Supporting Information Appendix

Hoang et al., PNAS 2016

SI Materials and Methods

Dilution of Y-adapter-ligated molecules. TruSeq adapter-ligated molecules were in a total volume of 20 µL. Five ten-fold serial dilutions were performed in 96-well PCR plates starting with 2 µL of adapter-ligated molecules (prior to PCR) in 18 µL of dilution buffer (TE containing 1 ng/µL pBlueScript). Samples were mixed by gently pipetting with a multichannel pipette. Two µL of each sample was then transferred into 18 µL of fresh dilution buffer using a multichannel pipette. The mixing and transferring was repeated for a total of five serial dilutions. Only 2 µL of each dilution (1/10 total volume) was used as template for each PCR. A 10^3-fold dilution was accomplished as follows: (i) use of 2 µL of the total 20 µL of adapter-ligated molecules (10-fold dilution); (ii) mixing 2 µL of adapter-ligated molecules with dilution buffer in a total volume of 20 µL (10-fold dilution); and (iii) use of 2 µL of diluted adapter-ligated molecules from the total 20 µL volume in the PCR reaction (10-fold dilution, see below). The five serial dilutions resulted in final dilution factors of 10^3, 10^4, 10^5, 10^6, and 10^7. The minimum amount of input DNA per library was 3.4 ng.

PCR amplification of diluted Y-adapter-ligated molecules. Custom HPLC-purified PCR primers (IDT), TS-PCR Oligo1 (5'-AATGATACGGCGACCACCGAG*A) and TS-PCR Oligo2 (5'-CAAGCAGAAGACGGCATACGA*G), were designed with one phosphorothioated bond (*) at the 3' end. PCR was performed in 50 µL total volume with 0.5 µM TS-PCR Oligo1, 0.5 µM TS-PCR Oligo2, Q5 2X HotStart High-Fidelity Master Mix (NEB) at 1X final concentration, and 2 µL of diluted adapter-ligated molecules as template. PCR was performed in Thermo HyBaid PCR Express HBPX Thermal Cycler. The following PCR program was used: 1) 98°C for 30 s 2) 98°C for 10 s, 69°C for 30 s, 72°C for 30 s for 18 or 20 cycles (see Table S1), and 3) 72° for 2 min. PCR reactions were purified with AMPure XP (Agilent) at 1.0X bead-to-sample ratio according to the manufacturer's protocol.

MiSeq run and analysis. A subset of amplified BotSeqS sequencing libraries was evaluated on an Illumina MiSeq instrument (~5 M clusters passed filter per library) to empirically deduce the optimal dilution. The "optimal dilution" was determined to result in 5 to 10 PCR duplicates per molecule when scaled to 1/2 lane of a HiSeq 2500 instrument (~70 M clusters passed filter per library in rapid run mode). For example, for an input of 500 ng gDNA into the TruSeq PCR-free library prep (selecting for 350 bp insert size), amplified libraries from the 10^4-, 10^5-, 10^6-fold dilutions were sequenced at 2 x 50 bp depth on MiSeq. Three different well-barcoded samples (which were also molecularly barcoded) were multiplexed in one MiSeq lane to test three dilutions of each sample. The .bam output files were uploaded into Galaxy, and Picard's Estimate Library Complexity Tool (Galaxy Tool Version 1.56.0) was executed using the default parameters. Optimal dilutions showed distributions ranging from one to four members per family with singletons comprising ~60-80% of total counts. In general, with an input of 500 ng of gDNA into the TruSeq PCR-free library prep, the 10^5-fold dilution yielded

~10 members per family on a subsequent HiSeq run used for BotSeqS. From our sequencing data, we estimate the average number of high quality clusters required to identify one rare mutation in colonic tissues was (1) 30 M in a normal child, (2) 12 M in a normal young adult, and (3) 5.8 M in a normal old adult.

Whole-genome sequencing. Thirty-two whole-genome sequencing (WGS) libraries were generated from the 34 individuals in this study. In the remaining two individuals without WGS, COL238 and COL239, Sanger sequence was performed to exclude clonal variants in the BotSeqS data. Of the final 20 µL of adapter-ligated molecules used to prepare BotSeqS libraries (prior to dilution), 10 µL was used to amplify a library for whole-genome sequencing using TruSeq PCR Primer Cocktail (Illumina) and TruSeq PCR Master Mix (Illumina) according to TruSeq PCR protocol. PCR reactions were purified with AMPure XP (Agilent) at 1.0X bead-to-sample ratio according to the manufacturer's instructions. The libraries were PE sequenced 2x100 bp on Illumina HiSeq 2500 at >30x coverage.

Characterization of BotSeqS sensitivity. Two DNA mixtures were prepared from the DNA of normal spleen samples PEN93 and PEN95. Whole genome sequence data was available from these two samples (1) and SNPs in PEN93 that were not present in PEN95 could be identified. Both mixtures contained the same amount of PEN95 DNA, but the low spike-in mix contained only 10% of the PEN93 DNA contained in the high spike-in mix. BotSeqS libraries from these samples were first analyzed using the normal BotSeqS pipeline to minimize clonal and germline mutations. Only a total of two mutations were detected among the two libraries; these two mutations likely represented rare mutations in the PEN95 sample, and suggest a mutation prevalence of ~8×10^{-7} mutation/bp. Next, the data were processed through the BotSeqS pipeline *without* filtering out mutations that were present in dbSNP (build 130 and 142). Seven PEN93-specific SNPs in the low spike-in and 89 PEN93-specific SNPs in the high spike-in mixtures were identified. After normalizing for the number of sequenced bases, the "mutation prevalence" (number of PEN93-specific SNPs/bp) was 2.7×10^{-6} for the low spike-in and 2.0×10^{-5} for the high spike-in samples. The difference between the low spike-in and the high spike-in was 7.4-fold, within the range expected from the 10-fold dilution given the relatively low number of mutations identified in the low spike-in sample.

Sensitivity is one to a few cells, as follows: one human cell has ~1.3e7 nuclear DNA "molecules", estimated based on each molecule being 500 bp in size (approximate library insert size). Based on the amount of DNA originally purified from each tissue (corresponding to a median of 1.2 M cells), the BotSeqS dilution process resulted in the assessment of ~60,000 nuclear DNA molecules per library. One way to assess sensitivity in terms of cells is to calculate the probability that, given we have picked a molecule from a specific cell, at least one other DNA molecule in the BotSeqS library was from the same cell. This can be modeled as a conditional binomial probability (using the *pbinom* functions of the *R* software): (1- pbinom(1,60000,1/1200000))/(1-pbinom(0,60000,1/1200000)) = 0.025. The probability that at least 3 molecules were from the same cell is then (1- pbinom(2,60000,1/1200000))/(1-

pbinom(0,60000,1/1200000)) = 0.00041. The probability that at least 4 molecules were from that same cell is (1- pbinom(3,60000,1/1200000))/(1- dbinom(0,60000,1/1200000)) = 5.1e-06. And the probability that at least 5 molecules will be from that same cell is (1- pbinom(4,60000,1/1200000))/(1- dbinom(0,60000,1/1200000)) = 5.1e-08.

As with the nuclear genome, we used a conditional binomial distribution to estimate the probability that at least two mtDNA molecules were from the same cell. We evaluated ~3,400 mtDNA molecules per BotSeqS library. The probability that, given we have picked a mtDNA molecule from a specific cell, at least another one of the 3,400 mtDNA molecules was from the same cell is (1- pbinom(1,3400,1/1200000))/(1- pbinom(0,3400,1/1200000)) = 0.0014. The probability that at least 3 molecules were from the same cell is then (1- pbinom(2,3400,1/1200000))/(1- pbinom(0,3400,1/1200000)) = 1.3e-06. The probability that at least 4 molecules were from that same cell is (1- pbinom(3,3400,1/1200000))/(1- dbinom(0,3400,1/1200000)) = 9.5e-10. And the probability that at least 5 molecules were from that same cell is (1- pbinom(4,3400,1/1200000))/(1- dbinom(0,3400,1/1200000)) = 5.5e-13.

Characterization of BotSeqS specificity. As one measure of specificity, we identified *rare* mutations as usual except that we used mutations that were present in only one strand rather than in both. Specifically, mutations were present in ≥ 90% of the Watson family members and the reference sequence was present in ≥ 90% of the Crick family members, or vice versa, but satisfied our other criteria for being "rare". We then created false Watson and Crick pairings, where the Watson strand had overlapping but different coordinates than the Crick strand, and vice versa, to determine if they contained the same mutation by chance. BotSeqS works by having low coverage throughout the genome, generated through the bottleneck dilution step, and precluded this analysis in the nuclear DNA. Instead, we used mtDNA because of the multiple copies of mtDNA per cell. The coverage of mtDNA with BotSeqS is much higher than that of nuclear DNA and facilitated the identification of overlapping molecules. We processed 30 BotSeqS control libraries this way and identified a total of 146 mtDNA mutations present in one strand only. Using this dataset, we then searched within each sample for overlapping molecules and identified 27 examples. None of the 27 false Watson and Crick pairs shared the same artifactual mutation.

Non-random shearing could produce another type of artifact, falsely suggesting that the Watson and Crick strands of a family were actually derived from two different molecules that coincidentally had the same genomic coordinate. To test for such artifacts, we identified Watson and Crick family pairs that contained the variant in the Watson strand and the reference sequence in the Crick strand, or vice versa, but this time included heterozygous germline variants rather than just the rare variants, and in nuclear DNA rather than in mtDNA. There are many more heterozygous variants in nuclear DNA than in mtDNA because the mtDNA is derived only from the oocyte. The discordances of interest could arise as a result of mispairing of a Watson strand with a Crick strand derived from a different template molecule - i.e., non-random shearing. Alternatively, discordances could result from an amplification error in one of the two strands during an early PCR cycle. Using our WGS data, we first identified 8,535,891 nuclear heterozygous variants observed among the 30 BotSeqS control libraries (median of 268,180 variants per library with range 121,850 to 529,920, with the same common variants present in many libraries). From the 8,535,891 nuclear heterozygous variants, we identified a total of 3,960,818 families (median of 123,130 families per library with range 65,832 to 222,140) for which both strands could be evaluated. Of these, 3,960,807 families had the concordant sequence at the variant position in both strands; only 11 heterozygous variants were discordant (i.e., the variant was present in ≥ 90% of the Watson family members and the reference sequence was present in ≥ 90% of the Crick family members, or vice versa). The rate of discordant germline heterozygous variants was thus 2.8×10^{-6} (11 out of 3,960,818) per bp. This rate is compatible with the known error rate of high fidelity DNA polymerases and could easily represent an amplification error that occurred in one of the two strands during the first PCR cycle, so represents an overestimate of shearing artifacts. Furthermore, it is important to note that BotSeqS eliminates such amplification errors by requiring mutations to be observed on both strands. Because BotSeqS requires mutations to be observed on both strands, the actual false positive rate can be estimated to be $\sim(1/3)(2.8 \times 10^{-6})(2.8 \times 10^{-6}) = 2.6 \times 10^{-12}$.

Generation of BotSeqS change and molecule tables. Sequence alignments and variant calling were performed with the Illumina secondary analysis package (CASAVA 1.8) using ELANDv2 matching to the GRCh37/hg19 human reference genome. High-quality reads were selected for further analysis only if they satisfied all of the following criteria: (i) passed chastity filter, (ii) read mapped in a proper pair, (iii) ≤ 5 mismatches to reference sequence (for 100 bp read), and (iv) perfect identity to reference sequence within the first and last five bases of each read. Sequencing reads were grouped into families based on identical paired-end endogenous barcodes. From our 44 BotSeqS libraries, we calculated that unfiltered mtDNA families represent a median of 0.4% (range 0.08 – 2%) of total unfiltered families (mtDNA+nuclear) per library. This is the expected range in sequencing data based on no enrichment of mtDNA. The members of a family were further subdivided into the two possible sequencing orientations to determine the number of Watson and Crick-derived family members. Watson and Crick families had identical genomic coordinates with each end sequenced in opposite reads. Quality scores of identical changes within a family were calculated as the average among the family members. The output for each BotSeqS library was two annotated tables of changes and template molecules (i.e., families). Details outlined in Bottleneck Sequencing System Pipeline Supplemental Document.

Selection of high quality changes and molecules

Custom algorithms were written in Microsoft SQL Server Management Studio to query the changes and molecules tables for each BotSeqS library. Selection criteria detailed in Table S2-S6. Selection was based on quality, clonality, and mappability of single-base pair substitutions. For example, it is known that one of the major sources of errors facing all short read alignment and variant callers are artifacts that arise when variants map to repetitive regions in the genome, including low complexity regions and copy number variants (2). The BotSeqS pipeline eliminates this universal error in a downstream step by filtering out the genomic noise from repetitive DNA and structural variants (detailed in Table S6). Indels were excluded because they are prone to alignment artifacts and are about 10 times less frequent than spontaneous point mutations. High quality single-base substitutions were defined as those with average quality scores (within the family) of ≥ Q30 and with ≥ 2 reads and ≥ 90% mutation fraction in both the Watson and Crick strands. Variants were considered to be clonal if the variant position was present in the WGS data from that sample or observed in > 1 template molecules (i.e., both strands of more than one UID). We also excluded any positions present in dbSNP130 or dbSNP142. We noticed that the dbSNP filtering drastically minimized recurrent sequencing or mapping artifacts and highly mutable regions. For example, homopolymer tracts (≥8 bp) are mutation hotspots that flood the mutation list. We observed that nearly all were filtered out with dbSNP142. Finally, families that harbored > 1 mutation were excluded as possible mapping artifacts. Details outlined in Bottleneck Sequencing System Pipeline Supplemental Document.

Calculation of mutation prevalence. Mutation prevalences were determined for each BotSeqS library (see Table S9) by dividing the total number of rare mutations by the total bp sequenced. The total bp sequenced was defined by number of families x 2 x read length of each family. The average length of the libraries was ~ 500 bp such that the 100 bp paired-end reads were unlikely to overlap. Only templates with perfect identity to the reference sequence in the first and last 5 bp of every read were considered. We further trimmed the reads by excluding cycle 6 and 7 to ensure quality. Therefore, the actual read length was 88 bases (100-7-5 = 88). For the samples from which technical replicate BotSeqS libraries were generated, the average mutation prevalence of the technical replicates was considered the mutation prevalence for the sample.

Validation of somatic mutations. All rare mutations from the nuclear and mtDNA genome passed visual inspection of the sequencing reads. For rare nuclear mutations, Sanger sequencing was performed on a representative set (514 out of 876 mutations). Of these, 514 of 514 (100%) were confirmed to be invisible by Sanger sequencing (excluding the COL238 and COL239 samples that did not have a matched WGS). This demonstrated that these mutations were neither present in the germline nor present in a highly clonal fashion. Mutations confirmed to be absent upon Sanger sequencing are indicated in Table S8.

Comparison to cancer genomes. MAF files representing nuclear somatic mutations from TCGA tumor types COADREAD and KIRC were downloaded at https://www.synapse.org/#!Synapse:syn1729383 (3). From the TCGA data, only single-base substitutions were considered and somatic mutations from ultra-mutated tumors were excluded. Mitochondrial DNA somatic mutations from colorectal and renal tumors were derived from supplementary file 2 of a previous report (4).

