Lab practice 2

25/26

6th and 9th October 2025

Lab Safety

- **Emergency exit**
- **No food**
- **No drinks**

•***Appropriate code of conduct***

- **No telephone, checking messages or smart phone calls or video, you can go outside**

Lab Safety

- **Wear Lab coat with name tag**
- **Wear goggles**
- **No open shoes and sandals**
- **No shorts, skirts**
- **No long pendents (earrings and neckless)**
- **No bracelets**
- **Tie your hairs**
- **Write your name on the foolscap (one per group)**
- **Take a break when you need it, inform your lab mates**

Vocabulary

Spectrophotometer
OD, optical density
Cuvette
Petri dish
Vials, tubes
Micropipettes
Tips
Waste
Replica plating
Bunsen burner
Alu-foil
Ethanol
Parafilm
Swarming

Proficiency in pipetting

1 mL = 1000 µL

0.1 mL = 100 µL

0.01 mL = 10 µL

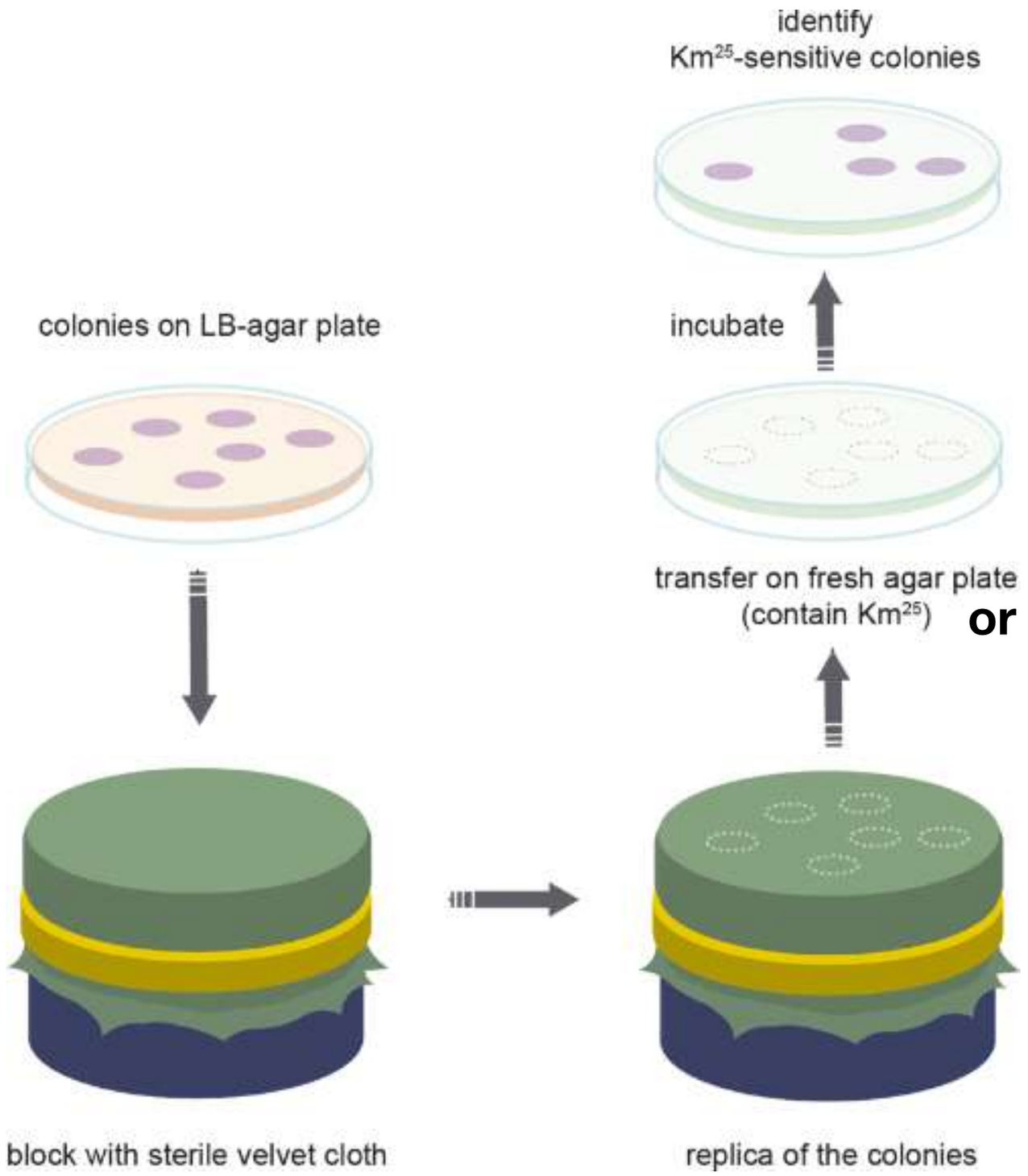
Color coding for tips and pipettes

Replica plating

Esther Ledergberg was an American microbiologist and pioneer of bacterial genetics. Her notable contributions include the discovery of the lambda phage, the transfer of genes between bacteria by specialized transduction, the development of replica plating, and the discovery of the bacterial fertility factor F (F plasmid). These contributions laid the foundation for much of the genetics work done in the latter half of the twentieth century. Lederberg also founded and directed the Plasmid Reference Center at Stanford University, where she maintained, named, and distributed plasmids of many types.

Replica plating, principle

https://app.jove.com/t/60749/quantification-plasmid-mediated-antibiotic-resistance-an-experimental

