

1/76

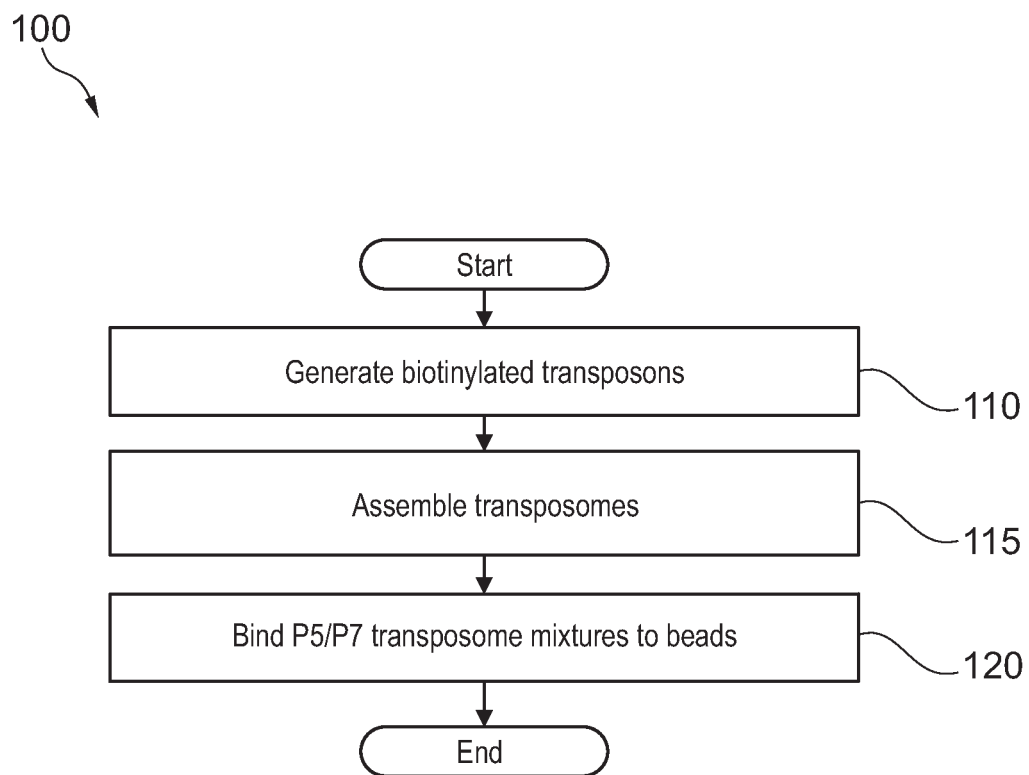


Fig. 1

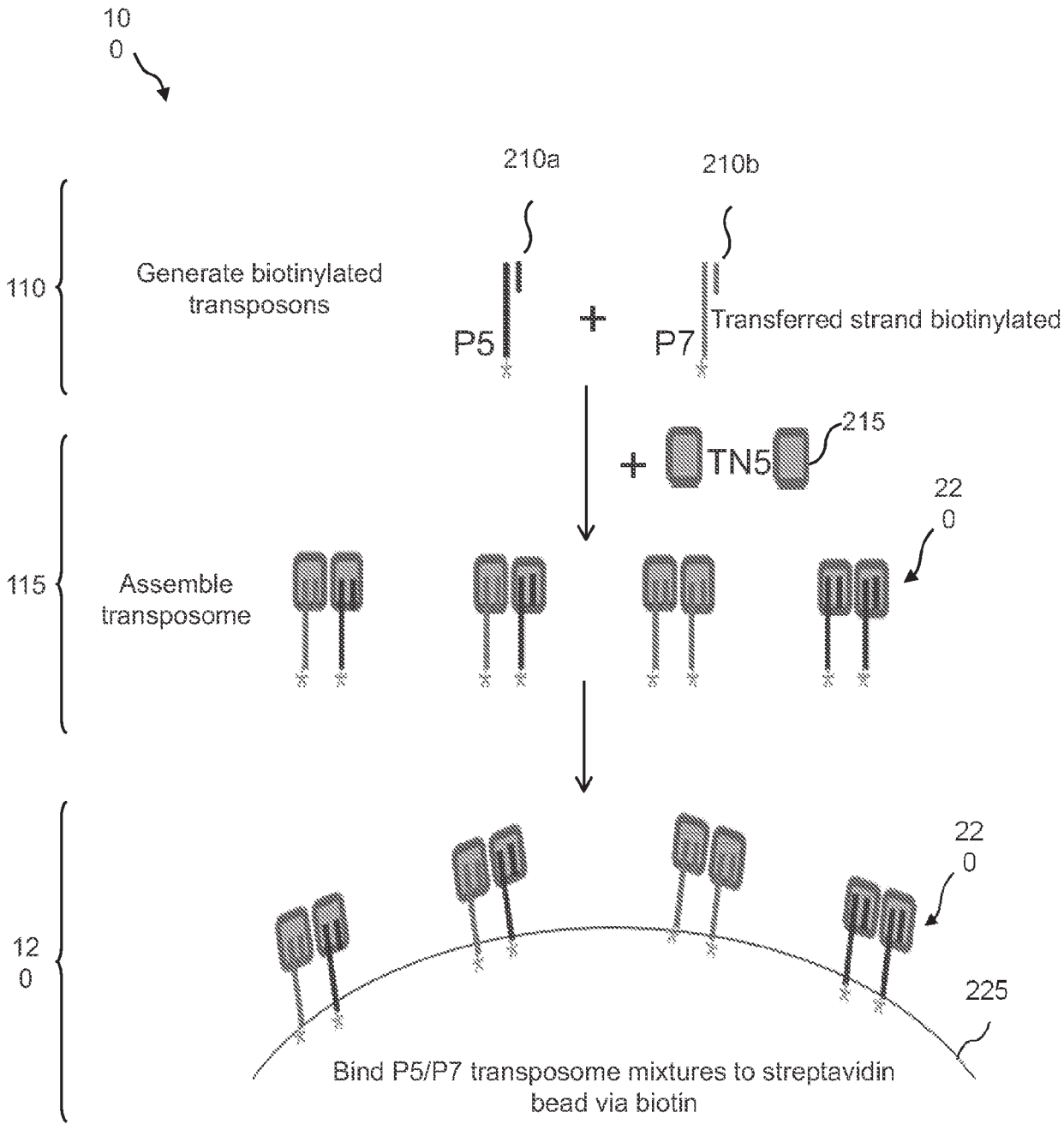


Figure 2

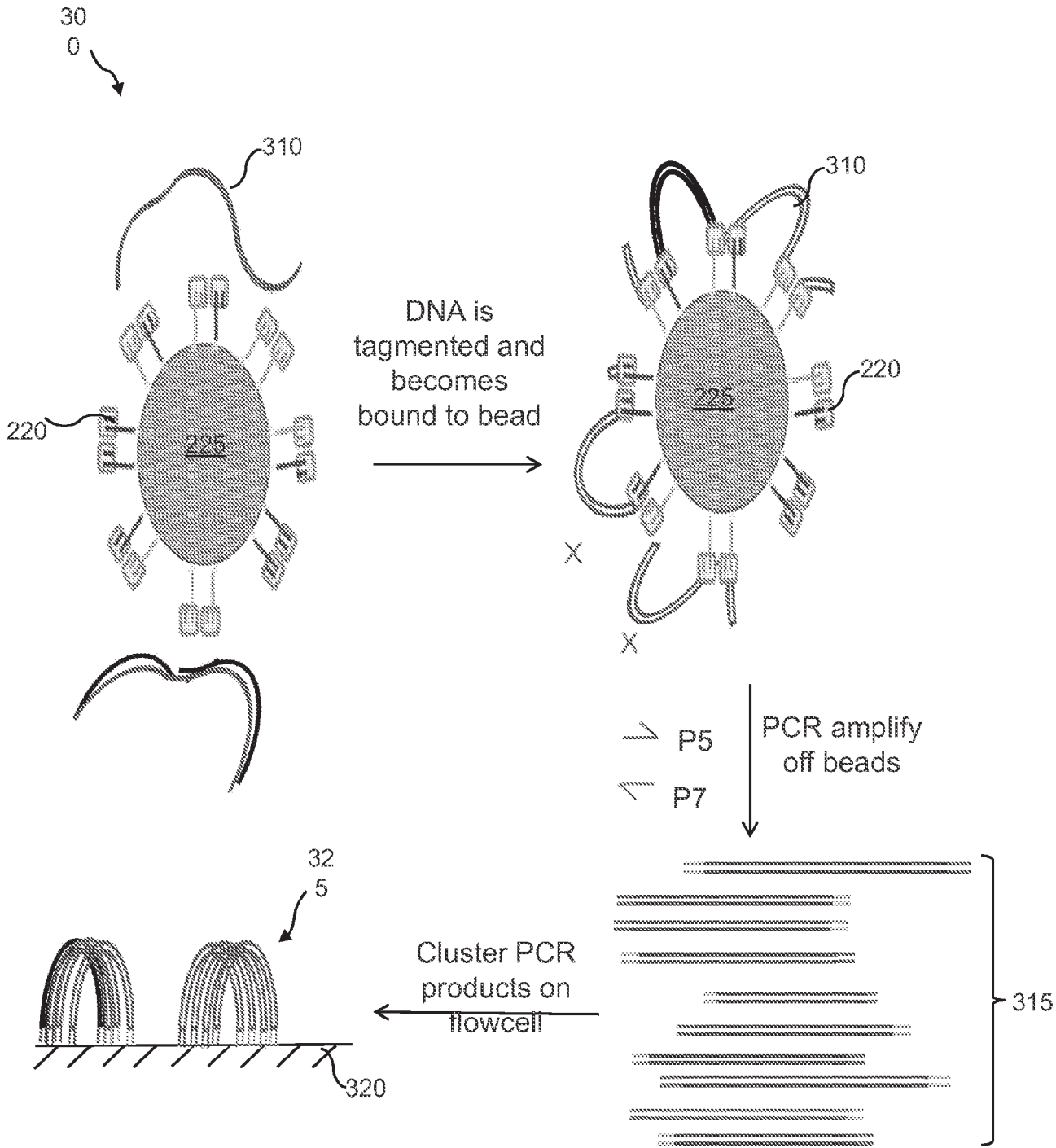


Figure 3

40
0



Sample	No of clusters with equal volume of PCR product							
pM used to cluster	15		19		21		24	
	K/m m2	%	K/m m2	%	K/m m2	%	K/m m2	%
50 ng	466	100	541	100	618	100	668	100
250ng	504	108	598	110	703	114	740	111
1000ng	512	109	602	111	670	108	716	107
*50ng full repeat	377	81	455	84	516	83	555	83

Figure 4

500 

	Median Insert Size	Mean Insert size
BBN50-index1	341	409.78
BBN500-index1	344	415.89
BBN50-index2	337	405.97
BBN500-index2	346	419.91
BBN50-index3	330	400.35
BBN500-index3	327	398.65
BBN50-index4	321	388.71
BBN500-index4	328	400.75
BBN50-index5	327	397.62
BBN500-index5	328	399.88
BBN50-index6	324	389.35
BBN500-index6	331	401.50

Figure 5

600 ↗

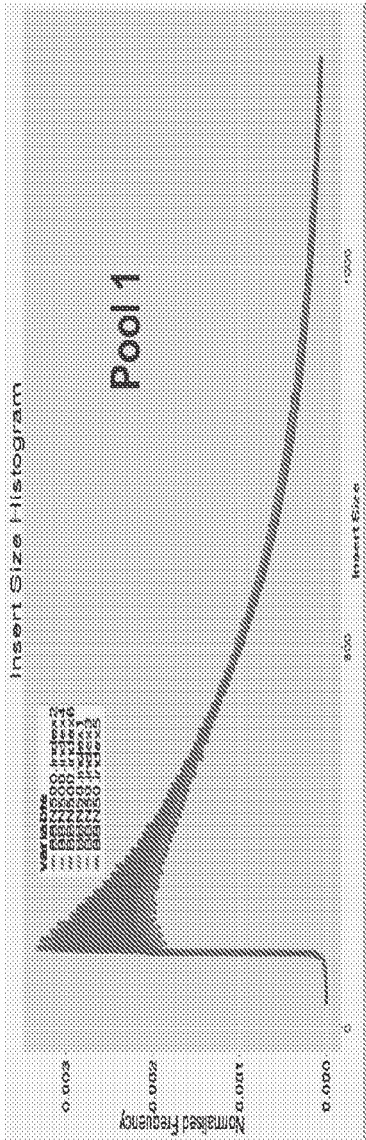


Figure 6A

650 ↗

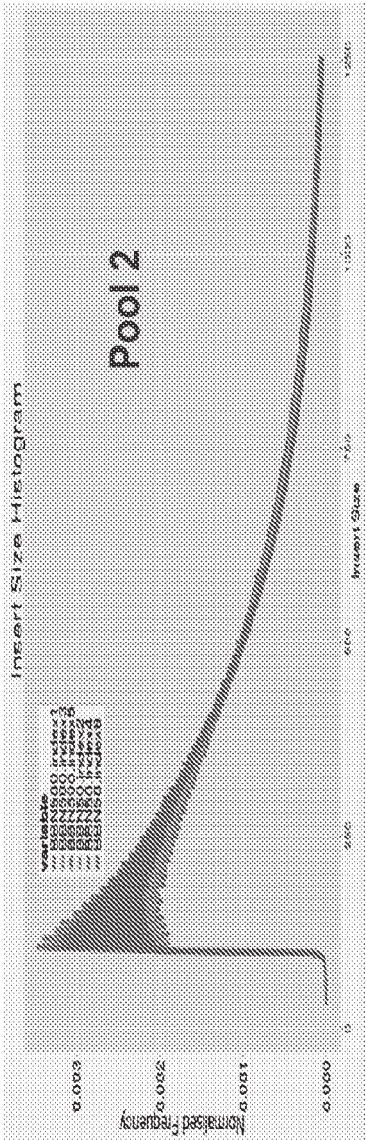


Figure 6B

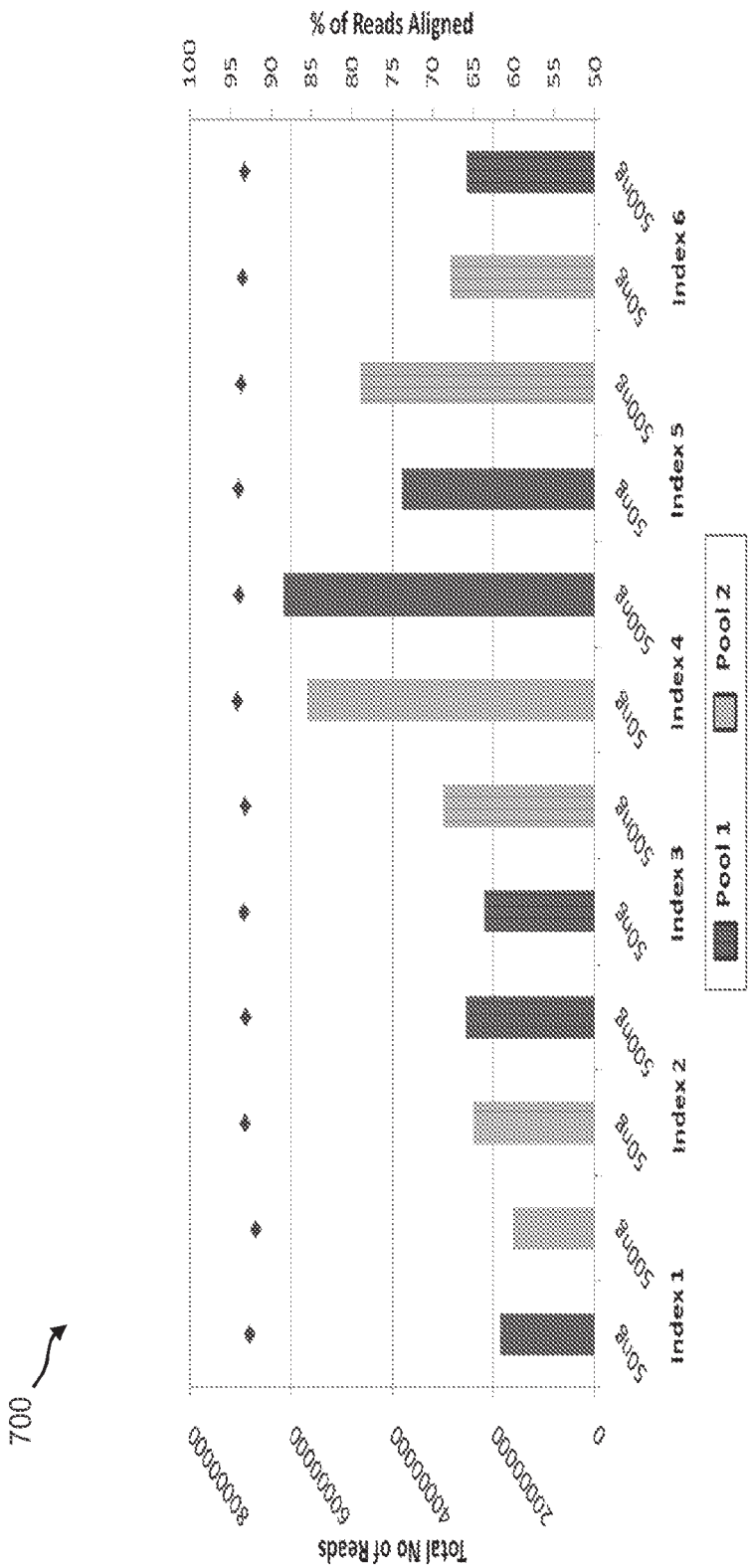
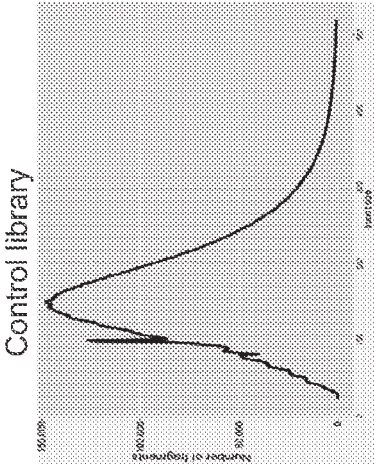


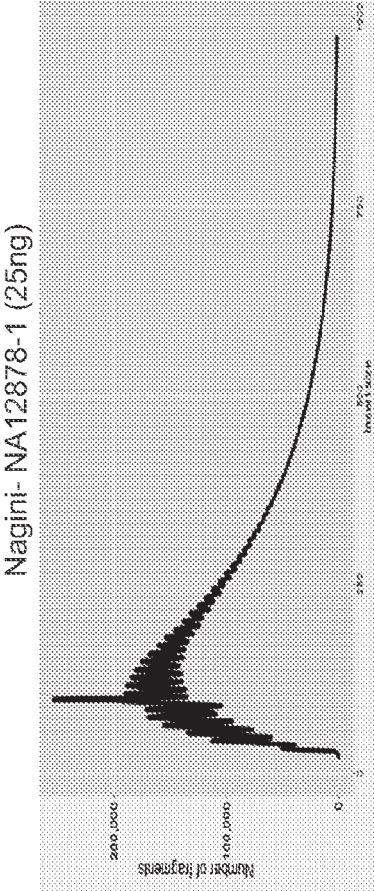
Figure 7

Figure 8A



800

Figure 8B



820

840

	Control	25ng	50ng	100ng	150ng	200ng	500ng
Mean	196.8	267.4	287.3	288.8	288.6	295.0	282.7
Median	163	206	222	224	223	230	220
mode	149	98	98	98	98	98	98

