FIG. 1

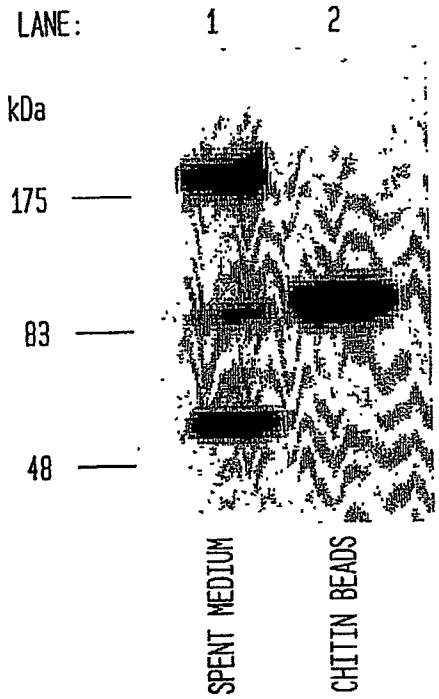


FIG. 2A

FIG. 2B

FAMILY 18 CHITINASE ACTIVE SITE

FIG. 2C

FIG. 3A

	GROWTH TIME			
	22 h	44 h	68 h	
WT	768	798	763	RFU min^{-1}
ΔKlcts1	0	0	0	

FIG. 3B

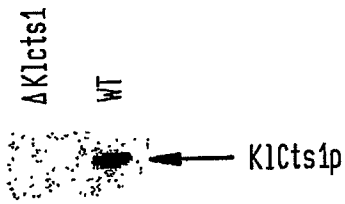


FIG. 4A

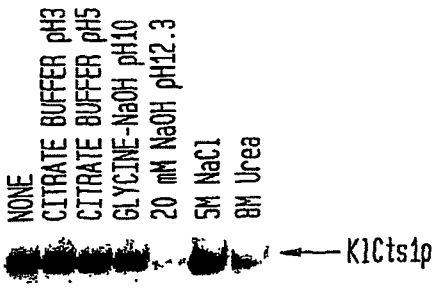


FIG. 4B

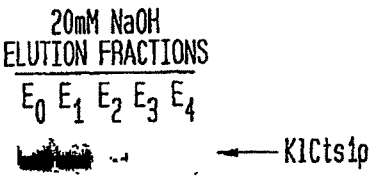


FIG. 4C

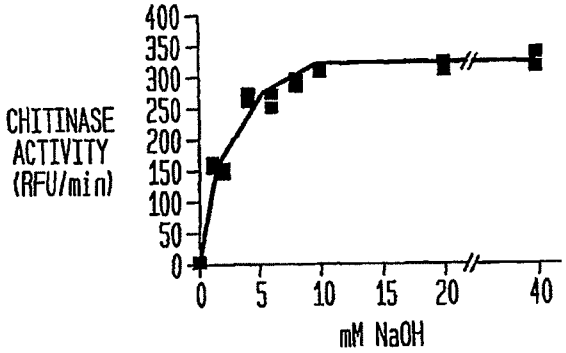


