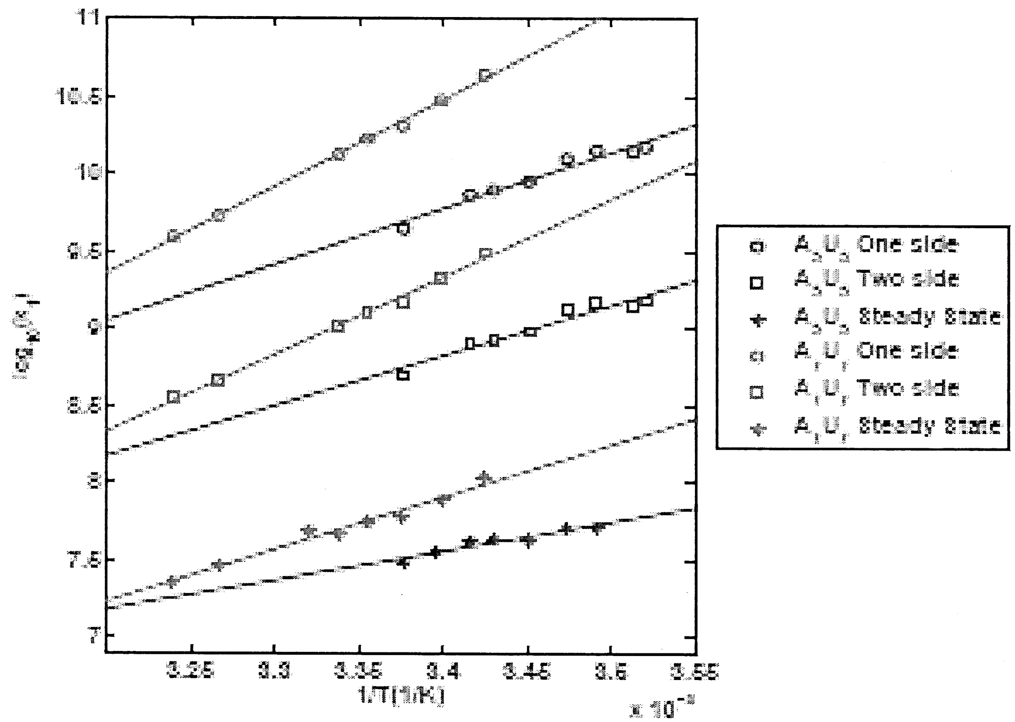
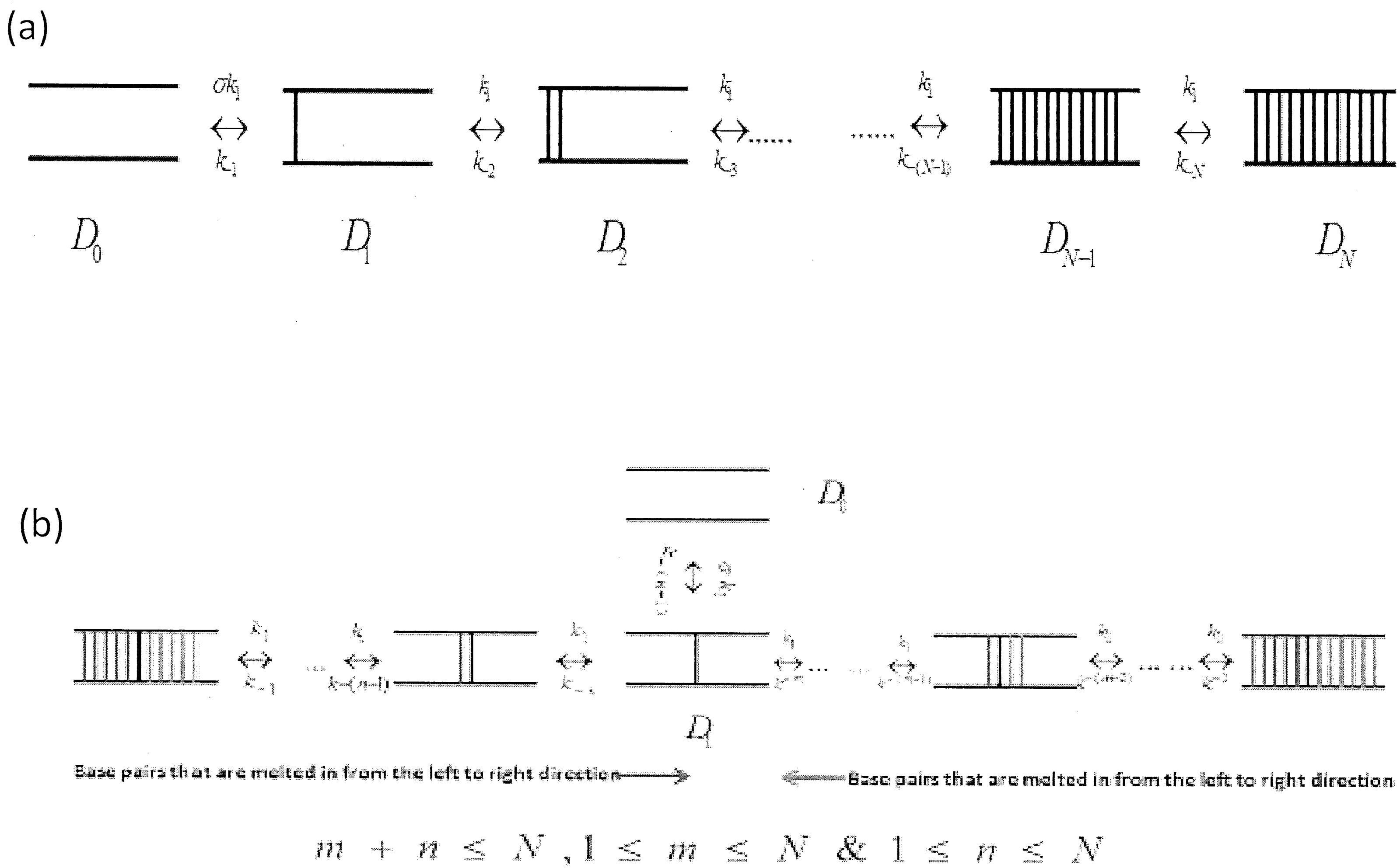


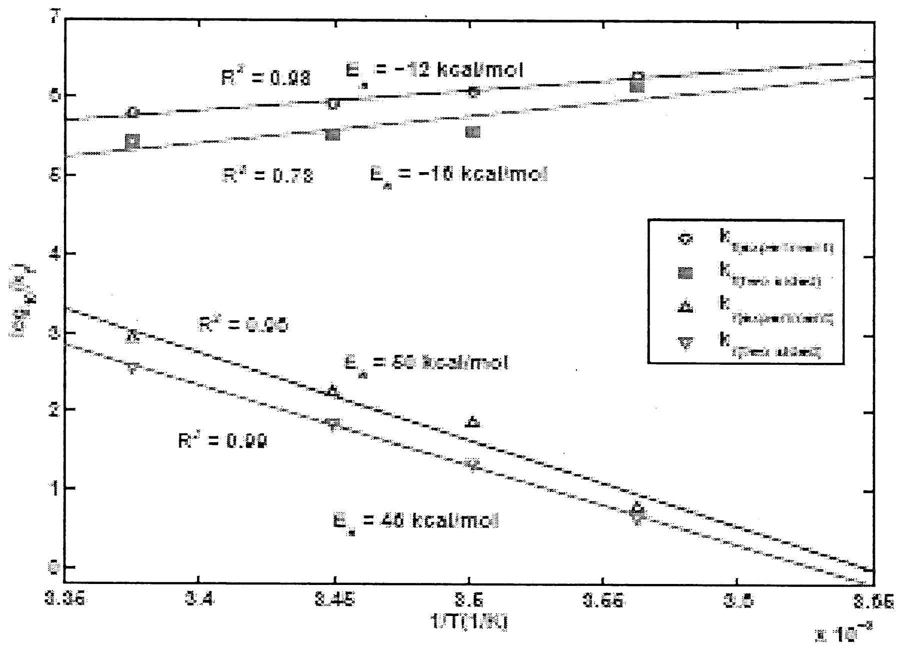
11
10.5
10
9.5
9
8.5
8
7.5
7
3.25
3.3
3.35
3.4
3.45
3.5
3.55
1/T(1/K)
x 10^-3
A2U2 One side
A2U2 Two side
A2U2 Steady State
A1U1 One side
A1U1 Two side
A1U1 Steady State

