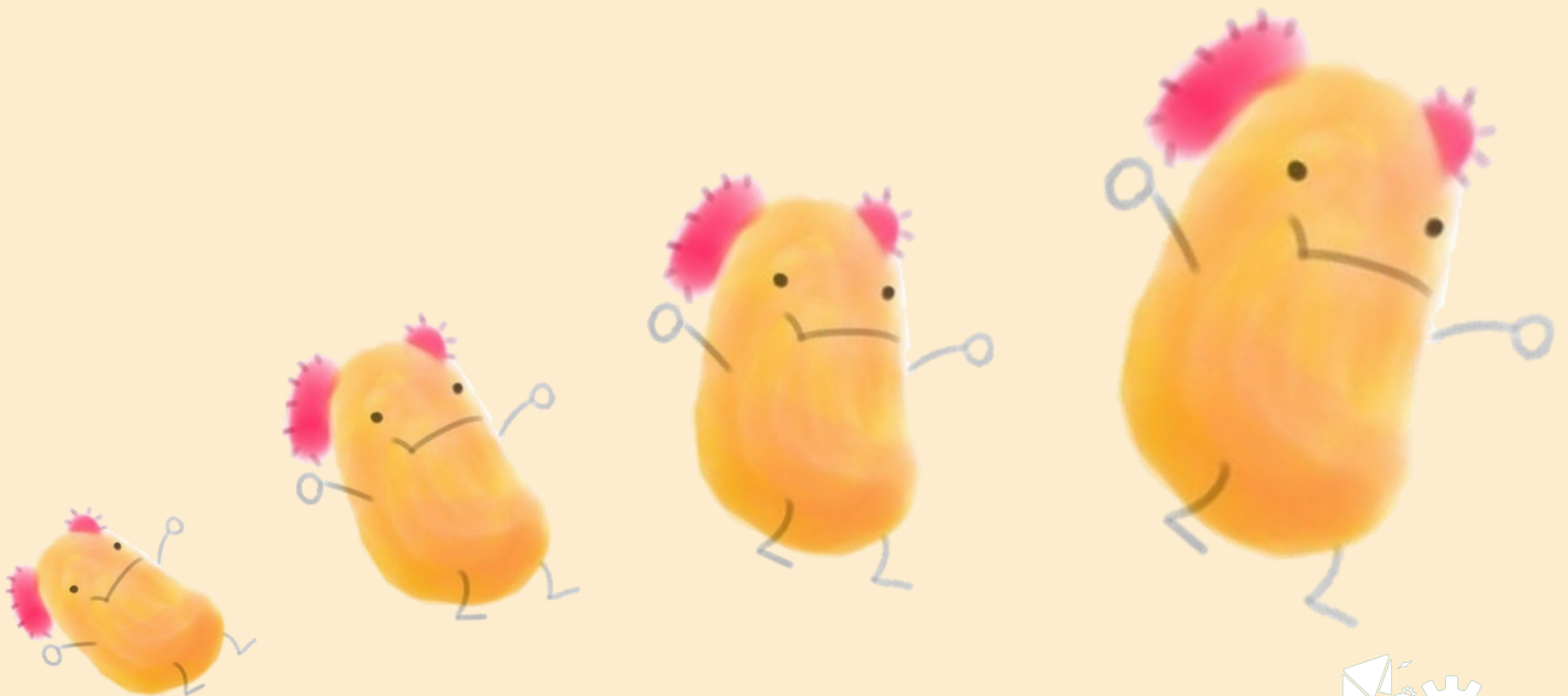


Fluotato teaches: Episode 1

Growth characteristics of E. coli

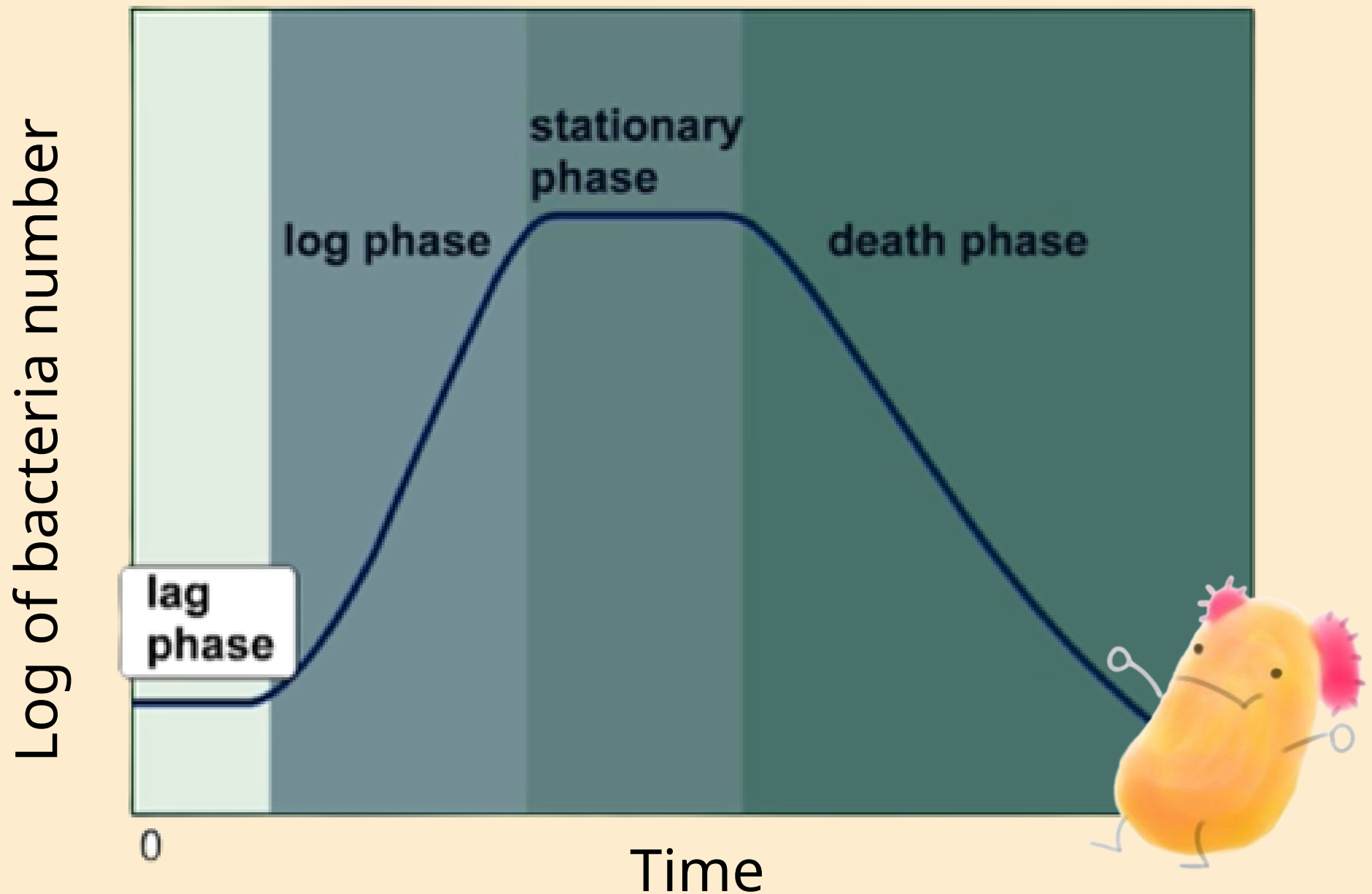


All organisms are able to **grow, reproduce,**
and **adapt** to their surrounding environments.



Bacteria, one of the fastest reproducing organisms, grows at an astonishing rate when there are plenty of nutrients. Gradually, the **growth rate decreases when nutrients are insufficient** to meet the demand of the increasing population. When **growth rate = death rate**, the **cell population remains constant**.

