



NYU

DEPARTMENT OF  
BIOLOGY



*Synthetic chromosomes in Yeast 2.0 –  
consolidation, debugging and beyond*

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August 5<sup>th</sup>, 2023

Build-A-Genome Workshop  
Hartwick College, NY

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Do not share or distribute. Only for 2023 Build A  
Genome Network Workshop participants.

*Saccharomyces cerevisiae*: “budding yeast” or “brewer yeast”

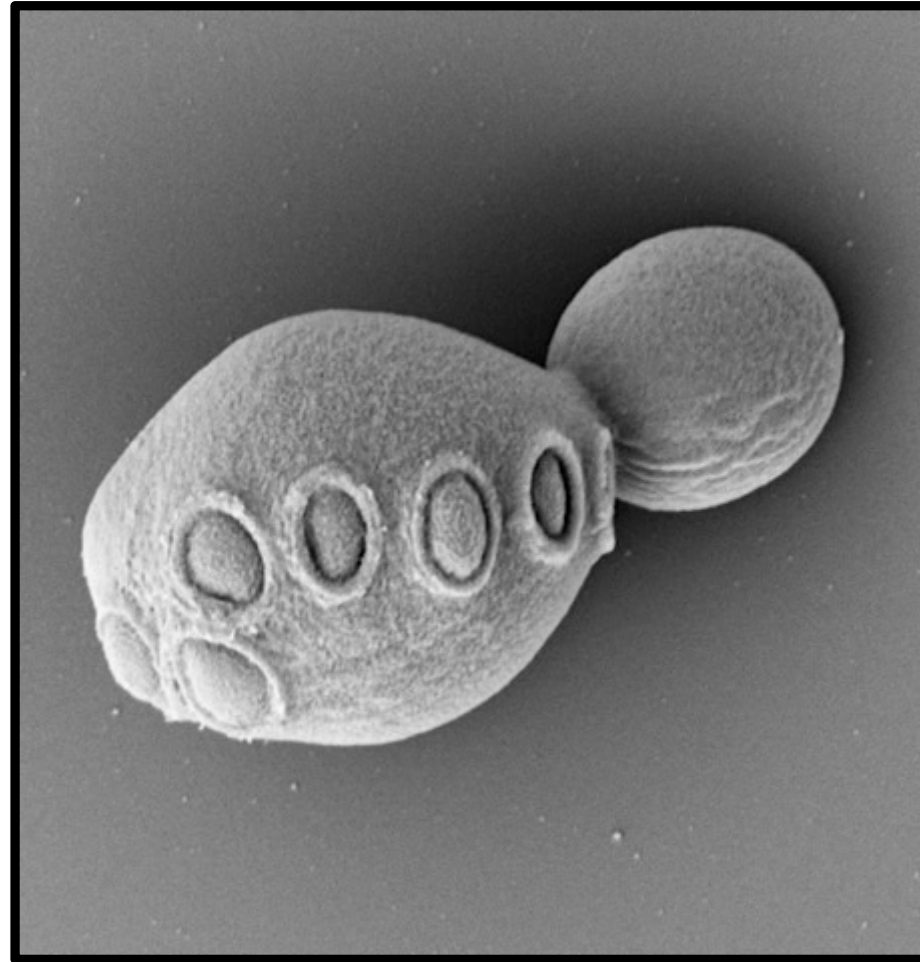


Emil Christian Hansen  
1883 single colony



2500 BC ancient Egypt

*Saccharomyces cerevisiae*: “budding yeast” or “brewer yeast”



# Yeast is an excellent tool for education

*Safe in lab*



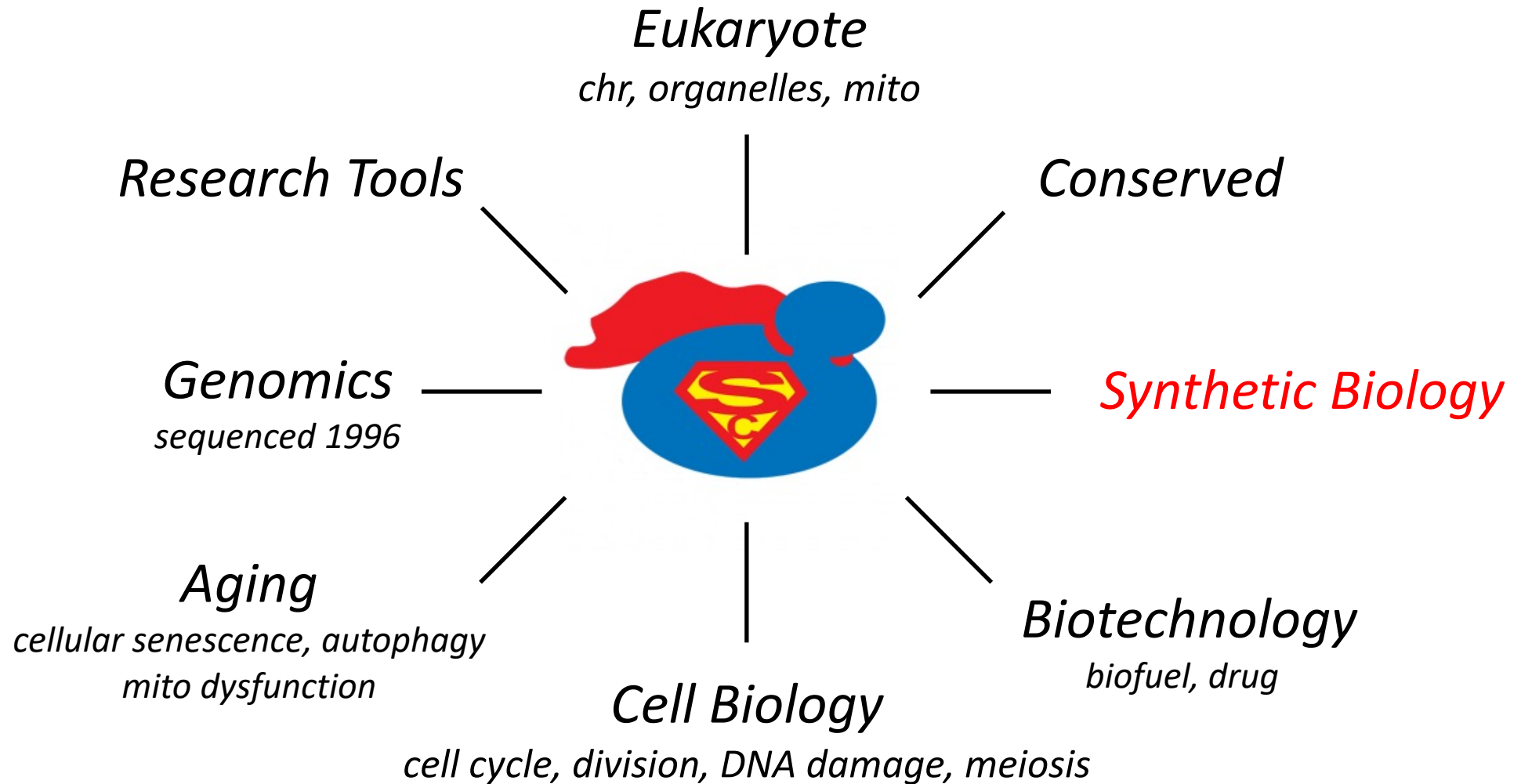
*Cheap*



*Robust*

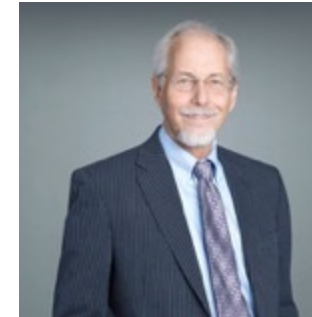


# Yeast is a powerful model organism for science



# Build-a-Genome class at NYU Biology

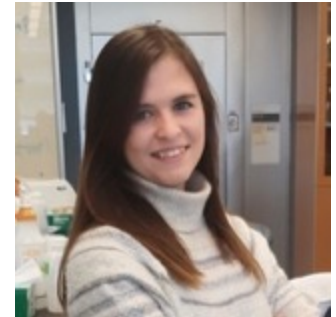
- Starting at 2020
- 18 trainees to date (6 this semester)
- 2020 - synthetic chromosome consolidation (endoreduplication intercross)
- 2022/2023 - next-gen strategy (chromosome substitution)
- Next year - big-DNA assembly (mouse/human)



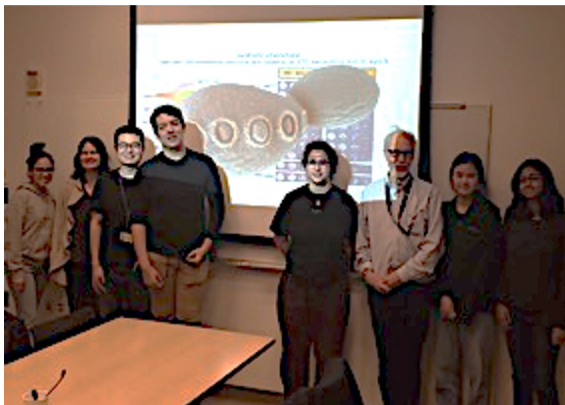
Jef Boeke



Stephanie Lauer  
St. Thomas Aquinas College  
(STAC)



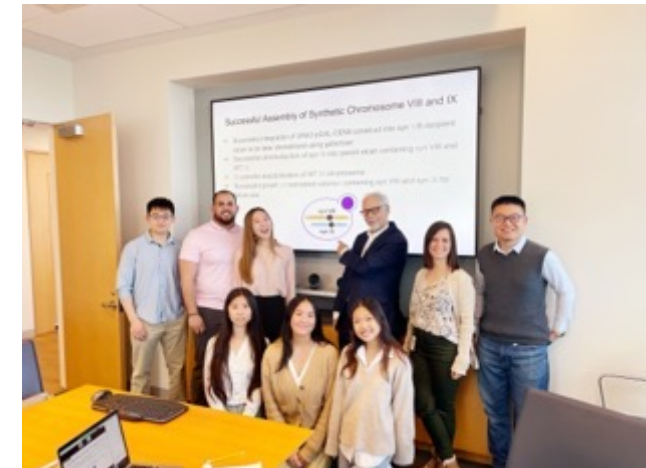
Raquel Ordoñez  
NYU ISG  
Maurano lab



first class (2020)



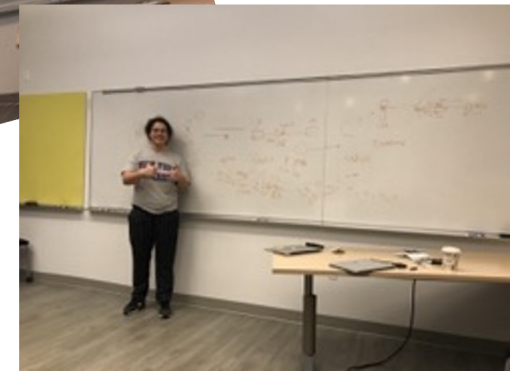
graduation! (2021)



recent class (2023 Spring)

## Build-a-Genome class at NYU

- Starting as “temporary” pilot class.
- In 2022, approved by NYU’s CAS Undergraduate Curriculum Committee as an official course: BIO-UA 957, Advanced Research in Synthetic Biology.
- Ongoing, active Sc2.0 project.
- Actual lab environment at medical campus.
- Acquire systematic training in
  - Genetics, molecular and synthetic biology.
  - Organizing their own data and prepare figures/diagram.
  - Benchling notebook, journal club, and “lab meeting” presentations.



## Goals for students – TEF

- Touch – comfortable with active learning, try to take advantage of the class.
- Explore – your interest and reach out in the lab!
- Fearless
  - practice by yourself, even with mistakes.
  - Communicate and present.
  - Build their confidence and ambitions.



*“the best course I have taken during my time at NYU so far”*

*“wanting to know more because these were all encouraged”*

*“this course really made me reignite my passion for research”*

*“My undergraduate experience would not be the same without this course”*



# Organizing & thinking: lab note using Benchling – what and why?

- *Intro*
- *Goal*
- *Controls*
- *Result*
- *Analysis*

TUESDAY, 2/7/2023

Today, we performed a standard lithium acetate sperm DNA yeast transformation protocol. We were to integrate our individual synthetic chromosomes (synVI for Gamal and I) into the recipient strain, ySLL376 - synVIII. The pYZ057 plasmid with the CEN construct of URA3-pGAL-CEN6 was used for the experimental control. We also had a positive control containing the pRS416 plasmid and a negative control containing no plasmid. The URA3-pGAL-CEN6 and the pRS416 plasmid both contain the URA3 marker, so we can select for the successfully transformed cells on SC-ura plates as they should be able to grow in the absence of uracil.

*Purpose:*

To successfully transform yeast cells with pYZ057 plasmid and to select for successfully transformed cells on SC-ura plates.

*Procedure:*

A standard LIOAc sperm DNA yeast transformation protocol was performed.

Once all solutions were prepared, they were plated onto SC-ura plates as follows:

SH1 - experimental control

- BY4741 + URA3-pGAL-CEN6 (from pYZ057)
- SH1 10%: 10% of SH1 mix plated
- SH1 90%: 90% of SH1 mix plated
- ura3 is expressed when integration is successful, so we expect the successfully transformed cells to grow on SC-ura plates

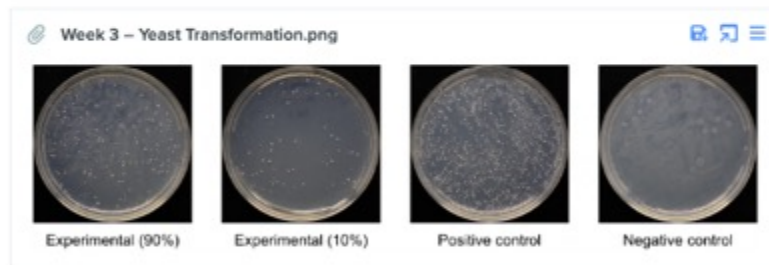
SH2 - positive control

- BY4741 + pRS416
- no exogenous DNA is contained, but the plasmid contains ura3 so we expect growth on SC-ura plates

SH3 - negative control

- BY4741
- no transformation and BY4741 contains the ura3 deletion so we expect no growth on SC-ura plates

*Results:*

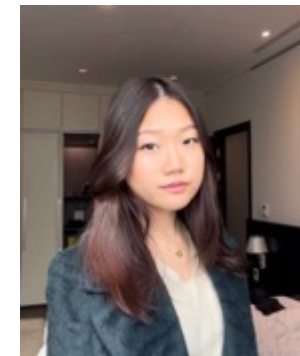


Taking a look at the experimental controls, we can see growth on both the 90% and 10% plates as expected. We can also see significant growth of the yeast cells that were transformed using pRS416 on the SC-ura plate as well, and the negative control which contained no plasmid showed no growth.

I counted about 1000 colonies on the positive control plate which means I had a transformation efficiency of about  $10^5$ .



# Benchling



Sunny Ham (NYU Class 2024)  
Student Intern at NYU ISG

# Learn from practice: auxotrophic marker and replica plating

*Journal club discussion:*

## Designer Deletion Strains derived from *Saccharomyces cerevisiae* S288C: a Useful set of Strains and Plasmids for PCR-mediated Gene Disruption and Other Applications

CARRIE BAKER BRACHMANN, ADRIAN JOACHIM LI†, PHILIP HIETER AND JEF I

Department of Molecular Biology and Genetics, MD 21205, U.S.A.

