

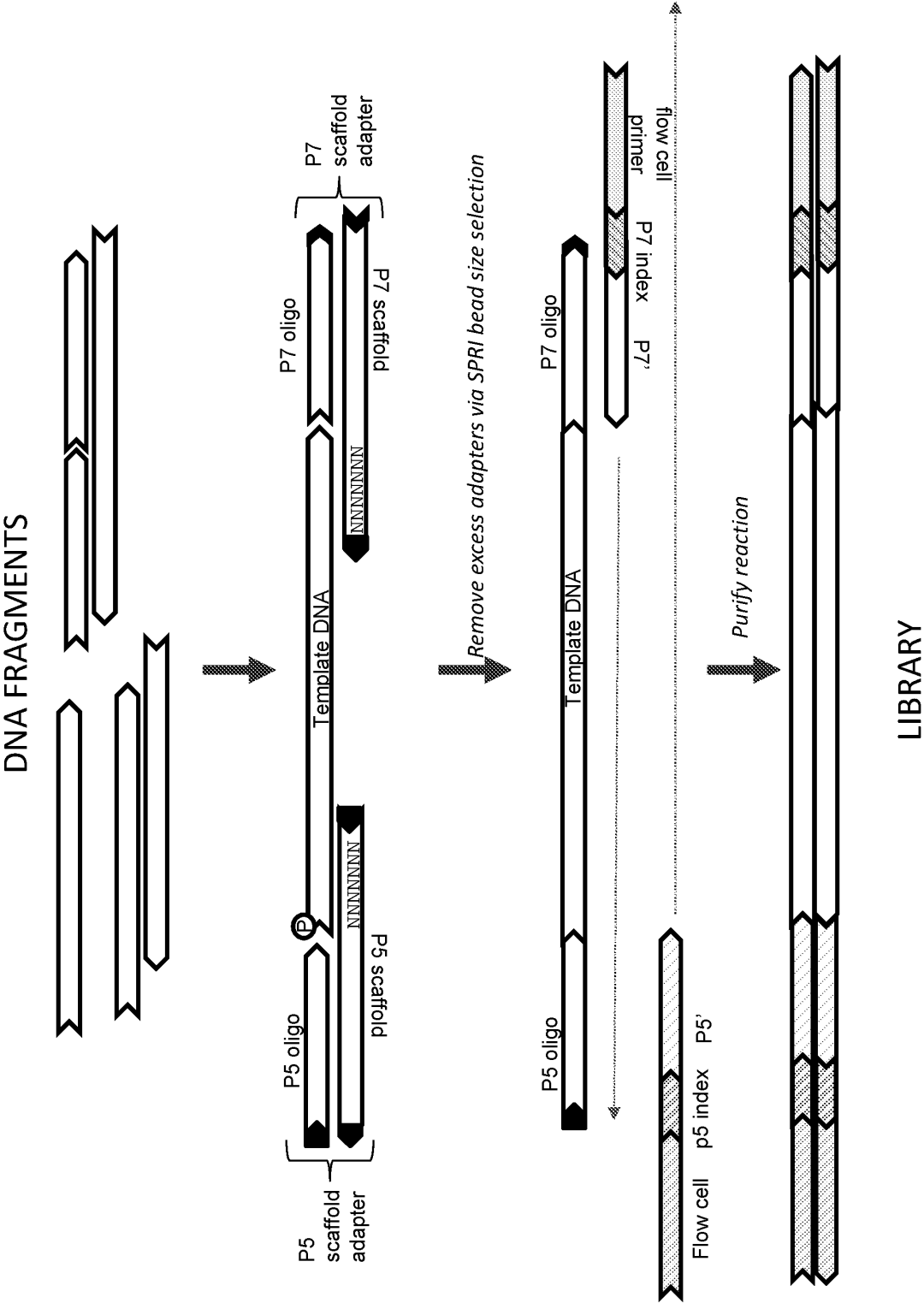


Fig. 1

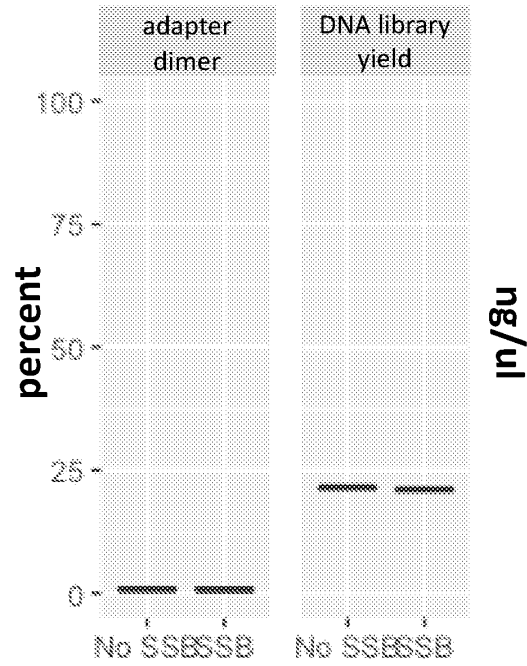


Fig. 2A

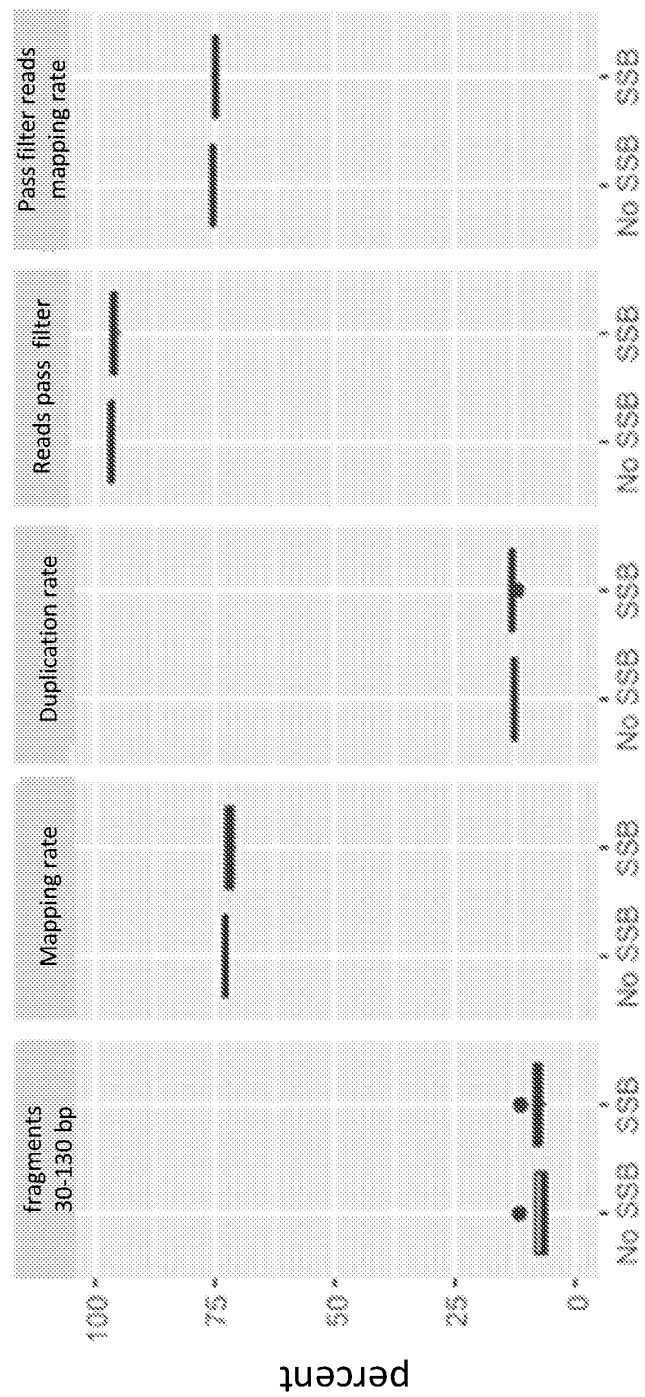


Fig. 2B

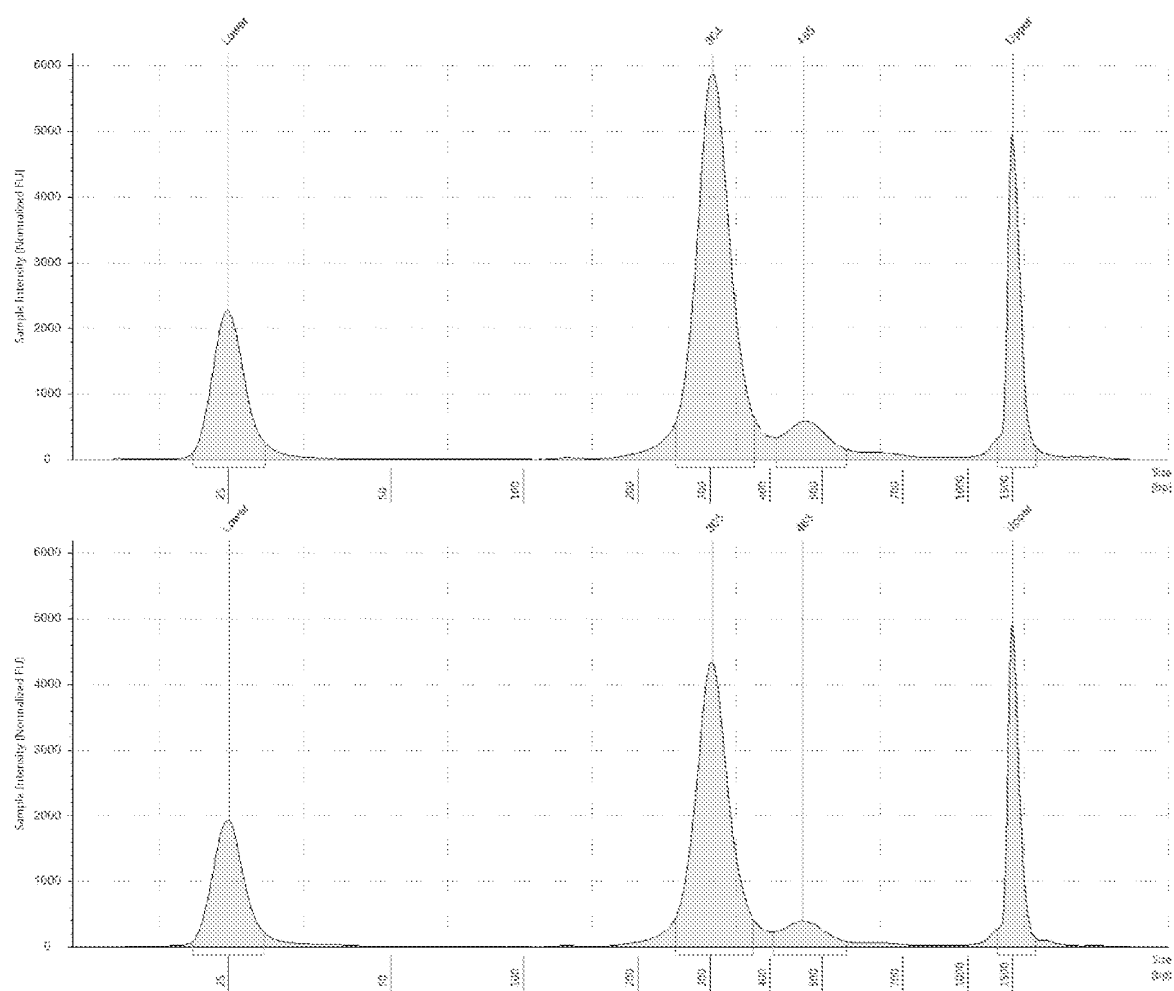


Fig. 3A

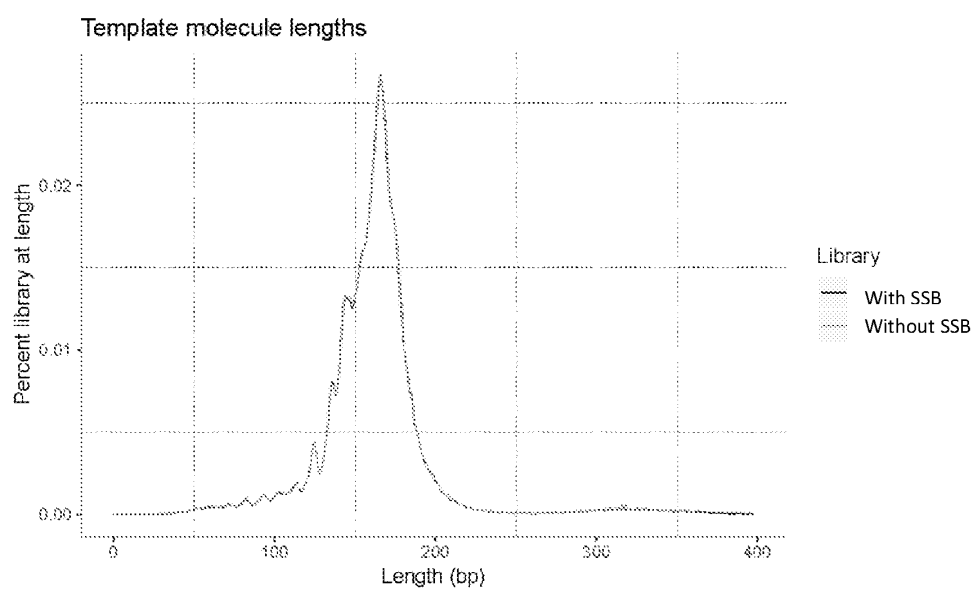


Fig. 3B

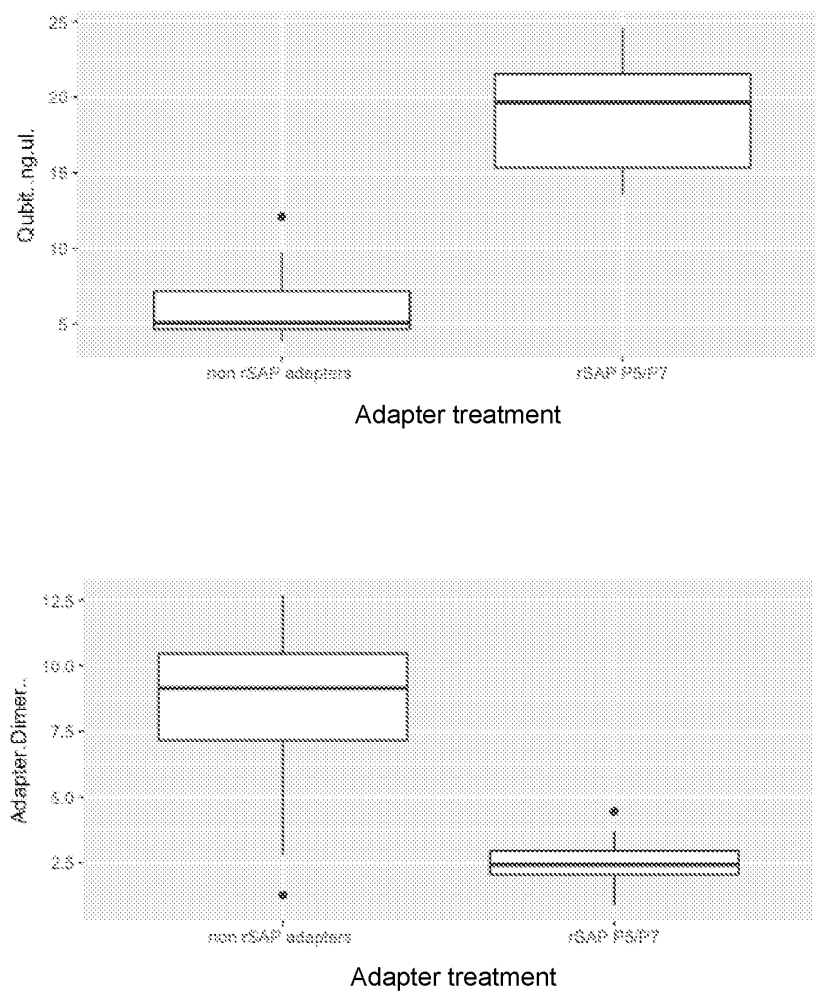


Fig. 4

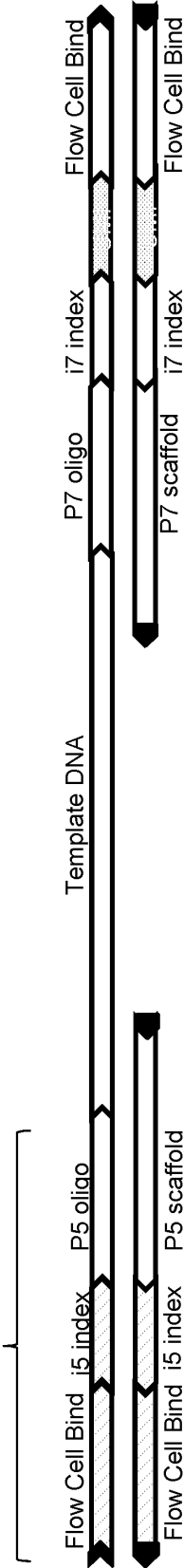


Fig. 5A

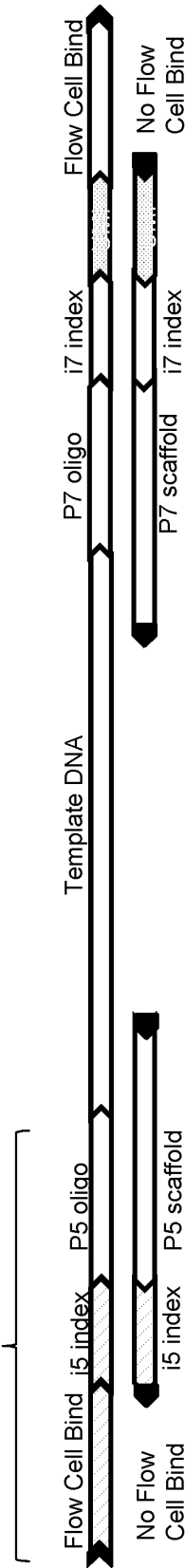


Fig. 5B

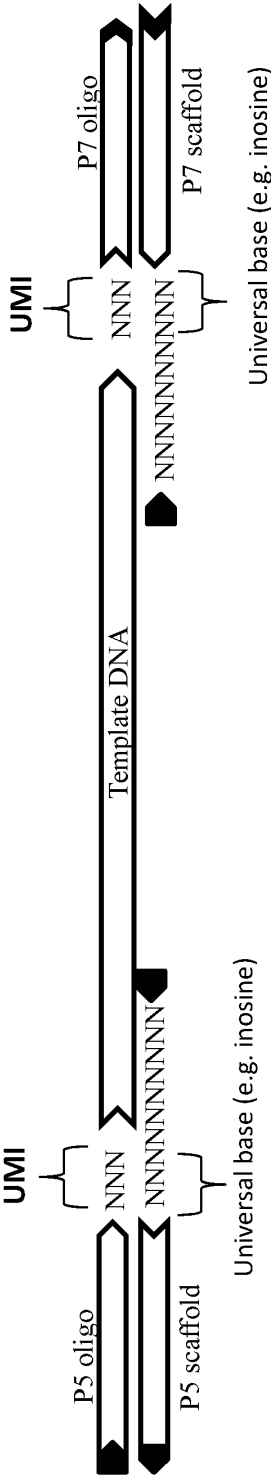


Fig. 6A

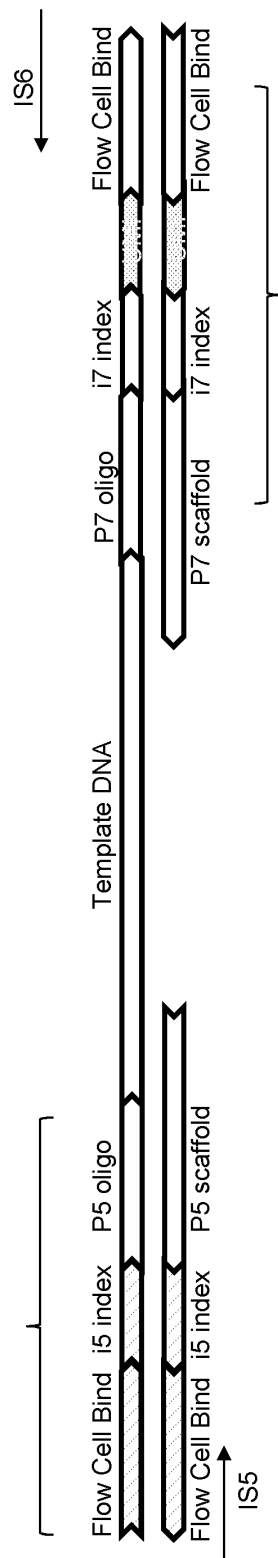
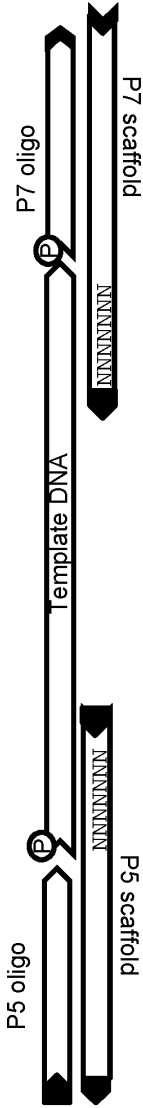
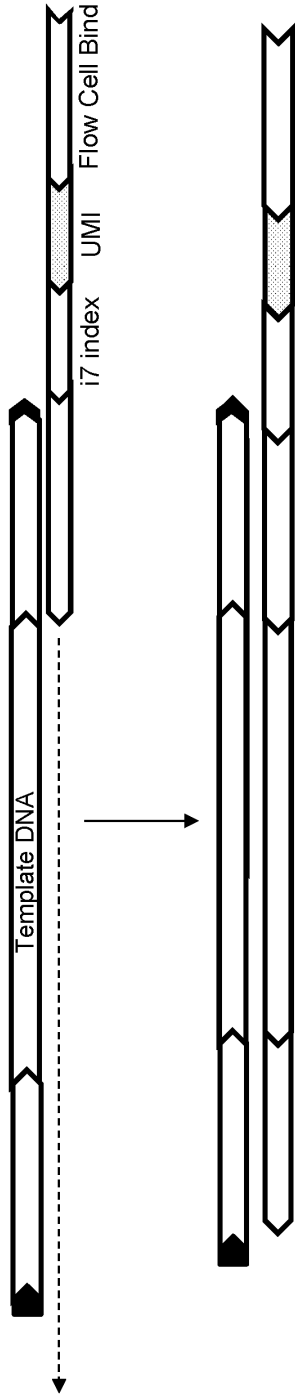


