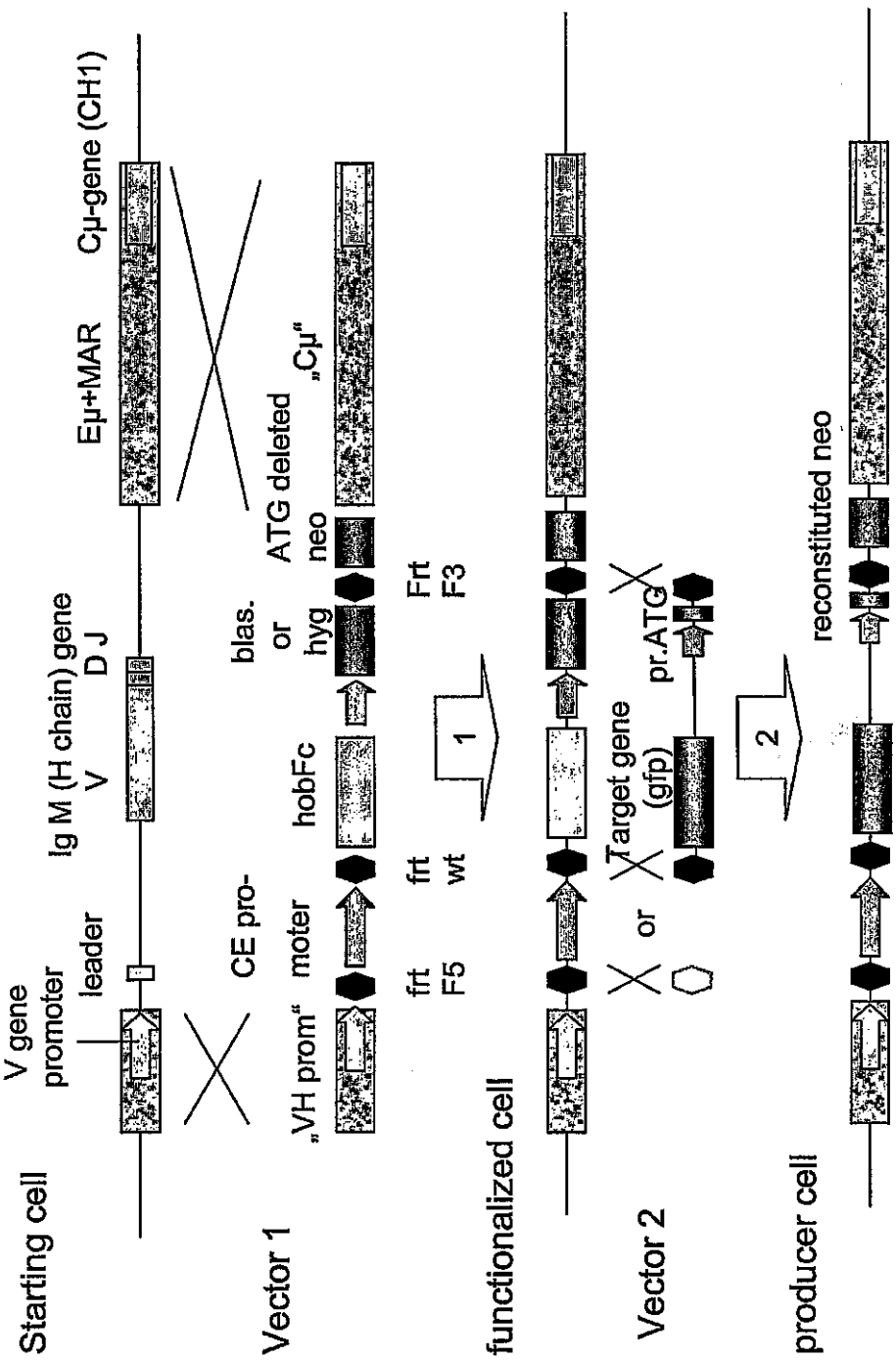

Starting cell
V gene promoter
leader
Ig M (H chain) gene
V
D J
Eμ+MAR
Cμ-gene (CH1)
Vector 1
„VH prom"
CE pro-moter
hobFc
blas. or hyg
ATG deleted neo
„Cμ"
frt F5
frt wt
Frt F3
1
functionalized cell
or
Target gene (gfp)
pr.ATG
Vector 2
2
producer cell
reconstituted neo

