

Supplemental Methods

Immunohistochemical staining

4µm FFPE sections were deparaffinized with xylene and rehydrated in ethanol. A 0.3% H₂O₂ solution was used to block endogenous peroxidase, and microwave-mediated antigen retrieval was performed in Tris-EDTA, pH 9.0. Sections were incubated overnight with primary antibodies against MLH1 (clone ES05, 1:50; Agilent, USA), MSH2 (clone FE11, 1:200, Agilent, USA), MSH6 (clone EPR3945, 1:200, Genetex, USA) or PMS2 (clone EP51, 1:40, Agilent, USA) at 4°C. After washing, they were then incubated for 30 minutes with poly-HRP (VWRKDPVM110HRP, ImmunoLogic), visualised using a DAB+ substrate chromogen system (K3468; Agilent) and counterstained with haematoxylin. Finally, the sections were dehydrated and mounted with coverslips.

Targeted Next Generation Sequencing (NGS)

Sequencing was performed using the Ion Torrent platform according to the manufacturer's recommendations. In brief, 21 ng/14 µl isolated DNA was used to prepare two primer pools. After the first PCR, the pools were combined and a new PCR run was performed to digest the primers. A third PCR was then performed to add barcodes to the samples. After purification using AMPureXP beads (A63882; Beckman Coulter), the NGS libraries were pooled, diluted to 60 pmol/L and loaded on a chip using the Ion Chef. Sequencing was subsequently performed in an Ion GeneStudio S5 Series sequencer.