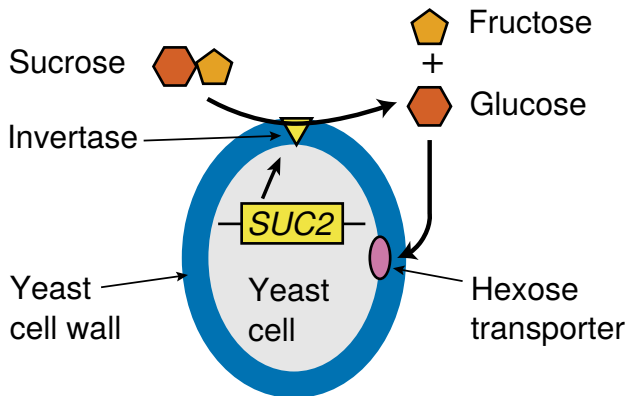
Yeast sucrose digestion

In low glucose, yeast secretes invertase, which remains in the cell wall.



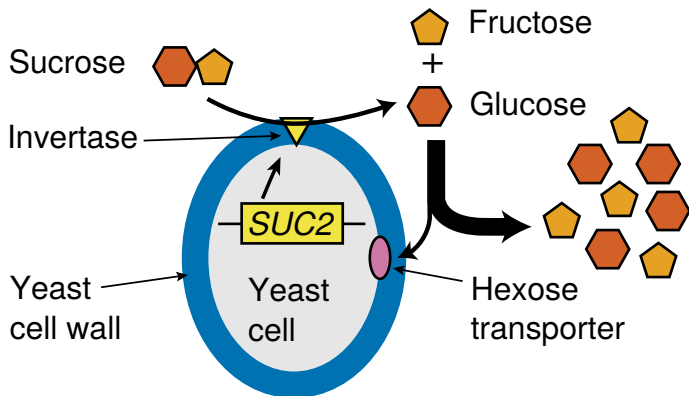
Yeast sucrose digestion

Sucrose is hydrolyzed by invertase.

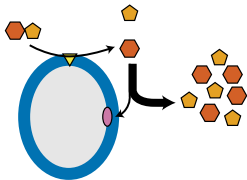


Yeast sucrose digestion

But glucose and fructose diffuse away.



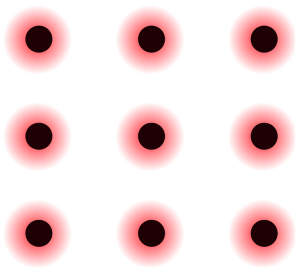
A cell can't capture enough sugar if...



- ▶ Low density of sucrose
- ▶ Low density of cells

Why do cells grow better at high density?

Spaced cells can't capture each other's diffusing sugars



Monosaccharide
concentration

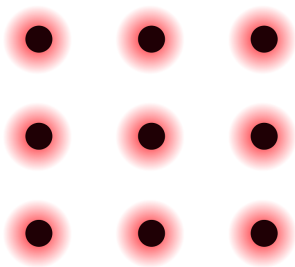
Low



High

Why do cells grow better at high density?

Cells in a clump can feed each other



Monosaccharide
concentration

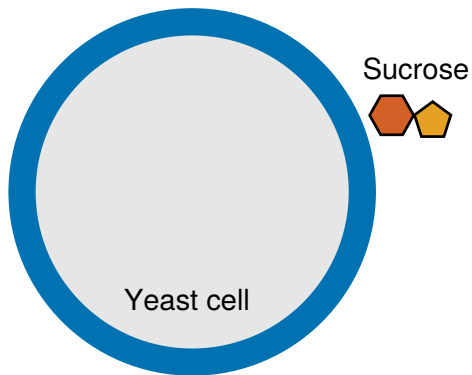
Low



High

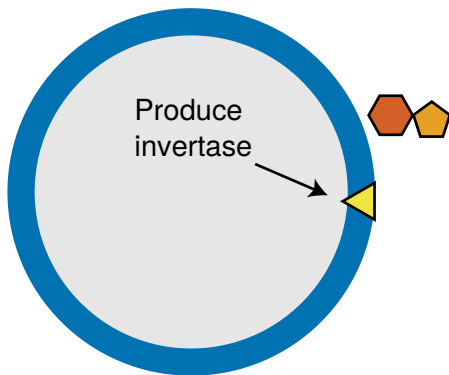
Model diffusion of sugar to a cell

Yeast cell inoculated into 150 μL of sucrose



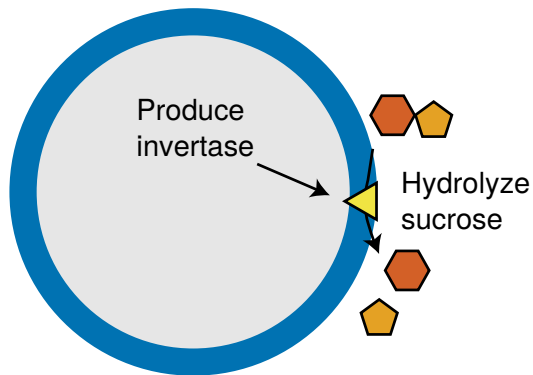
Model diffusion of sugar to a cell

At each time step, produce invertase,...



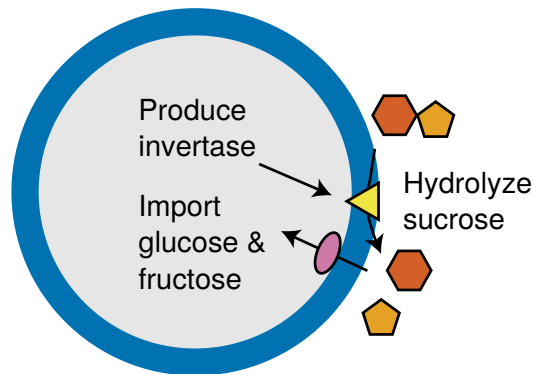
Model diffusion of sugar to a cell

...hydrolyze sucrose,...



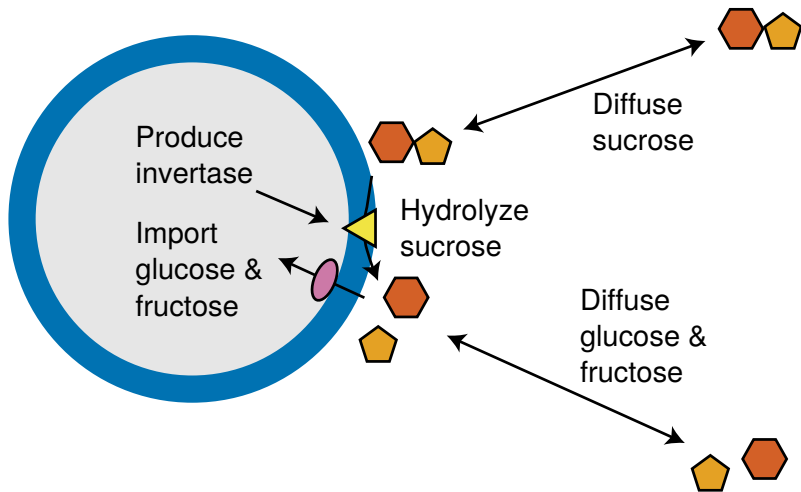
Model diffusion of sugar to a cell

...import glucose and fructose,...



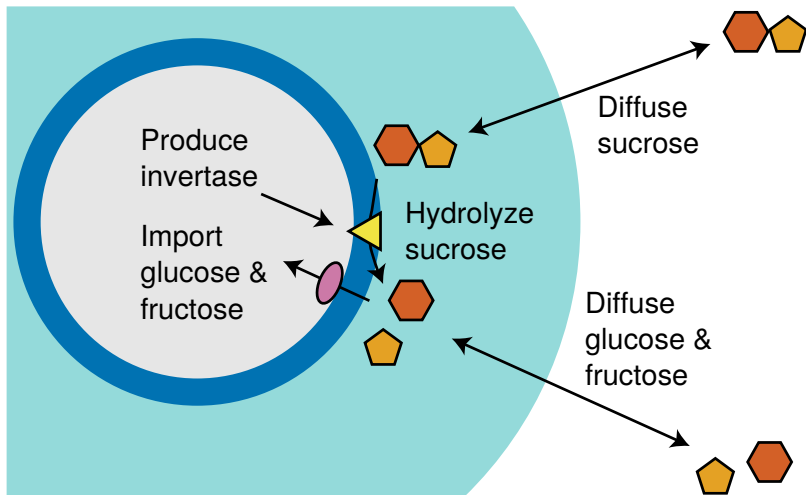
Model diffusion of sugar to a cell

...and diffuse all nutrients.



