

## Next-generation sequencing-based multigene mutational screening for acute myeloid leukemia using MiSeq: applicability for diagnostics and disease monitoring

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Manuscript received on June 24, 2013. Manuscript accepted on October 16, 2013.

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## Supplementary Methods

*Genes interrogated by TruSeq Cancer Amplicon Panel: AKT1, BRAF, FGFR1, GNAS, IDH1, FGFR2, KRAS, NRAS, PIK3CA, MET, RET, EGFR, JAK2, MPL, PDGFRA, PTEN, TP53, FGFR3, FLT3, KIT, ERBB2, ABL1, HNF1A, HRAS, ATM, RB1, CDH1, SMAD4, STK11, ALK, SRC, SMARCB1, VHL, MLH1, CTNNB1, KDR, FBXW7, APC, CSF1R, NPM1, SMO, ERBB4, CDKN2A, NOTCH1, JAK3, PTPN11, GNAQ and GNA11.*

*Library preparation for sequencing on MiSeq:* The genomic library was prepared using 250 ng of DNA template and customized Truseq Amplicon Cancer Panel (TSACP) kit (illumina) according to the manufacturer's protocol. Briefly, library preparation involved hybridization of the probe mixture to genomic DNA and areas of interest were captured by extension and ligation. In addition to areas complimentary to genomic DNA, each of the probes has a common sequence, which is used to PCR-amplify the captured sequences in a subsequent step. This PCR step also adds sequencing adapters (for binding of sequencing primers) and short stretches of identifier sequences or barcodes on the 3' and 5' ends of the amplicons to be used as unique sample identifiers, thus facilitating multiplexed sequencing of the samples.

*Sequencing using MiSeq.* Library generated using customized TSCAP was purified using AMPure magnetic beads (Agentcourt, Brea, CA) according to the manufacturer's protocol. From each library, equal quantities of the DNA were isolated and eluted using Library normalization beads (TSCAP kit) following the manufacturer's instructions and equal volumes were mixed. This ensures same representation of the library from each sample during multiplexed sequencing. Paired end sequencing of samples was performed using MiSeq Reagent Kit, V1 (300 cycles) using MiSeq sequencer (illumina).

On average, libraries from 10 samples per sequencing run were multiplexed. Base calling and the sequencing run summary were obtained using Real Time Analysis Software (V1.14.23) (illumina). Sequencing quality was evident by the Q30 scores (one error in 1000 bp sequence) and a cutoff of 85% sequence with Q30 score was used as an indication of successful sequencing.

*Sensitivity, inter-run and intra-run reproducibility.* Sensitivity was determined by serially diluting DNA from H2122 (ATCC CRL-5985), a human lung carcinoma cell line with a known homozygous mutation in *KRAS* and a heterozygous mutation in *MET* into DNA from HL60 cell line, a human promyelocytic leukemia cell line. Briefly, DNA isolated from H2122 was diluted into DNA from HL60, in ratios of 1:4, 1:9, 1:19 (H2122:HL60) resulting in 20%, 10% and 5% dilutions, respectively which were sequenced. Similarly, DLD1 cell line (ATCC CCL-221) DNA with 8 heterozygous (monoallelic) mutations was serially diluted into normal DNA control (Promega, Madison, WI) in ratios of 1:1, 1:3, 1:9 and 1: 19 (DLD1: normal) resulting in 50%, 25%, 10% and 5% dilutions of DLD1 DNA and sequenced in 6 independent runs. Intra-run reproducibility was tested by sequencing 1:9 diluted (10%) (DLD1: normal DNA) sample barcoded by 20 different barcodes on a single sequencing run and inter-run assay reproducibility was assessed by sequencing 10% DLD1 DNA diluted into normal DNA sequenced in 12 different runs.

*Library preparation for Ion Torrent Personal Genome Machine (IT-PGM).* Library preparation was performed using the Ion Torrent (IT) Ampliseq Kit 2.0 Beta (Life Technologies) and IT Ampliseq cancer panel primers (Life Technologies) to interrogate mutational hotspots in 46 cancer-related genes following the manufacturer's instructions as described previously.<sup>34</sup> Sequencing adaptors with short stretches of index sequences

(barcodes) that enable sample multiplexing were ligated to the amplicons using the Ion Express Barcode Adaptors Kit (Life Technologies). The library prepared was quantified using the Bioanalyzer highsensitivity DNA chip (Agilent Technologies Inc, Santa Clara, CA).

*Emulsion PCR and sequencing using IT-PGM.* Clonal amplification of the barcoded library DNA onto the ionspheres (ISPs) by emulsion PCR (E-PCR) and subsequent isolation of ISPs with DNA was performed using Ion Express Template Kit (Life Technologies) and IT OneTouch ES (Life Technologies) as described previously.<sup>34</sup> Enriched ISPs were subjected to sequencing on a 318 IonChip (8 samples) using the sequencing kit (Life Technologies) as per the manufacturer's instructions. A cutoff of 300,000 reads with a quality score of AQ20 (1 misaligned base per 100 bases) was used as a measure of successful sequencing of a sample. Torrent Suite V.2.0.1 and variant caller V.1.0 (Life Technologies) were used for alignment and variant calling, respectively using Human genome build 19 (hg19) as reference.

**Supplementary Table 1. Summary of mutations detected in the sample set by MiSeq and IT-PGM.** Tumor type used, the correlation of results from sequencing on MiSeq using Truseq Amplicon Cancer panel and on IT-PGM using Ampliseq Cancer Panel have been summarized (NC - Not Covered)

