Involvement of Yme1 Regulatory Proteins in F_1F_o-ATPase Assembly

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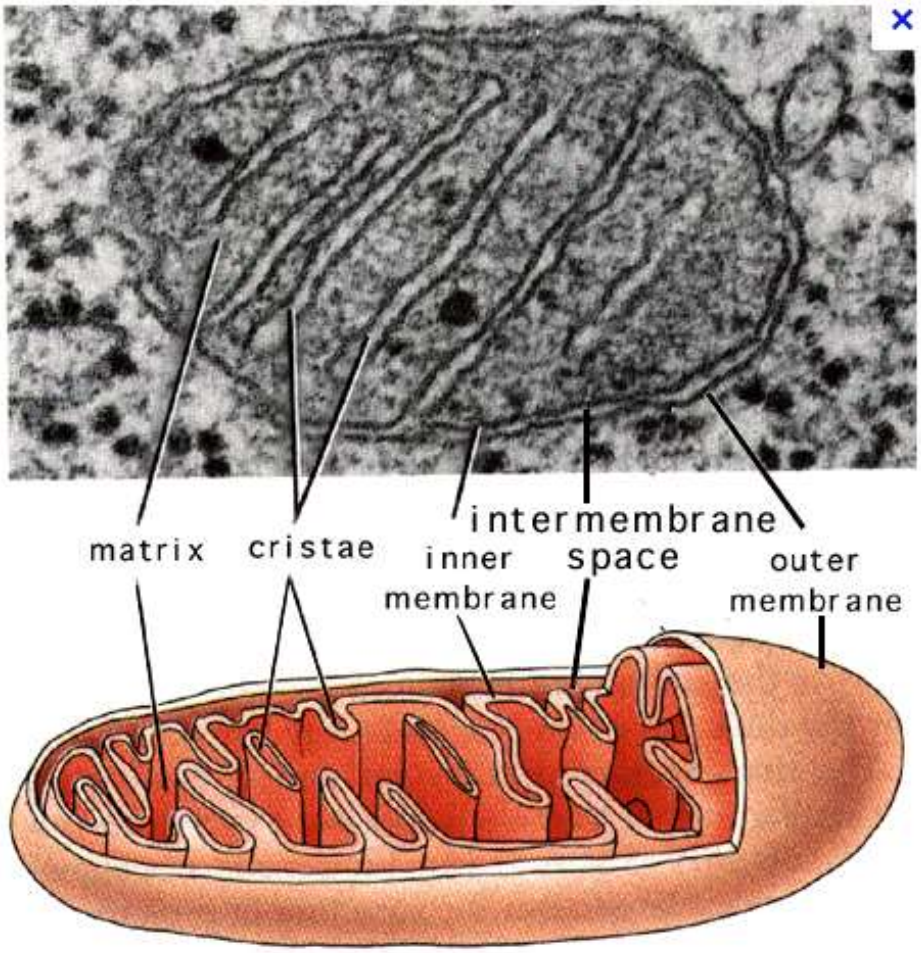
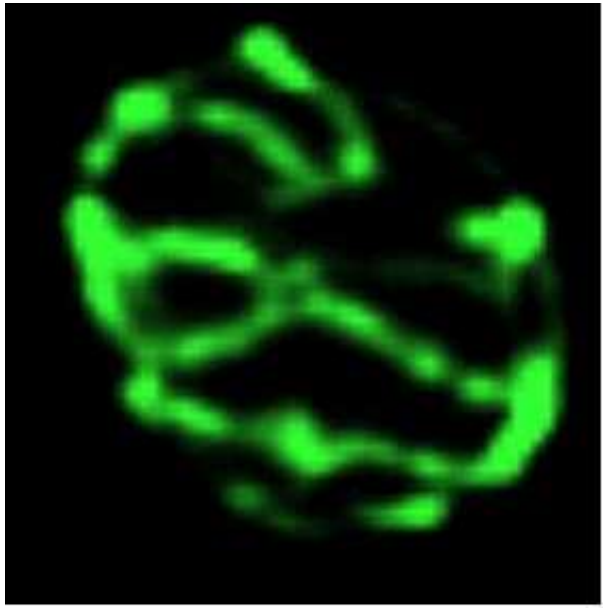
Mitochondria in yeast

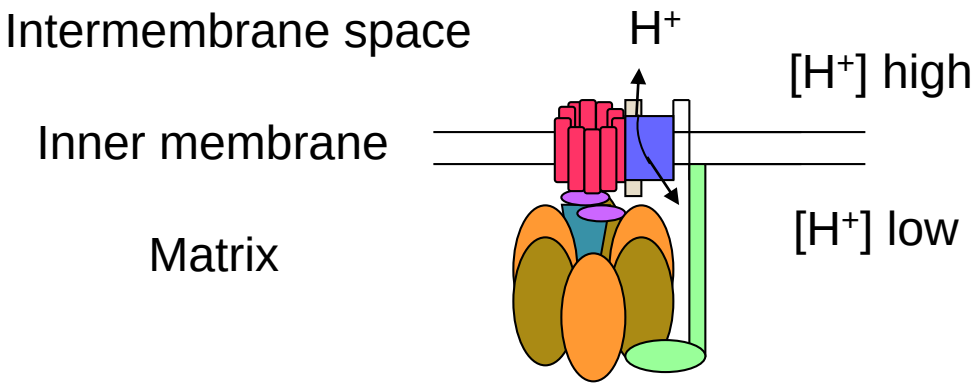


Mitochondrial compartments

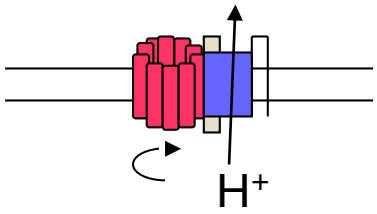


Reticulated yeast mitochondria

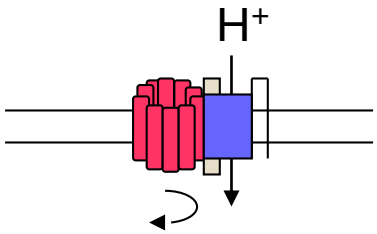
‘Nature’s marvelous motor’: ATP synthase = F_1F_o-ATPase



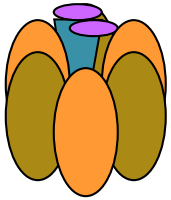
Rotor turns clockwise.
Every 120˚ , ATP → ADP + Pi
Growth by fermentation



