Bacterial Transformation

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Background

In this lab, you will be recreating a pivotal experiment in the history of genetics – in doing so, you will be using a technique used now routinely by synthetic biologists to modify the behavior of microbes. Bacterial transformation is the process of introducing new DNA into a bacterial cell, so that DNA can be expressed and change cell structure or behavior.

The fact that DNA is the transforming material was first demonstrated clearly by an experiment conducted by Avery, Macleod, and McCarthy in 1944. You can read more about this historic and important experiment below:

You will be carrying out a modified version of this experiment using a harmless strain of *Escherichia coli*. One strain is expressing a protein known as Red Fluorescent Protein, which gives the cells a characteristic red color. These cells have been heat-killed and all the proteins and DNA have been released from the cell. You will then use this lysate, either directly or after treatment, to transform another *E. coli* strain.

Pre-Lab Questions

Define the following terms:

Chemically Competent Cells: _____

Lysate: _____

Culture: _____

uL (Microliter): _____

Why was the Avery, Macleod, and McCarthy experiment important? How did it contribute to our understanding of genetics?

What is DNase? Why is it used in this experiment (as well as Avery, Macleod, and McCarthy's experiment)?

What is Red Fluorescent Protein? Where was it first identified, and how is it used in synthetic biology?

Materials

1mL of clarified cell lysate from *E. coli* with plasmid pBBa_J04450 (RFP plasmid). Keep refrigerated or frozen until ready.

Competent cells (3 microcentrifuge tubes with 100uL each), *E. coli* prepared by TSS method. Keep refrigerated or frozen until ready.

5uL fixed volume micropipette and tips

1mL Syringes

Empty 1.5mL microcentrifuge tubes

DNase solution (diluted in 50% glycerol). Keep refrigerated or frozen until ready.

3M NaOH

30% AcOH (Acetic Acid)

LB or SOC Media

5x LB Plates with Chloramphenicol (30ug/mL)

Plate spreader

Diluted bleach safety, for safe disposal or spill cleanup

Supplied at home:

Heating element or water bath at 42C

Ice bucket

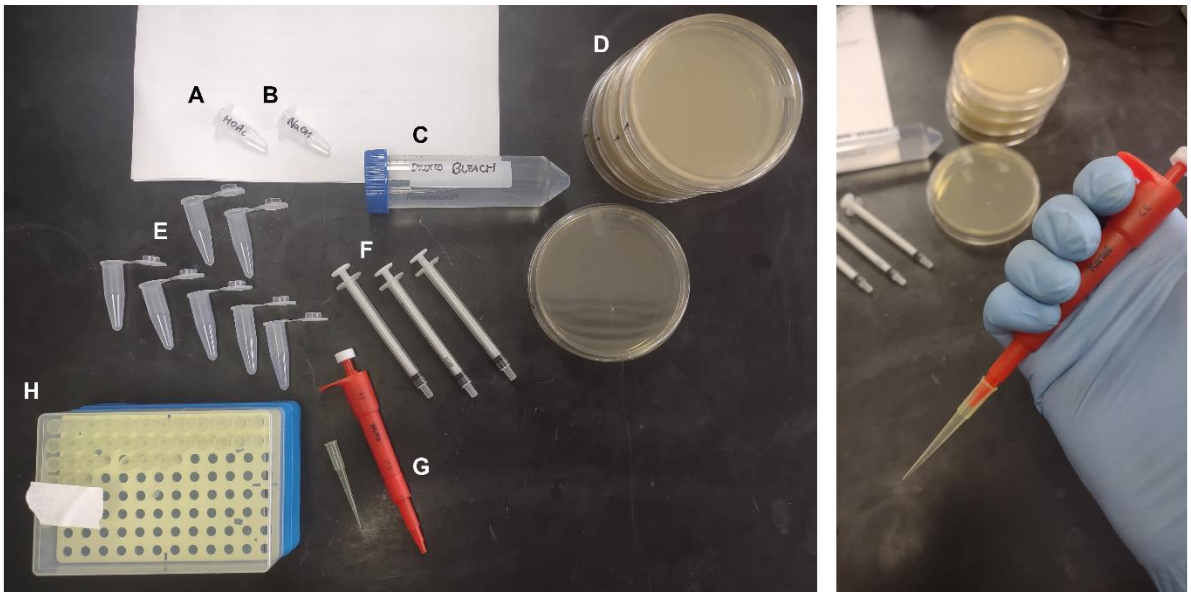

Fig 1. Materials. Left panel: Kit materials include Acetic Acid (A), NaOH (B), Diluted Bleach (C), LB + Chloramphenicol plates (D), 1.5mL Microcentrifuge tubes (E), 1mL Syringes (F), Fixed volume micropipettor (G) and micropipette tips (H). Right Panel: proper way to attach a pipette tip and hold the micropipette.

