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Supplemental information

**Expanded targeting scope of LbCas12a variants
allows editing of multiple oncogenic mutations**

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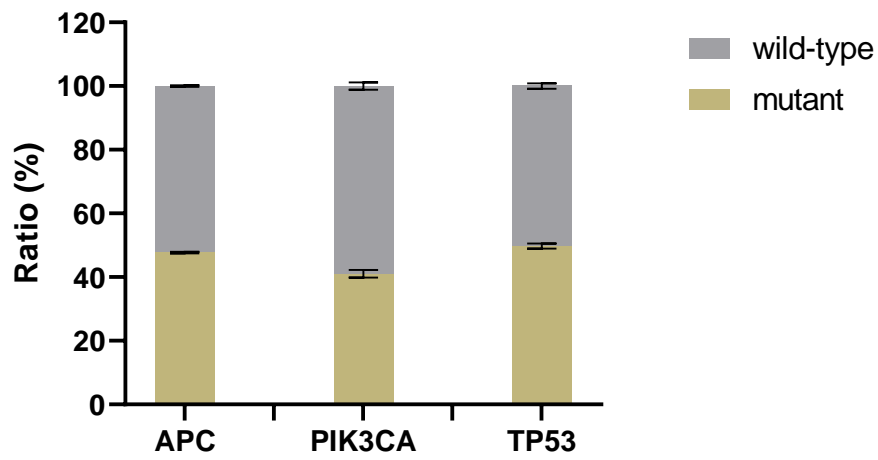


Figure S1. Ratios of wild-type vs mutant *APC*, *PIK3CA*, and *TP53* sequences in HCT-15 colon cancer cells. Genomic DNA was isolated from HCT-15 cells and analyzed using deep sequencing.