Rotor turns anticlockwise.
Every 120˚ , ADP + Pi → ATP
Growth by respiration

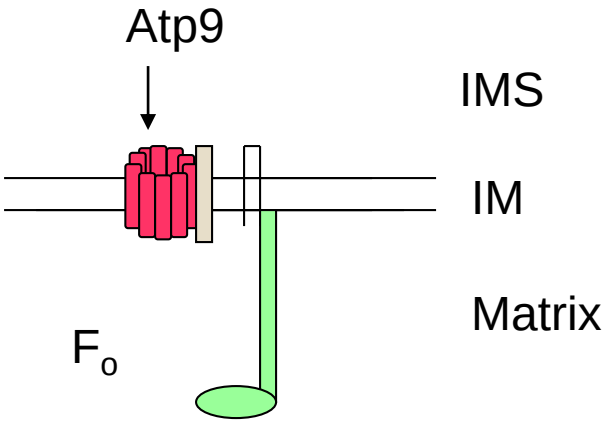


F_1F_o-ATPase can be separated into two components

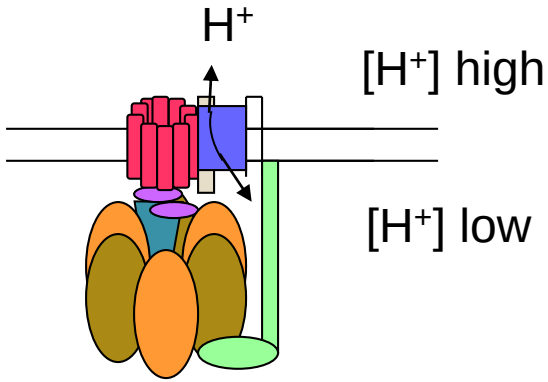


F_1-ATPase

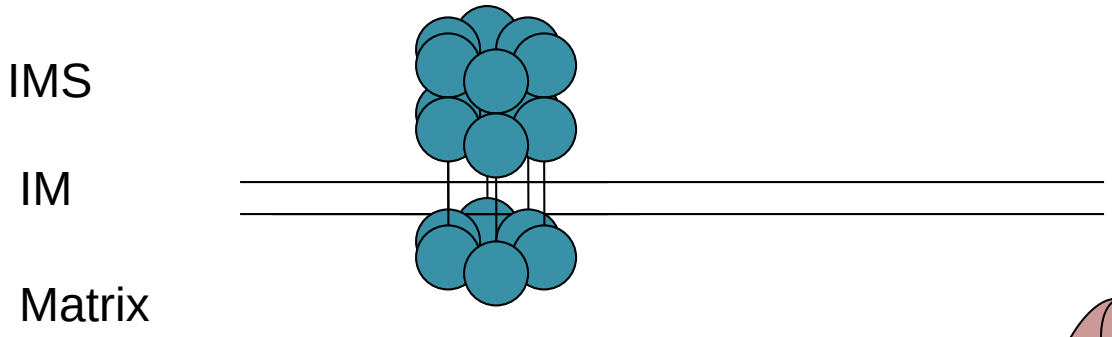
+



Yeast can grow in presence of F_1-ATPase and absence of F_o only in fermentation.

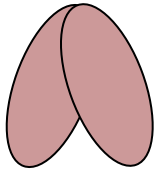


F_1F_o-ATPase



Yme1 homohexamer

Involved in quality control
for misfolded and
non-functional proteins



Hsc82 - Hsp82 homodimers
Hsp90 family - chaperone proteins

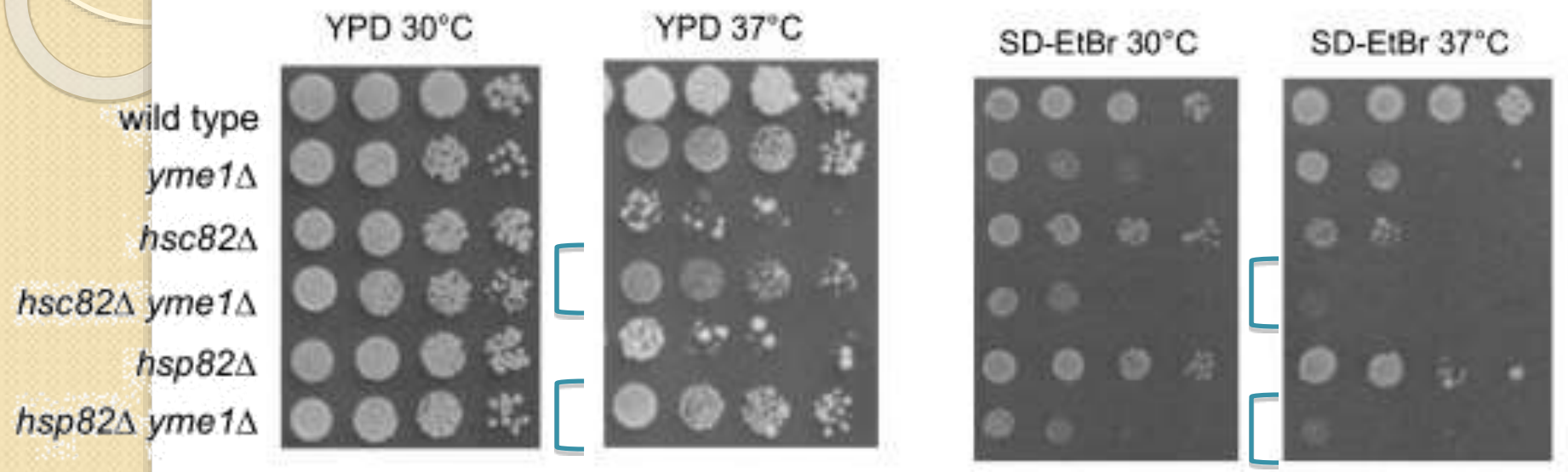
Involved in assembly of
multisubunit complexes

Yme1 and Hsc82/Hsp82 previously shown to be involved in assembly of F_1F_o-ATPase

(*Francis and Thorsness, 2011*)

Growth using F_1F_o-ATPase

Growth using F_1-ATPase



(Francis and Thorsness, 2011)

