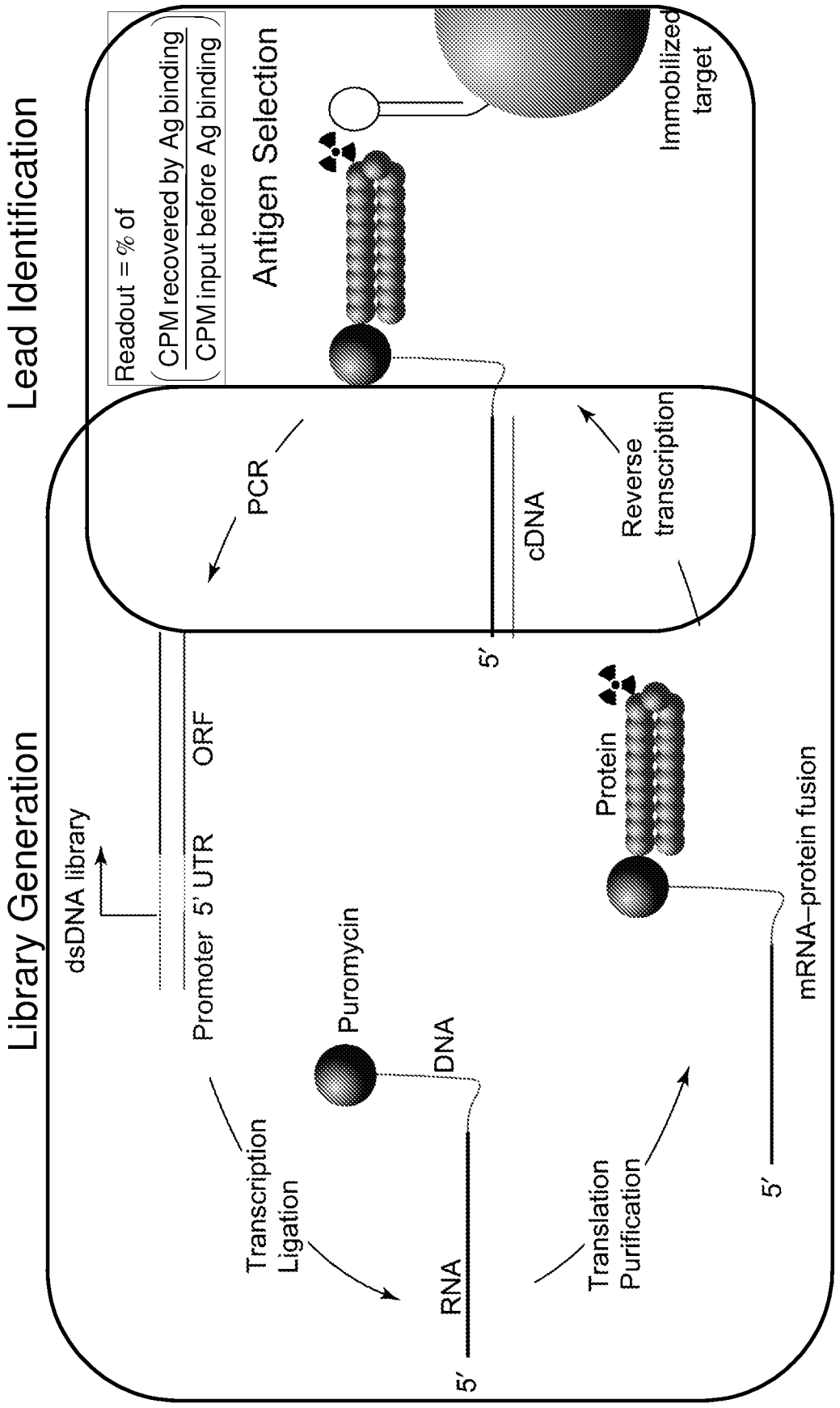

Lead Identification
Library Generation
Antigen Selection
Readout = % of
CPM recovered by Ag binding
CPM input before Ag binding
Immobilized target
PCR
cDNA
Reverse transcription
5'
dsDNA library
Promoter 5' UTR
ORF
Puromycin
DNA
RNA
Transcription
Ligation
Protein
Translation
Purification
mRNA–protein fusion

Fig. 1

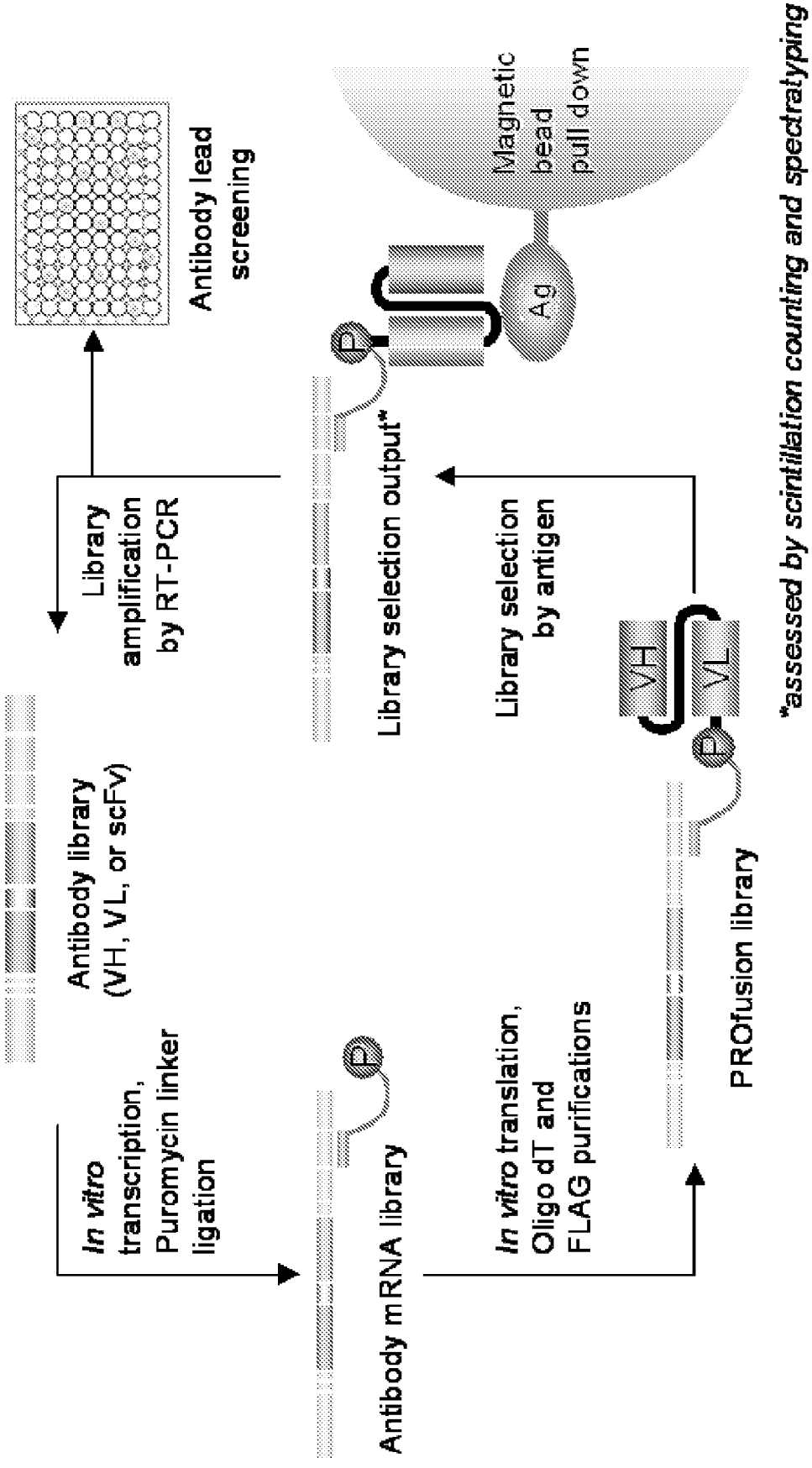

Antibody lead screening
Library amplification by RT-PCR
Library selection output*
Magnetic bead pull down
Ag
P
Library selection by antigen
VH
VL
P
PROfusion library
*assessed by scintillation counting and spectratyping
In vitro translation, Oligo dT and FLAG purifications
Antibody mRNA library
P
In vitro transcription, Puromycin linker ligation
Antibody library (VH, VL, or scFv)

