



LAB JOURNAL GENE EXTRACTION



Prepared by

bioiqs.igem@gmail.com

Laboratory of Biochemistry, IQS School of Engineering

Strategy 1: Gene extraction, PCRs



In this strategy, we considered obtaining the HLA-DQ from scratch. Based on former studies, only the exons 2 and 3 form each chain (α and β) codify for the extracellular domain of the HLA-DQ that interacts with gluten epitopes. With this in mind, we designed a robust model for the extraction of the α and β chains of the HLA-DQ from the genomic DNA of a celiac patient. A set of primers were designed to conduct 3 different PCRs (including 10 reactions) to obtain the α and β chains flanked with restriction sites for further cloning.

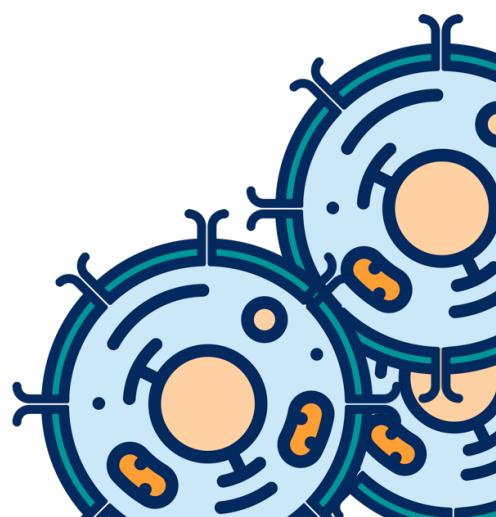
Strategy 2: Synthetic genes



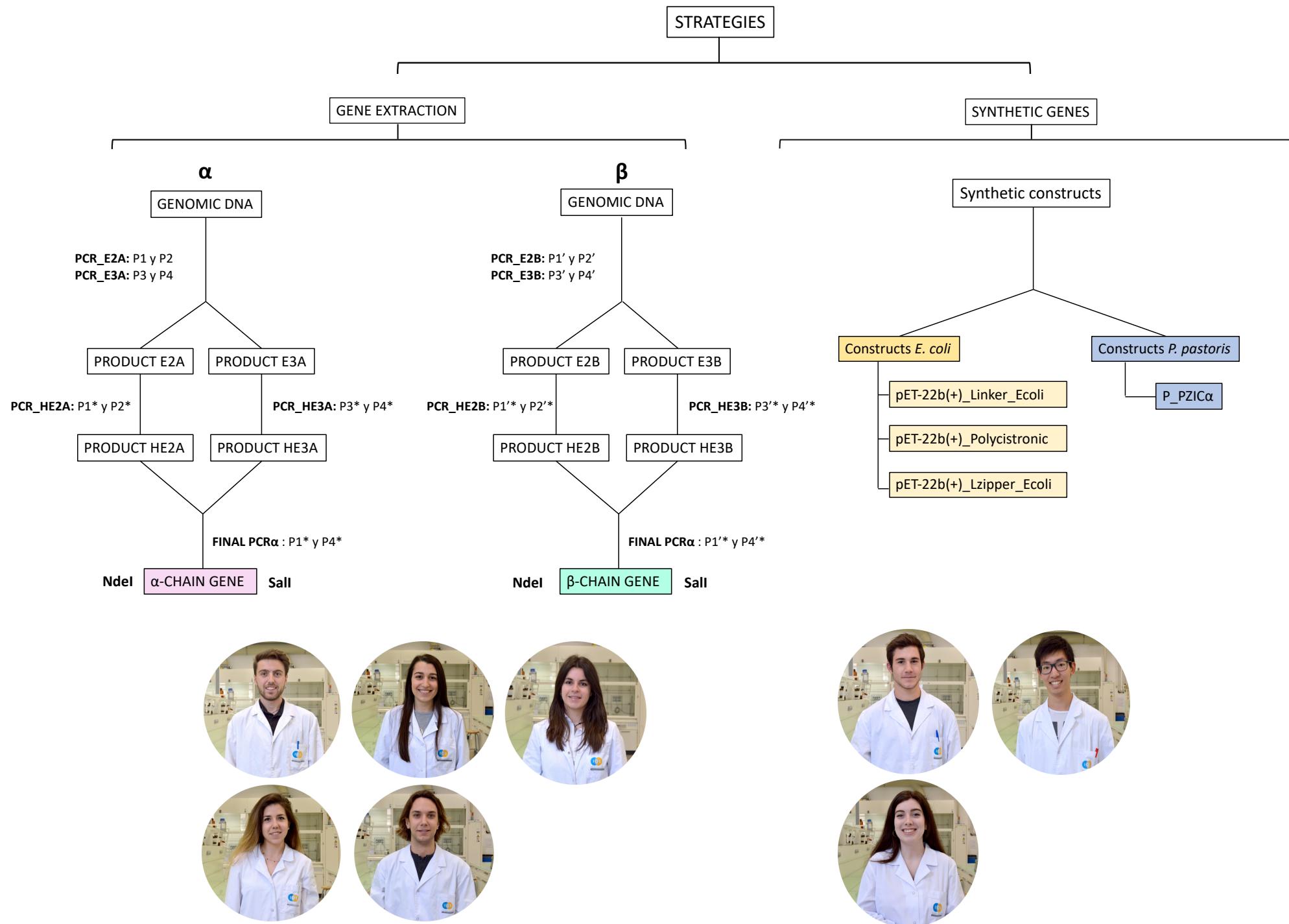
As one of the main objectives is to express the human HLA in a bacterial cell host, several approaches to conduct this were considered. Firstly, a structural analysis of the protein was made in order to visualize the different protein domains. Based on this analysis, we decided to express only the extracellular domain which was encoded by the exons 2 and 3 of the HLA gene. Next, as an attempt to improve the protein stability, we chose to add Leucine Zippers to the constructs. And finally, specific tags were added at the C-terminus of the gene sequences to enable the protein purification.

