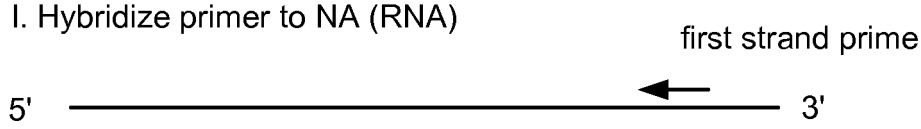
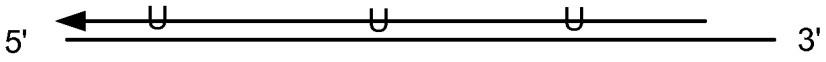
I. Hybridize primer to NA (RNA)



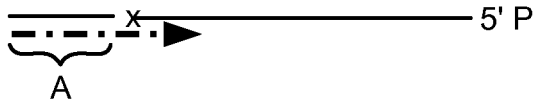
II. Primer extension incorporating dU



III. Degrade template NA and treat primer extension products with UNG and cleave to generate fragments of desired size range with blocked 3'-ends



IV. Anneal first adaptor comprising a 3'-overhang comprising random sequence at the 3'-end and sequence A (one exemplary fragment shown)

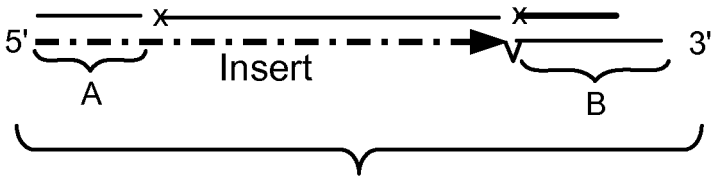


V. Extend hybridized 3'-end of the of adaptor along the fragment comprising a blocked 3'-end generated in step III with a DNA polymerase



Double stranded fragments with an appended sequence A

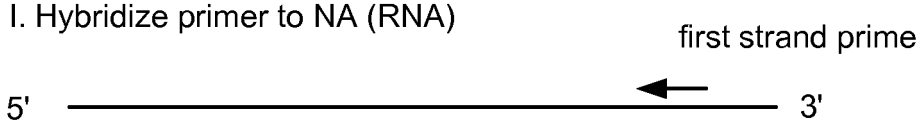
VI. Ligate a second adaptor comprising sequence B



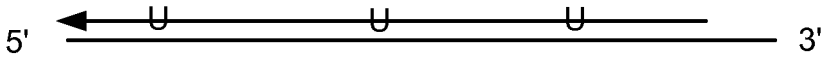
Strand specific product with defined sequences, A and B, at the 5' and 3'-ends of the insert(s) respectively

FIG. 1A

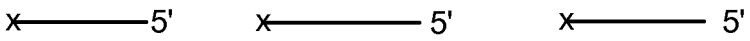
I. Hybridize primer to NA (RNA)



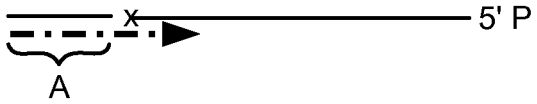
II. Primer extension incorporating dU



III. Degrade template NA and treat primer extension products with UNG and cleave to generate fragments of desired size range with blocked 3'-ends



IV. Anneal first adaptor comprising a 3'-overhang comprising random sequence at the 3'-end and sequence A (one exemplary fragment shown)

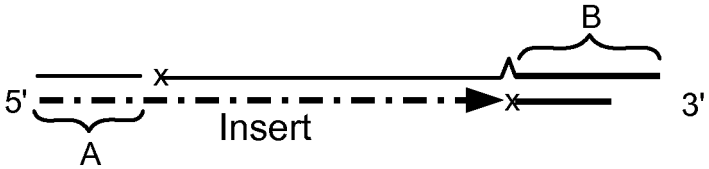


V. Extend hybridized 3'-end of the of adaptor along the fragment comprising a blocked 3'-end generated in step III with a DNA polymerase

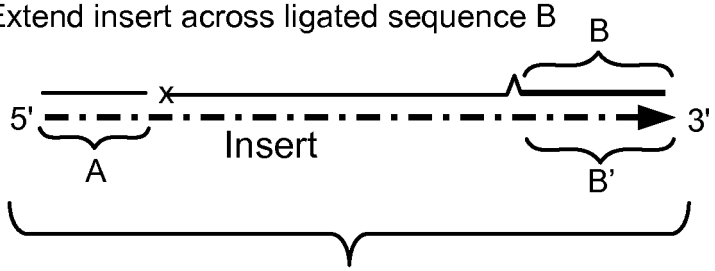


Double stranded fragments with an appended sequence A

VI. Ligate a second adaptor comprising sequence B



VII. Extend insert across ligated sequence B



Strand specific product with defined sequences, A and B, at the 5' and 3'-ends of the insert(s) respectively

