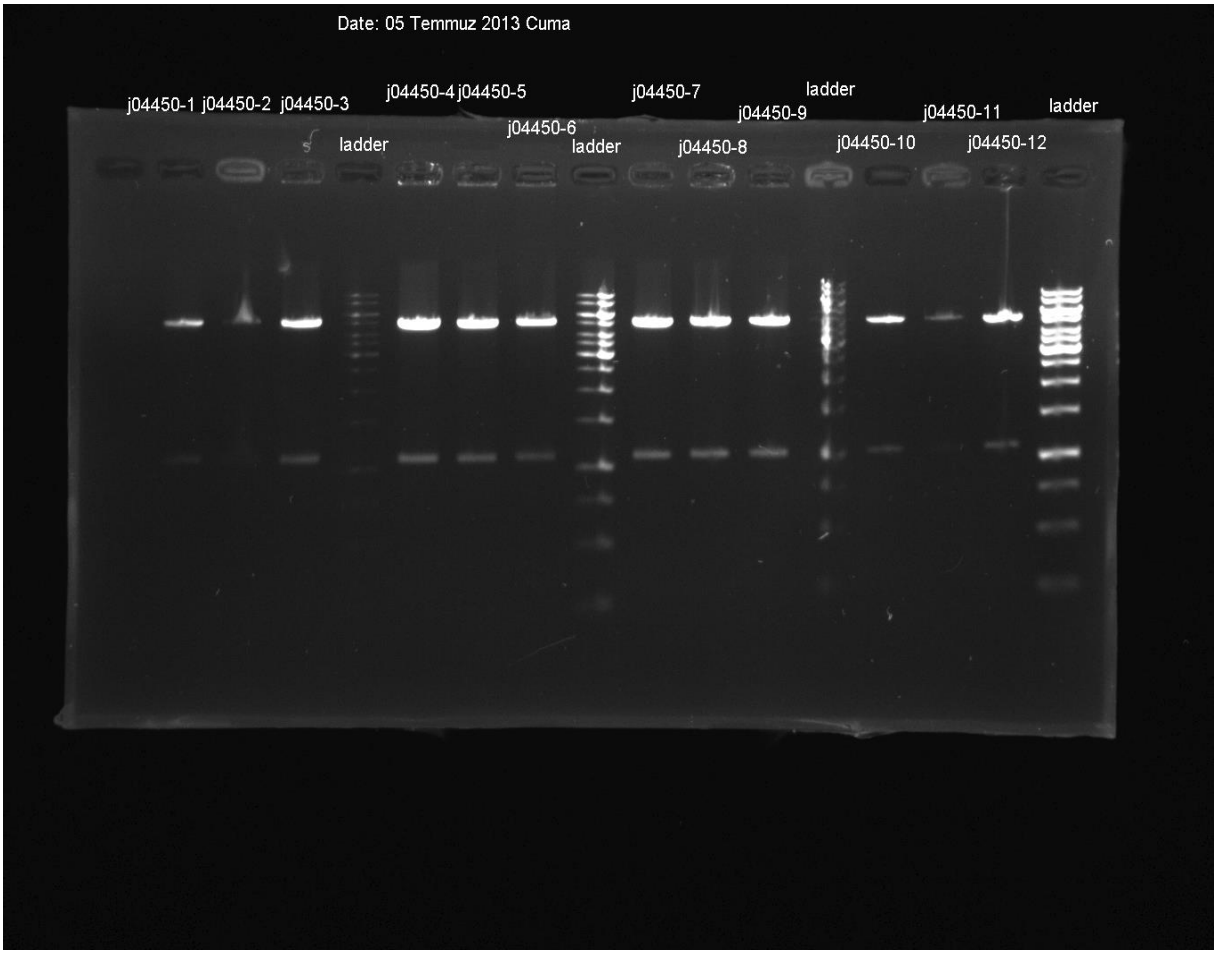
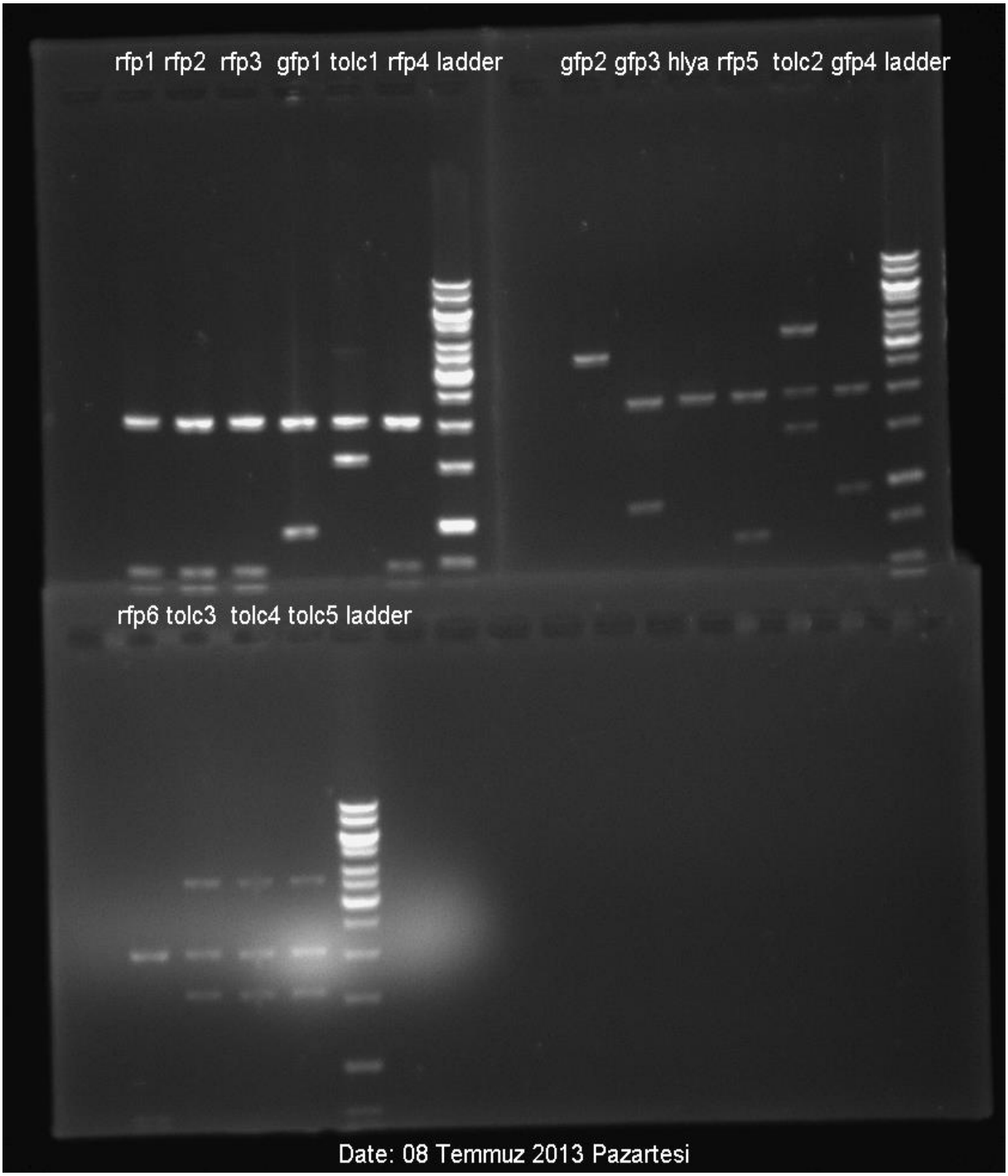
05.07.13



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

08.07.13



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

09.07.13

Here today is, the first day of the month of “Ramazan”. Ramazan means us to fasting for a whole month. we still worked, and we will still be working on. Believe that ramazan will bring us so many great things.

Brainstorm for the human practise

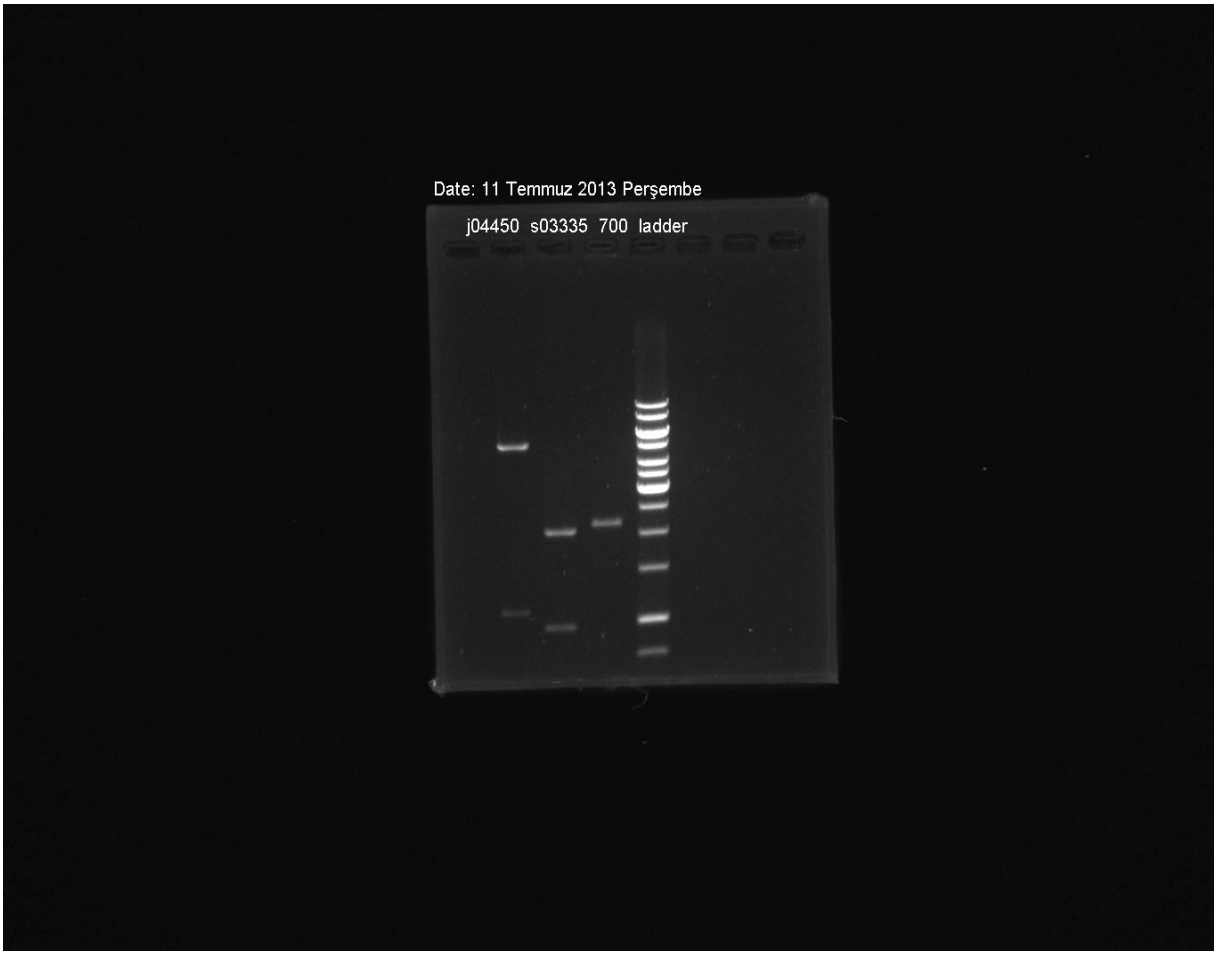
- Meeting with the expert of “oncology”
- Giving presentation to “Ülker” for a **novel production**
- an email to Mustafa Nevzat
- Educating new igemers at highschool
- Workshop at our school’s congress

We are learning some techniques while doing our project’s experiments, so, we did digestion, nanodrop measurement and electrophoresis.

- Digested s03335(with xs) and j04500(with ep)
- Take the digestion tubes into the thermosicle
- Nanodrop measurement
- Electrophoresis

11.07.13

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 except 700 were found correct

12.07.13

Date: Friday 12th July 2013

Morning:

- The day started off by partially re-organising and cleaning the Atoms iGEM lab. We then began our meeting by planning todays experiment schedule and debating about how we should enhance our English speaking and reading skills. The decision we finally arrived to was 1) trying our best to speak English in the lab unless we get stuck and 2) choosing a book to read at least 3 pages per day. Furthermore some of our team members were given responsibilities. These are:

❖**Nur Nihal, Safa, Mikail**: is in charge of finding the ideal coding system for our gene bank.

❖**Omer**: is in charge of obtaining an approval letter/form from the ethics committee so that we can work with mice in the later stages of our project.

- During the meeting, we received the great news each of us was impatiently waiting for! Most of the genes which we had ordered approximately two weeks ago arrived today! ☺ In addition to this, E. Coli Nissle 1917 sent by Professor M. Goulian of Penn University also entered our university boundaries! As expected this resulted in double joy of happiness for us Atoms iGEMers!

Afternoon:

- During the afternoon, we continued with the experiments we had begun by doing digestion and ligation yesterday. Some of us did transformation while others prepared a liquid culture. These experiments were conducted purely on the basis of gaining more experience and becoming at ease when it comes to following the instructions stated on the protocols as well as having more familiarity with lab equipment's.

- Following that, Aysenur, Nur Nihal and Aslihan then prepared ampicillin resistant LB agar petri-dishes.

- We then had another meeting where Human Practice ideas were discussed and an in-depth talk on finding the ideal novel approach transpired. We came up with the idea of bringing about a compulsory inspection on synthetic biology research and companies related to this area of the science field. Mustafa abi separated Human Practice into 5 sub-headings. These were: safety, ethics, outreach, ownership and future aspects. We had the opportunity to speak about what we already have accomplished for each one or planning to in the next 11 weeks of our remaining time. On the other hand, the thought of contacting the scientists whom have written the research papers/articles we have read until now arised. This will be to ask their views on our project and/or synthetic biology. Some questions initially coming to mind are of such: how can we improve our project, the ethics etc. (not sure about this part)

Evening:
- Finally we began working with our newly arrived genes by firstly diluting them which followed by their transformation ☺ We ended the day by cleaning our lab!

Important Deadlines:
Wiki writings assigned to each team member must be completed by Monday 15th July 2013.
Passports to be received by latest 1st August 2013

13.07.13

Morning:

- **Gel electrophoresis** with digestion of ligation products

  Product names: GFP2/4, GFP2/3, GFP2/2, GFP2/1, GFP2/3, GFP2/5,

  lig- GFP2/2, lig-GFP2/1, GFP1/2

  Result: Ligation did not work.

