Results & Future

Expression

The next few pages will guide you through the results of the characterization of our submitted parts. On this page, you will find short descriptions of our results, leading you to the final result. We hope you will you will find an interest in the results.

The aim of our project was to express one of our six Gln-H proteins.

The six mutant genes will be expressed in the bacterial system; the degree of expression will be tested for each of the genes. The protein that will be expressed with the correct amount should connect to the gluten and create a color reaction that proves the connection. The color reaction will only be created if the structure of the protein was done correct and accurate in designing the protein.

For this purpose We created 6 mutant proteins from 3 different bacterial sources. The six mutants are different from each other in the way they report on the presence of gluten – half of them them includes the GFP protein and half of them the HAD enzyme.

Stage 1: Cloning the six mutant genes of the Gln-H protein

The cloning is done into the pET-R 3 plasmid. These six parts will be found in the Igem library according to the following details. The name of the gene includes its source (the name of the bacteria we took the DNA sequence of the wanted protein) and the name of the reporter gene.

TM- HAD

BBa_k1790000- http://parts.igem.org/Part:BBa_K1790000:Design

TM-GFP

BBa_k1790001- http://parts.igem.org/Part:BBa_K1790001:Design

GBS- HAD

BBa_k1790002- http://parts.igem.org/cgi/partsdb/part_info.cgi?part_name=BBa_K1790002

GBS- GFP

BBa_k1790003- http://parts.igem.org/Part:BBa_K1790003

EC- GFP

BBa_k1790004- http://parts.igem.org/Part:BBa_K1790004

EC- HAD

BBa_k1790005- http://parts.igem.org/Part:BBa_K1790005

The genes were cloned to the Pet-R 3 plasmid and after that a transformation was implemented to the component bacteria – type: DH5 –alpha E.Coli.

For every ligation product (each one of the mutants) 2 types of transformation were performed: heat shock and electroporation. This action was performed in order to increase the chance of transformation feasibility.

At the end of the transformation the cells were seeded on a platform including – LB+amp+1%glu.

Stage 2- Expressing the genes after cloning

After purification of the proteins in the cells (using lysozyme and sonication), we carried out a specific separation of Gln-H using Ni-NTA Colonna Agarose (QIAGEN).

The Purification System is based on the remarkable selectivity of patented Ni-NTA (nickel-nitrilotriacetic acid) resin for proteins which contain an affinity tag of six or more histadine residues. This technology allows one-step purification of almost any His-tagged protein from any expression system under native or denaturing conditions.

We ran the collection of fractions of the entire extraction process of the protein Gln-H, on SDS PAGE. This stage finds the protein that expresses the best way possible the system we created.

Size of expected protein – 55KDa

Following are the results for each of the mutants.

Fig 1: SDS PAGE EC-HAD (Clone 1)

