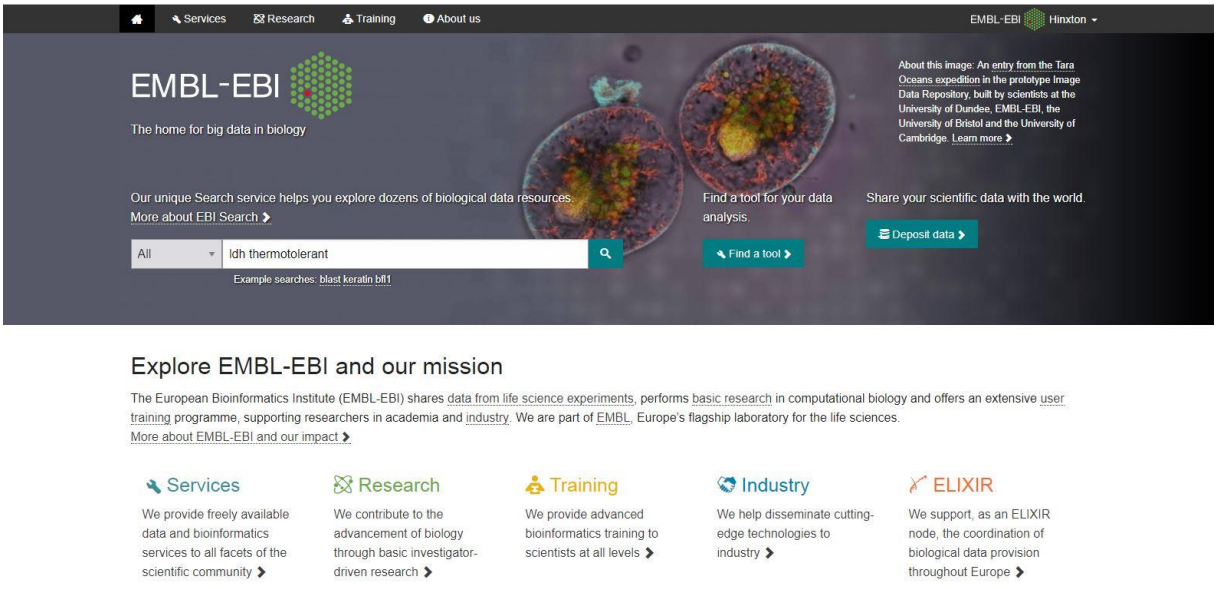
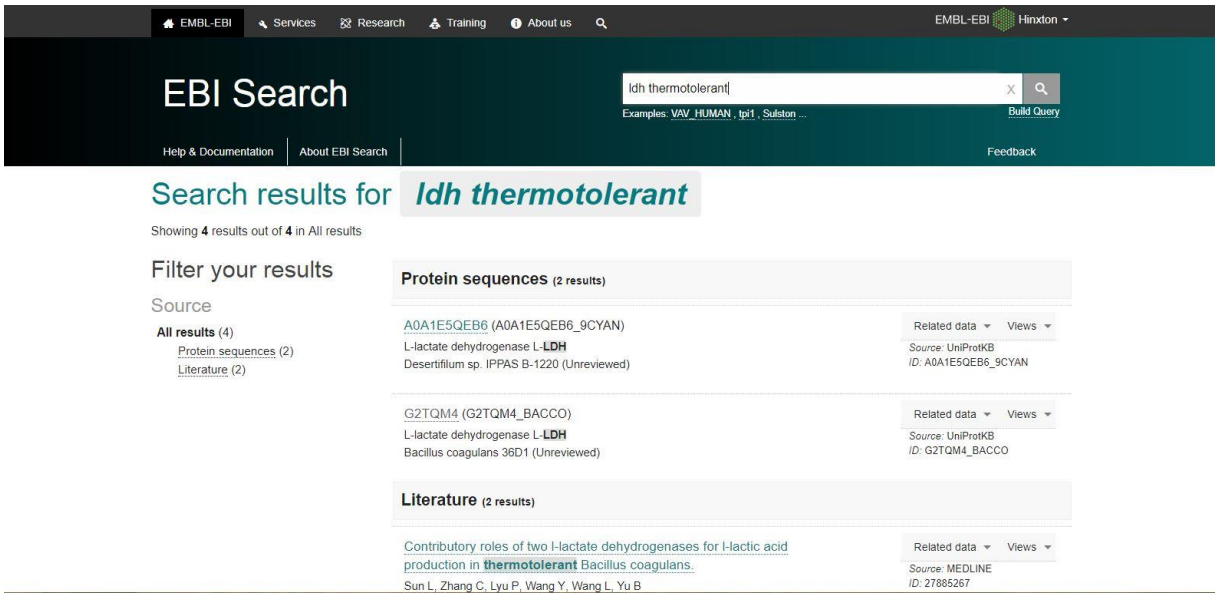
Bioinformatics