Received 9 June 1997; accepted 18 June 1997

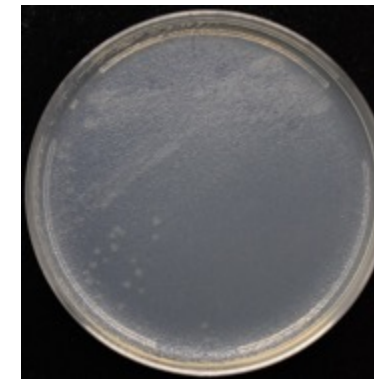
**URA3**  
**LEU2**  
**HIS3**  
**LYS2**  
**MET15**  
**ADE2**  
**TRP1**

Allele	ΔORF?	Reverts?	Notes	Molecular description*	References
<i>ade2-101</i>	No	Yes	Ochre mutation, red colonies		
<i>ade2-BglII</i>	No	No	Red colonies	Frameshift ( <i>BglII</i> site filled in at pos. 592)	Engelbrecht and Roeder (1990)
<i>his3Δ200</i>	Yes	No	Cold sensitive; high frequency of petite formation, especially during transformation	Δ 1 kb (– 205–835)	Struhl (1985); Fasullo and Davis (1988); Siram <i>et al.</i> YGM RNA processing mtg (1993)
<i>his3Δ1</i>	Partial	No		Δ 187 bp <i>HindIII-HindIII</i> internal (305–492)	Scherer and Davis (1979)
<i>his3-11,15</i>	No	No	Double mutant		Lau and G. R. Fink, unpublished
<i>leu2Δ1</i>	Partial	No		Δ 0.6 kb, <i>EcoRI-ClaI</i> internal (163–649)	Sikorski and Hieter (1989)
<i>leu2-3,112</i>	No	No	Double mutant	<i>leu2-3</i> is a +1 frameshift mutation	Hinnen <i>et al.</i> (1978); Gaber and Culbertson (1982)
<i>lys2-801</i>	No	Yes	Amber mutation		Winston <i>et al.</i> (1995)
<i>lys2Δ202</i>	Partial	No		Δ 1.0 kb, <i>XhoI-HpaI</i> internal (1813–2864)	
<i>trp1Δ1</i>	Yes	No	Cold sensitive <sup>b</sup> , weak galactose inducer (deletes <i>GAL3</i> UAS), removes <i>ARS1</i> , also called <i>trp1-901</i>	Δ 1.45 kb, <i>EcoRI-EcoRI</i> (– 102 to 1352)	Sikorski and Hieter (1989)
<i>trp1Δ63</i>	Partial	No	Cold sensitive <sup>b</sup>	Δ 0.6 kb, <i>EcoRI-HindIII</i> (– 102 to 513)	Sikorski and Hieter (1989)
<i>trp1-289</i>	No	Yes	Cold sensitive <sup>b</sup>		Rose and Winston (1984)
<i>ura3-52</i>	No	No		Ty1 insertion (transcribing left to right) at pos. 121	
<i>ura3-1</i>	No	Yes			
<i>Alleles described in this study</i>					
<i>ade2Δ::hisG</i>	No	No			Aparicio <i>et al.</i> (1991)
<i>leu2Δ0</i>	Yes	No	Designer deletion		This study
<i>lys2Δ0</i>	Yes	No	Designer deletion		This study
<i>met15Δ0</i>	Yes	No	Designer deletion		This study
<i>ura3Δ0</i>	Yes	No	Designer deletion		This study

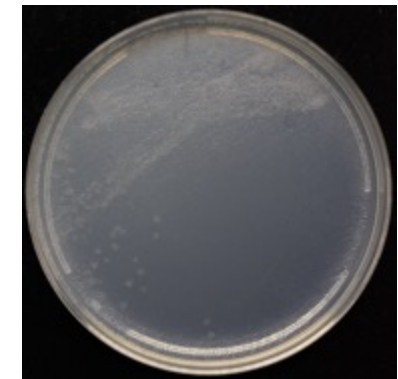
An updatable version of this table is maintained by SGD (<http://genome-www.stanford.edu/Saccharomyces/>).  
\*The sequence coordinates are relative to the first ATG of the selectable marker ORF, in which the A residue is defined as +1.  
<sup>b</sup>All Trp<sup>-</sup> strains are cold sensitive (Singh and Manney, 1974).

Strain: BY4741

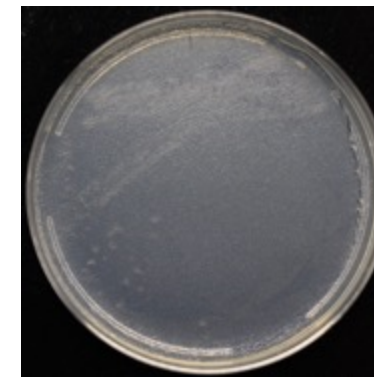
*MATα leu2Δ0 met15Δ0 ura3Δ0 his3Δ1*



SC–Leu



SC–Ura

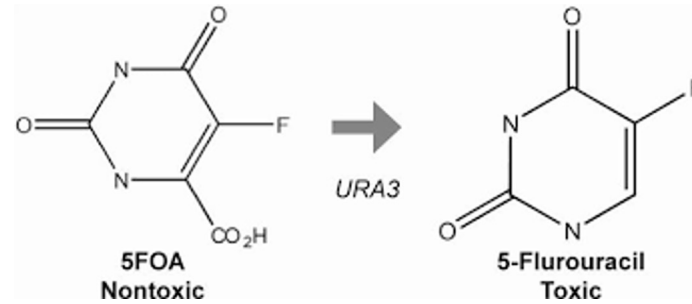






SC–His



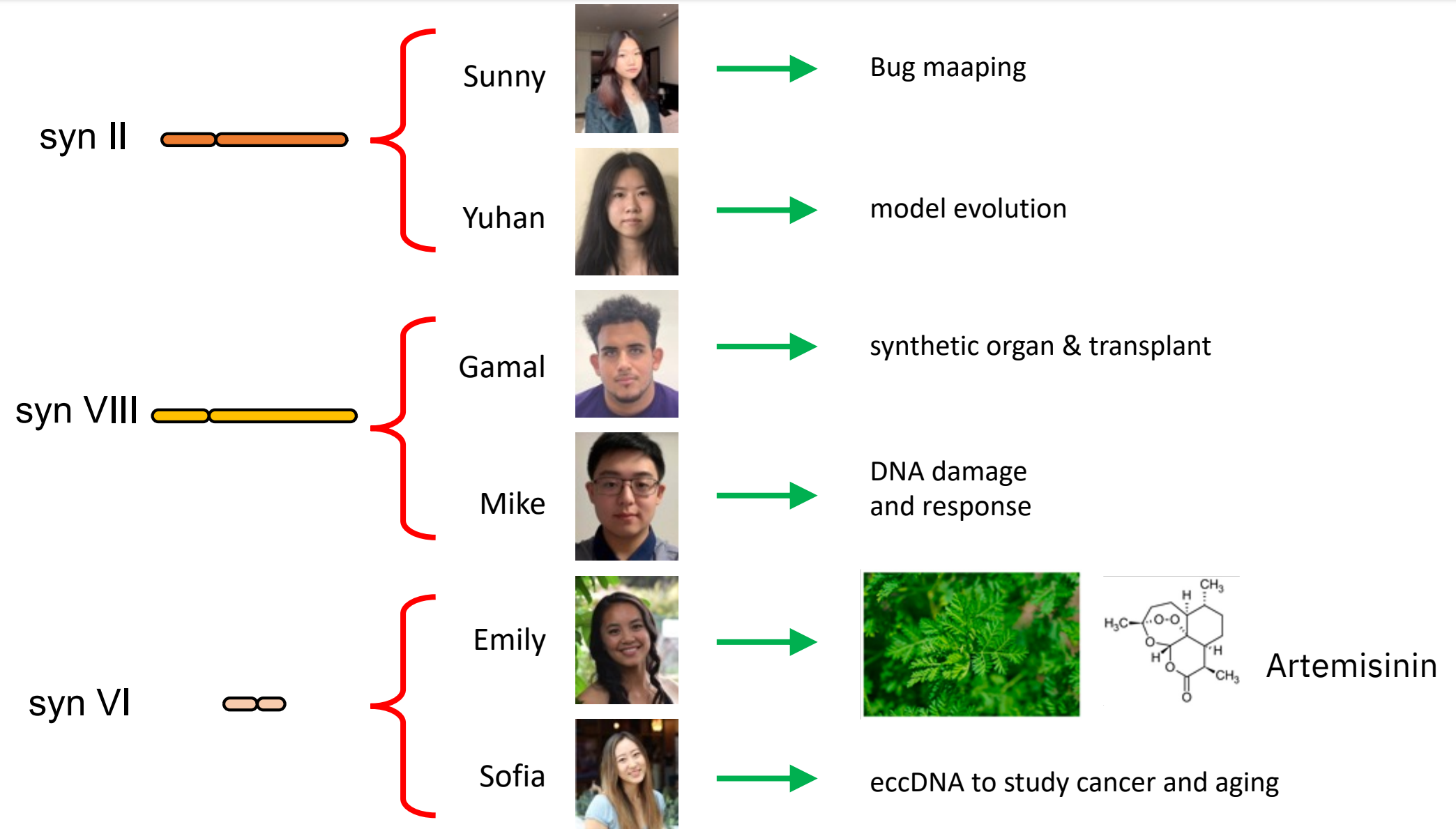
SC–Trp

## Learn from practice: auxotrophic marker and replica plating

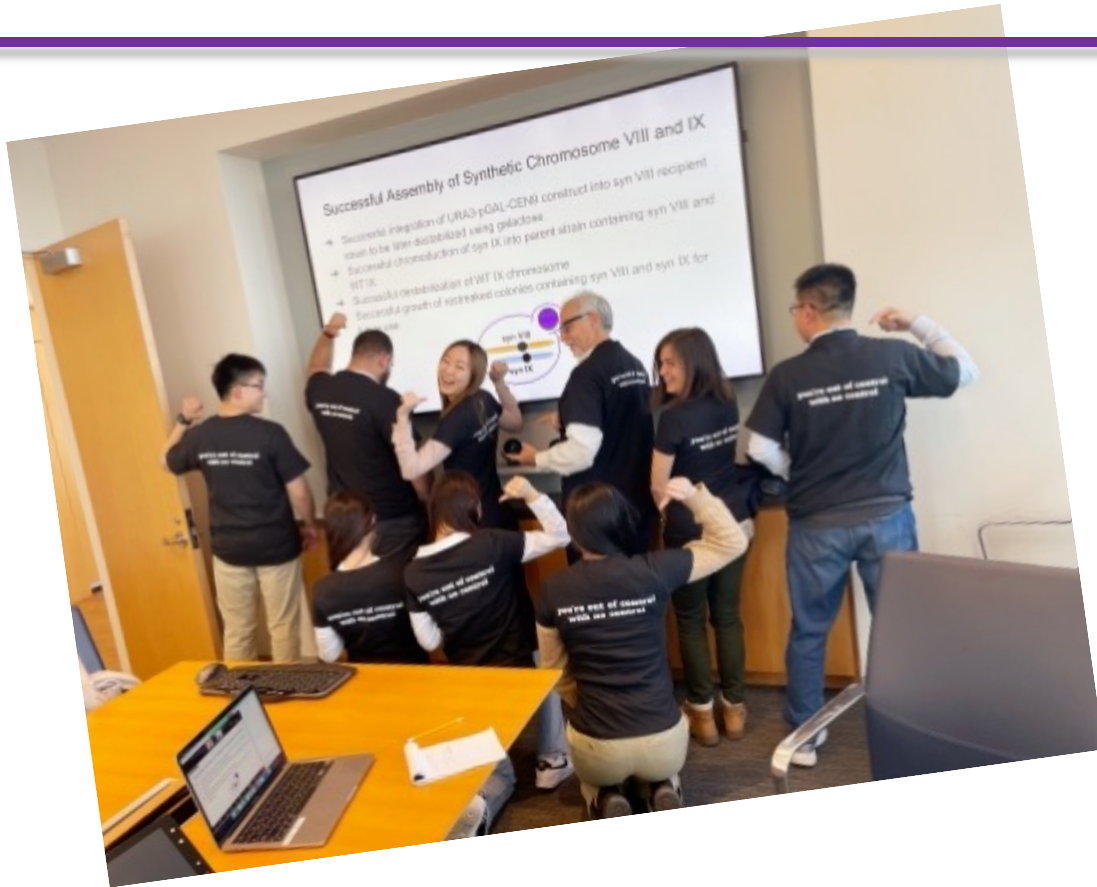


	Growth on SC-URA	Growth on 5-FOA
<i>URA3</i> <sup>+</sup>	 <i>positive selection</i>	
<i>ura3</i>		 <i>counter selection</i>

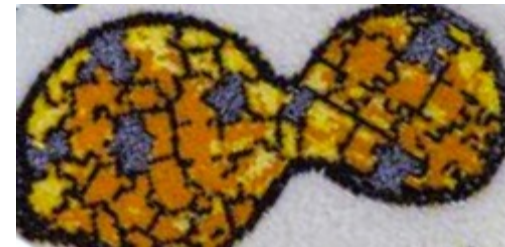
# Explore their interest and “kick them off”: be fearless



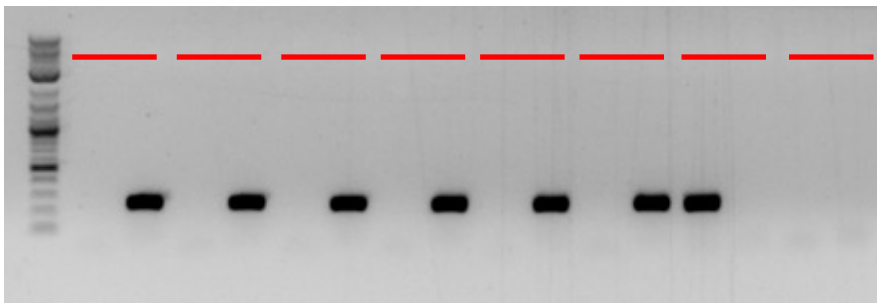
Pitch: if you don't have controls, you will be out of control



