Sex-determining regions as drivers for evolutionary potential and phenotypic plasticity in *Zygosaccharomyces rouxii* clade

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Ph.D. STEBA Workshop 2017-2018

UMCC
Unimore Microbial Culture Collection
bioprospecting
biobanking
biomics

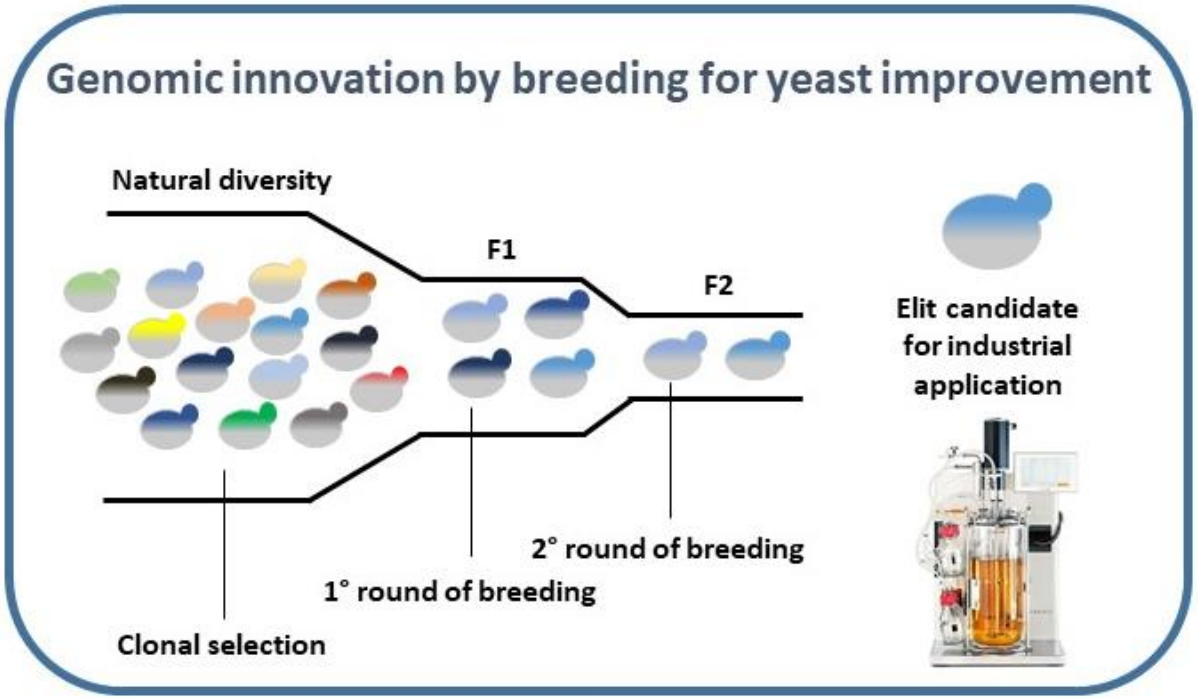
Zygosaccharomyces rouxii is a promising cell factory for food fermentation and white biotechnologies

