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a.

![Histogram of base observation frequencies](image)

He et al., Supplementary Figure 1a
Supplementary Figure 1. Sequencing coverage and uniformity. (a) PCR-based enrichment. (b) Capture-based enrichment.
Supplementary Figure 2. Variations in the frequency of heteroplasmic variants among 10 organs of the same individual. Fourteen variants had frequencies varying between 1.6% and 91% in at least one of the 10 organs analyzed. For each variant the maximal and minimal frequency (%) detected among the organs is indicated.
Skeletal muscle (7.4%)

Heart (43.6%)

Liver (70.9%)

Kidney (90.9%)

He et al., Supplementary Figure 3a
Supplementary Figure 3. Sequence chromatograms of heteroplasmic variants in different organs from the same individual. (a) Patient #11: base 16093 T> C. (b) Patient #11: base 72 T> C. (c) Patient #12: base 72 T> C. In each case the organ source of mtDNA and the variant allele frequency as determined by massively-parallel-sequencing is indicated.

He et al., Supplementary Figure 3c
Supplementary Figure 4. Circulating mutant DNA in the plasma of two colorectal cancer patients.

He et al., Supplementary Figure 4
FULL METHODS

**PCR-based enrichment strategy.** Three to six ng of total cellular DNA (equivalent to ~ 500 – 1000 cells) was used as a template for each PCR reaction. Amplicons generated using primer sets PCR1 and 2 (Fig. 1a and Supplementary Table 7) were in general produced using HotStart Phusion polymerase (NEB, Beverly, MA) in reactions of 30 ul containing 1 x Phusion HF buffer, 0.2 mM dNTPs, 0.5uM forward and 0.5 uM reverse primers, 5% DMSO and 0.6 u Phusion polymerase. The following cycling conditions were used: 1 cycle of 98°C for 30 sec; 25 cycles of 98°C for 10 sec, 60°C for 30 sec, 72°C for 30 sec. Amplicons generated with primer set PCR3 (Fig. 1a and previously described1) were produced in the same fashion, with the exception that the 72°C elongation step was for 90 sec.

For each DNA sample, PCR products representing 25 amplicons (PCR1 or PCR2) or nine amplicons (PCR3) were pooled. PCR1 and PCR2 amplicons were purified using a kit from Agencourt (Beverly, MA) while the longer PCR products generated with primer set PCR3 were purified with a kit from Omega Bio-Tek (Norcross, GA). Two to five ug of the pooled, purified PCR product was blunt-ended at 20°C for 30 min in a total volume of 100 ul containing 1 x T4 ligation buffer, 10 mM ATP, 1 mM of each dNTP, 5 ul T4 DNA polymerase, 1 ul Klenow polymerase, and 5 ul T4 PNK (all enzymes were obtained from NEB). Blunt-ended DNA was PCR-purified and ligated using Quick Ligase (NEB), in a total volume of 100ul, as described by the manufacturer. Ligated DNA was purified with a Qiagen PCR purification kit (Cat# 28104) and eluted with 100 ul of 70 °C elution buffer. Purified, ligated DNA was fragmented with a Bioruptor sonicator (Diagenode, Sparta, NJ) at low power, by cycling for 15 sec on, then 15 sec off, for 2x15 minutes while cooled in an ice bath. This sonication reduced the size of the ligated DNA from >12 kb to 100 to 400 bp. The fragmented DNA was purified and used for preparation of an Illumina DNA library as described in Illumina genomic DNA library preparation manual.

**Capture-based enrichment strategy.**

**Step 1. Preparation of probes.** Fifty amplicons were designed to cover the mtDNA genome (Supplementary table 8). For every amplicon, the 5’ end of the forward primer was tagged with M13-Forward sequence (5’-gtaaaacgacggccagt-3’). PCR was performed as described above using normal human colon mucosa DNA as template and primers without the biotin tag. These PCR products were then used as templates for a second PCR using double-biotinylated M13-Forward sequence as forward primers (Supplementary Table 7) to obtain biotinylated PCR products. The resultant PCR products were pooled and purified with a Qiagen kit (cat# 28104). The purified PCR products were then mixed with an equal volume of 2 x Bind-and-Wash buffer (10 mM Tris-Cl, pH7.5, 1
mM EDTA, 2 M NaCl) containing 250 ul Dynal MyOne streptavidin-coated beads (product# 650.02, Invitrogen). After incubation at room temperatures for one hour, the beads were washed twice with 1 x PCR buffer (20 mM Tris-Cl, pH 8.4, 50 mM KCl). Bead-attached DNA was then denatured in 0.2N NaOH for 5 min at room temperature to remove the non-biotinylated strands, and washed in 1xPCR buffer. The biotinylated single-stranded DNA remaining attached to the beads was released by incubation in 200 ul Hi-Di formamide (product# 4311320, ABI, Foster City, CA) at 95°C for three minutes. ssDNA probe mixture was precipitated by adding 3M Sodium Acetate, pH5.2, DNA carrier and 2.5 volumes of ethanol, then centrifuged for five minutes in a microfuge at ~12,000 g. After washing the pellet with 75% ethanol, the single strand DNA was resuspended in H2O.

**Step 2. Hybridization-based capture.** Total cellular DNA was used to prepare a paired-end library by slight modifications of the standard Illumina protocol. 2.5 ug of the library DNA was then mixed with 2.5 ug of the single-stranded biotinylated DNA in 50ul containing 0.2% SDS and 4.5 x SSPE (1xSSPE: 0.15 M NaCl, 0.01 M NaH2PO4, and 0.001 M EDTA; from Amresco, Solon, Ohio), covered with mineral oil and incubated at 95°C for 3 min, followed by incubation at 65°C for 12 hours to allow hybridization. DNA was then purified with NucleoSpin Extract II with NTB buffer (Clontech, Mountain View, CA). The DNA was eluted from the NucleoSpin column in 100 ul elution buffer and was captured on streptavidin-coated MyOne beads as described above. The beads were washed twice with 1 x PCR buffer; resuspended in washing buffer composed of 0.1% SDS in 2 x SSPE and incubated at 68°C with shaking for 30 min. The beads were collected on a magnet and washed with fresh washing buffer for another 30 min at 68°C, then washed twice with 1x PCR buffer. The captured target DNA was released by denaturation in 0.2N NaOH at room temperature for 5 min, ethanol-precipitated as described above, then resuspended in 15 ul elution buffer (5mM Tris-HCl, pH8.5). Two ul was used as template for HotStart Phusion PCR (18-cycle) amplification to obtain a 1°-enriched mtDNA library. Approximately three ug of 1°-enriched mtDNA library DNA was then mixed with 2.5 ug of the biotinylated ss DNA described above in 110 ul of 1xPCR buffer (from 10xPCR buffer, cat#53286, Invitrogen). Hybridization was performed by incubating the DNA at 95°C for 1 min; then cooled to 50°C at 0.1°C/sec, then incubated at 50°C for 1min. The hybrids were captured with MyOne beads, washed once in 1 x PCR buffer; twice with wash solution I (2 x SSC, 0.05% SDS), using a 15 min incubation at room temperature for each wash; beads were then washed twice, for 15 minutes each, with wash solution II (0.1 x SSC, 0.1% SDS) at 50°C, then washed once with 1 x PCR buffer. Captured target DNA was released and ethanol-purified as described above, resuspended in 15 ul elution buffer (5mM Tris-HCl, pH8.5). Three ul was used as a template for HotStart Phusion PCR (18-cycle) amplification to obtain a 2°-enriched mtDNA library, which was subsequently used for sequencing with a Genome Analyzer II instrument.
Genome Analyzer sequence data analysis