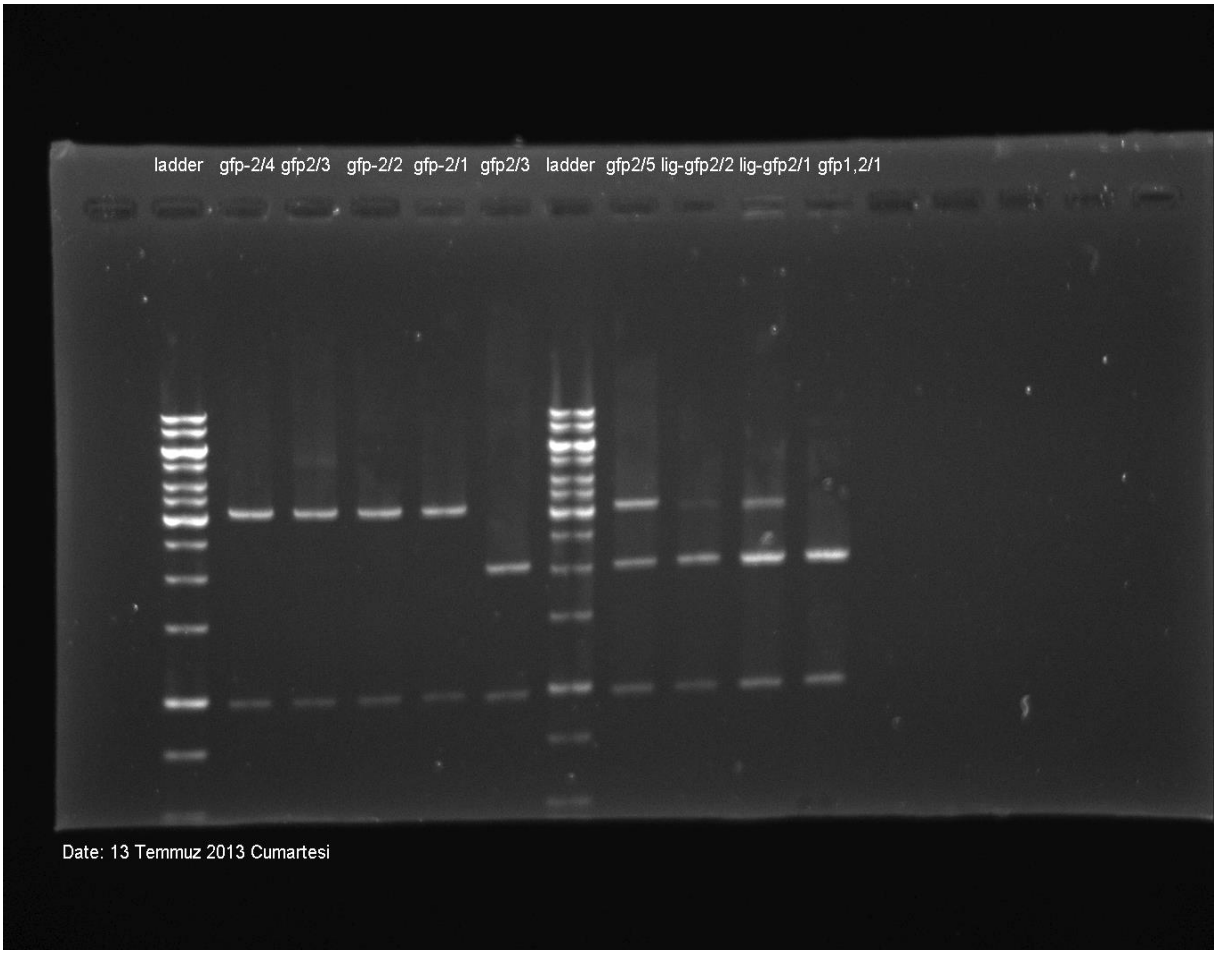
- **Meeting** about Mutaflor/other companies' collaboration strategy.
  Result: We should not apply right now, we should wait until September/or mid of September.
  Outline time schedule:
  July 15-30th: Ethics approval
  August: In vitro & in vivo experiments
  September: Apply patent & Get an appointment from Mutaflor, Novartis etc.

Afternoon:

- **Liquid culture** with our main 11 genes' transformation plates
- **Transformation** of promoters J04500, J23100 from IGEM kit.

Evening

- We've found project name: **Oncoli**



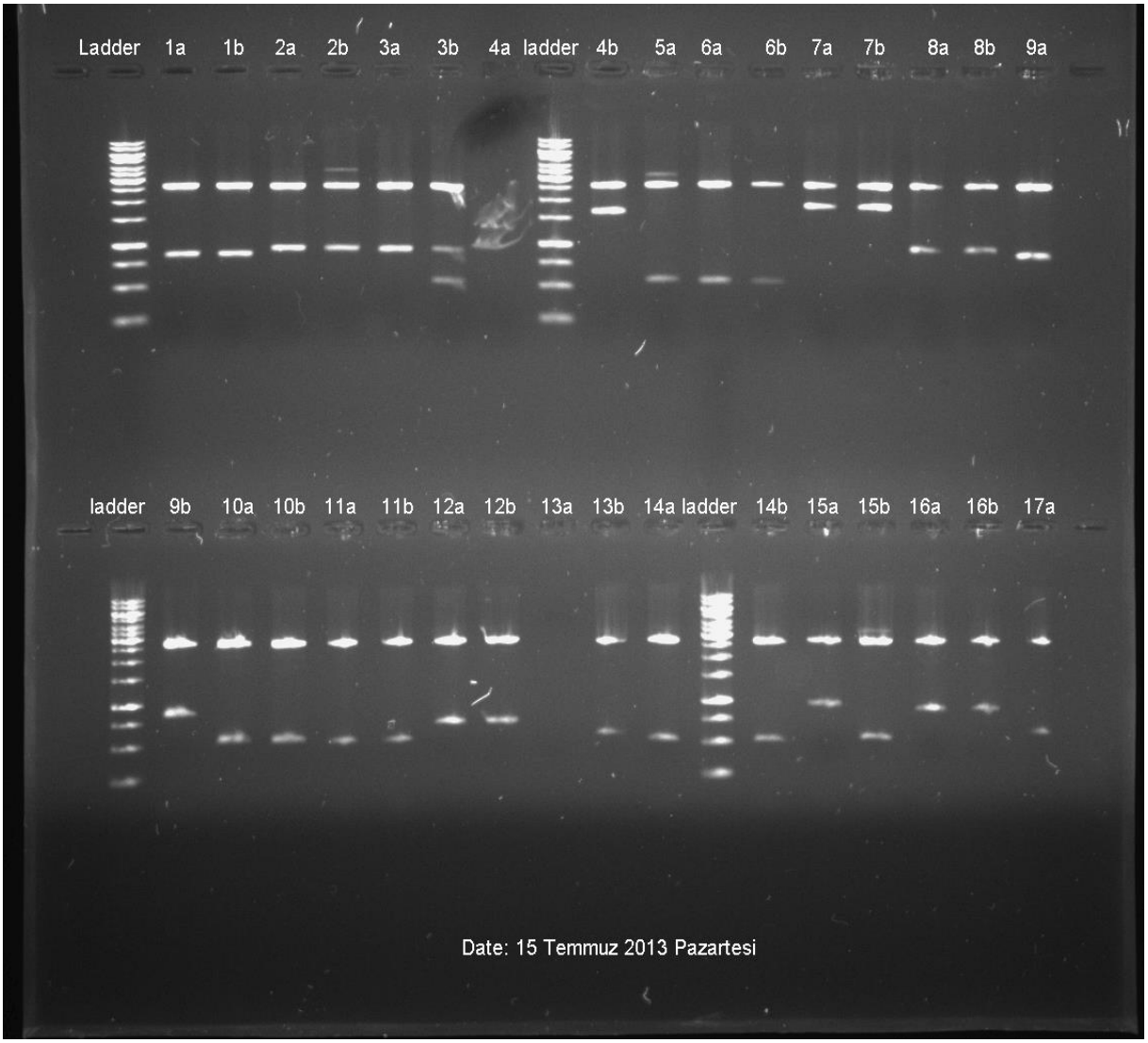
Parts were cloned to check ,via electrophoresis gel and all parts were found correct

15,07,13

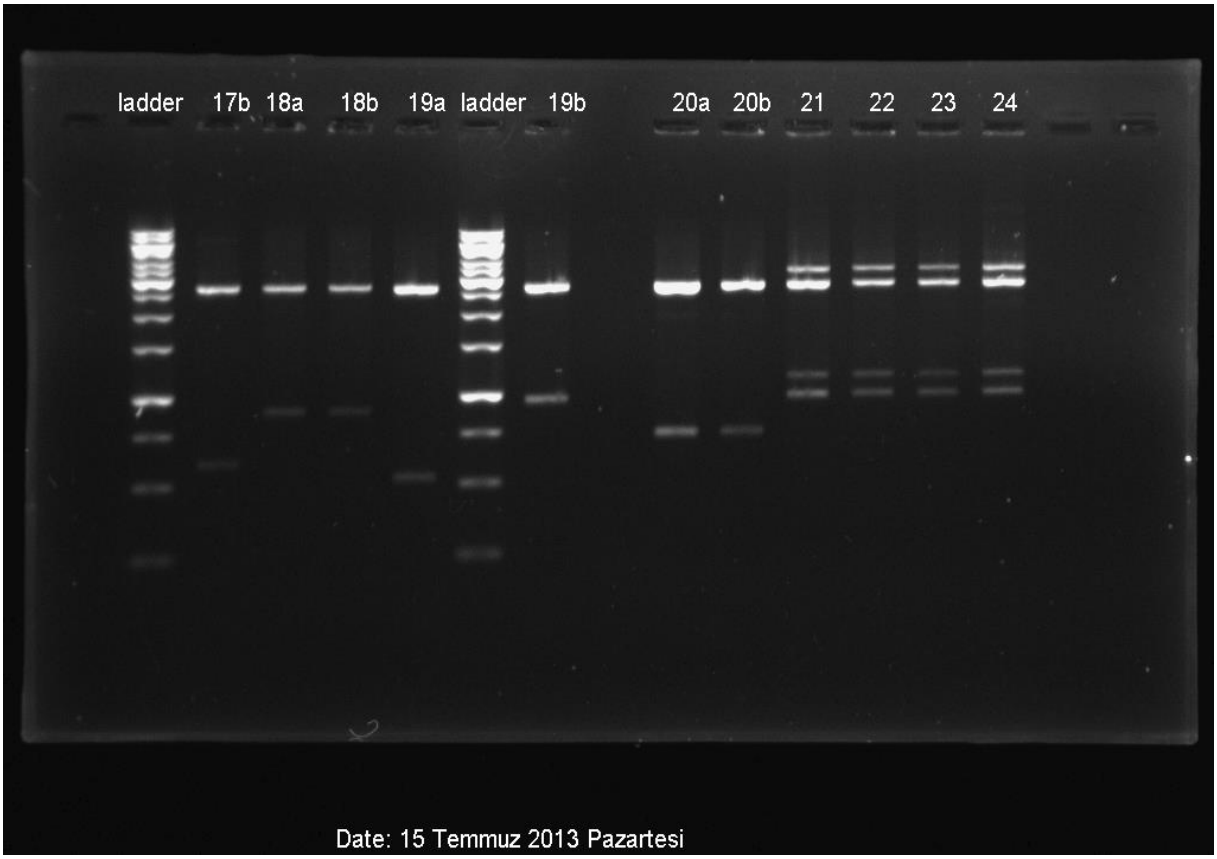
lig.Rfp-Gfp inserted in to e.coli

2.we mate digestion experiment for genes coming from genescript.

3.digested genes were performed gel Electrophoresis



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 correct

17.07.13

1.prepareTriton X,Glycine procedure .(Mikail,Fatih)

aim:secreted protein with chemical

2.digestion experiment(Ayşenur,Nihal)

aim: Ligation optimize

a.RFP-SP

b.GFP-XP

c.J04500-SP

d.J04500-ES

e.TolC-XP

3.PCR purification experiment(Safa,Aslıhan)

aim: Ligation optimize

LsrK—EP

psB1c3—EP

Ligation experiment

NOT:Human Practise----Skype

Nissle

Safety

Modeling

Catherine and Mail

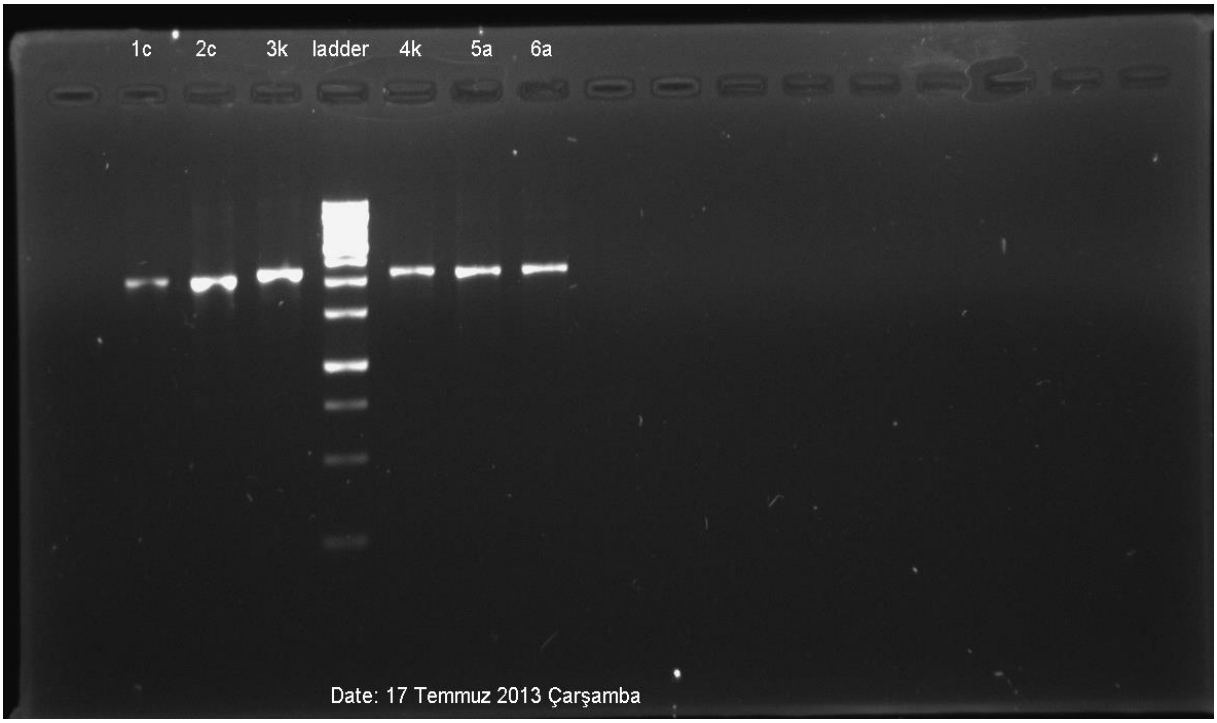
4.Compatent Cell

5.Ligation

6.J04500

S03335

cotransformation



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

18.07.13

Midnight:

- Competent Cells were prepared for E. Coli Nissle and N10.
- GFP, J04500 Transformation.
- Rfp transformation into Nissle and N10.

Morning:

- During the morning, we centrifuged the glycine-triton falcon tubes to observe the pellet and separate the TorA-RFP. However none produced any RFP. So we have excluded these for further experimental use.
- NurNihal & Aysenur isolated TolC and GFP then digested them which followed on to electrophoresis.
- Safa and Aslihan were in charge of doing pind2(lsrk)+psbrc3 ligation. They then ran electrophoresis on RFP-GFP 1 & 2 to see whether digestion took place. However the results showed that they were not cut.

