SUPPLEMENTAL FIGURES

Supplemental Figure S1

	CRISPR-DS	Time Required (min)	Active Time Required (min)	~TOTAL Time required (hrs)	Standard-DS	Time Required (min)	Active Time Required (min)	~TOTAL Time required (hrs)
	Preparation time: design of locus specific gRNA and biotinylated probes				**Preparation time: design of locus specific biotinylated probes**			
Day 1	Prepare Cas9 Digest	30	30	**0.5**	Prepare to Sonicate Sonicate Prepare ERAT End repair Prepare ligation Ligation 0.8X Ampure BW Prepare PCR PCR 0.8X Ampure BW Prepare Wash Buffers Prepare Samples Lyophilize Prepare to hybridize	45 3 10 60 10 15 20 10 20 20 10 10 60 20	15 3 10 - 10 - 20 10 - 20 10 10 - 20	**5.2**
	Overnight CRISPR-Cas9 Digestion				**Overnight Hybridization**			
Day 2	0.5X Ampure BW 1.8X Ampure BW Prepare ERAT End repair Prepare ligation Ligation 0.8X Ampure BW Prepare PCR PCR 0.8X Ampure BW Prepare Wash Buffers Prepare Samples Lyophilize Prepare to hybridize	15 20 10 60 10 15 20 10 20 20 10 10 60 20	15 20 10 - 10 - 20 10 - 20 10 10 - 20	**5**	Heated Washes Prepare PCR 0.8X Ampure BW Prepare Wash Buffers Prepare Samples Lyophilize Prepare to hybridize	120 10 60 20 10 10 60 20	120 10 - 20 10 10 - 20	**5.2**
	Overnight Hybridization				**Overnight Hybridization**			
Day 3	Heated Washes Prepare PCR PCR 0.8X Ampure BW	120 10 60 20	120 10 - 20	**3.5**	Heated Washes Prepare PCR PCR 0.8X Ampure BW	120 10 60 20	120 10 - 20	**3.5**
	*** Ready to Sequence***				*** Ready to Sequence***			

Figure S1. Timeline of library preparation for CRISPR-DS and standard-DS. CRISPR-DS reduces the time required for library preparation by nearly an entire day, with just a short set-up for the CRISPR/Cas9 digestion on Day 1. ERAT: End-Repair and A-Tailing; BW; bead wash. Note: These times are approximations