The 36 base tags obtained from the Genome Analyzer II reads aligned to the human mtDNA sequence of 16569 bp (AC_000021) using Eland software from Illumina. This alignment allowed up to two mismatches at the first 32 out of the total 36 bases in each tag. Four bases in the human mtDNA genome were excluded from analysis: bases 309-311 (in a long stretch of “Cs”) and base 3107 (“N” base in AC_000021). Three quality filters were used for selecting tags for further analysis: (i) all 36 bases in a tag were required to have a Phred score of at least 23 (meaning there was ~0.5% probability of a base calling error); (ii) no “N” base was allowed anywhere in the 36 bases; and (iii) no more than three mismatches were permitted in the 36 bases (e.g., the last four bases could have no more than 2 mismatches if there was one mismatch in the first 32 bases). Subsequent analysis was performed with database software SQL 2005. Each position of the mtDNA genome was assigned a coverage depth, representing the number of quality-filtered tags containing the base was observed, and a mutation fraction, representing the fraction of tags containing that base in mutant form. To be categorized as a mutation, the identical mutation had to be identified (i) in at least ten “distinct” tags, i.e., tags whose first base was at ten different positions (for clarity, there are 16569 distinct tags possible in the mitochondrial genome) and (ii) in at least three tags sequenced from the forward direction and at least three from the reverse direction. These criteria were applied to normal mucosa samples, cancer samples and samples from CEPH families. For analysis of mtDNA from normal human organs, mutations were defined by at least five “distinct” tags, and at least one tag sequenced from the forward direction and at least one from the reverse direction.

Determination of background error rates

PCR products used for Genome Analyzer II sequencing were generated through two PCR steps. In the first step, 96 pairs of PCR primers, each with a universal tag (5'-ACACGACGCTCTTCCGATCT-3' for forward primers, 5'-GCATACGAGCTCTTCCGATCT-3' for reverse primers), were designed to amplify 96 different regions on chromosome 13, 18 and 21. Genomic DNA from human B lymphoblastoid cells was used as PCR template. After 30 cycles of PCR using Phusion high fidelity polymerase (NEB), the PCR product was mixed and diluted 1000 times in TE buffer. In the second step PCR, one pair of PCR primers were designed to contain the universal sequence used in the first step and the Illumina grafting primers (Forward Primer: 5'—AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'; Reverse Primer: 5'-CAAGCAGAAGACGGCATACGAGCTCTTCCGATCT-3'). The PCR was conducted in a single PCR tube using this pair of primers and the diluted PCR product mixture from first step as template. After 15 cycles of PCR using Phusion Flash master mix (NEB), the product
was purified with a Qiaquick purification column (cat# 28104, Qiagen) and quantified by absorption at 600 nm.

For sequencing data analysis, a Phred score of at least 23 for each base in the 36-base tags was applied to select high quality tags for mutation analysis. For every non-heterozygous position, coverage of the non-reference base and reference base was extracted from the sequencing result. The sequencing error for each base was calculated as the coverage of non-referenced base divided by the coverage of the reference base. Analysis of 2746 bases by two independent experiments showed that the average fraction of mutations per base was 0.063% and 0.052%, with a standard deviation of 0.058% in both experiments, and the highest mutation rate was 0.82% and 0.7%, respectively.

Sanger sequencing and BEAMing confirmation of mutation

Three to six ng of total cellular DNA was used as a template for each PCR reaction using HotStart Phusion polymerase (NEB) in reactions of 30 ul containing 1 x Phusion HF buffer, 0.2 mM dNTPs, 0.5uM forward and 0.5 uM reverse primers, 5% DMSO and 0.6 u Phusion polymerase. The following cycling conditions were used: 1 cycle of 98°C for 30 sec; 25 cycles of 98°C for 10 sec, 60°C for 30 sec, 72°C for 30 sec. PCR products were purified with PCR purification kit (cat# 28104, Qiagen) and conventional dye terminator sequencing with forward and reverse PCR primers were performed by MWG biotech (High Point, NC). Beaming confirmation of mutation was performed as described previously.

Detection of circulating mutant mtDNA in plasma

Collection of plasma samples from colorectal cancer patients and isolation of circulating DNA was performed as previously described with modifications. Specifically, we used a virus vacuum kit (Qiagen #57714) for plasma DNA purification. Five hundred ul of plasma was mixed with 40 ul of proteinase K (Invitrogen #25530-049). 5.6 ul of 1ug/ ul RNA carrier was mixed with 500 ul of AL buffer and subsequently this mixture of 506 ul was added to the plasma/proteinase mixture, mixed by vortexing and incubated for one hour at 60°C. Following incubation, 600 ul of ice cold ethanol was added and the solution incubated at room temperature for 5 minutes. The mixture was then transferred into the virus vacuum kit column and spun in a microfuge at 7000 rpm for one minute. This loading and centrifugation step was repeated several times until the whole mixture was processed. The column was washed with 600 ul AW1 buffer, 700 ul AW2 buffer and 700 ul ethanol by spinning.
for one minute at 7000 rpm at each step. Finally, a spin at maximal speed for three minutes was performed to dry the column. DNA was finally eluted in 35 ul AVE buffer.

The nuclear DNA copy number (genome equivalent) per milliliter of plasma was determined by quantitative PCR as previously described\(^2\). To measure the copy number of circulating mtDNA in plasma, we used forward primer 5'-ctccagcgtctgcaatg-3' and reverse primer 5'-tcaggtctatcacc-3' to amplify and obtain mtDNA fragment of 101 bp from normal human total cellular DNA. This PCR product was quantified by Picogreen and subsequently used as a copy number standard for quantitative PCR using the same set of primers to determine mtDNA copy number per milliliter of plasma.

The determination of circulating mutant APC gene frequency was previously described\(^2\). Primers used for BEAMing detection of mutant mtDNA frequency: for patient #8 (base 4097) - forward primer 5'-TCCCGCGAAATTAATACGACcagcactctcctgaaact-3', and reverse primer 5'-GCTGGAGCTCTGAGCTAgtagccagtccgggtat-3'; for patient #9 (base 16291) – forward primer 5'-TCCCGCGAAATTAATACGACcaccctcacttaggtgat-3', and reverse primer 5'-GCTGGAGCTCTGAGCTAgggacgagaagggatttgac-3'. First step PCR was performed as described above with Phusion polymerase with an input template DNA of about 100 genome equivalent. Second step amplification (Beaming with universal primer – forward 5'-TCCCGCGAAATTAATACGAC-3', and reverse 5'-GCTGGAGCTCTGAGCTA-3') and detection of mutant mtDNA allele frequency by single base extension was performed as previously described\(^2\). Primers used for single base extension: for base 4097 5'-ctatgagtccagtctgaaagt-3'; and for base 16291 5'-tacagtctgtactgtttaaggtgt-3'. The percentages of circulating mutant mtDNA was determined to be 0.65% (before surgery) or 0.004% (after surgery) for patient #8; and 2.87% (before surgery) or 0.06% (after surgery) for patient #9.

