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Synthetic cDNA Construction of Granulocyte Stimulating Factor

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Annotation: **INTRODUCTION:** Granulocytes are a category of white blood cells characterized by the presence of granules in their cytoplasm. They are also called polymorphonuclear leukocytes (PMN or PML) because of the varying shapes of the nucleus, which is usually lobed into three segments. In common parlance, the term polymorphonuclear leukocyte often refers specifically to neutrophil granulocytes, the most abundant of the granulocytes. Granulocytes or PMN are released from the bone marrow by the regulatory complement proteins. There are three types of granulocytes, distinguished by their appearance under Wright's stain: Neutrophil granulocytes, Eosinophil granulocytes and Basophil granulocytes

Objectives: Cloning of GCSF cDNA

and expression of the granulocyte colony stimulating factor protein. Methodology Primer designing: Oligonucleotides were manually designed and synthesized, in even numbers, corresponding to the double stranded DNA, in a sequential manner. The G-CSF nucleotide sequence (Accession number NM_000759) of 522 bp, was divided in 12 short sequences of approximately 50 bp each. Oligonucleotides contained overlapping regions of about 10 bases at their 5'- and 3'-ends. The primers employed in the present study are given in Table 1. The primers P1 and P12 contained the restriction sites for, respectively, Nde I and Bam HI. The 522 bp final PCR product was agarose-gel purified and cloned into the pJET1.2 Blunt vector (Fermantas).

CONCLUSION: Full length cDNA was constructed and cloned into blunt end vector. Further the cloned product need to express to get the recombinant H-GCSF protein to purify it. Construction and cloning of h-GCSF cDNA was completed and the protein purification has to be standardized.

INTRODUCTION:

Granulocytes are a category of white blood cells characterized by the presence of granules in their cytoplasm. They are also called polymorphonuclear leukocytes (PMN or PML) because of the varying shapes of the nucleus, which is usually lobed into three segments. In common parlance, the term polymorphonuclear leukocyte often refers specifically to neutrophil granulocytes, the most abundant of the granulocytes.Granulocytes or PMN are released from the bone marrow by the regulatory complement proteins.There are three types of granulocytes, distinguished by their appearance under Wright's stain: Neutrophil granulocytes, Eosinophil granulocytes and Basophil granulocytes.[1-4]

Their names are derived from their staining characteristics; for example, the most abundant granulocyte is the neutrophil granulocyte, which has neutrally staining cytoplasmic granules.

Other white blood cells that are not granulocytes ("agranulocytes") are mainly lymphocytes and

monocytes.

