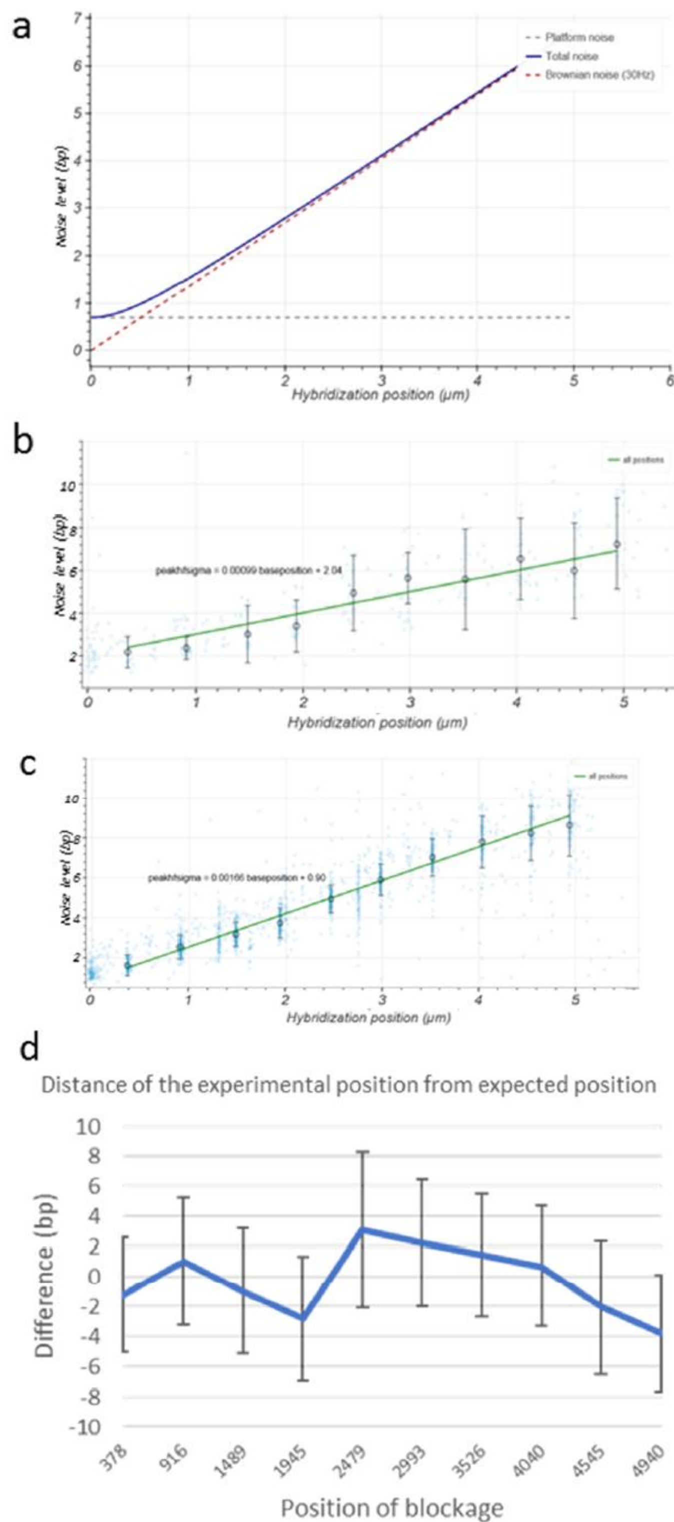
Supplemental material

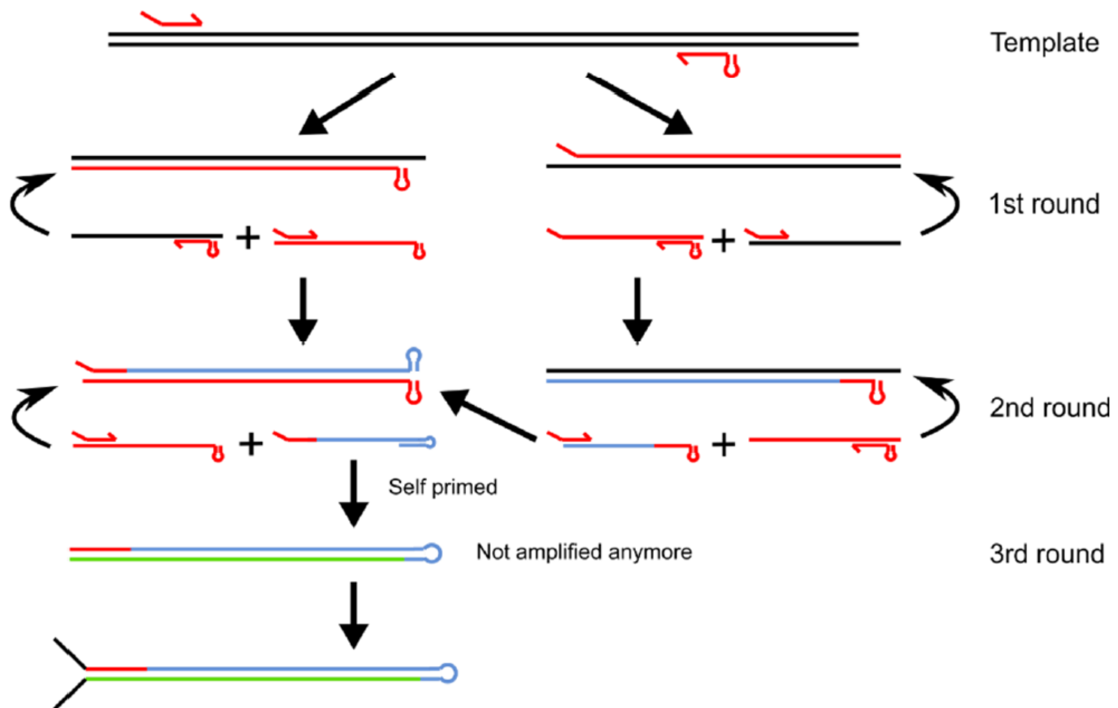
Figures and tables

Supplemental Figure 1. *Estimation of the platform noise.*



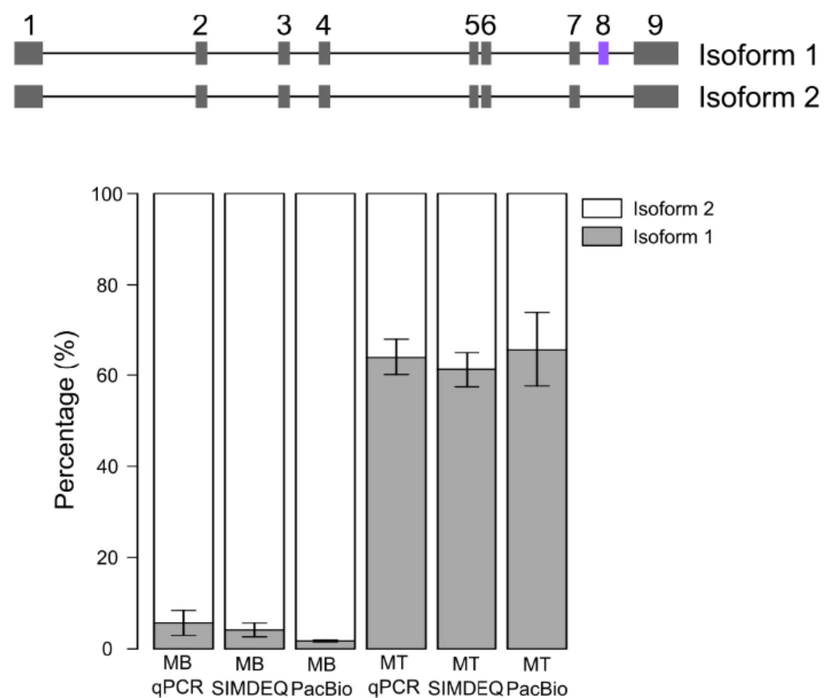
(a) Modelling of the measured total noise (dark blue line), which is composed of the platform noise (which is constant independently of the molecule length, black dotted line) plus the Brownian noise, which is dependent on the length of the molecule (red dotted line). A 5 kb hairpin was constructed to determine the platform noise by testing ten ten-base oligonucleotides along the length of the molecules. For each position, the noise was recorded at a frequency of 30 images per second and plotted against the position of blockage (each bleu point corresponds to a mapped blocking position). After fitting a linear regression, it is possible to determine the instrument noise (when the molecule length is 0), which is 2.04 bases for the original MT **(b)** and 0.90 bases for the new SDI platform **(c)**. **(d)** The difference in base pairs between the experimental and expected position for all the oligonucleotides along the 5 kb hairpin is plotted. For positions up to 1.5 kb from the Y-shape, the precision is less than 1 base and reached four bases at the 4940 bp position of the hairpin.