|   |                             | <i>MiSeq</i>  |                                      | <i>Ion-Torrent PGM</i>   |                                   |
|---|-----------------------------|---|--------------------------------------|--|-----------------------------------|
| Sample No<br>WHO diagnosis<br>(Blast Count) | Cytogenetic<br>profile      | Mutation Detected by<br>Miseq (Coverage)  | Variant<br>Frequency<br>(%)          | Mutation detected<br>by IT-PGM<br>(Coverage)   | Variant<br>Frequency<br>(%)       |
| 1<br>AMML (38%)                             | 46,XX[20]                   | <i>KDR p.Q472H (6193)</i><br><i>KRAS p.G12A (5745)</i><br><i>NRAS p. Q61R (765)</i>   | 49.4<br>18.3<br>14.6                 | Yes (3849)<br>Yes (1271)<br>Yes (3392)   | 54.0<br>49.8<br>19.6              |
| 2<br>AML-MRC (33%)                          | 46,XY[20]                   | <i>KDR p.Q472H (3035)</i><br><i>NPM1, Exon 11,</i><br><i>4bp Insertion (3231)</i>   | 47.4<br>29.4                         | Yes (2268)<br>Yes (2196)   | 47.0<br>33.8                      |
| 3<br>AMML (67%)                             | 46,XY,del(12)<br>(p11.2)[2] | <i>FLT3 p.D835H (1826)</i><br><i>KDR p.Q472H (3927)</i><br><i>MET p.T1010I (4415)</i><br><i>NPM1, Exon 11,</i><br><i>4bp Insertion (1190)</i><br><i>APC p.L1129S (3605)</i> | 24.7<br>49.1<br>51.6<br>34.2<br>46.0 | Yes (2584)<br>Yes (4660)<br>Yes (5780)<br>Yes (4448)<br>NC on IT-PGM<br>(Sanger Confirmed) | 48.4<br>47.0<br>49.7<br>31.8<br>- |
| 4<br>AMML (81%)                             | 46,XX[20]                   | <i>KDR p.Q472H (3034)</i><br><i>KIT p.M541L (6536)</i><br><i>NPM1, Exon 11,</i><br><i>4bp Insertion (937)</i><br><i>ABL1 p.L248R (275)</i>                                  | 49.1<br>50.1<br>35.1<br>5.09         | Yes (4083)<br>Yes (1608)<br>Yes (3018)<br>NC on IT-PGM<br>(Sanger Confirmed)               | 46.0<br>50.1<br>39.0<br>-         |
| 5<br>AML without<br>maturation (84%)        | 46,XX[20]                   | <i>IDH1 p.R132C (2801)</i><br><i>NPM1, Exon 11,</i><br><i>4bp Insertion (937)</i>   | 47.23<br>31.8                        | Yes (4252)<br>Yes (3108)   | 37.6<br>32.8                      |
| 6<br>t-AML (73%)                            | 46,XX[20]                   | <i>KIT p.M541L (5736)</i><br><i>NPM1, Exon 11,</i><br><i>4bp Insertion (937)</i>  | 49.9<br>31.8                         | Yes (1608)<br>Yes (2090)   | 50.1<br>25.3                      |
| 7<br>AML without<br>maturation (79%)        | 46,XX[20]                   | <i>ATM, p.V410A (5907)</i><br><i>KDR p.Q472H (4928)</i><br><i>MET p.N375S (1449)</i><br><i>NPM1, Exon 11,</i><br><i>4bp Insertion (1449)</i>                                | 50.1<br>50.2<br>52.8<br>39.1         | Yes (4648)<br>Yes (2832)<br>Yes (2335)<br>Yes (3166)                                       | 52<br>48.0<br>60.8<br>34.8        |
| 8<br>AML without<br>maturation (91%)        | 47,XX,+15[1]                | <i>NPM1, Exon 11,</i><br><i>4bp Insertion (1617)</i><br><i>KDR p.Q472H (4693)</i>   | 35.0<br>50.8                         | Yes(302)<br>Yes (452)  | 35.8<br>49.0                      |
| 9<br>Acute erythroid<br>leukemia (20%)      | 46,XX[20]                   | <i>NPM1, Exon 11,</i><br><i>4bp Insertion (981)</i><br><i>APC p.M1413V (2816)</i>   | 39.1<br>51.1                         | Yes (2502)<br>NC on IT-PGM<br>(Sanger Confirmed)   | 32.1<br>-                         |
| 10<br>AML-MRC (30%)                         | 47,XX,+8[10]                | <i>MET p.N375S (1449)</i><br><i>NRAS p.G12R (3376)</i>  | 56.3<br>37.5                         | Yes (2335)<br>Yes (4108)   | 60.8<br>21.2                      |

|                                    |                                  |  |                              |  |                              |
|------------------------------------|----------------------------------|--|------------------------------|--|------------------------------|
| 11<br>AML without maturation (93%) | 46,XY[20]                        | ATM p.A1039T (2868)<br>KDR p.Q472H (4856)  | 33.5<br>50.8                 | Yes (2346)<br>Yes (3065)                             | 62.4<br>46.0                 |
| 12<br>AMML (38%)                   | 46,XY[20]                        | KDR p.Q472H (4632)<br>NPM1, Exon 11,<br>4bp Insertion (1929)<br>NRAS p.G13D (2685)                         | 51.0<br>35.6<br>20.1         | Yes (2581)<br>Yes (2059)<br>Yes (3911)               | 48.0<br>28.8<br>27.4         |
| 13<br>AML (56%)                    | 46,XY[20]                        | IDH1 p. R132H (2607)<br>KDR p.Q472H (4700)<br>KIT p.M541L (9548)<br>NPM1, Exon 11,<br>4bp Insertion (2488) | 25.7<br>99.7<br>48.4<br>19.5 | Yes (4934)<br>Yes (3426)<br>Yes (1765)<br>Yes (3633) | 46.4<br>99.0<br>50.7<br>18.1 |
| 14<br>Persistent AML (82%)         | Complex                          | KRAS p.G12D (3984)   | 51.4                         | Yes (1271)   | 49.8                         |
| 15<br>AMML (24%)                   | 46,XX [20]                       | KRAS p.G12R (3574)   | 45.9                         | Yes (973)  | 42.5                         |
| 16<br>AML-MRC (56%)                | 46,XY,del(16)<br>(q13q21)[16]    | IDH1 p.R132H (3009)<br>KIT p.M541L (9081)<br>NPM1, Exon 11,<br>4bp Insertion (461)<br>KDR p.Q472H (4530)   | 44.7<br>49.0<br>31.8<br>58.2 | Yes (4055)<br>Yes (1097)<br>Yes (2092)<br>Yes (3496) | 51.3<br>47.3<br>31.6<br>52.0 |
| 17<br>AML (1%)                     | 46,XX[20]                        | KDR p.Q472H (3937)<br>ABL1 p. L248R (458)  | 47.8<br>6.11                 | Yes (2448)<br>NC on IT-PGM<br>(Sanger Confirmed)     | 50.0<br>-                    |
| 18<br>AML (0%)                     | 46,XY,t(11;21)<br>(p11.2;q22)[1] | MET p.N375S (1735)   | 37.9                         | Yes (2335)   | 60.8                         |
| 19<br>(AML) (3%)                   | 46,XX[20]                        | IDH1 p. R132L (3012)   | 22.9                         | Yes (4934)   | 46.4                         |
| 20<br>AML without maturation (80%) | 46,XY,del(20)<br>(q11.2q13.3)[5] | JAK2 p.V617F (950)   | 50.2                         | Yes (4701)   | 49.2                         |
| 21<br>MDS, RAEB-2 (10%)            | 46,XX[20]                        | KDR p.Q472H (3459)<br>NPM1, Exon 11,<br>4bp Insertion (1260)   | 48.1<br>30.2                 | Yes (1121)<br>Yes (1453)                             | 52.0<br>23.9                 |
| 22<br>MDS (1%)                     | 47, XY, +8[10]                   | APC p.R876Q (2791)   | 52.9                         | Yes (3345)   | 46.1                         |
| 23<br>AML-MRC (40%)                | 46,XX,del(5)(q13q33)[20]         | KRAS p.Q61H (2494)   | 27.5                         | Yes (2985)   | 37.02                        |
| 24<br>AML-MRC with CLL (53%)       | 46,XY[20]                        | NPM1, Exon 11,<br>4bp Insertion (297)  | 32.3                         | Yes (3971)   | 29.3                         |
| 25<br>AML-MRC (43%)                | 46,XY[18]                        | KDR p.Q472H (4794)   | 48.7                         | Yes (1146)   | 61.0                         |
| 26<br>AML-MRC (61%)                | 46,XX[20]                        | FLT3 p.D835Y (2939)<br>NPM1, Exon 11,<br>4bp Insertion (982)   | 49.1<br>36.4                 | Yes (2584)<br>Yes (1293)                             | 48.4<br>36.1                 |
| 27<br>AMML (61%)                   | 46,XX[20]                        | FLT3 p.D835A (592)<br>KIT p.M541L (3109)<br>NRAS p.G13D (911)  | 24.6<br>50.3<br>36.0         | Yes (4476)<br>Yes (1608)<br>Yes (3911)               | 12.5<br>50.1<br>27.4         |
| 28<br>AML-MRC (42%)                | 46,XY[20]                        | ATM p.P604S (1225)   | 55.1                         | Yes (555)  | 30.0                         |