Study design and statistical analysis. For study design, no prior power analysis or randomization was performed because the variance was initially unknown. The goal of the study was to find major, biologically meaningful differences between the cohorts. To find major differences, sample sizes can be small. The initial rationale for the sample size for colon and brain was to acquire at least three individuals in each age group in order to understand the average trend of somatic mutational patterns for each age group. Age groups for colon and brain were selected based on human body growth and maintenance: early body development at < 10 years, fully grown young adult body at ~20-40 years, and old, maintained adult body at > 90 years. For colon, one tissue from the young child age group (SIN230) was later determined to be duodenum, leaving only two individuals representing the young child age group for colon epithelium. For normal kidney, criteria for kidney acquisition were an age-matched and non-smoking control group for the kidneys of smokers and aristolochic acid-exposed samples. All normal kidney controls were Caucasian and therefore less likely to originate from a high risk AA-exposed population (e.g. Asia). From the same kidney tissue source, three aliquots of flash frozen, post-mortem normal kidney from a five month old individual were available as technical replicates and to further test an age-trend for non-carcinogen exposed normal kidneys. All analyzed samples were reported in the manuscript.

Even with the small sample size, no violations of the assumptions of the tests were detected, including violations about the homogeneity of variances. T-test and ANOVA analyses were performed using GraphPad Prism 5.0f. Fisher's exact test and principal component analysis was performed using R version 3.2.2.

1. Jiao Y, et al. (2011) DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* 331(6021):1199-1203.
2. Li H (2014) Toward better understanding of artifacts in variant calling from high-coverage samples. *Bioinformatics* 30(20):2843-2851.
3. Kandoth C, et al. (2013) Mutational landscape and significance across 12 major cancer types. *Nature* 502(7471):333-339.
4. Ju YS, et al. (2014) Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer. *eLife* 3.

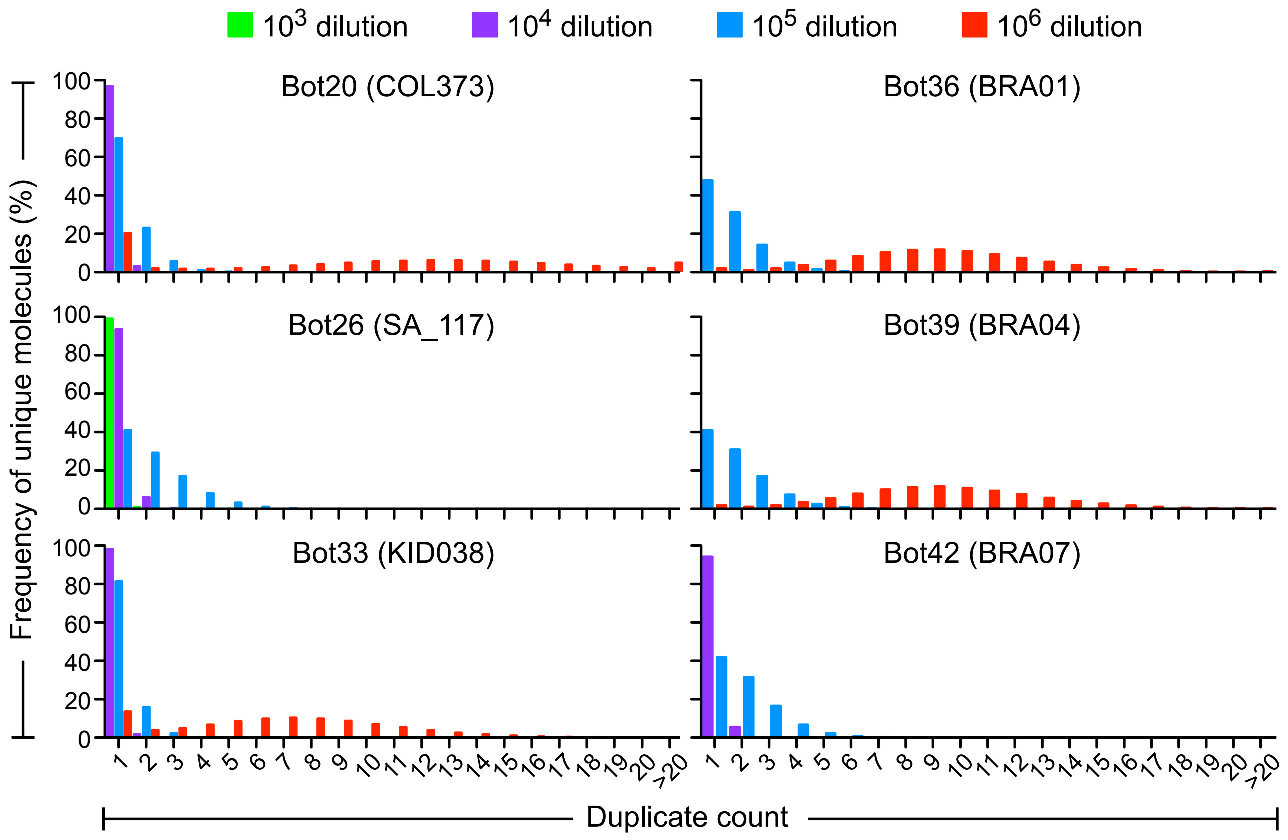


Fig. S1. Assessment of duplicate counts with MiSeq prerun. Histograms showing the distribution of UID family members (PCR duplicates from individual template molecules, shown on the x-axis). Either two or three serial dilutions (10^3, 10^4, 10^5, or 10^6) were evaluated on the MiSeq for six BotSeqS libraries (Bot20, Bot26, Bot33, Bot36, Bot39, Bot42) to generate ~5 M properly paired reads per library. Family member counts were determined here using Picard's Estimate Library Complexity program. Libraries generated from the 10^5 dilution (blue) were subsequently used for the final HiSeq run reported in this study. Note that the HiSeq distribution is expected to shift to the right compared to the MiSeq distribution due to the increase of clusters sequenced per library (~5 M clusters scaled to ~70 M clusters). For example, the BotSeqS libraries from the 10^6 dilution (red) were not used because the members per UID family would be too high on a HiSeq run, limiting the number of different families that could be evaluated with a given amount of sequencing.

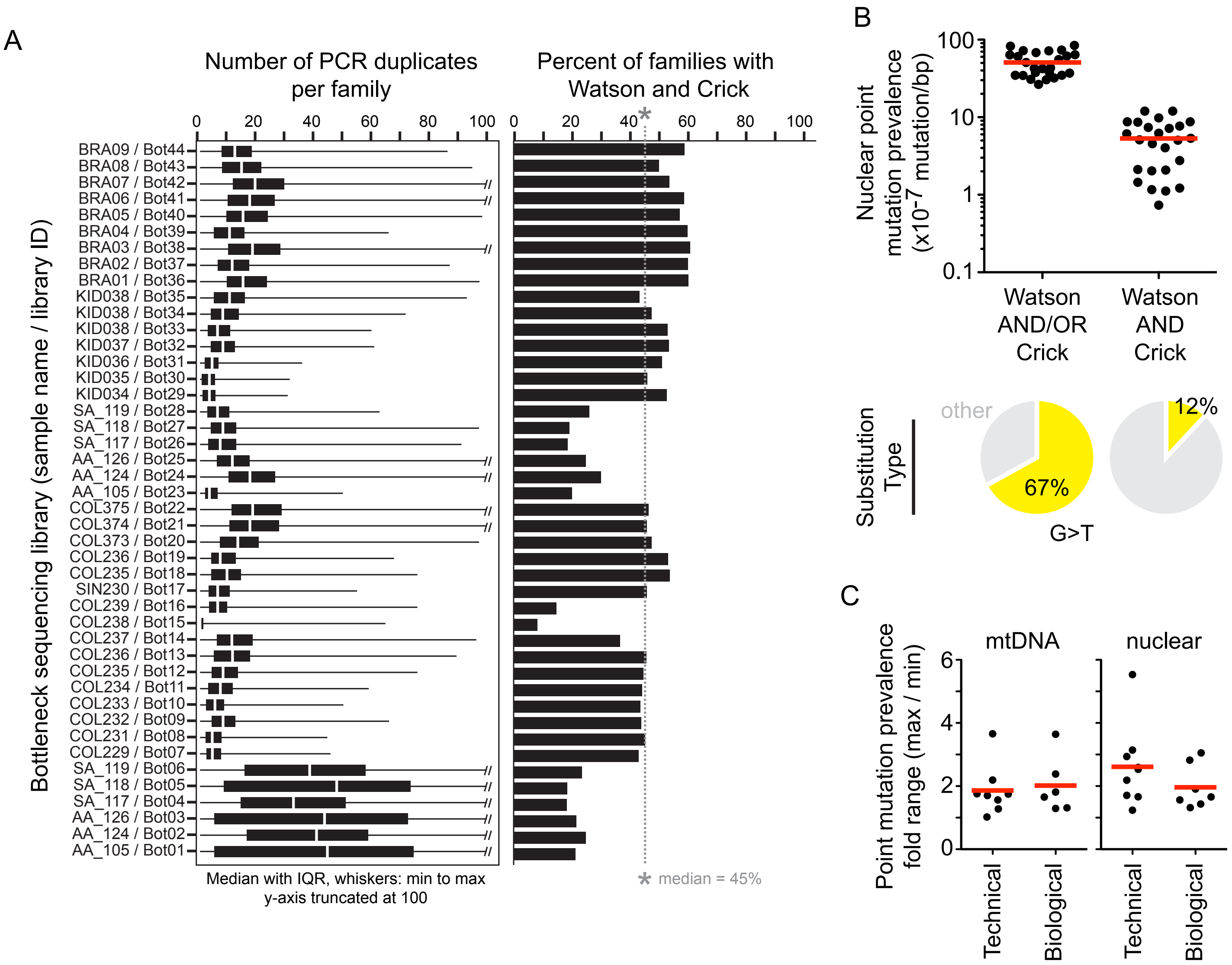


Fig. S2. Methodological parameters in BotSeqS (A) Left graph: horizontal box and whisker plots for 44 BotSeqS libraries (y-axis) and number of members per family (PCR duplicate count, x-axis). Filled boxes represent the first to third quartile range with hash mark indicating median. Whiskers represent minimum to maximum. Double slash of whisker indicates that maximum was truncated at 100. An average of 4 M (range 0.4 to 10 M) unfiltered families per library were assessed. Right graph: percent of unfiltered families with both Watson and Crick duplicate families present in each BotSeqS library. Grey dashed line indicates median. (B) Top graph: Nuclear point mutation prevalences (y-axis) considering mutations observed in “Watson AND/OR Crick” or “Watson AND Crick” families in normal tissues of 25 control individuals. “OR” mutations represent ≥ 90% mutation fraction in Watson family with a minimum of two Watson reads or ≥ 90% mutation fraction Crick family with a minimum of two Crick reads. “AND” mutations represent ≥ 90% mutation fraction in Watson family with a minimum of two Watson reads and ≥ 90% mutation fraction Crick family with a minimum of two Crick reads. “AND” mutations are an internal subset of the “AND/OR” dataset, which is a modified version of the BotSeqS pipeline. Red line indicates average. Bottom pie charts: frequency of G>T substitutions out of all possible substitution types (labeled as ‘other’) considering Watson AND/OR Crick (left pie chart) or Watson AND Crick (right pie chart) of 25 control individuals shown in top graph. Number of nuclear mutations generating mutational spectra for Watson AND/OR Crick was N=4415 and for Watson AND Crick was N=206. (C) Fold-range of point mutation prevalence of technical and biological BotSeqS replicates (details in Table S1) from mtDNA (left graph) and nuclear genome (right graph). Each point represents the ratio of the maximum to minimum of the replicate group. Red line indicates average.

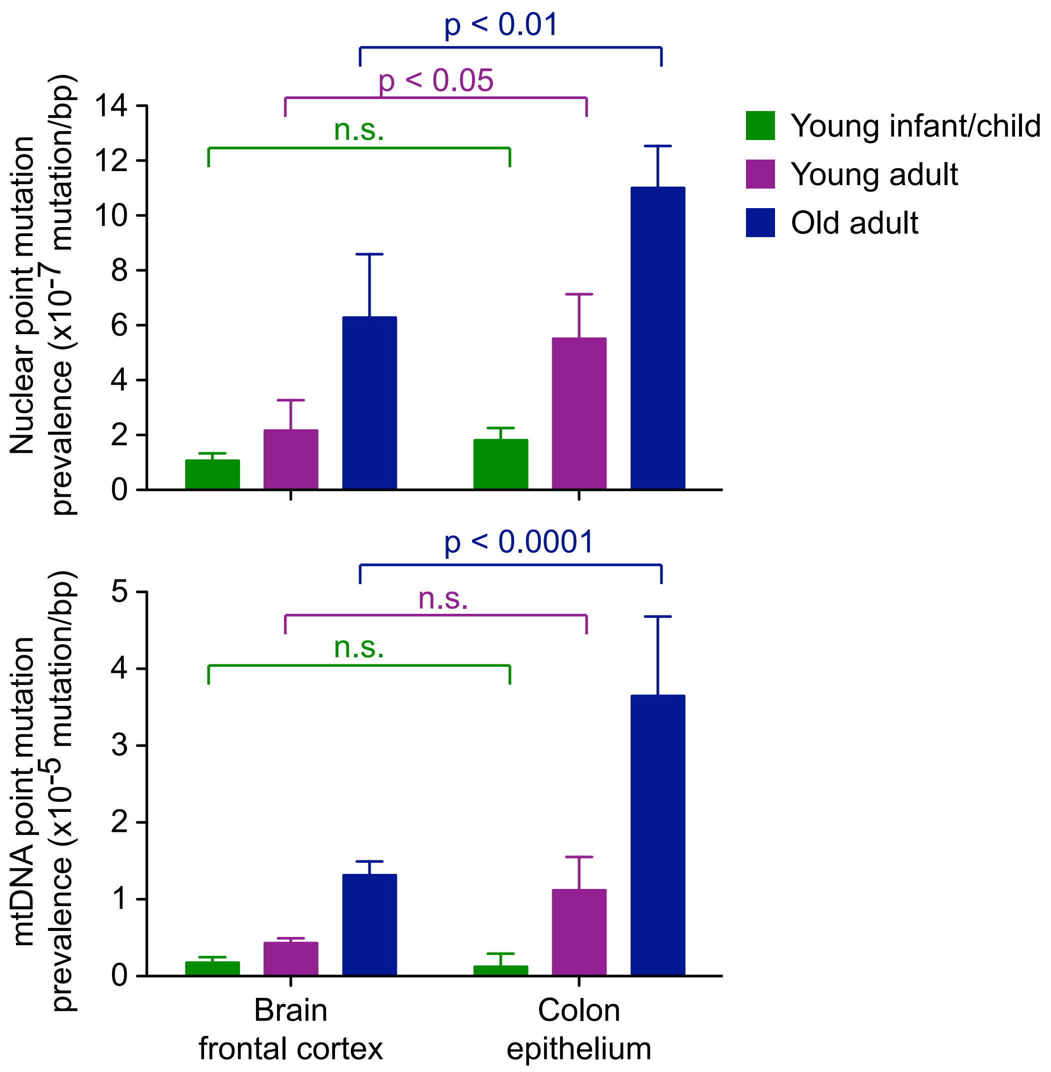


Fig. S3. Rare point mutations accumulate in normal tissues of the colon more than in brain. Point mutation prevalence (y-axis) in nuclear (top graph) and mitochondrial (bottom graph) genome in normal brain frontal cortex (left side) and normal colon epithelium (right side) grouped by age (young infant/child in green, young adult in purple, old adult in blue). Average +/- SD of each cohort shown. Two-way ANOVA with Bonferroni multiple comparison post-test was performed using GraphPad Prism 5.0f software with P values reported above bars. n.s. indicates P > 0.05. For brain, the number of individuals and average age of group are as follows- infant/child: N=3 (BRA01, BRA02, BRA03), 3.5 years old (y/o); young adult: N=3 (BRA04, BRA05, BRA06), 22 y/o; and old adult: N=3 (BRA07, BRA08, BRA09), 93 y/o. For colon, infant/child: N=2 (COL229, COL231), 5.5 y/o ; young adult: N=6 (COL235, COL236, COL237, COL373, COL374, COL375), 28 y/o; old adult: N=3 (COL232, COL233, COL234), 96 y/o.