FIGURE 1



(a)
σk_1
k_{-1}
k_1
k_{-2}
k_1
k_{-3}
k_1
$k_{-(N-1)}$
k_1
k_{-N}
D_0
D_1
D_2
D_{N-1}
D_N
(b)
D_0
D_1
Base pairs that are melted in from the left to right direction
Base pairs that are melted in from the left to right direction
$m + n \leq N, 1 \leq m \leq N \;\&\; 1 \leq n \leq N$

FIGURE 2



R^2 = 0.98
E_a = −12 kcal/mol
R^2 = 0.78
E_a = −16 kcal/mol
R^2 = 0.95
E_a = 56 kcal/mol
R^2 = 0.99
E_a = 46 kcal/mol
1/T(1/K)

FIGURE 3

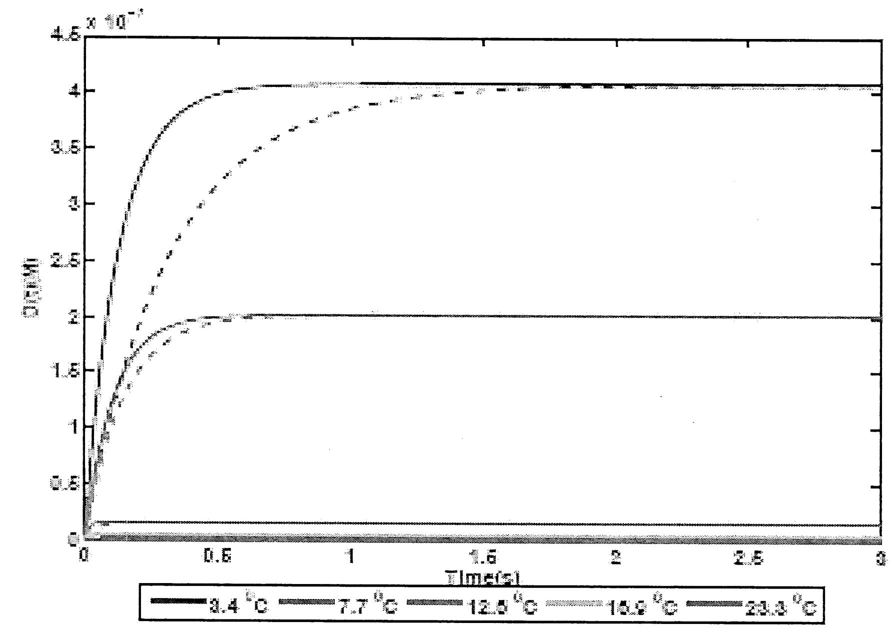

x 10^-7
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.5
1
1.5
2
2.5
3
Time(s)
3.4 °C
7.7 °C
12.5 °C
15.9 °C
23.3 °C

FIGURE 4

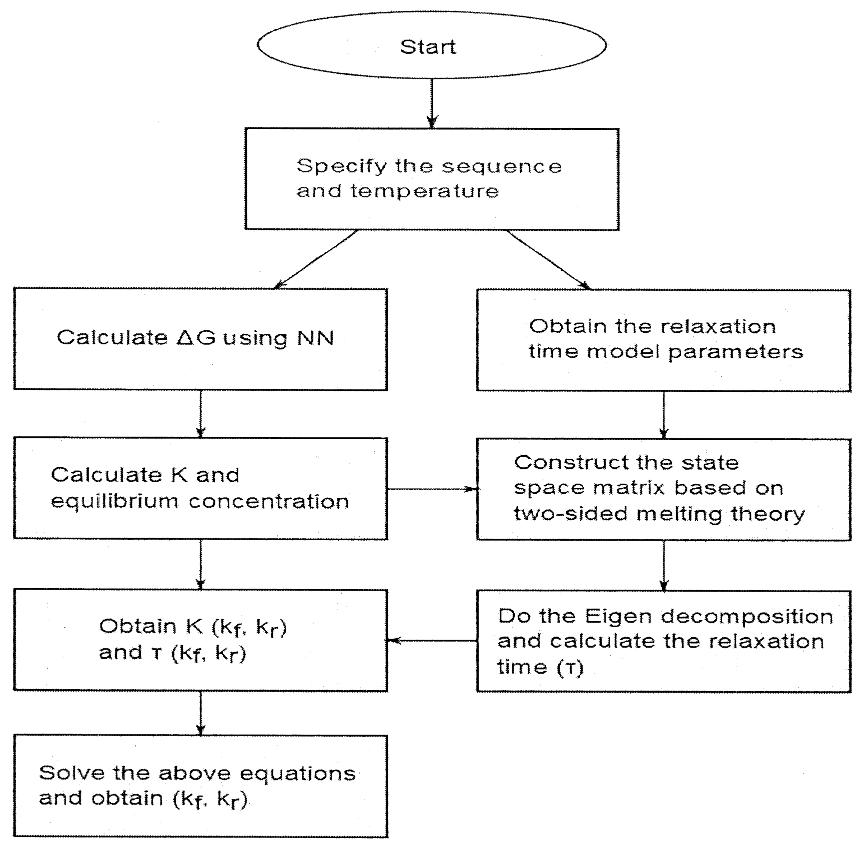
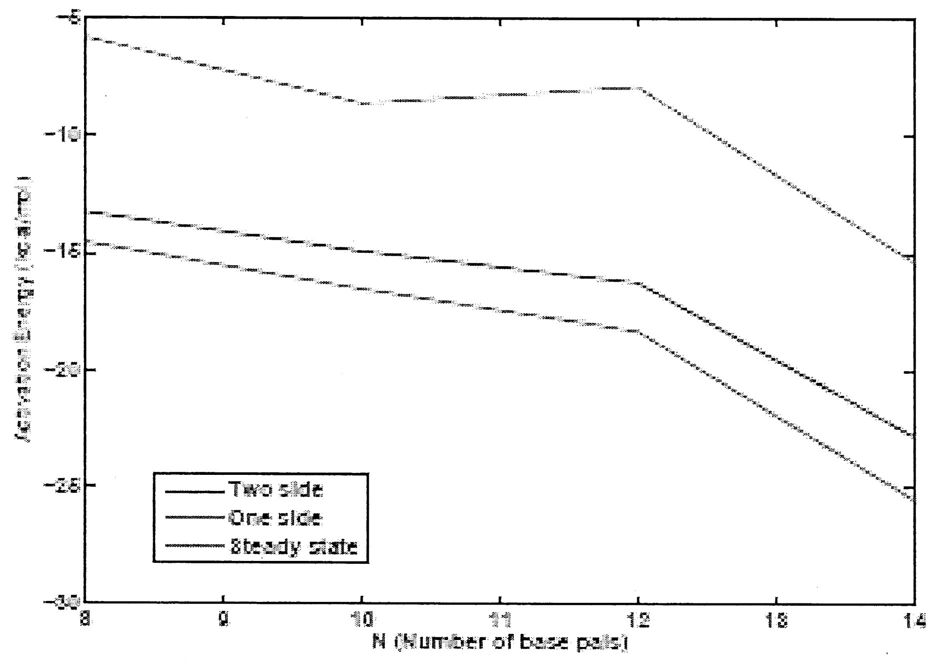


FIGURE 5. Flowchart for calculation of annealing rate constants



-5
-10
-15
-20
-25
-30
8
9
10
11
12
13
14
N (Number of base pairs)
Activation Energy (kcal/mol)
Two side
One side
Steady state

