Aims:

Geneticists used to call the cell’s reproduction rate as “cell growth rate”. Underlying cell growth are the duplication of genetic materials (DNA), accumulation of cell-building macromolecules (proteins, carbohydrates and lipids) and the division between the daughter cells and their parents. All these biological processes must be completed within the time frame of the “generation time”. How fast cells can complete the duplication of themselves? In ideal circumstances, some common bacterial cells can divide and double every 20 minutes! In this experiment we are going to determine the cell growth rate of the *Escherichia coli* strain TG1. We will

1. Follow the cell number changes during cell growth by measuring the optical density of cell culture.

2. Directly count the number of cells in the cultures by determining the CFU’s (colony forming units) on solid medium.

3. Determine the parameter which indicates the rate of bacterial growth: the mean generation (doubling) time.

in the presence of nutrients → cell proliferation, i.e. divide and increase in numbers
**Growth characteristics of E. coli:**

Population growth in batch culture - 4 distinct phases

- **Lag phase**
  - introduced into fresh medium
  - usually no immediate increase in cell no. / mass occurs
  - adjusting to new environment
  - varies in length - depends on strains and environmental conditions (e.g. older bacteria – takes longer to adjust)

- **Exponential (or log) phase**
  - grow and divide
  - population doubles in equal time intervals (exponential)
  - growth rate constant
  - most rapid and active phase

- **Stationary phase**
  - constant cell numbers but still may be "growing"
  - growth rate balances death rate
  - nutrients depleted and waste products increasing

- **Death / Decline phase**
  - decline in viable cell numbers
  - death rate > growth rate
  - nutrients depleted and toxic wastes increased

**Growth kinetics** (quantitative aspects of exponential growth):

- generation (or doubling) time \( g \) (or \( t_d \))
- population doubles in number during a specified length of time

\[
2^1 - 2^2 - 2^3 - 2^4
\]

\[
2 - 4 - 8 - 16
\]

\[
x_2 = x_1 \cdot 2^n
\]

\[
n = \log_2(x_2 / x_1)
\]

\[
g = t / n
\]

where:

- \( g = t_d \) = generation or doubling time
- \( t = \) time of exponential growth
- \( n = \) no. of generations
- \( x_1 = \) no. of cells at \( t = t_1 \) (initial no. of cells)
- \( x_2 = \) no. of cells at \( t = t_2 \) (final no. of cells)
Materials

General laboratory equipments:

- Autoclave
- Laminar flow cabinet (for drying plates)
- Shaking incubator (fitting with a total of 25 flasks of 50 ml)
- UV/VIS spectrophotometer
- Incubator (37°C)

Consumables and reagents (per pair):

- 1 flask with 25 ml LB medium in a 50 ml flask
- Eppendorf pipettes (1 ml and 200 µl)
- Sterile tips (1 ml, 200 µl)
- 1 Bunsen burner
- matches
- 6 Falcon tubes (5 ml)
- 8 LB plates
- Sterile 1.5 ml Eppendorf tubes (12)
- 1 rack for 1.5 ml Eppendorf tubes
- 1 rack for Falcon tubes
- 2 disposable spreaders
- Distilled water (13 ml in a Falcon tube)
- Sterile water (13 ml in a Falcon tube)
- Liquid LB medium as blank control (5 ml in a Falcon tube)
- 1 ice bucket
- 2 Plastic cuvettes (1 ml, passlength=1 cm)
- 1 log scale graph paper

L-Broth (LB medium):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% yeast extract</td>
<td>0.5%</td>
</tr>
<tr>
<td>1% Bacto Tryptone</td>
<td>1%</td>
</tr>
<tr>
<td>0.5% NaCl</td>
<td>0.5%</td>
</tr>
<tr>
<td>pH 7.2 (pH can be adjusted</td>
<td>0.3 ml</td>
</tr>
<tr>
<td>to 7.2-7.4 by adding 0.3</td>
<td></td>
</tr>
<tr>
<td>ml of 5 N NaOH before</td>
<td></td>
</tr>
<tr>
<td>autoclaving)</td>
<td></td>
</tr>
</tbody>
</table>

*E. coli* strain: TG1 (the strain TG1 is non-pathogenic and non-toxic, so it can be handled on normal bench without gloves)
Experimental Procedures

Day -1

1. Inoculate two conical flasks of 50 ml containing 25 ml of LB medium with a colony of the TG1 strain. The cultures are incubated at 37 °C for 16-18 hours with shaking at 250 rpm. 1.25 ml of the overnight culture is distributed into a sterile 5 ml Falcon tube. This step is done by the instructor.

I. Sampling your cell cultures.

Day 1

Each pair of students will be provided with a conical flask of 50 ml containing 25 ml of fresh LB medium and 1.25 ml of pre-culture for the strain TG1.