|  |   |   |                              |  |                              |
|--|---|---|------------------------------|--|------------------------------|
|  |   | <i>NPM1, Exon 11,<br/>4bp Insertion (1807)<br/>PTPN11 p.A72D</i>  | 32.6<br>35.1                 | Yes (961)<br>Yes (353)   | 22.0<br>79.6                 |
| 29<br>(Acute<br>monocytic<br>Leukemia) (74%) | 53,XX,+X,<br>der(1)t(1;1)<br>(p36.3;q12),+2,<br>+4,+5,+8,+12,+<br>16[4] | <i>ABL1 p.R307Q (2556)<br/>KRAS p.Q61H (1197)<br/>NPM1, Exon 11,<br/>4bp Insertion (1553)</i>                         | 44.6<br>37.9<br>20.2         | Yes (703)<br>Yes (2985)<br>Yes (2594)                          | 61.4<br>37.0<br>21.7         |
| 30<br>AML-MRC (26%)                          | 45,X,-Y[20]   | <i>NPM1, Exon 11,<br/>4bp Insertion (2129)<br/>KDR p.Q472H (4150)<br/>KIT p.M541L (8411)<br/>MPL p.W515R (331)</i>    | 32.0<br>48.7<br>99.5<br>32.3 | Yes (1735)<br>Yes (1134)<br>Yes (4188)<br>Yes (498)            | 24.7<br>58.0<br>99.9<br>41.3 |
| 31<br>t-AML (81%)                            | 46,XX[20]   | <i>NPM1, Exon 11,<br/>4bp Insertion (490)<br/>APC p.I1307K<br/>IDH2 p.R140Q (1047)</i>                                | 36.5<br>48.8<br>47.6         | Yes (3189)<br>Yes (657)<br>NC on IT-PGM<br>(Sanger Confirmed)  | 34.7<br>43.6<br>-            |
| 32<br>AML-MRC (88%)                          | 46,XY,inv(9)<br>(p12q13)[20]  | <i>KDR p.Q472H (4348)<br/>KIT p.M541L (6982)<br/>IDH2 p.R140Q (542)</i>   | 48.6<br>49.5<br>26.5         | Yes (3193)<br>Yes (933)<br>NC on IT-PGM<br>(Sanger Confirmed)  | 52.0<br>49.8<br>-            |
| 33<br>AML with<br>Inv16 (42%)                | 46,XX,inv(16)(<br>p13.1q22)[16]   | <i>KDR p.Q472H (4683)<br/>RET p.S649L (8513)<br/>TP53 p. R148T (781)</i>  | 47.7<br>47.6<br>92.5         | Yes (2564)<br>Yes (3713)<br>NC on IT-PGM<br>(Sanger Confirmed) | 56.0<br>40.4<br>-            |
| 34<br>AML with<br>Inv16 (53%)                | 46,XX,inv(16)<br>(p13.1q22)[19]   | <i>KDR p.Q472H (2304)<br/>KRAS p.G12D (1727)<br/>NRAS p.Q61R (325)</i>  | 52.7<br>7.7<br>19.0          | Yes (2858)<br>Yes (1271)<br>Yes (3392)                         | 52.0<br>49.8<br>19.6         |
| 35<br>AML-MRC (37%)                          | 46,XX,del(5)(q<br>13q33)[4]   | <i>TP53 p.V274F (795)<br/>TP53 p. H179R (1059)<br/>APC p.S1404F (874)</i>   | 39.1<br>24.8<br>5.15         | Yes (503)<br>Yes (1558)<br>NC on IT-PGM<br>(Sanger Confirmed)  | 26.8<br>41.7<br>-            |
| 36<br>AMML (65%)                             | 46,XY,del(7)(q<br>22),-10,-18[1]  | <i>KDR p.Q472H (4840)<br/>FLT3 p.D835Y (2365)<br/>IDH1 p.R132H (2975)<br/>NPM1, Exon 11,<br/>4bp Insertion (1164)</i> | 47.4<br>9.5<br>39.8<br>32.8  | Yes (3474)<br>Yes (3633)<br>Yes (1443)<br>Yes (2249)           | 56.0<br>18.1<br>29.1<br>26.5 |
| 37<br>AML (81%)                              | 46,XY,t(4;17)(q<br>13;p13)[11]  | <i>FLT3 p.Y842H (1968)<br/>NRAS p. G12C (1938)<br/>NRAS p.G13D (1939)</i>   | 15.7<br>21.5<br>18.0         | Yes (3233)<br>Yes (4108)<br>Yes (3911)                         | 9.3<br>21.2<br>27.4          |
| 38<br>AML (80%)                              | 47,XY,+Y[6]   | <i>ATM p.V410A (6016)<br/>IDH1 p.R132H (2960)<br/>KDR p.Q472H (6029)<br/>NPM1, Exon 11,<br/>4bp Insertion (2214)</i>  | 51.0<br>43.8<br>50.2<br>34.7 | Yes (4648)<br>Yes (4934)<br>Yes (4030)<br>Yes (3961)           | 46.4<br>46.4<br>46.0<br>34.4 |
| 39<br>AML (45%)                              | Complex   | <i>NPM1, Exon 11,<br/>4bp Insertion (1342)<br/>KIT p.D816V (3832)</i>   | 33.01<br>77.6                | Yes (2975)<br>Yes (4331)                                       | 31.6<br>77.8                 |

