

Multiparametric and accurate functional analysis of genetic sequence variants using CRISPR-Select

In the format provided by the
authors and unedited

Supplemental Table 1. Sequence of crRNAs.

crRNA name	20 nt guide sequence of crRNA
<i>PIK3CA</i> -H1047R	CAAATGAATGATGCACATCA
<i>PTEN</i> -L182*	AATCCAGATGATTCTTTAAC
<i>BRCA2</i> intron1	AGGCGGCGTTGGTCTCTAAC
<i>BRCA2</i> 3' intergenic	AGACAATAGCAGTTGTACCA
<i>BRCA2</i> -N289H	ACTTCATCTTCTAGGACATT
<i>BRCA2</i> -K513R	CTTGCAATTGAAAGTCTCTTT
<i>BRCA2</i> -C554W	ATCAATTAAATTTGGACATA
<i>BRCA2</i> -D946V	AGTGTCAATTAAAAAAGATT
<i>BRCA2</i> -T2403A	GGTGGAACAAAGACTTTGGT
<i>BRCA2</i> -L2510R	TTTGCAAGATACAGACTGCC
<i>BRCA2</i> -I2627F	ATCACTATAGATGGATCATA
<i>BRCA2</i> -I2627N	ATCACTATAGATGGATCATA
<i>BRCA2</i> -F2638L	TATGGAATGTGCCTTTCCCTA
<i>BRCA2</i> -Y2658H	TGCTTCTTCAACTAAAATAC
<i>BRCA2</i> -Y2660C	TTTCACTTTTAGATATGATA
<i>BRCA2</i> -I2675M	ATCGGCTATAAAAAAGATAA
<i>BRCA2</i> -T2722I	CCATTATTGAACTTACAGAT
<i>BRCA2</i> -T2722R	CCATTATTGAACTTACAGAT
<i>BRCA2</i> -D2723G	TTATTGAACTTACAGATGGG
<i>BRCA2</i> -G2748S	AAGAATGGCAGACTGACAGT
<i>BRCA2</i> -E3002G	TTATATTCTCTGTTAACAGA
<i>BRCA2</i> -A3140P	GGCCTTCTTACTTTATTTGC
<i>BRCA2</i> -67+1G>C	AAGACACGCTGCAACAAAGC
<i>BRCA2</i> -316+1G>T	GATAAATTCAAATTAGACTT
<i>BRCA2</i> -476-2A>G	TCCCCTTTTTTTTACCCCCAG
<i>BRCA2</i> -517-2A>G	TTTTGGTGTCTGACGACCCT
<i>KRAS</i> -C12D/G	GTAGTTGGAGCTTGTGGCGT
<i>FGFR4</i> -V550M	GCACTCCACGATCACGTACA
<i>ESR1</i> -Y537S	TCCAGCAGCAGGTCATAGAG
<i>EGFR</i> -Y69*	ATCATAATTCCTCTGCACAT
<i>NFKBIZ</i> -E201fs	ACCAACGCCCGGGGAGAGCA

All crRNAs are *H. sapiens* specific.

Supplemental Table 2. Sequence of ssODN repair templates.

