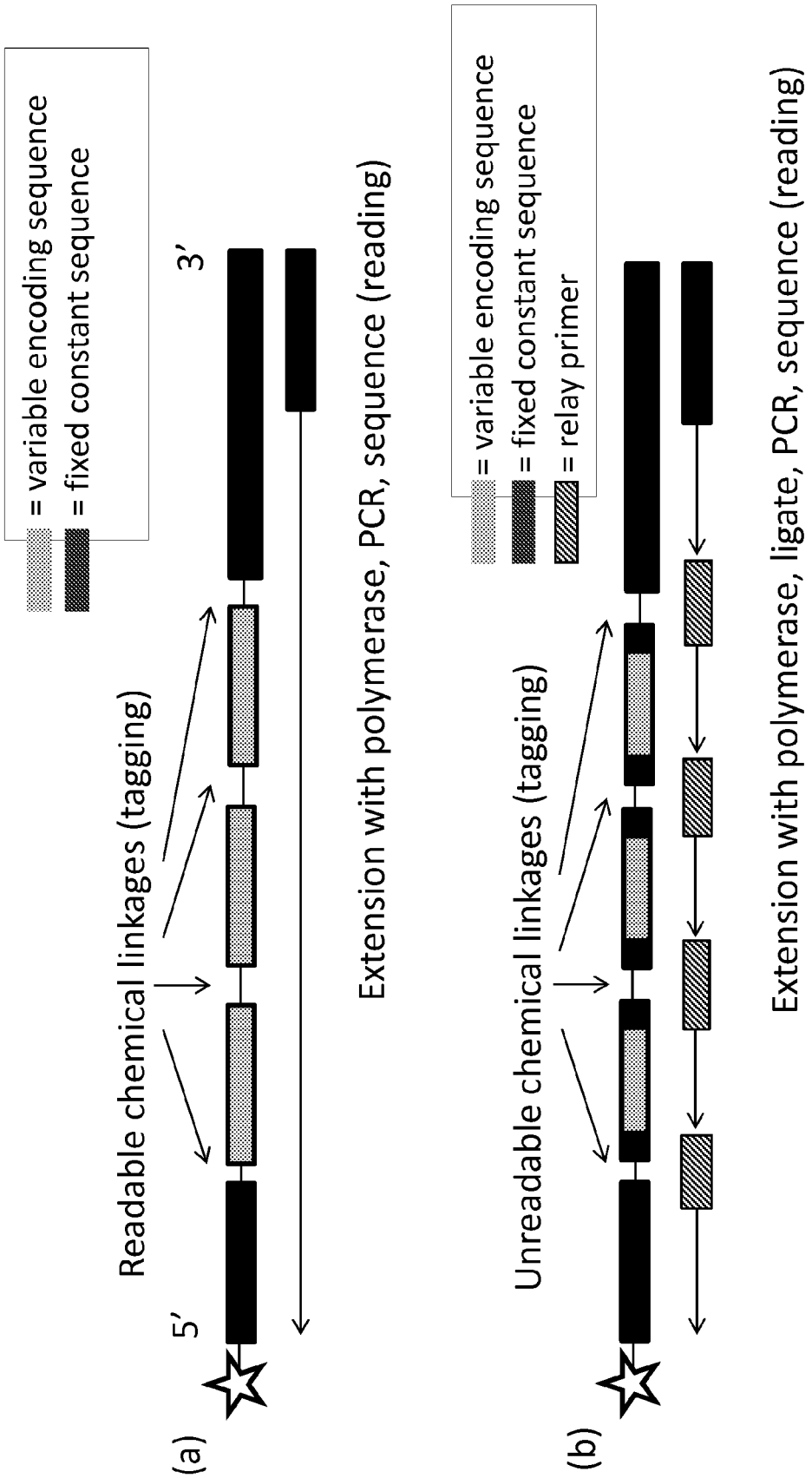

= variable encoding sequence
= fixed constant sequence
3'
5'
Readable chemical linkages (tagging)
Extension with polymerase, PCR, sequence (reading)
(a)
= variable encoding sequence
= fixed constant sequence
= relay primer
Unreadable chemical linkages (tagging)
Extension with polymerase, ligate, PCR, sequence (reading)
(b)

Figure 1

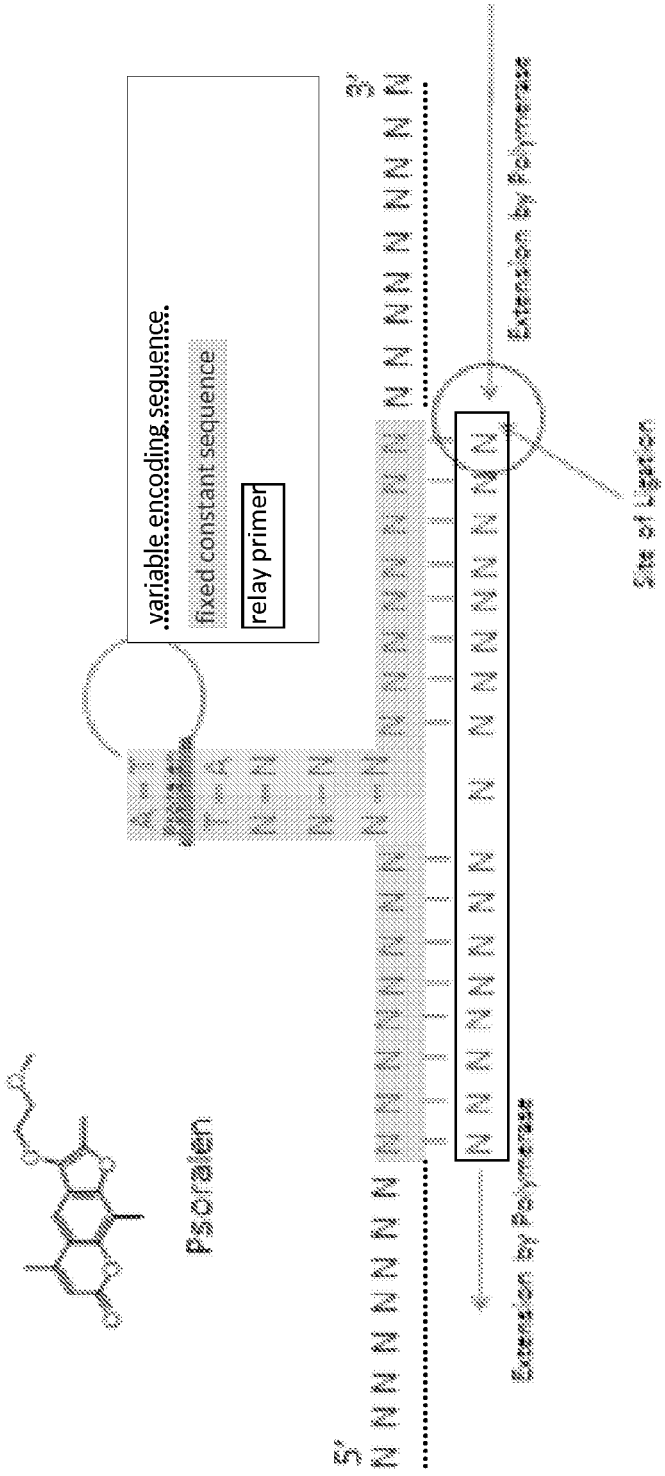

variable encoding sequence
fixed constant sequence
relay primer
Psoralen
5'
3'
Extension by Polymerase
Site of Ligation
Extension by Polymerase

Figure 2

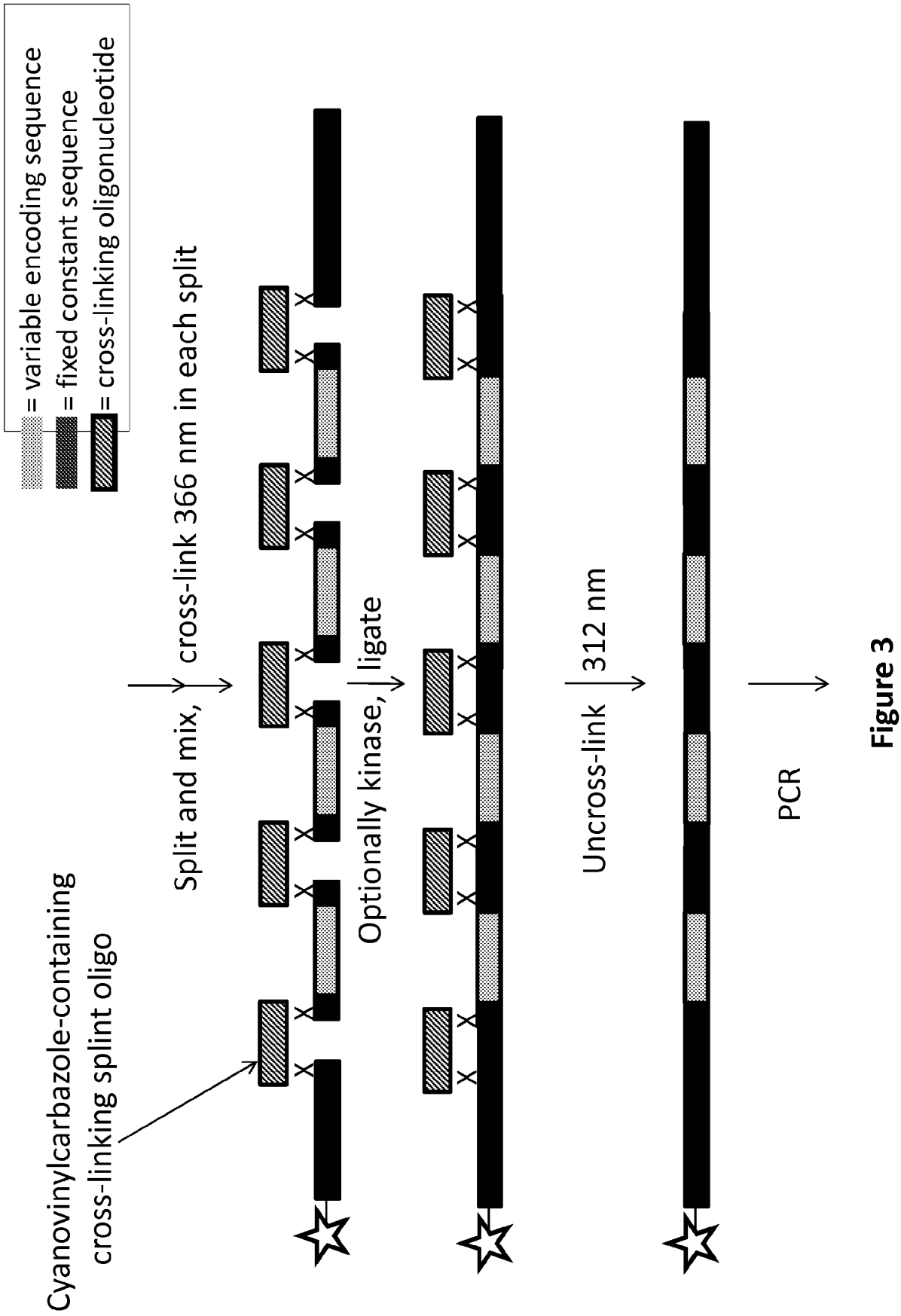

= variable encoding sequence
= fixed constant sequence
= cross-linking oligonucleotide
Cyanovinylcarbazole-containing cross-linking splint oligo
Split and mix, cross-link 366 nm in each split
Optionally kinase, ligate
Uncross-link 312 nm
PCR

Figure 3


366 nm
316 nm
X = Cyanovinylcarbazole
Cyanovinylcarbazole cross-linked to Thymidine
5' N N N N N N N N
N N N N N N N N



