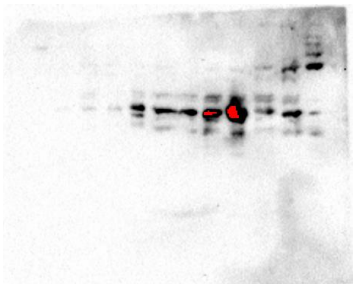
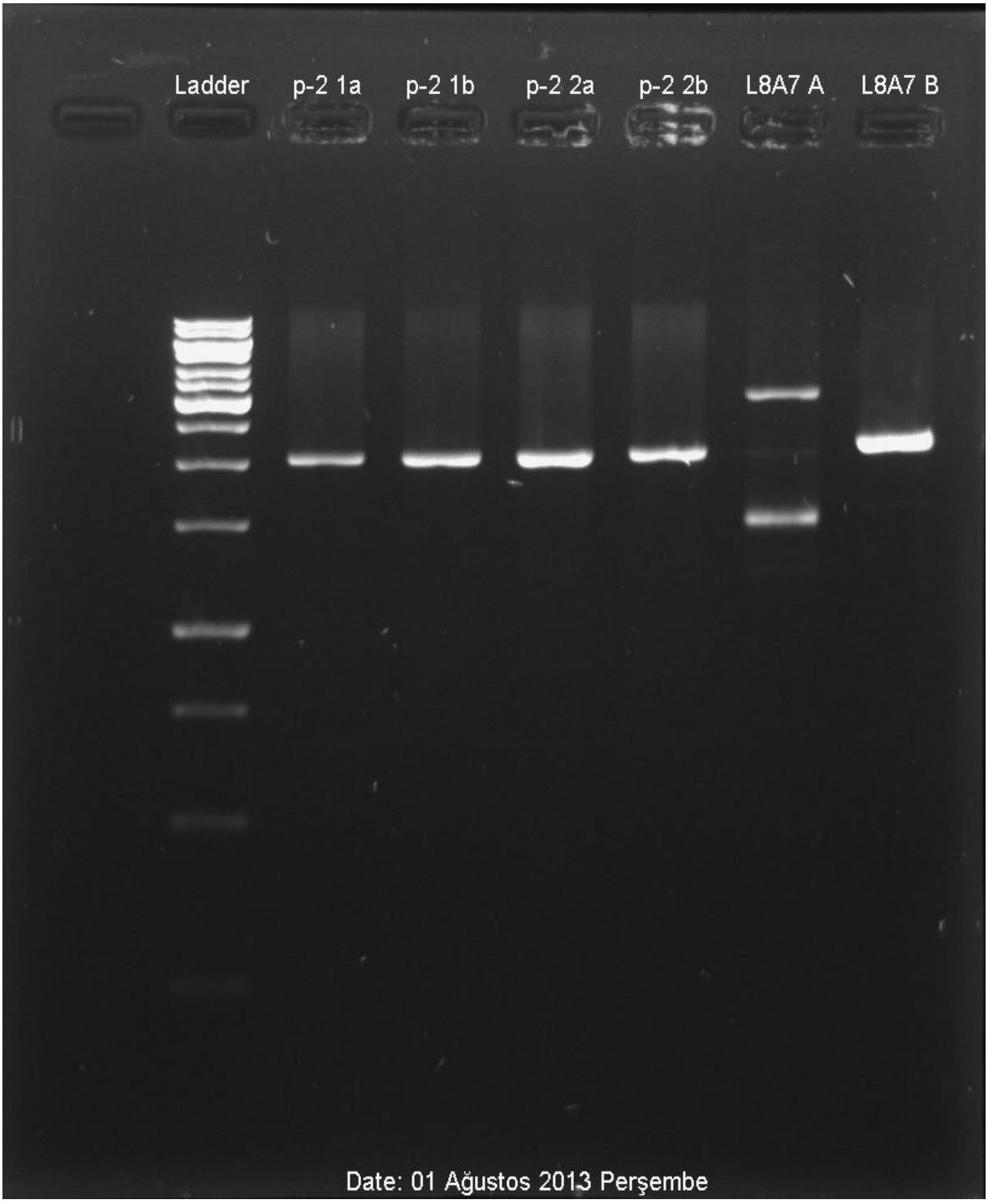
01.08.13

1) Complete Western → result: heated proteins' (at 95 C 5 min) band results are very similar to previous western. So, first western was successful!!
   Non-heated proteins give more bands, which could not be explained well.
   Result: Bradford assay is necessary to measure how much protein we have.
   Blocking solution should contain tween.
   (nihal, aysenur, aslihan, safa)
2) New DMEM preparation (+ 50 ml FBS, +5 ml penicilin/strep, +4 ml L-glutamine, +500 ul plazmosin) (safa)
3) 0.75 mg/ 15 ml dH2O Kanamycin stock preparation (taha,omer)
4) GFP (L8) Transformation with NEB10 (AC plate): **aim** test AC plate
   GFP (L8) Transformation with Nissle (Chl plate): **aim** test competent Nissle (Two plates of nissle: control & Nissle) (aysenur / taha –plate spreading)
5) Autoclave new LB – new plates with kanamycin antibiotics (taha, fethi, omer)
6) DNA isolation pind2 (ligation sample with psb1c) (aslihan, nihal)
7) Digestion Pind2 (two samples, four tubes), L8B7(one tube) (nihal) & gel electrophoresis (omer, fethi)
8) 50 ul TritonX + 0.1 g glycine → 5 ml LB (two samples: TorA+RFP & HlyA+RFP)
9) Responsible Atoms for Human Practice
   Abdulkerim
   Mustafa
   Mikail
   Esin
10) Meeting with Ferit Hoca- results:
    1. Modeling is promising
    2. Wiki seems not promising
    3. Animation & wiki depend on Ferit hoca meeting with his students
11) Immunoflorescence experiment until first antibody overnight incubation (His tag antibody dilution 1/500)
    Skip TZN Step in protocol because we could not remember what TZN is.
    Important note: Bacteria did not attach after centrifuge (4000 rpm 10 min & then 4000 rpm 30 min) question: how can bacteria attach?
    Triton-X & 10x PBS buffer preparation

We used western bloot experiment in order to observe boiled and unboiled tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.




Ladder
p-2 1a
p-2 1b
p-2 2a
p-2 2b
L8A7 A
L8A7 B
Date: 01 Ağustos 2013 Perşembe

Parts were cloned to check ,via electrophoresis gel and all parts expect L8A7 A were found wrong

02.08.13

1) Western experiment (safa, mikail, omer, nihal, aslihan)
   AIM: New antibody from Abcam (Best antibody company) has arrived! So, test antibody→ hopefully, it works!

   Western gel preparation- running (safa, mikail, omer) transfer buffer preparation & transfer overnight (nihal, aslihan)

   RESULT: Coming soon …

2) Immunofluorescence experiment (Mustafa, esin)
   AIM: To stain OmpA on cell surface
   RESULT: We achieved fixation of bacteria!!! But we could not stain them because first antibody is *rabbit* monoclonal anti-his tag antibody so the second antibody should be *anti-rabbit* … our second antibody was anti-mouse → wrong. (According to one idea: Even it was ok, staining would most likely not work because first antibody (santa cruz) is problematic)

   Experiment should be repeated with glutaraldehyde & formaldehyde fixation and with 500 ul bacteria. Also we need to search for *attachment* ways of bacteria to coverslip.

3) Transformation (Mikail, Omer, Fatih)
   AIM: To test ligation of L13 + B7 (17 °C- second experiment)
   New kanamycin plates were used (1.8.2013 plates by taha)
   RESULT: tomorrow

   Today's Important Events:
   1) Passport
   2) His tag columns
   3) Abcam antibody
   4) Primers (to get constructs from puc57 plasmid)
   5) Elman's assay materials

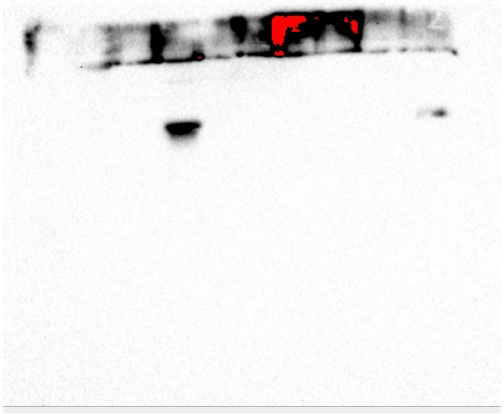
   Future Plan:

   6) Sponsor-sponsor-sponsor we need sponsor!!!!!! ☒☒☒☒☒☒☒
   7) Human practice – HP atoms should have a meeting!

