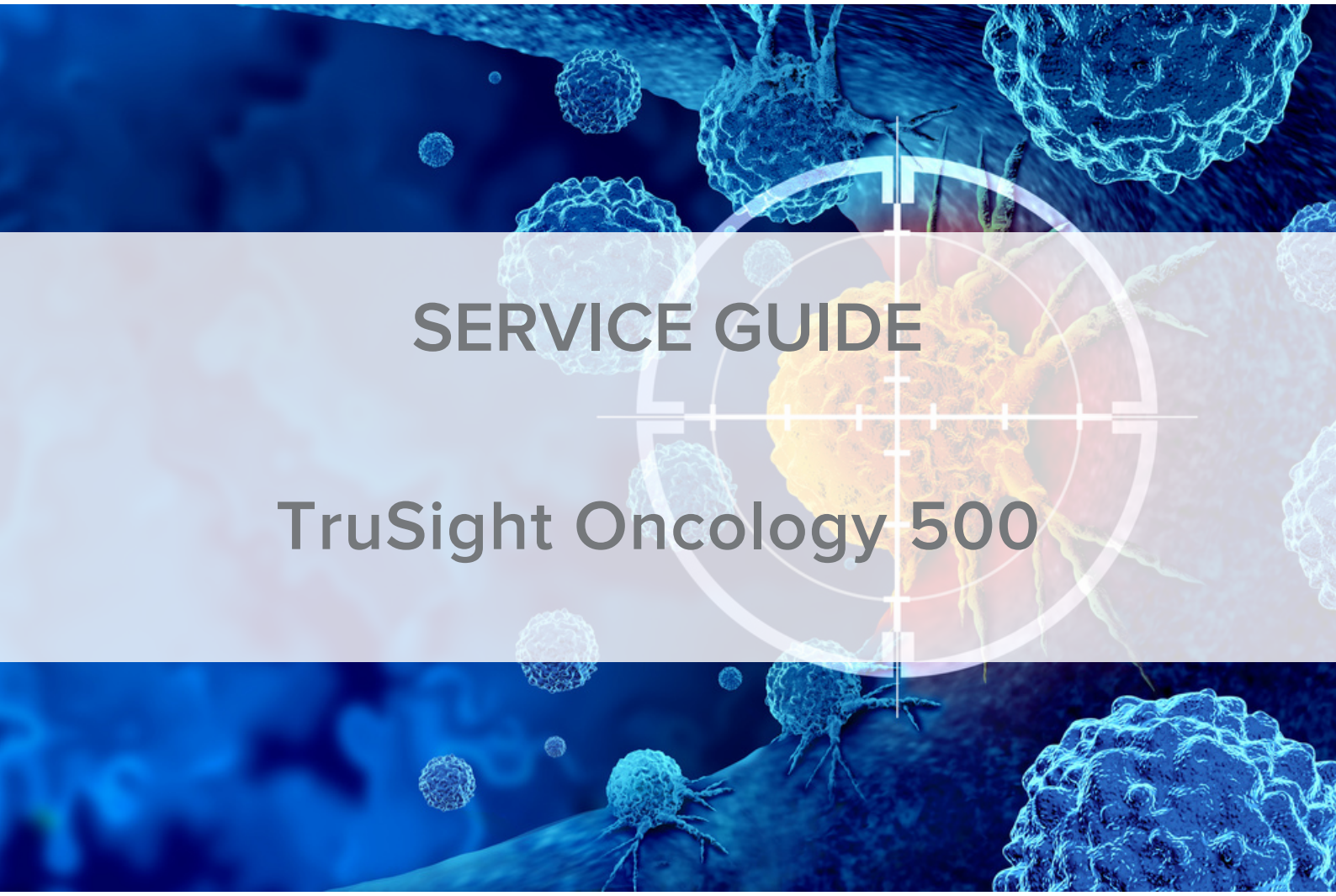

SERVICE GUIDE

TruSight Oncology 500

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www.agrf.org.au

SVG2402TSO

Service Guide
TSO500

Service Guide
TSO500

1.0 Overview

The TruSight Oncology 500 HT DNA/RNA assay is a single comprehensive NGS screening assay containing an excess of 500 DNA and RNA genes. The TSO500 assay uses a hybrid workflow for RNA and DNA samples, extracted from solid tumour samples, to provide screening with high sensitivity for low frequency somatic variants across 523 from DNA and 55 genes for known and novel fusion and splice variants from RNA. Therefore, saving sample and time, to maximise ability to find cancer relevant biomarkers. Biomarkers include;

- Single nucleotides variants (SNVs)
- Insertions
- Deletions
- Gene amplifications
- Multi-nucleotide variants (MNVs)
- Gene fusions
- Microsatellite instability (MSI)
- Tumour mutational burden (TMB)

AGRF Trusight Oncology 500 HT service is for processing RNA and DNA extracted from the same sample only. Data is analysed using Illumina DRAGEN TSO500 or LocalApp workflows with one comprehensive analysis report for concise reviewing.

2.0 Submission types for TSO500

Samples can be supplied to AGRF as either extracted RNA and DNA or FFPE samples for extraction. Please discuss your options with your account manager prior to quote being issued. If using our extraction service please supply duplicate submissions for RNA and DNA extractions. We require a DNA and RNA from each sample.

