

School of Health Science USM
GTB 204- Techniques in Molecular Biology

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Cloning Stimulation Project

Cloning and Expression of CSF-1 Gene: Towards its application in tooth growth promotion

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ABSTRACT

Tooth diseases and malformation has been a common disease in any part of the world. Within this study, we cloned the Colony Stimulating Factor 1 (CSF- 1) gene, which is important to stimulate the Bone Morphogenesis Protein and Epidermal Growth Factor to allow the secretion of Growth Hormone for differentiation of bone basal cells into the growth of tooth. We used pRSET, which has the properties of N- terminal 6- Histidine, T7 promoter and Ampicilin resistance gene. The procedure of cloning the gene involves the restriction of the pRSET plasmid using EcoR1 and then undergo ligation using DNA ligase to produce recombinant plasmid.

After that, the recombinant plasmid undergo transformation into B21 (DE3) *E. coli* host. The transformation procedure will be done by the Calcium Chloride method.

After transformation of the plasmid, the *E. coli* host, which contain recombinant plasmid, will be grown on LB agar plates with Ampicilin. Random colonies from the plate will be patched into new plate for further analysis.

The plasmid then is extracted from the lysate and screened using multiple restriction enzyme digestion and visualized with gel electrophoresis to select the plasmid contained our target insert in forward orientation. This selected colonies will then induced with IPTG to express CSF- 1 protein where it is harvested and screened using specific anti- Xpress antibody epitope.

Eventually, the protein product will undergo protein purification by using Nickel Metal Affinity Chromatography method.

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INTRODUCTION

Human tooth has been a very important asset not only for mastication and communication, but also for cosmetic purposes. But in whole human life span, there are only 2 dentitions occur- one during childhood and the permanent dentition. Therefore, if there is any accident, disease or malformation occur, this priceless asset will not be replaced. Even though our reconstructive and odontology technologies are very advanced, but it can only repair or create an artificial tooth which cannot be compared to the original that erupted from own flesh and bone.

In human physiological act, the primary dentition erupts at 6- 24 months of birth, while the permanent tooth started to take place at 6 years old onward. In molecular aspect of the tooth development, the same basal cells that formed bone are required to form the dental follicle. A type of growth factor- the **Colony Stimulating Factor 1** (CSF-1) is required to stimulate these basal cells for the process of tooth remodeling and differentiation of osteoclast¹. This factor is essential to stimulate the cells to release 2 important cytokines, which are Epidermal Growth Factor (EGF) and Transforming Growth Factor (TGF- β 1). These two factors are essential to stimulate Bone Morphogenesis Factors (BMP) and Fibroblast Growth Factor (FGF), which are expressed in dental epithelium and affect on the underlying mesenchyme². they up regulate the expression of many genes to produce other factors, which work in a cascade, and this will stimulate cell proliferation and act as epithelial signals transmitting epithelial-mesenchymal interactions². Protease is also released which assisted in breakdown of follicular tissue in eruption pathway. All these factors are expressed specifically in restricted and transient epithelial cell clusters, called enamel knots².

With the advanced molecular biology technology, and thanks to the effort of the Human Genome Project, we have been able to identify the specific CSF-1 gene and its sequence, which enable us to clone on a host and harvest the protein it expressed. This CSF-1 gene is located in chromosome1 locus 1p21- p13. With a total of 3939 base pairs. From the BLAST search and through website <http://www.ncbi.nlm.nih.gov>, we able to convert the protein sequence into cDNA which doesn't contain any introns. With these

gene, we insert it into a plasmid vector called pRSET and transformed it to *E. coli* B21 (DE3) host. Due to the T7 promoter gene in the vector, these host when induced with IPTG, it will start to express the CSF-1 protein. These protein expressed can be purified with Nickel Metal Affinity Chromatography (due to 6xHistidine gene in the vector) and able be detected using anti Xpress antibody epitope detection.

Objective of The Project

Our overall aim in the study is to produce a Tooth Growth Promoting Factor called CSF-1, which have the following criteria:

1. It should be harmless and compatible with human physiological activity.
2. It should be a purified protein without any other additional substances that could harm human body.
3. It should able to promote tooth growth regardless of age and sex of the subject.
4. It should optimally and specifically simulate human basal cell or dental epithelium cells.

PRINCIPLE AND METHODOLOGY

Choosing gene from Genbank website

We accessed the Genbank through <http://www.ncbi.nlm.nih.gov> to choose the interest of gene sequences. We search for the gene we need, that is Colony Stimulating Factor 1. We used various keyword, such as osteoprotegerin and CSF-1. Then we decide to choose *Homo sapiens* Colony Stimulating Factor 1 (macrophage) (CSF1), mRNA.

Result of this step

1. We chose the gene sequence *Homo sapiens* Colony Stimulating Factor 1 (macrophage) (CSF1), mRNA. The accession number of this gene sequence is **NM_000757**. The sequence has have 3939 base pairs.

