Whole Genome Sequencing and Analysis - Illumina

Acute Leukemias of Ambiguous Lineage (ALAL)

PCR-free whole genome sequencing:

To minimize library bias and coverage gaps associated with PCR amplification of high GC or AT-rich regions we have implemented a version of the TruSeq DNA PCR-free kit (E6875-6877B-GSC, New England Biolabs), automated on a Microlab NIMBUS liquid handling robot (Hamilton).

Briefly, 500ng of genomic DNA was arrayed in a 96-well microtitre plate and subjected to shearing by sonication (Covaris LE220). Sheared DNA was end-repaired, and size selected using paramagnetic PCRClean DX beads (C-1003-450, Aline Biosciences) targeting a 300-400bp fraction. After 3' A-tailing, full length TruSeq adapters were ligated. Libraries were purified using paramagnetic (Aline Biosciences) beads. PCR-free genome library concentrations were quantified using a qPCR Library Quantification kit (KAPA, KK4824) prior to sequencing with paired-end 125 base reads on the Illumina HiSeq2500 platform using V4 chemistry according to manufacturer recommendations.