A neutrophil with a segmented nucleus (center and surrounded by erythrocytes), the intra-cellular granules are visible in thecytoplasm (Giemsa-stained high magnification) Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte, constituting 50% to 60% of the total circulating white blood cells. One litre of human blood contains about five billion neutrophils 5x109, which are about 12-15 micrometers in diameter, and live approximately 6 hours. Once neutrophils have received the appropriate signals, it takes them about thirty minutes to leave the blood and reach the site of an infection. Neutrophils do not return to the blood; they turn into pus cells and die. Mature neutrophils are smaller than monocytes, and have a segmented nucleus with several sections(two to five segments); each section is connected by chromatin filaments. Neutrophils do not normally exit the bone marrow until maturity, but during an infection neutrophil precursors called myelocytes and promyelocytes are released. Neutrophils have three strategies for directly attacking micro-organisms: phagocytosis (ingestion), release of soluble anti-microbials (including granule proteins), and generation of neutrophil extracellular traps (NETs). Neutrophils are professional phagocytes: they are ferocious eaters and rapidly engulf invaders coated with antibodies and complement, and damaged cells or cellular debris. The intra-cellular granules of the human neutrophil have long been recognized for their protein-destroying and bactericidal properties. Neutrophils can secrete products that stimulate monocytes and macrophages; these secretions increase phagocytosis and the formation of reactive oxygen compounds involved in intracellular killing. Neutrophils have two types of granules; primary (azurophilic) granules (found in young cells) and specific granules(which are found in more mature cells) [5-8]. Primary granules contain cationic proteins and defensins that are used to kill bacteria, proteolytic enzymes and cathepsin G to break down (bacterial) proteins, lysozyme to break down bacterial cell walls, and myeloperoxidase (used to generate toxic bacteria-killing substances). In addition, secretions from the primary granules of neutrophils stimulate the phagocytosis ofIgG antibody-coated bacteria. The secondary granules contain compounds that are involved in the formation of toxic oxygen compounds, lysozyme, and lactoferrin (used to take essential iron from bacteria). Neutrophil extracellular traps (NETs) comprise a web of fibers composed of chromatin and serine proteases that trap and kill microbes extracellularly. Trapping of bacteria is a particularly important role for NETs in sepsis, where NET are formed within blood vessels. An eosinophil surrounded by erythrocytes Eosinophils also have lobed nuclei (two to four lobes). The number of granules in an eosinophil can vary because they have a tendency to degranulate while in the blood stream. Eosinophils play a crucial part in the killing of parasites (e.g., enteric nematodes) because their granules contain a unique, toxic basic protein and cationic protein (e.g., cathepsin) receptors that bind to IgE are used to help with this task. These cells also have a limited ability to participate in phagocytosis, they are professional antigen-presenting cells, they regulate other immune cell functions (e.g., CD4+ T cell,dendritic cell, B cell, mast cell, neutrophil, and basophil functions), they are involved in the destruction of tumor cells, and they promote the repair of damaged tissue. A polypeptide called interleukin-5 interacts with eosinophils and causes them to grow and differentiate; this polypeptide is produced by basophils. A basophil with lobed nuclei surrounded by erythrocytes Basophils are one of the least abundant cells in bone marrow and blood (occurring at less than two percent of all cells). Like neutrophils and eosinophils, they have lobed nuclei; however, they have only two lobes, and the chromatin filaments that connect them are not very visible. Basophils have receptors that can bind to IgE, IgG, complement, and histamine. The cytoplasm of basophils contains a varied amount of granules; these granules are usually numerous enough to partially conceal the nucleus. Granule contents of basophils are abundant with histamine, heparin, chondroitin sulfate, peroxidase,platelet-activating factor, and other substances[9-12].

When an infection occurs, mature basophils will be released from the bone marrow and travel to the site of infection. When basophils are injured, they will release histamine, which contributes to the inflammatory response that helps fight invading organisms. Histamine causes dilation and

increased permeability of capillaries close to the basophil. Injured basophils and other leukocytes will release another substance called prostaglandins that contributes to an increased blood flow to the site of infection.

Both of these mechanisms allow blood-clotting elements to be delivered to the infected area (this begins the recovery process and blocks the travel of microbes to other parts of the body). Increased permeability of the inflamed tissue also allows for more phagocyte migration to the site of infection so that they can consume microbes. Granulocytopenia is a problem commonly encountered in patients with HIV infection. Although low granulocyte counts usually reflect the toxicity of therapies for HIV infection or associated conditions, studies of untreated patients have also shown a high incidence of granulocytopenia, particularly in patients with more profound immunodeficiency. For example, the Multicenter AIDS Cohort Study found that 0.8% of HIV-seropositive patients with mean CD4+ T lymphocyte counts of greater than 700 cells/mm3 had abnormally low granulocyte counts, whereas granulocytopenia was present in 13.4% of those with mean CD4+ T lymphocyte counts below 249 cells/mm3.Zon and Groopman noted low granulocyte counts in 13% of asymptomatic HIV-seropositive patients and in 44% of those with frank CDC-defined AIDS.

The pathogenesis of granulocytopenia in patients with HIV infection is multifactorial. An autoimmune mechanism involving antigranulocyte antibodies and impaired granulopoiesis has been postulated, but not yet proved, to account for granulocytopenia in some patients. Any infiltrative process involving the bone marrow (infection, malignancy) may also produce granulocytopenia. In clinical practice, however, drug toxicity is responsible for most of the granulocytopenia seen in patients with HIV infection.

It is important to note that in a one study, investigators showed a good correlation between the level of the absolute granulocyte count and the risk of hospitalization for a significant bacterial infection in weeks immediately following the absolute neutrophil count (ANC)[13-16].

