

Project Report on

**Engineering a mammalian expression construct of**

**SSBP1 and SSBP1 $\Delta$ MTS**

Submitted by

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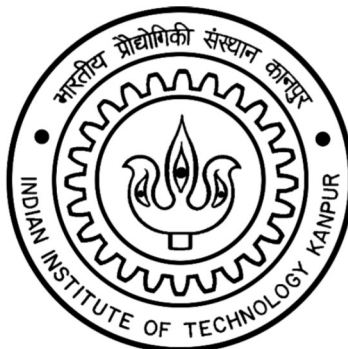
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## Acknowledgement

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Bearing in mind previous I am using this opportunity to express my deepest gratitude and special thanks to the **Prof. S Ganesh, Department of Biological Sciences and Bioengineering, IIT- Kanpur** who in spite of being extraordinarily busy with his duties, took time out to hear, guide and keep me on the correct path and allowing me to carry out my project at their esteemed organization and extending during the training.

I express my deepest thanks to **Dr. Rashmi Parihar and Miss Saloni Agarwal** for taking part in useful decision & giving necessary advices and guidance and arranged all facilities to make it easier. I choose this moment to acknowledge their contribution gratefully.

I perceive as this opportunity as a big milestone in my career development. I will strive to use gained skills and knowledge in the best possible way, and I will continue to work on their improvement, in order to attain desired career objectives. Hope to continue cooperation with all of you in the future.

**I would also like to thank my family, especially my mother for her support.**

Thank you for everything.

## **Introduction**

### **About SSBP1**

SSBP1 is a single-stranded DNA (ssDNA)-binding complex involved in the maintenance of mitochondrial genome stability and plays an important role in mitochondrial DNA replication, maintenance of mitochondrial DNA content (Pokrzywinski KL et al.; 2016) and plays a significant role in mitochondrial biogenesis. Since mitochondria is the powerhouse of the cell so it is important to maintain the healthy pool of mitochondria. Mitochondrial defects are reported in absence of SSBP1 protein. It is also reported that SSBP1 acts as a tumour suppressor (Li Q et al.; 2017). Since dysfunction of SSBP1 can cause the free radical generation (Wang Y et al.; 2017) and excessive free radicals is reported to be the cause of various neurodegenerative disorders which suggests SSBP1 could play a role in neurodegenerative disorder. So to understand the functional role played by SSBP1 it is important to overexpress this protein in any mammalian system so for that we need to engineer a construct in which SSBP1 is cloned downstream to the mammalian expression promoter.

Molecular Cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms.

In this, the DNA to be cloned is obtained from an organism of interest, then treated with enzymes in the test tube to generate smaller DNA fragments. Subsequently, these fragments are then combined with vector DNA to generate recombinant DNA molecules. The recombinant DNA is then introduced into a host organism. This will generate a population of organisms in which recombinant DNA molecules are replicated along with the host DNA. Because they contain foreign DNA fragments, these are transgenic or genetically modified microorganisms (GMO). This process takes advantage of the fact that a single bacterial cell can be induced to take up and replicate a single recombinant DNA molecule. This single cell can then be expanded exponentially to generate a large number of colonies of bacteria, each of which contain copies of the original recombinant molecule. Thus, both the resulting bacterial population, and the recombinant DNA molecule, are commonly referred to as "clones".

**Primary Aim:** To successfully clone Single-stranded DNA binding protein 1 (SSBP1)

**Secondary Aim:** To understand the common methods and techniques required.

# Objective

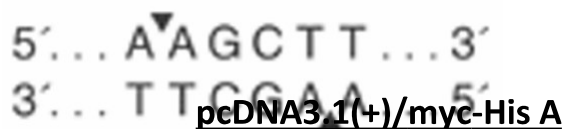
As discussed, to understand the significance of SSBP1 we need to engineer a construct in which SSBP1 will be downstream to the mammalian expression promoter which is done by molecular cloning.

