The ROYAL MARSDEN Clinical Genomics

TruSight RNA Pan_Cancer Panel for fusion detection

The TruSight RNA Pan Cancer panel (catalogue ID: RS-303-1002 and RS-303-1003) is used to detect cancer-related gene fusions in a selection of clinically relevant genes. The Illumina TruSight RNA Fusion Panel is a RNA-seq assay that uses capture-based enrichment to target 1385 cancer fusion-associated genes. Total RNA is extracted from fresh and FFPE samples and converted to double-stranded cDNA, followed by ligation of indexes. Enrichment steps are carried out by hybridisation of oligonucleotide probes targeting the 1,385 genes, followed by isolation from solution by magnetic pulldown. 21,043 exonic regions are targeted with 57,010 probes. Libraries are pooled and sequenced on the NextSeq 550 or MiSeq using 2x76bp paired-end conditions with single or dual indexing. Analysis is performed using RNA-Seq Alignment App v2.0.2 (BaseSpace Sequencing Hub) using STAR aligner (to RefSeq Homo Sapiens/ hg19 genome) and Manta for gene fusion calling with default parameters.
The protocol enables identification of fusions in a semi-agnostic approach, i.e. only 1 of the genes involved in the fusion needs to be covered by the panel design to allow detection. For example, NTRK3 is covered in the panel design, AKAP13 is not but an NTRK3–AKAP13 is detectable.

As per Illumina Pan Cancer Gene fusion panel targeting 1,385 cancer-associated genes and 21,043 exons: https://emea.support.illumina.com/downloads/trusight-rna-pan-cancer-target-regions-files.html. This kit targets 160 bp of the 5' and 3' UTR of every targeted gene, which ensures that the full gene of every targeted gene is covered.

Overall performance

Sensitivity: 100% (95%CI: 66.37% to 100.00%)
Specificity: 100% (95%CI: 78.20% to 100.00%)
Accuracy: 100% (95%CI: 85.75% to 100.00)

Trueness: 94.00% (95% CI: 83.45% to 98.75%)

Assay has a low failure rate of 7.5%.

Detection of fusions/translocations is currently undergoing validation, therefore any variants should be subject to confirmation by an orthogonal method. The method is not currently suitable for detection of *STIL-TAL1* fusions (or other fusions in genes on the same chromosome, at close proximity and in the same orientation).