LDH sequence

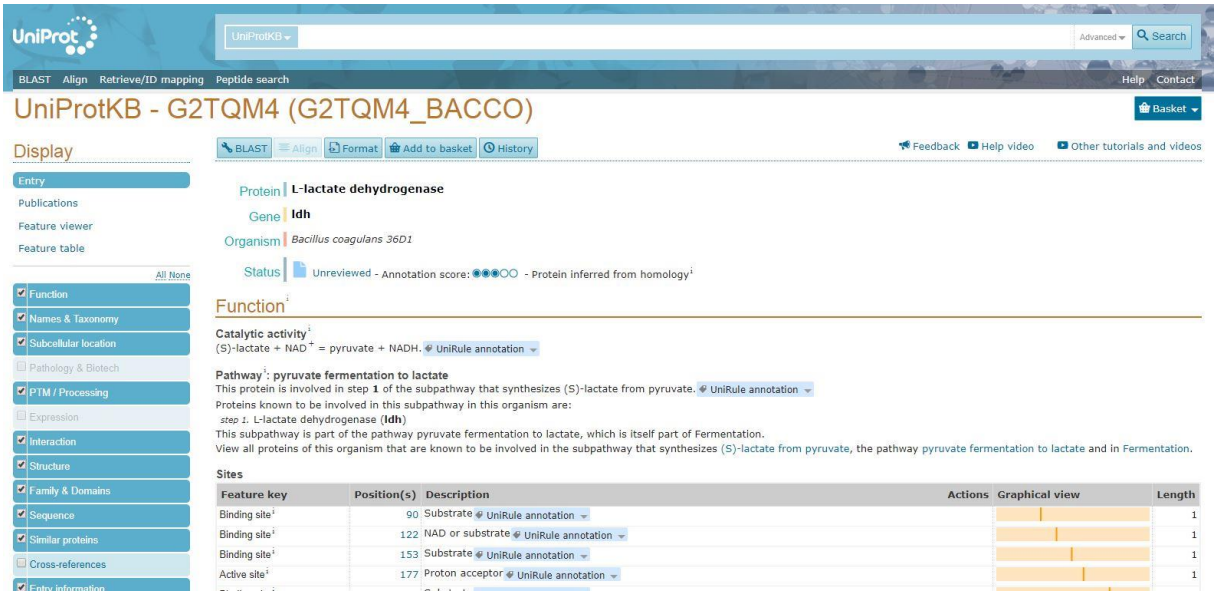
1. For creating lactate from pyruvate we needed an enzyme, called lactate-dehydrogenase (LDH). Therefore we used the database of the European Bioinformatics Institute (www.ebi.ac.uk) to find an appropriate enzyme. Since the Methylococcus capsulatus is a thermotolerant organism, we thought it would be worth searching for a thermotolerant LDH as well, so we made the search with the keywords “ldh thermotolerant”.



2. Searching for the keywords “ldh thermotolerant” two results were shown. One of them was the protein sequence from the Bacillus coagulans 36D1 bacterium and the other one from the algae species *Desertifilum*. For using it in a bacterium we chose the bacterium enzyme.