2. The nucleotide sequence of gene of interest gene is :

```
1 cctgggtcct ctcgggcgcca gagccgctct ccgcatocca ggacagcggg gcgccctcgc
61 gccggggcgc cactccgca gcagccagcg agccagctgc cccgtagtac cgcgccgggc
121 gccgccgggc gctgccctcc cagcacatgg ctgggctccc tgctgttgtt ggtctgtctc
181 ctggcggagca ggagtatcac cgaggagggtg tcggagtact gtagccacat gattgggagt
241 ggacacctgc agtctctgca ggggctgatt gacagtcaga tggagacctc gtgcaaaatt
301 acatattgagt ttgtagacca ggaacagttg aaagatccag tgtgtacctc taagaaggca
361 ttctcctcctg tacaagacat aatggaggac accatgcgct tcagagataa caccgccaat
421 cccatcgcca ttgtgcagct gcaggaactc tctttgaggc tgaagagctg cttcaccaag
481 gattatgaag agcatgacaa ggctgcgctc cgaactttct atgagacacc tctccagttg
541 ctggagaagg tcaagaatgt ctttaatgaa acaaagaatc tccttgacaa ggactgggat
601 attttcagca agaactgcaa caacagcttt gctgaatgct ccagccaaga tgtggtgacc
661 aagcctgatt gcaactgcct gtaccctaaa gccatcccta gcagtgacct ggcctctgtc
721 tcccctcatc agcccctcgc cccctccatg gccctgtggt ctggcttgac ctgggaggac
781 tctgagggaa ctgagggcag ctccctcttg cctggtgagc agcccctgca cacagtggat
841 ccaggcagtg ccaagcagcg gccaccaggg agcacctgcc agagctttga gccgccagag
901 accccagttg tcaaggacag caccatcggg ggctcaccac agcctcgccc ctctgtcggg
961 gccttcaacc ccgggatgga ggatattctt gactctgcaa tgggcactaa ttgggtccca
1021 gaagaagcct ctgggagaggc cagtgagatt cccgtacccc aaggggacaga gctttcccc
1081 tccaggccag gagggggcag catgcagaca gagcccgcca gaccagcaa ctctcttca
1141 gcatcttctc cactccctgc atcagcaaaag ggccaacagc cggcagatgt aactgctaca
1201 gccttgccca ggttgggccc cgtgatgccc actggccagg actggaatca cccccccag
1261 aagacagacc atccatctgc cctgctcaga gaccccccg agccaggctc tcccaggatc
1321 tcatcactgc gccccaggg cctcagcaac ccctccacc tctctgctca gccacagctt
1381 tccagaagcc actcctcggg cagcgtgctg ccccttgggg agctggaggg caggaggagc
1441 accagggatc ggacgagccc cgcagagcca gaagcagcac cagcaagtga aggggcagcc
1501 agggccctgc cccgttttaa ctccgttcc ttagctgaca caggccatga gaggcagctc
1561 gagggatcct ccagcccgca gctccaggag tctgtcttcc acctgctggt gccagtgctc
1621 atcctggctc tgctggctgt cggaggcctc ttgttctaca ggtggaggcg gcggagccat
1681 caagagcctc agagagcgga ttctcccttg gagcaaccag agggcagccc cctgactcag
1741 gatgacagac aggtggaact gccagtgtag agggaaattc aagctggagc cacagaacag
1801 tctctctcgt ggaggagaca ttatggggcg tccaccacca ccctccctg gccatctctc
1861 tggaatgtgg tctgccctcc accagagctc ctgctgcca ggactggacc agagcagcca
1921 ggctggggcc cctctgtctc aaccgcgaga cccttgactg aatgagagag gccagaggat
1981 gctccccatg ctgccactat ttattgtgag ccctggaggc tccatgtgct ttgaggagg
2041 ctggtgagcc cggctcagga ccctcttccc tcaggggctg cagcctctc tcaactcctt
2101 ccatgccgga acccagcca gggaccaccc ggctgtggt ttgtgggaaa gcagggtgca
2161 cgtgaggag tgaacaacc ctgcaccagc agggcctgcc tggtgccaag gtatcccagc
2221 ctggacaggg atggacctgt ctccagacagc agggacctga agttcgtggg gcgggacagc