Oligonucleotide CNVK2_P_TagB (MW 6,579)
5'-Monophospho-ACGTCGGTAAAAAAAAAAAA

Oligonucleotide CNVK_2_NP_TagB (MW 6,184)
ACGTCGGTAAAAAAAAAA

Oligonucleotide CNVK2_TagA (MW 5,558)
AAAAAAAAAAGTCGTGAC

Figure 5

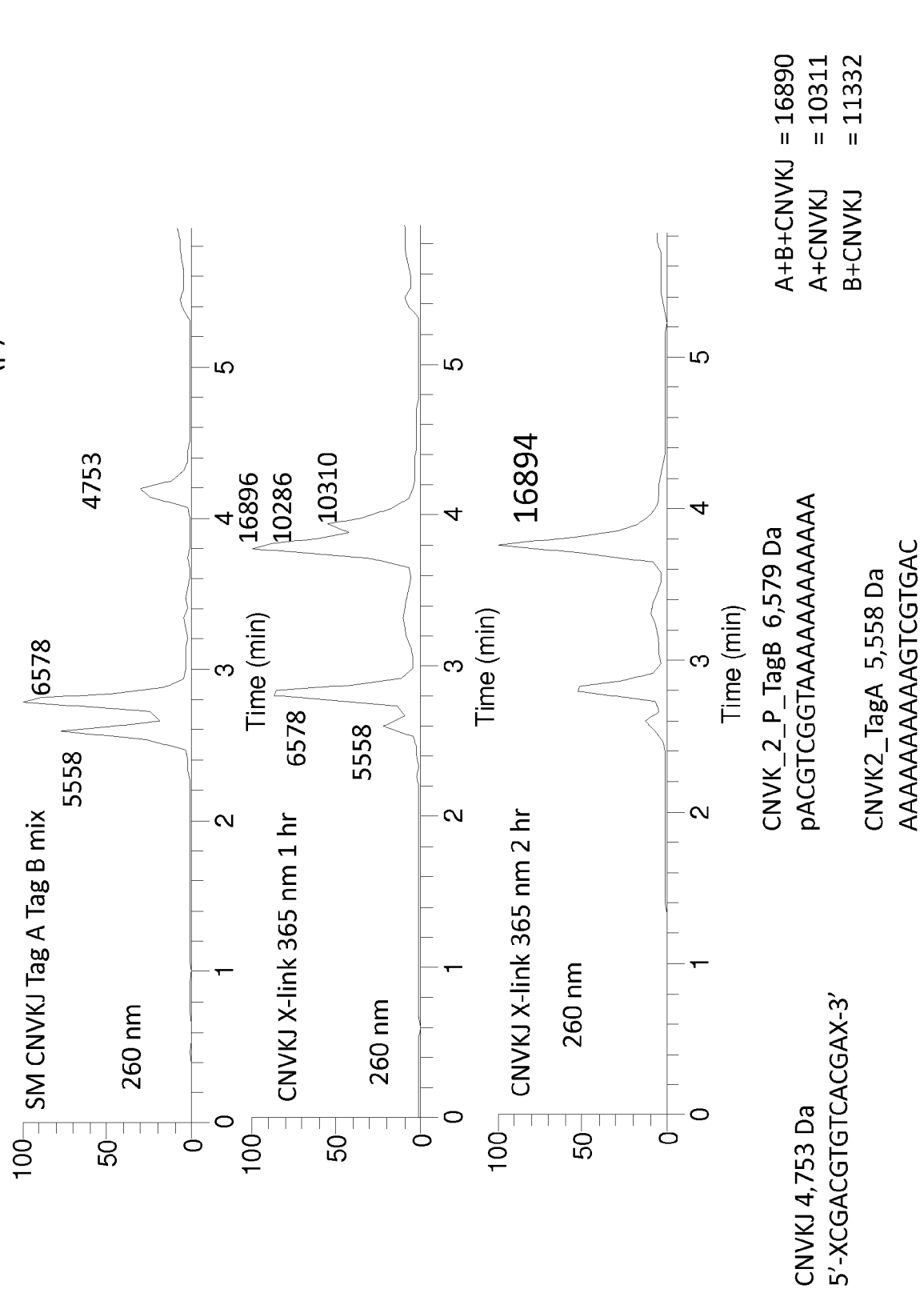
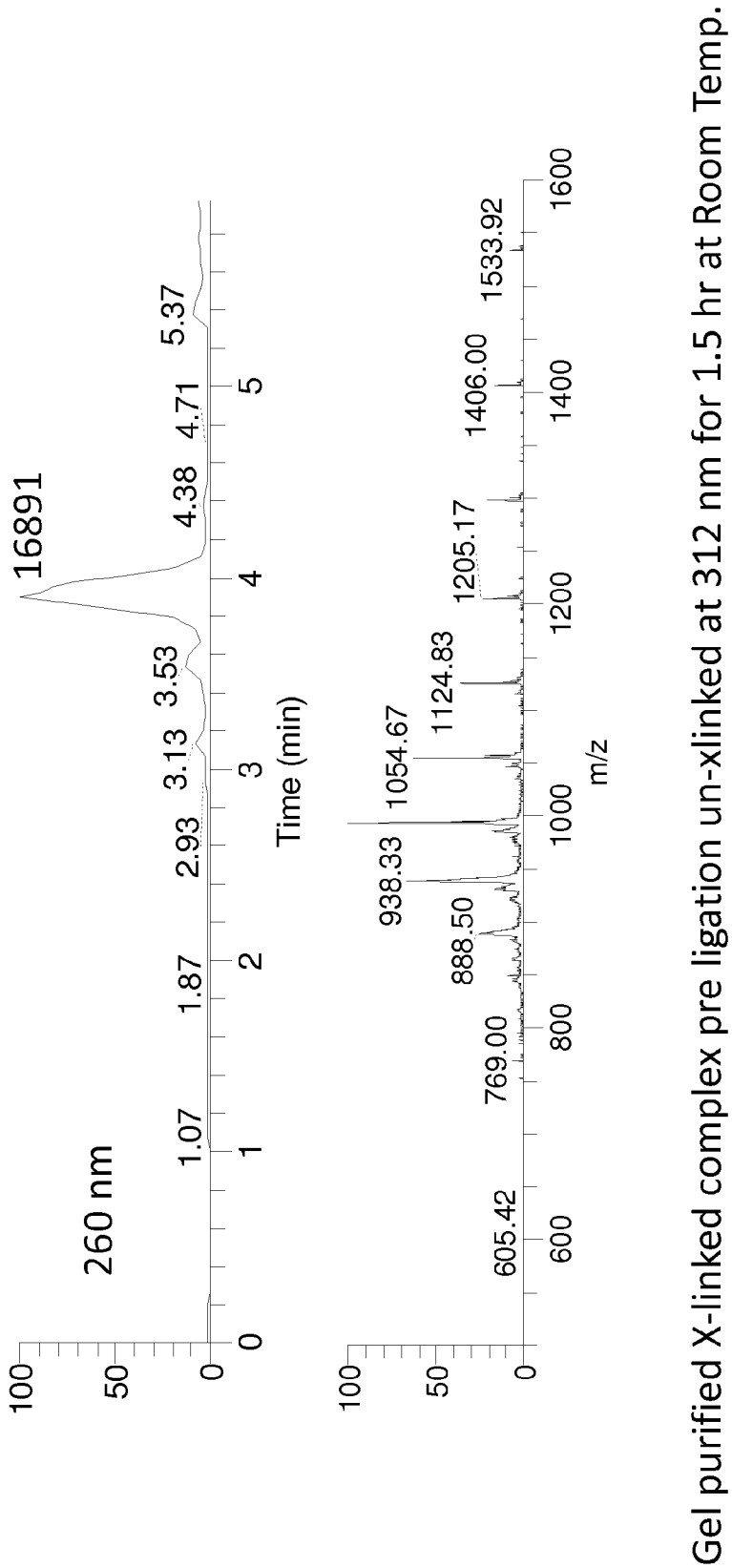

5'-XCGACGT---- GTCACGAX-3'
3'-AAAAAAAAAAATGGCTGCA(p) CAGTGCTGAAAAAAAA-5'
SM CNVKJ Tag A Tag B mix
260 nm
6578
5558
4753
CNVKJ X-link 365 nm 1 hr
260 nm
16896
10286
10310
6578
5558
CNVKJ X-link 365 nm 2 hr
260 nm
16894
Time (min)
CNVKJ 4,753 Da
5'-XCGACGTGTCACGAX-3'
CNVK_2_P_TagB 6,579 Da
pACGTCGGTAAAAAAAAAAAA
CNVK2_TagA 5,558 Da
AAAAAAAAAAGTCGTGAC
A+B+CNVKJ = 16890
A+CNVKJ = 10311
B+CNVKJ = 11332

Figure 6


Gel-purified X-linked complex pre-ligation
260 nm
16891
1.07
1.87
2.93
3.13
3.53
4.38
4.71
5.37
Time (min)
605.42
769.00
888.50
938.33
1054.67
1124.83
1205.17
1406.00
1533.92
m/z

Gel purified X-linked complex pre ligation un-xlinked at 312 nm for 1.5 hr at Room Temp.
260 nm
5558
6578
0.40
0.73
1.13
1.60
2.40
2.60
3.25
3.64
3.91
4.44
5.44
5.91
Time (min)

Figure 7

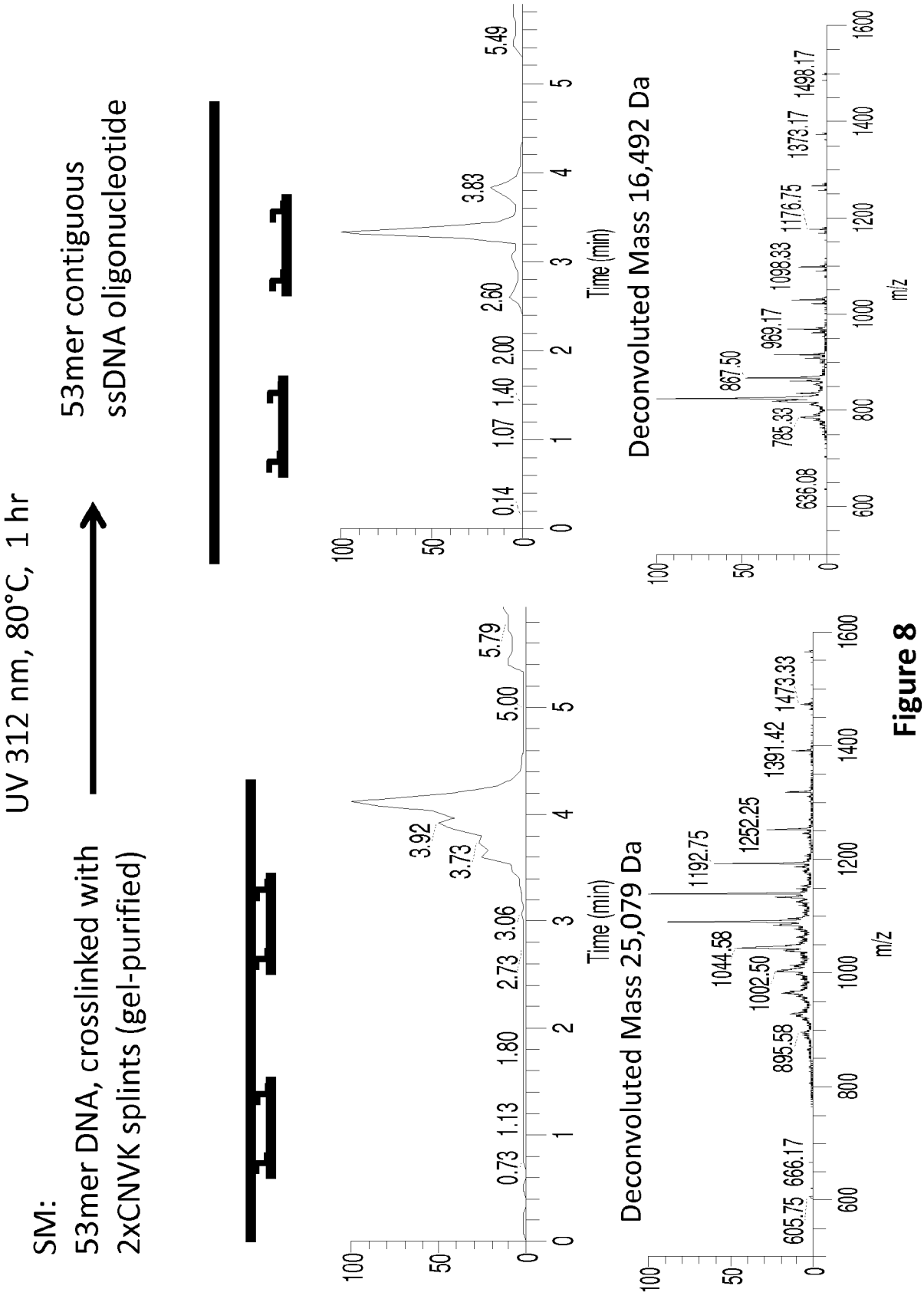

SM:
53mer DNA, crosslinked with
2xCNVK splints (gel-purified)
UV 312 nm, 80°C, 1 hr
53mer contiguous
ssDNA oligonucleotide
Time (min)
Deconvoluted Mass 25,079 Da
Deconvoluted Mass 16,492 Da
m/z

Figure 8

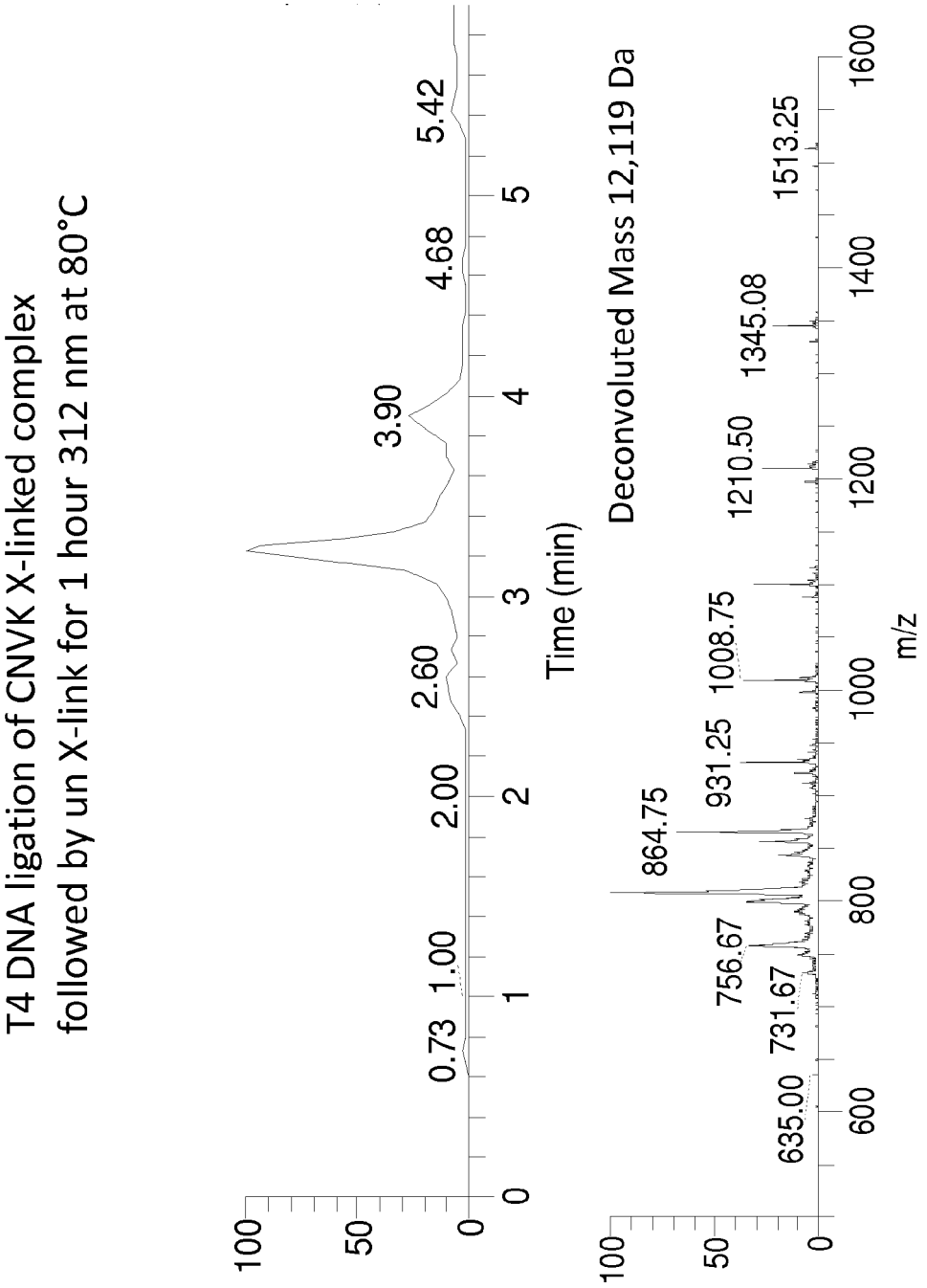

T4 DNA ligation of CNVK X-linked complex
followed by un X-link for 1 hour 312 nm at 80°C
100
50
0
0.73
1.00
2.00
2.60
3.90
4.68
5.42
0
1
2
3
4
5
Time (min)
Deconvoluted Mass 12,119 Da
635.00
731.67
756.67
864.75
931.25
1008.75
1210.50
1345.08
1513.25
600
800
1000
1200
1400
1600
m/z