Protocol / Procedure

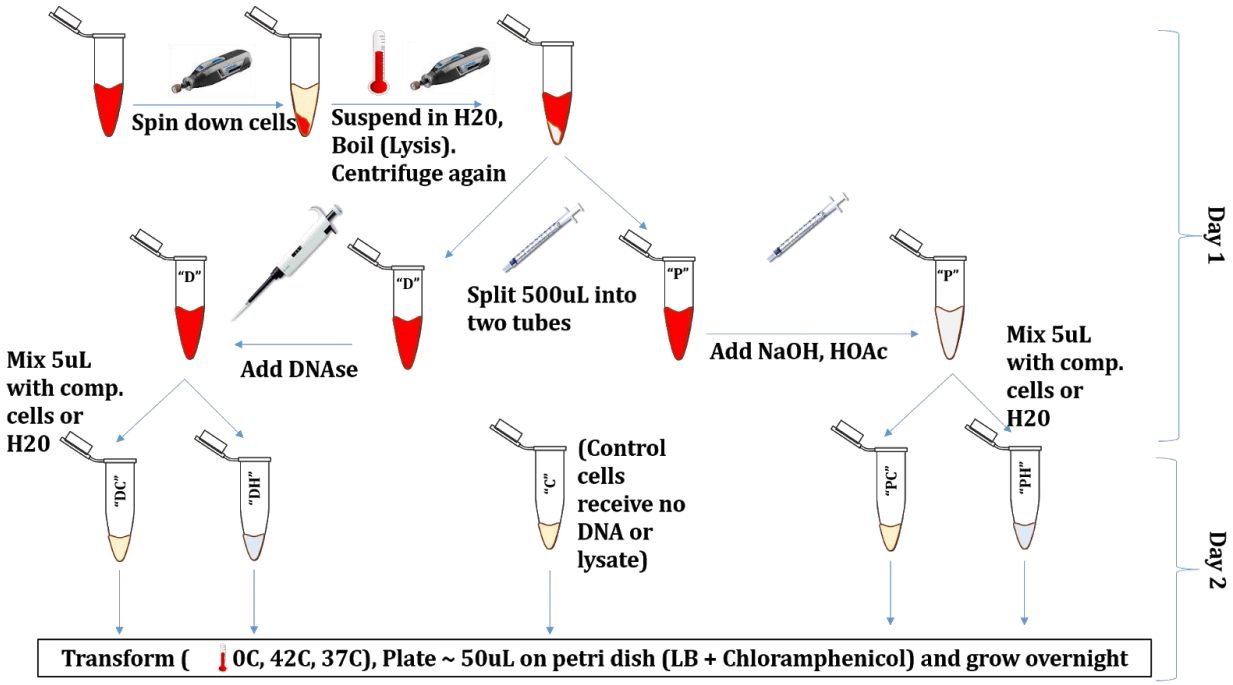

Fig 2. Graphical Protocol. In this protocol, the first few steps (which use either a centrifuge or a Dremel tool with a special adaptor to spin cells) have already been prepared and the lysate is provided directly.

Use of micropipette

Whenever 5uL of a solution needs to be transferred (or some multiple of this), you can use the fixed volume pipette. To do this, place a disposable tip on the pipette. Depress the plunger gently, put the tip into the solution to be transferred, and release the plunger. The liquid should now be in the pipette tip. Then, place the tip in the receiving vial and depress the plunger again to dispense the solution. Use a new tip for each pipetting task!

Lysate Treatment

Split the cell lysate into two separate tubes, labelled “D” and “P” – 0.5mL of cell lysate into each (use the syringe to transfer this volume).

> Tube D: 5uL of DNAse solution is added to this tube and incubated at room temperature for at least 10 minutes (a slightly higher temperature, up to 37C, can also be used). Record the color of the lysate after this treatment.