Fig. 6B

Library molecule after adapter + scaffold ligation reaction



Denature, add UMI and indexing adapter via single primer extension



Index PCR specifically for strand with UMI

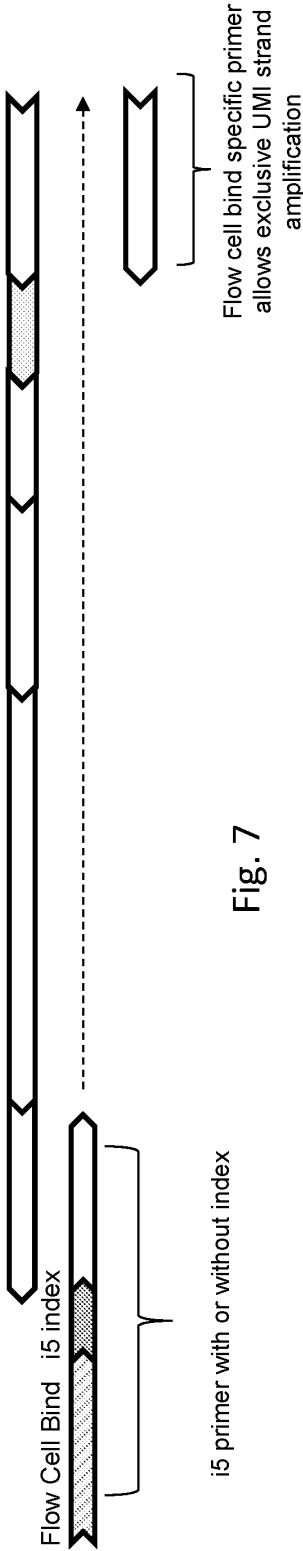


Fig. 7

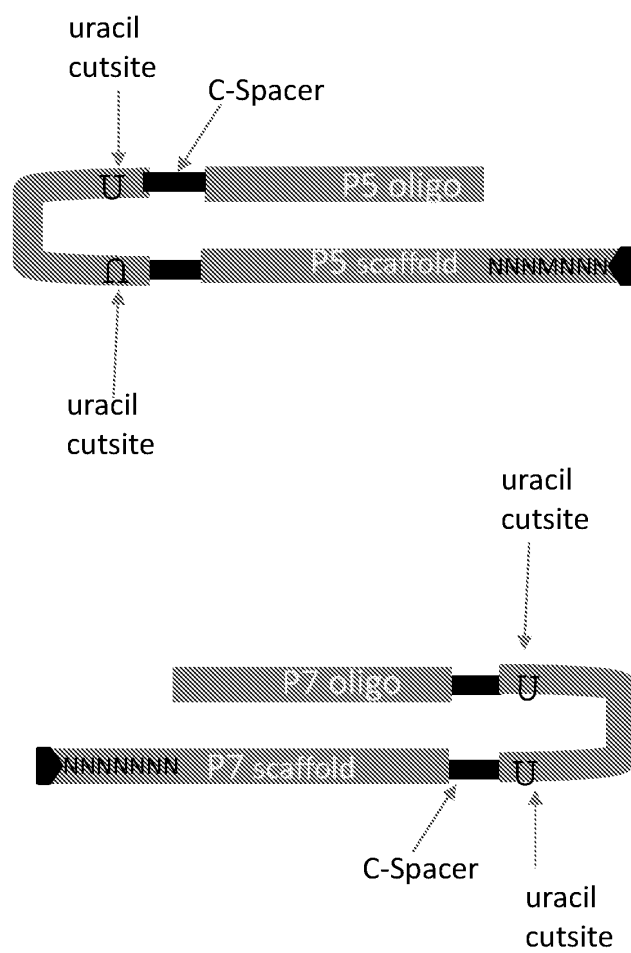


Fig. 8

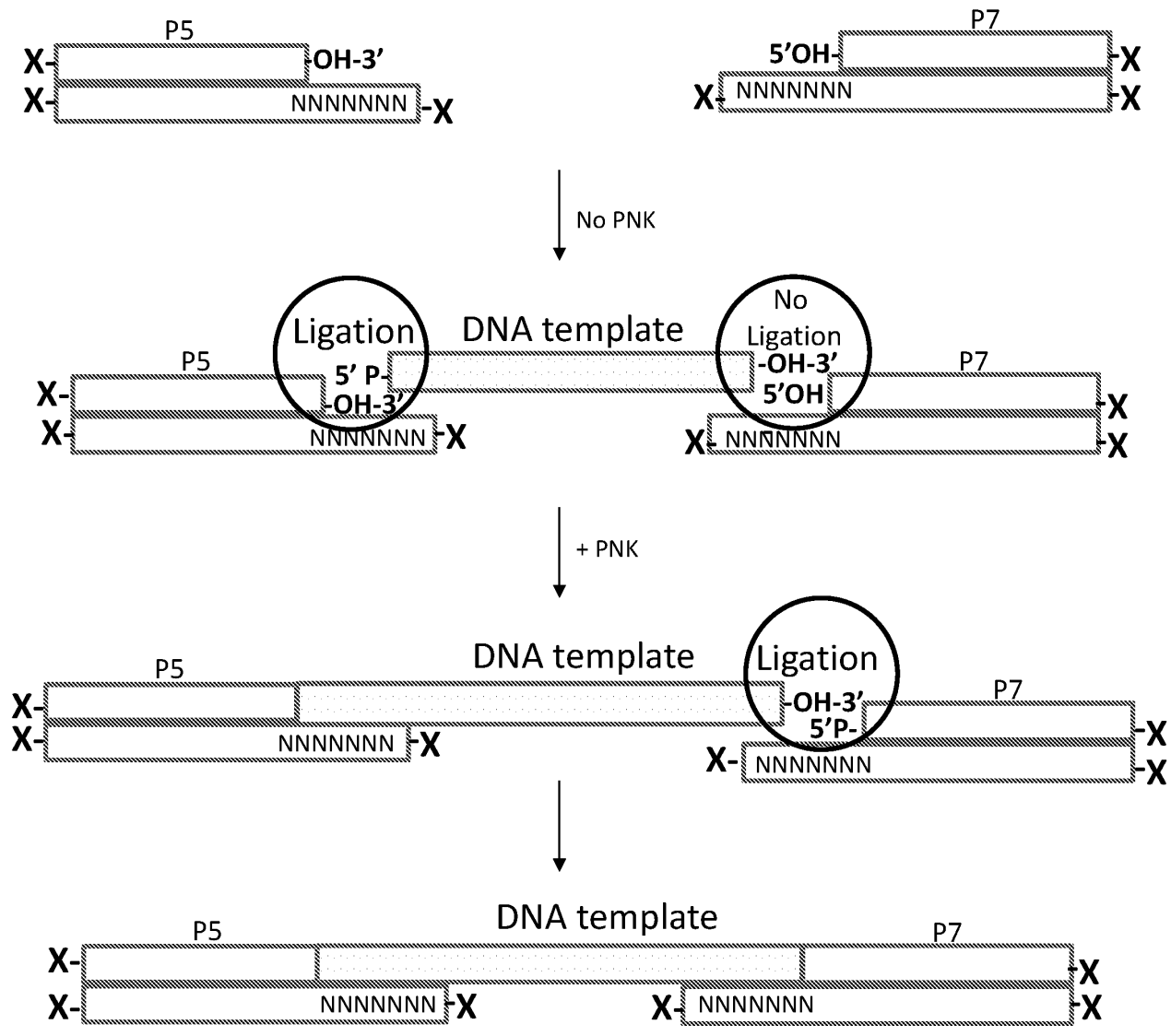


Fig. 9

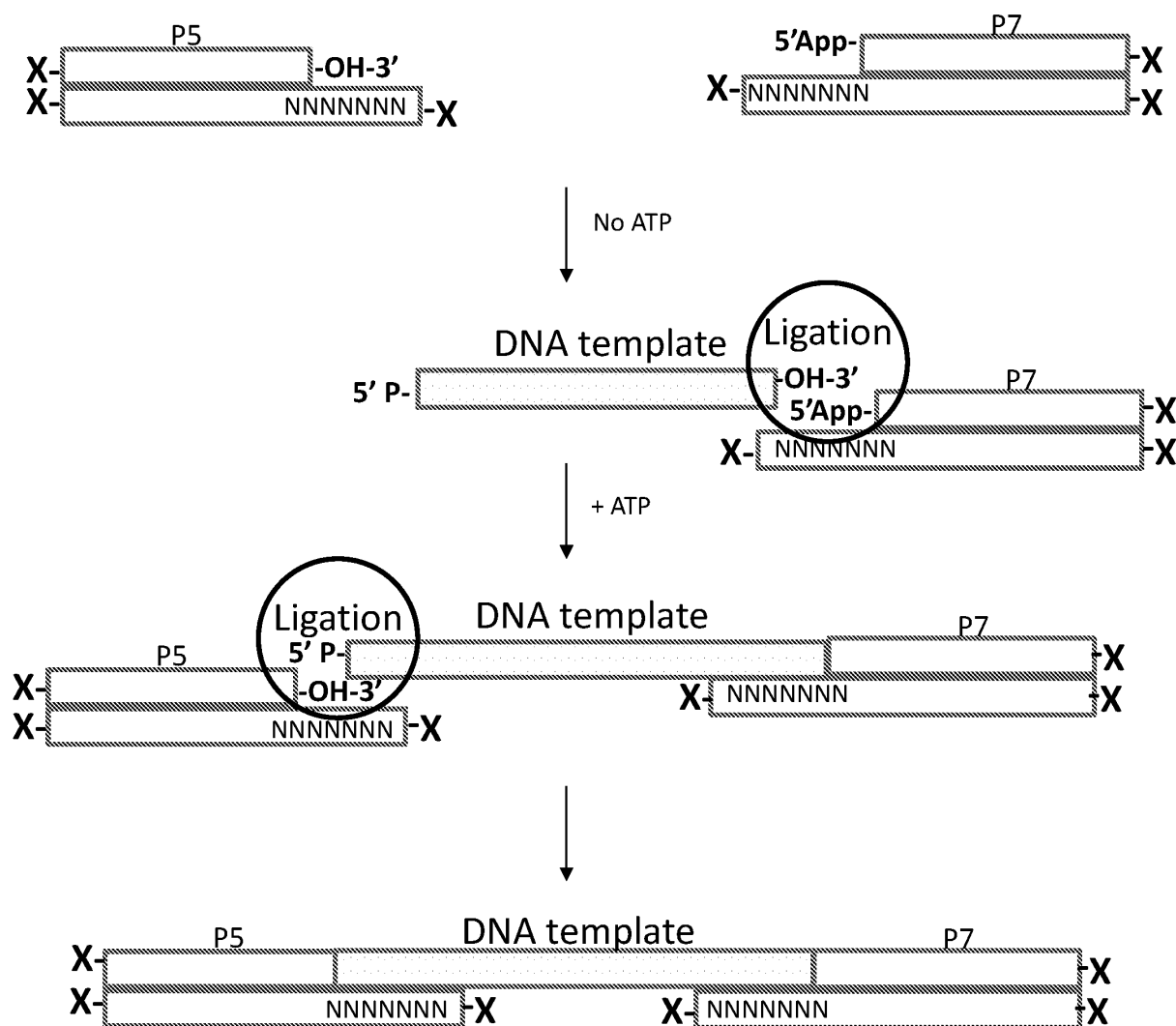


Fig. 10

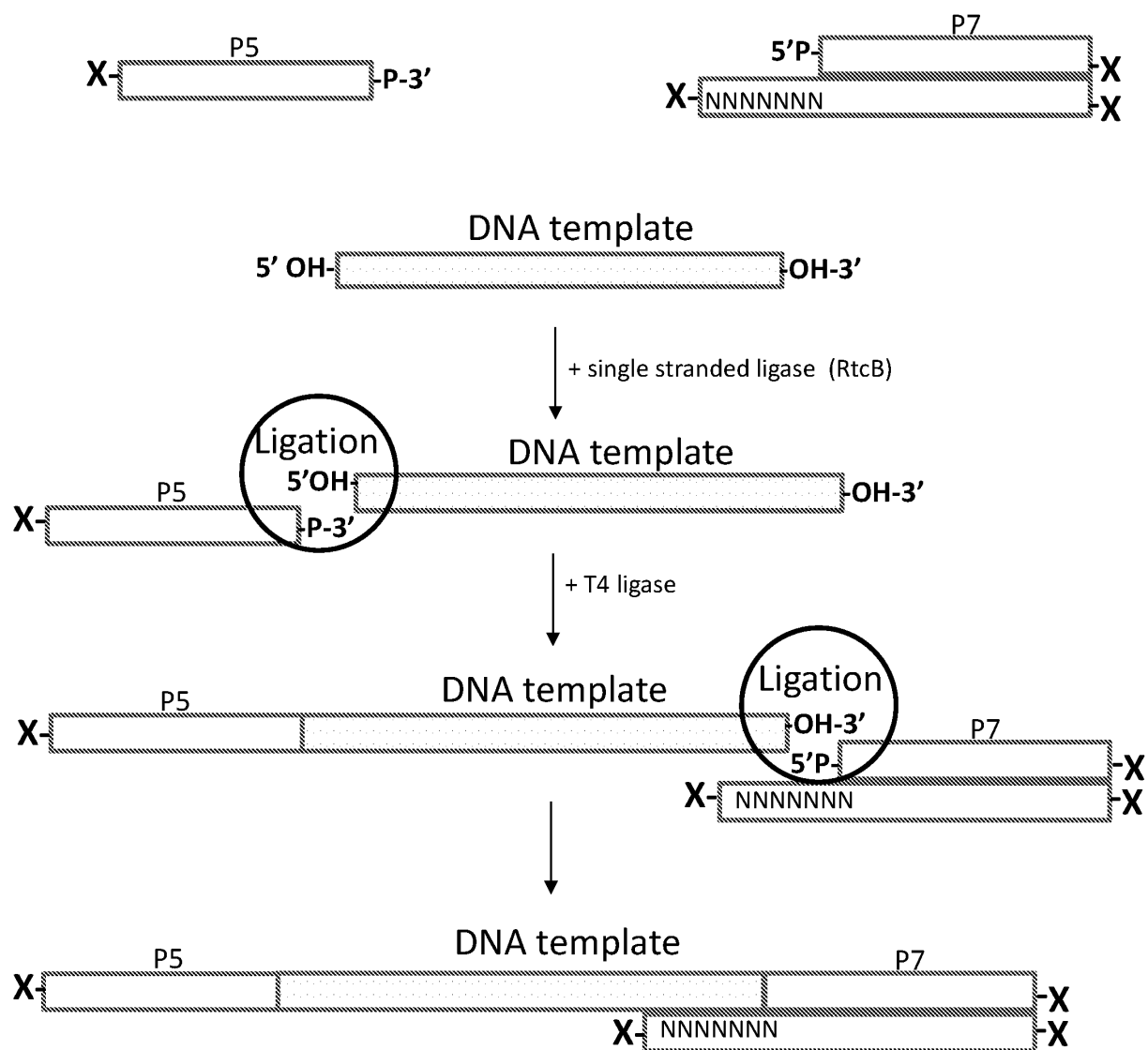


Fig. 11

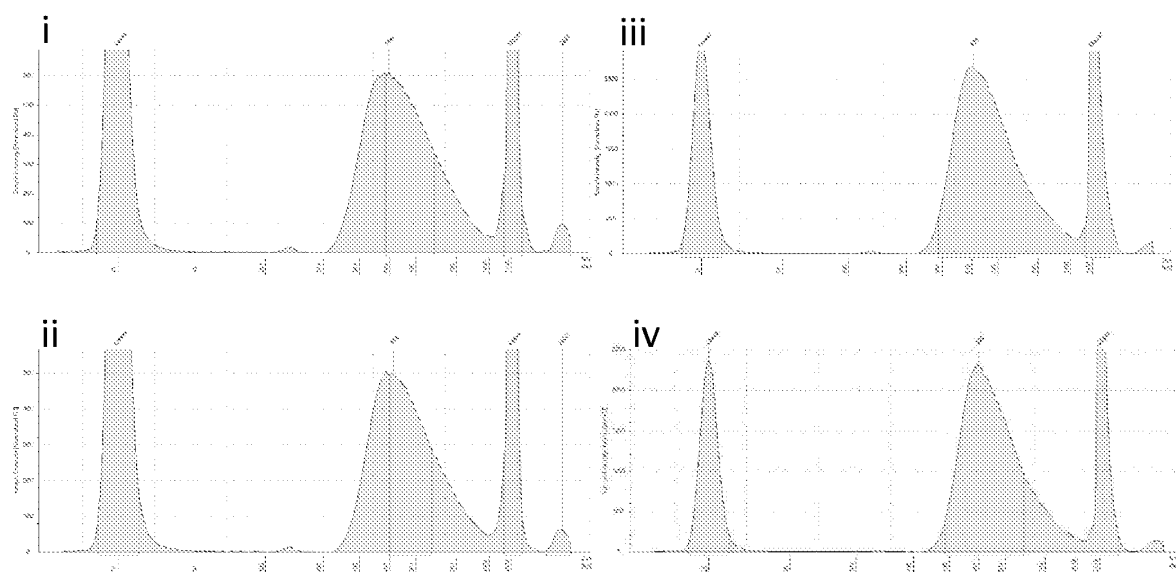
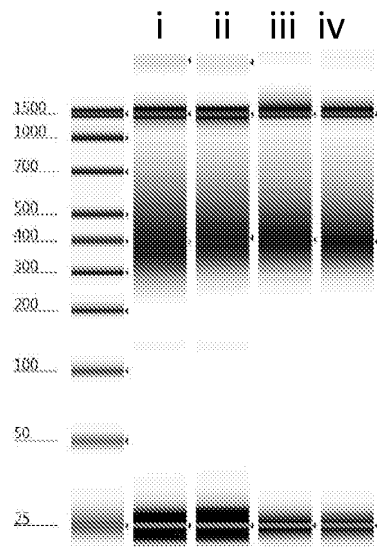


Fig. 12



	RNA input (ng)	Quantity (ng/μl)	Tapestation (bp)
i	10	12	398
ii	10	11.2	414
iii	10	28.2	406
iv	10	28.5	399