PRO

- Robustness
- Fermentative vigour
- Osmo- and halo- tolerance

CONTRA

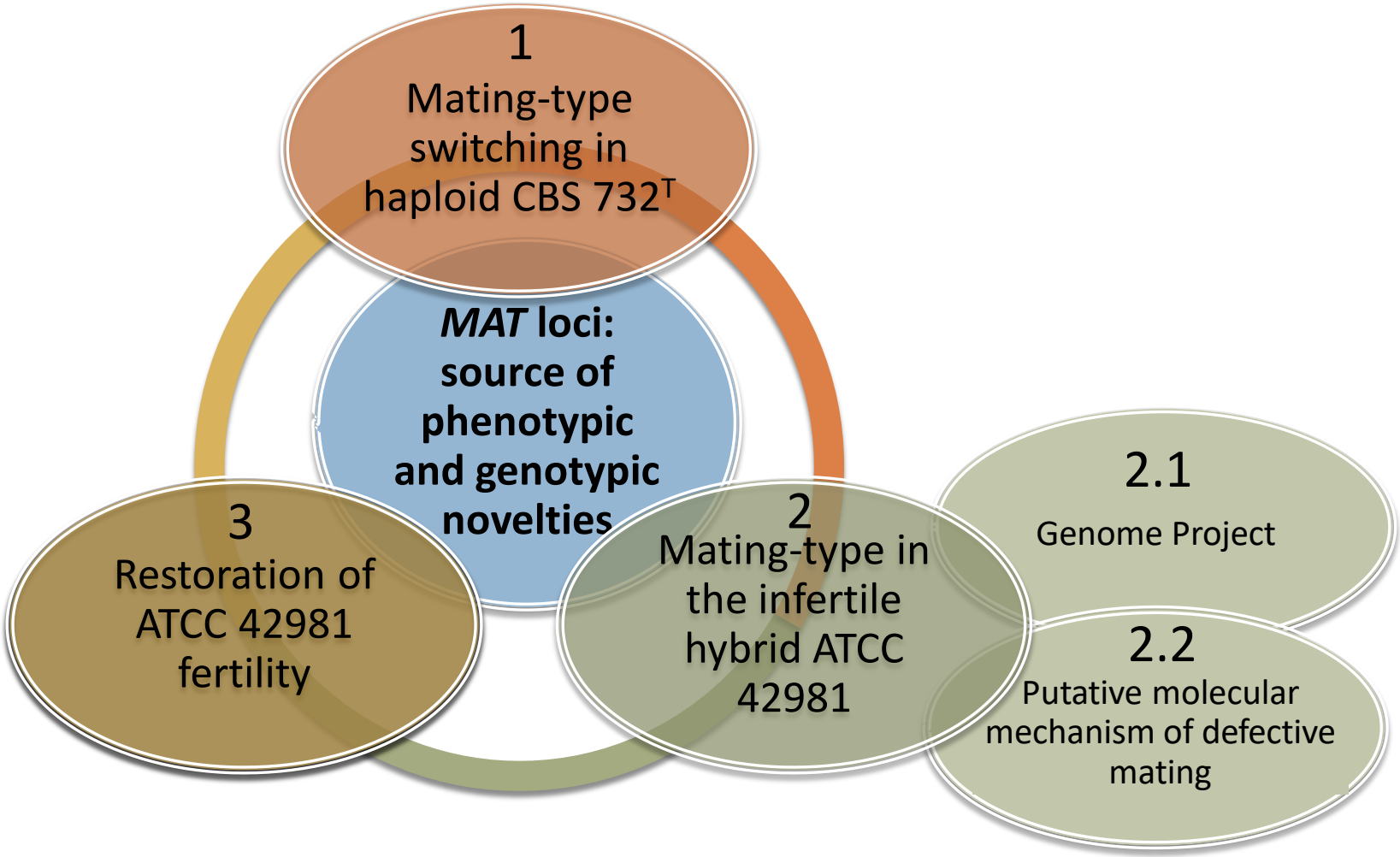
- Potential spoilage agent
- No knowlegde about cell cycle and sex determination system



Quantitative genetics of industrial traits

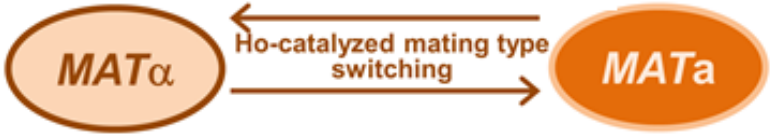
Study of hybrid infertility and its biotech restoration

Main experimental activities

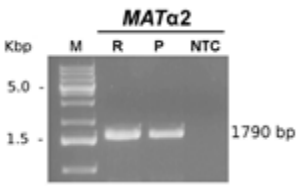


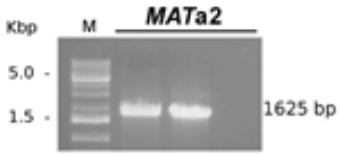
1. Mating-type switching in haploid CBS 732^{T}