Figure 8C

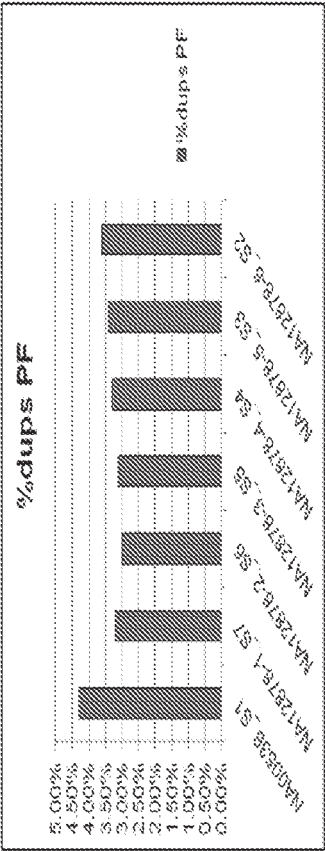


Figure 9A

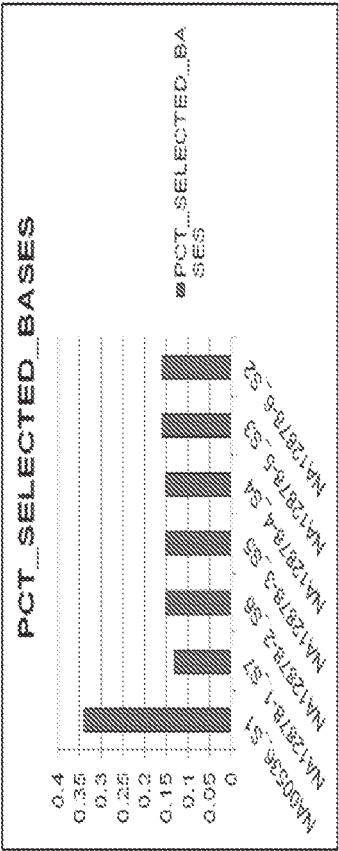


Figure 9B

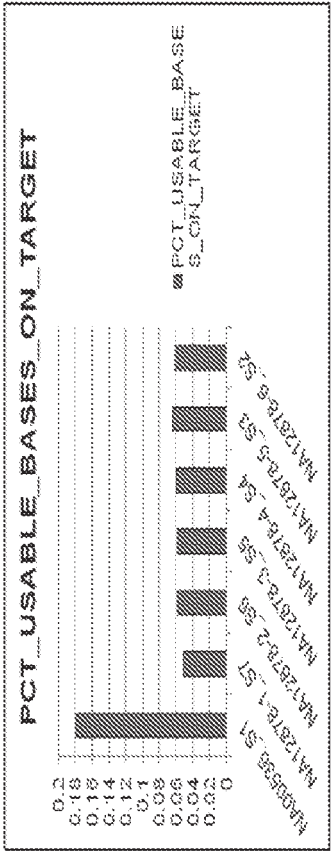


Figure 9C

900

920

940

1000 ↗

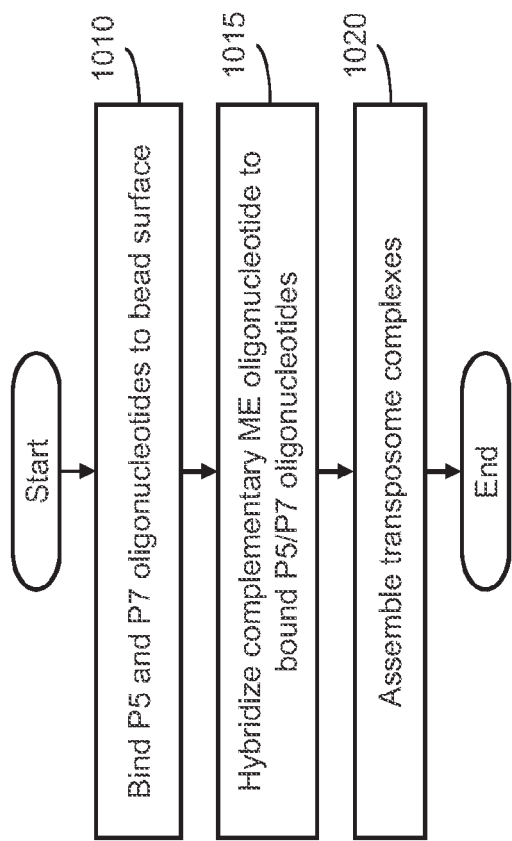


Figure 10

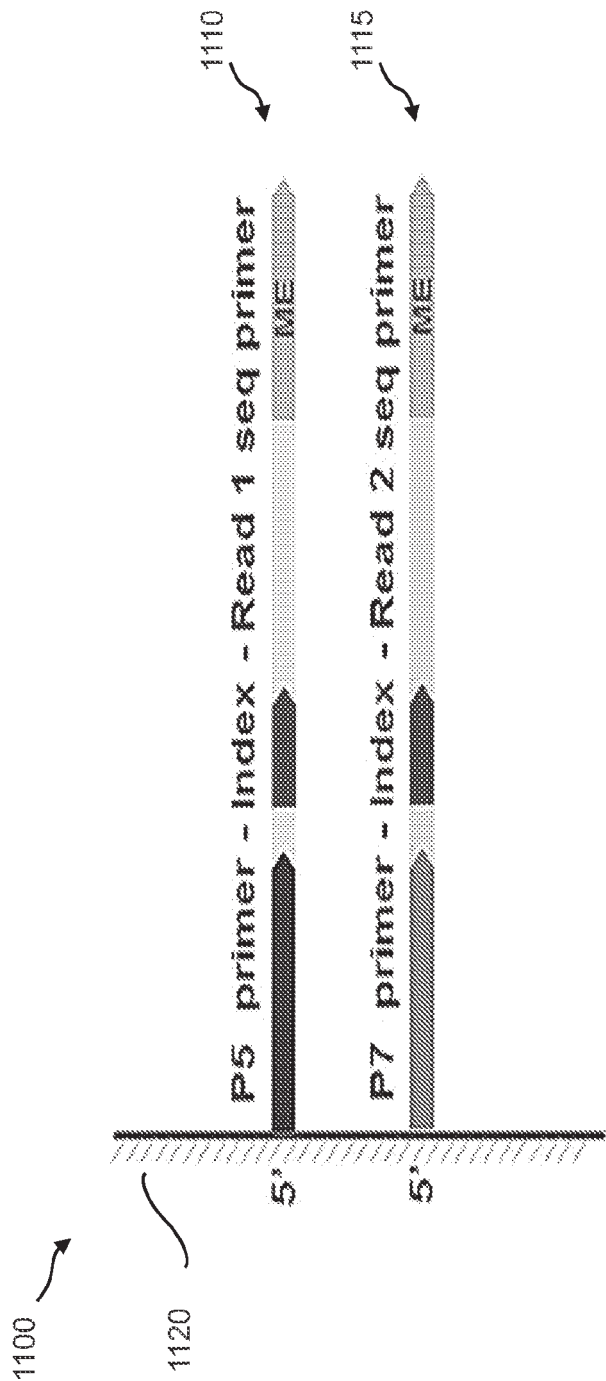


Figure 11

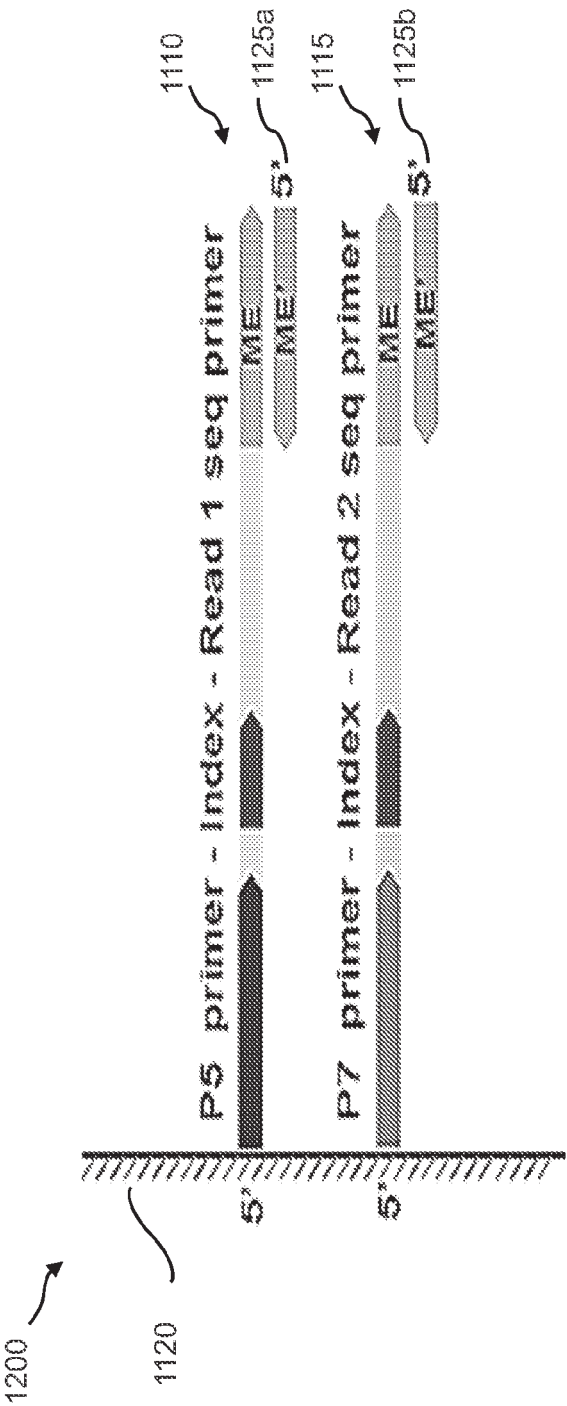


Figure 12

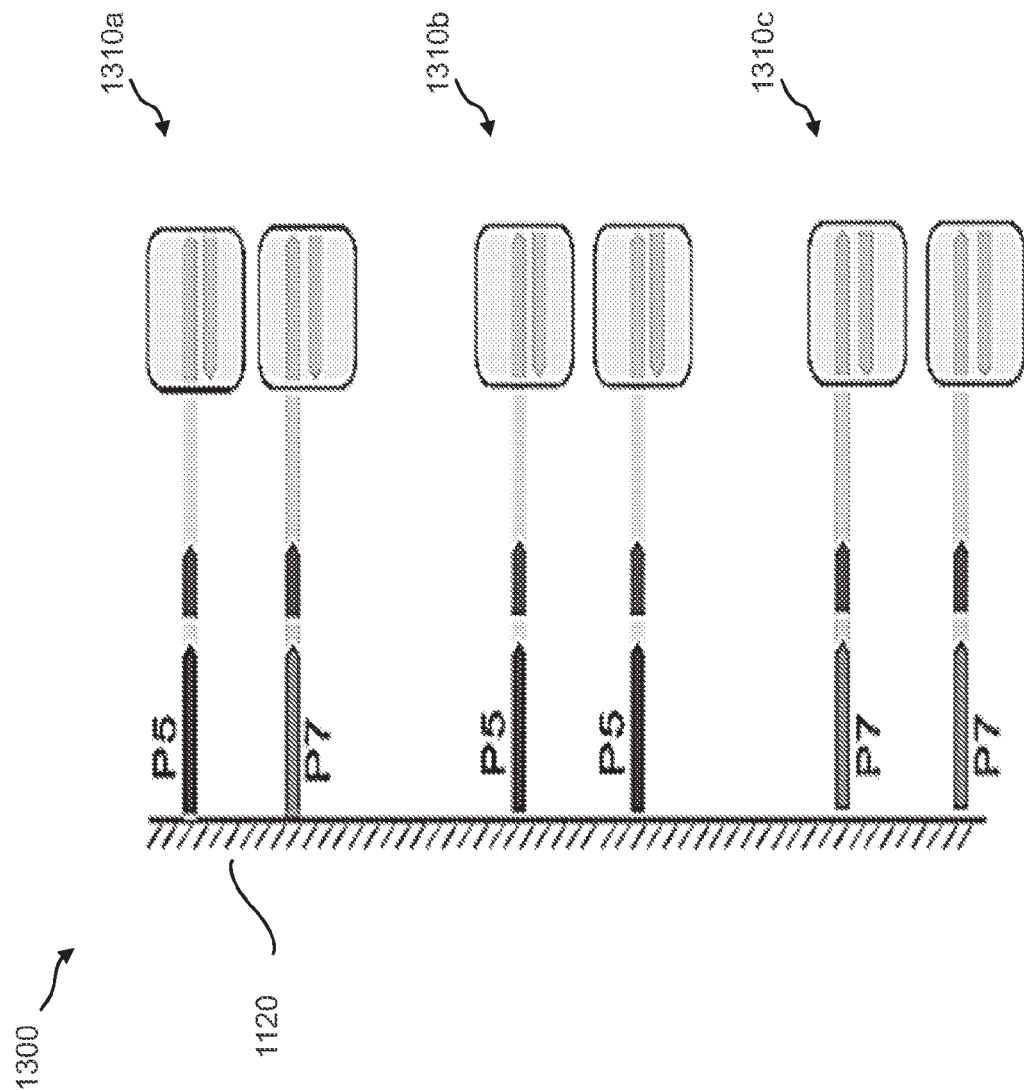


Figure 13

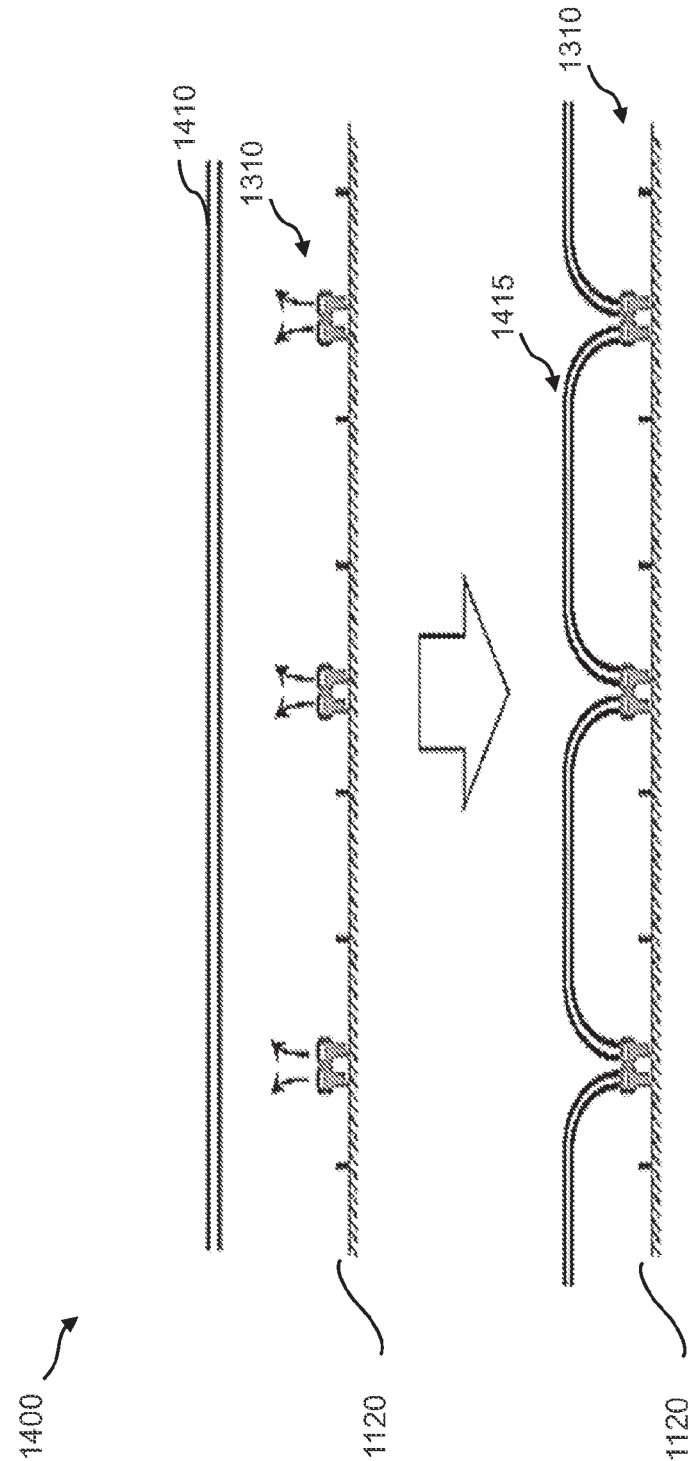


Figure 14

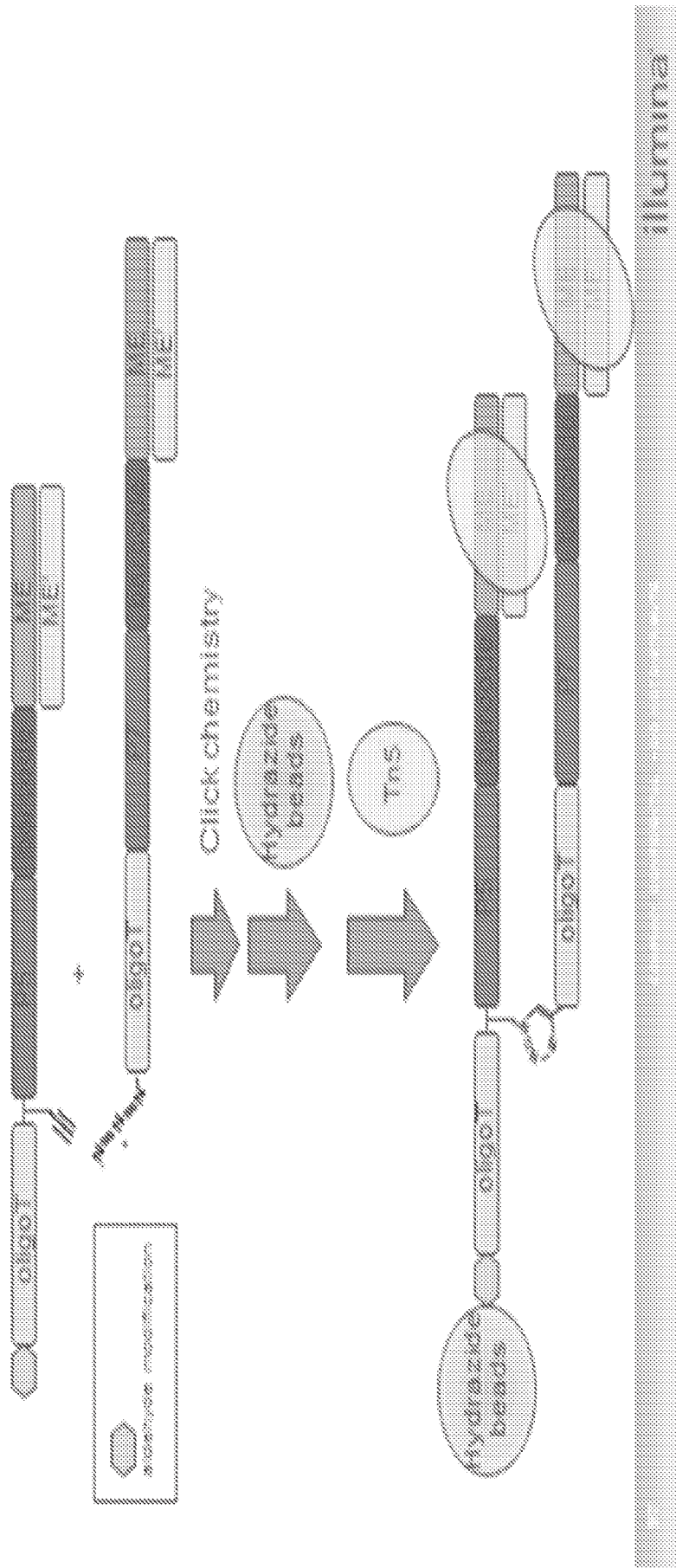


Figure 15

CPT on beads using hybridization based indexing

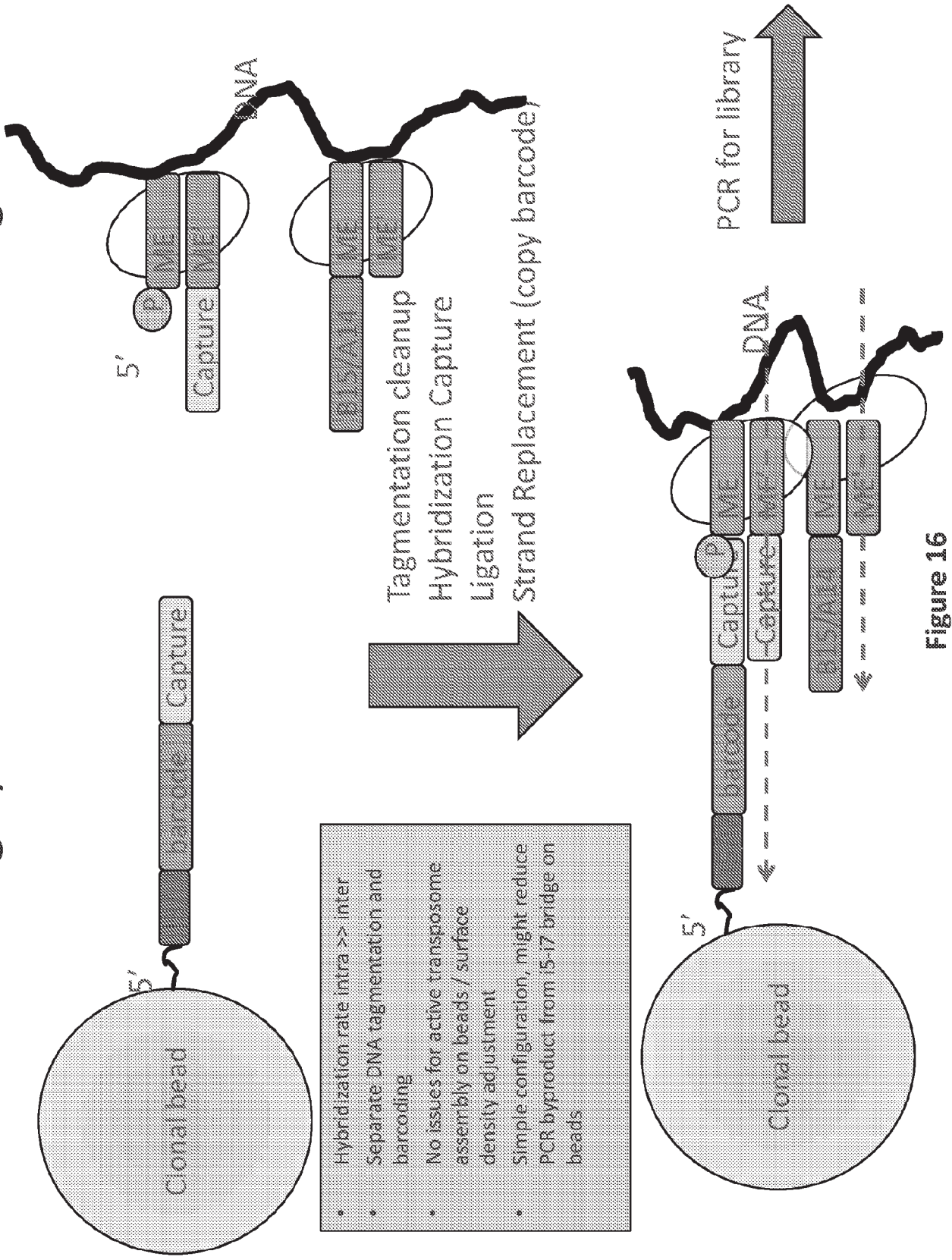


Figure 16

Contiguity Preserving Transposition (CPT) on Beads

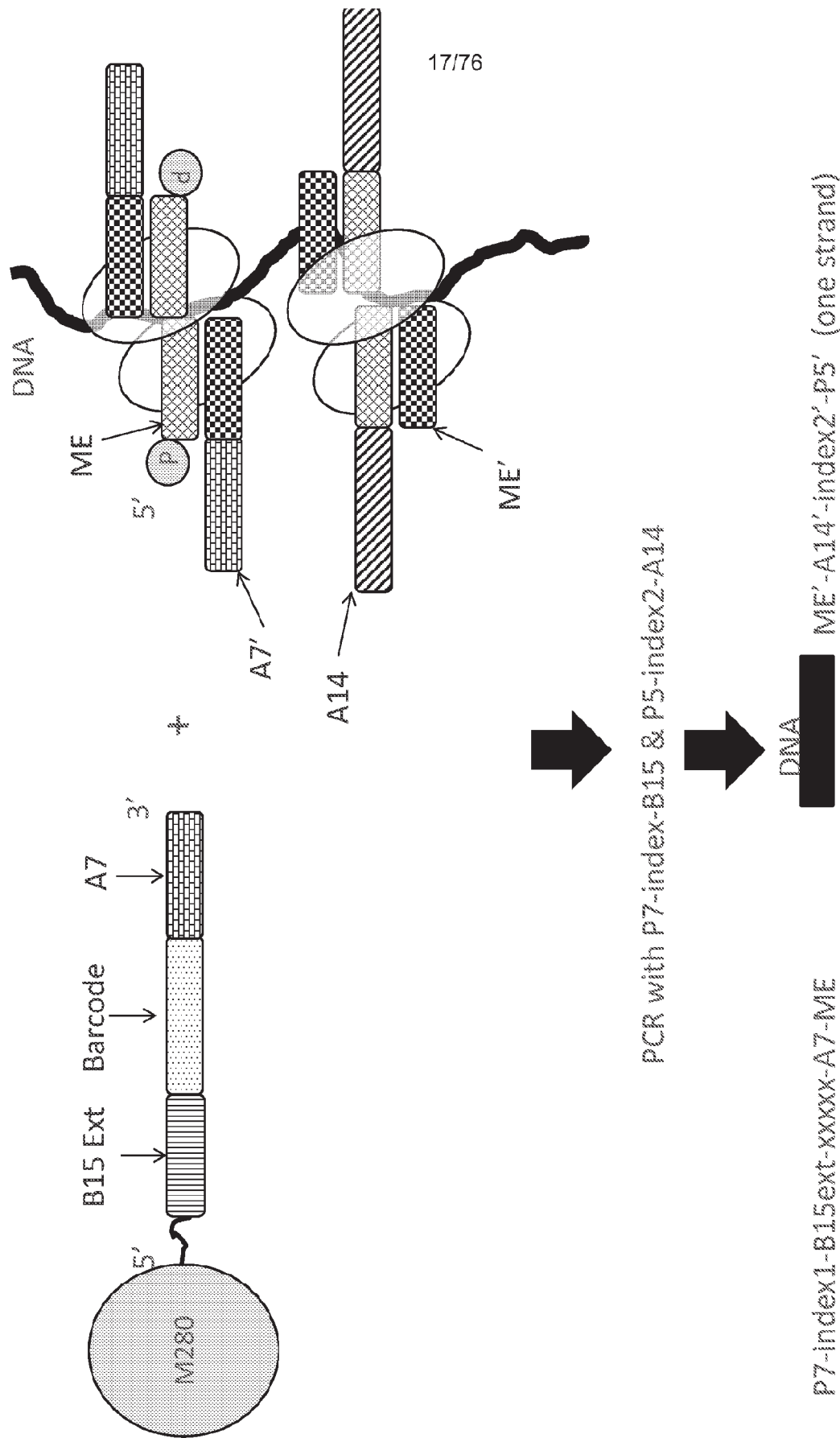


Figure 17

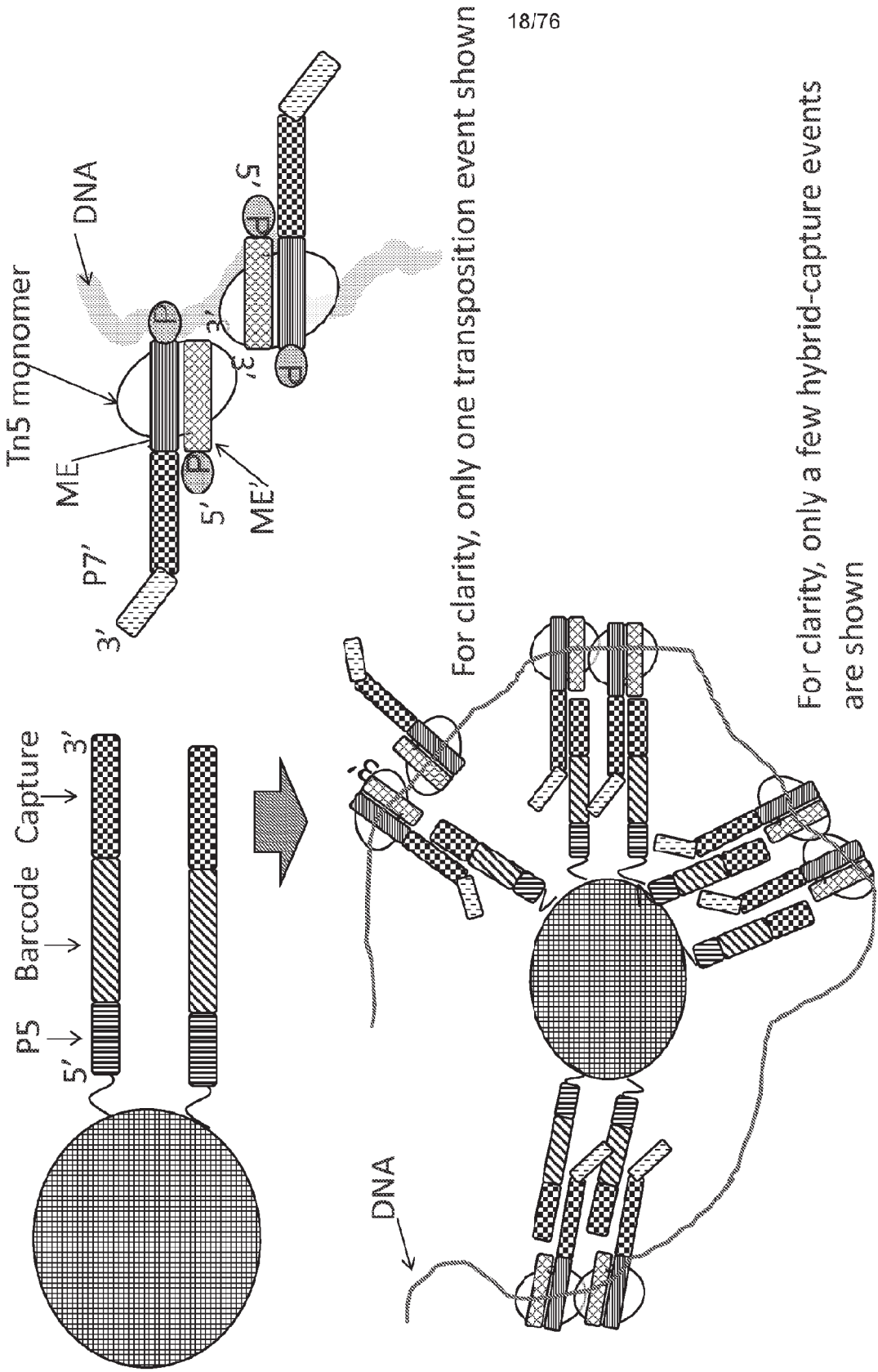
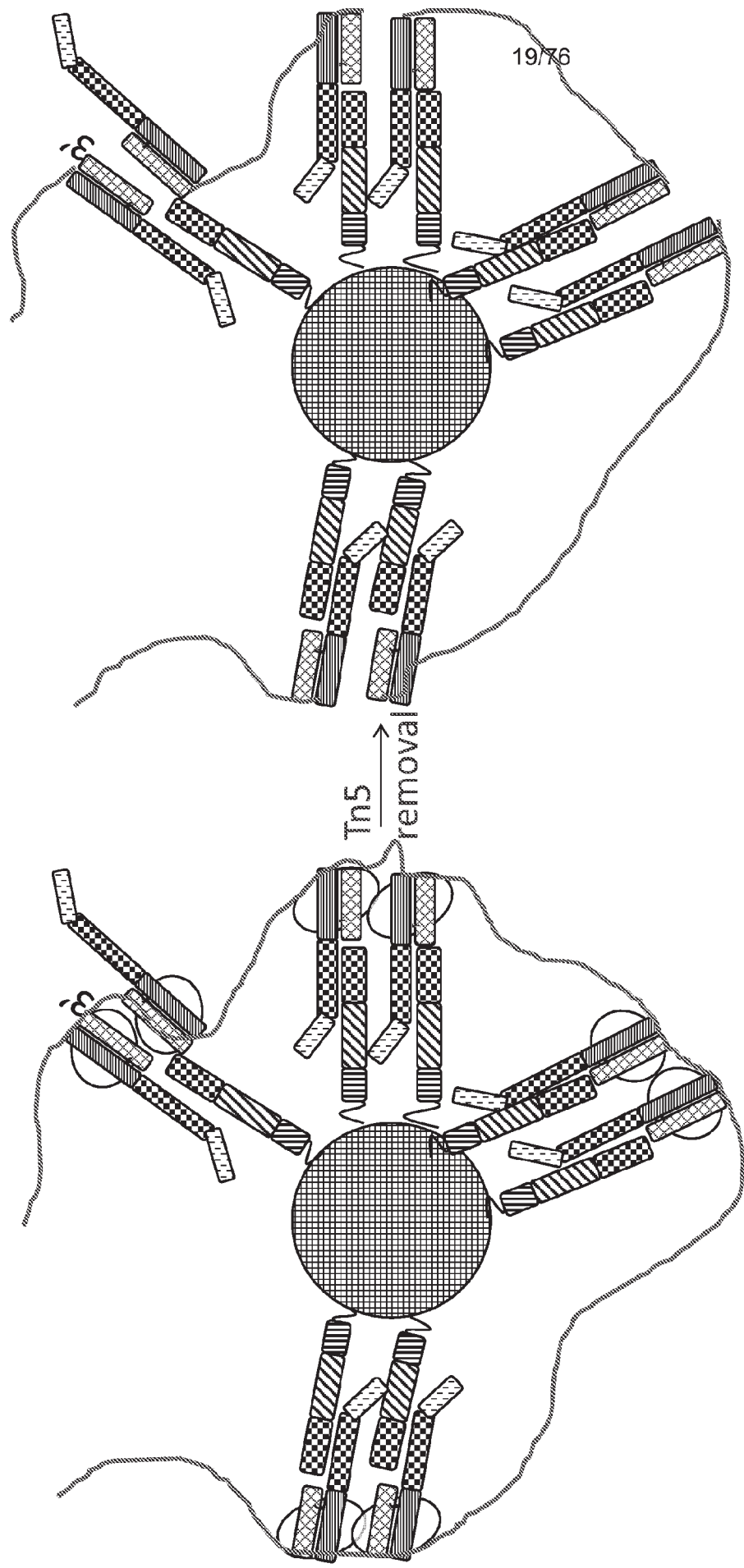


Figure 18



After hybridization capture, contiguity is maintained after Tn5 removal. DNA is hybridized to bead with indexed primers keeping all individual libraries together on a bead.