**Supplementary References**

1. Ramos, A., Santos, C., Alvarez, L., Nogues, R., & Aluja, M.P., Human mitochondrial DNA complete amplification and sequencing: a new validated primer set that prevents nuclear DNA sequences of mitochondrial origin co-amplification. *Electrophoresis* 30, 1587-1593 (2009).

2. Diehl, F. *et al.*, Circulating mutant DNA to assess tumor dynamics. *Nat Med* 14, 985-990 (2008).
| Patient # | Age | Position | Allele 1 | Allele 2 | Frequency of allele 1* |
|-----------|-----|----------|----------|----------|-----------------------|
| 1         | 66  | 60       | C        | T*       | 1.95%                 |
| 1         | 66  | 72       | C        | T*       | 4.50%                 |
| 1         | 66  | 94       | A        | G*       | 2.60%                 |
| 1         | 66  | 228      | G*       | A        | 2.0%                  |
| 1         | 66  | 1888     | A        | G*       | 2.45%                 |
| 1         | 66  | 14566    | G        | A*       | 29.6%                 |
| 1         | 66  | 16126    | T*       | C        | 4.6%                  |
| 2         | 77  | 60       | C        | T*       | 3.05%                 |
| 2         | 77  | 72       | C        | T*       | 5.70%                 |
| 2         | 77  | 94       | A        | G*       | 7.80%                 |
| 2         | 77  | 186      | T        | C*       | 2.10%                 |
| 2         | 77  | 16126    | T*       | C        | 2.5%                  |
| 3         | 46  | 1632     | C        | T*       | 2.95%                 |
| 3         | 46  | 3070     | A        | G*       | 16.55%                |
| 3         | 46  | 16385    | T        | A*       | 43.60%                |
| 4         | 50  | 72       | C        | T*       | 4.25%                 |
| 4         | 50  | 200      | A*       | G        | 2.7%                  |
| 4         | 50  | 16126    | T*       | C        | 2.0%                  |
| 4         | 50  | 16324    | T*       | C        | 1.8%                  |
| 5         | 35  | 72       | C        | T*       | 3.30%                 |
| 5         | 35  | 94       | A        | G*       | 1.60%                 |
| 6         | 53  | 72       | C        | T*       | 4.20%                 |
| 6         | 53  | 94       | A        | G*       | 2.10%                 |
| 7         | 48  | 59       | T*       | C        | 2.1%                  |
| 7         | 48  | 200      | A*       | G        | 8.2%                  |
| 7         | 48  | 225      | G*       | A        | 8.1%                  |
| 7         | 48  | 16183    | A*       | C        | 4.1%                  |
| 8         | 64  | 72       | C        | T*       | 2.80%                 |
| 8         | 64  | 9280     | C        | T*       | 5.35%                 |
| 8         | 64  | 10851    | A        | G*       | 1.65%                 |
| 9         | 42  | 60       | C        | T*       | 2.00%                 |
| 9         | 42  | 72       | C        | T*       | 6.30%                 |
| 9         | 42  | 94       | A        | G*       | 4.15%                 |
| 9         | 42  | 234      | G        | A*       | 4.05%                 |
| 9         | 42  | 9830     | T        | C*       | 32.10%                |
| 9         | 42  | 13858    | G        | A*       | 12.00%                |
| 10        | 59  | 60       | C        | T*       | 3.45%                 |
| 10        | 59  | 72       | C        | T*       | 16.75%                |
| 10        | 59  | 16093    | T*       | C        | 27.9%                 |
| 10        | 59  | 16337    | T        | C*       | 3.30%                 |

* Average of frequencies determined by PCR1 and PCR2 libraries.
Supplementary Table 2. Heteroplasmic variants in different organs of Patient #12 (78 year old)

| Position | allele 1 | allele 2 | Pancreas | Lung | Skeletal Muscle | Kidney | Liver | # tissues with variant | Min % | Max %  |
|----------|----------|----------|----------|------|----------------|--------|-------|-----------------------|-------|--------|
| 64       | A        | C*       | <0.35%   | <0.35%| 4.36%          | 0.78%  | <0.35%| 1                     | <0.35%| 4.36%  |
| 72       | C        | T*       | <0.35%   | <0.35%| 72.62%         | 20.92% | 13.23%| 3                     | <0.35%| 72.62% |
| 73       | G        | A*       | <0.35%   | <0.35%| 3.94%          | <0.35% | <0.35%| 1                     | <0.35%| 3.94%  |
| 189      | G        | A*       | <0.35%   | <0.35%| 11.97%         | <0.35% | 0.39% | 1                     | <0.35%| 11.97% |
| 408      | A        | T*       | <0.35%   | <0.35%| 4.52%          | <0.35% | <0.35%| 1                     | <0.35%| 4.52%  |
| 411      | A        | C*       | <0.35%   | <0.35%| 1.68%          | <0.35% | <0.35%| 1                     | <0.35%| 1.68%  |
| 5741     | A        | G*       | <0.35%   | <0.35%| <0.35%         | <0.35% | <0.35%| 2.02%                 | <0.35%| 2.02%  |
| 5760     | A        | G*       | <0.35%   | <0.35%| <0.35%         | <0.35% | <0.35%| 1.69%                 | <0.35%| 1.69%  |
| 8392     | A        | G*       | 0.81%    | 2.24% | <0.35%         | <0.35% | <0.35%| 1                     | <0.35%| 2.24%  |
| 10742    | G        | T*       | 1.67%    | <0.35%| <0.35%         | <0.35% | <0.35%| 1                     | <0.35%| 1.67%  |
| 16148    | T        | C*       | <0.35%   | <0.35%| 0.78%          | <0.35% | <0.35%| 1.69%                 | <0.35%| 1.69%  |