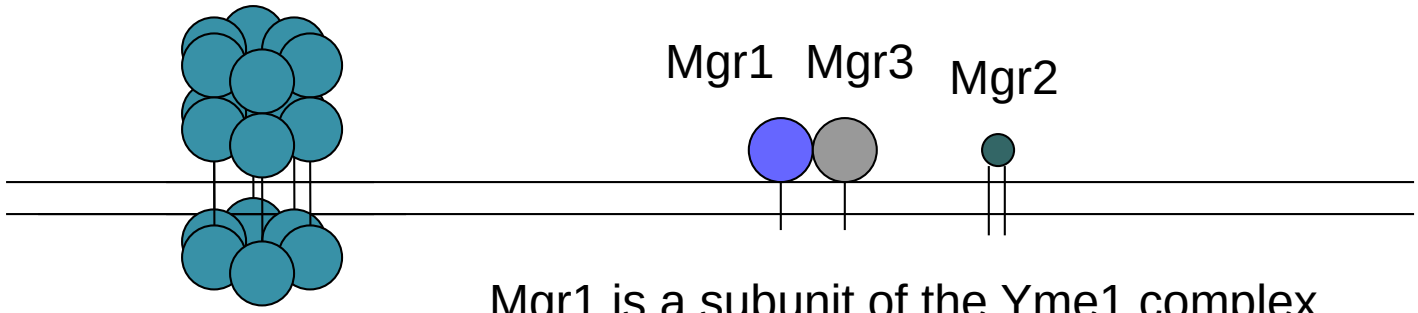
*yme1*Δ allows growth of *hsc82*Δ and *hsp82*Δ at 37° C using F_1F_o-ATPase

yme1 Δ *inhibits hsc82*Δ and *hsp82*Δ growth at 37° C using F_1-ATPase

IMS

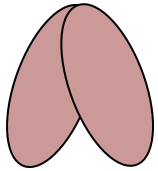
IM

Matrix



Mgr1 is a subunit of the Yme1 complex

Mgr1/Mgr3 form a subcomplex



Hsc82/Hsp82 homodimers

Objective: To determine whether loss of Mgr1, Mgr2, or Mgr3 has the same effect on growth of strains lacking Hsc82 or Hsp82 as loss of Yme1

Procedure :

Step 1.

Make single mutant strains lacking *MGR1*, *MGR2*, or *MGR3* in the Thorsness lab wild type strain.

Step 2.

Make double mutant strains lacking *MGR1*, *MGR2*, or *MGR3* and *HSC82* or *HSP82*.

Step 3.

Test by dilution assays whether loss of *MGR1*, *MGR2*, or *MGR3* allows growth by fermentation of strains lacking *HSC82* or *HSP82*

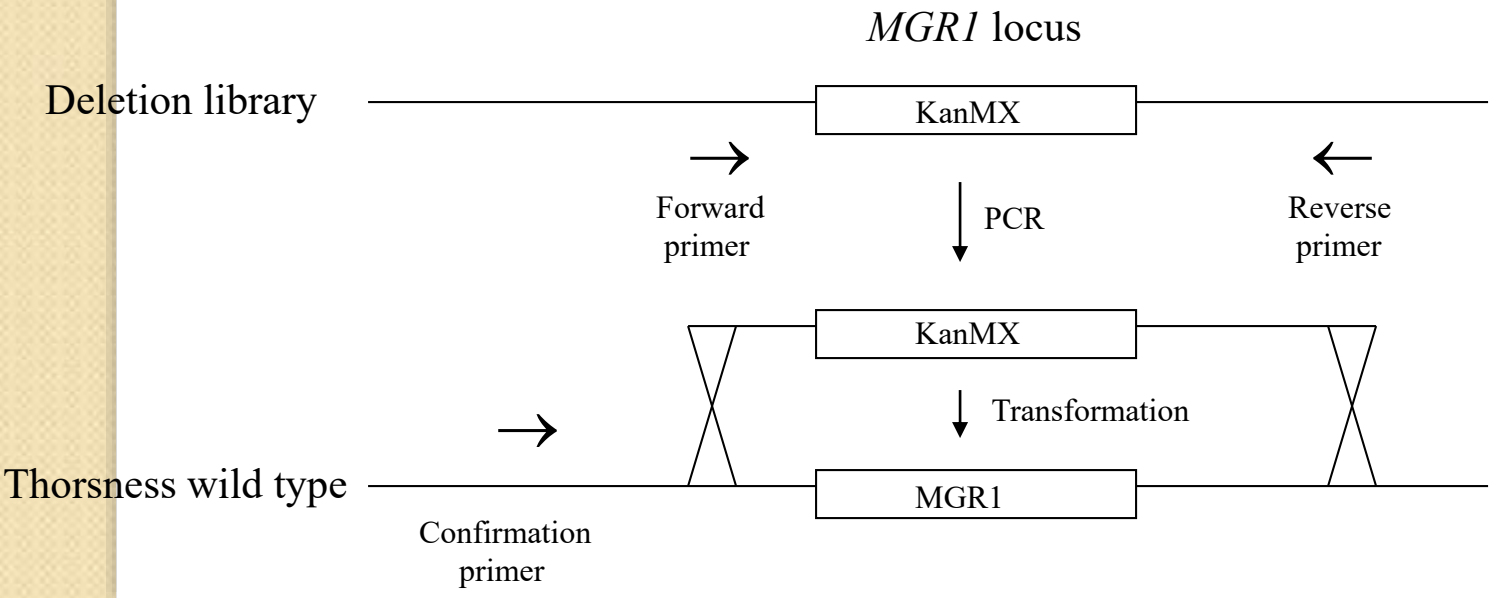
Step 1. Make yeast strains lacking genes for *MGR1*, *MGR2*, and *MGR3*

Deletion library has *MGR1*, *MGR2*, or *MGR3* replaced by the Kanomycin resistance gene.

Library background strain is different from Thorsness wild type.

Therefore, need to make deletion mutants in Thorsness wild type

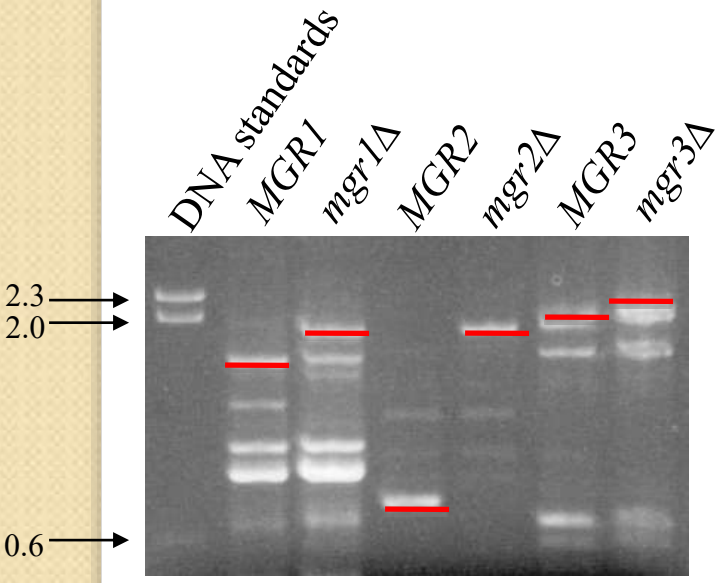
1. Use PCR to amplify DNA segments containing the Kanomycin resistance genes in the three library strains.
2. Transform Thorsness wild type strain.
3. Confirm transformations are correct with PCR.

