**LAB WORK 12.**

**Subject: Genetic recombination. Conjugation in Escherichia coli K-12.Session**

**Purpose:** To implementConjugation in Escherichia coli K-12.

**Objectives**:

1. Learn of how to make a Suspension hybridization.
2. Learn of how to make a Spot hybridization.
3. Performexercises 1, 2.

Conjugation requires cell-to-cell contact for DNA to be transferred from a donor to a recipient. Bacterial conjugation is plasmid-mediated gene transfer.A plasmid that can mediate gene transfer is termed the F (fertility) plasmid. A bacterial cell containing the F plasmid is called an $F^+$cell. A bacterial cell not containing a F plasmid is called the $F^-$cell. A bacterial cell containing a F plasmid integrated into the bacterial DNA is termed a Hfr (high frequency of recombination) cell.

**Lab Exercise 1.Suspension hybridization.**

**Methodical instructions:** Use the Met His Val SerStrains of Escherichia coli K-12.

| **Strain** | **Sex** | **genotype** |
|---|---|---|
| **1** | F- | Met – His- Val+ Ser+ |
| **2** | F+ | Met + His+ Val- Ser- |
| **3** | Hfr | Met + His+ Val- Ser- |

Make crossing between strains:F+ x F- andHfr x F-. Conjugation is carried out in liquid medium. The conjugation mix for this purpose prepares.

**Procedure:**

1. To culture of a strain 1 add culture of a strain 2.
2. To culture of a strain 1 add culture of a strain 3.
3. Place test tubes in the thermostat, $37^0$, 1hour.
4. Divide Petri's cup with the minimum medium into 2 sectors.
5. Sign each.
6. From the first test tube place 100 mcl of a conjunction mix in sector 1.
7. Distribute a mix with a spatial on surface of nutrient medium.
8. From the second test tube place 100 mcl of a conjunction mix in sector 1.
9. Distribute a mix with a spatula on surface of nutrient medium.
10. Place cups in the thermostat, $37^0$, 48 hours.

**Lab Exercise 2.Spot hybridization.**

**Methodical instructions:**Make crossing between strains:F+ x F- andHfr x F- .Transfer of a factor occurs not only by crossing in a liquid broth, but also on a cup surface with agar.

**Procedure:**

1. Divide a Petri dish with the minimum nutrient medium into 5 sectors.
2. Sign each.
3. Drop of suspension on sector 1 of a strain 1.
4. Drop of suspension on sector 2 of a strain 2.
5. Drop of suspension on sector 3 of a strain 3.
6. On sector 4, firstly a drop of a strain 1 and when it dry a drop of suspension a strain 2.

7. On sector 4, firstly a drop of a strain 1 and when it dry a drop of suspension a strain 3.
8. Petri dish put in the thermostat on $37^0$, 48 hours.

Write the results of experiment in the table. Make a conclusion.

Results of conjugation in E. Coli

| Method of hybridization | Type of hybridization | Presence of recombinants |
|---|---|---|
| Spot | F- x F- | |
| | F- x Hfr | |
| Suspension | F- x F+ | |
| | F- x Hfr | |

**Equipment:**

- Broth Culture of F-, F+, Hfr strains Escherichia coli K-12
- Petri dishes with minimum nutrient medium
- Disinfectant tray
- Inoculation loop
- Burnerflame
- Spatula