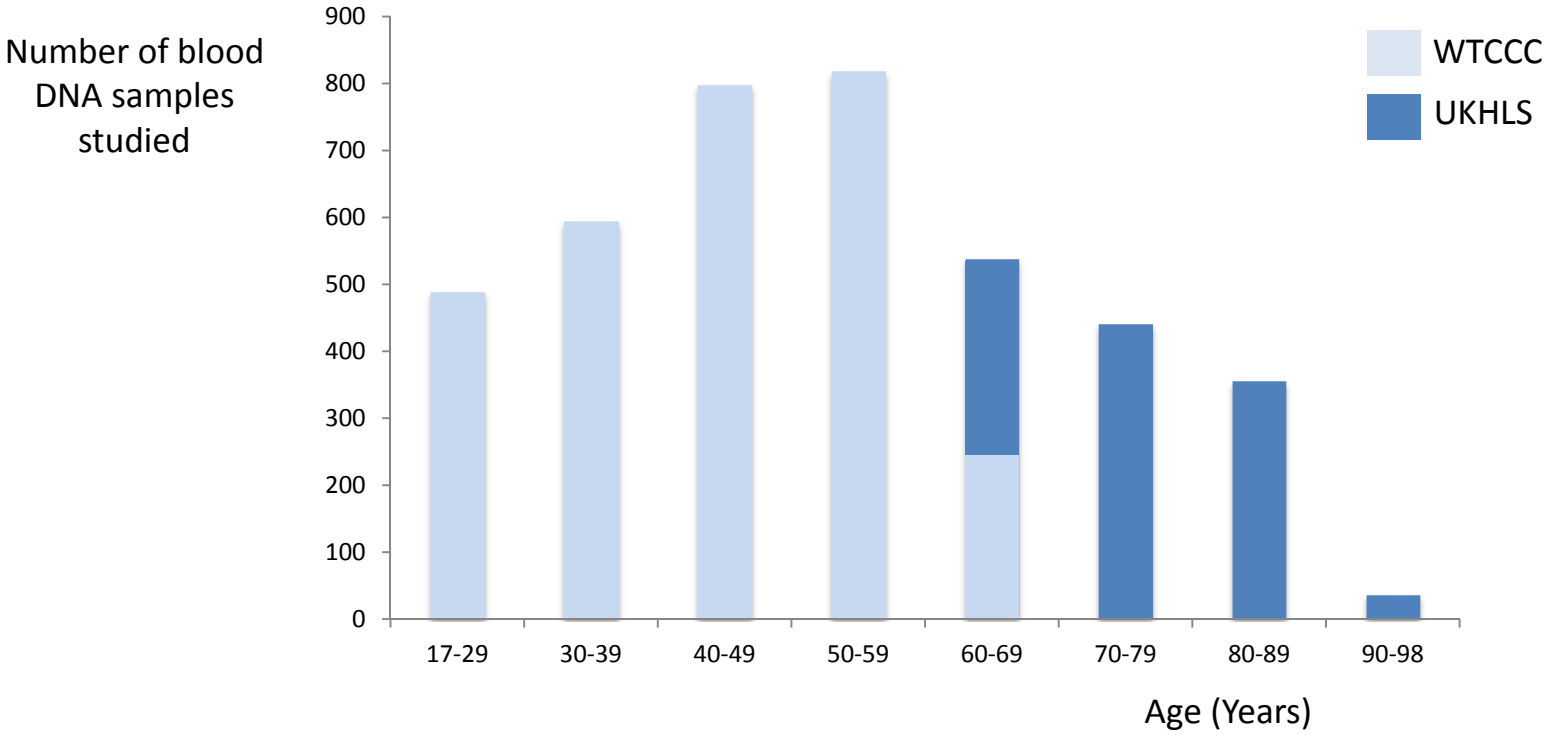
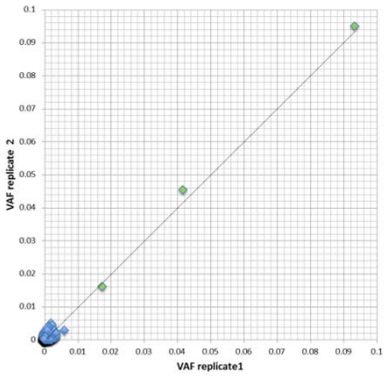
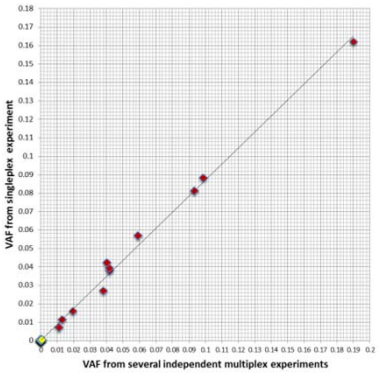

Number of blood DNA samples studied
900
800
700
600
500
400
300
200
100
0
17-29
30-39
40-49
50-59
60-69
70-79
80-89
90-98
Age (Years)
WTCCC
UKHLS