ssODN name	ssODN sequence
Reference sequence	AGCAAGAGGCTTTGGAGTATTTTCATGAAACAAATGAATGATGCACATCA <u>GGG</u> TGGCTGGACAACAAAAATGGATTGGATCTTCCACACAAT
PIK3CA-H1047H (WT ⁺)	AGCAAGAGGCTTTGGAGTATTTTCATGAAACAAATGAATGATGCACATCATGGTGGCTGGACAACAAAAATGGATTGGATCTTCCACACAAT
PIK3CA-H1047R (Variant)	AGCAAGAGGCTTTGGAGTATTTTCATGAAACAAATGAATGATGCACCTCATGGTGGCTGGACAACAAAAATGGATTGGATCTTCCACACAAT
Reference sequence	TATTCCCAGTCAGAGGCGCTATGTGTATTATTATAGCTA <u>CTT</u> GTAAAGAATCATCTGGATTATAGACCAGTGGCACTGTTGTTTCACAA
PTEN-L182L (WT ⁺)	TATTCCCAGTCAGAGGCGCTATGTGTATTATTATAGCTACCTGTTCAAGAATCATCTGGATTATAGACCAGTGGCACTGTTGTTTCACAA
PTEN-L182* (Variant)	TATTCCCAGTCAGAGGCGCTATGTGTATTATTATAGCTACCTGTAAAGAATCATCTGGATTATAGACCAGTGGCACTGTTGTTTCACAA
Reference sequence	TTTAAAGTAAATAGCTGCAAAGACCACATTGGAAAGTCAATG <u>CCA</u> ATGTCCTAGAAGATGAAGTATATGAAACAGTTGTAGATACCTCTGAA
BRCA2-N289N (WT ⁺)	TTTAAAGTAAATAGCTGCAAAGACCACATTGGAAAGTCAATGCCAACCTCCTAGAAGATGAAGTATATGAAACAGTTGTAGATACCTCTGAA
BRCA2-N289H (Variant)	TTTAAAGTAAATAGCTGCAAAGACCACATTGGAAAGTCAATGCCACATGTCCTAGAAGATGAAGTATATGAAACAGTTGTAGATACCTCTGAA
Reference sequence	TTCAGGGTATCAAAAAGTCTATATTCAAGAATAAGAGAATCA <u>CTT</u> AAAGAGACTTTCAATGCAAGTTTTTCAGGTCATATGACTGATCCAAAC
BRCA2-K513K (WT ⁺)	TTCAGGGTATCAAAAAGTCTATATTCAAGAATAAGAGAATCACCTAAGAGAGACTTTCAATGCAAGTTTTTCAGGTCATATGACTGATCCAAAC
BRCA2-K513R (Variant)	TTCAGGGTATCAAAAAGTCTATATTCAAGAATAAGAGAATCACCTAGAGAGACTTTCAATGCAAGTTTTTCAGGTCATATGACTGATCCAAAC
Reference sequence	ACTGGAAATACATACTGTTTGCTCACAGAAGGAGGACT <u>CTT</u> TAATGTCCTCAAAATTTAATTGATAATGGAAGCTGGCCAGCCACCACCACACAG
BRCA2-C554C (WT ⁺)	ACTGGAAATACATACTGTTTGCTCACAGAAGGAGGACTCCTTAATGCCAAATTTAATTGATAATGGAAGCTGGCCAGCCACCACCACACAG
BRCA2-C554W (Variant)	ACTGGAAATACATACTGTTTGCTCACAGAAGGAGGACTCCTTAATGGCCAAATTTAATTGATAATGGAAGCTGGCCAGCCACCACCACACAG
Reference sequence	GGAGACACAGGTGATAAACAAGCAACCCCAAGTGTCAATTAAAAAAGATT <u>GGG</u> TTTATGTTCTTGCAAGGAGAGAACAAAAATAGTGTAAGCAG
BRCA2-D946D (WT ⁺)	GGAGACACAGGTGATAAACAAGCAACCCCAAGTGTCAATTAAAAAAGACTTTGGTTTATGTTCTTGCAAGGAGAGAACAAAAATAGTGTAAGCAG
BRCA2-D946V (Variant)	GGAGACACAGGTGATAAACAAGCAACCCCAAGTGTCAATTAAAAAAGTTTGGTTTATGTTCTTGCAAGGAGAGAACAAAAATAGTGTAAGCAG
Reference sequence	ACAAGAAATGAAAAAATGAGACACTTGATTACTACAGGCAGAC <u>CCA</u> ACCAAAGTCTTTGTTCCACCTTTTAAAACTAAATCACATTTTCACAGA
BRCA2-T2403T (WT ⁺)	ACAAGAAATGAAAAAATGAGACACTTGATTACTACAGGCAGACCAACAAAGTCTTTGTTCCACCTTTTAAAACTAAATCACATTTTCACAGA
