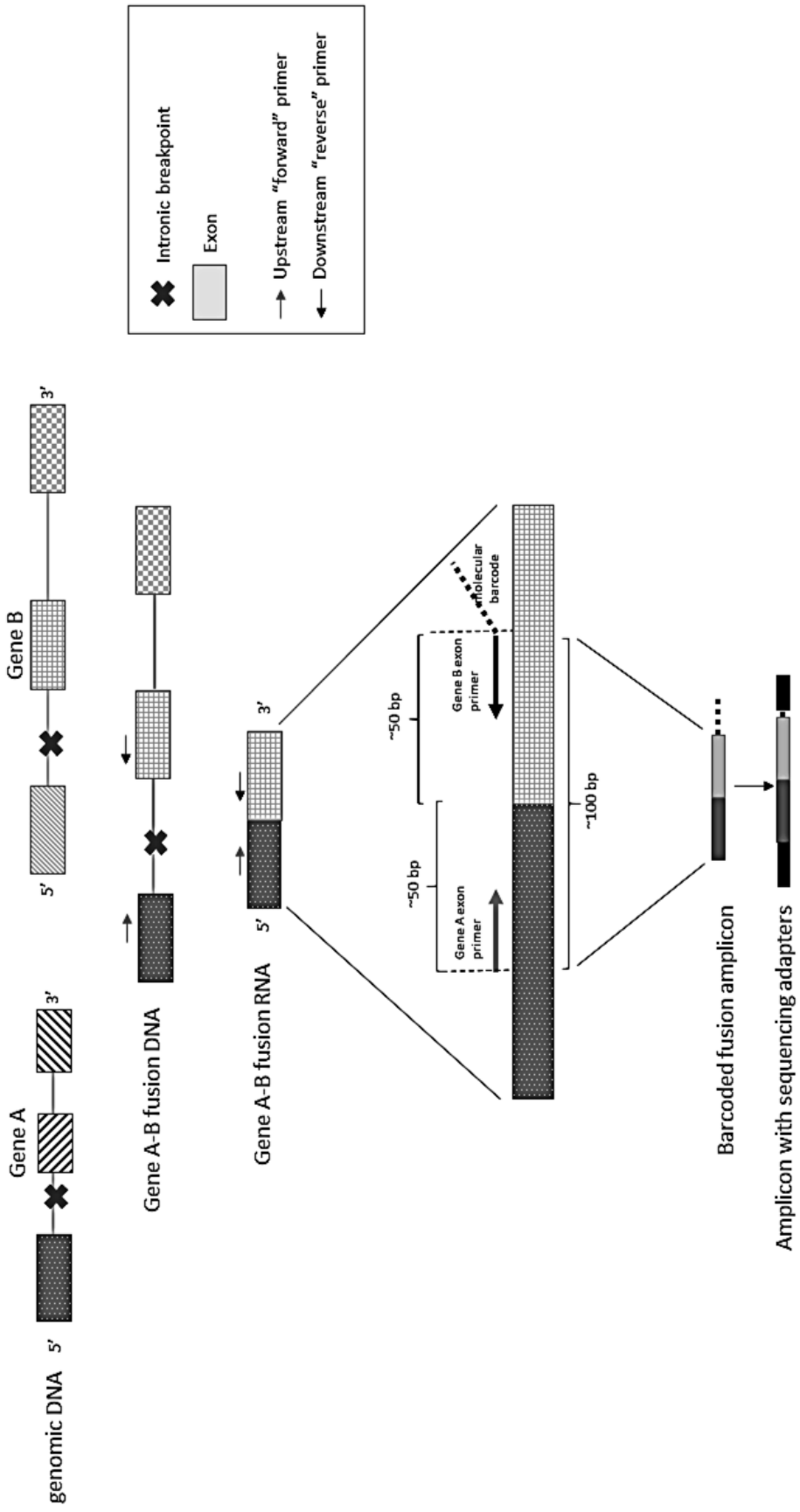

Intronic breakpoint
Exon
Upstream "forward" primer
Downstream "reverse" primer
genomic DNA
5'
Gene A
3'
Gene B
5'
3'
Gene A-B fusion DNA
Gene A-B fusion RNA
5'
3'
~50 bp
Gene A exon primer
~50 bp
Gene B exon primer
molecular barcode
~100 bp
Barcoded fusion amplicon
Amplicon with sequencing adapters

Fig. 1

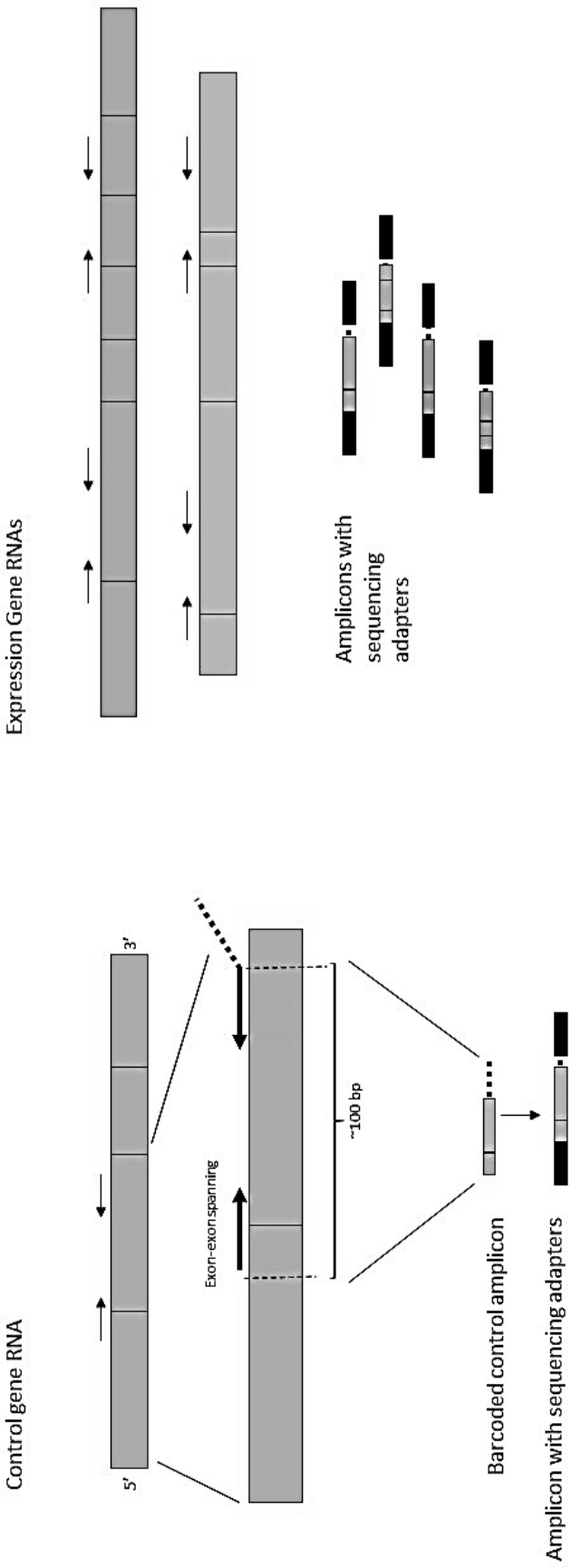

Expression Gene RNAs
Amplicons with sequencing adapters
Control gene RNA
5'
3'
Exon-exon spanning
~100 bp
Barcoded control amplicon
Amplicon with sequencing adapters

Fig. 2A

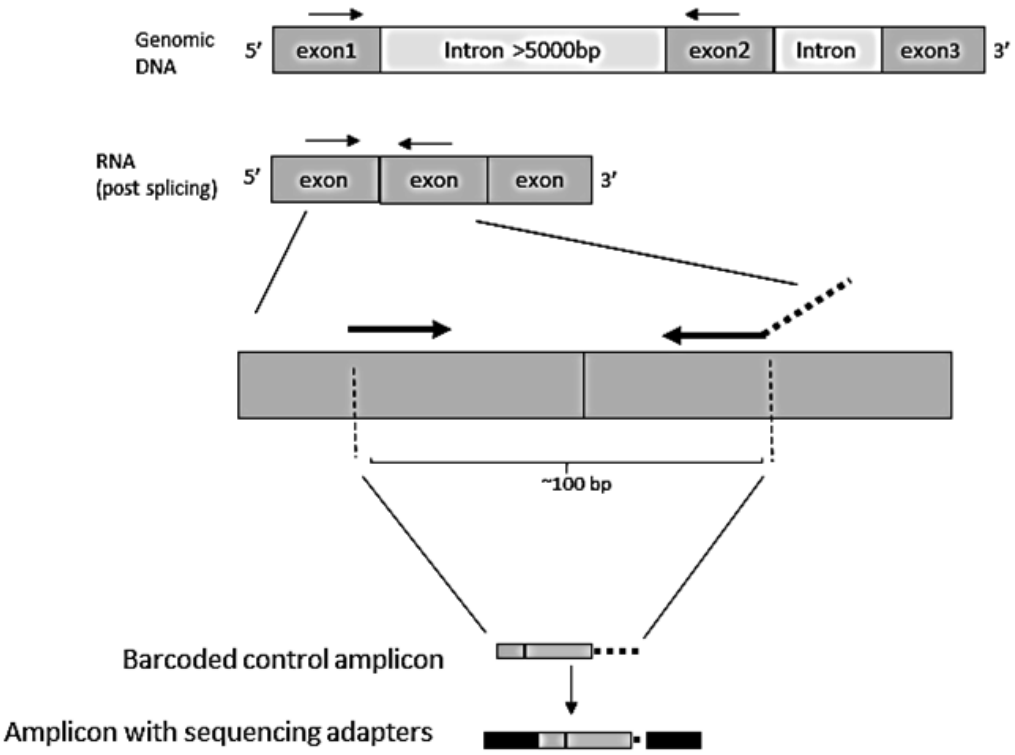

Genomic DNA
5′
exon1
Intron >5000bp
exon2
Intron
exon3
3′
RNA (post splicing)
5′
exon
exon
exon
3′
~100 bp
Barcoded control amplicon
Amplicon with sequencing adapters

Fig. 2B

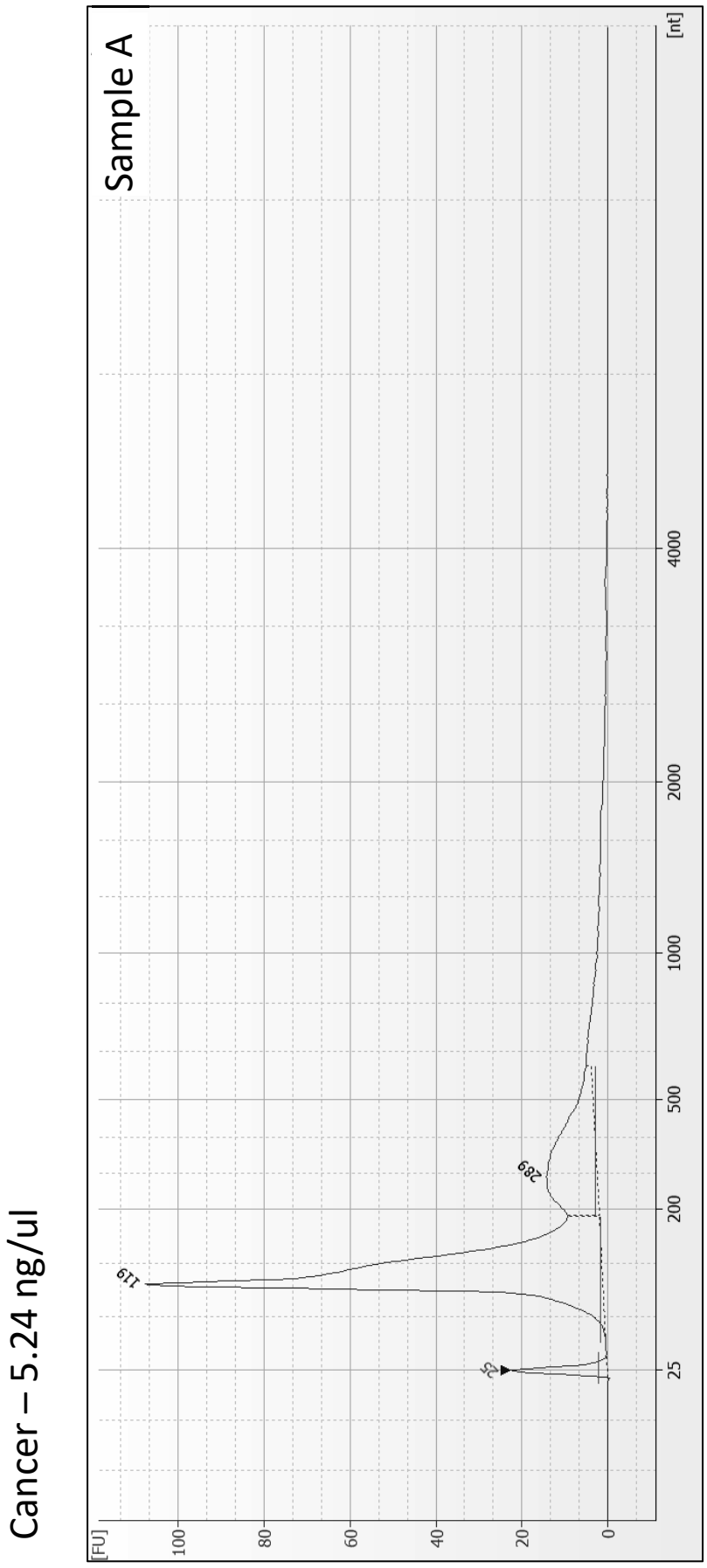

Cancer – 5.24 ng/ul
Sample A
[FU]
100
80
60
40
20
0
25
119
289
25
200
500
1000
2000
4000
[nt]