Mating-type switching mechanism

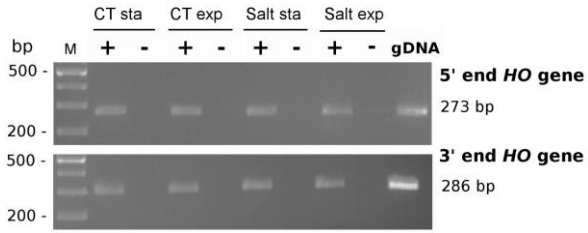


RT-PCR





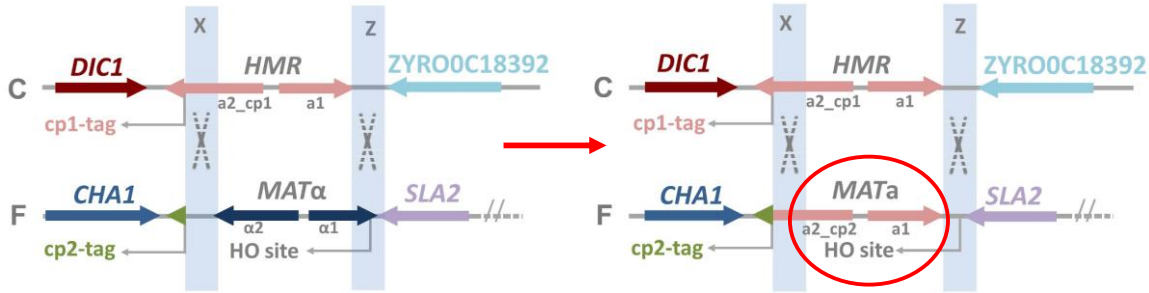
Cell-cycle relaxed transcriptional control



RT-PCR

- *MAT* switching is indipendent from the environmental conditions;
- Pure *MAT*α and *MAT***a** cultures suitable for breeding programs and pheromone-based studies on cell-to-cell communication;

α to a genotype switching generates a new *MAT*a2 gene

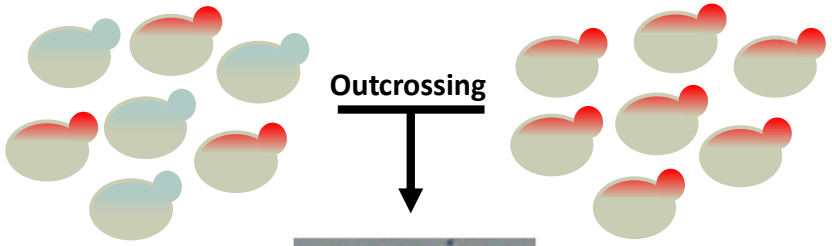


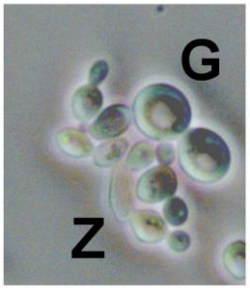
- Despite *HO* constitutive expression, mating-type switching occurs rarely or belatedly during colony formation.

1. CBS 732^{T} isogenic lines display distinct outcross fertility behaviour

CBS 732^{T}_P
α/**a** mixed population

CBS4837
mating-type a



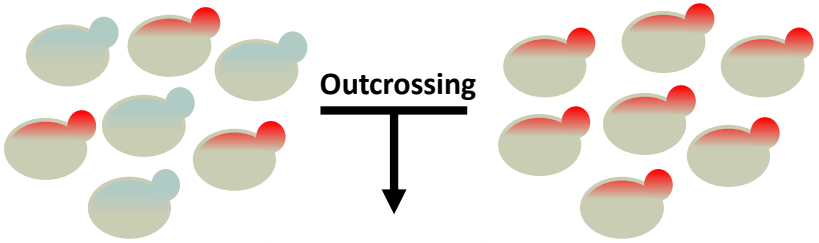


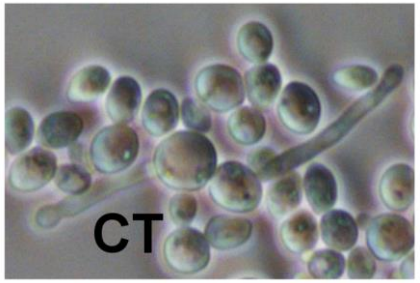
Z; emerging zygote
G; giant cells
CT; abnormal conjugation tubes