**Figure S1**

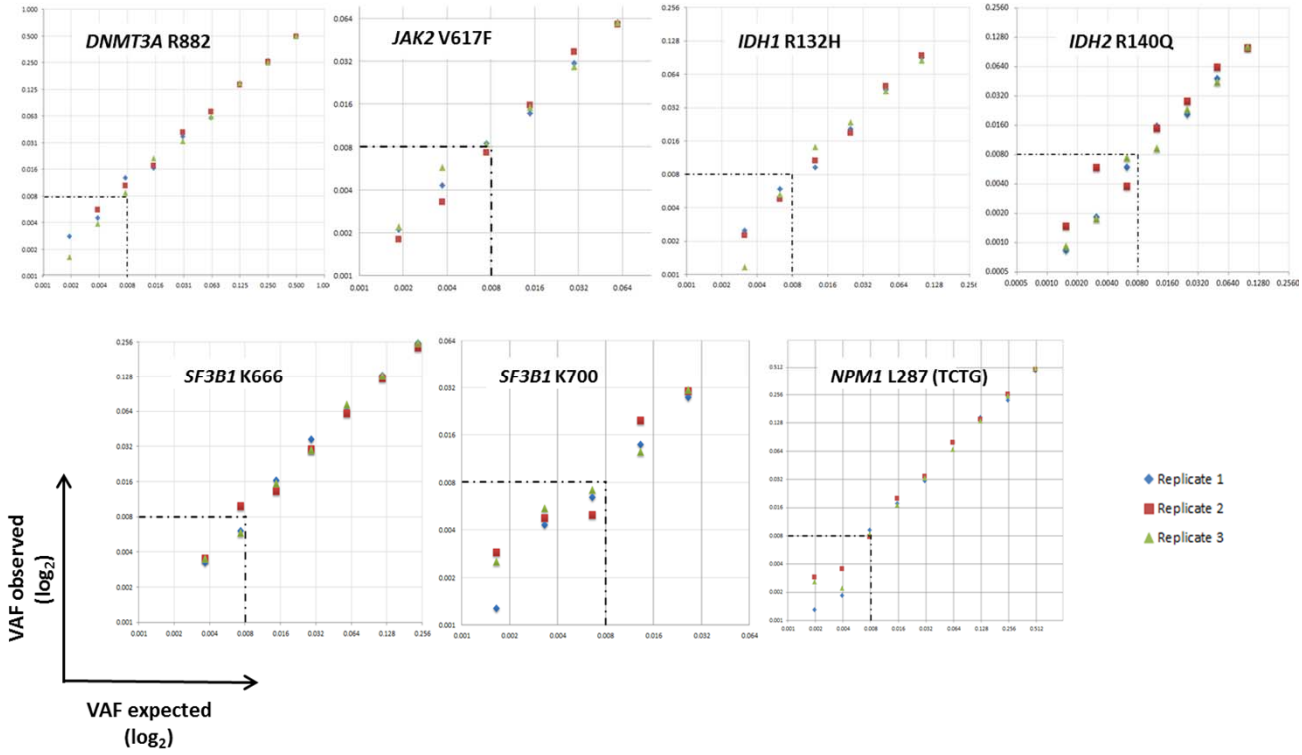
A




B




C




D

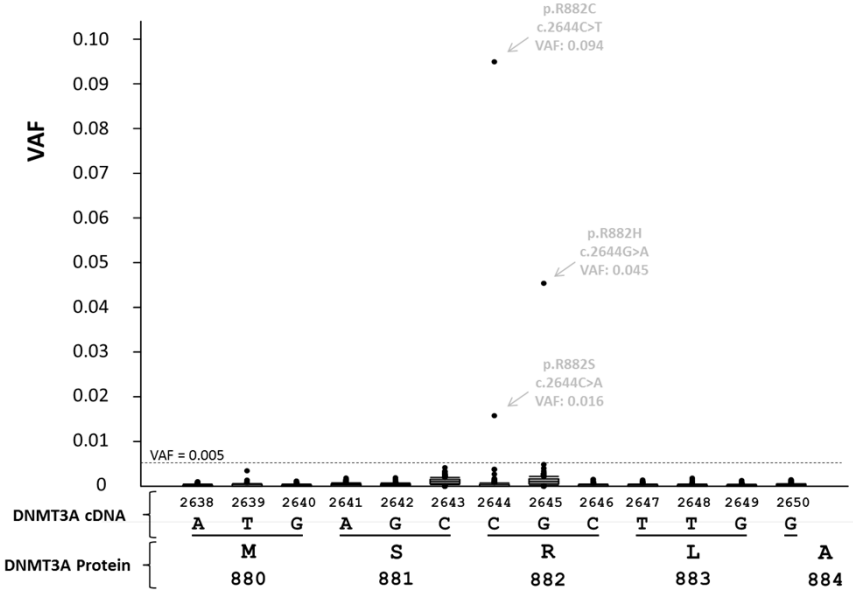
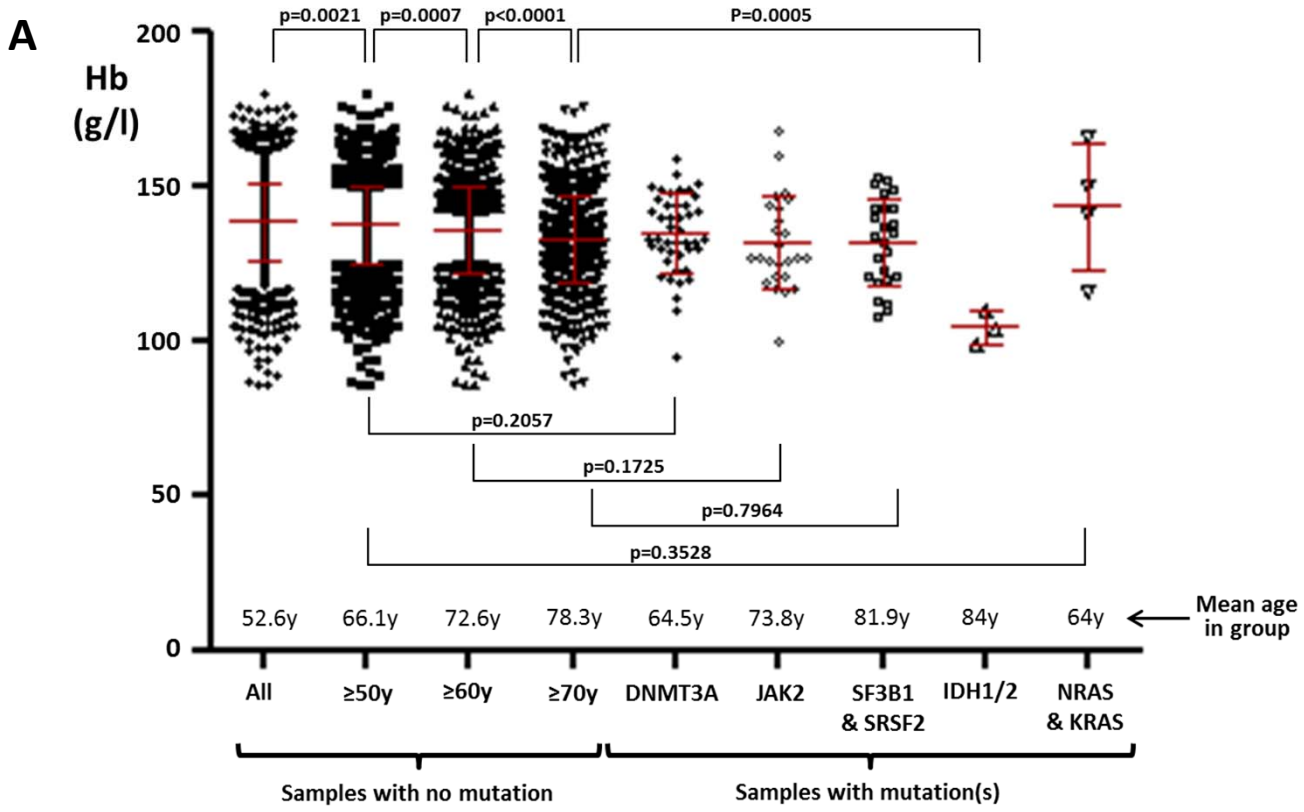