Fig. 2

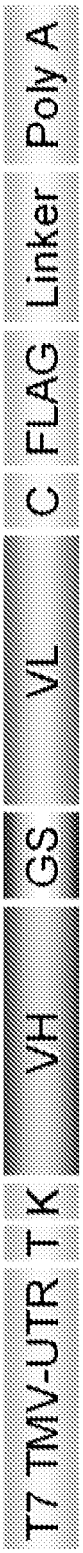


Fig. 3

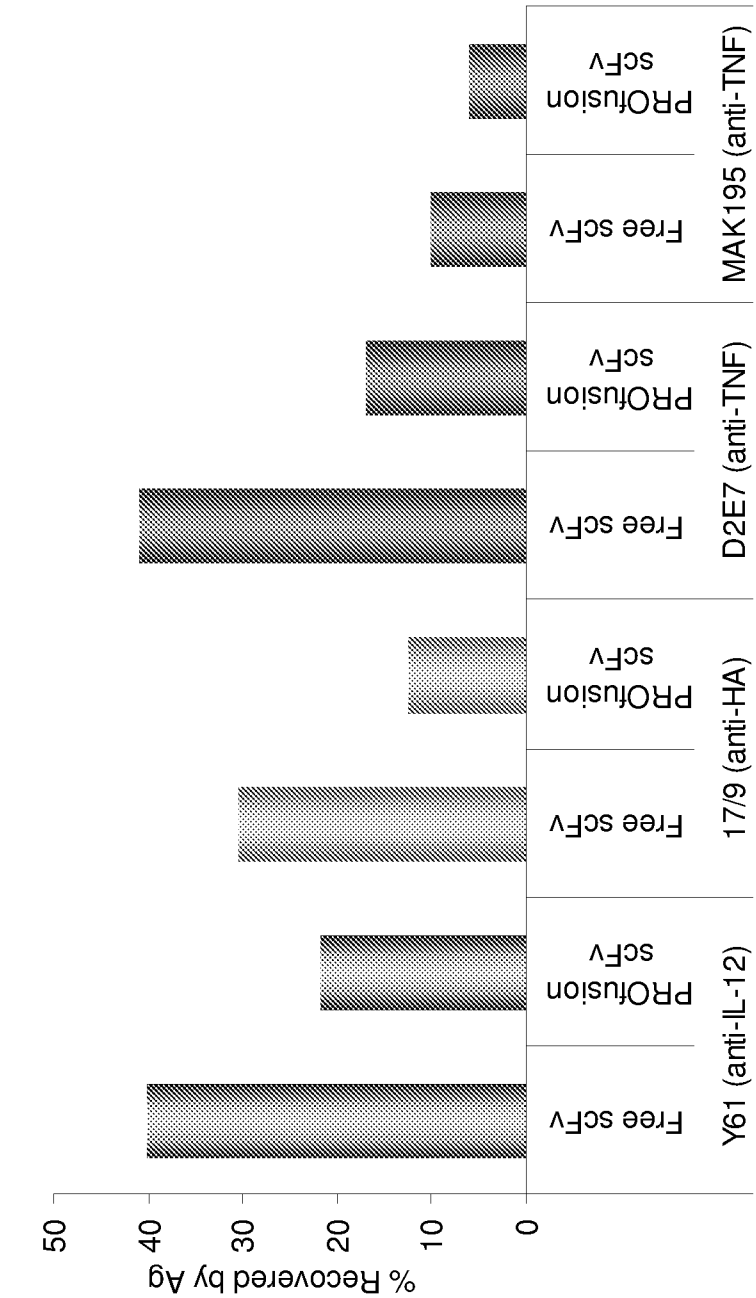

% Recovered by Ag
50
40
30
20
10
0
Free scFv
PROfusion scFv
Free scFv
PROfusion scFv
Free scFv
PROfusion scFv
Y61 (anti-IL-12)
17/9 (anti-HA)
D2E7 (anti-TNF)
MAK195 (anti-TNF)

Fig. 4a

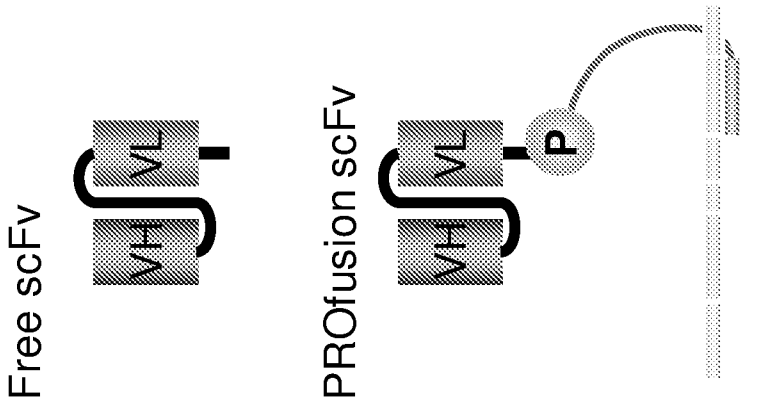
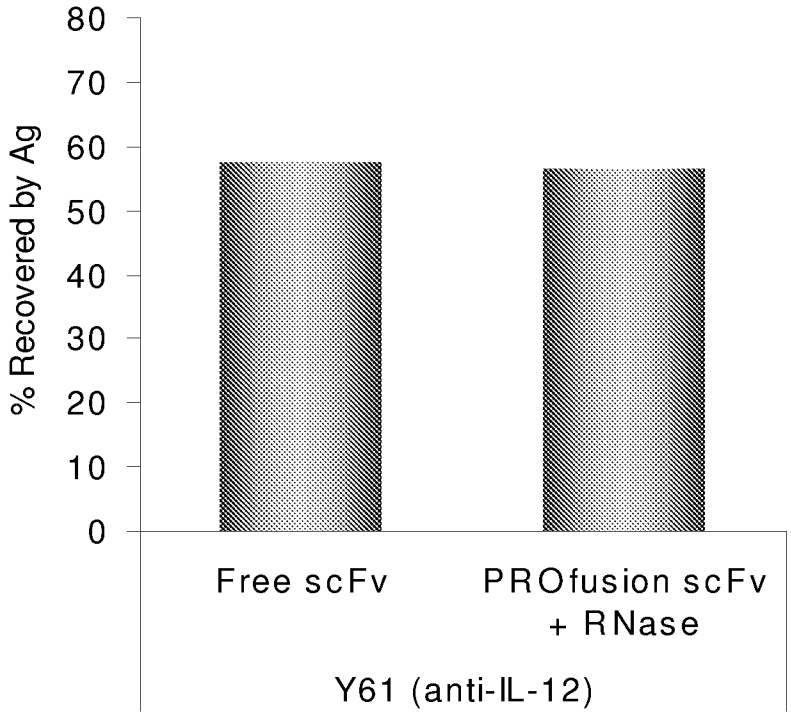

Free scFv
VH
VL
PROfusion scFv
VH
VL
P

Fig. 4b


80
70
60
50
40
30
20
10
0
% Recovered by Ag
Free scFv
PROfusion scFv + RNase
Y61 (anti-IL-12)

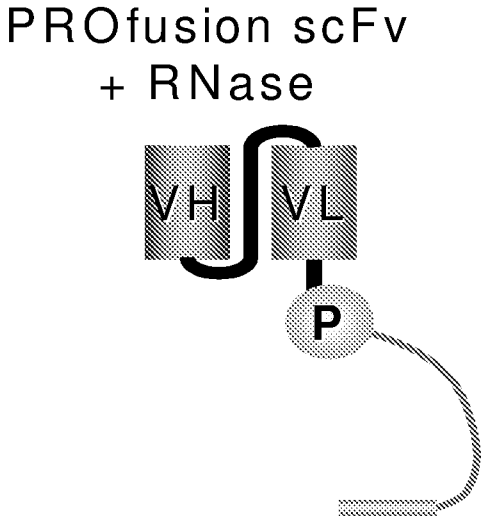

PROfusion scFv + RNase
VH
VL
P

Fig. 5

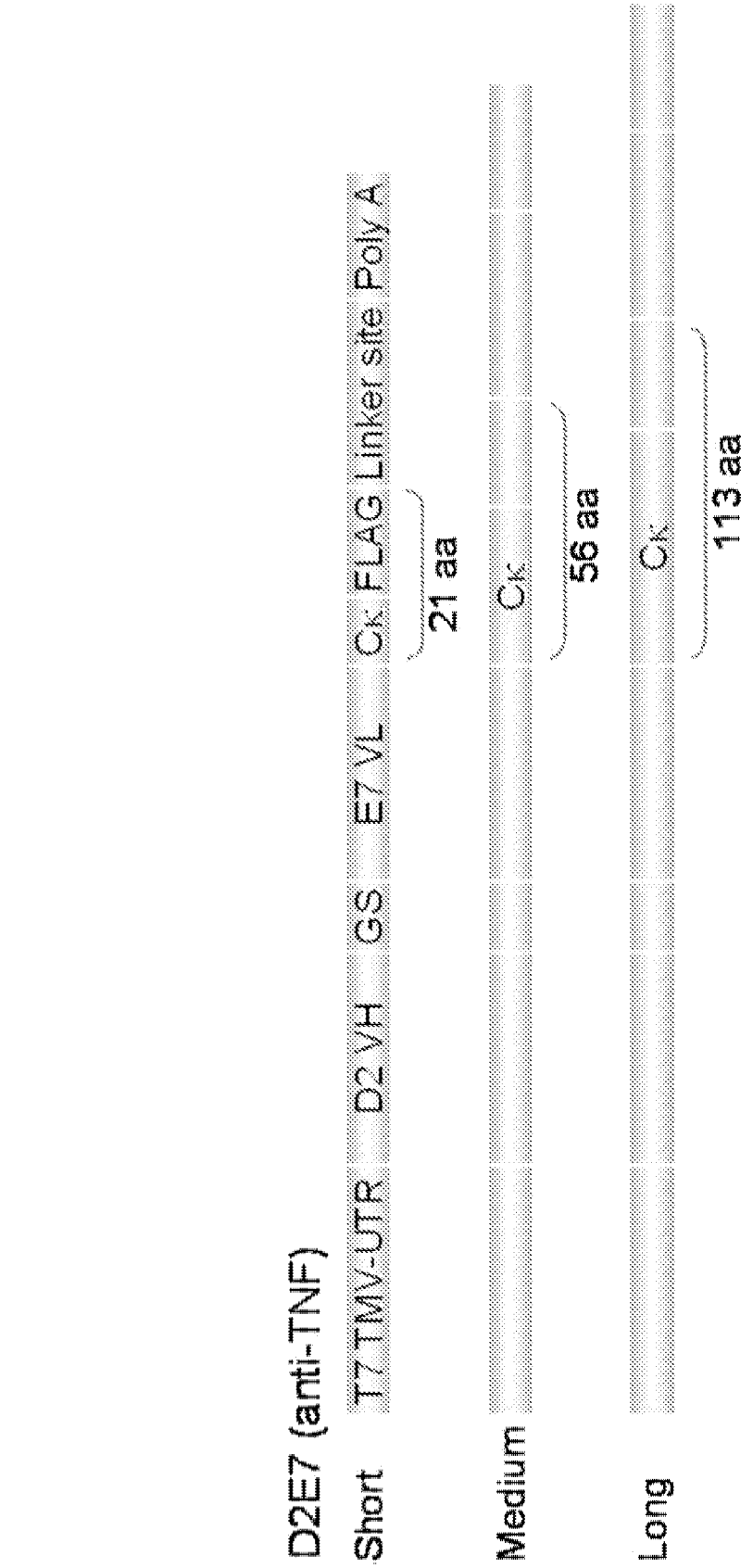

D2E7 (anti-TNF)
Short
T7 TMV-UTR
D2 VH
GS
E7 VL
Cκ FLAG Linker site Poly A
21 aa
Medium
Cκ
56 aa
Long
Cκ
113 aa

