Oxford Nanopore Technologies

Tumour Methylation Profiling FAQs

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Important: Tumour Methylation Profiling is for Research Use Only (RUO). It is not validated for clinical use, is not intended for diagnostic procedures, and must not be used to inform clinical decisions. Third-party analysis tools described in this FAQ are not part of the protocol suite and are not supported or validated by Oxford Nanopore.

Product Overview

1. What is the tumour methylation profiling product?

Tumour Methylation Profiling is a suite of three Research Use Only (RUO) protocols for molecular profiling of CNS and other solid tumours. Each protocol combines DNA methylation profiling with genomic information in a single assay. The three protocols are:

- Ultra-rapid singleplex adaptive sampling protocol
- Singleplex adaptive sampling protocol
- Multiplex methylation whole genome sequencing protocol

Each protocol offers a different balance of speed, targeted enrichment, coverage and multiplexing.

2. What are the differences between the tumour methylation profiling protocols?

The three protocols address different unmet needs. The two Singleplex protocols use Adaptive Sampling, an on-device targeted sequencing approach, together with the RUO Pan-Cancer Digital Panel to enrich for 723 cancer-associated genes.

- Ultra-rapid Singleplex Adaptive Sampling: ideal for time-critical applications when paired with extraction using the QIAamp Fast DNA Tissue Kit (Qiagen, 51404).
- Singleplex Adaptive Sampling: uses barcode-based indexing for simplified sample tracking and is compatible with lower sample inputs.
- Multiplex Methylation Whole Genome Sequencing: uses a low-pass WGS approach to sequence four samples per PromethION flow cell. It suits cost-sensitive applications that require only methylation classification and large-scale copy number variant analysis

3. What bundle configurations are available for tumour methylation profiling?

Tumour Methylation Profiling is available in four bundle configurations, covering the three protocols:

- Ultra-rapid Singleplex Adaptive Sampling: 6 samples
- Singleplex Adaptive Sampling: 6 samples
- Singleplex Adaptive Sampling: 18 samples
- Multiplex Methylation Whole Genome Sequencing: 24 samples

Each bundle includes the Oxford Nanopore library preparation reagents and flow cells needed for the stated number of samples. For pricing and ordering visit our bundles store page.

4. What does the Pan-Cancer Digital panel target?

The Pan-Cancer Digital Panel includes 723 cancer-associated genes, covering exons, introns and promoters, with 20 kb flanking buffers. It is designed to support the detection of:

- Single nucleotide variants (SNVs)
- Insertions and deletions (indels)
- Structural variants (SVs)

- Copy number variants (CNVs)
- Genome-wide methylation patterns

The table below is a non-exhaustive list of key biomarkers and the tumour types associated with them in published research studies.

Tumour Types	Genes
CNS tumours	IDH1, IDH2, ATRX, TP53, CIC, FUBP1, TERT, EGFR, PDGFRA, PTEN, NF1, CDKN2A, CDKN2B, MET, BRAF, FGFR1, MYC, MYCN, MGMT, H3-3A, NTRK1, NTRK2, NTRK3
Breast	BRCA1, BRCA2, PALB2, ATM, CHEK2, ESR1, ERBB2, PIK3CA, AKT1, PTEN, TP53, CDH1, CCND1, FOXA1, GATA3
Colorectal	APC, ALK, KRAS, NRAS, BRAF, PIK3CA, PTEN, TP53, SMAD4, ERBB2, FBXW7, RNF43, POLE, POLD1, MLH1, MSH2, MSH6, PMS2
Head and Neck	TP53, PIK3CA, EGFR, NOTCH1, HRAS, CDKN2A, FAT1, CCND1, CASP8
(Non-Small Cell) Lung	EGFR, ALK, ROS1, RET, MET, KRAS, BRAF, ERBB2, FGFR1, NTRK1, NTRK2, NTRK3, STK11, KEAP1, TP53, PIK3CA, MAP2K1, MAP2K2
Melanoma	ARID2, BRAF, CDKN2A, NRAS, KIT, NF1, PTEN, RAC1, MAP2K1, MAP2K2, TERT, POLD1
Ovarian	BRCA1, BRCA2, ERBB2, BRIP1, PALB2, RAD51B, RAD51C, RAD51D, ATM, TP53, ARID1A, NF1, CCNE1, PIK3CA, PTEN, MSH2
Pancreatic	KRAS, TP53, CDKN2A, SMAD4, BRCA1, BRCA2, PALB2, ATM, STK11
Prostate	AR, BRCA1, BRCA2, ATM, ATR, CDK12, CHEK2, ERG, PALB2, MYC, NBN, NKX3-1, RAD51B, RAD51C, RAD51D, PIK3CB, HOXB13, FOXA1, SPOP, PTEN, TP53, tumour methylation profilingRSS2, KMT2C, KMT2C
Sarcoma	EWSR1, FUS, BCOR, CIC, NAB2, PAX3, PAX7, NF1, TP53, CDK4, KIT, MDM2, PDGFRA
Hematologic Malignancies	ABL1, ASXL1, BCL2, BCL6, BCR, BTK, CALR, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, MYD88, NOTCH1, NPM1, RUNX1, STAT3, TET2, TP53
And others: Bladder, Cervical, Cholangiocarcinoma, Endometrial, Gastric/Gastroesophageal, Liver, Neuroendocrine, Pan-cancer, Renal Cell, Thyroid, etc	

