

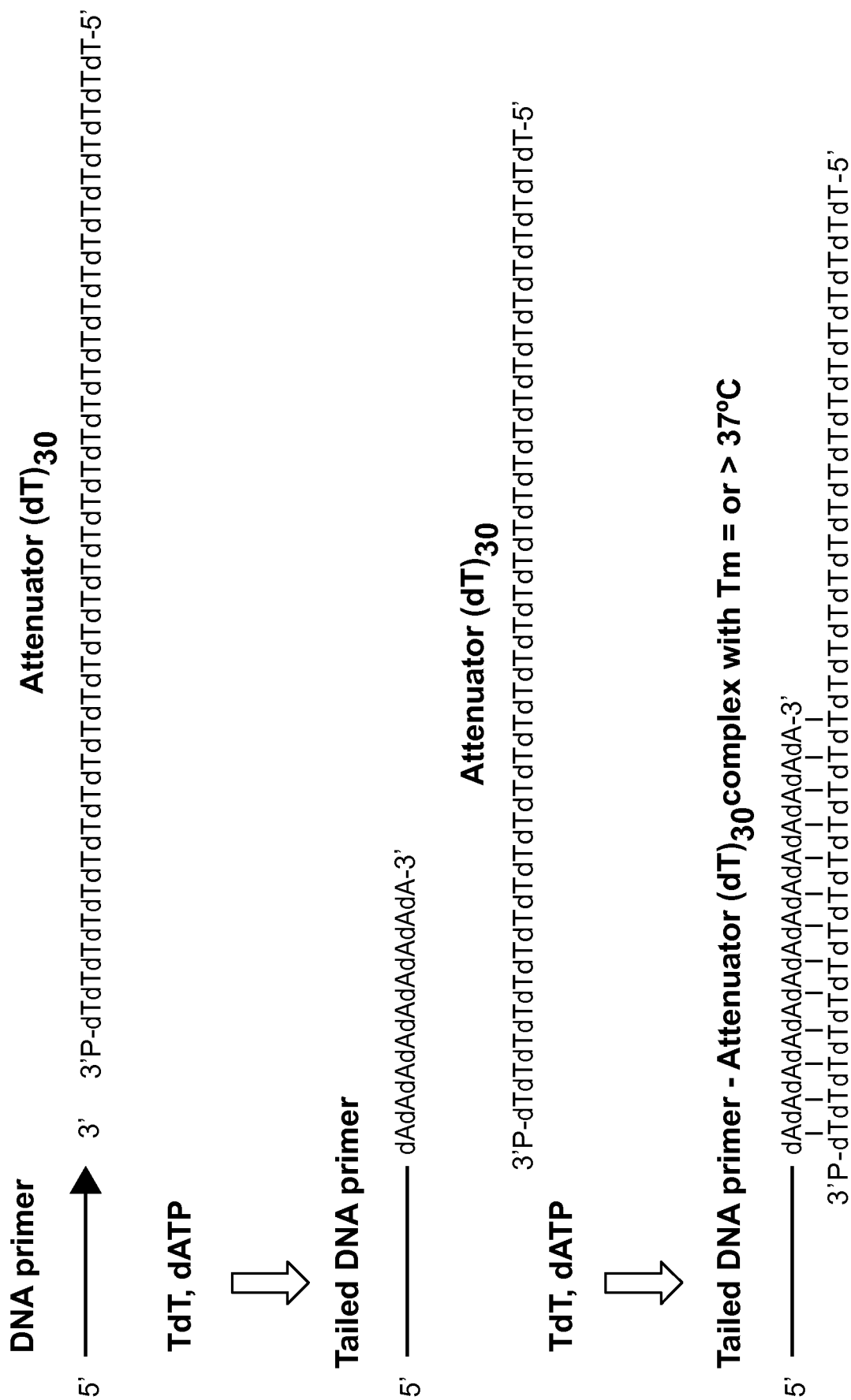
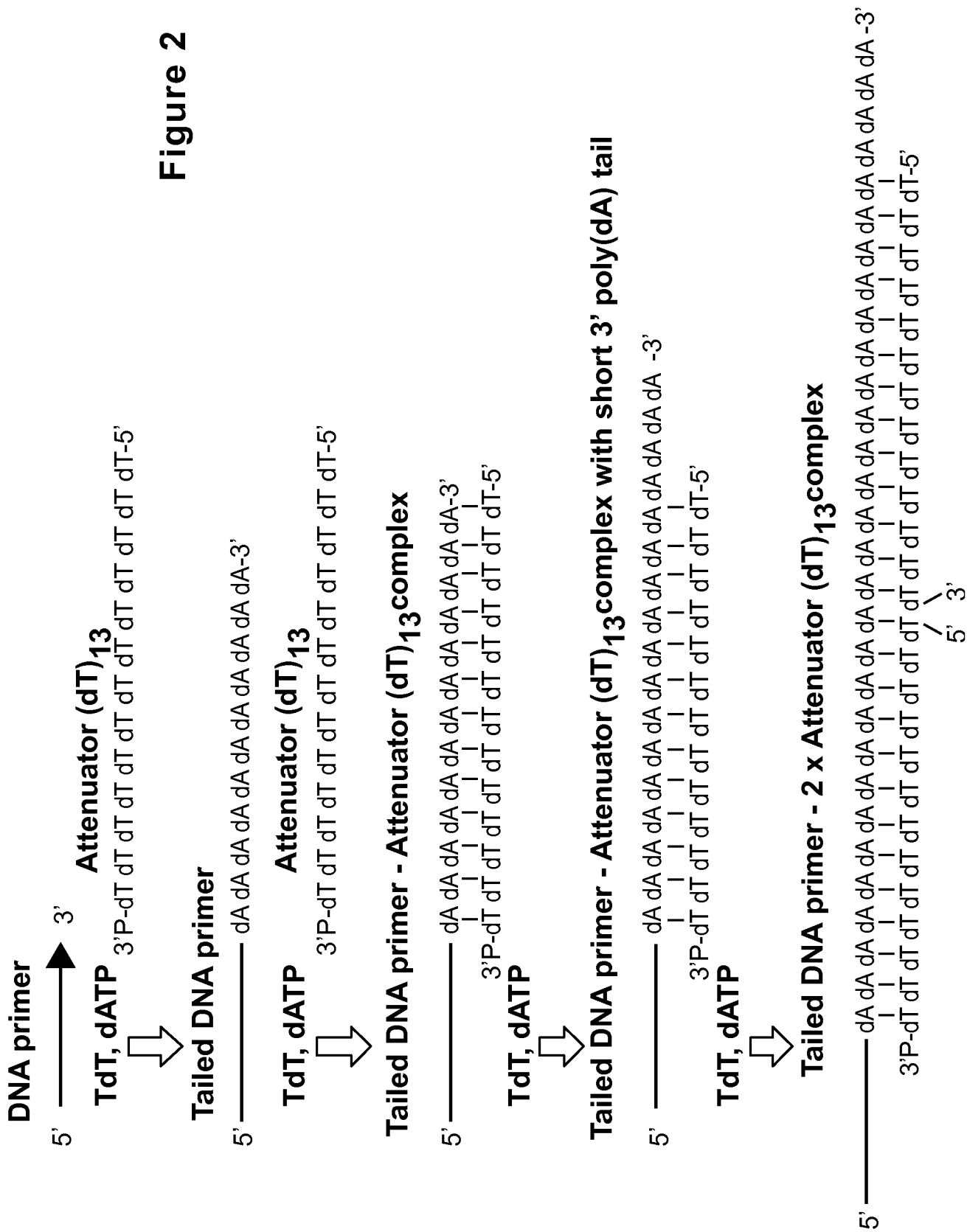


