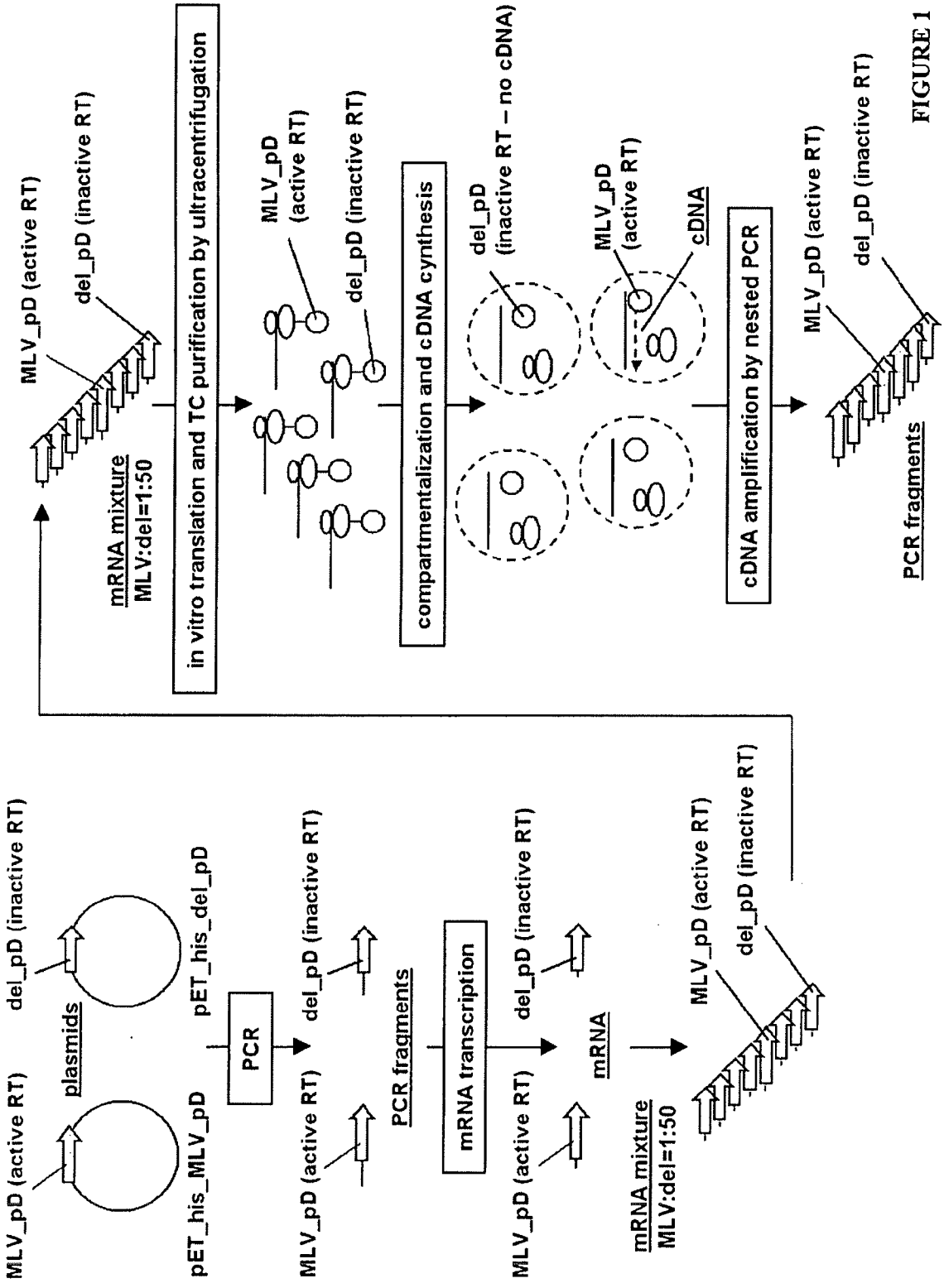

MLV_pD (active RT)
del_pD (inactive RT)
plasmids
pET_his_MLV_pD
pET_his_del_pD
PCR
MLV_pD (active RT)
del_pD (inactive RT)
PCR fragments
mRNA transcription
MLV_pD (active RT)
del_pD (inactive RT)
mRNA
mRNA mixture
MLV:del=1:50
MLV_pD (active RT)
del_pD (inactive RT)
in vitro translation and TC purification by ultracentrifugation
MLV_pD (active RT)
del_pD (inactive RT)
compartmentalization and cDNA cynthesis
del_pD (inactive RT – no cDNA)
MLV_pD (active RT)
cDNA
cDNA amplification by nested PCR
MLV_pD (active RT)
del_pD (inactive RT)
PCR fragments

FIGURE 1

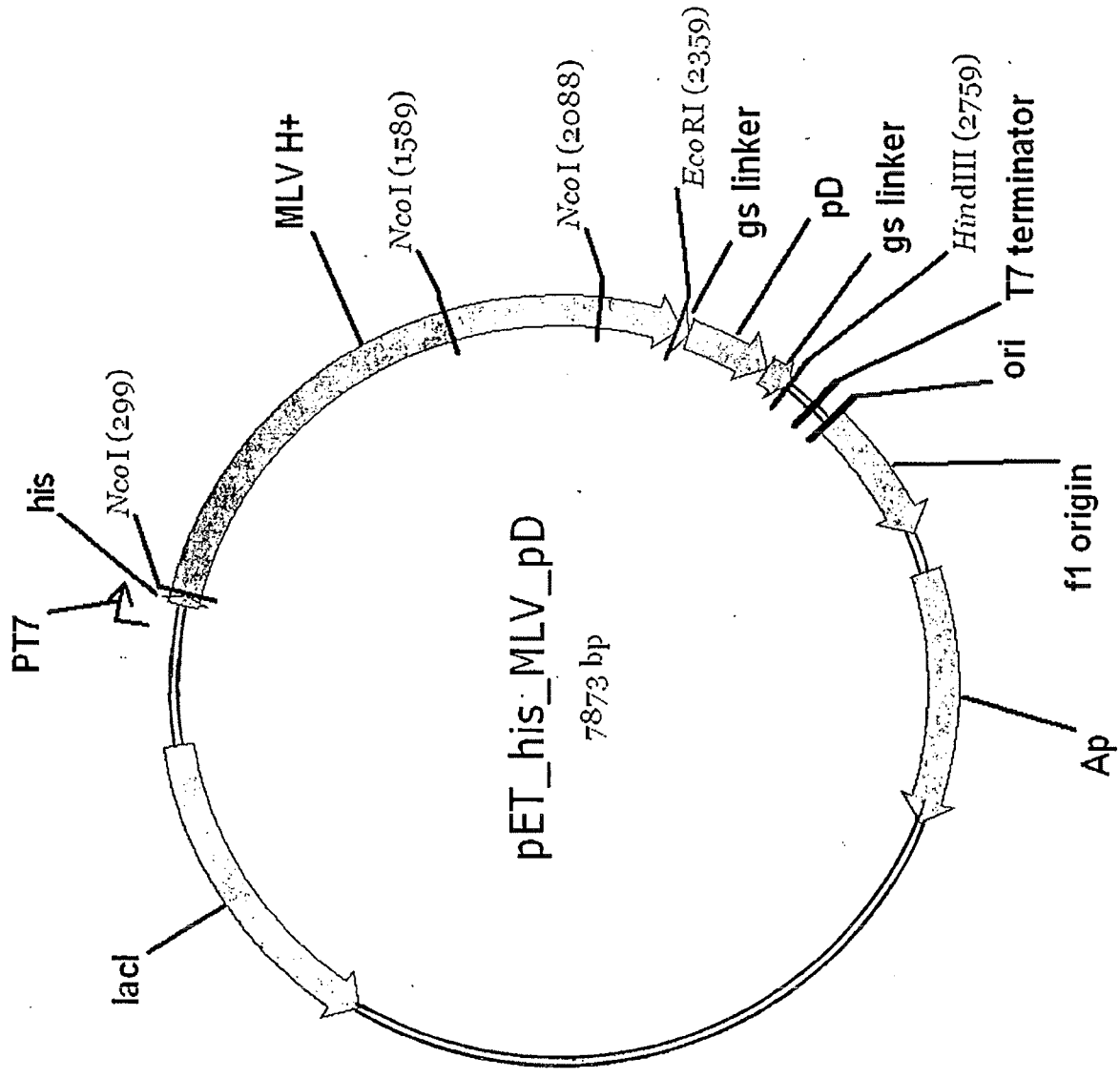

pET_his_MLV_pD
7873 bp
PT7
his
NcoI (299)
MLV H+
NcoI (1589)
NcoI (2088)
EcoRI (2359)
gs linker
pD
gs linker
HindIII (2759)
T7 terminator
ori
f1 origin
Ap
lacI

