Application of Biotechnology for Production of Recombinant Protein (Human Interferon Gamma)

Thesis

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Declaration

I declare that this thesis has been composed by myself and that work of which it is a record has been done by myself. It has not been submitted for a degree at this or any other university.

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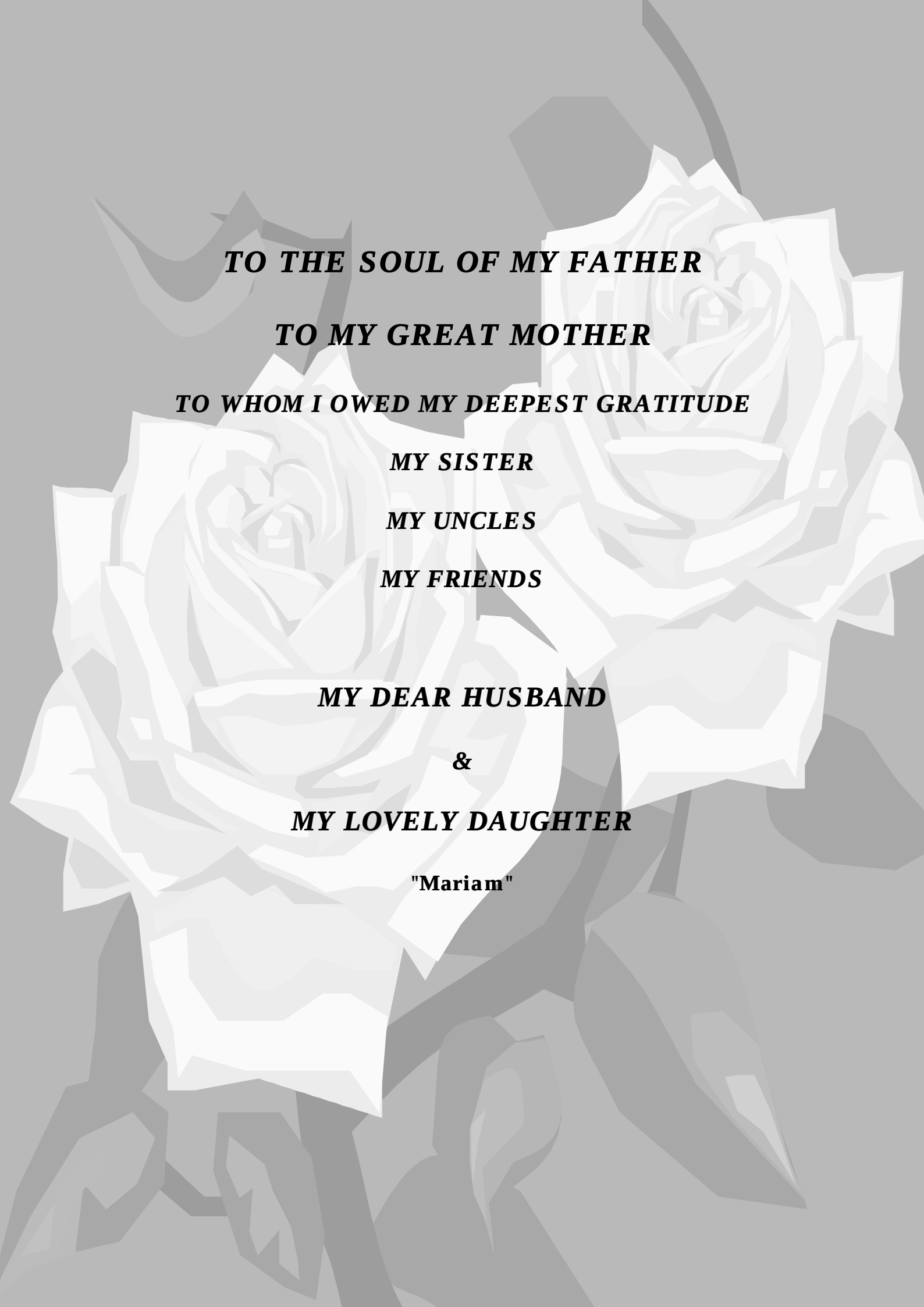

TO THE SOUL OF MY FATHER

TO MY GREAT MOTHER

TO WHOM I OWED MY DEEPEST GRATITUDE

MY SISTER

MY UNCLES

MY FRIENDS

MY DEAR HUSBAND

&

MY LOVELY DAUGHTER

"Mariam"

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ABSTRACT

Interferon gamma (IFN-γ), also called immune interferon, is chemically and pharmacologically distinct from IFN-α and IFN-β. It has weaker antiviral activity, more potent immunomodulator properties including macrophage activation, produced mainly by activated T-cells and natural killer.

