FIGURE 1

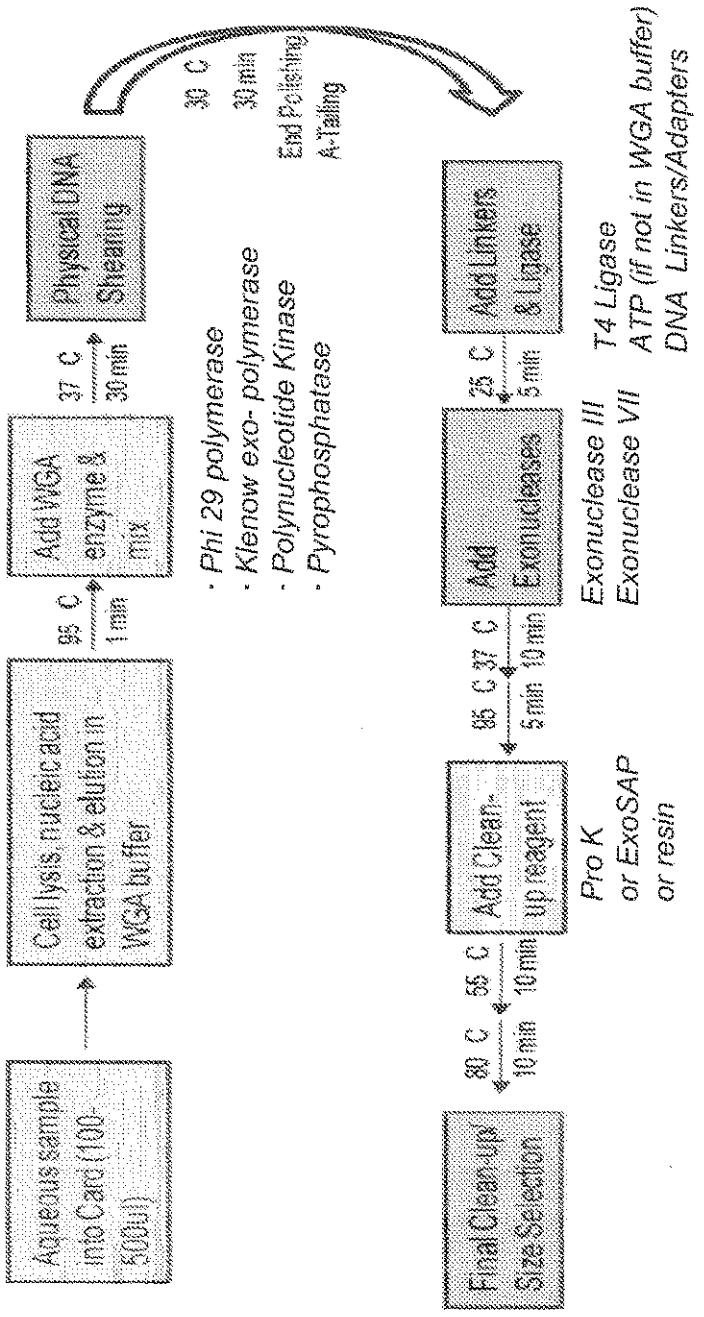

Aqueous sample into Carc (100-500ul)
Cell lysis, nucleic acid extraction & elution in WGA buffer
95 C 1 min
Add WGA enzyme & mix
37 C 30 min
Physical DNA Shearing
30 C 30 min
End Polishing A-Tailing
· Phi 29 polymerase
· Klenow exo- polymerase
· Polynucleotide Kinase
· Pyrophosphatase
Add Linkers & Ligase
25 C 5 min
T4 Ligase
ATP (if not in WGA buffer)
DNA Linkers/Adapters
Add Exonucleases
95 C 37 C 5 min 10 min
Exonuclease III
Exonuclease VII
Add Clean-up reagent
80 C 10 min
55 C 10 min
Pro K
or ExoSAP
or resin
Final Clean-up Size Selection

