**Supplementary methods**

**Whole-exome sequencing protocol**

**Preparation of Genomic DNA Libraries**

Genomic DNA libraries were prepared following Illumina's (Illumina, San Diego, CA) suggested protocol with the following modifications. **(1)** 3 µg of genomic DNA from tumor or normal cells in 100 µl of TE was fragmented in a Covaris sonicator (Covaris, Woburn, MA) to a size of 100–500 bp. DNA was purified with a PCR purification kit (Cat # 28104, Qiagen) and eluted in 35 µl of elution buffer included in the kit. **(2)** Purified, fragmented DNA was mixed with 40 µl of H2O, 10 µl of 10 x T4 ligase buffer with 10 mM ATP, 4 µl of 10 mM dNTP, 5 µl of T4 DNA polymerase, 1 µl of Klenow Polymerase, and 5 µl of T4 polynucleotide Kinase. All reagents used for this step and those described below were obtained from New England Biolabs (NEB, Ipswich, MA) unless otherwise specified. The 100-µl end-repair mixture was incubated at 20oC for 30 min, purified by a PCR purification kit (Cat # 28104, Qiagen) and eluted with 32 µl of elution buffer (EB). **(3)** To A-tail the DNA, all 32 µl of end-repaired DNA was mixed with 5 µl of 10 x Buffer (NEB buffer 2), 10 µl of 1 mM dATP and 3 µl of Klenow (exo-). The 50-µl mixture was incubated at 37oC for 30 min before DNA was purified with a MinElute PCR purification kit (Cat # 28004, Qiagen). Purified DNA was eluted with 12.5 µl of 70oC EB and obtained with 10 µl of EB. **(4)** For adaptor ligation, 10 µl of A-tailed DNA was mixed with 10 µl of PE-adaptor (Illumina), 25 µl of 2x Rapid ligase buffer and 5 µl of Rapid Ligase. The ligation mixture was incubated at room temperature (RT) or 20oC for 15 min. **(5)** To purify adaptor-ligated DNA, 50 µl of ligation mixture from step (4) was mixed with 200 µl of NT buffer from NucleoSpin Extract II kit (cat# 636972, Clontech, Mountain View, CA) and loaded into NucleoSpin column. The column was centrifuged at 14,000 g in a desktop centrifuge for 1 min, washed once with 600 µl of wash buffer (NT3 from Clontech), and centrifuged again for 2 min to dry completely. DNA was eluted in 50 µl elution buffer included in the kit. **(6)** To obtain an amplified library, ten PCRs of 25 µl each were set up, each including 13.25 µl of H2O, 5 µl of 5 x Phusion HF buffer, 0.5 µl of a dNTP mix containing 10 mM of each dNTP, 0.5 µl of Illumina PE primer #1, 0.5 µl of Illumina PE primer #2, 0.25 µl of Hotstart Phusion polymerase, and 5 µl of the DNA from step (5). The PCR program used was: 98oC 1 minute; 6 cycles of 98oC for 20 seconds, 65oC for 30 seconds, 72oC for 30 seconds; and 72oC for 5 min. To purify the PCR product, 250 µl PCR mixture (from the 10 PCR reactions) was mixed with 500 µl NT buffer from a NucleoSpin Extract II kit and purified as described in step (5). Library DNA was eluted with 70oC-warm elution buffer and the DNA concentration was estimated by absorption at 260 nm.

