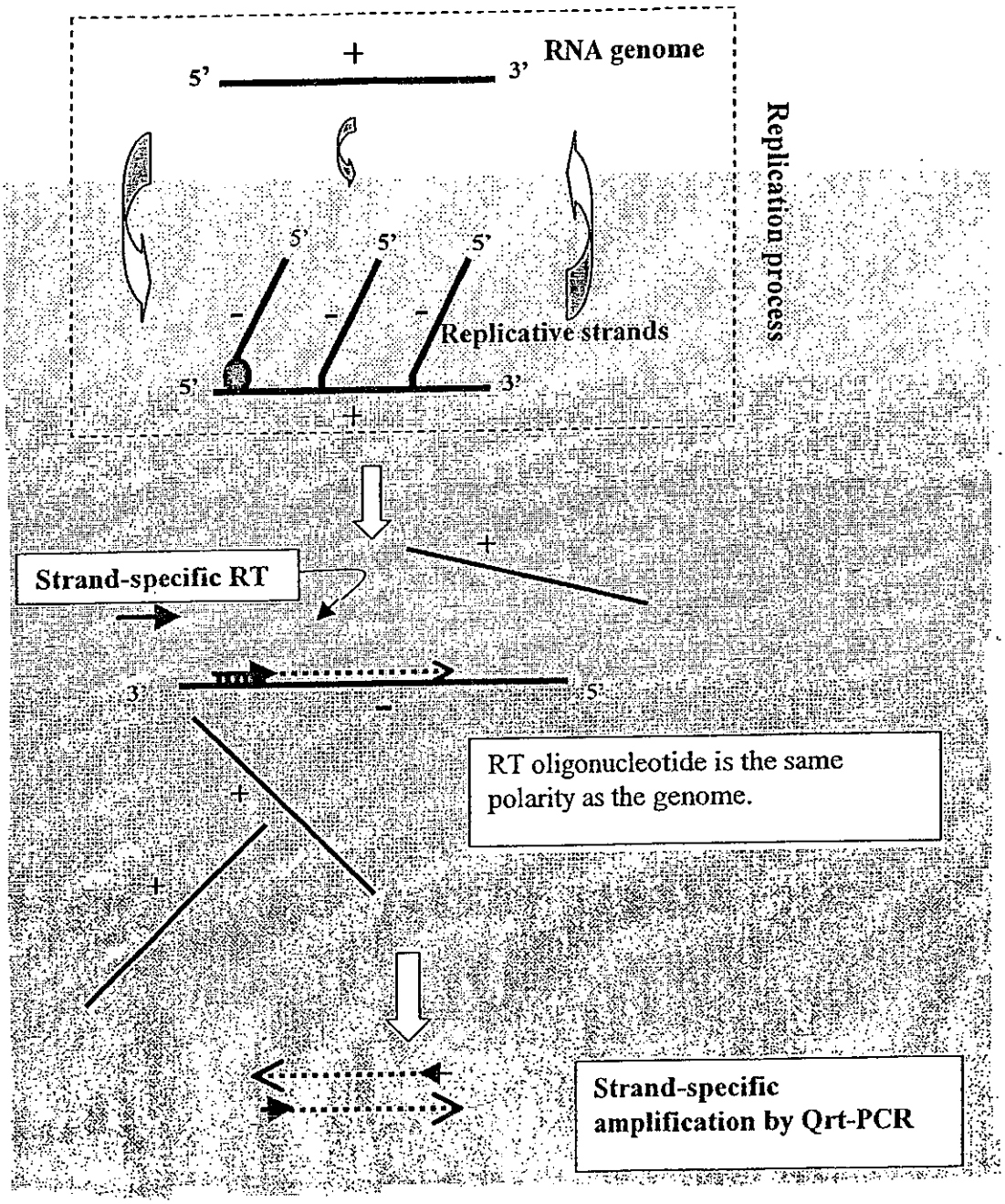


Figure 1
PRIOR ART

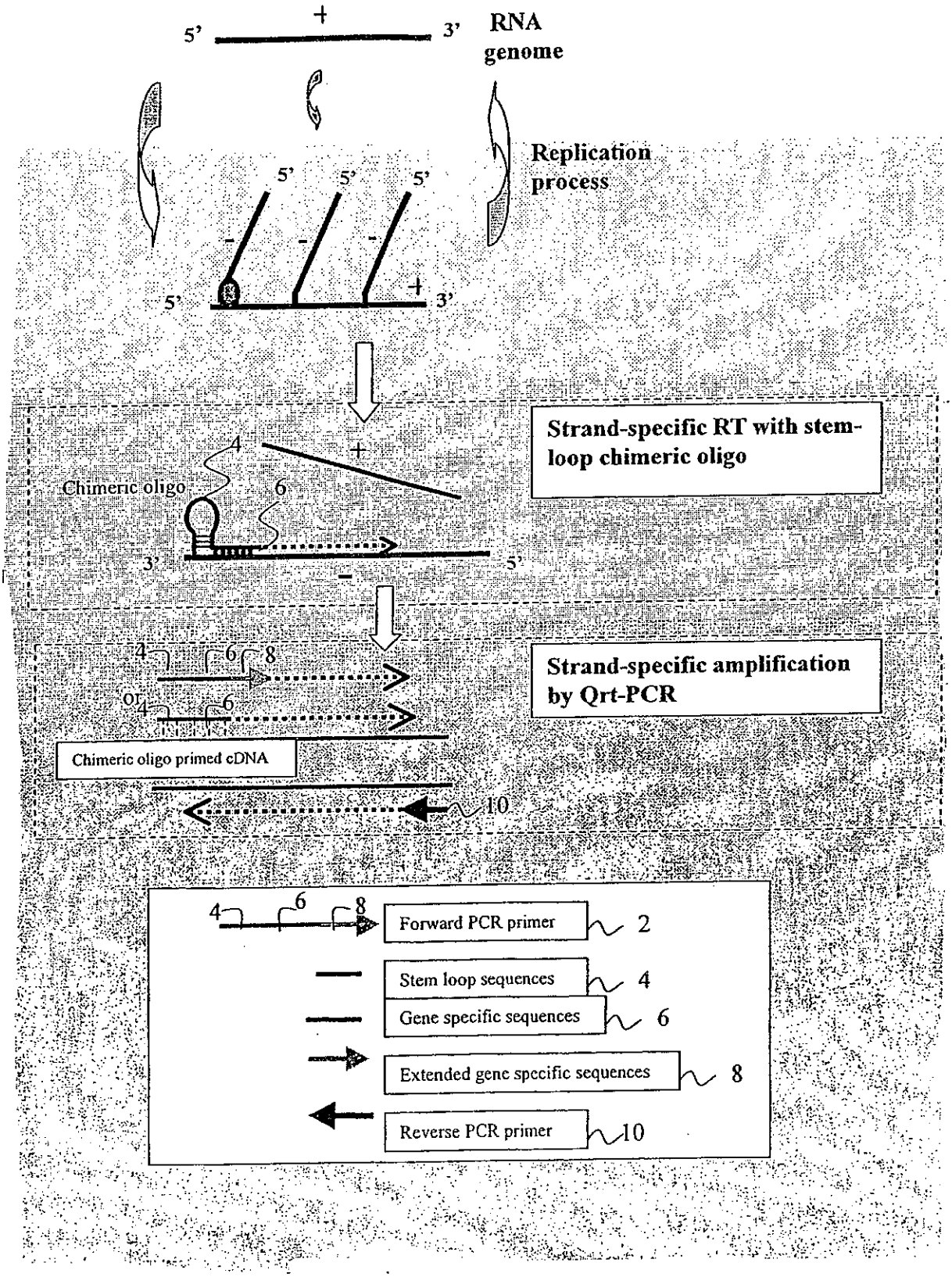

5'
+
3'
RNA genome
Replication process
5'
5'
5'
-
-
-
5'
+
3'
Strand-specific RT with stem-loop chimeric oligo
Chimeric oligo
4
6
+
3'
5'
-
Strand-specific amplification by Qrt-PCR
4
6
8
or
4
6
Chimeric oligo primed cDNA
10
4
6
8
Forward PCR primer
2
Stem loop sequences
4
Gene specific sequences
6
Extended gene specific sequences
8
Reverse PCR primer
10