Drug-Induced Granulocytopenia: AZT therapy is probably the most common cause of low granulocyte counts in patients with HIV infection. Severe granulocytopenia (< 500 cells/mm3) developed in 16% of AZT-treated patients in the original placebo-controlled study of AZT therapy for advanced HIV disease and symptomatic middle-stage HIV disease; only 2% of placebo-treated patients became granulocytopenic to this degree. Despite the relatively high frequency of AZT-induced granulocytopenia, there were no reported episodes of bacterial infection or sepsis in the study group. In subsequent studies of AZT therapy.the observed risk of bacterial infection was low, reflecting the brief duration of AZT-induced granulocytopenia; the dosage of AZT was reduced or discontinued when the granulocyte count fell below the range of 500 to 1,000 cells/mm3. As stated previously, low ANC is associated with increased risk of hospitalization for significant bacterial infection in weeks following the ANC. Shaunak and Bartlett described their experience in treating 30 patients with severe, recurrent (three or more episodes) AZT-induced granulocytopenia. The total follow-up time was 493 months (41.1 patient-years) and the granulocyte count was less than 1,000 cells/mm3 for 41% of that time. AZT therapy was reduced or discontinued when the granulocyte count fell below the range of 500 to 1,000 cells/mm3. Patients with granulocyte counts of less than 500 cells/mm3 had an incidence of bacterial infection that was 230% higher than in patients with granulocyte counts of 500 to 1,000 cells/mm3 (seven infections in 40 months vs. nine infections in 169 months). The authors concluded that AZT therapy can be continued despite granulocytopenia without a major increase in the incidence of bacterial infection provided that the granulocyte count is not lower than 500 cells/mm3.

Ganciclovir therapy for symptomatic cytomegalovirus infection is another common cause of granulocytopenia in patients with advanced HIV disease. Jacobson et al. observed absolute granulocyte counts of less than 800 cells/mm3 in 10 of 32 patients receiving chronic daily maintenance ganciclovir therapy.

Four patients developed central venous catheter-associated bacteremia; all had granulocyte counts

of greater than 1,200 cells/mm3 when bacteremia occurred. In other studies reviewed by Jacobson, bacterial infection was a very rare complication of ganciclovir-induced granulocytopenia, possibly reflecting the brief duration of ganciclovir-induced granulocytopenia attributable to the discontinuation of ganciclovir therapy when the granulocyte count falls below 500 to 1,000 cells/mm3. Recovery is generally prompt.

A number of other medications commonly used in the setting of HIV infection can cause granulocytopenia. Trimethoprim-sulfamethoxazole and pentamidine are standard therapy for PCP. Granulocytopenia has been reported in a high percentage of patients receiving these antibiotics in clinical trials, but bacterial infections have not occurred as a consequence. Interferon-alpha therapy, both alone and in combination with AZT, can also cause granulocytopenia[17-20].

Antineoplastic chemotherapy is probably the most common cause of low granulocyte counts in patients without HIV infection. Granulocytopenia secondary to cancer chemotherapy also complicates treatment of HIV-infected patients, perhaps to an even greater extent as a result of impaired bone marrow function. There are few reports, however, describing the types or incidence of granulocytopenia-related infections in this setting. With a relatively aggressive combination chemotherapy regimen (doxorubicin [Adriamycin] plus bleomycin plus vincristine ["ABV"]) in the treatment of advanced HIV-related Kaposi's sarcoma, granulocytopenia (less than 1,000 cells/mm3) occurred in 11 of 33 patients, and bacterial infections developed in 5. Patients with HIV-related non-Hodgkin's lymphoma frequently require hospitalization for empiric antibiotic therapy when granulocytopenia (< 500 cells/mm3) and fever develop following chemotherapy. In a review of 99 such hospitalizations, 23 episodes of bacteremia were identified. This frequency of bacteremia is similar to that seen in cancer patients without HIV infection who develop granulocytopenia following chemotherapy.

In summary, drug-induced granulocytopenia is common in patients with HIV infection. When the granulocyte count falls below 500 cells/mm3, the risk of infection and sepsis is significant, an observation in accord with similar findings in other disease states. Therefore, empiric antibiotic therapy need not be prescribed in cases of mild granulocytopenia resulting from drug therapy or underlying disease states. Rather, antibiotics are reserved for those situations in which frank evidence of bacterial infection is present, or the granulocyte count is below 500 cells/mm3 and is expected to remain at that level for a prolonged period of time, as in the aftermath of chemotherapy for HIV-associated malignancie

Qualitative functions of granulocytes from patients with HIV infection have been studied in vitro, and a number of abnormalities have been noted. Defective chemotaxis, deficient degranulation responses, and ineffective phagocytosis and killing have all been reported. The clinical importance of these observations has not been clearly established [21-24].