Supplemental Figure 2. *Principle of the loop PCR strategy.*



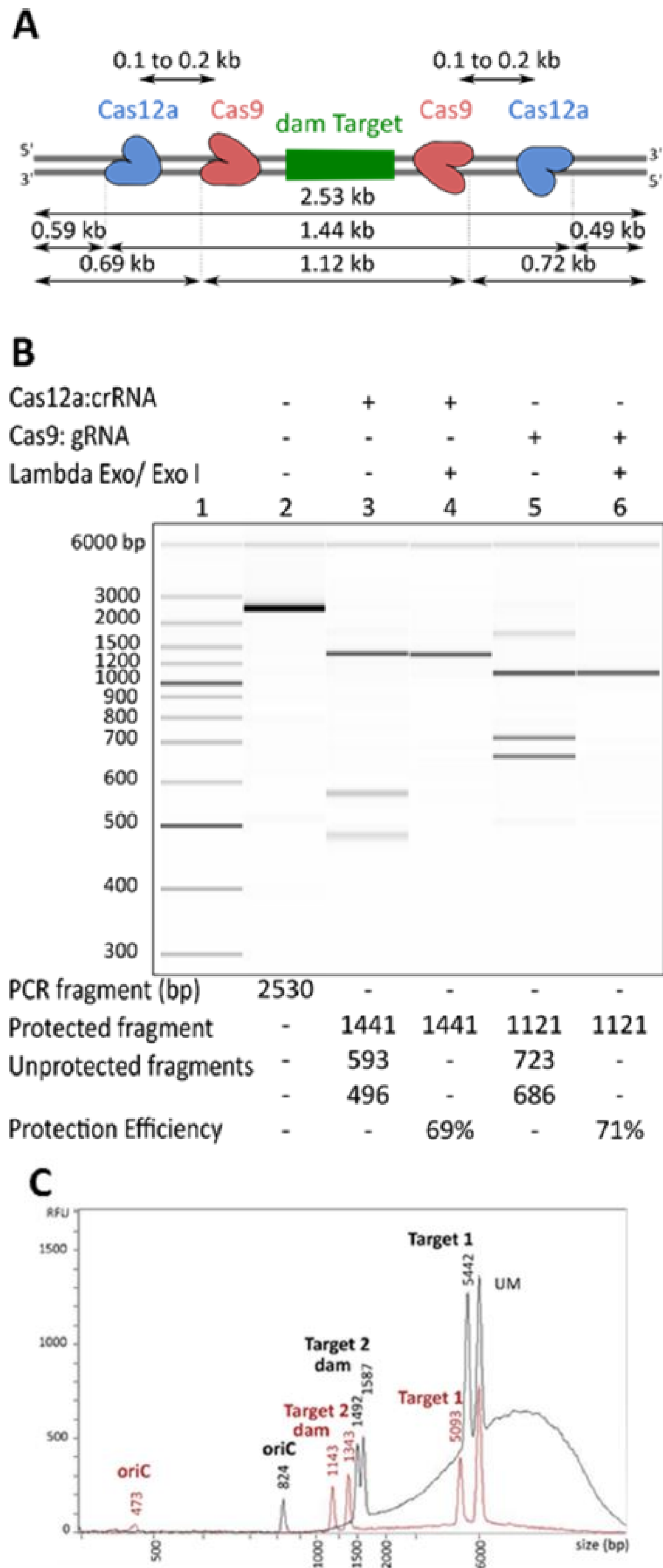
The loop PCR strategy uses a normal forward primer, but the reverse primer is designed so that it encompasses a loop. At the first cycle of the PCR, the reverse looped primer creates a DNA molecule which incorporates the loop at its 5' end, which cannot be extended. In the second cycle, when the forward primer is extended, it will copy the loop generated in the previous cycle. In the third cycle, the replicated loop, which is now at the 3’ end of the transcript, will fold back on itself and self-prime. Since this newly synthesized molecule is in a hairpin form it cannot be further amplified. The remaining single-stranded cDNAs with loops at their 5’ ends will act as templates to each form another 3' loop molecule, which leads to the desired double-stranded hairpin product.