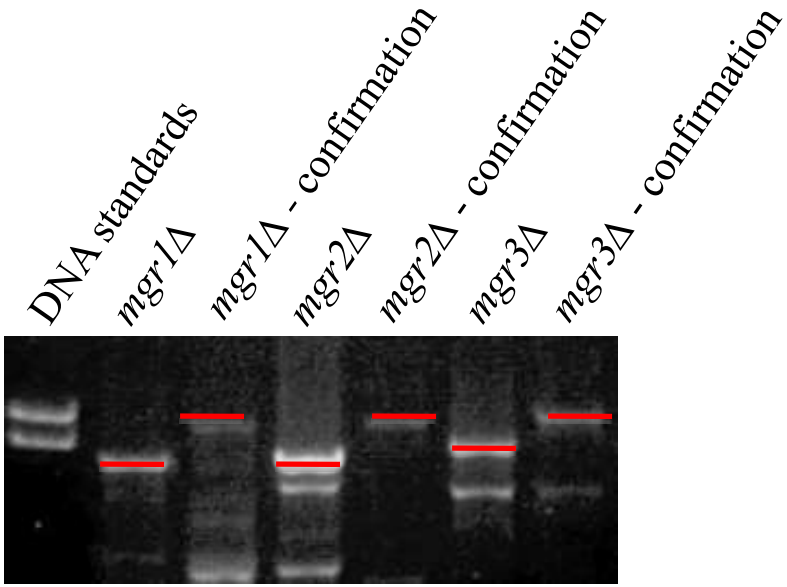


PCR amplification of deletion library and transformed strain DNA

Wild type and deletion library genomic DNA
+ forward and reverse primers

Wild type transformed strain genomic DNA
+ forward and reverse primers
or forward confirmation and reverse primers





Transformed strains have the kanomycin resistance gene incorporated at the correct locations

Sporulation in yeast - background

Single mutant haploid Mating type α aB

n

n

DNA replication

2n

Diploid

4n

Meiotic division I

2n

2n

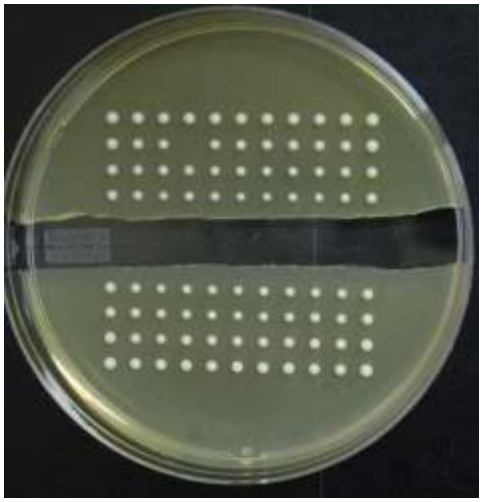
Meiotic division II

4 haploid spores = tetrad

n n n n

Single mutant haploid, mating type a Ab

wild type x wild type



n n n n

α α a a

AB aB Ab ab

wild type

single mutants

double mutant

Step 2 - make double mutants by crossing *mgr1Δ*, *mgr2Δ* or *mgr3Δ* strains with *hsc82Δ* or *hsp82Δ* strains

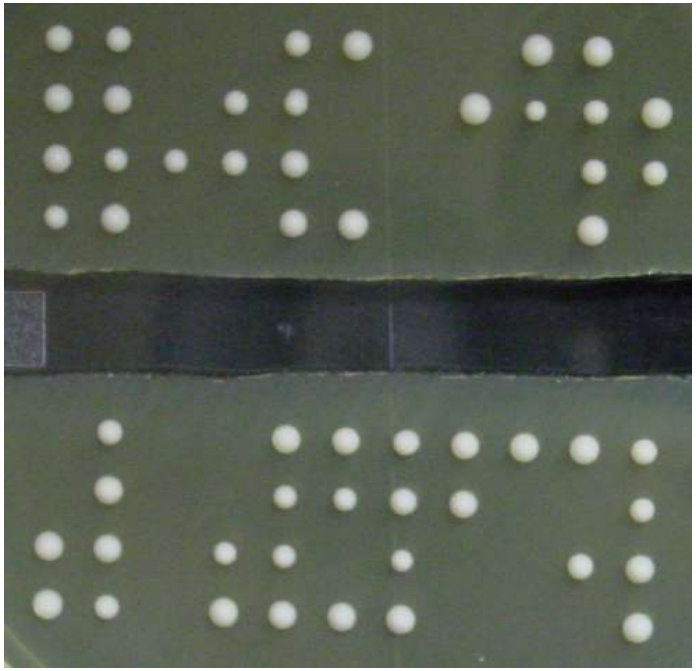
1. Mix α (*mgr1Δ*, *mgr2Δ* or *mgr3Δ)* and a (*hsc82Δ* or *hsp82Δ)* haploid strains to make diploids

2. Isolate and dissect tetrads.

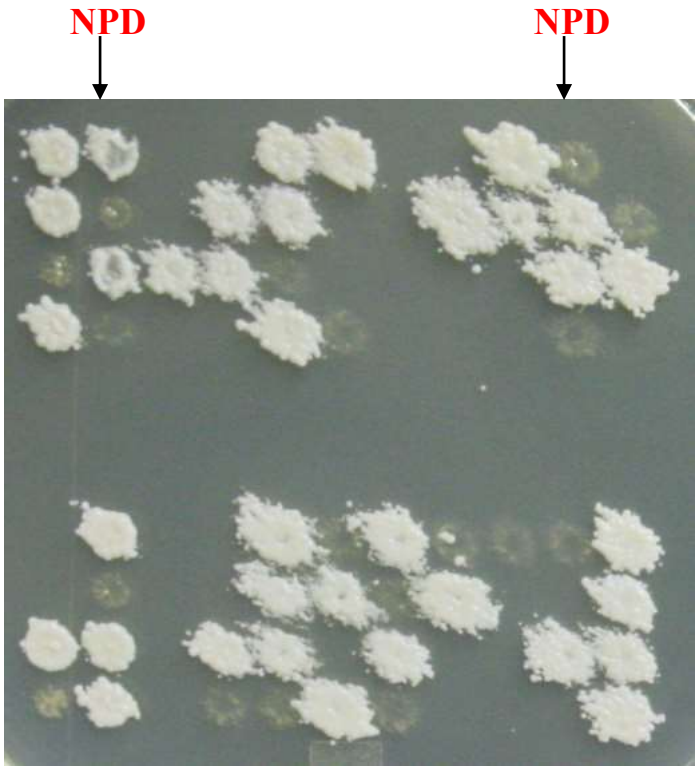
3. Screen for tetrads containing 2 kan+ and 2 kan- colonies (NPDs)
   - kan+ colonies must contain both mutations