BRCA2-T2403A (Variant)	ACAAGAAATGAAAAAATGAGACACTTGATTACTACAGGCAGACCAACCCAAAGTCTTTGTTCCACCTTTTAAAACTAAATCACATTTTCACAGA
Reference sequence	ATTAAGAAGAAACAAAGGCAACGCGTCTTTCCACAG <u>CGA</u> GGCAGTCTGTATCTTGCAAAAACATCCACTCTGCCTCGAATCTCTCTGAAAGCA
BRCA2-L2510L (WT ⁺)	ATTAAGAAGAAACAAAGGCAACGCGTCTTTCCACAGCCAGGCAGTTGTATCTTGCAAAAACATCCACTCTGCCTCGAATCTCTCTGAAAGCA
BRCA2-L2510R (Variant)	ATTAAGAAGAAACAAAGGCAACGCGTCTTTCCACAGCCAGGCAGTCGTATCTTGCAAAAACATCCACTCTGCCTCGAATCTCTCTGAAAGCA
Reference sequence	CCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGGATCATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTT
BRCA2-I2627I (WT ⁺)	CCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGGATTATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTT
BRCA2-I2627F (Variant)	CCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGGTTTCATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTT
Reference sequence	CCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGGATCATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTT
BRCA2-I2627I (WT ⁺)	CCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGGATTATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTT
BRCA2-I2627N (Variant)	CCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGGAAATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTT
Reference sequence	CACTATAGATGGATCATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTTGCTAATAGATGCCTAAGCCCAGAAAGGGTGCTT
BRCA2-F2638F (WT ⁺)	CACTATAGATGGATCATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTTGCTAATAGATGCCTAAGCCCAGAAAGGGTGCTT
BRCA2-F2638L (Variant)	CACTATAGATGGATCATATGGAAACTGGCAGCTATGGAATGTGCCTTTCTTAAGGAATTTGCTAATAGATGCCTAAGCCCAGAAAGGGTGCTT
Reference sequence	GCTAATAGATGCCTAAGCCCAGAAAGGGTGCTTCTTCAACTAAAAATACAGGCAAGTTTAAAGCATTACATTACGTAATCATATACGGCAGTAT
BRCA2-Y2658Y (WT ⁺)	GCTAATAGATGCCTAAGCCCAGAAAGGGTGCTTCTTCAACTAAAAATAGGCAAGTTTAAAGCATTACATTACGTAATCATATACGGCAGTAT
BRCA2-Y2658H (Variant)	GCTAATAGATGCCTAAGCCCAGAAAGGGTGCTTCTTCAACTAAAAACAGGCAAGTTTAAAGCATTACATTACGTAATCATATACGGCAGTAT
Reference sequence	AGAGTCACACTTCCTAAAAATATGCATTTTGTGTTTCACCTTTTAGATATGATACGGAAATTGATAGAAGCAGAAGATCGGCTATAAAAAAGATA
BRCA2-Y2660Y (WT ⁺)	AGAGTCACACTTCCTAAAAATATGCATTTTGTGTTTCACCTTTTAGATATCGATACGGAAATTGATAGAAGCAGAAGATCGGCTATAAAAAAGATA
BRCA2-Y2660C (Variant)	AGAGTCACACTTCCTAAAAATATGCATTTTGTGTTTCACCTTTTAGATATGATACGGAAATTGATAGAAGCAGAAGATCGGCTATAAAAAAGATA

