

Bacterial Conjugation

(BE-301)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students).

Checklist

- 1 vial Bac: E.C. BB4 (F⁺) Agar Stab
- 1 vial Bac: E.C. SCS1 (F⁻) Agar Stab
- 2 vials LB Broth
- 1 bottle LB Broth
- 20 Discs: Ampicillin Discs
- 20 Discs: Tetracycline Discs
- 1 vial Ampicillin and Tetracycline
- 1 vial TET Buffer
- 2 packs LB Agar
- 2 Culture tubes
- 9 Petri Dishes
- 30 Centrifuge Tubes (2ml)
- 6 Inoculating Loops

SPECIAL HANDLING INSTRUCTIONS

- Store Ampicillin Discs, Tetracycline Discs and the ampicillin/tetracycline mix at 4°C.
 - Store Bac: E.C. BB4 (F⁺) and Bac: E.C. SCS1 (F⁻) Agar Stabs at 4°C.
 - All other reagents can be stored at room temperature.

The majority of reagents and components supplied in the *BioScience Excellence*[™] kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
T061	TET Buffer	Flammable

ADDITIONAL EQUIPMENT REQUIRED

- Shaking Incubator
 - Incubator
 - Autoclave*
 - Forceps

* 100ml premade bottles of LB agar (L011), which is melted in a boiling waterbath, can be used if an autoclave is not available. You will require 2 x LB Agar bottles.

TIME REQUIRED

- **Day 1:** 2 hours
- **Day 2:** 30 minutes



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OBJECTIVES

- Understand the naturally occurring bacterial conjugation events
- Transfer antibiotic resistance from one bacteria to another.

BACKGROUND

Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating. Interestingly, conjugation is not actually sexual, as it does not involve the fusing of gametes and the creation of a zygote. Bacterial conjugation is in fact the simple exchange of genetic material and information from one bacteria to another. It is bacterial conjugation that allows bacteria to transfer drug resistance.

For bacteria to achieve conjugation one of the bacteria has to carry the F-plasmid, also known as the F factor, and “partner” bacteria must not have the F-factor

The F-plasmid is a specialized plasmid, known as an episome that is able to integrate itself into the bacterial chromosome and is about 100kbp in length. The F-plasmid has its own origin of replication (*oriV*), which is a specific sequence at which DNA replication is originated. Within a single bacterium there can only be a single copy of the F-plasmid, whether that copy is free or integrated into the bacterial chromosome.

The F-plasmid encodes for all the proteins necessary for bacterial conjugation, including the proteins necessary to form the pili, a finger like projection that attaches to the partner bacteria (Figure 1).

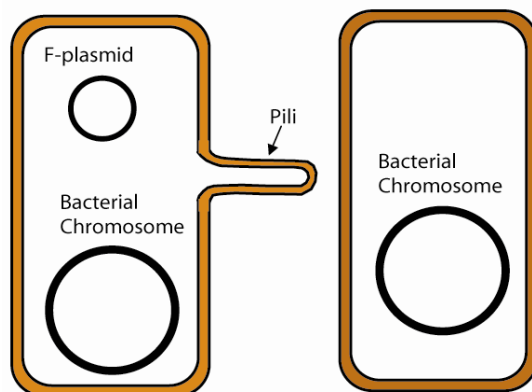


Figure 1: Bacterial conjugation.

TEACHER'S PRE EXPERIMENT SET UP



*Wear heat protective gloves throughout the autoclaving and pouring agar plate procedure
Make Agar plates the day before the experiment*

Prepare LB Agar plates

1. Empty the entire contents of one LB Agar packs into an autoclavable container and add 150ml distilled water. Autoclave for 15min at 121°C.

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2. Once the LB Agar has cooled to hand hot temperature (about 45°C), pour a ~0.5cm / ¼” layer of agar into 6 Petri dishes. This is approximately 20-25ml each plate.
3. Let the plate set for 20-30minute to solidify.
4. Distribute two LB agar plates to be shared by two groups of students.

