Bacterial genetic exchange :

Conjugation of *ina+ (ice nucleation active)* gene from *E. coli* into luminous *V. harveyi*

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Introduction:

We wanted to transfer the ice formation inducing gene (*ina*+) from *Escherichia coli* (pJL 1703) to a luminescent bacteria strain called *Vibrio harveyi*. Due to the lack of a plasmid transfer enabling gene (*tra*+) in *Escherichia coli* (pJL 1703) we need the helper strain *Escherichia coli* (pRK 2013) to transfer its (*tra*+) to *Escherichia coli* (pJL 1703) first. Equipped with the plasmid transfer ability our ice formation inducing strain will be able to pass on its (*ina*+) to *Vibrio harveyi*. The conjugation process involving the donor, the recipient and the helper strains is called *triparental mating*.

Full Instructions of this experiment:
http://www.microeco.unizh.ch/uni/kurs/bio3_05/pdf/10conjug.pdf

Please see these links for explanation of conjugation:
http://www.hhmi.org/biointeractive/animations/conjugation/conj_frames.htm [Animation!!!]
http://www.molgen.mpg.de/~ag_lanka/bacterial_conjugation.html
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/A/Avery.html

We use the following stems:
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)

Phenotype abilities:
tra+: Enables bacteria to transfer plasmids through pili.
ina+: Enables bacteria to build membrane proteins that catalyze water into ice formations.
lux+: Enables bacteria to emit light in visible range.
Kan^r: Enables resistance to antibiotic Kanamicin.
Amp^r: Enables resistance to antibiotic Ampicillin.

What we wanted to happen is, that the helper strain transfers its transferring ability to the donor. Then the donor should transfer its ice formation-inducing gene to the strain with luminescent abilities. At the end we should have a transconjugant strain of luminescent bacteria that has the ability to induce the ice formations. We used selective medium with lethal concentrations of Kanamycin and Ampicillin. When our donor is passing on the (*ina*+) gene the (*Kan^r*) is associated with it, so our recipient strain, which originally only possesses a resistance against Ampicillin, is expected to have resistance to both antibiotics.

We tested some mixtures of the strains then the one with all three of them on the media:

Treatment	R	R+D	R+H	D+H	R+D+H
Growth on Kan + Amp medium	-	-	-	-	+
Luminescence	-	-	-	-	+

It is interesting to look at R+H:
Why does this combination not allow the recipient to grow on our media? This is due to the suicide plasmid in the helper strain, in which the transfer in the recipient cell would have been occurred, but the DNA polymerase of *Vibrio harveyi* can not recognize their gene promotors.

After testing their luminescence ability of our transconjugants in the dark room, we had to also test their capability to catalyze water into ice formations. Therefore, we randomly selected some luminescence colonies and spreaded them onto a new plate, to have more cells available. After incubating for 48 hours, the cells on the selected medium were scratched and resuspended in the buffer solution. As controls, we also tested the solution containing buffer only (negative control), the solution containing donor cells (positive control) and recipient cells (negative control). After incubating at -5 °C we detected the ice formations in the positive control, as well as in the solution with our transconjugants. Other negative controls contained still all liquid solutions. As expected our experiment is a full success.

For further details on the glowing ability (luminescence), see report and instructions of experiment 12.