

Supplementary materials

Supp. Table 1 (S1)

Primers used to amplify *Ctnnb1* and *Kras* for Endpoint PCR and E-ice-COLD-PCR

Primer	Name	Length	Tm	Sequence	Product
Forward	Ctnnb1	20	60,37	CACTGGCAGCAGCAGTCTTA	111
Reverse		22	59,98	GGAGGTGTCAACATCTTCTTCC	
Forward	Kras	20	58,46	AGGCCTGCTGAAAATGACTG	135
Reverse		23	61,61	AGCAGCGTTACCTCTATCGTAGG	

Supp. Table 2 (S2)

Blocker probes used to *Ctnnb1* and *Kras* in E-ice-COLD-PCR

Probe	Sequence
Ctnnb1_BP_41	TCTTACTTG+G+AT+T+C+TG+G+AATCCAT+T+C+TGGTGCCACCA+C+C+AC/3Phos
Kras_BP_42	GACTGAGTATAAACTTGTGGTGGTTGGAGCTG+G+TGCGTAGG/3Phos

Locked nucleic acid (LNA): +A, +G, +C, and +T

Supp. Table 3 (S3)

Mutations detected in the tumor samples with E-ice-COLD-PCR and endpoint PCR together with massive parallel sequencing

ID	Method	Gene	Type of change	Transcript	Exon	cfDNA change	Protein change	Allelic fraction
EPCR1	E-PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.94G>A	p.D32N	18.44
						c.101G>A	p.G34E	2.04
						c.122C>T	p.T41I	19.92
EPCR3	E-PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.94G>A	p.D32N	22.69
						c.98C>T	p.S33F	2.85
						c.101G>A	p.G34E	3.14
EPCR8	E-PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.122C>T	p.T41I	13.4
						c.94G>A	p.D32N	5.84
						c.101G>A	p.G34E	3.22
EPCR10	E-PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.110C>T	p.S37F	9.05
						c.122C>T	p.T41I	11.79
						c.94G>A	p.D32N	22.43
EPCR16	E-PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	9.93
						c.98C>T	p.S33F	2.33
						c.110C>T	p.S37F	5.39
PCR1	PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.122C>T	p.T41I	1.0
						c.94G>A	p.D32N	5.57
						c.101G>A	p.G34E	13.71
PCR3	PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.122C>T	p.T41I	2.54
						c.94G>A	p.D32N	11.53
						c.98C>T	p.S33F	2.4
PCR8	PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	2.65
						c.122C>T	p.T41I	3.44
						c.94G>A	p.D32N	4.38
PCR10	PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	3.47
						c.110C>T	p.S37F	11.16
						c.122C>T	p.T41I	7.21
PCR16	PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.94G>A	p.D32N	9.85
						c.101G>A	p.G34E	14.67
						c.98C>T	p.S33F	2.56
PCR16	PCR	Ctnnb1	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	9.58
						c.110C>T	p.S37F	2.84
						c.122C>T	p.T41I	5.95

PCR: Endpoint PCR, E-PCR: E-ice-COLD-PCR

Supp. Table 4 (S4)

Mutations detected in the cfDNA samples with E-ice-COLD-PCR and endpoint PCR together with massive parallel sequencing

Day	Step	ID	Method	Gene	Type of change	Transcript	Exon	cfDNA change	Protein change	Allelic fraction	
01	Normal	M1	E-PCR	ND	ND	ND	ND	ND	ND	ND	
		M2	E-PCR								
		M3	E-PCR								
		M1	PCR	ND	ND	ND	ND	ND	ND	ND	
		M2	PCR								
		M3	PCR								
21	ACF	M4	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	9.66	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	12.32	
		M5	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	3.67	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	12.05	
		M6	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	7.55	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	34.75	
		M4	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_007614.3	3	c.101G>A	p.G34E	2.31	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	9.65	
		M5	PCR	ND	ND	ND	ND	ND	ND	ND	ND
		M6	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	3.42	
				<i>Kras</i>	nonsynonymous SNV	NM_007614.3	2	c.35G>A	p.G12D	12.23	
		28	Adenoma	M7	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.94G>A	p.D32N
<i>Ctnnb1</i>	nonsynonymous SNV					NM_021284.6	3	c.98C>T	p.S33F	8.6	
<i>Ctnnb1</i>	nonsynonymous SNV					NM_021284.6	3	c.110C>T	p.S37F	2	
<i>Ctnnb1</i>	nonsynonymous SNV					NM_021284.6	3	c.122C>T	p.T41I	1.99	
<i>Kras</i>	nonsynonymous SNV					NM_021284.6	2	c.35G>A	p.G12D	19.56	
M8	E-PCR			<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	2.48	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	28.4	
M9	E-PCR			<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.98C>T	p.S33F	2.39	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	29.08	
M7	PCR			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	13.14	
M8	PCR			<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	3.05	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	5.56	
M9	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.98C>T	p.S33F	4.14			
		<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	5.39			
35	Adenoma	M10	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.94G>A	p.D32N	7.68	
				<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.98C>T	p.S33F	1.6	
				<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	1.05	
				<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.122C>T	p.T41I	6.59	
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	36.36	