FIG. 1B

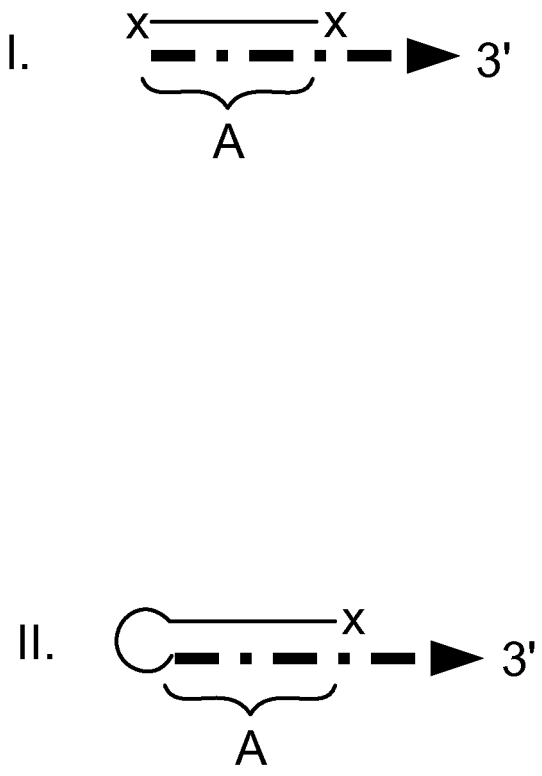


FIG. 2

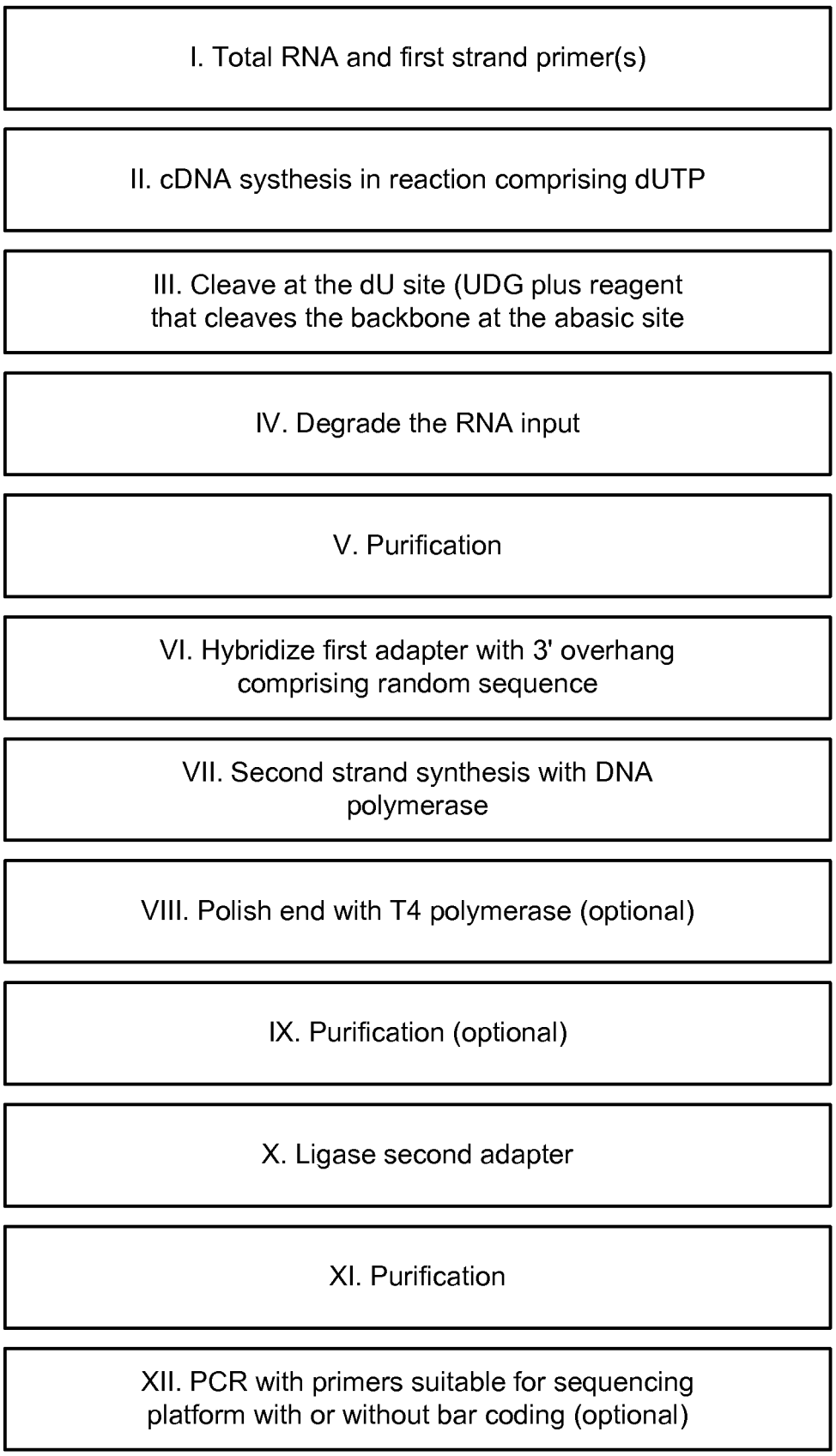

I. Total RNA and first strand primer(s)
II. cDNA systhesis in reaction comprising dUTP
III. Cleave at the dU site (UDG plus reagent that cleaves the backbone at the abasic site
IV. Degrade the RNA input
V. Purification
VI. Hybridize first adapter with 3' overhang comprising random sequence
VII. Second strand synthesis with DNA polymerase
VIII. Polish end with T4 polymerase (optional)
IX. Purification (optional)
X. Ligase second adapter
XI. Purification
XII. PCR with primers suitable for sequencing platform with or without bar coding (optional)