Figure 19

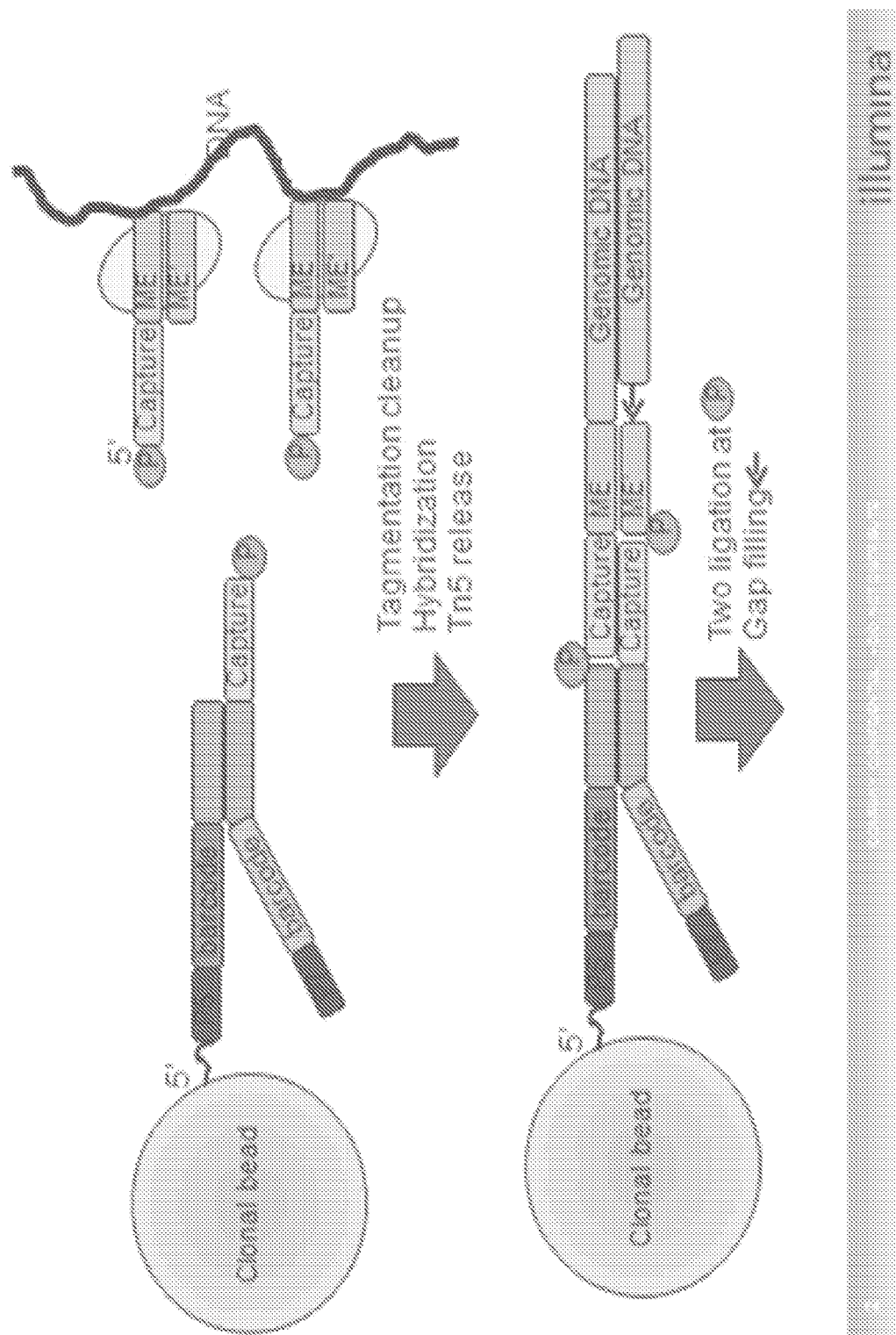


Figure 20

CPT-seq on beads (Y-adapter design)

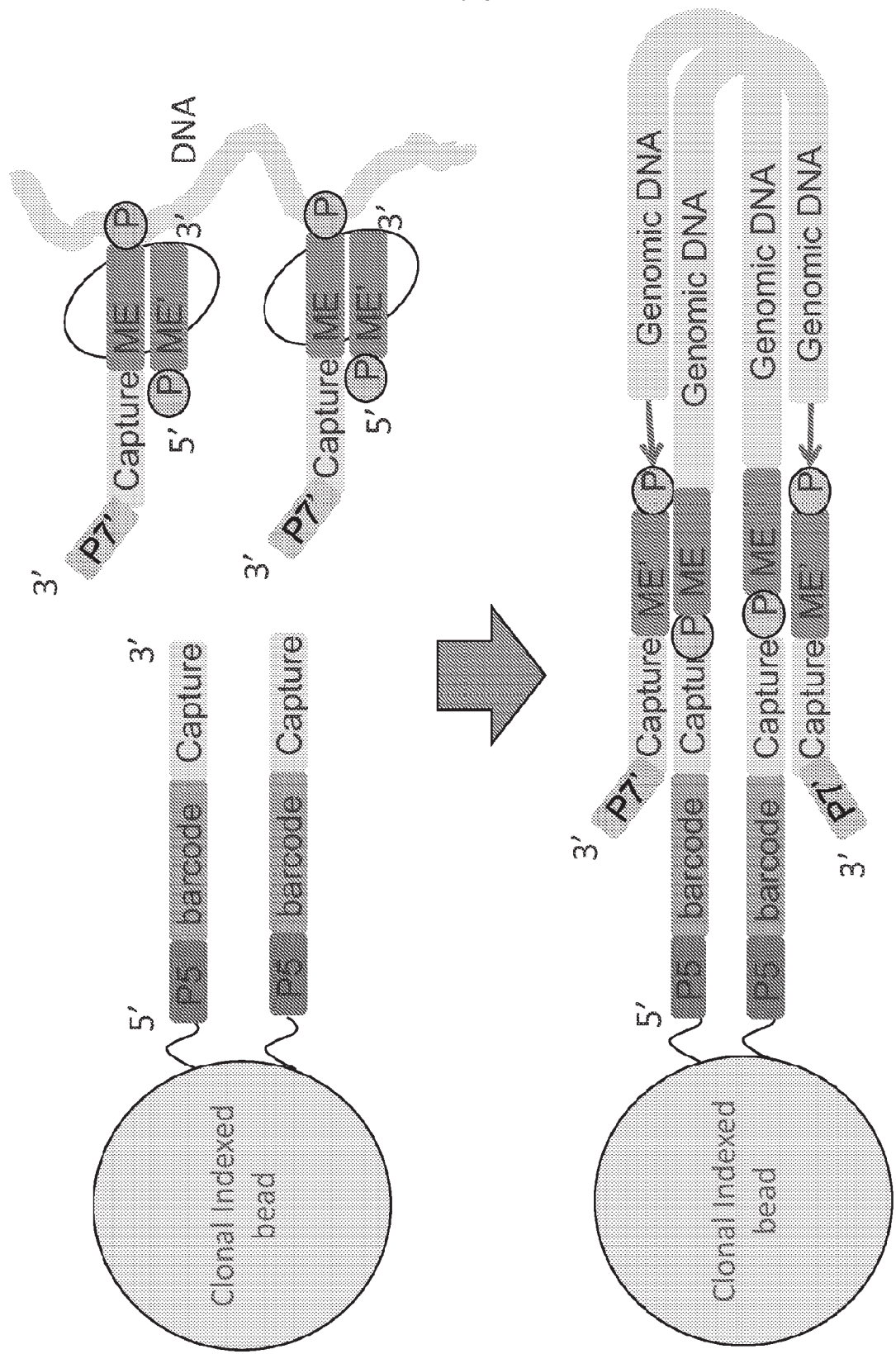


Figure 21

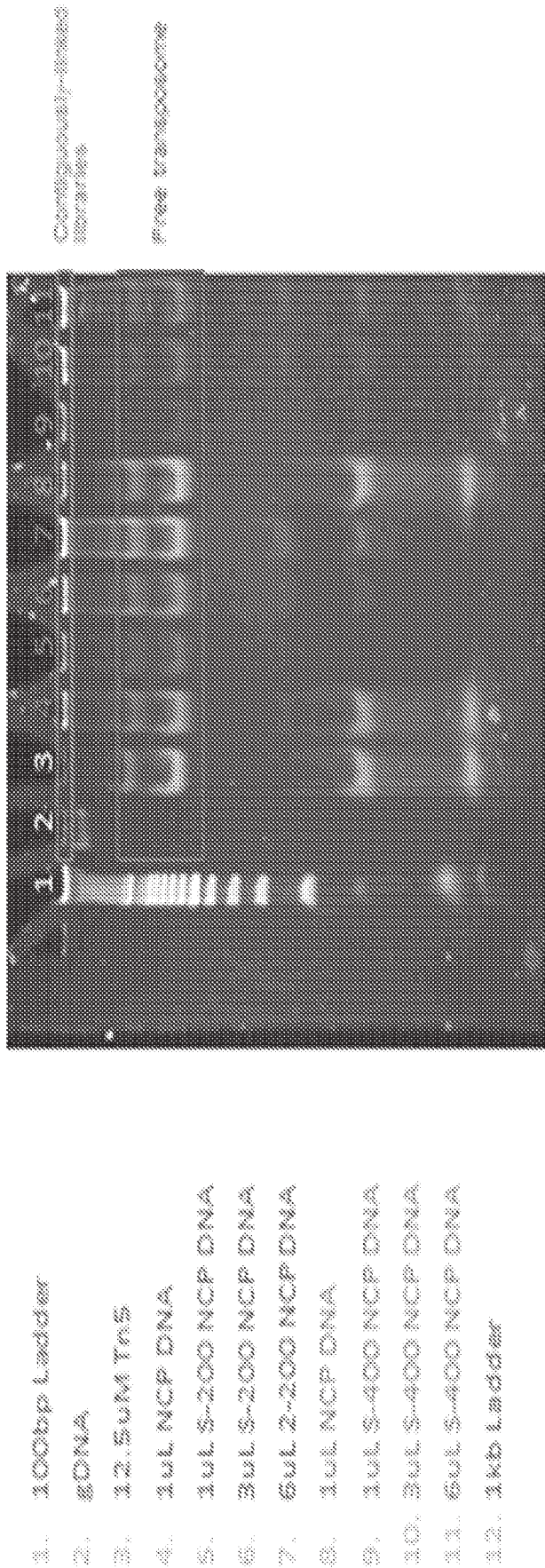


Figure 22

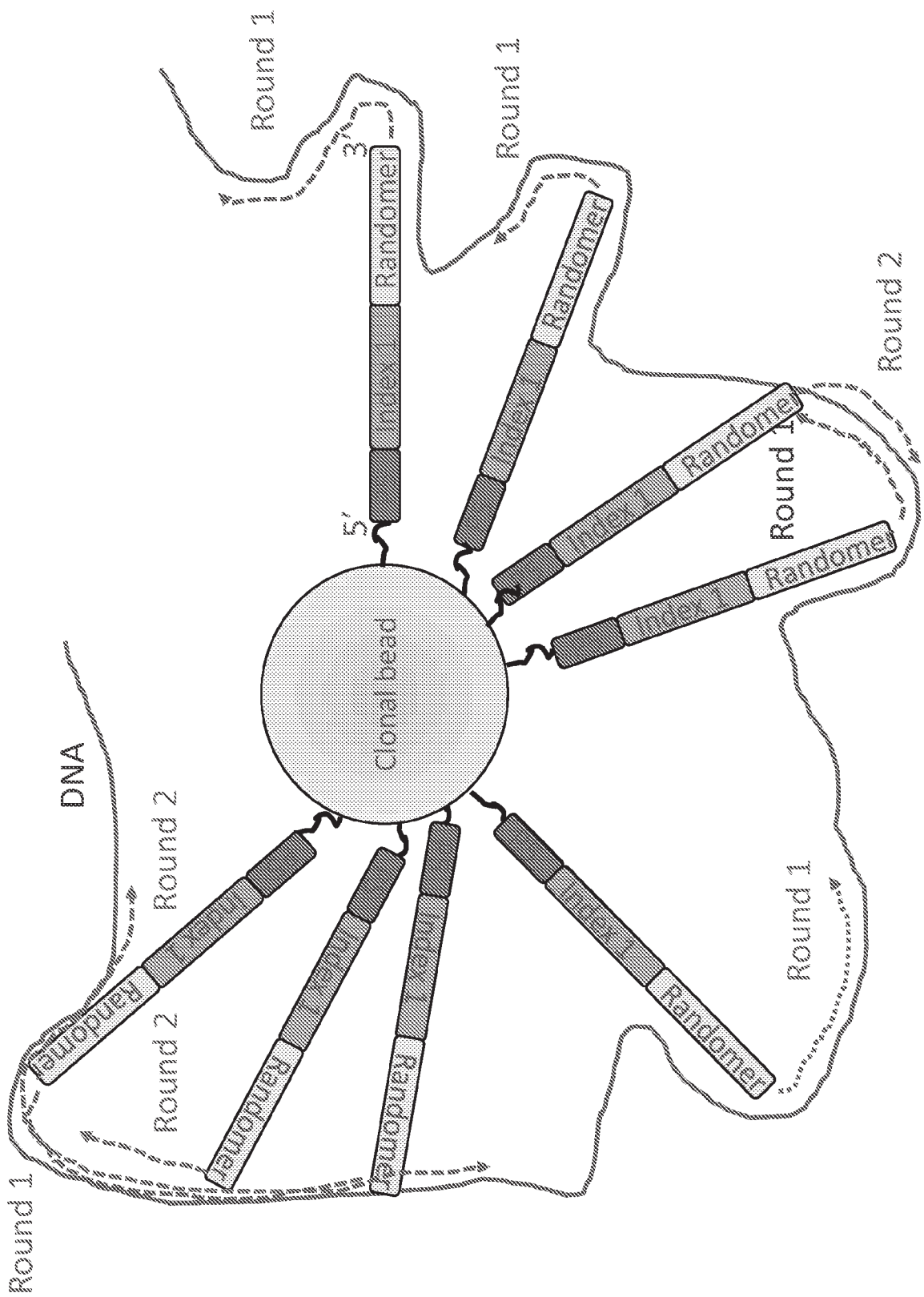


Figure 23

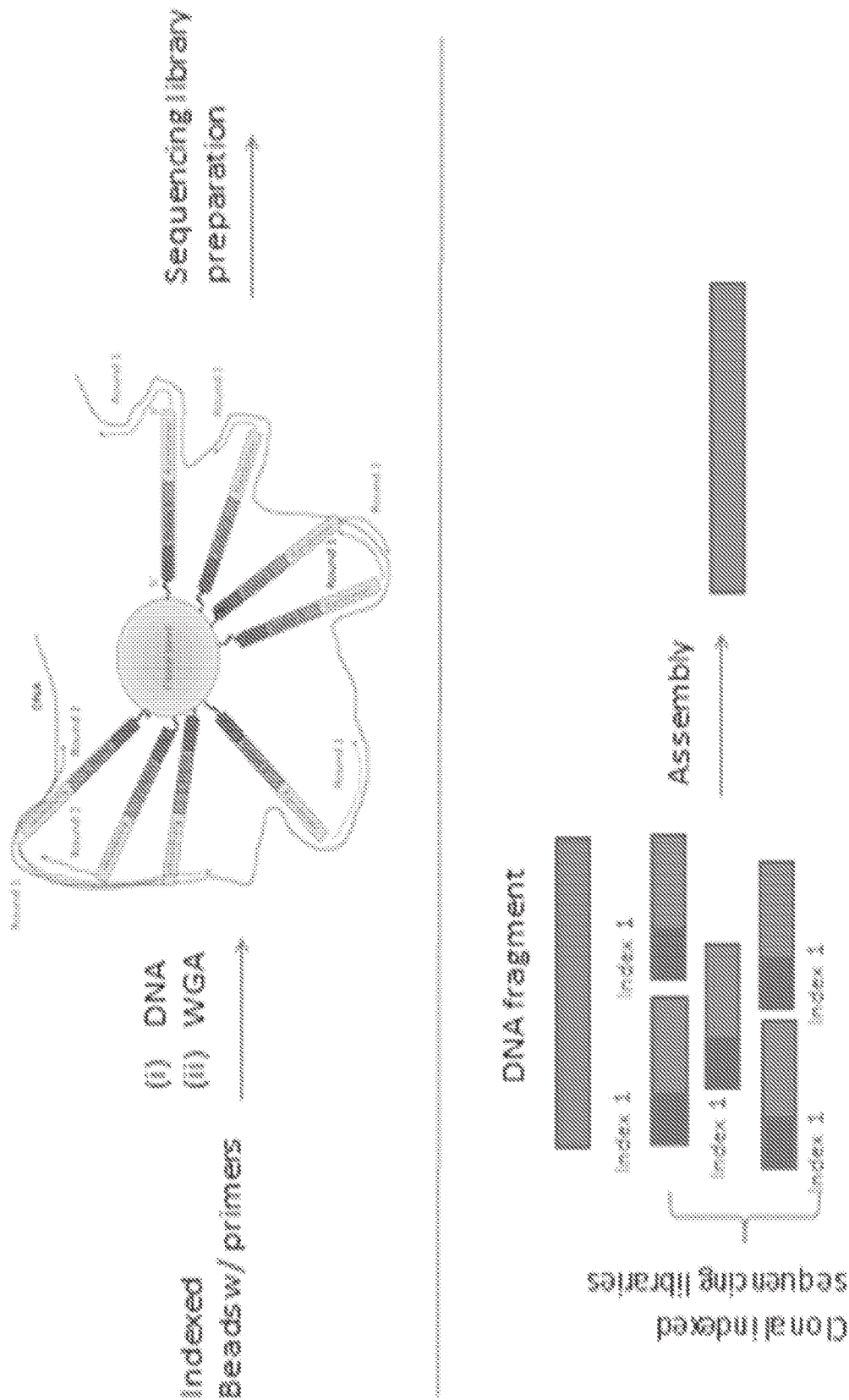
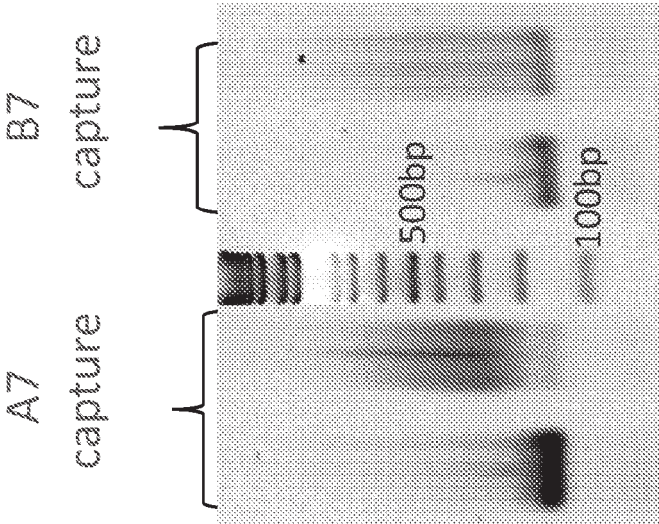


Figure 24

Optimization of Capture Probe Density on Beads



Probe Density (dilution)		Ligation
1	1x	No
2	10x	Yes
3	1x	No
4	10x	Yes

Figure 25

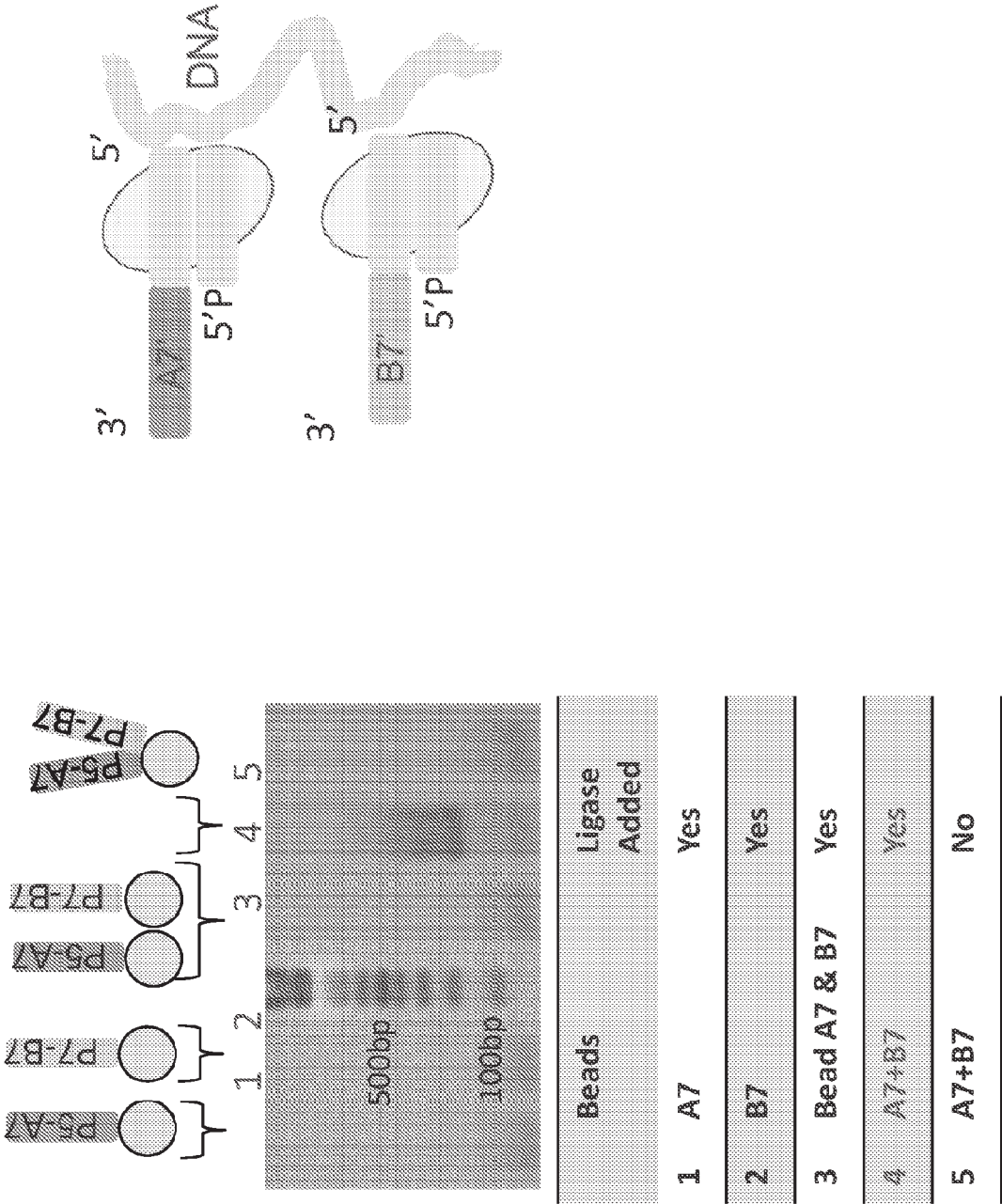


Figure 26

Feasibility of Clonal Indexing

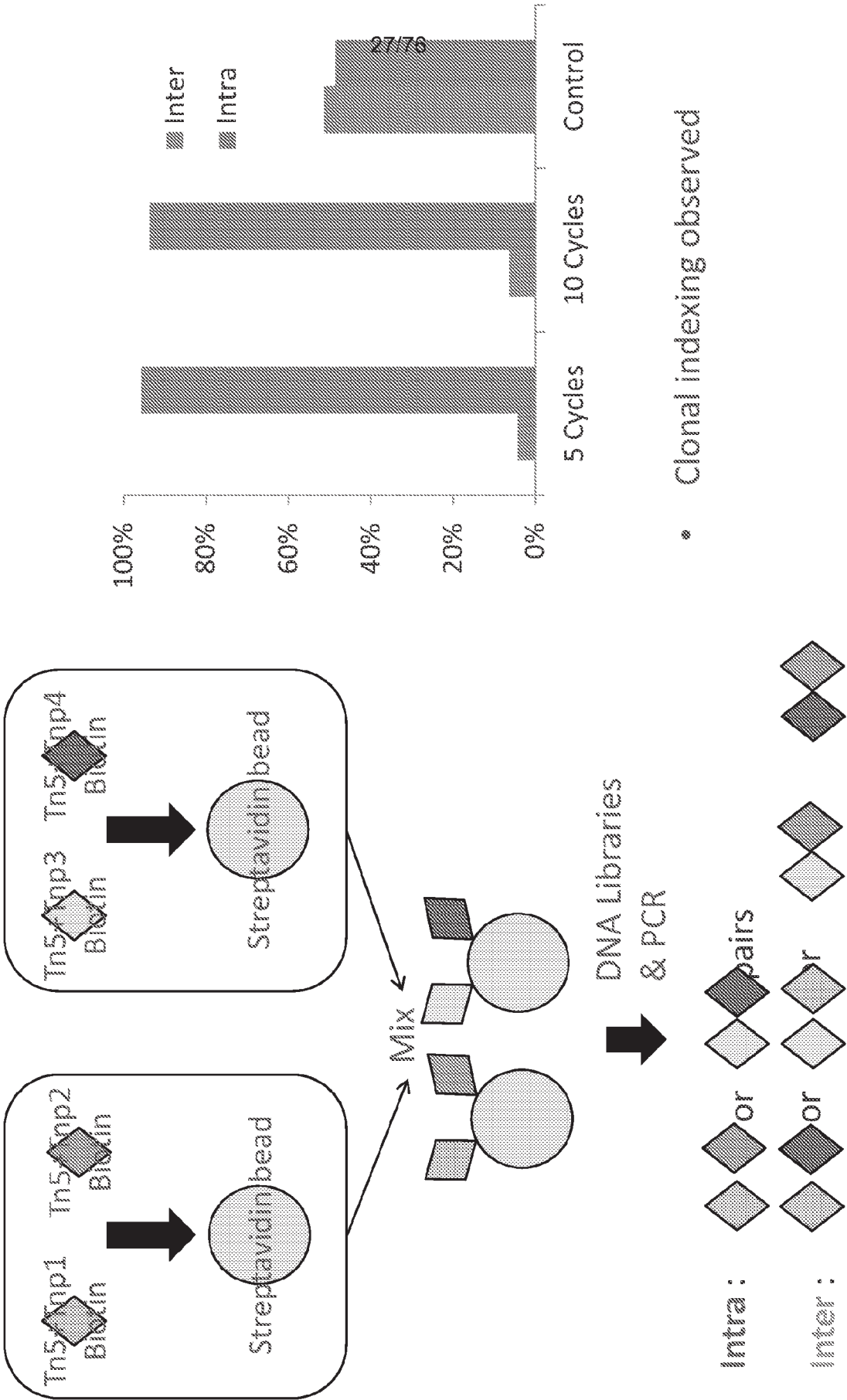


Figure 27

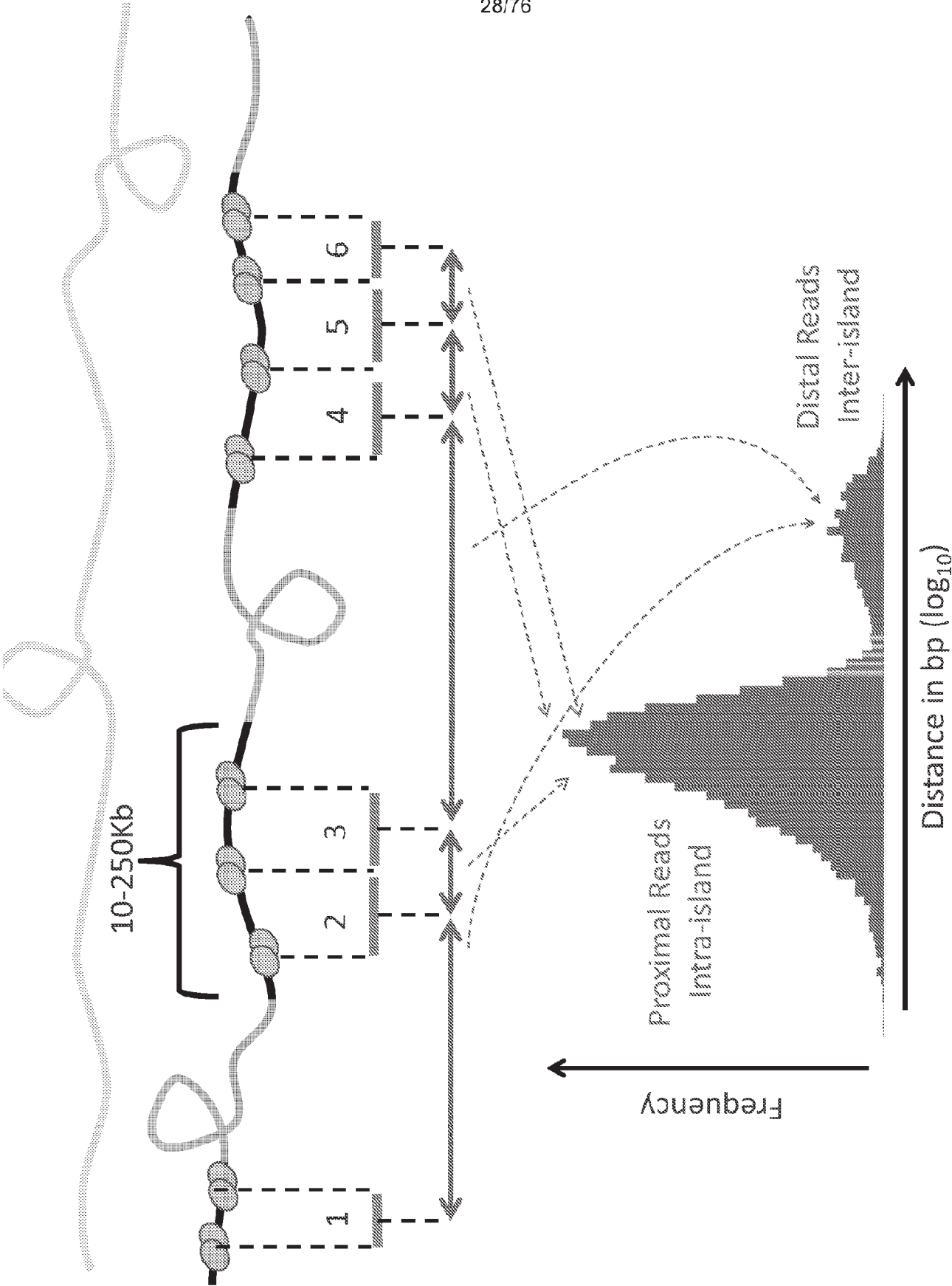
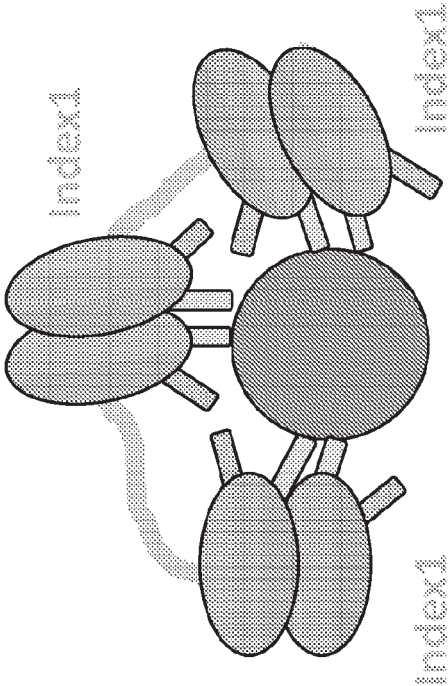