IFN-γ is a drug used to reduce the frequency and severity of serious infections associated with chronic granulomatous disease (CGD) and delays the effects of osteopetrosis in patients with severe malignant forms. IFN-γ is currently in late stage of clinical trials in treatment of HCV and Leshmaniasis.

In this study hIFN-γ was induced and isolated from human Peripheral blood mononuclear cells (PBMC's), analyzed by digestion, sequencing and cloned in pCRII TOPO TA cloning vector to maintain its integrity.

The hIFN-γ ORF was sub-cloned in pET-15b expression vector and analyzed by PCR. The recombinant pET-15b/hIFN-γ vector was transformed into several expression bacterial strains and was successfully expressed using Isopropylthio -D-galactoside (IPTG) as an inducer in Rosetta (DE3) bacterial strain and tested by Western blot.

The results of this work indicate that the expressed recombinant protein is the hIFN-γ protein which confirmed by the molecular weight and the immunogenicity against the hIFN-γ antibodies.

LIST OF ABBREVIATIONS

a.a	Amino acid
Ab	Antibody
AD	Atopic dermatitis
ampR	Ampicillin resistance
APCs	Antigen presenting cells
Arg	Arginine
Asp	Aspartic acid
AMV	Avian myeloblastosis virus
BCGF	B-cell growth factor
BHK	Baby hamster kidney
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
cfu	Colony forming per unit
CGD	Chronic granulomatous disease
CHO	Chinese hamster ovary
Claudin1	CLDN1
CMV	Cytomegalovirus
DAB	3, 3'-Diaminobenzidine
DEPC	Diethylpyrocarbonate
DNA	Deoxribonucleic acid
dNTP	Deoxy nucleotide tri-phosphate

E. coli	*Escherichia coli*
FDA	Food and drug administration
EDTA	Ethyline diamine tetraacetate
FBSA	Fetal bovine serum albumin
FCS	Fetal calf serum
GAF	Gamma interferon activator factor
GAS	Gamma interferon activation site
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
HBsAg	Hepatitis B surface antigen
HGH	Human growth hormone
hIFN-γ	Human interferon gamma
IBs	Inclusion bodies
IFN-α	Interferon alpha
IFN-β	Interferon beta
IFN-γR	Interferon gamma receptor
IFN-ω	Interferon omega
IFN-τ	Interferon tau
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
Ile	Isoleucine
IPTG	Isopropylthio -D-galactoside
IRF	Interferon transcription factor
ISGF	Interferon stimulated gene factor

ISRE	Interferon stimulated response element
JAK	Janus family kinase
KDa	Kilo-dalton
lac	Lactose utilization operon
LB medium	Luria-Bertani medium
Leu	Leucine
Lys	Lysine
MCS	Multiple cloning site
MHC	Major histocompatibility complex
MNC	Mononuclear cells
MOPS	3-[N-morpholino]propanesulfonic acid
mRNA	Messenger Ribonucleic Acid
MW	Molecular weight
NCBI	National Center for Biotechnology Information
NK	Natural killers
O.D	Optical density
ORF	Open reading frame
Ori	Origin of replication
OPC	Oropharyngeal candidiasis
PAGE	Polyacrylamide gel electrophoresis
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PHA	Phytohemagglutinin
PGK	3phosphoglycerate kinase promoter
pL	Phage lambda promoter

PMN or PML	Polymorphonuclear leukocytes
PMSF	Phenyl methyl sulfoxide
PPD	Purified protein derivatives
Pro	Proline
PSI-BLAST	Position-specific iterated BLAST
PTPs	Protein tyrosine phosphatases
PVDF	Polyvinylidene flouride
RBS	Ribosomal binding site
RNA	Ribonucleic acid
rpm	Round per minute
RPMI	Roswell park Memorial institute
RT-PCR	Reverse transcription PCR
S. cerevisiae	*Saccharomyces cerevisiae*
SDS	Sodium dodecyl sulfate
SEA	Staphylococcus enterotoxin A
SEB	Staphylococcus enterotoxin B
SOC	Super optimal catabolic repression medium
Stat	Signal transducer and activator of transcription
STD	Standard for DNA
Ta	Annealing temperature
TAE	Tris-acetate DNA electrode buffer
TE	Tris EDTA buffer
TEMED	N, N, N`, N` –tetra-methylenediamine
Th	T helper cell
$\boldsymbol{T_m}$	Melting temperature

TNF	Tumor necrosis factor
TPA	Tissue plasminogene activator
tRNA	Transfer Ribonucleic Acid

LIST OF FIGURES