Fig. 3A

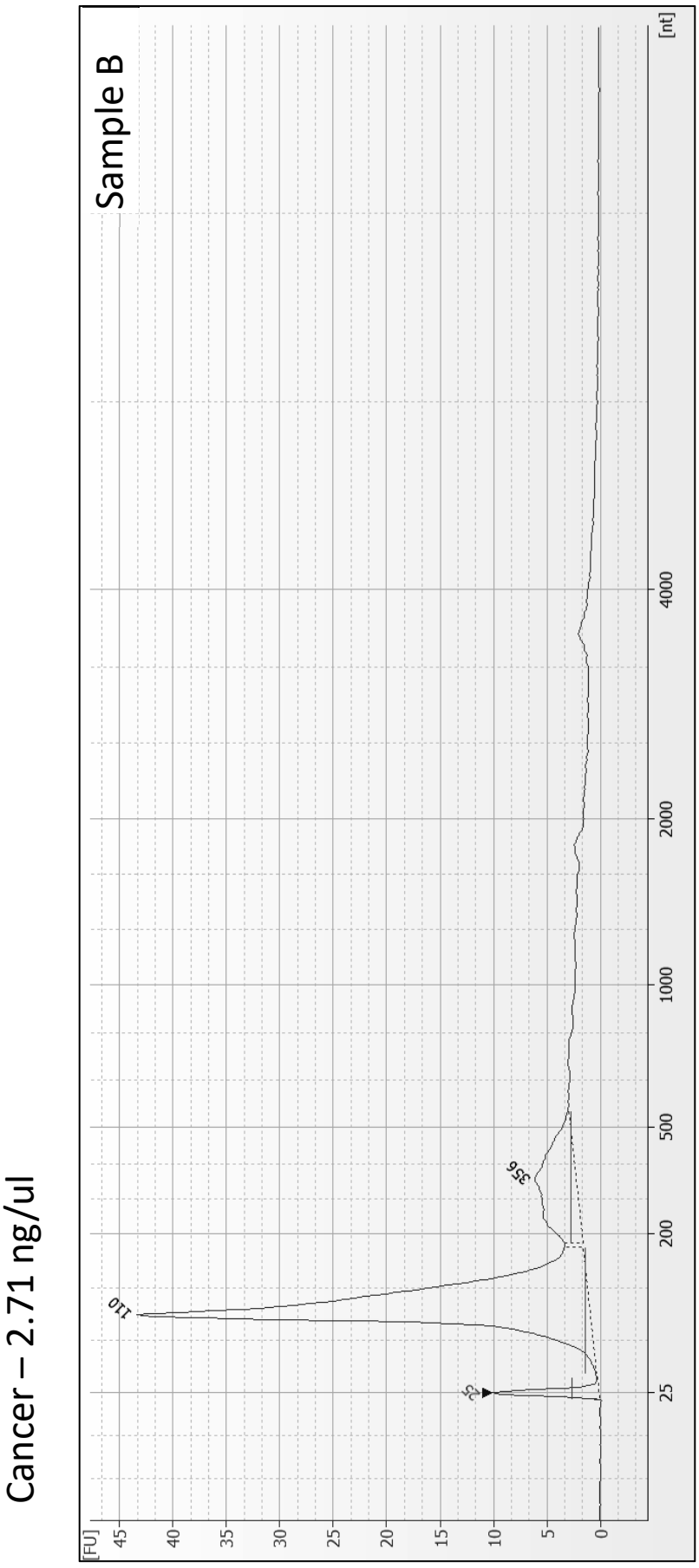

Cancer – 2.71 ng/ul
Sample B
[FU]
[nt]
45
40
35
30
25
20
15
10
5
0
25
200
500
1000
2000
4000
35
356
110

Fig. 3B

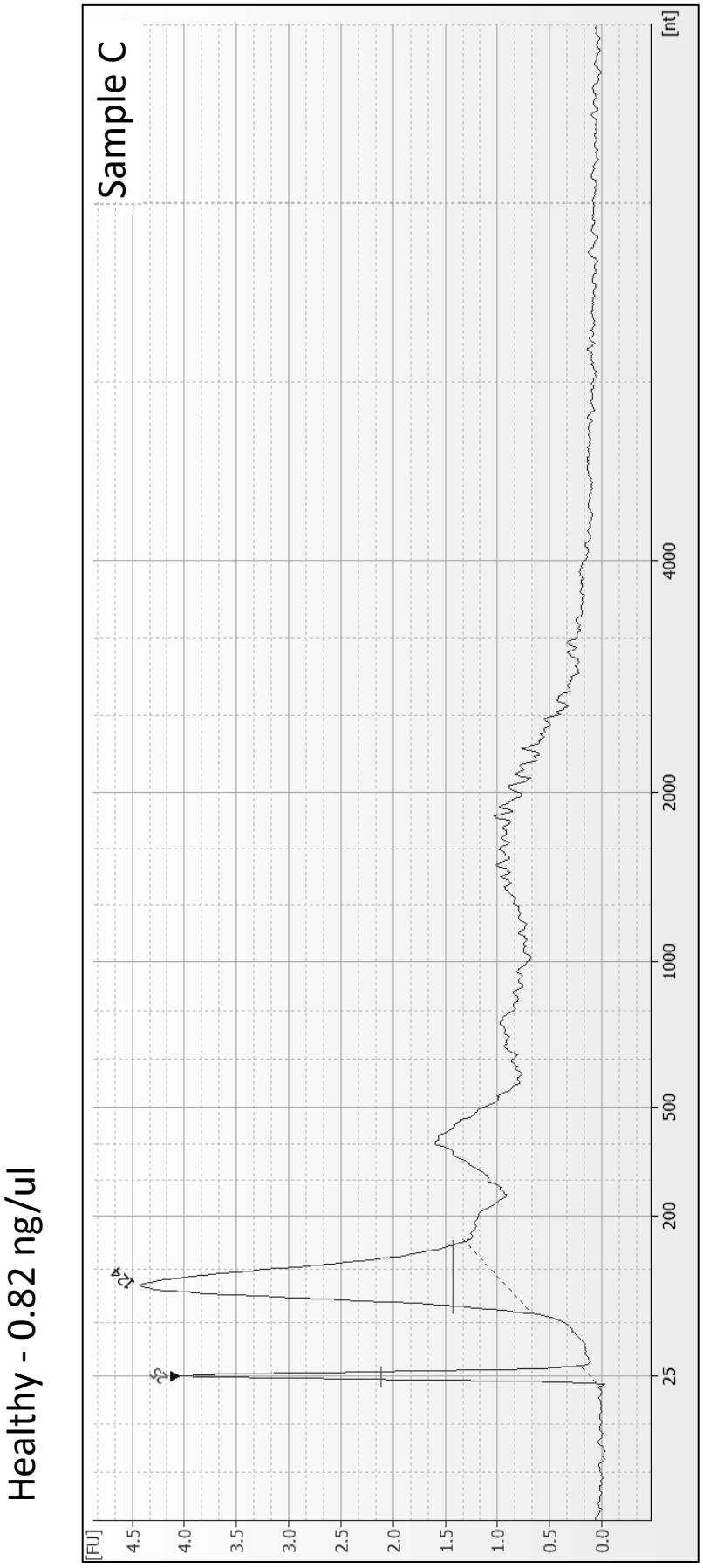
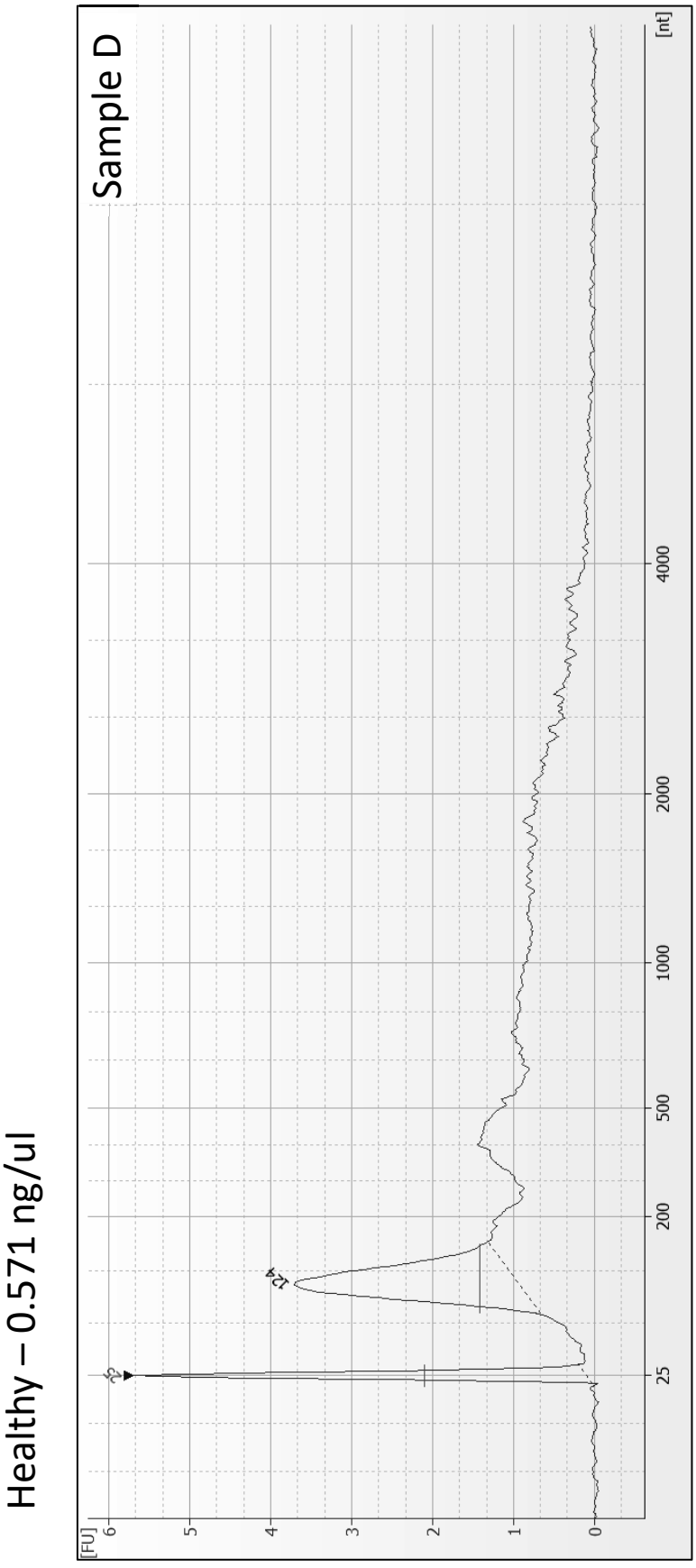

Sample C
Healthy - 0.82 ng/ul
[FU]
[nt]
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
25
200
500
1000
2000
4000
124
35

Fig. 3C



Healthy – 0.571 ng/ul

Fig. 3D

Sample	Diagnosis	cfDNA per ml plasma (ng)	cfRNA per ml plasma (ng)
Ca-1	Metastatic Prostate Cancer	145	43.7
Ca-2	Metastatic Lung Cancer	26.6	27.1
Ca-3	Metastatic Pancreatic Cancer	25.8	61.5
Ca-4	Metastatic Prostate Cancer	356.5	52.1
Ca-5	Metastatic Lung Cancer	2156	96.8
Hth-1	Healthy	7.92	8.2
Hth-2	Healthy	4.76	5.71
Hth-3	Healthy	7.37	3.33

