

SGA recipes

(need ~ 1 to 2 liters plates at each step = 20 to 40 plates)

Step 1: Pin the query. Also pin the Deletion Mutant Array (DMA) set (from refrig or -80).

Step 2: Mating: Query X DMA set.

Steps 1 and 2- NEED ~ 60 YPD OMNITRAYS TO GET THROUGH MATING STAGE.

YPD is 20g Peptone
10g Yeast Extract
20g Agar
900 ml H₂O

- Autoclave
- Add 100 ml 20% glucose
- Pour ~ 20 omnitrays per liter

Step 3: Diploid Select. Need ~ 1L (20 plates) of YPD + G418 + clonNAT. Recipe is same as above, except just prior to pouring add 200 mg/L of G418 **and** 1.0 ml of 100mg/ml NAT.

clonNAT stocks as follows (Amberg's recipe):

1. Add 0.5 g of NAT to 5.0 ml of sterile ddH₂O. Mix briefly.
2. filter sterilize
3. dispense into 1.0 ml sterile microfuge tubes.
4. store @ -80C.

Step 4: Sporulation- Need ~ 1L (20 plates) of Sporulation Plates (recipe- page 2)

Steps 5 + 6*: MATa Haploid Select- Need ~ 2L (40 plates) of H-A- Can plates (recipe- page 3)

Step 7*: Haploid Select + G418- Need ~ 1L (20 plates) of H-A- Can plates + G418 (page 3)

Step 8: Double Mutant Select- Need ~ 1L (20 plates) of H-A- Can plates + G418 + clonNAT (3)

H-A- Drop out Mix contains:

Ade	2.4g	Tyr	3.6g	Thr	24.0g
Ura	2.4g	Leu	7.2g	Asp	12.0g
Trp	2.4g	Lys	3.6g		
Met	2.4g	Phe	6.0g		

***SGA Hail Mary Option:** Omit Steps 5, 6 and 7! Pin directly from Step 4 (Spo) to Step 8 (dm).

SIMCHEN'S SPO MEDIUM (From Amberg)

1. In a 2 L flask add:
 - 900 ml ddH₂O (will add 100 ml 1X AA stock after autoclaving)
 - 15 g Potassium Acetate (1.5%)
 - 2.5 g Yeast Extract (0.25%)
 - 0.5 g Dextrose (0.05%)
2. Stir 30 min. (pH is reduced from ~ 8.0 to ~7.0 by Yeast Extract)
4. Add 20 g agar.
5. Autoclave 20'.

6. Add 1X Amino Acid Stock: 100 ml!!

7. Pour omnitray plates.

1X Amino Acid Stock:

	<u>100 ml</u>	<u>500 ml</u>
Adenine	40 mg	200 mg
Arginine	40 mg	200 mg
Histidine	20 mg	100 mg
Leucine	20 mg	100 mg
Lysine	20 mg	100 mg
Methionine	20 mg	100 mg
Threonine	350 mg	1.75 g
Tryptophan	20 mg	100 mg
Tyrosine (@4C)	40 mg	200 mg
Uracil	40 mg	200 mg

→ddH₂O to volume indicated; *filter sterilize. Store @ 4°C.*

STEPS 5 AND 6: His- Arg- Can Plates- S.C. (Boone protocol)

1000mL

Agar	21g!
Yeast Nitrogen Base without amino acids	6.7g
H-A- drop out mix (page 1)	2.0g
ddH ₂ O	900ml

NaOH pellet- add one only (brings final plate pH to 5.0)- Check pH/ agar rigidity after autoclaving

-> **Autoclave NOT MORE THAN 20 MINUTES!! REMOVE PROMPTLY AT END OF CYCLE (30 MINUTES)!**

- ➔ Add 100mL 20% glucose (2% final conc.) *immediately*
- ➔ When COOL, add:
 - 0.7 ml Canavanine (100 mg/ml in sterile H₂O)
 - 0.17g 3-AT (3-aminotriazole)