FIGURE 6

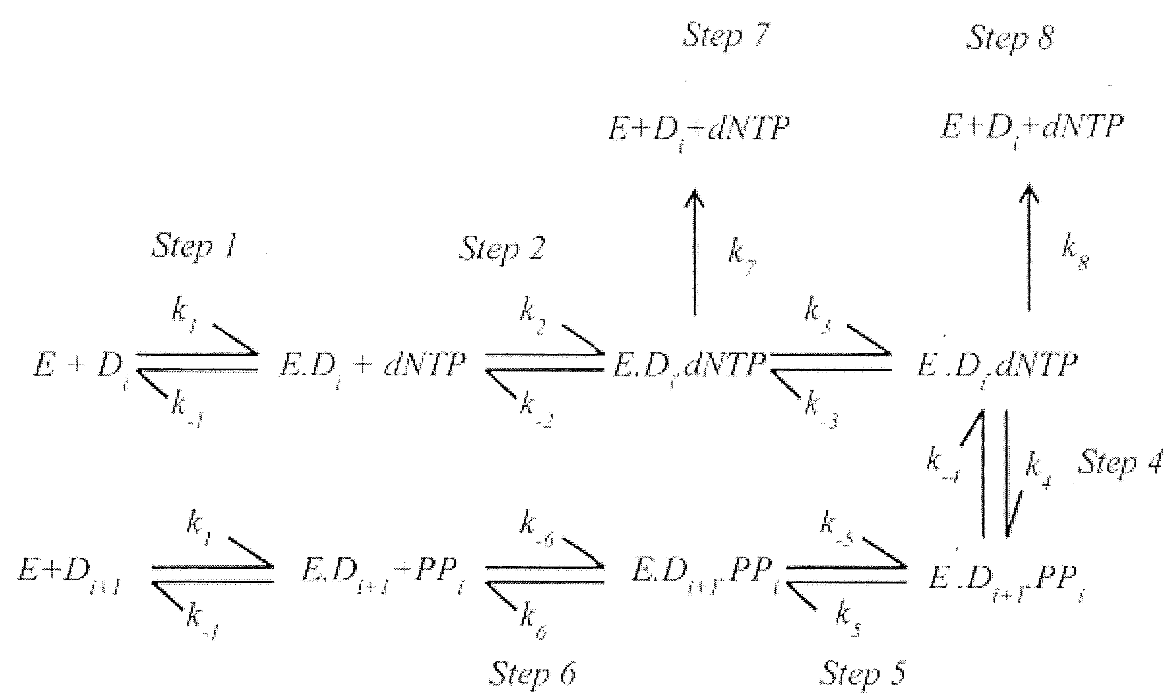


FIGURE 7. Reaction scheme for polymerase-mediated DNA extension. (k_1,k_{-1} in this scheme are denoted k_e,k_{-e} in the text.)

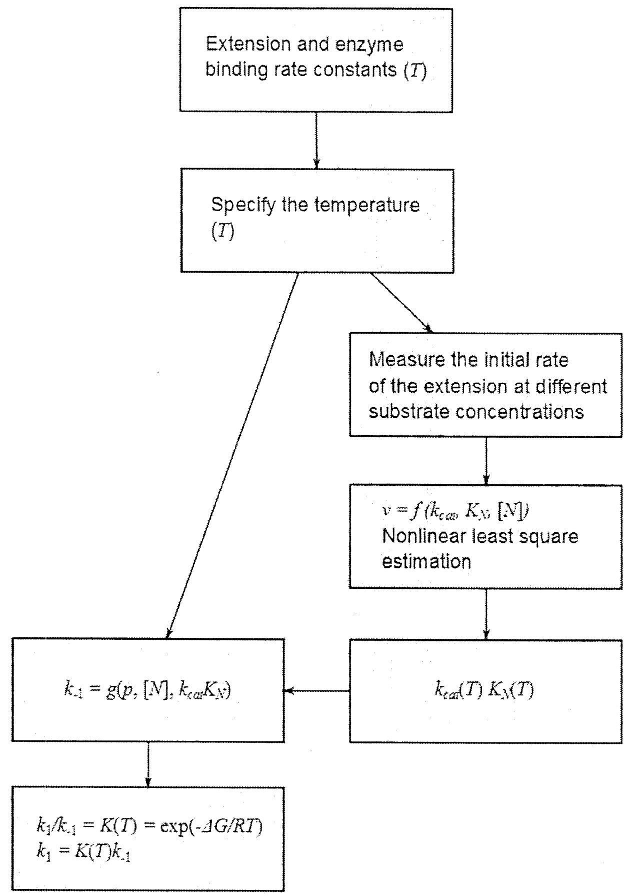


FIGURE 8. Flowchart for calculation of polymerase extension rate constants (k_1,k_{-1} in this scheme are denoted k_e,k_{-e} in the text.)

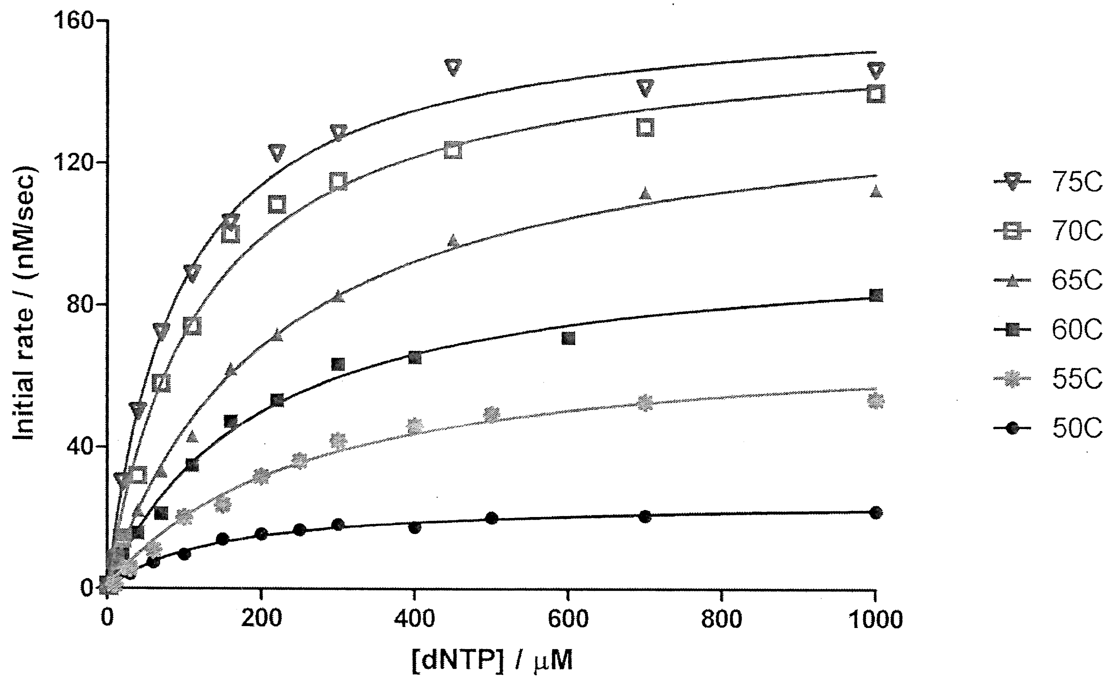
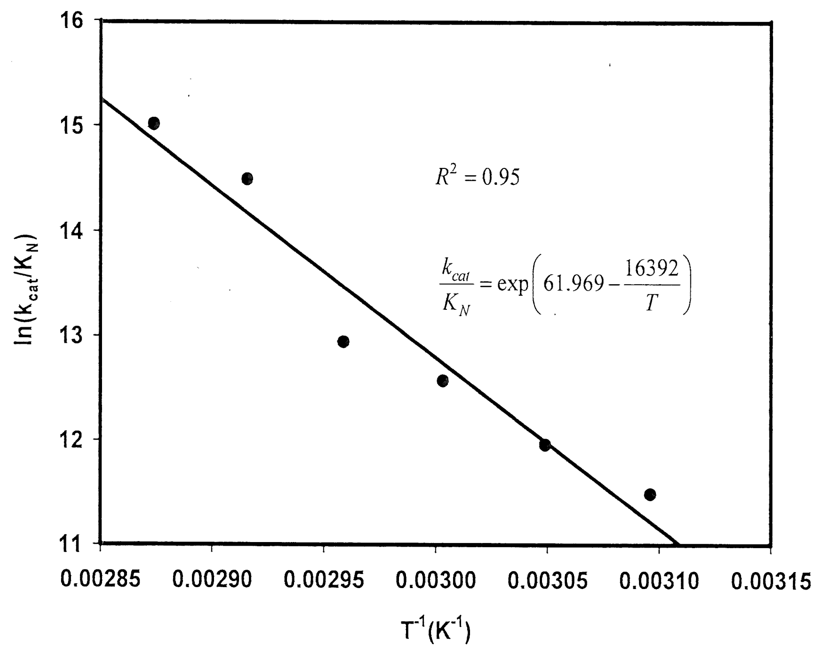
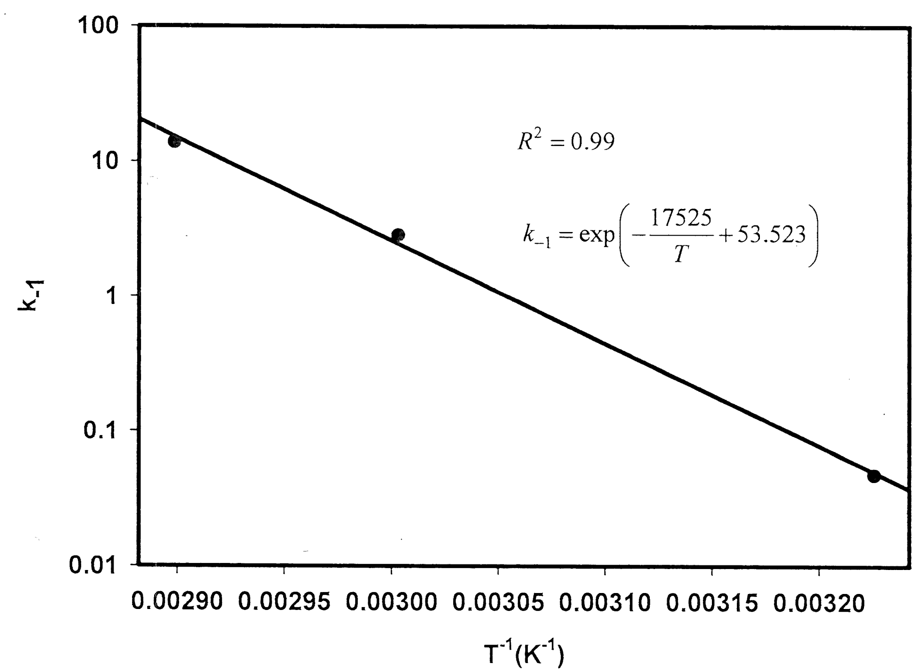