Total # heteroplasmic variants 1 1 7 2 4

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| Position | Allele 1* | Allele 2 | Allele 2 frequency in mother | Allele 2 frequency in father | Allele 2 frequency in child #1 | Allele 2 frequency in child #2 |
|----------|----------|----------|-----------------------------|-----------------------------|-------------------------------|-------------------------------|
| 4769     | A        | G        | >98.4%                       | >98.4%                      | >98.4%                        | >98.4%                        |
| 8860     | A        | G        | >98.4%                       | >98.4%                      | >98.4%                        | >98.4%                        |
| 5573     | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 15519    | T        | C        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 14233    | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 14766    | C        | T        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 16304    | T        | C        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 2706     | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 750      | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 10463    | T        | C        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 11251    | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 709      | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 16126    | T        | C        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 930      | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 5147     | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 4216     | T        | C        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 16344    | C        | T        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 10750    | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 73       | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 4917     | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 11812    | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 13668    | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 1888     | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 15928    | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 11719    | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 15607    | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 263      | A        | G        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 15452    | C        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 16294    | C        | T        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 16296    | C        | T        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 6608     | C        | T        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 8697     | G        | A        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 7028     | C        | T        | >98.4%                       | <0.35%                      | >98.4%                        | >98.4%                        |
| 4051     | G        | A        | 77.84%                       | <0.35%                      | <0.35%                        | <0.35%                        |
| 3861     | A        | G        | 19.57%                       | <0.35%                      | <0.35%                        | <0.35%                        |
| 12294    | G        | A        | 11.11%                       | <0.35%                      | <0.35%                        | <0.35%                        |
| 11090    | A        | C        | 4.00%                        | 4.52%                       | 3.08%                         | 3.15%                         |
| 9655     | G        | A        | 2.97%                        | <0.35%                      | <0.35%                        | <0.35%                        |
| 12775    | G        | A        | <0.35%                       | <0.35%                      | <0.35%                        | 25.78%                        |
| 8245     | A        | G        | <0.35%                       | >98.4%                      | <0.35%                        | <0.35%                        |
| 12618    | G        | A        | <0.35%                       | >98.4%                      | <0.35%                        | <0.35%                        |
| 3899     | T        | C        | <0.35%                       | <0.35%                      | <0.35%                        | 5.32%                         |
| 5043     | G        | A        | <0.35%                       | <0.35%                      | 7.61%                         | <0.35%                        |
| 14615    | G        | A        | <0.35%                       | <0.35%                      | 36.00%                        | <0.35%                        |
| 3679     | T        | C        | <0.35%                       | 13.42%                      | <0.35%                        | <0.35%                        |
| 13945    | A        | G        | <0.35%                       | 98.05%                      | <0.35%                        | <0.35%                        |
| 3010     | G        | A        | <0.35%                       | >98.4%                      | <0.35%                        | <0.35%                        |
| 567      | A        | C        | <0.35%                       | <0.35%                      | <0.35%                        | 5.72%                         |

* Reference allele.
| Position | Allele 1 | Allele 2 | Allele 2 frequency in mother | Allele 2 frequency in father | Allele 2 frequency in child #1 | Allele 2 frequency in child #2 |
|----------|----------|----------|-----------------------------|-----------------------------|-------------------------------|-------------------------------|
| 15452    | C        | A        | >98.4%                       | >98.4%                       | >98.4%                        | >98.4%                        |
| 4917     | A        | G        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 13368    | G        | A        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 4769     | A        | G        | >98.4%                       | >98.4%                       | >98.4%                        | >98.4%                        |
| 10463    | A        | T        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 8860     | A        | G        | >98.4%                       | >98.4%                       | >98.4%                        | >98.4%                        |
| 15607    | A        | G        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 1888     | G        | A        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 750      | A        | G        | >98.4%                       | >98.4%                       | >98.4%                        | >98.4%                        |
| 11719    | G        | A        | >98.4%                       | >98.4%                       | >98.4%                        | >98.4%                        |
| 73       | A        | G        | >98.4%                       | >98.4%                       | >98.4%                        | >98.4%                        |
| 16163    | A        | G        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 263      | A        | G        | >98.4%                       | >98.4%                       | >98.4%                        | >98.4%                        |
| 12633    | C        | A        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 11251    | A        | G        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 709      | G        | A        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 15928    | G        | A        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 2706     | A        | G        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 8697     | G        | A        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 16294    | C        | T        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 16126    | T        | C        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 16189    | T        | C        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 14766    | C        | T        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 4216     | T        | C        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 3867     | C        | T        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 7028     | C        | T        | >98.4%                       | <0.35%                       | >98.4%                        | >98.4%                        |
| 16187    | C        | T        | 56.53%                       | <0.35%                       | 56.72%                        | 56.35%                        |
| 16186    | C        | T        | 55.85%                       | <0.35%                       | 56.70%                        | 56.95%                        |
| 9055     | G        | A        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 13967    | C        | T        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 12308    | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 14798    | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 1811     | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 10398    | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 1189     | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 16093    | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 16319    | G        | A        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 16224    | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 16311    | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 11299    | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 10289    | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 12372    | G        | A        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 9962     | G        | A        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 9698     | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 10550    | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 1719     | G        | A        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 11467    | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 10861    | T        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 15257    | G        | A        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 13619    | T        | C        | <0.35%                       | <0.35%                       | 32.94%                        | <0.35%                        |
| 11923    | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 15946    | C        | T        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 3480     | A        | G        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 5913     | G        | A        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 3075     | G        | C        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 14167    | C        | T        | <0.35%                       | >98.4%                       | <0.35%                        | <0.35%                        |
| 12889    | G        | A        | <0.35%                       | >98.4%                       | 14.70%                        | <0.35%                        |

* Reference allele.
### Supplementary Table 5. Cancer-specific base substitutions identified in colorectal cancers

| Patient ID       | BasePos | WT base | Mutant base | Frequency in cancer* | Frequency in matched normal* |
|------------------|---------|---------|-------------|----------------------|-----------------------------|
| Patient #1 (Co109c) | 4126    | C       | A           | 7.7%                 | <0.35%                      |
|                  | 4770    | G       | A           | >98.4%               | <0.35%                      |
|                  | 16030   | C       | T           | 5.3%                 | <0.35%                      |
| Patient #2 (Co108c) | -       | -       | -           | -                    | -                           |
| Patient #3 (Co92c) | 1273    | G       | A           | >98.4%               | <0.35%                      |
|                  | 2119    | T       | C           | 19.3%                | <0.35%                      |
| Patient #4 (Hx401x) | 10726   | G       | A           | 8.1%                 | <0.35%                      |
| Patient #5 (Hx402x) | 3428    | G       | A           | 36.8%                | <0.35%                      |
|                  | 3977    | T       | C           | 65.3%                | <0.35%                      |
|                  | 5638    | T       | C           | 18.9%                | <0.35%                      |
|                  | 6912    | G       | A           | 15.5%                | <0.35%                      |
|                  | 9838    | G       | C           | 51.6%                | <0.35%                      |
|                  | 10704   | G       | A           | 17.0%                | <0.35%                      |
| Patient #6 (Hx403x) | 574     | 6Gs     | 7Gs         | 16.3%                | <0.35%                      |
| Patient #7 (Hx404x) | 1576    | G       | C           | 7.8%                 | <0.35%                      |
|                  | 2973    | T       | C           | 6.4%                 | <0.35%                      |
|                  | 12009   | G       | A           | 68.8%                | <0.35%                      |
| Patient #8 (Hx406x) | 4097    | T       | C           | 29.6%                | <0.35%                      |
| Patient #9 (Hx407x) | 12653   | G       | A           | 5.1%                 | <0.35%                      |
|                  | 14110   | T       | C           | 12.4%                | <0.35%                      |
|                  | 16291   | C       | T           | 86.1%                | <0.35%                      |
| Patient #10 (Hx408x) | 93      | A       | G           | 77.9%                | <0.35%                      |

