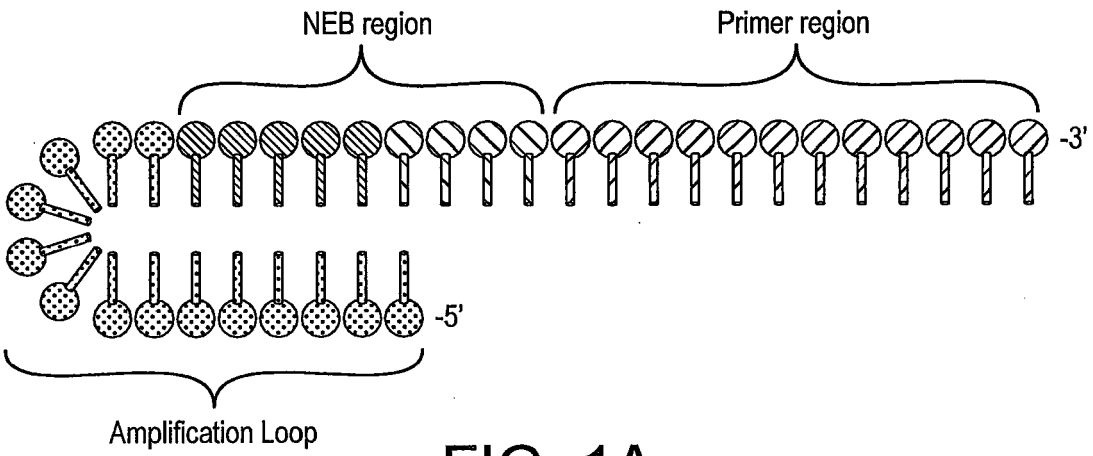

FIG. 1A

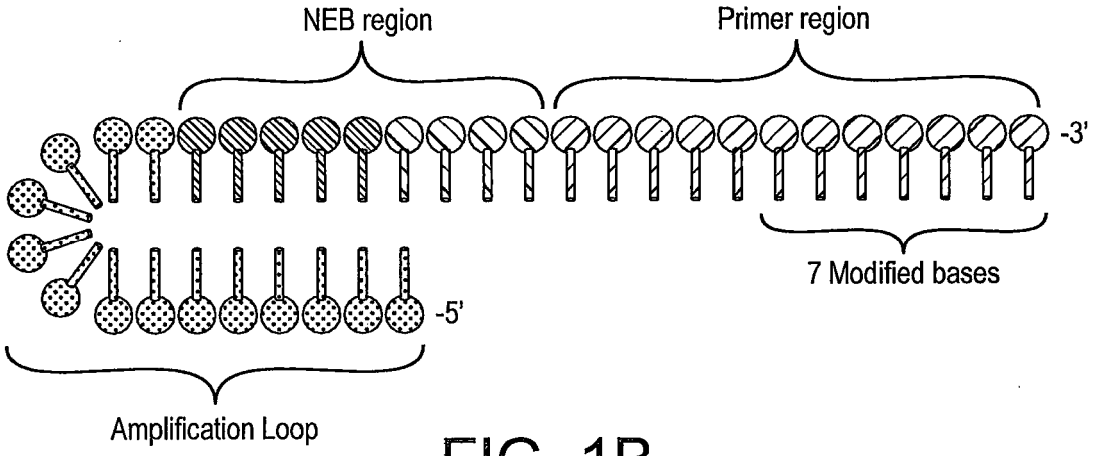

FIG. 1B

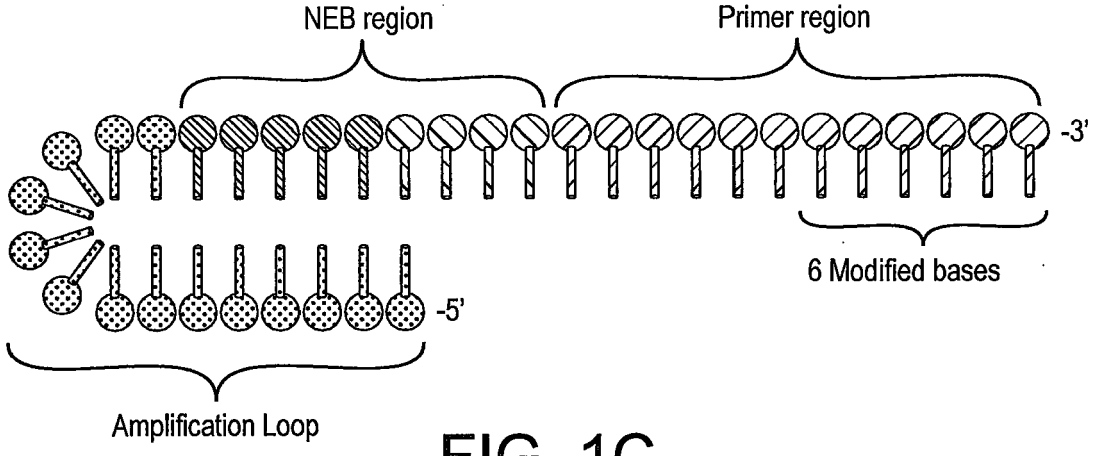

FIG. 1C

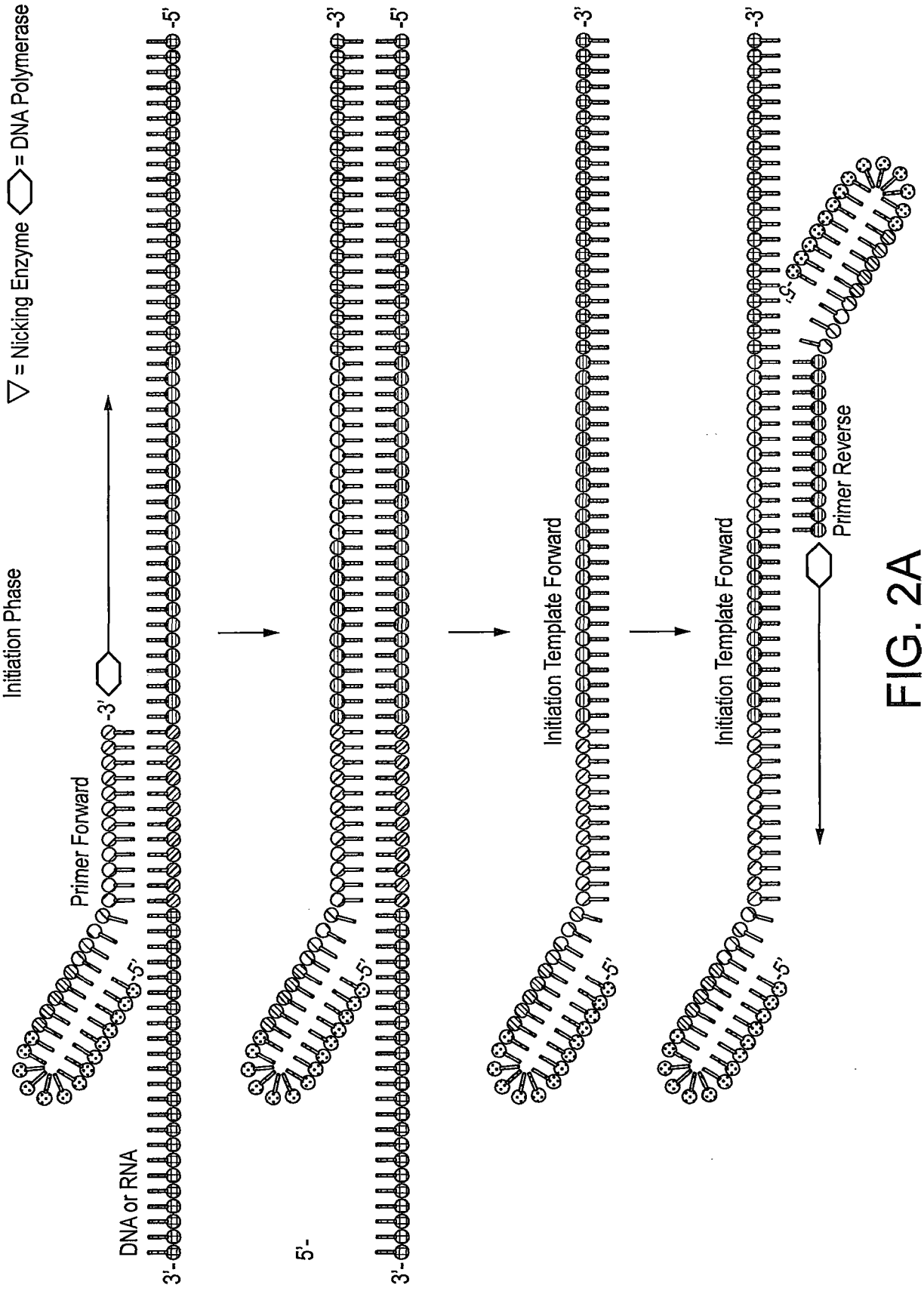
= Nicking Enzyme
= DNA Polymerase
Initiation Phase
Primer Forward
DNA or RNA
3'-
-5'
-3'
5'-
Initiation Template Forward
Primer Reverse

FIG. 2A

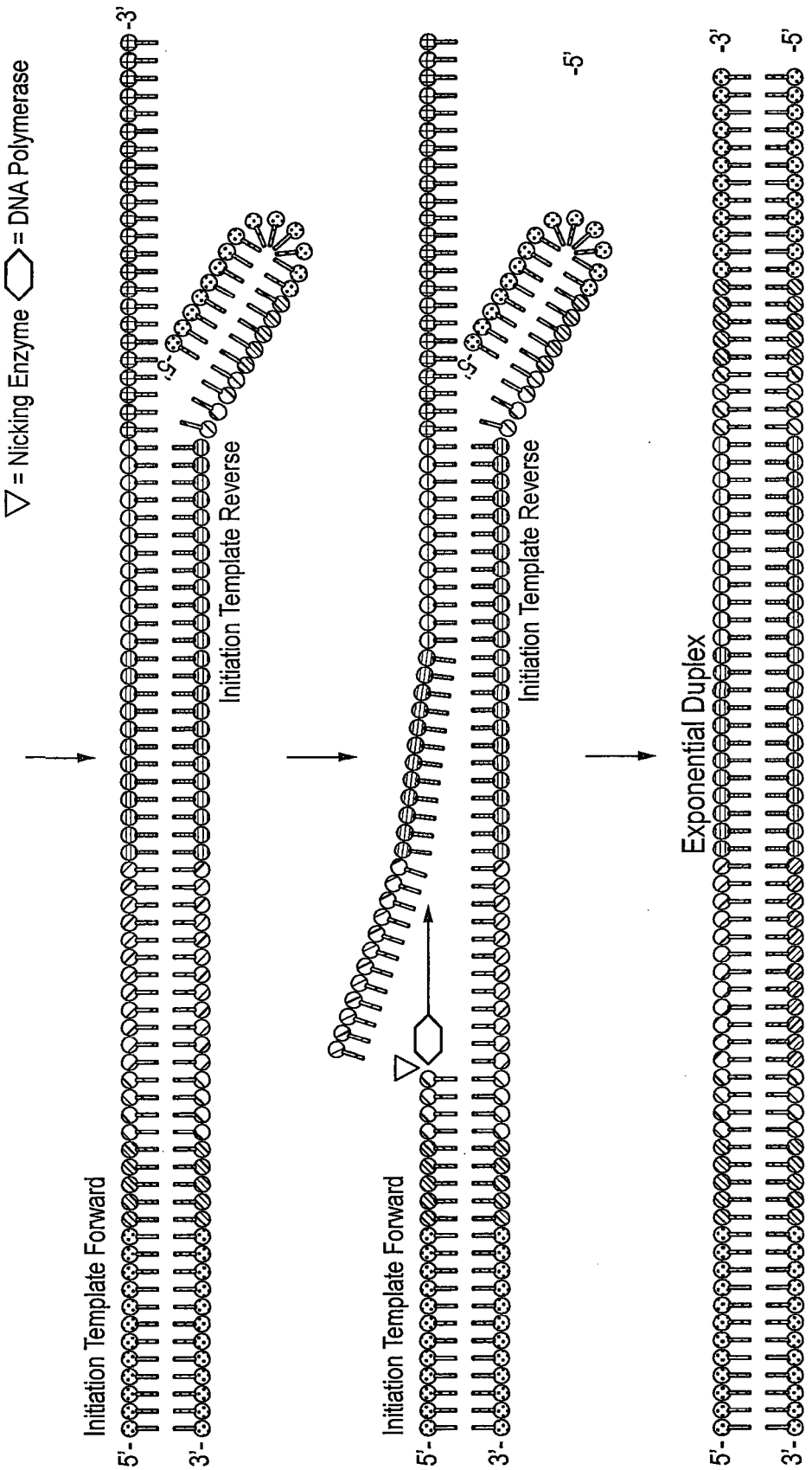
▽ = Nicking Enzyme ⬡ = DNA Polymerase
Initiation Template Forward
Initiation Template Reverse
Exponential Duplex
5'
3'
-3'
-5'

FIG. 2A
(CONT)

