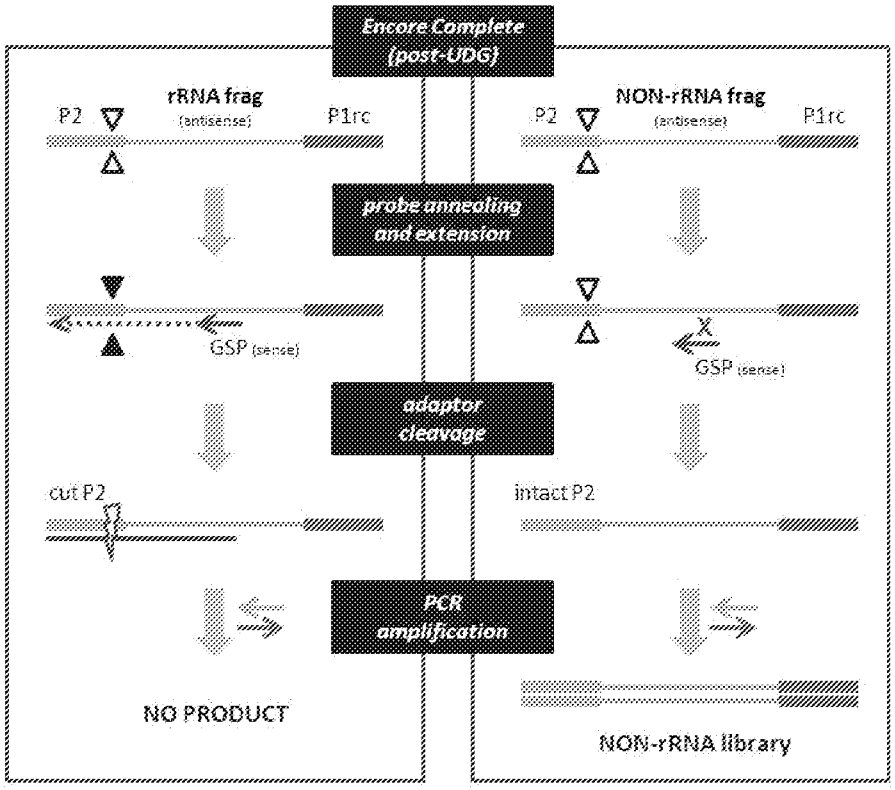
Figure 1. rRNA depletion from a nucleic acid library using insert-dependent adaptor cleavage (InDA-C).



[1] Encore Complete RNA-Seq library system is used to generate strand-specific cDNA libraries from 100 ng of *E.coli* total RNA.

Figure 2. Summary of results - Example 1.

library	probe	BspQ1	total number of reads	% of reads mapping to reference	% of mapped reads in ORFs	% of mapped reads in rRNA	% of rRNA reads mapping to sense strand	% of rRNA reads mapping to antisense strand
ctrl	-	-	27,161,879	99.3	1.0	99.0	99.7	0.3
test1	-	-	7,510,963	97.3	5.6	92.9	99.1	0.9
test2	+	-	8,679,815	97.5	5.6	92.9	99.1	0.9
test3	-	+	9,331,682	97.4	5.2	93.4	99.2	0.8
test4	+	+	9,355,578	95.1	11.7	86.1	98.1	1.9

