Protocol: Synthetic Complete and Drop-out Media

Unlike rich YPD medium, synthetic complete and synthetic drop-out media contain defined concentrations of each nutrient. Synthetic complete medium is a defined medium that contains all the essential nutrients. Synthetic drop-out medium lacks one or more amino acids and is used for selection after plasmid transformation (yeast plasmids contain genes that complement auxotrophies). The genotypes for three common laboratory yeast strains are listed below:

Strain	Genotype
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
BY4743	*MAT*a/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*

BY4741 and BY4742 are haploid (they only contain one copy of each gene). BY4741 has a histidine, leucine, methionine, and uracil auxotrophy, while BY4742 has a histidine, leucine, lysine, and uracil auxotrophy. The diploid strain BY4743 strain is a mating between these two strains. It has the mating type *MAT*a/α, and has a histidine, leucine, and uracil auxotrophy. Note that BY4743 can make lysine and methionine, because only one of the two alleles is mutated.

The following reagents are going to be required to make liquid and agar synthetic media:

Reagents
Basic Medium (this is the 'base' for all synthetic medium)
10X Drop-out Amino Acids (this contains most, but not all, of the required amino acids)
Various solutions of 10X amino acids
Glucose (40%)
Bacto Agar (for solid agar medium)
diH_2O

<u>Note:</u> When autoclaving liquid, the rule of thumb is to make sure the final volume of your liquid is no more than ½ the max volume of the container (e.g., no more than 500 ml of liquid in a 1 L Pyrex bottle.)

Synthetic Complete or Drop-out Medium (liquid)

1. To an autoclavable bottle, add:

Reagent		
	Amount to add per 500 mL	**Amount to add per 250 mL**
Basic Medium	Variable (up to 500 ml)	Variable (up to 250 ml)
10X Drop-out Amino Acids	50 ml	25 ml
10X Amino Acids	50 ml (each)	25 mL (each)
40% Glucose	25 ml	12.5 ml

Here is an example: if I want to make 500 mL of Synthetic Drop-out (-His, -Leu) medium:

Reagent	**Volume**
Basic Medium	325 ml
10X Drop-out Amino Acids (-His, -Leu, -Trp, -Ura)	50 ml
10X Trp	50 ml
10X Ura	50 ml
40% glucose	25 ml

For Synthetic Complete, you would add 10X His, 10X Leu, 10X Trp, 10X Ura, and adjust the volume of Basic Medium accordingly (225 ml).

2. Add reagents to the top chamber of a disposable of a bottle-top filter. For the basic medium, use a clean graduated cylinder; for the amino acids and glucose, use sterile technique and sterile serological pipettes.

3. Filter the media by vacuum source.

Synthetic Complete or Drop-out Agar (plates)

1. To an autoclavable bottle, add:

Reagent		
	Amount to add per 500 mL	**Amount to add per 250 mL**
Basic Medium	Variable (up to 500 ml)	Variable (up to 250 ml)
10X Drop-out Amino Acids	50 ml	25 ml
10X Amino Acids	50 ml (each)	25 mL (each)
Bacto Agar	20 g	10

2. Autoclave the mixture **(You <u>MUST</u> get trained on how to use the autoclave before doing this!)**
   a. Make sure the screw-caps are **<u>LOOSLEY</u>** screwed on, allowing gas to escape the bottle.
   b. Place a piece of autoclave tape across the cap, making sure one end is taped to the glass bottle.
   c. Autoclave for 25 minutes (use "SLOW EXHAUST" setting).

3. Place the bottle in a 55°C in a water bath to allow the temperature of the media to come down.

4. Add 25 ml of 40% glucose (per 500 ml final volume) or 12.5 ml of 40% glucose (per 250 ml final volume)
5. Gently swirl the medium to mix glucose (avoid bubbles).
6. While still warm, pipette media into 10cm diameter plastic petri dishes using a serological pipette.
7. Allow agar mixture to cool and solidify overnight.

10X Drop-out Amino Acids and 10X Amino Acids/Uracil

<u>NOTE:</u> Shake Sigma drop-out powder ***VIGOROUSLY*** to mix contents and break clumps with a scoopula before use.

1. Add 250 ml Basic Media to a 500 mL beaker using a clean graduated cylinder. Add **one** of the following reagents to make a 10X stock of that solution:

Reagent	**Amount to add per 250 ml final volume**
10X Drop-out Amino Acids	
Sigma Y2001 (-His, -Leu, -Trp, -Ura)	3.45 g
Sigma Y1501 (-Ura)	4.8 g
10X Amino Acids/Uracil	
Tryptophan	190 mg (0.19 g)
Histidine	190 mg (0.19 g)
Leucine	950 mg (0.95 g)
Uracil	190 mg (0.19 g)

2. Add a stir-bar to the beaker. Rinse a thermometer with diH2O and place it in the beaker. Stir with gentle heat, bringing the temperature up to 55-65°C. Do not exceed 65°C.

3. When the amino acids have been completely dissolved, place an aluminum foil cover over the beaker and remove the solution from the hot plate to let it cool to room temperature.

4. Filter sterilize the solution using a disposable bottle-top filter and vacuum source. Label as appropriate. If making 10X Drop-out Amino Acids, indicate whether the solution is -HIS, -LEU, -TRP, -URA or just -URA.