Supplemental Table 2. Sequence of ssODN repair templates (continued).

ssODN name	ssODN sequence
Reference sequence	TATGATACGGAATTGATAGAAGCAGAAGATCGGCTATAAAAAAGATAA Ile TGGAAAGGGATGACACAGCTGCAAAAACACTTGTTCCTCTGTGTT
BRCA2-I2675I (WT ⁺)	TATGATACGGAATTGATAGAAGCAGAAGATCGGCTATAAAAAAGATCATGGAAGGGATGACACAGCTGCAAAAACACTTGTTCCTCTGTGTT
BRCA2-I2675M (Variant)	TATGATACGGAATTGATAGAAGCAGAAGATCGGCTATAAAAAAGAT Met GATGGAAGGGATGACACAGCTGCAAAAACACTTGTTCCTCTGTGTT
Reference sequence	AAACTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Thr TACAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTA
BRCA2-T2722T (WT ⁺)	AAACTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Thr TACAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTA
BRCA2-T2722I (Variant)	AAACTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Ile TATAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTA
Reference sequence	AAACTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Thr TACAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTA
BRCA2-T2722T (WT ⁺)	AAACTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Thr TACAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTA
BRCA2-T2722R (Variant)	AAACTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Arg TAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTA
Reference sequence	CTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Asp TACAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTAGCT
BRCA2-D2723D (WT ⁺)	CTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Asp TACAGATGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTAGCT
BRCA2-D2723G (Variant)	CTAGTAGTGCAGATACCCAAAAAGTGGCCATTATTGAAC Gly TGGGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTAGCT
Reference sequence	GATCCTCCCTCTTAGCTGTCTTAAAGAATGGCAGACTGAC Thr GGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTAGCT
BRCA2-G2748G (WT ⁺)	GATCCTCCCTCTTAGCTGTCTTAAAGAATGGCAGACTGAC Thr GGTGGTATGCTGTTAAGGCCCAGTTAGATCCTCCCTCTTAGCT
BRCA2-G2748S (Variant)	GATCCTCCCTCTTAGCTGTCTTAAAGAATGGCAGACTGAC Ser AGTCAGAAGATTATTCTTCATGGAGCAGAAGTGGTGGGCTCTCCTGAT
Reference sequence	CTGAGTATTTGGCGTCCATCATCAGATTTATATTCTCTGTTA Thr AGGAAAGAGATACAGAATTTATCATCTTGCAACTTCAAAATCTAAA
BRCA2-T3001T (WT ⁺)	CTGAGTATTTGGCGTCCATCATCAGATTTATATTCTCTGTTA Thr AGGAAAGAGATACAGAATTTATCATCTTGCAACTTCAAAATCTAAA
BRCA2-E3002G (Variant)	CTGAGTATTTGGCGTCCATCATCAGATTTATATTCTCTGTTA Gly AGGAAAGAGATACAGAATTTATCATCTTGCAACTTCAAAATCTAAA
Reference sequence	CTCCAGTGGCGACCAGAATCCAAATCAGGCCTTCTTACTTTAT Phe GGAGATTTTCTGTGTTTCTGCTAGTCCAAAAGAGGGCCACTTT
BRCA2-A3140A (WT ⁺)	CTCCAGTGGCGACCAGAATCCAAATCAGGCCTTCTTACTTTAT Phe GGAGATTTTCTGTGTTTCTGCTAGTCCAAAAGAGGGCCACTTT
BRCA2-A3140P (Variant)	CTCCAGTGGCGACCAGAATCCAAATCAGGCCTTCTTACTTTAT Pro CTGGAGATTTTCTGTGTTTCTGCTAGTCCAAAAGAGGGCCACTTT
Reference sequence	GGCCAAACATTTTTGAAATTTTTAAGACACGCTGCAACAAAGC Lys AGGTATTGACAAATTTTATATAACTTTATAAAATTACACCGAGA
BRCA2-K21K (WT ⁺)	GGCCAAACATTTTTGAAATTTTTAAGACACGCTGCAACAAAGC Lys AGGCAGGTATTGACAAATTTTATATAACTTTATAAAATTACACCGAGA
BRCA2-67+1G>C (Variant)	GGCCAAACATTTTTGAAATTTTTAAGACACGCTGCAACAAAGC Arg TATTGACAAATTTTATATAACTTTATAAAATTACACCGAGA
Reference sequence	ACCAATCTCCTGTAAAAGAATTAGATAAAATTCAAATTAGACT Asp AGGTAAGTAATGCAATATGGTAGACTGGGGAGAACTACAAACT
BRCA2-N104N (WT ⁺)	ACCAATCTCCTGTAAAAGAATTAGATAAAATTCAAATTAGACT Asp TTAGGTAAGTAATGCAATATGGTAGACTGGGGAGAACTACAAACT
BRCA2-316+1G>T (Variant)	ACCAATCTCCTGTAAAAGAATTAGATAAAATTCAAATTAGACT Arg TAGTAAGTAATGCAATATGGTAGACTGGGGAGAACTACAAACT
Reference sequence	GTTAATAAAAATAAACTTAACAATTTTCCCTTTTTTTACCC Val AGTATGTGGGAGTTTGTTTCATACACCAAAGTTTGTGAAGGTAAAT
BRCA2-V159V (WT ⁺)	GTTAATAAAAATAAACTTAACAATTTTCCCTTTTTTTACCC Val CGTATGTGGGAGTTTGTTTCATACACCAAAGTTTGTGAAGGTAAAT
BRCA2-476-2A>G (Variant)	GTTAATAAAAATAAACTTAACAATTTTCCCTTTTTTTACCC Arg GTGATGTGGGAGTTTGTTTCATACACCAAAGTTTGTGAAGGTAAAT
Reference sequence	TGATCAGGGCATTCTATAAAAAATAAACTATTTTCTTTCTCC Arg AGGTTCGTCAGACACCAAAACATATTTCTGAAAGTCTAGGAGCTGA
BRCA2-R174R (WT ⁺)	TGATCAGGGCATTCTATAAAAAATAAACTATTTTCTTTCTCC Arg AGGTTCGTCAGACACCAAAACATATTTCTGAAAGTCTAGGAGCTGA
BRCA2-517-2A>G (Variant)	TGATCAGGGCATTCTATAAAAAATAAACTATTTTCTTTCTCC Arg GGTTCGTCAGACACCAAAACATATTTCTGAAAGTCTAGGAGCTGA
Reference sequence	GGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCT Cys AGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCAT
KRAS-C12C ⁺ (Variant ⁺)	GGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCT Cys GGCGTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCAT
KRAS-C12G ⁺ (WT ⁺)	GGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCT Gly AGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCAT
KRAS-C12D (Variant)	GGCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGAGCT Asp ATGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCAT

Supplemental Table 2. Sequence of ssODN repair templates (continued).

ssODN name	ssODN sequence
Reference sequence	GGGCCCCGAGGAACTCCCGCAGGTTTCCCTTGGCGGCGCACTCCACGATCACGTACAGGGGCCCTGCAGAGGGAGTGGAGGGAGCGTGGA
FGFR4-V550V (WT')	GGGCCCCGAGGAACTCCCGCAGGTTTCCCTTGGCGGCGCACTCAACGATCACGTACAGGGGCCCTGCAGAGGGAGTGGAGGGAGCGTGGA
FGFR4-V550M (Variant)	GGGCCCCGAGGAACTCCCGCAGGTTTCCCTTGGCGGCGCACTCCATGATCACGTACAGGGGCCCTGCAGAGGGAGTGGAGGGAGCGTGGA
Reference sequence	GGAGCATCTGTACAGCATGAAGTGCAAGAACGTGGTGCCCCCTCTATGACCTGCTGCTGGAGATGCTGGACGCCACCACCGCTACATGCGCC
ESR1-Y537Y (WT')	GGAGCATCTGTACAGCATGAAGTGCAAGAACGTGGTGCCCCCTCTACGACCTGCTGCTGGAGATGCTGGACGCCACCACCGCTACATGCGCC
ESR1-Y537S (Variant)	GGAGCATCTGTACAGCATGAAGTGCAAGAACGTGGTGCCCCCTCTCTGACCTGCTGCTGGAGATGCTGGACGCCACCACCGCTACATGCGCC
Reference sequence	GTTCAATAACTGTGAGGTGGTCCTTGGAATTTGGAAATTACCTATGTCAGAGGAATTATGATCTTTTCCTTCTTAAAGGTTGGTGACTT
EGFR-Y69Y (WT')	GTTCAATAACTGTGAGGTGGTCCTTGGAATTTGGAAATTACCTACGTGCAGAGGAATTATGATCTTTTCCTTCTTAAAGGTTGGTGACTT
EGFR-Y69* (Variant)	GTTCAATAACTGTGAGGTGGTCCTTGGAATTTGGAAATTACCTAGCTGCAGAGGAATTATGATCTTTTCCTTCTTAAAGGTTGGTGACTT
Reference sequence	TCTTTCCAGACACCACCTCAAACACCAACGCCCGGGGAGAGCATGGAAGATGTTCACTCAATGAACCGAAACAGGAGAGCAGTGCTGAT
NFKBIZ-E201E (WT')	TCTTTCCAGACACCACCTCAAACACCAACGCCCGGGGAGAGCATGGAAGATGTTCACTCAATGAACCGAAACAGGAGAGCAGTGCTGAT
NFKBIZ-E201fs (Variant)	TCTTTCCAGACACCACCTCAAACACCAACGCCCGGGGAGAGCATGGAAGAAGATGTTCACTCAATGAACCCAAACAGGAGAGCAGTGCTGAT

The codon affected by mutagenesis is boxed. WT' nucleotide and amino acid substitutions are indicated in blue fond. Variant nucleotide and amino acid substitutions are indicated in red fond. The crRNA target site is underlined in the reference sequence. The PAM is underlined and indicated in purple fond in the reference sequence. The position of the CRISPR-Cas9 elicited DNA double-strand break is indicated by a vertical

Supplemental Table 3. Sequence of PCR primers.