Figure 9

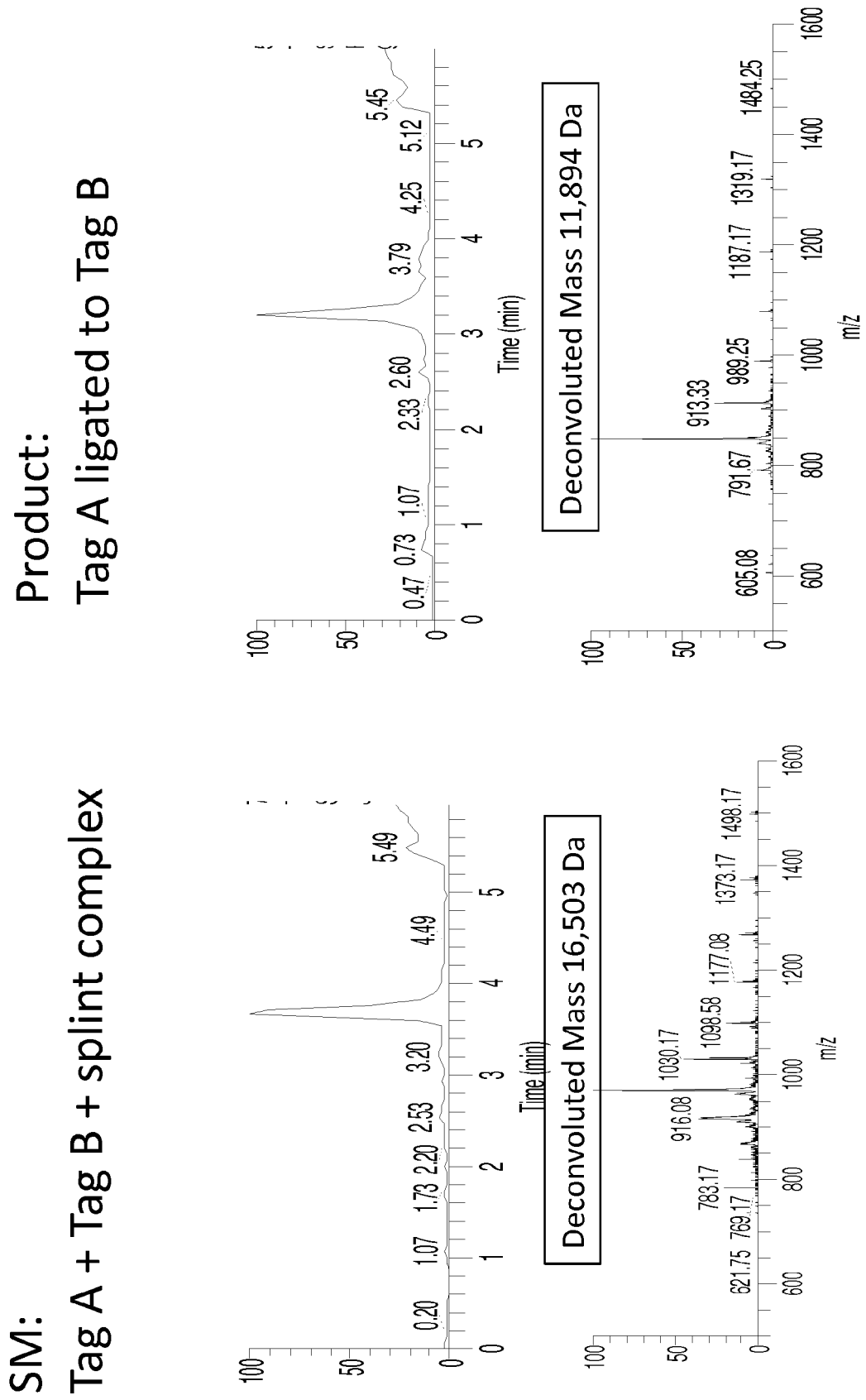

SM:
Tag A + Tag B + splint complex
Product:
Tag A ligated to Tag B
Time (min)
Deconvoluted Mass 16,503 Da
Deconvoluted Mass 11,894 Da
m/z

Figure 10

5PSO2_A9_TA (MW 11,556.3 Da)
5'-Psoralen (C2-linked)-TAGCGGATGCAAAAAAAAAAGGCGAGCT TGCGTACTG-3'

PSO_HP_A9_TCT (MW 6,087.1 Da)
5'-AAAAAA AAAGCATCCGCTCT -3'

Figure 11

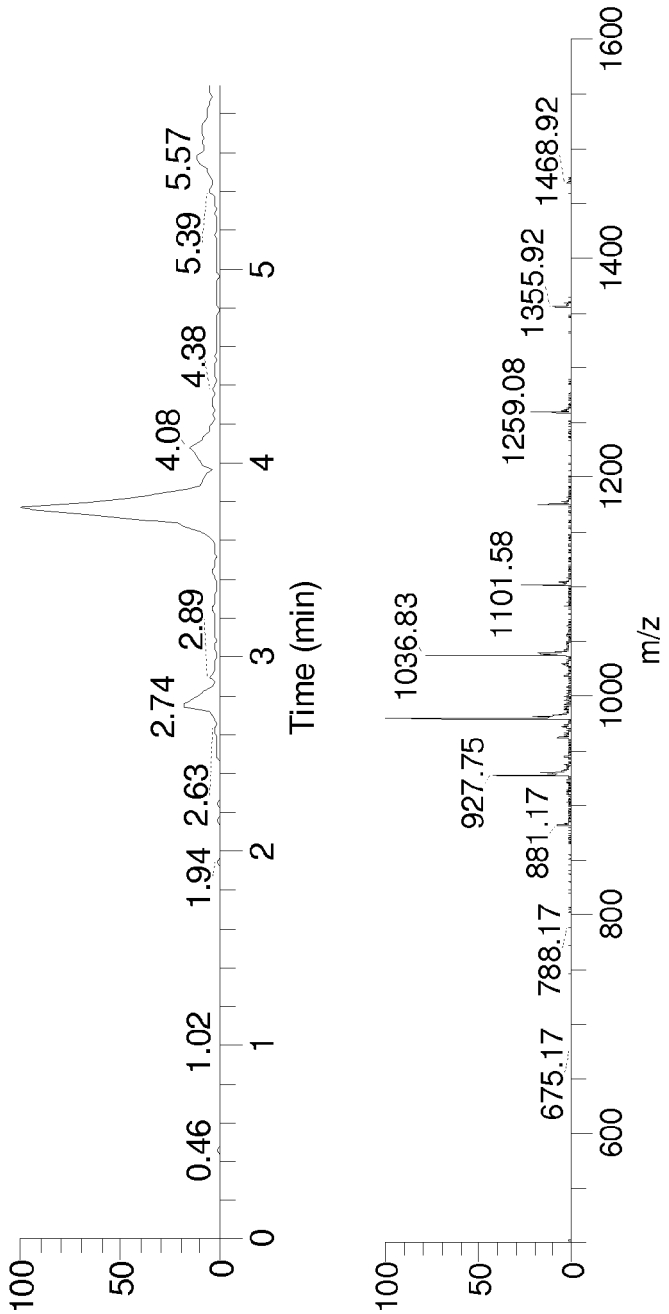

100
50
0
0
0.46
1.02
1.94
2.63
2.74
2.89
4.08
4.38
5.39
5.57
1
2
3
4
5
Time (min)
100
50
0
675.17
788.17
881.17
927.75
1036.83
1101.58
1259.08
1355.92
1468.92
600
800
1000
1200
1400
1600
m/z

Figure 12

Psoralen (C2) Phosphoramidite

Tag1_PsoCVU (MW 3,662.5 Da)
5’-AAAAACTCAGGT-3’

SplintC_PsoC2 (MW 4,529.0 Da)
5’-TCCGCTCGACCTGAG-3’

5PsoC2_A9_GA (MW 11,580.6 Da)
5’-Psoralen (C2)-GAGCGGATGCAAAAAAAAAAGGCGAGCTTGCGTACTG-3’

Figure 13

5′-NNNNNNNNNNNNT PNNNNNNNNNNNNNNNNNN-3′
3′-NNNAGNNNNNNNN-5′

P = 5′-Psoralen

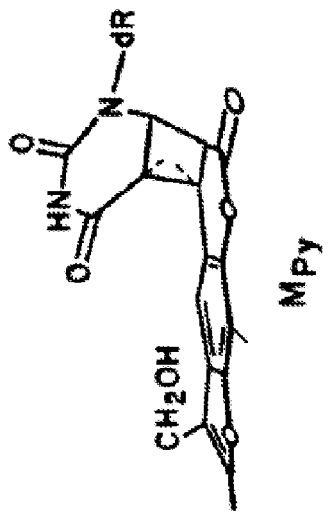


Figure 14

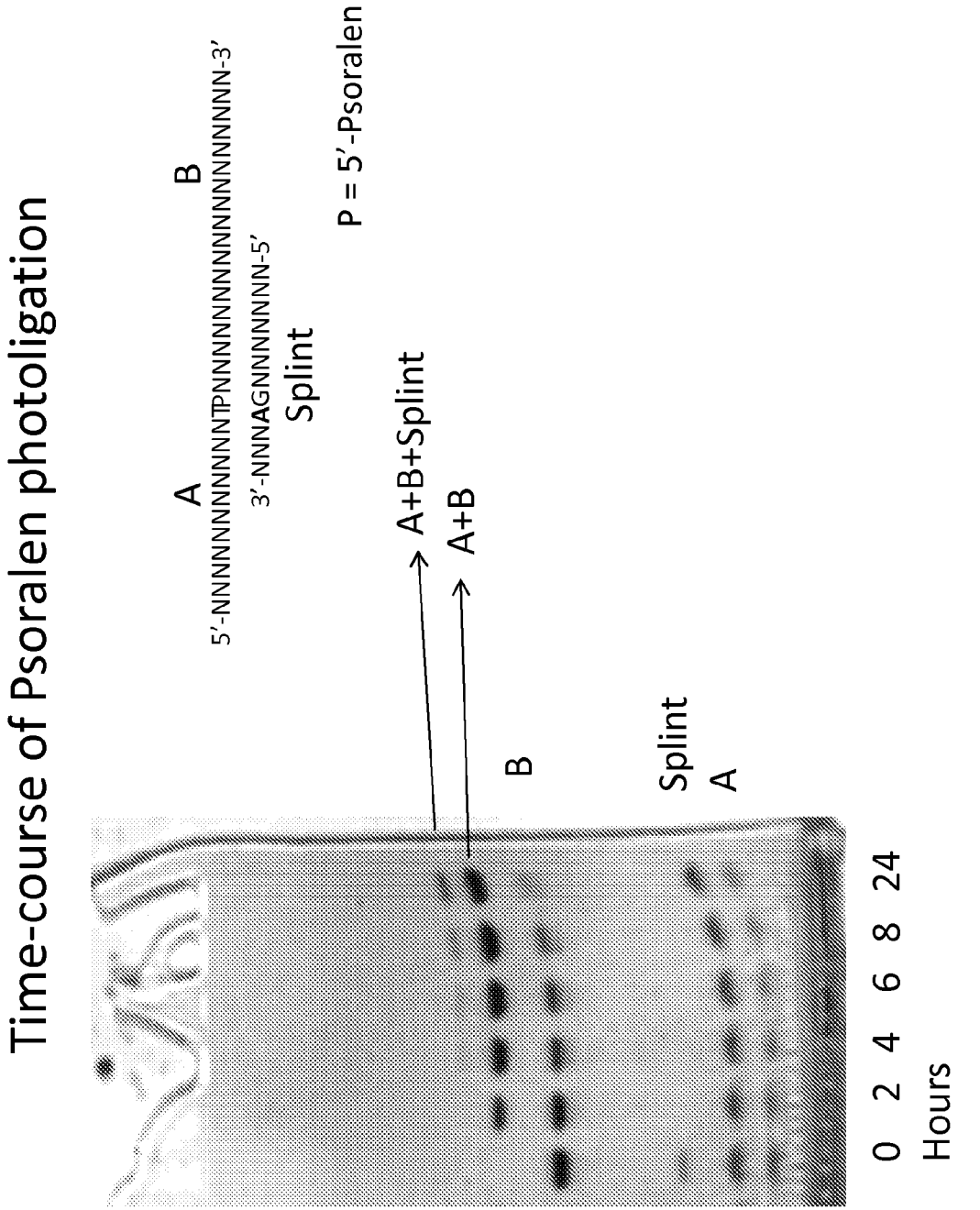

Time-course of Psoralen photoligation
A
5'-NNNNNNNNNNNNTPNNNNNNNNNNNNNNNNNNN-3'
3'-NNNAGNNNNNNNN-5'
Splint
B
P = 5'-Psoralen
A+B+Splint
A+B
B
Splint
A
0 2 4 6 8 24
Hours

Figure 15

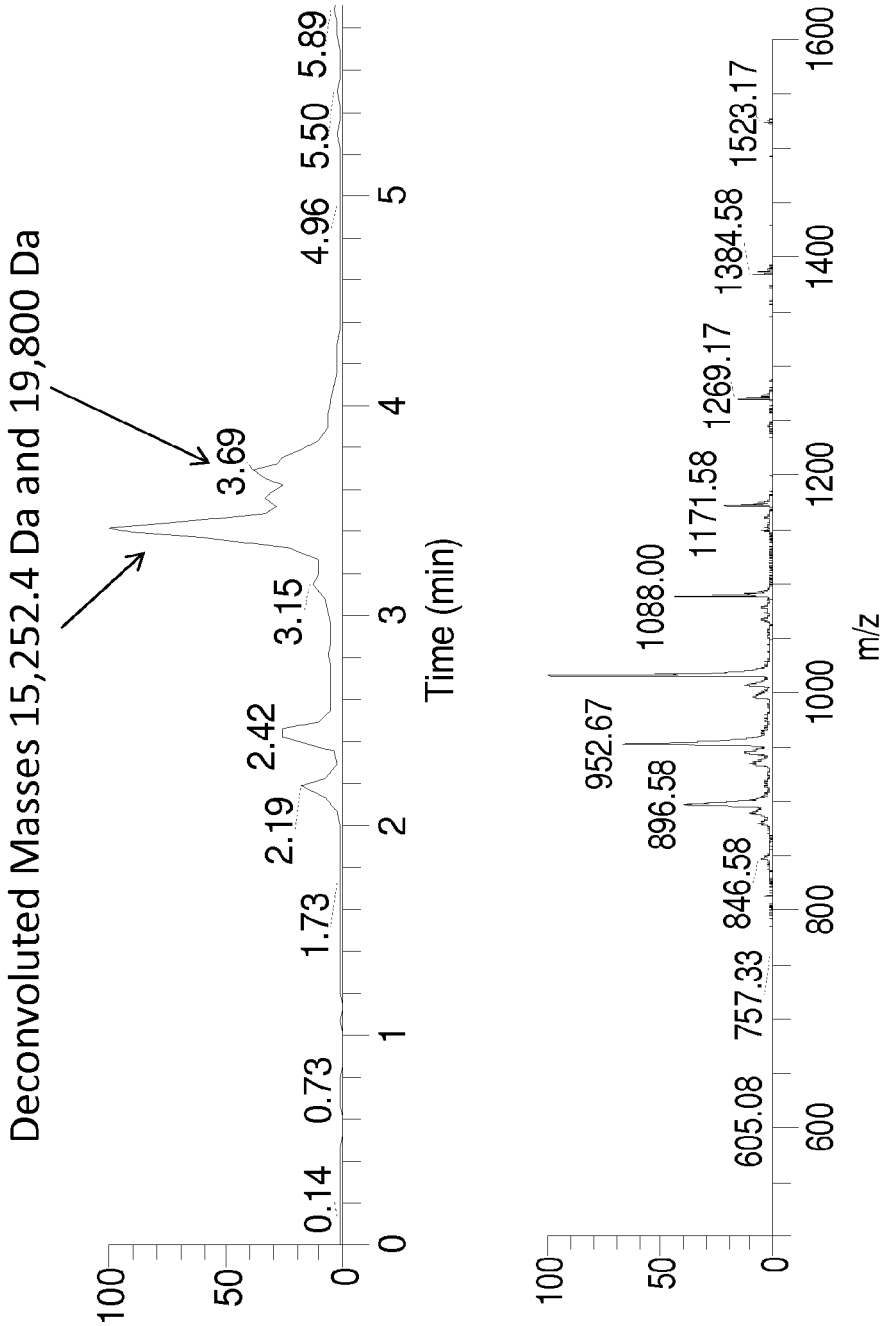

Deconvoluted Masses 15,252.4 Da and 19,800 Da
0.14
0.73
1.73
2.19
2.42
3.15
3.69
4.96
5.50
5.89
Time (min)
100
50
0
0
1
2
3
4
5
605.08
757.33
846.58
896.58
952.67
1088.00
1171.58
1269.17
1384.58
1523.17
m/z
600
800
1000
1200
1400
1600