5. Are the tumour methylation protocols validated for clinical use or diagnosis?

No. Tumour Methylation Profiling is a Research Use Only (RUO) product. It is not validated or authorised for use in clinical or diagnostic procedures.

Technical Details – Sample and library preparation

6. What type of samples have been tested with the protocols?

The protocols have been tested with DNA extracted from fresh or frozen tissue. Input requirements are defined for each protocol.

7. Does tumour methylation profiling support FFPE tumour samples?

No, tumour methylation profiling protocols do not support FFPE samples.

8. What extraction methods are compatible with the tumour methylation profiling protocols?

The protocols use DNA extracted from tissue and should be compatible with any extraction method that meets the quality and quantity specifications in the protocols. We have successfully tested compatibility with the following kits:

- QIAmp fast DNA tissue kit (Qiagen) – recommended for Ultra-Rapid AS protocol
- Quiagen Puregene Tissue kit
- MagAttract HMW DNA kit (Qiagen)

9. What input material is required and how much DNA is needed?

The standard input is high-quality genomic extracted for fresh or frozen tissue. The recommended amount varies by protocol:

Protocols	Standard input	Range
Ultra-rapid Singleplex AS	1 µg	maximum: 2 µg (recommended for optimal pore occupancy)
Singleplex AS	1 µg	minimum: 0.5 µg maximum: 2 µg (recommended for optimal pore occupancy)
Multiplex Methylation WGS	250 ng	maximum: 1ug (recommended for optimal pore occupancy)

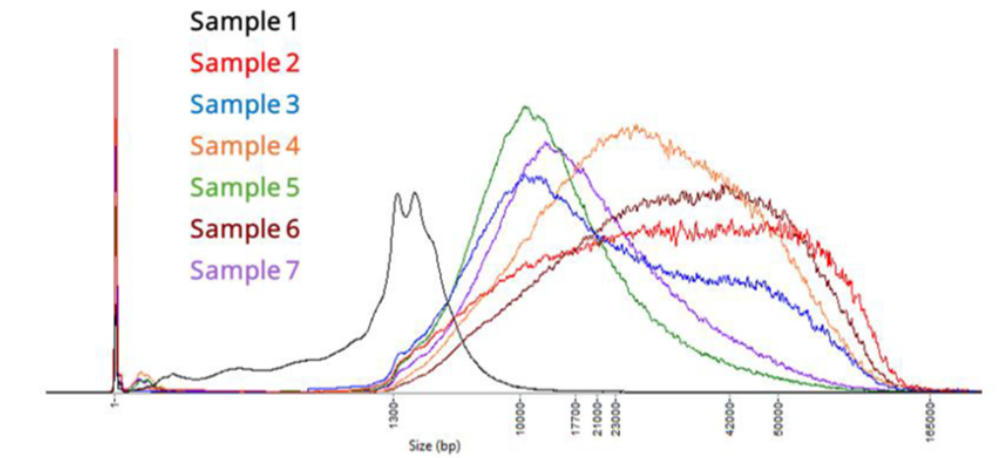
DNA should have N50 fragment lengths 6kb – 10kb assessed using the Agilent FemtoPulse system with the Genomic DNA 165 kb kit.

