Figure 1A

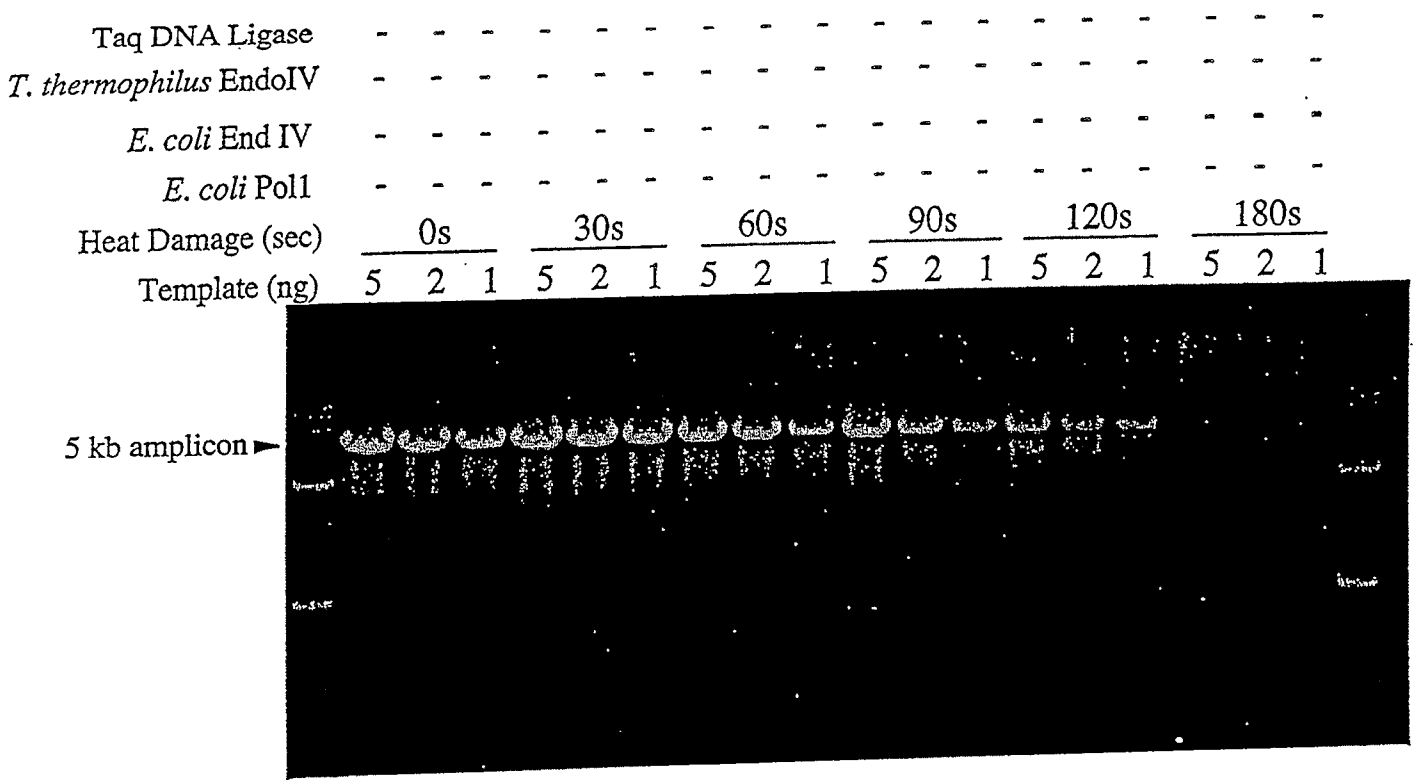

Taq DNA Ligase
T. thermophilus EndoIV
E. coli End IV
E. coli Pol1
Heat Damage (sec)
0s
30s
60s
90s
120s
180s
Template (ng)
5 2 1 5 2 1 5 2 1 5 2 1 5 2 1 5 2 1
5 kb amplicon