With these ideas, our team designed different synthetic genes following three strategies for the heterologous expression of the human HLA in *E.coli*.

- Expression of both α chain and β chain as a unique gene by using a fusion linker.
- Expression of the α and β chain as two separate genes in the same constructs by establishing a RBS for each gene.
- Expression of the α and β chain as two separate genes but adding the Leucine Zippers to increase its stability.

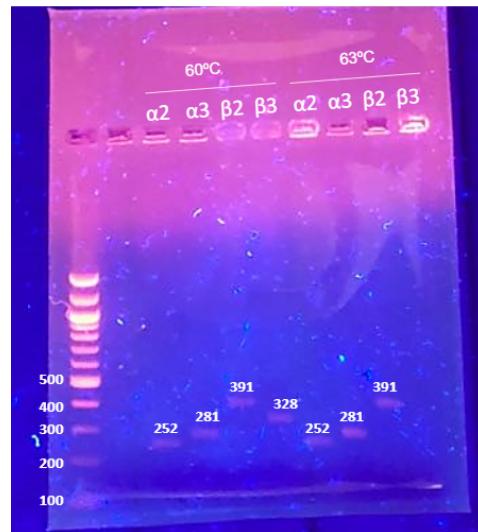


STRATEGY MAP

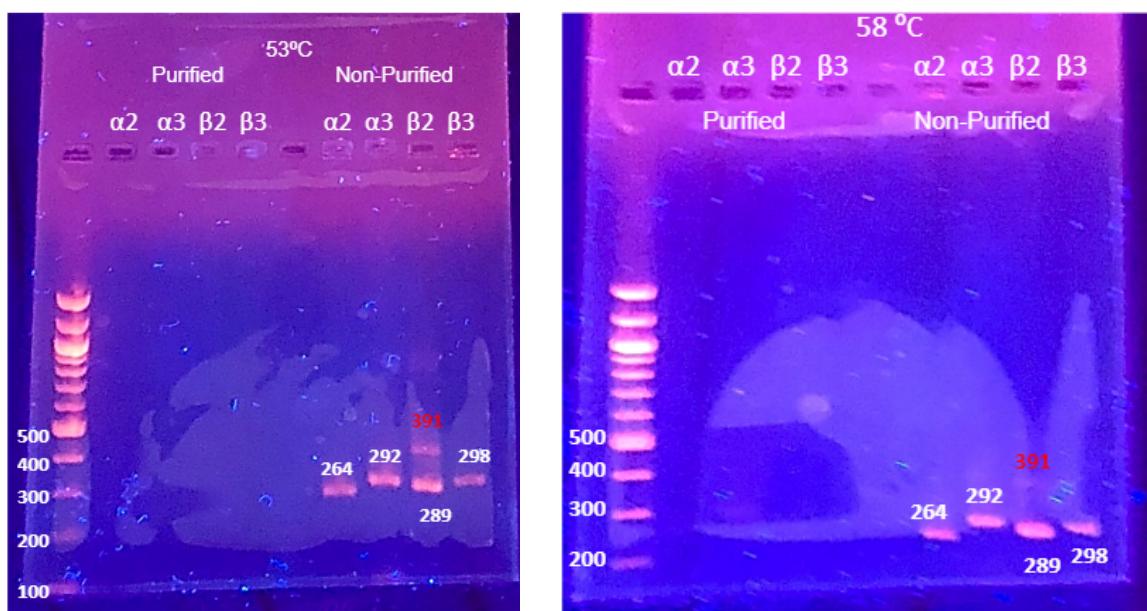


Week 1: 16/07/18 – 20/07/18

- **DNA genomic extraction:** from one of the members of the group who has been recently diagnosed as celiac. Genomic DNA extraction was done according to the Protocol 1 (DNA genomic extraction). All solutions were autoclaved. Proteinasa K stock (10X) was prepared in sterile PBS.
