LEGENDS TO THE SUPPLEMENTARY FIGURES

Supplementary Figure 1

Immunoprecipitation for CLIP from cerebellum extracts obtained from wild-type or *Fmr1*-null mice using the polyclonal anti-FMRP R60 antibody. After immunoprecipitation, as described in Materials and Methods, 1/10 of each extract was loaded on a 11% polyacrylamide denaturating gel and Western blot was performed using the 1C3 monoclonal anti-FMRP antibody. As shown in this figure, we revealed FMRP at 75 kDa and RNA/FMRP complex at 95 kDa only in wild-type animals, while no FMRP was detected in cerebellar extracts obtained from *Fmr1* knock-out mice, indicating the specificity of this antibody. The IgG heavy chains are indicated at 50 kDa.

Supplementary Figure 2

A. Cation-dependent termination of reverse transcription in N19 (positive control) and Δ35 (negative control). All purines of the G-quadruplex structure harboured by the N19 fragment (derived from FMR1) are indicated in red (G) and blue (A), respectively. The Δ35 probe was generated by deleting 35 nucleotides corresponding to the fragment included between positions 1613 and 1648 of the *FMR1* ISO1 isoform [16,23]. For N19, the two strongest pauses of reverse transcriptase in the presence of K+ are indicated by arrowheads. Three minor pauses are observable in the presence of K+. All pauses are not present in the Δ 35 fragment.

B. Cation-dependent termination of reverse transcription in the *Cod2A* fragment of the *GRK4* mRNA using two different primers in order to completely cover the Cod2 fragment. No pauses are observed in the presence of K+, Na+ or Li+, demonstrating the absence of G-quadruplex-forming structures in this RNA fragment bound by FMRP.

Supplementary Figure 3

10% PAGE gel showing the running of retro transcribed G4RIF; RNA after treatment with DMS, CMCT, RNAseA, T1 or V1.

Supplementary Figure 4.

QuShape analysis of the G4RIF A modified G4RIF RNA harboring a complementary sequence to M13rev primer at its' 3'end was PAGE purified and its structure was analysed by CMCT and DMS. Processed RNA were reverse transcribed with VIC-labeled M13rev primers, and the reactivities were compared to mock -treated RNA. Sequence alignement was achieved by resolving in the same capillary two dideoxy-nucleotide sequencing reactions (NED and PET labelled M13rev primers) and one cDNA from mock- or chemical-treated RNA reaction. Reactivities for CMCT- (A) or DMS-treated (B) samples were computed with the QuShape software. A 5'-3' oriented sequence is displayed at the bottom of each graph. The deducted secondary structure in (C) is numbered according to the two graphs.