Fig. 6

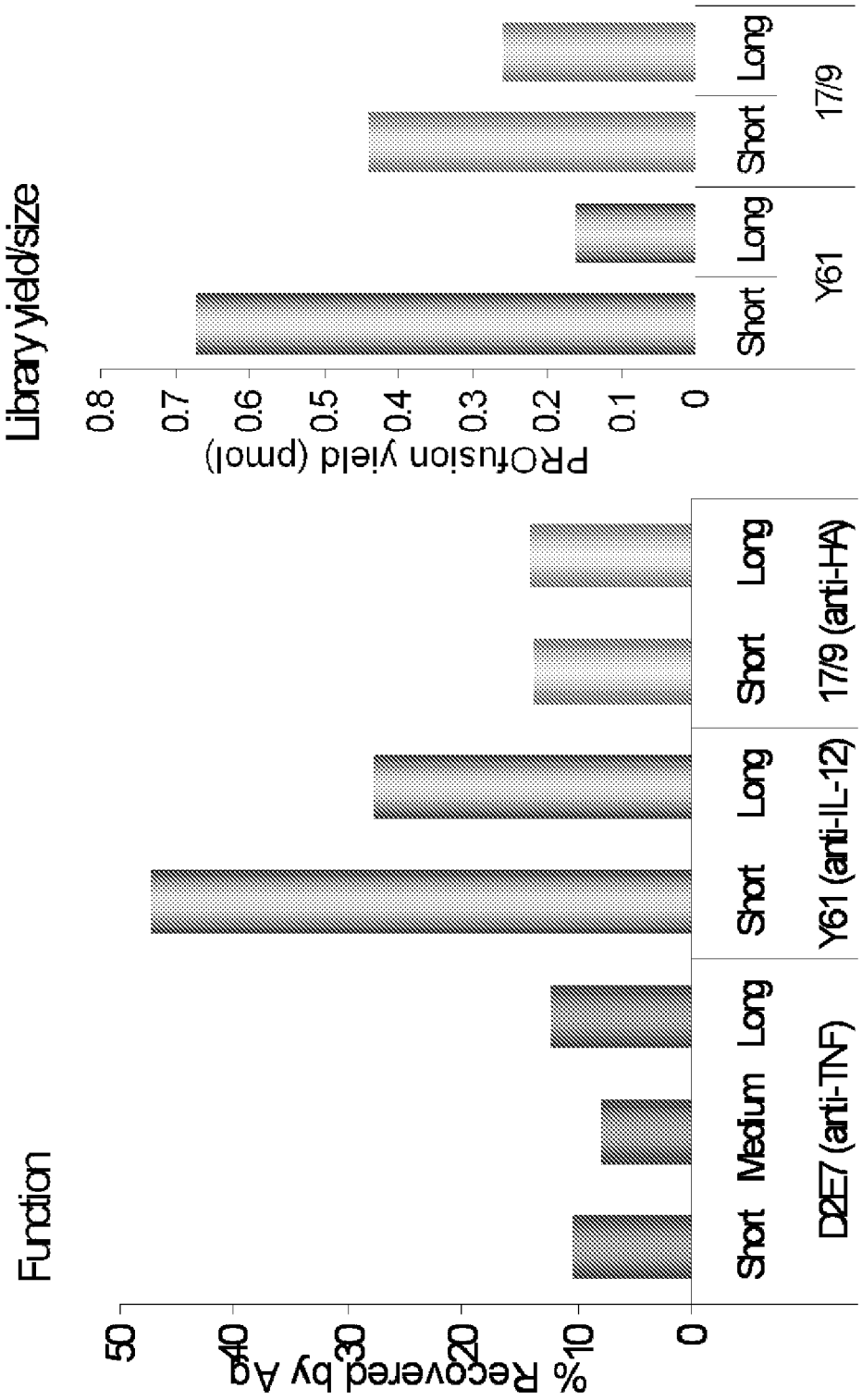

Library yield/size
PROfusion yield (pmol)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Short
Long
Y61
Short
Long
17/9
Function
% Recovered by Aq
50
40
30
20
10
0
Short
Medium
Long
D2E7 (anti-TNF)
Short
Long
Y61 (anti-IL-12)
Short
Long
17/9 (anti-HA)

Fig. 7

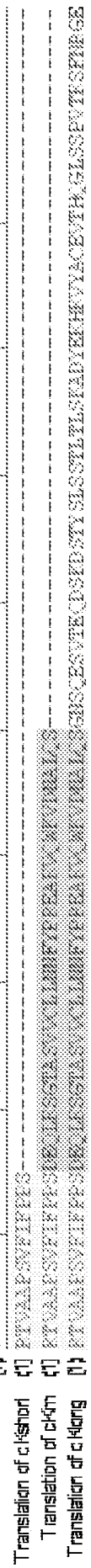

Translation of cHshon1
Translation of cHkm
Translation of cHdmg

Fig. 8

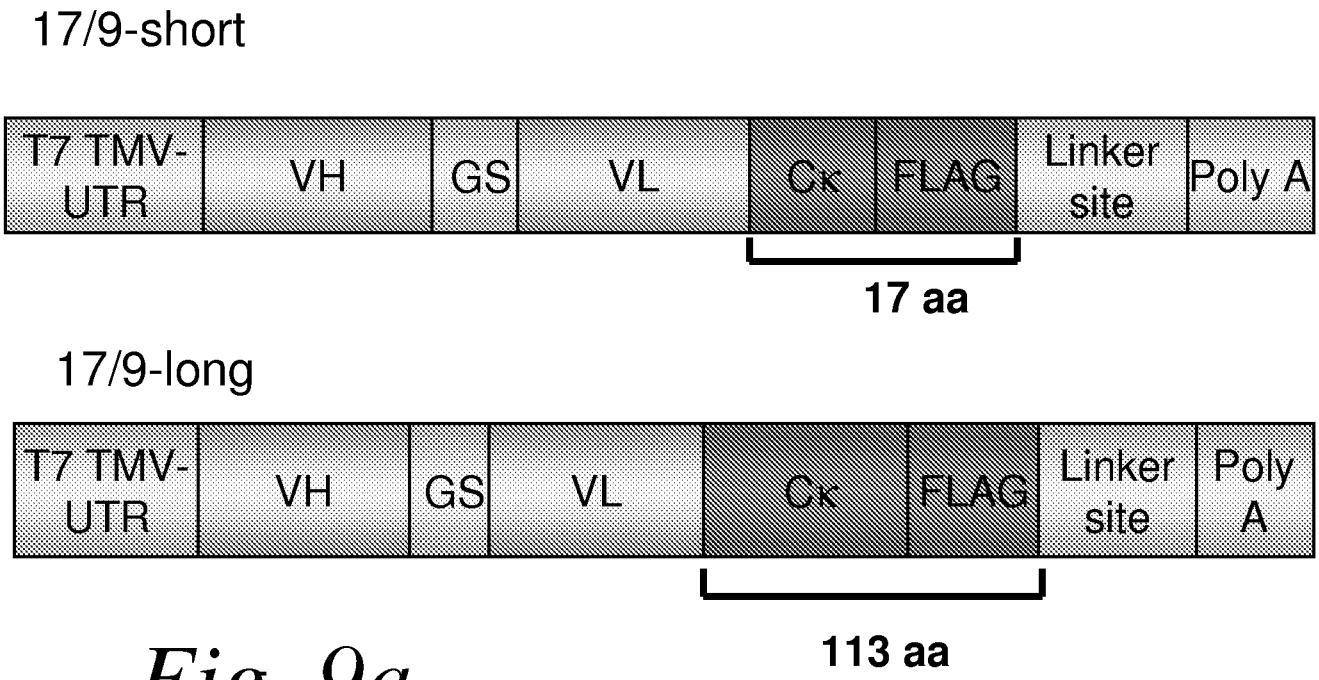

17/9-short
T7 TMV-UTR
VH
GS
VL
Cκ
FLAG
Linker site
Poly A
17 aa
17/9-long
T7 TMV-UTR
VH
GS
VL
Cκ
FLAG
Linker site
Poly A
113 aa

Fig. 9a

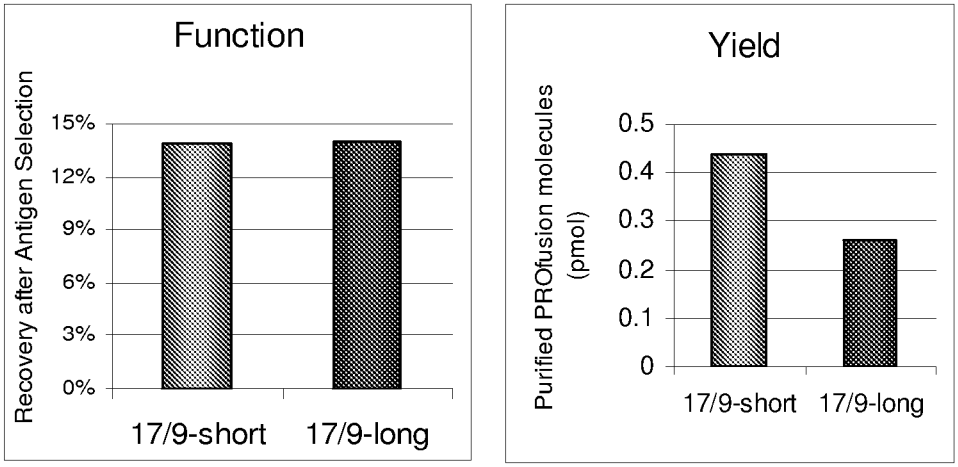

Function
Recovery after Antigen Selection
15%
12%
9%
6%
3%
0%
17/9-short
17/9-long
Yield
Purified PROfusion molecules (pmol)
0.5
0.4
0.3
0.2
0.1
0
17/9-short
17/9-long