160
120
80
40
0
Initial rate / (nM/sec)
0
200
400
600
800
1000
[dNTP] / μM
75C
70C
65C
60C
55C
50C

FIGURE 9



16
15
14
13
12
11
ln(kcat/KN)
R2 = 0.95
kcat/KN = exp(61.969 − 16392/T)
0.00285
0.00290
0.00295
0.00300
0.00305
0.00310
0.00315
T-1(K-1)

FIGURE 10



100
10
1
0.1
0.01
k-1
$R^2 = 0.99$
$k_{-1} = \exp\left(-\frac{17525}{T} + 53.523\right)$
0.00290 0.00295 0.00300 0.00305 0.00310 0.00315 0.00320
$T^{-1}(K^{-1})$

FIGURE 11

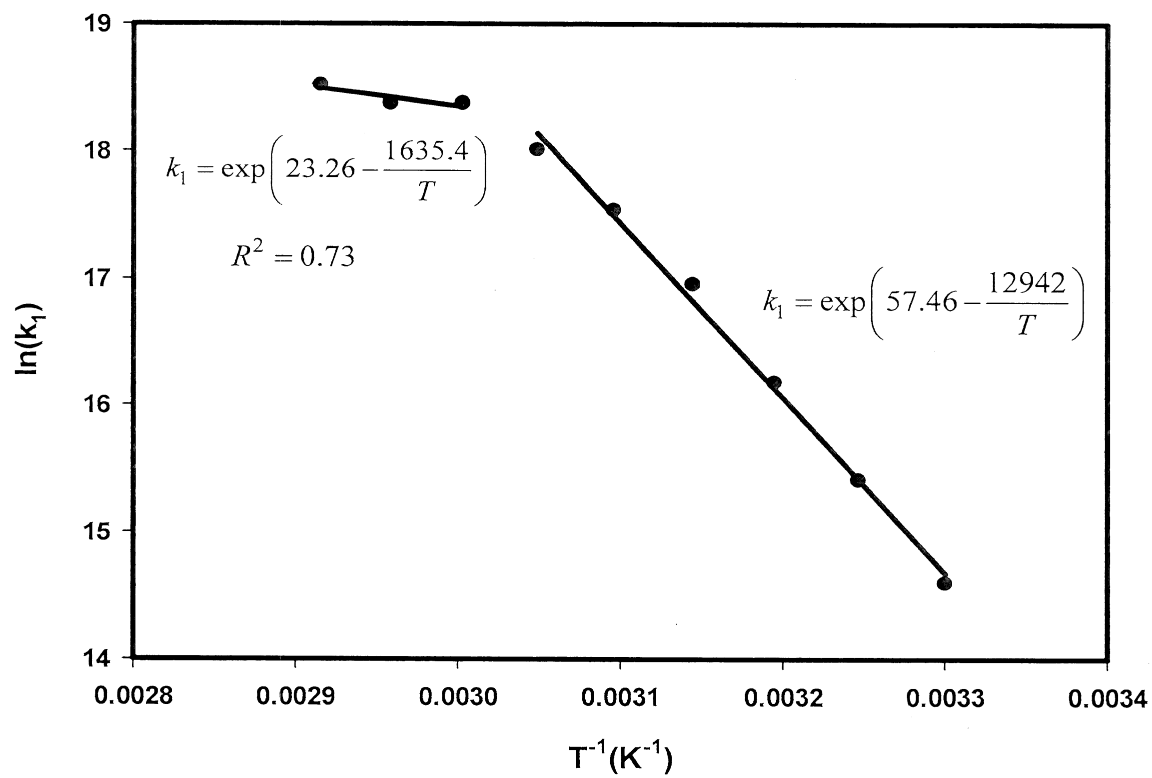

19
18
17
16
15
14
ln(k1)
0.0028
0.0029
0.0030
0.0031
0.0032
0.0033
0.0034
T-1(K-1)
$k_1 = \exp\left(23.26 - \frac{1635.4}{T}\right)$
$R^2 = 0.73$
$k_1 = \exp\left(57.46 - \frac{12942}{T}\right)$

FIGURE 12

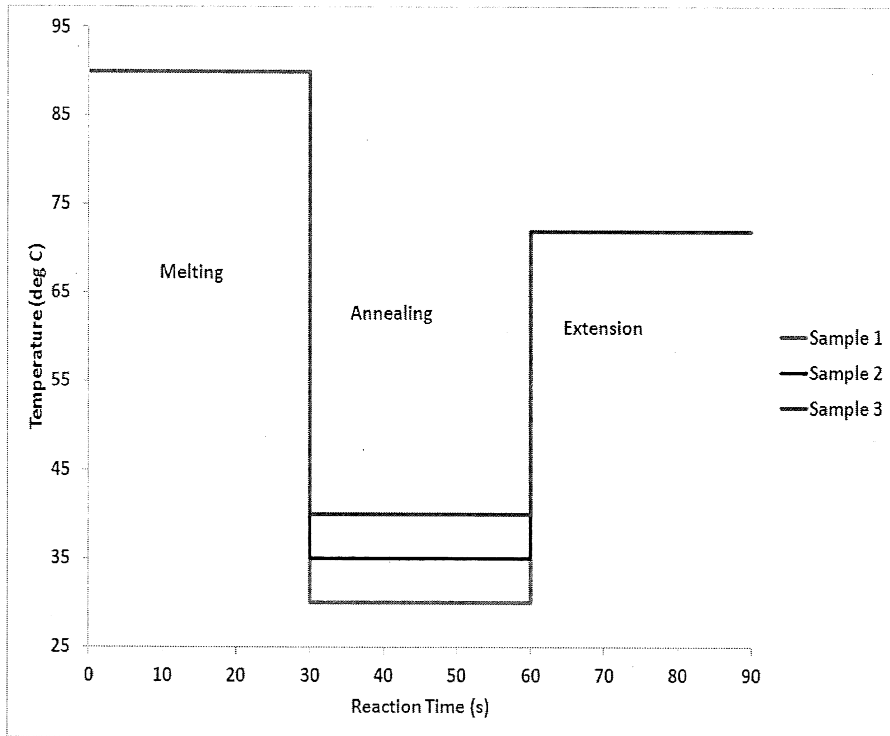

95
85
75
65
55
45
35
25
Temperature (deg C)
0
10
20
30
40
50
60
70
80
90
Reaction Time (s)
Melting
Annealing
Extension
Sample 1
Sample 2
Sample 3

