

Timeline working days (total days) day of week	Process	Comments
Day 1 (1) Tuesday	Material assessment	Methylation profiling requires high tumour content (>70% tumor cells of total cells) Hematoxylin and eosin staining for identification of optimal area. Scraping of tumour cell rich areas from unstained slides (10 slides of 10 µm each) or punch extraction from paraffin block (either 1.5 mm or 3 mm diameter). Cases with low tumor content are avoided when possible.
Day 2 (2) Wednesday	DNA extraction (1/2)	Day one of automated DNA extraction (Maxwell 16 FFPE plus LEV DNA purification kit, AS1135, Promega).
Day 3 (3) Thursday	DNA extraction (2/2) DNA quality control Bisulfite conversion (1/2)	Day two of DNA extraction. Concentration measurement using Qubit (Qubit dsDNA BR assay kit, Q32853, Invitrogen). Illumina FFPE QC kit only used optionally (e.g. for highly necrotic cases). Day one of bisulfite conversion of 250ng (in exceptional cases as little as 50-100ng) (Zymo EZ DNA methylation kit; D5002, Zymo).
Day 4 (4) Friday	Bisulfite conversion (2/2) DNA Restoration	Day two of bisulfite conversion. FFPE DNA Restore (Illumina). DNA cleanup. Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual. Freezing over weekend.
Day 5 (7) Monday	Whole genome amplification	Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual.
Day 6 (8) Tuesday	Fragmentation, precipitation Resuspension Array hybridization (1/2)	Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual.
Day 7 (9) Wednesday	Array hybridization (2/2) Washing and staining Scanning of array Technical quality control	Part of methylation array kit provided by Illumina. Performed strictly according to instruction manual. Technical quality control using assay internal system controls following the instruction manual. Eliminate bad samples.
Day 8 (10) Thursday	Data upload to Webportal Integration with pathological findings Writing of reports	Upload to <a href="http://www.molecularneuropathology.org">www.molecularneuropathology.org</a> Gender check. Checking for genotype matches if established. Exclusion of case swapping. Integration of pathological findings with methylation profiling. Possibly reconsideration of histology and possibly additional molecular investigations required.