|   |            |   |                      |  |                      |
|---|------------|---|----------------------|--|----------------------|
| 40<br>Acute monocytic<br>Leukemia (43%) | 46,XX [20] | <i>FLT3 p.D835V (1947)</i>  | 21.1                 | Yes (4476)                             | 12.1                 |
|   |            | <i>KDR p.Q472H (4861)</i><br><i>NPM1, Exon 11,<br/>4bp Insertion (3560)</i><br><i>PTPN11 p. E76K (7409)</i> | 46.3<br>32.1<br>24.4 | Yes (1899)<br>Yes (1443)<br>Yes (3270) | 47.0<br>12.1<br>17.1 |
| 41<br>AML (32%)                         | NA         | <i>IDH1 p.R132C (3520)</i><br><i>KIT p.M541L (12238)</i>  | 41.0<br>50.7         | Yes (4934)<br>Yes (1483)               | 46.4<br>48.6         |
| 42<br>AML-MRC (7%)                      | 46,XY[20]  | <i>KIT p.M541L (22765)</i><br><i>KRAS p.G60V (3931)</i>   | 99.8<br>45.6         | Yes (991)<br>Yes (8063)                | 99.8<br>38.5         |
| 43<br>MDS (15%)                         | 46,XY[19]  | <i>KIT p.M541L (16620)</i><br><i>NRAS p.G13R (531)</i><br><i>NRAS p.G12A(531)</i>                           | 49.4<br>23.5<br>21.2 | Yes (1608)<br>Yes (2804)<br>Yes (2827) | 50.1<br>23.6<br>23.6 |
| 44<br>AMML (61%)                        | 46,XX,[22] | <i>KDR p.Q472H (4218)</i><br><i>NPM1, Exon 11,<br/>4bp Insertion (1052)</i>                                 | 99.3<br>37.8         | Yes (1474)<br>Yes (1576)               | 100<br>22.8          |



**Supplementary Table 2. Sequencing of samples in parallel using MiSeq and conventional sequencing platforms.** A set of 11 samples were sequenced in parallel using MiSeq and other sequencing platforms used in the laboratory and compared.

| Diagnosis<br>Blast (%) | MiSeq   |               | Detection on<br>Parallel Platforms                 |
|------------------------|---|---------------|--|
|                        | Mutation detected<br>(Coverage)                             | Frequency (%) |  |
| 1<br>AML (81%)         | <i>DNMT3A</i> p. R882H (822)                                | 47.8          | Yes (Sanger)                                       |
| 2<br>AML (10%)         | <i>IDH1</i> p.R132C (4581)<br><i>JAK2</i> p. V617F (7283)   | 33.7<br>96.8  | Yes (Sanger)<br>Yes (Pyro)                         |
| 3<br>AML (1%)          | <i>DNMT3A</i><br>p. R882H (2188)                            | 25.8          | Yes (Sanger)                                       |
| 4<br>AML-MRC (49%)     | <i>JAK2</i> p. V617F (5202)                                 | 25.9          | Yes (Pyro)   |
| 5<br>AML-MRC (67%)     | <i>IDH1</i> p.R132C (3223)                                  | 34.3          | Yes (Sanger)                                       |
| 6<br>AML-MRC (50%)     | <i>NRAS</i> p.G12D (4905)                                   | 13.9          | Yes (Pyro)   |
| 7<br>t-AML (90%)       | <i>IDH1</i> p.R132L (4018)                                  | 40.9          | Yes (Sanger)                                       |
| 8<br>AMML (76%)        | <i>NPM1</i> (4bp Ins) (800)<br><i>DNMT3A</i> p. R882H (466) | 30.2<br>42.2  | Yes (Capillary<br>Electrophoresis)<br>Yes (Sanger) |
| 9<br>t-AML (35%)       | <i>IDH2</i> p.R140Q (1816)                                  | 31.5          | Yes (Sanger)                                       |
| 10<br>AML-MRC (90%)    | <i>NRAS</i> p.Q61K (1167)                                   | 31.1          | Yes (Pyro)   |
| 11<br>AML (30%)        | <i>IDH2</i> p.R140W (4522)<br><i>JAK2</i> p. V617F (1833)   | 31.2<br>23.6  | Yes (Sanger)<br>Yes (Pyro)                         |

**Supplementary Table 3. Detection of *FLT3* ITDs by MiSeq Reporter and Pindel.** 10 archival samples with known internal tandem duplications (ITDs) in exon 11 of *FLT3* were sequenced using MiSeq and the aligned sequencing data was reanalyzed via Pindel, a program designed to detect indels. (NC – Not called by MiSeq Reporter)

| <b><i>FLT3</i> ITD Detection</b>             |  |  |                       |                          |
|--|--|--|-----------------------|--------------------------|
| <b>Sample</b>                                | <b>Cytogenetics</b>                                  | <b>Capillary Electrophoresis (ITD Size and % allele frequency)</b> | <b>MiSeq Reporter</b> | <b>Pindel</b>            |
| <b>1</b><br>AMML (67%)                       | 46,XY,del(12)(p11.2)<br>[2] 47,XY,+8[1]<br>46,XY[37] | 18bp (23%)   | NC                    | Yes (18bp)               |
| <b>2</b><br>AMML (81%)                       | 46,XX[20]  | 31bp (39%)   | NC                    | Yes (30bp)               |
| <b>3</b><br>AML without maturation (56%)     | 46,XY,t(11;21)(p11.2;q22)[1]                         | 94bp (1%)  | NC                    | Yes (93bp)               |
| <b>4</b><br>Acute monocytic leukemia (86%)   | Complex  | 61bp (3%)  | NC                    | Yes (60bp)               |
| <b>5</b><br>AMML (61%)                       | 46,XX,[22]   | 42bp (3%)<br>58bp (35%)  | NC<br>NC              | Yes (42bp)<br>Yes (57bp) |
| <b>6</b><br>AML with maturation (38%)        | 46,XY[13]  | 31bp (32%)   | NC                    | Yes (30bp)               |
| <b>7</b><br>t-AML (81%)                      | 46,XX[20]  | 46bp (1%)  | NC                    | Yes (45bp)               |
| <b>8</b><br>Acute monoblastic leukemia (68%) | Complex  | 55bp (6%)<br>58bp (9%)   | NC<br>NC              | Yes (54bp)<br>Yes (57bp) |
| <b>9</b><br>AML with Maturation (32%)        | Complex  | 61bp (3%)  | NC                    | Yes (60bp)               |
| <b>10</b><br>AML (3%)                        | NA (PB)  | 100bp (34%)  | NC                    | Yes (99bp)               |

**Supplementary Table 4. Comparison of sequencing results from MiSeq Version 1 and Version 2 sequencing.** 12 samples were sequenced by both version 1 and Version 2 sequencing on MiSeq. The variants detected, their frequencies and sequencing coverage are compared.

