

## ***S. cerevisiae* Growth Media**

### **YPD (rich, undefined media)**

We use YPD for routine propagation of all our strains. With the exception of D-glucose, or dextrose (the **D** in YPD), the composition of YPD is undefined. The yeast extract (the **Y** in YPD) is the water-soluble extract generated from the autolysis of yeast. It includes a myriad of vitamins, salts, and small organic molecules important for growth. Peptone (the **P** in YPD) is generated by the proteolytic treatment of animal protein, and consequently, contains many amino acids and peptides. YPD can be used for liquid cultures or in plates. When used in plates, agar is included at a final concentration of 15-20 grams/liter.

<b>Composition of YPD</b>	
<b>Component</b>	<b>grams per liter</b>
Yeast extract	10
Peptone	20
D-glucose (dextrose)	20

### **Yeast Complete medium (YC)**

The yeast strains used in this experiment will grow in minimal media that contains just the supplements necessary to complement their auxotrophies, but they grow somewhat better in YC (Yeast Complete), a defined medium with a variety of amino acids or nucleotides. YC also contains YNB (Yeast Nitrogen Base), which is a complex mixture of vitamins, minerals and salts. The components of YC can be easily modified for individual experiments. See below for details and additional notes.

<b>Composition of Yeast Complete Medium</b>					
<b>Component</b>	<b>grams/liter</b>	<b>Component</b>	<b>mg/liter</b>	<b>Component</b>	<b>mg/liter</b>
YNB*	1.7	arginine	100	tyrosine	50
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	5	aspartic acid	50	lysine	100
D-glucose	20	isoleucine	50	methionine	50
		phenylalanine	50	tryptophan	100
		proline	50	leucine	100
		serine	50	histidine	50
		threonine	100	uracil	10
		valine	50	adenine	10

from the Gottschling lab protocols at:

<http://research.fhcrc.org/gottschling/en/protocols/yeast-protocols/yc-media.html>

***Amino acid and nucleotide bases:*** We find it convenient to prepare powdered mixtures of the amino acids and nucleotide bases that can be stored at room

temperature, eliminate the need to weigh out components individually for each experiment. Since the various powders can settle at different rates, it is a good idea to shake the mixtures well before using.

In addition to the complete mixture of amino acids and nucleotide bases, we also prepare "dropout" mixtures in which one or more components, e.g. methionine and/or uracil, are missing.

**YNB:** It is important not to autoclave YNB for long periods of time, as some of the components will degrade. Some labs prefer to make a 10X stock of YNB which is then filter sterilized and stored in the refrigerator. For the sulfur source experiment, we use a sulfate-free YNB that can be purchased from Sunrise Science Products.

**Sulfur sources:** YC complete contains both ammonium sulfate and methionine, which serve as sulfur sources. To substitute sulfur sources, we:

- replace ammonium sulfate with ammonium chloride (7.9 g/L)
- use sulfate-free YNB in the place of regular YNB (1.7 g/L)
- add filter-sterilized 100X stocks of sulfur sources:
  - 25 mM sodium sulfite (freshly prepared - do NOT autoclave)
  - 2 mg/mL cysteine (freshly prepared - do NOT autoclave)
  - 2 mg/mL methionine

Note that the sodium sulfite degrades over time. Plates with sodium sulfite should be used within a week of their preparation. Alternatively, these plates can be made without sodium sulfite, and the 100 X stock can be spread on the surface of poured plates shortly before they are used.

**BiGGY (Bismuth Glucose Glycine Yeast) agar:** BiGGY agar can be purchased from a number of suppliers. BiGGY contains yeast extract, which supplies enough methionine for all *met* mutants to grow. (Yeast are capable of making more methionine than they absolutely need to grow.) The principal sulfur source in BiGGY is sulfite. The colony color reflects the amount of sulfide that is generated by the strains, using sulfite as sulfur source. Follow the manufacturer's instructions to prepare the media. Note that the media should NOT be autoclaved. The components of BiGGY are:

Component *	grams/liter
bismuth ammonium citrate	5.0
sodium sulfite	3.0
glucose	10.0
glycine	10.0
yeast extract	1.0
agar	16.0

\*pH is adjusted to 6.8