Figure 2

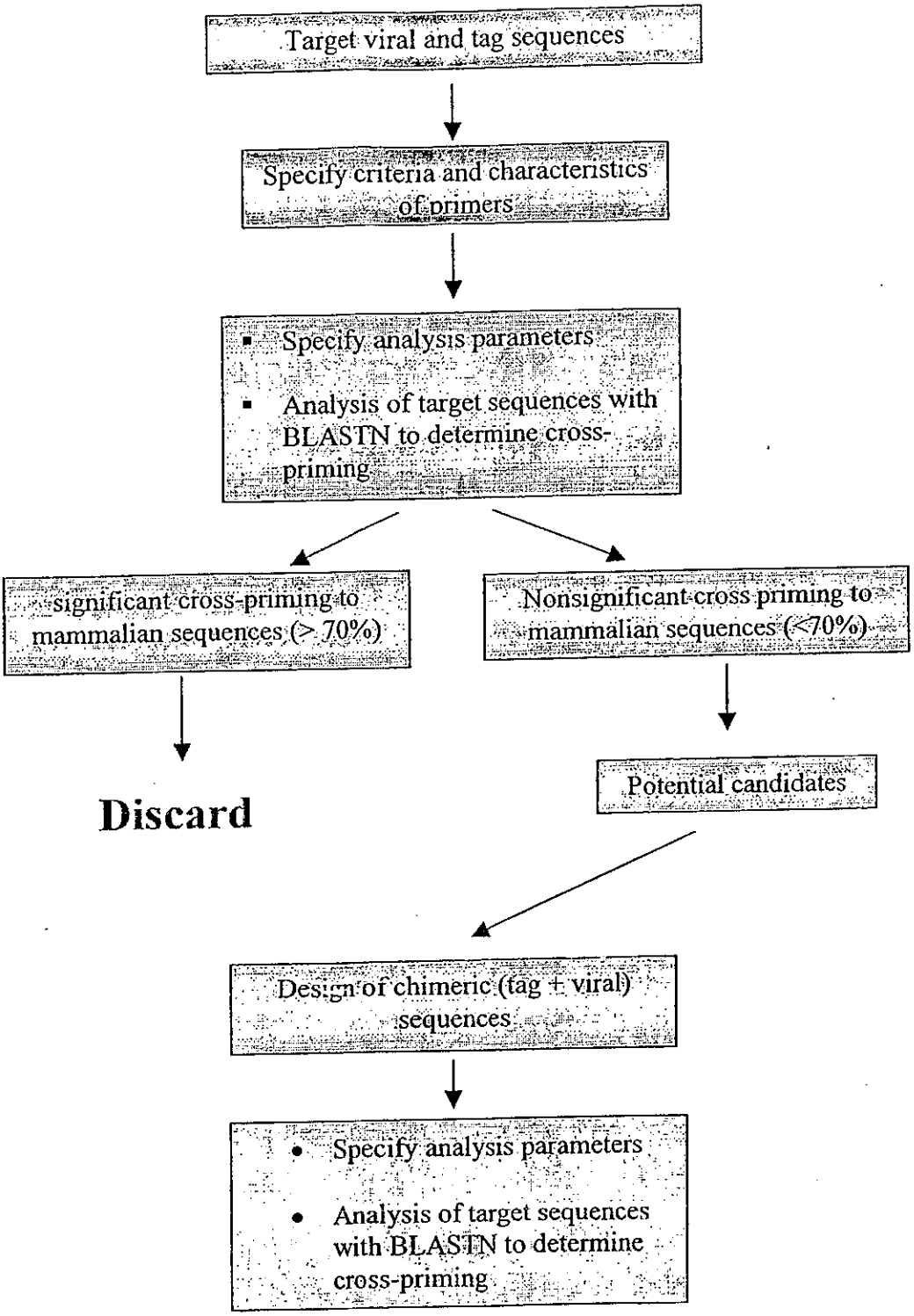

Target viral and tag sequences
Specify criteria and characteristics of primers
Specify analysis parameters
Analysis of target sequences with BLASTN to determine cross-priming
significant cross-priming to mammalian sequences (> 70%)
Nonsignificant cross priming to mammalian sequences (<70%)
Discard
Potential candidates
Design of chimeric (tag + viral) sequences
Specify analysis parameters
Analysis of target sequences with BLASTN to determine cross-priming

Figure 3

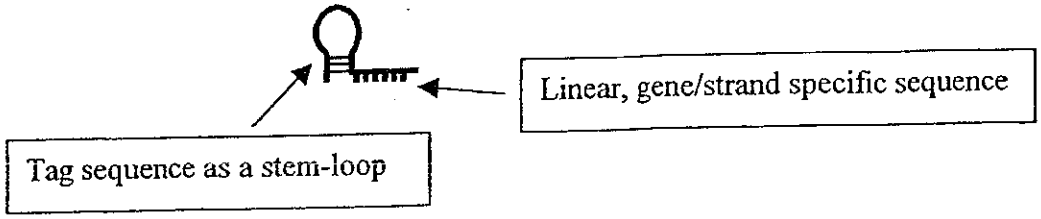


Figure 4

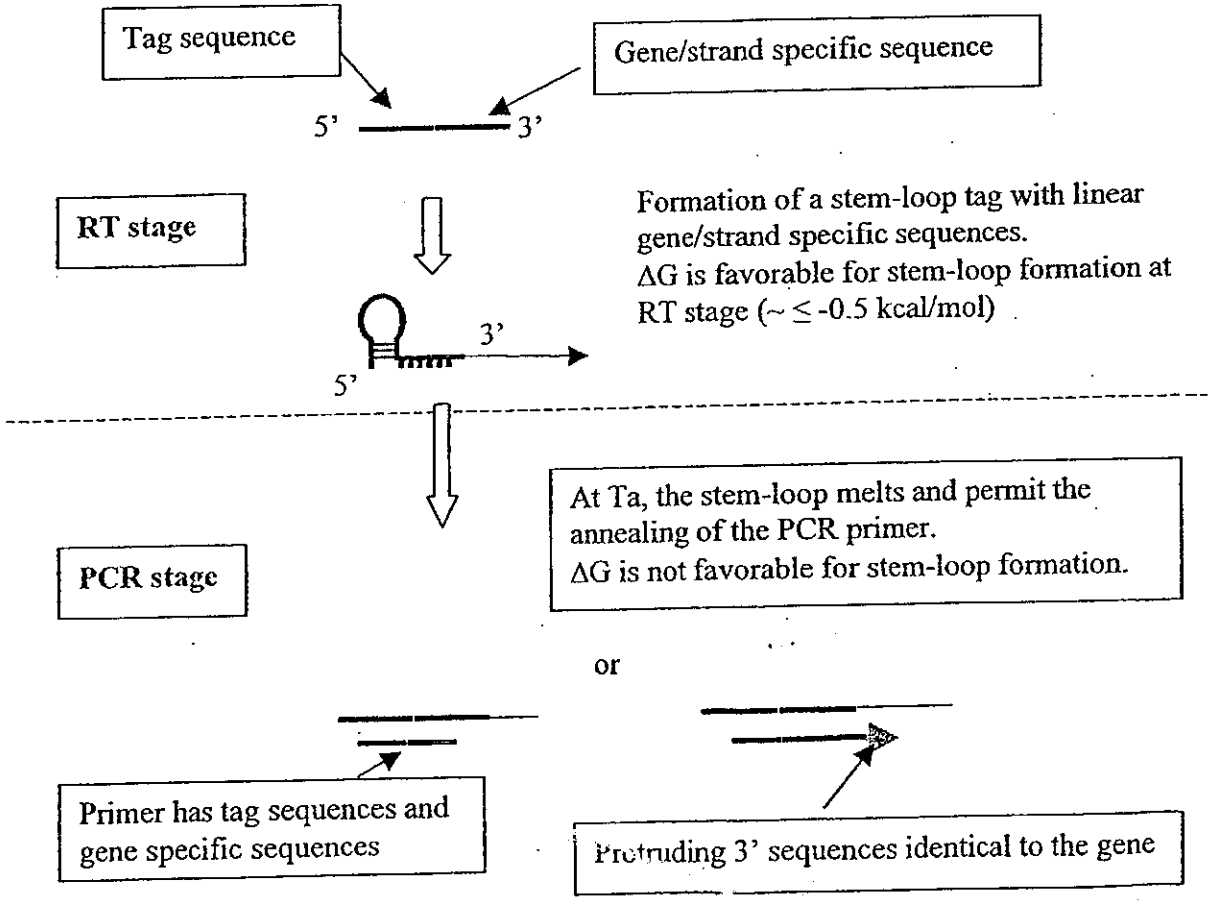


Figure 5

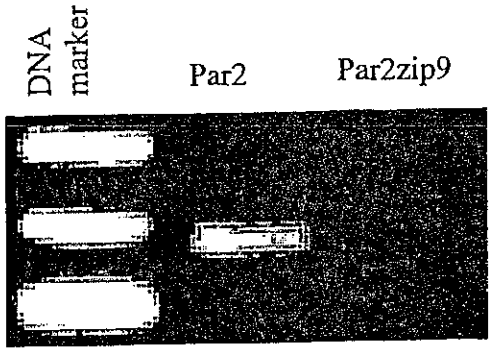


Figure 6

```
Max complementarity: 9 bp, dG=-4.20 Kcal/mol

5'-TCTACAAAGACAGCACACTTTGTAGAGACCTGGG-3'
   *********          *********

      C:::C
      A   A
       G*C          Par2zip9
       A*T
       A*T
       A*T
       C*G
       A*T
       T*A
       C*G
       T*A
    5'-   GACCTGGG -3'
```