Fig. 13

	i	ii	iii	iv
Number of input reads	441589	272701	250203	250592
Average input read length	202	202	202	202
Uniquely mapped reads number	337546	205866	208486	213879
Uniquely mapped reads %	76.44%	75.49%	83.33%	85.35%
Average mapped length	196.76	196.85	200.6	200.63
Number of reads mapped to multiple loci	22877	14759	7711	7877
% of reads mapped to multiple loci	5.18%	5.41%	3.08%	3.14%
Number of reads mapped to too many loci	445	286	110	86
% of reads mapped to too many loci	0.10%	0.10%	0.04%	0.03%
% of reads unmapped: too many mismatches	0.00%	0.00%	0.00%	0.00%
% of reads unmapped: too short	18.26%	18.97%	13.52%	11.45%
% of reads unmapped: other	0.02%	0.02%	0.02%	0.02%
Number of chimeric reads	0	0	0	0
% of chimeric reads	0.00%	0.00%	0.00%	0.00%

Fig. 14

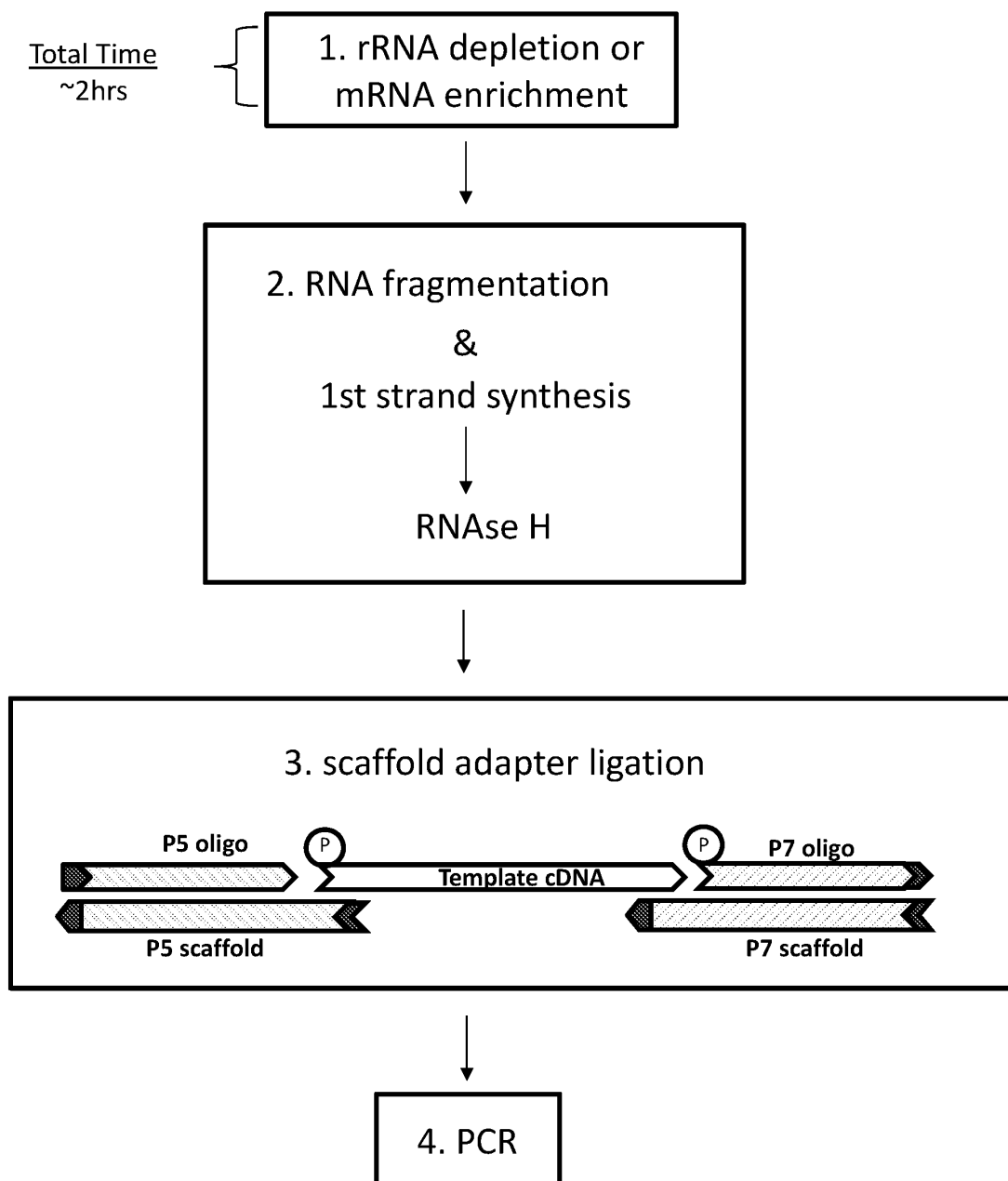


Fig. 15

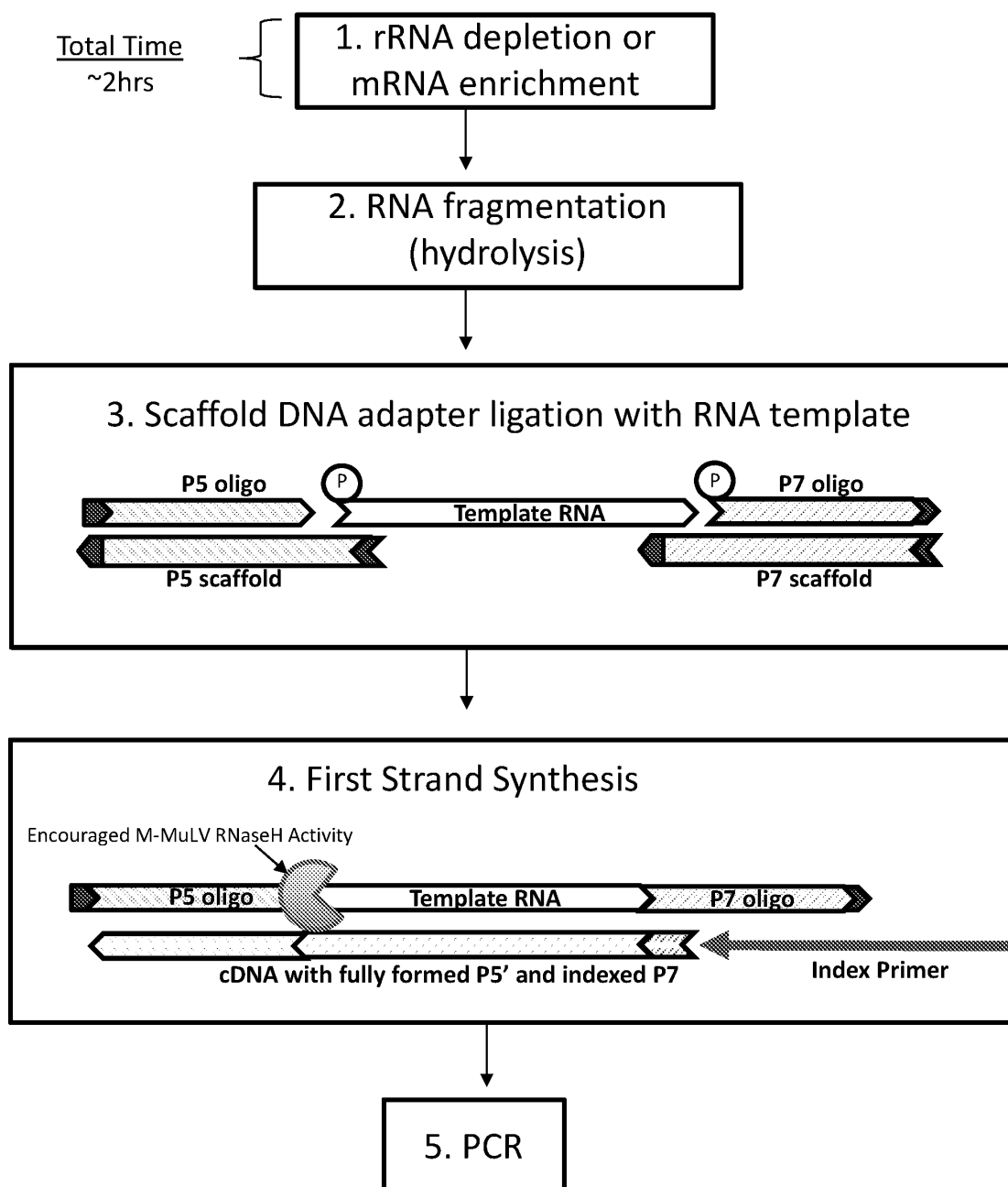


Fig. 16

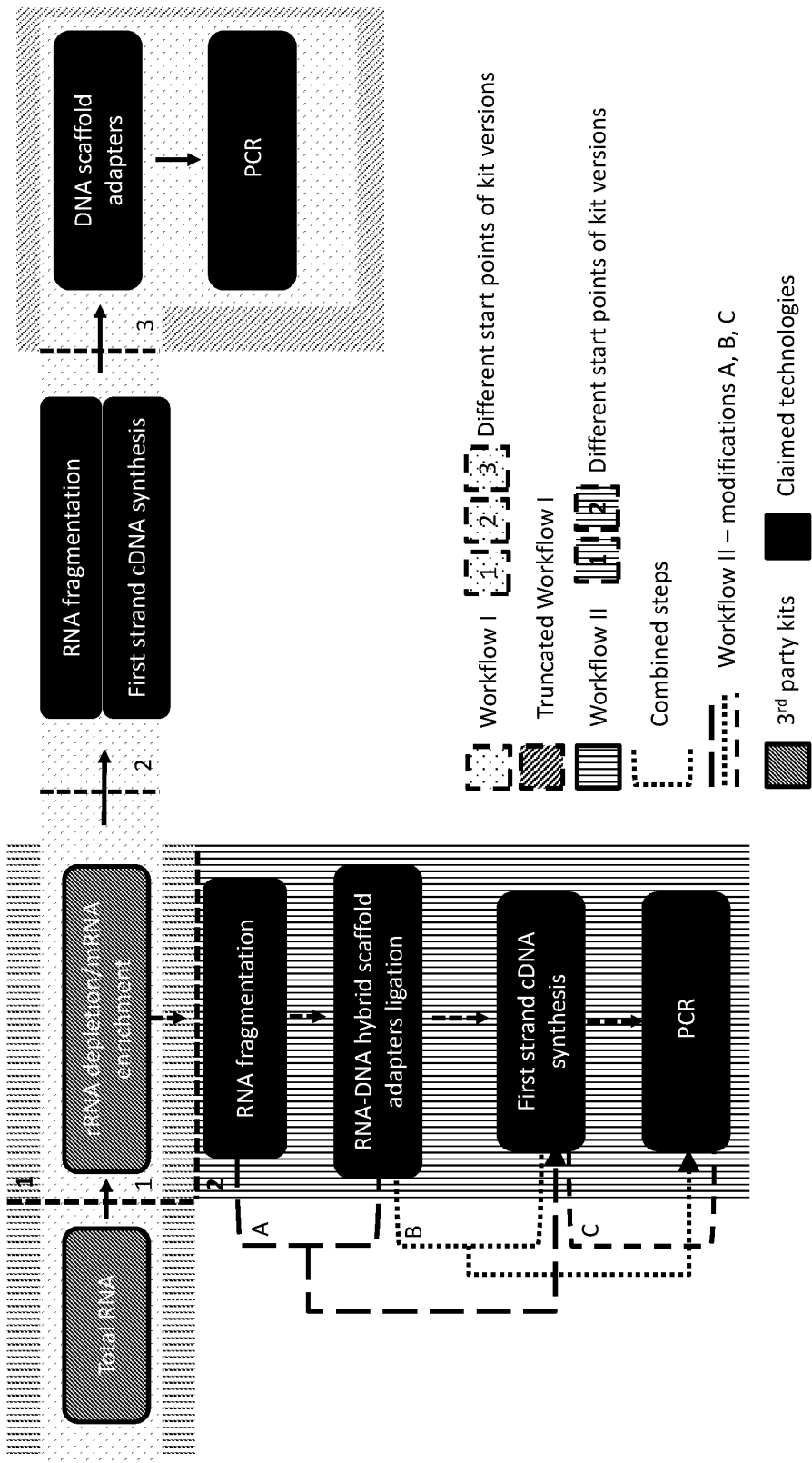


Fig. 17

Purpose	Input cfDNA	cfDNA Purification Kit	Original Tapestation Dimer %	1st Serial 1.2x SPRI Dimer %	2nd Serial 1.2x SPRI Dimer %	3rd Serial 1.2x SPRI Dimer %
1.2x Serial SPRI Clean x3	S0072	Zymo	87.55	52.13	12.97	5.48