Fig. S1

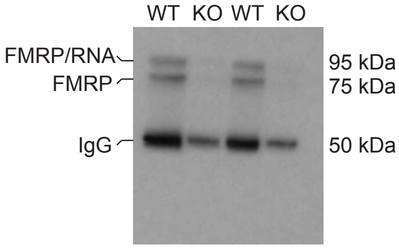


Fig. S2

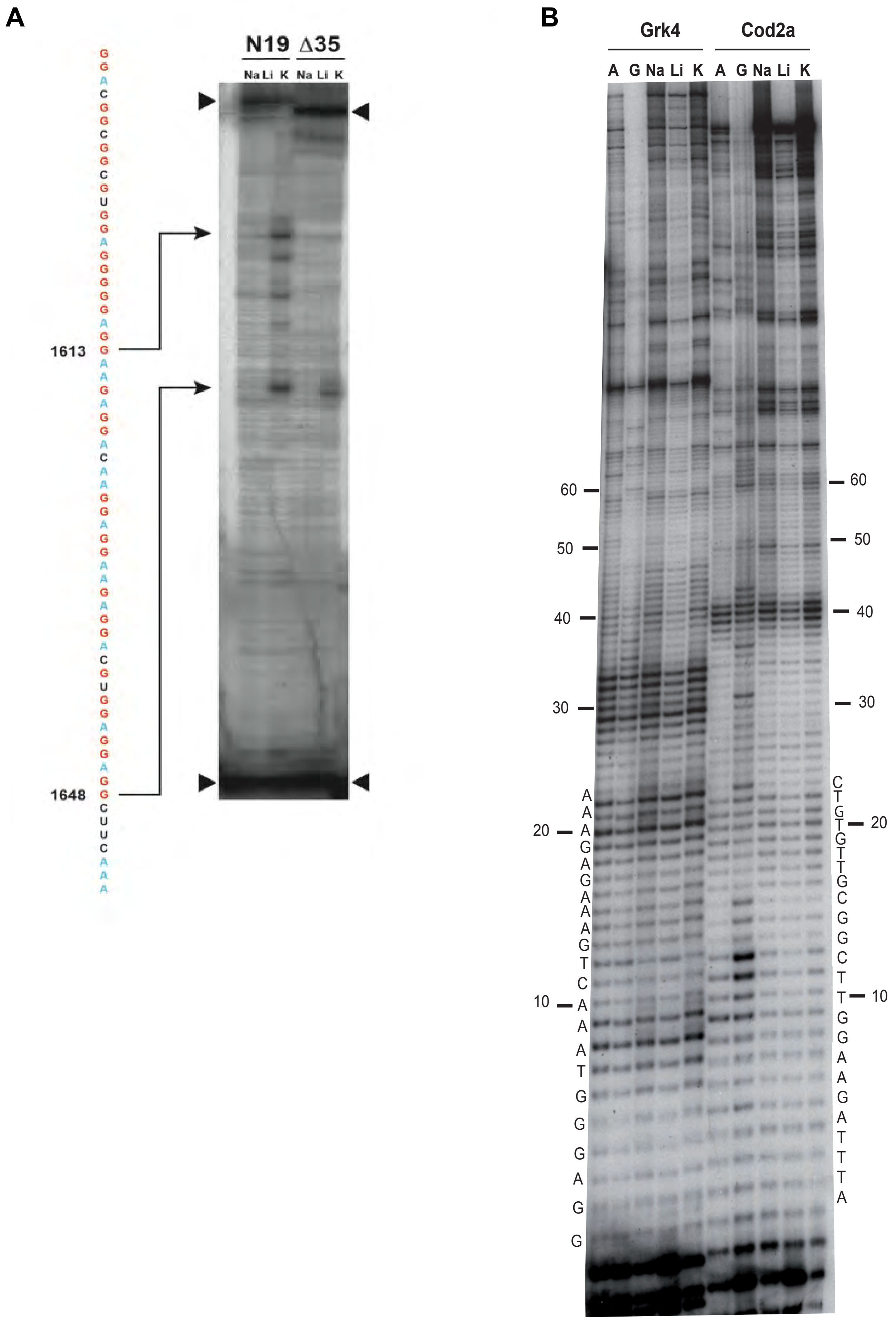

A
G
G
A
C
G
G
C
G
G
C
G
U
G
G
A
G
G
G
G
G
A
G
1613 G
A
A
G
A
G
G
A
C
A
A
G
G
A
G
G
A
A
G
A
G
G
A
C
G
U
G
G
A
G
G
A
G
1648 G
C
U
U
C
A
A
A
N19
Δ35
Na Li K Na Li K
B
Grk4
Cod2a
A G Na Li K A G Na Li K
60
50
40
30
A
A
20 A
G
A
G
A
A
A
G
T
C
10 A
A
A
T
G
G
G
A
G
G
60
50
40
30
C
T
G
T 20
G
T
T
G
C
G
G
C
T
T 10
G
G
A
A
G
A
T
T
T
A