Figure 1B

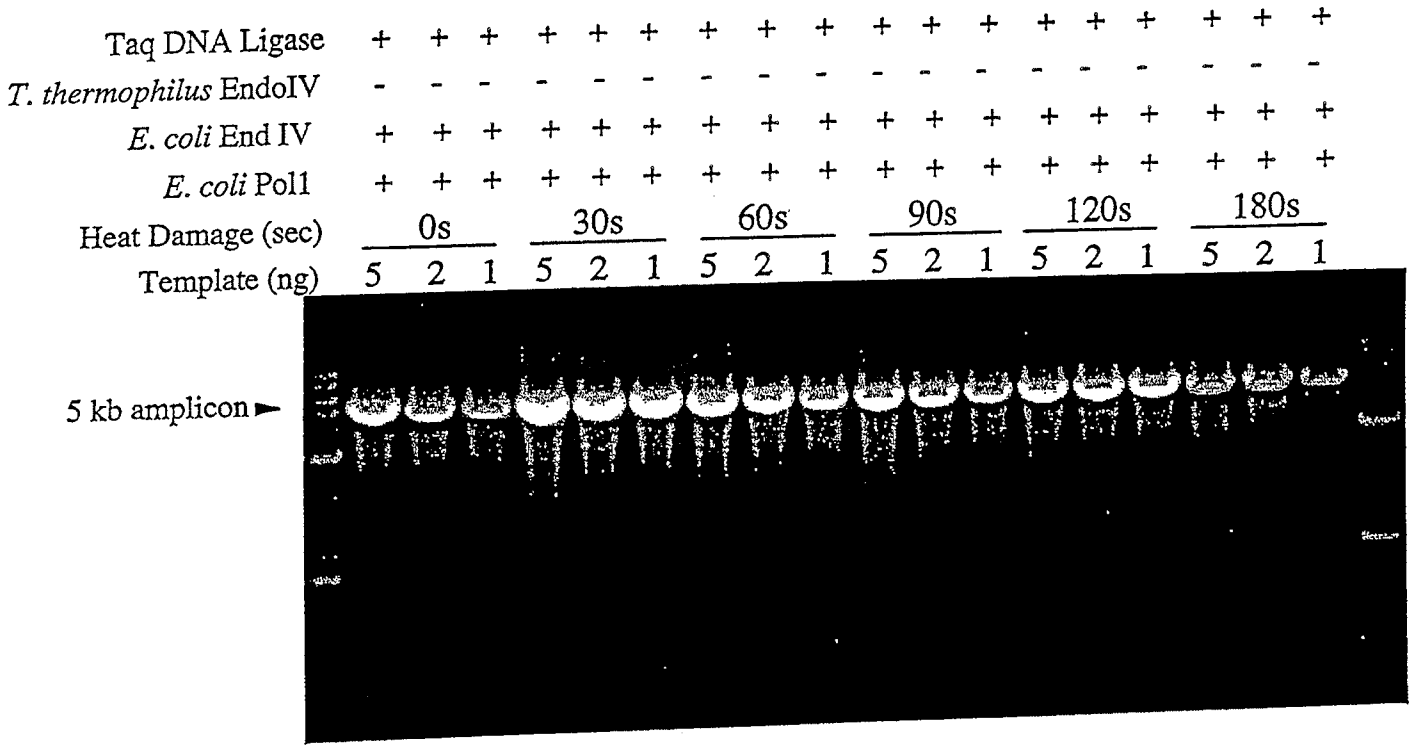

Taq DNA Ligase + + + + + + + + + + + + + + + + + +
T. thermophilus EndoIV - - - - - - - - - - - - - - - - - -
E. coli End IV + + + + + + + + + + + + + + + + + +
E. coli Pol1 + + + + + + + + + + + + + + + + + +
Heat Damage (sec) 0s 30s 60s 90s 120s 180s
Template (ng) 5 2 1 5 2 1 5 2 1 5 2 1 5 2 1 5 2 1
5 kb amplicon

Figure 1C

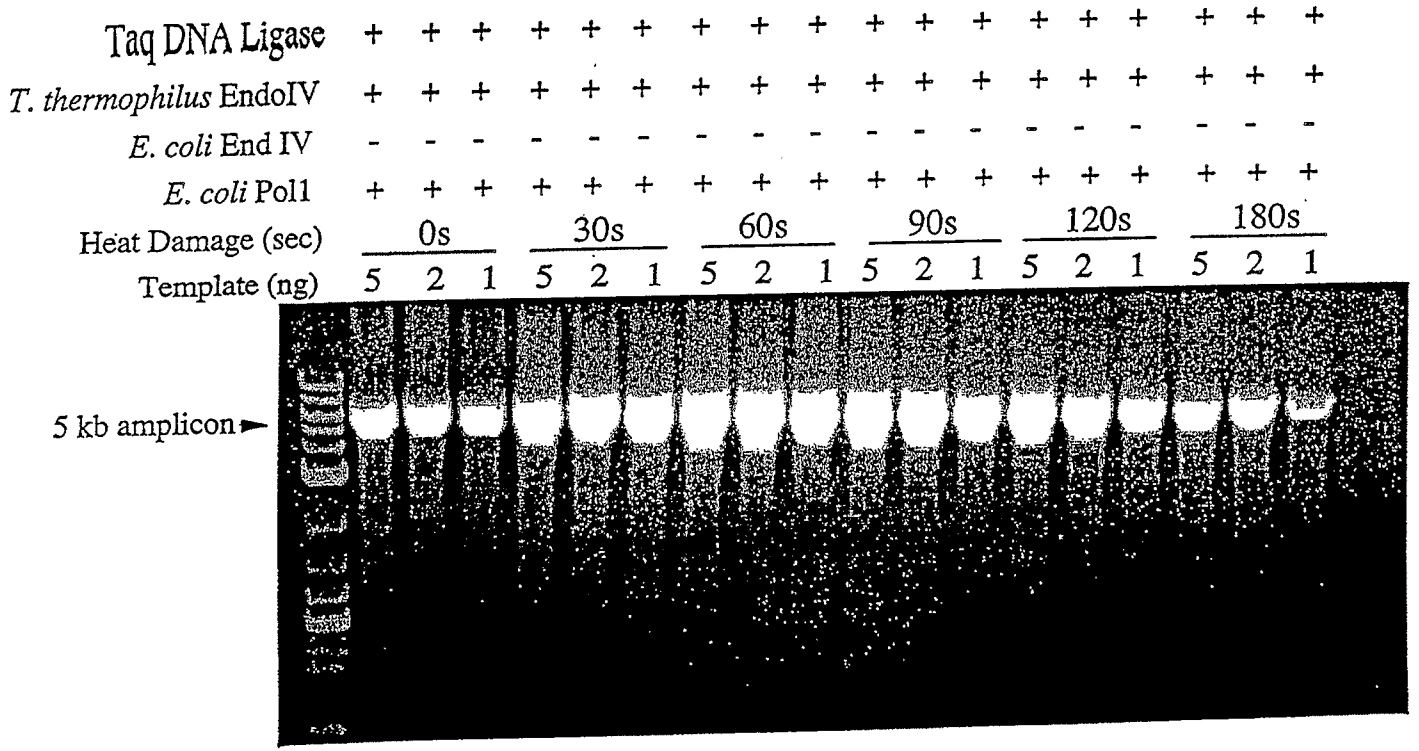

Taq DNA Ligase + + + + + + + + + + + + + + + + + +
T. thermophilus EndoIV + + + + + + + + + + + + + + + + + +
E. coli End IV - - - - - - - - - - - - - - - - - -
E. coli Pol1 + + + + + + + + + + + + + + + + + +
Heat Damage (sec) 0s 30s 60s 90s 120s 180s
Template (ng) 5 2 1 5 2 1 5 2 1 5 2 1 5 2 1 5 2 1
5 kb amplicon