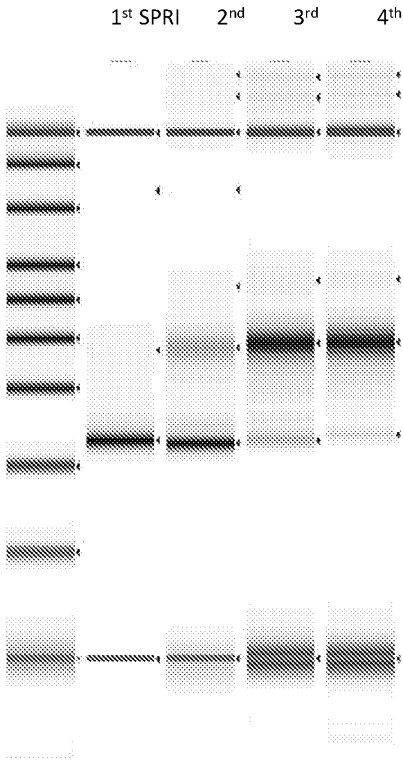


Fig. 18

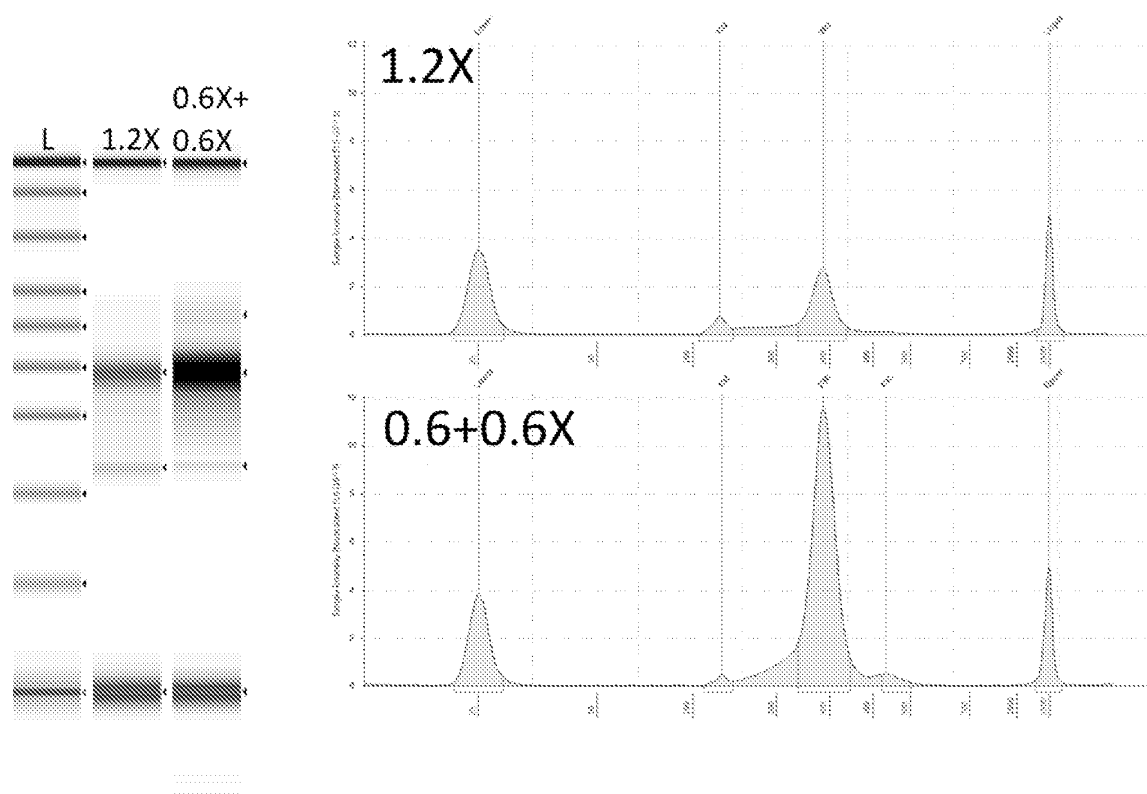


Fig. 19

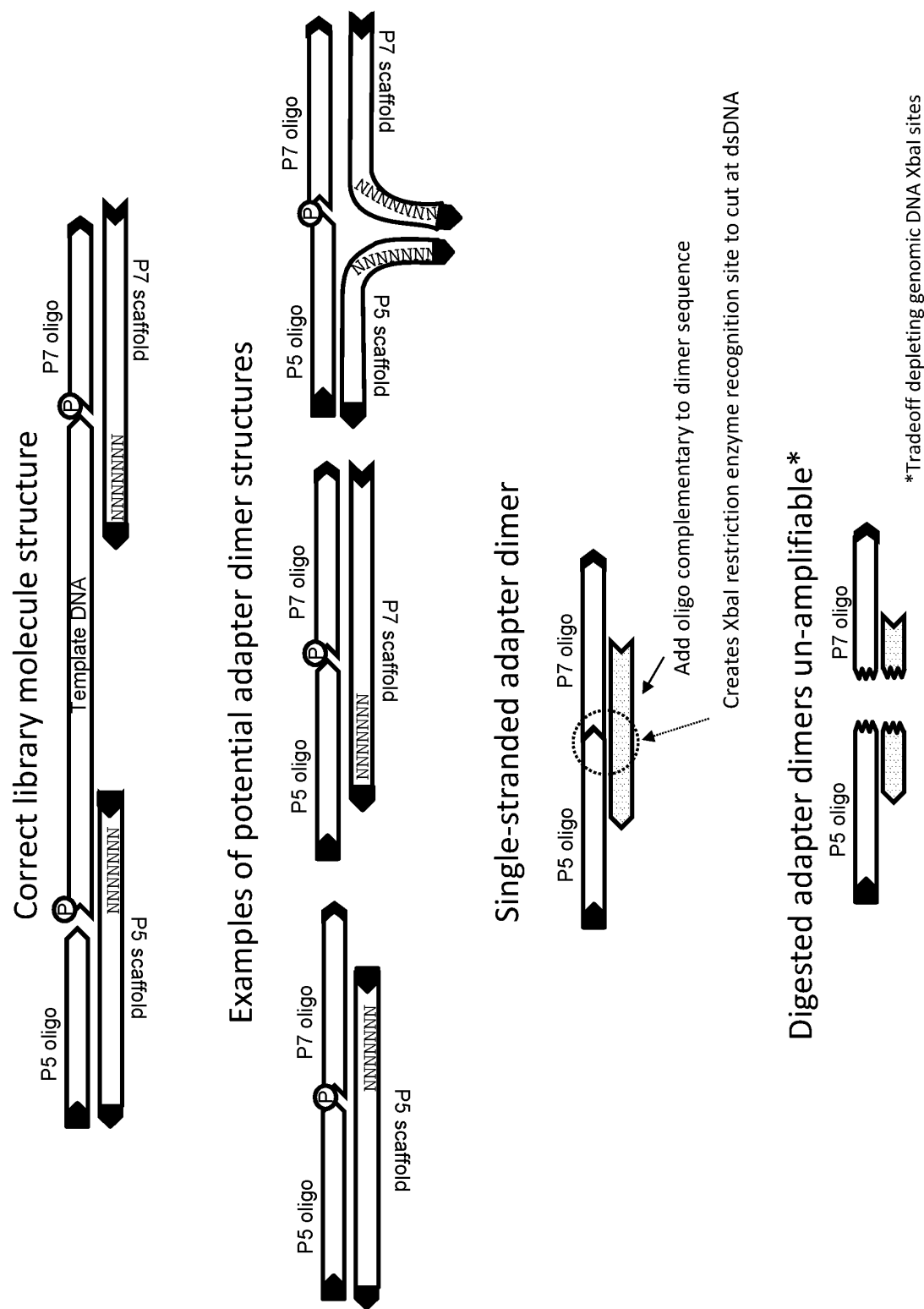


Fig. 20

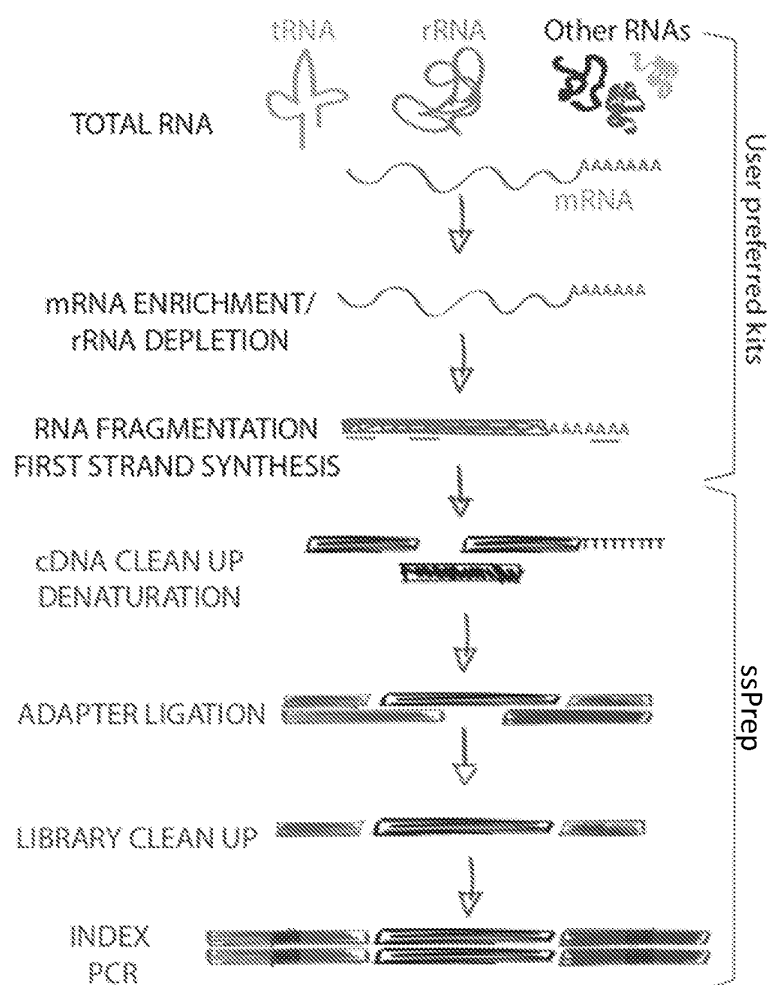


Fig. 21

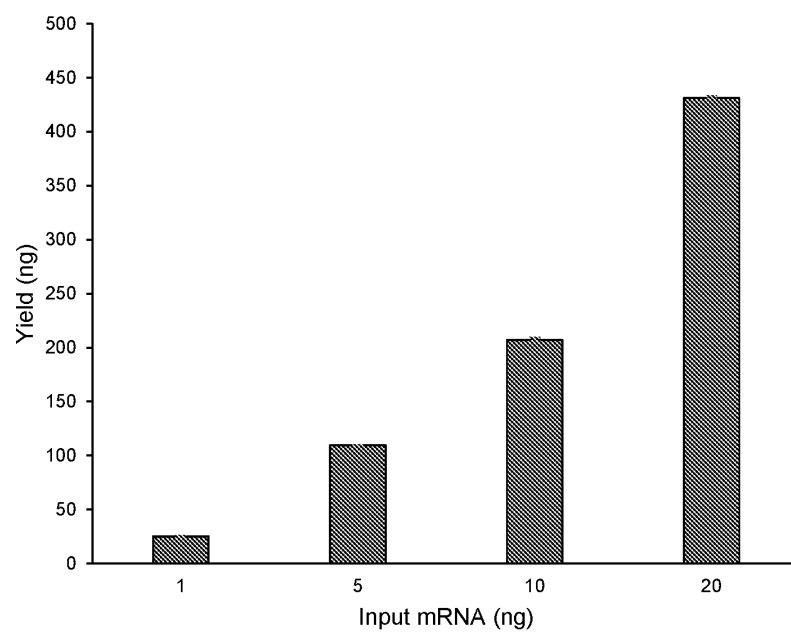


Fig. 22

Directional RNA-Seq Kits	Method Type	Input mRNA	Protocol time (hours)*	PCR cycles for 10ng input
ssPrep	Single-stranded	1-20ng	~2	9
commercial kit 1	Double-stranded	1-100ng	~5	9-10
commercial kit 2	Double-stranded	Up to 100ng	~5	>15
commercial kit 3	Double-stranded	10-100ng	~6.5	15

Fig. 23

Kit	Type of Prep Method	Sample	Yield	Input Reads	Uniquely mapped reads	Average mapped length
ssPrep	Single-stranded	ssPrep-1	378 ng	12,821,116	11,558,853	145
		ssPrep-2	370 ng	13,332,866	11,963,648	145
Commercial kit	Double-stranded	dsPrep-1	386 ng	15,247,791	14,059,492	149
		dsPrep-2	432 ng	15,716,512	14,488,921	149

Fig. 24

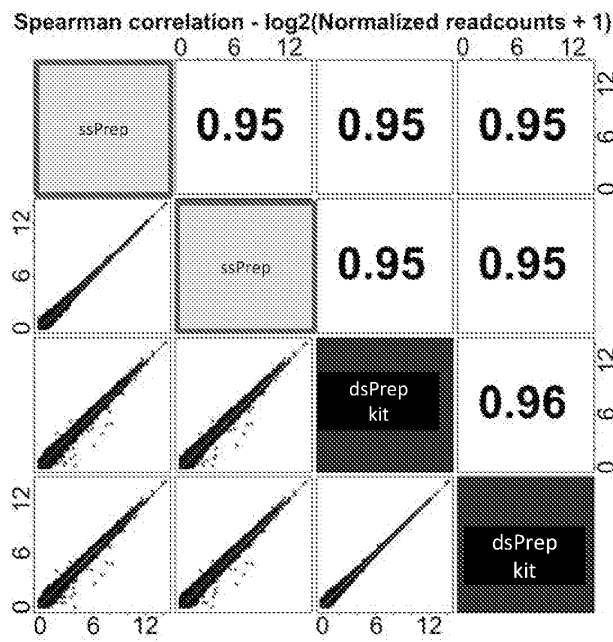
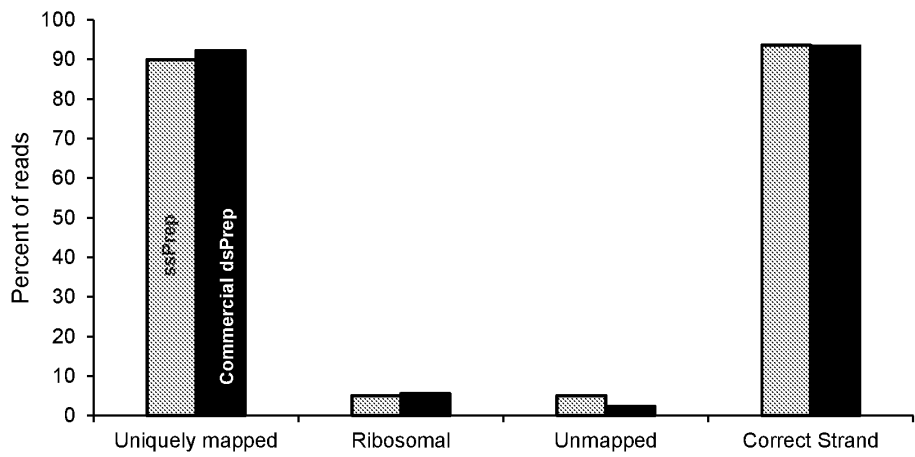


Fig. 25

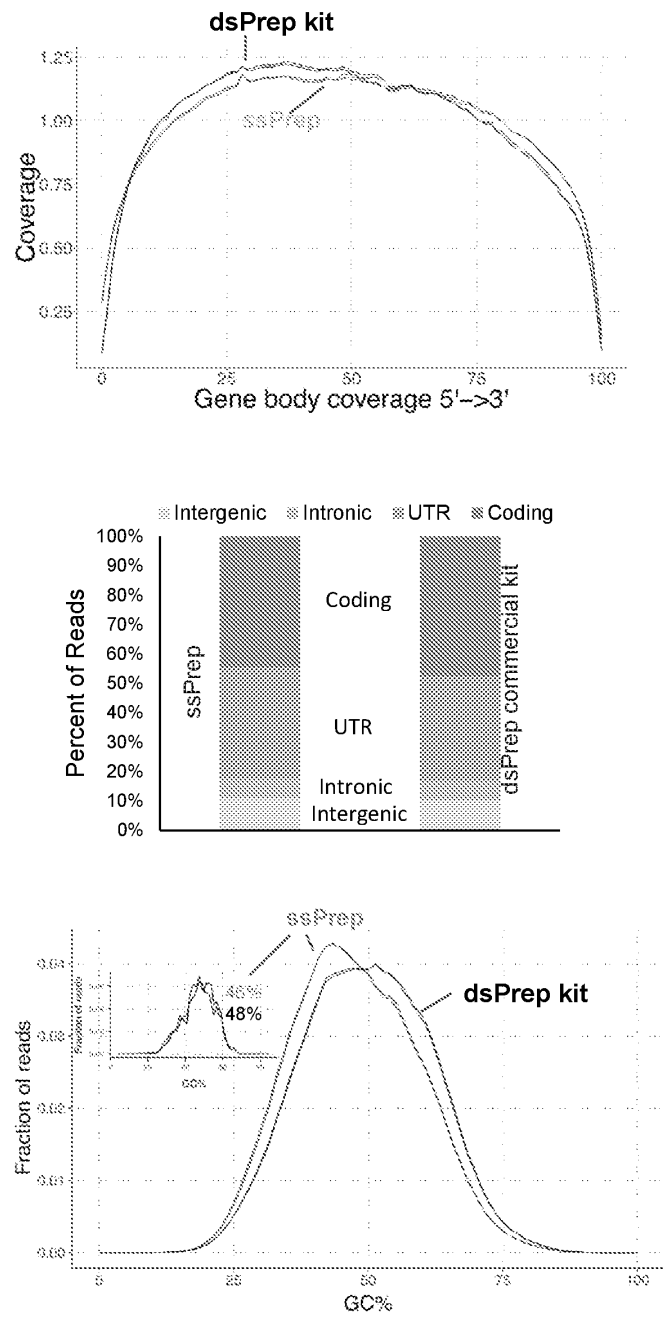


Fig. 26

Library	Total ERCC reads	Incorrect Strand	Correct Strand	Percent Correct Strand
ssPrep	349499	1845	347654	99.47
ssPrep	348261	2199	346062	99.37
dsPrep	384339	858	383481	99.78
dsPrep	389578	764	388814	99.80