Fig 1a

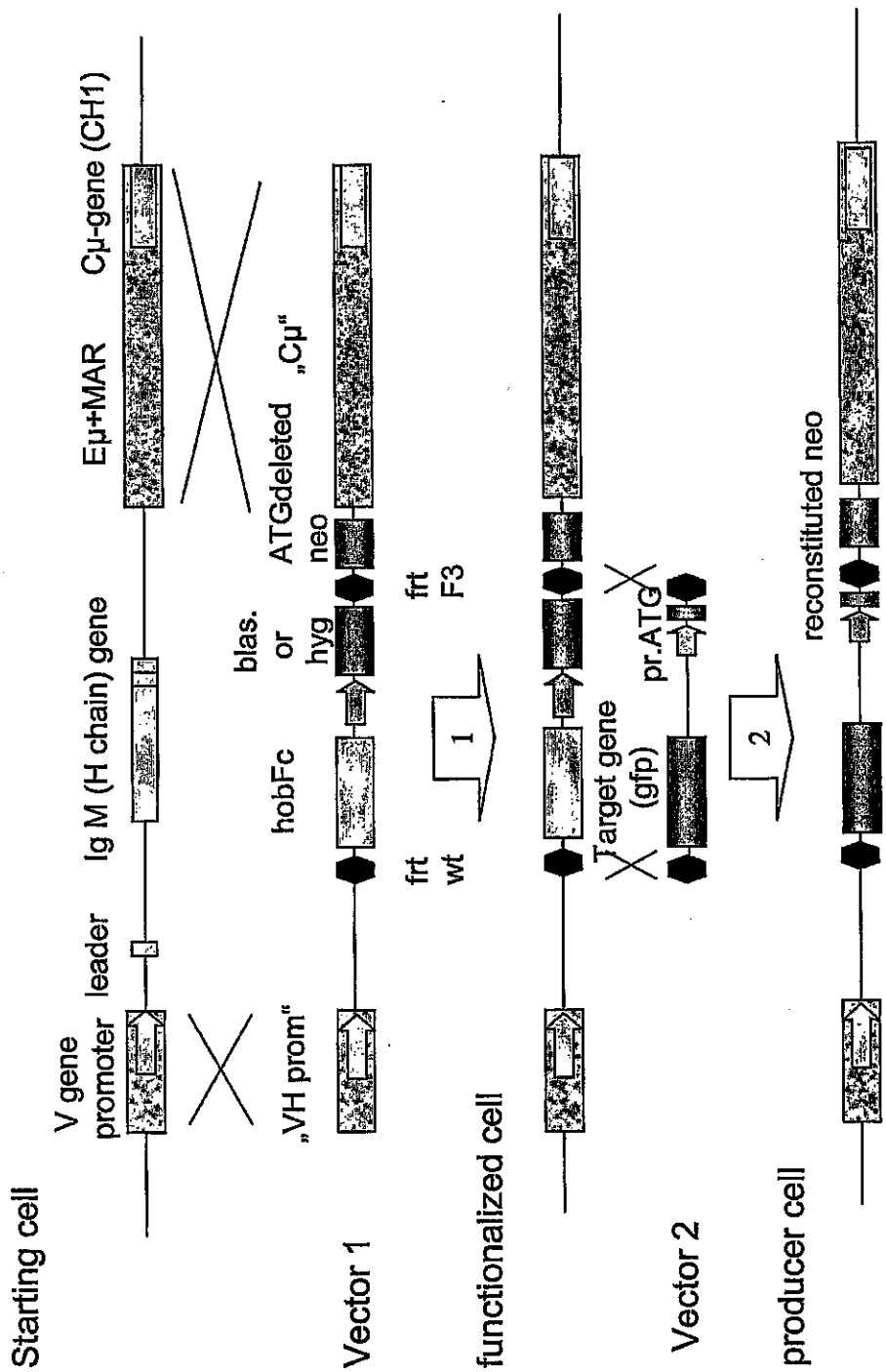

Starting cell
V gene promoter
leader
Ig M (H chain) gene
Eμ+MAR
Cμ-gene (CH1)
„VH prom"
hobFc
blas. or hyg
neo
ATGdeleted „Cμ"
Vector 1
frt wt
1
frt F3
functionalized cell
Target gene (gfp)
pr.ATG
Vector 2
2
reconstituted neo
producer cell

Fig 1b

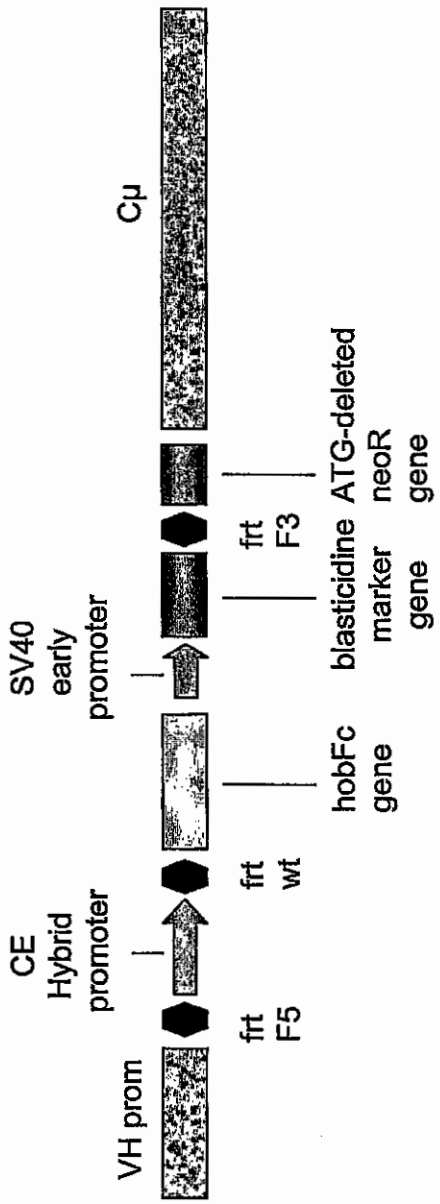

VH prom
frt F5
CE Hybrid promoter
frt wt
hobFc gene
SV40 early promoter
blasticidine marker gene
frt F3
ATG-deleted neoR gene
Cμ

Fig 2

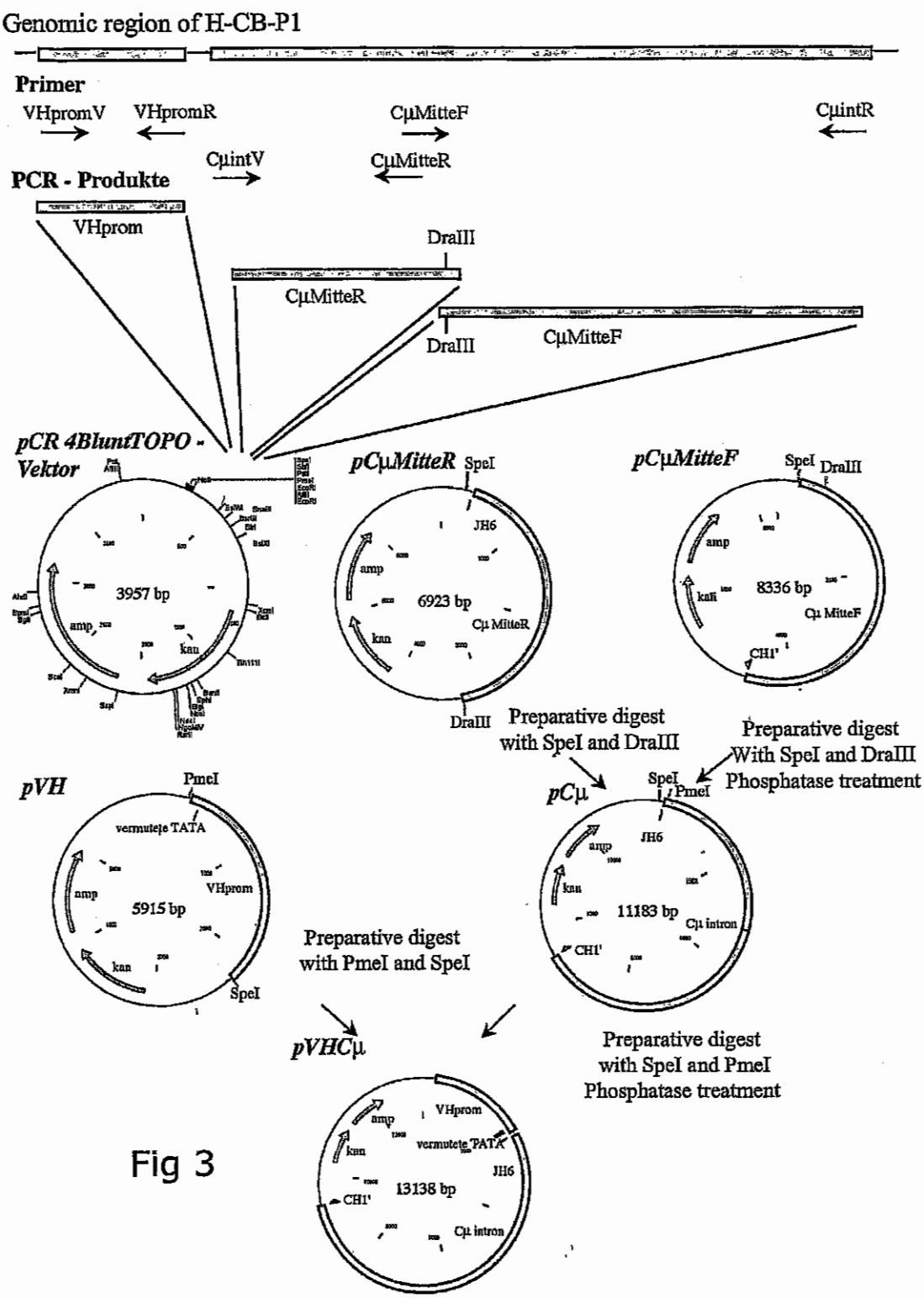

Genomic region of H-CB-P1
Primer
VHpromV
VHpromR
CμMitteF
CμintR
CμintV
CμMitteR
PCR - Produkte
VHprom
DraIII
CμMitteR
DraIII
CμMitteF
pCR 4BluntTOPO - Vektor
3957 bp
amp
kan
pCμMitteR
SpeI
JH6
6923 bp
Cμ MitteR
DraIII
pCμMitteF
SpeI
DraIII
amp
kan
8336 bp
Cμ MitteF
CH1'
Preparative digest with SpeI and DraIII
Preparative digest With SpeI and DraIII Phosphatase treatment
pVH
PmeI
vermutete TATA
VHprom
amp
5915 bp
kan
SpeI
pCμ
SpeI
PmeI
JH6
amp
kan
11183 bp
Cμ intron
CH1'
Preparative digest with PmeI and SpeI
pVHCμ
VHprom
amp
kan
vermutete TATA
JH6
13138 bp
CH1'
Cμ intron
Preparative digest with SpeI and PmeI Phosphatase treatment