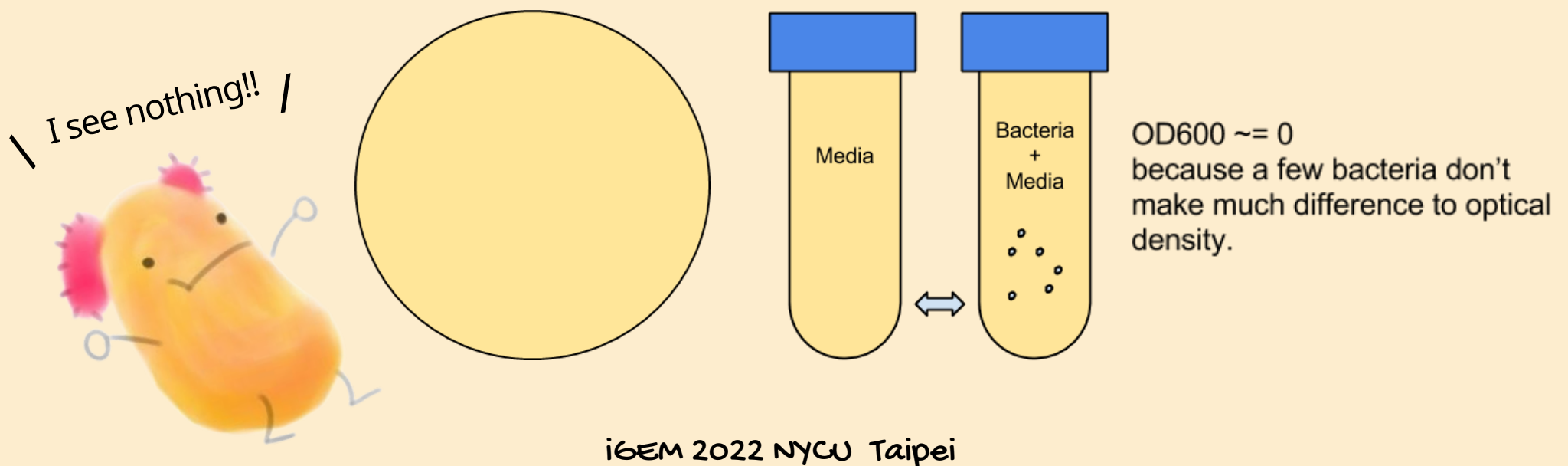
For a better understanding, let's draw out the **growth curve** of our model organism, *Escherichia coli* (E. coli) !



The growth curve can be divided into four main stages: **lag**, **log**, **stationary** and **death** phase.

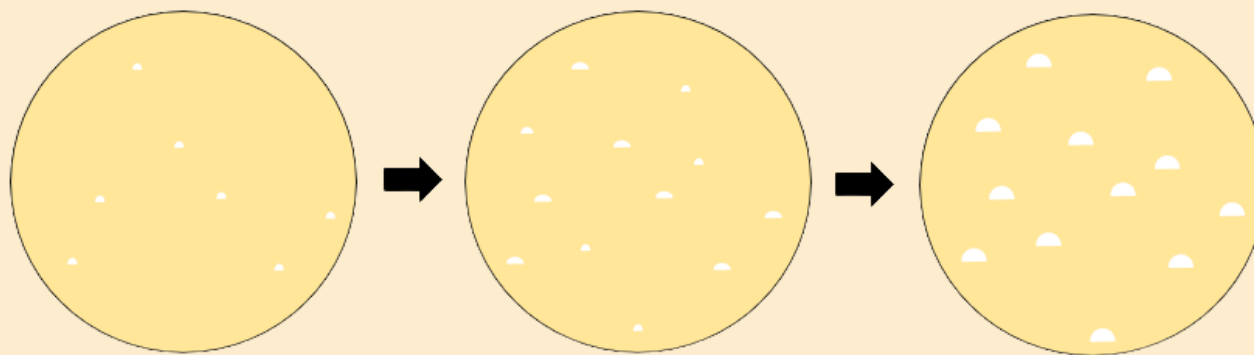
Lag phase

- Occurs when bacteria **adjust to environment change**
- **New enzymes are produced** to digest food, build biomass, and prepare for cell division, **increase in ribosomal factors**
- Sigma factor **$\sigma 70$** initiates expression of essential genes involved in **amino acid** and **metal metabolism**
- Lesser nutrients resulted in longer lag phase.
- **Longer lag phase** sometimes indicates **antibiotic tolerance**
- No observable colonies on solid culture, liquid culture clear with no turbidity (OD = 0 ~ 0.2)



Log phase (Exponential phase)

- Rapid division: double "exponentially" through **binary fission**
- **Doubling time** could be as less as **20 minutes** for E. coli
- Exhibit **constant growth rate** and **steady metabolic activity**
- Increase in **cell width** and **cell length**
- Increase in **flagellum length** and **density**
- Observable colonies on solid culture, liquid culture becomes turbid



Transition from early log to late log:
Colonies grow in size and amount

See the answer
at the last page!!