FIGURE 2

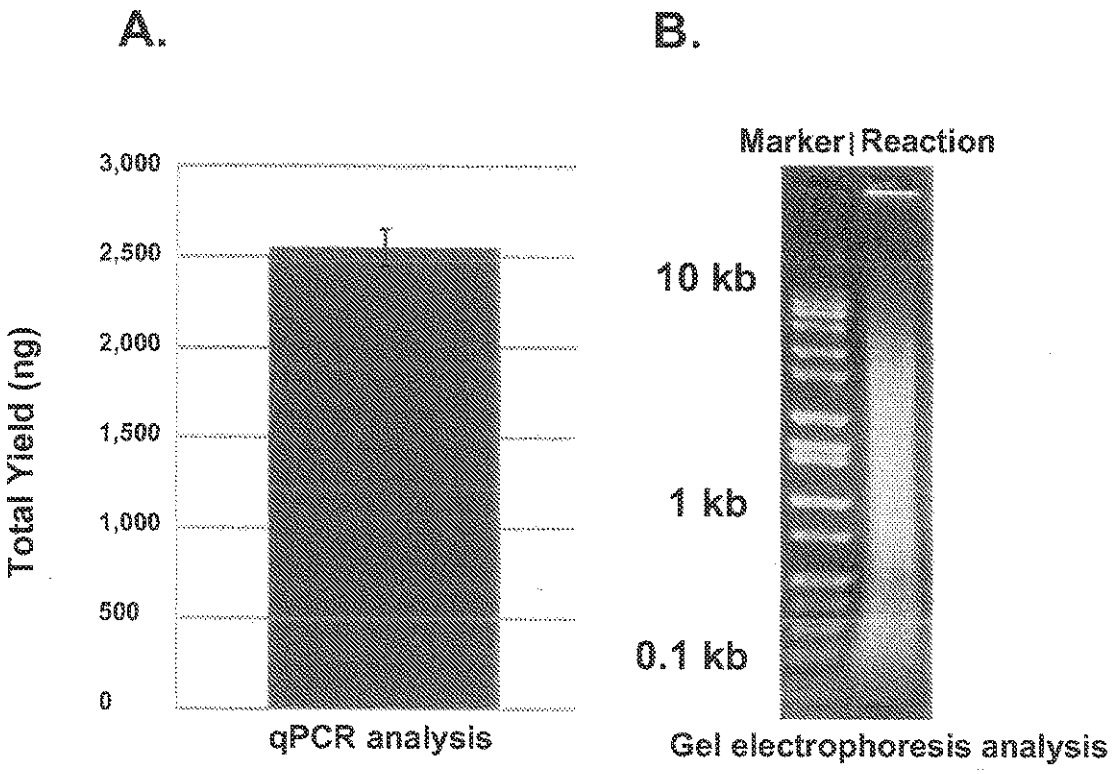

A.
B.
Total Yield (ng)
3,000
2,500
2,000
1,500
1,000
500
0
qPCR analysis
Marker
Reaction
10 kb
1 kb
0.1 kb
Gel electrophoresis analysis