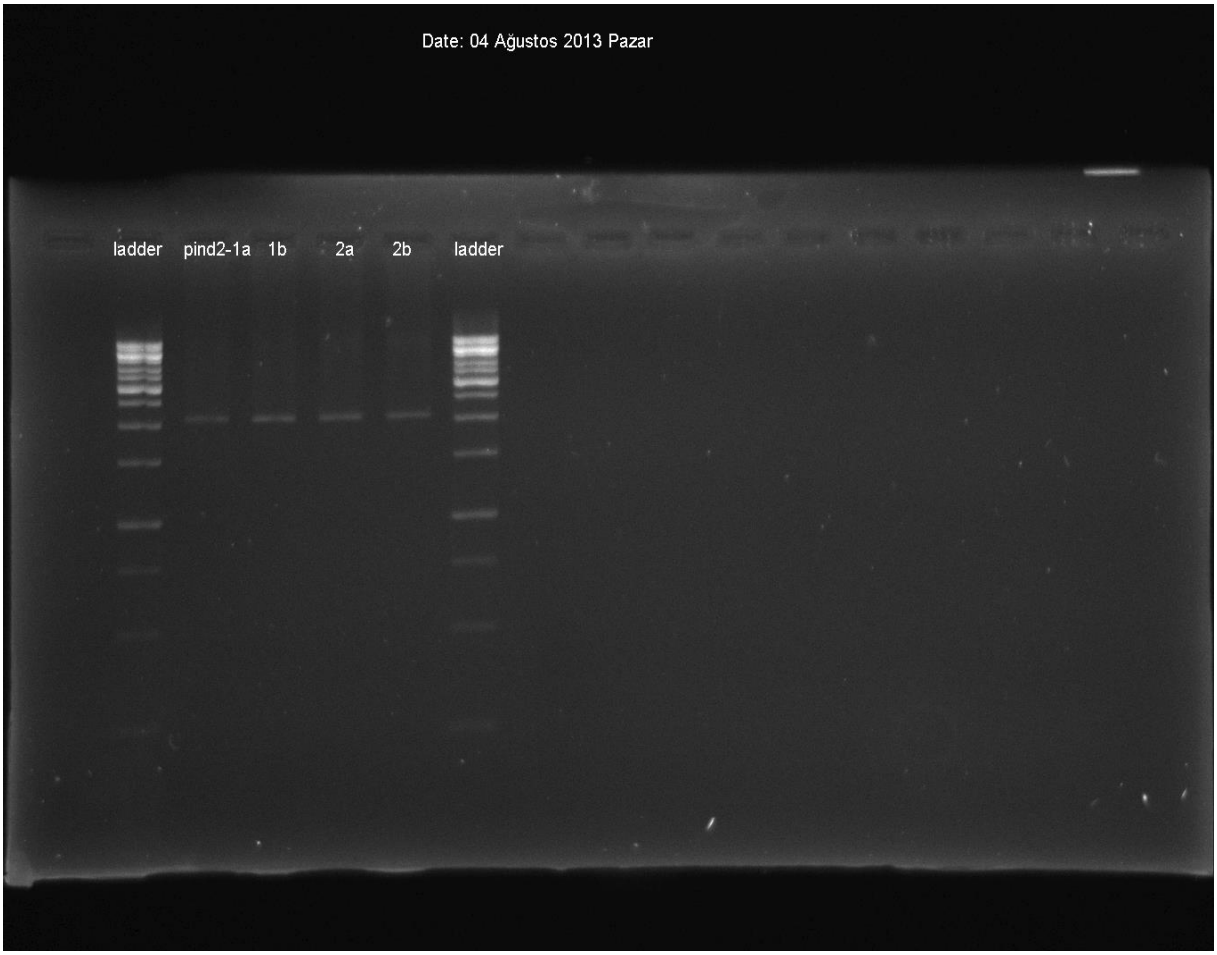
8) Column purification of TAT-Apoptin (saturday)
9) Grow MCF-7 cancer cell line & colon cancer cell line (saturday)
10) Learn XTT assay & caspase 3 assay protocol for apoptosis measurement of cancer cells. Also learn apoptin is caspase dependent or independent ?
11) Elman's assay standards & assay application (Sunday ?)

04.08.13

We used western bloot experiment in order to observe boiled and unboiled tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.



Parts were cloned to check ,via electrophoresis gel and all parts were found correct



Parts were cloned to check ,via electrophoresis gel and all parts were found wrong

5.8.2013

1) Pind2-psb1c ligation liquid culture > DNA isolation> digestion (Aslihan)
2) Elmann's assay (Mustafa, esin, safa)
   OD values at 412 nm
   His-Enzyme: 0.24
   LuxS-Pfs: 0.20
   Aim: These contructs are his-enzyme and luxs-pfs. So we are testing enzyme capability to convert SAH to AI-2. Elman's assay measures an intermediate substance of this reaction, which has sulfhydryl group.
   Results are very low so we should repeat the experiment
   1*Same OD bacteria cultures should have been done. So, Safa prepared liquid culture of two parts. We'll check OD values then we'll try to make same OD> so that Elman's assay measures effectively only enzyme activity.
   2*SAH can affect the result so when SAH comes, we should repeat the experiment.

3) Bradford protein assay (Mustafa, esin, safa)

Aim: Before protein purification, we should calculate how much protein we should add to columns since we cannot add more than 8 mg/ml concentration.
Results are low.
OD values at 595 nm
TAT-Apoptin: 0.646
TAT-E4ORF4: 0.708
Concentration calculation based on standard curve:
BSA Standards give the following equation: y = 0.5751x + 0.2082
Y=concentration, x= absorbance

After we compare the results according to the standard curve, we concluded that we should add as much as the column can take ☺
Also, Safa prepared new liquid cultures of TAT-Apoptin & TAT-E4Orf4 for 36-hour incubation to increase protein amount.

4) Liquid cultures of four constructs (Safa)
Details are written above

5) Kanamycin new stock & new plates (Aslihan)
Aim: Kanamycin control plate has bacteria today so we want to eliminate it!
Results: We do not have any results yet but this time, Aslihan filtered the kanamycin stock solution, which we've not done before.

6) **Human Practice**:
Mustafa searched for some pictures for game "Feeling a cancer cell" (possible name for game) but we could not figure out how we can find & print those organ pictures. However, we are hopeful because game seems like fantastic ☺
New collaboration offer came from iGEM Copenhagen team.
** We should do collaboration with other teams & at the same we should search for novel approach so that we can increase gold medal chance.

7) Nissle Competent Cell preparation according to NEB10 protocol (Mustafa)
Its OD is very low so we postponed the experiment till tomorrow.

8) **Ethic Approval**
Safa prepared Ethic document and he checked with Omer Faruk hoca & Esra hoca.
This application is for future parts of project.

06.8.2013

1) Nissle Competent cell according to NEB10 protocol (Mustafa, esin)

2) Pind2 digestion gel electrophoresis (Aslihan zimmet> Aysenur emanet)
   Aim: pind2-tolc ligation preparation
   Results: Digestion repeat

3) TolC-j04500 digestion samples' gel electrophoresis (Aysenur)
   Results: Tolc es & tolc1a & j04500 es

   These three samples can be used for further ligation experiment

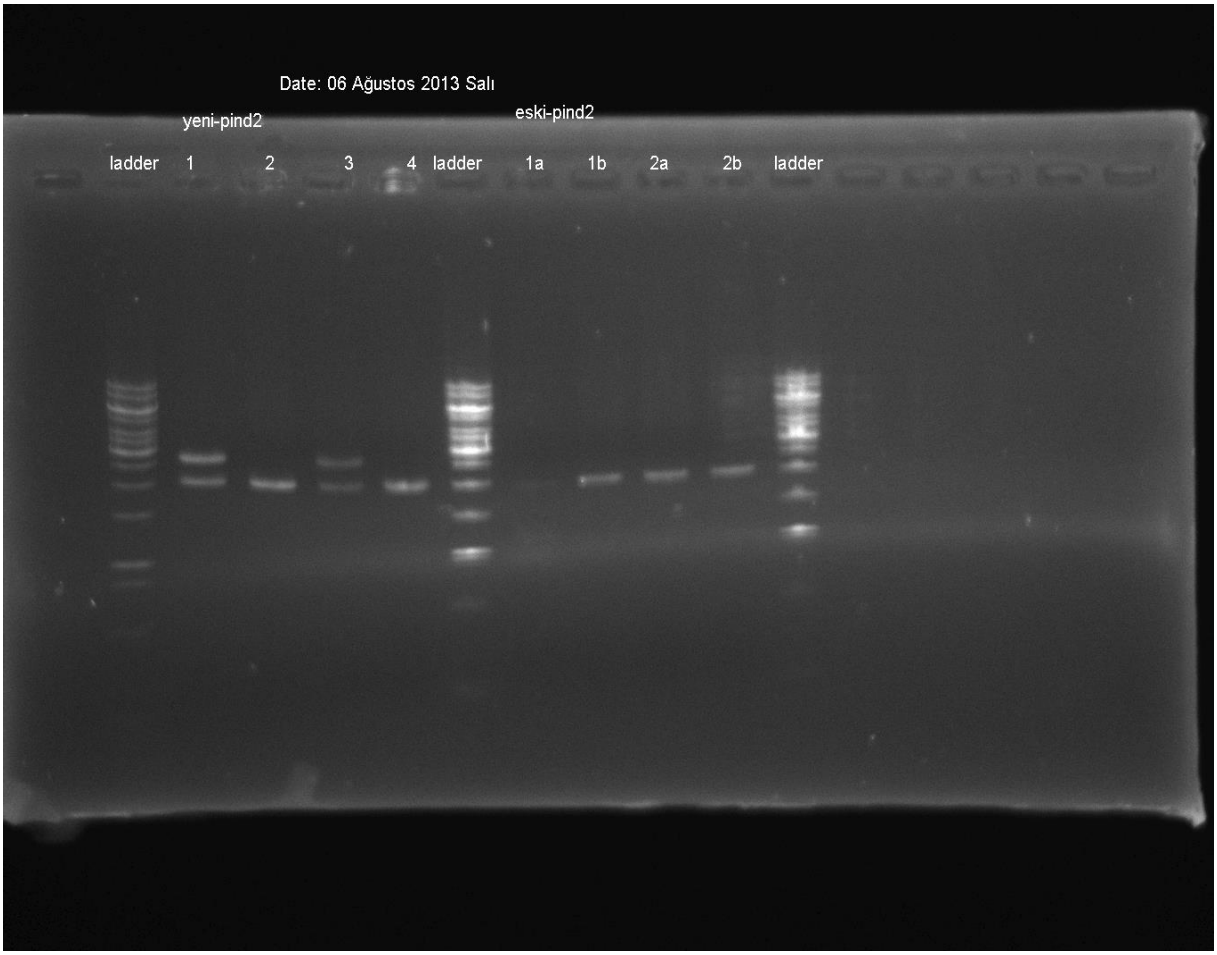
4) His tagged protein purification (Mustafa, esin)
   Aim: To purify his tagged proteins for further experiments (such as giving the proteins to cancer cells)
   Results: Bradford & OD280 nm measurement gives very low protein purity. Actually we had low amount of whole protein extract at the beginning of experiment so we've decided:
   ***1)** Increase the amount of bacteria in liquid culture (100 ml bacteria)
   ***2)** Increase the time of liquid culture from 16 h to 36 h
   ***3)** Try to do both methods: Sonication & chemical lysis (with PMSF proteinase inhibitor)

