Bacterial genetic exchange: Conjugation of *ina (ice nucleation active)* gene from *E.coli* into luminous *V.harveyi*

Authors: Patrick Brunner s0170821@access.unizh.ch
Angela Kölliker s0173033@access.unizh.ch
Andrea Schiess s0171034@access.unizh.ch

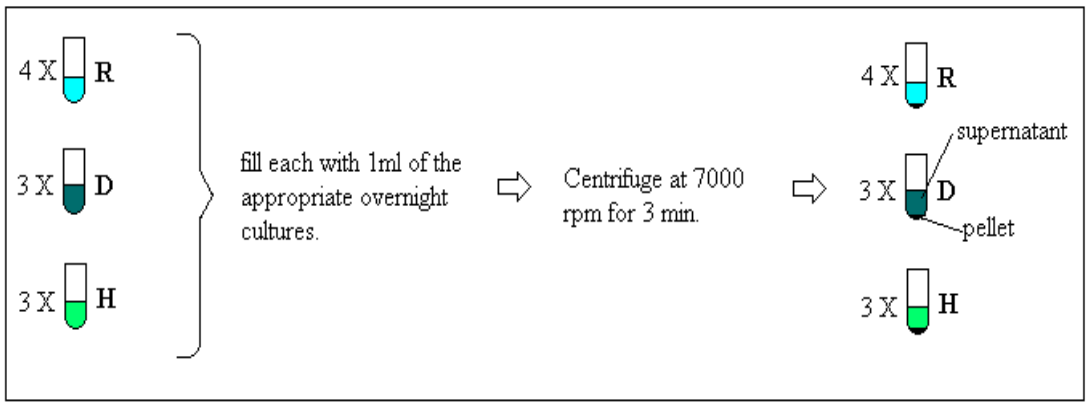
Tutor: Munti Yuhana myuhana@botinst.unizh.ch

Objectives of the experiment
To understand gene transfer by conjugation in bacteria (triparental mating) and to learn about *ina (ice nucleation active)* gene expression in recipient bacteria by performing an ice nucleation assay.

To „create" a transconjugant bacterium from a phenotype able to glow in darkness which acquires the *ina* gene enabling the organism to catalyze ice formation. This can be achieved through a conjugation experiment. The experiment involves the recipient with a luminescent phenotype and a donor which provides the ice nucleation active gene (*ina*$^+$) on plasmid pJL1703. The *ina*$^+$ gene is responsible for catalyzing water crystallization. The plasmid is mobilizable, but it is not self-transferable. Therefore, the helper strain *E.coli (pRK 2013)* is required. This strain provides the *tra* operon.

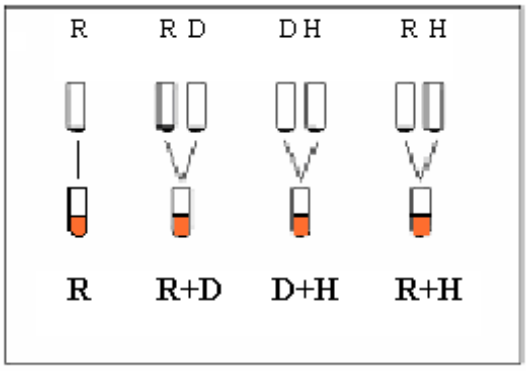
In this experiment we used 2 strains of *Escherichia coli* and 1 strain of *Vibrio harveyi* with the following genotypes:
1) *E.coli (pJL 1703): tra*$^-$, *ina*$^+$, *Kan*^{r}, *Amp*^{s}, *lux*$^-$ (**donor/D**)
2) *E.coli (pRK 2013): tra*$^+$, *ina*$^-$, *Kan*^{r}, *Amp*^{s}, *lux*$^-$ (**helper/H**)
3) *V.harveyi: tra*$^-$, *ina*$^-$, *Kan*^{s}, *Amp*^{r}, *lux*$^+$ (**recipient/R**)

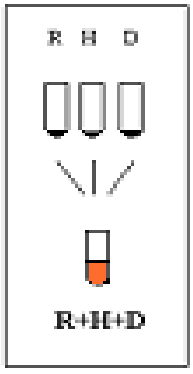
Practical work:
For the first step we prepared bacterial cultures in liquid Luria Bertani (LB) medium. The cultures were incubated overnight.