Fig 3

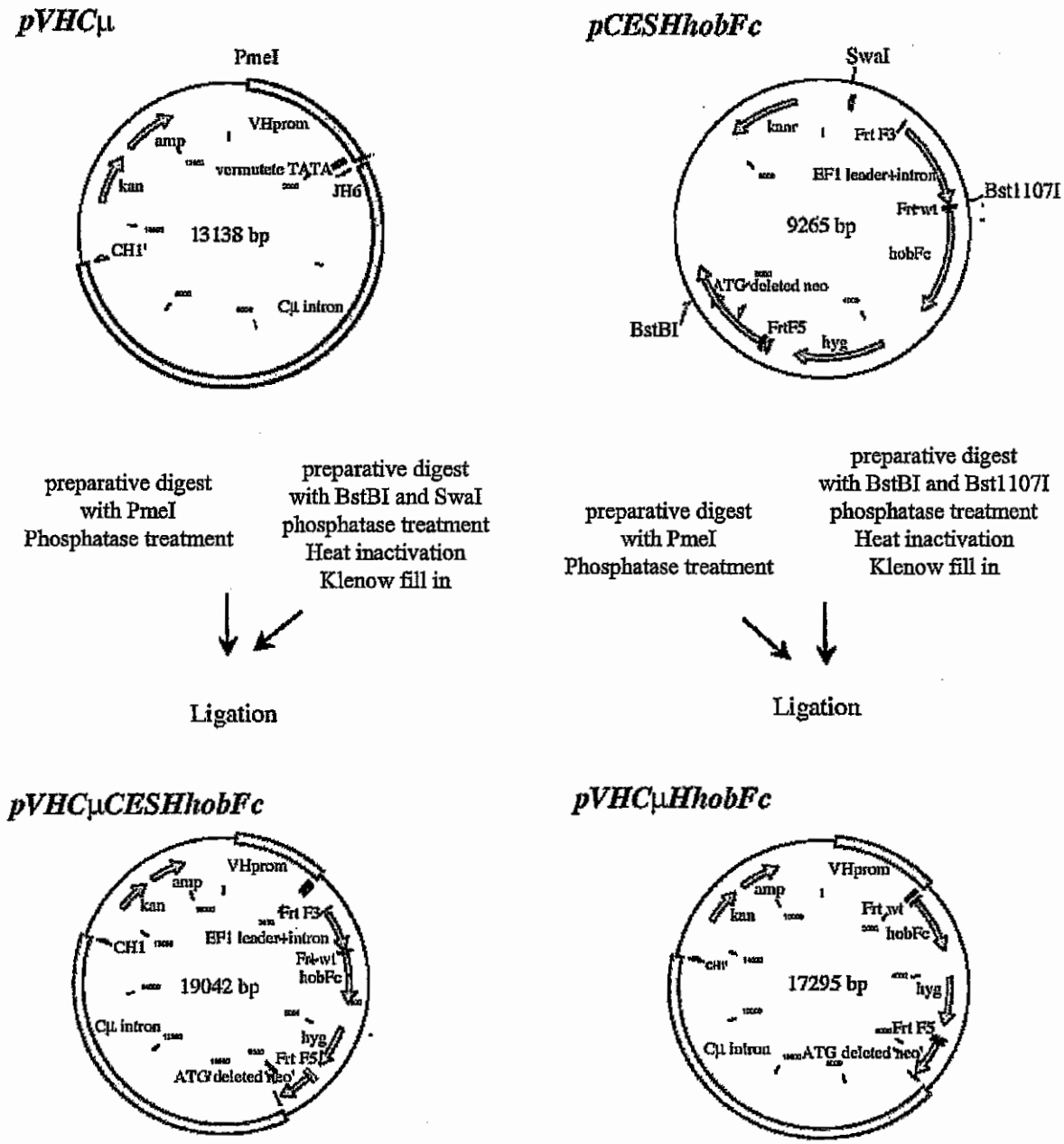

pVHCμ
PmeI
VHprom
amp
kan
vermutete TATA
JH6
13138 bp
CH1'
Cμ intron
pCESHhobFc
SwaI
kanr
Frt F3'
EF1 leader+intron
Bst1107I
Frt wt
9265 bp
hobFc
ATG deleted neo
BstBI
FrtF5
hyg
preparative digest
with PmeI
Phosphatase treatment
preparative digest
with BstBI and SwaI
phosphatase treatment
Heat inactivation
Klenow fill in
preparative digest
with PmeI
Phosphatase treatment
preparative digest
with BstBI and Bst1107I
phosphatase treatment
Heat inactivation
Klenow fill in
Ligation
Ligation
pVHCμCESHhobFc
VHprom
amp
kan
Frt F3
CH1'
EF1 leader+intron
Frt wt
hobFc
19042 bp
Cμ intron
hyg
Frt F5
ATG deleted neo'
pVHCμHhobFc
VHprom
amp
kan
Frt wt
hobFc
CH1'
17295 bp
hyg
Frt F5
Cμ intron
ATG deleted neo'