5) Immunofluorescence (Mustafa, esin, aysenur)
   Aim: To visualize OmpA on bacterial cell surface.
   Results: No result! We could not see colorful bacteria but we've seen some fixed bacteria. Bacteria amount is also very low so it is possible that we could not spread properly.

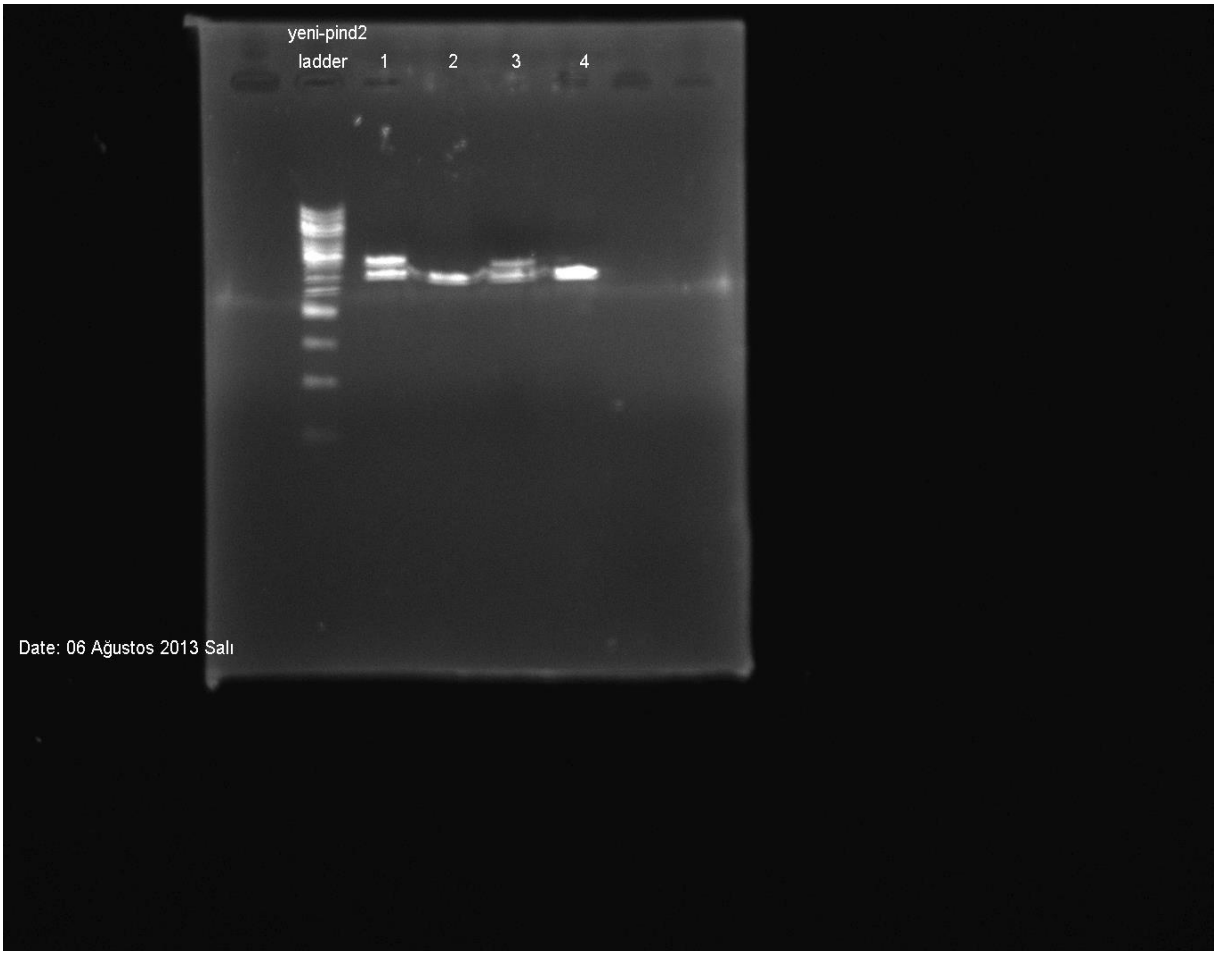
6) Liquid culture of Tat-Apoptin (~100 ml) → start 36 h incubation (Mustafa)

7) Cell culture experiments (Mustafa, esin)
   Aim: To grow breast cancer cell line MCF-7 and colon cancer cell line HT-29.
   Results: MCF-7 attached flask surface but HT-29 (with collagen gel) did not attach.

→ **<u>Today's important event: New Nissle (from Germany) arrived !!!</u>**



Parts were cloned to check ,via electrophoresis gel and all parts expect 1,3 were found wrong



Parts were cloned to check, via electrophoresis gel and 1,3 were found correct; 2,4 were found wrong

7.8.2013

Protein Isolation with Lysis Buffer & Sonication method

Aim: To isolate whole protein of bacteria liquid culture (10ml, 36 hour incubation, L29: His-TAT-Apoptin) before purification

1. Centrifuge 10 ml liquid culture at highest rpm (of IGEM big centrifuge machine) for 10 min.
2. Add 5 ml Lysis buffer

   Lysis Buffer:

   -50mM Tris pH 8.0

   -10% glycerol (for stabilization of the protein and prevention of aggregation)

   -0.1% Triton X-100 (for prevention of aggregation of hydrophobic and membrane proteins).

-1mM PMSF (in isopropanol)

2. Divide into 1.5 ml eppendorfs (1 ml to each tube- 5 eppendorf)

3. Incubate 30min on ice.

4. Sonicate 3x 20'' till sample is no longer viscous. After 20" sonication, wait 1 min.

5. Centrifuge 14,000rpm for 30min at 4 C.

6. Collect supernatants to new tubes→ supernatant should have protein !!

Bradford Assay:

Aim: To check the protein isolation quality via color change of Bradford reagent from brown to blue

Results: Unfortunately we could not see any color change. This means that we could not isolate protein or bacteria could not produce protein.

Possible Solutions:

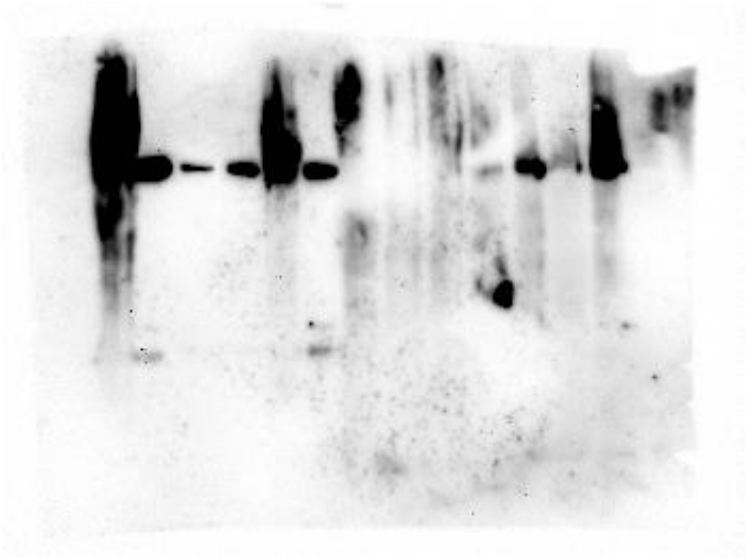
1. Search for bacteria effective protein production (from articles, google etc)
   Now, we are trying to do 36 h incubation of liquid cultures, then 4 C incubation
2. Search for new isolation procedures, it may change, we can add detergent to procedure

Main Result: We should definitely succeed protein isolation & purification , otherwise …

Human Practice

1. Now, we have a game path schema. There are organs on path. We should find a way of printing it! & We should decide on games!

We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.



