

CyPet-SUMO1: Cell Culture, Bioinformatic Analysis, DNA digestion and PCR Checking

Introduction:

In the 1st lab we introduced plasmids encoding fluorescent fusion protein inside E. coli cells. pET28(b)-CyPet-SUMO1. SUMO stands for Small Ubiquitin-related MOdifier. It is a crucial protein modifier and contains amino acids. SUMO is a conjugating protein that will make it ready for attachment with the target protein. SUMO can be in reverse in conjunction with the lysin residue of the targeted protein. Makes it ready for targeting protein attachment. Let's say E2-SH will bind SUMO or Ubc-9 so it will get associated with that as a Sumocongulating enzyme. E2 is a conjugating enzyme. E2 will be separated SUMO is responsible to make E2 ready for conjugation. E3-ligase is the last enzyme that separates the activated residue and gets associated with lysin(target protein) and mediates sumoylation. E1 will associate first then E1 will be separated and then E2 will be associated and then E2 will be separated as well. As E3 is a ligase so it will do ligation of the protein sequence and do sumoylation inside the nucleus. E1 an activating enzyme grabs ubiquitin floating around. E3 is the ligating enzyme that grabs both the target protein and the ubiquitin-bound E2. The whole process is repeated again and again until the protein has a long ubiquitin chain to be recognized by the proteasome and get degraded.

Gel Electrophoresis applies an electrical pulse to cell charge and is a technique used to detect and separate DNA, RNA or protein molecules based on size and electrical charge. When an electric field is applied fragments migrate by the gel. DNA isolation by the right gene is amplified through bacterial cells and isolation of amplified DNA molecules from bacterial cells. Use the NCMI gene bank to verify the sequencing results of pET25b clones using CLC sequence and chromas. CLC sequence is used to sequence the basic bioinformatics analyses and chromas traces from the automated Sanger sequence. Chromas can freely trace simple DNA sequences. Purifying the plasmid DNA from bacterial cells will be based on the alkaline lysis of bacterial cells followed by DNA separation by silica is done by molecules that are binding to the silica surface in presence of salt. Bacterial cells come together to make a lysate medicine and the cells are broken down for boosting immunity to fight infections. In this experiment, lysate will be cleared for the sample to be ready for purification on the QIAprep silica membrane. QIAprep is designed for the isolation of plasmid that is used in molecular biology applications which

includes fluorescent sequencing and cloning. DNA gets absorbed because of buffers in the lysis and also because of the presence of a silica membrane. High-quality plasmid DNA is eluted from QIAprep with a Buffer of 50-100 μ l. Thus the DNA is now purified and used in a wide range of applications.

Denaturation is by melting the bonds between complementary hydrogen bases of the DNA strand. After the annealing of the primers, stable hydrogen bonds are formed when the primer sequence matches the template sequence. The final step is extension and elongation where dNTPs are added to the DNA template strand.

PCR is used to amplify genes and using this technique we can check if the gene was joined together as a chemical bond with the plasmid. Enzymes such as restriction enzymes can cut DNA and then can digest it to check from the plasmid if the insert is ligated inside the vector. This can be verified by automated DNA sequencing.

Material and Methods:

Transform Plasmid DNA pET28(b)-CyPet-SUMO1 into bacterial cells by electroporation:

1. An 0.6 ml Eppendorf tube from a range of 50 μ l pipette was filled up to 40 μ l and then put it in the ice and then take 1 μ l of plasmid DNA inside bacterial cells.
2. Electroporation cuvette is used to transfer the bacterial cells and plasmid DNA to perform electroporation at 1.8 kV
3. Use a pipette that can take up to 250 μ l of SOC into corvette by taransferring the bacterial cells with SOC medium into the Eppendorf tube used in step 1.
4. Put the bacterial cells at 37°C for 1 hr inside the incubator.
5. Turn on the bunsen burn and heat the middle side and let it from 90 degrees two times to get a decent shape that can fit inside the agar plate to mix. Then preheat the 10 cm agar plate in a 37°C incubator. On the warm agar, palate mix the transformation mixture and then incubate overnight.
6. Culture a single colony in LB liquid medium and the TA will post the 3ml of LB medium with kanamycin on canvas the next day.