Fig. S3

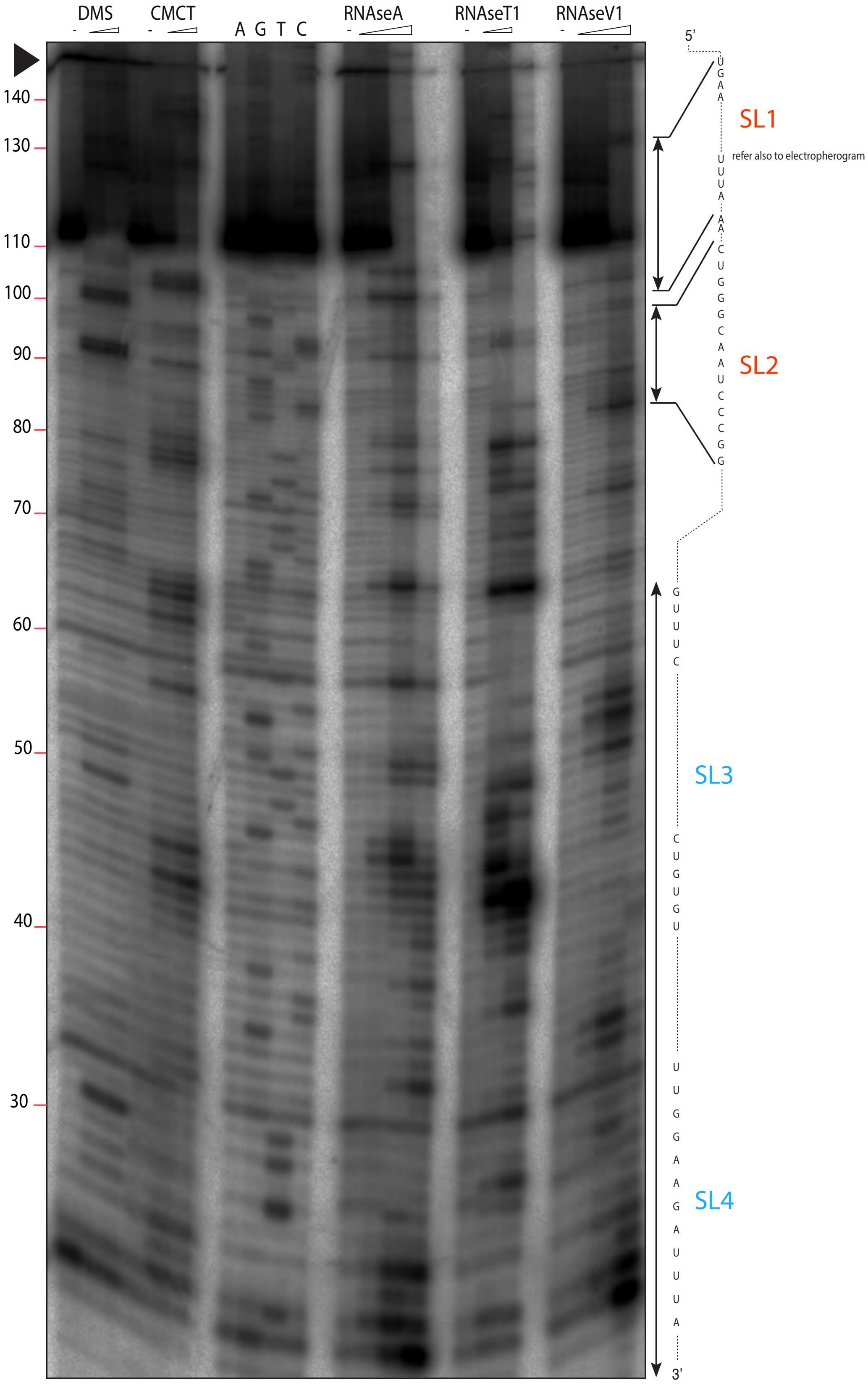