Non-parental ditypes have double mutants

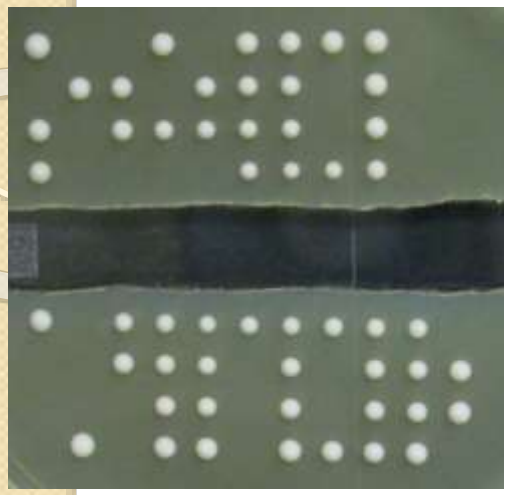
mgr1Δ x hsc82Δ
all colonies



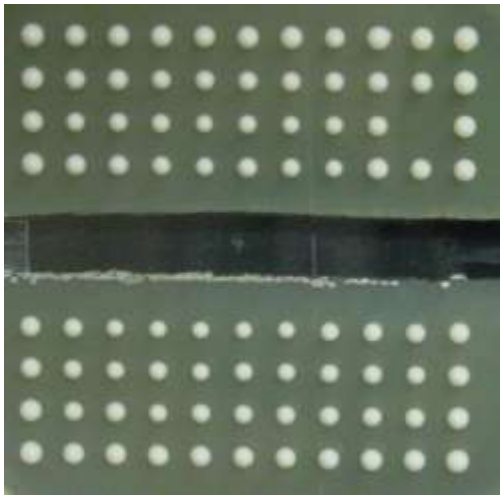
mgr1Δ x hsc82Δ
screen for kanomycin resistance



