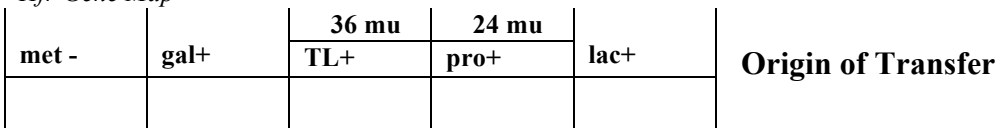
Bacterial Conjugation and Genetic Mapping in *E. Coli*

Abstract

In this experiment we studied the phenomenon of conjugation between two members of the *E.Coli* species, Hfr and F-. We used selective media to grow conjugated recombinants to determine genetic loci of these genes in relation to an origin of transfer. We used the method of uninterrupted mating. We then plated the original suspension on these specific media plates. The final viable colonies were counted and organized by recombined phenotype, allowing us to calculate genetic map distances of the genes in the bacterial chromosome and determine the order of entry of the genes into the F- bacteria from the Hfr strain. We determined that the order of entry was lac+ first, pro+ next, followed by TL+, gal+, and if met- had made it all the way through the conjugation tube it would be last. Below is a chromosomal map of our map distance findings for the genes transferred by the Hfr.

Hfr Gene Map



Introduction

We used bacterial conjugation to measure genetic distances and observe recombined phenotypes through the use of *E. Coli*, specifically, an Hfr strain that is F+. This categorization indicates that the bacteria contain a non-attached, transferable sex factor that can be donated to an F- strain, which we introduced through uninterrupted mating. This creates a donor pair, the male is known as a genetic donor, F+ and the female as a genetic recipient, F-. Bacterial conjugation is the formation of a very fragile conjugation tube that creates a canal for the F factor to be transferred. The canal is

created through contact of pili from one bacterium to the cell wall of another, known as collision. Next, the bacteria must identify each other as a donor-acceptor pair. Once the conjugation tube has been formed, the transfer occurs. This is an energy dependent, sequential transfer that is unidirectional and proceeds at a slow but constant rate. The selecting gene must then be expressed phenotypically, if they are dominant. Finally there must be integration of the gene into the replicated genome of the accepting bacteria. This specific order of operation sets limitations to conjugation. The gene that is closest to the origin of transfer has the greatest chance of getting into the F-cell's chromosome. The origin of transfer is the point at which a break occurs in the integrated F factor. This is the leading point of the chromosome to be transferred. By selecting for a certain gene that we expect to be transferred and analyzing the amount of bacteria that grew in the specified media, we can determine genetic distances between these genes and are able to tell where recombination about these genes took place.

Materials and Methods

Per student pair:

2 jars of sterile toothpicks (70ct. in each jar)
2 sterile velveteen pads
3 sterile test tubes
0.2ml Hfr culture
1.8ml F- culture
5 plates media #1
5 plates media #2
4 plates media #4
2 plates media #'s 3 & 5
1 bottle NB (20ml)
7 sterile, individually wrapped plastic pipettes: 1ct. 10ml, 6ct. 1ml

Table I

Genetic Markers of Two E. Coli Strains

	TL	pro	lac	gal	met	str	thi
Hfr	+	+	+	+	-	S	-
F-	-	-	-	-	+	R	-

Table II

In addition to thiamine, the following supplements were supplied to the corresponding plates:

Media No.	glucose	lactose	galactose	Thr-leu	met	pro	strep
1	+	-	-	-	+	+	+
2	+	-	-	+	+	-	+
3	-	+	-	+	+	+	+
4	-	-	+	+	+	+	+
5	+	-	-	+	-	+	+

To begin the process of conjugation, we mixed 0.2 ml of Hfr culture with 1.8ml of F- culture. We then incubated the mixture at 37^{o}C for 60-70 minutes, this is known as uninterrupted mating. We then took the mixture out of incubation and added 1ml of it to 9ml of nutrient broth for dilution. This created a concentration of 10^{-1}. With a pipette, we transferred 0.1ml from the original solution to three plates, media 1, 2 and 4. We then transferred 0.1ml from the diluted solution to three new plates, media1, 2, and 4. These six plates were then incubated at 37^{o}C for 48 hours. The following day, the plates were scored and 2 master plates were created from the viable colonies, one on media 1 the other on media 2. We chose to create our master plates from the original solution, and allow our dilution plates to be used by other students. These plates were then incubated at 37^{o}C for 24 hours. Once the incubation period had passed, we replicated the master plates onto the above mentioned media plates. The media 1 master plate was replicated to media 2,then 3,then 4,then 5, and finally to media 1. The media 2 master plate was replicated to media 1, then 3, then 4, then 5, and finally to media 2. Once results were observed, the following day, the plates were placed into cold storage until they could be viewed by the GSI.