Fig. 27

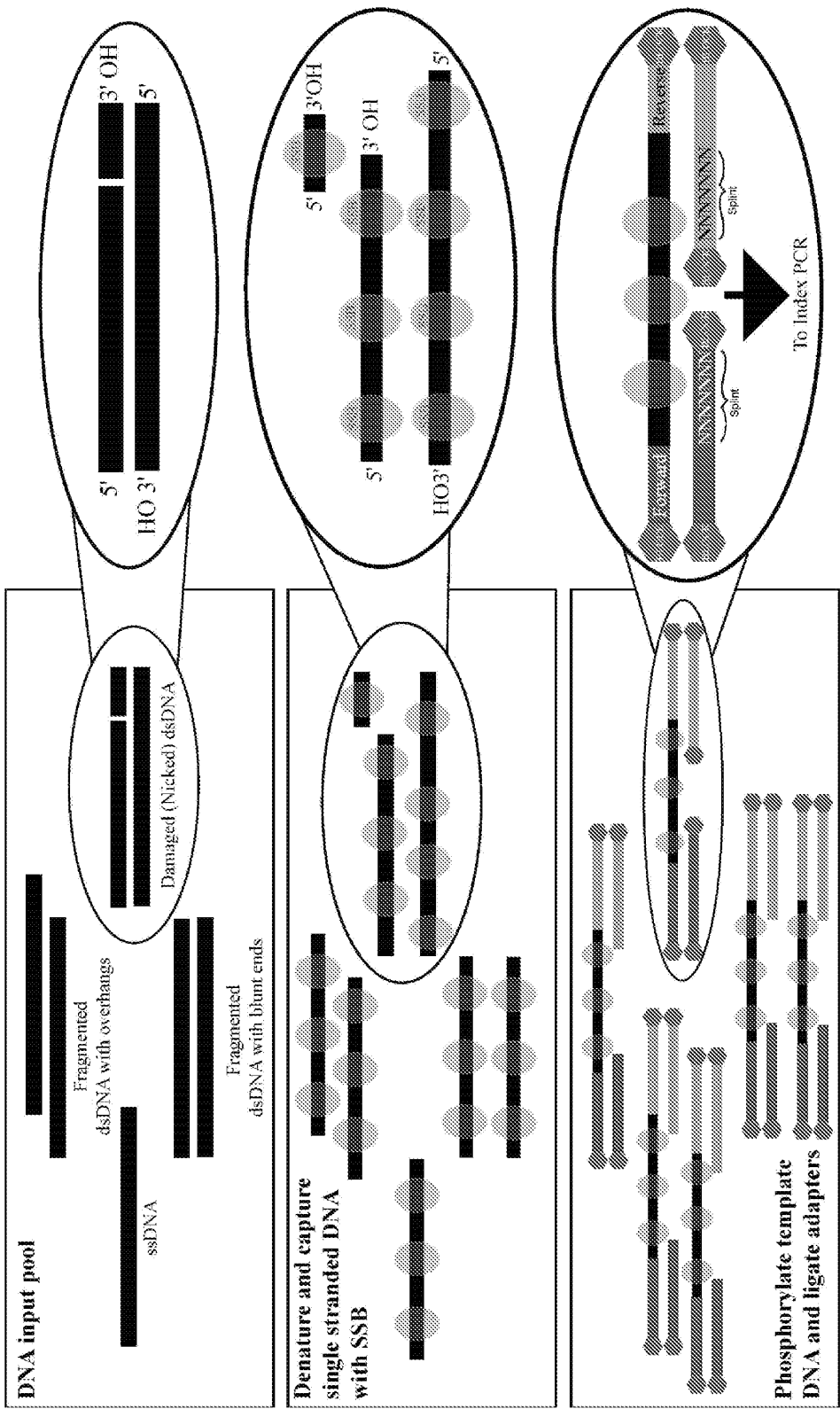


Fig. 28

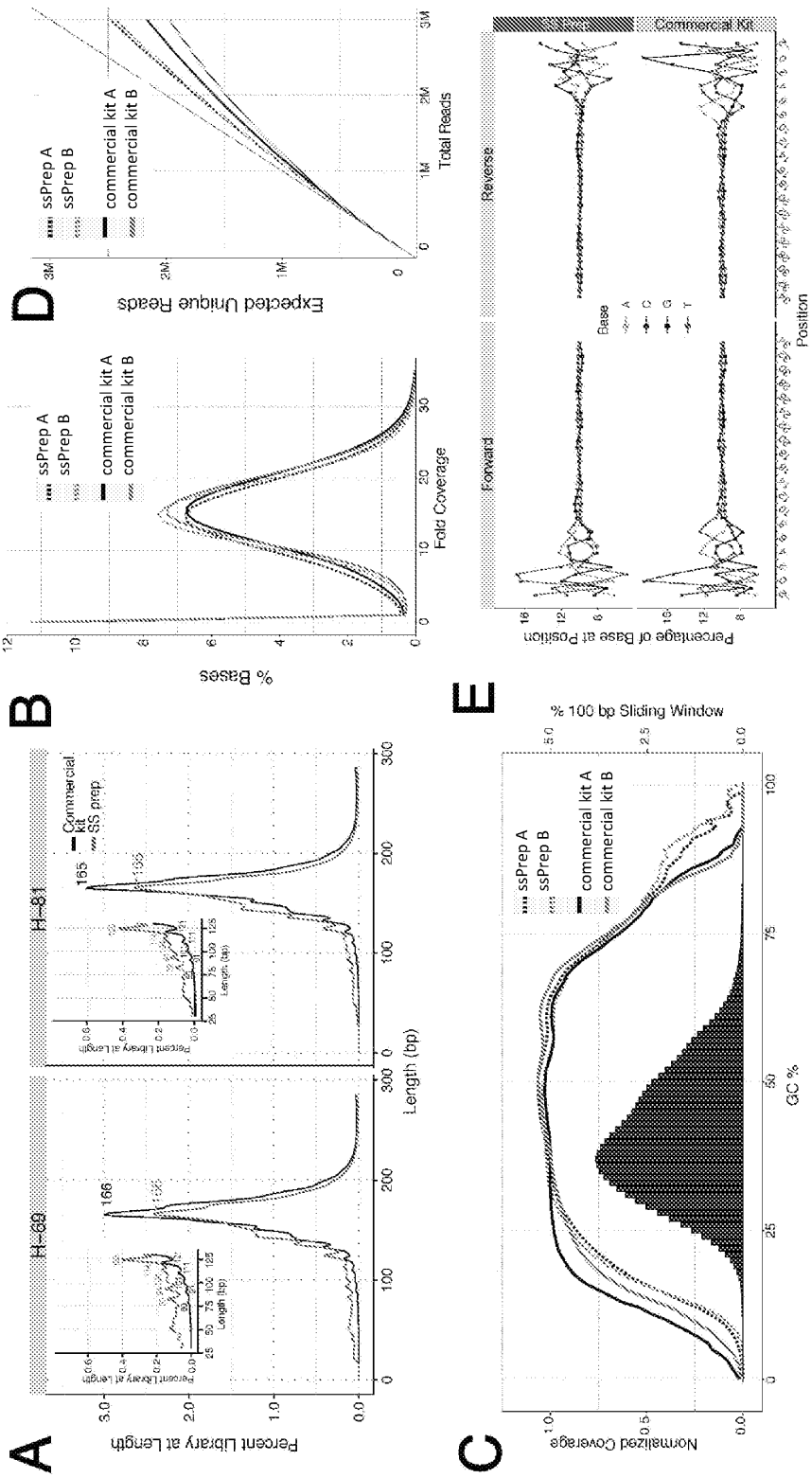


Fig. 29

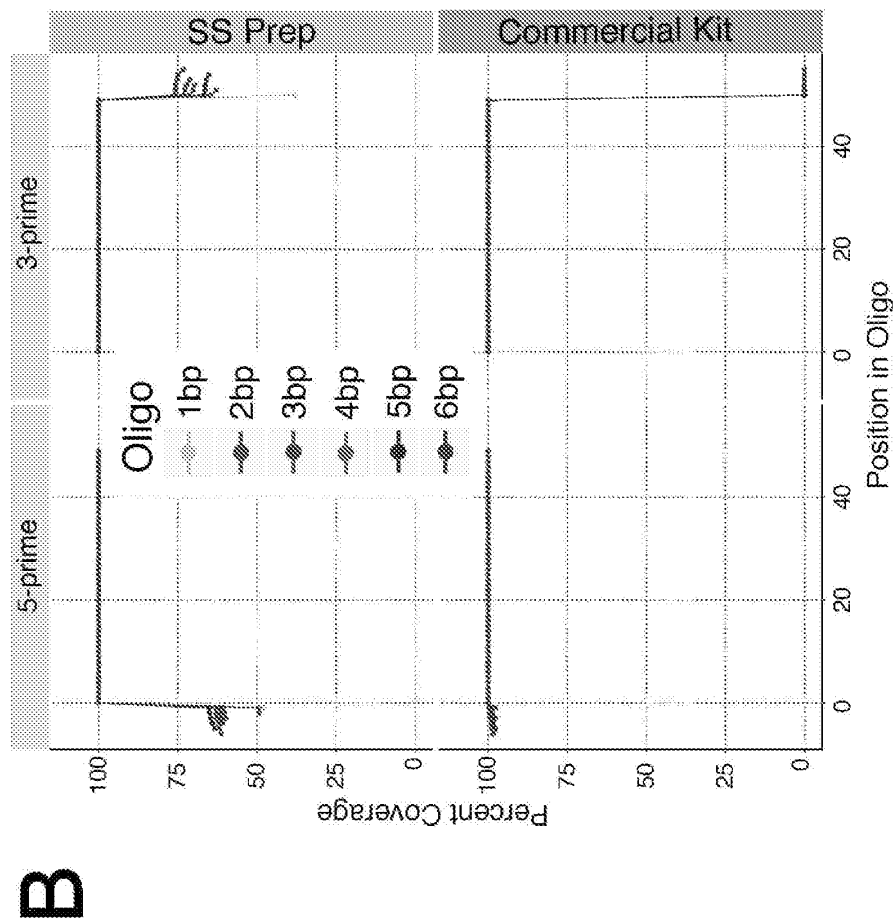
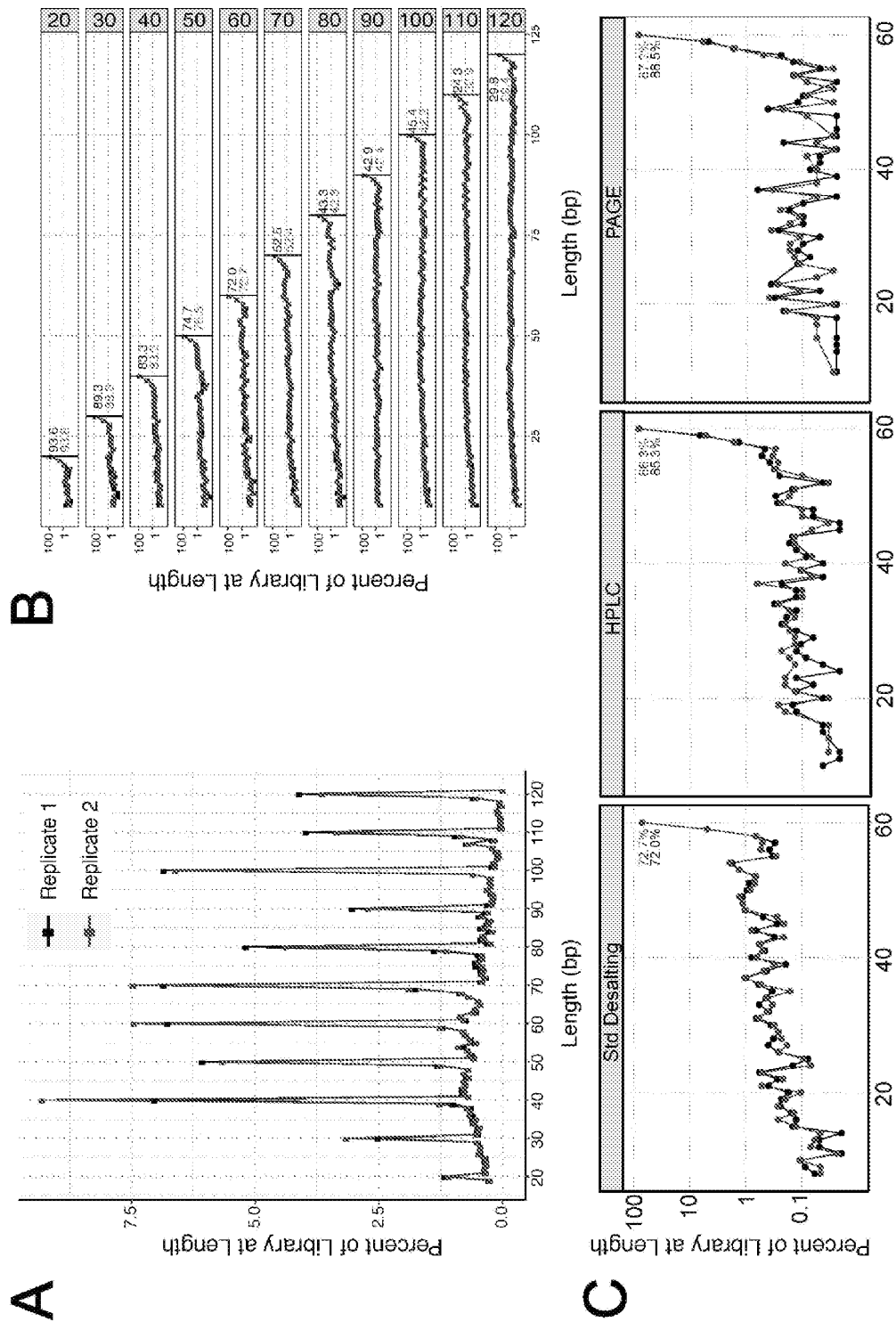


Fig. 30



Length (bp)
Fig. 31

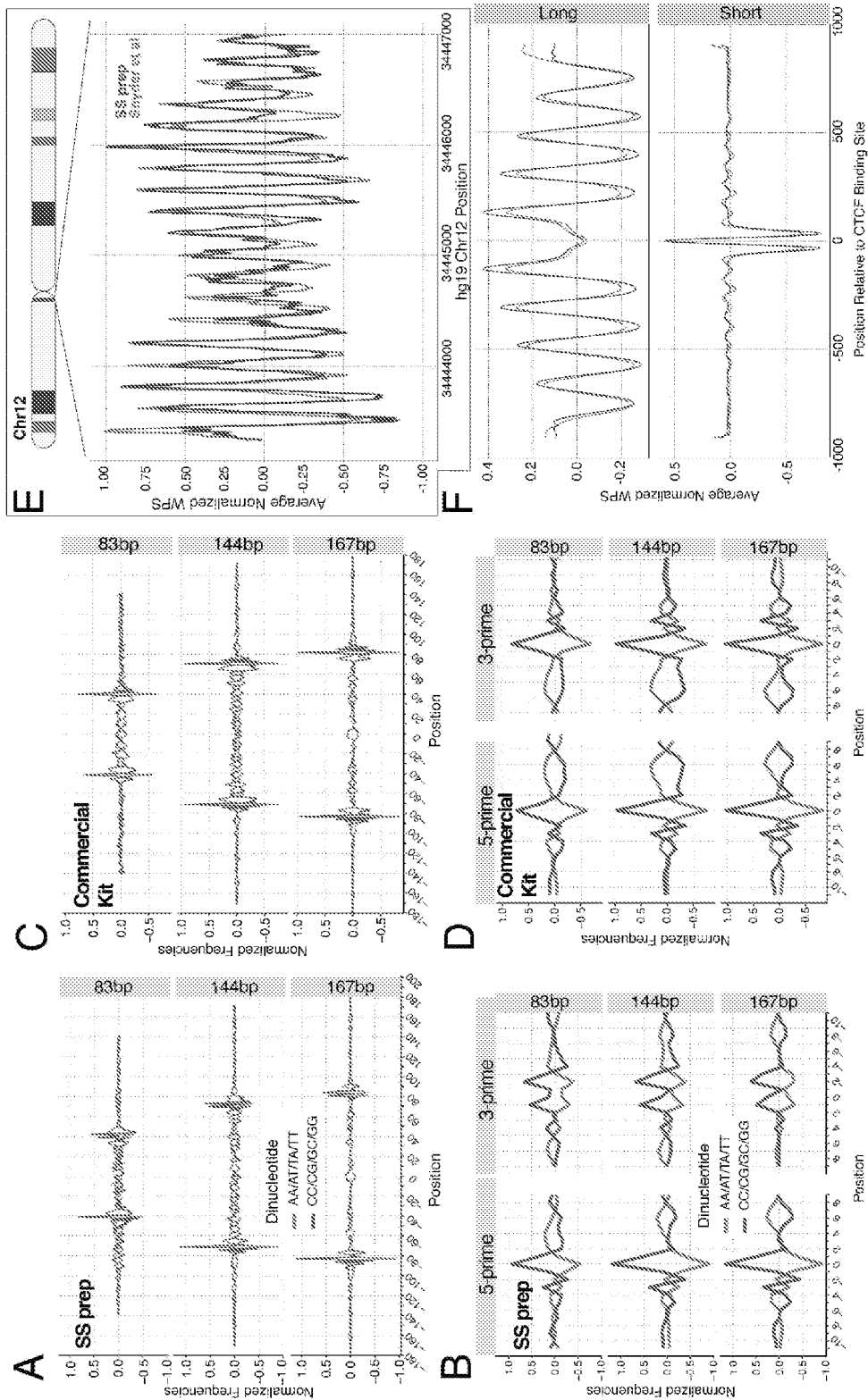


Fig. 32

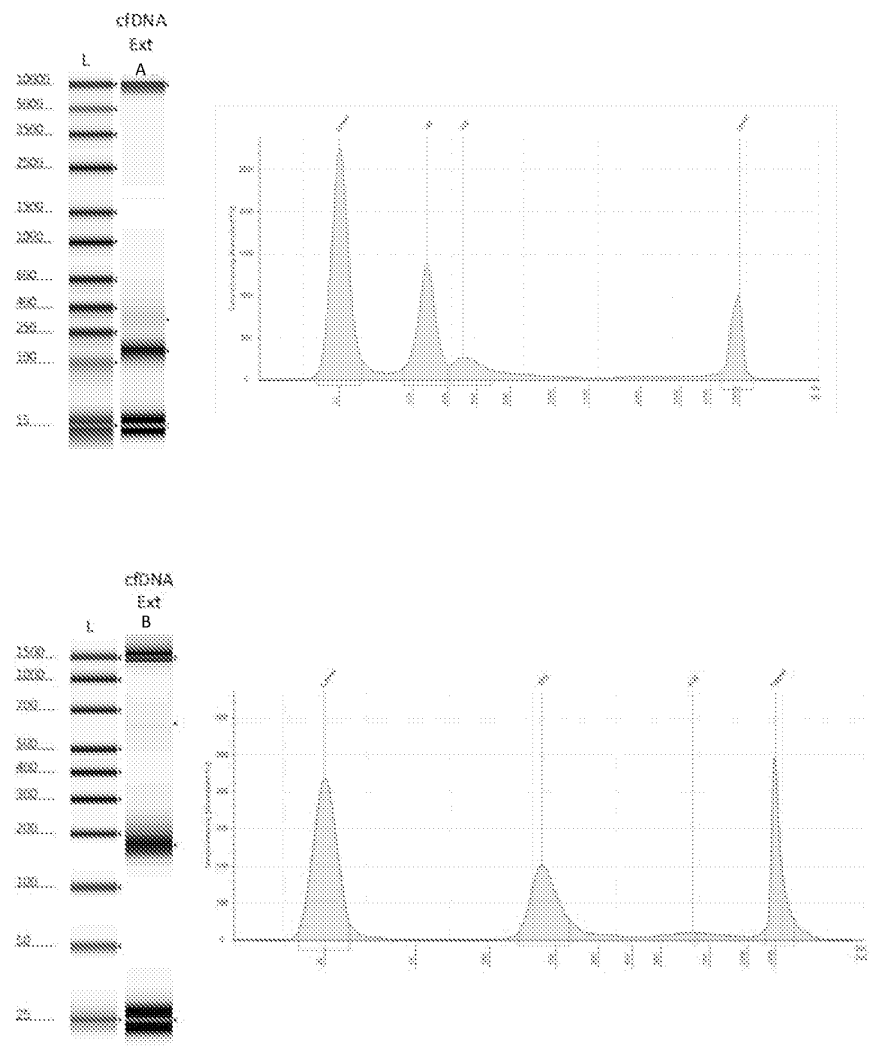


Fig. 33A

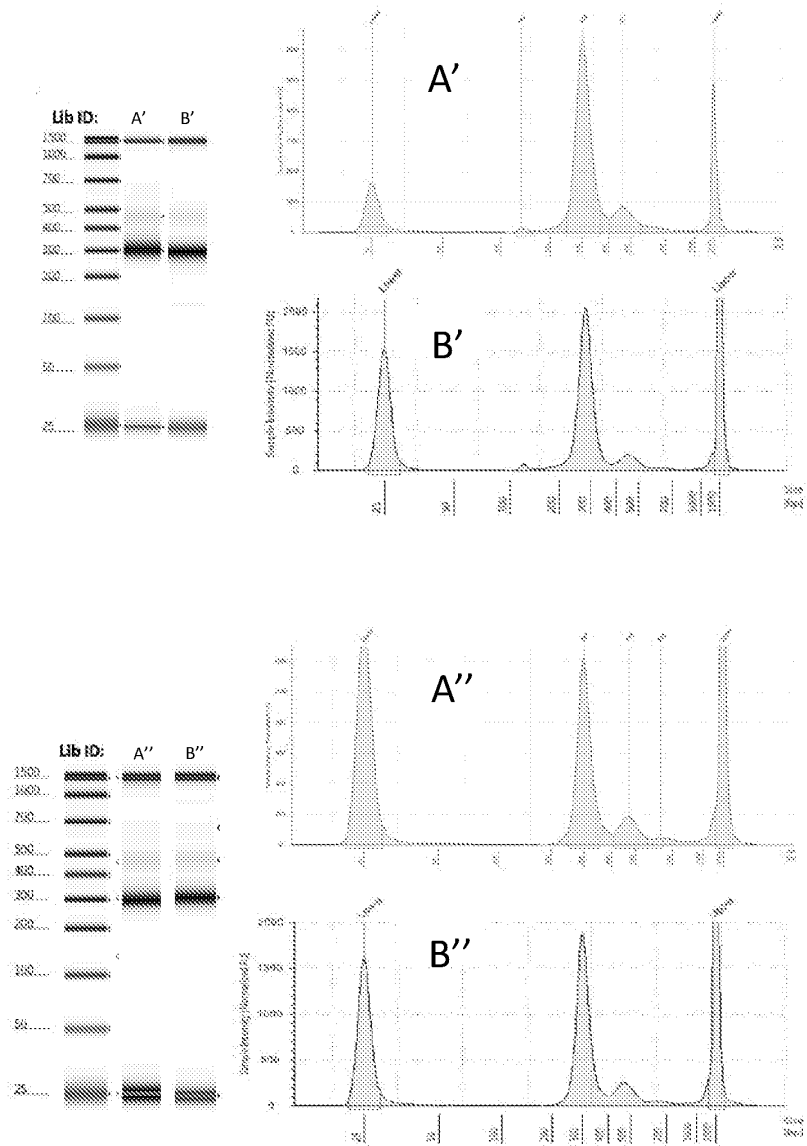


Fig. 33B

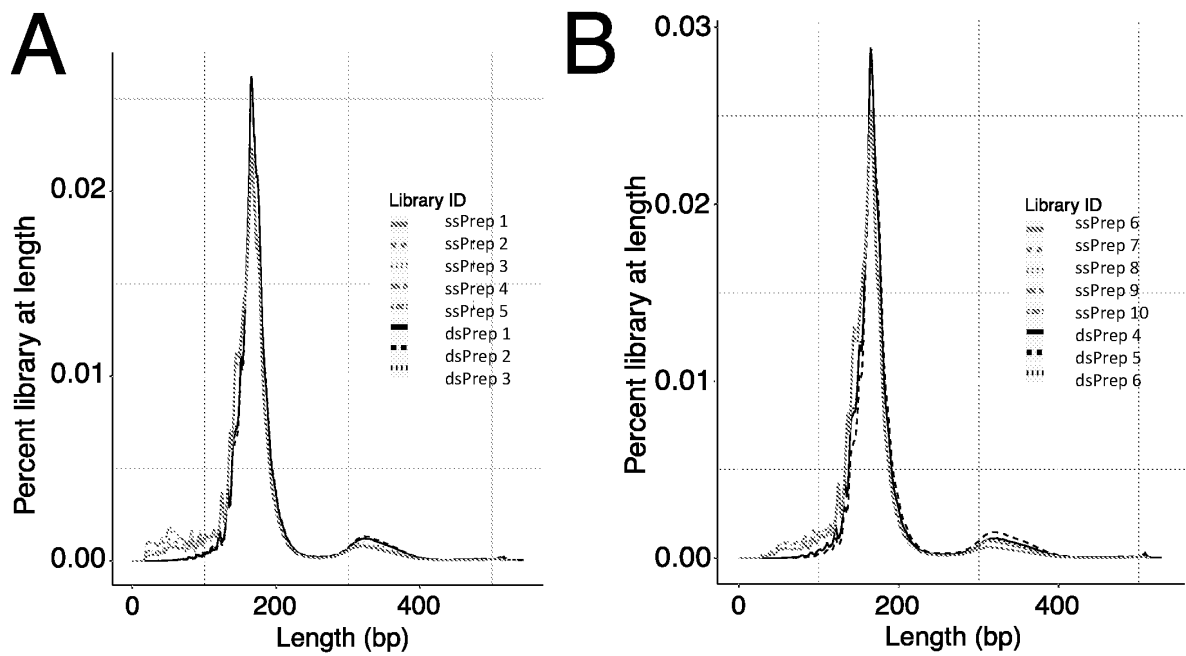


Fig. 34

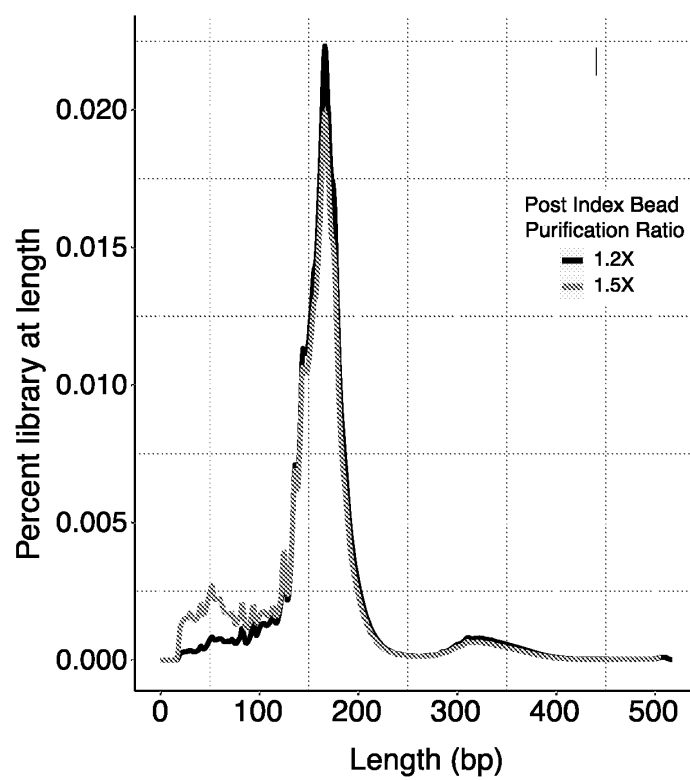


Fig. 35

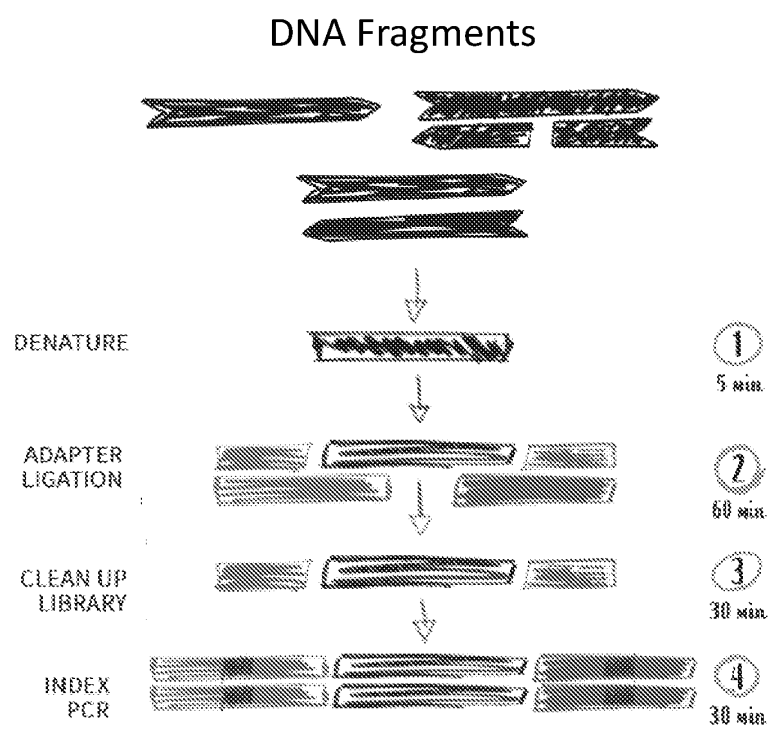


Fig. 36

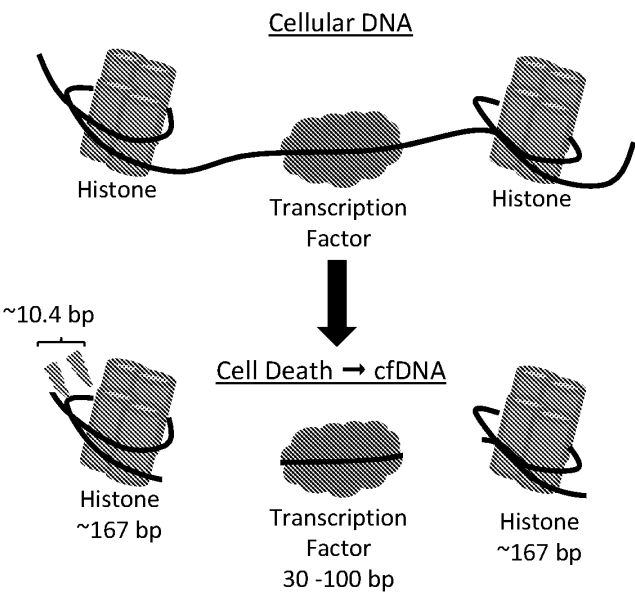


Fig. 37

Kit	Type of Prep Method	Input Amount	Total Time thru Index PCR
ssPrep	Single-stranded	1ng	2.5hrs
Commercial kit 1	Double-stranded	1ng	3hrs
Commercial kit 2	Double-stranded	1ng	2.5hrs
Commercial kit 3	Single-stranded	1ng	3.5hrs