FIGURE 13

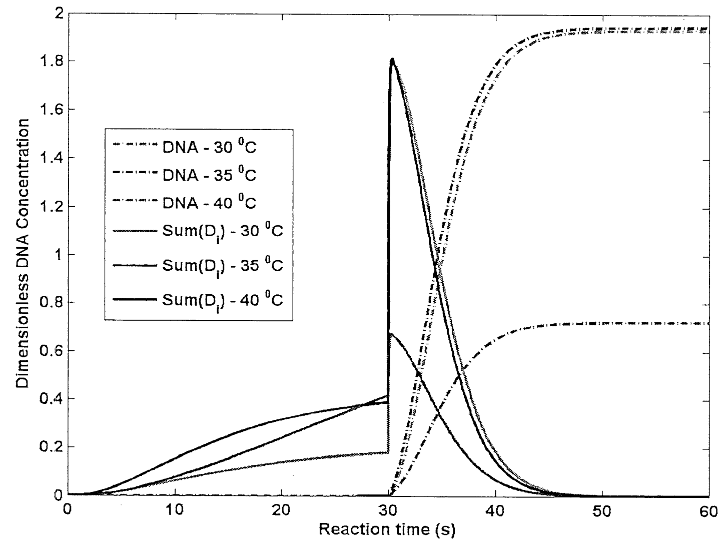
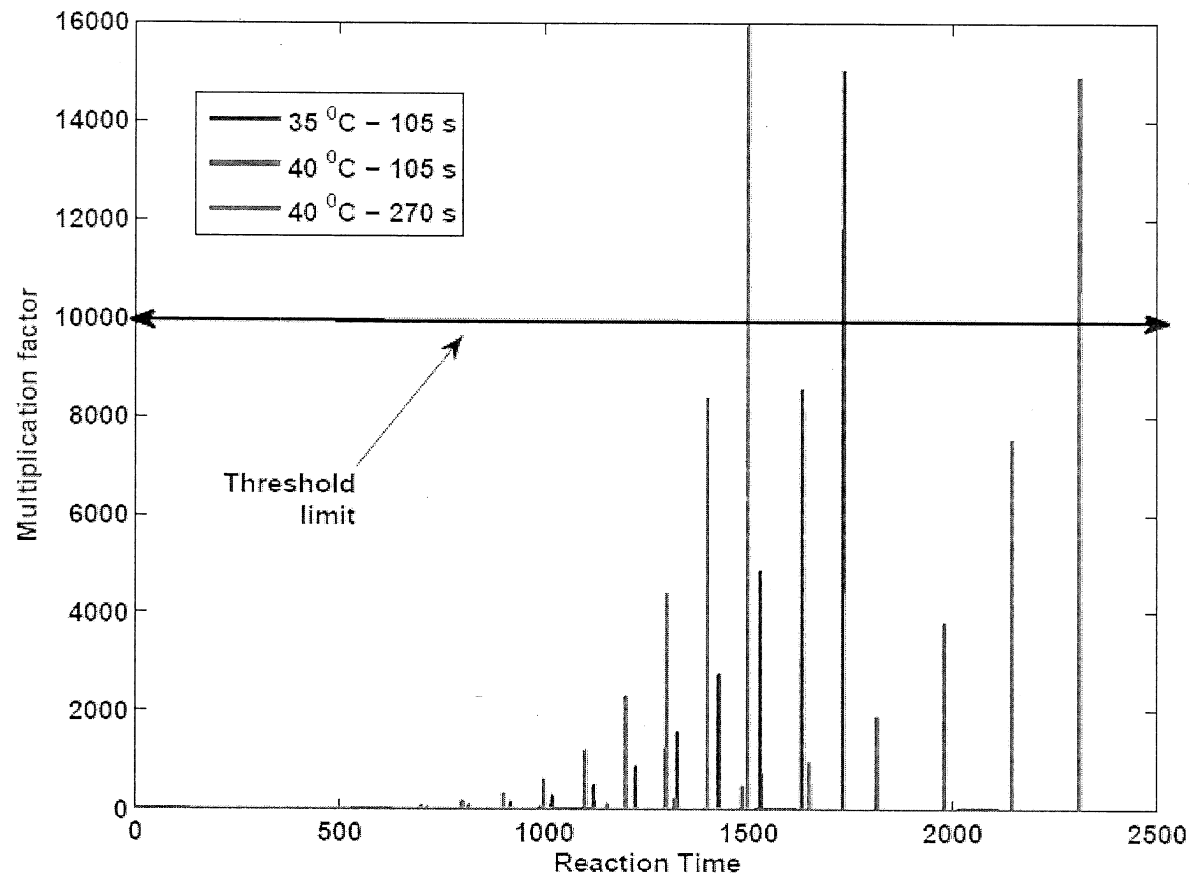
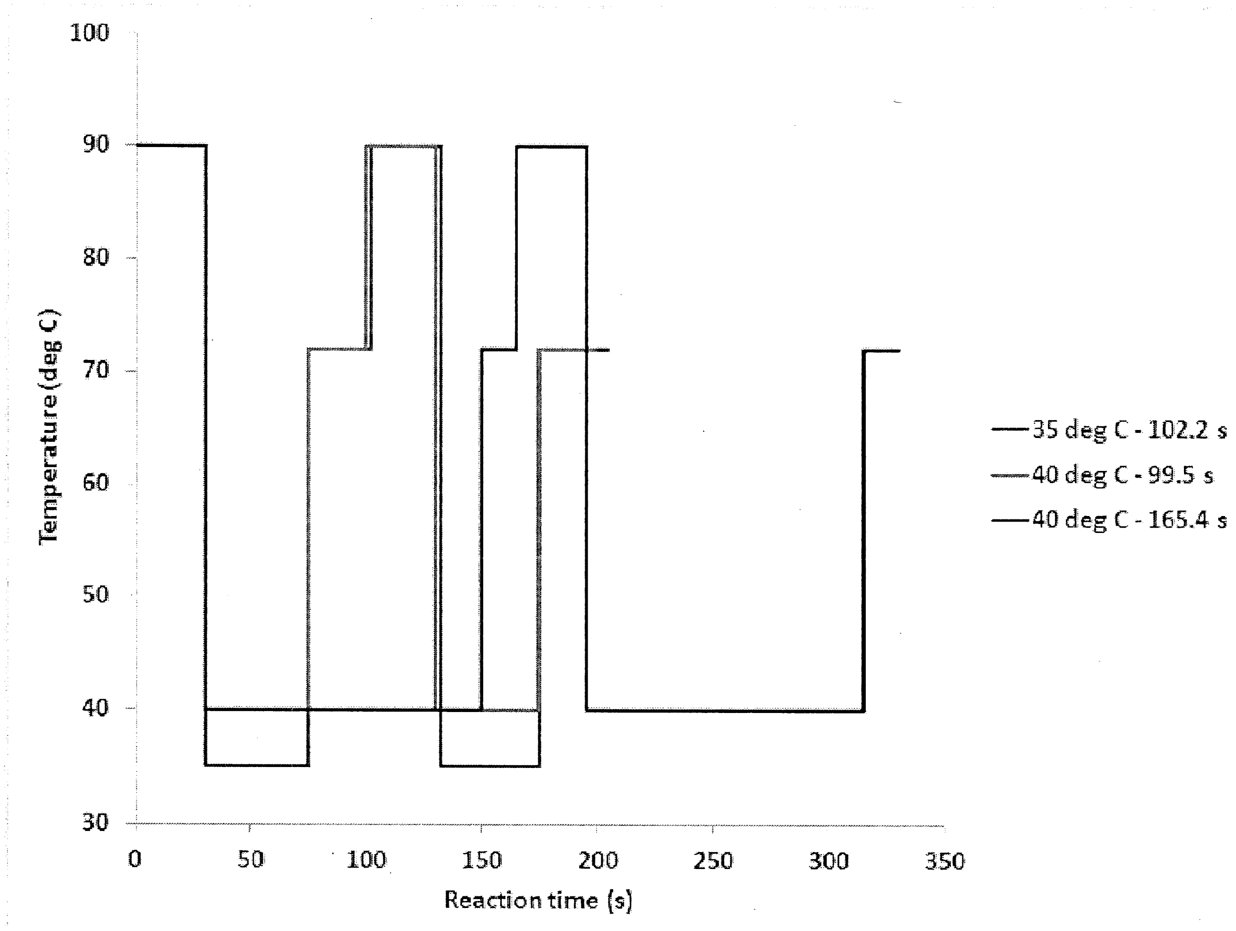