Primers used in first round PCR for amplicon sequencing

Primer name	Primer sequence
<i>PIK3CA</i> -H1047R-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAGGCTTTGGAGTATTTTCATGA
<i>PIK3CA</i> -H1047R-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTGTCTTTGCCTGCTGAGAGTT
<i>PTEN</i> - L182*-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGACCCAGTTACCATAGCA
<i>PTEN</i> - L182*-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTAGTGCCACTGGTCTATAATCCA
<i>BRCA2</i> -N289H-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACTTTAACAGGATTTGGAAAAACA
<i>BRCA2</i> -N289H-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTAGATTTTTCACATTCATCAGCGT
<i>BRCA2</i> -K513R-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGAGAGATGAAGAGCAGCAT
<i>BRCA2</i> -K513R-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTGAGTCCTCTTCTGTGAGCAA
<i>BRCA2</i> -C554W-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCAGAATAAGAGAATCACCTAAAGA
<i>BRCA2</i> -C554W-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTAGTGGATATTAAACCTGCATTCT
<i>BRCA2</i> -D946V-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTAACAAGCAACCCAAGTGTCA
<i>BRCA2</i> -D946V-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTAGAGTCCTGCCCCATTTGTTC
<i>BRCA2</i> -T2403A-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGACTTTGGAAAAATCTTCAAGC
<i>BRCA2</i> -T2403A-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTCTGTTCAACTCTGTGAAAATGTGA
<i>BRCA2</i> -L2510R-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACAAGTCTTCAGAATGCCAGAGA
<i>BRCA2</i> -L2510R-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTACACTCTGTCATAAAAAGCCATCA
<i>BRCA2</i> -I2627F-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGAATTCAGTATCATCCTATGTGGT
<i>BRCA2</i> -I2627F-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTCAGAAACCTTAACCATACTGCCG
<i>BRCA2</i> -I2627N-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTGTGGATCCAAAGCTTATTTTC
<i>BRCA2</i> -I2627N-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTTGTAAATGCTTTAAACTTGCCGTGT
<i>BRCA2</i> -F2638L-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGTGTGGATCCAAAGCTTATTTTC
<i>BRCA2</i> -F2638L-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTTGTAAATGCTTTAAACTTGCCGTGT
<i>BRCA2</i> -Y2658H-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTGGCAGCTATGGAATGTGC
<i>BRCA2</i> -Y2658H-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTAGACTACACAGAAACCTTAACCA
<i>BRCA2</i> -Y2660C-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAATTCTAGAGTCACACTTCCT
<i>BRCA2</i> -Y2660C-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTTTTGCAGCTGTGTCATCCC
<i>BRCA2</i> -I2675M-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAATTCTAGAGTCACACTTCCT
<i>BRCA2</i> -I2675M-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTTTTGCAGCTGTGTCATCCC
<i>BRCA2</i> -T2722I-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGACATAATTTTATTGAGCGCA
<i>BRCA2</i> -T2722I-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTCCACCAGTTCTGTCTCCATGA
<i>BRCA2</i> -T2722R-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGACATAATTTTATTGAGCGCA
<i>BRCA2</i> -T2722R-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTCCACCAGTTCTGTCTCCATGA
<i>BRCA2</i> -D2723G-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGACACAGCTGCAAAAACAC
<i>BRCA2</i> -D2723G-R	TGACTGGAGTTTACAGCGTGTGCTCTTCCGATCTGCTTCAAGAGGTGTACAGGCA
<i>BRCA2</i> -G2748S-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGGTATGCTGTTAAGGCCCA

<i>BRCA2</i> -G2748S-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTTGACTGATTTTACCAAGAGTGC
<i>BRCA2</i> -E3002G-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTTGCACTTTTCTCATCTTTCTCCA
<i>BRCA2</i> -E3002G-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTTTGTGCTGCTAACTGTATGT
<i>BRCA2</i> -A3140P-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTGGACTTGCCCCCTTCGTCTAT
<i>BRCA2</i> -A3140P-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTTGTCTCTTGAAAGTGGCCCTC
<i>BRCA2</i> -67+1G>C-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTTGGATCCAAAGAGAGGCCAAC
<i>BRCA2</i> -67+1G>C-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTTGTGGTTAACCTGCAAACGA
<i>BRCA2</i> -316+1G>T-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTTCTGCCGCTGTACCAATCTC
<i>BRCA2</i> -316+1G>T-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTAGAGACTGATTTGCCAGCA
<i>BRCA2</i> -476-2A>G-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTACCTAAGGGATTGCTTTGTTTTA
<i>BRCA2</i> -476-2A>G-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTTTGGTGTATGAAACAACTCCAC
<i>BRCA2</i> -517-2A>G-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTTCTTAATGATCAGGGCATTTC
<i>BRCA2</i> -517-2A>G-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTACCTCATCTGCTCTTTCTTGT
<i>KRAS</i> -C12-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTGGTACTGGTGGAGTATTTGATAGTG
<i>KRAS</i> -C12-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTACCTCTATTGTTGGATCATATTCTGT
<i>FGFR4</i> -V550M-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTCTCTCCACGCTCCCTCCA
<i>FGFR4</i> -V550M-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTACTCCAGATACTGCATGCCT
<i>ESR1</i> -Y537S-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTTTCCCTTCTAGGGATTTCAGC
<i>ESR1</i> -Y537S-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTGTGGGCGTCCAGCATCTC
<i>EGFR</i> -Y69*-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTTCAATAACTGTGAGGTGGTCC
<i>EGFR</i> -Y69*-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTTTCAAGTGAATTCTGCCCA
<i>NFKBIZ</i> -E201fs-F	<i>ACACTCTTTCCCT</i> TACACGACGCTCTTCCGATCTTTTCTTCTTCCAGACACCAC
<i>NFKBIZ</i> -E201fs-R	<i>TGACTGGAGTT</i> CAGACGCTGTGCTCTTCCGATCTGTTTCAAGGAAACGGGGCTG