10. How should I evaluate the quality of extracted DNA before shearing?

After gDNA extraction, assess sample quality on the Agilent Femto Pulse System to visualise the unsheared gDNA profile and confirm integrity and size distribution.

IMPORTANT: For the Ultra-rapid Singleplex AS protocol perform the QC analysis after completion of sample preparation and initiation of the sequencing run to minimise workflow time.

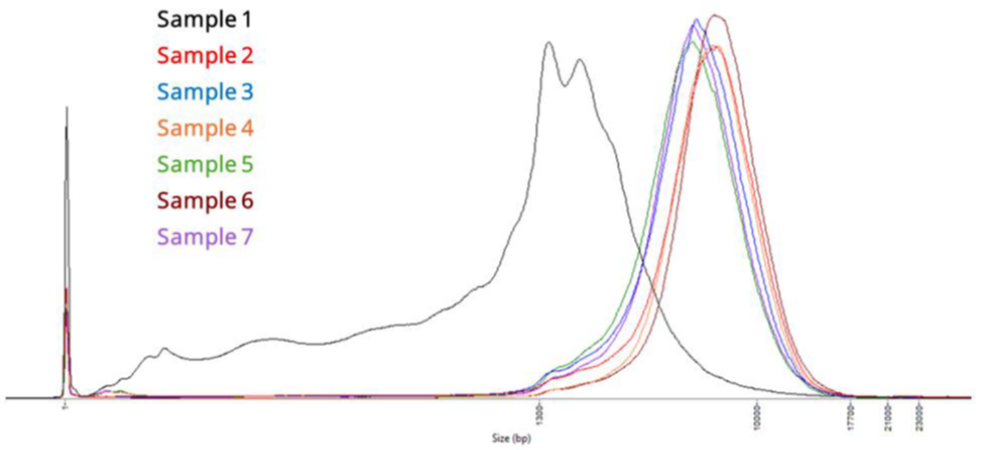
The figure below shows fragment length profiles for unsheared genomic DNA from seven commercial tumour samples, extracted using the QIAamp Fast DNA Tissue Kit. Sample 1 shows reduced fragment lengths, consistent with higher levels of necrosis. Samples 2–7 show acceptable fragment size distributions. Avoid degraded DNA or low-molecular-weight fragments, as these can reduce read lengths and adaptive sampling efficiency.



11. Recommendations for evaluating the quality of sheared DNA prior to sequencing?

After gDNA shearing, assess sample quality on the Agilent Femto Pulse System to confirm integrity and size distribution.

The figure below shows fragment length profiles for the same seven samples after shearing. Sample 1 continues to show reduced fragment lengths, consistent with higher necrosis levels or damaged samples. Avoid degraded DNA or low-molecular-weight fragments for the reasons above. Samples 2–7 show acceptable fragment size distributions.



12. How shearing efficiency affect enrichment and sequencing results?

Inefficient shearing with the recommended g-TUBE can cause pore blockage and reduced coverage.

Technical Details - Sequencing

13. What type of flow cell is tumour methylation profiling compatible with?

All protocols are compatible with, and have been tested on, PromethION R10.4.1 (FLO-PRO114M) flow cells. They can therefore be run on a PromethION 2 Integrated or a PromethION 24. Find out more about our compatible devices here.

14. Which MinKNOW software version is recommended for sequencing?

We recommend MinKNOW 26.01 LTS (Long-Term Support), the version used to generate the published Tumour Methylation Profiling data. Key characteristics of 26.01 LTS:

- A fixed major version with strict change control and timely change notifications
- Maintained for a minimum of 36 months, with at least 6 months' notice of changes
- Supported by a dedicated software development and QA effort

15. Where can the Pan-Cancer BED file be found?

You can access the Pan-Cancer BED file in the product pages for Tumour Methylation Profiling and the Adaptive Sampling. This includes the analysis BED file.

16. Is it possible to add or remove genes from the Pan-Cancer BED file?

The gene list can be edited to create a custom panel via our Adaptive Sampling Digital Panel Online Designer. Performance metrics may vary for custom panels.