**Figure S2**


A
Hb (g/l)
200
150
100
50
0
p=0.0021
p=0.0007
p<0.0001
P=0.0005
p=0.2057
p=0.1725
p=0.7964
p=0.3528
52.6y
66.1y
72.6y
78.3y
64.5y
73.8y
81.9y
84y
64y
Mean age in group
All
≥50y
≥60y
≥70y
DNMT3A
JAK2
SF3B1 & SRSF2
IDH1/2
NRAS & KRAS
Samples with no mutation
Samples with mutation(s)

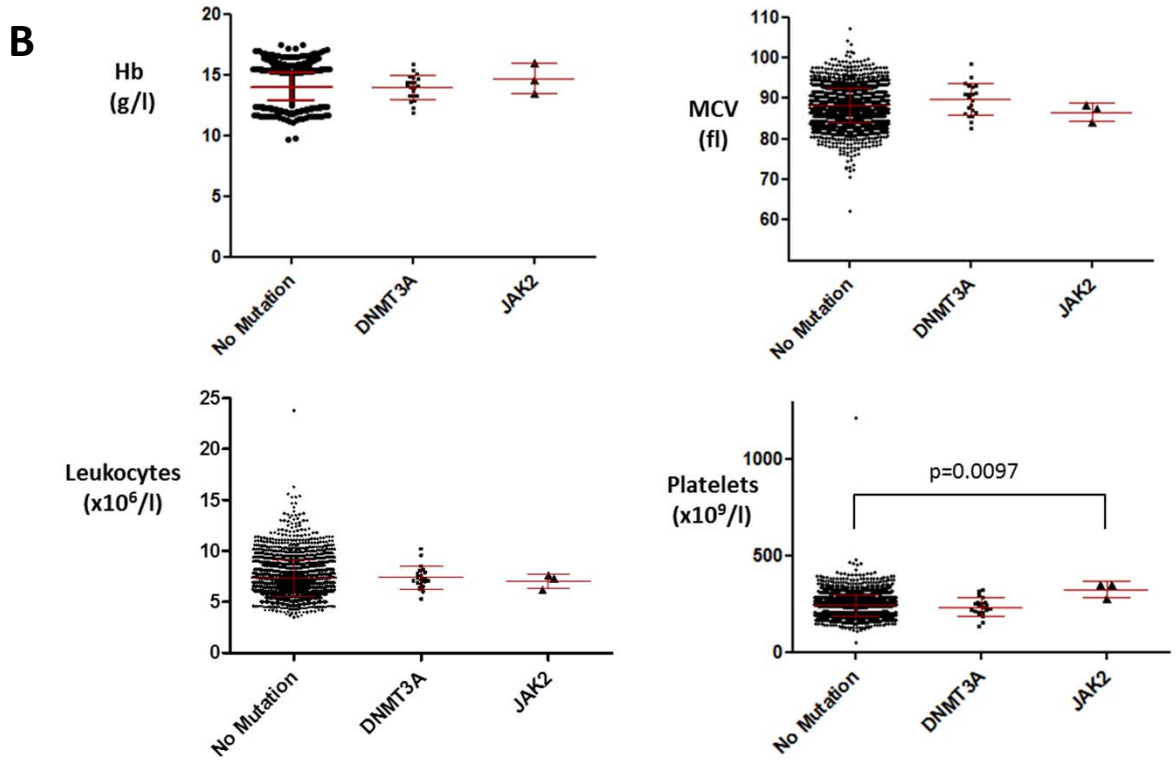

B
Hb (g/l)
MCV (fl)
Leukocytes (x10⁶/l)
Platelets (x10⁹/l)
p=0.0097
No Mutation
DNMT3A
JAK2