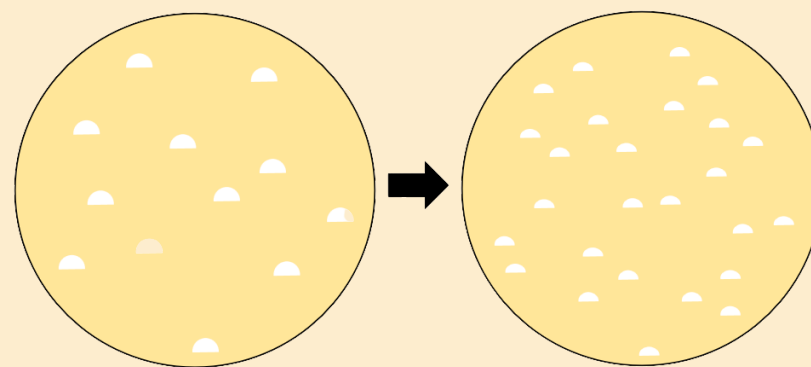
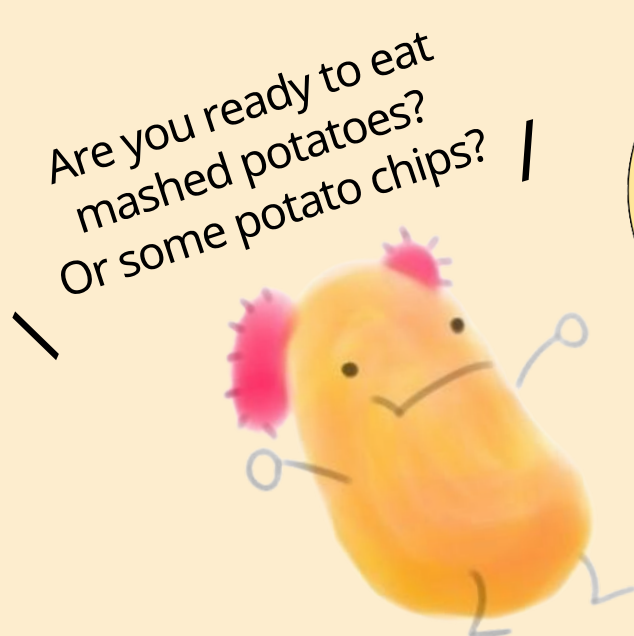


Think about it !

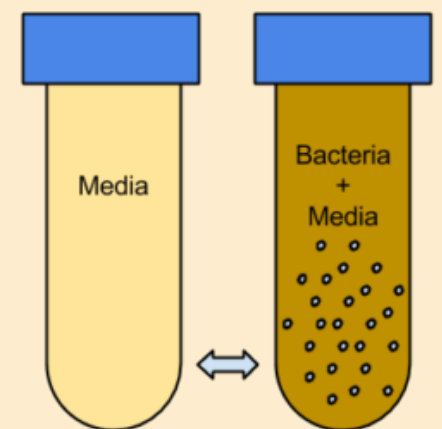
It takes 60–90 min for E. coli to replicate and segregate the chromosome. Since replication occurs before division, how does E. coli catch up for division if it has a slower speed?

Stationary phase

- Occurs during **nutrient limitation** or **toxic accumulation**
- **Dividing cells = dying cells**, cell number remains "stationary"
- Cells become **spherical** and **smaller** with a rigid cell envelope
- **Ribosome enters "hibernating" state** to prevent unnecessary waste of key elements
- Induction of **genes responsible for stress response** in order to deal with change in pH, temperature, osmolarity, etc.
- Observable colonies on solid culture, **ready to harvest!**