Fig. 38

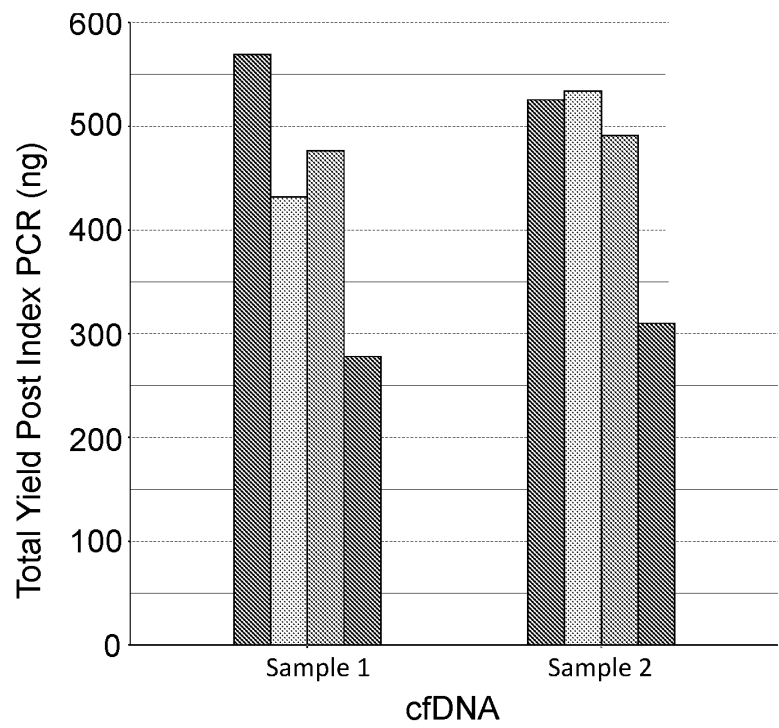


Fig. 39

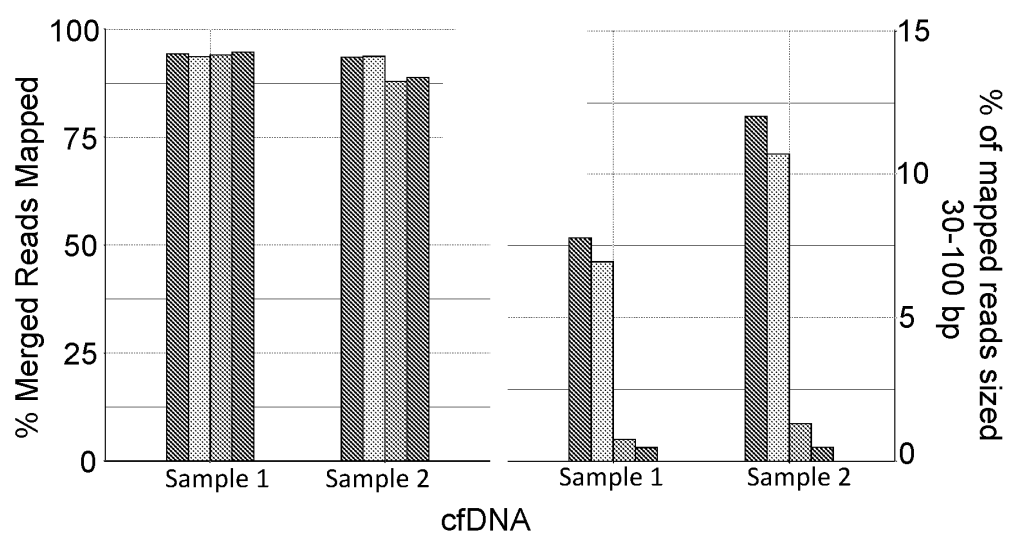


Fig. 40

Kit	Type of Prep Method	Input cDNA Extract ID	Yield Post 10c index PCR (total)	Merged Read Pairs Sequenced	Mapping Rate*	Mapped Reads in 30-100bp* Bin
ssPrep	Single-stranded	Sample 1	569ng	73,077,812	94.35%	7.77%
		Sample 2	525ng	63,449,167	93.52%	12.0%
Commercial kit 1	Double-stranded	Sample 1	278ng	78,705,033	94.66%	0.49%
		Sample 2	310ng	54,734,560	88.88%	0.49%
Commercial kit 2	Double-stranded	Sample 1	476ng	74,429,274	94.01%	0.77%
		Sample 2	470ng	71,294,525	87.97%	1.32%
Commercial kit 3	Single-stranded	Sample 1	432ng	78,018,897	93.66%	6.94%
		Sample 2	534ng	69,959,246	93.73%	10.68%