Leslie Mitchell

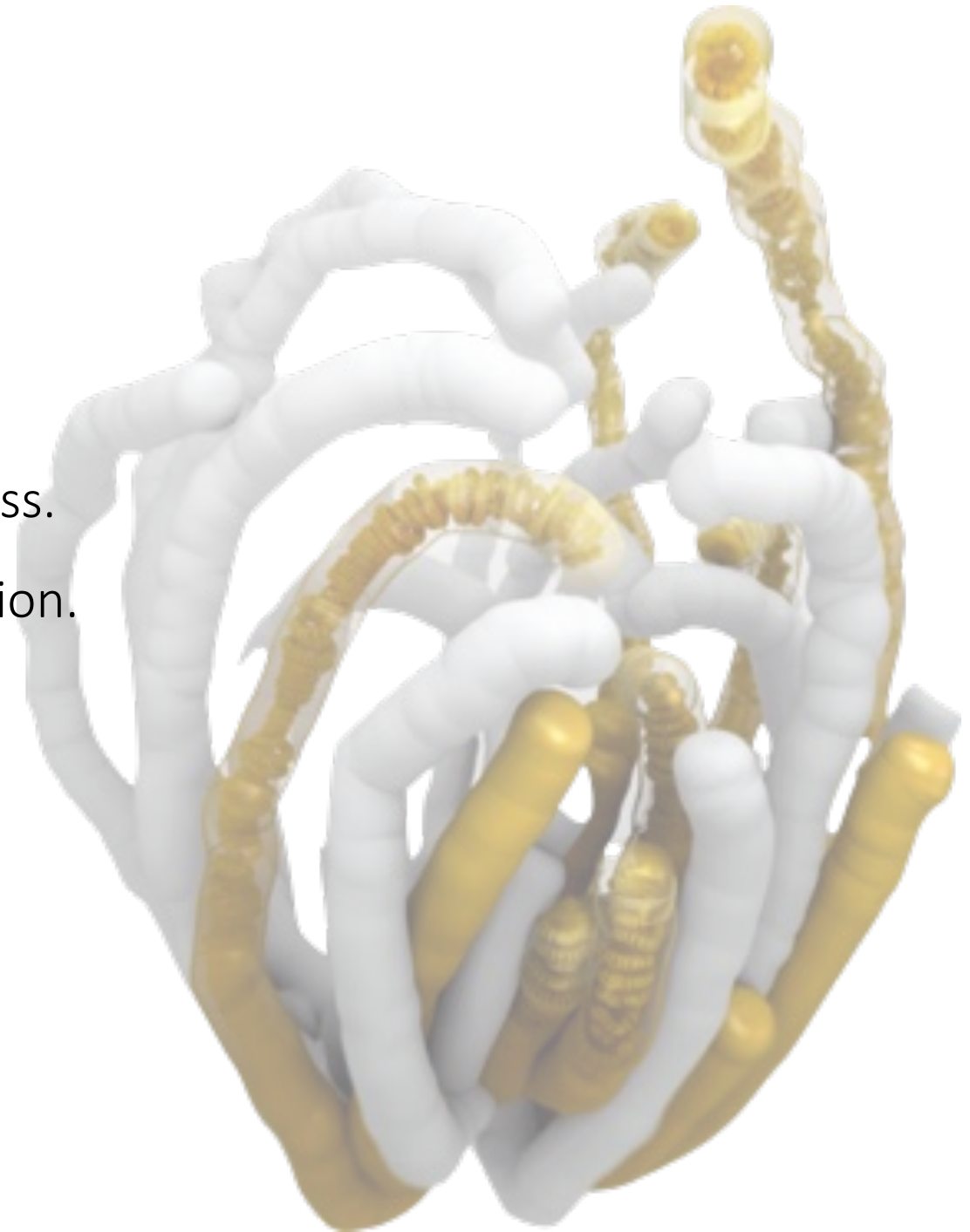


*Sc2.0 - the synthetic yeast genome project*

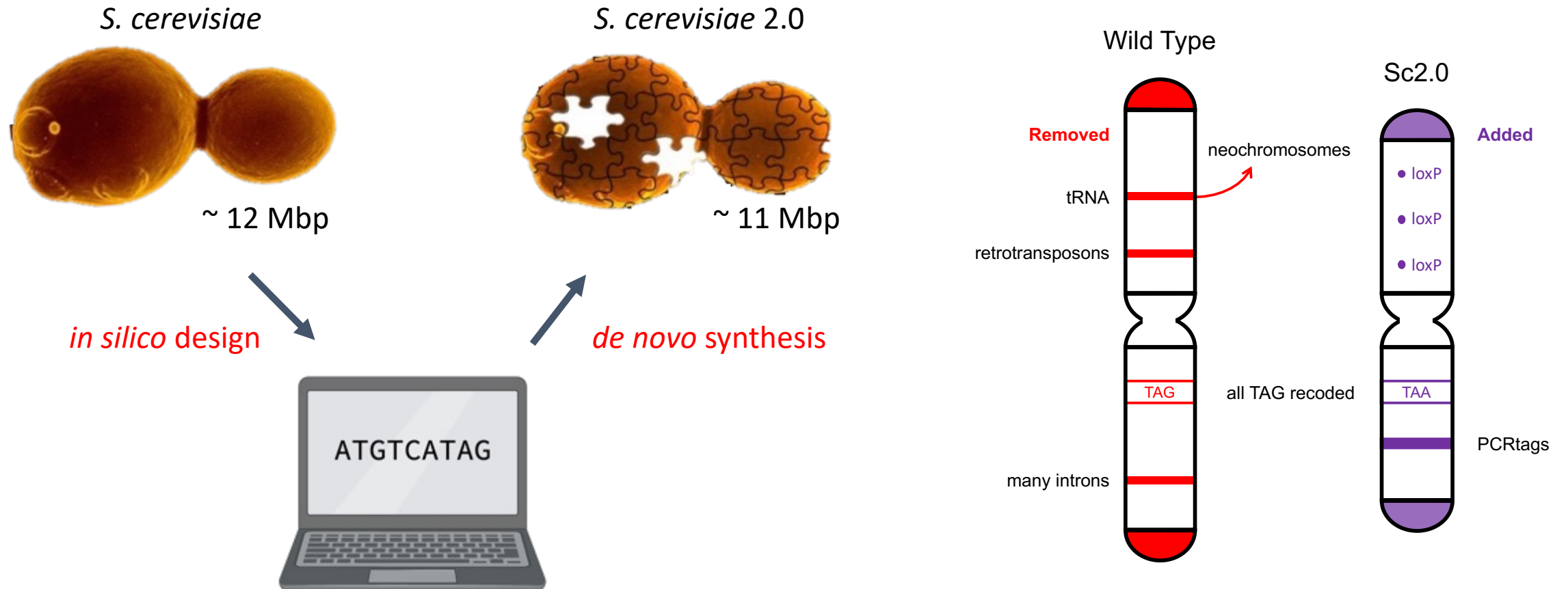


## *Outline of today's talk*

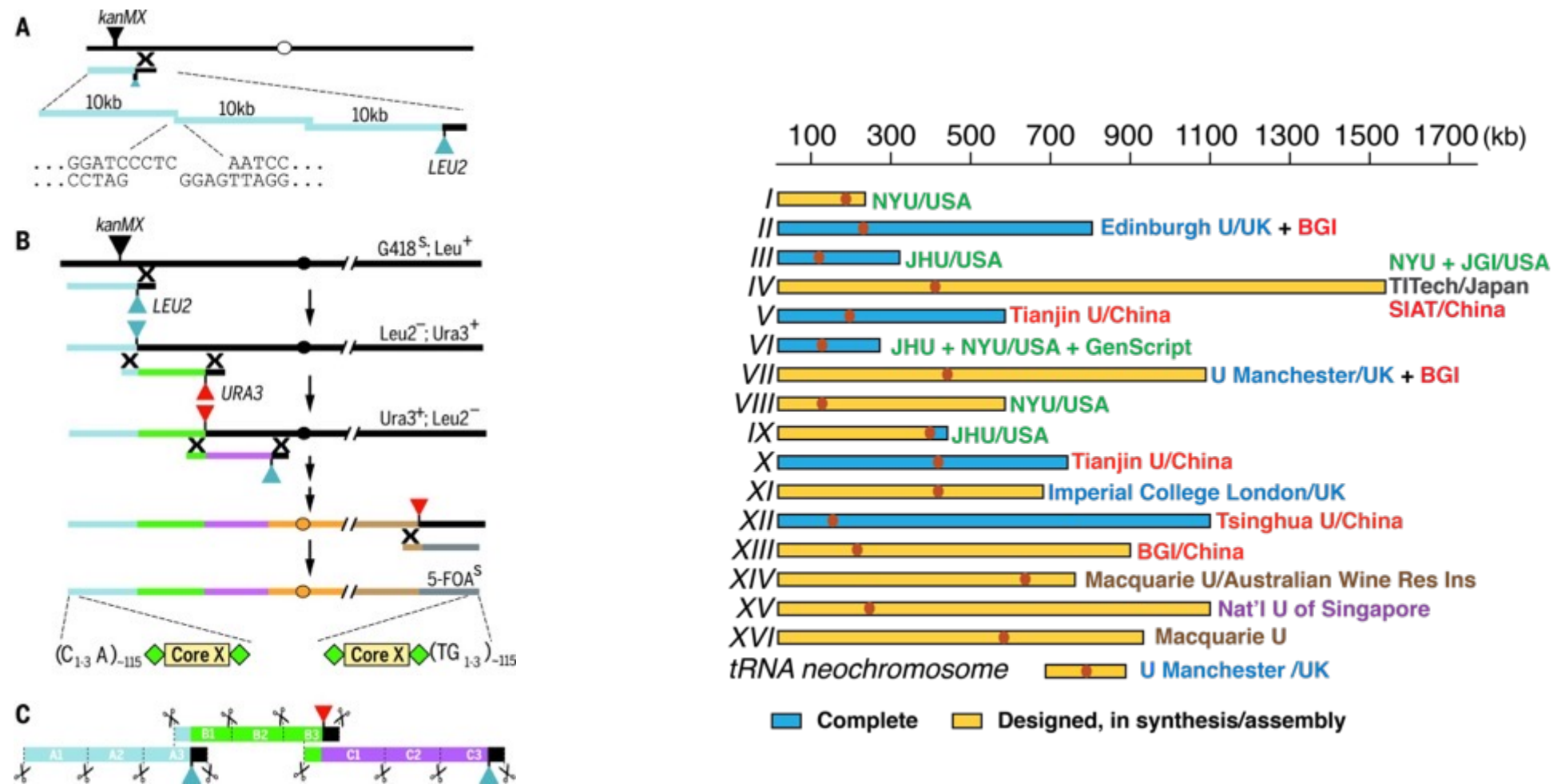
- Brief intro to **NYU BAG**.
- Synthetic chromosome **consolidation**.
  - syn6.5 using endoreduplication intercross.
  - finish Sc2.0 with chromosome substitution.
- Map designer “**bugs**”.
  - CRISPR D-BUGS
  - Single and combinatorial bugs.
- Synthetic yeast for **education**.



# Understand yeast genome from a bottom-up approach



# Individual synthetic chromosome was assembled by SwAP-In by Sc2.0 consortium

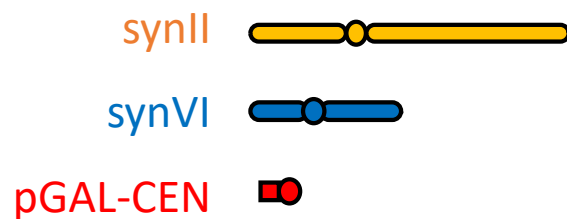
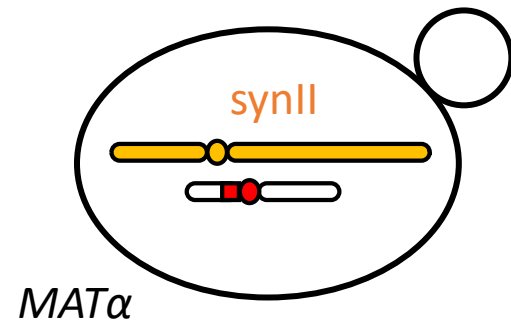


Switching Auxotrophies Progressively for Integration  
(SwAP-In)



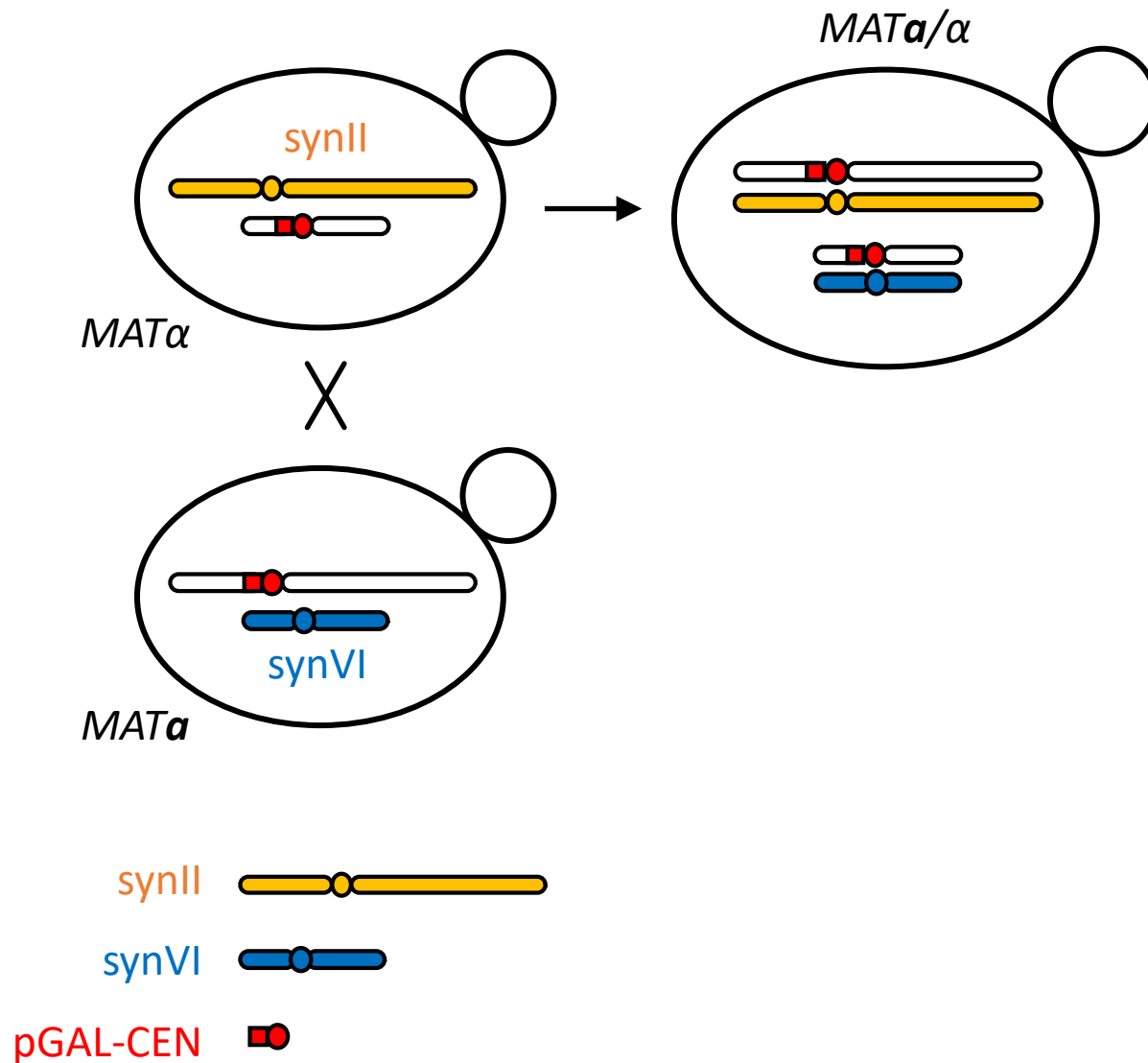
## Consolidation

Multiple synthetic chromosomes were consolidated using endoreduplication intercross