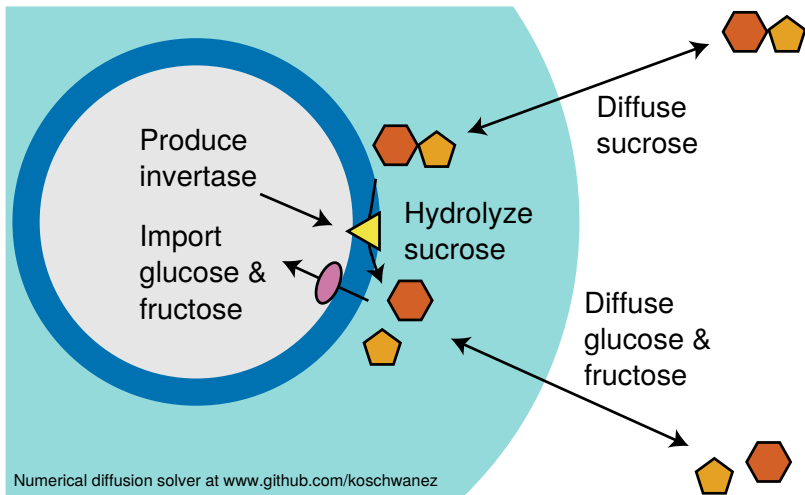
Model diffusion of sugar to a cell

Add a mean field of cells to account for other cells in the well.



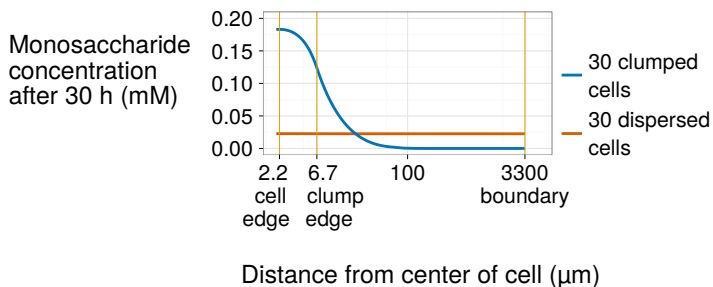
Model diffusion of sugar to a cell

All parameters measured or taken from published data (no free parameters.)



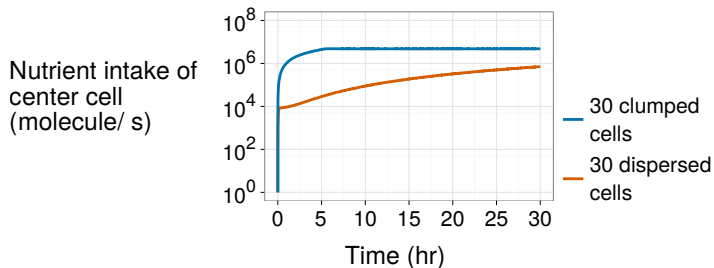
Model 30 cells in 8 mM sucrose for 30 hours

Monosaccharide concentration at the center cell is higher in a clump.



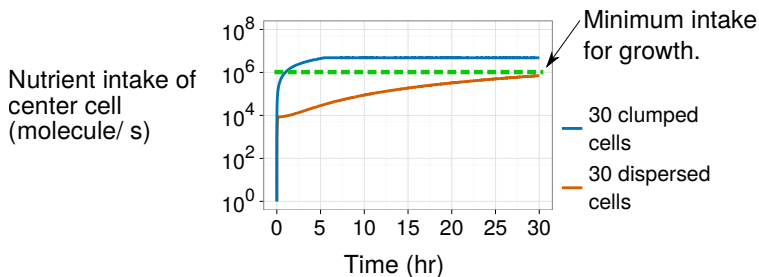
Model 30 cells in 8 mM sucrose for 30 hours

Nutrient intake at the center cell is higher in a clump.



Model 30 cells in 8 mM sucrose for 30 hours

A clump of cells will quickly exceed the minimum intake required for growth.

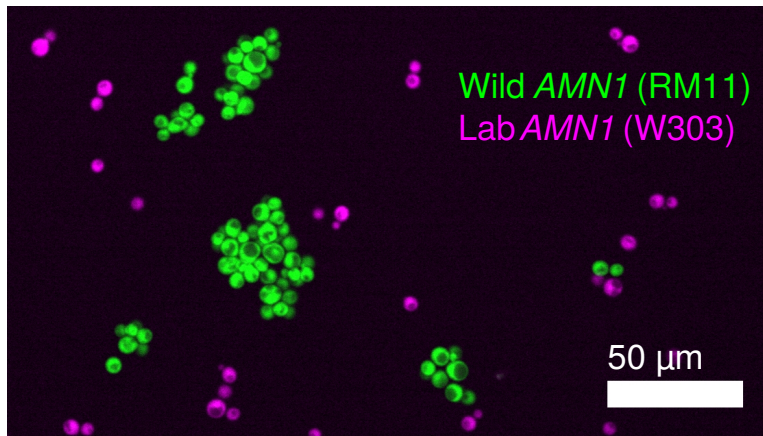


Prediction in low sucrose and low cell density

A clump of cells can grow, single cells cannot.

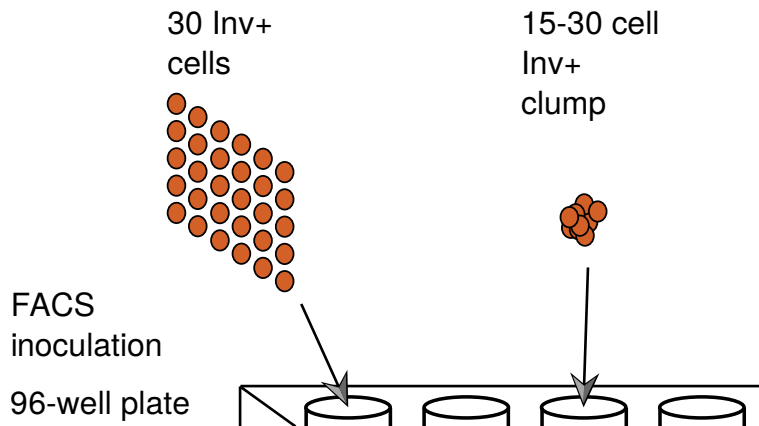
AMN1 controls clumpiness

Discovered in Kruglyak lab



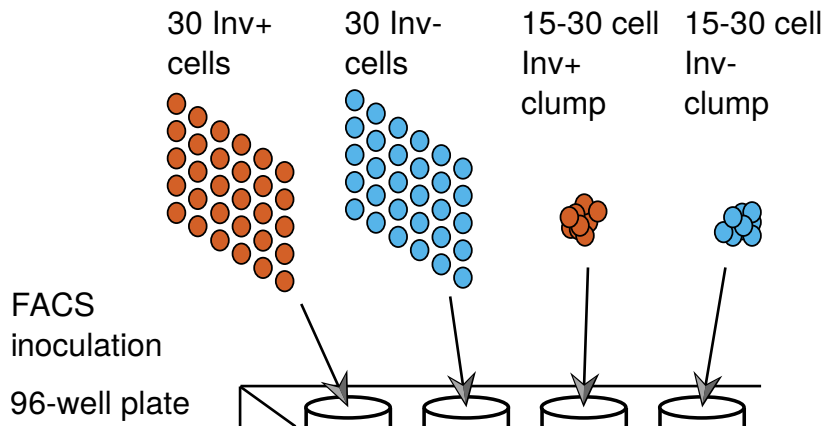
Can clumps grow where cells cannot?

Fluorescent Activated Cell Sorter (FACS) sorts cells or clumps from an *AMN1* strain.