| Sample | Variants<br>Mutation Detected by<br>Miseq and Coverage              | Version 1                |                      | Version 2                |                      |
|--------|---|--------------------------|----------------------|--------------------------|----------------------|
|        |   | Variant<br>Frequency (%) | Coverage (X)         | Variant<br>Frequency (%) | Coverage<br>(X)      |
| 1      | <i>FGFR3</i> p.F384L<br><i>KIT</i> , p.M541L<br><i>MET</i> , T1010I | 48.7<br>51.4<br>51.2     | 306<br>18034<br>8119 | 49.5<br>51.0<br>54.3     | 216<br>11994<br>5479 |
| 2      | <i>APC</i> p.E1317Q<br><i>JAK2</i> p.V617F                          | 50.7<br>59.7             | 9923<br>3015         | 49.9<br>59.3             | 12056<br>3416        |
| 3      | <i>KIT</i> p.M541L<br><i>TP53</i> p.Y220C                           | 99.8<br>54.8             | 21312<br>5348        | 99.8<br>57.5             | 22977<br>5358        |
| 4      | <i>KIT</i> p.M541L  | 51.8                     | 19410                | 50.9                     | 23113                |
| 5      | <i>KDR</i> p.Q472H  | 50.5                     | 9159                 | 50.2                     | 10275                |
| 6      | <i>KDR</i> p.Q472H<br><i>TP53</i> p.R273H                           | 49.9<br>36.6             | 6433<br>2151         | 50.5<br>35.4             | 5449<br>1948         |
| 7      | <i>TP53</i> p.N247I<br><i>TP53</i> p. R110P                         | 8.7<br>29.1              | 3702<br>999          | 7.0<br>22.9              | 4539<br>1938         |
| 8      | <i>TP53</i> p.N239S   | 11.0                     | 4460                 | 10.3                     | 4425                 |
| 9      | <i>NPM1</i> (4bp insertion)<br><i>PTPN11</i> p.S502L                | 33.9<br>36.1             | 1676<br>5772         | 40.5<br>38.9             | 2863<br>8682         |
| 10     | <i>KIT</i> p.M541L  | 48.5                     | 9159                 | 50.2                     | 10275                |
| 11     | <i>KDR</i> p.Q472H  | 99.5                     | 5046                 | 99.6                     | 6083                 |
| 12     | <i>KDR</i> p.Q472H  | 51.4                     | 6050                 | 51.2                     | 9207                 |

**Supplementary Table 5.** Sensitivity studies were conducted using two cell lines (H2122 and DLD1). Details regarding the various dilutions used, the average expected and detected variant frequencies of the mutations in six separate dilution studies are summarized below. The cytogenetic profile of the cell lines is also included when available. The information summarized is an average of 2 and 6 separate studies for H2122 and DLD1, respectively.

| DLD1<br>(Variants)   | Chromosome<br>positions | DLD1<br>(Undiluted)         | DLD1 (50%)<br>(1:1 diluted)  |                 | DLD1 (25%)<br>(1:3 diluted)  |                 | DLD1 (10%)<br>(1:9 diluted)  |                 | DLD1 (5%)<br>(1:19 diluted) |                 |
|--|-------------------------|-----------------------------|------------------------------|-----------------|------------------------------|-----------------|------------------------------|-----------------|-----------------------------|-----------------|
|  |                         | Variant<br>Frequency<br>(%) | Expected<br>(%)              | Detected<br>(%) | Expected<br>(%)              | Detected<br>(%) | Expected<br>(%)              | Detected<br>(%) | Expected<br>(%)             | Detected<br>(%) |
| <i>IDH1</i> p.G97D   | 2q33.3                  | 33.2                        | 16.6                         | 13.5            | 8.3                          | 7.1             | 3.3                          | 2.8             | 1.6                         | 1.5             |
| <i>PIK3CA</i> p.D549N  | 3q26.3                  | 48.8                        | 24.4                         | 25.0            | 12.2                         | 10.8            | 4.8                          | 4.6             | 2.4                         | 2.4             |
| <i>PIK3CA</i> p.E545K  | 3q26.3                  | 49.4                        | 24.7                         | 25.1            | 12.3                         | 11.2            | 4.9                          | 4.9             | 2.4                         | 2.5             |
| <i>KIT</i> p.V532I   | 4q11-q12                | 50.5                        | 25.2                         | 25.9            | 12.6                         | 12.7            | 5.0                          | 5.6             | 2.5                         | 2.9             |
| <i>XPO1</i> p.E510K  | 2p15                    | 66.8                        | 33.4                         | 42.4            | 16.7                         | 24.5            | 6.6                          | 11.3            | 3.3                         | 6.0             |
| <i>KRAS</i> p.G13D   | 12p12.1                 | 50.2                        | 25.1                         | 26.5            | 12.5                         | 14.5            | 5.0                          | 6.2             | 2.5                         | 3.5             |
| <i>TP53</i> p.S241F  | 17p13.1                 | 49.2                        | 24.6                         | 25.5            | 12.3                         | 12.6            | 4.9                          | 5.2             | 2.4                         | 2.5             |
| <i>SMO</i> p.T640A   | 7q32.3                  | 64.6                        | 32.3                         | 36.0            | 16.1                         | 23.0            | 6.4                          | 9.2             | 3.2                         | 6.4             |
| <p><b>DLD1 Cytogenetic analysis (ATCC CCL-221):</b> Karyotype: 46, XY, 2, + dir dup (2)(p13p23). Modal chromosome number of 46, occurring in 86% of cells.<br/>Rate of polyploidy (17.1%).</p> |                         |                             |                              |                 |                              |                 |                              |                 |                             |                 |
| H2122<br>(Variants)  | Chromosome<br>positions | H2122<br>(Undiluted)        | H2122 (20%)<br>(1:4 diluted) |                 | H2122 (10%)<br>(1:9 diluted) |                 | H2122 (5%)<br>(1:19 diluted) |                 |                             |                 |
|  |                         | Frequency<br>(%)            | Expected<br>(%)              | Detected<br>(%) | Expected<br>(%)              | Detected<br>(%) | Expected<br>(%)              | Detected<br>(%) | Expected<br>(%)             | Detected<br>(%) |
| <i>NRAS</i> p.G12C   | 1p13.2                  | 99.5                        | 19.9                         | 17.2            | 9.9                          | 7.6             | 4.9                          | 4.3             | 4.3                         | 2.1             |
| <i>MET</i> p.N375S   | 7q31                    | 50.4                        | 10.0                         | 7.2             | 5.0                          | 3.6             | 2.5                          | 2.1             | 2.1                         | 2.1             |
| <p><b>H2122 Cytogenetic analysis (ATCC CRL-5985) :</b> Not available</p>   |                         |                             |                              |                 |                              |                 |                              |                 |                             |                 |