- **Gene extraction:** The beforementioned genomic DNA samples were used to perform an analytical PCR_1 to obtain the α and β exons. PCR1 was tested at different temperatures: 60°C and 63°C.

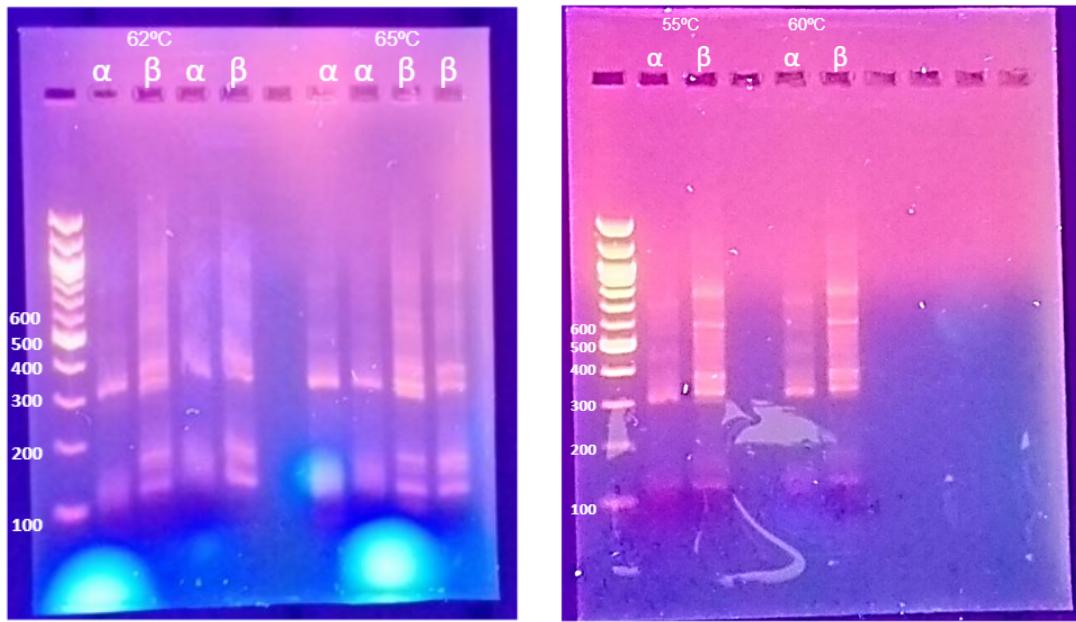


- **PCR products purification:** samples from PCR_1 at 63°C were purified using PCR and DNA Fragment Purification kit (Neo Biotech).
- **Analytical PCR_2** was performed in order to add homology tails at the terminal ends of each exon. The process is described in the Protocol 2. Purified and non-purified samples from PCR_1 were used as template. Two different temperatures (53°C and 58°C) were tested.



NOTEBOOK: STRATEGY 1, GENE EXTRACTION

- In order to obtain the α and β chains, an **analytical PCR_3** was conducted following the steps described in the Protocol 2. Different temperatures of annealing were tested, including 55°C, 60°C, 62°C and 65°C. Results: Smear.