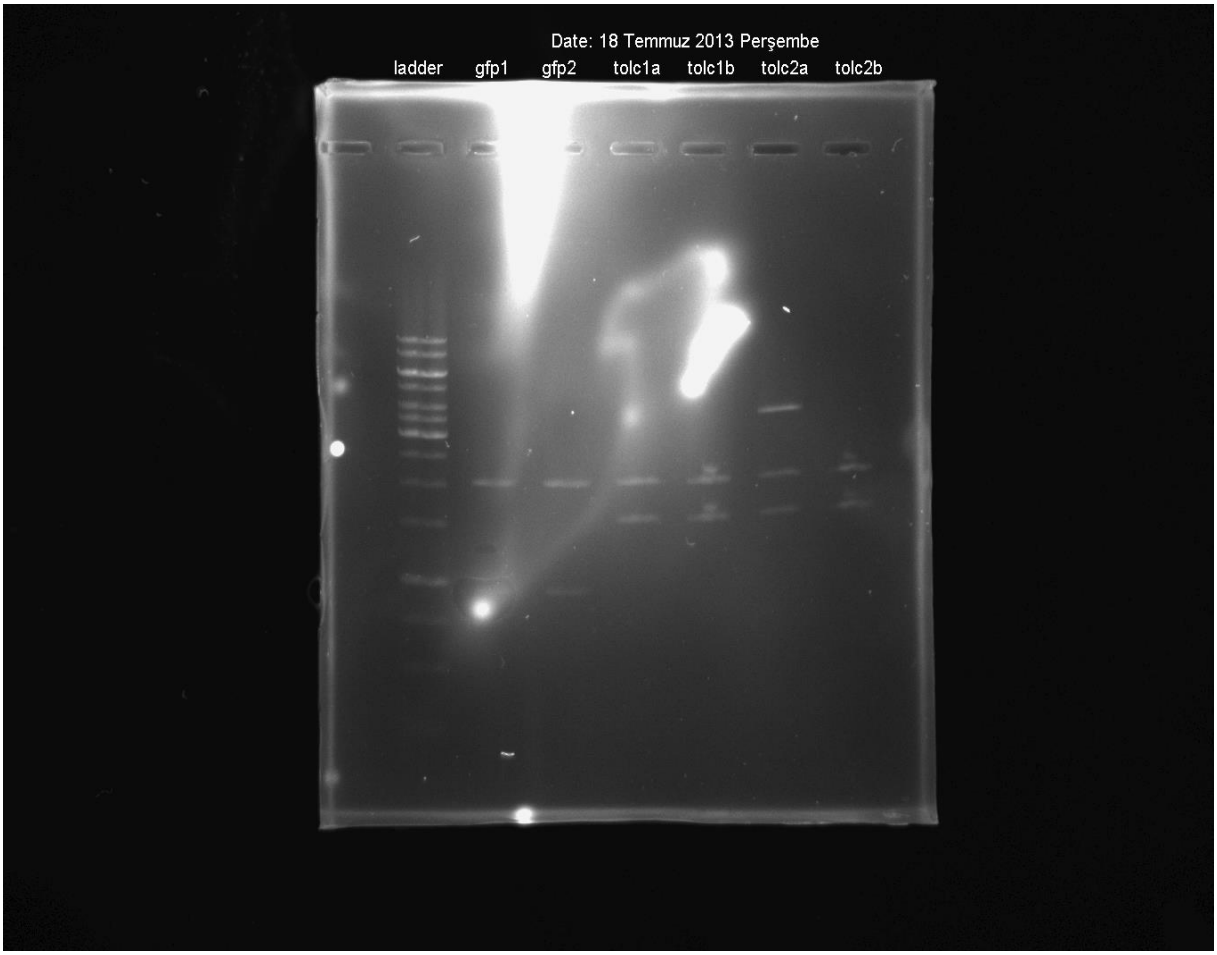
Afternoon:

- Safa and Aslihan completed transformation on PIND2/PSBRC3.
- We had a meeting on Human Practice. Mikail came up with the idea of writing a letter to the President regarding the very little sensitivity our country has towards Synthetic Biology and also form a miniature thought based iGEM website where universities/high schools can join in an internet based competition as teams. (Idea iGEM) Abdulkerim had an opinion of forming a 'Safety Form' where iGEM teams can share their thoughts and give advice on other teams projects. Atoms Girls thought that having a 'send your thoughts on our project/possible problems' selection on our wiki may bring the 'best wiki prize'.
- Liquid culture has been prepared for J04450 (RFP), J33350 (GFP) (Fethi & Safa) J04500 and S03335 (Aslihan & Nur Nihal)
- Safa diluted the genes which have newly arrived. (Protein G, LuxsPfs2, H-T/A (H))
- Ampicillin resistant plates have been prepared (Safa & Aslihan)

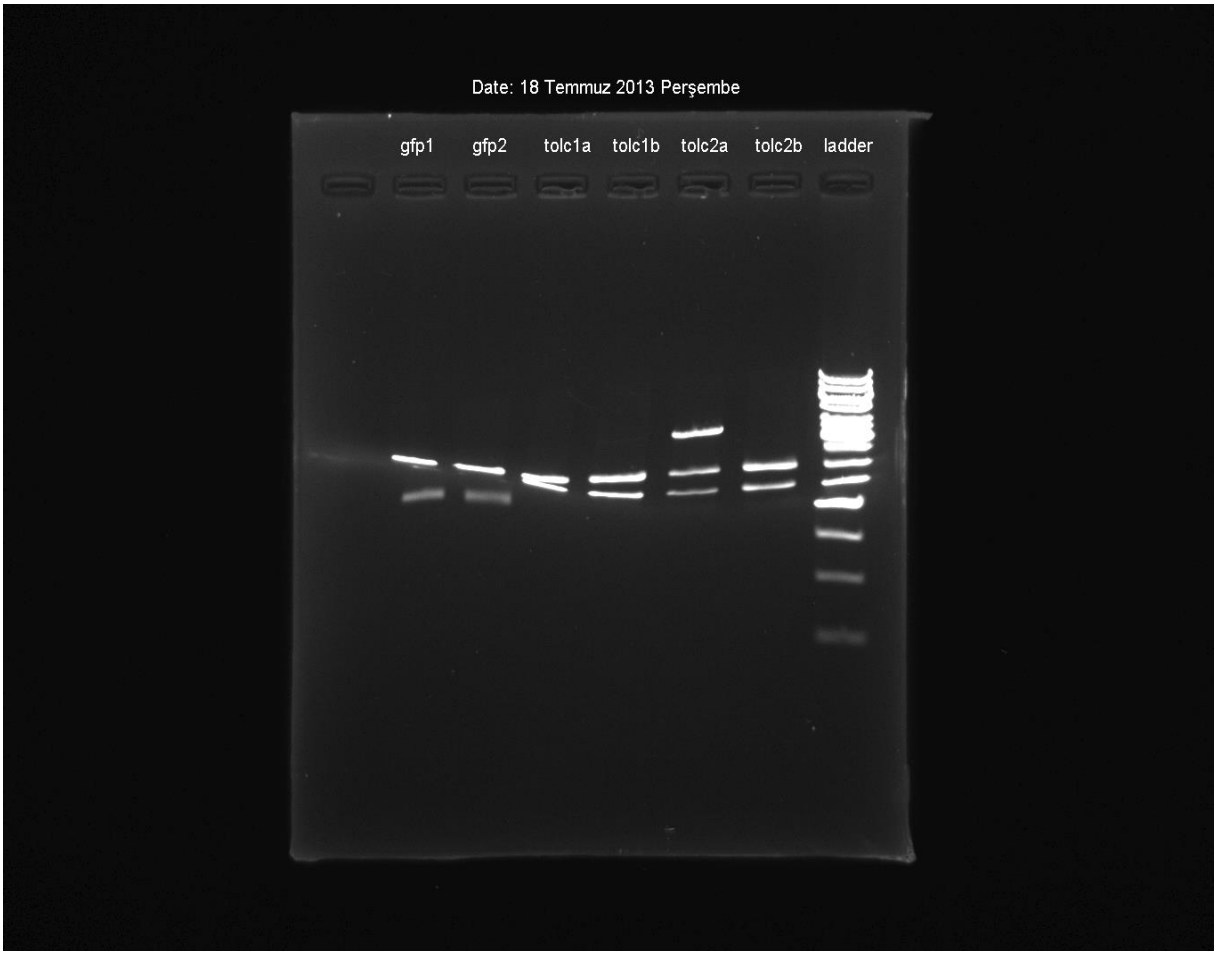
Evening:

- NurNihal & Aysenur prepared gel and ran electrophesis for TolC & GFP.
- Safa organised the cabinets. Fethi + Abdulkerim filled in the empty pipette tip boxes.

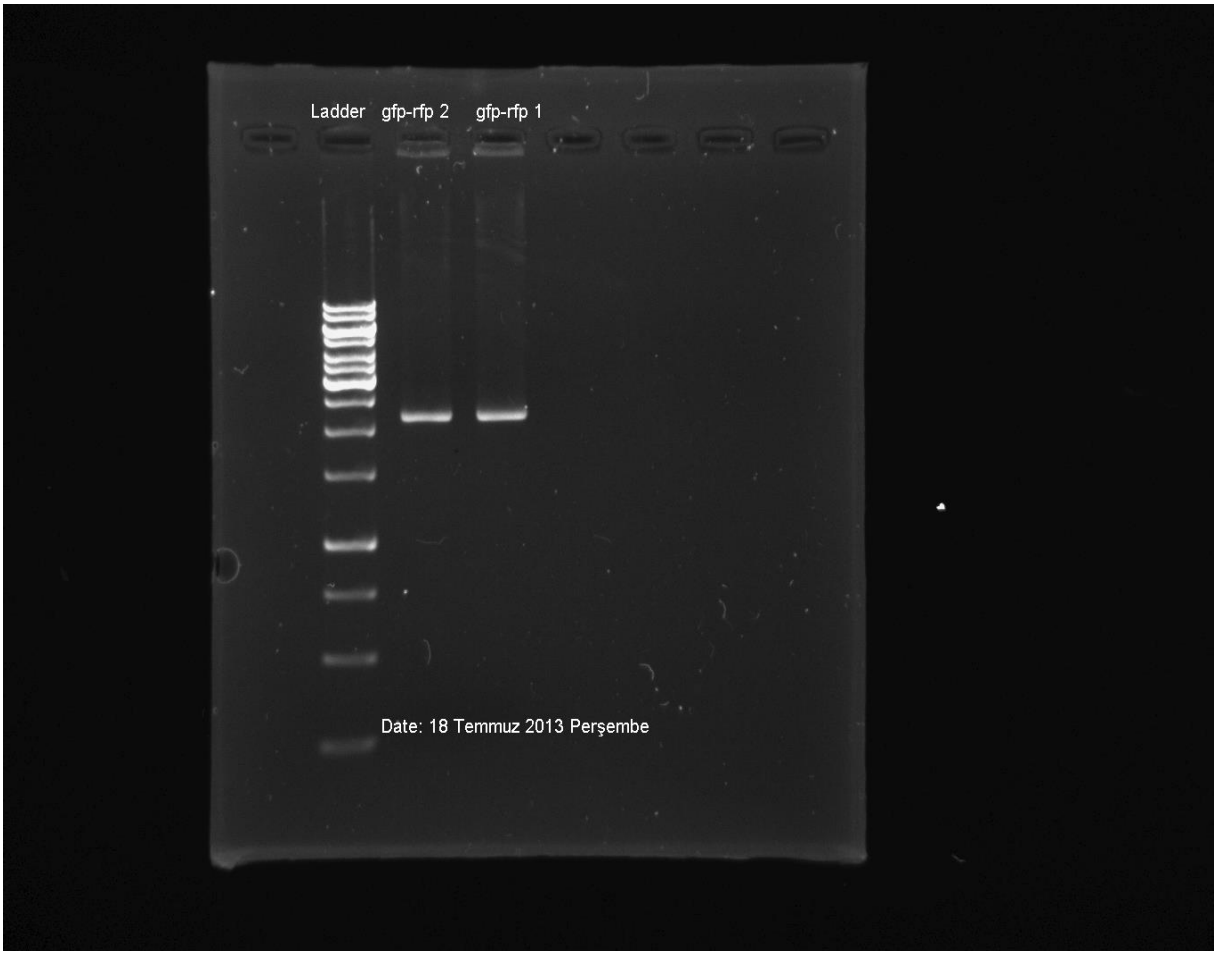
Reminder: Those who have not yet applied for Passports must do because we need to apply for schengen visa! ☺ Also please don't forget to send reports.



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 correct



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

19.07.2013

*pind2 liquid culture (16 hour incubation)

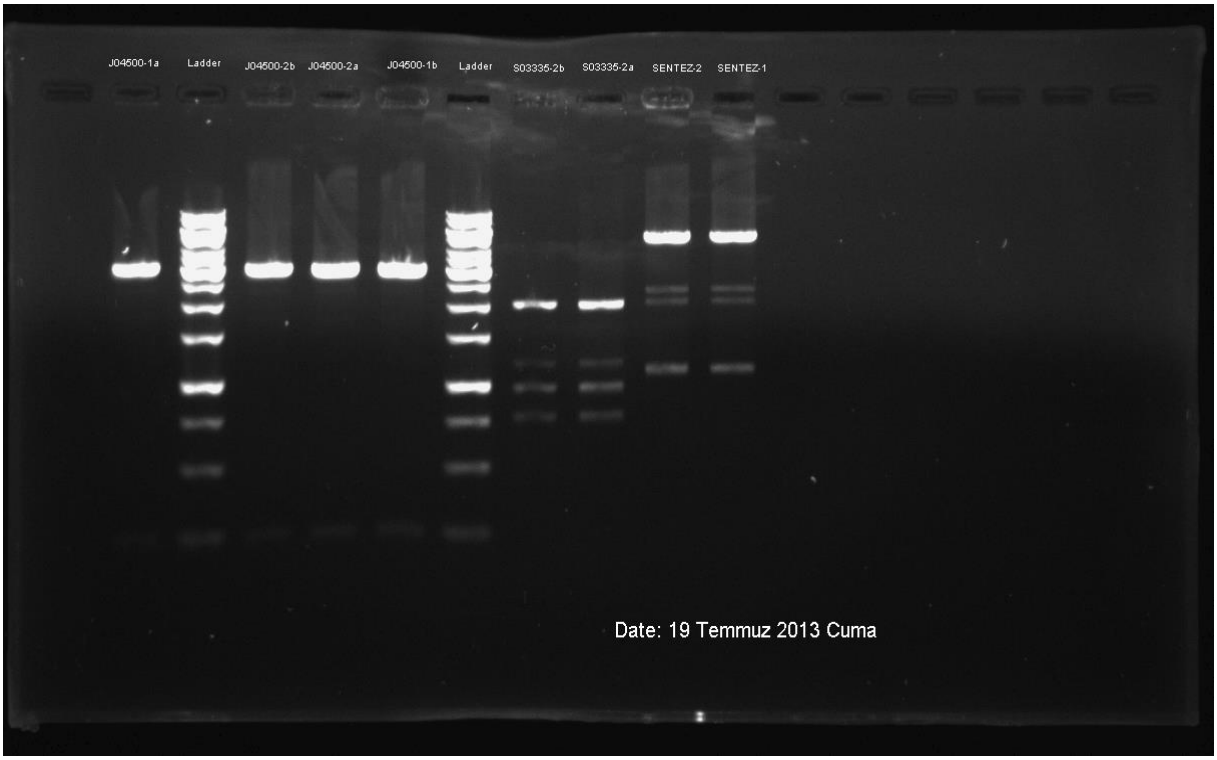
Aim: co-transformation with pind1 or firstly transform pind1/grow it/ then transform pind2.

