# Biofilm Formation — Crystal Violet (CV) Staining

## Introduction

Biofilms are surface-attached microbial communities protected by an extracellular matrix and known for their high antibiotic resistance. This protocol utilizes the high-throughput microtiter dish assay—quantified via crystal violet staining—to evaluate the early-stage biofilm formation dynamics of the model organism *Pseudomonas aeruginosa*.

Reference:
1. O'Toole, G. A. (2011). Microtiter dish biofilm formation assay. *Journal of Visualized Experiments (JoVE)*, (47), e2437.
2. Wu, K., Mo, Y., Duan, M., Zheng, Y., Chang, Y., Ding, M., ... & Duan, N. (2025). A structure-guided bifunctional aptamer mitigates Pseudomonas aeruginosa virulence and biofilms by disrupting las and rhl quorum sensing systems. *Analytical Chemistry*, *97*(37), 20291-20302.

## Materials

- Bacterial strain
  - Pseudomonas aeruginosa PAO1
- Aptamer
- 0.1% Crystal violet
- 30% acetic acid
- 1x PBS
- 96-well plates
- 37 °C incubator (static)
- Microplate reader ($OD_{550}$)

## Procedure

### Preculture

1. Culture bacterial strain in appropriate volume of LB broth overnight for the subsequent process.

### Subculture

2. Transfer the precultured bacteria into a 96-well.

   198 μL Bacterial suspension (confirm OD600=0.1) + 2 μL drug (concentration depends on demand)/per well

3. every group should be with more than triple repeat.

**reagent volume**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Reagent | Concentration | Per well | |
| 2 | Bacterial suspension (0.2) | $OD_{600}$ ≈ 0.1 | 198 µL | |
| 3 | Aptamer / control | 50 µM (→ 0.5 µM final) | 2 µL | |
| 4 | Crystal violet | 0.1% (v/v) | 200 µL | |
| 5 | Acetic acid | 30% | 200 µL | |

4. culture in 37 °C, 48 h
5. Discard the supernatant, Add 200 µL 1x PBS and gently shake the well for 1min.

   Never stick to the bottom of the well when sucking the supernatant, as some biofilm attached there might be destructed.
6. Discard supernatant, Add 200 µL 0.1% CV; stain 15 min at room temperature.
7. Discard supernatant, and add 200 µL 1x PBS and gently shake the well for 1min.

   Never stick to the bottom of the well when sucking the supernatant, as some biofilm attached there might be destructed.
8. Discard supernatant
9. Stand for 8h-overnight at room temperature until the well is dried out of any fluid.
10. and add 200 µL 30% acetic acid and shake the well for 10 min.
11. Gently pipet the liquid and transfer it to fresh wells.
12. Read $OD_{550}$.