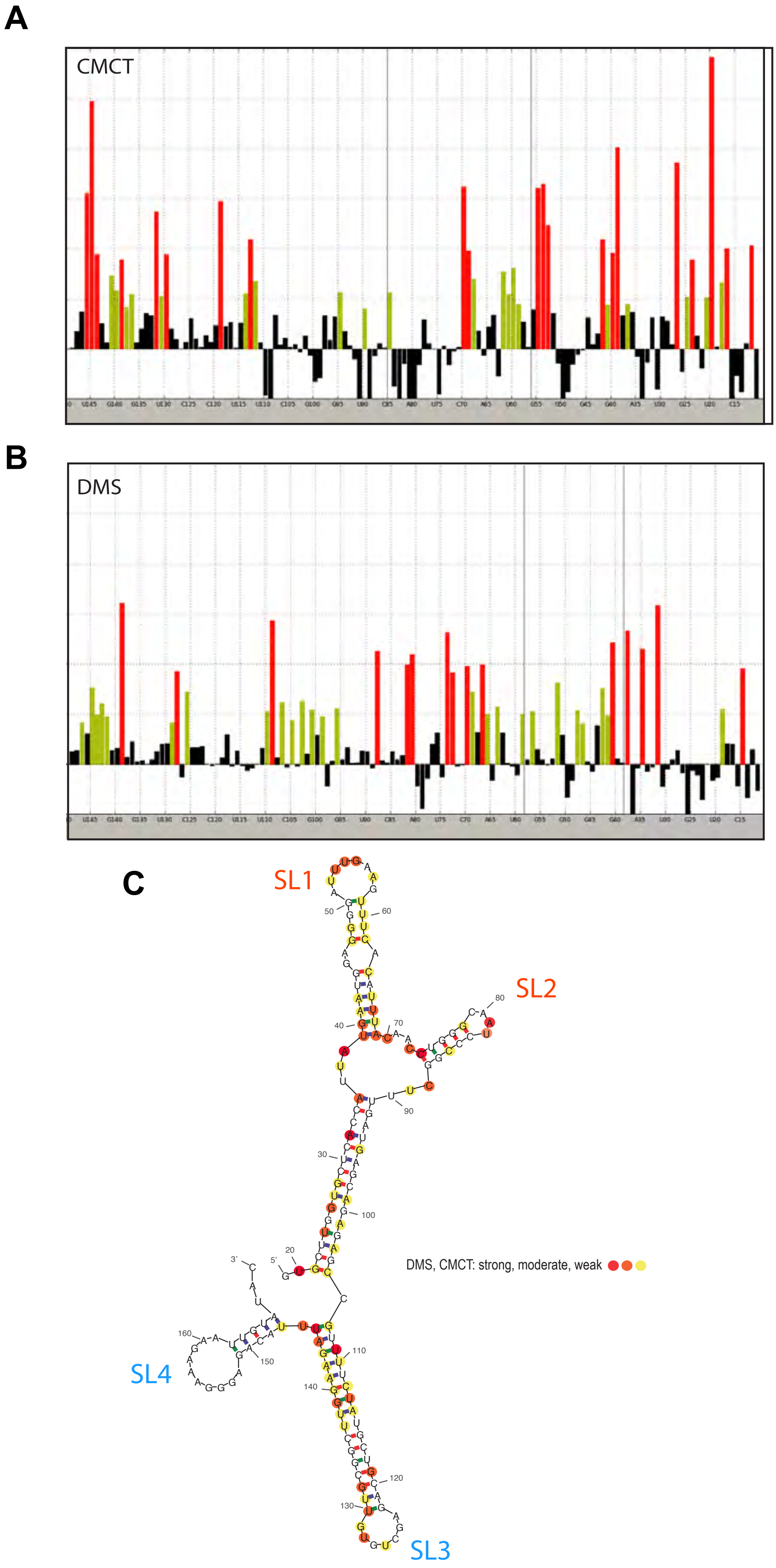