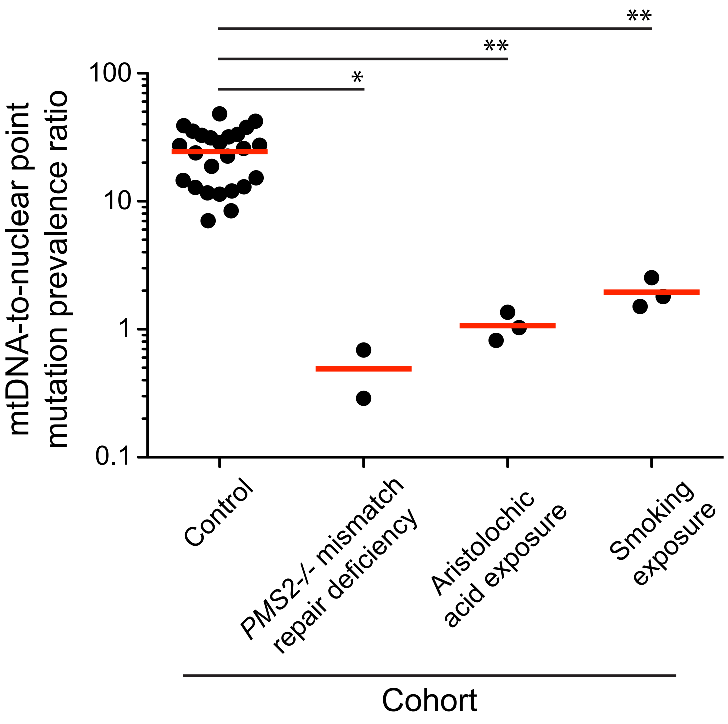
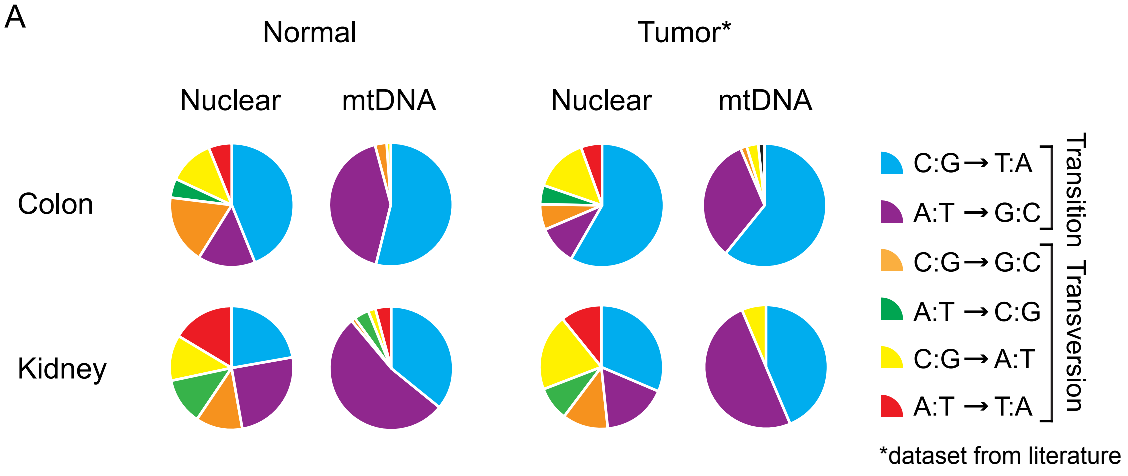


Fig. S4. Mitochondrial and nuclear point mutation prevalences in normal tissues from the same individual. Data points represent the ratio between mitochondrial to nuclear point mutation prevalences (y-axis) within the normal tissue of each individual. Individuals were grouped into four cohorts (x-axis) with N=24 for Control (see Table S9), N=2 (COL238, COL239) for *PMS2-/-* mismatch repair deficiency, N=3 (AA_105, AA_124, AA_126) for aristolochic acid exposure, and N=3 individuals (SA_117, SA_118, SA_119) for smoking exposure. One ratio from the control cohort (COL229) was zero and omitted from this analysis. Average (red line) ratio for each cohort is 25 for Control, 0.5 for DNA repair defect PMS2-/-, 1.1 for aristolochic acid exposure, and 2.0 for smoking exposure. *P < 0.05, **P < 0.01, one-way ANOVA with Bonferroni multiple comparison post-test.

A



B

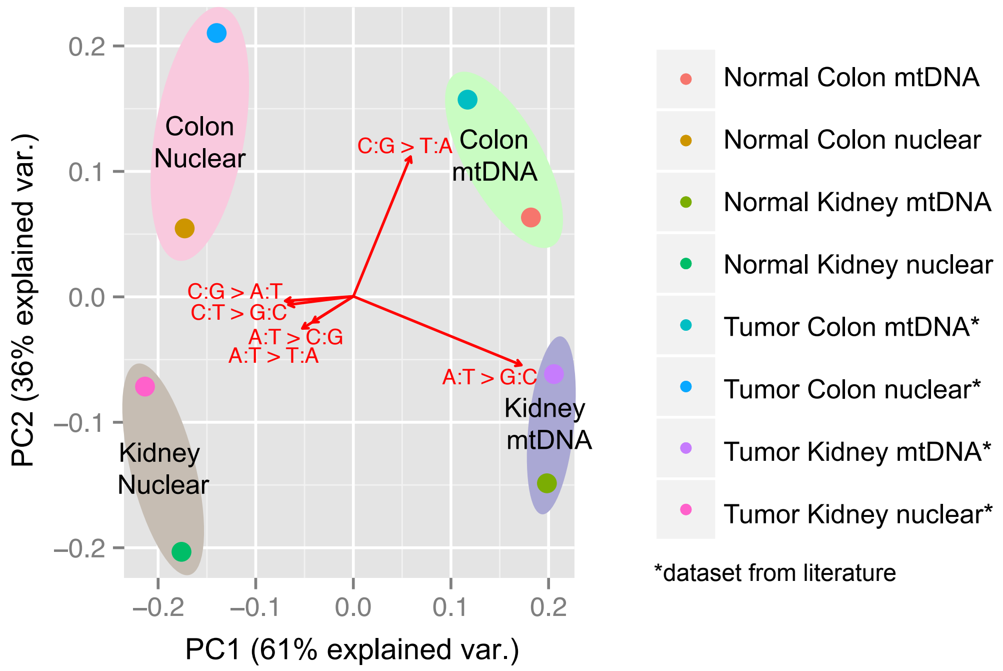


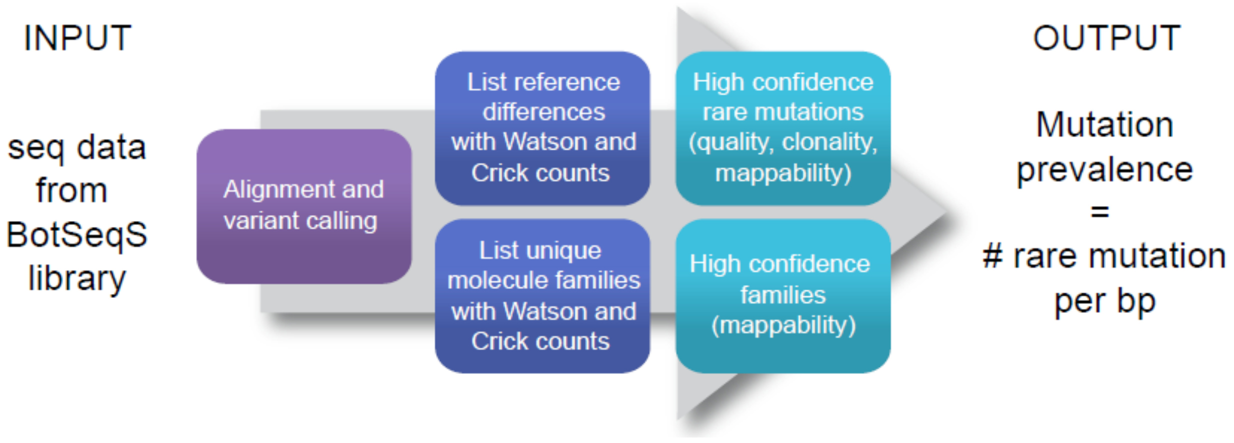
Fig. S5. Normal tissues and tumors derived from the same tissue type have similar mutation spectra (A) Pie charts of nuclear and mitochondrial substitution types out of six possible (see legend) comparing normal (left side) and tumors (right side) derived from colon (top) and kidney (bottom). "Normal" represents the rare mutational spectra data derived from normal tissues shown in Fig. 3. "Tumor" nuclear mutations represent clonal mutation data from colorectal carcinomas (COAD/READ) and clear cell renal carcinoma (KIRC) from the TCGA dataset acquired from www.synapse.org/#!Synapse:syn1729383. “Tumor” mtDNA mutations from colon and kidney were acquired from “colorectal” and “renal” tumor types in supplementary file 2 of Ju et al., 2014. For normal tissues, the number of substitutions assessed was as follows: colon nuclear N=94 from 13 individuals, colon mtDNA N=116 from 12 individuals, kidney nuclear N=73 from 7 individuals, and kidney mtDNA N=299 from five individuals. For tumor tissue, the number of substitutions assessed was as follows: colorectal carcinoma nuclear N=18,538 from 193 individuals, colorectal carcinoma mtDNA N=64 from 76 individuals, clear cell renal cell carcinoma nuclear N=24,559 from 417 individuals, and renal carcinoma mtDNA N=16 from 23 individuals. (B) Principal component analysis (PCA) of mutational spectra from the cohorts indicated in (A). PCA performed and graphed using R software.

SI BotSeqS Pipeline Supplement

1 Summary

Bottleneck Sequencing System (BotSeqS) is an unbiased next-generation sequencing (NGS) method designed to accurately quantitate extremely rare, or “nonclonal”, somatic mutations from a population of cells. Our initial application of BotSeqS was on human tissue samples using the Illumina sequencing platform. Therefore the BotSeqS pipeline described here is specific to human samples and Illumina sequencing. However, in principal, BotSeqS may be developed with any type of cell population, organism, and/or high throughput sequencing platform.

The goal of BotSeqS data processing is to accurately calculate the genome-wide mutation prevalence for nuclear DNA and mitochondrial DNA (mtDNA) per sample. The pipeline can be broken down into three phases as shown in Figure 1: (1) Alignment and variant calling, (2) Listing reference differences (or “changes”) and unique molecules annotated with counts and quality parameters, and (3) selection of high confidence rare mutations and molecules.


INPUT
seq data from BotSeqS library
Alignment and variant calling
List reference differences with Watson and Crick counts
List unique molecule families with Watson and Crick counts
High confidence rare mutations (quality, clonality, mappability)
High confidence families (mappability)
OUTPUT
Mutation prevalence = # rare mutation per bp

2 Illumina sequencing data outputs

Two libraries were generated for each sample, whole-genome sequencing (WGS) and bottleneck sequencing library (BOT).

1. WGS libraries were sequenced to >30x average coverage. Variants were aligned and called using Illumina’s Isaac algorithm. BotSeqS pipeline worked with variants tabulated in the genome variant call format (gVCF). This information facilitated the identification of somatic mutations in the BOT libraries. For example, about 90% of reference differences in BOT libraries were germline or clonal variants present in the WGS, and therefore filtered out.

2. BOT libraries were pair-ended sequenced at 2 x 100 bp with an average of 73 M clusters pass filter (about one-half lane of Illumina HiSeq 2500 on rapid run mode). Sequencing data were processed through Illumina's on machine secondary analysis CASAVA 1.8. This program included ELANDv2 alignment to hg19 and variant calling. The output of BOT data was an Illumina export txt file with 22 columns (for details of Illumina column information, please refer to *http://support.illumina.com/help/SequencingAnalysisWorkflow/Content/Vault/Informatics/Sequencing_Analysis/CASAVA/swSEQ_mCA_Exporttxt.htm*).

3 Data pre-processing and structure

Microsoft Sequel (SQL) Server Management Studio software was used for the majority of the data processing steps. BOT export files were first processed through our custom pipeline used for Illumina DNA sequencing studies. Because this pre-processing pipeline was developed specifically for our in-house hardware structure, it is not useful for off-site reproduction and therefore not included here. However, below we describe the output of our custom pipeline pre-processing. The output data structure is important to understand as it was the basis for the following SQL queries we provide further below.

SQL outputted the following 885 tables for each BOT library. Tables 1-6 include experimental information, processing criteria, and summary sheets. The remaining tables were organized into chromosomal regions, with each region associated with three tables: changes, coverage, and tags. The human genome was arbitrarily split into a number of different regions to increase processing speed.

1. ConsolidatedChanges
2. Experiment
3. ExperimentSummary
4. ImportedFiles
5. RoiCoverage
6. Sample
7. Zchr1_10Changes
8. Zchr1_10Coverage
9. Zchr1_10Tags
10. Zchr1_11Changes
11. Zchr1_11Coverage
12. Zchr1_11Tags
13. Zchr1_12Changes
14. Zchr1_12Coverage
15. Zchr1_12Tags
16. Zchr1_13Changes
17. Zchr1_13Coverage
18. Zchr1_13Tags
19. Zchr1_14Changes
20. Zchr1_14Coverage
21. Zchr1_14Tags
22. Zchr1_15Changes
23. Zchr1_15Coverage
24. Zchr1_15Tags
25. Zchr1_16Changes

26. Zchr1_16Coverage
27. Zchr1_16Tags
28. Zchr1_17Changes
29. Zchr1_17Coverage
30. Zchr1_17Tags
31. Zchr1_18Changes
32. Zchr1_18Coverage
33. Zchr1_18Tags
34. Zchr1_19Changes
35. Zchr1_19Coverage
36. Zchr1_19Tags
37. Zchr1_1Changes
38. Zchr1_1Coverage
39. Zchr1_1Tags
40. Zchr1_20Changes
41. Zchr1_20Coverage
42. Zchr1_20Tags
43. Zchr1_21Changes
44. Zchr1_21Coverage
45. Zchr1_21Tags
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861. ZchrY_2Tags
862. ZchrY_3Changes
863. ZchrY_3Coverage
864. ZchrY_3Tags
865. ZchrY_4Changes
866. ZchrY_4Coverage
867. ZchrY_4Tags
868. ZchrY_5Changes
869. ZchrY_5Coverage
870. ZchrY_5Tags
871. ZchrY_6Changes
872. ZchrY_6Coverage
873. ZchrY_6Tags
874. ZchrY_7Changes
875. ZchrY_7Coverage
876. ZchrY_7Tags
877. ZchrY_8Changes
878. ZchrY_8Coverage
879. ZchrY_8Tags
880. ZchrY_9Changes
881. ZchrY_9Coverage
882. ZchrY_9Tags
883. ZNMChanges
884. ZNMCoverage
885. ZNMTags

Below we describe the following tables from the output above:

(1) Consolidated Changes
(2) Zchr*_*Changes
(3) Zchr*_*Coverage
(4) Zchr*_*Tags

(1) ConsolidatedChanges- summarizes all variants called

Column header	**Example value**
UID	59173F25-9FFE-42B6-96F7-0006CF05D366
IndexString	0
Coverage	8
MutCount	8
ACount	0
CCount	0
GCount	0
TCount	8
SumQualityScore	311
MinQualityScore	30
MaxQualityScore	41
AverageQualityScore	38
Forward	3
Reverse	5
DistinctCoverage	2
DistinctTags	2
DistinctPairs	2
MutType	Homo-SBS
Chrom	chr4
Start	181198628
End	181198628
BaseFrom	C
BaseTo	T
Transcript	NULL
TxChangeStart	NULL
TxChangeEnd	NULL
TxBaseFrom	NULL
TxBaseTo	NULL
AminoStart	NULL
AminoEnd	NULL
AAFrom	NULL
AATo	NULL
SNP	rs2309255
Deleted	1
Comment	NULL