Figure 1D

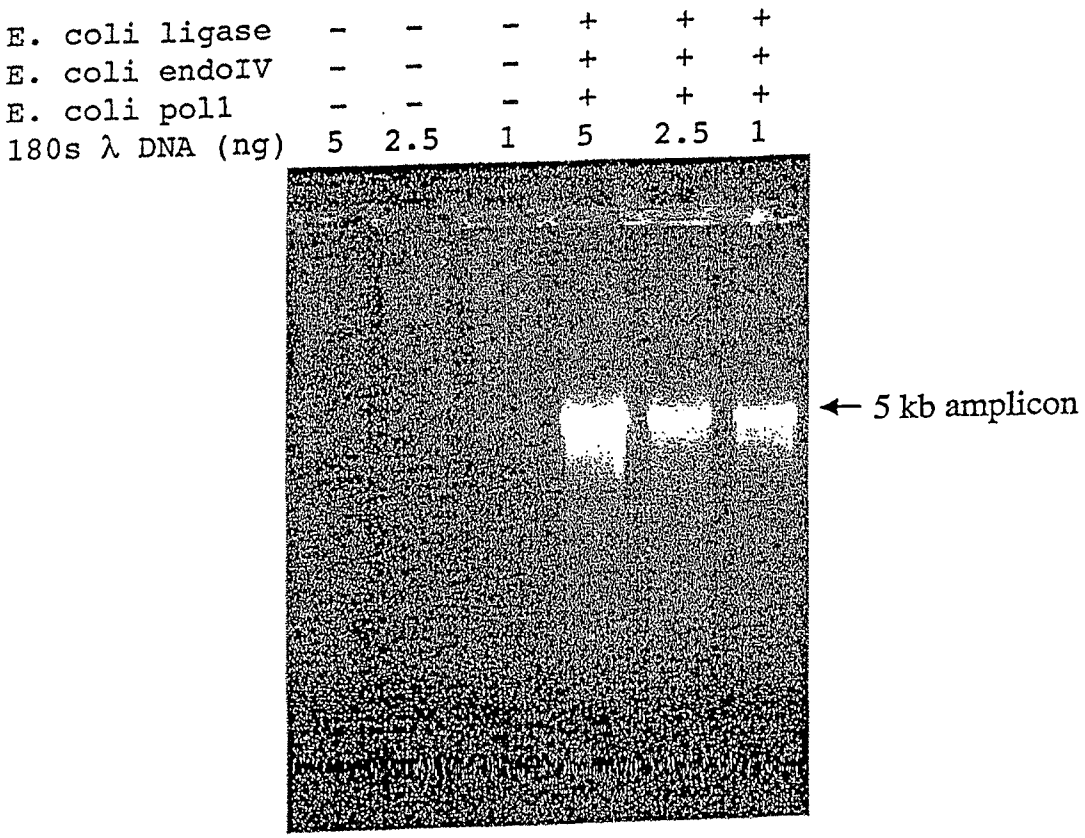

E. coli ligase - - - + + +
E. coli endoIV - - - + + +
E. coli pol1 - - - + + +
180s λ DNA (ng) 5 2.5 1 5 2.5 1
← 5 kb amplicon

Figure 2A

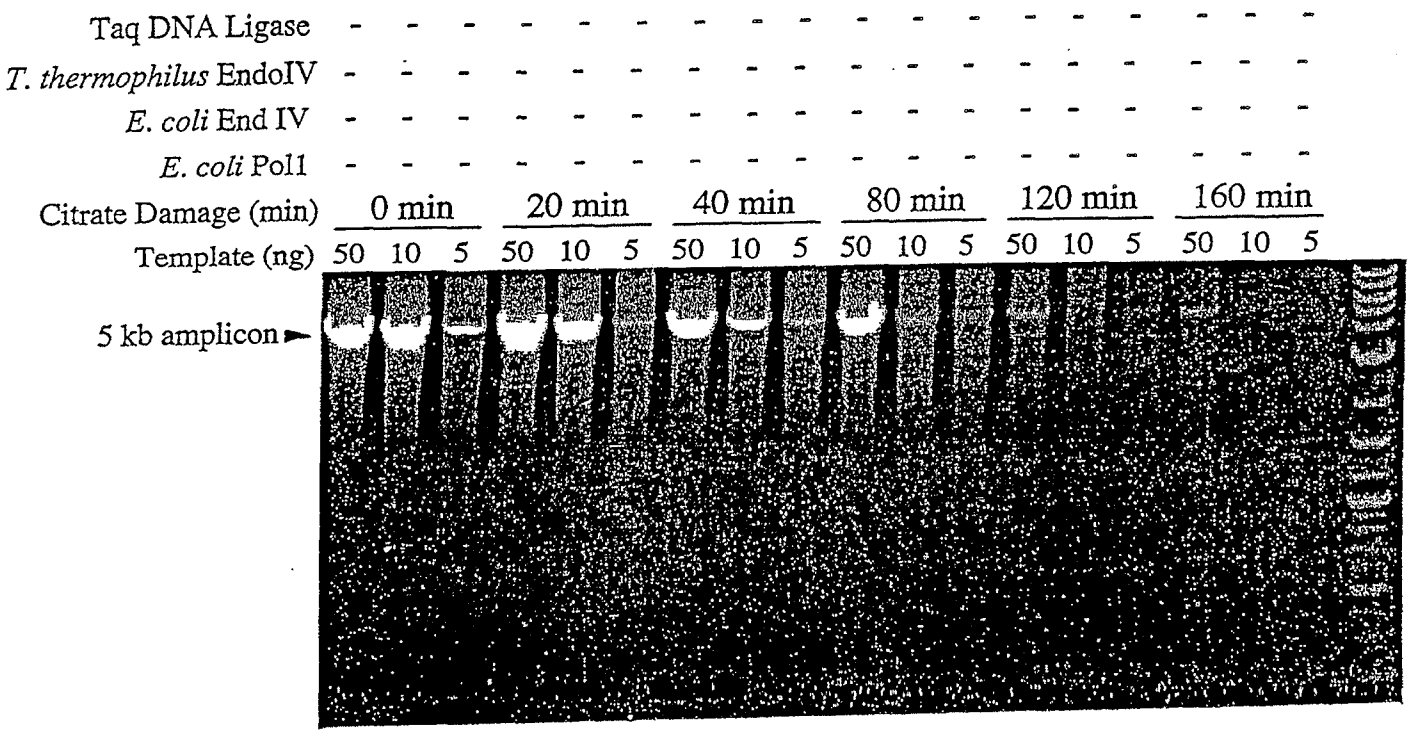

Taq DNA Ligase - - - - - - - - - - - - - - - - - -
T. thermophilus EndoIV - - - - - - - - - - - - - - - - - -
E. coli End IV - - - - - - - - - - - - - - - - - -
E. coli Pol1 - - - - - - - - - - - - - - - - - -
Citrate Damage (min) 0 min 20 min 40 min 80 min 120 min 160 min
Template (ng) 50 10 5 50 10 5 50 10 5 50 10 5 50 10 5 50 10 5
5 kb amplicon

Figure 2B

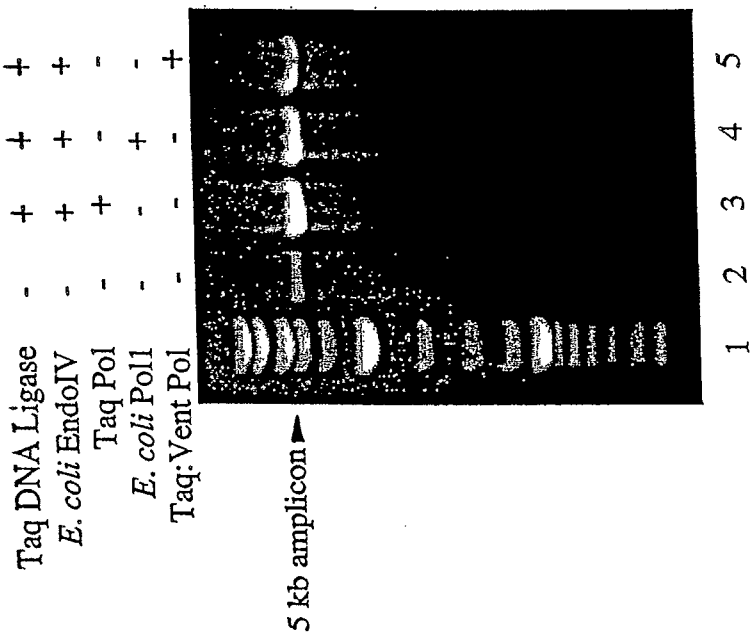

Taq DNA Ligase - + + +
E. coli EndoIV - + + +
Taq Pol - + - -
E. coli Pol1 - - + -
Taq:Vent Pol - - - +
5 kb amplicon
1 2 3 4 5