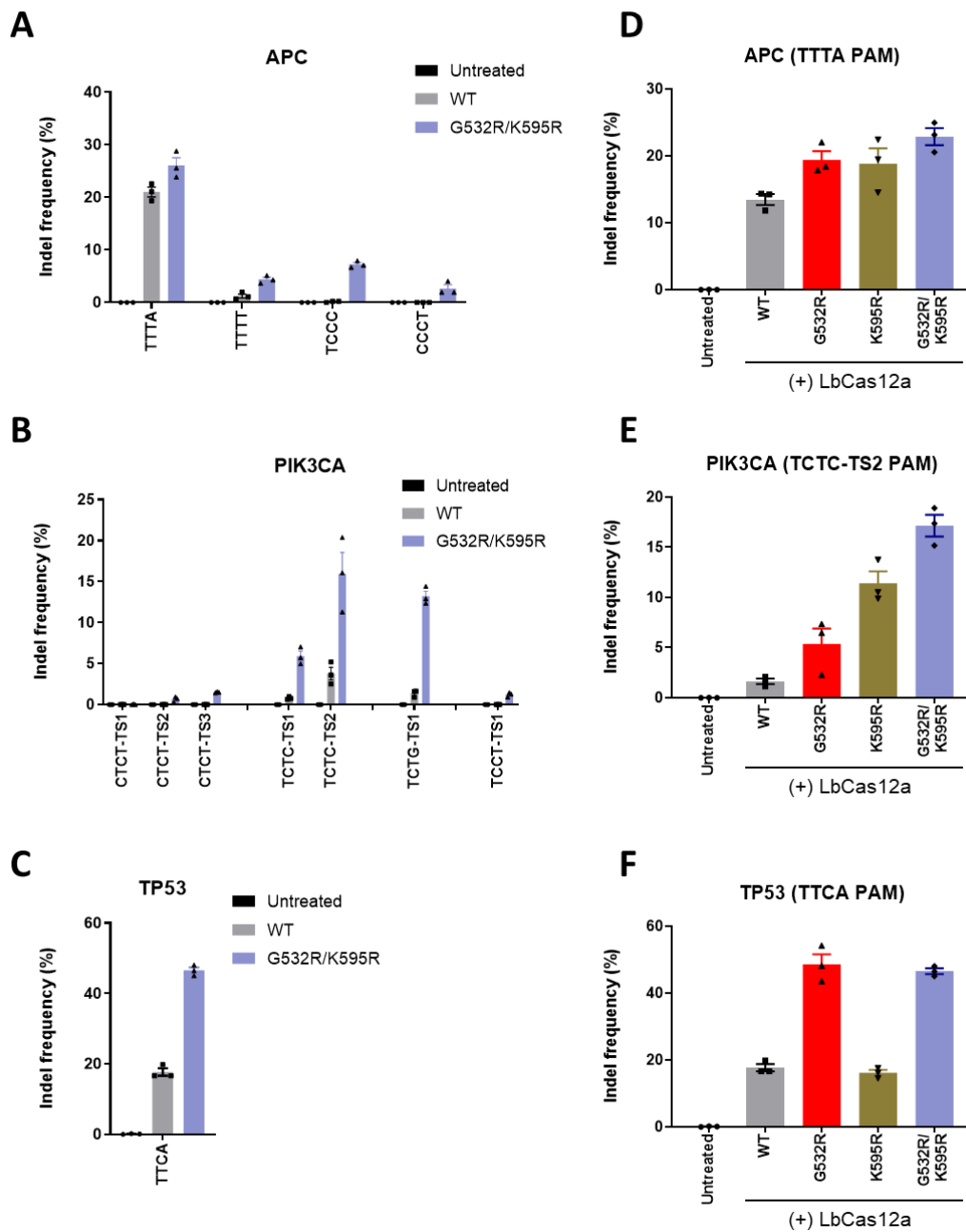


Figure S2. Indel frequencies mediated by engineered LbCas12a-mediated at oncogenic sites. (A-C) Efficiencies of LbCas12a and the LbCas12a-G532/K595R at endogenous target sites in the *APC* (A), *PIK3CA* (B), and *TP53* (C) genes in HCT-15 colon cancer cells. Mean indel frequencies \pm s.e.m. are shown. Dots represent individual data points. (D-F) Comparison of editing efficiencies of LbCas12a variants in HCT-15 cells transfected with crRNAs that resulted in the highest indel frequencies in the *APC* (D), *PIK3CA* (E), or *TP53* (F) genes.

Table S1. Potential off-target sites of LbCas12a identified by Digenome-seq.³⁰ Mismatched nucleotides are shown in red. On, on- target sites; Off, off-target site.

PAM variant	Number of cleavage sites
TTN	37
TTCN	2
TCTN	1
CTTN	1

Target	PAM	Location	Target sequence (5' to 3')
On	TTG	chr2	TCCTCCGGTTCTGGAACCACACC
Off	TTC	chr2	TCCTCCGGTTCTGGAACCgAtC
Off	TTC	chr17	TCCTCCGGTTCTGGAACCgAtt
On	TTC	chr19	CTGATGGTCCATGTCTGTACTC
Off	TTC	chr5	CTGATGGTCCATacCTGTTAacg
On	TTT	chr19	GTGGGCAACATGCTGGTCATCCT
Off	CTG	chr5	GTGGGCAACtcGcTGTCATgtT

Table S2. The crRNA target sequences for the experiments shown in Figures 1 and 2. Target sequences are shown in black and PAM sequences in blue.