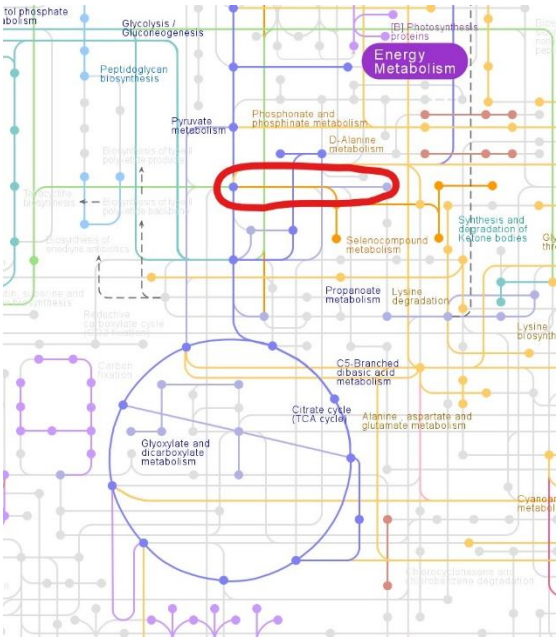


3. In the UniProt database (www.uniprot.org) we could find valuable information (e.g. function, names and taxonomy, structure, sequence etc.) about the chosen enzyme.

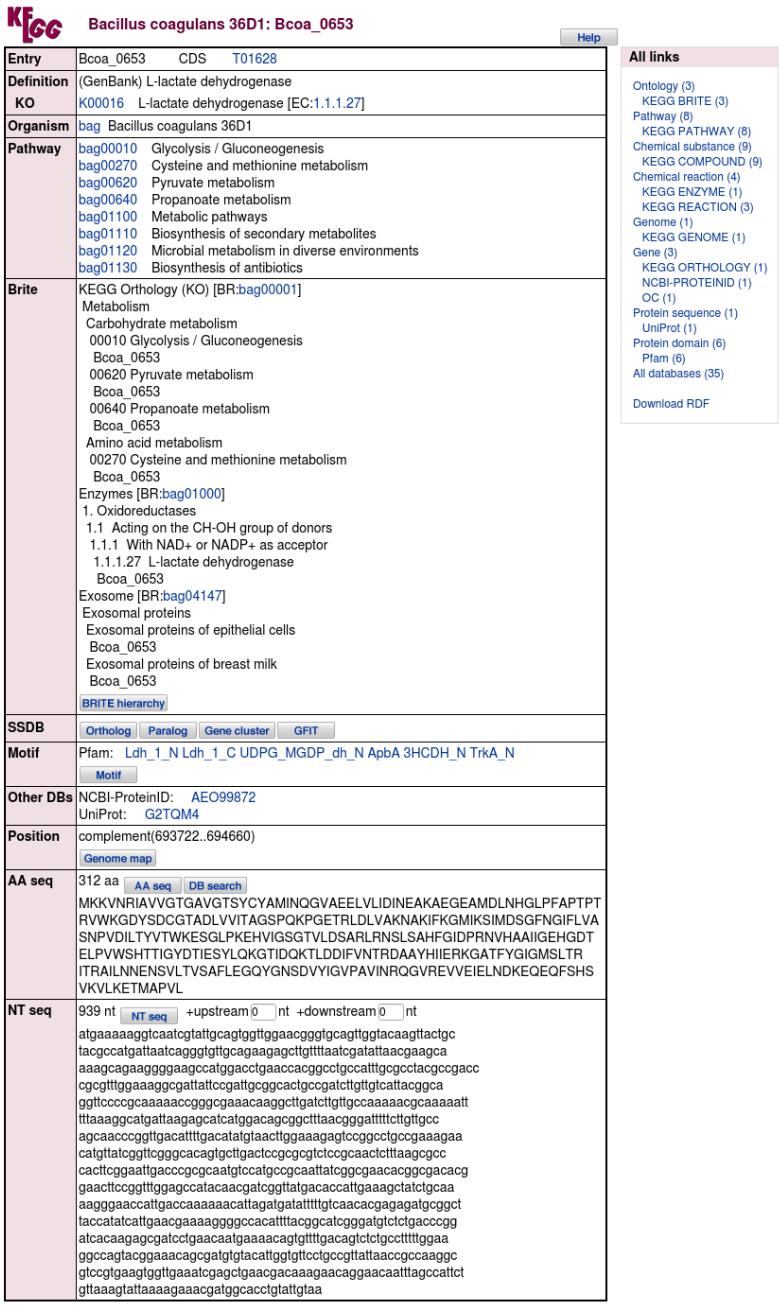


4. For finding the gene sequence of the LDH, we visited the website of the KEGG PATHWAY Database (http://www.genome.jp/kegg/pathway.html), where we looked for the metabolic pathway of Bacillus coagulans.

