

# Chapter 1

## Bacterial Transformation

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## Introduction

This experiment demonstrates the process of transformation by which the bacteria take up DNA from the surrounding medium, incorporate it, and acquire an altered genotype that is heritable. The students enjoy doing an experiment similar to that of Avery et al. (1944) that showed that DNA was the hereditary material.

This experiment is performed in a first-year Introductory Biology course and has been used in a Genetics and Biochemistry course. The experimental procedure can be completed in a 3-hour laboratory with the students working in pairs. The plates are incubated at 37°C for 2 days at which time they can be read or stored in the cold until the following laboratory period.

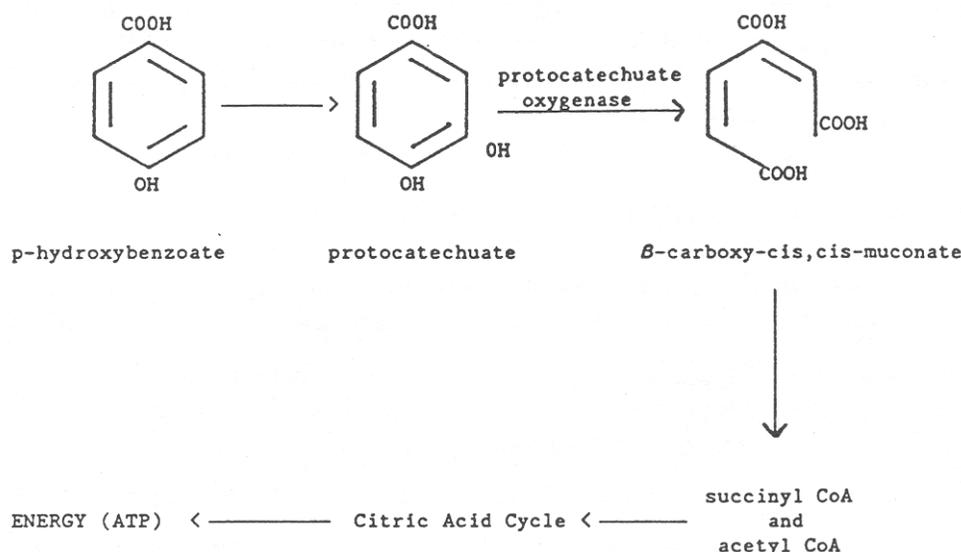
This laboratory introduces the student to selective growth conditions, sterile techniques, dilutions, and plating. Using p-hydroxybenzoic acid as a carbon source has advantages in that there is very little contamination of the plates. *Acinetobacter* is a stable strain; there are very few reversions and it is transformed more readily than other strains (approximately 10–20% transformation in the student labs).

The main preparation for this laboratory is making plates. The Hutner's "Metals 44" and the concentrated base can be prepared in advance and stored for at least 1 year. The other stock solutions can be made several days before being used. Because the unusual carbon source allows little contamination of the plates, they can be made ahead and stored. The two strains of *Acinetobacter* can be obtained directly from the authors.

## Student Outline

### Introduction

Bacterial cells in the proper physiological state can absorb molecules of DNA from their environment. Once inside the cell, these DNA molecules can recombine with the DNA of the cell and become a heritable portion of the bacterial chromosome. This process is called transformation and was the basis of the experiments presented in 1944 by Avery, McCarty, and MacLeod which demonstrated that deoxyribonucleic acid is the carrier of the genetic material. The process of transformation involves three steps: (1) Fragments of DNA from a donor bacterial cell bind to the surface of a recipient cell. (2) Following the initial binding, the DNA fragments are taken up by the recipient cell and are no longer exposed to the environment. (3) Inside the cell, the donor cell DNA fragments are incorporated into the recipient cell's genetic material by recombination. Recombinant bacterial cells can be identified by choosing donor cell DNA carrying genes that confer an advantage to the recipient cell under selective growth conditions. Under these conditions, only the recombinant bacterial cells grow and divide, eventually giving rise to colonies of transformants. The bacteria you will be working with today are derivatives of *Acinetobacter calcoaceticus*, a common soil bacterium. These bacteria are capable of utilizing aromatic compounds, like para-hydroxybenzoate, as their sole source of carbon and energy according to the metabolic pathway shown in Figure 1.1.