Fig. 4

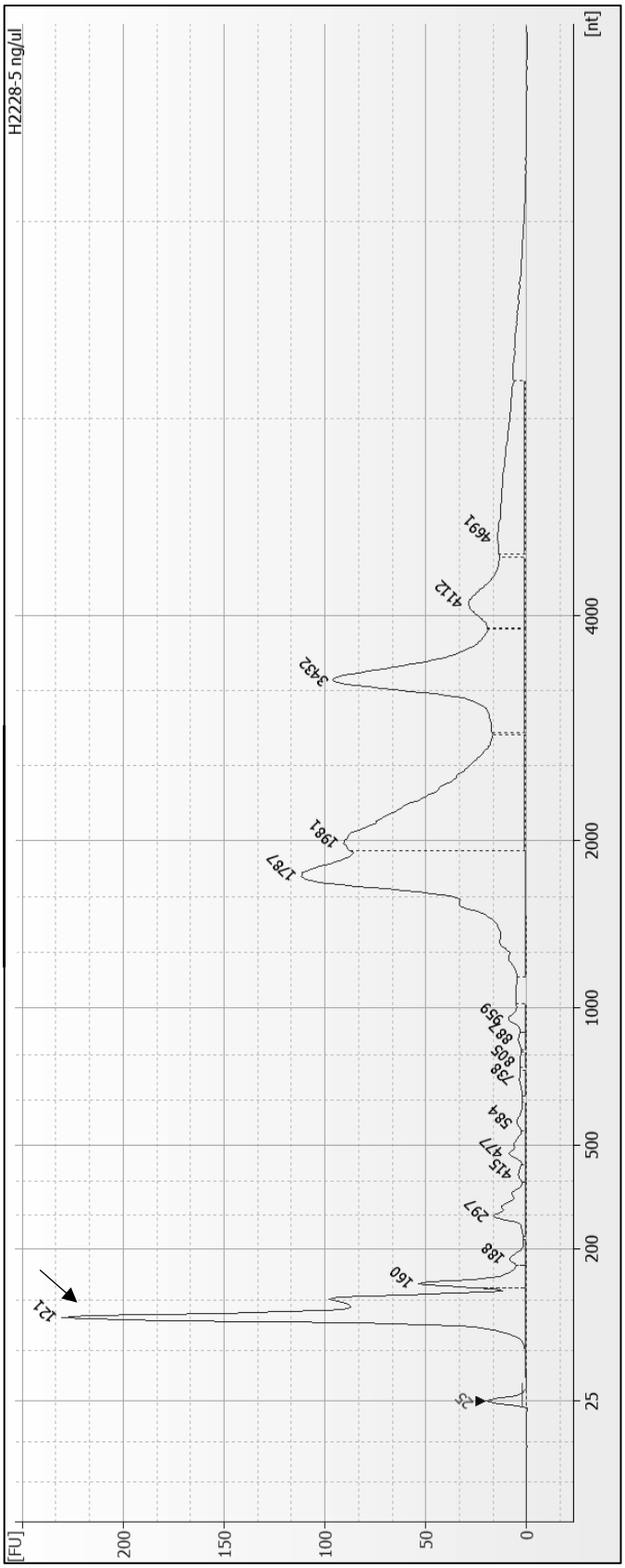

H2228 RNA
H2228-5 ng/ul
[FU]
[nt]
200
150
100
50
0
25
200
500
1000
2000
4000
21
25
160
188
297
415
477
564
758
805
887
959
1787
1981
3452
4112
4691

Fig. 5A

Fragmented H2228 RNA

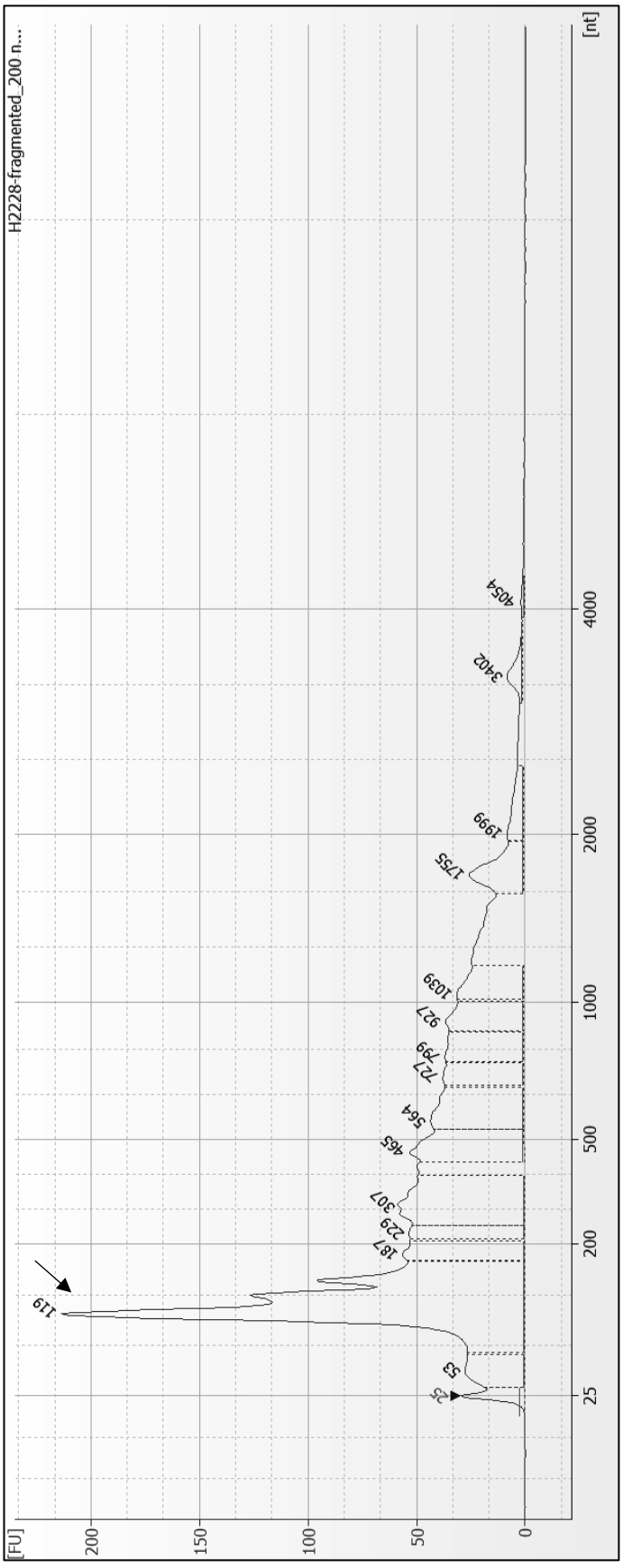


Fig. 5B

Plasma cfRNA

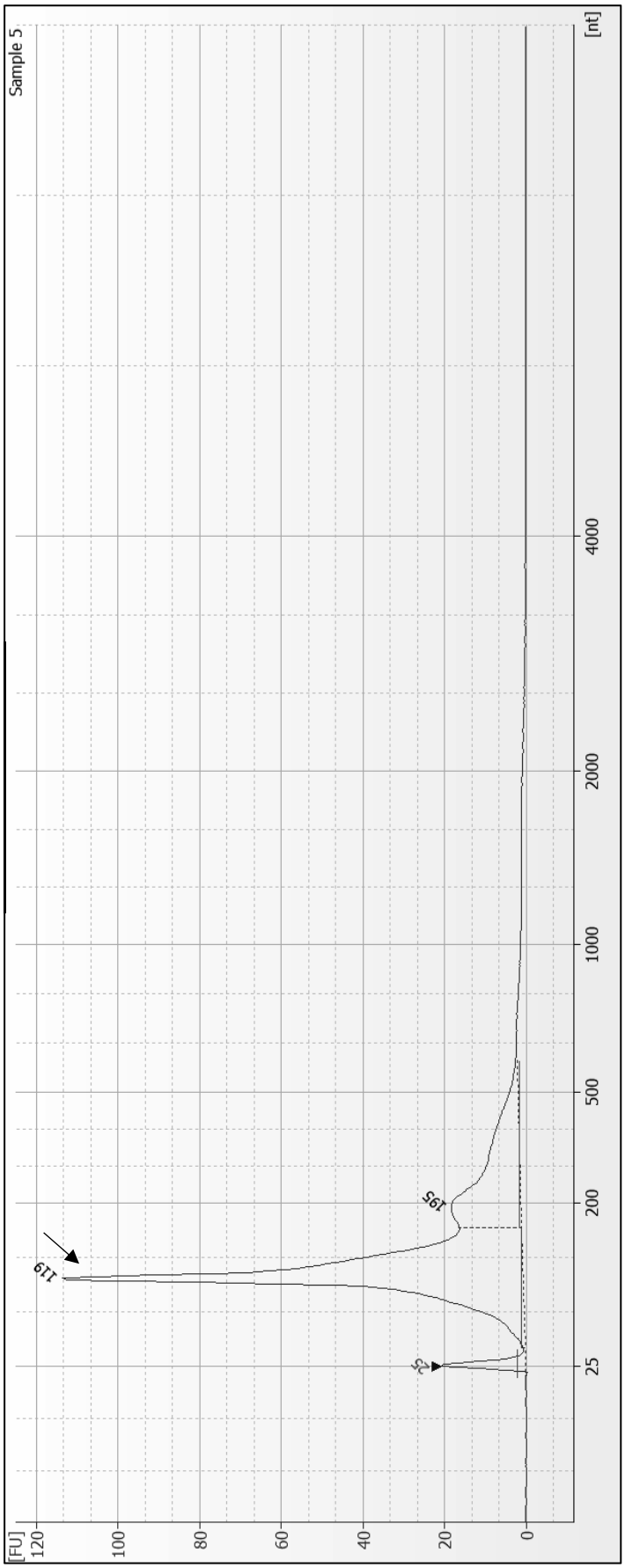


Fig. 5C

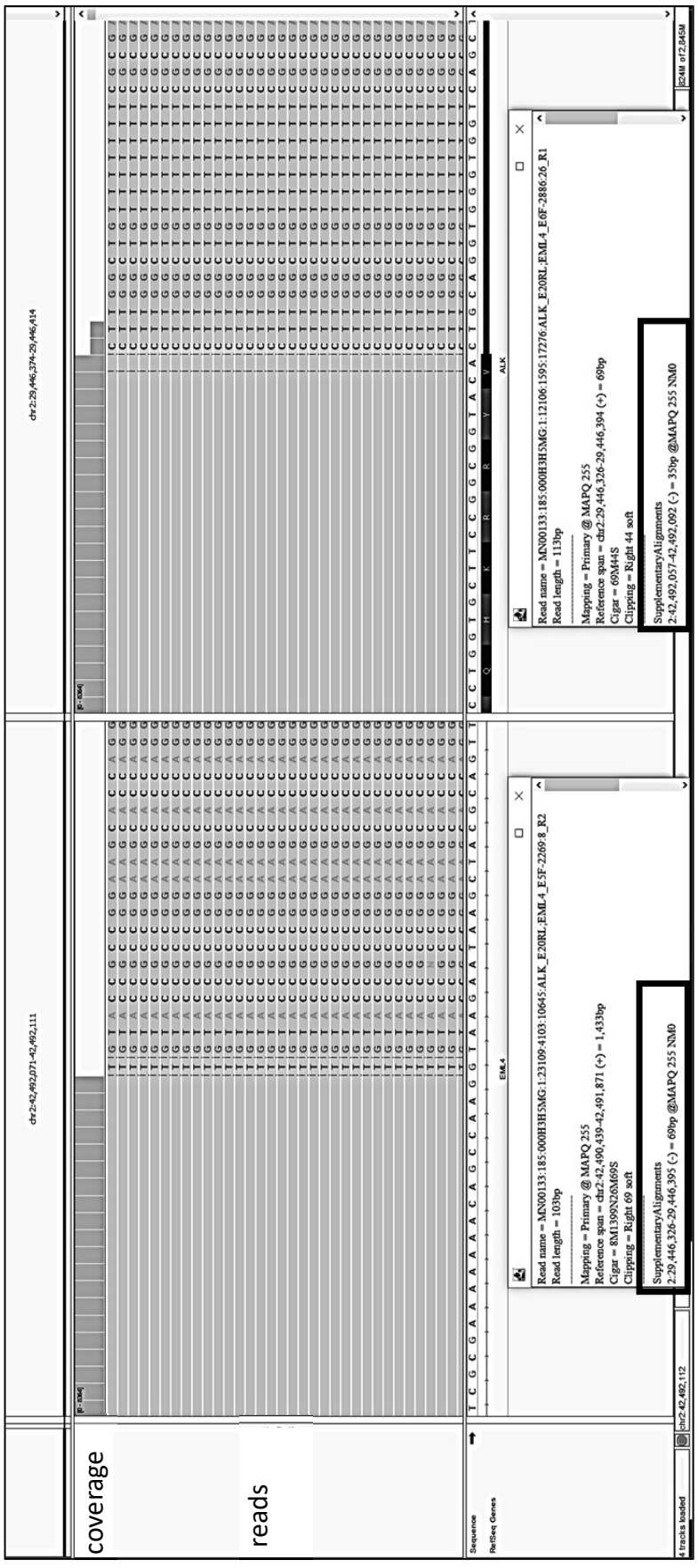

chr 2: 42,492,071-42,492,111
chr 2: 29,446,374-29,446,414
coverage
reads
EML4
ALK