Fig 4

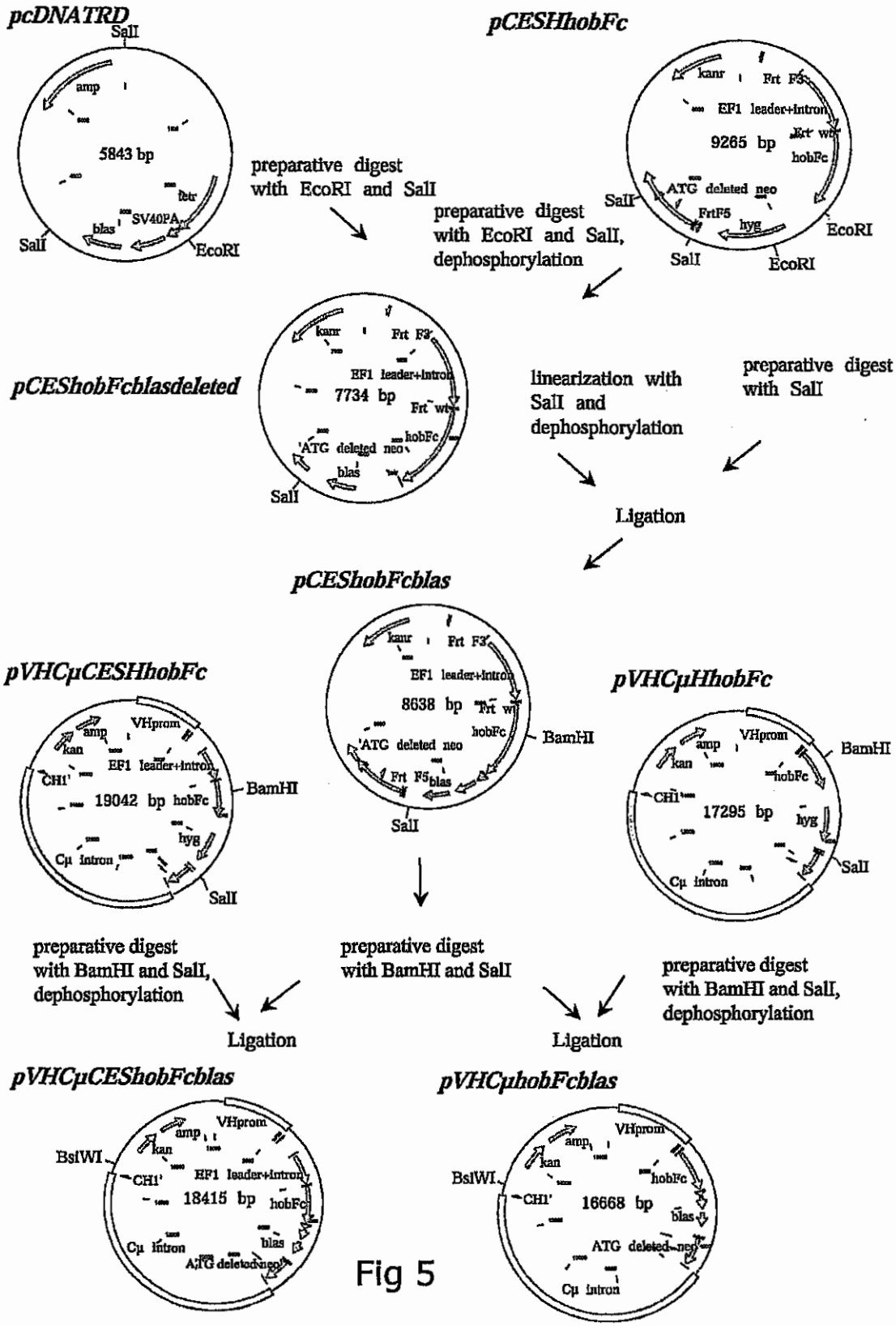

pcDNATRD
SalI
amp
5843 bp
tetr
SV40PA
blas
SalI
EcoRI
preparative digest
with EcoRI and SalI
pCESHhobFc
kanr
Frt F3'
EF1 leader+intron
9265 bp
Frt wt'
hobFc
ATG deleted neo
FrtF5
hyg
SalI
EcoRI
preparative digest
with EcoRI and SalI,
dephosphorylation
pCEShobFcblasdeleted
kanr
Frt F3'
EF1 leader+intron
7734 bp
Frt wt'
hobFc
'ATG deleted neo
blas
SalI
linearization with
SalI and
dephosphorylation
preparative digest
with SalI
Ligation
pCEShobFcblas
kanr
Frt F3'
EF1 leader+intron
8638 bp
Frt wt'
hobFc
'ATG deleted neo
Frt F5'
blas
BamHI
SalI
pVHCμCESHhobFc
amp
VHprom
kan
EF1 leader+intron
CH1'
19042 bp hobFc
BamHI
hyg
Cμ intron
SalI
pVHCμHhobFc
amp
VHprom
BamHI
kan
hobFc
CH1'
17295 bp
hyg
Cμ intron
SalI
preparative digest
with BamHI and SalI,
dephosphorylation
preparative digest
with BamHI and SalI
preparative digest
with BamHI and SalI,
dephosphorylation
Ligation
Ligation
pVHCμCEShobFcblas
BsiWI
amp
VHprom
kan
CH1'
EF1 leader+intron
18415 bp hobFc
Cμ intron
blas
ATG deleted neo
pVHCμhobFcblas
BsiWI
amp
VHprom
kan
hobFc
CH1'
16668 bp
blas
ATG deleted neo
Cμ intron