* Average of frequencies determined from PCR1 and PCR2 libraries. No somatic mutations were identified in Patient #2.
Supplementary Table 6. Variants identified in normal mucosae and cancer cells

| Patient # | Position | WT base | Mutant base | Average fraction in normal | Average fraction in cancer |
|-----------|----------|---------|-------------|---------------------------|---------------------------|
| 1         | 60       | T       | C           | 2.0%                       | <0.35%                    |
| 1         | 72       | T       | C           | 4.5%                       | <0.35%                    |
| 1         | 73       | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 94       | G       | A           | 2.6%                       | <0.35%                    |
| 1         | 189      | A       | G           | 1.6%                       | <0.35%                    |
| 1         | 228      | G       | A           | >98.0%                     | >98.4%                     |
| 1         | 263      | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 295      | C       | T           | >98.4%                     | >98.4%                     |
| 1         | 462      | C       | T           | >98.4%                     | >98.4%                     |
| 1         | 489      | T       | C           | >98.4%                     | >98.4%                     |
| 1         | 750      | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 1438     | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 1888     | G       | A           | 2.5%                       | 6.0%                      |
| 1         | 4769     | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 7028     | C       | T           | >98.4%                     | >98.4%                     |
| 1         | 7711     | T       | C           | >98.4%                     | >98.4%                     |
| 1         | 8860     | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 9548     | G       | A           | >98.4%                     | >98.4%                     |
| 1         | 10398    | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 11251    | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 11654    | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 11719    | G       | A           | >98.4%                     | >98.4%                     |
| 1         | 12612    | A       | G           | >98.4%                     | >98.4%                     |
| 1         | 13708    | G       | A           | >98.4%                     | >98.4%                     |
| 1         | 14566    | G       | A           | 29.7%                      | <0.35%                    |
| 1         | 14766    | C       | T           | >98.4%                     | >98.4%                     |
| 1         | 14798    | T       | C           | >98.4%                     | >98.4%                     |
| 1         | 15452    | C       | A           | >98.4%                     | >98.4%                     |
| 1         | 16069    | C       | T           | >98.4%                     | >98.4%                     |
| 1         | 16126    | T       | C           | 95.4%                      | >98.4%                     |
| 1         | 4126     | C       | A           | <0.35%                     | 7.7%                      |
| 1         | 4770     | G       | A           | <0.35%                     | >98.4%                     |
| 1         | 5245     | G       | A           | <0.35%                     | 2.5%                      |
| 1         | 16030    | C       | T           | <0.35%                     | 5.3%                      |
| 2         | 60       | C       | T           | 3.1%                       | <0.35%                    |
| 2         | 72       | T       | C           | 5.7%                       | <0.35%                    |
| 2         | 73       | A       | G           | >98.4%                     | >98.4%                     |
| 2         | 94       | G       | A           | 7.8%                       | <0.35%                    |
| 2         | 146      | T       | C           | >98.4%                     | >98.4%                     |
| 2         | 186      | C       | T           | 2.1%                       | <0.35%                    |
| 2         | 242      | C       | T           | >98.4%                     | >98.4%                     |
| 2         | 263      | A       | G           | >98.4%                     | >98.4%                     |
| 2         | 295      | C       | T           | >98.4%                     | >98.4%                     |
| 2         | 462      | C       | T           | >98.4%                     | >98.4%                     |
| 2         | 489      | T       | C           | >98.4%                     | >98.4%                     |
| 2         | 750      | A       | G           | >98.4%                     | >98.4%                     |
| 2         | 1438     | A       | G           | >98.4%                     | >98.4%                     |
| 2         | 2158     | T       | C           | >98.4%                     | >98.4%                     |
| 2         | 4769     | A       | G           | >98.4%                     | >98.4%                     |
| 2         | 7028     | C       | T           | >98.4%                     | >98.4%                     |
| 2         | 8269     | G       | A           | >98.4%                     | >98.4%                     |
| 2         | 8280     | A       | G           | 1.4%                       | 1.6%                      |
| 2         | 8557     | G       | A           | >98.4%                     | >98.4%                     |
| 2         | 8860     | A       | G           | >98.4%                     | >98.4%                     |
|      |     |     |        |        |
|------|-----|-----|--------|--------|
| 2    | 10398 | A   | G      | >98.4% |
| 2    | 11251 | A   | G      | >98.4% |
| 2    | 11719 | G   | A      | >98.4% |
| 2    | 12007 | G   | A      | >98.4% |
| 2    | 12612 | A   | G      | >98.4% |
| 2    | 13708 | G   | A      | >98.4% |
| 2    | 13879 | T   | C      | >98.4% |
| 2    | 14470 | T   | C      | >98.4% |
| 2    | 14766 | C   | T      | >98.4% |
| 2    | 15452 | C   | A      | >98.4% |
| 2    | 16069 | C   | T      | >98.4% |
| 2    | 16126 | T   | C      | 97.6%  |
| 2    | 16145 | G   | A      | >98.4% |
| 2    | 16172 | C   | T      | >98.4% |
| 3    | 73    | A   | G      | >98.4% |
| 3    | 185   | G   | A      | >98.4% |
| 3    | 228   | G   | A      | >98.4% |
| 3    | 263   | A   | G      | >98.4% |
| 3    | 295   | C   | T      | >98.4% |
| 3    | 462   | C   | T      | >98.4% |
| 3    | 489   | T   | C      | >98.4% |
| 3    | 750   | A   | G      | >98.4% |
| 3    | 1438  | A   | G      | >98.4% |
| 3    | 1632  | T   | C      | 3.0%   |
| 3    | 2280  | C   | T      | >98.4% |
| 3    | 3070  | G   | A      | 16.6%  |
| 3    | 4769  | A   | G      | >98.4% |
| 3    | 7028  | C   | T      | >98.4% |
| 3    | 8860  | A   | G      | >98.4% |
| 3    | 8865  | G   | A      | >98.4% |
| 3    | 10398 | A   | G      | >98.4% |
| 3    | 11251 | A   | G      | >98.4% |
| 3    | 11719 | G   | A      | >98.4% |
| 3    | 12612 | A   | G      | >98.4% |
| 3    | 13708 | G   | A      | >98.4% |
| 3    | 14766 | C   | T      | >98.4% |
| 3    | 14798 | T   | C      | >98.4% |
| 3    | 15452 | C   | A      | >98.4% |
| 3    | 15853 | C   | T      | >98.4% |
| 3    | 16069 | C   | T      | >98.4% |
| 3    | 16126 | T   | C      | >98.4% |
| 3    | 16311 | T   | C      | >98.4% |
| 3    | 16385 | A   | T      | 43.6%  |
| 3    | 16390 | G   | A      | >98.4% |
| 3    | 1273  | G   | A      | <0.35% |
| 3    | 2119  | T   | C      | <0.35% |
| 4    | 72    | T   | C      | 4.3%   |