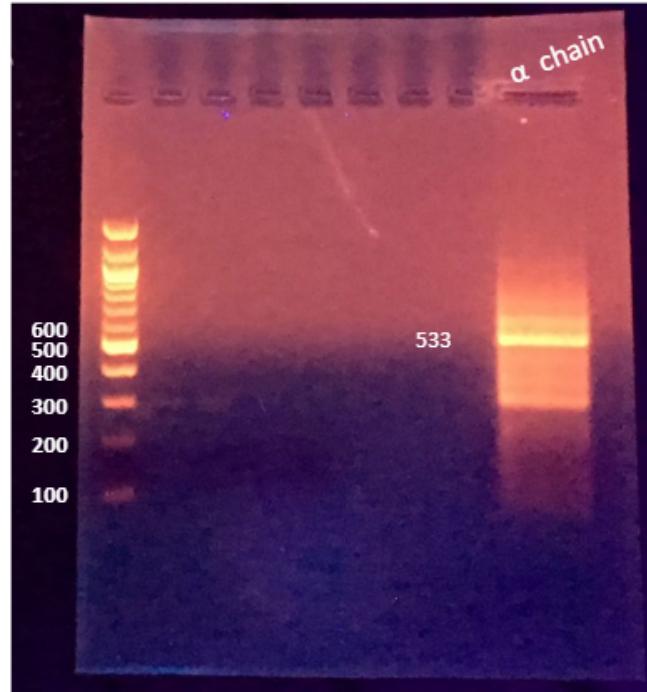


Week 2: 23/07/18 – 27/07/18

- **Band extraction:** α and β exons from the former PCR_2 (from Week 1) were purified using QIAquick Gel Extraction kit (QIAGEN).
- **Analytical PCR_3** was set at 62°C and 64°C using PCR_2 purified samples as a template. Results: α chain was obtained. No band belonging to the β chain was observed.



- **Preparative PCR_3_α Chain** was set at 62°C using PCR_2 purified samples as a template.
- **Band extraction:** the α chain was purified from the agarose gel using QIAquick Gel Extraction kit Protocol 3.



- **Electrocompetent cells** DH5 α were prepared according to the Protocol 4. Viability and transformation efficiency were tested. Electrocompetent cells are viable (O/N culture in LB+agar plates).

Week 3: 30/07/18 – 03/08/18

- **Miniprep pET22b vector** (using QIA Miniprep Kit).
- **pET22b vector and α chain digestion** as described in Protocol 5.



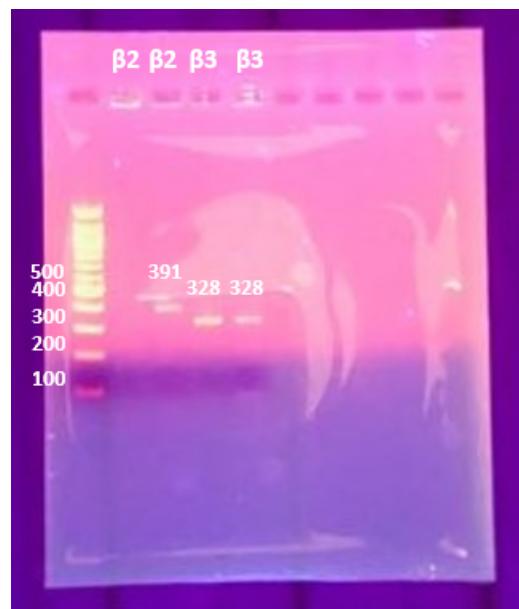
- **pET22b vector and α chain ligation** as described in Protocol 6. Different ratios of vector-insert were tested, including:

- ***E. coli DH5α* electroporation with pET22b- α chain.** *E. coli* cells were transformed as described in Protocol 7 and cultured in LB+Ampicillin plates at 37°C overnight. Results: Some colonies were observed.