Molecular cloning is a complex procedure consisting of many steps:

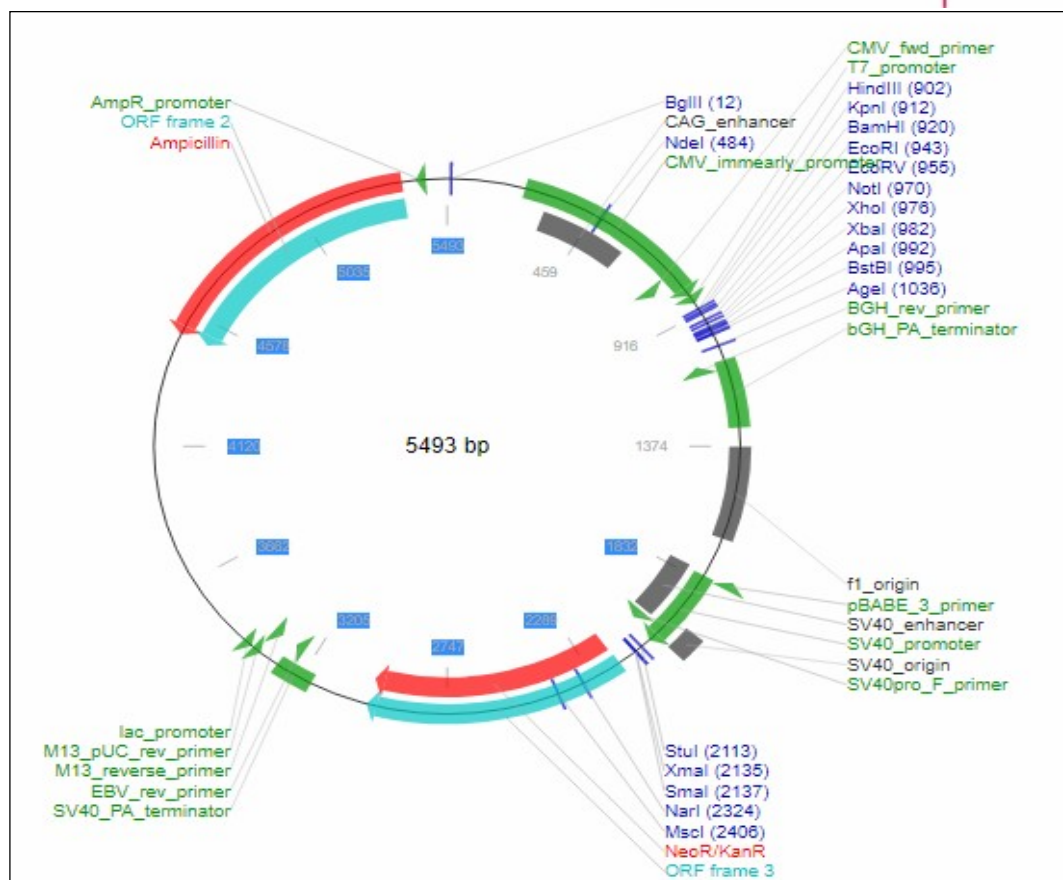
- HeLa Cells → RNA isolation → RT PCR → cDNA → PCR → SSBP1 / SSBP1  $\Delta$ MTS amplification → Restriction digestion (1)
- pcDNA transformation → plasmid isolation → Restriction digestion (2)
- Digested product (1) and (2) → Ligation → Transformation → Plating → Screening

## Restriction Enzymes:

### Hind III :



### Xba I



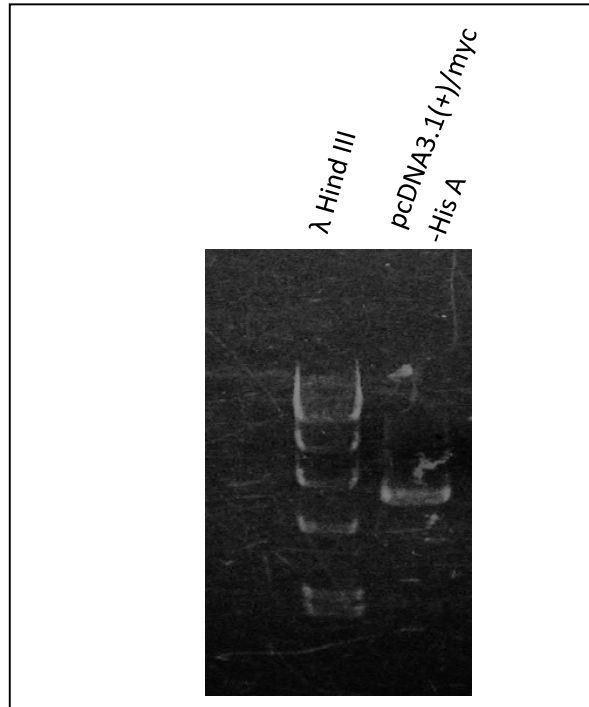
## Results

The cloning of SSBP1 consist of various steps:

### **Plasmid isolation:**

The vector plasmid (pcDNA3.1(+)/myc-His A) was isolated and Gel electrophoresis was done on 1.5% Agarose Gel.