DMS
-
CMCT
-
A G T C
RNAseA
-
RNAseT1
-
RNAseV1
-
140
130
110
100
90
80
70
60
50
40
30
5'
U
G
A
A
SL1
refer also to electropherogram
U
U
U
A
A
A
C
U
G
G
G
C
A
A
U
C
C
C
G
G
SL2
G
U
U
U
C
SL3
C
U
G
U
G
U
U
U
G
G
A
A
G
A
U
U
U
A
SL4
3'

Fig. S4


A
CMCT
U145
G140
G135
U130
C125
C120
U115
U110
C105
G100
G95
U90
C85
A80
U75
C70
A65
U60
G55
G50
G45
G40
A35
U30
G25
U20
C15
B
DMS
C
SL1
SL2
SL3
SL4
5'
3'
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
DMS, CMCT: strong, moderate, weak

seqNum	seqRNA	area BSA	area_FMRP	areaDiff	normDiff	centered 20-mer bin	binned -average
9	A	2002,32	2101,93	-99,66	-0,06		
10	A	2088,77	2634,94	-546,17	-0,33		
11	A	289,92	1555,44	-1265,52	-0,77		
12	G	0	332,23	-332,23	-0,2		
13	G	1147,43	841,54	305,89	0,18	**average 20-mer bin**	**6,250153846**
14	G	365,37	247,63	117,74	0,07		
15	A	528,79	299,57	229,22	0,14		
16	G	702,16	464,42	237,74	0,14		
17	A	1250,05	1176,41	73,64	0,04	4,47	-1,780153846
18	C	16154,08	13102,32	3051,76	1,85	4,65	-1,600153846
19	A	18964,55	17419,59	1544,96	0,93	4,99	-1,260153846
20	U	2137,22	1608,92	528,3	0,32	5,96	-0,290153846
21	U	310,02	261,89	48,12	0,03	6,38	0,129846154
22	U	780,9	577,27	203,63	0,12	7,12	0,869846154
23	A	4522,33	3367,79	1154,54	0,7	7,17	0,919846154
24	G	1416,17	1297,52	118,65	0,07	7,82	1,569846154
25	A	7117,32	6849,2	268,12	0,16	7,86	1,609846154
26	A	5988,2	5119,16	869,04	0,53	7,56	1,309846154
27	G	5171,33	4274,95	896,37	0,54	6,12	-0,130153846
28	G	1518,79	1504,67	14,12	0,01	5,38	-0,870153846
29	U	401,1	196,92	204,18	0,12	5,27	-0,980153846
30	U	426,69	403,18	23,51	0,01	5,17	-1,080153846
31	C	1619,2	1287,23	331,97	0,2	5,08	-1,170153846
32	G	1474,71	1114,99	359,72	0,22	4,38	-1,870153846
33	G	8789,24	7262,47	1526,77	0,92	4,27	-1,980153846
34	C	3217,25	3019,65	197,6	0,12	4,08	-2,170153846
35	G	7611,35	6307,57	1303,78	0,79	3,42	-2,830153846
36	U	1780,16	1481,13	299,03	0,18	4,6	-1,650153846
37	U	2489,54	2916,39	-426,85	-0,26	4,51	-1,740153846
38	G	3724,65	3040,14	684,51	0,41	4,23	-2,020153846
39	U	1060,66	749,16	311,5	0,19	4,55	-1,700153846
40	G	1676,95	1330,78	346,17	0,21	4,26	-1,990153846
41	U	434,17	541,91	-107,74	-0,07	4,29	-1,960153846
42	C	266,92	209,71	57,21	0,03	3,9	-2,350153846
43	G	29,58	26,3	3,28	0	4,5	-1,750153846
44	A	1013,69	1076,22	-62,52	-0,04	4,4	-1,850153846
45	G	891,9	938,59	-46,69	-0,03	5,42	-0,830153846
46	A	1584,12	1798,47	-214,34	-0,13	5,77	-0,480153846
47	C	9191,61	6352,81	2838,8	1,72	6,29	0,039846154
48	G	1631,18	1769,3	-138,12	-0,08	6,43	0,179846154
49	U	1907,69	2170,61	-262,92	-0,16	6,31	0,059846154
50	C	2400,45	1851,1	549,35	0,33	6,35	0,099846154
51	G	369,02	509,73	-140,7	-0,09	6,27	0,019846154
52	U	1557,46	1140,41	417,05	0,25	6,14	-0,110153846
53	A	4311,49	3435,51	875,98	0,53	5,83	-0,420153846
54	U	7105,32	5911,04	1194,28	0,72	5,82	-0,430153846
55	C	4476,19	3341,03	1135,16	0,69	6,18	-0,070153846