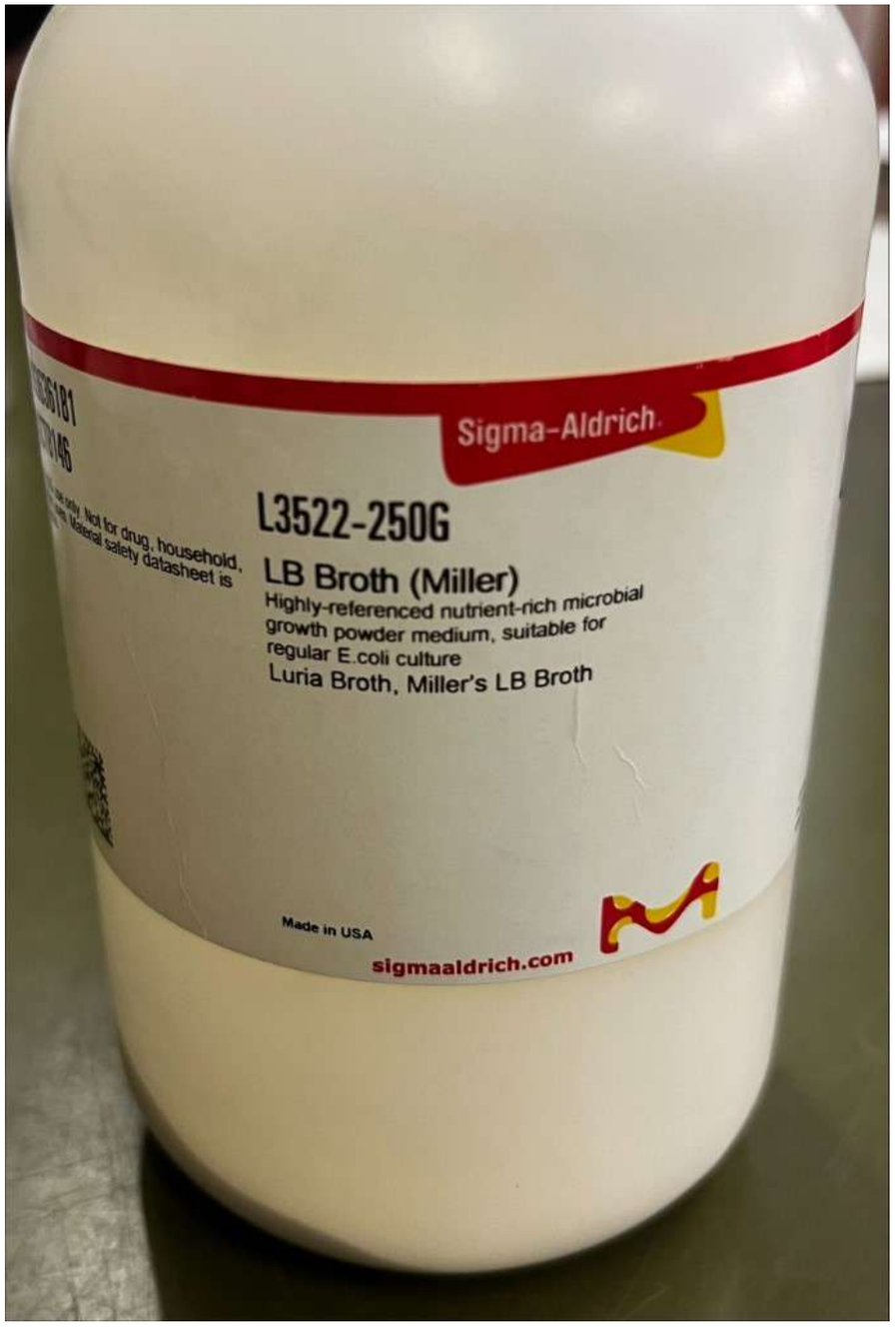


Colonies able to grow on diverse media

or diverse media

Mac Conkey Agar

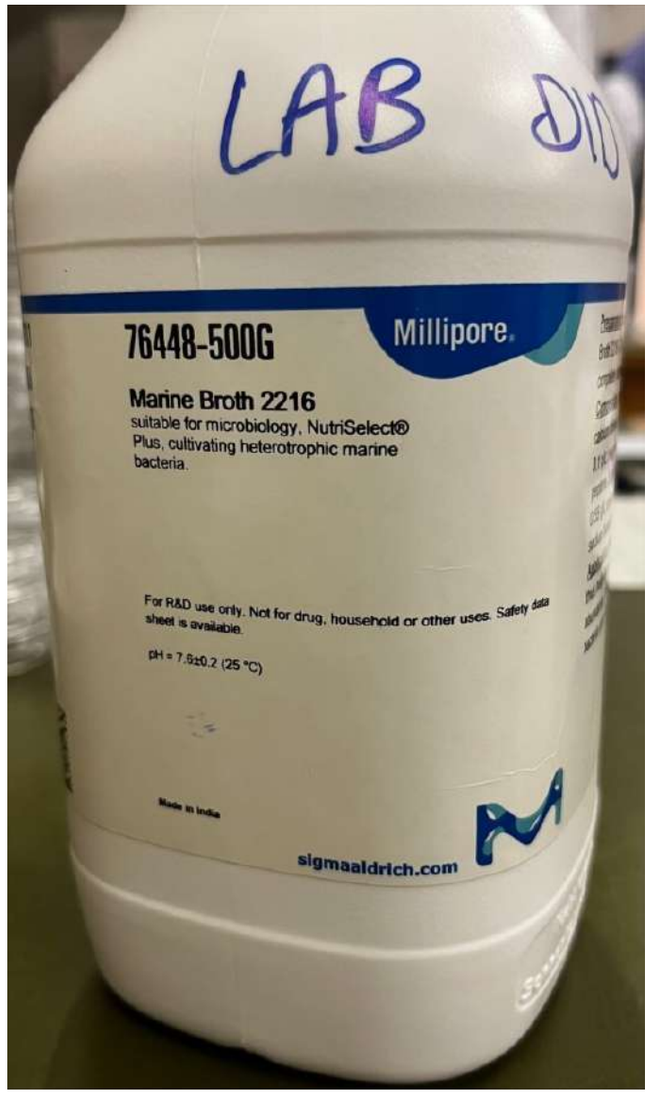
LB

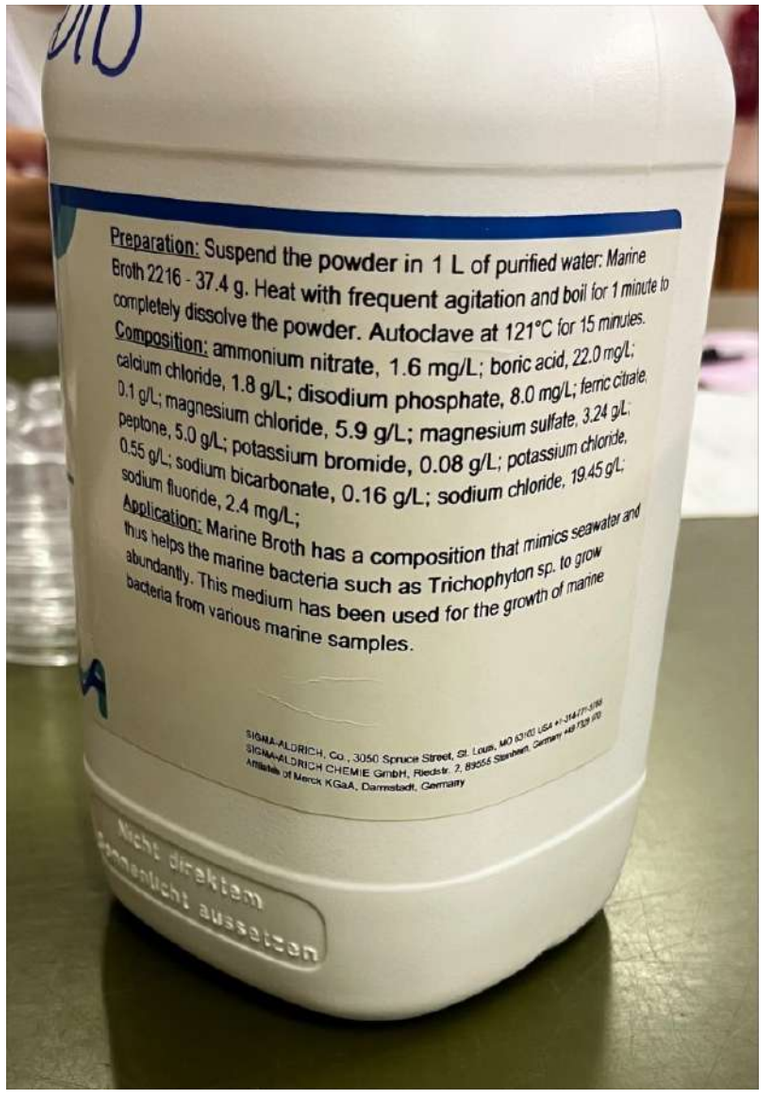

Sigma-Aldrich.
L3522-250G
LB Broth (Miller)
Highly-referenced nutrient-rich microbial growth powder medium, suitable for regular E.coli culture
Luria Broth, Miller's LB Broth
Made in USA
sigmaaldrich.com

MS


OXOID
CM0085
MANNITOL SALT
AGAR
IVD
500g makes 4.5 litres
Typical formula (g/l)
'Lab-Lemco' Powder 1.0; Peptone 10.0; Mannitol 10.0;
Sodium chloride 75.0; Phenol red 0.025; Agar 15.0.
pH 7.5 ± 0.2 at 25°C
Keep tightly closed, away from bright light
486246
2011/11
(01)05032384001507(17) 111100(10)486246
500 Grams
OXOID LTD., BASINGSTOKE, HAMPSHIRE, ENGLAND