2281 ctggcctgca tttcccgtaa aggtgtgcag cctgagagac ggggaagagg ggctctgca
2341 cctgctggtc tgcactgaca gctgaaggg tctacacctc cggctcacct aagtcctgt
2401 gctgggtgccc agggccagag gggaggccag ccctgccctc aggaactgcc tgactgcca
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2461 gtgatgccaa gagggggatc aagcactggc ctctgcccct cctccttcca gcacctgcca
2521 gagcttctcc agcaggccaa gcagaggctc ccctcatgaa ggaagccatt gcactgtgaa
2581 cactgtacct gcctgctgaa cagcctcccc ccgtccatcc atgagccagc atccgtccgt
2641 cctccactct ccagcctctc cccagcctcc tgcactgagc tggcctcacc agtgcactga
2701 gggagcccct cagccctgac cttctcctga cctggccttt gactccccgg agtggagtgg
2761 ggtggggagaa cctcctgggc cgccagccag agccgctctt taggctgtgt tcttcgcca
2821 ggtttctgca tcttccactt tgacattccc aagaggggaag ggactagtgg gagagagcaa
2881 gggaggggag ggcacagaca gagagcctac agggcgagct ctgactgaag atgggccttt
2941 gaaatatagg tatgcacctg aggttggggg aggggtctgca ctcccaaacc ccagcgagc
3001 gtcttttccc tgctgcccag aggaacctgg ggctgagcag gttatccctg tcaggagccc
3061 tggactgggc tgcactcag ccccactgac atgggatcca gctcccatcc acttctcacc
3121 cttctttcct ccctgacctg gtcagcagtg atgacctcca actctcacc accccctca
3181 ccatcacctc taaccaggca agccaggggt ggagagcaat caggagagcc aggcctcagc
3241 ttccaatgcc tggagggcct ccactttgtg gccagcctgt ggtgctggct ctgaggccta
3301 ggcaacgagc gacagggctg ccagttgccc ctgggttctt ttgtgctgct gtgtgcctcc
3361 tctcctcccg ccctttgtcc tccgctaaga gacctgccc tacctggccg ctgggcccgg
3421 tgactttccc ttctgcccc ggaaagtgag ggtcggtgg ccccacttc cctgtcctga
3481 tgccgacagc ttaggggaag gcaactgaact tgcatatggg gcttagcctt ctagtacag
3541 cctctatatt tgatgctaga aaacacatat ttttaaagg aagaaaaata aaaaggcatt
3601 ccccttcat cccctacct taacatata atattttaa ggtcaaaaa gcaatccaac
3661 ccaactgcaga agctctttt gagcacttg tggcatcaga gcaggaggag ccccagagcc
3721 acctctggtg tccccaggc tacctgctca ggaacccct ctgttctctg agaactcaac
3781 agaggacatt ggctcacgca ctgtgagatt ttgttttat acttgcaact ggtgaattat
3841 tttttataaa gtcattttaa tatctattta aaagatagga agctgcttat atatttaata
3901 ataaaagaag tgcacaagct gccgttgacg tagctcgag

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Open Reading Frame (ORF) Analysis

Open Reading Frame analysis is a DNA sequence containing series of codons, which can be translated into protein. ORF starts with a start codon . Start codon is ATG in DNA and AUG in mRNA, contain the actual sequence to code for a protein/ polypeptide and ends with a stop codon like TGA, TAG, and TAA (UGA, UAG,UAA in mRNA).

This analysis is to make sure that our gene can be expressed and to be cloned in real life application.

We analyze the gene sequences through ORF website <http://www.ncbi.nlm.nih.gov/gorf/gorf.html> .

Result of this step

1. By just examining the sequence of the gene, we found out that there are both start codon (ATG) and stop codon (TGA) in the sequence.