5. In the figure we had to find the reaction of the lactate-pyruvate converting for the enzyme details.



6. Opening the enzyme's datasheet we realized that two enzymes are in connection with this reaction. We chose the L-lactate-dehydrogenase because it was smaller in size and had the usual beginning ATG bases.



Bacillus coagulans 36D1: Bcoa_0653

Help

Entry	Bcoa_0653 CDS T01628
Definition	(GenBank) L-lactate dehydrogenase
KO	K00016 L-lactate dehydrogenase [EC:1.1.1.27]
Organism	bag Bacillus coagulans 36D1
Pathway	bag00010 Glycolysis / Gluconeogenesis bag00270 Cysteine and methionine metabolism bag00620 Pyruvate metabolism bag00640 Propanoate metabolism bag01100 Metabolic pathways bag01110 Biosynthesis of secondary metabolites bag01120 Microbial metabolism in diverse environments bag01130 Biosynthesis of antibiotics
Brite	KEGG Orthology (KO) [BR:bag00001] Metabolism Carbohydrate metabolism 00010 Glycolysis / Gluconeogenesis Bcoa_0653 00620 Pyruvate metabolism Bcoa_0653 00640 Propanoate metabolism Bcoa_0653 Amino acid metabolism 00270 Cysteine and methionine metabolism Bcoa_0653 Enzymes [BR:bag01000] 1. Oxidoreductases 1.1 Acting on the CH-OH group of donors 1.1.1 With NAD+ or NADP+ as acceptor 1.1.1.27 L-lactate dehydrogenase Bcoa_0653 Exosome [BR:bag04147] Exosomal proteins Exosomal proteins of epithelial cells Bcoa_0653 Exosomal proteins of breast milk Bcoa_0653 BRITE hierarchy
SSDB	Ortholog Paralog Gene cluster GFIT
Motif	Pfam: Ldh_1_N Ldh_1_C UDPG_MGDP_dh_N ApbA 3HCDH_N TrkA_N Motif
Other DBs	NCBI-ProteinID: AEO99872 UniProt: G2TQM4
Position	complement(693722..694660) Genome map
AA seq	312 aa AA seq DB search MKKVNRIAVVGTGAVGTSYCYAMINQGVAEELVLIDINEAKAEGEAMDLNHGLPFAPTPT RVWKGDYSDCGTADLVVITAGSPQKPGETRLDLVAKNAKIFKGMIKSIMDSGFNGIFLVA SNPVDILTYVTWKESGLPKEHVIGSGTVLDSARLRNSLSAHFGIDPRNVHAAIIGEHGDT ELPVWSHTTIGYDTIESYLQKGTIDQKTLDDIFVNTRDAAYHIIERKGATFYGIGMSLTR ITRAILNNENSVLTVSAFLEGQYGNSDVYIGVPAVINRQGVREVVEIELNDKEQEQFSHS VKVLKETMAPVL
NT seq	939 nt NT seq +upstream 0 nt +downstream 0 nt atgaaaaaggtcaatcgtattgcagtggttggaacgggtgcagttggtacaagttactgc tacgccatgattaatcagggtgttgcagaagagcttgttttaatcgatattaacgaagca aaagcagaaggggaagccatggacctgaaccacggcctgccatttgcgcctacgccgacc cgcgtttggaaaggcgattattccgattgcggcactgccgatcttgttgtcattacggca ggttccccgcaaaaaccgggcgaaacaaggcttgatcttgttgccaaaaacgcaaaaatt tttaaaggcatgattaagagcatcatggacagcggctttaacgggatttttcttgttgcc agcaacccggttgacattttgacatatgtaacttggaaagagtccggcctgccgaaagaa catgttatcggttcgggcacagtgcttgactccgcgcgtctccgcaactctttaagcgcc cacttcggaattgacccgcgcaatgtccatgccgcaattatcggcgaacacggcgacacg gaacttccggtttggagccatacaacgatcggttatgacaccattgaaagctatctgcaa aagggaaccattgaccaaaaaacattagatgatatttttgtcaacacgagagatgcggct taccatatcattgaacgaaaaggggccacattttacggcatcgggatgtctctgacccgg atcacaagagcgatcctgaacaatgaaaacagtgttttgacagtctctgccttttggaa ggccagtacggaaacagcgatgtgtacattggtgttcctgccgttattaaccgccaaggc gtccgtgaagtggttgaaatcgagctgaacgacaaagaacaggaacaatttagccattct gttaaagtattaaaagaaacgatggcacctgtattgtaa

