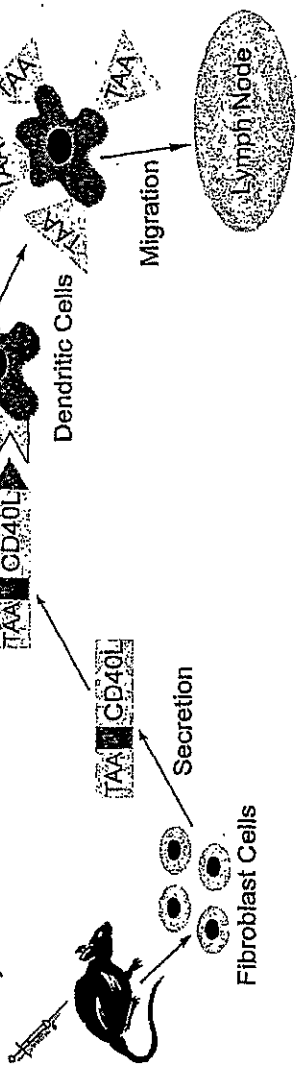
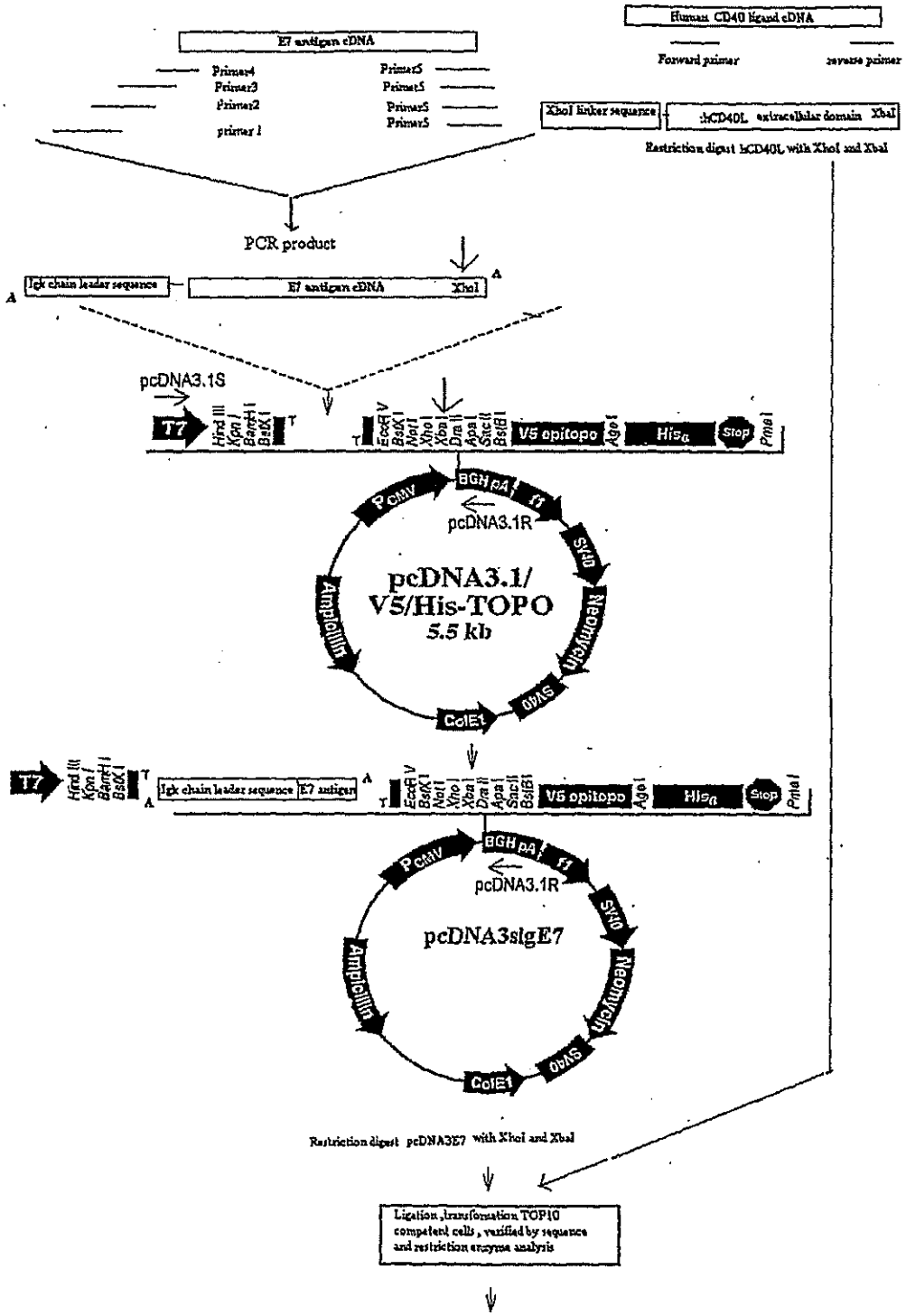
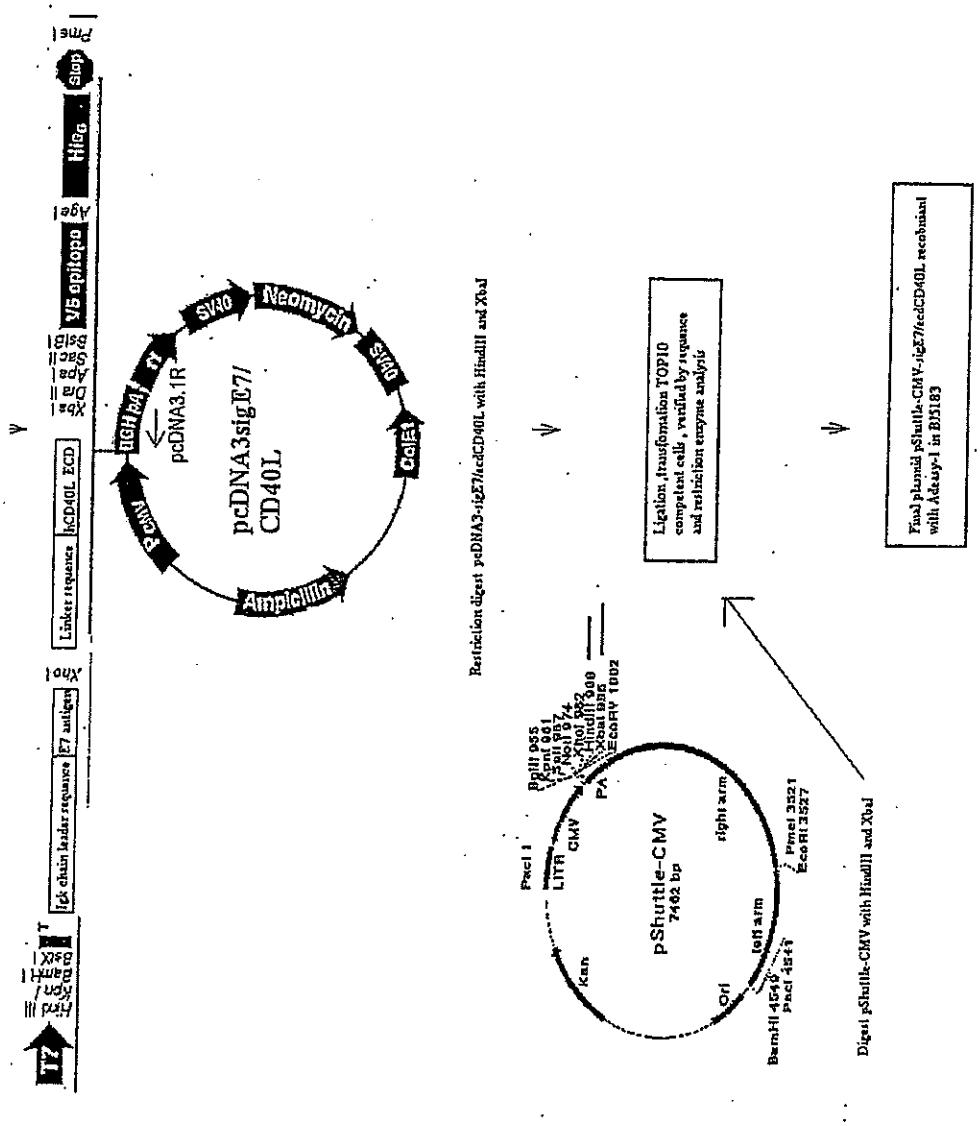
FIG. 1