Figure 16

5Bio_Tag_PsoCVU (MW 4,232.1 Da) /5BioTEG/ is a proprietary
5'-/5BioTEG/AAAAACTCAGGT-3' biotin modification

5PsoC2_A9_GA (MW 11,580.6 Da)
5'-Psoralen (C2)-GAGCGGATGCAAAAAAAAAGGCGAGCTTGCGTACTG-3'

SplintC_PsoC2 (MW 4,529.0 Da) FAMprimer (MW 5,099.5 Da)
5'-TCCGCTCGACCTGAG-3' 5'-/56-FAM/AGTACGCAAGCTCGC-3'

Phos-SplintC_PsoC2
5'-Monophosphate-TCCGCTCGACCTGAG-3'

Psoralen (C2) Phosphoramidite

/56-FAM/ Phosphoramidite

Figure 17

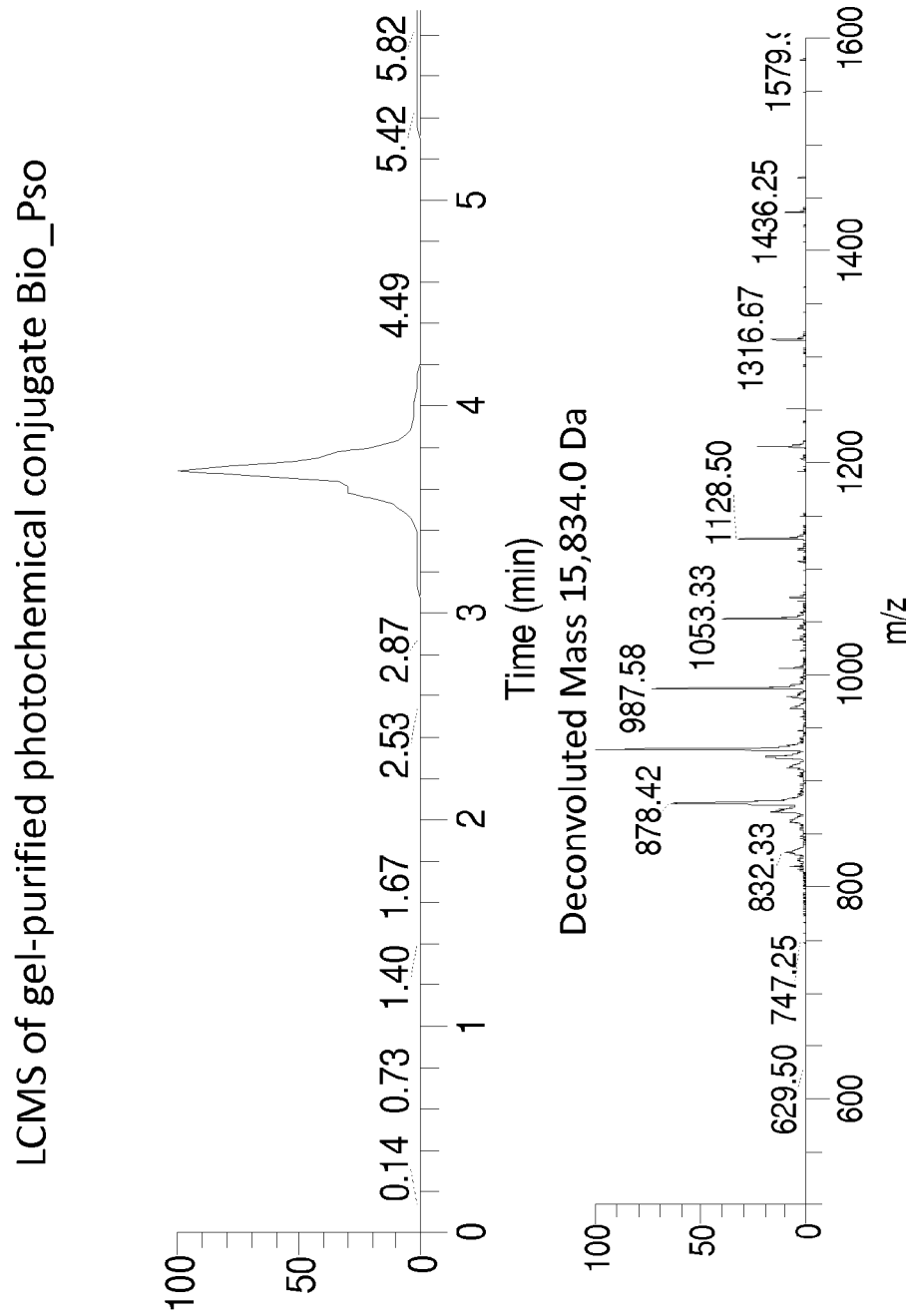

LCMS of gel-purified photochemical conjugate Bio_Pso
100
50
0
0.14 0.73 1.40 1.67 2.53 2.87 4.49 5.42 5.82
0 1 2 3 4 5
Time (min)
Deconvoluted Mass 15,834.0 Da
100
50
0
629.50 747.25 832.33 878.42 987.58 1053.33 1128.50 1316.67 1436.25
600 800 1000 1200 1400 1600
m/z

Figure 18

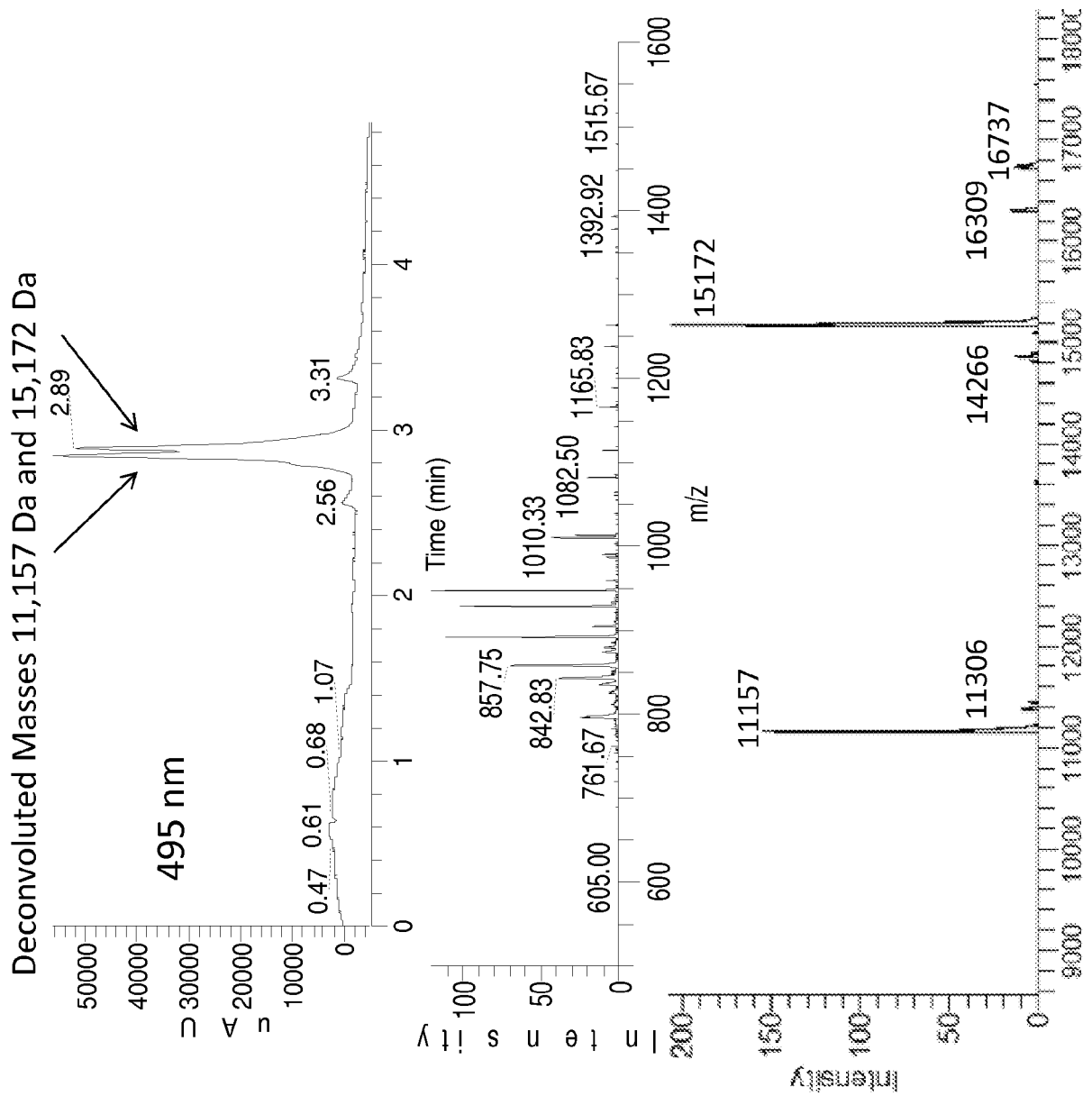

Deconvoluted Masses 11,157 Da and 15,172 Da
495 nm
2.89
3.31
2.56
1.07
0.68
0.61
0.47
Time (min)
857.75
842.83
761.67
605.00
1010.33
1082.50
1165.83
1392.92
1515.67
m/z
11157
11306
14266
15172
16309
16737
Mass, Da

Figure 19

LCMS of no-ligation control of cDNA generated from Bio_Pso using terminal and non-terminal primer extension

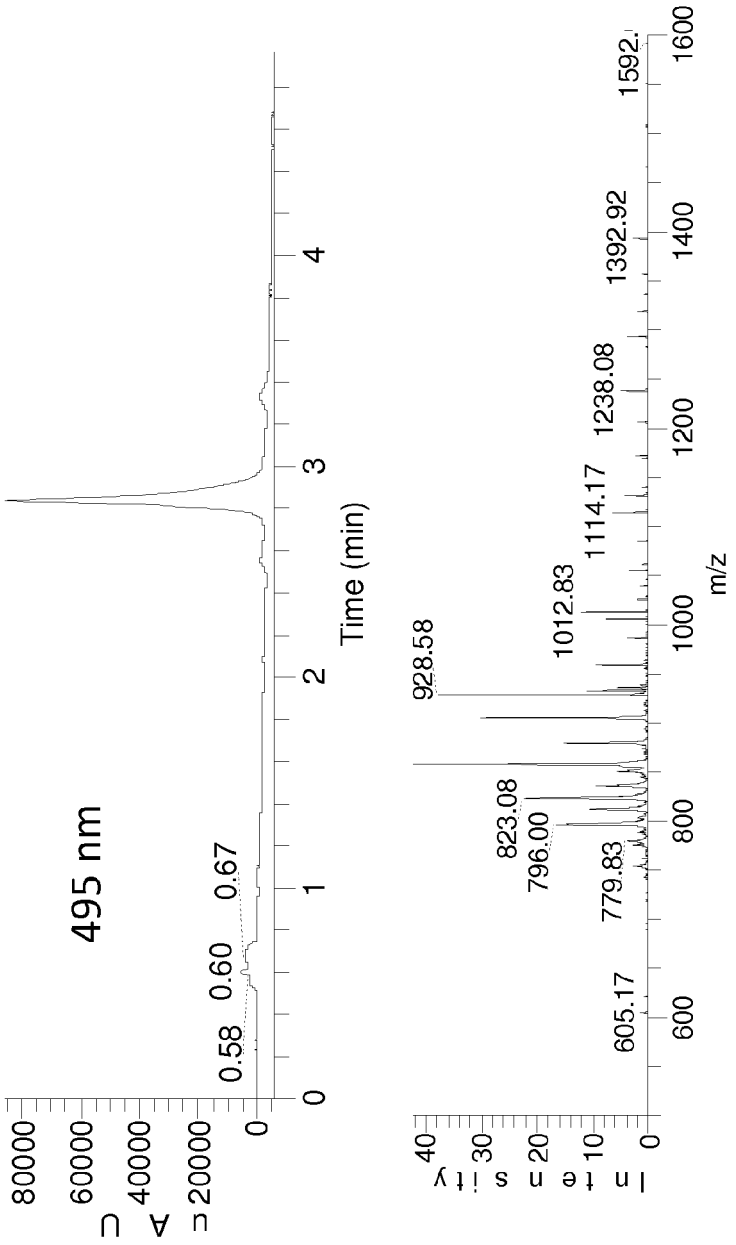
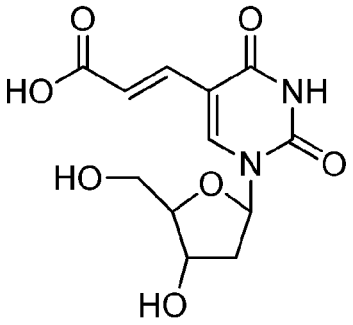


Figure 20



5-(Carboxy)vinyl-2'-deoxyuridine

SplintC_CVU
5’-TCCGCTCAACCTGAG-3’

Tag1_PsoCVU (MW 3,662.5 Da)
5’-AAAAACTCAGGT-3’

SplintA_CVU
5’-TCCGCTTAACCTGAG-3’

CVU_G (MW 11,576.2 Da)
5'- (5-(Carboxy)vinyl-2'-deoxyuridine)GAGCGGATGCAAAAAAAAAGGCGAGCTTGCGTACTG -3‘

CVU_A (MW 11560.2 Da)
5'- (5-(Carboxy)vinyl-2'-deoxyuridine)AAGCGGATGCAAAAAAAAAGGCGAGCTTGCGTACTG -3'