ZoBell/Marine Broth

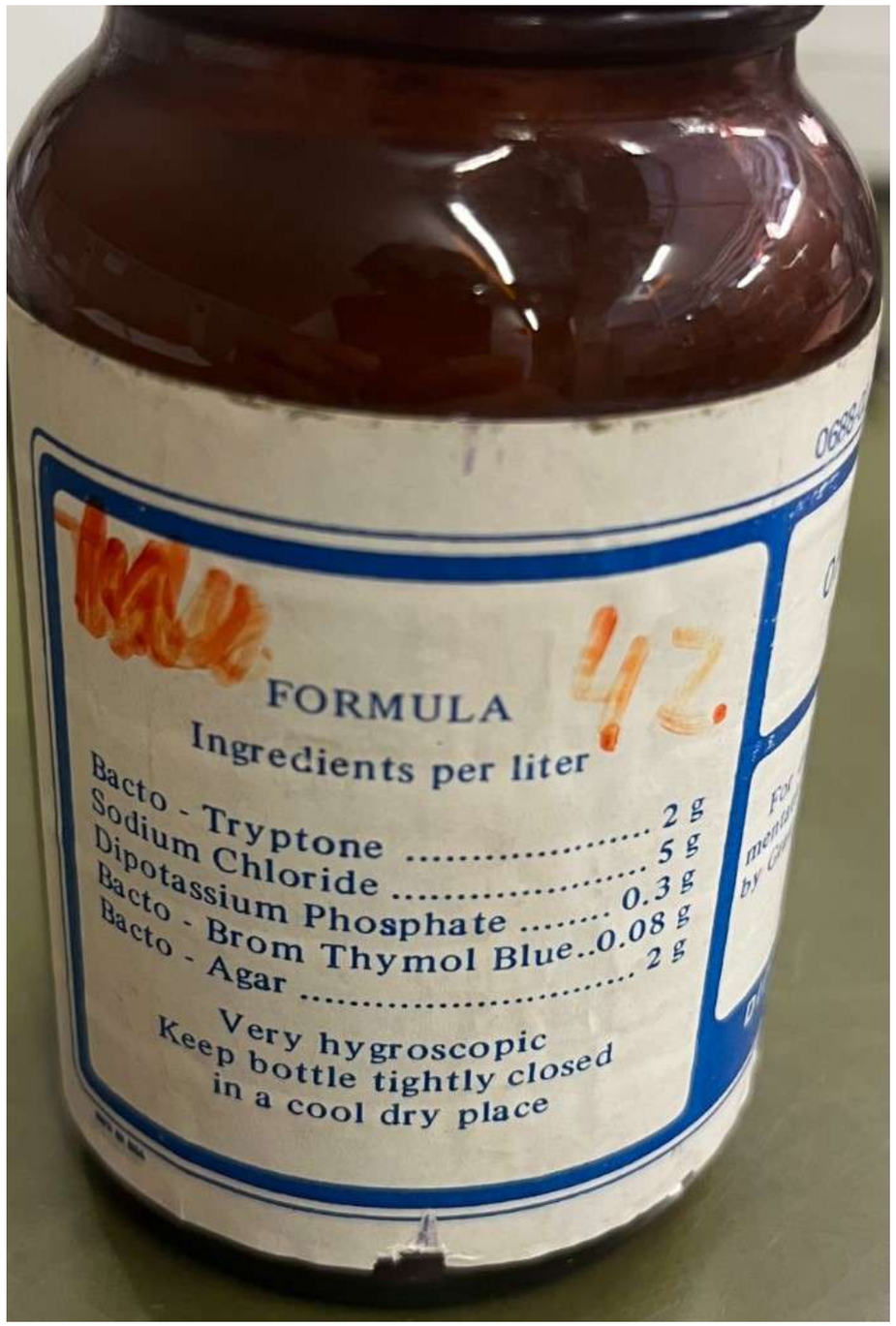


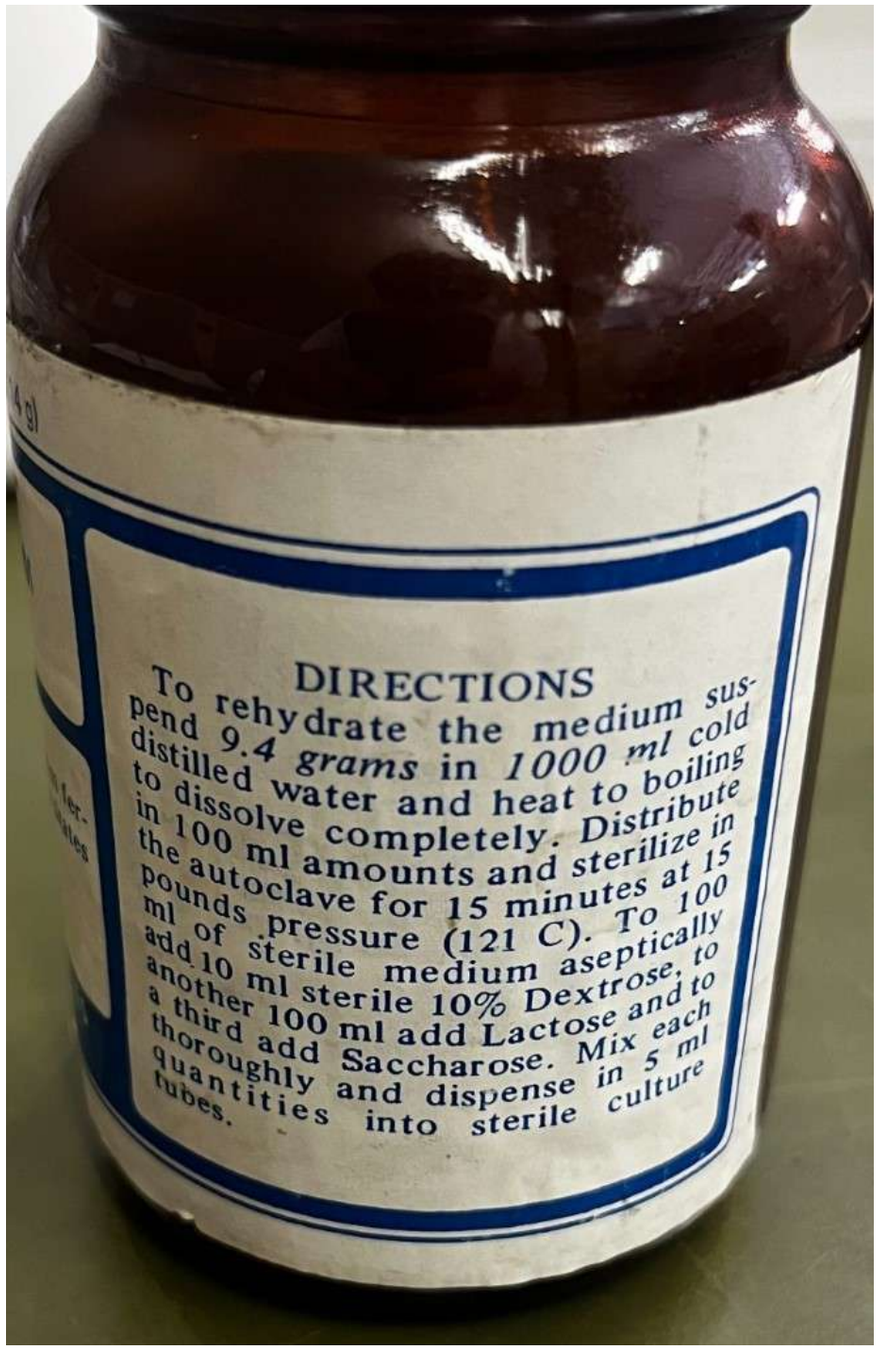


OF


0688-02
¼ lb (114 g)
DIFCO
BACTO
OF BASAL MEDIUM
DEHYDRATED
J. Bact. 66:24:1953
For differentiating oxidative from fermentative metabolism of carbohydrates by Gram negative bacteria.
Final pH 6.8 at 25 C.
Control 723225
DIFCO LABORATORIES
DETROIT MICHIGAN USA
Store at 15 - 30 C

OF


FORMULA
Ingredients per liter
Bacto - Tryptone ..... 2 g
Sodium Chloride ..... 5 g
Dipotassium Phosphate ..... 0.3 g
Bacto - Brom Thymol Blue..0.08 g
Bacto - Agar ..... 2 g
Very hygroscopic
Keep bottle tightly closed
in a cool dry place
42.


DIRECTIONS
To rehydrate the medium suspend 9.4 grams in 1000 ml cold distilled water and heat to boiling to dissolve completely. Distribute in 100 ml amounts and sterilize in the autoclave for 15 minutes at 15 pounds pressure (121 C). To 100 ml of sterile medium aseptically add 10 ml sterile 10% Dextrose, to another 100 ml add Lactose and to a third add Saccharose. Mix each thoroughly and dispense in 5 ml quantities into sterile culture tubes.