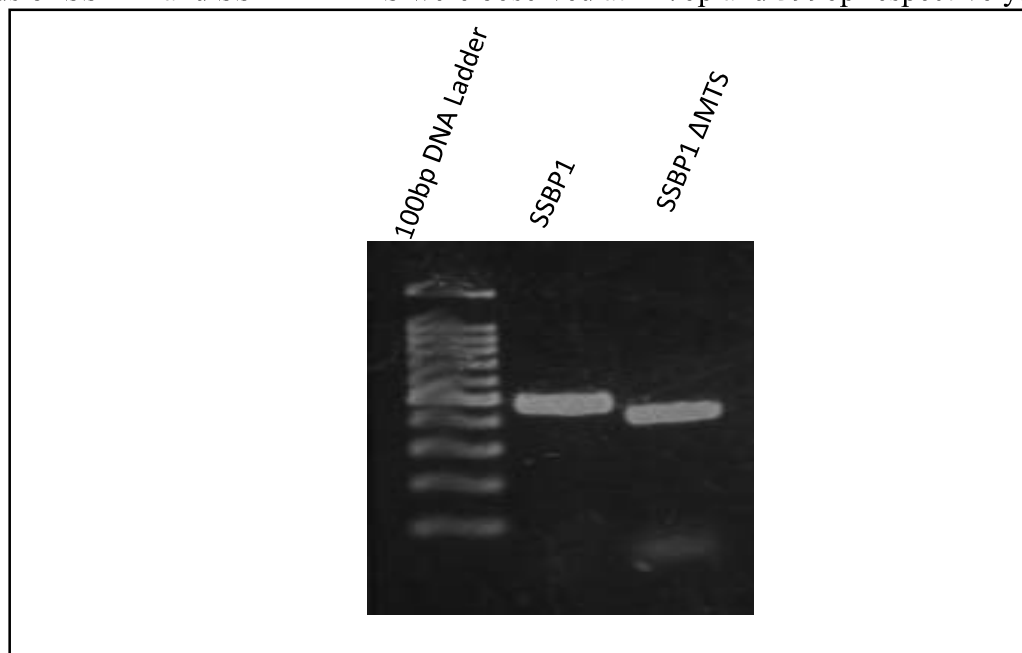
The band was observed between 3rd (6557bp) and 4th (4361bp) band of  $\lambda$  Hind III marker, which is our vector (6631bp).