2. From the result of the website analysis :

Frame	From	To	Length
+1	106	1770	1665

-1	1	882	882
+3	2952	3449	498
-1	1324	1821	498
-2	2439	2846	408
-2	729	1103	375
-2	3165	33515	351
-1	2800	3108	309
+3	2025	2300	276
-2	1	275	275
-3	2372	2632	261
-2	1368	1622	255
-3	1619	1855	237
+2	1742	1957	216
+2	1547	1735	189
-2	519	698	177
+2	485	658	174

3. For the purpose of our cloning stimulating project, we need the whole sequence for the expression of esterase. The +1 frame includes the whole frame from base pair 106 to 1770 base pair, which has the length of 1665.

Blast Analysis

The RID for analysis done in Gene Bank website: 1028552817-027371-22789

There are few similarities in the esterase gene sequence with other organisms but those similarities are very minor. These organisms have some gene sequence which bear similarities with part of esterase gene sequence : Human Macrophage- specific Colony Stimulating Factor, *Homo sapiens* colony stimulating factor, *Homo sapiens* macrophage Colony Stimulating Factor, *Mus musculus* Colony Stimulating Factor, Mouse macrophage colony-stimulating factor, *Mus musculus* Monoclonal Antibody, *Drosophila melanogaster*, *Homo sapiens* 3 BAC RP11-451B8,

Restriction Enzyme Analysis

This stage is to determine the site in the DNA sequence that allow any restriction enzyme to cut in the gene of interest. By knowing the restriction endonuclease enzyme that cuts in the middle of the strand, we must include the usage of such restriction enzyme site in the linker of later stage.

We used the website <http://rna.lundberg.gu.se/cgi-bin/cutter2/cutter> to analyze the restriction enzyme site in the sequence.

1. Only two restrictions enzyme cut in the sequence and they are **BamHI** and **PstI**

1. The are following endonucleases were selected but don't cut this sequence:

AatII, Acc16I, Acc65I, AccIII, AfeI, AflIII, AgeI, AhdI, Aor51HI, AscI, AseI, AsnI, Asp718I, AspEI, AspI, AtsI, AviII, BanIII, BbrPI, BclI, BglII, Bpu14I, Bsa29I, BsaAI, BsaBI, BsaWI, BscI, Bse8I, BseAI, BseCI, BsePI, Bsh1365I, BsiMI, BsiWI, Bsp106I, Bsp119I, Bsp13I, Bsp1407I, Bsp68I, BspCI, BspDI, BspEI, BspLU11I, BspXI, BsrBRI, BsrGI, BssHII, Bst1107I, BstBI, stSNI, Bsu15I, CciNI, Cfr42I, ClaI, CpoI, Csp45I, CspI, DrdI, Eam1105I, EclHKI, Eco105I, Eco32I, Eco47III, Eco72I, EcoRV, EcoT22I, FbaI, FseI, FspI, HindIII, HpaI, Kpn2I, KpnI, Ksp22I, KspI, LspI, MamI, MfeI, MluI, Mph1103I, MroI, MunI, NheI, NotI, NruI, NsiI, NspV, PacI, Pfl23II, PinAI, Ple19I, PmaCI, PmeI, PmlI, Ppu10I, PshBI, Psp1406I, PspLI, PstNHI, PvuI, RsrII, SacII, SbfI, SexAI, SfiI, Sfr303I, SfuI, SgfI, SgrAI, SnaBI, SplI, SrfI, Sse8387I, SspBI, SstII, SunI, Tth111I, VspI, XbaI, Zsp2I

Primer Sequence Analysis