Fig. 9b

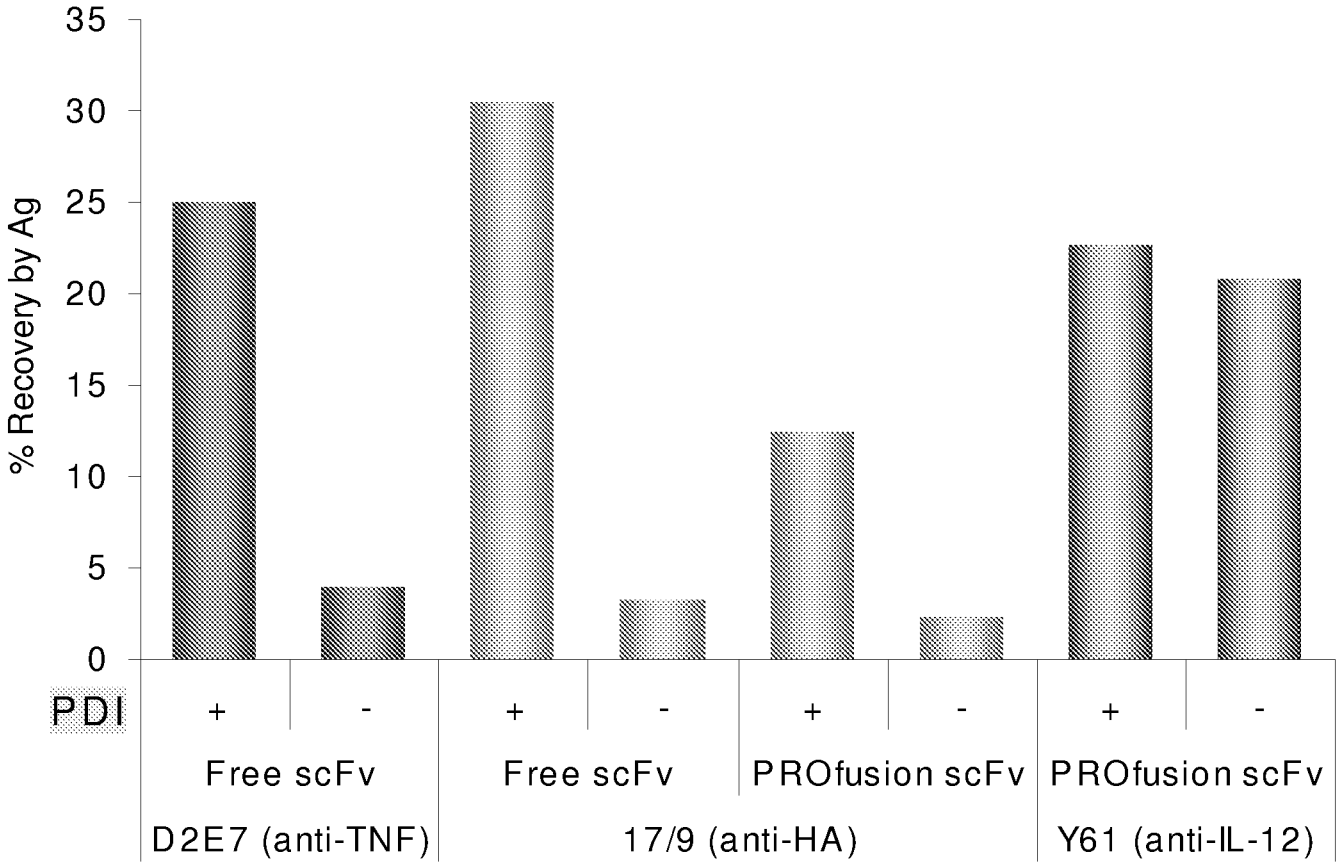

35
30
25
20
15
10
5
0
% Recovery by Ag
PDI
+
-
+
-
+
-
+
-
Free scFv
D2E7 (anti-TNF)
Free scFv
17/9 (anti-HA)
PROfusion scFv
PROfusion scFv
Y61 (anti-IL-12)

Fig. 10

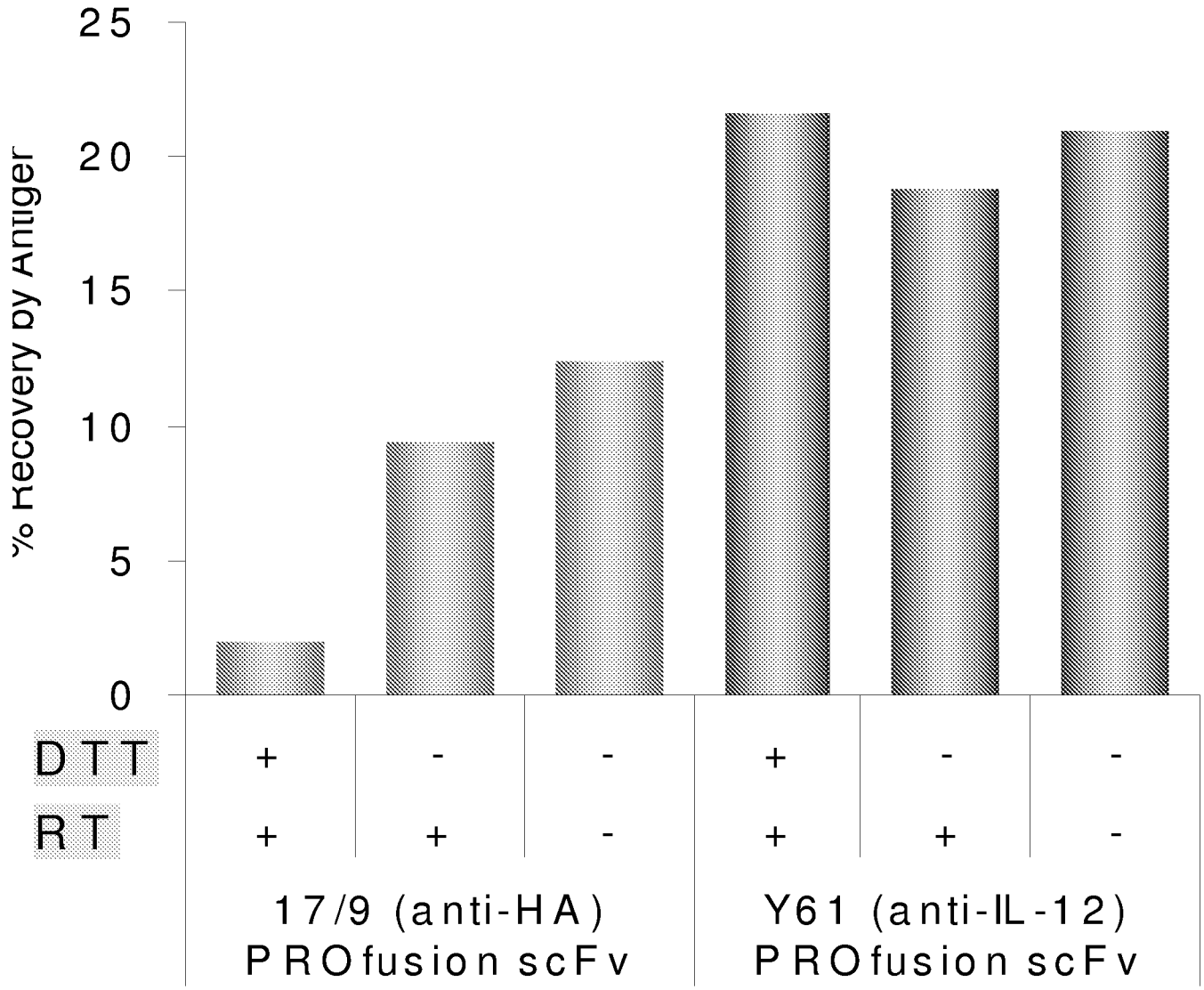
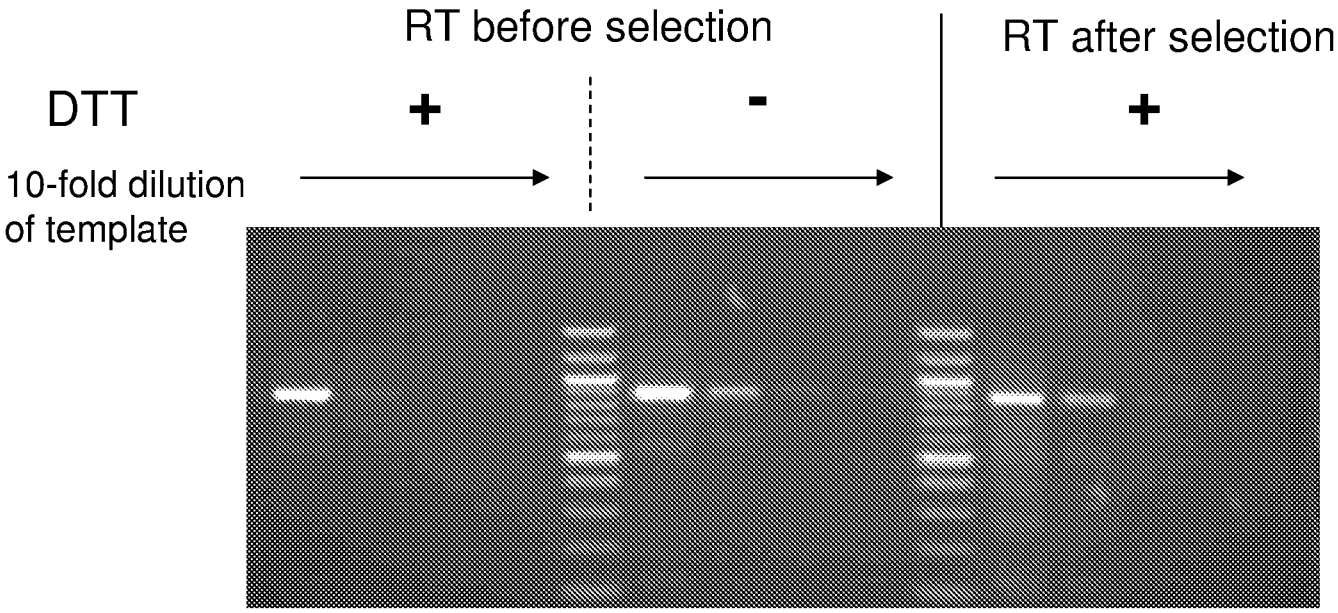

25
20
15
10
5
0
% Recovery by Antiger
DTT
RT
+
-
-
+
-
-
+
+
-
+
+
-
17/9 (anti-HA)
PROfusion scFv
Y61 (anti-IL-12)
PROfusion scFv