Figure 28

Approaches to Derive Contiguity Information on Solid Support

Support

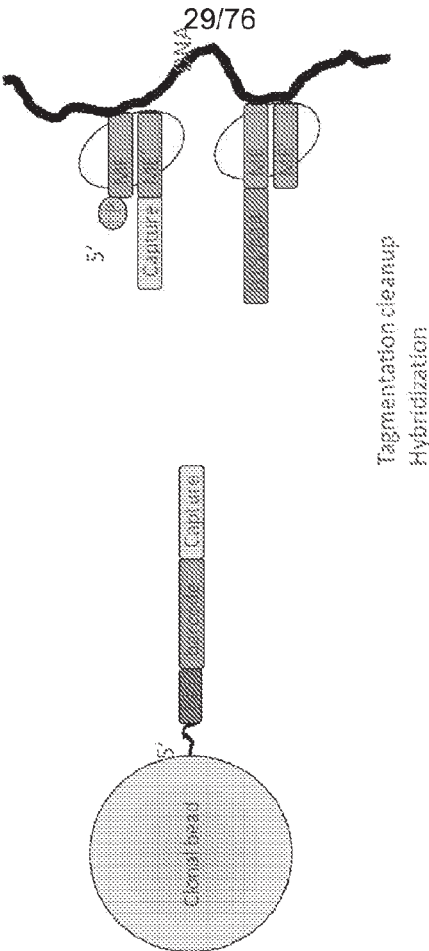
Clonal Indexed Transposomes on Beads



DNA (orange) is transposed by indexed transposomes on beads

FIG. 29A

Clonal indexed capture probes on beads & CPT – DNA

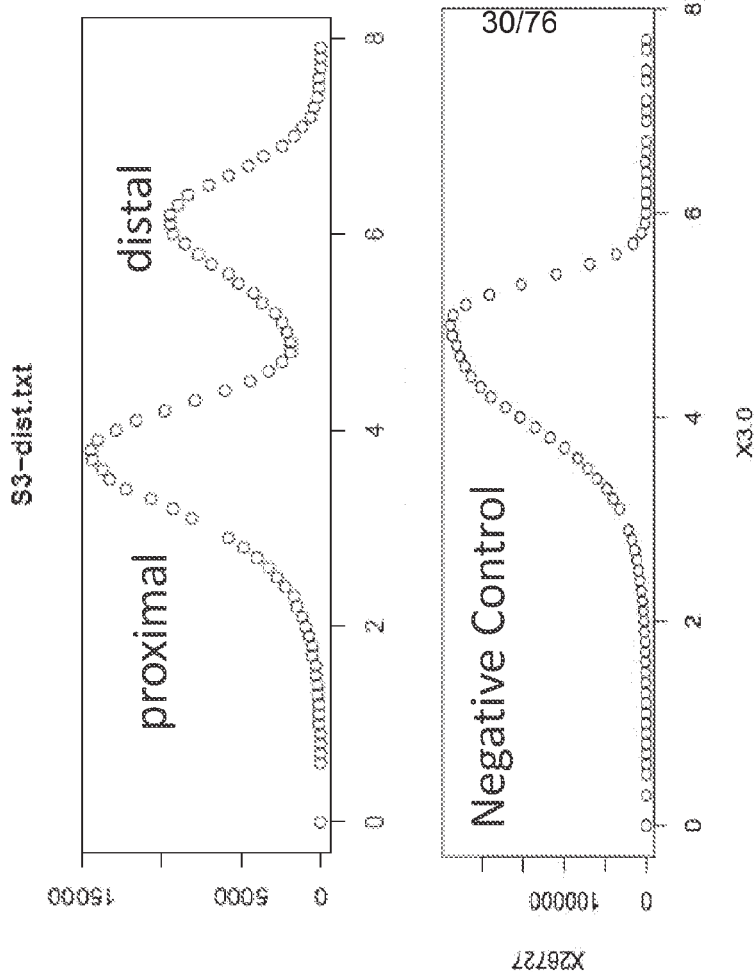
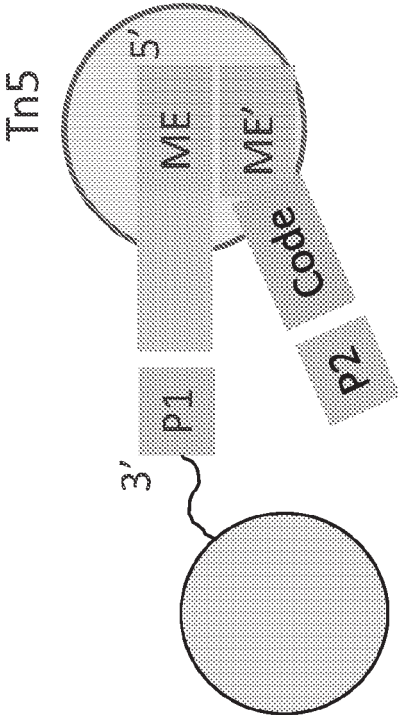


Tagmentation cleanup
Hybridization

Transposed DNA is captured by clonal indexed beads. Index is transferred to CPT- DNA

FIG.29B

One Pot 96 Indexed Clonal Bead Transposition



(only monomer shown for clarity)

Figure 30

Bead approach on 96plex (transposomes on beads)
(200nM surface density/100pg or 200pg DNA input)

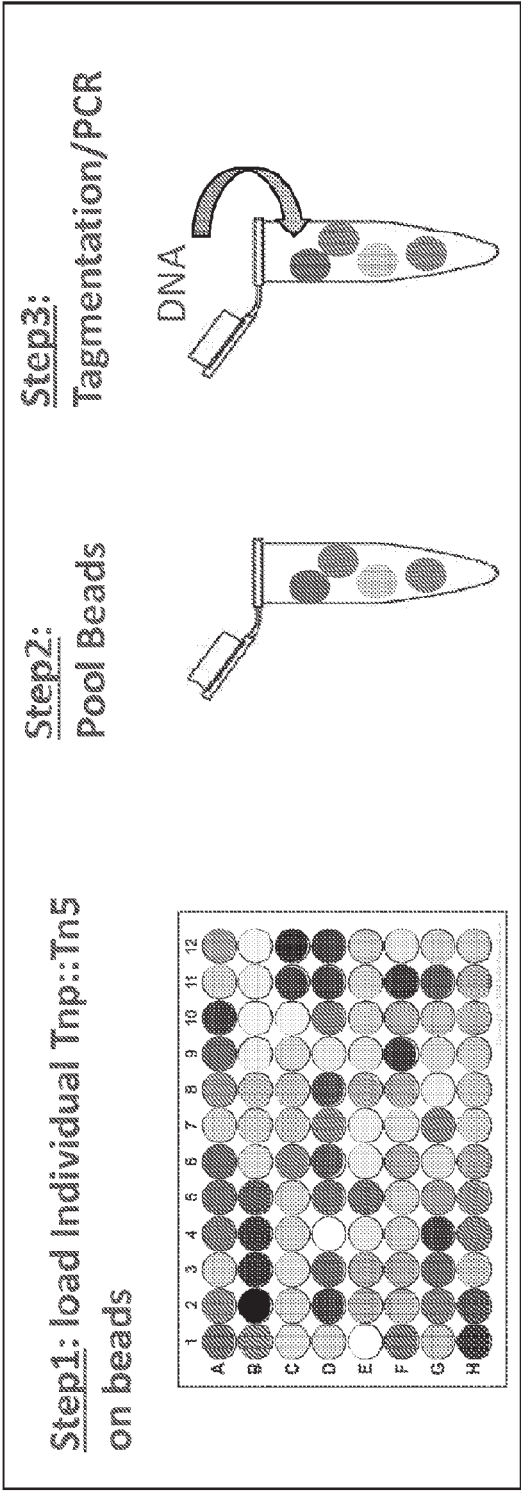
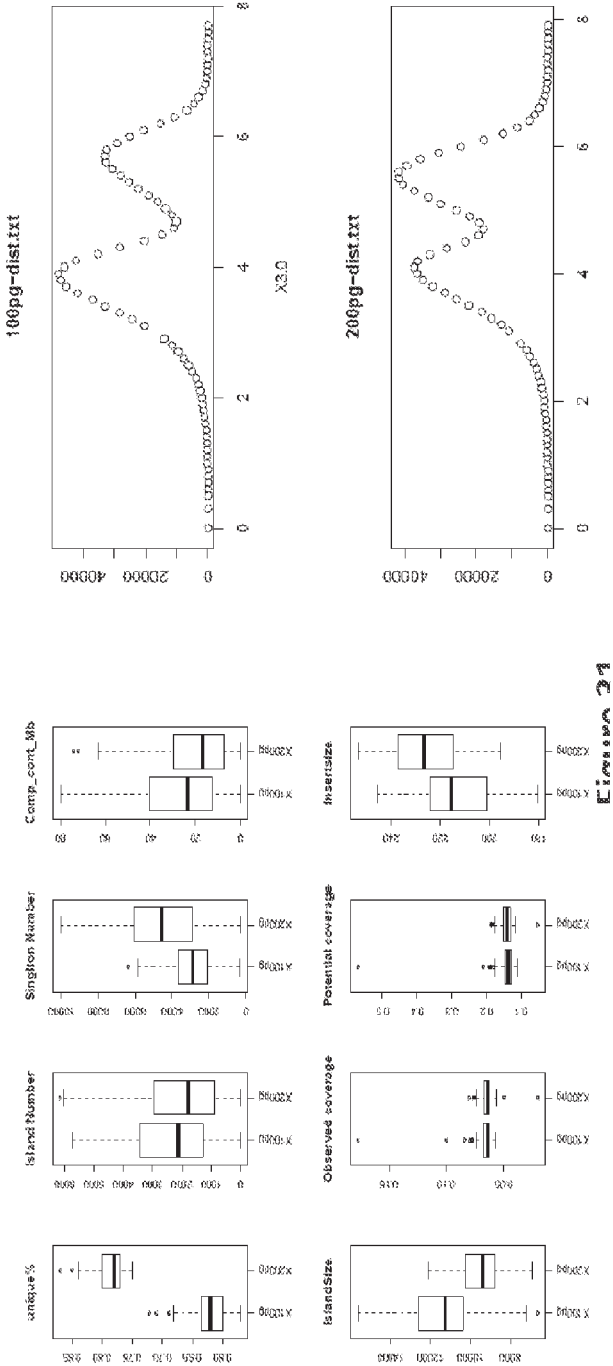


Figure 31

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5'biotin ME and 3'biotin ME' oligos on beads

- Assembly Tnp:Tn5 complex first
- Load on beads
- Wash beads
- Mix beads
- Add DNA and tagmentation
- PCR

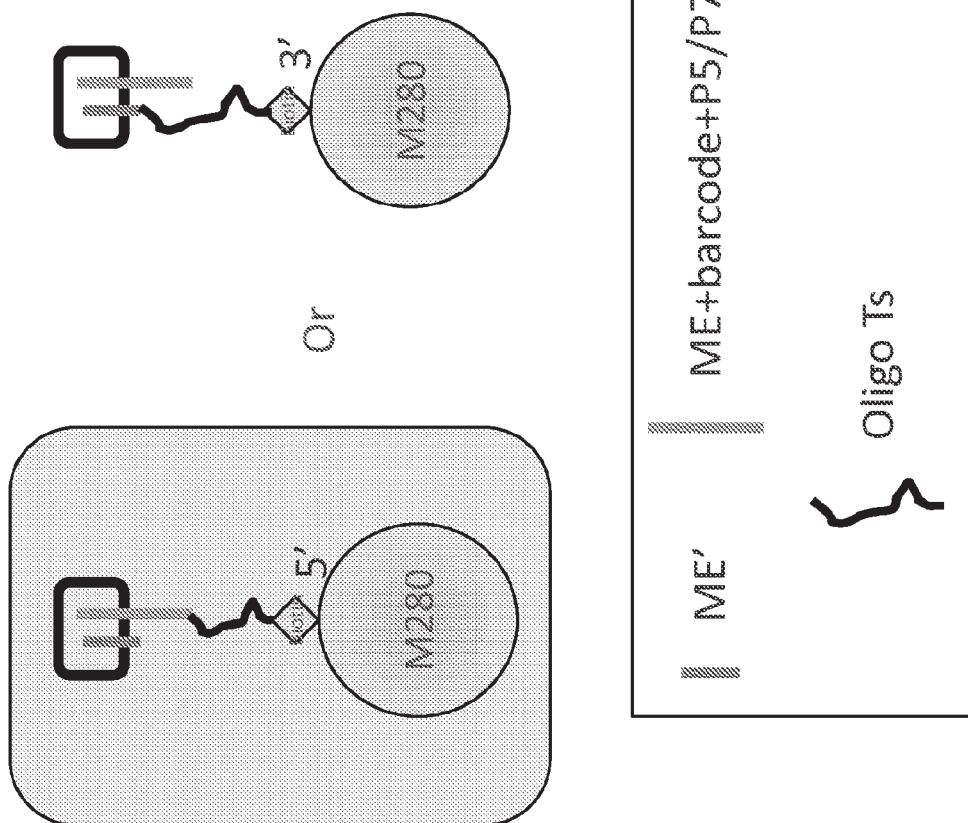


Figure 32

Library Sizes for Transposomes on Beads

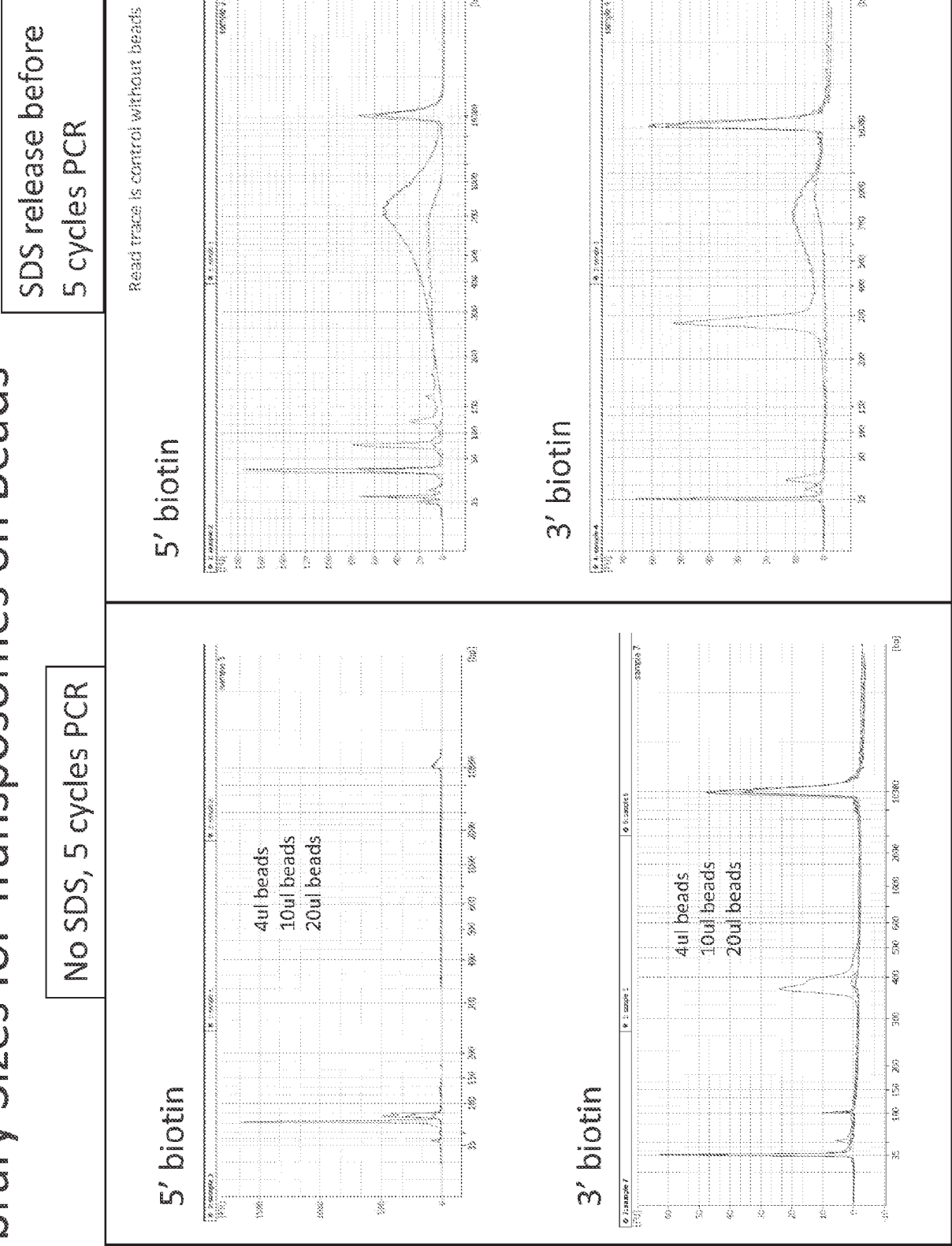


Figure 33

Transposome Surface density vs insertion size

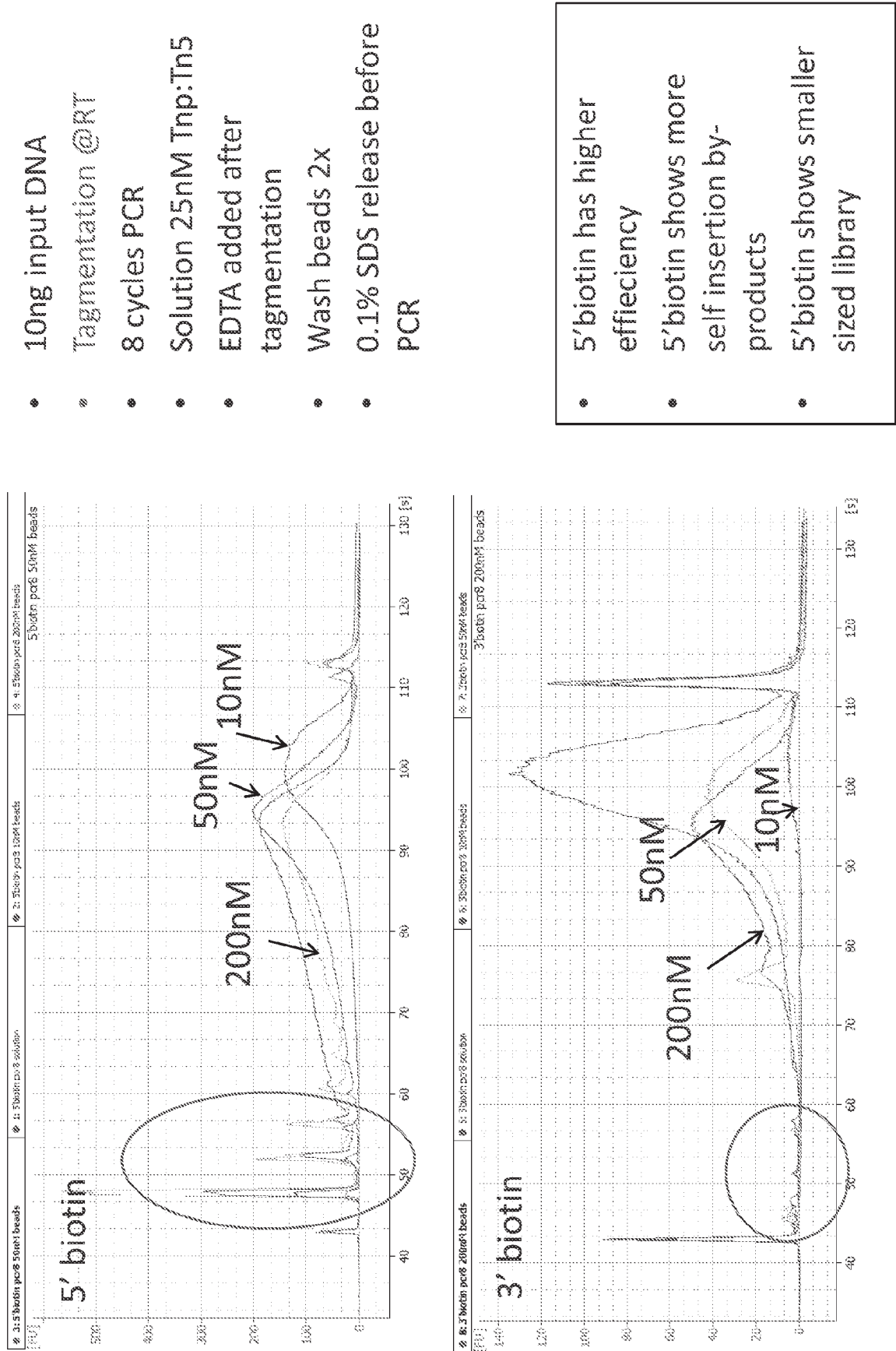
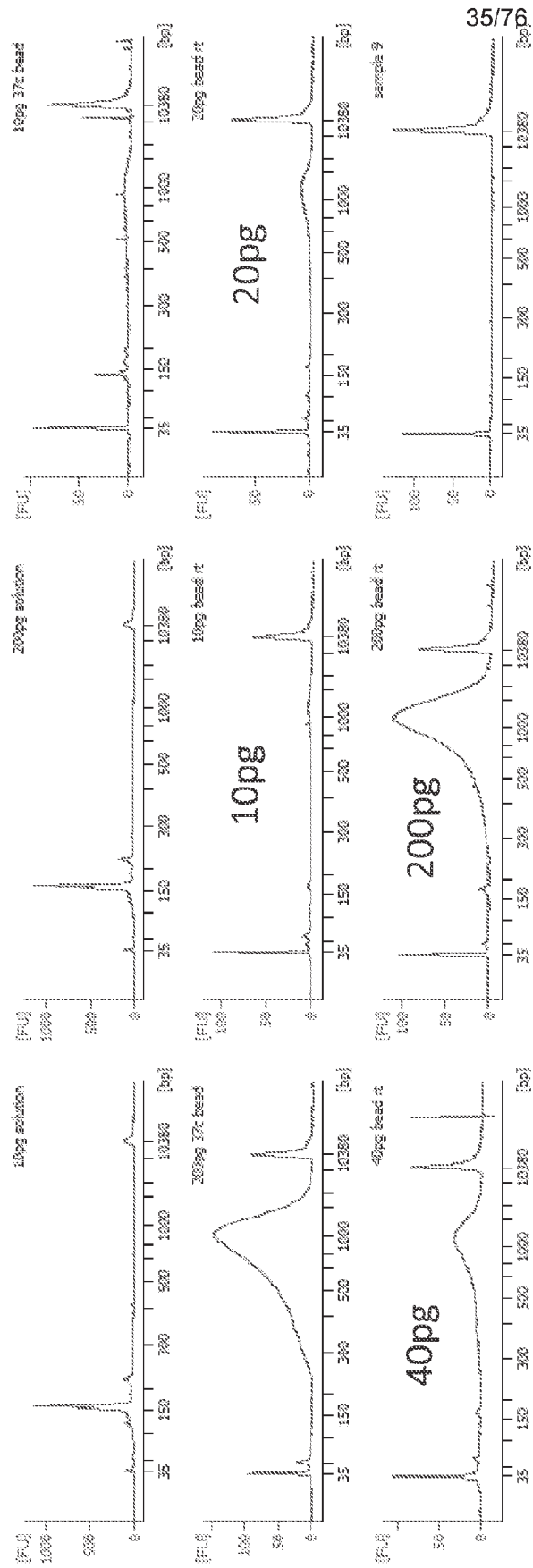


Figure 34

DNA input titration



- ✓ 10pg input might be too low
- ✓ Size distribution is independent on the input DNA amount
- ✓ 37c doesn't change the size distribution

Figure 35

Island Size & Distribution for One-pot on bead Indexing

Randomized
(neg. control)

Beads

Solution

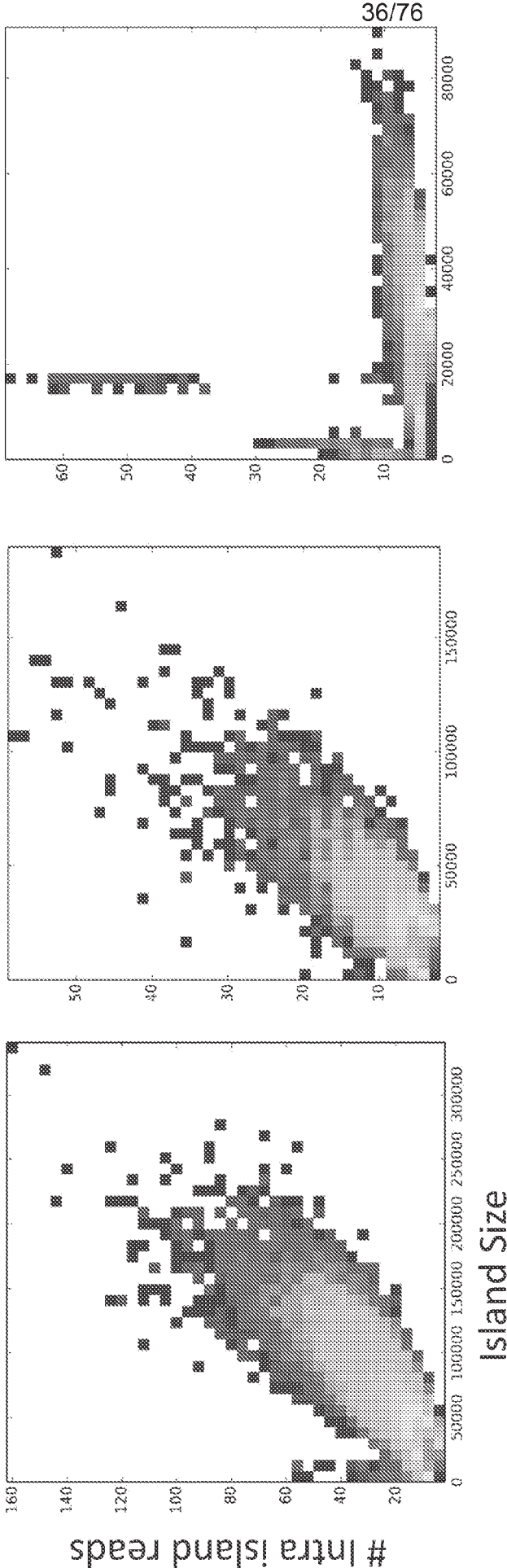
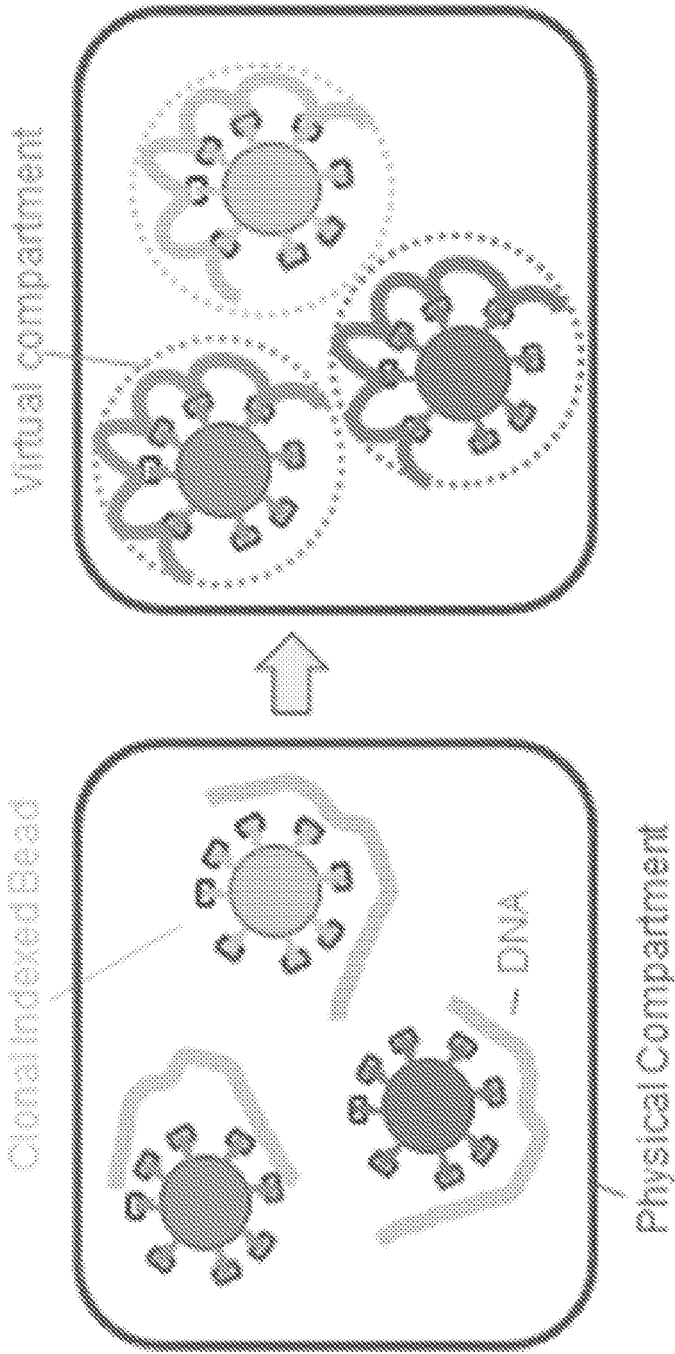


Figure 36

Clonal Indexing Across DNA Molecules



- DNA in proximity of clonal bead receives same index
- Intra >>> inter molecular indexing
- 5 um bead sufficient to capture 100kb contiguity (C=15 um, 100kb ~34um)