Supplemental Figure 3. *Quantification of CAPZB in different cellular state using different techniques.*



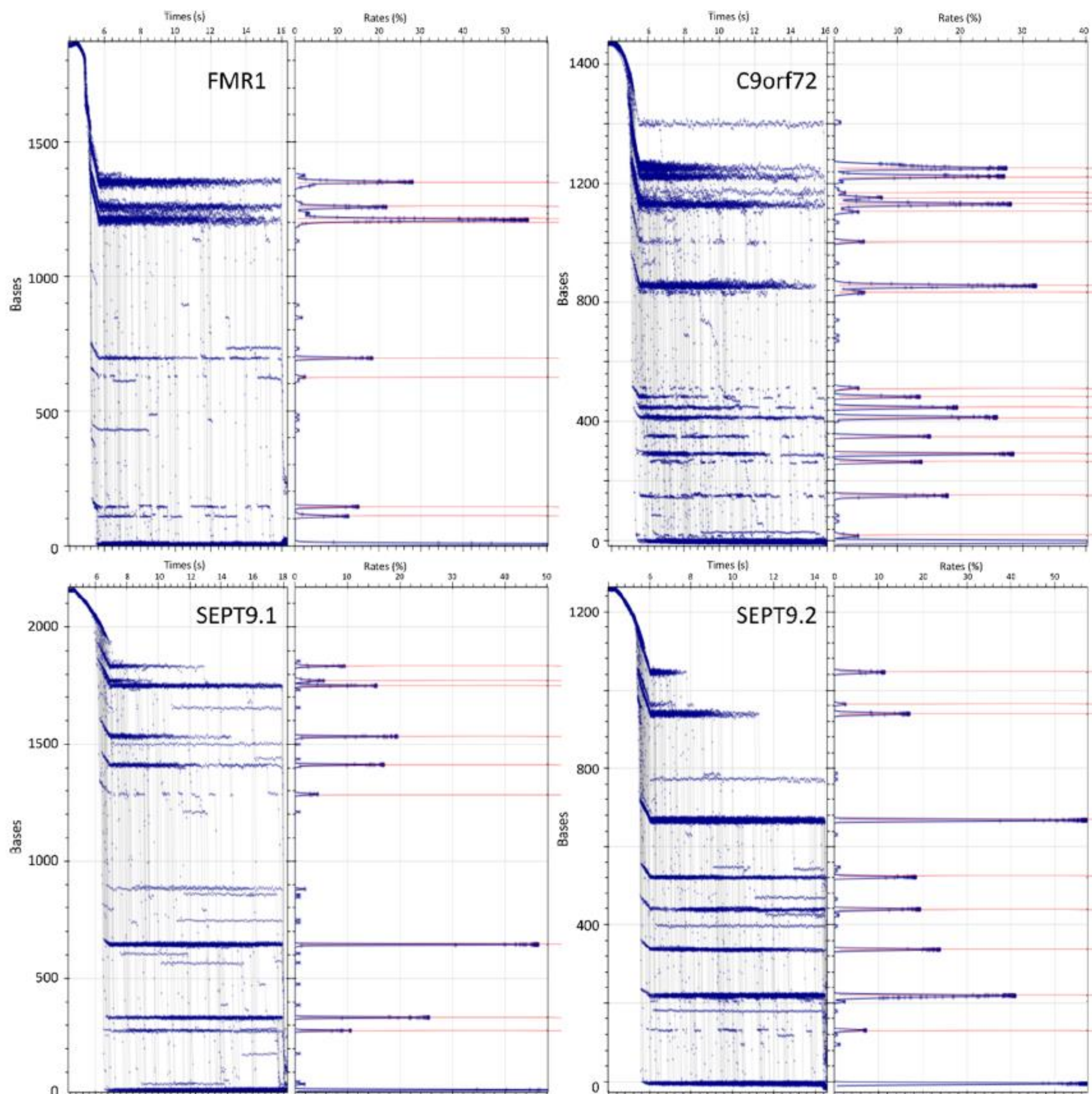
Both isoforms of CAPXB were quantified in both myoblast and myotube for the inclusion or exclusion of exon 8. Similar results were obtained independently of the technique used for quantification.