FIGURE 3

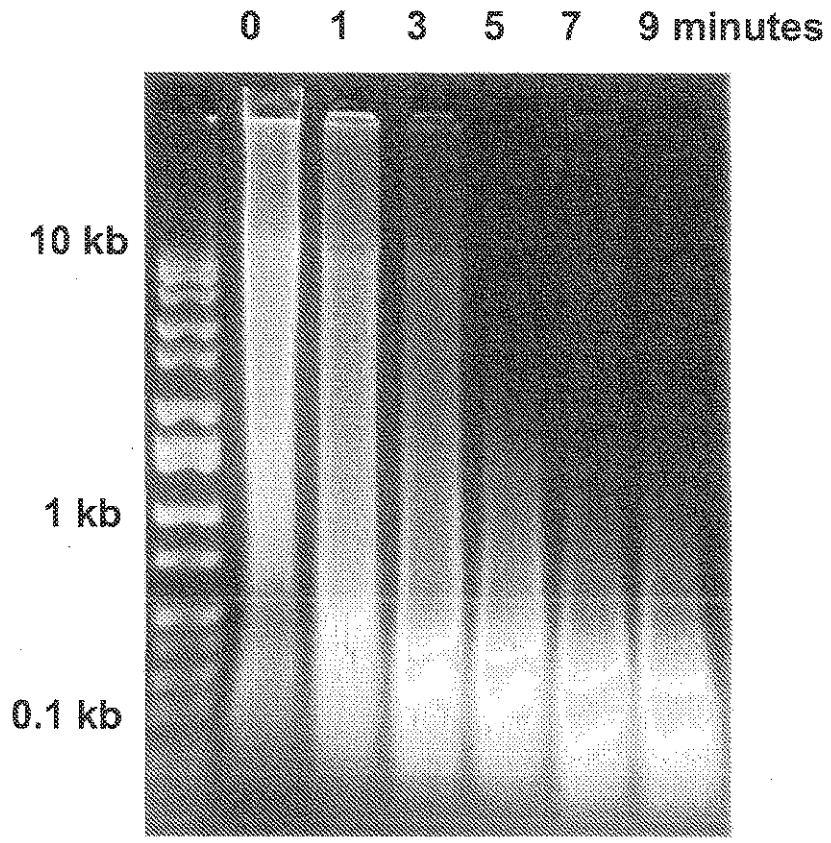


FIGURE 4

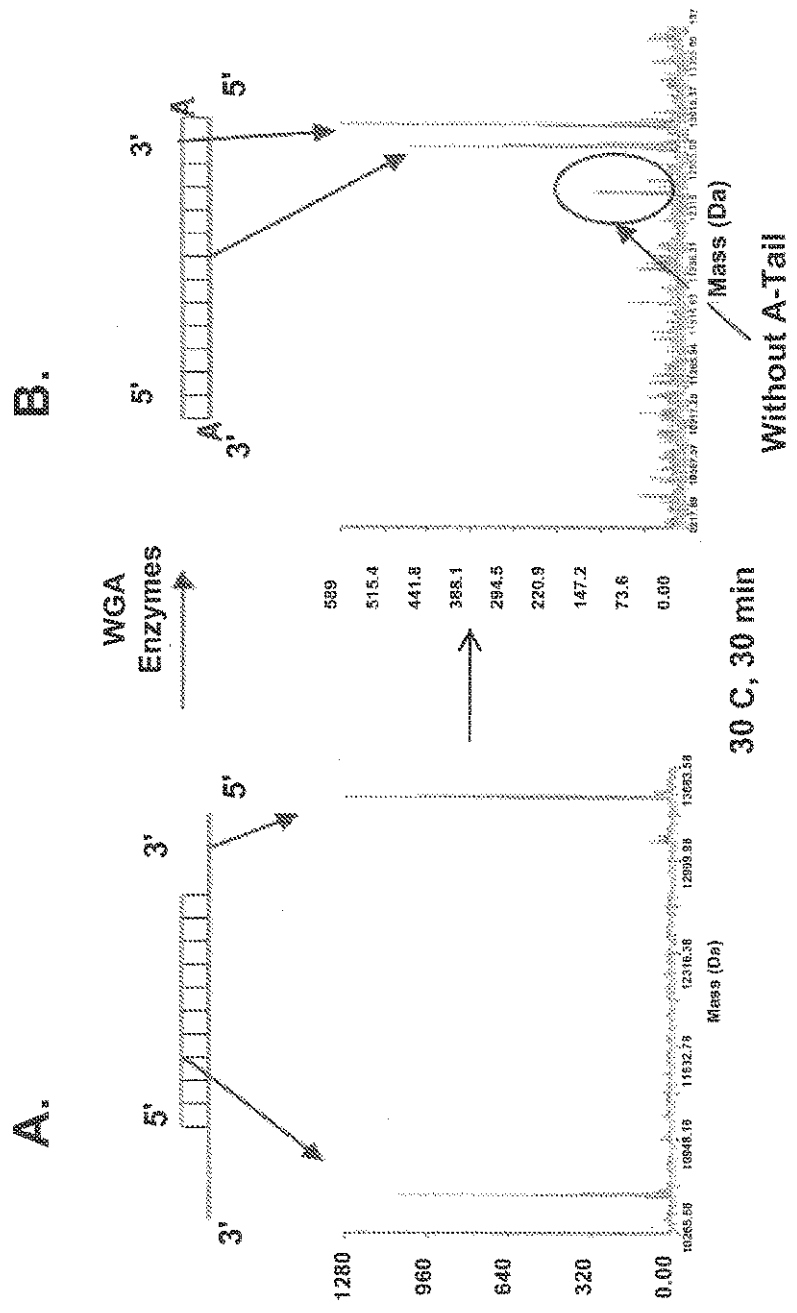

A.
B.
WGA Enzymes
30 C, 30 min
Without A-Tail
Mass (Da)
5'
3'
1280
960
640
320
0.00
589
515.4
441.8
368.1
294.5
220.9
147.2
73.6
0.00