Supplemental Figure S2

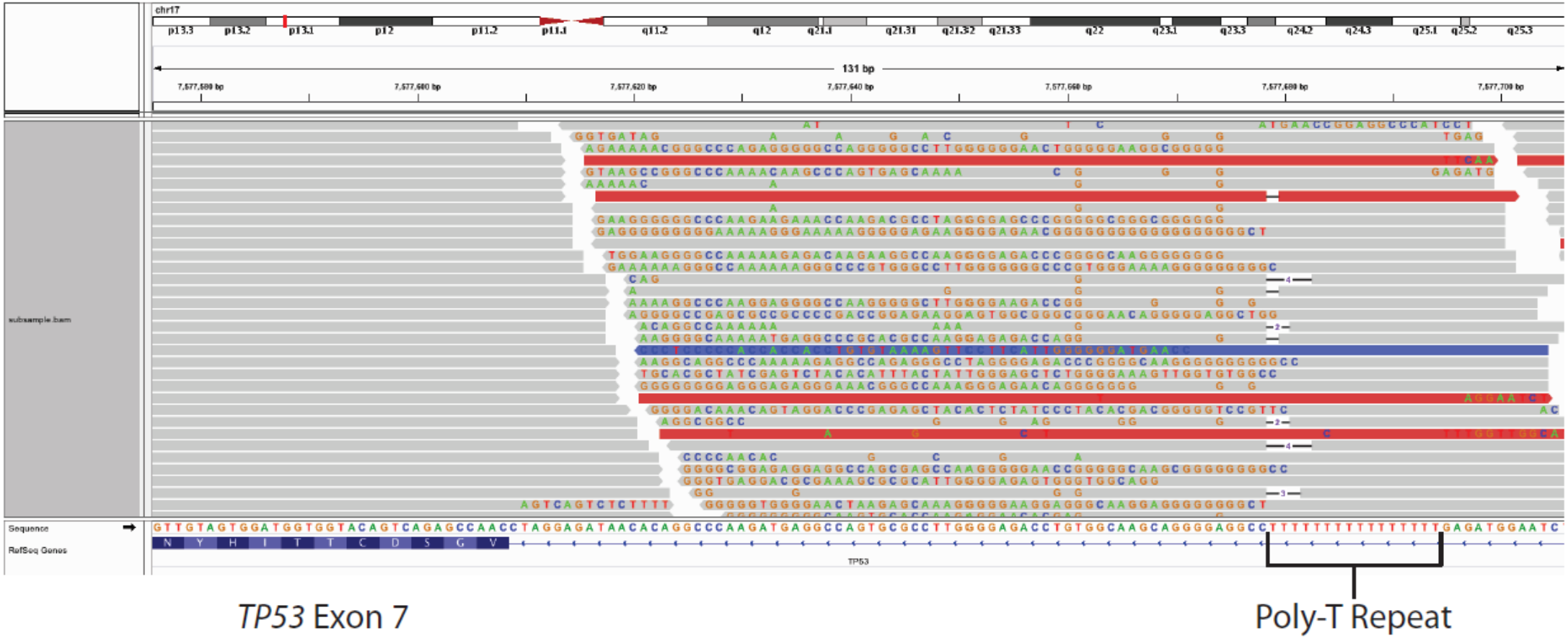


Figure S2. Homopolymer region produces suboptimal sequencing near *TP53* exon 7. The IGV plot shows suboptimal sequencing quality in standard-DS reads that contain the Poly-T repeat. To avoid this problem, the CRISPR-DS gRNA had to be placed in the small region between the Poly-T repeat and the beginning of exon 7, constraining the size of that fragment.