Figure 1



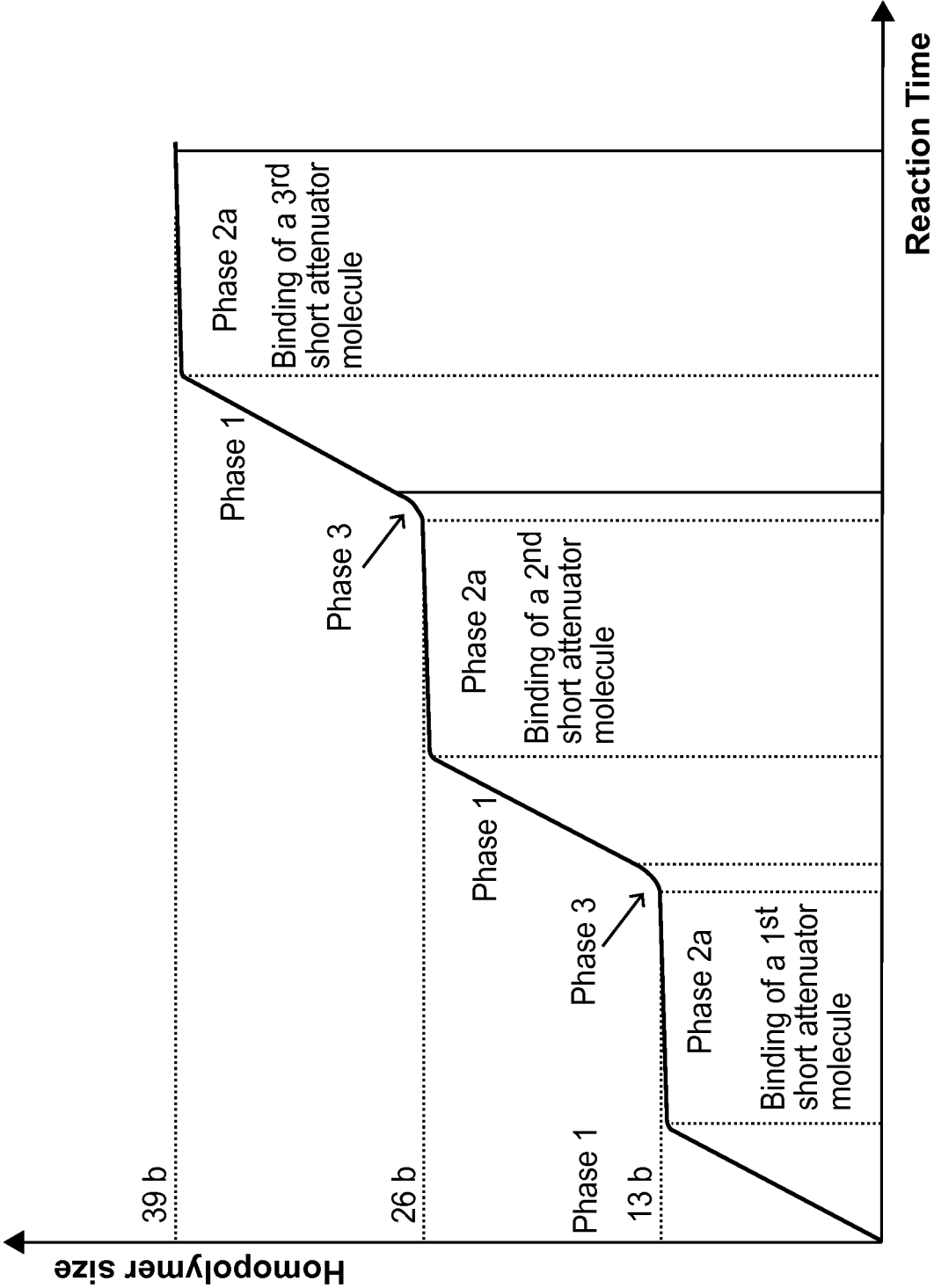


Figure 3

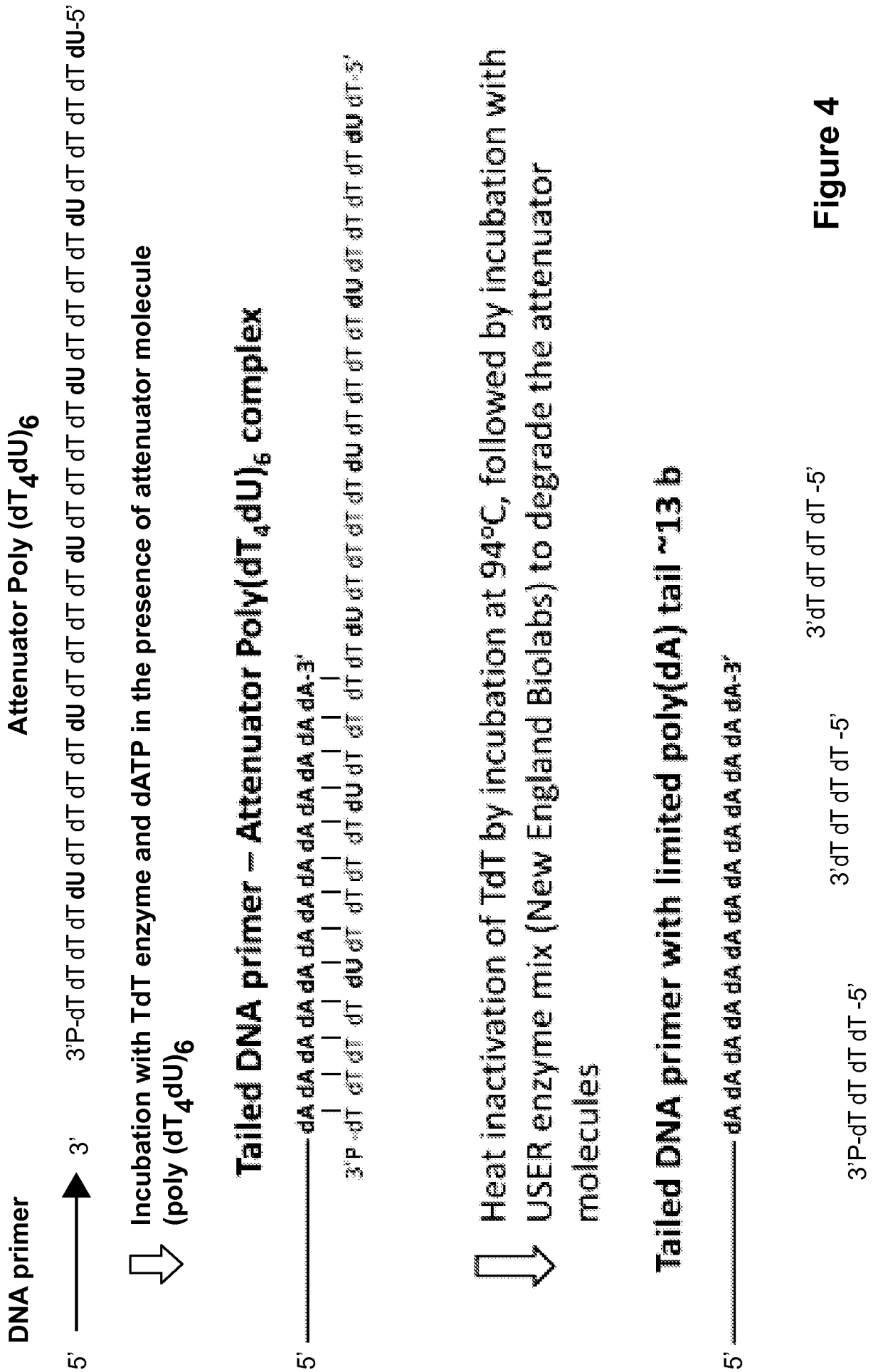


Figure 4

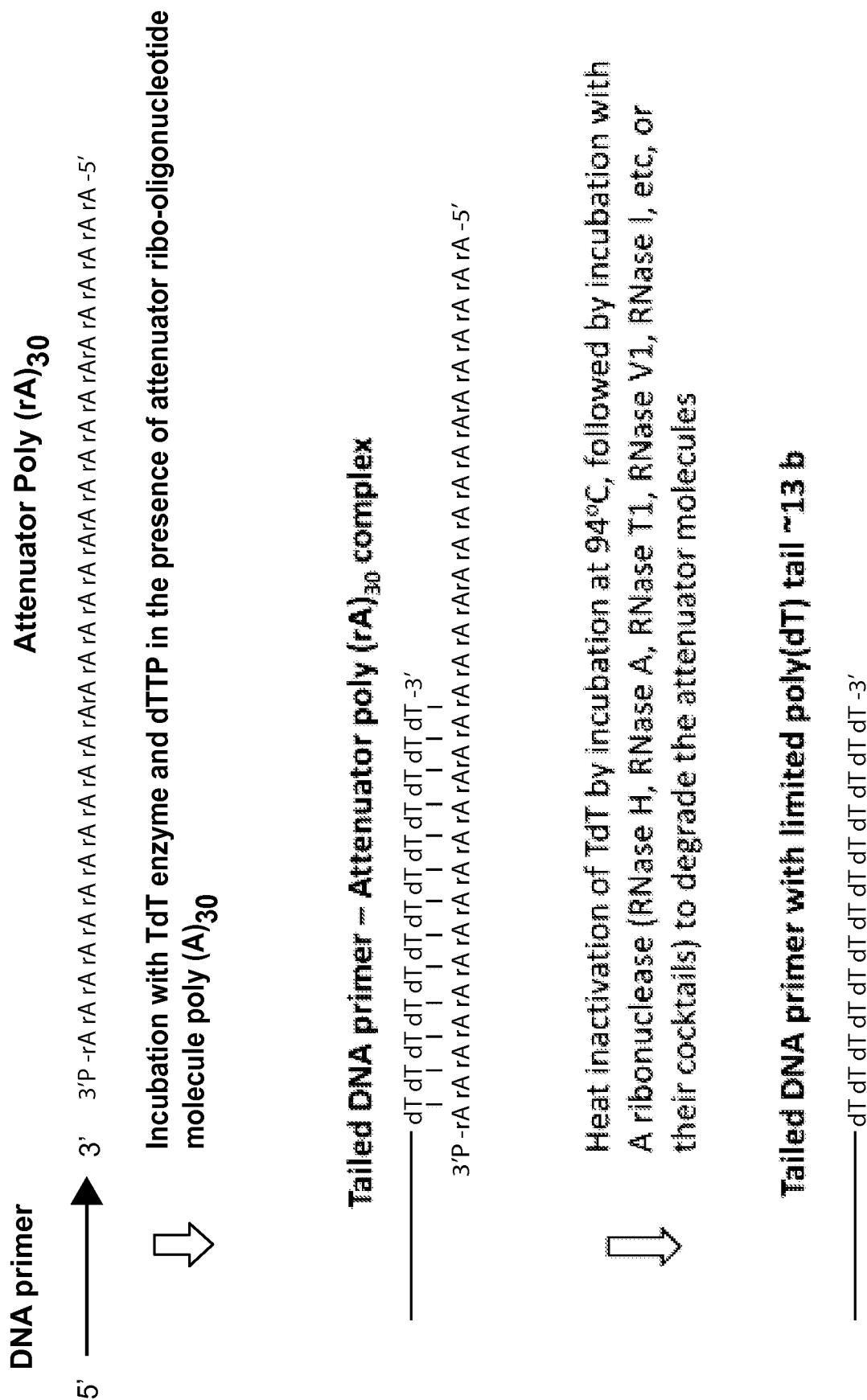


Figure 6

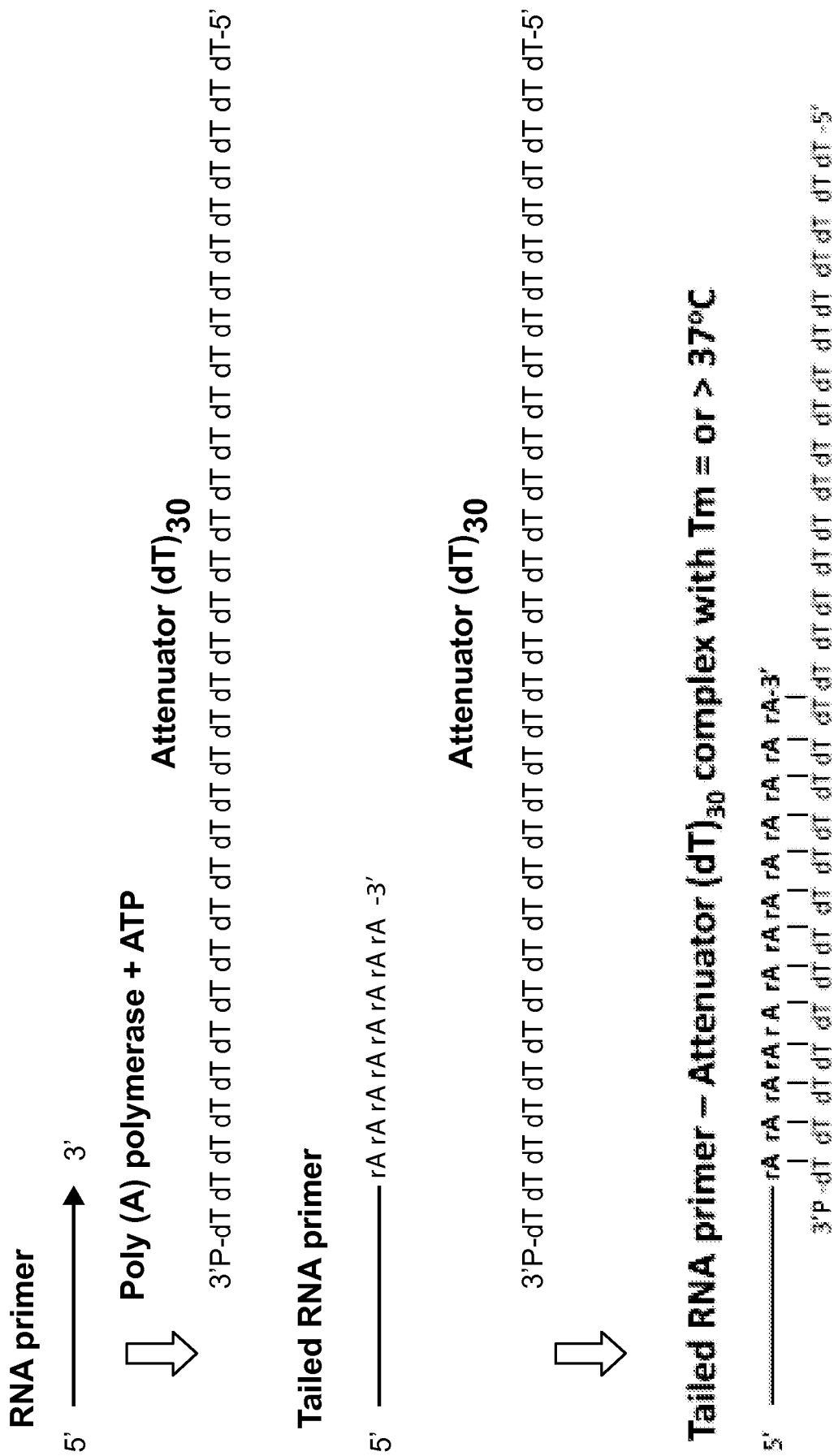
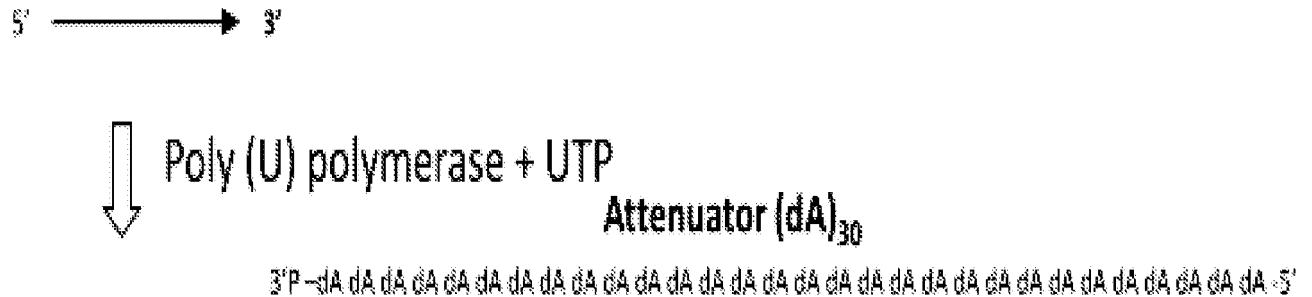
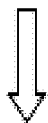


Figure 7

RNA primer



Tailed RNA primer



Tailed RNA primer – Attenuator (dA)₃₀ complex with Tm = or > 37°C

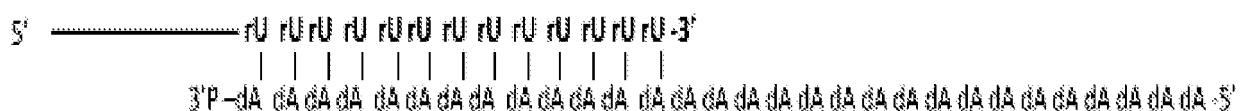
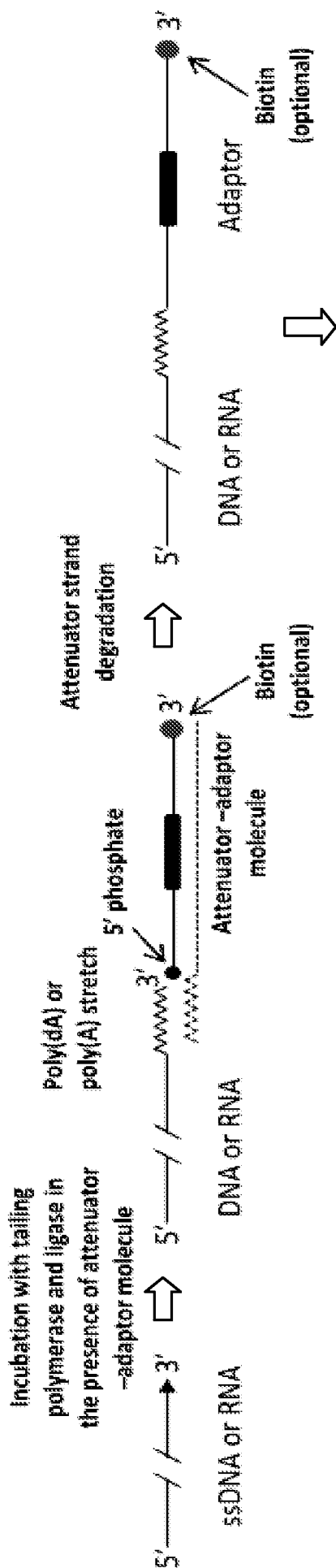


Figure 8

A. Limited tailing –ligation reaction



Immunize to streptavidin-coated magnetic beads (optional)

B. Limited tailing –polymerase-extension reaction

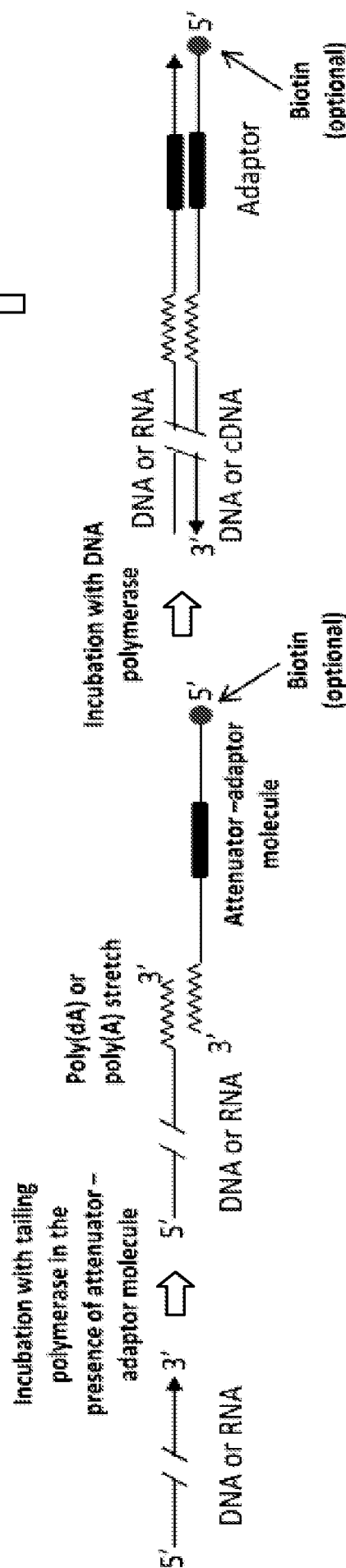
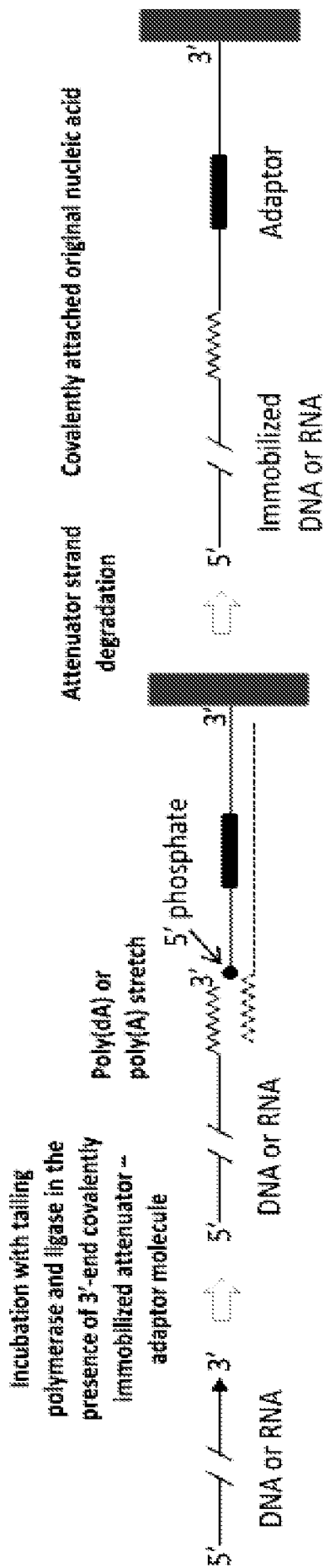


Figure 9

A. Immobilization by 3'-end using limited tailing –ligation reaction



B. Immobilization by 5'-end using limited tailing –polymerase-extension reaction

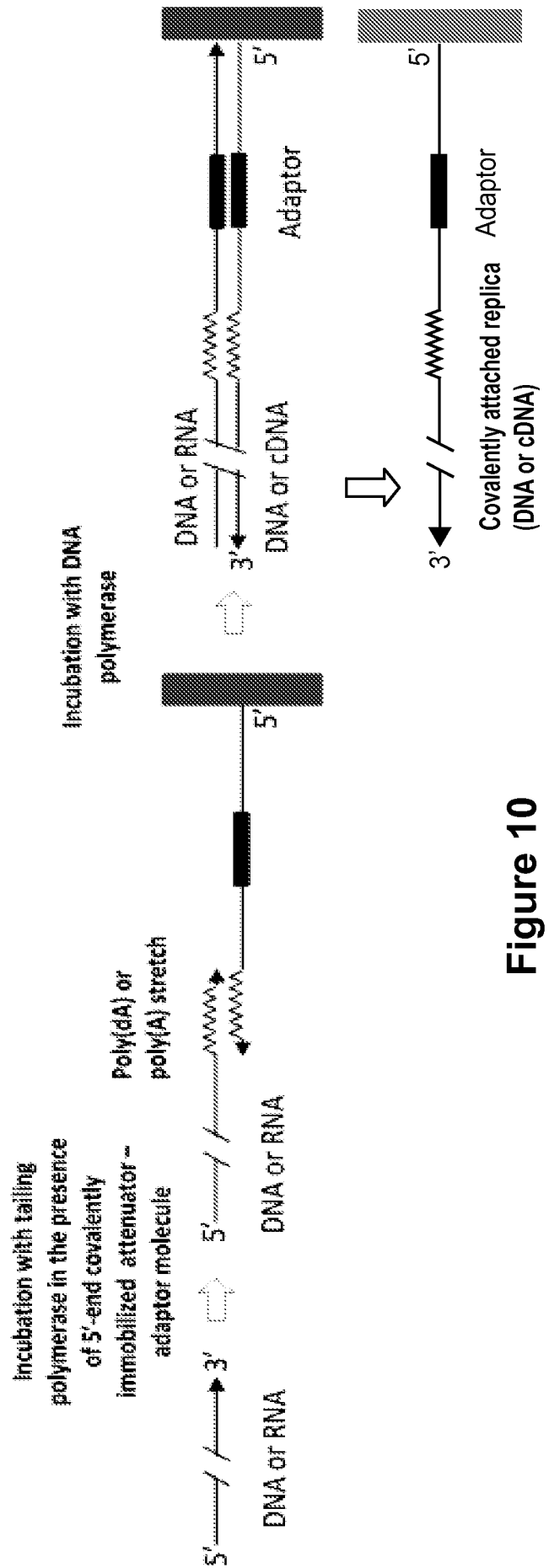
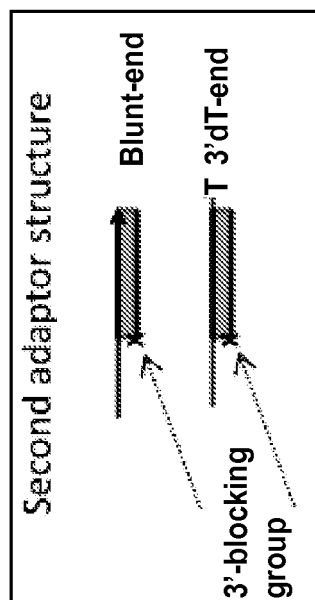
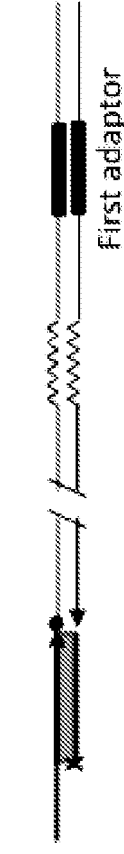


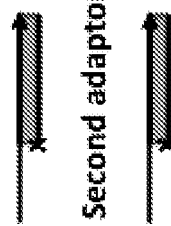
Figure 10



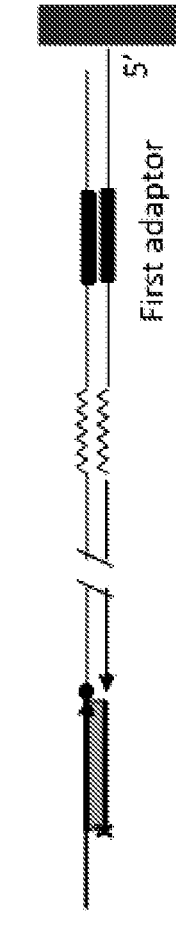
Second adaptor
second strand
synthesis



Second adaptor ligation



Blunt-end (after DNA polymerase with
proofreading activity) or end with the 3'-
single dA extension (after Taq, Klenow
(exo-), or reverse transcriptase)