This particular process in to determine the best sequence that can be use to produce forward and reverse primers for the usage of amplification of the gene with PCR.

We use the website <http://www.ncbi.nlm.nih.govt> to check for the best sequence and the result is as below:

LEFT PRIMER 1612 20 59.96 55.00 5.00 0.00 **cccagtgcatcctggtctt**

RIGHT PRIMER 1804 20 60.03 55.00 3.00 2.00 **gagactgttctgtgcgtcca**

SEQUENCE SIZE: 3939

INCLUDED REGION SIZE: 1665

Properties of pRSET Cloning Vector

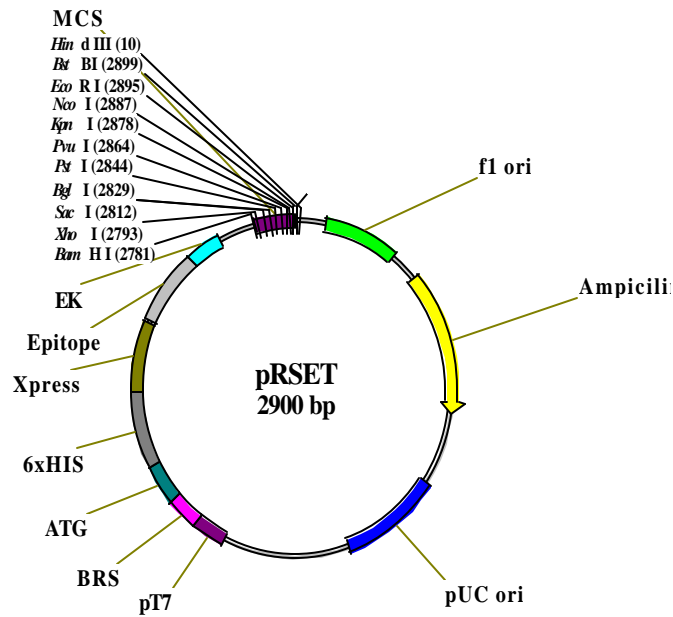


Figure 1: pRSET plasmid map

Description:

This pRSET vector is designed for high level prokaryotic expression controlled by a strong **bacteriophage T7 promoter**. Expression is induced by the production of T7 RNA polymerase in B21 (DE3) *E. coli* host. The pRSET vectors other properties such as:

1. T7 gene 10 sequence to provide protein stability.
2. N terminal polyhistidine (6xHis) tag for rapid purification with nickel resin and detection with an Anti- HisG antibody.
3. N- terminal Xpress epitope for protein detection with the Anti- Xpress Antibody.
4. Enterokinase cleavage site for removal of fusion tag.

Media Preparation

Preparation of LB Agar.

Trypton	15 gm
Yeast extracts	5 gm
Sodium chloride	10 gm
Agar	15 gm

pH was adjusted to 7.0 and the final volume was made up to 1000 ml and then autoclaved before pouring in plate with 25 ml each.

Preparation of LB Broth.

Tryptone	15gm
Yeast extracts	5 gm
Sodium chloride	10gm

pH was adjusted up to 7.0 and a final volume was made up to 1000ml and aliquoted 10 ml each in universal bottle before sent to autoclave.

Plasmid Extraction

Reagent .

Solution I	Tris pH 8.0 EDTA and glucose. RNAase. Lysozyme
Solution II	sodium hydroxide. SDS-Sodium dodecyl sulphate. (alkaline pH 12.0)
solution III	Sodium or potasium acetate

Protocol:

It was done using Miniprep protocol Organism were taken and inoculated into 10 ml LB Broth with antibiotics and grown overnight in a shaker .The next day the culture are ready to be extracted.

1. Transfer 1.5 ml of bacterial culture to a micro centrifuge tube.
2. Centrifuge at 12000rpm for 2 minutes.
3. Carefully remove the supernatant.
4. Then add 250ul of lysis solution/ RNAase and mixed gently. The solution will lyses the cell to make it expose the DNA.
5. Then add 250 ul of solution II. Mixed gently by inverting and rotating. Do not vortex. It will lyses completely
6. Leave at room temperature for 5 minutes.
7. Add 350 ml solution III.
8. Put on ice for 5 minutes.

9. Centrifuge for 12 minutes at 12000rpm.
10. Place a spin column in a collection tube.
11. Transfer the supernatant to the column.
12. Centrifuge the column and collection for 1 minute.
13. Discard the flow and replace the column.
14. Add 250 μ l of HB buffer and centrifuge for 1 minute at 12000rpm and discard the flow.
