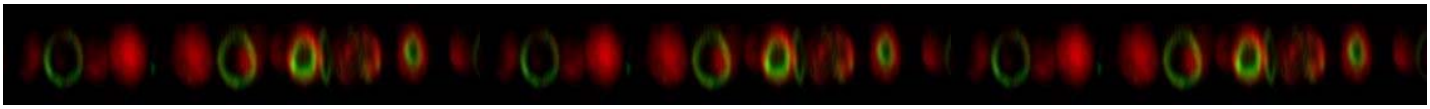


GROWING CELLS

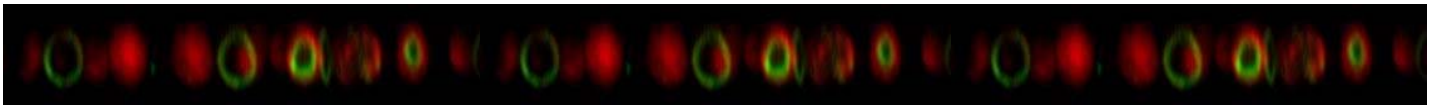
1. This is the most important technique you will use in the lab. This will ensure that your cells are healthy. So pay attention☺!
2. The most commonly used media in the lab is YE (rich media) and EMM (minimal media).
3. The optimum temperature for growing pombe cells is 32ºC. The permissive temperature for temperature sensitive mutants is 25ºC and the restrictive temperature is 35.5ºC. These temperatures may vary among different mutants so confirm the temperatures before use.
4. For 35.5ºC use the large shaking water bath. You can change the flask clamps as per your needs. You can also use this water bath for growing bacterial cells. The optimum temperature for bacterial cells is 37ºC.
5. For yeast cells the optimum speed for rotation in 180rpm. For bacterial cells use 220rpm.
6. Start a preculture from a plate of cells no more than 2 days old. The colonies on the plate need to be clearly visible but not too overgrown.
7. Take a small amount of cells, about 0.5ul, on a sterile toothpick and inoculate into 10ul of sterile media. If using EMM make sure to add all the supplements.
8. Grow at the suitable temperature overnight.
9. Next day in the morning measure the O.D. of the cells in the preculture.
10. To measure O.D. first take 100ul of cells in a clean microfuge tube and add 900ul of sterile water. Note that you have diluted your cells 10X here.
11. Use this diluted samples to measure O.D. in the spectrophotometer at 590nm. Use sterile water as blank. Please make sure you are trained to use this machine.
12. Since you diluted the samples 10X before measuring O.D. make sure to multiply your readings by 10 before proceeding. It is important to dilute the samples by a factor of 10 before measurement to ensure that the sample is within the optimum range of the spec.

DILUTING CELLS

1. Before you calculate you will need to know certain facts about growing pombe cells. Refer to the table below

Media	Temperature ºC	Generation time
YE	25	3h
	29	2h 30min
	32	2h 10min
	35.5	2h
minimal	25	4h
	29	3h
	32	2h 30min
	35.5	2h 20min

2. Do not grow the cells for more than 8 generations and beyond an O.D. 0.5.
3. To calculate the dilution of the cells first calculate the number of hours you want to grow the cells. If diluting in the morning then you will need to set up a fresh dilution in the evening for the next morning. That way you will not let the cells over grow. If the number of hours you plan to grow the cells is X, then you can calculate the no. of generations. However before you proceed it is important to note that pombe cells undergo a shock when diluted into fresh media and they need to recover before they start to grow. For cells in YE at 32ºC it takes about 1 hour to recover. So subtract this time from X to account for recovery time. Thus the number of generations are (X-1)/generation time. Refer to the table above to find the right generation time. Let G be the number of generations.
4. Next we will compute the starting O.D. that will give us a final O.D. of 0.5 when the cells undergo G generations. Starting O.D.= $0.5/2^G$
5. So now if you dilute your cells to the starting O.D. calculated above after G generations you will get an O.D. of 0.5.
6. To dilute the cells to the above O.D. We will use the $V_1C_1=V_2C_2$ formula.
7. We typically grow cells to a volume of 25ml.
8. Thus V_2 = (Starting O.D. X25ml)/0.5.
9. Take V_2ml of cells and add fresh media to make up the volume to 25ml.
10. In the evening as per your plan again measure the O.D. of the cells. And dilute it for the next morning. Make sure you are not growing the cells for more than 8 generations.
11. If the next day, the cells appear healthy under the microscope and has reached the right O.D. they are ready for your experiments.



PLEASE NOTE:

At every stage of growing cells and dilution make sure the cells appear fine under the microscope. Use the small light microscope in the clean bench to check your cells. This will also ensure that you are not diluting contaminated cells.

It is ABSOLUTELY essential to check your cells under the light microscope before you proceed with your experiments.

DO NOT use the excel calculator. Learn to do the dilutions manually. If you don't know how ASK Dr. DAS!!!