Supplemental Figure S3

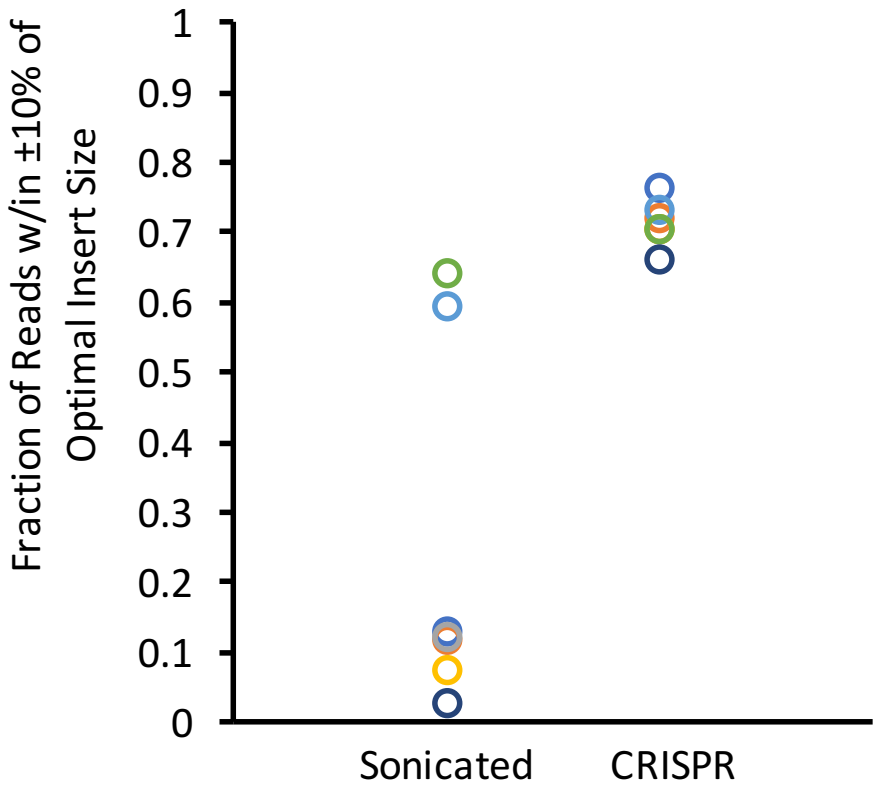


Figure S3. Fraction of reads within 10% of optimal insert size: CRISPR-DS vs standard-DS. The fraction of reads within 10% of optimal insert size corresponds to the yellow highlighted regions in Fig. 4b, c. Markers with matching colors between the two fragmentation methods represent the same sample. This comparison includes seven samples in the study that were analyzed with both methods: 4 peritoneal fluid DNAs (Table 2) and one normal bladder control DNA processed with 250ng, 100ng, and 25ng input DNA (Figure 5).

Supplemental Figure S4

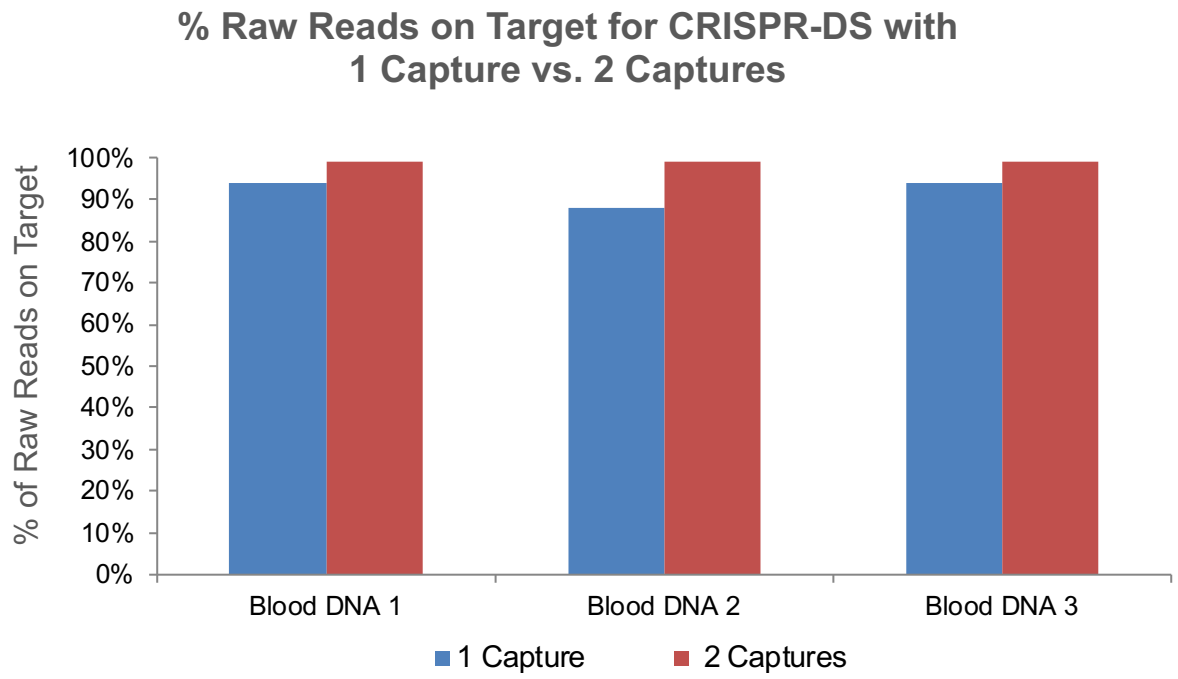


Figure S4. Target enrichment for CRISPR-DS with one vs. two captures. Three de-identified blood DNA samples were processed for CRISPR-DS and split in half after one hybridization capture. The first half was indexed and sequenced and the second half was subject to an additional round of capture, as required in the original DS protocol. After one capture, all three samples had nearly ~90% of raw reads on-target, indicating successful enrichment of the target region. Performing an additional capture slightly increased enrichment to 99%. However, this minimal gain is unnecessary as 90% of raw reads on-target is sufficient enrichment for successful analysis. Note that raw reads are converted to DCS reads, which are typically >98% on-target.