Second adaptor
second strand
synthesis

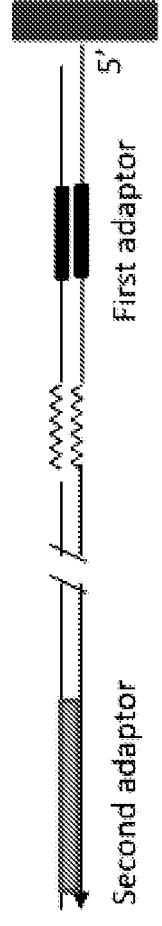


Figure 11

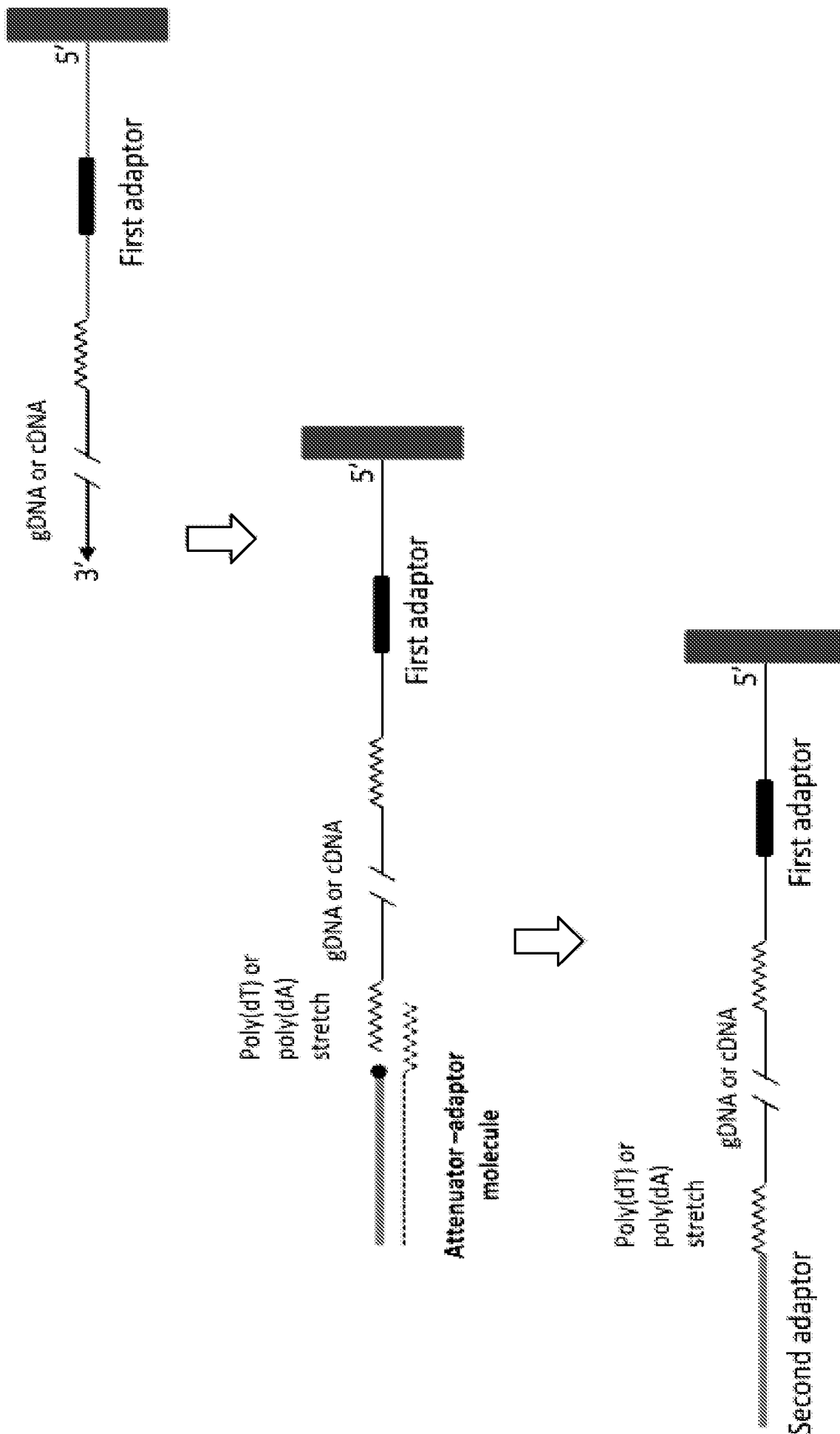


Figure 12

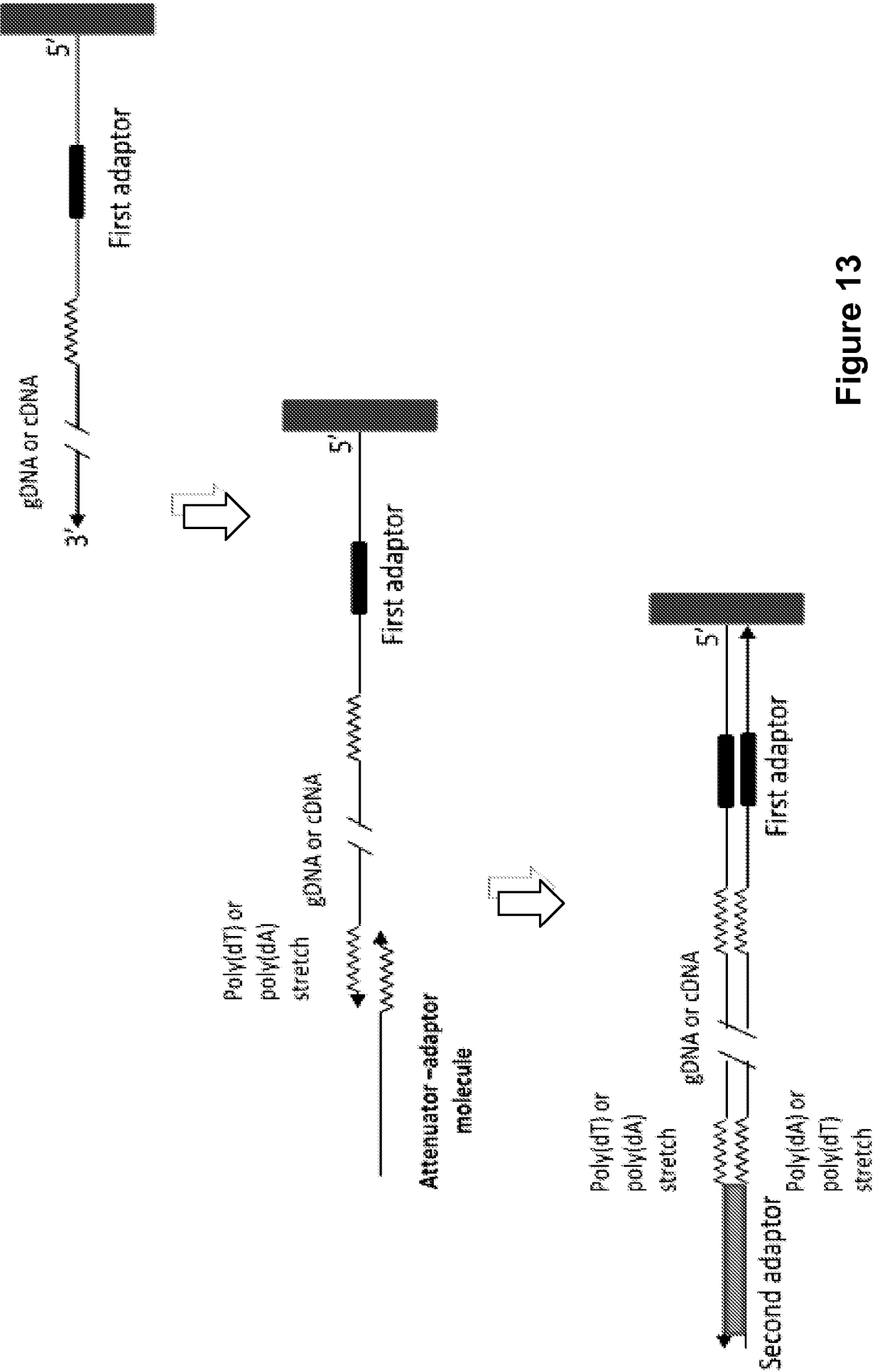


Figure 13

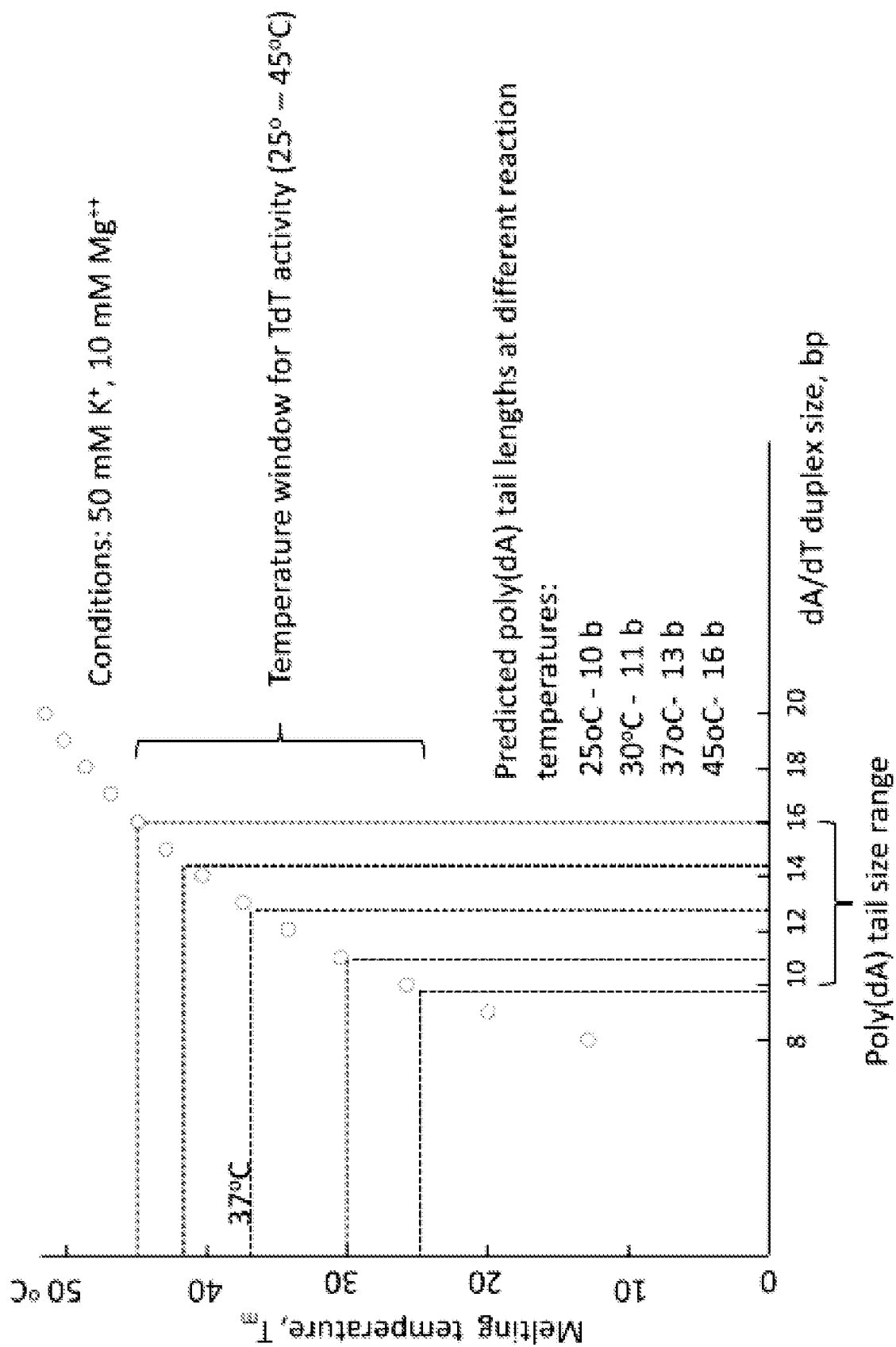


Figure 14

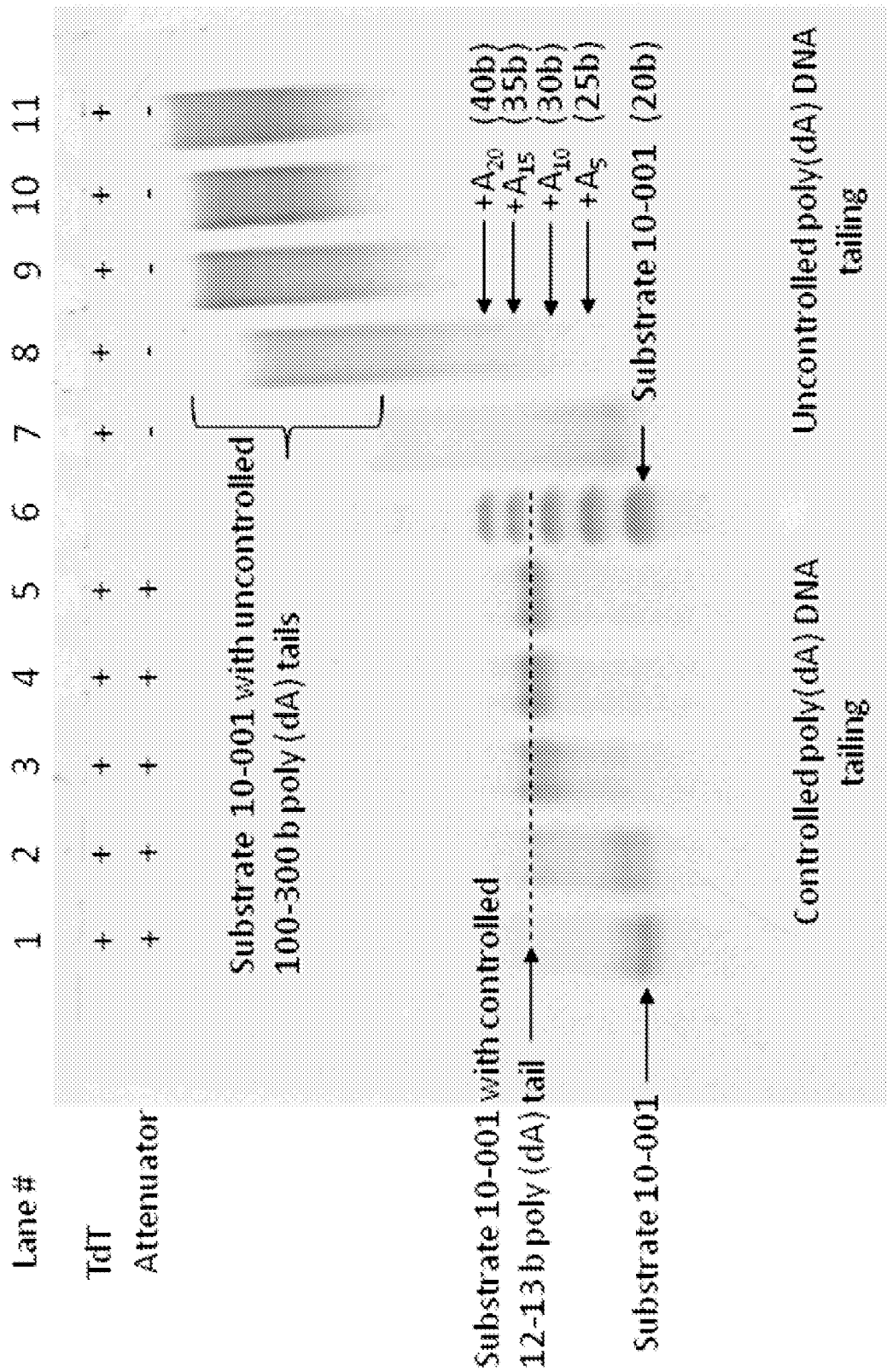


Figure 15

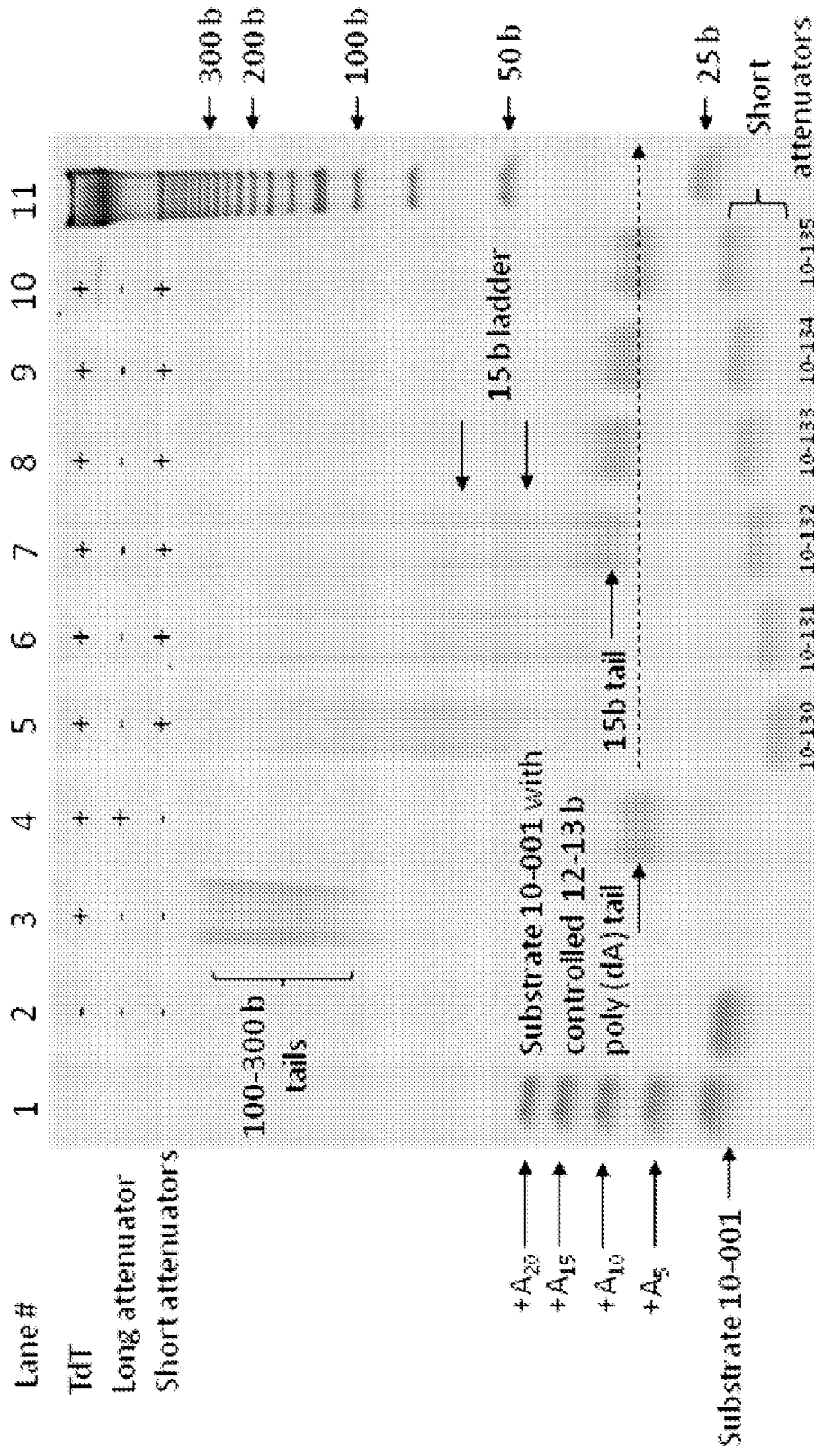


Figure 16A

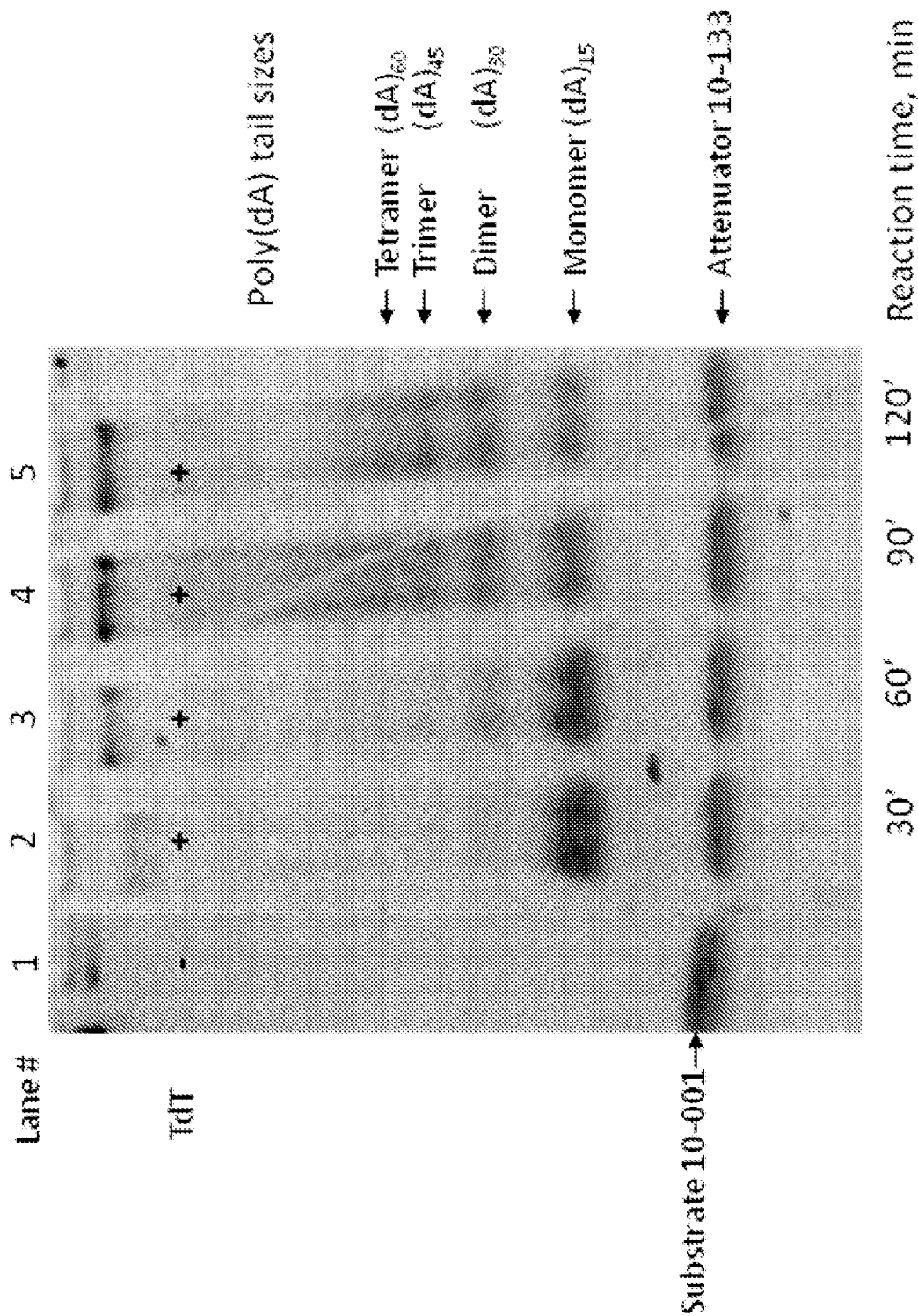


Figure 16B