### **RNA Isolation and SSBP1 and SSBP1 $\Delta$ MTS gene amplification:**

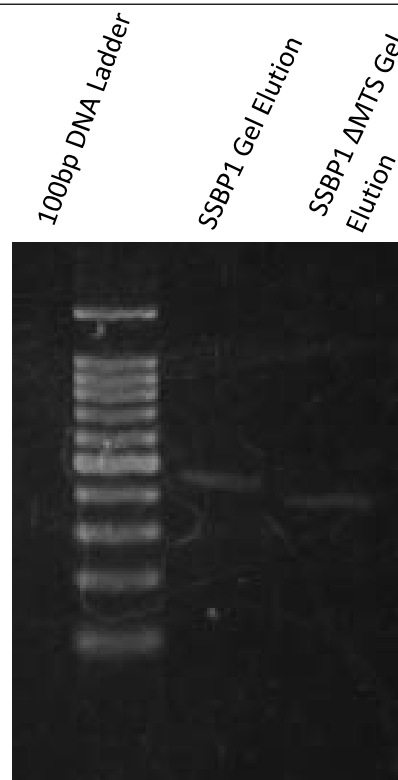
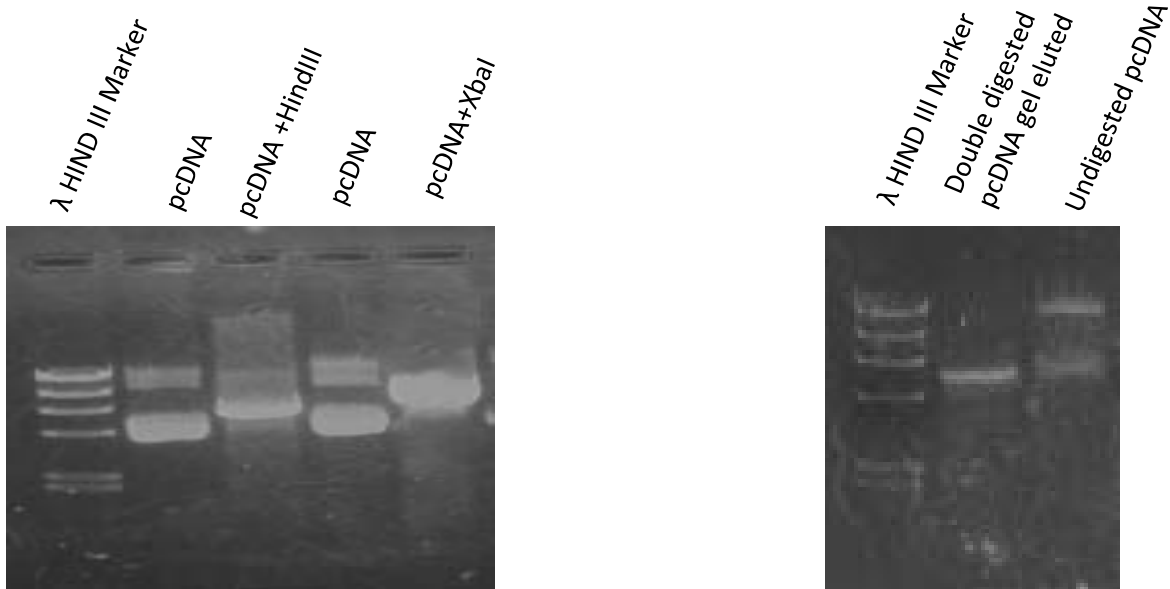
Since HeLa cells are human cancerous cells so to get the gene copy of SSBP1 RNA from human origin RNA were isolated from HeLa cells. Further cDNA, was obtained from which was amplified and product was run through gel electrophoresis (1.5% agarose gel).

Bands of SSBP1 and SSBP1  $\Delta$ MTS were observed at 447bp and 399bp respectively.



### **Restriction Digestion and Gel Elution:**

The PCR product of SSBP1 and SSBP1  $\Delta$ MTS and vector backbone pcDNA was digested using Xba I and Hind III and the digested product were run through gel electrophoresis and their respective bands were obtained. After the digestion, gel elution was done of the products obtained and eluted products were run through gel electrophoresis and their bands were observed

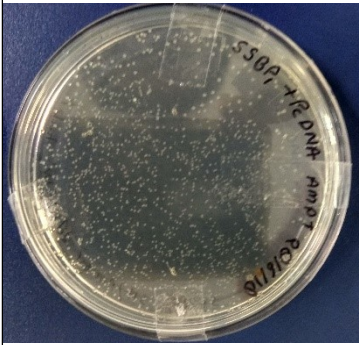


**Screening:**

After the digestion ligation was done and then transformation of the ligated products was done. Afterwards plating was done and then they were isolated at 37C for a day and then they were screened for positive colonies for which 5 random colonies were picked, colony PCR was done and they were run through gel electrophoresis. The screening showed four positive result out of five for SSBP1 and SSBP1 $\Delta$ MTS. **Thus, cloning of SSBP1 and SSBP1 $\Delta$ MTS was successfully done.**