Tube P: 10uL of the NaOH solution should be added and the tube inverted. Record the color of the lysate after this step, and again after addition of 10uL of AcOH.

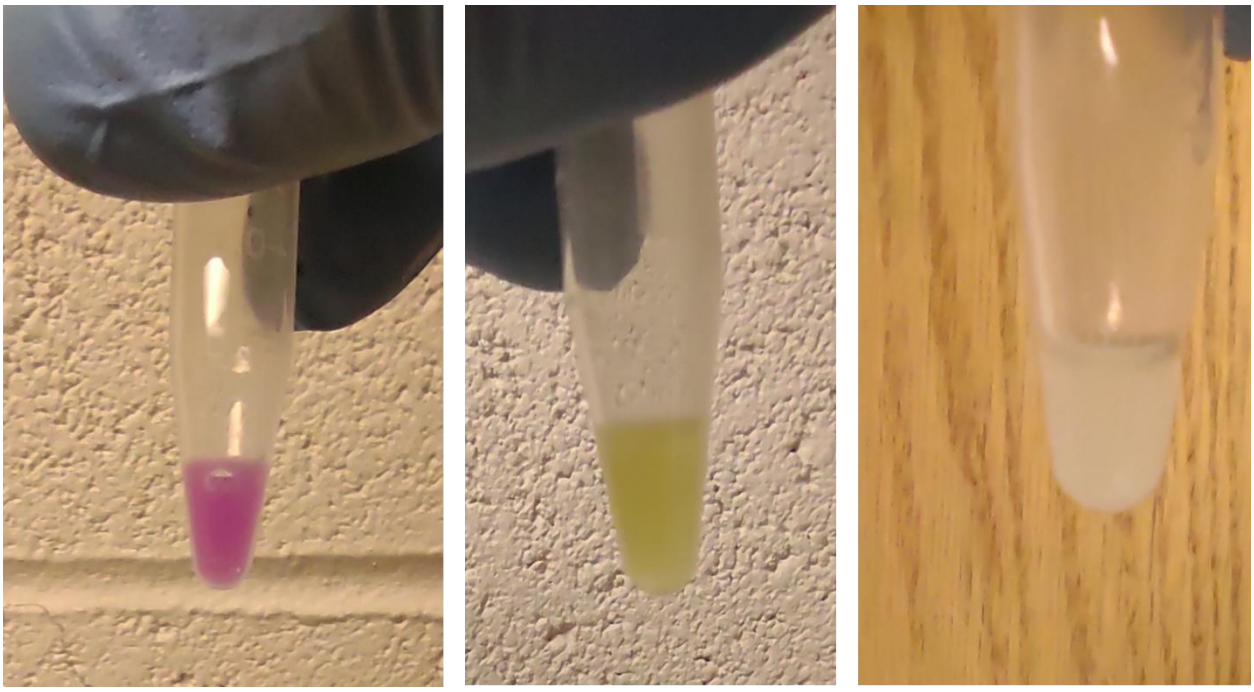

Fig 3. Lysate color. You should observe a pink/red solution (not cloudy if cell lysis is effective) at the outside, as the red fluorescent protein is intact (Left panel). Addition of NaOH will alter this protein and change the color (Middle panel). Further modulation of the pH with Acetic acid will finally abolish the color observed (Right panel).

Transformation Protocol

- 3 microcentrifuge tubes of competent cells (100uL each) should be labelled “DC”, “PC”, and “C” – the C in each stands for competent cells.
- 2 microcentrifuge tubes of water (100uL each) should be labelled “DH” and “PH” – the H stands for water (H_2O)
- To the “D_” tubes (“DC” and “DH”) add 5uL of the DNase treated lysate (from previous step).
- To the “P_” tubes (“PC” and “PH”) add 5uL of the pH-adjusted lysate.
- Keep these tubes on ice as they are assembled and until all ready for heat shock. Take this time to prepare a warm water bath (42C) if not already prepared.
- Heat shock by placing these tubes in the warm water for 30 seconds
- Add 1mL of SOC media (TB or LB as alternatives), and incubate for at least 30 minutes for 37C.