**Figure 1.1.** Metabolic pathway of p-hydroxybenzoate.

The two strains of *A. calcoaceticus* that will be used today are designated ADP-1 and ADP-6. ADP-1 is a wild-type strain capable of growing on p-hydroxybenzoate culture medium. These cells contain all the enzymes required to convert p-hydroxybenzoate to succinyl CoA and acetyl CoA. ADP-6 is a mutant strain in which the gene coding for the enzyme, protocatechuate oxygenase, is defective. As a result, ADP-6 cells do not contain any protocatechuate oxygenase enzyme molecules. Since the cells cannot convert protocatechuate to β-carboxy-cis, cis-muconate in the pathway shown in Figure 1.1, ADP-6 cannot grow on p-hydroxybenzoate. In today's experiment, you will prepare DNA from ADP-1 cells (containing the gene for protocatechuate oxygenase) and use this DNA to transform ADP-6 cells. Transformants of ADP-6 will be

identified based upon their ability to grow on p-hydroxybenzoate following incorporation of the protocatechuate oxygenase gene (from ADP-1 DNA) into their genetic material. Untransformed ADP-6 cells will not grow under these conditions.

### Protocol

Use good sterile technique at all times. Your TA will demonstrate sterile technique and dilution, spreading, and streaking of bacterial cells.

#### A: Verification of Growth Properties

*Acinetobacter calcoaceticus* is grown in petri dishes on solid agar gel. The agar is supplemented with nutrients (minerals and a carbon source) required by the bacteria for growth. In today's lab, the nutrient agar plates contain p-hydroxybenzoate (pHB) as the only carbon source.

Draw a line on the bottom of a pHB plate to divide it in thirds and label one third, ADP-1, the other third, ADP-6, and the remaining third, ADP-1 DNA. Using the technique demonstrated by your TA, streak out a loopful of ADP-1 cells in the labelled area; repeat with ADP-6 cells. After finishing step B5, streak a loopful of ADP-1 lysate on the remaining third of the plate. Label (name, lab day, room number) the top of the plate and set aside. The cells will be grown for 2 days at 37°C then stored in the cold until next week.

#### B: Preparation of Donor DNA from ADP-1

1. Spin the ADP-1 culture for 10 minutes in a table top centrifuge ( $\sim 500 \times g$ ). Be sure to use a balance tube in the opposite slot of the centrifuge. After centrifugation, discard the supernatant.
2. Thoroughly re-suspend the cell pellet in 2.0 ml of warm lysis buffer. Lysis buffer contains sodium citrate and a detergent (sodium dodecyl sulfate, SDS). The detergent dissolves the ADP-1 cell membrane releasing DNA into solution.
3. Incubate the cells in lysis buffer at 60°C for 30 minutes in a water bath.
4. After incubation, remove the tube containing the lysed cells and note that the solution has become more viscous. This is a common property of DNA and other large polymers in solution. Vigorously shake the tube several until the viscosity is decreased. This shears or snaps the DNA polymer into small fragments some of which will contain the gene for protocatechuate oxygenase.
5. To verify that all ADP-1 cells were lysed, streak a loopful on the verification plate in step A. The DNA solution will be diluted in SSC buffer containing saline (0.9% NaCl) and sodium citrate. Label six SSC tubes (containing 4.5 ml of SSC) 10-1, 10-2, 10-3, 10-4, 10-5, and 10-6. Make serial dilutions of your DNA solution as follows: with a 1.0 ml pipet, transfer 0.5 ml of DNA solution to the tube labelled  $10^{-1}$ , and mix. The DNA is now one-tenth ( $10^{-1}$ ) as concentrated as the original solution ( $0.5 \text{ ml DNA solution} + 4.5 \text{ ml SSC} = 0.5 \text{ ml DNA solution}/5.0 \text{ ml total solution} = 1/10 = 10^{-1}$ ). Repeat this procedure with a new pipet, transferring 0.5 ml of  $10^{-1}$  diluted DNA into the tube labelled  $10^{-2}$  and mix. Continue this procedure through the  $10^{-6}$  dilution tube. You should then have a set-up that matches that summarized in Table 1.1.

- Remember to use a clean pipet for each transfer and to mix the tube contents thoroughly between transfers.