FIG. 3

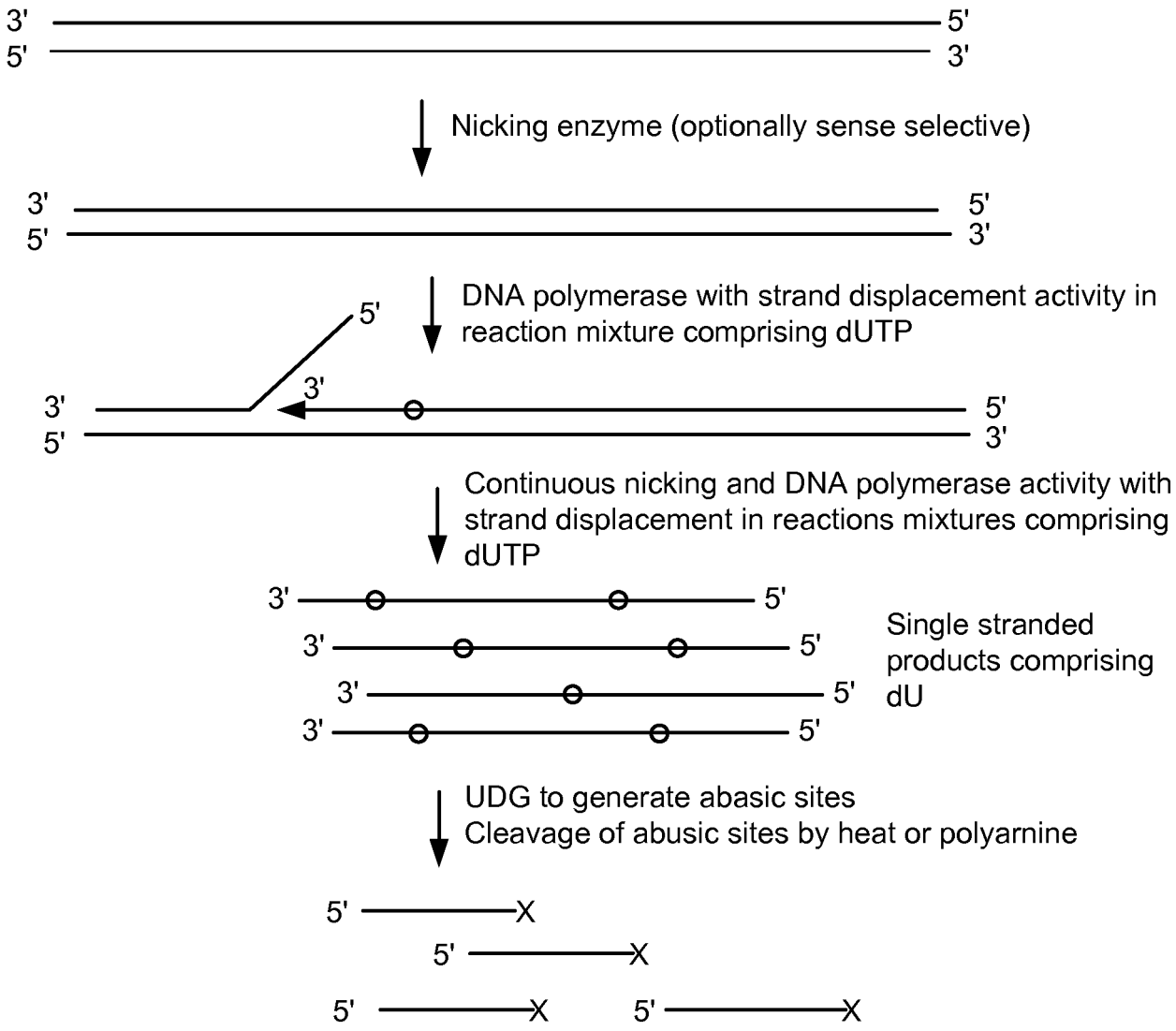

3'
5'
5'
3'
Nicking enzyme (optionally sense selective)
3'
5'
5'
3'
DNA polymerase with strand displacement activity in reaction mixture comprising dUTP
5'
3'
3'
5'
5'
3'
Continuous nicking and DNA polymerase activity with strand displacement in reactions mixtures comprising dUTP
3'
5'
3'
5'
3'
5'
3'
5'
Single stranded products comprising dU
UDG to generate abasic sites
Cleavage of abusic sites by heat or polyarnine
5'
X
5'
X
5'
X
5'
X
Multiple fragments of desired size range blocked at the 3'-end and suitable for generation of libraries as described in Figure 1.