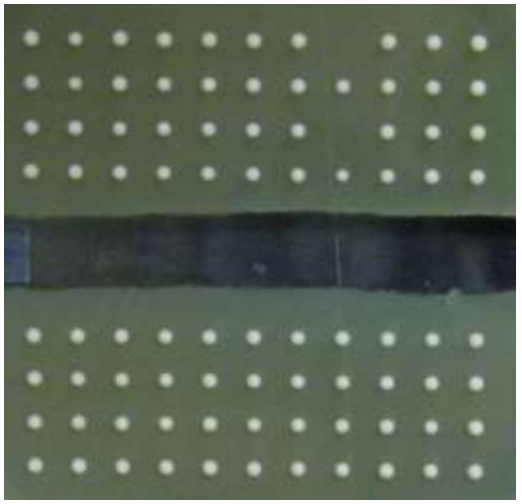
mgr1Δ x hsp82Δ



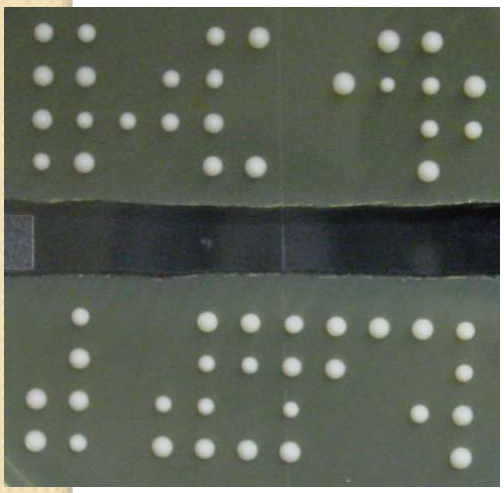
mgr2Δ x hsp82Δ



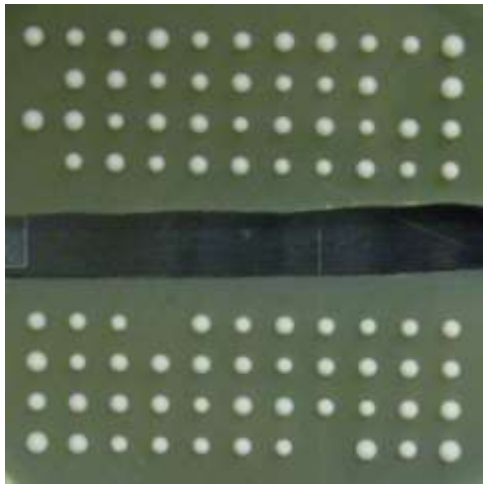
mgr3Δ x hsp82Δ



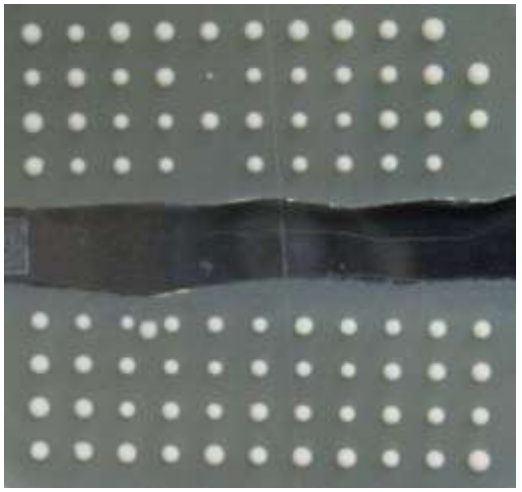
mgr1Δ x hsc82Δ



mgr2Δ x hsc82Δ

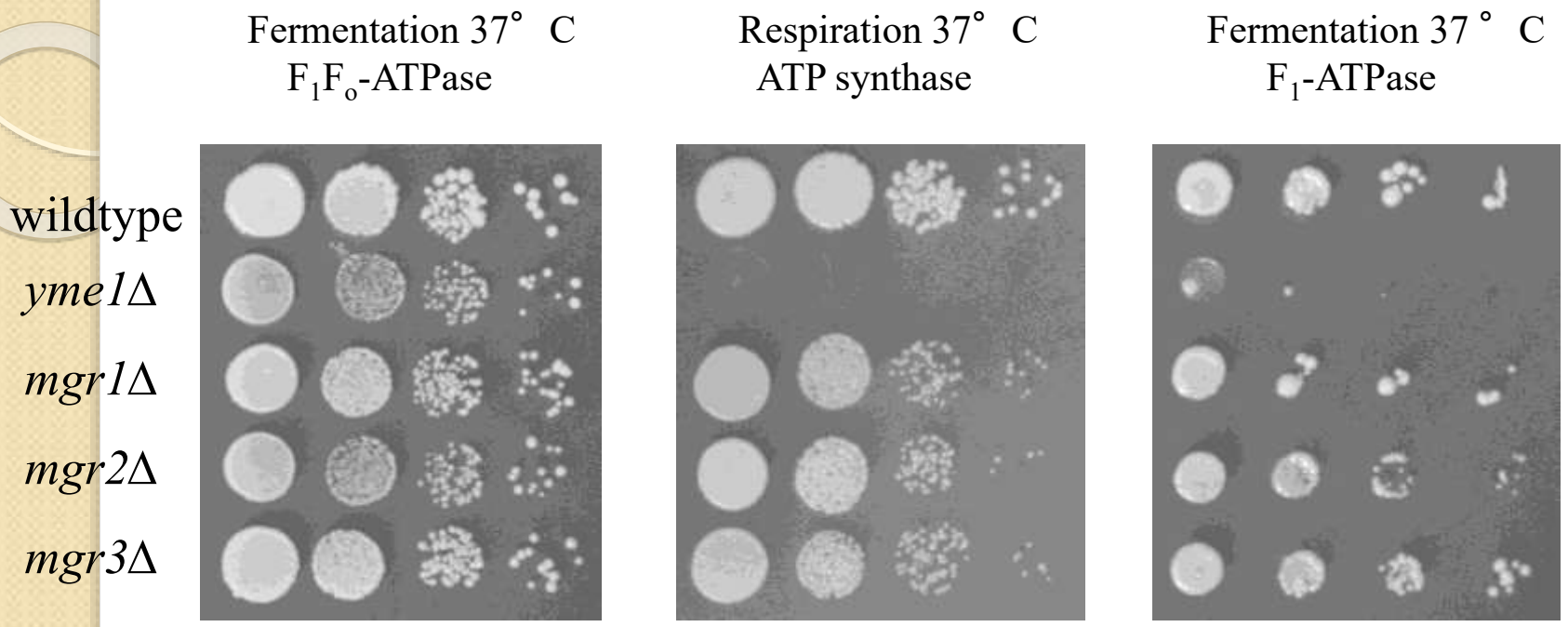


mgr3Δ x hsc82Δ

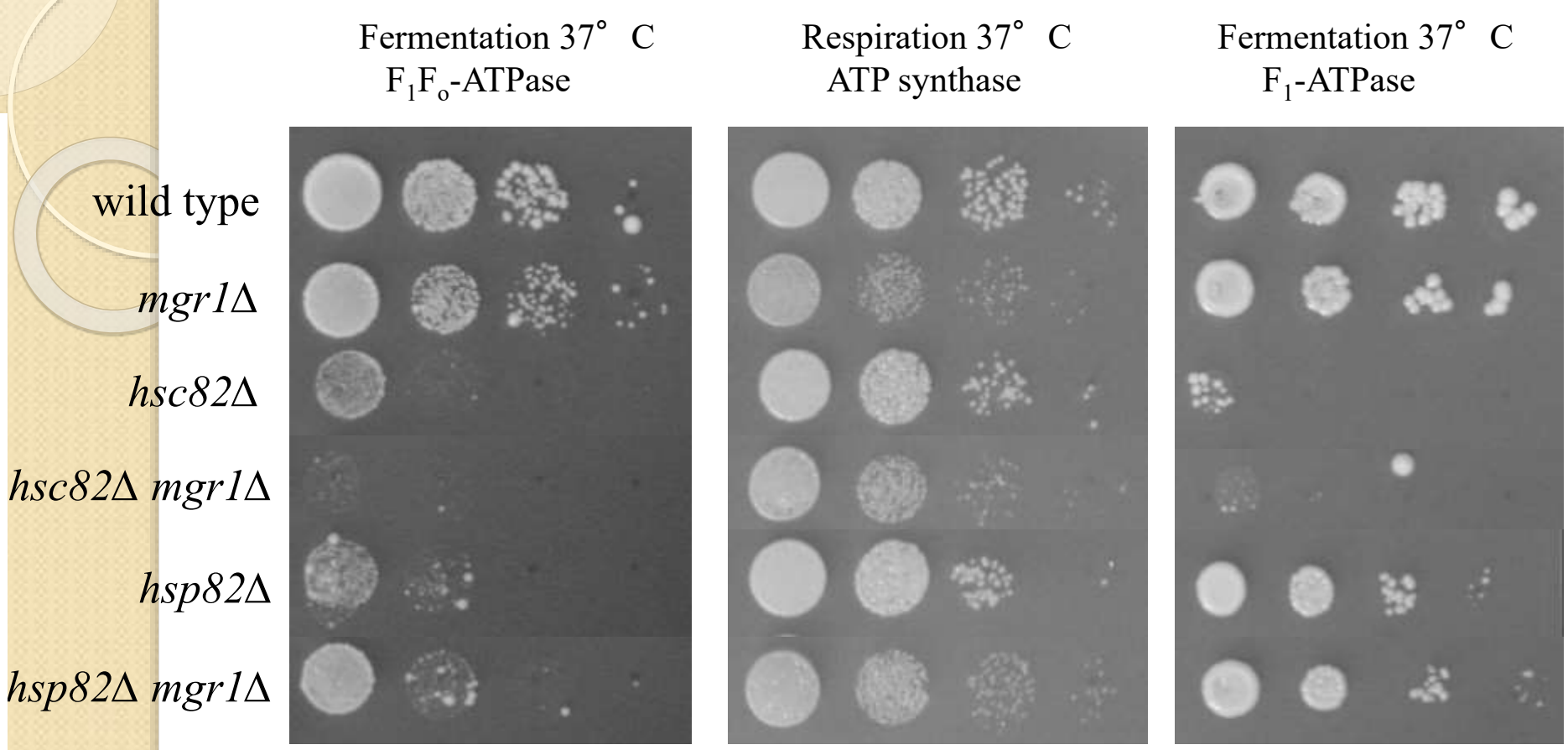


Loss of spore viability in crosses with *mgr1Δ*

Step 3 - dilution assays for growth on different media at 37° C



The first dilution performed was to compare the single deletions of *MGR1, MGR2*, and *MGR3* to the deletion of *YME1.*



Loss of Mgr1 did not increase growth of strains lacking Hsc82 or Hsp82 similar to loss of Yme1