Supplemental Figure 4. *Cas meganucleases can shield the end of DNA fragments from digestion with exonucleases.*



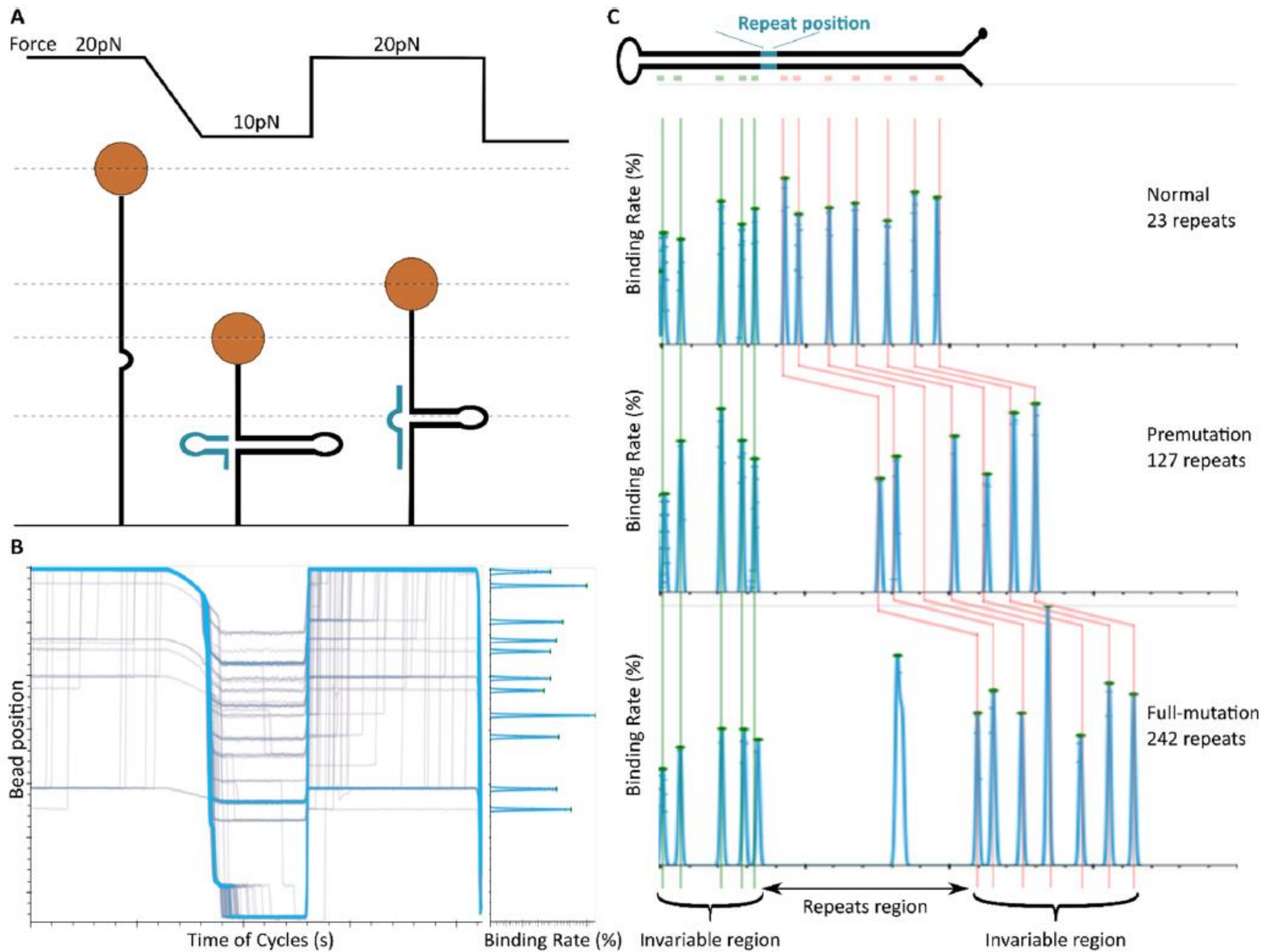
(A) Schematic representation of the experimental design of the protection assay. PCR fragment was generated such that the left- and right-side fragments resulting from digestion with Cas protein are of different sizes, allowing their simultaneous detection by capillary electrophoresis (schema not to scale). **(B)** The PCR product was incubated with either the Cas12a:crRNA complex (lanes 3-4) or the cas9:gRNA complex (lanes 5-6). After one hour of incubation, the reaction was supplemented with lambda Exo/ExoI nucleases (lanes 4 and 6) to digest all the DNA located outside the two Cas complexes. The expected fragments sizes and protection efficacy for each complex are listed below the Figure. **(C)** The protection of four targets from *E. coli* was achieved by incubating genomic DNA with all eight Cas12a:crRNA complexes corresponding to the four targets and after one hour, the reaction was supplemented with a mix of exonucleases (black outline). After this first protection step, 1/10th of the protected material was incubated with the eight dCas9:gRNA complexes and after one hour of incubation, exonucleases were added to the reaction tube (red outline). The peak corresponding to each target is marked as well as their estimated sizes. After the first protection step, a large quantity of undigested gDNA is present, indicated by the dome. This is eliminated with the second protection step. RFU: relative fluorescence unit. UM: Upper marker.

Supplemental Figure 5. *Signature produced by the oligonucleotide CAAG on the enriched targets for human gDNA.*



Expected and experimental positions of the CAAG oligonucleotide on the FMR1, C9orf72, SEPT9.1 and SEPT9.2 hairpin produced from the enriched fragments. Each red line represents one expected blockage. These specific patterns allowed us to identify and classify the functional hairpins as one of the four targets.