Fig 5

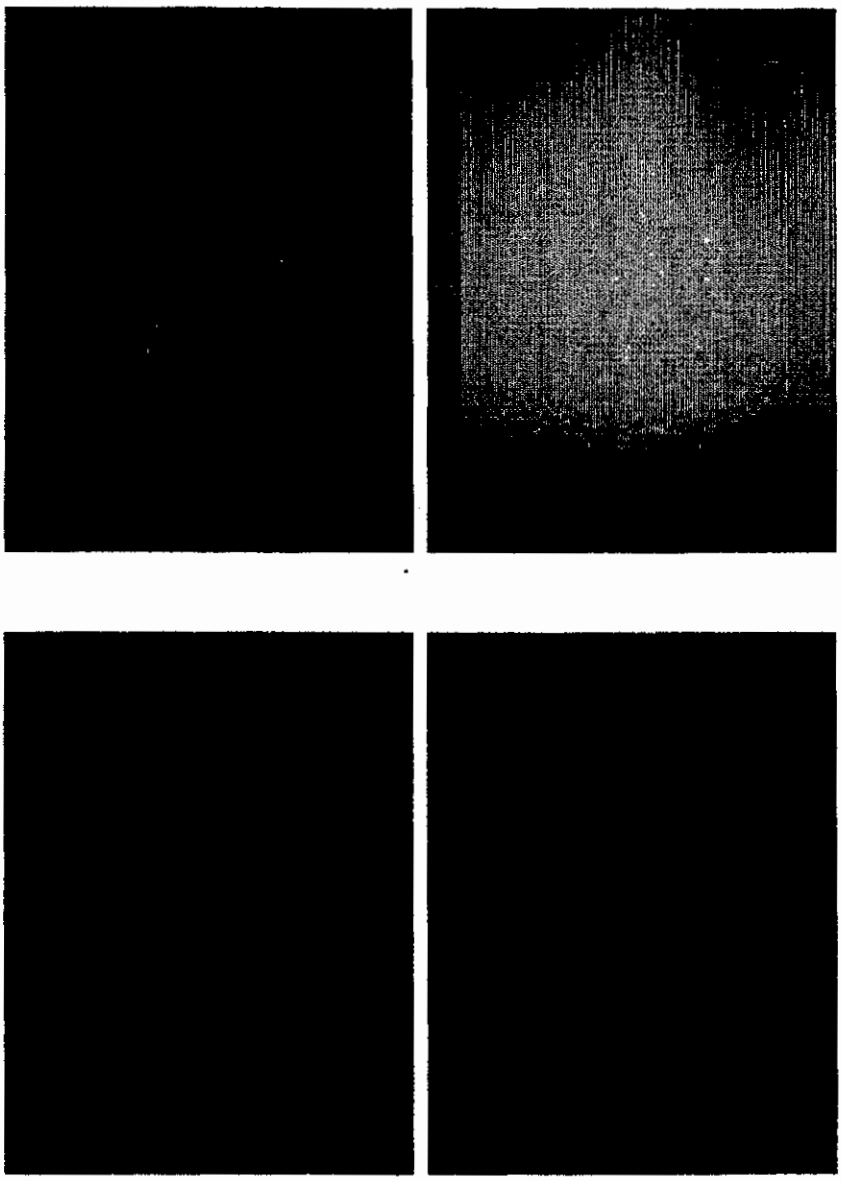


Fig 6

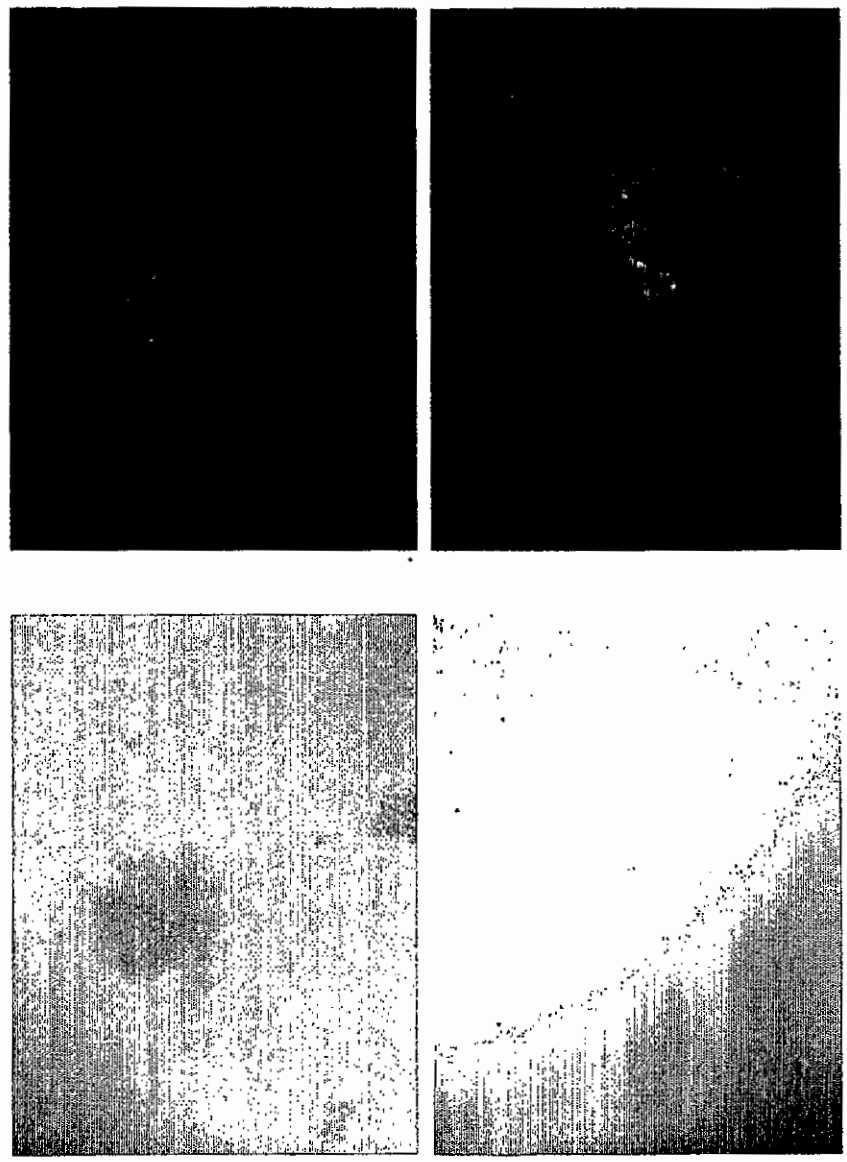


Fig 7

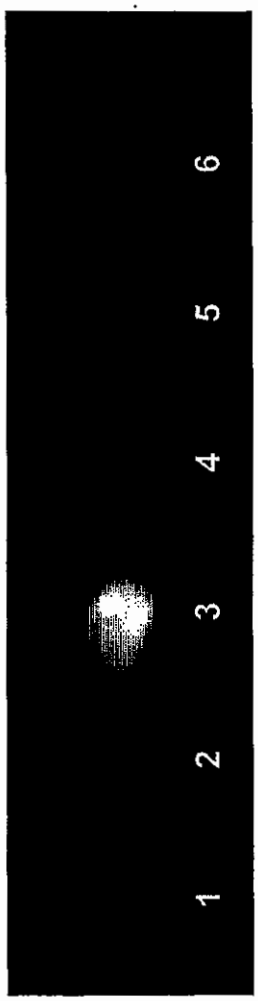


Fig. 8

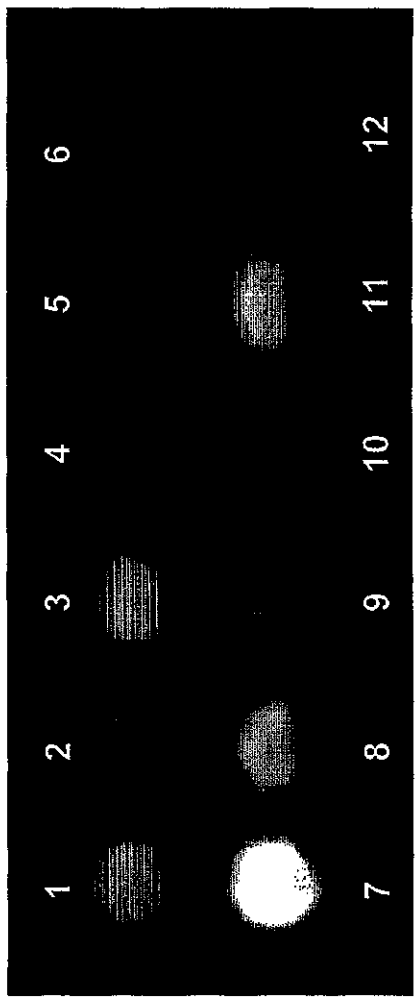


Fig. 9:

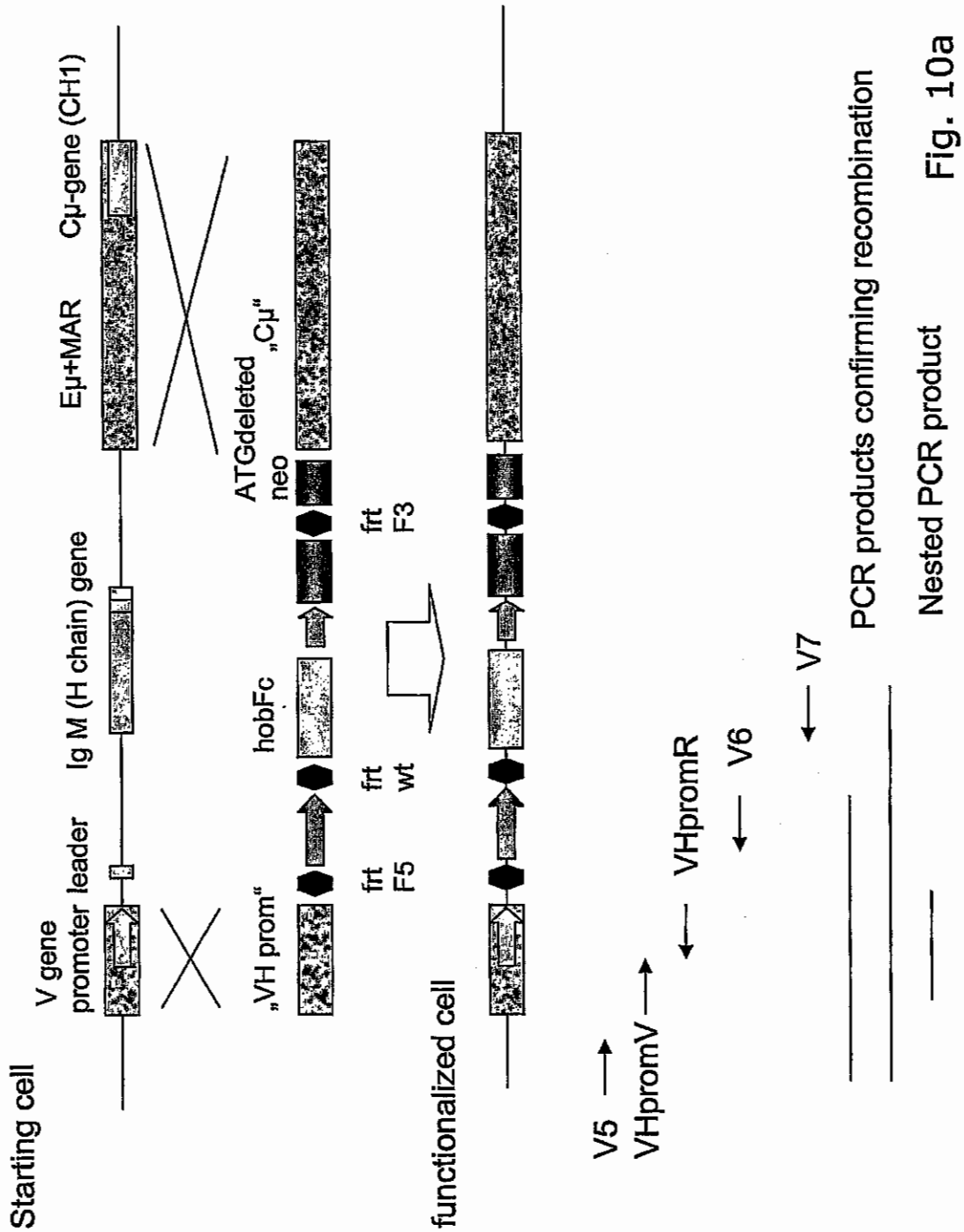
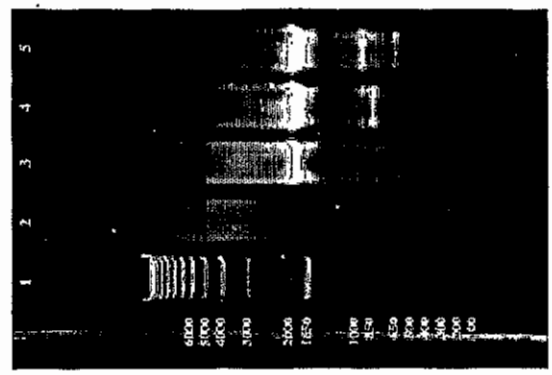

Starting cell
V gene
promoter leader
Ig M (H chain) gene
Eμ+MAR
Cμ-gene (CH1)
„VH prom"
hobFc
ATGdeleted „Cμ"
neo
frt
F5
frt
wt
frt
F3
functionalized cell
V5
VHpromV
VHpromR
V6
V7
PCR products confirming recombination
Nested PCR product