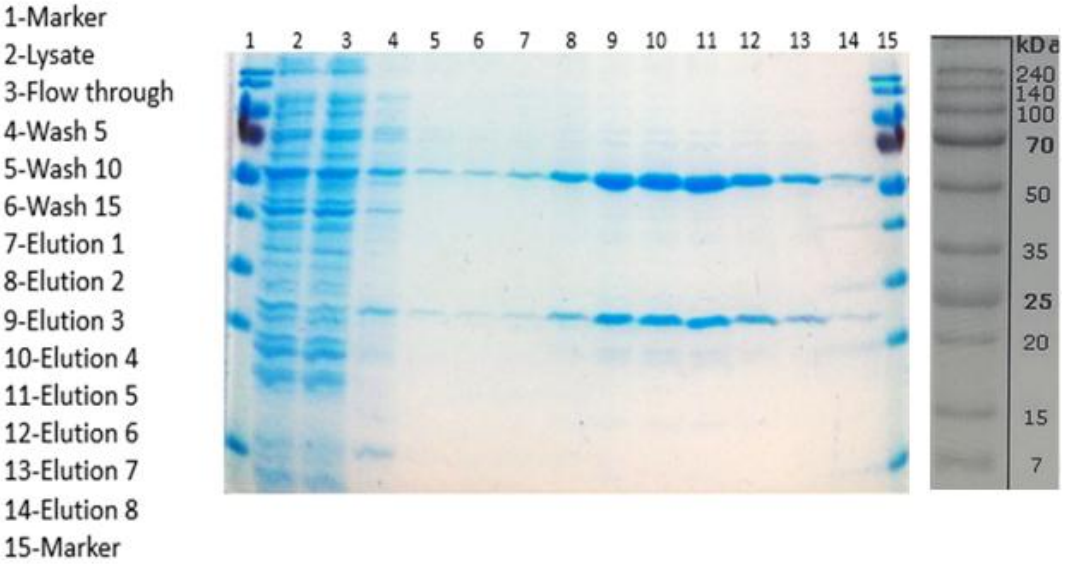


Figure1 - gene No. 1 EC_HAD is expressed in the system, apparently is split into two subunits, we assume that the reason lies in the separation process, changes in electric energy is due to the change in PH may cause protein breakdown in subunits. Another possibility is that there are more proteins we separated in the constraint process to release the Gln_H protein. However we can see that the protein is expressed well in the system.

Fig 2: SDS PAGE EC-GFP (Clone 2)

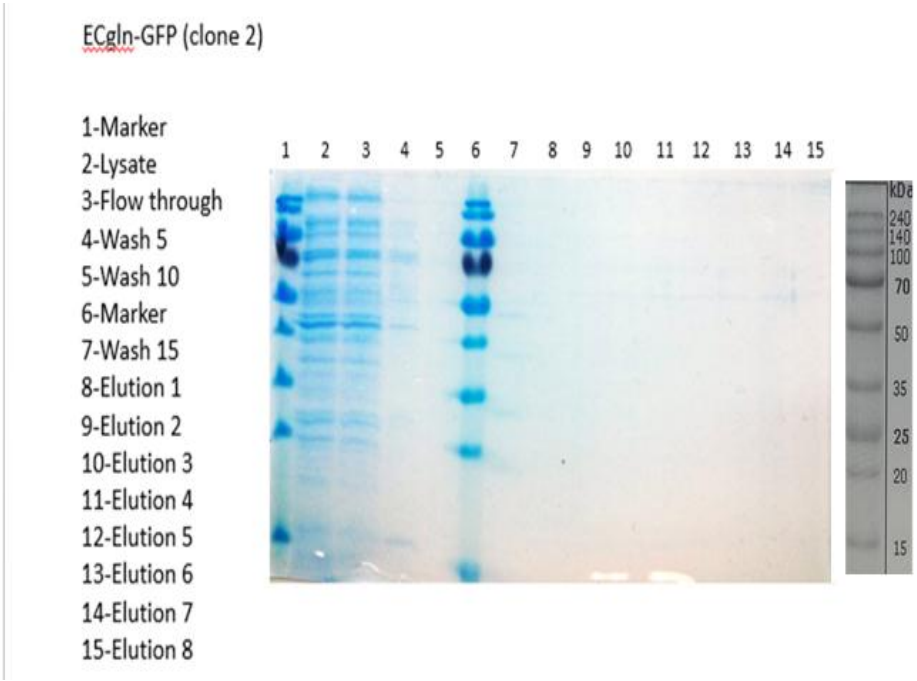


Figure 2 gene No. 2- EC-GFP shows the protein expression Gln_H channels 7-14. Protein expression in this gene is lower; the marker strength is very low relative to the protein extracted from gene No. 1.

Fig 3:SDS PAGE GBS- GFP (Clone 4)

