**INSILICO ANALYSIS**

Insilico analysis of any system is essential as it provides a better scientific understanding and serves as a guide for future physical experimentation. We have carried out insilico studies by using an online bioinformatics tool called RNA VIENNA FOLD to predict the secondary structures of the new parts that we are adding to the registry this year. This helped us to anticipate if our system would work as expected or not.

**Optimization of the ROSE RNA thermometer for B.subtilis:-** The functionality of the RNA thermometer is based on the intrinsic property of its structure. Hence just by altering the Shine Dalgarno sequence, it can be made functional across a variety of prokaryotic hosts. Since B.subtilis is the second most used chassis organism in iGEM, RNA Thermometer optimized for this bacterium will be a valuable addition to the Registry. The secondary structure of the RNA thermometer optimized for B.subtilis was obtained just by pasting the new sequence with the altered SD sequence in RNA VIENNA FOLD. This was very similar to the one optimized for E.coli and both had nearly equal thermodynamic free energies as shown in the figures below.

(Picture 1) (Picture 2)

**RNA pHmeter: -** Insilico analysis was done to obtain the 2D structure of this part, the sequence of which has been obtained from GENBANK. To make the pHmeter adaptable and easy to use we decided to replace its natural scar site with the BioBrick scar site. The modification to replace the scar site was carried out such that the secondary structure and Gibbs free energy change remained similar to that of the natural one as shown in the figure below. Both the variants of the RNA pHmeter i.e the wild type riboswitch with the natural scar site and a modified one with a replaced BioBrick scar site were then tested and their performances were compared.

(Picture 1) (Picture 2)