Supplemental Figure 6. *Principle of the repeat analysis of FMR1 using an opening assay.*



(A) An oligonucleotide designed to form a three-way junction is injected into the flow cell and the functional molecules are cycled between high and low forces. The oligonucleotides bind to the molecules during the phase of high force (20 pN) through a single-stranded end and cause a transient blockage when the force is reduced. When the force is increased to 20 pN, a three-way junction is formed and transiently prevents opening of the molecules. Measurement at high force prevents any secondary structures (for example, intra-molecule hairpins or G-quadruplexes) that could affect the measurement of the blocking position during the closing phase. **(B)** Typical opening and closing cycles for this assay (left part) and the corresponding histogram of the detected blocking positions (right part). **(C)** Schematic representation of the invariable positions of the reference oligonucleotides that bind on either side of the repeat region of the FMR1 hairpin. Green lines represent the theoretical binding position of the five oligonucleotides located upstream of the repeats and the red lines are the positions located downstream of the repeats. The extracted experimental peaks were fitted to these reference positions (X-axis) and used to calibrate the measurement of the repeat length. The graph represents three different molecules with either 23, 127 or 242 repeats. (The additional peak within the repeat region of the molecule with 242 repeats is due to a non-specific blockage of the molecule within the repeats.)

Supplemental Table 1. *CpG and Non-CpG sites on the FMR1 locus.*

				Methylated Molecules					
				WT n=11		Pre-Mutated n=9		Full-Mutated n=3	
Site Number	Position within HP	Sequence	Chr Position	Nb	Ratio	Nb	Ratio	Nb	Ratio
1	276	CG	147911340	1	0.09	0	0.00	0	0.00
2	300	CG	147911364	3	0.27	3	0.33	0	0.00
3	306	CG	147911370	1	0.09	3	0.33	0	0.00
4	321	CT	147911385	0	0.00	1	0.11	0	0.00
5	326	CG	147911390	2	0.18	1	0.11	0	0.00
6	346	CA	147911410	0	0.00	1	0.11	0	0.00
7	388	CT	147911452	0	0.00	2	0.22	0	0.00
8	397	CA	147911461	0	0.00	2	0.22	0	0.00
9	409	CG	147911473	3	0.27	2	0.22	1	0.33
10	428	CG	147911492	5	0.45	2	0.22	0	0.00
11	440	CT	147911504	0	0.00	1	0.11	0	0.00
12	443	CG	147911507	3	0.27	2	0.22	0	0.00
13	448	CT	147911512	0	0.00	1	0.11	0	0.00
14	456	CG	147911520	4	0.36	3	0.33	0	0.00
15	510	CG	147911574	4	0.36	4	0.44	0	0.00
16	512	CG	147911576	4	0.36	4	0.44	0	0.00
17	529	CG	147911593	3	0.27	2	0.22	1	0.33
18	543	CG	147911607	3	0.27	2	0.22	0	0.00
19	563	CG	147911627	6	0.55	2	0.22	0	0.00
20	593	CG	147911657	6	0.55	3	0.33	0	0.00
21	605	CG	147911669	5	0.45	2	0.22	0	0.00
22	607	CG	147911671	5	0.45	2	0.22	0	0.00