Figure 7

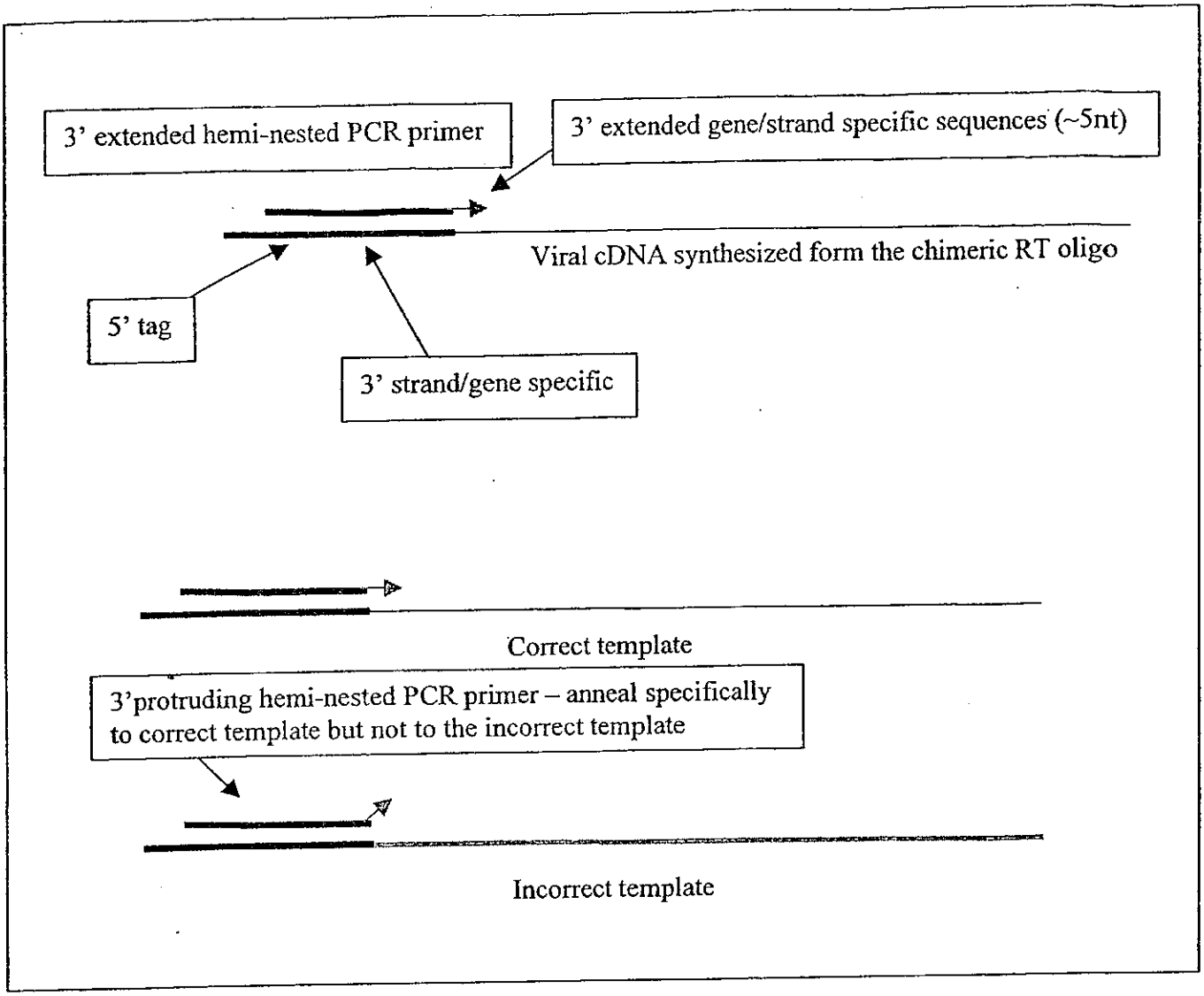


Figure 8

BOX 1

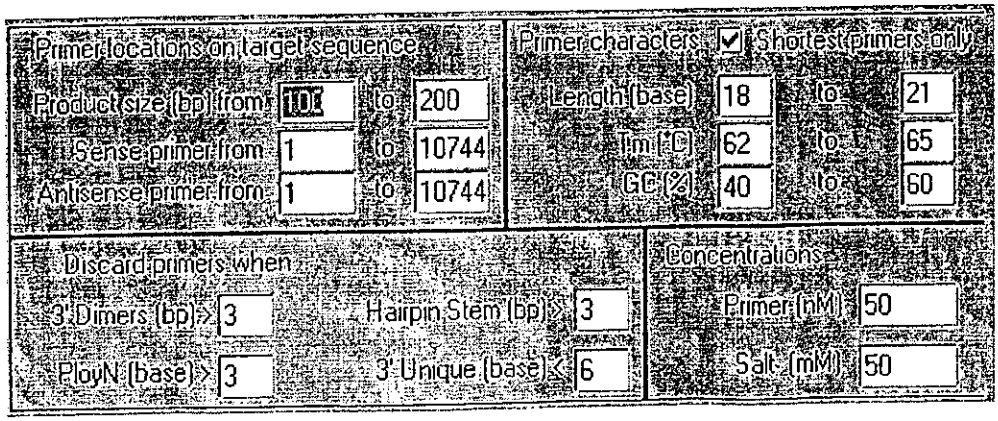


Figure 9

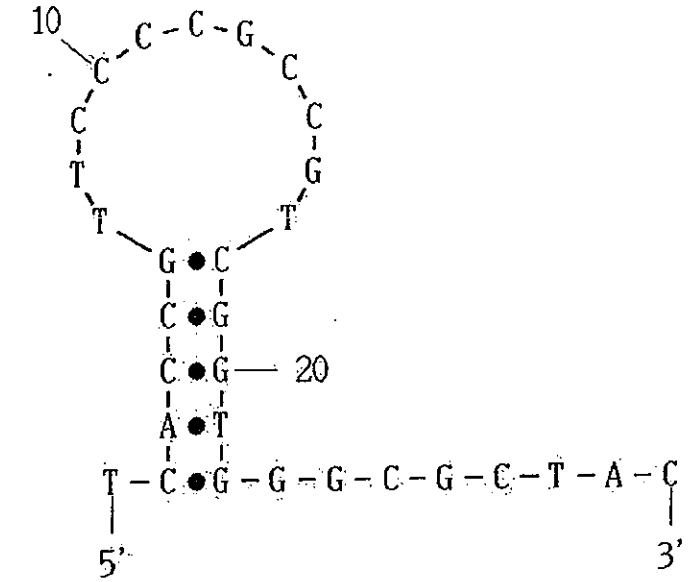


Figure 10

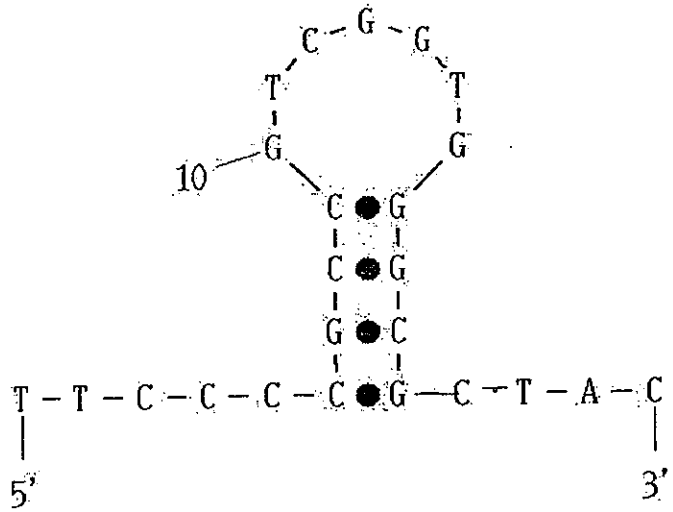


Figure 11

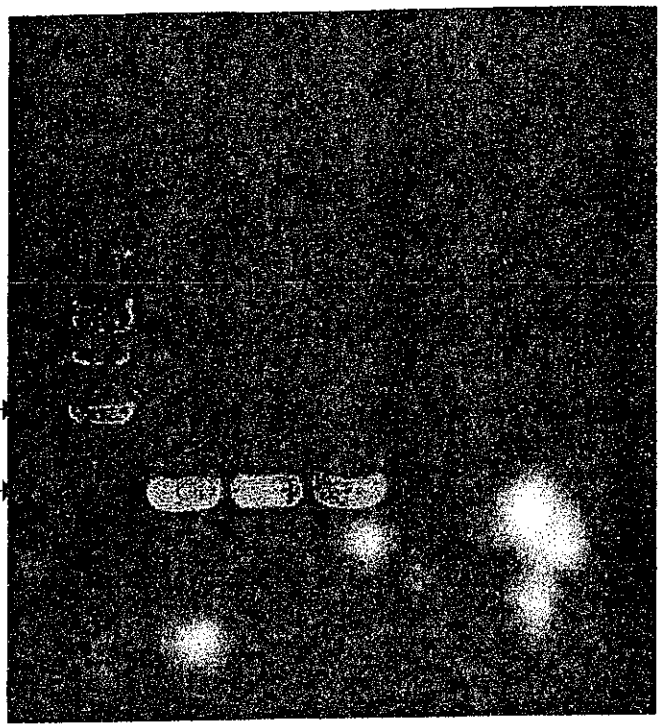


Figure 12

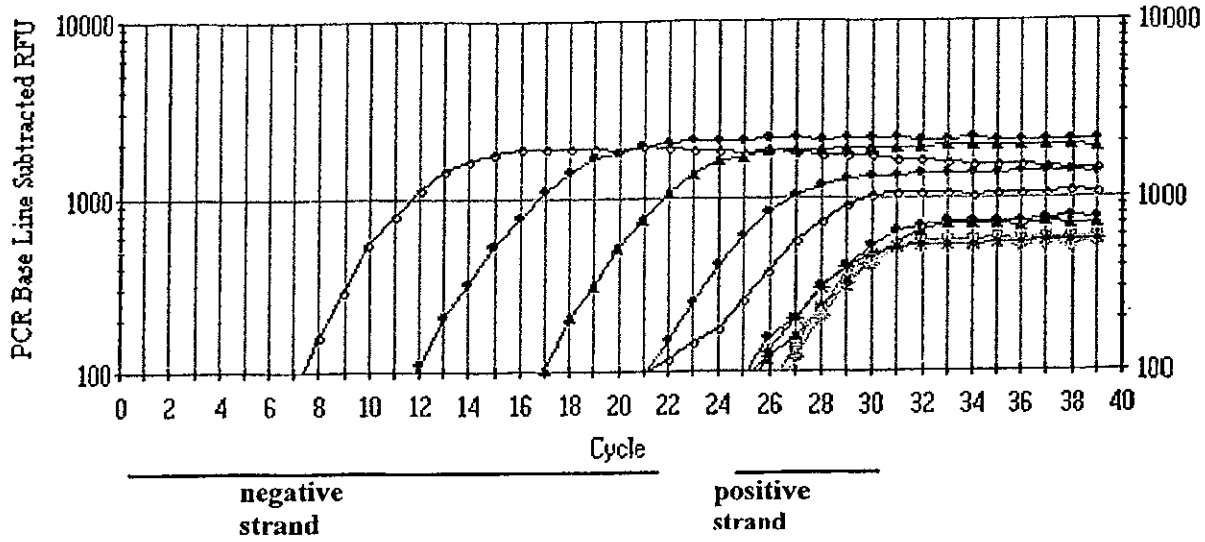