(2) Zchr*_*Changes

Column header	**Example value**
ClusterID	0_HISEQ_150_2_2109_10547_89283
IndexString	0
ReadNumber	1
Cycle	34

Filtered	Y
TotalMutationsInTag	1
QualityScore	9
MutType	SBS
Chrom	chr1
MatchOrientation	F
Start	29401957
End	29401957
BaseFrom	T
BaseTo	A
Deleted	1
PairCoordinates	chr1F29401924chr1R29402354

(3) Zchr*_*Coverage

Column header	**Example value**
IndexString	0
Position	17185422
RawCoverage	13
PhredCoverage	11
EffectiveCoverage	0.994141
DistinctRawCoverage	2
DistinctPhredCoverage	2
DistinctEffectiveCoverage	0.25

(4) Zchr*_*Tags

Column	**Example value**
ClusterID	0_HISEQ_150_2_2109_10045_85816
IndexString	0
ReadNumber	1
Sequence	CCCACCACCACACCCAGTTAATTTTTTGTATTTTTAGTAGAGGCAG
Quality	___eeeeegggfeffgffhghfhhihhhfhhiihihhfhefefdgfif_e`fcbbb\db^`dddeec
Match	chr1.fa
Chrom	chr1
MatchOrientat	F
LeftPosition	22162961
RightPosition	22163060
MatchContig	NULL
MatchDescript	100
MinQS	7
MaxQS	41
AverageQS	35
TotalMuations	0
AlignmentSco	11
PairedEndRe	888
PartnerChrom	NULL

PartnerContig	NULL
PartnerOffset	314
PartnerOrient	R
Filtered	Y
Exclude	0
PairCoordinat	chr1F22162961chr1R22163275

4 Organize data into change and molecule table with Watson and Crick family counts

The goal of this section is to use the data structure detailed above to reorganize the data into two tables per BOT library: changes and molecules. The outputs of QUERY 1 were two intermediate tables that are the input to QUERY 2. The outputs of QUERY 2 were the final tables with example names as follows:

Final molecule output of QUERY 2 (molecules table): *NgsNCS_Brain01NormalP30MM6F5Genome100D031615_SupermutantMoleculesSummaryFinal_022213v6*

Final changes output of QUERY 2 (changes table): *NgsNCS_Brain01NormalP30MM6F5Genome100D031615_SuperMutantsSummaryFinal_022213v6*

Below are Query 1 and Query 2. After the queries, examples of the **Final molecules output of QUERY 2** and **Final changes output of QUERY 2** are given.

QUERY 1

```
declare
@SQL varchar(8000),
@ChromSub varchar(50),
@exp varchar(255),
@dbTo varchar(255),
@serverFrom varchar(255),
@Version varchar(255),
@TagMutationsMax tinyint,
@TagLen_i int,
@TagLen_s varchar(3),
@Trim tinyint,
@lowlim_search varchar(3),
@maxgap varchar(3),
@lowlim_total varchar(3)

set @serverFrom = 'LUDSEQ7'
set @exp = 'NgsNCS_Brain09NormalP30MM6F5Genome100D031615'
set @dbTo = '[MH_Nonclonal_NCS]'
set @Version='022213v6'
set @TagMutationsMax = 5 -- Maximum number of mutations in both tags,
inclusive
set @TagLen_i = 100 -- Length of tag (integer)
set @TagLen_s = 100 -- Length of tag (string)
set @Trim = 5 -- Number of bases on each end of tag that must be perfect,
inclusive

set @lowlim_search = 2
set @maxgap = 1
set @lowlim_total = 8

create table #TempTag (
      [ClusterID] [varchar](250) not null,
      [IndexString] [varchar](25) null,
      [ReadNumber] [tinyint] not null,
      [Sequence] [varchar](100) null,
      [Quality] [varchar](100) null,
      [Match] [varchar](50) null,
      [Chrom] [varchar](50) null,
      [MatchOrientation] [varchar](1) null,
      [LeftPosition] [int] null,
      [RightPosition] [int] null,
      [MatchContig] [varchar](150) null,
      [MatchDescription] [varchar](150) null,
      [MinQS] [tinyint] null,
      [MaxQS] [tinyint] null,
      [AverageQS] [tinyint] null,
      [TotalMutationsInTag] [tinyint] null,
      [AlignmentScore] [int] null,
      [PairedEndReadAligmentScore] [int] null,
      [PartnerChrom] [varchar](150) null,
      [PartnerContig] [varchar](150) null,
      [PartnerOffset] [int] null,
      [PartnerOrientation] [char](1) null,
      [Filtered] [char](1) null,
      [Exclude] [bit] null,
      [PairCoordinates] [varchar](120) null,
      [GenePair] [varchar](50)
)

create table #TempChanges (
      [ClusterID] [varchar](250) not null,
      [IndexString] [varchar](25) null,
      [ReadNumber] [tinyint] not null,
      [Cycle] [tinyint] not null,
      [Filtered] [char](1) null,
      [TotalMutationsInTag] [tinyint] null,
      [QualityScore] [tinyint] null,
      [MutType] [varchar](25) null,
      [Chrom] [varchar](50) null,
      [MatchOrientation] [char](1) null,
      [Start] [int] null,
      [end] [int] null,
      [BaseFrom] [varchar](60) null,
      [BaseTo] [varchar](60) null,
      [Deleted] [bit] null,
      [PairCoordinates] [varchar](120) null
)

create table #TempTagsChangesInDels (
	[ClusterID] [varchar](250) not null,
	[ReadNumber] [tinyint] not null,
	[Sequence] [varchar](100) null,
	[Quality] [varchar](100) null,
	[Match] [varchar](50) null,
	[Chrom] [varchar](50) null,
	[MatchOrientation] [varchar](1) null,
	[LeftPosition] [int] null,
	[RightPosition] [int] null,
	[MinQS] [tinyint] null,
	[MaxQS] [tinyint] null,
	[AverageQS] [tinyint] null,
	[TotalMutationsInTag] [tinyint] null,
	[AlignmentScore] [int] null,
	[PairedEndReadAligmentScore] [int] null,
	[PartnerChrom] [varchar](150) null,
	[PartnerOrientation] [char](1) null,
	[Filtered] [char](1) null,
	[Exclude] [bit] null,
	[PairCoordinates] [varchar](120) null,
	[GenePair] [varchar](50) null,

	[Cycle] [tinyint] not null,
	[QualityScore] [tinyint] null,
	[MutType] [varchar](25) null,
	[Start] [int] null,
	[end] [int] null,
	[BaseFrom] [varchar](60) null,
	[BaseTo] [varchar](60) null,
	[Deleted] [bit] null,

	[HomoPolymerString] [varchar](100) null
)

create table #TempChangesWithHT (
	[ClusterID] [varchar](250) not null,
	[IndexString] [varchar](25) null,
	[ReadNumber] [tinyint] not null,
	[Cycle] [tinyint] not null,
	[Filtered] [char](1) null,
	[TotalMutationsInTag] [tinyint] null,
	[QualityScore] [tinyint] null,
	[MutType] [varchar](25) null,
	[Chrom] [varchar](50) null,
	[MatchOrientation] [char](1) null,
	[Start] [int] null,
	[end] [int] null,
	[BaseFrom] [varchar](60) null,
	[BaseTo] [varchar](60) null,
	[Deleted] [bit] null,
	[PairCoordinates] [varchar](120) null,

	[HomoPolymerString] [varchar](100) null
)
```

```
create table #TempMoleculesTable (
	[ClusterID] [varchar](250) not null,
	[ReadNumber] [tinyint] not null,
	[PairCoordinates] [varchar](120) null,
)

create table #TempMoleculesSummaryTable	(
	[PairCoordinates] [varchar](120) null,
	[ForRead1] [int] not null,
	[ForRead2] [int] not null,
	[TotalCount] [int]
)

set @SQL = 'create table
'+@dbTo+'.dbo.['+@exp+'_SupermutantMoleculesSummary'+@Version+'] '

set @SQL = @SQL+'([PairCoordinates] [varchar](120) null, ForRead1 [int] not
null, ForRead2 [int] not null, TotalCount [int])  '
exec(@SQL)

set @SQL = 'create table
'+@dbTo+'.dbo.['+@exp+'_SuperMutantsSummary'+@Version+'] '
set @SQL = @SQL+'([PairCoordinates] [varchar](120) null, [Chrom] [varchar](50)
null, [Start] [int] null, [end] [int] null, '
set @SQL = @SQL+'[BaseFrom] [varchar](60) null, [BaseTo] [varchar](60) null,
[Cycle] [tinyint] not null, [MatchOrientation] [char](1) null, [InHT]
[CHAR](1) null, '
set @SQL = @SQL+'[AverageQualityScore] [int] null, [MutRead1] [int] null,
[MutRead2] [int] null, [ForRead1s] [int] null, [ForRead2s] [int] null,
[TotalMutCount] [int] null ) '
exec(@SQL)

declare mycursor cursor forward_only for
select [ChromosomeSub] from [LudwigMasterGenes].[dbo].[Genome_hg19cForGenome]
order by [Chromosome], [CutOn]

open mycursor

fetch next from mycursor into @ChromSub

while @@fetch_status = 0
begin

set @SQL= '
insert into #TempTag
select *
from '+@serverFrom+'.['+@exp+'].[dbo].[Z'+@ChromSub+'Tags]
'
exec(@SQL)
update #TempTag set [ClusterID] = substring([ClusterID],3,len(ClusterID)-2)

set @SQL='
insert into #TempChanges
select *
from '+@serverFrom+'.['+@exp+'].dbo.[Z'+@ChromSub+'Changes]
'
```

```
exec(@SQL)
update #TempChanges set [ClusterID] = substring([ClusterID],3,len(ClusterID)-
2)

insert into #TempTagsChangesInDels (
	[ClusterID],
	[ReadNumber],
	[Sequence],
	[Quality],
	[Match],
	[Chrom],
	[MatchOrientation],
	[LeftPosition],
	[RightPosition],
	[MinQS],
	[MaxQS],
	[AverageQS],
	[TotalMutationsInTag],
	[AlignmentScore],
	[PairedEndReadAligmentScore],
	[PartnerChrom],
	[PartnerOrientation],
	[Filtered],
	[Exclude],
	[PairCoordinates],
	[GenePair],
	[Cycle],
	[QualityScore],
	[MutType],
	[Start],
	[end],
	[BaseFrom],
	[BaseTo],
	[Deleted]
)
select
	a.[ClusterID],
	a.[ReadNumber],
	a.[Sequence],
	a.[Quality],
	a.[Match],
	a.[Chrom],
	a.[MatchOrientation],
	a.[LeftPosition],
	a.[RightPosition],
	a.[MinQS],
	a.[MaxQS],
	a.[AverageQS],
	a.[TotalMutationsInTag],
	a.[AlignmentScore],
	a.[PairedEndReadAligmentScore],
	a.[PartnerChrom],
	a.[PartnerOrientation],
	a.[Filtered],
	a.[Exclude],
	a.[PairCoordinates],
	a.[GenePair],
	b.[Cycle],
	b.[QualityScore],
	b.[MutType],
	b.[Start],
	b.[end],
	b.[BaseFrom],
	b.[BaseTo],
	b.[Deleted]

from #TempTag a inner join #TempChanges b
on a.[ClusterID] = b.[ClusterID] AND a.[ReadNumber] = b.[ReadNumber]
where b.[MutType] = 'INS' OR b.[MutType] = 'DEL'

set @SQL = '
	use '+@dbTo+'
	update #TempTagsChangesInDels
	set [HomoPolymerString] = dbo.HomopolymerTractPosforSBS( [Sequence],
'+@lowlim_search+', '+@maxgap+', '+@lowlim_total+' )
	where [MutType] = ''INS'' or [MutType] = ''DEL''
	'
exec(@SQL)

insert into #TempChangesWithHT
select
	a.[ClusterID],
	a.[IndexString],
	a.[ReadNumber],
	a.[Cycle],
	a.[Filtered],
	a.[TotalMutationsInTag],
	a.[QualityScore],
	a.[MutType],
	a.[Chrom],
	a.[MatchOrientation],
	a.[Start],
	a.[end],
	a.[BaseFrom],
	a.[BaseTo],
	a.[Deleted],
	a.[PairCoordinates],
	b.[HomoPolymerString]
from #TempChanges a left outer join #TempTagsChangesInDels b
on a.[ClusterID] = b.[ClusterID] AND a.[ReadNumber] = b.[ReadNumber] AND
a.[Cycle] = b.[Cycle]

insert into #TempMoleculesTable
select
	FR.[ClusterID],
	FR.[ReadNumber],
	FR.[PairCoordinates]
from #TempTag FR
inner join #TempTag RR on FR.[ClusterID]=RR.[ClusterID] and FR.ReadNumber !=
RR.ReadNumber
left join #TempChanges C on C.ClusterID=FR.ClusterID and (C.Cycle <=@Trim or
C.Cycle >(@TagLen_i-@Trim))
where FR.MatchOrientation='F' and FR.PartnerOrientation ='R' and
FR.[PartnerChrom] is null
```

```
and FR.Exclude=0 and RR.Exclude=0 and
FR.TotalMutationsInTag<=@TagMutationsMax and
RR.TotalMutationsInTag<=@TagMutationsMax and C.ClusterID is null and
(RR.RightPosition-FR.LeftPosition)>(@TagLen_i*2-2)

insert into #TempMoleculesSummaryTable
select [PairCoordinates],
      sum(case ReadNumber when 1 then 1 else 0 end) as  ForRead1,
/*!*/ sum(case ReadNumber when 2 then 1 else 0 end) as  ForRead2,
      count(*) as TotalCount
from #TempMoleculesTable
group by [PairCoordinates]

set @SQL = 'insert into
'+@dbTo+'.dbo.['+@exp+'_SupermutantMoleculesSummary'+@Version+'] '
set @SQL = @SQL+'select * from #TempMoleculesSummaryTable '
exec(@SQL)

set @SQL = '
insert into '+@dbTo+'.dbo.['+@exp+'_SuperMutantsSummary'+@Version+']
select
      C2.PairCoordinates,
      C2.Chrom,
      C2.Start,
      C2.[end],
      C2.BaseFrom,
      C2.BaseTo,
      C2.Cycle,
      C2.MatchOrientation,
case
when ((C2.[MutType] = ''INS'' OR C2.[MutType] = ''DEL'') AND
C2.[MatchOrientation] = ''F'' AND substring(C2.[HomoPolymerString], C2.Cycle,
1) = ''X'') then ''Y''
when ((C2.[MutType] = ''INS'' OR C2.[MutType] = ''DEL'') AND
C2.[MatchOrientation] = ''R'' AND substring(C2.[HomoPolymerString],
('+@TagLen_s+'-C2.Cycle)+1, 1) = ''X'') then ''Y''
else ''N''
end as InHT,
      sum(C2.QualityScore)/count(*) as AverageQualityScore,
      sum(case C2.ReadNumber when 1 then 1 else 0 end) as  MutRead1,
/*!*/ sum(case C2.ReadNumber when 2 then 1 else 0 end) as  MutRead2,
      Min(MS.ForRead1) as ForRead1s,
      Min(MS.ForRead2) as  ForRead2s,
      count(*) as TotalMutCount
from #TempChangesWithHT C2 inner join #TempMoleculesTable M
on M.ClusterID=C2.ClusterID and C2.MatchOrientation=''F'' inner join
#TempMoleculesSummaryTable MS on MS.PairCoordinates=C2.PairCoordinates
group by
C2.PairCoordinates, C2.Chrom, C2.Start, C2.[end], C2.BaseFrom, C2.BaseTo,
C2.MutType, C2.Cycle, C2.MatchOrientation, C2.[HomoPolymerString]
order by C2.Chrom, C2.Start, C2.[end], C2.BaseFrom, C2.BaseTo, C2.Cycle,
C2.MatchOrientation, C2.PairCoordinates
'
exec(@SQL)

set @SQL = '
insert into '+@dbTo+'.dbo.['+@exp+'_SuperMutantsSummary'+@Version+']
```

```
select
C2.PairCoordinates, C2.Chrom, C2.Start, C2.[end], C2.BaseFrom, C2.BaseTo,
C2.Cycle, C2.MatchOrientation,
case
when ((C2.[MutType] = ''INS'' OR C2.[MutType] = ''DEL'') AND
C2.[MatchOrientation] = ''F'' AND substring(C2.[HomoPolymerString], C2.Cycle,
1) = ''X'') then ''Y''
when ((C2.[MutType] = ''INS'' OR C2.[MutType] = ''DEL'') AND
C2.[MatchOrientation] = ''R'' AND substring(C2.[HomoPolymerString],
('+@TagLen_s+'-C2.Cycle)+1, 1) = ''X'') then ''Y''
else ''N''
end as InHT,
      sum(C2.QualityScore)/count(*) as AverageQualityScore,
      sum(case C2.ReadNumber when 1 then 1 else 0 end) as  MutRead1,
/*!*/ sum(case C2.ReadNumber when 2 then 1 else 0 end) as  MutRead2,
      Min(MS.ForRead2) as ForRead1s,
      Min(MS.ForRead1) as  ForRead2s,
      count(*) as TotalMutCount
from #TempChangesWithHT C2 inner join #TempMoleculesTable M
on M.ClusterID=C2.ClusterID and C2.MatchOrientation=''R'' inner join
#TempMoleculesSummaryTable MS
on MS.PairCoordinates=C2.PairCoordinates
group by
C2.PairCoordinates, C2.Chrom, C2.Start, C2.[end], C2.BaseFrom, C2.BaseTo,
C2.MutType, C2.Cycle, C2.MatchOrientation, C2.[HomoPolymerString]
order by C2.Chrom, C2.Start, C2.[end], C2.BaseFrom, C2.BaseTo, C2.Cycle,
C2.MatchOrientation, C2.PairCoordinates
'
exec(@SQL)

delete from #TempTag
delete from #TempChanges
delete from #TempTagsChangesInDels
delete from #TempChangesWithHT
delete from #TempMoleculesTable
delete from #TempMoleculesSummaryTable

fetch next from mycursor into @ChromSub
end

close mycursor
deallocate mycursor

drop table #TempTag
drop table #TempChanges
drop table #TempMoleculesTable
drop table #TempMoleculesSummaryTable
drop table #TempTagsChangesInDels
drop table #TempChangesWithHT


----Remove NULLs from SuperMutantsSummary table (InDels)
set @SQL = '
update '+@dbTo+'.dbo.['+@exp+'_SuperMutantsSummary'+@Version+']
set
      BaseFrom = isnull(BaseFrom,''''),
      BaseTo = isnull(BaseTo,'''')
```

```
'
exec(@SQL)
```