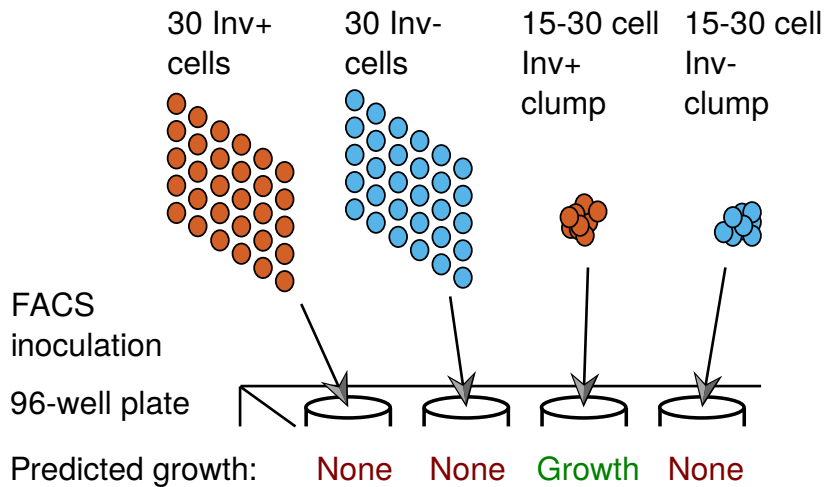
Can clumps grow where cells cannot?

Inv^- cells are used as a control.



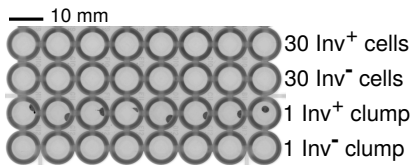
Can clumps grow where cells cannot?

Growth predicted in well only with Inv^+ clump



Clumps can grow where cells cannot.

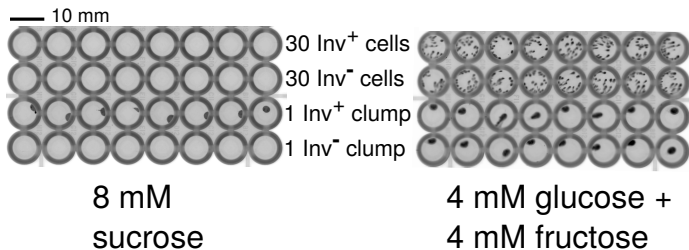
Growth differs in low concentrations of sucrose.



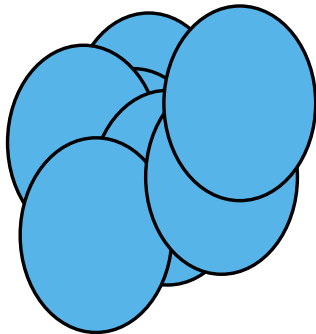
8 mM
sucrose

Clumps can grow where cells cannot.

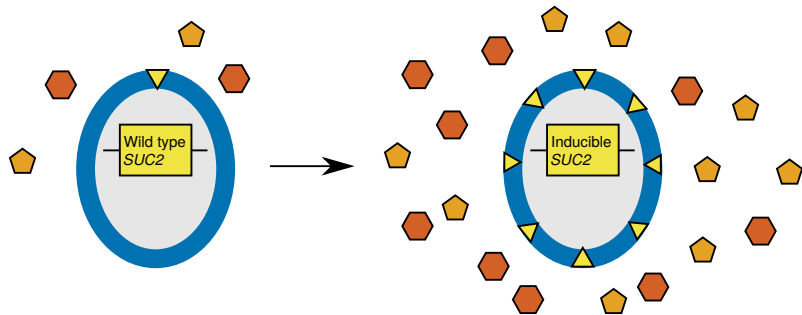
Growth in all wells in monosachharide.



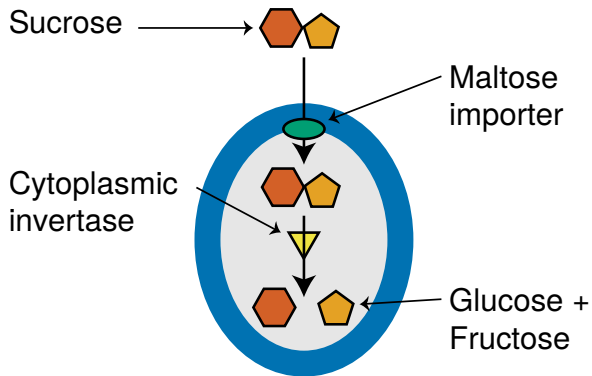
Strategy 1: Form multicellular clumps



Strategy 2: Make more invertase



Strategy 3: Import sucrose



Outline

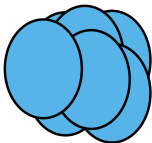
How could multicellularity have evolved?

Engineering growth in low sucrose

Evolving growth in low sucrose

Three strategies an engineer would take

Form multicellular clumps



Boost invertase expression

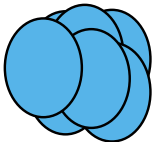


Import sucrose



WWED?

Form multicellular clumps



Boost invertase expression



Import sucrose



Experimental evolution schematic

10 parallel cultures

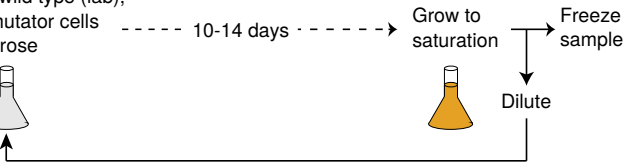
Inoculate wild type (lab),
haploid, mutator cells
in low sucrose

----- 10-14 days ----->

Grow to
saturation

Freeze
sample

Dilute



Experimental evolution schematic

10 parallel cultures

Inoculate wild type (lab),
haploid, mutator cells
in low sucrose

----- 10-14 days ----->

Grow to
saturation

Freeze
sample

Dilute



~230-310 generations per culture



Experimental evolution schematic

10 parallel cultures

Inoculate wild type (lab),
haploid, mutator cells
in low sucrose



----- 10-14 days ----->

Grow to
saturation



Freeze
sample

Dilute

~230-310 generations per culture

Inoculate
cells in low
sucrose



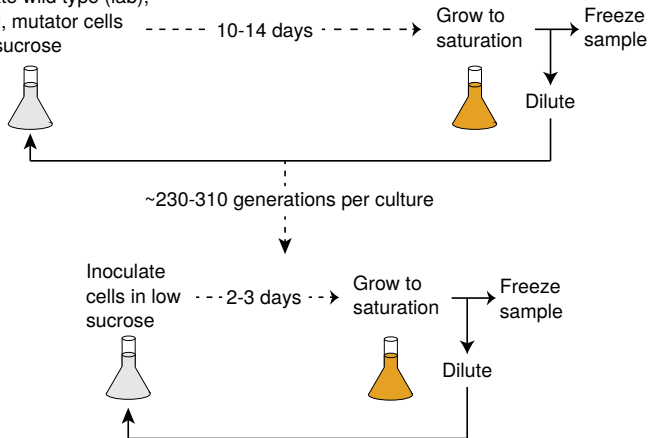
--- 2-3 days --->

Grow to
saturation



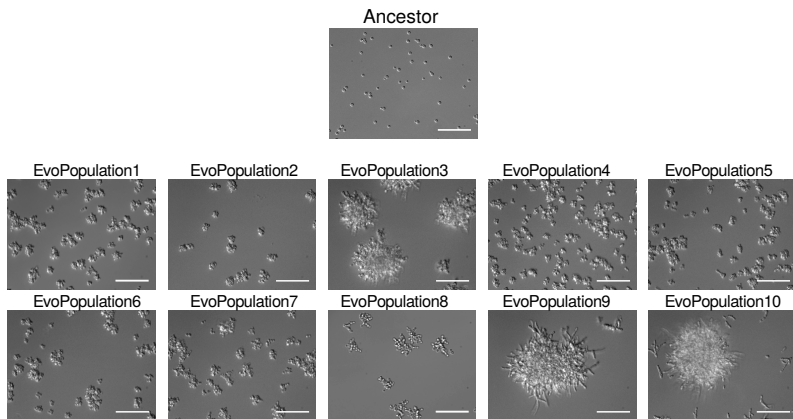
Freeze
sample

Dilute



The evolved populations

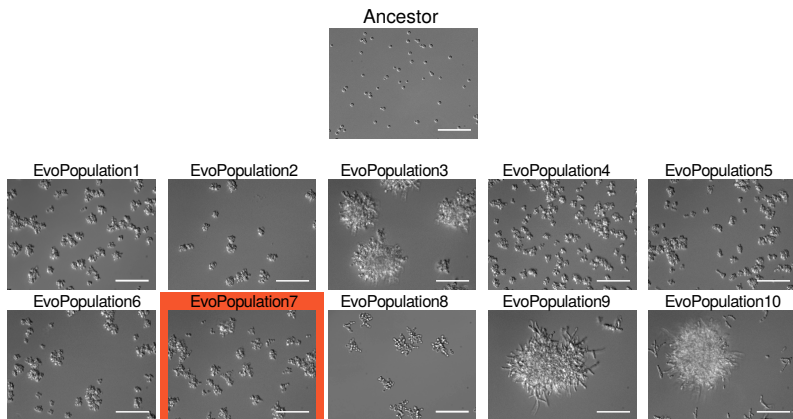
All populations are clumpy.



