Library Preparation and DNA Sequencing

Following initial sample dsDNA quality control for quantity with a Qubit fluorometer and size distribution with an Agilent Fragment Analyzer, 50 ng of gDNA is enzymatically fragmented using Twist Bioscience's Library Preparation EF Kit 2.0 (Twist Bioscience, 104207). Index adapters (Twist Bioscience, 100577) are ligated onto the DNA fragments, and then amplified with 10 cycles of PCR. Libraries are purified with Binding and Purification beads (Twist Bioscience, 100983), quantified with a Qubit fluorometer, and checked again on a Fragment Analyzer. Up to 8 libraries of like organisms are pooled together with a total mass of 1500 ng/pool. Human DNA is then hybridized with Twist's Comprehensive Exome probes (Twist Bioscience, 102032) and mouse DNA is hybridized with Twist's Mouse Exome probes (Twist Bioscience, 101239). Samples are hybridized for 30 minutes with Twist's Fast Hybridization Reagents (Twist Bioscience, 104180) before being bound to streptavidin beads and washed with Twist's Fast Hybridization and Wash Kit (Twist Bioscience, 104180). Following hybridization, hybridized pools are amplified with 8 cycles of PCR using primers and master mix from the Library Preparation Kit EF. Final libraries are purified with Twist's Binding and Purification beads, quantified with a Qubit fluorometer, and their size checked with a Fragment Analyzer. Libraries are sequenced on an Illumina NovaSeq X Plus, generating > 25 million 100 bp read pairs and analyzed using Illumina's Dragen enrichment pipeline.

Data Alignment and Analysis Methods

Reads were aligned using a functionally equivalent pipeline, as defined in [doi 10.1038/s41467-018-06159-4]. Specifically, each read pair was separately aligned to the appropriate reference (GRCh38, mm38) genome using bwa-mem version 0.7.17 with the -M option (Li 2009). The resulting SAM files are first sorted and converted to BAM with Picard SortSam (version 2.18.22), then all resulting BAMs are merged and duplicate marked with Picard MarkDuplicates (Picard 2019) using the "`OPTICAL_DUPLICATE_PIXEL_DISTANCE=2500`" command line flag.

References

"Picard Toolkit." 2019. Broad Institute, GitHub Repository. https://broadinstitute.github.io/picard/; Broad Institute

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform *Bioinformatics*, *25*(14), 1754–1760. doi:10.1093/bioinformatics/btp324