PAM	Gene	No.	Sequence (5' to 3')
TCTN	TP53	1	TCTACAAGCAGTCACAGCACATGACGG
		2	TCTTGGCCAGTTGGCAAAACATCTTGT
		3	TCTGAGCAGCGCTCATGGTGGGGCAG
		4	TCTCCTAGGTTGGCTCTGACTGTACCA
	APC	5	TCTATCCCCTGATTCAGAAAATTTGA
		6	TCTTAACAGAGGTCATCTCAGAACAAAG
		7	TCTGCCGCTCAGCATCATGTGAGCCGG
		8	TCTCAGAACAAGCATGAAACCGGCTCA
	PIK3CA	9	TCTACTATGAGGTGAATTGAGGTCCCT
		10	TCTTGGGGGCATCAAGTGGATGCCCA
		11	TCTGAACGTTTGTAAAGAAGCTGTGGA
		12	TCTCCAAATAATGACAAGCAGAAGTAT
TTCN	TP53	13	TTCATGCCGCCCATGCAGGAAGTCTTA
		14	TTCTGTTCATCCAAATACTCCACACGCA
		15	TTCCACATAGTGTGGTGGTCCCTATG
		16	TTCCACACCCCGCCCGGCACCCGCGT
	APC	17	TTCATTCTGCCGCTCAGCATCATGTGA
		18	TTCTGCCGCTCAGCATCATGTGAGCCG
		19	TTCCGACATATCATCCTTATCATGAG
		20	TTCCCGGGCAGTAAAGAGGCTCGGGC
	PIK3CA	21	TTCACTTGAAGAAGTTGATGGAGGGG
		22	TTCTTAAATAGTTTCATGCTTTATGGT
		23	TTCTGTAAGTGTACTCAAGAAGCAGAA
		24	TTCCGAAGAAATATTCTGAACGTTTGT
TCCN	TP53	25	TCCACACCCCGCCCGGCACCCGCGTC
		26	TCCTCAGCATCTTATCCGAGTGAAGG
		27	TCCGTCATGTGCTGTGACTGCTTGTAG
		28	TCCCCTGCCCTCAACAAGATGTTTGC
	APC	29	TCCATCTTAACAGAGGTCATCTCAGAA
		30	TCCTTGACCTTCAATTCTGCCGCTCAGC
		31	TCCGCAATGCCAAAAAGAAAAAGCCT
		32	TCCCAACAATACAGAGTCTTGTGCATT
	PIK3CA	33	TCCAACAACCTCTTCAAGATGAATCTT
		34	TCCTGATTGCTTCAGCAATTAAGTGT
		35	TCCGAAGAAATATTCTGAACGTTTGT
		36	TCCCCTTGGGTTATAAATAGTGCCTC
CTCN	TP53	37	CTCATGGTGGGGGCAGCGCCTCACAAC
		38	CTCTTAGGTCTGGCCCTCCTCAGCAT
		39	CTCGATAAGATGCTGAGGAGGGGCCA
		40	CTCCGTCATGTGCTGTGACTGCTTGTA
	APC	41	CTCACATGATGCTGAGCGGCAGAAATGA
		42	CTCTGTATTGTTGGGAAATCCCGGGG
		43	CTCGGATTCACGCCTGCCTCTCTTGT
		44	CTCCACTCCTTGACCTTCATTCTGCC
	PIK3CA	45	CTCAAGAAGCAGAAAGGGAAGAATTTT
		46	CTCTGAACAACATAAACTCTGTGTTTT
		47	CTCGAAGTATGTTGCTATCCTCTGAAC
		48	CTCCATCAACTTCTTCAAGATGAATCT
TTTN	TP53	49	TTTAAATGGGACAGGTAGGACCTGATT
		50	TTTTGCCAACTGGCCAAGACCTGCCCT
		51	TTTGCCAACCTGGCCAAGACCTGCCCTG
		52	TTTCCTTCCACTCGGATAAGATGCTGA
	APC	53	TTTACTGCCCGGGAATTTCCCAACAA
		54	TTTTTTGGCATTGGCGGAGCTTATACAT
		55	TTTGGCATGCGGAGCTTATACATTC
		56	TTTCTCCCACTCCTTGACCTTCATTCT
	PIK3CA	57	TTTACAAACGTTCAGAATATTTCTTCG
		58	TTTGTAGTTGTTTCAAGGATAGCAACAT
		59	TTTGATGAAACAAGACGACTTTGTGAC
		60	TTTCGTCTTCTTGGTAAACACTTACGA

Table S3. Summary of indel frequencies generated by the engineered LbCas12a variants at sites with non-canonical and canonical PAMs.