Molecular cloning and expression of cDNA for hG-CSF have been described. The mature human G-CSF is a 18.8 kDa protein of 174 amino-acid polypeptide chain with two intra-molecular disulphide bonds between residues Cys36-Cys42 and Cys64-Cys74 and one free cysteine at residue 17.Native hG-CSF has a single O-glycosylation site at Thr133, which protects the protein from aggregation but is not crucial for biological activity. The recombinant hG-CSF produced by E.coli, has identical biological activity to that of endogenous protein, but differs in that it contains an N-terminal methionine residue and is not glycosylated.

Recent publications describe various protocols of cloning, expression and purification of the rhG-CSF. These protocols involve use of several chromatography columns, high amount of detergents for the purification of G-CSF and some of them were not applicable to recombinant G-CSF expressed in E. coli as inclusion bodies In industry, production of biopharmaceuticals employing a simple and cost-efffective process involving fewer steps and yielding high levels of active protein is an essential prerequisite [25-29]. Here we describe the cloning of recombinant human granulocyte colony-stimulating factor gene, protein expression in E. coli cells, a straightforward purification protocol of the active recombinant protein from inclusion bodies and characterization

of rhG-CSF by analytical methods. We believe that the combination of the different experimental strategies presented here provides an efficient protocol that may be useful in the industrial process of rhG-CSF protein production.[30-32].

Objectives: Cloning of GCSF cDNA and expression of the granulocyte colony stimulating factor protein.

Methodology

Primer designing:

Oligonucleotides were manually designed and synthesized, in even numbers, corresponding to the double stranded DNA, in a sequential manner. The G-CSF nucleotide sequence (Accession number NM_000759) of 522 bp, was divided in 12 short sequences of approximately 50 bp each. Oligonucleotides contained overlapping regions of about 10 bases at their 5'- and 3'-ends. The primers employed in the present study are given in Table 1. The primers P1 and P12 contained the restriction sites for, respectively, Nde I and Bam HI. The 522 bp final PCR product was agarose-gel purified and cloned into the pJET1.2 Blunt vector (Fermantas).

PCR AMPLIFICATION:

The pairs of oligonucleotides were assembled and PCR amplified to yield the hG-CSF coding DNA sequence in a step-wise fashion. PCR-amplified DNA fragments with the expected sizes were detected by 2% agarose gel electrophoresis stained with 0.5 µg mL-1 of Ethidium Bromide, purified and another round of PCR amplification carried out.

AGAROSE GEL ELECTROPHORESIS:

1. The gel was prepared by adding 0.6 gm of agarose in 40ml of 1x TAE buffer. agarose was melted at 75°C and Add 4ul of ethidium bromide (0.5ug/ml- final concentration) when the gel was at 50°C. Transfer the content to casting tray.

2. The amplified product of target region (along with loading dye that also contains glycerol) was then loaded in to wells and was run at 100V for about 30 minutes.

3. After 30 minute run, observe gel under UV transilluminator and document the results using Gel DOC apparatus.

ELUTION OF DNA FROM GEL (Spin column method):

Principle:

The ethidium bromide stained fragment of interest is excised from the agarose gel.

The agarose gel slice is dissolved in a chaotropic solution with the addition of heat and then placed into a spin column where the DNA comes into contact with a glass fiber membrane and DNA binds to the membrane. Salts, organics and other contaminating molecules are washed away, and the purified DNA is then eluted with either Elution Buffer or Molecular Biology Grade Water.

Assay Procedure:

1. The separated DNA was cut with a new sterilized blade and then transformed in to a sterile 1.5ml tube.

2. Added 750ul of binding buffer (i.e three times the weight of the gel piece).

3. The eppendorf tube was then kept in a water bath that was preset at 60°C, for about 10 minutes.

5. The entire mix was then slowly transferred to the column placed in 2.0ml collection tube and was spin at 10000rpm for 2 minutes at room temperature.

6. Discarded the eluted solution and then added 750ul of wash buffer to the column and centrifuged at 10000rpm for 2 minutes at room temperature.