FIG. 4D

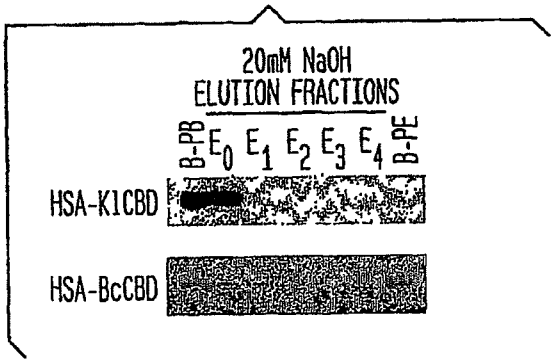


FIG. 5

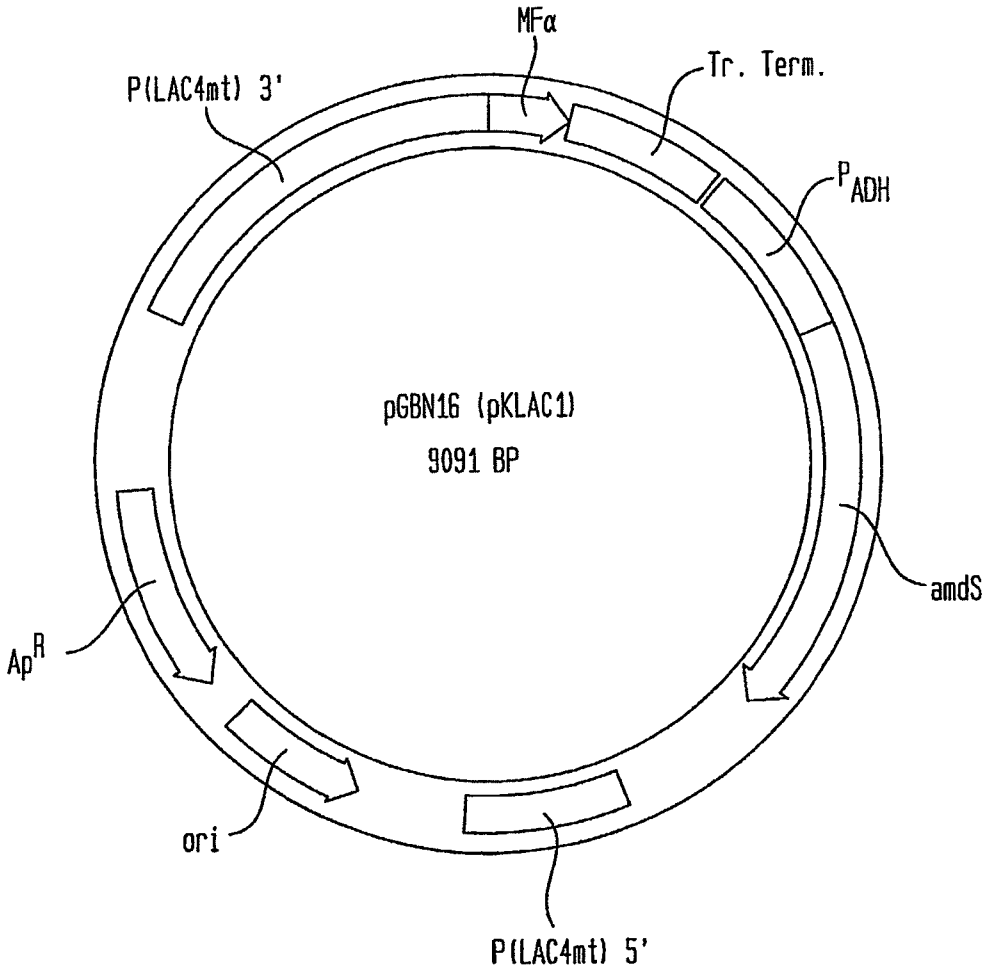

MFα
Tr. Term.
P(LAC4mt) 3'
P ADH
pGBN16 (pKLAC1)
9091 BP
amdS
Ap R
ori
P(LAC4mt) 5'

FIG. 6A

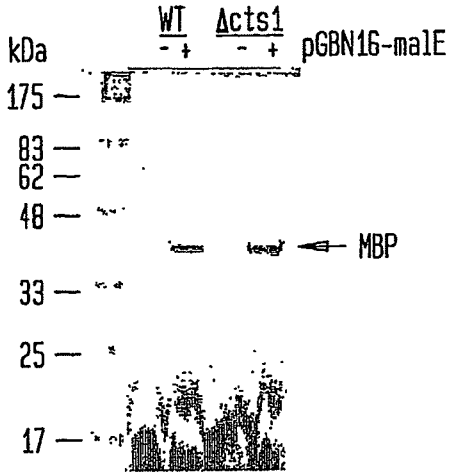

WT
Δcts1
- + - +
pGBN16-malE
kDa
175
83
62
48
33
25
17
MBP

FIG. 6B


B-PB WT
B-PB Δcts1
E0 E1 E2 E3
1 2 3 4 5 6
kDa
175
83
62
48
33
25
K1Cts1p (LANE 1 ONLY)
HSA-K1CBD