17. Which basecalling models are recommended?

We recommend running High Accuracy Basecalling (HAC) for the Multiplex Methylation WGS protocol and Super High Accuracy Basecalling (SUP) for both Adaptive Sampling protocols. The protocols have been validated using live basecalling models v5.2 integrated in MinKNOW 26.01 LTS. More details are available in the Somatic Analysis Bioinformatic Guide.

18. What are the sequencing runtimes recommendations?

Protocols	Methylation classification	Variants SVs, SNPs, CNVs
Ultra-rapid Singleplex AS	Minimum 30min*	Full length of the PromethION FC (~72h)
Singleplex AS	Minimum 1h (or > 0.75Gb)	
Multiplex Methylation WGS	Minimum 2h (or > 0.75Gb)	For CNVs: after least 16h of sequencing

* Methylation output files (BAMs) are generated approximately every 2 minutes, corresponding to 50,000 reads per BAM file, so you can monitor classification scores earlier if you wish. The time to a high-confidence classification depends on the classifier, sample quality (tumour content) and tumour type.

19. What is the recommended genome assembly?

We recommend the GIABv3 GRCh38 alt masked reference genome which improves mapping in this build. Other reference genomes can contain false duplications that reduce adaptive sampling coverage and the fidelity of variant calls. The Genome in a Bottle (GIAB) masked reference corrects for these while maintaining GRCh38 coordinates.

Technical Details - Analysis

20. What is the expected coverage and uniformity for the Pan-cancer panel?

In our testing on a 5k-pore PromethION flow cell, the Pan-Cancer Digital Panel achieved more than 75x coverage on target regions and more than 7x low-pass coverage across the genome, with a fold-80 of 1.18.

21. What are the recommended analysis pipelines for tumour methylation profiling?

We recommend the Somatic Analysis Bioinformatics Guide which covers a range of third-party somatic variant callers and methylation classifiers.

22. What are the compute requirements for using 3rd party analysis tools?

Compute requirements vary by tool, dataset size, and intended application. Users should refer to the documentation provided by the tool developers or providers and contact the respective authors for recommendations.

A high-level guidance table is available in the Somatic Analysis Bioinformatic Guide for informational purposes only and should not be considered a substitute for tool-specific guidance.

23. Where can I find the tumour classification and variant call outputs?

The variant calling and classification sections of the Somatic Analysis Bioinformatics Guide describe where each tool writes its output files. BAM files output by MinKNOW contain modification call information when a modified-base model was used during basecalling.

24. What variant callers does tumour methylation profiling recommend?

Tumour methylation profiling recommends:

- Severus v1.7 for SVs
- ichorCNA v.0.6.0. for CNVs
- DeepSomatic v1.10.0 for low variant VAF SNVs and indels.

For more information, please consult the Somatic Analysis Bioinformatics Guide.

25. Which methylation classifiers for CNS tumours should the customers use?

Tumour Methylation Profiling is compatible with several methylation classifiers. Choosing the classifier that best fits your research need is up to you. Compatibility by protocol is shown below:

Methylation Classifiers	Protocols
CrossNN (nanoDX)	All protocols
SturgeonV1 and V2	All protocols
Epinn (Argus-CNS)	All protocols
Rapid-CNS2	Singleplex Adaptive Sampling and Methylation Multiplex WGS
MNP-flex	Singleplex Adaptive Sampling and Methylation Multiplex WGS
ROBIN	All protocols but settings need adjusting (see following question)

For more information, please consult the Somatic Analysis Bioinformatics Guide.

26. Which BAM output frequency should I select when running ROBIN as methylation-based classifier?

ROBIN v0.5 supports BAM files containing up to 50,000 reads, including supplementary alignments. As MinKNOW excludes supplementary alignments from read counts, we recommend configuring MinKNOW to write 35,000 reads per file. Exceeding the supported read count may result in delayed classifications or out of memory errors. For more detailed information, please consult the Somatic Analysis Bioinformatics Guide.

27. Where can I find CNS tumour sample datasets?

Sequencing datasets generated with the Tumour Methylation Profiling protocols are available for download here. The data is organised by protocol and run, so you can download individual datasets or replicates on their own. The release covers all three protocols (Multiplex Methylation Whole Genome Sequencing, Singleplex Adaptive Sampling, and Ultra-rapid Singleplex Adaptive Sampling) and includes raw POD5 files and basecalled BAM files.

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