	M11	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	11.7	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	50.4	
	M12	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	27.37	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	54.54	
	M10	PCR	ND	ND	ND	ND	ND	ND	ND	
	M11	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	2.8	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	27.47	
	M12	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	6.97	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	7.92	
	42	M13	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	25.18
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	43.4
		M14	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.122C>T	p.T41I	20.71
M15		E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.122C>T	p.T41I	20.81	
M13		PCR	ND	ND	ND	ND	ND	ND	ND	
M14		PCR	ND	ND	ND	ND	ND	ND	ND	
M15		PCR	ND	ND	ND	ND	ND	ND	ND	
49	M16	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	8.7	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	59.72	
	M17	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	6.59	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	60.79	
	M18	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	15.37	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	20.65	
	M16	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	4.38	
	M17	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	6.489	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	13.07	
	M18	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	6.7	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	23.34	
	56	M19	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.94G>A	p.D32N	1.43
<i>Ctnnb1</i>				nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	1.66	
<i>Ctnnb1</i>				nonsynonymous SNV	NM_021284.6	3	c.110C>T	p.S37F	1.37	
<i>Kras</i>				nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	10.12	
M20		E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.94G>A	p.D32N	6.8	
			<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.98C>T	p.S33F	2.11	
			<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	3.18	
M21		E-PCR	<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	1.23	
			<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	2.23	
M19		PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.110C>T	p.S37F	1.4	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	35.49	
M19	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	3.68		

			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	5.19	
	M20	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	3.51	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	7.68	
	M21	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	3.55	
			<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	2.21	
70	Carcinoma	M22	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	3.4
		M23	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	1.0
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	18.91
		M24	E-PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	11.04
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	3
		M22	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	2.9
		M23	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	2.8
				<i>Kras</i>	nonsynonymous SNV	NM_021284.6	2	c.35G>A	p.G12D	2.17
		M24	PCR	<i>Ctnnb1</i>	nonsynonymous SNV	NM_021284.6	3	c.101G>A	p.G34E	6.48

PCR: Endpoint PCR, E-PCR: E-ice-COLD-PCR

Supp. Table 5 (S5)

Mutations detected in the tumor samples with endpoint PCR together with massive parallel sequencing

Sample	ID	Chr	Star	REF	ALT	Func.refGene	Gene.refGene	ExonicFunc.refGene	AChange.refGene	GT	AF	DP
Tumor	EPCR1	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.1844	491
Tumor	EPCR1	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0204	499
Tumor	EPCR1	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.1992	505
Tumor	EPCR3	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.2269	411
Tumor	EPCR3	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T:p.S33F	0/1	0.0285	420
Tumor	EPCR3	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0314	419
Tumor	EPCR3	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.134	430
Tumor	EPCR8	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.0548	433
Tumor	EPCR8	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0322	445
Tumor	EPCR8	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T:p.S37F	0/1	0.0905	456
Tumor	EPCR8	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.1179	456
Tumor	EPCR10	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.2243	374
Tumor	EPCR10	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0993	383
Tumor	EPCR16	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T:p.S33F	0/1	0.0233	457
Tumor	EPCR16	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0539	436
Tumor	EPCR16	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T:p.S37F	0/1	0.01	446
Tumor	EPCR16	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.0557	441

Tumor	PCR1	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.1371	467
Tumor	PCR1	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0254	479
Tumor	PCR1	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.1153	483
Tumor	PCR3	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.1137	440
Tumor	PCR3	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T:p.S33F	0/1	0.024	436
Tumor	PCR3	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0265	459
Tumor	PCR3	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.0344	465
Tumor	PCR8	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.0438	501
Tumor	PCR8	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0347	506
Tumor	PCR8	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T:p.S37F	0/1	0.1116	513
Tumor	PCR8	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.0721	519
Tumor	PCR10	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.0985	507
Tumor	PCR10	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.1467	521
Tumor	PCR16	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T:p.S33F	0/1	0.0256	543
Tumor	PCR16	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0958	547
Tumor	PCR16	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T:p.S37F	0/1	0.0284	565
Tumor	PCR16	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T:p.T41I	0/1	0.0595	564