STEPS 7 AND 8: His- Arg- Can Plates + G418 (+ NAT)-S.C. (Boone protocol)

1000mL

Agar	21g!
<i>Yeast Nitrogen Base without amino acids, and without NH₄SO₄</i>	1.7g
Monosodium glutamate	1.0g
H-A- drop out mix (page 1)	2.0g
ddH ₂ O	900mL

NaOH pellet- add one only (brings final plate pH to 5.0)- Check pH/ agar rigidity after autoclaving

-> **Autoclave NOT MORE THAN 20 MINUTES!! REMOVE PROMPTLY!**

- ➔ Add 100mL 20% glucose (2% final conc.) *immediately*
- ➔ When COOL, add:
 1. 0.7 ml Canavanine (100 mg/ml in sterile H₂O)
 2. 0.17 g 3-AT (3-aminotriazole)
 3. 0.2 g G418 (causes a ppt- *ignore!*)
 4. 1.0 ml of clonNAT (100 mg/ml in H₂O; 100 ug total)

STEP 5*: S.D. His- Arg- Can Plates (*Amberg protocol*)

	<u>1000mL</u>
Agar	≥ 21g
Yeast Nitrogen Base without amino acids	6.7g
ddH ₂ O	900mL

-> Autoclave NOT MORE THAN 20 MINUTES!! REMOVE PROMPTLY AT END OF CYCLE (30 MINUTES)!

➔ Add *IMMEDIATELY*:

1. 100 mL 20% glucose (2% final conc.)
2. **0.5 g of Amino Acid Add-In Mix (1.2g Ura: 1.2g Met: 1.8g Lys: 3.6g Leu = 7.8g Total in mix)**

➔ When COOL, add:

1. 0.7 ml Canavanine (100 mg/ml in H₂O)
2. 0.17 g 3-AT

***Control for Step 8!**

STEP 8: S.D. His- Arg- Can Plates + G418 + NAT (*Amberg protocol*)

	<u>1000mL*</u>
Agar	≥ 21g
<i>Yeast Nitrogen Base w/o amino acids, and without NH₄SO₄</i>	1.7g
Monosodium glutamate	1.0g
ddH ₂ O	900mL

-> Autoclave NOT MORE THAN 20 MINUTES!! REMOVE PROMPTLY

➔ Add *IMMEDIATELY*:

1. 100 mL 20% glucose (2% final conc.)
2. **0.5 g of Amino Acid Add-In Mix (1.2g Ura: 1.2g Met: 1.8g Lys: 3.6g Leu = 7.8g Total in mix)**

➔ When COOL, add:

1. 0.7 ml Canavanine (100 mg/ml in H₂O)
2. 0.17 g 3-AT
3. 0.2 g G418 (causes a ppt- *ignore!*)
4. 1.0 ml of clonNAT (100 mg/ml in H₂O; 100 ug total)

***Addition of even a *small* NaOH pellet darkens the medium considerably and causes a ppt (subsequent addition of 0.5 g AA mix causes the medium to re-**

clarify somewhat). Darkness does not noticeably affect cell growth but does make it more difficult to score the final results.

N.B. Autoclaving a Step 7/8 medium of high pH (e.g. > 6.0) causes a ppt and variable blackness in color (depending on the size of the NaOH pellet added); by contrast, autoclaving a Step 7/8 medium of low pH (e.g. < 5.0) causes agar mushiness that cannot withstand the force of 0.5 mm pins. Thus, pH is critical, and should be maintained between 5.0 and 6.0. Note that the amount (weight) of amino acid (AA) mix added is a respective factor (lowers the pH), and that the amount of AA mix and NaOH pellet are offsetting factors regarding pH. Thus, either add both the AA mix AND NaOH pellet before autoclaving, or if adding AA mix after autoclaving omit the NaOH pellet altogether.