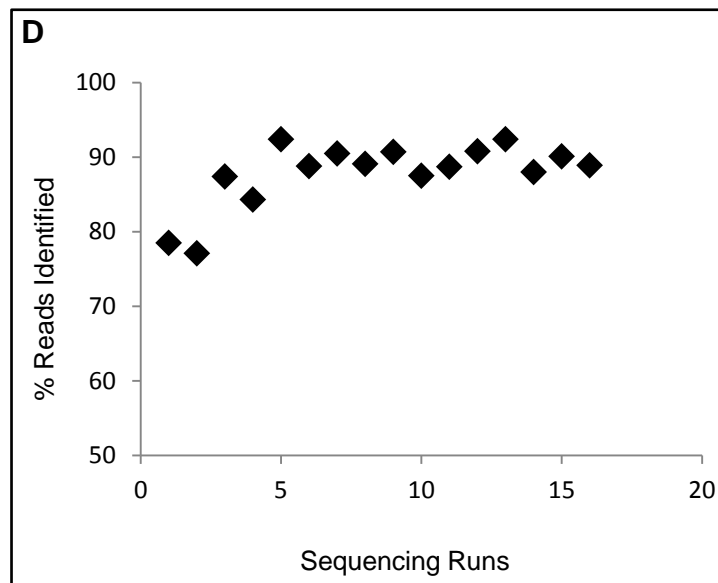
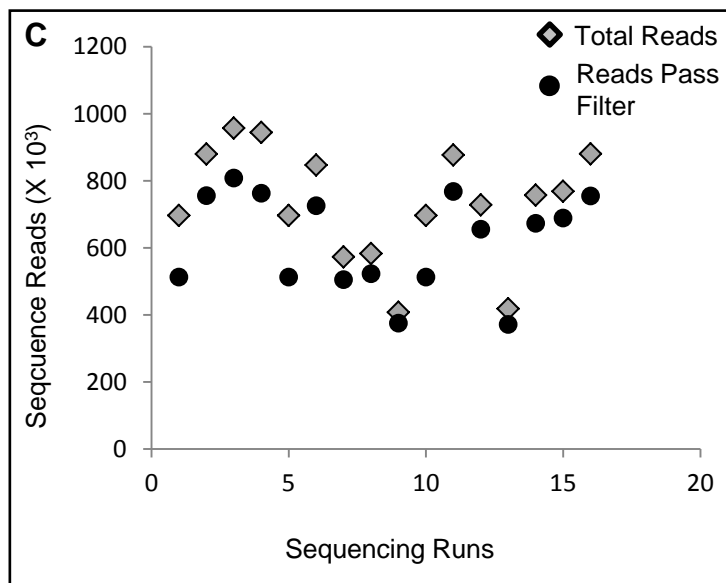
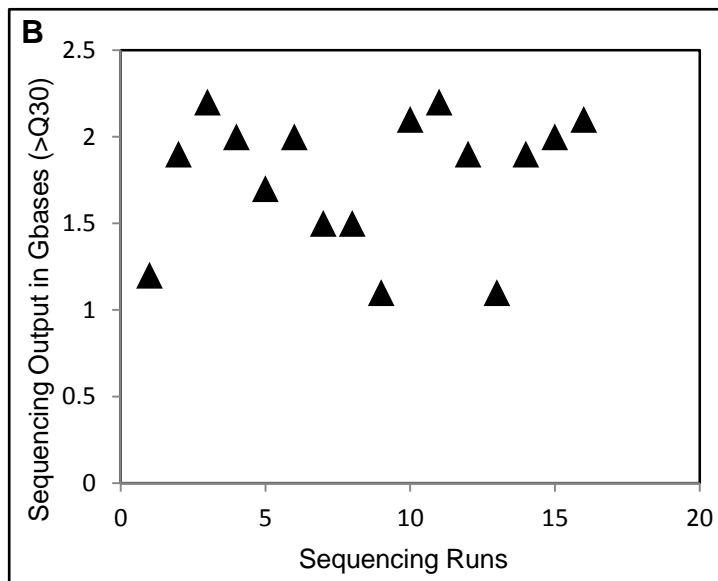
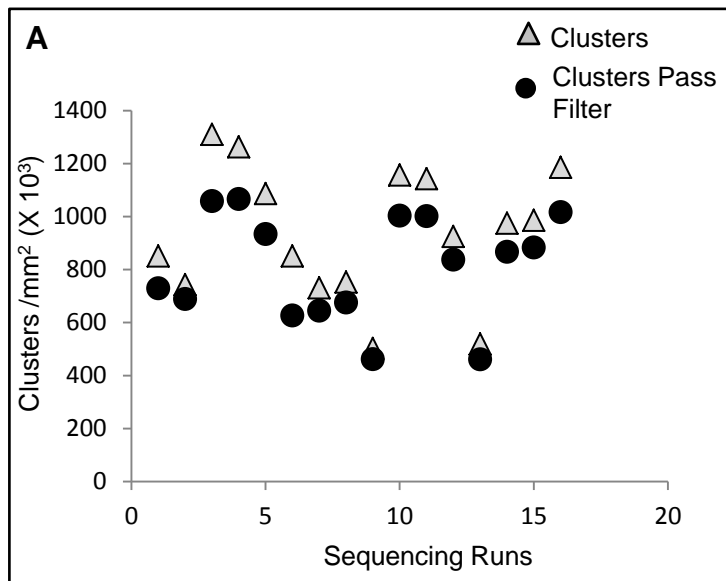
## Supplementary Figure Legends

**Supplementary Figure 1.** *MiSeq Sequencing run metrics.* The performance of 16 sequencing runs are summarized. (A) The cluster formation efficiency across the runs is reflected by the cluster density and the clusters pass filter. (B) Plot of total sequencing outputs with a base calling quality of more than Q30 (one error in 1000 bases) for the sequencing runs. (C) Total sequencing reads and reads pass filter which provide useful sequencing information (with chastity scores of  $\geq 0.6$ ) are shown. (D) The total number of reads that could be identified or assigned to the barcode sequence ID associated with each of the multiplexed samples.

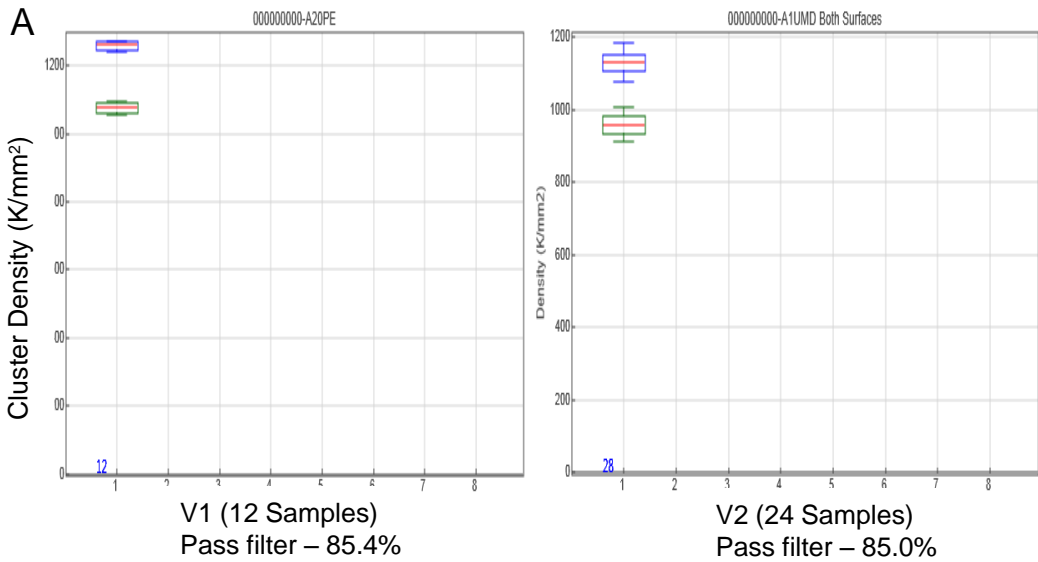
**Supplementary Figure 2.** *Validation of MiSeq Version 2 upgrade.* (A) A comparison of the cluster generation density and clusters pass filter of the sequencing runs with Version1 (V1) (12 samples) and Version 2 (V2) (with the same 12 samples and additional 12 samples). (B) A doubling of sequencing capacity of MiSeq V2 upgrade (4.7Gb) was evident in comparison to the V1 (2.1Gb) with comparable sequencing quality (Q30). (C) Higher sequencing capacity of V2 also evident in higher sequencing reads and reads pass filter (upper panel). The 12 samples common to runs of both versions had comparable sequencing reads and average per base coverage (lower panel).