Supp. Table 6 (S6)

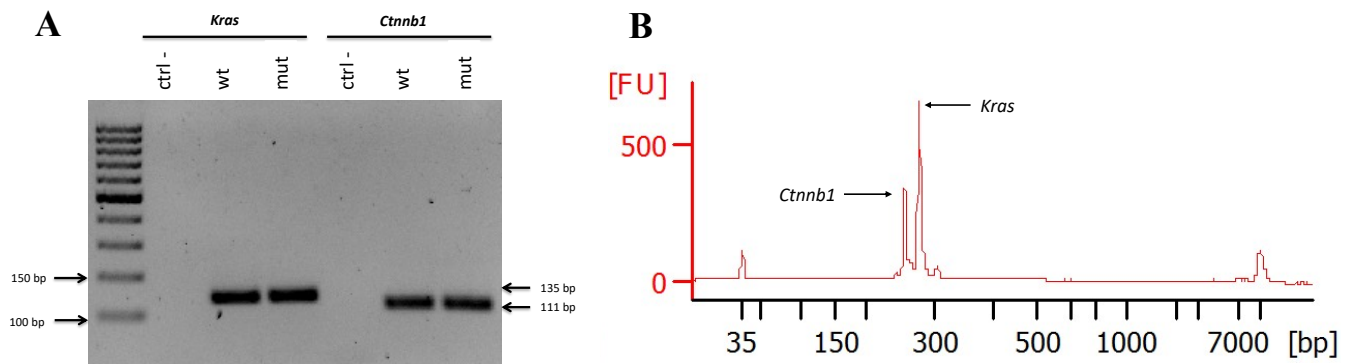
Mutations detected in the cfDNA samples with E-ice-COLD-PCR and endpoint PCR together with massive parallel sequencing

Sample	ID	Chr	Star	REF	ALT	Func.refGene	Gene.refGene	ExonicFunc.refGene	AAChange.refGene	GT	AF	DP
cfDNA	EPCR4	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.1232	1437
cfDNA	EPCR4	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0966	863
cfDNA	EPCR5	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.1205	1780
cfDNA	EPCR5	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0367	1063
cfDNA	EPCR6	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.3475	1127
cfDNA	EPCR6	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0755	1292
cfDNA	EPCR7	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.1956	1806
cfDNA	EPCR7	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.089	808
cfDNA	EPCR7	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T;p.S33F	0/1	0.086	925
cfDNA	EPCR7	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T;p.S37F	0/1	0.02	987
cfDNA	EPCR7	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T;p.T41I	0/1	0.019	803
cfDNA	EPCR8	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.284	1072
cfDNA	EPCR8	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0239	1042
cfDNA	EPCR9	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.2908	1828
cfDNA	EPCR9	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T;p.S33F	0/1	0.0239	758
cfDNA	EPCR10	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.3636	1483
cfDNA	EPCR10	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A:p.D32N	0/1	0.0768	467
cfDNA	EPCR10	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T;p.S33F	0/1	0.016	467
cfDNA	EPCR10	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0105	483
cfDNA	EPCR10	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T;p.T41I	0/1	0.0659	483
cfDNA	EPCR11	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.504	1664
cfDNA	EPCR11	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.117	1237
cfDNA	EPCR12	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.5454	1759
cfDNA	EPCR12	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.2737	923