Figure 13A

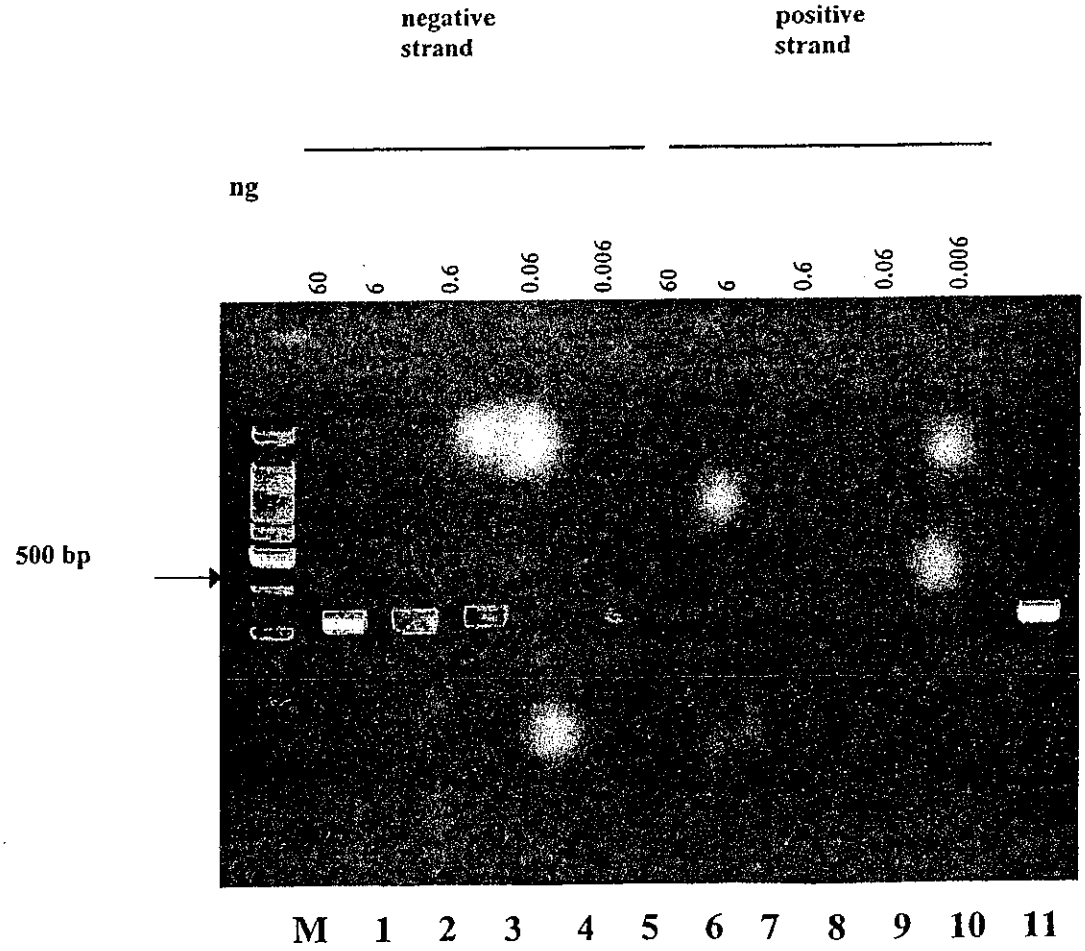


Figure 13B

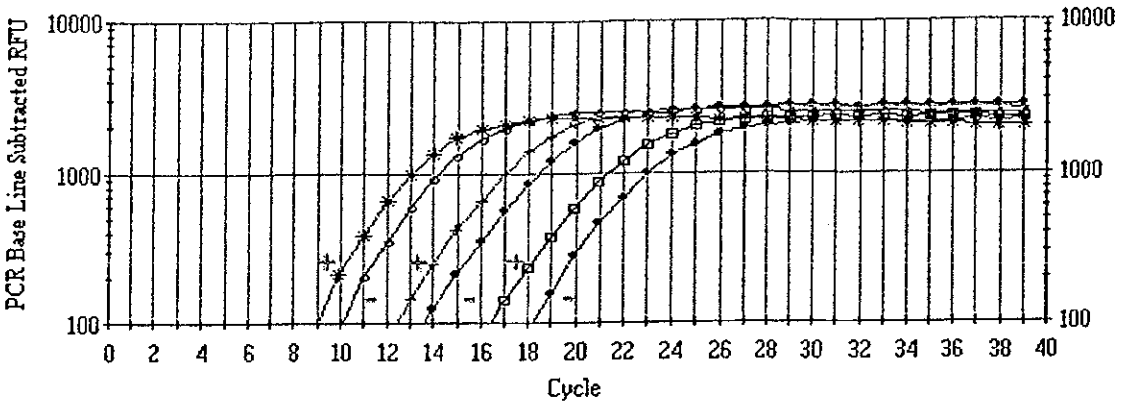


Figure 13C

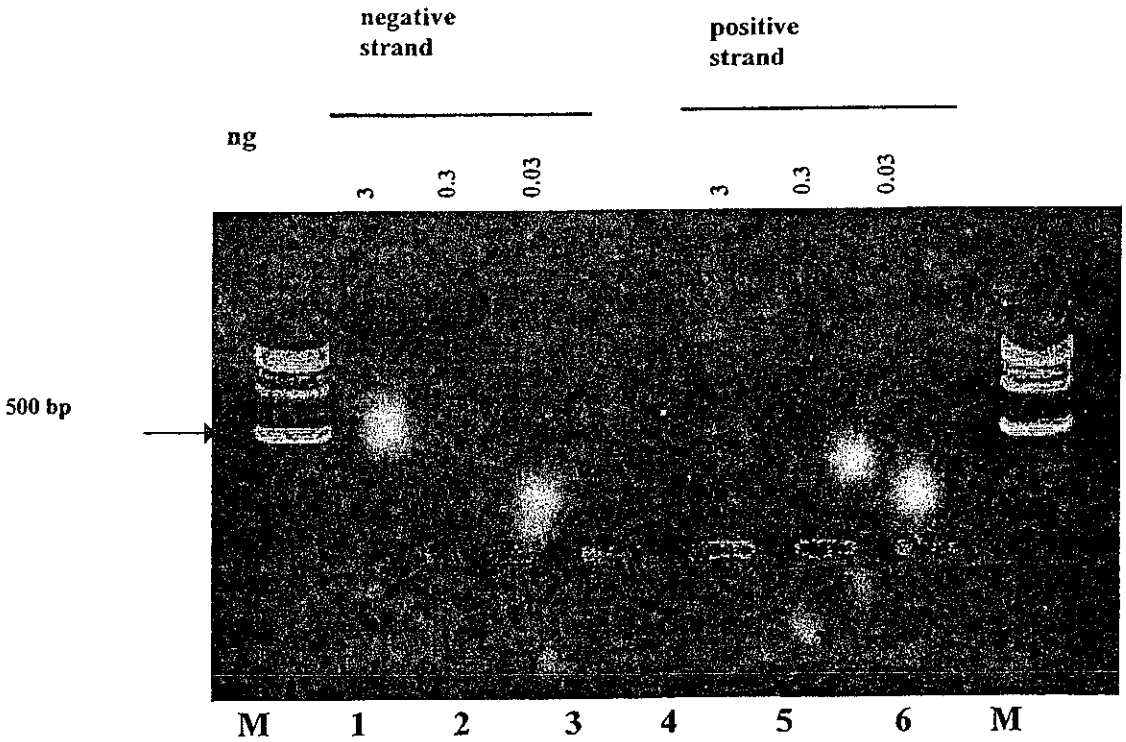


Figure 13D

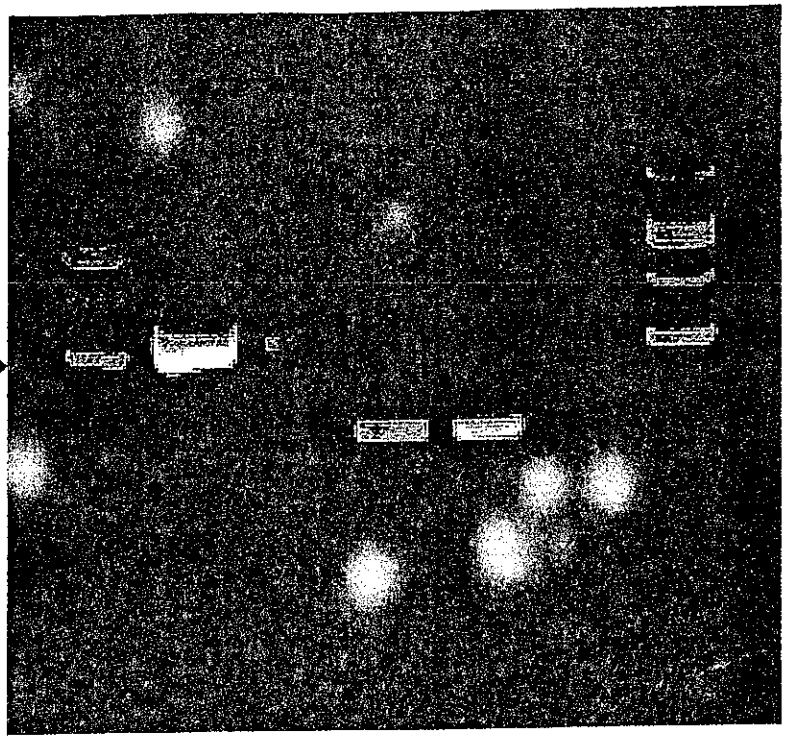


Figure 14

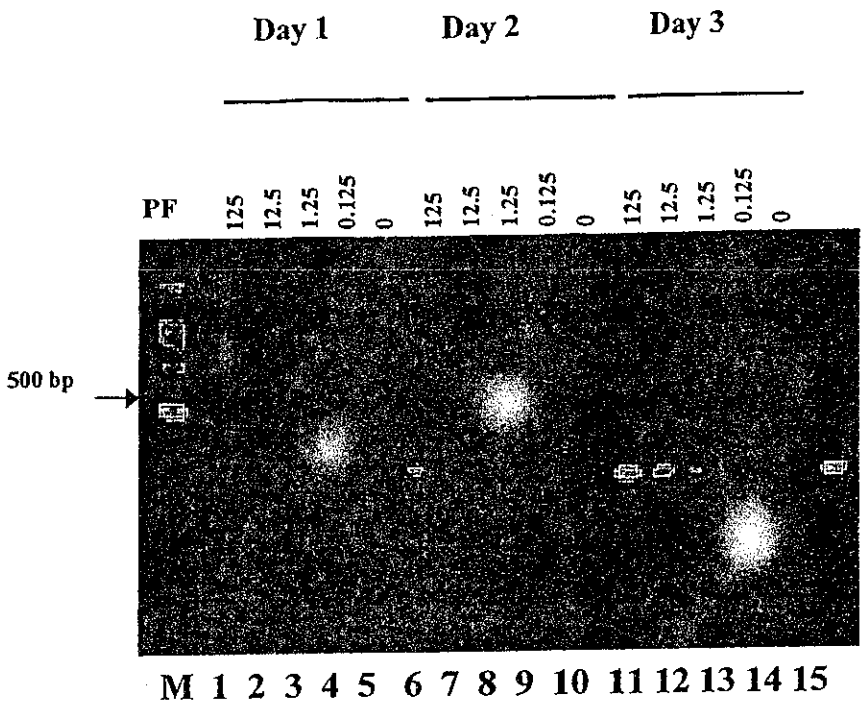


Figure 15A