**Figure S3**

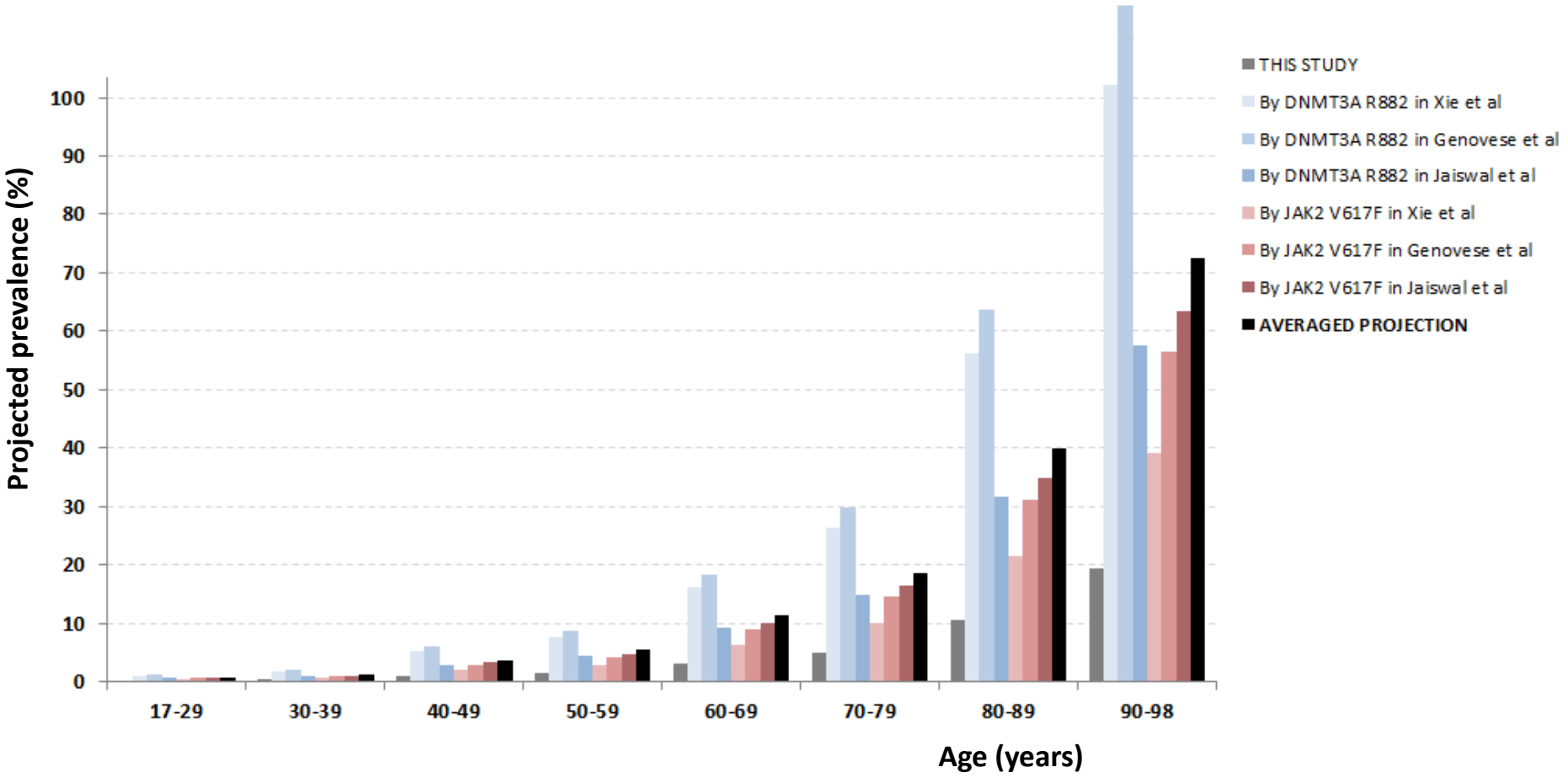

Projected prevalence (%)
100
90
80
70
60
50
40
30
20
10
0
17-29
30-39
40-49
50-59
60-69
70-79
80-89
90-98
Age (years)
THIS STUDY
By DNMT3A R882 in Xie et al
By DNMT3A R882 in Genovese et al
By DNMT3A R882 in Jaiswal et al
By JAK2 V617F in Xie et al
By JAK2 V617F in Genovese et al
By JAK2 V617F in Jaiswal et al
AVERAGED PROJECTION

**Figure S4**

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1. Numbers of individuals/DNA samples studied for the presence of clonal hemopoiesis**

The sample group from which blood DNA samples were obtained is indicated. Samples were studied with different but overlapping sets of multiplex PCR primers (Plex 1-3, see Table S1). WTCC= Wellcome Trust Case Control Consortium, UKHLS= United Kingdom Household Longitudinal Study.

***Related to experimental procedures***

**Figure S2. Methodological validation of multiplex PCR-MiSeq**

**A.** To validate the reproducibility, accuracy and error rate of our multiplex PCR-MiSeq sequencing protocol, we studied the same 384 blood DNA samples twice in independent experiments and derived VAF values for the two *DNMT3A* R882 mutation hotspot positions, c.2644 and c.2645. The 371 samples with more than 1000 reads in both experiments are plotted (i.e. 2 replicates of 742 VAF datapoints). VAFs from the same sample and position from experiment 1 are plotted against the equivalent value from experiment 2. The 739 samples with VAFs ≤0.006 (blue diamonds) show relatively poor correlation in keeping with PCR/sequencing error at these very low VAF values. Nevertheless, no VAF values >0.006 are seen in either replicate except for the three real mutant samples (green diamonds) whose higher VAFs correlate extremely well**.**

**B.** To validate the reproducibility of our protocol for quantifying real mutations with VAFs ≥0.008, we re-analyzed 11 samples carrying subclonal DNA mutations with VAFs from 0.01 to 0.19 (i.e. 1-19% mutant reads) and 14 samples without evidence of subclonal mutations determined in several independent multiplex PCR experiments/batches , using an independent singleplex PCR for *DNMT3A* R882 (different primers). As shown, there was excellent correlation between multiplex and singleplex VAFs for all 11 mutant samples (red diamonds). The 14 samples without evidence of subclonal mutations according to the multiplex PCR, again gave very low VAFs (<0.0004) with singleplex analysis (yellow diamonds), therefore demonstrating the robust VAF quantitation achieved using our approach.

**C.** To validate the linearity of VAF quantitation of our protocol for the detection of low level subclonal mutations, we analyzed serial 2-fold dilutions of primary leukaemic or cell line (OCI-AML3) DNA into unmutated cord blood DNA for the following codons: *DNMT3A* R882, *JAK2* V617F, *IDH1* R132, *IDH2* R140, *SF3B1* K666, *SF3B1* K700 and *NPM1* L287. The "expected" VAF values were derived using the VAF obtained for the neat DNA of primary leukaemic or OCI-AML3 DNA in each experiment. Results of 3 independent replicate experiments using the same diluted DNA samples are shown. All hotspot loci studied show very good correlation between replicates for VAFs ≥0.008 (0.8%), our cut-off for "calling" mutant clones.

**D.** To derive the likelihood of obtaining a false positive mutation call we calculated the VAF for nucleotide positions surrounding the *DNMT3A* R882 codon outside of the mutation hotspots (c.2644 and c.2645) in 371 samples. Across all samples analyzed in this way, nucleotide positions outside the mutation hotspots gave VAFs lower than 0.005 (maximum VAF was 0.0041 at position c.2643). At the two hotspot positions we observed 3 samples with VAFs much higher than 0.005 (arrows). Near identical results were obtained when the same samples were analyzed for a second time using independent PCR amplifications and sequencing. These data were used to derive the likelihood of obtaining an erroneous VAF value greater than 0.008 (i.e. a false positive mutation call).

***Related to experimental procedures***

**Figure S3. Comparisons of blood results between participants with different mutations**

**A.** Hemoglobin concentrations (Hb) in different age and mutation groups for all participants (WTCCC & UKHLS). Individual values are plotted and red bars represent mean +/‐ standard deviation for each group. Paired t‐tests were performed for the indicated comparisons and, amongst individuals with no mutations, values were found to differ significantly between age groups (p values for each comparison indicated). Therefore, for each mutation group the "no mutation" group with the most similar mean age was used as control. Only IDH1/2 mutant samples had significantly lower Hb compared to age-matched controls. The 6 samples with more than one mutation were classified according to the mutation with the highest VAF.

**B.** Blood count results in different WTCCC participant groups. Individual values are plotted and red bars represent mean +/- standard deviation for each group. Paired t-tests were performed comparing "No mutation" samples with the DNMT3A mutant group (n=24) and, separately, with the JAK2 mutant group (n=3). The only significant comparison ($p<0.05$) was for platelet counts, which were higher in the JAK2 group. However, values for all 3 JAK2 mutant samples were within the reference range. The one KRAS and one NRAS mutant samples identified in the WTCCC sample group were not included in these comparisons. MCV=mean corpuscular volume (of erythrocytes).