Figure 21

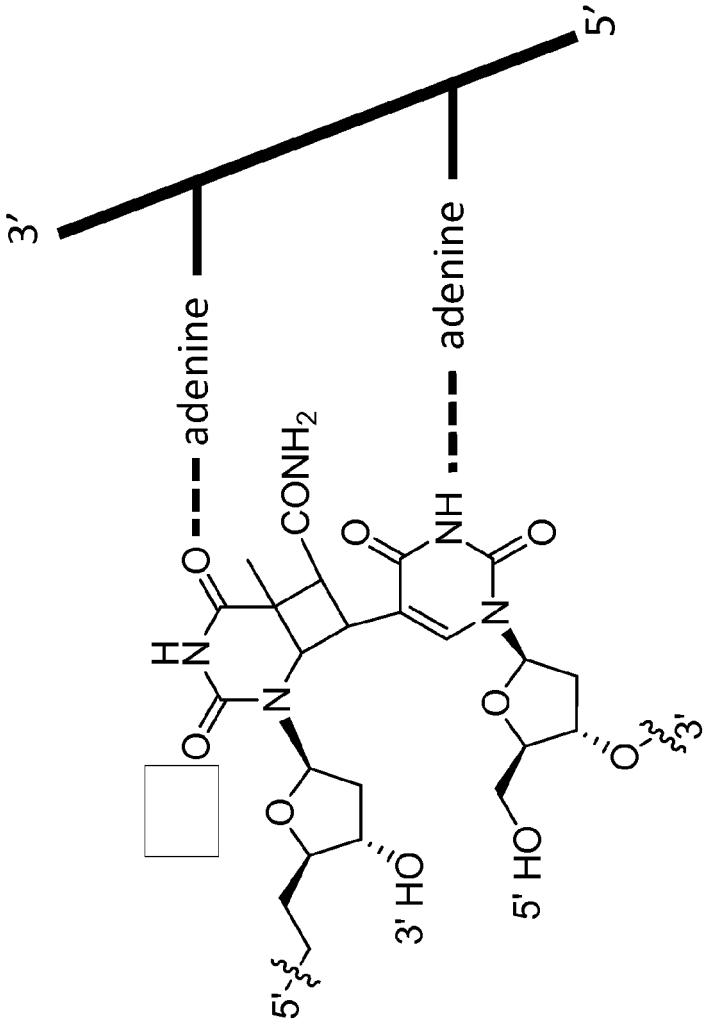


Figure 22

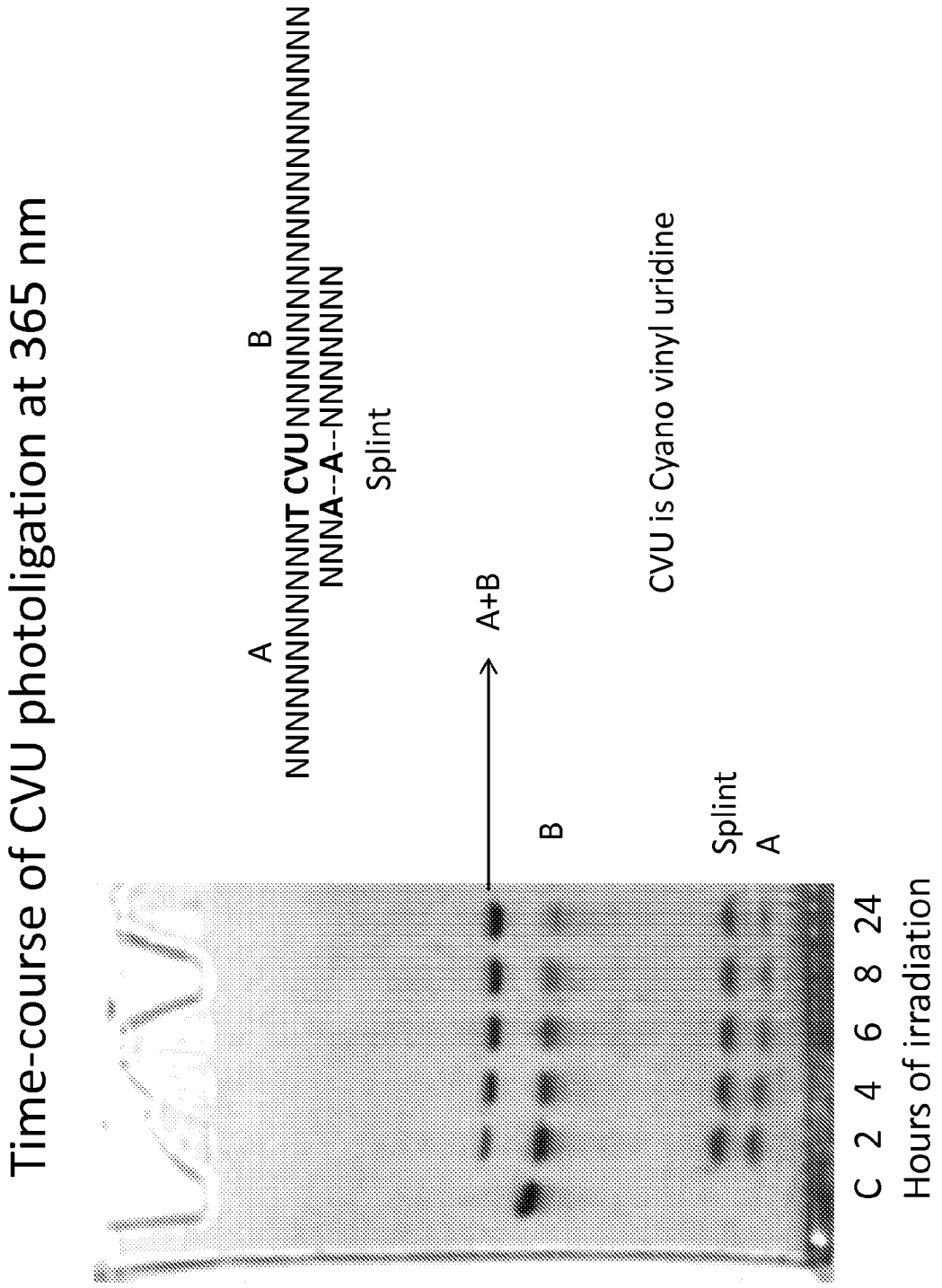

Time-course of CVU photoligation at 365 nm
A
NNNNNNNNNNNNT CVUNNNNNNNNNNNNNNNNNN
B
NNNA--A--NNNNNNN
Splint
A+B
B
CVU is Cyano vinyl uridine
Splint
A
C 2 4 6 8 24
Hours of irradiation

Figure 23

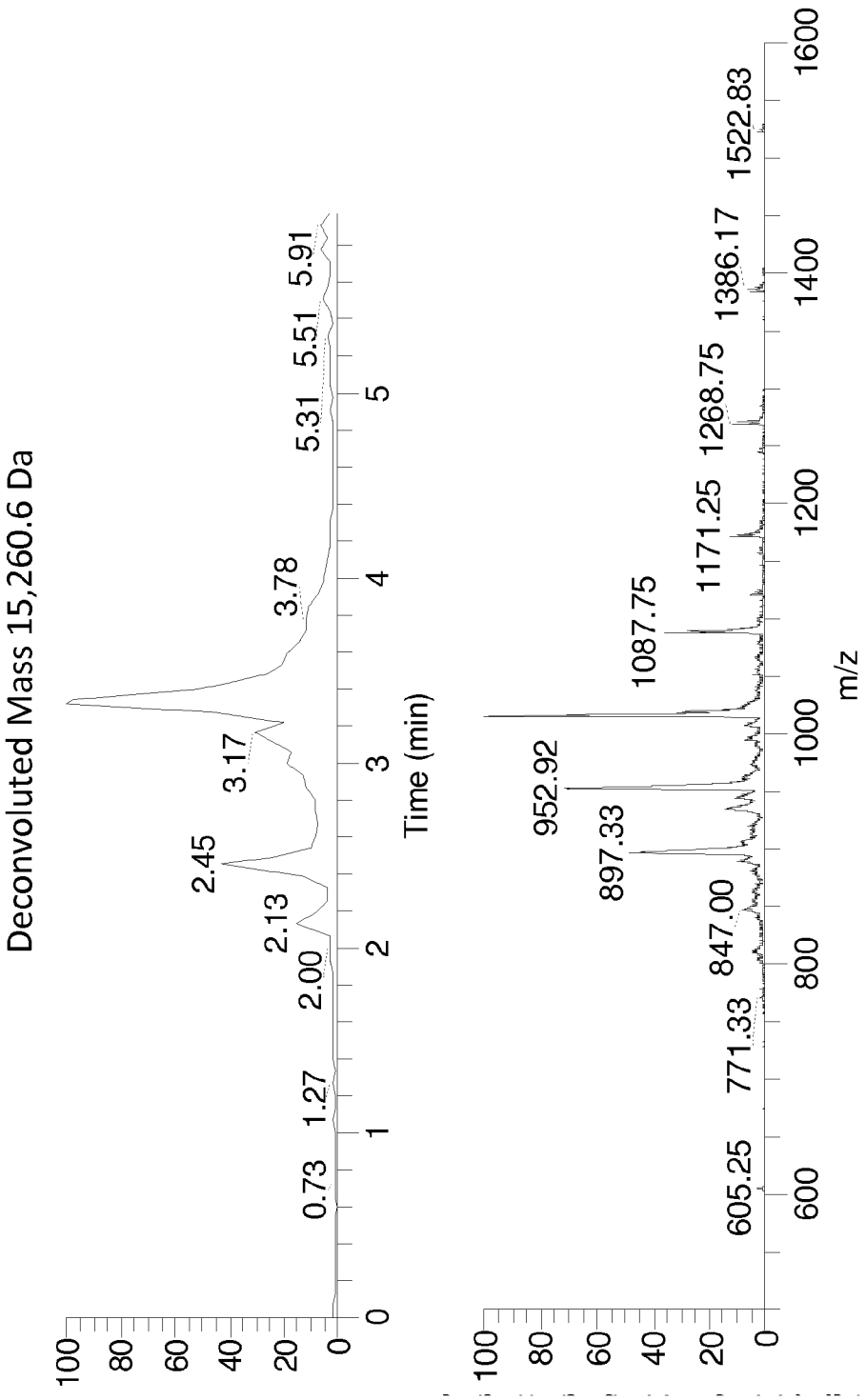

Deconvoluted Mass 15,260.6 Da
Time (min)
0.73
1.27
2.00
2.13
2.45
3.17
3.78
5.31
5.51
5.91
m/z
605.25
771.33
847.00
897.33
952.92
1087.75
1171.25
1268.75
1386.17
1522.83

Figure 24

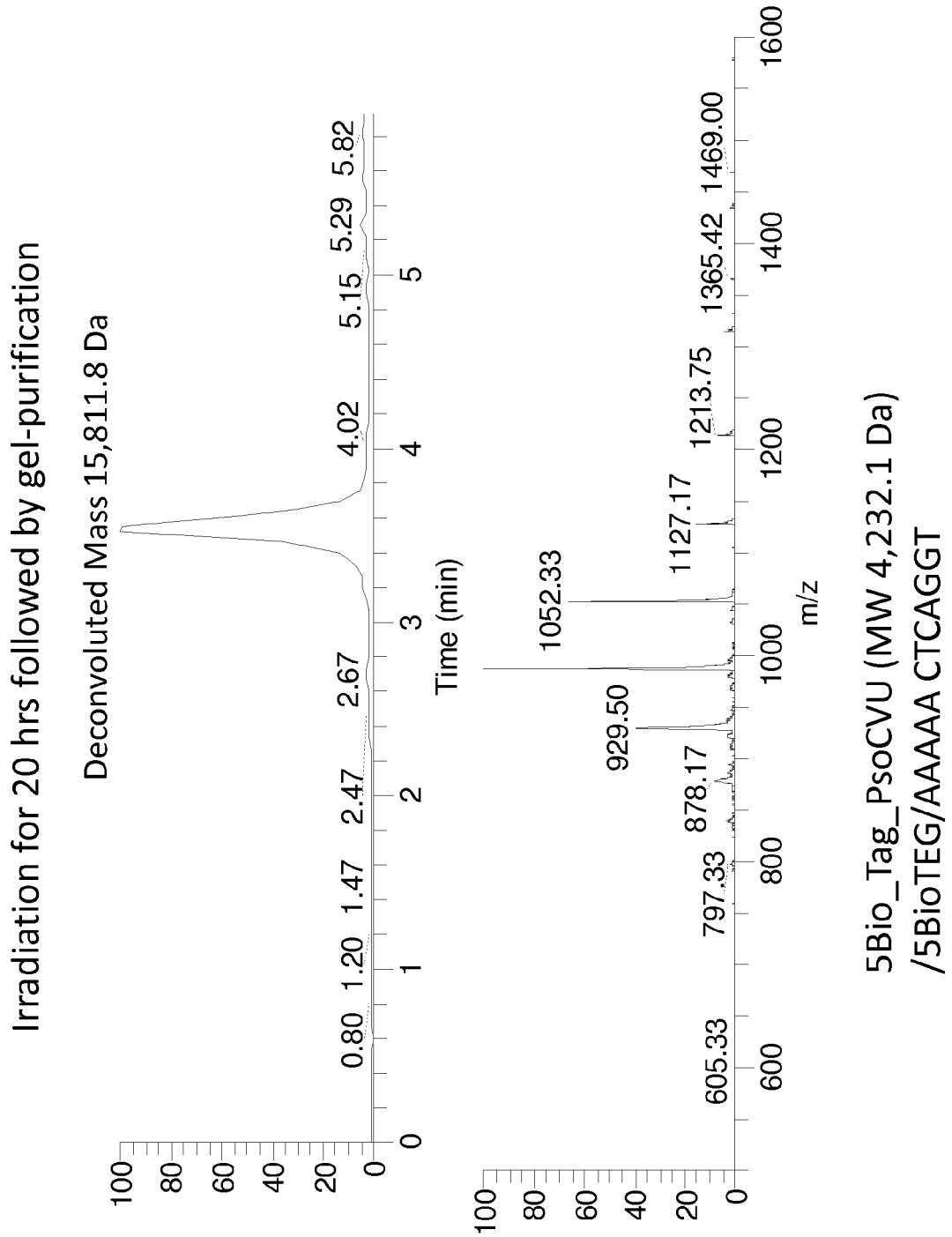

Irradiation for 20 hrs followed by gel-purification
Deconvoluted Mass 15,811.8 Da
100
80
60
40
20
0
0.80
1.20
1.47
2.47
2.67
4.02
5.15
5.29
5.82
0
1
2
3
4
5
Time (min)
5Bio_Tag_PsoCVU (MW 4,232.1 Da)
/5BioTEG/AAAAA CTCAGGT
605.33
797.33
878.17
929.50
1052.33
1127.17
1213.75
1365.42
1469.00
600
800
1000
1200
1400
1600
m/z