| 4    | 73    | A   | G      | >98.4% |
| 4    | 200   | A   | G      | 97.3%  |
| 4    | 263   | A   | G      | >98.4% |
| 4    | 709   | G   | A      | >98.4% |
| 4    | 750   | A   | G      | >98.4% |
| 4    | 1420  | T   | C      | >98.4% |
| 4    | 1438  | A   | G      | >98.4% |
| 4    | 1888  | G   | A      | >98.4% |
| 4    | 2141  | T   | C      | >98.4% |
| 4    | 4769  | A   | G      | >98.4% |
| 4    | 6249  | G   | A      | >98.4% |
| 4    | 6524  | T   | C      | >98.4% |
|   |   |   |   |   |
|---|---|---|---|---|
| 4 | 7028 | C | T | >98.4% |
| 4 | 8860 | A | G | >98.4% |
| 4 | 9117 | T | C | >98.4% |
| 4 | 11251 | A | G | >98.4% |
| 4 | 11719 | G | A | >98.4% |
| 4 | 11764 | A | G | >98.4% |
| 4 | 11812 | A | G | >98.4% |
| 4 | 12741 | C | T | >98.4% |
| 4 | 13368 | G | A | >98.4% |
| 4 | 14233 | A | G | >98.4% |
| 4 | 14687 | A | G | >98.4% |
| 4 | 14766 | C | T | >98.4% |
| 4 | 14905 | G | A | >98.4% |
| 4 | 15452 | C | A | >98.4% |
| 4 | 16126 | T | C | >98.4% |
| 4 | 16296 | C | T | >98.4% |
| 4 | 16324 | T | C | >98.4% |
| 4 | 16519 | T | C | >98.4% |
| 4 | 10726 | G | A | <0.35% |
| 5 | 73 | T | C | >98.4% |
| 5 | 150 | C | G | >98.4% |
| 5 | 263 | A | G | >98.4% |
| 5 | 4769 | A | G | >98.4% |
| 5 | 6671 | T | C | >98.4% |
| 5 | 7028 | C | G | >98.4% |
| 5 | 7158 | A | G | >98.4% |
| 5 | 2833 | A | A | >98.4% |
| 5 | 4769 | A | A | >98.4% |
| 5 | 6671 | T | G | >98.4% |
| 5 | 7028 | C | T | >98.4% |
| 5 | 11365 | T | C | >98.4% |
| 5 | 11719 | G | T | >98.4% |
| 5 | 12705 | C | T | >98.4% |
| 5 | 14766 | C | T | >98.4% |
| 5 | 16168 | C | T | >98.4% |
| 5 | 16311 | T | T | >98.4% |
| 5 | 3428 | G | A | <0.35% |
| 5 | 3977 | T | C | <0.35% |
| 5 | 5638 | T | C | >98.4% |
| 5 | 6912 | G | A | <0.35% |
| 5 | 9838 | G | C | <0.35% |
| 5 | 10704 | G | A | <0.35% |
| 6 | 72 | T | C | >98.4% |
| 6 | 73 | A | G | >98.4% |
| 6 | 94 | G | A | >98.4% |
| 6 | 263 | A | G | >98.4% |
| 6 | 750 | A | A | >98.4% |
| 6 | 1438 | A | G | >98.4% |
| 6 | 3197 | T | C | >98.4% |
| 6 | 4769 | A | A | >98.4% |
| 6 | 7028 | C | T | >98.4% |
| 6 | 8860 | A | G | >98.4% |
| 6 | 9477 | G | A | >98.4% |
| 6 | 11467 | A | G | >98.4% |
| 6 | 11719 | G | A | >98.4% |
| 6 | 12103 | C | A | >98.4% |
|     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|
| 6   | 12308 | A   | G   | >98.4% | >98.4% |
| 6   | 12372 | G   | A   | >98.4% | >98.4% |
| 6   | 14766 | C   | T   | >98.4% | >98.4% |
| 6   | 14793 | A   | G   | >98.4% | >98.4% |
| 6   | 14893 | A   | G   | >98.4% | >98.4% |
| 6   | 15218 | A   | G   | >98.4% | >98.4% |
| 6   | 16192 | C   | T   | >98.4% | >98.4% |
| 6   | 16399 | A   | G   | >98.4% | >98.4% |
| 7   | 59   | T   | C   | 97.9%  | >98.4% |
| 7   | 73   | A   | G   | >98.4% | >98.4% |
| 7   | 195  | T   | C   | >98.4% | >98.4% |
| 7   | 200  | A   | G   | >98.4% | >98.4% |
| 7   | 225  | G   | A   | 92.0%  | >98.4% |
| 7   | 263  | A   | G   | >98.4% | >98.4% |
| 7   | 750  | A   | G   | >98.4% | >98.4% |
| 7   | 1438 | A   | G   | >98.4% | >98.4% |
| 7   | 4769 | A   | G   | >98.4% | >98.4% |
| 7   | 6221 | T   | C   | >98.4% | >98.4% |
| 7   | 6371 | C   | T   | >98.4% | >98.4% |
| 7   | 8860 | A   | G   | >98.4% | >98.4% |
| 7   | 10256| T   | C   | >98.4% | >98.4% |
| 7   | 11719| G   | A   | >98.4% | >98.4% |
| 7   | 12705| C   | T   | >98.4% | >98.4% |
| 7   | 14470| T   | C   | >98.4% | >98.4% |
| 7   | 14766| C   | T   | >98.4% | >98.4% |
| 7   | 16183| A   | C   | 95.9%  | 96.6%  |
| 7   | 16189| T   | C   | >98.4% | >98.4% |
| 7   | 16519| T   | C   | >98.4% | >98.4% |
| 7   | 1576 | G   | C   | <0.35% | 7.8%   |
| 7   | 12009| G   | A   | <0.35% | 68.8%  |
| 8   | 72   | T   | C   | 2.8%   | <0.35% |
| 8   | 73   | A   | G   | >98.4% | >98.4% |
| 8   | 228  | G   | A   | >98.4% | >98.4% |
| 8   | 263  | A   | G   | >98.4% | >98.4% |
| 8   | 295  | C   | T   | >98.4% | >98.4% |
| 8   | 462  | C   | T   | >98.4% | >98.4% |
| 8   | 489  | T   | C   | >98.4% | >98.4% |
| 8   | 750  | A   | G   | >98.4% | >98.4% |
| 8   | 1438 | A   | G   | >98.4% | >98.4% |
| 8   | 4769 | A   | G   | >98.4% | >98.4% |
| 8   | 7028 | C   | T   | >98.4% | >98.4% |
| 8   | 8860 | A   | G   | >98.4% | >98.4% |
| 8   | 9280 | T   | C   | 5.4%   | <0.35% |
| 8   | 9548 | G   | A   | >98.4% | >98.4% |
| 8   | 9836 | T   | C   | >98.4% | >98.4% |
| 8   | 10398| A   | G   | >98.4% | >98.4% |
| 8   | 10851| G   | A   | 1.7%   | <0.35% |
| 8   | 11251| A   | G   | >98.4% | >98.4% |
| 8   | 11719| G   | A   | >98.4% | >98.4% |
| 8   | 12612| A   | G   | >98.4% | >98.4% |
| 8   | 13708| G   | A   | >98.4% | >98.4% |
| 8   | 14766| C   | T   | >98.4% | >98.4% |
| 8   | 14798| T   | C   | >98.4% | >98.4% |
| 8   | 15452| C   | A   | >98.4% | >98.4% |
| 8   | 16069| C   | T   | >98.4% | >98.4% |
| 8   | 16126| T   | C   | >98.4% | >98.4% |
| 8   | 4097 | T   | C   | <0.35% | 29.6%  |
| 9   | 60   | T   | C   | 2.0%   | <0.35% |
| 9   | 72   | T   | C   | 6.3%   | 0.5%   |
| # | Position | Base 1 | Frequency 1 | Base 2 | Frequency 2 |
|---|----------|--------|-------------|--------|-------------|
| 9 | 73       | A      | >98.4%      | G      | >98.4%      |
| 9 | 94       | G      | 4.2%        | A      | <0.35%      |
| 9 | 228      | G      | >98.4%      | A      | >98.4%      |
| 9 | 234      | A      | 4.1%        | G      | 3.4%        |
| 9 | 263      | A      | >98.4%      | G      | >98.4%      |
| 9 | 295      | C      | >98.4%      | T      | >98.4%      |
| 9 | 462      | C      | >98.4%      | T      | >98.4%      |
| 9 | 489      | T      | >98.4%      | C      | >98.4%      |
| 9 | 750      | A      | >98.4%      | G      | >98.4%      |
| 9 | 1343     | A      | G           | >98.4% | >98.4%      |
| 9 | 4769     | A      | >98.4%      | G      | >98.4%      |
| 9 | 7028     | C      | >98.4%      | T      | >98.4%      |
| 9 | 8860     | A      | >98.4%      | G      | >98.4%      |
| 9 | 9830     | C      | 32.1%       | T      | 5.3%        |
| 9 | 10172    | G      | >98.4%      | A      | >98.4%      |
| 9 | 10398    | A      | >98.4%      | G      | >98.4%      |
| 9 | 11251    | A      | >98.4%      | G      | >98.4%      |
| 9 | 11719    | G      | >98.4%      | A      | >98.4%      |
| 9 | 12477    | T      | >98.4%      | C      | >98.4%      |
| 9 | 12612    | A      | >98.4%      | G      | >98.4%      |
| 9 | 13708    | A      | >98.4%      | G      | >98.4%      |
| 9 | 13858    | A      | >98.4%      | G      | >98.4%      |
| 9 | 14766    | C      | >98.4%      | T      | >98.4%      |
| 9 | 14976    | C      | >98.4%      | T      | >98.4%      |
| 9 | 15452    | C      | >98.4%      | A      | >98.4%      |
| 9 | 16063    | C      | >98.4%      | T      | >98.4%      |
| 9 | 16069    | C      | >98.4%      | T      | >98.4%      |
| 9 | 16126    | T      | >98.4%      | C      | >98.4%      |
| 9 | 12653    | G      | <0.35%      | A      | >98.4%      |
| 9 | 14110    | T      | <0.35%      | C      | 12.4%       |
| 10 | 60       | T      | <0.35%      | C      | <0.35%      |
| 10 | 72       | T      | 3.5%        | C      | <0.35%      |
| 10 | 263      | A      | >98.4%      | G      | >98.4%      |
| 10 | 750      | A      | >98.4%      | G      | >98.4%      |
| 10 | 1438     | A      | >98.4%      | G      | >98.4%      |
| 10 | 4769     | A      | >98.4%      | G      | >98.4%      |
| 10 | 6296     | C      | >98.4%      | A      | >98.4%      |
| 10 | 8860     | A      | >98.4%      | G      | >98.4%      |
| 10 | 16093    | T      | 72.2%       | C      | >98.4%      |
| 10 | 16337    | C      | 3.3%        | T      | <0.35%      |
| 10 | 16519    | T      | >98.4%      | C      | >98.4%      |
| 10 | 93       | A      | <0.35%      | G      | 77.9%       |
### Supplementary Table 7. Primers for preparation of PCR1 and PCR2 libraries