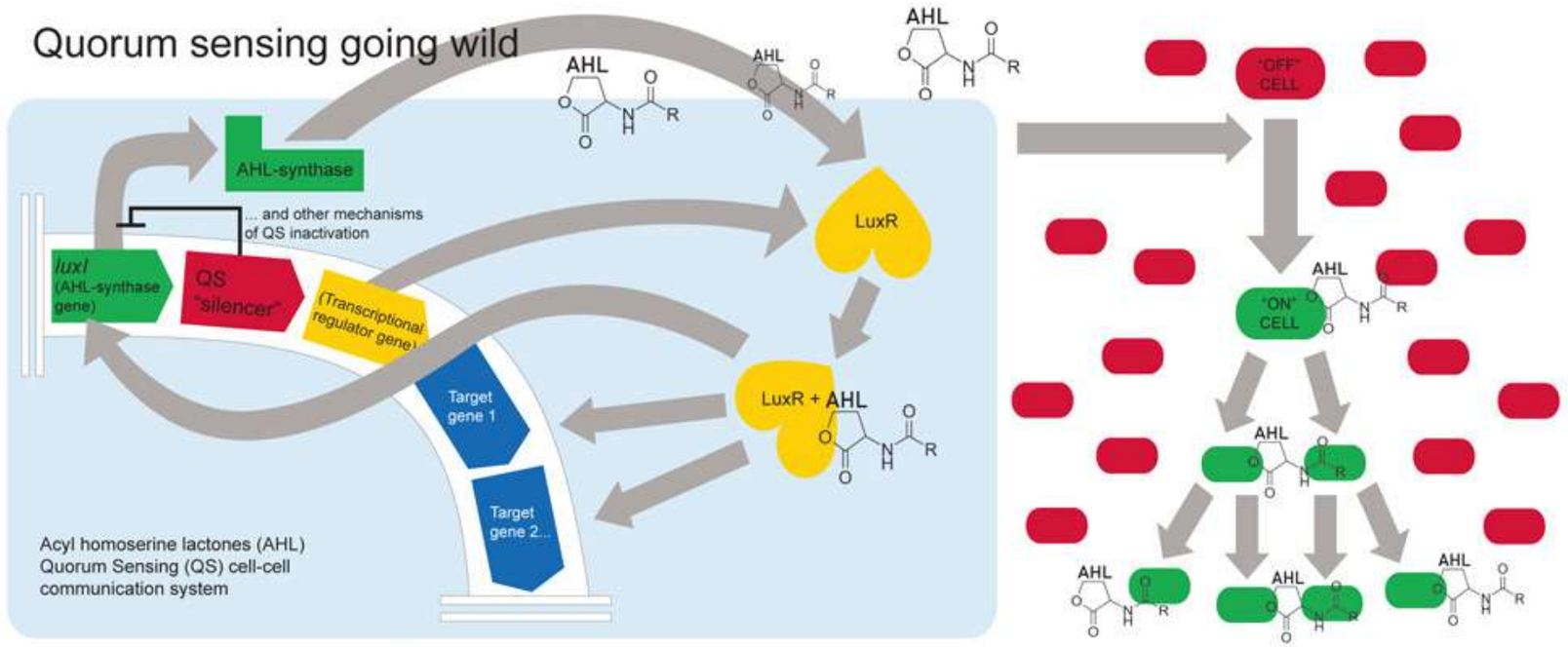
Experiment flow 1

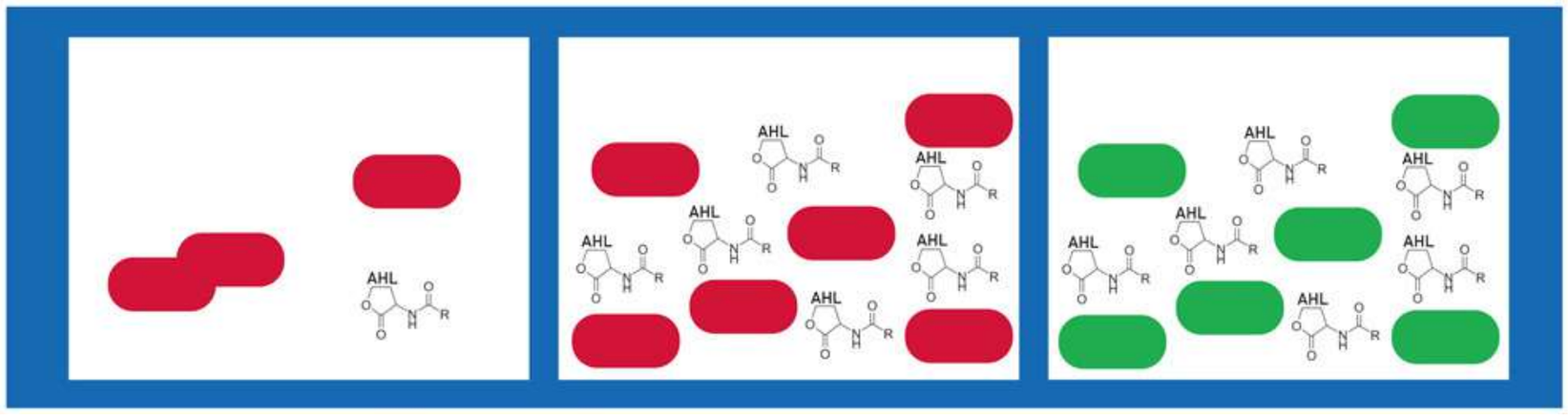
A. A LB plate with microbial colonies
B. Take a picture of plate and draw the colonies and number them
C. Replica plating on different media (LB, OF, MS, MAC, ZoBell)
D. Turn in the foolscap paper
E. Incubating for 3 days and taking pictures (in plastic bag + wet wipe)
F. Counting colonies and plotting growth dynamic
G. Writing final RESULT report on e-book (pictures and number) and answer questions by next Monday or Thursday
H. Bring back plates

Replica plating questions

1. How many colonies did grow on the different media in comparison to the control plate?
2. What colonies did grow?
3. What can you infer from these colonies?

