Experiment 3: Bacterial genetics 2 (interrupted mating)

Protocol 3.1 Interrupted mating

Materials:
Bunsen burner
Box of sterile blue tips
Box of sterile yellow tips
100-1000µl pipette
10-100µl or 20-200µl pipette (to pipette 100µl)
1.5 ml microcentrifuge tubes (eppendorfs) in 1 bag of 20
Marker pen to write on eppendorfs and plates
Eppendorf rack
Vortexer
Disposable spreaders (five packs of 5)

Minimal media agar plates (MM):
4 plates labelled A: MM – cys (contains histidine, tryptophan, serine and streptomycin)
4 plates labelled B: MM – ser (contains histidine, tryptophan, cysteine and streptomycin)
4 plates labelled C: MM – trp (contains histidine, cysteine, serine and streptomycin)
4 plates labelled D: MM – his (contains cysteine, tryptophan, serine and streptomycin)

***Escherichia coli* strains**:

Hfr (donor strain)
F^- (recipient strain)

Protocol

1. Label the **Eppendorf tubes <u>and</u> plates** as outlined below:

	Time of mating (in minutes)			
Marker				
cysteine	A5	A20	A30	A50
serine	B5	B20	B30	B50
tryptophan	C5	C20	C30	C50
histidine	D5	D20	D30	D50

2. Add 500 µl of the F^- strain to each labelled Eppendorf tube

3. Add 100 µl of the Hfr strain to each labelled Eppendorf tube. **Mix well** by gently pipetting up and down

4. Place all your 16 Eppendorf tubes in rack on your bench at room temperature

5. Mating will begin when you mix the strains in your first 4 tubes so make a note of the time. **This is your time 0**

6. At each time period, remove your tubes from the rack and vortex for 10 seconds to interrupt the mating. **i.e.** After 5 minutes remove A5, B5, C5, and D5 from the rack and vortex each Eppendorf tube for 10 seconds

7. After vortexing, plate 100 µl from each Eppendorf on to the corresponding plate by pipetting the 100 µl onto the centre of the plate and using the spreader to distribute the liquid evenly across the plate. **i.e.** 100 µl of A5 to plate A5, 100 µl of B5 on B5 and so on

8. After 20 min from time 0, repeat steps 6 and 7 with the tubes/plates labelled 20

9. After 30 min from time 0, repeat steps 6 and 7 with the tubes/plates labelled 30

10. After 50 min from time 0, repeat steps 6 and 7 with the tubes/plates labelled 50

11. When you have finished, leave all of your plates on your bench, make sure you label them with your initials. Your plates will be grown at 37 ^{0}C overnight.

Protocol 3.2 – Counting colonies. In the following laboratory session **(Week 8)**

Count the colonies on your plates and record your observations in Table 3.1. (See the section for Lab 3 Data Analysis.)

Table 3.1 Colonies counted on the interrupted mating plates. TNTC = Too Numerous To Count; TFTC = Too Few To Count.

	5 min	**20 min**	**30 min**	**50 min**
Cysteine				
Serine				
Tryptophan				
Histidine				
Tetracycline				