| set1_F  | set1_F_s tart | set1_F_e nd | set1_R  | set1_R_s tart | set1_R_e nd | set2_F  | set2_F_s tart | set2_F_e nd | set2_R  | set2_R_s tart | set2_R_e nd |
|---------|---------------|-------------|---------|---------------|-------------|---------|---------------|-------------|---------|---------------|-------------|
| tcacccatatcaacactcaag | 14 | 34 | aacacttttattttaggggtatg | 632 | 656 | caaaaaaagaagccttaaccc | 356 | 378 | gggagggggtgactaaac | 945 | 964 |
| aggttggtgcctcttcctct | 651 | 671 | aactttctgcctctcctc | 1297 | 1316 | tccccataaagctaaa | 961 | 982 | gtttagtctagagcgcttc | 1659 | 1678 |
| aaggtgtacccatgaggtg | 1331 | 1350 | tcgaaacacttcacataca | 2007 | 2026 | acgaaaccatttaccaaa | 1718 | 1737 | ttaatcctgacagctggttca | 2334 | 2355 |
| tgcccaaacagccctctaa | 2055 | 2074 | gcagttgcctttcgccttgg | 2669 | 2688 | gcataagctgcgtcagatt | 2333 | 2352 | ttgctctgcatcaacctcagag | 2986 | 3005 |
| gcctaatccagcaagcagca | 2701 | 2720 | tcgccatgagttgtgcttc | 3300 | 3319 | tcgagccgctatagcactcgaa | 3015 | 3034 | gaggccctgtgaggctgtga | 3593 | 3612 |
| acaacattacagcgcgc | 3302 | 3321 | gccgtcttcgtctctcctga | 3875 | 3894 | ccrrttacaccaacacgtt | 3587 | 3606 | gggagagctggagatgtagtga | 4233 | 4252 |
| ggcttcacatcatgccgtg | 3934 | 3953 | gctacgcgtcttgtagtgtg | 4519 | 4538 | tcacccatcaacctctcagat | 4228 | 4247 | tccagatgtaaaggggtcata | 4792 | 4811 |
| tctttgcagcagcaactcact | 4507 | 4526 | tcgctctgctcttgagttaaag | 5129 | 5148 | gcgccaaggtttcctaaaggg | 4812 | 4831 | gttgaaagccggagtgttcttt | 5415 | 5470 |
| tcgcaacctgaaacagctaa | 5162 | 5181 | ttgaaatgccttcagcagaa | 5785 | 5805 | ctcctacagctactctctca | 5461 | 5480 | gcctccgattatgaggttagtttc | 6114 | 6134 |
| aaagacggtcactccctgctc | 5832 | 5851 | gaagggggtgtttggtttttgatt | 6436 | 6455 | gaggctttgcacactgactt | 6129 | 6148 | tgtgagtctcacacagatagaa | 6756 | 6775 |
| aatacacaagccccccttcc | 6436 | 6455 | aagcctctcatatgtgcaaa | 7062 | 7081 | tggcttctaggtttttgatt | 6743 | 6762 | gggcttctcagatggtggttt | 7408 | 7427 |
| caacacctcaacccctaca | 7122 | 7141 | ttctgtcgccctgctcagttt | 7746 | 7766 | cccctacacacattcggaagaa | 7404 | 7424 | gcagctctcgctgcaagttggc | 8062 | 8081 |
| catctcagctgcagggaaa | 7747 | 7766 | tgaagtgcgggtgctcttcttc | 8382 | 8401 | catagctgtcccacacat | 8070 | 8089 | tcaggttctgctctttaggttgc | 8709 | 8730 |
| tatacctgatgctggcc | 8380 | 8399 | gttggcttccatgaattt | 9041 | 9059 | caaactaaagagcagaaacct | 8704 | 8729 | gttgtccttggatgtggttc | 9397 | 9416 |
| tcaacccatatccctctctt | 9070 | 9090 | aagctggagagacgccagaa | 9750 | 9769 | gcggctttgataggggtgatt | 9438 | 9457 | aagcglagaggggtggtgatt | 10082 | 10102 |
| ggcatctggggcctcaattt | 9777 | 9796 | tcgaaacactccctcctgcttct | 10418 | 10438 | tcaccaoccttacaccttc | 10084 | 10103 | tgggacagatattttttagctg | 10578 | 10780 |
| ccaaatgccccccattacca | 10466 | 10485 | caagttggagagagctttggtggttg | 11130 | 11149 | ccctgcagctgcttgttccaa | 10796 | 10817 | ggggttgagtcagaggtggtt | 11497 | 11516 |
| atgacggccacgccgacaga | 11164 | 11181 | gggagagcggctttagctgagc | 11852 | 11870 | acctgctccacactccctgg | 11527 | 11546 | tcgctgtaaaagggggtctg | 12190 | 12210 |
| cccccatataaacactctgg | 11888 | 11888 | aacactctctgctgctcaggggtag | 12499 | 12522 | tccaqggaagcctcactgaagaa | 12202 | 12223 | atcgcaagccgagagatagaa | 12879 | 12898 |
| acaacccatatcaacgcgtct | 12546 | 12565 | ttgtgtaaggagcgagactc | 13203 | 13222 | ttcaattctgcctctagtttcg | 12879 | 12898 | cagggagagcgagagagatag | 13571 | 13590 |
| tcgccacctccactcaag | 13238 | 13258 | tatacgggttggcgtggtgtg | 13935 | 13953 | agggccttactagcctgagaa | 13593 | 13612 | aggggcttagctagcctggctct | 14268 | 14287 |
| acgcgcacctctctctcda | 13976 | 13995 | gggttggggtgggttttcctc | 14612 | 14631 | aactacccagtctccctccttcttc | 14269 | 14288 | ctglagggctagcgagatgg | 14977 | 14996 |
| ccacaactcacaagaacacagag | 14643 | 14664 | gaggtgggtttcgagctg | 15318 | 15337 | caagtgccgctcactat | 14989 | 15017 | gcagagagcctcctgctcttt | 15605 | 15624 |
| atgacggcttcagccact | 15311 | 15330 | tcggtttcaacagtgcgtg | 15910 | 15929 | agggcgtctgcctatttac | 15613 | 15632 | gtagcgtcctgtgctcagtt | 16240 | 16259 |
| ctttttccaagggacaaatca | 15938 | 15958 | cgtgatgctttatttgaaggggaac | 16543 | 16566 | cacccotcaccacactagt | 16257 | 16276 | ggggttggcaggagtgtctg | 336 | 355 |
Supplementary Table 8. Primers for preparation of mtDNA capturing probes