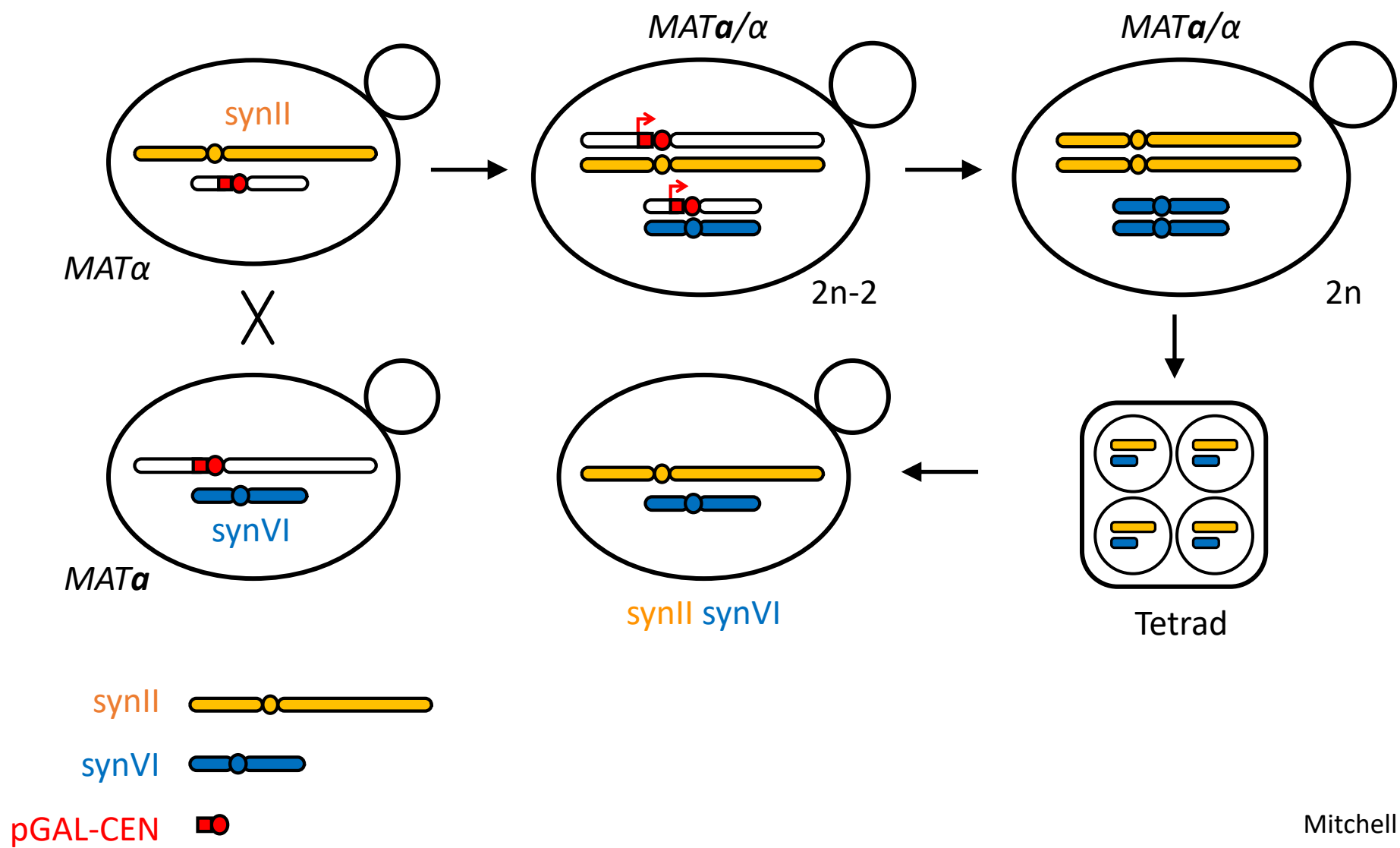
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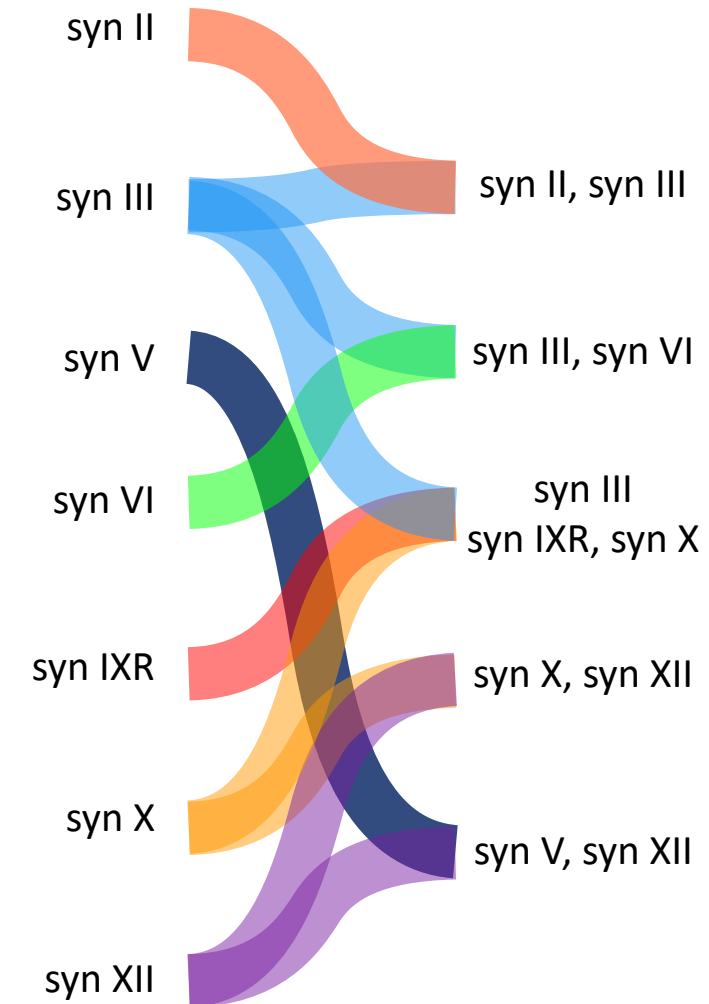
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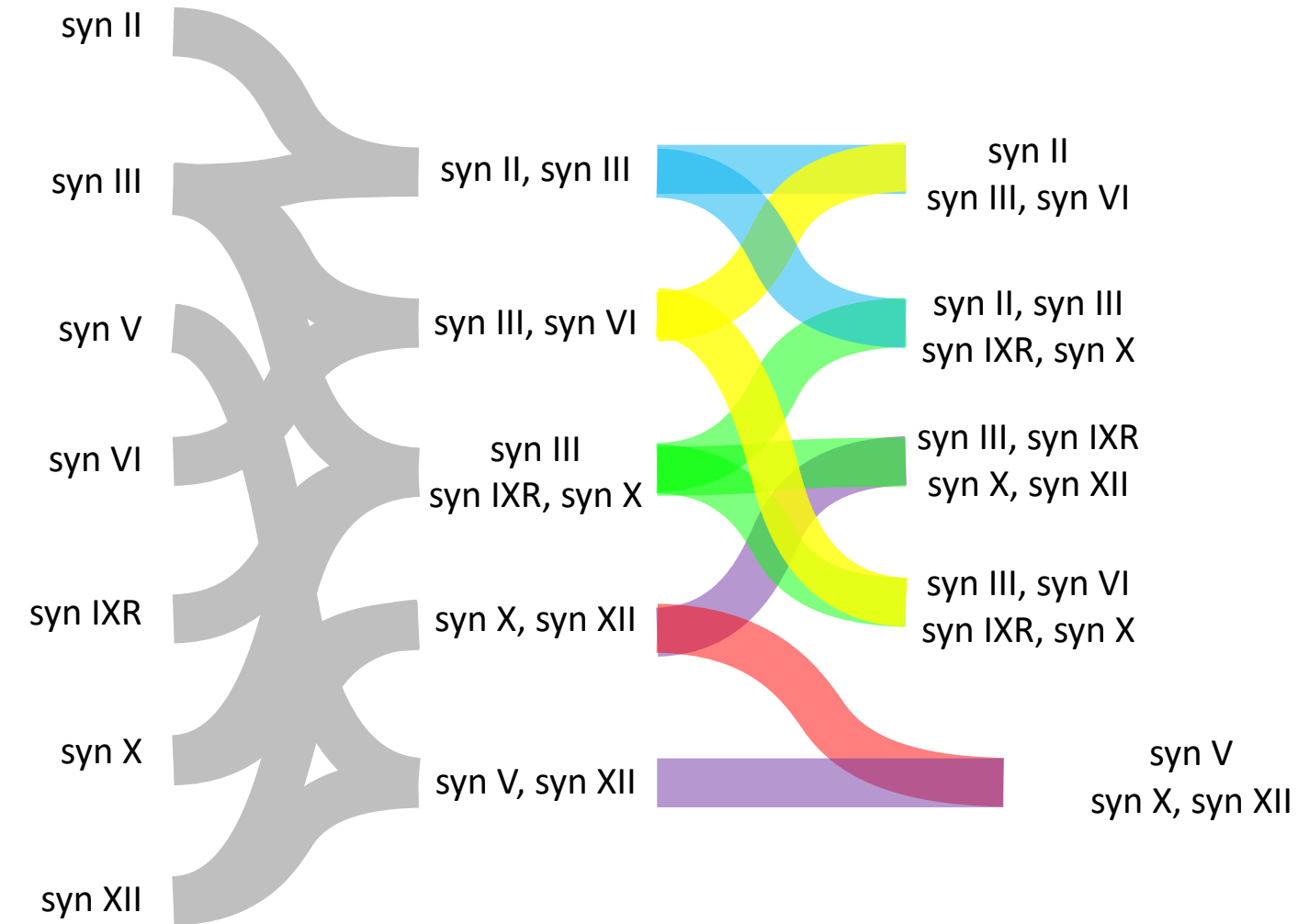
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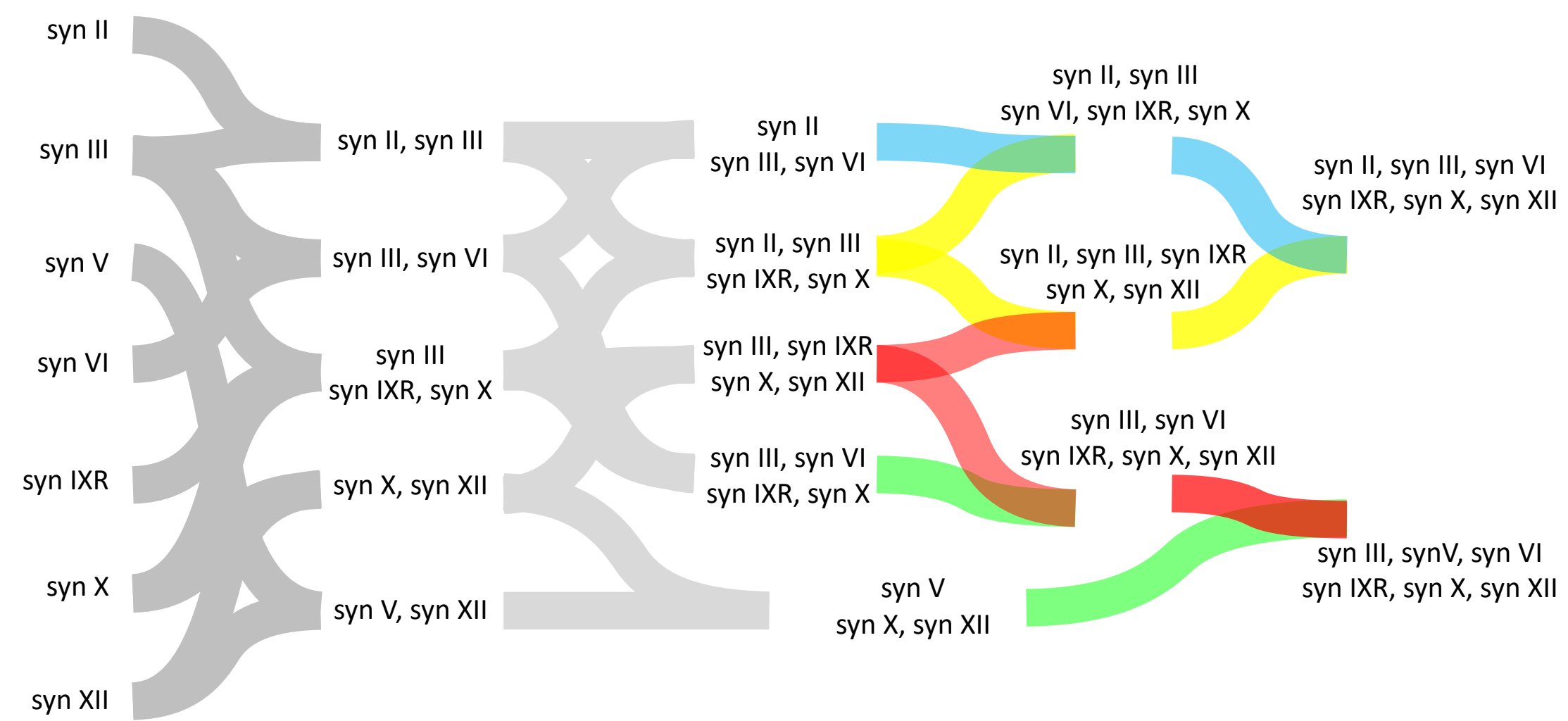
# Consolidation

Multiple synthetic chromosomes were consolidated using endoreduplication intercross



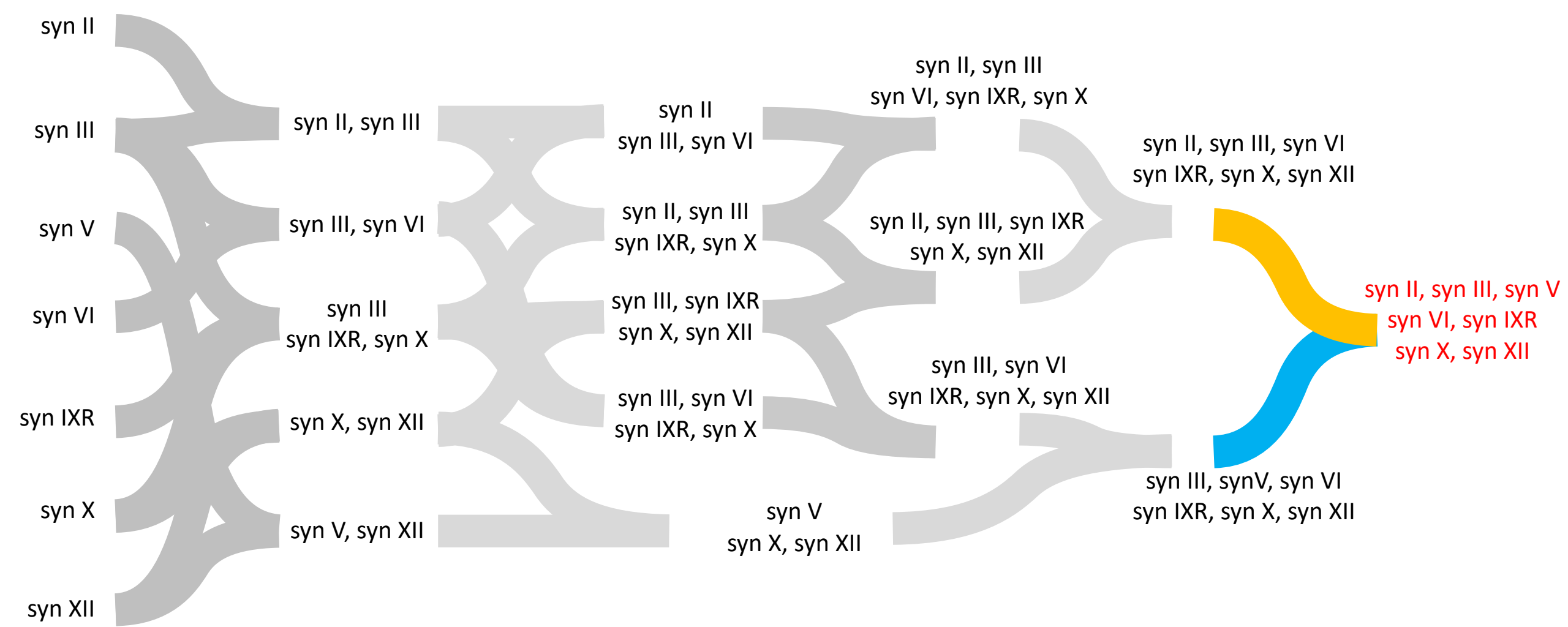
# Consolidation

Multiple synthetic chromosomes were consolidated using endoreduplication intercross



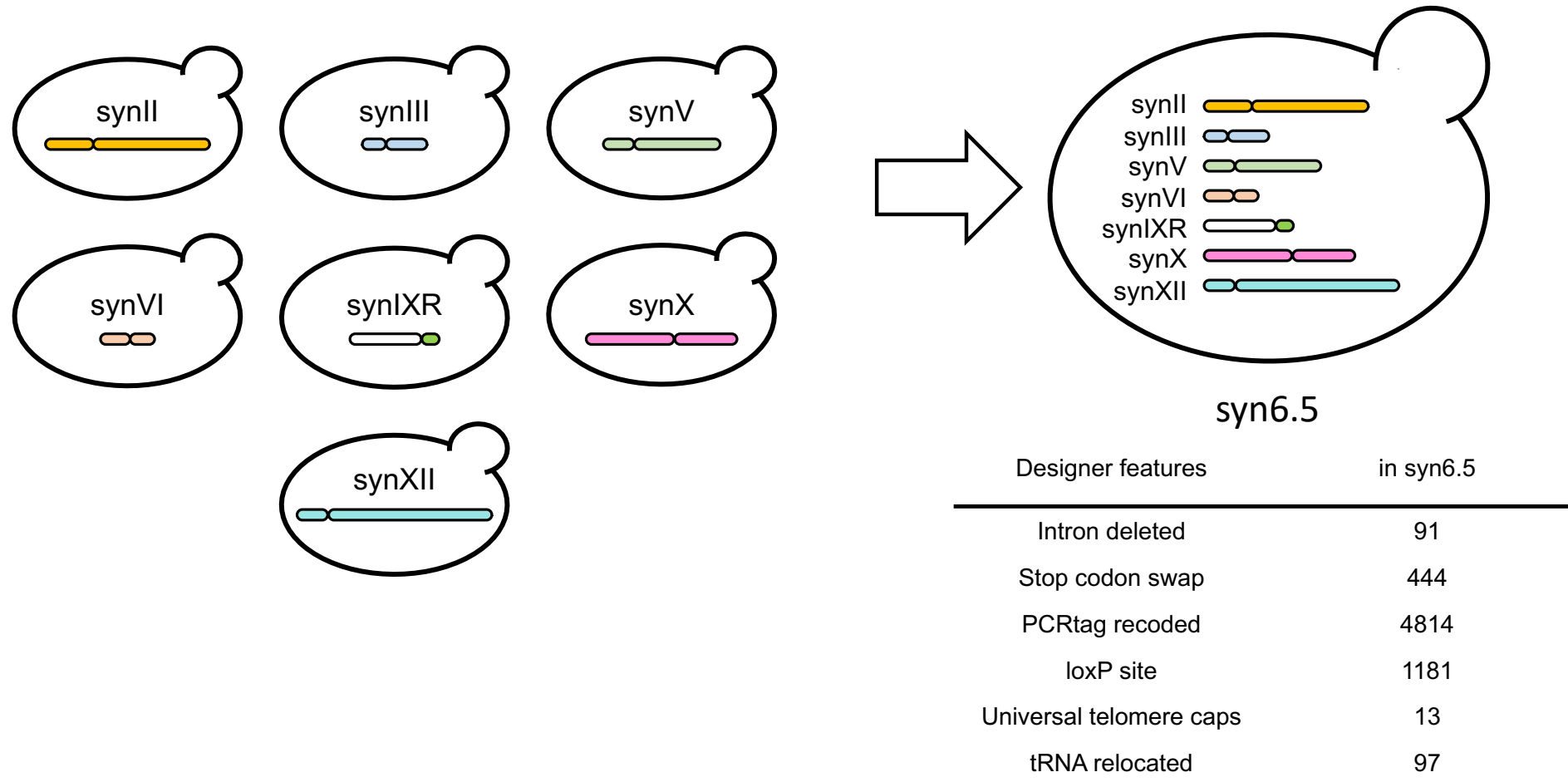
# Consolidation

Multiple synthetic chromosomes were consolidated using endoreduplication intercross