Fig. 10a



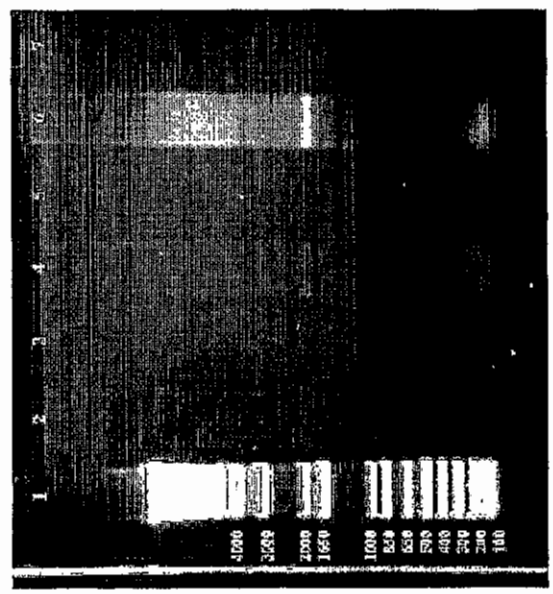


Fig 10b

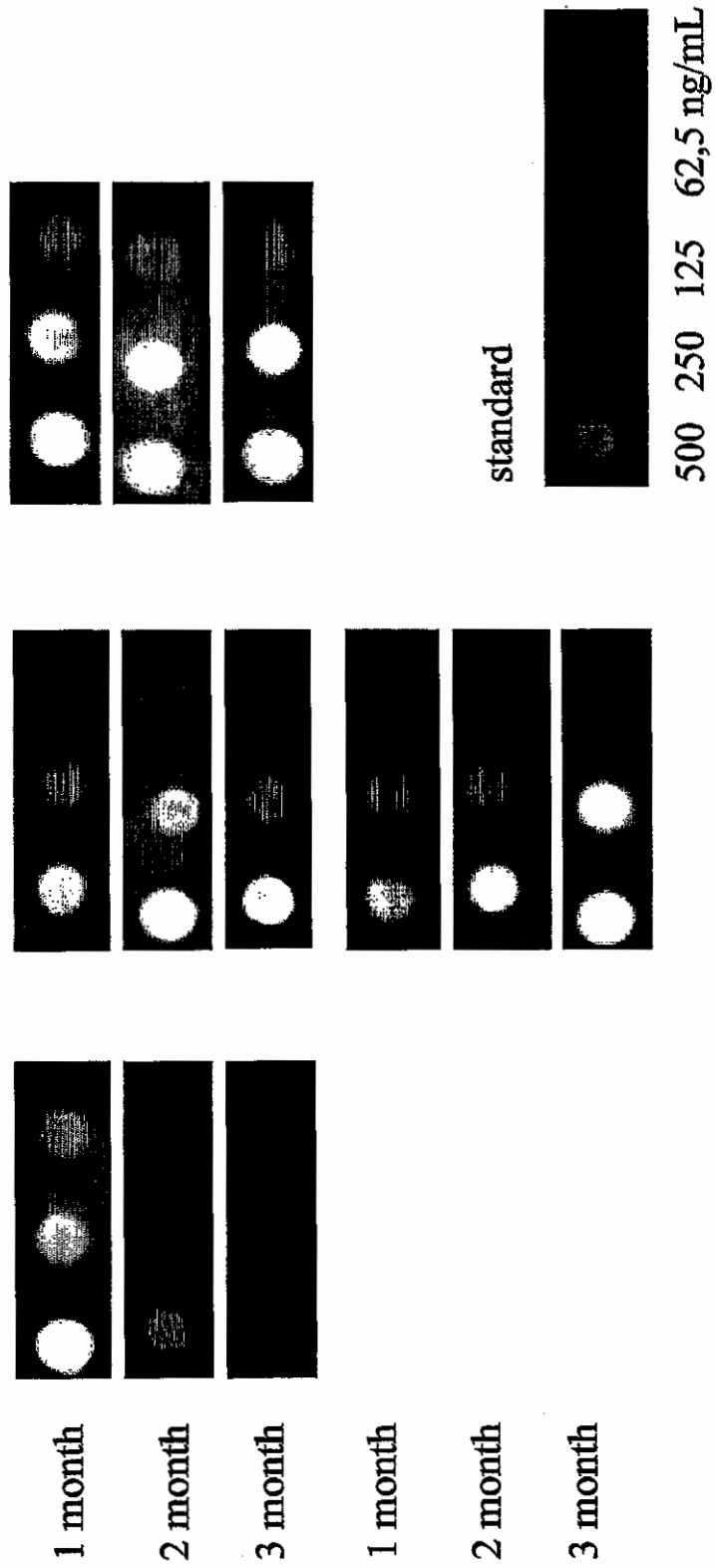

1 month
2 month
3 month
1 month
2 month
3 month
standard
500 250 125 62,5 ng/mL

Fig. 11

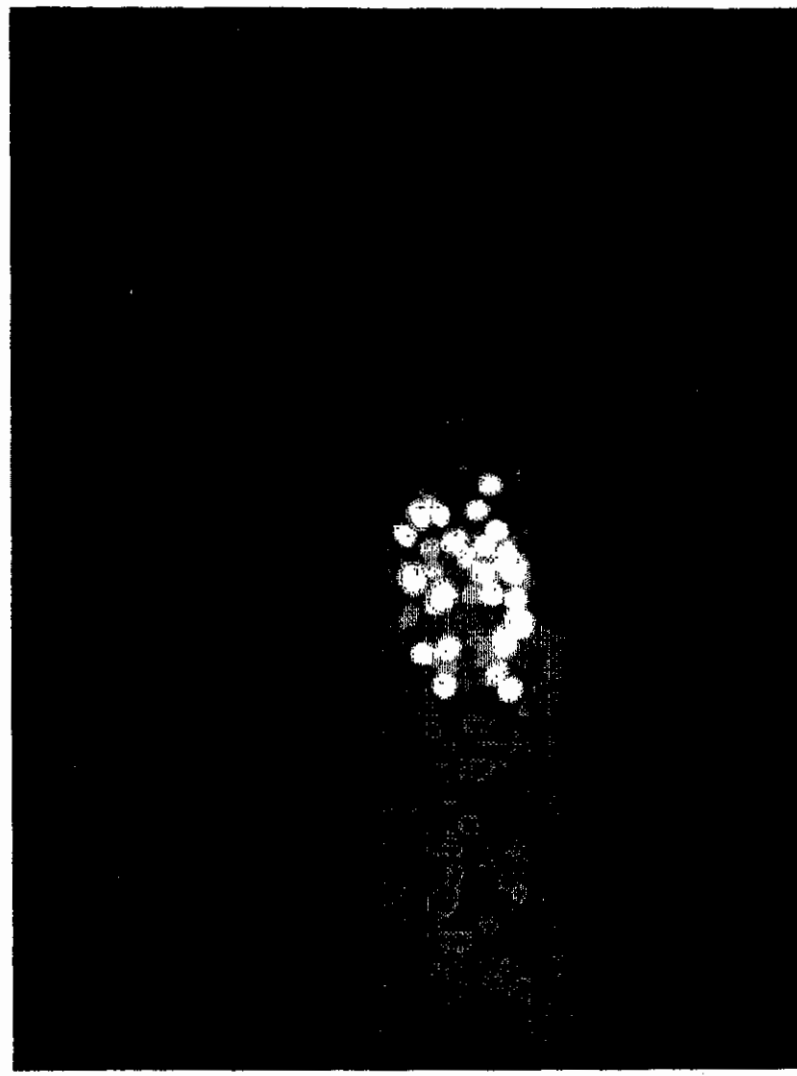


Fig. 12

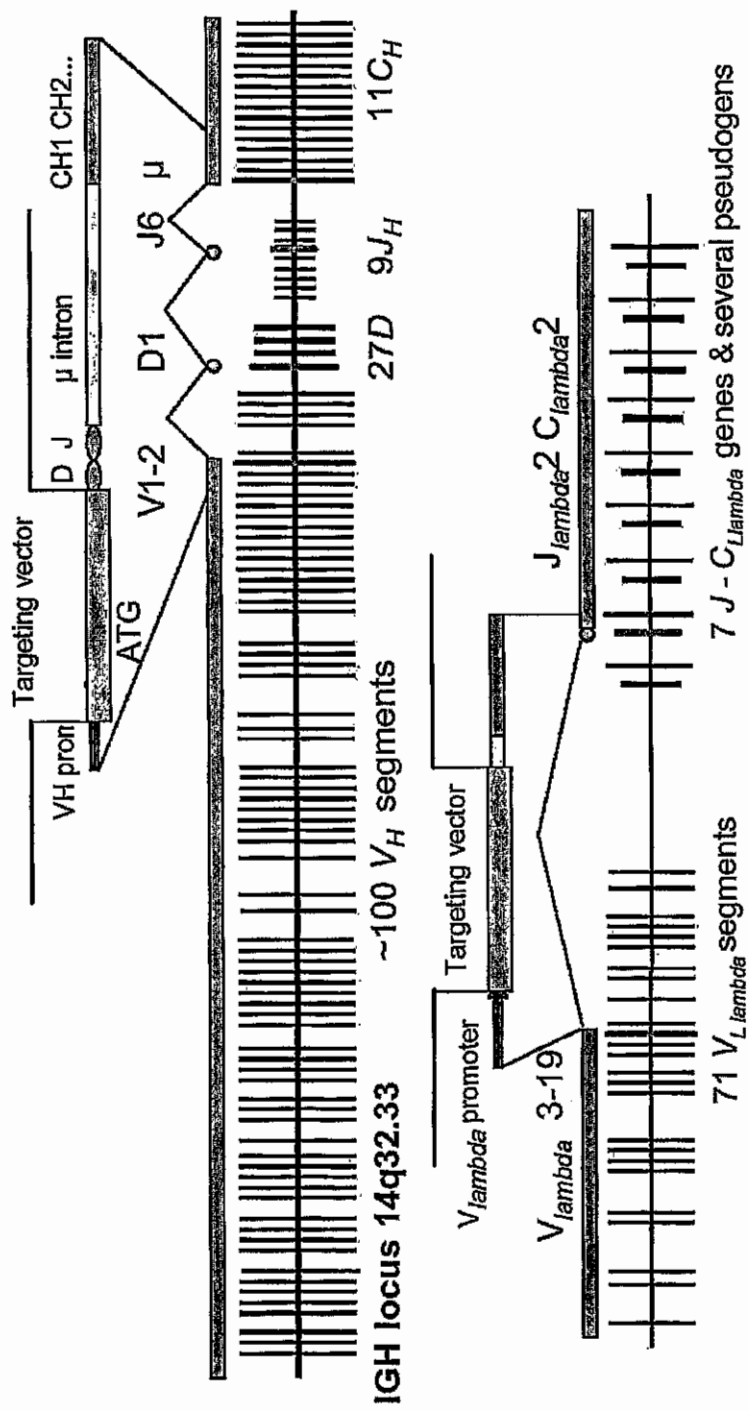

Targeting vector
VH prom
ATG
V1-2
D J
μ intron
CH1 CH2...
D1
J6
μ
IGH locus 14q32.33
~100 V_H segments
27D
9J_H
11C_H
Targeting vector
V_{lambda} promoter
V_{lambda} 3-19
71 $V_{L\,lambda}$ segments
$J_{lambda}2$ $C_{lambda}2$
7 J - $C_{Llambda}$ genes & several pseudogens
IG_{lambda} locus 22q11.2