***Related to Figure 1***

**Figure S4. Projected overall prevalence of Age-Related Clonal Haemopoiesis driven by leukemia-associated mutations**

Our methodology for detecting hotspot mutations was much more sensitive than approaches used by others to date. In order to derive approximate estimates of the overall prevalence of ARCH driven by leukemia-associated mutations, we projected our findings onto those of published datasets from three recent studies that used whole-exome sequencing of blood DNA to identify individuals with ARCH (Xi et al, 2014; Genovese et al; Jaiswal et al). As the age-distribution of participants varied significantly between studies and details of age-distribution of individuals mutations were not given by the two largest studies (Genovese et al; Jaiswal et al), we used the fraction of all mutations represented by DNMT3A R882 and by JAK2 V617F in each study to derive estimates of the overall prevalence of ARCH at the sensitivity of our study (i.e. VAF≥0.008). We chose these two mutations as they are the two commonest recurrent events in our study and also because they were identified in most age groups, albeit at varying frequencies. A notable limitation of this approach is the fact that exome sequencing detects different mutations with different sensitivities. In fact sequence coverage for DNMT3A R882 was lower, whilst that for JAK2 V617 was higher than average. Also each study set a different minimum VAF for "calling" mutations (Xie et al, VAF≥0.1; Genovese et al, VAF≥0.05 and Jaiswal et al, VAF≥0.03). Nevertheless, even the most conservative of our projections indicate that ARCH is much commoner than previously considered and is likely to occur in the majority of people aged over 90 years.

***Related to Figure 1***

| Gene | Target codon | Plex 1 | Plex 2 | Plex 3 | Numbers studied at each locus | Number of mutations per locus | Incidence of mutations per locus (%) |
|---|---|---|---|---|---|---|---|
| DNMT3A | R882 | ✓ | ✓ | ✓ | 4067 | 47 | 1.16 |
| JAK2 | V617 | ✓ | ✓ | ✓ | 4067 | 25 | 0.61 |
| NPM1 | L287 | ✓ | ✓ | ✓ | 4067 | 0 | 0.00 |
| SRSF2 | P95 | ✓ | | ✓ | 2577 | 13 | 0.50 |
| SF3B1 | K666 | ✓ | | ✓ | 2577 | 10 | 0.39 |
| SF3B1 | K700 | ✓ | | ✓ | 2577 | 8 | 0.31 |
| IDH1 | R132 | ✓ | | ✓ | 2577 | 2 | 0.08 |
| IDH2 | R140 | ✓ | | ✓ | 2577 | 1 | 0.04 |
| IDH2 | R172 | ✓ | | ✓ | 2577 | 0 | 0.00 |
| KRAS | G12 | | ✓ | ✓ | 2606 | 2 | 0.08 |
| NRAS | G12 | | ✓ | ✓ | 2606 | 2 | 0.08 |
| NRAS | Q61 | | ✓ | ✓ | 2606 | 0 | 0.00 |
| KIT | D816 | | ✓ | ✓ | 2606 | 0 | 0.00 |
| FLT3 | D835 | | ✓ | ✓ | 2606 | 0 | 0.00 |
| FLT3 | N676 | | ✓ | | 1490 | 0 | 0.00 |
| Number of individuals screened using each Plex design | | 1531 | 1536 | 1152 | | | |
| Number of individuals with adequate coverage for analysis* | | 1461 | 1490 | 1116 | | | |

**Table S1**

| Study | Mutation Group | Mutation number | Read depth (coverage)* | | | |
|---|---|---|---|---|---|---|
| | | | Mean | SD** | Minimum | Maximum |
| Genovese et al, 2014 | All mutations | 327 | 91.7 | 51.0 | 11 | 371 |
| | DNMT3A (all) | 190 | 91.2 | 49.2 | 21 | 255 |
| | DNMT3A R882 | 123 | 58.6 | 17.2 | 30 | 101 |
| | JAK2 V617F | 24 | 111.0 | 27.1 | 79 | 191 |
| | SF3B1 K666 | 3 | 162.0 | 89.3 | 72 | 234 |
| | SF3B1 K700 | 9 | 89.3 | 32.7 | 59 | 164 |
| | SRSF2P95 | 5 | 59.0 | 10.3 | 47 | 69 |
| Jaiswal et al, 2014 | All mutations | 805 | 94.0 | 53.9 | 16 | 432 |
| | DNMT3A (all) | 403 | 92.6 | 50.7 | 18 | 384 |
| | DNMT3A R882 | 67 | 61.9 | 15.9 | 34 | 95 |
| | JAK2 V617F | 31 | 121.8 | 38.8 | 81 | 265 |
| | SF3B1 K666 | 11 | 69.5 | 15.7 | 52 | 107 |
| | SF3B1K700 | 12 | 82.5 | 26.7 | 38 | 133 |
| | SRSF2 P95 | 10 | 51.8 | 11.3 | 35 | 72 |
| Xie et al, 2014 | All mutations | 77 | 107.8 | 69.0 | 22 | 387 |
| | DNMT3A (all) | 18 | 109.3 | 86.8 | 28 | 387 |
| | DNMT3A R882 | 6 | 65.5 | 31.6 | 28 | 115 |
| | JAK2 V617 | 8 | 156.0 | 66.0 | 63 | 237 |
| | SF3B1K666 | 0 | n/a | n/a | n/a | n/a |
| | SF3B1K700 | 2 | 90.0 | 15.6 | 79 | 101 |
| | SRSF2 P95 | 0 | n/a | n/a | n/a | n/a |

**Table S2**

| Patient age | Gender | Indication for autologous HSCT | Time since HSCT (months) |
|---|---|---|---|
| 61 | M | Myeloma | 12 |
| 68 | M | Hodgkins Lymphoma | 4.5 |
| 63 | M | Mantle Cell Lymphoma | 1 |
| 27 | M | Hodgkins Lymphoma | 17 |
| 53 | F | Follicular Lymphoma | 8 |
| 41 | F | Diffuse Large B Cell Lymphoma | 12 |
| 66 | F | Mantle cell Lymphoma | 24 |
| 63 | M | Myeloma | 34 |
| 57 | F | Diffuse Large B Cell Lymphoma | 15 |
| 55 | M | Myeloma | 26 |
| 51 | M | Myeloma | 65 |
| 49 | M | NK T cell Lymphoma | 15 |

