Molecular cloning and genetic engineering – Inoculating Bacterial Culture

Aim

Growing up sufficient amount of bacteria for experimental use. Here three different methods for inoculating.

Materials

LB medium

Antibiotic for selection

Bacterial colony

Procedure

1. Bacterial culture solid medium plate culture:

(1) Use a sterilized inoculation ring to pick a little Escherichia coli preservation solution. Hold the plate near fire with left hand and spread the solution on AGAR plate back and forth to make a evenly painted film (about 1/10 of the tablet total surface area.

(2) Burn the inoculation ring to kill the residual bacteria on the ring. After it has cooled, take bacteria solution from the film and draw continuous parallel line (about 1/5 of the surface of the plate).

(3) Burn inoculation ring again before draw the third parallel line.

(4) With the same method of 2 and 3 for the fourth, fifth line, the surface of the flat plate.

(5) Cover the lid of the plate and make it bottom up.

(6) Mark the bacteria name test number and date with the label.

(7) Incubate at 37℃ for 24 hours and collect the results.

2. Liquid medium culture:

(1) Add 10 μL bacterial liquid to the flask carefully and gently vibrate it to well blend the bacteria with the medium.

(2) Plug the flask and pack it with kraft paper.

(3) Culture is then conducted on shaker at 37 ℃ for 24 h.

3. Inclined medium inoculation:

(1) Sterilize the seed ring and its stem by passing it through flame.

(2) Let the mouth of strain tube pass through flame, too.

(3) Dip few bacteria liquid with the seed ring.

(4) Stick the seed ring into the medium and windingly paint the liquid on the slope.

(5) Pass the mouth of the strain tube through flame again and plug the

tube.

(6) Sterilize the seed ring again.