	(+) Cas12a				
	Untreated	WT	G532R	K595R	G532R/ K595R
TCTN	0.0	2.7	5.5	9.6	12.4
TTCN	0.0	10.1	23.5	11.3	23.2
TCCN	0.0	2.1	13.9	6.3	16.9
CTCN	0.0	0.4	6.9	0.8	6.6
TTTN	0.0	29.9	28.0	32.8	28.9

Indel frequency (%)

Table S4. The crRNA target sequences for the experiments shown in Figure 3. Target sequences are shown in black and PAM sequences in blue.

PAM	Gene	No.	Sequence (5' to 3')
TCCT	<i>TP53</i>	1	TCCTCAGCATCTTATCCGAGTGGGAAGG
	<i>APC</i>	2	TCCTTGACCTTCATTTCTGCCGCTCAGC
	<i>CTNNB1</i>	3	TCCTTCGGGCTGGTGACAGGGAAGACA
	<i>SRSF3</i>	4	TCCTGTCCATTGGACTGTAAGGTTTAT
TTCT	<i>TP53</i>	5	TTCTGTCATCCAAATACTCCACACGCA
	<i>APC</i>	6	TTCTGCCGCTCAGCATCATGTGAGCCG
	<i>CTNNB1</i>	7	TTCTGCATCATCTTGATAGTTAATCAA
TCTT	<i>SRSF3</i>	8	TTCTAGAGATAGGAGAAGAGAGAGATC
	<i>TP53</i>	9	TCTTGGCCAGTTGGCAAAACATCTTGT
	<i>APC</i>	10	TCTTAAACAGAGGTCATCTCAGAACAAG
CTCT	<i>CTNNB1</i>	11	TCTTACCTGGACTCTGGAATCCATTCT
	<i>SRSF3</i>	12	TCTTGGAAACAATGGCAACAAGACGGA
	<i>TP53</i>	13	CTCTTAGGTCTGGCCCTCCTCAGCAT
CCTT	<i>APC</i>	14	CTCTGTATGTTGGGAAATCCCAGGGG
	<i>CTNNB1</i>	15	CTCTGTTCAGCTTCTGGGTTGATG
	<i>SRSF3</i>	16	CTCTTACACGGCAGCCACATAGTGTT
CCTT	<i>TP53</i>	17	CCTTCCACTCGGATAAGATGCTGAGGA
	<i>APC</i>	18	CCTTCATTCTGCCGCTCAGCATCATGT
	<i>CTNNB1</i>	19	CCTTAGTCCAAAGGCTCAGGCCAGAAA
CTTT	<i>SRSF3</i>	20	CCTTACAGTCCAATGGACAGGAATCAC
	<i>TP53</i>	21	CTTTTCGACATAGTGTGGTGGTGCCT
	<i>APC</i>	22	CTTTACTGCCCCGGGAATTTCCAACA
CCCT	<i>CTNNB1</i>	23	CTTTACCACTCAGAGAAGGAGCTGTGG
	<i>SRSF3</i>	24	CTTTGGCTACTATGGACCACTCCGAA
	<i>TP53</i>	25	CCCTGTGCAGCTGTGGGTTGATTCCAC
TTTT	<i>APC</i>	26	CCCTGGCCCGAGCCTCTTTACTGCCCC
	<i>CTNNB1</i>	27	CCCTTAGTCCAAAGGCTCAGGCCAGAA
	<i>SRSF3</i>	28	CCCTCAGAACACTATGTGGCTGCCGTG
TTTT	<i>TP53</i>	29	TTTTGCCAACTGGCCAAGACCTGCCCT
	<i>APC</i>	30	TTTTTGGCATTGCGGAGCTTATACAT
	<i>PIK3CA</i>	31	TTTTAGTTGTTTCCAGAGGATAGCAACAT
TTTT	<i>CTNNB1</i>	32	TTTTCCCTTCCGGAGCAGGGTTCTGCC
	<i>SRSF3</i>	33	TTTTGGCTACTATGGACCACTCCGAAG

Table S5. The crRNA target sequences for the experiments shown in Figure 4. Target sequences are shown in black and PAM sequences in blue. Adenines (As) shown in red in the sequences represent targetable nucleotides in the LbABE8e target window (positions 6 to 16 in the protospacer, counting from the PAM-proximal base).