QUERY 2

```
declare
@SQL varchar(8000),
@chromSub varchar(50),
@exp varchar(255),
@dbData varchar(255),
@dbFilter varchar(255),
@tableBins varchar(255),
@tableStructVars varchar(255),
@tableIntRepeats varchar(255),
@tableRM varchar(255),
@tableSimRepeats varchar(255),
@tableSuperDups varchar(255),
@version varchar(255),
@tagLen varchar(3)

set @exp = 'NgsNCS_Brain09NormalP30MM6F5Genome100D031615'
set @dbData = '[MH_NonClonal_NCS]'
set @dbFilter = '[MH_Filtering]'
set @tableBins = '[hg18_RepDNA_Bins_Master]'
set @tableStructVars = '[hg19__RepDNA_dgvMerged_042814]'
set @tableIntRepeats = '[hg19__RepDNA_nestedRepeats_042814]'
set @tableRM = '[hg19__RepDNA_rmsk_042814]'
set @tableSimRepeats = '[hg19_RepDNA_simpleRepeat_042814]'
set @tableSuperDups = '[hg19__RepDNA_genomicSuperDups_042814]'
set @version='022213v6'
set @tagLen = '100'
--MH revision 5/8/14: imported hg19 repetitive tables and replaced old hg18
files above

--Calculate Molecule Span for Filtering
set @SQL = 'alter table
'+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummary'+ @version +']
      add
            chrom varchar(50),
            MoleculeLeft int,
            MoleculeRight int
'
exec(@SQL)

set @SQL = 'update '+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummary'+
@version +']
      set
      chrom =
            substring(PairCoordinates,1,charindex( ''F'', PairCoordinates
            collate Latin1_General_CS_AS ) - 1),
      MoleculeLeft =
            substring(PairCoordinates,charindex( ''F'', PairCoordinates
            collate Latin1_General_CS_AS ) + 1, charindex( ''c'',
            PairCoordinates collate Latin1_General_CS_AS, 2) -
            charindex( ''F'', PairCoordinates collate Latin1_General_CS_AS )
            - 1),  --note: 1-based start
      MoleculeRight =
            substring(PairCoordinates,charindex( ''R'', PairCoordinates
            collate Latin1_General_CS_AS ) + 1 ,( len(PairCoordinates) -
            charindex( ''R'', PairCoordinates collate
            Latin1_General_CS_AS ) )) + '+@tagLen+' - 1 -- note: 1-based end
'
exec(@SQL)

--Assign Bins to Molecules
set @SQL = '
create table '+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummaryFinal_'+
@version +'] (
      [PairCoordinates] [varchar](120) null, [ForRead1] [int] not null,
[ForRead2] [int] not null, [TotalCount] [int], [chrom] [varchar](50) not null,
[MoleculeLeft] [int] not null, [MoleculeRight] [int] not null, [#bin] bigint
null
)
'
exec(@SQL)

set @SQL = '
insert into '+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummaryFinal_'+
@version +']
select
      a.[PairCoordinates],
      a.[ForRead1],
      a.[ForRead2],
      a.[TotalCount],
      a.[chrom],
      a.[MoleculeLeft],
      a.[MoleculeRight],
      b.[#bin]

from '+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummary'+ @version +'] a
      left outer join '+@dbFilter+'.dbo.'+@tableBins+' b
on a.chrom = b.chrom
      and(a.MoleculeLeft between b.#bin_MIN + 1 and b.#bin_MAX and
      a.MoleculeRight between b.#bin_MIN + 1 and b.#bin_MAX
)
'
exec(@SQL)

--Begin Filtering
set @SQL = ' alter table
'+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummaryFinal_'+ @version +']
add RepDNA varchar(25) ' exec(@SQL)

--Filter Molecules that DON'T have a bin
set @SQL = '
update SM
set SM.RepDNA =
case
-- Filter against Segmental Duplications (SuperDups)
when exists (
      select *
```

```
       from '+@dbFilter+'.dbo.'+@tableSuperDups+' SD
       where (
             SM.chrom = SD.chrom
             and SM.MoleculeLeft between SD.chromStart + 1 and SD.chromEnd
             ) or (
             SM.chrom = SD.otherchrom
             and SM.MoleculeLeft between SD.otherStart + 1 and SD.otherEnd
             ) or (
             SM.chrom = SD.chrom
             and SM.MoleculeRight between SD.chromStart + 1 and SD.chromEnd
             ) or (
             SM.chrom = SD.otherchrom
             and SM.MoleculeRight between SD.otherStart + 1 and SD.otherEnd
             )
             ) then ''SuperDup''

--Exclude Copy Number variants
when exists(
       select *
       from '+@dbFilter+'.dbo.'+@tableStructVars+' CN
       where (
             SM.chrom = CN.chrom
             and   (
             (SM.MoleculeLeft  between CN.chromStart + 1 and CN.chromEnd)
             or   (SM.MoleculeRight between CN.chromStart + 1 and CN.chromEnd)
             )
             and CN.varType = ''CopyNumber''
             )
             ) then ''StructVar''

--Exclude interrupted Repeats
when exists(
       select *
       from '+@dbFilter+'.dbo.'+@tableIntRepeats+' IR
       where (
             SM.chrom = IR.chrom
             and   (
             (SM.MoleculeLeft  between IR.chromStart + 1 and IR.chromEnd)
             or
             (SM.MoleculeRight between IR.chromStart + 1 and IR.chromEnd)
             )
             )
             ) then ''InterruptedRepeat''

--Exclude Simple Repeats
when exists(
       select *
       from '+@dbFilter+'.dbo.'+@tableSimRepeats+' SR
       where (
             SM.chrom = SR.chrom
             and   (
             (SM.MoleculeLeft  between SR.chromStart + 1 and SR.chromEnd)
             or
             (SM.MoleculeRight between SR.chromStart + 1 and SR.chromEnd)
             )
             )
             ) then ''SimpleRepeat''
```

```
-- Filter against Repeat Masker 3.27
when exists (
      select *
      from '+@dbFilter+'.dbo.'+@tableRM+' RM
      where
            SM.chrom = RM.genoName
            and (
            (SM.MoleculeLeft  between RM.genoStart + 1 and RM.genoEnd)
            or
            (SM.MoleculeRight between RM.genoStart + 1 and RM.genoEnd)
            )
            ) then ''RM327''

else ''Unique''
end
from '+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummaryFinal_'+ @version
+'] SM
where SM.[#bin] is null
'
exec(@SQL)

--Filter Molecules that DO have an assigned bin
set @SQL = '
update SM
set SM.RepDNA =
case
-- Filter against Segmental Duplications (SuperDups)
      when exists (
      select *
      from '+@dbFilter+'.dbo.'+@tableSuperDups+' SD
      where SM.[#bin] = SD.[#bin] and (
            SM.chrom = SD.chrom
            and SM.MoleculeLeft between SD.chromStart + 1 and SD.chromEnd
            ) or (
            SM.chrom = SD.otherchrom
            and SM.MoleculeLeft between SD.otherStart + 1 and SD.otherEnd
            ) or (
            SM.chrom = SD.chrom
            and SM.MoleculeRight between SD.chromStart + 1 and SD.chromEnd
            ) or (
            SM.chrom = SD.otherchrom
            and SM.MoleculeRight between SD.otherStart + 1 and SD.otherEnd
            )
            ) then ''SuperDup''

--Exclude Copy Number variants
      when exists(
      select *
      from '+@dbFilter+'.dbo.'+@tableStructVars+' CN
      where SM.[#bin] = CN.[#bin] and (
            SM.chrom = CN.chrom
            and (
            (SM.MoleculeLeft  between CN.chromStart + 1 and CN.chromEnd)
            or
            (SM.MoleculeRight between CN.chromStart + 1 and CN.chromEnd)
            )
            and CN.varType = ''CopyNumber''
            )
            ) then ''StructVar''

--Exclude interrupted Repeats
      when exists(
      select *
      from '+@dbFilter+'.dbo.'+@tableIntRepeats+' IR
      where SM.[#bin] = IR.[#bin] and (
            SM.chrom = IR.chrom
            and (
            (SM.MoleculeLeft  between IR.chromStart + 1 and IR.chromEnd)
            or
            (SM.MoleculeRight between IR.chromStart + 1 and IR.chromEnd)
            )
            )
            ) then ''InterruptedRepeat''

--Exclude Simple Repeats
      when exists(
      select *
      from '+@dbFilter+'.dbo.'+@tableSimRepeats+' SR
      where SM.[#bin] = SR.[#bin] and (
            SM.chrom = SR.chrom
            and   (
            (SM.MoleculeLeft  between SR.chromStart + 1 and SR.chromEnd)
            or
            (SM.MoleculeRight between SR.chromStart + 1 and SR.chromEnd)
            )
            )
            ) then ''SimpleRepeat''

-- Filter against Repeat Masker 3.27
      when exists (
      select *
      from '+@dbFilter+'.dbo.'+@tableRM+' RM
      where SM.[#bin] = RM.[#bin] and (
            SM.chrom = RM.genoName
            and (
            (SM.MoleculeLeft  between RM.genoStart + 1 and RM.genoEnd)
            or
            (SM.MoleculeRight between RM.genoStart + 1 and RM.genoEnd)
            )
            )
            ) then ''RM327''

else ''Unique''
end
from '+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummaryFinal_'+ @version
+'] SM
where SM.[#bin] is not null
'
exec(@SQL)

--add RepDNA filtering info to SuperMutantsSummary table
set @SQL = '
```

```
create table '+@dbData+'.dbo.['+@exp+'_SuperMutantsSummaryFinal_'+ @version
+'] (
      [PairCoordinates] varchar(250), [RepDNA] varchar(25), [chrom]
varchar(50), [Start] int, [end] int, [BaseFrom] varchar(60), [Baseto]
varchar(60), [Cycle] TINYint, [MatchOrientation] varchar(1), [InHT]
[CHAR](1),[AverageQualityScore] TINYint, [MutRead1] int, [MutRead2] int,
[ForRead1s] int, [ForRead2s] int, [TotalMutCount] int
)
'
exec(@SQL)

set @SQL = '
insert into '+@dbData+'.dbo.['+@exp+'_SuperMutantsSummaryFinal_'+ @version +']
      select
            a.[PairCoordinates], b.[RepDNA], a.[chrom], a.[Start], a.[end],
            a.[BaseFrom], a.[Baseto], a.[Cycle], a.[MatchOrientation],
            a.[InHT], a.[AverageQualityScore], a.[MutRead1], a.[MutRead2],
            a.[ForRead1s], a.[ForRead2s], a.[TotalMutCount]
from '+@dbData+'.dbo.['+@exp+'_SuperMutantsSummary'+ @version +'] a
left outer join (
--Subquery below prevents duplicating rows when two molecules share the same
pair coordinates
      select c.PairCoordinates, c.RepDNA
      from '+@dbData+'.dbo.['+@exp+'_SupermutantMoleculesSummaryFinal_'+
@version +'] c
      group by c.PairCoordinates, c.RepDNA
      ) b
      on a.[PairCoordinates] = b.[PairCoordinates]
'
exec(@SQL)
```

Final molecule output of QUERY 2

Column header	Description	Example value
Paircoordinates	Leftmost coordinate of each paired-end read of the template; used as Unique Identifier (UID)	chr21F28219909chr21R28220135
ForRead1	“Watson” family read counts	19
ForRead2	“Crick” family read counts	22
TotalCount	Total sum of counts (Watson + Crick)	41
Chrom	chromosome	chr21
MoleculeLeft	hg19 leftmost coordinate of DNA molecule	28219909
MoleculeRight	hg19 rightmost coordinate of DNA molecule	28220234
#bin	UCSC Human browser hg19 coordinate bin system	800

RepDNA	Unique or Repetitive DNA; values for RepDNA are ‘InterruptedRepeat’, ‘SimpleRepeat’, ‘SuperDup’, ‘StructVar’, ‘RM327’	Unique

Final changes output of QUERY 2

Column header	**Description**	**Example value**
PairCoordinates	Leftmost coordinate of each paired-end read of the template; used as Unique Identifier (UID)	chr1F20528671chr1R20529090
RepDNA	Annotated ‘Unique’ or Repetitive DNA	RM327
chrom	Chromosome	chr1
Start	hg19 Start coordinate, 1-start	20528702
end	hg19 end coordinate, 1-end	20528702
BaseFrom	Reference Base	C
Baseto	Non-reference Base	G
Cycle	Cycle number in read	32
MatchOrientation	Reference strand orientation	F
InHT	In Homopolymer with >= 8 ntd	N
AverageQualityScore	Average Quality Score of all family duplicates	41
MutRead1	“Watson” family read counts with mutation	1
MutRead2	“Crick” family read counts with mutation	0
ForRead1s	Total “Watson” family read counts	8
ForRead2s	Total “Crick” family read counts	4
TotalMutCount	Sum total of read counts with mutation (Watson + Crick mutation read counts)	1

5 Filter and select high confidence rare mutations and high confidence molecules

The goal of this section is to:

(1) Use **Final molecules output of QUERY 2** to filter and select for high confidence DNA molecules based on mappability. To do this, we used QUERY 3 for nuclear genome and QUERY 4 for mtDNA.