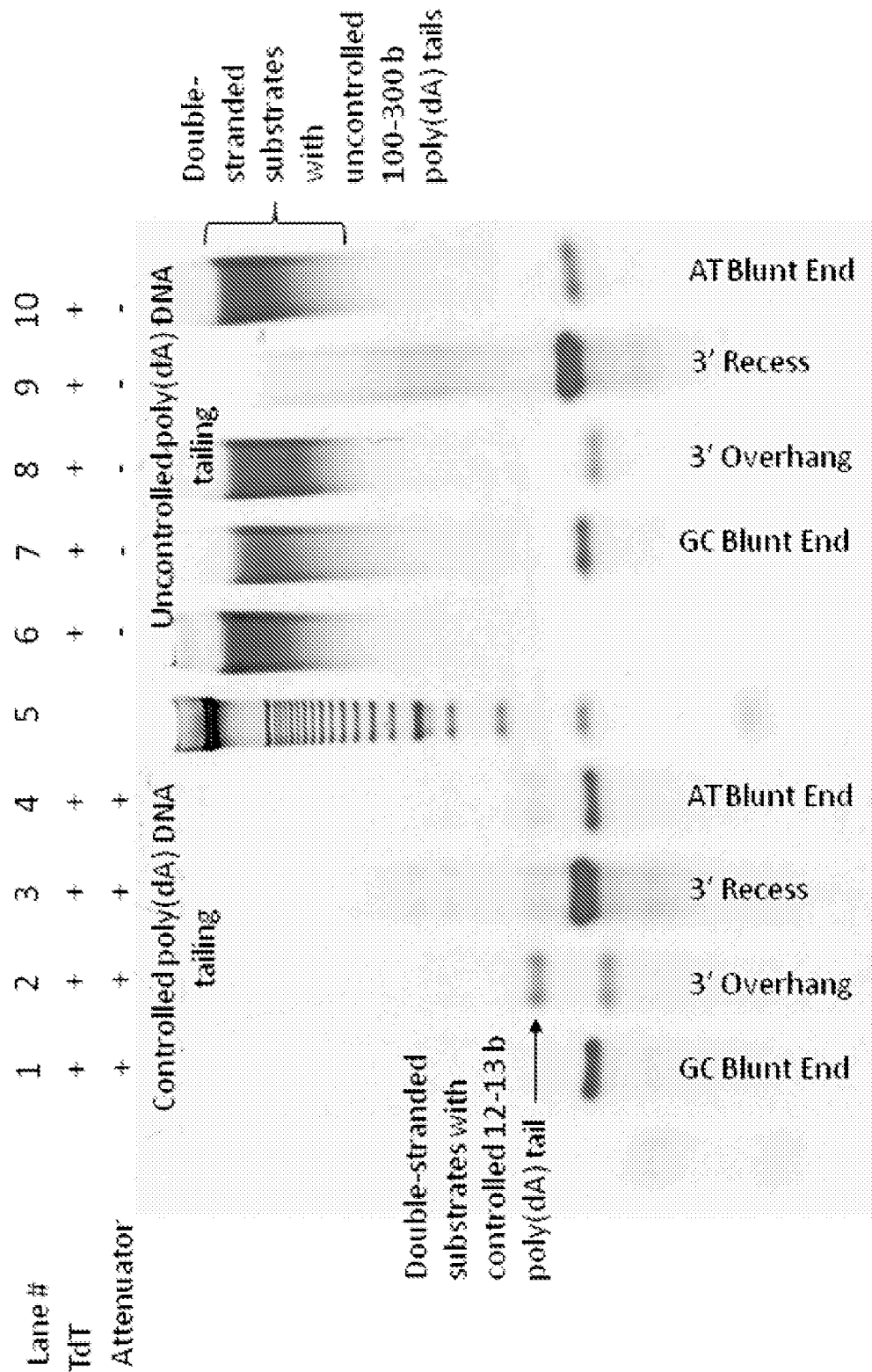


Figure 17

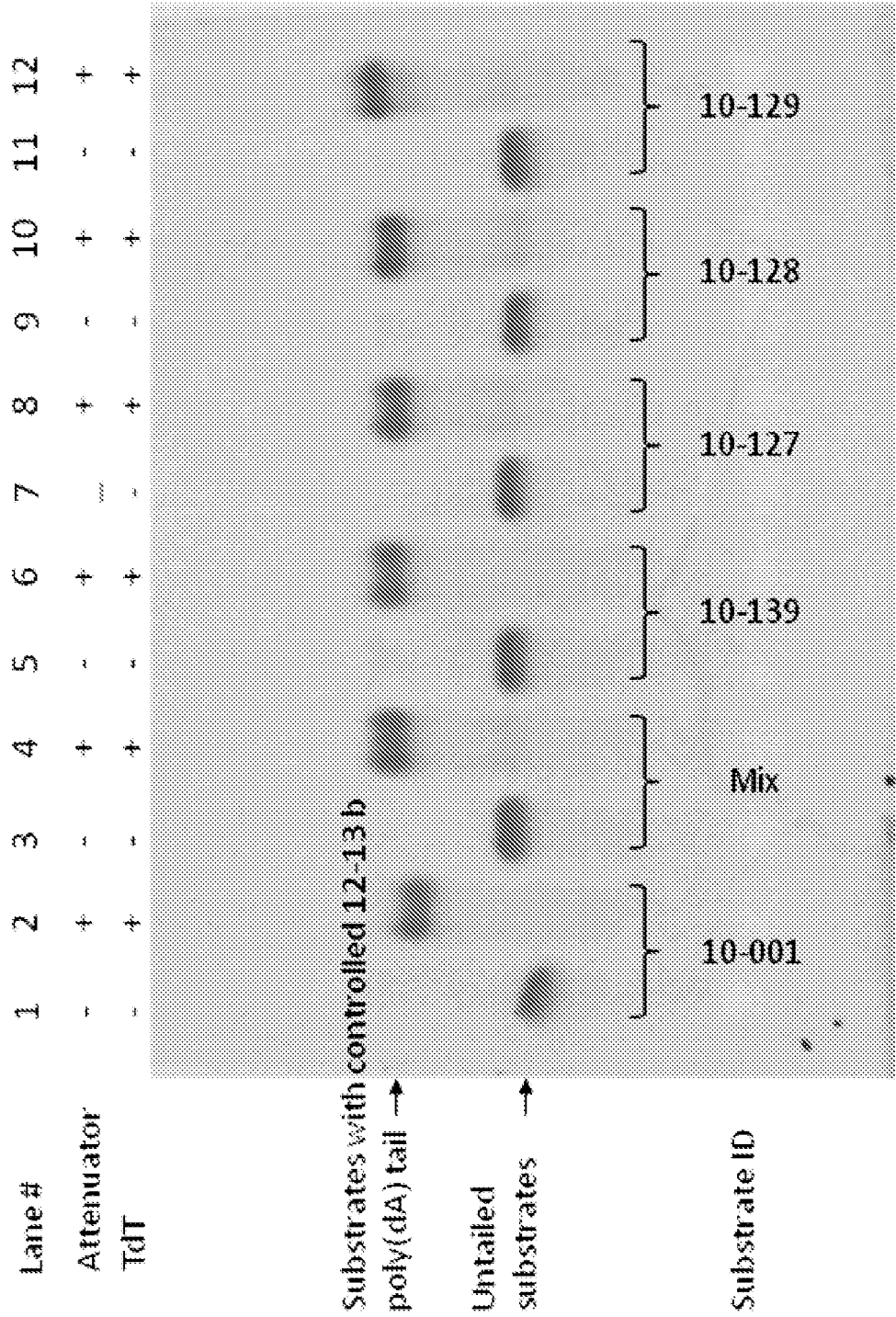


Figure 18

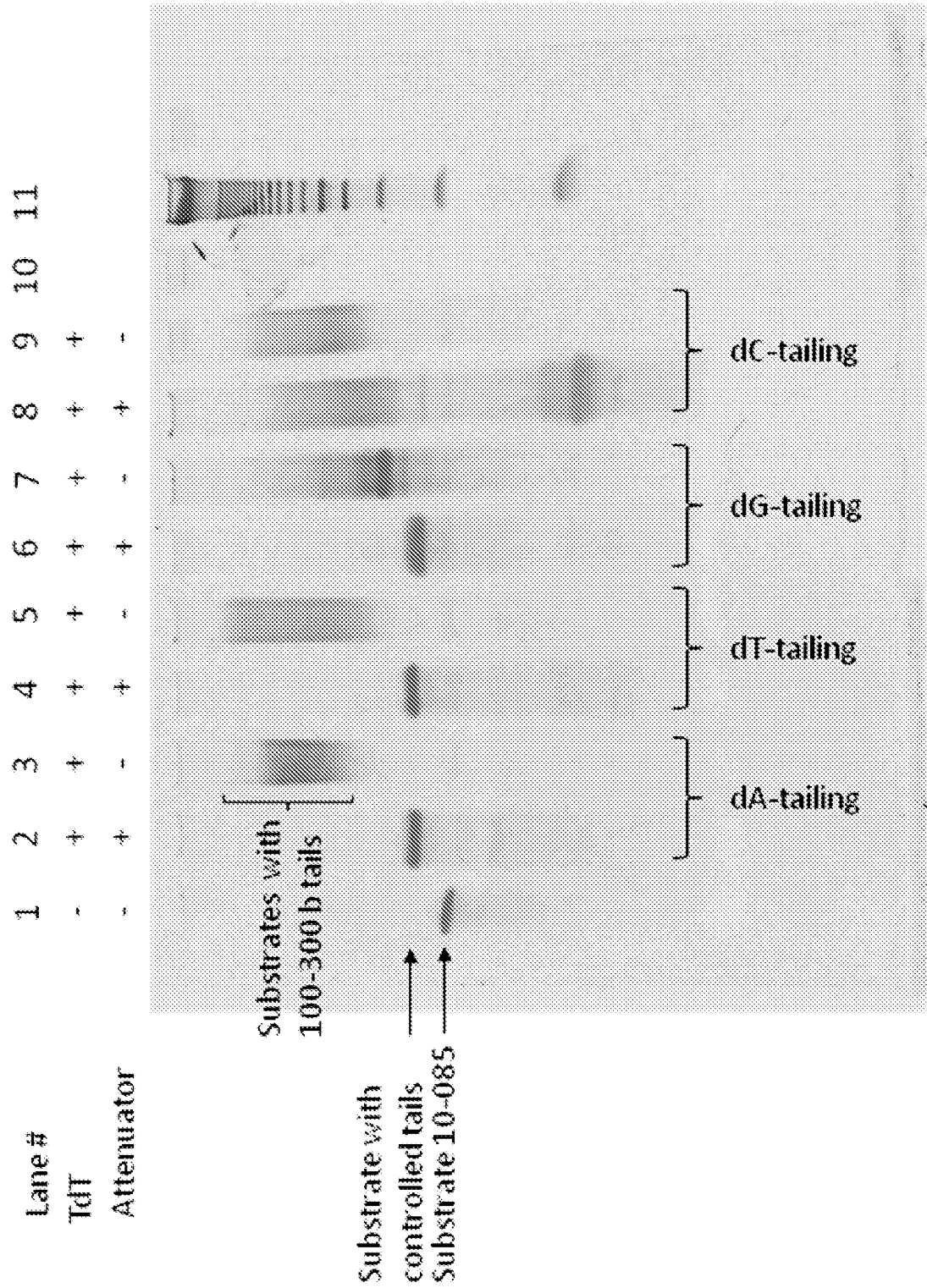


Figure 19

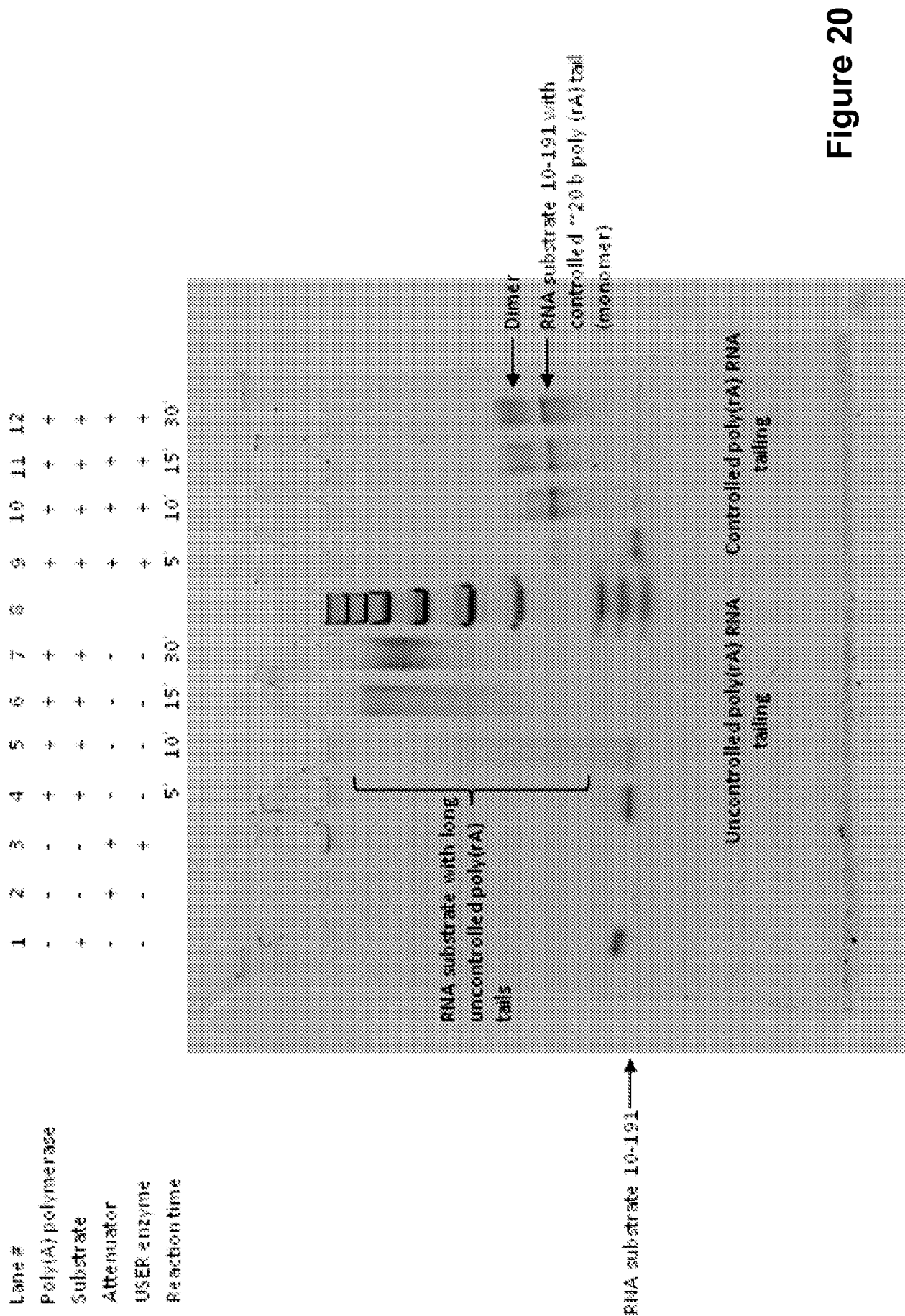


Figure 20

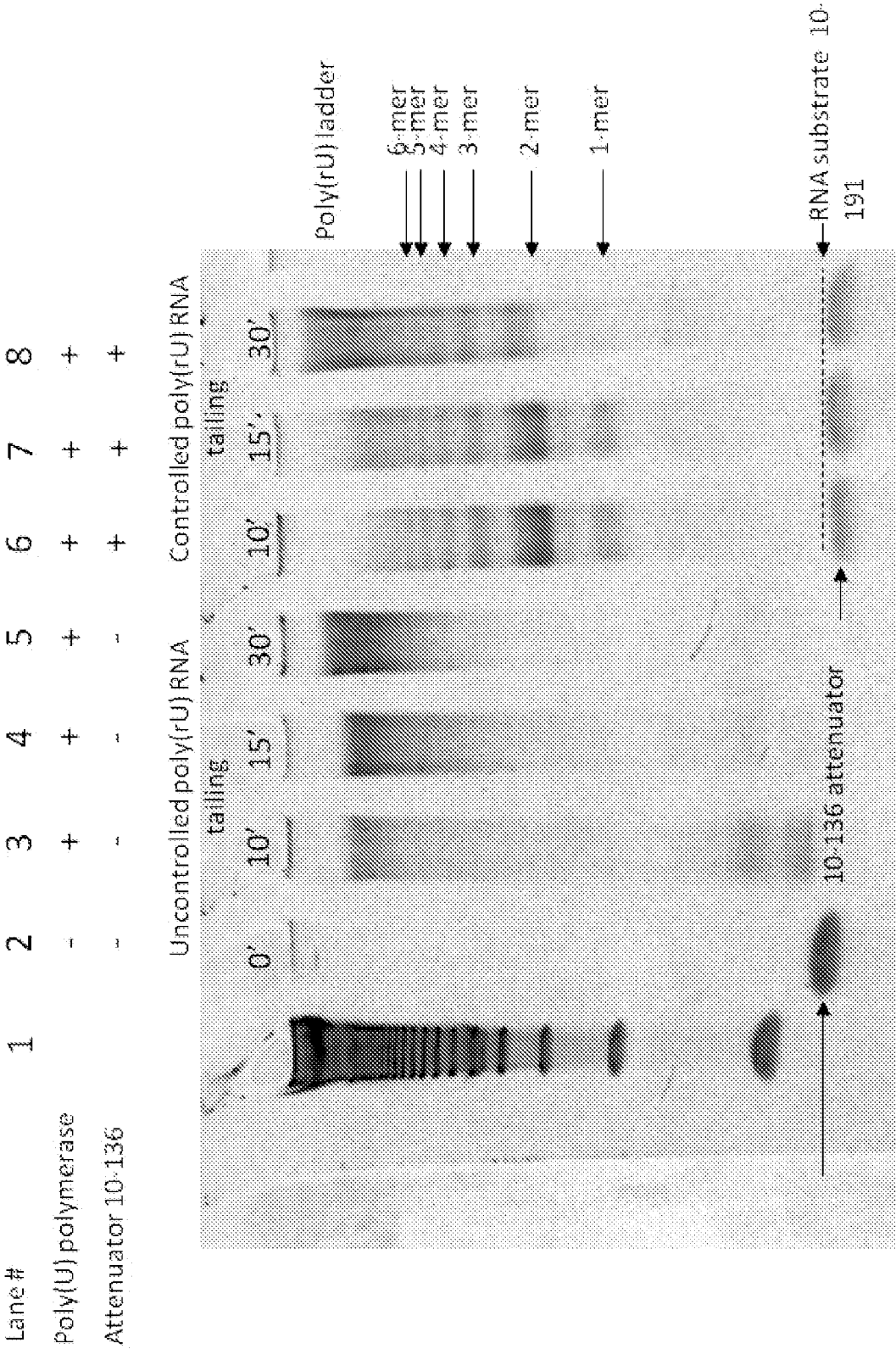


Figure 21a

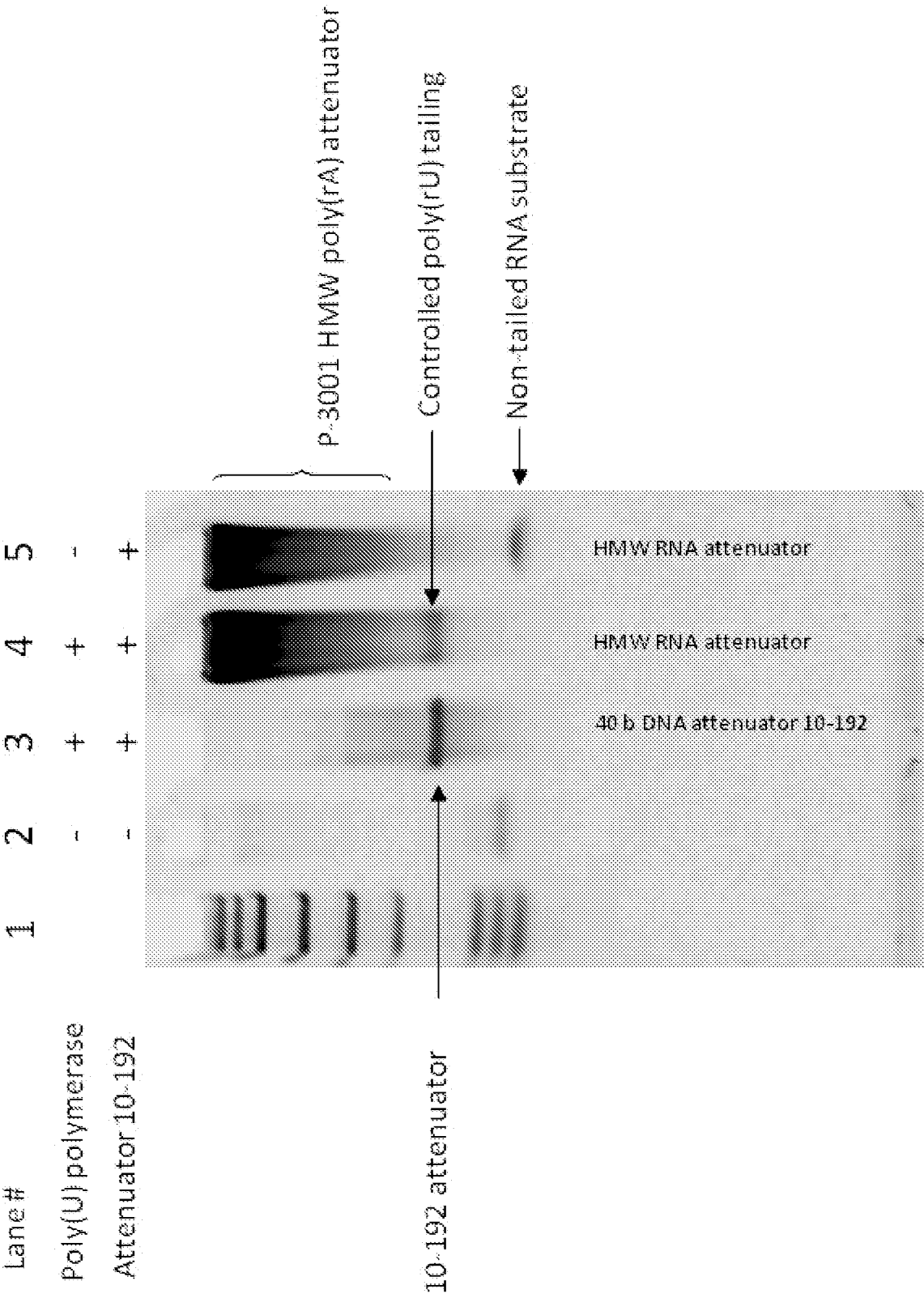


Figure 21b

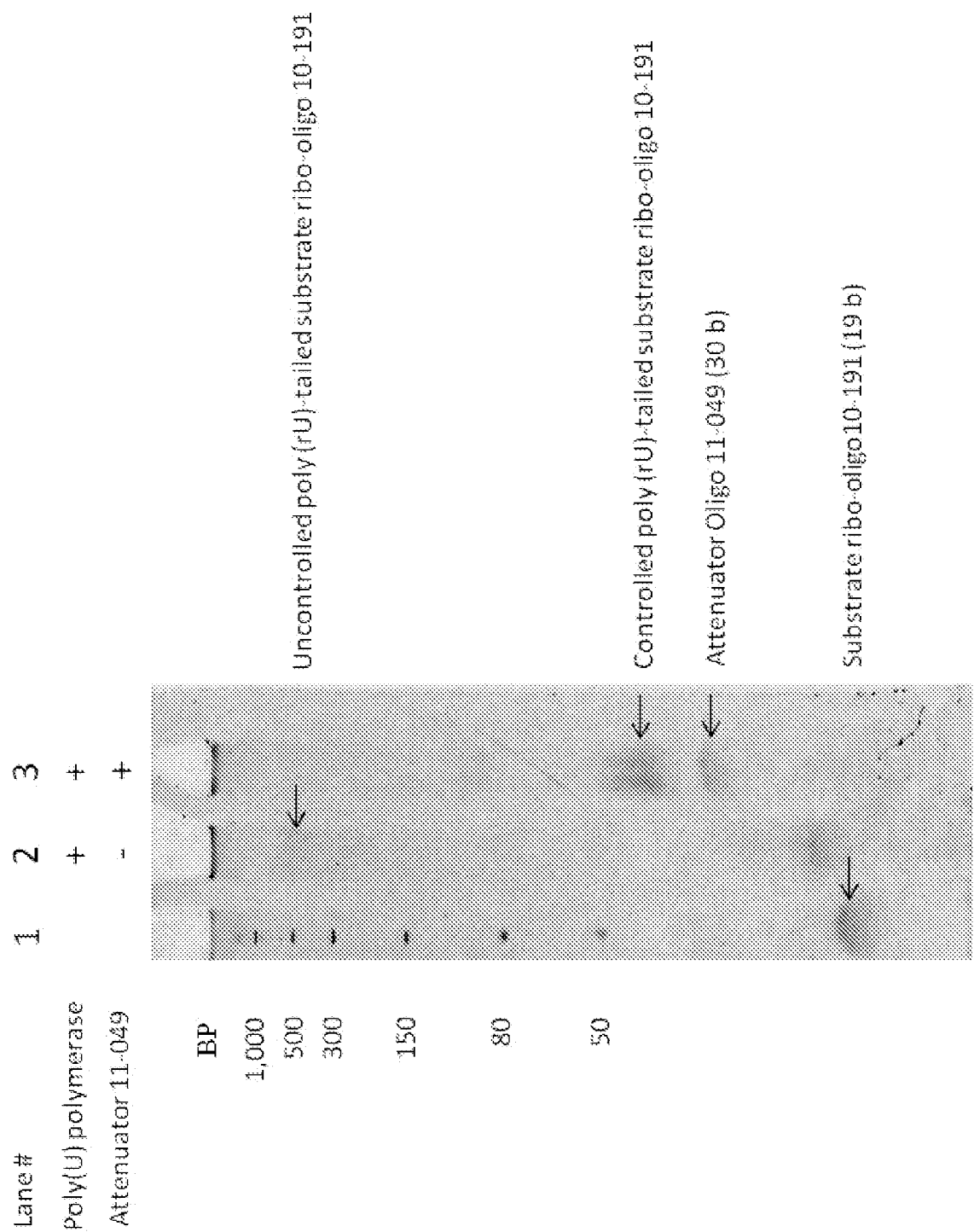


Figure 21c

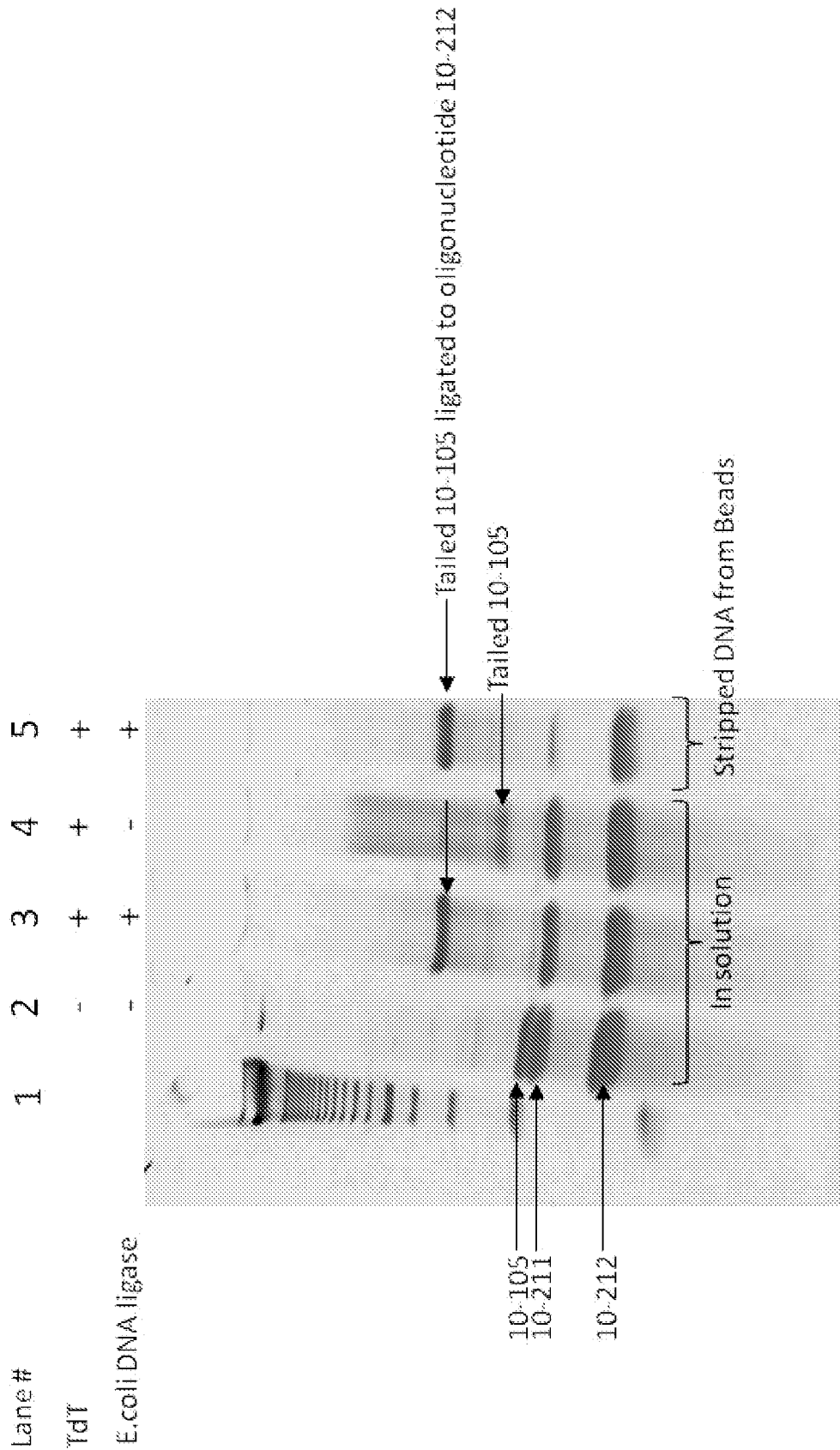


Figure 22