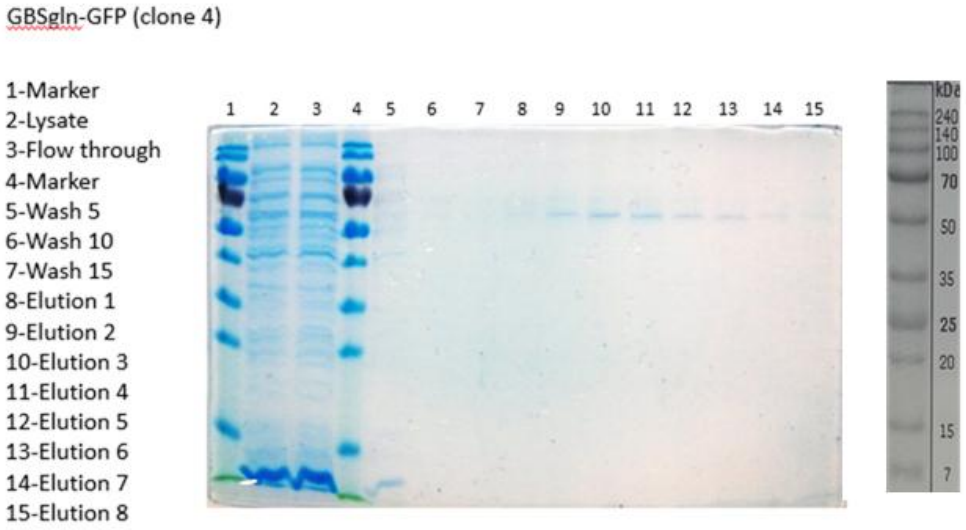


Figure 3- gene No. 4 GBS- GFP is expressed in channels 8-14, protein Gln-H expression in this gene is lower than the expression of the protein than in gene No.1 however, higher than the expression of protein extracted from gene No. 2.

(Protein Activity Test - EC-HAD OD depending on time (SEC

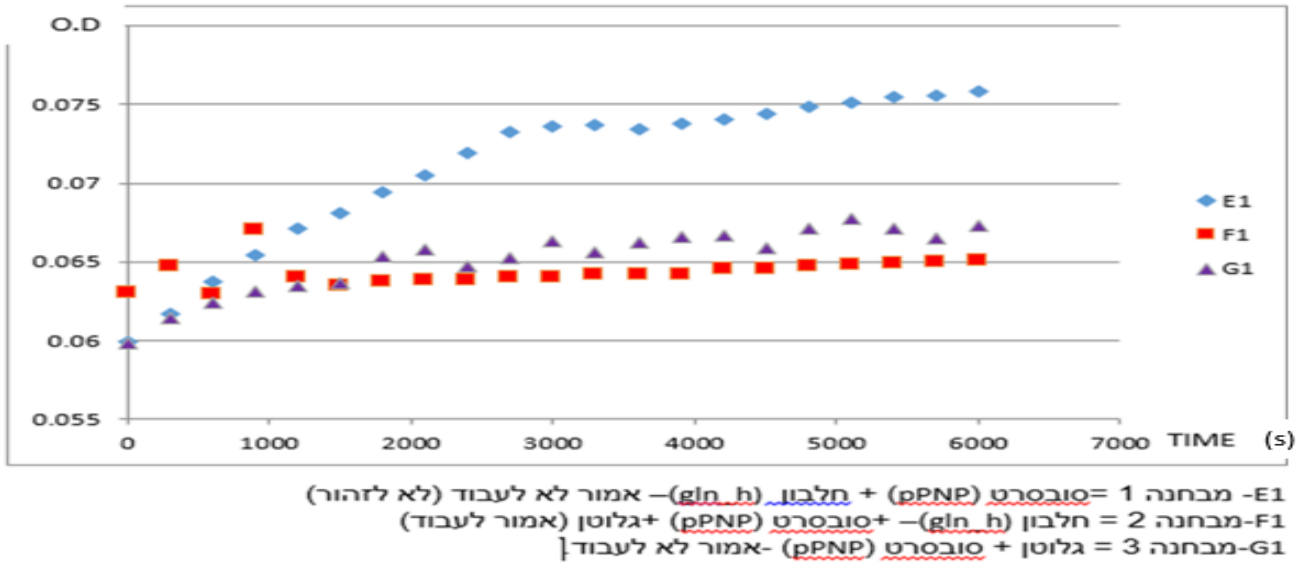


Fig 4: SDS PAGE GBS-HAD (Clone3)