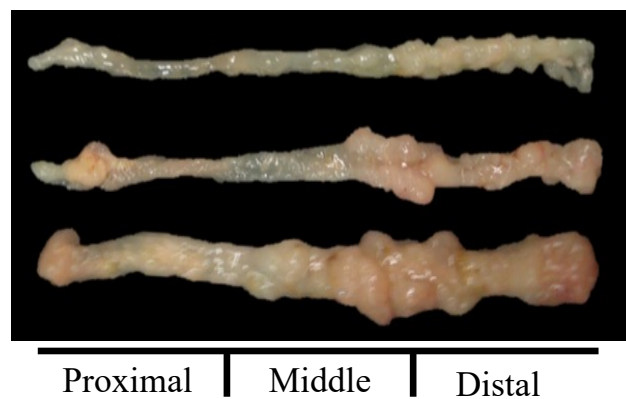
cfDNA	EPCR13	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.434	1440
cfDNA	EPCR13	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.2518	1114
cfDNA	EPCR14	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T;p.T41I	0/1	0.2071	1518
cfDNA	EPCR15	chr9	120950630	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C122T;p.T41I	0/1	0.2081	1428
cfDNA	EPCR16	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.5972	1325
cfDNA	EPCR16	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.087	1263
cfDNA	EPCR17	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.6079	2305
cfDNA	EPCR17	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.0659	846
cfDNA	EPCR18	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.2065	1025
cfDNA	EPCR18	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.1537	1025
cfDNA	EPCR19	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.1012	1711
cfDNA	EPCR19	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A;p.D32N	0/1	0.0143	940
cfDNA	EPCR19	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.0166	1157
cfDNA	EPCR19	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T;p.S37F	0/1	0.0137	1462
cfDNA	EPCR20	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.0123	1348
cfDNA	EPCR20	chr9	120950602	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G94A;p.D32N	0/1	0.068	1486
cfDNA	EPCR20	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T;p.S33F	0/1	0.0211	1398
cfDNA	EPCR20	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.0318	1142
cfDNA	EPCR21	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.3549	1758
cfDNA	EPCR21	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.0223	874
cfDNA	EPCR21	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T;p.S37F	0/1	0.014	1154
cfDNA	EPCR22	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.034	1068
cfDNA	EPCR23	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.1891	909
cfDNA	EPCR23	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.01	1461
cfDNA	EPCR24	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.03	1437
cfDNA	EPCR24	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A;p.G34E	0/1	0.1104	1243
cfDNA	PCR4	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T;p.G12D	0/1	0.0965	1518

cfDNA	PCR4	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0231	893
cfDNA	PCR6	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.1223	1494
cfDNA	PCR6	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0342	1007
cfDNA	PCR7	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.1314	1113
cfDNA	PCR8	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.0556	1830
cfDNA	PCR8	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0305	1089
cfDNA	PCR9	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.0539	924
cfDNA	PCR9	chr9	120950606	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C98T:p.S33F	0/1	0.0414	1099
cfDNA	PCR11	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.2747	118
cfDNA	PCR11	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.028	1270
cfDNA	PCR12	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.0792	1428
cfDNA	PCR12	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0697	954
cfDNA	PCR16	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0438	880
cfDNA	PCR17	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.13	1056
cfDNA	PCR17	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.06489	1031
cfDNA	PCR18	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.2334	921
cfDNA	PCR18	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.067	819
cfDNA	PCR19	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.0519	1592
cfDNA	PCR19	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0368	1024
cfDNA	PCR20	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.0768	985
cfDNA	PCR20	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0351	1132
cfDNA	PCR21	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.0221	1158
cfDNA	PCR21	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.0355	1026
cfDNA	PCR21	chr9	120950618	C	T	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.C110T:p.S37F	0/1	0.01	1398
cfDNA	PCR22	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.029	1154
cfDNA	PCR23	chr6	145246771	C	T	exonic	KRAS	nonsynonymous SNV	KRAS:NM_021284:exon2:c.C35T:p.G12D	0/1	0.0217	1051
cfDNA	PCR23	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	0.028	1552