Prepare LB Agar plates with antibiotic

1. Empty the entire contents of the remaining LB Agar pack in to an autoclavable container and add 150ml distilled water. Autoclave for 15min at 121°C.
2. Prepare the antibiotic: Add 200µl Tetracycline Buffer to the vial of ampicillin and tetracycline mix.
3. Once the LB Agar has cooled to hand hot temperature (about 45°C), add the entire contents of the ampicillin and tetracycline mix vial and gently swirl the LB agar to thoroughly mix.
4. Label the bottom of 3 Petri dishes with “Antibiotic” and then pour a ~0.5cm / ¼” layer of agar into each Petri dish. This is approximately 20-25ml each plate.
5. Let the plate set for 20-30minute to solidify.
6. Distribute one antibiotic LB agar plate to be shared by two groups of students.

Prepare overnight cultures of bacteria

1. Label one culture tube with “BB4” and another with “SCS1”.
2. Using aseptic techniques transfer 7ml LB Broth provided in the kit to each of the labeled culture tubes.
3. Pipette 0.8ml LB Broth into the E.C. BB4 Agar Stab and incubate at 37°C for 30 minutes.
4. Vigorously shake or vortex for 1-2 minutes, then transfer 0.5ml LB broth from the agarose stab to the BB4 labeled culture tube.
5. Pipette 0.8ml LB Broth into the E.C. SCS1 Agar Stab and incubate at 37°C for 30 minutes.
6. Vigorously shake or vortex for 1-2 minutes, then transfer 0.5ml LB broth from the agarose stab to the SCS1 labeled culture tube.
7. Culture the bacteria in a 37°C shaker overnight.
8. The following day, label six Centrifuge Tubes (2ml) with “BB4” and six Centrifuge Tubes (2ml) with “SCS1”. Dispense 1ml of each overnight culture into the respective labeled six Centrifuge Tubes (2ml).

MATERIALS FOR EACH GROUP

Supply each group with the following components. The LB Agar plates are shared between two groups of four students.

- 1 vial 1ml E.C. BB4 (F⁺) overnight culture
- 1 vial 1ml E.C. SCS1 (F⁻) overnight culture
- 2 Ampicillin Discs
- 2 Tetracycline Discs
- 2 LB Agar Plates (shared between two groups)
- 1 Ampicillin Tetracycline LB Agar Plate (shared between two groups)
- 1 Pair of Forceps



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- 1 Centrifuge Tube (2ml)
- 1 Inoculating Loop

PROCEDURE



Wear gloves throughout the experiment procedure.

Do not carry out any hand to mouth actions during the experiment. Use aseptic techniques throughout.

The aim of this experiment is to pass the drug resistance of one bacteria to another bacteria by the process of bacterial conjugation.

1. Label the Centrifuge Tube (2ml) with your group name.
2. Add 0.5ml of E.C. BB4 (F⁺) to the labeled 2ml tube.
3. With a clean pipette tip, add 0.5ml E.C. SCS1 (F⁻) to the 2ml centrifuge tube with the BB4 bacteria. Gently mix by inverting the tube a few times.
4. Incubate the mixture in a 37°C incubator for 60 minutes without any shaking.

Steps 5-11 are performed with another group of students.

5. In the meantime, on the bottom of the LB agar plates label one with “BB4” and the other with “SCS1”. Divide each plate into quarters and label two quarters with “Ampicillin” and the others with “Tetracycline” (See figure 2).
6. One group adds the remaining E.C. BB4 (F⁺) overnight culture to the “BB4” plate and gently tilt the plate back and forth and side to side to spread the liquid over the entire surface of the plate. Use a pipette to remove any excess liquid.
7. The other group adds the remaining E.C. SCS1 (F⁻) overnight culture to the “SCS1” plate and gently tilt the plate back and forth and side to side to spread the liquid over the entire surface of the plate. Use a pipette to remove any excess liquid.
8. Allow both plates to dry for 15-20 minutes.
9. Each group adds their appropriate antibiotic discs to the two LB Agar plates. Using forceps, carefully place an ampicillin disc and a tetracycline disc in the center of the appropriately labeled quarter of each LB agar plate. (See figure 2 for placement)

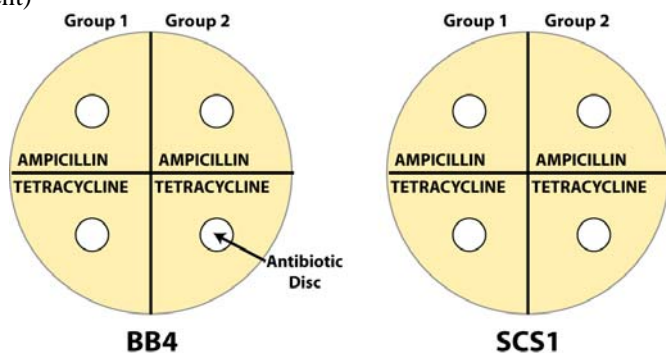


Figure 2

10. Once the plates are dry, turn the plates upside down, so that the agar is at the top, and place in a 37°C incubator for 18-24 hours.