Fig. 6A

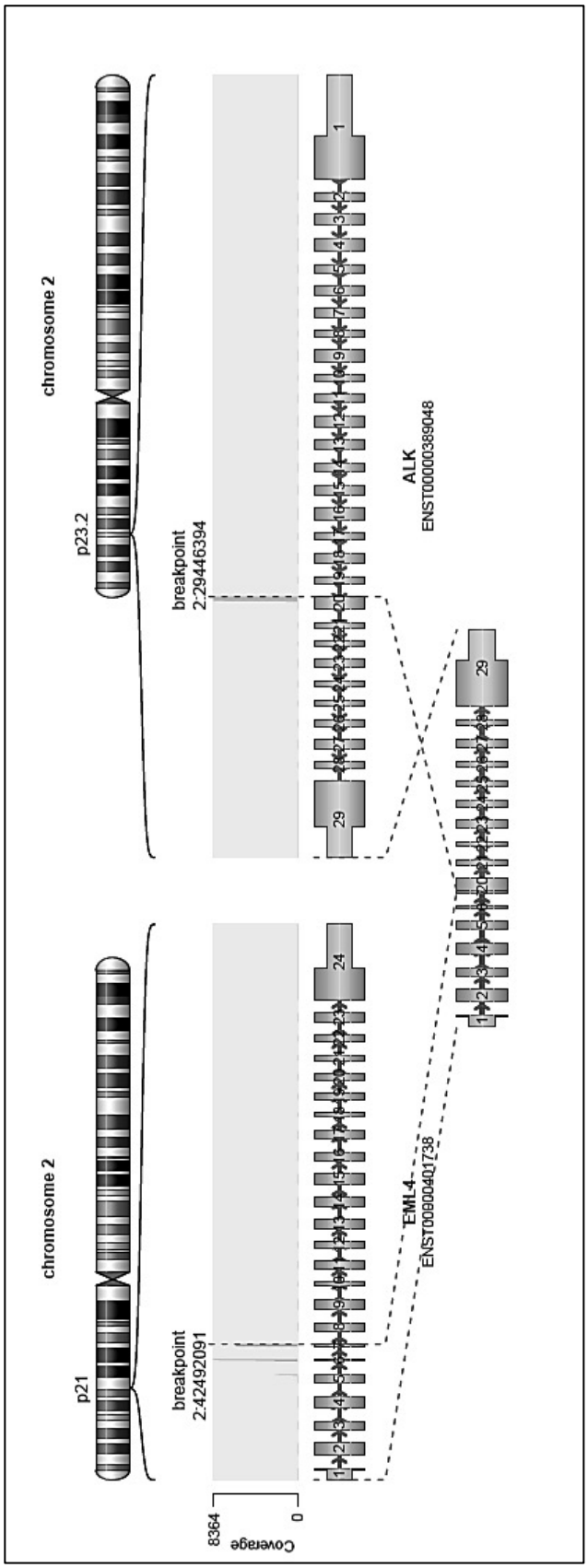

chromosome 2
p23.2
breakpoint
2:29446394
ALK
ENST00000389048
chromosome 2
p21
breakpoint
2:42492091
EML4
ENST00000401738
Coverage
8364
0

Fig. 6B

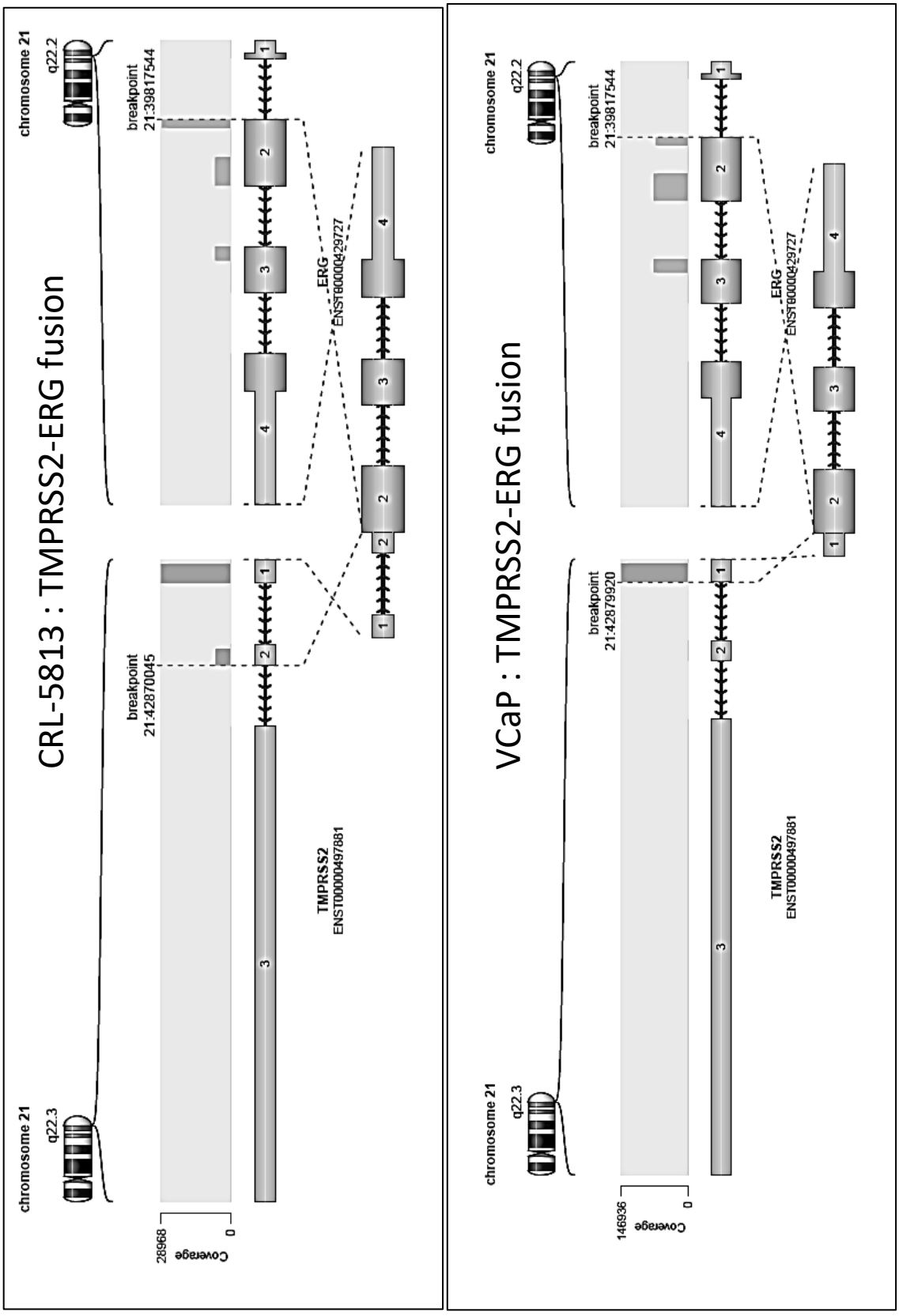

CRL-5813 : TMPRSS2-ERG fusion
chromosome 21
q22.3
q22.2
breakpoint
21:42870045
breakpoint
21:39817544
TMPRSS2
ENST00000497881
ERG
ENST00000429727
Coverage
28968
0
VCaP : TMPRSS2-ERG fusion
chromosome 21
q22.3
q22.2
breakpoint
21:42879920
breakpoint
21:39817544
TMPRSS2
ENST00000497881
ERG
ENST00000429727
Coverage
146936
0

Fig. 7

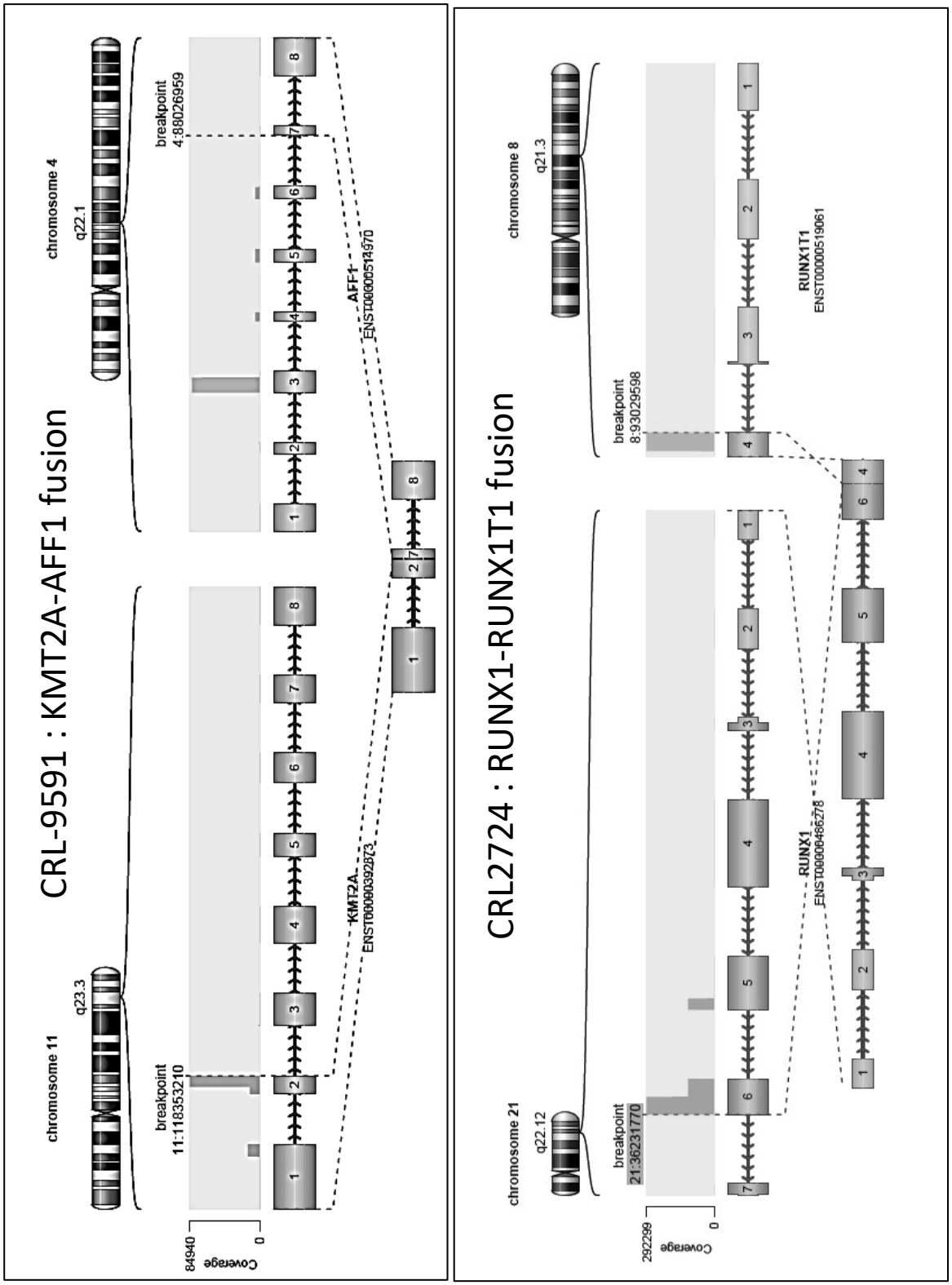


Fig. 7 (Continued)