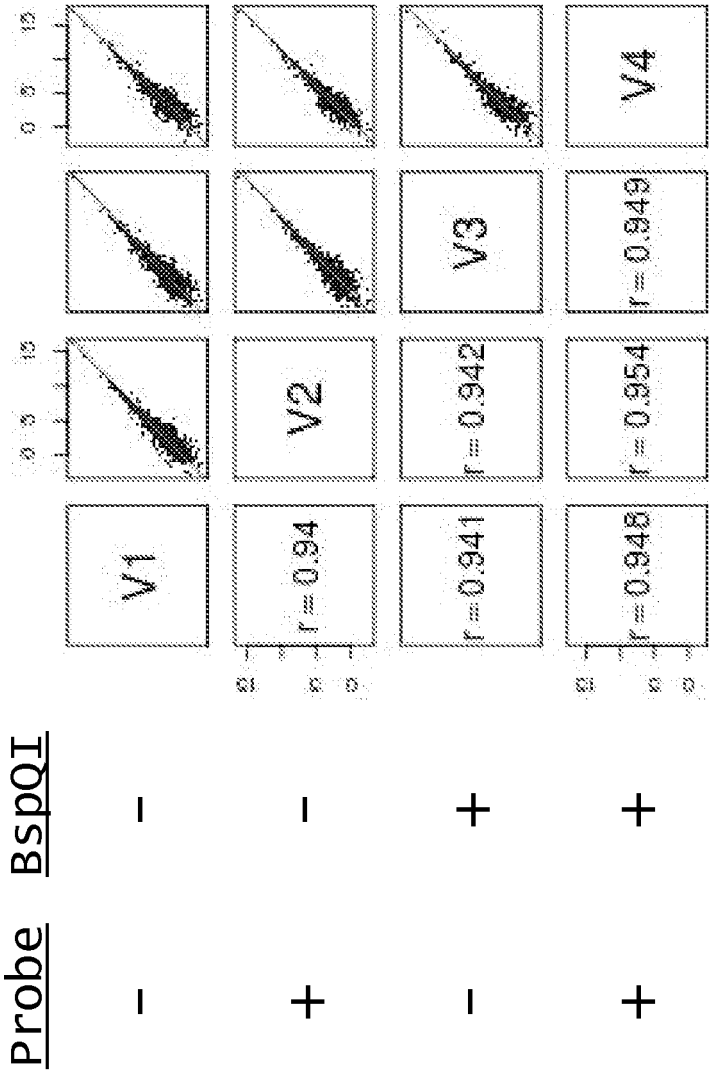


Figure 3. Comparison of expression profiles from the test libraries 1-4 in Example 1. InDA-C does not perturb transcript abundance.