Expected zygote formation

CBS 732^{T}_R
α/**a** mixed population

CBS4837
mating-type a



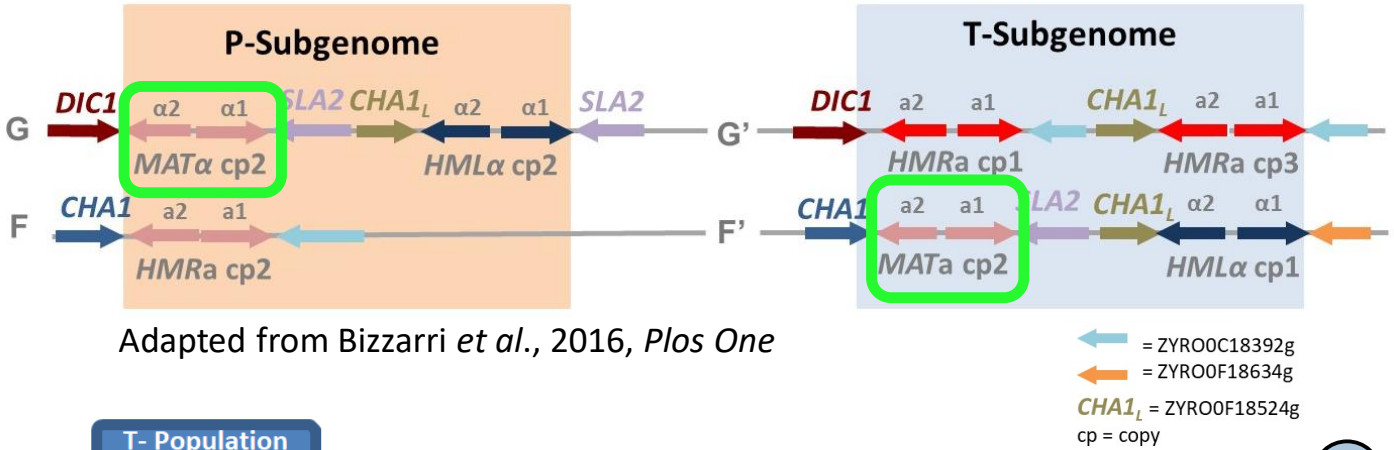


Hyperelongated conjugation tubes

Mating-type switching is a source of instability inside microbial culture collections