Clone 3

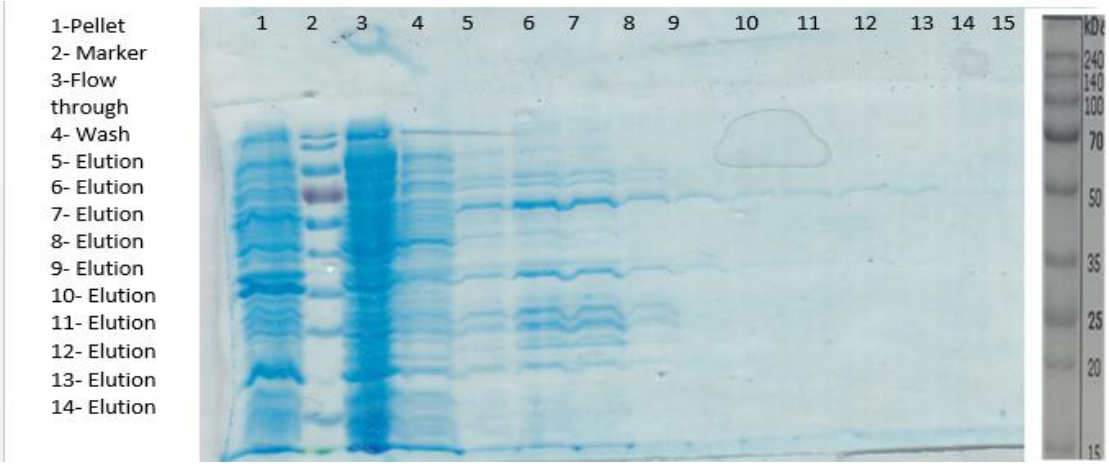


Figure 4 gene No. 3 - EC-HAD expressed in channels 5-14, protein Gln-H expression in this gene is significantly lower than the protein expression from gene No. 5 but higher than protein expression extracted from gene No. 6.

Fig 5: SDS PAGE TM-HAD (Clone 5)

Clone 5

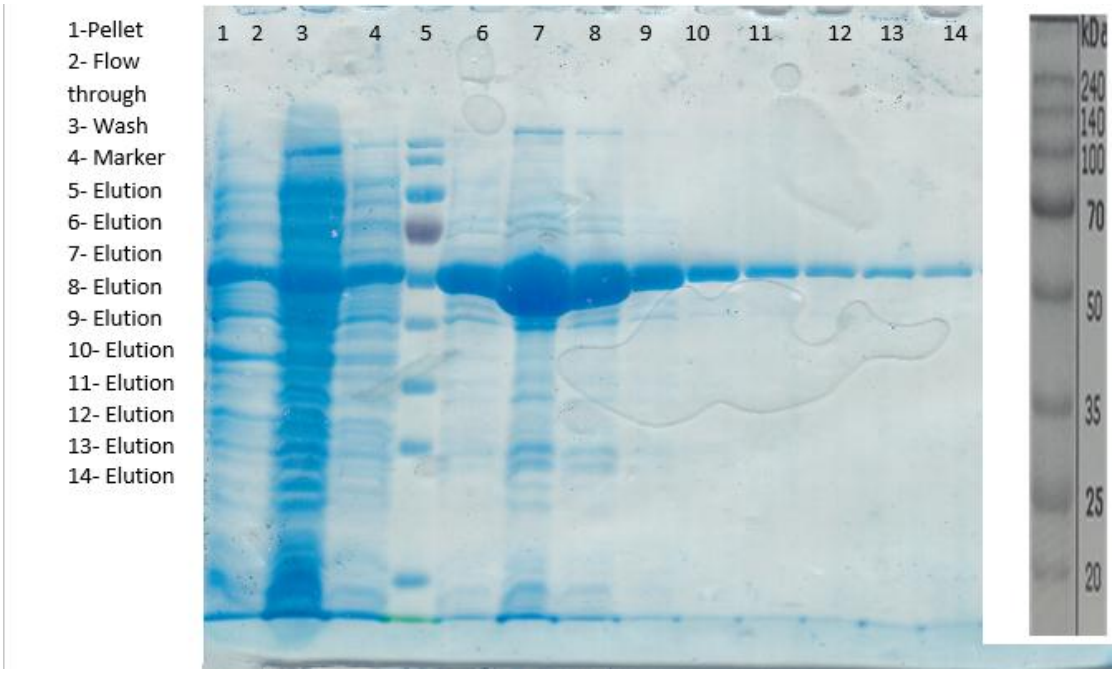


Figure 5- gene No. 4 TM-HAD is expressed starting channel 5 in all the channels, 5-.14 protein Gln-H expression in this gene was highest observed.