09.08.2013 Report

1. Transformation for competent cell control procedure was completed. Nissle 1917 competent cells had been prepared before via using the protocol of rubidium chloride including competent cell preparation protocol. For Nissle 1917, one negative control was also transformed. Additionally, a NEB10 strain is also used in the experiment in order to check the efficiency of experiment and eliminate the possibility of human error.
2. The LB cultures of S03335, J04450 and E1010 were prepared in order to use them in the immunofluorescence assay. E1010 was prepared as negative control during the assay. GFP and RFP were prepared separately to measure and to compare the fluorescence of red or green fluorescent proteins.
3. The DMEM medium of MCF7 breast cancer cells was replaced with new one.
4. Project description of Project Oncoli was added in our wiki page.

10.08.2013 Report

1. The protein extraction was done from 50 ml L29 culture. Afterwards, Bradford's Assay was performed to the samples. No significant color change was observed. However, we wanted to perform an SDS-PAGE assay, because we decided that we cannot distinguish the existence of the protein in the tubes by only color change due to the very little amount of the proteins.
2. L25 and L34 were exposed to the Ellmann's Reagent. The experiment was done by exposing the half of the samples to SAH in order to allow them to produce more AI-2. Half of the other was diluted with PBS to equal the amount of the solutions. Eventually, Ellmann's Assay was performed. The results will be received tomorrow, The LB culture of competent control plate was prepared. Note that the colonies were **not red.** That indicated colonies had no plasmid we wanted. We will do plasmid isolation to ensure this reality. The reason how these colonies without plasmid were survived in the agar plate including ampicillin is that maybe the region they have lived has little antibiotic because of the bad distribution of ampicillin.

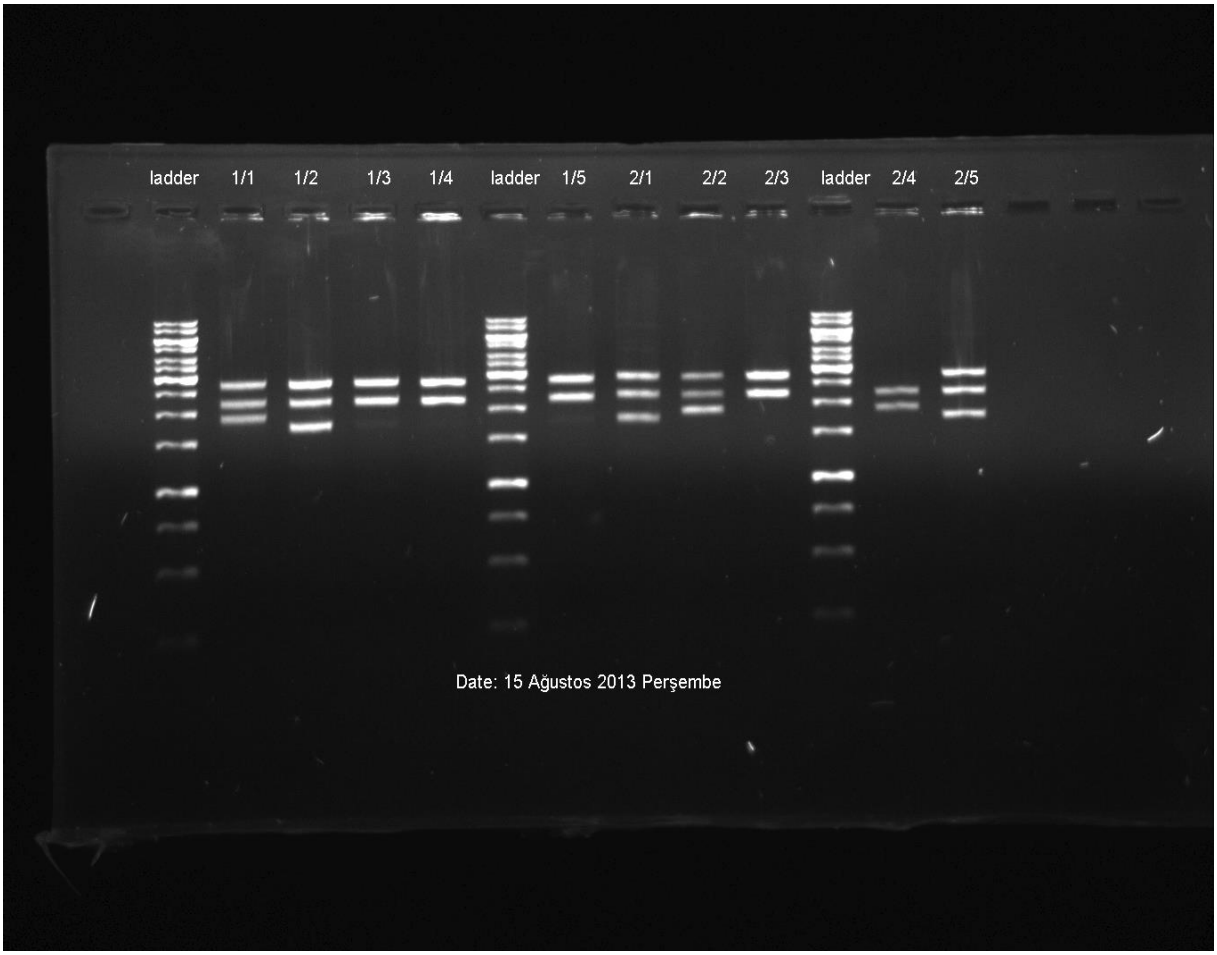
11.08.13

- Ellmans Assay for Luxs pfs and his enzyme was accomplished. Control was competent. Unfortunately there were no positive results.
- Protein Purification and SDS-PAGE Assay was performed on L29. Results to be recieved tomorrow.
- Nissle Control and Competent control liquid cultures were isolated. They are to be digested tomorrow in order to see whether they have the RFP generator.
- Since Pind2's (ligation) electrophoresis result of date 6th august wasnt as expected, we decided to throw them away and have repeated the ligation procedure to give it another try. They will be transformed tomorrow.
- New ideas for human practice have arised; still under development. Much to tell when all the rest of the atoms return to the lab which will really interest them too ☺

15.08.13

- L29-10ml (TAT-Apoptin) protein purification experiment was done, we will meausure it tomorrow.
- İmmunoflourescence experiment was started. Our goal is observing the C215 in arround of bacteria, as an aura on outer membrane.
- In the cancer cell culture room, MCF27 Cells's medium was changed . Cells are growing regularly.
- Electrophoresis from isolated and digested liquid culture called PIND2-Chp was done.

We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.



Parts were cloned to check ,via electrophoresis gel and 1/3, 1/4, 1/5, 2/3, 2/4 were found correct; 1/1, 1/2, 2/1, 2/2, 2/5 were found wrong

16.08.13

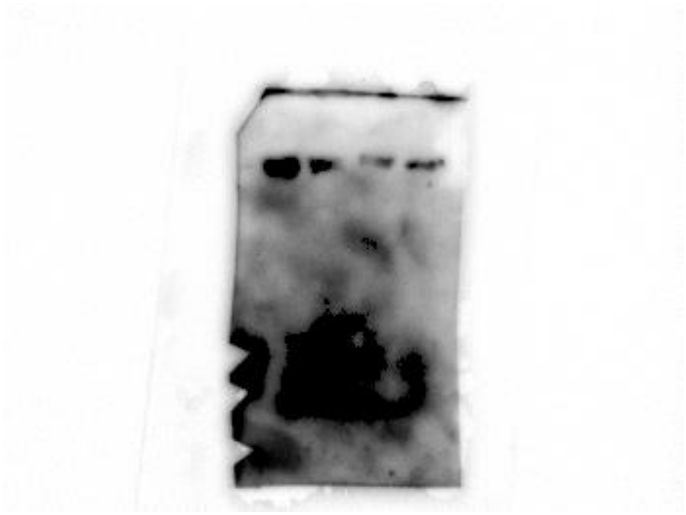
- Nur Nihal ran electrophoresis on Pind2-PSB1C3 ligation results. A couple of us tried commenting on it but wasn't sure whether what we were saying was correct. Therefore it needs approval!
- Mikail and Taha were in charge of doing western blotting. One gel was for SDS the other one was transferred to a membrane which then followed on to introducing the 1st antibody. Results are to be received in the following days.
- A group of Atoms have done transformation with Ecoli BL21 and Bacillus.I believe competent for Bacillus were being produced too [?!]
- Nur Nihal also did gradient PCR for sequencing; we are waiting for the results.
- Aysenur prepared liquid cultures for HlyA-RFP, TorA-RFP, His-TAT-Apoptin, OmpA, C215, His-link-enzyme, Luxs-pfs.