(2) Use **Final changes output of QUERY 2** to filter and select for high confidence rare somatic mutations based on quality, clonality, and mappability. To do this, we used QUERY 5 for nuclear genome and QUERY 6 for mtDNA.

QUERY 3 (for nuclear molecules)

```
create table #sm_select (
      pairCoordinates varchar(250), repDNA varchar(25), chrom varchar(50),
start bigint, [end] bigint, baseFrom varchar(60), baseTo varchar(60), cycle
tinyint, matchOrientation varchar(1), inHT char(1), averageQualityScore
tinyint, mutRead1 int, mutRead2 int, forRead1s int, forRead2s int,
totalMutCount int
)

insert #sm_select
select
      sm.pairCoordinates, sm.repDNA, sm.chrom, sm.start, sm.[end],
sm.baseFrom, sm.baseTo, sm.cycle, sm.matchOrientation, sm.inHT,
sm.averageQualityScore,sm.mutRead1, sm.mutRead2, sm.forRead1s, sm.forRead2s,
sm.totalMutCount

from[MH_Nonclonal_NCS].[dbo].[NgsNCS_SB118NN2NormalP30MM6F5Genome100D063014_1
20714_SuperMutantsSummaryFinal_022213v6] sm
where sm.averageQualityScore >= 20
      and sm.averageQualityScore != 100
      and sm.forRead1s >= 1
      and sm.forRead2s >= 1
      and sm.inHT = 'N'
      and sm.chrom != 'chrM'
      and cast(sm.mutRead1 as float)/cast(sm.forRead1s as float) > 0.5
      and cast(sm.mutRead2 as float)/cast(sm.forRead2s as float) > 0.5

--WGS snp option 1:
      and not exists (
            select *
            from [MH_Nonclonal_WGS].[dbo].[LP6005897-DNA_F02.SNPs] wgs1_snp
            where sm.chrom = wgs1_snp.#chrom
                  and sm.start = wgs1_snp.pos
      )
--WGS snp option 2:
      --and not exists (
      --select *
      --from [MH_Nonclonal_WGS].[dbo].[Brain06_variants] wgs2_snp
      --where len(wgs2_snp.ref) = 1
      --    and   (
      --    len(wgs2_snp.alt) = 1
      --    or (wgs2_snp.alt like '%,%' and len(wgs2_snp.alt) = 3)
      --    )
      --    and sm.chrom = wgs2_snp.#chrom
      --    and (sm.start = wgs2_snp.pos or sm.[end] = wgs2_snp.pos)
      --)

      and not exists (
            select *
            from [LudwigMasterGenes].[dbo].[SNP_SNP130_hg19] snp130
            where sm.[chrom] = snp130.chromosome
                  and   sm.[start] = snp130.position
                  and snp130.posReferenceAllele is not null
                  and snp130.posOtherAllele is not null
      )

create table #mutCountPairCoo (
      pairCoordinates varchar(250), mutPerPairCoo int
)
insert #mutCountPairCoo
select paircoordinates, count(*) as mutPerPairCoo
from #sm_select
group by pairCoordinates

create table #uid_select (
pairCoordinates varchar(120), forRead1 int, forRead2 int, totalCount int,
chrom varchar(50), moleculeLeft int, moleculeRight int, #bin bigint, repDNA
varchar(25)
)
insert #uid_select
select pairCoordinates, forRead1, forRead2, totalCount, chrom, moleculeLeft,
moleculeRight,#bin, repDNA
from
[MH_Nonclonal_NCS].[dbo].[NgsNCS_SB118NN2NormalP30MM6F5Genome100D063014_12071
4_SupermutantMoleculesSummaryFinal_022213v6] uids
where uids.chrom != 'chrM'
      and uids.forRead1 >= 2
      and uids.forRead2 >= 2
      and not exists (
            select *
            from
[MH_Nonclonal_NCS].[dbo].[NgsNCS_SB118NN2NormalP30MM6F5Genome100D063014_12071
4_SuperMutantsSummaryFinal_022213v6] hp
            where hp.inHT = 'Y' and
                  hp.pairCoordinates = uids.pairCoordinates
            )
      and not exists (
            select *
            from #mutCountPairCoo mc
            where mutPerPairCoo > 1
                  and mc.pairCoordinates = uids.PairCoordinates
      )

create table #uid_oldrepFilter (
      pairCoordinates varchar(120), forRead1 int, forRead2 int, totalCount
int, chrom varchar(50), moleculeLeft int, moleculeRight int, #bin bigint,
repDNA varchar(25)
)
insert #uid_oldrepFilter
select pairCoordinates, forRead1, forRead2, totalCount, chrom, moleculeLeft,
moleculeRight, #bin, repDNA
from #uid_select uids

where not exists (
      select *
```

```
from [MH_Filtering].[dbo].[hg19__RepDNA_dgvMerged_042814] dgv
where uids.chrom = dgv.chrom
and (
uids.moleculeLeft between dgv.chromStart + 1 and dgv.chromEnd
or
uids.moleculeRight between dgv.chromStart + 1 and dgv.chromEnd
)
)
and not exists (
select *
from [MH_Filtering].[dbo].[hg19__RepDNA_genomicSuperDups_042814] sd
where (
uids.chrom = sd.chrom
and   (
uids.moleculeLeft between sd.chromStart + 1 and sd.chromEnd
or
uids.moleculeRight between sd.chromStart + 1 and sd.chromEnd
)
) or (
uids.chrom = sd.otherchrom
and (
uids.moleculeLeft between sd.otherStart + 1 and sd.otherEnd
or
uids.moleculeRight between sd.otherStart + 1 and sd.otherEnd
)
)
)
and not exists (
select *
from [MH_Filtering].[dbo].[hg19__RepDNA_nestedRepeats_042814] nr
where uids.chrom = nr.chrom
and   (
      uids.moleculeLeft  between nr.chromStart + 1 and nr.chromEnd
      or
      uids.moleculeRight  between nr.chromStart + 1 and nr.chromEnd
      )
)
and not exists (
select *
from [MH_Filtering].[dbo].[hg19__RepDNA_rmsk_042814] rm
where uids.chrom = rm.genoName
and (
uids.moleculeLeft between rm.genoStart + 1 and rm.genoEnd
or
uids.moleculeRight between rm.genoStart + 1 and rm.genoEnd
)
)
and not exists (
select *
from [MH_Filtering].[dbo].[hg19_RepDNA_simpleRepeat_042814] sr
where uids.chrom = sr.chrom
and (
uids.moleculeLeft between sr.chromStart + 1 and sr.chromEnd
or
uids.moleculeRight between sr.chromStart + 1 and sr.chromEnd
)
)
```

```
create table #uid_newrepFilter (
pairCoordinates varchar(120), forRead1 int, forRead2 int, totalCount int,
chrom varchar(50),moleculeLeft int, moleculeRight int, #bin bigint, repDNA
varchar(25)
)
insert #uid_newrepFilter
select pairCoordinates, forRead1, forRead2, totalCount, chrom, moleculeLeft,
moleculeRight,#bin, repDNA
from #uid_oldRepFilter uids
where
      not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvMerged_c1toc14]
dgvM
      where uids.chrom = dgvM.chrom
      and (
      uids.moleculeLeft between dgvM.chromStart + 1 and dgvM.chromEnd
      or
      uids.moleculeRight between dgvM.chromStart + 1 and dgvM.chromEnd
      )
      )
      and not exists (
      select *
     from  [MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvSupporting_c1toc14]
dgvS
      where uids.chrom = dgvS.chrom
      and (
      uids.moleculeLeft between dgvS.chromStart + 1 and dgvS.chromEnd
      or
      uids.moleculeRight between dgvS.chromStart + 1 and dgvS.chromEnd
      )
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_genomicSuperDups] sd
      where (     uids.chrom = sd.chrom
      and   uids.moleculeLeft between sd.chromStart + 1 and sd.chromEnd
      ) or (
      uids.chrom = sd.otherChrom
      and uids.moleculeLeft between sd.otherStart + 1 and sd.otherEnd
      ) or (
      uids.chrom = sd.chrom
      and uids.moleculeRight between sd.chromStart + 1 and sd.chromEnd
      ) or (
      uids.chrom = sd.otherChrom
      and uids.moleculeRight between sd.otherStart + 1 and sd.otherEnd
      )
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_nestedRepeats] nr
      where uids.chrom = nr.chrom
      and (
      uids.moleculeLeft between nr.chromStart + 1 and nr.chromEnd
      or
      uids.moleculeRight between nr.chromStart + 1 and nr.chromEnd
      )
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_simpleRepeat] sr
      where uids.chrom = sr.chrom
      and   (
      uids.moleculeLeft between sr.chromStart - 99  and sr.chromEnd + 100 --
MH UPDATED 5/14/15
      or
      uids.moleculeRight between sr.chromStart - 99  and sr.chromEnd + 100 --
MH UPDATED 5/14/15
      )
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm
      where uids.chrom = rm.genoName
      and (
      uids.moleculeLeft between rm.genoStart + 1 and rm.genoEnd
      or
      uids.moleculeRight between rm.genoStart + 1 and rm.genoEnd
      )
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sine
      where rm_sine.repClass = 'SINE'
      and uids.chrom = rm_sine.genoName
      and (
      uids.moleculeLeft between rm_sine.genoStart - 99 and rm_sine.genoEnd +
100
      or
      uids.moleculeRight between rm_sine.genoStart - 99 and rm_sine.genoEnd +
100
      )
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_lc
      where rm_lc.repClass = 'low_complexity'
      and   uids.chrom = rm_lc.genoName
      and (
      uids.moleculeLeft between rm_lc.genoStart - 99 and rm_lc.genoEnd + 100
      or
      uids.moleculeRight between rm_lc.genoStart - 99 and rm_lc.genoEnd + 100
      )
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sr
      where rm_sr.repClass = 'simple_repeat'
      and   uids.chrom = rm_sr.genoName
      and (
      uids.moleculeLeft between rm_sr.genoStart - 99 and rm_sr.genoEnd + 100
      or
      uids.moleculeRight between rm_sr.genoStart - 99 and rm_sr.genoEnd + 100
      )
      )

select COUNT(*)
from #sm_select
select COUNT(*)
from #mutCountPairCoo
select COUNT(*)
from #uid_select
select COUNT(*)
from #uid_oldRepFilter
select COUNT(*)
from #uid_newRepFilter

drop table #sm_select
drop table #mutCountPairCoo
drop table #uid_select
drop table #uid_oldRepFilter
drop table #uid_newRepFilter
```