Figure 3

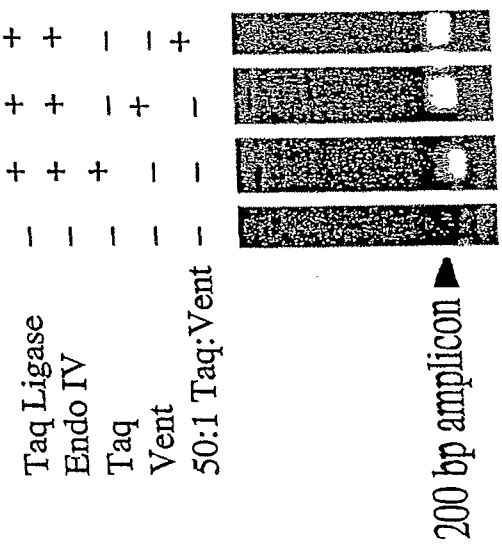


Figure 4

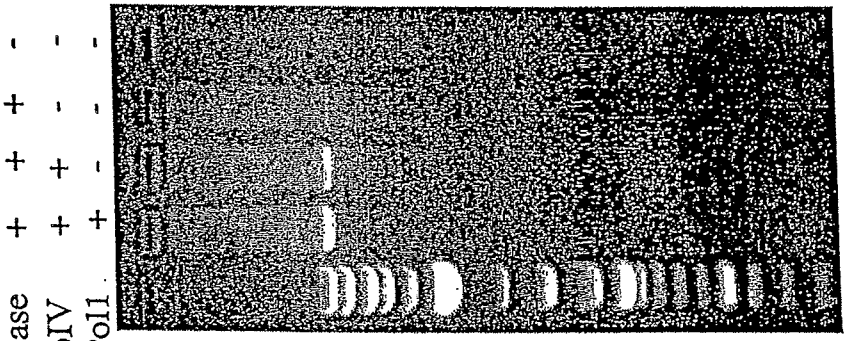


Figure 5

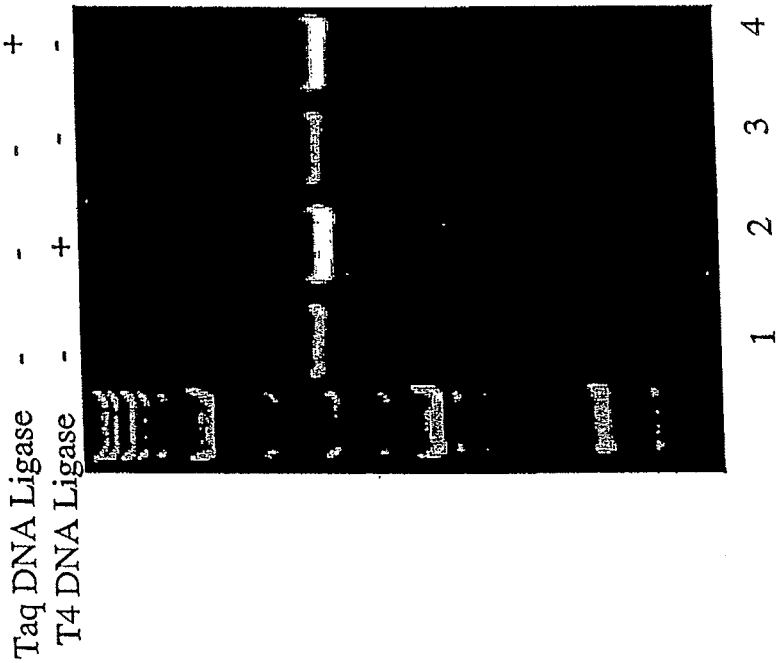


Figure 6-1

- 5 38 88 1092 >gi|2258283|gb|AAB63574.1| replication factor C like protein [Emericella nidulans]
+ 5 38 88 1092 >gi|27735373|gb|AAB63523.2| replication factor C like protein [Emericella nidulans]
+ 5 38 88 1092 >gi|40739497|gb|EAA58687.1| hypothetical protein AN6303.2 [Aspergillus nidulans FGSC A4]
+ 5 38 88 1092 >gi|49097960|ref|XP_410440.1| hypothetical protein AN6303.2 [Aspergillus nidulans FGSC A4]
+ 5 38 88 1092 >gi|7493935|pir||T18305 replication factor C like protein - Emericella nidulans
+ 5 38 88 1092 >gi|7493936|pir||T18306 replication factor C protein - Emericella nidulans
- 5 63 166 623 >gi|48141611|ref|XP_397246.1| similar to Activator 1 140 kDa subunit (Replication factor C large subunit) (Germline transcription factor 1) [Apis mellifera]
- 5 63 173 1051 >gi|31242853|ref|XP_321857.1| ENSANGP00000020306 [Anopheles gambiae]
- 5 75 214 952 >gi|49646861|emb|CAG83248.1| unnamed protein product [Yarrowia lipolytica CLIB99]
- 5 75 214 952 >gi|50547051|ref|XP_500995.1| hypothetical protein [Yarrowia lipolytica]
- 5 78 233 603 >gi|55234052|gb|EAA01207.3| ENSANGP00000020306 [Anopheles gambiae str. PEST]
- 5 102 307 1147 >gi|2827257|gb|AAB99788.1| DNA binding protein [Homo sapiens]
+ 5 102 307 1147 >gi|296908|emb|CAA80355.1| PO-GA [Homo sapiens]
+ 5 102 307 1147 >gi|30354564|gb|AAH51751.1| Replication factor C large subunit [Homo sapiens]
- 5 102 307 1147 >gi|32528306|ref|NP_002904.3| replication factor C large subunit [Homo sapiens]
+ 5 102 307 1147 >gi|422807|pir||JN0599 DNA-binding protein PO-GA - human
+ 5 102 307 1147 >gi|46430941|gb|AAS94325.1| replication factor C (activator 1) 1, 145kDa [Homo sapiens]
+ 5 102 307 1147 >gi|53972062|gb|AAV20814.1| Sequence 1074 from patent US 6753314
- 5 102 307 1148 >gi|2136100|pir||A49651 replication factor C large subunit - human
- 5 102 307 1148 >gi|30353858|gb|AAH51786.1| RFC1 protein [Homo sapiens]
+ 5 102 307 1148 >gi|410218|gb|AAA16121.1| replication factor C large subunit
- 5 102 307 1148 >gi|52632416|gb|AAH35297.1| Replication factor C large subunit [Homo sapiens]