2. Pre-warm the 50 ml LB medium (a rich bacterial growth medium) at 37 °C in a shaker for 15 minutes.

3. Start the experiment by adding 1.25 ml of the overnight E. coli pre-culture to the pre-warmed LB medium.

4. Mix well the culture and take 2 ml of the diluted culture using the 1 ml Eppendorf pipette into a 5 ml Falcon tube. Label the tube as t=0. Keep the tube on ice and ready for measuring OD$_{600}$ as t = 0. The flask is returned to the shaker at 37°C and is incubated with shaking at 250 rpm.

5. Take a 2 ml sample of the culture into a Falcon tube every 30 minutes. Remember to label the tubes before transferring the cultures. So you will have the cultures for t = 0.5, 1.0, 1.5, 2.0 and 2.5 hours.

II. Measuring optical density of your samples by spectrophotometer.

This is based on the principle that the extent of light absorption by the cells in suspension is linear with cell mass over the approximate range. However, when the cell concentration is too high, internal light scattering between the cells in suspension reduces the accuracy of measurement.
6. Let the Shimadzu 1240 spectrophotometer "warm up" for at least 15 minutes so you get the correct reading.
7. Choose the photometric option (option 1).
8. To adjust the wavelength to 600nm (green light), press first GOTO WL, then type 600 and press enter.
9. Pipette 1 ml of LB medium (without cells) into a plastic cuvette of 1 ml (passlength=1 cm). Place the cuvette in the machine, close the cover and press Auto-Zero. Confirm that the reading is zero. This serves as the blank reading.
10. Transfer 1 ml of your samples in a fresh plastic cuvette (mix well the cultures before pipetting into the cuvette). Place the cuvette in the machine, close the cover and read the optical density (OD$_{600}$). Remember to keep the remaining of your samples (1 ml) on ice for experiment in the section III (see below).

(N.B. You can only accurately read OD up to a value of about 1.0-1.2. Above this level readings are not accurate. If the reading from your sample is higher than 2.0, make a 10-fold dilution (as before) and record the OD of this)

11. Trace the OD values on the provided paper graph and make comments on the growth curve. Define the different stage of cell growth.

III. Viable counting of bacterial cells.

The usual method employed is to make dilution series of a suspension of bacteria, take a representative volume of the dilutions, plate on the LB medium, incubate these and count the colonies developing after incubation. Providing only those plates containing between about 300 and 30 colonies are counted, the method is reasonably reliable. At colony densities above 300/plate there may be underestimates of the true colony numbers due to competition for and depletion of nutrients; below colony densities of about 30/plate there are problems associated with the statistical errors present in counting such small numbers.

You are asked to count the cell numbers in the cultures at t=1.0 and t=2.0 hours.

12. Set up 12 sterile 1.5 ml Eppendorf tubes containing 0.9 ml of sterile water. You need 6 tubes for the series of t=1.0 and t=2.0 hours. Label the tubes as $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ for each series. Using a pipette with a sterile tip, aseptically transfer 0.1 ml of your culture into the first tube ($10^{-1}$ dilution). Discard the tip.

13. Mix well by reverting the tube 5-6 times and using a fresh pipette tip, transfer 0.1 ml to the next dilution tube ($10^{-2}$ dilution). Discard pipette tip. Proceed in this way with your dilutions down to $10^{-6}$. You will have 6 dilutions for the cultures at t=1.0 h and t=2.0 h respectively.

14. Mix well the diluted cultures and using a fresh pipette tip, take 0.1 ml of suspension at the $10^{-6}$, $10^{-7}$, $10^{-8}$ and $10^{-9}$ dilutions and transfer it to the surface of an LB agar plate. You may use the same 0.1 ml pipette for each sample, as long as you plate the most diluted sample first. Remember that you need first to label the plates with
your name and the dilutions before plating your cells. So you will have a total of 8 plates.

15. Take a spreader and spread out your bacterial dilution over the whole surface of the plate. Start with the most diluted sample so that you can use the same spreader for the 4 plates of the same dilution series.

16. Once dry, place the plates in appropriate box for incubation at 37°C for overnight.

17. In the next lab period, select plates of any dilution with between 30 – 300 colonies and accurately score the number of colonies. Present the results along with the corresponding OD data in the format shown below:

<table>
<thead>
<tr>
<th>Time (h):</th>
<th>1 (or 2) hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD\textsubscript{600} :</td>
<td></td>
</tr>
<tr>
<td>Viable Count:</td>
<td></td>
</tr>
<tr>
<td>\textbf{10}^{-3} dilution:</td>
<td></td>
</tr>
<tr>
<td>\textbf{10}^{-4} dilution:</td>
<td></td>
</tr>
<tr>
<td>\textbf{10}^{-5} dilution:</td>
<td></td>
</tr>
<tr>
<td>\textbf{10}^{-6} dilution:</td>
<td></td>
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</tbody>
</table>

Then calculate:
- The cell density (cells/ml) at t=1 and t=2 hours
- No of cells in 1 OD\textsubscript{600} based on the average cell density (cells/ml) at t=1 and t=2 hours
- The generation (doubling) time of TG1

**IV. Experimental report**
1. Aims and experimental principles (keep to about half a page – straight copying of the protocol is strongly discouraged!)
2. Results
3. Discussions