FIG. 4

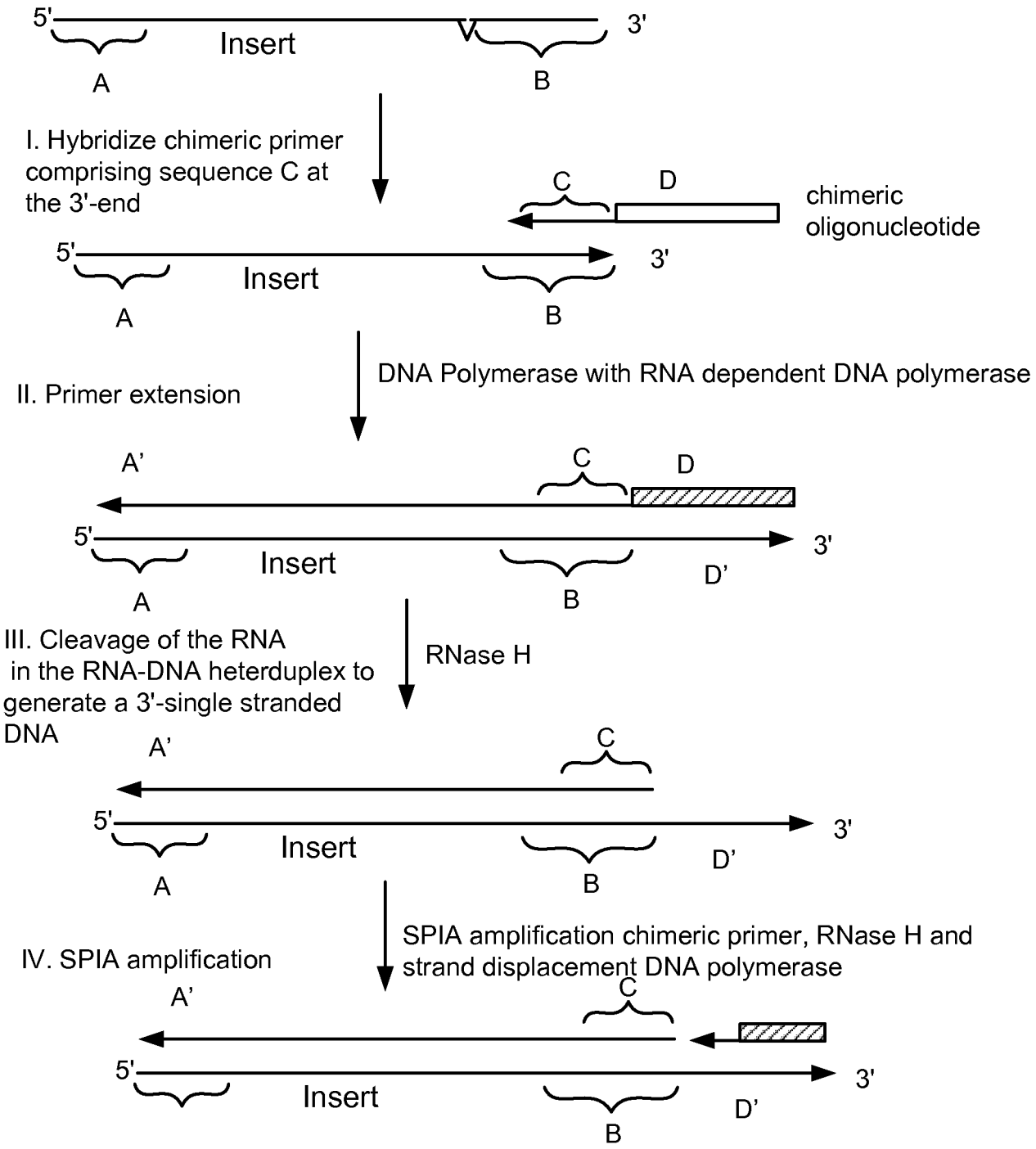

5'
Insert
3'
A
B
I. Hybridize chimeric primer comprising sequence C at the 3'-end
C
D
chimeric oligonucleotide
5'
Insert
3'
A
B
II. Primer extension
DNA Polymerase with RNA dependent DNA polymerase
A'
C
D
5'
Insert
3'
D'
A
B
III. Cleavage of the RNA in the RNA-DNA heterduplex to generate a 3'-single stranded DNA
RNase H
A'
C
5'
Insert
3'
D'
A
B
IV. SPIA amplification
SPIA amplification chimeric primer, RNase H and strand displacement DNA polymerase
A'
C
5'
Insert
3'
D'
B