15. Add 100 Wash buffer into the column and centrifuge for 1 minute at 12000 rpm and discard the flow.
16. Centrifuge again for 90 second to remove the residual buffer.
17. Place the column in a sterile 1.5 ml tube.
18. Add 50 μ l deionised water or TE buffer .
19. Centrifuge 12000rpm for 1 minute.
20. Discard the column. Plasmid DNA is in the deionised water or TE buffer will be collected in the 1.5 ml tube. Place it in -20°C further used.

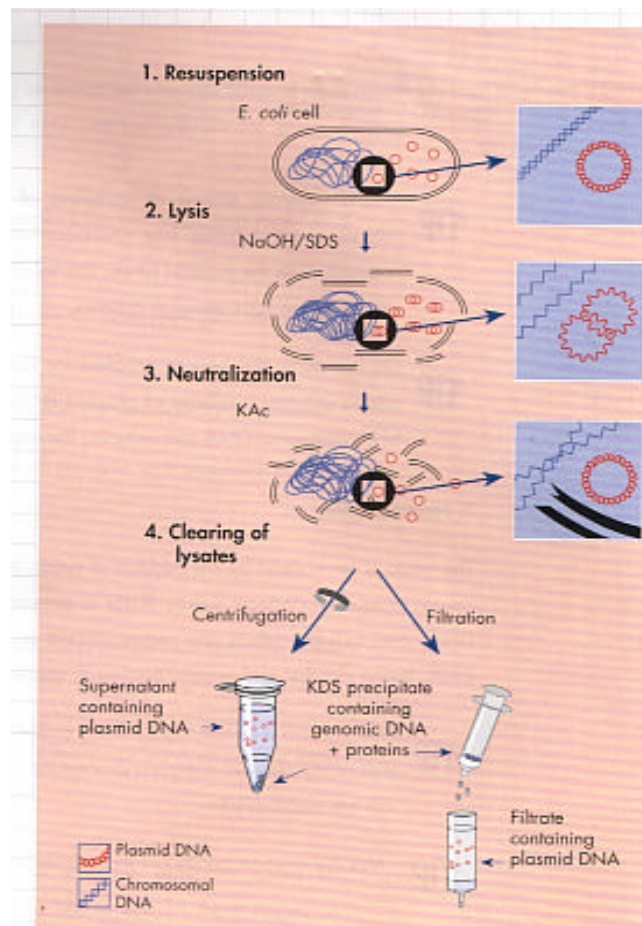


Figure 2: Flow chart of plasmid extraction process.

PCR Screening

Principle:

Polymerase chain reaction (PCR) is a technique used to amplify specific region of DNA, in order to produce enough DNA for further investigation .

The number of nucleotides in the DNA strand are variable depending on the gene. PCR method comes in 3 main steps.

- i. Denaturation where the DNA was heated to denature the DNA paired strands into two.
- ii. Annealing is a process where the forward and reverse primers anneal to separated DNA template strand.
- iii. Extension came with enzyme called Taq polymerase which binding up all the nucleotide of the separated strand with its opposite partner.
One cycle was counted when all these three steps occurred.

Methodology:

PCR screening was done by adding up a mixture of reagents necessary as mentioned.

10x TEbuffer
MgCl (25 mmol/l
dNTP (20mmol/l)
Primers 1 (forward)
Primers 2(reverse)
Taq polymerase
Template
Distilled water

} mixture pipette into appendorf tube.

After mixing , add mineral oil and put inside thermal cycle for 30 cycle.

Run the PCR product on agarose gel

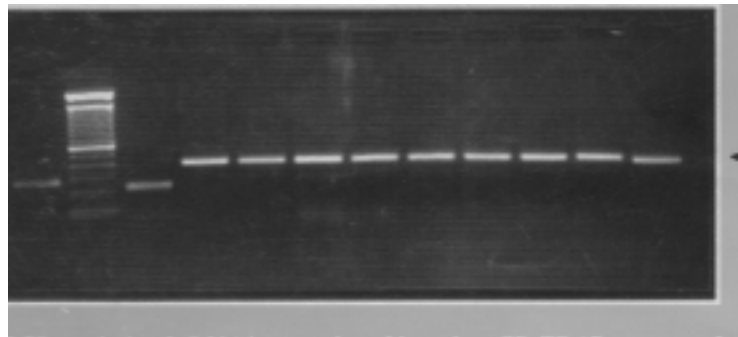


Figure 3: Example of PCR product stained with Ethidium Bromide

Adding EcoR1 Linker to CSF-1 Insert

Reverse Transcriptase Polymerase Chain Reaction (RT- PCR) is done on the ORF of CSF-1 gene to amplify the sequence for the purpose of further analysis. During the design of the primer, an EcoRI linker sequence is added so that later when it is cut with the selected enzyme, it will have EcoRI site on both end for the purpose of ligation to the plasmid vector.

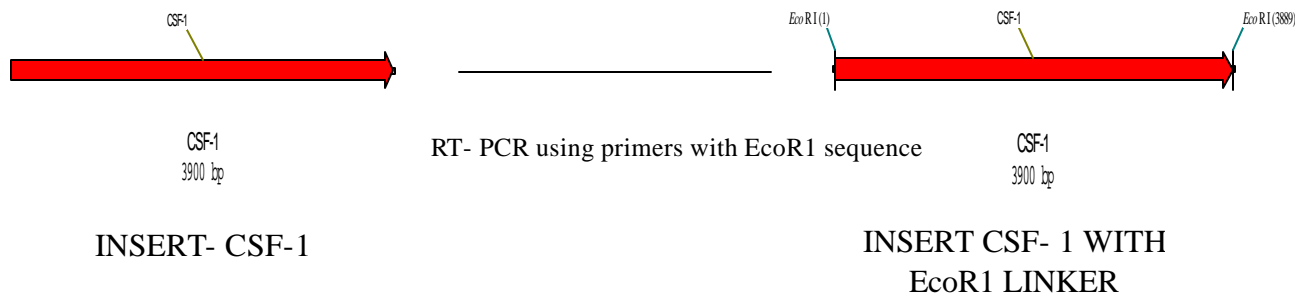


Figure 4: RT- PCR of CSF-1 using primers containing EcoR1 sequence.

Calculation of the Annealing Temperature

There is a formula to calculate the annealing temperature, which required the sequence of the primer because the amounts of specific nucleotides were needed. The formula is as below:

Melting Temperature (T_m):

$$T_m = (A+T)2 + (G+C)4$$

Annealing Temperature (T_a):

$$T_a = \text{Melting temperature}(T_m) - 2 \text{ to } 5 \text{ } ^\circ\text{C}$$

Using the formula above, the melting temperature for the forward primer is 62°C and reverse primer is also 62°C. Therefore, the annealing temperature is 57°C.

