yMAT_1v1

Random Spore analysis in 96 Well Format (UCSF)

SET-UP:

- Streak out Kan strains to test on YPAD + G418 in 48 well plates
- Prepare NAT KO strain with haploid selection markers and selection against the diploid.
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STAGE1:

- Mate strains on 48 well plates. Leave at RT overnight.

STAGE2:

- Streak onto diploid selection: YPAD+G418+NAT in 48 well plates and incubate at 30ºC overnight.

STAGE3:

- Streak onto pre-SPO in 48 well plates, and incubate at 30ºC overnight

STAGE4:

- transfer to liquid spo in 98 well format and incubate for 5 days at 22ºC-30ºC. Cells must be well aerated for this stage.

STAGE5:

1. Take 20μl from the Spo liquid and transfer to a 96well PCR plate.
2. Spin down (1minute 2500rcf) and remove liquid.
3. Re-suspend in 20μl Zymolase (5μg/μl in 1Msorbitol and 10mM Tris 7.5)
4. Incubate for 45min at 30ºC (best in PCR machine)
5. Add 200μl 1M sorbitol to stop reaction and keep at 4ºC until plating.
6. Plate onto each single and the double selection plate with appropriate selection against the diploids and appropriate selection for one of the mating types and count differences in colony number and size. Take care to choose the right media at this stage. For example, we use SD-LEU (To select for the alpha haplotype) –LYS +S-AEC (to select against the diploid) –ARG +Can (To select against the diploid) and add Nat, Kan or Nat + Kan.

Media as used for the Synthetic lethality screens