FIG. 5

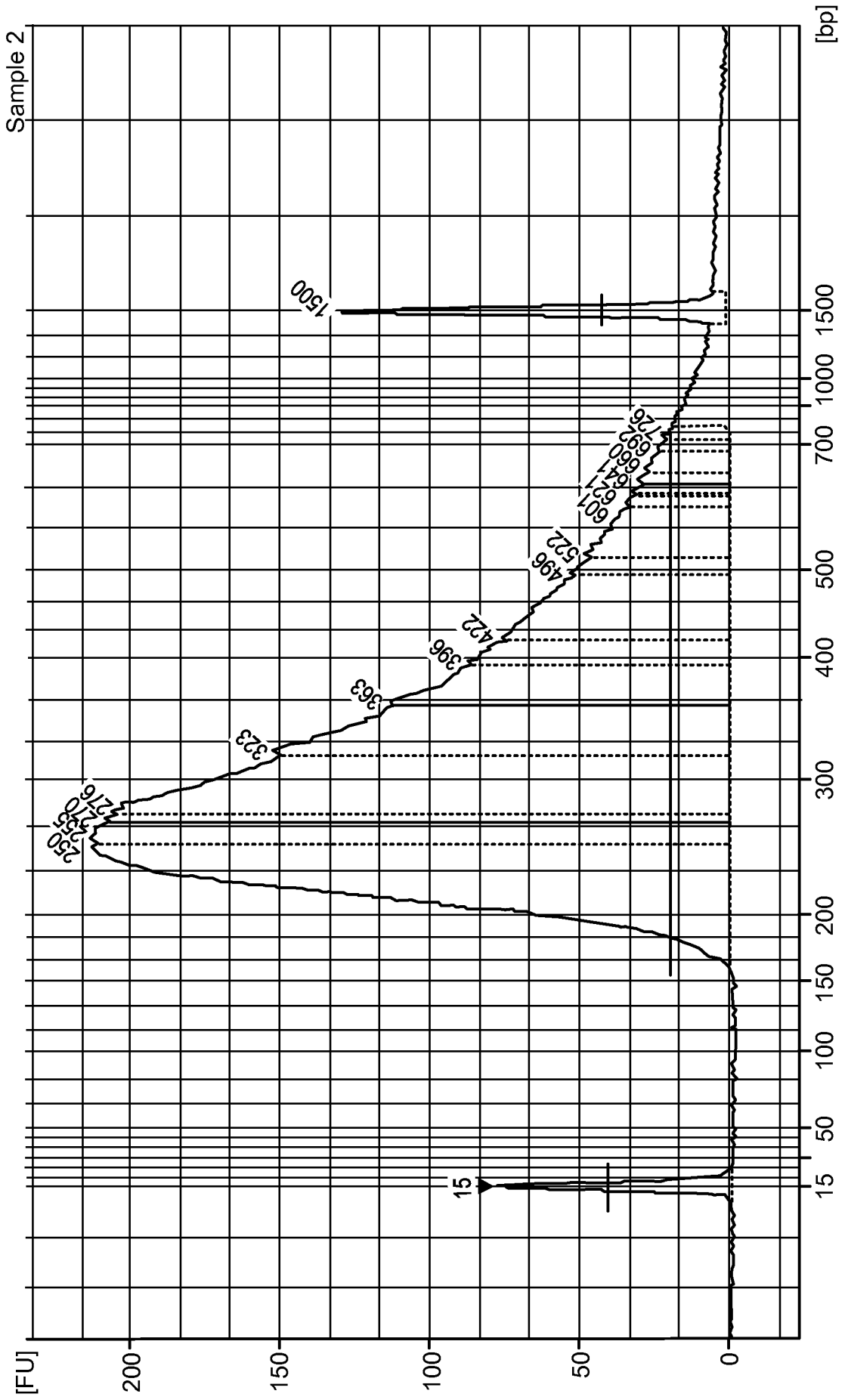

Sample 2
[FU]
200
150
100
50
0
[bp]
15
50
100
150
200
300
400
500
700
1000
1500
15
250
255
270
276
323
363
396
422
496
522
601
621
641
660
692