We filled four Eppendorf tubes with 1 ml of the **Recipient** culture, three tubes with 1ml of the **Donor** culture and another three tubes with 1 ml of the **Helper** culture. The tubes were centrifuged at 7000 rpm for 3 minutes to collect the cells.
As controls we took four Eppendorf tubes, each containing 750 µl LB medium and marked them R, R+D, D+H and R+H, respectively.

We divided the pellet-containing tubes into those four groups referring to those labels. Then we resuspended the pelleted-cells with 100 µl sterile LB medium. The cultures were incubated overnight at 30º C.





For the main experiment (triparental mating) we took one Eppendorf tube, containing 750 µl LB marked with R+D+H.

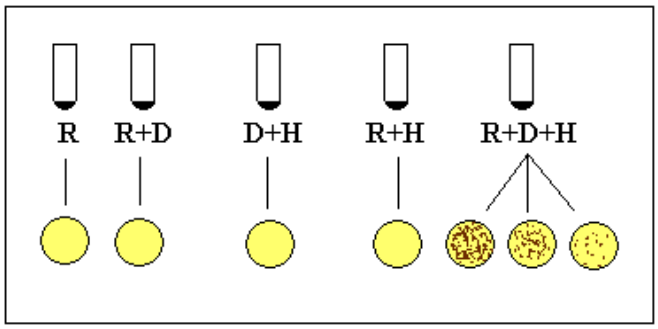
The pelleted-cells of the R tube were resuspended with 100 µl sterile LB liquid medium, mixed with D, and H cells, and put into the R+D+H Eppendorf tube.

All of R, R+D, D+H, R+H and R+D+H tubes were incubated overnight at 30º C.

Plating of the cells on Ampicillin, Kanamycin and LB medium.

The controls were plated undiluted onto LB +Amp + Kan medium.

The triparental mating (R+D+H) cells were diluted with 0.9ml LB medium also containing Ampicillin and Kanamycin. We plated 25 µl, 50 µl and 100 µl onto different LB + Amp + Kan media.



Testing the growing transconjugants for luminescent bacteria: We selected 10 individual colonies, picked and streaked them individually onto LB + Amp + Kan Agar medium and incubated overnight at 30º C. For assaying the ice nucleation capability, we scraped two loops full from the plate and resuspended the cells into a test tube containing 10 ml of sterile PBS (phosphate-buffered saline). We then put the assay tubes into the cooling bath (at -5º C) for 3 to 5 minutes.

The transconjugants were able to glow in darkness. After we diluted them in 10 ml phosphate-buffered saline solution and exposed them at –5°C in a cooling bath for 3 to 5 minutes the solution quickly freezes (= indication for the presence of *ina*).

Discussion

Why did it happen?

Let's remember the genotypes of the two strains of *E. coli* and the one of *V. harveyi*:

E.coli (pJL 1703): tra$^-$*, ina*$^+$*, Kan*^{r}*, Amp*^{s}*, lux*$^-$ (**donor/D**)
E.coli (pRK 2013): tra$^+$*, ina*$^-$*, Kan*^{r}*, Amp*^{s}*, lux*$^-$ (**helper/H**)
V.harveyi: tra$^-$*, ina*$^-$*, Kan*^{s}*, Amp*^{r}*, lux*$^+$ (**recipient/R**)

The plasmid of *E. coli* (pRK 2013) cannot be maintained in other bacteria since it belongs to the narrow-host-range-plasmid group. It is possible, however, to transfer it into another *E.coli* (pJL1703), because the recipient *E.coli* belong to the same species, and it carries the transfer operon (*tra*$^+$). At the beginning of our experiment we prepared a few controls. First we just incubated PBS solutions containing *V. harveyi* cells in the cooling bath. As expected, the bacteria weren't able to initiate freezing the solution at this temperature. The reason is that *Vibrio* does not have an *ina*$^+$ gene.