Other primers

Primer name	Primer sequence
<i>BRCA2</i> -T2722R-SingleCell-F	TGATACGGAAATTGATAGAAGCAGA
<i>BRCA2</i> -T2722R-SingleCell-R	GGGCTTCAAGAGGTGTACAGG
<i>BRCA2</i> -T2722R-SingleCell-seq	ATTGATAGAAGCAGAAGATCGGC
<i>BRCA2</i> -1F	GTGTTTTACAGCTGCTGGGC
<i>BRCA2</i> -1R	CATTAAATTGTCACTTTTGAGGGGA
<i>BRCA2</i> -2F	ATGGCAGGTTGTTACGAGGC
<i>BRCA2</i> -2R	TGAGCTGGTCTGAATGTTCTGT
<i>BRCA2</i> -3F	TGGCCAAAAGGAAGTCTGTT
<i>BRCA2</i> -3R	GTACTGGCCTGGGAACTCTC

All primers are *H. sapiens* specific. F, forward primer. R, reverse primer. The common overhangs in NGS primers are indicated by italics.

Supplemental Note. Estimation of minimum number of sequencing reads needed

a. Formula for estimation of minimum read number

We derived below formula to estimate the minimum number of amplicon NGS reads needed for a desired P value with a given number of experimental replicates, for a given effect size and for given knockin frequencies in a CRISPR-Select analysis (see **c**, for derivation of the formula). It should be noted that the formula serves as a guideline only, as the model incorporates certain assumptions and approximations that may not be valid for all experiments.

$$N > \left(\frac{T_s(2n - 2) \sqrt{\frac{2 + es^2 - 2es}{n}}}{es} \right)^2 \left(\frac{(1 - p_1)}{p_1} + \frac{(1 - p_2)}{p_2} \right)$$

, where

N : Read number

- Total amplicon NGS reads for the given CRISPR-Select target site in the sample given as a number, e.g., 22949

n : Experimental replicates

- Experimental replicates given as a number, e.g., 3

$T_s(2n - 2)$:

- To find the critical T value, consult a t -distribution table (two-tailed; e.g. <https://www.medcalc.org/manual/t-distribution-table.php>) according to desired P value (S) and degrees of freedom, calculated by the $(2n-2)$ formula

n : Experimental replicates

- Experimental replicates given as a number, e.g., 3

es : Effect size

- The variant:WT' ratio change given as a fraction, e.g., 0.80, meaning an 80% loss of variant alleles

p_1 : Knockin frequency of variant-of-interest

- Given as a fraction, e.g., 0.06

p_2 : Knockin frequency of WT'.

- Given as a fraction, e.g., 0.07

b. Two examples of estimation of minimum read number

In below example one, a P value of 0.05 was desired for a CRISPR-Select experiment performed 3 times, where variant and WT' were knocked in at 2% and 3% frequencies,

respectively and the effect size of variant was 0.2, meaning a 20% loss of variant alleles. Degrees of freedom is $(2*3-2=)$ 4 and with desired P value (S) of 0.05, T is 2.776 according to t-distribution table (<https://www.medcalc.org/manual/t-distribution-table.php>). Thereby, the minimum number of reads needed are:

$$N > \left(\frac{2.776 \sqrt{\frac{2 + 0.2^2 - 2 * 0.2}{3}}}{0.2} \right)^2 \left(\frac{(1 - 0.02)}{0.02} + \frac{(1 - 0.03)}{0.03} \right) \approx 8566$$

In below example two, a P value of 0.05 was desired for a CRISPR-Select experiment performed 3 times, where variant and WT' were knocked in at 2% and 3% frequencies, respectively and the effect size of variant was 0.8, meaning an 80% loss of variant alleles. Degrees of freedom is $(2*3-2=)$ 4 and with desired P value (S) of 0.05, T is 2.776 according to t-distribution table (<https://www.medcalc.org/manual/t-distribution-table.php>). Thereby, the minimum number of reads needed are:

$$N > \left(\frac{2.776 \sqrt{\frac{2 + 0.8^2 - 2 * 0.8}{3}}}{0.8} \right)^2 \left(\frac{(1 - 0.02)}{0.02} + \frac{(1 - 0.03)}{0.03} \right) \approx 339$$