Fig. 41

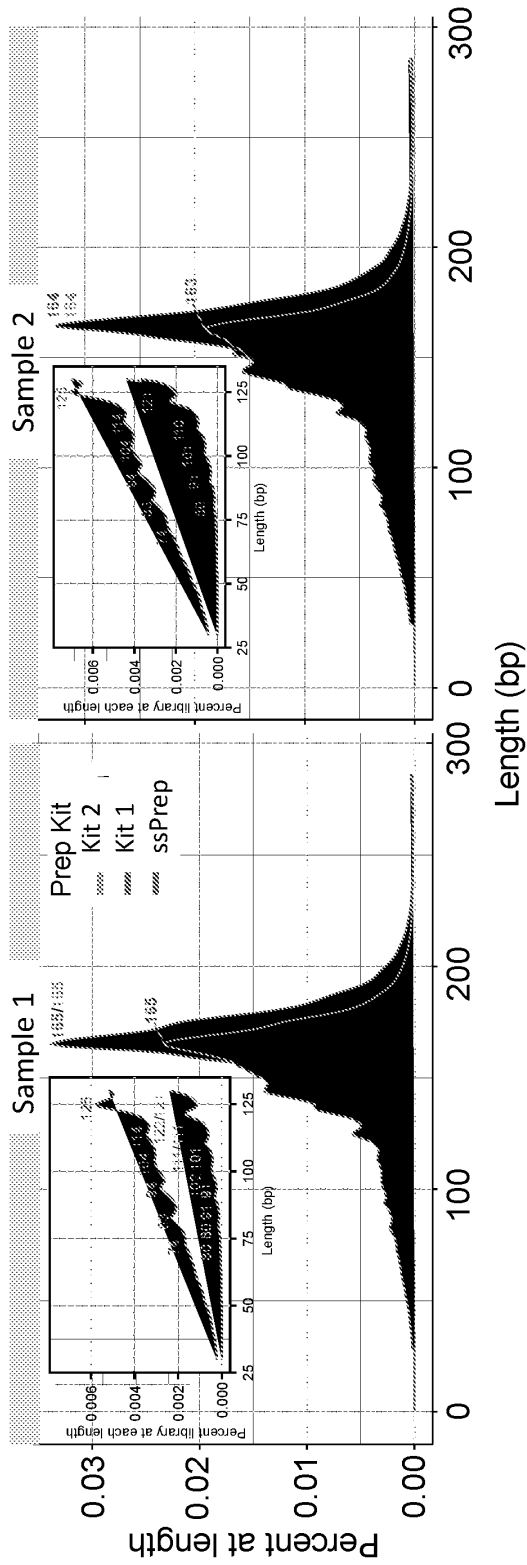


Fig. 42A

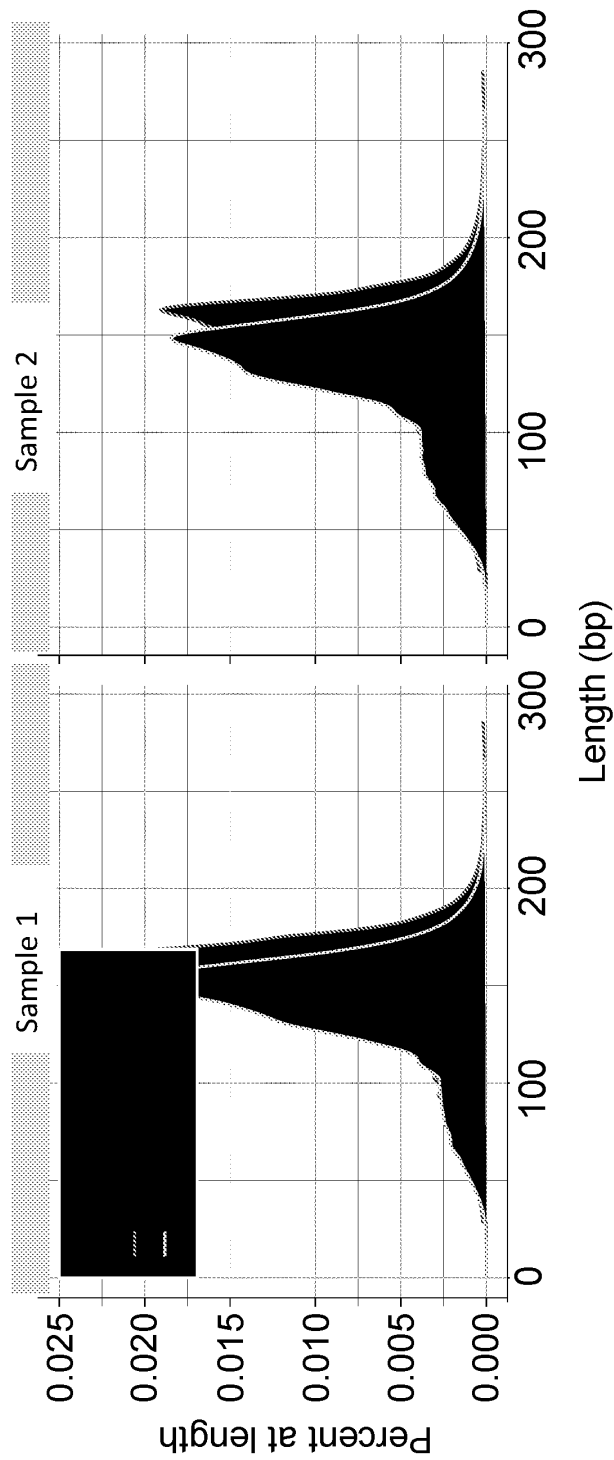


Fig. 42B

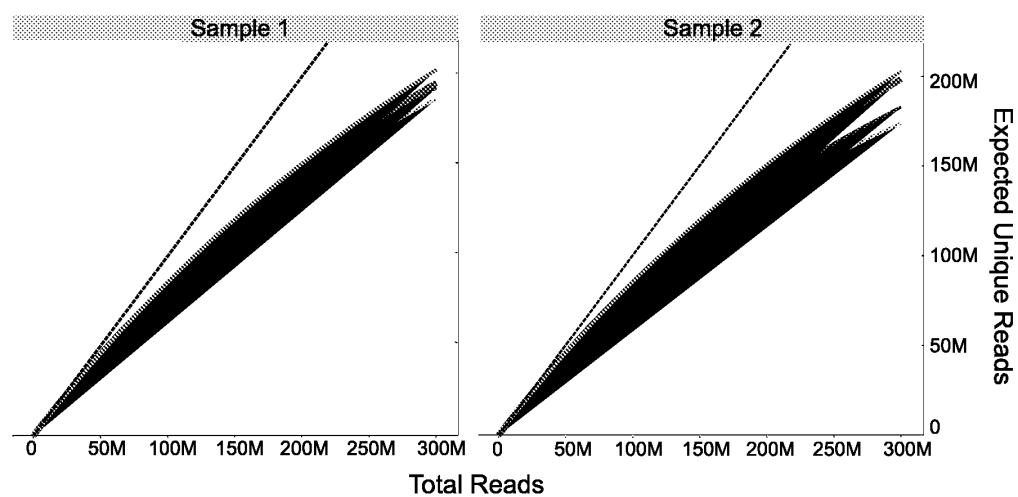


Fig. 43

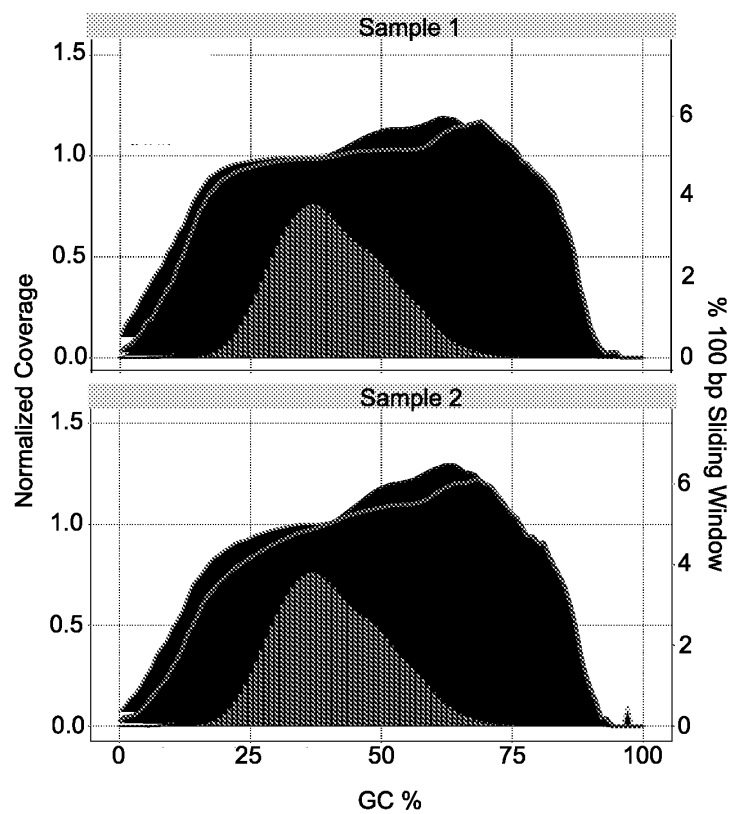


Fig. 44

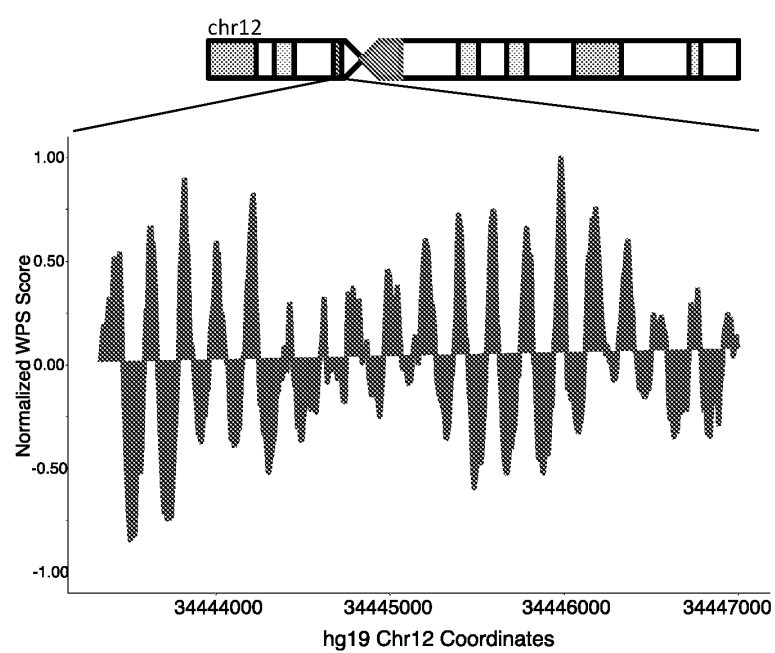


Fig. 45

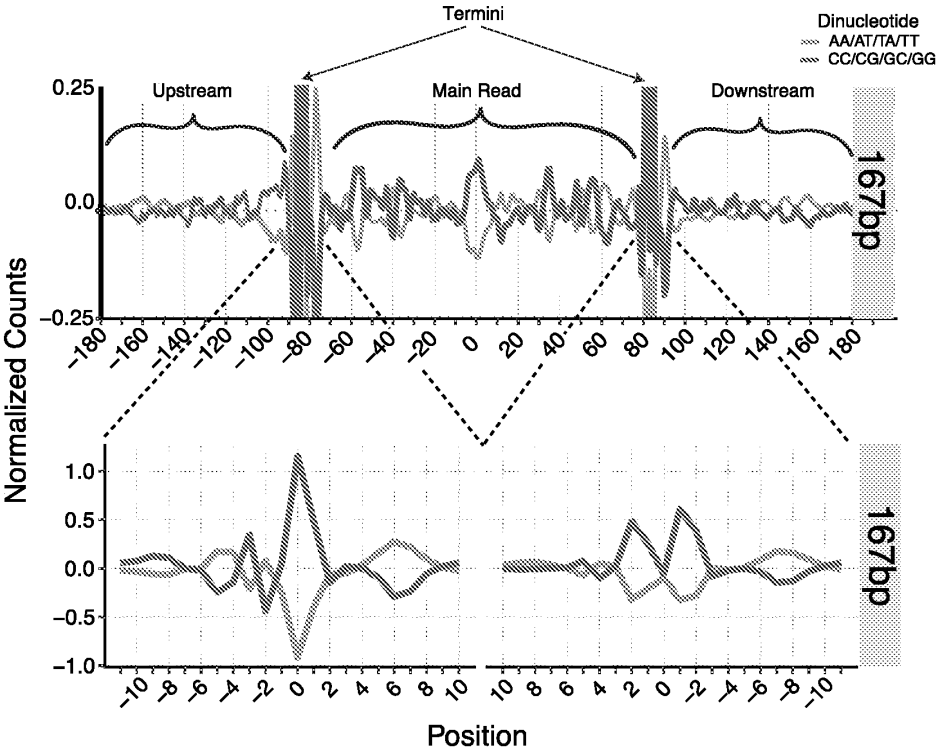


Fig. 46

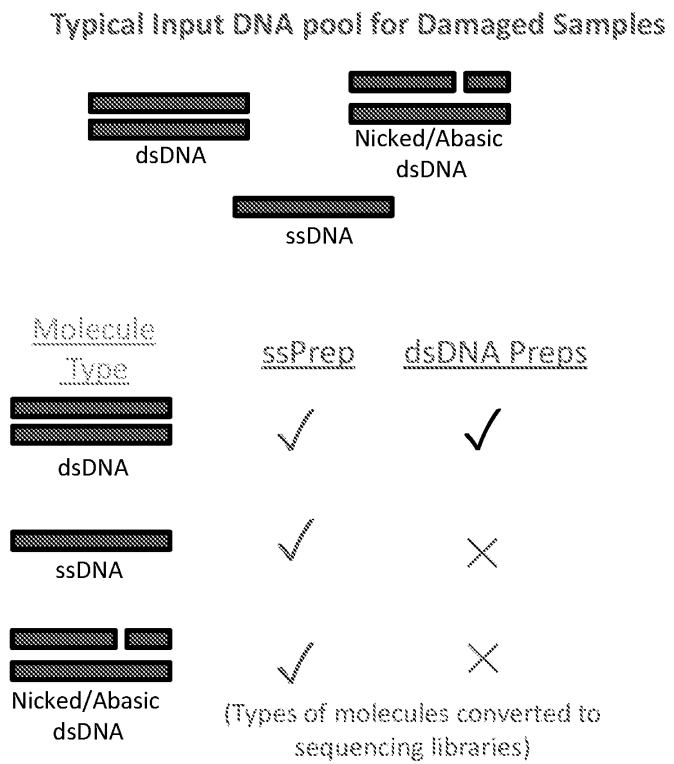


Fig. 47

14.3% adapter dimers, 206 bp average length, 60.7 nmol/ μ l

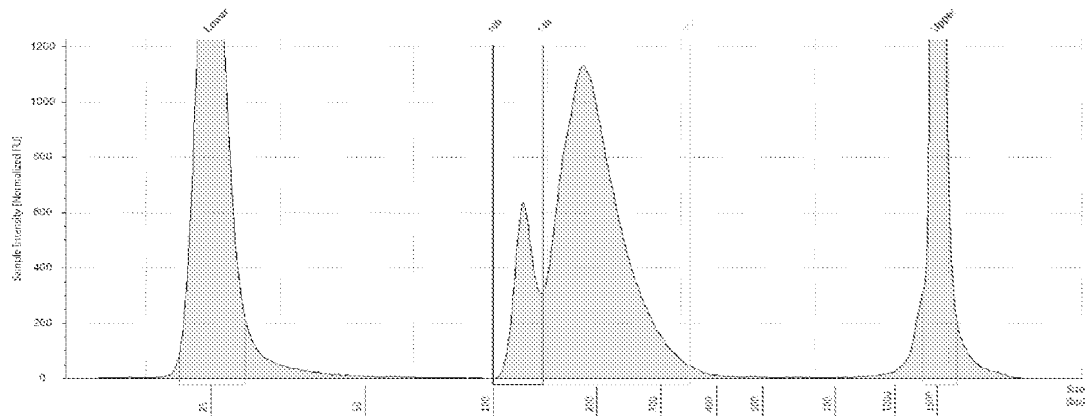


Fig. 48A

26.4% adapter dimers, 197 bp average length, 28.5 nmol/ μ l

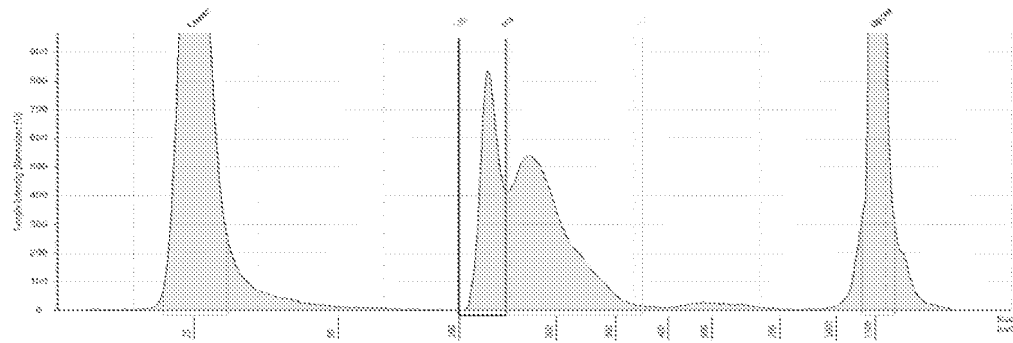


Fig. 48B

26.9% adapter dimers, 193 bp average length, 27.8 nmol/ μ l

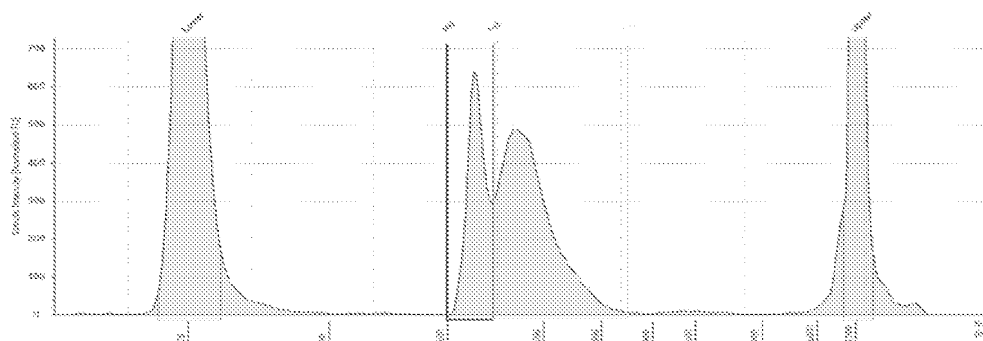


Fig. 48C

27.8% adapter dimers, 192 bp average length, 32.8 nmol/ μ l

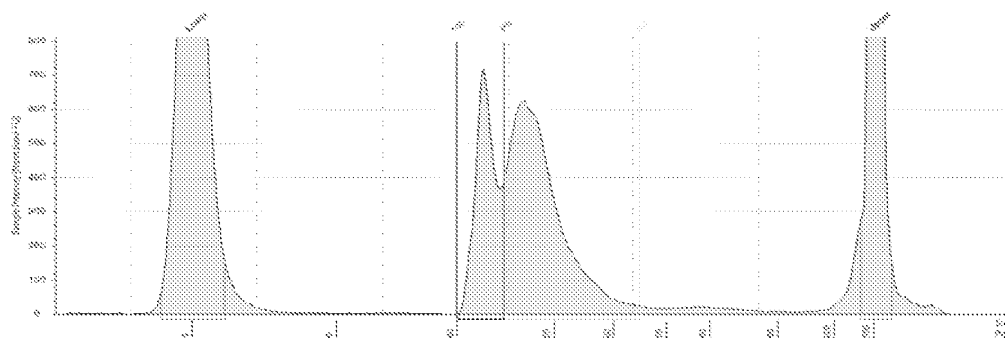


Fig. 48D

50 μ l EB - SPRI

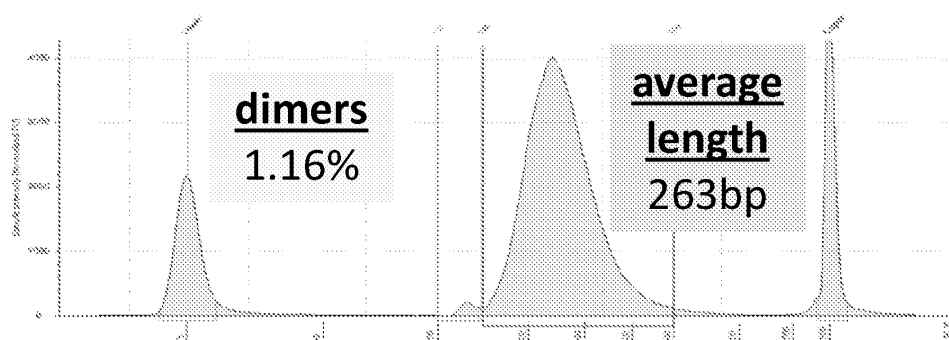


Fig. 49A

column

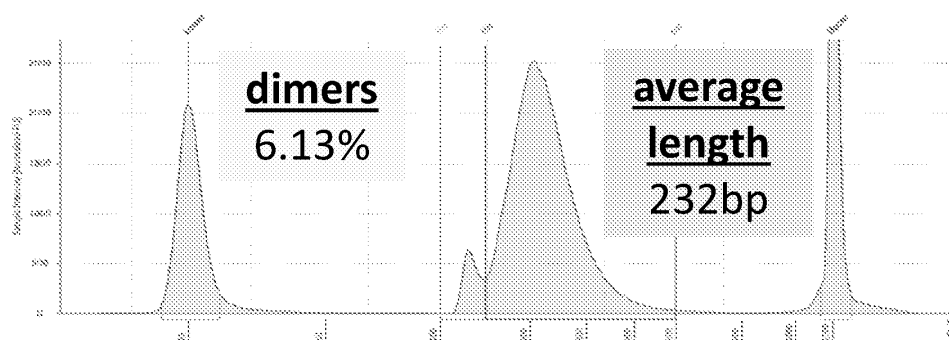


Fig. 49B

5 μ l isopropanol, 45 μ l EB - SPRI

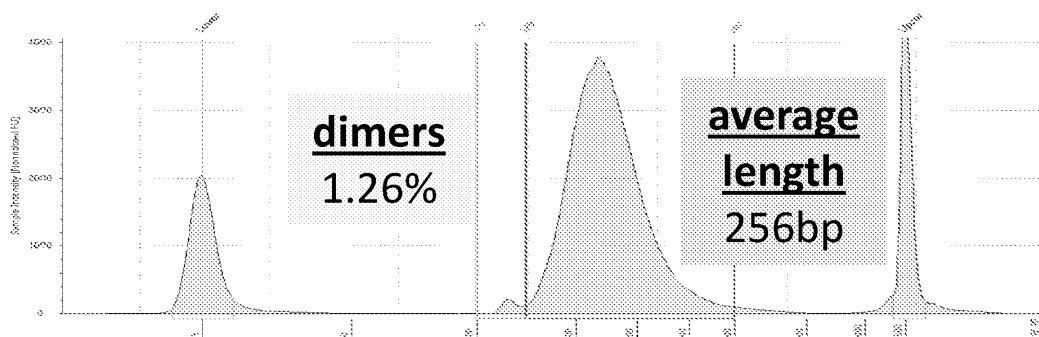


Fig. 49C

10 μ l isopropanol, 40 μ l EB - SPRI

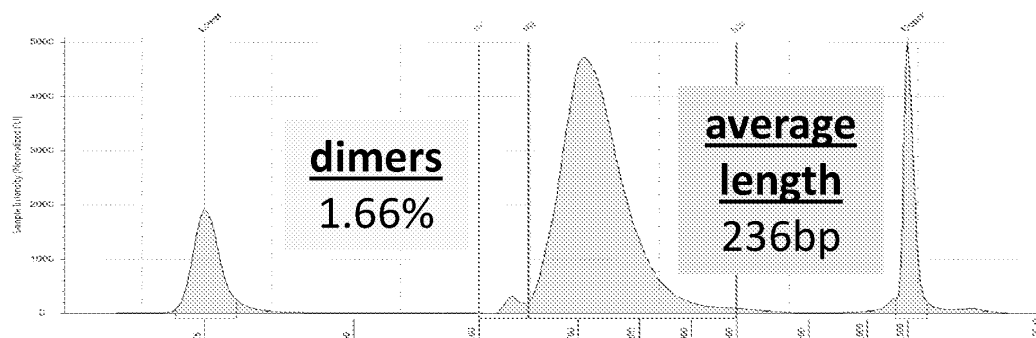


Fig. 49D

20 μ l isopropanol, 30 μ l EB - SPRI

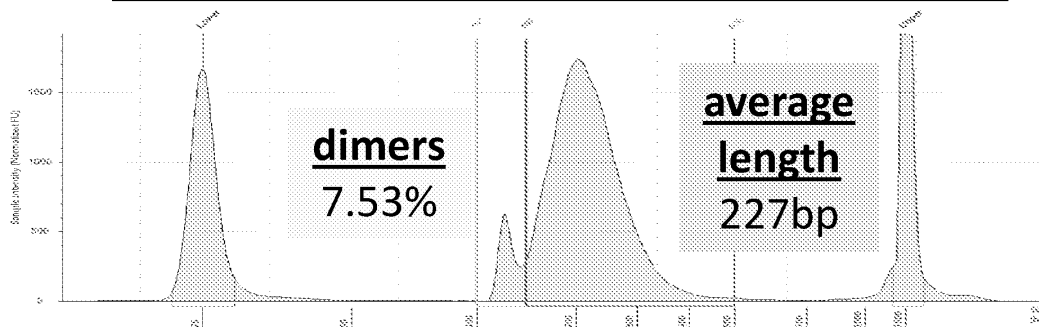


Fig. 49E

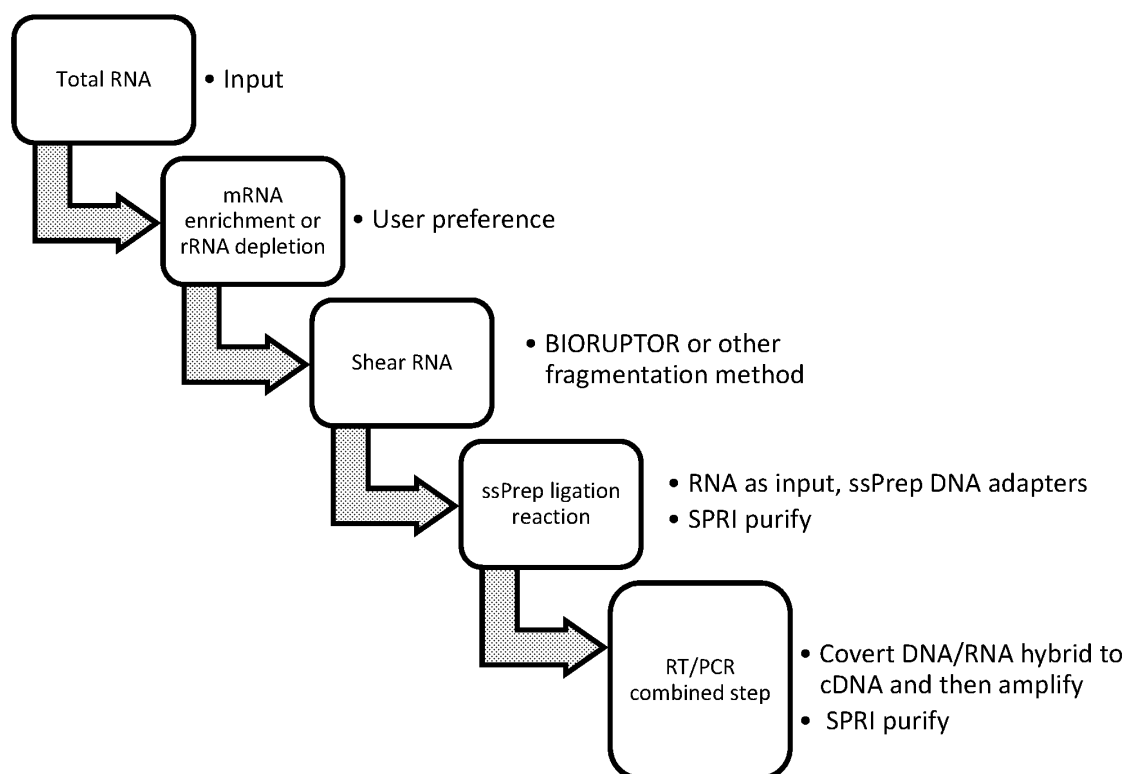
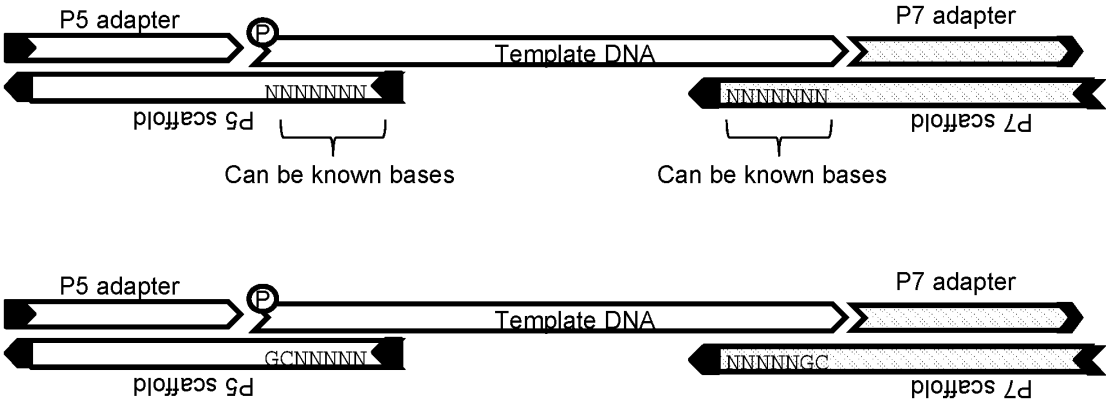


Fig. 50



Library Information	Percent of Reads that Start with CG	Percent of Reads that End with CG
No Enriched Scaffolds	1.7	1.1
CG Dinucleotide Enriched Scaffolds	5.2	19.6