Figure 6-2

- 5 102 307 1148 >gi|56757608|sp|P35251|RFC1_HUMAN Activator 1 140 kDa subunit (Replication factor C large subunit) (A1 140 kDa subunit) (RF-C 140 kDa subunit) (Activator 1 large subunit) (DNA-binding protein PO-GA)
- 5 102 357 748 >gi|14591755|ref|NP_143722.1| ERCC4-like helicase/ERCC4-type nuclease [Pyrococcus horikoshii OT3]
- 5 125 416 1001 >gi|50255468|gb|EAL18203.1| hypothetical protein CNBK2210 [Cryptococcus neoformans var. neoformans B-3501A]
- 5 153 672 636 >gi|55416925|gb|AAV50575.1| NAD-dependent DNA ligase [Mimivirus]
- 5 153 672 636 >gi|55819181|ref|YP_142657.1| NAD-dependent DNA ligase [Mimivirus]
- 5 159 540 656 >gi|48837044|ref|ZP_00294039.1| COG0322: Nuclease subunit of the excinuclease complex [Thermobifida fusca]
- 5 162 568 1084 >gi|38105148|gb|EAA51609.1| hypothetical protein MG03204.4 [Magnaporthe grisea 70-15]
+ 5 162 568 1084 >gi|39942248|ref|XP_360661.1| hypothetical protein MG03204.4 [Magnaporthe grisea 70-15]
- 5 185 653 679 >gi|50875895|emb|CAG35735.1| related to NAD-dependent DNA ligase [Desulfotalea psychrophila LSv54]
+ 5 185 653 679 >gi|51244858|ref|YP_064742.1| related to NAD-dependent DNA ligase [Desulfotalea psychrophila LSv54]
- 5 192 735 860 >gi|49654048|emb|CAG86464.1| unnamed protein product [Debaryomyces hansenii CBS767]
+ 5 192 735 860 >gi|50419709|ref|XP_458382.1| unnamed protein product [Debaryomyces hansenii]
- 5 220 688 679 >gi|14089803|emb|CAC13562.1| DNA LIGASE (POLYDEOXYRIBONUCLEOTIDE SYNTHASE [NAD+]) [Mycoplasma pulmonis]
- 5 220 688 679 >gi|15828860|ref|NP_326220.1| DNA LIGASE (POLYDEOXYRIBONUCLEOTIDE SYNTHASE [NAD+]) [Mycoplasma pulmonis UAB CTIP]
+ 5 220 688 679 >gi|25293995|pir||E90560 hypothetical protein MYPU_3890 [imported] - Mycoplasma pulmonis (strain UAB CTIP)
- 5 226 749 823 >gi|15639622|ref|NP_219072.1| DNA ligase (lig) [Treponema pallidum subsp. pallidum str. Nichols]
+ 5 226 749 823 >gi|3322933|gb|AAC65609.1| DNA ligase (lig) [Treponema pallidum subsp. pallidum str. Nichols]
- 5 226 749 823 >gi|6014987|sp|O83642|DNLJ_TREPA DNA ligase (Polydeoxyribonucleotide synthase [NAD+])
- 5 226 749 823 >gi|7521016|pir||H71300 probable DNA ligase (lig) - syphilis spirochete
- 5 230 678 660 >gi|15594897|ref|NP_212686.1| DNA ligase (lig)

Figure 6-3

[Borrelia burgdorferi B31]
+ 5 230 678 660 >gi|2688477|gb|AAC66923.1| DNA ligase (lig) [Borrelia burgdorferi B31]
+ 5 230 678 660 >gi|7428521|pir||G70168 DNA ligase (NAD) (EC 6.5.1.2) - Lyme disease spirochete
- 5 230 678 660 >gi|7673988|sp|O51502|DNLJ_BORBU DNA ligase (Polydeoxyribonucleotide synthase [NAD+])
- 5 236 938 867 >gi|28376351|gb|AA04-1087.1| major surface protein 3 [Anaplasma marginale]
- 5 457 1834 984 >gi|14325803|gb|AAK60014.1| topoisomerase V [Methanopyrus kandleri]
- 5 457 1834 984 >gi|19888102|gb|AAM02649.1| Topoisomerase V [Methanopyrus kandleri AV19]
- 5 457 1834 984 >gi|20094872|ref|NP_614719.1| Topoisomerase V [Methanopyrus kandleri AV19]

Figure 7 (SEQ ID NO:11)