**Table 1.1.** DNA dilution summary.

Tube dilution	SSC (ml)	DNA solution
$10^{-1}$	4.5	0.5 ml DNA
$10^{-2}$	4.5	0.5 ml $10^{-1}$ dilution
$10^{-3}$	4.5	0.5 ml $10^{-2}$ dilution
$10^{-4}$	4.5	0.5 ml $10^{-3}$ dilution
$10^{-5}$	4.5	0.5 ml $10^{-4}$ dilution
$10^{-6}$	4.5	0.5 ml $10^{-5}$ dilution

### C: Transformation of ADP-6

- Spin the ADP-6 culture as you did in step B1 for the donor culture and discard the supernatant.
- Pour the contents of an SSC tube (4.5 ml) onto the cell pellet and re-suspend the cells. Be sure the pellet is completely suspended. Use a vortex mixer if necessary.
- Take seven pHB plates and label them as follows: ADP-6/0 (no DNA), ADP-1 DNA (no ADP-6), ADP-6/100 (undiluted DNA), ADP-6/10-3, ADP-6/10-4, ADP-6/10-5 and ADP-6/10-6. The number after the slash mark refers to the ADP-1 donor DNA dilution. See Table 1.2.
- With a 1.0 ml pipet, place 0.1 ml of the ADP-6 cell suspension from step 2 on the agar. Using a clean pipet for each dilution, place 0.1 ml of the appropriate DNA dilution (or 0.1 ml SSC for the ADP-6/0 plate) next to the cells.
- Using the technique demonstrated by your TA, mix the cells and DNA and spread them evenly over the agar with a sterile (flamed), triangular glass rod. Flame and cool the rod after each spread.
- Label the plates (name, lab day, room number). Place all 10 plates, including two plates from part D, in a stack (growth properties, transformation, DNase) and tape securely. Put plates on the front bench. Your TA will place the plates upside down in a 37°C incubator. The plates will be incubated for 2 days then stored in the cold for counting next week.

**Table 1.2.** Transformation plate summary

Plate label	ADP-6 cell suspension (ml)	ADP-1 DNA
ADP-6/O	0.1	0.1 ml SSC--no DNA
ADP-1 DNA	–	0.1 ml undiluted DNA
ADP-6/10 <sup>0</sup>	0.1	0.1 ml undiluted DNA
ADP-6/10 <sup>-3</sup>	0.1	0.1 ml 10 <sup>-3</sup> dilution
ADP-6/10 <sup>-4</sup>	0.1	0.1 ml 10 <sup>-4</sup> dilution
ADP-6/10 <sup>-5</sup>	0.1	0.1 ml 10 <sup>-5</sup> dilution
ADP-6/10 <sup>-6</sup>	0.1	0.1 ml 10 <sup>-6</sup> dilution

**D: Effect of DNase on Transformation**

1. Set up and spread two additional ADP-6/10-3 plates and label them DNase 0 and DNase 30.
2. Immediately after spreading, spray the plate labelled DNase 0 with a DNase solution. DNase or deoxyribonuclease is a hydrolytic enzyme that digests DNA.
3. Place both plates in the 37°C incubator. After 30 minutes of incubation, remove the DNase 30 plate and spray with DNase as in step 2.
4. Label the DNase plates (name, lab day, room number), and place them in the stack for incubation.
5. DNA uptake exercise: Plan a time course experiment to determine when the maximum amount of DNA is taken up by the recipient cells.

**E: DNA Isolation**

For DNA to come out of solution, one needs a relatively high concentration of DNA in a high salt solution and a ratio of 2.5 volumes of ethanol to 1 volume of DNA solution.

To 1.0 ml of DNA solution (after ADP-1 lysis in SDS, step B4), add 2.5 ml of 95% ethanol. Hold the test tube at an angle and let the ethanol slowly run down the side. Cloudiness appears at the ethanol interface. Gently shake the solution, what happens?

**F: Counting** (*next week*)

1. Check the growth properties plate from part A noting which strain grew and which didn't on the pHB plate
2. Count the number of transformed colonies (each arising from a single transformed cell) on the ADP-6/10<sup>-5</sup> and 10<sup>-6</sup> plates in part C. Count the colonies by turning the plate over and marking the plate bottom with a marking pen as each colony is counted. If possible, estimate

the number of colonies on the ADP-6/10<sup>-3</sup> and ADP-6/10<sup>-4</sup> plates by counting one quarter of the plate and multiplying that number by four (try to pick a representative quadrant if the colonies are not evenly distributed over the plate). Count the number of colonies on the ADP-6/0 plate and the ADP-1 DNA plate.