56	U	8754,08	6764,31	1989,76	1,2	4,46	-1,790153846
57	U	5233,54	5082,85	150,69	0,09	4,58	-1,670153846
58	U	7727,95	6196,82	1531,13	0,93	6,8	0,549846154
59	U	700,41	149,61	550,79	0,33	6,68	0,429846154
60	G	1780,27	1623,9	156,38	0,09	6,89	0,639846154
61	C	552,58	606,01	-53,43	-0,03	7,31	1,059846154
62	C	264,08	350,71	-86,63	-0,05	6,99	0,739846154
63	G	816,51	1033,47	-216,96	-0,13	6,34	0,089846154
64	A	607,76	1182,72	-574,95	-0,35	5,68	-0,570153846
65	G	2748,77	2820,28	-71,51	-0,04	4,45	-1,800153846
66	A	2743,26	2365,64	377,62	0,23	3,67	-2,580153846
67	G	3308,18	3303,73	4,45	0	4,84	-1,410153846
68	A	3924,22	3856,38	67,84	0,04	4,8	-1,450153846
69	C	10190,87	6790,16	3400,71	2,06	4,93	-1,320153846
70	G	1124,53	774,35	350,18	0,21	4,91	-1,340153846
71	A	1298,96	1099,75	199,21	0,12	5,75	-0,500153846
72	G	3680,16	2577,86	1102,3	0,67	5,87	-0,380153846
73	U	1429,57	1083,12	346,46	0,21	5,8	-0,450153846
74	A	721,65	606,68	114,97	0,07	5,79	-0,460153846
75	G	479,98	433,77	46,22	0,03	5,8	-0,450153846
76	U	3932,71	3984,23	-51,53	-0,03	5,75	-0,500153846
77	U	4114,83	5250,67	-1135,84	-0,69	5,82	-0,430153846
78	U	13952,1	10478,46	3473,64	2,1	4,23	-2,020153846
79	C	1251,31	771,61	479,7	0,29	4,07	-2,180153846
80	G	1747,7	1388,48	359,22	0,22	3,99	-2,260153846
81	G	694,97	772,25	-77,27	-0,05	3,33	-2,920153846
82	C	3137,82	1838,38	1299,44	0,79	3,01	-3,240153846
83	C	0	21,37	-21,37	-0,01	3,05	-3,200153846
84	C	1258,28	1945,95	-687,68	-0,42	3,11	-3,140153846
85	U	4382,74	4460,57	-77,83	-0,05	3,28	-2,970153846
86	A	1374,24	972,72	401,51	0,24	6,14	-0,110153846
87	A	2556,01	2643,83	-87,83	-0,05	5,16	-1,090153846
88	C	3217,55	3040,61	176,94	0,11	6,06	-0,190153846
89	G	3368,55	2592,44	776,11	0,47	5,79	-0,460153846
90	G	775,19	699,52	75,67	0,05	6,25	-0,000153846
91	G	99,23	31,91	67,33	0,04	5,88	-0,370153846
92	U	30,69	15,5	15,2	0,01	6,38	0,129846154
93	C	1656,16	1832,54	-176,38	-0,11	6,94	0,689846154
94	C	1725,86	1550,13	175,73	0,11	7,93	1,679846154
95	A	682,16	540,15	142	0,09	8,61	2,359846154
96	A	536,01	297,72	238,3	0,14	9,14	2,889846154
97	C	10380,42	6800,37	3580,05	2,17	9,44	3,189846154
98	A	6702,46	4844,76	1857,7	1,12	9,28	3,029846154
99	U	5080,31	3112,07	1968,24	1,19	9,4	3,149846154
100	U	77,44	163,79	-86,35	-0,05	9,98	3,729846154
101	U	4598,25	3925,57	672,68	0,41	11,71	5,459846154
102	A	4061,84	3372,54	689,3	0,42	13,64	7,389846154
103	C	3862,75	3044,55	818,19	0,49	14,77	8,519846154