All links

Ontology (3)
KEGG BRITE (3)
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KEGG PATHWAY (8)
Chemical substance (9)
KEGG COMPOUND (9)
Chemical reaction (4)
KEGG ENZYME (1)
KEGG REACTION (3)
Genome (1)
KEGG GENOME (1)
Gene (3)
KEGG ORTHOLOGY (1)
NCBI-PROTEINID (1)
OC (1)
Protein sequence (1)
UniProt (1)
Protein domain (6)
Pfam (6)
All databases (35)

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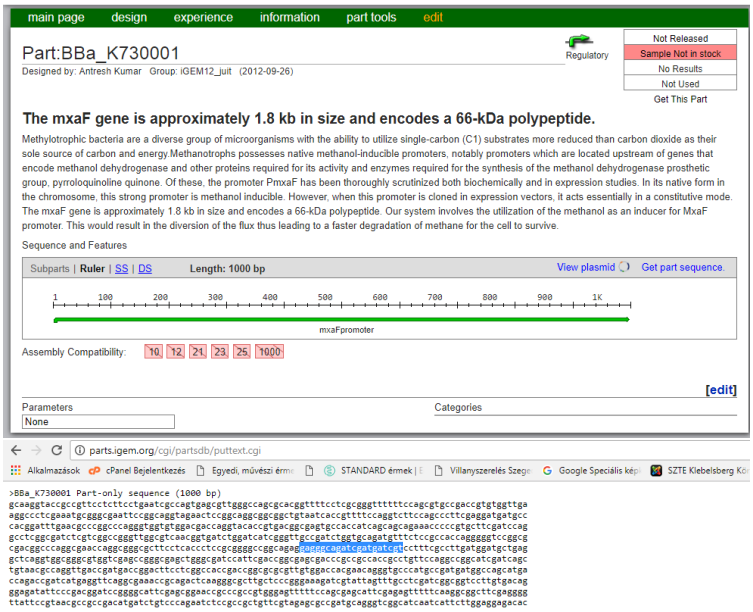
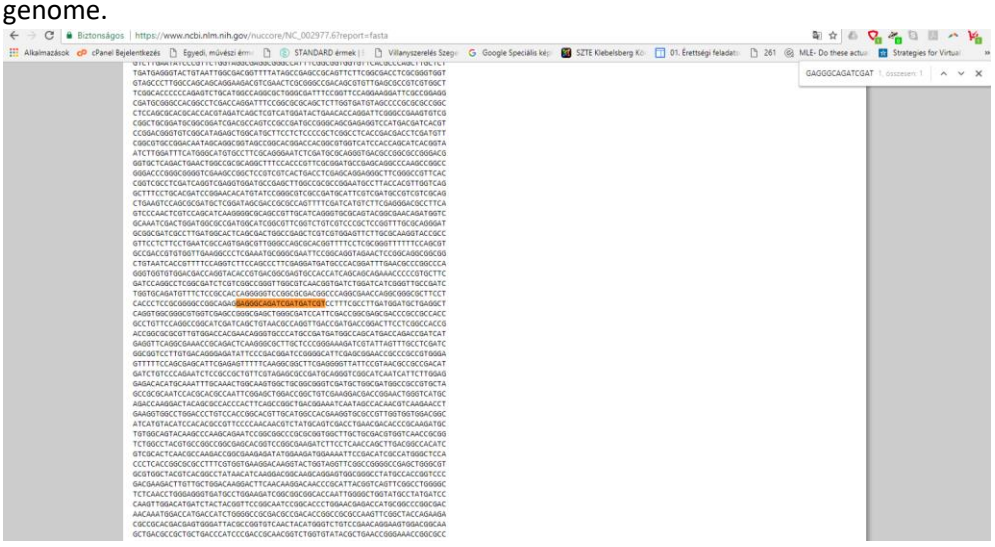
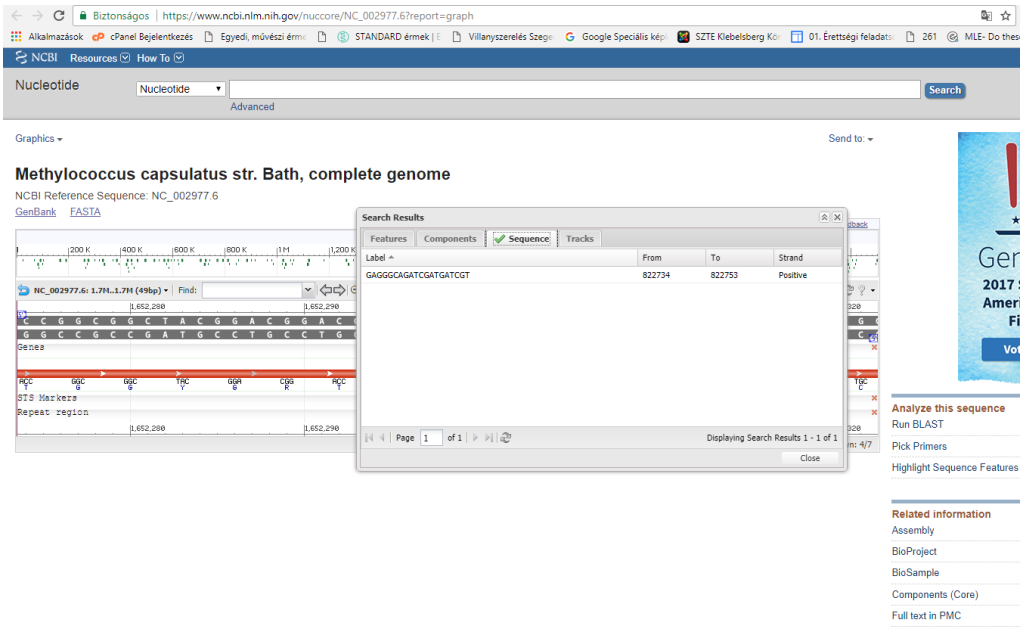
Assembling the final construct