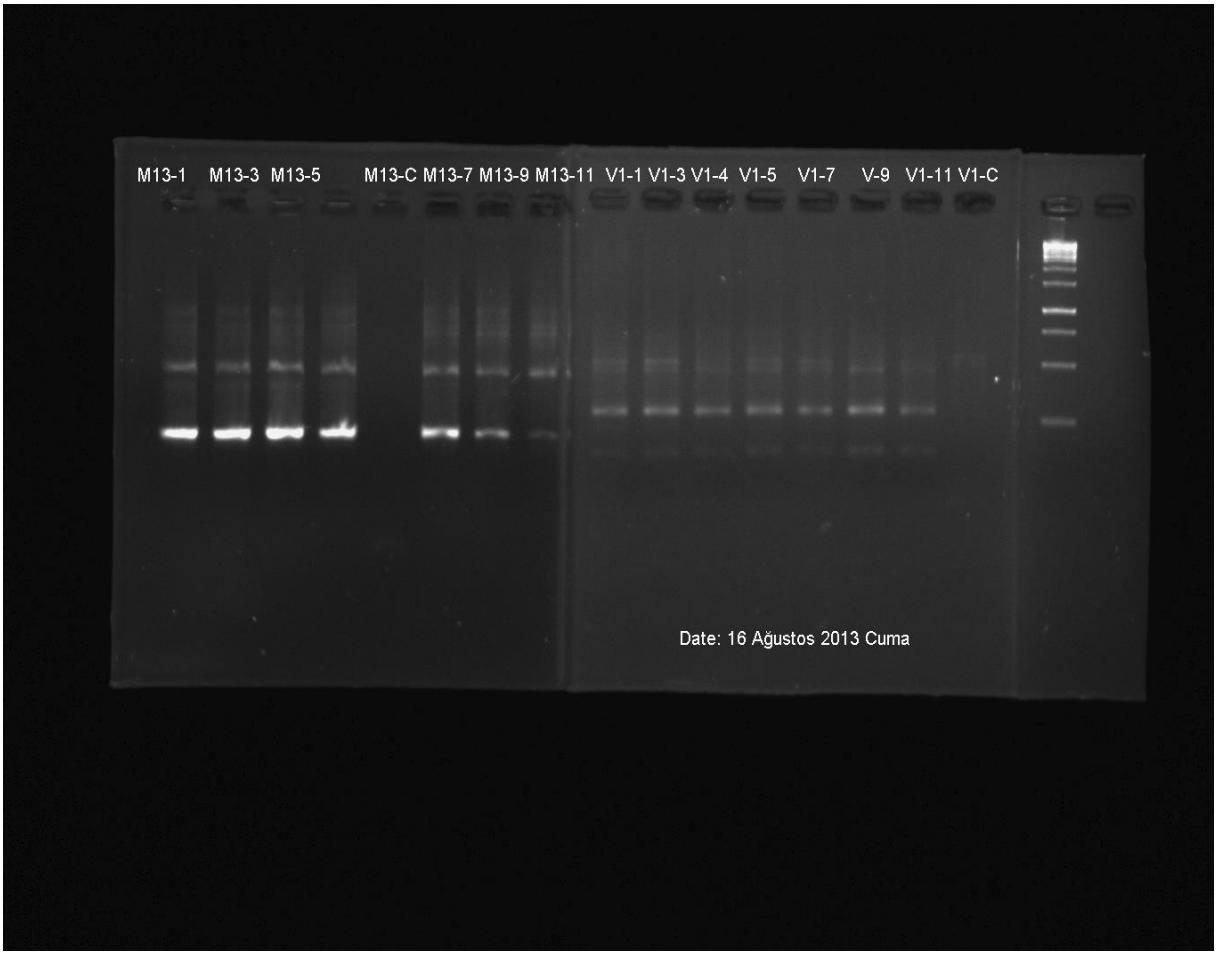
- Esin abla, Furkan, Abdulkerim and Aslihan completed the final procedures of immune-fluorescent colouring, when observed under the microscope very little bacteria were seen on the cover slip and fluorescence couldn't be seen either. The maximum zoom our microscope can do it 40x which might be inhibiting us from seeing it [?]. This is just one of the few possible reasons why.

- HP committee had a meeting.

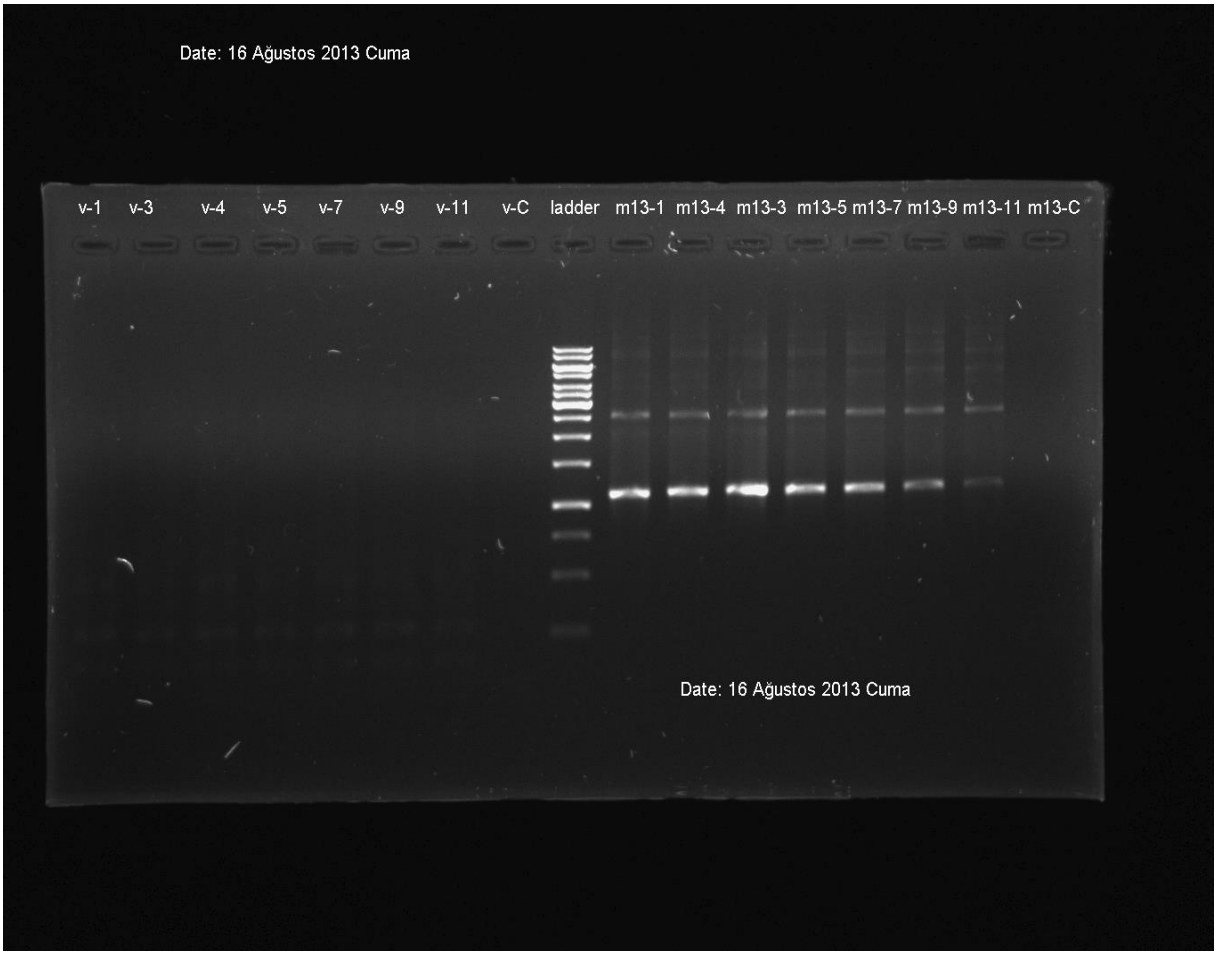
Reminder: Fatih is in charge of sharing the required documents for visa application. We need to fill in the schengen form and complete our checklist of documents to book an appointment.

We used western bloot experiment





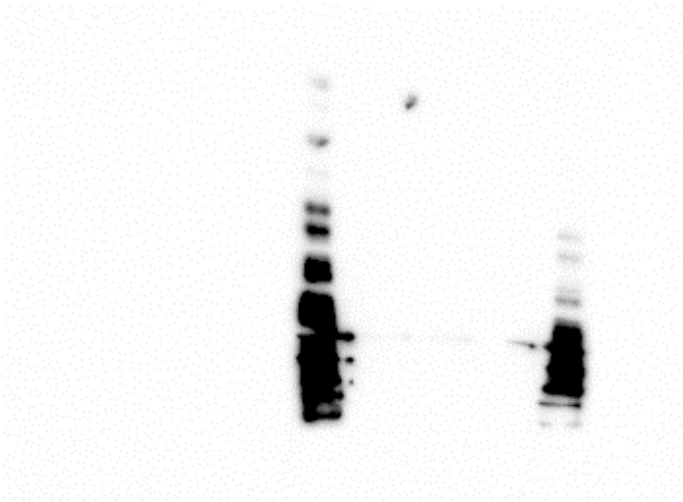
Parts were cloned pcr to check ,via electrophoresis gel and all parts were found correct



Parts were cloned to check ,via electrophoresis gel and all parts were found correct

17.08.13

We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.



18.8.2013

1) C215-linker-his tag & Tat-e4orf4- his tag constructs' liquid culture (Mikail)
   Aim: To isolate proteins, which are different from Apoptin

2) LB-Agar LB-Broth (with ampicillin) (mikail)
   Note: LB-Broth ampicillin is sensitive to light so we covered it with aluminum foil.

3) B. subtilis Transformation with GFP (taha)
   Note: After 30 min ice incubation, medium B was added.

4) Ligation test: J04500-TolC-psb1C3 Ligation (Aysenur)
   Results: Plate lost! Liquid culture ?

21.08.13

We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.