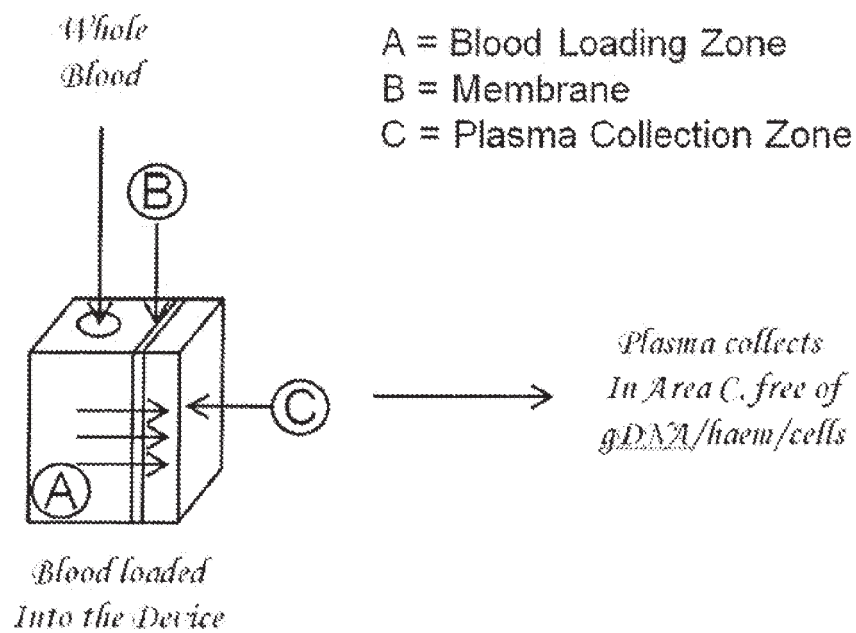
illumina

CONFIDENTIAL - INTERNAL USE ONLY

8

Figure 37

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**Figure 38**

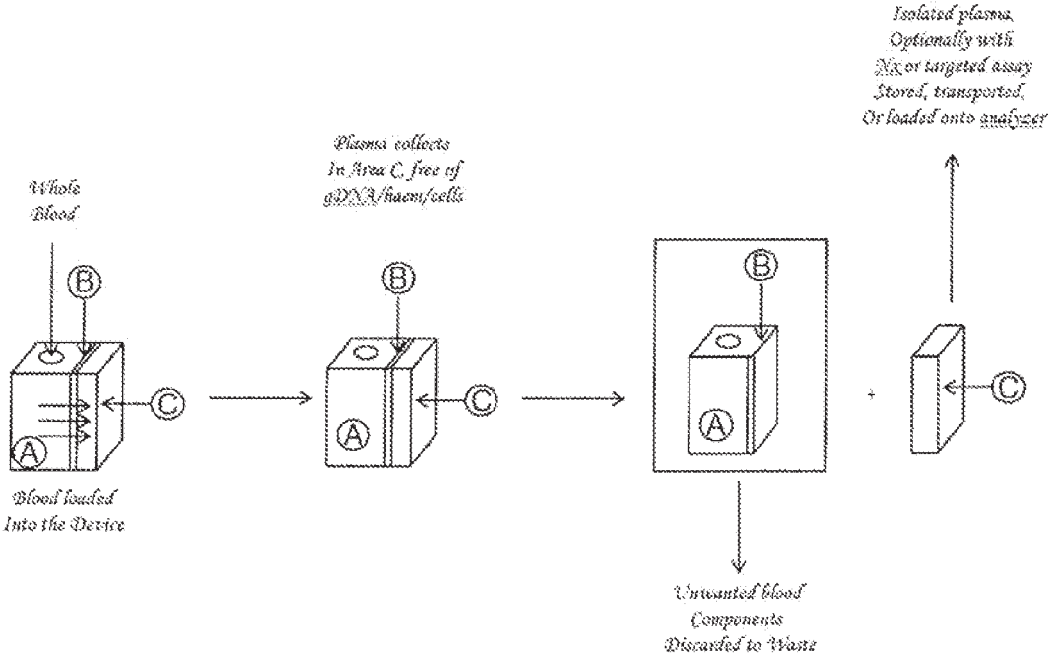
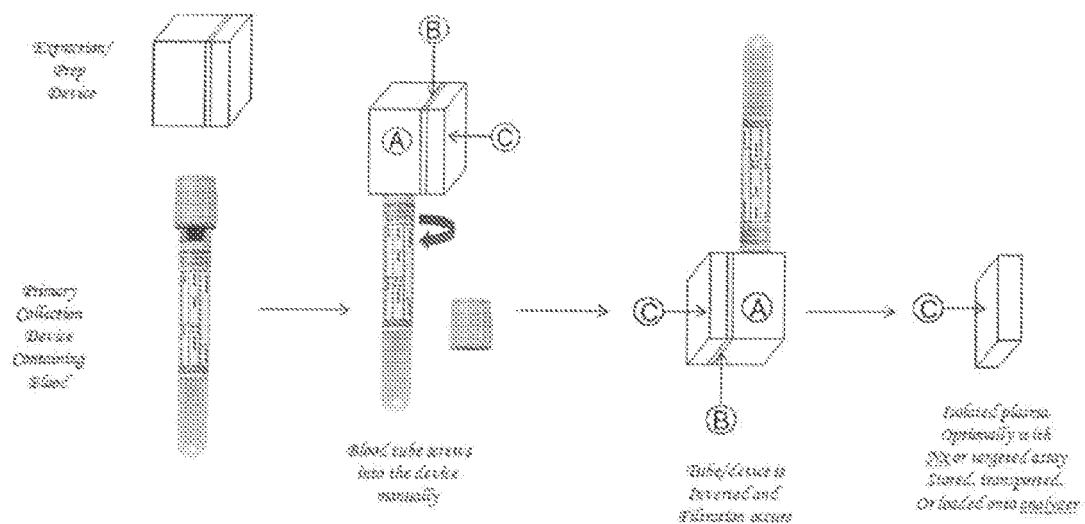


Figure 39

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**Figure 40**

CPT-Seq Enrichment

- Targeted enrichment of whole-genome indexed-linked libraries
- Sample indexing + enrichment compatible

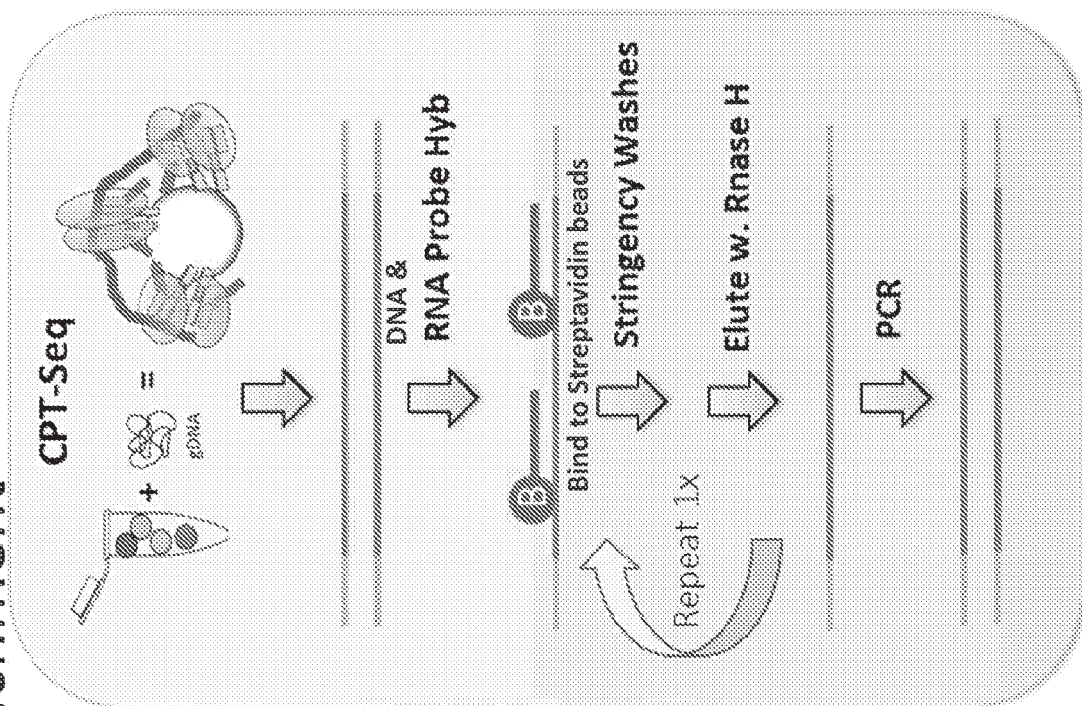


Fig. 41

Phased Exome: Exome enrichment of CPT-seq libraries

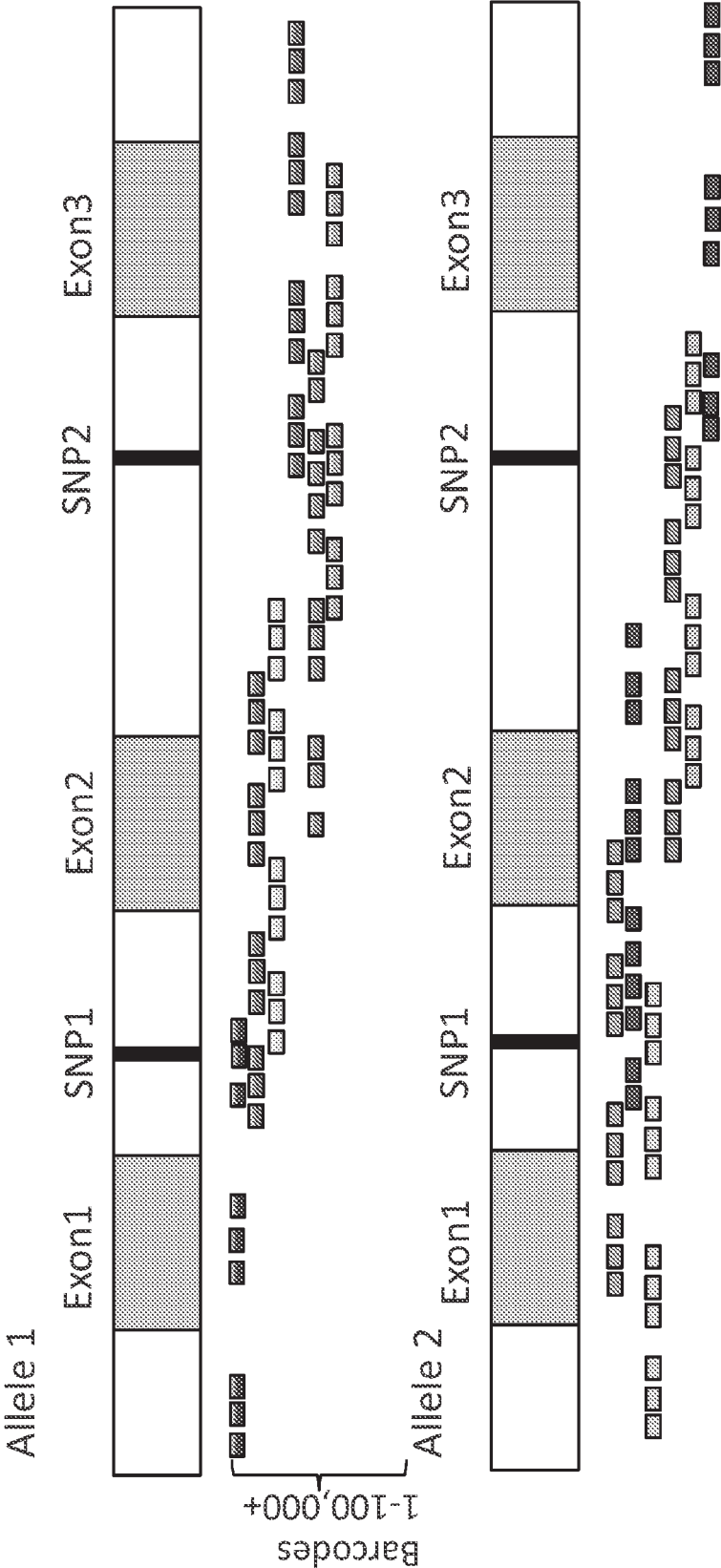


Fig. 42

Methyl CPT-Seq Workflow 1

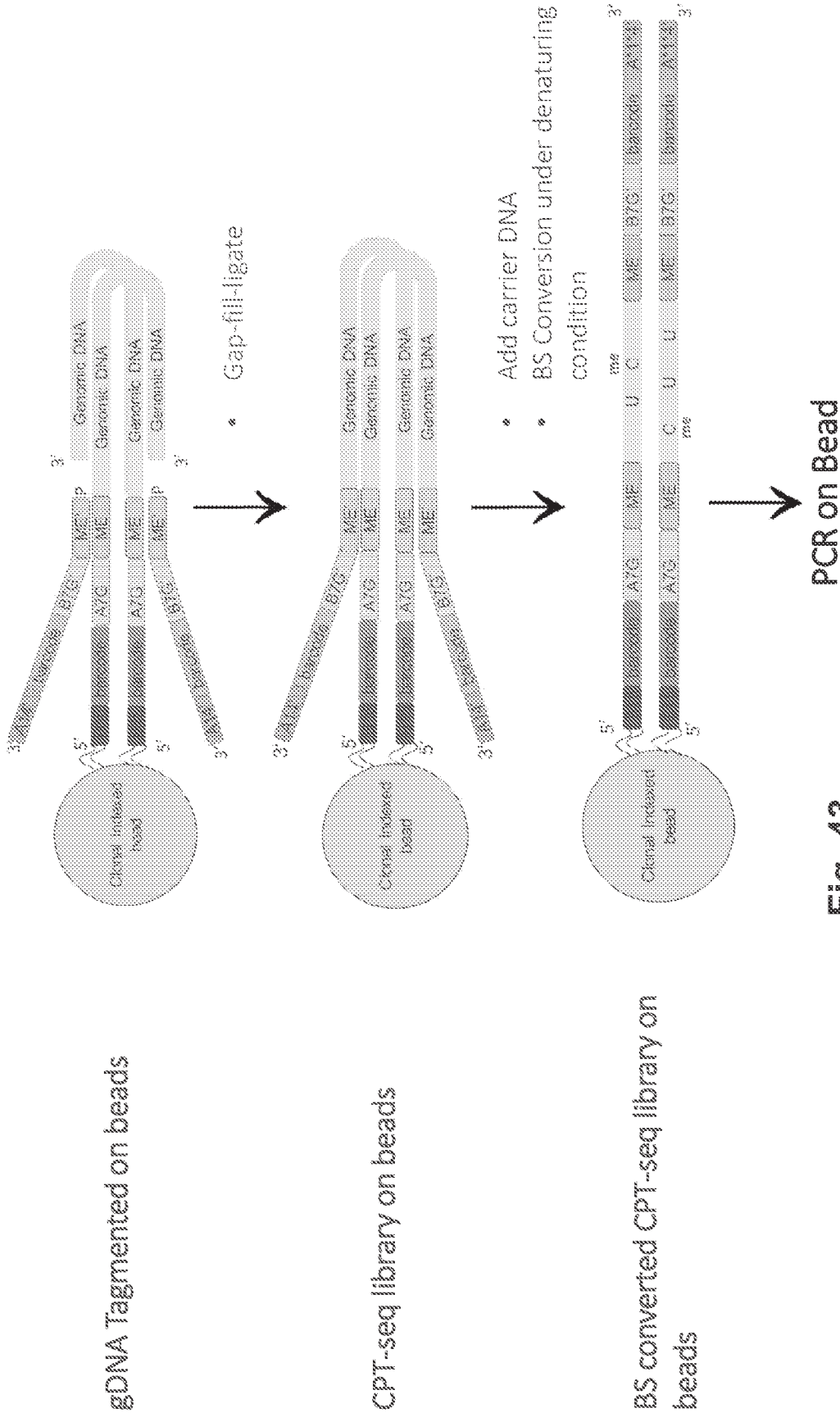
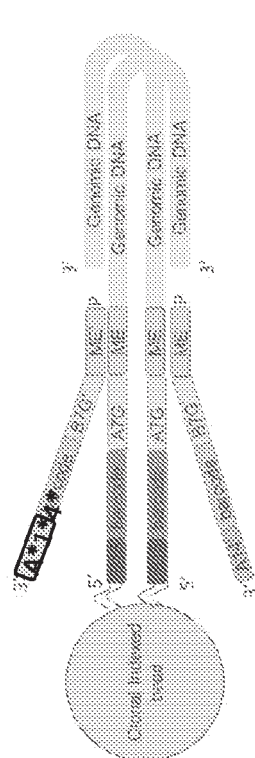


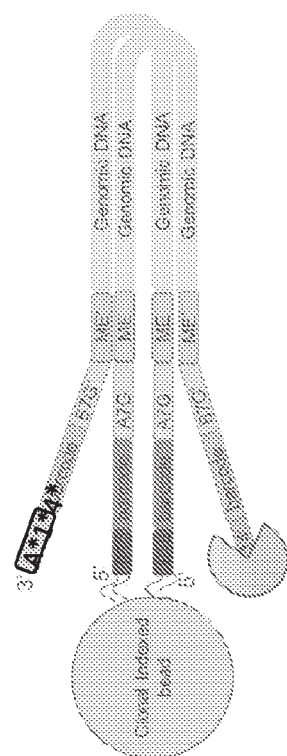
Fig. 43

Example Methyl CPT-Seq Workflow 2

Genomic DNA tagged on beads.
50% of 3' ends contain thio-phosphate
backbone.

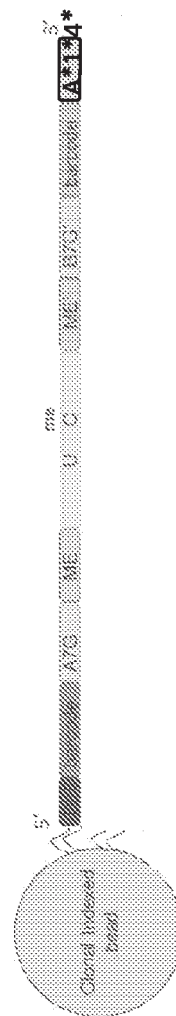


* Gap-filling



CPT-seq library on beads

- Exo I and/or Exo III Digestion
- Add carrier DNA
- BS Conversion



BS converted CPT-seq library on beads

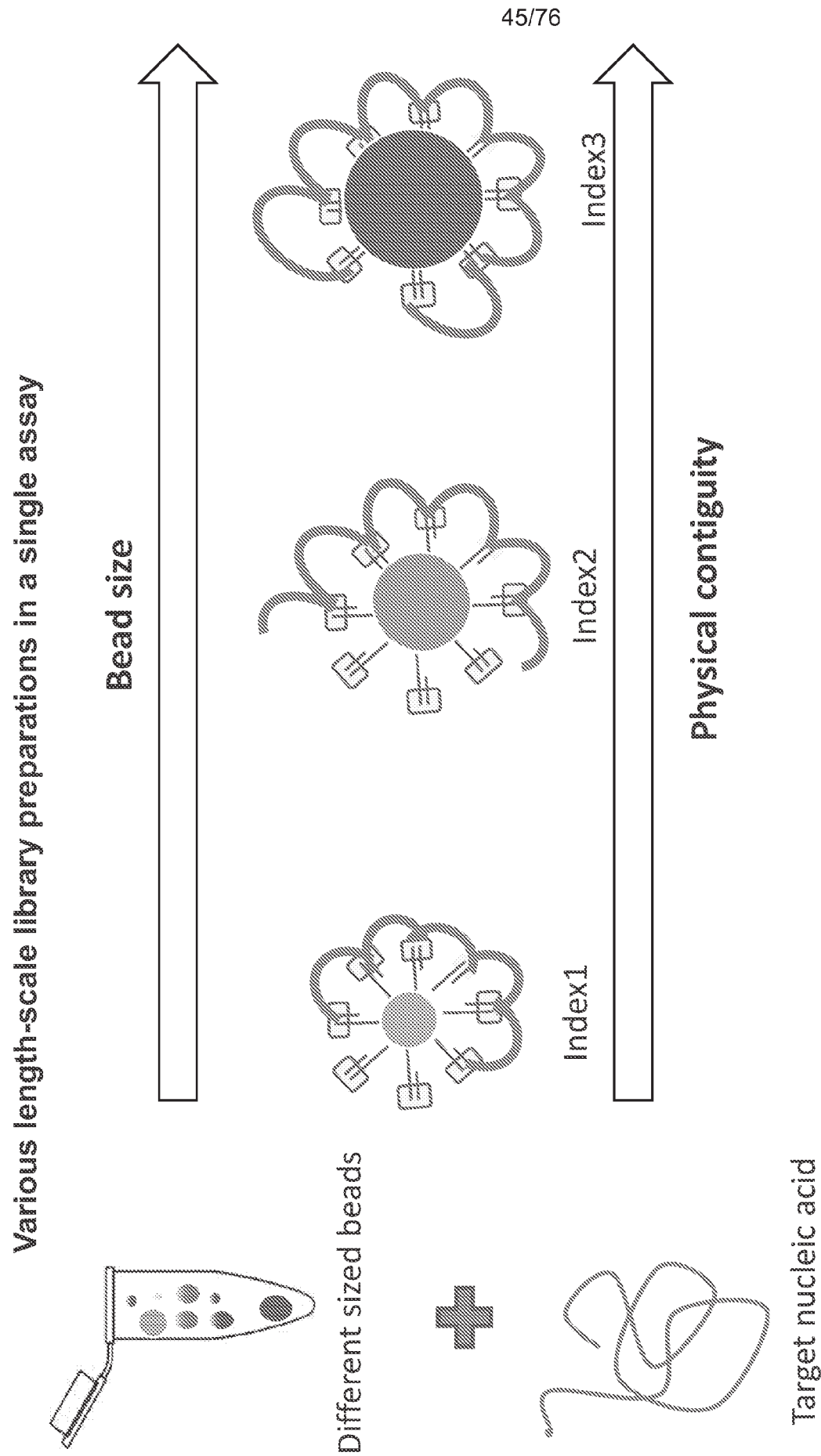
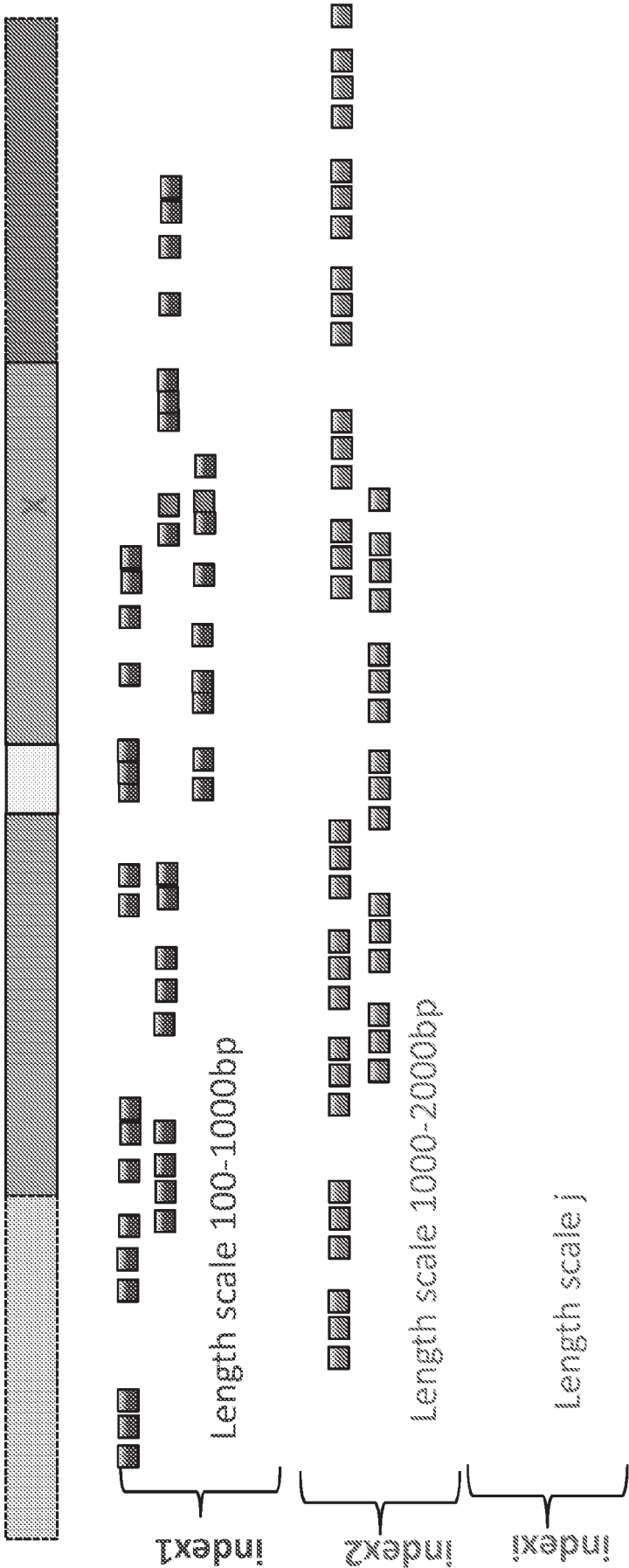


Fig. 45

Example of assembly/SV characterization w/ different length scale libraries

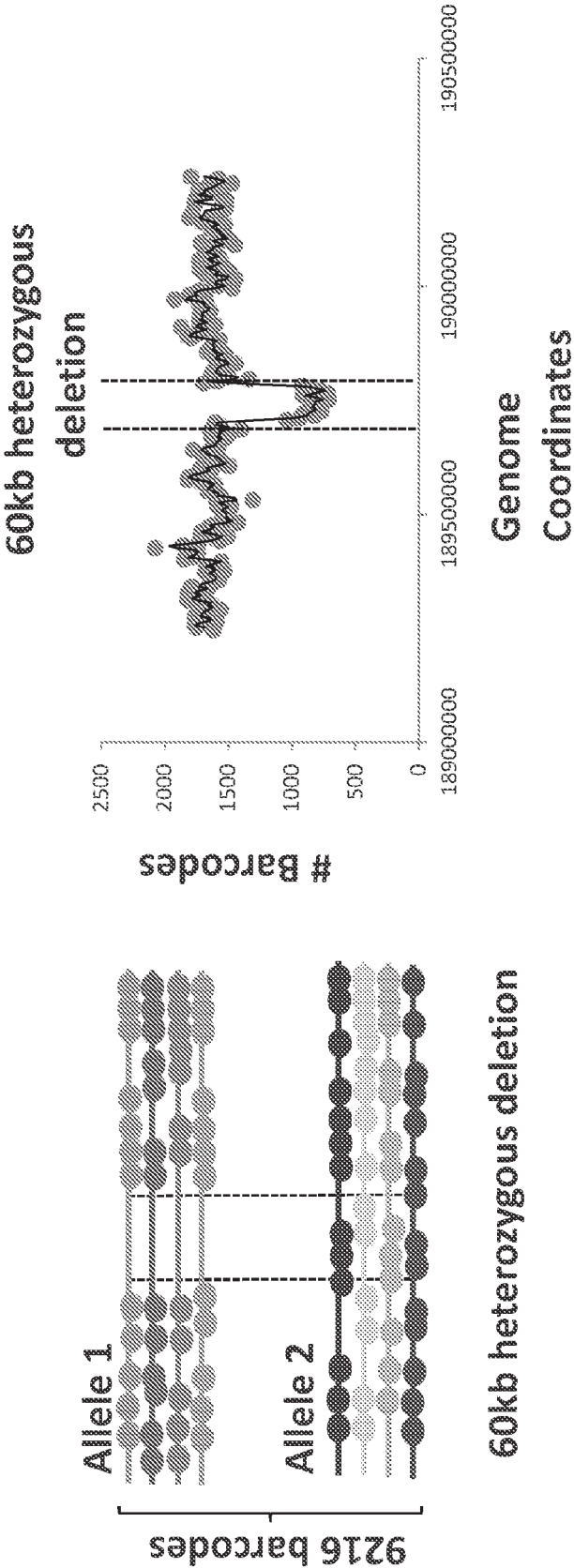
Gene A Pseudo Gene A (X= variant)



Range of contiguity can facilitate assembly and structural variant calling

Fig. 46

Structural variant analysis with CPT-seq



» Heterozygous 60kb deletion on Chr 1 detection with CPT-seq.