FIGURE 5

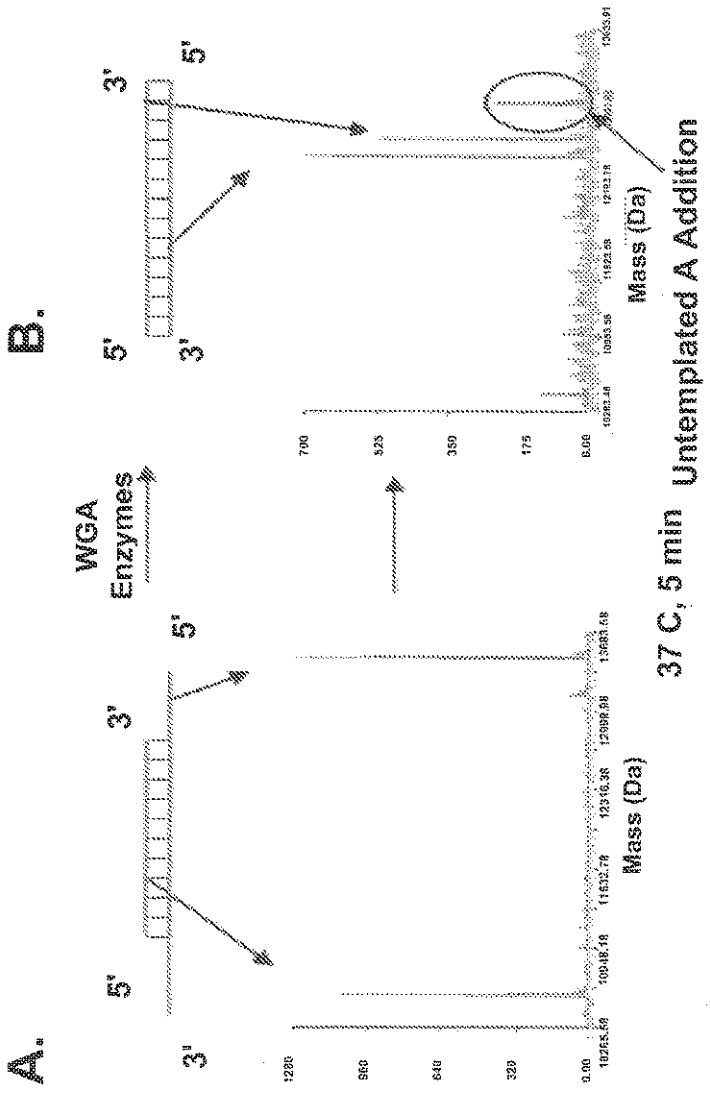

A.
5'
3'
3'
5'
Mass (Da)
WGA
Enzymes
37 C, 5 min
B.
3'
5'
5'
3'
Mass (Da)