3. Count the number of colonies on the ADP-6/10<sup>-3</sup> plates treated with DNase at 0 and 30 minutes after spreading. Compare to the untreated ADP-6/10<sup>-3</sup> plate in part C.

### G: Laboratory Report

1. Which strain of *Acinetobacter calcoaceticus* grew on the pHB plate in part A? Explain the results on this plate.
2. As the concentration of ADP-1 donor DNA added to the mutant ADP-6 strain was increased from 10<sup>-6</sup> to 10<sup>0</sup> dilution in part C, what happened to the number of transformed colonies observed? Why? Is the number of colonies proportional to the DNA concentration? What result would you expect, on the basis of your results, from adding more DNA? Explain.
3. Suggest two reasons why it did not contain any donor DNA. Would you expect any growth on the ADP-1 DNA plate? Why is this plate included as a control?
4. Compare the number of transformed colonies on the ADP-6/10<sup>-3</sup> plate in part C with the ADP-6/10<sup>-3</sup> plate treated with DNase at 0 and 30 minutes in part D. How do you account for the different numbers of transformants on each plate? Assuming that DNase, RNase, and protease cannot enter the bacterial cells, would you expect to see the same results if the plates were sprayed with RNase or protease instead of DNase? Why?
5. Graph and discuss the results of the DNA uptake exercise.
6. When you made your DNA preparation in part B, the initial concentration of ADP-1 cells in lysis buffer was about  $5 \times 10^8$  cells/ml.
  - (a) Assuming that each cell contains one protocatechuate oxygenase gene, what is the theoretical number of protocatechuate oxygenase genes available for transforming ADP-6 in 0.1 ml of 10<sup>-5</sup> dilution of the DNA solution you prepared?
  - (b) What percent of this theoretical number actually gave rise to transformants on the ADP-6/10<sup>-5</sup> plate?
  - (c) Give two reasons why the number of transformants observed on the ADP-6/10<sup>-5</sup> plate was less than the theoretical maximum.

## Sterile Technique

Genetic studies with bacteria are complicated by the presence of many potential contaminating microorganisms in the air and on all laboratory furniture and equipment. It is therefore necessary to use sterile containers, media and other equipment in order to obtain satisfactory results. Wash your hands before and after working with bacteria.

**Lab bench:** Always wipe off your bench area with 70% alcohol before you begin to work.

**Petri plates:** Do not open petri plates until you are ready to use them. When you open a plate, raise the lid only as high as necessary to pipet or streak cells. Close the lid as soon as you are finished. When plates are placed into the incubator, they should be put in upside down. This prevents water from condensing on the lid and “raining” onto the cells growing on the agar surface. Always label your plates. Write on the bottom of the plate and include your name, lab day, and room numbers.

**Pipets:** Individually wrapped, sterile, plastic pipets should be used at all times. Each pipet should be unwrapped only when it is needed. Never touch the delivery tip region of the pipet with your fingers. Never put pipets on the lab table: if they have already been used, they will contaminate the surface with bacteria; if they are sterile, they will be contaminated. Containers will be provided for used pipets.

**Other sterile material:** You will be provided with capped tubes of sterile SSC buffer for dilution. Again, minimize the time that these tubes are open during dilution. Always flame the mouth of the tubes before making any transfers (described later).