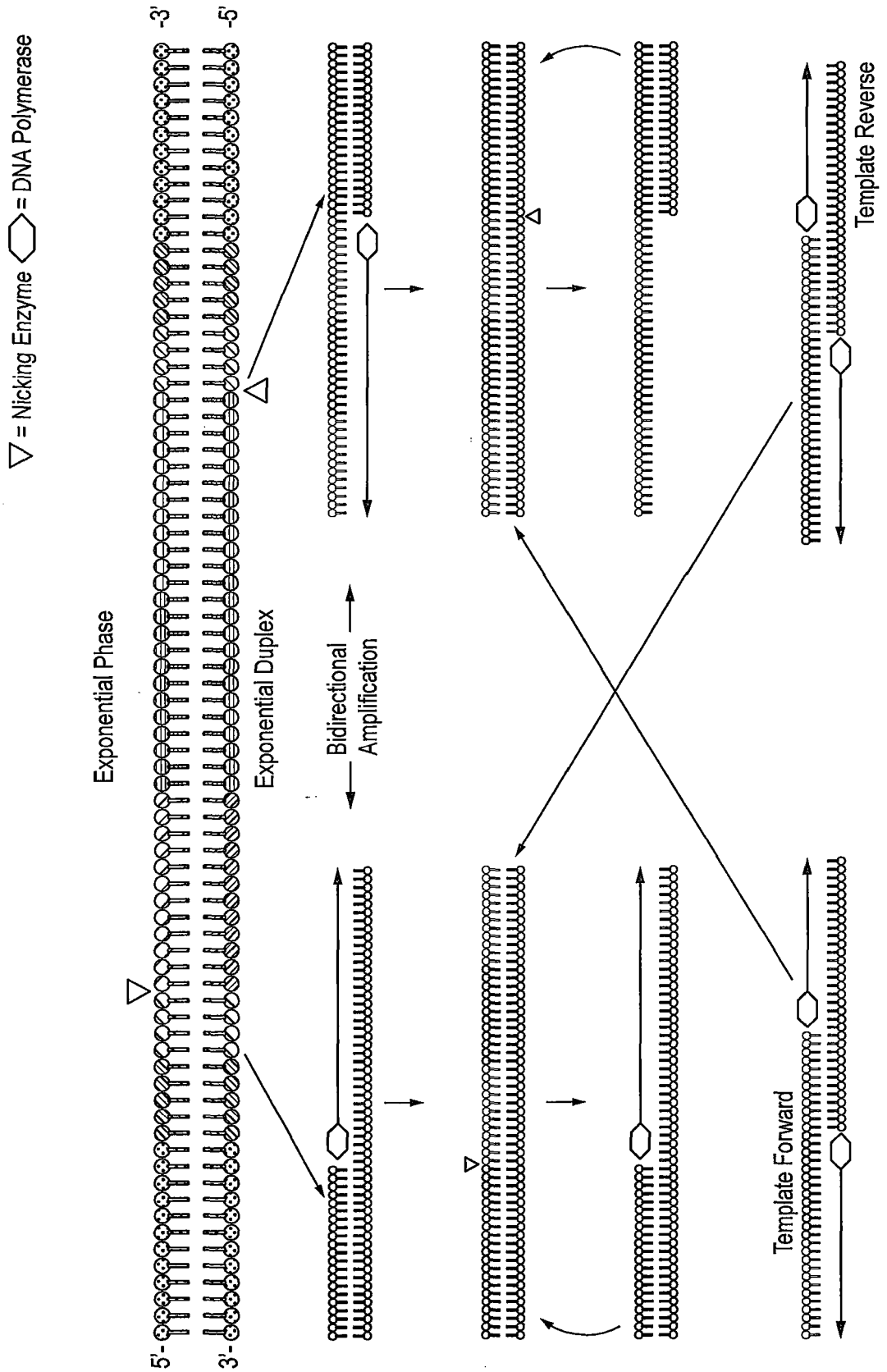
= Nicking Enzyme
= DNA Polymerase
Exponential Phase
Exponential Duplex
Bidirectional Amplification
Template Forward
Template Reverse
5'-
3'-
-3'
-5'

FIG. 2B

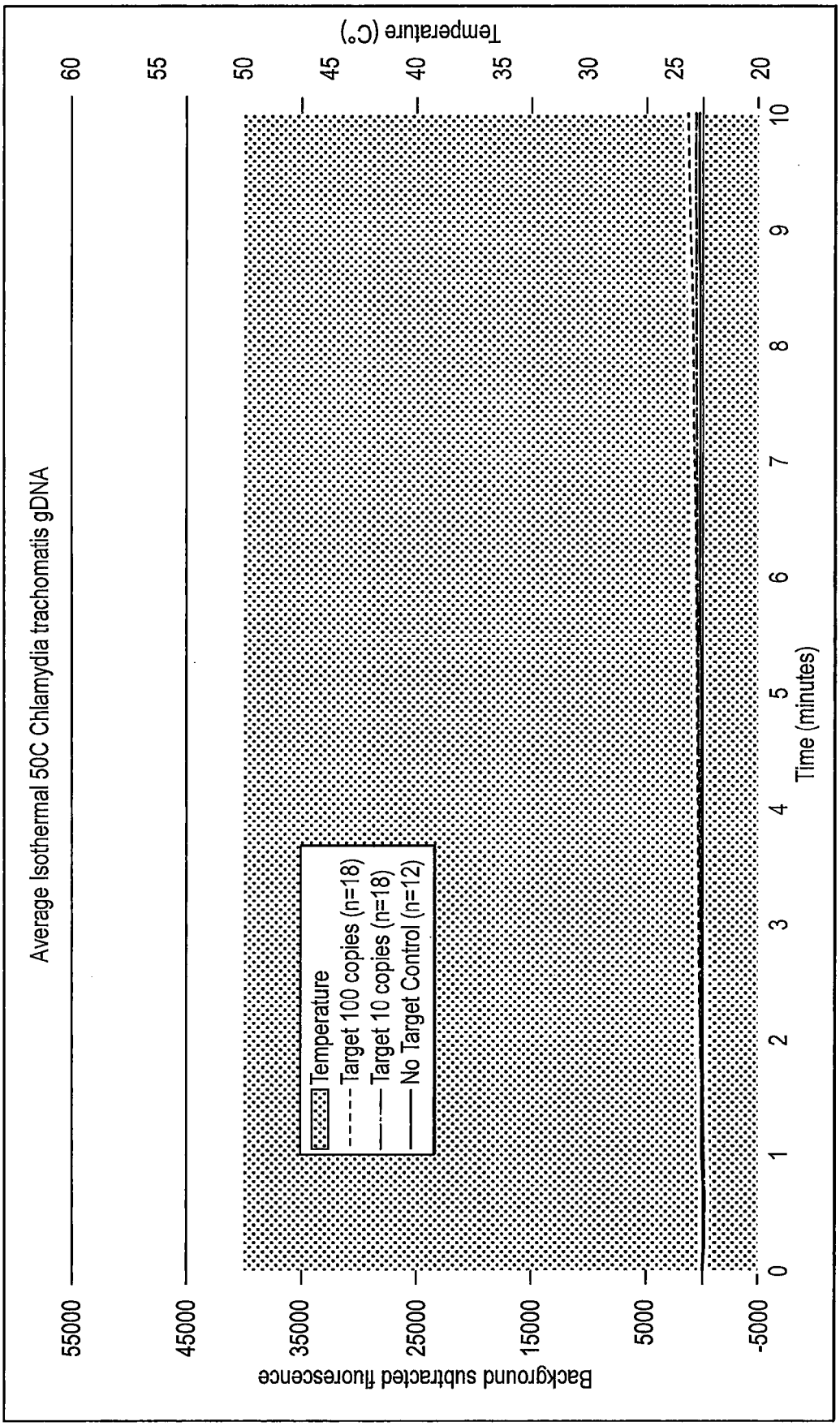
Average Isothermal 50C Chlamydia trachomatis gDNA
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)
Background subtracted fluorescence
Time (minutes)
Temperature (C°)
55000
45000
35000
25000
15000
5000
-5000
0
1
2
3
4
5
6
7
8
9
10
20
25
30
35
40
45
50
55
60

FIG. 3

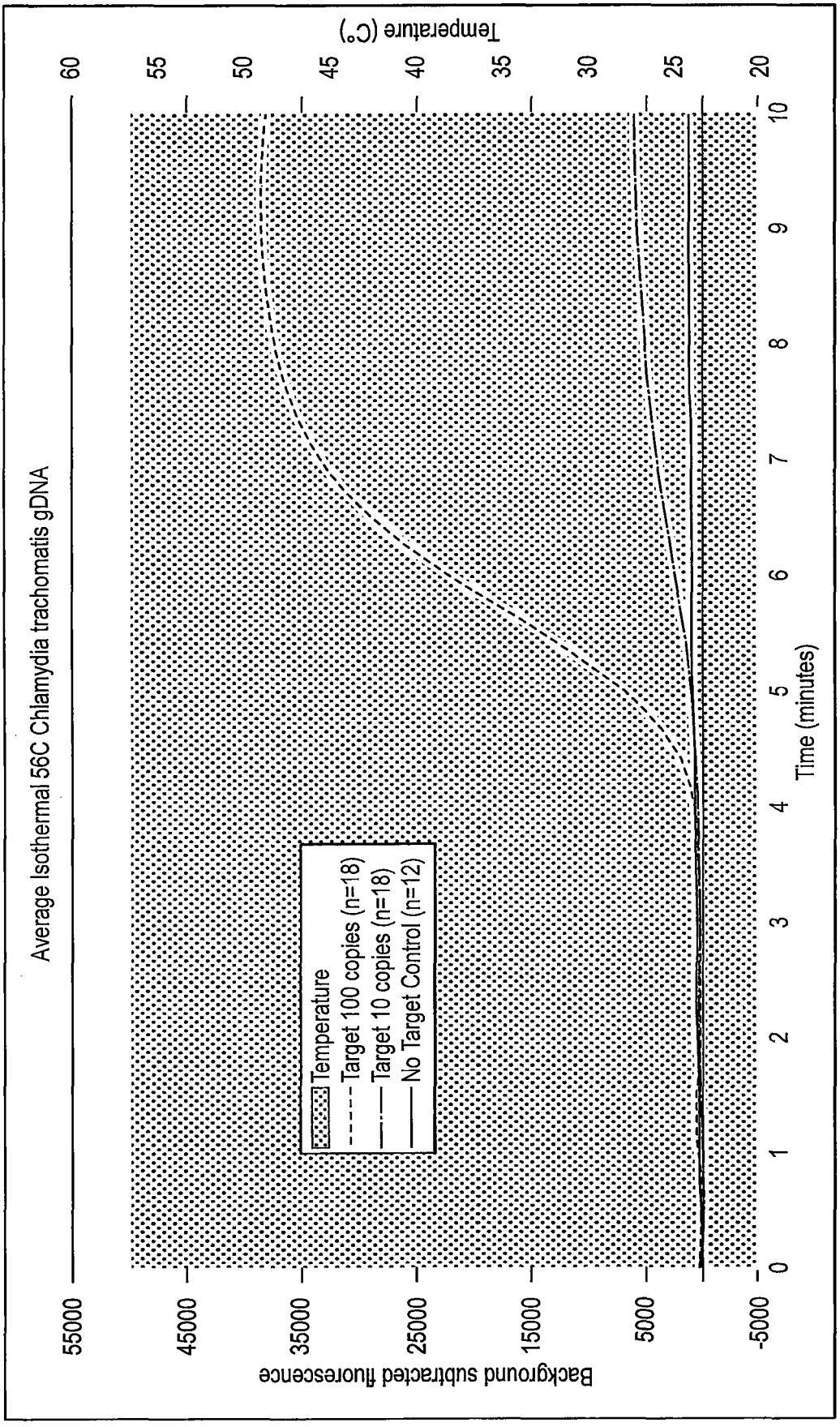
Average Isothermal 56C Chlamydia trachomatis gDNA
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
Temperature (C°)
60
55
50
45
40
35
30
25
20
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)

FIG. 4

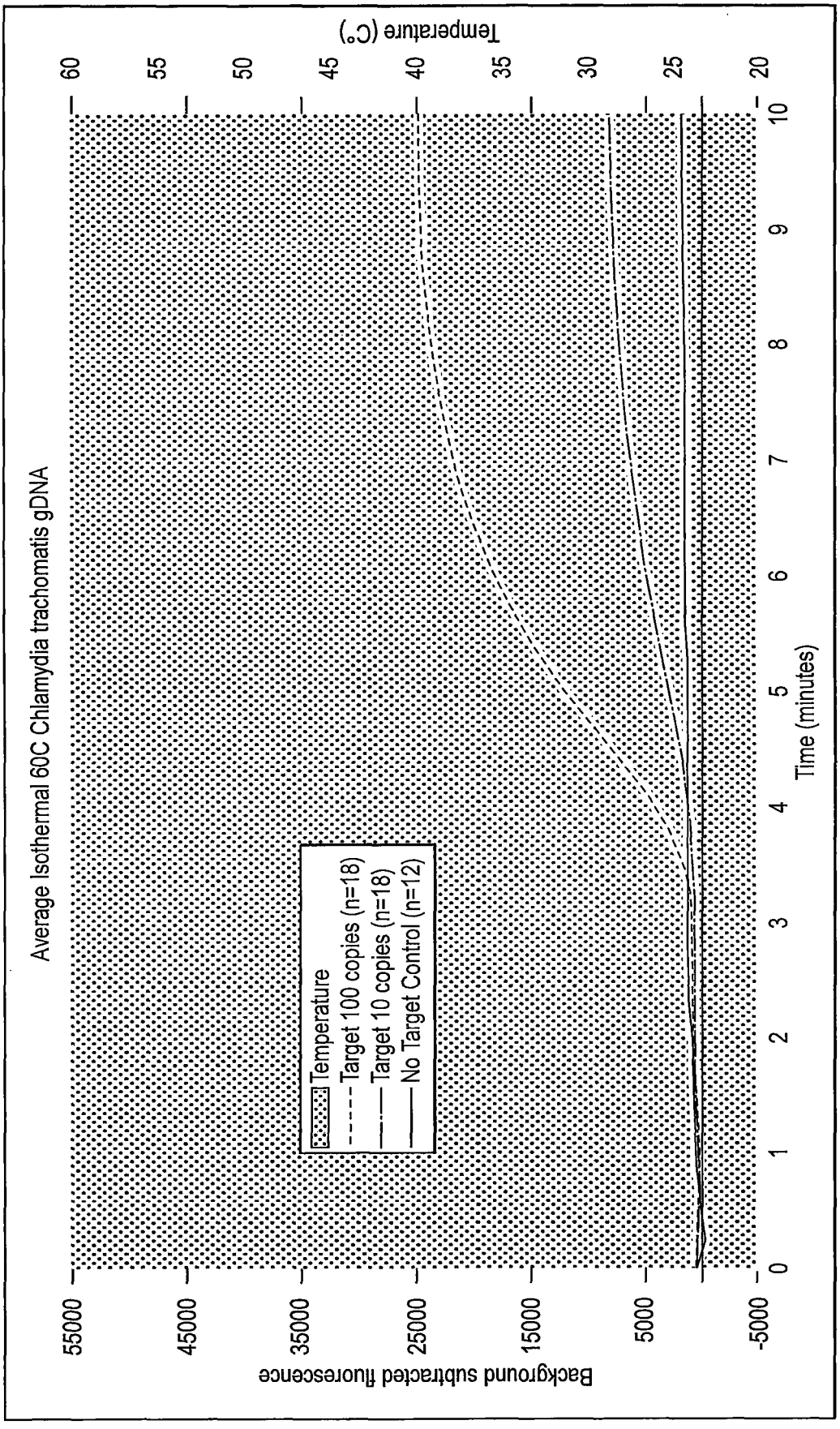
Average Isothermal 60C Chlamydia trachomatis gDNA
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
Temperature (C°)
60
55
50
45
40
35
30
25
20
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)