* GFP
J04500
GFP-RF

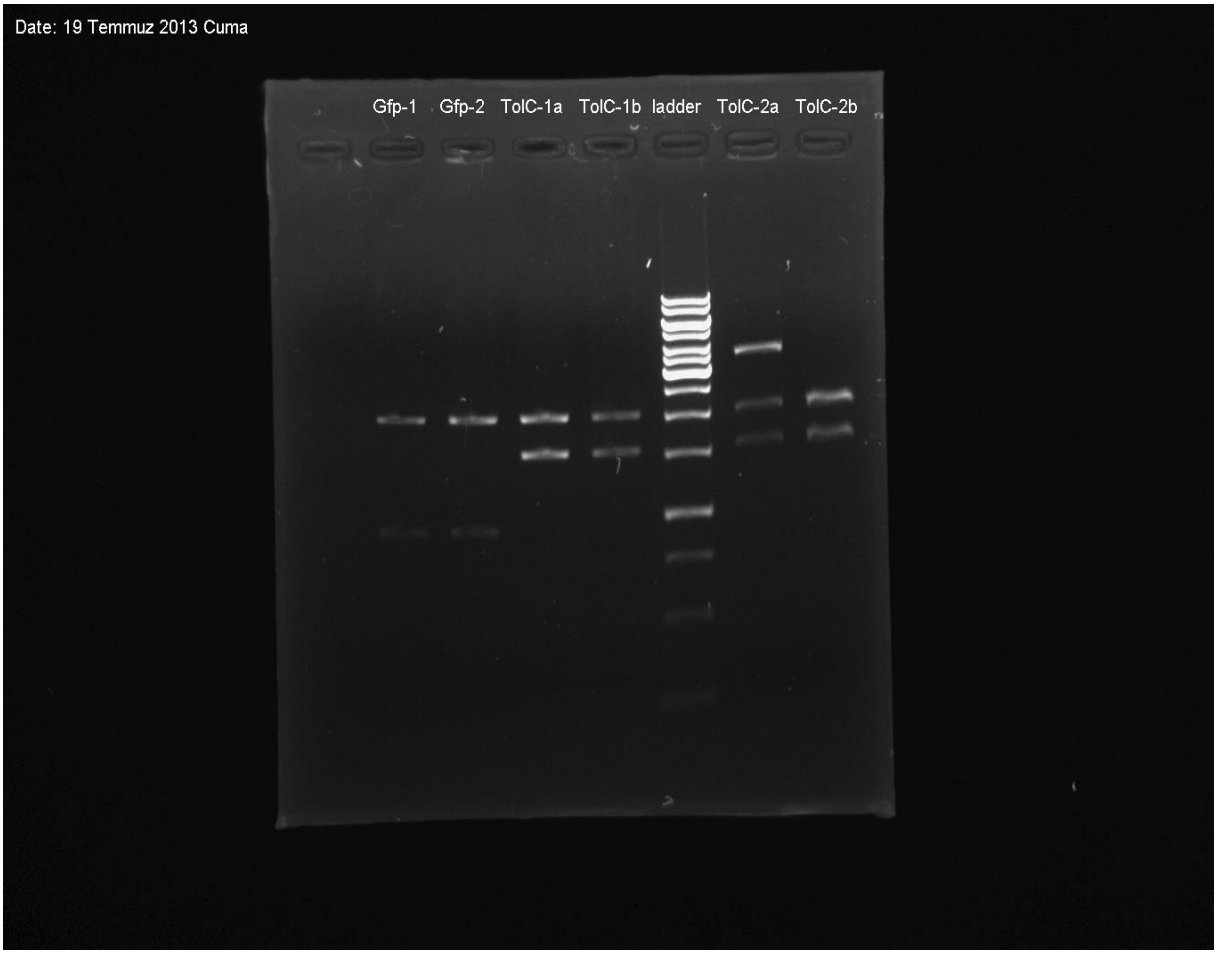
} Isolation, digestion, gel electrophoresis

Result: interesting (!) GFP-RFP band sizes (bigger than expected).

*All Experiments' algorithm



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



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

20.07.13

Midnight:

- GFP (EP), J04500 (ES), J04500+GFP (EP) have been digested and ran electrophoresis.
- NEB10 competent trial #3 -> S03335 and Control (CHL resistant)
- Terrific Broth (TB) has been prepared:
  1. 90ml Deionised H_20, 1.2g Tryptone, 2.4g yeast extract (Autoclaved)
  2. 10ml Deionised H_20, 0.17m of KH_2PO_4, 0.72m of K_2HPO_4 (Autoclaved)
  3. Once autoclaved, both prepared solutions are mixed to produce TB.
- 4ml of TorA-RFP liquid culture has been prepared.

Morning:

- In the morning, NurNihal and Aslihan isolated Pind2 which followed on to digestion and electrophoresis.
- Abdulkerim prepared the liquid culture for HLYA-RFP.

Afternoon:

- We had a meeting:
  - Safa explained how you do western blotting and also touched on its certain difficulties whilst doing it. (We have an optimised protocol)
  - Fethi was in charge of researching for immunocytochemistry, so informed us on how it works.
  - Abdulkerim was going to research how much apoptin we should give to the cancer cells but was unable to us he was occupied with something else to do.
  - Aysenur found the protocol for ellmans essay and the required equipments for it.

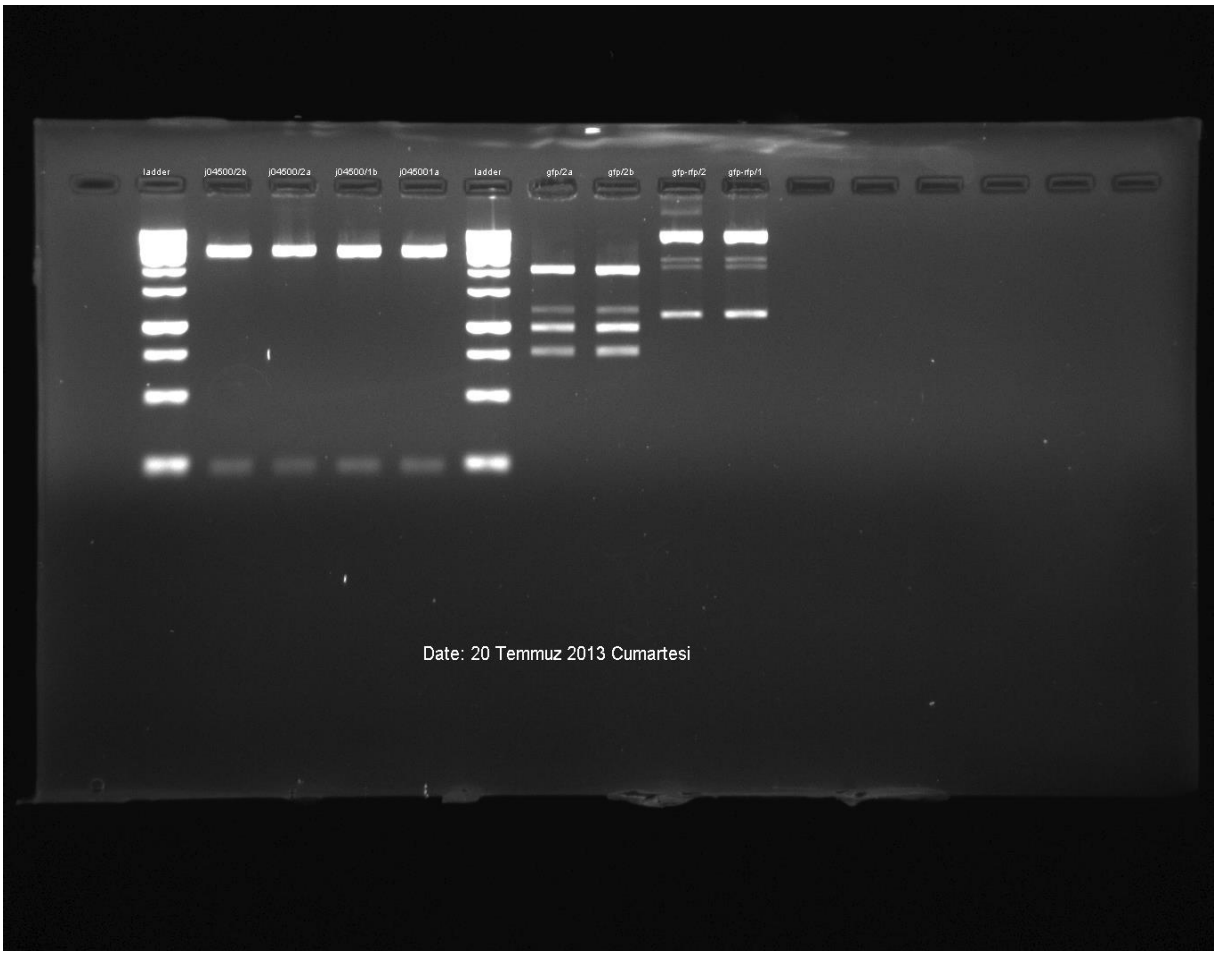
- Aslihan prepared a liquid culture of SO3335 (GFP).

Evening:

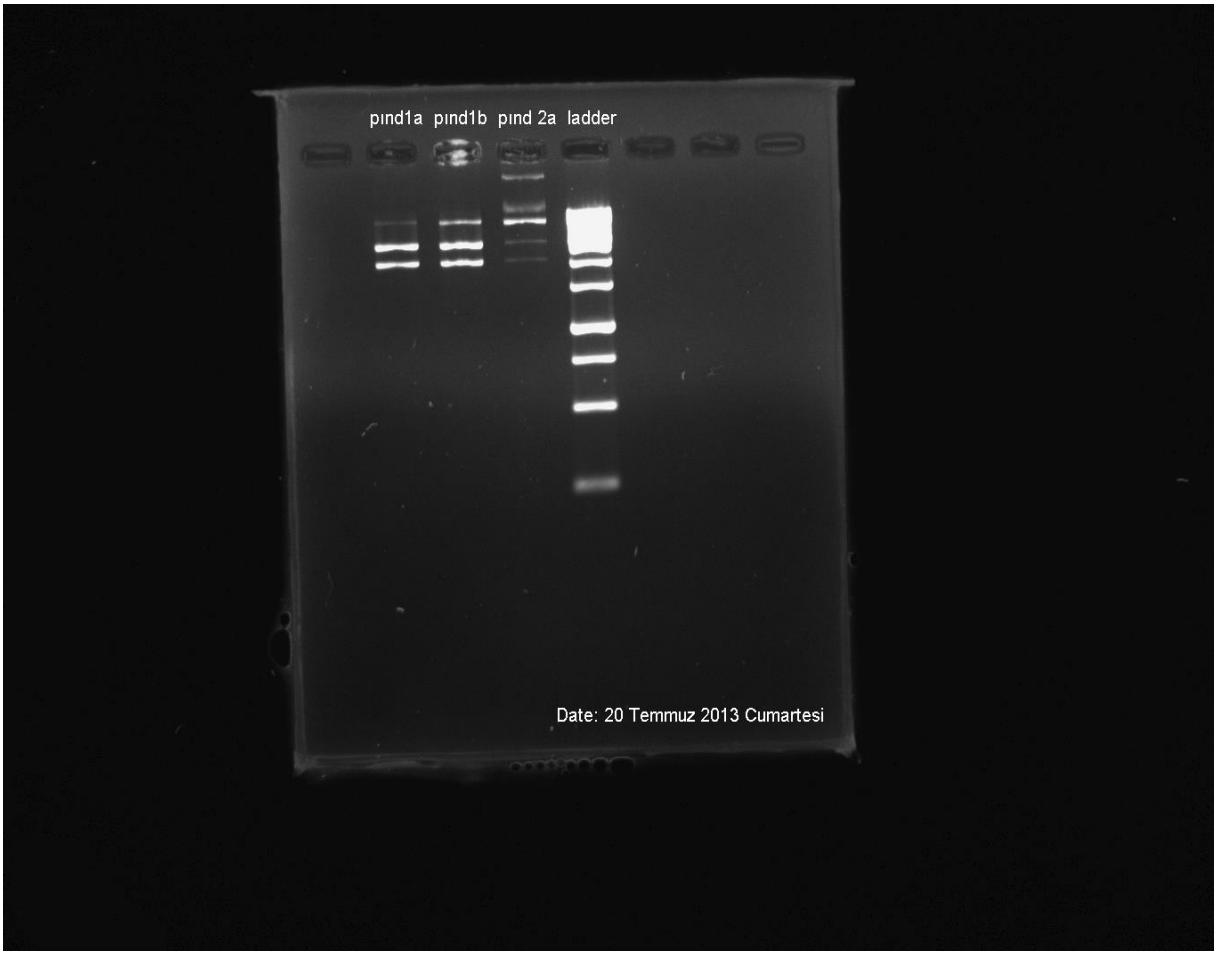
- Fethi and Safa fermented TorA-RFP with triton and glycine in 4 falcon tubes. From 2ml ofTorA-RFP -> 200 ul fermentation.
  1. **1st falcon tube:** 5ml TB + 200ul TorA-RFP
  2. **2nd falcon tube:** 5ml TB + 200ul TorA-RFP + 1% Triton
  3. **3rd falcon tube:** 5ml TB + 200ul TorA-RFP + 2% Glycine
  4. **4th falcon tube:** 5ml +200ul TorA-RFP + 1% Triton +2% Glycine

- Tests to see if competent cells are working:

Results	Gene	Competent	Resistance
Colony in control (-)	New 3 genes	Old	Amp (A.G.P)
Colony in control (-)	RFP	New	Amp (A.G.P)
Colony in control (-)	RFP	New	Amp (Fethi plate)
Colony in control (-)	GFP	New	Amp (Fethi Plate)