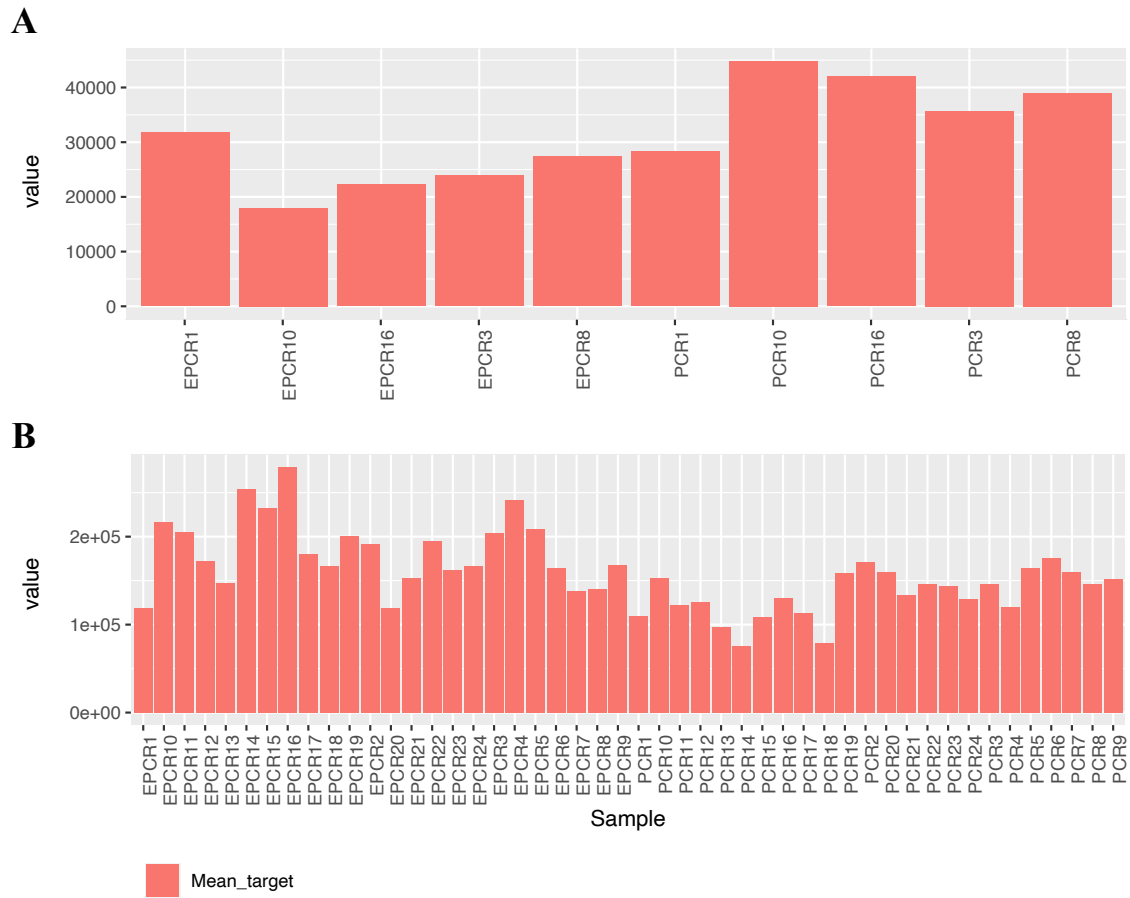
cfDNA	PCR24	chr9	120950609	G	A	exonic	CTNNB1	nonsynonymous SNV	CTNNB1:NM_007614:exon3:c.G101A:p.G34E	0/1	6.48E-02	873
-------	-------	------	-----------	---	---	--------	--------	-------------------	---------------------------------------	-----	----------	-----