Quorum Sensing



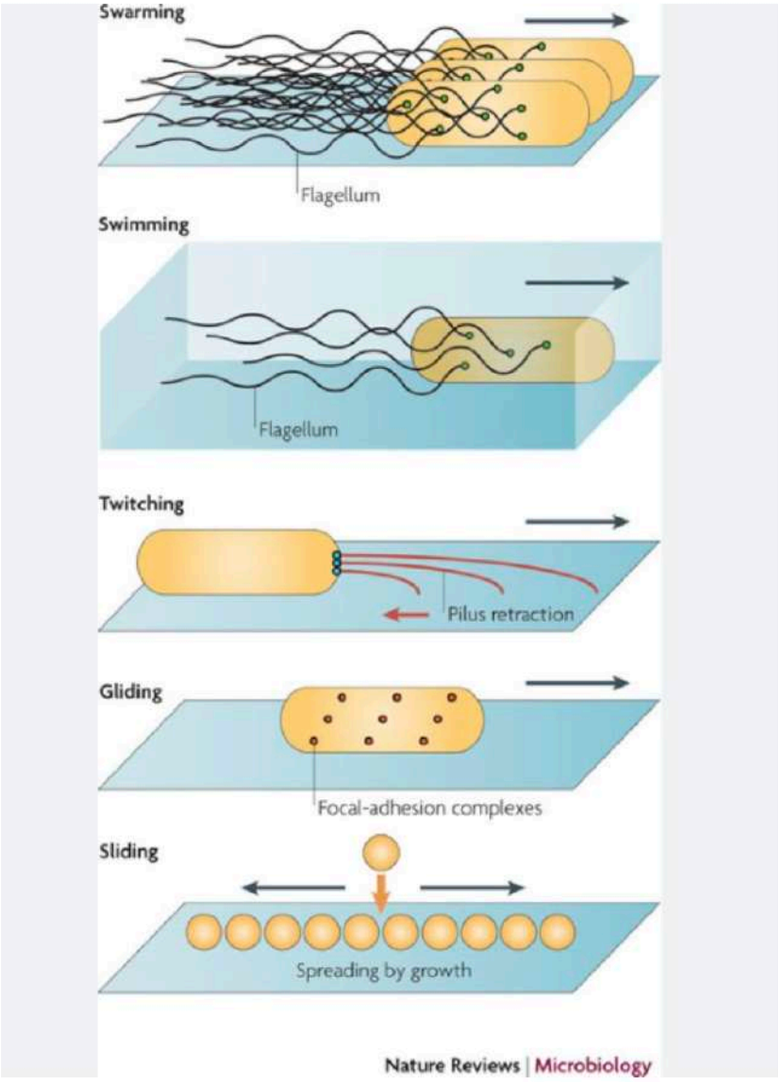


Spacapan, Mihael et al.
iScience, Volume 26, Issue 1, 108000

Coordinated behaviour, depending on how many cells there are in the vicinity —> gene activation

Swarming motility

https://app.jove.com/v/60915/time-lapse-imaging-bacterial-swarms-collective-stress

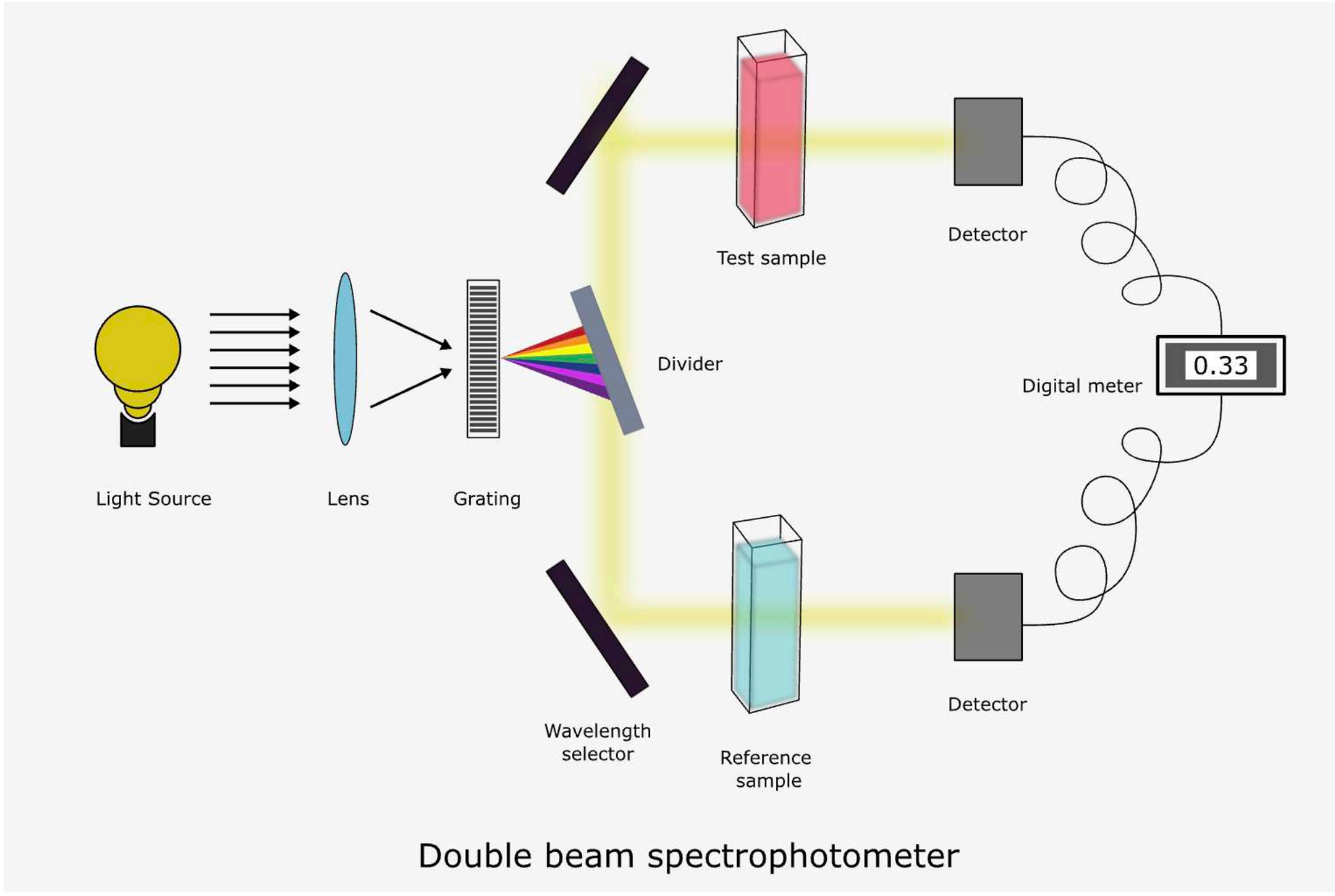


Experiment flow 2

A. Measure OD of SWAT3 bacterium at 660 nm
B. Pierce the center of the ZoBell plates at 0.3% and 1.5% agar
C. Turn in the foolscap paper
D. Take picture (ALSO IN THE DARKNESS) of T0 and a picture of the petri dish with a ruler
E. Incubating for 5 days and taking pictures (in plastic bag + wet wipe)
F. Measure the diameter
G. Writing final RESULT report on e-book (pictures and number) and answer questions
H. Bring back plates