FIGURE 2

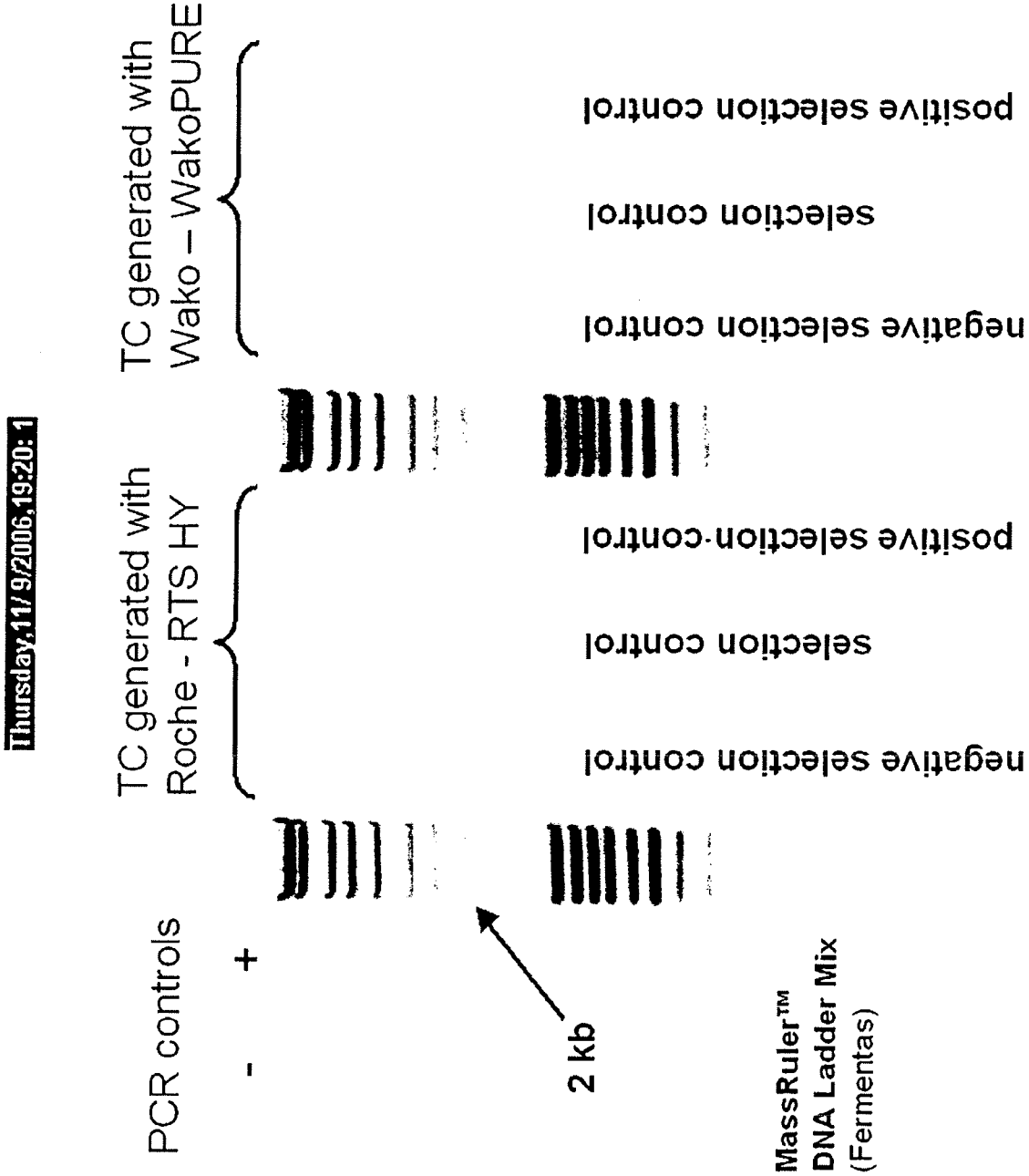

Thursday, 11/9/2006, 19:20: 1
PCR controls
+
-
TC generated with Roche - RTS HY
TC generated with Wako – WakoPURE
2 kb
MassRuler™ DNA Ladder Mix (Fermentas)
negative selection control
selection control
positive selection control
negative selection control
selection control
positive selection control