FIG. 5

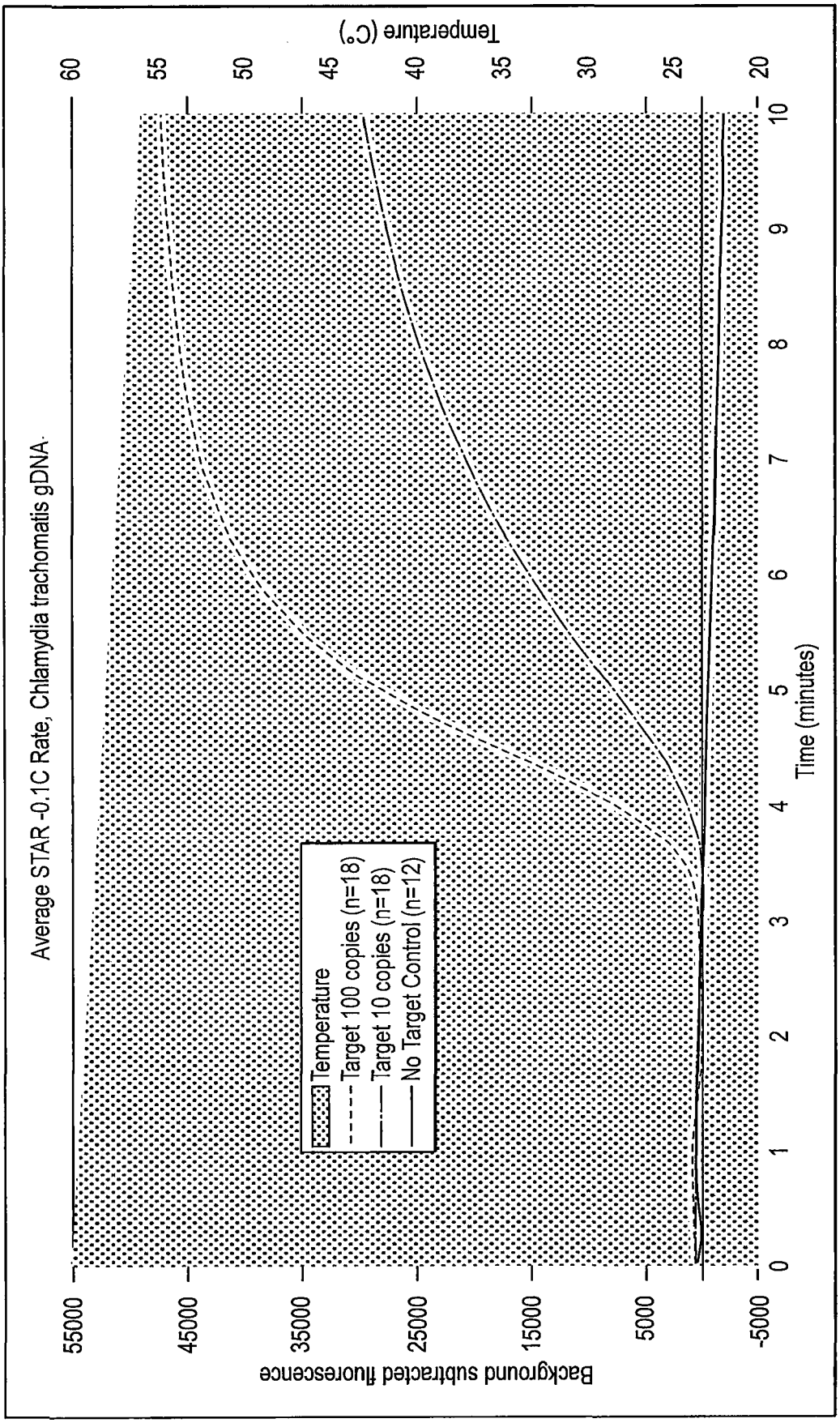
Average STAR -0.1C Rate, Chlamydia trachomatis gDNA
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
Temperature (C°)
60
55
50
45
40
35
30
25
20
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)

FIG. 6

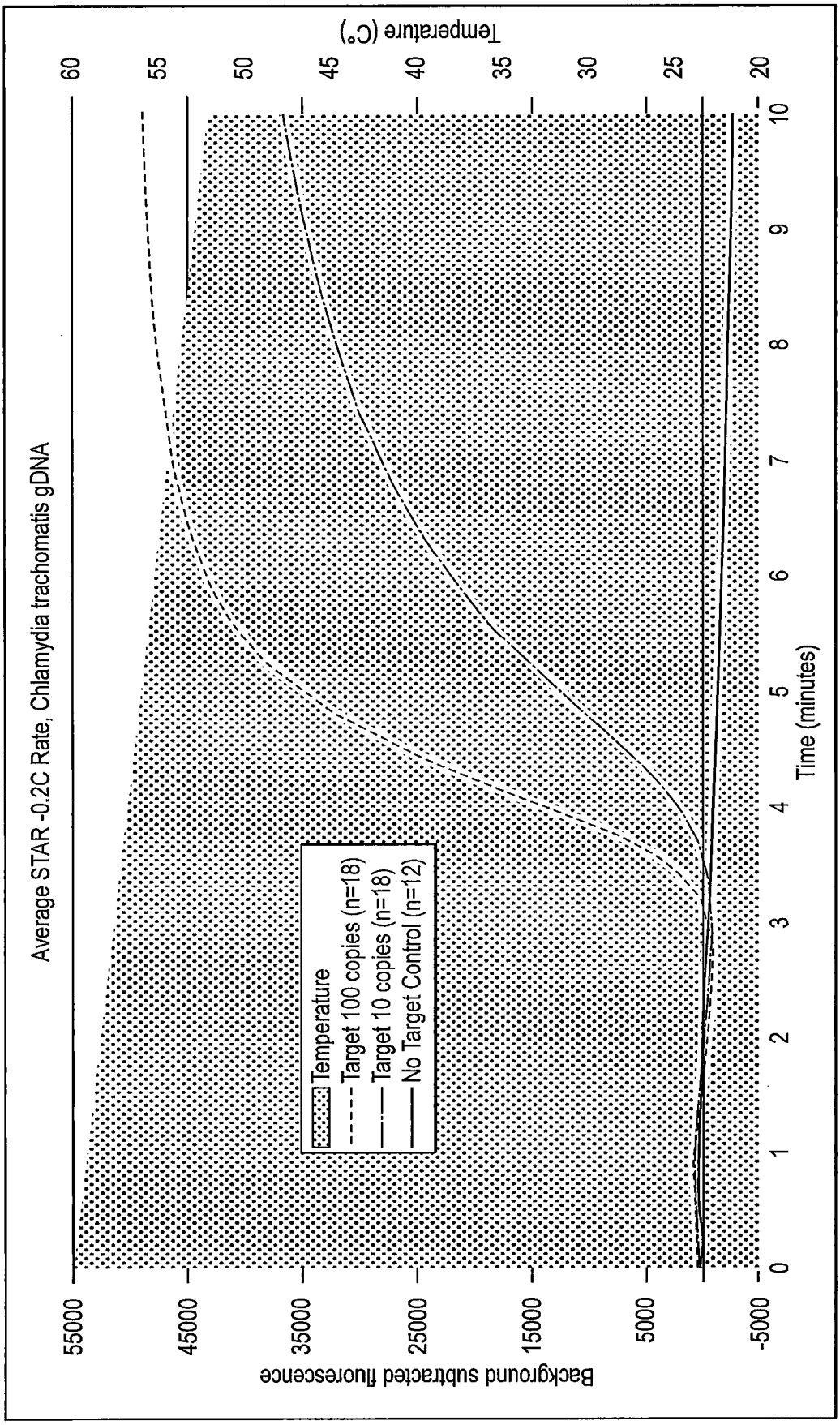
Average STAR -0.2C Rate, Chlamydia trachomatis gDNA
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
Temperature (C°)
60
55
50
45
40
35
30
25
20
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)

FIG. 7

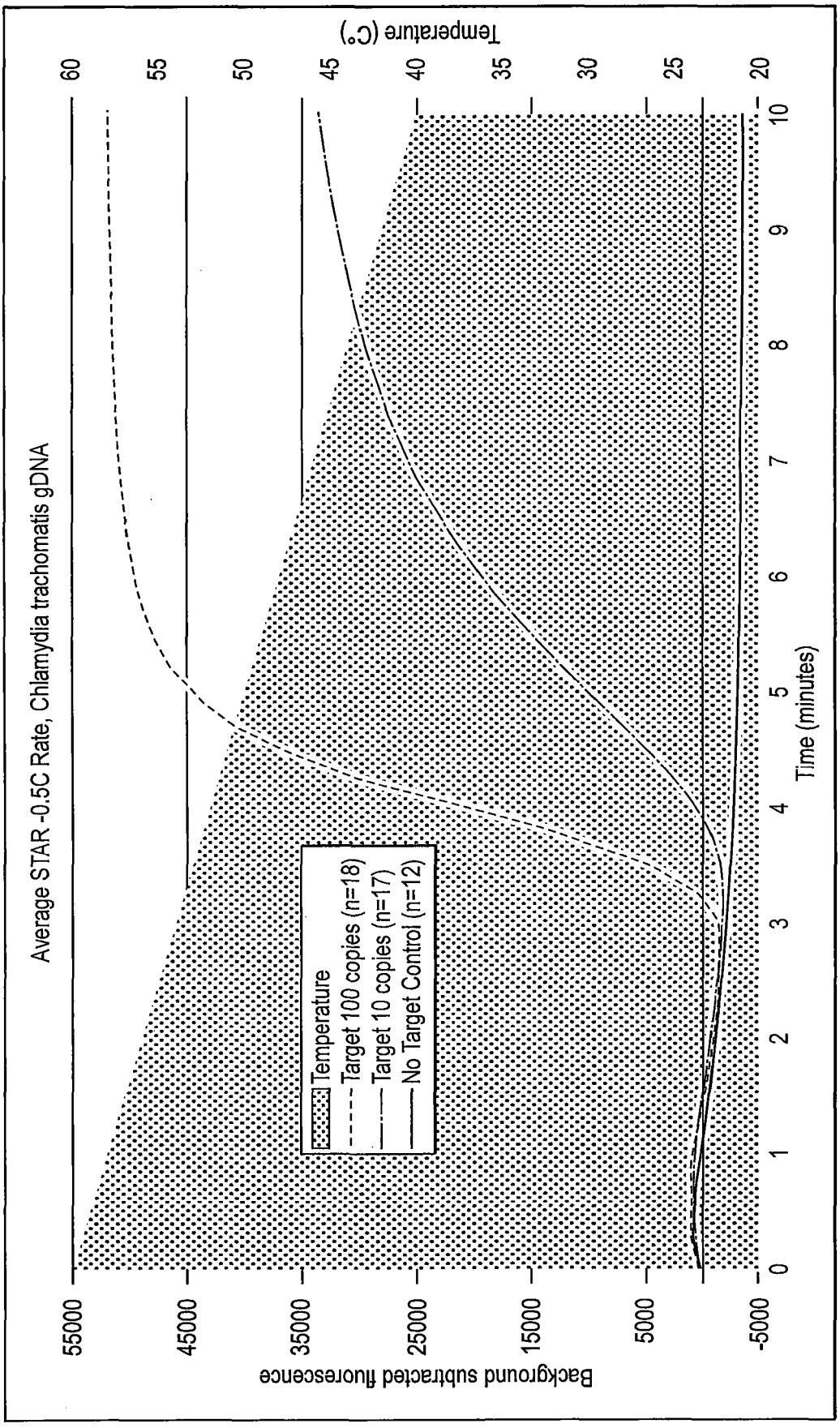
Average STAR -0.5C Rate, Chlamydia trachomatis gDNA
Temperature
Target 100 copies (n=18)
Target 10 copies (n=17)
No Target Control (n=12)
Background subtracted fluorescence
Time (minutes)
Temperature (C°)
55000
45000
35000
25000
15000
5000
-5000
0
1
2
3
4
5
6
7
8
9
10
20
25
30
35
40
45
50
55
60

