# Sporulation and Dissection of Yeast a/alpha Diploids

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These conditions work well for the Hieter yeast strains

1. Isolate diploid colonies. Inoculate 2 ml YPD + Ade with a single, large diploid colony and grow O/N at 30 degrees C to stationary phase.

2. Put 200 ul of stationary phase cells into 5 ml sterile, distilled H2O. Spin down 2 min. in clinical centrifuge. Wash again with 5 ml H2O.

3. Resuspend in 2 ml 0.5% (50 mM) KOAc (pH 7.0) + 0.5X nutrients for auxotrophic markers (usually Ade, Ura, Trp, Leu, His, Lys, Met).

4. Incubate on tube roller at rm. temp. for 3-7 days.

5. Check for sporulation under microscope. Spin down tetrads in a small glass tube and wash 3 times with 5 ml sterile H2O. Resuspend in 2 ml H2O.

6. Spin down 180 ul cells. Resuspend in 90 ul Zymolyase, 0.5 mg/ml in 1M sorbitol. Incubate at 30 degrees for 5-10 minutes.

7. Slowly add 0.3 ml H2O on ice to stop the reaction.

8. Plate 30 ul cells onto YPD + Ade, Trp, Ura and dissect.