FIGURE 3

FIGURE 4

Thursday, 11/ 9/2006, 13:25:53

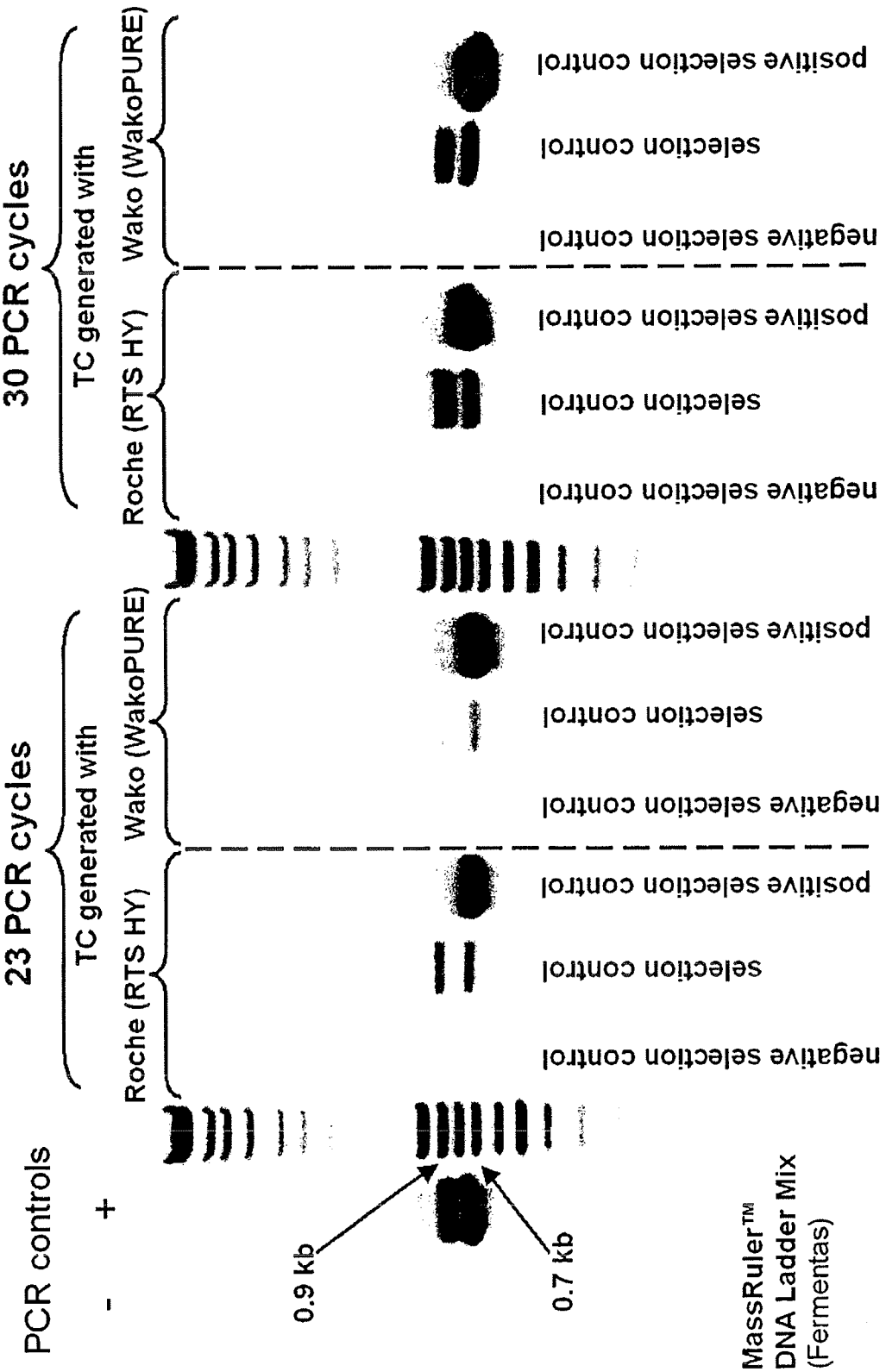
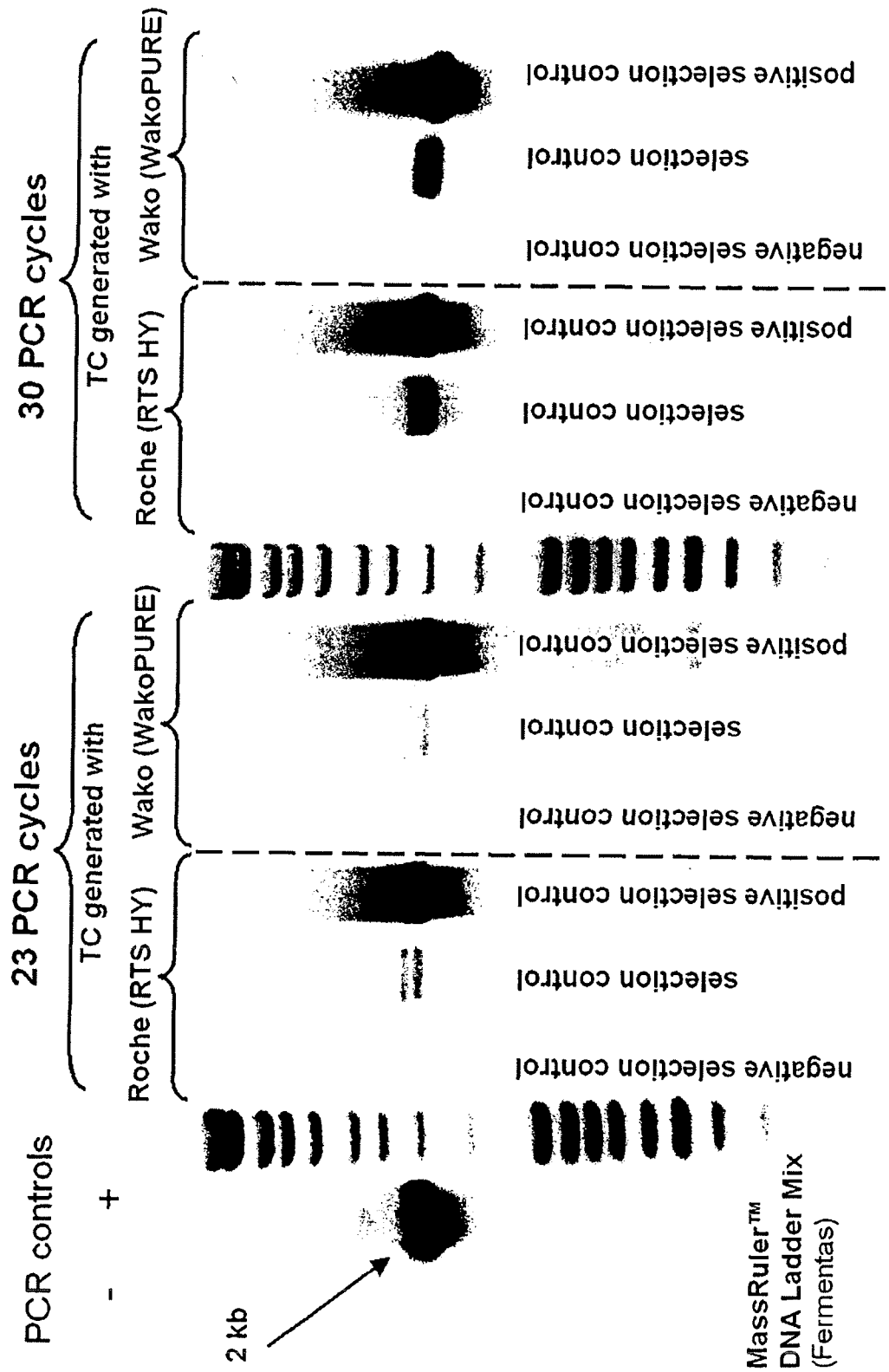


FIGURE 5

Thursday, 11/ 9/2006, 16:11:16



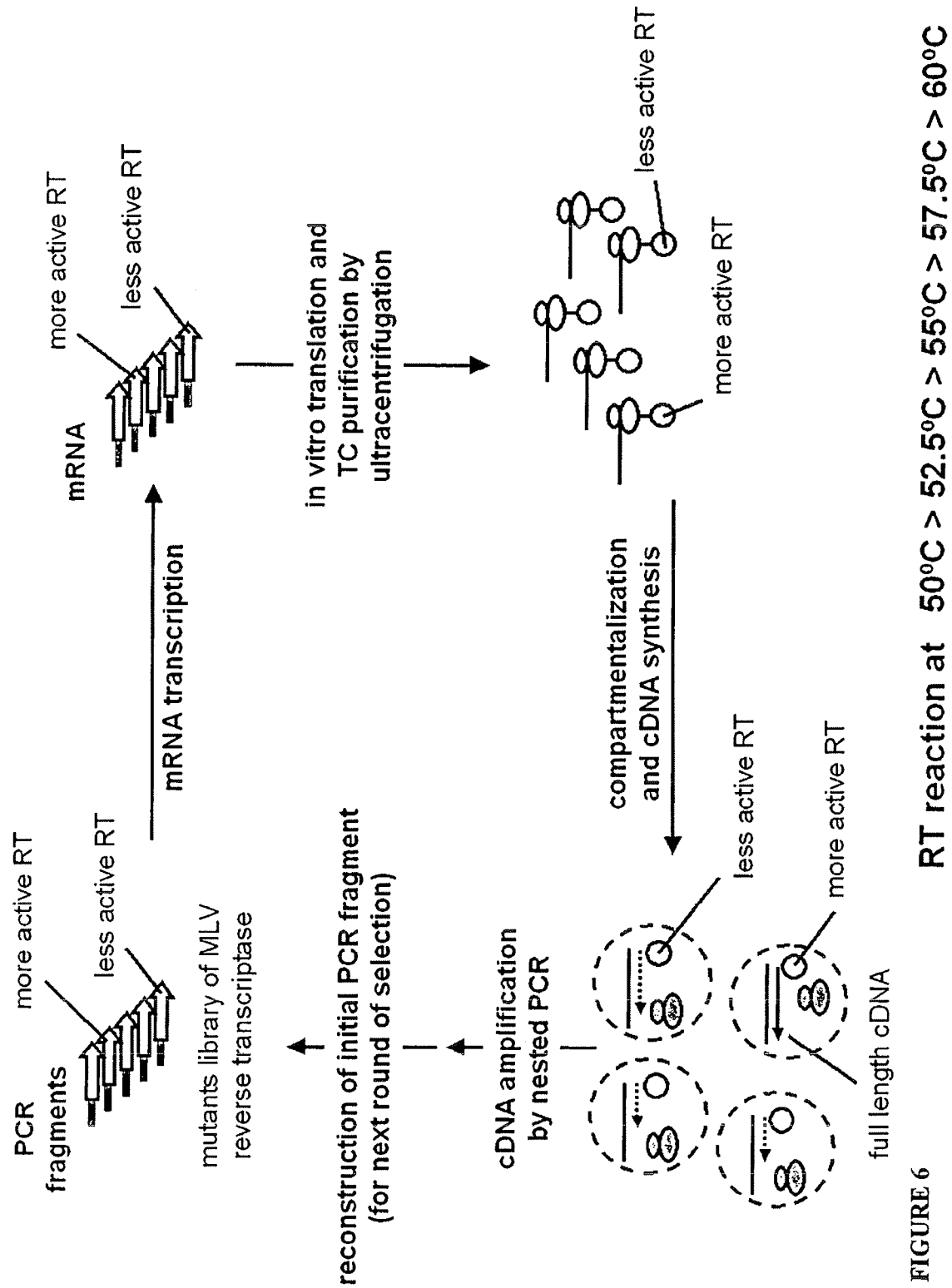

PCR fragments
more active RT
less active RT
mutants library of MLV reverse transcriptase
mRNA transcription
mRNA
more active RT
less active RT
in vitro translation and TC purification by ultracentrifugation
reconstruction of initial PCR fragment (for next round of selection)
cDNA amplification by nested PCR
compartmentalization and cDNA synthesis
less active RT
more active RT
less active RT
more active RT
full length cDNA
RT reaction at 50°C > 52.5°C > 55°C > 57.5°C > 60°C