C. Derivation of formula for estimation of minimum read number

The formula was derived as follows: We used the ratio of variant reads to WT' reads to test, whether the difference in ratios of two conditions (e.g., two time points) is significant. The variant: WT' ratio of the i -th sample in one condition is x_i and the variant:WT ratio of the i -th sample in another condition is y_i . Ratios are given as fractions, e.g., 0.8. To test whether the means of x and y are different in n experimental replicates, we used equal variance t-test as follows:

$$t = \frac{\bar{x} - \bar{y}}{s_p \cdot \sqrt{\frac{2}{n}}} \quad (1)$$

, where

$$s_p = \sqrt{\frac{\sigma_x^2 + \sigma_y^2}{2}} \quad (2)$$

Effect size (es) is incorporated as follows:

$$\bar{y} = (1 - es)\bar{x} \quad (3)$$

Because x and y are paired, we assume that x and y have the same relative variance (α):

$$\alpha = \frac{\sigma_x}{\bar{x}} = \frac{\sigma_y}{\bar{y}} \quad (4)$$

Then,

$$\sigma_x = \bar{x} \cdot \alpha \quad (5)$$

$$\sigma_y = \bar{y} \cdot \alpha \quad (6)$$

$$t = \frac{es}{\alpha \sqrt{\frac{2+es^2-2es}{n}}} \quad (7)$$

The desired P value (S) for significance as well as number of experimental replicates (n) are incorporated as follows:

$$|t| > T_S(2n - 2) \quad (8)$$

, which equals:

$$\left| \frac{es}{\alpha \sqrt{\frac{2+es^2-2es}{n}}} \right| > T_S(2n - 2) \quad (9)$$

$$\alpha < \frac{|es|}{T_S(2n-2) \sqrt{\frac{2+es^2-2es}{n}}} \quad (10)$$

Among the total N reads of each sample, the number of m variant reads follows binomial distribution: $m \sim B(N, p_1)$, where p_1 is frequency of variant knockin given as a fraction, e.g., 0.02. The number of w WT' reads also follows binomial distribution: $w \sim B(N, p_2)$, where p_2 is frequency of WT' knockin given as a fraction, e.g., 0.02.

$$x = \frac{m}{w} \quad (11)$$

$$\sigma_m^2 = Np_1(1 - p_1) \quad (12)$$

$$\sigma_w^2 = Np_2(1 - p_2) \quad (13)$$

The relative variance α^2 of x is:

$$\sigma_x^2 = \sigma_{\frac{m}{w}}^2 = \sigma_m^2 \left(\frac{\partial x}{\partial m} \right)_{\bar{m}}^2 + \sigma_w^2 \left(\frac{\partial x}{\partial w} \right)_{\bar{w}}^2 = \frac{\sigma_m^2}{\bar{w}^2} + \frac{\sigma_w^2 \bar{m}^2}{\bar{w}^4} \quad (14)$$

$$\alpha^2 = \left(\frac{\sigma_x}{\bar{x}} \right)^2 = \left(\frac{\sigma_m}{\bar{m}} \right)^2 = \left(\frac{\sigma_m}{\bar{m}} \right)^2 + \left(\frac{\sigma_w}{\bar{w}} \right)^2 \quad (15)$$

When $N \rightarrow +\infty$; $\bar{m} \rightarrow Np_1$, $\bar{w} \rightarrow Np_2$, the Eq. 15 can be written as:

$$\alpha^2 \rightarrow \frac{(1-p_1)}{Np_1} + \frac{(1-p_2)}{Np_2}, (N \rightarrow +\infty) \quad (16)$$

The Eq. 10 can be written as:

$$\frac{(1-p_1)}{Np_1} + \frac{(1-p_2)}{Np_2} < \left(\frac{es}{T_S(2n-2) \sqrt{\frac{2+es^2-2es}{n}}} \right)^2, (N \rightarrow +\infty) \quad (17)$$

$$N > \left(\frac{T_S(2n-2) \sqrt{\frac{2+es^2-2es}{n}}}{es} \right)^2 \left(\frac{(1-p_1)}{p_1} + \frac{(1-p_2)}{p_2} \right), (N \rightarrow +\infty) \quad (18)$$