

Resource Summary Report

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An error-corrected panel-based next-generation sequencing assay for ultra-sensitive somatic mutation detection in ctDNA

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.bb3siqne>

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Summary: Circulating tumour DNA (ctDNA), fragmented DNA shed into the circulation from tumour cells, holds the potential to become a minimally-invasive biopsy material with which to interrogate the tumour landscape. However, ctDNA can be highly diluted in normal cell-free DNA (cfDNA) (released into the blood primarily by hematopoietic cells), and thus robust liquid biopsy assays with high analytical sensitivity are required for ctDNA to reach its full clinical potential in molecular genotyping. This protocol uses optimised off-the-shelf reagents in order to generate indexed and molecularly-tagged cfDNA and germline DNA libraries compatible with Illumina short-read sequencing. This protocol involves the hybridisation-capture of targeted regions of the genome using RNA baits - and has been used previously with a 42-gene panel 447 kbp in size. Also described here is the bioinformatic tools used for data processing and variant identification. The combination of deep-sequencing and error-correction based on molecular barcoding allows high sensitivity and specificity to be generated at low variant frequencies.

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