7. Centrifugation step was repeated to remove excess isopropanol from the sample.

8. The column was then transferred to a fresh collection tube and added 50ul of autoclaved distilled water. Spin at 10000rpm for 2 minutes at room temperature.

9. Again added 10ul of autoclaved distilled water and centrifuged at 10000rpm.

10. Transferred the eluted product in to clean 1.5ml eppendorf tube and preserved at -20°C.

PCR PRODUCT CLONING:

For cloning PCR products with 3'-dA overhangs generated by Taq DNA polymerase,

the DNA Blunting Enzyme is a proprietary thermostable DNA polymerase with proofreading activity. It will remove 3'- overhangs and fill-in 5'-overhangs. Nucleotides for the blunting reaction are included in the reaction buffer.

METHODOLOGY:

Designing primers from cDNA

↓

Aligning 12 different primers together by anchor PCR

↓

Gel elution of PCR amplified product at each step to remove primers

↓

Ligation of aligned product into blunt end cloning vector

↓

Transformation of ligated product into host cell

↓

Isolation of plasmid DNA

↓

Confirmation of recombinants by PCR assay

RESULTS:

Primers designed were shown bellow

P1-5' AA CATATGACCCCCCTGGGCCCTGCCAGCTCCCTGCCCCAGAGCTTCCTGCTC AAGTG 3'

P2-5' GCGCTGCGCCATCGCCCTGGATCTTCCTCACTTGCTCTAAGCACTTGAGCA 3'

P3-5' GGCGCAGCGCTCCAGGAGAAGCTGTGTGCCACCTACAAGCT 3'

P4-5' CCAGAGAGTGTCCGAGCAGCACCAGCTCCTCGGGGTGGCACAGCTTGTAGG 3'

P5-5' CACTCTCTGGGCATCCCCTGGGCTCCCCTGAGCAGCTGCCCCAGCCAGGCCCTG CAG 3'

P6-5' GGTAGAGGAAAAGGCCGCTATGGAGTTGGCTCAAGCAGCCTGCCAGCTGCA GGGCC 3'

P7-5' TTCCTCTACCAGGGGCTCCTGCAGGCCCTGGAAGGGATCTCCCCCGAGTTGGGT C 3'

P8-5' TGGTGGCAAAGTCGGCGACGTCCAGCTGCAGTGTGTCCAAGGTGGGACCCAACT C 3'

P9-5' TTTGCCACCACCATCTGGCAGCAGATGGAAGAACTGGGAATGGCCCCT GCC CT 3'

P10-5' AAAGCAGAGGCGAAGGCCGGCATGGCACCCTGGGTGGGCTGCAGGGCAGGGG 3'

P11-5' CCTCTGCTTTCCAGCGCCGGGCAGGAGGGGTCCTGGTTGCCTCCCATCTGC AGAGCTTCC 3'

P12-
5'AAGATCCTCAGGGCTGGGCAAGGTGGCGTAGAACGCGGTACGACACCTCCAGGAA GCTCTG 3'

Primer sequences for construction of hG-CSF. The G-CSF nucleotide sequence (Accession number NM_000759) of 522 bp, was divided into 12 DNA sequences of approximately 50 bp each, containing 10-bp overlapping regions at their 5'- and 3'-ends for adjacent oligonucleotides. The restriction sites, *Nde* I and *Bam* HI, are underlined in primers P1 and P12, respectively.

PCR Result:

A full length cDNA was constructed by using anchor PCR as shown in figure 4. The full length cDNA was cloned into pJET1.2 as shown in figure 7. The clones were confirmed by PCR as shown in figure 8

DISCUSSION:

The human G-CSF was formally first been applied to the leukopenia in US in 1991. At present, the hG-CSF is the most widely-used and clinically effective haematopoietic growth factors. The randomized studies using rhG-CSF versus placebo after chemotherapy for cancers resulted in faster neutrophil recovery, less severe neutropenia, and infections reduced. we constructed the prokaryotic expression vector PJET1.2 containing human G-CSFb cDNA, and need to achieve high level expression of the hG-CSF in E. coli.