Sequencing from NCBI gene bank:

1. Download the data from the canvas and download the CLC sequence viewer and chromas. CLC. Load all the CyPet-SUMO1 files on CLC. Copy and paste the given CyPet on to the CLC sequence. Assemble the CYPet-SUMO1 file as a standard sequence in word
2. Remove the Stop at the end of SUMO and add Sall and copy-paste the sequence from the online database. Yellow long given sequence of CyPet followed by green which is the Sall and the red is from the database: **CAAAGTCGAC****TCTGAC**
3. In the CLC sequence do the reverse complementary of all three reverse complementary sequences. Go to nucleotide analysis→ reverse complementary sequence and SAVE them so they will appear on the CLC data while navigating between different files.
4. Next we need to align the sequences with the standard given data. So there will be three sequences that we will be looking at i.e forward, standard, and reverse files. And then the expected results would be whether mutation(more or less), computer error, or pure sequence. Use Chromas to identify the peaks by entering the sequence in “Find sequence”. A full-colored square means the software has full confidence about the outcome and less colored square means that there is low confidence in the peak as it might get hidden under a much higher peak that will be of the following letter.

Design, Setup, and Procedure for the purification of Plasmid DNA from Bacterial Cells:

First we add 1.5ml of Bacteria into a 1.5ml Eppendorf tube from the three given samples then centrifuge the tube for 1min at 13,0000rpm. Repeat these steps again but dispose of the previous liquid. Add 250 μ l of suspension A1, and 250 μ l of buffer B1 incubate for 5 mins in room temp, and add 350 μ l of Neutralizing buffer N1 and spin them for 10 min at the same rpm while centrifuging. Transfer it to the DNA column and spin it for 1 min. Dispose of the flow through and add 500 μ l of wash Buffer spin it for 1min and transfer the column into a 1.5ml Eppendorf tube.

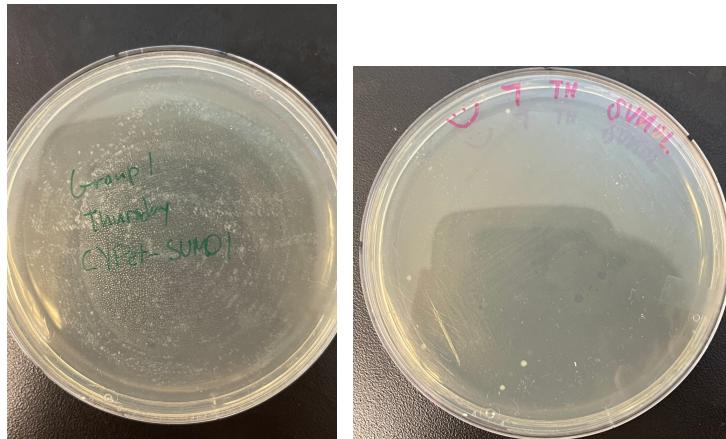
Add 50 μ l of ddH₂O and spin for a minute and what you then get is plasmid DNA. There will be 65 μ l of digestion mixed with buffer, nhel, not I, and ddH₂O. So prepare three 0.6ml Eppendorf tubes. Add 20 μ l of digestion mix and 30 μ l of DNA. Now incubate for 30 mins at 37C after that you will run the sample in 1% agarose gel and check the DNA under UV light. Dilute the DNA

template for mini-prep in a 0.6mL Eppendorf tube and add 1 μ l DNA and 9 μ l ddH₂O. In a 0.2mL PCR tube mix 49 μ l of PCR Master Mix and 1 μ l of diluted DNA. Use your pipet by pulling in and out to thoroughly mix them. A setup of PCR will be available under these conditions:

Step		Temperature (°C)	Time (min:sec)
1	Initial Denaturation	94	0:30
2	Denaturation	94	0:15
3	Annealing	54	0:30
4	Elongation/Extension	72	2:00
5	Cycling	Go To Step 2	25 times
6	Cooling/ Storage	4	30:00

Carefully load the reaction onto a 1% Agarose gel as guided by the TA using a pipette. The sequence will be available for analysis within a week.