Supplemental Figure S5

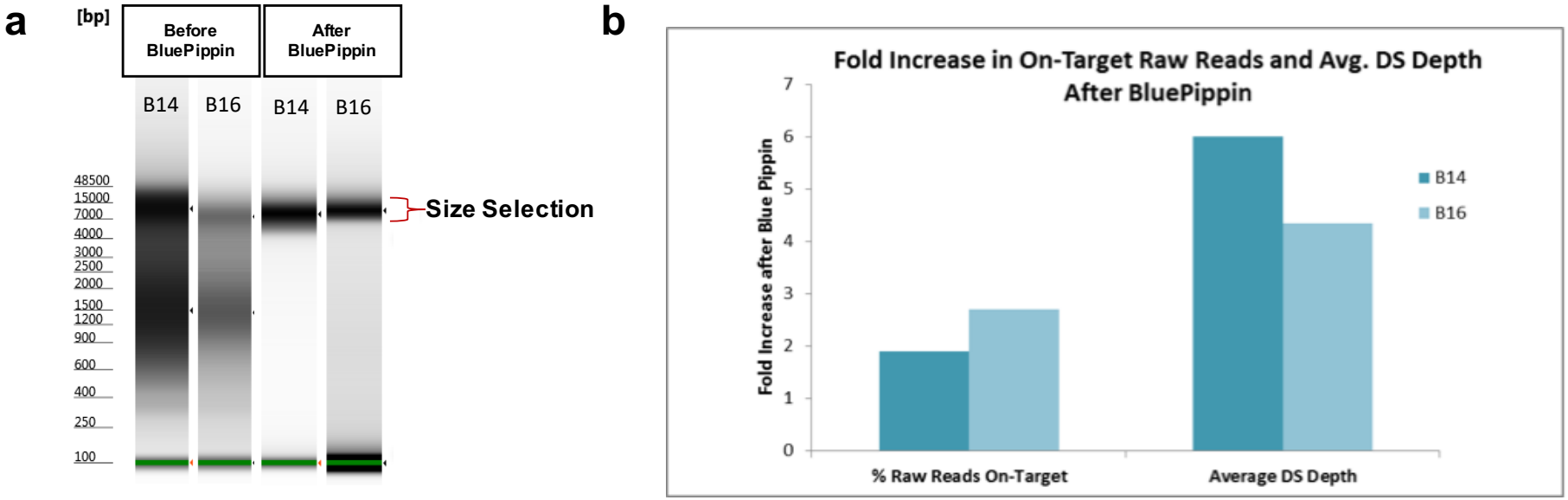


Figure S5. Pre-enrichment for high molecular weight (MW) DNA with BluePippin. Two samples with degraded DNA (B14, DIN=6; B16, DIN=4) were run on a BluePippin 0.75% gel cassette using high-pass setting to obtain >8kb fragments. (a) Genomic TapeStation demonstrating successful removal of lower MW DNA products by BluePippin. (b) Comparison of percentage of on-target raw reads and DCS depth for the same DNA sequenced before and after BluePippin pre-enrichment for high MW DNA. Pre-enrichment resulted in ~2-fold increase in percentage of on-target raw reads and about a 5-fold increase on average DCS depth.