### Screening

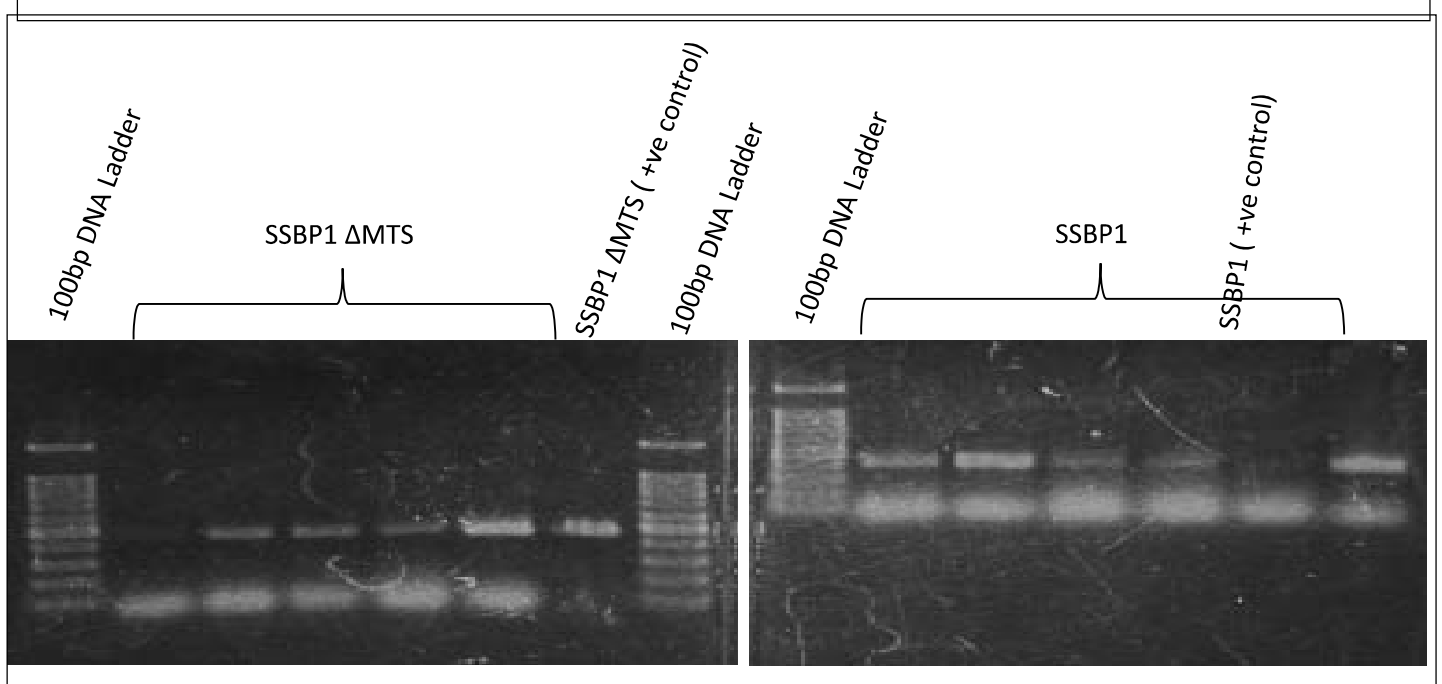
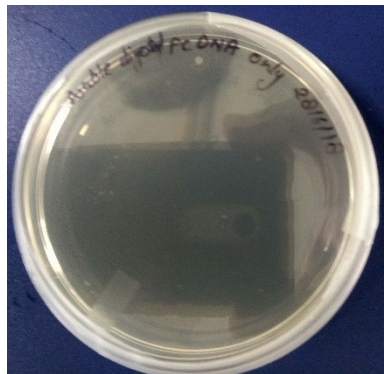
SSBP1



SSBP1 $\Delta$ MTS



pC DNA



### Materials and Methods



### **RNA Isolation**

1. The medium(supernatant) was carefully removed and 1ml TRIzol was added and left for 5 minutes.
2. It was then mixed and transferred into a 1.5 ml Eppendorf tube and left for 5 min.
3. 200µL of chloroform was added and tube was shaken vigorously for 20 seconds and left at Room temperature for 5 minutes.
4. It was then centrifuged at 12000 RPM for 15 minutes at 4° C.
5. At this point, there were three layers in each tube:  
**Top layer:** aqueous  
**Middle layer/interphase:** white precipitated DNA  
**Bottom layer:** pink organic phase
6. The aqueous phase was taken out which was fresh RNA, without disturbing other layers.
7. 500 µL isopropanol was added and solution was mixed and left at RT for 10 min.
8. It was then centrifuged at 12000 RPM for 10 min at 4° C and 1 mL chilled alcohol (75%) was added.
9. Then it was centrifuged at 7500 RPM for 5 minutes and wash was discarded.
10. 75% alcohol was added and RNA pellet was stored at -80° C for further use.