**Table S3**

| Patient age | Gender | Indication for Allogeneic HSCT | Time since HSCT (months) | Donor age at sampling | Donor Gender | Donor Chimerism (%) |
|---|---|---|---|---|---|---|
| 63 | M | Diffuse Large B Cell Lymphoma | 5 | 67 | F | 95 |
| 52 | F | Chronic Lymphocytic Leukemia | 36 | Unknown | M | 99 |
| 52 | M | Angioimmunoblastic lymphoma | 18 | 47 | F | 100 |
| 61 | M | Myelodysplastic syndrome (RAEB) | 63 | 27 | M | 100 |
| 33 | M | Non-Hodgkin's Lymphoma | 94 | 37 | F | Unknown |
| 58 | M | Acute Myeloid Leukemia | 15 | Unknown | M | 100 |
| 59 | M | Acute Myeloid Leukemia | 25 | Unknown | M | 100 |
| 44 | F | Acute Myeloid Leukemia | 34 | Unknown | F | 100 |
| 41 | M | Myeloma | 22 | Unknown | M | 100 |
| 49 | M | Acute Myeloid Leukemia | 105 | Unknown | M | Unknown |
| 56 | F | Acute Myeloid Leukemia | 63 | 42 | M | 96 |
| 47 | F | Chronic Myeloid Leukemia | 170 | Unknown | Unknown | Unknown |
| 50 | M | Blast crisis of Chronic Myeloid Leukemia | 30 | Unknown | M | Unknown |
| 19 | M | Aplastic Anaemia | 13 | 15 | F | 98 |
| 67 | M | Secondary Acute Myeloid Leukemia | 45 | Unknown | M | 100 |
| 25 | M | Hodgkin's Lymphoma | 42 | 27 | F | 100 |
| 65 | F | Acute Myeloid Leukemia | 62 | 44 | M | 99 |
| 61 | M | Acute Myeloid Leukemia | 13 | Unknown | M | 100 |
| 58 | F | Secondary Acute Myeloid Leukemia | 14 | Unknown | M | 100 |
| 48 | F | Acute Lymphoblastic Leukemia | 62 | 58 | M | 100 |

**Table S4**

**SUPPLEMENTAL TABLE LEGENDS**

**Table S1. Multiplex PCR reactions and numbers of individuals (blood DNA samples) studied**

WTCCC samples were screened with Plex 1 or Plex2. UKHLS samples were screened with Plex 3

* Only samples with ≥1000 reads at all studied loci were interrogated for the presence of mutations

***Related to Table 1***

**Table S2. Read depth statistics for selected mutation calls in three recent studies using exome sequencing to identify individuals with ARCH**

* Total read count (reference reads + mutant reads)
** SD = standard deviation

NB: These statistics are for coverage at called mutations. Numerical read depth (coverage) values for samples without identified mutations at these loci were not provided.

***Related to Figure 1 & Table 2***

**Table S3. Characteristics of individuals sampled after autologous hematopoietic stem cell transplantation (HSCT)**

***Related to experimental procedures***

**Table S4. Characteristics of individuals sampled after allogeneic hematopoietic stem cell transplantation (HSCT) and their respective donors**

***Related to experimental procedures***

# Supplemental Experimental Procedures

### Targeted re-sequencing

Multiplex primer combinations were tested and their concentrations adjusted to give similar levels of amplification for each of the target positions. First round multiplex PCR amplifications were performed with tailed gene primers on batches of up to 384 samples and individually barcoded by second round PCR with 384 pre-validated "MiSeq-ready" primers[1]; using a high fidelity polymerase (KAPA HiFi, Anachem or Q5 Hot Start HF, New England Biolabs). PCR reaction conditions used were as follows: 95$^{o}$C for 3min, [98$^{o}$C for 20s, 65$^{o}$C for 60s, 60$^{o}$C for 60s, 55$^{o}$C for 60s, 50$^{o}$C for 60s, 70$^{o}$C for 60s] x6 cycles, held at 4$^{o}$C until addition of barcoded second round primers, then [98$^{o}$C for 20s, 62$^{o}$C for 15s, 72$^{o}$C for 30s] x19 cycles, 72$^{o}$C 60s. For each batch, equal volumes of each sample were pooled, double SPRI size selected (X0.55 and X0.75) and quantified before storage at -20$^{o}$C until sequencing. A total of 11 MiSeq runs (250nt paired-end) were used for mutation identification. One of the 11 sample batches was repeated from PCR to sequencing for experimental validation purposes (Supplemental Figure S2A). Also, the reproducibility of our assay in quantifying variant allele fractions (VAFs) was further confirmed by studying 11 unselected samples harboring *DNMT3A*-R882 mutant clones of varying sizes (VAF 0.01-0.18), using a different *DNMT3A*-R882 primer set in a singleplex PCR using the following conditions: 98$^{o}$ for 30s [98$^{o}$ for 20s, 60oC for 30s, 72$^{o}$C for 60s] x6 cycles, held at 4oC until addition of barcoded second round primers, then [98$^{o}$C for 20s, 62$^{o}$C for 15s, 72$^{o}$C for 30s] x19 cycles, 72$^{o}$C 60s (Supplemental Figure S2B). Finally, the linearity of VAF calling was confirmed, using serial dilutions of leukemia or cell line DNA into cord blood DNA, for specific mutations including *DNMT3A*-R882, *JAK2*-V617F, *IDH1*-R132H, *IDH2*-R140Q, *SF3B1*-K666N, *SF3B1*-K700 and *NPM1*_mutation_A (TCTG duplication) (Supplemental figures S2C). The first 1571 samples (1531 WTCC, 32 post-transplant and 18 cord blood) were amplified using "Plex 1", the next 1554 samples (1536 WTCC, and 18 cord blood) using "Plex 2" and the final 1152 samples (UKHLS) using Plex 3 primer sets.

### Samples with two mutations at the same or neighboring loci

Amongst all samples, we identified 5 individuals with two independent spliceosome gene mutations of different VAFs (Figure 1B), 3 of which harbored the mutations at the same or at neighboring loci. Two of these, #760 and #565, harbored *SRSF2* P95H and *SRSF2* P95L, and another, #424, harbored *SF3B1* K666 and *SF3B1* K700. In an attempt to determine whether mutations were acquired on the same or on different alleles (maternal vs paternal), we looked for neighboring SNPs that could be used to "phase" the variants. We searched the Ensembl database for SNPs near *SRSF2* P95 and *SF3B1* K666/K700 and identified nearby polymorphisms for both locations, namely rs237057 (A/G MAF(G)=0.19) near *SRSF2* P95 and rs113023355 (A/G MAF(G)=0.012) near *SF3B1* K666 and K700. However, all three individuals were homozygous for the common alleles (A/A) and regrettably we were unable to phase the somatic variants.

**Bioinformatic Analysis** – Perl script for detecting *NPM1* mutations

In order to detect *NPM1* mutations with high sensitivity, we wrote a new Perl script to extract from each sample the reads covering the *NPM1* mutation hotspot and align these against the reference genome. Subsequently, the script individually parses each read looking for insertions at the hotspot position. The number of reads reporting the reference is recorded and so is the number reporting any variants and the sequence of this variant. Using sequencing data from normal samples manually spiked with *NPM1*-mutant DNA (OCI-AML3 cell line, mutation A), we determined that mutant reads with an expected VAF ≥ 0.002 (0.2%) were reliably detected (Supplemental Figure S2C).