FIG. 7

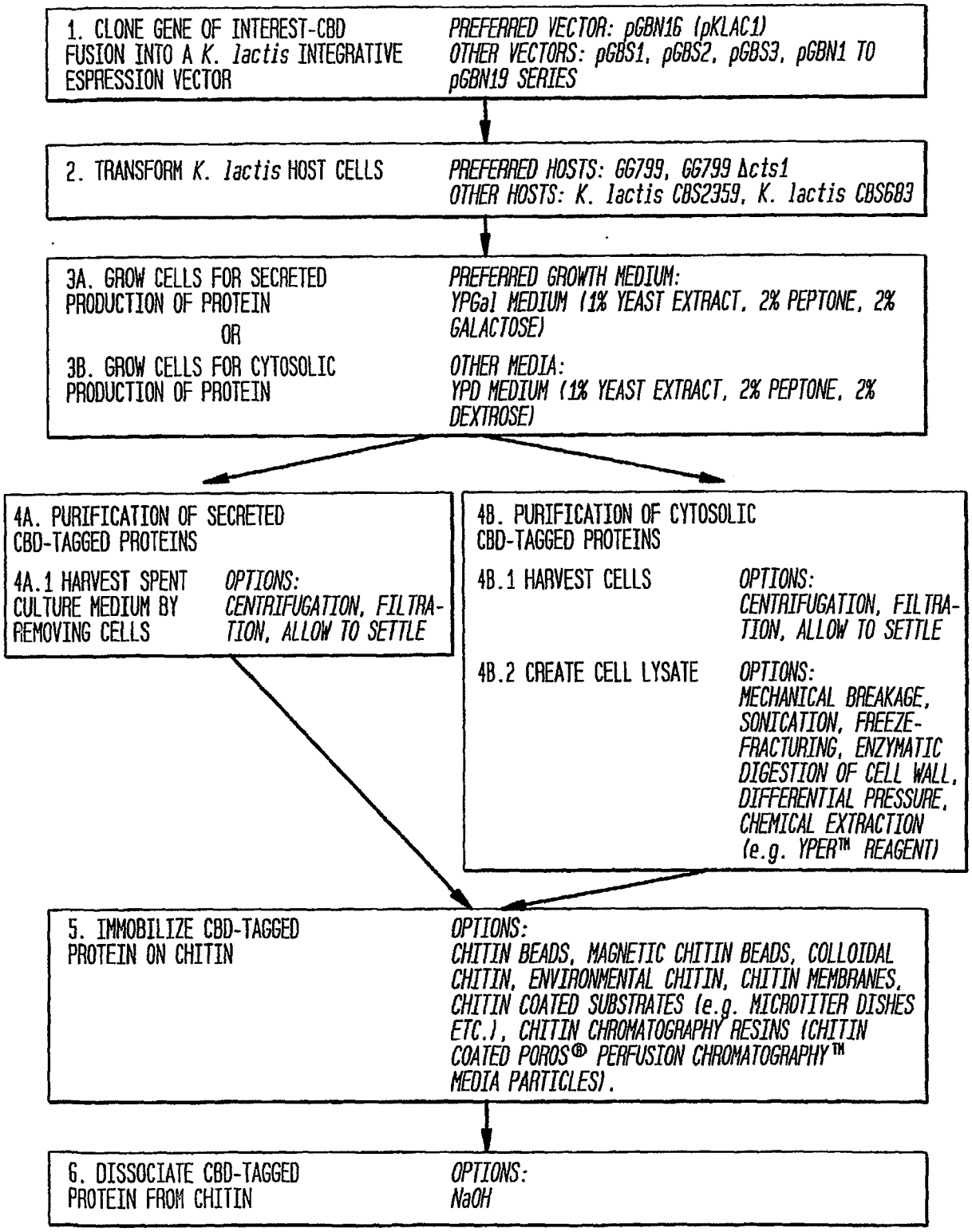

1. CLONE GENE OF INTEREST-CBD FUSION INTO A K. lactis INTEGRATIVE ESPRESSION VECTOR
PREFERRED VECTOR: pGBN16 (pKLAC1)
OTHER VECTORS: pGBS1, pGBS2, pGBS3, pGBN1 TO pGBN19 SERIES
2. TRANSFORM K. lactis HOST CELLS
PREFERRED HOSTS: GG799, GG799 Δcts1
OTHER HOSTS: K. lactis CBS2359, K. lactis CBS683
3A. GROW CELLS FOR SECRETED PRODUCTION OF PROTEIN
OR
3B. GROW CELLS FOR CYTOSOLIC PRODUCTION OF PROTEIN
PREFERRED GROWTH MEDIUM:
YPGal MEDIUM (1% YEAST EXTRACT, 2% PEPTONE, 2% GALACTOSE)
OTHER MEDIA:
YPD MEDIUM (1% YEAST EXTRACT, 2% PEPTONE, 2% DEXTROSE)
4A. PURIFICATION OF SECRETED CBD-TAGGED PROTEINS
4A.1 HARVEST SPENT CULTURE MEDIUM BY REMOVING CELLS
OPTIONS:
CENTRIFUGATION, FILTRATION, ALLOW TO SETTLE
4B. PURIFICATION OF CYTOSOLIC CBD-TAGGED PROTEINS
4B.1 HARVEST CELLS
OPTIONS:
CENTRIFUGATION, FILTRATION, ALLOW TO SETTLE
4B.2 CREATE CELL LYSATE
OPTIONS:
MECHANICAL BREAKAGE, SONICATION, FREEZE-FRACTURING, ENZYMATIC DIGESTION OF CELL WALL, DIFFERENTIAL PRESSURE, CHEMICAL EXTRACTION (e.g. YPER™ REAGENT)
5. IMMOBILIZE CBD-TAGGED PROTEIN ON CHITIN
OPTIONS:
CHITIN BEADS, MAGNETIC CHITIN BEADS, COLLOIDAL CHITIN, ENVIRONMENTAL CHITIN, CHITIN MEMBRANES, CHITIN COATED SUBSTRATES (e.g. MICROTITER DISHES ETC.), CHITIN CHROMATOGRAPHY RESINS (CHITIN COATED POROS® PERFUSION CHROMATOGRAPHY™ MEDIA PARTICLES).
6. DISSOCIATE CBD-TAGGED PROTEIN FROM CHITIN
OPTIONS:
NaOH

