Selecting blocks, marking up slides and assessing for molecular testing

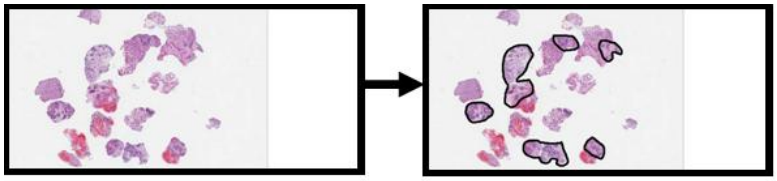
- Molecular testing is undertaken for diagnostic, prognostic and predictive reasons on a range of cancers.
- All cases should be selected, marked up and assessed by a pathologist before sending to the genomic lab.

Selecting blocks

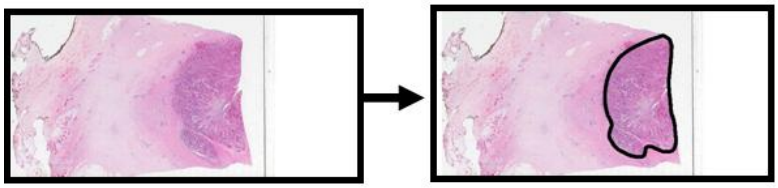
- Primary or metastatic tumour, and cell block, biopsy or resection specimens are all ok to use for testing. Resection mega-blocks can be used if no tumour exists in standard blocks.
- Oncologists might specify which sample to test e.g. the most recent case if looking for emerging resistance mutations, otherwise chose a sample with maximal tumour content (see below).
- The minimum tumour content for testing depends on the technology being used:
    - For next generation sequencing, at least 5% tumour cells in the area marked for extraction/testing.
    - For methylation testing, at least 15% tumour cells in the area marked for extraction/testing.
    - For whole genome sequencing, at least 30% tumour cells in the area marked for extraction/testing.
- Aiming for >30% tumour in all cases is preferable, if necessary select a different block/case if one exists.
- Samples should aim to contain minimal necrosis (for WGS there is a requirement of <20% by area).
- Samples should contain sufficient overall cellularity for the technology being used:
    - For FISH, a minimum of 50 tumour cells per section but ideally >100.
    - For sequencing, samples that have very low cellularity (<700 cells/section) are usually unsuitable.
- For colorectal cancer, select up to five tumour blocks to pool together to ensure adequate sampling.

Marking up the slide(s)

- For tests requiring nucleic acid extraction, macro-dissection maximises tumour content and increases the chances of detecting molecular events.
- For FISH, marking up the area of maximal tumour content facilitates focusing on tumour rich areas.
- A “before” and “after” H&E are cut either side of the unstained sections to be tested.
- Mark the area for extraction/FISH reporting on the “before” H&E looking to maximise tumour content. This should be done on all “before” slides if multiple blocks are used. If there are multiple areas of tumour, they can be marked separately as shown below. Aim to minimise non-tumour areas, though marked up areas should ideally not be smaller than 3 mm in width to allow accurate macro-dissection.
- If all of the tissue on the slide should be used, mark around the whole area (do not leave the slide blank).
- Check that the tumour has not cut out on the “after” H&E (if so, write this on the slide).



Biopsy with tumour rich areas marked



Resection with tumour rich area marked

Assessing tumour %, necrosis % and overall cellularity

- In order to interpret the test result, it is essential to document tumour %, necrosis % and overall cellularity.
- For tumour %, estimate the percentage of tumour cells out of all nucleated cells within the marked up area (average this across the total area if multiple blocks/areas have been used). Note that this is **not** an area assessment. Samples cannot be 100% tumour unless they are pure tumour cell lines – there are always some background cells e.g. stroma/inflammatory cells. See examples over the page.
- Necrosis % is done as an % area assessment within the marked up area. Aim to exclude necrosis from the marked up areas such that it totals less than 20% (samples with more than 20% necrosis are not suitable for whole genome sequencing). Again average this across the total area if multiple blocks have been used.
- Overall cellularity assesses the total number of nucleated cells per section. There are 5 categories: very low (<700), low (700-4,000), medium (4,000-10,000), high (10,000-50,000) and very high (>50,000).

- These assessments are well recognised to be subjective. Reproducibility can be improved by undertaking the recommended HEE training (https://www.genomicseducation.hee.nhs.uk/education/online-courses/tumour-assessment-in-the-genomic-era/) and completing the regular GenQA Tissue-I scheme (https://genqa.org/eqa).

Completing the request form

- Check that the genomic request form is complete and contains the clinical details, test(s) required, sample type, tumour type, tumour %, necrosis %, overall cellularity, and name of the assessing pathologist.
- The request form, marked H&E's and unstained sections can now be sent to the genomics lab.

Example tumour percentage assessments