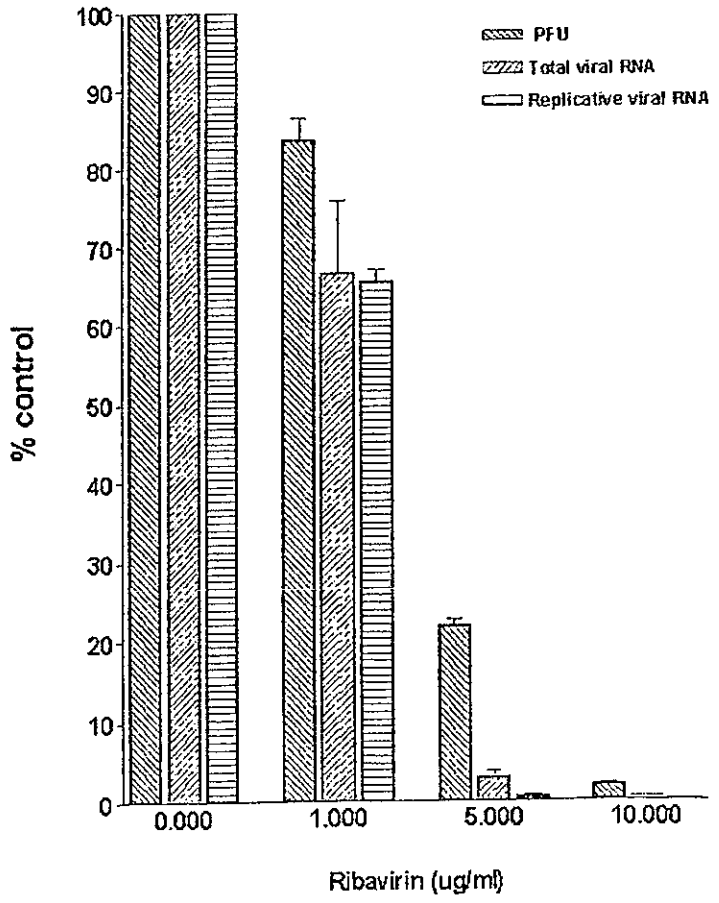


Figure 15B

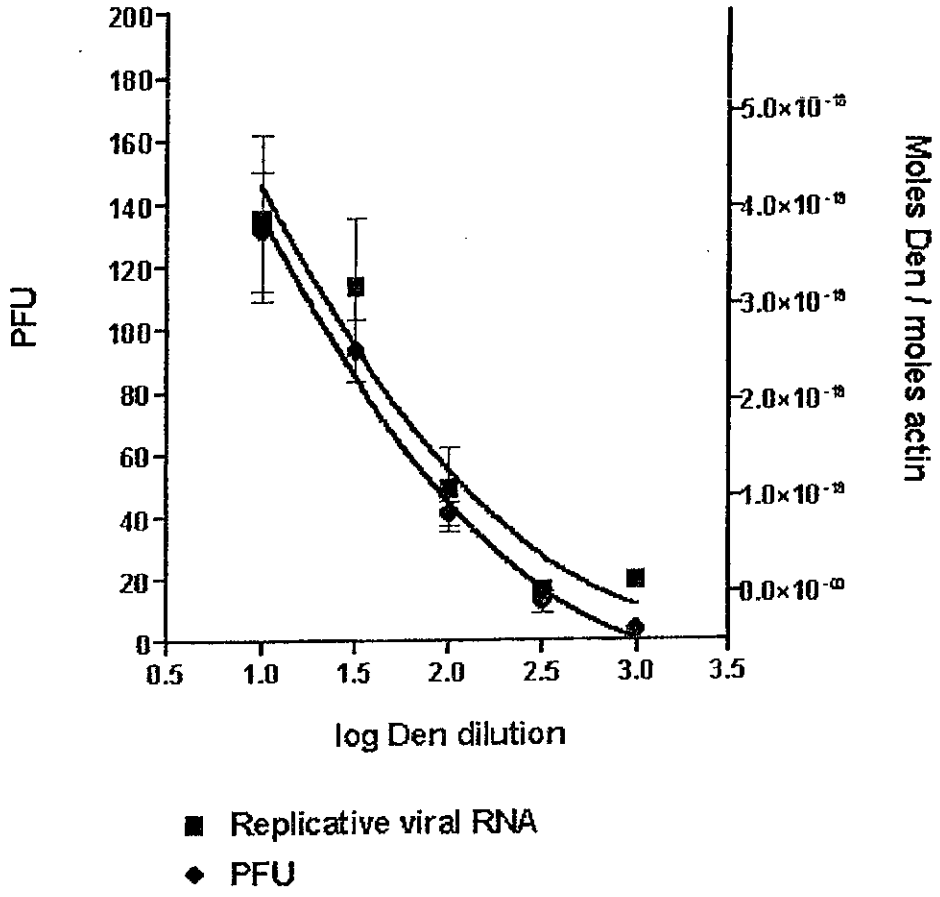

PFU
200
180
160
140
120
100
80
60
40
20
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
log Den dilution
5.0×10⁻¹⁸
4.0×10⁻¹⁸
3.0×10⁻¹⁸
2.0×10⁻¹⁸
1.0×10⁻¹⁸
0.0×10⁻⁰⁰
Moles Den / moles actin
Replicative viral RNA
PFU