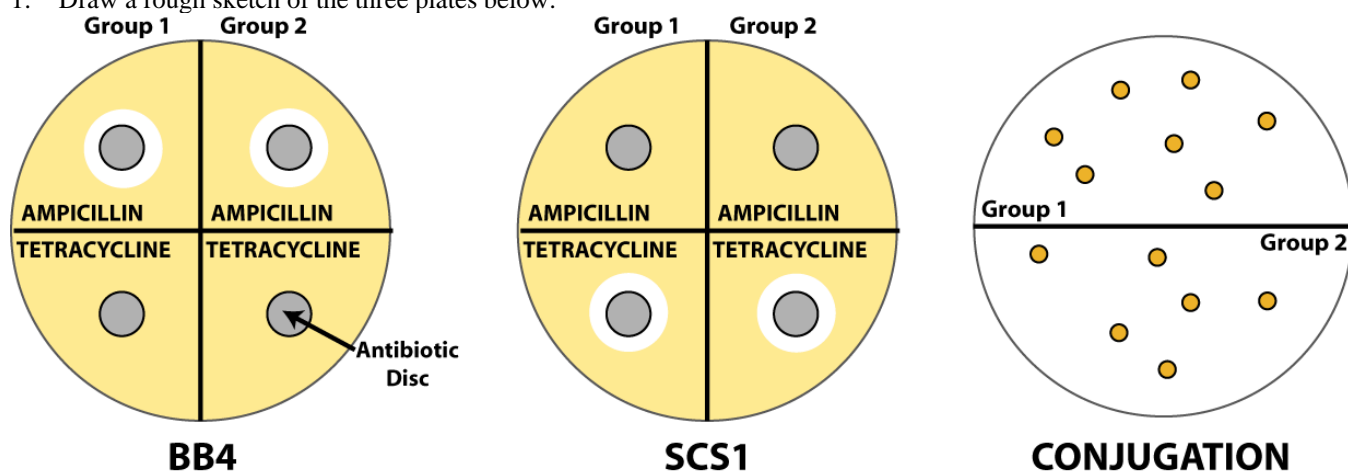
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- Label the bottom of the Ampicillin Tetracycline antibiotic LB agar plate with “Bacterial conjugation”. Divide the plate in half and label each half with the respective group name.
- At the end of the incubation (step 4), vortex, or vigorously shake, the tube for 1 minute to stop the conjugation process.
- Pipette 0.5ml bacterial conjugation mix on to your half of the Ampicillin and Tetracycline antibiotic LB agar plate and use the loop end of the inoculating loop to spread the liquid over the entire surface of your half of the plate. Use a pipette to remove any excess liquid.
- Allow the plate to dry for 15-20 minutes.
- Once the plates are dry, turn the plates upside down, so that the agar is at the top, and place in a 37°C incubator for 18-24 hours.
- The following day, examine the plates and record your results in the following section.

RESULTS, ANALYSIS & ASSESSMENT

- Draw a rough sketch of the three plates below:



The number of colonies on the conjugation plate may vary depending on how many bacteria conjugated.

- Did bacterial conjugation occur? Explain why you reached this decision.

Yes, because colonies grew on the bacterial plate containing both ampicillin and tetracycline antibiotics. If conjugation failed, then the original bacteria stocks would not be able to grow in the presence of both antibiotics. This is confirmed by the controls.

- Which bacteria strain donated its resistance marker and which bacteria strain was the recipient? Explain your decision.

BB4 strain donated its tetracycline resistance to the SCS1 strain. The reason is that the BB4 strain carries the F plasmid and conjugation always occurs in the direction of F^+ to F^- strains.