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

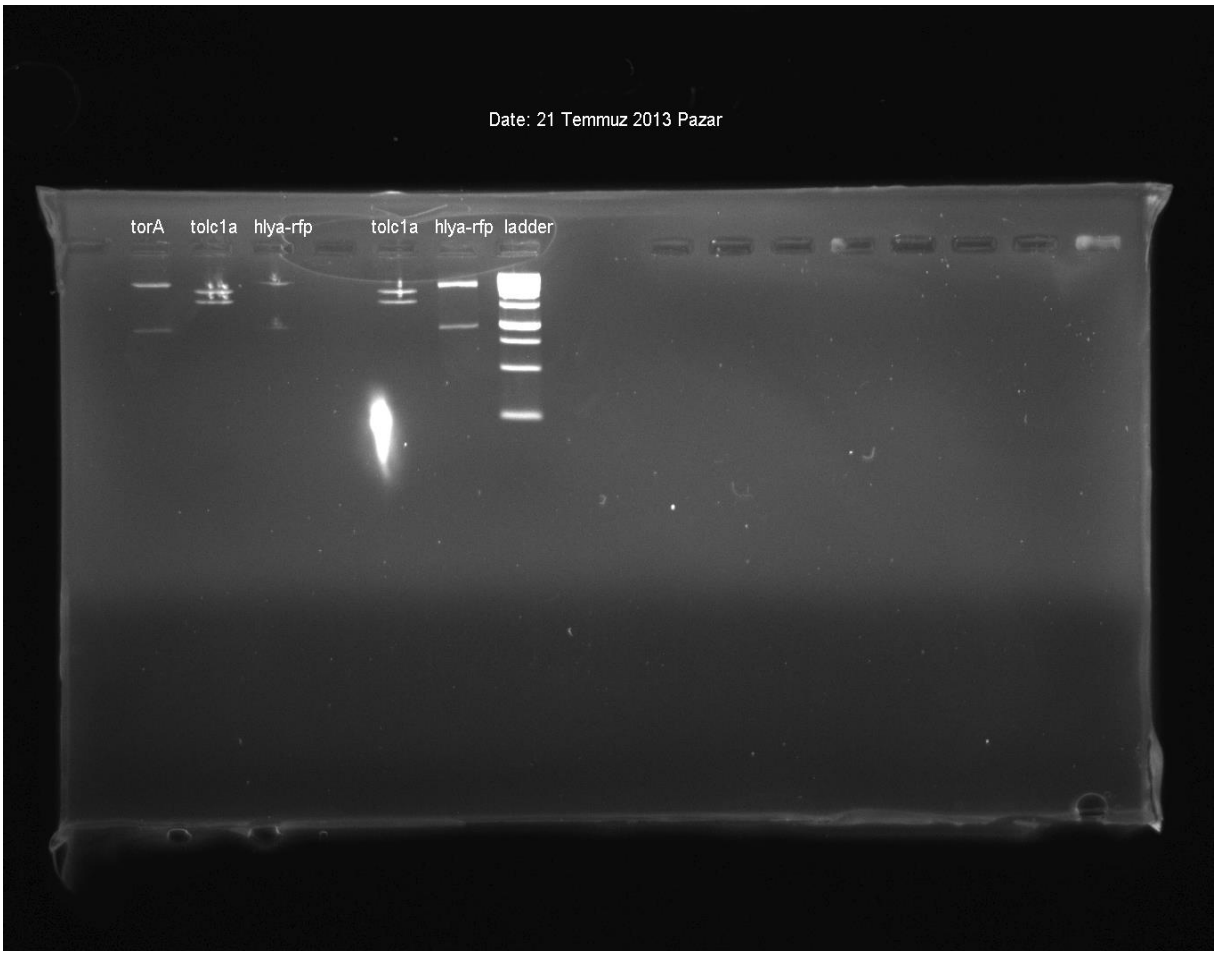


Parts were cloned to check ,via electrophoresis gel and all parts were found correct

21.07.13

Morning/Afternoon:

- Aysenur and NurNihal digested TorA, HlyA-RFP, TolC and ran electrophoresis.
- Fethi, Safa diluted Amp and made a glycerol stock of GFP.
- We attempted doing amp resistant agar plates but it may have been a failure! :/
- Aslihan isolated GFP.
- Yesterday, we used one of the new competents' to test whether it is resistant to CHL (agar plates) and there were no colonies visible in the control plate meaning that there are two possibilities:
    1. Either the competent is resistant to Amp.
    2. The Amp we used to make the Petri-dishes is not working or contaminated. (This is more likely to be true)



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

22.07.13

Morning:

- Amp resistant LB/Agar Plates were prepared by Nur Nihal, Safa and Aslihan.
- TorA and RFP were centrifuged again, very tiny pink like pellet was seen but we couldn't be sure.
- Aysenur and Abdulkerim were spectators of the team who were doing western blotting.

Afternoon:

- TorA and RFP problem was partially solved. Gokhan abi enlightened us on the reason why we are not seeing the red colour that should be emitted by RFP. The non-existence of RBS before and between TorA and RFP seemed to be the problem. However, later it was realized that if RBS is to be placed then two different proteins will be produced and this is not favored. So the idea of placing a linker between TorA and RFP arised. The following genes will be re-ordered as (like TorA-RFP) they are not working either:
    - ❖ Hlya-RFP

- ❖ Tat-RFP-Histag
- Nur Nihal and Aslihan did TolC-HlyA-RFP transformation.
- Gokhan Abi and Safa used PCR to replicate TolC and RFP using the iGEM primers.

Evening:

- Abdulkerim went into cell culture department with Esin Abla to gain some familiarity.

24.07.13

Morning:

- Two plates from Nissle culture were implemented by Esin Abla and Mustafa Abi. One plate didn't include any antibiotics.
- Nitrogen was ordered for competent cells.
- Esin Abla and Mustafa Abi has re-ordered TorA-GFP and GFP-HlyA.
- Aysenur, Nur Nihal and Aslihan did co-transformation for Pind/Pind2.
- Nur Nihal has done further research on HlyA.
- The requirements for western blotting (Sonicator and Lysis buffer) has been prepared which will be used for His tag-TAT-X, MPG-X.
- The immunocytochemistry protocol for OmpA-C215-His tag has been accomplished.

Afternoon:

- We had a meeting. Gene bank still seems to be a problem; unnecessary tubes which will not be in need for future use should not be stored. We need to organise the isolation and digestion boxes in the freezer and place them according to their coordinates as well as taking notes of them on the electronic system. Reporting system has changed. From now on excel tables will be present in the lab where each person will note down which experiment has been conducted on the day, the results and their names which then will be typed on the computer.
- **Revision meeting:** Starting early on wiki's has put us on a good stead. Glycerol stocks should be made for store and future use (…) Wiki texts need revisiting to determine the missing ones.
- NEB10 competent control plates gave good results. Pink colour could be seen which means the AMP we used was troubled.
- 3ml Amp resistant liquid cultures were prepared for all His-x-apoptin.
- Gokhan abi and Safa performed PCR again to do digestion as results were not as expected.

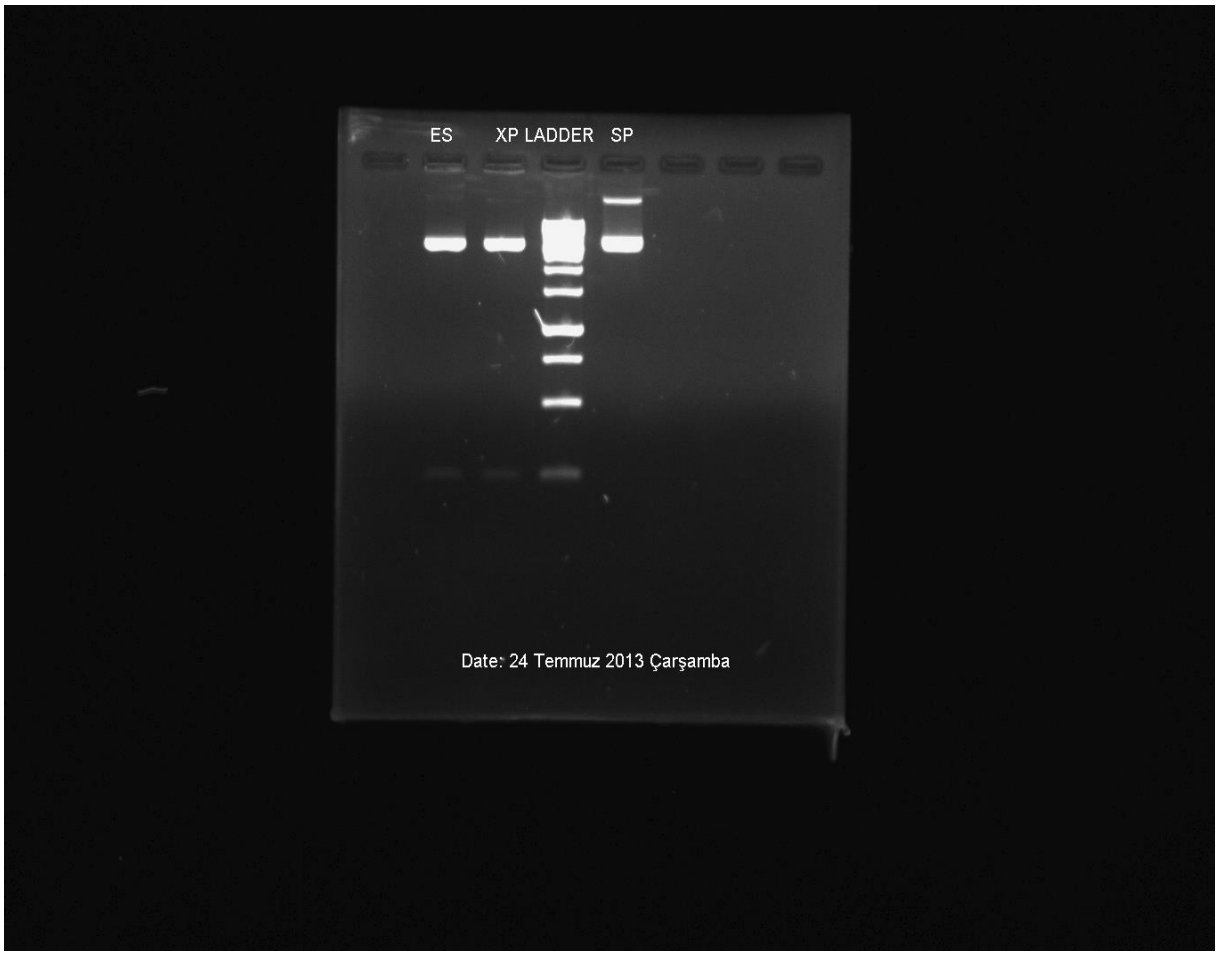
To be done:

- Ligation plates which were prepared overnight (classic) on 23.07.2013 needs to be transformed on CHL resistant plates.

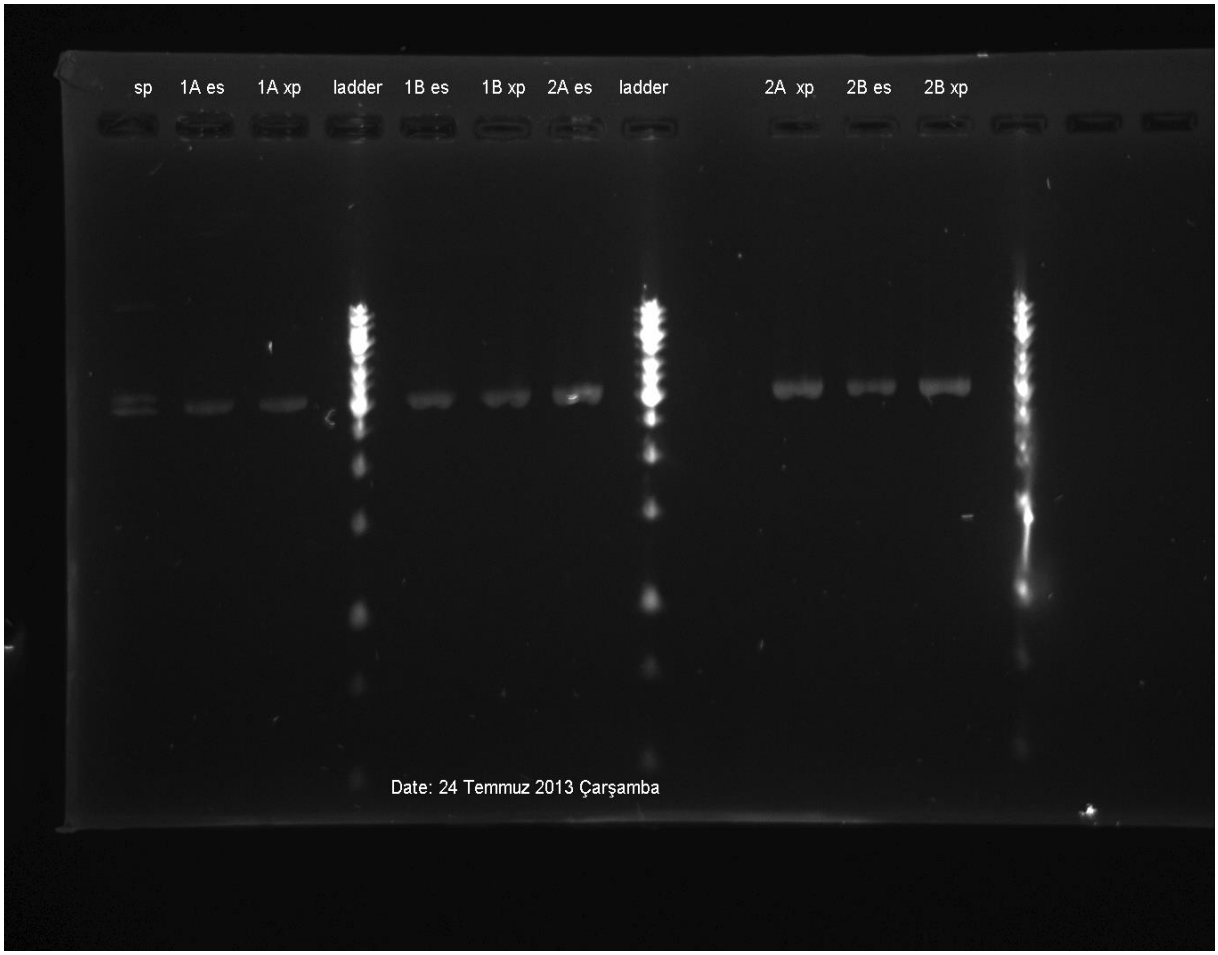
- SP J04500 digested for 2A Assembly should be ran electrophoresis again.
- Safa and Mustafa abi will digest J04500 (es, xp, sp) with pSB1C3 (ep) and 50ul should be ran electrophoresis.
- Parts need to be added on the registry.
- Ligation should be performed using the following processes:
    - ❖ 2A Assemply
    - ❖ GEX Overnight
    - ❖ GEX classic
    - ❖ Insert PCR

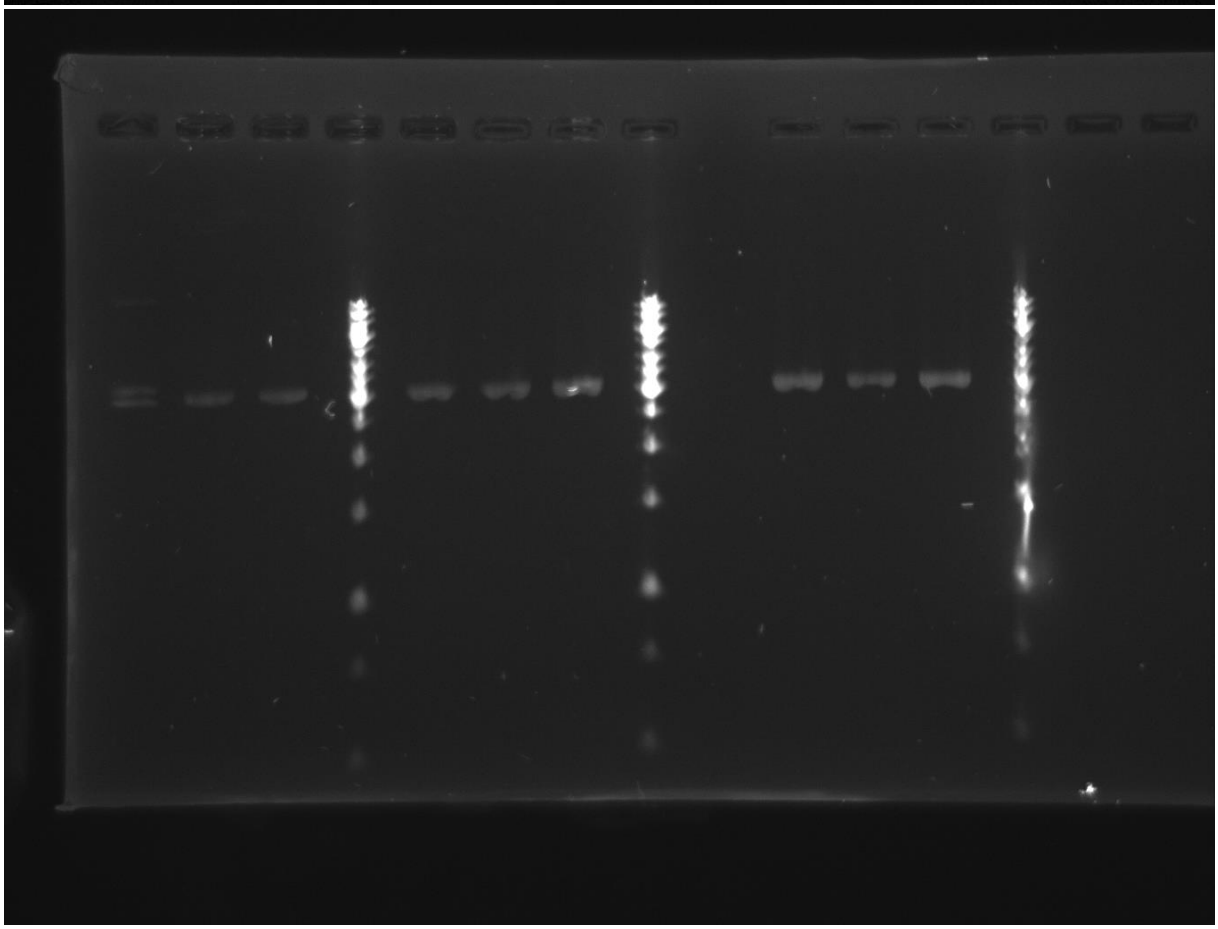
<u>The following needs to be debated at the next meeting:</u>