Figure 25

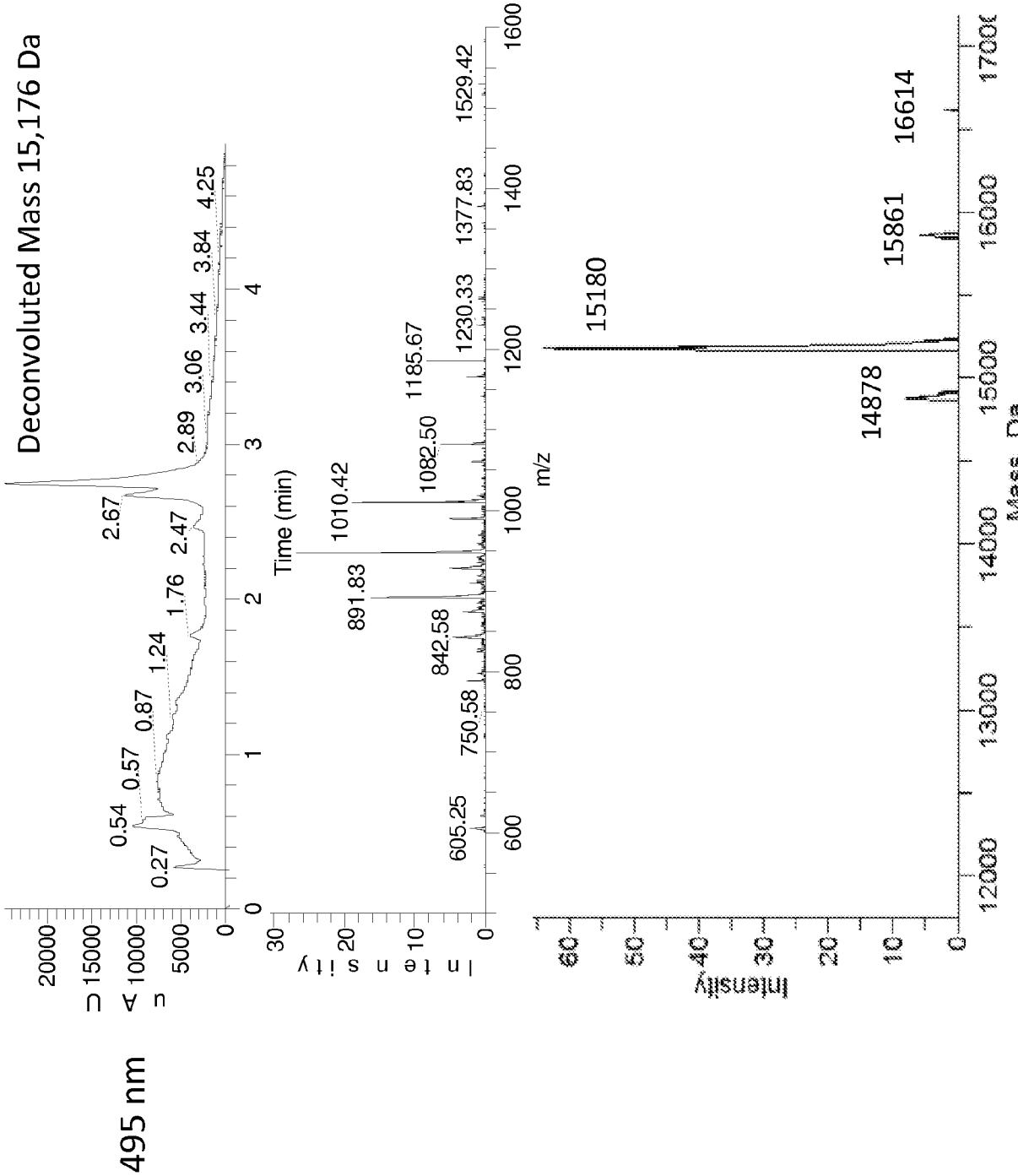

Deconvoluted Mass 15,176 Da
495 nm
Time (min)
m/z
Mass, Da
Intensity
15180
14878
15861
16614
2.89
3.06
3.44
3.84
4.25
2.67
2.47
1.76
1.24
0.87
0.57
0.54
0.27
1010.42
891.83
1082.50
1185.67
1230.33
1377.83
1529.42
842.58
750.58
605.25

Figure 26

S_IDTN$_3$

TGCGGTCTAACTGTCTAG TTCACCTTCTCCGGAATGAACAGG/3AzideN/

S_IDTalkyne

/5Hexynyl/TACCGAATTGCCGCCTGATTGAAGCAATCGGGTGCTATGCTT

5Hexynyl Modification

Figure 27

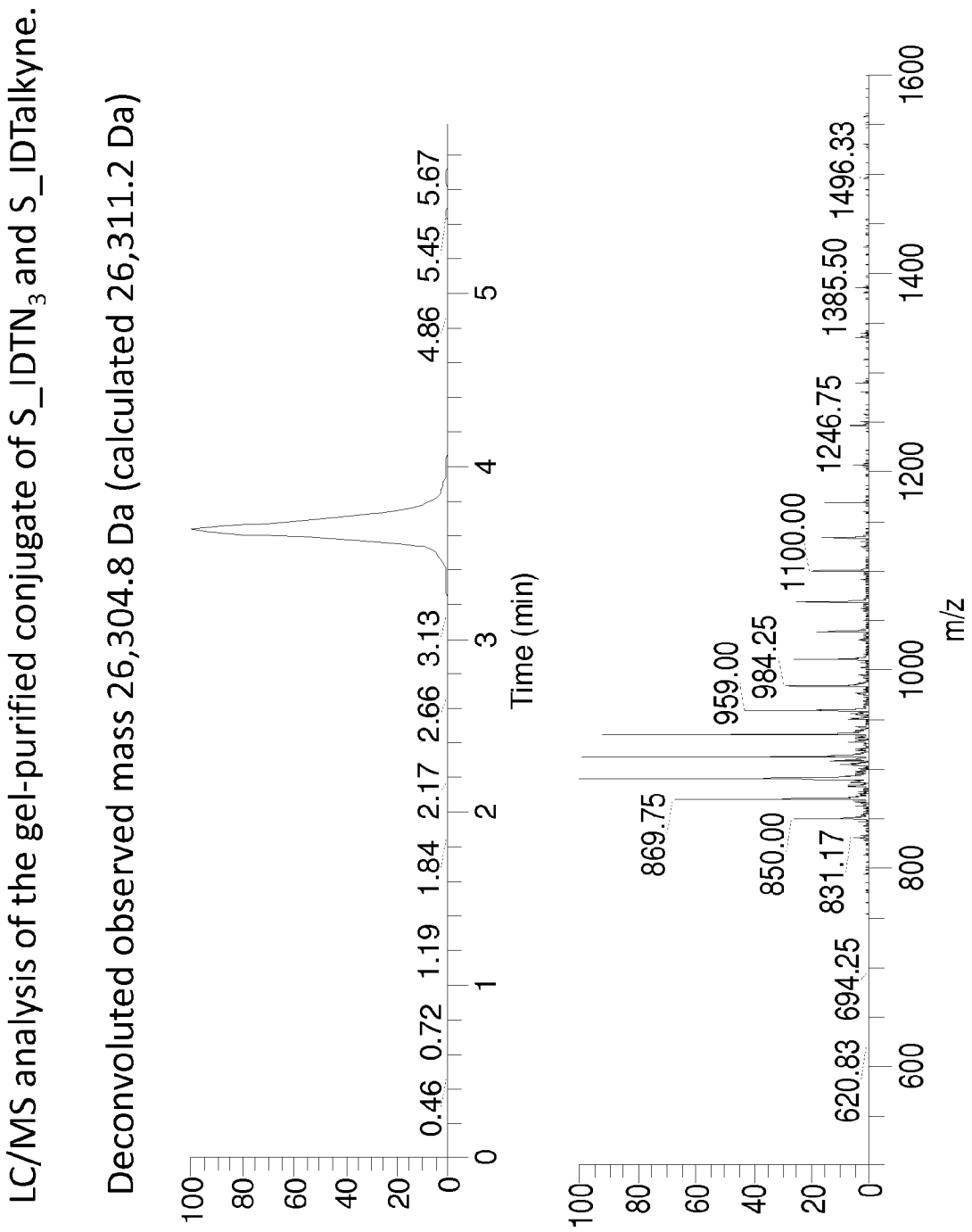

LC/MS analysis of the gel-purified conjugate of S_IDTN3 and S_IDTalkyne.
Deconvoluted observed mass 26,304.8 Da (calculated 26,311.2 Da)
Time (min)
m/z
0.46
0.72
1.19
1.84
2.17
2.66
3.13
4.86
5.45
5.67
620.83
694.25
831.17
850.00
869.75
959.00
984.25
1100.00
1246.75
1385.50
1496.33

Figure 28

TKR_Central: /5Hexynyl/GGAATGAACAGG **GTAAGCTGGAGT**GAAGGCGTTATG/3AzideN/

S_IDTN$_3$: TGCGGTCTAACTGTCTAGTTCACCTTCTCCGGAATGAACAGG /3AzideN/

TKR_DBCO_S: /DBCO/GAAGGCGTTATGTCCGTACTCTTGCAATCGGGTGCTATGCTT

DBCO Phosphoramidite

Figure 29

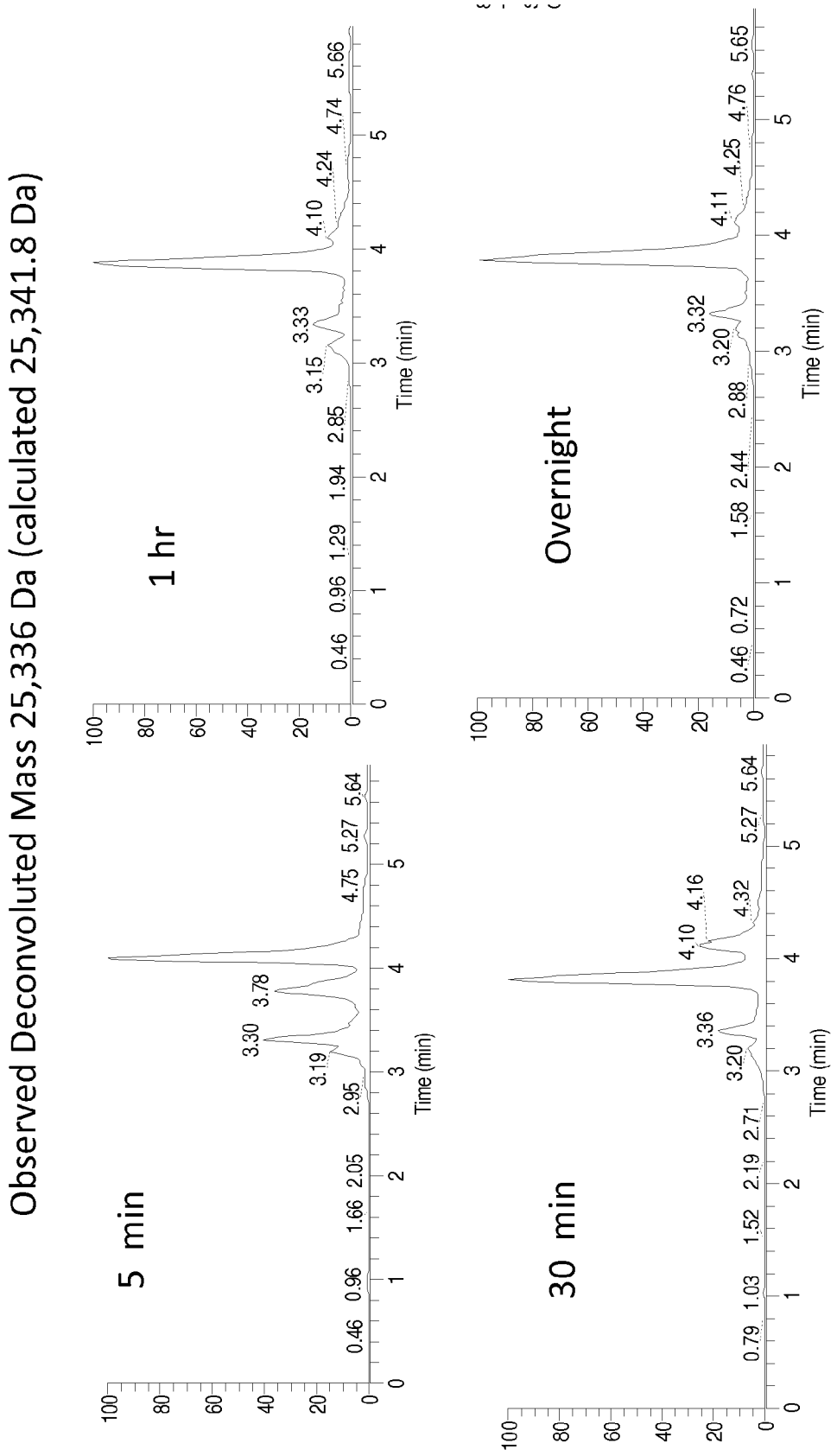

Observed Deconvoluted Mass 25,336 Da (calculated 25,341.8 Da)
5 min
1 hr
30 min
Overnight
Time (min)
Time (min)
Time (min)
Time (min)