### **Plasmid Isolation**

1. The culture was vortexed and transferred to 2 mL Eppendorf tubes.
2. Then they were centrifuged at 12000RPM for 1 minute and supernatant was removed.
3. 200 µL resuspension buffer was added and was vortexed for 1 minute.
4. 200µL freshly prepared Lysis buffer was added very slowly and slowly inverted for 5-6 times.
5. 350µL neutralization buffer was added and tube was inverted for 5-6 times.
6. Then it was centrifuged for 10 minutes for 12000 RPM.
7. The supernatant was taken and transferred to spin-win columns which were incubated earlier with QBT.
8. Then they were centrifuged at 12000 RPM for 1 minute, the flow through was removed.
9. 700µL wash buffer was added and then they were centrifuged at 12000RPM for 1 minute and flow through was removed.
10. Then they were centrifuged for 2 minutes at 12000RPM and flow through was removed.
11. Then the columns were transferred to 2 mL Eppendorf and 80µL elution buffer was added and incubated for 30 minutes.
12. Then they were centrifuged at 12000RPM for 2 mins and the plasmids were stored at -20°C.
13. Check on 1.5 x Agarose gel.

#### **Preparation of silica column:**

1. The column was taken and 700µL of QBT was added and left for 10 minutes.
2. Then they were centrifuged at 12000 RPM for 1 min and flow through was discarded.

#### **Lysis Buffer: For 2mL**

10% SDS : 200µL | 2 N NaOH : 200µL | ADW : 1600µL

#### **Composition of other buffers:**

**Resuspension buffer:** 50mM Tris HCl, 10mM EDTA, RNase

**Neutralisation buffe:** 4 M Guanidine Hydrochloride, 0.5M Potassium acetate

### **Transformation**

1. Competent cells (*E. coli* DH5α) were taken and were placed on ice (to avoid temperature stress).

2. 1.5 mL Eppendorf tube was taken with about 500ngDNA (6μL).
3. 60μL of competent cells were added to the tube and mixed slowly to avoid any stress.
4. Then tube was incubated on ice for 20 minutes.
5. Heat shock was given for 90 seconds at 42°C.
6. The tube was again placed on ice for 20 minutes.
7. 600μL Lysogeny Broth was added and tube was kept 37°C on shaking incubator at 90 RPM for 1 hour.
8. Then it was centrifuged at 12000 RPM for 1 minute.
9. The supernatant was removed but small amount was left.
10. Then it can either be plated or incubated into Lysogeny Broth for overnight.
11. For Incubate: 10 ml LB + 1 ml Ampicillin.
12. For Plating: 100ml ADW + 1.5 Agar + 2.5 LB → autoclave → Add ampicillin and Cool at RT → Do plating.

### **Restriction Digestion**

For 20μL using Hind III and Xba I:

DNA	1μg	:	5μL
Enzyme	1 Unit	:	.1 μL for each
Buffer (Tango Buffer)	10x → 1x	:	1μL
ADW		:	13.8μL

- Incubate at 37 °C for overnight.
- Check on 1x Agarose gel.

### **DNA Precipitation**

1. Add 1/10<sup>th</sup> of CH<sub>3</sub>COONa in total volume of DNA, 5μl for 50 μL.
2. 2μL Glycogen (stored at -20°C) was added and the tube was vortexed.
3. 500μL absolute ethanol was added and mixture was left at -20°C for half an hour.
4. Then it was centrifuged at 12000 RPM for 30 minutes at 4°C and supernatant was discarded.
5. 500μL of 70% Ethanol was added and then it centrifuged for 10 minutes at 12000 RPM.
6. The alcohol was aspirated and incubated for 20 minutes and DNA was dissolved in 50μL 1xTE.

### **Gel Elution**

1. The gel favourable part of gel was taken out.
2. The mass of 1.5 ml Eppendorf tube was noted and the gel was transferred into it and mass was again noted, and mass of gel was calculated.
3. 200μL of Binding buffer was added per 100mg of Gel and gel was dissolved in buffer using dry bath at 50°C.
4. The solution was transferred into spin win column and incubated at 15 minutes and was centrifuged at 12000 RPM for 1 min.
5. The flow through was poured again in column and centrifuged for 1 minute at 12000RPM and this step was repeated for 3 times.
6. At last the flow through was discarded and 700 μL Wash buffer was added and centrifuged at 1 minute for 12000 RPM and flow through was discarded.
7. Then it was centrifuged at 12000RPM for 2 minutes.
8. The column was transferred into 2 ml Eppendorf and then eluted with 50 μL ADW and incubated for 30 min. Then it was centrifuged for 1 min at 12000RPM and DNA was collected.

### **Ligation**

For 20  $\mu$ L Reaction:

		SSBP1	SSBP1 $\Delta$ MTS
Vector DNA	13 ng	5 $\mu$ L	5 $\mu$ L
Insert DNA	calculated	3 $\mu$ L	3 $\mu$ L
Water		11 $\mu$ L	11 $\mu$ L
T4 DNA Ligase		1 $\mu$ L	1 $\mu$ L

- Then the reaction mixture was left at 16C for overnight.
- Then it was dry heated at 65C for 10 minutes.
- Then it was transformed and plated.