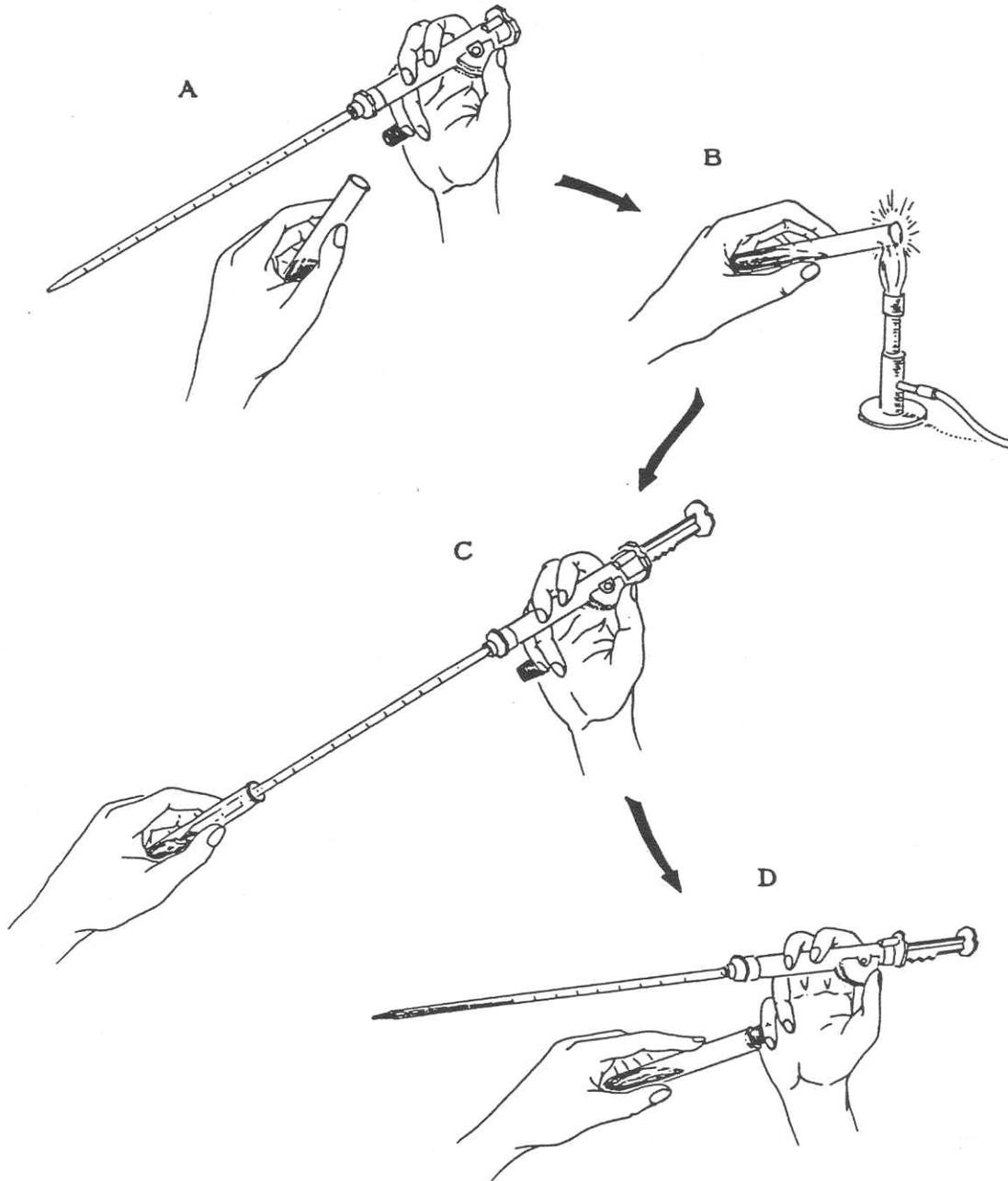
## Spreading and Streaking of Bacterial Cells

Cells are spread on petri plates with a triangular glass spreader. After pipetting a solution of cells onto the agar surface, the cells should be spread out over the surface with a flame sterilized spreader. (Be sure spreader is cool before touching bacteria!) The objective is to obtain an even distribution of cells over the surface of the plate. Alternatively, cells can be streaked across an agar surface. A flame-sterilized, wire inoculating loop is cooled then dipped into a bacterial culture and a loopful of cell suspension is gently streaked in a zig-zag pattern across the surface of the plate without backtracking over the path.

## Sterile Transfer of Cells and Solutions

1. Pick up the tube containing the cell suspension or solution and holding a pipet or inoculating loop in your right hand, remove the plug or cap of the tube with the fourth and fifth fingers of your right hand as illustrated in Figure 1.2A. Keep the tube slanted as much as possible to prevent contaminants in the air from falling into the tube.
2. Pass the open end of the tube through a flame. It is not necessary to heat the tube; any contaminating microorganisms lurking on the edge ready to fall in will be destroyed by the flame (Figure 1.2B).
3. Insert the pipet or inoculating loop into the solution and withdraw the sample (Figure 1.2C). Replace the cap on the tube (Figure 1.2D).

4. You are transferring to another tube, remove its cap or plug, pass the open end through a flame and deliver the sample. Replace the cap. If you are transferring to a petri plate, raise the lid slightly, streak or pipet the sample and replace the lid.