2.1 Extracted RNA and DNA

AGRF requires a minimum 500 ng of RNA, (20 ng/µl) and 200 ng, (10 ng/µl) DNA, to allow for processing and quality control of samples. Prior to processing RNA will be quality accessed on the Agilent Tape Station and quantified by qubit. We would recommend not processing RNA samples with a DV200 of less than 20%.
DNA will be quality accessed by Agilent Tape Station and quantified by qubit. We would recommend not processing DNA samples with a DIN of less than 1.5. AGRF are happy to review your QC data prior to submitting. Forward files to techsupport@agrf.org.au

2.2 FFPE tissue for DNA and RNA extraction

Sample material for DNA and RNA extraction from FFPE tissue needs to be supplied in an eppendorf. Sections, scrolls, need to be no more than 5 to 10 µm in thickness, cut from a FFPE block using a microtome. The total area of actual tissue, excluding wax, needs to be no less than 4 mm^3 for each extraction. Please provide no less than 8 mm^3 in total, 4 mm^3 in separate eppendorfs, to streamline RNA and DNA. If calculating the amount is not possible, please provide 20 sections (10 for DNA and 10 for RNA) of 5 µm thickness or an equivalent volume. Do NOT submit whole paraffin blocks.

RNA and DNA yield from FFPE samples varies greatly, depending on the tissue type, as well as fixation and embedding conditions. Samples will be quality checked prior to processing and any concerns reported prior to processing.

3.0 Technical Consideration

The solid tumour assay processes RNA and DNA extracted from formalin-fixed parafilm embedded (FFPE) samples. Samples will be processed in batches of 16, 8 x RNA and 8 x DNA.

4.0 Sample Returns/Discards

Samples are stored with AGRF for 3 months after you receive your data. If you wish for your samples to be returned, you must discuss this with your account manager during quoting or contact us after you receive your data. At the completion of your project, we can either:

- Return your samples by courier at ambient (please ask your account manager for a quote).
- Return samples by courier with dry ice (please ask your account manager for a quote).

If we are not notified within the specified time frame, samples will be automatically discarded.

5.0 How to Submit Samples

Online Submission:

- Submit your sample details online.
- Select: “Next-Generation Sequencing” as the Service Type.
- 0≤23 samples and all tissue samples requiring extraction:
- Please complete tube submissions.
- ≥24 RNA/DNA (samples not requiring extraction);
- Please complete plate submissions, (an additional handling charge of $1.50 per sample will occur if tubes are used).
- We recommend shipping plates that are heat-sealed, or strip-cap sealed on dry ice.
- AGRF can organise dry ice shipping for your samples, please contact us for further details.
- Submission Format – by selecting tube or plate, the “SampleFile” template link will appear. Click “Download Template” and enter your sample details:
- Sample Name can be up to 40 characters in length.
- Please include the ‘gender at birth’ to allow us to perform a sex chromosomal ploidy check.
- You can use numbers, letters, underscores and hyphens.
- The first character must be a letter or number. For example: Sample-1_b.
- An error will occur if a name is duplicated.
- Save and close completed Template File locally, select “Browse” to upload file.
- Submit and print a paper copy of your sample submission receipt that will be generated as a PDF file.

This receipt must be included with your sample package.

AGRF can organise dry ice shipment for your samples as part of your quoted services or you can use our free shipping between nodes once a week service. For information on this service go to Free Shipping.

Post/send/deliver samples to the addresses below:

Melbourne TSO500 service:
AUSTRALIAN GENOME RESEARCH FACILITY
LEVEL 13,
VICTORIAN COMPREHENSIVE CANCER CENTRE
305 GRATTAN STREET
MELBOURNE, VIC, 3000

6.0 Results and Data Outputs

Sequencing is performed on the NovaSeq X plus, 150 bp paired end, 50 million reads per library.

The bioinformatic analysis may utilise either the Illumina LocalApp or Illumina TSO500 DRAGEN workflows. These analysis pipelines will perform alignment to the hg19 human reference genome, small variant calling and filtering, copy number calling, gene fusion calling, annotation, estimate tumour mutational burden and microsatellite instability, as well as detect human to human DNA contamination. Output files will consist of alignment files (.bam), variant call format files (.vcf) and additional quality metric and variant information. The Illumina TSO500 DRAGEN workflow will also perform BRCA Exon-level copy number calling. The clinical service encompasses sample gender confirmation checks.

7.0 Quality Statement

All clinical works carried out by AGRF follow the strict requirements of ISO15189. AGRF Ltd is accredited by the National Association of Testing Authorities (NATA) in the field of Medical Testing (Scope: Investigation of constitutional genetic variants - Diagnostic Testing. Whole exome sequencing studies for inherited (germline) DNA/RNA changes). Staff and analysis processes follow Standard Operating Procedures, which define responsibilities and quality checks to achieve reported standards. Compliance is monitored at regular reviews and during internal audits. The work is supervised by a person with relevant qualifications and checked while in progress and upon completion to ensure that it meets the necessary ISO15189 standards.