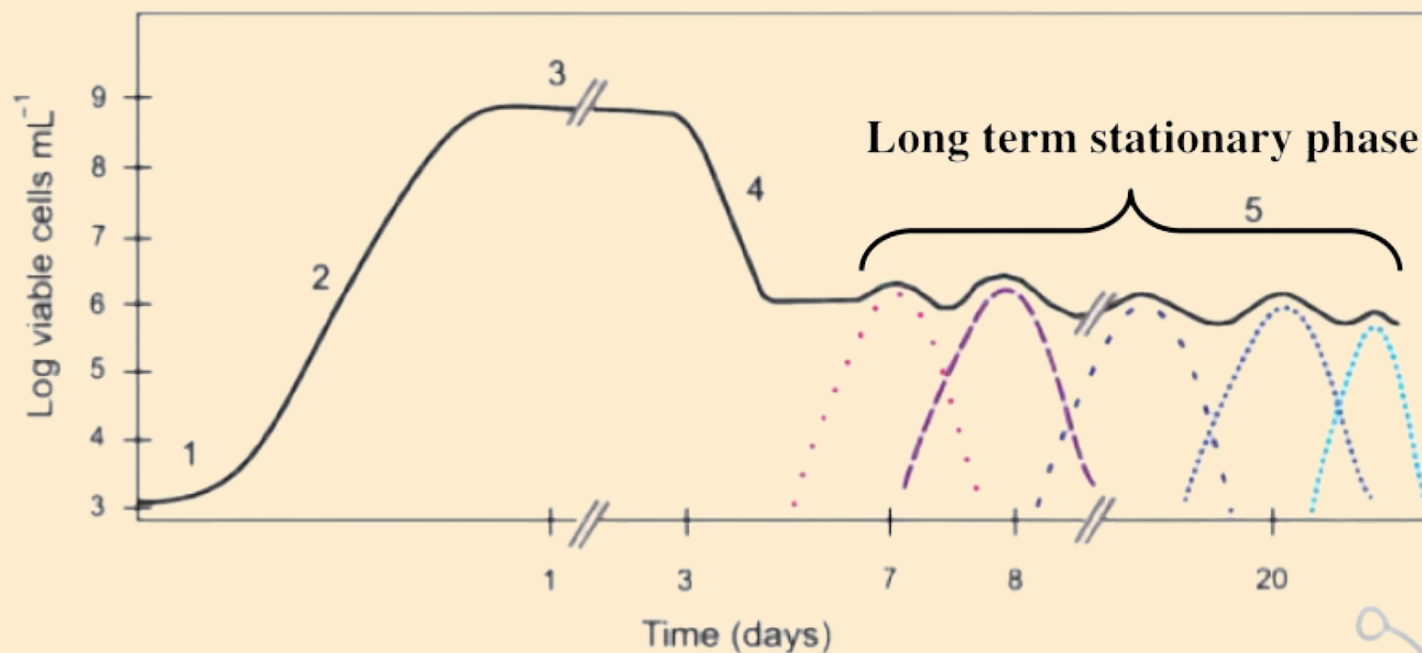
Transition from log to stationary



Color becomes dark after OD exceeds 0.5 (about mid-log phase)

Death phase

- Bacteria can no longer replicate due to **lack of nutrients** and **cellular damage**, resulting in a sharp decline in population
- **Contents inside dead bacteria** are released into the environment after membrane lysis, making these nutrients **available to other bacteria**. The continuous fluctuation of remaining living bacteria forms the **long-term stationary phase (LTSP)**.

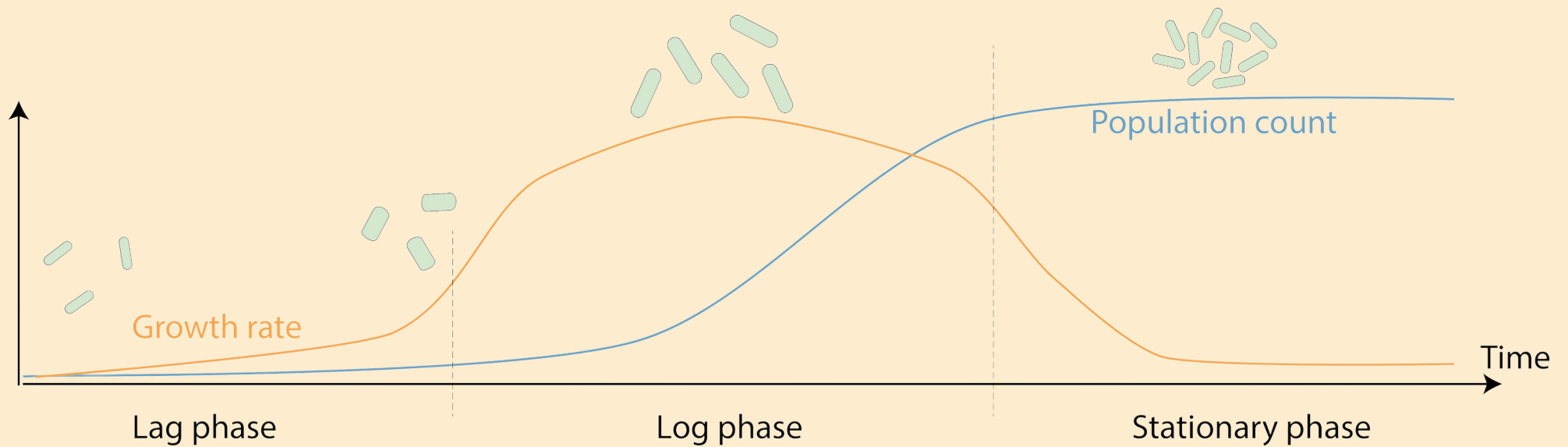


1: lag phase
2: log phase
3: stationary phase
4: death phase

Yay! They survived
it after all~



What we learned today:



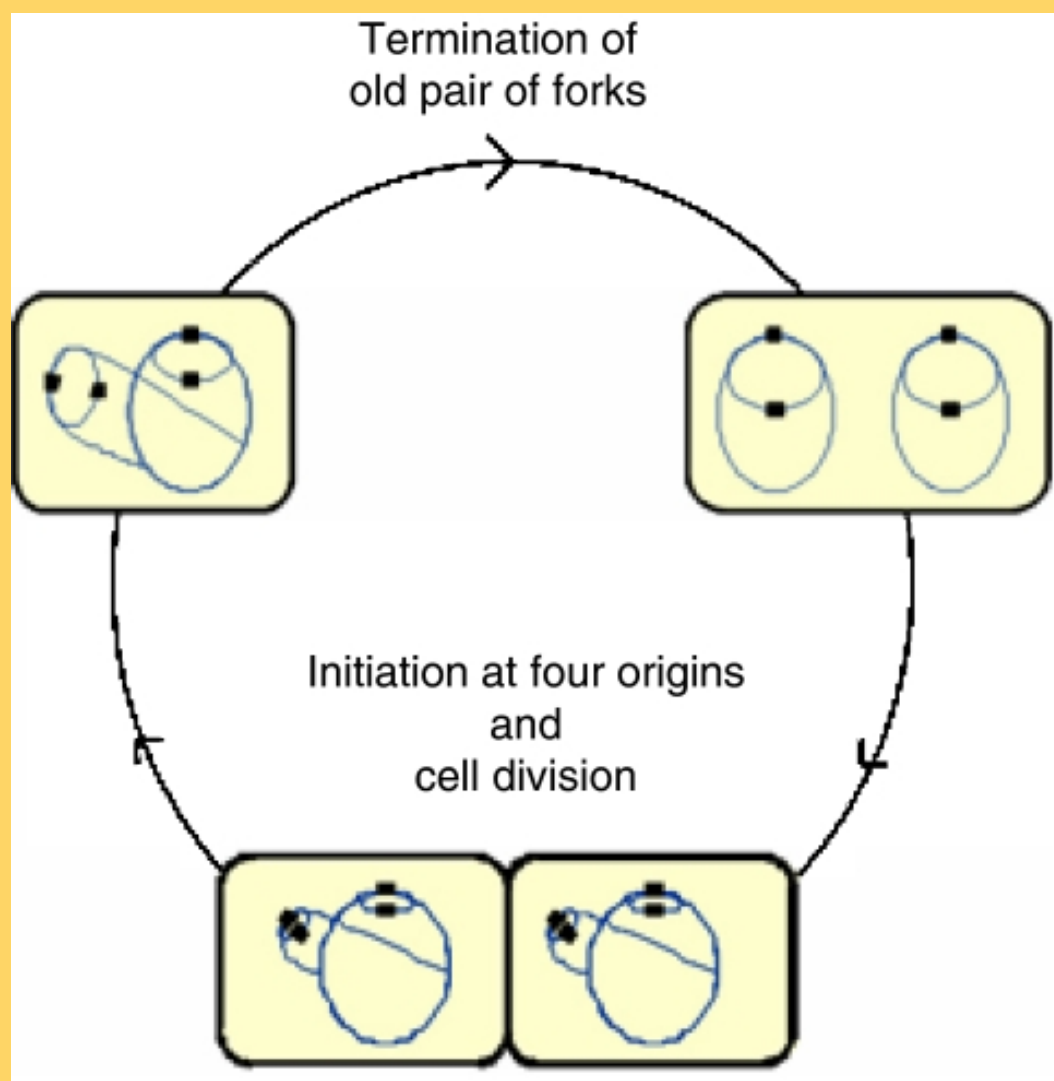
1. The bacterial growth curve consists of lag, log, stationary and death phase, each with different morphology and gene expression profile.
2. Bacterial population increases throughout the growth curve, with maximum growth rate in log phase. Cells become wider and longer when entering log phase and reduce their size after entering stationary phase.
3. Growth curve may differ according to strain, medium, temperature and type of plasmid, but they follow a similar pattern.