Fig. 11



* Template was normalized by beads volume, not by counts

Fig. 12

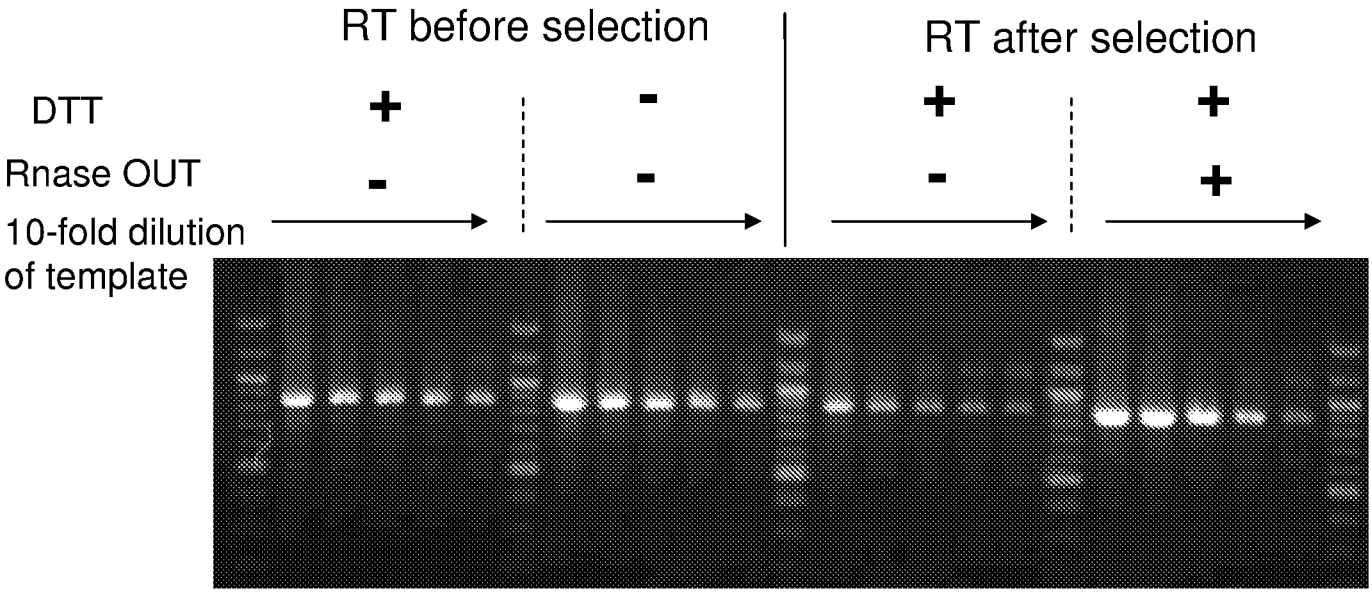

RT before selection
RT after selection
DTT
Rnase OUT
10-fold dilution of template
+
-
-
-
+
-
+
+

Fig. 13

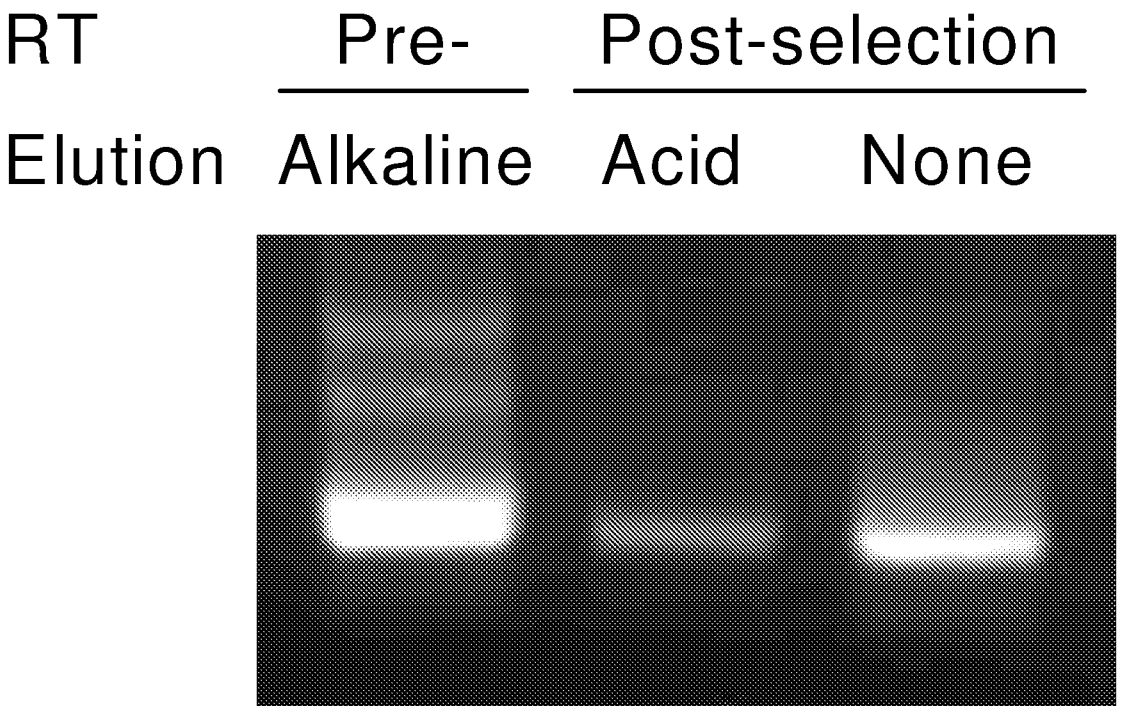


Fig. 14

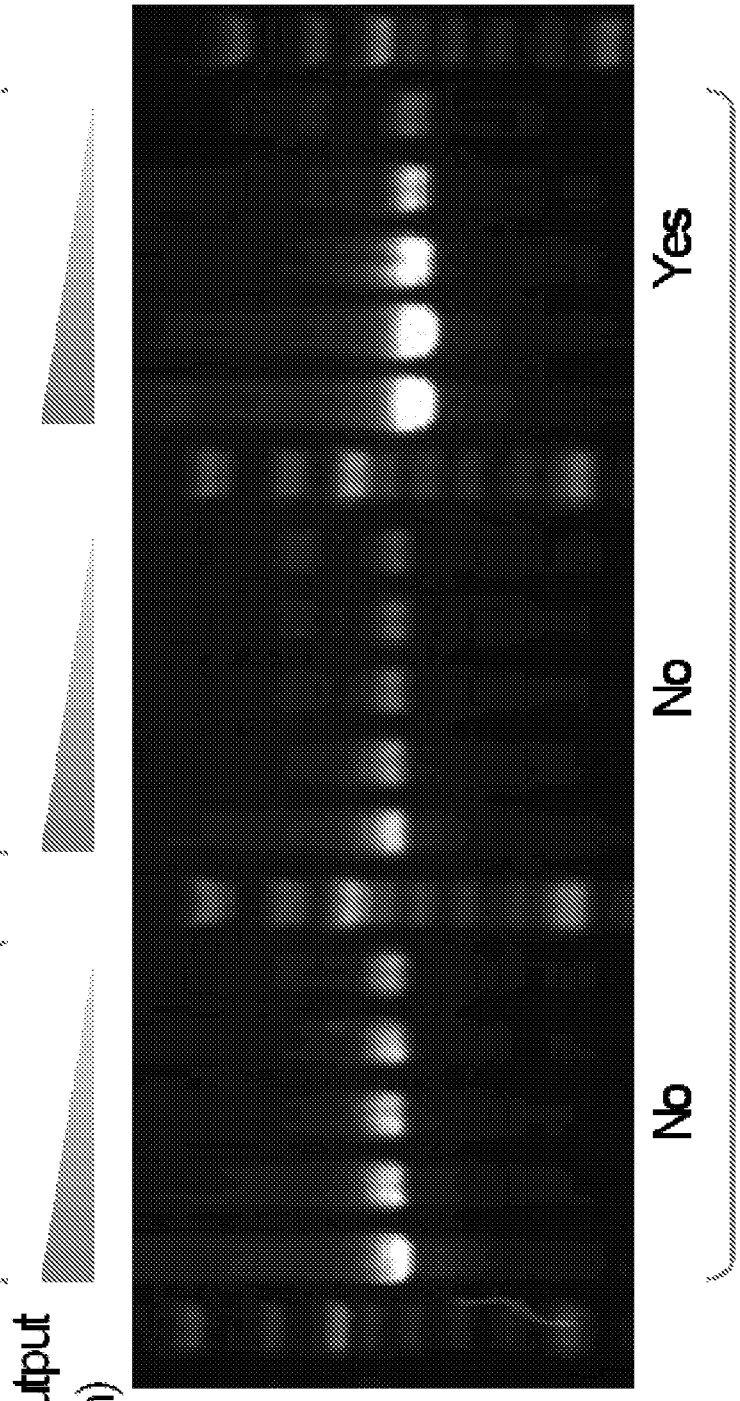

RT before Ag selection
RT after Ag selection
Selection Output
(10X dilution)
No
No
Yes
RNase Inhibitor during Ag Selection

Fig. 15

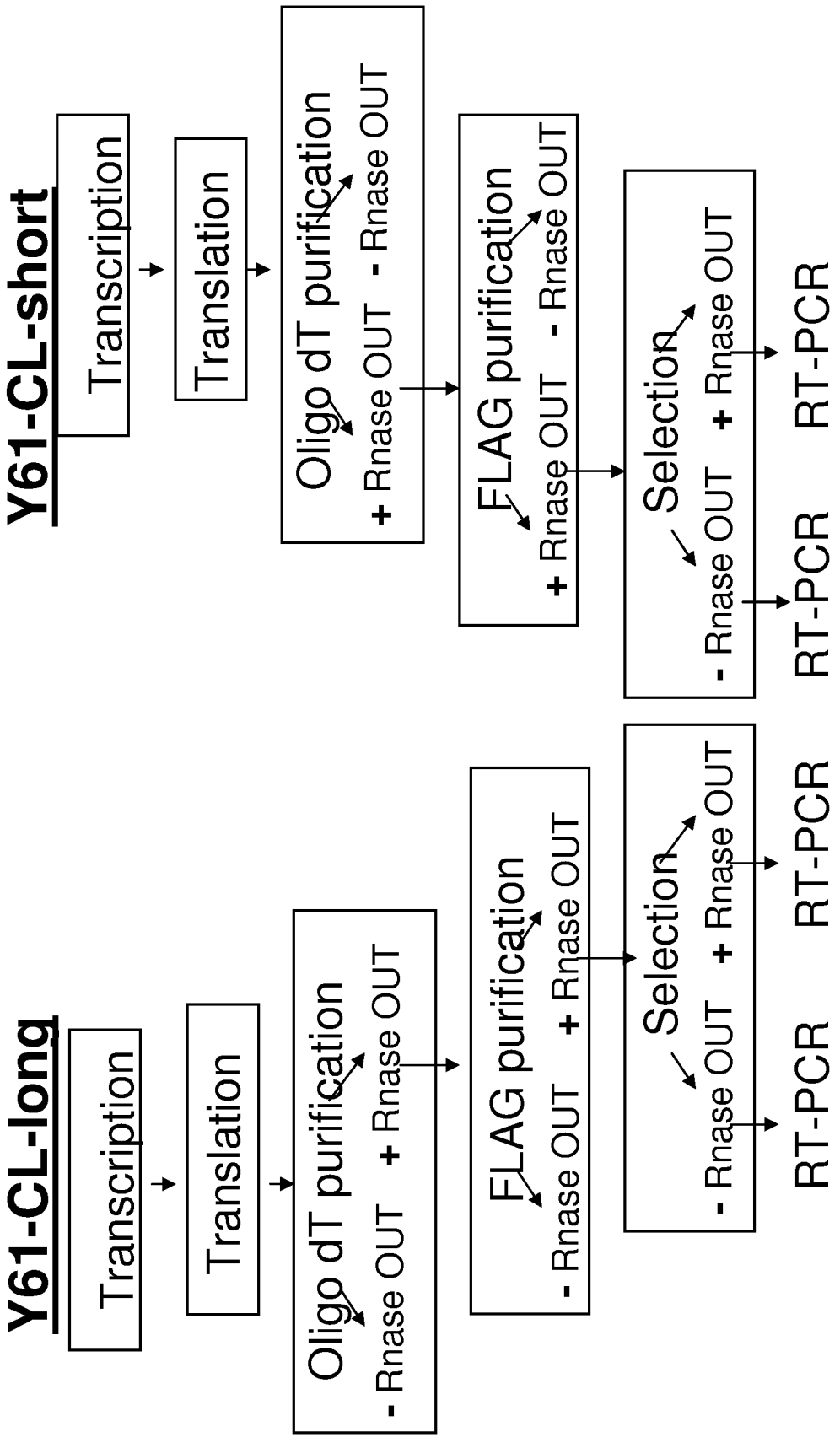

Y61-CL-short
Transcription
Translation
Oligo dT purification
+ Rnase OUT - Rnase OUT
FLAG purification
+ Rnase OUT - Rnase OUT
Selection
- Rnase OUT + Rnase OUT
RT-PCR
RT-PCR
Y61-CL-long
Transcription
Translation
Oligo dT purification
- Rnase OUT + Rnase OUT
FLAG purification
- Rnase OUT + Rnase OUT
Selection
- Rnase OUT + Rnase OUT
RT-PCR
RT-PCR