FIGURE 6

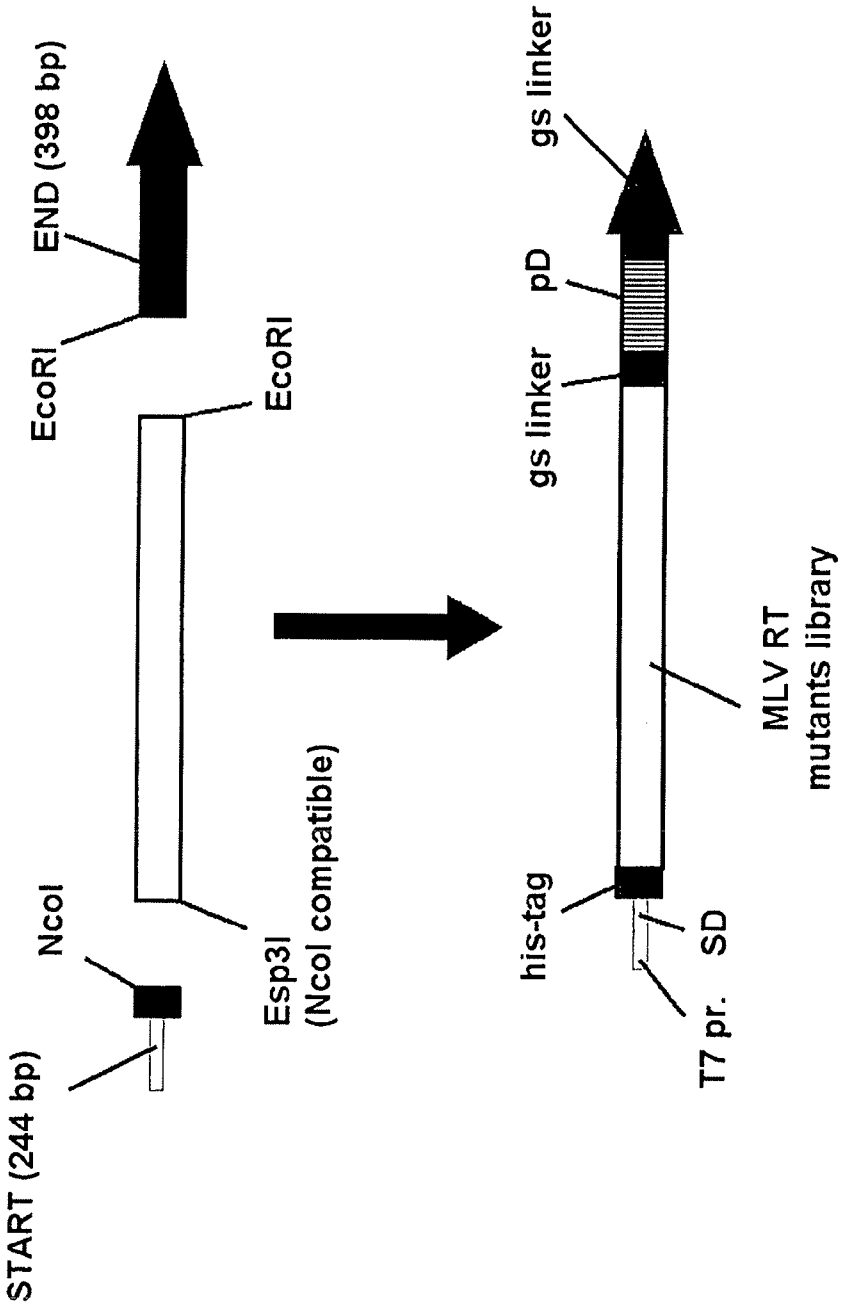

START (244 bp)
NcoI
Esp3I
(NcoI compatible)
EcoRI
EcoRI
END (398 bp)
his-tag
T7 pr.
SD
MLV RT
mutants library
gs linker
pD
gs linker
gs linker

FIGURE 7

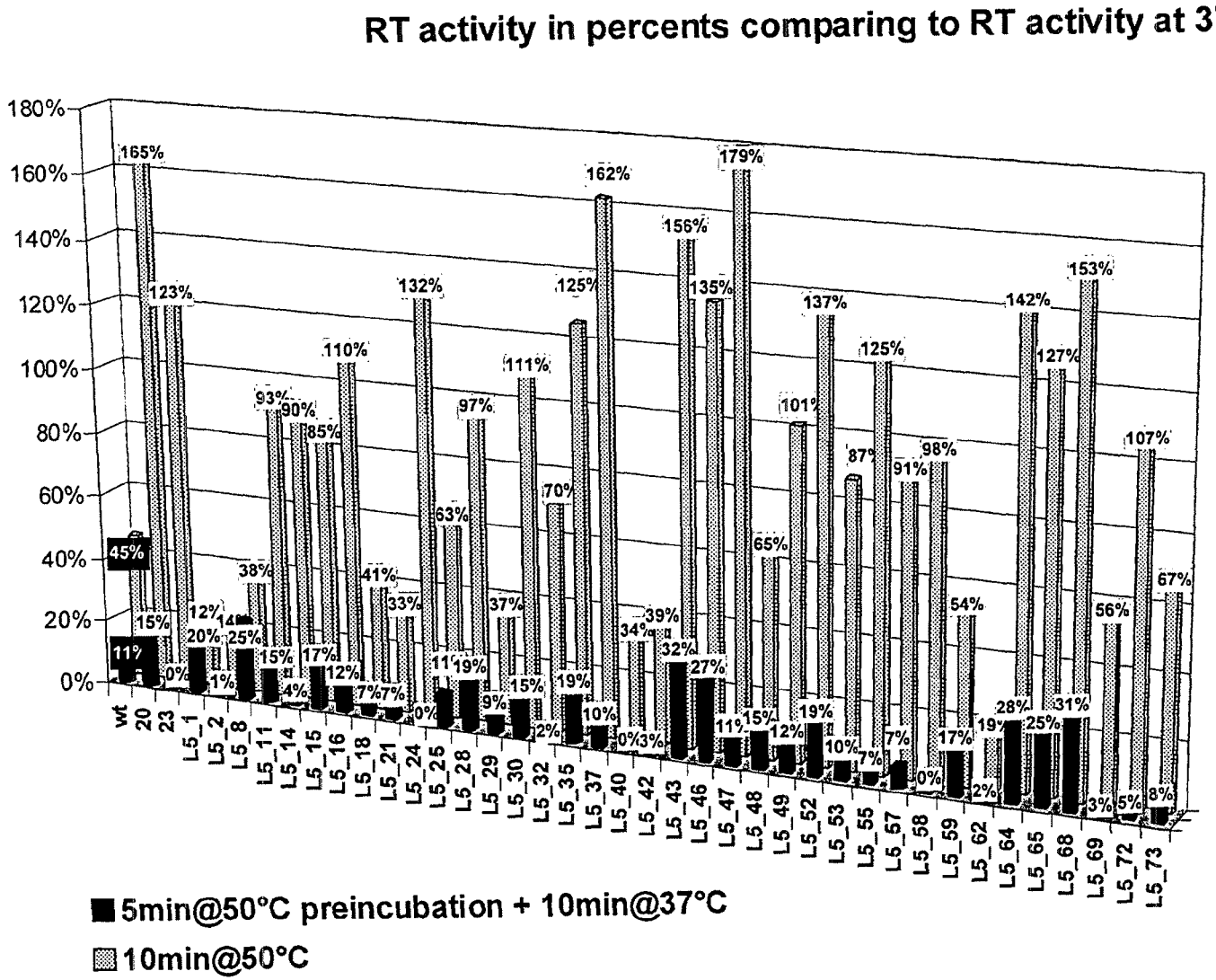

RT activity in percents comparing to RT activity at 37°C
180%
160%
140%
120%
100%
80%
60%
40%
20%
0%
wt
20
23
L5_1
L5_2
L5_8
L5_11
L5_14
L5_15
L5_16
L5_18
L5_21
L5_24
L5_25
L5_28
L5_29
L5_30
L5_32
L5_35
L5_37
L5_40
L5_42
L5_43
L5_46
L5_47
L5_48
L5_49
L5_52
L5_53
L5_55
L5_57
L5_58
L5_59
L5_62
L5_64
L5_65
L5_68
L5_69
L5_72
L5_73
5min@50°C preincubation + 10min@37°C
10min@50°C