PAM	Gene	No.	Sequence (5' to 3')
TCTN	<i>TP53</i>	1	TCTACAAGCAGTCA CAGCA CATGACGG
	<i>PIK3CA</i>	2	TCTACTATGAGGTGAATTGAGGTCCCT
	<i>APC</i>	3	TCTTAACAGAGGTCA TCTCAGA ACAAG
	<i>SRSF3</i>	4	TCTTGAAACA ATGGCAACA AAGACGGA
TTCN	<i>TP53</i>	5	TT CATG CCGCC CA TGCAGGA ACT GTTA
	<i>APC</i>	6	TT CAT TCTGCCGCT CAGCA TCATGTGA
	<i>SRSF3</i>	7	TTCTAGAGATAGG AGAAGA GAGAGATC
	<i>TP53</i>	8	TTCTGTCATCC AAATA CTCCACACGCA
TTCN	<i>CTNNB1</i>	9	TTCTGCATCA TCTTGA TAGTTAATCAA
	<i>APC</i>	10	TT CGG GACAT ATCA TCCT TAT CATGAG
	<i>PIK3CA</i>	11	TT CGT AAAGT TTACTCA AGAAGCAGAA
	<i>TP53</i>	12	TTCCACACCCCGCCCGGC ACCC GGGT
TCCN	<i>APC</i>	13	TTCCCGGGGC AGTAAAG AGGCTCGGGC
	<i>TP53</i>	14	TCCACACCCCGCCCGGC ACCC GGTC
	<i>APC</i>	15	TCC ATCTTAA CAGAGGT CA TCTCAGAA
	<i>SRSF3</i>	16	TCCTGTCCATGG ACTGTAA GGTTTAT
TCCN	<i>CTNNB1</i>	17	TCCTTCGGGCTGGT GACAGGGA AGACA
	<i>TP53</i>	18	TCCGTCATGTGCTGT ACTGCT TGTAG
	<i>APC</i>	19	TCCGCAATGCC AAAAAGAA AAAGCCT
	<i>APC</i>	20	TCCCAACA ATACAG AGTCTTTGT CATT
CTCN	<i>SRSF3</i>	21	CTCTTACACGGC AGCCACA TAGTG TTC
	<i>TP53</i>	22	CTCCGTCATGTGCTGT ACTGCT TGTA

Table S6. Base editing window of LbABE8e variants at six target sites with non-canonical PAMs.

PAM (Site #)	LbABE8e										
	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
TTCA (Site 5)				3.31				2.16			0.07
TTCT (Site 8)			3.33	1.17	0.93		5.07				
TTCG (Site 11)				2.67				0.27	0.00		0.03
TTCC (Site 13)		0.41			3.62	0.54	0.35		0.05		
TCCA (Site 15)	0.02		1.81		0.88					0.11	
TCCT (Site 16)					1.48					1.32	0.06

PAM (Site #)	LbABE8e-G532R										
	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
TTCA (Site 5)				4.05				2.23			0.10
TTCT (Site 8)			5.67	1.47	1.40		7.43				
TTCG (Site 11)				5.50				0.80	0.03		0.03
TTCC (Site 13)		1.40			14.59	2.22	1.13		0.11		
TCCA (Site 15)	0.01		4.23		2.45					0.42	
TCCT (Site 16)					4.97					4.40	0.26

PAM (Site #)	LbABE8e-K595R										
	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
TTCA (Site 5)				2.49				1.78			0.02
TTCT (Site 8)			1.97	0.63	0.53		3.03				
TTCG (Site 11)				2.73				0.33	0.00		0.07
TTCC (Site 13)		0.48			6.17	0.90	0.64		0.08		
TCCA (Site 15)	0.00		1.89		0.79					0.14	
TCCT (Site 16)					4.97					4.40	0.26