726
1500

FIG. 6

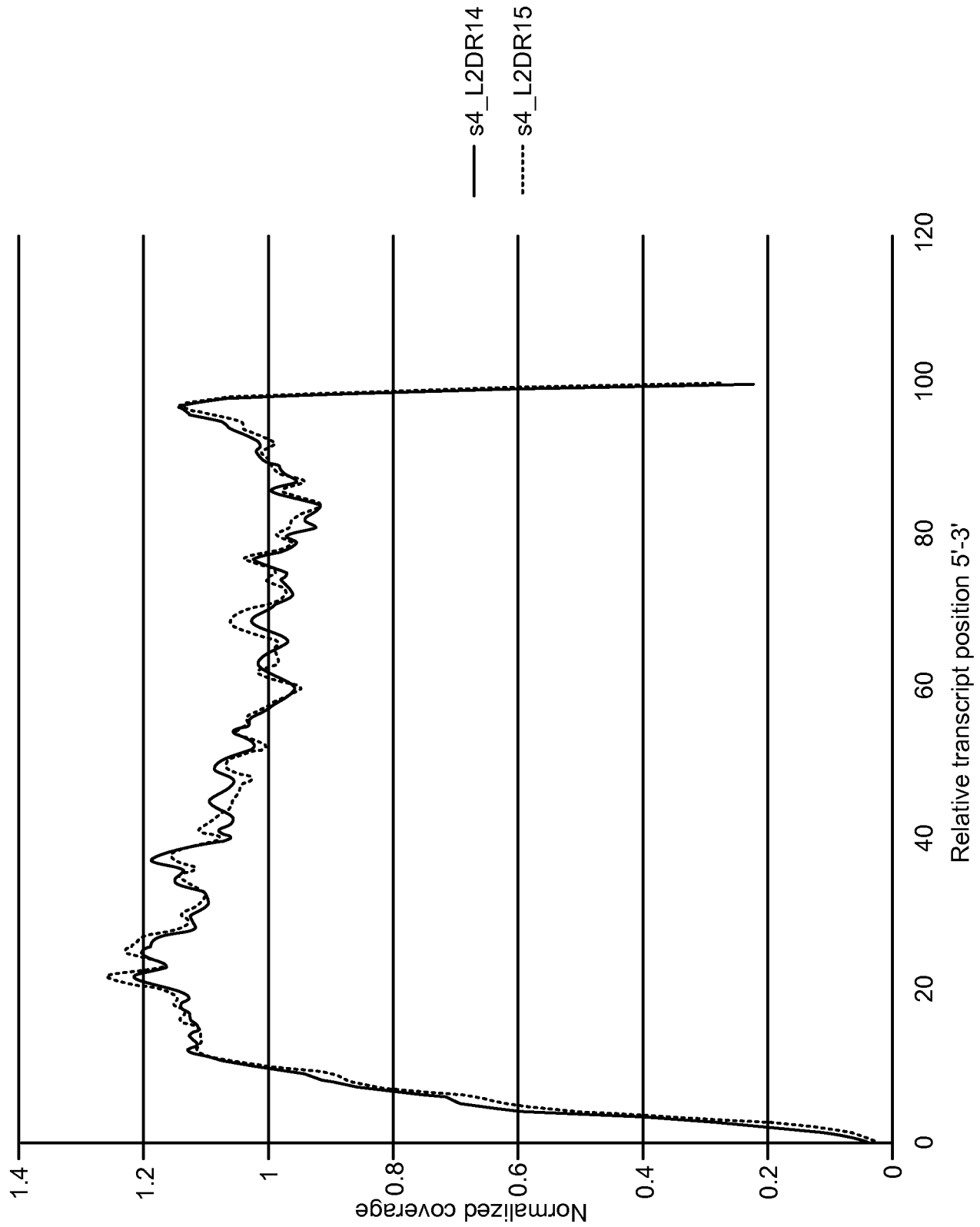

s4_L2DR14
s4_L2DR15
Normalized coverage
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
Relative transcript position 5'-3'