DNA - 30 °C
DNA - 35 °C
DNA - 40 °C
Sum(D_i) - 30 °C
Sum(D_i) - 35 °C
Sum(D_i) - 40 °C
Dimensionless DNA Concentration
Reaction time (s)
0
10
20
30
40
50
60
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2

FIGURE 14



35 °C – 105 s
40 °C – 105 s
40 °C – 270 s
Threshold
limit
Multiplication factor
Reaction Time
0
2000
4000
6000
8000
10000
12000
14000
16000
0
500
1000
1500
2000
2500

FIGURE 15



100
90
80
70
60
50
40
30
Temperature (deg C)
0
50
100
150
200
250
300
350
Reaction time (s)
35 deg C - 102.2 s
40 deg C - 99.5 s
40 deg C - 165.4 s

FIGURE 16

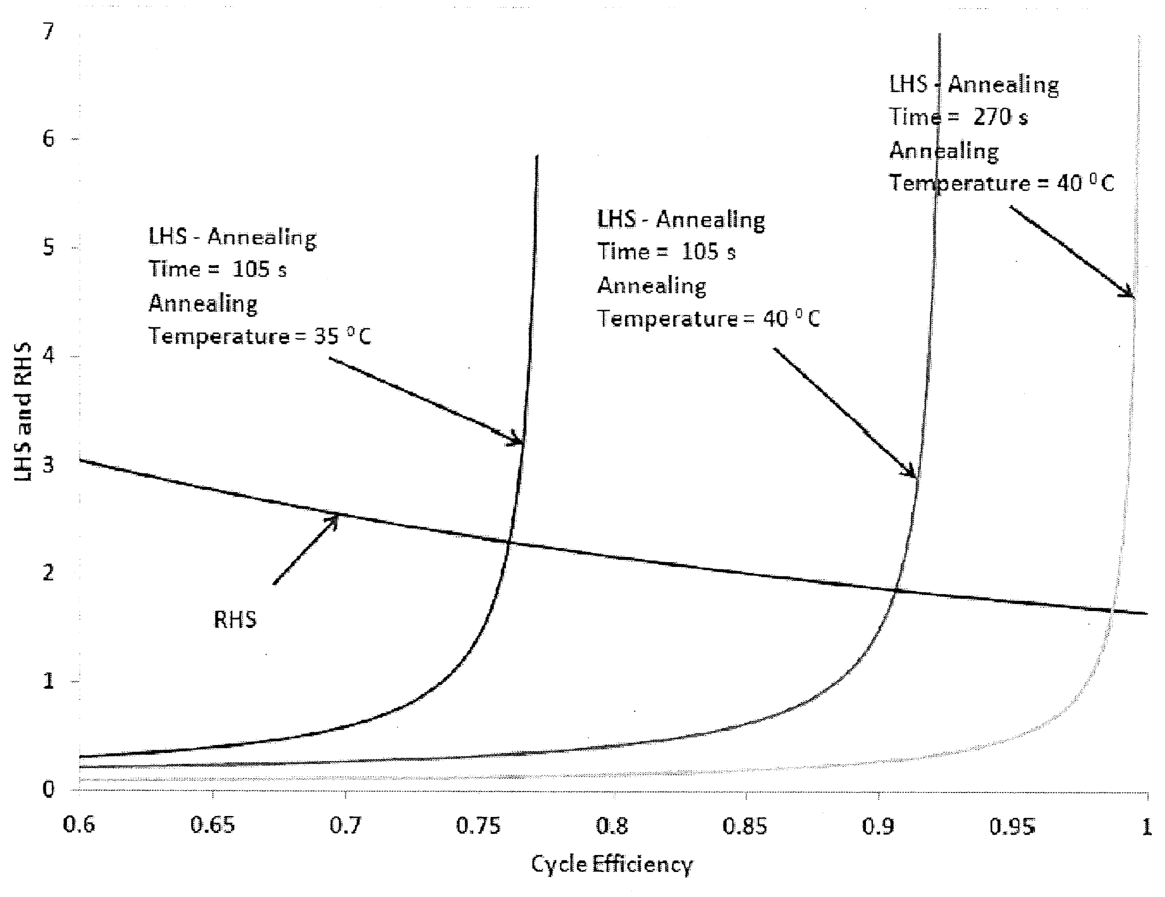

LHS - Annealing
Time = 105 s
Annealing
Temperature = 35 °C
LHS - Annealing
Time = 105 s
Annealing
Temperature = 40 °C
LHS - Annealing
Time = 270 s
Annealing
Temperature = 40 °C
RHS
LHS and RHS
Cycle Efficiency
0
1
2
3
4
5
6
7
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1

FIGURE 17

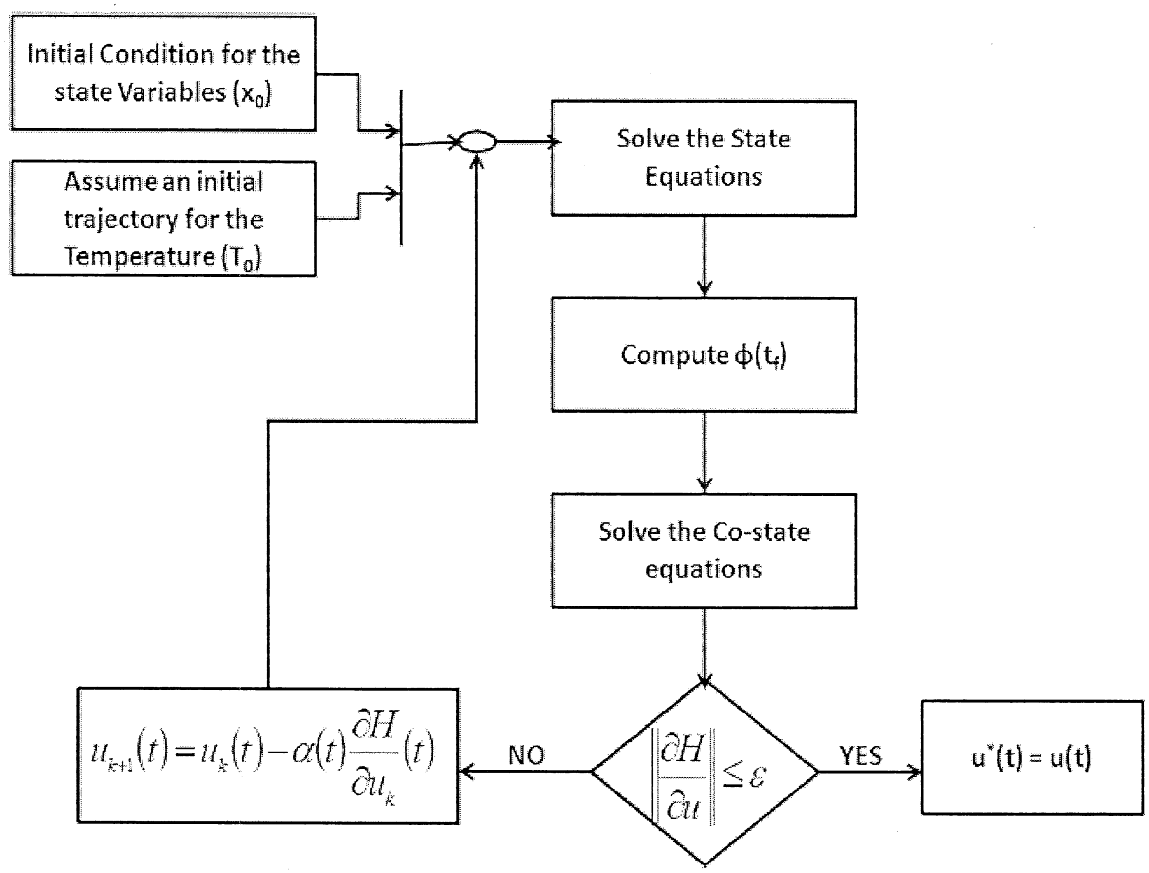
$$
\left.\begin{aligned}
g_1(x) &= [x_{15}\ 0\ 0\ 0\ 0\ 0\ 0\ x_{15}\ 0\ 0\ 0\ 0\ 0\ 0\ -x_{15}\ 0\ 0]^T \\
g_2(x) &= [-x_1x_8\ 0\ 0\ 0\ 0\ 0\ 0\ -x_1x_8\ 0\ 0\ 0\ 0\ 0\ 0\ x_1x_8\ 0\ 0]^T
\end{aligned}\right\}\text{sequence-dependent melting}
$$