Figure 4

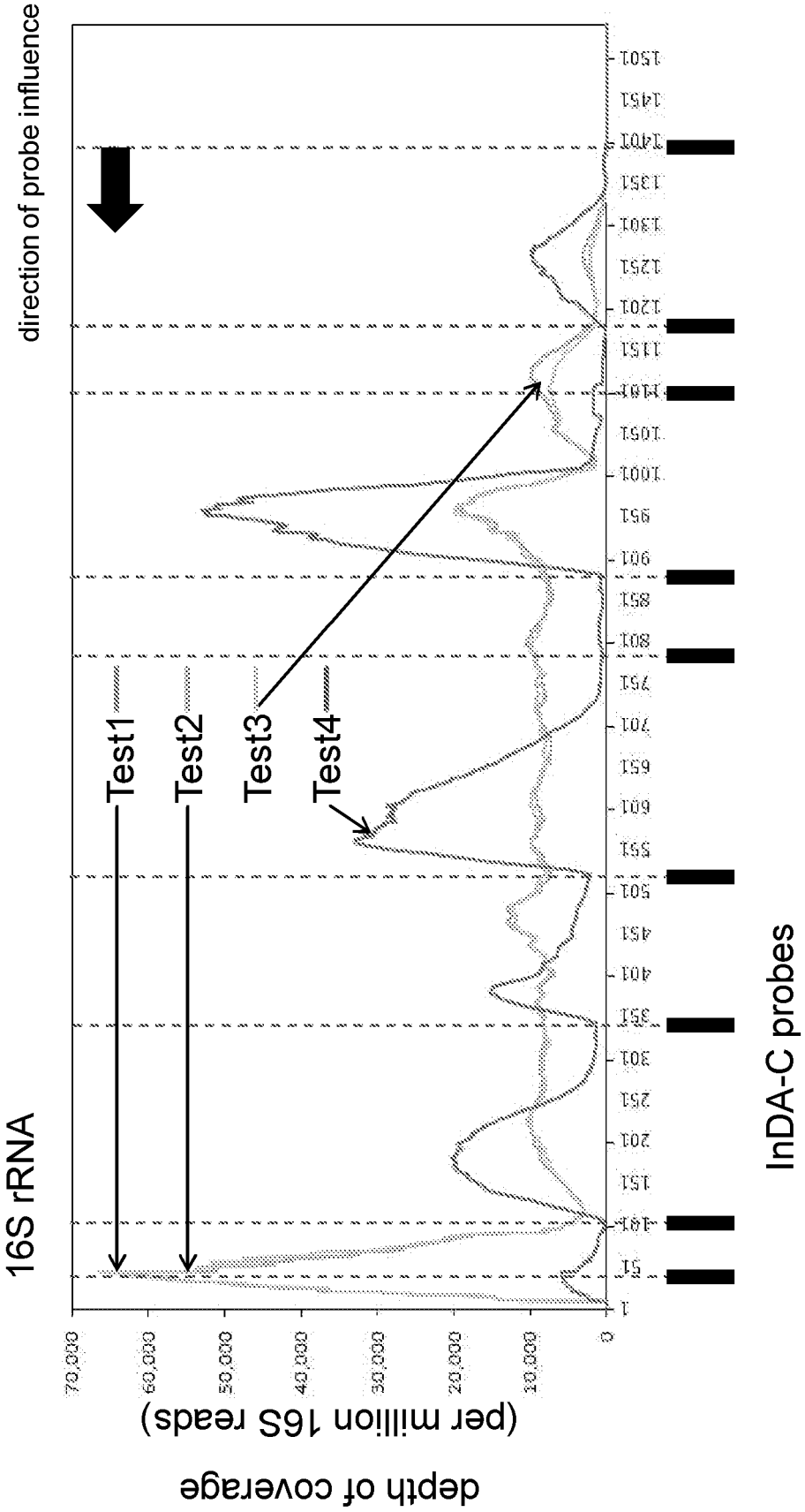

direction of probe influence
16S rRNA
Test1
Test2
Test3
Test4
depth of coverage
(per million 16S reads)
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
1
51
101
151
201
251
301
351
401
451
501
551
601
651
701
751
801
851
901
951
1001
1051
1101
1151
1201
1251
1301
1351
1401
1451
1501
InDA-C probes