1. It was also essential to find an appropriate promoter with high efficiency to enable the gene to work. Therefore, we looked for a promoter which can be found originally in Methylococcus capsulatus. The former iGEM team, iGEM12_juit found a nearly 1.8 kb long sequence which contained a promoter but they could not determine the exact location of the promoter.

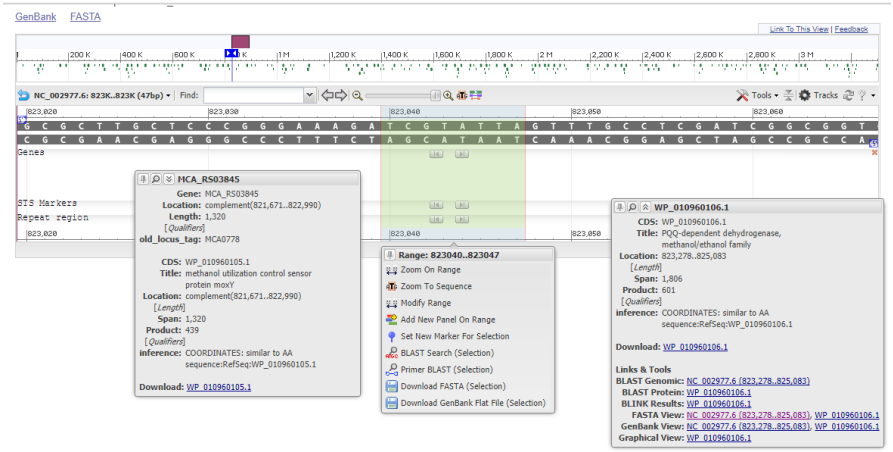


2. We did a search to find the 1.8 kb long sequence in Methylococcus capsulatus' complete genome.



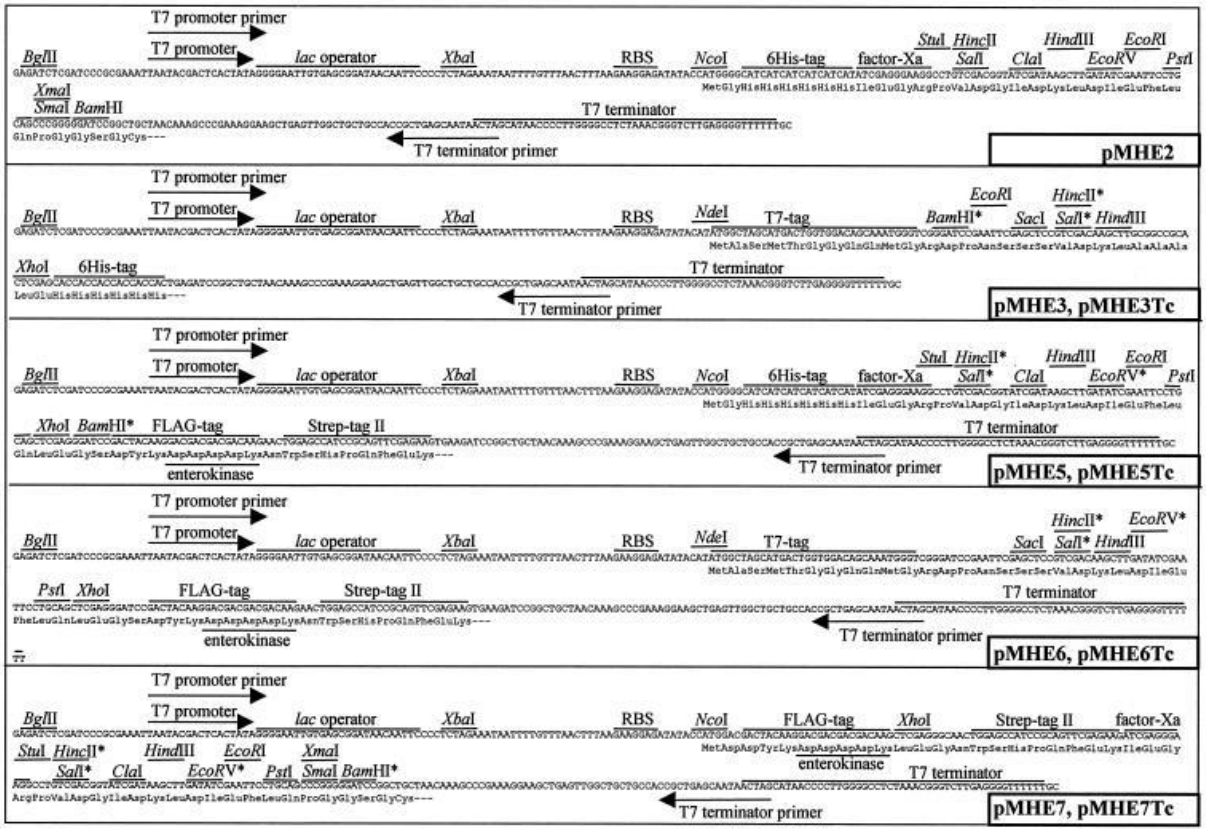


3. This sequence contained not only the nucleotide sequence between two genes (the moxY and the mxaF) but did contain a partial part of each of the two genes.



4. The putative moxY or mxaF used in this study did only contain the nucleotide sequence between the two genes, which must contain the promoter of either the moxY or the mxaF gene. Unfortunately, the orientation of the promoter is not known because the moxY and the mxaF genes are in different directions, therefore we could not determine neither the exact orientation of the promoter nor the exact sequence but we managed to approach the exact sequence of the promoter and apply it in such a way that the orientation was not needed to know.

5. Having the sequence of the LDH gene and an appropriate promoter, only a usable vector was needed to transfer the gene and the promoter into Methylococcus capsulatus. An article wrote about the vectors pMHE2, pMHE3, pMHE5, pMHE6 and pMHE7 (figure). We chose the pMHE5 and pMHE7 vectors because they were available for us in short time.