Scale bar = 50 μ m

The evolved populations

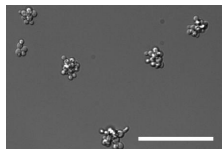
All populations but one are clonal.



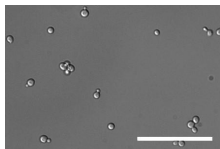
Scale bar = 50 μm

One population had three different clones

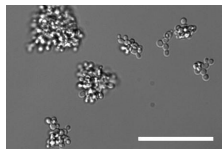
12 total clones: 11 are clumpy



EvoClone7A

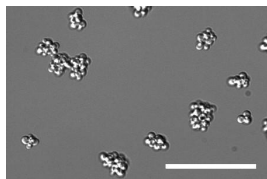


EvoClone7B

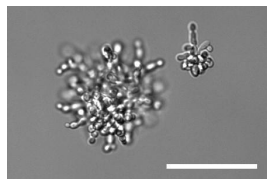


EvoClone7C

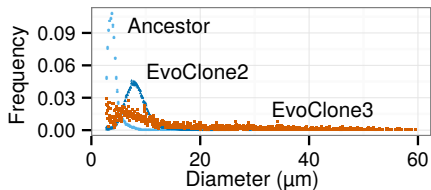
Clump size and variation varies between strains



EvoClone2



EvoClone3



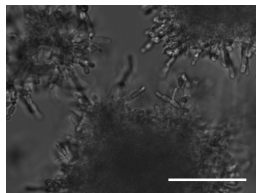
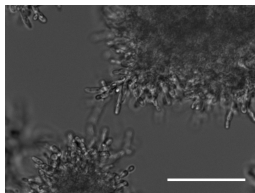
Scale bar = 50 μm

Clump size regulation varies between strains

EvoClone10

EvoClone9

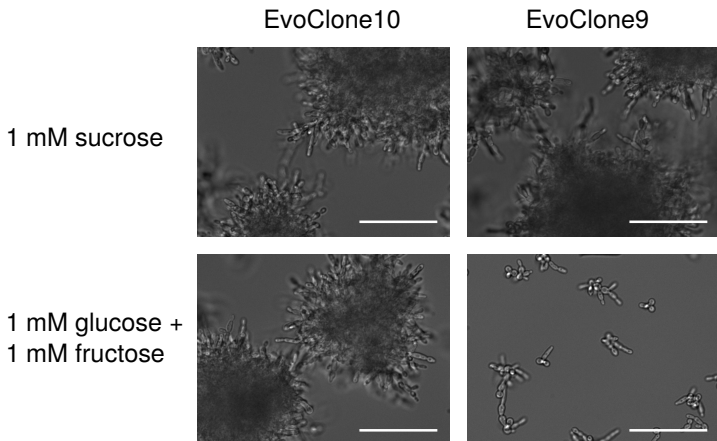
1 mM sucrose



1 mM glucose +
1 mM fructose

Scale bar = 50 μm

Clump size regulation varies between strains



Scale bar = 50 μ m

The big questions

1. What strategies were used to answer the selection?
2. What are the mutations behind these strategies?

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Evolution: 2 months

Analysis: 2 years

The big questions

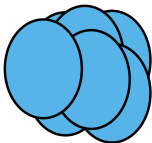
1. What strategies were used to answer the selection?
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Evolution: 2 months

Analysis: 2 years

What strategies were used?

Form multicellular clumps



Boost invertase expression

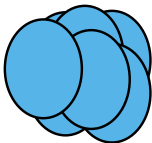


Import sucrose



What strategies were used?

Form multicellular clumps



11/12

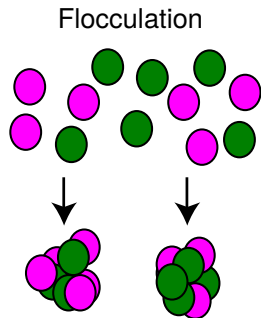
Boost invertase expression



Import sucrose

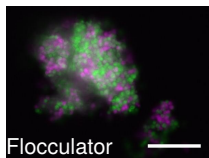
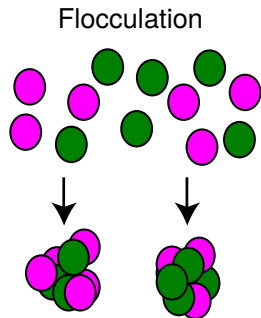


Two ways that yeast form clumps



Scale bar = 50 μm

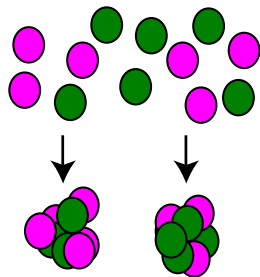
Two ways that yeast form clumps



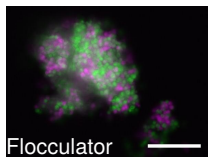
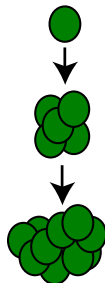
Scale bar = 50 μm

Two ways that yeast form clumps

Flocculation

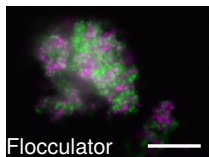
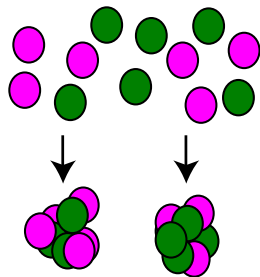


Incomplete separation

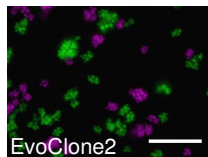
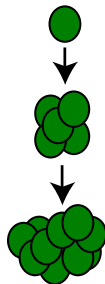


Two ways that yeast form clumps

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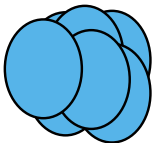
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What strategies were used?

Form multicellular clumps



11/12

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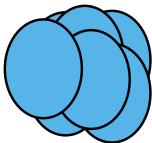


Import sucrose



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Form multicellular clumps



11/12

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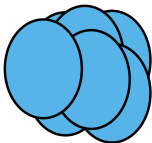
10/12

Import sucrose



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11/12

Boost invertase expression



10/12

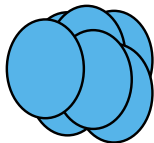
Import sucrose



0/12

What strategies were used?

Form multicellular clumps



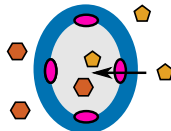
11/12

Boost invertase expression



10/12

Boost hexose transporter expression



11/12

The big questions

1. What strategies were used to answer the selection?
2. What are the mutations behind these strategies?

The big questions

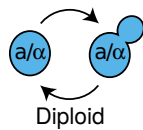
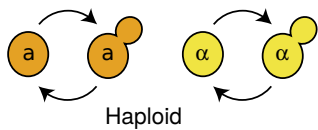
1. What strategies were used to answer the selection?
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The big questions

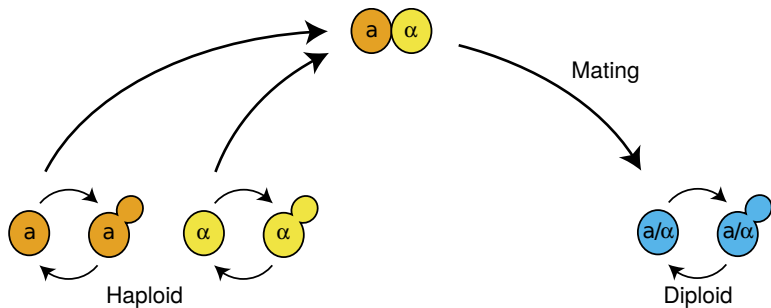
1. What strategies were used to answer the selection?
2. What are the mutations behind these strategies?