Don't forget to give
me a big hug!



Answer to **Think about it !**

When we think about eukaryotic cells, only one round of replication occurs before division. However, in rapidly-growing bacteria, replication starts at multiple origins at the same time as cell division. Therefore, the fast speed of division does not interfere with the the slow speed of replication.



Reference

1. Research paper

(1) Ryabov, A., Kerimoglu, O., Litchman, E., Olenina, I., Roselli, L., Basset, A., Stanca, E., & Blasius, B. (2021). Shape matters: the relationship between cell geometry and diversity in phytoplankton. *Ecology letters*, 24(4), 847–861.

(2) Shi, H., Hu, Y., Odermatt, P.D. et al. Precise regulation of the relative rates of surface area and volume synthesis in bacterial cells growing in dynamic environments. *Nat Commun* 12, 1975 (2021).

(3) Fossum, S., Crooke, E., & Skarstad, K. (2007). Organization of sister origins and replisomes during multifork DNA replication in *Escherichia coli*. *The EMBO journal*, 26(21), 4514–4522.

(4) Jaishankar, J., & Srivastava, P. (2017). Molecular Basis of Stationary Phase Survival and Applications. *Frontiers in microbiology*, 8, 2000.

2. Website

(1) London Biohackspace: OD600 Spectrophotometer

<https://biohackspace.org/projects/od600-spectrophotometer/>

Bye bye! See you next time.
Follow us for more info!



Fluotato teaches: Episode 2

Methods for monitoring bacterial growth status



In **[Fluotato teaches: Episode 1]**, we understood the basic growth characteristics of bacteria. But... have you ever wondered:

Why is it important to know which growth phase your bacteria is in?



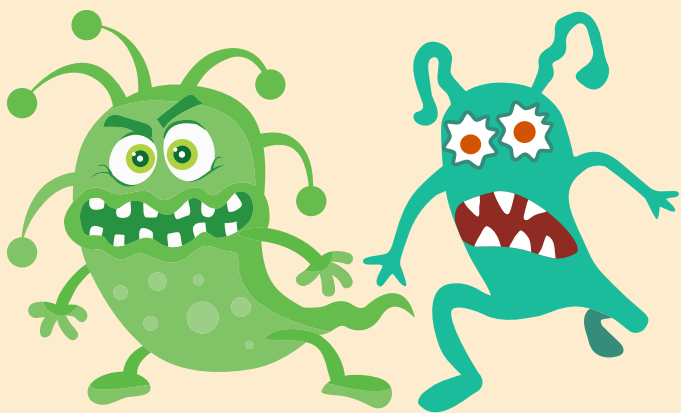
How can this be applied in laboratory research?

In this episode, Fluotato will introduce the importance of monitoring bacterial growth status, as well as common methods used for detection. Let's start by talking about the **applications of E. coli!**

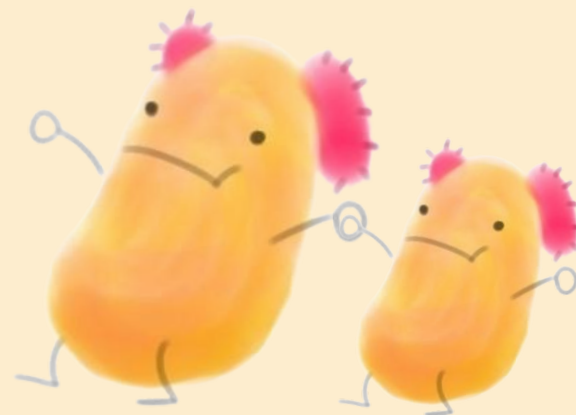
Applications of E. coli

When we speak of E. coli, the first thing that comes to mind may be the **pathogenic strains** that **cause illness**. Yet, there is another group of **laboratory E. coli strains** that are non-pathogenic and beneficial for research usage.

Scientists develop mutant strains from wild type strains in order to achieve a certain function. For example, the **DH5α strain** is designed to enable **easier plasmid insertion**; the **BL21(DE3) strain** is designed for **high-level recombinant protein expression**.