Fig. 51

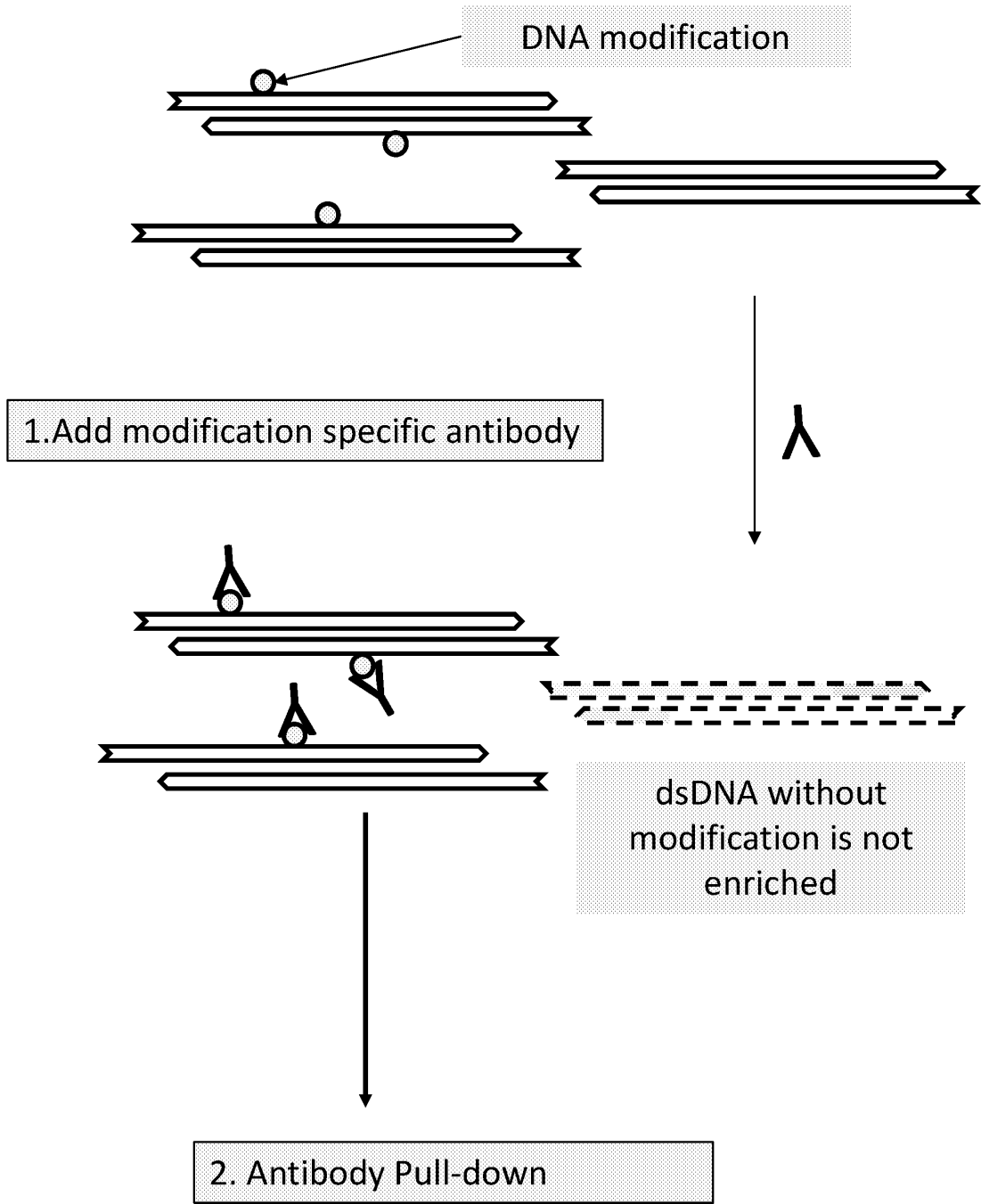


Fig. 52A

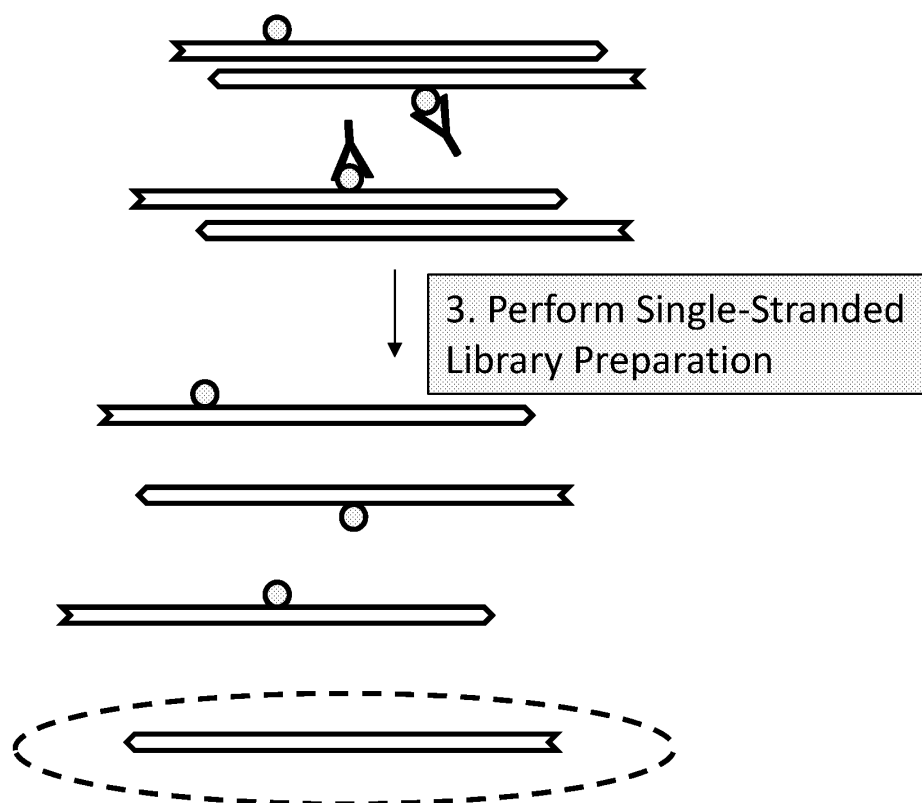


Fig. 52B

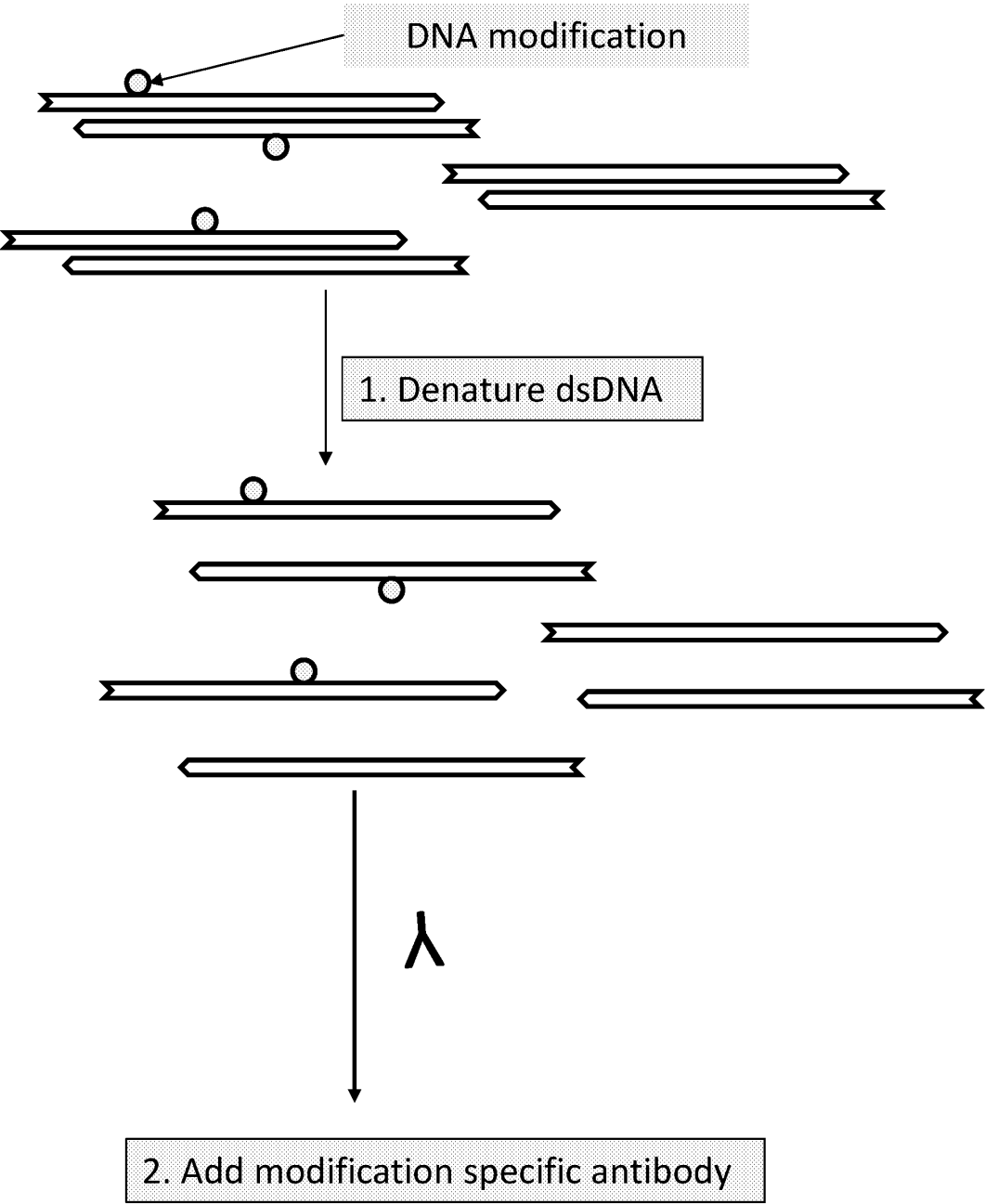


Fig. 52C

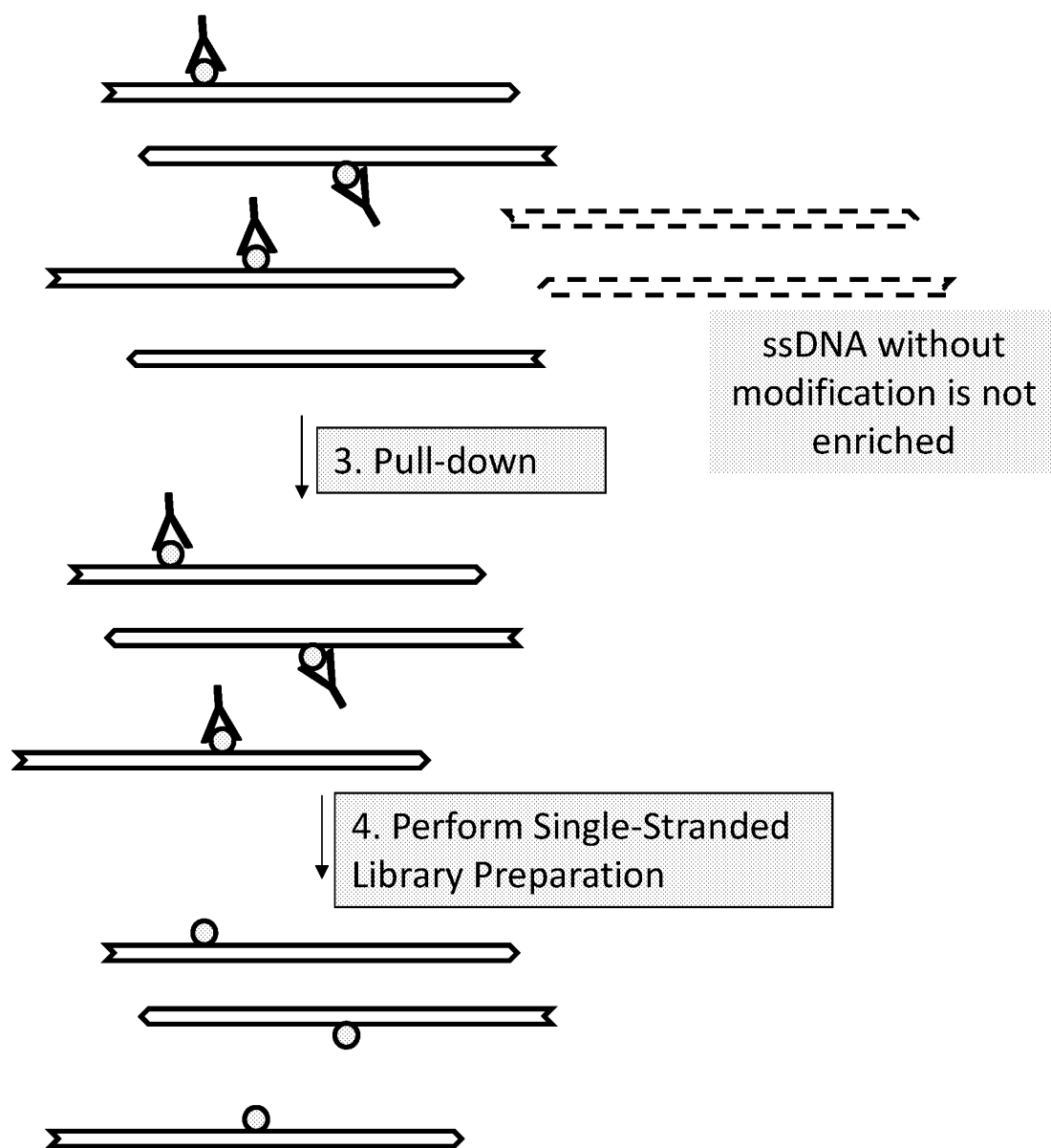


Fig. 52D

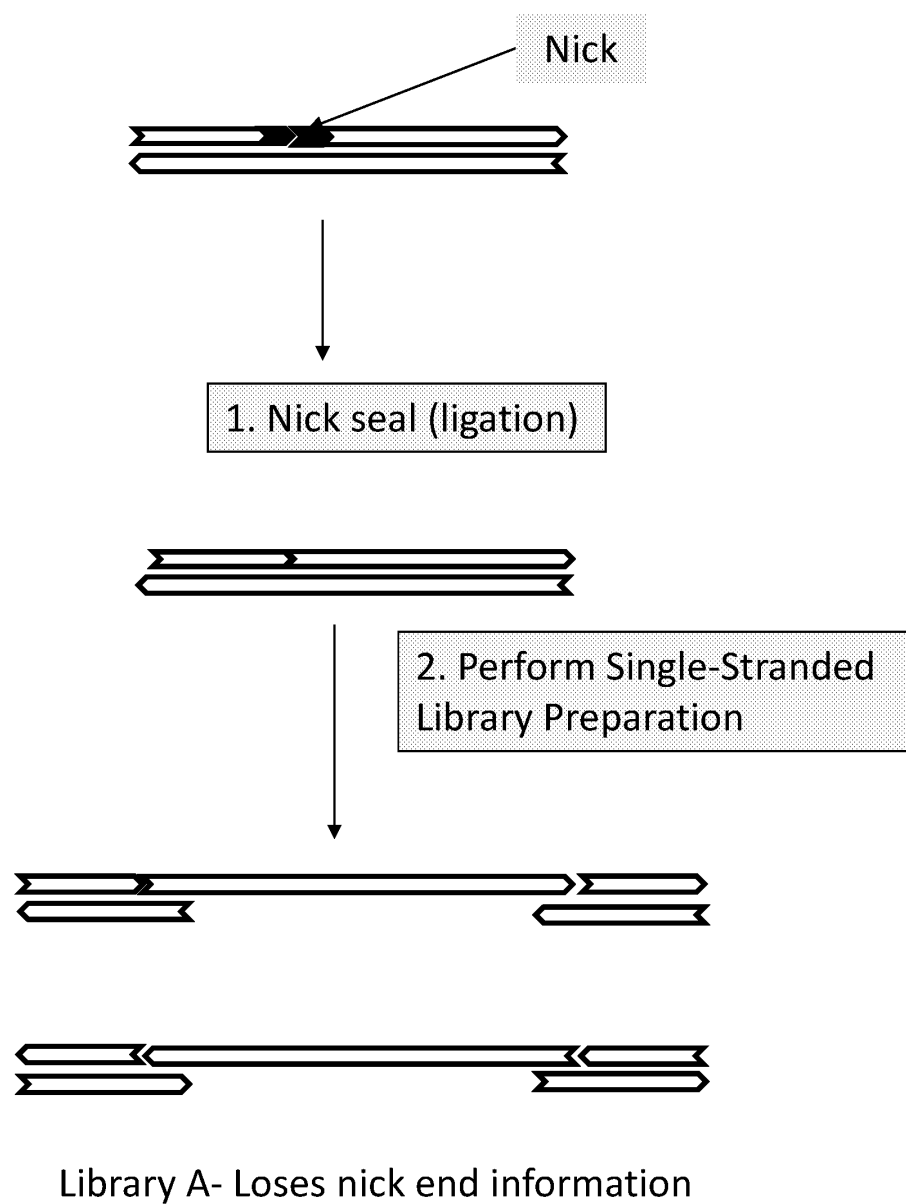


Fig. 53A

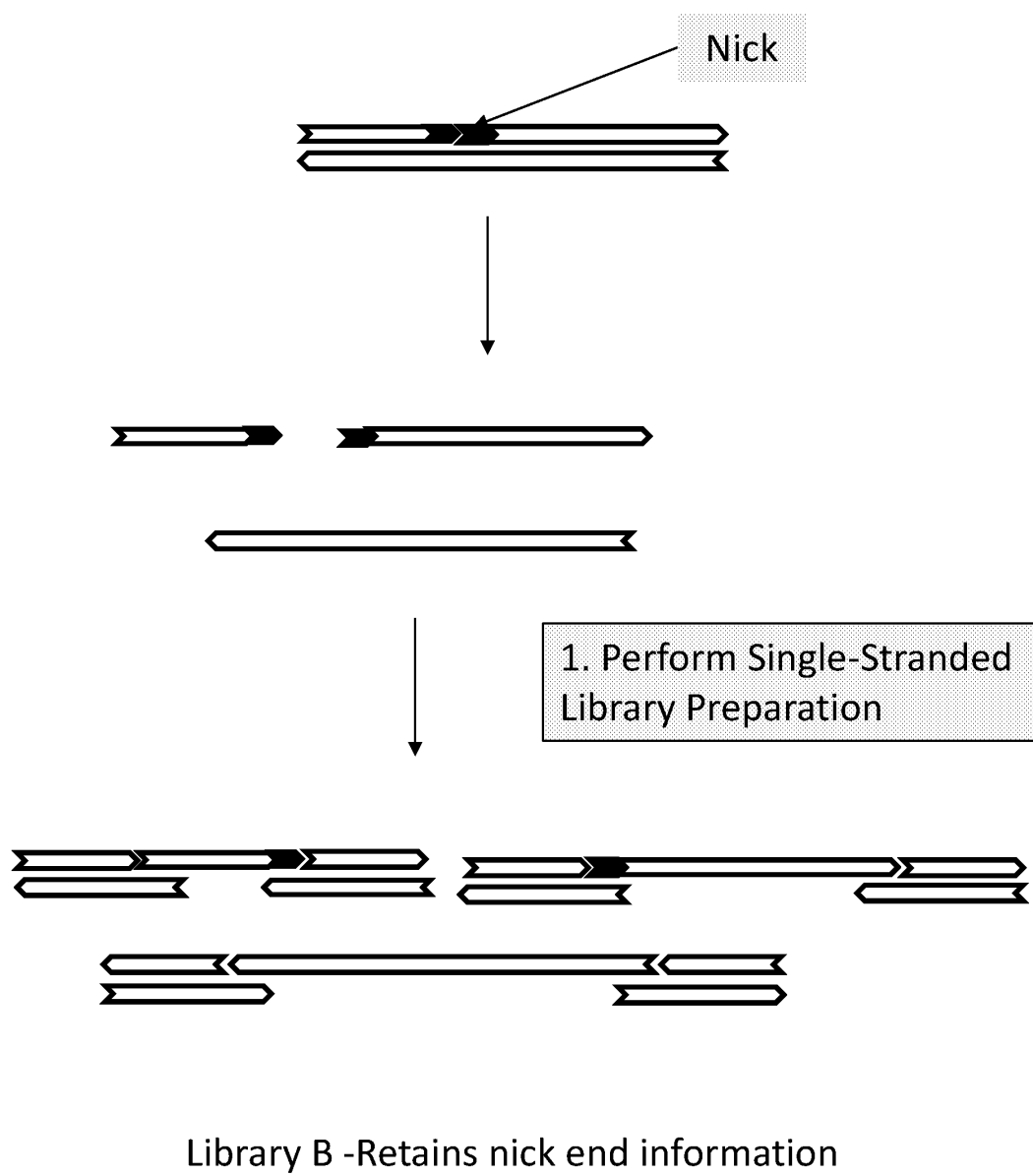


Fig. 53B

Oligonucleotide DNA and
Scaffold Polynucleotide DNA:



Oligonucleotide RNA and
Scaffold Polynucleotide DNA:



Oligonucleotide RNA and
Scaffold Polynucleotide RNA:



Fig. 54

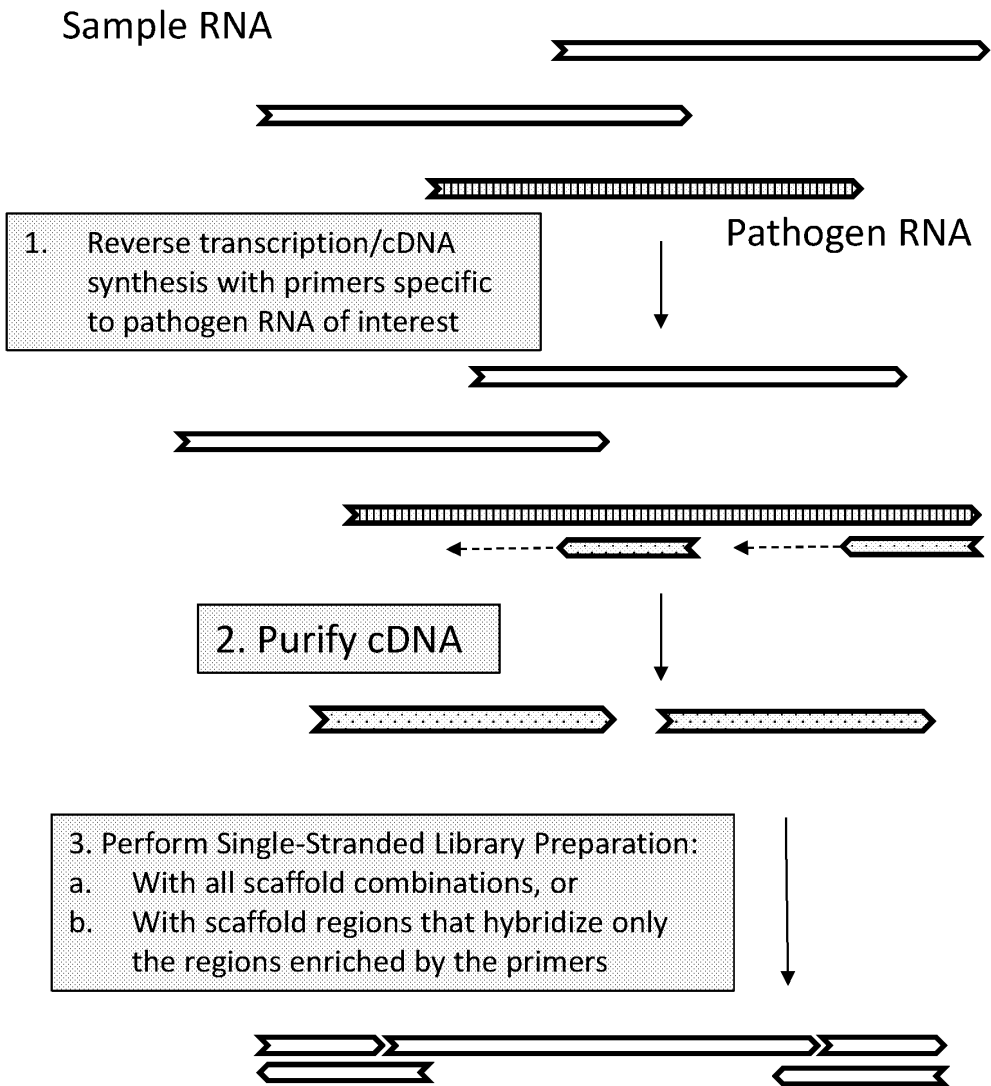


Fig. 55