Supplemental Figure S6

a

MIXTURE	POSITION	VARIANT	EXPECTED MAF	CRISPR-DS MAF	STANDARD-DS MAF
1:1	7577407	A>C	0.610	0.596	0.607
1:1	7577427	G>A	0.610	0.595	0.626
1:1	7578275	G>A	0.219	0.218	0.199
1:1	7579472	G>C	0.338	0.330	0.349
1:1	7579619	G>T	0.052	0.046	0.047
1:1	7579801	G>C	0.346	0.365	0.342
1:10	7577407	A>C	0.522	0.509	0.528
1:10	7577427	G>A	0.522	0.509	0.566
1:10	7578275	G>A	0.044	0.032	0.027
1:10	7579472	G>C	0.466	0.473	0.472
1:10	7579619	G>T	0.010	0.009	0.008
1:10	7579801	G>C	0.477	0.445	0.438
1:100	7577407	A>C	0.502	0.513	0.492
1:100	7577427	G>A	0.502	0.514	0.476
1:100	7578275	G>A	0.004	0.001	0.002
1:100	7579472	G>C	0.494	0.505	0.488
1:100	7579619	G>T	0.001	0.002	0.001
1:100	7579801	G>C	0.506	0.524	0.463
1:1000	7577407	A>C	0.500	0.486	0.458
1:1000	7577427	G>A	0.501	0.487	0.445
1:1000	7578275	G>A	0.0004	0.0005	ND
1:1000	7579472	G>C	0.497	0.488	0.510
1:1000	7579619	G>T	0.0001	ND	ND
1:1000	7579801	G>C	0.509	0.495	0.489

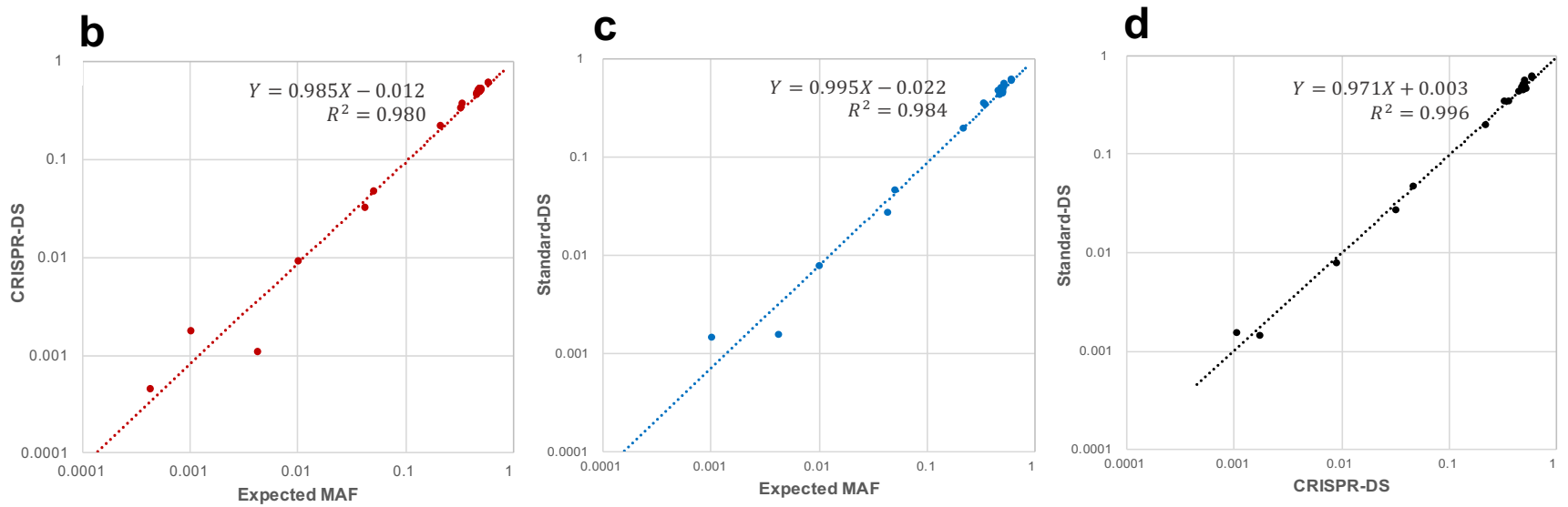


Figure S6. Comparison of mutant allele fraction (MAF) detected by CRISPR-DS and standard-DS. Two samples with known MAF for 6 different variants were mixed at 1:1, 1:10, 1:100 and 1:1000. The resulting mixtures were sequenced with both CRISPR-DS and standard-DS. (a) The expected MAF as well as the resulting MAF by each technique, for each variant, are reported in this table. ND: not detected. Note that the mutation at frequency 0.0001 was not expected to be detected with either method because the mean depth of sequencing was ~2,000x. (b-c) Expected MAF of mixture samples vs. MAF from sequencing samples with CRISPR-DS (red) and standard-DS (blue), with least-squares regression line shown. $R^2 = 0.980$ and 0.984 respectively. (d) MAF of mixture samples sequenced with CRISPR-DS on x-axis and MAF by standard-DS on y-axis, least-squares regression line shown, $R^2 = 0.996$.