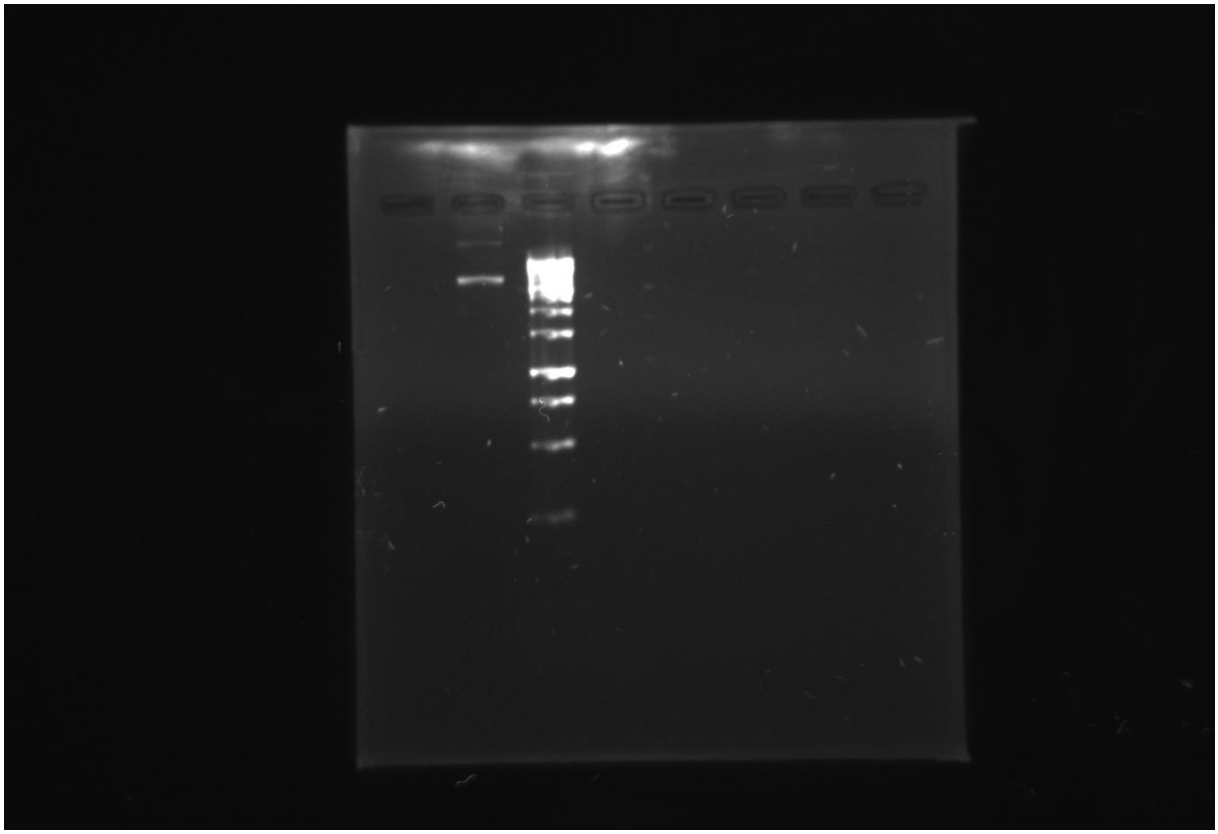
- Human Practice (Needs urgent kick in)
- Sponsorship (!!!)
- We need a designer for the wiki.
- Template for wiki is required.

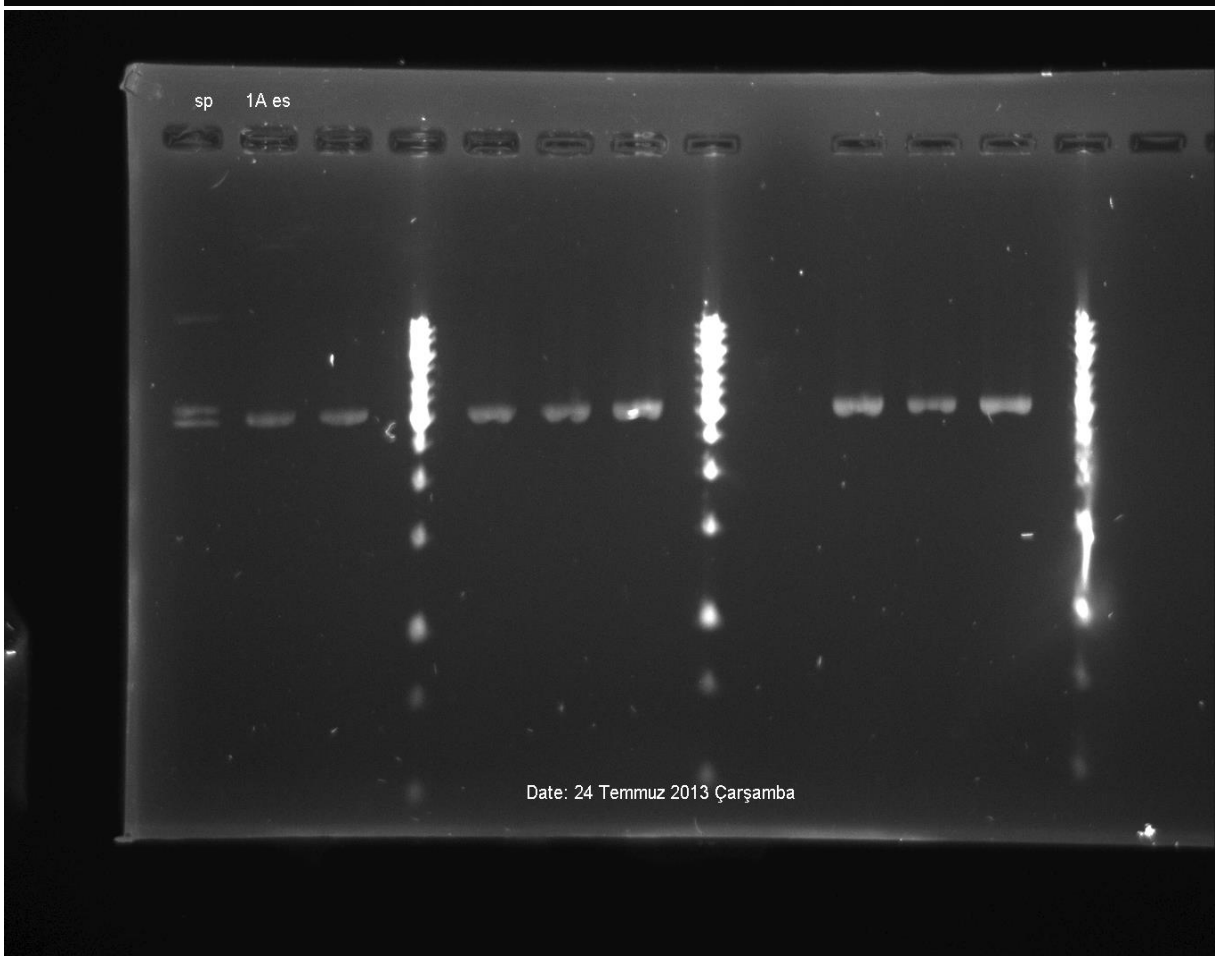


Parts were cloned to check ,via electrophoresis gel and all parts were found wrong


sp
1A es
1A xp
ladder
1B es
1B xp
2A es
ladder
2A xp
2B es
2B xp
Date: 24 Temmuz 2013 Çarşamba

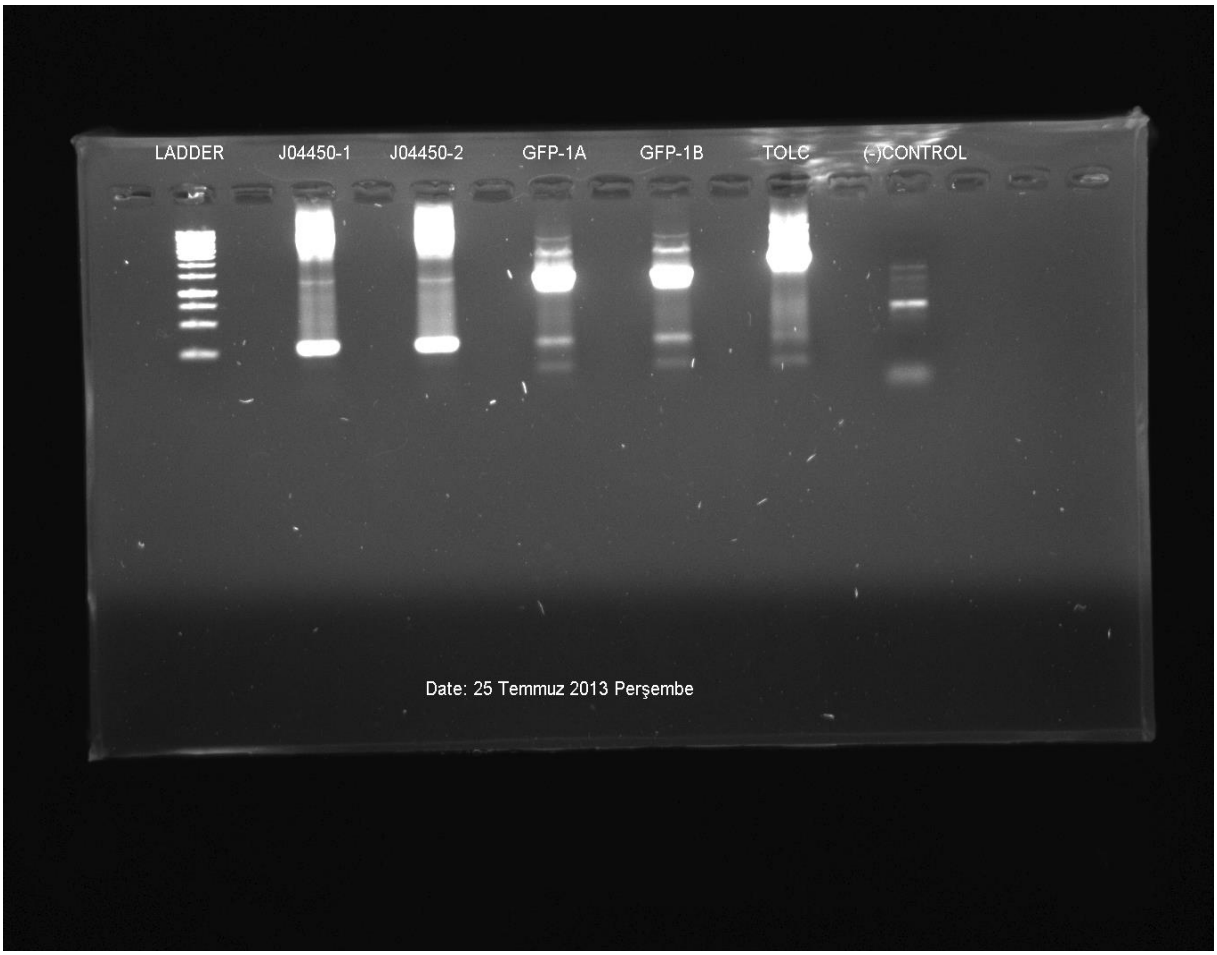




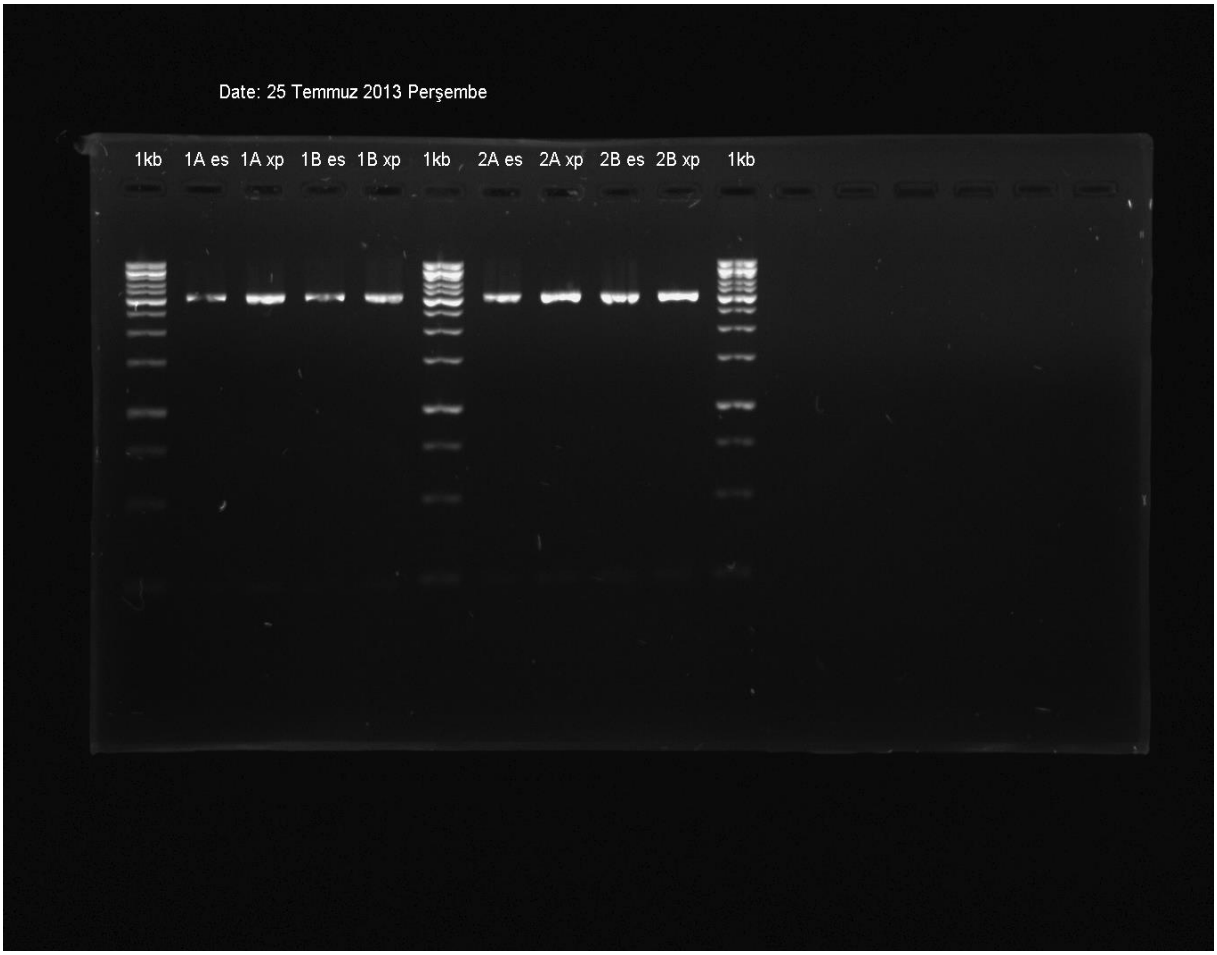


Parts were cloned to check ,via electrophoresis gel and all parts were found wrong