Results and Discussion:



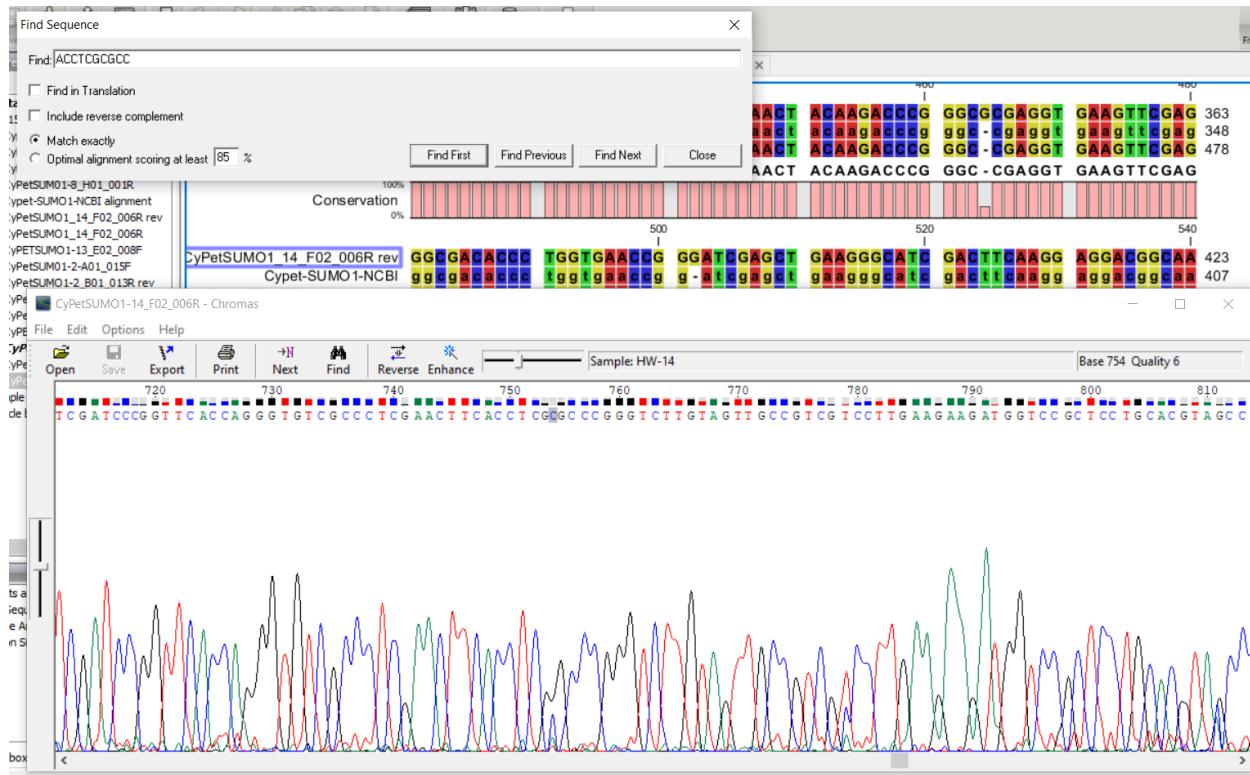
The sample is quite spread out and there are multiple dots across the agar plate. When comparing it to the neighboring group's cultured colonies ours has gone under more growth as there is abundant growth visible therefore higher rate for transformation hence this plate will be the ideal colony when compared to the others for several factors such as physical condition active growth, health, temperature, density, concentration.



