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 (MATα) and WDHY18 (MATα) 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 MATα and strains that mate with WDHY18 are MATa.