**Supplementary Figure 3.** *Inter- and intra-run assay reproducibility.* (A) Inter-run reproducibility was assessed by sequencing the 1:9 diluted (10%) sample of DLD1 in 11 separate sequencing runs and the detection of 8 mutations was tested. (B) Intra-run reproducibility was estimated by sequencing 24 DLD1 (10%) samples, each indexed with a distinct barcode combination and sequenced on a single sequencing run. Detection of the 8 mutations in DLD1 was found to be consistent in each of the 24 samples sequenced.

**Supplementary Figure 4.** *Summary of somatic mutations detected.* A pie chart depicting different genes and the number of somatic mutations detected in the AML/MDS cohort tested by sequencing on MiSeq (includes all samples listed in supplementary Tables 1, 2, 3 and 4). The mutations detected in our study found in genes not routinely tested for AML have also been summarized (right panel). Information regarding the number of mutations detected in each gene and their relative percentages are provided next to the gene names. Germline polymorphisms were not included in this summary.

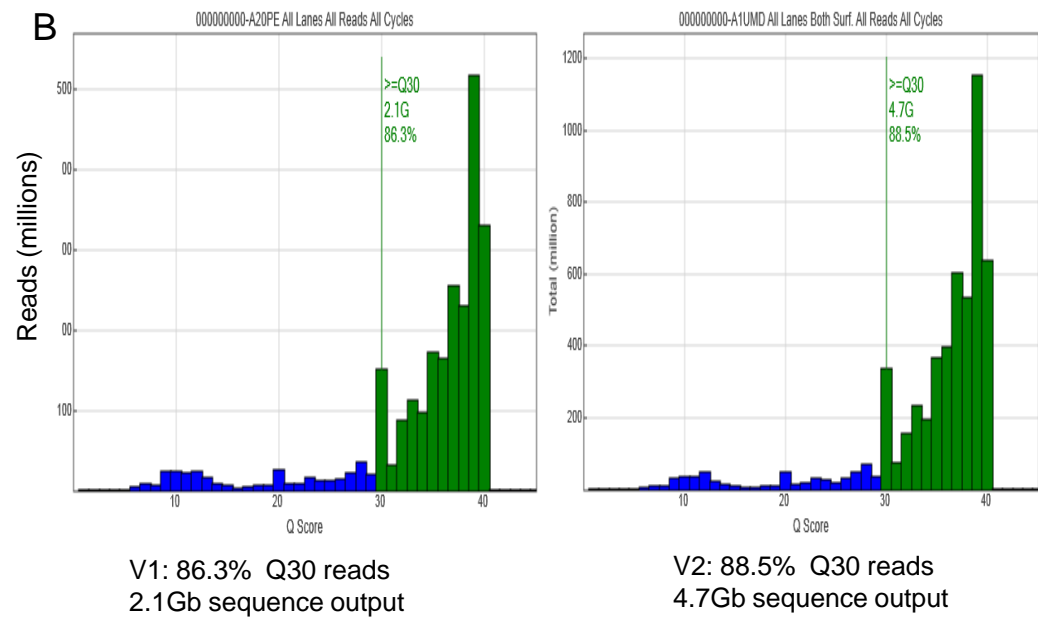


Supplementary Figure 1



**C**

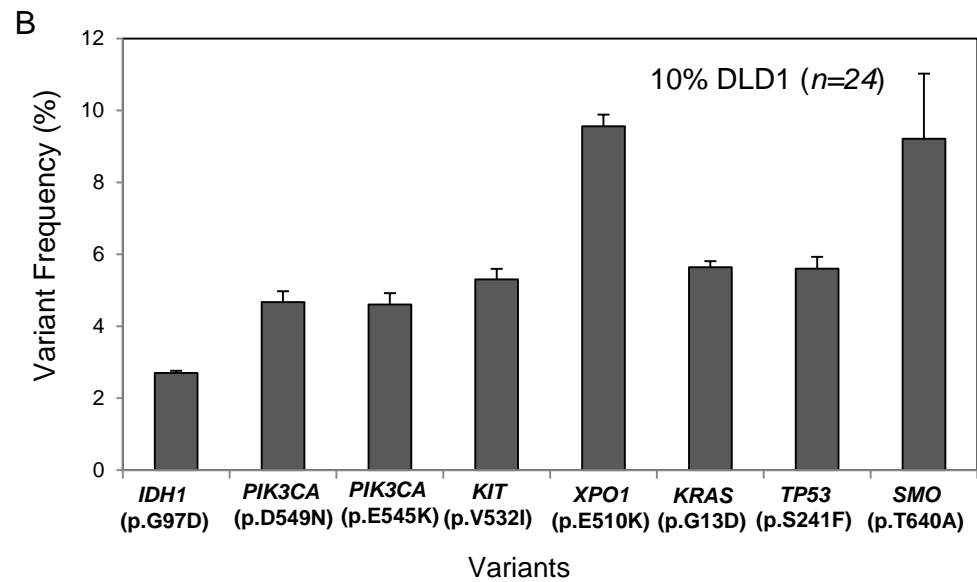
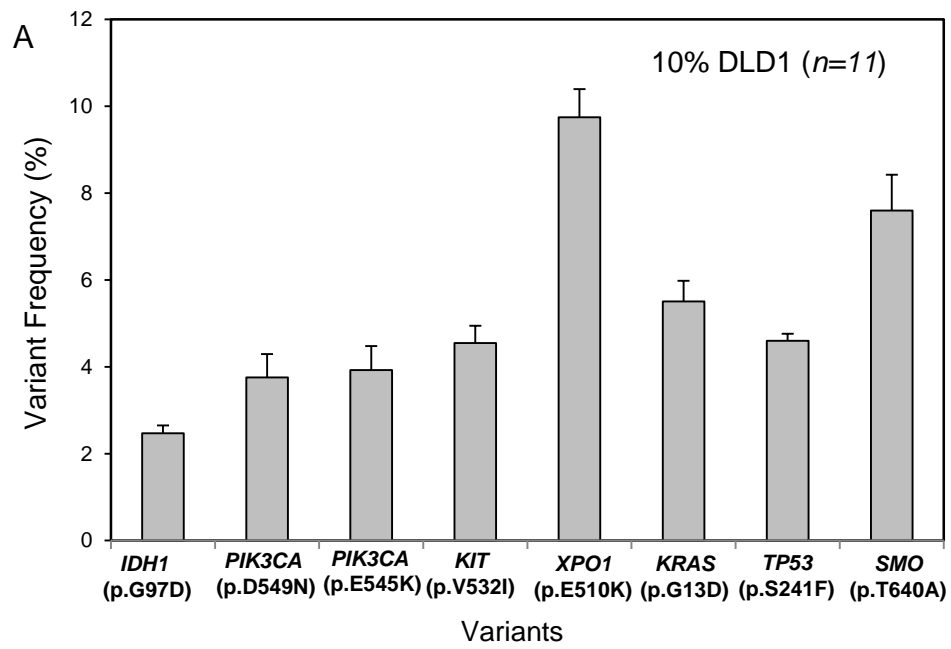
|           | TOTAL READS | PF READS | % READS IDENTIFIED (PF) |
|-----------|-------------|----------|-------------------------|
| Version 1 | 9162448     | 7830961  | 96.3212                 |
| Version 2 | 19598832    | 16657210 | 96.5842                 |