**Figure 1.2.** The proper technique for sterile transfer of cells and solutions.

**Materials**

## 1. Prepared material:

- pHB plates (9 plates per pair)
- SSC dilution tubes (5 tubes per pair)
- Lysis buffer (50 ml in 60° water bath)
- Atomizer with DNase (1 per lab, in refrigerator or on ice)
- ADP-1 culture (1 tube per pair)
- ADP-6 culture (1 tube per pair)

## 2. General laboratory equipment:

- 37°C incubator
- Water bath, 60°C (1)
- Clinical centrifuges, horizontal (swinging bucket) rotor, 15-ml shields (1 per 4–6 students)
- Vortex mixers (1 or more)
- Bunsen burners, matches or striker (1 per 2–4 students)
- Bacteriological loops for streaking (1 per pair)
- Glass spreaders (1 per pair)
- Pipets, sterile 1-ml and 5-ml (individually wrapped, plastic, disposable) (12 per pair)
- 70% alcohol, to sterilize glass spreaders (250-ml beaker, 1/3 full, 1 per 4 students)
- Beakers, 125-ml, for bacterial supernatants (1 per pair)
- Test tube racks
- Masking tape
- Marking pens

3. To grow *Acinetobacter calcoaceticus* (ADP) cultures

ADP-1: Stationary overnight culture

ADP-6: Logarithmic culture (1:10 dilution of an overnight culture grown 4 hours before the laboratory)

Daily cultures: All daily cultures are grown at 37°C, shaking, in Basal Medium + 1% yeast extract. Initial cultures are started 48 hours prior to the laboratory by transferring a loop of cells to 5 ml Basal Medium + 1% yeast extract, 37°C, shaking, overnight. 16 × 100-mm screw-capped tubes are used to grow *Acinetobacter* in the Basal Medium with 1% yeast extract. The bacteria can be centrifuged directly in these tubes in the IEC clinical centrifuge. The day preceding each laboratory (at about 4 or 5 p.m.), overnight cultures are started by inoculating 0.1 ml of the cells into 5 ml of medium at 37°C, shaking.

Stock cultures: ADP-stock cultures are grown on 10 mM sodium succinate plates. Use the same recipe for pHB plates (minus p-hydroxybenzoic acid) using 10 ml per liter of 1 M sodium succinate. The stocks are grown at room temperature and transferred every 6–8 weeks.

## 4. Standard saline citrate (SSC): 0.15 M NaCl + 0.015 M Na.citrate, pH 7.0

For 1 liter: Dissolve 8.8 g NaCl, 4.4 g Na.citrate.2H<sub>2</sub>O, adjust pH to 7.0. Dispense 4.5 ml in test tubes with caps, autoclave for 15 minutes.

5. Lysis buffer: SSC, pH 7.0 + 0.1% sodium dodecyl sulfate (SDS). To 1 liter of SSC, pH 7.0 add 1 g SDS. Dispense 50 ml in screw-capped bottles. Autoclave for 15 minutes.

6. Pancreatic DNase: 250 µg/ml DNase in 0.025 M NaCl. Make new solutions daily. Keep cold.

Directions: Sterilize a solution of 0.025 M NaCl (0.73 g NaCl in 500 ml distilled water). Add DNase directly to sterile 0.025 M NaCl and place in sterile atomizer bottles. For example, dissolve 10 mg of pancreatic DNase (Sigma DN:100 from bovine pancreas) in 40 ml of sterile 0.025 M NaCl, dispense into four sterile atomizer bottles and refrigerate until used. (Alternatively, aliquots of DNase can be frozen: stock solution of 1 mg/ml DNase in 0.1 M NaCl. For daily use, thaw and dilute 1:4 in sterile water. Store in refrigerator.)

7. pHB plates:

For 1 liter of media (makes approximately 40 plates):

Flask A: Add the following to 420 ml distilled water in 1 liter flask (see directions below to make a 0.5 M solution):

25 ml of 0.5 M Na<sub>2</sub>HPO<sub>4</sub>  
25 ml of 0.5 M KH<sub>2</sub>PO<sub>4</sub>  
10 ml of 10% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
10 ml of concentrated base  
10 ml of 0.5 M p-hydroxybenzoic acid

Flask B: Add agar to water, do not mix:

15 g of Bactoagar  
500 ml distilled water

Loosely cover and autoclave both flasks for 15 minutes. Using sterile techniques, swirl flask B to mix the agar and then mix flasks A and B together. Dispense 25 ml into each of 40 sterile petri plates (100 × 15 mm). Plates should dry for several days and then can be stored in the cold..