FIG. 8

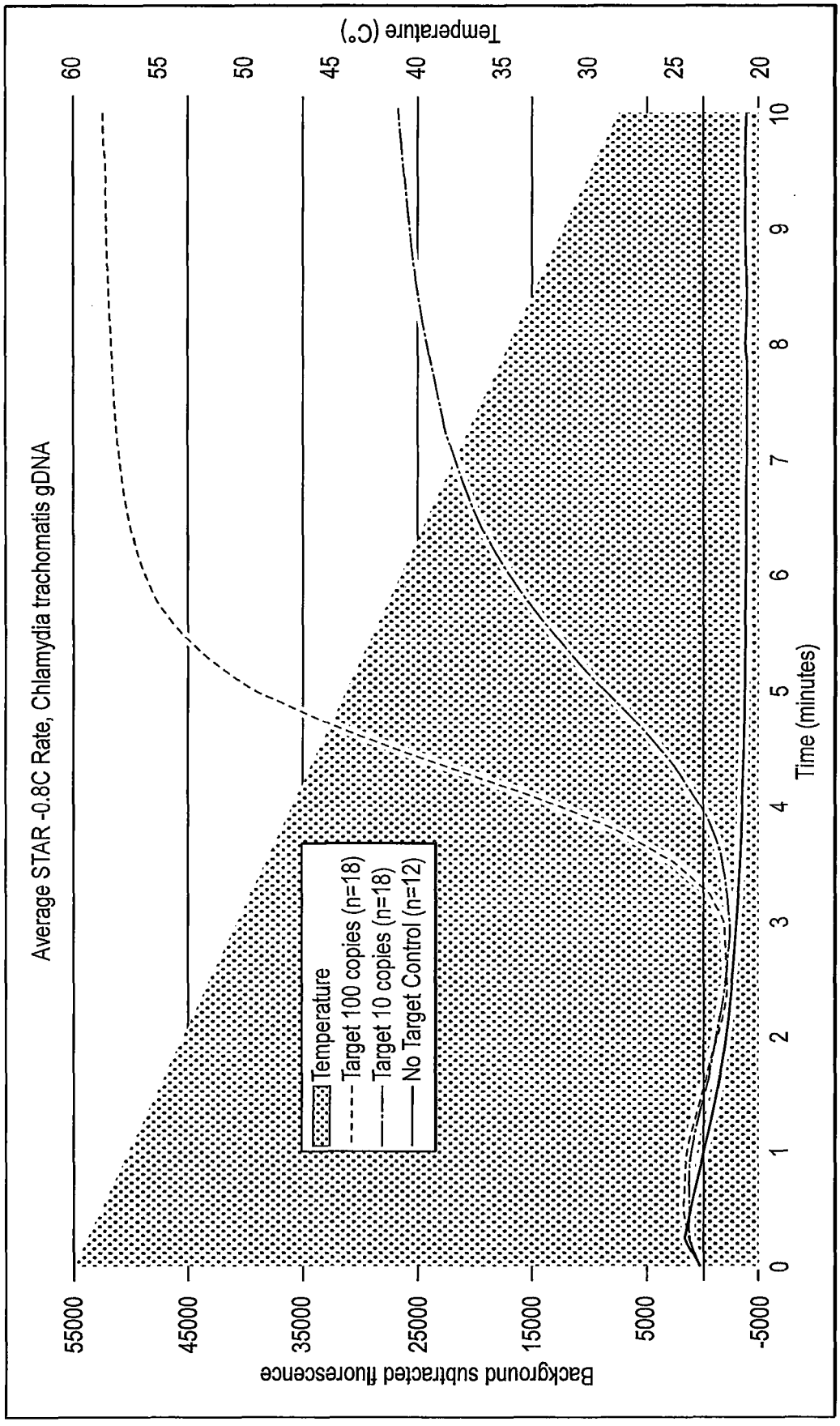
Average STAR -0.8C Rate, Chlamydia trachomatis gDNA
Temperature (C°)
60
55
50
45
40
35
30
25
20
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)

FIG. 9

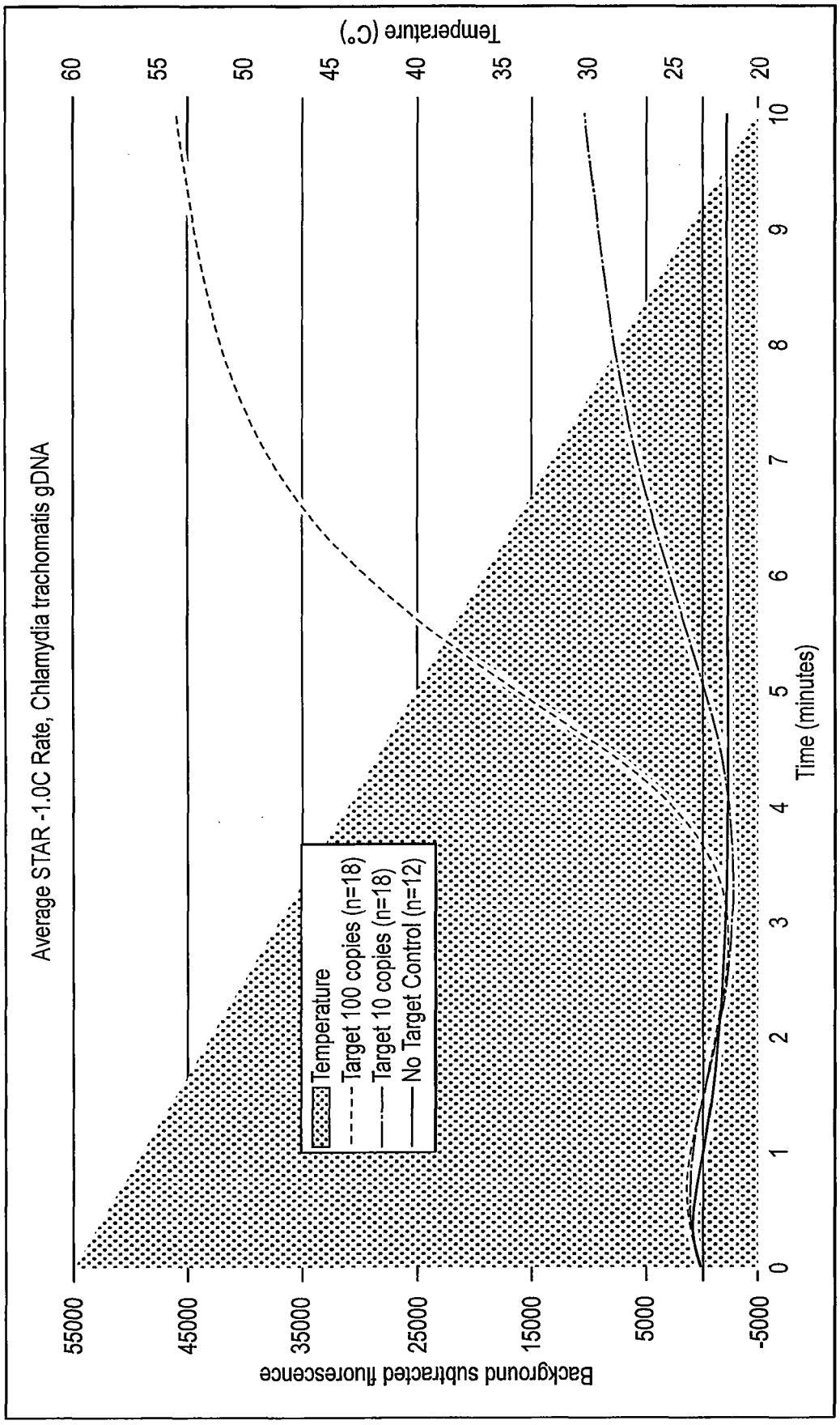
Average STAR -1.0C Rate, Chlamydia trachomatis gDNA
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
Temperature (C°)
60
55
50
45
40
35
30
25
20

FIG. 10

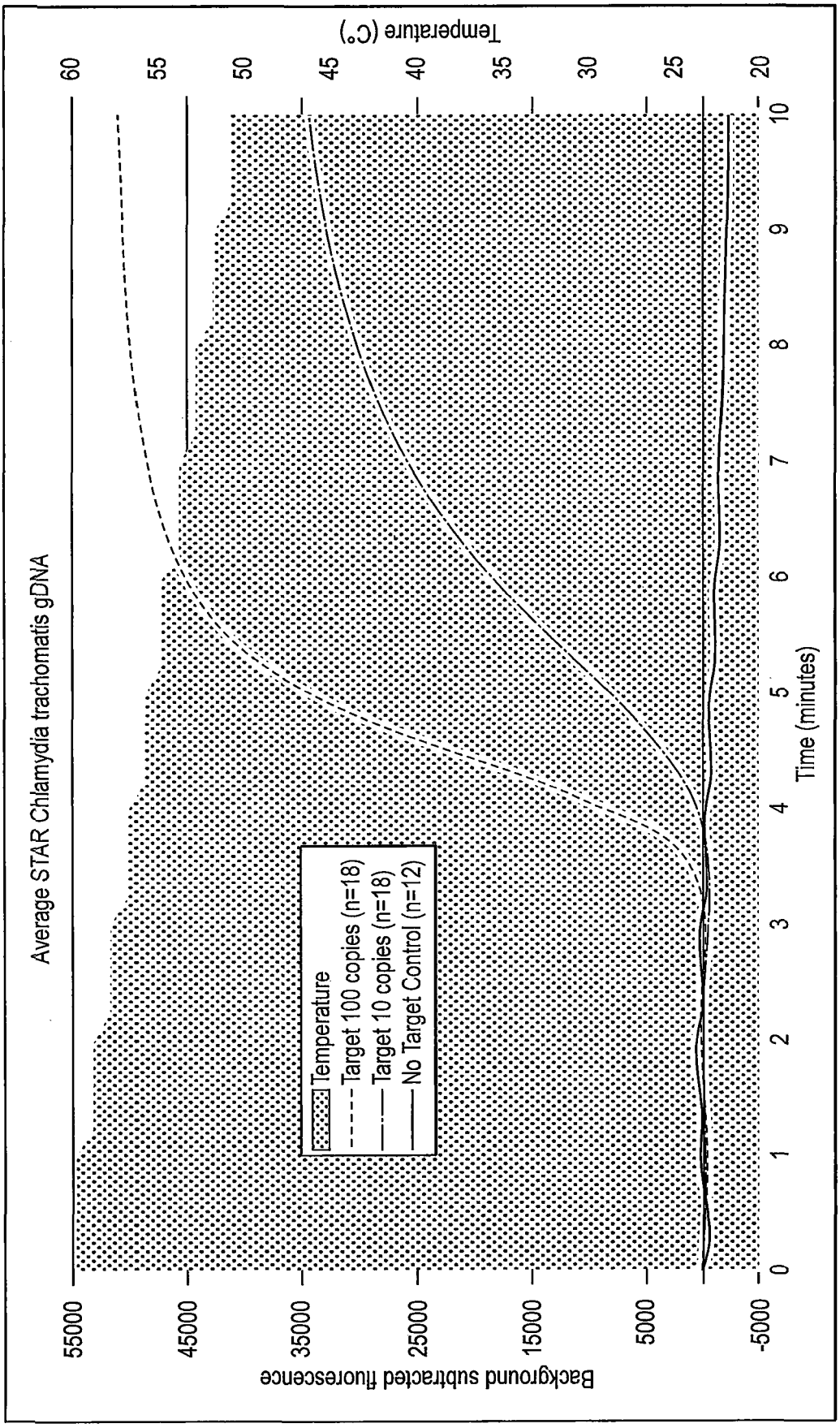
Average STAR Chlamydia trachomatis gDNA
Temperature
Target 100 copies (n=18)
Target 10 copies (n=18)
No Target Control (n=12)
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
Temperature (C°)
60
55
50
45
40
35
30
25
20

FIG. 11

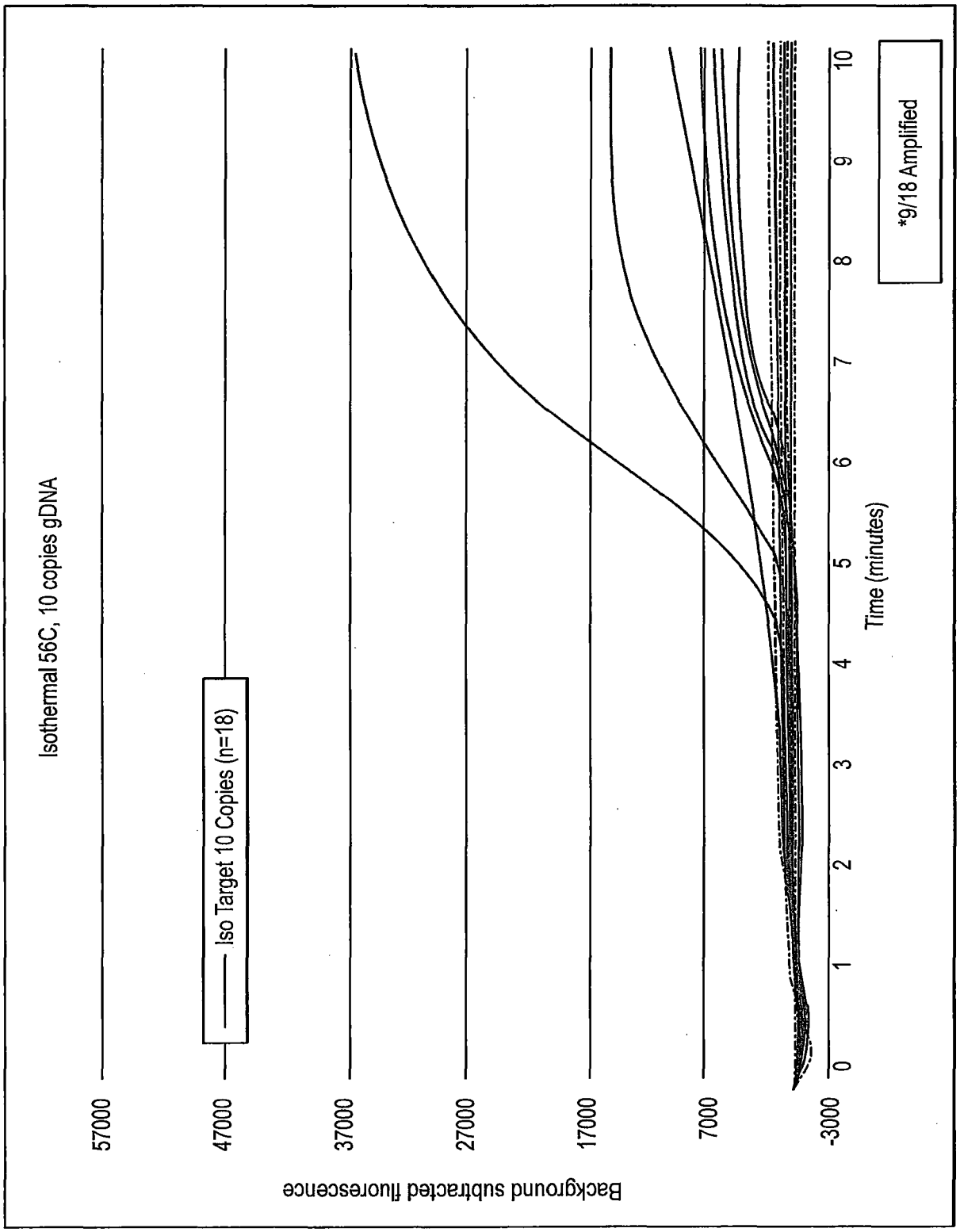
Isothermal 56C, 10 copies gDNA
Iso Target 10 Copies (n=18)
*9/18 Amplified
Background subtracted fluorescence
57000
47000
37000
27000
17000
7000
-3000
0
1
2
3
4
5
6
7
8
9
10
Time (minutes)