### Polymerase Chain Reaction

**Reaction Mixture for 20 mL RT PCR:**

RNA	5 $\mu$ L
Primer	1 $\mu$ L
RT enzyme	1 $\mu$ L
RiboLock	0.5 $\mu$ L
dNTP (10mM)	2 $\mu$ L
Buffer	4 $\mu$ L
ADW	6.5 $\mu$ L

**Thermo regulation for RT PCR:**

25C	5 minutes
42C	90 minutes
70C	5 minutes
4C	$\infty$

**Reaction Mixture for 20  $\mu$ L PCR:**

cDNA	2 $\mu$ L
Forward Primer	0.5 $\mu$ L
Reverse Primer	0.5 $\mu$ L
dNTP(2mM)	1 $\mu$ L
<i>Taq</i> Enzyme	0.1 $\mu$ L
Buffer	1 $\mu$ L
ADW	4.8 $\mu$ L

**Thermo Regulation for PCR:**

95C	5 minutes	} X 34
95C	30 minutes	
60C	30 minutes	
72C	1.5 minutes	
72C	5 minutes	
12C	$\infty$	

## **Extra Methods and techniques Learned**

### **SDS- PAGE**

1. Stand was settled up and the Resolution buffer was made and poured.
2. When the buffer was solidified, stacking buffer was made and poured and slit maker was inserted.
3. Loading dye was added into the protein lysate and protein lysate were given water bath for 5 minutes.
4. Then the protein lysate were loaded and transfer buffer was poured into SDS-PAGE.
5. SDS-PAGE ran for about 1.5 hours at 30mA.
6. Then western blotting was done and blot was prepared.

Resolution Buffer: for 15 mL 12%

ADW	5.9ml
30% Acrylamide mix	5.0 mL
1.0 M Tris pH 8.8	3.8mL
10%SDS	0.15mL
10% Ammonium persulfate	0.15mL
TEMED	0.006mL

Stacking Buffer: 8 mL

ADW	5.5mL
30% Acrylamide mix	1.3mL
2.0 M Tris pH 6.8	1.0mL
10%SDS	0.08
10% Ammonium persulfate	0.08
TEMED	0.008

Transfer Buffer: for 800 mL

ADW	640mL
Methanol	160mL
Glycine	11.2g
Tris Base	2.4g
SDS	0.8g

### **Developing Blot**

1. The blot was taken from the transfer assembly and washed with 1xTBST for 5 minutes.
2. Then it was blocked in milk blocking for 1 hour at 37C at 34 RPM.
3. Then blot was treated with Tubulin for 1 hour at room temperature.
4. Then it was washed with 1xTBST for 5 minutes for 2 times and one time with milk blocking for 5 minutes.
5. Then the blot was treated with 2<sup>nd</sup> anti-body for 1 hour at room temperature.
6. Then it was washed with 1x TBST 2 times for 7 minutes and one time with 1xTBS.
7. Then the blot was developed.

#### **1x TBST: 1L**

100 ml 10xTBS + 900 mL ADW → 1x TBS

1xTBS + 1 ml Tweek20 → 1x TBST

#### **5% Milk Blocking:**

5g Milk Blocking

100mL 1xTBST

### Designing Primer

>**NM\_001256510.1:436-882 Homo sapiens single stranded DNA binding protein 1 (SSBP1), transcript variant 1, mRNA**

**ATG TTT CGA AGA CCT GTA TTA CAG GTA CTT CGT CAG TTT GTA AGA CAT** GAG TCC GAA  
ACA ACT ACC AGT TTG GTT CTT GAA AGA TCC CTG AAT CGT GTG CAC TTA CTT GGG CGA  
GTG GGT CAG GAC CCT GTC TTG AGA CAG GTG GAA GGA AAA AAT CCA GTC ACA ATA TTT  
TCT CTA GCA ACT AAT GAG ATG TGG CGA TCA GGG GAT AGT GAA GTT TAC CAA CTG GGT  
GAT GTC AGT CAA AAG ACA ACA TGG CAC AGA ATA TCA GTA TTC CGG CCA GGC CTC AGA  
GAC GTG GCA TAT CAA TAT GTG AAA AAG GGG TCT CGA ATT TAT TTG GAA GGG AAA ATA  
GAC TAT GGT GAA TAC ATG GAT AAA AAT AAT GTG AGG CGA CAA GCA ACA ACA ATC ATA  
GCT GAT AAT ATT ATA TTT CTG AGT GAC CAG ACG AAA GAG AAG GAG TAG