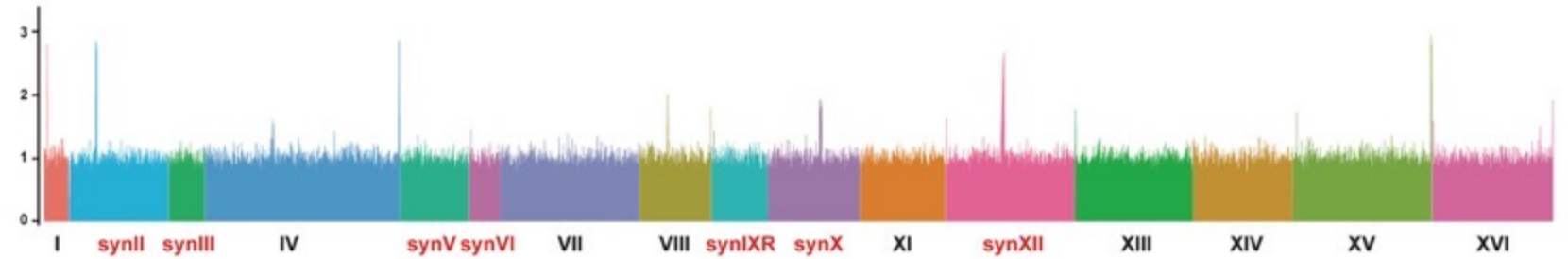
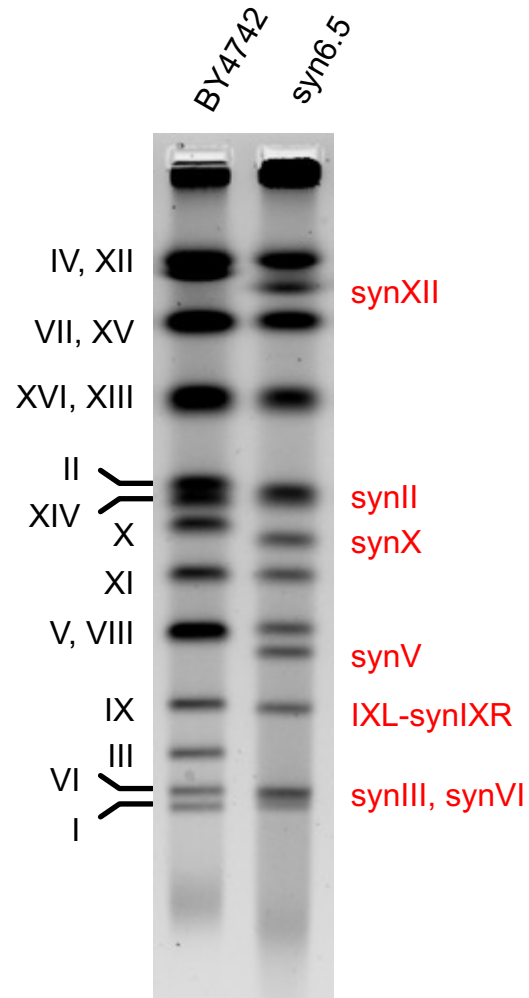
## Consolidation

Multiple synthetic chromosomes were consolidated using endoreduplication intercross





# Multiple synthetic chromosomes characterizations by PFGL and sequencing

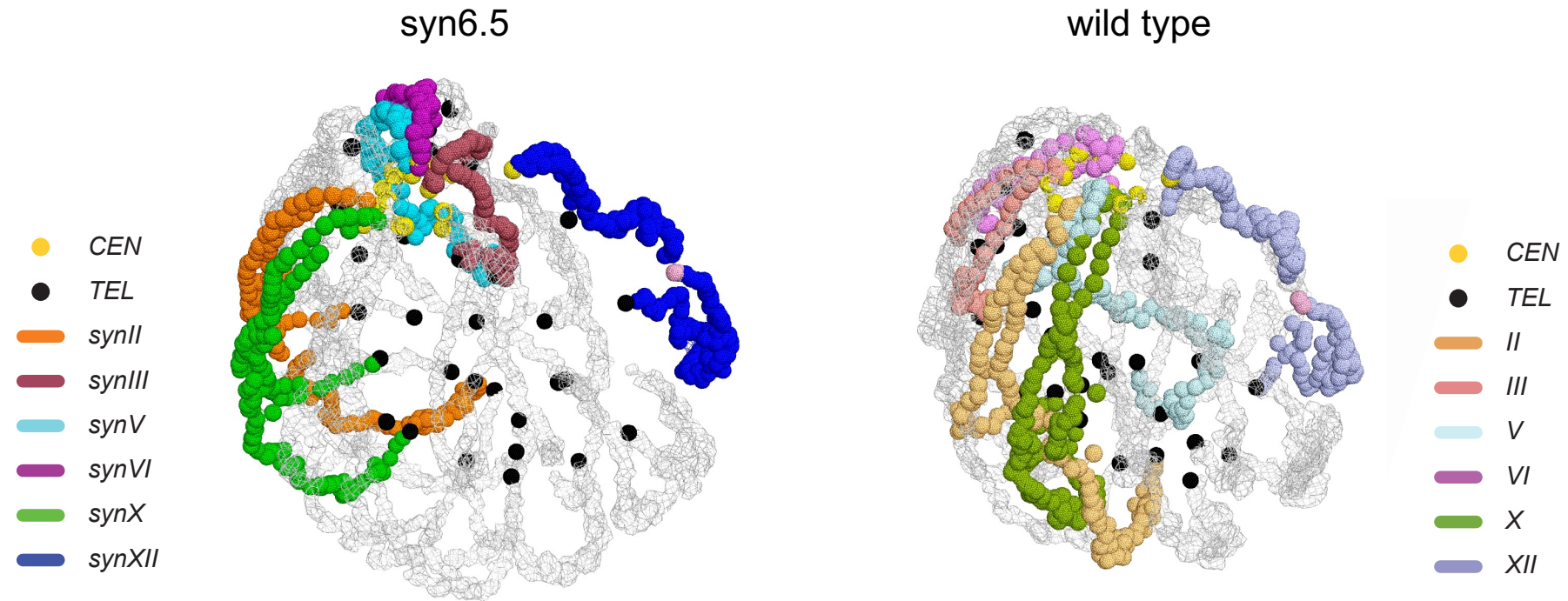


Whole genome sequencing coverage by Illumina

## 3D chromosome organization of multiple synthetic chromosomes

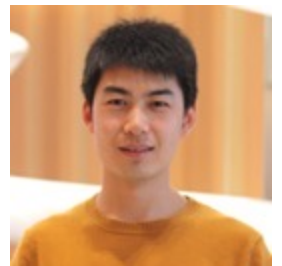
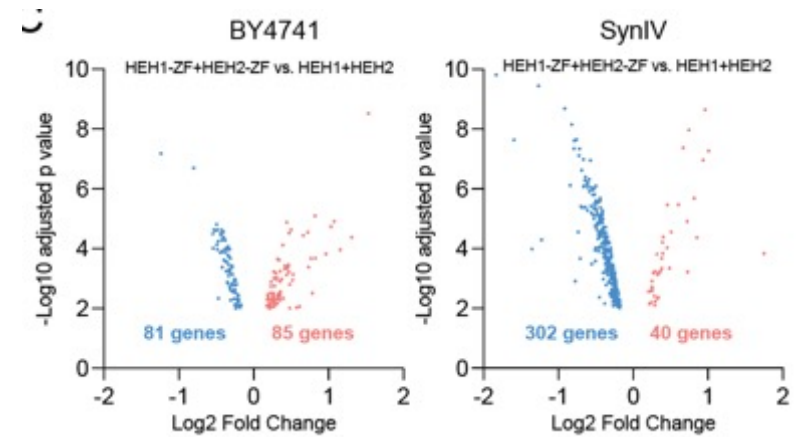
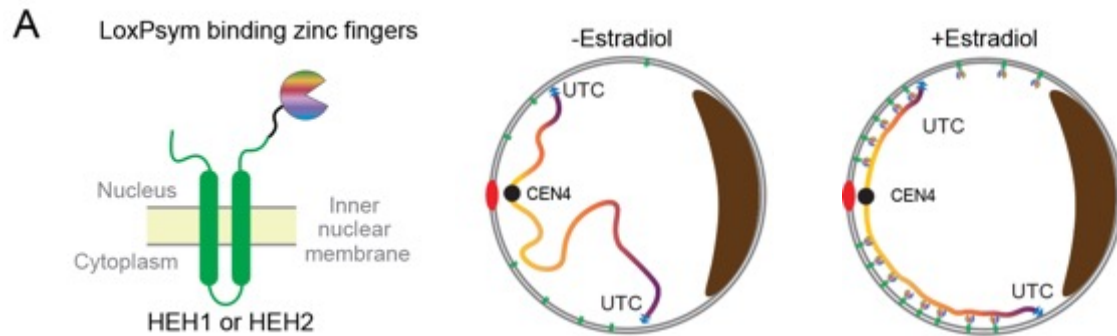
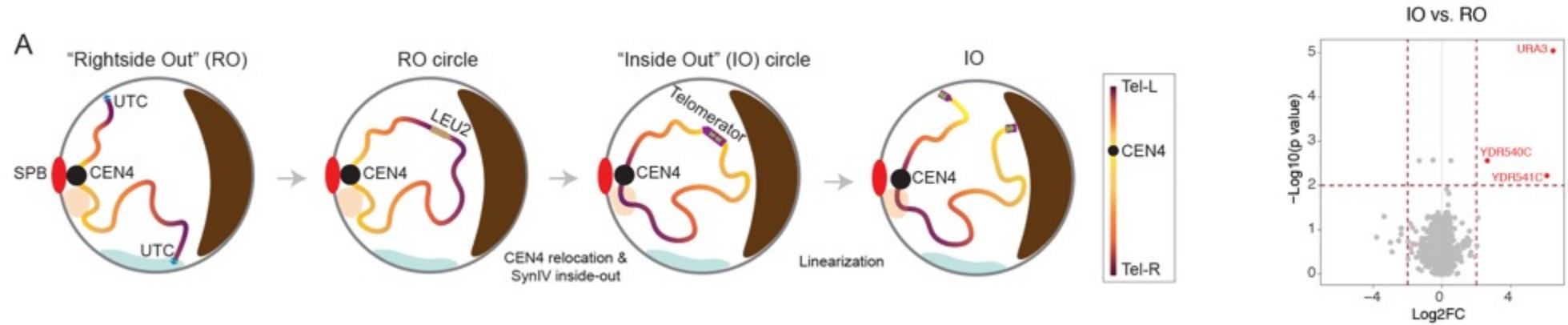
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## 3D chromosome organization of multiple synthetic chromosomes



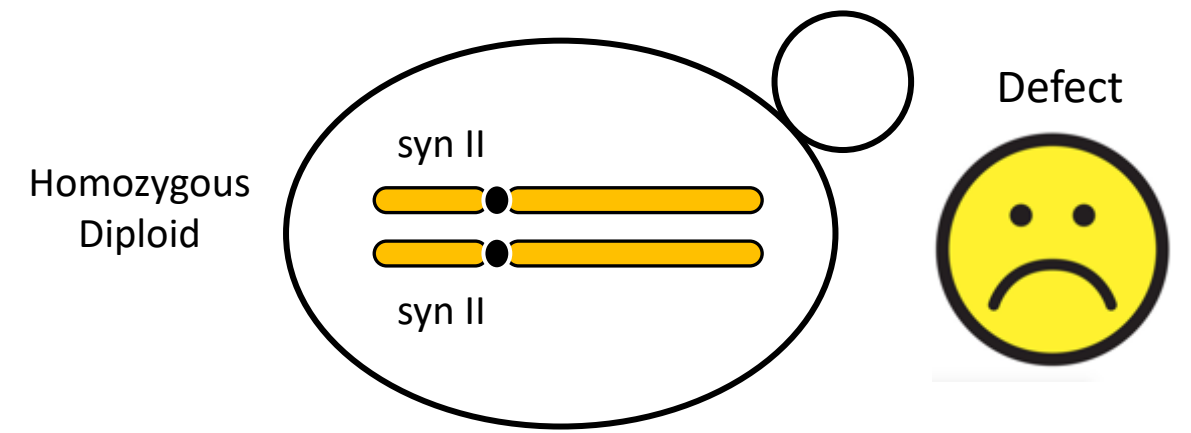
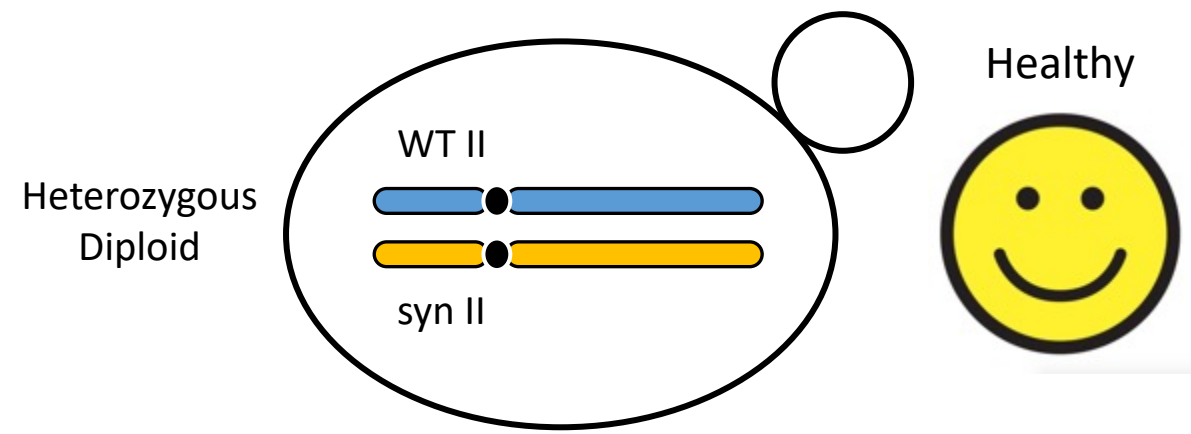
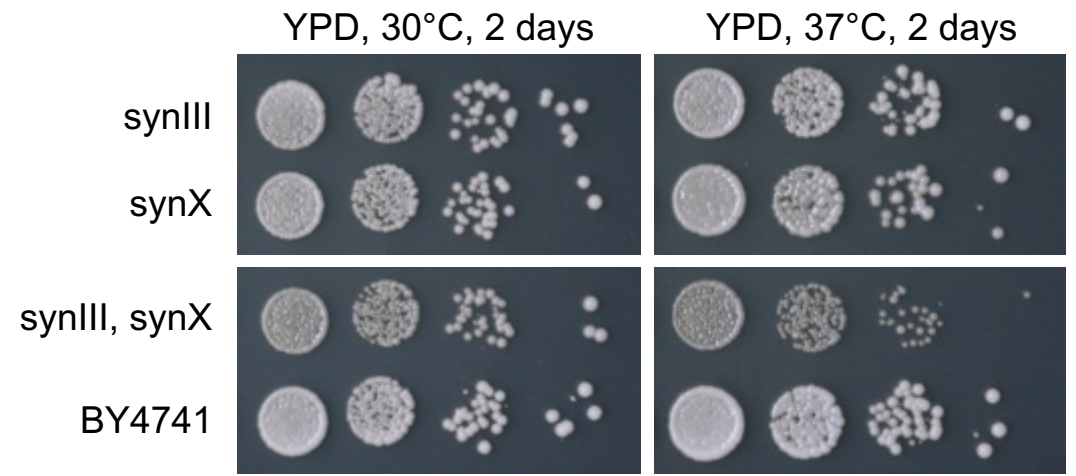
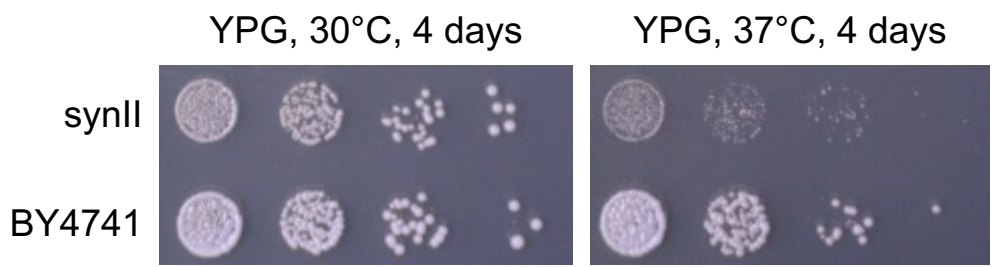
3D chromosome organization of multiple synthetic chromosomes