22.08.13

1) over expression group made protein isolation from bacteria (Abdulkerim, Fatih, Ömer)

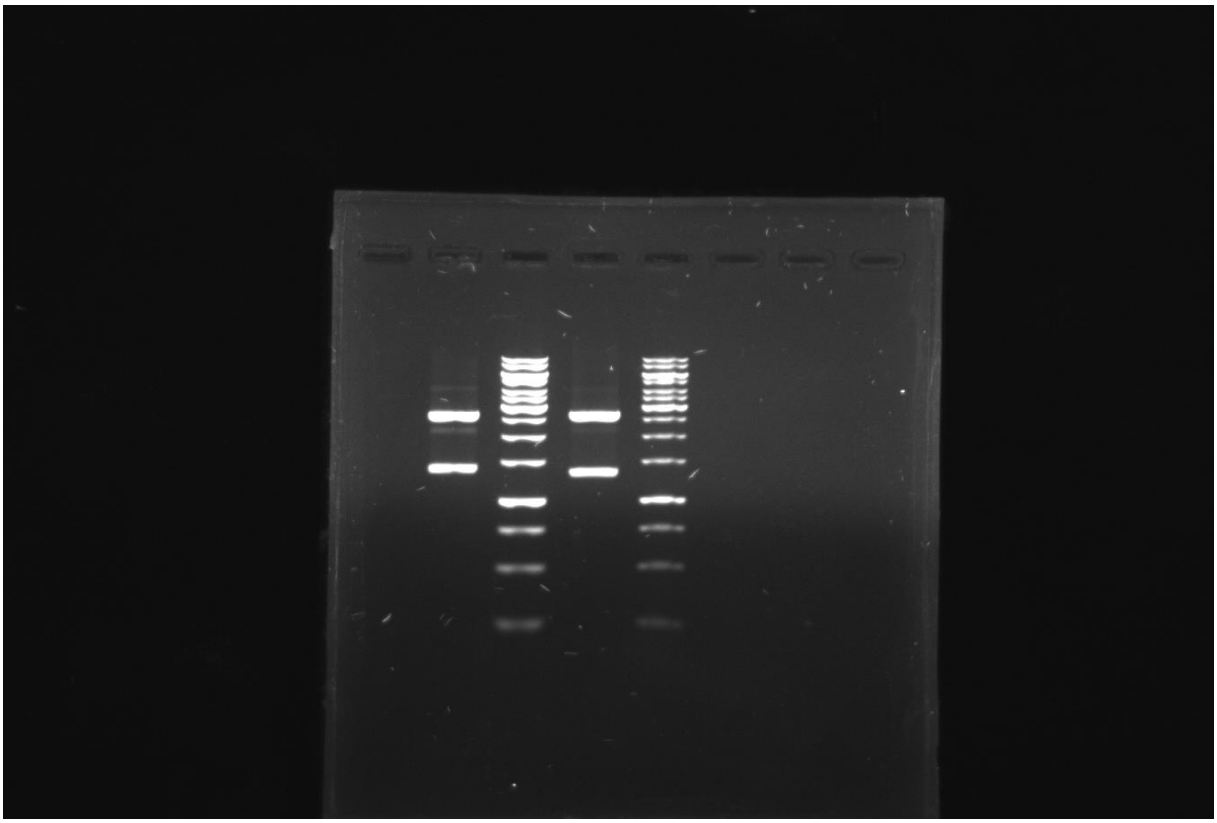
3)prepare competent bactria from BL21.(Mustafa Abi,Fethi,Ömer,Fatih)

4)performed transformation from new competent BL21.(Fethi, Mustafa Abi)

6)performed western for apoptin sample .(Safa)

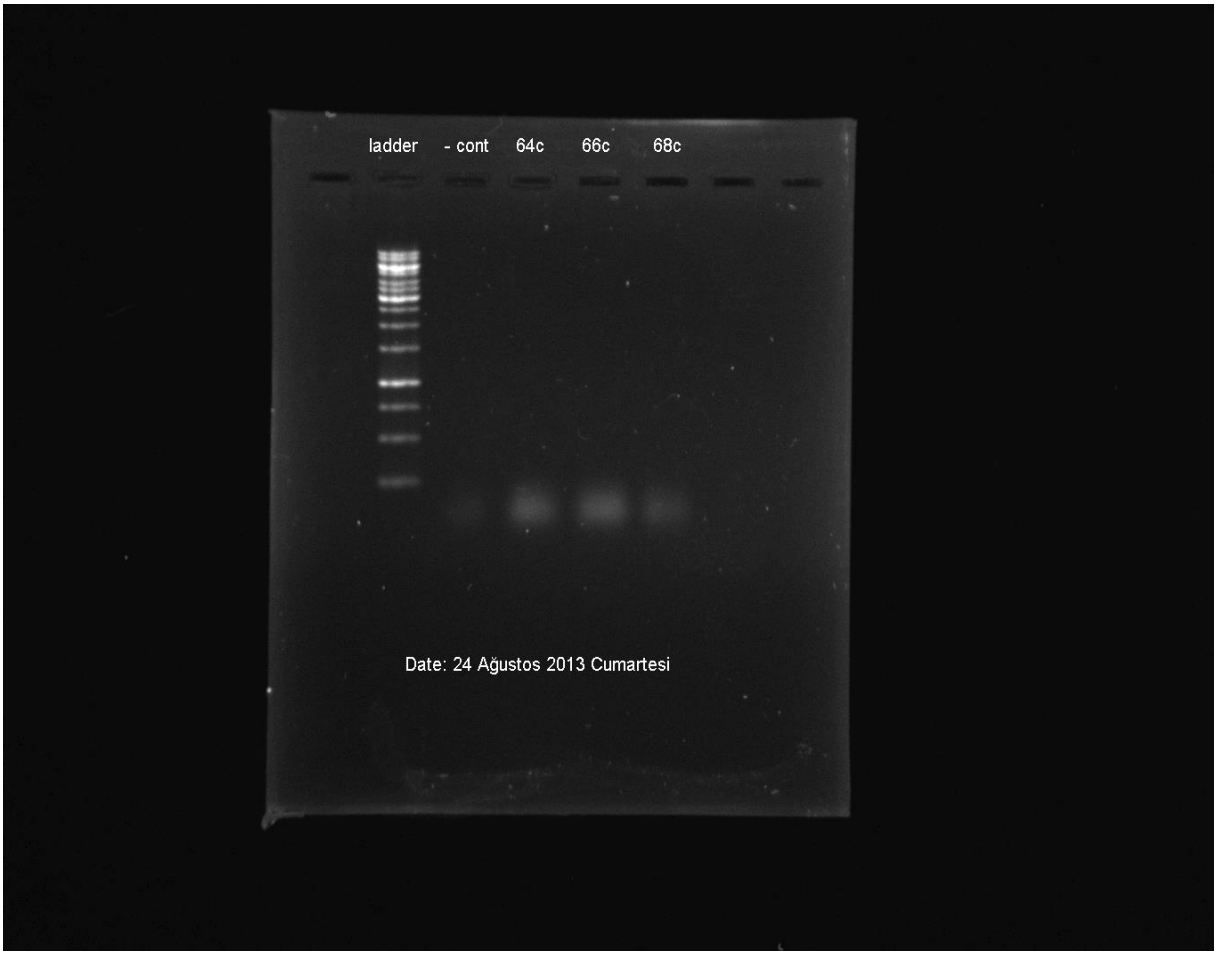
We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.

23.08.13

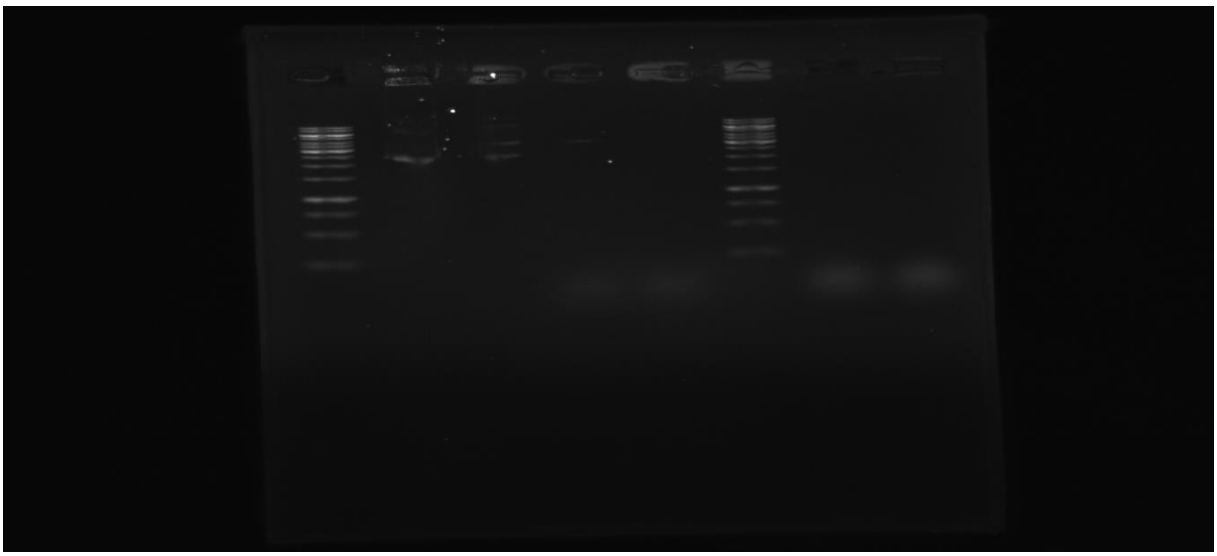


Parts were cloned to check ,via electrophoresis gel and all parts were found correct

24.08.13



Parts were cloned pcr to check ,via electrophoresis gel and all parts were found wrong