Fig. 16

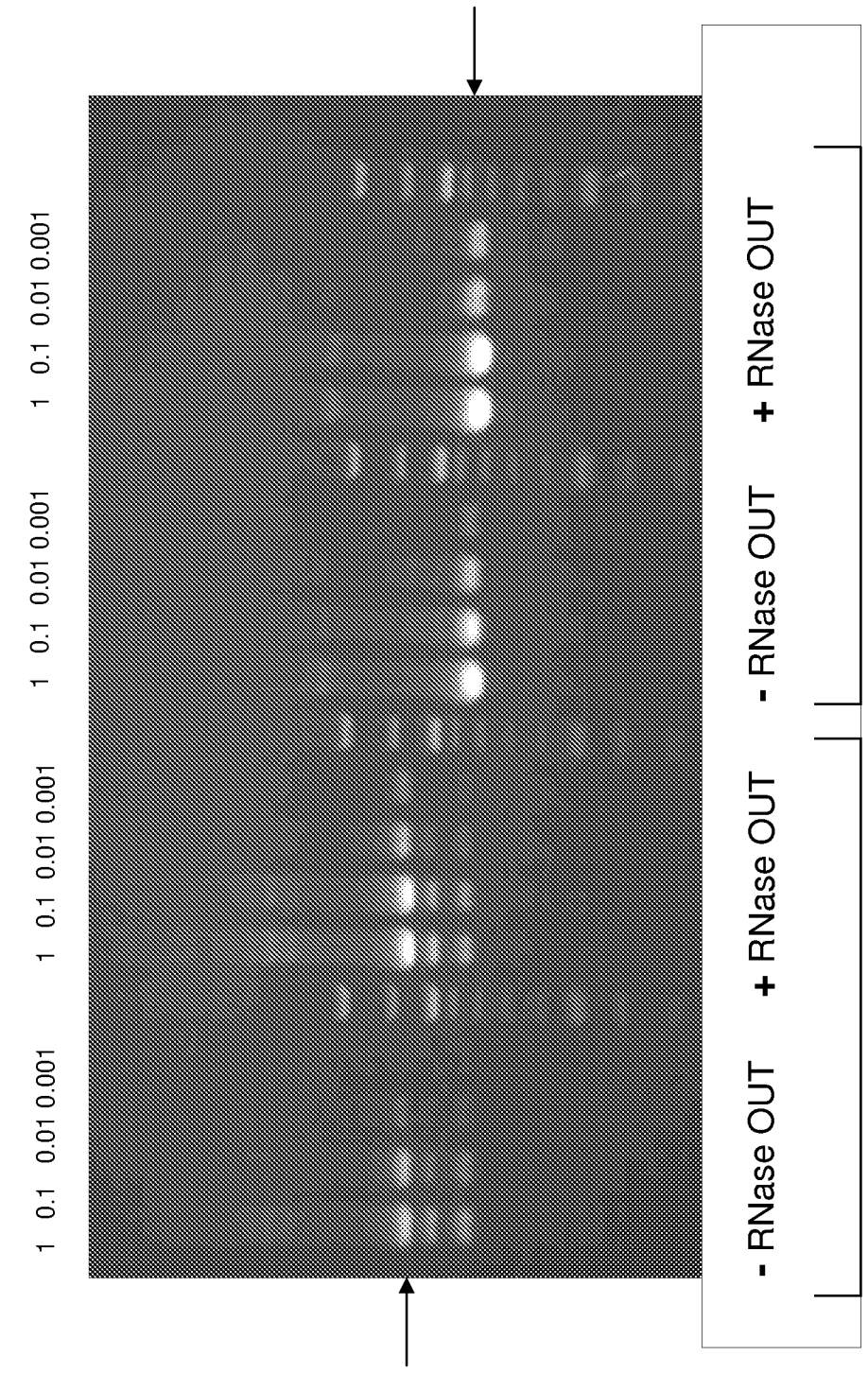

µl of RT product
In PCR reaction
1 0.1 0.01 0.001
1 0.1 0.01 0.001
1 0.1 0.01 0.001
1 0.1 0.01 0.001
- RNase OUT
+ RNase OUT
- RNase OUT
+ RNase OUT
Y61-CL-long
Y61-CL-short