| Amplicon# | Forward primer | Reverse primer | First PCR | Second PCR |
|-----------|----------------|----------------|-----------|------------|
| 1         | M13-tcaaccttaaaacaactcagc | gcctccgattatgatgggta | M13-Forward | gcctccgattatgatgggta |
| 2         | M13-ccaaaccaaaagacctccacc | aaacctatttgtttatggggtgat | M13-Forward | aaacctatttgtttatggggtgat |
| 3         | M13-aggttctgtcctacgctcttc | gggggaatgctggagattgt | M13-Forward | gggggaatgctggagattgt |
| 4         | M13-tcccaatataagctaatcactac | aagctggattcttcgcttcac | M13-Forward | aagctggattcttcgcttcac |
| 5         | M13-aagttcgacccatgtagt | gccttcagctcagagcggtca | M13-Forward | gccttcagctcagagcggtca |
| 6         | M13-aggcacaaccctttacaaa | gaggcttcggtcctttagtgtt | M13-Forward | gaggcttcggtcctttagtgtt |
| 7         | M13-tgccccacaagacctctaa | gggtttcgaatgtgtgg | M13-Forward | gggtttcgaatgtgtgg |
| 8         | M13-gcactagttcgctcagat | gcctccgattatgatgggta | M13-Forward | gcctccgattatgatgggta |
| 9         | M13-ggcttaacacagcgaagcga | tcaggttcgtcctttagtgtt | M13-Forward | tcaggttcgtcctttagtgtt |
| 10        | M13-tgcagccgctattaagagtt | gccttcagctcagagcggtca | M13-Forward | gccttcagctcagagcggtca |
| 11        | M13-acaactaaccctgagccaaac | gagcctctgctttctttagtgga | M13-Forward | gagcctctgctttctttagtgga |
| 12        | M13-ccctcgtcaactcactaaccctctcta | ggtttcttcgaatgtgtgg | M13-Forward | ggtttcttcgaatgtgtgg |
| 13        | M13-aagagcggctaacccctcgtcggc | gggtttcgaatgtgtgg | M13-Forward | gggtttcgaatgtgtgg |
| 14        | M13-ccccctactcttcacatctct | gggtttcgaatgtgtgg | M13-Forward | gggtttcgaatgtgtgg |
| 15        | M13-ccccctactcttcacatctct | gggtttcgaatgtgtgg | M13-Forward | gggtttcgaatgtgtgg |
| 16        | M13-gcctcagctcactcttcacatctct | gggtttcgaatgtgtgg | M13-Forward | gggtttcgaatgtgtgg |
| 17        | M13-tcagctgtgctctctctcctc | tcaggttcgtcctttagtgtt | M13-Forward | tcaggttcgtcctttagtgtt |
| 18        | M13-ccataataaacccttcccttc | tgggctttggagttgcagtt | M13-Forward | tgggctttggagttgcagtt |
| 19        | M13-tcataatcctgtagtaata | tgggctttggagttgcagtt | M13-Forward | tgggctttggagttgcagtt |
| 20        | M13-gtcctcatctgcagctc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 21        | M13-ccataaacccttcggcttctc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 22        | M13-aaggagccgagcttcggt | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 23        | M13-ccctaaggccatcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 24        | M13-tgacctagctgctctctcctc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 25        | M13-taactatcaccactctcggt | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 26        | M13-tcacaaccttgctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 27        | M13-tcaactctggcatcttcgctc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 28        | M13-ccctgtctcagctactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 29        | M13-tcaacctttcagctactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 30        | M13-gcctctgtcgctactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 31        | M13-ggcatctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 32        | M13-tcaactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 33        | M13-tcaacctgtcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 34        | M13-tcaactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 35        | M13-tcaactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 36        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 37        | M13-tcactactattaaactcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 38        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 39        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 40        | M13-ttactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 41        | M13-tcactactattaaactcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 42        | M13-tcactactattaaactcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 43        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 44        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 45        | M13-tcactactattaaactcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 46        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 47        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 48        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 49        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |
| 50        | M13-taactctggctcactcttc | tgcagccgctttccaattaggt | M13-Forward | tgcagccgctttccaattaggt |