FIG. 8

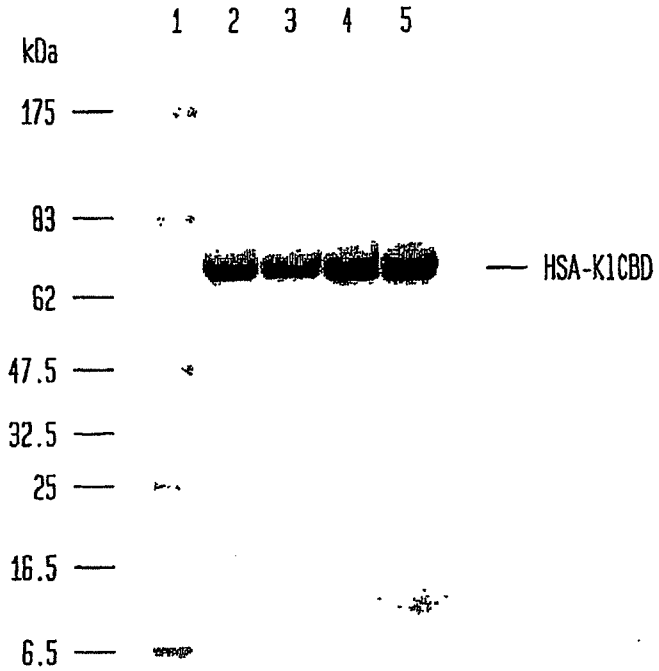


FIG. 9A

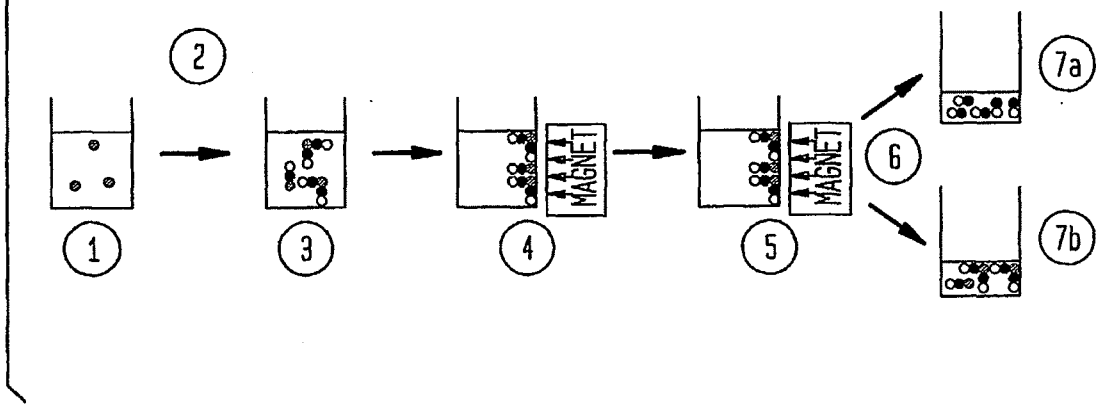

2
1
3
4
MAGNET
5
MAGNET
6
7a
7b

FIG. 9B

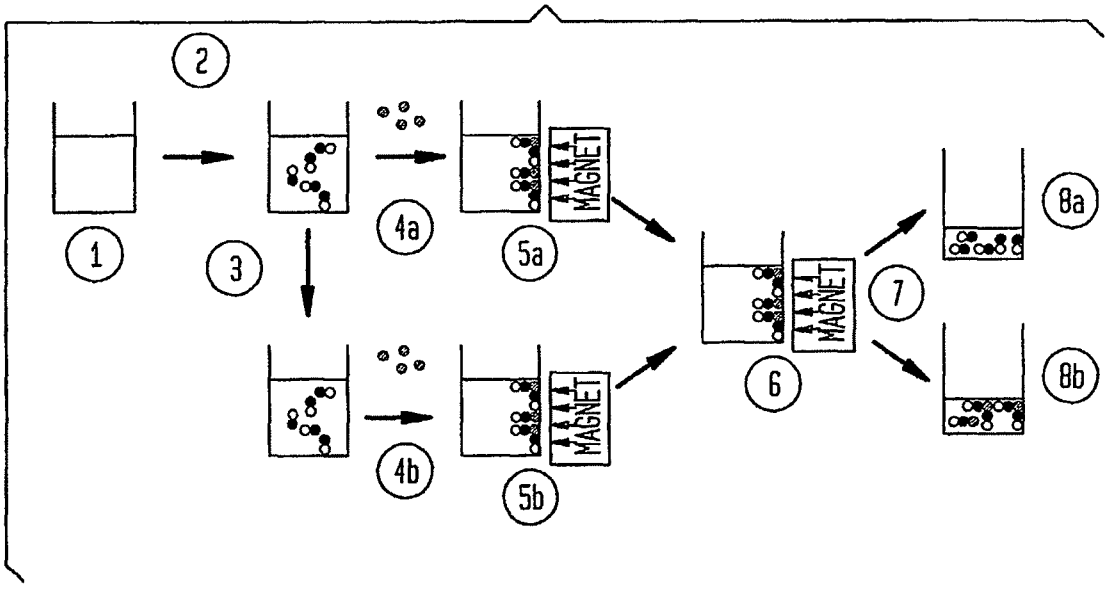

2
1
3
4a
5a
MAGNET
4b
5b
MAGNET
6
MAGNET
7
8a
8b

FIG. 10A

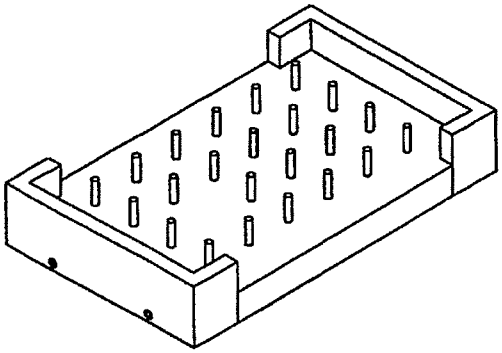