FIGURE 8

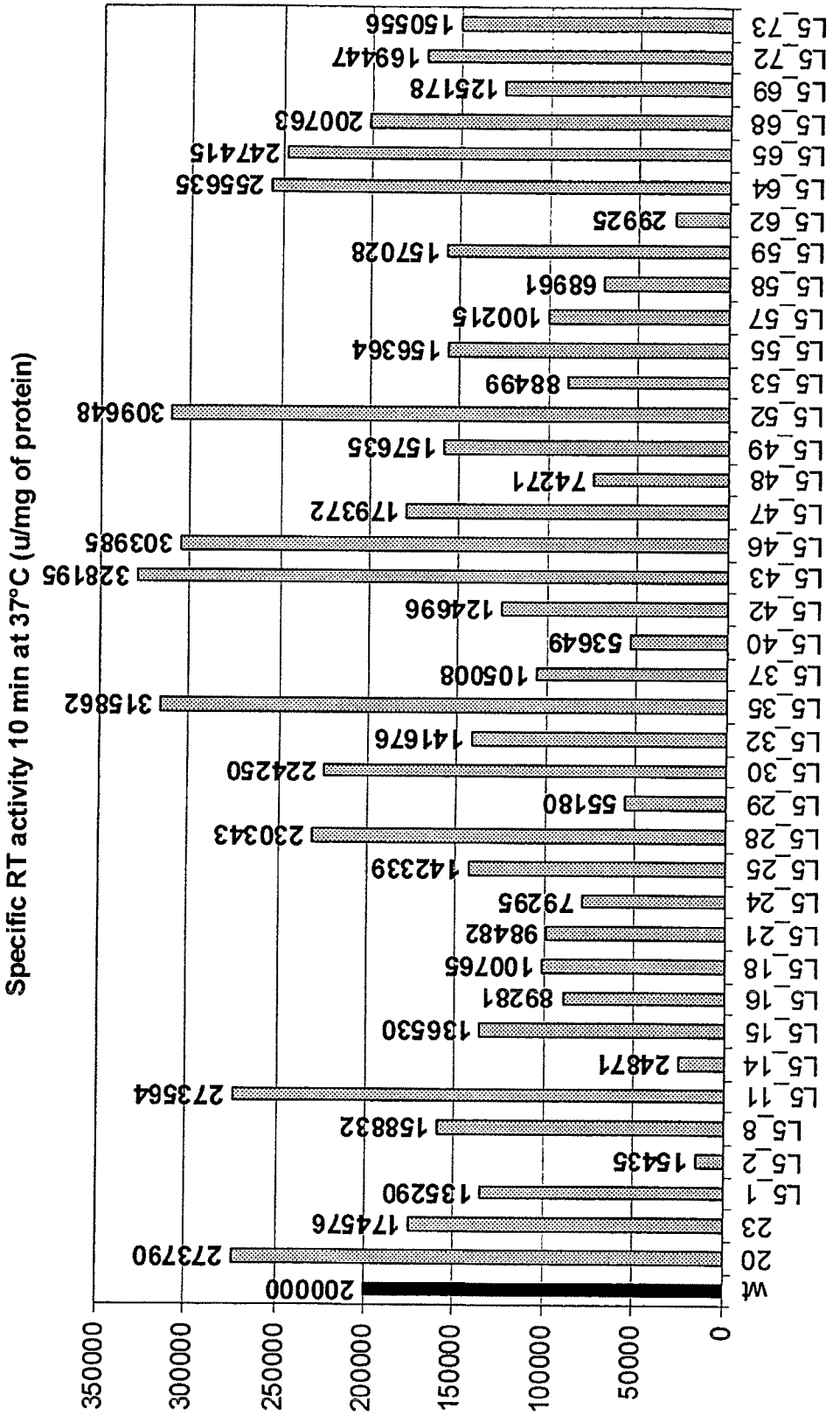

Specific RT activity 10 min at 37°C (u/mg of protein)
350000
300000
250000
200000
150000
100000
50000
0
wt
20
23
L5_1
L5_2
L5_8
L5_11
L5_14
L5_15
L5_16
L5_18
L5_21
L5_24
L5_25
L5_28
L5_29
L5_30
L5_32
L5_35
L5_37
L5_40
L5_42
L5_43
L5_46
L5_47
L5_48
L5_49
L5_52
L5_53
L5_55
L5_57
L5_58
L5_59
L5_62
L5_64
L5_65
L5_68
L5_69
L5_72
L5_73
200000
273790
174576
135290
15435
158832
273564
24871
136530
89281
100765
98482
79295
142339
230343
55180
224250
141676
315862
105008
53649
124696
328195
303985
179372
74271
157635
309648
88499
156364
100215
68961
157028
29925
255635
247415
200763
125178
169447
150556

FIGURE 9

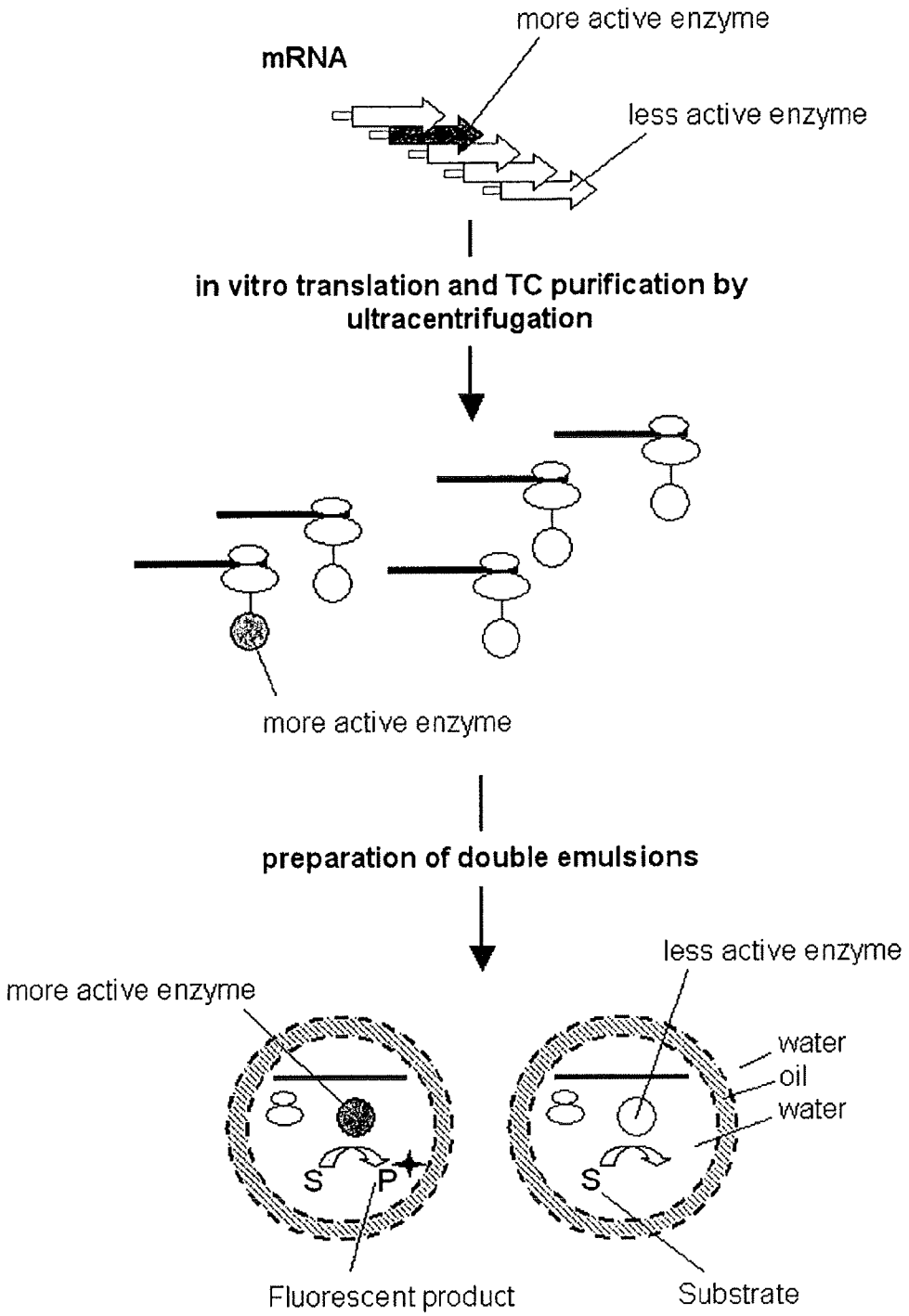

more active enzyme
mRNA
less active enzyme
in vitro translation and TC purification by ultracentrifugation
more active enzyme
preparation of double emulsions
less active enzyme
more active enzyme
water
oil
water
S
P
S
Fluorescent product
Substrate
Sorting in FACS