PAM (Site #)	LbABE8e-G532R/K595R										
	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
TTCA (Site 5)				5.05				2.92			0.07
TTCT (Site 8)			8.50	3.07	2.77		11.17				
TTCG (Site 11)				6.43				0.97	0.10		0.13
TTCC (Site 13)		1.12			15.01	2.32	1.10		0.11		
TCCA (Site 15)	0.01		5.84		2.92					0.52	
TCCT (Site 16)					7.48					6.23	0.48

PAM (target gene)	LbABE8e-G532R/K595R										
	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16
TTTA (APC)		2.29									
TCTC (PIK3CA)	0		0			12.7	0			0	
TTCA (TP53)				0.33				0.1		2.59	0

Table S7. The crRNA target sequences in Figure 5 and Figure S2 are shown in black and PAM sequences in blue. Red colored adenine (A) in the sequences represents a missense mutation in *APC*, *PIK3CA*, and *TP53* gene which are corresponding to 6496C(G)/T(A), 1633G/A, and 722C(G)/T(A), respectively. The crRNA sequences used in the CRISPR array are marked in yellow.

Gene	PAM	No.	Sequence (5' to 3')
<i>APC</i>	TTTA	-	TTTAGAATTCATGGGCCTTTATTACTT
	TTTT	-	TTTTAGAATTCATGGGCCTTTATTACT
	TCCC	-	TCCCCTGGTTTTAGAAATTCATGGGCCT
	CCCT	-	CCCTGGTTTTAGAAATTCATGGGCCTT
<i>PIK3CA</i>		TS1	CTCTCTCTGAAATCACTAAGCAGGAGA
	CTCT	TS2	CTCTCTGAAATCACTAAGCAGGAGAAA
		TS3	CTCTGAAATCACTAAGCAGGAGAAAGA
	TCTC	TS1	TCTCTCTGAAATCACTAAGCAGGAGAA
		TS2	TCTCTGAAATCACTAAGCAGGAGAAAAG
	TCTG	TS1	TCTGAAATCACTAAGCAGGAGAAAGAT
TCCT	TS1	TCCTCTCTCTGAAATCACTAAGCAGGA	
<i>TP53</i>	TTCA	-	TTCATGCCGCCCATGCAGAAACTGTTA

Table S8. The crRNA sequences in Figure 5 are presented. The crRNA sequence targeting *APC*, *PIK3CA*, and *TP53* gene containing a missense mutation (adenine; a) are highlighted with yellow, green, and blue color, respectively. As control, the non-targeting crRNA sequence that does not target human genome are highlighted with pink color, respectively. The direct repeat and separator sequences are marked with black and red, respectively.

Multiplexed crRNA	Sequence (5' to 3')
V1	aatttctactaagtgtagatGAATTCaTGGGCCTTATTACTT aatttctactaagtgtagatTGAAATCACTaAGCAGGAGAAAGaatttctactaagtgtagatTGCCGCCCATGCAGaAA CTGTTA
V2	aatttctactaagtgtagatGAATTCaTGGGCCTTATTACTT AAATaatttctactaagtgtagatTGAAATCACTaAGCAGGAGAAAGAAATaatttctactaagtgtagatTGCCGCCCA TGCAGaAACTGTTA
V3	AAATaatttctactaagtgtagatGAATTCaTGGGCCTTATTACTTAAATaatttctactaagtgtagatTGAAATCAG TaAGCAGGAGAAAGAAATaatttctactaagtgtagatTGCCGCCCATGCAGaAACTGTTA
Non-targeting	AAATaatttctactaagtgtagatCACCTGTTCAATCCCTGCAGGAATaatttctactaagtgtagatTGAGGAGCC GGAGCCCCAAGCAAAATaatttctactaagtgtagatATTCCCTGCAGGACAACGCCCA

Table S9. List of primers used for targeted deep sequencing.