Fig 6: SDS PAGE TM-GFP (Clone6)

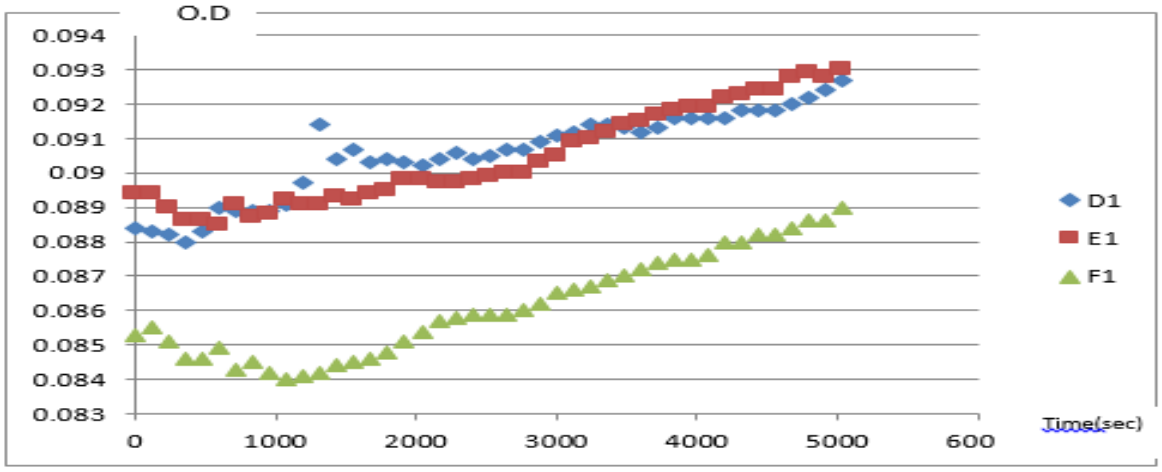
Clone 6

Figure 6 gene No. 6 TM GFP is expressed in channels 5-15, protein Gln-H .expression in this gene is lower than the protein expression in the rest of the genes

Avtivity Tests:

No activity was seen in proteins 3,5,6

Protein activity test-GBS-HAD O.D depending on time (SEC)



D1- מבחנה 1 =סובסרט (pPNP) + חלבון (gln_h)– אמור לא לעבוד (לא לזהור)
D1-מבחנה 2 = חלבון (gln_h)– +סובסרט (pPNP) +גלוטן (אמור לעבוד)
F1-מבחנה 3 = גלוטן + סובסרט (pPNP) -אמור לא לעבוד.

Throughout the process we made sure of the existence of control.

1. Transformation stage, the control plate had bacterial cells without the recombinant plasmid and their seeding on a selective platform containing Amp, and of course the control plate was clean from bacteria.
2. Protein cleaning stage, the control plate was a selective platform containing Amp but without IPTG molecule and therefore, we did not see protein, lactose-free that activates the T7- insert encoded to the Gln_H protein will not be expressed.

Appendix 1

Difficulties on the way

1. Throughout the process we encountered difficulties with genes 3,5,6. First, the ligation process did not succeed- the genes did not enter into plasmid expression, the transformation process was done in a different way in order to increase the chances of success. In consultation with the laboratory team leader, Dr. Itamar Yadid, it was decided that we will perform a different process with these genes. First we cut them, then we increased them by phusion PCR reaction, then we performed ligation and sedimentation of ethanol and performed a transformation using electroporation method for bacteria bl21 (de3), the next step we performed PCR colony and continued the process as we did with genes 1,2,4.