Average of more than 100 mutations per strain

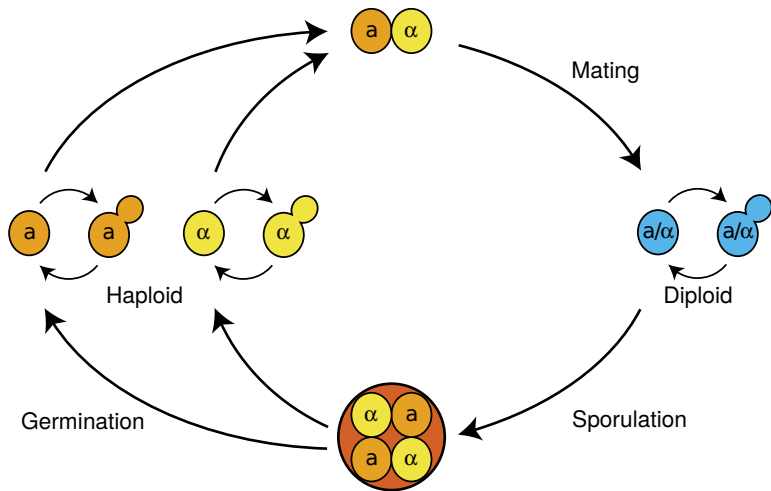
Life cycle of yeast



Life cycle of yeast



Life cycle of yeast



Bulk segregant analysis

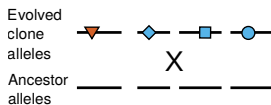
Backcross evolved clone to ancestor and isolate progeny with selected phenotype.

Evolved clone alleles



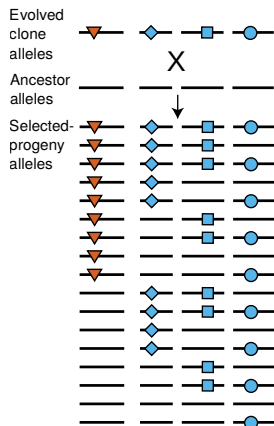
Bulk segregant analysis

Backcross evolved clone to ancestor and isolate progeny with selected phenotype.



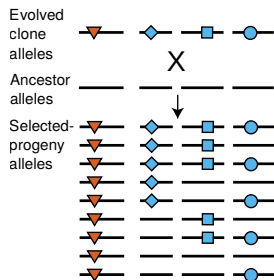
Bulk segregant analysis

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Bulk segregant analysis

Backcross evolved clone to ancestor and isolate progeny with selected phenotype.

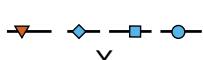


Bulk segregant analysis

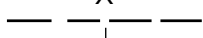
Backcross evolved clone to ancestor and isolate progeny with selected phenotype.

Sequence evolved clone, ancestor, and pooled progeny to find putative causal mutations.

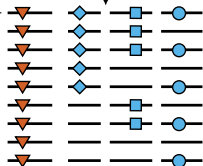
Evolved clone alleles



Ancestor alleles



Selected-progeny alleles



GCCCAGAAAGTAATGGAT**A**GAACCTTTTTCCCTCAACA

TGCAGAGTTGGAGATAGT**C**TAACCGTGACTTCATTTTCAA

GCCCAGAAAGTAATGGAT**T**GAACCTTTTTCCCTCAACA

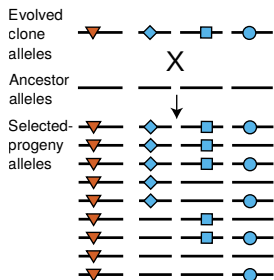
TGCAGAGTTGGAGATAGT**G**TAACCGTGACTTCATTTTCAA

GCCCAGAAAGTAATGGAT**A**GAACCTTTTT
 CCCAGAAAGTAATGGAT**A**GAACCTTTTT
 CCAGAAAGTAATGGAT**A**GAACCTTTTTCC
 CCAGAAAGTAATGGAT**A**GAACCTTTTTCC
 AGAAAGTAATGGAT**A**GAACCTTTTTCCCT
 GAAAGTAATGGAT**A**GAACCTTTTTCCCT
 AAAGTAATGGAT**A**GAACCTTTTTCCCT
 AAAGTAATGGAT**A**GAACCTTTTTCCCT
 AAGTAATGGAT**A**GAACCTTTTTCCCTCA
 AGTAATGGAT**A**GAACCTTTTTCCCTCAA
 GTAATGGAT**A**GAACCTTTTTCCCTCAAC
 TAATGGAT**A**GAACCTTTTTCCCTCAACA

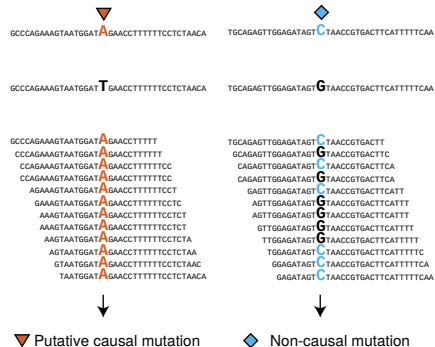
TGCAGAGTTGGAGATAGT**C**TAACCGTGACTT
 GCAGAGTTGGAGATAGT**C**TAACCGTGACTTC
 CAGAGTTGGAGATAGT**C**TAACCGTGACTTCA
 CAGAGTTGGAGATAGT**C**TAACCGTGACTTCA
 GAGTTGGAGATAGT**C**TAACCGTGACTTCATT
 AGTTGGAGATAGT**G**TAACCGTGACTTCATT
 AGTTGGAGATAGT**G**TAACCGTGACTTCATT
 GTTGGAGATAGT**G**TAACCGTGACTTCATT
 TTGGAGATAGT**C**TAACCGTGACTTCATTTT
 TGGAGATAGT**C**TAACCGTGACTTCATTTTC
 GGAGATAGT**C**TAACCGTGACTTCATTTTCA
 GAGATAGT**C**TAACCGTGACTTCATTTTCAA

Bulk segregant analysis

Backcross evolved clone to ancestor and isolate progeny with selected phenotype.



Sequence evolved clone, ancestor, and pooled progeny to find putative causal mutations.



From 1521 total mutations, 80 putative causal

Lie in or near 53 genes.

ACE2 WHI2 ECM5 PRC1 HXK1 MIT1
PUF4 MPT5 GCN2 MTH1 AXL2 DNF2
ENP2 NAT1 PDR1 HTZ1 SKS1
IRA2 ERG1 UBR1 PHO8 SAN1 IFM1
GCN3 SNF2 GCR2 GPB2 MCD1 CSE2
RGT1 SIN4 KEM1 SAC6 RAM1
SYP1 GAC1 NRG1 IRA1 GIN4 WTM2 SNF3 MED1
IRC8 NUT1 ARO2 ARE1 BPH1
TOP3 UBC5

From 1521 total mutations, 80 putative causal

Lie in or near 53 genes.

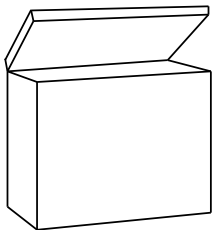
ACE2 WHI2 ECM5 PRC1 HXK1 MIT1
PUF4 MPT5 GCN2 MTH1 AXL2 DNF2
ENP2 NAT1 PDR1 HTZ1 SKS1
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RGT1 SIN4 KEM1 SAC6 RAM1
SYP1 GAC1 NRG1 IRA1 GIN4 WTM2 SNF3 MED1
IRC8 NUT1 ARO2 ARE1 BPH1
TOP3 UBC5

AMN1 was not found.