Fig. 47A

NA12878 Chr1 60kb heterozygous

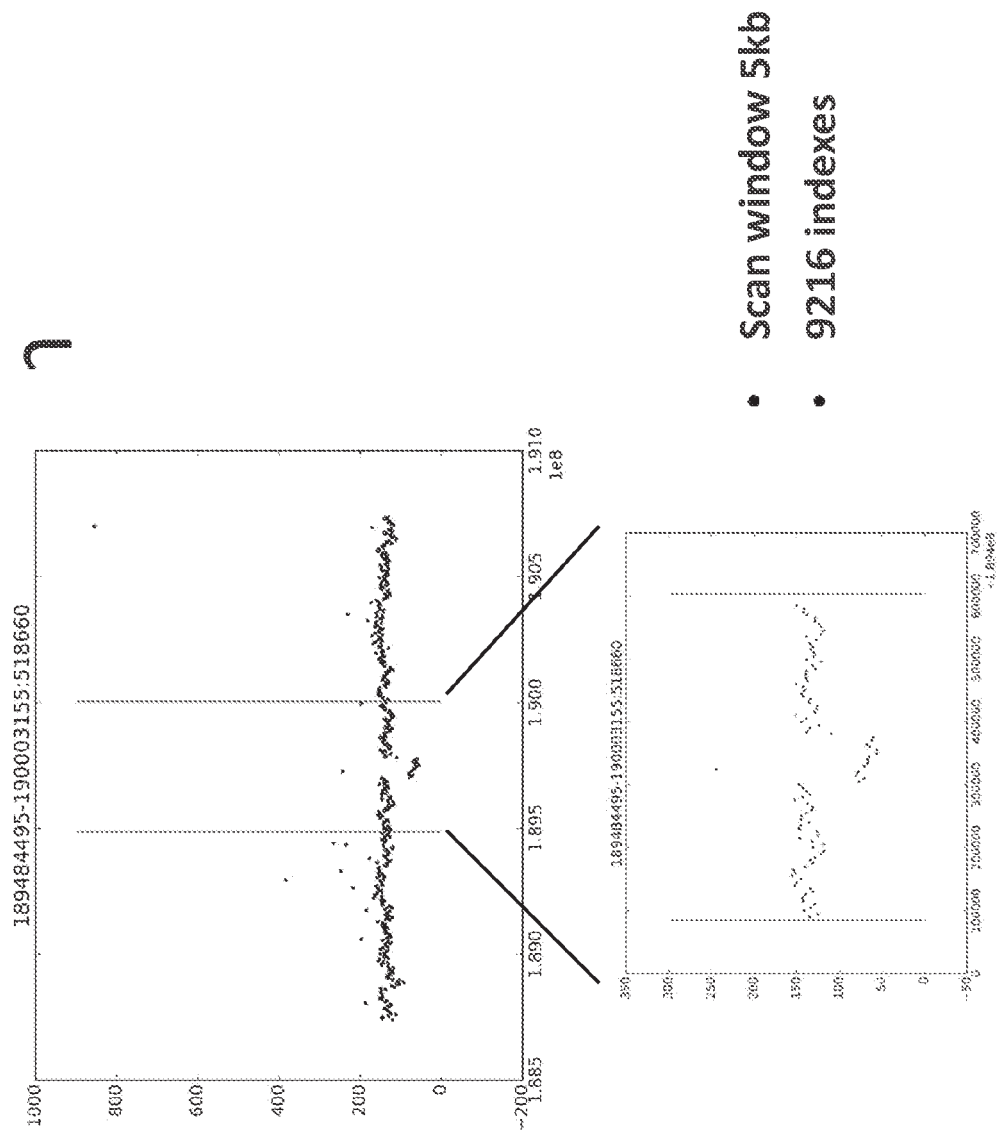
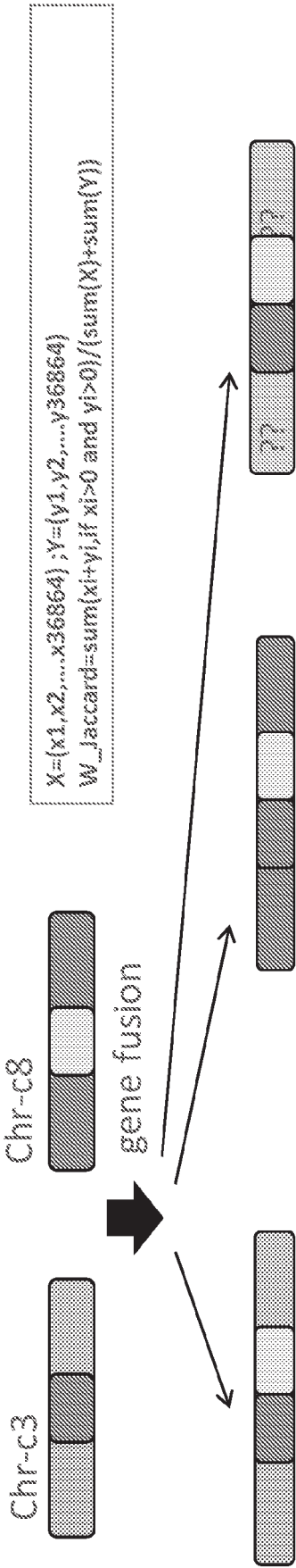
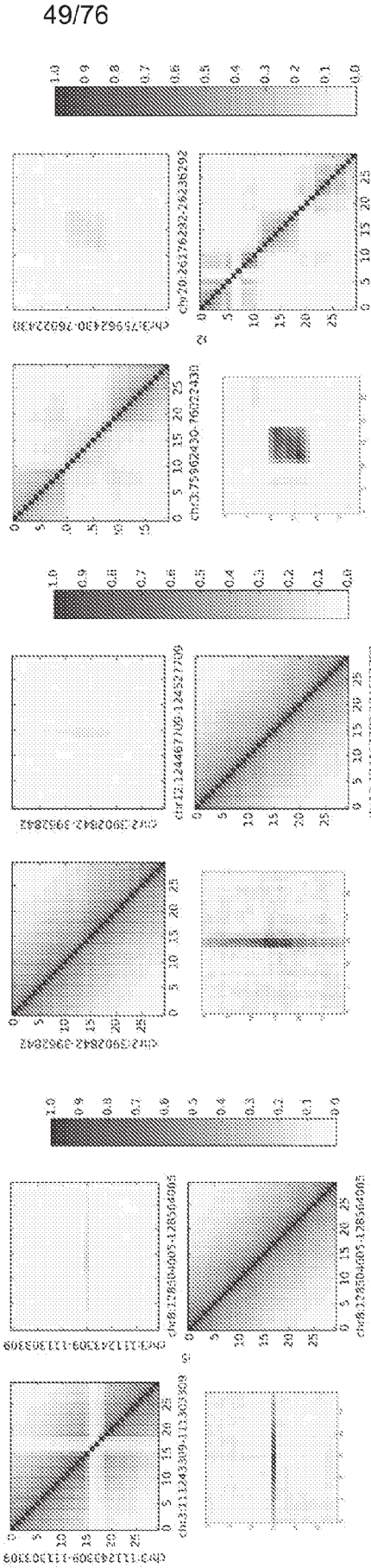


Fig. 47B

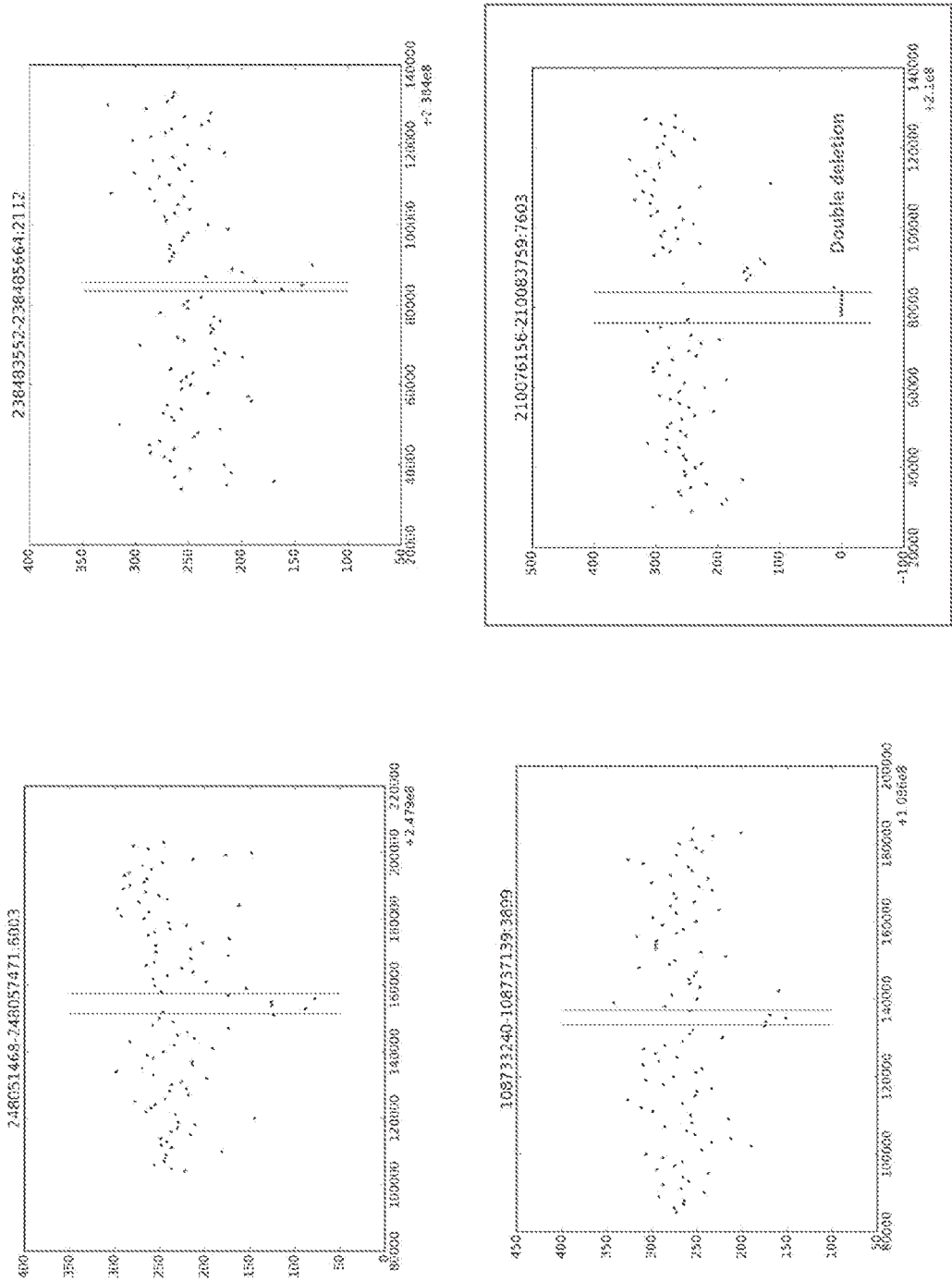
Gene fusion detection with CPT-seq



- Scan window is 2kb w/ each window a 36864 vector in which each element records how many reads have been found in the window.
- For each window pair (X,Y), the weighted Jaccard index is calculated and displayed as a heatmap.

Fig. 48

Deletions detection with CPT-seq



• Scan window 1kb

Fig. 49

Bisulfite Conversion Efficiency Optimization

- Optimized Promega's MethyEdge Bisulfite Conversion system to improve conversion efficiency.

Cond	DNA	Beads	BSC Treatment
1	10ng	No	1hr @ 60°C / 0.3M NaOH
2		Yes	1hr @ 60°C / 0.3M NaOH
3			1hr @ 60°C / 1M NaOH
4		Yes	1hr @ 65°C / 0.3M NaOH

- ME sequence analyzed to determine efficiency of bisulfite conversion treatments.

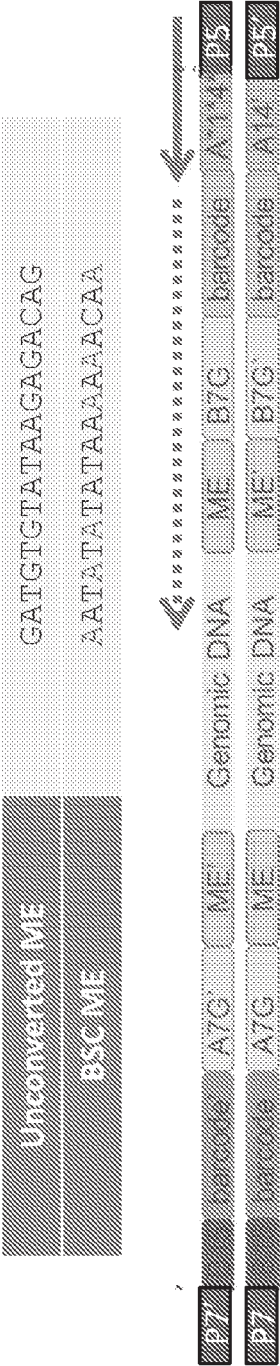


Fig. 50

Bisulfite Conversion Efficiency Optimization

Results

- 95% bisulfite conversion (BSC) of indexed-linked libraries attached to beads.
- Similar PCR yields observed between bisulfite conditions > Harsher bisulfite treatment did not appear to degrade libraries.

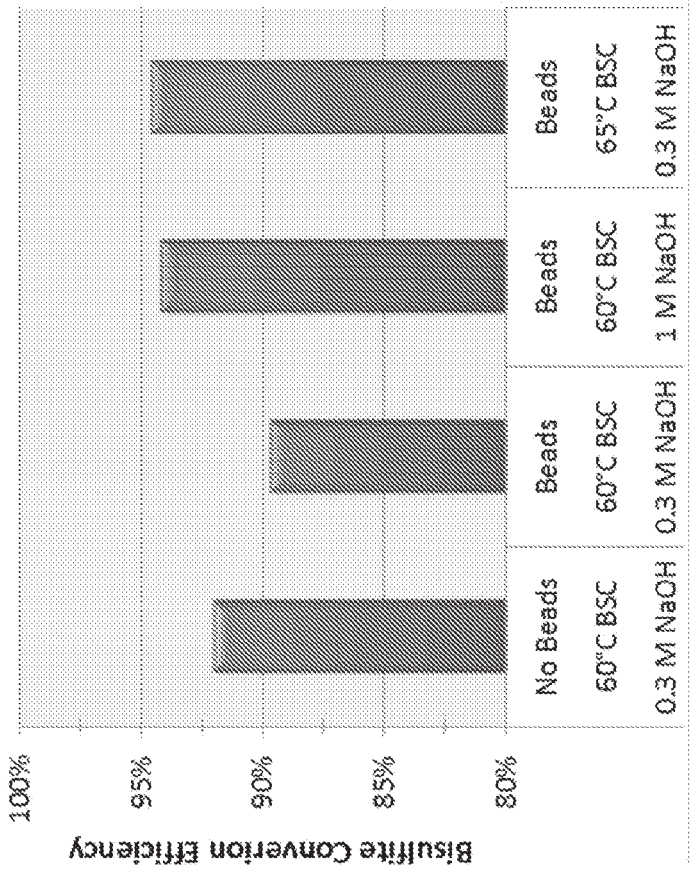


Fig. 51

IVC plot (intensity versus cycles per individual base)

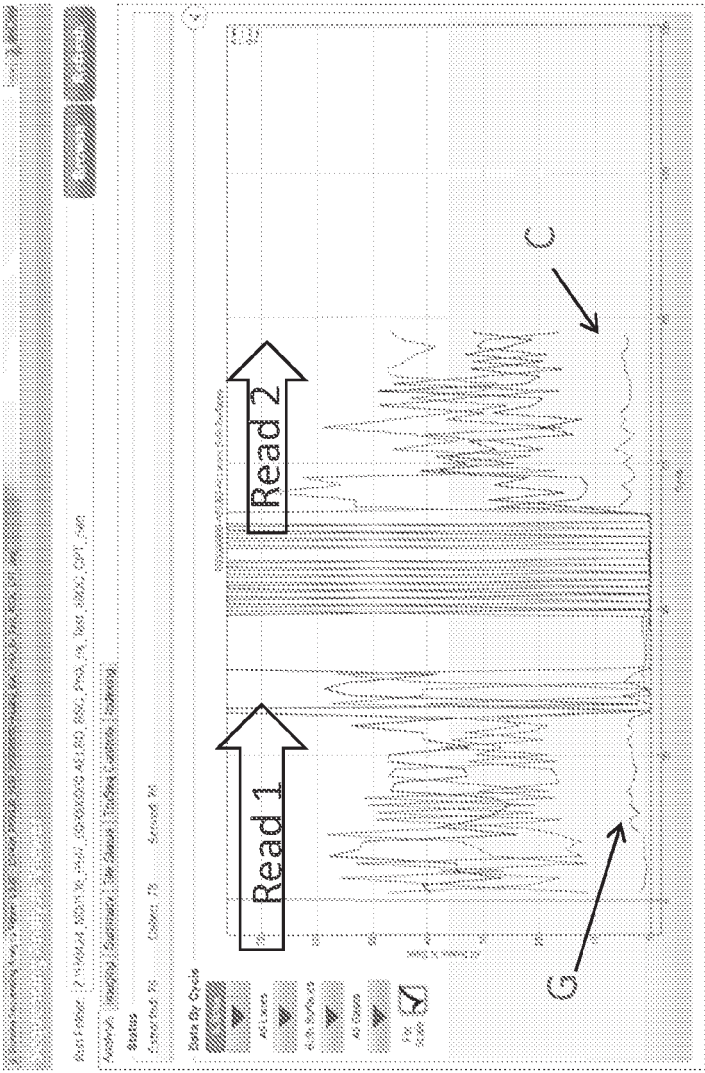
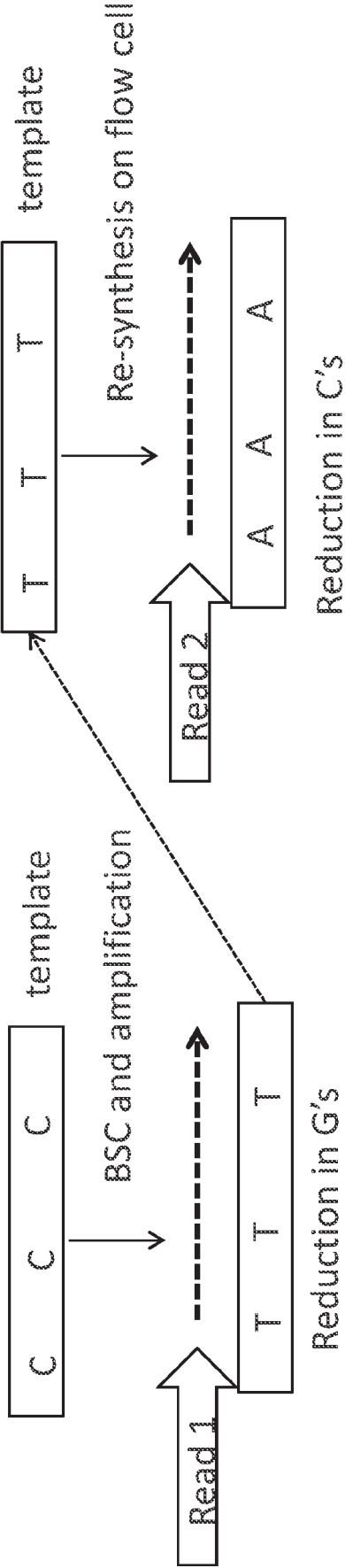


Fig. 52

Bisulfite Conversion Efficiency Optimization

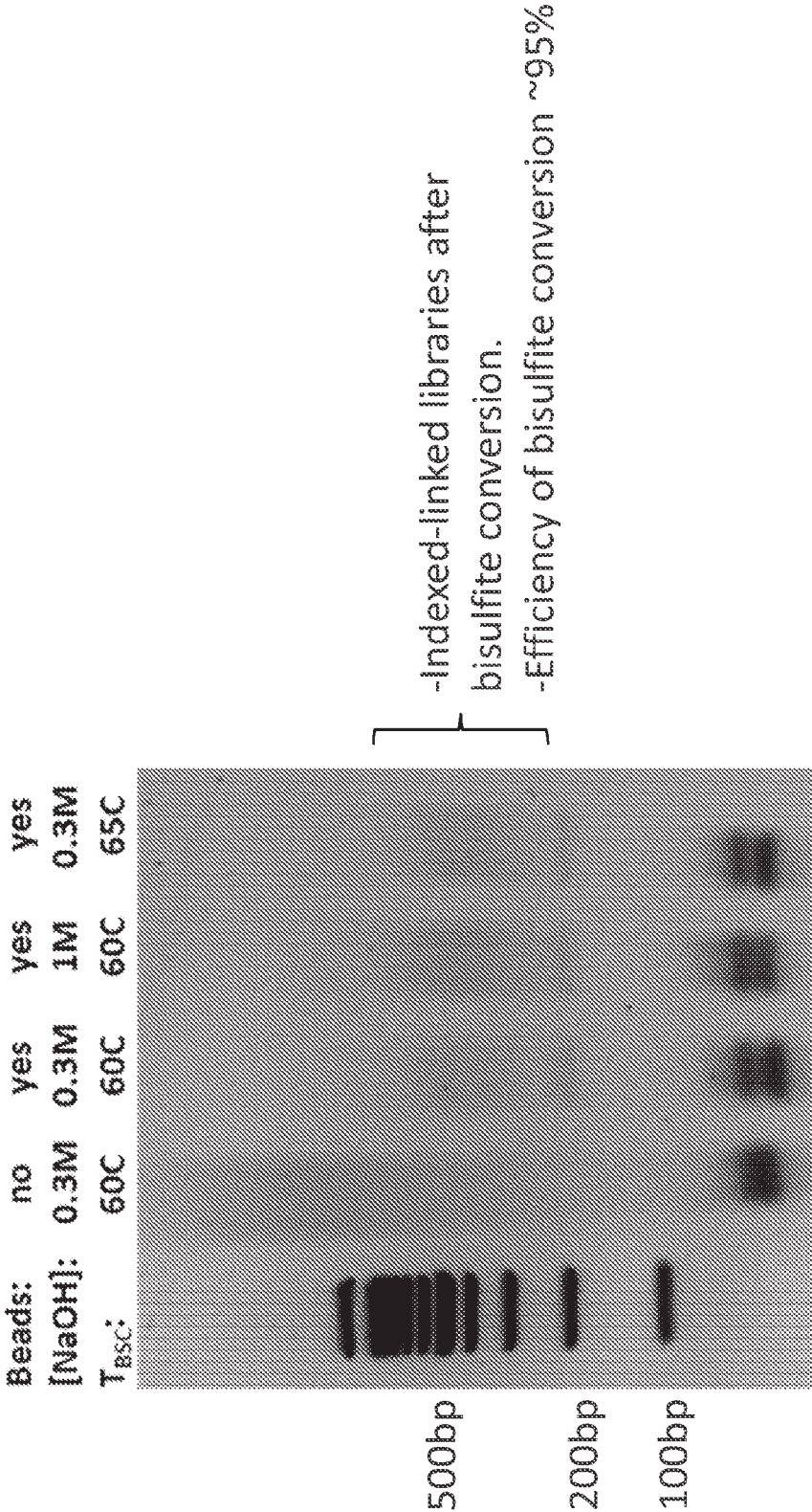
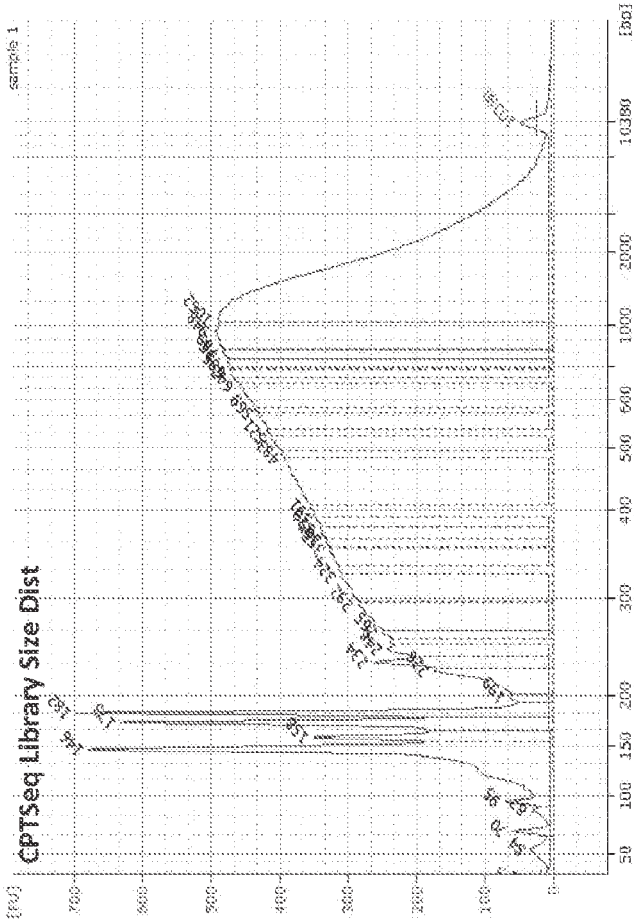


Fig. 53

Enrichment of CPT-Seq libraries

Cond	Library	Probe Set	[Probe] pM/ga	Hyb Volume	Hyb Ramp	EHB	Elution	Washes	SVB
1	1.1ug CPTSeq	DNA Dunn A, B, K	100pM	25uL	95 > 58 (O/N)	Form, Nxt Bkrs	ET1	Std	3x
2	1.1ug CPTSeq	EEX	2.5uL	25uL	95 > 58 (O/N)	Form, Nxt Bkrs	ET1	Std	3x
3	1.1ug CPTSeq	RNA Dunn A, B, K	100pM	25uL	95 > 65 (O/N)	Form, Nxt Bkrs	RNase H	Std	3x

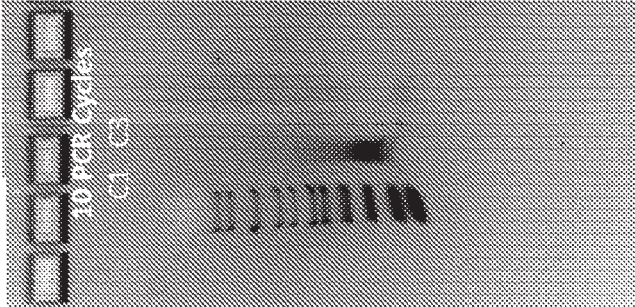


CPT-Seq Library

≈ 1ug/library prep

Fig. 54

CPT-Seq Libraries Post Enrichment



- Targeted indexed-linked sequencing libraries

Fig. 55

Target Haplotyping, Applications to

HLA

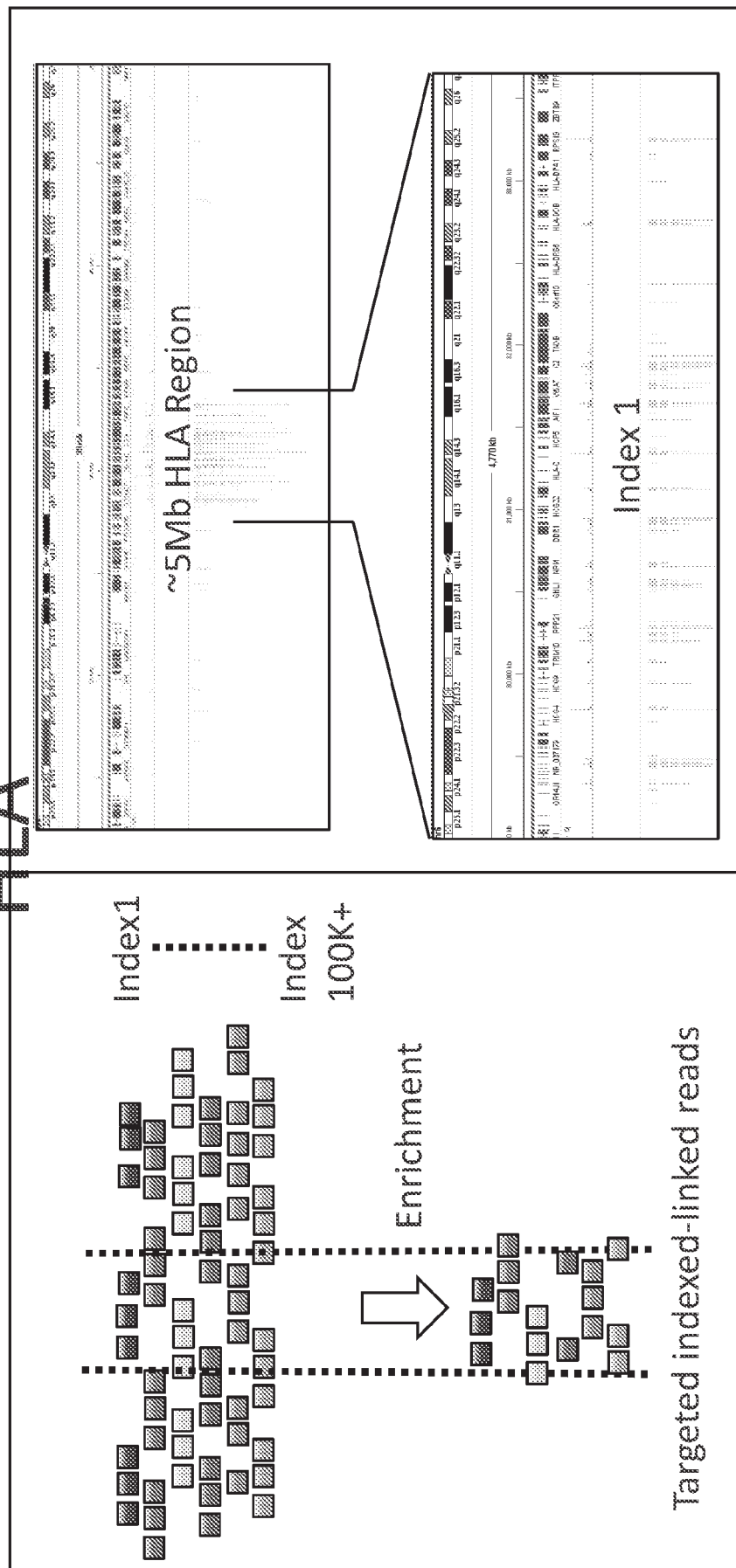


Fig. 56

Mechanisms for Swapping

1 - DNA binds to two differently indexed beads

Possible solutions:

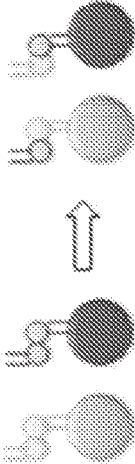
- More space between beads (dilute DNA more before adding)
- Add dummy beads to space complex-beads out more



2 - Complexes come apart and re-form

Possible solutions:

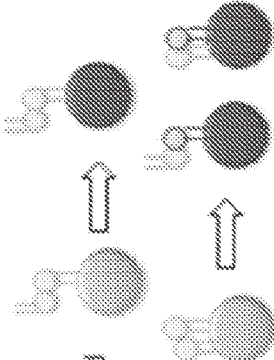
- Be more gentle with mixing steps
- Wash away monomers not bound by SA
- Crosslink?
- Form complexes fresh?