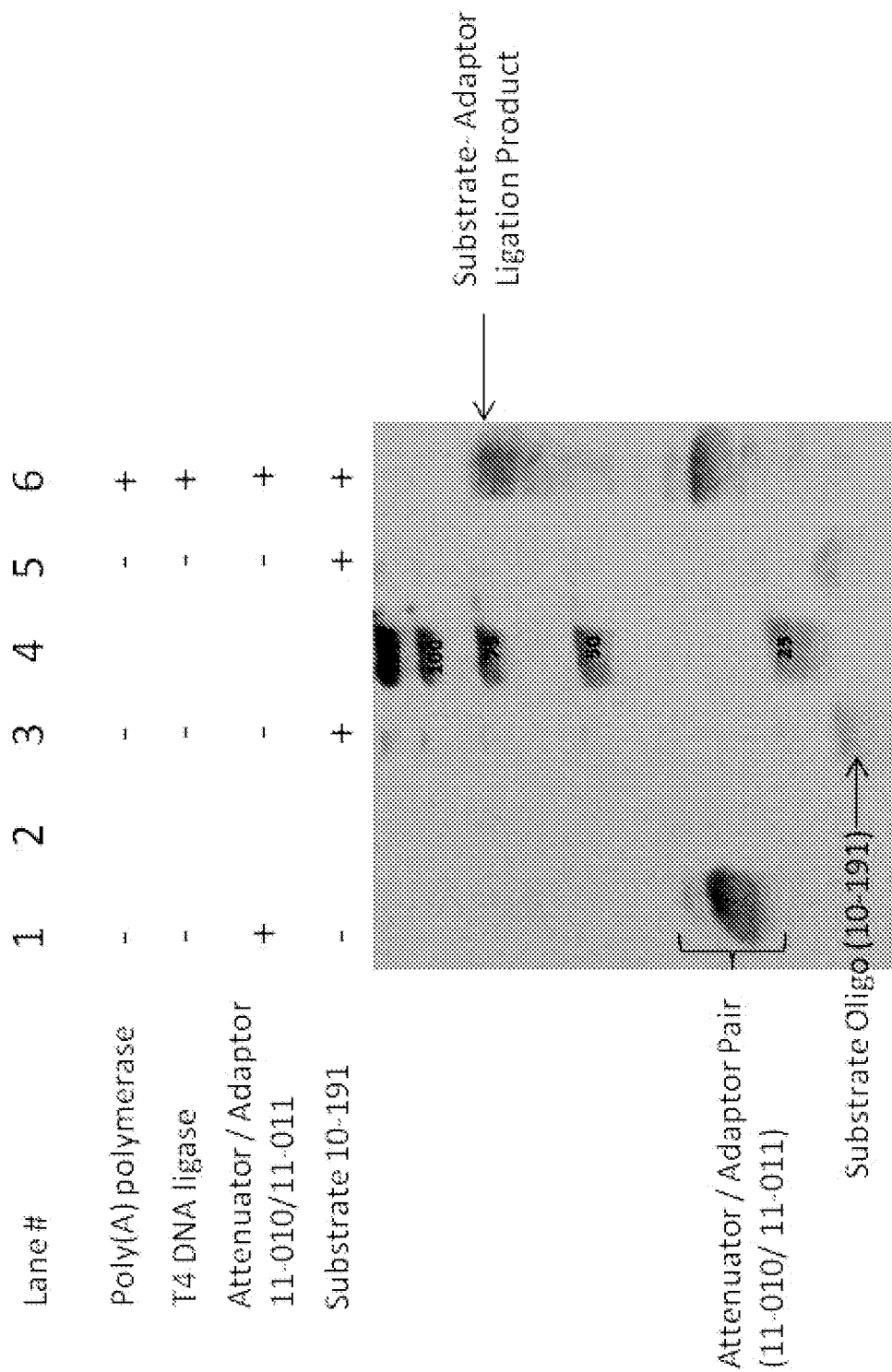


Figure 23

Substrate 10-105

5'-TAG TTG CTG CAA CCT AGT CTA GAT CTA TAA ACG CAC CTT TGG ACA CGG GG

Attenuator-adaptor

P-GATCGGAAGACGTCGTGTAGGGAAAGAGTGT A-P
UUUUTTTTTTTCTIAGCC TTCT CGCAGCACATC C CTTT CTCACAT - B

$TdT + dATP$

5'-TAG TTG CTG CAA CCT AGT CTA GAT CTA TAA ACG CAC CTT TGG ACA CGG GG AAAAAAAAAA
 P-GATCGGAAGAGCGTCGTGTAGGGAAAGAGTGA-P
 rUUTTTTTTTTTTCTAGCC TTCT CGCAGCACATC C CTTT CTCACAT - B