**Statistical Analysis and mutation calling threshold**

We observed an apparent sequencing + PCR error rate < 0.13% after quality filters, which is broadly in line with sequencing errors observed elsewhere with current Illumina sequencing pipelines[2] and corresponds to a phred-scaled base call quality of 30. Postulating a binomial distribution of variant allele counts with this error probability and a total allele count (read depth) ≥ 1000, one would expect a false positive call rate $<10^{-5}$ when calling variants at VAF ≥ 0.008. To test this, we analyzed the range of VAFs derived from the study of 384 WTCC samples at 13 nucleotide positions at and around *DNMT3A* codon R882. Only amplicons giving ≥1000 reads were included in analyses. We found that the 3710 VAF values (10 positions x 371 samples) at positions outside the R882 hotspot (i.e. non-targets of known leukemia-associated mutations) were always ≤0.0045 indicating a very small combined PCR-MiSeq error rate. The 3 real subclonal samples in this group of 384 were easily distinguishable from error (Supplemental Figure S2D)

## Supplemental References


1 Quail, M. A. *et al.* SASI-Seq: sample assurance Spike-Ins, and highly differentiating 384 barcoding for Illumina sequencing. *BMC genomics* **15**, 110, doi:10.1186/1471-2164-15-110 (2014).

2 Ekblom, R., Smeds, L. & Ellegren, H. Patterns of sequencing coverage bias revealed by ultra-deep sequencing of vertebrate mitochondria. *BMC genomics* **15**, 467, doi:10.1186/1471-2164-15-467 (2014).

| Primer Name | Chromosome | Start coordinate (GRCh37) | Primer Sequence† |
|---|---|---|---|
| 5247756-DNMT3A_p.R882_F | 2 | 25457060 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT CCT CAT GTT CTT GGT GTT TTmA T ‡ |
| 5247757-DNMT3A_p.R882_R | 2 | 25457302 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT TTT CTC CCC CAG GGT MTT mUG |
| 5247759-IDH1_p.R132_F | 2 | 209112927 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TAA ATG TGT GTA AAT ATA CAG TTmA T |
| 5247760-IDH1_p.R132_R | 2 | 209113173 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTR TTA TCT GCA AAA ATA TCY CmCC |
| 5247762-IDH2_p.R140_R172_F | 15 | 90631745 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AAG ARG ATG KCT AGG YGA GmGA |
| 5247764-IDH2_p.R140_R172_R | 15 | 90631986 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTC TCA MAG AGT TCA AGC TGA mAG |
| 5247766-SRSF2_p.P95_F | 17 | 74732797 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGC TTC GCC GCG GAC CTT TmGT |
| 5247767-SRSF2_p.P95_R | 17 | 74733038 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTG AGG ACG CTA TGG ATG CCA mUG |
| 5247768-SF3B1_p.K700_F | 2 | 198266642 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TAG TAA TTT AGA TTT ATG TCG mCC |
| 5247769-SF3B1_p.K700_R | 2 | 198266886 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTG GCA TAG TTA AAA CCT GTG TmUT |
| 5247770-SF3B1_p.K666_F | 2 | 198267228 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT ACC CTG TCT CCT AAA GAA AAmA A |
| 5247771-SF3B1_p.K666_R | 2 | 198267470 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT AGA GCT TTT GCT GTT GTA mGC |
| 5247772-NPM1_p.L287fsX_F | 5 | 170837352 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGT TTG GAA TTA AAT TAC ATC TmGA |
| 5247773-NPM1_p.L287fsX_R | 5 | 170837602 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA AAA TTT TTT AAC AAA TTG TTT AAA mCT |
| 5247774-repeat_CAG_F1 | X | 67545198 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AGG TGG ACC AGA AAT GGA AmAT |
| 5247775-repeat_CAG_R1 | X | 67545441 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT GTG GTC TTT ATC CAA AAG TTmU A |
| 5739576-JAK2V617_F | 9 | 5073696 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AGT CTT TCT TTG AAG CAG CAmA G |
| 5739764-JAK2V617_R | 9 | 5073887 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA GTT TAC ACT GAC ACC TAG CmUG |

**Nucleotide sequences for multiplexed primers used in Plex 1**

* Consecutive primers constitute forward (F) and reverse (R) primer pairs for the indicated loci

† Forward primers format: 5’ ACACTCTTTCCCTACACGACGCTCTTCCGATCT-[gene-specific forward] 3’, Reverse primerformat:5’ TCGGCATTCCTGCTGAACCGCTCTTCCGATCT-[gene-specific reverse] 3’

‡ "m" denotes a single 2'-O-Methyl base in place of the DNA base, used in order to minimise potential primer dimers

| Primer Name* | Chromosome | Start coordinate (GRCh37) | Primer Sequence† |
|---|---|---|---|
| 6029105-JAK2_V617_F | 9 | 5073696 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AGT CTT TCT TTG AAG CAG CAmA G ‡ |
| 6029106-JAK2_V617_R | 9 | 5073887 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA GTT TAC ACT GAC ACC TAG CmUG |
| 6029123-DNMT3A_R882_F | 2 | 25457051 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TCT CTC CAT CCT CAT GTT CTmU G |
| 6029124-DNMT3A_R882_R | 2 | 25457284 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT TGG TTT CCC AGT CCA CTA TmAC |
| 6029109-TET2_H880_F | 4 | 106157575 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AGT GCA TGC AAA ATA CAG GTmU T |
| 6029110-TET2_H880_R | 4 | 106157784 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA ACT GAA GCT TGT TGT RAC TmUC |
| 6029111-TET2_R1214_F | 4 | 106164665 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGA CCC TTG TTT TGT TTT GGmU T |
| 6029112-TET2_R1214_F | 4 | 106164877 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT AAG CTC CGA GTA GAG TTT GmUC |
| 6029113-KIT_exon8_F | 4 | 55589690 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AGT GAA TGT TGC TGA GGT TTmU C |
| 6029114-KIT_exon8_R | 4 | 55589911 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTG TCC TTC CCC TCT GCA TTA TmAA |
| 6029103-KIT_exon17_F | 4 | 55599207 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGG TTT TCT TTT CTC CTC CAmA C |
| 6029104-KIT_exon17_R | 4 | 55599396 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT CCT TTG CAG GAC WGT CAmA G |
| 6029115-NRAS_G12_F | 1 | 115258606 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT ATG GGT AAA GAT GAT CCG ACmA A |
| 6029116-NRAS_G12_R | 1 | 115258831 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTC GCC AAT TAA CCC TGA TTA CmUG |
| 6029121-NRAS_Q61_F | 1 | 115256340 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT CCC TAG TGT GGT AAC CTC ATmU T |
| 6029122-NRAS_Q61_R | 1 | 115256573 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA GAT GGT GAA ACC TGT TTG TmUR |
| 6029107-KRAS_G12_F | 12 | 25398214 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGT TSG ATC ATA TTC RTC CAmC A |
| 6029108-KRAS_G12_R | 12 | 25398416 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA AGG TAC TGG TGG AGT ATT TmGA |
| 6029117-TET2_exon8_F | 4 | 106182816 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGG GAT TCA AAA TGT AAG GGmG A |
| 6029118-TET2_exon8_R | 4 | 106183041 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT TGT TAC AAT TGC TGC CAA TmGA |
| 6029119-FLT3_N676_F | 13 | 28602158 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGC TCA GTG TCT AAT TCC ACmU T |
| 6029120-FLT3_N676_R | 13 | 28602388 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA GAA CTC AAG ATG ATG ACC CmAG |
| 6029125-FLT3_D835_F | 13 | 28592585 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TAG GAA ATA GCA GCC TCA CAmU T |
| 6029126-FLT3_D835_F | 13 | 28592819 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTG GTA CCT CCT ACT GAA GTT GmAG |
| 6029127-ASXL1_F | 20 | 31022393 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT GGC GAG AGG TCA CCA CmUG |
| 6029128-ASXL1_R | 20 | 31022630 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTC TCC CYA TTT AGA GGA TAA GmGC |
| 6029129-RUNX1_F | 21 | 36252791 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TTT TGA AAT GTG GGT TTG TTmG C |
| 6029130-RUNX1_R | 21 | 36253035 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTC ATT TGT CCT TTG ACT GGT GmUT |
| NPM1_p.L287fsX_F | 5 | 170837352 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGT TTG GAA TTA AAT TAC ATC TmGA |
| NPM1_p.L287fsX_R | 5 | 170837602 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA AAA TTT TTT AAC AAA TTG TTT AAA mCT |