23	617	CG	147911681	4	0.36	3	0.33	0	0.00
24	625	CA	147911689	1	0.09	1	0.11	0	0.00
25	628	CG	147911692	5	0.45	5	0.56	0	0.00
26	639	CT	147911703	0	0.00	1	0.11	0	0.00
27	644	CG	147911708	5	0.45	4	0.44	0	0.00
28	660	CG	147911724	4	0.36	3	0.33	0	0.00
29	662	CG	147911726	3	0.27	3	0.33	0	0.00
30	666	CG	147911730	4	0.36	2	0.22	0	0.00
31	670	CG	147911734	2	0.18	3	0.33	0	0.00
32	675	CG	147911739	4	0.36	3	0.33	0	0.00
33	677	CG	147911741	4	0.36	3	0.33	0	0.00
34	679	CG	147911743	4	0.36	3	0.33	0	0.00
35	690	CG	147911754	5	0.45	2	0.22	0	0.00
36	695	CG	147911759	2	0.18	2	0.22	0	0.00
37	703	CG	147911767	4	0.36	2	0.22	0	0.00
38	707	CG	147911771	3	0.27	3	0.33	0	0.00
39	714	CA	147911778	1	0.09	2	0.22	0	0.00
40	720	CG	147911784	2	0.18	2	0.22	0	0.00
41	722	CG	147911786	1	0.09	2	0.22	0	0.00
42	728	CG	147911792	2	0.18	2	0.22	0	0.00
43	730	CG	147911794	2	0.18	0	0.00	0	0.00
44	732	CG	147911796	2	0.18	1	0.11	0	0.00
45	746	CA	147911810	0	0.00	3	0.33	0	0.00
46	750	CT	147911814	5	0.45	1	0.11	0	0.00
47	760	CG	147911824	5	0.45	4	0.44	0	0.00
48	766	CG	147911830	5	0.45	5	0.56	0	0.00
49	780	CG	147911844	3	0.27	3	0.33	0	0.00
50	782	CG	147911846	3	0.27	3	0.33	0	0.00
51	798	CG	147911862	3	0.27	4	0.44	0	0.00
52	807	CG	147911871	3	0.27	2	0.22	2	0.67
53	812	CG	147911876	4	0.36	2	0.22	2	0.67
54	815	CA	147911879	1	0.09	1	0.11	0	0.00
55	820	CA	147911884	3	0.27	2	0.22	0	0.00
56	829	CA	147911893	4	0.36	3	0.33	0	0.00
57	833	CG	147911897	3	0.27	2	0.22	0	0.00
58	838	CG	147911902	4	0.36	3	0.33	0	0.00
59	843	CG	147911907	3	0.27	4	0.44	0	0.00
60	851	CG	147911915	4	0.36	4	0.44	0	0.00
61	856	CA	147911920	2	0.18	4	0.44	0	0.00
62	865	CG	147911929	3	0.27	5	0.56	0	0.00
63	875	CG	147911939	6	0.55	6	0.67	0	0.00
64	880	CG	147911944	5	0.45	6	0.67	0	0.00
65	892	CG	147911956	4	0.36	5	0.56	0	0.00
66	899	CT	147911963	0	0.00	4	0.44	0	0.00
67	903	CG	147911967	4	0.36	5	0.56	0	0.00
68	913	CG	147911977	5	0.45	7	0.78	0	0.00
69	917	CG	147911981	4	0.36	4	0.44	0	0.00
70	920	CG	147911984	3	0.27	2	0.22	0	0.00
71	925	CG	147911989	4	0.36	5	0.56	0	0.00
72	928	CG	147911992	4	0.36	5	0.56	0	0.00
73	931	CG	147911995	5	0.45	3	0.33	0	0.00
74	935	CG	147911999	1	0.09	5	0.56	0	0.00
75	937	CG	147912001	0	0.00	2	0.22	0	0.00
76	941	CG	147912005	3	0.27	4	0.44	0	0.00
77	944	CG	147912008	3	0.27	3	0.33	0	0.00
78	947	CG	147912011	3	0.27	3	0.33	0	0.00
79	953	CG	147912017	4	0.36	5	0.56	0	0.00
80	957	CT	147912021	2	0.18	1	0.11	0	0.00
81	959	CG	147912023	4	0.36	3	0.33	0	0.00
82	962	CG	147912026	3	0.27	5	0.56	0	0.00
83	963	CT	147912027	3	0.27	2	0.22	0	0.00
84	968	CA	147912032	3	0.27	3	0.33	0	0.00
85	975	CG	147912039	4	0.36	3	0.33	0	0.00
86	979	CG	147912043	3	0.27	5	0.56	0	0.00
87	985	CG	147912049	4	0.36	5	0.56	0	0.00

List of all the positions and the rate of methylation across the molecule of the DNA sample NA06896.