Paper accepted for publication in *FEMS Microbiology Letters.*

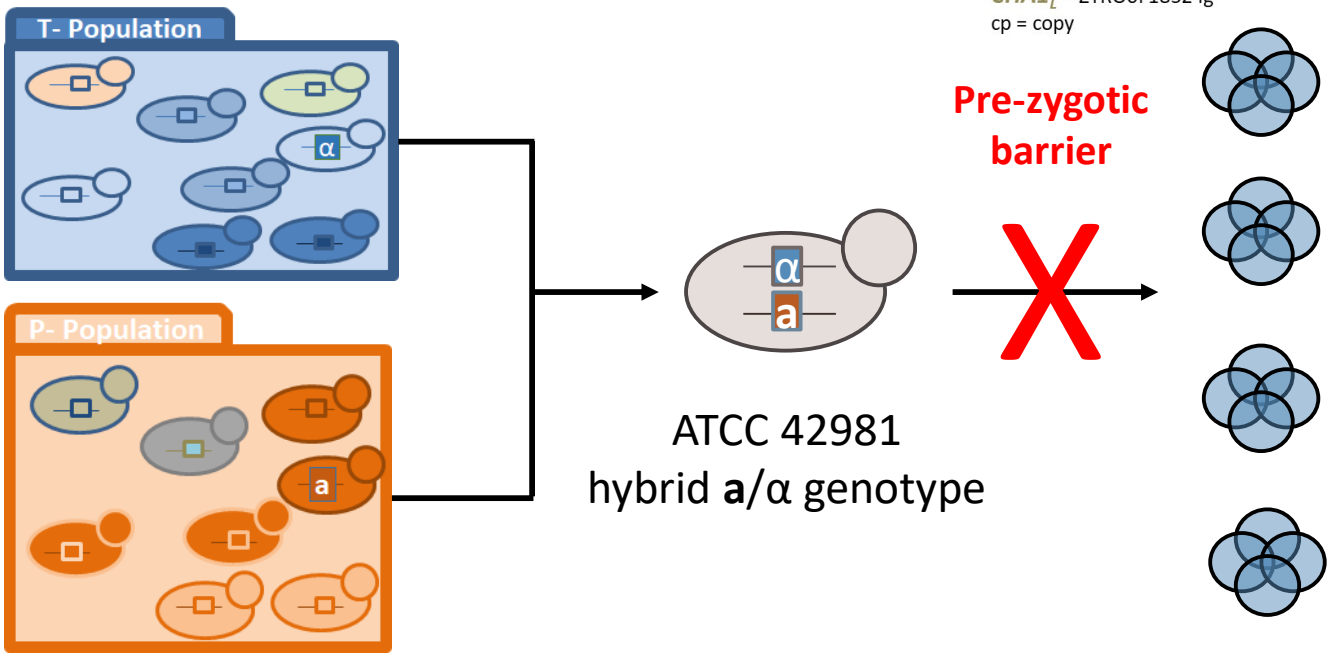
2. Characterization of mating-type in the sterile hybrid ATCC 42981



Adapted from Bizzarri *et al.*, 2016, *Plos One*

Mating-type loci

*MAT***a**1 and *MAT*α2 genes are from two different parental sub-genomes



ATCC 42981
hybrid **a**/α genotype

Gordon and Wolfe, 2008

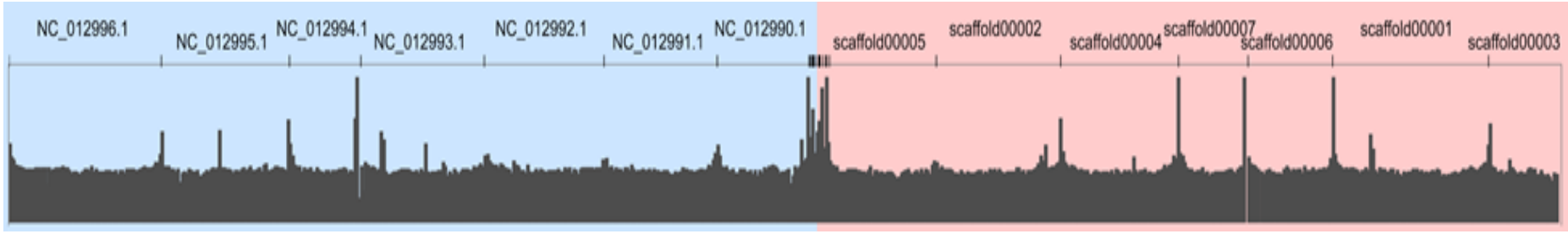
Sub-genomes incompatibility

Phylogenetic divergence between the parental species prevents meiosis and/or sporification