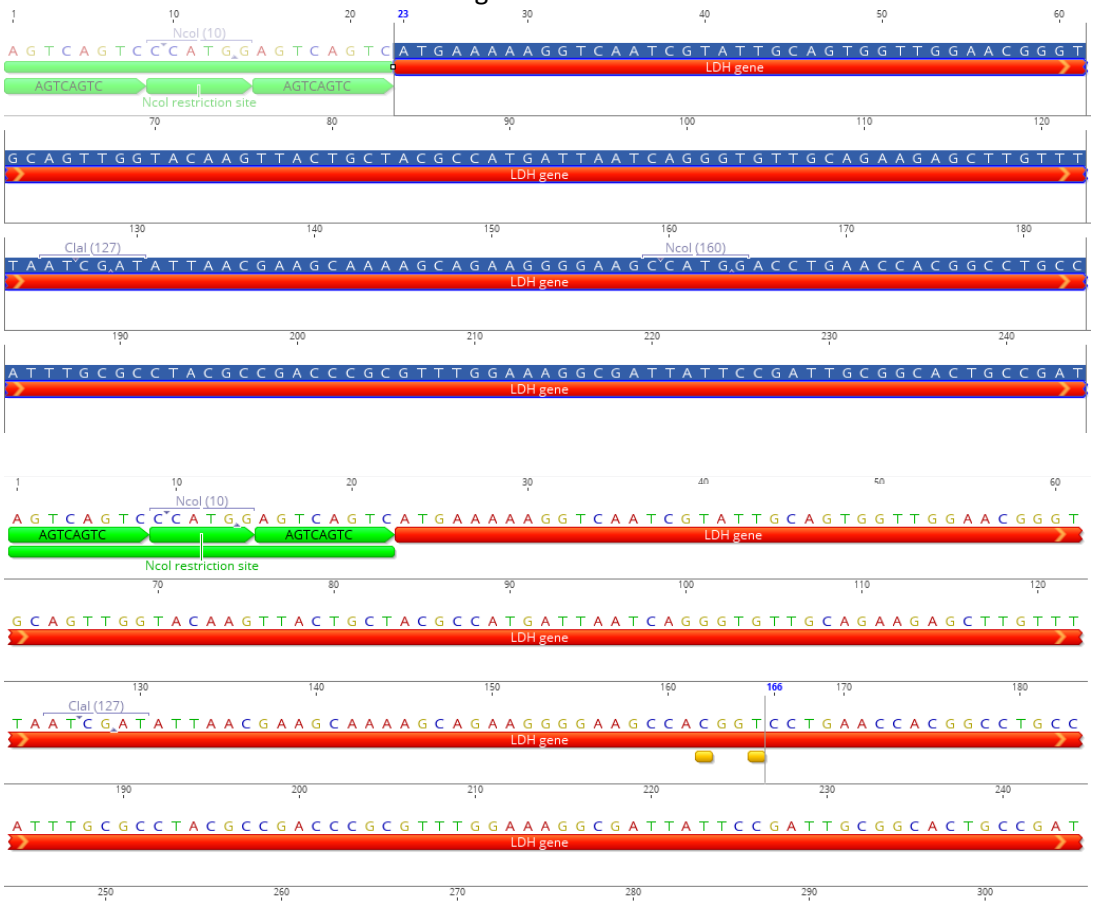
Figure

6. The LDH gene was intended to ligate between the NcoI and EcoRV restriction sites, therefore, it was supplied with an NcoI restriction site and an addition AGTCAGTC nucleotide sequence before and after the NcoI restriction site, in order to minimalize the possible damage made by the restriction enzymes (figure).

Figure: The basic structure of the synthesized gene

1. Addition AGTCAGTC bases
2. NcoI restriction site
3. LDH gene
4. EcoRV restriction site

7. The LDH gene contained an NcoI restriction site in itself, therefore, we replaced the bases so that the coded amino-acid did not changed.



8. The promoter was intended to ligate between the BglII restriction sites. This could enable the promoter to ligate in both orientations, randomly. The promoter was supplied with BglII restriction sites at both ends and addition AGTCAGTC nucleotides before and after each added AGTCAGTC nucleotides (figure).

Figure: The basic structure of the synthesized promoter

1. Addition AGTCAGTC bases
2. BglII restriction site
3. Promoter