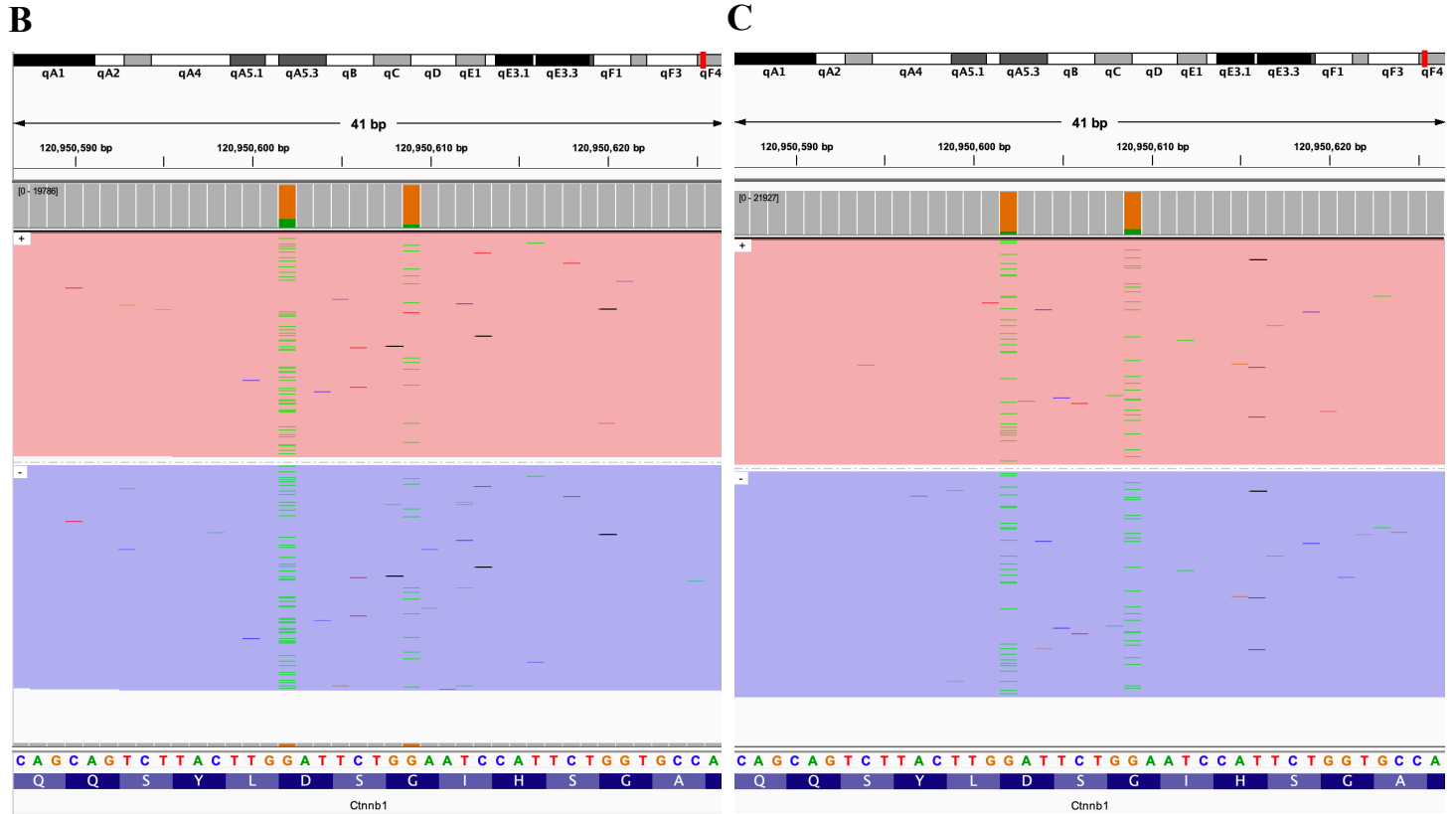
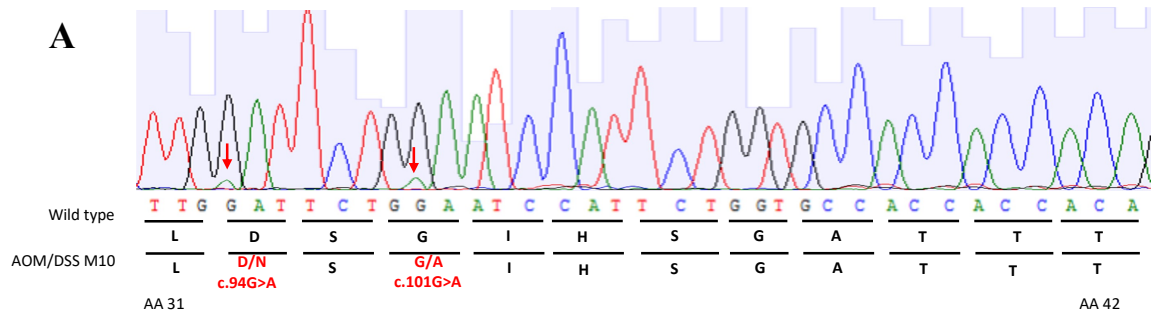
Supp. Figure 1. A) Representative image of the size of the PCR products for *Kras* and *Ctnnb1* used for library preparation. B) Final library of *Kras* and *Ctnnb1* evaluated in bioanalyzer. Barcodes were added to the PCR products at 5' and 3', increasing their size by 120 base pairs.



Supp. Figure 2. Representative image of CRC development in the AOM/DSS model taken at the end of the experiment. The tumors were developed in the middle and distal part of the colon.



Supp. Figure 3. Depth of sequencing for all the samples. A) Sequencing depth in tumor samples (mean $31,310.68 \pm 8,924.165$). B) sequencing depth in cfDNA (mean $159,509.8 \pm 43,030.71$).



Supp. Figure 4. Representative images of the mutational profiling of the tumor of mouse M10. A) Sanger sequencing electropherogram showing a mutation in the *Ctnnb1* gene. B) Local mutation profile of the *Ctnnb1* gene in the Integrative Genomic Viewer after E-ice-COLD-PCR amplification and massive parallel sequencing. C) Local mutation profile of the *Ctnnb1* gene in the Integrative Genomic Viewer after PCR and massive parallel sequencing.