$$
\left.\begin{aligned}
g_3(x) &= [-x_1x_2\ -x_1x_2\ x_1x_2\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0]^T \\
g_4(x) &= [x_3\ x_3\ -x_3\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0]^T \\
g_5(x) &= [0\ 0\ 0\ 0\ 0\ 0\ 0\ -x_8x_9\ -x_8x_9\ x_8x_9\ 0\ 0\ 0\ 0\ 0\ 0\ 0]^T \\
g_6(x) &= [0\ 0\ 0\ 0\ 0\ 0\ 0\ x_{10}\ x_{10}\ -x_{10}\ 0\ 0\ 0\ 0\ 0\ 0\ 0]^T
\end{aligned}\right\}\text{sequence-dependent annealing}
$$

$$
\left.\begin{aligned}
g_7(x) &= x_{17}[0\ 0\ -x_3\ x_3\ -x_5\ x_5\ 0\ 0\ 0\ 0\ -x_{10}\ x_{10}\ -x_{12}\ x_{12}\ 0\ 0\ -(x_3+x_5+x_{10}+x_{12})]^T \\
g_8(x) &= [0\ 0\ x_4\ -x_4\ x_6\ -x_6\ 0\ 0\ 0\ 0\ x_{11}\ -x_{11}\ x_{13}\ -x_{13}\ 0\ 0\ (x_4+x_6+x_{11}+x_{13})]^T
\end{aligned}\right\}\text{sequence-dependent enzyme binding}
$$

$$
\left.\begin{aligned}
g_9(x) &= x_{16}[0\ 0\ 0\ -x_4\ 0\ x_4-x_6\ x_6\ 0\ 0\ 0\ -x_{11}\ x_{11}-x_{13}\ x_{13}\ 0\ (x_4+x_6+x_{11}+x_{13})\ 0]^T \\
g_{10}(x) &= [0\ 0\ 0\ 0\ 0\ 0\ -x_7\ 0\ 0\ 0\ 0\ 0\ 0\ -x_{14}\ 0\ 0\ x_7+x_{14}]^T
\end{aligned}\right\}\text{extension}
$$

FIGURE 18



Initial Condition for the state Variables (x_0)
Assume an initial trajectory for the Temperature (T_0)
Solve the State Equations
Compute $\phi(t_f)$
Solve the Co-state equations
$\left\|\frac{\partial H}{\partial u}\right\| \leq \varepsilon$
NO
YES
$u_{k+1}(t) = u_k(t) - \alpha(t)\frac{\partial H}{\partial u_k}(t)$
$u^*(t) = u(t)$

FIGURE 19

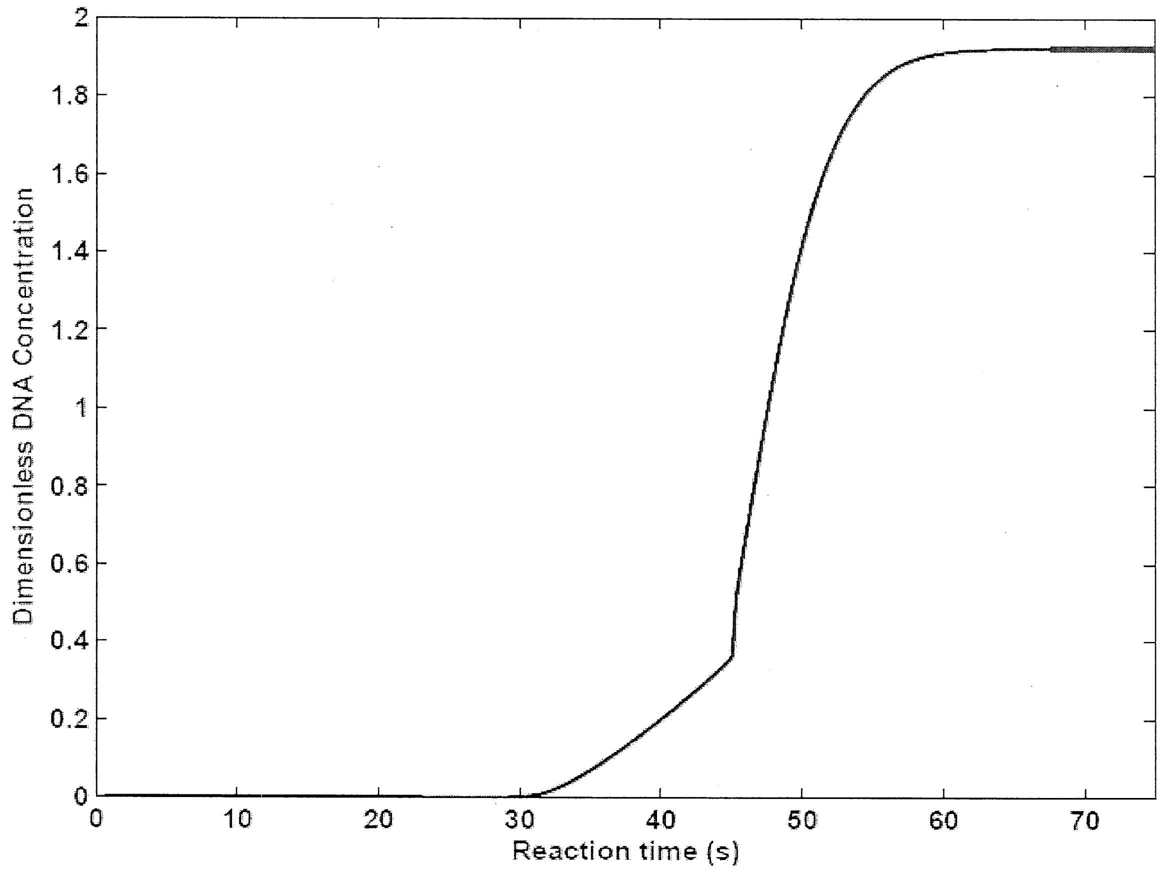

Dimensionless DNA Concentration
Reaction time (s)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
10
20
30
40
50
60
70