Fermentation 37° C
F_1F_o-ATPase

Respiration 37° C
ATP synthase

Fermentation 37° C
F_1-ATPase

wild type

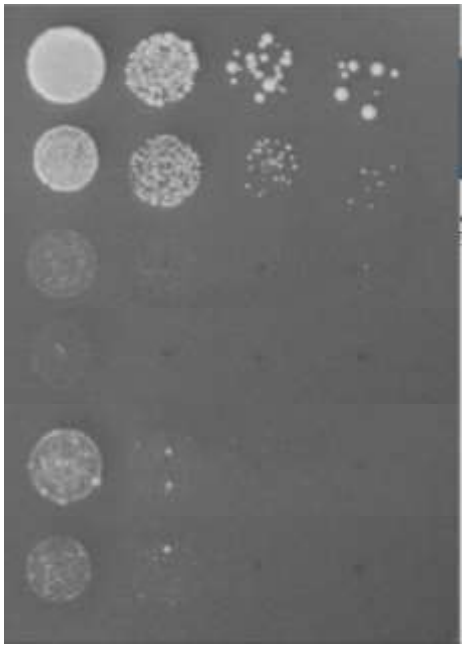
mgr2Δ

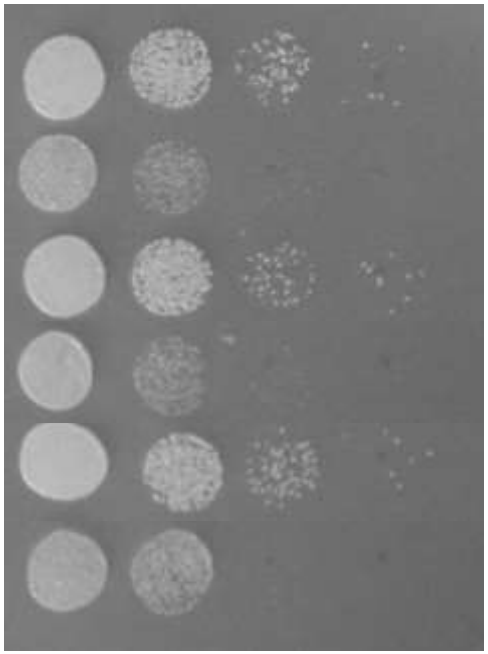
hsc82Δ

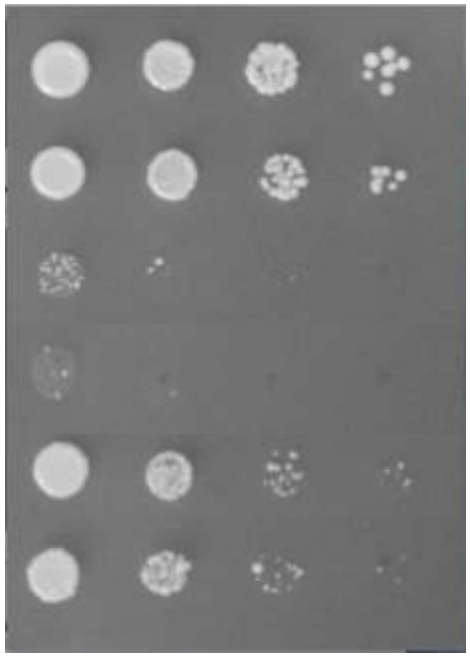
hsc82Δ mgr2Δ

hsp82Δ

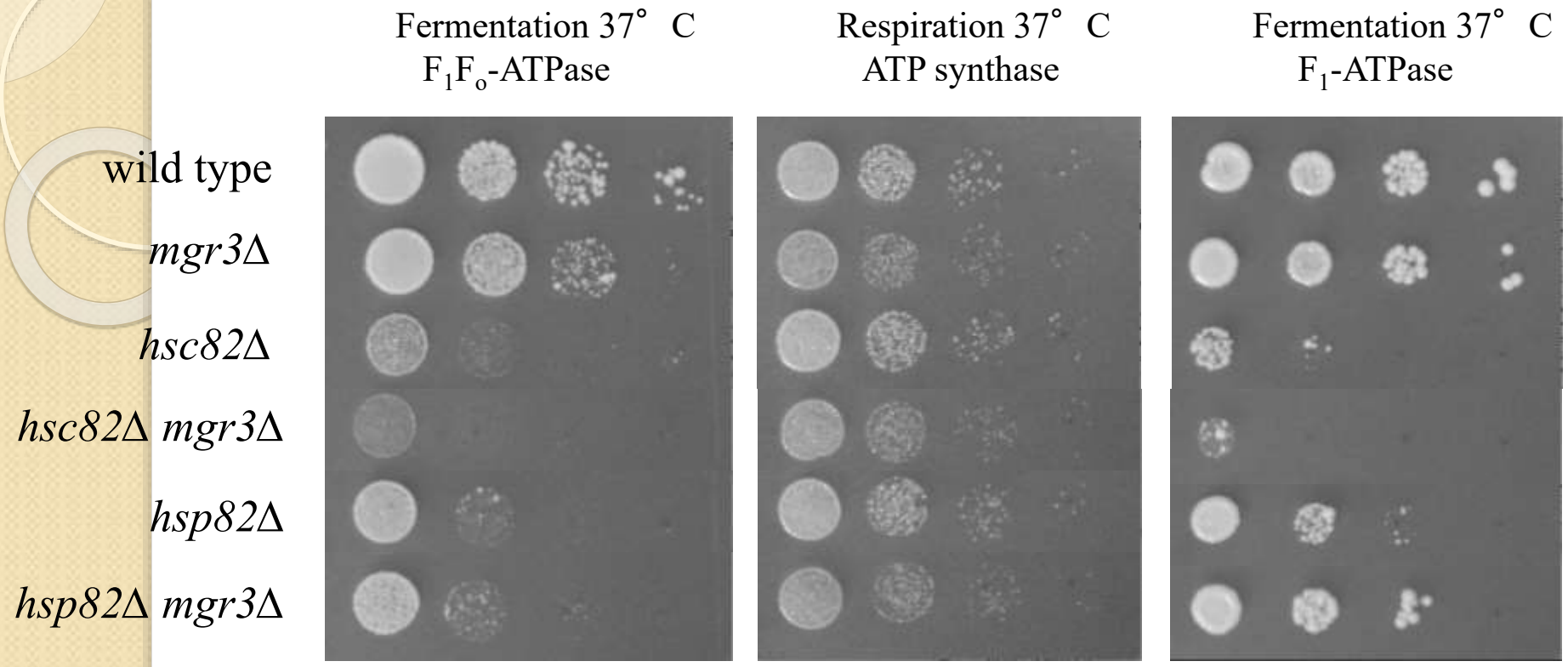
hsp82Δ mgr2Δ







Loss of Mgr2 did not increase growth of strains lacking Hsc82 or Hsp82 similar to loss of Yme1