atgccgcgct acgggttcca cctttccatc gccgggaaaa aggcgtggc

cggggcggtg gaggaggcca ccgccctcgg cctcaccgct ttccagatct

tcgccaaaag cccgcggagc tggcgcccaa gggccctctc ccggccgag

gtggaggcct tccgcgcctt aagggaggcc tccgggggcc tccccgccgt

gatccacgcc tcctacctgg tcaacctggg ggcggagggg gagctttggg

agaagagcgt ggcgagcctg gcggacgacc tggagaaggc cgccctcctc

ggggtggagt acgtggtcgt ccaccccggc tcgggccgcc ccgagcgggt

caaggaaggg gccctcaagg ccctgcgcct cgccggcgtc cgctcccgcc

ccgtcctcct cgtggagaac accgccgggg gcggggagaa ggtgggggcg

cggtttgagg agctcgcctg gctcgtggcg gacacccccc tccaggtctg

cctggacacc tgccacgcct acgccgccgg gtacgacgtg gccgaggacc

ccttgggggt cctggacgcc ctggaccggg ccgtgggcct ggagcgggtg

cccgtggtcc acctcaacga ctccgtgggc ggcctcggaa gccgcgtgga

ccaccacgcc cacctcctcc agggaaagat cggggagggg ctcaagcgcg

tcttcttgga cccgaggctc aaggaccggg tcttcatcct ggaaaccccc

aggggaccgg aggaggacgc ctggaacctc cgggtcctca gggcctggct

cgaggaggcc taa

Figure 8

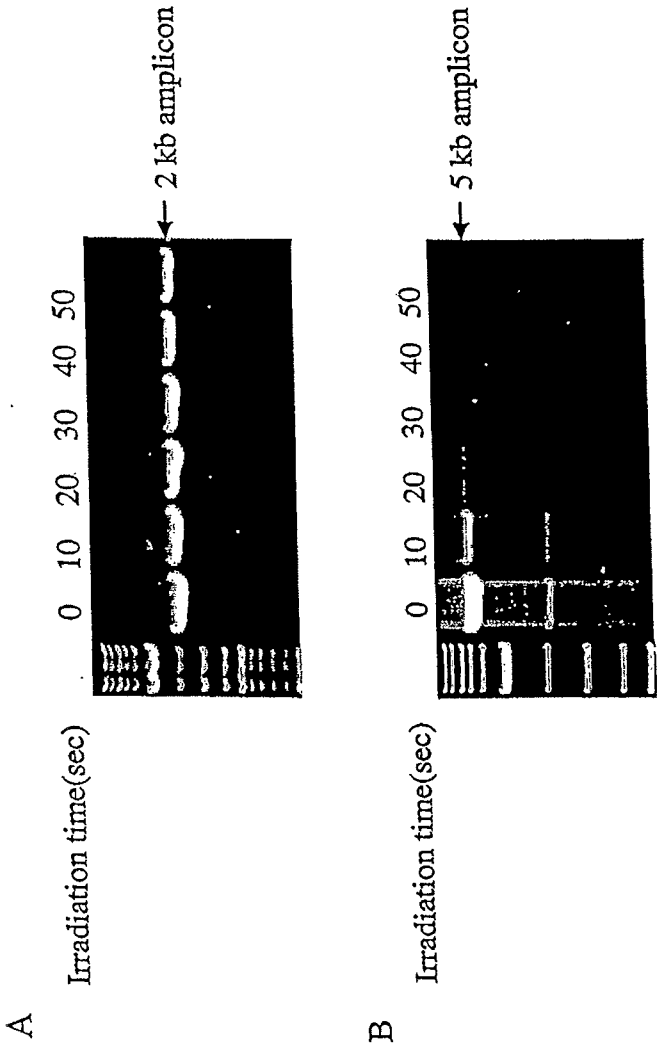

A
Irradiation time(sec)
0 10 20 30 40 50
2 kb amplicon
B
Irradiation time(sec)
0 10 20 30 40 50
5 kb amplicon

Figure 8

C

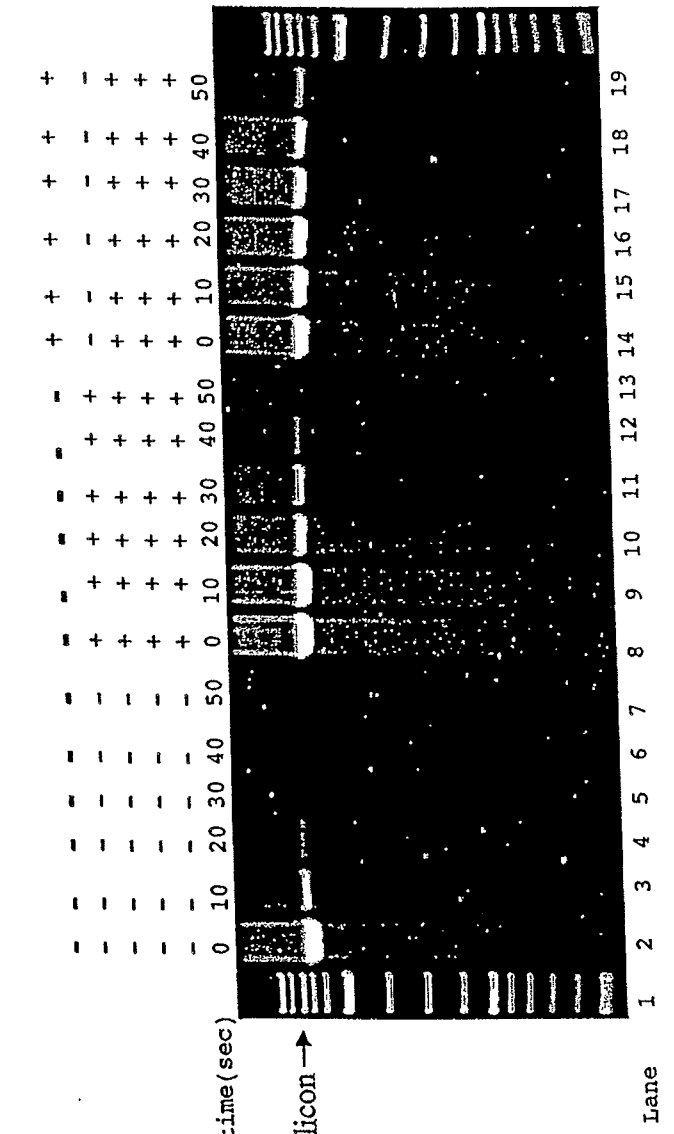
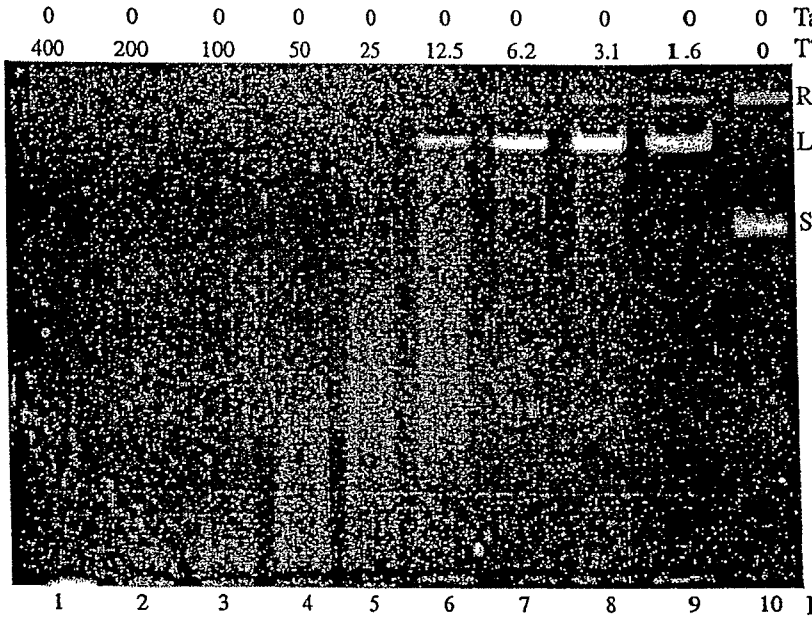