Figure 5: Directional Library Construction

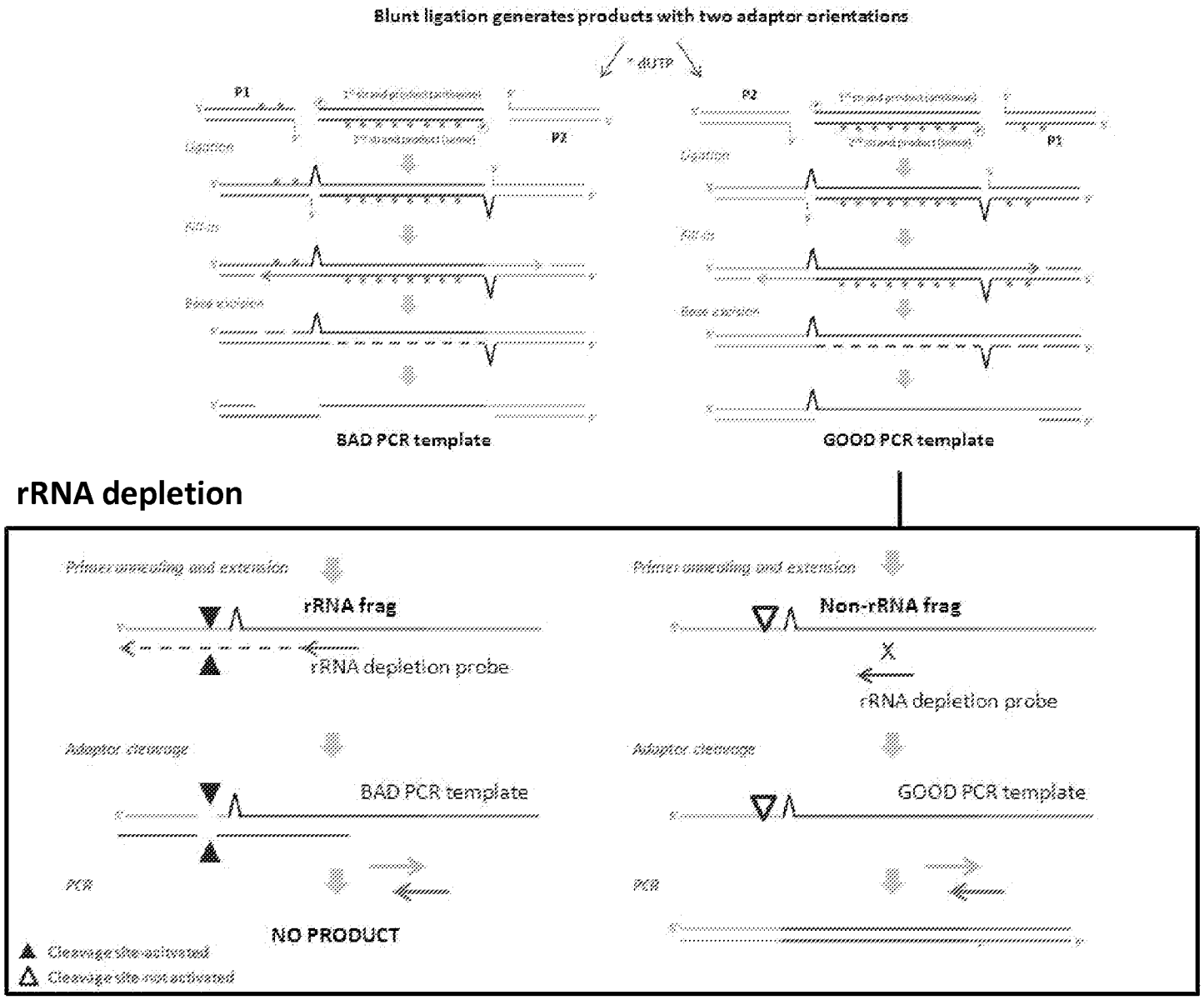


Figure 6: InDA-C with Single cell, cDNA double hydrolysis

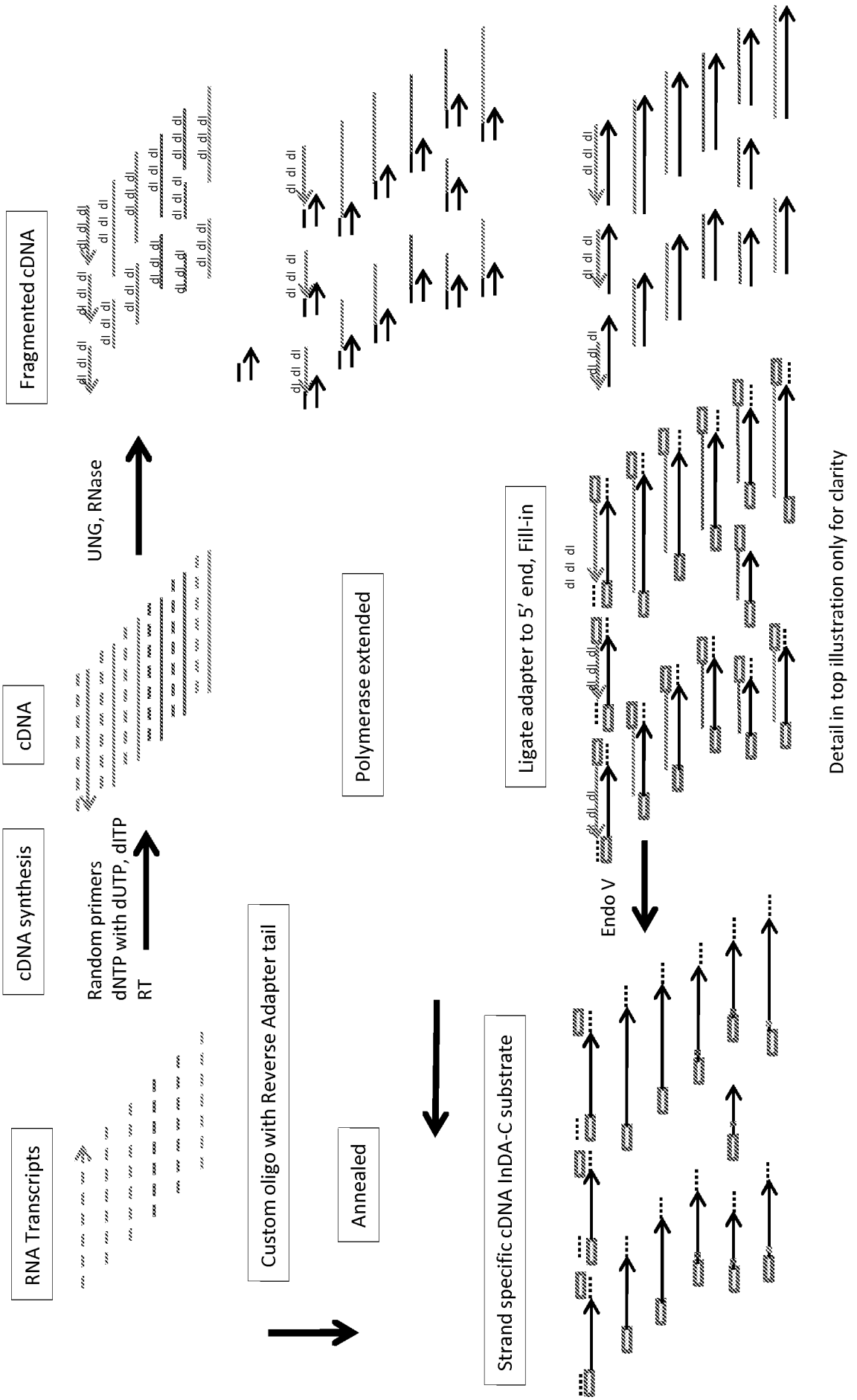