8. Stock solutions for pHB plates:

Prepare ahead and store in refrigerator. If any solutions precipitate, warm to redissolve before use. Quantities listed are for 40 liters of media.

1 liter of 0.5 M Na<sub>2</sub>HPO<sub>4</sub>  
1 liter of 0.5 M KH<sub>2</sub>PO<sub>4</sub>  
500 ml of 10% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
500 ml of concentrated base  
500 ml of 0.5 M p-hydroxybenzoic acid: Dissolve 34.5 g in 400 ml water on magnetic stirrer (suspension). Bring pH to 7.0 with 5 M NaOH to put into solution, then bring the final volume to 500 ml.

## 9. Basal Medium + 1% yeast extract (quantities for 100 ml of media):

2.5 ml of 0.5 M  $\text{Na}_2\text{HPO}_4$   
 2.5 ml of 0.5 M  $\text{KH}_2\text{PO}_4$   
 1 ml of 10%  $(\text{NH}_4)_2\text{SO}_4$   
 1 g of yeast extract (Difco)

Dissolve in distilled water and bring final volume to 100 ml. Dispense 5 ml in  $16 \times 100$  mm screw-capped tubes. These tubes fit the 3-3/4 inch shields in the swinging bucket head of the clinical centrifuge. Autoclave for 15 minutes. Tighten caps when cool.

## 10. Concentrated base (per liter):

Nitrilotriacetic acid (NTA-free acid)	20.0 g
$\text{MgSO}_4$ anhydrous	28.9 g
(or $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )	59.3 g)
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	6.67 g
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	18.50 mg
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	198.00 mg
“Metals 44”	100.00 ml

Dissolve NTA separately in 600 ml water and neutralize with KOH (14.6 g KOH); add other components and dissolve in order given. Adjust pH to 6.8 before making to final volume of 1 liter. A precipitate forms when adjusting the pH from the acid side of 6.8 with KOH (need about 100 ml 1 M KOH), but eventually redissolves with stirring. When the pH is near 6.8, the color of the solution changes from a deep yellow to straw color. Pour a layer of toluene over the final base. It may be kept for at least 1 year in the cold.

## 11. Hutner's “Metals 44”:

Dissolve the following in the order given (do not add components until the previous one has completely dissolved):

In 800 ml of distilled water dissolve:

EDTA ( <i>free acid</i> , not sodium salt, warm to dissolve)	2.50 g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	10.95 g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	5.00 g
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	1.54 g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	392.00 mg
(or $\text{CuSO}_4$ anhydrous 251.00 mg)	
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	250.00 mg
$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$	177.00 mg

Add a few drops of concentrated  $\text{H}_2\text{SO}_4$  to retard precipitation; this stores indefinitely. Made to a final volume of 1 liter, it should be a clear, solution lime green in colour.

## Results

Plate label	Number of colonies
ADP-6/0 (no DNA)	0 (range: 0–4)
ADP-6/10 <sup>0</sup>	Too many
ADP-6/10 <sup>-1</sup>	Too many
ADP-6/10 <sup>-2</sup>	Too many
ADP-6/10 <sup>-3</sup>	1200
ADP-6/10 <sup>-4</sup>	280 (range: 100–400)
DNase 0 minutes	0 (range: 0–4)
DNase 30 minutes	548 (range: 200–confluent)
Streaking ADP-1	Growth
Streaking ADP-6	No growth

Sometimes the polysaccharide coat genes are co-transformed and two types of colonies appear: larger irregular and small round colonies. Occasionally, there is a background haze of mini-colonies. ADP-6 goes through about 10 divisions before using up the residual nutrients of the liquid growth media and relying on p-hydroxybenzoic acid as the carbon source. If this should be a problem, an additional washing step could be added (step C1 and C2).

If there are numerous colonies on the DNase 0 minute, the plate was probably not sprayed at 0 minutes, but later, allowing some transformations to occur. If the colonies are all in the center of the plate, either the cells and/or DNA were in the center spot too long before spreading of the mixture.

## Acknowledgments

The transformation laboratory originated from work done by Dr. Nicholas Ornston, Yale University.

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