2.1 Genome Project

Haplotypes dissection

Sequence coverage



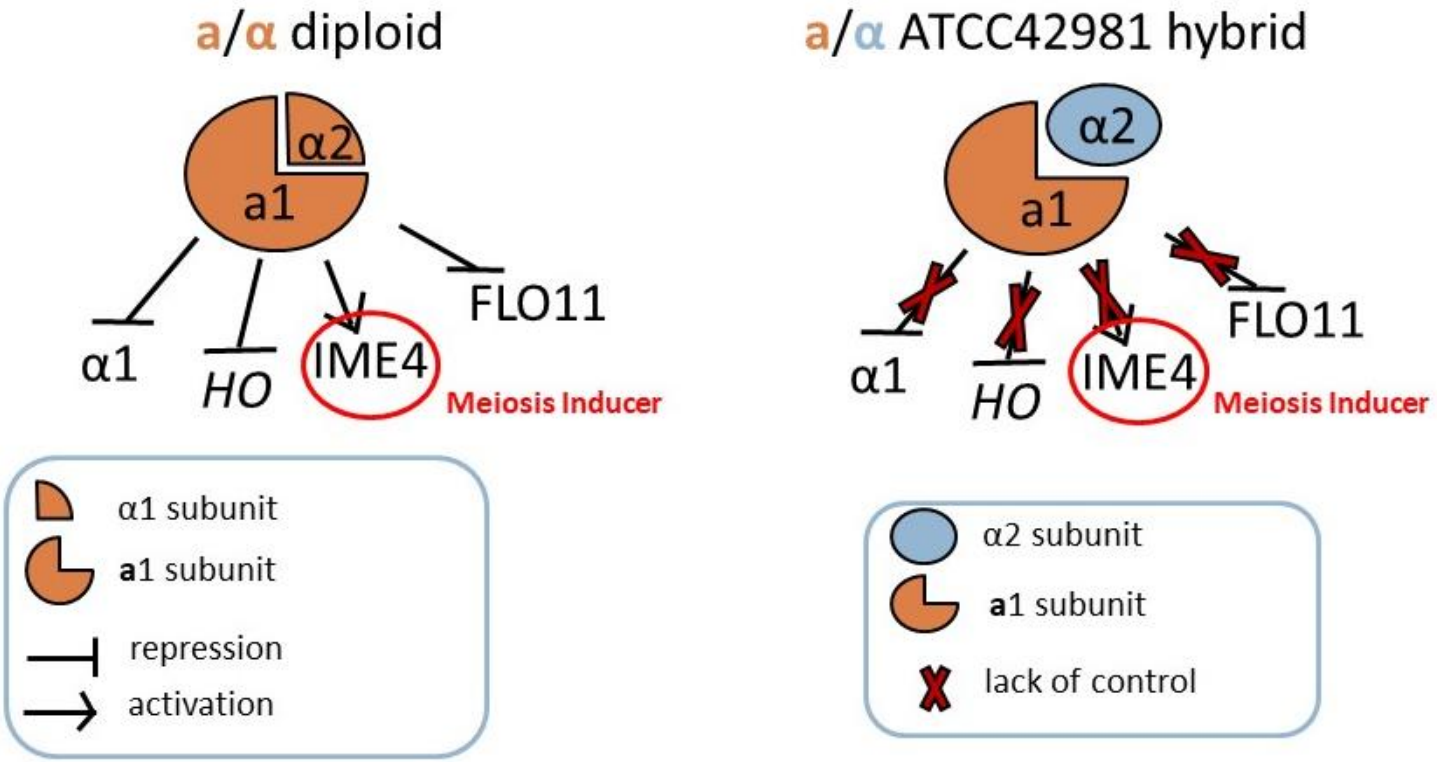
T Sub-genome

100% identical to *Z. rouxii* type-strain CBS 732^{T}

P Sub-genome

15% divergent to *Z. rouxii* type-strain CBS 732^{T}

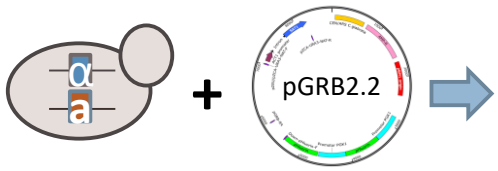
2.2 Putative molecular mechanism of defective mating

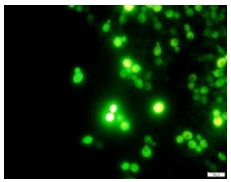


The different phylogenetic origin of **a**1 and α2 heterodimer subunits could generate negative epistasis accounting for ATCC 42981 infertility and dysregulation of cell identity.