104	A	1786,91	1552,22	234,69	0,14	14,66	8,409846154
105	C	4896,44	3348,11	1548,33	0,94	14,27	8,019846154
106	U	4738,5	3215,47	1523,03	0,92	11,87	5,619846154
107	U	5199,33	4407,53	791,8	0,48	10,66	4,409846154
108	U	4002,25	3331,82	670,43	0,41	9,42	3,169846154
109	G	2224,53	1709,39	515,15	0,31	9,49	3,239846154
110	A	683,42	402,5	280,92	0,17	9,27	3,019846154
111	A	2439,6	1417,5	1022,1	0,62	8,83	2,579846154
112	G	8468,82	5598,64	2870,18	1,74	8,34	2,089846154
113	U	10127,62	7119,77	3007,85	1,82	7,76	1,509846154
114	U	7189,33	5138,29	2051,04	1,24	7,49	1,239846154
115	U	2245	2284,7	-39,7	-0,02	6,72	0,469846154
116	A	453,52	874,28	-420,76	-0,25	6,97	0,719846154
117	G	1531,7	1910,18	-378,48	-0,23	7,5	1,249846154
118	G	949,28	1093,81	-144,53	-0,09	7,72	1,469846154
119	G	213,41	300,25	-86,83	-0,05	8	1,749846154
120	G	316,51	278,08	38,43	0,02	8,09	1,839846154
121	A	2658,1	2344,5	313,59	0,19	6,71	0,459846154
122	G	483,42	509,69	-26,28	-0,02	4,9	-1,350153846
123	G	677,3	676,75	0,55	0	3,68	-2,570153846
124	U	958,65	1687,71	-729,05	-0,44	3,62	-2,630153846
125	A	7851,43	6739,99	1111,43	0,67	3,77	-2,480153846
126	A	3223,61	2976,27	247,34	0,15	4,29	-1,960153846
127	G	8459,56	7258,51	1201,05	0,73	4,23	-2,020153846
128	U	11111,96	9550,17	1561,79	0,94	4,53	-1,720153846
129	A	2951,63	2077,41	874,22	0,53	4,64	-1,610153846
130	U	2209,17	1457,42	751,75	0,45	4,67	-1,580153846
131	U	8398,57	7218,42	1180,15	0,71	4,87	-1,380153846
132	A	3493,94	2897,93	596,01	0,36	4,96	-1,290153846
133	C	648,3	634,41	13,89	0,01	6,28	0,029846154
134	C	646,92	622,08	24,84	0,02	5,74	-0,510153846
135	A	2106,5	2241,83	-135,34	-0,08	6,08	-0,170153846
136	C	24,9	190,42	-165,53	-0,1	6,18	-0,070153846
137	U	2714,78	2233,63	481,15	0,29	5,64	-0,610153846
138	C	1482,02	1730,79	-248,77	-0,15	5,33	-0,920153846
139	G	1060,67	640,07	420,59	0,25	5,83	-0,420153846
140	U	2254,55	2040,91	213,64	0,13	6,33	0,079846154
141	G	1319,91	950,84	369,07	0,22	6,28	0,029846154
142	G	424,02	127,88	296,13	0,18	6,25	-0,000153846
143	U	769,39	617,92	151,47	0,09	5,92	-0,330153846
144	U	10764	9303,5	1460,5	0,88	5,77	-0,480153846
145	C	4277,97	4066,6	211,37	0,13	5,82	-0,430153846
146	G	3808,17	2993	815,16	0,49	5,46	-0,790153846
147	U	6755,31	5389,67	1365,64	0,83		
148	G	3320,67	2662,11	658,56	0,4		
149	U	1226,89	862,81	364,08	0,22		
150	U	4917,26	3339,76	1577,51	0,95		
151	C	13890,65	11897,54	1993,11	1,21		

152	C	2197,49	1681,44	516,06	0,31
153	G	2731,73	2771,61	-39,89	-0,02
154	U	3680,92	4192,47	-511,55	-0,31
155	A	187,25	566,01	-378,76	-0,23
156	G	32,54	107,45	-74,91	-0,05
157	A	260,84	368,84	-107,99	-0,07
158	A	1923,71	1439,49	484,22	0,29
159	A	5037,28	4629,08	408,2	0,25