

Sporulation of *S.cerevisiae*

Production of diploid strains

- always start with freshly grown cells !!!

Method 1 (mexican hats):

- mix strain 1 and strain 2 in a drop of sterile H₂O on a YPD plate
- incubate for 4-6 h at 30°C
- check under microscope for zygotes with first bud (mexican hats)
- put mated cells on tetrad plate and separate zygotes
- incubate at 30°C for 2-3 days
- check colonies for mating type (nonmaters are diploids)

Method 2 (manual mating):

- put both strains separately on the same tetrad plate
- separate pairs of cells (one from each strain) so that they are touching
- incubate for 2-3 days at 30°C
- test colonies for mating type (nonmaters are diploids)

Method 3 (with markers):

- if the strains carry mutations in different marker genes, diploids can be selected on drop out media lacking both amino acids
- mix strain 1 and strain 2 in a drop of sterile H₂O on a YPD plate
- incubate over night at 30°C
- streak cells on double drop out plates
- test single colonies for mating type (diploids do not mate)

Sporulation of diploid *S.cerevisiae* strains

- media:
 - YPAc
 - SPM⁺

- incubate diploid cells in 3 ml YPAc over night at 30°C
- spin down YPAc culture for 5 min at 3500 rpm
- wash pellet in 2 ml SPM⁺
- resuspend pellet in 1.5 ml SPM⁺
- incubate at least 24 h at 30°C (up to 5 days)
- check for sporulation under microscope

Tetrad dissection

- zymolyase solution:
 - 1 mg zymolyase T20 (=20 U/ml)
 - 0.5 ml 2 M sorbitol (=1 M)
 - 0.5 ml H₂O

- material:
 - tetrad plates: YPD with high quality pure agar

- spin down 200 µl sporulated cells (eppendorf centrifuge)
- resuspend in 50 µl zymolyase solution
- incubate for 10 min at 37°C
- add 0.8 ml sterile H₂O
- put 10 µl on tetrad plate
- separate spores
- incubate at 30°C for 2-3 days

Mating type determination

- prepare tester plates:
 - incubate tester strains WDHY17 (MAT_a) and WDHY18 (MAT_a)
in 5 ml YPD over night at 30°C
 - plate onto YPD plates to get a regular lawn and incubate over night at 30°C
 - store tester plates at 4°C

- mating type determination:
 - replica plate the strain to be determined on to two YPD plates
(marked with 17 and 18)
 - replica plate onto the same plates the respective tester strain
 - incubate at 30°C over night (mating)
 - replica plate the mated cells onto SD -all
 - strains that mate with WDHY17 are MAT_a and
strains that mate with WDHY18 are MAT_a.