The most commonly mutated pathways

Pathway	Mutations
<i>ACE2</i>	8
<i>UBR1</i>	6
<i>RGT1</i> pathway	8
Mediator	5
<i>IRA1/2</i>	5

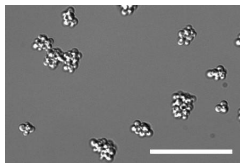
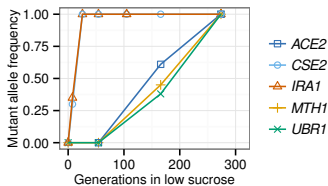
Yeast can be frozen and thawed



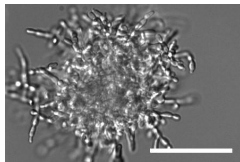
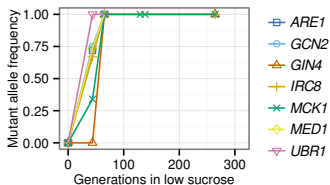
-80 °C

Tracking allele frequency reveals mutational sweeps.

EvoClone2

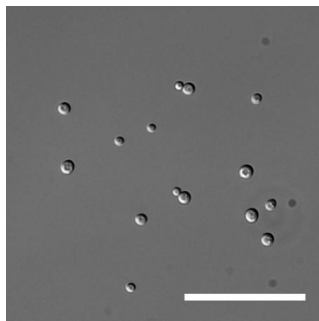


EvoClone9

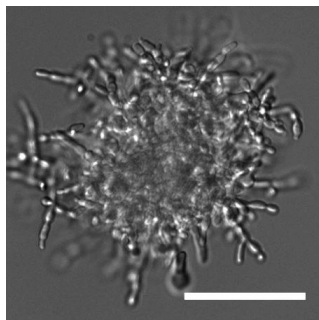


Scale bar = 50 μ m

Recreating strains



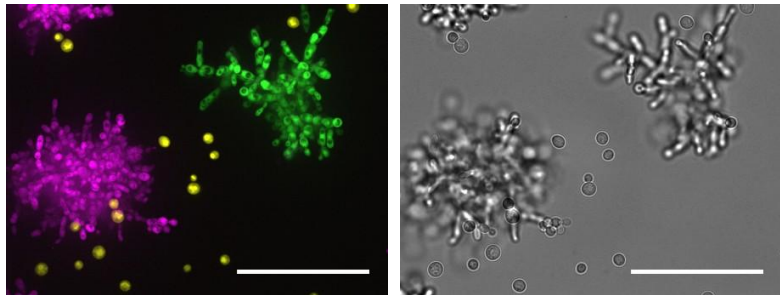
Ancestor



EvoClone9 (8 mutations)

Scale bar = 50 μm

Two strains recreated

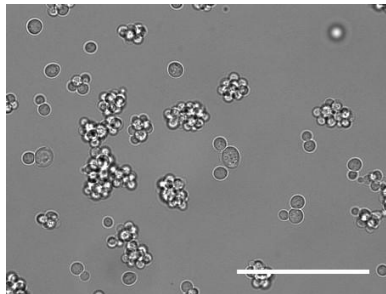
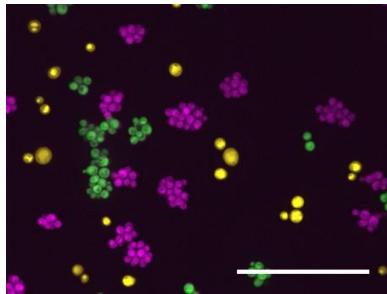


Ancestor in yellow
EvoClone9 in green
Recreate9 in magenta (8 mutations)

Excellent growth in sucrose

Scale bar = 50 μm

Two strains recreated

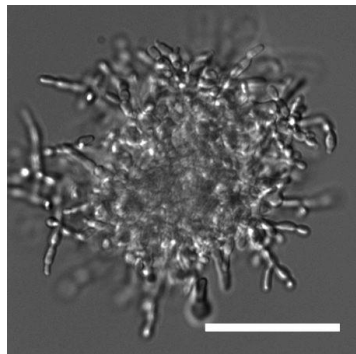


Ancestor in yellow
EvoClone2 in green
Recreate2 in magenta (5 mutations)

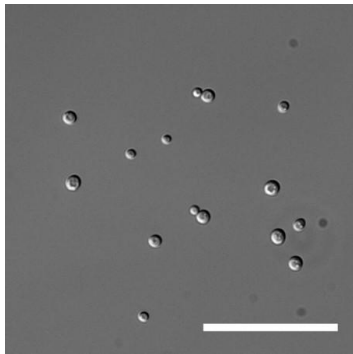
Excellent growth in sucrose

Scale bar = 50 μm

Reverting strains



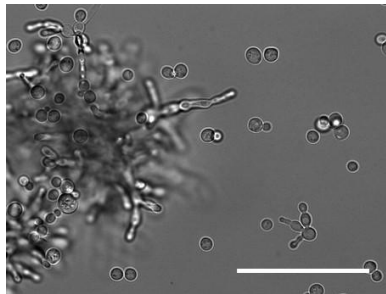
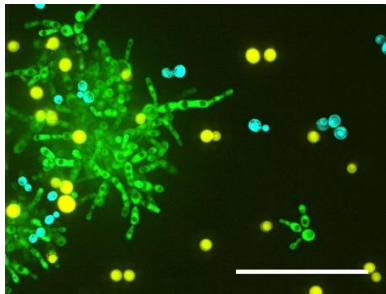
EvoClone9 (8 mutations)



Ancestor

Scale bar = 50 μm

Two strains reverted



Ancestor in yellow

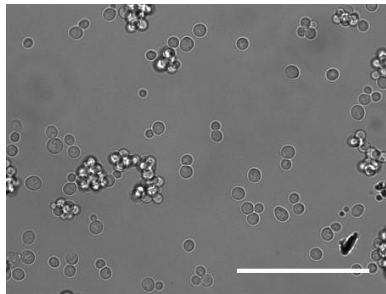
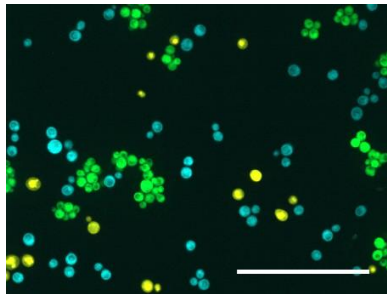
EvoClone9 in green

Reverted9 in cyan (8 reverted mutations)

Very poor growth in sucrose

Scale bar = 50 μm

Two strains reverted



Ancestor in yellow

EvoClone2 in green

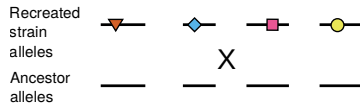
Reverted2 in cyan (5 reverted mutations).

Very poor growth in sucrose

Scale bar = 50 μm

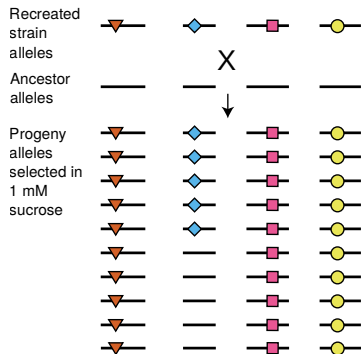
Are individual mutations causal?

Backcross recreated strain to ancestor and isolate progeny with selected phenotype.



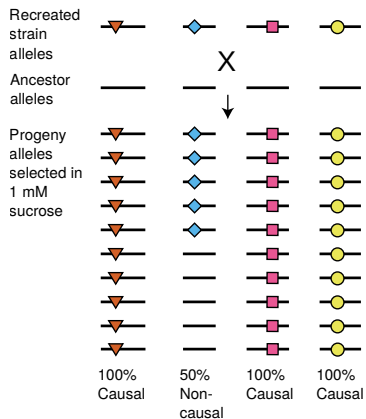
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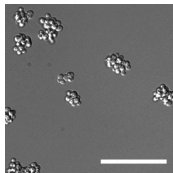
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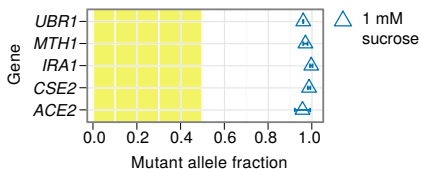


Are individual mutations causal?