Pathogenic strain



Laboratory strain

- DH5α
- BL21(DE3)

Applications of E. coli

Basic research



- Gene cloning
- Protein expression

Industrial



- Mass production of recombinant proteins

E. coli is often used as a **vector for gene cloning** experiments in basic research settings due to its ease of culture and fast replication rate. It is also used in the industrial field for **mass production of recombinant protein / pharmaceuticals**.

Factors that affect protein production

Many factors affect the **yield and quality of the protein product** in protein expression experiments. Often, an **inductor** (usually IPTG) is added to the bacterial culture at a certain time point to induce high protein expression. Researchers or manufacturers have to **try out to find the optimal condition for induction**.

Inductor concentration

Induction duration

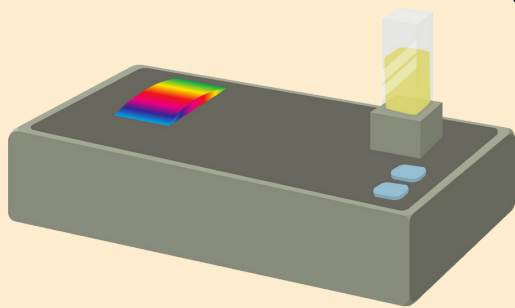
Induction time point

Temperature of induction

Usually, IPTG is added during the **log phase** of bacterial growth, however the accurate time point differs due to protein characteristics. **This is when monitoring growth status comes in handy.** How is it measured?

Methods for monitoring bacterial growth

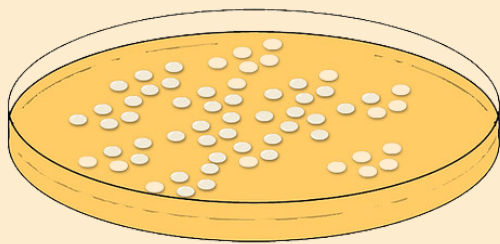
There are many common methods for evaluating bacterial growth, each with pros and cons.



OD₆₀₀ measurement

- The most common method used nowadays
- Pros: **Fast**, sample is recoverable, low cost
- Cons: **Precipitation in liquid culture** (dead bacteria, metabolites, etc.) **reduces accuracy**

Culture cell count



- Pros: **Easy to quantify**, easy to determine presence on contamination, low cost
- Cons: **Requires time for bacteria to grow into colony**, very limited number of species can be grown

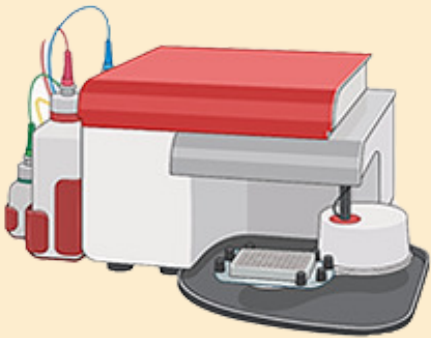
Methods for monitoring bacterial growth



Biochemical assay (ATP, DNA assay)

- Pros: **Fast**, measures ATP from **all living cells**
- Cons: Expensive reagents, **does not provide true cell count**

Flow cytometry



- Pros: **Very accurate enumeration**, combining different stains enables counting of multiple types of cells
- Cons: **Expensive equipment**, not applicable to environmental samples

Quantitative PCR



- Pros: Enumeration can be as broad or specific as desired based on primers used
- Cons: **Expensive and technical-demanding**

Project motivation

Now we understand how researchers evaluate bacterial growth. We have heard from some researchers that:

To test the optimal time point for IPTG induction, I have to **continuously measure OD value every 30 minutes**. I hope there is a more **time-saving** method.



I work in a biopharmaceutical company that produces recombinant drugs. Developing an alternative method that enables **automatic color detection** of different bacterial growth status would **save manpower**.

The decision

Motivated by the need to develop a **time-saving, automatic** method of detecting bacterial growth, with **low cost and low technical threshold**, our team decided to work on this issue as our project goal.

Follow Episode 3 to understand
the details of our project design!



Reference

1. Research paper

(1) Brown DC, Turner RJ. (2022). Assessing Microbial Monitoring Methods for Challenging Environmental Strains and Cultures. *Microbiology Research*, 13(2):235-257.

2. Website

(1) Gold Biotechnology: How Does IPTG Induction Work?

<https://www.goldbio.com/articles/article/how-does-iptg-induction-work>

Bye bye! See you next time.
Follow us for more info!