Gel Electrophoresis

Preparation of Reagent.

Preparation of 10X TAE stock solution buffer.

Tris 48.0gm(40mM)
Glacial acetic acid 11.42ml
0.5M EDTA 2.0ML (1Mm) diluted to 1000 ml with distilled water and pH was set to pH 8.0

Preparation of 5X TBE stock solution buffer.

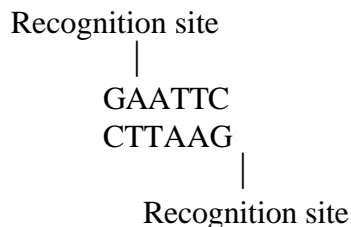
Tris 54.0 gm
Boric acid 27.5gm
0.5 M EDTA (pH 8.0) 20.0ml (0.111Mm) diluted up to 1000ml with distilled water.

Preparation of 1% TAE / TBE gel.

100 ml of 1X TAE/ 0.5X TBE buffer was poured into a 250 ml conical bottle and 1 gm of agarose gel powder was weighed and added into it. After a brief mix, it was boiled in microwave oven for 5 minutes until it turned clear. Then it was let cold by dipping in water for a while and 1.5 ul μ g Ethidium bromide (10mg/ ml) was added. Then the gel was poured into a clean gel electrophoresis plate with combs.

Restriction Enzyme Digestion

This process is to line arise the vector by cutting it with restriction enzyme that is located in the sequence in the vector (Multiple Cbning Site) so that it uptake the insert during ligation process. In this case, we use EcoR1 enzyme, which is an endonuclease enzyme that cuts in the middle of the strand with the sequence GAATTC.



The EcoRI enzyme will cut at the palindromic sequence of the vector and produce the sticky end at the both side as it is shown in the diagram below:

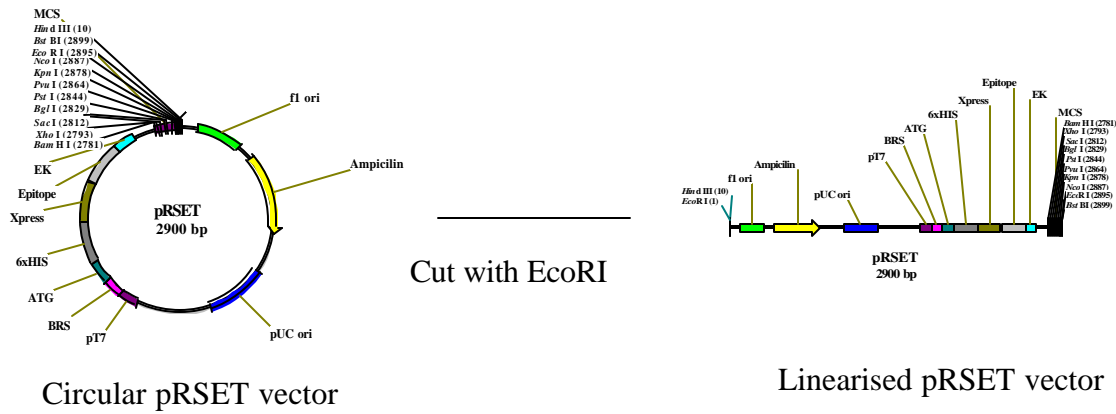


Figure 5: pRSET vector cut with EcoR1 enzyme

Ligation of Insert into pRSET Vector

EcoRI is used to linearise the vector and it is then ligated with the CSF-1 insert using T4 DNA Ligase. The process is shown in the flow chart below:

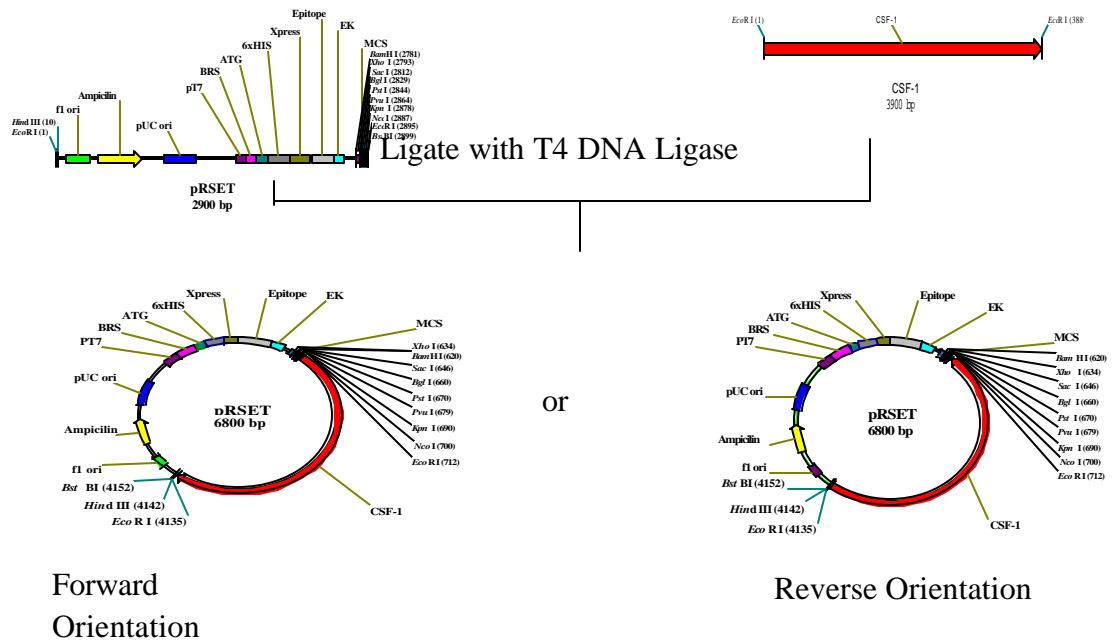


Figure 6: Insertion of CSF-1 insert into pRSET vector.

Transformation

The recombinant plasmid with CSF-1 gene will be transformed into BL 21 (DE3) *E. coli* host at this stage. In order to make this host competent, the BL 21 will be treated either with calcium chloride method or by means of electroporation. But, in this case, we are using calcium chloride method. The process is shown in the flow chart below:

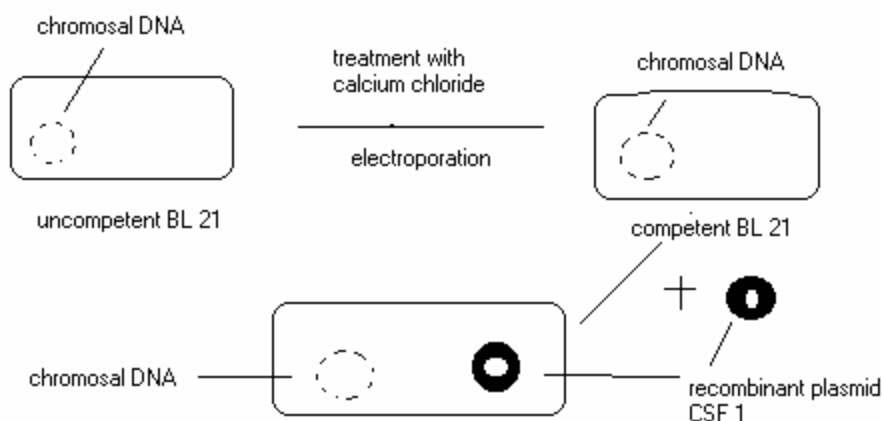


Figure 7: Transformation of plasmid vector into B21 (DE3) *E. coli* host.

Patching

Patching was done to keep the isolated selected colonies as a stock for further analysis.

Procedure:-

1. Around 20-50 colonies were chosen randomly.
2. Selected colony was patched on a LB agar plate with appropriate antibiotics with wire loop and the same was suspended in the same 20 ml distilled water for preparing the cell lysate
3. LB agar plate was incubated overnight at 37 °C while the cell lysates were used for PCR screening.