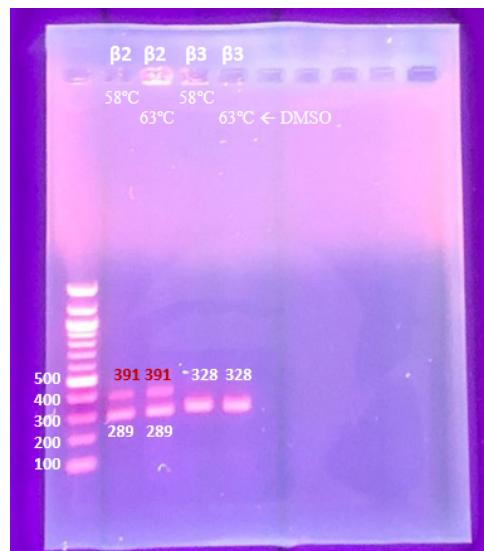
Week 4: 27/08/18 – 31/08/18

HLA β chain: amplification of exons 2 and 3

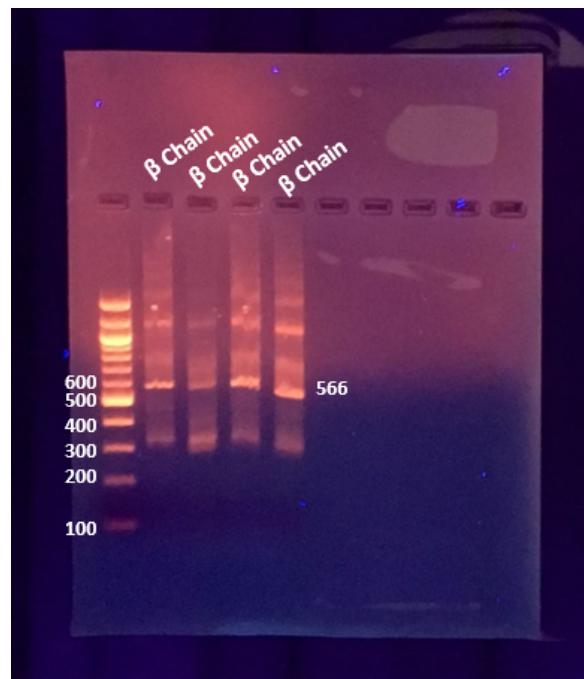
- **Analytical PCR_1- β** was performed at 60°C using Roger's genomic DNA as described in Protocol 2. The result was as expected. Two bands were obtained at ~ 400bp and 300bp respectively, corresponding to the expected molecular weight of exons 2 and 3.



- **Analytical PCR_2- β** was performed using PCR_1- β samples in order to add homology tails to the exons 2 and 3. Two different PCR conditions were tested: one PCR at 58°C w/o DMSO and another PCR at 60°C with DMSO. In the case of the exon 2, there were unspecific amplifications (one single extra band was observed in the agarose gel) were reported at both temperatures. On the other hand the exon 3 was successfully amplified.



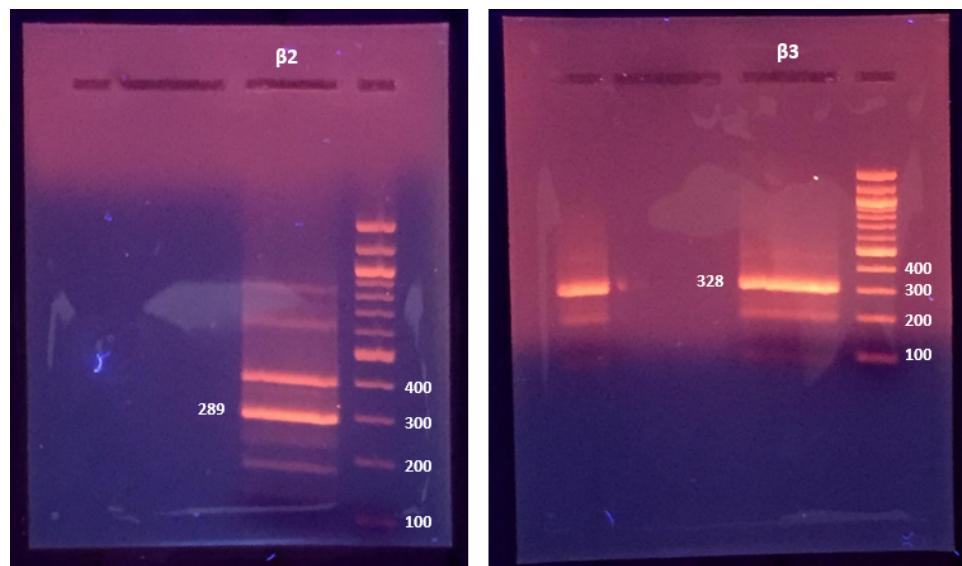
- **Analytical PCR_3_β** was performed as described in Protocol 2 using PCR_2_β sample in order to obtain the β chain (exon 2 and 3 ligation). PCR was performed at 60°C. All replicates (a total of 4) showed that there were unspecific amplifications. Although this fact, there was a band at 600bp which may correspond to HLA β chain.