**Yellow highlighted region** is mitochondrial targeting sequence (MTS).

Forward primer 5' **AAGCTT**GCCATGTTTCGAAGA3' (Hind III enzyme)

Reverse primer 5' **TCTAGA**CTCCTTCTCTTTCGT3' (Xba I)

SSBP1 ΔMTS

Forward primer 5' **AAGCTT**GCCATGGAGTCCGAAACA3'

**Green highlighted regions** are restriction sites.

### Vector

>**pcDNA3.1(+)/myc-His A**

GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCA  
TAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAAT  
TTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGT  
TTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTA  
ATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTA  
CGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTA  
TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA  
CTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGAC  
GGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTA  
CATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTG  
GATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATGACGTCAATGGGAGTTTGT  
TGGCACCAAAATCAACGGGACTTTCGAAGTGTCTGTAACAACTCCGCCCCATTGACGCAAAATGG  
GCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCAC  
TGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGTT**AAGCT**  
**T**ATGGCGGACCCTAAATACGCCGACCTTCCCGGCATTGCCAGGAATGAGCCAGATGTTTATGAA  
ACTAGCGACCTACCTGAGGATGATCAAGCGGAGTTCGATGCGGAGCTGGAGGAGCTGACAAGC

ACAAGTGTGGAACACATCATTGTCAATCCTAATGCTGCCTATGACAAGTTCAAGGACAAGAGAG  
TGGGGACAAAGGGACTTGATTTCTCAGATCGTATTGGAAAAACCAAGAGGACAGGATATGAATC  
TGGAGAATATGAGATGCTTGGAGAGGGTCTGGGAGTGAAGGAGACACCCACGAAAAGTACCA  
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GGAGTCAGCCACAGAGGAGAAGCTGACCCCTGTGTTGCTGGCTAAACAGCTGGCAGCCCTGAAG  
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ACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTAT  
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GATTTAACAAAAATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTC  
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 CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTG  
 GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA  
 CCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTT  
 TTTTGTGTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT  
 CTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATC  
 AAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATAT  
 ATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGT  
 CTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTT  
 ACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA

CCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGA  
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 TAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCT  
 CGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCC  
 ATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGC  
 AGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGAT  
 GCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGT  
 TGCTCTTGCCCGGCGTCAATACGGGATAAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCAT  
 CATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGA  
 TGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAG  
 CAAAAACAGGAAGGCAAAATGCCGCAAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATAC  
 TCATACTCTTCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA  
 TATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCGCGCACATTTCCCCGAAAAGTGCC  
 ACCTGACGTC

Green highlighted regions are restriction sites.

Blue highlighted region is myc tag.

### **References**

- Li Q, Qu F, Li R, He X, Zhai Y, Chen W, Zheng Y. A functional polymorphism of SSBP1 gene predicts prognosis and response to chemotherapy in resected gastric cancer patients. *Oncotarget*. 2017 Dec 2;8(67):110861-110876
- Pokrzywinski KL, Biel TG, Kryndushkin D, Rao VA. Therapeutic Targeting of the Mitochondria Initiates Excessive Superoxide Production and Mitochondrial Depolarization Causing Decreased mtDNA Integrity. *PLoS One*. 2016 Dec 28;11(12): e0168283
- Tiranti V., Rocchi M., Didonato S., Zeviani M. "Cloning of human and rat cDNAs encoding the mitochondrial single-stranded DNA-binding protein (SSB)." Gene 126:219-225(1993)
- Wang Y, Hu L, Zhang X, Zhao H, Xu H, Wei Y, Jiang H, Xie C, Zhou Y, Zhou F. Downregulation of Mitochondrial Single Stranded DNA Binding Protein (SSBP1) Induces Mitochondrial Dysfunction and Increases the Radiosensitivity in Non-Small Cell Lung Cancer Cells. *J Cancer*. 2017 May 12;8(8):1400-1409
- <https://www.addgene.org/vector-database/2094/>
- <https://www.ncbi.nlm.nih.gov/gene/6742>
- <https://www.uniprot.org/uniprot/Q04837>