Supplemental Figure S7

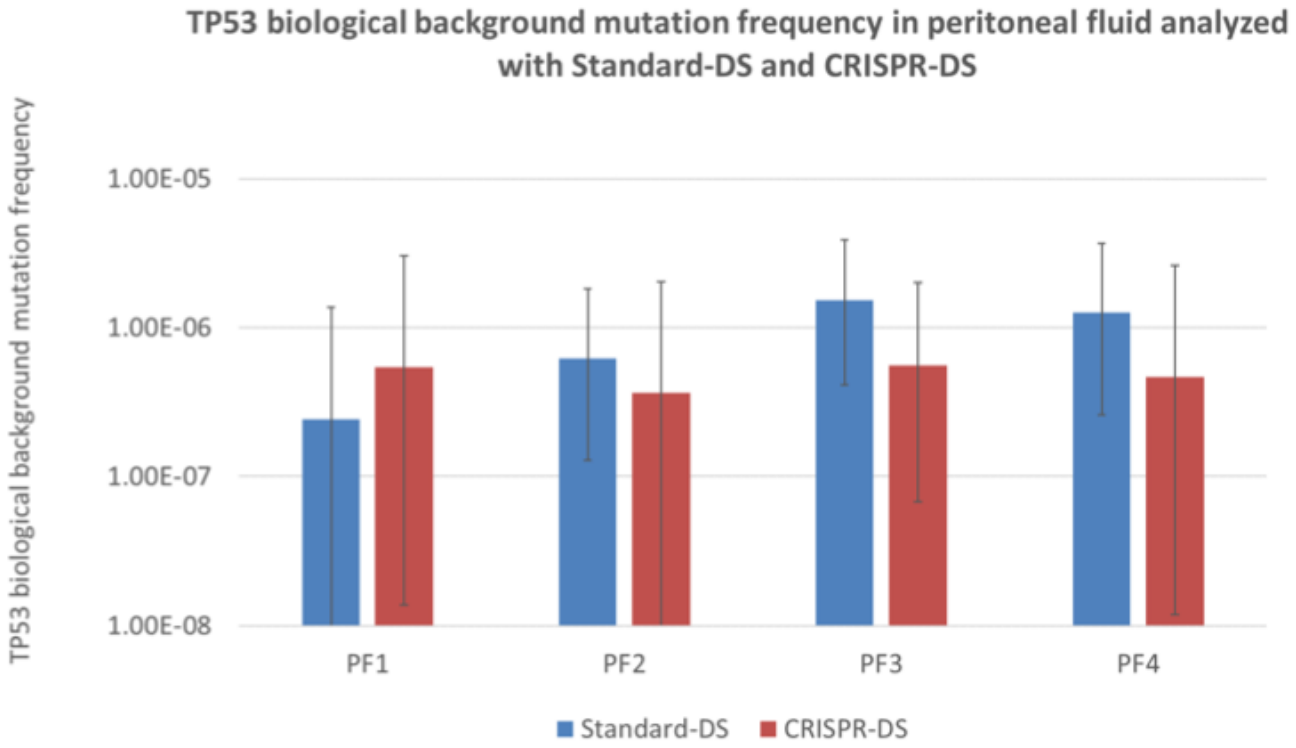


Figure S7. Comparison of *TP53* biological background mutation frequency measured by Standard-DS and CRISPR-DS. Four peritoneal fluid samples previously analyzed with Standard-DS were processed with CRISPR-DS. *TP53* biological background mutation was calculated as the number of *TP53* mutations not including the tumor mutation divided by the total number of nucleotides sequenced. Error bars correspond to the 95% confidence intervals calculated using the Clopper-Pearson 'exact' method for binomial distributions.