FIG. 10B

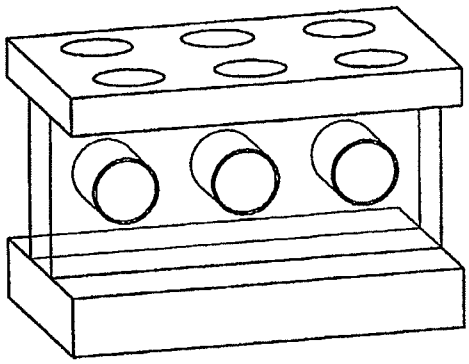


FIG. 10C

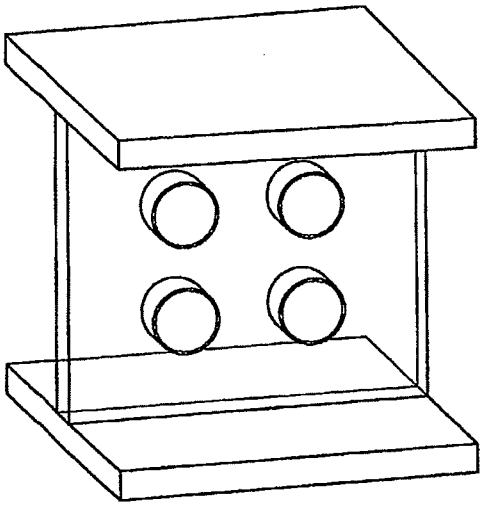


FIG. 10D

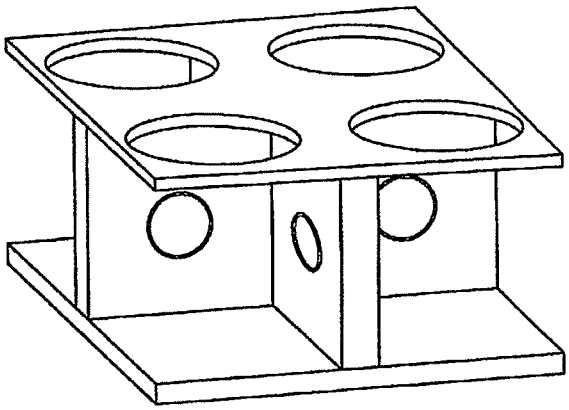


FIG. 11A

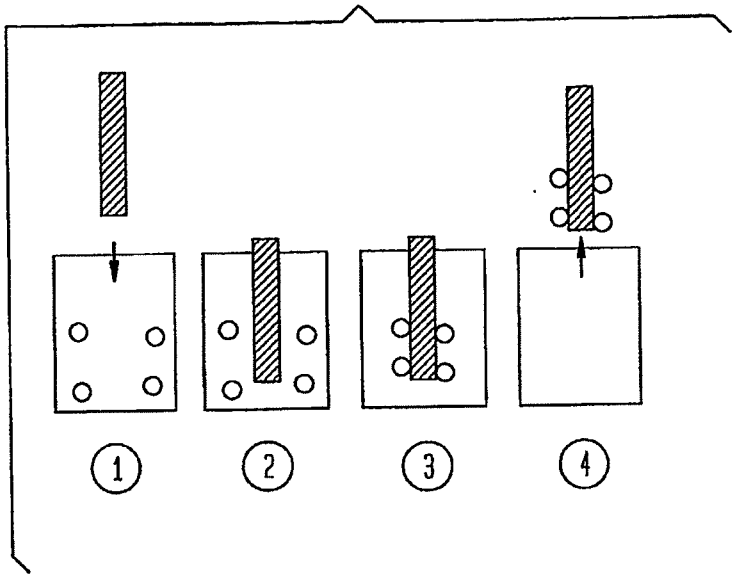


FIG. 11B

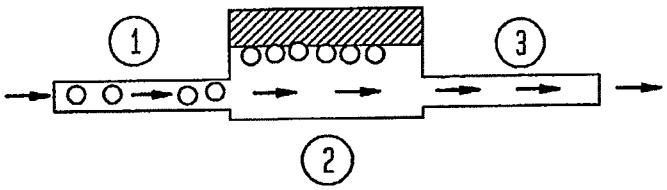