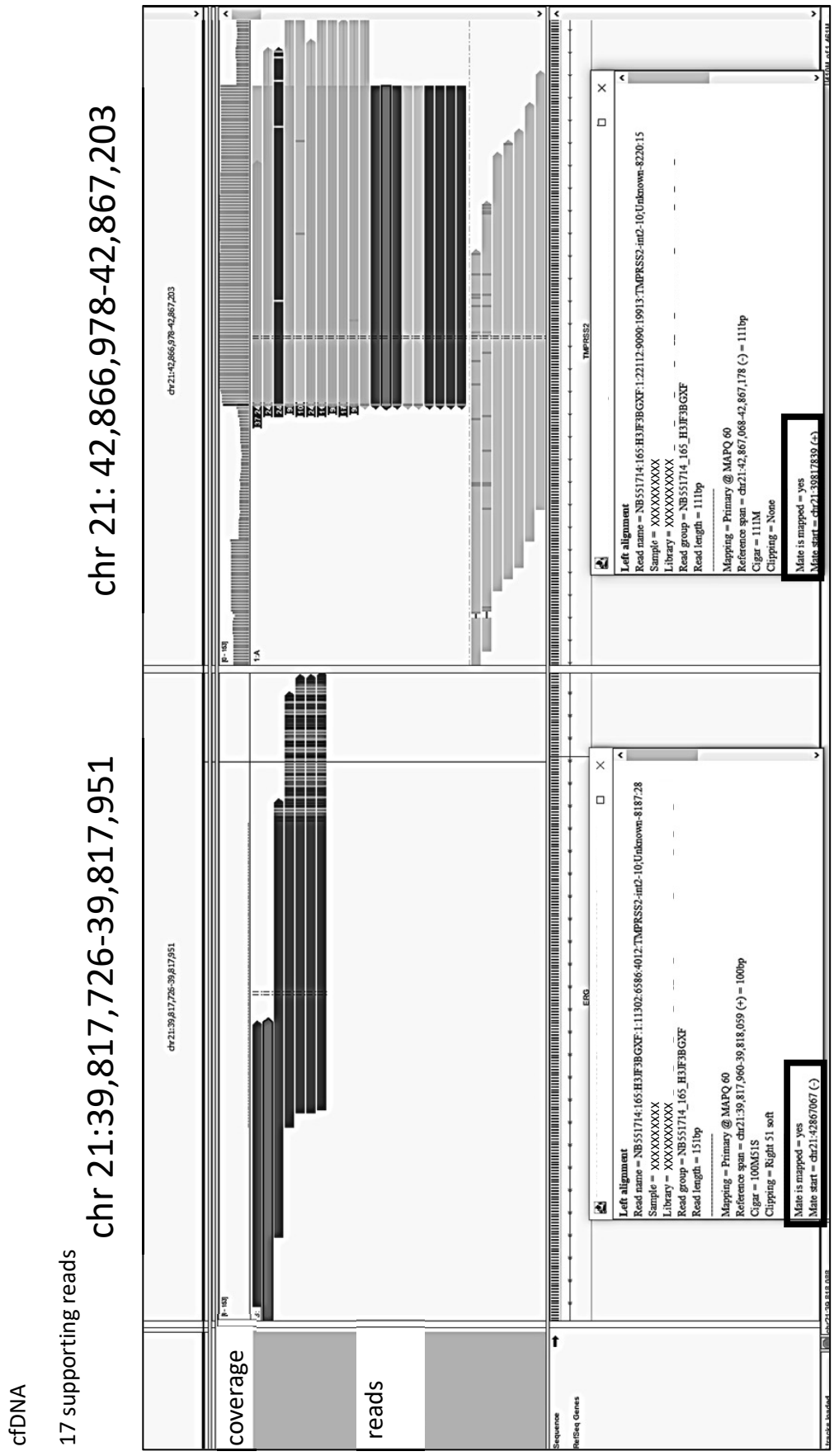

cfDNA
17 supporting reads
chr 21:39,817,726-39,817,951
chr 21: 42,866,978-42,867,203
coverage
reads
ERG
TMPRSS2

Fig. 8A

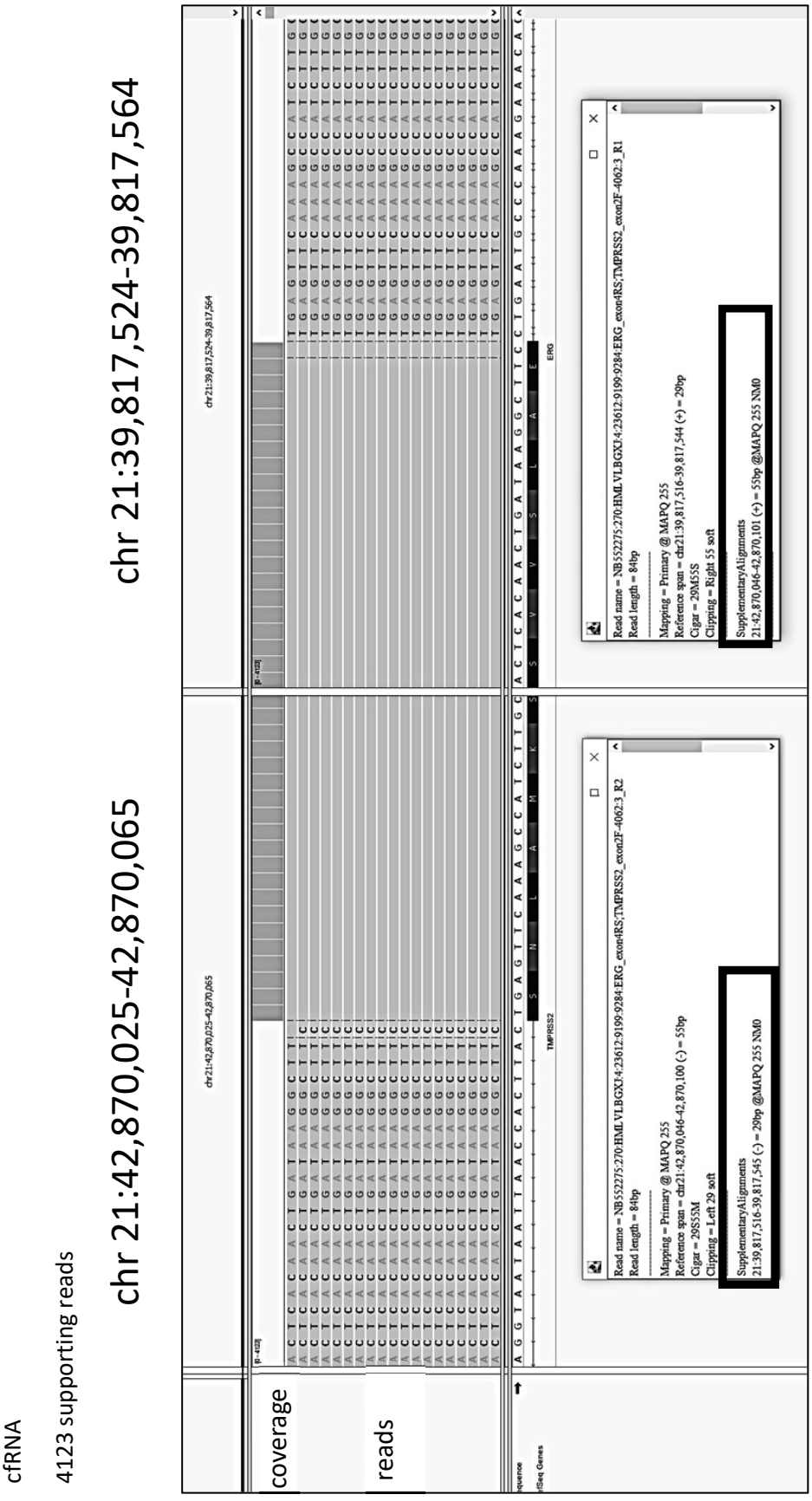

cfRNA
4123 supporting reads
chr 21:42,870,025-42,870,065
chr 21:39,817,524-39,817,564
coverage
reads
TMPRSS2
ERG

Fig. 8B

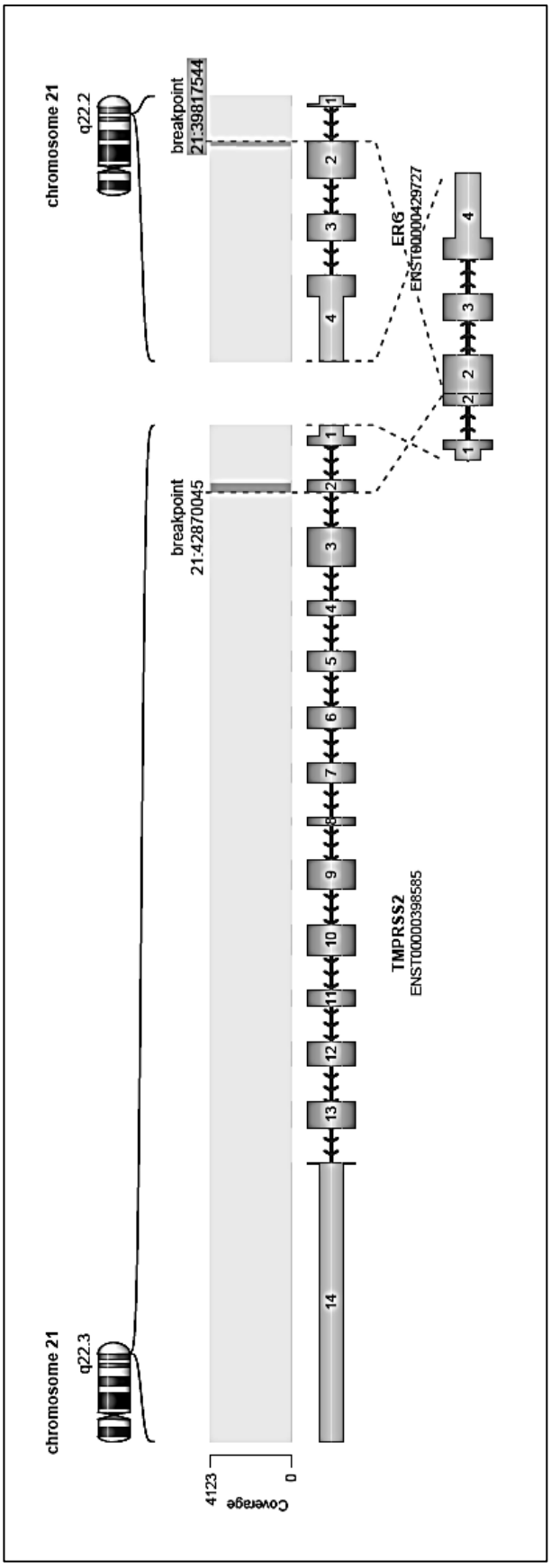

chromosome 21
q22.2
breakpoint
21:39817544
ERG
ENST00000429727
breakpoint
21:42870045
TMPRSS2
ENST00000398585
chromosome 21
q22.3
Coverage
4123
0

Fig. 8C

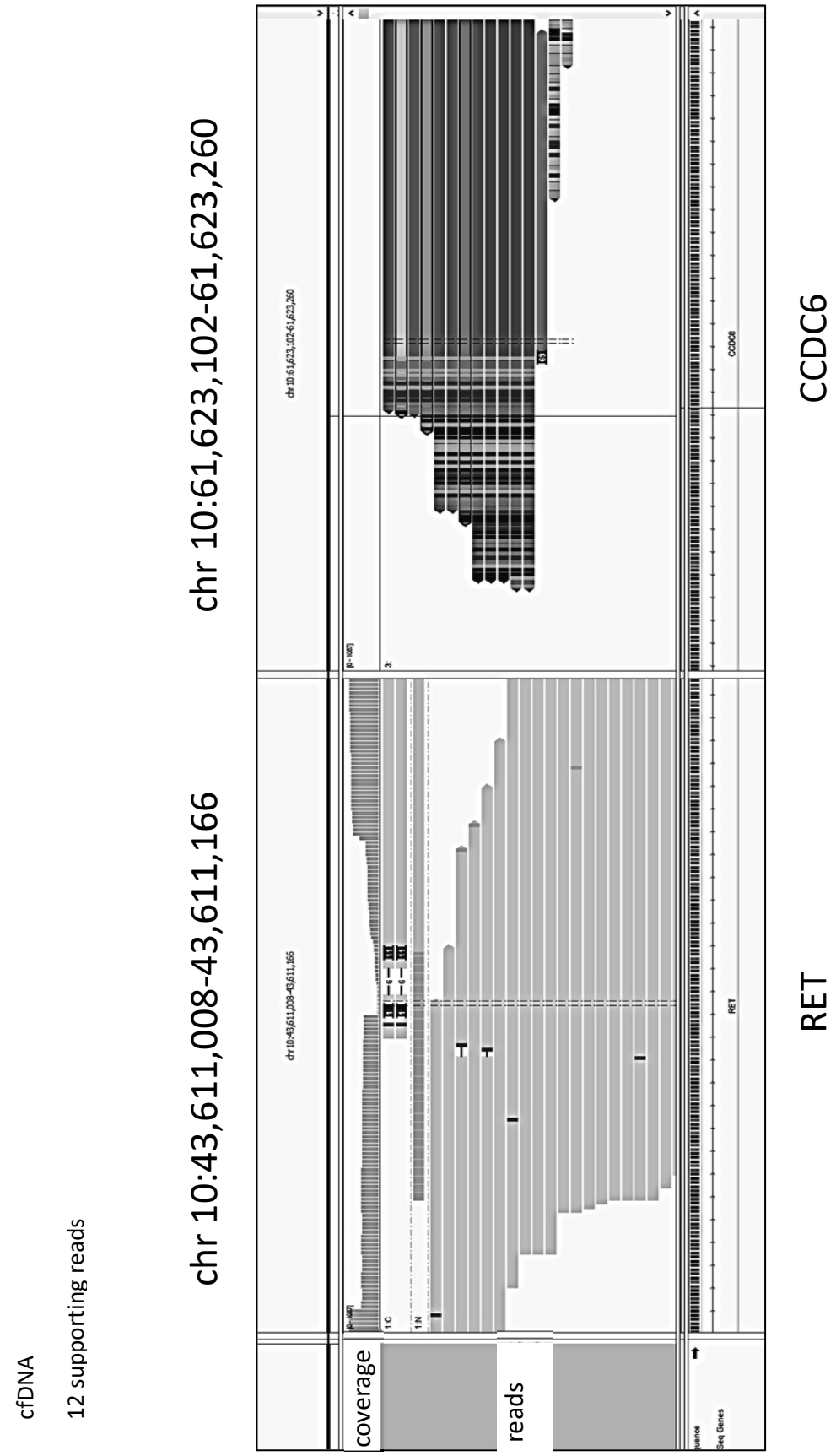

cfDNA
12 supporting reads
chr 10:43,611,008-43,611,166
chr 10:61,623,102-61,623,260
coverage
reads
RET
CCDC6