↓
E.coli ligase + NAD

Substrate 10-105 with attached poly(dA)₁₂ linker and adaptor 10-212 / 10-211

5'-TAG TTG CTG CAA CCT AGT CTA GAT CTA TAA ACG CAC CTT TGG ACA CGG GG AAAAAAAAAAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAA-P
 rUUTTTTTTTTTTCTAGCC TTCT CGCAGCACATC C CTTT CTCACAT -B

Figure 24a

Substrate 10-105

5'-TAG TTG CTG CAA CCT AGT CTA GAT CTA TAA ACG CAC CTT TGG ACA CGG GG

Immobilized attenuator-adaptor

P-GATCGGAGAGCGTCGTAGGGAAGAGGTGA-P

UUTTTTTTTTTTCTAGCC TTCT CGCAGCACATC C CTT CTCACAT - (B)

TdT + dATP



5'-TAG TTG CTG CAA CCT AGT CTA GAT CTA TAA ACG CAC CTT TGG ACA CGG GG AAAAAAAAAA

P-GATCGGAAGAGCGTCGTGTAGGAAAGAGTGA-P

TTTTCTTTTCTAGCC TTCT CGCAGCACATC C CTTT CTCACAT-

E.coli ligase + NAD



immobilized substrate—adaptor complex

5'-TAG TTG CTG CAA CCT AGT CTA GAT CTA TAA ACG CAC CTT TGG ACA CGG GG AAAAAAAAAAAGATCGAAGAGCGTCGTAGGGAAAGAGTGTAA-P

...TTTTTTTCTAGCC TTCT CGCAGCACATC C CTTT CTCACAT-

Non-reacted immobilized adaptor

P-GATCGGAAGAGCGTCGTGTAGGAAAGAGTGA-P

...UUUUUUUUUUUUAAGCC TTCT CGCAGCACATC C CTTT CTCACAT -

Figure 24b

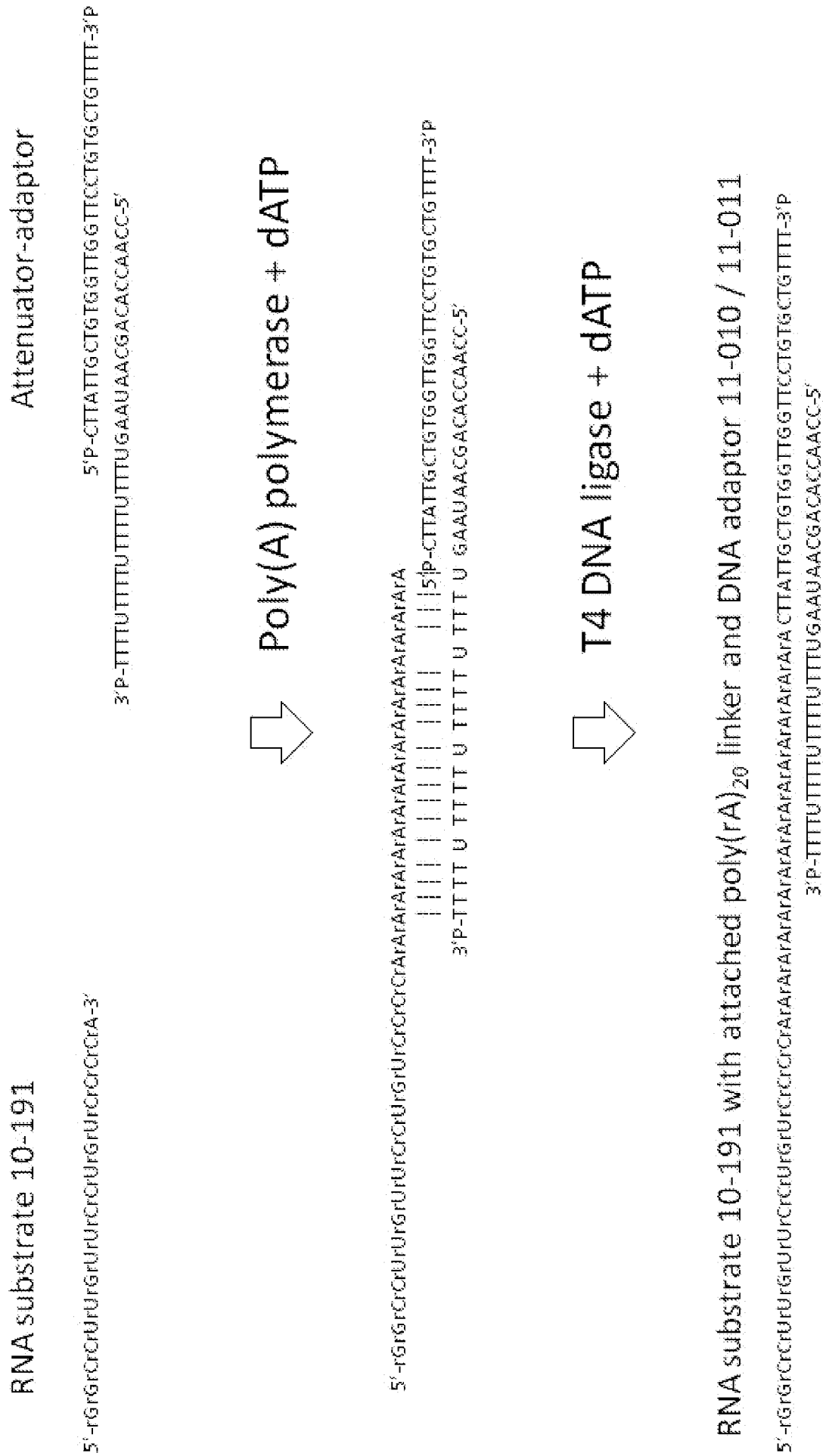


Figure 24c

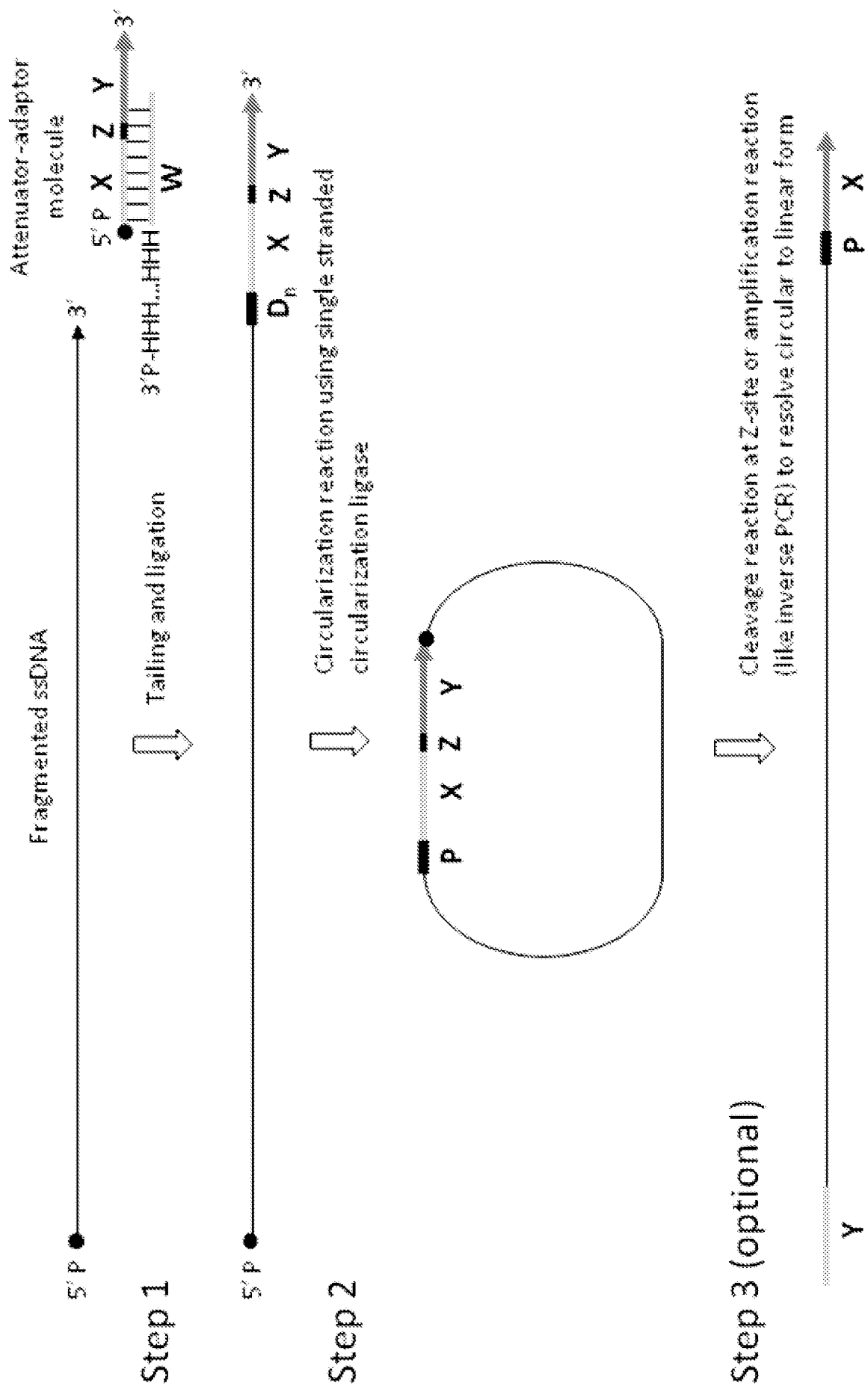


Figure 25

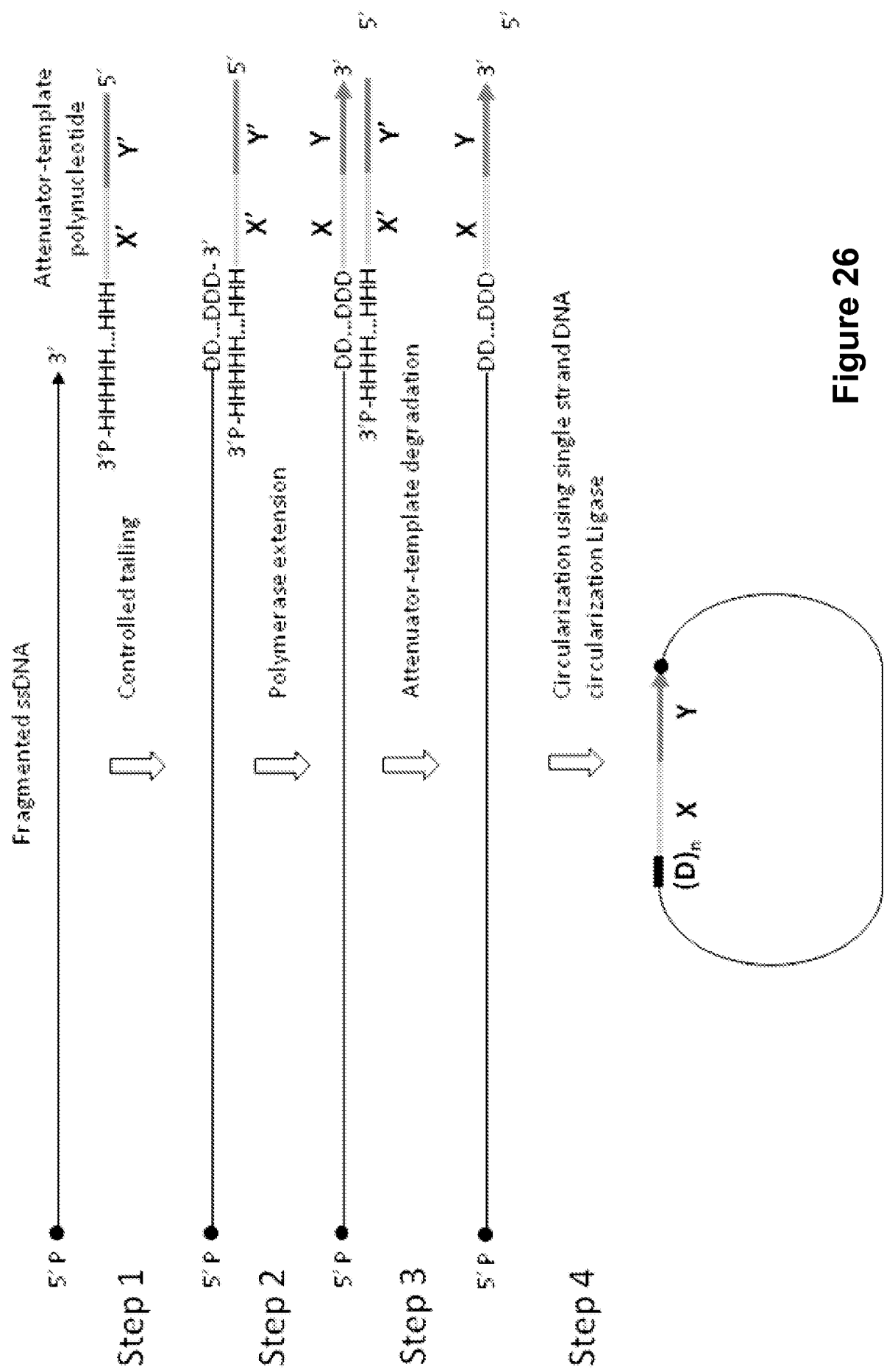


Figure 26

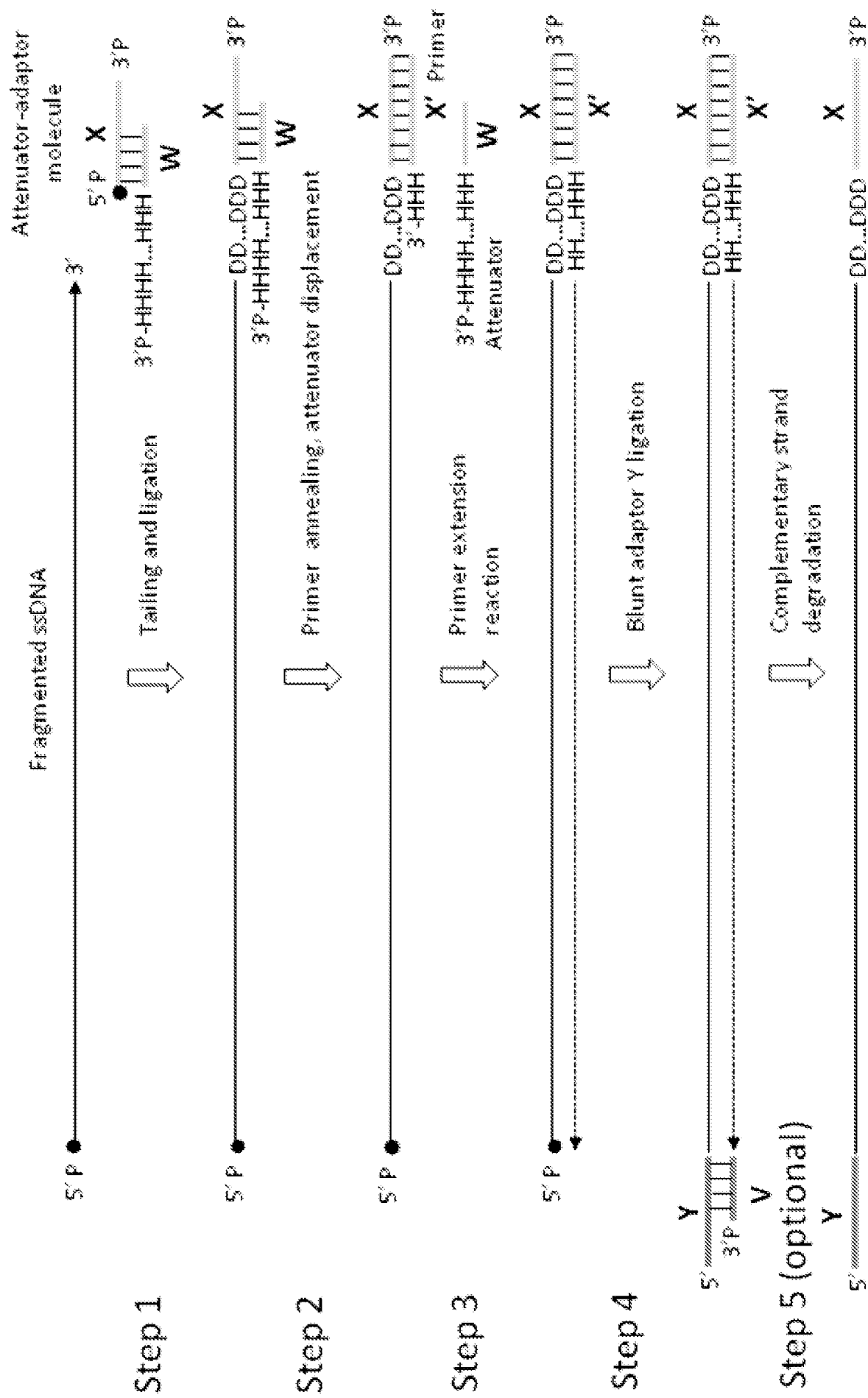


Figure 27

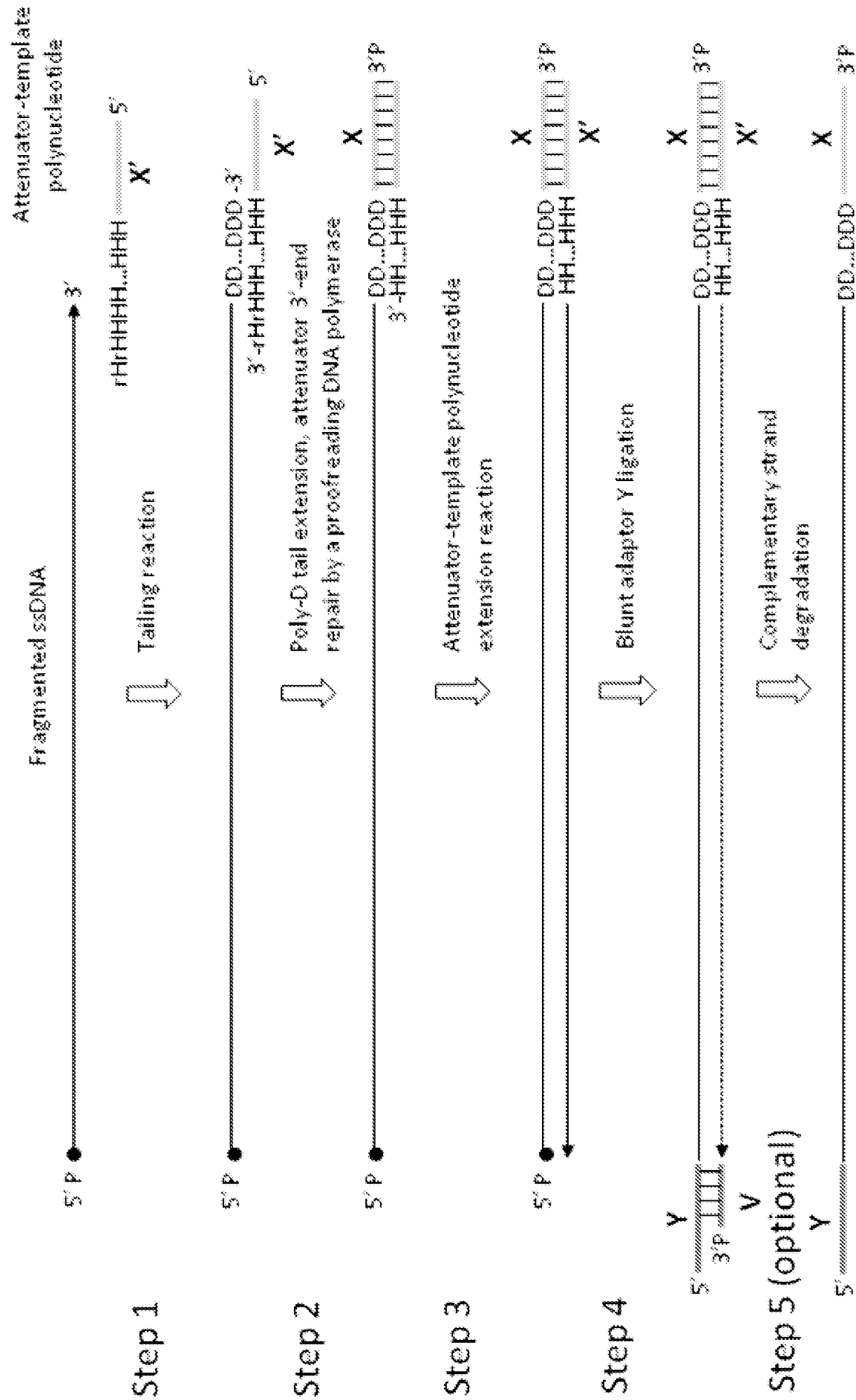


Figure 28

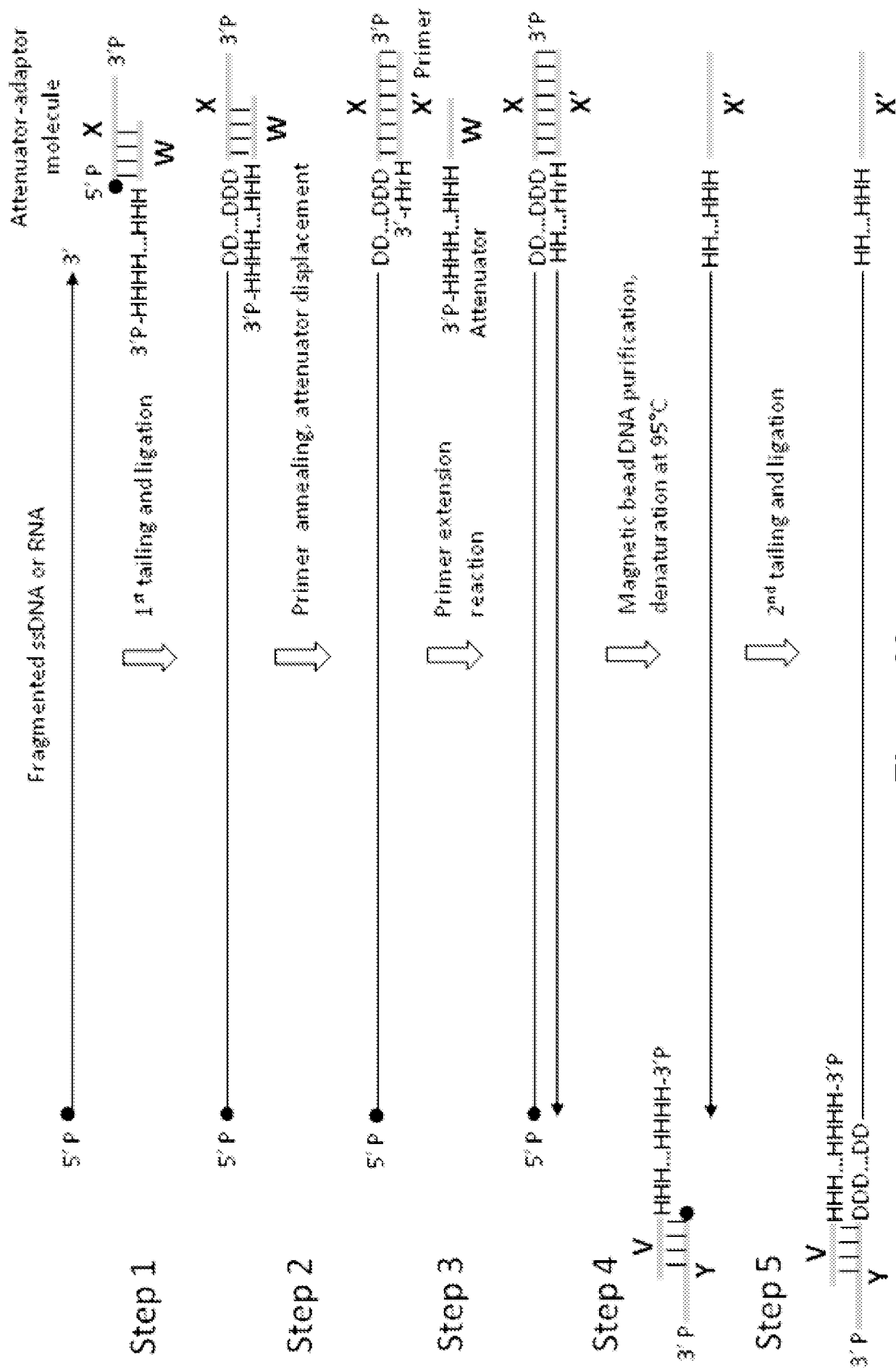
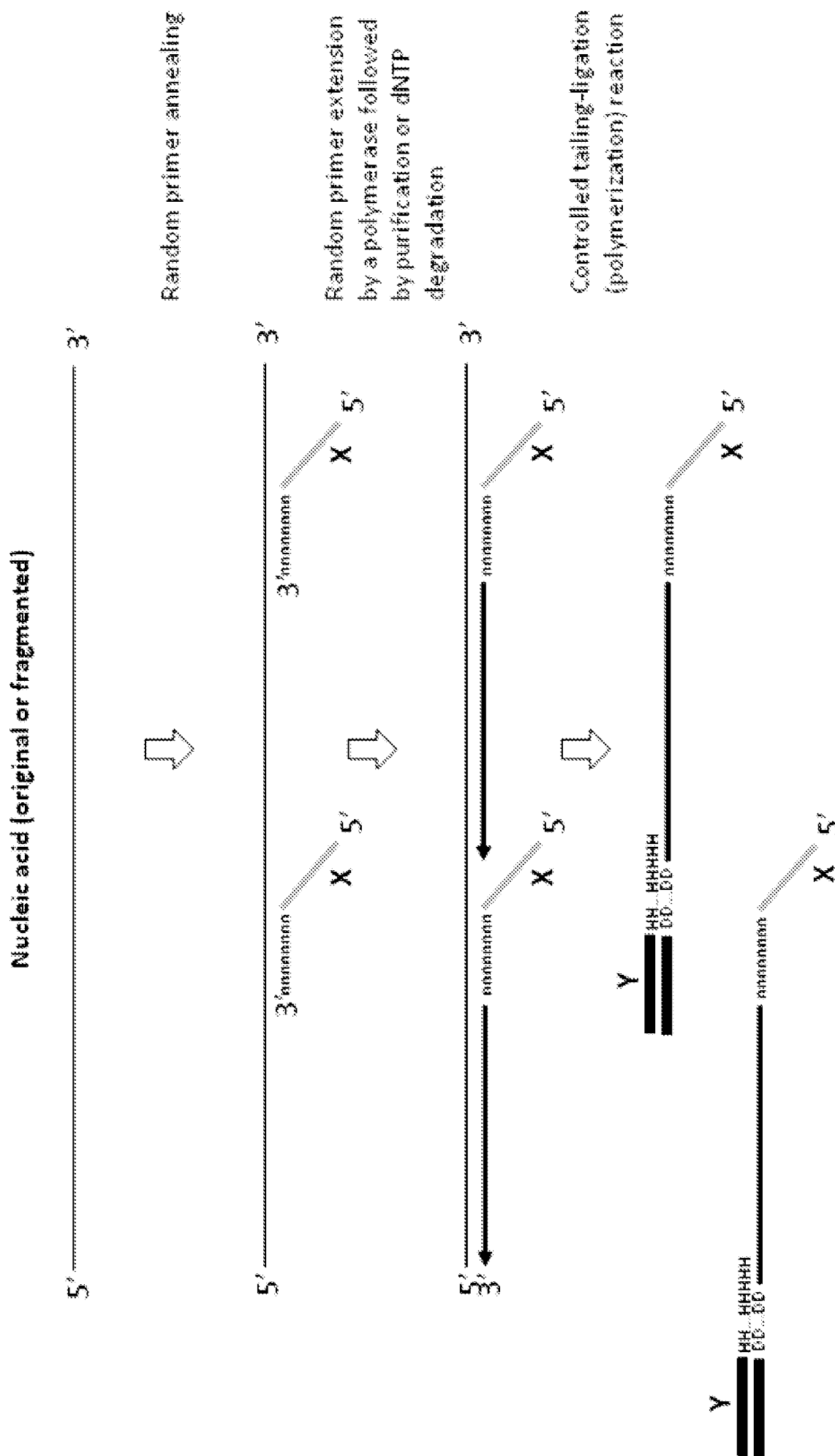


Figure 29



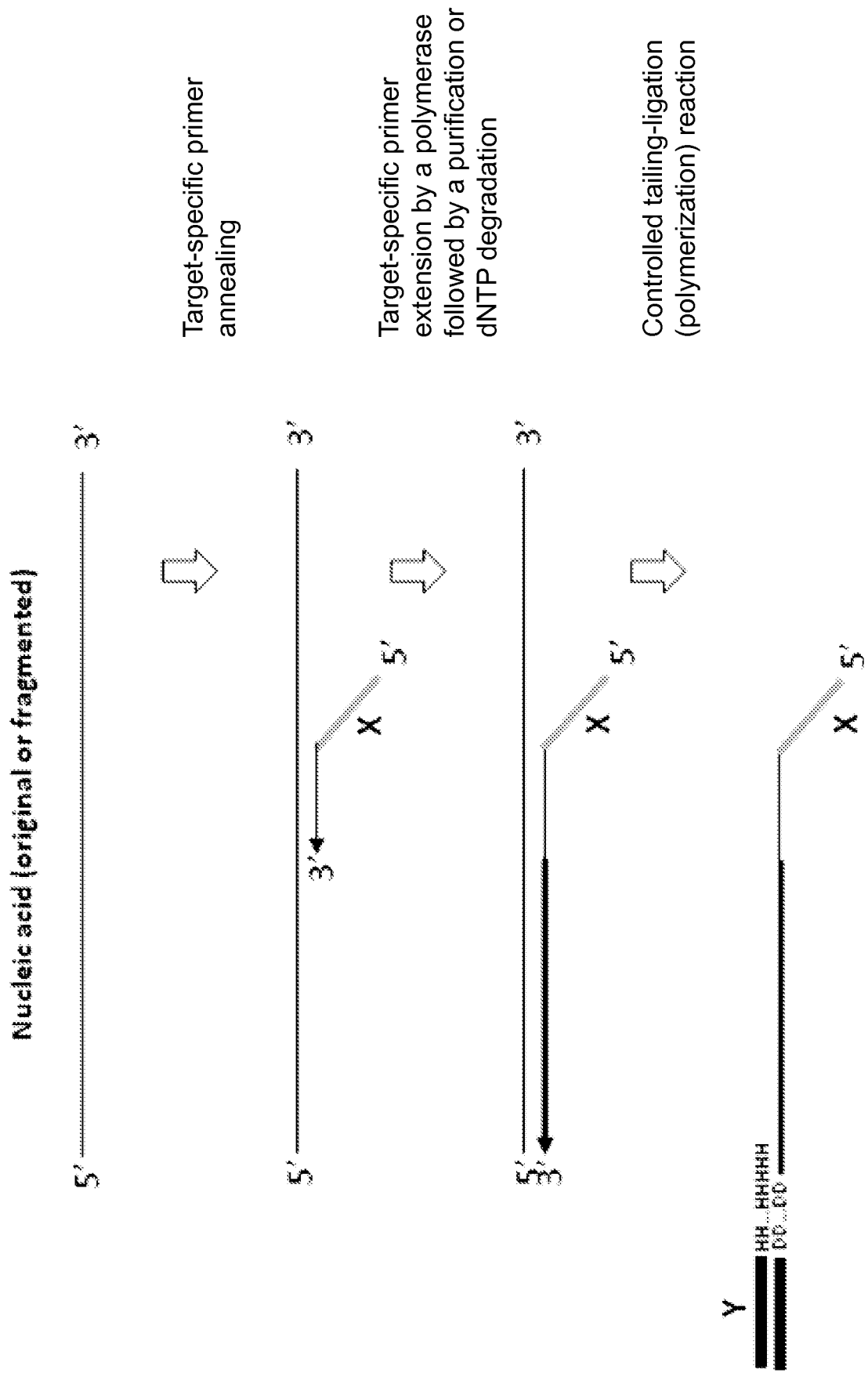
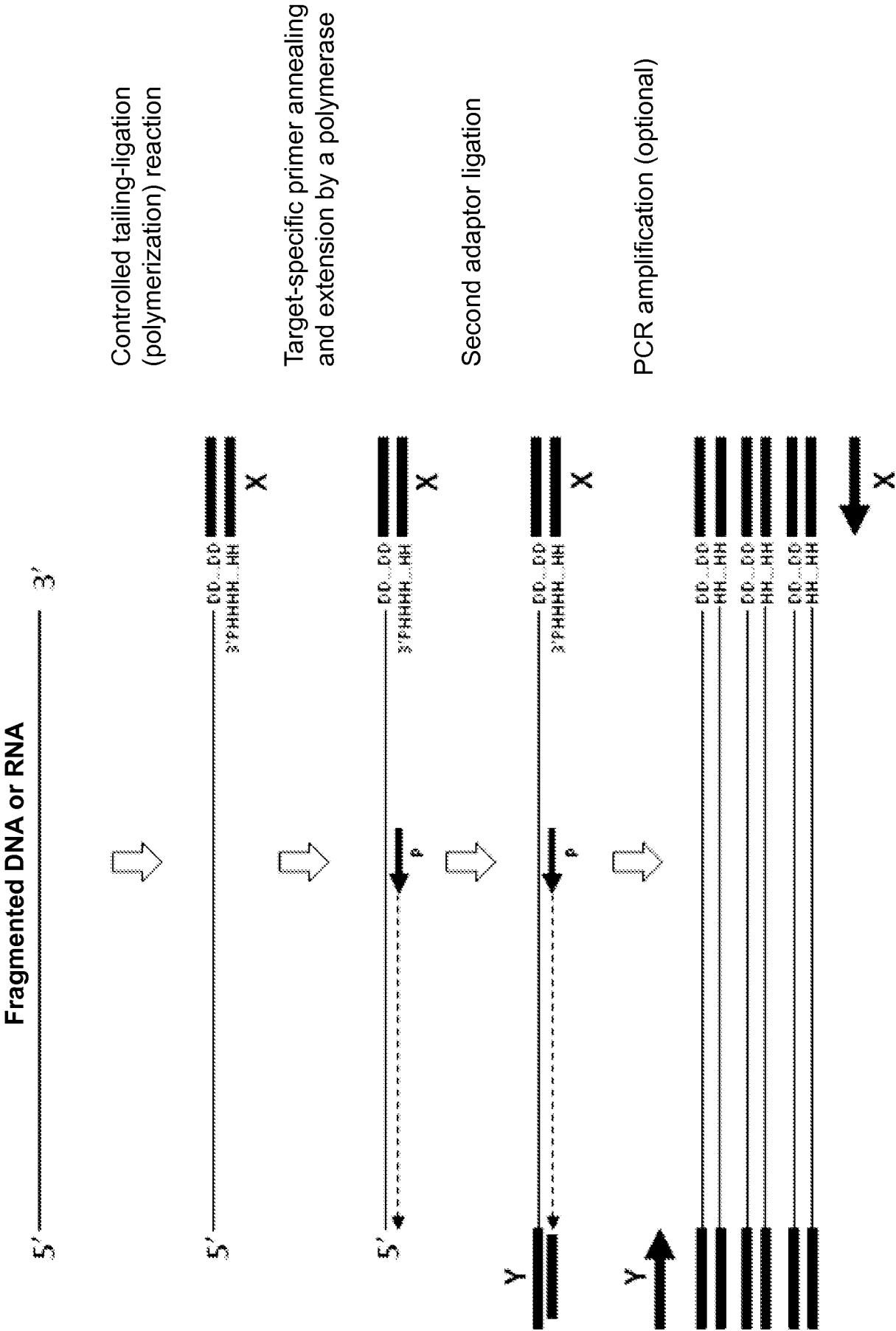


