

Supplementary Materials: APC Mutations Are Not Confined to Hotspot Regions in Early-Onset Colorectal Cancer

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Table S1. APC amplicon specific primers for next generation sequencing

Name	Sequence (5'-3')	Product Size (bp)
APC Exon 5 F APC	ACGACGCTCTTCCGATCTCACCATGACTGACGTATTTGCT	389
Exon 5 R APC Exon	CGTGTGCTCTTCCGATCTAGAGCCAAAATAAACACAGCCTT	
6 F APC Exon 6 R	ACGACGCTCTTCCGATCTCCTGAGCTTTTAAAGTGGTAGCC	458
APC Exon 8 F APC	CGTGTGCTCTTCCGATCTTGTAAGTACAGCTAAAGTAAGGT	
Exon 8 R APC Exon	ACGACGCTCTTCCGATCTTCTGCAGTTAATGCTCATATGCAA	402
9 F1 APC Exon 9 R1	CGTGTGCTCTTCCGATCTTGGCATTAGTGACCAGGGTTT	397
APC Exon 9 F2 APC	ACGACGCTCTTCCGATCTTCATCACTTAATTGGTTTTTGGCTT	
Exon 9 R2 APC Exon	CGTGTGCTCTTCCGATCTAAGGACTCGGATTTACAGCC	374
11 F1 APC Exon 11	ACGACGCTCTTCCGATCTGGAAATCCCGGGGAGTAA	
R1 APC Exon 11 F2	CGTGTGCTCTTCCGATCTTGAGTAGCACAAATGGCTGA	406
APC Exon 11 R2	ACGACGCTCTTCCGATCTGGGGTGGAGAACTGGCATA	
APC Exon 12 F APC	CGTGTGCTCTTCCGATCTACCTTGTGGCTACATCTCCAAA	320
Exon 12 R APC Exon	ACGACGCTCTTCCGATCTTAGGGGGACTACAGGCCATT	
13 F APC Exon 13 R	CGTGTGCTCTTCCGATCTGCGAATGTGAAGCACAGGTT	511
APC Exon 15 F1	ACGACGCTCTTCCGATCTTGGCTTCAAGTTGTCTTTTTAATG	476
APC Exon 15 R1	CGTGTGCTCTTCCGATCTTGAGATACTAAATACTGAGCAACAA	
APC Exon 15 F2	ACGACGCTCTTCCGATCTAGTGATAGGATTACAGGCGTG	387
APC Exon 15 R2	CGTGTGCTCTTCCGATCTGAAATTAGGGAATCTCATGG	
APC Exon 15 F3	ACGACGCTCTTCCGATCTTCCCAAGGCATCTCATCGT	387
APC Exon 15 R3	CGTGTGCTCTTCCGATCTATGGCTGACACTTCTTCCATGA	436
APC Exon 15 F4	ACGACGCTCTTCCGATCTAGAAGCTCTGCTGCCCATAC	
APC Exon 15 R4	CGTGTGCTCTTCCGATCTCCTTCCAGAGTTCAACTGCTCA	449
APC Exon 15 F5	ACGACGCTCTTCCGATCTTCCATACAGGTCACGGGGAG	
APC Exon 15 R5	CGTGTGCTCTTCCGATCTTGGATGGAGCTGATTCTGCC	470
APC Exon 15 F6	ACGACGCTCTTCCGATCTAGCAGTGAGAATACGTCCACA	
APC Exon 15 R6	CGTGTGCTCTTCCGATCTGCTTTGTGCTGGCTGATT	504
	ACGACGCTCTTCCGATCTGCAGACTGCAGGGTTCTAGT	
	CGTGTGCTCTTCCGATCTGACCCTCTGAACTGCAGCAT	
	ACGACGCTCTTCCGATCTAACAGCTCAAACCAAGCGAG	
	CGTGTGCTCTTCCGATCTTTTGAAGCAGTCTGGGCTGG	

Amplicon-specific primers (black bases) designed with non-specific 18bp sequences at the 5' end of primers (red bases). Two non-specific sequences used, one for forward primers, one for reverse primers. bp, base pairs.

Table S2. Forward adapter primers for creating next generation sequencing libraries

Name	Sequence (5'-3')
FPI1	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CGTGAT ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI2	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC ACATCG ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI3	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GCCTAA ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI4	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TGGTCA ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI5	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CACTGT ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI6	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC ATTGGC ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI7	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GATCTG ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI8	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TCAAGT ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI9	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC CTGATC ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI10	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC AAGCTA ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI11	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GTAGCC ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI12	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TACAAG ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI13	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TTGACT ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI14	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GGAACT ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI15	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC TGACAT ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T
FPI16	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC GGACGG ACAC T CTT TCC CTA CAC GAC GCT CTT CCG ATC T

Forward adapter primers with unique index sequences shown in **red**.

Table S3. Reverse adapter primers for creating next generation sequencing libraries

Name	Sequence (5'-3')
RPI1	CAA GCA GAA GAC GGC ATA CGA GAT CGTGAT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI2	CAA GCA GAA GAC GGC ATA CGA GAT ACATCG GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI3	CAA GCA GAA GAC GGC ATA CGA GAT GCCTAA GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI4	CAA GCA GAA GAC GGC ATA CGA GAT TGGTCA GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI5	CAA GCA GAA GAC GGC ATA CGA GAT CACTGT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI6	CAA GCA GAA GAC GGC ATA CGA GAT ATTGGC GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI7	CAA GCA GAA GAC GGC ATA CGA GAT GATCTG GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI8	CAA GCA GAA GAC GGC ATA CGA GAT TCAAGT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI9	CAA GCA GAA GAC GGC ATA CGA GAT CTGATC GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI10	CAA GCA GAA GAC GGC ATA CGA GAT AAGCTA GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI11	CAA GCA GAA GAC GGC ATA CGA GAT GTAGCC GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI12	CAA GCA GAA GAC GGC ATA CGA GAT TACAAG GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI13	CAA GCA GAA GAC GGC ATA CGA GAT TTGACT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI14	CAA GCA GAA GAC GGC ATA CGA GAT GGAACT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI15	CAA GCA GAA GAC GGC ATA CGA GAT TGACAT GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC
RPI16	CAA GCA GAA GAC GGC ATA CGA GAT GGACGG GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC

Reverse adapter primers with unique index sequences shown in **red**