AWMGS
All Wales Medical Genomics Service

Request for Molecular Analysis of Tumour Sample
All Wales Medical Genomics Laboratory

Patient forename		Surname	Consultant
Date of birth	Sex	Hospital (***essential for report***)	Requested by
Address Postcode		Hospital number NHS number *(or affix addressogram)* Alternative hospital number Date requested	Email addresses for reports (*NHS Wales or NHS.net*): Oncologist (s) Pathologist (s) MDT Co-ordinator (s)

Please note: Gene analysis relies on sampling tumour tissue only.
Tissue blocks for genomic analysis can no longer be accepted.

Pathologist	Pathology Hospital	Block number
Sampling method, biopsy type, and fixation method		Date block sent to Lab Genetics
Relevant clinical details (e.g. tumour histology) ***Please also attach appropriate pathology report***		

Please indicate PRIMARY tumour type (this determines which NGS gene panel analysis is applied):

☐ Lung ☐ Colorectal ☐ Melanoma ☐ Glioma ☐ GIST ☐ Thyroid

Other (please specify) _____

See reverse of form for DNA- and RNA-based gene panel analysis performed on each tumour type.

Please indicate analysis required (>1 can be ticked):

☐ DNA-based NGS ☐ MGMT promoter methylation (glioma only) ☐ 1p/19q FISH (glioma only)

☐ RNA-based NGS (replaces FISH testing for ALK and ROS1, and covers NTRK1/2/3)*

*If <30ng RNA is obtained, the FISH salvage pathway will be used in place of RNA-based NGS. Should this salvage pathway be required and insufficient material be available to perform all relevant analyses, tests will be performed in accordance with the published gene fusion frequencies within the tumour type, with the most frequently rearranged gene being the first to be tested.

Please provide: For ALL requests: **1 x H&E** stained slide with area of highest neoplastic cell content CLEARLY circled (use of fine black marker recommended).

Please state the ~ % neoplastic cell content present in the H&E highlighted tumour area _____%.

AND: For DNA-based NGS panel testing (& MGMT for Gliomas): **60 µM** (preferably 6x10µM) air dried unstained sections mounted on slides

AND: For 1p/19q FISH analysis: **4 x 3-4µM** sections (singly mounted) on charged/adhesion slides

AND: For RNA-based NGS panel testing: **50 µM** (preferably 5x10µM) air dried unstained sections mounted on slides. Note: slides for RNA ideally prepared in an RNase-free environment.

AND: For salvage FISH testing (in the event that RNA-based NGS cannot be performed/is unsuccessful):

ALK or ROS1 FISH analysis: **2 x 3-4µM** sections (singly mounted) on charged/adhesion slides PER GENE

NTRK1, NTRK2, and NTRK3 FISH analysis: 2 x 3-4µM sections (singly mounted) on charged/adhesion slides PER GENE

For all RNA-based NGS panel testing requests, we ask that additional slides for FISH analysis are **provided upfront** at point of test request to allow activation (with minimal delay) of the salvage FISH pathway for clinically relevant gene rearrangements in the event that insufficient RNA is obtained for NGS testing/NGS testing is unsuccessful.

Samples should be dispatched as soon as possible as the patient's treatment is dependent upon the molecular analysis.

Funding (information mandatory for testing):

☐ Welsh NHS patient – WHSSC-funded ☐ Other (please specify) _____

Please complete this request form and send with the sample to:
All Wales Medical Genomics Laboratory, Institute of Medical Genetics,
University Hospital of Wales, Heath Park, Cardiff CF14 4XW

Laboratory contact details for enquiries: Phone - 02920 742641 Email – lab.genetics@wales.nhs.uk
For further information on testing, refer to the AWGL website http://www.wales.nhs.uk/AWMGS

DNA-based Next Generation Sequencing (NGS) gene panel analysis for the detection of single nucleotide genetic variations and small indels

DNA-based NGS testing comprises the analysis of the following genes if >50ng DNA is obtained from the FFPE sample provided:

Lung EGFR (clinically relevant hotspots) KRAS NRAS PIK3CA CDKN2A PTEN RET BRAF ERBB2 (HER2)	**Colorectal** KRAS NRAS BRAF PTEN TP53 EGFR PIK3CA	**All other tumour types are analysed across the complete panel:** Androgen receptor ARID1A ATRX BRAF BRCA1 BRCA2 CDKN2A EGFR ESR1 ERBB2 H3F3A HRAS IDH1 IDH2 KIT KRAS NRAS PDGFRA PIK3CA PTEN RET TERT TP53
Melanoma BRAF KIT NRAS TP53 CDKN2A PTEN	**GIST** KIT PDGFRA	
Glioma IDH1 IDH2 BRAF EGFR ATRX H3F3A TERT PTEN TP53 CDKN2A	**Thyroid** BRAF EGFR KRAS NRAS PIK3CA TP53 HRAS RET TERT	

Note: if <50ng of DNA is obtained from the FFPE sample provided, an alternative NGS panel will be utilised for testing, which provides hotspot analysis of all clinically actionable genes relevant to the tumour type but overall a less comprehensive gene analysis.

RNA-based Next Generation Sequencing (NGS) gene panel analysis for the detection of gene rearrangements (replaces ALK and ROS1 FISH service)

RNA-based NGS testing comprises the analysis of the following genes if >30ng RNA is obtained from the FFPE sample provided:

Lung EGFR* ALK MET* RET ROS1 NTRK1 NTRK2 NTRK3	**All other tumour types:** NTRK1 NTRK2 NTRK3
Glioma EGFR* BRAF NTRK1 NTRK2 NTRK3	**Tumour types can be analysed for the whole panel on request:** ALK BRAF EGFR* MET* NTRK1 NTRK2 NTRK3 RET ROS1
Thyroid EGFR* RET NTRK1 NTRK2 NTRK3	

*Gene rearrangements within EGFR relate to EGFRvIII, which is an in-frame deletion of exons 2-7; clinically relevant EGFR hotspot variant analysis for lung cancer samples requires DNA-based NGS analysis. Gene rearrangements within MET relate to MET exon 14 skipping events.

Note: if <30ng of RNA is obtained from the FFPE sample provided, FISH analysis will be performed** as an alternative for NTRK1, NTRK2, and NTRK3 analysis, as well as ALK and ROS1 analysis (where requested).
**providing that sufficient material is provided.