**Exome and Targeted Subgenomic DNA Capture**

The human exome was captured following a protocol from Agilent's SureSelect Paired-End Version 2.0 Human Exome Kit, or using custom kits with probes designed to capture the desired genomic regions (Agilent, Santa Clara, CA) with the following modifications. **(1)** A hybridization mixture was prepared containing 25 µl of SureSelect Hyb # 1, 1 µl of SureSelect Hyb # 2, 10 µl of SureSelect Hyb # 3, and 13 µl of SureSelect Hyb # 4. **(2)** 3.4 µl (0.5 µg) of the PE-library DNA described above, 2.5 µl of SureSelect Block #1, 2.5 µl of SureSelect Block #2, and 0.6 µl of Block #3; was loaded into one well in a 384-well Diamond PCR plate (cat# AB-1111, Thermo-Scientific, Lafayette, CO), sealed with microAmp clear adhesive film (cat# 4306311; ABI, Carlsbad, CA) and placed in GeneAmp PCR system 9700 thermocycler (Life Sciences, Carlsbad CA) for 5 min at 95°C, then held at 65°C (with the heated lid on). **(3)** 25–30 µl of hybridization buffer from step (1) was heated for at least 5 min at 65°C in another sealed plate with heated lid on. **(4)** 5 µl of SureSelect Oligo Capture Library, 1 µl of nuclease-free water, and 1 µl of diluted RNase Block (prepared by diluting RNase Block 1: 1 with nuclease-free water) were mixed and heated at 65oC for 2 min in another sealed 384-well plate. **(5)** While keeping all reactions at 65°C, 13 µl of Hybridization Buffer from Step (3) was added to the 7 µl of the SureSelect Capture Library Mix from Step (4) and then the entire contents (9 µl) of the library from Step (2). The mixture was slowly pipetted up and down 8 to 10 times. **(6)** The 384-well plate was sealed tightly and the hybridization mixture was incubated for 24 hours at 65°C with a heated lid.

After hybridization, five steps were performed to recover and amplify captured DNA library: **(1)** Magnetic beads for recovering captured DNA: 50 µl of Dynal MyOne Streptavidin C1 magnetic beads (Cat # 650.02, Invitrogen Dynal, AS Oslo, Norway) was placed in a 1.5-ml microfuge tube and vigorously resuspended on a vortex mixer. Beads were washed three times by adding 200 µl of SureSelect Binding buffer, mixing on a vortex for 5 seconds and then removing the supernatant after placing the tubes in a Dynal magnetic separator. After the third wash, beads were resuspended in 200 µl of SureSelect Binding buffer. **(2)** To bind captured DNA, the entire hybridization mixture described above (29 µl) was transferred directly from the thermocycler to the bead solution and mixed gently; the hybridization mix /bead solution was incubated in an Eppendorf thermomixer at 850 rpm for 30 min at room temperature. **(3)** To wash the beads, the supernatant was removed from beads after applying a Dynal magnetic separator and the beads were resuspended in 500 µl SureSelect Wash Buffer #1 by mixing on vortex mixer for 5 seconds and incubated for 15 min at room temperature. Wash Buffer#1 was then removed from beads after magnetic separation. The beads were further washed three times, each with 500 µl pre-warmed SureSelect Wash Buffer #2 after incubation at 65°C for 10 min. After the final wash, SureSelect Wash Buffer #2 was completely removed. **(4)** To elute captured DNA, the beads were suspended in 50 µl SureSelect Elution Buffer, vortex-mixed and incubated for 10 min at room temperature. The supernatant was removed after magnetic separation, collected in a new 1.5-ml microcentrifuge tube, and mixed with 50 µl of SureSelect Neutralization Buffer. DNA was purified with a Qiagen MinElute column and eluted in 17 µl of 70oC EB to obtain 15 µl of captured DNA library. **(5)** The captured DNA library was amplified in the following way: 15 PCR reactions each containing 9.5 µl of H2O, 3 µl of 5 x Phusion HF buffer, 0.3 µl of 10 mM dNTP, 0.75 µl of DMSO, 0.15 µl of Illumina PE primer #1, 0.15 µl of Illumina PE primer #2, 0.15 µl of Hotstart Phusion polymerase, and 1 µl of captured exome library were set up. The PCR program used was: 98oC for 30 seconds; 14 cycles of 98oC for 10 seconds, 65oC for 30 seconds, 72oC for 30 seconds; and 72oC for 5 min. To purify PCR products, 225 µl of PCR mixture (from 15 PCR reactions) was mixed with 450 µl of NT buffer from NucleoSpin Extract II kit and purified as described above. The final library DNA was eluted with 30 µl of 70oC elution buffer and DNA concentration was estimated by OD260 measurement.