FIG. 12A

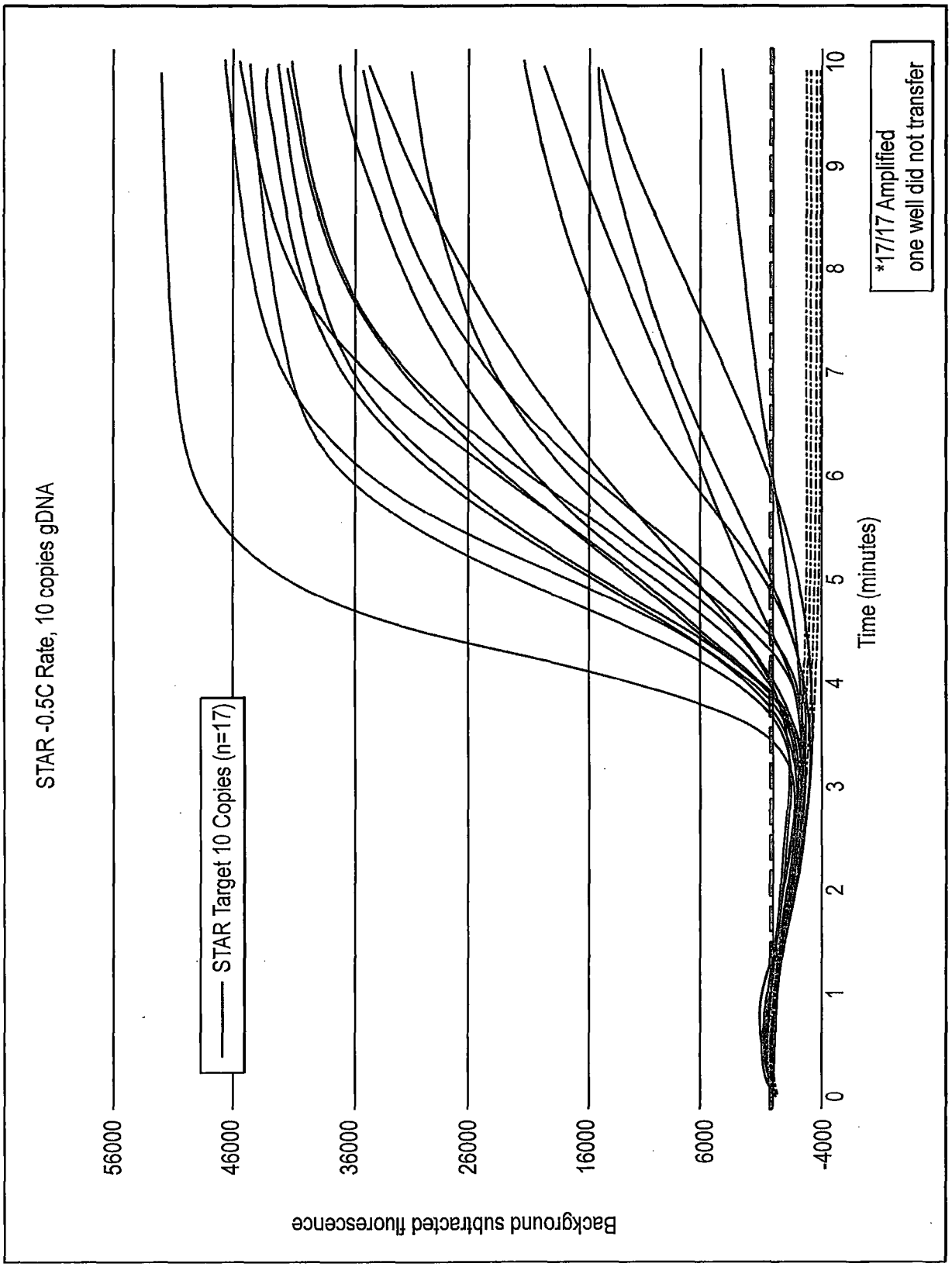
STAR -0.5C Rate, 10 copies gDNA
STAR Target 10 Copies (n=17)
*17/17 Amplified
one well did not transfer
Background subtracted fluorescence
Time (minutes)
56000
46000
36000
26000
16000
6000
-4000
0
1
2
3
4
5
6
7
8
9
10

FIG. 12B

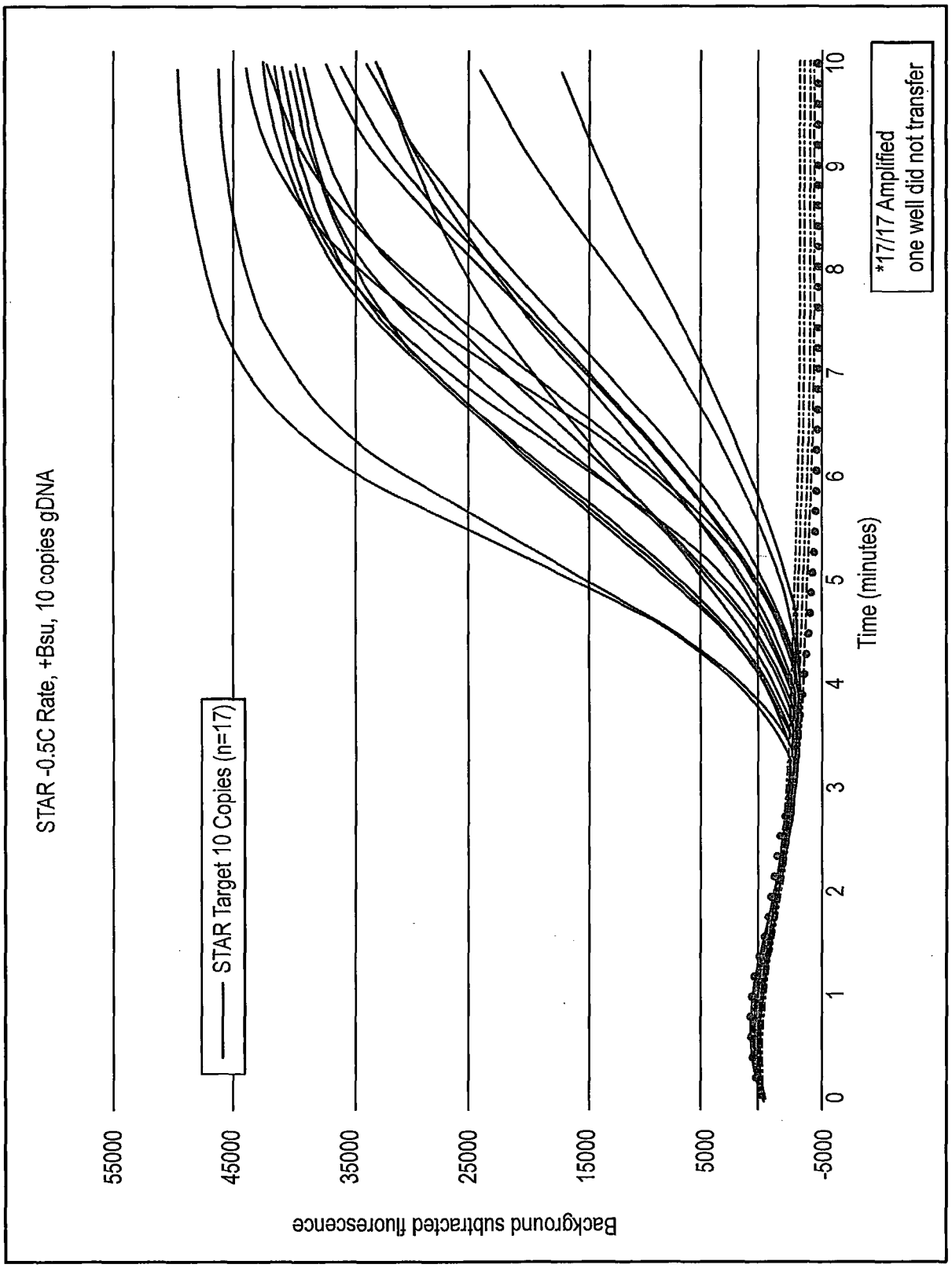
STAR -0.5C Rate, +Bsu, 10 copies gDNA
STAR Target 10 Copies (n=17)
*17/17 Amplified
one well did not transfer
Background subtracted fluorescence
55000
45000
35000
25000
15000
5000
-5000
0
1
2
3
4
5
6
7
8
9
10
Time (minutes)

FIG. 12C

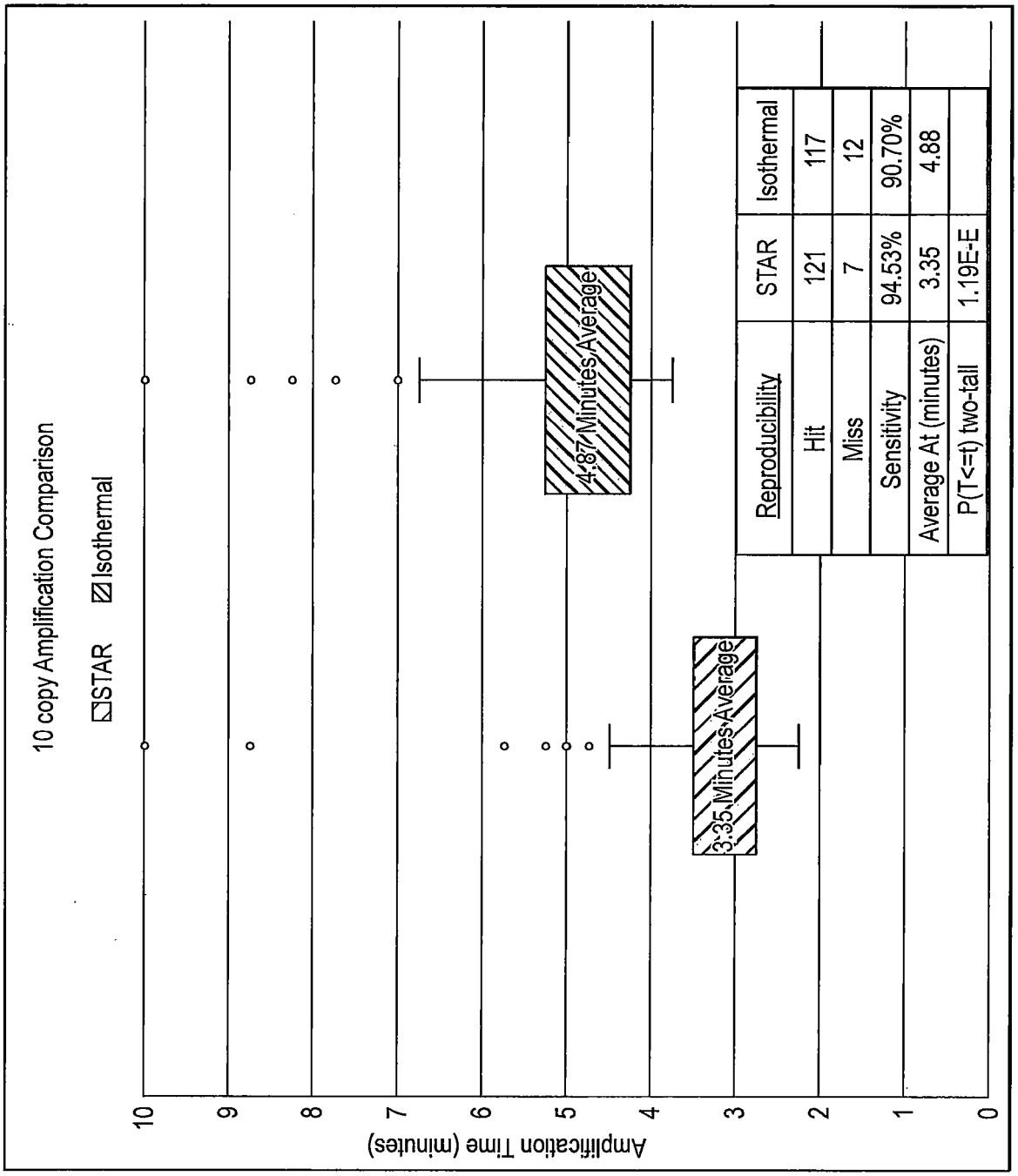
10 copy Amplification Comparison
STAR
Isothermal
Amplification Time (minutes)
0
1
2
3
4
5
6
7
8
9
10
4.87 Minutes Average
3.35 Minutes Average
Reproducibility
STAR
Isothermal
Hit
121
117
Miss
7
12
Sensitivity
94.53%
90.70%
Average At (minutes)
3.35
4.88
P(T<=t) two-tail
1.19E-E