Figure 7: InDA-C with Single cell, 3 base code in second strand primer

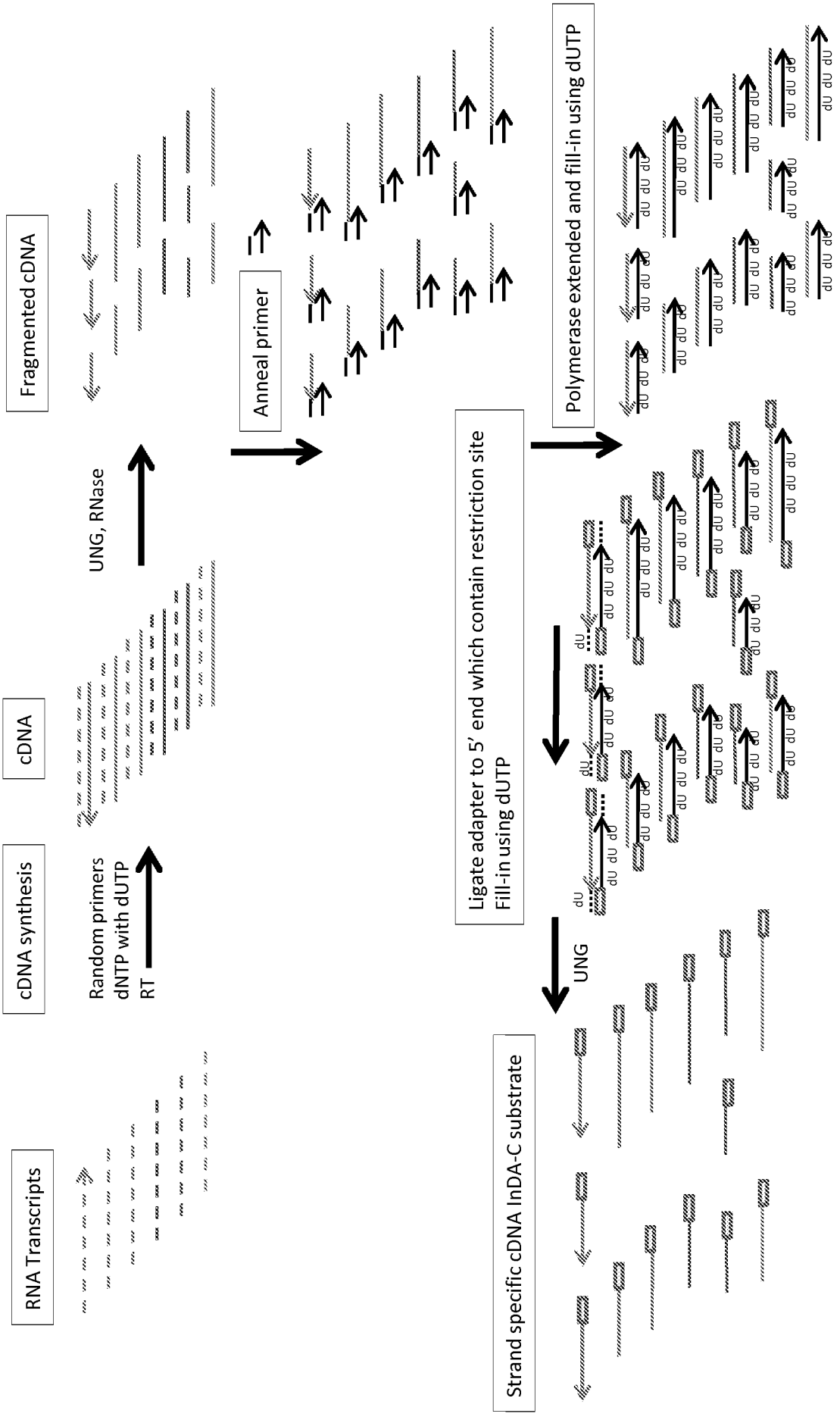
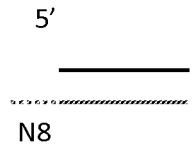
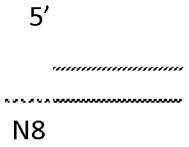