FIG. 12

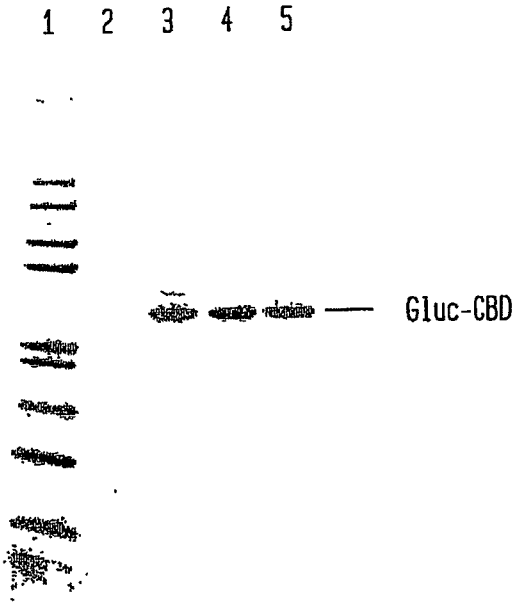
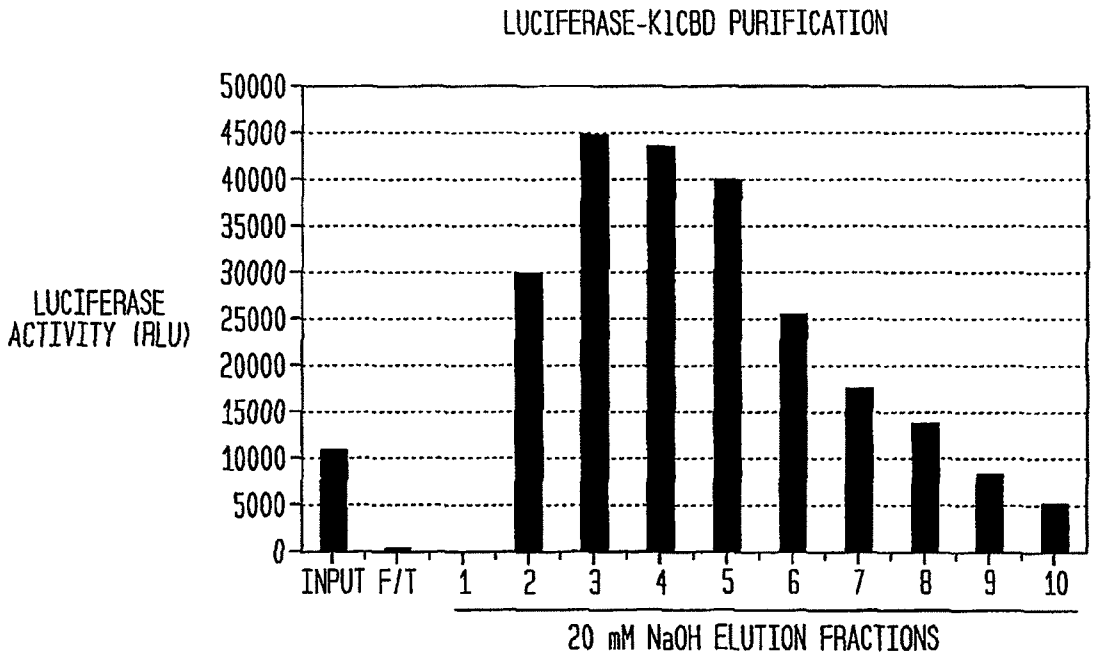


FIG. 13


LUCIFERASE-K1CBD PURIFICATION
LUCIFERASE ACTIVITY (RLU)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
INPUT
F/T
1
2
3
4
5
6
7
8
9
10
20 mM NaOH ELUTION FRACTIONS