# Spatial regulation of synthetic eukaryotic chromosome in yeast

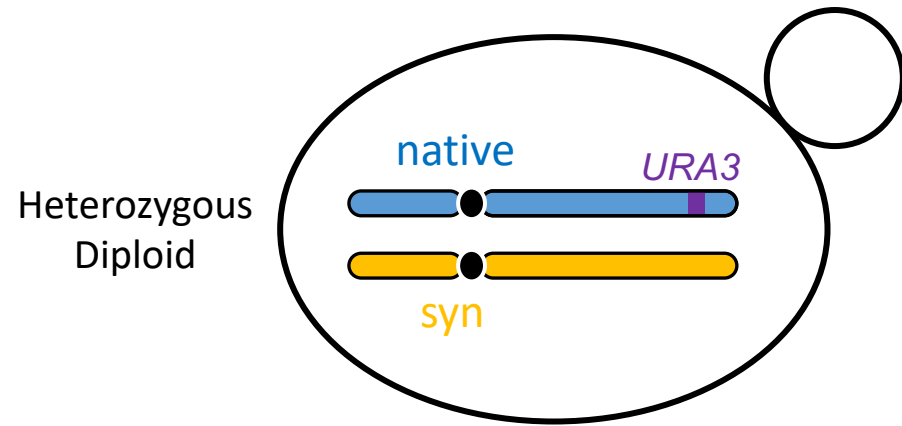


Weimin Zhang

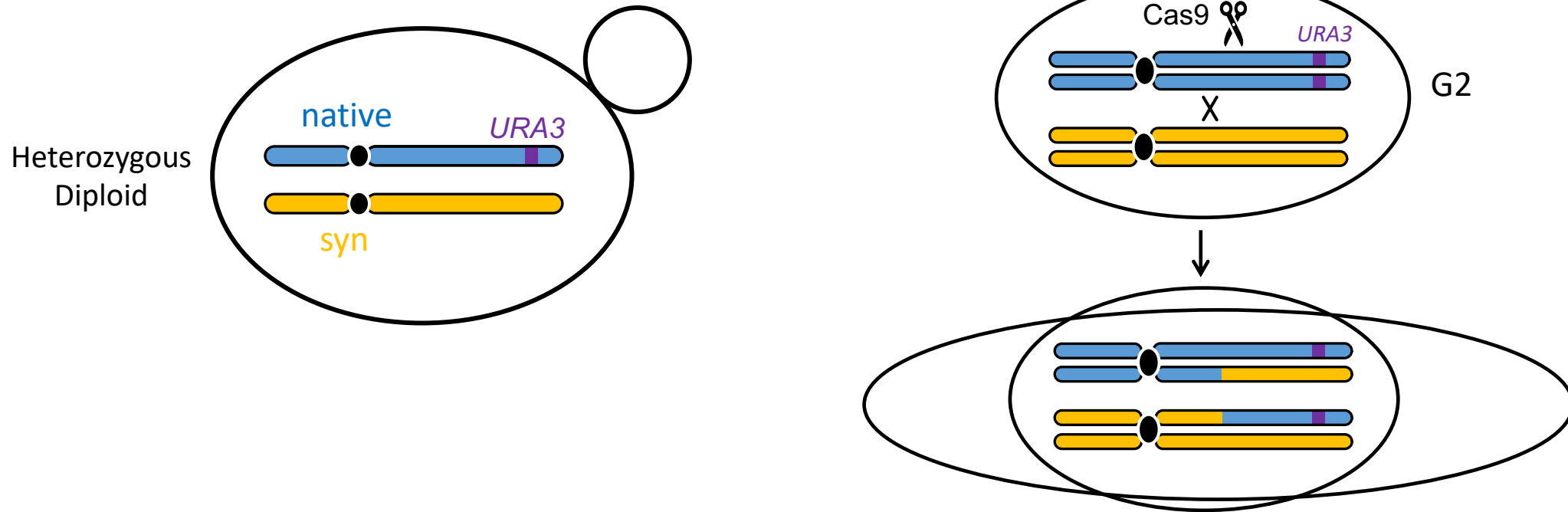
# Map designer “bugs” that caused growth defect of yeast strains with synthetic chromosomes



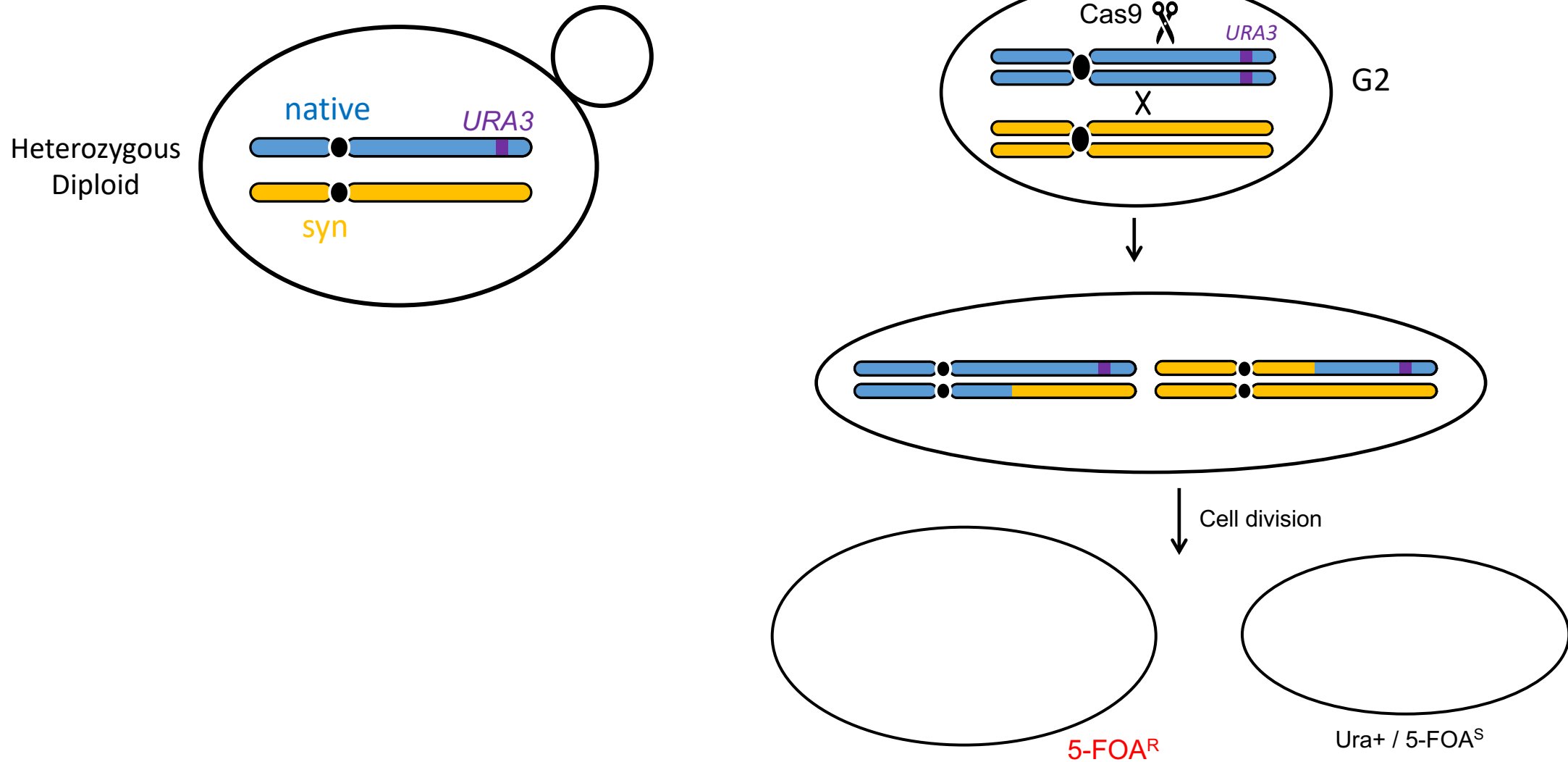
# CRISPR D-BUGS or CRISPR Directed Biallelic URA3-assisted Genome Scan



# CRISPR D-BUGS or CRISPR Directed Biallelic URA3-assisted Genome Scan

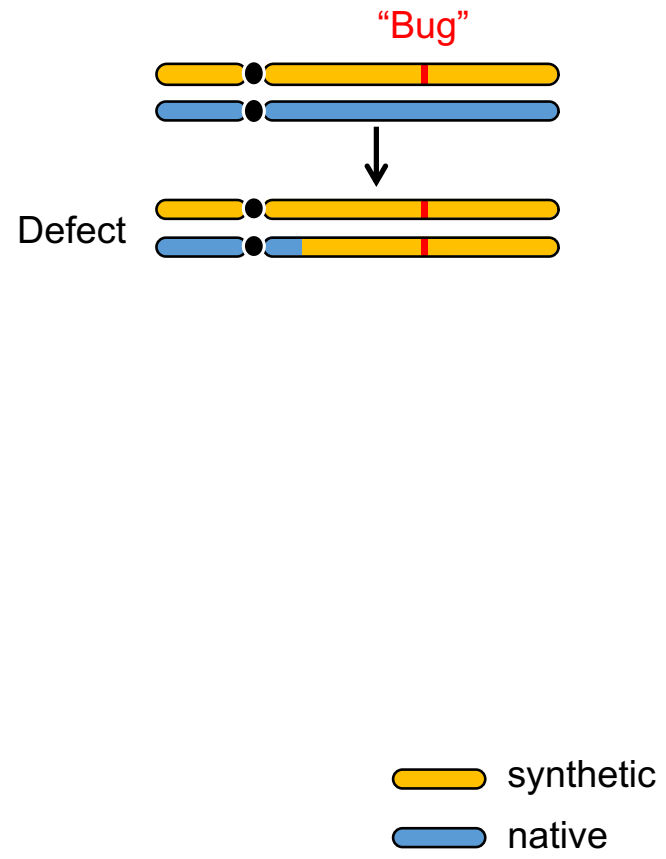


# CRISPR D-BUGS or CRISPR Directed Biallelic *URA3*-assisted Genome Scan

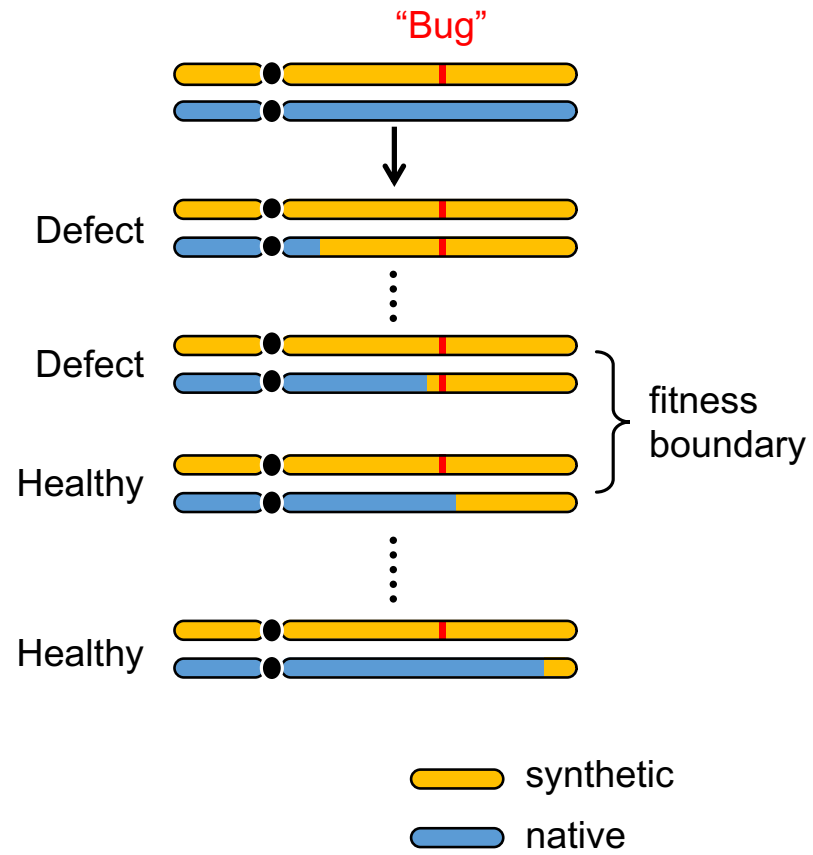




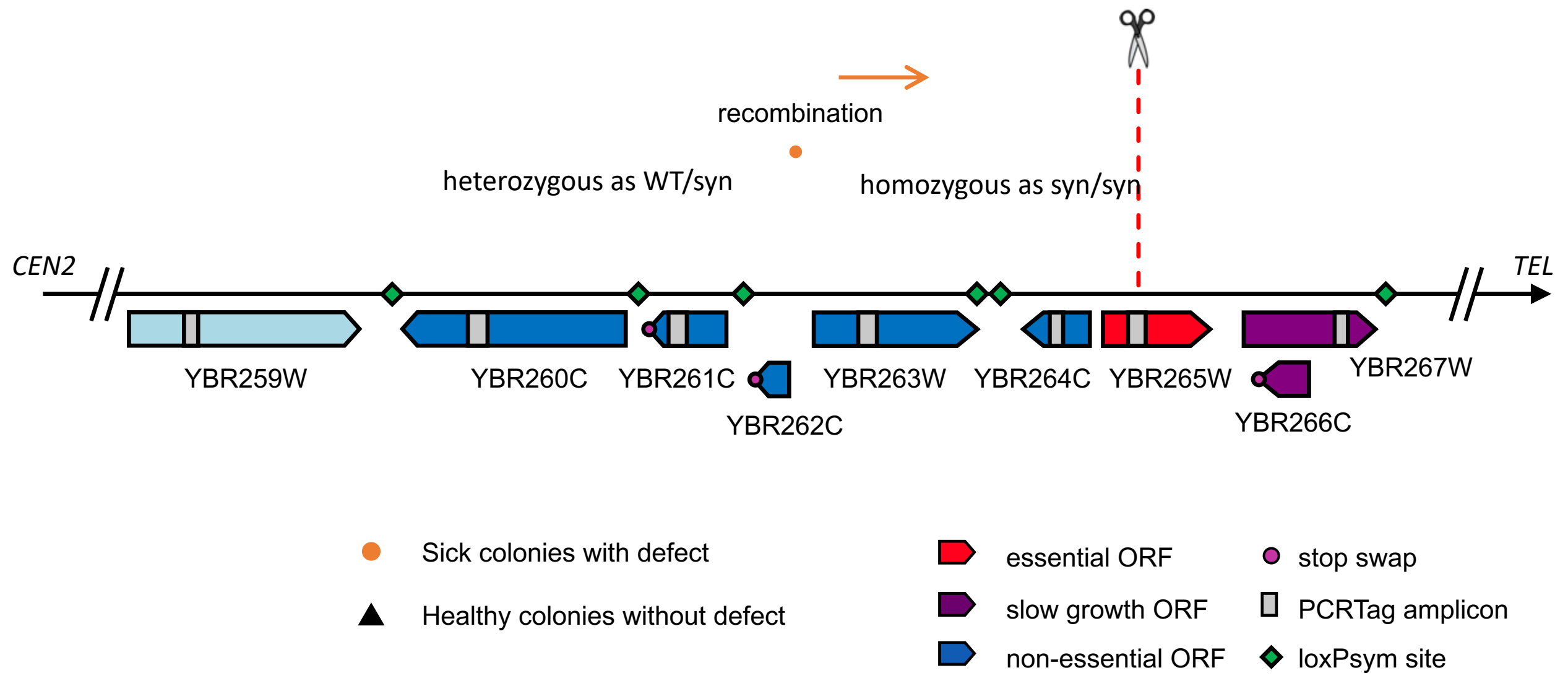
# CRISPR D-BUGS or CRISPR Directed Biallelic *URA3*-assisted Genome Scan



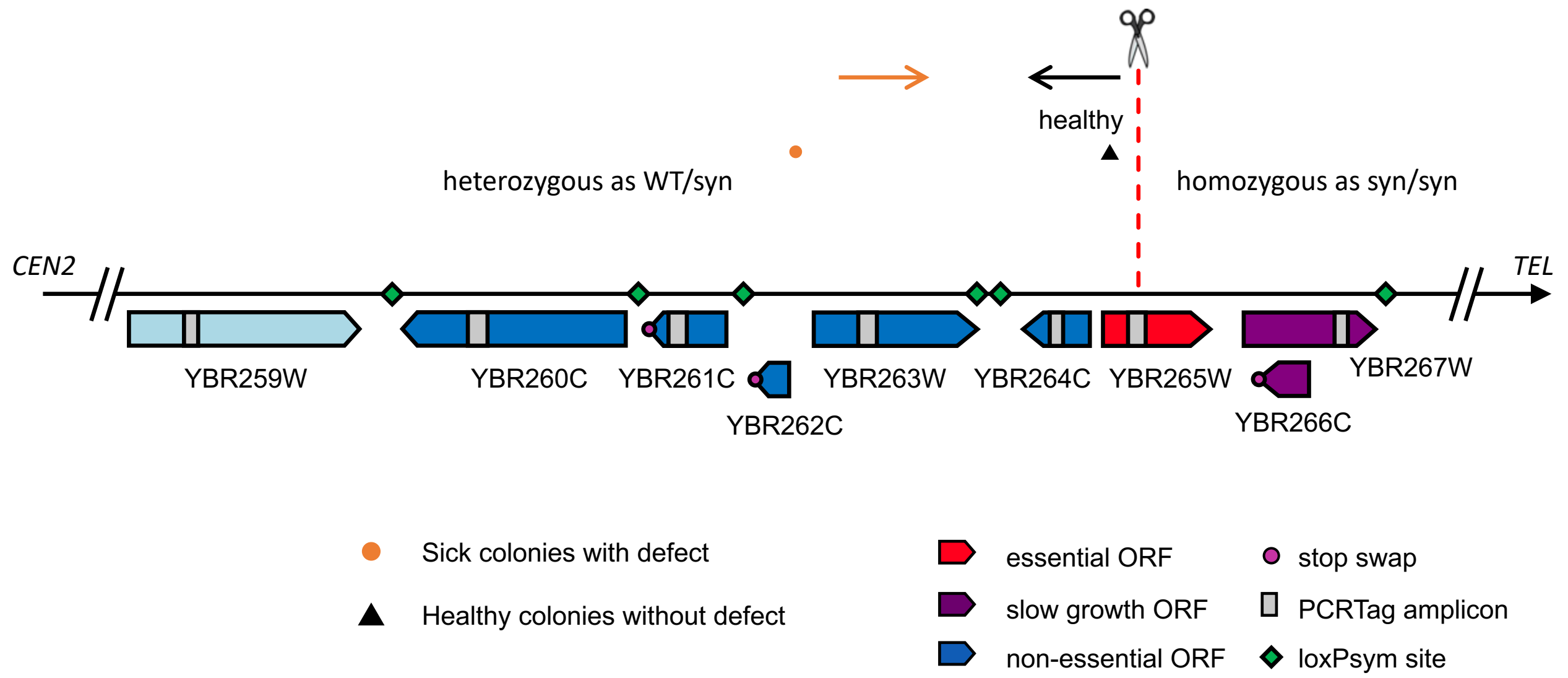
# CRISPR D-BUGS or CRISPR Directed Biallelic *URA3*-assisted Genome Scan