QUERY 4 (for mtDNA molecules)

```
create table #sm_select (
pairCoordinates varchar(250), repDNA varchar(25), chrom varchar(50), start
bigint, [end] bigint, baseFrom varchar(60), baseTo varchar(60), cycle tinyint,
matchOrientation varchar(1), inHT char(1), averageQualityScore tinyint,
mutRead1 int, mutRead2 int, forRead1s int, forRead2s int, totalMutCount int
)
insert #sm_select
select sm.pairCoordinates, sm.repDNA, sm.chrom, sm.start, sm.[end],
sm.baseFrom, sm.baseTo, sm.cycle, sm.matchOrientation, sm.inHT,
sm.averageQualityScore,sm.mutRead1, sm.mutRead2, sm.forRead1s, sm.forRead2s,
sm.totalMutCount
from
[MH_Nonclonal_NCS].[dbo].[NgsNCS_CRC238N1NormalP30MM6F5Genome100D042814_Super
MutantsSummaryFinal_022213v6] sm
where sm.averageQualityScore >= 20
      and sm.averageQualityScore != 100
      and   sm.forRead1s >= 1
      and sm.forRead2s >= 1
      and sm.inHT = 'N'
      and sm.chrom = 'chrM' --for mtDNA
      and cast(sm.mutRead1 as float)/cast(sm.forRead1s as float) > 0.5
      and cast(sm.mutRead2 as float)/cast(sm.forRead2s as float) > 0.5

--WGS snp option 1:
      --and not exists (
      --    select *
      --    from [MH_Nonclonal_WGS].[dbo].[LP6005897-DNA_H01.SNPs] wgs1_snp
      --    where wgs1_snp.#chrom = 'chrM' --for mtDNA
      --          and sm.start = wgs1_snp.pos
      --)

--WGS snp option 2:
      and not exists (
	select *
	from [MH_Nonclonal_WGS].[dbo].[CRC238N_variants] wgs2_snp
	where wgs2_snp.#chrom = 'chrM'  --for mtDNA
		and   len(wgs2_snp.ref) = 1
		and   (
		len(wgs2_snp.alt) = 1
		or (wgs2_snp.alt like '%,%' and len(wgs2_snp.alt) = 3)
		)
		and (sm.start = wgs2_snp.pos or sm.[end] = wgs2_snp.pos)
	)

	--and not exists (
	--	select *
	--	from [LudwigMasterGenes].[dbo].[SNP_SNP130_hg19] snp130
	--	where sm.[chrom] = snp130.chromosome
	--		and   sm.[start] = snp130.position
	--		and snp130.posReferenceAllele is not null
	--		and snp130.posOtherAllele is not null
	--)  --dbSNP does not have mitoSNP


create table #mutCountPairCoo (
	pairCoordinates varchar(250), mutPerPairCoo int
)
insert #mutCountPairCoo
select paircoordinates, count(*) as mutPerPairCoo
from #sm_select
group by pairCoordinates


create table #uid_select (
	pairCoordinates varchar(120), forRead1 int, forRead2 int, totalCount
int, chrom varchar(50), moleculeLeft int, moleculeRight int, #bin bigint,
repDNA varchar(25)
	)
insert #uid_select
select pairCoordinates, forRead1, forRead2, totalCount, chrom, moleculeLeft,
moleculeRight, #bin, repDNA
from
[MH_Nonclonal_NCS].[dbo].[NgsNCS_CRC238N1NormalP30MM6F5Genome100D042814_Super
mutantMoleculesSummaryFinal_022213v6] uids
where uids.chrom = 'chrM' --for mtDNA
	and uids.forRead1 >= 2
	and uids.forRead2 >= 2
	and not exists (
	select *
	from
[MH_Nonclonal_NCS].[dbo].[NgsNCS_CRC238N1NormalP30MM6F5Genome100D042814_Super
MutantsSummaryFinal_022213v6] hp
	where hp.inHT = 'Y' and
		hp.pairCoordinates = uids.pairCoordinates
		)
	and not exists (
		select *
		from #mutCountPairCoo mc
		where mutPerPairCoo > 1
	and mc.pairCoordinates = uids.PairCoordinates
```

```
      )

--ALL oldRepDNA tables do not include chrM
--create table #uid_oldrepFilter (
--    pairCoordinates varchar(120), forRead1 int, forRead2 int,
--    totalCount int, chrom varchar(50),
--    moleculeLeft int, moleculeRight int, #bin bigint, repDNA varchar(25)
--)
--insert #uid_oldrepFilter
--select
--    pairCoordinates, forRead1, forRead2, totalCount, chrom,
--    moleculeLeft, moleculeRight,
--    #bin, repDNA
--from #uid_select uids
--where not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19__RepDNA_dgvMerged_042814] dgv
--    where uids.chrom = dgv.chrom
--    and (
--    uids.moleculeLeft between dgv.chromStart + 1 and dgv.chromEnd
--    or
--    uids.moleculeRight between dgv.chromStart + 1 and dgv.chromEnd
--                      )
--    )
--    and not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19__RepDNA_genomicSuperDups_042814] sd
--          where (
--    uids.chrom = sd.chrom
--    and   (
--    uids.moleculeLeft between sd.chromStart + 1 and sd.chromEnd
--    or
--    uids.moleculeRight between sd.chromStart + 1 and sd.chromEnd
--    )
--    ) or (
--    uids.chrom = sd.otherchrom
--    and (
--    uids.moleculeLeft between sd.otherStart + 1 and sd.otherEnd
--    or
--    uids.moleculeRight between sd.otherStart + 1 and sd.otherEnd
--    )
--    )
--    )
--    and not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19__RepDNA_nestedRepeats_042814] nr
--    where uids.chrom = nr.chrom
--    and   (
--    uids.moleculeLeft  between nr.chromStart + 1 and nr.chromEnd
--    or
--    uids.moleculeRight  between nr.chromStart + 1 and nr.chromEnd
--    )
--    )
--    and not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19__RepDNA_rmsk_042814] rm
--    where uids.chrom = rm.genoName
--    and (
--    uids.moleculeLeft between rm.genoStart + 1 and rm.genoEnd
--    or
--    uids.moleculeRight between rm.genoStart + 1 and rm.genoEnd
--    )
--    )
--    and not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19_RepDNA_simpleRepeat_042814] sr
--    where uids.chrom = sr.chrom
--    and (
--    uids.moleculeLeft between sr.chromStart + 1 and sr.chromEnd
--    or
--    uids.moleculeRight between sr.chromStart + 1 and sr.chromEnd
--    )
--    )

--FOUR tRNA sites in RepeatMasker; all others do not have chrM
create table #uid_newrepFilter (
      pairCoordinates varchar(120), forRead1 int, forRead2 int, totalCount
int, chrom varchar(50), moleculeLeft int, moleculeRight int, #bin bigint,
repDNA varchar(25)
)
insert #uid_newrepFilter
select pairCoordinates, forRead1, forRead2, totalCount, chrom, moleculeLeft,
moleculeRight,#bin, repDNA
from #uid_select uids
where
--not exists (
--    select *
--    from
--    [MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvMerged_c1toc14] dgvM
--    where uids.chrom = dgvM.chrom
--    and (
--    uids.moleculeLeft between dgvM.chromStart + 1 and dgvM.chromEnd
--    or
--    uids.moleculeRight between dgvM.chromStart + 1 and dgvM.chromEnd
--                )
--)
--and not exists (
--    select *
--    from
--[MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvSupporting_c1toc14] dgvS
--    where uids.chrom = dgvS.chrom
--    and (
--    uids.moleculeLeft between dgvS.chromStart + 1 and dgvS.chromEnd
--    or
--    uids.moleculeRight between dgvS.chromStart + 1 and dgvS.chromEnd
--                )
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[031015_goldenPath_hg19_genomicSuperDups] sd
--    where (     uids.chrom = sd.chrom
--    and   uids.moleculeLeft between sd.chromStart + 1 and sd.chromEnd
--    ) or (
--    uids.chrom = sd.otherChrom
--    and uids.moleculeLeft between sd.otherStart + 1 and sd.otherEnd
--    ) or (
--    uids.chrom = sd.chrom
--    and uids.moleculeRight between sd.chromStart + 1 and sd.chromEnd
--    ) or (
--    uids.chrom = sd.otherChrom
--    and uids.moleculeRight between sd.otherStart + 1 and sd.otherEnd
--                )
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[031015_goldenPath_hg19_nestedRepeats] nr
--    where uids.chrom = nr.chrom
--    and (
--    uids.moleculeLeft between nr.chromStart + 1 and nr.chromEnd
--    or
--    uids.moleculeRight between nr.chromStart + 1 and nr.chromEnd
--          )
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[031015_goldenPath_hg19_simpleRepeat] sr
--    where uids.chrom = sr.chrom
--    and   (
--    uids.moleculeLeft between sr.chromStart + 1 and sr.chromEnd
--    or
--    uids.moleculeRight between sr.chromStart + 1 and sr.chromEnd
--          )
--)
not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm
      where rm.genoName = 'chrM'
      and (
      uids.moleculeLeft between rm.genoStart + 1 and rm.genoEnd
      or
      uids.moleculeRight between rm.genoStart + 1 and rm.genoEnd
      )
      )
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sine
--    where rm_sine.repClass = 'SINE'
--    and uids.chrom = rm_sine.genoName
--    and (
--    uids.moleculeLeft between rm_sine.genoStart - 99 and
--    rm_sine.genoEnd + 100
--    or
--    uids.moleculeRight between rm_sine.genoStart - 99
--    and rm_sine.genoEnd + 100
--          )
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_lc
--    where rm_lc.repClass = 'low_complexity'
--    and   uids.chrom = rm_lc.genoName
--      and (
--      uids.moleculeLeft between rm_lc.genoStart - 99 and rm_lc.genoEnd + 100
--      or
--      uids.moleculeRight between rm_lc.genoStart - 99 and rm_lc.genoEnd + 100
--            )
--)
--and not exists (
--      select *
--      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sr
--      where rm_sr.repClass = 'simple_repeat'
--      and   uids.chrom = rm_sr.genoName
--      and (
--      uids.moleculeLeft between rm_sr.genoStart - 99 and rm_sr.genoEnd + 100
--      or
--      uids.moleculeRight between rm_sr.genoStart - 99 and rm_sr.genoEnd + 100
--)
--)

select COUNT(*)
from #sm_select
select COUNT(*)
from #mutCountPairCoo
select COUNT(*)
from #uid_select
--select COUNT(*)
--from #uid_oldRepFilter
select COUNT(*)
from #uid_newRepFilter

drop table #sm_select
drop table #mutCountPairCoo
drop table #uid_select
--drop table #uid_oldRepFilter
drop table #uid_newRepFilter
```

QUERY 5 (nuclear mutations)

```
create table #sm_select (pairCoordinates varchar(250), repDNA varchar(25),
chrom varchar(50), start bigint, [end] bigint, baseFrom varchar(60), baseTo
varchar(60), cycle tinyint, matchOrientation varchar(1), inHT char(1),
averageQualityScore tinyint, mutRead1 int, mutRead2 int, forRead1s int,
forRead2s int, totalMutCount int
)
insert #sm_select
select sm.pairCoordinates, sm.repDNA, sm.chrom, sm.start, sm.[end],
sm.baseFrom, sm.baseTo, sm.cycle, sm.matchOrientation, sm.inHT,
sm.averageQualityScore,sm.mutRead1, sm.mutRead2, sm.forRead1s, sm.forRead2s,
sm.totalMutCount
from
[MH_Nonclonal_NCS].[dbo].[NgsNCS_Barin07NormalP30MM6F5Genome100D031615_SuperM
utantsSummaryFinal_022213v6] sm
where sm.averageQualityScore >= 20
      and sm.averageQualityScore != 100
      and   sm.forRead1s >= 1
      and sm.forRead2s >= 1
      and sm.inHT = 'N'
      and sm.chrom != 'chrM'
      and cast(sm.mutRead1 as float)/cast(sm.forRead1s as float) > 0.5
      and cast(sm.mutRead2 as float)/cast(sm.forRead2s as float) > 0.5

--WGS snp option 1:
      --and not exists (
      --    select *
      --    from [MH_Nonclonal_WGS].[dbo].[LP6005897-DNA_H01.SNPs] wgs1_snp
      --    where sm.chrom = wgs1_snp.#chrom
      --          and sm.start = wgs1_snp.pos
      --)
--WGS snp option 2:
      and not exists (
      select *
      from [MH_Nonclonal_WGS].[dbo].[Brain07_variants] wgs2_snp
      where len(wgs2_snp.ref) = 1
      and   (
      len(wgs2_snp.alt) = 1
      or (wgs2_snp.alt like '%,%' and len(wgs2_snp.alt) = 3)
      )
      and sm.chrom = wgs2_snp.#chrom
      and (sm.start = wgs2_snp.pos or sm.[end] = wgs2_snp.pos)
      )

      and not exists (
      select *
      from [LudwigMasterGenes].[dbo].[SNP_SNP130_hg19] snp130
      where sm.[chrom] = snp130.chromosome
      and   sm.[start] = snp130.position
      and snp130.posReferenceAllele is not null
      and snp130.posOtherAllele is not null
      )


create table #mutCountPairCoo (
      pairCoordinates varchar(250), mutPerPairCoo int
)
insert #mutCountPairCoo
select paircoordinates, count(*) as mutPerPairCoo
from #sm_select
group by pairCoordinates


create table #sm_select_filtered (
      pairCoordinates varchar(250), repDNA varchar(25), chrom varchar(50),
start bigint, [end] bigint, baseFrom varchar(60), baseTo varchar(60), cycle
tinyint, matchOrientation varchar(1), inHT char(1), averageQualityScore
tinyint, mutRead1 int, mutRead2 int, forRead1s int, forRead2s int,
totalMutCount int, mutPerPairCoo int
)
insert #sm_select_filtered
select
sm.pairCoordinates, sm.repDNA, sm.chrom, sm.start, sm.[end], sm.baseFrom,
sm.baseTo, sm.cycle, sm.matchOrientation, sm.inHT,
sm.averageQualityScore,sm.mutRead1, sm.mutRead2, sm.forRead1s, sm.forRead2s,
sm.totalMutCount, mc.MutPerPairCoo
```

```
from #sm_select sm left outer join #mutCountPairCoo mc
on sm.pairCoordinates = mc.pairCoordinates
where sm.averageQualityScore >= 30
      and cast(sm.mutRead1 as float)/cast(sm.forRead1s as float) >= 0.9
      and cast(sm.mutRead2 as float)/cast(sm.forRead2s as float) >= 0.9
      and   sm.cycle != 6
      and sm.cycle != 7
      and mc.mutPerPairCoo = 1
      and not exists (
            select *
            from [MH_Filtering].[dbo].[hg19__RepDNA_dgvMerged_042814] dgv
            where sm.chrom = dgv.chrom
      and sm.start between dgv.chromStart + 1 and dgv.chromEnd
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[hg19__RepDNA_genomicSuperDups_042814] sd
      where (
      sm.chrom = sd.chrom
      and   sm.start between sd.chromStart + 1 and sd.chromEnd
      ) or (
      sm.chrom = sd.otherchrom
      and sm.start between sd.otherStart + 1 and sd.otherEnd
      )
      )
      and not exists (
            select *
            from [MH_Filtering].[dbo].[hg19__RepDNA_nestedRepeats_042814] nr
            where sm.chrom = nr.chrom
                  and   sm.start  between nr.chromStart + 1 and nr.chromEnd
      )
      and not exists (
            select *
            from [MH_Filtering].[dbo].[hg19__RepDNA_rmsk_042814] rm
            where sm.chrom = rm.genoName
                  and sm.start between rm.genoStart + 1 and rm.genoEnd
      )
      and not exists (
            select *
            from [MH_Filtering].[dbo].[hg19_RepDNA_simpleRepeat_042814] sr
            where sm.chrom = sr.chrom
                  and sm.start between sr.chromStart + 1 and sr.chromEnd
      )


select count(*)
from #sm_select
select count(*)
from #mutCountPairCoo
select count(*)
from #sm_select_filtered


select *
from #sm_select_filtered sm
where forRead1s >= 2 and forRead2s >= 2
```

```
--WGS indel option 1:
--and not exists (
--    select *
--    from [MH_Nonclonal_WGS].[dbo].[LP6005897-DNA_H01.Indels] wgs1_indel
--    where sm.chrom = wgs1_indel.#chrom
--    and ( sm.start between wgs1_indel.pos + 1 and wgs1_indel.pos + len(ref)
--    or
--    sm.[end] between wgs1_indel.pos + 1 and wgs1_indel.pos + len(ref)
--          )
--)
--WGS indel option 2:
      and not exists (
      select *
      from [MH_Nonclonal_WGS].[dbo].[Brain07_variants] wgs2_indel
      where (     len(wgs2_indel.ref) > 1
      or
      (           len(wgs2_indel.ref) = 1
      and len(wgs2_indel.ref) != len(wgs2_indel.alt)
      and wgs2_indel.alt not like 'A,%'
      and wgs2_indel.alt not like 'T,%'
      and wgs2_indel.alt not like 'C,%'
      and wgs2_indel.alt not like 'G,%'
      )
      )
      and sm.chrom = wgs2_indel.#chrom
      and (
      sm.start between wgs2_indel.pos + 1 and wgs2_indel.pos + len(ref)
      or    sm.[end] between wgs2_indel.pos + 1 and wgs2_indel.pos + len(ref)
      )
      )

      and not exists (
      select *
      from [MH_filtering].[dbo].[031015_goldenPath_hg19_snp142_snps] snp142
      where sm.chrom = snp142.chrom
      and sm.start = snp142.posEnd
      )
      and not exists (
      select *
      from [MH_filtering].[dbo].[031015_goldenPath_hg19_snp142_other]
      other142
      where sm.chrom = other142.chrom
      and sm.Start between other142.posStart and other142.posEnd
      )
      and not exists (
      select *
      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvMerged_c1toc14]
      dgvM
      where sm.chrom = dgvM.chrom
      and sm.start between dgvM.chromStart + 1 and dgvM.chromEnd
      )
      and not exists (
      select *
     from  [MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvSupporting_c1toc14]
dgvS
      where sm.chrom = dgvS.chrom
      and sm.start between dgvS.chromStart + 1 and dgvS.chromEnd
```

```
        )
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_genomicSuperDups] sd
        where (
        sm.chrom = sd.chrom
        and sm.start between sd.chromStart + 1 and sd.chromEnd
        ) or (
        sm.chrom = sd.otherchrom
        and sm.start between sd.otherStart + 1 and sd.otherEnd
        )
        )
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_nestedRepeats] nr
        where sm.chrom = nr.chrom
        and   sm.start between nr.chromStart + 1 and nr.chromEnd
        )
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_simpleRepeat] sr
        where sm.chrom = sr.chrom
        and   sm.start between sr.chromStart - 99 and sr.chromEnd + 100
        )
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm
        where       sm.chrom = rm.genoName
        and sm.start between rm.genoStart + 1 and rm.genoEnd
        )
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sine
        where rm_sine.repClass = 'SINE'
        and sm.chrom = rm_sine.genoName
        and sm.start between rm_sine.genoStart - 99 and rm_sine.genoEnd + 100
        )
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_lc
        where rm_lc.repClass = 'low_complexity'
        and   sm.chrom = rm_lc.genoName
        and sm.start between rm_lc.genoStart - 99 and rm_lc.genoEnd + 100
        )
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sr
        where rm_sr.repClass = 'simple_repeat'
        and   sm.chrom = rm_sr.genoName
        and sm.start between rm_sr.genoStart - 99 and rm_sr.genoEnd + 100
        )
order by chrom, start


drop table #sm_select
drop table #mutCountPairCoo
drop table #sm_select_filtered
```