FIGURE 10

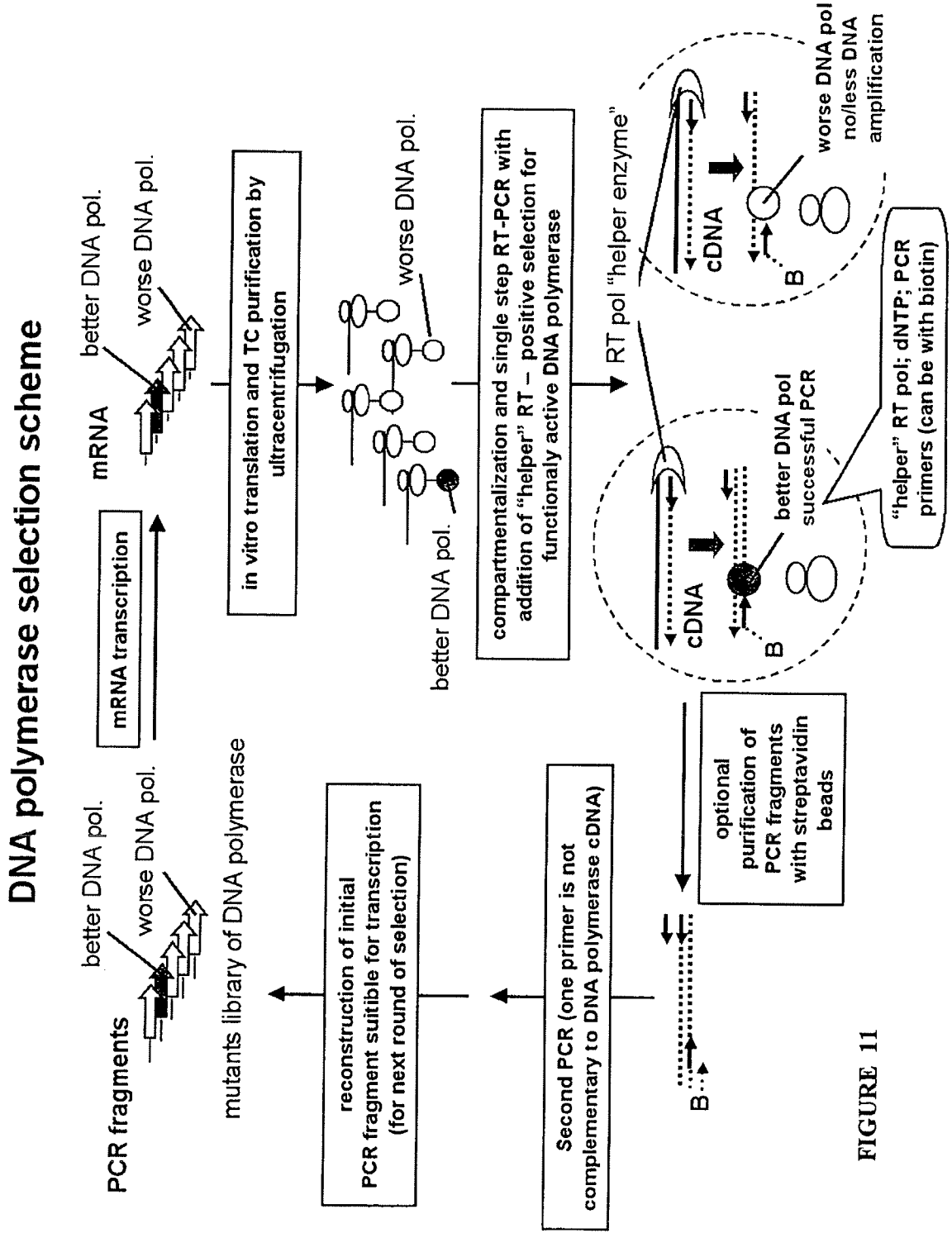

DNA polymerase selection scheme
PCR fragments
better DNA pol.
worse DNA pol.
mutants library of DNA polymerase
reconstruction of initial PCR fragment suitible for transcription (for next round of selection)
Second PCR (one primer is not complementary to DNA polymerase cDNA)
optional purification of PCR fragments with streptavidin beads
B
mRNA transcription
mRNA
in vitro translation and TC purification by ultracentrifugation
compartmentalization and single step RT-PCR with addition of "helper" RT – positive selection for functionaly active DNA polymerase
RT pol "helper enzyme"
cDNA
better DNA pol successful PCR
worse DNA pol no/less DNA amplification
"helper" RT pol; dNTP; PCR primers (can be with biotin)