4. To the cell lysate, 2 drops of mineral oil was added and boiled for 10 minutes and centrifuged briefly for 2 minutes at 12000 rpm. 2ml of the cell lysate was used as template for PCR screening.

Determination of the Orientation for the Insert

The target CSF-1 gene must be inserted properly in forward orientation, in order for it to express the correct protein. Since we are using the same one enzyme to ligate the insert into the vector, which is EcoR1, there is a possibility that the insert is ligated in a reverse orientation. In order to screen the plasmid for the correct orientation, there are few techniques can be done:

1. Multiple restriction enzyme analysis and screen it with gel electrophoresis.
2. Send the plasmid for sequencing by other company.

Protein Purification

Nickel metal affinity chromatography method is used to purify our protein. The principle of this method is to separate the proteins based on isoelectrical charge and the biological activity of the protein.

Our recombinant protein is attach with 6 Histidine tag, is passed through a Nickel affinity column. The target protein will bind with the Nickel that is attached to the resin while the unbounded protein or protein without 6His tag are washed through the column with the buffer.

After that, pH of the buffer is lower down to reduce the binding affinity of the protein to the resin. This will cause the target protein flow down through the column, hence able to be eluted and harvested. Finally, His-Tag protein is treated with the specific protease to cleave off the His-Tag. The recombinant protein is freed of the His-Tag peptide by running it over the metal-chelate column again.

Summary of the Cloning Project

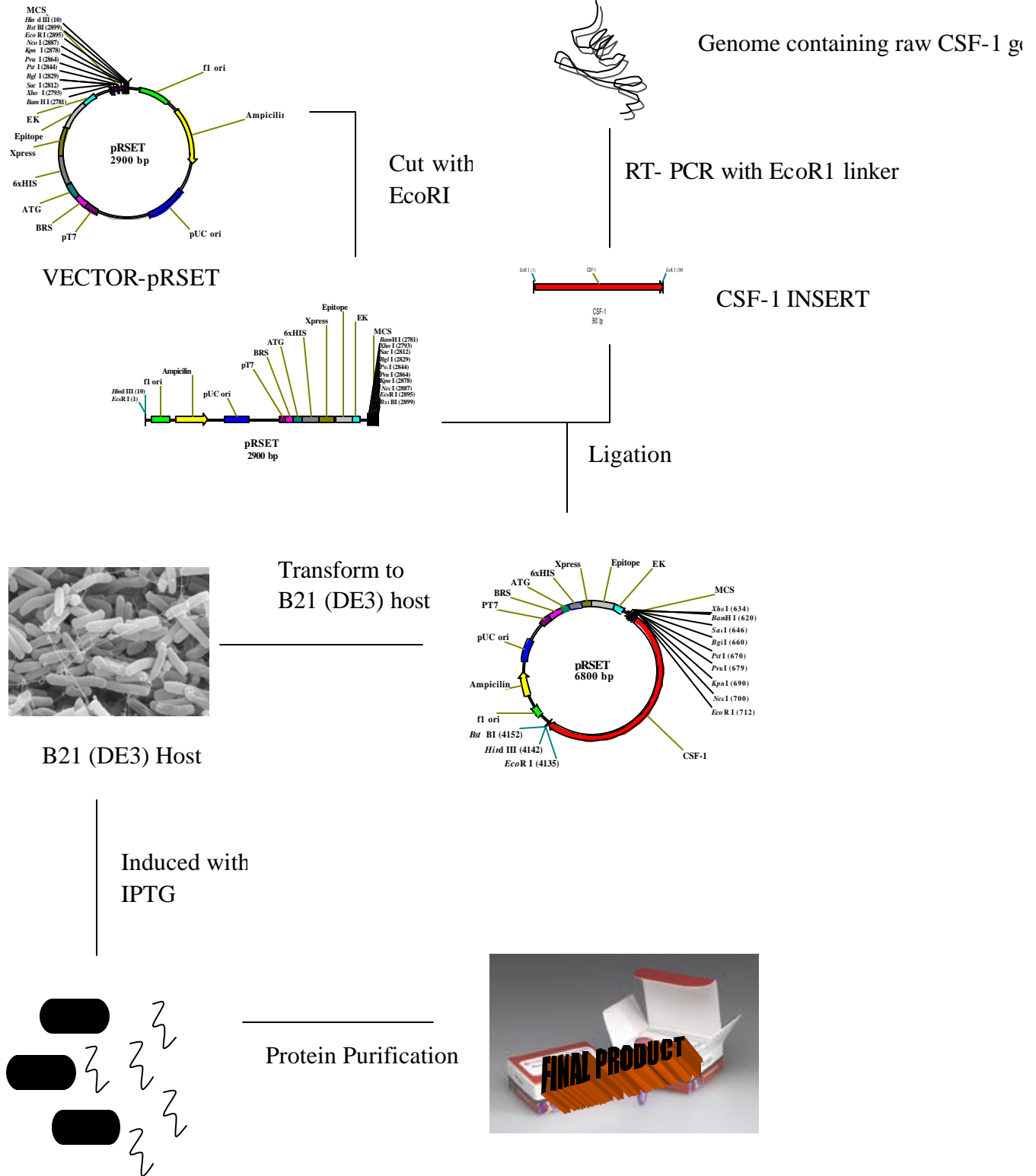


Figure 8: Flow chart of summary for whole cloning project.

CONCLUSION

During this project, we have successfully cloned CSF-1 insert into vector pRSET. With the result we have achieved, confidently CSF-1 has high potential to be use for the benefit of people over the world.

In future, this gene will be evaluated because it can promote tooth eruption for the patient with dental problem due to disease, accident or malformation, .It offers new clinical option beside old method we have such as prostodontics and implantation of teeth. We already knows that, these kind of old method such as removable denture is not as effective as the original teeth. CFS-1 also have cosmetic value because it promote growth of new teeth after we lost our second set of teeth. The physical and function of the teeth will be as optimum as teeth we have before.

So, we hope that by developing CSF-1, it will give a new choice in oral health treatment and a new revolution in molecular science achievement.

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