3. Experimental strategy

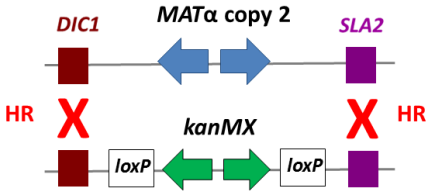
1. • Optimization electroporation protocol to transform *Zygosaccharomyces*

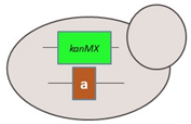




Fluorescent transformed cells

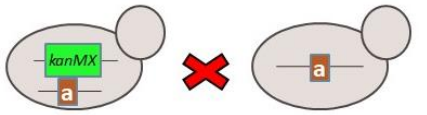
2. • Targeted *MATα* disruption by *loxP-kanMX-loxP* cassette and construction of Δ*MATα* mutants

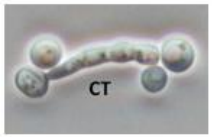




KanMX resistance interated in the genome

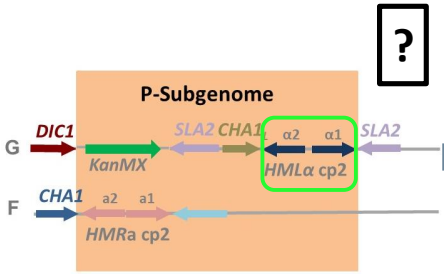
3. • Analysis of phenotypic effects: salt-stress tolerance and fertility assays

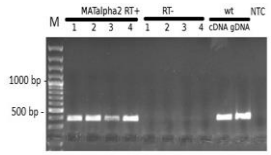




CT: conjugation tubes

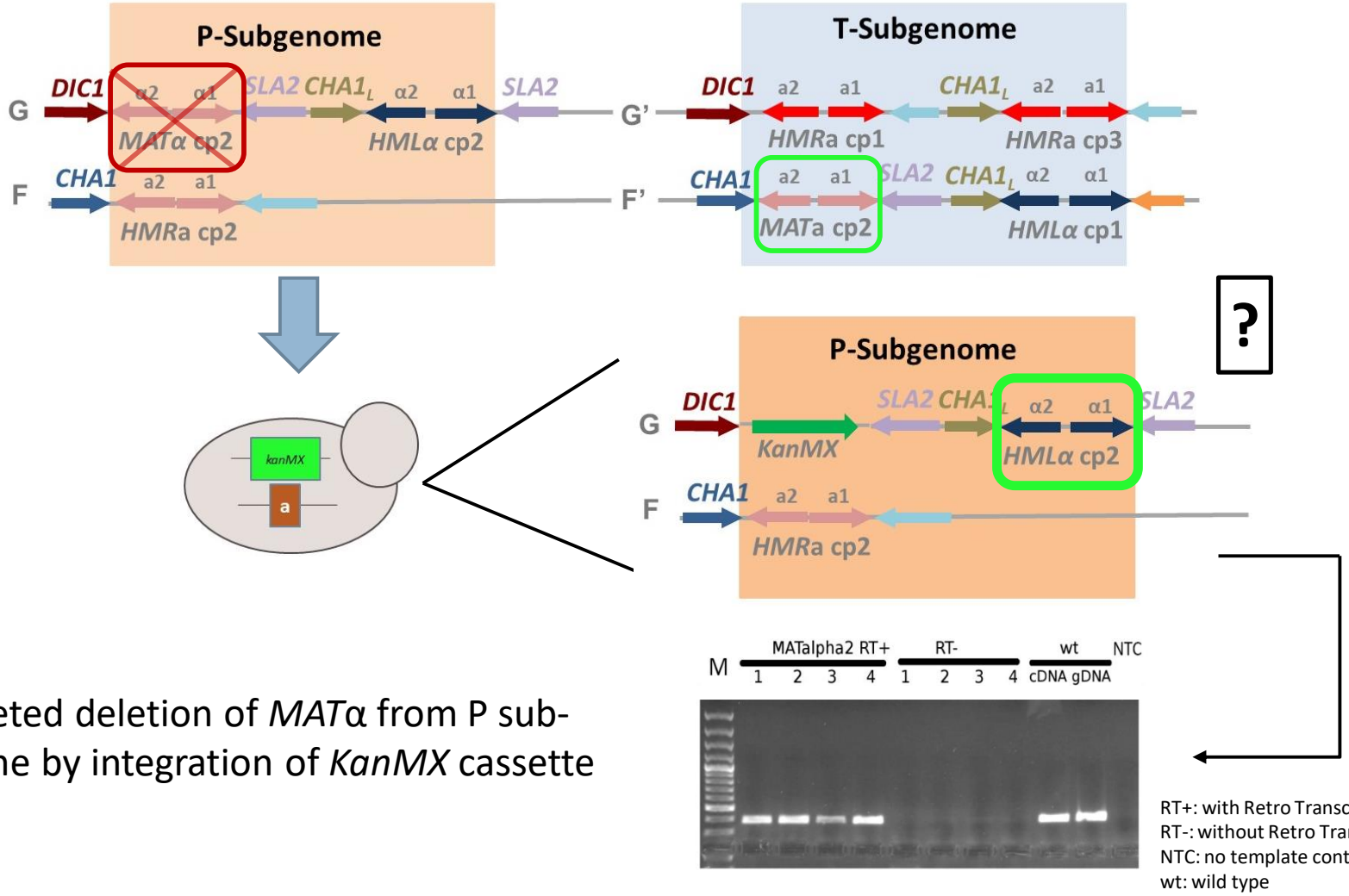
4. • Cell-identity verification: *MATα* and *HO* expression analysis