**Nucleotide sequences for multiplexed primers used in Plex 2**

* Consecutive primers constitute forward (F) and reverse (R) primer pairs for the indicated loci

† Forward primers format: 5’ ACACTCTTTCCCTACACGACGCTCTTCCGATCT-[gene-specific forward] 3’, Reverse primerformat:5’ TCGGCATTCCTGCTGAACCGCTCTTCCGATCT-[gene-specific reverse] 3’

‡ "m" denotes a single 2'-O-Methyl base in place of the DNA base, used in order to minimise potential primer dimers

| Primer Name* | Chromosome | Start coordinate (GRCh37) | Primer Sequence† |
|---|---|---|---|
| 5247756-DNMT3A_p.R882_F | 2 | 25457060 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT CCT CAT GTT CTT GGT GTT TTmA T ‡ |
| 5247757-DNMT3A_p.R882_R | 2 | 25457302 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT TTT CTC CCC CAG GGT MTT mUG |
| 5247759-IDH1_p.R132H_1_F | 2 | 209112927 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TAA ATG TGT GTA AAT ATA CAG TTmA T |
| 5247760-IDH1_p.R132H_1_R | 2 | 209113173 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTR TTA TCT GCA AAA ATA TCY CmCC |
| 5247762-IDH2_p.R140_R172_F | 15 | 90631745 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AAG ARG ATG KCT AGG YGA GmGA |
| 5247764-IDH2_p.R140_R172_R | 15 | 90631986 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTC TCA MAG AGT TCA AGC TGA mAG |
| 5247766-SRSF2_p.P95_F | 17 | 74732797 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGC TTC GCC GCG GAC CTT TmGT |
| 5247767-SRSF2_p.P95_R | 17 | 74733038 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTG AGG ACG CTA TGG ATG CCA mUG |
| 5247768-SF3B1_p.K700_F | 2 | 198266642 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TAG TAA TTT AGA TTT ATG TCG mCC |
| 5247769-SF3B1_p.K700_R | 2 | 198266886 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTG GCA TAG TTA AAA CCT GTG TmUT |
| 5247770-SF3B1_p.K666_F | 2 | 198267228 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT ACC CTG TCT CCT AAA GAA AAmA A |
| 5247771-SF3B1_p.K666_R | 2 | 198267470 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT AGA GCT TTT GCT GTT GTA mGC |
| 5247772-NPM1_p.L287fsX_F | 5 | 170837352 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGT TTG GAA TTA AAT TAC ATC TmGA |
| 5247773-NPM1_p.L287fsX_R | 5 | 170837602 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA AAA TTT TTT AAC AAA TTG TTT AAA mCT |
| 5739576-JAK2V617_F | 9 | 5073696 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT AGT CTT TCT TTG AAG CAG CAmA G |
| 5739764-JAK2V617_R | 9 | 5073887 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA GTT TAC ACT GAC ACC TAG CmUG |
| 6029103-KIT_exon17_F | 4 | 55599207 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGG TTT TCT TTT CTC CTC CAmA C |
| 6029104-KIT_exon17_R | 4 | 55599396 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTT CCT TTG CAG GAC WGT CAmA G |
| 6029107-KRAS_G12_F | 12 | 25398214 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TGT TSG ATC ATA TTC RTC CAmC A |
| 6029108-KRAS_G12_R | 12 | 25398416 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA AGG TAC TGG TGG AGT ATT TmGA |
| 6029115-NRAS_G12_F | 1 | 115258606 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT ATG GGT AAA GAT GAT CCG ACmA A |
| 6029116-NRAS_G12_R | 1 | 115258831 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTC GCC AAT TAA CCC TGA TTA CmUG |
| 6029121-NRAS_Q61_F | 1 | 115256340 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT CCC TAG TGT GGT AAC CTC ATmU T |
| 6029122-NRAS_Q61_R | 1 | 115256573 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTA GAT GGT GAA ACC TGT TTG TmUR |
| 6029125-FLT3_D835_F | 13 | 28592585 | ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT TAG GAA ATA GCA GCC TCA CAmU T |
| 6029126-FLT3_D835_R | 13 | 28592819 | TCG GCA TTC CTG CTG AAC CGC TCT TCC GAT CTG GTA CCT CCT ACT GAA GTT GmAG |

**Nucleotide sequences for multiplexed primers used in Plex 3**

* Consecutive primers constitute forward (F) and reverse (R) primer pairs for the indicated loci

† Forward primers format: 5’ ACACTCTTTCCCTACACGACGCTCTTCCGATCT-[gene-specific forward] 3’, Reverse primerformat:5’ TCGGCATTCCTGCTGAACCGCTCTTCCGATCT-[gene-specific reverse] 3’

‡ "m" denotes a single 2'-O-Methyl base in place of the DNA base, used in order to minimise potential primer dimers