FIG. 13

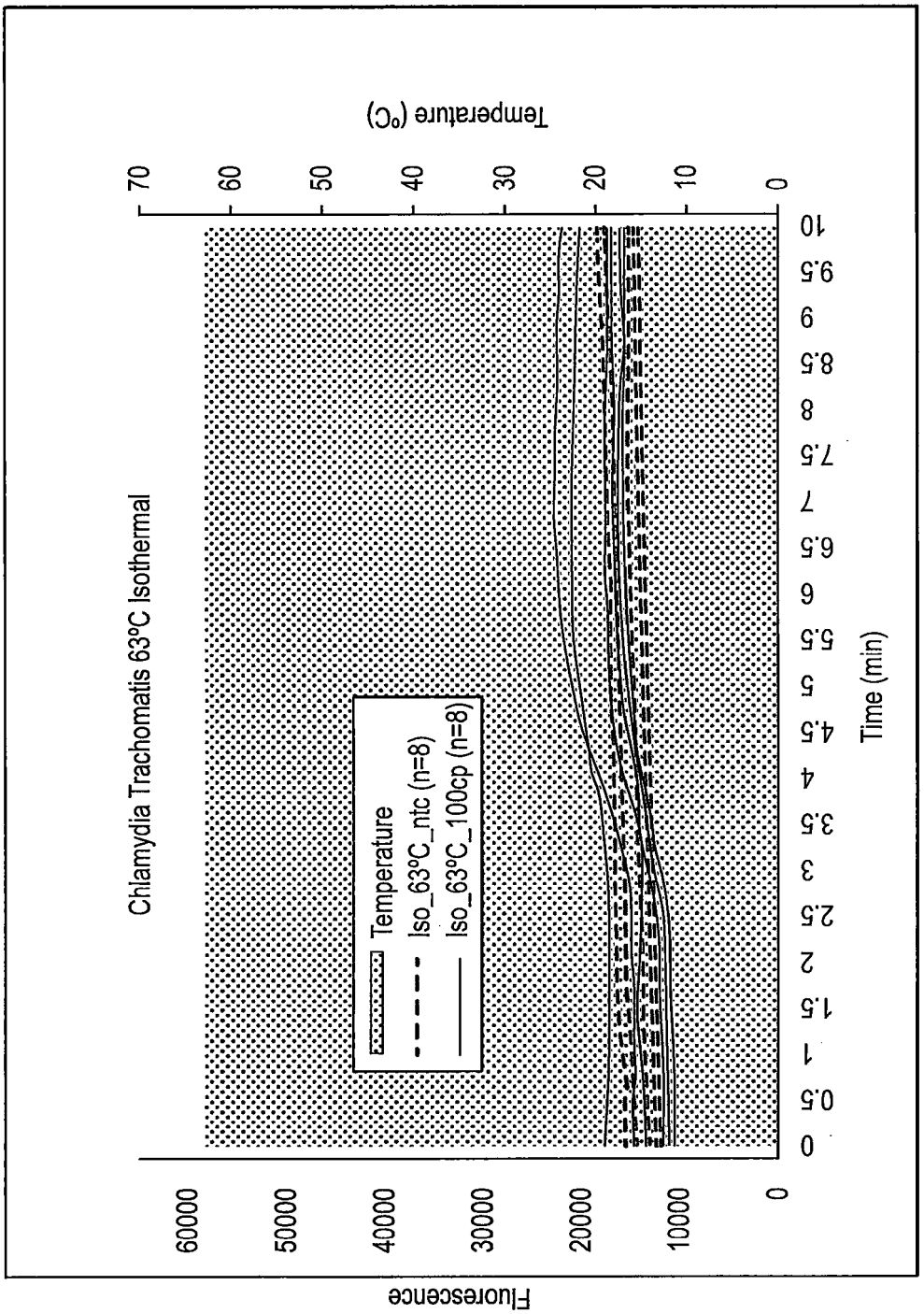
Chlamydia Trachomatis 63°C Isothermal
Temperature
Iso_63°C_ntc (n=8)
Iso_63°C_100cp (n=8)
Fluorescence
Temperature (°C)
Time (min)

FIG. 14A

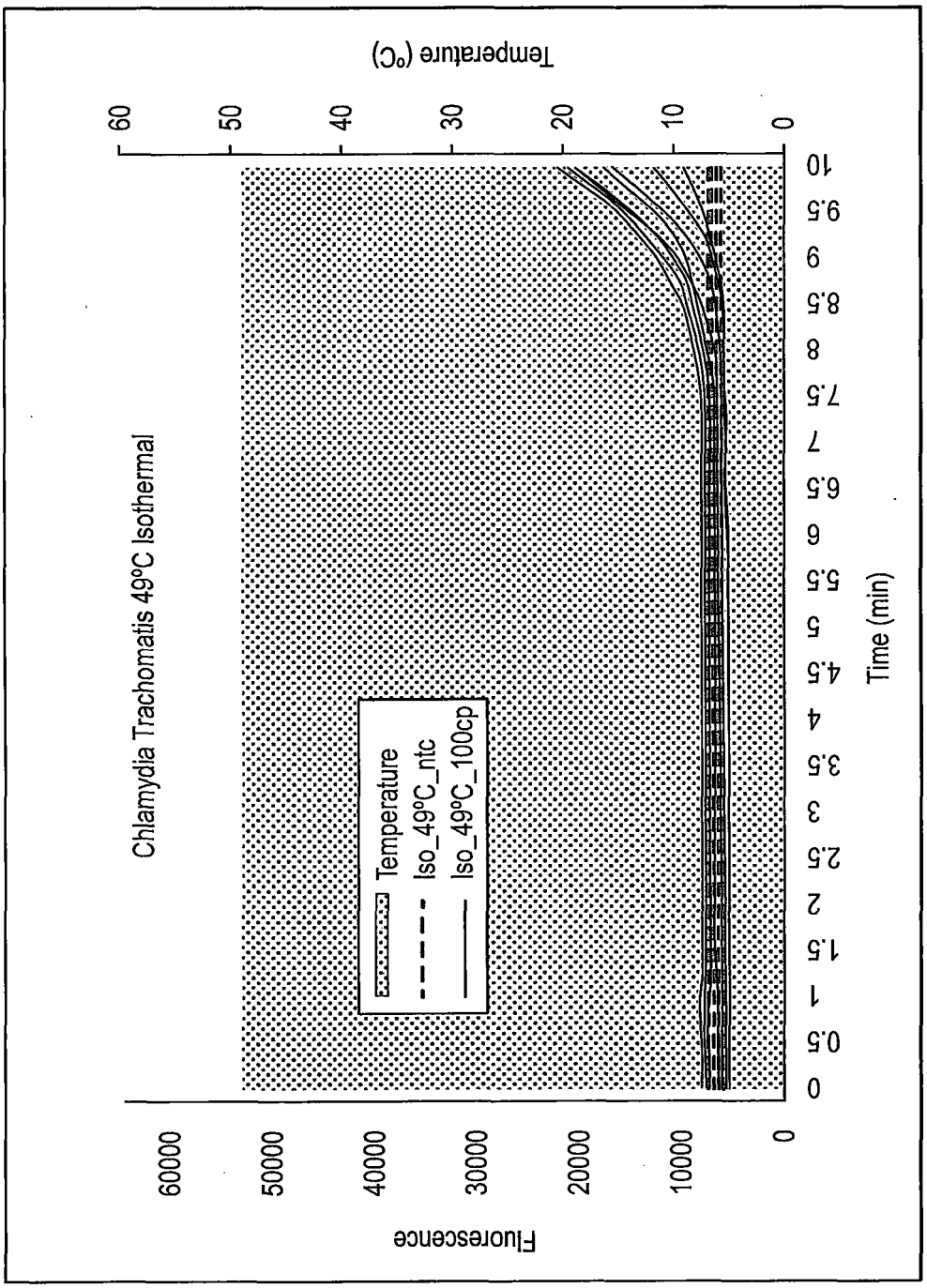
Chlamydia Trachomatis 49°C Isothermal
Temperature
Iso_49°C_ntc
Iso_49°C_100cp
Fluorescence
Temperature (°C)
Time (min)

FIG. 14B

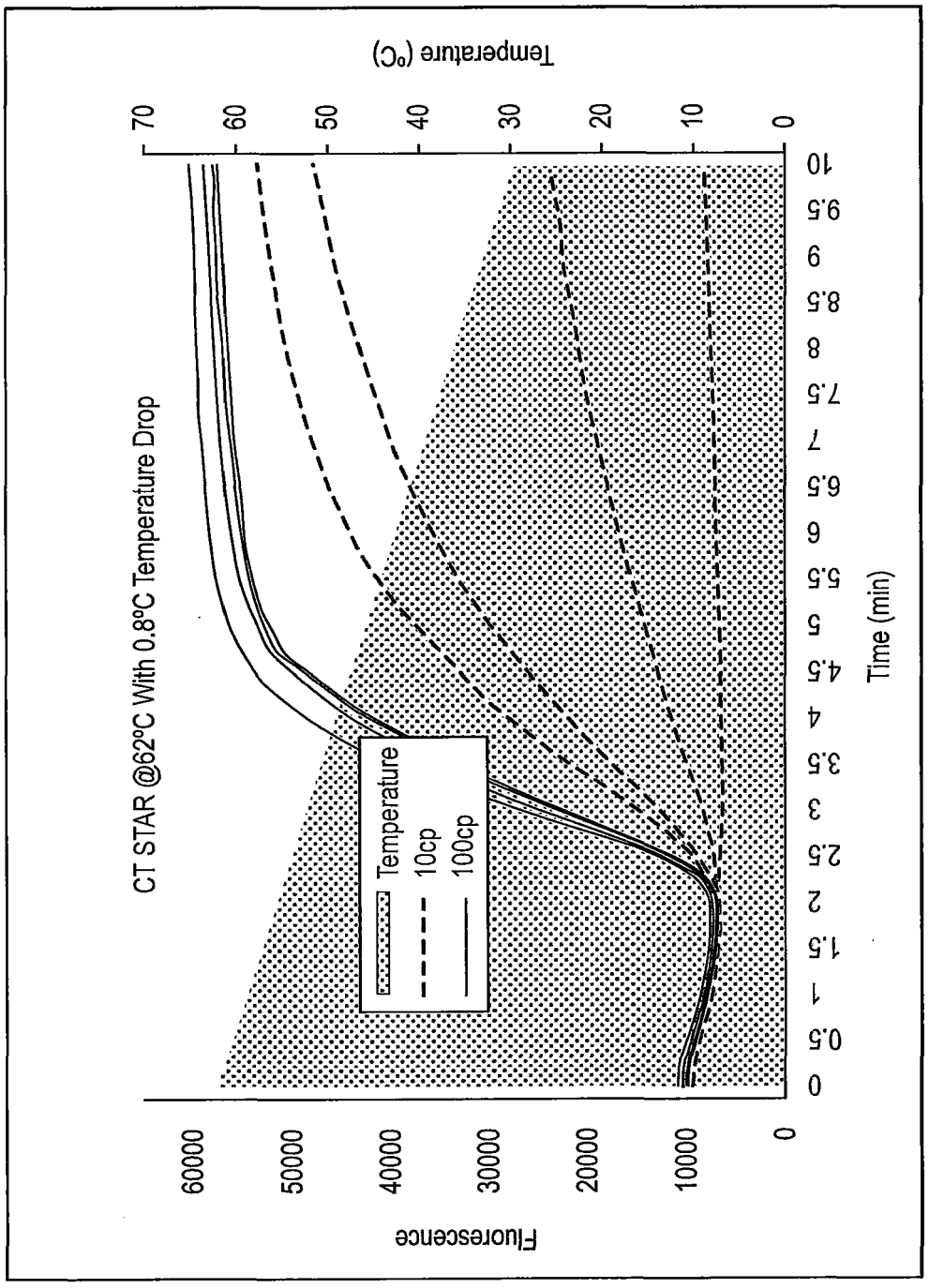
CT STAR @62°C With 0.8°C Temperature Drop
Temperature
10cp
100cp
Fluorescence
Temperature (°C)
Time (min)

