

Targeted DNA transfer in eukaryotic cells based on laser technology

**A Thesis Submitted in the Partial Fulfillment of the
Requirements for the degree of
Master of Science**

**In
Laser Interaction with Matter Group**

Department of Laser Sciences and Interactions

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2001**

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Abstract

We have modified our previously designed system for plant cells using laser microbeam cell surgery to be matched with animal cells.

These modifications included: 1- Using different laser source (low power He-Cd at 441.5 nm wavelength in the visible region instead of Excimer laser at 308 nm wavelength in the near UV region). 2- Instead of using pulsed laser, we have used here CW He-Cd laser chopped by electrical chopper which is synchronized with the mechanical motion of the moving stage. The main advantage of the modified laser setup for gene transfer: it was less damaging to the sensitive animal cells which have thin cell membranes.

The present work aimed to: 1- Design a modified laser microbeam cell surgery setup, applicable to animal cells such as fibroblast cells. 2- Introduce pBK-CMV phagemid vector, containing *LacZ* and neomycin resistance genes into fibroblast cell line (BHK-21). 3- Assure gene transfer and its expression in the used cells. 4- Examine the efficiency of such system. 5- Evaluate the resulting cell damage using the laser beam for the gene transfer.

The main results of this work: 1- Our modified laser microbeam setup proved its efficiency as a tool for gene transfer into fibroblast cells. 2- We have achieved transfection efficiency (8.97- 12%). 3-No damages or fragmentations for any component or organelle of transfected fibroblast cell resulted from applying laser microbeam compared with control cell as indicated by transmission electron microscope technique.

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List of abbreviations

AcMNPV	<i>Autographa californica</i> multiple nuclear polyhedrosisvirus
ACTH	Adenocorticotropic hormone
AdPirin	Adenovirus pirin
ADV	Adenoviruses
AF	Annulus fibrosus
AIG	Anthrax Immune Globulin
ANEPPS	fast responding membrane potential dyes
ANF	Atrial Natriuretic Factor
AP	Action Potential
Ar ⁺	Argon ion laser
Ar F	Argon Floride laser
ATP	Adenosine Triphosphate
BALB/c	albino strain of laboratory mouse
BALB/3T3	mouse embryonic stem cells
BALB/MK-2	mouse keratinocytes
BEAS-2B	bronchial epithelial cells
BHK-21	Baby Hamster Kidney fibroblast cells
bp	base pair
BR14	third-generation microbubble
c	speed of light
C	Chromatin
°C	degree centigrate
Ca P _i	Calcium Phosphate
CCD	Charge Coupled Device
CD16	Fc receptor
cDNA	complementary Deoxy Nucleotide Acid Transferase
CEF	Chicken Embryo Fibroblasts
CHO	Chinese Hamster Ovary cell line
CHO-K1	Chinese Hamster Ovary cell line
Chol	Cholestrol
CLSM	Confocal Laser Scanning Microscopy
cm	centimeter
Cm	Cell membrane
CMV	Cytomegalovirus
COS-1	african green monkey fibroblast-like cell line
COS-7	cell line derived from kidney cells of African green monkey
CO ₂	Carbon dioxide
CTL	Cytotoxic T Lymphocyte
CW	Continuous Wave
C57BL/6	inbred strain of lab mouse
dbcAMP	Dibutyryl cyclic Adenosine Monophosphate
DC-Chol-DOPE	cationic lipooome
ddH ₂ O	double distilled water

DEAE	Diethylaminoethyl
<i>dhfr</i>	dihydrofolate reductase gene
DH5 α	strain of <i>E.coli</i> bacteria
DK1	Dolphin epithelial cells
DMF	Dimethylformamide
DNA	Deoxy ribonucleic acid
dNTPs	deoxy Nucleotide Triphosphate
DOPE	Dodecyl phosphatidylethanolamine
DOTAP	1,2-Dioleoyl-3-Trimethylammonium-Propane (Chloride Salt)
DsRed	Red fluorescent protein
e	electron
EBV	Epestein-Barr virus
ECs	Endothelial cells
EDTA	Ethylene Diamine Tetra- Acetic acid
EGFP	Enhanced Green Fluorescence Protein
<i>EGFP</i>	Enhanced Green Fluorescence encoded gene
EMEM	Earle's Minimal Essential Medium
EPO	Erythropoietin
Er: YAG	Erbium doped in Yttrium Aluminum Garnet
Et Br	Ethidium bromide stain
ET-1	Endothelin-1
EYFP	Encoding Yellow Fluorescent Protein
ΔE	Energy
F1	first family
F2	second family
FACS	Facial Action Coding System
FCS	Feotal Calf Serum
FISH	Fluorescence <i>In Situ</i> Hybridization
FRAT	Frequently Rearranged in Advanced T-cell lymphomas
FRAT1	Frequently Rearranged in Advanced T-cell lymphomas 1
FRAT2	Frequently Rearranged in Advanced T-cell lymphomas 2
G418	aminoglycoside antibiotic (Geneticin)
GATA4	GATA binding protein 4
GFP	Green Fluorescent Protein
GHRH	Growth Hormone Releasing Hormone
gPGCs	gonadal Primordial Germ Cells
G1/S	a stage in the cell cycle at the boundary between the G1 phase and the S phase
GSH	reduced form of L-glutathione
GSK-3	Glycogen Synthesis Kinase-3
h	hour
H-2Kb	type of antibody
HB	Human Hepatoblastoma
He-Cd	Helium Cadmium laser