Gene.	1st PCR		2nd PCR	
	Forward (5' to 3')	Reverse (5' to 3')	Forward (5' to 3')	Reverse (5' to 3')
APC				
exon 8	GATAGTCGACCGCCAATC GT	ACAGCACATTGGTAC TGAATGC	ACACTCTTCCCTACACGACG CTCTCCGATCTAGCCTACAC CATTTTTGCATGT	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTAC CATCTTGCTTCATACTTT TCTGA
exon 11	TTTTTTTTTTGGCGGGGG GG	CTGGTCCATGCCTGG TTCAT	ACACTCTTCCCTACACGACG CTCTCCGATCTCCATGCGAC AGTCTGGATGT	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTGA TTTCACGCTGCCTCTCT
CTNNB1				
exon 1	CTCTCCACCCTATCCCTA GTTT	TGTGTGGGCTGACCT AGTAA	ACACTCTTCCCTACACGACG CTCTCCGATCTGAGGAGAGA CAGCCCTTAGT	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTCT ATGAGGTGAAGGAGGTA AA
exon 3	GCTTTCCTCTCTCCCTGC TT	CTGACTTTCAGTAAG GCAATGAA	ACACTCTTCCCTACACGACG CTCTCCGATCTCCAATCTAC TAATGCTAATACTGTTTCG	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTAC TCTTACCAGCTACTTGT CT
exon 4	CAAGAACAAGTAGCTGGT AAGA	CATGATAGCGTGTCT GGAAG	ACACTCTTCCCTACACGACG CTCTCCGATCTGAACGTGG ATAGTGAGTGTTG	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTTA CCTGGTCTCGTCATTTA G
exon 9	CTTACCTGACAGATCCA AGTC	CGCAGCCATACTTCC TACTTAC	ACACTCTTCCCTACACGACG CTCTCCGATCTATAGGAAGG GATGGAAGGTCTC	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTCA GATGACGAAGCACAGA TG
PIK3CA				
exon 2	TGCCCCAAGAATCCTAG TAGA	GCAAAGGCAGCAAAC ATTCC	ACACTCTTCCCTACACGACG CTCTCCGATCTAGCAAGAAA ATACCCCTCCA	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTTC ACGGTTGCTACTGGTTC
exon 4	GGCAGCCCGCTCAGATAT AA	CTGGGCGAGAGTGAG ATTCC	ACACTCTTCCCTACACGACG CTCTCCGATCTGGCAAATAA TAGTGGTGATCTGGG	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTAG GAAGTATTCATCACATCC ACACA
SRSF3				
exon 2	GGCTTCCTGAGGGTTAGG AG	CAGTGATTGGGAAAG CCATC	ACACTCTTCCCTACACGACG CTCTCCGATCTAGAAATGCA TCGTGATTCTCG	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTGC CATCTAAGAGCCACATAT CC
exon 3	GCCTCATAAAGTGTGGG ATTAC	CCAACATGAACAGTG ACCTAAAG	ACACTCTTCCCTACACGACG CTCTCCGATCTGCCTCATAA AGTGTGGGATTAC	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTGG GCCACGATTTCTACTTCT
exon 5	GGAGTGCAGTGGTGTGAT TA	CATCTGTAACCTGGT GACACTTTAG	ACACTCTTCCCTACACGACG CTCTCCGATCTGAGTACCA TGCCAGATCAA	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTGG CTTAGAAGACAGGCTTA G
TP53				
exon 4	GCACCACCACACTATGTC GA	CGCCAACTCTCTCTA GCTCG	ACACTCTTCCCTACACGACG CTCTCCGATCTGTGAGGAAT CAGAGGCCTGG	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTCC CTGCCCAACAAGATGT
exon 5	TAAGCAGCAGGAGAAAGC CC	CTACAGTACTCCCT GCCCT	ACACTCTTCCCTACACGACG CTCTCCGATCTTAAGCAGCA GGAGAAAGCCC	GTGACTGGAGTTCAGACG TGTGCTCTCCGATCTCC AGGCCTCTGATTCCTCAC