FIG. 15A

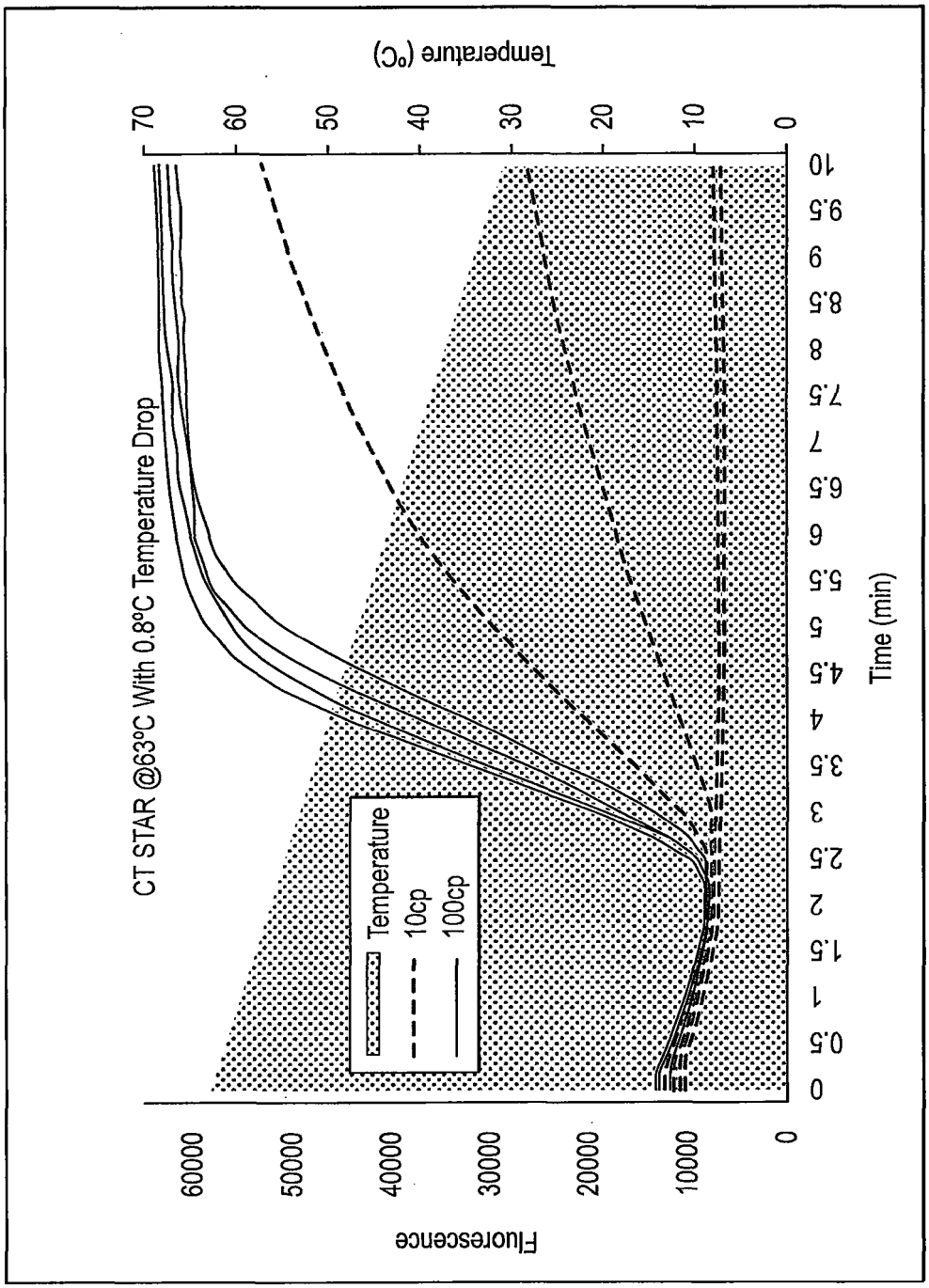
CT STAR @63°C With 0.8°C Temperature Drop
Temperature
10cp
100cp
Fluorescence
60000
50000
40000
30000
20000
10000
0
Temperature (°C)
70
60
50
40
30
20
10
0
Time (min)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
9
9.5
10

FIG. 15B

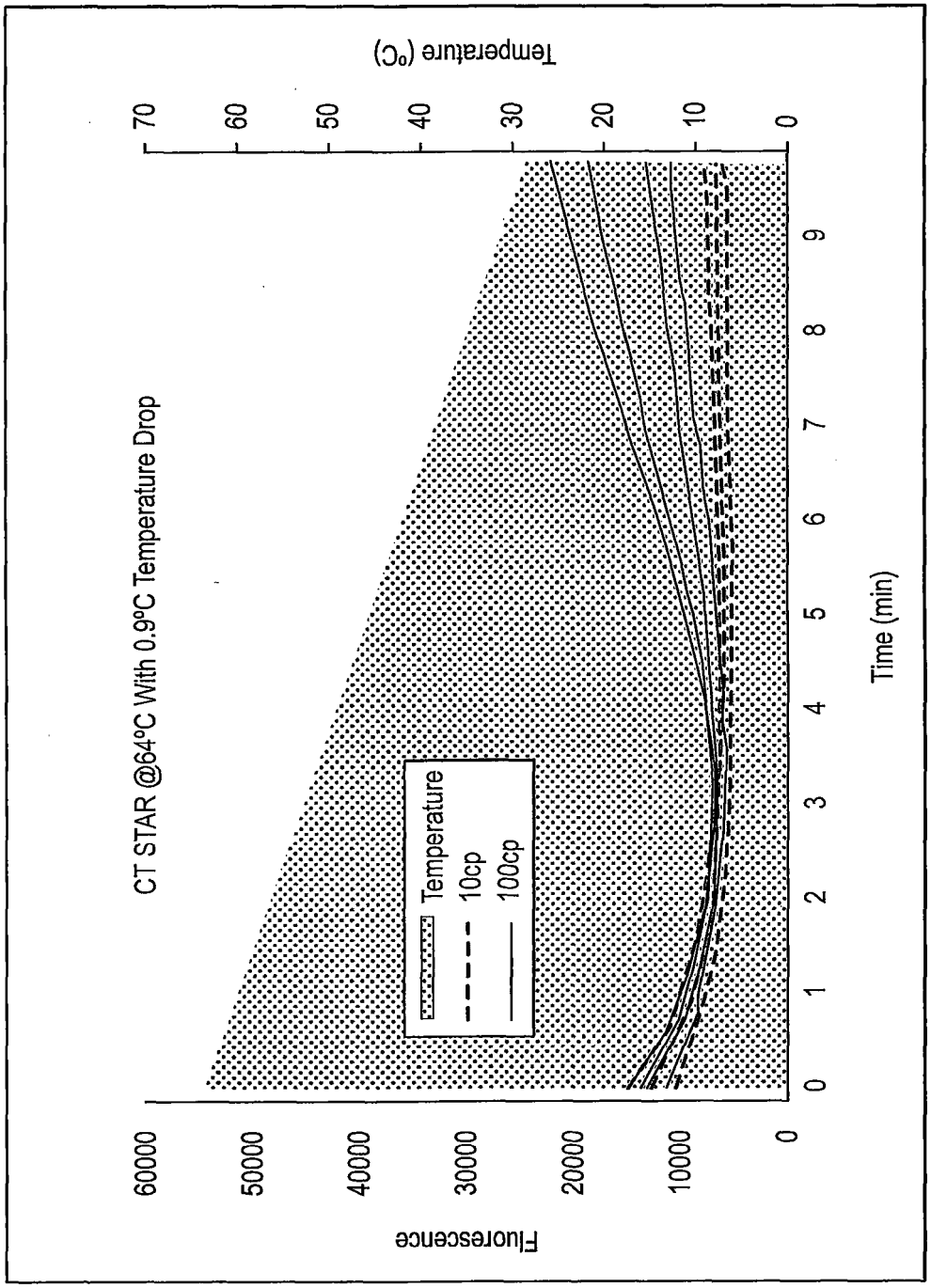
CT STAR @64°C With 0.9°C Temperature Drop
Temperature
10cp
100cp
Fluorescence
60000
50000
40000
30000
20000
10000
0
Temperature (°C)
70
60
50
40
30
20
10
0
Time (min)
0
1
2
3
4
5
6
7
8
9

FIG. 15C

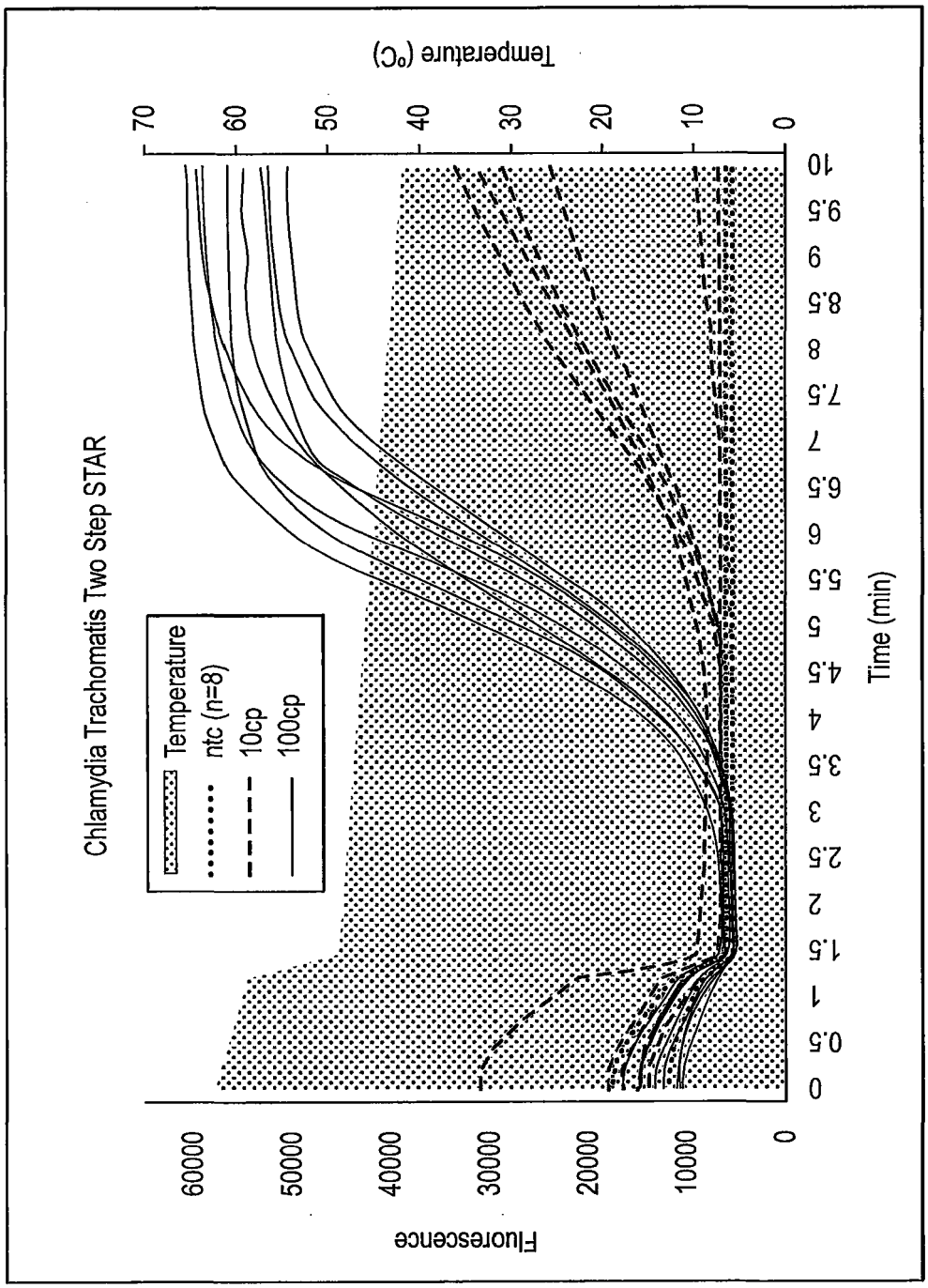
Chlamydia Trachomatis Two Step STAR
Temperature
ntc (n=8)
10cp
100cp
Fluorescence
60000
50000
40000
30000
20000
10000
0
Temperature (°C)
70
60
50
40
30
20
10
0
Time (min)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
8.5
9
9.5
10

FIG. 16A

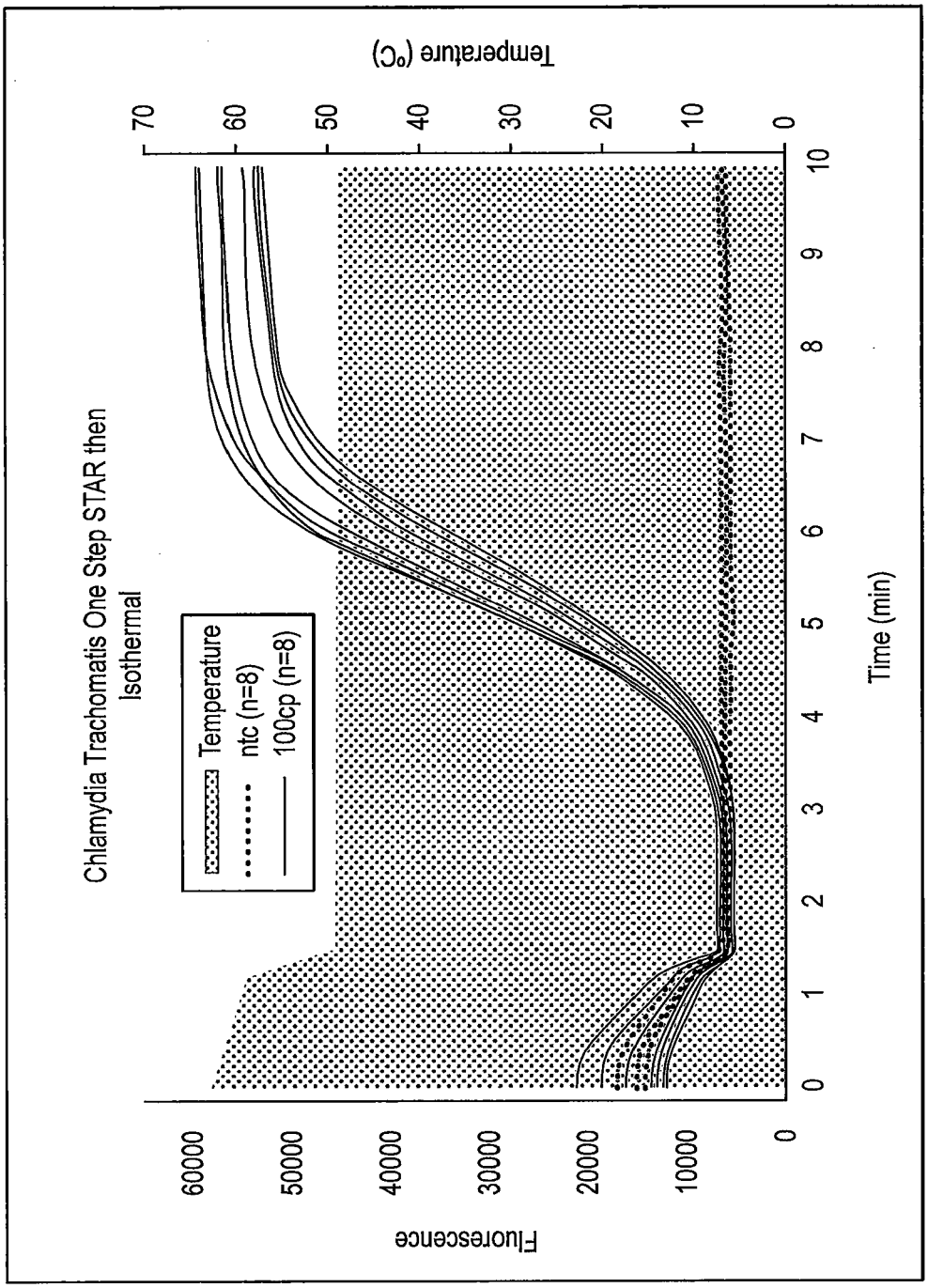
Chlamydia Trachomatis One Step STAR then Isothermal
Temperature
ntc (n=8)
100cp (n=8)
Fluorescence
60000
50000
40000
30000
20000
10000
0
Time (min)
0
1
2
3
4
5
6
7
8
9
10
Temperature (°C)
70
60
50
40
30
20
10
0