Figure 16

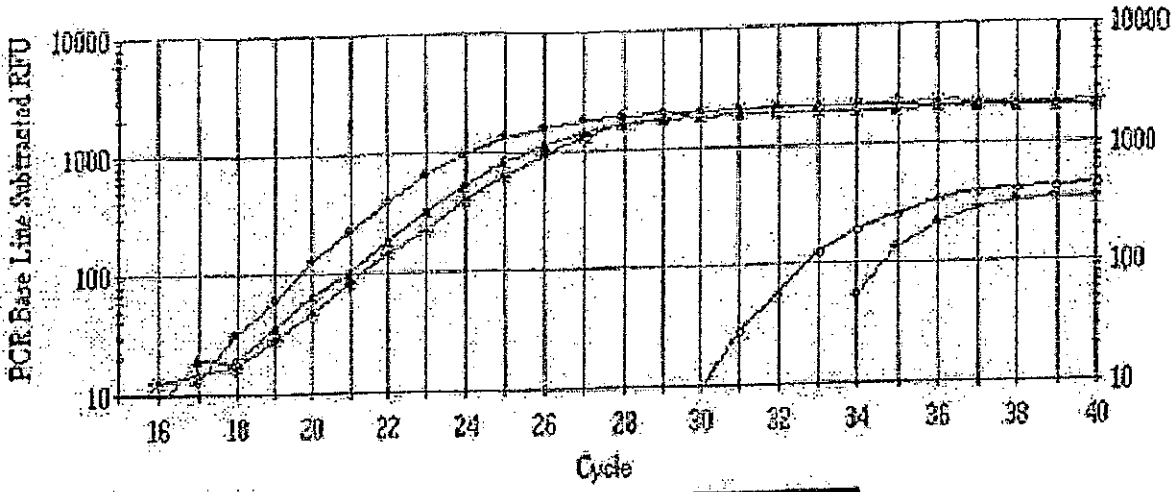


Figure 17A

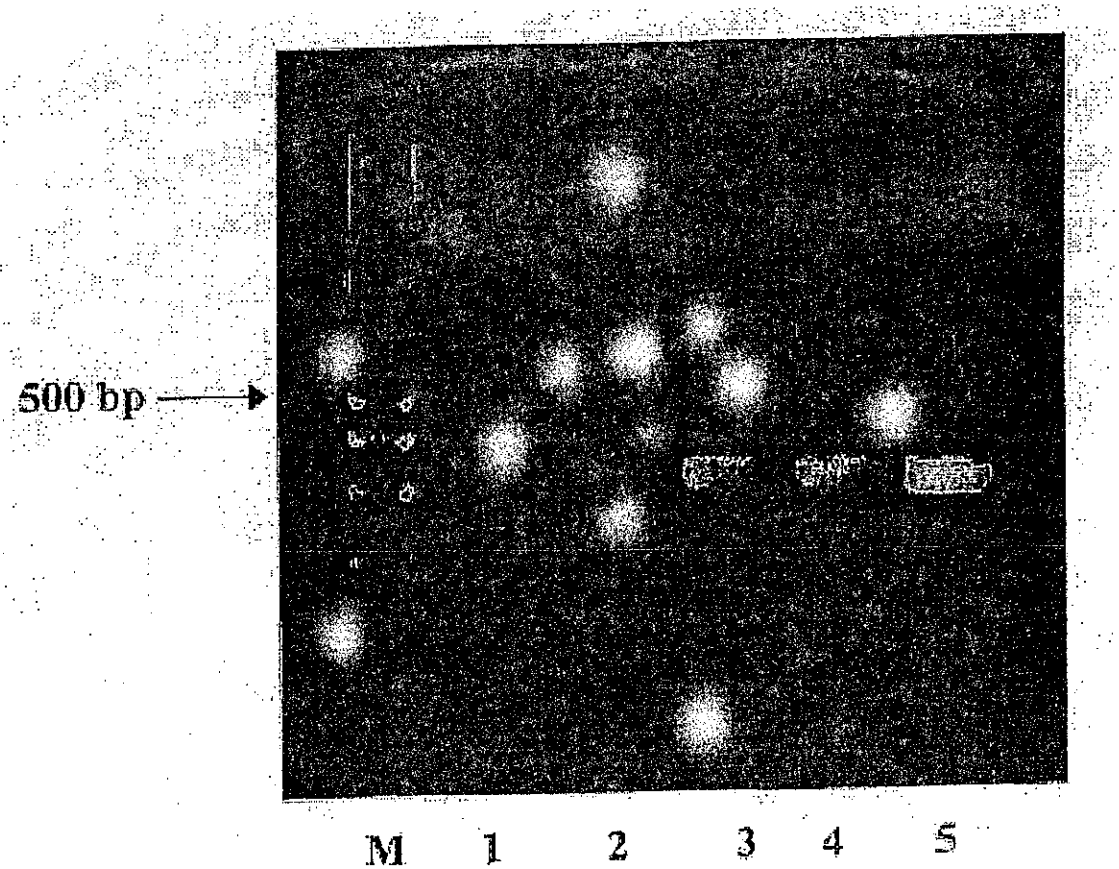


Figure 17B

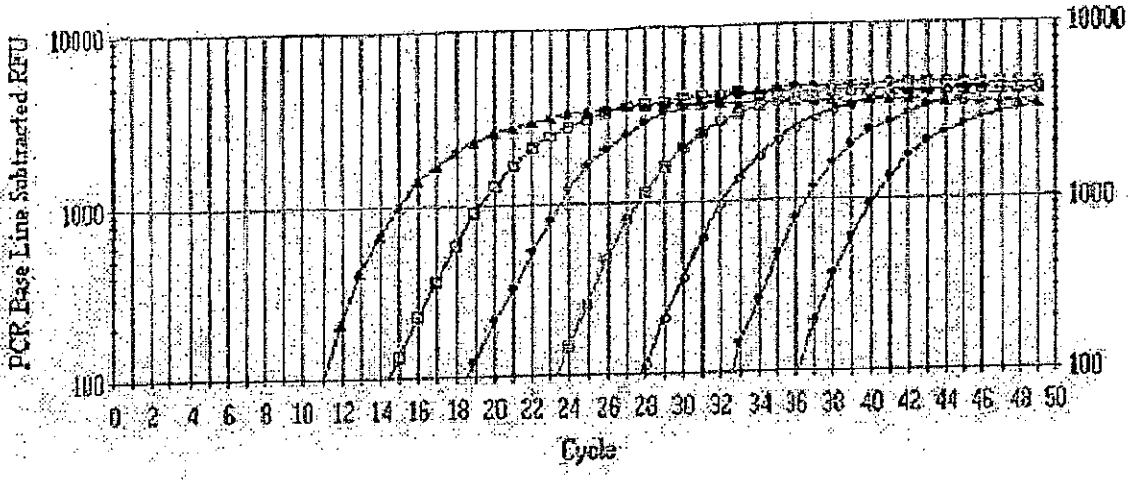


Figure 18A

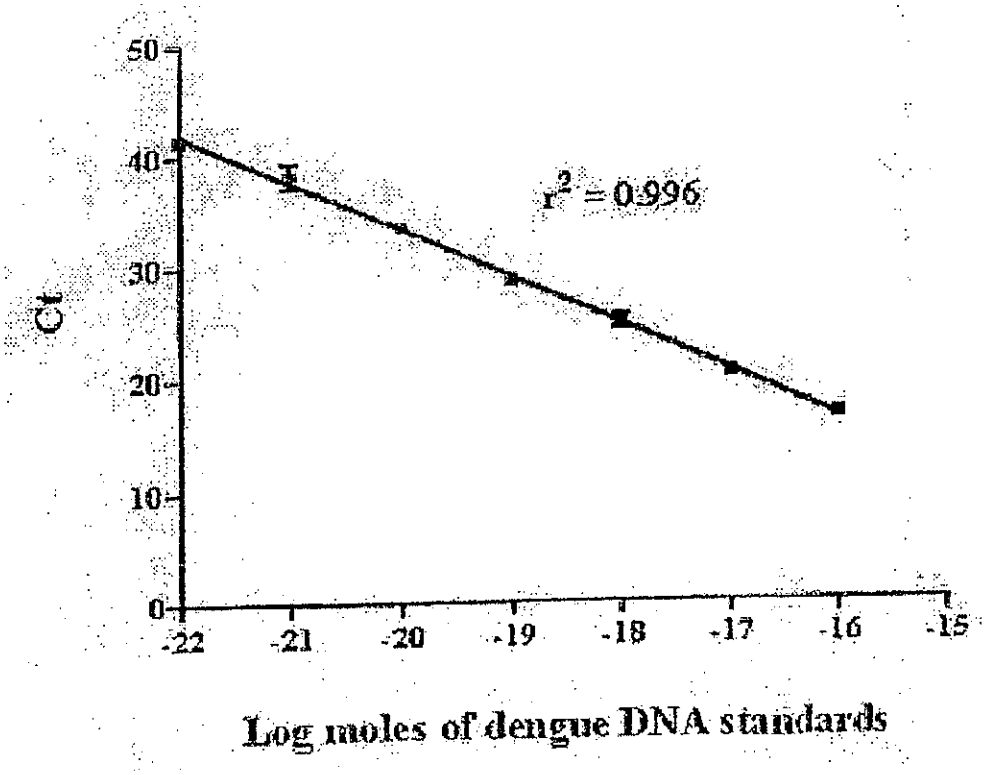


Figure 18B

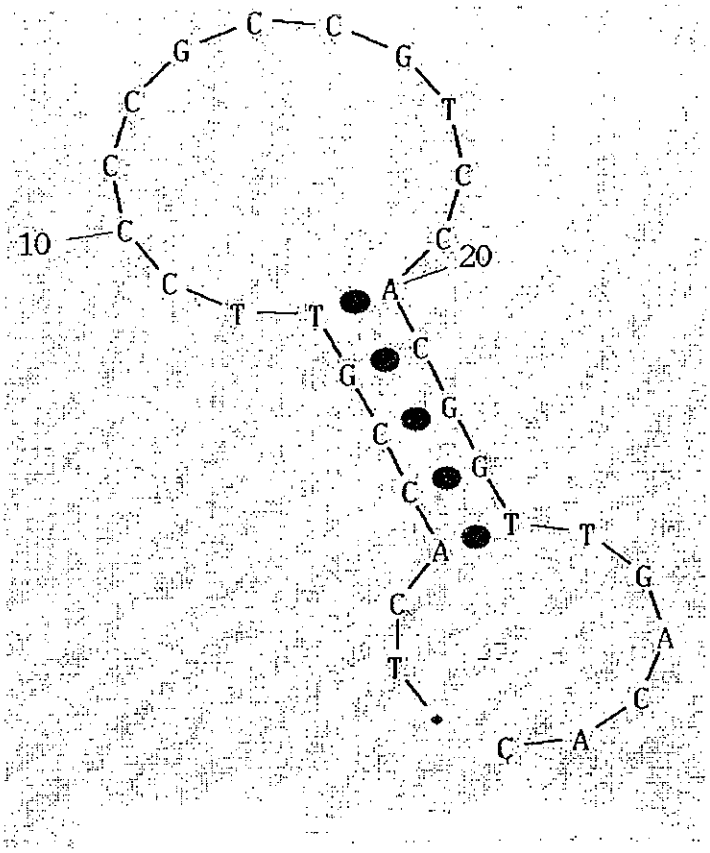


Figure 19

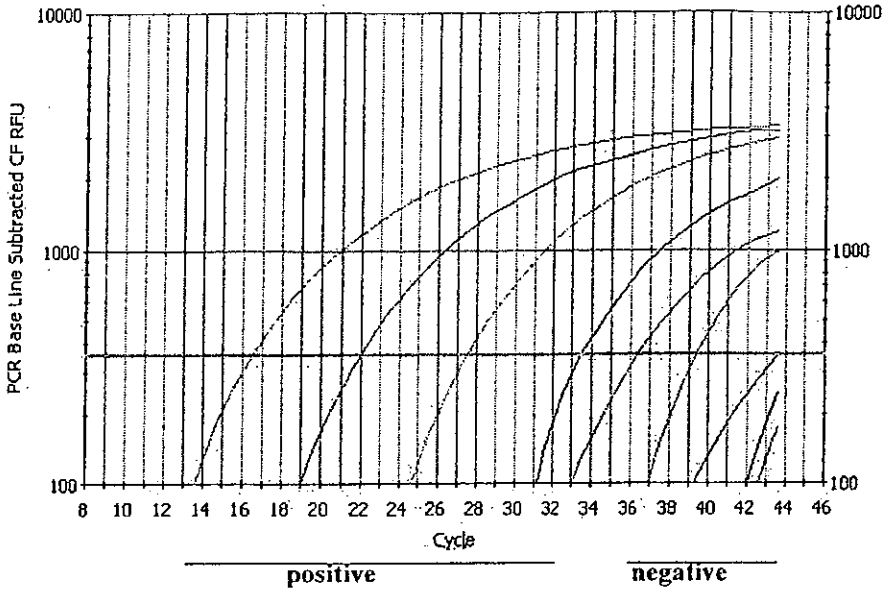