For comparison we made 3 different diparental matings as controls:
recipient + donor (R + D),
donor + helper (D + H),
recipient + helper (R + H)
No growth was observed when we plated the controls undiluted onto LB (Luria Bertani medium) + Ampicilin + Kanamicin Agar plates. None of the D, H, or R has is resistant to both antibiotics. We propose the following explanations for these observations:

R + D: R is sensitive to Kanamicin (*Kan*^{s}), D is sensitive to Ampicillin (*Amp*^{s}) and both are not able to transfer their plasmids to each other because they lack a transfer operon (*tra).*

D + H: D is sensitive to Ampicillin (*Amp*^{s}), H is sensitive to Ampicillin (*Amp*^{s}) too, so they are not able to grow on an Ampicillin-containing medium. They are able to exchange plasmids, however, since H contains transfer operon (*tra).*

R + H: R is Kanamicin sensitive (*Kan*^{s}), H is Ampicillin sensitive (*Amp*^{s}) and H is narrow host range. Although H carries a *tra*$^+$ operon neither R nor H can grow on media containing Kanamycin and Ampicillin.

With this knowledge we started our main experiment:The triparental mating. Bacterial mating (conjugation) is a process of genetic transfer that involves cell-to-cell contact. A conjugative plasmid uses the helper mechanism (*tra*$^+$ operon) to transfer a copy of its plasmid to a new host.

Transconjugant bacteria are able to grow on the LB+Kan+Amp medium, if they acquired resistance to Kanamycin in addition. If they are able to glow we know that they are *V. harveyi* and if they are also able to quickly freeze the buffer solution, we have clear indication that they successfully acquired the ina gene from the *E.coli* donor.

Mating between *E.coli* (**donor**) and *E.coli* (**helper**): The donor receives the *tra*$^+$ operon from the helper.
Transconjugant *E.coli* (D+H) contain: *tra*$^+$*, ina*$^+$*, Kan*^{r}*, Amp*^{s}*, lux*$^-$
Besides carrying the *tra*$^+$ operon they will not glow and grow on LB+Kanamycin but not on LB+Ampicillin containing plates. Since the transconjugant donor is now *tra*$^+$, it is capable of conjugating with the final recipient.

Mating between the transconjugant *E.coli* (*tra*$^+$) and *V.harveyi*:
V.harveyi transconjugants then contains: *tra*$^+$*, ina*$^+$*, Kan*^{r}*, Amp*^{r}*, lux*$^+$
V.harveyi transconjugants (*Kan*^{r}*, Amp*^{r}) will grow on LB+Kan+Amp containing plates, while both, the original donor (*Kan*^{r}*, Amp*^{s}), the helper (*Kan*^{r}*, Amp*^{s}) und the *E.coli* D+H transconjugant (*Kan*^{r}*, Amp*^{s}) will not be able to grow on LB+Kan+Amp containing media. Original recipient *V.harveyi (Kan*^{s}*, Amp*^{r}) will also not grow on LB+Kan+Amp plates.
This way the recipient gets *tra*$^+$*, ina*$^+$ and *Kan*^{r} from the transconjnugant donor (D+H). The findings of the triparental mating was a bacterial strain which is able to catalyze ice formation and to glow.

Appendix:
www.Links http://www.mun.ca/biochem/courses/4103/topics/conjugations.html
http://www.hhmi.org/lectures/biointeractive/animation/conjugation/conj_tranes.html
Reading Chapters 9.1, 9.5, 9.9 in BBOM 9th