Week 5: 03/09/18 – 09/09/18

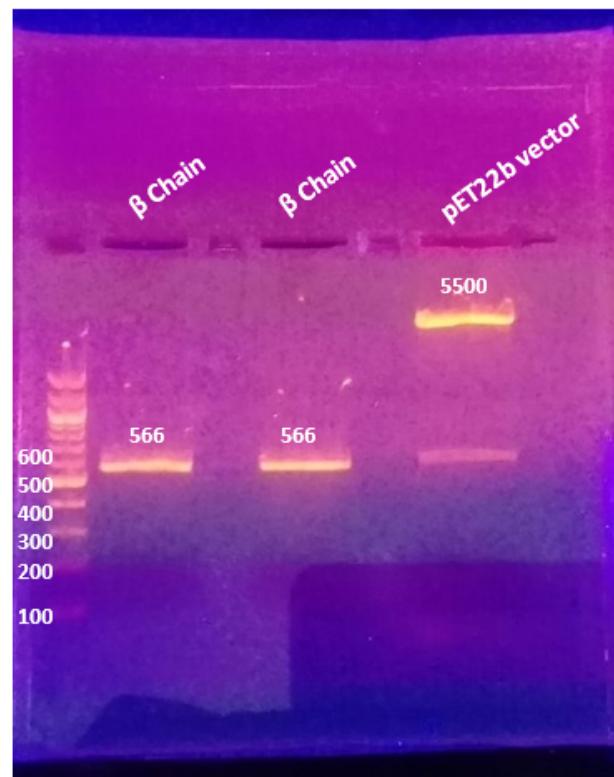
Obtention of the β chain

- **Preparative PCR_2_β** was performed using PCR_1_β samples (week 9) in order to obtain higher amount of exon 2 and 3 of the β chain. PCR was performed at 60°C. Results: Exon 2 and 3 together with smear.

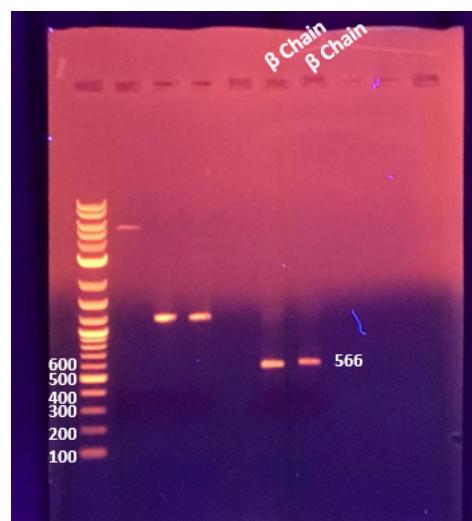


NOTEBOOK: STRATEGY 1, GENE EXTRACTION

- **Band extraction:** Exons 2 and 3 were purified from the agarose gel using QIAquick Gel Extraction kit (QIAGEN).
- **Exon 2 and 3 samples Quantification** using Qubit (Invitrogen). **Results:** **Exon 2: 6,69 ng/µL. Exon 3: 11,1 ng/µL.**
- **PCR Colony:** *E. coli* pET22- α colonies from eek 3 were analyzed as described in Protocol 7. Results: α chain (insert) was not amplified, meaning either the vector was not digested properly or the plates were contaminated.
- **Preparative PCR_3- β** was performed as described in Protocol 2 at 60°C. Results: Specific band at 600pb belonging to the β chain is observed. No smear was observed.



- **Band extraction:** β chain replicates were purified from agarose gel using QIAquick Gel Extraction kit (QIAGEN).
- **Analytical gel of β chain after purification** (wells 6,7).