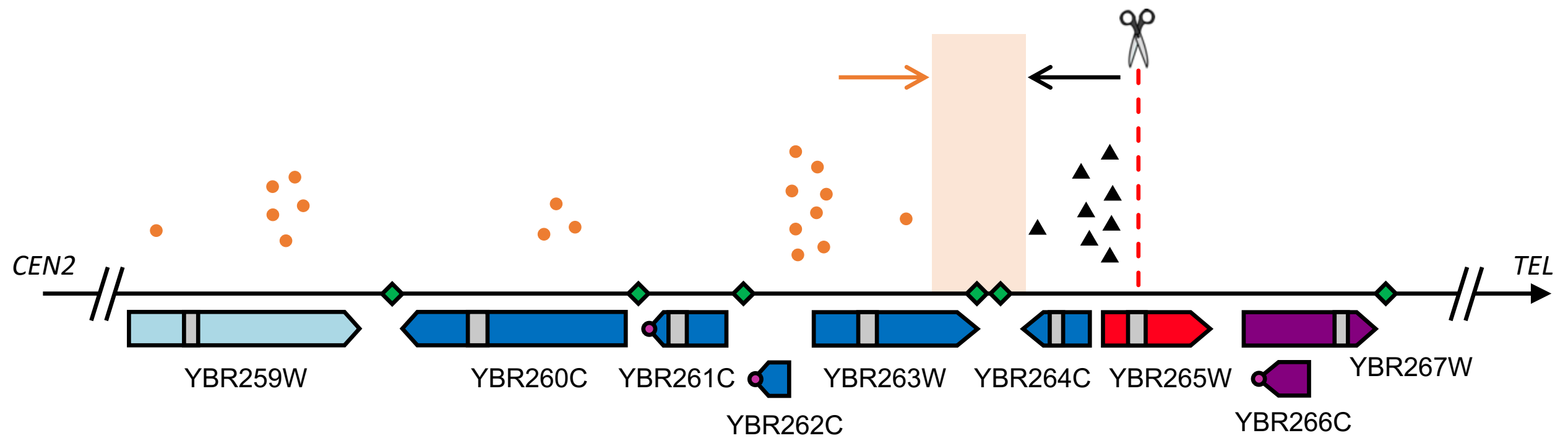
Each recombination site of single colonies was aligned to synthetic chromosome II



Each recombination site of single colonies was aligned to synthetic chromosome II



Each recombination site of single colonies was aligned to synthetic chromosome II



● Sick colonies with defect

▲ Healthy colonies without defect

➤ essential ORF

➤ slow growth ORF

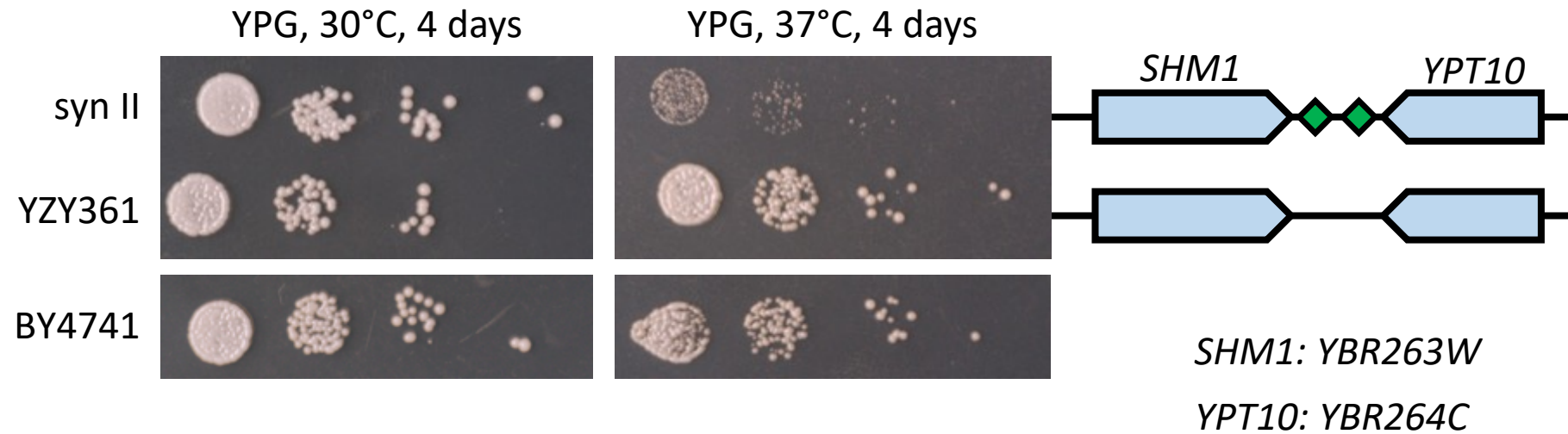
➤ non-essential ORF

● stop swap

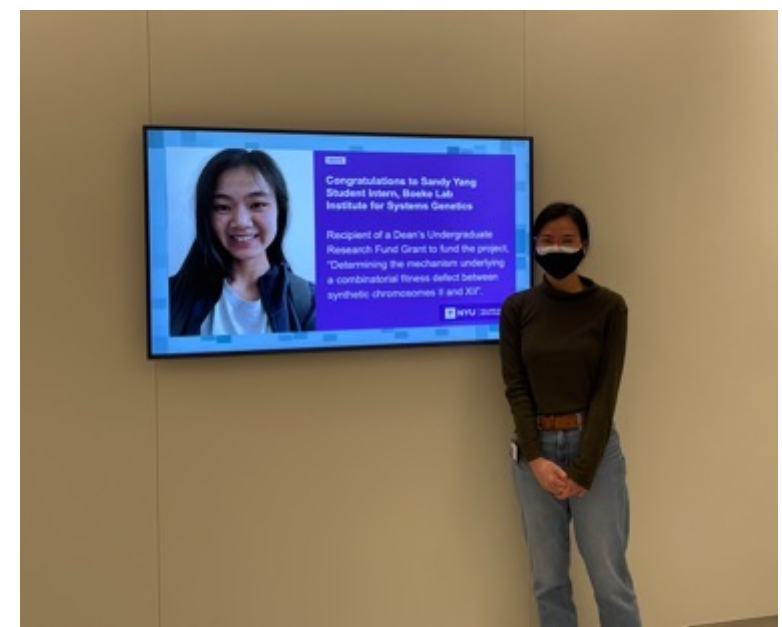
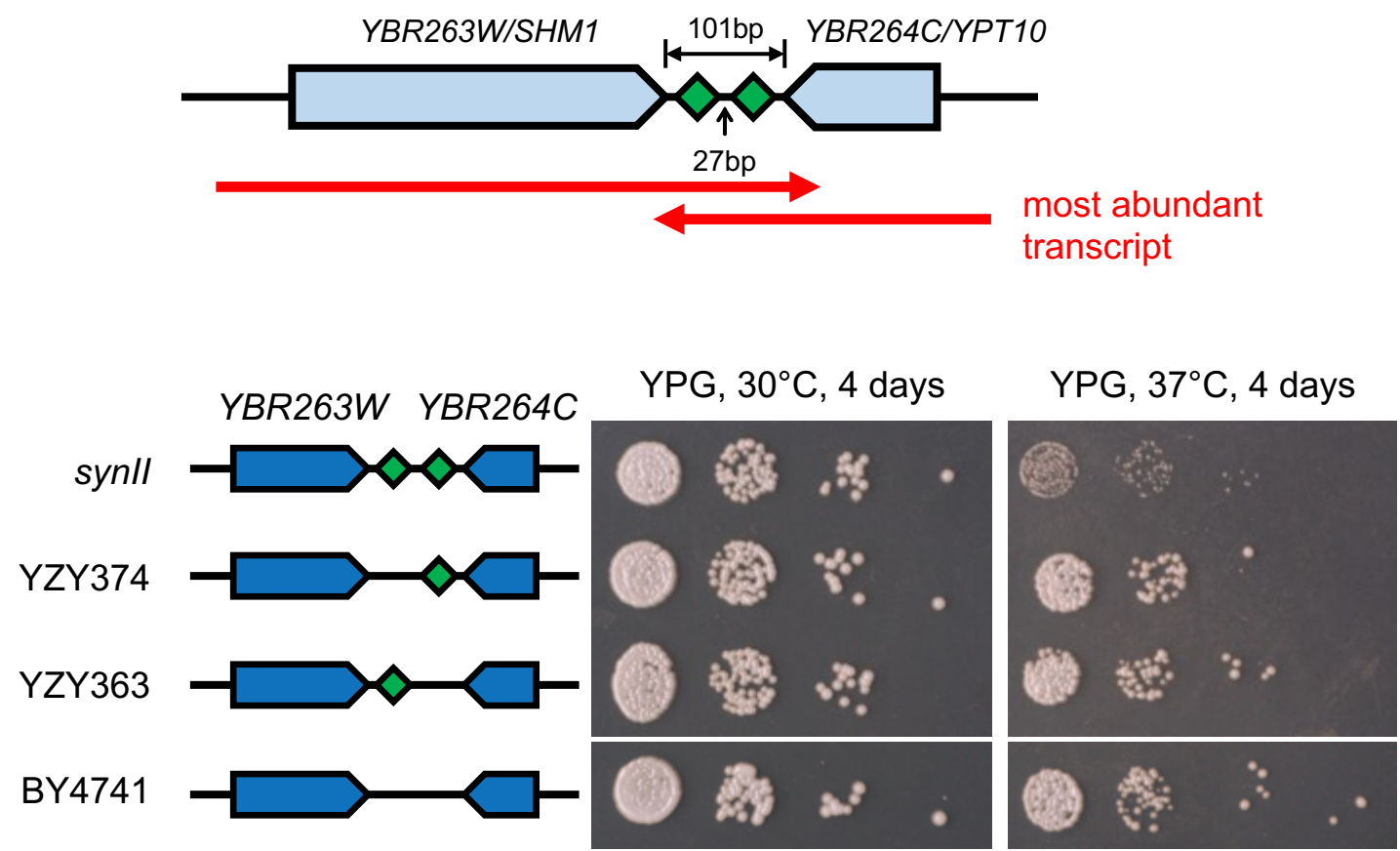
▭ PCRTag amplicon

◆ loxPsym site

The syn II growth defect was successfully rescued by deleting those two loxPsym sites



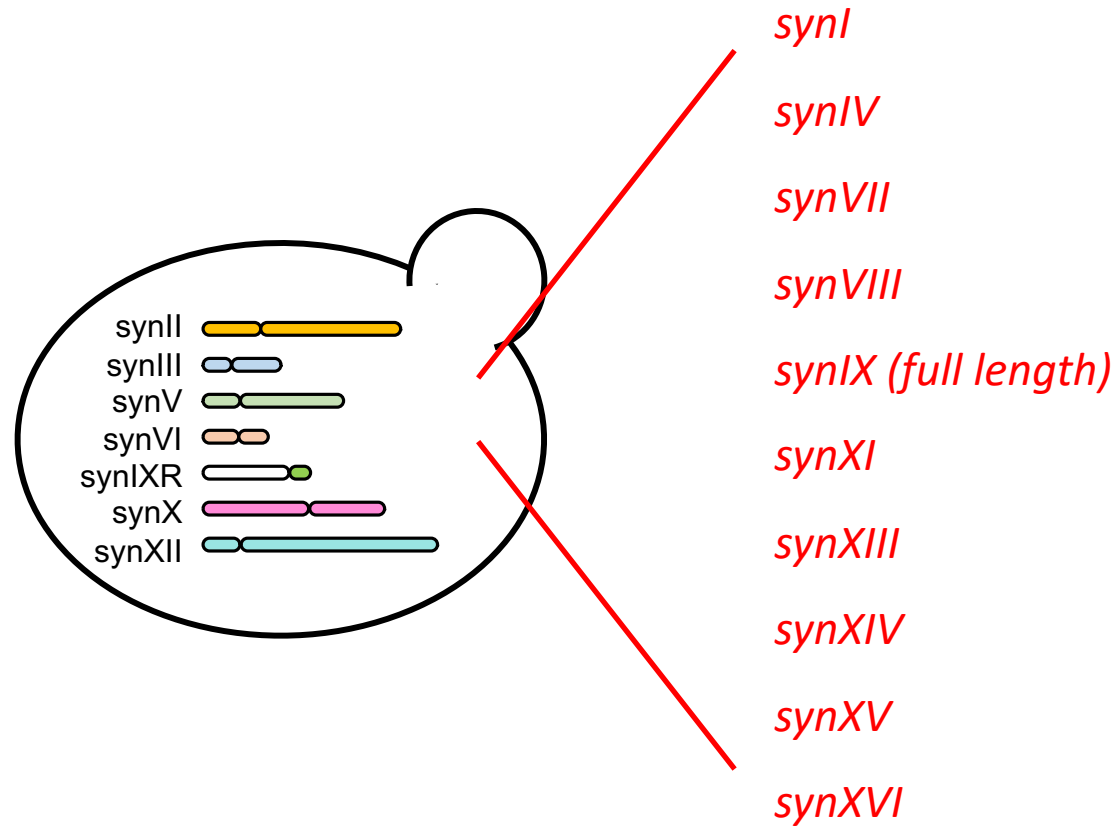
The *synII* YPG growth defect was caused by two *loxP* sites existing in the 3' UTR



Sandy Yang from BAG  
NYU Class 2021  
DURF and Horace W. Stunkard Prize in Biology

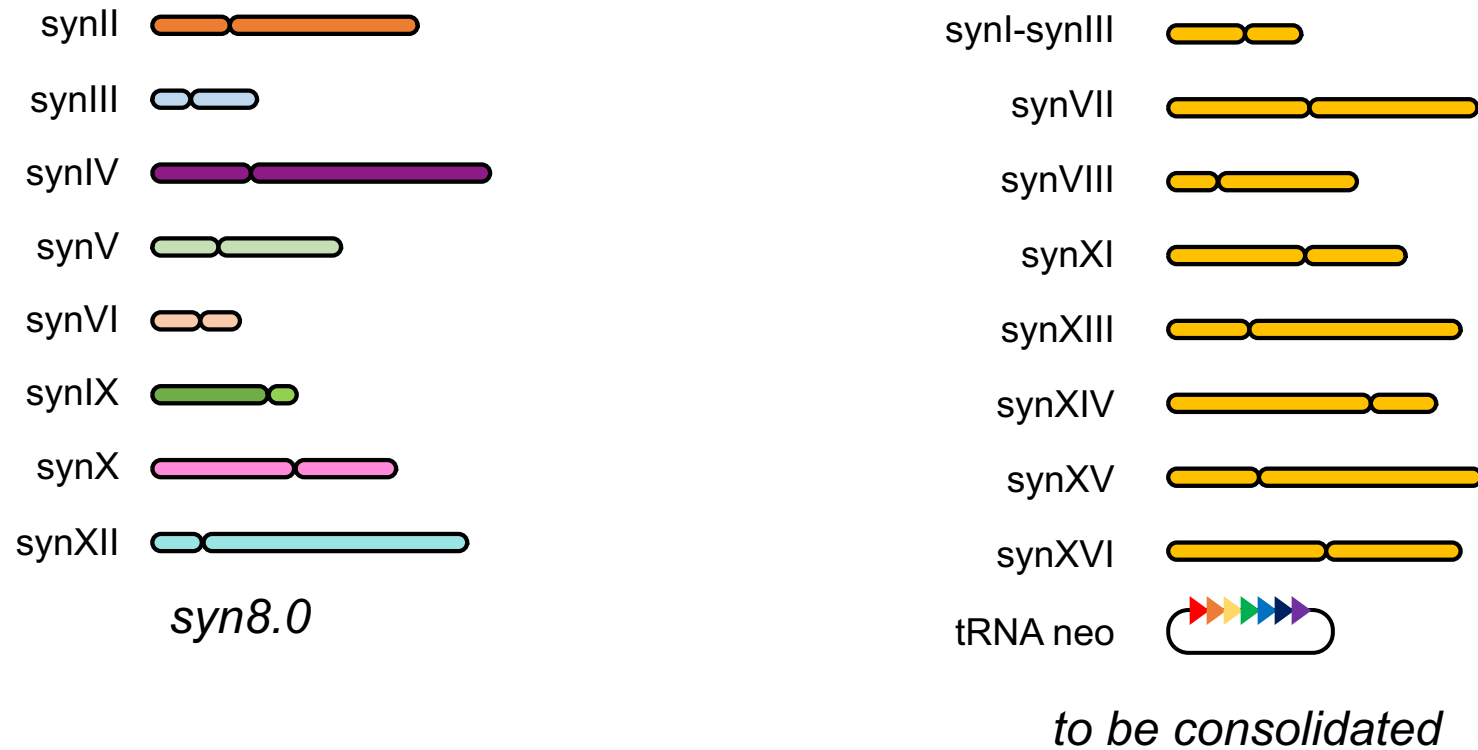
# Next generation consolidation strategy – chromosome substitution