Spectrophotometer

https://psiberg.com/spectrophotometers/

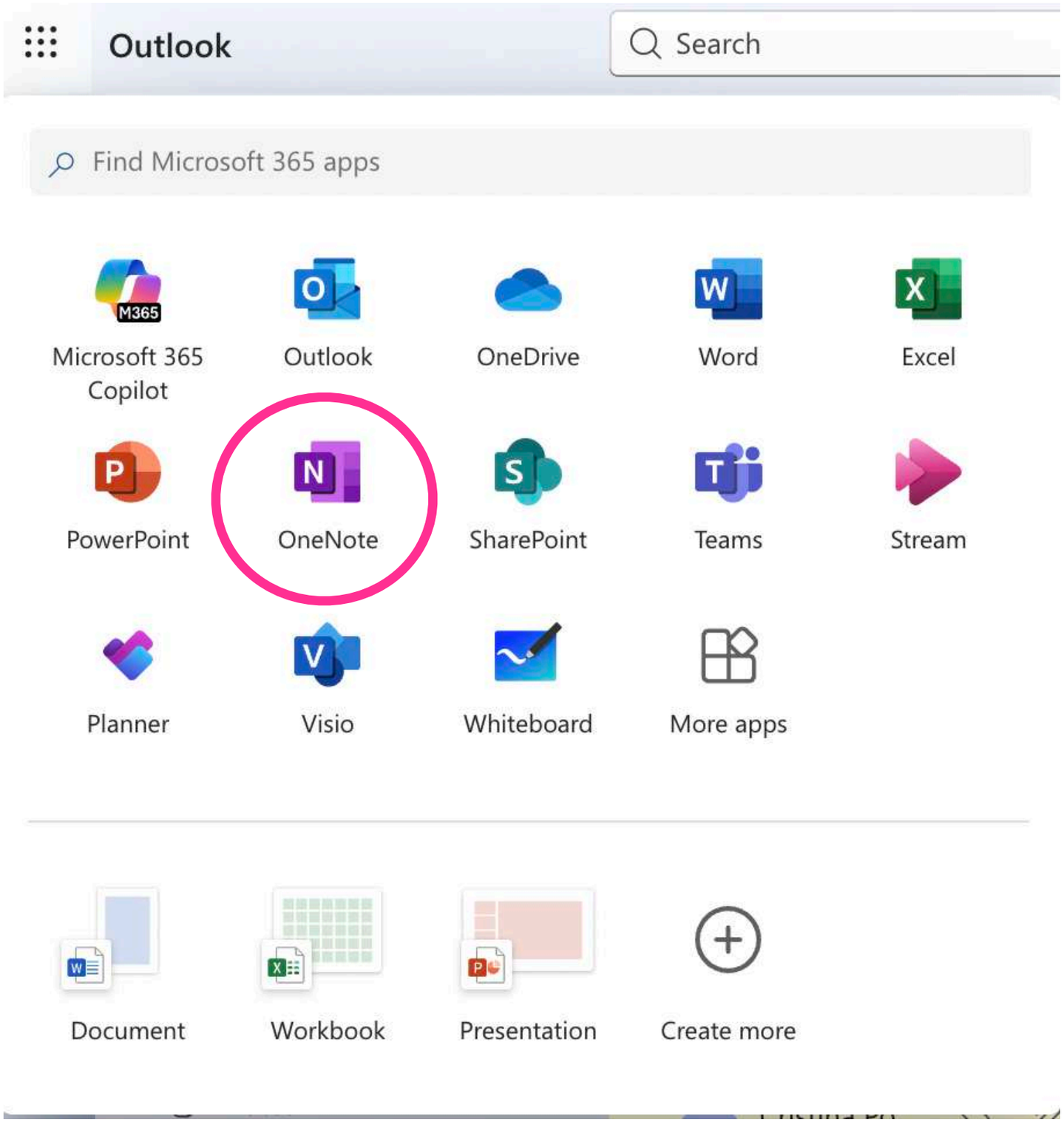


Double beam spectrophotometer

Quorum sensing questions

1. Did you notice a difference in the growth on 0.3% vs 1.5%? If yes, explain.
2. How fast did the colony on 0.3% spread vs the one on 1.5%?

e-BOOK



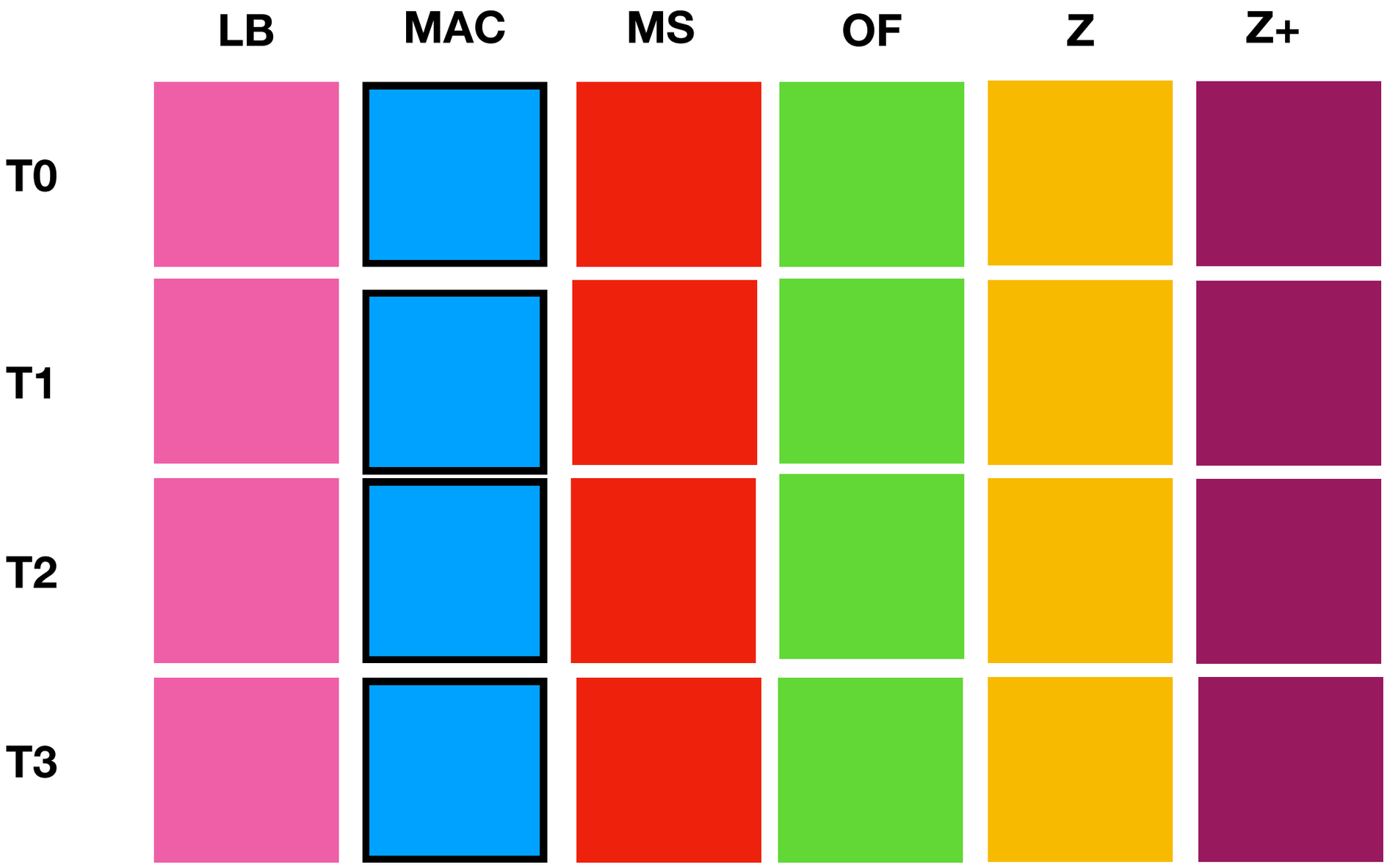
- **OneNote**
- **Create and share a notebook**