T4 pdg 80U
T4 pdg 10U
Taq Ligase
Endo IV
Pol I
Irradiation time(sec)
0 10 20 30 40 50 0 10 20 30 40 50 0 10 20 30 40 50
5 kb amplicon
Lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Figure 9

A


0 0 0 0 0 0 0 0 0 0 Taq DNA Ligase (units)
400 200 100 50 25 12.5 6.2 3.1 1.6 0 T7 endo I (units)
Relaxed Circular
Linear
Supercoiled Circular
1 2 3 4 5 6 7 8 9 10 Lane

B

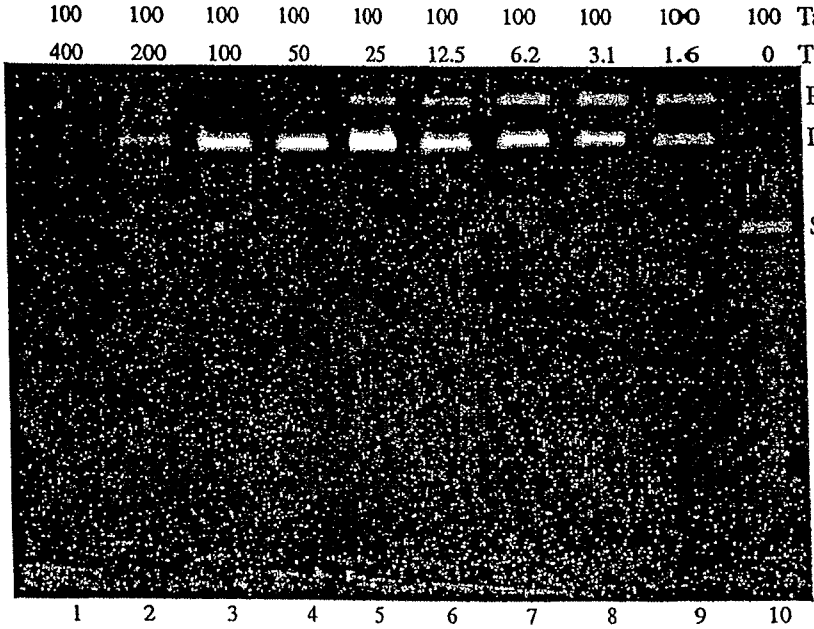

100 100 100 100 100 100 100 100 100 100 Taq DNA Ligase (units)
400 200 100 50 25 12.5 6.2 3.1 1.6 0 T7 endo I (units)
Relaxed Circular
Linear
Supercoiled Circular
1 2 3 4 5 6 7 8 9 10 Lane

Figure 10

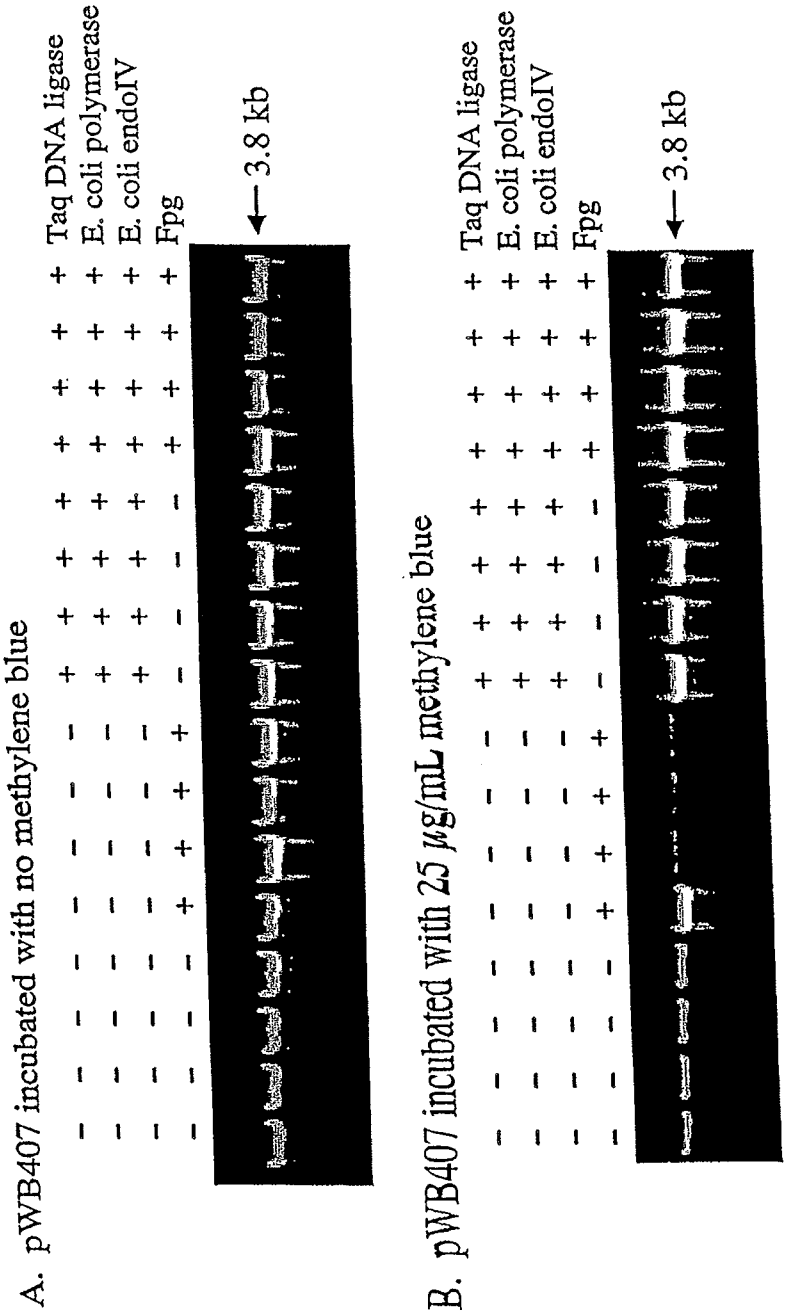

A. pWB407 incubated with no methylene blue
Taq DNA ligase
E. coli polymerase
E. coli endoIV
Fpg
3.8 kb
B. pWB407 incubated with 25 μg/mL methylene blue
Taq DNA ligase
E. coli polymerase
E. coli endoIV
Fpg
3.8 kb