Fig. 9A

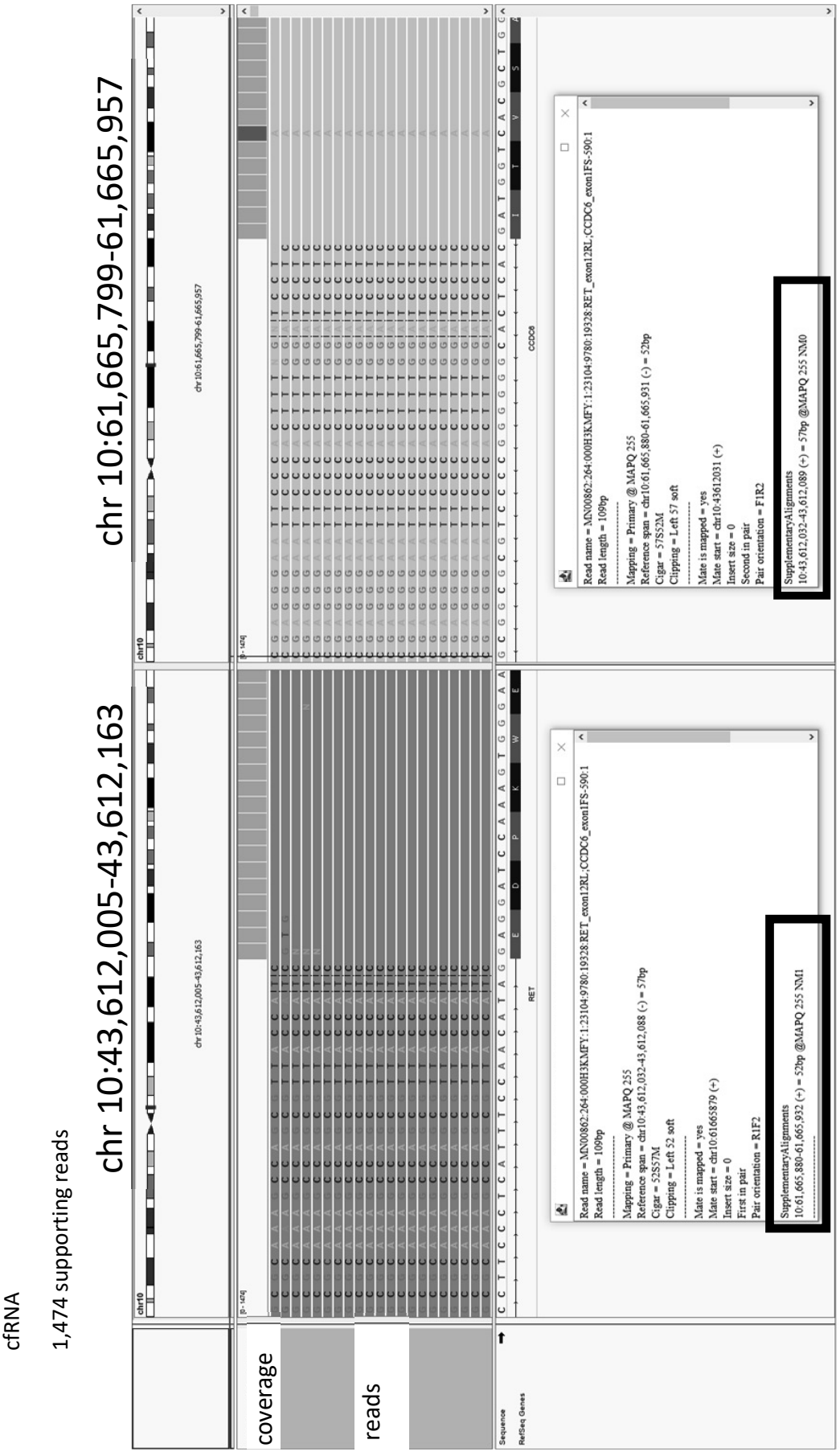

cfRNA
1,474 supporting reads
chr 10:43,612,005-43,612,163
chr 10:61,665,799-61,665,957
coverage
reads
RET
CCDC6

Fig. 9B

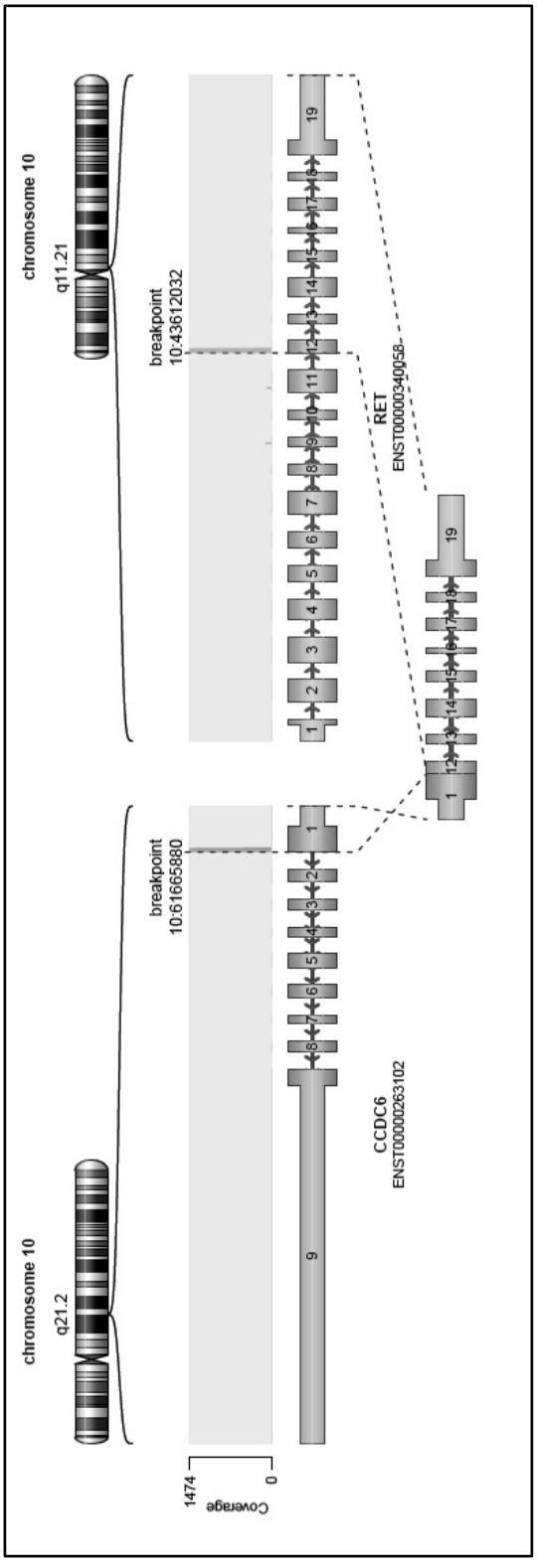

chromosome 10
q11.21
breakpoint
10:43612032
RET
ENST00000340058
chromosome 10
q21.2
breakpoint
10:61665880
CCDC6
ENST00000263102
Coverage
1474
0

Fig. 9C

Cellular RNA

159,106 supporting reads

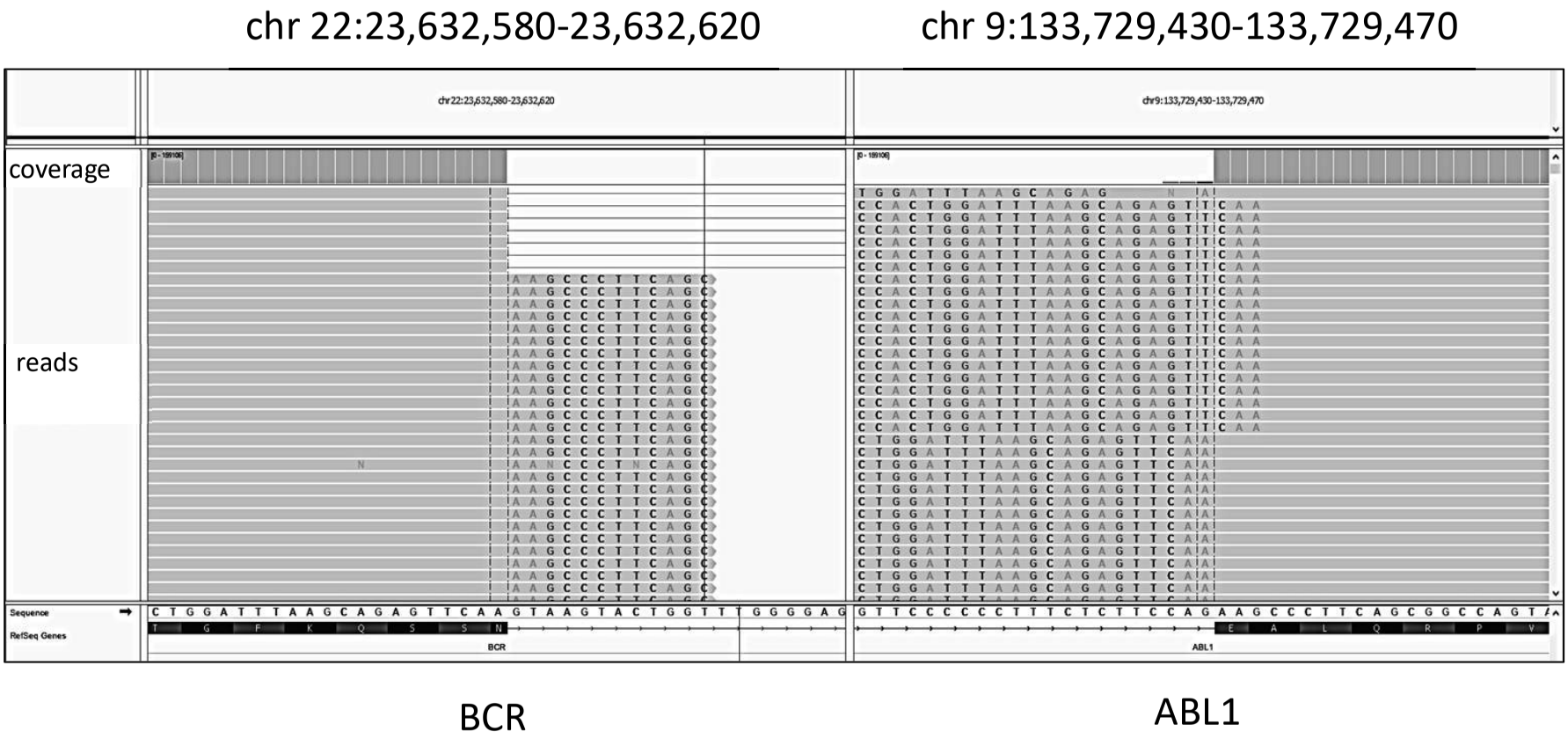


Fig. 10A

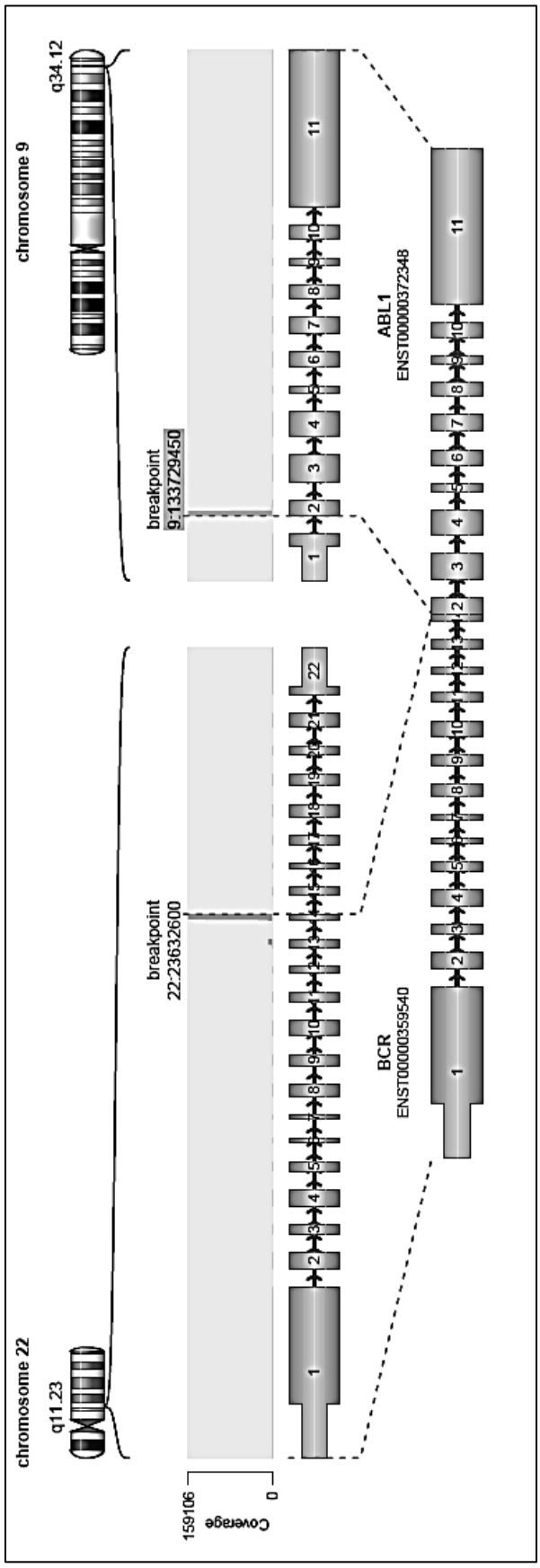

chromosome 9
q34.12
chromosome 22
q11.23
breakpoint
9:133729450
breakpoint
22:23632600
ABL1
ENST00000372348
BCR
ENST00000359540
Coverage
159106
0