Figure 20A

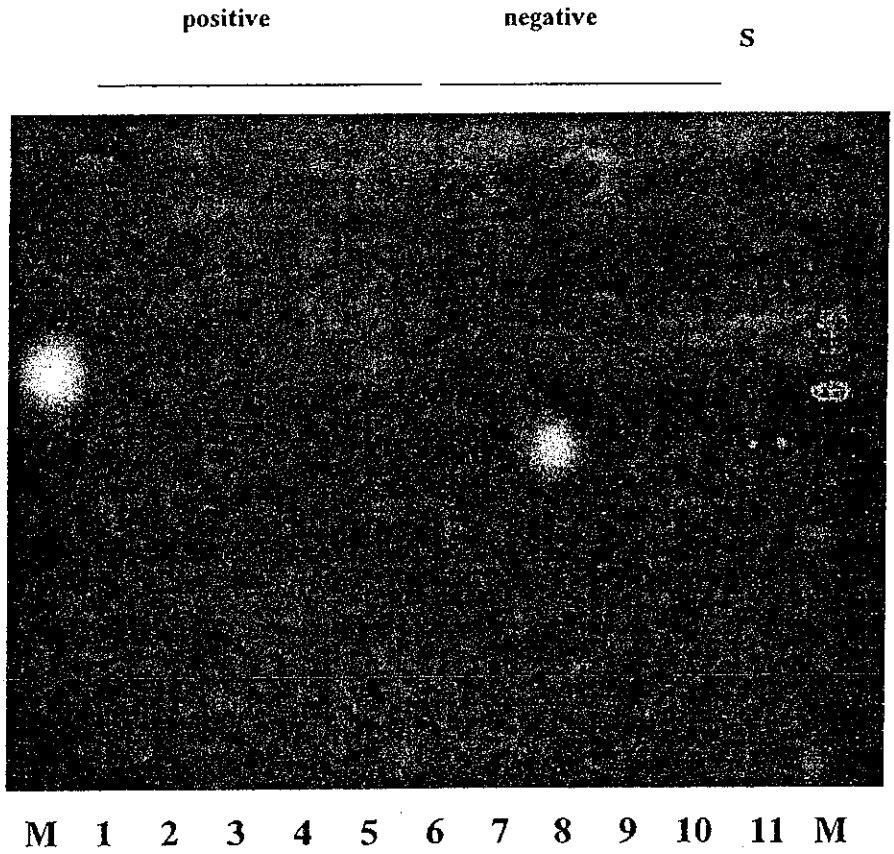


Figure 20B

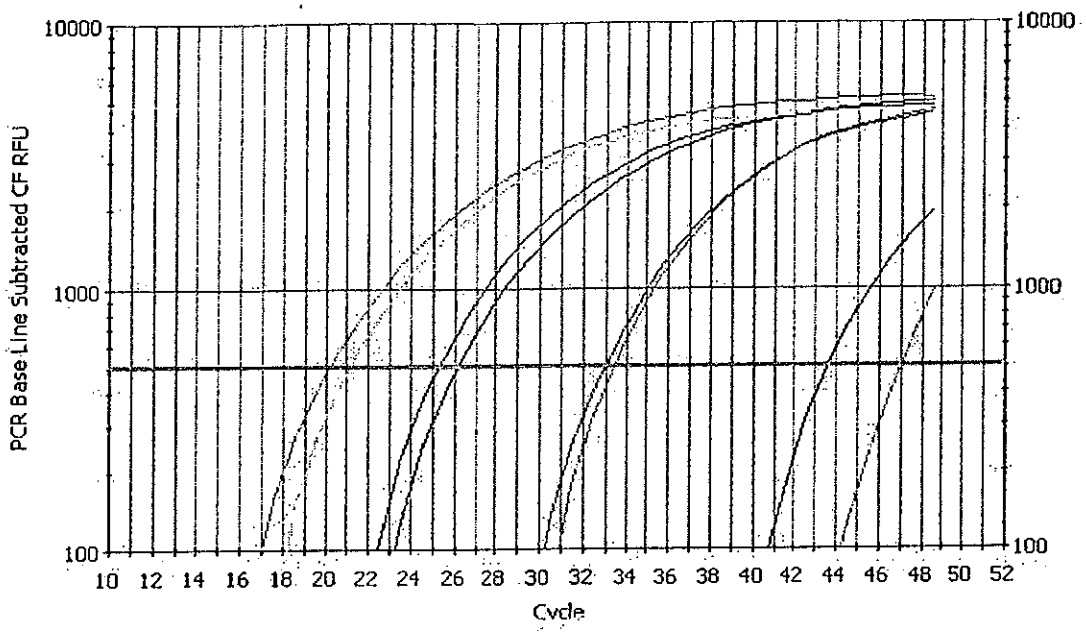


Figure 20C

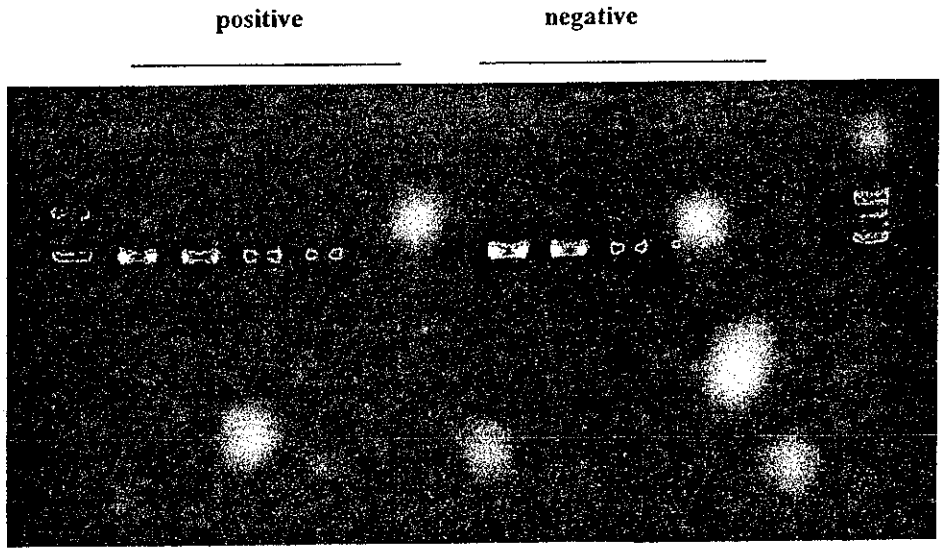


Figure 20D

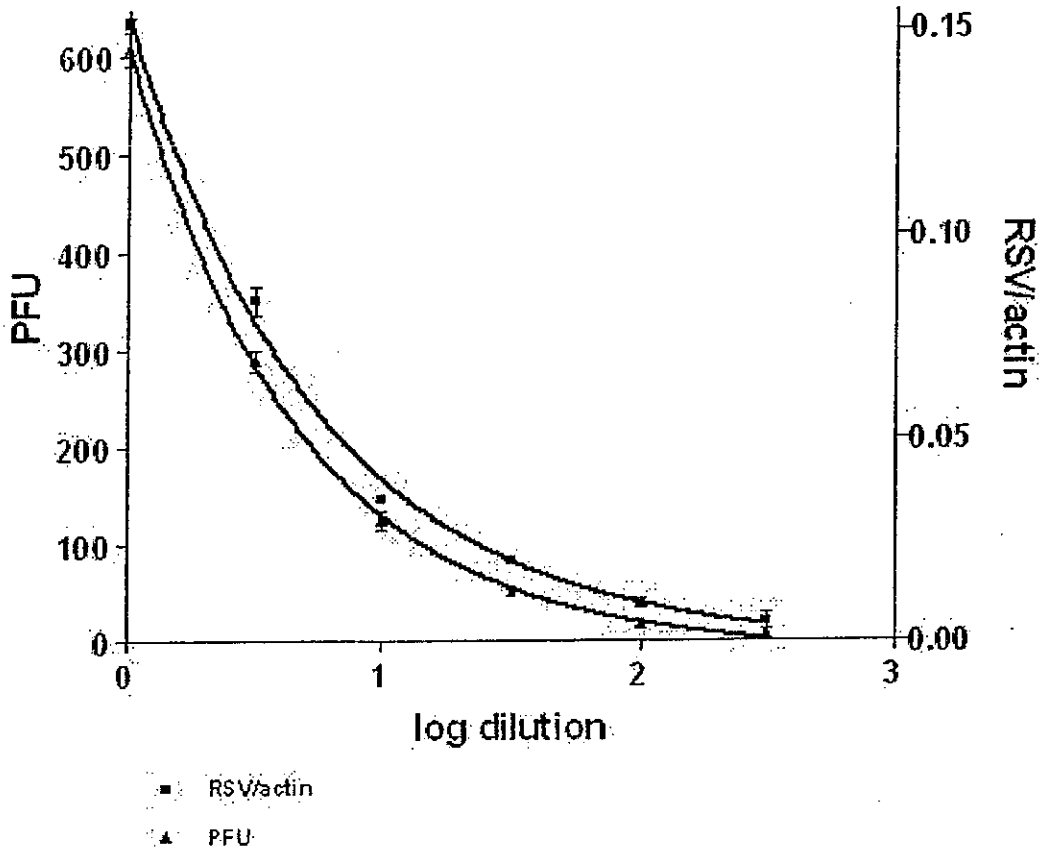

PFU
600
500
400
300
200
100
0
0.15
0.10
0.05
0.00
RSV/actin
0
1
2
3
log dilution
RSV/actin
PFU

Figure 21