FIGURE 11

DNA dependent DNA polymerase evolution scheme

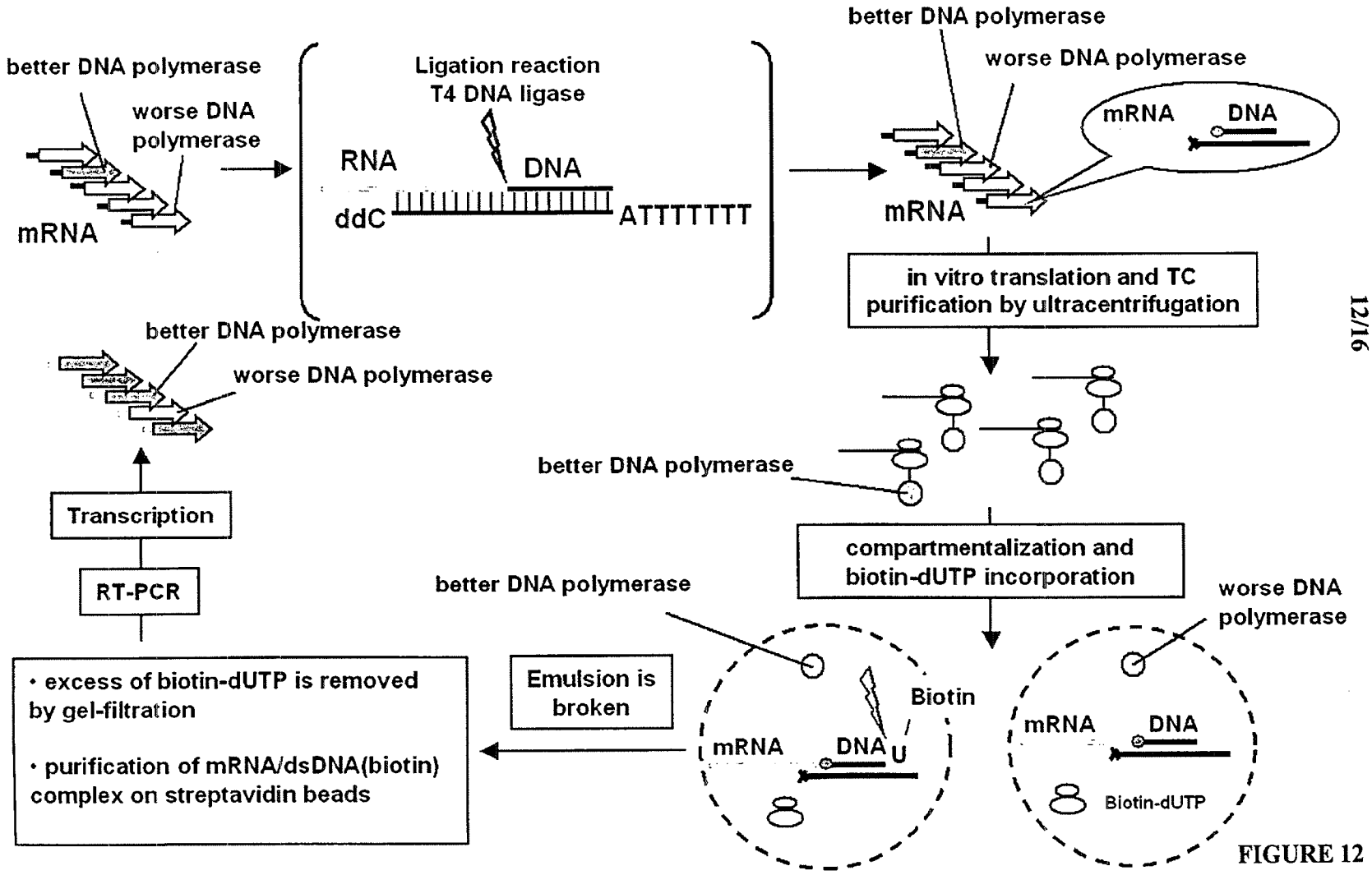


FIGURE 12

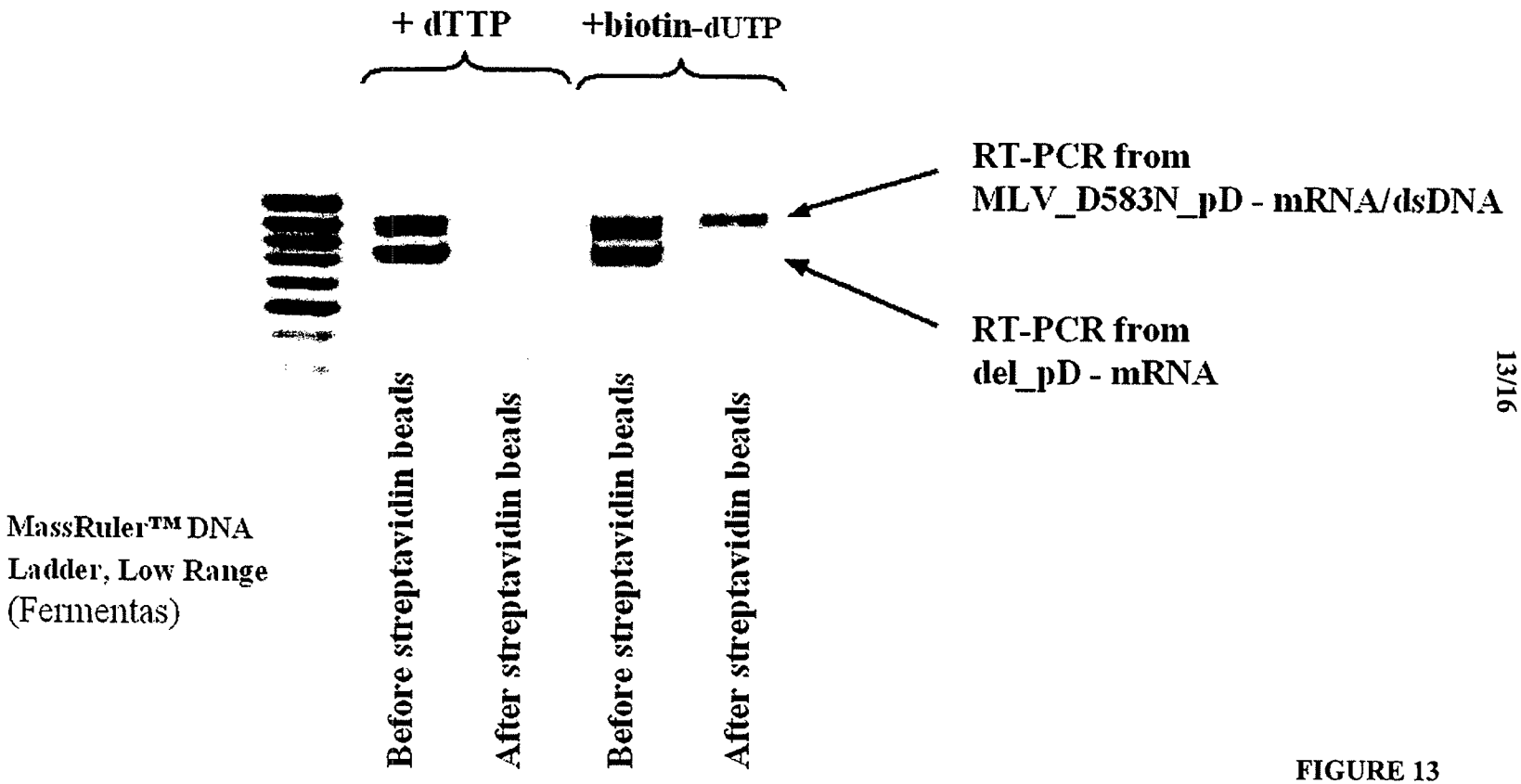
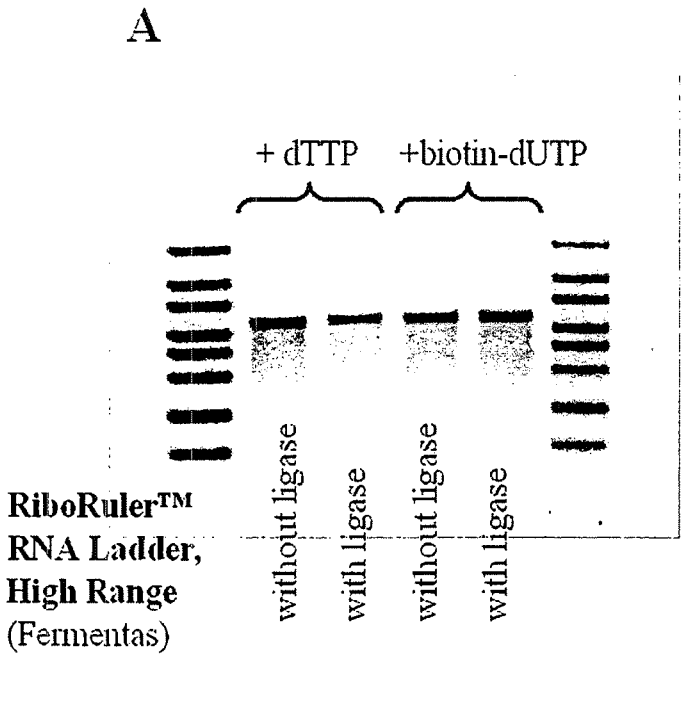
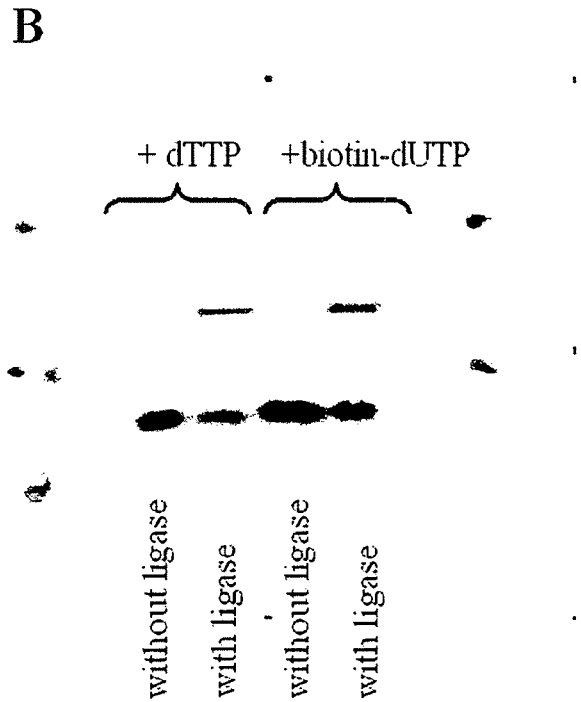

+ dTTP
+biotin-dUTP
RT-PCR from
MLV_D583N_pD - mRNA/dsDNA
RT-PCR from
del_pD - mRNA
MassRuler™ DNA
Ladder, Low Range
(Fermentas)
Before streptavidin beads
After streptavidin beads
Before streptavidin beads
After streptavidin beads