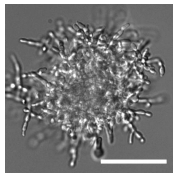
EvoClone2



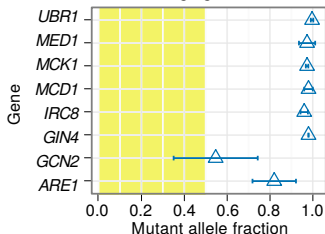
Recreated2 allele segregation



EvoClone9



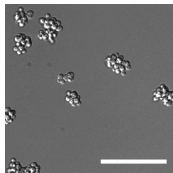
Recreated9 allele segregation



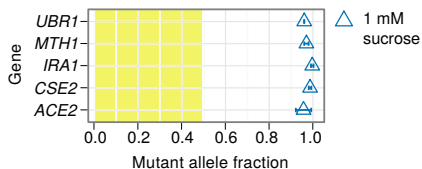
Error bar = 2 x stdev,
3 independent trials

Are individual mutations causal?

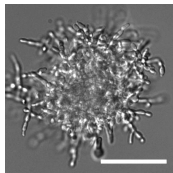
EvoClone2



Recreated2 allele segregation

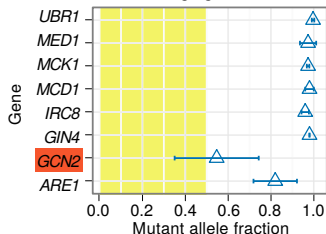


EvoClone9



Error bar = 2 x stdev,
3 independent trials

Recreated9 allele segregation



Fitness varies in different environments

4 of 12 clones grow poorly in low monosaccharide.

Others grow about as well as the ancestor.

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Alleles that are detrimental in other environments either:

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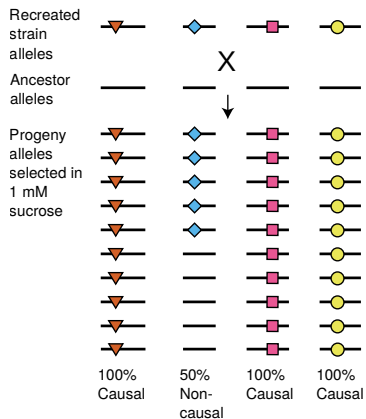
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Alleles that are detrimental in other environments either:

1. Have no effect in sucrose (i.e are non-causal)
2. Are selected for in sucrose

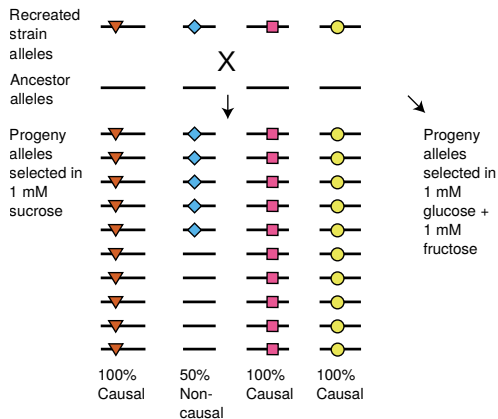
Segregate alleles in other environments

Backcross recreated strain to ancestor and isolate progeny with selected phenotype.



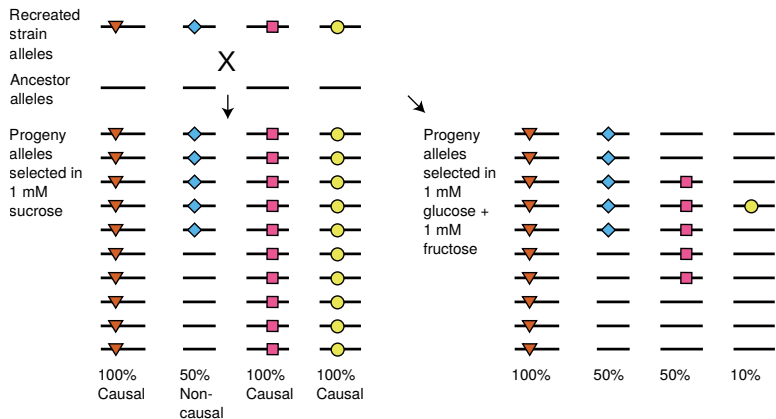
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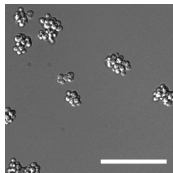
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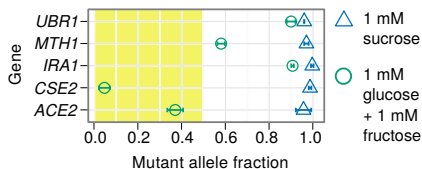


Selection in low monosaccharide

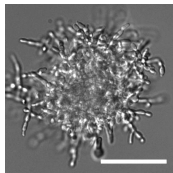
EvoClone2



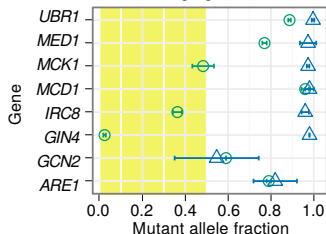
Recreated2 allele segregation



EvoClone9



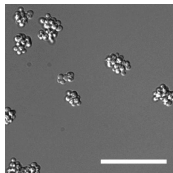
Recreated9 allele segregation



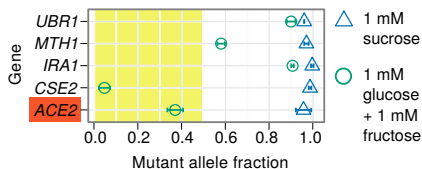
Error bar = 2 x stdev,
3 independent trials

Selection in low monosaccharide

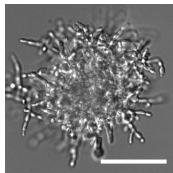
EvoClone2



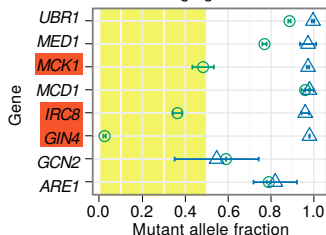
Recreated2 allele segregation



EvoClone9



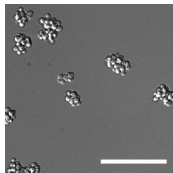
Recreated9 allele segregation



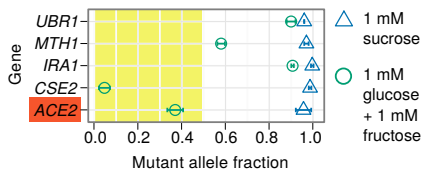
Error bar = 2 x stdev,
3 independent trials