s.c. injection of Adenovirus
Fibroblast Cells
Secretion
TAA
CD40L
TAA
CD40L
Dendritic Cells
Dendritic Cells
present TAA Antigen
TAA
TAA
TAA
TAA
Migration
Lymph Node


Human CD40 ligand cDNA
Forward primer
reverse primer
E7 antigen cDNA
Primer4
Primer3
Primer2
primer 1
Primer6
Primer5
Primer6
Primer5
XhoI linker sequence
hCD40L extracellular domain XbaI
Restriction digest hCD40L with XhoI and XbaI
PCR product
Igk chain leader sequence
E7 antigen cDNA
XhoI
pcDNA3.1S
T7
Hind III
KpnI
BamH I
BstX I
EcoR V
BstX I
Not I
Xho I
Xba I
Dra II
Apa I
Sac II
BstB I
V5 epitope
Age I
His6
Stop
Pme I
pcDNA3.1R
pcDNA3.1/
V5/His-TOPO
5.5 kb
pcDNA3sIgE7
Restriction digest pcDNA3E7 with XhoI and XbaI
Ligation ,transformation TOP10
competent cells , verified by sequence
and restriction enzyme analysis


pcDNA3sigE7/
CD40L
pcDNA3.1R
Neomycin
SV40
Ampicillin
CMV
T7
BGH pA
Ig k chain leader sequence
E7 antigen
hCD40L ECD
V5 epitope
His6
Stop
Restriction digest pcDNA3-sigE7/hcdCD40L with HindIII and XbaI
pShuttle-CMV
7462 bp
Ligation, transformation TOP10
competent cells , verified by sequence
and restriction enzyme analysis
Final plasmid pShuttle-CMV-sigE7/hcdCD40L recombinant
with Adeasy-1 in BJ5183
Digest pShuttle-CMV with HindIII and XbaI