Figure 30

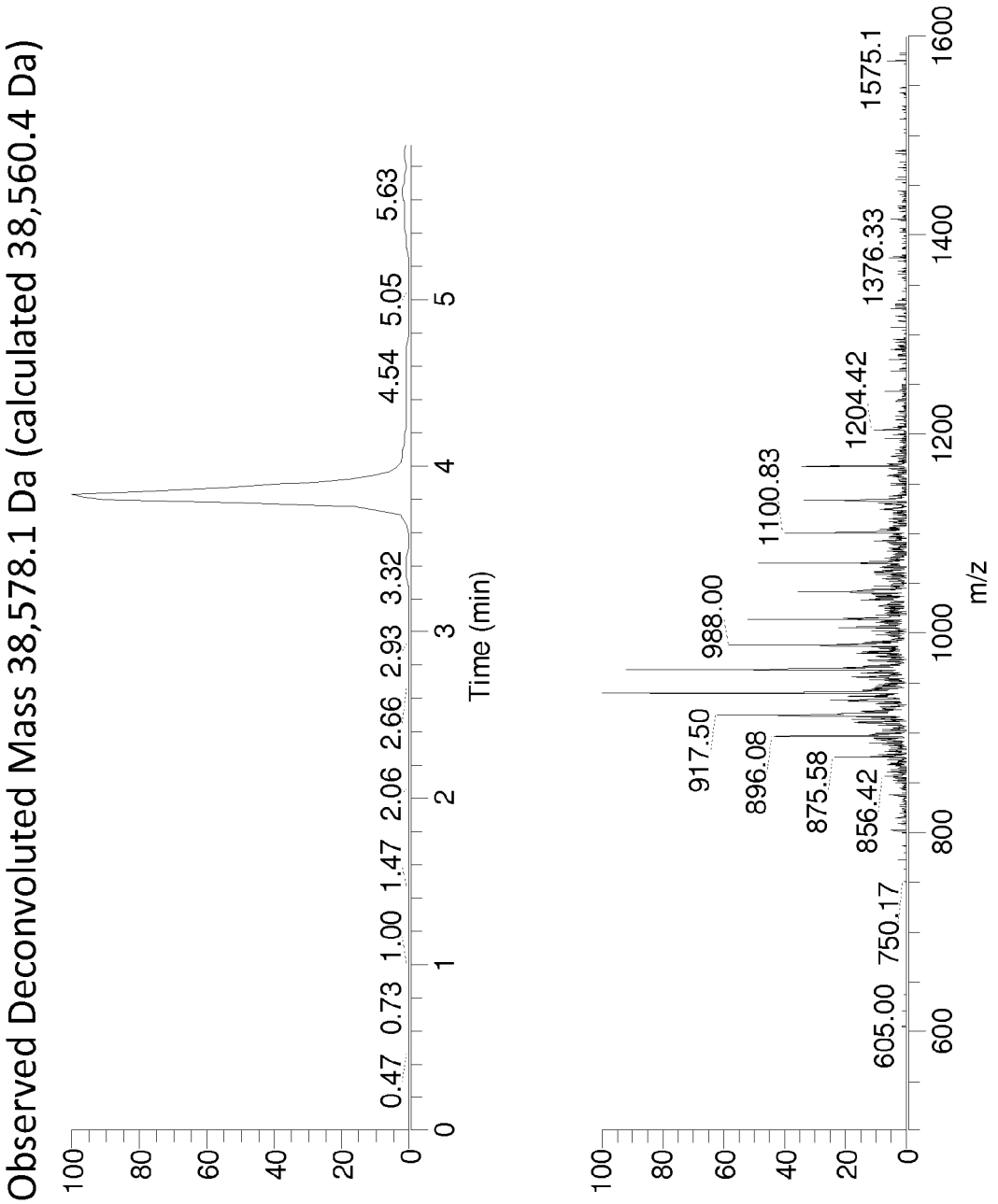

Observed Deconvoluted Mass 38,578.1 Da (calculated 38,560.4 Da)
100
80
60
40
20
0
0.47
0.73
1.00
1.47
2.06
2.66
2.93
3.32
4.54
5.05
5.63
0
1
2
3
4
5
Time (min)
605.00
750.17
856.42
875.58
896.08
917.50
988.00
1100.83
1204.42
1376.33
1575.1
600
800
1000
1200
1400
1600
m/z

Figure 31

Conjugate_Click_S
TGCGGTCTAACTGTCTAGTTCACCTTCTCCGGAATGAACAGG(click)TACCGAATTGCCGCCTGATTGAAG
-CAATCGGGTGCTATGCTT

Conjugate_Click_L
TGCGGTCTAACTGTCTAGGCACTTGTTCGTTTGCCAGTGTGAGGAATGAACAGG(click)TACCGAATTGC
CTTCCTCG-TACAGTTCTAAGGCGCTTGGACACCACTTCAATCGGGTGCTATGCTT

Figure 32

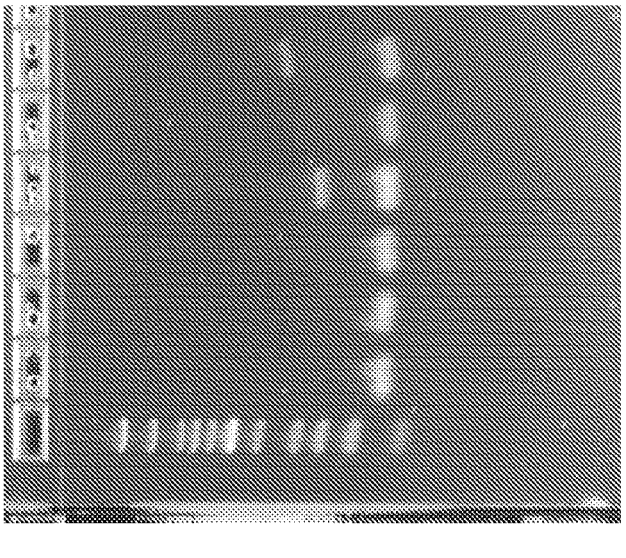


Figure 33

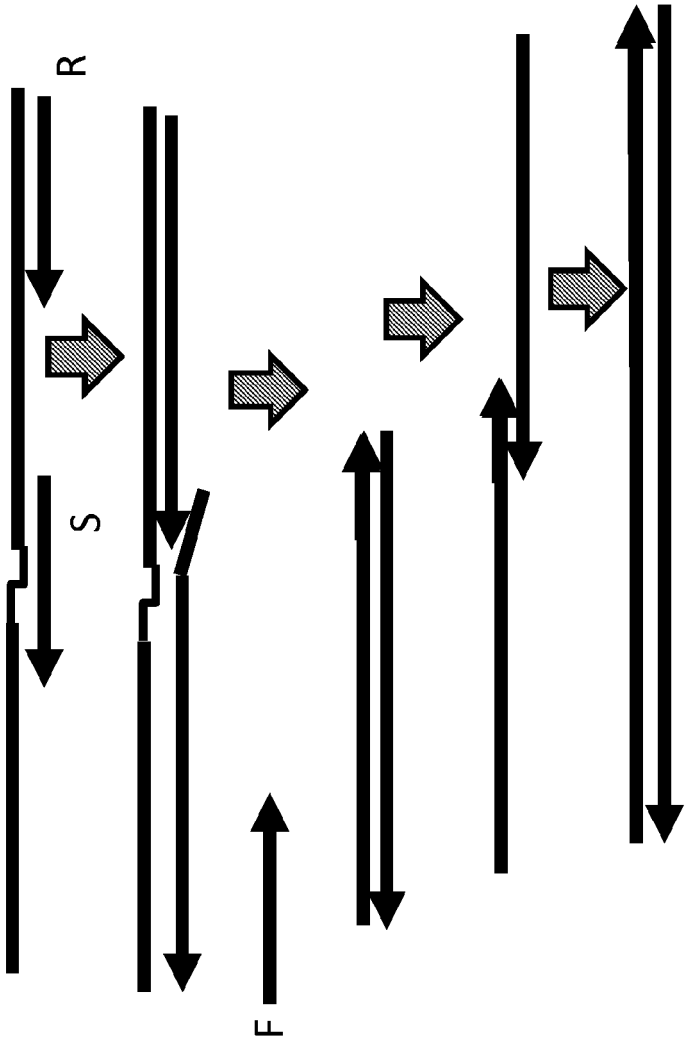
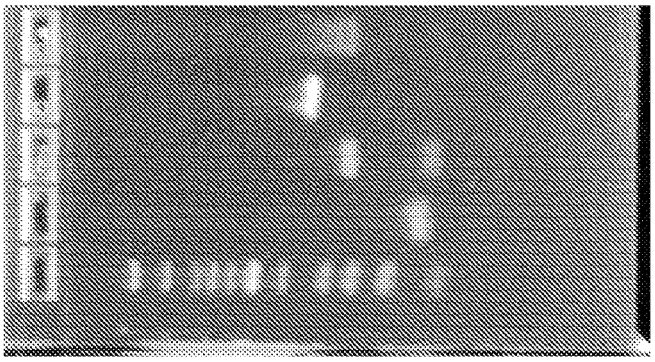


Figure 34



Lane 1 2 3 4 5

Figure 35

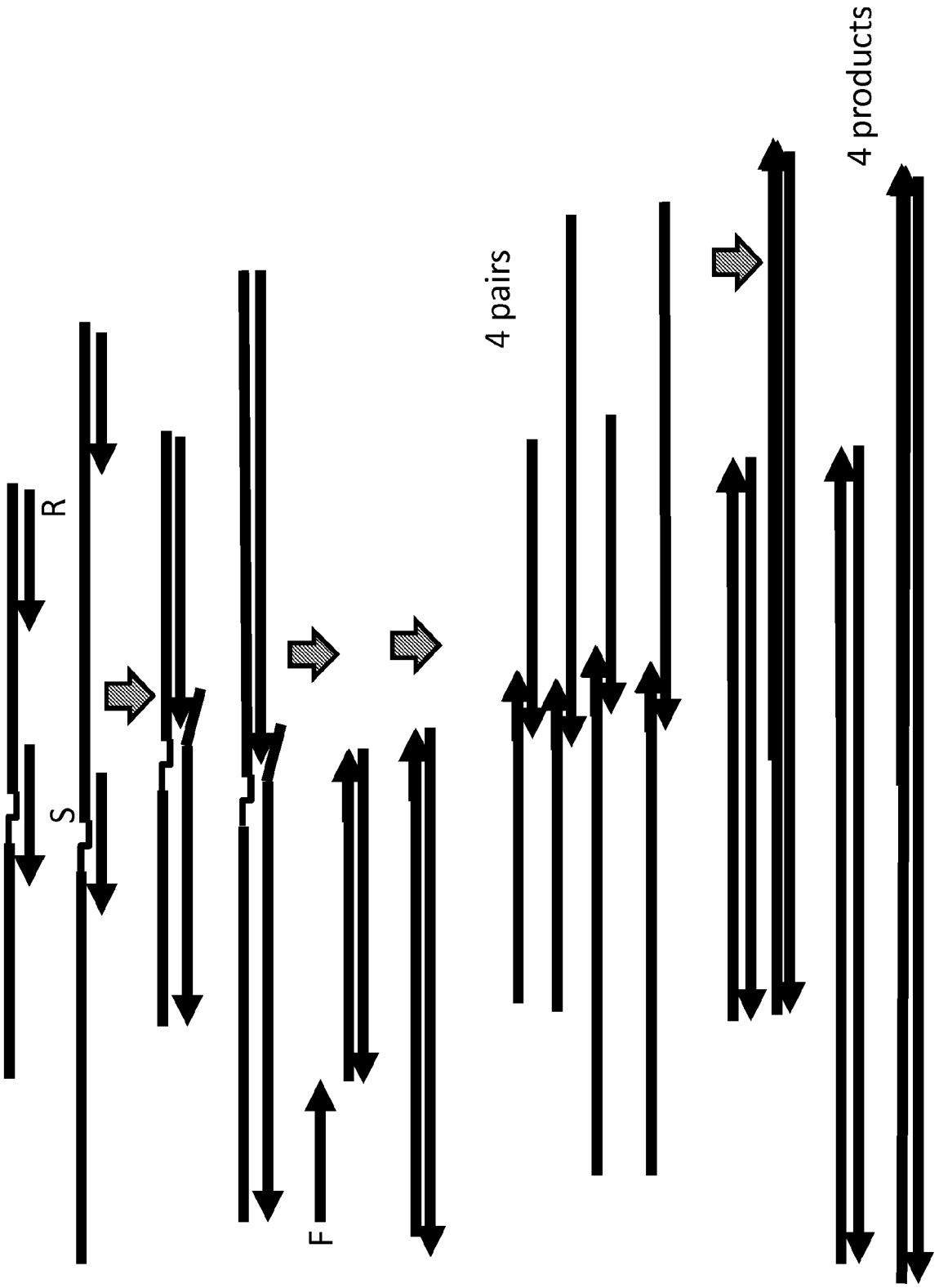


Figure 36

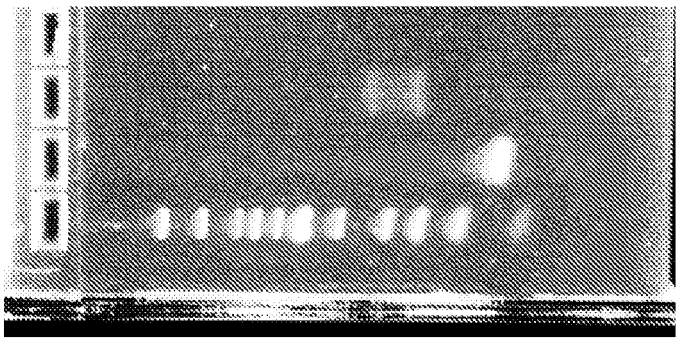

32 cycles, starting conc 5 pM
1-Marker
2- Solution PCR
3- Emulsion PCR
Lane 1 2 3 4

Figure 37

TKR_S:
TGCGGTCTAACTGTCTAGTTCACCTTCTCCGGAATGAACAGG(click)GGAATGAACAGGGCCTGATTGAAGCAATCGGGTGCTATGCTT

TKR_L:
TGCGGTCTAACTGTCTAGGCACTTGTTCGTTTGCCAGTGTGATGGACACCACTTGGAATGAACAGG(click)GGAATGAACAGGGGAATGAACAG
GTTCCTCGTACAGTTCTAAGGCGCTCAATCGGGTGCTATGCTT