Fig. 10B

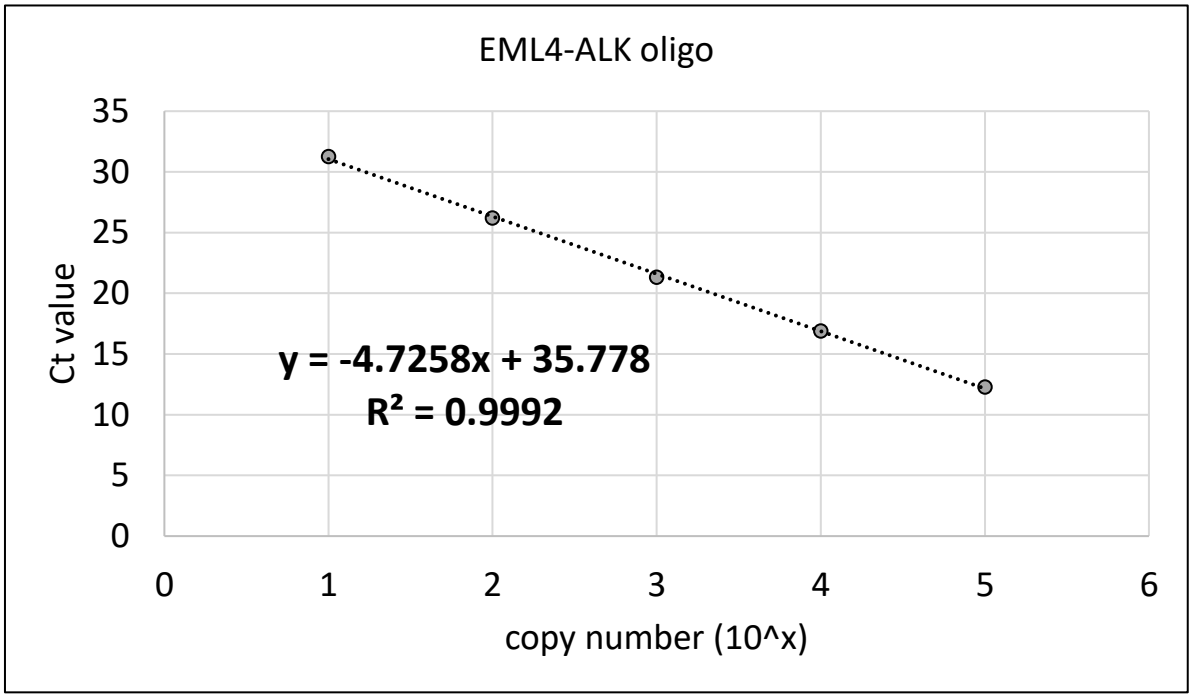

EML4-ALK oligo
Ct value
copy number (10^x)
y = -4.7258x + 35.778
R² = 0.9992
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6

Fig. 11

Sample	Type	RNA (ng)
C_20-146	Metastatic Lung Cancer	35
C_20-347	Metastatic Prostate Cancer	24
C_20-126.1	Metastatic Lung Cancer	33
C_20-126.2		83
C_20-1069.1	Metastatic Lung Cancer	10.9
C_20-1069.2		27
C_21-7	Metastatic Breast Cancer	25
C_21-93	Metastatic Lung Cancer	30.2
H_A_1	Healthy	4.1
H_A_2	Healthy	3.5
H_B	Healthy	2.43
H_C	Healthy	3.7

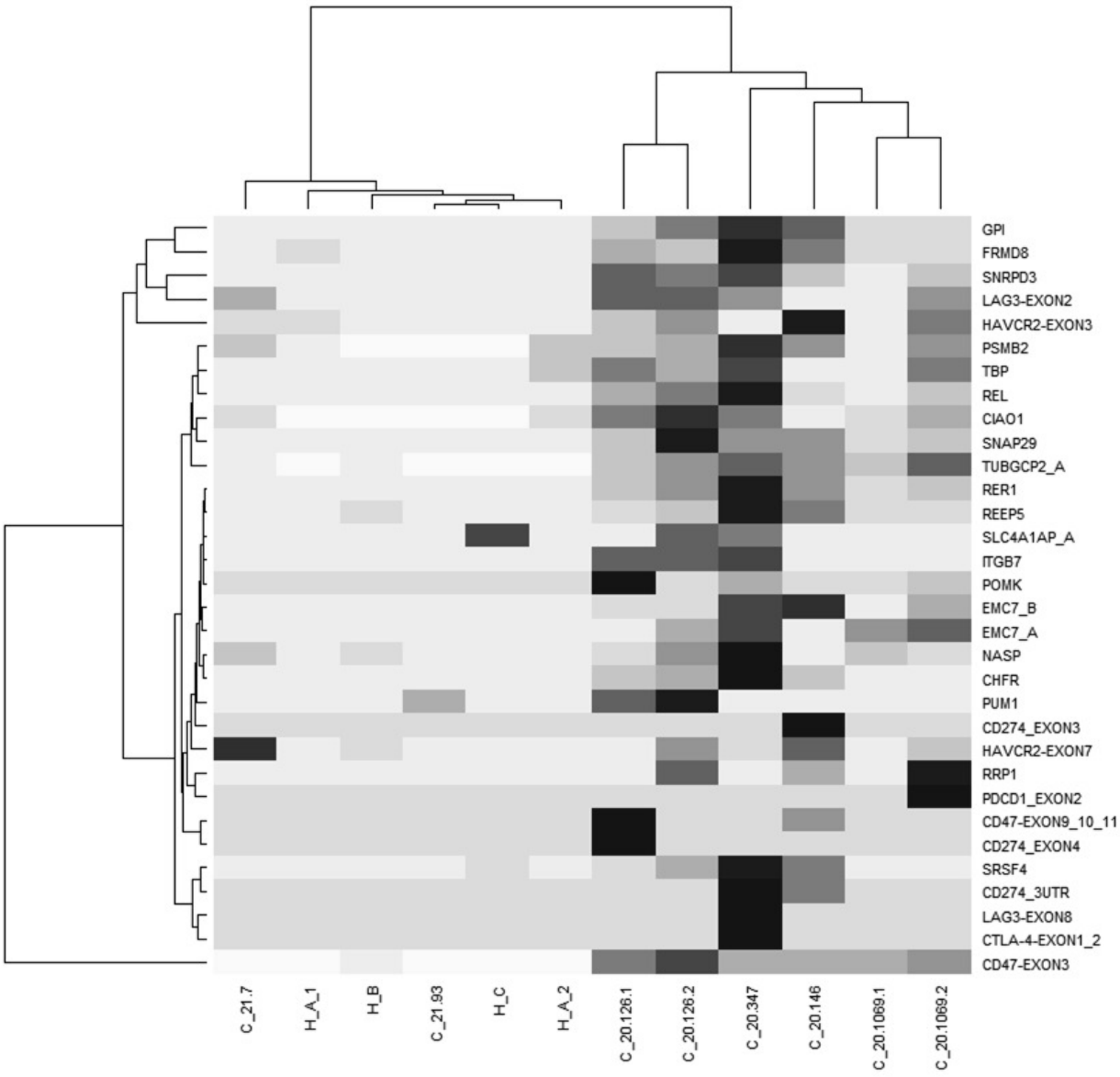


Fig. 12

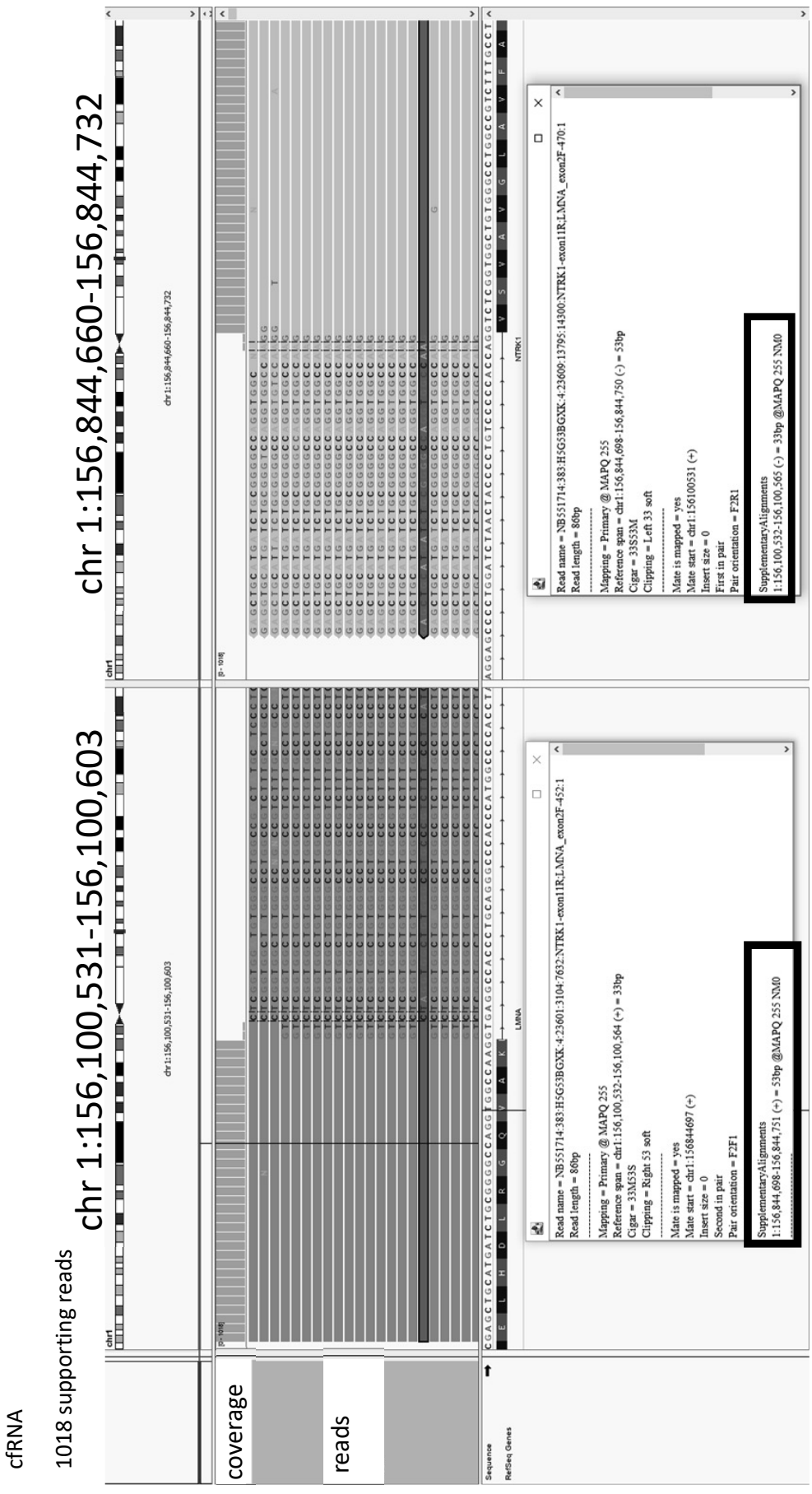

cfRNA
1018 supporting reads
chr 1:156,100,531-156,100,603
chr 1:156,844,660-156,844,732
coverage
reads
LMNA
NTRK1