Figure 11

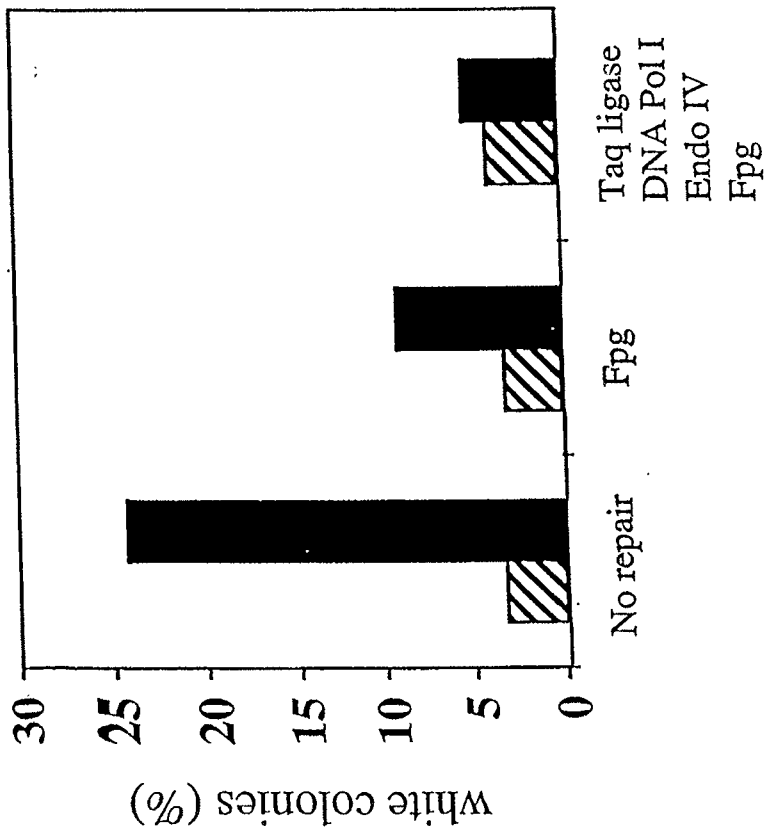
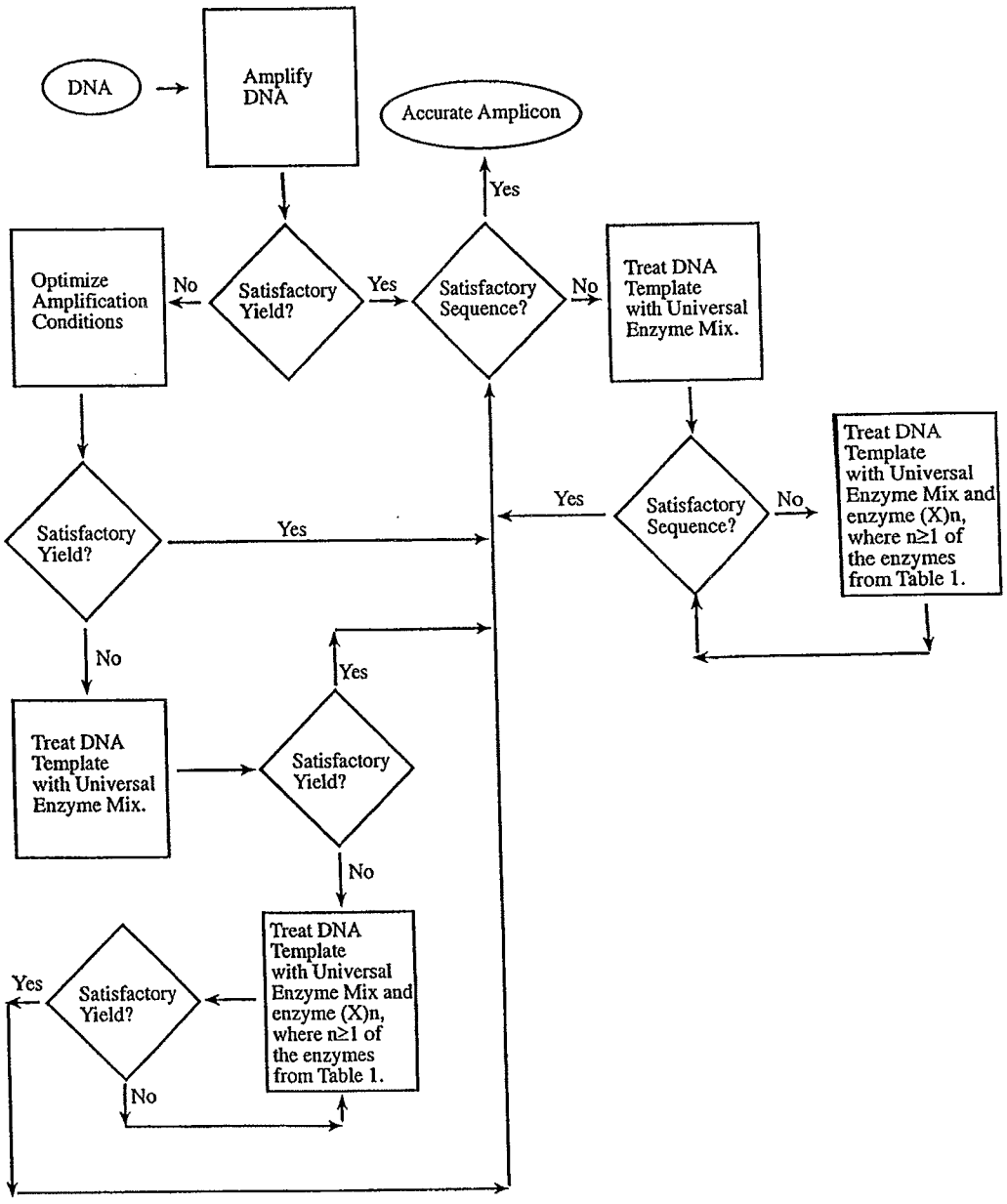

0 μg/mL Methylene Blue
25 μg/mL Methylene Blue
white colonies (%)
30
25
20
15
10
5
0
No repair
Fpg
Taq ligase
DNA Pol I
Endo IV
Fpg

Figure 12


DNA
Amplify DNA
Accurate Amplicon
Yes
Optimize Amplification Conditions
No
Satisfactory Yield?
Yes
Satisfactory Sequence?
No
Treat DNA Template with Universal Enzyme Mix.
Satisfactory Yield?
Yes
Yes
Satisfactory Sequence?
No
Treat DNA Template with Universal Enzyme Mix and enzyme (X)n, where n≥1 of the enzymes from Table 1.
No
Yes
Treat DNA Template with Universal Enzyme Mix.
Satisfactory Yield?
No
Yes
Satisfactory Yield?
Treat DNA Template with Universal Enzyme Mix and enzyme (X)n, where n≥1 of the enzymes from Table 1.
No