QUERY 6 (mtDNA mutations)

```
create table #sm_select (
pairCoordinates varchar(250), repDNA varchar(25), chrom varchar(50), start
bigint, [end] bigint, baseFrom varchar(60), baseTo varchar(60), cycle tinyint,
matchOrientation varchar(1), inHT char(1), averageQualityScore tinyint,
mutRead1 int, mutRead2 int, forRead1s int, forRead2s int, totalMutCount int
)
insert #sm_select
select
sm.pairCoordinates, sm.repDNA, sm.chrom, sm.start, sm.[end], sm.baseFrom,
sm.baseTo, sm.cycle, sm.matchOrientation, sm.inHT,
sm.averageQualityScore,sm.mutRead1, sm.mutRead2, sm.forRead1s, sm.forRead2s,
sm.totalMutCount
from
[MH_Nonclonal_NCS].[dbo].NgsNCS_CRC238N1NormalP30MM6F5Genome100D042814_SuperM
utantsSummaryFinal_022213v6 sm
where sm.averageQualityScore >= 20
      and sm.averageQualityScore != 100
      and   sm.forRead1s >= 1
      and sm.forRead2s >= 1
      and sm.inHT = 'N'
      and sm.chrom = 'chrM' --for mtDNA
      and cast(sm.mutRead1 as float)/cast(sm.forRead1s as float) > 0.5
      and cast(sm.mutRead2 as float)/cast(sm.forRead2s as float) > 0.5

--WGS snp option 1:
      --and not exists (
      --    select *
      --    from [MH_Nonclonal_WGS].[dbo].[LP6005897-DNA_B03.SNPs] wgs1_snp
      --    where wgs1_snp.#chrom = 'chrM'
      --          and sm.start = wgs1_snp.pos
      --)

--WGS snp option 2:
      and not exists (
      select *
      from [MH_Nonclonal_WGS].[dbo].[CRC238N_variants] wgs2_snp
      where wgs2_snp.#chrom = 'chrM' --for chrM
            and len(wgs2_snp.ref) = 1
            and   (
            len(wgs2_snp.alt) = 1
            or (wgs2_snp.alt like '%,%' and len(wgs2_snp.alt) = 3)
            )
            and (sm.start = wgs2_snp.pos or sm.[end] = wgs2_snp.pos)
      )
--and not exists (
--    select *
--    from [LudwigMasterGenes].[dbo].[SNP_SNP130_hg19] snp130
--    where sm.[chrom] = snp130.chromosome
--          and   sm.[start] = snp130.position
--          and snp130.posReferenceAllele is not null
--    and snp130.posOtherAllele is not null
--)
```

```
create table #mutCountPairCoo (
      pairCoordinates varchar(250), mutPerPairCoo int
)
insert #mutCountPairCoo
select paircoordinates, count(*) as mutPerPairCoo
from #sm_select
group by pairCoordinates


create table #sm_select_filtered (
pairCoordinates varchar(250), repDNA varchar(25), chrom varchar(50), start
bigint, [end] bigint, baseFrom varchar(60), baseTo varchar(60), cycle tinyint,
matchOrientation varchar(1), inHT char(1), averageQualityScore tinyint,
mutRead1 int, mutRead2 int, forRead1s int, forRead2s int, totalMutCount int,
mutPerPairCoo int
)
insert #sm_select_filtered
select
sm.pairCoordinates, sm.repDNA, sm.chrom, sm.start, sm.[end], sm.baseFrom,
sm.baseTo, sm.cycle, sm.matchOrientation, sm.inHT,
sm.averageQualityScore,sm.mutRead1, sm.mutRead2, sm.forRead1s, sm.forRead2s,
sm.totalMutCount, mc.MutPerPairCoo
from #sm_select sm left outer join #mutCountPairCoo mc
on sm.pairCoordinates = mc.pairCoordinates
where sm.averageQualityScore >= 30
      and cast(sm.mutRead1 as float)/cast(sm.forRead1s as float) >= 0.9
      and cast(sm.mutRead2 as float)/cast(sm.forRead2s as float) >= 0.9
      and   sm.cycle != 6
      and sm.cycle != 7
      and mc.mutPerPairCoo = 1
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19__RepDNA_dgvMerged_042814] dgv
--    where sm.chrom = dgv.chrom
--          and sm.start between dgv.chromStart + 1 and dgv.chromEnd
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19__RepDNA_genomicSuperDups_042814] sd
--    where (
--          sm.chrom = sd.chrom
--          and   sm.start between sd.chromStart + 1 and sd.chromEnd
--    ) or (
--          sm.chrom = sd.otherchrom
--          and sm.start between sd.otherStart + 1 and sd.otherEnd
--    )
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[hg19__RepDNA_nestedRepeats_042814] nr
--    where sm.chrom = nr.chrom
--          and   sm.start  between nr.chromStart + 1 and nr.chromEnd
--)
--    and not exists (
--    select *
--      from [MH_Filtering].[dbo].[hg19__RepDNA_rmsk_042814] rm
--      where sm.chrom = rm.genoName
--            and sm.start between rm.genoStart + 1 and rm.genoEnd
--)
--      and not exists (
--      select *
--      from [MH_Filtering].[dbo].[hg19_RepDNA_simpleRepeat_042814] sr
--      where sm.chrom = sr.chrom
--            and sm.start between sr.chromStart + 1 and sr.chromEnd
--)


select count(*)
from #sm_select
select count(*)
from #mutCountPairCoo
select count(*)
from #sm_select_filtered


select *
from #sm_select_filtered sm
where forRead1s >= 2 and forRead2s >= 2

--WGS indel option 1:
--and not exists (
--      select *
--      from [MH_Nonclonal_WGS].[dbo].[LP6005897-DNA_B03.Indels] wgs1_indel
--      where wgs1_indel.#chrom = 'chrM' --for mtDNA
--      and ( sm.start between wgs1_indel.pos + 1 and wgs1_indel.pos + len(ref)
--      or    sm.[end] between wgs1_indel.pos + 1 and wgs1_indel.pos + len(ref)
--            )
--)

--WGS indel option 2:
      and not exists (
      select *
      from [MH_Nonclonal_WGS].[dbo].[CRC238N_variants] wgs2_indel
      where wgs2_indel.#chrom = 'chrM' --for mtDNA
      and ( len(wgs2_indel.ref) > 1
      or
      (     len(wgs2_indel.ref) = 1
            and len(wgs2_indel.ref) != len(wgs2_indel.alt)
            and wgs2_indel.alt not like 'A,%'
            and wgs2_indel.alt not like 'T,%'
            and wgs2_indel.alt not like 'C,%'
            and wgs2_indel.alt not like 'G,%'
      )
      )
      and (
      sm.start between wgs2_indel.pos + 1 and wgs2_indel.pos + len(ref)
      or    sm.[end] between wgs2_indel.pos + 1 and wgs2_indel.pos + len(ref)
      )
      )

      and not exists (
      select *
```

```
        from [MH_filtering].[dbo].[031015_goldenPath_hg19_snp142_snps] snp142
        where snp142.chrom = 'chrM' --for mtDNA
              and sm.start = snp142.posEnd
        )
        and not exists (
        select *
        from
        [MH_filtering].[dbo].[031015_goldenPath_hg19_snp142_other] other142
        where other142.chrom = 'chrM' --for mtDNA
        and sm.Start between other142.posStart and other142.posEnd
        )
--and not exists (
--      select *
--      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvMerged_c1toc14]
dgvM
--      where sm.chrom = dgvM.chrom
--            and sm.start between dgvM.chromStart + 1 and dgvM.chromEnd
--)
--and not exists (
--      select *
--      from
--      [MH_Filtering].[dbo].[031015_goldenPath_hg19_dgvSupporting_c1toc14]dgvS
--      where sm.chrom = dgvS.chrom
--            and sm.start between dgvS.chromStart + 1 and dgvS.chromEnd
--)
--and not exists (
--      select *
--      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_genomicSuperDups] sd
--      where (
--                  sm.chrom = sd.chrom
--            and sm.start between sd.chromStart + 1 and sd.chromEnd
--            ) or (
--                  sm.chrom = sd.otherchrom
--            and sm.start between sd.otherStart + 1 and sd.otherEnd
--            )
--)
--and not exists (
--      select *
--      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_nestedRepeats] nr
--      where sm.chrom = nr.chrom
--            and   sm.start between nr.chromStart + 1 and nr.chromEnd
--)
--and not exists (
--      select *
--      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_simpleRepeat] sr
--      where sm.chrom = sr.chrom
--            and   sm.start between sr.chromStart - 99 and sr.chromEnd + 100
--)
        and not exists (
        select *
        from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm
        where       rm.genoName = 'chrM'
                    and sm.start between rm.genoStart + 1 and rm.genoEnd
        )
--and not exists (
--      select *
--      from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sine
--    where rm_sine.repClass = 'SINE'
--    and sm.chrom = rm_sine.genoName
--    and sm.start between rm_sine.genoStart - 99 and rm_sine.genoEnd + 100
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_lc
--    where rm_lc.repClass = 'low_complexity'
--          and   sm.chrom = rm_lc.genoName
--          and sm.start between rm_lc.genoStart - 99 and rm_lc.genoEnd + 100
--)
--and not exists (
--    select *
--    from [MH_Filtering].[dbo].[031015_goldenPath_hg19_rmsk] rm_sr
--    where rm_sr.repClass = 'simple_repeat'
--          and   sm.chrom = rm_sr.genoName
--          and sm.start between rm_sr.genoStart - 99 and rm_sr.genoEnd + 100
--)
order by chrom, start


drop table #sm_select
drop table #mutCountPairCoo
drop table #sm_select_filtered
```

6 Validation of mutation calls

From QUERY 5 and QUERY 6, we get a candidate mutation list for nuclear DNA and mtDNA, respectively.

For nuclear DNA mutations candidates:

1. All candidate calls were visually inspected in a mutation viewer to verify call.
2. Subset of candidate mutations were Sanger sequenced from the original gDNA sample to verify that they were not present at clonal frequencies (e.g. germline).

For mtDNA mutation candidates:

1. All candidate calls were visually inspected in a mutation viewer to verify call.
2. Any candidate observed in the mutation viewer to be present in more than one UID family was eliminated.

7 Addendum to QUERY 1

Integrated into QUERY 1 is the following homopolymer code written in C#. This code identifies mutations that occur within homopolymers that are 8 or more nucleotides in length.

```
using System;
using System.Data;
using System.Data.SqlClient;
using System.Data.SqlTypes;
using Microsoft.SqlServer.Server;
using System.Text.RegularExpressions;
using System.Collections.Generic;

public static partial class UserDefinedFunctions
{

    [SqlFunction(IsDeterministic = true, IsPrecise = true)]
    public static string HomopolymerTractPosforSBS(string matchString, int
lowlim_search, int maxgap, int lowlim_total)
    {
           /* Define search patterns for homopolymers */
        string ht_pos_pat =     "([A]{" + lowlim_search + ",})|" +
                                "([C]{" + lowlim_search + ",})|" +
                                "([T]{" + lowlim_search + ",})|" +
                                "([G]{" + lowlim_search + ",})";
        Regex ht_pos = new Regex(ht_pos_pat, RegexOptions.IgnoreCase |
RegexOptions.Compiled);

           /* Search for homopolymers */
           MatchCollection ht_pos_matches = ht_pos.Matches(matchString);
        int[,] ht_pos_key = new int[ht_pos_matches.Count, 3];
        int ht_pos_key_cursor = 0;
        int nuc_num;
        foreach (Match hpMatch in ht_pos_matches)
        {
            if      (hpMatch.ToString().Substring(0, 1) == "A")
            {
                nuc_num = 1;
            }
            else if (hpMatch.ToString().Substring(0, 1) == "C")
            {
                nuc_num = 2;
            }
            else if (hpMatch.ToString().Substring(0, 1) == "T")
            {
                nuc_num = 3;
            }
            else if (hpMatch.ToString().Substring(0, 1) == "G")
            {
                nuc_num = 4;
            }
            else
            {
                nuc_num = 0;
            }

            ht_pos_key[ht_pos_key_cursor, 0] = nuc_num;
            ht_pos_key[ht_pos_key_cursor, 1] = hpMatch.Index;
            ht_pos_key[ht_pos_key_cursor, 2] = hpMatch.Length;
            if (ht_pos_key_cursor > 0 && ht_pos_key_cursor <
ht_pos_matches.Count - 1) /*not first and not last?*/
            {
                if (ht_pos_key[ht_pos_key_cursor - 1, 2] < lowlim_total &&
ht_pos_key[ht_pos_key_cursor - 1, 0] != nuc_num)
                {
                    ht_pos_key[ht_pos_key_cursor - 1, 2] = 0;
                }
                else if (ht_pos_key[ht_pos_key_cursor - 1, 0] == nuc_num)
                {
                    if (hpMatch.Index - (ht_pos_key[ht_pos_key_cursor - 1,
1] + ht_pos_key[ht_pos_key_cursor - 1, 2]) <= maxgap)
                    {/* "merge" the items */
                        ht_pos_key[ht_pos_key_cursor - 1, 2] = 0;
                        ht_pos_key[ht_pos_key_cursor, 1] =
ht_pos_key[ht_pos_key_cursor - 1, 1];
                        ht_pos_key[ht_pos_key_cursor, 2] = (hpMatch.Index
- ht_pos_key[ht_pos_key_cursor - 1, 1]) + hpMatch.Length;
                    }
                    else if (ht_pos_key[ht_pos_key_cursor - 1, 2] >=
lowlim_total)
                    {
                        ht_pos_key_cursor++;
                        continue;
                    }
                    else
                    {
                        ht_pos_key[ht_pos_key_cursor - 1, 2] = 0;
                    }
                }

            }
            else if (ht_pos_matches.Count == 1 && ht_pos_key_cursor == 0)
/*first and only?*/
            {
                if (hpMatch.Length < lowlim_total)
                {
                    ht_pos_key[ht_pos_key_cursor, 2] = 0;
                }
            }
            else if (ht_pos_key_cursor > 0 && ht_pos_key_cursor ==
ht_pos_matches.Count - 1)
            {
                if (ht_pos_key[ht_pos_key_cursor - 1, 2] < lowlim_total &&
ht_pos_key[ht_pos_key_cursor - 1, 0] != nuc_num)
                {
                    ht_pos_key[ht_pos_key_cursor - 1, 2] = 0;
                }
                else if (ht_pos_key[ht_pos_key_cursor - 1, 0] == nuc_num)
                {
                    if (hpMatch.Index - (ht_pos_key[ht_pos_key_cursor - 1,
1] + ht_pos_key[ht_pos_key_cursor - 1, 2]) <= maxgap)
                    {/* "merge" the items */
                        ht_pos_key[ht_pos_key_cursor - 1, 2] = 0;
                        ht_pos_key[ht_pos_key_cursor, 1] =
ht_pos_key[ht_pos_key_cursor - 1, 1];
                        ht_pos_key[ht_pos_key_cursor, 2] = (hpMatch.Index
- ht_pos_key[ht_pos_key_cursor - 1, 1]) + hpMatch.Length;
                        if (ht_pos_key[ht_pos_key_cursor, 2] <
lowlim_total)     /*new block unique for last item only*/
                        {
                            ht_pos_key[ht_pos_key_cursor, 2] = 0;
                        }

                    }
                    else if (ht_pos_key[ht_pos_key_cursor - 1, 2] >=
lowlim_total)
                    {
                        ht_pos_key_cursor++;
                        continue;
                    }
                    else
                    {
                        ht_pos_key[ht_pos_key_cursor - 1, 2] = 0;
                    }
                }
            }

            ht_pos_key_cursor++;
        }


        /* Build output string */
        string ht_pos_out = new string(' ', matchString.Length);
        int j;
        for (j = 0; j < ht_pos_matches.Count; j++)
        {
            if (ht_pos_key[j, 2] >= lowlim_total)
            {
                ht_pos_out = ht_pos_out.Insert(ht_pos_key[j, 1], new
string(' ', ht_pos_key[j, 2]).Replace(" ", "X"));
                ht_pos_out = ht_pos_out.Remove(matchString.Length);
            }
        }

        return ht_pos_out;
    }

}
```