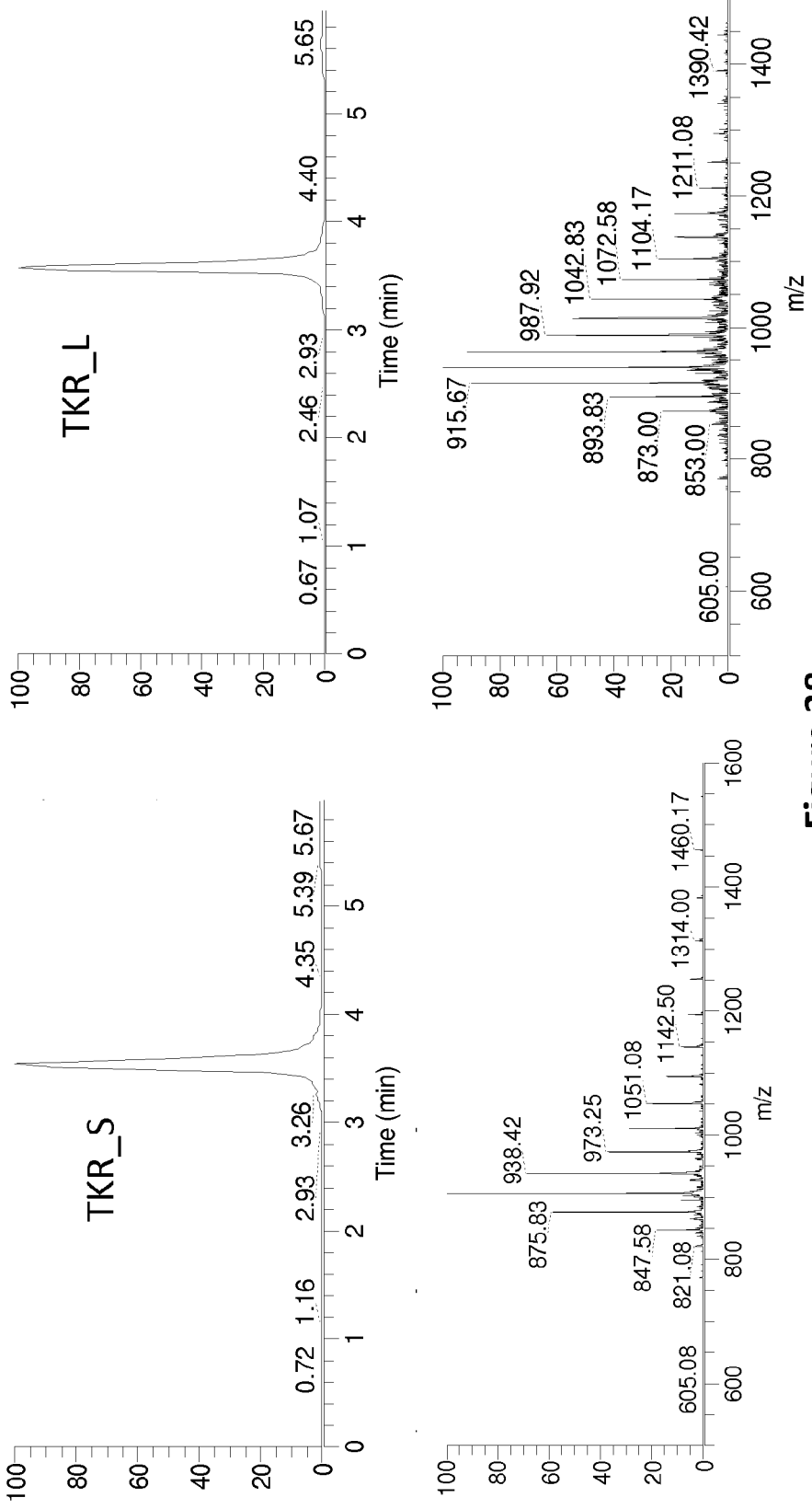


Figure 38

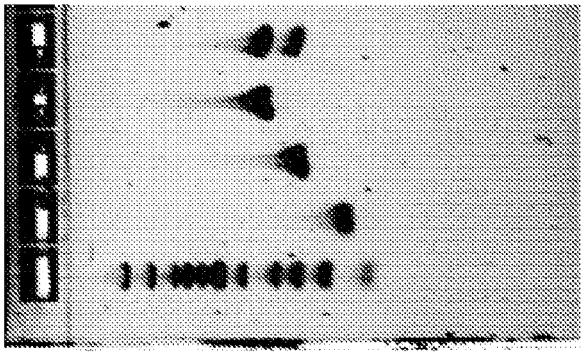


Figure 39

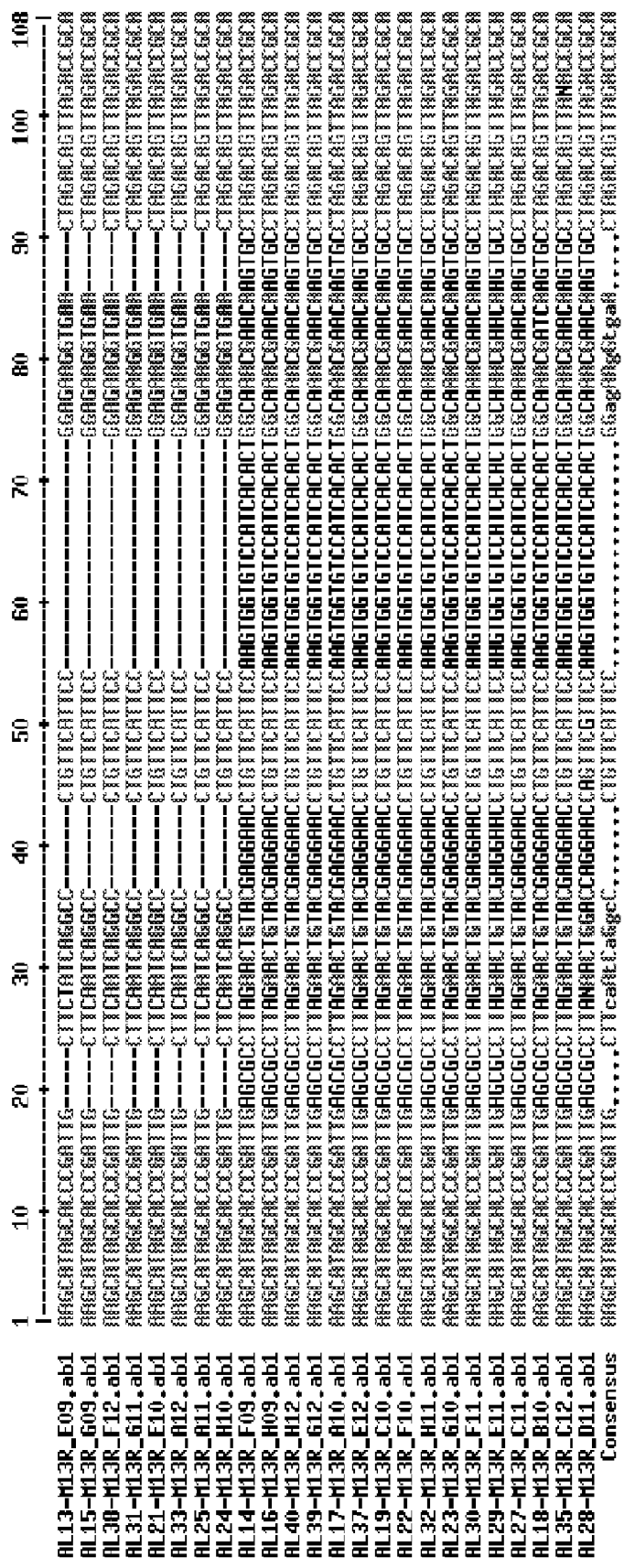


Figure 40

TKR_2_click_S
TGCGGTCTAACTGTCTAGTTCACCTTCTCCGGAATGAACAGG(CLICK)GGAATGAACAGGGTAAGCTGGAGTGAAGGCGTTATG(DBCO)
GAAGGCGTTATGTCCGTACTCTTGCAATCGGGTGCTATGCTT

TKR_2_click_L
TGCGGTCTAACTGTCTAGGCACTTGTTCGTTTGCCAGTGTGATGGACACCACTTGGAATGAACAGG(CLICK)GGAATGAACAGGGTAAGC
TGGAGTGAAGGCGTTATG(DBCO)GAAGGCGTTATGTGATATCCGTGGTGTCGTGAGTTCCAATCGGGTGCTATGCTT

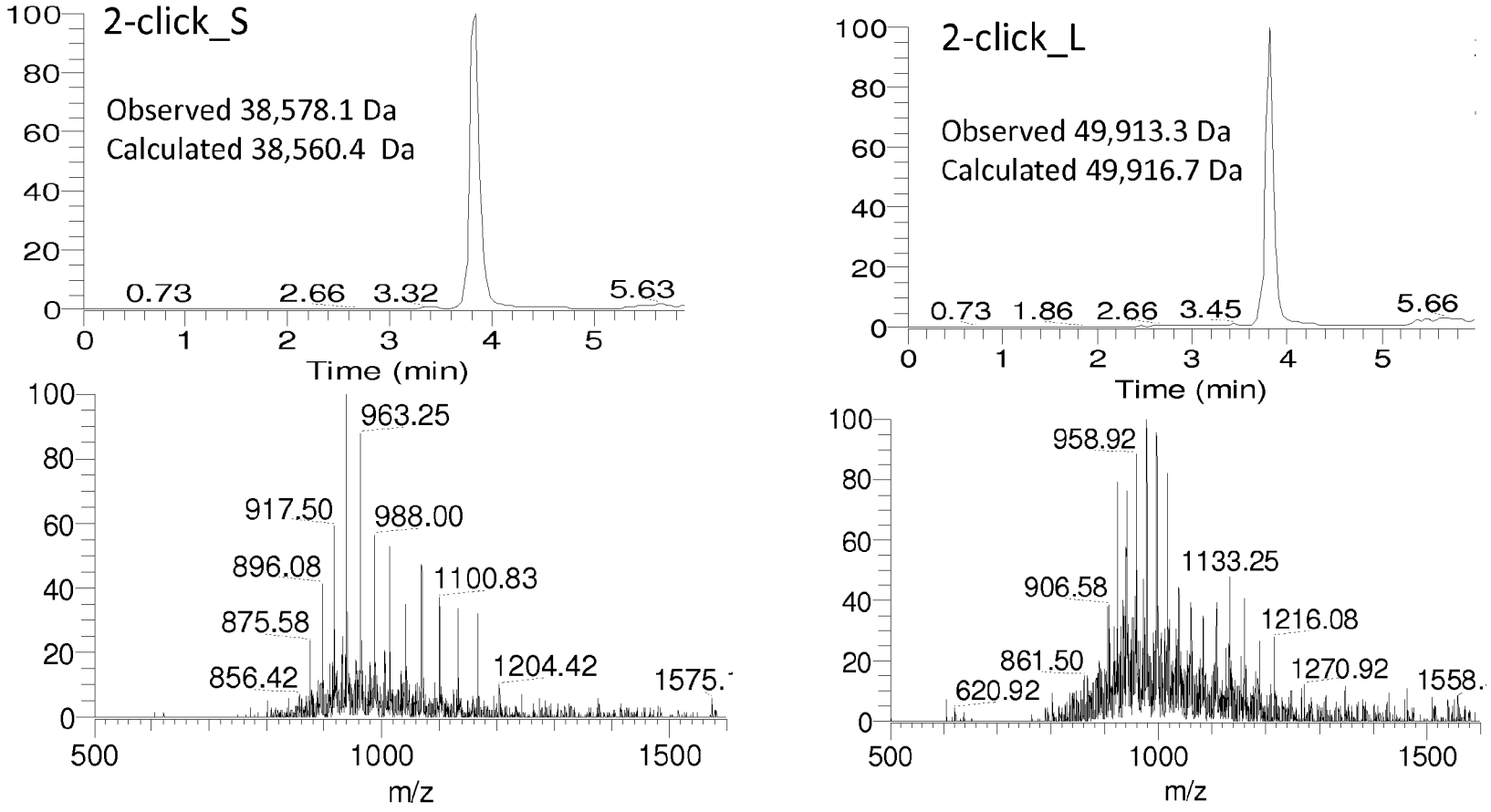


Figure 41

SUBSTITUTE SHEET (RULE 26)

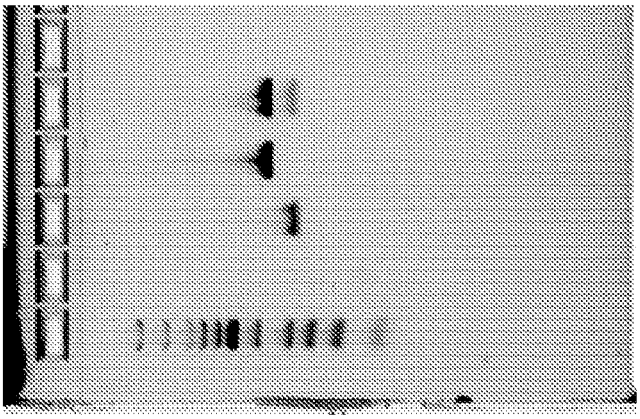


Figure 42

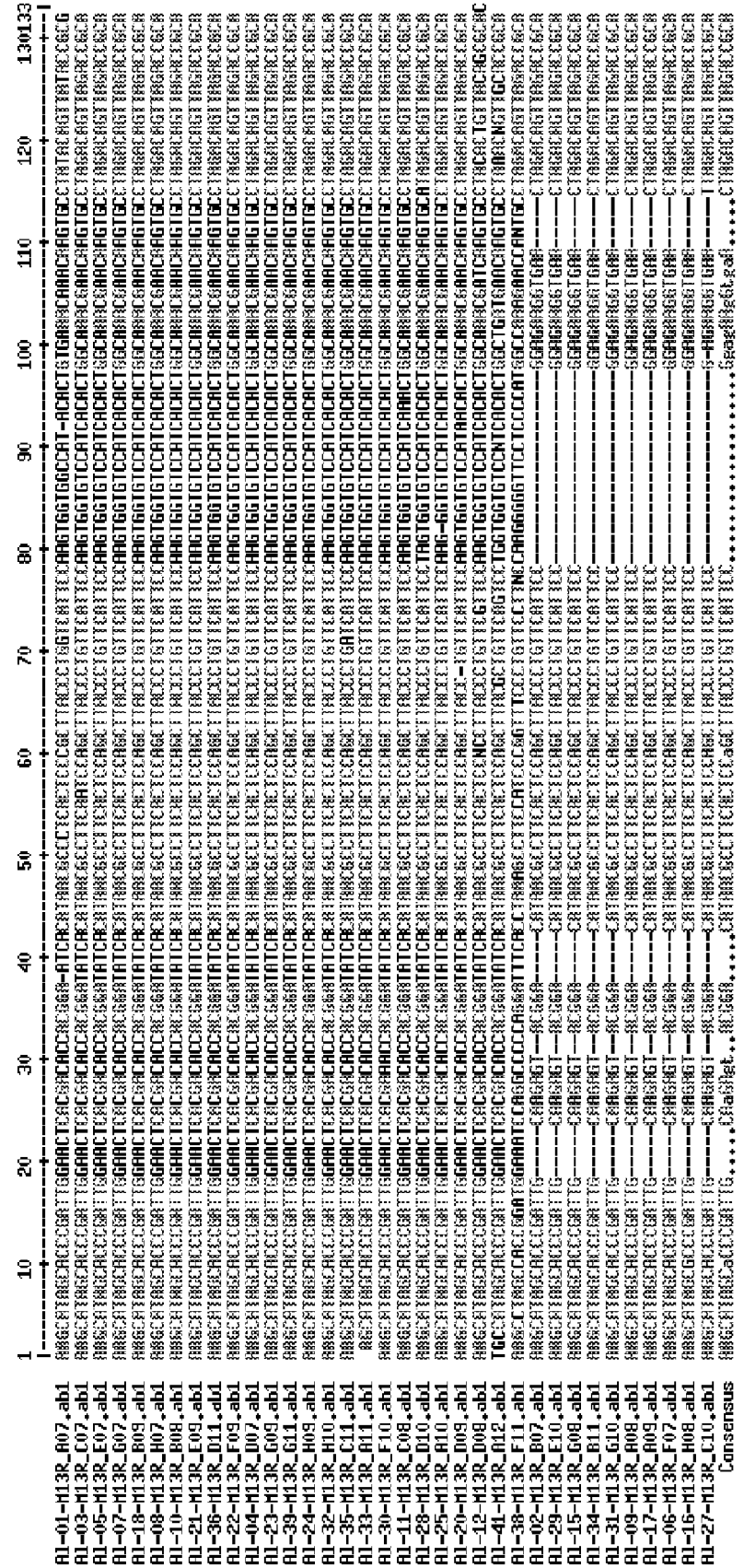


Figure 43