Supplemental Figure S8

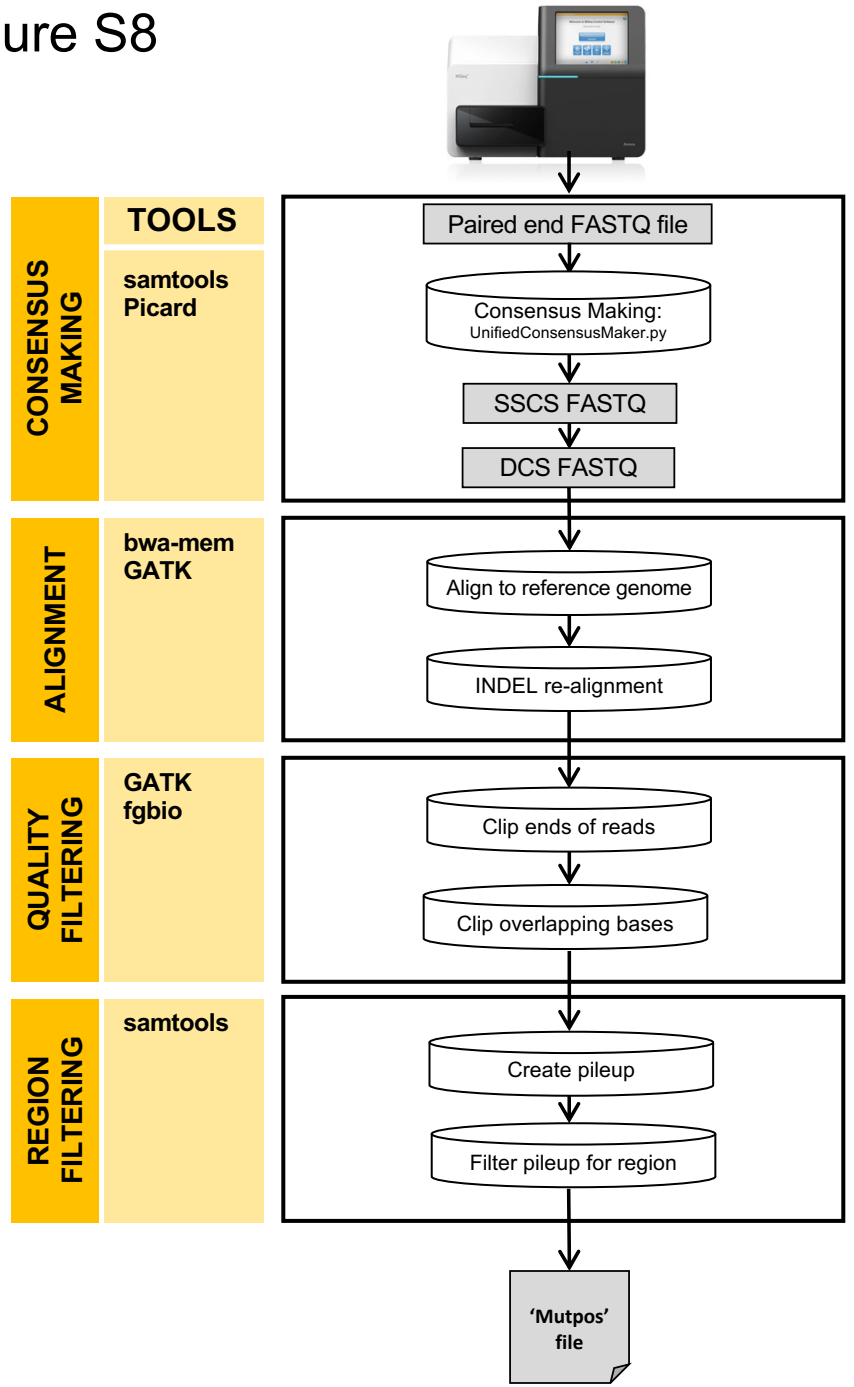
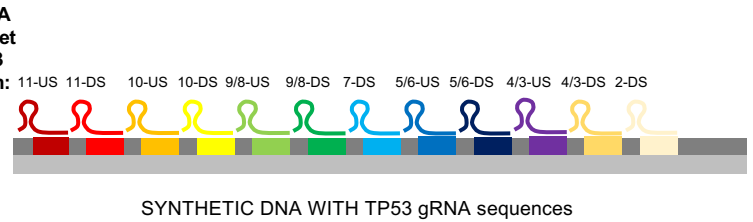