FIGURE 13





C

long+

5′-.....mRNA.....CGAACGTGGCGAGAAAGGAAGGGAAGAAAGAAGTC +dTTP(biotin-dUTP)+[α-P^{33}]dATP

ddC-GCCTTGGCCGCCACCTAGCACAGAAGAAGCTTGCACCGCTCTTTCCTTCCCTTCTTTCTTCAGATTTTTTT-5′

ddC-Long2

FIGURE 14

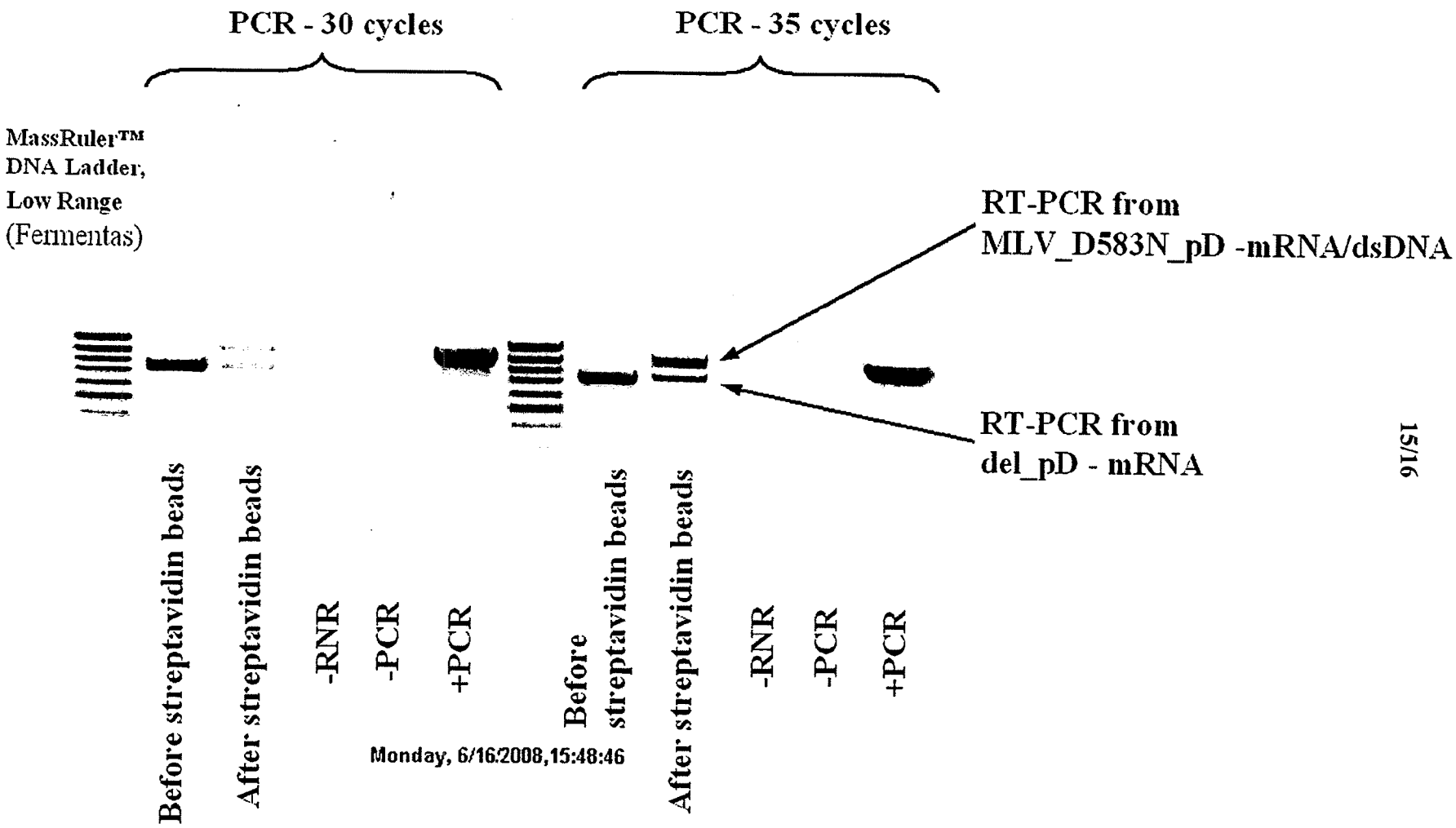

PCR - 30 cycles
PCR - 35 cycles
MassRuler™ DNA Ladder, Low Range (Fermentas)
RT-PCR from MLV_D583N_pD -mRNA/dsDNA
RT-PCR from del_pD - mRNA
Before streptavidin beads
After streptavidin beads
-RNR
-PCR
+PCR
Before streptavidin beads
After streptavidin beads
-RNR
-PCR
+PCR
Monday, 6/16/2008, 15:48:46

FIGURE 15

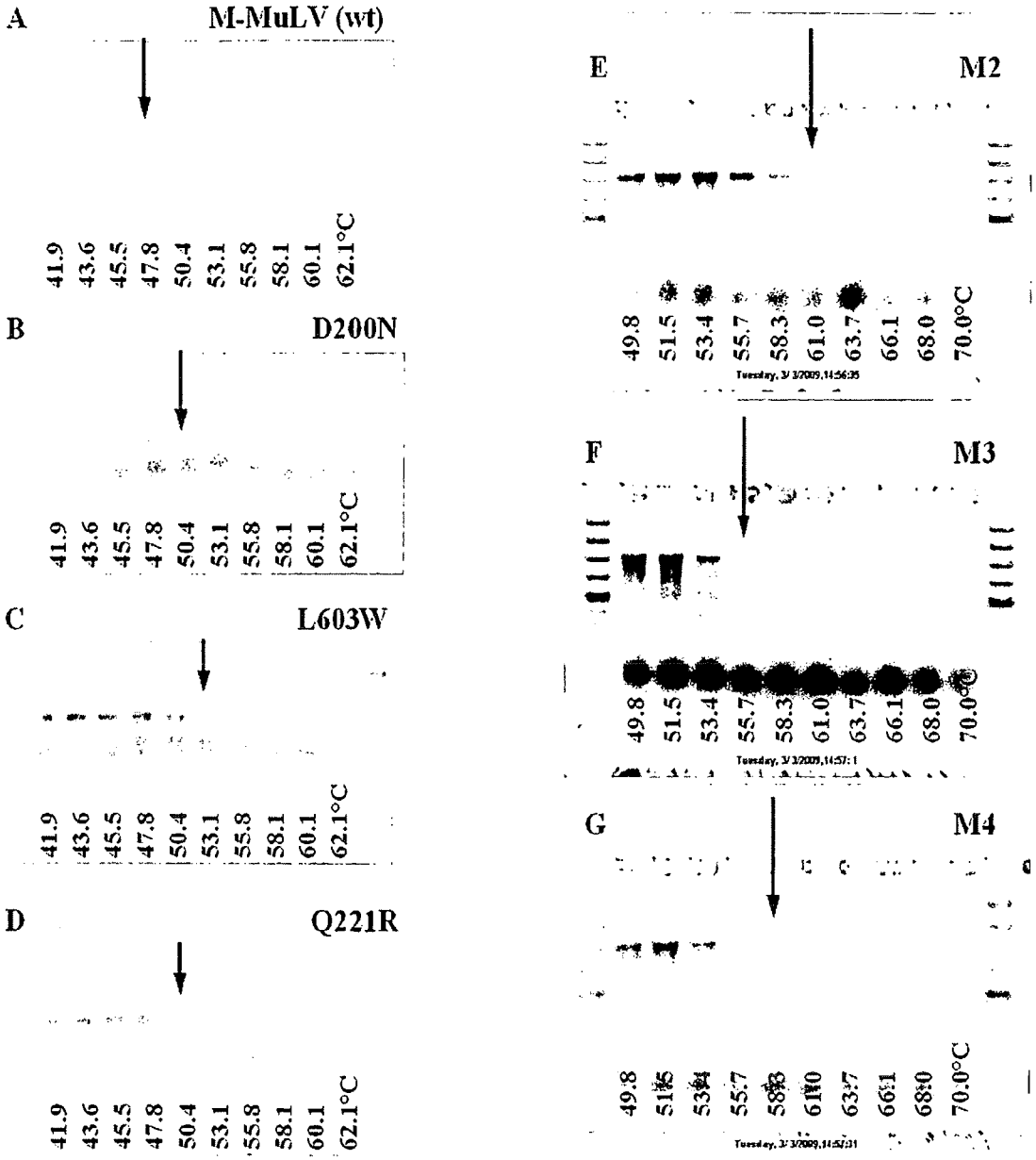

A
M-MuLV (wt)
41.9
43.6
45.5
47.8
50.4
53.1
55.8
58.1
60.1
62.1°C
B
D200N
C
L603W
D
Q221R
E
M2
49.8
51.5
53.4
55.7
58.3
61.0
63.7
66.1
68.0
70.0°C
F
M3
G
M4

FIGURE 16