As it is visible that most of the above does not match so this is a junk DNA (015F and 0.13R rev)



The following combination has two mutations that appear in the CLC sequencer but when checking with the chromatogram we find out that the peak for C which is actually the reverse complimentary does occur but it is just hidden because the next one C has a much higher peak. So this combination of sequence(008F and 006R rev complimentary) is pure it only has a software error. An example that was checked: is “AGCTCGATCC” There is no reverse counting in Chromas and that is why we have to check it by doing a complimentary when trying to find the mutation.

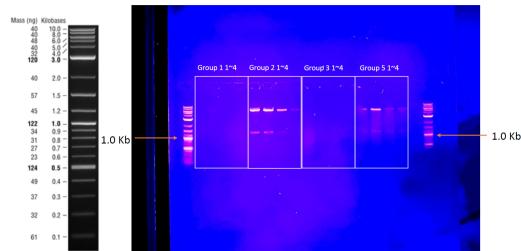


Another example from the same sequence combinations there is a small clear peak that is hidden under the nearby G's for the missing C in the following reverse complementary search “ACCTCG**C**GCC”. Again a software error and thus this combination of sequences can be called a pure sequence if we disregard the computer errors.

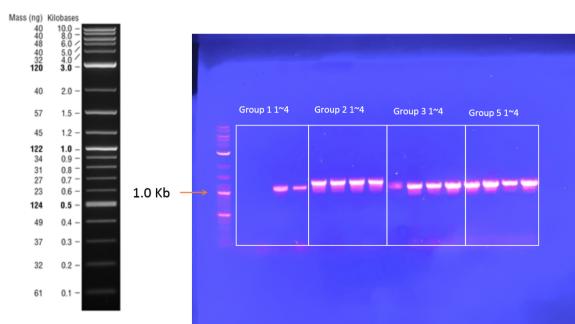


The above actually has a mutation as there is an absent T in all the files but the reverse complimentary sequence in CLC shows that the T was present when confirming with Chromas we can conclude that the T never existed in 001R. So this sequence has mutations.

Digest checking 1



PCR checking 1



The visible lights represent the top and bottom lights where the top one is undigested and the lower one is the genetic material.

DNA extraction happened from E.coli which appears purified and the length is 1kB. For Digest checking there does not appear to be a frame for Group 1 when compared to group 2 but when looking at the top there appears to be some rise of violet color light indicating that the experiment sample did rise up the ladder.

When looking at the PCR there appear to be two lights indicating that PCR did take place. We see two lanes confirming the presence of Cy-Pet-SUMO1. The bands in each lane depict the presence of the plasmid of 1kB length.

Discussion, Summary, and Conclusion:

In Lab1 we introduced E.Coli cells in a process of transformation. An Antibiotic called Kanamycin was there in the agar plate where culturing happened and it was successful as per the results indicate. Then in lab 2 where we used Bioinformatics analysis tools by using CLC Sequence Viewer and Chromas to understand and observe the alignments, reverse complimentary, and mutation identification between three files with one constant standard file. Some had fewer mutations, some had more mutations, some modifications were not actually present but just a software error, and one was a junk sequence. In lab 3 we obtained results for the DNA digestion and PCR checking for the visible bands where the top band shows the undigested material and the lower shows the genetic material.

Works Cited:

1. Laboratory 1: Transform pET28(b)-CyPet-SUMO1 or pET28(b)-Ypet-Ubc9 fusion genes into Top10 E. coli bacteria., UCR, BIEN 155 Lab Manual, 2022
2. Purify Plasmid DNA from Bacterial Cells and Submit for Sequencing, UCR, BIEN 155 Lab Manual, 2022
3. Verify the sequencing results of clones in comparison with genes from the NCBI gene bank, <https://www.ncbi.nlm.nih.gov/>, UCR, BIEN 155 Lab Manual, 2022