What happens if we express tRNA which are absent in all bacteria?

Gaurav Diwan, Zarina Bikmullina, Deepa Agashe, Artai Lage - Lab of Bacterial Genome Evolution

Background: Genes that express tRNA are very important for protein synthesis. Although some tRNA anticodons are not present across all sequenced bacteria. This is surprising since these tRNA can simply arise as a result of a single point mutation in another isoacceptor tRNA. It is possible that these tRNA face negative selection. Thus, we tested how the expression of such tRNA in bacteria could affect its growth.

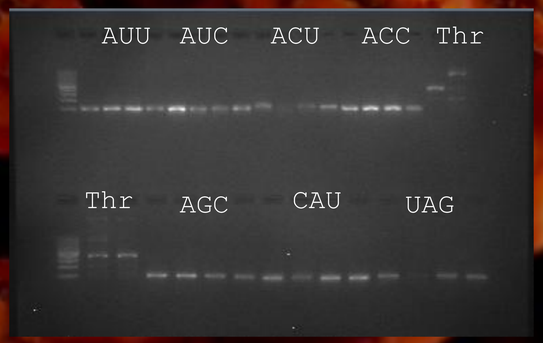
At first we made PCR in order to obtain copies of the DNA sequence that code the absent tRNA anticodons

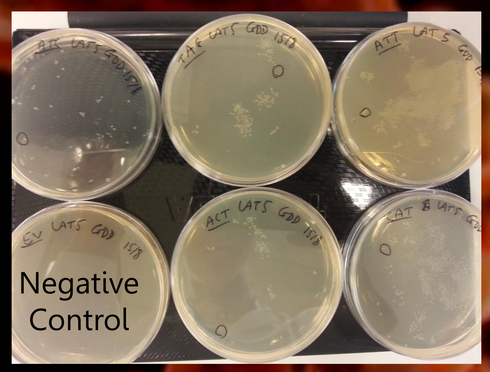
AUU AUC ACU ACC AGC; Control - CAU UAG

When PCR worked we had to purify the DNA and we obtained 260/280 ratio of 1,75-1,86

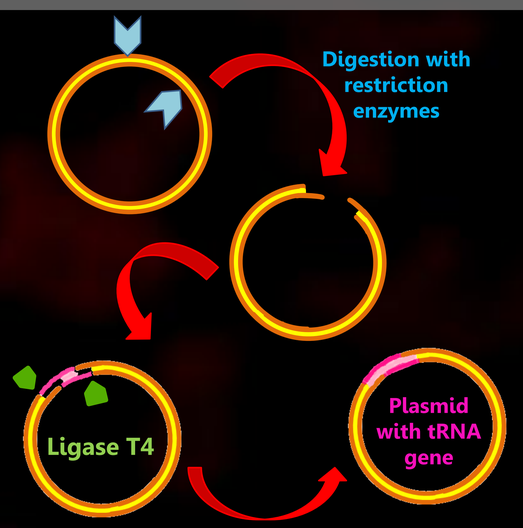
Then, we had to digest the PCR product and plasmid with restriction enzymes, EcoRI and NotI

Results





Ligation: after the digestion of the plasmid we ligated the DNA insertion with the vector. We made a negative control to make sure that the plasmid was digested.



When the ligation was completed we transformed *E. coli* to get clones for each anticodon

Conclusions