Untemplated A Addition

FIGURE 6

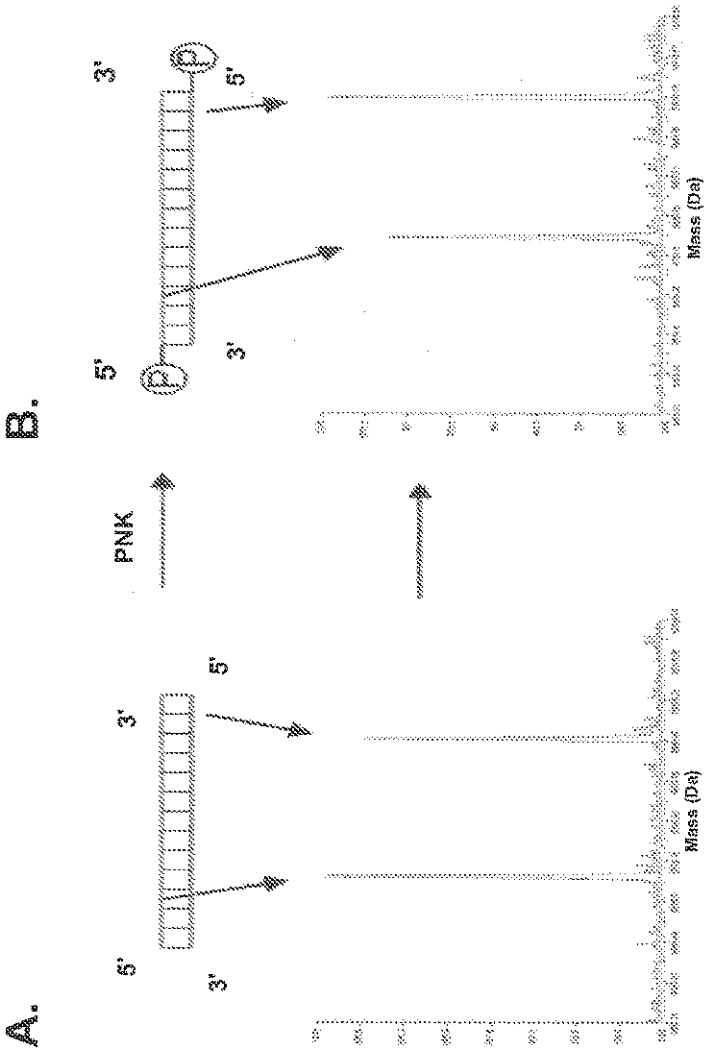
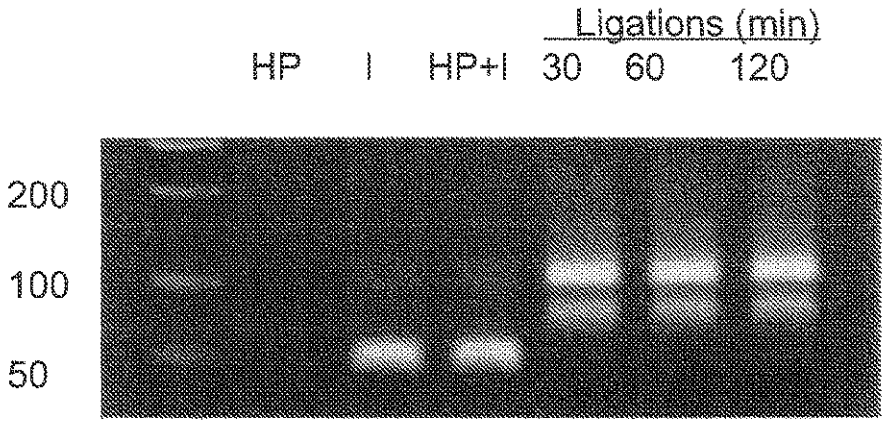