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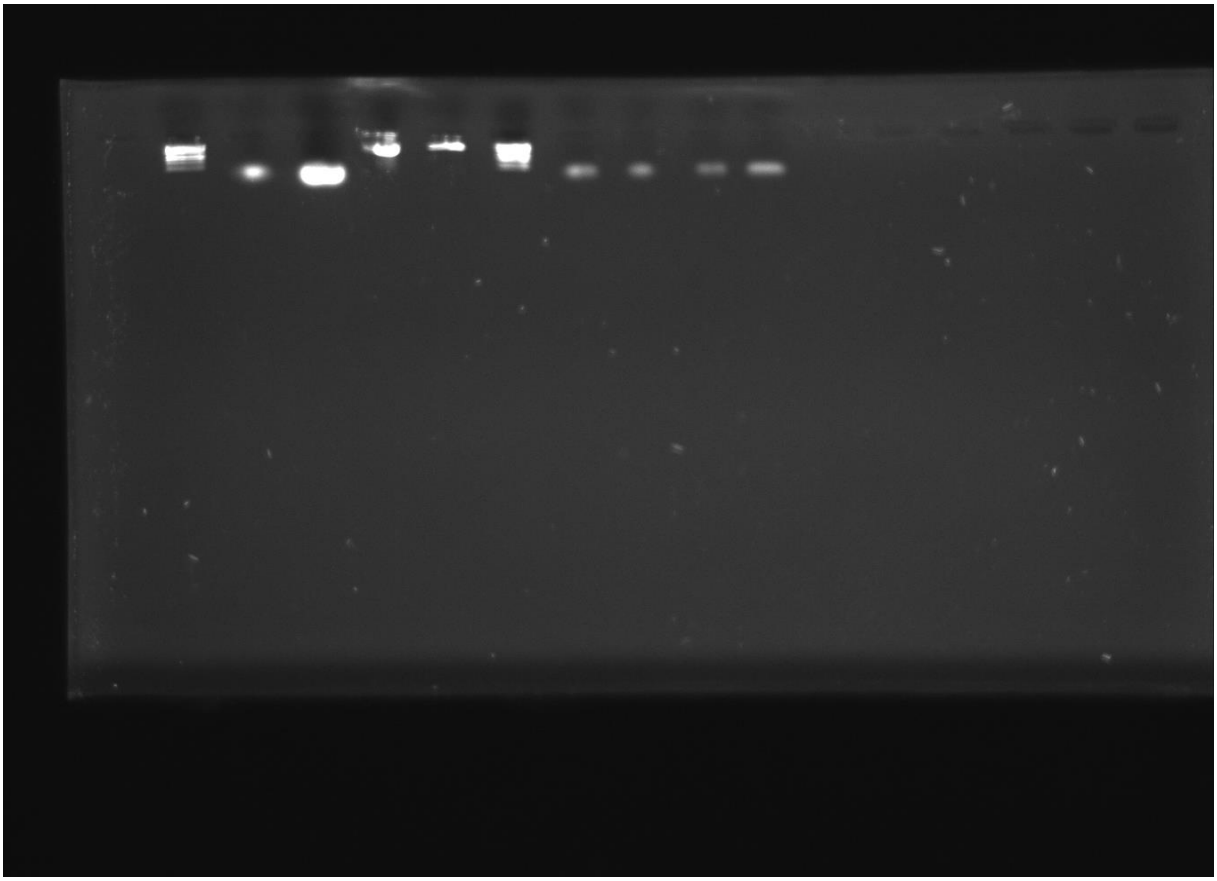


primer concentration testing

26.8.2013

Basic Cloning : (Taha, Ömer, Safa)

psb1C3-genes transformation: Results, today

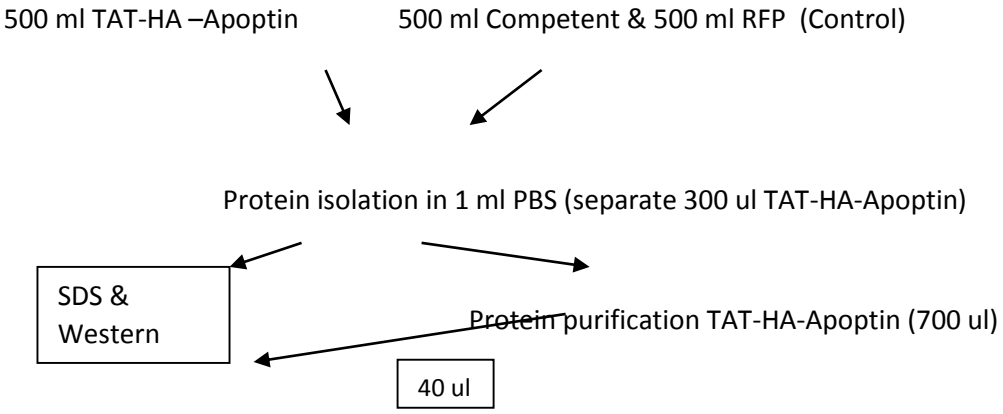
8 NEB10 > 7 ☻// 5 TOP10>1 ☻

We should repeat 5 of them.

Protein Exposure :(Nihal)

Below scheme is just a plan, we could only isolate it today. We'll repeat the experiment tomorow with TAT-HA-Apoptin & RFP control.

Note: *Ampicillin is contaminated because we've seen bacteria in competent although it has antibiotics. Today, we've prepared new ampicillin plates.*



Isolated & purified samples--> 6 well plate (trypan blue), 96 well plate (XTT)

Protein Overexpression (Mikail, Fethi, Fatih)

SDS result:

BL21 & TNY 37 C 24 hour seems the best. Others should also be evaluated. There are some thick bands!!!

Transformations **(with old BL21 & new BL21)**

Tat-Apoptin

Tat-ha- Apoptin

Tat-E4of4

C215

OmpA (Furkan should follow !!!!)

luxS-pfs (Ayşenur should follow !!!!)

His-linker-Enzyme (Ayşenur should follow !!!!)

Control

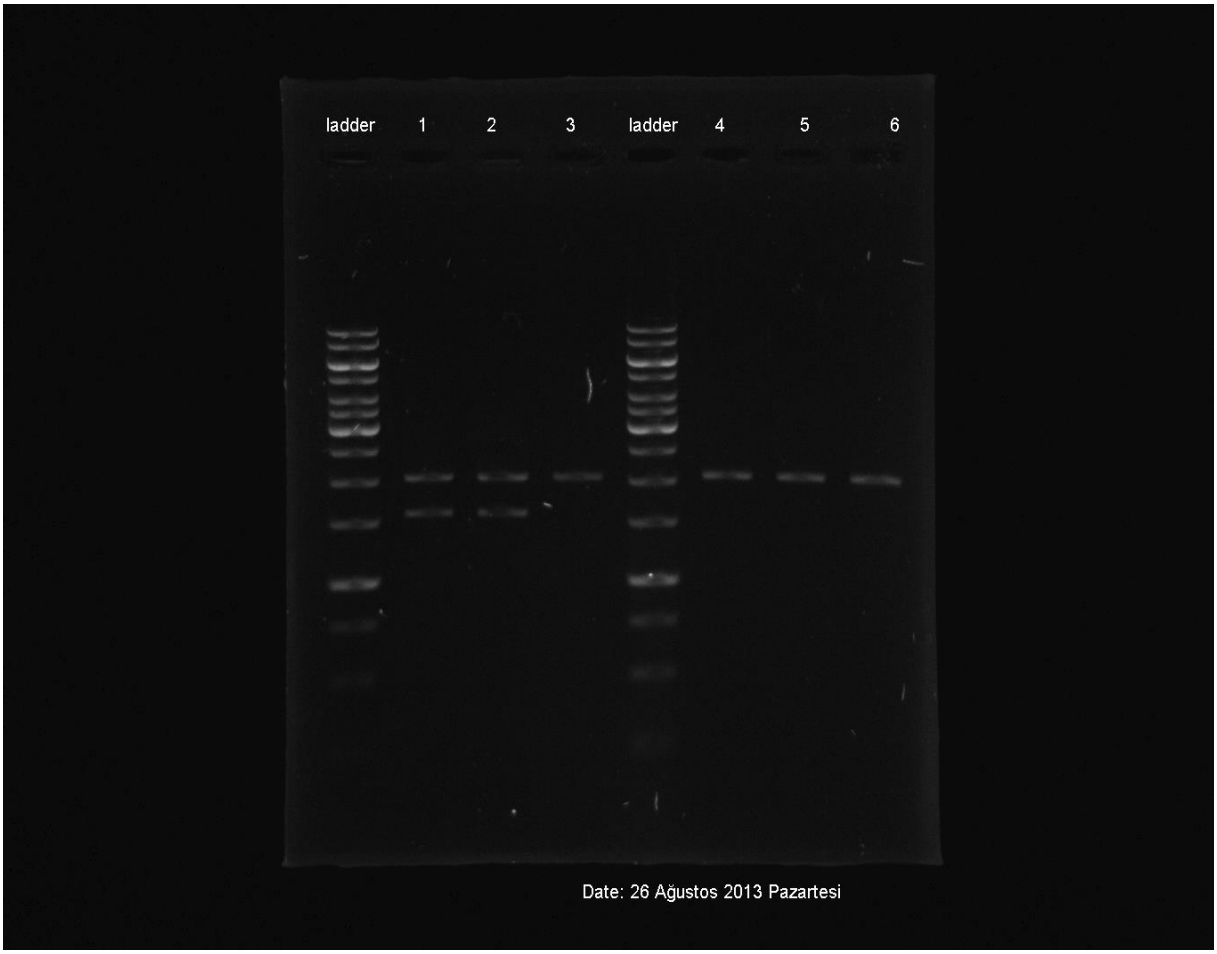
Immunofluorescence (Furkan)