Fig. 13A

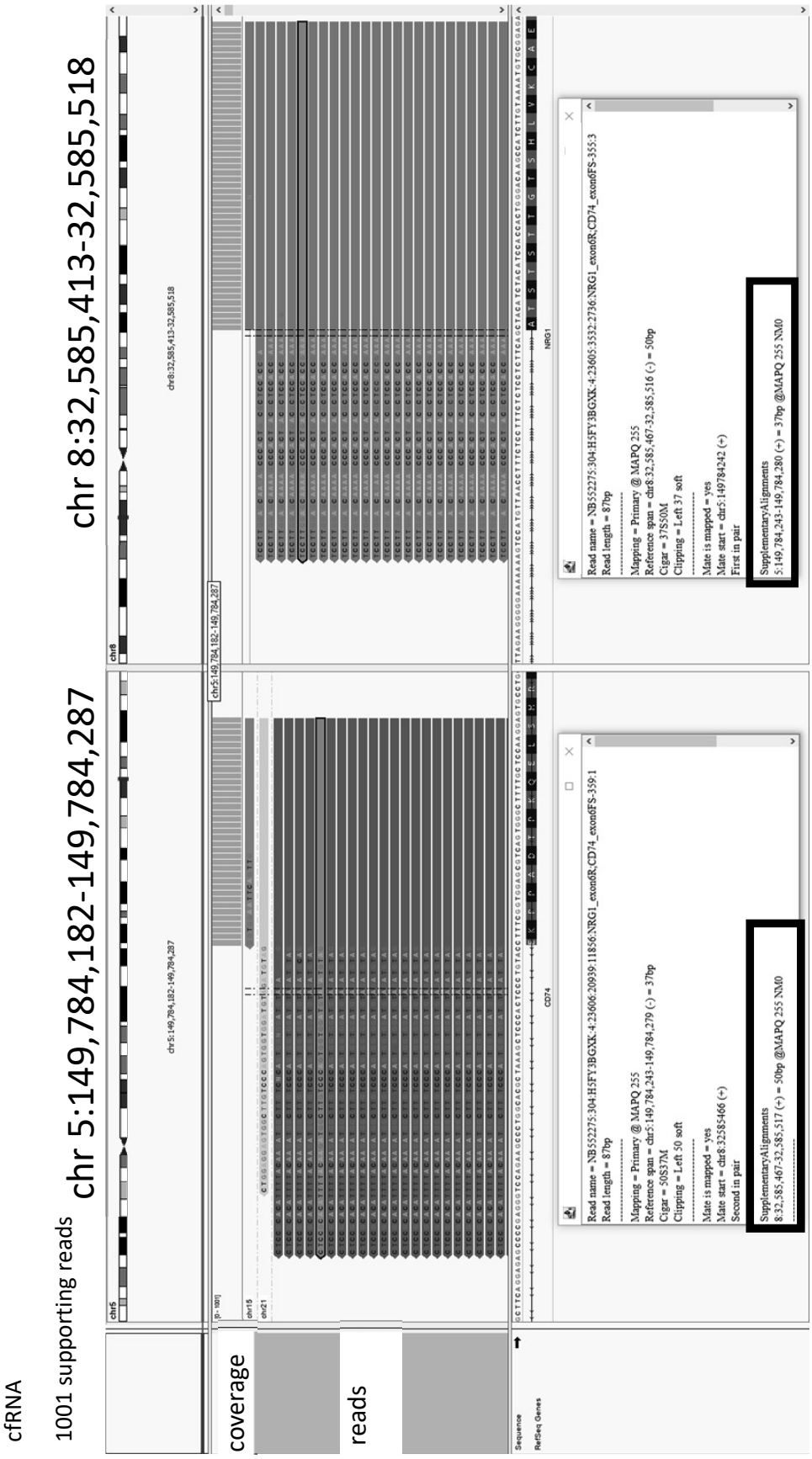

cfRNA
1001 supporting reads
chr 5:149,784,182-149,784,287
chr 8:32,585,413-32,585,518
coverage
reads
CD74
NRG1

Fig. 13B

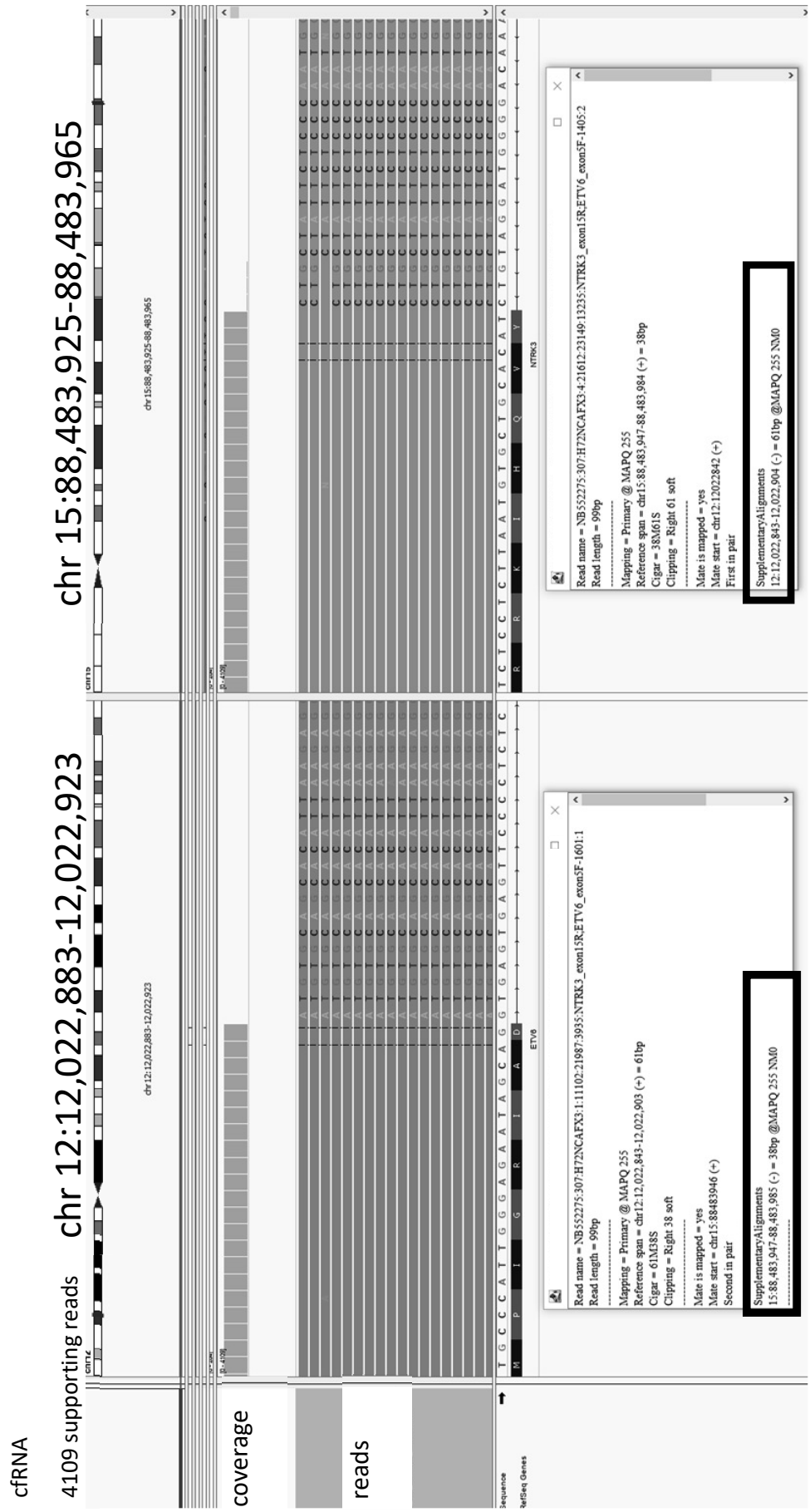

cfRNA
4109 supporting reads
chr 12:12,022,883-12,022,923
chr 15:88,483,925-88,483,965
coverage
reads
ETV6
NTRK3

Fig. 13C

(A)

	cfDNA fusion+	cfDNA fusion-
cfRNA fusion+	12	5
cfRNA fusion-	1	27

(B)

cfDNA and cfRNA fusion+	Only cfRNA fusion+	Only cfDNA fusion-
EML4-ALK (n = 4)	LMNA-NTRK1 (n = 2)	KIF5B-RET (n = 1)
MET exon 14 skipping (n = 3)	CD74-NRG1 (n = 1)	
CD74-ROS1 (n = 2)	ETV6-MET (n = 1)	
NCOA4-RET (n =1)	ETV6-NTRK3 (n = 1)	
CCDC6-RET (n=1)		
FGFR3-TACC3 (n=1)		
Co-occurring fusions detected in cfRNA		
EML4-ALK* and EWSR1-FLI1		
EML4-ALK*, SQSTM1-MET and CCDC6-RET		
CD74-ROS1* and SND1-BRAF		

Fig. 14

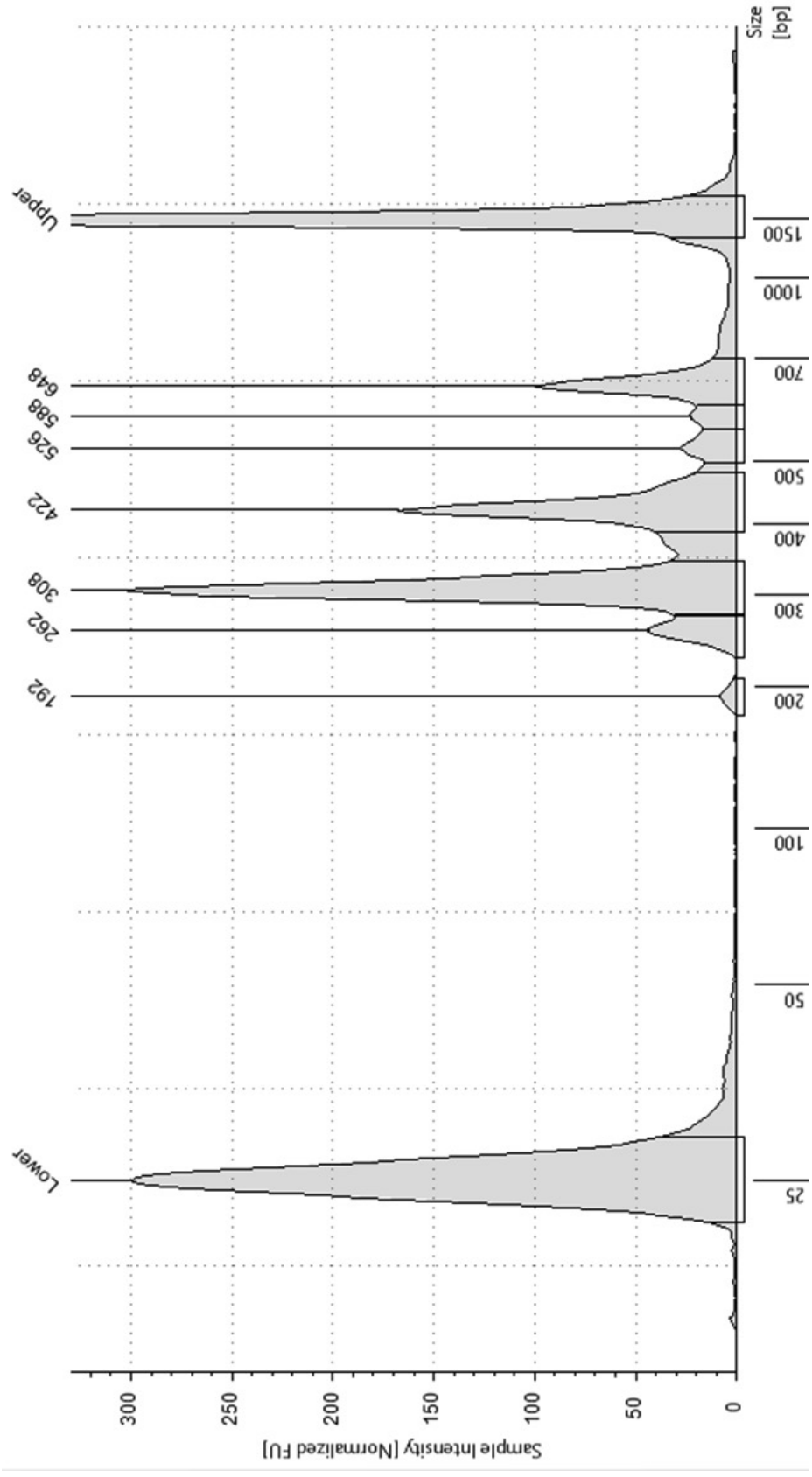

Sample Intensity [Normalized FU]
300
250
200
150
100
50
0
Size [bp]
25
50
100
200
300
400
500
700
1000
1500
Lower
Upper
192
262
308
422
526
588
648

Fig. 15