*Other synthetic chromosomes assembled*

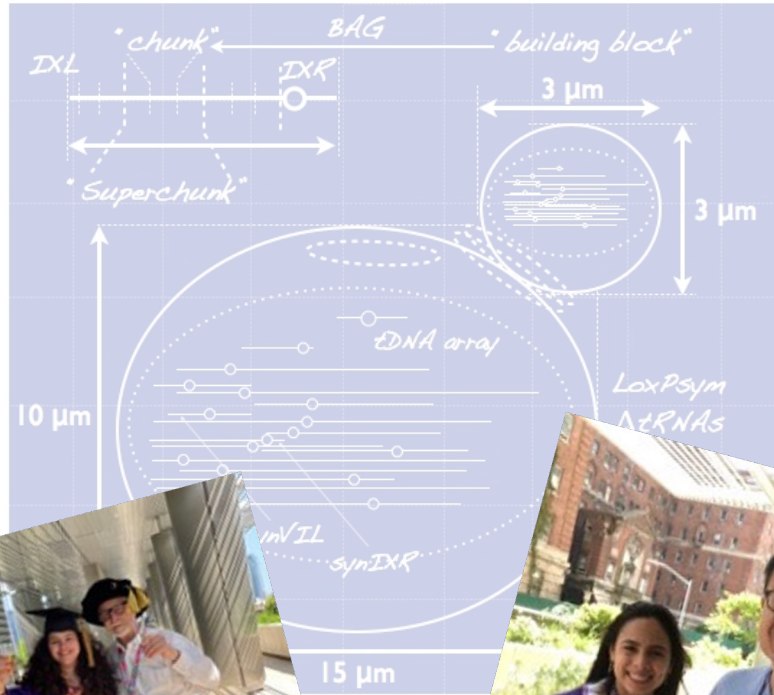




# Next generation consolidation strategy – chromosome substitution



# Build-A-Genome: yeast is an amazing tool for education



Camila Coelho



Cindy Hernandez

- Build-A-Genome: JHU, Tianjin U, NYU.
- Global network: Lisa, Eric, Rob.
- Labs working on synthetic biology.
  - Sc2.0
  - metabolic engineering
  - mammalian genome writing
- Industrial & Biotech
- 100s of trainees.

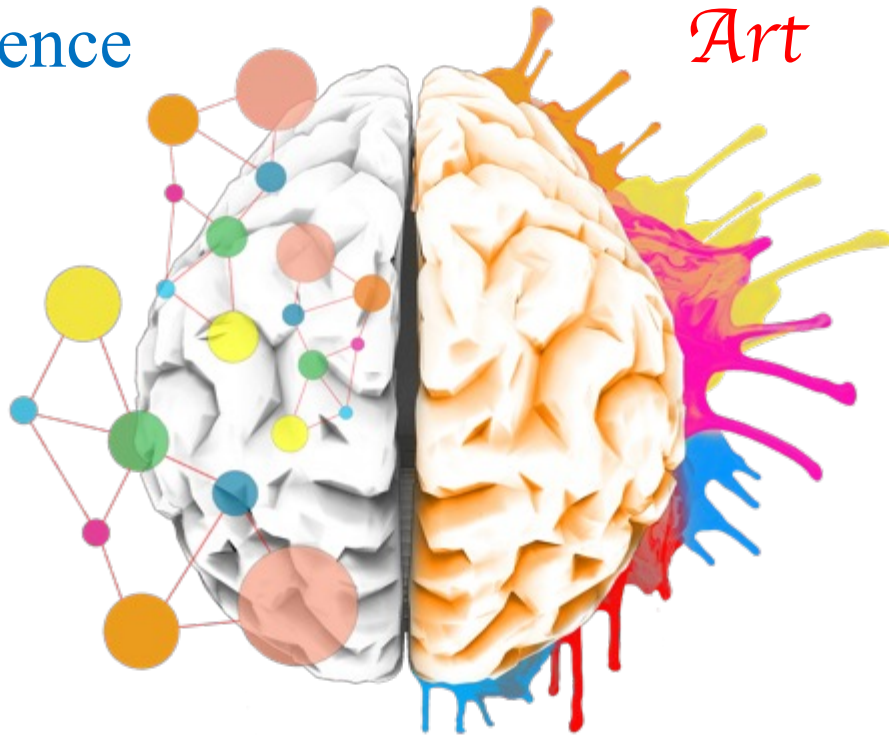
Email:

[yu.zhao@nyulangone.org](mailto:yu.zhao@nyulangone.org)

(two-year commitment)

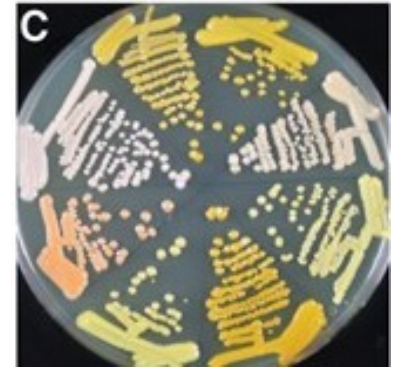
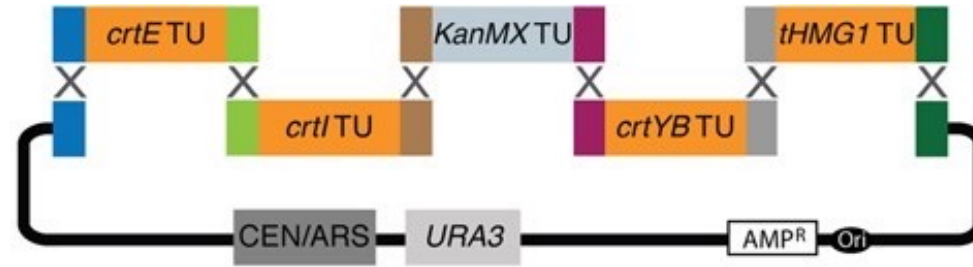
# Yeast Art: where the science is married with art

Science

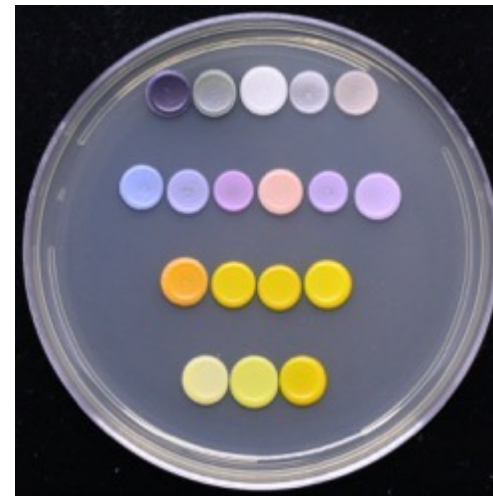


Art

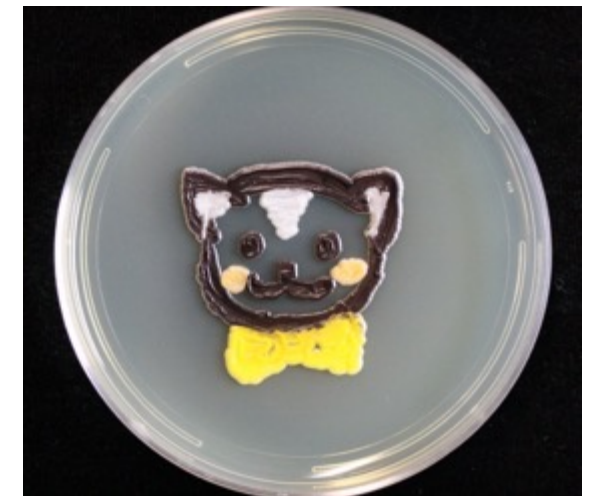
$\beta$ -carotene biosynthesis pathway



Mitchell, et al. NAR 2015



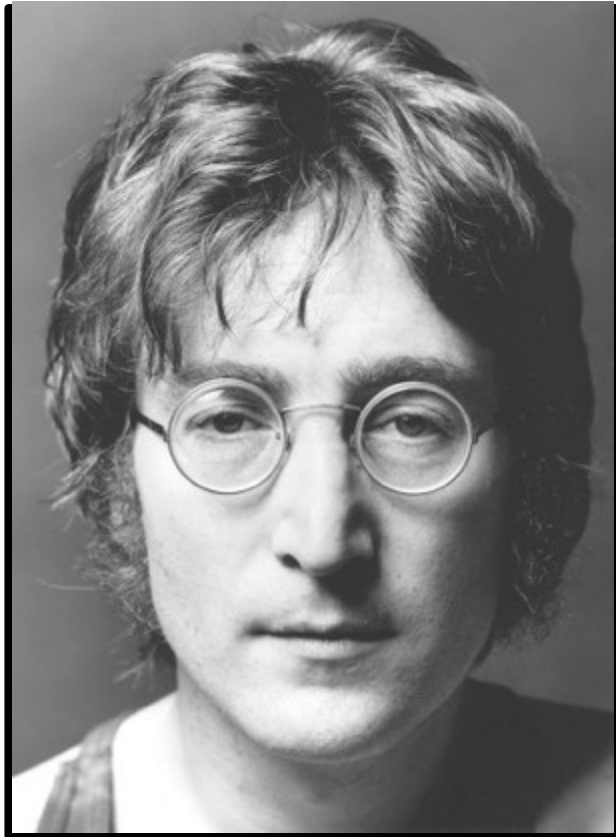
Yeast art palette



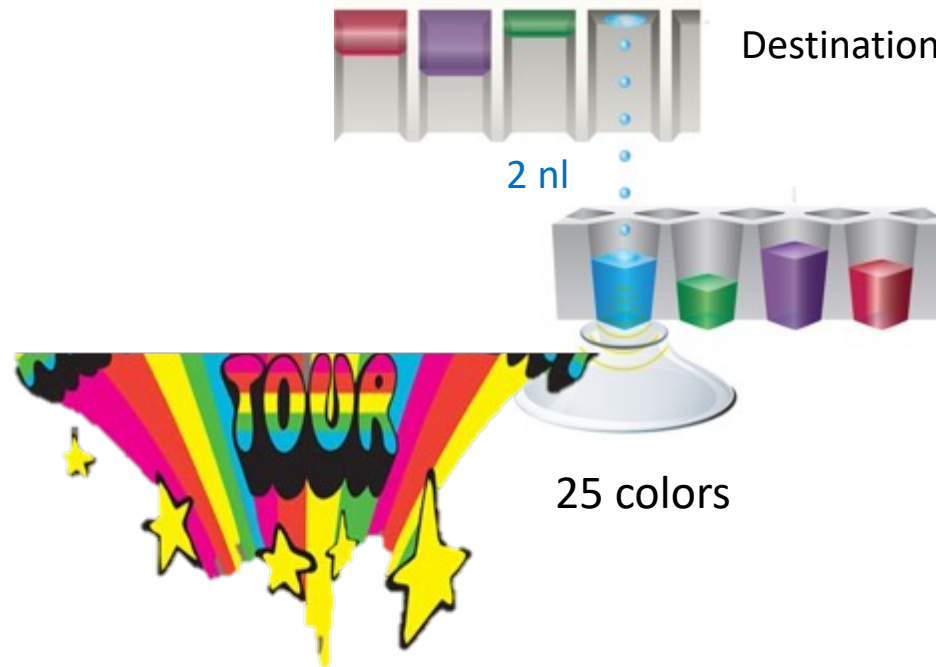
picture source:  
The Graduate Center, CUNY

find more: Yeast Art <http://www.yeastart.org/>

# Yeast Art: where the science is married with art



by Alexander Lund on The Yeast Art  
24,576 biopixels

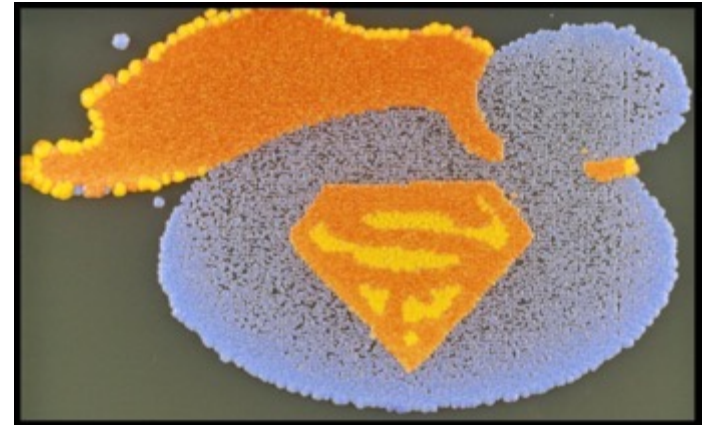
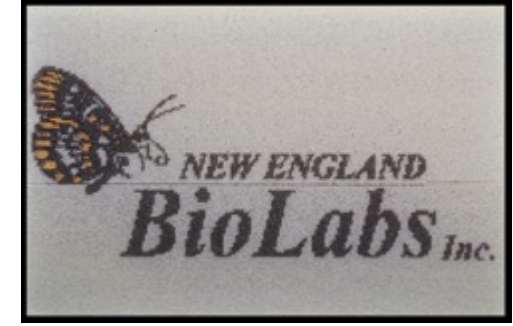
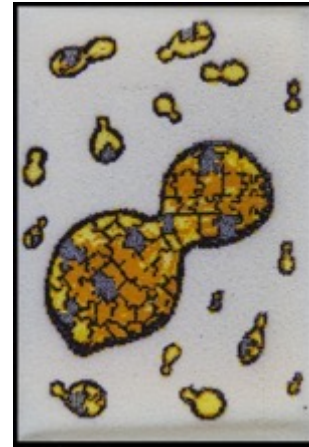


Echo liquid handler

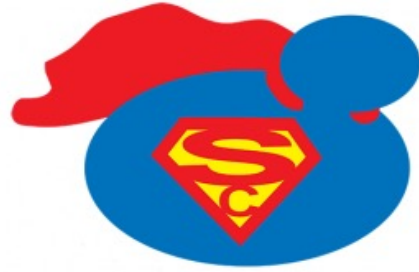
# Yeast art: where the science is married with art



Yeast chromosomes painted by yeast

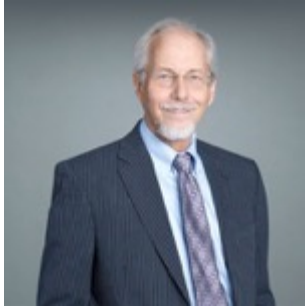


## *Recap of today's talk*



- Brief intro to **NYU BAG**.
- Synthetic chromosome **consolidation**.
  - syn6.5 using endoreduplication intercross.
  - finish Sc2.0 with chromosome substitution.
- Map designer “**bugs**”.
  - CRISPR D-BUGS
  - Single and combinatorial bugs.
- Synthetic yeast for **education**.





Jef Boeke



Leslie Mitchell



Neta Agmon



Stephanie Lauer



Raquel Ordoñez



Automation team!  
(A-team)



Boeke lab

**Sc2.0 teams**

**Luciana Lazar Stefanita**

**Weimin Zhang**

Laura McCulloch

Max Hasse

**Aleksandra Wudzinska**

All Boeke lab members

**Sc2.0 Consortium**

**Lars Steinmetz**

**Amanda Hughes**

Aaron Brooks

Giovanni Stracquadanio

Tom Ellis

**Students mentored in labs**

Sandy Yang

Camila Coelho

Cindy Hernandez

Milosz Majewski

Sunny Ham

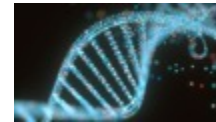
**Yizhi “Patrick” Cai**

Junbiao Dai

Yingjin Yuan

Yue Shen

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CEGS

(Centers of Excellence in Genomic Science)



**Benchling**