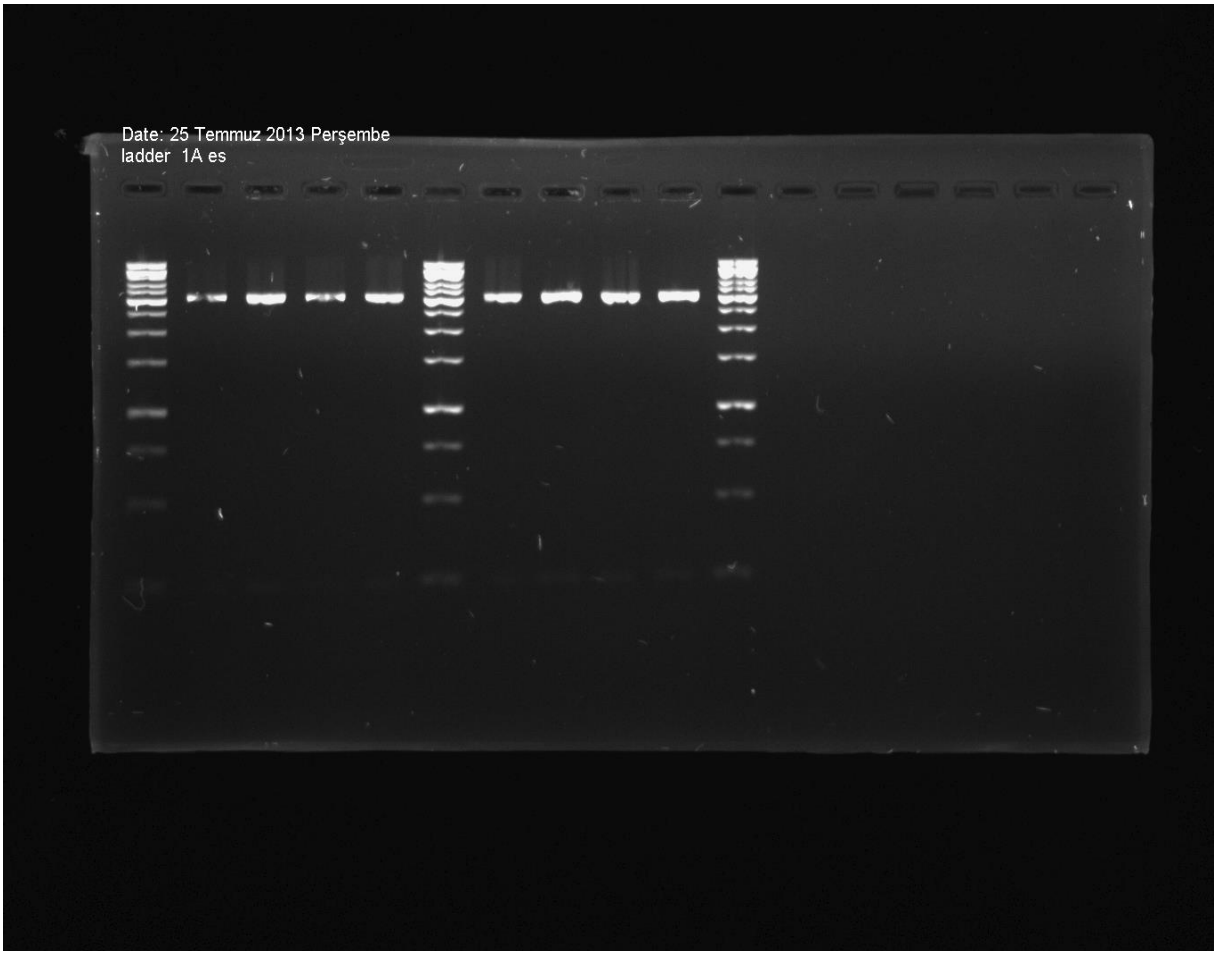
25.07.13



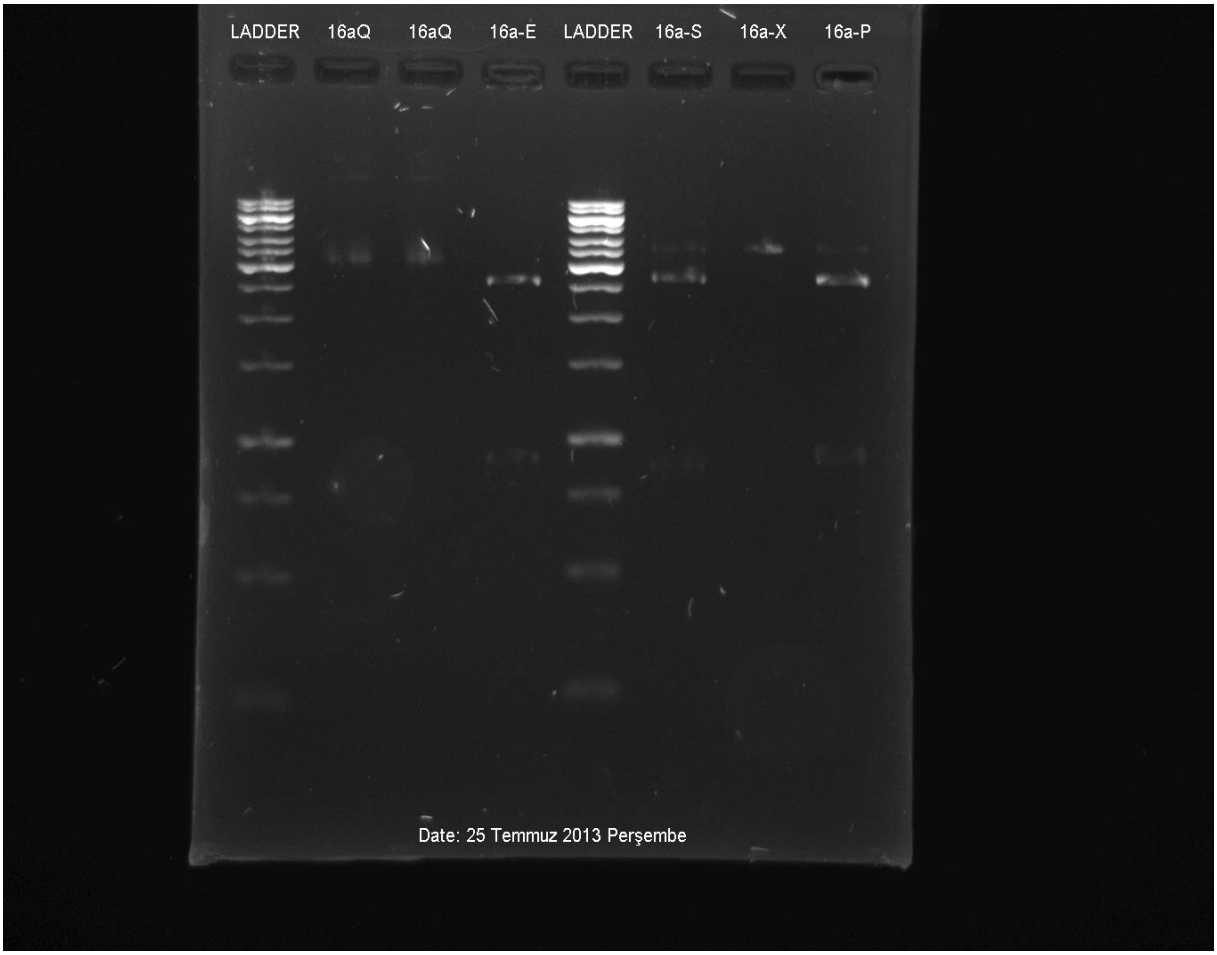
Parts were cloned pcr to check ,via electrophoresis gel and all parts were found wrong because (-) control was contaminated



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



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



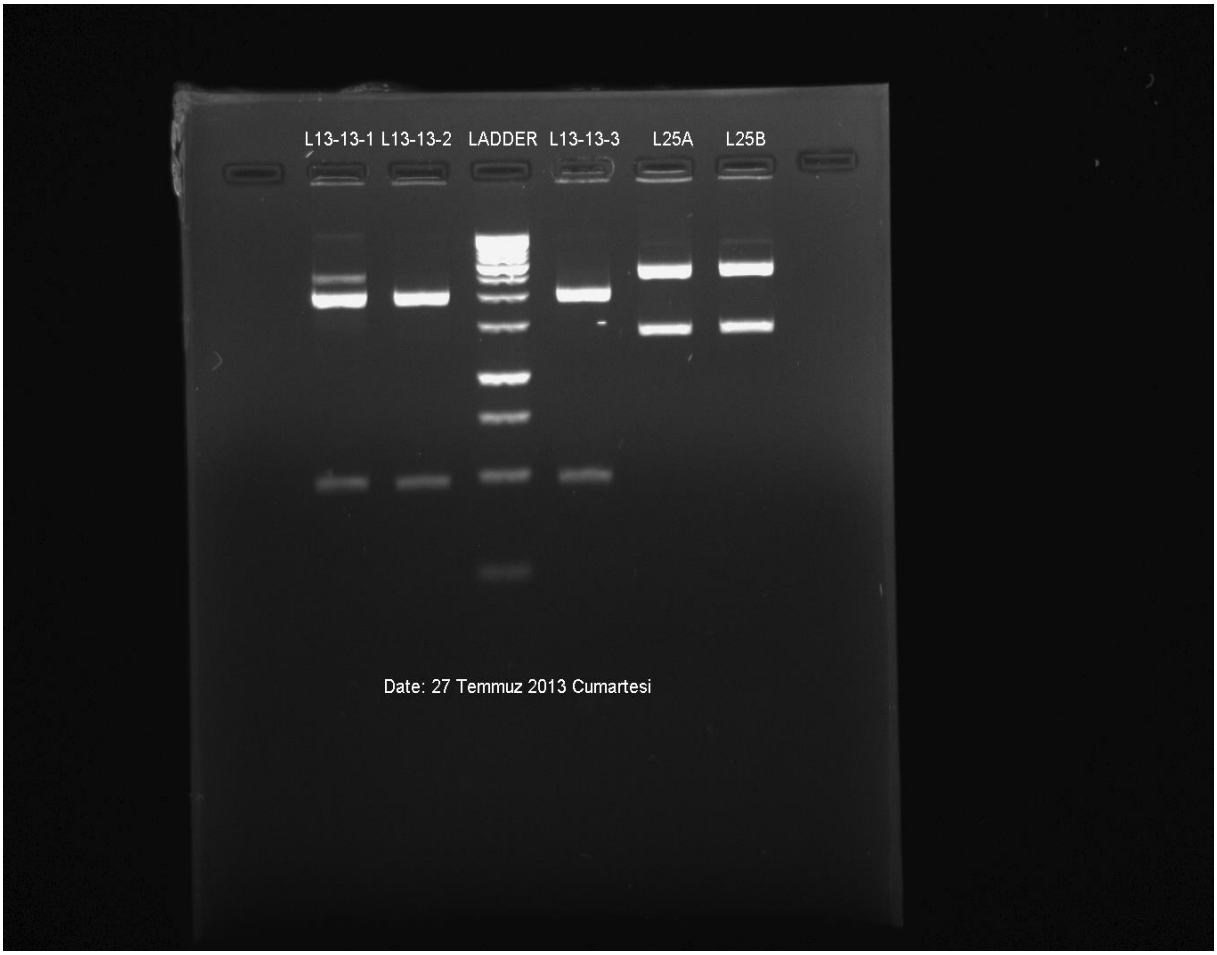
Parts were cloned to check ,via electrophoresis gel and all parts were found wrong

26.07.2013

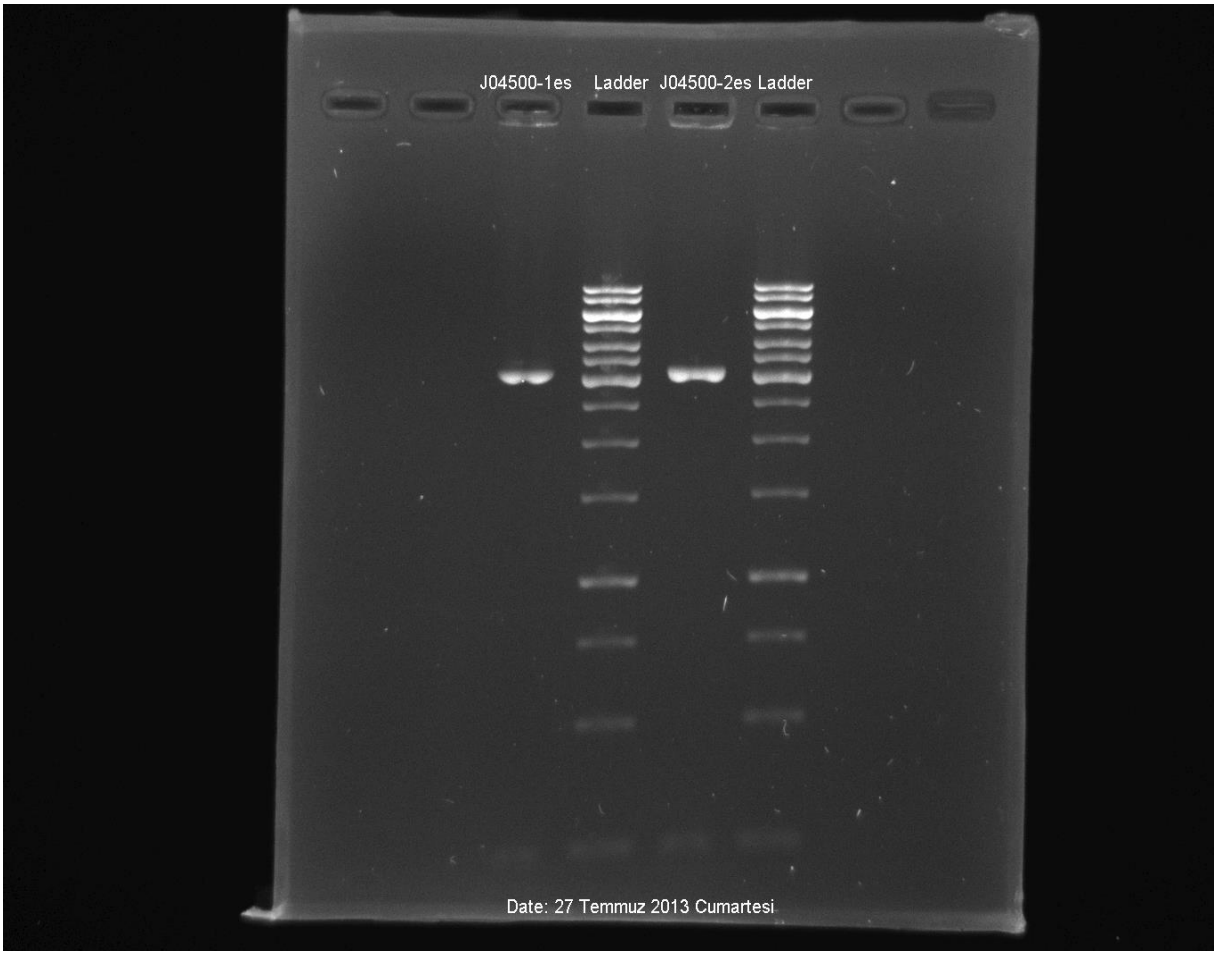
1. J04500 part was planned to be digested as ES, XP and SP; but again, it was believed that it was half-digested which the result of the gel indicated.
2. Because of this, the planned ligations could not been performed.
3. The liquid cultures of L32/L33 co-transformation and overnight ligation/16 C ligation parts were prepared.
4. The preparation of Nissle 1917 competent cell protocol was done; but it was unsuccessful to measure the OD value after 3 hour incubation. At last, it was diluted with additional LB to equal the final solution to 100 ml.
5. The parts including His tags were again isolated from bacterial cultures via sonication. It is planned to perform a Western blotting, unfortunately an unexpected error was occurred which resulted with the delay of the assay.
6. TolC and J04500 were copied via PCR and digested with correct enzymes in order to use them in the ligation protocol. After the gel extraction was done; the ligation was performed.
7. Our new genes were ordered from GeneScript.
8. Nissle 1917 plates were prepared in order to send them to microbiology lab to be investigated whether it contains our strain.