No selection for clumps in low monosaccharide

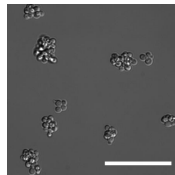
EvoClone2



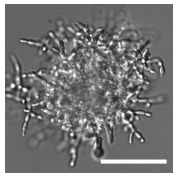
Recreated2 allele segregation



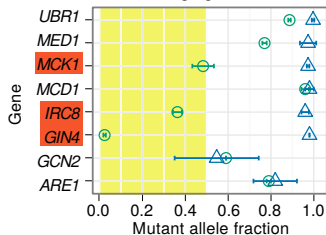
*ace2-L323**



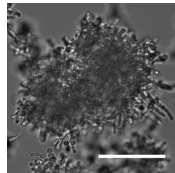
EvoClone9



Recreated9 allele segregation



gin4-W19 irc8-G57V*
mck1-G227Vfs249

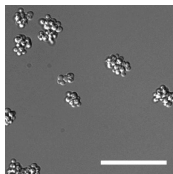


Error bar = 2 x stdev,
3 independent trials

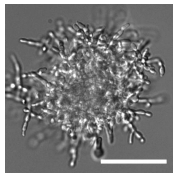
Scale bar = 50 μ m

Selection in high glucose

EvoClone2

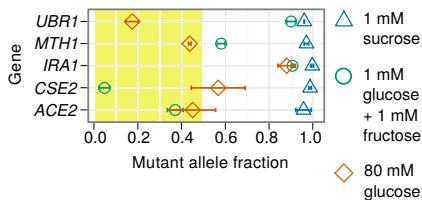


EvoClone9

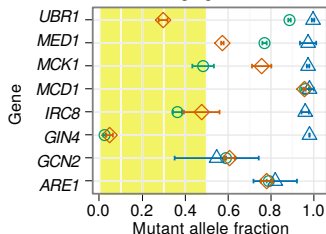


Error bar = 2 x stdev,
3 independent trials

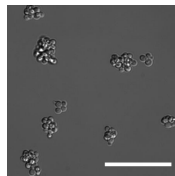
Recreated2 allele segregation



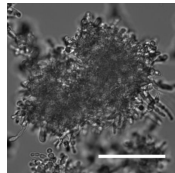
Recreated9 allele segregation



*ace2-L323**



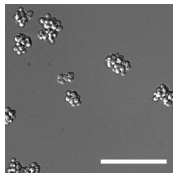
gin4-W19 irc8-G57V
mck1-G227Vs249*



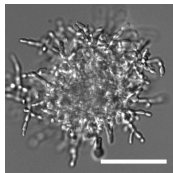
Scale bar = 50 μ m

Selection in high glucose

EvoClone2



EvoClone9

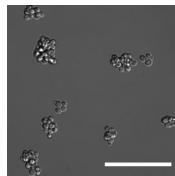


Error bar = 2 x stdev,
3 independent trials

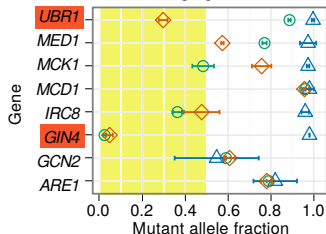
Recreated2 allele segregation



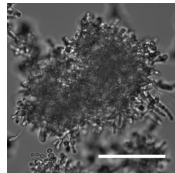
*ace2-L323**



Recreated9 allele segregation



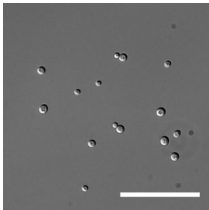
gin4-W19 irc8-G57V*
mck1-G227Vs249



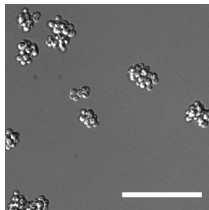
Scale bar = 50 μ m

ace2 is responsible for clumpiness

Ancestor



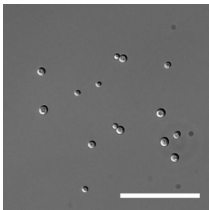
EvoClone2



Scale bar = 50 μm

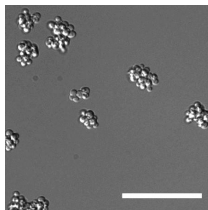
ace2 is responsible for clumpiness

Ancestor



<i>ACE2</i>	<i>ACE2</i>	<i>ACE2</i>	<i>ACE2</i>	<i>ACE2</i>
<i>CSE2</i>	<i>CSE2</i>	<i>CSE2</i>	<i>CSE2</i>	<i>CSE2</i>
<i>IRA1</i>	<i>ira1</i>	<i>IRA1</i>	<i>IRA1</i>	<i>ira1</i>
<i>MTH1</i>	<i>MTH1</i>	<i>MTH1</i>	<i>mth1</i>	<i>mth1</i>
<i>UBR1</i>	<i>UBR1</i>	<i>ubr1</i>	<i>UBR1</i>	<i>UBR1</i>

EvoClone2

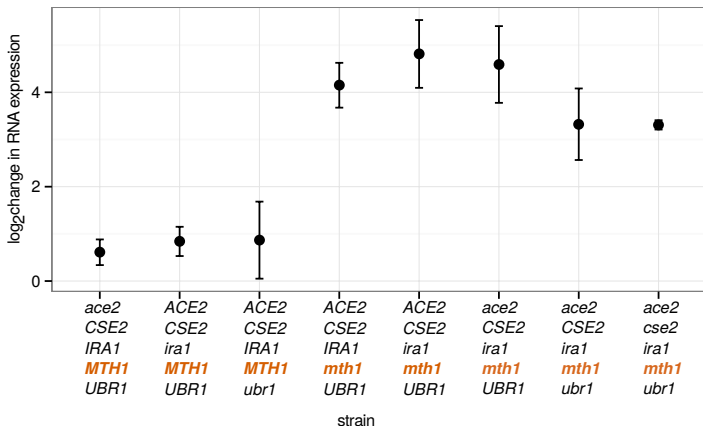


<i>ace2</i>	<i>ace2</i>	<i>ace2</i>	<i>ace2</i>
<i>CSE2</i>	<i>CSE2</i>	<i>CSE2</i>	<i>cse2</i>
<i>IRA1</i>	<i>ira1</i>	<i>ira1</i>	<i>ira1</i>
<i>MTH1</i>	<i>mth1</i>	<i>mth1</i>	<i>mth1</i>
<i>UBR1</i>	<i>UBR1</i>	<i>ubr1</i>	<i>ubr1</i>

Scale bar = 50 μ m

mth1 is responsible for increased *HXT4* expression

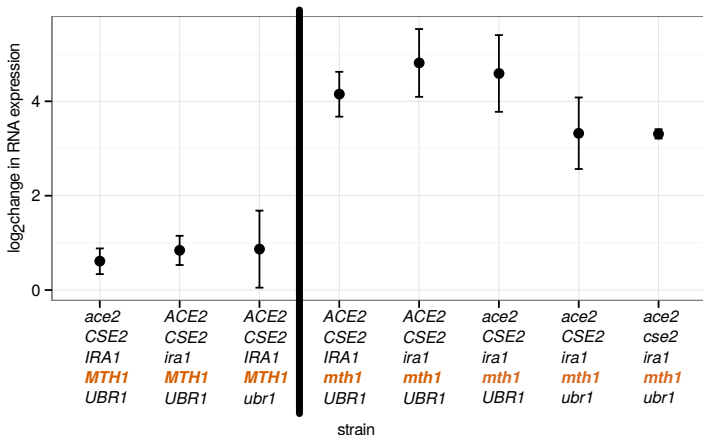
Change in *HXT4* expression compared to wild-type ancestor



Error bar = 2 x stdev over 3 independent trials

mth1 is responsible for increased *HXT4* expression

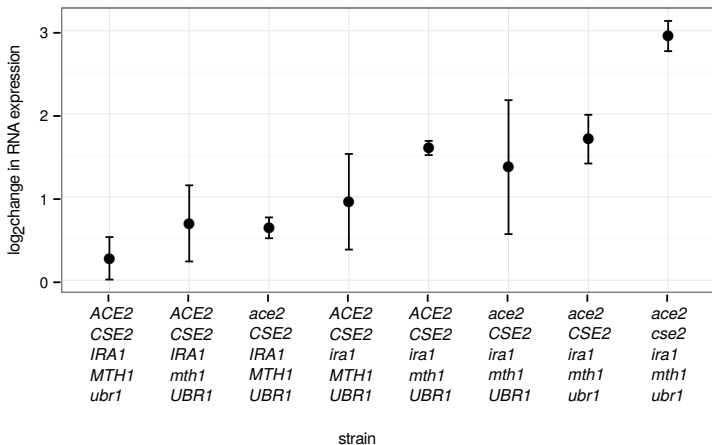
Change in *HXT4* expression compared to wild-type ancestor



Error bar = 2 x stdev over 3 independent trials

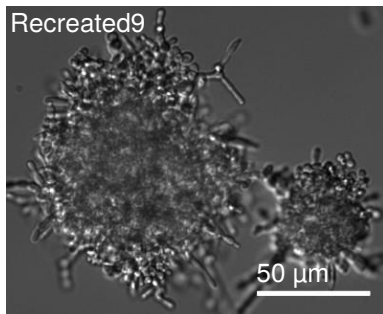
SUC2 is more complex

Change in *SUC2* expression compared to wild-type ancestor



Error bar = 2 x stdev over 3 independent trials

Continuing work with evolved strains



Find mutations responsible for:

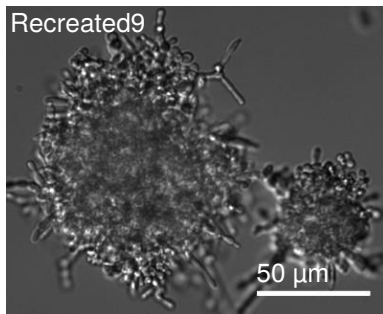
Size and size regulation

irc8, mck1, gin4

Hexose transporter increase

Invertase increase

Continuing work with evolved strains



Find mutations responsible for:

Size and size regulation

irc8, mck1, gin4

Hexose transporter increase

Invertase increase

Recreate other strains and find mutations underlying the strategies.

Thank you