Fig. 13

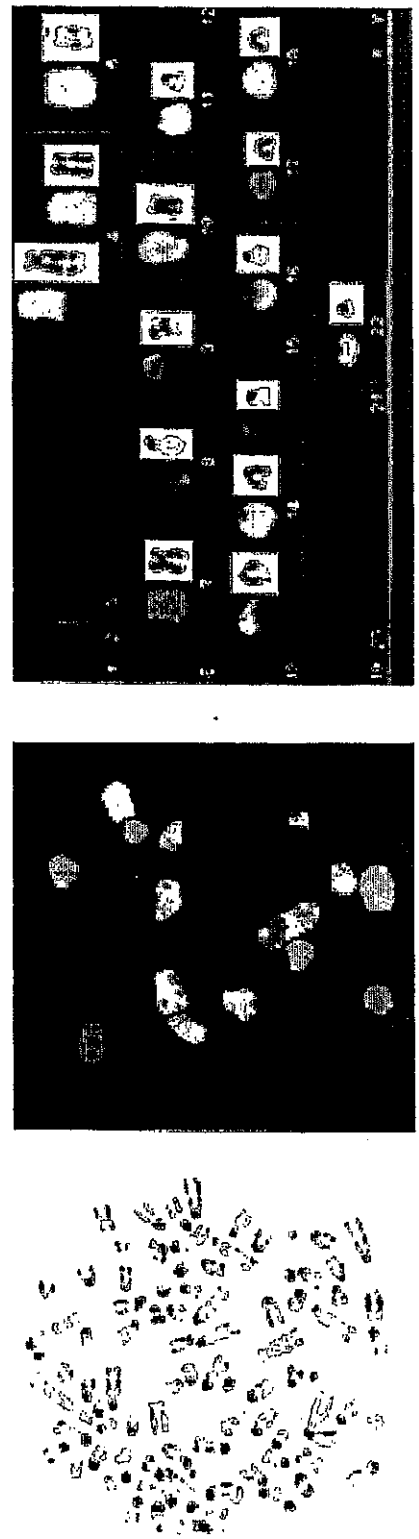


Fig. 14

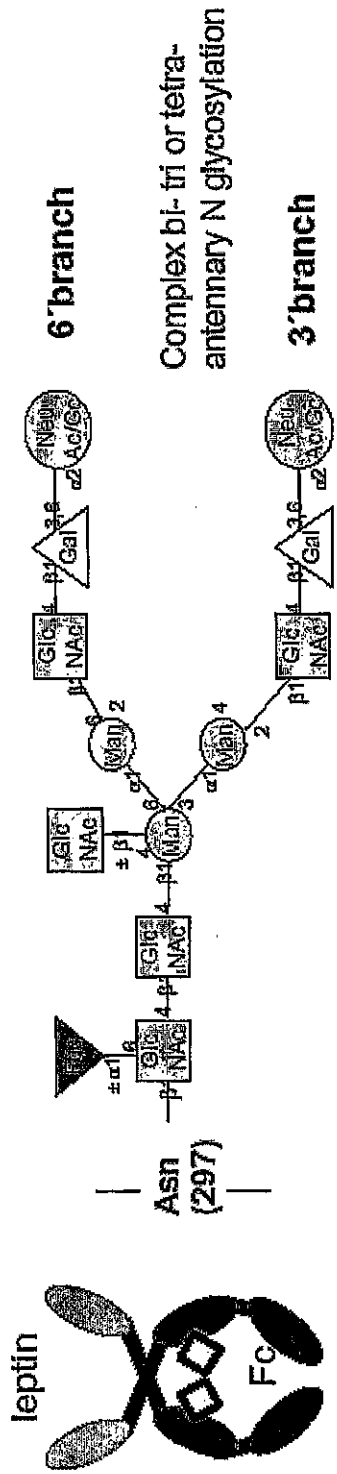

6'branch
Complex bi- tri or tetra-antennary N glycosylation
3'branch
Asn (297)
leptin
Fc

Fig. 15

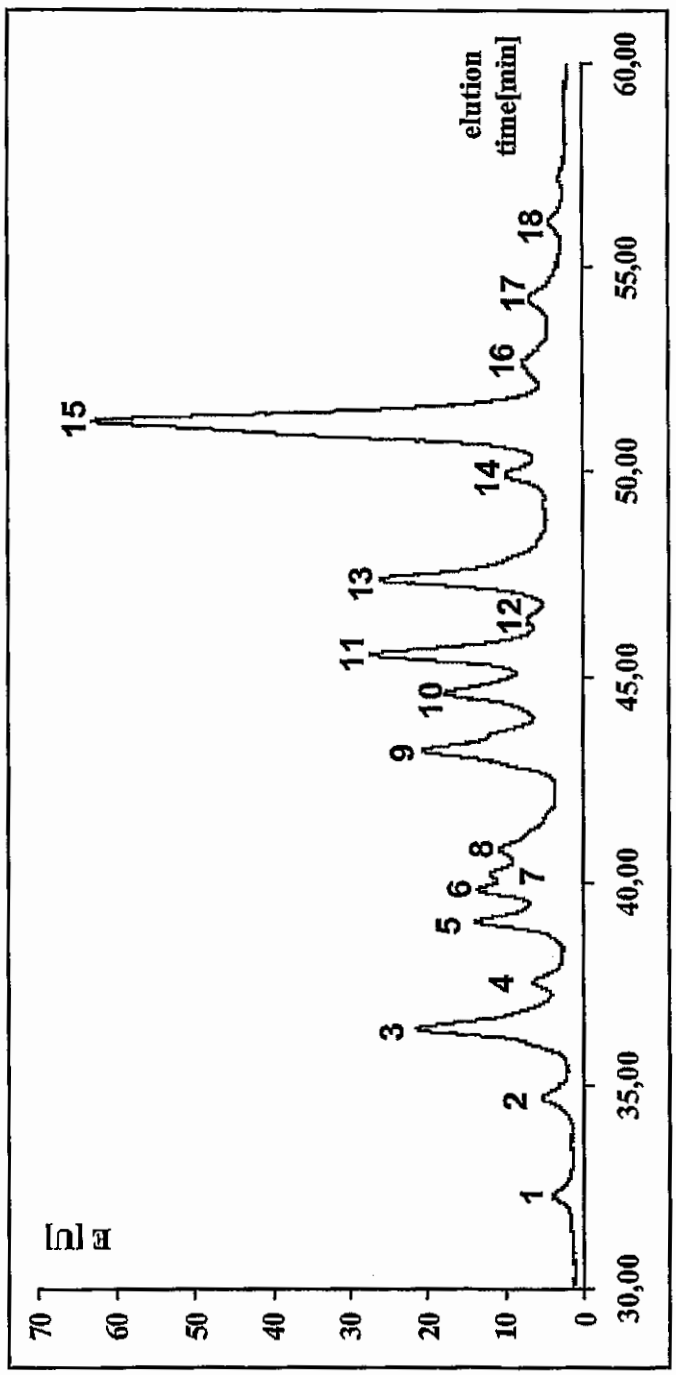

mAU
70
60
50
40
30
20
10
0
30,00
35,00
40,00
45,00
50,00
55,00
60,00
elution time[min]
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Fig. 16

SEQUENCE LISTING

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<140>
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<170> PatentIn Ver. 2.1

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<220>
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<220>
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<400> 19
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