Fig. 17

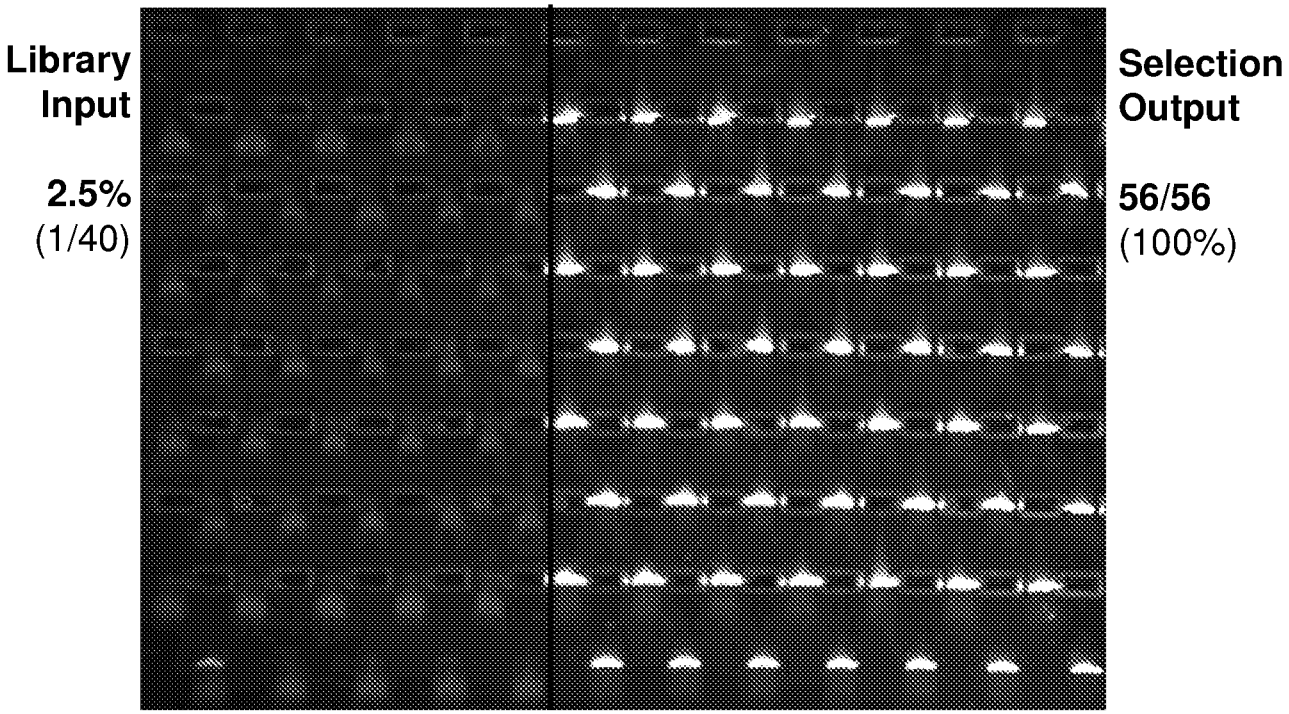


Fig. 18

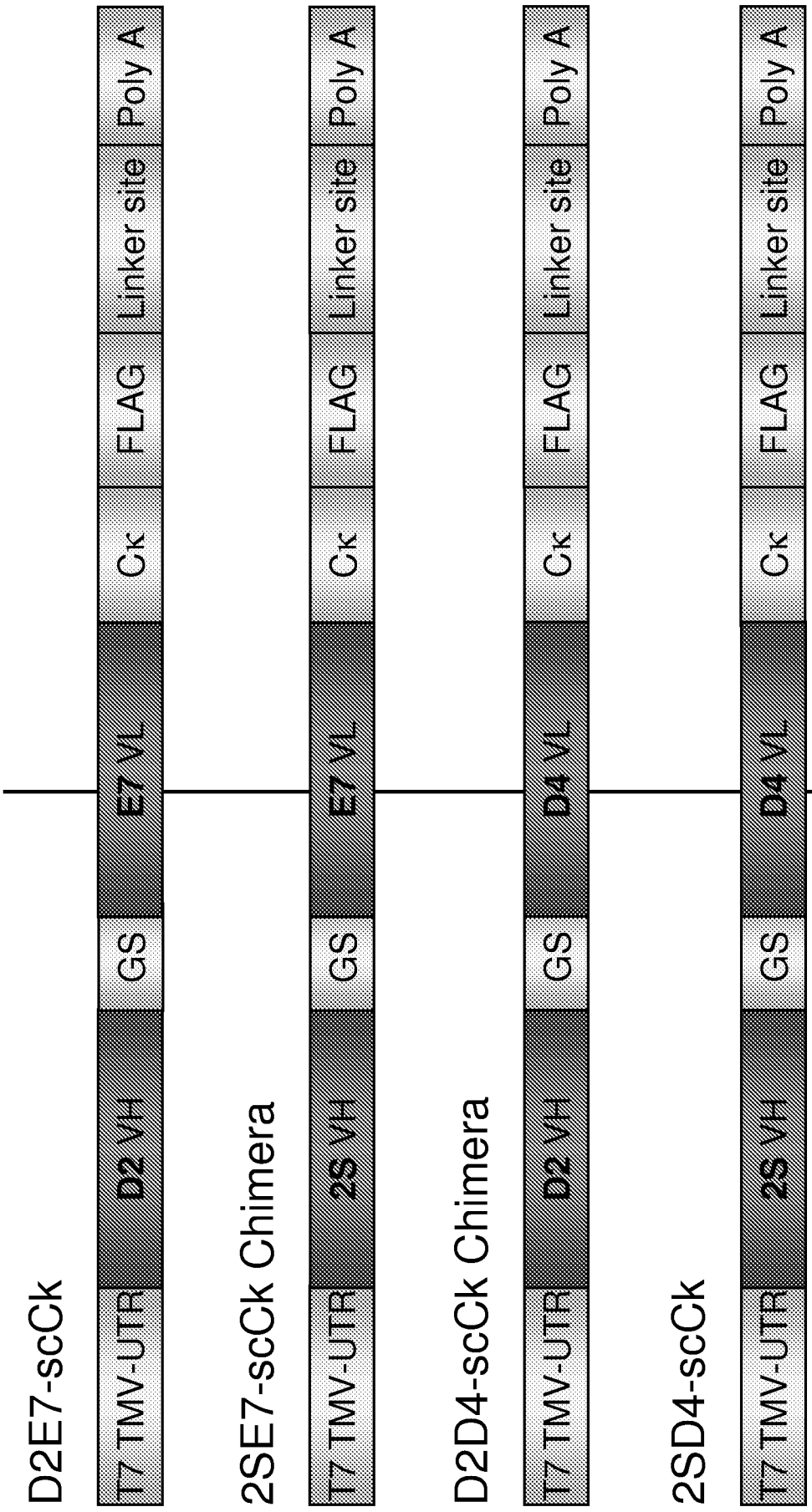
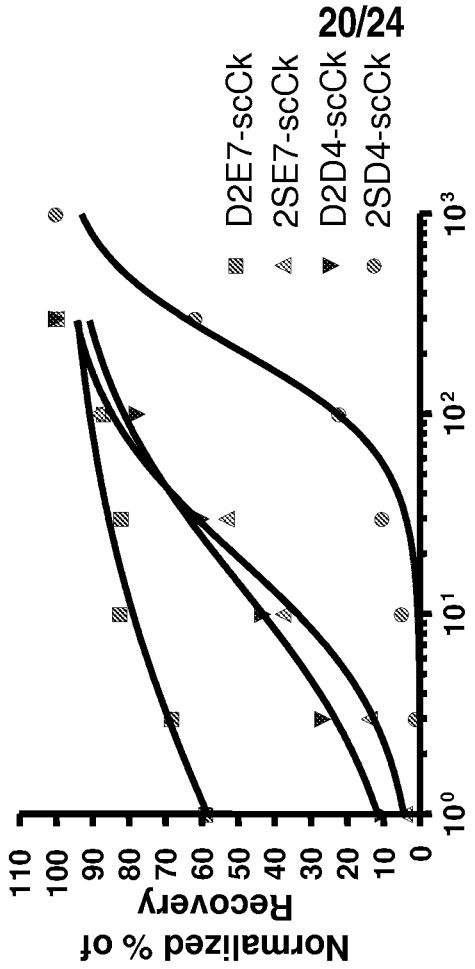

D2E7-scCk
T7 TMV-UTR
D2 VH
GS
E7 VL
Cκ
FLAG
Linker site
Poly A
2SE7-scCk Chimera
T7 TMV-UTR
2S VH
GS
E7 VL
Cκ
FLAG
Linker site
Poly A
D2D4-scCk Chimera
T7 TMV-UTR
D2 VH
GS
D4 VL
Cκ
FLAG
Linker site
Poly A
2SD4-scCk
T7 TMV-UTR
2S VH
GS
D4 VL
Cκ
FLAG
Linker site
Poly A

Fig. 19



EC50	D2E7-scCk	2SE7-scCk	D2D4-scCk	2SD4-scCk
	0.4506	19.36	14.45	209.8

Fig. 20b

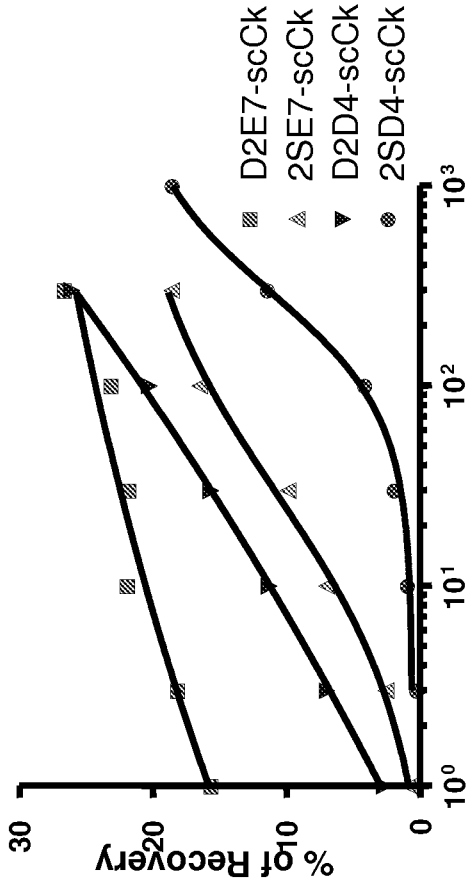


Fig. 20a

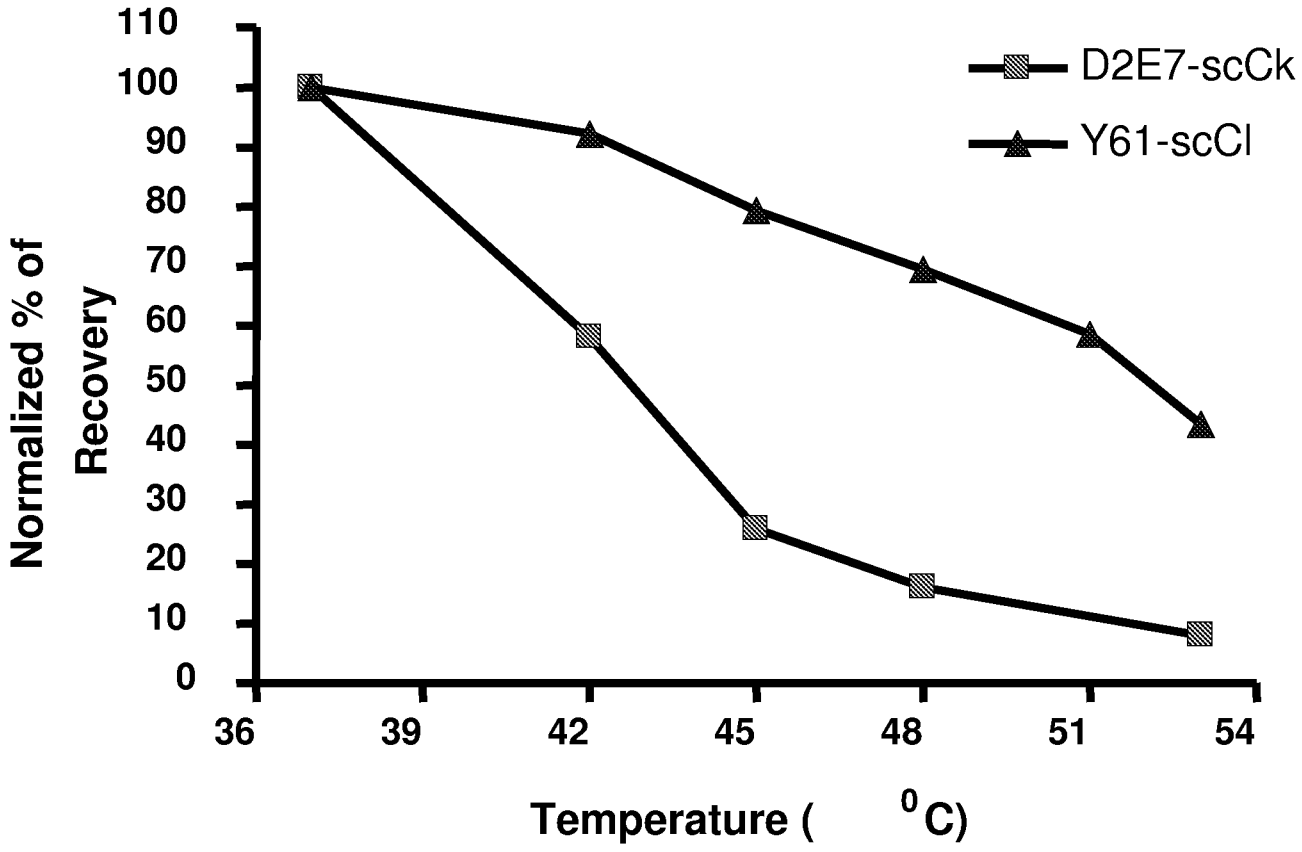

110
100
90
80
70
60
50
40
30
20
10
0
Normalized % of Recovery
36
39
42
45
48
51
54
Temperature (0C)
D2E7-scCk
Y61-scCl