Unexpected *HMLα* copy 2 transcription

3. Goals achieved till now

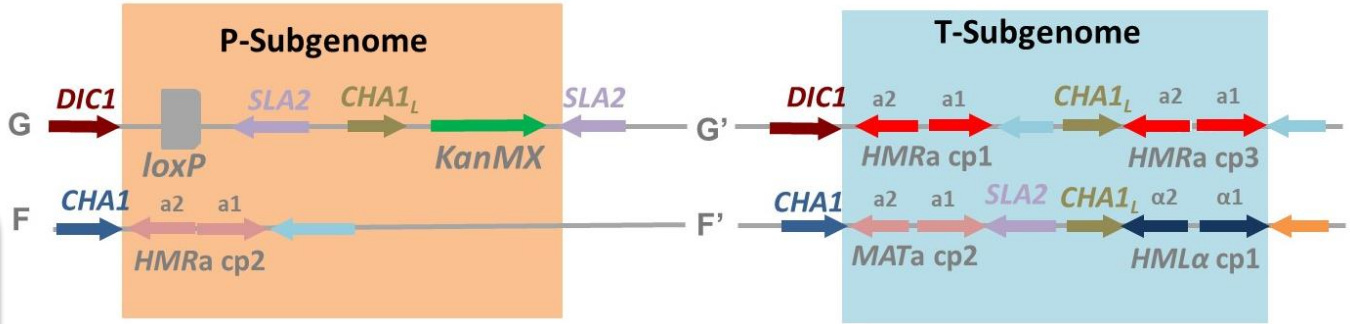


Targeted deletion of *MATα* from P sub-genome by integration of *KanMX* cassette

RT+: with Retro Transcriptase
RT-: without Retro Transcriptase
NTC: no template control
wt: wild type

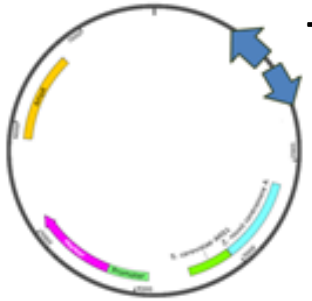
3. Next steps

Further deletion of P-type *HMLα* silent cassette



• 1

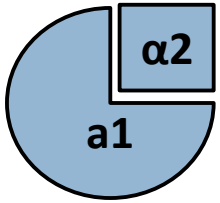
+



T- type *MATα*

Functional complementation of P-type *MATα* by a expression vector (episomal or centromeric) hosting T-type *MATα*

• 2



Phylogenetic congruent and functional heterodimer

Thank you for your attention!

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