Title:

MGP Panel: a comprehensive targeted genomics panel for molecular profiling of multiple myeloma patients

Supplementary Data

Methods

Sample Processing (version 1)

100 ng of tumor and normal control DNA in low EDTA buffer was used to prepare libraries using the HyperPlus kit (KAPA Biosystems). Briefly, DNA was enzymatically fragmented, followed by end repair and A-tailing, before ligation with dual index adapters. Ligated products were cleaned up using AMPureXP Beads (Beckman). After bead cleanup, the prepared libraries underwent dual size selection to remove products of unwanted size before being amplified with a universal primer for 6 cycles. Amplified libraries were purified using AMPure XP beads and were hybridized overnight to the mutation and translocation captures (SeqCap EZ target enrichment; Nimblegen) mixed at a ratio of 8:1 (4.0 µl plus 0.5 µl). Up to 16 samples (8 patients with tumor/control pairs) with unique indices were combined to 1500 ng DNA for hybridization. After hybridization cleanup, the eluted DNA was further amplified for 11 cycles. After bead cleanup of amplified DNA, the concentration and size distribution of the hybridized libraries was determined to allow for optimal sequencing. The HiSeq 2500 or NextSeq500 (Illumina, San Diego, CA, USA) were used for sequencing with 75 bp paired-end reads. The first 185 tumor samples were processed using this version of reagents.

Sample Processing (version 2.1)

Due to manufacturing changes in the way probes were deigned and made, the protocol was updated for the newer HyperCap EZ target enrichment reagents (KAPA Biosystems). The overall process is highly similar, with the following modifications. HyperPure beads (KAPA Biosystems) were used instead of AMPure XP (Beckman Coulter) beads. At the ligation stage, a universal adapter was added to the sample DNA instead of unique dual

index adapters. Conversely, at the PCR stage, unique dual index adapters were used for each sample. The dual size selection was moved from before the PCR step to afterwards. Hybridization was essentially identical, but used the newer HyperCap reagents instead of the deprecated SeqCap reagents and the ratio of panels was modified to 7:1 (3.5 µl + 0.5 µl) to accommodate new reaction volumes.

16 samples were repeated using the newer reagents to ensure consistency between the methodologies (**Supplementary Figure 2 and 3**). The full HyperCap protocol can be found in the **Supplementary Protocol**.

Mutation allele standards

To test the accuracy of mutation detection by both the panel and the bioinformatic pipeline we utilized a set of standards with known mutations and allele frequencies. Five DNA standards were used with varying allele frequencies: Tru-Q1 (5% tier), Tru-Q3 (5% tier), Tru-Q7 (1.3% tier), Tru-Q0 (30% tier), and the Myeloid DNA reference standard (5% tier) (Horizon Discovery). These five samples were interrogated by the panel and a random germline sample was used as the non-tumor DNA for analysis purposes. In total, 74 mutations in 16 genes were compared for variant allele frequency (VAF) between the expected (standards) and observed (panel) values. Expected VAFs varied from 1.3-40%.

Multiplex Ligation Dependent Probe Amplification (MLPA)

MLPA was performed on 13 MM cell lines and 101 patient specimens using the Salsa MLPA Probemix P425 Multiple Myeloma (MRC Holland, Netherlands) based on the modified protocol of Schouten et al.. For MLPA experiments, 50-250 ng genomic DNA was denatured at 98°C for 5 minutes followed by hybridization at 60°C for 16-20 hours in hybridization mixture. A ligation reaction was performed using ligase 65 master mix at 54°C for 15 minutes. PCR was performed using master mix containing Salsa PCR primer mix and Salsa polymerase with the following PCR reaction: 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds and final extension at 72°C for 20 minutes. Fragment separation was performed by capillary electrophoresis on a SeqStudio™ Genetic Analyzer System. Coffalyser.Net software, in combination with the appropriate lot-specific Coffalyser sheet, was used for MLPA data analysis. For intrasample normalization, within each sample, each probe peak was compared to the

peaks of the reference probes. Reference probes detect sequences that are expected to have a normal copy number in all samples. MLPA probe-mixes contained 8 or more reference probes located on various chromosomes. For inter-sample normalization, final probe ratios were determined by comparing the relative probe peak in the DNA sample of interest to all reference samples. Genomic DNA from Lonza™ Human Lung Fibroblasts (NHLF), Human Dermal Fibroblasts, neonatal (HDFn) and human female genomic DNA (Promega) were used as reference DNA samples, which are expected to have a normal copy number for both the reference and target probes.

Whole Genome Sequencing

Genomic DNA from tumor and non-tumor samples were first evaluated for quantity, and quality, using the TapeStation 4200 (Agilent) and Qubit Fluorometer 3.0 (ThermoFisher). About 300 ng high quality genomic DNA was used for library preparation. Briefly, DNA was prepared using the DNA PCR-free Library Prep Tagmentation Kit (Illumina). Libraries were pooled and sequenced in 150 bp paired-end read format on a NovaSeq 6000 sequencer (Illumina, Inc.) to a mean depth of 73x. Data were processed and aligned to hg38. Translocations were detected in the same way as for the panel using Manta (v1.6.0), and copy number abnormalities were detected using AscatNgs.[1] For homozygous deletion detection, Control-FREEC[2] or Battenburg[3] was also used to confirm events in *CDKN2C*, *RB1*, or *TP53* which were detected on the panel. Mutations were determined using Strelka2 and filtered using fpfilter as before.

References

1. Raine KM, Van Loo P, Wedge DC, et al. ascatNgs: Identifying Somatically Acquired Copy-Number Alterations from Whole-Genome Sequencing Data. *Curr Protoc Bioinformatics*. 2016;56:15 19 11-15 19 17.
2. Boeva V, Popova T, Bleakley K, et al. Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics*. 2012;28(3):423-425.
3. Nik-Zainal S, Alexandrov LB, Wedge DC, et al. Mutational processes molding the genomes of 21 breast cancers. *Cell*. 2012;149(5):979-993.