3 - SA-biotin bond breaks and complex re-hybs to another bead

Possible solutions:

- Traptavidin beads
- Block free streptavidin sites so that biotinylated oligos can't bind



4 - Contamination

4

complex complexes - complex, all only

illumina

Figure 57

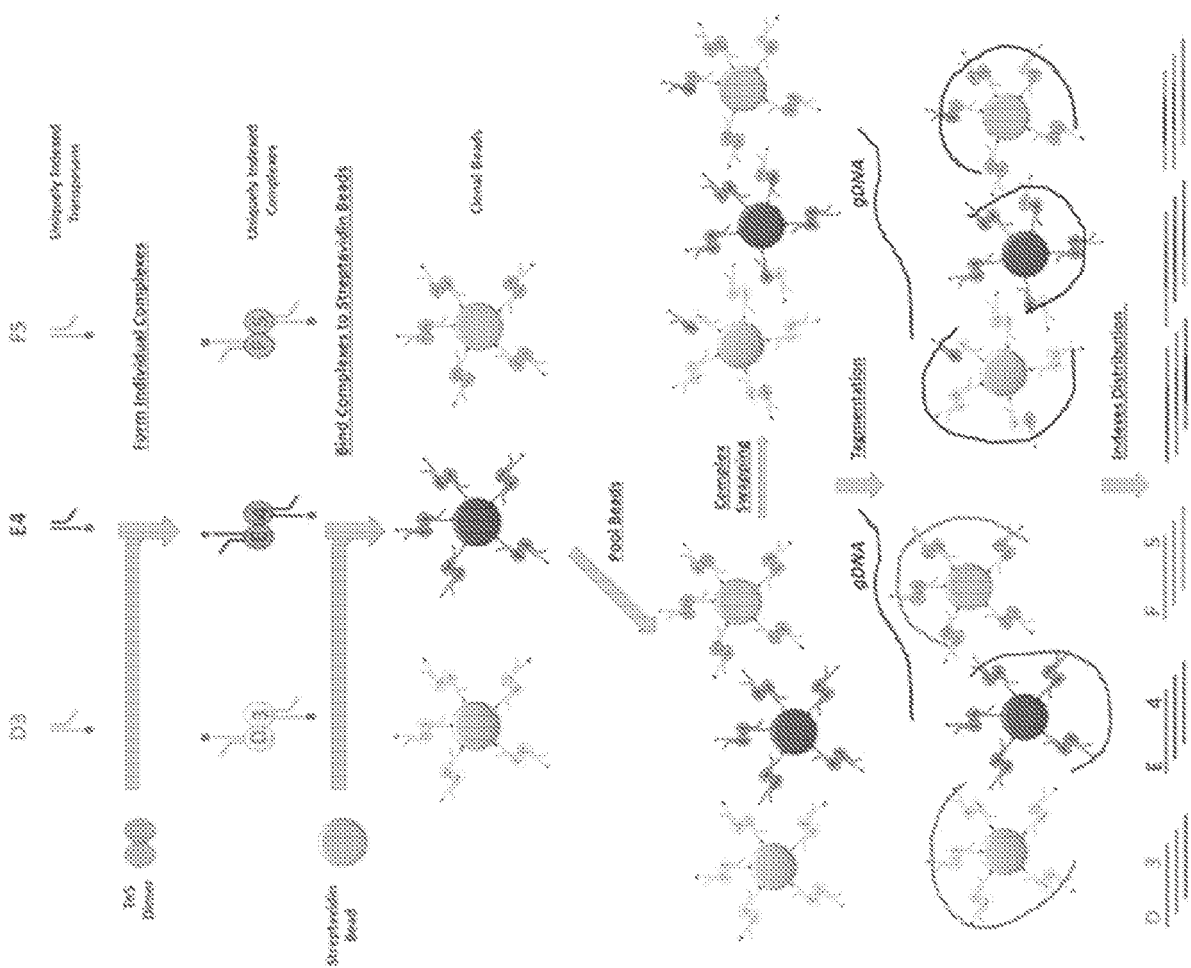


Figure 58

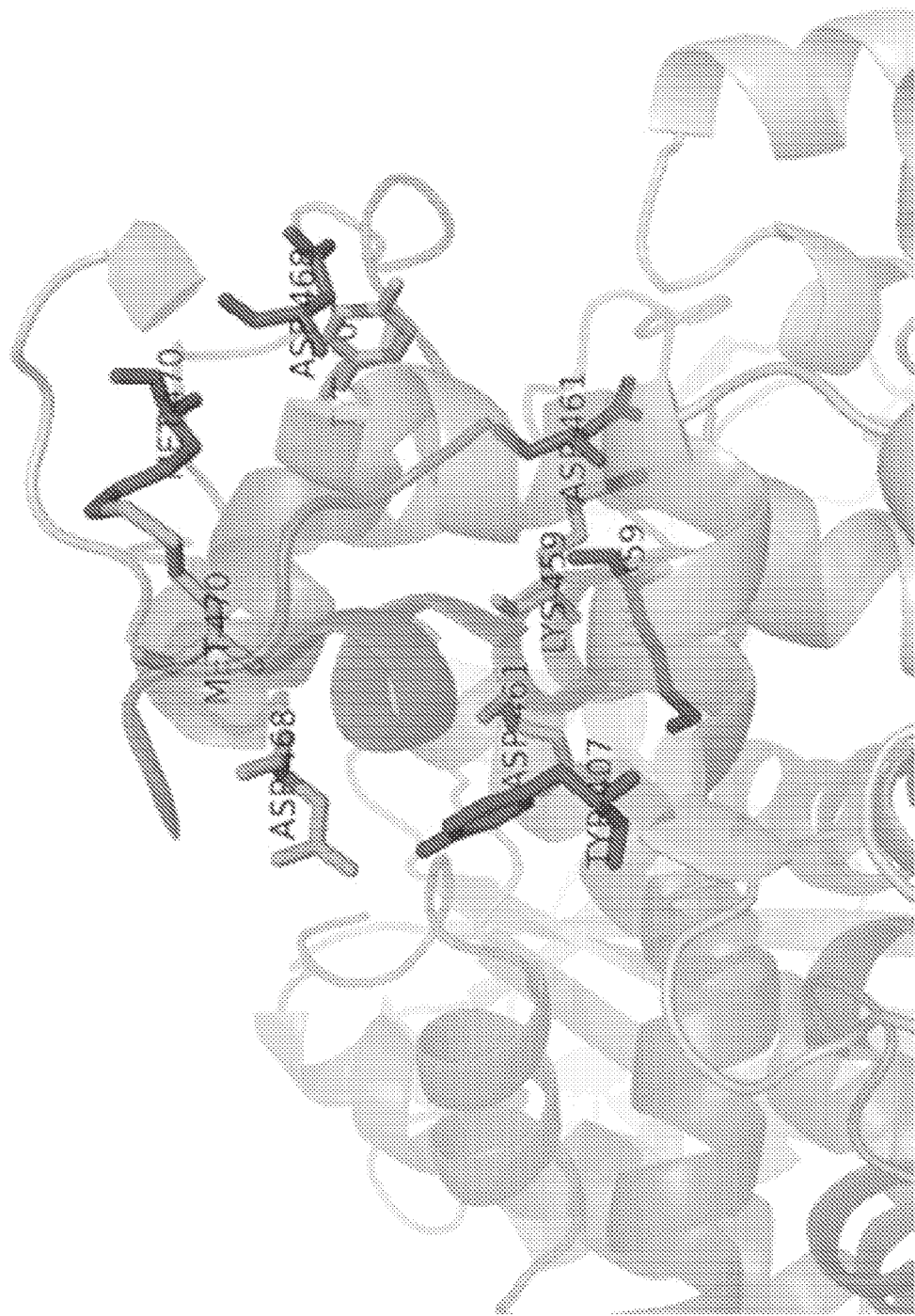


Fig. 59

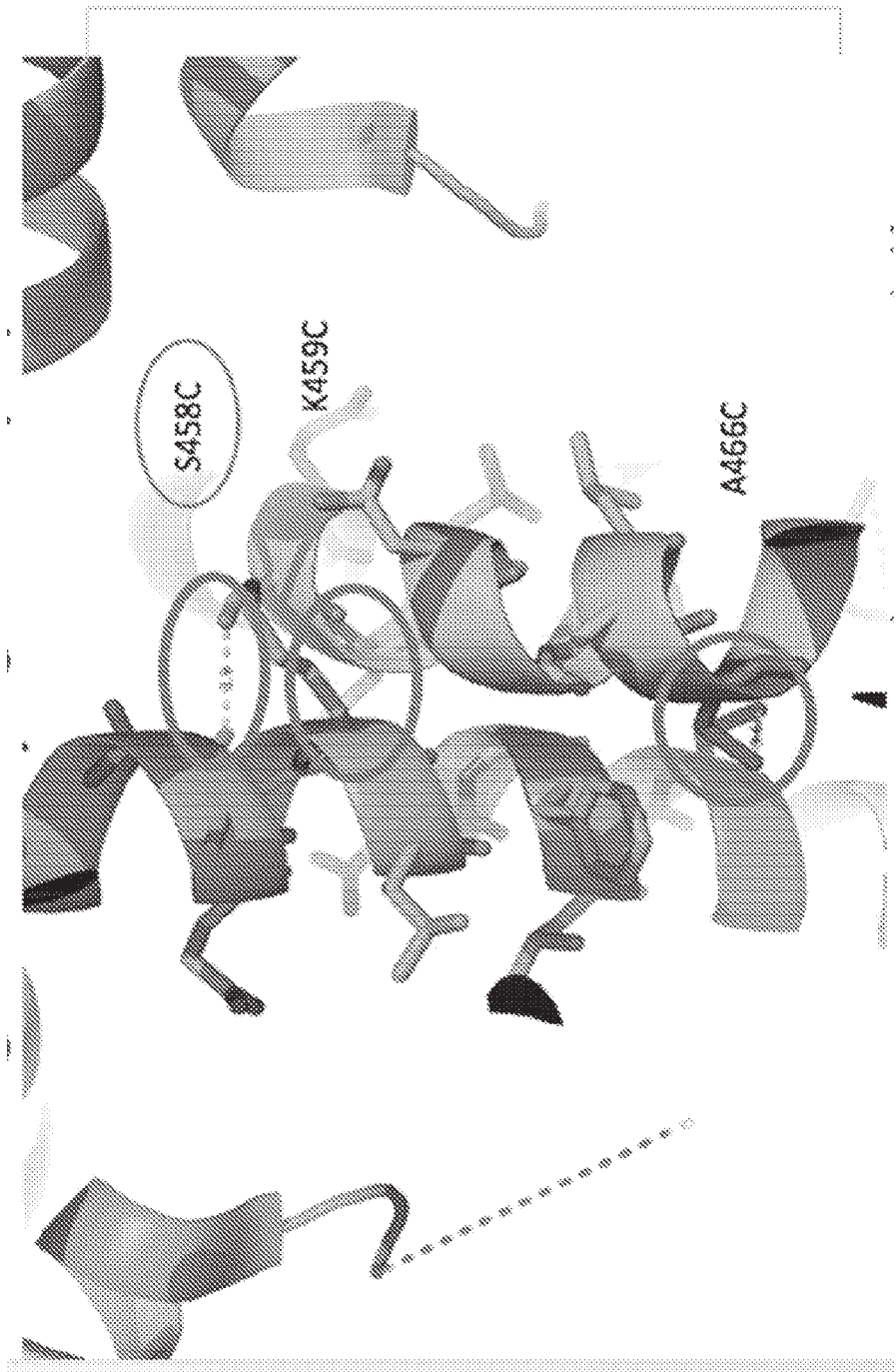


Fig. 60

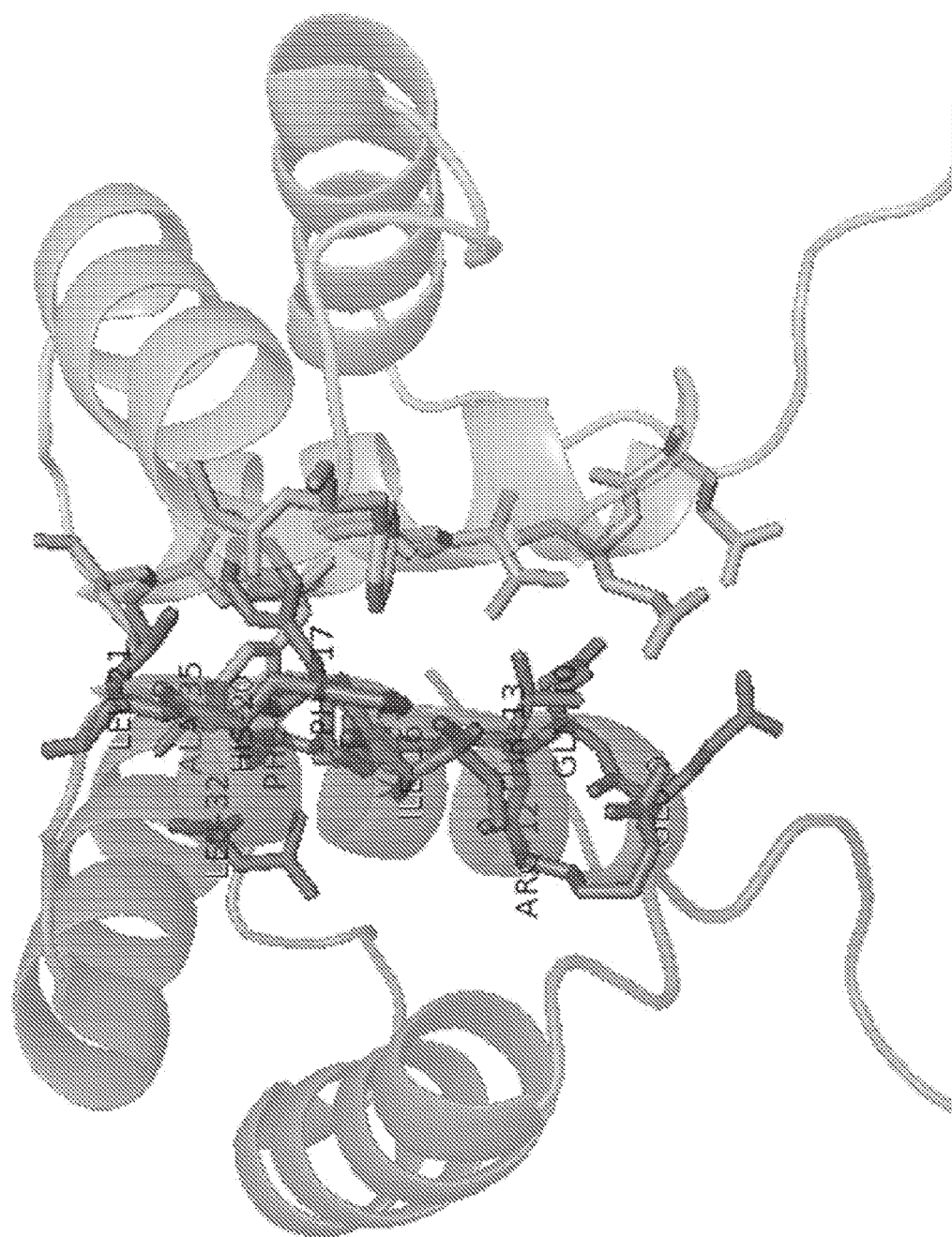


Fig. 61

Fig 5. Dimer transposase nanoparticle bioconjugate (dTnp-NP)

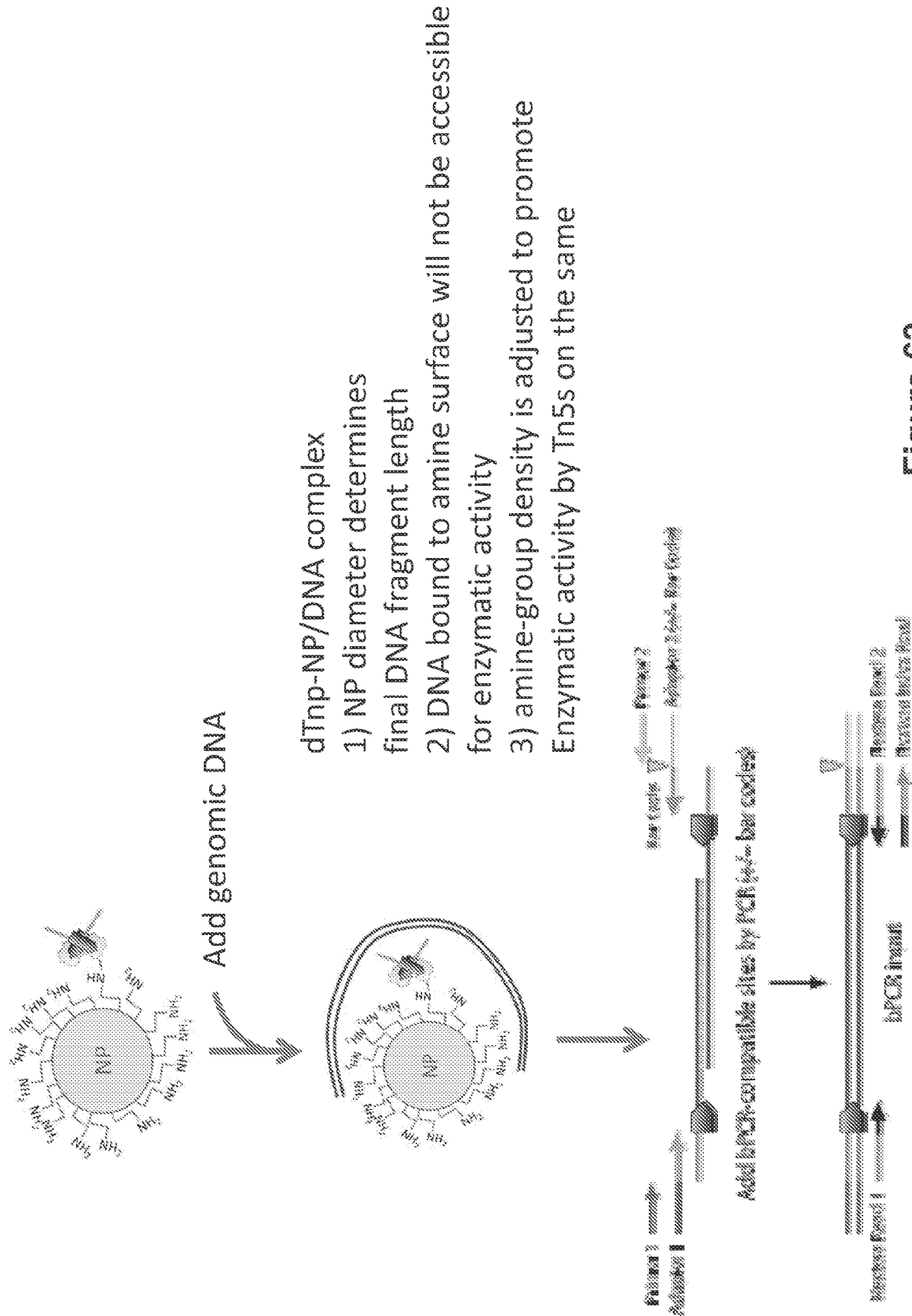


Figure 62

Mu-looped Complexes

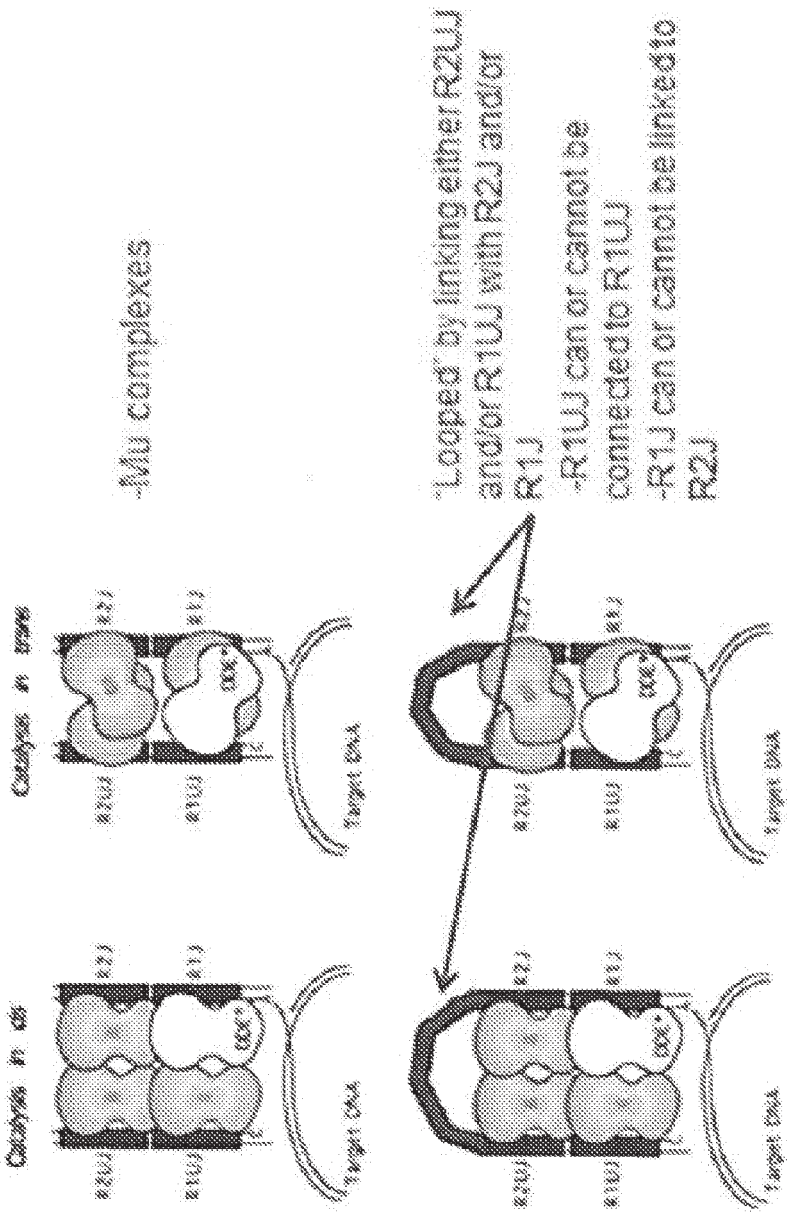
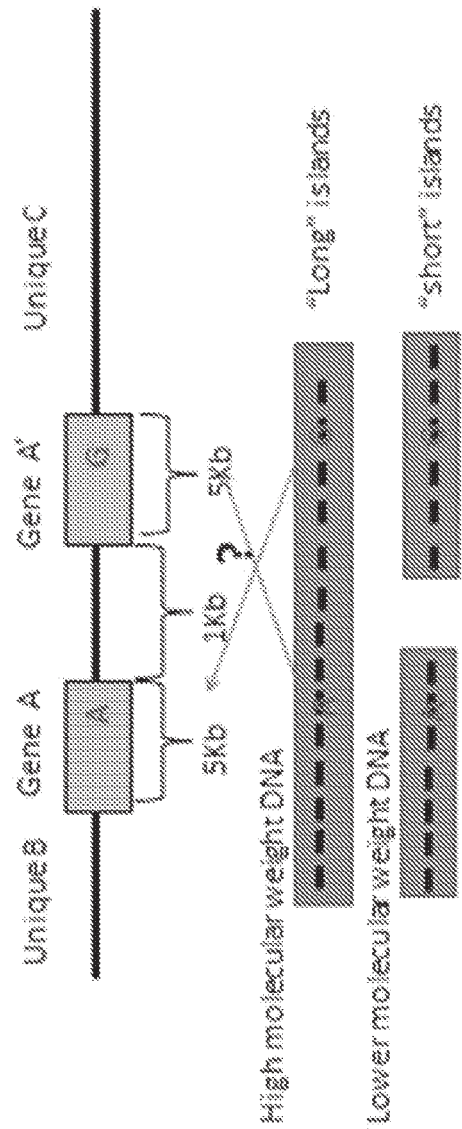


Fig. 63

Indexed linked reads for assembly/phasing of pseudo-genes



- Example, CYP2D6
 - Long islands (>30kb) of indexed linked reads will span the pseudogenes, gene A and A'
 - Due to the high sequence, it is not possible to determine which variant belongs to Gene A or Gene A'
 - Shorter islands will only link one variant in one of the pseudogenes with unique surrounding sequence.
- > This can be accomplished by shearing the DNA before CPT-seq or use combination of sheared and unshredded DNA

Fig. 64

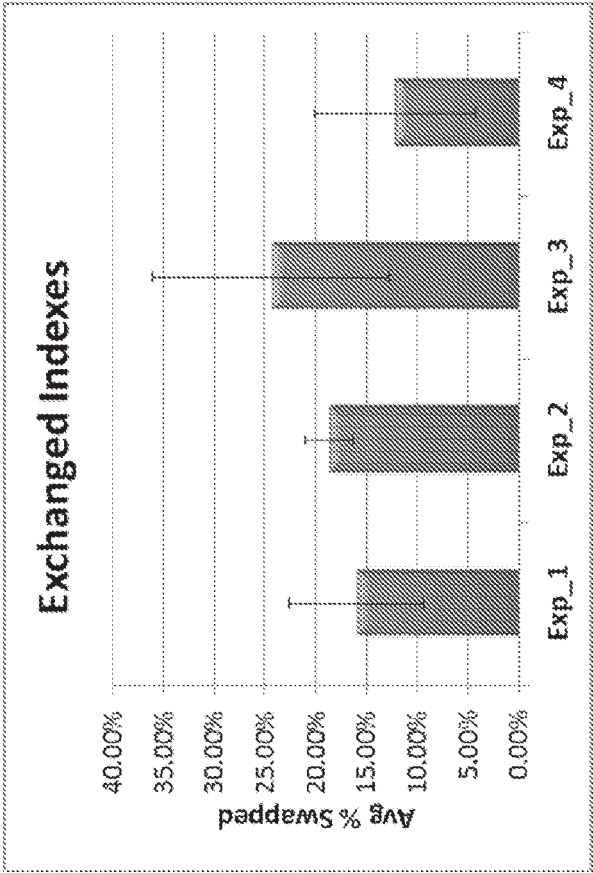


Figure 1: Index exchange. Bead with different indices were prepared. After mixing, Index exchange was determine by sequencing the libraries and reporting the indices for each library. % “swapped” was calculated as $(D4+D5+E3+E5+f4)/(\text{sum of all } 96)$ and reported in the figure.