Figure 31



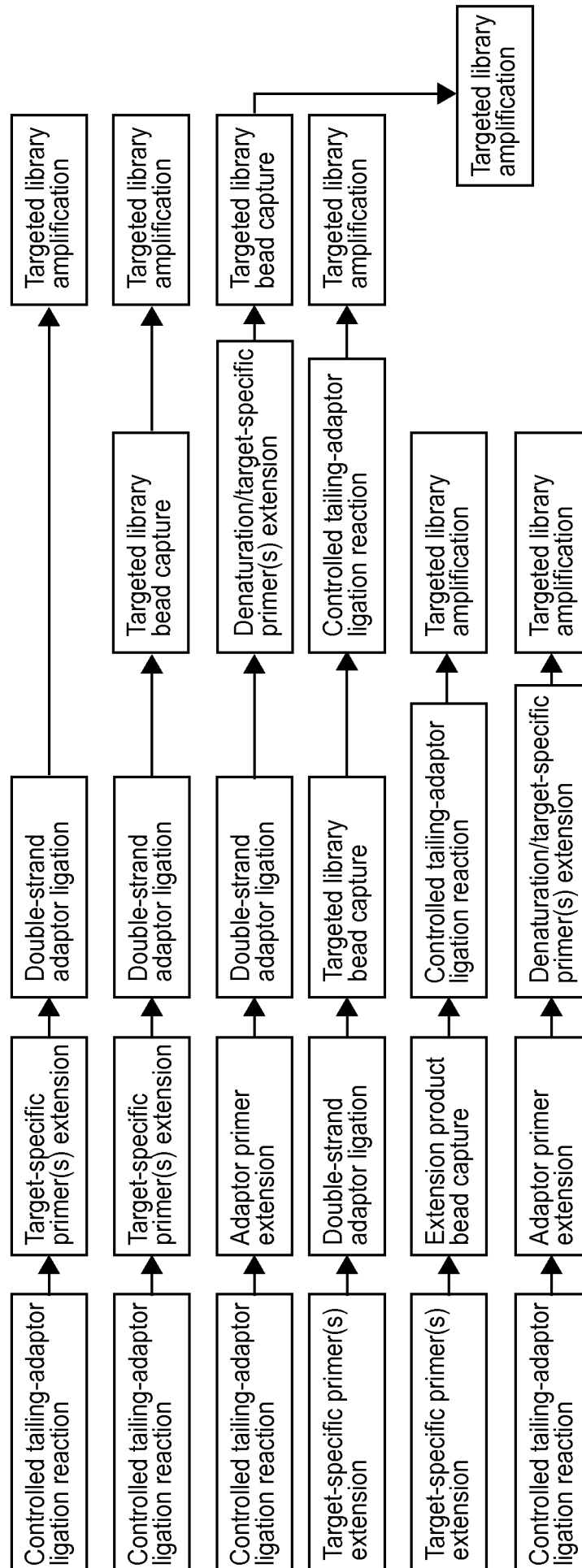
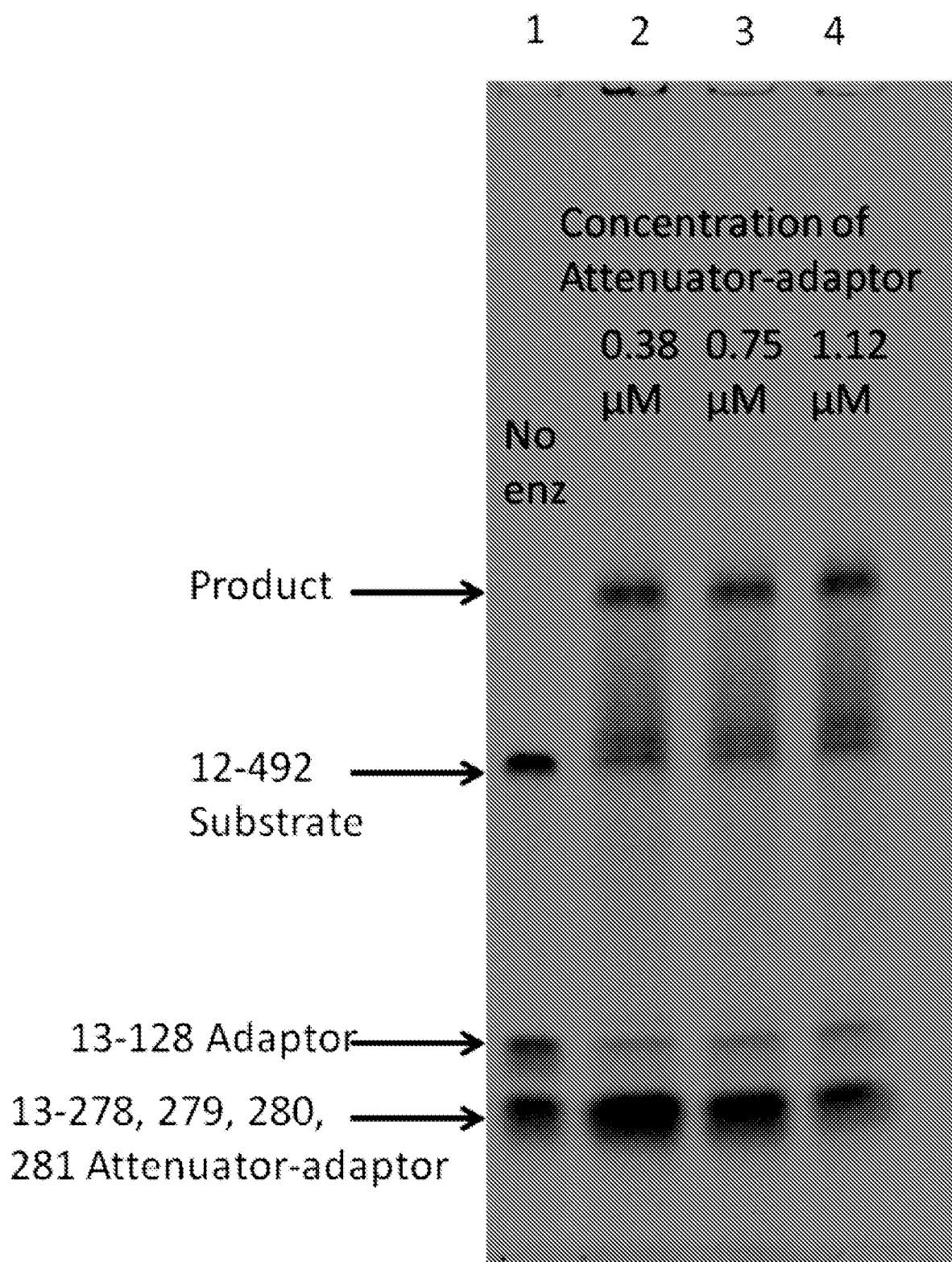


Figure 33

39/42

**Figure 34**

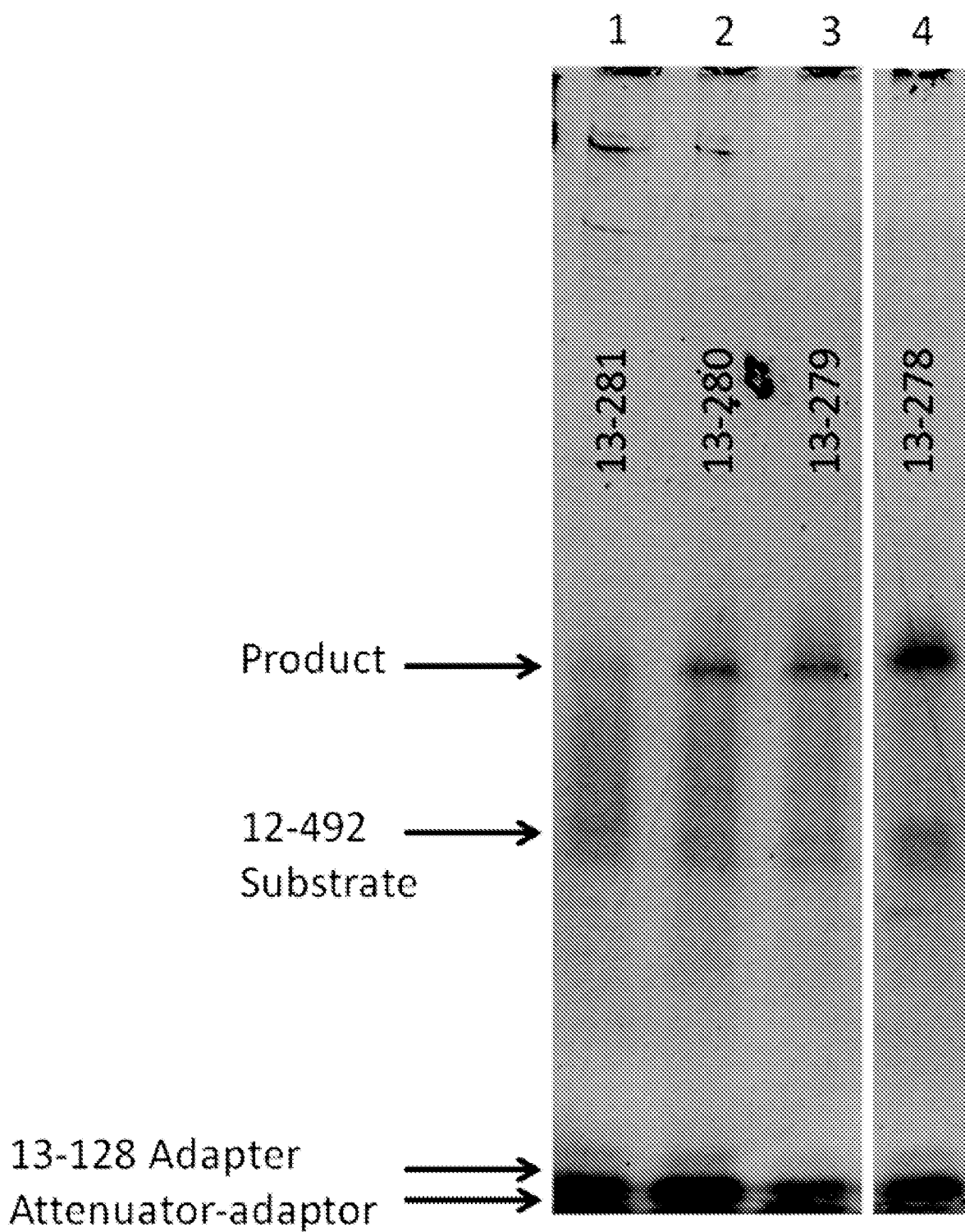


Figure 35

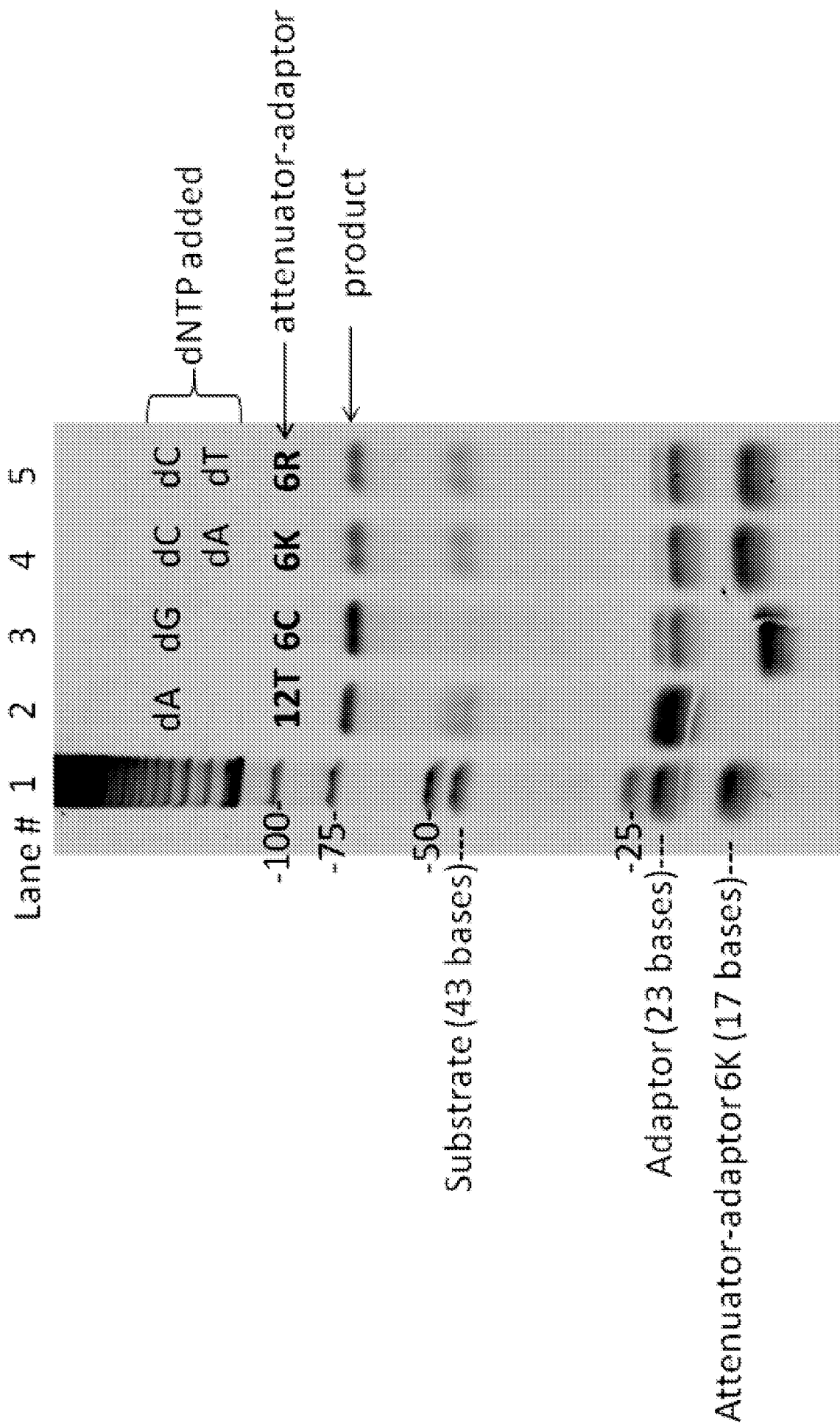


Figure 36

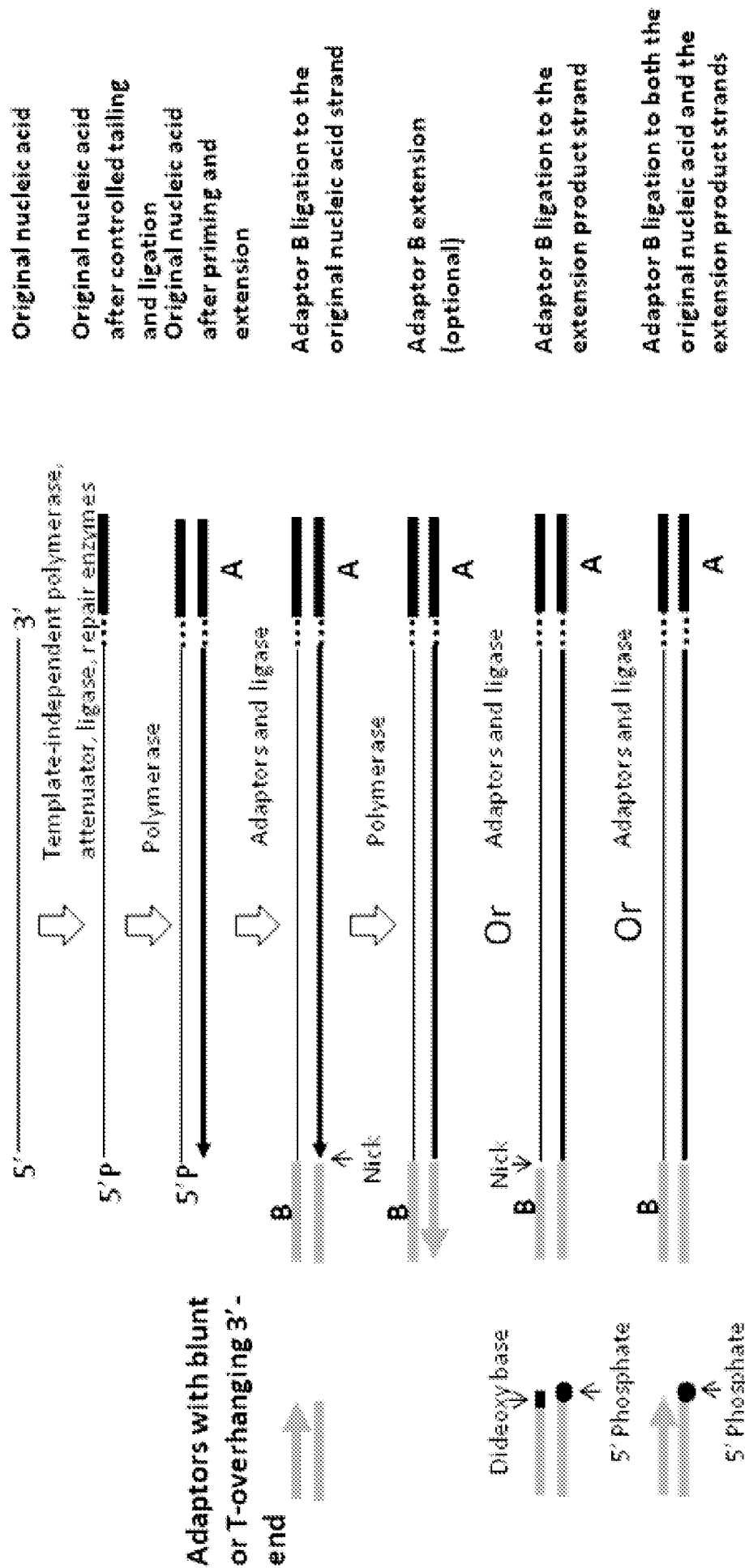


Figure 37