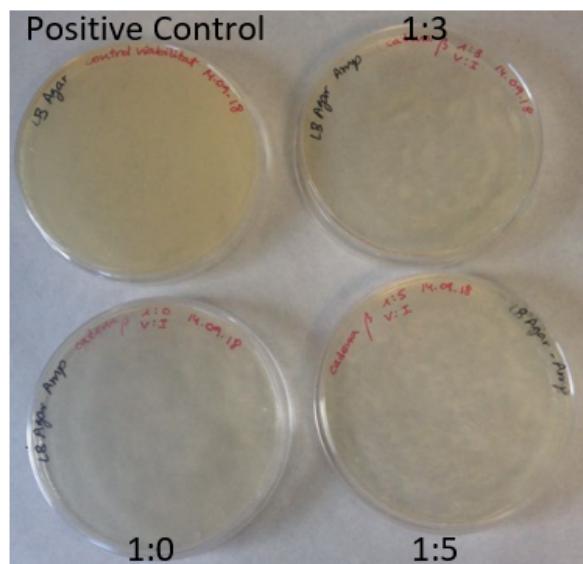


NOTEBOOK: STRATEGY 1, GENE EXTRACTION

- **β chain quantification** using Qubit (invitrogen). Results: 13,3 ng/ μ L; 14,1 ng/ μ L (replicates).
- **pET22 vector and β chain digestion** as described in Protocol 5.
- **pET22b vector and β chain ligation** as described in Protocol 6. Ratios Vector:Insert -> 1:0, 1:3 and 1:5.
- ***E. coli* DH5 α electroporation with pET22b- β chain.** *E. coli* cells were transformed and cultured in LB+Ampicillin plates at 37°C.

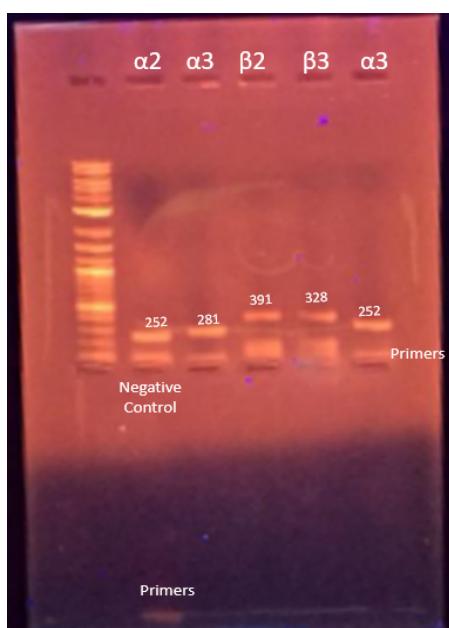
Week 6: 10/09/18 – 14/09/18

- **Results *E. coli* DH5 α transformation with pET22b- β chain.** Results: Positive Control: Bacterial lawn. No colonies were observed in *E. coli* pET22b- β chain plates.



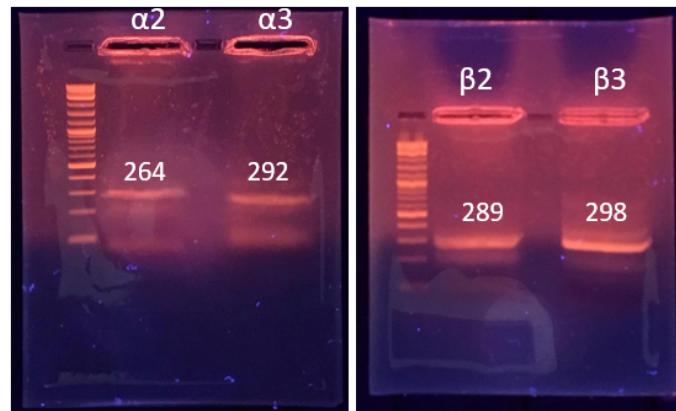
Week 7: 17/09/18 – 21/09/18

- **Analytical PCR_1** was performed at 60°C using Roger's genomic DNA as described in Protocol 2. The results obtained were as expected. However, there were unspecific bands at ~ 100 bp in each sample that belong to the primers as it was also present in negative control sample.



NOTEBOOK: STRATEGY 1, GENE EXTRACTION

- **PCR products purification:** Samples from PCR_1 were purified using PCR and DNA Fragment Purification kit (Neo Biotech).
- **Preparative PCR_2** was performed at 60°C. Results were as expected.



- **Band extraction:** Both, α and β exons, were purified from agarose gel using QIAquick Gel Extraction kit (QIAGEN).

Week 8: 24/09/18 – 29/09/18

- **Preparative PCR_3** was performed at 60°C. Results: Specific band at 566pb corresponding to the β chain. No band was observed for the α chain.
- **Band extraction:** β chain was purified from the agarose gel using QIAquick Gel Extraction kit (QIAGEN).