FIG. 7

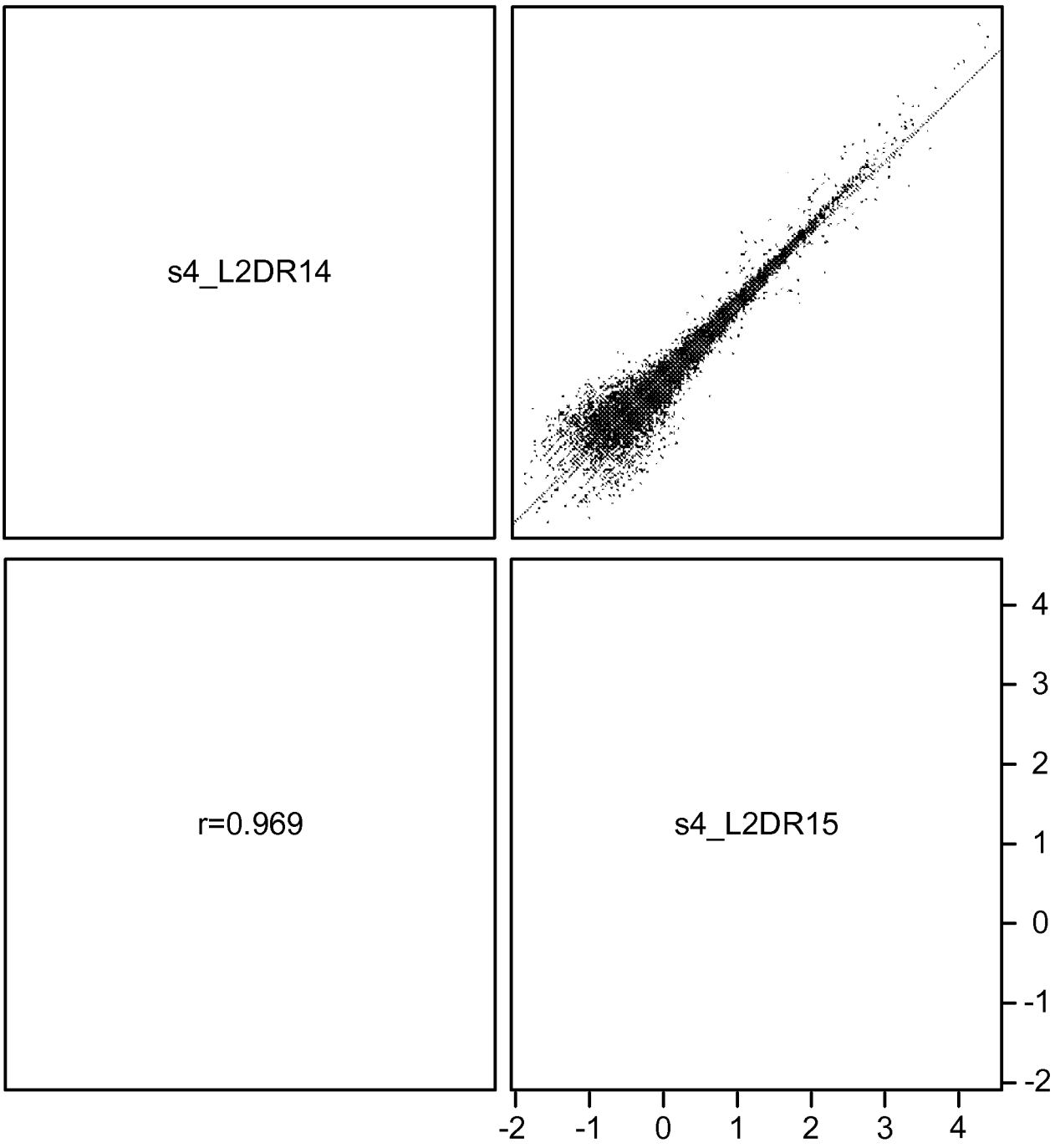


FIG. 8

Sample	Input	% of all		% of mapped				RefSeq breakdown (counts)			RefSeq strand retention			
		Not aligned	aligned	rRNA	non-rRNA	mito rRNA	cyto rRNAA	exon	intron	intergenic	coding	5'UTR	3'UTR	rRNA
1	100 ng	12%	88%	6.6%	93.4%	1.6%	5.0%	27.7%	38.6%	33.7%	97.2%	96.5%	95.1%	99.5%
2	100 ng	7%	93%	7.1%	92.9%	1.5%	5.6%	31.0%	39.0%	30.1%	97.8%	97.1%	95.8%	99.6%
3	1 ng	13%	87%	9.2%	90.8%	0.95%	8.23%	25.5%	38.4%	36.1%	97.5%	96.6%	95.7%	99.6%