Fig. 65

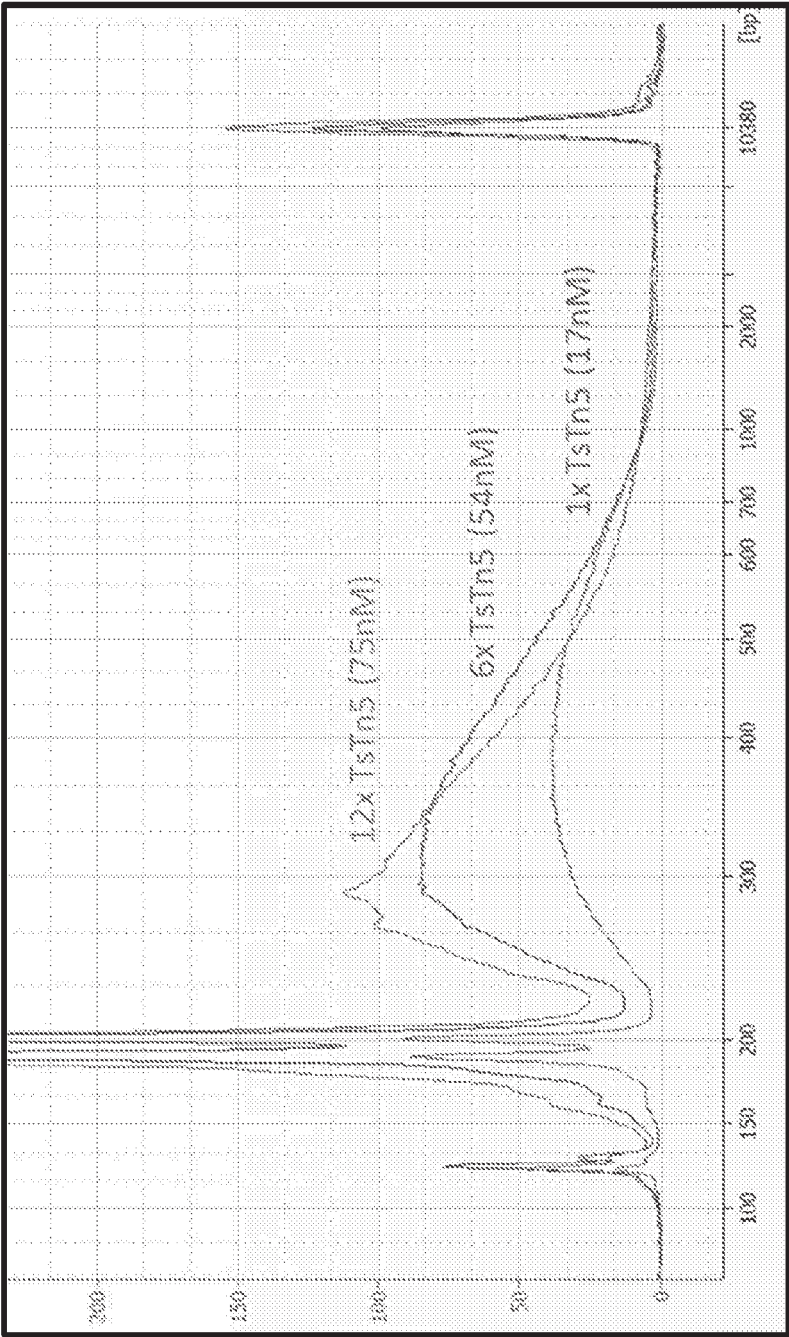
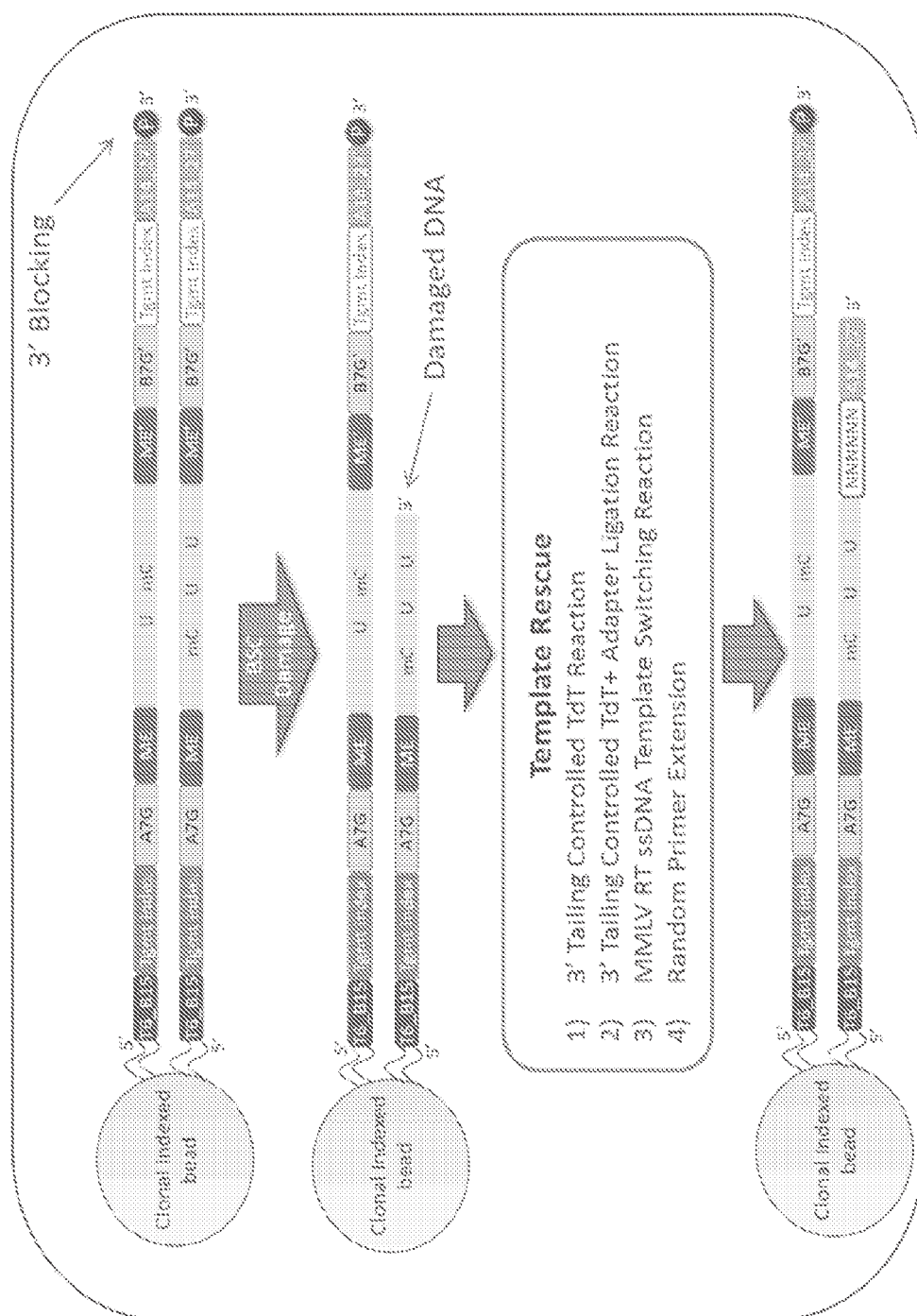


Fig. 66



79

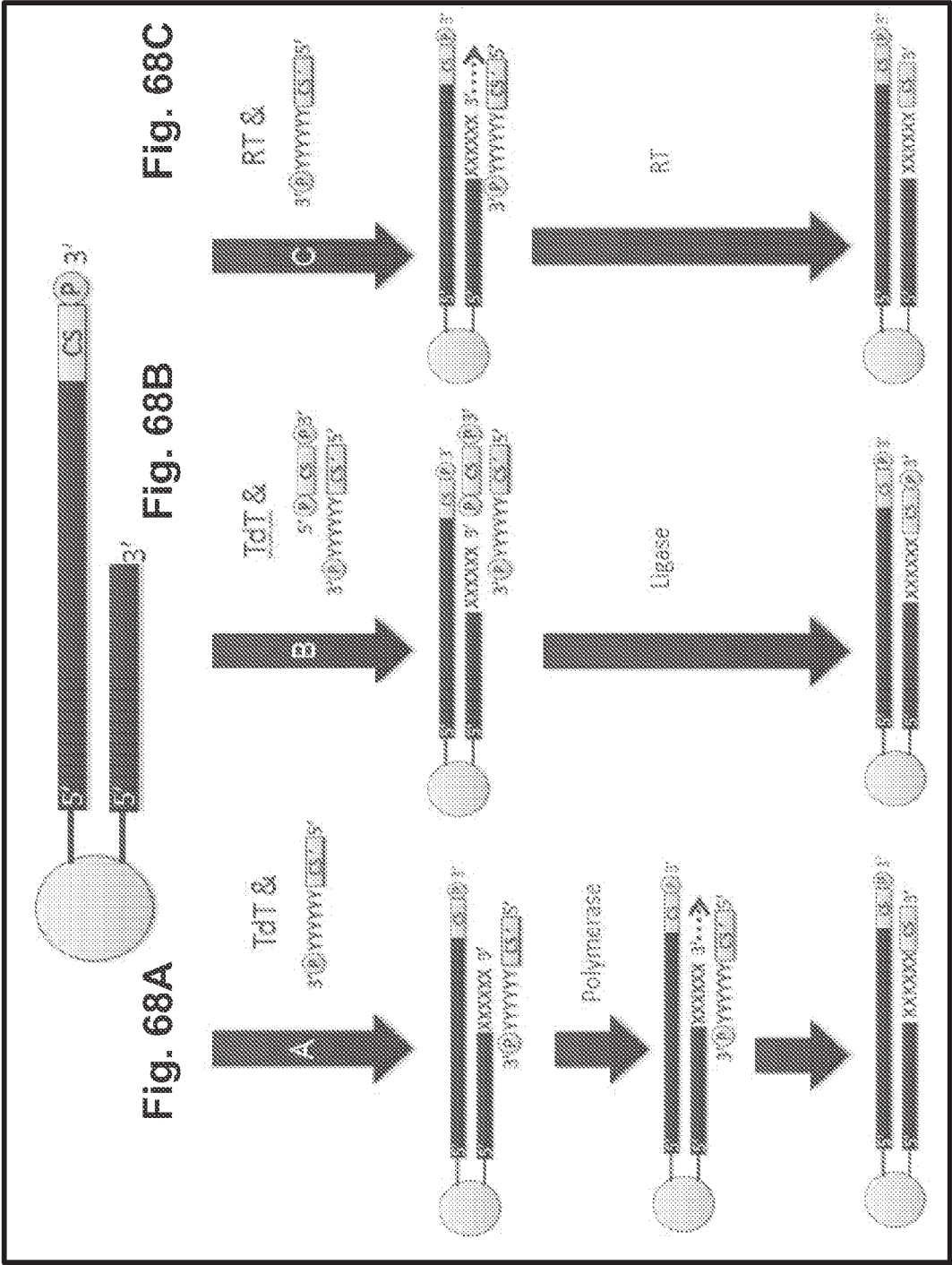


Fig. 68

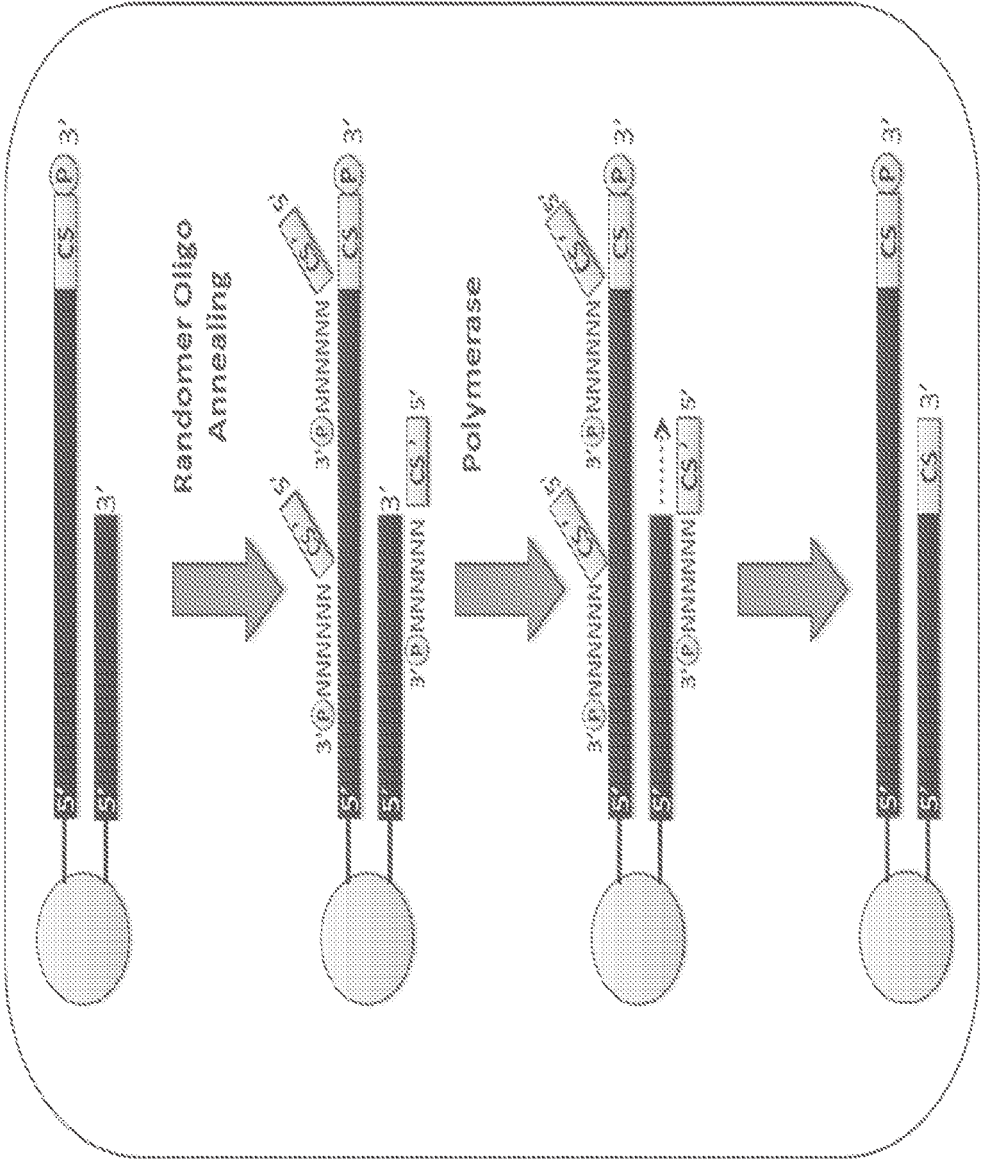


Fig. 69

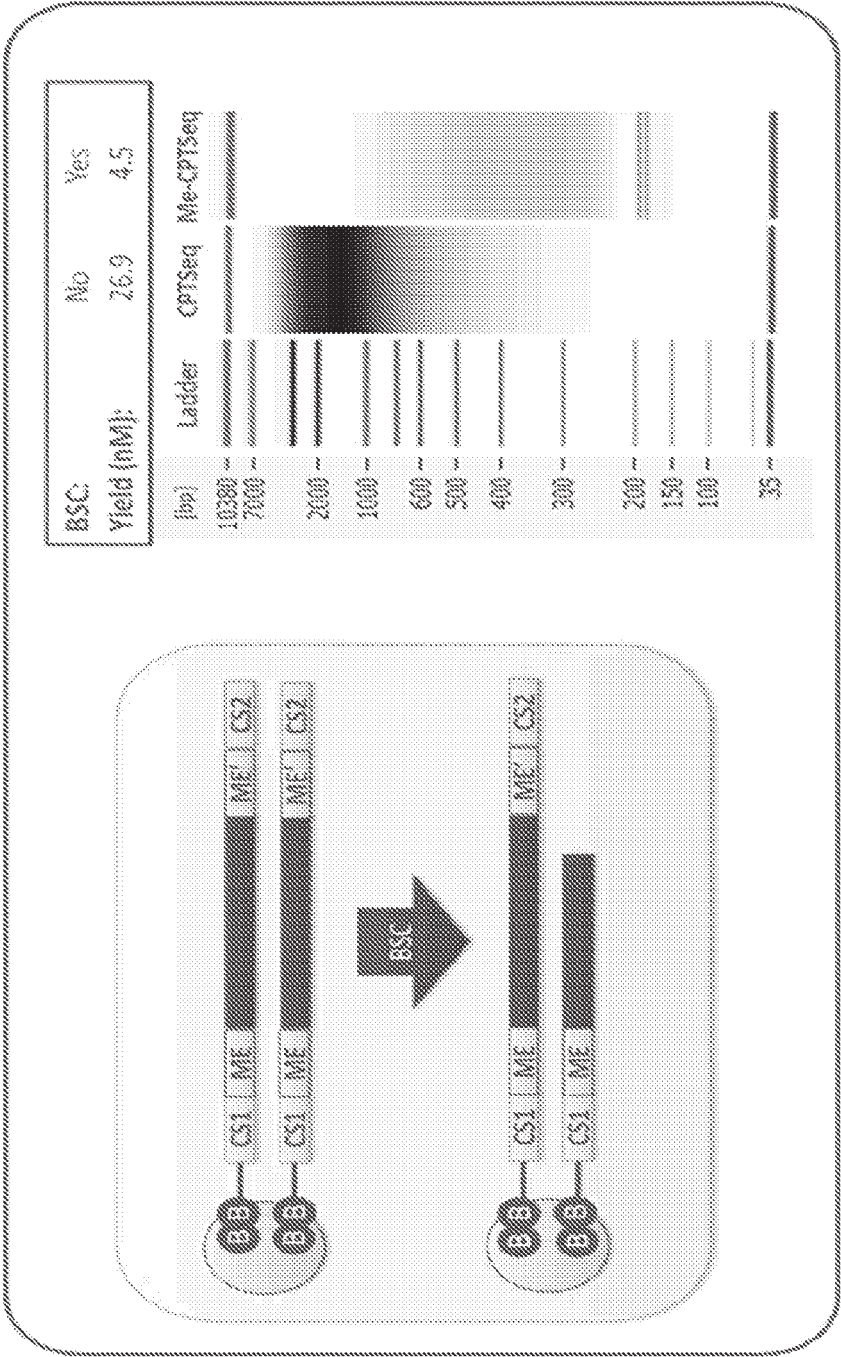


Fig. 70

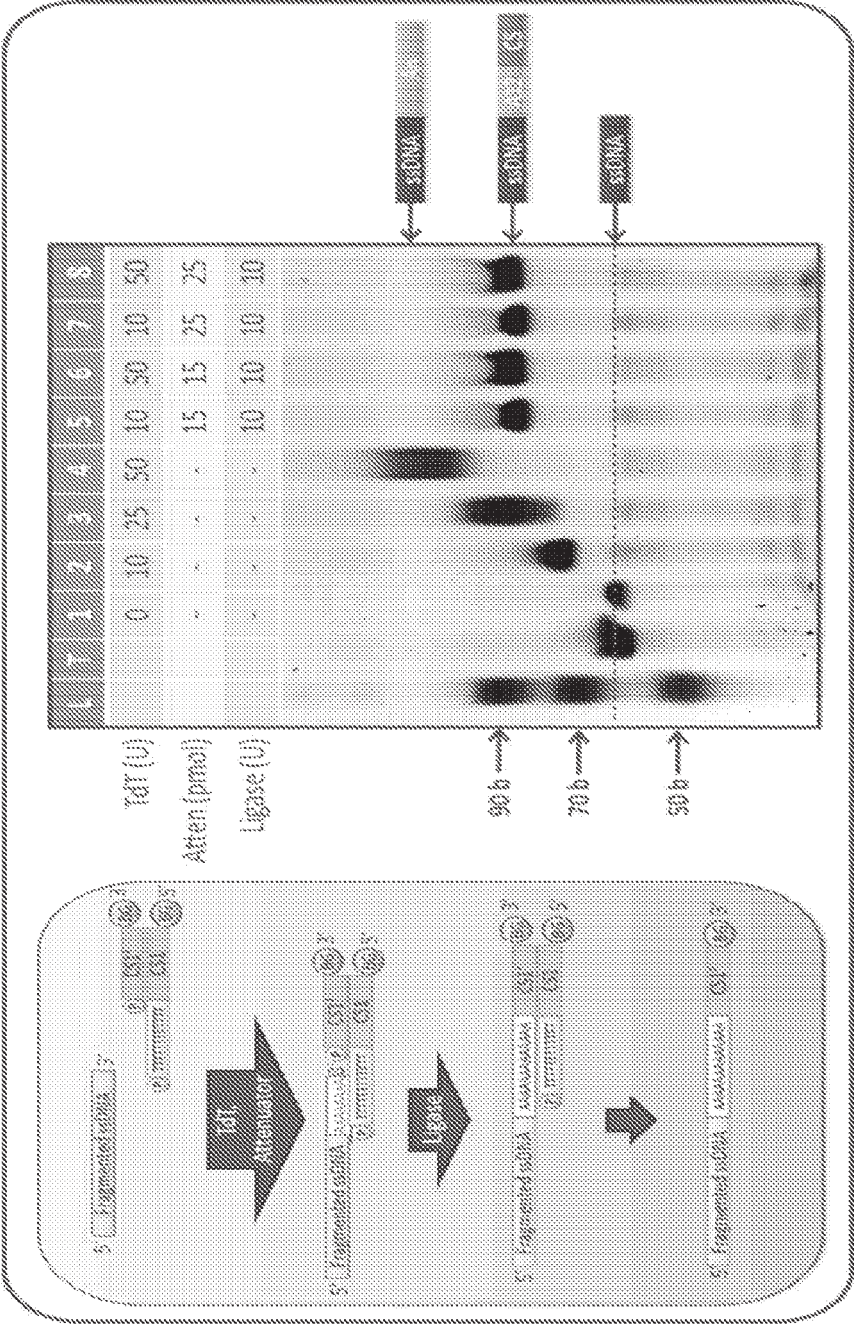


Fig. 71

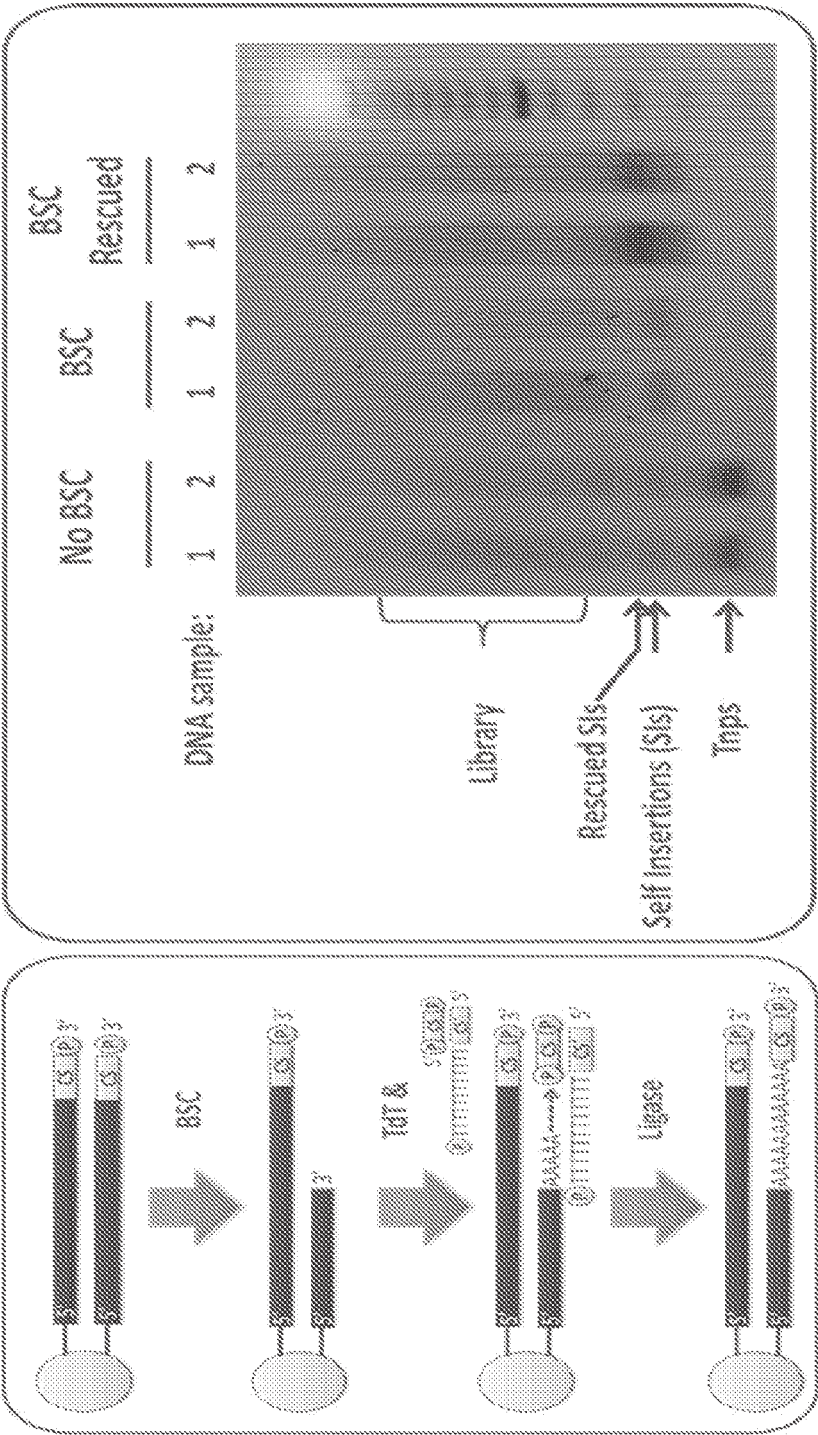


Fig. 72

MiSeq Methyl CPT-Seq Statistics

- Observed phased reads “islands”
 - Average island size 17 kb
 - >50% unique reads
 - 88% of Cs are converted to Us

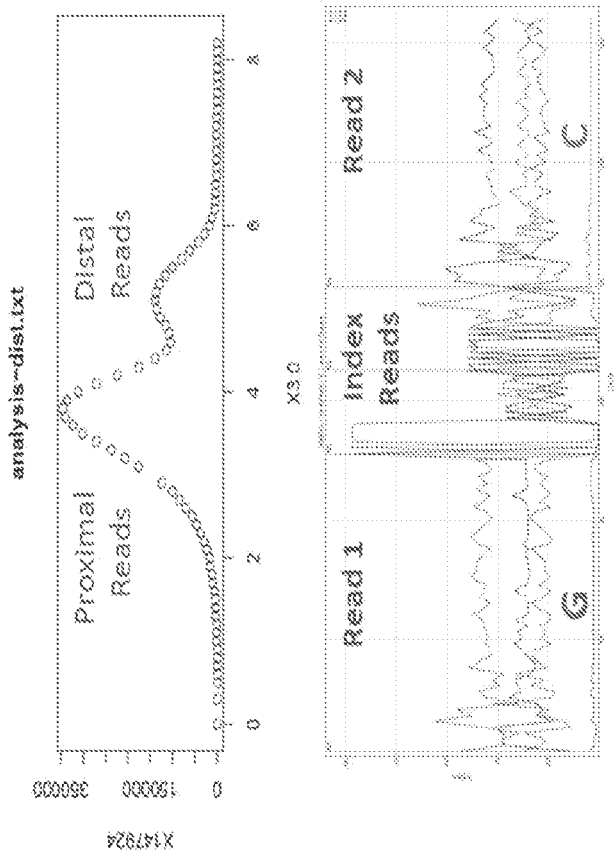


Fig. 73

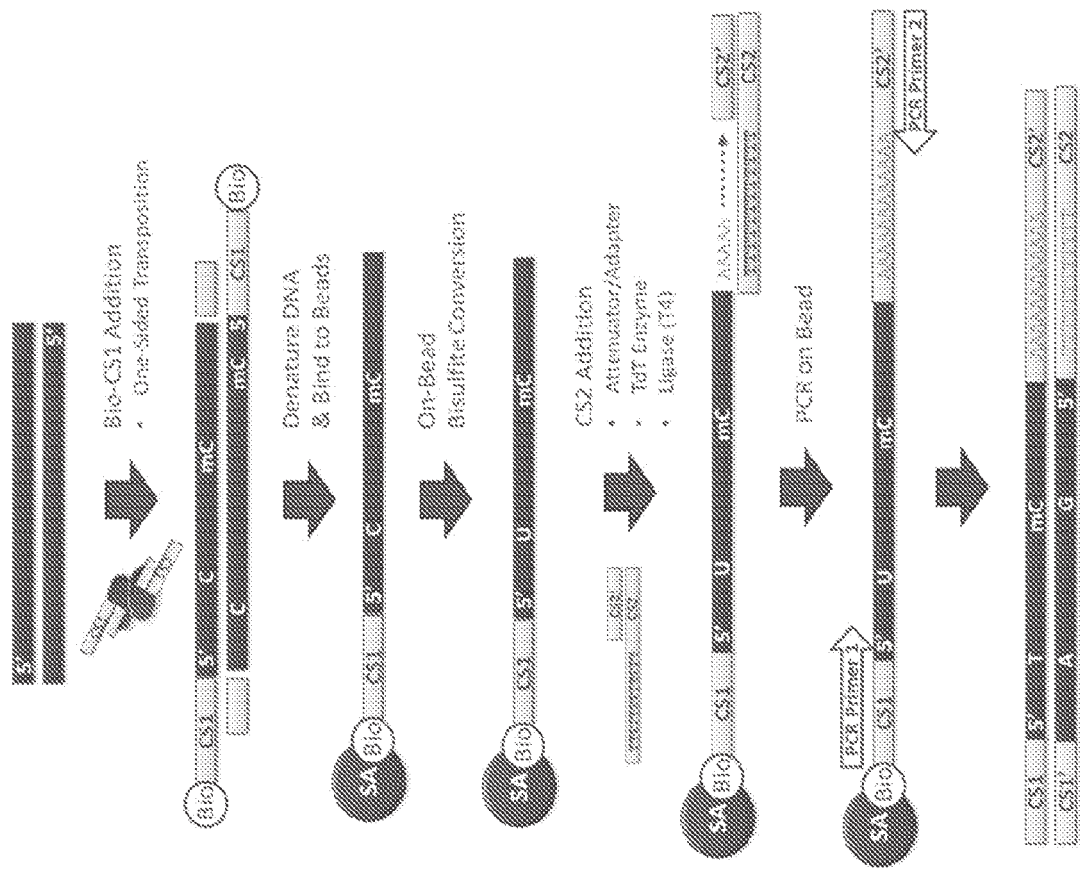


Fig. 74

Bisulphite Conversion Efficiency Optimization

Cryst.	DMF	Reagent	Reaction conditions
1		NaO	1 hr @ 60°C / 0.3M NaOH
2			1 hr @ 60°C / 0.3M NaOH
3	20%	NaO	1 hr @ 60°C / 0.3M NaOH
4			1 hr @ 60°C / 0.3M NaOH

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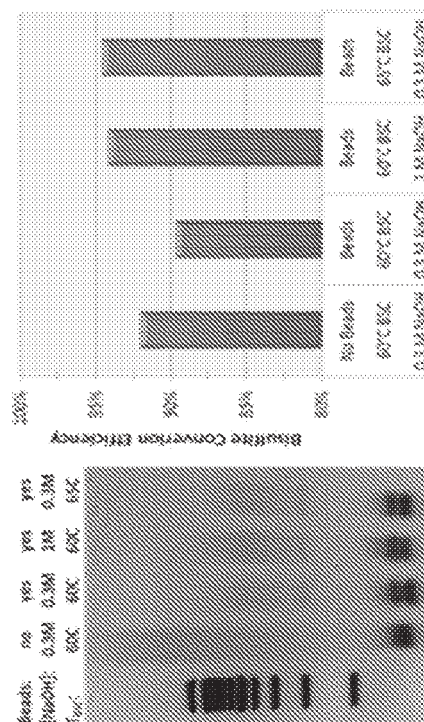
are associated with the occurrence of this disease. Coverage of the genome.

[illegible]

Fig. 75A

155

- 93% bisulfite conversion with 1M NaOH at 65°C heat treatment.



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5767