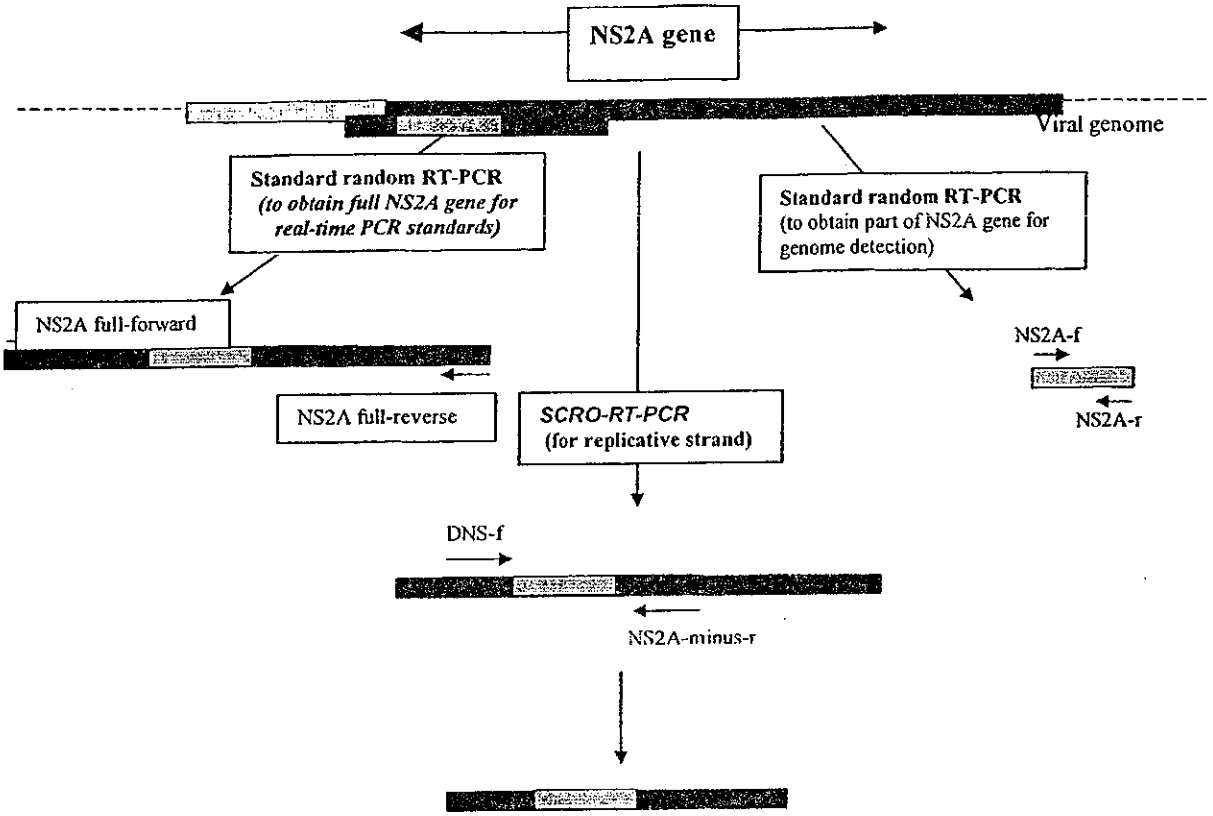

NS2A gene
Viral genome
Standard random RT-PCR
(to obtain full NS2A gene for real-time PCR standards)
Standard random RT-PCR
(to obtain part of NS2A gene for genome detection)
NS2A full-forward
NS2A full-reverse
SCRO-RT-PCR
(for replicative strand)
NS2A-f
NS2A-r
DNS-f
NS2A-minus-r

Figure 22