Results

In this experiment we found that through uninterrupted mating of the Hfr and F-, we get the highest number of viable colonies from the media 2 plate, which selects for

Pro+. We then see that the media 1 plate, which selects for TL+, exhibits the next highest number of colonies and finally that the least number of colonies grows on media 4, which selects for Gal+, as supported by the Table III of the appendix. We see from the media 1 master selection plate series that the most number of colonies grew on media 1, selecting as previously mentioned, and media 5, which selects for Met+, followed by media 2, also selects as previously mentioned, then media 3, which selects for Lac+. The least amount of colonies grew on media 4, the Gal+ selecting plate. The numerical data for the media 1 master plate selections can be found in Table IV of the appendix. The data from the media 2 master selection plate series shows the highest amount of growth on the media 2, as well as media 5. Media 3 plate had the next highest amount of colonies followed by media 1, to leave only media 4 as the plate having the fewest colonies. This information can be found with numerical values for colonies in Table V of the appendix.

Discussion

Through the methods of selection we conclude that the first gene to enter the F- cell is Lac+ gene, which indicates its location as being the closest to origin of transfer. Following the Lac+ gene, in order, we see that Pro+, TL+, and Gal+ enter successively. We determine that the Met- gene from the Hfr was not expressed phenotypically in our recombinants. This is because any bacteria containing this gene, assuming it is dominant, would not have survived our selective media 5. The order of entry of these genes was determined through three criteria found through the analyses of the plates. The first criteria came from our initial uninterrupted data that told us through order of decreasing quantity that Pro+ must be closer to the origin of transfer Than TL+ and Gal+ because the

media 2 plate had the most colonies. TL+ comes next followed by Gal+. The second criteria comes from our analyses of the TL+ selection plates which tells us that in this selection, Pro+ must be closer to TL+ than Lac+. The third criteria comes from the Pro+ selection plate series that tells us when selecting for Pro+, Lac+ must be closer to Pro+ than TL+. Through creation of organized tables (please see appendix) of the recombinant phenotypes, we are able to calculate map distances between the genes selected for and those that come between it and the origin of transfer. Table VI in the appendix has the data and calculations for the map distances carefully outlined. Below is a map of our found genetic order of entry as well as the calculated map distances of the selected genes. The discrepancy in the two calculated distances between the TL+ and Lac+ in Table VI is 10 mu. This could be due to the fact that we had more recombinants between crosses 1 and 4 of the media 1 selection than expected and less recombination between crosses 1 and 3 of the media 1 selection. In order to find a perfect correlation between the calculations, we would have needed more colonies that had recombined to give us a TL+ Pro+ Lac- phenotype. We could repeat the experiment to determine whether this was the case or whether there was a discrepancy in the media 2 master selection series. We chose to use the values for the map distances that were shorter because it is known that when we determine map units that the direct distance between genes is more accurate that when calculating a distance that passes over genes.

		60mu			
		36 mu	24 mu		
Met +	Gal+	TL+	Pro+	Lac+	**Origin of Transfer**

Conclusion

We can conclude from this experiment that the order of entry is Lac+ first, Pro+ second, TL+ third, Gal+ fourth and Met+ fifth. We can also conclude that the genetic map distance between Lac+ and Pro+ is 24 mu, the distance between TL+ and Pro+ is 36 mu, and consequently the distance between TL+ and Lac+ is 60 mu. We cannot conclude any distances from the origin for Gal+ or Met+ since they entered the F- bacteria after the genes that we selected for.

Appendix

Table III *Uninterrupted Mating Results of Suspension*

	Media 1	Media 2	Media 4
Number of Colonies	200	256	19

Table IV *Media 1 Master Plate Selection for TL+ Recombinants*

	Media 2	Media 3	Media 4	Media 5	Media 1
Selection	Pro+	Lac+	Gal+	Met+	TL+
# Of colonies	34	29	1	50	50
# Of recombinants	34	29	1	0	50

Table V *Media 2 Master Plate Selection for Pro+ Recombinants*

	Media 1	Media 3	Media 4	Media 5	Media 2
Selection	TL+	Lac+	Gal+	Met+	Pro+
# Of colonies	17	38	1	50	50
# Of recombinants	17	38	1	0	50

Table VI *Map Distance Calculations with TL+ Selection*

Crosses	Phenotype	Recombinants
1&2	TL+ Pro- Lac-	14
1&3	TL+ Pro+ Lac-	7
1&4	TL+ Pro+ Lac+	27
1, 2, 3, & 4	TL+ Pro- Lac+	2
		50 total

TL+→Lac+
=((14+7+2(2))/50)*100 =50 mu

TL+→Pro+
=((14+2(2))/50)*100 = 36 mu

Map Distance Calculations with Pro+ Selection

Crosses	Phenotype	Recombinants
1&2	Pro+ Lac-	12
1&3	Pro+ Lac+	38

Pro+→Lac+ = (12/50)*100 = 24 mu