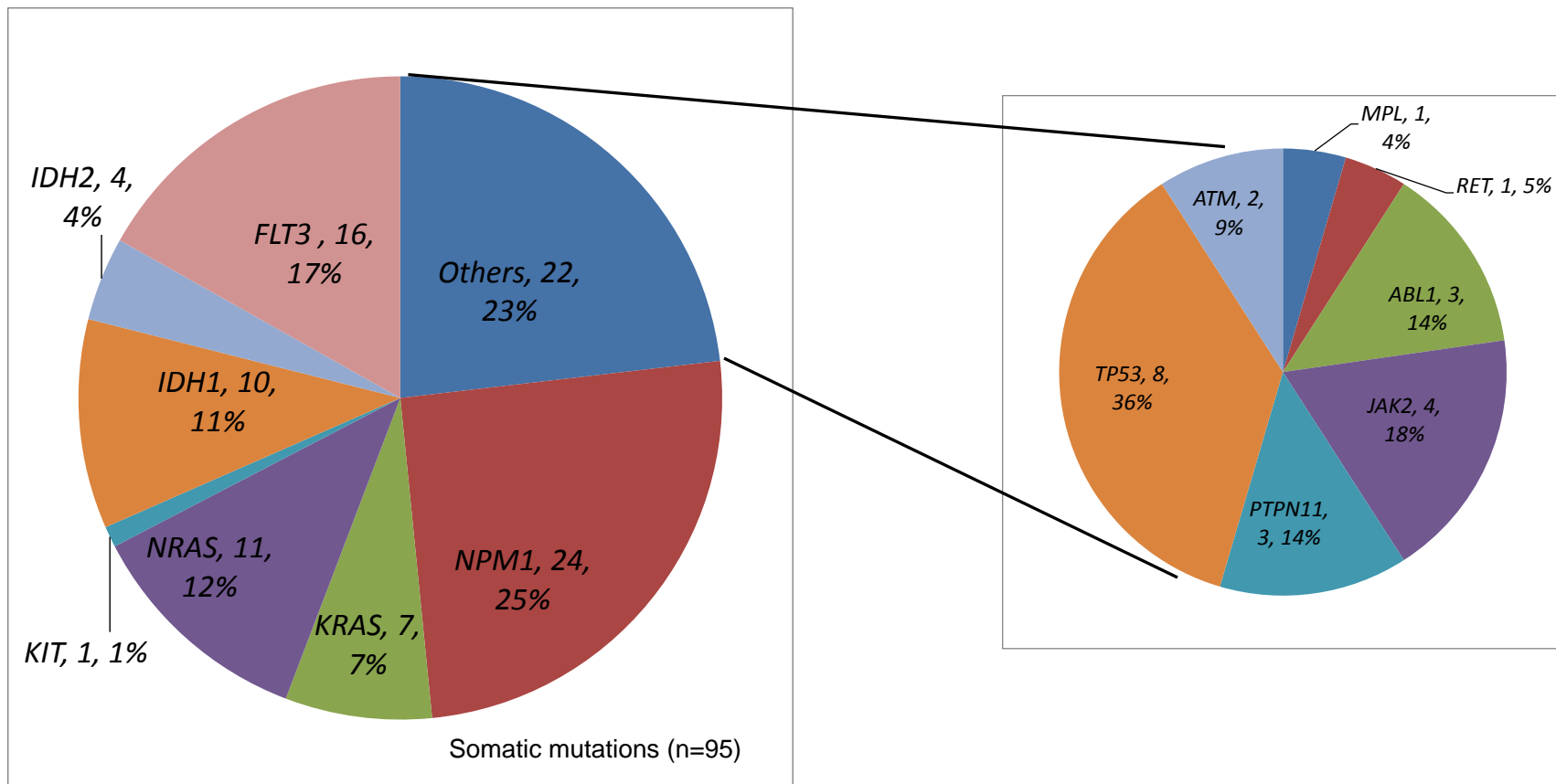
| SAMPLE | Version 1               |             |                                  | Version 2               |             |                                  |
|--------|-------------------------|-------------|----------------------------------|-------------------------|-------------|----------------------------------|
|        | % READS IDENTIFIED (PF) | TOTAL READS | AVERAGE COVERAGE OF BASE /SAMPLE | % READS IDENTIFIED (PF) | TOTAL READS | AVERAGE COVERAGE OF BASE /SAMPLE |
| 1      | 7.1422                  | 559,303     | 2397                             | 2.5257                  | 420,711     | 1803                             |
| 2      | 6.8349                  | 535,238     | 2294                             | 3.6059                  | 600,642     | 2574                             |
| 3      | 8.3392                  | 653,039     | 2799                             | 4.4798                  | 746,210     | 3198                             |
| 4      | 10.0348                 | 785,821     | 3368                             | 6.0122                  | 1,001,465   | 4292                             |
| 5      | 8.5265                  | 667,707     | 2862                             | 4.6352                  | 772,095     | 3309                             |
| 6      | 8.1914                  | 641,465     | 2749                             | 4.2802                  | 712,962     | 3056                             |
| 7      | 8.4891                  | 664,778     | 2849                             | 4.6742                  | 778,591     | 3337                             |
| 8      | 8.5949                  | 673,063     | 2885                             | 2.793                   | 465,236     | 1994                             |
| 9      | 7.9823                  | 625,091     | 2679                             | 4.025                   | 670,453     | 2873                             |
| 10     | 8.0036                  | 626,759     | 2686                             | 3.8701                  | 644,651     | 2763                             |
| 11     | 6.2272                  | 487,650     | 2090                             | 2.613                   | 435,253     | 1865                             |
| 12     | 7.9549                  | 622,945     | 2670                             | 2.7595                  | 459,656     | 1970                             |

Supplementary Figure 2





Supplementary Figure 3



Supplementary Figure 4