Replica plating questions

1. How many colonies did grow on the different media in comparison to the control plate?
2. What colonies did grow?
3. What can you infer from these colonies?

Reporting

Replica plating



Replica plating

	Colony 1	**Colony 2**	**Colony 3**	**Colony 4**	**Colony 5**	**Colony etc**			
Z	Y								
OF		Y							
Z+					Y				
LB	Y	Y	Y	Y	Y	Y			
MS		Y							
MAC			Y	Y					

Replica plating: answer

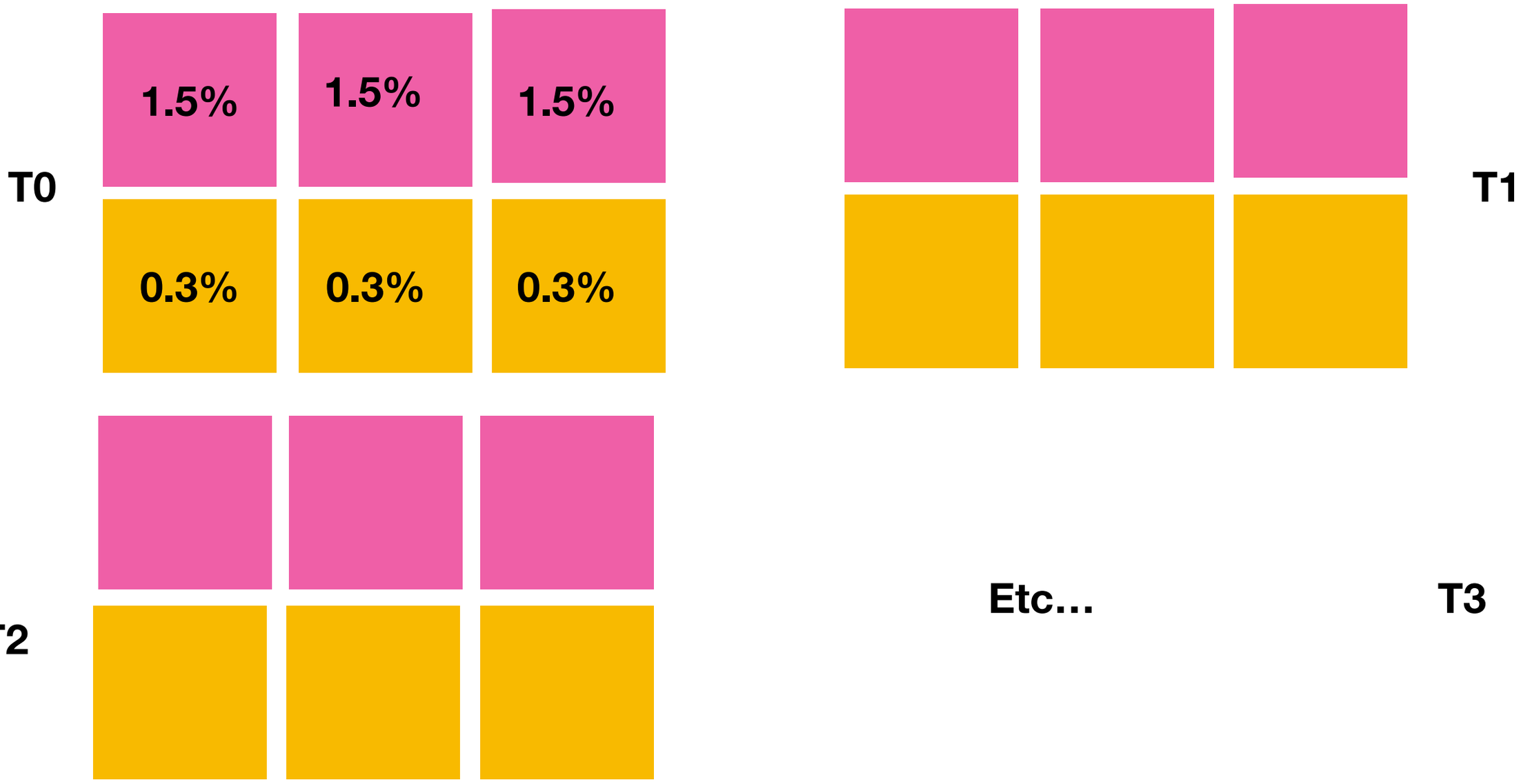
1. How many colonies did grow on the different media in comparison to the control plate?
2. What colonies did grow?
3. What can you infer from these colonies?

Quorum sensing questions

1. Did you notice a difference in the growth on 0.3% vs 1.5%? If yes, explain.
2. How fast did the colony on 0.3% spread vs the one on 1.5%?

Reporting

Quorum sensing



Diameter growth (mm)

	T1	T2	T3	T4	T5
0.3%_a					
0.3%_b					
0.3%_c					
1.5%_a					
1.5%_a					
1.5%_a					

Quorum sensing: answer

1. Did you notice a difference in the growth on 0.3% vs 1.5%? If yes, explain.
2. How fast did the colony on 0.3% spread vs the one on 1.5%?