FIG. 9

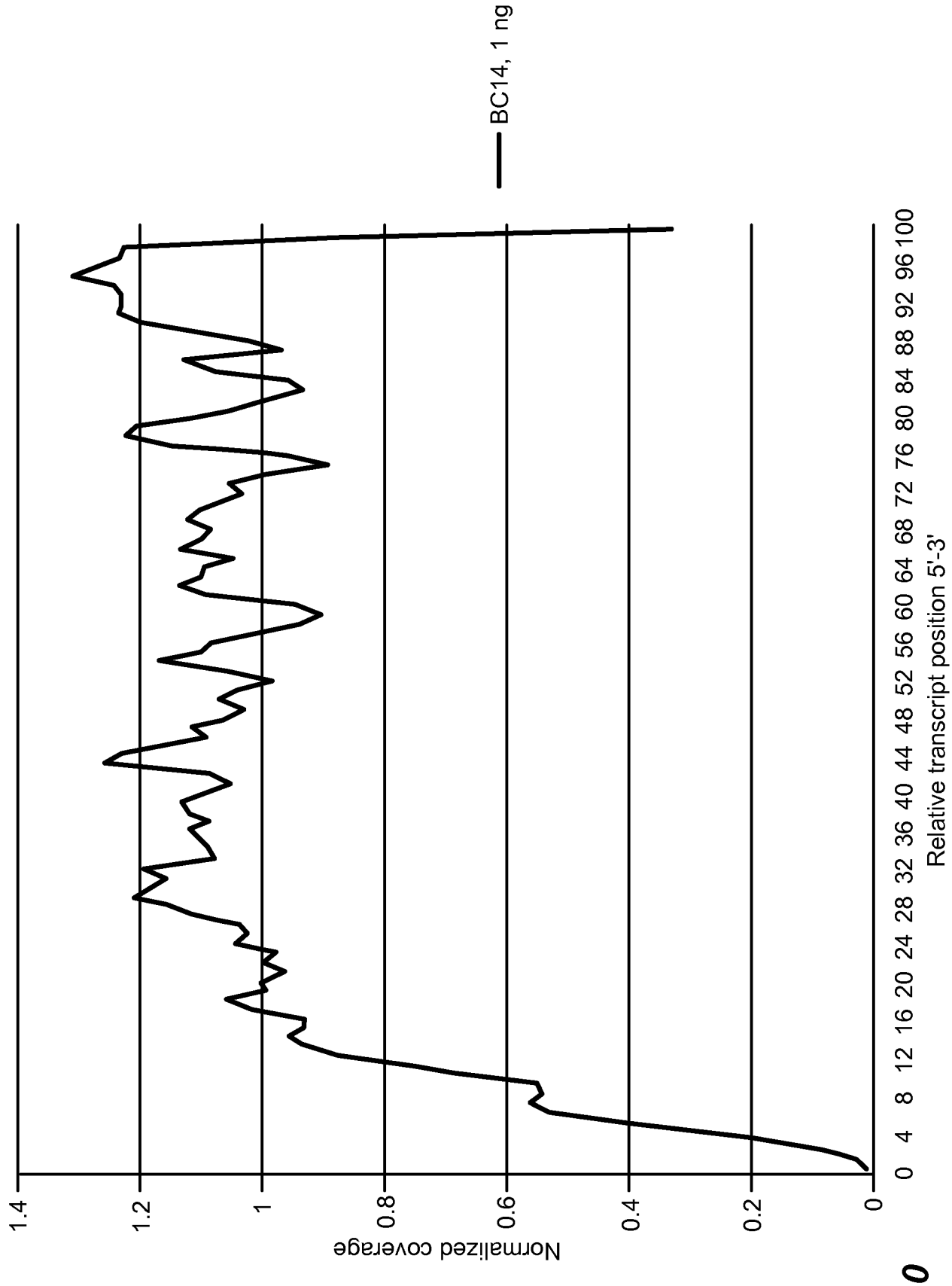

BC14, 1 ng
Normalized coverage
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100
Relative transcript position 5'-3'

FIG. 10