Figure 8: Primer construct

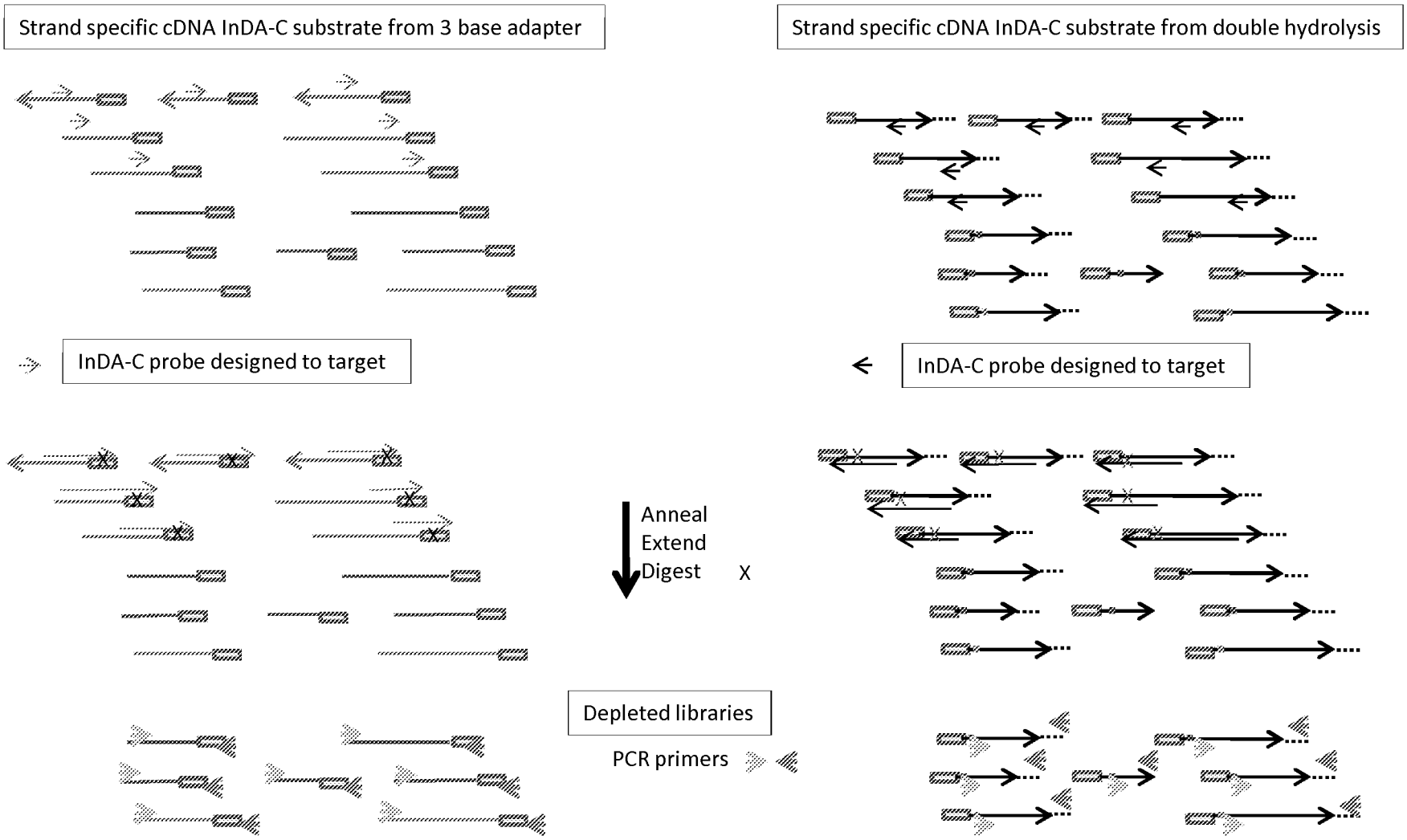


Partial duplex primer according to Example 7:
33 base double stranded region with 8 random nucleotides on lower strand
Double stranded region of lower strand is 3 base code, there are no Adenosine residues
33 base region contains sequence amenable to specific priming during PCR library enrichment



Partial duplex primer according to Example 6:
33 base double stranded region with 8 random nucleotides on lower strand
33 base region contains Type II restriction enzyme recognition site
33 base region contains sequence amenable to specific priming during PCR library enrichment

Figure 9


Strand specific cDNA InDA-C substrate from 3 base adapter
Strand specific cDNA InDA-C substrate from double hydrolysis
InDA-C probe designed to target
InDA-C probe designed to target
Anneal
Extend
Digest
X
Depleted libraries
PCR primers