Tables:

Table 1: Thermocycling conditions1:

Stage 01		**Stage 02**		**Stage 03**		**Stage 04**	
Hold **(Initial Denaturation)**		**Three Temperature cycle** (Repeat 5 times)		**Two Temperature cycle** (Repeat 39 times from step 6)		Hold	
Temperature	**Time**	**Temperature**	**Time**	**Temperature**	**Time**	**Temperature**	**Time**
95°C	1 min	95°C	15Sec	95°C	15Sec	4°C	∞
		45°C	30Sec	68	45		
		72°C	1min				

Table 2: Thermocycling conditions2:

Stage 01		**Stage 02**		**Stage 03**		**Stage 04**	
Hold **(Initial Denaturation)**		**Three Temperature cycle** (Repeat 40 times)		**OneTemperature**		Hold	
Temperature	**Time**	**Temperature**	**Time**	**Temperature**	**Time**	**Temperature**	**Time**
95°C	1 min	95°C	15Sec	72°C	5min	4°C	∞
		45°C	30Se				
		72°C	1min				

Table 3: Reaction Setup:

Component	25 µl reaction	50µl reaction	Final Concentration
10X Standard *Taq* Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Template DNA	2ul	4.0ul	<1,000 ng
Taq DNA Polymerase (5000U/ml)	0.3 µl	0.6 µl	1. unit/R PCR
Nuclease-free water	to 25 µl	to 50 µl	

Table 4: APPROACH 1

Steps		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
I ST STEP	PRIMERS	P1& P2		P3& P4		P5& P6		P7& P8		P9& P10		P11& P12	
	PRODUCT	P1		P2		P3		P4		P5		P6	
2nd STEP	PRIMERS	P1 & P4				P5&P8				P9&P12			
	TEMPLATE	P1 & P2				P3&P4				P5&P6			
	2ndPRODUCT	P7				P8				P9			
3rd STEP	PRIMERS	P1 & P8											
	TEMPLATE	P7&P8											
	3rdPRODUCT	P10											
4th STEP	PRIMERS	P1 & P12											
	TEMPLATE	P10 & P9											
	4th RODUCT	No Amplification											

Table 5: APPROACH 2

Steps		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
I ST STEP	PRIMERS	P1& P2		P3& P4		P5& P6		P7& P8		P9& P10		P11& P12	
	PRODUCT	P1		P2		P3		P4		P5		P6	
2nd STEP	PRIMERS	P1 & P4				P5&P8				P9&P12			
	TEMPLATE	P1 & P2				P3&P4				P5&P6			
	2ndPRODUCT	P7				P8				P9			
3rd STEP	PRIMERS					P5&P12							
	TEMPLATE					P8&P9							
	3rd RODUCT					P10							
4th STEP	PRIMERS	P1 & P12											
	TEMPLATE	P10 & P7											
	4thPRODUCT	No Amplification											

Table 6: APPROACH 3

Steps		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
I ST STEP	PRIMERS	P1& P2		P3& P4		P5& P6		P7& P8		P9& P10		P11& P12	
	PRODUCT	P1		P2		P3		P4		P5		P6	
2nd STEP	PRIMERS	P1 & P4								P9&P12			
	TEMPLATE	P1 & P2								P5&P6			
	2ndPRODUCT	P7								P8			
3rd STEP	PRIMERS	P1&P6						P7&p12					
	TEMPLATE	P7&P3						P4&p8					
	3rd RODUCT	P9						P10					
4th	PRIMERS	P1 & P12											

STEP	TEMPLATE	P9 & P10
	4th RODUCT	With gradient at 58°C amplification is present

For approach 1 and 2 till the last step we used thermal cycling conditions 1 and at the last step we used Thermal cycling conditions 3. Approach three was performed with Thermal cycling conditions 1 and 3

SYNOPSIS

INTRODUCTION: Granulocyte colony-stimulating factor (**G-CSF** or **GCSF**) is a colony-stimulating factor hormone. G-CSF is also known as**colony-stimulating factor 3** (**CSF 3**).It is a glycoprotein, growth factor and cytokine produced by a number of different tissues to stimulate the bone marrow to produce granulocytes and stem cells. G-CSF then stimulates the bone marrow to release them into the blood. G-CSF also stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils.

CONCLUSION:

Full length cDNA was constructed and cloned into blunt end vector. Further the cloned product need to express to get the recombinant H-GCSF protein to purify it. Construction and cloning of h-GCSF cDNA was completed and the protein purification has to be standardized.

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