Figure S8. Overview of CRISPR-DS data processing. Raw FastQ files from the Illumina platform MiSeq sequencer are used to create Single Stranded Consensus Sequence (SSCS) FastQ files and double-stranded consensus sequence (DCS) FastQ files. DCS FastQ files are then aligned to the reference genome using BWA-mem, and then post-processed using GATK and fgbio software. After pileup and filtering for the region of interest, a custom python script creates a text file ('mutpos' file) with mutant calls.

Supplemental Figure S9

a



b

TP53 guide RNA	TP53 Exon(s) Targeted	Left Fragment (bp)	Right Fragment (bp)
TP5e11_US	11	24	476
TP5e11_DS	11	64	436
TP5e10_US	10	104	396
TP5e10_DS	10	144	356
TP5e9-8_US	9,8	184	316
TP5e9-8_DS	9,8	224	276
TP5e7_DS.v2	7	264	236
TP5e6-5_US	5,6	304	196
TP5e6-5_DS	5,6	344	156
TP5e4-3_US.v2	4,3	384	116
TP5e4-3_DS	4,3	424	76
TP5e2_DS	2	464	36

c

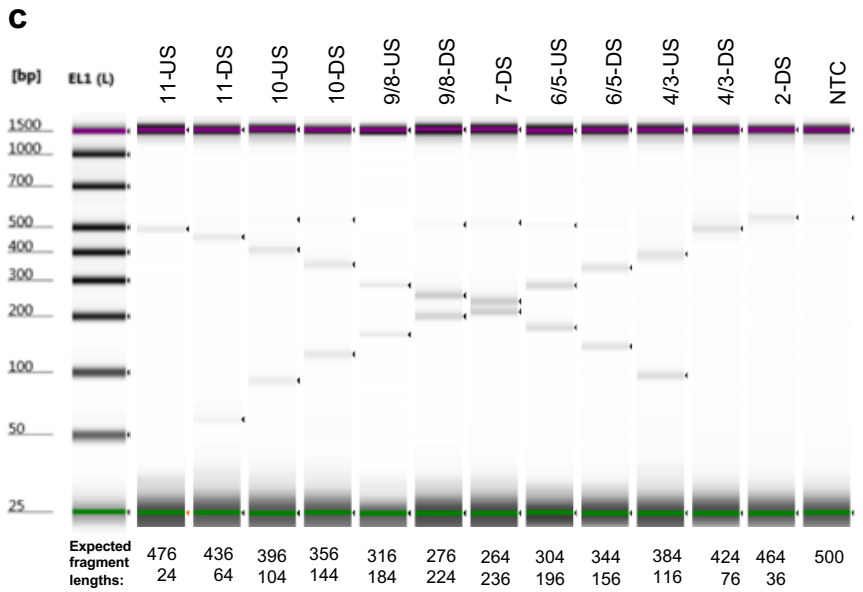


Figure S9. Control CRISPR/Cas9 digestion of *TP53* gRNAs. (a) A 500-bp synthetic dsDNA was designed to contain the 12 *TP53* gRNA sequences used in the study. Each guide is 23-bp long, and they are separated by 17 bp of spacer sequence. The colored boxes represent the gRNA sequence and the grey boxes represent spacer DNA sequence. (b) List of the 12 *TP53* gRNAs used in the study (as in Supplemental Table S1) and their predicted fragment lengths after cutting the synthetic DNA. (c) TapeStation gel image shows distinct bands corresponding to the expected fragment lengths for each of the gRNAs, demonstrating successful cutting.