FIGURE 7





HP = Hairping oligo, I= insert, ligation was carried out with T4 ligase

FIGURE 8

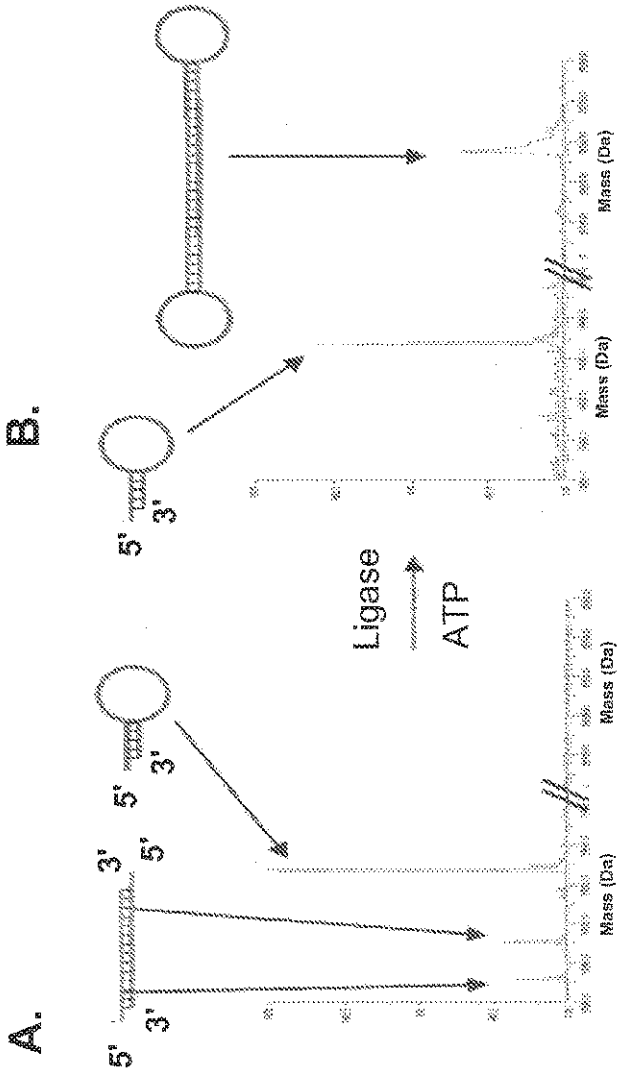


FIGURE 9

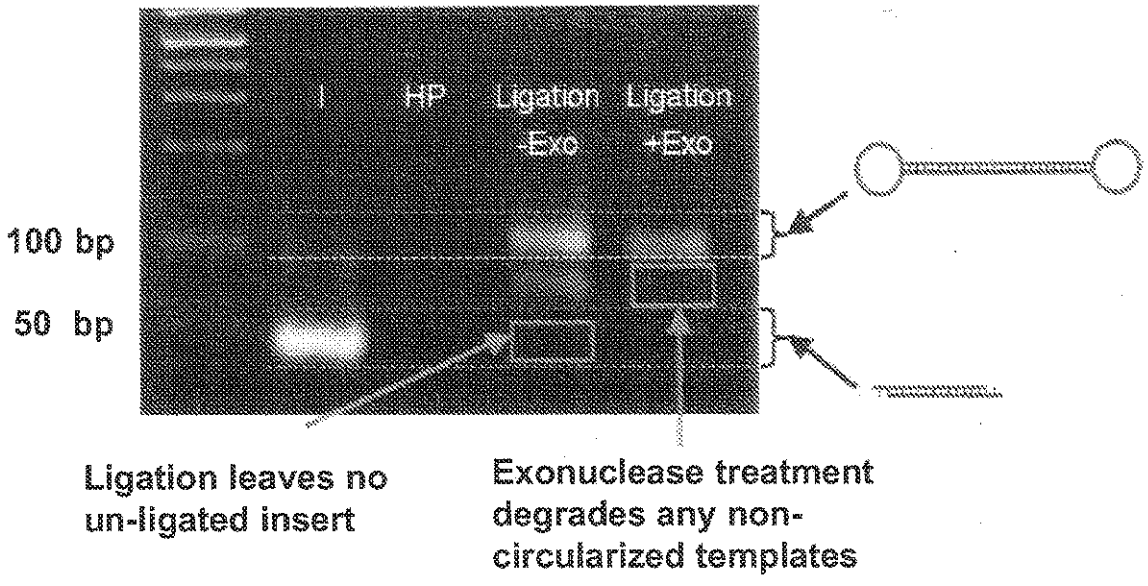

I
HP
Ligation -Exo
Ligation +Exo
100 bp
50 bp
Ligation leaves no un-ligated insert
Exonuclease treatment degrades any non-circularized templates

FIGURE 10