Fig. 21

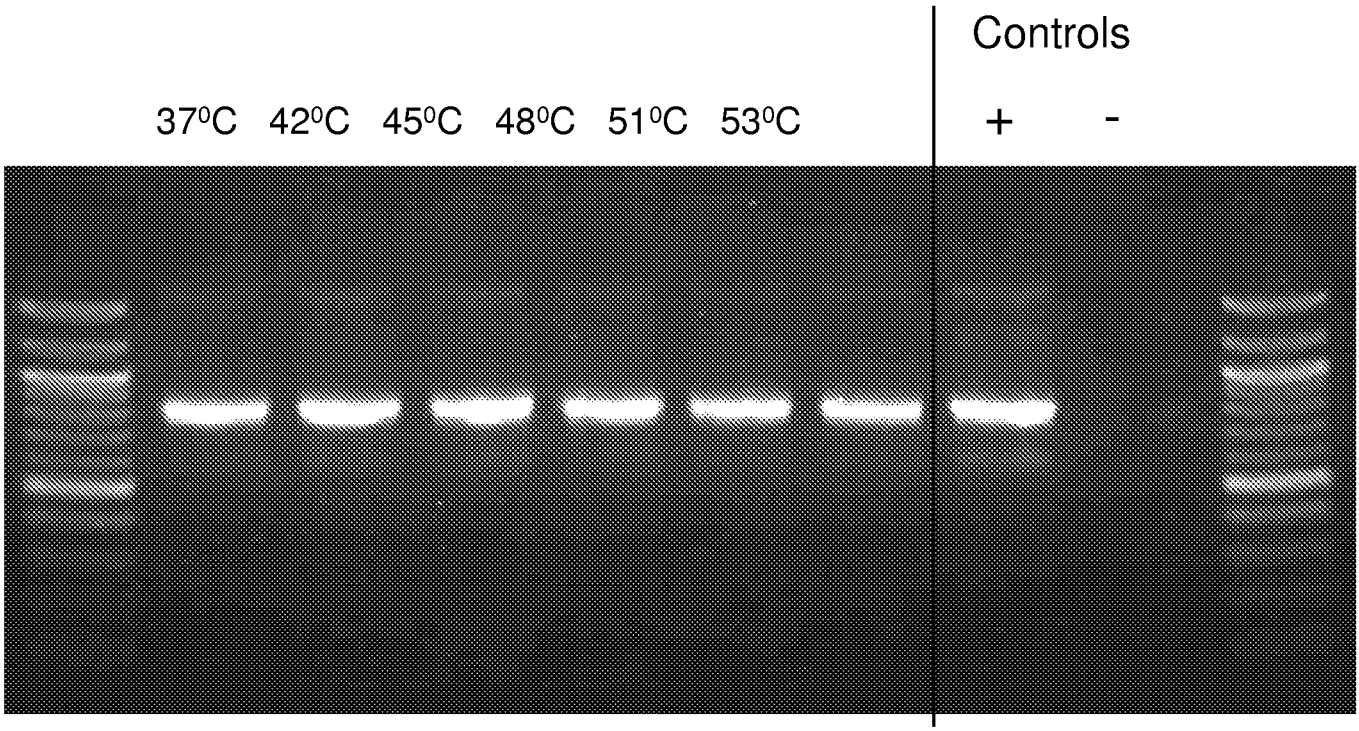


Fig. 22

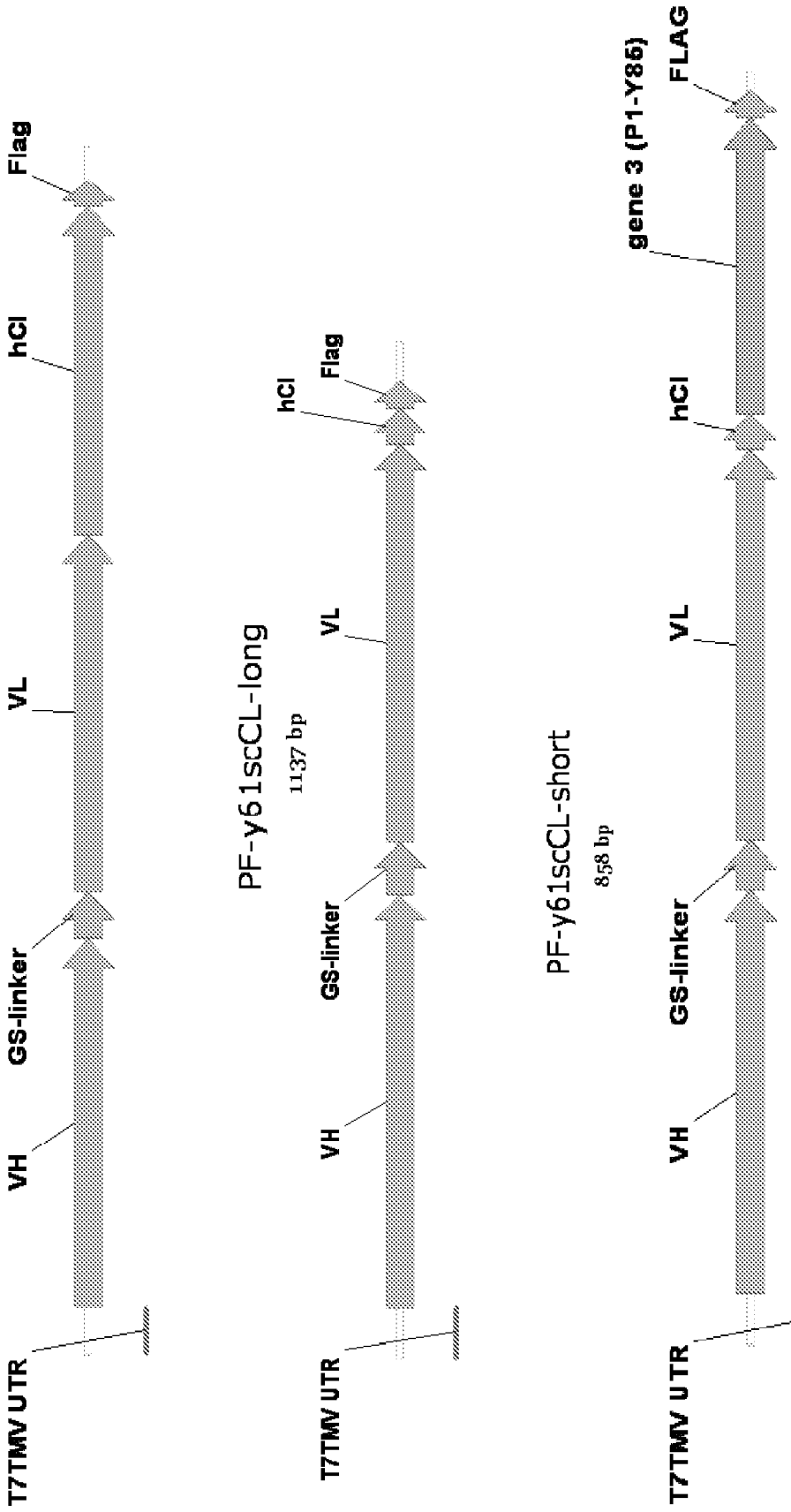

Generated Y61 Single Chain constructs to be tested in PROfusion format:
T7TMV UTR
VH
GS-linker
VL
hCl
Flag
PF-y61scCL-long
1137 bp
T7TMV UTR
VH
GS-linker
VL
hCl
Flag
PF-y61scCL-short
858 bp
T7TMV UTR
VH
GS-linker
VL
hCl
gene 3 (P1-Y86)
FLAG
PF-y61scGene3
1097 bp

Fig. 23

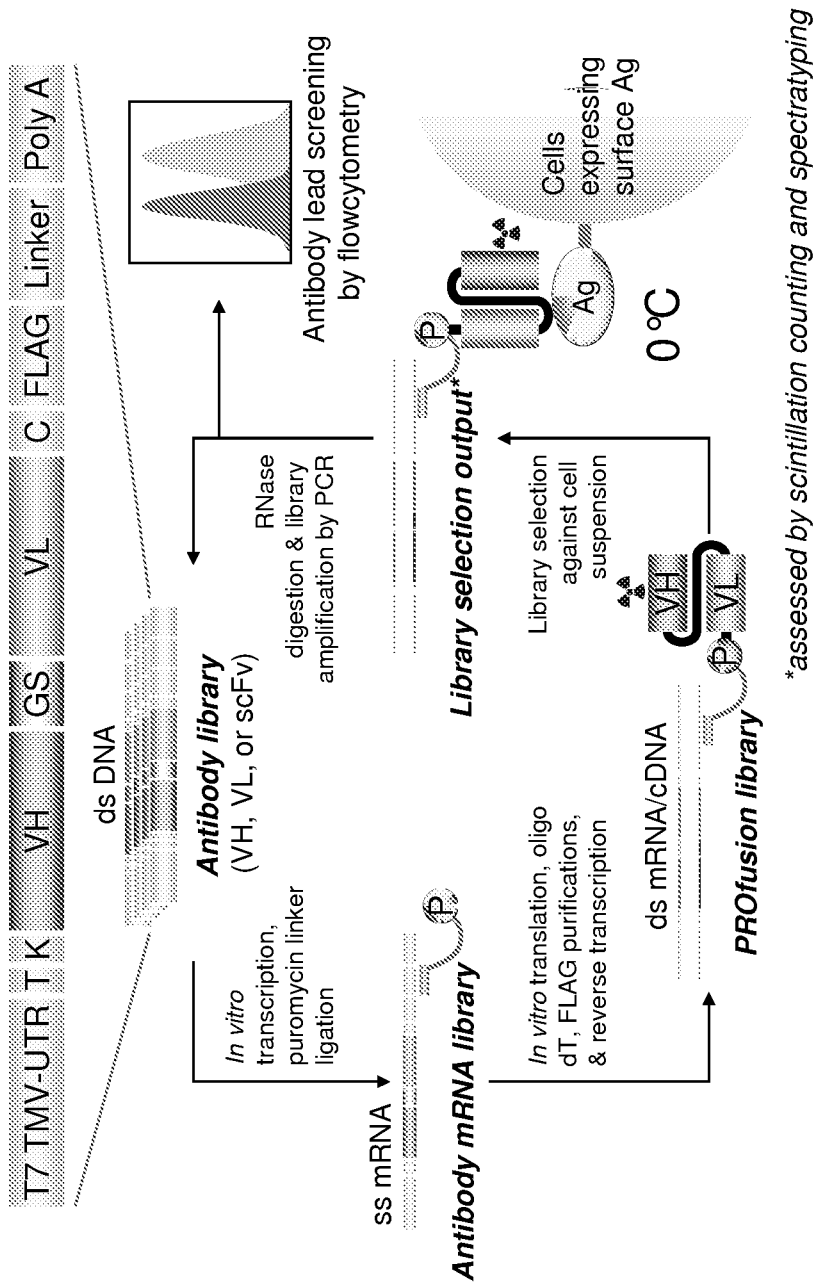

T7 TMV-UTR T K VH GS VL C FLAG Linker Poly A
ds DNA
Antibody library
(VH, VL, or scFv)
In vitro transcription, puromycin linker ligation
RNase digestion & library amplification by PCR
Antibody lead screening by flowcytometry
ss mRNA
Antibody mRNA library
Library selection output*
In vitro translation, oligo dT, FLAG purifications, & reverse transcription
Library selection against cell suspension
Cells expressing surface Ag
Ag
0 °C
ds mRNA/cDNA
PROfusion library
*assessed by scintillation counting and spectratyping

Fig. 24