FIG. 16B

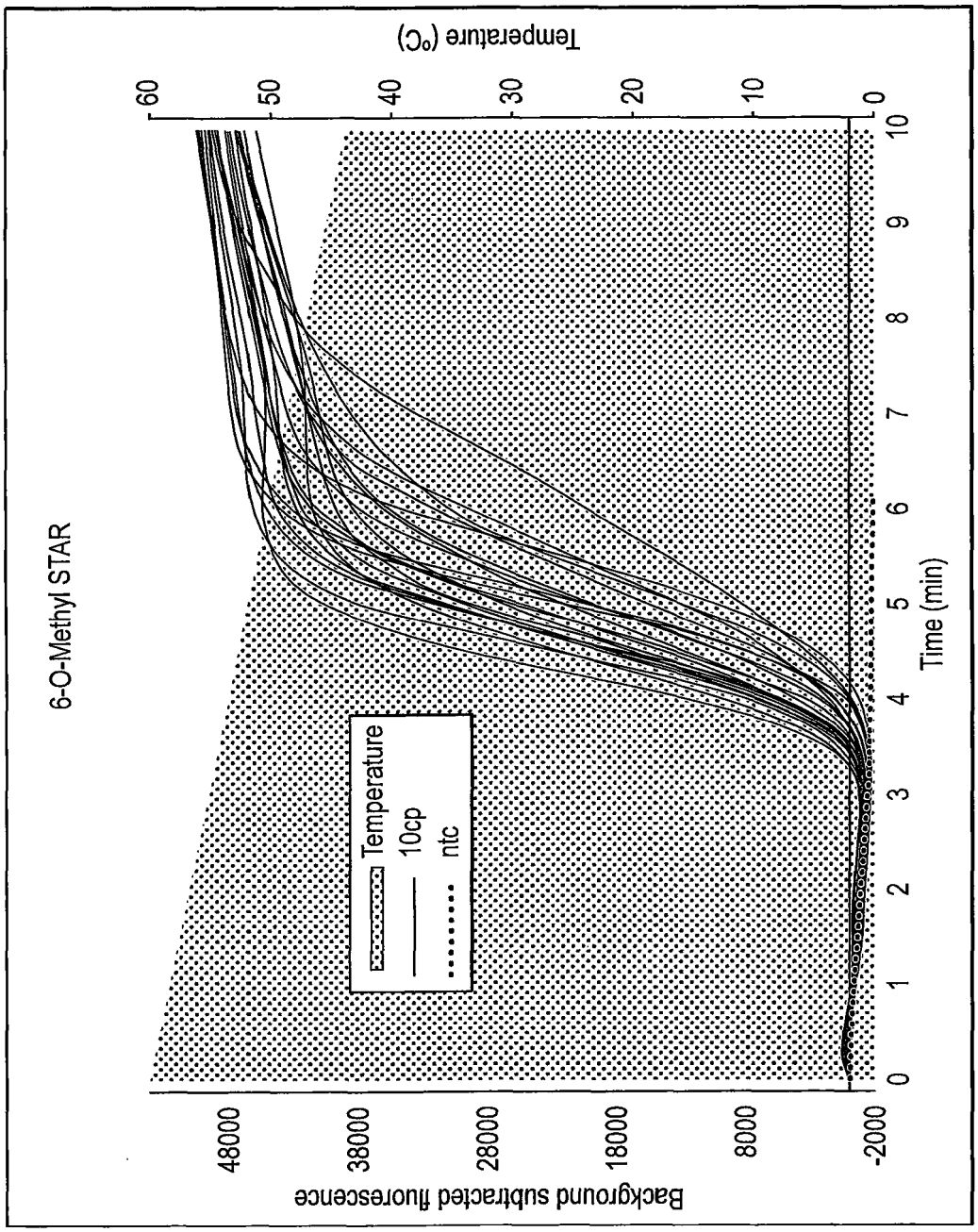
6-O-Methyl STAR
Temperature
10cp
ntc
Background subtracted fluorescence
48000
38000
28000
18000
8000
-2000
Time (min)
0
1
2
3
4
5
6
7
8
9
10
Temperature (°C)
60
50
40
30
20
10
0

FIG. 17A

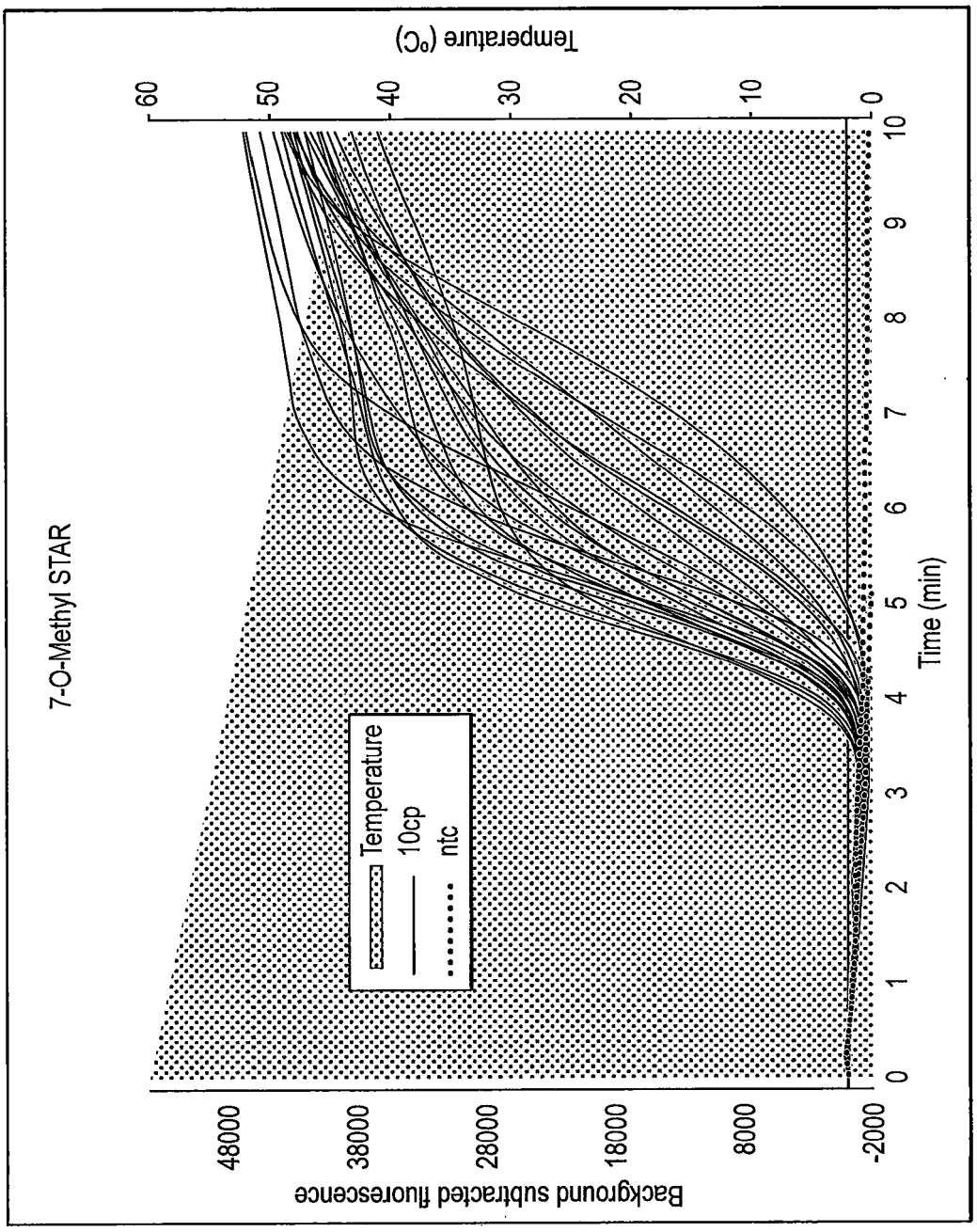
7-O-Methyl STAR
Temperature
10cp
ntc
Background subtracted fluorescence
48000
38000
28000
18000
8000
-2000
Time (min)
0
1
2
3
4
5
6
7
8
9
10
Temperature (°C)
60
50
40
30
20
10
0

FIG. 17B

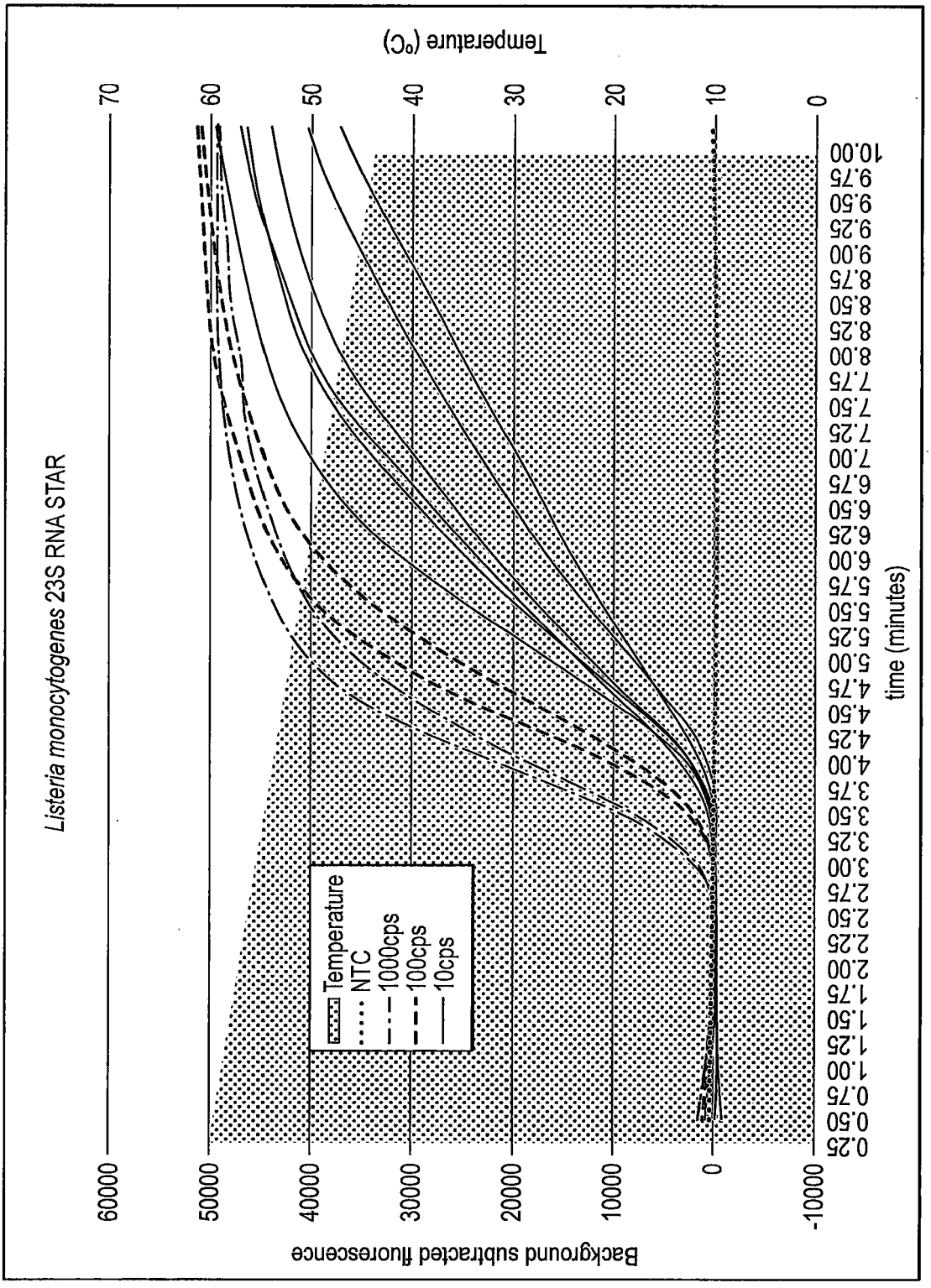
Listeria monocytogenes 23S RNA STAR
Background subtracted fluorescence
Temperature (°C)
time (minutes)
Temperature
NTC
1000cps
100cps
10cps
60000
50000
40000
30000
20000
10000
0
-10000
70
60
50
40
30
20
10
0
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
3.25
3.50
3.75
4.00
4.25
4.50
4.75
5.00
5.25
5.50
5.75
6.00
6.25
6.50
6.75
7.00
7.25
7.50
7.75
8.00
8.25
8.50
8.75
9.00
9.25
9.50
9.75
10.00

FIG. 18