They wait for BL21 transformation

Ellman's assay (Ayşenur,Aslıhan)

LuxS-Pfs & His-linker-enzyme –> isolate in 200 ul PBS

20 ul for SDS, 180 ul for Ellman's assay



Parts were cloned to check, via electrophoresis gel and all parts expect 1,2 were found wrong

28.08.13

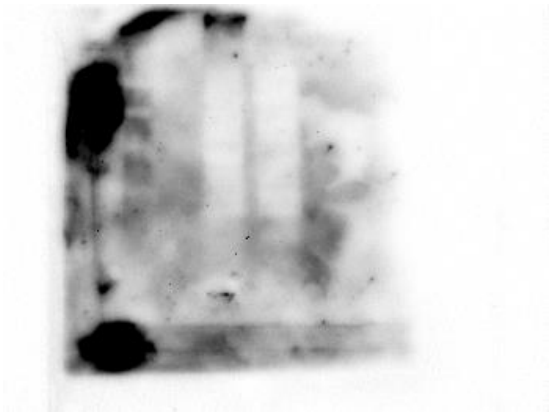
We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.

29.08.13

- 6 falcon tubes of liquid culture for each of the four genes: luxs-pfs, his-enzim, pind2 lig and ompa-c215 was prepared. Today they were isolated. These will be used for Ellmans Assay.
- Esin Abla and Nur Nihal approached to count the number of cancer cells dead by TAT-Apoptin they introduced the day before, but an error occured. For this reason, they will repeat this experiment once planning a more advanced version which will involve performin different kinds of exp like sds, western blotting of tat apoptin at the same time as the app of apoptin to cancer cells.
- Vectors for order have been researched. A few extras, similar to pET22b(+) have been devised, an email has been sent to Gokhan abi in order to determine whether they can be ordered from embl together with the 22 vector aswell: PGAT2, Phat2 (?)
- A 'wake up' meeting was done!

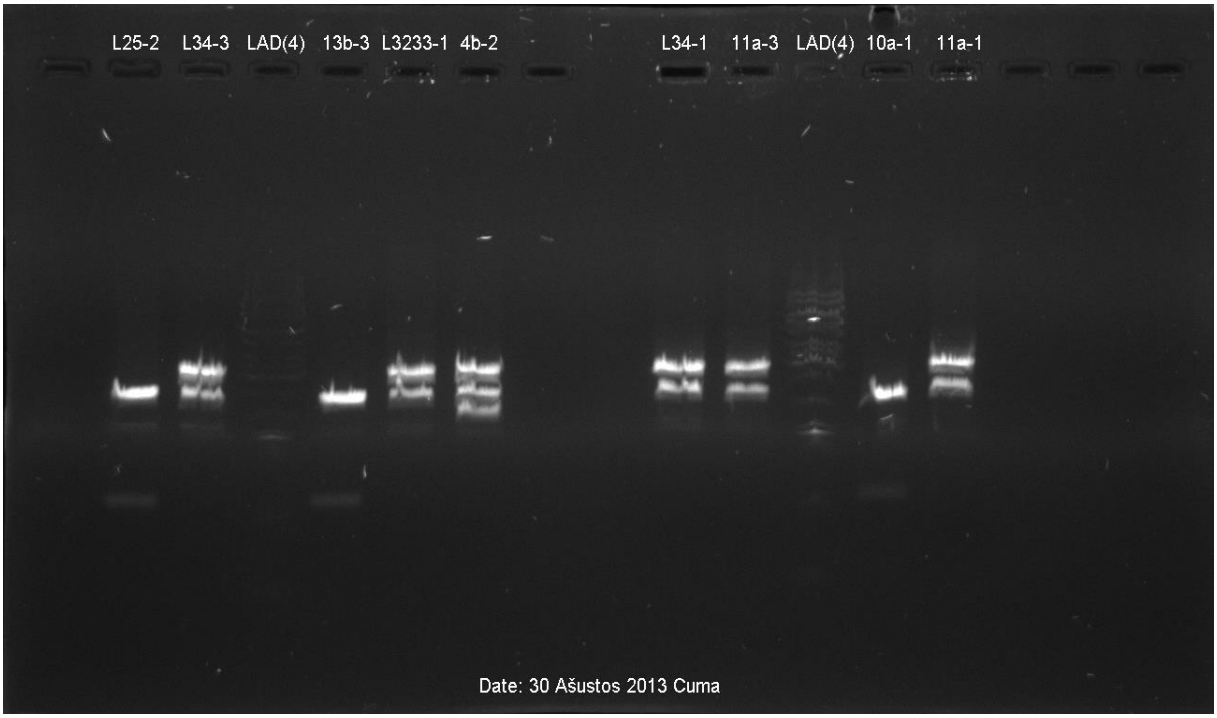
(Apologies if i have missed other exp. From today)

We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.



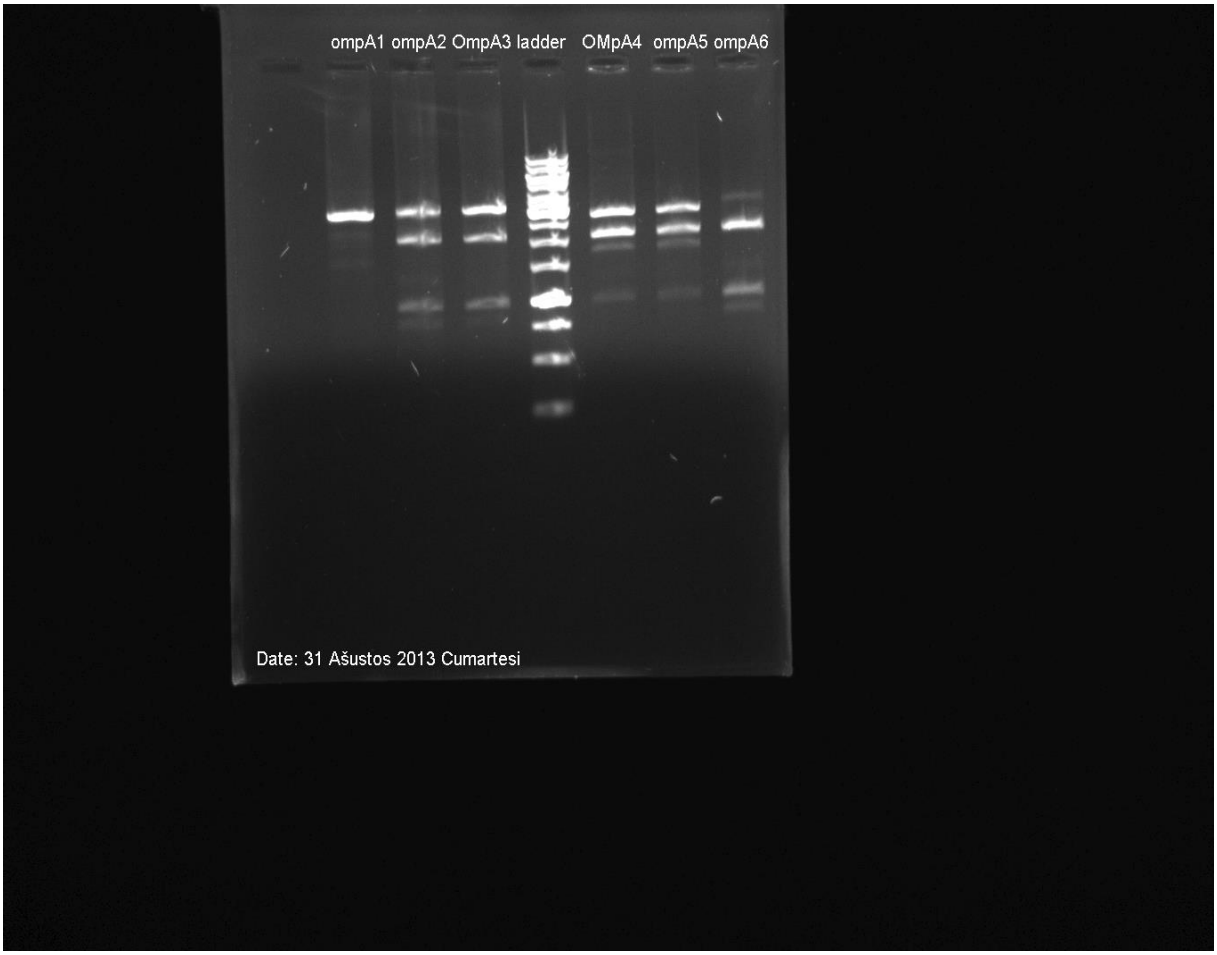
30.08.13

We used western bloot experiment in order to observe tat-apoptin (BBa_K1202105) but we didn't observe BBa_K1202105.



Parts were cloned to check ,via electrophoresis gel and L25-2, 13b-3, 4b-2,10a-1 were found wrong,L34-3,L32/33-1, L34-1, 11a-3,11a-1 were found correct

31.08.13



Parts were cloned to check, via electrophoresis gel and all parts expect ompa1,ompa6 were found correct