- Plate 0.1mL of each transformation onto a separate LB + Chloramphenicol agar plate
- Predict the outcome of each treatment, and record the results after incubation either overnight at 37C or after at least 48 hours at room temperature

Transformation	Expected Result	Observed Result	Conclusion
DC			
DH			
PC			
PH			
C			

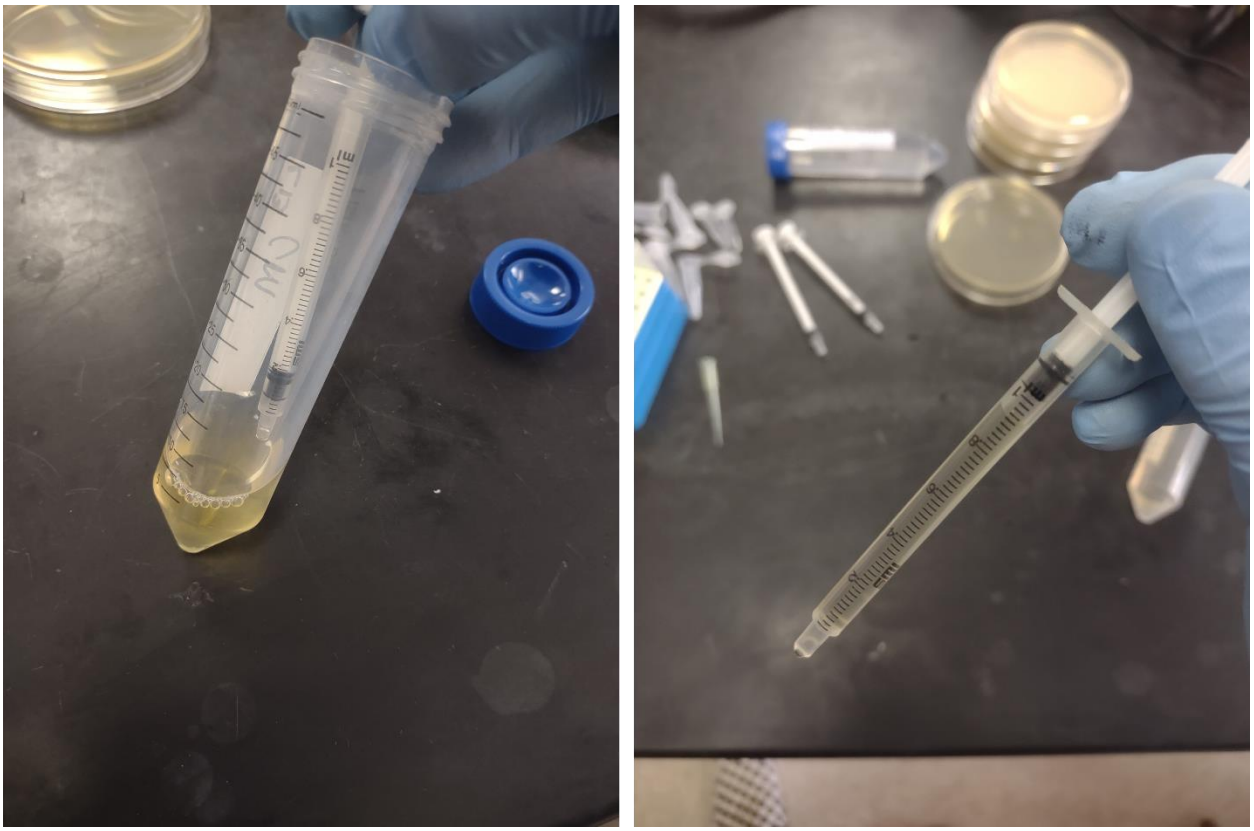

Fig 4. Addition of SOC media and use of the 1mL syringe. For any steps in the protocol that require transfer of 0.1 to 1mL, you can use the disposable syringe. For example, during the transformation protocol, you can place this into the SOC media container (Left panel) and draw up on the plunger until the liquid fills to the 1mL mark (Right panel).

Post-Lab Questions

What is the composition of the clarified cell lysate?

Explain the color change observed on the addition of NaOH first, and Acetic acid second. What is changing, and why?

Why is tube “C” included? What does this represent?

Why are tubes “DH” and “PH” included? What does this represent?

Why is transformation important? How is it used by synthetic biologists?

How does transformation impact the spread of disease and antibiotic resistance?

Claim-Evidence-Reasoning

What is the transforming principle or molecule in this experiment?

Claim:

Evidence:

Reasoning:

Notes