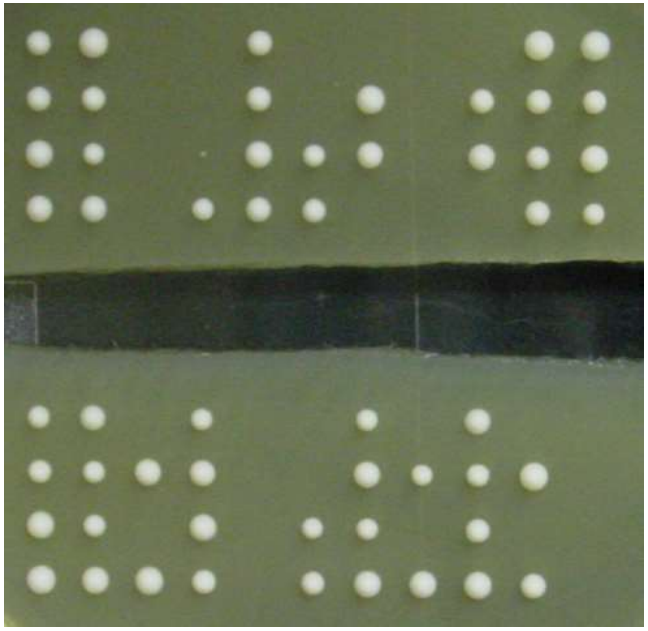


Loss of Mgr3 did not increase growth of strains lacking Hsc82 or Hsp82 similar to loss of Yme1

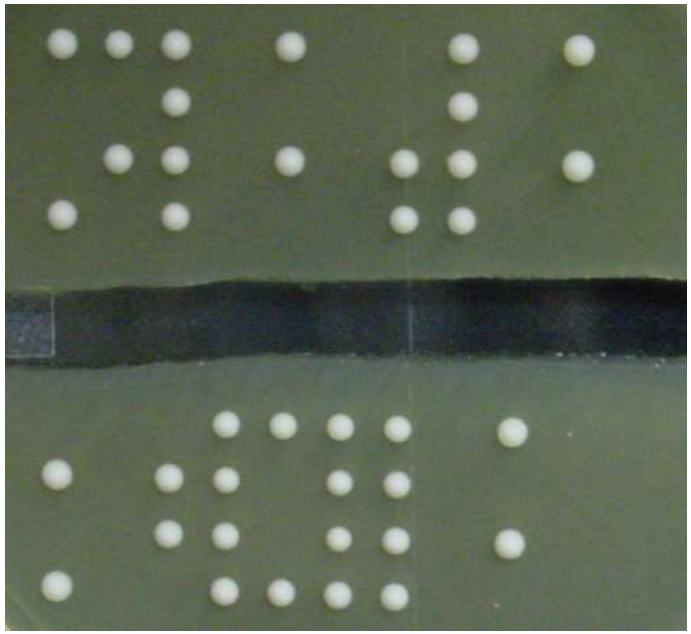
Loss of spore viability from crosses involving *mgr1Δ*

Unusual observations:

mgr1Δ HSC82 x *MGR1 hsc82Δ*



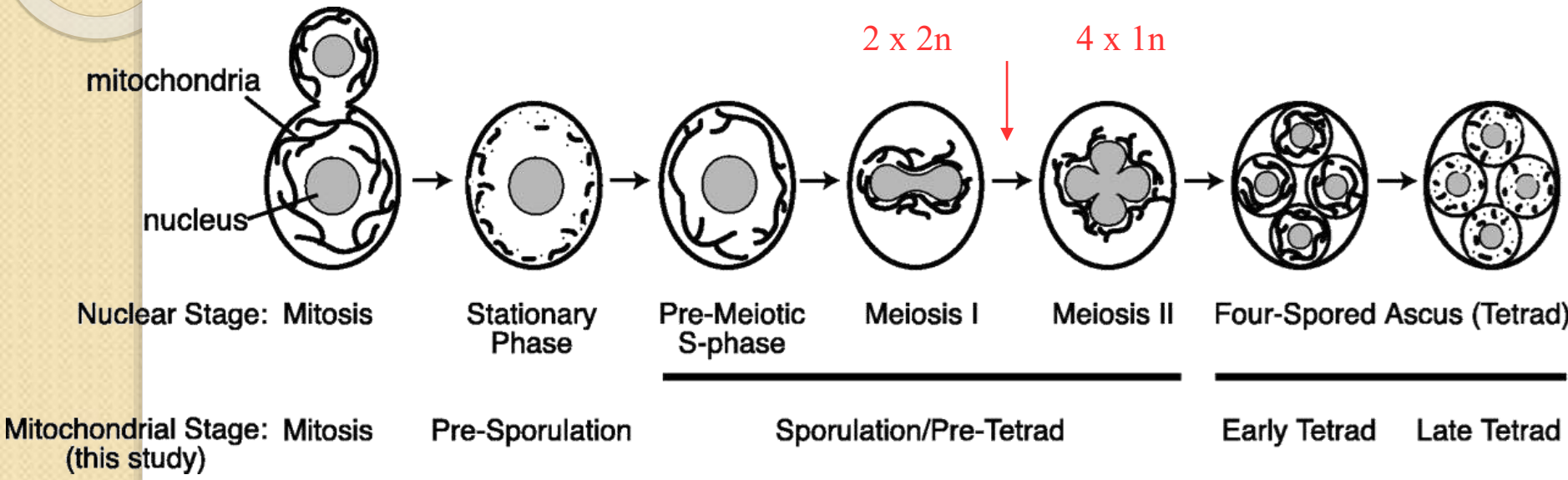
mgr1Δ HSP82 x *MGR1 hsp82Δ*



1. Reduced spore viability
2. Sporulation produces tetrads with mostly 0, 2, or 4 viable spores

Mgr1 may have a role in organization/assembly of F_1F_o-ATPase in mitochondria of spores

Figure 1. Mitochondrial dynamics during meiosis and sporulation in budding yeast.



Gorsich S W , Shaw J M, Mol. Biol. Cell 2004;15:4369-4381

Effect of *mgr1*Δ occurs after Meiosis I
- observation unlike anything previously reported

Conclusions

1. Deletion of Mgr1, Mgr2, or Mgr3 does not permit growth of strains lacking Hsc82 and Hsp82 at 37° C on fermentation media. Therefore, not the same effect as loss of Yme1.

2. Mgr1 may play a role with Hsc82/Hsp82, in creating spores that can germinate on fermentation media, perhaps in assembly/organization of F_1F_o-ATPase in spores.

Thanks to Peter Thorsness and Brian Francis