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

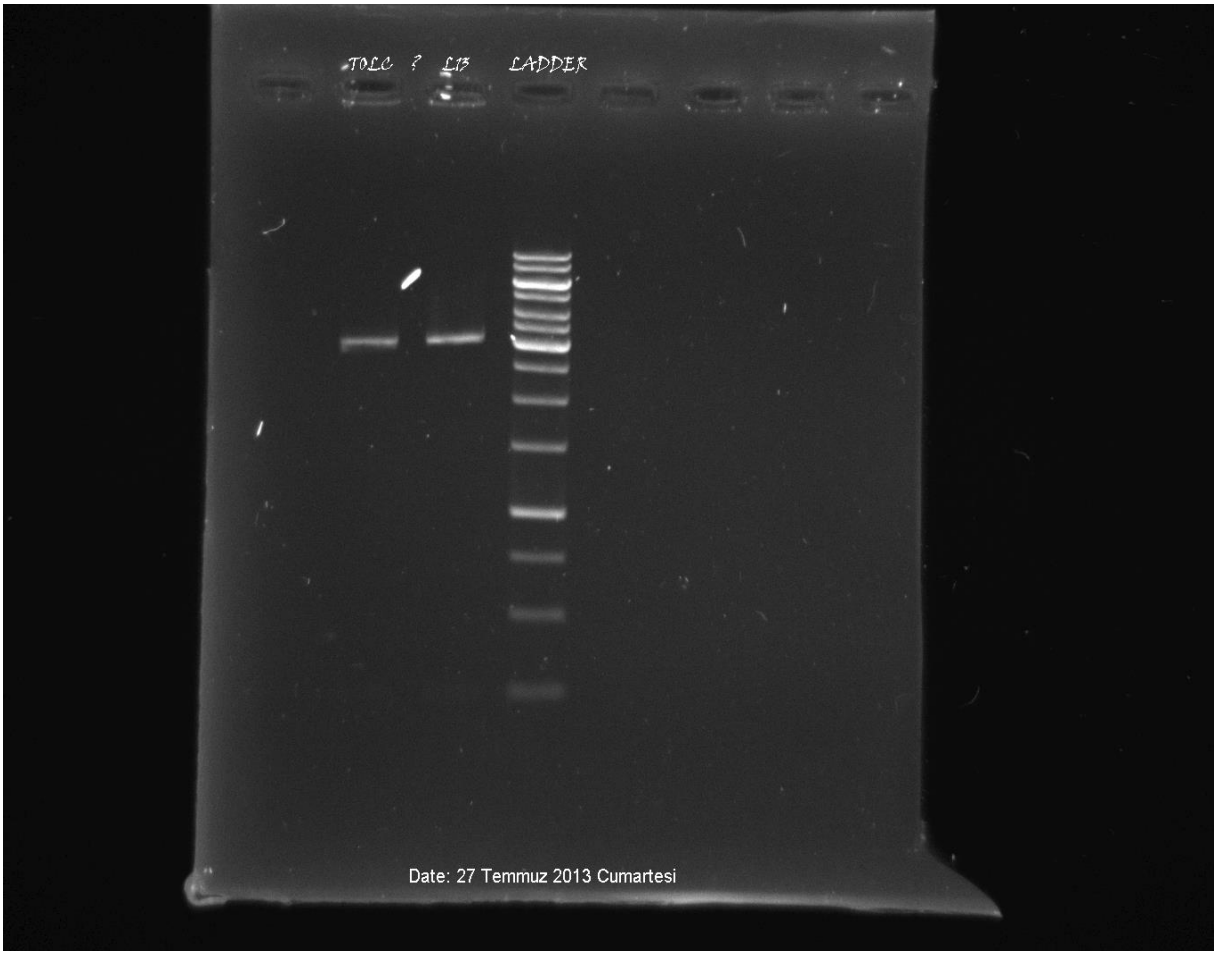
27.07.13



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

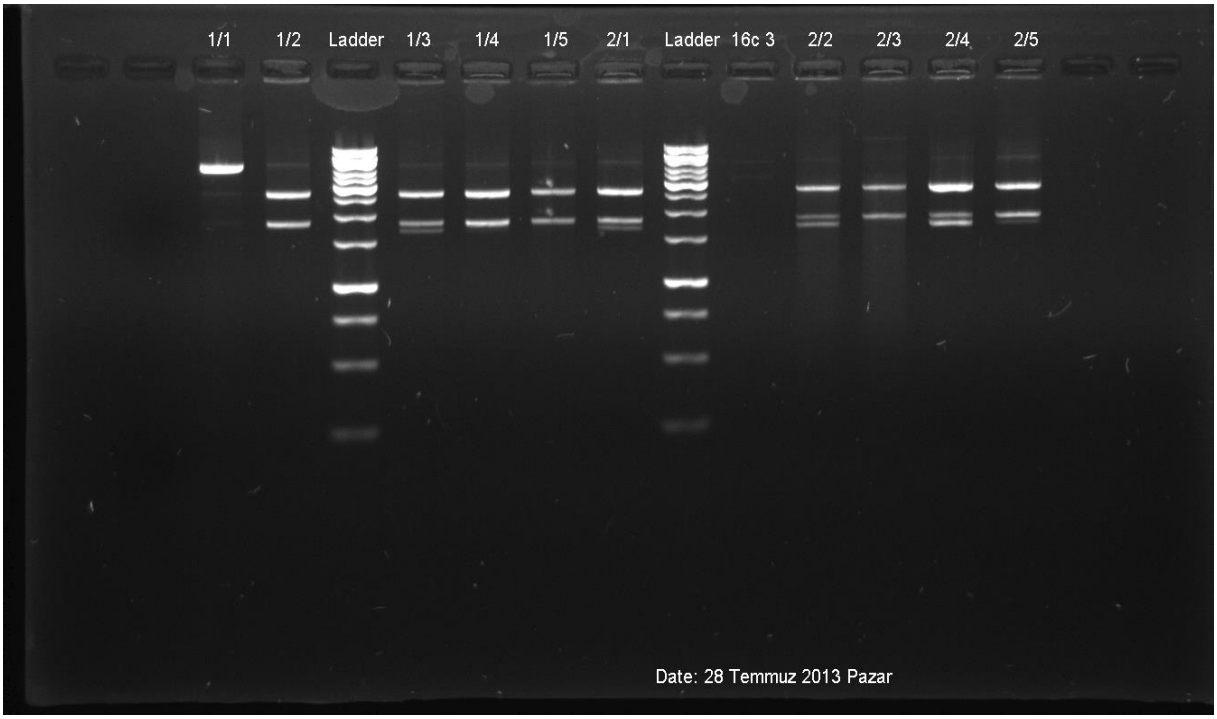


Parts were cloned to check ,via electrophoresis gel and all parts were found correct



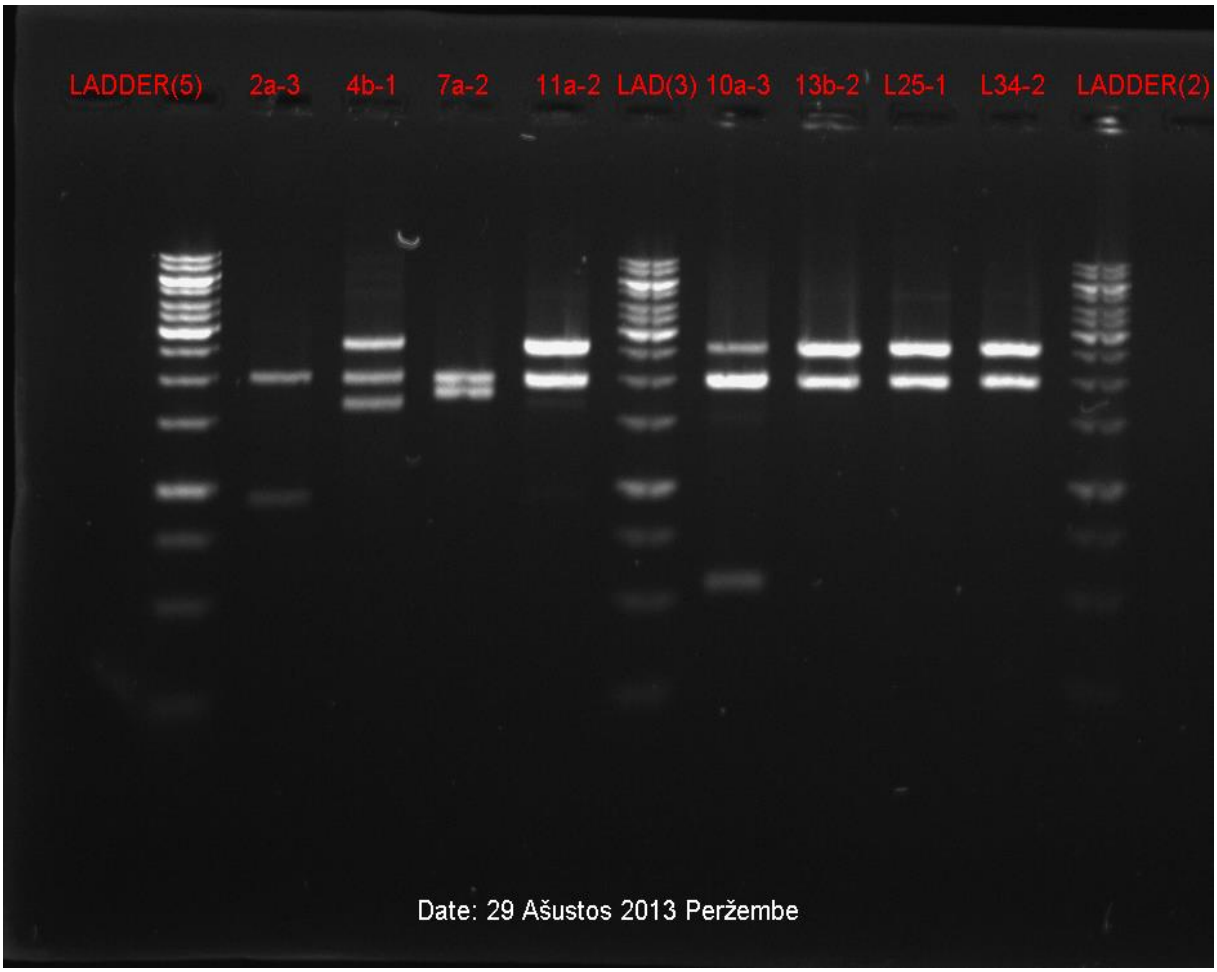
Parts were cloned to check ,via electrophoresis gel and all parts were found wrong

28.07.13



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

29.07.13



30.07.13

Date: 30th July 2013

Morning/Afternoon:

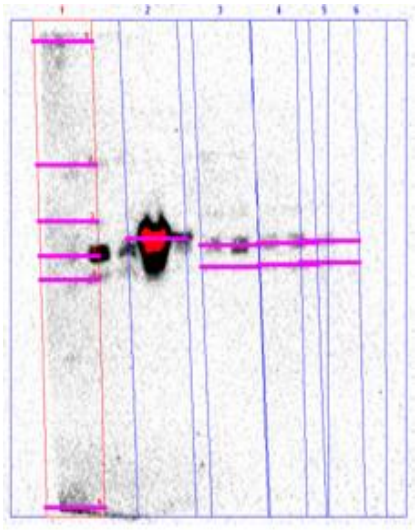
- The western blotting of isolated proteins was successfully accomplished today. Despite seeing the results, we had a little trouble commenting on it as all proteins were seen on the same level/line which was not as expected. After speaking with Omer Faruk Hoca we were informed on the use of 15% gel. According to him the reason why they appeared to be similar is due to using 10% gel so kDa difference couldn't be distinguished. We have therefore began another western experiment using the same protein stock! Safa prepared a 15% gel. (Aysenur,Safa, Nur, Aslihan)
- Mikail went into cell culture and prepared collagen gel.
- Aslihan has prepared AC agar petri-dishes and liquid culture of L8A7.
- Aysenur diluted AMP.
- Mustafa abi and Esin abla went to bilkent to get trained on immunofluorescent colouring.

To be done:

- Best modelling research: Safa
- Wiki templates: Aysenur

- Human Practice:
  - ❖ Cancer cell game
  - ❖ Short film
  - ❖ Ipad project presentation-live
  - ❖ Placebo
- Research on the amount of TAT-RFP, TAT-Apoptin that should be given to cancer cell: Nur

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



31.07.13

1) Repeat Western with degenerated & non-degenerated protein samples (gel-transfer-1st antibody incubation)
2) Liquid culture of Pind2-psb1C from ligation plate (aslihan) →2 falcons
3) OmpA (L38), TorA (L37), HlyA (L36) liquid culture (nihal, aysenur)
4) GFP (L8) Transformation with NEB10 (AC plate): **aim** test AC plate
   GFP (L8) Transformation with Nissle (Chl plate): **aim** test competent Nissle (nihal, aysenur, omer) → control & Nissle plate
5) Ligation product (Promoter-TolC) transformation (mikail, omer) →control & ligation plates
6) PCR & then, ligation product DNA isolation (safa)