**SafeSeq Protocol**

**Preparation of genomic libraries**

Templates were prepared for sequencing as described previously (1), with modifications noted below that facilitated the amplification of multiple gene regions in a single well of a 96-well PCR plate. In brief, each strand of each template molecule was encoded with a 14 base unique identifier (UID) – comprised of degenerate “N” bases (equal probability of being an “A,” “C,” “G,” or “T”) - with two to four cycles of amplicon-specific PCR (“UID assignment PCR cycles”). Both forward and reverse gene-specific primers contained universal tag sequences at their 5' ends, providing the primer binding sites for the second-round amplification, but only the forward primer contained the UID, which was positioned between the 5' universal tag and the 3’ gene-specific sequences. Four “N” bases were additionally included in the reverse primer to facilitate sequencing analysis of paired-end libraries. The UID assignment PCR cycles were performed on 66 ng of DNA in a 50 µL reaction containing 1X Phusion HF buffer, 0.25 mM dNTPs, 0.5 µM each of forward (containing 14 “N” bases) and reverse primers, and 2 U of Phusion Hot Start II Polymerase (Thermo Scientific). Carryover of residual UID-containing primers to the second-round amplification, which can complicate template quantification (1), was minimized through a 15 s exonuclease digestion at 370C to degrade unincorporated primers. In Kinde *et al*. (1), Exonuclease-I (Enzymatics) was chosen to eliminate the residual UID-containing primers, however we found that a different exonuclease – RecJf (New England Biolabs) – followed by purification with AMPure XP beads (Beckman) and elution in 10 µL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), more extensively removed the UID-containing primers and yielded more robust amplification products. The eluted templates were amplified in a second-round PCR with primers containing the grafting sequences necessary for hybridization to the Illumina GA IIx flow cell at their 5’ ends (Fig. 2). The reverse amplification primer additionally contained an index sequence between the 5 ’grafting and 3’ universal tag sequences to enable the PCR products from multiple individuals to be simultaneously analyzed in the same flow cell compartment of the sequencer (1). The second-round amplification reactions contained 1X Phusion HF buffer, 0.25 mM dNTPs, 0.5 µM each of forward and reverse primers, and 2 U of Phusion Hot Start II Polymerase in a total of 50 µL. After an initial heat activation step at 980 C for 2 minutes, twenty-three cycles of PCR were performed with the following cycling conditions: 980C for 10 s, 650C for 15 s, and 720C for 15 s. The multiplexed assay was performed in similar fashion utilizing six independent amplifications – each containing 66 ng of DNA (i.e.,~400 ng total) – per sample. The PCR products were purified with AMPure XP beads and used directly for sequencing on either Illumina MiSeq or GA IIx instruments, with equivalent results.

*Data Analysis*. High quality sequence reads were analyzed as previously described (1). Briefly, we selected reads that contained high quality basecalls in their UID region (i.e., the first 14 cycles) by utilizing the quality scores generated by default, which indicate the probability that an individual base call was made in error (2). Reads in which each of the 14 bases comprising the UID (representing one original template strand) had a quality score ≥15 were grouped by their UIDs and only the UIDs supported by more than one read were retained for further analysis. The template-specific portion of the reads that contained the sequence of an expected amplification primer was matched to a reference sequence set with a custom script (available from the authors upon request). Artifactual mutations introduced during the sample preparation or sequencing steps were eliminated by requiring that >90% of reads sharing the same UID contained the identical mutation (a “supermutant,”). For the 46 assays querying a single amplicon, we required that the fraction of mutant alleles was significantly different from the background mutation levels determined from a negative control (*P* <0.001, binomial test). As mutations are not known *a priori* in a screening environment, we used a more agnostic metric to detect mutations in the multiplexed assay. A threshold supermutant frequency was defined for each sample as equaling the mean frequency of all supermutants plus six standard deviations of the mean. Only supermutants exceeding this threshold were designated as mutations.

References:

1. Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proceedings of the National Academy of Sciences of the United States of America 2011;108(23):9530-5.

2. Ewing B, Green P. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome research 1998;8(3):186-94.