FIGURE 20

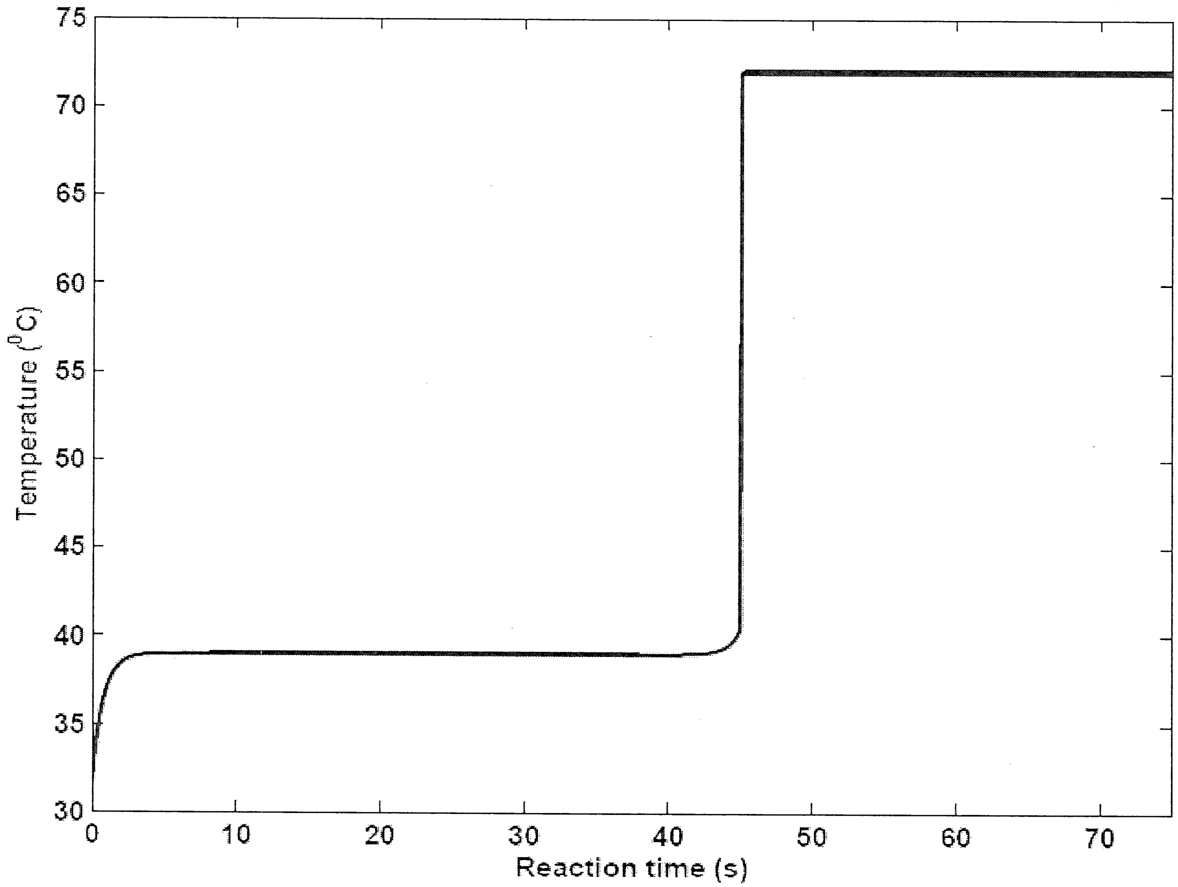
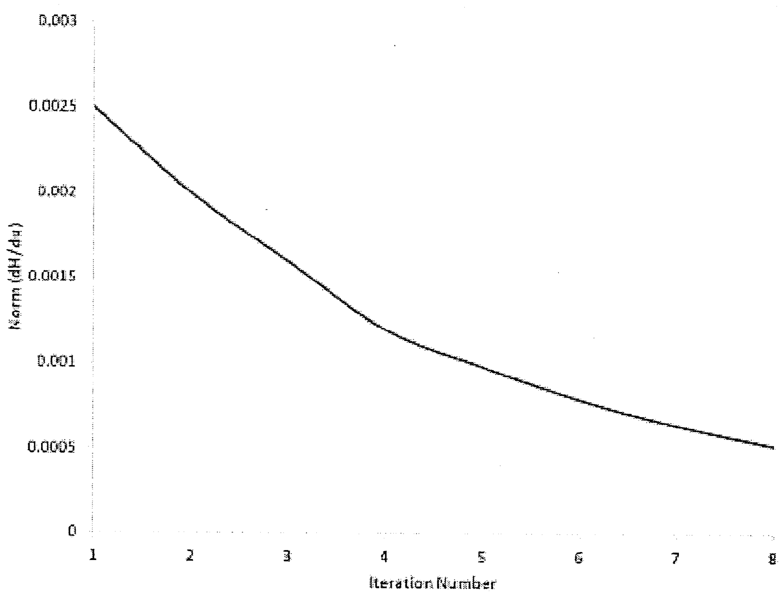
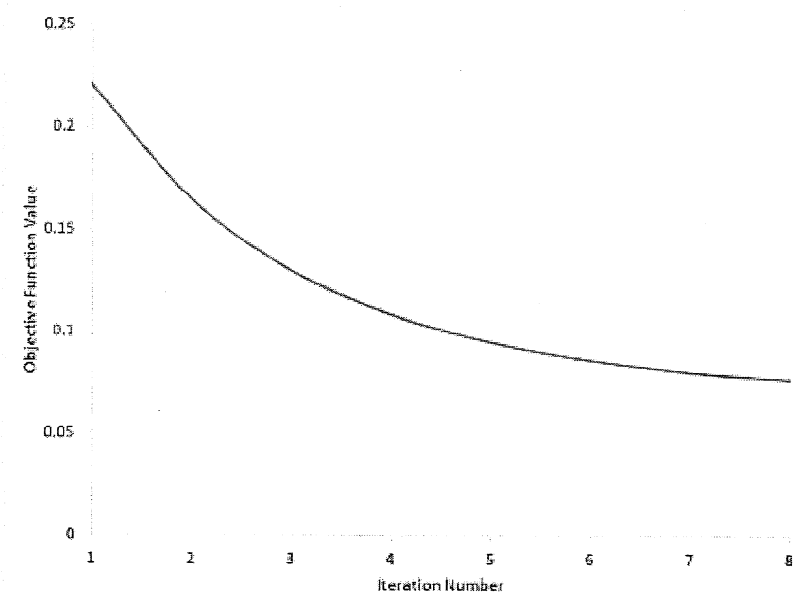

Temperature (^0C)
75
70
65
60
55
50
45
40
35
30
0
10
20
30
40
50
60
70
Reaction time (s)

FIGURE 21


0.003
0.0025
0.002
0.0015
0.001
0.0005
0
Norm (dH/du)
1
2
3
4
5
6
7
8
Iteration Number

(a)


0.25
0.2
0.15
0.1
0.05
0
Objective Function Value
1
2
3
4
5
6
7
8
Iteration Number

(b)

FIGURE 22

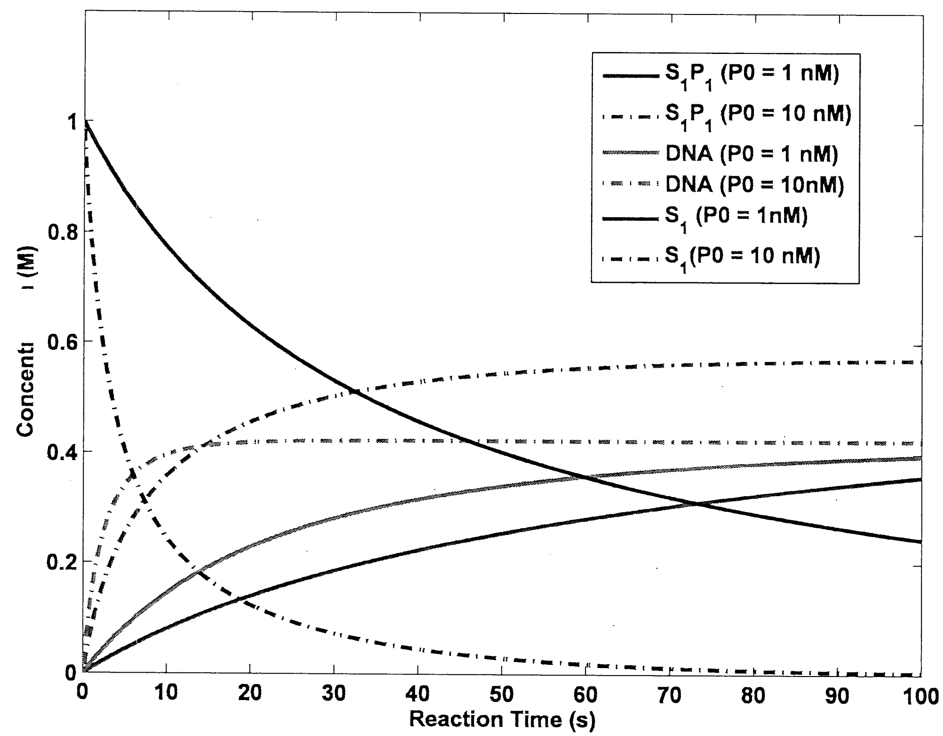


FIGURE 23. Predicted evolution of reaction species during a typical primer annealing reaction